Universidad CEU San Pablo
CEINDO – CEU Escuela Internacional de Doctorado

PROGRAMA en CIENCIA Y TECNOLOGÍA DE LA SALUD

Nuevas aplicaciones del anillo de ciclopropano: iminoazúcares rigidificados como inhibidores de glicosidasas y nuevos reordenamientos de sustratos cicloprpénicos

TESIS DOCTORAL
Presentada por:
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A mi familia

La presente memoria titulada: *"Nuevas aplicaciones del anillo de ciclopropano: iminoazúcares rigidificados como inhibidores de glicosidasas y nuevos reordenamientos de sustratos ciclopropénicos"*, se presenta por compendio de publicaciones con el visto bueno de mis supervisores, los profesores Javier Pérez Castells y Gema Domínguez Martín. Hemos seguido los requerimientos aprobados por el comité directivo de CEINDO el 30 de noviembre de 2017. Con el objetivo de conseguir la mención internacional, está escrita en español y se incluye el resumen y las conclusiones en inglés. Se ha llevado a cabo en el Departamento de Química y Bioquímica, en la Facultad de Farmacia de la Universidad San Pablo CEU (Madrid, España). He realizado una estancia de un mes en el grupo de investigación del profesor F. Javier Cañada en el CIB, CSIC (Madrid, España). Además, realicé otra estancia de 4 meses en Georg-August-Universität, en el laboratorio del profesor Lutz. Ackermann (Göttingen, Alemania).

Esta tesis ha dado lugar a las siguientes publicaciones:

- Puet, A.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J. Amino Acid-Based Synthesis and Glycosidase Inhibition of Cyclopropane-Containing Iminosugars. *ACS Omega* **2020**, *5*, 31821-31830. DOI: 10.1021/acsomega.0c04589.
- Puet, A.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J. Synthesis and Evaluation of Novel Iminosugars Prepared from Natural Amino Acids. *Molecules* **2021**, *26*, 394. DOI: 10.3390/molecules26020394.
- Puet, A.; Domínguez, G.; Pérez-Castells, J. Straightforward Synthesis of Highly Functionalized Indanes and Tetralines Through Ene-Cyclopropene Rearrangement Mediated by Ruthenium. *J. Org. Chem.* **2022**, *87*, 2686-2696. DOI: 10.1021/acs.joc.1c02636.
- Puet, A.; Giona, E.; Domínguez, G.; Pérez-Castells, J. One Pot Synthesis of Spirocycles and Cyclopropa[*b*]pyrans by Alkenylation-Rearrangement of Cyclopropenes. *J. Org. Chem.* **2022**, *87*, 12470-12476. DOI: 10.1021/acs.joc.2c01420.
- Martínez, A. M.; Puet, A.; Domínguez, G.; Alonso, I.; Castro-Biondo, R.; Pérez-Castells, J. Intramolecular Diels-Alder Reaction of Cyclopropenyl Vinylarenes: Access to Benzonorcarane Derivatives. *Org. Lett.* **2023**, *25*, 5923-5928. DOI: 10.1021/acs.joc.2c01420.

La siguiente publicación incluye el trabajo que llevé a cabo durante mi estancia en el laboratorio del Prof. Lutz Ackermann.

- Kaplaneris, N.; Puet, A.; Kallert, F.; Pohlmann, J.; Ackermann, L. Late-Stage Functionalization of Tryptophan-Containing Peptides with Thianthrenium Salts: Conjugation and Ligation. *Angew. Chem. Int. Ed.* **2023**, *62*, e202216661. DOI: 10.1002/anie.202216661.

Esta tesis ha sido supervisada por los profesores Javier Pérez Castells y Gema Domínguez Martín. Primero me gustaría agradecerles la oportunidad que me brindaron de poder trabajar en su laboratorio: gracias a ellos me he formado y aprendido tanto, no sólo en el entorno químico. Estoy agradecido de muchas cosas, pero quiero destacar alguna de ellas: la primera es la confianza que han depositado siempre en mí, para cualquier labor en el laboratorio; la segunda el apoyo y la paciencia que siempre me han demostrado, incluso en los momentos más difíciles. A todo hay que sumar el gran ambiente de trabajo que se respira en su laboratorio.

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Me gustaría agradecer también la oportunidad que me brindó el Prof. Lutz Ackermann al dejarme realizar una estancia de 4 meses en su laboratorio, junto con el resto de los investigadores. Sobre todo, al Dr. Nikolaos Kaplaneris por la confianza y apoyo a la hora de trabajar codo con codo en un mismo proyecto; a Lina, por su alegría y ayuda en el laboratorio y al Dr. Efraím Reyes por los dos últimos meses de mi estancia en Alemania.

Alberto, José María y Jorge, mis compañeros de doctorado, con los cuales he compartido muchos momentos tanto dentro como fuera del laboratorio, creando una atmósfera inmejorable. Quiero agradecerle a Manu el buen humor y lo agradable que es trabajar a su lado, además de su predisposición a ayudar en cualquier momento. Tengo que agradecer también al grupo de Química Médica de nuestra universidad, por los recursos que me han proporcionado, imprescindibles para la realización de esta tesis. A Lourdes, con la que he compartido mucho haciendo que este tiempo fuera más ameno y agradable con su inacabable felicidad. A Alba por estar siempre, tanto en el laboratorio como fuera de él, todos los desayunos, cenas y momentos donde no sólo ha sido un consuelo, sino también una liberación y distracción en los momentos difíciles.

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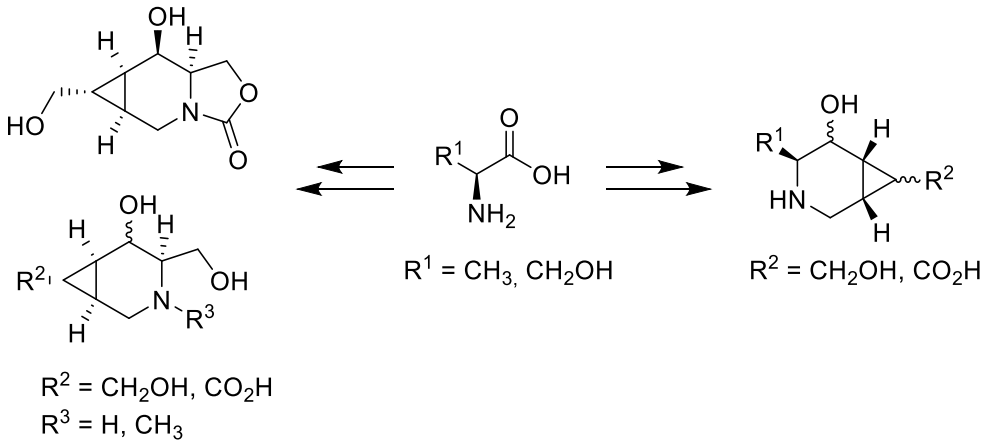
ABREVIATURAS

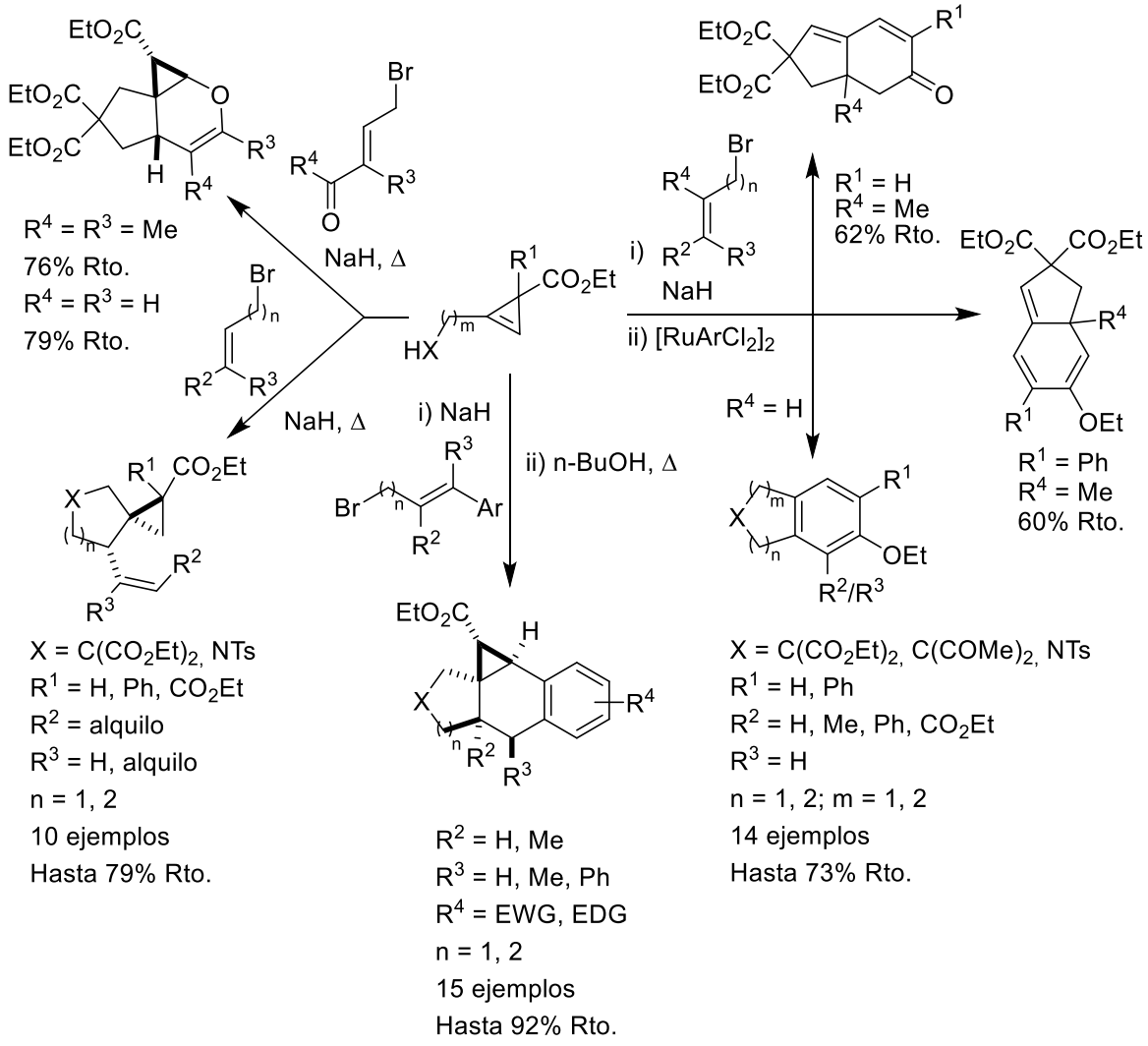
1-(DNJ)	1-deoxinojirimicina
DAB	1,4-didesoxi-1,4-imino-D-arabinitol
DCE	Dicloroetano
DMDP	*N*-carboxymethyl-2,5-dideoxy-2,5-imino-D-mannitol
DMSO	Dimetilsulfóxido
EDG	Grupo electrodonador
EMA	Agencia Europea del Medicamento
esp	Ácido $\alpha,\alpha,\alpha',\alpha'$-tetrametil-1,3-bencenodipropiónico
EWG	Grupo electroatractor
Gb3	Globotriasoilceramida
GH	Glicosil hidrolasa
GT	Glicosil transferasa
HOMO	*Highest Occupied Molecular Orbital*
IUBMB	*International Union of Biochemistry and Molecular Biology*
LAB	1,4-Didesoxi-1,4-imino-L-arabinitol Iminosazúcar
LED	Diodo Emisor de Luz
LSF	Funcionalización de última etapa
LUMO	*Lowest Unoccupied Molecular Orbital*
RCM	Metátesis de Cierre de Anillo
RMN	Resonancia Magnética Nuclear
$Ru_3(CO)_{12}$	Dodecacarbonil trirutenio (0)
SOI	Interacción del Orbital Secundario
TBAF	Fluoruro de tetrabutilamonio
TBATB	Tribromuro de tetrabutilamonio
TsOH	Ácido *p*-toluensulfónico
VIH	Virus de Inmunodeficiencia Humana

INTRODUCCIÓN GENERAL

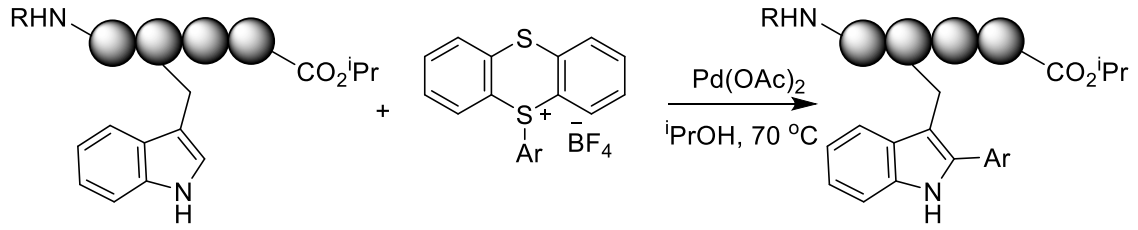
El primer capítulo de esta memoria trata de la síntesis de iminoazúcares fusionados a un anillo de ciclopropano y de su evaluación como posibles inhibidores de glicosidasas. Fue llevado a cabo en colaboración con el grupo del profesor F. Javier Cañada, en el Centro de Investigaciones Biológicas (CIB) en el CSIC en Madrid, donde se realizaron los ensayos enzimáticos de los compuestos sintetizados previamente. De este proyecto surgieron dos publicaciones científicas en las que se recogen las síntesis orgánicas de los compuestos y los resultados de los ensayos enzimáticos. Dichas revistas fueron elegidas por ser de química multidisciplinar y de acceso abierto: *ACS Omega*, con un índice de impacto de 3,613 en 2020 (Q2) publicado por la *American Chemical Society* y en *Molecules*, con un índice de impacto de 5,110 en 2021 (Q2) publicado por el *MDPI*.



El segundo capítulo se enmarca en una línea de investigación de nuestro grupo encaminada al estudio de la reactividad de ciclopropenos catalizada por complejos de rutenio. Partiendo de ciclopropenos como productos de partida, se obtuvieron, por un lado compuestos aromáticos funcionalizados, y por otro, cambiando las condiciones de reacción, productos espirocícliclos, mediante una reacción Alder-énica. Ambas síntesis fueron publicadas en sendos trabajos en el *Journal of Organic Chemistry*, una revista de alto impacto en el campo de la química orgánica (3,608 de índice de impacto en 2022, Q1). Un último trabajo ha sido aceptado por la revista *Organic Letters* (5,214 de índice de impacto en 2022, Q1). En este trabajo se presenta el reordenamiento de ciclopropenos, mediante una reacción de Diels-Alder, para obtener compuestos tetracíclicos de manera diastereoselectiva.



En el último capítulo se recoge el trabajo realizado durante mi estancia en el laboratorio del Prof. Lutz Ackermann, en Alemania. En dicho se trabajo se consiguió la activación C-H del anillo de indol en el triptófano con el objetivo de poder modificar péptidos de una manera sencilla. Los resultados se publicaron en *Angewandte Chemie International Edition*, una revista de química multidisciplinar con un índice de impacto de 16,592 en 2022 (Q1).



OBJETIVOS

En el primer capítulo de esta tesis se presenta la síntesis de diversos iminoazúcares rigidificados por un anillo de ciclopropano y su posterior estudio como posibles inhibidores de glicosidasas. La evaluación de los productos sintetizados se hizo en colaboración con el Prof. F. Javier Cañada en el CIB, CSIC. Los objetivos de este capítulo son los siguientes:

- Síntesis del mayor número posible de iminoazúcares fusionados a un anillo de ciclopropano como elemento rigidificador, empezando con la L-serina y la L-alanina como materiales de partida.
- Estudio de la capacidad inhibitoria de los productos obtenidos frente a 8 glicosidasas diferentes (α,β-glucosidasas, α,β-galactosidasas, α,β-manosidasas, α-L-fucosidasa y una neuraminidasa) mediante ensayos *in vitro* y técnicas de RMN.

El segundo capítulo está dedicado al estudio de la reactividad de sistemas ciclopropen-énicos dentro de una línea de trabajo del grupo que ya llevaba varios años desarrollándose. Una parte consiste en un reordenamiento catalizado por complejos dímeros de rutenio. Otra parte, que surge durante el estudio de la reacción de reordenamiento, consiste en reacciones pericíclicas de estas estructuras. Los objetivos de este capítulo son los siguientes:

- Optimización de la reacción de reordenamiento de estructuras ciclopropen-énicas hacia sistemas aromáticos catalizada por dímeros de rutenio.
- Estudio del alcance de la reacción mediante la creación de una quimioteca de compuestos con diferente sustitución.
- Propuesta de un curso razonable para la reacción.
- Desarrollo y optimización de la síntesis *"one pot"* de espirociclos mediante una reacción Alder-énica, con el estudio de la influencia de la estereoquímica del doble enlace en el producto final y alcance de la reacción.
- Estudio de la reacción intramolecular de Diels-Alder en vinilarenos y ciclopropenos con especial atención al resultado estereoquímico de la misma. Para ello se utilizarán cálculos computacionales y difracción de Rayos X.

En el tercer capítulo de esta memoria se recoge el trabajo realizado durante la estancia en Alemania. Se presenta la activación C-H del anillo de indol en triptófano para ser ligado con derivados de fármacos, aminoácidos u otros péptidos usando sales de tiantreno. Los objetivos propuestos en este proyecto son los siguientes:

- Optimización de la activación C-H en triptófano y síntesis de la sales usando fármacos y otros aminoácidos como fenilalanina y tirosina.
- Estudio de la compatibilidad de la reacción con diferentes grupos funcionales.
- Desarrollar una quimioteca donde se usen, a parte de diferentes anillos aromático, derivados de fármacos y aminoácidos, péptidos cíclicos y lineales.

CAPÍTULO I: NUEVOS IMINOAZÚCARES RIGIDIFICADOS COMO POTENCIALES INHIBIDORES DE GLICOSIDASAS

1. ANTECENDENTES

Los carbohidratos son conocidos por su importante papel estructural y de almacenamiento de energía estando, además, implicados en el reconocimiento molecular. Esta última función es clave en procesos como las infecciones bacterianas o víricas, la señalización y diferenciación celular o la metástasis de tumores entre otros.[1]

Los monosacáridos pueden variar en la estereoquímica, la configuración anomérica (α o β) y el tamaño del anillo, y pueden estar unidos con otros monosacáridos de manera lineal o ramificada. Esta variabilidad hace que un simple hexasacárido pueda tener más de 10^{12} posibles isómeros.[2] Esta enorme diversidad estructural supone la posibilidad de contener gran cantidad de información en moléculas relativamente pequeñas. Es lo que se conoce como "glicocódigo". Las uniones entre monosacáridos se denominan enlaces glilcosídicos. En ocasiones son enormemente estables, como sucede con la celulosa que tiene una vida media muy larga. Los sistemas biológicos recurren para las hidrólisis de estos enlaces a las enzimas glicosidasas que son capaces de aumentar la velocidad de hidrólisis hasta 10^{17} veces.[3] Este proceso enzimático se da en multitud de rutas metabólicas. Las glicosidasas suelen ser muy específicas, y se especializan en aspectos como pueden ser la configuración anomérica, la estereoquímica del producto tras la reacción enzimática, el lugar donde ocurre la hidrólisis en la cadena del polisacárido o el tipo de azúcar a hidrolizar. Esto explica el gran número de ellas que hay en el organismo, hasta el punto de que entre el 1% y el 3% de la información contenida en el código genético codifique estas enzimas.[4]

En 1965, Johnson y Phillips elucidaron por primera vez la estructura de una glicosidasa, más concretamente una lisozima, mediante el uso de difracción de rayos X (Figura 1).[5] Tras este trabajo, se fueron elucidando diferentes estructuras, con lo que se revelaron los aspectos estructurales comunes de esta familia de enzimas.

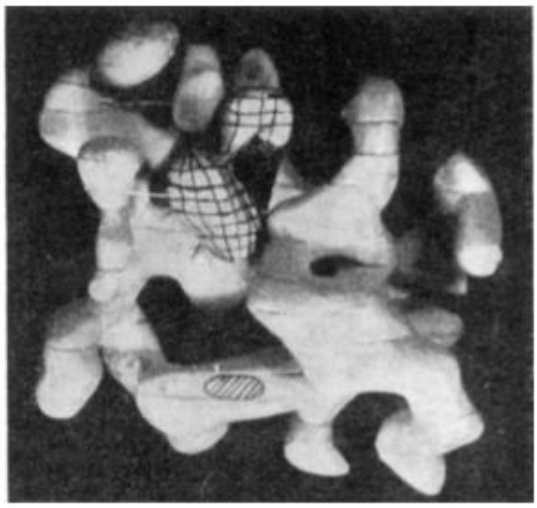


Figura 1. Estructura 3D de una lisozima.[5]

[1] Kötzler, M. P.; Hancock, S. M.; Withers, S. G. Glycosidases: Functions, Families and Folds, **2014**, In: eLS. John Wiley & Sons, Ltd: Chichester.
[2] Hancock, S. M.; Withers, S. G. Glycosidases: Functions, Families and Folds, **2007**, In: eLS. John Wiley & Sons, Ltd.
[3] Wolfenden, R.; Lu, X.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 6814-6815.
[4] Henrissat, B.; Davies, G. *Curr. Opin. Struc. Biol.* **1997**, *7*, 637-644.
[5] Johnson, L.; Phillips, D. *Nature* **1965**, *206*, 761-763.

La nomenclatura utilizada por la IUBMB (*International Union of Biochemistry and Molecular Biology*) para las glicosidasas se basa en su especificidad, pero no refleja sus aspectos estructurales. En 1990, Henrissat las clasificó por su secuencia de aminoácidos, que hizo posible predecir su relación estructura-actividad. Actualmente, esta clasificación es la más utilizada y sigue siendo actualizada, contando con más de 130 familias diferentes y plasmando sus aspectos funcionales, estructurales y mecanísticos.[6] A lo largo de los años se ha determinado que, a pesar de haber muchas familias de glicosidasas, sólo hay 4 centros catalíticos diferentes, que dependen del tipo de polisacárido a hidrolizar (Figura 2).

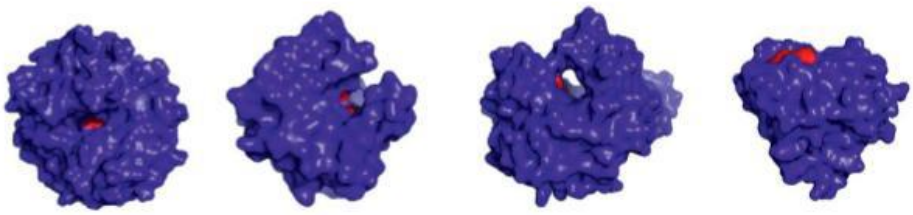


Figura 2. Diferentes centros catalíticos conocidos en glicosidasas.[1]

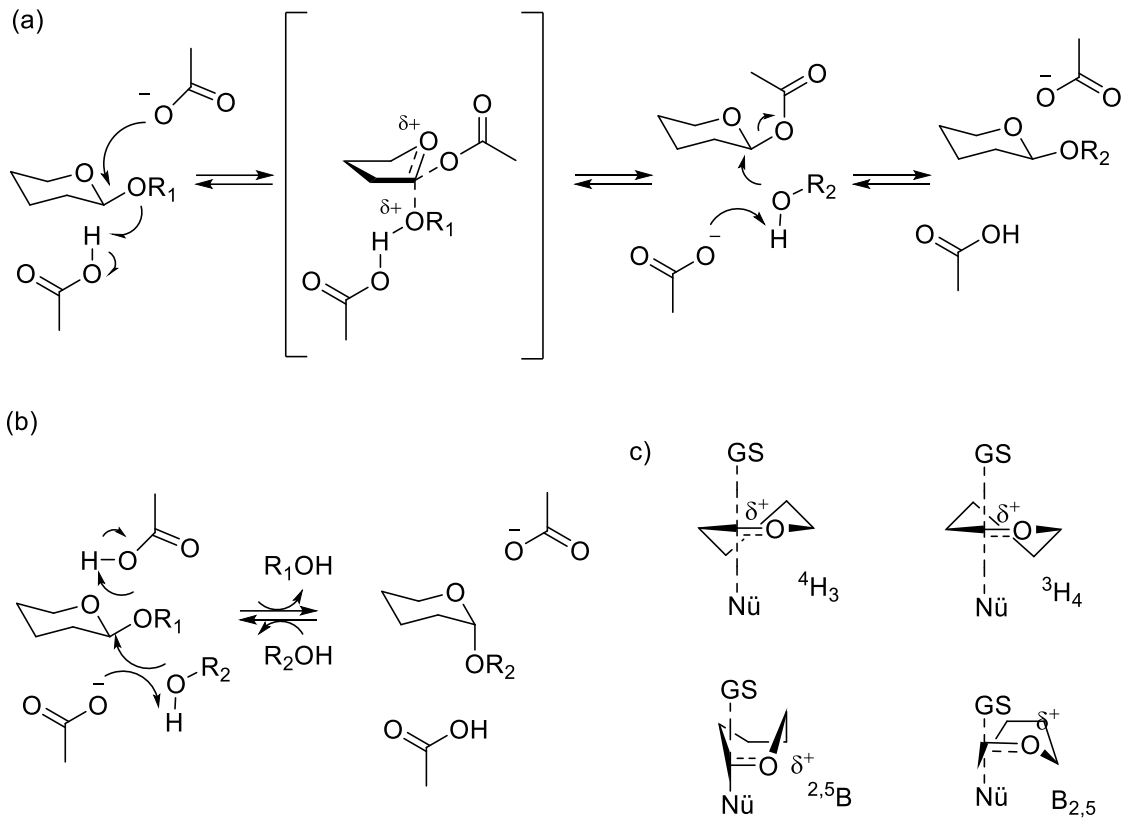
El nombre de las glicosidasas también hace referencia a la reacción que catalizan. Si hidrolizan el enlace glicosídico para obtener un monosacárido libre se llaman glicosil hidrolasas (GHs); si dan lugar a una transglicosidación, en la que el producto obtenido es otro azúcar diferente, se llaman glicosil transferasas (GTs); las fosforilasas introducen un grupo fosforilo en un monosacárido, y cuando el producto obtenido es un compuesto insaturado vía eliminación, se denominan liasas.[7]

1.1. MECANISMOS CATALÍTICOS

La hidrólisis de carbohidratos es una reacción fundamental que se ve acelerada por una catálisis ácida mediante un estado de transición electro-deficiente. Los azúcares son estables a pH básico y son las glicosidasas las encargadas de aportar el medio ácido mediante la cesión de un protón.[8]

Se han propuesto muchos mecanismos diferentes. En el Esquema 1 se muestran dos ejemplos, el primero transcurre con retención (Esquema 1a) y el segundo ocurre con inversión de la configuración en el carbono anomérico (Esquema 1b). En todos los casos participa un residuo ácido (ácido aspártico o glutámico) junto a un nucleófilo (una molécula de agua en caso de GHs u otro monosacárido en caso de GTs) que rompe el enlace glicosídico. Otro aspecto común que tienen estos mecanismos es el desarrollo de carga positiva en el oxígeno como intermedio y la pérdida de la conformación de silla del azúcar.[9] Sinnot fue el primero en describir este cambio en la estructura durante el estado de transición a las 2 estructuras de semi-sillas $^{4}H_{3}$ y $^{3}H_{4}$ (o las conformaciones relacionadas de sobre ^{4}E y ^{3}E) a través de una conformación de bote (Esquema 1c). En estas conformaciones el solapamiento orbital ayuda a estabilizar dicho ion.[8]

[6] www.cazy.org (visited on 08/06/2023).
[7] Bourne, Y.; Henrissat, B. *Curr. Opin. Struct. Biol.* **2001**, *11*, 593-600.
[8] Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171-1202.
[9] Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515-553.



Esquema 1. a) Hidrólisis con retención de la configuración. b) Hidrólisis con inversión de la configuración. c) Cambios en la estructura de silla durante el estado de transición.

1.2. INHIBICIÓN

El elevado número de glicosidasas presente en los organismos provoca que los carbohidratos posean muy baja estabilidad metabólica. La manera más común de inhibir estas enzimas es utilizando compuestos que mimeticen a los azúcares (glicomiméticos) y posean una mayor estabilidad frente a la hidrólisis. Los glicomiméticos son, generalmente, ciclos con múltiples grupos hidroxilo. La principal diferencia estructural con los carbohidratos naturales es que para lograr mayor estabilidad metabólica se reemplaza el oxígeno endocíclico de los azúcares, por otro átomo.[10] Estos compuestos se clasifican según el átomo sustituto en: tioazúcares, carbazúcares e iminoazúcares. Otros glicomiméticos son de tipo disacárido y hay también compuestos no glicosídicos (Figura 3).

[10] (a) Asano, N. *Glycobiology* **2003**, *13*, 93R-104R. (b) Cipolla, L.; Araújo, A. C.; Bini, D.; Gabrielli, L.; Russo, L.; Shaikh, N. *Expert Opin. Drug Discov.* **2010**, *5*, 721-737.

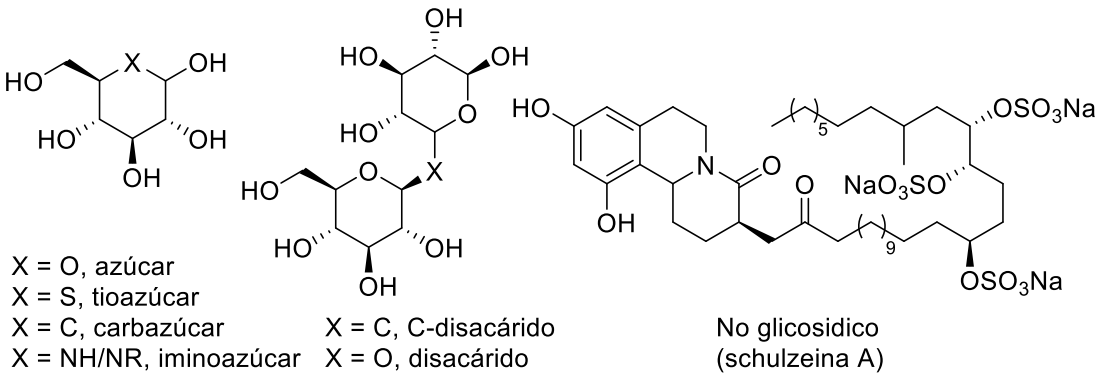


Figura 3. Miméticos de carbohidratos.

1.2.1. TIOAZÚCARES

En los tioazúcares el átomo de oxígeno del ciclo ha sido sustituido por uno de azufre, aunque también se denominan tioazúcares a disacáridos unidos por un puente sulfuro. Las propiedades biológicas diferenciadoras de estos compuestos son atribuidas al tamaño y densidad electrónica del azufre comparado con el oxígeno. Una de sus principales ventajas es su buena biodisponibilidad.[11] Los tioazúcares y sus derivados han mostrado propiedades inhibitorias y especificidad frente a algunas glicosidasas debido a que la interacción hidrofóbica con la enzima es más fuerte con el azufre que con el oxígeno.[12] Han mostrado actividad antineoplásica, antidiabética, antiviral y antitrombótica entre otras.[13] Sivapriya y colaboradores demostraron que la actividad de alguno de estos compuestos se encontraba en el rango de micro- a milimolar.[14] Para la preparación de estas estructuras se usan tanto reacciones enzimáticas como síntesis química clásica.[15]

Los tioazúcares no son muy abundantes en la naturaleza. El primer tioazúcar aislado de una fuente natural, la esponja marina *Clathria pyramida*, fue la 5-tio-D-manosa en 1987.[16] El salacinol[17] y el kotalanol[18] son 2 potentes inhibidores de la α-glucosidasa intestinal. Fueron aislados de una planta india y contienen un anillo de tetrahidrotiofeno que forma una sal de sulfonio (Figura 4).

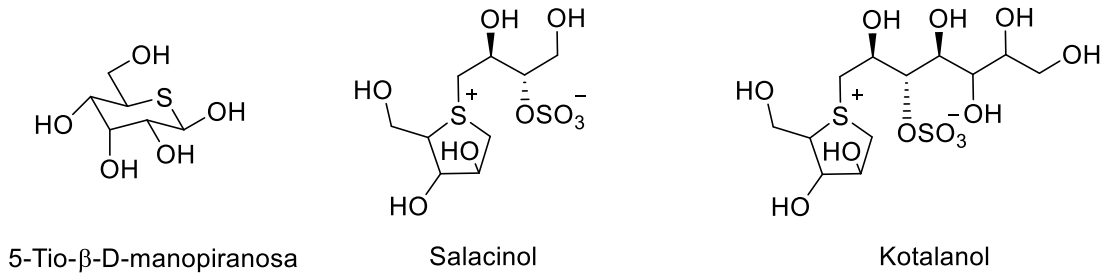


Figura 4. Ejemplo de tioazúcares de origen natural.

[11] Brás, N. F.; Cerqueira, N.; Ramos, M. J.; Fernandes, P. A. *Expert Opin. Ther. Patents* **2014**, *24*, 857-874.
[12] Kajimoto, T.; Liu, K. K. C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco., J. A. Jr.; Wong, C. H. *J. Am. Chem. Soc.* **1991**, *113*, 6187-6196.
[13] a) Hudlicky, T.; Price, J. D.; Rulin, F.; Tsunoda T. *J. Am. Chem. Soc.* **1990**, *112*, 9439-9440. b) Bozo, E.; Boros, S.; Kuszmann, J. *Carbohydr. Res.* **1998**, *311*, 191-202.
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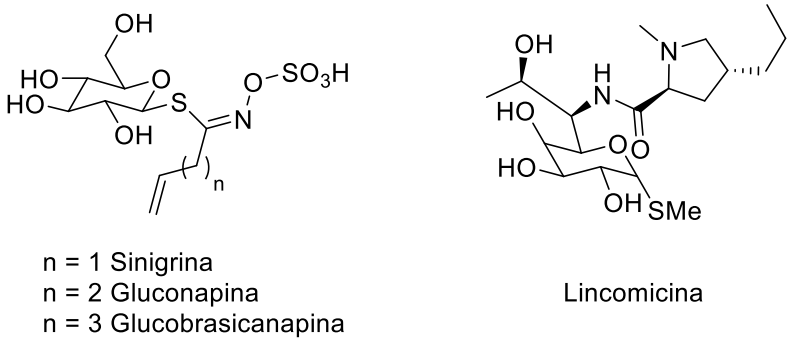
[16] Capon, R. J.; MacLeod, J. K. *J. Chem. Soc., Chem. Commun.* **1987**, 1200-1201.
[17] Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron* **1997**, *38*, 8367-8370.
[18] Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339-1340.

Los glucosinolatos son otro tipo de tioazúcares que, además del azufre, poseen un átomo de nitrógeno. Son metabolitos secundarios de plantas, encontrados principalmente en la familia *Brassicaceae*, como pueden ser el brócoli, las coles de Bruselas y la coliflor. Los más representativos son la sinigrina, gluconapina o glucobrasicanapina entre otros.[19] Tienen poder antioxidante, anticarcinogénico, antimicrobiano y pueden ser utilizados para preservar alimentos.[20] La actividad antimicrobiana de estos compuestos ha sido demostrada en diferentes trabajos, siendo eficaces contra bacterias y hongos.[21] Otros estudios han demostrado su capacidad antivírica frente a diversos coronavirus.[22] La lincomicina es el ejemplo más representativo de un tioazúcar con actividad biológica, cuya función principal no es la de interactuar con glicosidasas (Figura 5). Es un compuesto aislado de *Streptomyces lincolnensis*[23] que actúa por unión al ribosoma bacteriano, impidiendo que se traduzcan las proteínas. Debido a que puede causar efectos secundarios, no es actualmente el antibacteriano utilizado como primera opción a menos que el paciente presente alergias a las penicilinas.[24]



n = 1 Sinigrina
n = 2 Gluconapina
n = 3 Glucobrasicanapina

Lincomicina

Figura 5. Estructuras de glucosinolatos y de la lincomicina.

Algunos tioazúcares sintéticos han demostrado poseer actividad inhibitoria frente a glicosidasas. El mecanismo de acción no está establecido completamente, y parece que además de inhibir estas enzimas también interfieren en el transporte de carbohidratos a través de la pared intestinal. La mayoría de los estudios de su modo de acción han sido realizados computacionalmente.

1.2.2. CARBAZÚCARES

Los carbazúcares son derivados de carbohidratos en los que el oxígeno ha sido reemplazado por un átomo de carbono. Son por tanto poli-hidroxiciclohexanos, también llamados ciclitoles, y poli-hicroxiciclopentanos. Estas moléculas se comportan de forma muy diferente a sus análogos oxigenados puesto que no poseen función acetal o hemiacetal, lo que los convierte en compuestos con mayor estabilidad metabólica y mayor movilidad conformacional.[25] Aun así, son capaces de mimetizar casi a la perfección a sus correspondientes análogos oxigenados. Por ejemplo, las enzimas encargadas del metabolismo de la glucosa no

[19] Horbowicz, M. *Veg. Crops Res. Bull.* **2003**, *58*, 23-40.
[20] Saavedra, M. J.; Borges, A.; Días, C.; Aires, A.; Bennett, R. N.; Rosa, E. S.; Simoes, M. *Med. Chem.* **2010**, *6*, 174-183.
[21] a) Días, C.; Aires, A.; Bennet, R. N.; Rosa, E. A.; Saavedra, M. J. *Med. Chem.* **2012**, *8*, 474-480. b) Borges, A.; Abreu, A. C.; Ferreira, C.; Saavedra, M. J.; Simoes, L. C.; Simoes, M. *J. Food Sci. Technol.* **2015**, *52*, 4737-4748.
[22] Del Valle Mendoza, J.; Pumarola, T.; Gonzales, L. A.; del Valle, L. J. *Asian Pac. J. Trop. Med.* **2014**, *7*, S415-S420.
[23] Mason, D. J.; Dietz, A.; DeBoer, C. *Antimicrob. Agents. Chemother.* **1963**, *1962*, 554-559.
[24] Yatoo, M. *et al. J Pure Appl. Microbiol.* **2019**, *13*, 27-44.
[25] Zorin, A.; Klenk, L.; Mack, T.; Deigner, H.-S.; Schmidt, M. S. *Top. Curr. Chem.* **2022**, *380*, 12.

diferencian la carba-β-DL-glucopiranosa de la propia glucosa. La 6a-carba-β-DL-fructopiranosa demostró ser igual de dulce que la D-fructosa.[26]

McCasland y colaboradores fueron los primeros en describir la síntesis de carbazúcares, concretamente la 5a-carba-R-DL-talopiranosa, la 5a-carba-*R*-DL-galactopiranosa y la 5a-carba-α-DL-glucopiranosa.[27] El primer carbazúcar natural fue aislado en 1973 de la fermentación de *Streptomyces sp.*[28] A partir de este trabajo, se publicaron diversos estudios sobre la inhibición de enzimas o para el tratamiento de VIH y cáncer basados en el comportamiento conformacional de este compuesto.[29] Sólo algunos ciclitoles, como la cariosa y el calditol, han sido aislados como productos naturales, y no hay otros ejemplos de carbaciclos de 5 eslabones procedentes de fuentes naturales. En cambio, compuestos como la carba-α-D-galactopiranosa o el ciclofelitol, son ejemplos de carbapiranosas encontradas en la naturaleza,[30] mientras que las valienaminas puedes ser encontradas como subunidades en moléculas más complejas (Figura 6).

El ciclofelitol es un potente inhibidor de β-glucosidasas, y también tiene actividad frente al VIH y posible actividad antimetastática.[31] Su diastereómero no natural, (1*R*,6*S*)-ciclofelitol, es capaz de inhibir α-glucosidasas.[32] Otro compuesto con actividad biológica es la carba-α-D-galactopiranosa, que posee actividad antibacteriana contra *Klebsiella pneumonia*,.[33] Un estudio sobre la liberación de insulina estimulada por la D-glucosa mostró que la 5a-carba-α-DL-glucopiranosa inhibe la glucoquinasa.[34]

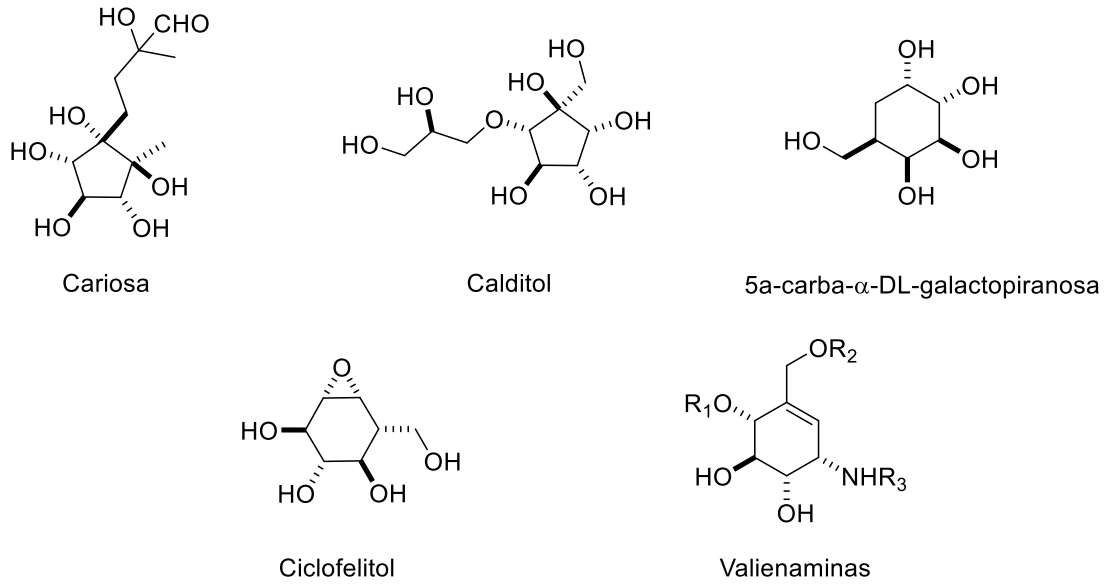


Figura 6. Carbafuranosas y carbapiranosas encontradas en la naturaleza.

Para la síntesis de estos de compuestos se han desarrollado diversas metodologías, la mayoría de ellas empleando monosacáridos como productos de partida, como puede ser la

[26] Ogawa, S.; Uematsu, Y.; Yoshida, S.; Sasaki, N.; Suami, T. *J. Carbohydr. Chem.* **1987**, *6*, 471-478.
[27] (a) McCasland, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1966**, *31*, 1516-1521. (b) McCasland, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1968**, *33*, 2841-2844.
[28] Miller, T. W.; Arison, B. H.; Albers-Schonberg, G. *Biotechnol. Bioeng.* **1973**, *15*, 1075-1080.
[29] (a) Arjona, O.; Gómez, A. M.; López, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919-2036. (b) Lahiri, R.; Ansari, A. A.; Vankar, Y. D. *Chem. Soc. Rev.* **2013**, *42*, 5102-5118. (c) Leerman, T.; Block, O.; Podeschwa, M. A. L.; Pfueller, U.; Altenbach, H.-J. *Org. Biomol. Chem.* **2010**, *8*, 3965-3974.
[30] Marco-Contelles, J. *Eur. J. Org. Chem*. **2001**, 1607-1618.
[31] Atsumi, S.; Iinuma, H.; Nosaka, C.; Umezawa, K. *J. Antibiot.* **1990**, *43*, 1579-1585.
[32] Tatsuta, K.; Niwata, Y.; Umezawa, K.; Nakata, M. *J. Antibiot.* **1991**, *44*, 456-458.
[33] Suami, T.; Ogawa, S. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 21-90.
[34] Miwa, I.; Hara, H.; Okuda, J.; Suami, T.; Ogawa, S. *Biochem. Int.* **1985**, *11*, 809-816.

glucosamina. Estas rutas de síntesis requieren muchos pasos, y los rendimientos globales no son elevados.[35] Alternativamente, se han desarrollado otras aproximaciones más rentables como pueden ser las basadas en ciclohexanodiol, en norborneno, en la reacción de Diels-Alder, etc.[36]

1.2.3. DISACÁRIDOS

Los principales disacáridos que destacan por su poder inhibitorio son la kojibiosa y la nigerosa (Figura 7). Estos compuestos son inhibidores de la α-glucosidasa I y II respectivamente.[37] La kojibiosa fue aislada por primera vez de extractos de sake en 1957.[38] La nigerosa puede ser obtenida a través de la hidrólisis de la amilopectina.[39] Tras el descubrimiento de estos compuestos, se pensó en el uso de "pseudodisacáridos", como pueden ser los C-disacáridos, para el tratamiento del VIH. Los C-disacáridos no poseen la función cetal, ya que están unidos mediante un átomo de carbono, lo que les da una mayor estabilidad metabólica, haciendo que se distribuyan mejor en el organismo. Postema y colaboradores sintetizaron diferentes C-disacáridos que fueron capaces de inhibir β-glicosidasas.[40]

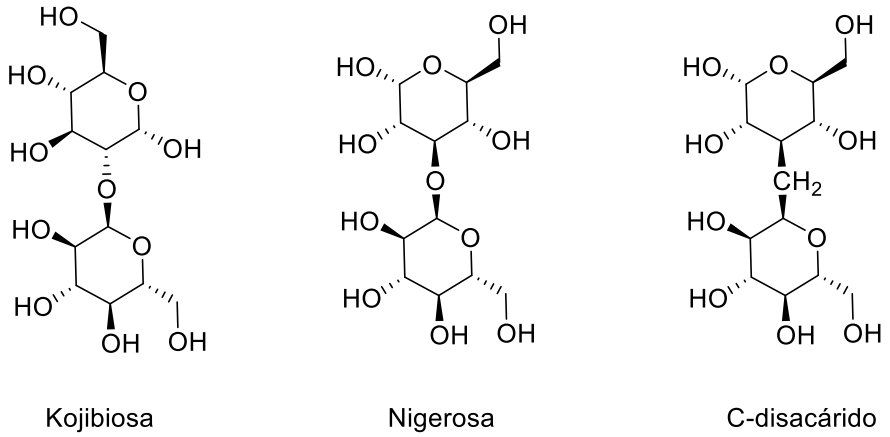


Figura 7. Estructuras de kojibiosa, nigerosa y C-disacáridos.

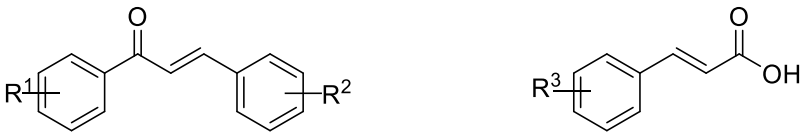
1.2.4. NO GLICOSÍDICOS

El centro activo de las glicosidasas normalmente posee triptófano. Este amino ácido aromático, interactúa con los azúcares mediante interacciones π que compensan la deficiencia electrónica del átomo de oxígeno en el estado de transición. La magnitud de dicha interacción ha sido calculada entre -0,5 y -0,8 kcal/mol, mientras que la estabilización usando residuos alifáticos se reduce hasta -0,1 kcal/mol.[41] Esto implica que los compuestos aromáticos pueden interactuar con las glicosidasas. Así, por ejemplo, estudios computacionales revelaron que el benzimidazol interacciona a través de los anillos de fenilo y de imidazol con diferentes residuos de fenilalanina y arginina en α-glucosidasas.[42] Se estudiaron algunos benzimidazoles tricíclicos frente diferentes glicosidasas, demostrando que este tipo de compuestos llegan a ser selectivos y sus interacciones puede ser racionalizadas.[43] Normalmente poseen una constante de

[35] Sun, Y.; Nitz, M. *J. Org. Chem.* **2012**, *77*, 7401-7410.
[36] Donohoe, T. J.; Blades, K.; Helliwell, M.; Waring, M. J.; Newcombe, N. J. *Tetrahedron Lett.* **1998**, *39*, 8755-8758.
[37] Ugalde, R. A.; Staneloni, R. J.; Leloir, L. F. *Eur. J. Biochem.* **1980**, *113*, 97-103.
[38] Sato, A.; Aso, K. *Nature* **1957**, *180*, 984-985.
[39] Wolfrom, M. L.; Thompson, A. *J. Am. Chem. Soc.* **1955**, *77*, 6403.
[40] Postema, M. H. D.; Piper, J. L.; Liu, L.; Shen, J.; Faust, M.; Andreana, P. *J. Org. Chem.* **2003**, *68*, 4748-4754.
[41] Laughrey, Z. R.; Kiehna, S. E.; Riemen, A. J.; Waters, M. L. *J. Am. Chem. Soc.* **2008**, *130*, 14625-14633.
[42] Arshad, T. *et al. Med. Chem. Res.* **2016**, *25*, 2058-2069.
[43] Prichard, K.; Campkin, D.; O´Brien, N.; Kato, A.; Fleet, G. W. J.; Simone, M. I. *Chem. Biol. Drug Des.* **2018**, *92*, 1171-1197.

inhibición baja, en el rango de μM. Se observó que cuando el anillo poseía un grupo nitro en cualquier posición, podía establecer puentes salinos con la enzima.[44]

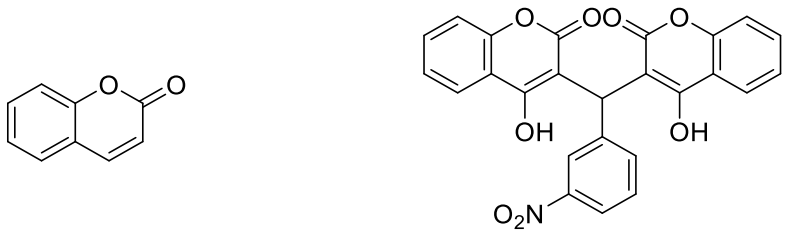
Las chalconas son compuestos capaces de inhibir glicosidasas. Se pueden encontrar en plantas y presentan diferentes propiedades farmacológicas, como antitumorales, anti-VIH, anti protozoarias, etc.[45] El ácido cinámico y sus derivados representan una clase en auge de inhibidores. Se ha demostrado la actividad del ácido *trans*-cinámico y algunos de sus derivados frente a α-glucosidasa,[46] de manera competitiva o no en función del compuesto estudiado (Figura 8).



Chalconas Ácido *trans*-cinámico

Figura 8. Chalconas y ácidos *trans*-cinámicos.

Khan y colaboradores sintetizaron derivados de cumarinas y *bis*-cumarinas, que resultaron ser inhibidores de α-glucosidasas. Estudios computacionales revelaron que los grupos hidroxilo en la dihidroxi *bis*-cumarina podían establecer hasta 6 enlaces de hidrógeno con los ácidos aspártico y glutámico y con argininas en el centro activo. Se predijo que el anillo aromático sería capaz de establecer interacciones con residuos de arginina además de diferentes interacciones hidrofóbicas con valinas y fenilalaninas. Los cálculos mostraron que cuanto mayor poder electroatractor posee el sustituyente en el anillo aromático, mayor es la fuerza de unión. Los compuestos que poseían grupos nitro llegaban a inhibir con un IC_{50} de 27 μM (Figura 9).[47]



Cumarina Derivado de *bis*-cumarina

Figura 9. Estructura de la cumarina y derivado de *bis*-cumarina.

Los flavonoides son inhibidores de α-amilasas y α-glucosidasas.[48] Lo Piparo y colaborades estudiaron los requisitos estructurales para que estos compuestos tuvieran actividad inhibitoria. Encontraron siete compuestos con IC_{50} de entre 10 y 50 μM. Estudios computacionales demostraron la formación de enlaces de hidrógeno con residuos de ácido aspártico y glutámico,

[44] (a) Taha, M.; Ismail, H.; Imran, S.; Mohamad, M. H.; Wadood, A.; Rahim, F.; Saad, S. M.; Rehman, A.; Khan, K. M. *Bioorg. Chem.* **2016**, *65*, 100-109. (b) Özil, M.; Emirik, M.; Etlik, S. Y.; Ulker, S.; Kahveci, B. *Bioorg. Chem.* **2016**, *68*, 226-235. (c) Singh, G.; Singh, A.; Singh, V.; Verma, R. K.; Tomar, J.; Mall, R. *Med. Chem. Res.* **2020**, *29*, 1846-1866.

[45] (a) Won, S.-J.; Liu, C.-T.; Tsao, L.-T.; Weng, J.-R.; Ko, H.-H.; Wang, J.-P.; Lin, C.-N. *Eur. J. Med. Chem.* **2005**, *40*, 103-112. (b) Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; Khan, S. R. *J. Med. Chem.* **2003**, *46*, 2813-2815.

[46] Adisakwattana, S.; Sookkongwaree, K.; Roengsumran, S.; Petsom, A.; Ngamrojnavanich, N.; Chavasiri, W.; Deesamer, S.; Yibchok-Anun, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2893-2896.

[47] Khan, K. M. *et al. Eur. J. Med. Chem.* **2014**, *81*, 245-252.

[48] (a) Xiao, J.; Kai, G.; Yamamoto, K.; Chen, X. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 818-836. (b) Proença, C.; Freitas, M.; Ribeiro, D.; Oliveira, E. F. T.; Sousa, J. L. C.; Tome, S. M.; Ramos, M. J.; Silva, A. M. S.; Fernandes, P. A.; Fernandes, E. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 1216-1228.

al igual que una fuerte interacción π-π con una tirosina.[49] Algunos flavonoides naturales, como los encontrados en *Adhatoa vasica Ness* y *Durante repens*, mostraron inhibición frente a glicosidasas.

1.2.5. IMINOAZÚCARES

Los iminoazúcares son derivados polihidroxilados de piperidina y pirrolidina. El potencial inhibitorio de estos compuestos es atribuido a su capacidad de mimetizar el ion oxocarbenio en el estado de transición de la reacción enzimática debido a la protonación del nitrógeno a pH fisiológico.[50] Los iminoazúcares se pueden obtener de numerosas fuentes naturales como plantas o bacterias, aunque como sucedió con otros glicomiméticos, los iminoazúcares naturales se encontraron después de que se hubieran descrito numerosos iminoazúcares sintéticos. Se clasifican en cinco grupos mayoritarios: piperidinas, pirrolidinas, indolizidinas, pirrolizidinas y nortropanos (Figura 10).

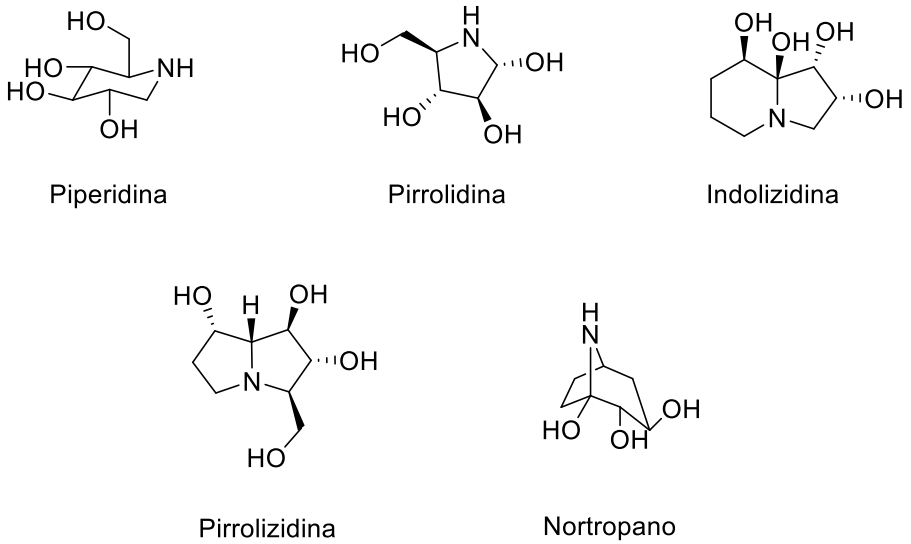


Figura 10. Grupos mayoritarios de iminoazúcares.

En los años 60, Paulsen, Jones y Hanessian publicaron las primeras síntesis de iminoazúcares.[51] En 1968 Inouye aisló e identificó la 1-desoxinojirimicina (1-DNJ) de *Streptomices nojiriensis* y describió sus propiedades antibacterianas.[52] Este compuesto es una piperidina polihidroxilada y carece de funcionalización en C1. Esto hace que sea más estable metabólicamente que otros iminoazúcares conservando sus propiedades inhibitorias. Durante los siguientes años, este producto natural se ha seguido estudiando y se le han encontrado diferentes propiedades como antihiperglucémico, antiobesidad y antiviral.[53] La manojirimicina y la galactonojirimicina fueron aisladas posteriormente.[54] Desde entonces, el interés en este tipo de compuestos fue aumentando. Paulsen describió en 1966 la primera síntesis total de 1-DNJ[55]

[49] Lo Piparo, E.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C. J. *J. Med. Chem.* **2008**, *51*, 3555-3561.
[50] Zamoner, L. O. B.; Aragao-Leoneti, V.; Carvalho, I. *Pharmaceuticals* **2019**, *12*, 108.
[51] (a) Paulsen, H. *Angew. Chem.* **1962**, *74*, 585-586. (b) Jones, J. K. N.; Szarek, W. A. *Can. J. Chem.* **1963**, *41*, 636-640. (c) Hanessian S. *Chem. Commun.* **1966**, *51*, 796-798.
[52] Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *24*, 2125-2144.
[53] Do, H. J.; Chung, J. H.; Hwang, J. W.; Kim, O. Y.; Lee, J.-Y.; Shin, M.-J. *Food Chem. Toxicol.* **2015**, *75*, 1-7.
[54] (a) Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Itoh, J.; Inouye, S.; Yamada, Y.; Niida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984**, *37*, 1579-1586. (b) Miyake, Y.; Ebata, M. *Agric. Biol. Chem.* **1988**, *52*, 661-666.
[55] Paulsen, H. *Angew. Chem. Int. Ed.* **1966**, *5*, 495-511.

y, a día de hoy, existen más de 30 rutas sintéticas diferentes.[56] Los iminoazúcares han sido ampliamente estudiados no sólo por su potencial para inhibir glicosidasas, sino por su capacidad para modular la actividad de otros tipos de enzimas relacionadas con carbohidratos.[57]

Varios derivados sintéticos de la 1-DNJ están entre los pocos iminoazúcares que han alcanzado el mercado farmacéutico. El primer medicamento con estructura de iminoazúcar aprobado como fármaco fue Glyset® (miglitol) para el tratamiento de la diabetes tipo II. Este compuesto actúa inhibiendo la α-glucosidasa, lo que impide el catabolismo de azúcares complejos. En 2003, Zavesca® (miglustat) fue usado como el primer tratamiento oral para las enfermedades de Gaucher y de Niemann-Pick C.[58] Estas enfermedades se caracterizan por unos niveles bajos de β-glucosidasa, lo que conlleva una acumulación de glucocerebrósidos en el organismo. Miglustat inhibe las enzimas encargadas de la biosíntesis de estos compuestos (Figura 11).

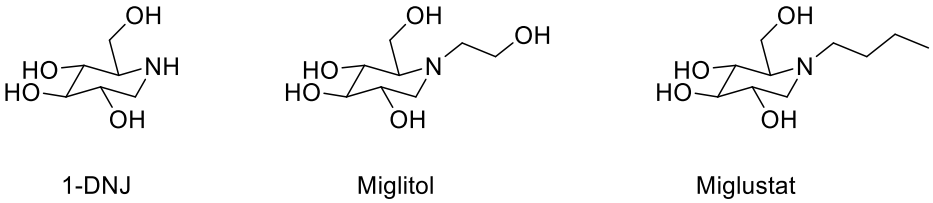


Figura 11. Estructuras de 1-DNJ, miglitol y miglustat.

Galafold® (migalastat) fue aprobado por la EMA en 2016 como el primer tratamiento basado en chaperonas contra la enfermedad de Fabry. Su mecanismo de acción se basa en la interacción con α-galactosidasas A que no han sufrido un pliegue correcto.[59] Cuando esto ocurre, la enzima cambia a la conformación que le hace ser activa. Lucerastat, la "forma galactosa" de miglustat, ha sido probada en ensayos clínicos de fase I contra la misma enfermedad. Ha demostrado ser capaz de disminuir los niveles en suero de globotriasoilceramida (Gb3), que se acumula como resultado de la ausencia o una errónea conformación de esta enzima (Figura 12).[60] Otros iminoazúcares como MON-DNJ, compuestos modificados desde iminoazúcares naturales, también se encuentran en diferentes fases de ensayos clínicos frente a la hepatitis C, el VIH y el virus del dengue.[61]

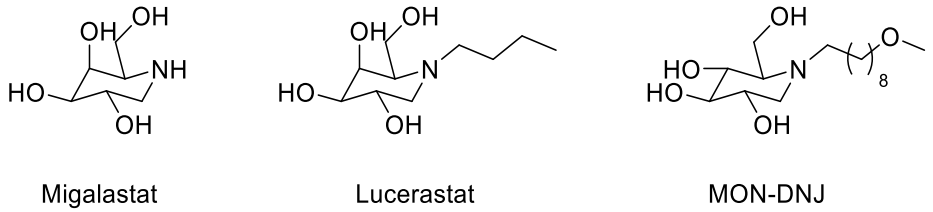


Figura 12. Estructuras de migalastat, lucerastat y MON-DNJ.

En 1976 se aisló de una planta india llamada *Derris Elliptica*, un nuevo alcaloide, la 2,5-dihidroximetil-3,4-dihidroxipirrolidina (DMDP), que demostró ser un potente inhibidor de glicosidasas.[62] Fleet publicó en 1985 la primera síntesis total de dicho compuesto a partir de D-

[56] (a) Hughes, A. B.; Rudge, A. J. *Nat. Prod. Rep.* **1994**, *11*, 135-162. (b) Afarinkia, K.; Bahar, A. *Tetrahedron: Assymetry* **2005**, *16*, 1239-1287.
[57] Henke, B. R.; Sparks, S. M. *Mini-Rev. Med. Chem.* **2006**, *6*, 845-847.
[58] Bennett, L. L.; Fellner, C. *Pharm. Therapeut.* **2018**, *43*, 274-309.
[59] Sánchez-Fernández, E. M.; García Fernández, J. M.; Mellet, C. O. *Chem. Comm.* **2016**, *52*, 5497-5515.
[60] Rodríguez-Monguio, R.; Spargo, T.; Seoane-Vázques, E. *Orphanet J. Rare Dis.* **2017**, *12*, 1-10.
[61] Alonzi, D. S.; Scott, K. A.; Dwek, R. A.; Zitzmann, N. *Biochem. Soc. Trans.* **2017**, *45*, 571-582.
[62] Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, *15*, 747-479.

glucosa como producto de partida.[63] Al compuesto que surge tras la eliminación de uno de los grupos hidroximetilenos se le conoce como DAB, y se encontró en *Angylocalyx boutiqueanus*.[64] Este compuesto presenta una fuerte capacidad inhibitoria de la enzima glucógeno fosforilasa. Actualmente se está estudiando como un posible fármaco para el tratamiento de la diabetes tipo II.[65] El enantiómero de DAB, conocido como LAB, ha demostrado ser un inhibidor más potente. En 2012, Kato y colaboradores mostraron el potencial de nuevos inhibidores de α-glucosidasa basados en LAB que eran capaces de reducir el nivel de glucosa en sangre. Además, las radicaminas A y B poseen una potente capacidad inhibitoria de dicha enzima lo que tiene gran potencial terapéutico (Figura 13).[66]

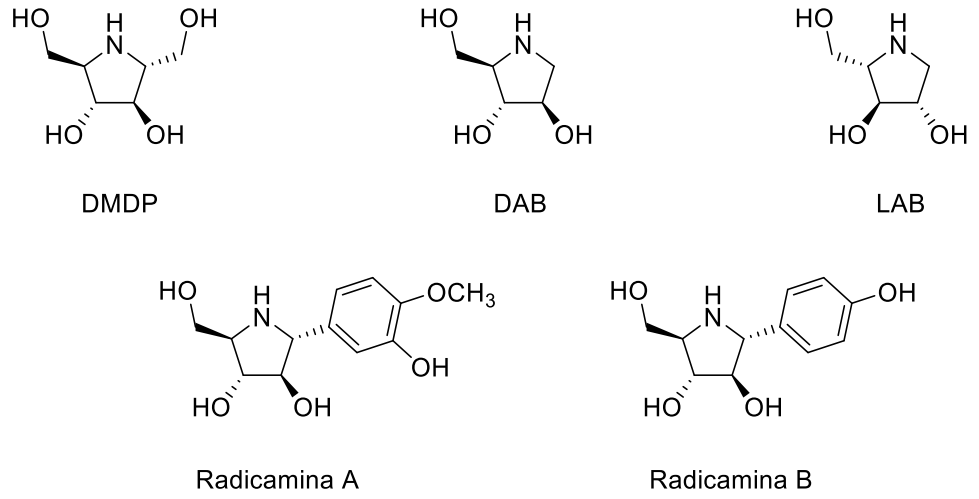


Figura 13. Ejemplos de iminoazúcares naturales con estructura de pirrolidina.

Los iminoazúcares de tipo indolizidina son compuestos bicíclicos importantes debido a su potencial para inhibir e interactuar con glicosidasas. La castanospermina fue el primer ejemplo descrito, aislado de *Castanospermu australe* en 1981,[67] pero no fue hasta el año 1984 cuando se publicó por primera vez la síntesis total de este compuesto.[68] Actualmente se sigue trabajando en rutas más rentables para la síntesis de la castanospermina, utilizando aminoácidos y otros productos naturales como sustratos de partida.[69] La castanospermina demostró ser inhibidora de las α- y β-manosidasas humanas, y tiene actividad antiviral contra VIH, herpes y hepatitis C entre otros.[70] A partir de este descubrimiento se han preparado muchos derivados que se han probado frente a diferentes enfermedades como cáncer, hepatitis C, enfermedades relacionadas con los lisosomas, etc.[71] Así, el celgosivir se encuentra en ensayos clínicos contra infecciones víricas. Otro iminoazúcar natural con actividad biológica es la (-)-swainsonina. Este compuesto ha sido capaz de reducir ciertos tumores y tratar alguna disfunción hematológica.[72] La lentiginosina, otro compuesto natural aislado de *Astralagus lentiginosus*, es

[63] Fleet, G. W. J.; Smith, P. W. *Tetrahedron Lett.* **1985**, *26*, 1469-1472.
[64] Nash, R. J.; Bell, E. A.; Williams, J. M. *Phytochemistry* **1985**, *24*, 1620-1622.
[65] Wang, J.-Z.; Cheng, B.; Kato, A.; Kise, M.; Shimadate, Y.; Jia, Y.-M-; Li, Y.-X., Fleet, G. W. J.; Yu, C.-Y. *Eur. J. Med. Chem.* **2022**, *233*, 114230.
[66] a) Kato, A. *et al. J. Med. Chem.* **2012**, *55*, 10347-10362. b) Li, Y.-X.; Ren, I.; Kato, A.; Jia, Y.-M.; Fleet, G. W. J.; Zhao, X.; Xiao, M.; Yu, C.-Y. *Eur. J. Org. Chem.* **2016**, *2016*, 1429-1438.
[67] Hohenschutz, L. D.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.; Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981**, *20*, 811-814.
[68] Bernotas, R. C.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 165-168.
[69] Myeong, I.-S.; Lee, Y.-T.; Kang, J.; Ham, W.-H. *J. Org. Chem.* **2019**, *84*, 4211-4220.
[70] Sasak, V. W.; Ordovas, J. M.; Elbein, A. D.; Berninger, R. W. *Biochem. J.* **1985**, *232*, 759-766.
[71] Li, Z.; Li, T.; Dai, S.; Xie, X.; Ma, X.; Zhao, W.; Zhang, W.; Li, J.; Wang, P. G. *ChemBioChem* **2013**, *14*, 1239-1247.
[72] Rose, D. R. *Curr. Opin. Struct. Biol.* **2012**, *22*, 558-562.

un potente anti-VIH, antitumoral y con ciertas propiedades inmunomoduladoras. También es capaz de inhibir la amiloglucosidasa fúngica (Figura 14).[73]

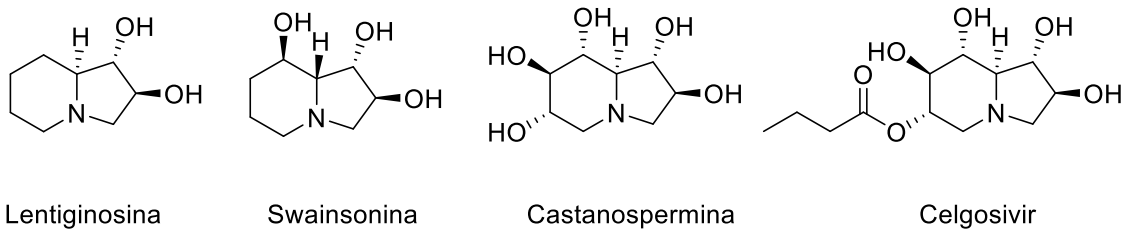


Figura 14. Diferentes iminoazúcares con estructura de indolizidina.

Otro tipo de iminoazúcares bicíclicos son las pirrolizidinas polihidroxiladas. La australina fue el primer ejemplo descrito de esta familia, y se aisló de *Castanospermum australe*.[74] La alexina fue aislada más tarde de especies *Alexa*.[75] Posteriormente se identificaron diferentes isómeros de la australina. La casuarina, aislada de *Myrtus communis*, y la unifluorina A, junto con sus epímeros, son las pirrolizidinas pentahidroxiladas más representativas.[76] Las hiacintacinas, nombradas así después de haber sido descubiertas en *Hyacinthoides non-scripta*, son otro grupo de estos iminoazúcares bicíclicos.[77] Las hiacintacinas A poseen tres grupos hidroxilo; las B tienen cuatro y las C son alcaloides pentahidroxilados (Figura 15).

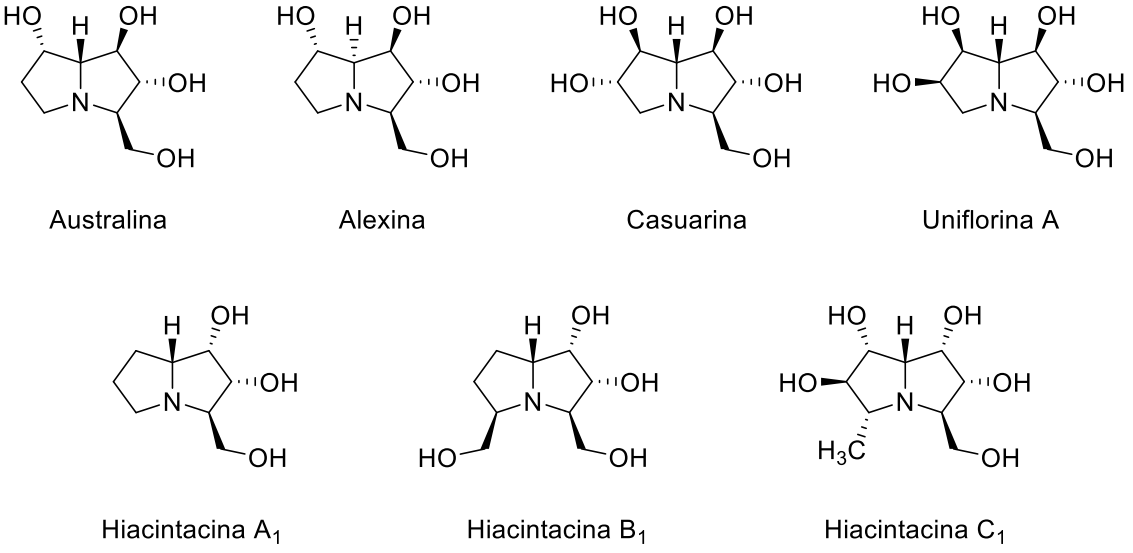


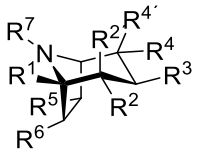
Figura 15. Iminoazúcares con estructura de pirrolizidina.

En el grupo de los nortropanos se encuentran las calisteginas. Estos compuestos fueron descritos por primera vez en el 1988, aislados de *Calistegia sepium*, pero no fueron caracterizados hasta 1990.[78] Todos ellos poseen un esqueleto de nortropano y grupos hidroxilo en diferentes posiciones y son potentes inhibidores de glicosidasas. Se dividen en diferentes

[73] Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265-295.
[74] Molyneux, R. J.; Benson, M.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J. Nat. Prod.* **1988**, *51*, 1198-1206.
[75] Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Derome, A. E.; Hamor, T. A.; Scofield, A. M.; Watkin, D. J. *Tetrahedron Lett.* **1988**, *29*, 2487-2490.
[76] Matsumura, T.; Kasai, M.; Hayashi, T.; Arisawa, M.; Momose, Y.; Arai, I.; Amagaya, S.; Komatsu, Y. *Pharm. Biol.* **2000**, *38*, 302-307.
[77] Kato, A.; Adachi, I.; Miyauchi, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Wormald, M. R.; Fleet, G. W. J.; Asano, N. *Carbohydr. Res.* **1999**, *316*, 95-103.
[78] (a) Tepfer, D.; Goldmann, A.; Pamboukdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Denarie, J.; Rosenberg, C. *J. Bacteriol.* **1988**, *170*, 1153-1161. (b) Goldmann, A.; Milat, M. L.; Ducrot, P. H.; Lallemand, J. Y.; Maille, M.; Lepingle, A.; Charpin, I.; Tepfer, D. *Phytochemistry* **1990**, *29*, 2125-2127.

grupos según el número de grupos hidroxilos presentes en su estructura: tres grupos hidroxilos forman el grupo de las calisteginas A; las calisteginas B son las que poseen cuatro y las pentahidroxiladas son las calisteginas C. Todas tienen un grupo hidroxilo en un carbono terciario formando la función hemiaminal (Tabla 1).

Tabla 1. Calisteginas naturales.[79]



Calisteginas	R^1	R^2	$R^{2'}$	R^3	R^4	$R^{4'}$	R^5	R^6	R^7
A_3	OH	OH		OH					
B_1	OH	OH		OH			OH		
B_2	OH	OH		OH	OH				
C_1	OH	OH		OH	OH		OH		
N-Me-C_1	OH	OH		OH	OH		OH		CH_3
N_1	NH_2	OH		OH	OH				

Las calisteginas pueden ser consideradas como piperizinas polihidroxiladas con un puente etileno o hidroxietileno entre C_1 y C_5, lo que las hace atractivas para aplicaciones médicas si bien las bases moleculares de su capacidad de inhibición de glicosidasas no se comprenden del todo aún.[80] Estudios mecanísticos en una β-glucosidasa mostraron que la calistegina B_2 se une en el centro catalítico de la enzima con el átomo de nitrógeno en la posición donde estaría el carbono anomérico en un azúcar nativo, orientación espacial diferente a la que adoptan otros iminoazúcares.[81] Las calisteginas normalmente son inhibidores de exoglicosidasas mientras que no alteran las endoglicosidasas. Las calisteginas B_1 y C_1 inhibien la β-galactosidasa, inhibición que puede o no ser competitiva.[82] Las primeras síntesis descritas son del año 1992, cuando se publicaron las síntesis racémicas y asimétricas de las calistegina A_3 y B_2.[83] El núcleo bicíclico, se obtuvo mediante reacciones de metátesis de cierre de anillo (RCM), expansiones de anillo y cicloadiciones entre otras.[84] También han sido utilizadas resoluciones enzimáticas para la obtención de los isómeros deseados.[85]

Aunque durante los últimos años se hayan desarrollado nuevas metodologías sintéticas para este tipo de compuestos, como pueden ser las basadas en química combinatoria o el uso de enzimas, la obtención de iminoazúcares sintéticos nuevos sigue siendo un desafío.[86] Esto se debe a que tienen muchos centros estereogénicos, normalmente poseen pocos grupos cromóforos y muchos grupos polares, lo que les convierte en compuestos poco solubles en disolventes orgánicos y de difícil detección, complicando los métodos de purificación y

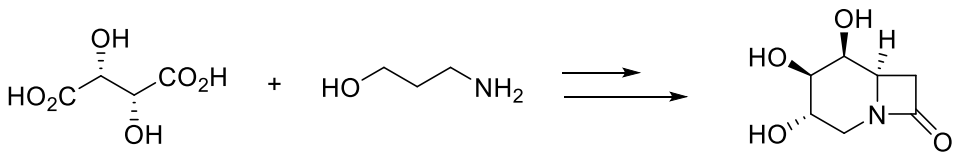
[79] Pino-Gonzalez, M. S.; Oña, N.; Romero-Carrasco, A. *Mini-Rev. Med. Chem.* **2012**, *12*, 1477-1484.
[80] (a) Milner, S. E.; Brunton, N. P.; Jones, P. W.; O´Brien, N. M.; Collins, S. G.; Maguire, A. R. *J. Agricul. Food Chem.* **2011**, *59*, 3454-3484. (b) Kaliappan, K. P.; Das, P.; Chavan, S. T.; Sabharwal, S. G. *J. Org. Chem.* **2009**, *74*, 6266-6274.
[81] Gloster, T. M.; Madsen, R.; Davies, G. J. *ChemBioChem.* **2006**, *7*, 738-742.
[82] Dräger, B. *Nat. Prod. Rep.* **2004**, *21*, 211-223.
[83] Duclos, O.; Mondange, M.; Duréault, A.; Depezay, J. C. *Tetrahedron Lett.* **1992**, *33*, 8061-8064.
[84] (a) Boyer, F.-D.; Hanna, I. *Tetrahedron Lett.* **2001**, *42*, 1275-1277. (b) Boyer, F. D.; Lallemand, J. Y. *Synlett* **1992**, 969-971. (c) Soulié, J.; Faitg, T.; Betzer, J. F.; Lallemand, J. Y. *Tetrahedron* **1996**, *52*, 15137-15146. (d) Marco-Contelles, J.; de Opazo, E. *J. Org. Chem.* **2002**, *67*, 3705-3717. (d) Kim, I. S.; Jung, Y. H. *Heterocycles* **2011**, *83*, 2489-2507.
[85] Johnson, C. R.; Bis, S. J. *J. Org. Chem.* **1995**, *60*, 615-623.
[86] Concia, A.; Lozano, C.; Castillo, J. A.; Parella, T.; Joglar, J.; Clapés, P. *Chem. Eur. J.* **2009**, *15*, 3808-3816.

aislamiento que requieren de técnicas especiales.[87] Las síntesis de iminoazúcares comparten las mismas dificultades que las de los carbohidratos, ya que normalmente éstos son usados como productos de partida, y el átomo de nitrógeno es añadido tras numerosas etapas. Por otro lado, las síntesis totales desde productos naturales sencillos como aminoácidos son más eficientes. En estos casos se obtienen diversos isómeros del mismo compuesto ya que muchas de las reacciones no son totalmente diastereoselectivas. En el campo de la búsqueda de productos con actividad biológica esto suele suponer una desventaja, pero en estas síntesis largas y tediosas obtener varios isómeros permite aumentar las posibilidades de encontrar compuestos que sean activos y potentes.

1.2.5.1. IMINOAZÚCARES RESTRINGIDOS CONFORMACIONALMENTE

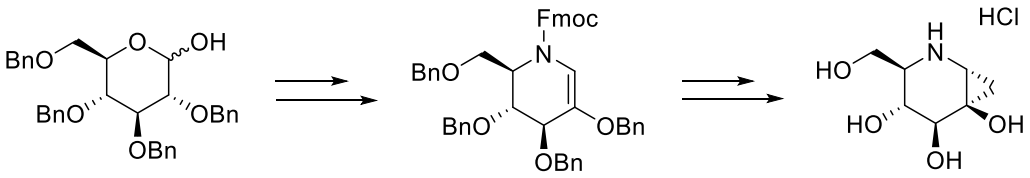
El mayor problema que presentan los iminoazúcares como punto de partida para el desarrollo de nuevos fármacos, es normalmente la falta de selectividad entre diferentes glicosidasas, especialmente en iminoazúcares monocíclicos, lo que da lugar a efectos secundarios no deseados. Los iminoazúcares pequeños son flexibles, por lo que pueden adoptar diferentes conformaciones, haciendo que estos compuestos interactúen con varias glicosidasas. Se podría solventar este problema mediante la rigidificación de la estructura fusionando el anillo principal a anillos más pequeños como ciclobutanos o ciclopropanos.

En 2006, Pandey y colaboradores describieron la síntesis de un híbrido entre una β-lactama y un iminoazúcar usando como productos de partida ácido tartárico y 3-aminopropanol.[88] Con la introducción de este pequeño anillo se restringe la libertad conformacional como demostraron los estudios de inhibición. Este compuesto resultó ser activo frente a β-galactosidasa (K_i=172 μM) mientras que contra α-galactosidasa lo era muy poco (K_i=900 μM). No mostró actividad frente a otras glicosidasas como manosidasas y glucosidasas (Esquema 2).



Esquema 2. Síntesis de una piperidina-β-lactama descrita por Pandey.

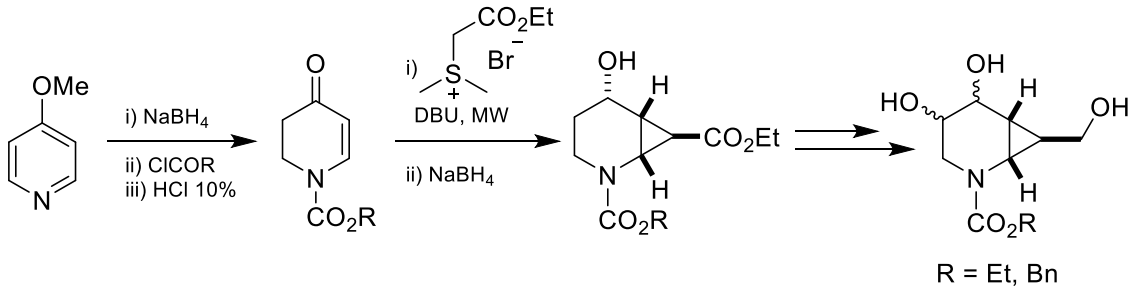
Desiré y Shipman publicaron en el 2001 la síntesis de un iminoazúcar fusionado a un anillo de ciclopropano.[89] En dicho trabajo se sintetizaron diversos derivados de desoxinojirimicina. La reacción clave era una reacción de Simmons-Smith para la construcción del anillo de ciclopropano, usando glucopiranosa como producto de partida (Esquema 3). Demostraron como este pequeño anillo era capaz de distorsionar la estructura de silla, rigidificando y fijando la conformación.



Esquema 3. Síntesis de Shipman y Desiré de un iminoazúcar fusionado a un anillo de ciclopropano.

[87] Nakagawa, K.; Ogawa, K.; Higuchi, O.; Kimura, T.; Miyazawa, T.; Hori, M. *Anal. Biochem.* **2010**, *404*, 217-222.
[88] Pandey, G.; Dumbre, S. G.; Khan, M. I.; Shabab, M.; Puranik, V. G. *Tetrahedron Lett.* **2006**, *47*, 7923-7926.
[89] Desiré, J.; Shipman, M. *Synlett* **2001**, 1332-1334.

Nuestro grupo sintetizó diversos isómeros de iminoazúcares *N*-sustituidos fusionados a un anillo de ciclopropano. El anillo de ciclopropano se construyó generando *in situ* un iluro de azufre, que reaccionaba con una cetona α,β-insaturada preparada previamente desde la 4-metoxipiridina. (Esquema 4).[90] Se sintetizaron 4 compuestos diferentes como mezclas racémicas en los que el nitrógeno del iminoazúcar estaba protegido como carbamato. La desprotección fue imposible debido a la proximidad del anillo de ciclopropano. En los diferentes intentos lo que se observó fue la apertura de éste. Tras este precedente, sintetizamos nuevos iminoazúcares partiendo en este caso de aminoácidos naturales, que es el trabajo que se presenta en esta memoria. Con esto conseguiríamos evitar, además de las dificultades mencionadas, las mezclas racémicas.



Esquema 4. Síntesis de iminoazúcares fusionados a ciclopropanos descritos por Pérez-Castells.

1.2.6. MIMÉTICOS DEL ESTADO DE TRANSICIÓN

Otra manera de inhibir las glicosidasas es utilizar compuestos capaces de mimetizar el estado de transición generado en la reacción enzimática. Como se muestra en el Esquema 1, se trata de un estado deficiente en electrones sin la estructura de silla del azúcar. En este grupo se encuentran los derivados carbonílicos y los glicales.[91]

1.2.6.1. DERIVADOS CARBONÍLICOS

Ezaki en 1940[92] y Horikoshi en 1942[93] mostraron que las lactonas derivadas de carbohidratos eran potentes inhibidores de glicosidasas. Levvy, en 1952, demostró que la inhibición de la β-glucuronidasa se debía a las lactonas presentes en algunos carbohidratos.[94] Además se ha observado que estas lactonas tienen un importante efecto antitumoral en ratones.[95] Su potencial inhibitorio es atribuido a la hibridación sp^2 del carbono anomérico, que puede mimetizar el estado de transición de la hidrólisis. Esta distorsión puede beneficiar interacciones electrostáticas en el centro activo de la enzima mejor que las aminas análogas. Por ejemplo, la lactama derivada del ácido glucurónico inhibe mejor la β-glucuronidasa que la correspondiente amina.[96] El oxígeno del grupo carbonilo de las lactamas, donde el nitrógeno reemplaza al carbono anomérico, participa más efectivamente en enlaces de hidrógeno que estabilizan la unión al receptor. Una amida es capaz de formar estructuras de tipo aminocetal menos estabilizadas que las que se forman con un nitrógeno en un carbono sp^3. García-

[90] Lopez-Rodriguez, A.; Dominguez, G.; Perez-Castells, J. *Synthesis* **2017**, *49*, 4606-4612.
[91] Houston, T. A.; Blanchfield, J. T. *Mini-Rev. Med. Chem.* **2003**, *3*, 669-678.
[92] Ezaki, S. *J. Biochem.* **1940**, *32*, 91-105.
[93] Horikoshi, K. *J. Biochem.* **1942**, *35*, 39-59.
[94] Levvy, G. A. *Biochem. J.* **1952**, *52*, 464-472.
[95] Carr, A. J. *Nature* **1963**, *198*, 1104-1105
[96] Niwa, T.; Tsuruoka, T.; Inouye, S.; Naito, Y.; Koeda, T.; Niida, T. *J. Biochem.* **1972**, *72*, 207-211.

Fernández sintetizó un derivado de la castanospermina que contiene un uretano.[97] La kifunensina, de origen natural, es similar estructuralmente y tiene poder inmunomodular cuando inhibe la α-manosidasa (Figura 16).[98]

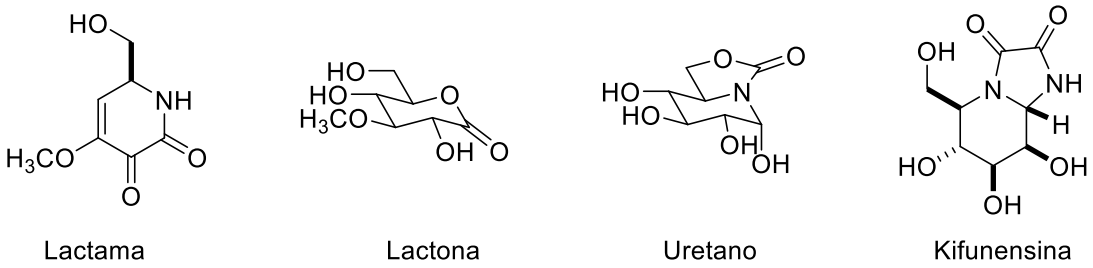


Figura 16. Ejemplos de derivados carbonilos de azúcares.

1.2.6.2. GLICALES

Los glicales son miméticos de carbohidratos que poseen un doble enlace carbono-carbono en el carbono anomérico. Se les relaciona con un nuevo tipo de aldehídos derivados de los carbohidratos ya que el carbono anomérico posee el mismo estado de oxidación y tiene casi la misma reactividad.[99] Se considera a estos compuestos miméticos del estado de transición debido a la estructura de semi-silla que presentan. Kiss y Somsák sintetizaron una serie de galactales C1 sustituidos que eran capaces de inhibir la β-galactosidasa de *E. Coli*.[100]

El campo donde estos compuestos han tenido mayor éxito es en la inhibición de la sialidasa o neuraminidasa, enzima encargada de hidrolizar los residuos de ácido siálico. Varios compuestos han demostrado ser capaces de actuar contra el virus de la gripe al inhibir esta enzima, ya que es vital en el ciclo de replicación del virus. El zanamivir (Ralenza®) fue diseñado como un inhibidor potente y selectivo de la sialidasa través de un diseño racional.[101] El oseltamivir (Tamiflu®), otro fármaco usado en el tratamiento de la gripe, fue obtenido al eliminar la cadena de glicerol y sustituirla por grupos hidrofóbicos (Figura 17). Estos dos compuestos fueron el inicio del posterior descubrimiento de otros análogos.[102]

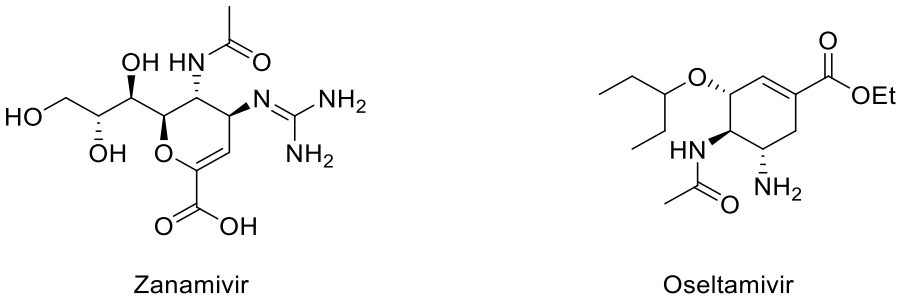


Figura 17. Estructuras de zanamivir y oseltamivir.

[97] Jiménez Blanco, J. L.; Díaz Pérez, V. M.; Ortiz Mellet, C.; Fuentes, J.; García Fernández, J. M.; Díaz Arribas, J. C.; Cañada, F. J. *Chem. Commun.* **1997**, *20*, 1969-1970.
[98] Kayakiri, H.; Takase, S.; Shibata, T.; Okamoto, M.; Terano H.; Hashimoto, M.; Tada, T.; Koda, S. *J. Org. Chem.* **1989**, *54*, 4015-4016.
[99] Fraser-Reid, B. *Accts. Chem. Res.* **1975**, *8*, 192-201.
[100] Kiss, L.; Somsák, L. *Carbohydr. Res.* **1996**, *291*, 43-52.
[101] von Itzstein, M. et al. *Nature* **1993**, *363*, 418-423.
[102] Wyatt, P. G.; Coomber, B. A.; Evans, D. N.; Jack, T. I.; Fulton, H. E.; Wonacott, A. J.; Colman, P.; Varghese, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 669-673.

2. AMINO ACID-BASED SYNTHESIS AND GLYCOSIDASE INHIBITION OF CYCLOPROPANE-CONTAINING IMINOSUGARS.

This work shows the first part of the synthesis of novel iminosugars rigidified by a cyclopropane ring and the study of their ability to inhibit glycosidases. The synthesis of 4 final compounds is described, starting from L-serine as the chiral pool. During the synthesis racemization was not observed. The key step was the cyclopropanation reaction of an α,β–unsaturated ketone using a previously synthetized sulfonium ylide. The enzimatic assays were carried out against different glycosidases in Prof. Javier Cañada´s laboratory. Some of these compounds presented inhibition in the range of mM. Even though this modest result, they were selective. In some cases, there was activation of neuraminidase up to 200%.

In this work I did all the experimental work including the synthesis and characterization of the compounds, the enzymatic assays and the analysis of the results. I collaborated in the elaboration of the manuscript with my supervisors, mainly in the experimental part and supplementary information.

En este trabajo se presenta la primera parte de la síntesis de nuevos iminoazúcares rigidificados por un anillo de ciclopropano y su estudio como posibles inhibidores de glicosidasas. Se describe la síntesis de 4 productos finales, usando la L-serina como producto de partida. No se observó racemización en ninguna etapa durante la síntesis. La reacción clave fue la ciclopropanación de una cetona α,β–insaturada usando un iluro de azufre previamente sintetizado y aislado. Los ensayos enzimáticos se llevaron a cabo en el laboratorio del Prof. Javier Cañada frente a diferentes glicosidasas. Algunos de estos compuestos presentaron inhibición en el rango de mM. A pesar de este modesto resultado, eran selectivos. En algunos casos, se observó la activación de la neuraminidasa en hasta un 200%.

En este trabajo he realizado toda la parte experimental incluyendo la síntesis y caracterización de los compuestos, los ensayos enzimáticos y la interpretación de los resultados obtenidos. He colaborado en la redacción del manuscrito junto a mis supervisores, especialmente en la parte experimental y la información suplementaria.

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Article

Amino Acid-Based Synthesis and Glycosidase Inhibition of Cyclopropane-Containing Iminosugars

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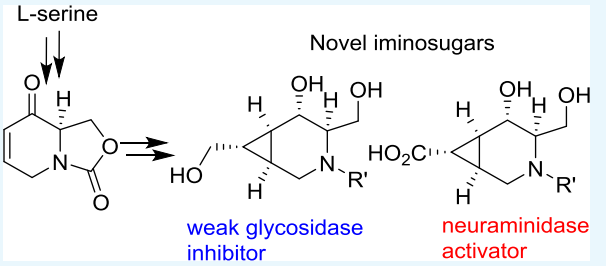
ABSTRACT: Synthesis of four iminosugars fused to a cyclopropane ring is described using L-serine as the chiral pool. The key steps are large-scale preparation of an α,β-unsaturated piperidinone followed by completely stereoselective sulfur ylide cyclopropanation. Stereochemistry of compounds has been studied by nuclear Overhauser effect spectroscopy (NOESY) experiments and ^{1}H homonuclear decoupling to measure constant couplings. The activity of these compounds against different glycosidases has been evaluated. Although inhibition activity was low (compound **8a** presents a (K_i) of 1.18 mM against β-galactosidase from *Escherichia coli*), interestingly, we found that compounds **8a** and **8b** increase the activity of neuraminidase from *Vibrio cholerae* up to 100%.



INTRODUCTION

Iminosugars are azaheterocycles with promising biological activities such as glycosidase and glycosyltransferase inhibition and modulation.[1] Many iminosugars are natural or synthetic polyhydroxylated piperidines, which can act as biomimetics of their corresponding pyranose analogues. Some of the most important natural piperidine iminosugars are nojirimycin (Figure 1, **I**) and its epimers, which, together with their deoxy analogues have turned out to be the lead molecules for drug design. Thus, stereochemical changes and functional group variation have led to iminosugars that can modulate glycosidase enzymes, exhibiting immunosuppressive, antiviral, or anti-inflammatory activities.[2] Bicyclic iminosugars, such as swainsonine (**II**), lentiginosine (**III**), castanospermine (**IV**), and their derivatives exhibit antitumor and immunosuppressive activities.[3]

Several iminosugars, miglitol (Glyset, **V**),[4] migalastat (Galafold, **VI**),[5] and miglustat (Zavesca, **VII**)[6] are commercially available for the treatment of type II diabetes and Fabry disease, and as the first oral treatment for Gaucher disease, respectively. Several other competitive inhibitors of glycosidases are being developed as new drugs and are in different phases of clinical trials.

The mechanism associated with glycosidase activity modulation is generally attributed to structural similarity to the oxacarbenium ion-like transition-state, formed during the hydrolysis of carbohydrates.[7] These transition states present diverse conformational pathways for different glycosidases,[8] making selective inhibition possible. In this context, designing conformationally restricted inhibitors seems to be an interesting approach. In addition, adequate metabolic stability is needed, which may be achieved with more rigid compounds. Recently, a study on α-mannanases showed how the enzyme surface restricts the conformational landscape of the substrate, rendering the $B_{2,5}$ conformation the most stable on-enzyme (Figure 2a).[9] In another study, a cyclopropane containing a cyclophellitol analogue, was designed as a specific β-glucosidase inhibitor for enzymes reacting through the $^4H_3$ transition-state conformation (Figure 2b).[10]

With these precedents, we expected that the introduction of a three-membered ring annulated to a piperidine ring would render novel iminosugars with a locked conformation that may be the starting point for finding the therapeutic compounds (Figure 3). The substituted cyclopropane moiety renders a fixed conformation and allows many different configurations that could increase selectivity to specific glycosidases.

The development of efficient routes for the preparation of iminosugars has received much attention from the synthetic community.[11] Most of the methods use carbohydrates as the chiral pool, which are transformed using reductive aminations[12] or other transformation strategies.[13] Alternatively, some asymmetric or biocatalysed approaches have been used.[14] But, there are fewer reports on approaches where amino acids are used as the chiral pool for the synthesis of iminosugar derivatives.[15]

Herein, we envisioned the preparation of novel bicyclic iminosugars that include the cyclopropane motif fused with piperidine starting from the natural amino acid L-serine as the

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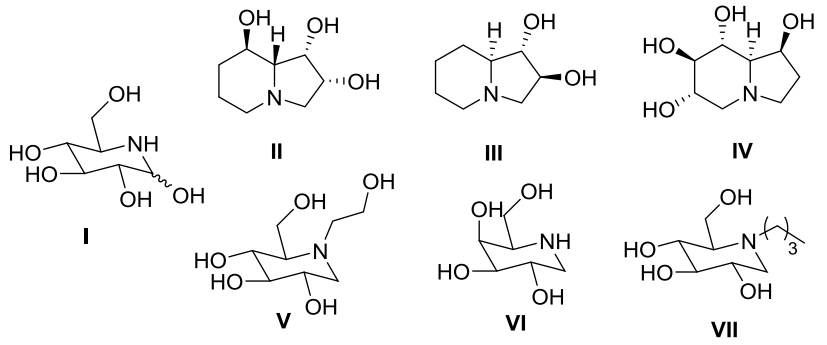


Figure 1. Structures of nojirimycin (**I**), swainsonine (**II**), lentiginosine (**III**), castanospermine (**IV**), miglitol (**V**), migalastat (**VI**), and miglustat (**VII**).

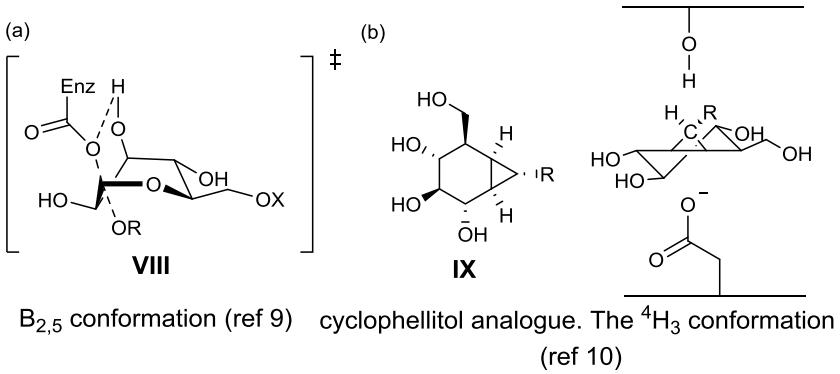


Figure 2. Transition-state conformations on-enzyme: (a) mannose $B_{2,5}$ conformation (**VIII**, reprinted with permission from ref 9) and (b) cyclophellitol analogue and its $^4H_3$ conformation (**IX**, ref 10, copyright 2017 American Chemical Society).

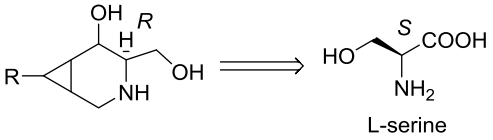


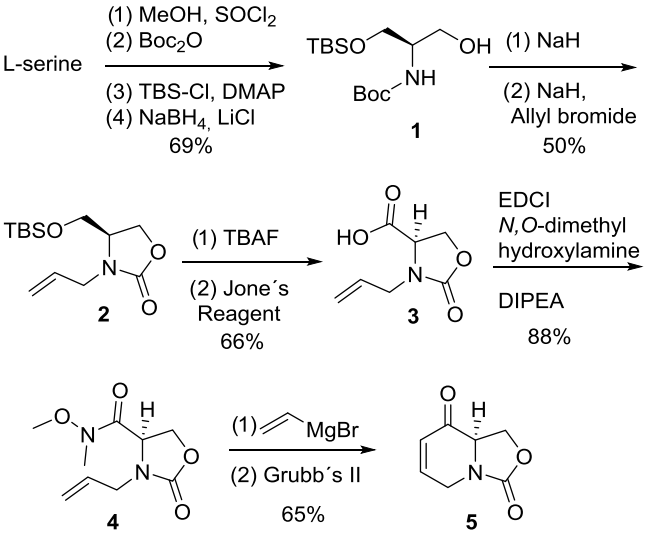
Figure 3. Structure of the target compound.

chiral pool. The final compounds present five stereogenic centers, and the synthesis involves the inversion of the configuration of the starting L-serine (*S*-configuration) into the C5 configuration of D-carbohydrates (*R*).[16] Preliminary glycosidase inhibition evaluation is shown.

RESULTS AND DISCUSSION

Our first goal was the synthesis of α,β-unsaturated ketone **5** in which the chiral center has *R* configuration. This configuration was selected as it corresponds to C5 in natural sugars and iminosugars, which share the *R* configuration in that position. This compound has already been prepared from D-serine and described.[17] In our case, we developed a synthesis approach using cheaper and natural L-serine, as depicted in Scheme 1. From this intermediate, a cyclopropanation reaction and further transformations resulted in a new family of piperidines fused to cyclopropanes. L-Serine was esterified and protected with Boc_2O, and the resulting intermediate was further protected and reduced to give desymmetrized alcohol **1** in which the configuration has changed from *S* to *R* in a few steps. This compound **1** was transformed into oxazolidinone by reaction with a base followed by allylation to give compound **2**. Following the previously reported methodology,[18] **2** was deprotected and oxidized into carboxylic acid **3**. This was converted into Weinreb amide **4**, which was treated with vinylmagnesium bromide and subjected to a ring-closing metathesis, RCM, using second-generation Grubbs' catalyst (Grubbs Catalyst M204), giving the starting material **5**.[19] This precursor containing the piperidine core was obtained in 13% global yield after 11 steps. No racemization was observed during the synthesis.

Scheme 1. Synthesis of Compound 5



The cyclopropanation reaction of **5** was performed using sulfur ylide. Interestingly, only one reaction product was observed and isolated in 70% yield. This product was designated as structure **6**, as a result of NMR analysis (nuclear

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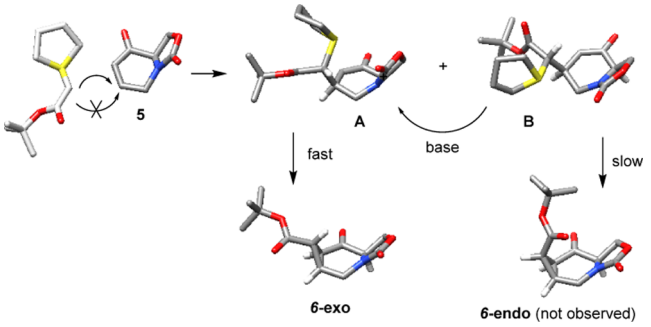
Overhauser effect (NOE) and coupling constants) of compounds **7a–b** (*vide infra*). Cyclopropanation occurred on the same side of oxazolidinone (endo attack) and the subsequent ring-closing step exclusively gave *exo*-cyclopropane. The ylide mediated cyclopropanation is a stepwise reaction in which the formation of the first C–C bond is the rate-determining step.[20] The attack of the ylide on **5** is more favored from the opposite face to the nitrogen lone electron pair as depicted in Scheme2; therefore, it occurs through the same face of the oxazolidinone ring (endo). This selectivity was observed previously in one unsaturated γ-lactam cyclopropanation,[21] although other precedents have described mixtures of endo and exo attacks.[22] Then, the stereoselectivity of cyclopropane is determined in the second step. Studies reported by Aggarwal's group,[23] showed that two intermediate betaines **A** and **B** are formed in a 1:1 ratio after the nucleophilic addition. The cyclization of betaine **A** is faster than that of **B**. Moreover, **B** can epimerize to give **A** before it closes the three-membered ring generally leading to high diastereoselectivity of exo-cyclopropane.

Scheme 2. Stereoselectivity of the Cyclopropanation Reaction of 5[24]



With the cyclopropane containing compound **6** in hand, the reduction of the ketone afforded a (3:2) mixture of the two diastereomers **7a–b** (Scheme 3). These alcohols were separated, characterized, and separately transformed into the final products. The stereochemistry of **7a** and **7b** was determined using NOE experiments and coupling constant values. Figure 4 shows the main correlations observed for **7a** and **7b** that allowed assigning the relative configuration of H6, H7, and H7a. The coupling constants between H7a, H7, and H6a were determined using homonuclear decoupling experiments. Values are shown in Figure 4, and the model agrees with the calculated angles for these couplings.

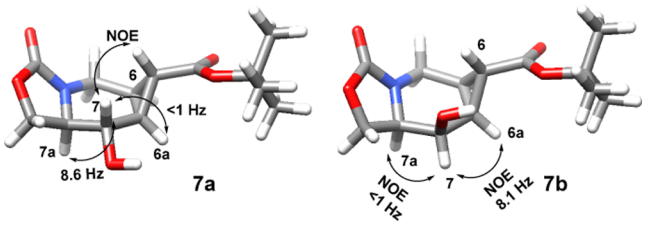
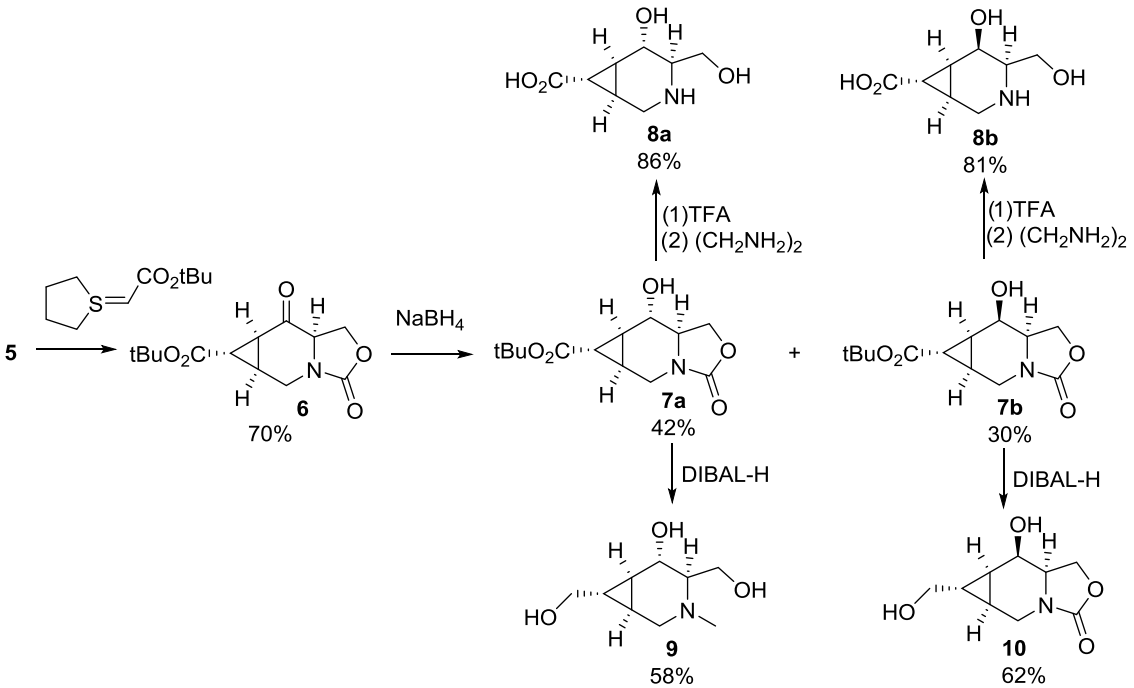


Figure 4. NOE signals and constant couplings in products **7a** and **7b**, respectively.[24]

In continuation of the synthesis, treatment with trifluoroacetyl (TFA) and further reaction with ethylendiamine gave products **8a** and **8b**, respectively, in excellent yields. On the other hand, compounds **7a** and **7b** gave different products on reacting with DIBAL-H, whereas **7b** gave the expected alcohol **10** (62%), additionally, the reaction of **7a** caused the cleavage of the oxazolidinone ring giving **9** in 58% yield. This behavior has been described previously (Scheme 3).[25]

Final compounds were screened for glycosidase inhibition activities (α-glucosidase from *Bacillus stearothermophilus*, β-glucosidase from almonds, α-galactosidase from green coffee beans, β-galactosidase from *Escherichia coli*, α-mannosidase

Scheme 3. Synthesis of Final Compounds from 5



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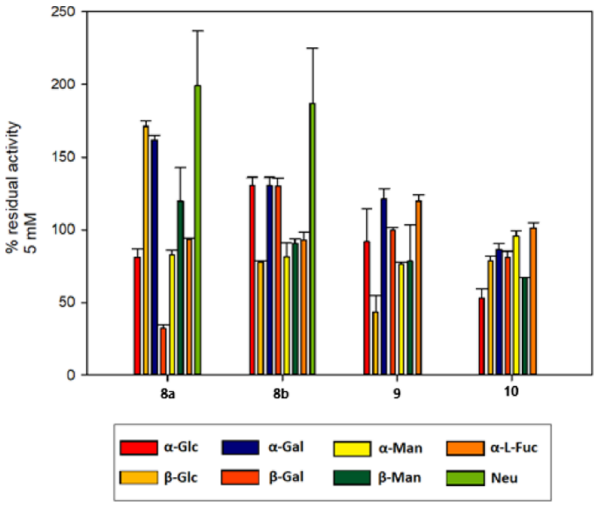
from *Jack beans*, β-mannosidase from *Helix pomatia,* and α-L-fucosidase from *Homo sapiens*) using *p*-nitrophenyl monosaccharides as substrates.

Carboxylic containing compounds **8a** and **8b**, could resemble the zwitterionic form of oseltamivir and zanamivir, well-known inhibitors of neuraminidase from Influenza virus[26] with carboxyl-amino and carboxyl-guanidine moieties, respectively. Preliminary docking calculations using AutoDock[27] showed that **8a** and **8b** could fit in the binding site of neuraminidase. Thus they were also evaluated as possible inhibitors of neuraminidase from *Vibrio cholerae*.

The enzymatic activities were calculated by measuring the absorbance of the phenoxide released in the enzymatic reaction at 405 nm. The compounds were initially screened at 1, 5, and 25 mM concentrations. With compounds **8a** and **9**, inhibition over 50% was observed with selected enzymes at 5 mM; **8b** and **10** did not show any significant inhibition of any glycosidase (Chart 1). Inhibition constants (K_i) were

Chart 1. Residual Glycosidase Activities in the Presence of 5 mM Synthesized Compounds



estimated assuming a competitive type inhibition in the cases of compound **8a** against β-galactosidase (K_i = 1.18 mM) and **9** against β-glucosidase (K_i = 4.43 mM). These two compounds exhibit some selectivity such that even at 25 mM no significant inhibition was observed against other glycosidases.

However, the observed inhibition was very weak compared to other iminosugar-based glycosidase inhibition, for example the measured K_i for deoxinojirimycin (DNJ) is 0.44 μM for α-glucosidase from *B. stearothermophilus*. The inhibition constant changes depending on the species that is studied, even for the same glycosidase of other species.[28] Other iminosugars present great activity against mannosidases.[29]

On the other hand, we found that compounds **8a** and **8b**, bearing a carboxylate group, also did not show any inhibition against neuraminidase but unexpectedly produced activation of the enzyme; these two compounds increased neuraminidase activity up to 100%. The possibility that the compounds act as favorable transglycosylation acceptors causing an increase of nitrophenol release was considered. NMR experiments were performed continuously following the reaction, but potential transient transglycosylation products could not be observed. Further research to explain this behavior is needed. Interestingly there are not many precedents on glycosidase activation by iminosugars. Two reports have accounted for this activation behavior. Thus, up to 70-fold activation of some of glycosidases was detected with multivalent iminosugars.[30] In another study, thienopyrimidines were found to activate certain glycosidases.[31] The activation mechanism could be explained by the stabilization of the active structure of the enzyme by the introduction of a small molecule adjacent/close to the substrate-binding site, locking the reactive form. Alternatively, if the activation is of the allosteric type occurring in a site different from the active site, it could be interesting to check if the activators have any pharmacological chaperone activity but avoid the temporal inhibition of the enzymatic activity, unlike the aforementioned migalastat and other proposed pharmacological chaperones that help maintaining the correct fold of the protein although temporally blocking the active site of the enzyme.

CONCLUSIONS

We described a multigram synthesis of an α,β-unsaturated ketone, which upon a stereoselective cyclopropanation reaction and further transformations gave a novel series of bicyclic piperidine-based iminosugars. The final products were studied against different glycosidases. Inhibition in most cases was low, but interestingly, the activation of neuraminidase was observed with products **8**. Possible explanations of this behavior, for example, allosteric activation, enzyme stabilization, or transglycosylation acceptor activity can be proposed. Current studies in our lab will provide insight into these possible mechanisms, and their potential applications will be explored/pursued.

EXPERIMENTAL SECTION

General Information. All chemicals were obtained from Aldrich/Merck, VWR, Fluorochem, and ABCR. Thin-layer chromatography (TLC) analyses were performed on Merck silica gel 60 F254 plates using phosphomolybdic acid or anisaldehyde and heat for detection. Silica gel NORMASIL 60 40−63 μm was used for flash chromatography. NMR spectra were recorded on a Bruker spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in δ ppm referenced to $CDCl_3$ (δ = 7.26 for ^{1}H and 77.00 for ^{13}C), CD_3OD (δ = 3.31 for ^{1}H and 49.00 for ^{13}C), or D_2O (δ = 4.79 for ^{1}H). Bidimensional spectra (heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond coherence (HMBC), correlated spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY)) were recorded in order to carry out the assignment. IR spectra were recorded on a Perkin-Elmer Spectrum 100. Specific optical rotation was measured using a polarimeter Anton Parr MCP 100. Melting points of solid compounds were determined using a Stuart Scientific Melting Point Apparatus SMP3. The absorbance of *p*-nitrophenoxide released in the enzymatic reactions was measured at 405 nm in a Perkin-Elmer Lamba 25.

Methyl (tert-Butoxycarbonyl)-L-serinate. Thionyl chloride (83 mL, 1.1 mol) was added to methanol (280 mL) at 0 °C, then L-serine (60.00 g, 571 mmol) is added. After 10 min, at 0 °C, the solution is heated at 65 °C for 2 h. The solvent is evaporated *in vacuo*, and 600 mL of AcOEt and a saturated solution of $NaHCO_3$ (until basic pH) are added. Di-*tert*-butyl dicarbonate (124.62 g, 0.571 mmol) in 265 mL of AcOEt is added. The reaction is stirred overnight at room temperature.

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The aqueous layer is extracted with AcOEt (2 × 300 mL). The combined organic layers are washed with brine (200 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude product is filtered through a pad of silica gel using Hex/AcOEt (9:1) to Hex/AcOEt (3:1) as eluents. A colorless oil is obtained (101.6 g, 81% after two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (brs, 1H, NH), 4.39 (brs, 1H, CH), 3.99−3.89 (m, 2H, CH_2O), 3.78 (s, 3H, OMe), 2.47−2.32 (m, 1H, OH), 1.45 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 155.9, 80.3, 63.3, 55.8, 52.8, 28.3 (3C). IR (NaCl): 3378, 2984, 2868, 1740, 1708 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.13 in dichloromethane (DCM)): +4.14. Found: C, 49.1; H, 7.9%. Calc. for $C_9H_{17}NO_5$: C, 49.3; H, 7.8%.

*tert-Butyl (R)-(1-((tert-Butyldimethylsilyl)oxy)-3-hydroxypropan-2-yl)carbamate (**1**).* To a solution of methyl (*tert*-butoxycarbonyl)-L-serinate (101.4 g, 463 mmol) in 400 mL of dimethylformamide (DMF) cooled to 0 °C is added imidazole (37.8 g, 555 mmol) and 4-dimethylaminopyridine (DMAP; 5.6 g, 46 mmol). After 10 min, *tert*-butyldimethylsilyl chloride (73.2 g, 486 mmol) is added. The reaction is stirred for 30 min at room temperature. AcOEt (400 mL) is added, and the organic layer is washed with water (3 × 1 L) and brine (400 mL), dried over $MgSO_4$, and evaporated *in vacuo*. To a suspension of $NaBH_4$ (35.0 g, 926 mmol) and LiCl (39.3 g, 926 mmol) in 800 mL of ethanol cooled to 0 °C, a solution of the crude in 190 mL of ethanol is added slowly. The reaction is stirred at 0 °C for 10 min, at room temperature for 30 min, and at 50 °C for 2.5 h. The reaction is cooled to 0 °C, and a saturated solution of NH_4Cl is added (until salts are dissolved, 450 mL). The aqueous layer is extracted with AcOEt (3 × 350 mL). The combined organic layers are washed with brine (200 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The reaction crude is filtered through a pad of silica gel using Hex/AcOEt (19:1) to Hex/AcOEt (1:1) as eluents. A colorless oil is obtained (123.2 g, 85% after two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (brs, 1H, NH), 3.86−3.66 (m, 5H, 2 × CH_2O + CH), 2.70 (brs, 1H, OH), 1.45 (s, 9H, 3 × CH_3), 0.90 (s, 9H, 3 × CH_3), 0.08 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 79.6, 64.0 (2C), 52.7, 28.5 (3C), 25.9 (3C), 18.3, −5.5 (2C). IR (NaCl): 3371, 2952, 2861, 1707 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.28 in DCM): +9.30. Found: C, 55.1, H, 10.4%. Calc. for $C_{14}H_{31}NO_4Si$: C, 55.0; H, 10.2%.

(R)-4-(((tert-Butyldimethylsilyl)oxy)methyl)oxazolidin-2-one. To a suspension of NaH 60% w/w (18.9 g, 472 mmol) in 300 mL of THF cooled to 0 °C is added a solution of **1** (123.2 g, 403 mmol) in 550 mL of THF. The reaction is stirred at 0 °C for 15 min, at room temperature for 25 min, and at 40 °C for 2.5 h. The reaction is cooled down to 0 °C, and a saturated solution of NH_4Cl is added until all salts are dissolved (250 mL). The aqueous layer is extracted with AcOEt (2 × 500 mL). The combined organic layers are washed with brine (300 mL), dried over $MgSO_4$, and evaporated *in vacuo*. A colorless wax is obtained (78.9 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (brs, 1H, NH), 4.42 (t, *J* = 8.6 Hz, 1H, CH_2O), 4.18 (dd, *J* = 8.8, 4.8 Hz, 1H, CH_2O), 3.94−3.88 (m, 1H, CH), 3.60 (d, *J* = 5.4 Hz, 2H, CH_2OSi), 0.87 (s, 9H, 3 × CH_3), 0.05 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 67.3, 64.8, 53.8, 25.9 (3C), 18.3, −5.4 (2C). IR (NaCl): 3315, 2959, 2848, 1745 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.32 in DCM): −15.94. Found: C, 52.2, H, 9.0%. Calc. for $C_{10}H_{21}NO_3Si$: C, 51.9; H, 9.2%.

*(R)-3-Allyl-4-(((tert-butyldimethylsilyl)oxy)methyl)-oxazolidin-2-one (**2**).* To a suspension of NaH 60% w/w (16.4 g, 409.1 mmol) in 500 mL of THF at 0 °C, a solution of (*R*)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)oxazolidin-2-one (78.9 g, 340.9 mmol) in 500 mL of THF is added slowly. Allyl bromide (29.5 mL, 340.9 mmol) is added and stirred for 15 min at 0 °C, 30 min at room temperature, and 2 h at 50 °C. A saturated solution of NH_4Cl is added until the salts are dissolved. The aqueous layer is extracted with AcOEt (3 × 200 mL). The combined organic layers are washed with brine (150 mL) and dried over $MgSO_4$. The solvent is evaporated *in vacuo*, and the residue is filtered through a pad of silica gel using Hex/AcOEt (19:1) to Hex/AcOEt (1:1) as eluents. A yellow oil is obtained (64.8 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84−5.74 (m, 1H, HC═), 5.27−5.21 (m, 2H, H_2C═), 4.33 (t, *J* = 8.7 Hz, 1H, CH_2O), 4.18−4.13 (m, 2H, CH_2O + CH_2N), 3.86−3.80 (m, 1H, CH), 3.69−3.62 (m, 3H, CH_2OSi + CH_2N), 0.89 (s, 9H, 3 × CH_3), 0.06 (s, 6H, 2 × CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 132.7, 118.5, 65.0, 62.2, 56.0, 45.3, 25.9 (3C), 18.3, −5.4 (2C). IR (NaCl): 3084, 2948, 2866, 1744 cm^{-1}. $[\alpha]_D^{25}$ (*c* 0.21 in $CHCl_3$): −11.84. Found: C, 57.1, H, 9.1%. Calc. for $C_{13}H_{25}NO_3Si$: C, 57.5; H,9.3%.

(S)-3-Allyl-4-(hydroxymethyl)oxazolidin-2-one. To a solution of **2** (55.5 g, 204.6 mmol) in 220 mL of THF is added TBAF·3H_2O (58.8 g, 225.0 mmol). The mixture is stirred for 30 min at room temperature. The solvent is evaporated *in vacuo* and filtered through a pad of silica gel using Hex/AcOEt (2:1) to Hex/AcOEt (1:2) as eluents. A colorless oil is obtained (28.9 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86−5.76 (m, 1H, HC═), 5.30−5.24 (m, 2H, H_2C═), 4.36 (t, *J* = 8.8 Hz, 1H, CH_2O), 4.25 (dd, *J* = 8.7, 6.0 Hz, 1H, CH_2O), 4.09 (ddt, *J* = 15.7, 5.3, 1.6 Hz, 1H, CH_2N), 3.90−3.84 (m, 1H, CHN), 3.80−3.73 (m, 2H, CH_2N + CH_2OH), 3.65 (dd, *J* = 11.9, 3.3 Hz, 1H, CH_2OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 132.5, 118.9, 64.7, 60.9, 56.3, 45.4. IR (NaCl): 3427, 3048, 2975, 2851, 1753 cm^{-1}. $[\alpha]_D^{25}$ (0.25 in $CHCl_3$): −44.28. Found: C, 53.8; H, 7.3%. Calc. for $C_7H_{11}NO_3$: C, 53.5; H, 7.1%.

*(R)-3-Allyl-2-oxo-oxazolidine-4-carboxylic Acid (**3**).* A solution of (*S*)-3-allyl-4-(hydroxymethyl)oxazolidin-2-one (28.9 g, 184.1 mmol) in 1 L of acetone is cooled to 0 °C, and 92 mL of Jones' reagent is added slowly. The reaction is stirred for 1.5 h at 0 °C. Isopropanol is added until the solution turns blue. The mixture is filtered through a pad of celite. The solvent is evaporated *in vacuo* and the residue is filtered through a pad of silica gel using Hex/AcOEt (1:1) to AcOEt 100% as eluents. A pale yellow oil is obtained (23.0 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (brs, 1H, OH), 5.80−5.70 (m, 1H, HC═), 5.27−5.24 (m, 2H, H_2C═), 4.52 (t, *J* = 9.4 Hz, 1H, CH_2O), 4.42 (dd, *J* = 9.0, 4.5 Hz, 1H, CH_2O), 4.36 (dd, *J* = 9.7, 4.6 Hz, 1H, CHN), 4.27 (dd, *J* = 15.4, 4.8 Hz, 1H, CH_2N), 3.75 (dd, *J* = 15.4, 8.0 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 158.5, 131.0, 120.1, 65.1, 56.0, 46.0. IR (NaCl): 3454, 2933, 2839, 1731 cm^{-1}. $[\alpha]_D^{25}$ (0.30 in $CHCl_3$): +8.98. Found: C, 48.8; H, 5.4%. Calc. for $C_7H_9NO_4$: C, 49.1; H, 5.3%.

*(R)-3-Allyl-N-methoxy-N-methyl-2-oxo-oxazolidine-4-carboxamide (**4**).* A solution of **3** (23.0 g, 134.4 mmol) in 500 mL of DCM is cooled to 0 °C. Diisopropylethylamine (DIPEA; 23.5 mL, 134.4 mmol), EDCI (25.8 g, 134.4 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (13.1 g, 134.4 mmol) are added. The reaction is stirred for 2 h at 0 °C. The solvent is evaporated *in vacuo* and filtered through a pad of silica gel using Hex/AcOEt (1:4) as the eluent. A yellow oil is obtained (25.3 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83−5.73 (m,

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1H, HC═), 5.25−5.21 (m, 2H, H_2C═), 4.66 (dd, J = 9.8, 5.6 Hz, 1H, CHN), 4.50 (t, J = 9.3 Hz, 1H, CH_2O), 4.32 (ddt, J = 15.4, 4.7, 1.7 Hz, 1H, CH_2N), 4.18 (dd, J = 8.8, 5.6 Hz, 1H, CH_2O), 3.69 (s, 3H, OCH_3), 3.68 (dd, J = 15.4, 8.3 Hz, 1H, CH_2N), 3.22 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 158.0, 132.2, 119.3, 64.5, 61.7, 54.8, 45.9, 32.7. IR (NaCl): 3088, 2979, 2928, 1754, 1672 cm^{-1}. $[\alpha]_D^{25}$ (c 0.37 in DCM): +31.11. Found: C, 50.4; H, 6.9%. Calc. for $C_9H_{14}N_2O_4$: C, 50.5; H, 6.6%.

(R)-4-Acryloyl-3-allyloxazolidin-2-one. To a solution of **4** (12.0 g, 56.0 mmol) in 270 mL of THF cooled to −30 °C, 0.7 M vinylmagnesium bromide (200 mL) is added slowly, keeping the temperature below −25 °C. When the addition is finished, the reaction is stirred for another 30 min at −30 °C. The reaction mixture is poured into a mixture of 200 mL of HCl 10% and 100 mL of MeOH cooled in a bath at −15 °C. This mixture is stirred for another 15 min. The aqueous layer is extracted with AcOEt (3 × 150 mL). The combined organic layers are washed with a solution of 1 M HCl (200 mL), with a saturated solution of $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude is used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (dd, J = 17.5, 10.4 Hz, 1H, ═CHCO), 6.39 (d, J = 17.4 Hz, 1H, H_2C ═ CHCO, trans), 6.01 (d, J = 10.4 Hz, 1H, H_2C ═ CHCO, cis), 5.78−5.68 (m, 1H, HC═), 5.24−5.16 (m, 2H, H_2C═), 4.59 (dd, J = 10.0, 5.2 Hz, 1H, CHN), 4.52 (t, J = 9.3 Hz, 1H, CH_2O), 4.26 (dd, J = 15.3, 4.6 Hz, 1H, CH_2N), 4.15 (dd, J = 8.6, 5.2 Hz, 1H, CH_2O), 3.59 (dd, J = 15.3, 8.0 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.1, 157.7, 132.3, 131.8, 131.4, 120.1, 63.8, 60.5, 46.2.

*(R)-1,8a-Dihydro-3H-oxazolo[3,4-a]pyridine-3,8(5H)-dione (**5**).* The crude of the previous reaction is dissolved in 180 mL of DCM and heated to reflux. Grubb's second-generation catalyst is added (1.2 g, 1.4 mmol). The reaction is stirred under reflux for 1.5 h. The solvent is evaporated *in vacuo*. The crude is purified in silica gel in Hex/AcOEt (1:1). A brown oil is obtained (5.6 g, 65% after two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (ddd, J = 10.5, 4.4, 2.0 Hz, 1H, ═ C**H**CH_2), 6.26 (dt, J = 10.5, 2.3 Hz, 1H, ═CHCO), 4.69 (dd, J = 9.0, 4.4 Hz, 1H, CHN), 4.61−4.50 (m, 2H, CH_2O + CH_2N), 4.27 (ddd, J = 9.4, 4.4, 2.1 Hz, 1H, CH_2O), 4.02 (dq, J = 20.5, 2.3 Hz, 1H, CH_2N). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 157.4, 146.5, 127.7, 64.2, 57.9, 41.8. IR (NaCl): 3081, 2959, 2920, 2854, 1752, 1748 cm^{-1}. $[\alpha]_D^{25}$ (c 0.09 in DCM): +65.02. Found: C, 55.1; H, 4.5%. Calc. for $C_7H_7NO_3$: C, 54.9; H, 4.6%.

*tert-Butyl (5aR,6S,6aS,7aR)-3,7-Dioxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylate (**6**).* To a solution of **5** (2.4 g, 15.9 mmol) in 13 mL of DCM at 0 °C is added a solution of *tert*-butyl (tetrahydrothiophenylidene)-acetate (9.6 g, 47.6 mmol) in 207 mL of DCM slowly. The reaction is stirred at room temperature for 30 min. Deionized water (20 mL) is added. The aqueous layer is extracted with DCM (2 × 30 mL). The combined organic phases are washed with brine (20 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude is purified in silica gel using Hex/AcOEt (2:1) as the eluent. A yellow wax is obtained (3.0 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (t, J = 9.7 Hz, 1H, CH_2O), 4.32 (dd, J = 9.3, 5.7 Hz, 1H, CH_2O), 4.23 (d, J = 14.1 Hz, 1H, CH_2N), 4.04 (dd, J = 10.3, 5.7 Hz, 1H, CHN), 3.53 (d, J = 14.0 Hz, 1H, CH_2N), 2.39 (dd, J = 7.9, 4.2 Hz, 1H, CHCO), 2.21−2.18 (m, 1H, C**H**CH_2N) 2.10 (t, J = 4.5 Hz, 1H, $CHCO_2$), 1.44 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0, 168.9, 156.9, 82.8, 64.2, 58.8, 37.2, 31.6, 28.1, 24.5, 22.7. IR (NaCl): 2975, 2863, 1748, 1736, 1719 cm^{-1}. $[\alpha]_D^{25}$ (c 0.11 in DCM): +38.02. Found: C, 58.0; H, 4.9%. Calc. for $C_{13}H_{17}NO_5$: C, 58.4; H, 4.6%.

*tert-Butyl (5aR,6S,6aS,7S,7aR) and tert-butyl (5aR,6-S,6aS,7R,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylate (**7a** and **7b**).* To a solution of **6** (1.1 g, 4.1 mmol) in 35 mL of absolute ethanol at 0 °C is added $NaBH_4$ (312 mg, 8.2 mmol). The reaction is stirred for 1 h at room temperature. A solution of saturated NH_4Cl (20 mL) and water (until salts dissolve) is added. The aqueous phase is extracted with AcOEt (3 × 60 mL). The combined organic layers are washed with brine (50 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude contained a 3:2 mixture of isomers **7a**/**7b** as determined by the integration of signals in the ^{1}H NMR spectrum of the reaction crude. This mixture was separated by silica gel chromatography using Hex/AcOEt (1:1) to Hex/AcOEt (1:2) as eluents. A yellow wax is obtained for isomer **7a** (463 mg, 42%). A yellow solid is obtained for isomer **7b** (330 mg, 30%).

Spectroscopic data for *tert*-butyl (5a*R*,6*S*,6a*S*,7*S*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]-pyridine-6-carboxylate **7a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (t, J = 8.6 Hz, 1H, CH_2O), 4.12 (dd, J = 9.1, 4.8 Hz, 1H, CH_2O), 4.00 (d, J = 13.6 Hz, 1H, CH_2N), 3.80 (dd, J = 8.5, 4.5 Hz, 1H, C**H**OH), 3.40 (dd, J = 13.6, 4.1 Hz, 1H, CH_2N), 3.33 (td, J = 8.3, 4.9 Hz, 1H, CHN), 2.41 (d, J = 5.0 Hz, 1H, OH), 1.76−1.70 (m, 1H, C**H**CH_2N), 1.67 (dd, J = 9.1, 5.0 Hz, 1H, C**H**CHOH), 1.44 (s, 9H, 3 × CH_3), 1.40 (t, J = 4.9 Hz, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 157.5, 81.4, 69.0, 68.3, 56.8, 38.5, 28.2 (3C), 27.3, 24.5, 20.3. IR (NaCl): 3361, 2975, 2863, 1748, 1736 cm^{-1}. $[\alpha]_D^{25}$ (c 0.14 in DCM): −2.20. Found: C, 58.2; H, 6.8%. Calc. for $C_{13}H_{19}NO_5$: C, 58.0; H, 7.1%.

Spectroscopic data for *tert*-butyl (5a*R*,6*S*,6a*S*,7*R*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]-pyridine-6-carboxylate **7b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (dd, J 0 8.5, 6.4 Hz, 1H, CH_2O), 4.29 (t, J = 8.8 Hz, 1H, CH_2O), 4.24−4.20 (m, 1H, C**H**OH), 4.00 (d, J = 13.4 Hz, 1H, CH_2N), 3.61−3.55 (m, 1H, CHN), 3.29 (dd, J = 13.4, 4.0 Hz, 1H, CH_2N), 2.11 (td, J = 8.4, 5.0 Hz, 1H, C**H**CHOH), 1.97 (d, J = 4.1 Hz, 1H, OH), 1.84−1.74 (m, 2H, 2 × CH cyclopropane), 1.44 (s, 9H, 3 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 157.8, 81.5, 63.1, 60.4, 55.7, 38.8, 28.3 (3C), 25.9, 21.2, 20.5. IR (KBr): 3361, 2975, 2863, 1748, 1736 cm^{-1}. $[\alpha]_D^{25}$ (c 0.04 in DCM): −10.46. Found: C, 58.3; H, 7.0%. Calc. for $C_{13}H_{19}NO_5$: C, 58.0; H, 7.1%. Mp > 180.0 °C, dec.

(5aR,6S,6aS,7S,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylic Acid. To a solution of **7a** (250 mg, 0.9 mmol) in 1.5 mL of DCM is added 9.3 mL of TFA at room temperature. The reaction is stirred for 30 min. The solvent is evaporated *in vacuo*. Toluene is added until all TFA is evaporated. The crude is purified in silica gel in 10% DCM/MeOH. A brown wax is obtained (187 mg, 94%). ^{1}H NMR (400 MHz, MeOD) δ 4.54 (t, J = 8.5 Hz, 1H, CH_2O), 4.14 (dd, J = 8.9, 5.2 Hz, 1H, CH_2O), 3.87 (d, J = 13.6 Hz, 1H, CH_2N), 3.82 (d, J = 8.7 Hz, 1H, C**H**OH), 3.44 (dd, J = 13.5, 4.2 Hz, 1H, CH_2N), 3.37 (td, J = 8.5, 5.2 Hz, 1H, CHN), 1.84−1.75 (m, 1H, C**H**CH_2N), 1.70 (dd, J = 9.3, 4.7 Hz, 1H, C**H**CHOH), 1.38 (brs, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, D_2O) δ 176.6, 159.5, 69.8, 69.6, 58.2, 39.5, 29.0, 24.5, 21.9. IR (NaCl): 3396, 2984, 2851, 1748, 1729 cm^{-1}. $[\alpha]_D^{25}$ (c

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0.05 in MeOH): −3.68. Found: C, 51.1; H, 5.3%. Calc. for $C_9H_{11}NO_5$: C, 50.7; H, 5.2%.

(5aR,6S,6aS,7R,7aR)-7-Hydroxy-3-oxohexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridine-6-carboxylic Acid. To a solution of **7b** (86 mg, 0.3 mmol) in 1 mL of DCM is added 3.2 mL of TFA at room temperature. The reaction is stirred for 30 min. The solvent is evaporated *in vacuo*. Toluene is added until all TFA is evaporated. The crude is purified in silica gel in 10% DCM/MeOH. A brown wax is obtained (60 mg, 90%). ^{1}H NMR (400 MHz, D_2O) δ 4.47 (dd, J = 8.5, 5.8 Hz, 1H, CH_2O), 4.31 (t, J = 8.8 Hz, 1H, CH_2O), 4.17 (dd, J = 8.0, 3.8 Hz, 1H, C**H**OH), 3.86 (d, J = 13.4 Hz, 1H, CH_2N), 3.71 (ddd, J = 9.5, 5.9, 3.9 Hz, 1H, CHN), 3.38 (dd, J = 13.4, 4.4 Hz, 1H, CH_2N), 2.09 (td, J = 8.4, 4.7 Hz, 1H, C**H**CHOH), 1.82 (t, J = 4.9 Hz, 1H, $CHCO_2$), 1.80−1.75 (m, 1H, C**H**CH_2N). ^{13}C NMR (100 MHz, D_2O) δ: 177.7, 159.6, 64.2, 59.8, 55.4, 38.4, 26.0, 21.2, 20.0. IR (KBr): 3388, 2991, 2867, 1740, 1732 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): −48.87. Found: C, 51.0; H, 4.9%. Calc. for $C_9H_{11}NO_5$: C, 50.7; H, 5.2%. Mp > 205.4 °C, dec.

*(1R,4R,5S,6S,7S)-5-Hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-7-carboxylic Acid (**8a**).* To a solution of (5a*R*,6*S*,6a*S*,7*S*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]pyridine-6-carboxylic acid (187 mg, 0.9 mmol) in 2 mL of MeOH is added ethylenediamine (0.18 mL, 2.6 mmol) at room temperature and heated at 60 °C for 1.5 h. The solvent is evaporated *in vacuo* and methanol is added to evaporate excess amine. A solution of HCl 4 N in dioxane (5 mL) is added and stirred for 30 min. A yellow wax is obtained (150 mg, 91%).^{1}H NMR (400 MHz, MeOD) δ 4.54 (t, J = 8.5 Hz, 1H, CH_2O), 4.14 (dd, J = 8.9, 5.3 Hz, 1H, CH_2O), 3.86 (d, J = 13.5 Hz, 1H, CH_2N), 3.72 (d, J = 8.7 Hz, 1H, C**H**OH), 3.44 (dd, J = 13.5, 4.3 Hz, 1H, CH_2N), 3.36 (td, J = 8.4, 5.2 Hz, 1H, CHN), 1.78−1.70 (m, 1H, C**H**CH_2N), 1.67 (dd, J = 9.2, 4.9 Hz, 1H, C**H**CHOH), 1.32 (t, J = 4.9 Hz, 1H, $CHCO_2$). ^{13}C NMR (100 MHz, MeOD) δ 159.5, 70.1, 69.6, 58.2, 39.6, 28.5, 25.7, 21.3. IR (NaCl): 3405, 2996, 2895, 1736 cm^{-1}. $[\alpha]_D^{25}$ (c 0.05 in MeOH): −5.41. Found: C, 51.0; H, 7.2%. Calc. for $C_8H_{13}NO_4$: C, 51.3; H, 7.0%.

*(1R,4R,5R,6S,7S)-5-Hydroxy-4-(hydroxymethyl)-3-azabicyclo [4.1.0]heptane-7-carboxylic Acid (**8b**).* To a solution of (5a*R*,6*S*,6a*S*,7*R*,7a*R*)-7-hydroxy-3-oxohexahydro-1*H*,3*H*-cyclopropa[*d*]oxazolo[3,4-*a*]pyridine-6-carboxylic acid (75 mg, 0.4 mmol) in 1 mL of MeOH is added ethylenediamine (0.07 mL, 1.1 mmol) and heated at 60 °C for 1.5 h. The solvent is evaporated *in vacuo* and methanol is added to evaporate excess amine. A solution of HCl 4 N in dioxane (1 mL) is added and stirred for 30 min. A yellow wax is obtained (60 mg, 90%). ^{1}H NMR (400 MHz, MeOD) δ 4.47 (dd, J = 8.5, 6.0 Hz, 1H, CH_2O), 4.30 (t, J = 8.8 Hz, 1H, CH_2O), 4.16 (dd, J = 8.0, 3.9 Hz, 1H, C**H**OH), 3.84 (d, J = 13.3 Hz, 1H, CH_2N), 3.75−3.62 (m, 1H, CHN), 3.37 (dd, J = 13.2, 4.4 Hz, 1H, CH_2N), 2.11−1.93 (m, 1H, C**H**CHOH), 1.79−1.64 (m, 2H, 2 × CH cyclopropane). ^{13}C NMR (100 MHz, MeOD) δ 158.80, 63.53, 59.64, 55.65, 38.46, 25.01, 21.50, 19.60. IR (NaCl): 3402, 2984, 2890, 1733 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): −32.0. Found: C, 51.4; H, 7.2%. Calc. for $C_8H_{13}NO_4$: C, 51.3; H, 7.0%.

*(1S,4R,5S,6S,7S)-4,7-bis(Hydroxymethyl)-3-methyl-3-azabicyclo[4.1.0]heptan-5-ol (**9**).* To a solution of **7a** (120 mg, 0.5 mmol) in 3 mL of DCM is added 1.86 mL of DIBAL-H 1,2M in toluene at 0 °C. The reaction is stirred for 4 h at room temperature. Methanol is added (10 mL). The salts are filtered and rinsed with methanol (2 × 10 mL). The solvent is evaporated *in vacuo*. The crude is purified in silica gel using MeCN/H_2O (9:1) as the eluent. A yellow wax is obtained (49 mg, 58%). ^{1}H NMR (400 MHz, MeOD) δ 3.81 (dd, J = 11.7, 3.2 Hz, 1H, CH_2O), 3.77−3.71 (m, 2H, CH_2O + C**H**OH), 3.45 (dd, J = 11.3, 6.7 Hz, 1H; HOC**H**$_2$Ccyclopropane), 3.37 (dd, J = 11.3, 6.8 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.07 (d, J = 11.7 Hz, 1H, CH_2N), 2.63 (dd, J = 11.6, 3.9 Hz, 1H, CH_2N), 2.38 (s, 3H, NCH_3), 1.75 (dt, J = 7.9, 2.9 Hz, 1H, CHN), 1.23−1.15 (m, 1H, HOCH_2C**H**cyclopropane), 1.12−1.03 (m, 1H, C**H**CH_2N), 0.99 (dd, J = 9.0, 4.6 Hz, 1H, C**H**CHOH). ^{13}C NMR (100 MHz, MeOD) δ 70.2, 67.1, 65.9, 60.1, 55.6, 43.0, 23.24, 23.22, 17.3. IR (NaCl): 3357, 2993, 2892 cm^{-1}. $[\alpha]_D^{25}$ (c 0.03 in MeOH): −4.65. Found: C, 57.5, H, 9.5%. Calc. for $C_9H_{17}NO_3$: C, 57.7; H, 9.2%.

*(5aS,6S,6aS,7R,7aR)-7-Hydroxy-6-(hydroxymethyl)-hexahydro-1H,3H-cyclopropa[d]oxazolo[3,4-a]pyridin-3-one (**10**).* To a solution of **7b** (117 mg, 0.4 mmol) in 3 mL of DCM at 0 °C is added 1.80 mL of 1,2M DIBAL-H in toluene. The reaction is stirred for 4 h at room temperature. Methanol is added (10 mL). The salts are filtered and rinsed with methanol (2 × 10 mL). The solvent is evaporated *in vacuo*. The crude is purified in silica gel using MeCN as the eluent. A yellow wax is obtained (54 mg, 62%). ^{1}H NMR (400 MHz, MeOD) δ 4.48 (dd, J = 8.5, 5.8 Hz, 1H, CH_2O), 4.32 (t, J = 8.8 Hz, 1H, CH_2O), 4.16 (dd, J = 8.1, 3.8 Hz, 1H, C**H**OH), 3.83 (d, J = 12.9 Hz, 1H, CH_2N), 3.69 (ddd, J = 9.4, 5.8, 3.8 Hz, 1H, CHN), 3.54 (dd, J = 11.2, 6.4 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.42 (dd, J = 11.3, 6.7 Hz, 1H, HOC**H**$_2$Ccyclopropane), 3.36 (dd, 12.9, 4.7 Hz, 1H, CH_2N), 1.42 (td, J = 8.4, 4.9 Hz, 1H, C**H**CHOH), 1.30−1.20 (m, 1H, HOCH_2C**H** cyclopropane), 1.19−1.09 (m, 1H, C**H**CH_2N). ^{13}C NMR (100 MHz, MeOD) δ 160.4, 65.6, 65.0, 61.4, 57.2, 40.2, 21.9, 21.0, 16.5. IR (NaCl): 3384, 2991, 2888, 1705 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): −35.3. Found: C, 54.5; H, 6.5%. Calc. for $C_9H_{13}NO_4$: C, 54.3; H, 6.6%.

General Procedure for Enzymatic Reactions. Glycosidase activities were assessed in 80 μL reaction volumes in Eppendorf vials. Buffer composition and enzyme concentration were adjusted depending on the enzyme assayed: 20 mM Na_2HPO_4 at pH 7.3 for β-glucosidase (3 μg/mL) and β-galactosidase (1 μg/mL); 20 mM Na_2HPO_4 at pH 6.8 for α-glucosidase (1 μg/mL) and α-galactosidase (20 μM); 20 mM NaH_2PO_4 at pH 5.5 for α- and β-mannosidase (7 and 2 μM respectively); 0.1 M NaOAc at pH 4.0 with 1 mg/mL of bovine serum albumin (BSA) for α-L-fucosidase (2 μM); and 50 mM NaOAc at pH 5.0 for neuraminidase (6 μM). The inhibitors were tested at 1, 5, and 25 mM final concentrations in the assays. Each enzyme mixture and inhibitor were homogenized and preincubated for 10 min at 37 or 40 °C (α-L-fucosidase). Each reaction was initiated and brought to a final volume of 80 μL, by addition of an aliquot of the corresponding *p*-nitrophenyl glycoside substrate to obtain the following final concentrations in the reaction mixtures: *p*-nitrophenyl α- and β-D-glucopyranoside (1 mM), *p*-nitrophenyl α- and β-D-galactopyranoside (0.5 mM), *p*-nitrophenyl α- and β-D-mannopyranoside (1 mM), *p*-nitrophenyl α-L-fucopyranoside (1 mM), or *p*-nitrophenyl neuraminic acid (1 mM). After 10 min of incubation time at the same temperature, each reaction was quenched with 400 μL of 1.0 M Na_2CO_3, and the absorbance at 405 nm was measured. Assays were repeated twice and data were averaged.

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The residual activity of each enzyme was calculated by the ratio of the absorbance measured after 10 min of reaction in the presence and absence of synthesized compounds. The equation used to calculate K_i was derived from Michaelis–Menten, where V_i is the absorbance measured in the absence of the synthesized compounds; V is the absorbance when the compounds were added to the enzymatic reaction; K_m indicates the Michaelis–Menten constant for each enzyme; and [I] is the concentration of the synthesized compounds (5 mM) and [S] is the concentration of the substrate (eq 1).

Calculated K_i for compounds 8a and 9

$$K_i = \frac{K_m[I]}{(K_m + [S])(V/V_i - 1)} \quad (1)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04589.

^{1}H NMR, ^{13}C NMR, and IR spectra of synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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3. SYNTHESIS AND EVALUATION OF NOVEL IMINOSUGARS PREPARED FROM NATURAL AMINO ACIDS.

This work shows the second part of the synthesis of novel iminosugars rigidified by a cyclopropane ring and the study of their ability to inhibit glycosidases. The synthesis of 7 final compounds is described, from L-serine and L-alanine as chiral pools, following a different synthetic strategy from the previous work. No racemization was observed in any of the reactions. The key step was the cyclopropanation reaction of an α,β–unsaturated ketone using a previously synthetized sulfonium ylide. The enzymatic assays were carried out against different glycosidases in Prof. Javier Cañada´s laboratory. Some of these compounds presented inhibition in the range of mM. Even though this modest result they were selective. In some cases, there was activation of glycosidases. Studies of possible transglicosidation reactions were additionally carried out using NMR.

In this work I did all the experimental work including the synthesis and characterization of the compounds, the enzymatic assays and the analysis of the results. I collaborated in the elaboration of the manuscript with my supervisors, mainly in the experimental part and supplementary information.

En este trabajo se presenta la segunda parte de la síntesis de nuevos iminoazúcares rigidificados por un anillo de ciclopropano y su estudio como posibles inhibidores de glicosidasas. Se describe la síntesis de 7 compuestos finales, usando L-serina y L-alanina como productos de partida, siguiendo una estrategia de síntesis diferente a la del trabajo anterior. No se observa racemización en ninguna etapa de la síntesis. La reacción clave fue la ciclopropanación de una cetona α,β–insaturada usando un iluro de azufre previamente sintetizado y aislado. Los ensayos enzimáticos se llevaron a cabo en el laboratorio del Prof. Javier Cañada frente a diferentes glicosidasas. Algunos de estos compuestos presentaban inhibición en el rango de mM. A pesar de este modesto resultado, eran selectivos. En algunos casos había activación de alguna glicosidasa. Adicionalmente, se realizaron estudios de RMN para estudiar una posible transglicosidación.

En este trabajo he realizado toda la parte experimental incluyendo la síntesis y caracterización de los compuestos, los ensayos enzimáticos y la interpretación de los resultados obtenidos. He colaborado en la redacción del manuscrito junto a mis supervisores, especialmente en la parte experimental y la información suplementaria.

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Synthesis and Evaluation of Novel Iminosugars Prepared from Natural Amino Acids

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Abstract: Cyclopropanated iminosugars have a locked conformation that may enhance the inhibitory activity and selectivity against different glycosidases. We show the synthesis of new cyclopropane-containing piperidines bearing five stereogenic centers from natural amino acids L-serine and L-alanine. Those prepared from the latter amino acid may mimic L-fucose, a natural-occurring monosaccharide involved in many molecular recognition events. Final compounds prepared from L-serine bear *S* configurations on the C5 position. The synthesis involved a stereoselective cyclopropanation reaction of an α,β-unsaturated piperidone, which was prepared through a ring-closing metathesis. The final compounds were tested as possible inhibitors of different glycosidases. The results, although, in general, with low inhibition activity, showed selectivity, depending on the compound and enzyme, and in some cases, an unexpected activity enhancement was observed.

Keywords: piperidine iminosugars; glycosidase inhibition; metathesis; sulfur ylide cyclopropanation

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1. Introduction

Natural or synthetic polyhydroxylated piperidines are iminosugars able to act as biomimetics of their corresponding pyranose analogs. For instance, nojirimycin, its epimers, and their deoxyanalogs have been used as lead molecules to design glycosidase and glycosyl transferase inhibitors and modulators [1–3]. Some iminosugars such as miglitol (Glyset®) [4], migalastat (Galafold®) [5], and miglustat (Zavesca®) [6] are commercially available, and others are actually in different clinical phases (Figure 1). The interaction with glycosidases is generally attributed to a structural similarity to diverse conformational oxocarbenium transition states formed during the hydrolysis of carbohydrates [7]. There are important variations in these transition states for different glycosidases [8,9]. Thus, there is a great interest in designing conformationally restricted inhibitors in order to achieve selective inhibition and adequate metabolic stability.

Our group engaged in the synthesis of novel piperidine iminosugars fused to a cyclopropane ring, resulting in structures with a locked conformation [10,11]. The cyclopropane renders a twist-like conformation to the piperidine ring that is found, as preferred for interactions with certain glycosidases [12,13]. We expect these compounds to be starting points for the finding of products of pharmacological interest (Figure 2). The possibility of variations in the substitution pattern of the cyclopropane allows different configurations that could direct their selectivity to different glycosidases. Although the development of synthetic routes to iminosugars has received much attention in the synthetic community [14,15], the preferred chiral pools are carbohydrates, which are transformed using reductive aminations [16,17], or other transformation strategies [18]. In our case, we developed synthetic approaches from natural amino acids, which have less precedents [19,20].

Other asymmetric or biocatalyzed approaches have been used [21]. In our previous work, starting from natural amino acid L-serine, we synthesized iminosugars bearing the *R* configuration on the carbon adjacent to nitrogen, which was supposed to mimic C5 in natural carbohydrates and iminosugars.

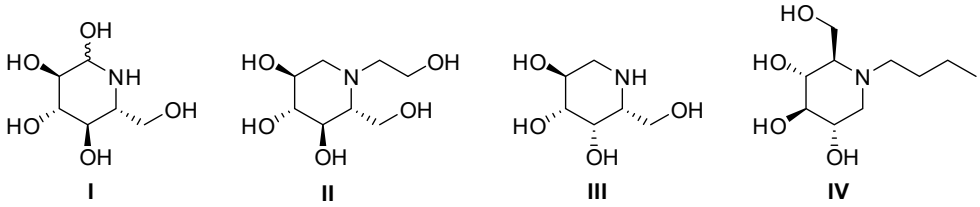


Figure 1. Structures of nojirimycin (**I**), miglitol (**II**), migalastat (**III**), and miglustat (**IV**).

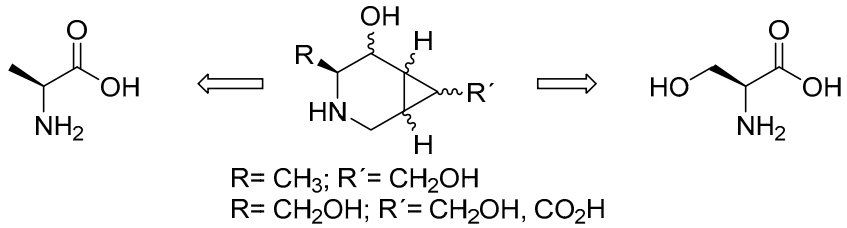


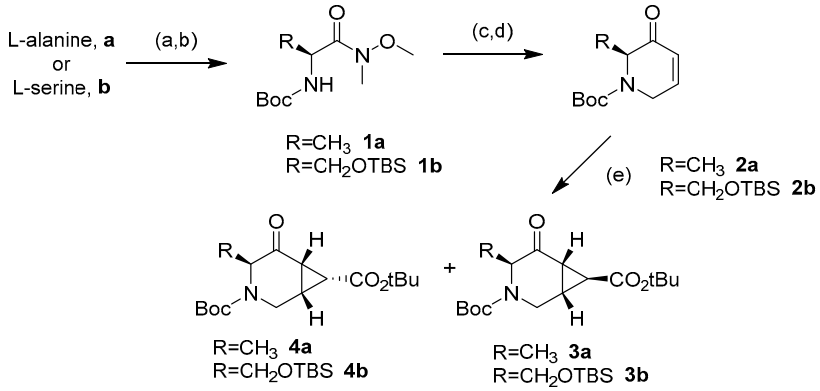
Figure 2. Structures of the targeted compounds.

The present work is focused on new compounds prepared both from L-alanine and L-serine. In the case of L-alanine, the derived compounds could mimic 6-dehydroxylated sugars as fucose. Fucose-containing glycans, such as in blood groups and Lewis oligosaccharides and related ones, are critical for a wide range of cell events [22]. These include cell–cell adhesion, immune response, viral and bacterial infection, and tumor progression. We prepared new bicyclic iminosugars that include the cyclopropane motif fused with a piperidine that may mimic the L-fucose ring and evaluated them against fucosidase and other glycosidases. In addition, we prepared cyclopropane-containing piperidine iminosugars starting from L-serine but now with an *S* configuration at the carbon that mimics C5. A preliminary glycosidase inhibition evaluation is shown. The synthesis implies building final compounds with five stereogenic centers [23–25].

2. Results and Discussion

2.1. Chemistry

The synthesis of the novel iminosugars started from L-serine or L-alanine as the chiral pool. Both amino acids were protected using di-*tert*-butyl dicarbonate (Boc_2O) [26]. Without further purification, these latter intermediates were submitted to coupling with *N*,*O*-dimethylhydroxylamine and, for the L-serine derivative, protection with *tert*-butyldimethylsilyl of the hydroxy group. Thus, intermediates **1a** (78%) and **1b** (76%) were obtained in good yields. Treatment with a base and allyl bromide, followed by a reaction with vinylmagnesium bromide at −30 °C, gave the precursors of the ring-closing metathesis reaction (RCM). For the RCM, a second-generation Grubbs' catalyst (Grubbs' Catalyst® M204, 3 mol %) was used, affording the α,β-unsaturated ketones **2a** and **2b** in 76% and 60% yield, respectively (Scheme 1) [27]. These compounds reacted with *tert*-butyl 2-(tetrahydro-1λ^4-thiophen-1-ylidene)acetate to give a mixture (as seen in ^{1}H-NMR) of cyclopropane exo:endo isomers **3a** (77%) and **4a** (5%) from **2a** in a 15:1 ratio, while **3b** (71%) and **4b** (18%) from **2b** in a 4:1 exo:endo ratio [28]. These isomers were isolated and separately characterized. ^{1}H-NMR at 90 °C in DMSO-d_6 determined that compounds **3b** and **4b** were obtained as a mixture of conformers due to the slow rotation of the Boc group in a 55:45 ratio in both cases (Figure 3).



Scheme 1. Reagents and conditions: (**a**) Boc_2O, NaOH 1M, Dioxane, 0 °C to r.t.; then, diisopropilehtyl amine (DIPEA), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), *N*,*O*-dimethylhydroxilamine, dichloromethane (DCM), 0 °C, 78% from L-serine (**1a**). (**b**) For the product derived from L-serine: Imidazole, 4-(dimethylamino)pyridine (DMAP), *tert*-butyldimthylsilyl chloride (TBSCl), dimethylformamide (DMF), 0 °C to r.t., 76% from L-serine (**1b**). (**c**) NaH, allyl bromide, DMF, 0 °C to 50 °C, 78% (from **1a**) and 58% (from **1b**). (**d**) Vinylmagnesium bromide, tetrahydrofurane (THF) −30 °C; then, second-generation Grubbs' catalyst (3 mol %), DCM, 50 °C, 76% (**2a**) and 60% (**2b**). (**e**) *tert*-Butyl (tetrahydrothiophenylidene)acetate, DCM, 0 °C to r.t., 77% (**3a**), 5% (**4a**), 71% (**3b**), and 18% (**4b**).

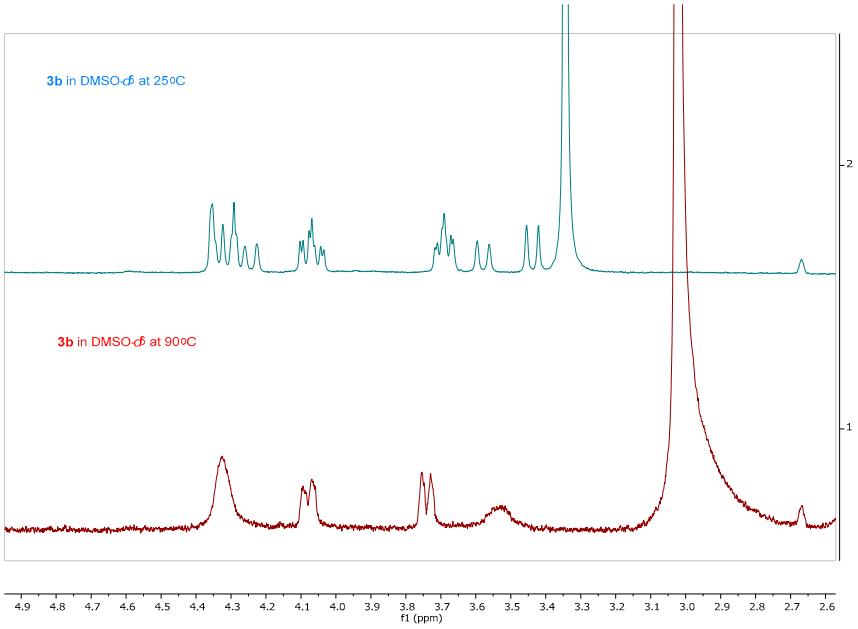
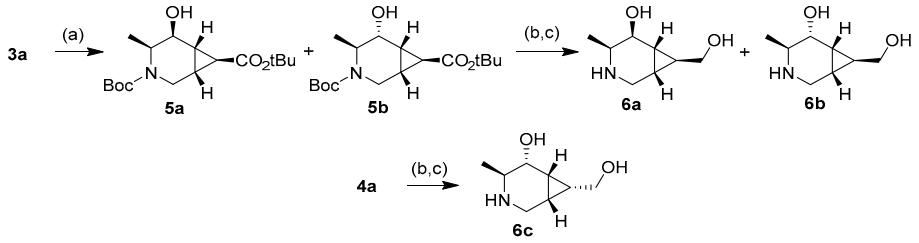


Figure 3. ^{1}H-NMR (400 MHz, $CDCl_3$) of **3b** in DMSO-d_6 at 25 °C (blue) and 90 °C (red).

The cyclopropanation reaction has two steps, ylide addition to the double bond and ring closure. It is known that the sulfur ylide attack is nonselective, and the exo:endo selectivity is determined in the second step [29–31]. The result depends on many different issues, such as temperature, reagent concentration, and the presence of a base. For instance, in the cyclopropanation reaction of **2a**, the 15:1 exo:endo ratio was observed when carrying out the reaction at 0 °C and a low concentration of ylide (0.15 M), while a higher temperature and ylide concentration promoted higher ratios in favor of an *exo* isomer. In the case of the cyclopropanation of compound **2b** with an ylide concentration of 2M and temperature of 20 °C, the ratio of *exo/endo* isomers can increase up to 20:1, as seen by NMR. Similar behavior has already been reported [32].

With the intermediates **3** and **4** in hand, the final products were prepared by a reduction of the ketone and further hydrolysis or reduction of the ester group. Thus, compound **3a** was treated with $NaBH_4$ to give isomers **5a** and **5b** in a 3:2 ratio (Scheme 2). These alcohols were isolated in 55% (**5a**) and 19% (**5b**) yields and separately treated with diisobutylaluminium hydride (DIBAL-H), giving the corresponding intermediates in 55% and 67% yields, respectively. A final treatment with trifluoroacetic acid (TFA) afforded compounds **6a** (85%) and **6b** (83%) after a final elution through a basic DOWEX resin. This two-step reduction allowed the separation of the isomers **5a** and **5b**. When using stronger conditions to perform the reduction in one step from **3a**, a mixture of diols was obtained but could not be separated. On the other hand, isomer **4a** reacted with DIBAL-H to give only a product in 32% yield, which, after hydrolysis with TFA and further elution through a basic DOWEX resin, afforded free amine **6c** (81%).



Scheme 2. Reagents and conditions: (**a**) $NaBH_4$, EtOH, 0 °C to r.t., 55% (**5a**) and 19% (**5b**). (**b**) DIBAL-H, DCM, 0 °C to r.t., 55% (from **5a**), 67% (from **5b**), and 32% (from **4a**). (**c**) Trifluoroacetic acid (TFA), MeOH, r.t., 85% (**6a**), 83% (**6b**), and 81% (**6c**).

Compound **3b** diastereoselectively reacted with $NaBH_4$, resulting in **7** as the only reaction product. **4b** reacted, giving a separable mixture of compounds **8** and **9**. The selectivity of this reduction is governed by the configuration of the cyclopropane ring but not by the location of the bulky *tert*-butyldimthylsilyl (OTBS) group; as in the case of **4b**, the hydride reacts by the face of the OTBS group. The Felkin-Anh model corroborates these results: on the *exo* isomer **3b**, the *tert*-butyl ester group gets far from the carbonyl, but the OTBS is the one making the steric hindrance to determine the stereochemistry of the reaction. On the other hand, the *endo* isomer **4b** has both bulky groups near the carbonyl, staying close to the ester. Moreover, CH_2OTBS can rotate to get farther from carbonyl, while the *tert*-butyl ester cannot (Figure 4).

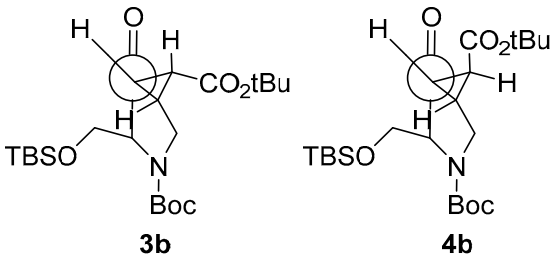
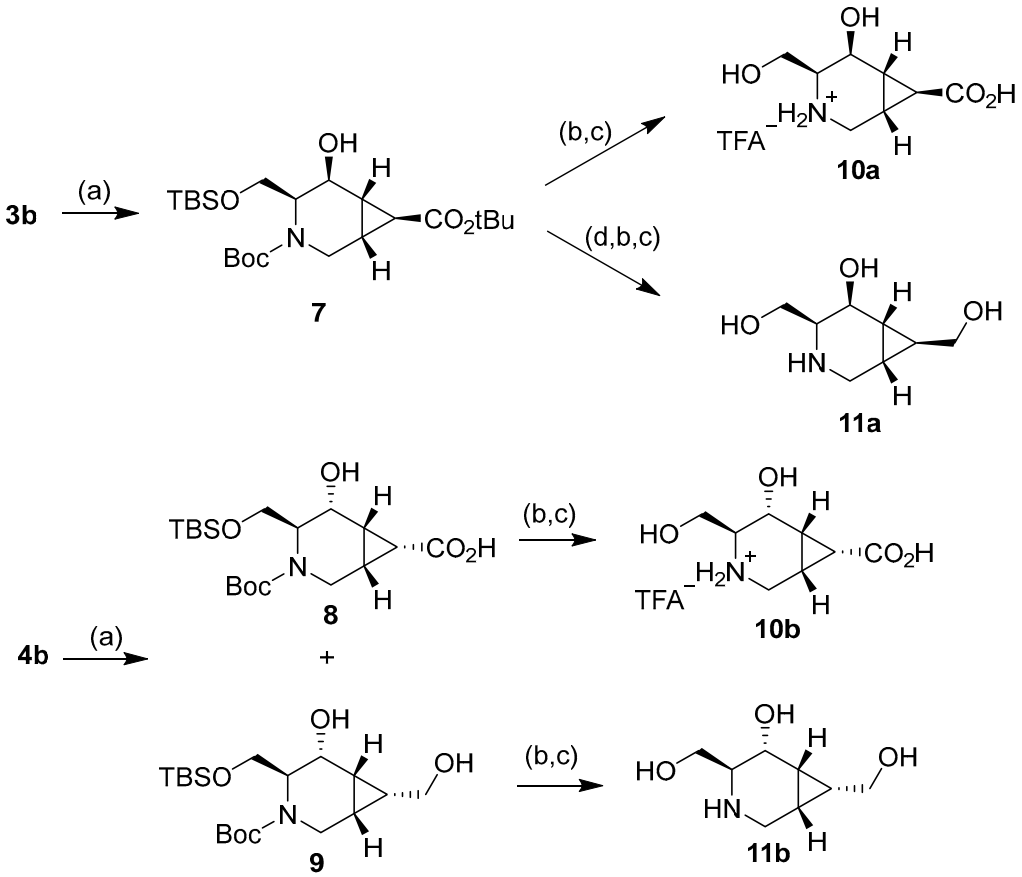


Figure 4. The Felkin-Anh model for compounds **3b** and **4b**.

Deprotection of the hydroxyl group of compound **7** using tetrabutylammonium fluoride trihydrate ($TBAF.3H_2O$) and further treatment with trifluoroacetic acid (TFA) resulted in the final product **10a** as a trifluoroacetate salt (Scheme 3) [33]. On the other hand, the reduction of the *tert*-butyl ester in **7** with DIBAL-H gave the *N*-Boc and OTBS protected intermediate, which was treated as previous to give the corresponding trifluoroacetate salt. After treatment of this salt with a basic DOWEX resin, the final compound **11a** was obtained. Compound **8** was deprotected to give compound **10b** as a trifluoroacetate salt. Finally, compound **9** was treated similarly to afford final compound **11b**.



Scheme 3. Reagents and conditions: (**a**) $NaBH_4$, EtOH, 0 °C to r.t., 74% (**7**), 30% (**8**), and 50% (9). (**b**) TBAF.$3H_2O$, THF, r.t., 85% (from **7** to **10a**), 68% (from **7** to **11a**), 88% (from **8**), and 86% (from **9**). (**c**) TFA, MeOH, r.t., 87% (**10a**), 91% (**11a**), 92% (**10b**), and 93% (**11b**). (**d**) DIBAL-H, DCM, 0 °C to r.t., 70%.

2.2. Modeling and NOE Experiments

The stereochemistry of all the synthesized products was assigned by means of NOESY experiments and coupling constant calculations. All compounds were first modeled on Chimera 1.13.1, using ANTECHAMBER for the computing charges [34]. With these models, we could calculate the relevant dihedral angles and predict the expected NOE effects, which were checked with those experimentally obtained. Figure 5 shows the models and main NOE effects of compounds **5a** and **b** and compound **6c** derived from L-alanine. H5 in compound **5a** shows a NOE interaction with H4 and H7. On the other hand, the other reduction isomer, **5b**, gave a NOE signal between H5 and H6 and an intense effect between H5 and the methyl group. The final product **6c** obtained from the *endo* isomer, after the cyclopropanation reaction, gave analog signals as **5b**.

The models and NOE interactions for compounds **7** and **11b** are shown in Figure 6. Compound **7** was assigned with the NOE effects observed between H7 and H5, H5 and H4, and between H7 and H4. The constant couplings measured in ^{1}H-NMR also agreed with the modeled angles. On the other hand, compound **11b** only showed one NOE signal between H5 and H6 (Figure 6). The measured coupling constant agreed with the calculated ones from models using the Carplus equation (see Supporting Information Table S1).

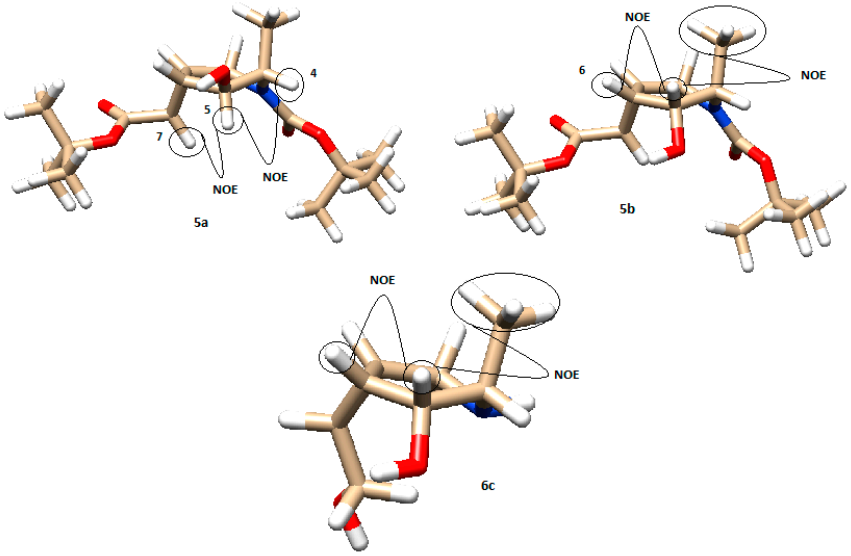


Figure 5. Model of compounds **5a, 5b**, and **6c** with their main NOE interactions (brown: carbon; white: hydrogen; red: oxygen; blue: nitrogen).

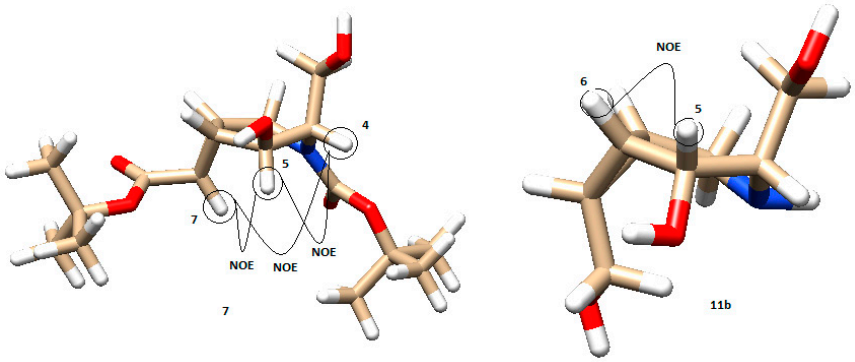


Figure 6. Model of compounds **7** and **11b**, respectively, with their main NOE interactions (brown: carbon; white: hydrogen; red: oxygen; blue: nitrogen).

2.3. Enzymatic Assays

Glycosidase activities were assessed in 80-µL reaction volumes in Eppendorf vials. Buffer composition and enzyme concentrations were adjusted depending on the enzyme: 20-mM Na_2HPO_4 at pH 7.3 for β-glucosidase from Almonds (3 µg/mL) and β-galactosidase from *Escherichia coli* (1 µg/mL), 20-mM Na_2HPO_4 at pH 6.8 for α-glucosidase from *Bacillus stearothermophilus* (1 µg/mL) and α-galactosidase from Green coffee (20 µM), 20-mM NaH_2PO_4 at pH 5.5 for α- and β-mannosidase from Jack beans and *Helix pomatia*, respectively (7 µM and 2 µM, respectively), 0.1-M NaOAc at pH 4.0 with 1 mg/mL of BSA (bovine serum albumin) for α-L-fucosidase from *Homo sapiens* (2 µM), and 50-mM NaOAc at pH 5.0 for neuraminidase from *Vibrio cholerae* (6 µM). The inhibitors were tested at 1-, 5-, and 25-mM final concentrations in the assays. Each mixture of enzyme and inhibitor was homogenized and preincubated for 10 min at 37 °C or 40 °C (α-L-fucosidase). Each reaction was initiated and brought to a final volume of 80 µL by the addition of an aliquot of the corresponding *p*-nitrophenyl glycoside substrate to obtain the following final concentrations in the reaction mixtures: *p*-nitrophenyl α- and β-D-glucopyranoside (1 mM), *p*-nitrophenyl α- and β-D-galactopyranoside (0.5 mM), *p*-nitrophenyl α- and β-D-mannopyranoside (1 mM), *p*-nitrophenyl α-L-fucopyranoside (1 mM), or *p*-nitrophenyl neuraminic acid (1 mM). After 10 min of incubation time at the same temperature, each reaction was quenched with 400 µL of 1.0-M Na_2CO_3, and the absorbance at 405 nm was measured. Assays were repeated twice, and the data was averaged.

The enzymatic activity was calculated by the ratio in the absorbance measured at 405 nm of the phenoxide released in the enzymatic reaction. The final compounds were screened at 1, 5, and 25 mM. The assays at 1 mM of synthesized compounds did not give clear results. Thus, the activity detected at 5 mM is shown in Table 1.

Table 1. Residual activity of enzymes at 5 mM of the active final compounds.

	α-Glc	β-Glc	α-Gal	β-Gal	α-Man	β-Man	α-L-Fuc
6a	*	*	*	*	*	*	*
6b	*	*	*	*	*	148%	142%
6c	*	43%	*	*	*	*	*
10a	*	*	154%	*	*	155%	*
10b	*	39%	*	*	*	*	*
11a	*	*	*	*	*	*	*
11b	*	*	*	25%	*	*	*

* Activity not detected even at 25 mM.

None of the synthesized compounds showed activity against α-glucosidase, α-mannosidase, or neuraminidase at the concentrations used. In the case of products **11a** and **6a**, no activity was observed against any of the enzymes. Interestingly, products **6c** and **10b** inhibited only one enzyme, β-glucosidase, decreasing their activity to 43% and 39% at 5 mM, respectively. The results at 25 mM were 20% and 13% of the residual activity, respectively. Product **11b** inhibited the activity of β-galactosidase to 25% at 5 mM but without an inhibition increase at 25 mM.

On the other hand, some assays showed an enhancement in the enzyme activity. Thus, compound **6b** increased the activity of β-mannosidase and α-L-fucosidase up to 148% and 142% at 5 mM, respectively. This increase raised up to 240% at 25 mM. Compound **10a** activated α-galactosidase and β-mannosidase up to around 155% at 5 mM.

Regarding the inhibitory results, we can conclude that these compounds are very weak inhibitors only against certain enzymes far from the inhibition values of well-known iminosugars such as deoxinojirimycin [35] or castanospermine analogs [36]. However, the activation observed in certain cases deserves some comments, as there are few precedents of this behavior [37,38], including our previous results with similar compounds [10]. This activation does not have a clear explanation. The possibility that the compounds could work as efficient transglycosidation acceptors and, thus, accelerate the nitrophenol release was checked following the enzymatic reaction in a NMR tube and recorded spectra each 5 min. However, no potential transglycosilation product was detected (see the Supporting Information). Other cases of glycosidase activity enhancements were described; thus, some glycosidases were found to activate when using multivalent iminosugars [37]. Other reports explained the activation mechanism by the introduction of a small molecule in the active site, locking the reactive form of the glycosidase [38], or through an allosteric-type interaction that changed the conformation of the enzyme into the active one. These observations need further research to explain this behavior.

3. Materials and Methods

3.1. General Information

All chemicals were obtained from Aldrich/Merck (St. Louis, MO, USA), VWR (Radnor, PA, USA), Fluorochem (Derbyshire, UK), and ABCR (Karlsruhe, Germany). TLC analyses were performed on Merck silica gel 60 F254 plates using phosphomolybdic acid or anisaldehyde and heat for detection. Silica gel NORMASIL 60 40–63 μm was used for flash chromatography. NMR spectra were recorded on a Bruker spectrometer (400 MHz or 300 MHz for ^{1}H and 100 MHz or 75 MHz for ^{13}C), (Billerica, MA, USA). Chemical shifts are reported in δ ppm referenced to $CDCl_3$ (δ = 7.26 for ^{1}H and 77.00 for ^{13}C), CD_3OD (δ = 3.31

for ^{1}H and 49.00 for ^{13}C), or D_2O (δ = 4.79 for ^{1}H). Bidimensional spectra (HMQC, HMBC, COSY, and NOESY) were recorded in order to carry out the assignment. Infrared spectra were done in a Perkin-Elmer spectrum 100 (Agilent, Santa Clara, CA, USA). Specific optical rotation was measured in a Polarimeter Anton Paar MCP 100 (Anton Paar, Graz, Austria). Melting points of solid compounds were determined using a Stuart Scientific Melting Point Apparatus SMP3 (Stuart, Staffordshire, UK). Microanalyses were done on a LECO CHNS-932 (LECO, St. Joseph, MI, USA). Absorbance of *p*-nitrophenoxyde released in the enzymatic reactions was measured at 405 nm in a Perkin-Elmer Lamba25 (PerkinElmer, Waltham, MA, USA).

3.2. Synthesis

Synthesis of **1**–**11**

Tert-butyl (S)-(1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (**1a**): To a solution of L-alanine (7.00 g, 78.57 mmol) in 175 mL of NaOH 1M at 0 °C was added a solution of di-*tert*-butyl dicarbonate (20.58 g, 94.29 mmol) in 77 mL of dioxane. The reaction was stirred 4 h at room temperature. The reaction was quenched with $KHSO_4$ 1M until pH 1 to 2. The aqueous layer was extracted with AcOEt (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude was dissolved in 300 mL of DCM and cooled to 0 °C. DIPEA (13.7 mL, 78.57 mmol), EDCI (15.06 g, 78.57 mmol), and *N*,*O*-dimethylhydroxilamine hydrochloride (7.67 g, 78.57 mmol) were added. The reaction was stirred at 0 °C for 1.5 h. One hundred milliliters of a solution of HCl 1M was added to the reaction. The aqueous phase was extracted with DCM (2 × 150 mL). The combined organic layers were washed with $NaHCO_3$ (150 mL) and brine (150 mL) and dried over $MgSO_4$. The solvent was evaporated in vacuo. A colorless oil was obtained (14.23 g, 78% after two steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.27 (d, *J* = 8.7 Hz, 1H, NH), 4.57 (brs, 1H, CHN), 3.66 (s, 3H, OCH_3), 3.10 (s, 3H, NCH_3), 1.33 (s, 9H, 3×CH_3), 1.20 (d, *J* = 6.9 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6, 155.1, 79.3, 67.0, 61.5, 46.4, 28.3 (3C), 18.5 ppm. IR (NaCl): 3065, 2986, 2935, 1761, 1684 cm^{-1}. $[\alpha]_D^{25}$ (c 0.23 in $CHCl_3$): −4.12. Anal. Calc. for $C_{10}H_{20}N_2O_4$: C, 51.7; H, 8.7; N, 12.1%. Found: C, 51.5; H, 9.0; N, 12.2%.

Tert-butyl (S)-allyl(1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate: To a suspension of **1a** (14.23 g, 61.30 mmol) in 200 mL of DMF at 0 °C was added slowly NaH 60% *w*/*w* (4.90 g, 122.6 mmol). After 10 min, allyl bromide was added (16 mL, 183.9 mmol). The reaction was stirred at 50 °C for 2.5 h. The reaction was quenched with 200 mL of a saturated solution of NH_4Cl. The aqueous phase was extracted with AcOEt (2 × 350 mL). The combined organic layers were washed with a saturated solution of NH_4Cl (150 mL), a saturated solution of $NaHCO_3$ (150 mL), and brine (150 mL) and dried over $MgSO_4$. The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex/AcOEt (9:1). A yellow oil was obtained (12.95 g, 78%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.67–5.57 (m, 1H, HC=), 5.06–4.98 (m, 1H, CHN), 4.89–4.82 (m, 2H, =CH_2), 3.75–3.59 (m, 2H, CH_2N), 3.53 (s, 3H, OCH_3), 2.94 (s, 3H, NCH_3), 1.23 (s, 9H, 3×CH_3), 1.09 (d, *J* = 7.2 Hz, 3H, CH_3) ppm. Two conformers were observed in ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.8, 155.1 (major), 154.4 (minor), 135.8 (major), 135.2 (minor), 115.3 (minor), 114.8 (major), 79.7 (minor), 79.4 (major), 61.2 (major), 61.0 (minor), 51.5 (minor), 49.6 (major), 46.5 (minor), 45.9 (major), 32.1 (minor), 31.9 (major), 28.0 (3C), 15.2 (minor), 15.0 (major) ppm. IR (NaCl): 3065, 2986, 2935, 1761, 1684 cm^{-1}. $[\alpha]_D^{25}$ (c 0.22 in DCM): −38.64. Anal. Calc. for $C_{13}H_{24}N_2O_4$: C, 57.3; H, 8.9; N, 10.3%. Found: C, 57.1; H, 8.7; N, 10.5%.

Tert-butyl (S)-2-methyl-3-oxo-3,6-dihydropyridine-1(2H)-carboxylate (**2a**): A solution of tert-butyl (S)-allyl(1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (8.66 g, 31.82 mmol) in 50 mL of THF was cooled to −30 °C. A solution of vinylmagnesium bromide 0.7 M in THF (100 mL) was added slowly, keeping the temperature below −25 °C. When the addition was finished, the reaction was stirred at the same temperature for 30 min more. The reaction was poured on a mixture of 60 mL of HCl 10% and 120 mL of MeOH cooled

in a bath at −15 °C. This mixture was stirred for another 15 min. The aqueous phase was extracted with AcOEt (2 × 100 mL). The combined organic layers were washed with a saturated solution of NH_4Cl (100 mL), a saturated solution of $NaHCO_3$ (100 mL), and brine (60 mL); dried over $MgSO_4$; and evaporated in vacuo. The crude was dissolved in 150 mL of DCM and heated to reflux. When reflux began, Grubbs' catalyst 2nd generation (812 mg, 0.96 mmol) was added. The reaction was stirred for 1.5 h. The reaction was filtered through a pad of celite. The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex/AcOEt (4:1). A yellow oil was obtained as 2 conformers in a ratio 60:40 (5.11 g, 76% after two steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.95 (brs, 1H, CH_2CH=major + minor), 6.05 (dt, *J* = 10.3, 2.3 Hz, 1H, COCH=, major + minor), 4.72–4.54 (m, 2H, CHN+CH_2N, major + minor), 3.84 (brs, 1H, CH_2N major), 3.79 (brs, 1H, CH_2N minor), 1.44 (s, 9H, 3×CH_3, major + minor), 1.22 (d, *J* = 7.2 Hz, 3H, CH_3, major + minor) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 196.8, 154.1, 146.3, 125.9, 80.9, 56.5, 39.8, 28.4 (3C), 15.9 ppm. IR (NaCl): 3058, 2979, 2931, 1748, 1681 cm^{-1}. $[\alpha]_D^{25}$ (c 0.08 in DCM): +73.37. Anal. Calc. for $C_{11}H_{17}NO_3$: C, 62.5; H, 8.1; N, 6.6%. Found: C, 62.7; H, 8.4; N, 6.4%.

Di-tert-butyl (1R,4S,6S)-4-methyl-5-oxo-3-azabicyclo[4.1.0]heptane-3,7-dicarboxylate (**3a** and **4a**): To a solution of **2a** (3.48 g, 16.46 mmol) in 17 mL of DCM at 0 °C was added a solution of *tert-butyl* 2-(tetrahydro-1λ^4-thiophen-1-ylidene)acetate (9.98 g, 49.38 mmol) in 270 mL of DCM. The reaction was stirred 20 min at 0 °C and 20 more minutes at room temperature. Deionized water was added (150 mL), and layers were separated. The aqueous layer was extracted with DCM (2 × 100 mL). The combined organic layers were washed with brine (80 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified in silica gel in Hex/AcOEt (9:1). *Exo* isomer (**3a**) was obtained as a pale brown solid (4.10 g, 77%). *Endo* isomer (**4a**) was obtained as an orange wax (280 mg, 5%). Other 2 isomers were obtained as a brown oil (10 mg, 0.2%).

Spectroscopic data for *di-tert-butyl (1R,4S,6S,7S)-4-methyl-5-oxo-3-azabicyclo[4.1.0] heptane-3,7-dicarboxylate* (*Exo-n*, **3a**): 2 conformers in a ratio (64:36) were observed. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.56 (q, *J* = 7.2 Hz, 1H, minor, CHN), 4.42 (d, *J* = 14.1 Hz, 1H, major, CH_2N), 4.36 (q, *J* = 7.1 Hz, 1H, major, CHN), 4.26 (d, *J* = 14.1 Hz, 1H, minor, CH_2N), 3.29 (d, *J* = 14.1 Hz, 1H, minor, CH_2N), 3.20 (d, *J* = 14.2 Hz, 1H, major, CH_2N), 2.32–2.25 (m, 4H, major + minor, CHCO + $CHCO_2$), 2.20–2.12 (m, 2H, major + minor, $CHCH_2N$), 1.47 (s, 9H, 3×CH_3, minor), 1.45 (s, 9H, 3×CH_3, major), 1.43 (s, 18H, 3×CH_3, major + minor), 1.23 (d, *J* = 7.2 Hz, 6H, major + minor, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.1 (major), 204.0 (minor), 169.4, 154.5, 82.1 (major), 82.0 (minor), 81.2, 56.9 (major), 55.9 (minor), 36.1 (minor), 34.7 (major), 32.2, 28.4 (3C), 28.1 (3C), 24.9 (major), 24.7 (minor), 24.6 (minor), 24.4 (major), 15.9 (major), 15.4 (minor) ppm. IR (KBr): 2975, 2863, 1739, 1731, 1714 cm^{-1}. $[\alpha]_D^{25}$ (c 0.16 in DCM): +88.22. m.p.: 98.5 °C–101.2 °C. Anal. Calc. for $C_{17}H_{27}NO_5$: C, 62.8; H, 8.4; N, 4.3%. Found: C, 63.0; H, 8.2; N, 4.6%.

Spectroscopic data for *di-tert-butyl (1R,4S,6S,7R)-4-methyl-5-oxo-3-azabicyclo[4.1.0] heptane-3,7-dicarboxylate* (*Endo-n*, **4a**): 2 conformers in a ratio (93:7) were observed. ^{1}H-NMR data given of the major conformer. ^{1}H-NMR (400 MHz, $CDCl_3$) δ4.46–4.36 (m, 2H, CHN+CH_2N), 3.38 (d, *J* = 13.4 Hz, 1H, CH_2N), 2.26 (t, *J* = 9.3 Hz, 1H, $CHCO_2$), 2.03 (dd, *J* = 9.6, 7.8 Hz, 1H, CHCO), 1.92-1.89 (m, 1H, $CHCH_2N$), 1.40 (s, 9H, 3×CH_3), 1.38 (s, 9H, 3×CH_3), 1.22 (d, *J* = 7.2 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.9 (major), 202.8 (minor), 168.6 (minor), 166.9 (major), 154.6, 82.3 (major), 82.2 (minor), 80.9, 57.2 (major), 56.5 (minor), 35.0 (minor), 33.9 (major), 28.7 (minor), 28.4 (3C), 28.4 (major), 28.1 (3C, minor), 28.0 (3C, major), 25.7 (major), 25.4 (minor), 21.6, 15.9 (major), 15.0 (minor) ppm. IR (NaCl): 2982, 2874, 1737, 1728, 1715 cm^{-1}. $[\alpha]_D^{25}$ (c 0.14 in DCM): +33.04. Anal. Calc. for $C_{17}H_{27}NO_5$: C, 62.8; H, 8.4; N, 4.3%. Found: C, 63.1; H, 8.0; N, 4.2%.

Tert-butyl (S)-(3-hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate: To a solution of L-serine (10 g, 95.16 mmol) in 200 mL of NaOH 1M at 0 °C was added a solution of di-*tert*-butyl dicarbonate (24.74 g, 113.36 mmol) in 90 mL of dioxane. The reaction was stirred

for 4 h at room temperature. The reaction was quenched with a solution of $KHSO_4$ 1M until pH 1 to 2. The aqueous phase was extracted with AcOEt (3 × 300 mL). The combined organic layers were dried over $MgSO_4$. The solvent was evaporated in vacuo, obtaining a colorless oil. The crude was dissolved in 360 mL of DCM and cooled to 0 °C. DIPEA (16.6 mL, 95.16 mmol), EDCI (18.24 g, 95.16 mmol), and *N*,*O*-dimethylhydroxilamine hydrochloride (9.28 g, 95.16 mmol) were added. The reaction was stirred at 0 °C for 1.5 h. One hundred milliliters of a solution of HCl 1M was added. The aqueous phase was extracted with DCM (2 × 150 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, and evaporated in vacuo. A white solid was obtained (21.14 g, 90% after 2 steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.65 (d, *J* = 8.7 Hz, 1H, NH), 4.77 (brs, 1H, CHN), 3.81–3.78 (m, 2H, CH_2O), 3.76 (s, 3H, OCH_3), 3.21 (s, 3H, NCH_3), 1.42 (s, 9H, 3×CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 156.0, 80.1, 67.2, 63.7, 61.7, 52.5, 28.4 (3C) ppm. IR (KBr): 3378, 2984, 2868, 1740, 1708 cm^{-1}. $[\alpha]_D^{25}$ (c 0.24 in $CHCl_3$): +2.59. m.p.: 110.1–115.6 °C. Anal. Calc. for $C_{10}H_{20}N_2O_5$: C, 48.4; H, 8.1; N, 11.3%. Found: C, 48.7; H, 7.9; N, 11.6%.

Tert-butyl (S)-(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate (**1b**): To a solution of *tert*-butyl (*S*)-(3-hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (21.14 g, 85.19 mmol) in 70 mL of DMF at 0 °C was added imidazole (17.40 g, 255.58 mmol), DMAP (520 mg, 4.26 mmol), and TBSCl (15.41 g, 102.23 mmol). The reaction was stirred for 40 min at room temperature. Deionized water (500 mL) and AcOEt (300 mL) were added. The aqueous layer was extracted with AcOEt (2 × 150 mL). The combined organic layers were washed with water (3 × 300 mL) and brine (150 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified in silica gel in Hex/AcOEt (9:1). A yellow oil was obtained (26.31 g, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ5.31 (d, *J* = 9.0 Hz, 1H, NH), 4.69–4.64 (m, 1H, CHN), 3.77 (dd, *J* = 10.1, 4.7 Hz, 1H, CH_2O), 3.71 (dd, *J* = 10.0, 5.2 Hz, 1H, CH_2O), 3.67 (s, 3H, OCH_3), 3.13 (s, 3H, NCH_3), 1.35 (s, 9H, 3×CH_3), 0.78 (s, 9H, 3×CH_3), −0.06 (s, 6H, 2×CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 155.4, 79.4, 63.5, 61.4, 52.4, 32.1, 28.3 (3C), 25.8 (3C), 18.2, −5.6 (2C) ppm. IR (NaCl): 2988, 2879, 1738, 1706 cm^{-1}. $[\alpha]_D^{25}$ (c 0.24 in DCM): +11.08. Anal. Calc. for $C_{16}H_{34}N_2O_5Si$: C, 53.0; H, 9.5; N, 7.7%. Found: C, 53.1; H, 9.8; N, 7.6%.

Tert-butyl (S)-allyl(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate: To a suspension of NaH 60% *w*/*w* (4.35g, 108.86 mmol) at 0 °C in 90 mL of DMF was added a solution of **1b** (19.72 g, 54.53 mmol) in 100 mL of DMF. Then, allyl bromide (16 mL, 183.9 mmol) was added. The reaction was stirred for 30 min at room temperature and 2 h at 50 °C. A solution of saturated NH_4Cl was added, until all salts were dissolved. The aqueous layer was extracted with AcOEt (3 × 150 mL). The combined organic layers were washed with a saturated solution of NH_4Cl (150 mL) and a saturated solution of $NaHCO_3$ (150 mL) and brine (100 mL), dried over $MgSO_4$, and evaporated in vacuo. The crude was purified in silica gel in Hex/AcOEt (9:1). A yellow oil was obtained (12.81 g, 58%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.92–5.71 (m, 1H), 5.34–4.93 (m, 3H), 4.02–3.76 (m, 4H), 3.72 (s, 3H), 3.15 (s, 3H), 1.43 (s, 9H), 0.86 (s, 9H), 0.04 (s, 6H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.6, 155.6 (major), 155.0 (minor), 136.0 (major), 135.3 (minor), 115.4 (minor), 115.1 (major), 80.1 (minor), 79.8 (major), 61.6, 61.0 (minor), 60.7 (major), 56.9 (minor), 55.5 (major), 46.5, 32.2, 28.4 (3C), 25.8 (3C), 18.2 (minor), 18.1 (major), −5.5 (2C) ppm. IR (NaCl): 3024, 2992, 2889, 1731, 1710 cm^{-1}. $[\alpha]_D^{25}$ (c 0.26 in DCM): −35.63. Anal. Calc. for $C_{19}H_{38}N_2O_5Si$: C, 56.7; H, 9.5; N, 7.0%. Found: C, 56.3; H, 9.2; N, 7.3%.

Tert-butyl-(S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-3-oxo-3,6-dihydropyridine-1(2H)-carboxylate (**2b**): A solution of tert-butyl(*S*)-allyl(3,8,8,9,9-pentamethyl-4-oxo-2,7-dioxa-3-aza-8-siladecan-5-yl)carbamate (2.18 g, 5.42 mmol) in THF (10 mL) was cooled to −30 °C. A solution of vinylmagnesium bromide 1M (12 mL) was added slowly, keeping the temperature below −25 °C. When the addition finished, the reaction was stirred for a further 30 min. The reaction was poured into a mixture of 10mL of HCl 10% and MeOH (20mL) cooled in a bath at −15 °C. This mixture was stirred for another 30 min at −15 °C. The aqueous layer

was extracted with AcOEt (3 × 40 mL). The combined organic layers were washed with a saturated solution of NH_4Cl (40 mL), a saturated solution of $NaHCO_3$ (40 mL), and brine (40 mL). The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude mixture was dissolved in 9 mL of DCM and heated to reflux. Grubbs' 2nd generation catalyst (179 mg, 0.21 mmol) was then added. The reaction was stirred for 1.5 h. The reaction was filtered through a pad of celite. The solvent was evaporated in vacuo, and the crude was purified in silica gel in Hex:AcOEt (9:1). An orange solid was obtained as 2 conformers in the ratio 63:37 (1.11 g, 60% after 2 steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.01 (ddd, *J* = 10.4, 5.0, 2.0 Hz, 1H, =$CHCH_2$, major), 6.90 (ddd, *J* = 10.4, 5.0, 2.0 Hz, 1H, =$CHCH_2$, minor), 6.17 (d, *J* = 10.4 Hz, 2H, =CHCO, major + minor), 4.69 –4.60 (m, 2H, CH_2N, major and CHN, minor), 4.55–4.47 (m, 2H, CH_2N, minor and CHN, major), 4.00–3.92 (m, 5H, CH_2N, major + minor and CH_2O, major + minor), 3.82 (dd, *J* = 10.2, 3.1 Hz, 1H, CH_2O, major), 1.49 (s, 9H, 3×CH_3, minor), 1.47 (s, 9H, 3×CH_3, major), 0.81 (s, 18H, 3×CH_3, major + minor), −0.02 (s, 6H, CH_3, major + minor), −0.04 (s, 6H, CH_3, major + minor) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 195.5, 154.4 (major), 154.3 (minor), 147.4 (major), 146.2 (minor), 127.8 (minor), 127.6 (major), 81.0 (major), 80.9 (minor), 65.9 (major), 65.7 (minor), 62.8 (major), 61.7 (minor), 44.2 (minor), 43.1 (major), 28.5 (3C), 25.9 (3C), 18.2, −5.6 (minor, 2C), −5.7 (major, 2C) ppm. IR (KBr): 3044, 2987, 2895, 1741, 1696 cm^{-1}. $[\alpha]_D^{25}$ (c 0.14 in DCM): +84.93. MP: 50.8 °C –55.6 °C. Anal. Calc. for $C_{17}H_{31}NO_4Si$: C, 59.8; H, 9.2; N, 4.1%. Found: C, 60.0; H, 9.4; N, 3.9%.

Di-tert-butyl (1S,4S,6R)-4-(((tert-butyldimethylsilyl)oxy)methyl)-5-oxo-3-azabicyclo[4.1.0] heptane-3,7-dicarboxylate (**3b** and **4b**): To a solution of 2b (1.11 g, 3.25 mmol) in 3 mL of DCM at 0 °C was slowly added a solution of tert-butyl 2-(tetrahydro-1λ^4-thiophen-1-ylidene)acetate (1.97 g, 9.75 mmol) in 55 mL of DCM. The reaction was stirred for 20 min at 0 °C and 20 more minutes at room temperature. Deionized water was added (50 mL), and the layers were separated. The aqueous phase was extracted with DCM (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude was separated in silica gel in Hex:AcOEt (9:1). *Exo* isomer was obtained as 2 conformers in a ratio 58:42 as an orange solid (1.05 g, 71%). *Endo* isomer was obtained as 2 conformers in a ratio 52:48 as a brown wax (260 mg, 18%).

Spectroscopic data for *di-tert-butyl (1R,4S,6S,7S)-4-(([tert-butyldimethylsilyl]oxy)methyl)-5-oxo-3-azabicyclo[4.1.0]heptane-3,7-dicarboxylate* (**3b**): ^{1}H-NMR (400 MHz, $CDCl_3$) δ4.47 (dd, *J* = 13.5, 1.9 Hz, 1H, CH_2N, major), 4.38 (t, *J* = 2.9 Hz, 1H, CHN, minor), 4.29 (dd, *J* = 13.6, 2.0 Hz, 1H, CH_2N, minor), 4.21 (t, *J* = 3.0 Hz, 1H, CHN, major), 4.15 (dt, *J* = 10.0, 3.1 Hz, 2H, CH_2O, major + minor), 3.79 (dd, *J* = 10.2, 2.7 Hz, 1H, CH_2O, minor), 3.74 (d, *J* = 12.4 Hz, 1H, CH_2N, minor), 3.71 (dd, *J* = 10.1, 2.7 Hz, 1H, CH_2O, major), 3.63 (dd, *J* = 13.6, 1.8 Hz, 1H, CH_2N, major), 2.34–2.31 (m, 2H, CHCO, major + minor), 2.19 (t, *J* = 4.4 Hz, 2H, $CHCO_2$, major + minor), 2.18–2.09 (m, 2H, $CHCH_2N$, major + minor), 1.42 (s, 18H, 3×CH_3, major + minor), 1.415 (s, 9H, 3×CH_3, minor), 1.411 (s, 9H, 3×CH_3, major), 0.82 (s, 18H, 3×CH_3, major + minor), −0.02 (s, 12H, 2×CH_3, major + minor) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 202.5 (major), 202.3 (minor), 169.5, 155.0 (minor), 154.7 (major), 82.0 (major), 81.9 (minor), 81.1, 66.0 (major), 65.6 (minor), 62.4 (major), 61.5 (minor), 39.4 (minor), 37.9 (major), 32.90 (minor), 32.87 (major), 28.5 (3C), 28.10 (3C, minor), 28.08 (3C, major), 25.9 (3C, minor), 25.8 (3C, major), 24.8 (major), 24.7 (minor), 24.6 (minor), 24.4 (major), 18.16, −5.5 (2C, minor), −5.7 (2C, major) ppm. IR (KBr): 2991, 2887, 1724, 1711, 1691 cm^{-1}. $[\alpha]_D^{25}$ (c 0.15 in DCM): +6.60. m.p.: 74.2 °C–79.0 °C. Anal. Calc. for $C_{23}H_{41}NO_6Si$: C, 60.6; H, 9.1; N, 3.1%. Found: C, 60.3; H, 9.4; N, 3.3%.

Spectroscopic data for *di-tert-butyl (1R,4S,6S,7R)-4-(([tert-butyldimethylsilyl]oxy)methyl)-5-oxo-3-azabicyclo[4.1.0]heptane-3,7-dicarboxylate* (**4b**): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (d, *J* = 13.4 Hz, 1H, CH_2N, major), 4.43 (d, *J* = 13.2 Hz, 1H, CH_2N, minor), 4.24 (t, *J* = 2.7 Hz, 1H, CHN, minor), 4.21–4.12 (m, 3H, CHN, major and CH_2O, major + minor), 3.92–3.85 (m, 2H, CH_2N, minor and CH_2O, minor), 3.82 (dd, *J* = 13.5, 3.2 Hz, 1H, CH_2N, major), 3.77 (dd, *J* = 10.4, 2.9 Hz, 1H, CH_2O, major), 2.29–2.20 (m, 2H, CHCO, major + minor), 2.09 (t,

J = 8.6 Hz, 2H, $CHCO_2$, major + minor), 1.95–1.81 (m, 2H, $CHCH_2N$, major + minor), 1.45 (s, 9H, 3×CH_3, minor), 1.40 (s, 9H, 3×CH_3, major), 1.39 (s, 9H, 3×CH_3, major), 1.38 (s, 9H, 3×CH_3, minor), 0.84 (s, 18H, 3×CH_3, major + minor), 0.00 (s, 6H, CH_3, major + minor), −0.01 (s, 6H, CH_3, major + minor) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.0 (major), 203.4 (minor), 167.4 (major), 167.0 (minor), 154.7 (minor), 154.6 (major), 82.5 (minor), 82.1 (major), 80.9 (minor), 80.7 (major), 67.5 (major), 66.3 (minor), 62.8 (major), 62.0 (minor), 38.5 (minor), 37.5 (major), 28.9, 28.6 (3C,), 28.2 (3C, major), 28.0 (3C, minor), 27.8, 25.91 (3C, minor), 25.89 (3C major), 22.0 (major), 21.9 (minor), 18.16 (minor), 18.13 (major), −5.6 (major + minor), −5.7 (major + minor) ppm. IR (NaCl): 2995, 2894, 1721, 1716, 1699 cm^{-1}. $[\alpha]_D^{25}$ (c 0.09 in DCM): +34.09. Anal. Calc. for $C_{23}H_{41}NO_6Si$: C, 60.6; H, 9.1; N, 3.1%. Found: C, 60.1; H, 8.9; N, 3.4%.

Di-tert-butyl (1R,4S,6S,7S)-5-hydroxy-4-methyl-3-azabicyclo[4.1.0]heptane-3,7-dicarboxylate (**5a** and **5b**): To a solution of **3a** (4.10 g, 12.61 mmol) in absolute ethanol (90 mL) at 0 °C was added $NaBH_4$ (954 mg, 25.22 mmol). The reaction was stirred for 1 h at room temperature. Then, a saturated solution of NH_4Cl was added (until salts were dissolved). The aqueous phase was extracted with AcOEt (3 × 50 mL). The combined organic layers were washed with brine (60 mL). A solid was obtained as a mixture of two isomers in a 3:2 ratio, that were separated by silica gel column chromatography using Hex:AcOEt (5:1) to Hex/AcOEt (2:1) as eluents (3.10 g, 55% (**5a**) and 19% (**5b**)).

Spectroscopic data from *di-tert-butyl (1R,4S,5S,6S,7S)-5-hydroxy-4-methyl-3-azabicyclo [4.1.0] heptane-3,7-dicarboxylate* (**5a**): White solid (2.27 g, 55%).^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.16 (brs, 1H, CHN), 4.01 (d, *J* = 14.1 Hz, 1H, CH_2N), 3.84 (td, *J* = 5.9, 5.4, 2.0 Hz, 1H, C**H**OH), 3.28 (dd, *J* = 14.1, 4.1 Hz, 1H, CH_2N), 1.63–1.58 (m, 1H, $CHCH_2N$), 1.55 (ddd, *J* = 9.4, 4.5, 2.0 Hz, 1H, CHCHOH), 1.434 (s, 9H, 3×CH_3), 1.431 (s, 9H, 3×CH_3), 1.22 (t, *J* = 4.6 Hz, 1H, $CHCO_2$), 1.15 (d, *J* = 6.9 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.6, 155.1, 80.9, 80.3, 67.5, 48.4, 36.1, 28.6 (3C), 28.3 (3C), 25.2, 25.0, 20.2, 11.4 ppm. IR (KBr): 3389, 2986, 2884, 1727, 1720 cm^{-1}. $[\alpha]_D^{25}$ (c 0.13 in DCM): +7.43. m.p.: 123.8 °C–127.1 °C. Anal. Calc. for $C_{17}H_{29}NO_5$: C, 62.4; H, 8.9; N, 4.3%. Found: C, 62.6; H, 9.2; N, 4.1%.

Spectroscopic data from *di-tert-butyl (1R,4S,5R,6S,7S)-5-hydroxy-4-methyl-3-azabicyclo [4.1.0] heptane-3,7-dicarboxylate* (**5b**): White solid (770 mg, 19%).^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.15–4.08 (m, 2H, CHN+CH_2N), 3.99–3.97 (m, 1H, CHOH), 3.20 (d, *J* = 13.8 Hz, 1H, CH_2N), 1.95–1.90 (m, 1H, CHCHOH), 1.67–1.63 (m, 2H, 2×CH cyclopropane), 1.46 (s, 9H, 3×CH_3), 1.44 (s, 9H, 3×CH_3), 1.13 (d, *J* = 7.2 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.9, 155.9, 80.8, 80.3, 66.3, 51.3, 35.4, 28.5 (3C), 28.3 (3C), 23.6, 20.6, 19.7, 16.1 ppm. IR (KBr): 3395, 2974, 2891, 1729, 1718 cm^{-1}. $[\alpha]_D^{25}$ (c 0.12 in DCM): −2.61. m.p.: 96.0 °C–98.6 °C. Anal. Calc. for $C_{17}H_{29}NO_5$: C, 62.4; H, 8.9; N, 4.3%. Found: C, 62.2; H, 9.1; N, 4.0%.

Tert-butyl (1S,4S,6S,7S)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptane-3-carboxylate: To a solution of **5a** (2.172 g, 6.62 mmol) in 55 mL of DCM cooled to 0 °C was added DIBAL-H 1.2M in toluene (22 mL). The reaction was stirred at room temperature for 1 h. The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex:AcOEt (1:3). A yellow wax was obtained as two conformers in a ratio 1:1 (931 mg, 55%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ4.05 (brs, 1H, CHN), 3.81 (dd, *J* = 13.8, 1.7 Hz, 1H, CH_2N), 3.76–3.69 (m, 2H, CH_2O+CHOH), 3.33 (dd, *J* = 13.8, 4.7 Hz, 1H, CH_2N), 3.21 (t, *J* = 9.7 Hz, 1H, CH_2O), 1.42 (s, 9H, 3×CH_3), 1.14 (d, *J* = 6.7 Hz, 3H, CH_3), 0.97-0.88 (m, 2H, 2×CH cyclopropane), 0.81–0.75 (m, 1H, $CHCH_2O$) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.4, 80.1, 68.6 (β), 68.5 (α), 65.5 (β), 65.4 (α), 48.7, 37.0, 28.6 (3C), 25.2, 20.23 (β), 20.19 (α), 14.7, 11.8 ppm. IR (NaCl): 3402, 2974, 2884, 1729 cm^{-1}. $[\alpha]_D^{25}$ (c 0.17 in DCM): +14.68. Anal. Calc. for $C_{13}H_{23}NO_4$: C, 60.7; H, 9.0; N, 5.4%. Found: C, 60.3; H, 9.2; N, 5.7%.

(1S,4S,5S,6S,7S)-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptan-5-ol (**6a**): To a solution of tert-butyl (1S,4S,6S,7S)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptane-3-carboxylate (931 mg, 3.62 mmol) in 0.5 mL of DCM and 1 mL of MeOH was added 30 mL of TFA. The reaction was stirred 30 min at room temperature. The solvent was

evaporated in vacuo. The crude was eluted through a basic DOWEX resin. A brown wax was obtained (484 mg, 85%). ^{1}H-NMR (300 MHz, MeOD) δ 4.09 (t, *J* = 1.7 Hz, 1H), 3.64 (dd, *J* = 13.6, 8.5 Hz, 1H), 3.48 (dd, *J* = 11.4, 6.0 Hz, 1H), 3.40 (dd, *J* = 11.4, 6.5 Hz, 1H), 3.13 (dd, *J* = 13.6, 2.5 Hz, 1H), 2.94 (qd, *J* = 6.6, 1.7 Hz, 1H), 1.34 (ddd, *J* = 9.1, 5.2, 1.7 Hz, 1H), 1.28 (d, *J* = 6.6 Hz, 3H), 1.25–1.18 (m, 1H), 1.18–1.07 (m, 1H) ppm. ^{13}C-NMR (75 MHz, MeOD) δ 65.0, 64.8, 52.2, 44.0, 26.7, 23.9, 15.3, 11.4 ppm. IR (NaCl): 3419, 2986, 2899 cm^{-1}. $[\alpha]_D^{25}$ (c 0.04 in MeOH): +10.75. Anal. Calc. for $C_8H_{15}NO_2$: C, 61.1%; H, 9.1; N, 8.9%. Found: C, 61.2; H, 9.4; N, 8.7%.

Tert-butyl (1S,4S,5R,6S,7S)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0] heptane-3-carboxylate: To a solution of **5b** (770 mg, 2.34 mmol) in 55 mL of DCM cooled to 0 °C was added DIBAL-H 1.2M in toluene (22 mL). The reaction was stirred at room temperature for 1 h. The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex:AcOEt (1:3). A yellow wax was obtained (400 mg, 67%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.19–3.98 (m, 3H, OH+CH_2N+CHN), 3.93 (d, *J* = 7.2 Hz, 1H, CHOH), 3.87 (dd, *J* = 11.2, 4.8 Hz, 1H, CH_2O), 3.13 (d, *J* = 13.0 Hz, 1H; CH_2N), 3.00 (dd, *J* = 11.2, 9.1 Hz, 1H, CH_2O), 2.66 (brs, 1H, OH), 1.43 (s, 9H, 3×CH_3), 1.28–1.23 (m, 1H, CHCHOH), 1.17–1.12 (m, 1H, $CHCH_2$N), 1.09 (d, *J* = 7.1 Hz, 3H, CH_3), 0.97–0.92 (m, 1H, $CHCH_2$OH) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.1, 80.1, 66.9, 65.6, 51.2, 35.7, 28.5 (3C), 19.7, 18.6, 16.5, 15.3 ppm. IR (NaCl): 3409, 2991, 2892, 1726 cm^{-1}. $[\alpha]_D^{25}$ (c 0.14 in DCM): −6.84. Anal. Calc. for $C_{13}H_{23}NO_4$: C, 60.7; H, 9.0; N, 5.4%. Found: C, 60.3; H, 9.2; N, 5.2%.

(1S,4S,5R,6S,7S)-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptan-5-ol (**6b**): To a solution of tert-butyl (1S,4S,5R,6S,7S)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0] heptane-3-carboxylate (400 mg, 1.55 mmol) in 1 mL of DCM and 2 mL of MeOH was added 12 mL of TFA. The reaction was stirred for 30 min at room temperature. The solvent was evaporated in vacuo. The crude was eluted through a basic DOWEX resin. A brown wax was obtained (202 mg, 83%). ^{1}H-NMR (400 MHz, MeOD) δ 3.98 (dd, *J* = 9.5, 5.4 Hz, 1H, CHOH), 3.62–3.54 (m, 2H, CH_2O+CH_2N), 3.35-3.27 (m, 1H, CH_2O), 3.03–2.99 (m, 1H, CH_2N), 2.63 (dq, *J* = 9.5, 6.4 Hz, 1H, CHN), 1.42–1.38 (m, 2H, 2×CH cyclopropane), 1.34–1.30 (m, 4H, CH_3+C**H**CH_2OH) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 68.2, 65.0, 53.4, 43.3, 26.6, 21.9, 15.9, 15.7 ppm. IR (NaCl): 3391, 2995, 2899 cm^{-1}. $[\alpha]_D^{25}$ (c 0.03 in MeOH): −51.91. Anal. Calc. for $C_8H_{15}NO_2$: C, 61.1%; H, 9.6; N, 8.9%. Found: C, 60.8; H, 9.4; N, 9.1%.

Tert-butyl (1S,4S,5R,6S,7R)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptane-3-carboxylate: To a solution of **4a** (280 mg, 0.86 mmol) in 10 mL of DCM at 0 °C was added DIBAL-H 1.2M in toluene (3.6 mL). The reaction was stirred 1 h at room temperature. Methanol was added (12 mL). The salts were filtered through a pad of celite and rinsed with methanol. The solvent was evaporated in vacuo and purified in silica gel in Hex:AcOEt (1:2). A yellow wax was obtained (71 mg, 32%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.24–4.18 (m, 2H, CHN+CHOH), 3.94 (d, *J* = 14.3 Hz, 1H, CH_2N), 3.89 (dd, *J* = 11.6, 5.9 Hz, 1H, CH_2O), 3.54 (t, *J* = 11.4 Hz, 1H, CH_2O), 3.31 (brs, 1H, OH), 3.25 (dd, *J* = 14.2, 5.7 Hz, 1H, CH_2N), 2.02 (brs, 1H, OH), 1.43 (s, 10H, 3×CH_3+CHCHOH), 1.33–1.26 (m, 1H, C**H**CH_2OH), 1.14–1.11 (m, 4H, CH_3+$CHCH_2$OH) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.5, 80.2, 68.1, 58.8, 51.2, 34.3, 28.6 (3C), 21.0, 16.0, 14.5, 11.3 ppm. IR (NaCl): 3392, 2981, 2896, 1719 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in DCM): +60.0. Anal. Calc. for $C_{13}H_{23}NO_4$: C, 60.7; H, 9.0; N, 5.4%. Found: C, 60.5; H, 9.3; N, 5.1%.

(1S,4S,5R,6S,7R)-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0]heptan-5-ol (**6c**): To a solution of tert-butyl (1S,4S,5R,6S,7R)-5-hydroxy-7-(hydroxymethyl)-4-methyl-3-azabicyclo[4.1.0] heptane-3-carboxylate (112 mg, 0.44 mmol) in 0.5 mL of DCM and 0.5 mL of MeOH was added 4 mL of TFA. The reaction was stirred for 30 min at room temperature. The solvent was evaporated in vacuo. The crude was eluted through a basic DOWEX resin. A brown wax was obtained (56 mg, 81%). ^{1}H-NMR (400 MHz, MeOD) δ 4.08 (dd, *J* = 9.8, 7.2 Hz, 1H, CHOH), 3.87 (dd, *J* = 12.5, 7.2 Hz, 1H, CH_2O), 3.80 (dd, *J* = 12.5, 9.2 Hz, 1H, CH_2O), 3.69

(dd, *J* = 14.3, 9.6 Hz, 1H, CH_2N), 2.95 (dd, *J* = 14.3, 5.1 Hz, 1H, CH_2N), 2.60 (dq, *J* = 9.7, 6.3 Hz, 1H, CHN), 1.75 (qd, *J* = 9.4, 5.1 Hz, 1H, $CHCH_2N$), 1.60 (td, J, 9.2, 7.2 Hz, 1H, CHCHOH), 1.53–1.44 (m, 1H, $CHCH_2OH$), 1.39 (d, *J* = 6.3 Hz, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 69.3, 58.4, 56.0, 40.1, 24.7, 18.7, 17.2, 14.8 ppm. IR (NaCl): 3413, 2992, 2905 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): −72.95. Anal. Calc. for $C_8H_{15}NO_2$: C, 61.1%; H, 9.6; N, 8.9%. Found: C, 61.3; H, 9.9; N, 8.7%.

Di-tert-butyl (1R,4S,5S,6S,7S)-4-(((tert-butyldimethylsilyl)oxy)methyl)-5-hydroxy-3-azabicyclo [4.1.0]heptane-3,7-dicarboxylate (**7**): To a solution of **3b** (970 mg, 2.13 mmol) in 11 mL of absolute ethanol at 0 °C was added $NaBH_4$ (161 mg, 4.26 mmol). The reaction was stirred for 1 h at room temperature. A saturated solution of NH_4Cl was added (30 mL). The aqueous phase was extracted with AcOEt (3 × 40 mL). The combined organic layers were washed with brine (40 mL). Only one isomer was observed, which was purified in silica gel in Hex:AcOEt (4:1). A yellow wax was obtained (716 mg, 74%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.16 (brs, 1H, CHOH), 4.06–3.90 (m, 3H, CH_2N+CH_2O+CHN), 3.89–3.78 (m, 1H, CH_2O), 3.34 (brs, 1H, CH_2N), 1.78 (dt, *J* = 9.3, 3.8 Hz, 1H, CHCHOH), 1.71–1.65 (m, 1H, $CHCH_2N$), 1.44 (s, 9H, 3×CH_3), 1.43 (s, 9H, 3×CH_3), 1.37 (t, *J* = 4.1 Hz, 1H, $CHCO_2$), 0.89 (s, 9H, 3×CH_3), 0.083 (s, 3H, CH_3), 0.076 (s, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.1, 155.2, 80.8, 80.4, 67.2, 61.9, 53.4, 37.2, 28.6 (3C), 28.2 (3C), 25.9 (3C), 25.3, 23.8, 20.2, 18.1, −5.4 (2C) ppm. IR (NaCl): 3429, 2988, 2892, 1714, 1696 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in DCM): +16.49. Anal. Calc. for $C_{23}H_{43}NO_6Si$: C, 60.4; H, 9.5; N, 3.1%. Found: C, 60.6; H, 9.4; N, 3.4%.

Di-tert-butyl (1R,4S,5S,6S,7S)-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo [4.1.0]heptane-3,7- dicarboxylate: To a solution of **7** (300 mg, 0.66 mmol) in 3 mL of THF was added TBAF.$3H_2O$ (303 mg, 0.96 mmol). The reaction was stirred for 1 h at room temperature. The solvent was evaporated in vacuo and purified in silica gel in Hex/AcOEt (1:1). A white solid was obtained (192 mg, 85%). ^{1}H-NMR (400 MHz, MeOD) δ 4.14 (brs, 1H, CHN), 4.10–4.01 (m, 1H, CH_2N), 3.92 (dd, *J* = 6.1, 1.7 Hz, 1H, CHOH), 3.88-3.84 (m, 1H, CH_2O), 3.73 (dd, *J* = 11.5, 9.7 Hz, 1H, CH_2O), 3.42–3.31 (m, 1H, CH_2N), 1.66–1.59 (m, 1H, $CHCH_2N$), 1.56 (ddd, *J* = 9.4, 4.5, 2.1 Hz, 1H, CHCHOH), 1.46 (s, 9H, 3×CH_3), 1.44 (s, 9H, 3×CH_3), 1.20 (t, *J* = 4.5 Hz, 1H, $CHCO_2$) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 172.7, 156.1, 80.6, 80.2, 65.7 (major), 65.5 (minor), 56.7, 54.9 (major), 53.7 (minor), 36.6 (minor), 35.4 (major), 27.2 (3C), 26.9 (3C), 25.4, 24.4, 20.5 (minor), 20.3 (major) ppm. IR (NaCl): 3435, 2991, 2884, 1716, 1692 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): +6.02. m.p.: 173.8 °C –172.5 °C. Anal. Calc. for $C_{17}H_{29}NO_6$: C, 59.5; H, 8.5; N, 4.1%. Found: C, 59.2; H, 8.8; N, 4.2%.

(1R,4S,5S,6S,7S)-7-carboxy-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptan-3-ium trifluoroacetate (**10a**): To a solution of di-*tert*-butyl (1*R*,4*S*,5*S*,6*S*,7*S*)-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3,7-dicarboxylate (192mg, 0.56mmol) in a mixture of 3 mL of DCM and 0.7 mL of MeOH was added TFA (5.6 mL). The reaction was stirred for 20 min. The solvent was evaporated in vacuo. A brown wax was obtained (140 mg, 87%). ^{1}H-NMR (400 MHz, D_2O) δ 4.47 (brs, 1H, CHOH), 3.89–3.81 (m, 2H, CH_2N+CH_2O), 3.78 (dd, *J* = 12.2, 8.5 Hz, 1H, CH_2O), 3.31 (d, *J* = 13.5 Hz, 1H, CH_2N), 3.03 (ddd, *J* = 8.2, 4.4, 1.5 Hz, 1H, CHN), 2.05–1.99 (m, 2H, 2×CH cyclopropane), 1.88 (t, *J* = 4.6 Hz, 1H, $CHCO_2$) ppm. ^{13}C-NMR (100 MHz, D_2O) δ 175.9, 163.0 (q, *J* = 35.5 Hz), 116.3 (q, *J* = 291.7 Hz), 60.6, 59.4, 56.3, 41.6, 26.1, 24.3, 15.8 ppm. IR (NaCl): 3398, 2991, 2884, 1710, cm^{-1}. $[\alpha]_D^{25}$ (c 0.03 in MeOH): +4.19. Anal. Calc. for $C_{10}H_{14}F_3NO_5$: C, 42.1; H, 5.0; N, 4.9%. Found: C, 41.9; H, 5.4; N, 5.1%.

Tert-butyl (1S,4S,5S,6S,7S)-4-(([tert-butyldimethylsilyl]oxy)methyl)-5-hydroxy-7-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate: To a solution of **7** (400 mg, 0.87 mmol) at 0 °C in 3 mL of DCM was added 2.2 mL of DIBAL-H 1.2M in toluene. The reaction was stirred 1 h at room temperature. Methanol was added (5 mL). The salts were filtered and rinsed with methanol (3 × 15 mL). The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex:AcOEt (1:1). A yellow wax was obtained (270 mg, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.11 (brs, 1H, CHOH), 3.95–3.71 (m, 4H, 2CH_2O+CHN+CH_2N), 3.56

(dd, *J* = 11.2, 6.2 Hz, 1H, CH_2OH), 3.39–3.26 (m, 2H, CH_2OH+CH_2N), 1.41 (s, 9H, 3×CH_3), 1.13–1.05 (m, 1H, CHCHOH), 1.06–0.99 (m, 1H, $CHCH_2N$), 0.99–0.90 (m, 1H, $CHCH_2OH$), 0.85 (s, 9H, 3×CH_3), 0.05 (s, 3H, CH_3), 0.04 (s, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.5, 80.2, 67.8, 65.0, 61.7, 54.0, 37.8, 28.5 (3C), 25.8 (3C), 23.3, 20.5, 18.1, 15.1, −5.4 (2C) ppm. IR (NaCl): 3384, 2984, 2896, 1698 cm^{-1}. $[\alpha]_D^{25}$ (c 0.10 in $CHCl_3$): +23.77. Anal. Calc. for $C_{19}H_{37}NO_5Si$: C, 58.9; H, 9.6; N, 3.6%. Found: C, 59.1; H, 9.3; N, 3.5%.

Tert-butyl (1S,4S,5S,6S,7S)-5-hydroxy-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate: To a solution of tert-butyl (1*S*,4*S*,5*S*,6*S*,7*S*)-4-(([*tert*-butyldimethylsilyl]oxy)methyl)-5-hydroxy-7-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate (270 mg, 0.70 mmol) in 7 mL of THF was added TBAF.3H_2O (243 mg, 0.77 mmol). The reaction was stirred at room temperature for 1 h. The solvent was evaporated in vacuo and purified in silica gel in AcOEt 100%. A yellow wax was obtained (185 mg, 68%). ^{1}H-NMR (400 MHz, MeOD) δ 4.05–3.98 (m, 2H, CHN+CH_2N), 3.95 (dd, *J* = 5.8, 2.6 Hz, 1H, C**H**OH), 3.91–3.81 (m, 1H, NCHC**H**$_2$OH), 3.74 (dd, *J* = 11.5, 8.6 Hz, 1H, NCHC**H**$_2$OH), 3.47–3.26 (m, 3H, 2CH_2OH+CH_2N), 1.45 (s, 9H, 3×CH_3), 1.06–0.98 (m, 1H, $CHCH_2N$), 1.00–0.91 (m, 1H, CHCHOH), 0.75 (tt, *J* = 6.8, 4.6 Hz, 1H, $CHCH_2OH$) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 157.7, 81.3, 68.0, 65.5, 58.9, 56.5 (major), 55.5 (minor), 38.9 (minor), 37.7 (major), 28.7 (3C), 25.0, 21.6, 16.7 (minor), 16.4 (major) ppm. IR (NaCl): 3413, 2992, 2887, 1702 cm^{-1}. $[\alpha]_D^{25}$ (c 0.05 in MeOH): +13.02. Anal. Calc. for $C_{13}H_{23}NO_5$: C, 57.1; H, 8.5; N, 5.1%. Found: C, 57.3; H, 8.3; N, 5.0%.

((1S,4S,5S,6S,7S)-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-5-ol (**11a**): To a solution of tert-butyl (1*S*,4*S*,5*S*,6*S*,7*S*)-5-hydroxy-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate (185 mg, 0.68 mmol) in 3 mL of DCM and 0.7 mL of MeOH was added TFA (6.8 mL). The reaction was stirred for 20 min. The solvent was evaporated in vacuo. The crude was filtered through a basic DOWEX resin. A brown wax was obtained (107 mg, 91%). ^{1}H-NMR (400 MHz, MeOD) δ 4.28 (brs, 1H, CHOH), 3.77–3.74 (m, 2H, $NCHCH_2OH$), 3.70 (dd, *J* = 13.7, 8.3 Hz, 1H, CH_2N), 3.48 (dd, *J* = 11.4, 6.1 Hz, 1H, CH_2OH), 3.41 (dd, *J* = 11.4, 6.5 Hz, 1H, CH_2OH), 3.12 (dd, *J* = 13.7, 2.4 Hz, 1H, CH_2N), 2.86 (t, *J* = 5.9 Hz, 1H, CHN), 1.33-1.23 (m, 2H, 2×CH cyclopropane), 1.14 (p, *J* = 5.6 Hz, 1H, $CHCH_2OH$) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 64.8, 62.7, 61.2, 58.5, 44.3, 26.7, 23.7, 11.9 ppm. IR (NaCl): 3429, 2988, 2892 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): +8.51. Anal. Calc. for $C_8H_{15}NO_3$: C, 55.5; H, 8.7; N, 8.1%. Found: C, 55.9; H, 8.2; N, 8.3%.

(1R,4S,5S,6S,7R)-3-(tert-Butoxycarbonyl)-4-(([tert-butyldimethylsilyl]oxy)methyl)-5-hydroxy-3-azabicyclo[4.1.0]heptane-7-carboxylic acid (**8**): To a solution of **4b** (260 mg, 0.57 mmol) in 3 mL of absolute ethanol was added $NaBH_4$ (43 mg, 1.14 mmol). The reaction was stirred for 48 h. Methanol was added (5 mL). The salts were filtered and rinsed with methanol (3 × 10 mL). The solvent was evaporated in vacuo. The crude was purified in silica gel from Hex:AcOEt (4:1) to Hex:AcOEt (2:1). A yellow wax was obtained as 2 conformers in a ratio 56:44 (70 mg, 30%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.04 (brs, 2H, CHOH, major + minor), 4.18 (brs, 1H, CHN, minor), 4.13–4.04 (m, 1H, CHN, major), 3.78–3.48(m, 8H, CH_2O and CH_2N, major + minor), 2.56–2.47 (m, 2H, CHCHOH, major + minor), 2.22 (dd, *J* = 8.5, 6.2 Hz, 2H, $CHCO_2$, major + minor), 1.82–1.70 (m, 2H, $CHCH_2N$, major + minor), 1.44 (s, 18H, 3×CH_3, major + minor), 0.89 (s, 18H, 3×CH_3, major + minor), 0.07 (s, 6H, CH_3, major + minor), 0.06 (s, 6H, CH_3, major + minor) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.7 (minor), 173.5 (major), 155.0, 80.6, 80.4, 74.1 (minor), 73.4 (major), 61.5 (major), 61.2 (minor), 52.4 (major), 51.6 (minor), 36.9 (minor), 35.9 (major), 28.4 (3C), 25.8 (3C), 23.9 (minor), 23.8 (major), 20.4 (minor), 19.8 (major), 18.1 (major), 16.3 (minor), −5.4, −5.5 ppm. IR (NaCl): 3408, 2995, 2891, 1712, 1698 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): +25.10. Anal. Calc. for $C_{19}H_{35}NO_6Si$: C, 56.8; H, 8.8; N, 3.5%. Found: C, 56.4; H, 8.5; N, 3.7%.

(1R,4S,5S,6S,7R)-3-(tert-butoxycarbonyl)-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-7-carboxylic acid: To a solution of **8** (70 mg, 0.17 mmol) in 1 mL of THF was added TBAF.3H_2O (59 mg, 0.19 mmol). The reaction was stirred for 30 min. The solvent was evaporated in vacuo. The crude was purified in silica gel in Hex:AcOEt (1:4). A yellow wax was obtained

(48 mg, 88%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.03 (brs, 1H, CHOH), 4.24–4.15 (m, 1H, CHN), 3.84–3.74 (m, 2H, CH_2O+CH_2N), 3.73–3.63 (m, 1H, CH_2O), 3.50 (d, *J* = 14.5 Hz, 1H, CH_2N), 2.60–2.50 (m, 1H, CHCHOH), 2.23 (dd, *J* = 8.5, 6.2 Hz, 1H, $CHCO_2$), 1.78 (qd, *J* = 8.0, 2.5 Hz, 1H, $CHCH_2N$), 1.42 (s, 9H, 3×CH_3) ppm.^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6, 156.1, 81.1, 73.3, 61.3, 52.6, 36.6, 28.3 (3C), 23.9, 19.9, 16.2 ppm. IR (NaCl): 3419, 2992, 2901, 1701 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): +15.41. Anal. Calc. for $C_{13}H_{21}NO_6$: C, 54.4; H, 7.4; N, 4.9%. Found: C, 54.6; H, 7.1; N, 5.1%.

(1R,4S,5S,6S,7R)-7-carboxy-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptan-3-ium trifluoroacetate (**10b**): To a solution of (1*R*,4*S*,5*S*,6*S*,7*R*)-3-(*tert*-butoxycarbonyl)-5-hydroxy-4-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-7-carboxylic acid (48 mg, 0.17 mmol) in a mixture of 1 mL of DCM and 0.5 mL of MeOH was added TFA (1.7 mL). The reaction was stirred for 20 min. The solvent was evaporated in vacuo. A brown wax was obtained (44 mg, 92%). ^{1}H-NMR (400 MHz, D_2O) δ 5.19 (d, *J* = 5.5 Hz, 1H, CHOH), 4.00 (dd, *J* = 14.7, 8.7 Hz, 1H, CH_2N), 3.93 (dd, *J* = 10.7, 5.4 Hz, 2H, CH_2O), 3.71 (dd, *J* = 6.3, 4.5 Hz, 1H, CHN), 2.86–2.74 (m, 2H, CH_2N+CHCHOH), 2.65 (dd, *J* = 8.4, 5.9 Hz, 1H, $CHCO_2$), 2.30 (qd, *J* = 8.3, 6.4 Hz, 1H, C**H**CH_2N) ppm.^{13}C-NMR (100 MHz, D_2O) δ 175.2, 163.0 (q, *J* = 35.5 Hz), 116.4 (q, *J* = 291.9 Hz), 73.6, 58.9, 54.3, 36.9, 24.7, 18.4, 14.3 ppm. IR (NaCl): 3385, 2997, 2899, 1707, cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): +10.19. Anal. Calc. for $C_{10}H_{14}F_3NO_5$: C, 42.1; H, 5.0; N, 4.9%. Found: C, 41.8; H, 5.2; N, 5.0%.

Tert-butyl (1S,4S,5S,6S,7R)-4-(([tert-butyldimethylsilyl]oxy)methyl)-5-hydroxy-7-(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate (**9**): To a solution of **4b** (260 mg, 0.57 mmol) in 3 mL of absolute ethanol was added $NaBH_4$ (43 mg, 1.14 mmol). The reaction was stirred for 48 h. Methanol was added (5 mL). The salts were filtered and rinsed with methanol (3 × 10 mL). The solvent was evaporated in vacuo. The crude was purified in silica gel from Hex:AcOEt (4:1) to Hex:AcOEt (2:1). A yellow wax was obtained (110 mg, 50%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.35 (dd, *J* = 8.8, 1.4 Hz, 1H, C**H**OH), 4.11–4.03 (m, 1H, CHN), 3.93 (d, *J* = 14.2 Hz, 1H, CH_2N), 3.86 (dd, *J* = 11.5, 5.7 Hz, 1H, CH_2OH), 3.59 (dd, *J* = 10.1, 7.5 Hz, 1H, CH_2O), 3.56–3.43 (m, 2H, CH_2O+CH_2OH), 3.12 (dd, *J* = 14.3, 5.3 Hz, 1H, CH_2N), 2.87 (brs, 1H, OH), 1.67 (brs, 1H, OH), 1.43 (q, *J* = 8.9 Hz, 1H, CHCHOH), 1.38 (s, 9H, 3×CH_3), 1.32–1.23 (m, 1H, $CHCH_2OH$), 1.13–1.02 (m, 1H, $CHCH_2N$), 0.82 (s, 9H, 3×CH_3), 0.000 (s, 3H, CH_3), −0.004 (s, 3H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.6, 80.3, 64.3, 61.6, 58.8, 57.4, 35.2, 28.6 (3C), 26.0 (3C), 21.6, 18.3, 15.3, 12.0, −5.30, −5.32 ppm. IR (NaCl): 3372, 2991, 2906, 1706 cm^{-1}. $[\alpha]_D^{25}$ (c 0.03 in $CHCl_3$): +24.04. Anal. Calc. for $C_{19}H_{37}NO_5Si$: C, 58.9; H, 9.6; N, 3.6%. Found: C, 59.2; H, 9.2; N, 3.4%.

Tert-butyl (1S,4S,5S,6S,7R)-5-hydroxy-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3- carboxylate: To a solution of **9** (110 mg, 0.29 mmol) in 2 mL of THF was added TBAF.3H_2O (101 mg, 0.32 mmol). The reaction was stirred for 30 min. The solvent was evaporated in vacuo. The crude was purified in silica gel in AcOEt. A yellow wax was obtained (68 mg, 86%). ^{1}H-NMR (400 MHz, MeOD) δ 4.29 (d, *J* = 8.9 Hz, 1H, CHOH), 4.14 (t, *J* = 7.6 Hz, 1H, $NCHCH_2OH$), 4.03 (d, *J* = 14.1 Hz, 1H, CH_2N), 3.78 (dd, *J* = 11.7, 7.3 Hz, 1H, $NCHCH_2OH$), 3.73–3.65 (m, 1H, CHN), 3.61 (dd, *J* = 11.3, 7.1 Hz, 1H, C**H**$_2$OH), 3.52 (dd, *J* = 11.3, 8.3 Hz, 1H, CH_2OH), 3.28–3.16 (m, 1H, CH_2N), 1.51 (q, *J* = 9.1 Hz, 1H, CHCHOH), 1.46 (s, 9H, 3×CH_3), 1.33–1.11 (m, 2H, 2×CH cyclopropane) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 157.6, 81.4, 64.4, 61.0, 59.6, 59.2 (major), 57.9(minor), 36.3 (minor), 35.1 (major), 28.7 (3C), 22.3, 16.2, 12.4 ppm. IR (NaCl): 3385, 2988, 2892, 1698 cm^{-1}. $[\alpha]_D^{25}$ (c 0.02 in MeOH): +10.68. Anal. Calc. for $C_{13}H_{23}NO_5$: C, 57.1; H, 8.5; N, 5.1%. Found: C, 56.8; H, 8.3; N, 5.3%.

((1S,4S,5S,6S,7R)-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-5-ol (**11b**): To a solution of tert-butyl (1S,4S,5S,6S,7R)-5-hydroxy-4,7-bis(hydroxymethyl)-3-azabicyclo[4.1.0]heptane-3-carboxylate (68 mg, 0.25mmol) in 1.5 mL of DCM and 0.7 mL of MeOH was added TFA (2.5 mL). The reaction was stirred for 20 min. The solvent was evaporated in vacuo. The crude was filtered through a basic DOWEX resin. A brown wax was obtained (40 mg, 93%). ^{1}H-NMR (400 MHz, MeOD) δ 4.34 (dd, *J* = 10.1, 7.3 Hz, 1H, CHOH), 3.94 (dd, *J* = 11.9,

3.0 Hz, 1H, $NCHCH_2OH$), 3.85 (d, *J* = 8.3 Hz, 2H, CH_2OH), 3.72 (dd, *J* = 12.0, 5.1 Hz, 1H, $NCHCH_2OH$), 3.65 (dd, *J* = 14.3, 9.6 Hz, 1H, CH_2N), 2.94 (dd, *J* = 14.2, 5.0 Hz, 1H, CH_2N), 2.57–2.48 (m, 1H, CHN), 1.80–1.67 (m, 1H, $CHCH_2N$), 1.60 (q, *J* = 8.9 Hz, 1H, CHCHOH), 1.46 (p, *J* = 8.4 Hz, 1H, $CHCH_2OH$) ppm. ^{13}C-NMR (100 MHz, MeOD) δ 63.7, 61.7, 60.4, 58.6, 40.1, 24.7, 18.9, 15.6 ppm. IR (NaCl): 3441, 2995, 2899 cm^{-1}. $[\alpha]_D^{25}$ (c 0.01 in MeOH): +6.38. Anal. Calc. for $C_8H_{15}NO_3$: C, 55.5; H, 8.7; N, 8.1%. Found: C, 55.3; H, 8.9; N, 8.3%.

4. Conclusions

We described the synthesis of bicyclic piperidine-based iminosugars from natural amino acids L-alanine and L-serine. The procedure involves the preparation of enantiomerically pure α,β-unsaturated ketones in four steps and high yields from the natural amino acids. These intermediates, upon a stereoselective cyclopropanation reaction and further straightforward transformations, give the final products, which contain five stereogenic centers. The synthetic methodology used allows the obtention of different configurations at some of the asymmetric carbons, which, in this project, is interesting, because selectivity towards different enzymes could be achieved. The behavior of the products against different glycosidases showed that inhibition was generally low but selective towards one or two enzymes. The activation of the target enzymes was observed in some cases.

Supplementary Materials: The following are available online, 1D and 2D NMR spectra of all new compounds (Figures S1–S72). Figure S41: Transglycosidation monitorization by ^{1}H-NMR). Table S1: Measured constant coupling from products derived from L-serine.

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Sample Availability: Not available.

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CAPÍTULO II:NUEVOS REORDENAMIENTOS DE SUSTRATOS CICLOPROPÉNICOS

4. ANTECEDENTES

Los ciclopropenos son los ciclos insaturados más pequeños. Esta estructura ha atraído a la comunidad científica debido a su elevada tensión de enlace (228 kJ mol^{-1}),[103] lo que lo convierte en un sistema altamente reactivo, sin dejar de ser estable. Esta estructura ha sido estudiada por muchos químicos teóricos y computacionales y su tensión se debe al ángulo extremadamente forzado que adoptan los carbonos sp^2 del doble enlace.[104]

La primera síntesis y posterior aislamiento fueron descritos en 1922 por Demi´yanov y Doyarenko.[105] Desde entonces, el interés por los ciclopropenos ha ido en aumento, especialmente desde el descubrimiento de que algunos productos naturales poseen estos anillos en su estructura, como puede ser el ácido estercúlico. La frecuente presencia de los ciclopropanos en productos naturales y fármacos como el ácido crisantémico o el forbol entre otros, contribuye a elevar la utilidad de los ciclopropenos como intermedios sintéticos (Figura 18).

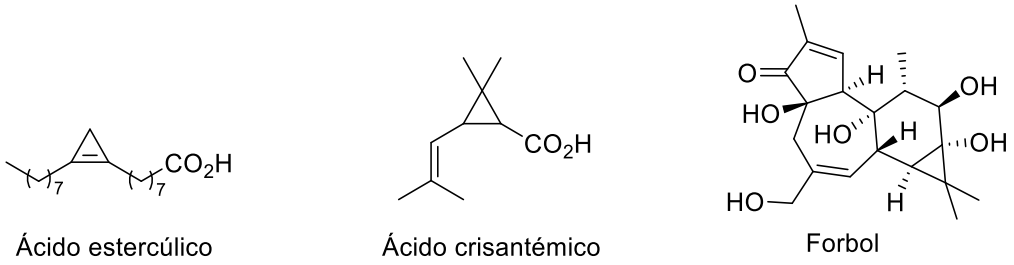
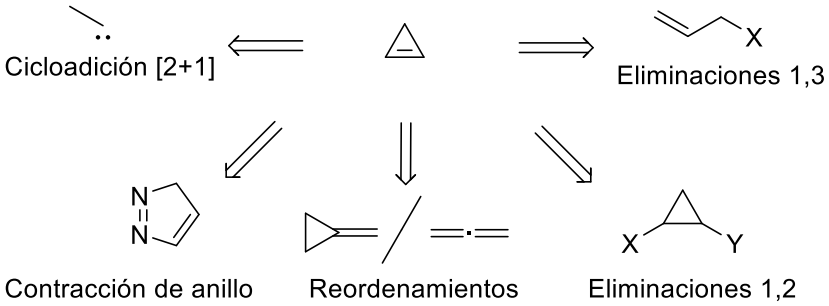


Figura 18. Estructuras de los ácidos estercúlico, crisantémico y del forbol.

4.1. SÍNTESIS DE CICLOPROPENOS

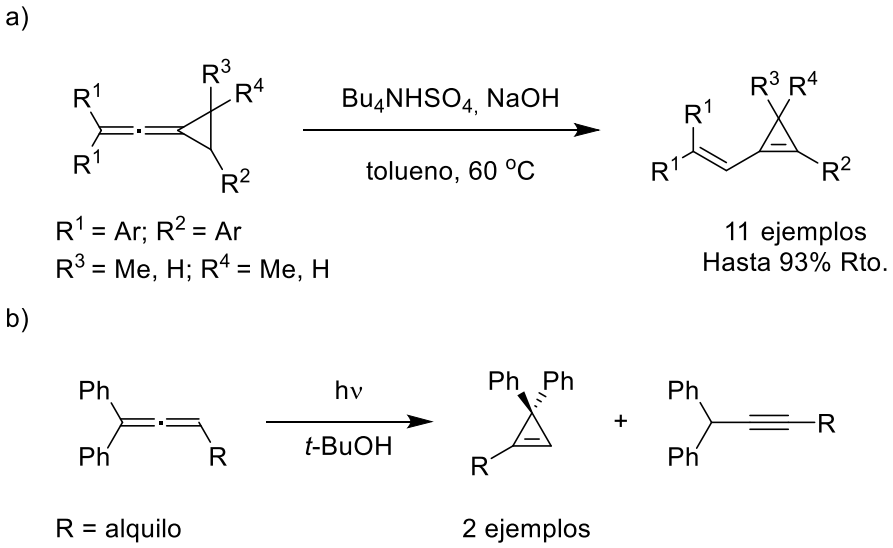
Aunque la manera más común de sintetizar ciclopropenos sea a través de una reacción de cicloadición [2+1], donde intervienen un triple enlace, un catalizador metálico y un compuesto que sea capaz de generar carbenos (normalmente un diazocompuesto), existen otras formas, entre las que destacan los reordenamientos, eliminaciones y contracciones de anillo (Esquema 5).[106]



Esquema 5. Diferentes síntesis de ciclopropenos.

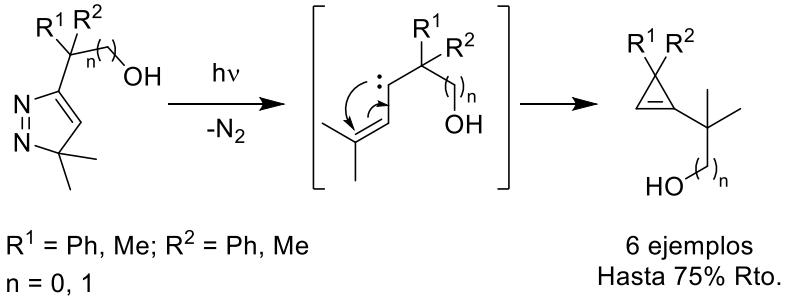
[103] a) Bingham, R. C.; Dewar, M. J. S. and Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1294-1301. b) Schleyer, P. v. R.; Williams, J. E.; Blanchard, K. R. *J. Am. Chem. Soc.* **1970**, *92*, 2377-2386.
[104] Johnson, W. T. G.; Borden, W. T. *J. Am. Chem. Soc.* **1997**, *119*, 5930-5933.
[105] Demi´yanov, N. Y.; Doyarenko, M. N. *Bull. Acad. Sci. Russ.* **1922**, *16*, 297-320.
[106] a) Baird, M. S. *Sci. Synth.*, **2010**, 47b, 1111-1133. b) Vicente, R. *Synthesis* **2016**, *48*, 2343-2360.

Los metilen-ciclopropanos son compuestos que pueden sufrir una reacción de reordenamiento para dar lugar a ciclopropenos. El grupo de Shi mostró que tratándolos con hidrogenosulfato de tetrabutilamonio (Bu_4NHSO_4) e hidróxido sódico (NaOH), en tolueno, se reorganizaban para dar lugar a ciclopropenos con rendimientos muy buenos (en torno al 90%, Esquema 6a).[107] En un trabajo previo, se estudió la reactividad fotoquímica de alenos. Se observó, que, entre otros productos de reordenamiento, se obtenían un alquino y un ciclopropeno como productos (Esquema 6b).[108]



Esquema 6. a) Reordenamiento de metilenciclopropanos. b) Reordenamiento fotocatalítico de alenos.

Los ciclopropenos se pueden obtener mediante una reacción de contracción de anillo. Estas reacciones están favorecidas cuando se elimina nitrógeno o dióxido de carbono. Dixneuf estudió el comportamiento fotoquímico de 3*H*-pirazoles, sintetizados a partir de alcoholes propargílicos. Observó que estos compuestos podían dar lugar a un cierre de anillo con eliminación de nitrógeno, obteniendo un ciclopropeno como producto final, con buenos rendimientos (Esquema 7).[109]

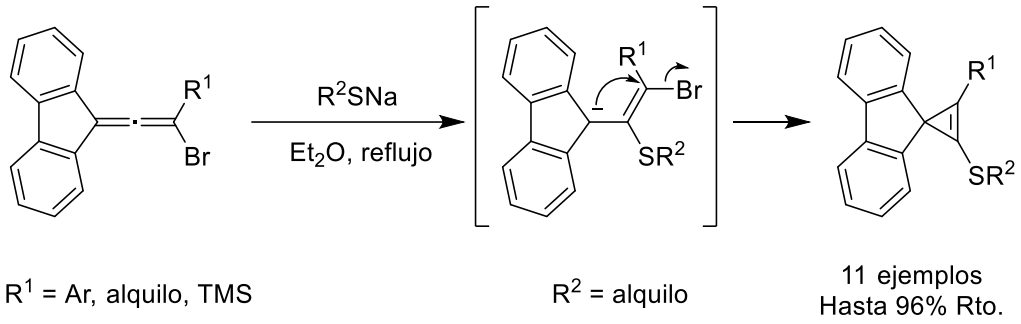


Esquema 7. Reacción fotoquímica de contracción de anillo.

Hay reacciones de eliminación 1,3 o eliminación 1,2 que también dan lugar a ciclopropenos. En el primer caso encontramos la deshidrohalogenación de haluros alílicos. Cuando el cloruro de alilo es tratado con una base como el hexametildisilamiduro de sodio (NaHMDS), se forma el anillo de ciclopropeno.[110] Otra alternativa interesante se observó en la adición de nucleófilos a

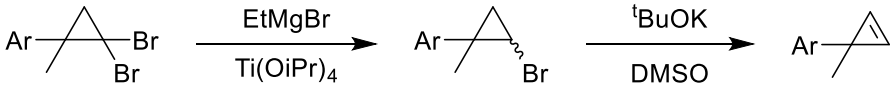
[107] Shao, L.-X.; Zhang, Y.-P.; Qi, M.-H.; Shi, M. *Org. Lett.* **2007**, *9*, 117-120.
[108] Steinmetz, M. G.; Mayes, R. T.; Yang, J.-G. *J. Am. Chem. Soc.* **1982**, *104*, 3518-3520.
[109] Hamdi, N.; Dixneuf, P. H.; Khemiss, A. *Eur. J. Org. Chem.* **2005**, 3526-3529.
[110] Binger, P.; Wedemann, P.; Goddard, R.; Brinker, U. H. *J. Org. Chem.* **1996**, *61*, 6462-6464.

alenos con grupos aromáticos, que ayudan a estabilizar el anión intermedio, que luego da lugar a una eliminación de bromuro cerrando el anillo (Esquema 8).[111]



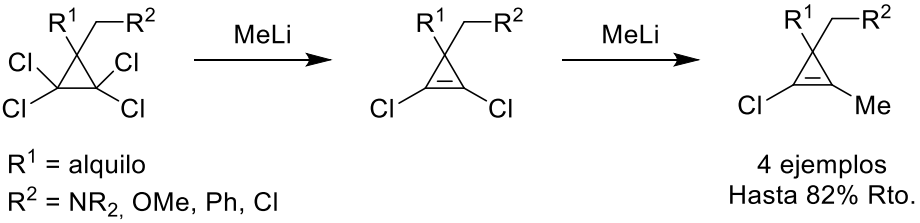
Esquema 8. Adición a alenos para formar ciclopropenos.

Otra manera de sintetizar ciclopropenos es a través una eliminación 1,2 en ciclopropanos. Este tipo de reacciones han sido descritas con diversos grupos funcionales, como, por ejemplo, a partir de monobromociclopropanos, en presencia de *terc*-butóxido potásico en DMSO anhidro.[112] Este compuesto monobromado normalmente es obtenido a partir de dibromociclopropano. Muchos grupos han estudiado como formar ciclopropenos a partir de compuestos dibromados, llegando a la conclusión de que el paso limitante es la eliminación de uno de los bromos. Esto se consigue eficientemente con el uso de bromuro de etilmagnesio (EtMgBr) en presencia de $Ti(O^iPr)_4$ (Esquema 9).[113] La deshidrobromación de bromociclopropanos se ha aplicado en la síntesis de ciclopropenos ferrocenil sustituidos como intermedios en diversas reacciones de expansión de anillo.[114] También se han descrito reacciones de deshidrocloración..[115]



Esquema 9. Formación de monobromociclopropenos y posterior eliminación.

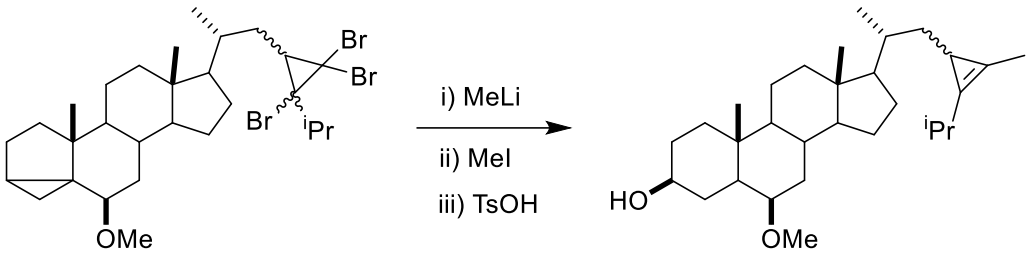
Otra posibilidad basada en la eliminación es la reacción de deshalogenación 1,2 de dihalociclopropanos. En estos casos, se utiliza MeLi (metil-litio) como base para la eliminación, dando lugar a un ciclopropeno litiado, que puede ser usado para su posterior funcionalización con diferentes electrófilos (Esquema 10).[116]



Esquema 10. Deshalogenación 1,2 en ciclopropanos.

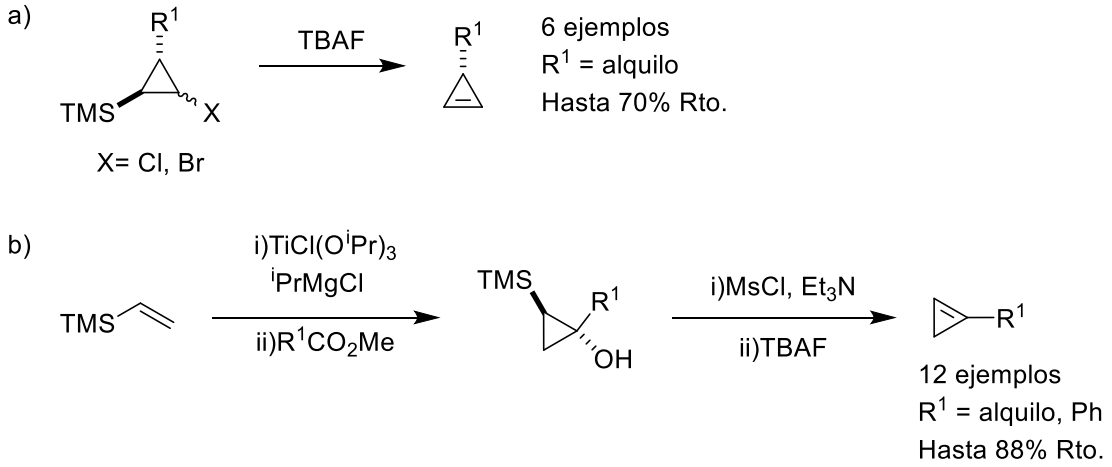
[111] Toda, T.; Kuwana, M.; Ohhashi, Y.; Yoshida, M. *Chem. Lett.* **1997**, 21-22.
[112] Al Dulayymi, J. R.; Baird, M. S.; Bolesov, I. G.; Tverezovskii, V.; Rubin, M. *Tetrahedron Lett.* **1996**, *37*, 8933-8936.
[113] Nizovtsev, A. V.; Al Dulayymi, J. R.; Baird, M. S.; Bolesov, I. G.; Tverezovskii, V. V. *Kinet. Catal.* **2003**, *44*, 151-164.
[114] Méndez, I. D.; Klimova, E.; Klimova, T.; Hernández, O. S.; Martínez García, M. *J. Organomet. Chem.* **2003**, *681*, 115-119.
[115] Shavrin, K. N.; Gvozdev, V. D.; Budanov, D. V.; Yurov, S. V.; Nefedov, O. M. *Mendeleev Commun.* **2006**, *2*, 73-76.
[116] Al-Dulayymi, J.; Baird, M. S. *Tetrahedron Lett.* **1988**, *29*, 6147-6148.

Esta reacción ha sido utilizada para obtener un compuesto tribromado intermedio, que con MeLi, y sin necesidad de aislamiento, origina el ciclopropeno final tras un tratamiento con cloruro de trimetilsililo.[117] Esta estrategia también se ha aplicado en la síntesis de derivados de isocalisteroles, un tipo de esteroides marinos (Esquema 11).[118]



Esquema 11. Síntesis de derivados de isocalisteroles.

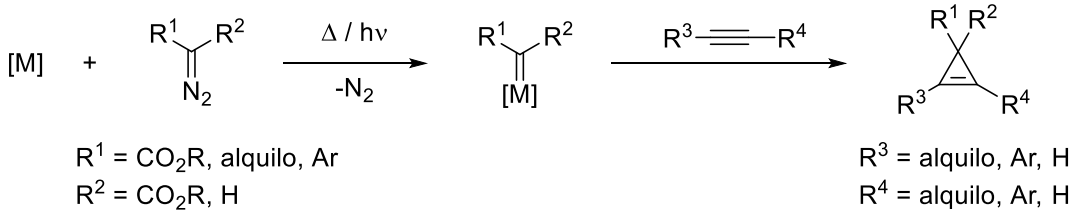
Un tipo de eliminación frecuentemente utilizada es la del grupo trimetilsililo, partiendo de 2-halotrimetilsililciclopropanos o de 2-trimetilisililciclopropanoles. Las deshalosililaciones y las deshidroxisililaciones se llevan a cabo con fluoruro de tetrabutilamonio (TBAF) (Esquema 12a).[119] Una desbromosililación se utilizó para la síntesis de los anteriormente mencionados isocalisteroles.[118] Respecto a las deshidroxisililaciones, es necesario convertir el grupo hidroxilo en un buen grupo saliente, por ejemplo, vía mesilación, para después efectuar la eliminación con TBAF. Los ciclopropanoles pueden ser obtenidos de manera sencilla a partir de trimetilsililalquenos y catalizadores de titanio. (Esquema 12b).[120]



Esquema 12. a) Deshalosililación. b) Deshidrosililación de ciclopropanos.

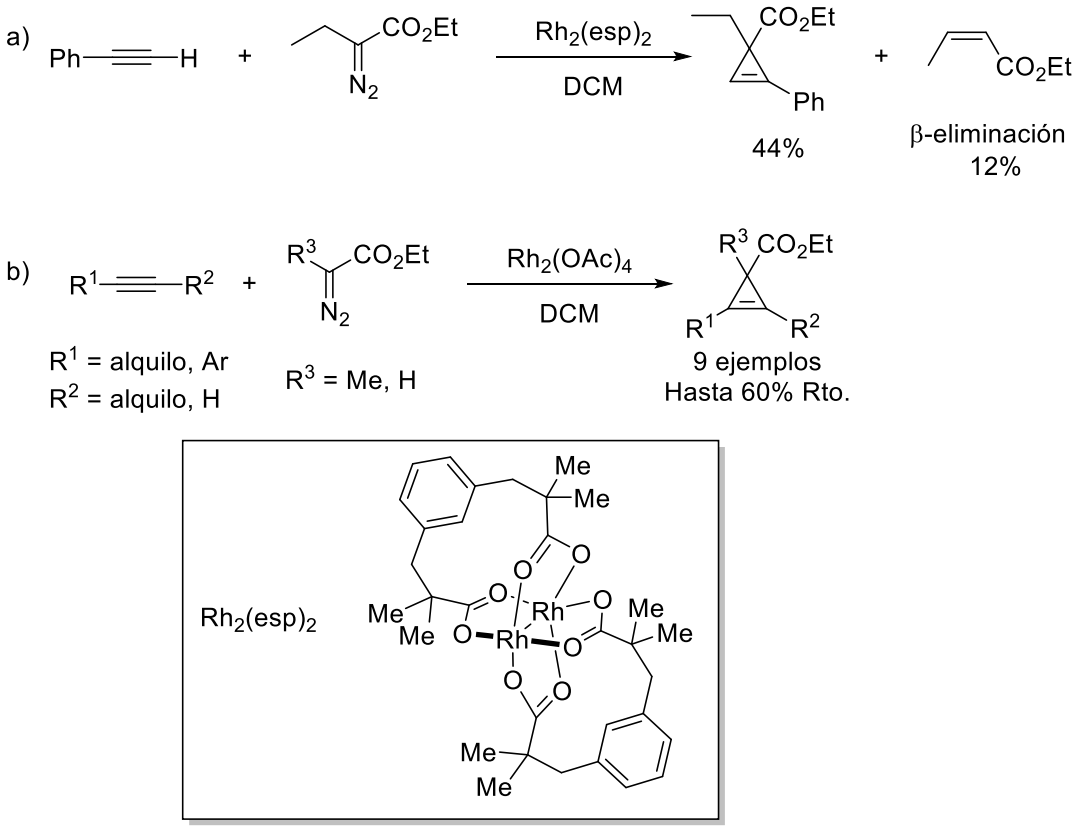
El método más habitual para la síntesis de ciclopropenos es la cicloadición [2+1] que fue descrita por primera vez en el año 1956, por D´jakonov y colaboradores. Este grupo hizo reaccionar el 1-fenilpropino con diazoacetato de etilo en presencia de un catalizador de cobre.[121] Los diazocompuestos generan carbenos fácilmente por eliminación de nitrógeno en presencia del metal, ya sea térmica o fotoquímicamente. Se obtiene así un carbeno metálico con mucha afinidad por electrones π (Esquema 13).

[117] Sheshenev, A. E.; Baird, M. S.; Croft, A. K.; Bolesov, I. G. *Medeleev Commun.* **2004**, *6*, 299-301.
[118] Kurek-Tyrlik, A.; Minksztym, K.; Wicha, J. *Eur. J. Org. Chem.* **2000**, *6*, 1027-1036.
[119] Haley, M. M.; Biggs, B.; Looney, W. A.; Gilberston, R. D. *Tetrahedron Lett.* **1995**, *36*, 3457-3460.
[120] a) Mizoriji, R.; Urabe, H.; Sato, F. *Tetrahedron Lett.* **1999**, *40*, 2557-2560. b) Mizoriji, R.; Urabe, H.; Sato, F. *J. Org. Chem.* **2000**, *65*, 6217-6222.
[121] D´jakonov, I. A.; Komendatov, M. I. *Vestnik Leningrad. Univ.* **1956**, *22*, 166-169.



Esquema 13. Esquema general de síntesis de ciclopropenos mediante cicloadición [2+1].

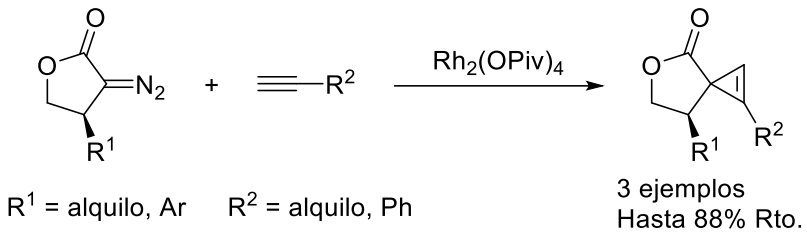
Los catalizadores más utilizados son los basados en rodio, como el tetraacetato de dirodio, debido sobre todo a la baja carga necesaria y la elevada reactividad del rodio frente a otros metales.[122] Como principal limitación de estos catalizadores, está su ineficacia con α-alquil-α-diazoacetatos, ya que aparece competencia entre la ciclopropenación y la β-eliminación de hidruro (Esquema 14a). Entre los pocos ejemplos descritos con este tipo de diazocompuestos, están los debidos a Müller (Esquema 14b)[123] y Padwa.[124] No fue hasta más tarde cuando se descubrió la β-eliminación en este tipo de compuestos.[125]



Esquema 14. a) β-Eliminación. b) Síntesis de Müller.

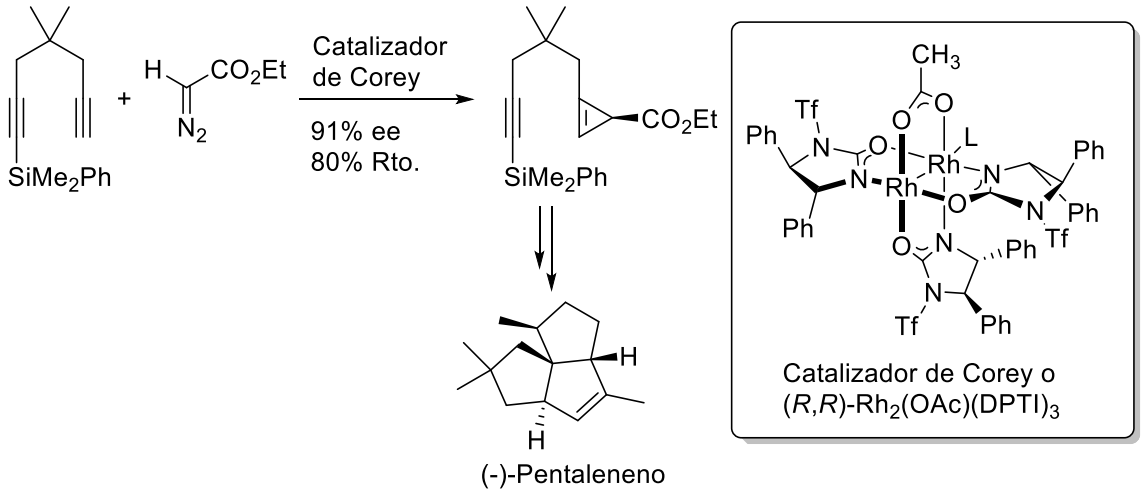
DeAngelis y colaboradores, evitaron la reacción de β-eliminación utilizando α-diazocarbolactonas. El carbeno se generaba *in situ* con un catalizador de rodio (Esquema 15).[126]

[122] Rubin, M.; Rubina, M. and Gevorgyan, V. *Synthesis* **2006**, *8*, 1221-1245.
[123] Müller, P. and Granicher, C. *Helv. Chim. Acta* **1995**, *78*, 129-144.
[124] Padwa, A.; Kassir, J. M. and Xu, S. L. *J. Org. Chem.* **1997**, *62*, 1642-1652.
[125] Panne, P. and Fox, J. M. *J. Am. Chem. Soc.* **2007**, *129*, 22-23.
[126] DeAngelis, A.; Dmitrenko, O. and Fox, J. M. *J. Am. Chem. Soc.* **2012**, *134*, 11035-11043.



Esquema 15. Síntesis de DeAngelis.

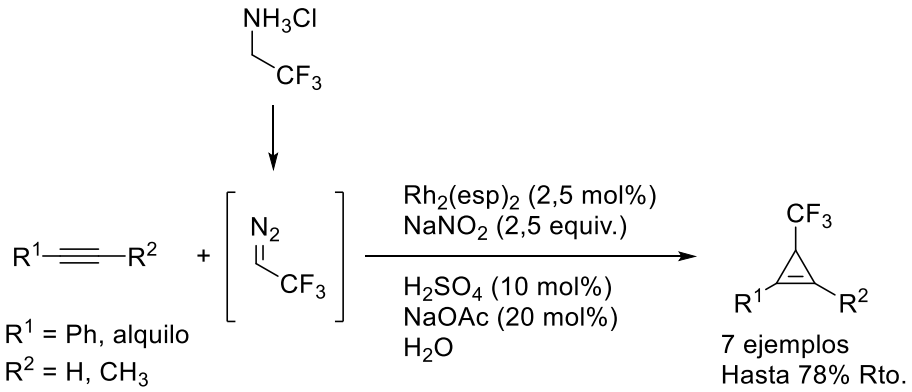
La otra limitación que poseen estos catalizadores de rodio es que no son reactivos frente a alquinos internos. González-Bobes y colaboradores, consiguieron el primer ejemplo de ciclopropenación de un alquino interno, usando diazomalonato de dietilo, 1-fenilpropino y $Rh_2(esp)_2$ como catalizador.[127] Aunque esto suponga una limitación, puede aprovecharse para la ciclopropenación selectiva de compuestos que posean diferentes triples enlaces en su estructura. Pallerla y Fox aprovecharon esta característica en un intermedio en la síntesis total del (-)-pentaleneno, donde consiguieron sintetizar el ciclopropeno selectivamente a partir de un diino y un catalizador de rodio quiral (Esquema 16).[128] El rodio puede ser usado como catalizador para ciclopropenaciones estereoselectivas cuando se usa un ligando quiral. El grupo de Corey desarrolló un catalizador con un ligando quiral, obteniendo ciclopropenos con rendimientos de hasta el 90% y enantioselectividades mayores al 90%.[129]



Esquema 16. Ciclopropenación selectiva de alquinos terminales.

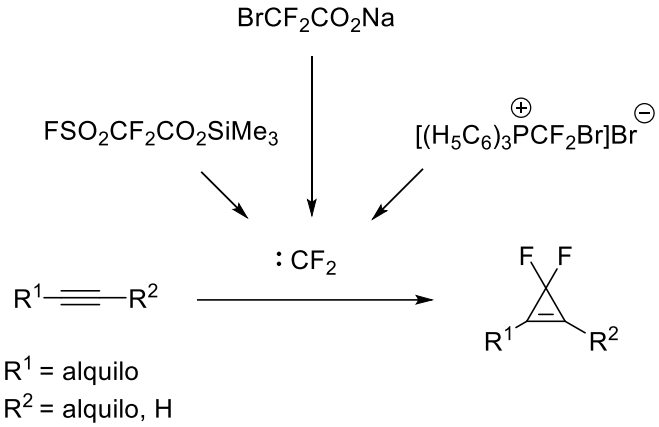
El grupo CF_3 es importante en diversos campos de la química, sobre todo en química médica, pero su introducción como sustituyente en ciclopropenos ha sido poco explotada. Müller y colaboradores, describieron en el 2004 una cicloadición [2+1] asimétrica de 1-hexino catalizada por Rh(II).[130] Como una extensión al trabajo previo donde sintetizaban ciclopropanos, Carreira demostró la posibilidad de sintetizar estos trifluorometil ciclopropenos a partir de una amina, generando *in situ* y en medio acuoso el diazocompuesto (Esquema 17). Esta variante, diferente a la de Müller, permitía obtener ciclopropenos monosustituidos.[131]

[127] González-Bobes, F.; Fenster, M. D. B.; Kiau, S.; Kolla, L.; Kolotuchin, S.; Soumeillant, M. *Adv. Synth. Catal.* **2008**, *350*, 813-816.
[128] Pallerla, M. K.; Fox, J. M. *Org. Lett.* **2007**, *9*, 5625-5628.
[129] Lou, Y; Horikawa, M.; Kloster, R. A.; Hawryluk, N. A.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 8916-8918.
[130] Müller, P.; Grass, S.; Shahi, S. P.; Bernardinelli, G. *Tetrahedron* **2004**, *60*, 4755-4763.
[131] Morandi, B.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 4294-4296.



Esquema 17. Síntesis de Carreira de ciclopropenos trifluorometil sustituidos.

Una metodología que no requiere de catalizador metálico es la síntesis de difluorociclopropenos. En estos casos, se da una cicloadición [2+1] entre un alquino y un difluorocarbeno generado *in situ*. Existen tres compuestos que pueden generar difluorocarbenos: 2,2-difluoro-2-(fluorosulfonil)acetato de trimetilsililo,[132] bromuro de bromodifluoro(trimetilfosfonio)metano[133] y bromodifluoroacetato de sodio.[134] Los difluorocarbenos son capaces de reaccionar con alquinos internos y terminales con buenos rendimientos (Esquema 18).



Esquema 18. Síntesis de difluorociclopropenos.

Aunque el rodio sea el metal más utilizado en estas reacciones, existen ejemplos del uso de otros catalizadores, que también pueden tener versiones asimétricas, como son el iridio,[135] cobalto,[136] sales de plata,[137] complejos de oro activados por plata[138] o catálisis por cobre o BF_3.[139]

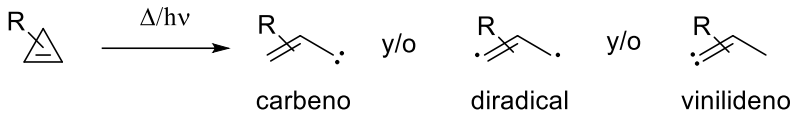
4.2. REACTIVIDAD

Aunque la energía de tensión del anillo de ciclopropeno sea muy elevada, las reacciones de apertura (en ausencia de otros reactivos) no están cinéticamente favorecidas pues la energía de

[132] Xu, W.; Chen, Q. Y. *J. Org. Chem.* **2002**, *67*, 9421-9427.
[133] Bessard, Y.; Schlosser, M. *Tetrahedron*, **1991**, 47, 7323-7328.
[134] Oshiro, K.; Morimoto, Y.; Amii, H. *Synthesis* **2010**, 2080-2084.
[135] Uehara, M.; Suematsu, H.; Yasutomi, Y.; Katsumi, T. *J. Am. Chem. Soc.* **2011**, *133*, 170-171.
[136] Cui, X.; Xu, X.; Lu, H.; Zhu, S.; Wotjas, L.; Zhang, X. P. *J. Am. Chem. Soc.* **2011**, *133*, 3304-3307.
[137] Marchueta, I.; Verdaguer, X.; Moyano, A.; Pericàs, M. A.; Riera, A. *Org. Lett.* **2001**, *3*, 3193-3196.
[138] Briones, J. F.; Davies H. M. L. *J. Am. Chem. Soc.* **2012**, *134*, 11916-11919.
[139] Gong, J.; Zhao, Z.; Zhang, F.; Wu, S.; Yan, G.; Quan Y.; Ma, B. *Org. Lett.* **2014**, *16*, 5524-5527.

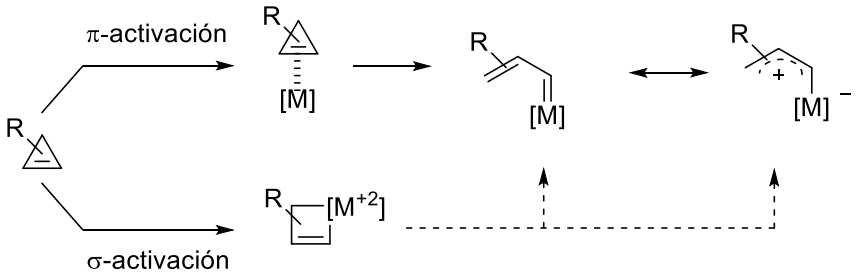
activación necesaria es de 140 kJ mol^{-1}.[140] Esto provoca que se necesiten altas temperaturas o luz ultravioleta para excitar el anillo. La inestabilidad de los ciclopropenos, por tanto, no viene dada por la tendencia a la ruptura del anillo, sino que es debida a oligomerizaciones o reacciones énicas cuando existe un hidrógeno en la posición 3.[141] Esto cambia en presencia de otros reactivos o bajo condiciones de catálisis, en las que son otros mecanismos los que predominan.

En ausencia de catalizadores metálicos, las reacciones de apertura de anillo en estos compuestos dan lugar a una ruptura del enlace σ C-C. Se forman tres intermedios muy reactivos: carbenos alílicos, especies diradicálicas o vinilidenos (Esquema 19). Normalmente, se obtiene una mezcla de productos.



Esquema 19. Intermedios formados en la apertura de anillo de ciclopropeno.

En presencia de catalizadores metálicos, la apertura de ciclopropenos puede ocurrir de diferentes formas. Así, los metales pueden coordinarse al enlace π C=C, dando lugar a un reordenamiento del enlace σ C-C, lo que genera un vinil carbeno, mediante un mecanismo que aún está siendo estudiado.[142] Por otro lado, los metales capaces de sufrir adiciones oxidativas pueden insertarse directamente en el enlace σ C-C. Esto genera un metalaciclobuteno, que evoluciona hacia los mismos intermedios (Esquema 20).[143] La reacción de metátesis es la única en la que se rompe el enlace π C=C.



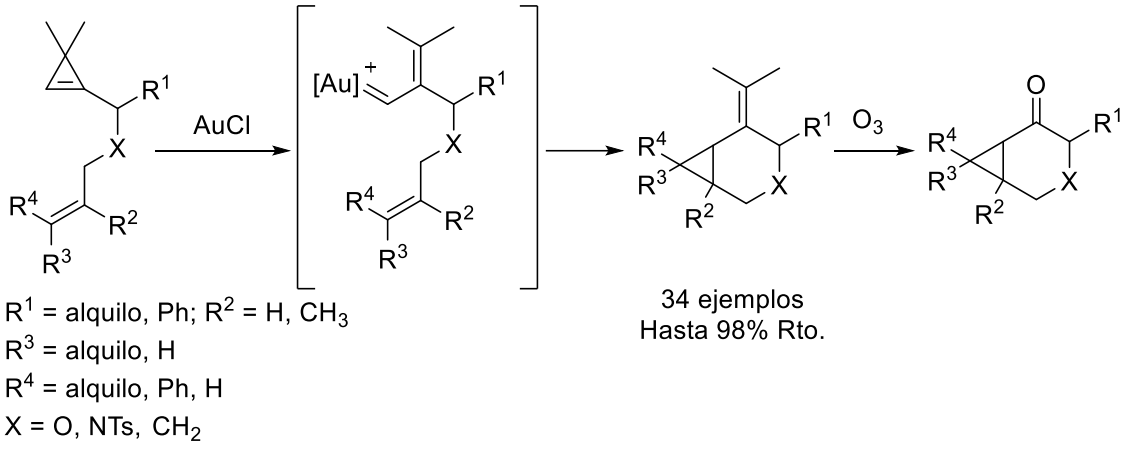
Esquema 20. Activación del anillo catalizada por metales.

4.2.1. REACCIONES CATALIZADAS POR METALES

Hay muchas reacciones de ciclopropenos catalizadas por diferentes metales de transición, como pueden ser niquel,[144] cobre,[145] plata[146], rodio[147] o cobalto[148] entre otros. Nos centraremos en los procesos catalizados por complejos de oro y rutenio. El grupo de Cossy ha estudiado en detalle el comportamiento de estructuras ciclopropen-énicas frente a catalizadores de oro. En 2010, observaron que estos compuestos sufrían una cicloisomerización obteniendo oxa- y

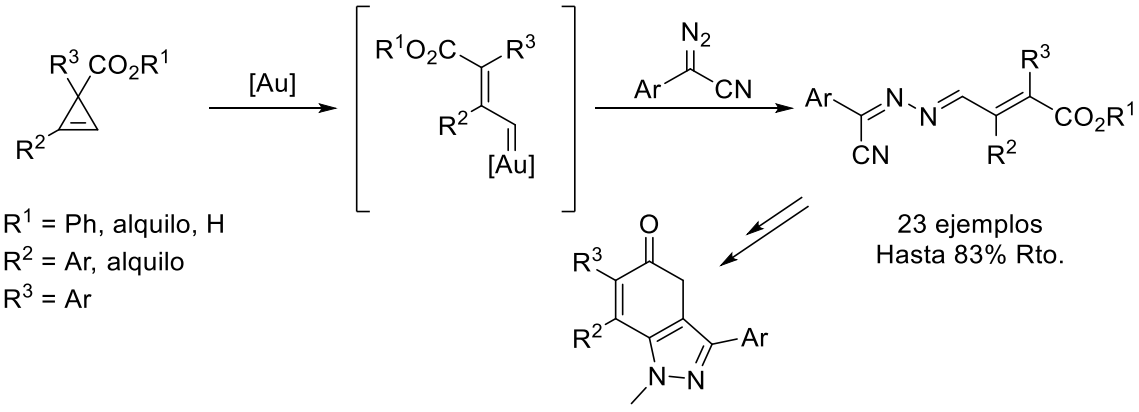
[140] Srinivasan, R. *J. Am. Chem. Soc.* **1969**, *91*, 6250-6253.
[141] Lin, H.-C.; Tsai, R.-T.; Wu, H.-P., Lee, H.-Y.; Lee, G.-A. *Tetrahedron* **2016**, *72*, 184-191.
[142] Ishikawa, A.; Tanimura, Y.; Nakao, Y.; Sato, H.; Sakaki, S. *Organometallics* **2012**, *31*, 8189-8199.
[143] Binger, P.; Müller, P.; Benn, R.; Mynott, R. *Angew. Chem. Int. Ed.* **1989**, *28*, 610-611.
[144] Binger, P.; McMeeking, J. *Angew. Chem. Int. Ed.. Engl.* **1974**, *13*, 466-467.
[145] Tomilov, Y. V.; Tsvetkova, N. M.; Nefedov, O. M. *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1989**, *38*, 1456-1465.
[146] Padwa, A.; Blacklock, T. J.; Loza, R. *J. Am. Chem. Soc.* **1981**, *103*, 2404-2405.
[147] Miege, F.; Meyer, C.; Cossy, J. *Angew. Chem. Int. Ed.* **2011**, *50*, 5932-5937.
[148] Li, C.; Zhang, H.; Feng, J.; Zhang, Y.; Wang, J. *Org. Lett.* **2010**, *12*, 3082-3085.

azabiciclo[4.1.0]heptanos con muy buenos rendimientos y diastereoselctividades.[149] El catalizador de oro, AuCl, generaba un vinil carbeno en el carbono más externo del ciclopropeno. Este carbeno, ciclopropanaba intramolecularmente el alqueno. Más tarde, demostraron la posibilidad de sintetizar α-diazocetonas mediante ozonolisis del producto obtenido tras la cicloadición (Esquema 21).[150]



Esquema 21. Reordenamiento de sistemas ciclopropen-énicos descrito por Cossy.

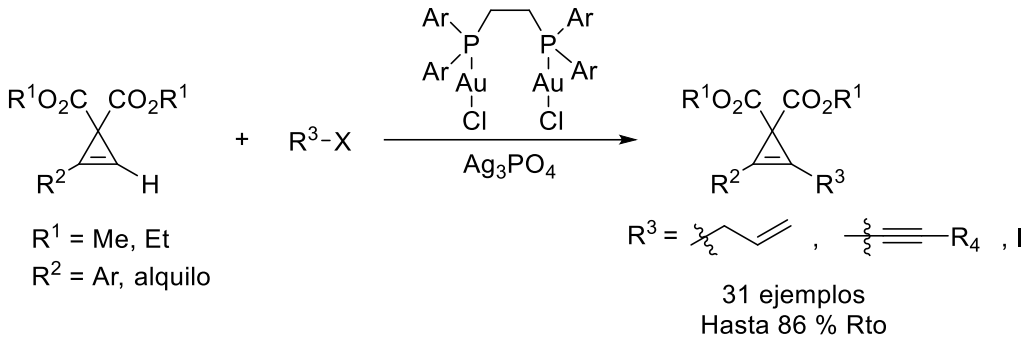
Li y colaboradores desarrollaron otra reacción catalizada por oro, que se podía dirigir hacia la formación de furanos o de naftoles tras un reordenamiento y posterior reacción de Friedel-Crafts.[151] En el 2021, el grupo de Liu publicó la síntesis de 1*H*-pirazolo[4,3-*b*]piridin-5-onas en la cual la etapa clave era la reacción entre un ciclopropeno y un diazocompuesto catalizada por un complejo de oro (Esquema 22).[152] En todos los casos, el oro forma el carbeno en el carbono más externo del ciclopropeno.



Esquema 22. Reacción entre ciclopropenos y diazocompuestos catalizada por oro.

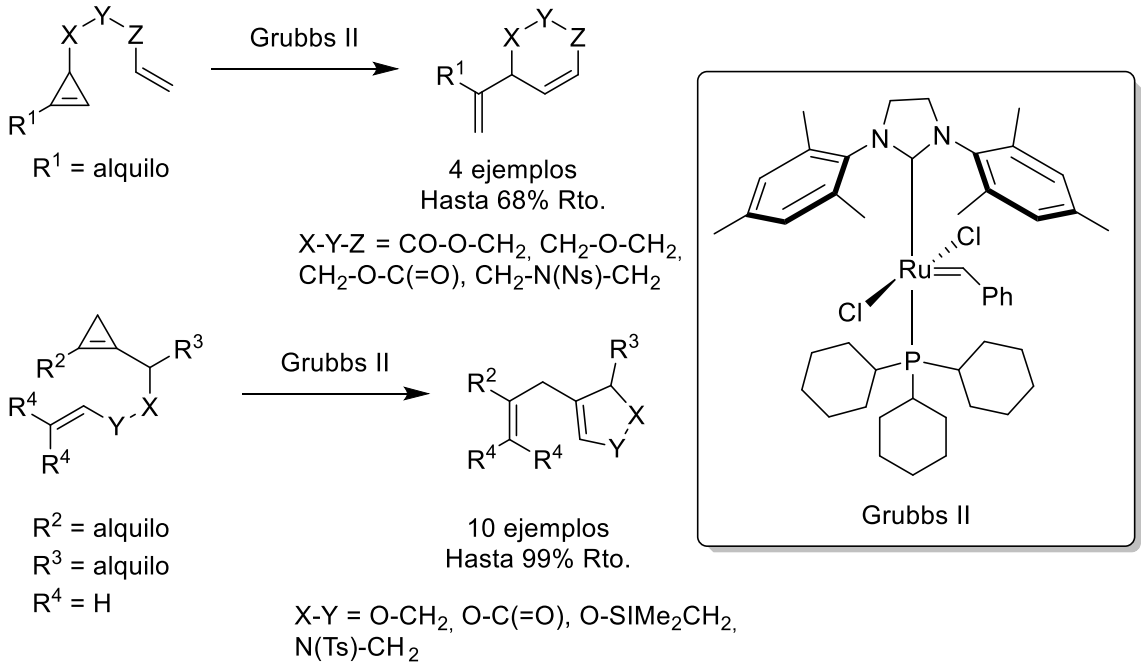
En la mayoría de los ejemplos descritos usando catalizadores de oro, los ciclopropenos sufren una apertura de anillo y posteriormente un reordenamiento que evoluciona hacia diferentes productos. El grupo de Xie observó, usando un catalizador de oro dinuclear, una activación C-H del anillo, dando lugar a un ciclopropeno nuevo. Esta reacción necesitaba un derivado halogenado y admitía tanto grupos alilo como alquinos terminales (Esquema 23).[153]

[149] Miege, F.; Meyer, C.; Cossy, J. *Org. Lett.* **2010**, *12*, 3082-3085.
[150] Miege, F.; Meyer, C.; Cossy, J. *Chem. Eur. J.* **2012**, *18*, 7810-7822.
[151] Li, T.; Julaiti, Y.; Wu, X.; Han, J.; Xie, J. *Chem. Eur. J.* **2022**, *28*, e202202851.
[152] Sadaphal, V. A.; Liu, R.-S. *Org. Lett.* **2021**, *23*, 5496-5500.
[153] Liu, K.; Li, T.; Liu, D.-Y.; Li, W.; Han, J.; Zhu, C.; Xie, J. *Sci. China Chem.* **2021**, *64*, 1958-1963.



Esquema 23. Activación C-H de ciclopropenos catalizadas por oro dinuclear.

El rutenio es otro metal que presenta gran afinidad por enlaces múltiples y es capaz de reaccionar con ciclopropenos. El grupo de Shi publicó en 2010 reacciones de metátesis en ciclopropen-inos catalizadas por el carbeno de Grubbs de primera generación.[154] Propusieron diferentes mecanismos, en función de donde se coordinara primero el rutenio: al triple enlace o al ciclopropeno. Observaron que la coordinación al ciclopropeno se daba por el carbono más sustituido, a diferencia de lo observado al usar catalizadores de oro. Cossy también realizó reacciones de metátesis de reordenamiento de anillo con ciclopropenos, en este caso usando carbenos de Grubbs de segunda generación. Como en el caso anterior, la reacción podía transcurrir por diferentes mecanismos (Esquema 24).[155]

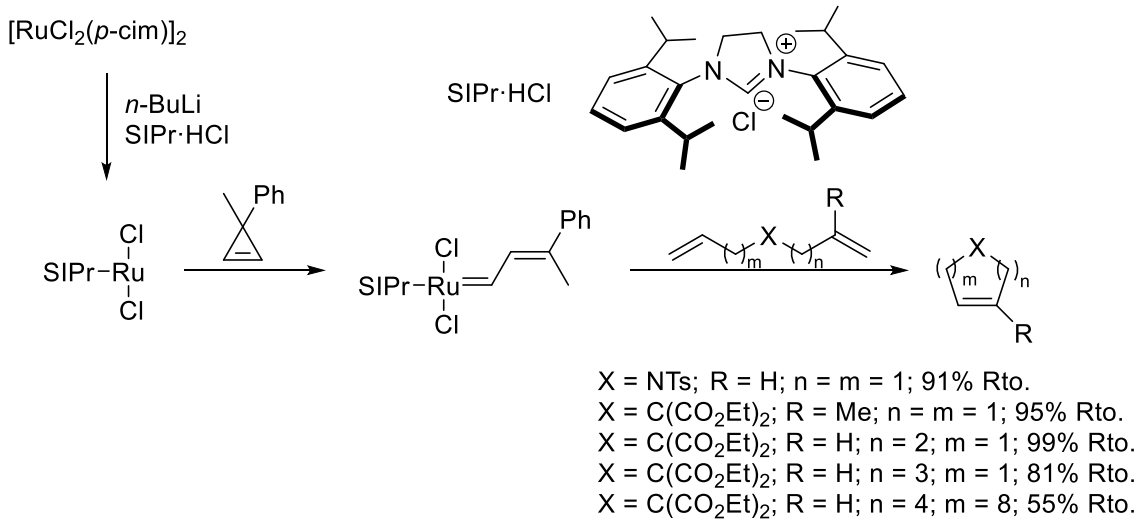


Esquema 24. Reacciones de metátesis en ciclopropenos.

Müller y colaboradores generaron *in-situ* un catalizador de rutenio de 14 electrones, para reacciones de RCM, usando ciclopropenos como ligandos. Estos compuestos actuaban como una fuente de carbenos al reaccionar con el complejo metálico. Para ello, usaban un dímero de

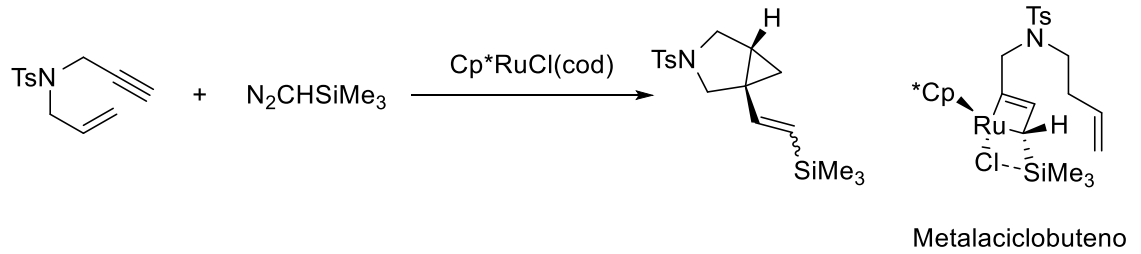
[154] Zhu, Z.-B.; Shi, M. *Org. Lett.* **2010**, *12*, 4462-4465.
[155] Miege, F.; Meyer, C.; Cossy, J. *Org. Lett.* **2010**, *12*, 248-251.

rutenio comercial ($[Ru_2Cl_2(p\text{-cim})]_2$). Observaron que los ciclopropenos ricos en electrones eran mejores para dicha coordinación al metal (Esquema 25).[156]



Esquema 25. Ciclopropenos como ligandos en reacciones de metátesis.

Un catalizador de rutenio que se caracteriza por no dar reacciones de metátesis, sino cicloadiciones, es el $Cp^*RuCl(cod)$.[157] Este catalizador tiende a coordinarse con enlaces múltiples y hay muchos ejemplos descritos de su uso con eninos. Uno de los primeros fue descrito por Monnier y colaboradores entre eninos y diazocompuestos.[158] Más adelante, estudiaron la estereoselectividad de la reacción. Llegaron a la conclusión de que la estereoquímica final quedaba fijada por la repulsión estérica generada entre el metal y los sustituyentes. La selectividad *Z* o *E* del doble enlace formado en la reacción dependía de las repulsiones o interacciones entre los sustituyentes en el metalaciclobuteno que se genera al reaccionar con el triple enlace (Esquema 26).[159]

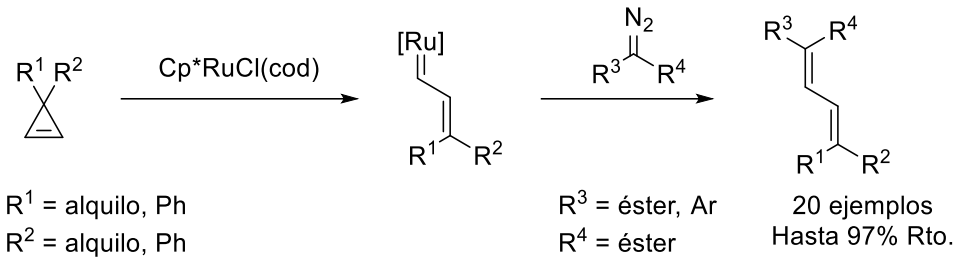


Esquema 26. Reactividad de Cp*RuCl(cod) frente a eninos.

Uno de los pocos precedentes en los que el complejo Cp*RuCl(cod) se ha utilizado con ciclopropenos se publicó el 2018. Wang y colaboradores describieron una reacción de acoplamiento cruzado entre un ciclopropeno y diazocompuestos. Consiguieron los correspondientes butadienos con buenos rendimientos y estereoselectividades mayores a 20:1. El carbeno se generaba primero mediante reacción del catalizador con el ciclopropeno, y

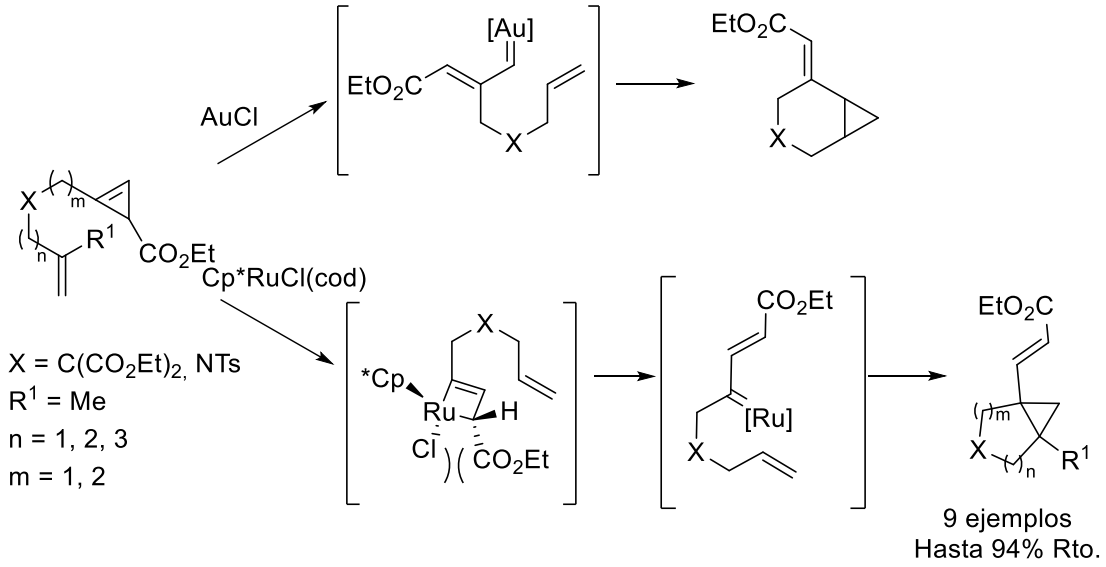
[156] Muller, D. S.; Raoul, Y.; Le Notre, J.; Basle, O.; Mauduit, M. *ACS Catal*. **2019**, *9*, 3511-3518.
[157] Yamamoto, Y. *Tetrahedron Lett.* **2017**, *58*, 3787-3794.
[158] Monnier, F.; Castillo, D.; Dérien, S.; Toupet, L.; Dixneuf, P. H. *Angew. Chem. Int. Ed.* **2003**, *42*, 5474-5477.
[159] Monnier, F.; Vovard-Le Bray, C.; Castillo, D.; Aubert, V.; Dérien, S.; Dixneuf, P. H.; Toupet, L.; Ienco, A.; Mealli, C. *J. Am. Chem. Soc.* **2007**, *129*, 6037-6049.

posteriormente reaccionaba el diazocompuesto, liberando nitrógeno, para dar lugar al producto final (Esquema 27).[160]



Esquema 27. Reacción entre ciclopropenos y diazocompuestos.

Nuestro grupo sintetizó una serie de sistemas ciclopropen-énicos mediante reacciones de alquinos con diazocompuestos catalizadas por $Rh_2(OAc)_4$ y posterior reacción con bromuros de alquenilo. Estos compuestos se trataron con los catalizadores de oro usados por Cossy y con Cp*RuCl(cod). Se observó, que mientras que el oro forma el carbeno en el carbono más externo del ciclopropeno, el rutenio lo hace en el carbono interno, dando lugar a alquenilbiciclo[3.1.0]hexanos. En ambos casos, se observó que se obtenía exclusivamente un isómero del doble enlace formado. En el caso del rutenio, esto se debe a la repulsión generada entre el ligando y el grupo éster del ciclopropeno, lo que provoca que se aleje lo máximo posible, dando lugar a la apertura del metalaciclo con configuración *E* (Esquema 28).[161]

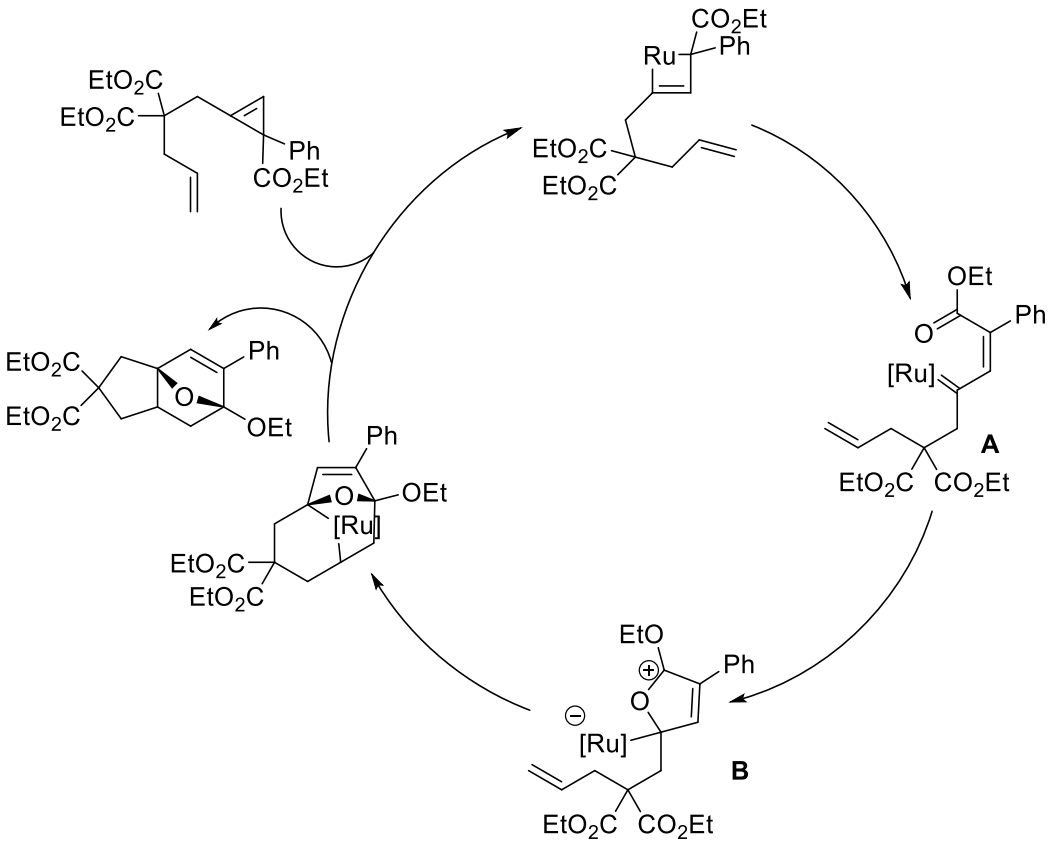


Esquema 28. Reacción regiodivergente de sistemas ciclopropen-énicos.

El grupo de Dixneuf no consiguió sintetizar los anillos de 6 con buenos rendimientos, y los ciclos de 7 eslabones no fueron posibles usando eninos como productos de partida.[162] En nuestro trabajo, se sintetizaron hasta 12 ejemplos, entre ellos anillos de 6 y 7 eslabones fusionados a ciclopropanos con buenos rendimientos. Cuando el anillo de ciclopropeno estaba disustituido, se observó un producto diferente. En estos casos, el grupo fenilo, que es más voluminoso que el éster, es el que se aleja del centro metálico, dejando al éster cerca (Esquema 29, **A**). Cuando la temperatura de la reacción no es suficiente, este intermedio no es capaz de

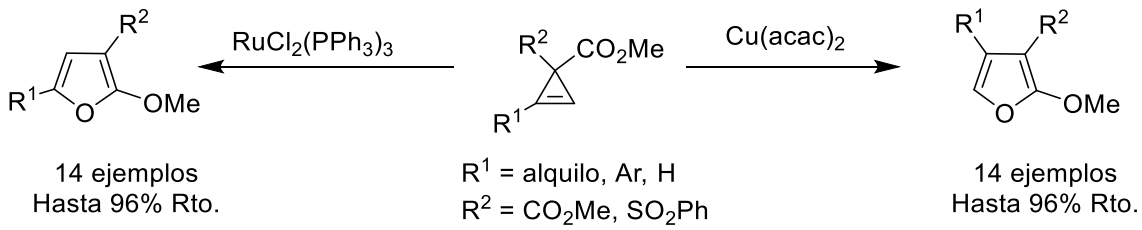
[160] Wang, B.; Yi, H.; Zhang, H.; Sun, T.; Zhang, Y.; Wang, J. *J. Org. Chem.* **2018**, *83*, 1026-1032.
[161] López-Rodríguez, A.; Domínguez, G.; Pérez-Castells, J. *J. Org. Chem.* **2019**, *84*, 924-933.
[162] Bray, C. V.-L.; Klein, H.; Dixneuf, P. H.; Macé, A.; Berreé, F.; Carboni, B.; Derién, S. *Adv. Synth. Catal.* **2012**, *354*, 1919-1925.

seguir reaccionando y se forma un dímero. Si el ciclo continúa, se genera el dihidrofurano **B** por un ataque nucleófilo del oxígeno. Una ciclación y posterior eliminación del catalizador da como producto un triciclo altamente conectado. Este tipo de intermedio fue descrito por Meyer y colaboradores en una reacción de isomerización de ciclopropenilmetil ésteres catalizadas por rodio.[163] Estos resultados fueron el antecedente al trabajo presentado en esta memoria.



Esquema 29. Ciclo catalítico con ciclopropenos disustituidos.

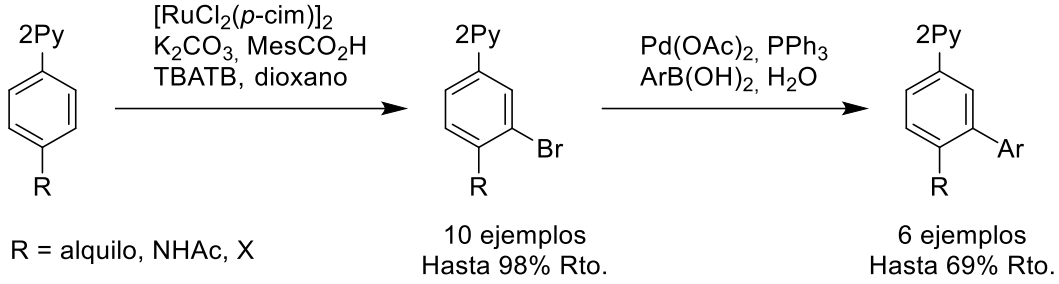
Chen y colaboradores estudiaron la reactividad de ciclopropenos con grupos carbonilo frente a diferentes catalizadores. Obtuvieron anillos de furano con rendimientos en torno al 90% en muchos casos. Observaron que se podía seleccionar el regioisómero resultante en función del metal usado. El cobre se coordinaba al carbono más externo del doble enlace mientras que el rutenio lo hacía en el interno.[164] Esta cicloisomerización funcionaba también con cetonas (Esquema 30).



Esquema 30. Reordenamiento de carbonilciclopropenos con diferentes metales.

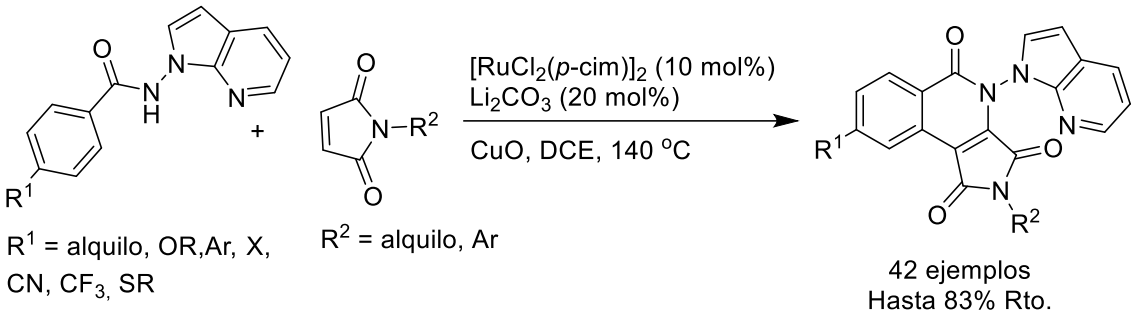
[163] Archambeau, A.; Nguyen, D.; Meyer, M.; Cossy, J. *Chem. Eur. J.* **2016**, *22*, 6100-6110.
[164] Chen, J.; Ma, S. *Chem. Asian J.* **2010**, *5*, 2415-2421.

Otros catalizadores que han sido usados para reacciones que no sean de metátesis son los dímeros de rutenio aunque, como en el caso anterior, no hay muchos ejemplos descritos con ciclopropenos. Estos complejos han sido utilizados en su mayoría en reacciones de activación C-H. Mientras que metales como rodio, paladio o cobre, así como algunas especies de rutenio como $Ru_3(CO)_{12}$,[165] activan el enlace C-H de los anillos aromáticos en posición orto respecto al grupo director,[166] el grupo de Greany consiguió por primera vez una meta activación usando $[RuCl_2(p\text{-cim})]_2$.[167] Además, demostraron la posibilidad de realizar una bromación y posterior funcionalización "*one pot*" (Esquema 31).



Esquema 31. Meta activación de anillos aromáticos.

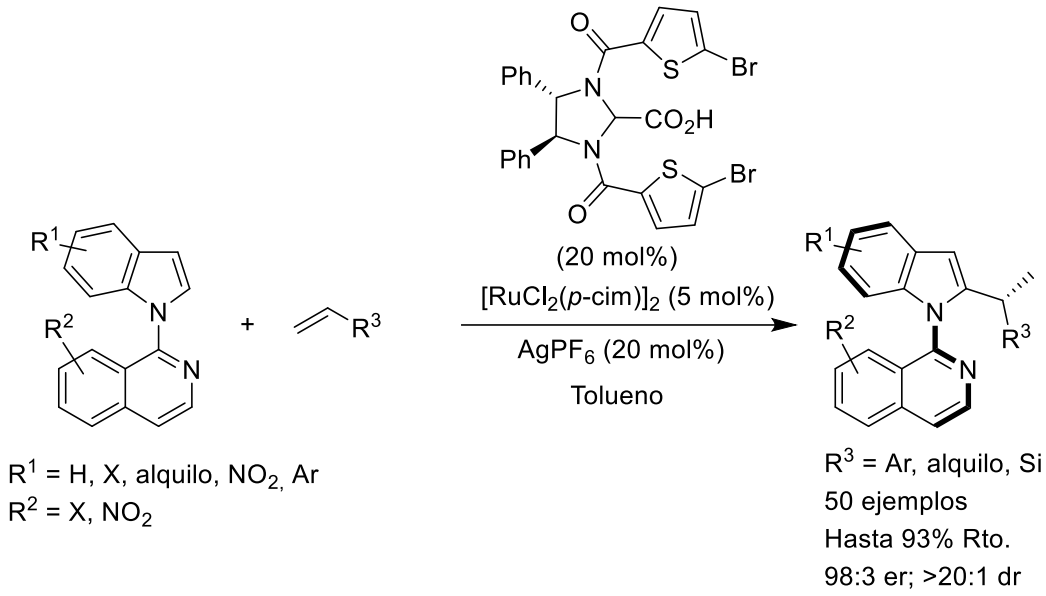
En el año 2021, Pati y colaboradores desarrollaron un protocolo para la obtención de isoquinolonas basadas en 7-azaindoles usando $[RuCl_2(p\text{-cim})]_2$.[168] A diferencia de trabajos previos, en los que se usaban catalizadores de rodio o cobre, el uso de rutenio daba como único producto el resultante de una anulación [4+2] (Esquema 32), evitando la [3+2] o la ciclación 1,1.[169] Otro uso ha sido la síntesis de eninos mediante activación de un triple y un doble enlace, con formación exclusiva del isómero *Z*.[170]



Esquema 32. Anulación [4+2] catalizada por rutenio.

Con $[RuCl_2(p\text{-cim})]_2$ se pueden hacer reacciones enantioselectivas al usar ligandos quirales, como demostró el grupo de Ackermann. Mediante el uso de un ligando quiral sintetizado en su laboratorio, pudieron hacer reaccionar doble enlaces con la posición 3 de un indol, con un grupo director como sustituyente. Consiguieron tanto diastereoselectividades como enantioselectividades muy altas (Esquema 33).[171]

[165] Wang, L.; Ackermann, L. *Chem. Comm.* **2014**, *50*, 1083-1085.
[166] a) Li, B.; Liu, B.; Shi, B.-F. *Chem. Comm.* **2015**, *51*, 5093-5096. b) Wang, X.-C.; Hu, Y.; Bonacorsi, S.; Hong, Y.; Burrell, R.; Yu, J.-Q. *J. Am. Chem. Soc.* **2013**, *135*, 10326-10329. c) Schroeder, N.; Lied, F.; Glorius, F. *J. Am. Chem. Soc.* **2015**, *137*, 1448-1451.
[167] Tesky, C: J.; Lui, A.; Greaney, M. F. *Angew. Chem. Int. Ed.* **2015**, *54*, 11677-11680.
[168] Pati, B.; Sagara, P.; Gosh, A.; Mohanti, S.; Ravikumar, P. *J. Org. Chem.***2021**, *86*, 6551-6565.
[169]a) Li, H.; Zhang, S.; Feng, X. J.; Yu, X. Q.; Yamamoto, Y.; Bao, M. *Org. Lett.* **2019**, *21*, 8563-8567. b) Guo, C.; Li, B.; Liu, H.; Zhang, X.; Fan, X. *Org. Lett.* **2019**, *21*, 7189-7193.
[170] Miao, H.; Wang, Z.-X. *Asian J. Org. Chem.* **2022**, *11*, e202200172.
[171] Li, Y.; Liou, Y.-C.; Oliveira, J.; Ackermann, L. *Angew. Chem. Int. Ed.* **2022**, *61*, e202212595.

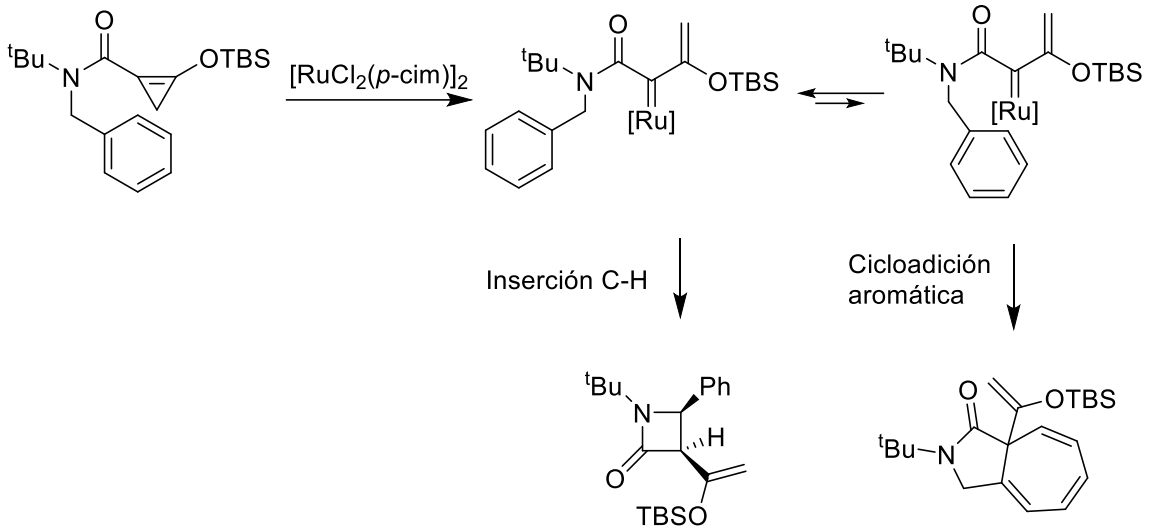


Esquema 33. Activación C-H de indoles catalizada por un dímero de rutenio descrita por Ackermann.

El $[RuCl_2(p\text{-cim})]_2$ está siendo utilizado en diferentes técnicas como son la electroquímica y la fotoquímica. Shen y colaboradores publicaron la síntesis de indolinas a través de una anulación [3+2] catalizada por este complejo bajo condiciones electrocatalíticas. Así se evita el uso de oxidantes como sales de plata o cobre.[172] El grupo de Ackermann, usando luz azul LED, consiguió un acoplamiento entre anillos aromáticos y carbonos sp^3. Observaron que la luz era necesaria para la reacción, que transcurría vía radicales.[173] Recientemente, Cheng y colaboradores, demostraron la capacidad de este tipo de catalizadores para activar selectivamente la posición *para* en un anillo aromático, bajo luz visible, obteniendo compuestos difluorados.[174]

Deng y colaboradores, estudiaron en 2016, la reactividad de ciclopropenos y de diazocompuestos frente a diferentes catalizadores. Obtenían dos productos diferentes, el correspondiente a una inserción C-H o el resultante de una cicloadición aromática. Usando el catalizador dímero de rutenio podían obtener casi exclusivamente (95:5) el producto de inserción C-H frente a la cicloadición con un rendimiento del 97% (Esquema 34).[175]

[172] Shen, H.; Liu, T.; Cheng, D.; Yi, X.; Wang, Z.; Liu, L.; Song, D.; Ling, F.; Zhong, W. *J. Org. Chem.* **2020**, *85*, 13735-13746.
[173] Wang, Y.; Chen, S.; Chen, X.; Zangarelli, A.; Ackermann, L. *Angew. Chem. Int. Ed.* **2022**, *61*, e202205562.
[174] Cheng, Y.; Zhang, X.; An, G.; Li, G.; Yang, Z. *Chin. Chem. Lett.* **2023**, *34*, 107625.
[175] Deng, Y.; Jing, C.; Arman, H.; Doyle, M. P. *Organomet.* **2016**, *35*, 3413-3420.

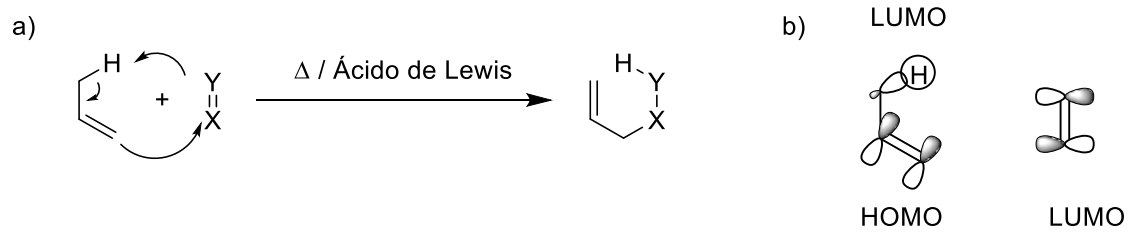


Esquema 34. Inserción C-H frente a cicloadición aromática promovida por rutenio.

4.2.2. REACCIONES PERICÍCLICAS

Las reacciones pericíclicas son reacciones concertadas, donde en el estado de transición hay un movimiento de electrones con una geometría cíclica. En dicho estado se forman y rompen a la vez enlaces π y σ.

La reacción Alder-énica, o reacción énica, es una reacción pericíclica que involucra 6 electrones. El componente denominado "eno" aporta 4 electrones: 2 electrones del sistema π de un doble enlace y 2 del enlace σ C-H alílico; los otros 2 electrones vienen del conocido como "enófilo", normalmente un doble enlace electrodeficiente. Se da una trasposición del doble enlace C=C, lo que lleva a la formación tres nuevos enlaces, 2σ C-C y C-H, y uno π (Esquema 35a). Estas reacciones pueden estar catalizadas por ácidos de Lewis o pueden ser térmicas.[176] En estas últimas interaccionan 3 orbitales: el HOMO del doble enlace alílico, el LUMO del σ C-H y el LUMO del enófilo (Esquema 35b).[177]



Esquema35. a) Mecanismo de reacción énica concertada. b) Orbitales moleculares implicados del eno y del enófilo respectivamente.

Las reacciones énicas en las que un ciclopropeno actúa como enófilo no poseen energías de activación tan elevadas. Esto se debe a la elevada tensión que posee el anillo. Durante el estado de transición, la aproximación *endo* suele ser más favorable que la *exo* debido a lo que se conoce como la interacción del orbital secundario (SOI): los orbitales del átomo de carbono

[176] Mikami, K.; Shimizu, M. *Chem. Rev.* **1992**, *92*, 1021-1050.
[177] Inagaki, S.; Fujimoto, H.; Fukui, K. *J. Am. Chem. Soc.* **1976**, *98*, 4693-4701.

del ciclopropeno que no intervienen en la reacción interaccionan con otros orbitales del "eno" estabilizando dicho estado de transición (Figura 19).[178]

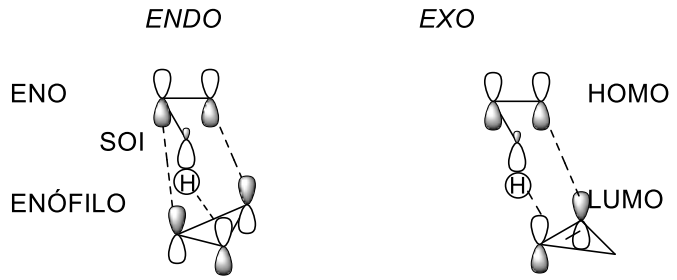
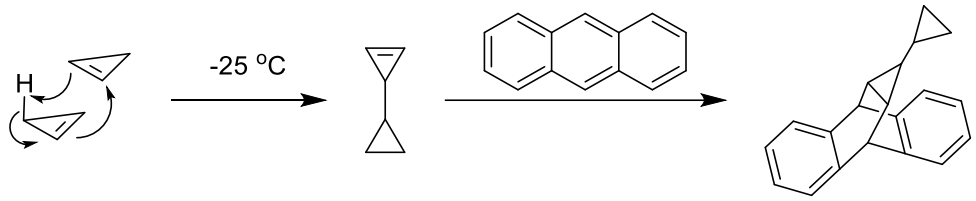


Figura 19. Aproximaciones *endo* y *exo* en la reacción énica de ciclopropenos.

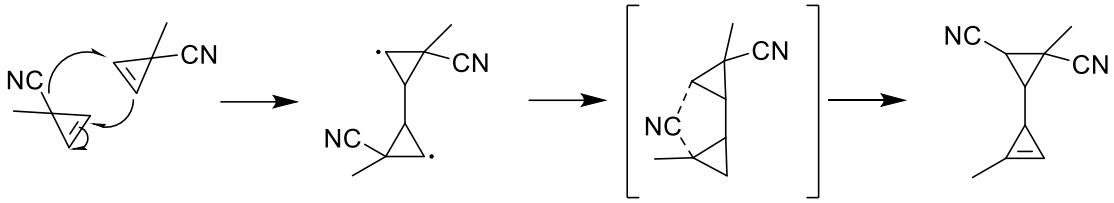
Como se ha comentado anteriormente, la inestabilidad de los ciclopropenos que tengan algún hidrógeno en C3 puede deberse a que experimenten reacciones énicas. En diferentes trabajos de los años 60 se observó que cuando el trifenilciclopropeno era calentado a reflujo de xileno se formaba un dímero. Tras usar un ciclopropeno con un átomo de deuterio en posición 3, dedujeron que se daba una reacción énica.[179] En un trabajo posterior, observaron la elevada reactividad del ciclopropeno, que empezaba a dimerizar a -25 ^{o}C y debía ser almacenado a -78 ^{o}C para evitarlo. Propusieron un mecanismo pericíclico para explicar la formación del dímero, que pudo ser detectado atrapándolo con antraceno (Esquema 36).[180]



Esquema 36. Dimerización del ciclopropeno y captura con antraceno.

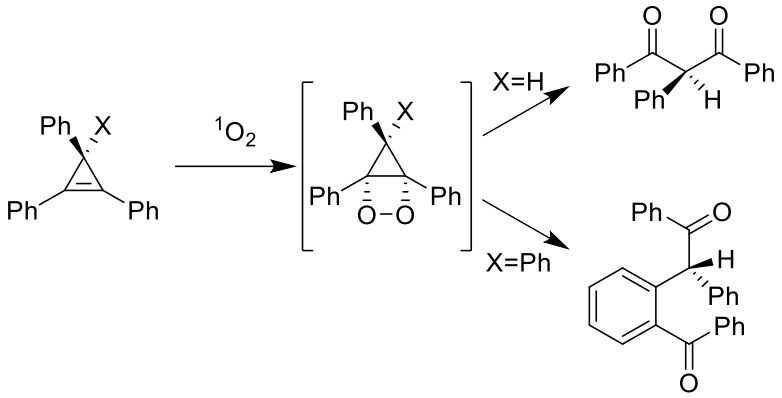
Aunque la reacción énica suele ser una reacción concertada, en el caso de los ciclopropenos, debido a su reactividad, no siempre es así. En el año 2006, Sakai estudió computacionalmente el mecanismo de esta reacción. Concluyó que, al reaccionar el ciclopropeno con el etileno, tanto en sus aproximaciones *endo* o *exo*, el mecanismo por etapas es más favorable que el mecanismo concertado.[181] Ashirov y colaboradores, observaron en 2008 la tetramerización del 3-metil-3-cianociclopropeno. En este caso, al no existir un hidrógeno en C3, se necesitaban temperaturas más elevadas. En dicho trabajo realizaron cálculos computacionales para explicar el mecanismo. En la formación del trímero, a parte del mecanismo concertado, observaron uno por etapas, donde se podía formar una especie diracálica (Esquema 37).[182] Este mecanismo se sustenta en una necesaria migración del grupo CN, que involucra radicales.[183] El mismo grupo, en un trabajo posterior, realizó una reacción tándem énica/Diels-Alder entre el mismo ciclopropeno y hexadieno.[184]

[178] Deng, Q.; Thomas, B. E.; Houk, K. N.; Dowd, P. *J. Am. Chem. Soc.* **1997**, *119*, 6902-6908.
[179] Breslow, R.; Dowd, P. *J. Am. Chem. Soc.* **1963**, *85*, 2729-2735.
[180] Dowd, P.; Gold, A. *Tetrahderon Lett.* **1969**, 85-86.
[181] Sakai, S. *J. Phys. Chem. A* **2006**, *110*, 12891-12899.
[182] Ashirov, R. V.; Shamov, G. A.; Lodochnikova, O. A.; Litvynov, I. A.; Appolonova, S. A.; Plemenkov, V. V. *J. Org. Chem.* **2008**, *73*, 5985-5988.
[183] Cekovic, Z. *J. Ser. Chem. Soc.* **2005**, *70*, 287-318.
[184] Lodochnikova, O. A.; Ashirov, R. V.; Appolonova, S. A.; Litvinov, I. A.; Plemenkov, V. V. *Russian J. Org. Chem.* **2010**, *46*, 49-53.



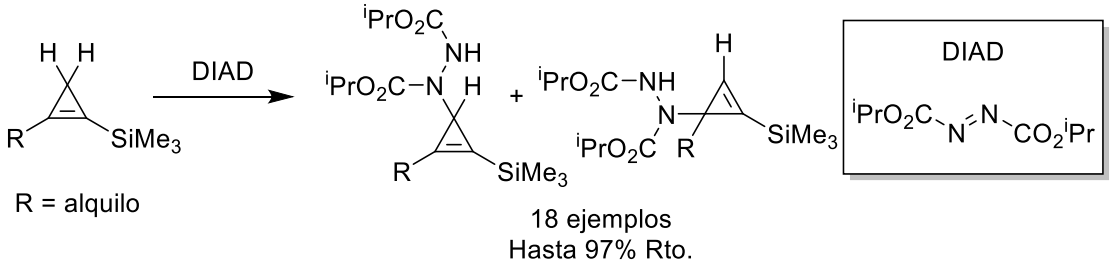
Esquema 37. Oligomerización de cianociclopropenos.

Lin y colaboradores observaron oligomerizaciones basadas en reacciones énicas.[185] En este trabajo, observaron diferentes productos cuando el ciclopropeno era expuesto al aire. Dedujeron que el oxígeno podía reaccionar con el ciclopropeno dando lugar a diferentes productos (Esquema 38).



Esquema 38. Inestabilidad del trifenilciclopropeno frente a oxígeno.

Sun y colaboradores publicaron una aminación de ciclopropenos a través de una reacción énica usando diazocompuestos.[186] Observaron que se obtenían dos productos, siendo mayoritario el correspondiente a una trasposición alílica tras la reacción énica (Esquema 39). Estudiaron computacionalmente dicha reacción, llegando a la conclusión de que la reacción énica no era concertada, sino por etapas. La trasposición correspondía a una migración [1,3] del diazocarboxilato.[187]

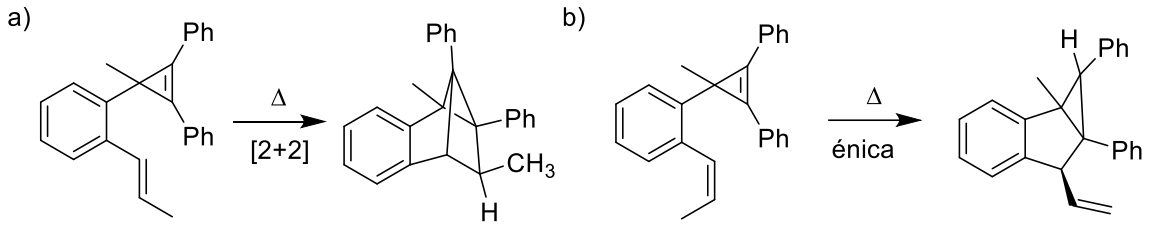


Esquema 39. Aminación de ciclopropenos a través de una reacción énica.

Un ejemplo de competición entre la reacción énica intramolecular y una cicloadición [2+2] fue estudiado por Padwa y colaboradores.[188] En este trabajo se dieron cuenta que la estereoquímica del doble enlace influía en que ocurriera una reacción u otra: el isómero *E*

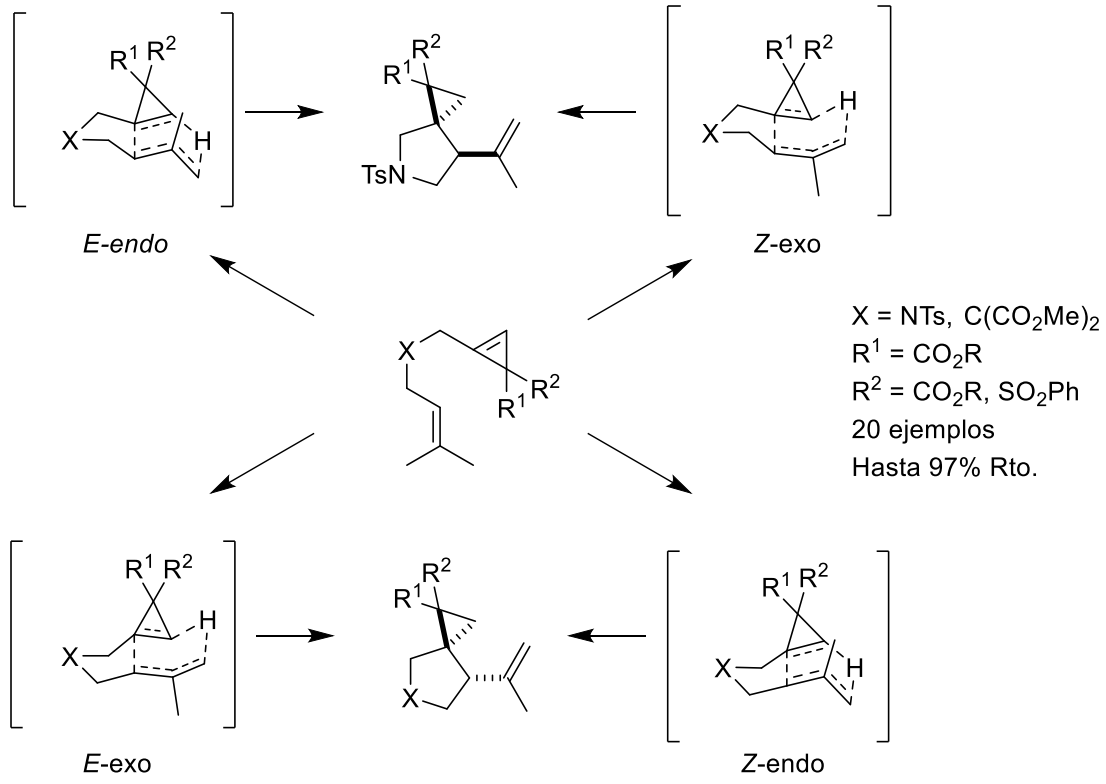
[185] Lin, H.-C.; Tsai, R.-T.; Wu, H.-P.; Lee, H.-Y.; Lee, G.-A. *Tetrahedron* **2016**, *72*, 184-191.
[186] Sun, C.; Li, J.; Lee, D.; Huang, G.; Xia, Y. *Chem. Comm.* **2012**, *48*, 10990-10992.
[187] Huang, G.; Xia, Y.; Sun, C.; Li, J.; Lee, D. *J. Org. Chem.* **2013**, *78*, 988-995.
[188] Padwa, A.; Rieker, W. F.; Rosenthal, R. J. *J. Am. Chem. Soc.* **1985**, *107*, 1710-1717.

reaccionaba a través de una cicloadición [2+2] (Esquema 40a), mientras que el *Z* sufría una reacción Alder-énica (Esquema 40b).



Esquema 40. a) Cicloadición intramolecular [2+2] de doble enlace *E*. b) Reacción énica del isómero *Z*.

En esta memoria hemos desarrollado nuevos ejemplos de la reacción Alder-énica en ciclopropenos. Durante el desarrollo de nuestro trabajo, Fan y colaboradores publicaron la síntesis de espirociclos mediante una reacción énica a partir de ciclopropen-enos.[189] Obtenían mayoritariamente sólo uno de los posibles isómeros. Mediante cálculos computacionales explicaron las energías de los estados de transición que llevan a los posibles isómeros (Esquema 41). En los casos en los que la cadena de doble enlace estaba disustituida terminalmente, llegaron a la conclusión de que la aproximación más favorable era con el sustituyente en *E* en la forma *exo*. El *E-endo* estaba próximo en energía y de ahí el observar un isómero minoritario. Las aproximaciones *endo* y *exo* del isómero *Z*, eran más desfavorables.



Esquema 41. Síntesis de espirociclos mediante reacción énica de ciclopropenos.

Los ciclopropenos han sido reconocidos como excelentes dienófilos en la reacción de Diels-Alder desde los años 60, gracias a los trabajos debidos al grupo de Wiberg.[190] Esta reacción ha

[189] Fan, P.; Liu, T.-T.; Qu, H.-Y.; Tao, P.; Liu, C.-X.; Liu, X.-Q.; Shen, M.-H.; Bao, X.; Xu, H.-D. *Org. Chem. Front.* **2021**, *8*, 4799-4804.
[190] Wiberg, K. B.; Bartley, W. J. *J. Am. Chem. Soc.* **1960**, *82*, 6375-6380.

sido estudiada computacionalmente por Apeloig y otros, para explicar la teoría del orbital secundario que tiene un papel importante para explicar a la estereoquímica del producto final.

En ciclopropenos sin sustitución, predomina la aproximación *endo* ya que se aumentan las interacciones orbitalarias.[191] Xidos y colaboradores, en 2001, estudiaron dichas aproximaciones con ciclopropenos sustituidos en C3. Llegaron a la conclusión que en estos casos la estereoquímica no sólo la determina el SOI sino que hay que tener en cuenta efectos estéricos que se pueden producir en las aproximaciones *endo* y *exo*. Estudiaron la posibilidad de orientaciones *sin* y *anti* en dichas aproximaciones, viendo que la *anti* siempre está más favorecida (Figura 20).[192]

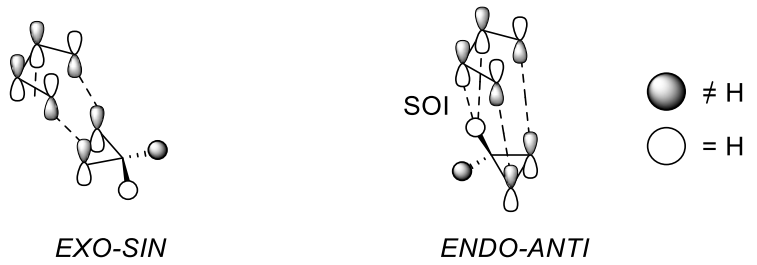
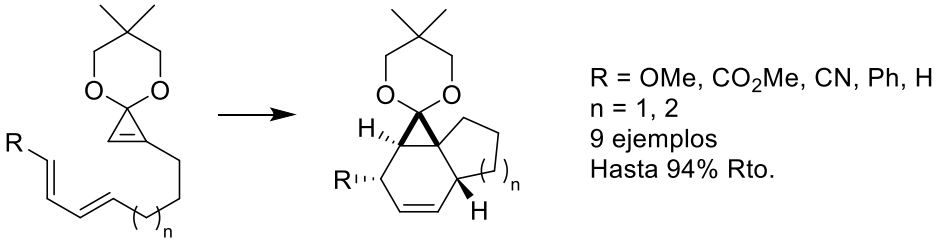


Figura 20. Aproximaciones *exo* y *endo* con diferentes orientaciones.

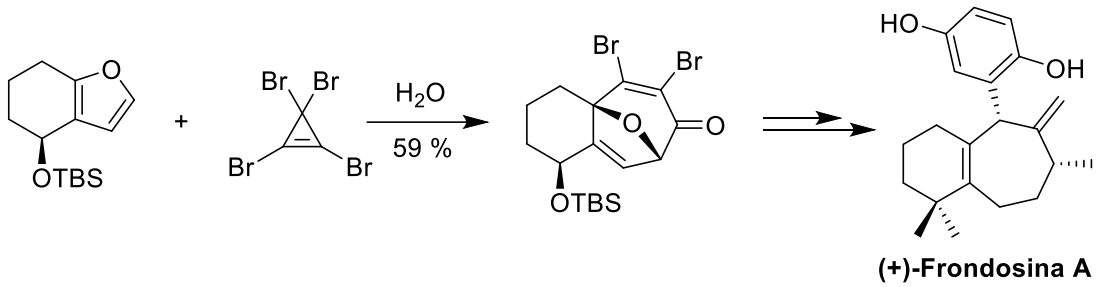
La primera reacción intramolecular de Diels-Alder con ciclopropenos fue publicada por Patel y Boger en 2010.[193] Aunque puedan tener lugar diferentes cicloadiciones [1+2], [3+2] y [3+4] sólo se obtienen los productos resultantes de una [4+2]. Tanto en la reacción normal, neutra e inversa, sólo se obtenía el isómero formado en la aproximación *exo* (Esquema 42).



Esquema 42. Reacción de Diels-Alder intramolecular descrita por Patel y Boger.

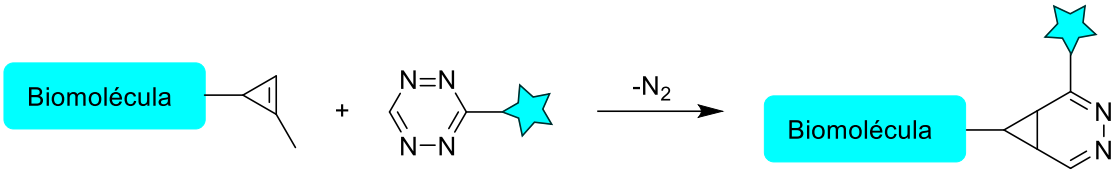
La reacción Diels-Alder ha sido aplicada a la síntesis de diferentes productos naturales. Shi y colaboradores publicaron la síntesis de alcaloides utilizados en la medicina tradicional china y japonesa. Una de las primeras etapas era una reacción de Diels-Alder entre un ciclopropeno y un ciclopentadieno, ambos inestables, en la que obtenían una mezcla de isómeros.[194] El año siguiente, Oblak y colaboradores publicaron la síntesis de terpenos de origen marino. El paso clave era una reacción de Diels-Alder entre un tetrabromociclopropeno y un furano, creando el núcleo central de una manera totalmente diastereoselectiva (Esquema 43).[195]

[191] Apeloig, Y.; Matzner, E. *J. Am. Chem. Soc.* **1995**, *117*, 5375-5376.
[192] Xidos, J. D.; Gosse, T. L.; Burke, E. D.; Poirier, R. A.; Burnell, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 5482-5488.
[193] Patel, P. R.; Boger, D. L. *Org. Lett.* **2010**, *12*, 3540-3543.
[194] Shi, Y.; Wilmot, J. T.; Nordstorm, L. U.; Tan, D. S.; Gin, D. Y. *J. Am. Chem. Soc.* **2013**, *135*, 14313-14320.
[195] Oblak, E. Z.; VanHeyst, M. D.; Li, J.; Wiemer, A. J.; Wright, D. L. *J. Am. Chem. Soc.* **2014**, *136*, 4309-4315.



Esquema 43. Síntesis de alcaloides a través de una reacción de Diels-Alder de ciclopropenos.

La reacción Diels-Alder ha tenido mucho uso en el campo de la química médica como reacción bioortogonal. En el 2012, Liang y colaboradores estudiaron esta reacción entre diferentes alquenos y tetrazinas o triazinas. Los cálculos computacionales previeron que la cicloadición entre ciclopropenos y tetrazinas sería muy favorable.[196] En 2013, Kamber y colaboradores demostraron la posibilidad de marcar biomoléculas con compuestos fluorescentes mediante esta técnica (Esquema 44).[197] El grupo de Devaraj desarrolló sondas fluorescentes, basadas en rodamina o BODIPY, para marcar ARN.[198]



Esquema 44. Reacción bioortogonal de marcaje de biomoléculas con sondas fluorescentes.

[196] Liang, Y.; Mackey, J. L.; Lopez, S. A.; Liu, F.; Houk, K. N. *J. Am. Chem. Soc.* **2012**, *134*, 17904-17907.
[197] Kamber, D. N.; Nazarova, L. A.; Liang, Y.; Lopez, S. A.; Patterson, D. M.; Shih, H.-W.; Houk, K. N.; Prescher, J. A. *J. Am. Chem. Soc.* **2013**, *135*, 13680-13683.
[198] Wu, H.; Devaraj, N. K. *Acc. Chem. Res.* **2018**, *51*, 1249-1259.

5. STRAIGHTFORWARD SYNTHESIS OF HIGHLY FUNCTIONALIZED INDANES AND TETRALINES THROUGH ENE-CYCLOPROPENE REARRANGEMENT MEDIATED BY RUTHENIUM.

This work involves the rearrangement of ene-cyclopropenes, catalyzed by ruthenium dimers, to form highly functionalized indanes and tetralines. The optimal conditions were in toluene at 180 ^{o}C, with only a 5 mol% of dichloro(hexamethylbenzene)ruthenium(II) dimer under microwave irradiation. Other dimers gave similar results. We propose a reaction pathway based on the different by products obtained.

In this paper I did all the experimental work. I collaborated in the manuscript elaboration with my supervisors, mainly in the experimental part and supplementary information.

En este trabajo se presenta el reordenamiento de ene-ciclopropenos, catalizado por complejos diméricos de rutenio, para formar indanos y tetralinas altamente funcionalizados. Las condiciones óptimas se dieron en tolueno a 180 ^{o}C, con solo un 5 mol% de dicloro(hexametilbenceno)rutenio (II) bajo radiación de microondas. Otros dímeros dieron resultados similares. Propusimos un mecanismo de reacción basado en los diferentes productos secundarios obtenidos.

En este trabajo realicé todo el trabajo experimental. He colaborado en la redacción del manuscrito junto a mis supervisores, principalmente en la parte experimental y la información suplementaria.

Puet, A.; Domínguez, G.; Pérez-Castells, J. Straightforward Synthesis of Highly Functionalized Indanes and Tetralines Through Ene-Cyclopropene Rearrangement Mediated by Ruthenium. *J. Org. Chem.* **2022**, *87*, 2686-2696. **Impact factor** (2022): 3.608. **Category**: Organic chemistry (Q1, 10/52). Source: JCR (Journal of Citations Reports).

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Straightforward Synthesis of Highly Functionalized Indanes and Tetralines through Ene-Cyclopropene Rearrangement Mediated by Ruthenium

Alejandro Puet, Gema Domínguez, and Javier Pérez-Castells*

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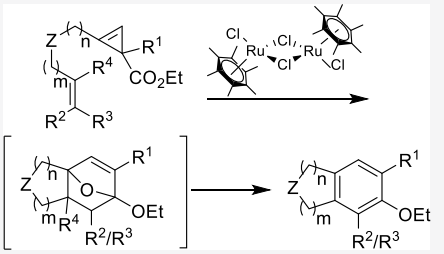
ABSTRACT: Ene-cyclopropenes give functionalized indanes and tetralines in the presence of ruthenium dimeric catalysts. This reaction involves the cyclopropene opening by the metal catalysts with a different regioselectivity respective to gold chlorides and produces totally different products than when using semisandwich ruthenium complexes. Here, the process leads to a bridged 7-oxanorbornene-type intermediate that is converted into a functionalized aromatic ring through deoxygenative aromatization. Alternative reaction pathways occur with substrates with no possible aromatization.



INTRODUCTION

Cyclopropenes are highly strained but stable compounds that are able to undergo different transformations,[1] which many times involve a C−C bond cleavage.[2] In particular, different types of cycloisomerizations have been described leading to furans or pyrroles.[3] Aryl- and vinylcyclopropenes have been transformed into indenes and naphthalenes by cycloisomerizations catalyzed by gold or Brönsted-acids.[4] The cyclopropene ring cleavage is not kinetically favored and frequently needs promotion by high temperatures/irradiation or by the use of a metal catalyst. In the first case, single C−C bonds are generally cleaved leading to radical species, whereas when using metal catalysts, intermediate vinylcarbenes are frequently formed. The increased π-density of the cyclopropene double bond facilitates the coordination with transition metals including those with high alkynophilicity. The regioselectivity of this ring opening depends on the substitution pattern and on the metal complex used. The generated metal vinylcarbenes can be described with their other resonance structure as allylic cations stabilized by the metal and may cyclopropanate double bonds inter- or intramolecularly. In a previous work, we described the divergent reaction pathway of ene-cyclopropene rearrangements catalyzed by ruthenium complexes compared with the same process under gold catalysis (Scheme 1).[5] Thus, substrates of type **1** gave bicyclo[*n*.1.0]alkanes **3** upon reaction with Cp*RuCl(cod) through the formation of the internal carbene **B**. This intermediate is formed due to the reverse regioselectivity of the cyclopropene opening with regard to that observed with gold or rhodium catalysts which produces external carbene **A** and, subsequently, products **2**.[6] In that previous work, we observed two examples, with gem-disubstituted cyclopropenes, in which compounds **4** were isolated. We believe this latter process proceeded through the attack of the ester oxygen to form **C** and then reaction with the double bond to give **4** as an only diastereomer. Herein, we show the study of the conditions which favor this novel transformation not only with disubstituted cyclopropenes but also with a wide variety of substrates. Under the developed conditions, highly functionalized aromatic products **5** were generally formed presumably upon evolution of the bicyclic intermediates **4**. When intermediate **4** cannot easily be aromatized ($R^5 \neq H$), other products such as **6** or **7** are formed. The mechanistic aspects are discussed.

RESULTS AND DISCUSSION

We prepared ene-cyclopropenes **1a−q** by the reaction of the already cyclopropene-containing substrate with different bromo alkenes. This procedure avoids the formation of the undesired cyclopropane which happens when the enyne is prepared first and then cyclopropenated. Thus, as described in our previous work, propargylmalonate was cyclopropenated[5] and then submitted to the reaction with bromo alkenes in good yields (Table 1). Compounds **1b**, **1e**, and **1h** were prepared from commercial crotyl bromide (*E*/*Z* 4:1) and **1c** from ethyl-4-bromocrotonate (*E*/*Z* 3:1), thus obtaining the products as *E*/*Z* mixtures in identical ratio as the starting material. These mixtures were used for the subsequent cycloisomerization as both isomers produce the same final product.

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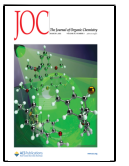

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Scheme 1. Summary of Previous Works and the Results of This Work

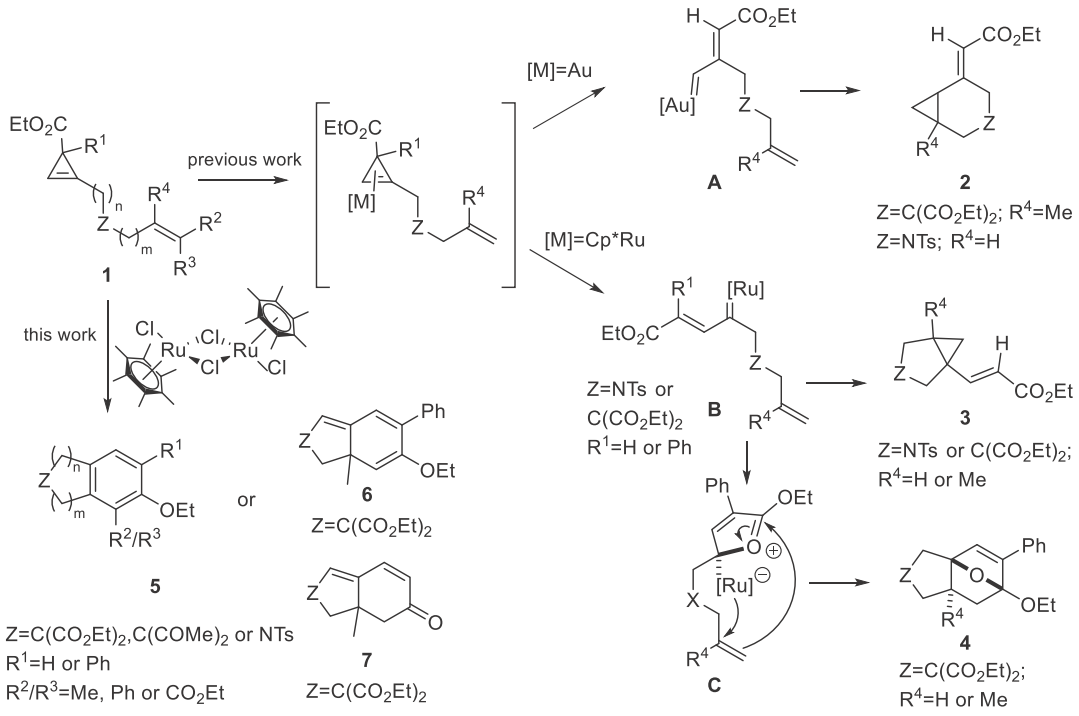
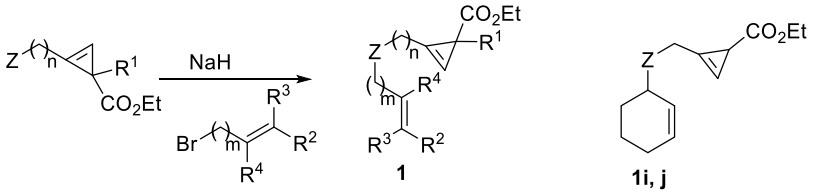


Table 1. Synthesis of Ene-Cyclopropenes 1



no.	Z	R^1	R^2	R^3	R^4	*n*	*m*	yield (%)[a]
1a	$C(CO_2Et)_2$	H	H	H	H	1	1	70
1b[b]	$C(CO_2Et)_2$	H	Me	H	H	1	1	64
1c[c]	$C(CO_2Et)_2$	H	CO_2Et	H	H	1	1	42
1d	$C(CO_2Et)_2$	Ph	H	H	H	1	1	73
1e[b]	$C(CO_2Et)_2$	Ph	Me	H	H	1	1	66
1f	$C(CO_2Et)_2$	Ph	Ph	H	H	1	1	61
1g	$C(COMe)_2$	H	H	H	H	1	1	75
1h[b]	$C(COMe)_2$	H	Me	H	H	1	1	69
1i	$C(CO_2Et)_2$							66
1j	$C(COMe)_2$							64
1k	$C(CO_2Et)_2$	H	Ph	H	H	2	1	63
1l	$C(CO_2Et)_2$	H	H	H	H	1	2	60
1m	$C(CO_2Et)_2$	Ph	H	H	H	1	2	63
1n	$C(CO_2Et)_2$	H	H	H	H	1	3	59
1o	$C(CO_2Et)_2$	H	H	H	Me	1	1	69
1p	$C(CO_2Et)_2$	Ph	H	H	Me	1	1	67
1q	NTs	H	H	H	H	1	1	68

[a]In pure product. [b]*E*/*Z* mixture (4:1). [c]*E*/*Z* mixture (3:1).

In this work, we studied the behavior of these ene-cyclopropenes when reacting with dimeric ruthenium(II) catalysts ($Ru_2Ar_2Cl_4$). The search of the best conditions is summarized in Table 2 using substrate **1a** as the model. We began by using toluene as the solvent at 160 °C under microwave (MW) irradiation using 10 mol % of the catalyst (**L1**) and 45 min of reaction. We found that the crude reaction showed total conversion of the starting material into a 3:2 mixture of compounds **5a** and **3a**. We tried different solvents (entries 2−7), observing that the formation of **5a** was favored with nonpolar solvents, toluene being the best solvent. Using a higher dilution did not improve the yield as the conversion was not complete after 45 min and gave the same ratio of final products (entry 8). On the other hand, increasing the temperature to 180 °C allowed an improvement in the amount of **5a**, lowering the reaction time needed for

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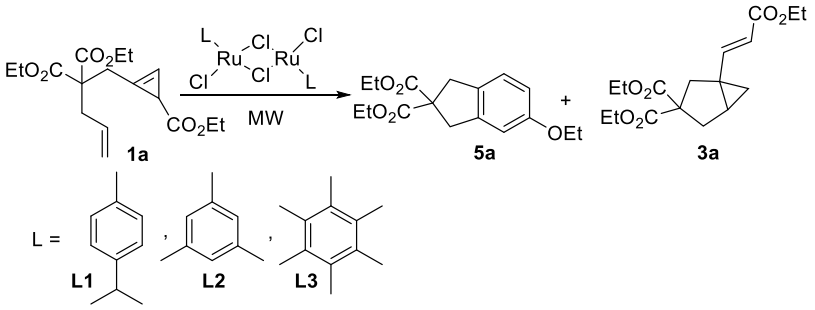
Table 2. Optimization of Conditions for the Obtention of 5a



no.	solvent	T (°C)	conc. (M)	time (min)	cat. (L, %)	conv[a] (%)	ratio[a] **5a**:**3a**
1	toluene	160	0.2	45	**L1**, 10%	100	3:2
2	NMP	160	0.2	45	**L1**, 10%	100	1:3
3	DCE	160	0.2	45	**L1**, 10%	100	1:1
4	dioxane	160	0.2	45	**L1**, 10%	100	1:1
5	acetone	160	0.2	45	**L1**, 10%	100	1:4
6	hexane	160	0.2	45	**L1**, 10%	60	3:2
7	xylene	160	0.2	45	**L1**, 10%	100	1:1
8	toluene	160	0.1	45	**L1**, 10%	80	3:2
9	toluene	160	0.2	30	**L1**, 10%	100	3:2
10	toluene	180	0.2	30	**L1**, 10%	100	3:1
11	toluene	180	0.2	15	**L1**, 10%	85	3:1
12	toluene	180	0.2	30	**L1**, 5%	100	3:1
13	toluene	180	0.2	30	**L2**, 5%	100	2:1
14	toluene	180	0.2	30	**L3**, 5%	100	4:1
15	toluene	180	0.2	45	**L3**, 2.5%	75	4:1
16	toluene	180	0.2	45	Cp*RuCl(cod)	100	0:1
17	toluene	160	0.2	120		0	

[a]Conversion and ratio measured by analysis of well-resolved signals of the starting material and the products in the ^{1}H NMR spectra of the crude.

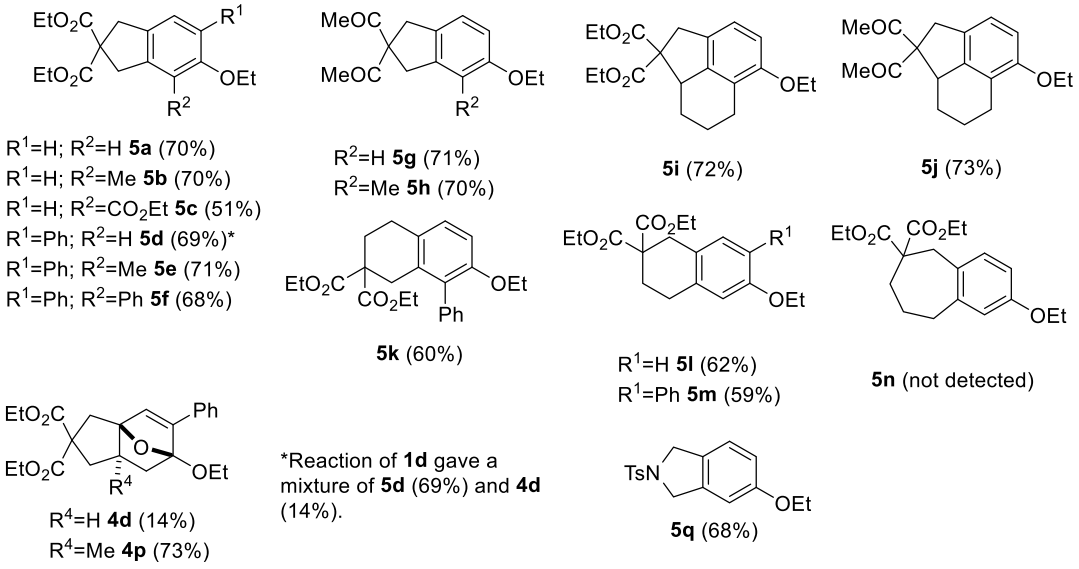


Figure 1. Synthetized products **4** and **5** with isolated yields.

completion to 30 min (entries 9−10). Moreover, decreasing the catalyst load to 5 mol % did not have any deleterious effect (entry 12). Finally, a further improvement in the ratio in favor of **5a** was achieved by changing the catalyst to hexamethylbenzene ruthenium(II) dimer (**L3**, entry 14). An attempt to lower the catalyst loading to 2.5 mol % (entry 15) revealed lack of total conversion, so we kept 5 mol % in the dimer for further reactions (10 mol % of Ru metal). With these optimized conditions, the **5a**:**3a** ratio was 4:1 in the crude mixture, and **5a** was isolated in 70% yield (plus 23% of **3a**). Toluene has a low dielectric constant and is generally not a good choice to reach high temperatures in the MW, which in any case is only achieved after long times. However, when the ruthenium complex is added, it helps in absorbing the MWs,[7] making it possible to reach 180 °C in no more than 6 min, and the pressure did not surpass 2 bars. Once optimized conditions

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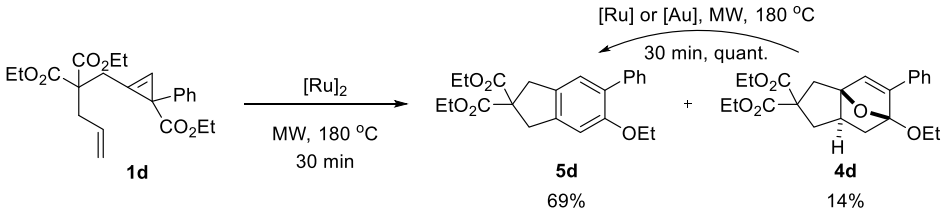
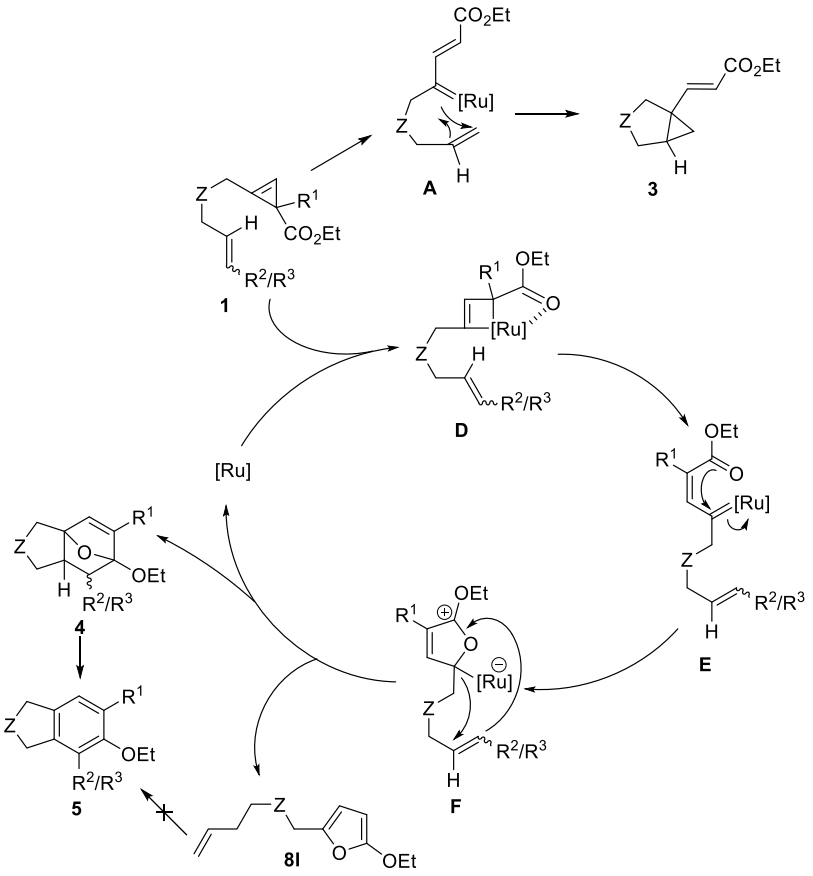
Scheme 2. Conversion of 4d into the Final Product 5d



Scheme 3. Proposed Reaction Pathway for the Transformation of Ene-Cyclopropenes 1



were found, we checked the reaction using Cp*RuCl(cod) as the catalyst (entry 16). Although total conversion was observed, the only identifiable product in the crude mixture was **3a**, not detecting the presence of **5a**. In addition, we checked the possibility of a thermal reaction (entry 17). With toluene alone, the maximum temperature reached was 160 °C, and after 120 min, no transformation was observed nor decomposition of the starting material.

The optimized conditions (entry 14) were used with the rest of the substrates leading to the products depicted in Figure 1. The procedure allowed the formation of 2,3-dehydro-1*H*-indenes (**5a**–**h**), 1,2,2a,3,4,5-hexahydroacenaphthylenes (**5i**–**j**), 1,2,3,4-tetrahydronaphtalenes (**5k**–**m**), and isoindoline **5q**, with different substitutions in different positions in yields ranging from 51 to 73%. In the reaction of **1l**, the desired product **5l** (62% yield) and, additionally, 18% of furan **8l** were isolated (see Scheme 3). Compound **1n** did not react, and the desired product **5n** bearing a seven-membered ring was not detected. Noteworthy, in none of these reactions could we detect the formation of products of type **3**. When using substrate **1p** (R^4 = Me), product **4p** was isolated in 73% yield, which is, as mentioned above, a presumable intermediate in the formation of products **5**.

Compound **1d** gave a separable mixture of compounds **5d** and **4d**.[5] In order to check if **4d** is an intermediate in the formation of **5d**, this compound was isolated and heated in the presence of the ruthenium dimer catalyst, observing its total conversion into **5d** (Scheme 2). This latter transformation also happened when using other ruthenium catalysts or with gold(I) chloride but not by simple heating of **4d** in the absence of a catalyst. This transformation known as deoxygenative aromatization is supported in the literature by the combination of the oxorhenium catalyst with triphenyl phosphite to give aromatic rings,[8] as well as with the use of different metals[9] or other noncatalytic conditions.[10] This transformation is favored if the epoxy bridge connects tertiary carbons and there is an electrodonor group.[11]

Scheme 3 shows a plausible reaction course that explains the formation of products **5**. As we had observed in our previous work, ruthenium catalysts tend to open the cyclopropene ring, producing a carbene intermediate in the most substituted side, where the emerging double bond can appear with *E* or *Z*

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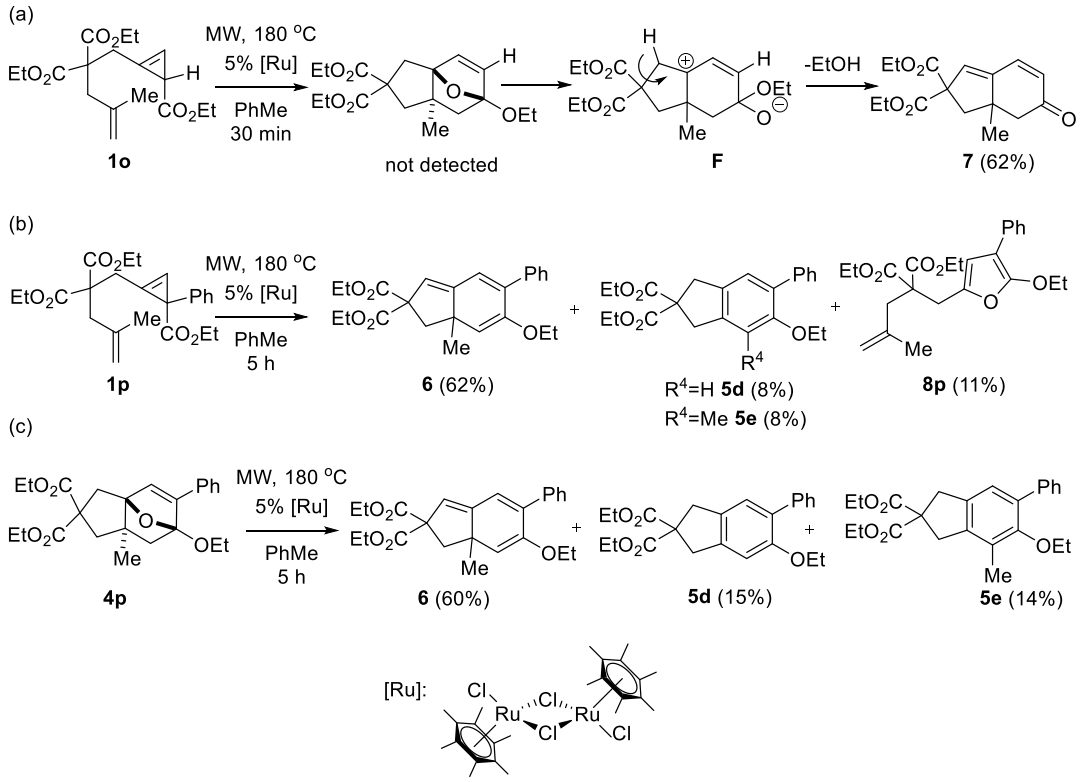
Scheme 4. Different Products Observed with Substrates 1o−p and from 4p



configuration (**A** or **E**). Intermediate **A** can react with the double bond in the chain producing the fused cyclopropene **3**. This process is the only one observed when using Cp*RuCl(cod) as the catalyst. However, when using dimeric ruthenium complexes $(RuArCl_2)_2$, it was only observed, as a minor way, with substrate **1a**. As this substrate lacks substituents at the olefinic bond, it may be favored for the formation of **3**, which is not observed with more crowded substrates or if the tether is longer. We understand that these ruthenium complexes may be cleaved at the reaction temperature into two monomers that will have the possibility of coordination with the oxygen in the carbonyl group of the ester as in **D**, which evolves into the intermediate carbene **E** with the *Z* configuration. This coordination would be the key to favor **E** over **A** and polar solvents may find it difficult, thus directing the process onto **A** and ultimately products **3** (see Table 2). The nucleophilic attack of the ester oxygen renders **F** which can give an intramolecular 1,3-dipolar cycloaddition[12] onto products **4** which, in most cases, suffer an aromatization process in the reaction medium to produce **5**. Alternatively, from **F**, a dihydrofuran can be formed as it was observed in the reaction of **1l**, where **8l** was isolated as a subproduct (18%).[13] In principle, **8l** could follow an intramolecular Diels−Alder reaction to give **5l**. To check this possibility, we submitted **8l** to the reaction conditions used with **1l** but only observed an extensive decomposition, not detecting **5l** at all.

The reaction of substrate **1o** gave, interestingly, a compound identified as **7** (62%) as the only reaction product (Scheme 4a). The formation of **7** may go through the carbocationic intermediate **F** which is transformed into **7** by ethanol elimination. When submitting substrate **1p** to the same forced conditions, we could isolate **6** (62%), **5d** (8%) and **5e** (8%). Additionally, furan **8p** was detected in the crude mixture. The formation of **6** and **7** is explained because of the presence of the methyl group that precludes the aromatization step, but the formation of **5d** and **5e** shows the possibility of a rearrangement where the methyl group can migrate to allow the formation of the more stable aromatic ring (Scheme 4b). In order to check that the formation of these products occurred during the deoxygenative aromatization step, we submitted intermediate **4p** to forced conditions (180 °C, 5 h in the presence of 5% of the catalyst). The main product of this reaction was **6** (isolated in 60% yield), and small amounts of **5d** and **5e** were isolated (Scheme 4c).

CONCLUSIONS

In summary, we show a novel reactivity of ene-cyclopropenes under dimeric ruthenium catalysis. The transformation of compounds **1** into **5** is an interesting process allowing the synthesis of five- and six-membered rings fused to functionalized aromatic rings. Thus, synthesis of 2,3-dehydro-1*H*-indenes, 1,2,2a,3,4,5-hexahydroacenaphthylenes, 1,2,3,4-tetrahydronaphtalenes, and an isoindoline is presented. The formation of compounds **6** and **7**, which arise from intermediates that cannot aromatize, gives mechanistic insights into the aromatization process.

EXPERIMENTAL SECTION

Materials and Methods. All chemicals were purchased from Merck, VWR, Fluorochem, and ABCR and were used without further purification. All MW reactions were carried out in an Initiator + MW reactor with an infrared probe for controlling the temperature and a pressure sensor. The reaction progress was monitored by ^{1}H NMR spectra or by using analytical thin-layer chromatography on silica gel 60 F-254 plates. Visualization was achieved by UV light (254 nm) or phosphomolybdic acid and heating. NMR spectra were recorded on a Bruker spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in δ ppm referenced to $CDCl_3$ (δ = 7.26

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for ^{1}H and 77.00 for ^{13}C). Bidimensional spectra heteronuclear multiple quantum coherence, heteronuclear multiple bond coherence, and correlation spectroscopy were recorded in order to carry out the assignment. Purification was done by flash chromatography on silica gel NORMASIL 60 40−63 μm.

The following chemicals were prepared according to the literature procedure:[5] diethyl 2-allyl-2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate, **1a**, diethyl 2-allyl-2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)malonate, **1d**, diethyl 2-(but-3-en-1-yl)-2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate, **1l**, diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)-2-(pent-4-en-1-yl)malonate, **1n**, diethyl 2-((3-(ethoxycarbonyl)-cycloprop-1-en-1-yl)methyl)-2-(2-methylallyl)malonate, **1o**, diethyl 2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)-2-(2-methylallyl)malonate, **1p**, ethyl 2-(((*N*-allyl-4-methylphenyl)-sulfonamido)methyl)cycloprop-2-ene-1-carboxylate, **1q**, diethyl 6-ethoxy-5-phenyl-1,6,7,7a-tetrahydro-3a,6-epoxyindene-2,2(3*H*)-dicarboxylate, **4d**, and diethyl 6-ethoxy-7a-methyl-5-phenyl-1,6,7,7a-tetrahydro-3a,6-epoxyindene-2,2(3*H*)-dicarboxylate, **4p**.

General Procedure A for the Synthesis of Ene-Cyclopropenes 1. To a 0.1 M solution in dry tetrahydrofuran (THF) of the cyclopropene (1 equiv) at 0 °C is added slowly NaH 60% w/w (1.1 equiv). After 10 min, bromo alkene (1.2 equiv) is added and stirred overnight at room temperature. A saturated solution of NH_4Cl is added. The aqueous phase is extracted with AcOEt (3×). The combined organic layers are washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The product is purified by silica gel column chromatography in a mixture of hexane and ethyl acetate.

General Procedure B for the Synthesis of Ene-Cyclopropenes 1. To a 0.05 M solution of cyclopropene (1 equiv) in acetone is added K_2CO_3 (1.2 equiv) at room temperature. After 10 min, bromo alkene (1.3 equiv) is added and stirred for 48 h at room temperature. The solution is filtered, and the solvent is evaporated *in vacuo*. The crude is purified by silica gel column chromatography in a mixture of hexane and ethyl acetate.

General Procedure for Cycloisomerization Reaction. To a dried MW reactor is added under argon 5 mol % dichloro-(hexamethylbenzene) ruthenium(II) dimer (purchased from Merck) and a 0.2 M solution in toluene of ene-cyclopropene. The reaction is heated under MW irradiation at 180 °C for 30 min in a sealed vessel. The crude is filtered through a pad of celite, and the solvent is evaporated *in vacuo*. The product is purified by silica gel column chromatography in a mixture of hexane and ethyl acetate.

Diethyl 2-(But-2-en-1-yl)-2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate, 1b. **1b** was prepared from diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (50 mg, 0.18 mmol) in 1.8 mL of THF, NaH 60% w/w (8 mg, 0.19 mmol), and crotyl bromide (22 μL, 0.21 mmol, *E*/*Z* 4:1) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 39 mg of a yellow oil (64%)as a mixture of isomers in a 4:1 ratio (*E*/*Z*). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.47 (q, *J* = 1.4 Hz, 1H, C═CH, *Z*), 6.46 (q, *J* = 1.5 Hz, 1H, C═CH, *E*), 5.66−5.57 (m, 1H, ═C**H**CH$_3$, *Z*), 5.56−5.47 (m, 1H, ═C**H**CH$_3$, *E*), 5.27−5.18 (m, 2H, CH$_2$═C**H**, *E* + *Z*), 4.22−4.15 (m, 8H, 2× OC**H**$_2$CH$_3$, *E* + *Z*), 4.14−4.08 (m, 4H, OC**H**$_2$CH$_3$, *E* + *Z*), 3.14−3.04 (m, 4H, CCH$_2$C, *E* + *Z*), 2.82−2.59 (m, 4H, C**H**$_2$CH═CH, *E* + *Z*), 2.10 (d, *J* = 1.5 Hz, 2H, CHCO$_2$Et, *E* + *Z*), 1.65−1.61 (m, 3H, CH$_3$, *E*), 1.60−1.58 (m, 3H, CH$_3$, *Z*), 1.26−1.21 (m, 18H, 3× CH$_2$C**H**$_3$, *E* + *Z*). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 175.80 (C, *E*), 175.76 (C, *Z*), 170.41 (C, *Z*), 170.35 (C, *E*), 170.31 (C, *Z*), 170.25 (C, *E*), 130.7 (CH, *E*), 128.8 (CH, *Z*), 124.2 (CH, *E*), 123.3 (CH, *Z*), 111.74 (C, *Z*), 111.67 (C, *E*), 97.7 (CH, *Z*), 97.6 (CH, *E*), 61.8 (CH$_2$, *Z*), 61.74 (CH$_2$, *Z*), 61.69 (CH$_2$, *E*), 61.67 (CH$_2$, *E*), 60.4 (CH$_2$, *E* + *Z*), 56.6 (C, *E*), 56.4 (C, *Z*), 35.8 (CH$_2$, *E*), 30.0 (CH$_2$, *Z*), 28.3 (CH$_2$, *Z*), 28.2 (CH$_2$, *E*), 19.9 (CH$_3$, *Z*), 19.8 (CH$_3$, *E*), 18.2 (CH, *E* + *Z*), 14.5 (CH$_3$, *E*), 14.20 (2× CH$_3$, *E*), 14.16 (2× CH$_3$, *Z*), 14.1 (CH$_3$, *Z*). IR (NaCl): 3098, 2984, 1660, 1652 cm^{-1}. Anal. Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74. Found: C, 64.02; H, 7.61.

Triethyl 5-(3-(Ethoxycarbonyl)cycloprop-1-en-1-yl)pent-1-ene-1,4,4-tricarboxylate, 1c. **1c** was prepared from diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (50 mg, 0.18 mmol) in 1.8 mL of THF, NaH 60% w/w (8 mg, 0.19 mmol), and ethyl-4-bromocrotonate (29 μL, 0.21 mmol, *E*/*Z* 3:1) following general procedure **A**. It was purified in hexane/AcOEt (4:1) to give 29 mg of a yellow oil (42%) as a mixture of isomers in a 3:1 ratio (*E*/*Z*). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77−6.66 (m, 2H,C**H**═CHCO$_2$Et, *E* + *Z*), 6.50 (q, *J* = 1.4 Hz, 2H, C═C**H**, *E* + *Z*), 5.85 (dt, *J* = 15.5, 1.4 Hz, 1H, CH═C**H**CO$_2$Et, *E*), 5.79 (dt, *J* = 15.5, 1.5 Hz, 1H, CH═C**H**CO$_2$Et, *Z*), 4.22−4.07 (m, 16H, 4× C**H**$_2$CH$_3$, *E* + *Z*), 3.10 (br s, 4H, CC**H**$_2$C, E + Z), 2.91−2.76 (m, 4H, CC**H**$_2$CH, *E* + *Z*), 2.10 (d, *J* = 1.5 Hz, 2H, CHCO$_2$Et, *E* + *Z*), 1.25−1.21 (m, 24H, 4× CH$_2$C**H**$_3$, *E* + *Z*). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 175.5 (C, *E*), 170.3 (C, *Z*), 169.6 (C, *E* + *Z*), 169.5 (C, *E* + *Z*), 167.4 (C, *Z*), 165.8 (C, *E*), 142.4 (CH, *Z*), 141.9 (CH, *E*), 125.8 (CH, *E*), 125.0 (CH, *Z*), 111.1 (C, *E* + *Z*), 98.3 (CH, *E* + *Z*), 62.10 (CH$_2$, *E*), 62.07 (CH$_2$, *E*), 61.8 (CH$_2$, *Z*), 61.0 (CH$_2$, *Z*), 60.48 (CH$_2$, *E* + *Z*), 60.47 (CH$_2$, *E* + *Z*), 57.7 (C, *Z*), 56.0 (C, *E*), 36.3 (CH$_2$, *Z*), 35.3 (CH$_2$, *E*), 28.7 (CH$_2$, *E* + *Z*), 19.8 (CH, *E* + *Z*), 14.4 (CH$_3$, *Z*), 14.30 (CH$_3$, *E* + *Z*), 14.25 (CH$_3$, *E*), 14.10 (CH$_3$, *Z*), 14.07 (CH$_3$, *E* + *Z*), 14.05 (CH$_3$, *E*). IR (NaCl): 3091, 2992, 1664, 1656 cm^{-1}. Anal. Calcd for $C_{20}H_{28}O_8$: C, 60.59; H, 7.12. Found: C, 60.34; H, 6.99.

Diethyl-2-(But-2-en-1-yl)-2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)malonate, 1e. **1e** was prepared from diethyl 2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)-malonate (50 mg, 0.14 mmol) in 1.4 mL of THF, NaH 60% w/w (6 mg, 0.15 mmol), and crotyl bromide (17 μL, 0.17 mmol, *E*/*Z* 4:1) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 38 mg of a yellow oil (66%) as a mixture of isomers in a 4:1 ratio (*E*/*Z*). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.18 (m, 10H, Ph, *E* + *Z*), 6.80 (t, *J* = 1.7 Hz, 1H, C═CH, *Z*), 6.78 (t, *J* = 1.6 Hz, 1H, C═CH, *E*), 5.65−5.56 (m, 1H, CH═C**H**, *Z*), 5.45−5.35 (m, 1H, CH═C**H**, *E*), 5.25−5.15 (m, 2H, C**H**═CH, *E* + *Z*), 4.24−4.03 (m, 12H, 3× C**H**$_2$CH$_3$, *E* + *Z*), 3.22 (dd, *J* = 17.8, 1.8 Hz, 1H, CCH$_2$, *Z*), 3.20 (dd, *J* = 17.8, 1.9 Hz, 1H, CCH$_2$, *E*), 3.12 (dd, *J* = 17.8, 1.3 Hz, 1H, CCH$_2$, *Z*), 3.10 (dd, *J* = 17.9, 1.5 Hz, 1H, CCH$_2$, *E*), 2.85−2.83 (m, 2H, CC**H**$_2$CH, *Z*), 2.77−2.66 (m, 2H, CC**H**$_2$CH, *E*), 1.58 (dd, *J* = 6.4, 1.5 Hz, 3H, CH$_3$, *E*), 1.53 (dd, *J* = 6.9, 1.8 Hz, 3H, CH$_3$, *Z*), 1.28−1.22 (m, 12H, 2× CH$_2$C**H**$_3$, *E* + *Z*), 1.15 (t, *J* = 7.1 Hz, 3H, CH$_2$C**H**$_3$, *E*), 1.14 (t, *J* = 7.1 Hz, 3H, CH$_2$C**H**$_3$, *Z*). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 174.8 (C, *E*), 174.7 (C, *Z*), 170.3 (2× C, *Z*), 170.22 (C, *E*), 170.21 (C, *E*), 141.3 (C, *E*), 141.2 (C, *Z*), 130.8 (CH, *E*), 128.8 (CH, *Z*), 128.3 (2× CH, *E* + *Z*), 128.0 (2× CH, *E* + *Z*), 126.43 (CH, *Z*), 126.40 (CH, *E*), 124.0 (CH, *E*), 123.2 (CH, *Z*), 117.1 (C, *Z*), 117.0 (C, *E*), 100.2 (CH, *Z*), 100.0 (CH, *E*), 61.8 (CH$_2$, *Z*), 61.7 (CH$_2$, *E* + *Z*), 61.6 (CH$_2$, *E*), 60.8 (CH$_2$, *E* + *Z*), 56.52 (C, *E*), 56.48 (C, *Z*), 35.5 (CH$_2$, *E*), 32.6 (C, *Z*), 32.5 (C, *E*), 29.7 (CH$_2$, *Z*), 27.7 (CH$_2$, *E*), 27.6 (CH$_2$, *Z*), 18.1 (CH$_3$, *E*), 14.4 (CH$_3$, *E* + *Z*), 14.2 (CH$_3$, *E*), 14.11 (CH$_3$, *Z*), 14.07 (CH$_3$, *E*), 14.0 (CH$_3$, *Z*), 13.0 (CH$_3$, *Z*). IR (NaCl): 3080, 2995, 1667, 1658 cm^{-1}. Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.27; H, 7.14.

Diethyl (*E*)-2-Cinnamyl-2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)malonate, 1f. **1f** was prepared from diethyl 2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)-malonate (50 mg, 0.14 mmol) in 1.4 mL of THF, NaH 60% w/w (6 mg, 0.15 mmol), and cinnamyl bromide (33 mg, 0.17 mmol) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 40 mg of a yellow oil (61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29−7.18 (m, 10H, Ph), 6.83 (t, *J* = 1.4 Hz, 1H, C═CH), 6.30 (d, *J* = 15.7 Hz, 1H, CH═C**H**Ph), 5.95 (dt, *J* = 15.5, 7.6 Hz, 1H, C**H**═CHPh), 4.25−4.07 (m, 6H, 3× C**H**$_2$CH$_3$), 3.28−3.13 (m, 2H, CH$_2$C), 2.98−2.87 (m, 2H, C**H**$_2$CH), 1.24 (t, *J* = 7.1 Hz, 3H, CH$_2$C**H**$_3$), 1.21 (t, *J* = 7.1 Hz, 3H, CH$_2$C**H**$_3$), 1.16 (t, *J* = 7.1 Hz, 3H, CH$_2$C**H**$_3$). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 174.7 (C), 170.06 (C), 170.05 (C), 141.2 (C), 137.0 (C), 134.8 (CH), 128.6 (2× CH), 128.3 (2× CH), 128.1 (2× CH), 127.6 (CH), 126.5 (CH), 126.4 (2× CH), 123.3 (CH), 117.0 (C), 100.4 (CH), 61.9 (CH$_2$), 61.8 (CH$_2$), 60.9 (CH$_2$), 56.7 (C), 36.0 (CH$_2$), 32.7 (C), 27.9 (CH$_2$), 14.4 (CH$_3$), 14.2 (CH$_3$), 14.1 (CH$_3$). IR (NaCl): 3081, 2987, 1658, 1651

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cm^{-1}. Anal. Calcd. for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.12; H, 6.59.

Ethyl 2-(2,2-Diacetylpent-4-en-1-yl)cycloprop-2-ene-1-carboxylate, 1g. 1g was prepared from ethyl 2-(2-acetyl-3-oxobutyl)-cycloprop-2-ene-1-carboxylate (50 mg, 0.22 mmol) in 4.4 mL of acetone, K_2CO_3 (37 mg, 0.27 mmol), and allyl bromide (25 μL, 0.29 mmol) following general procedure **B**. It was purified in hexane/AcOEt (9:1) to give 44 mg of a yellow oil (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (q, J = 1.5 Hz, 1H, C=CH), 5.52−5.42 (m, 1H, C**H**=CH_2), 5.13−5.08 (m, 2H, CH=C**H$_2$**), 4.10 (q, J = 7.1 Hz, 2H, C**H$_2$**CH_3), 3.18−3.03 (m, 2H, CCH_2C), 2.82−2.72 (m, 2H, C**H$_2$**CH), 2.14 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.09 (d, J = 1.5 Hz, 1H,$CHCO_2Et$), 1.23 (t, J = 7.1 Hz, 3H, CH_2C**H$_3$**). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 204.64 (C), 204.61 (C), 175.7 (C), 131.6 (CH), 120.1 (CH_2), 111.4 (C), 98.1 (CH), 70.0 (C), 60.6 (CH_2), 35.2 (CH_2), 26.8 (CH_3) 26.7 (CH_3), 26.5 (CH_2), 19.6 (CH), 14.5 (CH_3). IR (NaCl): 3071, 2994, 1712, 1660 cm^{-1}. Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.39; H, 7.79.

Ethyl-2-(2,2-Diacetylhex-4-en-1-yl)cycloprop-2-ene-1-carboxylate, 1h. 1h was prepared from ethyl 2-(2-acetyl-3-oxobutyl)-cycloprop-2-ene-1-carboxylate (50 mg, 0.22 mmol) in 4.4 mL of acetone, K_2CO_3 (37 mg, 0.27 mmol), and crotyl bromide (30 μL, 0.29 mmol, E/Z 4:1) following general procedure **B**. It was purified in hexane/AcOEt (9:1) to give 43 mg of a yellow oil (69%) as a mixture of isomers in a 4:1 ratio (E/Z). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.39 (q, J = 1.6 Hz, 2H, C=CH, E + Z), 5.62−5.53 (m, 1H, CH=C**H**, Z), 5.52−5.43 (m, 1H, CH=C**H**, E), 5.04 (m, 2H, C**H**=CH, E + Z), 4.07 (q, J = 7.1 Hz, 4H, C**H$_2$**CH_3, E + Z), 3.14−2.98 (m, 4H, CCH_2C, E + Z), 2.77−2.73 (m, 2H, CC**H$_2$**CH Z), 2.68−2.64 (m, 2H, CC**H$_2$**CH, E), 2.10 (s, 3H, $COCH_3$ Z), 2.09 (s, 3H, $COCH_3$ E), 2.07 (s, 3H, $COCH_3$ Z), 2.06 (s, 3H, $COCH_3$ E), 2.05 (d, J = 1.5 Hz, 2H, $CHCO_2Et$, E + Z) 1.58 (dd, J = 6.6, 1.5 Hz, 3H, CHC**H$_3$**, E), 1.55 (dd, J = 7.0, 1.6 Hz, 3H, CHC**H$_3$**, Z), 1.20 (t, J = 7.2 Hz, 6H, CH_2C**H$_3$**, E + Z). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 204.82 (C, Z), 204.81 (C, Z), 204.76 (C, E), 204.7 (C, E), 175.6 (C, E), 175.5 (C, Z), 130.6 (CH, E), 128.9 (CH, Z), 123.7 (CH, E), 122.8 (CH, Z), 111.5 (C, Z), 111.4 (C, E), 97.8 (CH, Z), 97.7 (CH, E), 70.0 (C, E), 69.9 (C, Z), 60.4 (CH_2, E + Z), 33.9 (CH_2, E), 28.2 (CH_2, Z), 26.7 (CH_3, E), 26.6 (CH_3, Z), 26.53 (CH_3, E), 26.47 (CH_2, Z) 26.4 (CH_3, Z), 26.3 (CH_2, E), 19.52 (CH, Z), 19.48 (CH, E), 18.0 (CH_3, E) 14.3 (CH_3, E + Z), 13.0 (CH_3, Z). IR (NaCl): 3080, 2987, 1710, 1663 cm^{-1}. Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 68.87; H, 7.85.

Diethyl 2-(Cyclohex-2-en-1-yl)-2-((3-(ethoxycarbonyl)-cycloprop-1-en-1-yl)methyl)malonate, 1i. 1i was prepared from diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (50 mg, 0.18 mmol) in 1.8 mL of THF, NaH 60% w/w (8 mg, 0.19 mmol), and 3-bromocyclohexene (24 μL, 0.21 mmol) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 43 mg of a yellow oil (66%) containing a mixture of diastereomers. Signals were only separated in the ^{13}C{^{1}H} NMR spectrum: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.42 (q, J = 1.5 Hz, 1H, C=CH), 5.75−5.65 (m, 2H, CH=CH), 4.25−4.07 (m, 6H, 3× C**H$_2$**CH_3), 3.25−3.04 (m, 3H, CCH_2C + CC**H**CH), 2.11 (dd, J = 6.8, 1.5 Hz, 1H, $CHCO_2Et$), 1.98−1.89 (m, 2H, CH_2), 1.84−1.71 (m, 2H, CH_2), 1.58−1.48 (m, 1H, CH_2), 1.41−1.33 (m, 1H, CH_2), 1.29−1.20 (m, 9H, 3× CH_2C**H$_3$**). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.7 (2× C, a + b), 169.1 (C, b), 169.0 (C, a), 168.8 (C, b), 168.7 (C, a), 128.1 (CH, a), 127.9 (CH, b), 126.6 (CH, b), 126.3 (CH, a), 111.04 (C, a), 111.01 (C, b), 96.2 (CH, a), 96.1 (CH, b), 60.40 (CH_2, b), 60.35 (CH_2, a), 60.32 (CH_2, a), 60.30 (CH_2, b), 59.2 (2× CH_2, a + b), 58.69 (C, a), 58.67 (C, b), 38.07 (CH, b), 38.05 (CH, a), 27.1 (CH_2, b), 26.9 (CH_2, a), 23.86 (CH_2, b), 23.85 (CH_2, a), 23.5 (CH_2, a), 23.1 (CH_2, b), 21.30 (CH_2, b), 21.25 (CH_2, a), 19.3 (CH, a), 19.0 (CH, b), 13.34 (CH_3, a), 13.33 (CH_3, b), 13.1 (CH_3, b), 13.02 (2× CH_3, a), 12.98 (CH_3, b). IR (NaCl): 3085, 2993, 1671, 1659 cm^{-1}. Anal. Calcd for $C_{20}H_{28}O_6$: C, 65.92; H, 7.74. Found: C, 66.17; H, 7.63.

Ethyl 2-(2-Acetyl-2-(cyclohex-2-en-1-yl)-3-oxobutyl)-cycloprop-2-ene-1-carboxylate, 1j. 1j was prepared from ethyl 2-(2-acetyl-3-oxobutyl)cycloprop-2-ene-1-carboxylate (50 mg, 0.22 mmol) in 4.4 mL of acetone, K_2CO_3 (37 mg, 0.27 mmol), and 3-bromocyclohexene (33 μL, 0.29 mmol) following general procedure **B**. It was purified in hexane/AcOEt (9:1) to give 43 mg of a yellow oil (64%) as a mixture of diastereomers in a ratio 1:1: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.35 (q, J = 1.5 Hz, 1H, C=CH, a), 6.31 (q, J = 1.5 Hz, 1H, C=CH, b), 5.82−5.74 (m, 2H, CH=CH, a + b), 5.52−5.48 (m, 1H, CH=CH, a or b), 5.46−5.42 (m, 1H, CH=CH, a or b), 4.15−4.05 (m, 4H, C**H$_2$**CH_3, a + b), 3.25−2.96 (m, 6H, CC**H$_2$**C + CC**H**CH, a + b), 2.18 (s, 3H, $COCH_3$, a or b), 2.17 (s, 3H, $COCH_3$, a or b), 2.16 (s, 3H, $COCH_3$, a or b), 2.14 (s, 3H, $COCH_3$, a or b), 2.11 (d, J = 1.5 Hz, 2H, $CHCO_2Et$, a + b), 2.02−1.91 (m, 4H, CH_2, a + b), 1.84−1.68 (m, 4H, CH_2, a + b), 1.62−1.49 (m, 2H, CH_2, a or b), 1.24 (t, J = 7.2, 6H, CH_2C**H$_3$**, a + b), 1.19−1.07 (m, 2H, CH_2, a or b). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 206.1 (C, b), 205.7 (C, b), 205.64 (C, a), 205.63 (C, a), 175.9 (C, b), 175.8 (C, a), 130.8 (CH, a), 206.1 (C), 205.7 (C), 205.64 (C), 205.63 (C), 175.9 (C), 175.8 (C), 130.8 (CH), 130.4 (CH), 127.3 (CH), 127.0 (CH), 112.5 (C), 112.4 (C), 97.62 (CH), 97.55 (CH), 72.8 (C), 72.7 (C), 60.50 (CH_2), 60.47 (CH_2), 39.4 (CH), 38.8 (CH), 29.3 (CH_3), 28.8 (CH_3), 27.9 (CH_3), 27.4 (CH_3), 26.6 (CH_2), 26.2 (CH_2), 25.1 (CH_2), 25.0, (CH_2) 24.9 (CH_2), 24.4 (CH_2), 22.5 (CH_2), 22.4 (CH_2), 20.50 (CH), 20.45 (CH), 14.5 (CH_3, a + b). IR (NaCl): 3088, 2994, 1708, 1657 cm^{-1}. Anal. Calcd for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 70.86; H, 8.06.

Diethyl (*E*)-2-Cinnamyl-2-(2-(3-(ethoxycarbonyl)cycloprop-1-en-1-yl)ethyl)malonate, 1k. 1k was prepared from diethyl 2-(2-(3-(ethoxycarbonyl)cycloprop-1-en-1-yl)ethyl)malonate (50 mg, 0.17 mmol) in 1.7 mL of THF, NaH 60% w/w (7 mg, 0.18 mmol), and cinnamylbromide (40 mg, 0.20 mmol) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 44 mg of a yellow oil (63%).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29−7.22 (m, 4H, Ar), 7.19−7.15 (m, 1H, Ar), 6.41 (d, J = 15.7 Hz, 1H, CH=C**H**Ph), 6.34 (q, J = 1.4 Hz, 1H, C=CH), 6.00 (dt, J = 15.4, 7.5 Hz, 1H, C**H**=CHPh), 4.20−4.12 (m, 4H, 2× C**H$_2$**CH_3), 4.10−4.00 (m, 2H, C**H$_2$**CH_3), 2.78 (dd, J = 7.6, 1.4 Hz, 2H, CC**H$_2$**CH), 2.51−2.46 (m, 2H, C**H$_2$**CH_2C), 2.20−2.16 (m, 2H, CH_2C**H$_2$**C), 2.11 (d, J = 1.5 Hz, 1H, $CHCO_2Et$), 1.23−1.16 (m, 9H, 3× CH_2C**H$_3$**). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 176.2 (C), 170.9 (2× C), 137.1 (C), 134.2 (CH), 128.6 (2× CH), 127.6 (CH), 126.3 (2× CH), 123.7 (CH), 114.9 (C), 95.2 (CH), 61.5 (2× CH_2), 60.3 (CH_2), 57.4 (C), 36.9 (CH_2), 30.0 (CH_2), 20.5 (CH_2), 20.0 (CH), 14.4 (CH_3), 14.2 (2× CH_3). IR (NaCl): 3098, 2984, 1660, 1652 cm^{-1}. Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.39; H, 7.31.

Diethyl 2-(But-3-en-1-yl)-2-((3-(ethoxycarbonyl)-3-phenyl-cycloprop-1-en-1-yl)methyl)malonate, 1m. 1m was prepared from diethyl 2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)-methyl)malonate (50 mg, 0.14 mmol) in 1.4 mL of THF, NaH 60% w/w (6 mg, 0.15 mmol), and 4-bromo-1-butene (17 μL, 0.17 mmol) following general procedure **A**. It was purified in hexane/AcOEt (9:1) to give 36 mg of a yellow oil (63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.19 (m, 5H, Ph), 6.78 (t, J = 1.6 Hz, 1H, C=CH), 5.78−5.68 (m, 1H, C**H**=CH_2), 5.01−4.94 (m, 2H, CH=C**H$_2$**), 4.23−4.05 (m, 6H, 3× C**H$_2$**CH_3), 3.28−3.14 (m, 2H, CH_2C), 2.18−1.87 (m, 4H, CCH_2CH_2), 1.28−1.22 (m, 6H, 2× CH_2C**H$_3$**), 1.15 (t, J = 7.1 Hz, 3H, CH_2C**H$_3$**). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.7 (C), 170.5 (C), 170.4 (C), 141.2 (C), 137.4 (CH), 128.2 (2× CH), 128.1 (2× CH), 126.5 (CH), 116.9 (C), 115.4 (CH_2), 100.0 (CH), 61.8 (CH_2), 61.7 (CH_2), 60.9 (CH_2), 56.3 (C), 32.6 (CH_2), 31.4 (CH_2), 28.5 (CH_2), 27.9 (C), 14.4 (CH_3), 14.2 (CH_3), 14.1 (CH_3). IR (NaCl): 3090, 2989, 1661, 1648 cm^{-1}. Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.79; H, 7.35.

Diethyl 5-Ethoxy-1,3-dihydro-2*H*-indene-2,2-dicarboxylate, 5a. 5a was prepared from **1a** (41 mg, 0.13 mmol) in 0.6 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 6.3 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 27 mg of a yellow oil (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06 (d, J = 8.2 Hz, 1H, Ar), 6.73 (s, 1H, Ar), 6.70 (d, J = 8.2 Hz, 1H, Ar), 4.20 (q, J = 7.1 Hz, 4H, 2× $COOCH_2CH_3$), 3.98 (q,

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J = 7.0 Hz, 2H, OC**H**$_2$CH$_3$), 3.54 (s, 2H, CH$_2$), 3.50 (s, 2H, CH$_2$), 1.38 (t, J = 7.0 Hz, 3H, OCH$_2$C**H**$_3$), 1.25 (t, J = 7.1 Hz, 6H, 2× COOCH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.8 (2× C), 158.6 (C), 141.6 (C), 131.9 (C), 124.9 (CH), 113.7 (CH), 110.3 (CH), 63.7 (CH$_2$), 61.8 (2× CH$_2$), 60.9 (C), 40.8 (CH$_2$), 39.8 (CH$_2$), 15.0 (CH$_3$), 14.2 (2× CH$_3$). IR (NaCl): 3070, 2990, 2975, 1682 cm^{-1}. Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.81; H, 7.31.

Diethyl 5-Ethoxy-4-methyl-1,3-dihydro-2*H*-indene-2,2-dicarboxylate, 5b. **5b** was prepared from **1b** (39 mg, 0.12 mmol) in 0.6 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 5.7 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 27 mg of a yellow oil (70%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.94 (d, J = 8.2 Hz, 1H, Ar), 6.66 (d, J = 8.2 Hz, 1H, Ar), 4.20 (q, J = 7.1 Hz, 4H, COOC**H**$_2$CH$_3$), 3.98 (q, J = 6.9 Hz, 2H, OC**H**$_2$CH$_3$), 3.53 (s, 2H, CH$_2$), 3.50 (s, 2H, CH$_2$), 2.13 (s, 3H, CH$_3$), 1.39 (t, J = 7.0 Hz, 3H, OCH$_2$C**H**$_3$), 1.27−1.23 (m, 6H, COOCH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 172.0 (2× C), 156.3 (C), 140.6 (C), 131.6 (C), 122.7 (C), 121.4 (CH), 110.7 (CH), 64.3 (CH$_2$), 61.8 (2× CH$_2$), 60.6 (C), 40.2 (CH$_2$), 39.7 (CH$_2$), 15.2 (CH$_3$), 14.2 (2× CH$_3$), 12.5 (CH$_3$). IR (NaCl): 3065, 2992, 2980, 1679 cm^{-1}. Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found: C, 67.57; H, 7.60.

Triethyl 5-Ethoxy-1,3-dihydro-2*H*-indene-2,2,4-tricarboxylate, 5c. **5c** was prepared from triethyl **1c** (22 mg, 0.06 mmol) in 0.3 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (2 mg, 2.7 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 10 mg of a yellow oil (51%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.17 (d, J = 8.4 Hz, 1H, Ar), 6.76 (d, J = 8.3 Hz, 1H, Ar), 4.38 (q, J = 7.2 Hz, 2H, COOC**H**$_2$CH$_3$), 4.19 (q, J = 7.1 Hz, 4H, 2× COOC**H**$_2$H$_3$), 4.04 (q, J = 6.9 Hz, 2H, OC**H**$_2$CH$_3$), 3.68 (s, 2H, CH$_2$), 3.51 (s, 2H, CH$_2$), 1.39 (t, J = 7.0 Hz, 3H, OCH$_2$C**H**$_3$), 1.38 (t, J = 7.1 Hz, 3H, OCH$_2$C**H**$_3$), 1.24 (t, J = 7.1 Hz, 6H, 2× COOCH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.6 (2× C), 166.9 (C), 156.7 (C), 141.3 (C), 132.7 (C), 127.1 (CH), 119.4 (C), 112.4 (CH), 65.2 (CH$_2$), 61.9 (2× CH$_2$), 61.0 (CH$_2$), 60.6 (C), 40.5 (CH$_2$), 39.7 (CH$_2$), 15.0 (CH$_3$), 14.5 (CH$_3$), 14.2 (2× CH$_3$). IR (NaCl): 3071, 2989, 2971, 1762, 1680 cm^{-1}. Anal. Calcd for $C_{20}H_{26}O_7$: C, 63.48; H, 6.93. Found: C, 63.66; H, 7.01.

Diethyl 5-Ethoxy-6-phenyl-1,3-dihydro-2*H*-indene-2,2-dicarboxylate, 5d. **5d** was prepared from **1d** (41 mg, 0.10 mmol) in 0.5 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (3 mg, 5.1 μmol) following the general procedure for cycloisomerization. A 5:1 mixture of **5d** and **4d** was detected in the crude and separated by silica gel column chromatography in hexane/AcOEt (9:1) to afford 29 mg of **5d** as a yellow oil (69%) and 6 mg of **4d** as a yellow oil (14%). Data for **5d:** ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.52 (d, J = 7.5 Hz, 2H, Ph), 7.38 (t, J = 7.5 Hz, 2H, Ph), 7.30 (t, J = 7.3 Hz, 1H, Ph), 7.14 (s, 1H, Ar), 6.82 (s, 1H, Ar), 4.23 (q, J = 7.1 Hz, 4H, 2× COOCH$_2$CH$_3$), 3.98 (q, J = 6.9 Hz, 2H, OCH$_2$CH$_3$), 3.61 (s, 2H, CH$_2$), 3.57 (s, 2H, CH$_2$), 1.31 (t, J = 6.9 Hz, 3H, OCH$_2$CH$_3$), 1.27 (t, J = 7.1 Hz, 6H, 2× COOCH$_2$CH$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.9 (2× C), 155.5 (C), 140.5 (C), 139.0 (C), 132.1 (C), 130.1 (C), 129.7 (2× CH), 127.9 (2× CH), 126.7 (CH), 126.5 (CH), 108.9 (CH), 64.4 (CH$_2$), 61.9 (2× CH$_2$), 60.8 (C), 40.9 (CH$_2$), 40.0 (CH$_2$), 14.9 (CH$_3$), 14.2 (2× CH$_3$). IR (NaCl): 3091, 3080, 2990, 2979, 1685. Anal. Calcd for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85. Found: C, 71.97; H, 6.96.

Diethyl 5-Ethoxy-4-methyl-6-phenyl-1,3-dihydro-2*H*-indene-2,2-dicarboxylate, 5e. **5e** was prepared from **1e** (38 mg, 0.09 mmol) in 0.5 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (3 mg, 4.6 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 25 mg of a yellow oil (71%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.46 (d, J = 7.7 Hz, 2H, Ph), 7.30 (t, J = 7.5 Hz, 2H, Ph), 7.21 (t, J = 7.5 Hz, 1H, Ph), 6.92 (s, 1H, Ar), 4.15 (q, J = 7.1 Hz, 4H, 2× COOC**H**$_2$CH$_3$), 3.53 (s, 2H, CH$_2$), 3.48 (s, 2H, CH$_2$), 3.35 (q, J = 7.0 Hz, 2H, OC**H**$_2$CH$_3$), 2.16 (s, 3H, CH$_3$), 1.19 (t, J = 7.0 Hz, 6H, 2× COOCH$_2$C**H**$_3$), 0.99 (t, J = 7.0 Hz, 3H, OCH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.9 (2× C), 154.1 (C), 140.0 (C), 139.5 (C), 135.0 (C), 134.2 (C), 129.3 (2× CH), 128.1 (2× CH), 127.5 (C), 126.9 (CH), 123.6 (CH), 68.6 (CH$_2$), 61.9 (2× CH$_2$), 60.3 (C), 40.5 (CH$_2$), 39.9 (CH$_2$), 15.6 (CH$_3$), 14.2 (2× CH$_3$), 13.1 (CH$_3$). IR (NaCl): 3091, 3076, 2995, 2986, 1685. Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.64; H, 7.20.

Diethyl 5-Ethoxy-4,6-diphenyl-1,3-dihydro-2*H*-indene-2,2-dicarboxylate, 5f. **5f** was prepared from **1f** (40 mg, 0.08 mmol) in 0.4 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (3 mg, 4.2 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 26 mg of a yellow oil (68%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.58 (d, J = 7.5 Hz, 2H, Ar), 7.43−7.29 (m, 8H, Ar), 7.16 (s, 1H, Ar), 4.22−4.16 (m, 4H, 2× C**H**$_2$CH$_3$), 3.65 (s, 2H, CH$_2$), 3.45 (s, 2H, CH$_2$), 3.21 (q, J = 7.0 Hz, 2H, C**H**$_2$CH$_3$), 1.23 (t, J = 7.1 Hz, 6H, 2× CH$_2$C**H**$_3$), 0.68 (t, J = 7.0 Hz, 3H, CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.6 (2× C), 153.2 (C), 139.5 (C), 139.2 (C), 137.0 (C), 135.4 (C), 134.8 (C), 132.8 (C), 129.7 (2× CH), 129.4 (2× CH), 128.03 (2× CH), 127.97 (2× CH), 127.0 (CH), 126.9 (CH), 125.4 (CH), 68.9 (CH$_2$), 61.7 (2× CH$_2$), 60.5 (C), 40.4 (CH$_2$), 40.3 (CH$_2$), 15.1 (CH$_3$), 14.0 (2× CH$_3$). IR (NaCl): 3089, 3070, 2991, 2980, 1690. Anal. Calcd for $C_{29}H_{30}O_5$: C, 75.96; H, 6.59. Found: C, 76.13; H, 6.61.

1,1′-(5-Ethoxy-2,3-dihydro-1*H*-indene-2,2-diyl)bis(ethan-1-one), 5g. **5g** was prepared from **1g** (44 mg, 0.17 mmol) in 0.8 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (6 mg, 8.3 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 29 mg of a yellow oil (71%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.07 (d, J = 8.1 Hz, 1H, Ar), 6.73 (br s, 1H, Ar), 6.70 (dd, J = 8.1, 2.4 Hz, 1H, Ar), 3.98 (q, J = 7.0 Hz, 2H, C**H**$_2$CH$_3$), 3.46 (s, 2H, CH$_2$), 3.43 (s, 2H, CH$_2$), 2.16 (s, 6H, 2× CH$_3$), 1.39 (t, J = 7.0 Hz, 3H, CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 205.1 (2× C), 158.8 (C), 141.3 (C), 131.6 (C), 125.2 (CH), 113.9 (CH), 110.5 (CH), 75.3 (C), 63.8 (CH$_2$), 38.0 (CH$_2$), 37.1 (CH$_2$), 26.7 (2× CH$_3$), 15.0 (CH$_3$). IR (NaCl): 3088, 2996, 2980, 1725. Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.32; H, 7.41.

1,1′-(5-Ethoxy-4-methyl-2,3-dihydro-1*H*-indene-2,2-diyl)bis(ethan-1-one), 5h. **5h** was prepared from **1h** (43 mg, 0.16 mmol) in 0.8 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (5 mg, 7.7 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 28 mg of a yellow oil (70%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.94 (d, J = 8.2 Hz, 1H, Ar), 6.66 (d, J = 8.2 Hz, 1H, Ar), 3.98 (q, J = 6.9 Hz, 2H, C**H**$_2$CH$_3$), 3.45 (s, 2H, CH$_2$), 3.41 (s, 2H, CH$_2$), 2.17 (s, 6H, 2× CH$_3$), 2.15 (s, 3H, CH$_3$), 1.40 (t, J = 6.9 Hz, 3H, CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 205.3 (2× C), 156.4 (C), 140.4 (C), 131.3 (C), 122.9 (C), 121.8 (CH), 110.8 (CH), 74.9 (C), 64.3 (CH$_2$), 37.5 (CH$_2$), 36.9 (CH$_2$), 26.7 (2× CH$_3$), 15.2 (CH$_3$), 12.5 (CH$_3$). IR (NaCl): 3091, 2990, 2982, 1722. Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.82; H, 7.74. Found: C, 73.95; H, 7.81.

Diethyl 5-Ethoxy-6,7,8,8a-tetrahydroacenaphthylene-1,1(2*H*)-dicarboxylate, 5i. **5i** was prepared from **1i** (43 mg, 0.12 mmol) in 0.6 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 5.9 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 30 mg of a yellow oil (72%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.95 (d, J = 8.0 Hz, 1H, Ar), 6.59 (d, J = 8.1 Hz, 1H, Ar), 4.31−4.20 (m, 2H, COOC**H**$_2$CH$_3$), 4.15−4.04 (m, 2H, COOC**H**$_2$CH$_3$), 3.98 (q, J = 7.0 Hz, 2H, OC**H**$_2$CH$_3$), 3.72 (dd, J = 12.0, 4.6 Hz, 1H, CCH), 3.44 (d, J = 15.5 Hz, 1H, CC**H**$_2$C), 3.15 (d, J = 15.5 Hz, 1H, CC**H**$_2$C), 2.75 (dd, J = 18.0, 6.4 Hz, 1H, CH$_2$), 2.45−2.36 (m, 1H, CH$_2$), 2.27−2.21 (m, 1H, CH$_2$), 2.16−2.09 (m, 1H, CH$_2$), 1.81−1.69 (m, 1H, CH$_2$), 1.38 (t, J = 7.0 Hz, 3H, COOCH$_2$C**H**$_3$), 1.29 (t, J = 7.1 Hz, 3H, COOCH$_2$C**H**$_3$), 1.22−1.14 (m, 4H, OCH$_2$C**H**$_3$ + CH$_2$). ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$): δ 171.7 (C), 170.5 (C), 155.5 (C), 141.6

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(C), 131.0 (C), 123.5 (C), 121.1 (CH), 109.6 (CH), 65.8 (C), 63.6 (CH_2), 61.4 (CH_2), 61.1 (CH_2), 47.9 (CH), 39.7 (CH_2), 25.9 (CH_2), 23.5 (CH_2), 21.9 (CH_2), 15.1 (CH_3), 14.3 (2× CH_3). IR (NaCl): 3078, 2993, 2985, 1686. Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.50; H, 7.66.

1,1′-(5-Ethoxy-1,2,6,7,8,8a-hexahydroacenaphthylene-1,1-diyl)bis(ethan-1-one), 5j. **5j** was prepared from **1j** (43 mg, 0.14 mmol) in 0.7 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (5 mg, 7.1 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 30 mg of a yellow oil (73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.98 (d, J = 8.0 Hz, 1H, Ar), 6.62 (d, J = 8.0 Hz, 1H, Ar), 4.00 (q, J = 7.0 Hz, 2H, C**H**$_2$CH$_3$), 3.74−3.70 (m, 1H, CCH), 3.33 (d, J = 15.7 Hz, 1H, CCH$_2$C), 3.20 (d, J = 15.7 Hz, 1H, CCH$_2$C), 2.77 (dd, J = 18.1, 6.4 Hz, 1H, CH_2), 2.46−2.36 (m, 1H, CH_2), 2.20 (s, 3H, CH_3), 2.15−2.11 (m, 2H, CH_2), 1.83 (s, 3H, CH_3), 1.79−1.70 (m, 1H), 1.40 (t, J = 7.0 Hz, 3H, CH$_2$C**H**$_3$), 1.10−1.01 (m, 1H, CH_2). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 207.8 (C), 206.1 (C), 156.0 (C), 142.5 (C), 129.9 (C), 124.1 (C), 121.6 (CH), 109.8 (CH), 78.8 (C), 63.7 (CH_2), 45.9 (CH), 38.1 (CH_2), 28.7 (CH_3), 28.4 (CH_3), 25.7 (CH_2), 23.6 (CH_2), 21.9 (CH_2), 15.1 (CH_3). IR (NaCl): 3087, 2991, 2985, 1718. Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.69; H, 7.59.

Diethyl 7-Ethoxy-8-phenyl-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate, 5k. **5k** was prepared from **1k** (44 mg, 0.11 mmol) in 0.5 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 5.3 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 25 mg of a yellow oil (60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42−7.38 (m, 2H, Ph), 7.33−7.29 (m, 1H, Ph), 7.22−7.19 (m, 2H, Ph), 7.01 (d, J = 8.4 Hz, 1H, Ar), 6.79 (d, J = 8.4 Hz, 1H, Ar), 4.15−4.06 (m, 4H, 2× COOC**H**$_2$CH$_3$), 3.89 (q, J = 7.0 Hz, 2H, OC**H**$_2$CH$_3$), 2.89 (s, 2H, CCH$_2$C), 2.83 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$), 2.28 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$), 1.18−1.12 (m, 9H, 3× CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 171.5 (2× C), 154.4 (C), 137.3 (C), 133.1 (C), 131.4 (C), 130.0 (2× CH), 128.3 (CH), 128.0 (2× CH), 127.3 (C), 126.6 (CH), 111.5 (CH), 64.6 (CH_2), 61.2 (2× CH_2), 54.0 (C), 33.2 (CH_2), 28.1 (CH_2), 25.8 (CH_2), 14.7 (CH_3), 13.9 (2× CH_3). IR (NaCl): 3091, 3084, 2995, 2987, 1672. Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.56; H, 7.08.

Diethyl 6-Ethoxy-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate, 5l, and Diethyl 2-(but-3-en-1-yl)-2-((5-ethoxyfuran-2-yl)methyl)malonate, 8l. **8l** was prepared from **1l** (36 mg, 0.11 mmol) in 0.5 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 5.3 μmol) following the general procedure for cycloisomerization. The products were separated by silica gel column chromatography in hexane/AcOEt (9:1) to afford 21 mg of **5l** as a yellow oil (62%) and 7 mg of **8l** as a yellow oil (18%). Data for **5l:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, J = 8.5 Hz, 1H, Ar), 6.68 (dd, J = 8.5, 2.3 Hz, 1H, Ar), 6.58 (br s, 1H, Ar), 4.17 (q, J = 7.1 Hz, 4H, 2× COOC**H**$_2$CH$_3$), 3.97 (q, J = 6.9 Hz, 2H, OC**H**$_2$CH$_3$), 3.19 (s, 2H, CCH$_2$C), 2.80 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$), 2.29 (t, J = 6.7 Hz, 2H, CH$_2$CH$_2$), 1.38 (t, J = 7.0 Hz, 3H, OCH$_2$C**H**$_3$), 1.22 (t, J = 7.1 Hz, 6H, 2× COOCH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 171.6 (2× C), 157.3 (C), 135.8 (C), 129.8 (CH), 125.7 (C), 113.9 (CH), 113.1 (CH), 63.5 (CH_2), 61.5 (2× CH_2), 53.9 (C), 34.1 (CH_2), 28.2 (CH_2), 26.4 (CH_2), 15.1 (CH_3), 14.2 (2× CH_3). IR (NaCl): 3093, 2994, 2981, 1674. Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found: C, 67.32; H, 7.65. Data for **8l:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88 (d, J = 3.1 Hz, 1H, C═CH), 5.83−5.73 (m, 1H, CH$_2$C**H**), 5.05−4.94 (m, 3H, CH═C + CH═C**H**$_2$), 4.20 (q, J = 7.1 Hz, 4H, 2× C**H**$_2$CH$_3$), 4.01 (q, J = 7.1 Hz, 2H, C**H**$_2$CH$_3$), 3.17 (s, 2H, CCH$_2$), 2.05−1.99 (m, 2H, CH$_2$CH$_2$), 1.96−1.91 (m, 2H, CH$_2$CH$_2$), 1.35 (t, J = 7.1 Hz, 3H, CH$_2$C**H**$_3$), 1.26 (t, J = 7.1 Hz, 6H, 2× CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 171.0 (2× C), 160.0 (C), 140.5 (C), 137.8 (CH), 115.1 (CH_2), 109.4 (CH), 81.4 (CH), 66.9 (CH_2), 61.5 (2× CH_2), 57.6 (C), 31.4 (CH_2), 31.2 (CH_2), 28.6 (CH_2), 14.8 (2× CH_3), 14.2 (CH_3). IR (NaCl): 3094, 3080, 2990, 2984, 1691. Anal. Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74. Found: C, 63.71; H, 7.80.

Diethyl 6-Ethoxy-7-phenyl-3,4-dihydronaphthalene-2,2(1*H*)-dicarboxylate, 5m. **5m** was prepared from **1m** (36 mg, 0.09 mmol) in 0.4 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (3 mg, 4.3 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 21 mg of a yellow oil (59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (d, J = 7.6 Hz, 2H, Ph), 7.38 (t, J = 7.5 Hz, 2H, Ph), 7.30 (d, J = 7.3 Hz, 1H, Ph), 7.08 (s, 1H, Ar), 6.65 (s, 1H, Ar), 4.19 (q, J = 7.1 Hz, 4H, 2× C**H**$_2$CH$_3$), 3.97 (q, J = 6.9 Hz, 2H, C**H**$_2$CH$_3$), 3.24 (s, 2H, CH_2), 2.86 (t, J = 6.7 Hz, 2H, CH$_2$CH$_2$), 2.34 (t, J = 6.7 Hz, 2H, CH$_2$CH$_2$), 1.32 (t, J = 7.0 Hz, 3H, CH$_2$C**H**$_3$), 1.24 (t, J = 7.2 Hz, 6H, 2× CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 171.6 (2× C), 154.3 (C), 138.6 (C), 134.9 (C), 131.2 (CH), 129.6 (2× CH), 129.1 (C), 128.0 (2× CH), 126.7 (CH), 125.8 (C), 112.7 (CH), 64.1 (CH_2), 61.6 (2× CH_2), 53.9 (C), 34.0 (CH_2), 28.2 (CH_2), 26.3 (CH_2), 14.9 (CH_3), 14.2 (2× CH_3). IR (NaCl): 3092, 3085, 2993, 2981, 1682. Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.93; H, 7.16.

5-Ethoxy-2-tosylisoindoline, 5q. **5q** was prepared from **1q** (50 mg, 0.15 mmol) in 0.8 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (5 mg, 7.5 μmol) following the general procedure for cycloisomerization. It was purified in silica gel in hexane/AcOEt (9:1) to afford 32 mg of a yellow oil (68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 8.3 Hz, 2H, 2× CH Ts), 7.30 (d, J = 7.9 Hz, 2H, 2× CH Ts), 7.04 (d, J = 8.4 Hz, 1H, Ar), 6.76 (dd, J = 8.4, 2.4 Hz, 1H, Ar), 6.67 (d, J = 2.3 Hz, 1H, Ar), 4.57 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 3.97 (q, J = 7.0 Hz, 2H, C**H**$_2$CH$_3$), 2.40 (s, 3H, CH_3), 1.38 (t, J = 7.0 Hz, 3H, CH$_2$C**H**$_3$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 159.1 (C), 143.7 (C), 137.6 (C), 134.0 (C), 129.9 (2× CH), 128.0 (C), 127.8 (2× CH), 123.5 (CH), 114.8 (CH), 108.4 (CH), 63.9 (CH_2), 54.0 (CH_2), 53.4 (CH_2), 21.6 (CH_3), 14.9 (CH_3). IR (NaCl): 3085, 3074, 2990, 2983. Anal. Calcd for $C_{17}H_{19}NO_3S$: C, 64.33; N, 4.41; H, 6.03. Found: C, 64.49; N, 4.50; H, 5.91.

Diethyl 6-Ethoxy-7a-methyl-5-phenyl-1,7a-dihydro-2*H*-indene-2,2-dicarboxylate, 6. **6** was prepared from **1p** (50 mg, 0.12 mmol) in 0.6 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 6 μmol) following the general procedure for cycloisomerization but in 5 h of reaction. It was purified in silica gel in hexane/AcOEt (19:1) to obtain 30 mg of **6** (62%) impurified with a small amount of **8p** as a yellow oil (ratio by ^{1}H NMR, **6**:**8p**; 92:8). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40−7.38 (m, 2H, Ph), 7.33−7.27 (m, 3H, Ph), 6.39 (s, 1H, CH═CPh), 5.74 (s, 1H, CCH═C), 5.05 (s, 1H, CH═COEt), 4.25−4.17 (m, 4H, 2× C**H**$_2$CH$_3$), 3.76−3.67 (m, 2H, C**H**$_2$CH$_3$), 2.67 (d, J = 13.0 Hz, 1H, CH_2), 2.54 (d, J = 13.0 Hz, 1H, CH_2), 1.29−1.20 (m, 9H, 3× CH$_2$C**H**$_3$), 1.16 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 171.8 (C), 171.2 (C), 151.2 (C), 150.3 (C), 138.7 (C), 138.1 (C), 129.0 (2× CH), 127.7 (2× CH), 127.4 (CH), 123.4 (CH), 122.0 (CH), 107.3 (CH), 64.7 (C), 62.8 (CH_2), 61.87 (CH_2), 61.85 (CH_2), 47.6 (C), 46.4 (CH), 29.0 (CH_3), 14.5 (CH_3), 14.22 (CH_3), 14.20 (CH_3). IR (NaCl): 3081, 2988, 2980, 1691. Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.56; H, 7.23.

Diethyl 7a-Methyl-6-oxo-1,6,7,7a-tetrahydro-2*H*-indene-2,2-dicarboxylate, 7. **7** was prepared from **1o** (41 mg, 0.12 mmol) in 0.6 mL of toluene and dichloro(hexamethylbenzene) ruthenium(II) dimer (4 mg, 6.0 μmol) following the general procedure for cycloisomerization. It was purified by silica gel column chromatography in hexane/AcOEt (9:1) to afford 21 mg of a yellow oil (62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 (d, J = 10.0 Hz, 1H, C**H**═CHCO), 5.99 (d, J = 10.0 Hz, 1H, CH═C**H**CO), 5.98 (s, 1H, CH═C), 4.29−4.16 (m, 4H, 2× C**H**$_2$CH$_3$), 2.66 (d, J = 13.4 Hz, 1H, CH_2), 2.62 (d, J = 15.2 Hz, 1H, CH_2CO), 2.52 (d, J = 15.6 Hz, 1H, CH_2CO), 2.38 (d, J = 13.5 Hz, 1H, CH_2), 1.27 (t, J = 7.1 Hz, 6H, 2× CH$_2$C**H**$_3$), 1.17 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 199.1 (C), 170.6 (C), 170.3 (C), 147.6 (C), 138.1 (CH), 129.3 (CH), 127.4 (CH), 66.4 (C), 62.3 (CH_2), 62.1 (CH_2), 52.4 (CH_2), 47.6 (C), 46.2 (CH_2), 25.9 (CH_3), 14.18 (CH_3), 14.17 (CH_3). IR

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(NaCl): 3087, 2993, 2982, 1728, 1684. Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.53; H, 6.80.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c02636.

Copies of ^1H and $^{13}C\{^1H\}$ NMR spectra of all products (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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6. ONE POT SYNTHESIS OF SPIROCYCLES AND CYCLOPROPA[*B*]PYRANS BY ALKENYLATION-REARRANGEMENT OF CYCLOPROPENES.

This work shows a one pot procedure to access spirocycles and cyclopropa[*b*]pyrans by alkenylation-rearrangement of cyclopropenes. This reaction took place without any catalyst, through a pericyclic mechanism. The optimal conditions were achieved using 2-MeTHF as solvent at reflux. We could synthetize [2.4.0] and [2.5.0] spirocycles. We also studied the influence of the stereochemistry of the double bond in the outcome of the reaction. When using α,β-unsaturated bromocarbonyl compounds cyclopropa[*b*]pyrans were obtained.

In this work I did the synthesis of some bromine derivatives together with Eleonora Giona (Erasmus undergraduate student), the synthesis of spirocycles and the characterization of final products. I collaborated in the manuscript elaboration with my supervisors, mainly in the experimental part and supplementary information.

En este trabajo se presenta un procedimiento "*one pot*" para la obtención de espirociclos y ciclopropa[*b*]piranos por una alquenilación y posterior reordenamiento de ciclopropenos. Esta reacción tiene lugar en ausencia de catalizadores, a través de un mecanismo pericíclico. Las condiciones óptimas se lograron utilizando 2-MeTHF como disolvente a reflujo. Pudimos sintetizar [2.4.0] y [2.5.0] espirociclos. También estudiamos la influencia de la estereoquímica del doble enlace en el producto de la reacción. Cuando se utilizaron compuestos bromocarbonílicos α,β-insaturados se obtuvieron ciclopropa[*b*]piranos.

En este trabajo realicé la síntesis de diversos derivados bromados junto a Eleonora Giona (estudiante Erasmus pre-grado) y la síntesis de los espirociclos, además de su caracterización. He colaborado en la redacción del manuscrito junto a mis supervisores, principalmente en la parte experimental y la información suplementaria.

Puet, A.; Giona, E.; Dominguez, G.; Pérez-Castells, J. One Pot Synthesis of Spirocycles and Cyclopropa[*b*]pyrans by Alkenylation-Rearrangement of Cyclopropenes. *J. Org. Chem.* **2022**, *87*, 12470-12476. **Impact factor** (2022): 3.608. **Category**: Organic chemistry (Q1, 10/52). Source: JCR (Journal of Citations Reports).

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One Pot Synthesis of Spirocycles and Cyclopropa[*b*]pyrans by Alkenylation-Rearrangement of Cyclopropenes

Alejandro Puet, Eleonora Giona, Gema Domínguez, and Javier Pérez-Castells*

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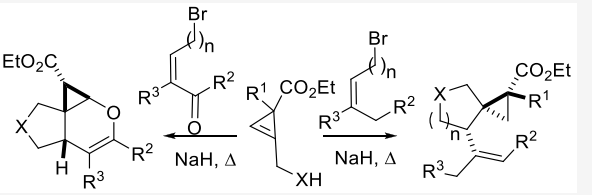
ABSTRACT: A one pot alkenylation followed by a stereoselective Alder-ene cycloisomerization of cyclopropenes give (aza)spiro[2.4]-heptanes and spiro[2.5]octanes in high yields. Total *trans* diastereoselectivity is achieved for spiro[2.4]heptanes if the cyclopropene is monosubstituted in C3. When an *α,β*-unsaturatedcarbonyl-containing bromide is used, an alternative cyclization takes place giving cyclopenta-[*c*]cyclopropa[*b*]pyrans.



Due to their high strain, cyclopropenes are able to undergo different types of cycloisomerizations involving carbon−carbon bond cleavages.[1] Ene-cyclopropenes can afford furans or pyrroles,[2] whereas vinylcyclopropenes have been transformed into indenes and naphthalenes by cycloisomerizations.[3] The cyclopropene ring opening is achieved by simple heating/irradiation or by metal catalysis. In the latter case, ring cleavage frequently gives intermediate vinylcarbenes, which can cyclopropanate double bonds inter- or intramolecularly.[4] We have shown that the regioselectivity of the ring opening depends on the substitution pattern and on the metal complex used.[5] Thus, when using gold catalysts with substrates **2** a metal carbene is formed at the external carbon atom of the cyclopropene. Conversely, upon reaction with Cp*RuCl(cod) the intermediate is the internal carbene **A**, which affords bicyclo-[*n*.1.0]alkanes **3** by intramolecular cyclopropanation.[6] With *gem*-disubstituted cyclopropenes, compounds **4** were isolated. If the catalysts was switched to dimeric ruthenium(II) catalysts $[RuArCl_2]_2$, highly functionalized indanes and tetralines **5** were generally formed through ene-cyclopropene rearrangement, presumably upon evolution of the bicyclic intermediates **4** (Scheme 1).[7] During the synthesis of the starting ene-cyclopropenes, **2**, carried out by allylation of **1**, we had observed a minor side reaction when the allyl derivative was disubstituted at the alkene terminal. The side products formed were the spirocycles **6**, which arise as a result of a thermal intramolecular Alder-ene reaction after the alkylation process. We envisioned the possibility of making this the main reaction pathway as these spirocycles are highly interesting products related with approved and in development drugs.[8] Not many general synthetic pathways are available to date for the synthesis of these compounds.[9] In particular, while we were developing the present work, the group of Xu disclosed the Alder-ene reaction of ene-cyclopropenes gem-disubstituted at the cyclopropene to produce spiro[2.4]heptane derivatives.[10] Herein we show a one pot procedure which transforms monosubstituted cyclopropenes into both spiro[2.4]heptanes and spiro[2.5]octanes **6** in one step without isolating the intermediate ene-cyclopropene. The presence of a carbonyl group conjugated with the alkene renders cyclopenta[*c*]-cyclopropa[*b*]pyrans **9** as a result of a cyclization process.

In order to optimize this transformation we used diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate **1a** as substrate, which was submitted to reaction with 3,3-dimethylallyl bromide and NaH under different conditions (Table 1). The formation of **6a** had been observed when the reaction was carried out in THF at rt, and this side process could only be led to 10% conversion by extending the reaction time to 24 h (entry 1). The same conditions, under reflux, led to total conversion of **1a** into **6a** only after 24 h of reaction (entries 2 and 3). Given that this is a thermal rearrangement, we next turned to solvents with the same characteristics as THF but with a higher boiling point. Thus, using 2-MeTHF under reflux conditions the reaction was completed after 6 h (entries 4−6). Less coordinating solvents like 1,2-dichloroethane did not give total conversion (entry 7). This also happened when using dioxane (entry 8). Increasing the reaction concentration did not affect either the conversion or the yield (entries 9 and 10). Thus, under the conditions of entry 10, we isolated product **6a** in a 78% yield. In all these reactions, **6a**, in which three new stereocenters are created, was detected as an only diastereoisomer with *trans* configuration at the cyclopropane ring as a presumable result of the reaction of intermediate **A**. The stereochemistry was assigned following the NOE correlation seen between the highlighted protons (Table 1).[11] For comparison reasons we synthesized and

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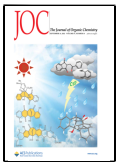
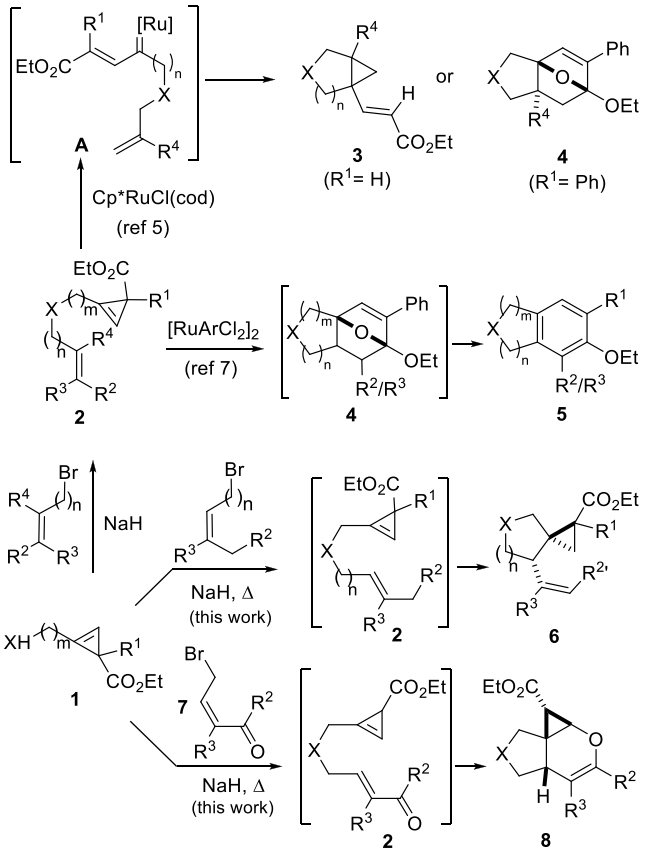


Scheme 1. Summary of Previous Works and of the Results of This Work



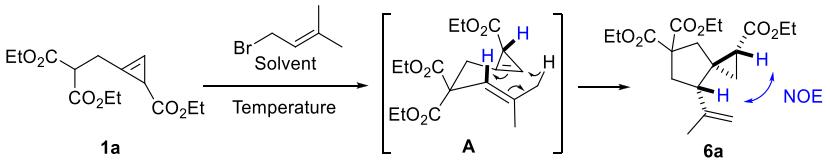
isolated the intermediate ene-cyclopropene (**A**: 72% yield),[6] and then heated this compound at reflux in 2-MeTHF, isolating **6a** in 92%. This makes a 66% overall yield, lower than the 78% achieved with the one pot procedure. In order to show the synthetic utility of the method, we carried out a reaction with 3.15 mmol of **1a** under the optimized conditions of entry 10, which led to a 72% yield in **6a** (entry 11).

With the optimized conditions in hand, different spirocycles were prepared (Table 2). We only detected one *trans* diastereoisomer in products **6a**, **6b**, and **6c**, while **6d**, which was prepared from an 85:15 commercial mixture of *trans*:*cis* 2-butenyl bromide, resulted in a 4:1 mixture of isomers (entries 1−4). This result could suggest a stereochemistry transfer of the starting material to the final product, giving different stereoisomers at the cyclopentane ring. However, this possibility was ruled out in favor of the *trans*:*cis* mixture of cyclopropanes shown (*vide infra*). Interestingly, it was possible to extend the method to the synthesis of the spiro[2.5]octanes **6e**, which were isolated as a 2:1 mixture of *trans*:*cis* isomers (entry 5). The formation of the six-membered ring needed 60 h of reaction time to be completed. When using *N*-tosyl derivative **1b** as substrate, we obtained **6f** and **6g** as a 4:1 *trans*:*cis* mixture showing the lack of total diastereoselectivity in this case (entries 6−7). With *gem*-disubstituted cyclopropenes **1c**,**d**, the reaction needed 24 h to be completed, achieving a 4:1 mixture of isomers in the case of **6h** and only *trans* isomer **6i** (entries 8−9). Interestingly, this latter substrate, **1d**, gave an only product, **6j**, when it was submitted to reaction with the 85:15 mixture of *trans*:*cis* 2-butenyl bromide.

The stereochemical outcome in the reaction of entry 10 (product **6j**) suggests that the mixtures of stereoisomers **6d** differ in the configuration of the cyclopropane stereocenter, sharing the same relative stereochemistry at the cyclopentane. In order to gain more insight in this point, we prepared *E* and *Z* isomers of 2-butenyl bromide as shown in Scheme 2. These bromides were converted into the substrates **2d***E* and **2d***Z*, which retained the stereochemistry of the double bond.

In the reaction of compound **2d***Z*, the rearrangement needed 2 days to be completed, leading to an only isomer that was assigned as **6da** because of the correlation seen in a NOE experiment between the protons shown. Interestingly, **2d***E*, after only 3 h of reaction was totally converted into a 4:1 mixture of isomers **6da** and **6db**. As reported by Xu,[10] this difference in reaction rate is due to the higher energy of the possible transition states coming from the *Z*-isomer, making this reaction more selective.

Table 1. Optimization of the One Pot Conversion of 1a into 6a



no.[a]	solvent	temp. (°C)	time (h)	[M]	conv. (%)[b]	yield (%)[c]
1	THF	rt	24	0.1	10	
2	THF	reflux	3	0.1	20	
3	THF	reflux	24	0.1	>99	(68)
4	2-MeTHF	reflux	3	0.1	70	
5	2-MeTHF	reflux	4	0.1	85	
6	2-MeTHF	reflux	6	0.1	>99	(79)
7	$ClCH_2CH_2Cl$	reflux	6	0.1	80	(51)
8	Dioxane	reflux	6	0.1	90	(48)
9	2-MeTHF	reflux	6	0.2	>99	(78)
10	2-MeTHF	reflux	6	0.3	>99	(78)
11[d]	2-MeTHF	reflux	6	0.3	>99	(72)

[a]Conditions: NaH (1.2 equiv), KI (0.1 equiv), 3,3-dimethylallyl bromide (1.3 equiv). [b]Conversion was measured by ^{1}H NMR. [c]In pure product. [d]Reaction with 3.15 mmol (900 mg) of **1a**, yielded 789 mg of **6a** (72%).

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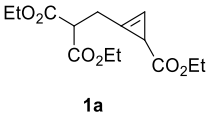
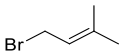
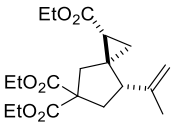
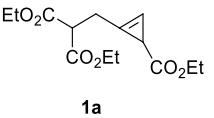
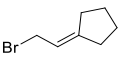
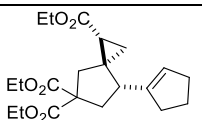
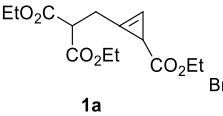
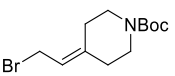
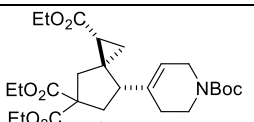
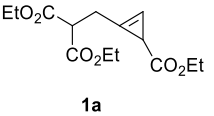
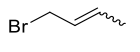
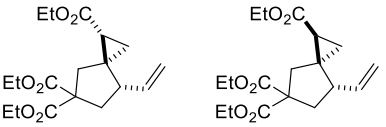
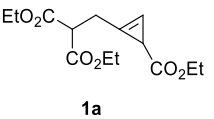
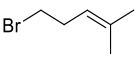
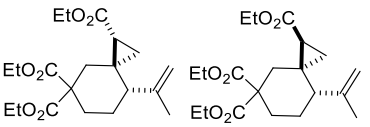
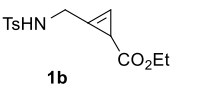
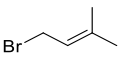
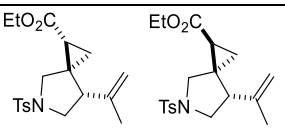
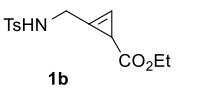
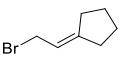
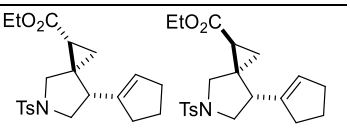
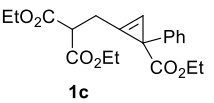
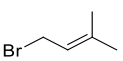
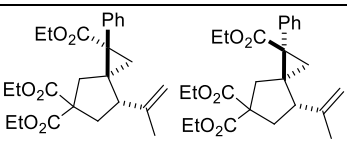
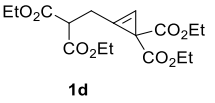
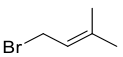
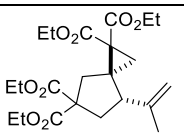
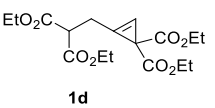
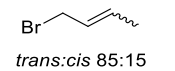
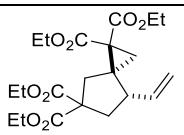
Table 2. Synthesis of Spiro Compounds 6[a]

Entry	Cyclopropene	Bromide	Product, (Yield)[b]
1	 **1a**		 **6a** (78%)
2	 **1a**		 **6b** (73%)
3	 **1a**		 **6c** (74%)
4	 **1a**	 *trans:cis* 85:15	 **6da** (4 :1, 76%) **6db**
5	 **1a**		 **6e**, (2 :1, 65%)
6	 **1b**		 **6f**, (4 :1, 79%)
7	 **1b**		 **6g**, (4 :1, 71%)
8	 **1c**		 **6h**, (4 :1, 70%)
9	 **1d**		 **6i** (72%)
10	 **1d**	 *trans:cis* 85:15	 **6j** (43%)

[a]Reaction conditions: cyclopropene (1 equiv, 0.3 M in 2-MeTHF at 0 °C), NaH 60% (1.2 equiv), and KI (0.1 equiv), reaction time: 10 min. Then bromoalkene (1.3 equiv, 6 h reflux). [b]In pure product except **6j**, which was contaminated with a 5% of an inseparable impurity.

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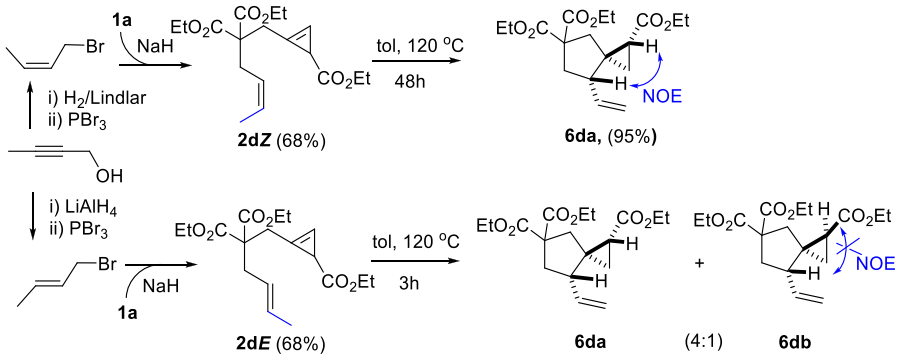
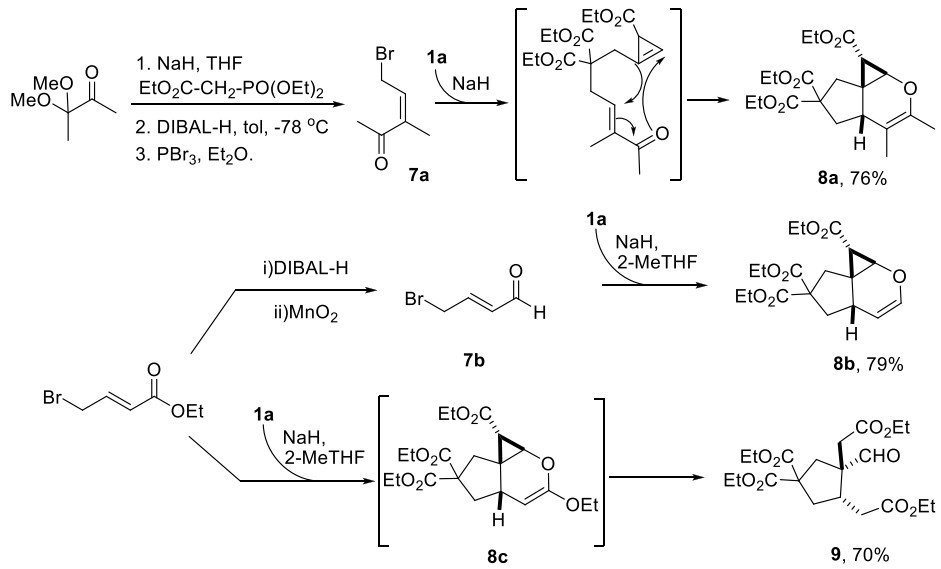
Scheme 2. Synthesis of 2d*Z* and 2d*E* and Rearrangement into Products 6d



Scheme 3. Synthesis of Products 8a,b and 9 by Reaction of 1a with Carbonylallyl Bromides



In order to extend the scope of the methodology we prepared bromo-ketone **7a**, from 3,3-dimethoxybutan-2-one through a Horner−Wadsworth−Emmons reaction, followed by reduction and bromination of the resulting alcohol. This bromide is unstable and totally decomposes after 24 h, so it was used in the next step just after the synthesis, reacting with cyclopropane **1a**. This reaction produced product **8a** in 76% of yield. Formation of **8a** can be explained by an attack of the carbonyl oxygen to the cyclopropene as shown in Scheme 3. Some examples of intermolecular cyclizations of this type were previously reported.[12] The parent (*E*)-4-bromo-2-butenal **7b**, prepared from ethyl 4-bromocrotonate using DIBAL-H and further oxidation with MnO_2,[13] led to product **8b**, which was isolated in 79% yield. The relative stereochemistry of these products was assigned based on the coupling constants of the cyclopropane hydrogens, which were typically *trans* (1.3−1.7 Hz), and with the NOESY spectrum of **8b**. We next submitted ethyl 4-bromocrotonate to the same reaction conditions with **1a**. In this case product **8c** was not isolated, but structure **9** (70% of yield) was a result of the ketal hydrolysis.

In summary, we have developed a one-pot strategy of alkenylation of cyclopropenes and further Alder-ene rearrangement that produces (aza)spiro[2.4]heptanes **6** with high yields and general good diastereoselectivity. Interestingly, extension to spiro[2.5]octanes is possible. A novel process that produces cyclopenta[*c*]cyclopropa[*b*]pyrans **8** is observed when using *α*,*β*-unsaturated bromocarbonyl compounds for the alkenylation.

EXPERIMENTAL SECTION

General Procedure for Spirocycle Formation. A solution of the cyclopropene (1 equiv) in 2-MeTHF (0.3 M) is cooled at 0 °C in an ice−water bath. NaH 60% (1.2 equiv) and KI (0.1 equiv) are added. Ten min after, the corresponding bromoalkene is added (1.3 equiv). After 10 min the reaction is heated at reflux for 6 h in an oil bath. A saturated solution of NH_4Cl (5 mL/mmol) is then added. The aqueous phase is extracted with AcOEt (2 × 25 mL/mmol). The combined organic layers are washed with brine and dried over $MgSO_4$, filtered, and concentrated in vacuo. The product is purified by column chromatography using mixtures of Hex/AcOEt as eluent.

Triethyl (1*R*,3*R*,7*S*)-7-(prop-1-en-2-yl)spiro[2.4]heptane-1,5,5-tricarboxylate, 6a. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (**1a**, 100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and 1-bromo-3-methylbut-2-ene (0.05 mL, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil (95 mg, 78%). A larger scale reaction was performed using 900 mg (3.15 equiv) of **1a** in 11 mL of 2-MeTHF, NaH 60% w/w (153 mg, 3.78 mmol), KI (54 mg, 0.315 mmol), and 1-bromo-3-methylbut-2-ene (0.45 mL, 4,14 mmol). After purification, 788 mg (72%) of **6a** were isolated. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (t, 1H, J = 1.8 Hz), 4.66 (s, 1H), 4.24−4.09 (m, 6H), 2.74 (dd, 1H, J = 10.2, 7.9 Hz), 2.62 (dd, 1H, J = 14.3, 1.6 Hz), 2.55 (ddd, 1H, J = 13.3, 7.8, 1.6 Hz), 2.34 (d, 1H, J = 14.3 Hz), 2.27 (dd, 1H, J =

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13.2, 10.3 Hz), 1.65 (s, 3H), 1.46 (dd, 1H, J = 8.5, 6.0 Hz), 1.30−1.22 (m, 9H), 1.15 (dd, 1H, J = 6.0, 4.7 Hz), 0.97 (dd, 1H, J = 8.6, 4.7 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.7, 171.8, 171.7, 143.7, 114.2, 61.63, 61.56, 60.5, 59.7, 52.3, 38.5, 37.8, 33.8, 27.0, 19.4, 17.9, 14.5, 14.22, 14.17. IR (NaCl) 3018, 2990, 2978, 1742, 1728 cm^{-1}. Anal. Calcd for $C_{19}H_{28}O_6$: C, 64.75; H, 8.01. Found: C, 64.61; H, 8.10.

Triethyl (1*R*,3*R*,7*S*)7-(cyclopent-1-en-1-yl)spiro[2.4]-heptane-1,5,5-tricarboxylate, 6b. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and (2-bromoethylidene)-cyclopentane[14] (80 mg, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil (96 mg, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, 1H), 4.26−4.04 (m, 6H), 2.87 (dd, 1H, J = 9.7, 8.1 Hz), 2.58 (dd, 1H, J = 14.3, 1.0 Hz), 2.51 (ddd, 1H, J = 13.2, 7.6, 1.0 Hz), 2.37 (d, 1H, J = 14.4 Hz), 2.27 (dd, 1H, J = 13.2, 10.1 Hz) 2.27−2.10 (m, 4H), 1.91−1.74 (m, 2H), 1.46 (dd, 1H, J = 8.5, 5.9 Hz), 1.28−1.21 (m, 9H), 1.09 (dd, 1H, J = 5.8, 4.8 Hz), 0.92 (dd, 1H, J = 8.5, 4.6 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.8, 172.0, 171.8, 142.7, 127.9, 61.53, 61.49, 60.4, 59.3, 46.0, 38.8, 37.7, 34.4, 32.8, 32.2, 25.7, 23.6, 17.9, 14.5, 14.2, 14.1. IR (NaCl) 3021, 2991, 2980, 1739, 1727 cm^{-1}. Anal. Calcd for $C_{21}H_{30}O_6$: C, 66.65; H, 7.99. Found: C, 66.53; H, 8.06.

Triethyl (1*R*,3*R*,7*S*)-7-(1-(*tert*-butoxycarbonyl)-1,2,3,6-tetra-hydropyridin-4-yl)spiro[2.4]heptane-1,5,5-tricarboxylate, 6c. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)-malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and *tert*-butyl 4-(2-bromoethylidene)piperidine-1-carboxylate[15] (133 mg, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil (127 mg, 74%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.36 (s, 1H), 4.24−4.07 (m, 6H), 3.95 (d, 1H, J = 14.9 Hz), 3.78−3.67 (m, 1H), 3.70 (dq, 1H, J = 18.2, 2.1 Hz), 3.19−3.13 (m, 1H), 2.73 (t, 1H, J = 9.3 Hz), 2.61 (d, 1H, J = 14.3 Hz), 2.51 (ddd, 1H, J = 13.3, 7.8, 1.3 Hz), 2.32 (d, 1H, J = 14.3 Hz), 2.24 (dd, 1H, J = 13.2, 10.3 Hz), 2.14−2.94 (m, 1H), 1.95 (d, 1H, J = 16.1 Hz), 1.45−1.42 (m, 10H), 1.29−1.21 (m, 9H), 1.14 (t, 1H, J = 5.3 Hz), 0.87 (dd, 1H, J = 8.6, 4.7 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.5, 171.7, 171.6, 155.0, 135.1, 122.3, 79.8, 61.7, 61.6, 60.6, 59.6, 51.8, 43.4, 39.6, 38.1, 37.9, 33.8, 28.6, 26.7, 25.8, 17.8, 14.5, 14.2, 14.1. IR (NaCl) 3011, 2991, 2985, 1736, 1730, 1725 cm^{-1}. Anal. Calcd for $C_{26}H_{39}NO_8$: C, 63.27; H, 7.96. Found: C, 63.40; H, 8.02.

Triethyl 7-vinylspiro[2.4]heptane-1,5,5-tricarboxylate, 6d. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)-malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and 1-bromo-but-2-ene (85:15 *E*:*Z* mixture, 0.05 mL, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil containing a mixture of isomers **6da**:**6db** in a 4:1 ratio (109 mg, 76%). As this mixture could not be separated, data for **6da** are given from its synthesis from **2d***Z* (see below) and data for **6db** were taken from the spectra of the mixture as follows.

Triethyl (1*S*,3*R*,7*S*)-7-vinylspiro[2.4]heptane-1,5,5-tricar-boxylate, 6db. ^1H NMR (400 MHz, $CDCl_3$) 5.49 (ddd, 1H, J = 17.1, 10.2, 8.5 Hz,), 5.02−4.91 (m, 2H), 4.21−4.05 (m, 6H), 2.68−2.59 (m, 1H), 2.58−2.49 (m, 2H), 2.46−2.39 (m, 1H), 2.08 (dd, 1H, J = 12.9, 9.4 Hz), 1.67 (dd, 1H, J = 8.6, 5.5 Hz), 1.26−1.18 (m, 10H), 0.85 (dd, 1H, J = 8.5, 4.7 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.6, 172.1, 171.7, 137.5, 117.1, 61.57, 61.49, 60.46, 58.9, 48.6, 40.07, 38.0, 34.9, 21.7, 17.6, 14.4, 14.12, 14.08.

Triethyl (1*R*,3*R*,7*S*)-7-vinylspiro[2.4]heptane-1,5,5-tricar-boxylate, 6da. A solution of **2d***Z* (50 mg, 0.15 mmol) in 1.5 mL of toluene was heated to 120 °C (oil bath) for 2 days in a sealed vessel. The solvent was evaporated in vacuo. The crude reaction was purified in silica gel in Hex/AcOEt (19:1) to afford a yellow oil (45 mg, 90%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.42 (ddd, 1H, J = 16.9, 10.2, 8.7 Hz), 5.03−4.96 (m, 2H), 4.21−4.10 (m, 6H), 2.71−2.64 (m, 1H), 2.58−2.53 (m, 2H), 2.43 (d, 1H, J = 14.4 Hz), 2.20 (dd, 1H, J = 13.3, 10.0 Hz), 1.51 (dd, 1H, J = 8.5, 5.9 Hz), 1.29−1.22 (m, 9H), 1.10 (dd, 1H, J = 8.5, 4.7 Hz), 1.04 (t, 1H, J = 5.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.7, 172.0, 171.8, 137.0, 117.4, 61.49, 61.47, 60.4, 58.6, 48.0, 40.0, 38.3, 34.6, 22.6, 16.9, 14.3, 14.02, 13.99. IR (NaCl) 3013, 2993, 2987, 1736, 1728 cm^{-1}. Anal. Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74. Found: C, 64.05; H, 7.62.

Triethyl 8-(prop-1-en-2-yl)spiro[2.5]octane-1,5,5-tricarbox-ylate, 6e. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)-methyl)malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and 5-bromo-2-methylpent-2-ene (0.06 mL, 0.46 mmol) during 3 days of reaction. The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil containing a mixture of isomers in a ratio 2:1 (83 mg, 65%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.04 (bs, 1H a), 4.99 (s, 1H b), 4.92 (t, 1H b, J = 1.5 Hz), 4.69 (s, 1H b,), 4.30−4.07 (m, 12H a+b), 2.52 (dd, 1H b, J = 14.1, 1.8 Hz), 2.41−2.34 (m, 1H b), 2.31 (d, 1H a, J = 14.4 Hz), 2.25−2.13 (m, 4H a+b), 2.02−1.92 (m, 4H a +b), 1.90−1.83 (m, 1H b) 1.74−1.66 (m, 1H b), 1.72 (s, 3H a), 1.70 (s, 3H b), 1.63 (dd, 1H a, J = 8.0, 5.8 Hz), 1.53 (dd, 1H b, J = 8.3, 5.9 Hz), 1.45−1.40 (m, 1H a), 1.31−1.19 (m, 18H a+b), 1.16 (dd, 1H a, J = 5.8, 4.5 Hz), 1.03 (t, 1H b, J = 5.1 Hz), 0.91−0.88 (m, 1H b), 0.72 (ddd, 1H a, J = 8.0, 4.4, 1.1 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 172.3, 172.13, 172.09, 171.7, 171.40, 171.38, 144.3, 143.4, 114.1, 113.8, 61.5, 61.2, 60.6, 60.5, 55.2, 55.0, 49.3, 48.3, 33.7, 31.1, 29.0, 28.6, 27.8, 27.6, 26.9, 26.8, 24.5, 23.3, 22.9, 19.7, 16.2, 14.6, 14.5, 14.20, 14.15. IR (NaCl) 3012, 2993, 2987, 1735, 1727 cm^{-1}. Anal. Calcd for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25. Found: C, 65.41; H, 8.35.

Ethyl (1*R*,3*R*,7*S*)-7-(prop-1-en-2-yl)-5-tosyl-5-azaspiro[2.4]-heptane-1-carboxylate, 6f.[10] Title compound was synthesized following the general procedure using ethyl 2-(((4-methylphenyl)-sulfonamido)methyl)cycloprop-2-ene-1-carboxylate (100 mg, 0.34 mmol) in 1.1 mL of 2-MeTHF, NaH 60% w/w (16 mg, 0.41 mmol), KI (6 mg, 0.034 mmol) and 1-bromo-3-methylbut-2-ene (0.05 mL, 0.44 mmol). The crude was purified in silica gel using Hex/AcOEt (4:1) as eluent to afford a yellow oil containing a 4:1 mixture of isomers (97 mg, 79%). Spectroscopic data for the major isomer: ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, 2H, J = 8.2 Hz), 7.32 (d, 2H, J = 8.0 Hz), 4.77 (s, 1H), 4.62 (s, 1H), 4.16−4.01 (m, 2H), 3.43 (dd, 1H, J = 9.8, 7.9 Hz), 3.38−3.32 (m, 2H), 3.31 (dd, 1H, J = 9.9, 5.7 Hz), 2.62 (dd, 1H, J = 7.4, 6.1 Hz), 2.43 (s, 3H), 1.62 (s, 3H), 1.53 (dd, 1H, J = 8.7, 5.9 Hz), 1.23 (t, 3H, J = 7.2 Hz), 1.16 (t, 1H, J = 5.5 Hz), 1.01 (dd, 1H, J = 8.7, 5.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 171.9, 143.7, 142.0, 133.1, 129.7, 128.0, 114.9, 60.9, 51.6, 51.5, 51.4, 33.2, 25.5, 21.7, 19.9, 17.4, 14.4. IR (NaCl) 3015, 2992, 2981, 1730 cm^{-1}. Anal. Calcd for $C_{19}H_{25}NO_4S$: C, 62.79; H, 6.93; N, 3.85; S, 8.82. Found: C, 62.92; H, 6.98; N, 3.74 S, 8.71.

Ethyl (1*R*,3*R*,7*S*)-7-(cyclopent-1-en-1-yl)-5-tosyl-5-azaspiro-[2.4]heptane-1-carboxylate, 6g. Title compound was synthesized following the general procedure using ethyl 2-(((4-methylphenyl)-sulfonamido)methyl)cycloprop-2-ene-1-carboxylate (100 mg, 0.34 mmol) in 1.1 mL of 2-MeTHF, NaH 60% w/w (16 mg, 0.41 mmol), KI (6 mg, 0.034 mmol) and (2-bromoethylidene)-cyclopentane[14] (76 mg, 0.044 mmol). The crude was purified in silica gel using Hex/AcOEt (4:1) as eluent to afford a yellow oil containing a 4:1 mixture of isomers (94 mg, 71%). Spectroscopic data for the major isomer: ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, 2H, J = 8.2 Hz), 7.31 (d, 2H, J = 8.0 Hz), 5.37 (s, 1H), 4.14−3.98 (m, 2H), 3.47 (dd, 1H, J = 9.6, 7.3 Hz), 3.41 (d, 1H, J = 10.9 Hz), 3.36 (d, 1H, J = 10.9 Hz), 3.28 (dd, 1H, J = 9.7, 6.4 Hz), 2.74 (t, 1H, J = 6.8 Hz), 2.42 (s, 3H), 2.26−2.03 (m, 4H), 1.86−1.70 (m, 2H), 1.52 (dd, 1H, J = 8.6, 5.8 Hz), 1.22 (t, 3H, J = 7.1 Hz), 1.07 (t, 1H, J = 5.4 Hz), 0.95 (dd, 1H, J = 8.7, 5.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 171.9, 143.5, 140.4, 133.5, 129.7, 128.7, 127.8, 60.8, 51.9, 51.0, 45.7, 33.8, 33.4, 32.2, 24.6, 23.4, 21.6, 16.9, 14.4. IR (NaCl) 3015, 2992, 2981, 1730 cm^{-1}. Anal. Calcd for $C_{21}H_{27}NO_4S$: C, 64.76; H, 6.99; N, 3.60; S, 8.23. Found: C, 64.86; H, 6.89; N, 3.69; S, 8.13.

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Triethyl (3*R*,7*S*)-1-phenyl-7-(prop-1-en-2-yl)spiro[2.4]-heptane-1,5,5-tricarboxylate, 6h. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)-3-phenylcycloprop-1-en-1-yl)methyl)malonate (100 mg, 0.28 mmol) in 1 mL of 2-MeTHF, NaH 60% w/w (13 mg, 0.33 mmol), KI (6 mg, 0.028 mmol), and 1-bromo-3-methylbut-2-ene (81 mg, 0.36 mmol) in 24 h of reaction. The crude was purified in silica gel using Hex/AcOEt (4:1) as eluent to afford a yellow oil containing a mixture of isomers in a ratio 4:1. (83 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.20 (m, 10H a+b), 4.82−4.79 (m, 1H b), 4.74 (s, 1H b), 4.41−3.95 (m, 12H a+b), 4.35 (s, 1H a), 3.49 (d, 1H a, J = 1.46 Hz), 2.95 (t, 1H b, J = 7.9 Hz) 2.74 (ddd, 1H b, J = 13.4, 8.4, 0.8 Hz), 2.66 (dd, 1H a, J = 14.6, 1.3 Hz), 2.59−2.51 (m, 2H a), 2.47 (d, 1H a, J = 14.5, 1.3 Hz) 2.36 (d, 1H b, J = 14.8 Hz), 2.21−2.11 (m, 2H, a+b), 1.87 (d, 1H a, J = 5.1 Hz), 1.79 (d, 1H b, J = 5.6 Hz), 1.75 (d, 1H b, J = 15.6 Hz), 1.72 (s, 3H b), 1.49 (s, 3H a), 1.44 (d, 1H b, J = 5.6 Hz), 1.34 (d, 1H a, J = 5.1 Hz), 1.32−1.17 (m, 18H a+b). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.8, 171.73, 171.68, 171.63, 171.60, 170.9, 145.2, 144.3, 136.8, 135.6, 131.8, 131.0, 128.0, 127.8, 127.3, 127.1, 114.2, 113.5, 61.6, 61.53, 61.51, 61.1, 61.0, 60.0, 59.5, 48.9, 47.8, 40.9, 40.1, 39.7, 39.4, 39.2, 38.7, 38.5, 38.4, 21.9, 21.0, 20.0, 18.6, 14.3, 14.2, 14.1, 14.0. IR (NaCl) 3015, 2995, 2988, 1731, 1725 cm^{-1}. Anal. Calcd for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 70.20; H, 7.43.

Tetraethyl (3*R*,7*S*)-7-(prop-1-en-2-yl)spiro[2.4]heptane-1,1,5,5-tetracarboxylate, 6i. Title compound was synthesized following the general procedure using diethyl 2-(3-ethoxy-2-(ethoxycarbonyl)-3-oxopropyl)cycloprop-2-ene-1,1-dicarboxylate (100 mg, 0.28 mmol) in 1 mL of 2-MeTHF, NaH 60% w/w (13 mg, 0.33 mmol), KI (6 mg, 0.028 mmol), and 1-bromo-3-methylbut-2-ene (81 mg, 0.36 mmol). The crude was purified in silica gel using Hex/AcOEt (4:1) as eluent to afford a yellow oil (85 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.74 (bs, 1H), 4.58 (bs, 1H), 4.28−4.10 (m, 8H), 2.85 (t, 1H, J = 8.5 Hz), 2.77 (dd, 1H, J = 14.5, 1.6 Hz), 2.63 (ddd, 1H, J = 13.1, 8.4, 1.8 Hz), 2.34 (d, 1H, J = 14.5 Hz), 2.25 (dd, 1H, J = 13.2, 8.6 Hz), 1.66 (s, 3H), 1.59 (d, 1H, J = 5.4 Hz), 1.44 (d, 1H, J = 5.3 Hz), 1.29−1.19 (m, 12H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.5, 170.9, 169.0, 167.3, 144.2, 114.6, 61.7, 61.6, 61.5, 61.2, 60.0, 48.4, 39.9, 39.6, 38.9, 38.5, 22.8, 19.0, 14.2, 14.14, 14.10, 14.05. IR (NaCl) 3012, 2990, 2986, 1734, 1727 cm^{-1}. Anal. Calcd for $C_{22}H_{32}O_8$: C, 62.25; H, 7.60. Found: C, 62.36; H, 7.50.

Tetraethyl (3*R*,7*S*)-7-vinylspiro[2.4]heptane-1,1,5,5-tetracarboxylate, 6j. Title compound was synthesized following the general procedure using diethyl 2-(3-ethoxy-2-(ethoxycarbonyl)-3-oxopropyl)cycloprop-2-ene-1,1-dicarboxylate (100 mg, 0.28 mmol) in 1 mL of 2-MeTHF, NaH 60% w/w (13 mg, 0.34 mmol), KI (6 mg, 0.028 mmol), and 1-bromo-but-2-ene (85:15 *E*:*Z* mixture, 0.04 mL, 0.36 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a colorless oil (49 mg, 43%). The product contained an impurity (ca. 5%) that could not be separated. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (ddd, 1H, J = 17.0, 10.0, 9.1 Hz), 4.98 (dd, 1H, J = 10.2, 1.6 Hz), 4.90 (dd, 1H, J = 17.0, 1.0 Hz), 4.22−4.15 (m, 8H), 2.88−2.80 (m, 1H), 2.68 (d, 1H, J = 14.6 Hz), 2.62 (dd, 1H, J = 13.5, 8.0 Hz), 2.46 (d, 1H, J = 14.6 Hz), 2.22 (dd, 1H, J = 13.4, 8.4 Hz), 1.59 (d, 1H, J = 5.4 Hz), 1.47 (d, 1H, J = 5.4 Hz), 1.29−1.22 (m, 12H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171,8, 171.5, 169.1, 167.3, 137.9, 117.2, 61.8, 61.7, 61.6, 61.2, 58.8, 45.3, 40.7, 40.1, 39.1, 37.5, 22.5, 14.22, 14.17, 14.1. IR (NaCl) 3010, 2988, 1735, 1730 cm^{-1}.

Triethyl (1*S*,1a*S*,4a*R*,7a*S*)-3,4-dimethyl-1,1a,4a,5-tetrahydrocyclopenta[*c*]cyclopropa[*b*]pyran-1,6,6(7*H*)-tricarboxylate, 8a. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)-methyl)malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and 5-bromo-3-methylpent-3-en-2-one (0.04 mL, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil (97 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 (d, 1H, J = 1.3 Hz), 4.21−4.07 (m, 6H), 2.80 (d, 1H, J = 14.1 Hz), 2.72−2.63 (m, 2H), 2.43 (d, 1H, J = 1.4 Hz), 2.40 (d, 1H, J = 14.1 Hz), 2.00−1.93 (m, 1H), 1.64 (s, 3H), 1.59 (s, 3H), 1.28−1.19 (m, 9H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 172.3, 171.8, 171.0, 140.5, 104.6, 62.6, 61.7, 61.5, 60.5, 58.1, 41.5, 40.9, 36.5, 34.9, 22.0, 15.6, 14.4, 14.3, 14.2, 14.1. IR (NaCl) 2994, 2988, 1726, 1721 cm^{-1}. Anal. Calcd for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.05; H, 7.50.

Triethyl (1*S*,1a*R*,4a*S*,7a*S*)-1,1a,4a,5-tetrahydrocyclopenta-[*c*]cyclopropa[*b*]pyran-1,6,6(7*H*)-tricarboxylate, 8b. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and 4-bromobut-2-enal (62 mg, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (9:1) as eluent to afford a yellow oil (97 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (dd, 1H, J = 6.1, 2.9 Hz), 4.87 (dd, 1H, J = 6.1, 1.6 Hz), 4.47 (d, 1H, J = 1.7 Hz), 4.21−4.08 (m, 6H), 2.83 (d, 1H, J = 14.0 Hz), 2.73−2.64 (m, 2H), 2.62 (d, 1H, J = 1.6 Hz), 2.37 (d, 1H, J = 14.0 Hz), 1.89 (t, 1H, J = 11.8 Hz), 1.23 (m, 9H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 172.1, 171.4, 170.5, 139.7, 102.8, 63.0, 61.6, 61.4, 60.4, 57.5, 41.0, 37.1, 35.7, 34.7, 21.5, 14.2, 14.00, 13.97. IR (NaCl) 3013, 2995, 2987, 1730, 1725 cm^{-1}. Anal. Calcd for $C_{18}H_{24}O_7$: C, 61.35; H, 6.87. Found: C, 61.48; H, 6.80.

Diethyl (3*R*,4*S*)-3,4-bis(2-ethoxy-2-oxoethyl)-3-formylcyclo-pentane-1,1-dicarboxylate, 9. Title compound was synthesized following the general procedure using diethyl 2-((3-(ethoxycarbonyl)-cycloprop-1-en-1-yl)methyl)malonate (100 mg, 0.35 mmol) in 1.2 mL of 2-MeTHF, NaH 60% w/w (17 mg, 0.42 mmol), KI (6 mg, 0.035 mmol), and ethyl 4-bromocrotonate (0.06 mL, 0.46 mmol). The crude was purified in silica gel using Hex/AcOEt (4:1) as eluent to afford a yellow oil (100 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.52 (s, 1H), 4.25−4.06 (m, 8H), 2.91 (d, 1H, J = 15.1 Hz), 2.76 (d, 1H, J = 16.7 Hz), 2.68−2.58 (m, 3H), 2.39−2.30 (m, 2H), 2.19 (dd, 1H, J = 15.8, 8.9 Hz), 2.08 (t, 1H, J = 11.7 Hz), 1.28−1.22 (m, 12H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 201.7, 171.7, 171.6, 171.3, 170.9, 62.0, 61.9, 60.84, 60.81, 58.4, 56.4, 40.3, 39.9, 38.8, 34.1, 33.7, 14.1, 14.0, 13.9. IR (NaCl) 2995, 2987, 2821 1733, 1729, 1725 cm^{-1}. Anal. Calcd for $C_{20}H_{30}O_9$: C, 57.96; H, 7.30. Found: C, 57.88; H, 7.36.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.2c01420.

General experimental procedures, data for compounds **2d***Z*, **2d***E*, and **7a**, and copies of ^{1}H, ^{13}C{^{1}H} NMR spectra of all new products, NOESY for **6a**, **6da**, and **8b**, and HMQC for **6c** (PDF)

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Notes

The authors declare no competing financial interest.

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7. INTRAMOLECULAR DIELS-ALDER REACTION OF CYCLOPROPENYL VINYLARENES: ACCESS TO BENZONORCARANE DERIVATIVES.

This work shows an intramolecular Diels-Alder reaction to access polycyclic compounds from cyclopropenyl vinylarene derivatives. This reaction was promoted by heat without any catalyst. The optimal conditions were achieved using *n*-BuOH as solvent at 140 ^{o}C. We could synthetize polyfused systems in a totally stereoselective way. We also studied the mechanism using NMR techniques. To determine the stereochemistry outcome of the reaction we characterized one product using X-Ray diffraction analysis.

In this work I did the synthesis of some bromine derivatives and some of the final products. I helped to assign the stereogenic centers and during the NMR experiments in the mechanistic studies.

En este trabajo se muestra una reacción intramolecular de Diels-Alder para formar compuestos policíclicos a partir de derivados de ciclopropenil vinilarenos. Esta reacción se da con calefacción y en ausencia de catalizadores. Las condiciones óptimas se obtuvieron usando *n*-BuOH como disolvente a 140 ^{o}C. Pudimos sintetizar sistemas fusionados de una manera totalmente estereoselectiva. También estudiamos el mecanismo de la reacción usando técnicas de RMN. Para determinar la estereoquímica del producto final, se caracterizó uno de ellos mediante difracción de Rayos-X.

En este trabajo realicé la síntesis de algunos derivados bromados y de algunos de los productos finales. Ayudé en la asignación de los centros estereogénicos y durante los experimentos de RMN en el estudio mecanístico.

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Intramolecular Diels–Alder Reaction of Cyclopropenyl Vinylarenes: Access to Benzonorcarane Derivatives

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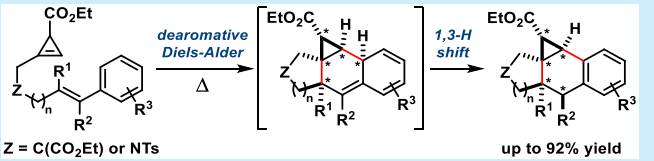
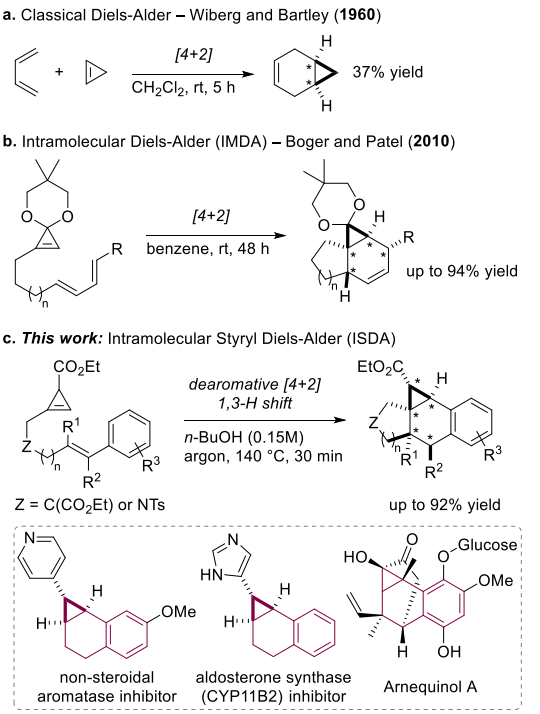
ABSTRACT: Intramolecular Diels–Alder vinylarene reaction (IMDAV) is a [4 + 2] cycloaddition that employs styrene derivatives as conjugated dienes, whose poor reactivity arises from the required loss of aromaticity, which is recovered by a subsequent [1,3]-H shift. Herein, we describe the use of cyclopropene as a dienophile, harnessing its strain energy to drive the IMDAV reaction. Benzonorcarane scaffolds form in good yields, excellent stereoselectivity, and broad functional tolerance. Theoretical calculations and NMR studies have revealed significant mechanistic insights.



Cyclopropenes are the smallest unsaturated cyclic molecules, highly strained,[1] but easily accessible substances with great utility in synthetic methodologies toward natural products, medicines, and novel materials.[2] Their unique and unpredictable reactivity[3] has prompted a diversity of reactivity that exploits the strain energy contained in these small-ring systems to provide a high thermodynamic driving force.[4] For instance, cyclopropenes are known to be exceptional dienophiles in strain-release-driven cycloadditions, such as the Diels–Alder reaction (DA),[5] which represents a powerful stereospecific, diastereo-, and regioselective synthetic method that allows up to two contiguous stereocenters to be generated in a single step (Scheme 1a).[6] In fact, high-strain energy release enables intramolecular Diels–Alder reaction (IMDA) to proceed even at low temperatures, especially with highly reactive substrates that can participate in normal, inverse electron-demand, or neutral Diels–Alder reactions.[7] Thus, Boger and Patel reported in 2010 the first IMDA reaction with cyclopropenes,[8] by using cyclopropenone ketals with tethered monosubstituted dienes (Scheme 1b). Nevertheless, although Diels–Alder reactions with cyclopropenes continue to be reported,[9] their use in the challenging IMDA reaction of vinylarenes (IMDAV) has not yet been described.[10] This reaction employs a styrene-like moiety as the diene, implying a high enthalpy barrier due to loss of aromaticity, usually restored via 1,3-H shift or H_2-extrusion.[11]

In this context, our previous studies on the Ru-catalyzed rearrangement of ene-cyclopropenes had led us to suspect that the vinylarene unit might be involved in a [4 + 2] cycloaddition process leading to the formation of a benzonorcarane derivative.[12] On this basis, we envisioned that it could be feasible to access benzonorcarane structures through an IMDA transformation between a cyclopropene unit and a styrene motif. In this transformation, the harnessing of

Scheme 1. Diels-Alder Reaction with Cyclopropenes



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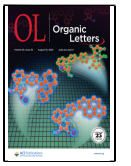

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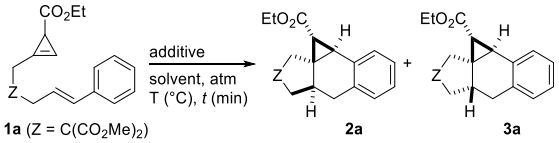
strain energy should be high enough to overcome the energy barrier required for the dearomative cycloaddition, even in the absence of metallic catalysts. Thus, successfully implementing this idea would open a synthetic pathway complementary to existing metal-catalyzed intramolecular cyclopropanations.[13]

Accordingly, we herein describe the first example of an intramolecular Diels–Alder reaction of cyclopropenyl vinylarenes. The method efficiently leads to the generation of unreported polyfused ring systems through the stereoselective generation of up to five stereogenic centers, allowing access to challenging tetracyclic benzonorcaranes (Scheme 1c). These scaffolds are found in many biologically active molecules, such as nonsteroidal aromatase inhibitors (NSAIs) with potential antitumoral activity,[14] or natural compounds such as the recently elucidated Arnequinol A (Scheme 1c).[15]

As mentioned above, when heating diethyl 2-cinnamyl-2-((3-(ethoxycarbonyl)cycloprop-1-en-1-yl)methyl)malonate (**1a**) at 160 °C in toluene under microwave irradiation for 30 min in the presence of 5 mol % of $[(C_6Me_6)RuCl_2]_2$, **1a** did not undergo the expected metal-catalyzed opening of the cyclopropene moiety, and two new products were formed instead. Their isolation and exhaustive NMR analyses (see SI) confirmed the formation, presumably via [4 + 2]-cycloaddition, of two diastereomers: the *endo–anti* **2a** (26% yield) and the *exo–anti* **3a** (4% yield) cycloadducts (Table 1, entry 1). Since this reactivity had no precedent in the literature, we decided to explore its extent, and to that end, it was first necessary to optimize the reaction conditions. Toward that end, we initially evaluated the role of the ruthenium catalyst, and we realized that the absence of ruthenium boosted the yields toward **2a** and **3a** up to 79 and 18%, respectively (Table 1, entry 2). Morevover, microwave irradiation was unnecessary (Table 1, entry 3), increasing the selectivity toward the *endo–anti* isomer **2a** at 140 °C with traditional heating (Table 1, entry 4). Unfortunately, lower temperatures prevented complete conversion of **1a** (Table 1, entry 5).

Table 1. Optimization of Reaction Conditions[a]



#	solvent	temp (°C)	time (min)	conv.[b] (%)	2a[b] (%)	3a[b] (%)
1[c,d]	toluene	160	30	>98	26	4
2[c]	″	″	″	>98	79	18
3	″	″	″	>98	80	16
4	″	140	″	>98	88	10
5	″	120	″	86	74	9
6[e]	″	140	″	>98	<2	<2
7[f]	″	″	″	>98	85	10
8	″	″	60	>98	86	11
9	″	″	15	84	72	10
10	THF	″	″	>98	79	12
11	DMF	″	″	>98	62	8
12	DMSO	″	″	>98	76	9
13	*n*-BuOH	″	″	>98	92	6
14	water	″	″	>98	70	9
15[g]	*n*-BuOH	″	″	>98	87[h]	4

[a]Reaction conditions: 0.15 mmol of **1a** was stirred in solvent (0.15M) under Ar at 140 °C for 30 min. [b]Conversion and yields were determined by ^{1}H NMR using 4-benzyloxybromobenzene as the internal standard. [c]Microwave irradiation. [d]In the presence of $[(C_6Me_6)RuCl_2]_2$ (5 mol %). [e]Under air. [f]In the presence of water (10 equiv). [g]Scale-up to 2.5 mmol of **1a**. [h]Isolated yield upon chromatographic purification.

Likewise, we also detected that oxygen had a detrimental effect given the high degree of decomposition of **1a** when performing the reaction under air (Table 1, entry 6). In contrast, the moisture influence was minimal, and the reaction proceeded well even in the presence of 10 equiv of water (Table 1, entry 7). The inalterability of the outcome when the reaction time was doubled confirmed the thermal stability of products (Table 1, entry 8). However, shorter reaction times prevented the starting substrate from fully reacting (Table 1, entry 9). We also screened different solvents and found a slight decrease in yield when carrying out the reaction in aprotic polar solvents (Table 1, entries 10–12). Greener solvents were also suitable, being able to carry out the reaction in water in acceptable yields (Table 1, entries 13–14). Polar protic *n*-butanol showed the best performance, giving rise to the *endo–anti* isomer **2a** selectively in 92% yield (Table 1, entry 13) and obtaining just traces of the *exo–anti* isomer **3a** under these conditions. It is worth mentioning that this method can be scaled up to 2.5 mmol without an appreciable loss of efficiency, which is essential for practical convenience (entry 15).

Therefore, heating a 0.15 M solution of the substrate (**1**) in *n*-BuOH under an argon atmosphere for 30 min was optimal for further investigations on the structural scope of the reaction (Scheme 2).[16] Substitution on the aromatic moiety was well tolerated, providing the corresponding benzonorcaranes in good to excellent yields (**2b–i**, 66–92% yield). Specifically, good yields were obtained in the presence of electron-donating substituents, such as methoxy (**2b**, 66%) or methyl groups (**2c**, 75%), even in the *ortho* position (**2i**, 75%), obtaining higher yields in the presence of electron-withdrawing substituents (**2d–2h**; 77–92%). These results are in agreement with an inverse electron demand Diels–Alder reaction (IEDDA). In this sense, substrates with reduced aromaticity, such as naphthalene derivatives, showed even higher reactivity, leading directly to final product **2j** (67% yield) during the synthesis of its precursor **1j** at room temperature (see SI). The structure of **2g** was confirmed by X-ray diffraction, unambiguously establishing the stereoselectivity of this reaction.[17] Concerning the olefin moiety, substitution at the internal position of the double bond (**2k**) was tolerated, albeit requiring the addition of a non-nucleophilic base, such as DBU. Likewise, aliphatic and aromatic substituents at the terminal position were also suitable and provided derivatives **2l** and **2n** in 72% and 63% yield, respectively. Remarkably, the reaction with (*E*)-isomer **1l** must be carried out in toluene since *n*-butanol favored the formation of spiro[2.4]heptane **4m** through an Alder-ene mechanism.[18] Unfortunately, the use of *Z*-isomers inevitably led to the aforementioned spiro compounds under the optimized reaction conditions (**4m**, 76% yield). The use of diphenyl derivative **1n** gave rise to the expected product without any additive or solvent variation (**2n**, 63%). The stereochemistry of position-5 was determined by NOESY of derivatives **2l** and **2n**, indicating that rearomatization occurs stereoselectively.

The effect of the tethering group was examined, as well. The elongation of the tether in **1o** required 180 °C for complete conversion. Higher temperatures and flexibility of the anchor caused a light loss of selectivity of the *endo–anti*

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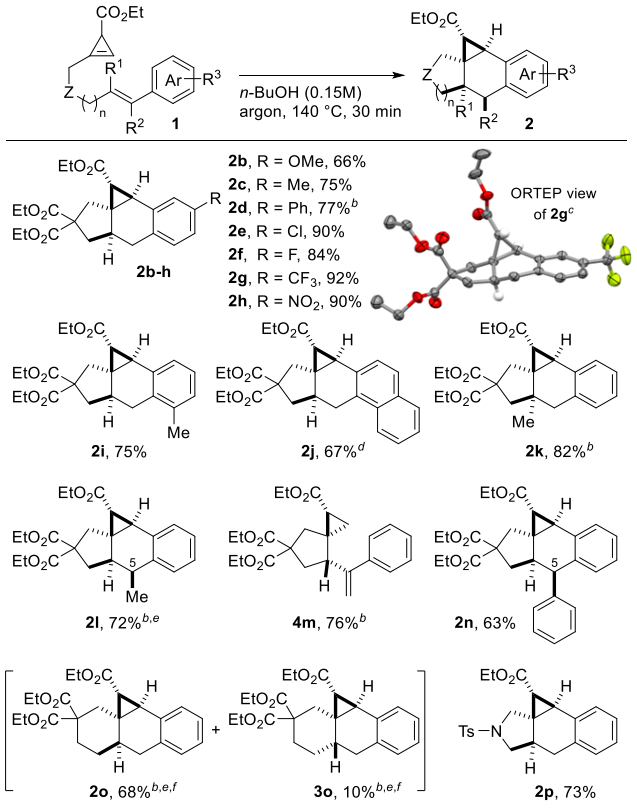
Scheme 2. Substrate Scope[a]



[a]Reaction conditions: 0.15 mmol of **1** was stirred in *n*-BuOH (0.15M) under argon at 140 °C for 30 min. [b]In the presence of 1.2 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). [c]Polar hydrogens were removed for clarity. [d]Generated in the last step of synthesis of **1j**. [e]In toluene. [f]180 °C.

diastereoisomer (**2o**, 68%) in favor of the *exo*−*anti* (**3o**, 10%). Additionally, it was proven that different tethering, e.g., a sulfonamide (**1p**), remained unaltered under the reaction conditions to afford the corresponding dihydroisoindoline **2p** in 73% yield.

The remarkable diastereoselectivity observed attracted our curiosity about the reaction mechanism of both cycloaddition and rearomatization. Since similar transformations have been proved to be stepwise formal [4 + 2] cycloadditions,[19] our reaction was monitored by nuclear magnetic resonance to ensure it proceeds concertedly through a Diels−Alder cycloadduct intermediate (Figure 1). Surprisingly, although this mechanism is widely accepted for IMDAV reactions, evidence is limited.[20] Monitoring could be performed since comparable results were obtained using dimethyl sulfoxide as the solvent (Table 1, entry 12). A first analysis was conducted at room temperature to verify the optimal conditions of **1a**, thus ruling out premature reactivity or decomposition (Figure 1, spectrum 1). While the temperature of the sample was raised, it was observed that **1a** started reacting at ca. 80 °C. After 6 min of reaction (2 min at 115 °C; Figure 1, spectrum 2), three different species were present: starting material **1a**, final product **2a** (Figure 1, spectrum 5), and a new species with characteristic signals in the range from 5.5 to 6.0 ppm. Subsequent spectra showed a gradual disappearance of **1a** over time with an inversely proportional increase of **2a** (Figure 1, spectra 2−4). It should be noted that while aliphatic and aromatic signals of **1a** and **2a** varied appreciably, the new

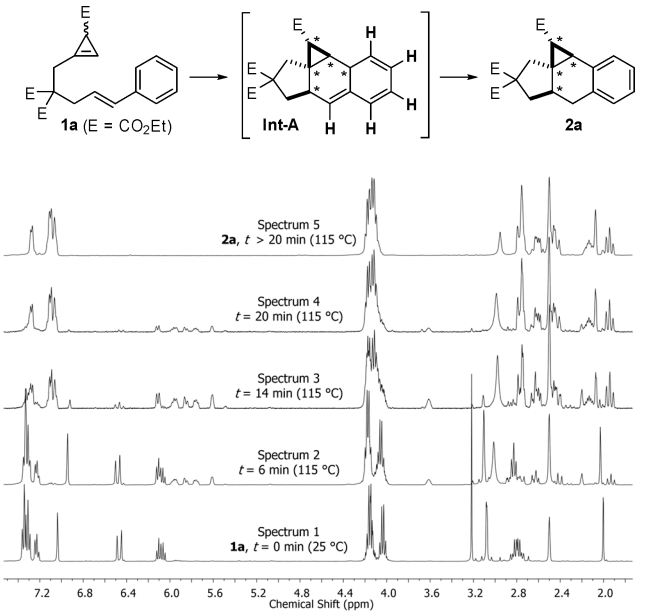


Figure 1. NMR-monitorization.

olefinic signals (5.0 to 6.0 ppm) remained constant over time (see SI). This fact, along with the absence of aromatic signals other than those of **1a** and **2a**, strongly suggests the temporary presence of a nonaromatic intermediate species with multiple olefinic protons, according to a typical Diels−Alder cycloadduct (**Int-A**).

Additionally, to explain the origin of the diastereoselectivity observed, the possible transition states that would afford the Diels−Alder cycloadducts were studied by DFT calculations, using **1a** as the model starting material (Figure 2, see SI for details). According to the lengths of the bonds being formed, all structures correspond to slightly asynchronous concerted processes.

The steric hindrance present in the *syn*-approach of the cyclopropane substituent concerning the diene moiety (higher for *endo* than for the *exo* approach) increases the free energy barrier to values that justify the absence of those products. In the case of the *anti*-approach, the *endo* cycloaddition is favored by 1.2 kcal·mol^{-1}, which predicts a ratio of 88:12, in relatively

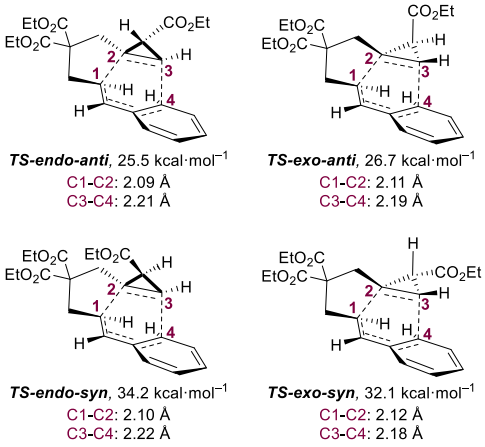


Figure 2. Spatial representation of the most stable transition states derived from **1a**, LC-ωPBE-D3BJ/6-311++G(d,p)/SMD(1-butanol)//LC-ωPBE-D3BJ/6-31G(d)/SMD(1-butanol).

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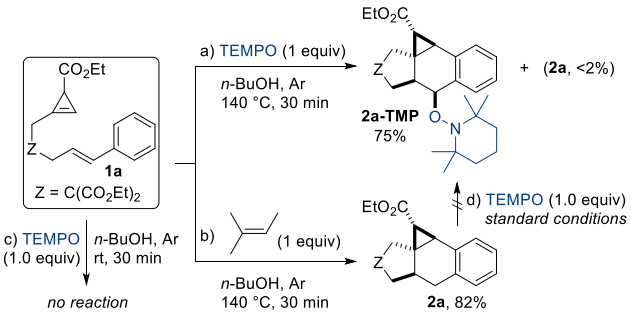
good agreement with the experimental stereoselectivity observed. This selectivity results from favorable geometry for the secondary orbital overlap and attractive electrostatic interactions in the *endo* transition state. The C3−C4 distance (2.21 Å) and the angle at that *syn* hydrogen is distorted from the plane of the cyclopropene ring (122°) are in the expected range.[21]

After a favored *endo*−*anti* [4 + 2] dearomative cycloaddition to give **Int-A**, a fifth new stereocenter is selectively generated at carbon-5 of final product **2a** via a [1,3]-hydrogen shift. Although such a stereoselective step has been observed previously,[22] it is still a matter of debate. Intramolecular suprafacial [1,3]-H shift is geometrically feasible since it does not require a constrained Möbius system of the orbitals involved;[23] however, it is symmetry-forbidden by the Woodward−Hoffmann rules.[24] Some control experiments were carried out to shed light on this aspect (Scheme 3). Under

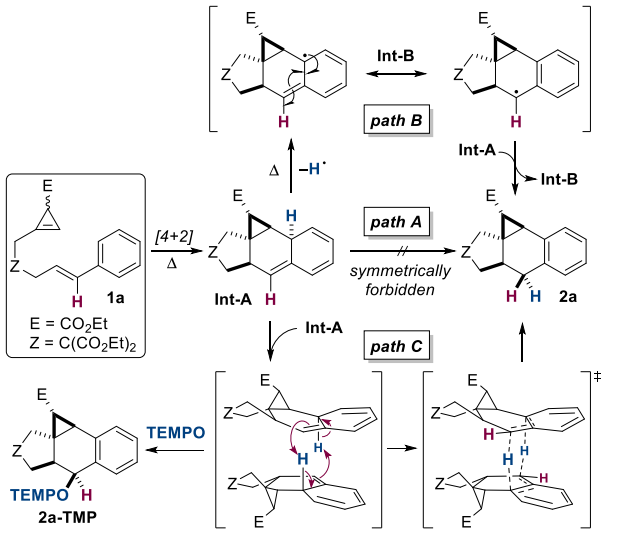
Scheme 3. Control Experiments



the optimized reaction conditions, the presence of a radical scavenger (TEMPO) led to the diastereoselective formation of intercepted product **2a-TMP** in 75% yield (Scheme 3a). Although such a trapped product strongly suggests the generation and intermediation of radical species during the reaction, the invariability observed when using 2-methyl-2-butene as a radical acceptor points in the opposite direction (Scheme 3b). Further experiments were performed to rule out premature coupling of TEMPO and **1a** or any late addition to **2a** (Scheme 3c,d). NMR studies also showed that [4 + 2] cycloaddition occurs before the formation of **2a-TMP** (see SI).

For a related transformation, an elegant alternative to symmetrically forbidden intramolecular 1,3-H shift (Scheme 4, path A) was proposed by Tantillo and Brummond, in which the diastereoselectivity and intermolecularity of rearomatization are rationalized by an intermolecular radical-chain mechanism (Scheme 4, path B).[25] According to this proposal, upon formation of cycloadduct **Int-A**, its C−H bond at position-9a is prone to undergo thermal cleavage,[26] providing the allylic carbon-centered radical **Int-B** capable of promoting an intermolecular hydrogen atom transfer (HAT) with a second **Int-A** molecule. Unfortunately, such an approximation would not explain the observed selective coupling of TEMPO. Alternatively, our mechanistic proposal involves a concerted bimolecular hydrogen rearrangement applicable to both scenarios (Scheme 4, path C). In consequence, two **Int-A** units can exchange hydrogens in a concerted fashion achieving a diastereoselective HAT and joint rearomatization in a single step. Within this scenario, the addition of TEMPO over **Int-A** would proceed on the least hindered face of the olefin moiety, thus promoting a radical rearomatization and explaining the diastereoselective addition of a radical species.

Scheme 4. Mechanistic Proposal



To conclude, we have developed the first example of an intramolecular Diels−Alder reaction through which we can use cyclopropenyl vinylarenes to produce hitherto unreported polyfused ring systems. This transformation proceeds efficiently, generating up to five new stereogenic centers in a stereoselective fashion to access rare but potentially attractive benzonorcarane scaffolds. NMR monitoring has strengthened the hypothesis of a concerted dearomative [4 + 2] mechanism, and theoretical calculations have revealed information about the diastereoselectivity of this reaction. Likewise, mechanistic studies have provided additional information on the rearomatization step, pointing toward a concerted bimolecular mechanism. Besides overcoming the challenge of reacting the styrene moiety with cyclopropene, the reaction is operationally simple as metal catalysts, Lewis acids, or oxidants are not required. Ongoing work is focused on expanding the scope of this chemistry, further derivatization of the cyclopropane unit, its challenging intermolecular version, and its implications in the synthesis of complex molecules.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c01864.

General methods, general experimental procedures, characterization data for new substrates and products (NMR, IR, and MS), details of the mechanistic studies, X-ray data of **2g**, NMR spectra, and theoretical calculations (PDF)

Accession Codes

Deposition Number 2265998 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystal-lographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: + 44 1223 336033.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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(16) It is strongly recommended to use substrates as soon as possible since they can slowly decompose (or react) even at low temperatures (see SI).

(17) Deposition Numbers 2265998 (for **2g**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre.

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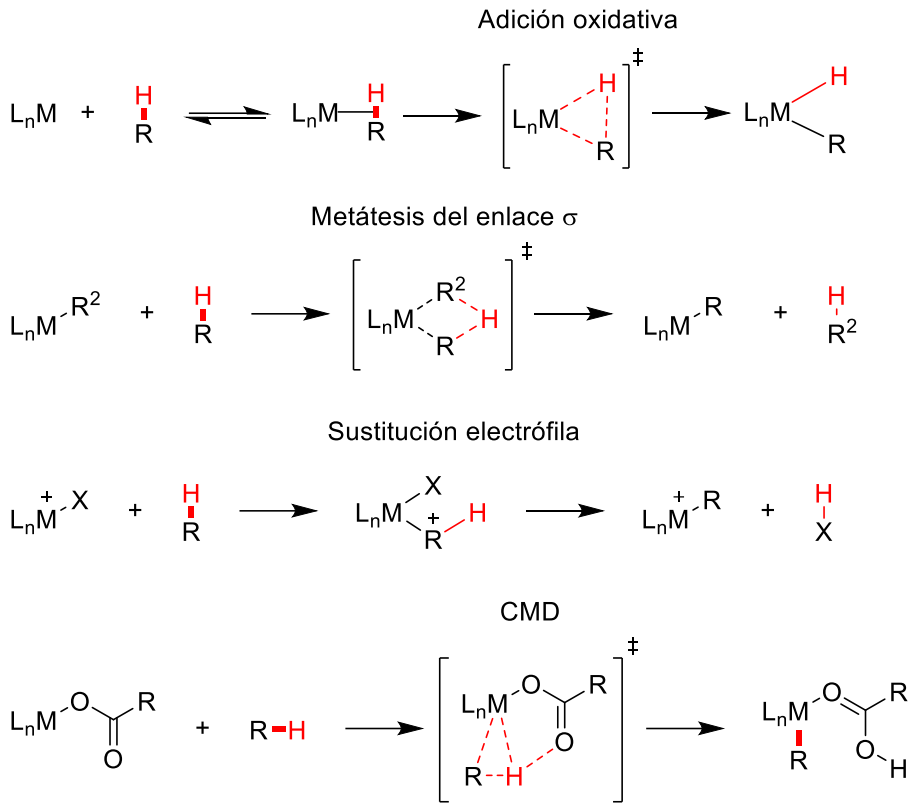
CAPÍTULO III: FUNCIONALIZACIÓN DE ÚLTIMA ETAPA POR ACTIVACIÓN C-H DEL TRIPTÓFANO.

8. ANTECEDENTES

La metodología denominada funcionalización de Última Etapa (LSF por sus siglas en inglés), ha sido definida por Ritter como una transformación quimioselectiva de una molécula compleja para obtener un análogo con la suficiente pureza y cantidad para un propósito concreto. Trata de evitar la prefuncionalización encaminada a preparar dicha transformación en etapas tempranas de la síntesis. Se puede así partir de fármacos conocidos o de productos naturales complejos y obtener derivados en una sola etapa sin realizar una síntesis completa desde productos sencillos.[199] La definición que dio Ackermann de LSF subraya que la transformación más habitual en esta metodología son las activaciones C-H. Al ser realizadas en una molécula de alta complejidad estructural, requieren un excelente nivel de quimioselectividad y, a ser posible, regioselectividad.[200]

Las activaciones C-H son reacciones en las que un enlace carbono-hidrógeno, normalmente inerte, reacciona como si de un grupo funcional se tratase. Permite reducir significativamente el número de etapas de síntesis ya que no es necesaria ninguna reacción de funcionalización previa.[201] Su gran desafío es la selectividad puesto que deben conseguir que reaccione sólo uno de los múltiples enlaces C-H del sustrato. Las activaciones C-H están consideradas como respetuosas con el medioambiente y atractivas económicamente y se han constituido como una poderosa herramienta en síntesis orgánica, ciencias de materiales, etc.[202] Frecuentemente, y sobre todo si el enlace C-H tiene una energía de disociación alta, están catalizada por complejos metálicos y en ese caso, el mecanismo se basa en la conversión del enlace C-H en enlace C-M. Los mecanismos habitualmente aceptados para esta trasformación son la adición oxidativa, la metátesis del enlace σ o la sustitución electrófila.[203] También se han propuesto, basándose en cálculos computacionales, procesos de metalación-desprotonación concertada (CMD) (Esquema 45)[204] o activaciones metal-ligando ambifílicos (AMLA).[205] Los enlaces de menos energía pueden reaccionar a través de mecanismos radicalarios, incluso sin la presencia de complejos metálicos.[206]

[199] Börgel, J.; Ritter, T. *Chem.* **2020**, *6*, 1877-1887.
[200] Guillemard, L.; Kaplaneris, N.; Ackermann, L.; Johansson, M. J. *Nat. Rev. Chem.* **2021**, *5*, 522-545.
[201] Gandeepan, P.; Müller, T.; Zell, D.; Cera, G.; Warratz, S.; Ackermann, L. *Chem. Rev.* **2019**, *119*, 2192-2452.
[202] a) Mercier, L. G.; Leclerc, M. *Acc. Chem. Res.* **2013**, *46*, 1597-1605. b) Li, J.; De Sarkar, S.; Ackermann, L. *Top. Organomet. Chem.* **2015**, *55*, 217-257.
[203] Dyker, G. *Handbook of C-H Transformations: Applications is Organic Synthesis*; Wiley-VCH Verlag GmbH: Weinheim, Germany, **2008**.
[204] a) García-Cuadrado, D.; Braga, A. A. C.; Maseras, F.; Echavarren, A. M. *J. Am. Chem. Soc.* **2006**, *128*, 1066-1067. b) Ackermann, L. *Chem. Rev.* **2011**, *111*, 1315-1345.
[205] Boutadla, Y.; Davies, D. L.; Macgregor, S. A.; Poblador-Bahamonde, A. I. *Dalton Trans.* **2009**, 5820-5831.
[206] Yi, H.; Zhang, G.; Wang, H.; Huang, Z.; Wang, J.; Singh, A.; Lei, A. *Chem. Rev.* **2017**, *117*, 9016-9085.



Esquema 45. Diferentes mecanismos en activaciones C-H.

Hasta ahora, la mayoría de activaciones C-H se han hecho con metales de transición de las series 4 y 5, como paladio, iridio, rodio y rutenio.[207] La principal limitación de estos metales en este tipo de reacciones, además de su elevado coste, es que generalmente son tóxicos. La cantidad permitida de trazas metálicas en los productos farmacéuticos y agroalimentarios constituyen un reto importante a la hora de implementar reacciones catalíticas con dichos metales. Por todo esto, existe una alta demanda en el desarrollo de procedimientos catalíticos que impliquen metales de transición de la tercera serie. Estos metales son menos tóxicos y más abundantes, lo que se traduce en un coste menor,[208] provocando muchos progresos en cuanto al uso de metales como titanio, vanadio, hierro, etc.[209]

Las reacciones selectivas de activación C-H no son las únicas que se usan en LSF. Gracias a los avances en el desarrollo de reacciones quimioselectivas con amplia tolerancia de grupos funcionales,[210] también se utilizan algunos acoplamientos cruzados catalizados por complejos metálicos y luz,[211] química radicalaria, fotoredox, etc. Sin embargo, las reacciones de interconversión de grupos funcionales, o las reacciones de acoplamiento cruzado catalizadas por

[207] a) Choy, P. Y.; Wong, S. M.; Kapdi, A.; Kwong, F. Y. *Org. Chem. Front.* **2018**, *5*, 288-321. b) Sharninghausen, L. S.; Crabtree, R. H. *Isr. J. Chem.* **2017**, *57*, 937-944. c) Wencel-Delord, J.; Patureau, F. W.; Glorius, F. *Top. Organomet. Chem.* **2015**, *55*, 1-27. d) Arockiam, P. B.; Bruneau, C.; Dixneuf, P. H. *Chem. Rev.* **2012**, *112*, 5879-5918.

[208] a) Miao, J.; Ge, H. *Eur. J. Org. Chem.* **2015**, *2015*, 7859-7868. b) Li, Y.; Hu, Y.; Wu, X.-F. *Chem. Soc. Rev.* **2018**, *47*, 172-194. c) Nagae, H.; Kundu, A.; Inoue, M.; Tsurugi, H.; Mashima, K. *Asian J. Org. Chem.* **2018**, *7*, 1256-1269.

[209] a) Kubiak, R.; Prochnow, I.; Doye, S. *Angew. Chem. Int. Ed.* **2010**, *49*, 2626-2629. b) Reis, P. M.; Silva, J. A. L.; Palavra, A. F.; Fraústo da Silva, J. J. R.; Kitamura, T.; Fujiwara, Y.; Pombeiro, A. J. L. *Angew. Chem. Int. Ed.* **2003**, *42*, 821-823. c) Wong, M. Y.; Yamakawa, T.; Yoshikai, N. *Org. Lett.* **2015**, *17*, 442-445. d) Kimura, N.; Kochi, T.; Kakiuchi, F. *J. Am. Chem. Soc.* **2017**, *139*, 14849-14852.

[210] a) Gutekunst, W. R.; Baran, P. S. *Chem. Soc. Rev.* **2011**, *40*, 1976-1991. b) Wencel-Delord, J. Glorius, F. *Nat. Chem.* **2013**, *5*, 369-375.

[211] a) Tellis, J. C.; Kelly, C. B.; Primer, D. N.; Jouffroy, M.; Patel, N. R.; Molander, G. A. *Acc. Chem. Res.* **2016**, *49*, 1429-1439. b) Twilton, J.; Le, C.; Zhang, P.; Shaw, M. H.; Evans, R. W.; MacMillan, D. W. C. *Nat. Rev. Chem.* **2017**, *1*, 0052. c) Milligan, J. A.; Phelan, J. P.; Badir, S. O.; Molander, G. A. *Angew. Chem. Int. Ed.* **2019**, *58*, 6152-6163.

metales, cuando el sustrato requiere la introducción de algún grupo funcional, no entran en la definición de LSF (Figura 21).

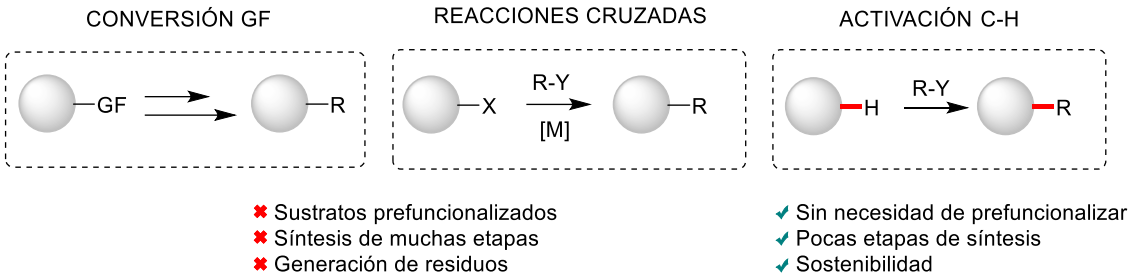
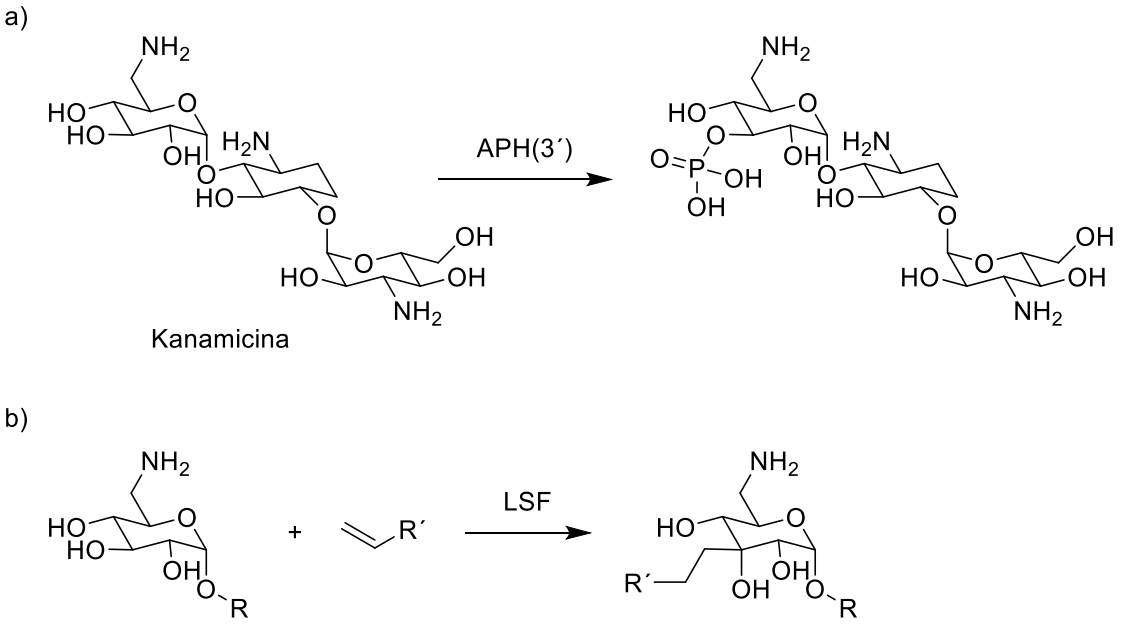


Figura 21. Diferencias entre conversión de grupos funcionales, reacciones cruzadas y activaciones C-H.

La metodología LSF tiene mucho interés en química médica. Por ejemplo, ha sido utilizada por Guo y colaboradores para la modificación química de la kanamicina. Trataban de obtener derivados capaces de combatir la resistencia de ciertas bacterias,[212] debida a la sobreexpresión de una enzima llamada APH(3´), la cual introduce un grupo fosfato en la posición 3´ del azúcar, lo que le hace ser inactivo (Esquema 46a).[213] Este grupo, desarrolló un protocolo para la sustitución selectiva en dicho carbono mediante catálisis fotoquímica, sin necesidad de proteger el resto de grupos hidroxilo presentes en la molécula (Esquema 46b).



Esquema 46. LSF en el antiobiótico kanamicina descrita por Guo.

Otra aplicación es la obtención, con una simple reacción, de derivados potencialmente activos de fármacos. Se reduce así el tiempo de síntesis y, por tanto, el coste.[214] Hay diversos ejemplos descritos, usando diferentes metodologías. Por ejemplo, Ackermann, mediante una reacción de activación C-H dirigida, consiguió un derivado metilado del paclitaxel.[215] Otra activación C-H, pero esta vez selectiva debido a la naturaleza electrónica de los reactivos, la

[212] Guo, T.; Yan, X.; Cao, H.; Lu, L.; Gao, L.; Tang, S.; Liu, J.; Wang, X. *ChemRxiv* **2022**, DOI: 10.26434/chemrxiv-2022-s2p4k-v2
[213] a) Tsuchiya, T.; Takahashi, Y.; Kobayashi, Y.; Umezawa, S.; Umezawa, H. *J. Antibiot.* **1985**, *38*, 1287-1290. b) Mingelot-Leclercq, M. P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 727-737.
[214] a) Zhang, L.; Ritter, T. *J. Am. Chem. Soc.* **2022**, *144*, 2399-2414. b) Degruyter, J. N.; Malins, L. R.; Baran, P. S. *Biochemistry* **2017**, *56*, 3863-3873.
[215] Friis, S. D.; Johansson, M. J.; Ackermann, L. *Nat. Chem.* **2020**, *12*, 511-519.

consiguió Ritter usando S-óxido de tiantreno con el ácido meclofenámico, reactividad en la que nos basamos para la obtención de los productos de partida en este capítulo (Figura 22).[216] Usando química radicalaria, el grupo de Gopalakrishnan, consiguió introducir un grupo difluorometilo en el anillo de imidazol de histidinas presentes en péptidos sin proteger.[217]

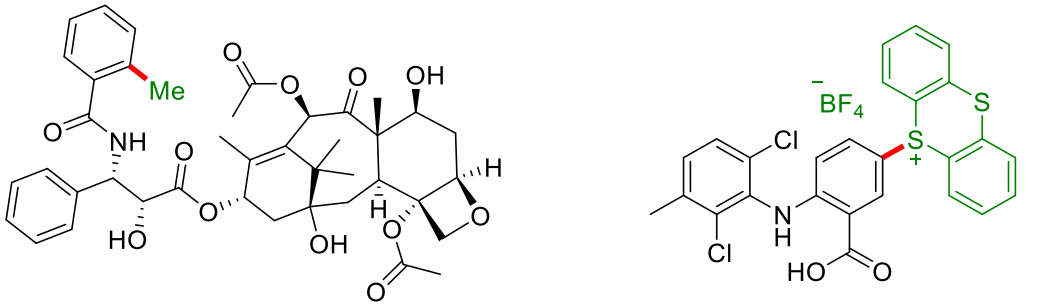


Figura 22. Derivados de fármacos mediante activación C-H.

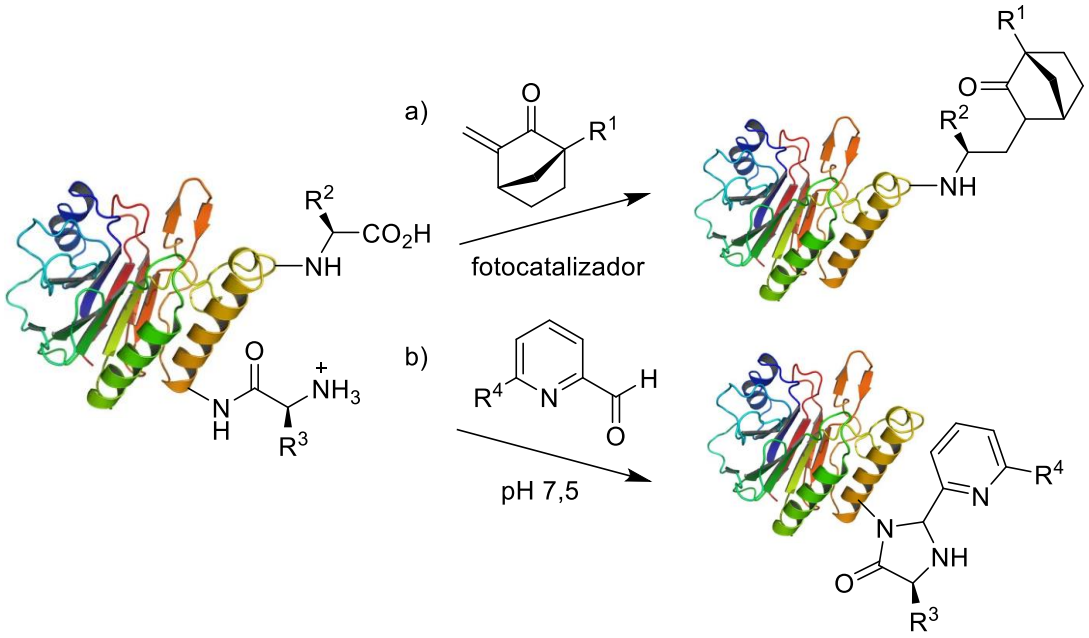
8.1. FUNCIONALIZACIÓN DE PÉPTIDOS

Las reacciones bioortogonales en péptidos de elevada complejidad estructural tienen un enorme potencial para el descubrimiento de fármacos y en imagen molecular, entre otras. La diversificación regio y quimioselectiva de péptidos y proteínas es de vital importancia en la industria farmacéutica. Por ejemplo, los péptidos que presentan aminoácidos no naturales presentan una conformación única, lo que normalmente confiere mayor estabilidad frente a la degradación.[218] Debido a la alta complejidad estructural de las proteínas, las LSF en estos compuestos requieren métodos suaves, robustos y que puedan ser predecibles en cuanto al producto obtenido. Hasta ahora, para la funcionalización de péptidos se han usado sobre todo condensaciones clásicas, cicloadiciones[219] o reacciones de acoplamiento cruzadas catalizadas por metales.[220] Durante la última década, se han ido implementando diferentes metodologías para la LSF en péptidos basadas en activaciones C-H.[221]

Las modificaciones terminales en proteínas son normalmente las más aplicables ya que los extremos son más accesibles y poseen un entorno químico diferente a los aminoácidos de la cadena.[222] De las modificaciones en los extremos de las proteínas, las que se efectúan en las zonas C-terminales se basan, normalmente, en la diferencia en el potencial de oxidación de este carboxilo frente a los ácidos de los residuos de aspártico y glutámico en la cadena. MacMillan y colaboradores aprovecharon este hecho para desarrollar un método, usando luz visible, basado en SET para una alquilación descarboxilativa del residuo C-terminal (Esquema 47a).[223] En cuanto a las modificaciones *N*-terminales, la diferente reactividad radica en el diferente pK_a de este grupo amino respecto al de las lisinas presentes en la cadena. En condiciones ligeramente

[216] Berger, F.; Plutschack, M. B.; Riegger, J.; Yu, W.; Speicher, S.; Ho, M.; Frank, N.; Ritter, T. *Nature* **2019**, *567*, 223-228.
[217] Noisier, A. F. M.; Johansson, M. J.; Knerr, L.; Hayes, M. A.; Drury III, W. J.; Valeur, E.; Malins, L. R.; Gopalakrishnan, R. *Angew. Chem. Int. Ed.*, **2019**, *58*, 19096-19102.
[218] Lenci, E.; Trabocchi, A. *Chem. Soc. Rev.* **2020**, *49*, 3262-3277.
[219] Hoyt, E. A.; Cal, P. M. S. D.; Oliveira, B. L.; Bernardes, G. J. L. *Nat. Rev. Chem.* **2019**, *3*, 147-171.
[220] Zhang, C.; Vinogradova, E. V.; Spokoyny, A. M.; Buchwald, S. L.; Pentelute, B. L. *Angew. Chem. Int. Ed.* **2019**, *58*, 4810-4839.
[221] Wei, W.; Lorion, M. M.; Shah, J.; Kapdi, A. R.; Ackermann, L. *Angew. Chem. Int. Ed.* **2018**, *57*, 14700-14717.
[222] Rosen, C. B.; Francis, M. B. *Nat. Chem. Biol.* **2017**, *13*, 697-705.
[223] a) Bloom, S.; Liu, C.; Kolmel, D. K.; Qiao, J. X.; Zhang, Y.; Poss, M. A.; Ewing, W. R.; MacMillan, D. W. C. *Nat. Chem.* **2018**, *10*, 205-211. b) Zuo, Z.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2014**, *136*, 5257-5260.

básicas, el NH_3^+ del aminoácido N-terminal se desprotona más fácilmente y es más nucleófilo (Esquema 47b).[224]

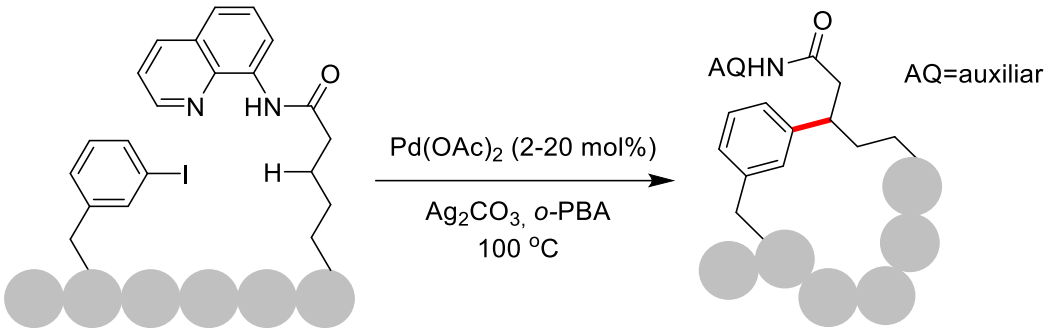


Esquema 47. Modificaciones clásicas de aminoácidos terminales en proteínas.

La modificación de los aminoácidos de cadena es una forma de modular la actividad de los péptidos naturales y enzimas, ya sea inhibiéndolos o modificando la actividad del centro activo.[225] Los métodos clásicos se enfocaban en la modificación de lisinas y cisteínas, debido a su nucleofilia, a la accesibilidad del disolvente y a la abundancia relativa de dichos aminoácidos. Las reacciones más utilizadas eran adiciones de Michael o aminaciones reductoras.[226] Ajustando la electrofilia de aceptores de Michael, se pueden seleccionar las lisinas más reactivas debido a que la estructura terciaria de los péptidos permite niveles altos de especificidad entre diferentes lisinas.[227] Las cisteínas suelen ser objetivo de modificaciones mediante reacciones de reducción de los puentes disulfuro que se forman entre cadenas. Cuando se encuentran cerca de las zonas de unión de metales, estas cisteínas son objetivo de reacciones de arilación catalizadas por Pd(II).

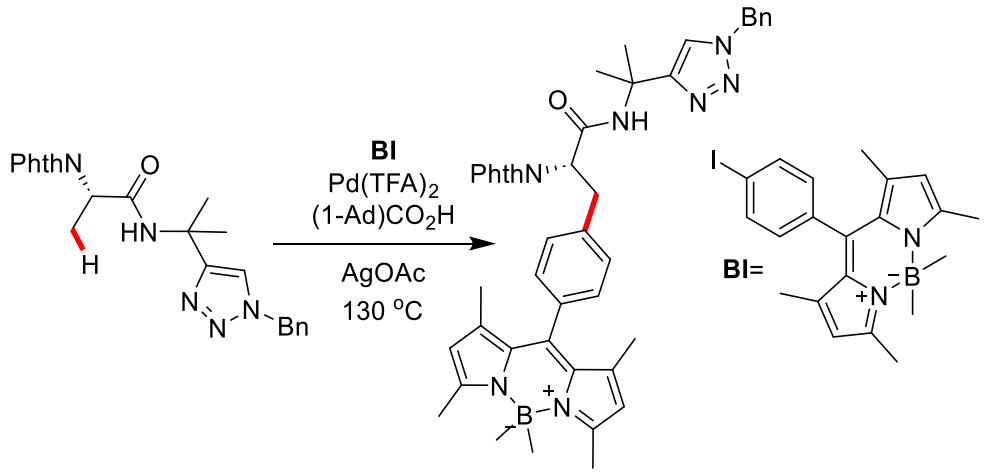
El grupo de Chen, usando paladio como catalizador, consiguió la activación C-H de carbonos sp^3, haciendo que reaccionaran con anillos aromáticos que tuvieran un átomo de iodo. Estos péptidos necesitaban de un grupo director. Mediante esta aproximación, podían transformar péptidos lineales en macrociclos con gran interés biológico, como el celogentin C, un compuesto antitumoral.[228] Esta reacción en algunos casos requería altas cargas de catalizador (en torno al 20 mol%), elevadas temperaturas y cantidades estequiométricas de oxidantes (Esquema 48).

[224] MacDonald, J. I.; Munch, H. K.; Moore, T.; Francis, M. B. *Nat. Chem. Biol.* **2015**, *11*, 326-331.
[225] Willwacher, J.; Raj, R.; Mohammed, S.; Davis, B. G. *J. Am. Chem. Soc.* **2016**, *138*, 8678-8681.
[226] a) Gunno, S. B.; Madder, A. *ChemBioChem* **2016**, *17*, 529-553. b) Chalker, J. M.; Bernardes, G. J. L.; Davis, B. G. A. *Acc. Chem. Res.* **2011**, *44*, 730-741.
[227] Huang, F.; Nie, Y.; Ye, F.; Zhang, M.; Xia, J. *Bioconjug. Chem.* **2015**, *26*, 1613-1622.
[228] a) Zhang, X.; Lu, G.; Sun, M.; Mahankali, M.; Ma, Y.; Zhang, M.; Hua, W.; Hu, Y.; Wang, Q.; Chen, J.; He, G.; Qi, X.; Shen, W.; Liu, P.; Chen, G. *Nat. Chem.* **2018**, *10*, 540-548. b) Feng, Y.; Chen, G. *Angew. Chem. Int. Ed.* **2010**, *49*, 958-961.



Esquema 48. Activación C-H en carbonos sp^3 en péptidos descrita por Chen.

Otros ejemplos de LSF mediante activación C-H en aminoácidos de cadena han sido descritos por el grupo de Ackermann. En el 2018, consiguieron una activación C-H de un carbono sp^3 dirigida por un triazol. En dicho trabajo unieron, a través del carbono que tenía un átomo de iodo, sondas fluorescentes como los BODIPYs (Esquema 49).[229] Posteriormente, se acoplaron azúcares protegidos usando también un catalizador de paladio.[230] En ambos trabajos, era necesario el uso de cantidades estequiométricas de sales de plata entre otros aditivos y altas temperaturas.

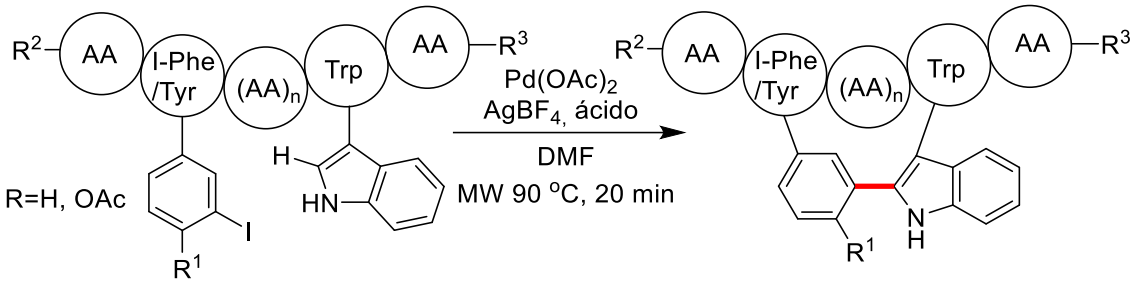


Esquema 49. Activación C-H en carbonos sp^3 en péptidos descrita por Ackermann.

De entre todos los aminoácidos que forman las proteínas, el triptófano es el menos abundante, entre el 1-2% de los aminoácidos expresados.[231] Este aminoácido posee el sistema π más rico en electrones. Esto provoca que intervenga en numerosas interacciones y que se encuentre frecuentemente en los centros activos.[232] El triptófano es pues un excelente objetivo para reacciones de LSF. Los esfuerzos se han centrado en buscar métodos quimioselectivos y que no requieran de condiciones drásticas bajo el uso de catálisis fotoquímica[233] o por metales de transición, preferiblemente, de la serie 3.[234]

[229] Wang, W.; Lorion, M. M.; Martinazzoli, O.; Ackermann, L. *Angew. Chem. Int. Ed.* **2018**, *57*, 10554-10558.
[230] Wu, J.; Kaplaneris, N.; Ni, S.; Kaltenhäuser, F.; Ackermann, L. *Chem. Sci.* **2020**, *11*, 6521-6526.
[231] Santiveri, C. M.; Jiménez, M. A. *Biopolymers* **2010**, *94*, 779-790.
[232] Barik, S. *Int. J. Mol. Sci.* **2020**, *21*, 8776.
[233] Lima, R. N.; Delgado, J. A. C.; Bernardi, D. I.; Berlinck, R. G. S.; Kaplaneris, N.; Ackermann, L.; Paixao, M. W. *Chem. Commun.* **2021**, *57*, 5758-5761.
[234] a) Kaplaneris, N.; Son, J.; Mendive-Tapia, L.; Kopp, A.; Barth, N. D.; Maksso, I.; Vendrell, M, Ackermann, L. *Nat. Commun.* **2021**, *12*, 3389. b) Kaplaneris, N.; Kaltenhäuser, F.; Sirvinskaite, G.; Fan, S.; De Oliveira, T.; Conradi, L.-C.; Ackermann, L. *Sci. Adv.* **2021**, *7*, eabe6202.

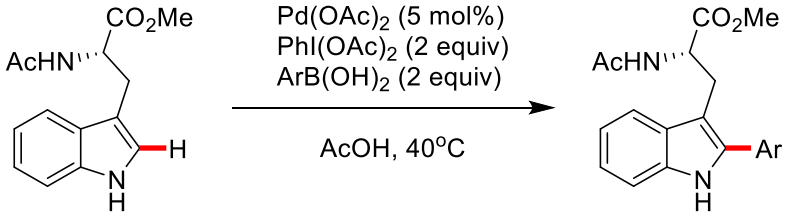
El grupo de Lavilla estudió la activación C-H en el anillo de indol,[235] para posteriormente aplicar la versión intramolecular de su metodología al triptófano (Esquema 50).[236] La reactividad consistía en la activación catalizada por $Pd(OAc)_2$ del anillo de indol del triptófano que reaccionaba con tirosinas o fenilalaninas en las que se había introducido un átomo de iodo en el anillo aromático. Aunque en algunos casos pudieron emplear medios acuosos, los inconvenientes eran que en determinados ejemplos debían usar hasta un 40 mol% de catalizador y cantidades estequiométricas de sales de plata que actuaran como oxidantes. Además, eran necesarias altas temperaturas y radiación microondas para que la reacción fuera rápida y así evitar epimerizaciones.



Esquema 50. Activación C-H en el anillo de indol de triptófano descrita por Lavilla.

Los sustratos iodados comerciales son los más utilizados como agentes de acoplamiento en activaciones C-H, aunque como se ve en todos los ejemplos mencionados, se necesitan cantidades estequiométricas de sales oxidantes y temperaturas de reacción elevadas. Para evitar el uso de aditivos se estudió el empleo de reactivos electrófilos cargados, que en principio facilitarían la adición oxidativa al catalizador metálico.

El grupo de Fairlamb desarrolló un protocolo utilizando ácidos arilborónicos como agentes de acoplamiento. Aunque aún era necesario el uso de oxidantes en cantidades estequiométricas ($PhI(OAc)_2$) o el uso de otros metales como co-catalizadores ($Cu(OAc)_2$), la temperatura necesaria para la transformación era menor que con los derivados de iodo (Esquema 51).[237]

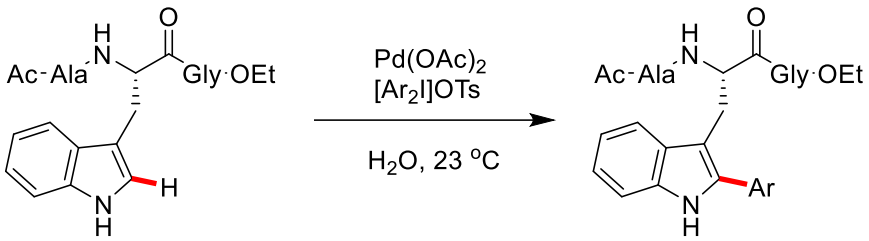


Esquema 51. Activación C-H en triptófano descrita por Fairlamb.

El grupo de Ackermann desarrolló un protocolo para la arilación selectiva de los residuos de triptófano en péptidos usando sales de diariliodonio mediante catálisis de paladio (Esquema 52).[238] Estas sales no precisaban utilizar ningún tipo de aditivo. La reacción transcurría a temperatura ambiente y, aunque con un rendimiento ligeramente menor, se podía dar en

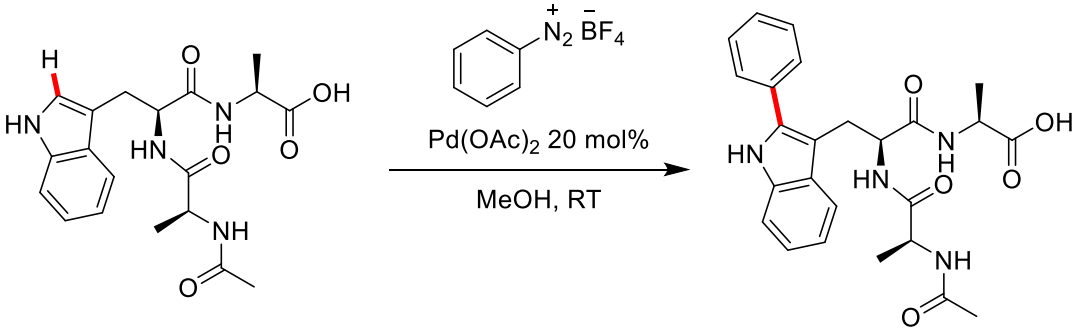
[235] a) Preciado, S.; Mendive-Tapia, L.; Albericio, F.; Lavilla, R. *J. Org. Chem.* **2013**, *78*, 8129-8135. b) Ruíz-Rodríguez, J.; Albericio, F.; Lavilla, R. *Chem. Eur. J.* **2010**, *16*, 1124-1127.
[236] Mendive-Tapia, L.; Preciado, S.; García, J.; Ramón, R.; Kielland, N.; Albericio, F.; Lavilla, R. *Nat. Commun.* **2015**, *6*, 7160.
[237] a) Reay, A. J.; Williams, T. J.; Fairlamb, I. J. S. *Org. Biomol. Chem.* **2015**, *13*, 8298-8309. b) Williams, T. J.; Reay, A. J.; Whitwood, A. C.; Fairlamb, I. J. S. *Chem. Commun.* **2014**, *50*, 3052-3054.
[238] Zhu, Y.; Bauer, M.; Ackermann, L. *Chem. Eur. J.* **2015**, *21*, 9980-9983.

medios acuosos. A pesar de este avance, las condiciones para la preparación de las sales requerían condiciones duras.



Esquema 52. Activación C-H del anillo de indol en triptófano descrita por Ackermann.

Más tarde, el grupo de Fairlamb estudió la posibilidad de usar sales de arildiazonio como agentes de acoplamiento debido a la similitud que presentan con los diariliodonio descritos previamente.[239] La síntesis de dichas sales seguía necesitando varias etapas y condiciones duras, pero la catálisis con paladio podía ser llevada a cabo a temperatura ambiente sin necesidad de ningún tipo de aditivo y con una carga del 5 mol% del catalizador. Mediante este protocolo, Fairlamb demostró la posibilidad de realizar la reacción de arilación en dos péptidos que habían sido descritos previamente como especies sensibles a la oxidación si se utilizaba catálisis dual Pd(0)/Cu(II), aunque en este caso se necesitaba aumentar la carga de catalizador hasta el 20 mol% (Esquema 53).[237a]



Esquema 53. Arilación mediante activación C-H en triptófano descrita por Fairlamb.

En 2019 Ritter publicó una activación C-H de anillos aromáticos totalmente selectiva sin el uso de ningún tipo de catalizador metálico. La selectividad se basaba en que la reacción ocurre vía radicalaria: el S-óxido de tiantreno o el S-óxido de dibenzotiofeno, cuando son tratados con anhídrido trifluoroacético y $HBF_4{\cdot}Et_2O$, forman un radical que es capaz de reaccionar regioselectivamente con un enlace C-H tanto en anillos aromáticos[240] como en alquenos.[241] El grupo de Wang, usando estas sales, desarrolló una reacción fotocatalítica, para la arilación de anillos de indol, con paladio e iridio como fotocatalizadores (Esquema 54).[242] En dicho trabajo era necesaria la presencia de una pirimidina que actuara como grupo director, además de necesitar una alta carga de catalizador (20 mol%).

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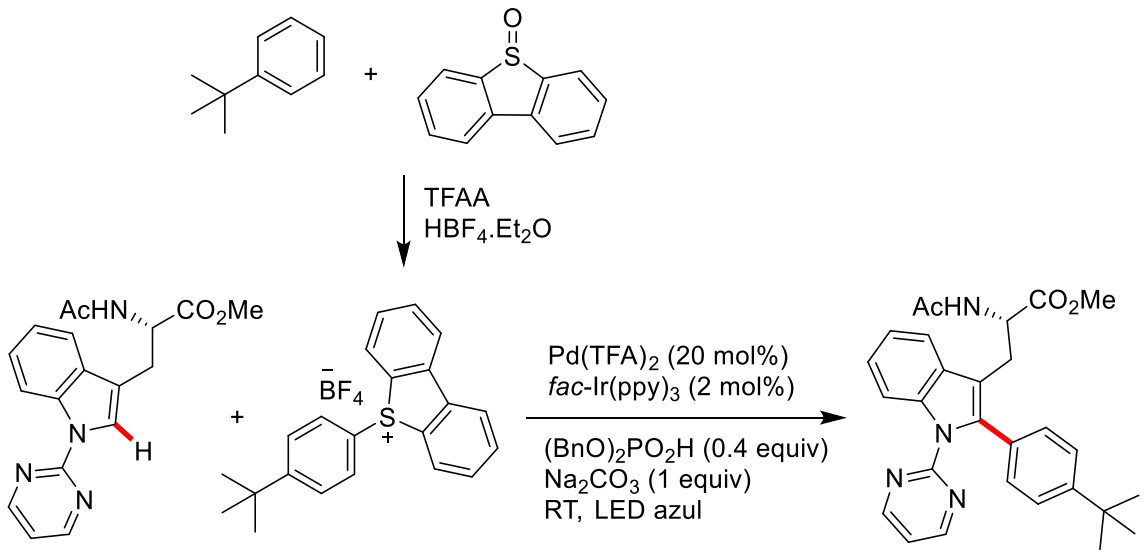

Esquema 54. Activación C-H fotocatalítica en el anillo de indol descrita por Wang.

9. LATE-STAGE FUNCTIONALIZATION OF TRYPTOPHAN-CONTAINING PEPTIDES WITH THIANTHRENIUM SALTS: CONJUGATION AND LIGATION.

This work describes the C-H activation of indol moieties in tryptophan derivatives. The optimal conditions were using isopropanol as solvent at 70 ^{o}C with 10 mol% of $Pd(OAc)_2$ as the catalyst. Using arylthianthrenium salts we could introduce diverse aromatic rings in tryptophan and in larger peptides, as well as drug derivatives and aminoacids such as phenylalanine and tyrosine.

In this work I was in charge of the synthesis and characterization of the thianthrenium salts used as starting materials and the reaction with tryptophan. I also collaborated writing the supplementary information and experimental part during the manuscript elaboration.

En este trabajo se presenta la activación C-H del anillo de indol en derivados de triptófano. Las condiciones óptimas se obtuvieron usando isopropanol como disolvente a 70 ^{o}C con una carga catalítica del 10 mol% de $Pd(OAc)_2$. Usando sales de ariltiantreno pudimos introducir en el triptófano y en péptidos más grandes diferentes anillos aromáticos, así como derivados de fármacos y otros aminoácidos como la fenilalanina y la tirosina.

En este trabajo me encargué de la síntesis y caracterización de las sales de tiantreno, usadas como productos de partida, y en la reacción con el triptófano. También he colaborado en la redacción de la parte experimental y la información suplementaria.

Kaplaneris, N.; Puet, A.; Kallert, F.; Pohlmann, J.; Ackermann, L. Late-Stage Functionalization of Tryptophan-Containing Peptides with Thianthrenium Salts: Conjugation and Ligation. *Angew. Chem Int. Ed.* **2023**, *62*, e202216661. **Impact factor** (2021): 16.592. **Category**: Multidisciplinary chemistry (Q1, 13/178). Source: JCR (Journal of Citations Reports).

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Peptide Modification

Late-stage C–H Functionalization of Tryptophan-Containing Peptides with Thianthrenium Salts: Conjugation and Ligation

*Nikolaos Kaplaneris, Alejandro Puet, Felix Kallert, Julia Pöhlmann, and Lutz Ackermann**

Abstract: Bioorthogonal late-stage diversification of structurally complex peptides bears enormous potential for drug discovery and molecular imaging, among other applications. Herein, we report on a palladium-catalyzed C–H arylation of tryptophan-containing peptides with readily accessible and modular arylthianthrenium salts. Under exceedingly mild reaction conditions, the late-stage diversification of structurally complex peptides was accomplished. The tunability and ease of preparation of arylthianthrenium salts allowed the expedient stitching of tryptophan-containing peptides with drug, natural product, and peptidic scaffolds by forging sterically congested biaryl linkages. The robustness of the palladium catalysis regime was reflected by the full tolerance of a plethora of sensitive and coordinating functional groups. Hence, our manifold enabled efficient access to highly decorated, labelled, conjugated, and ligated linear and cyclic peptides.

Late-stage functionalization of biomolecules has emerged as an efficient method for the expansion of the accessible chemical space, without reliance on cost- and time-intensive de novo manifolds.[1] Thus, the site- and chemoselective diversification of peptides and proteins is of prime importance in academia and pharmaceutical industries. In this context, peptides featuring unnatural amino acids hold a unique conformational space and feature distinct bioactivities, concurrently being more stable towards proteolytic degradation.[2] Efficient late-stage diversification of peptides and proteins, that are not hindered by the myriad of functional groups found on such complex biomolecules, require predictable, robust, and mild methods. Until recently, predominantly classical condensations and (cyclo)additions,[3] or metal-catalyzed cross-couplings[4] have been exploited for the functionalization of peptides. Despite these major advances, palladium-catalyzed cross-couplings require two pre-functionalized substrates, leading to cost- and time-intensive multistep syntheses. During the last decade, C–H activation has been recognized as a transformative platform in molecular syntheses. Thus, late-stage diversification of peptides by noble-metal-catalyzed C–H activation[5] was established by Lavilla/Albericio,[6] Chen,[7] Shi,[8] Ackermann,[9] and Yu,[10] among others[11] and more recently through 3d transition metal catalysis.[12] Among the proteinogenic amino acids, tryptophan is the least abundant residue accounting for only 1–2 % of the amino acids expressed.[13] The indole moiety of tryptophan is the most electron-rich π-system present in amino acids, a property that enables tryptophan to engage in a variety of interactions; thus, tryptophan is generally enriched at centers of biochemical significance.[14] These properties render tryptophan an excellent target for late-stage functionalization as a tool for establishing expedient structure–activity relationships of various peptides and proteins. Thus, significant recent efforts have been devoted to developing mild and chemoselective methods for the functionalization of tryptophan residues, under photochemical[15] and transition metal-catalyzed manifolds.[6,12,16] Among these methods palladium-catalyzed arylation of tryptophan has surfaced for the introduction of various (hetero)aryl moieties utilizing various arylating agents, under distinct reaction conditions (Figure 1a). Among these arylating agents, aryl iodides are the most well-established, yet they typically require stoichiometric amounts of silver salts and elevated reaction temperatures.[6c] To bypass the need for additives and harsh reaction conditions charged electrophiles were utilized, presumably facilitating the oxidative addition, such as aryldiazonium salts[16d] and diaryliodonium salts.[16e] Remarkably, the site- and chemoselective arylation with the diaryliodonium salts was achieved in H_2O at room temperature. In addition, under an oxidative manifold utilizing arylboronic acids, the arylation was achieved by Fairlamb and coworkers under mild reaction conditions, albeit with the requirement of stoichiometric amounts of oxidants.[16f,g] Despite these undisputed advances, the preparation of such arylating agents require rather harsh reaction conditions, thus hindering their use for the merging of structurally

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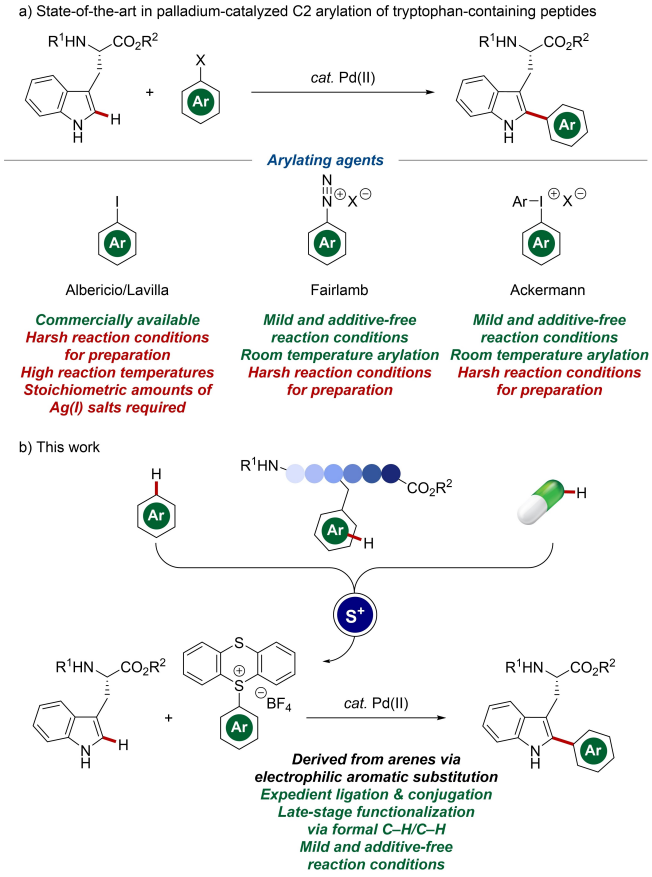
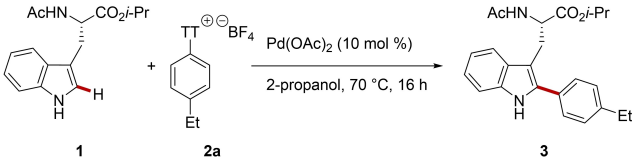


Figure 1. a) Arylating agents for C2 arylation of tryptophan residues. b) Expedient entry to conjugated and ligated peptides by palladium-catalyzed C–H arylation with arylthianthrenium salts.

Table 1: Optimization of the C2 arylation of tryptophan derivative **1** with arylthianthrenium salt **2a**.[a]



1 **2a** **3**

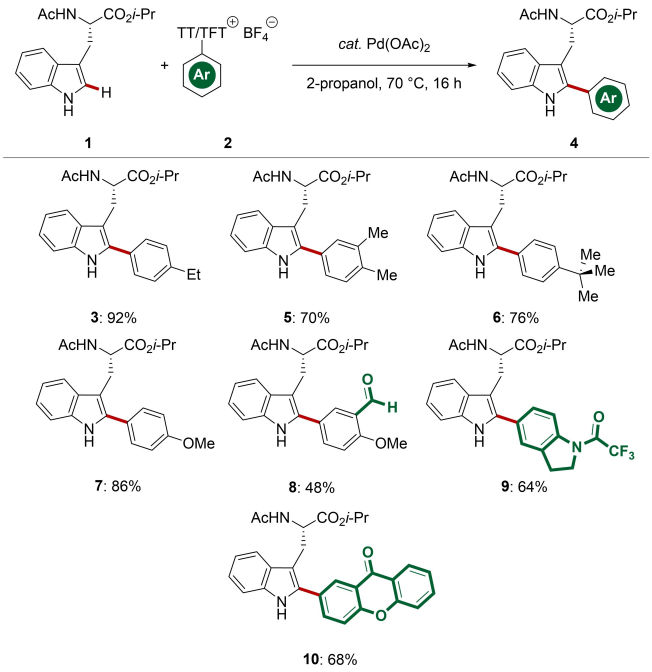
Entry	Deviation from the standard conditions	Yield [%][b]
1	none	92
2	toluene as solvent	52
3	1,4-dioxane/DME as solvent	34/33
4	EtOAc as solvent	42
5	DMA/DMF/MeCN as solvent	nd/nd/nd
6	MeOH/TFE/HFIP as solvent	16/nd/nd
7	at 50 °C	54
8	$Pd(OAc)_2$ (5.0 mol %)	75
9	$MnBr(CO)_5$/$[RuCl_2(p\text{-cymene})]_2$/ $[Cp^*RhCl_2]_2$/$[Cp^*IrCl_2]_2$ as catalyst	nd/nd/nd/nd
10	without $Pd(OAc)_2$	nd
11	Under light irradiation 456 nm/390 nm[c]	54/24
12	Under light irradiation, without $Pd(OAc)_2$ 456 nm/390 nm[c]	nd/nd

[a] Reaction conditions: **1** (0.10 mmol), **2a** (0.15 mmol), $Pd(OAc)_2$ (10 mol %), solvent (1.0 mL), 70 °C, 16 h. [b] Yield of isolated product. [c] Reaction at 25 °C. TT: thianthrene, DME: 1,2-dimethoxyethane, DMA: *N*,*N*-dimethylacetamide, DMF: *N*,*N*-dimethylformamide, TFE: 2,2,2-trifluoroethanol, HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol, Cp*: 1,2,3,4,5-pentamethylcyclopentadienyl.

complex motifs to tryptophan residues. This impediment leads to lengthy and costly multistep de novo approaches. Hence, we probed whether a more versatile arylating agent could be employed to overcome those limitations. Recently, elegant studies by Ritter,[17] Procter,[18] and others[19] showcased that the site- and chemoselective preparation of arylsulfonium salts provides expedient access to structurally complex aryl electrophiles for cross-couplings and photochemical reactions. As part of our program on late-stage functionalization through C–H activation, we now report on the arylation of tryptophan-containing peptides with arylthianthrenium salts without the aid of directing groups (Figure 1b).[20] Salient features of our findings include 1) epimerization-free C–H arylation of structurally complex peptides under additive-free and exceedingly mild reaction conditions, 2) expedient access to peptide/drug conjugates with drug-derived thianthrenium salts and 3) ligation of peptides by the stitching of tryptophan residues with tyrosine or phenylalanine residues to forge sterically demanding biaryl motifs.

We commenced our studies towards the desired C2 arylation of directing group-free tryptophan derivative **1** with arylthianthrenium salt **2a** and $Pd(OAc)_2$ by probing various solvents under additive-free reaction conditions (Table 1). Among the solvents that were tested, 2-propanol provided the desired product in 92 % at 70 °C (entry 1). Interestingly, typical solvents, such as EtOAc, DMF, and DMA, utilized for the C2 arylation of tryptophan derivatives with other arylating agents, led to significantly lower yields (entries 4 and 5). Furthermore, other alcoholic solvents, such as MeOH, TFE, and HFIP, resulted in complete suppression of the reaction (entry 6). Lowering the reaction temperature to 50 °C or the catalyst loading led to slightly diminished yields of the isolated product (entries 7 and 8). Interestingly, various transition-metal catalysts that have been utilized in the C2 functionalization of tryptophan residues proved completely inefficient in the directing-group-free C–H arylation (entry 9). A control experiment demonstrated the essential nature of the palladium catalyst (entry 10). While arylthianthrenium salts have previously been employed as aryl radical precursors under photochemical conditions,[21] reactions promoted solely by light irradiation were not viable (entries 11 and 12).

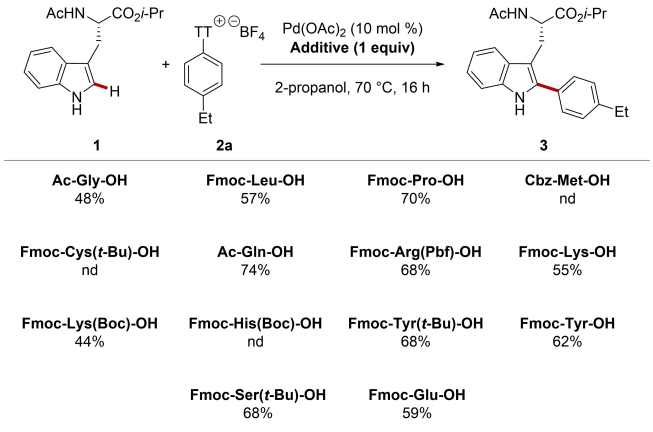
Having optimized the conditions for the palladium-catalyzed C2 arylation of tryptophan residues, we explored the generality of our methodology by probing various arylthianthrenium salts **2** (Scheme 1). Alkyl and alkoxy substituents on the arene moiety of the arylthianthrenium salt **2** were well tolerated leading to the desired arylated tryptophan derivatives **5**–**7** in good to excellent yields. The electrophilic aldehyde functional group on the arylthianthrenium salt **2e** was well tolerated, leading to the functionalized tryptophan derivative **8**, destined



Scheme 1. Chemoselective palladium-catalyzed arylation of tryptophan **1** with arylthianthrenium salts. TFT: tetrafluorothianthrene.

for further condensation-based post-synthetic manipulations. Furthermore, protected indoline derived thianthrenium salt **2f** enabled the efficient construction of the biaryl indole/indoline motif in **9**. The facile preparation of the labeled tryptophan derivative **10**, featuring the fluorescent xanthone scaffold, was also achieved with high efficacy. Under otherwise identical reaction conditions, pyridine- or benzothiophene-derived thianthrenium salts did thus far lead to less satisfactory results.

After having established the initial reaction scope with respect to the arylthianthrenium salts, we conducted an expedient chemoselectivity test[16c] with various amino acid additives (Scheme 2). Thus, hydrophobic amino acids, such as glycine, leucine and proline, were well



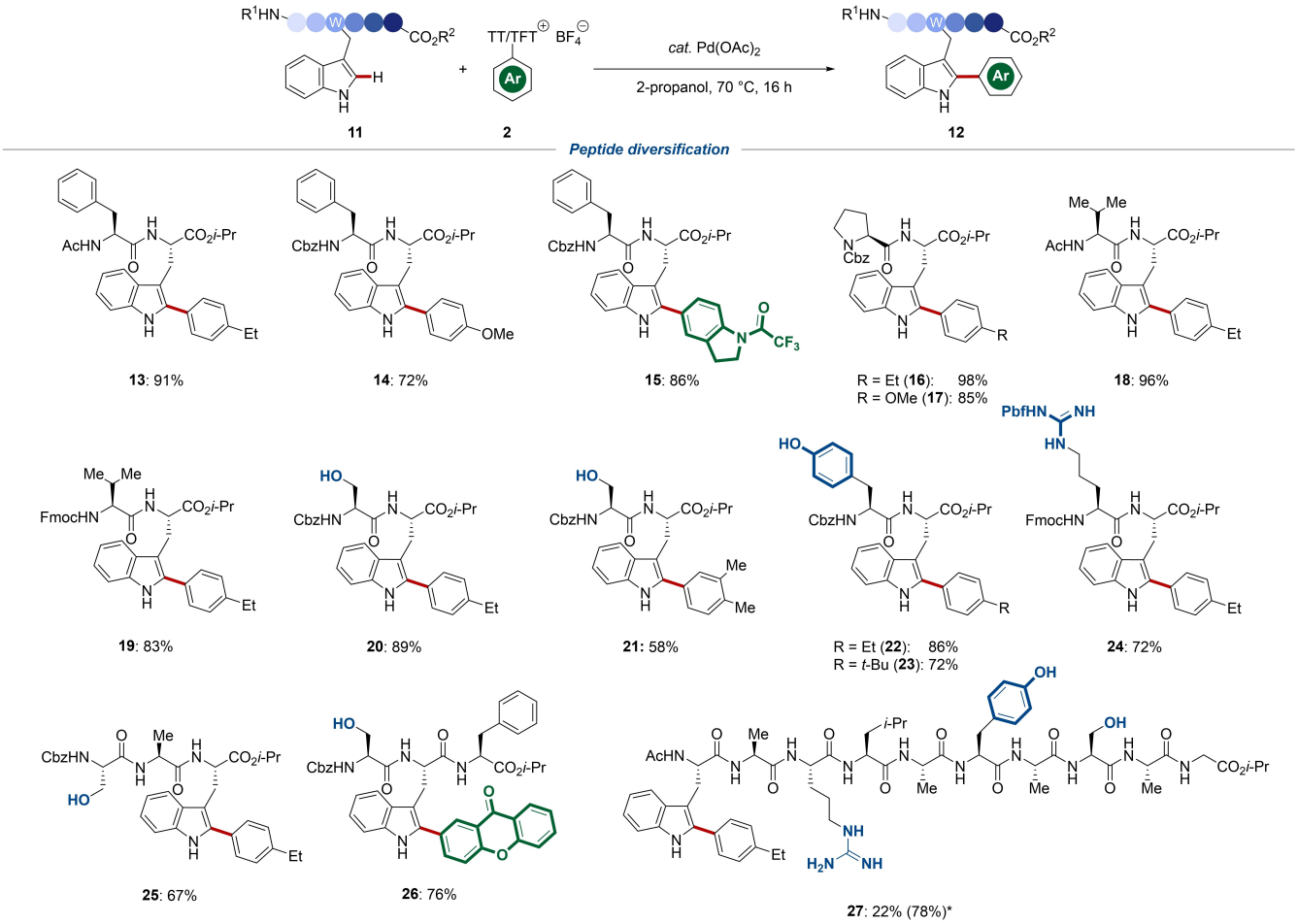
Scheme 2. Chemoselectivity test for palladium-catalyzed C–H arylation of tryptophan **1**.

tolerated, while sulfur-containing amino acids proved to be more challenging. Notably, basic free amino NH_2-groups in lysine and free hydroxyl-groups were found to be fully compatible.

Next, the late-stage arylation of larger peptides **11** using arylthianthrenium salts **2** was probed under our optimized reaction conditions (Scheme 3). Various dipeptides featuring hydrophobic amino acids, such as phenylalanine, proline and valine, were readily arylated providing the desired functionalized dipeptides **13**–**19** in good to excellent yields. The site- and chemoselectivity of the palladium-catalyzed C–H arylation was reflected by complete tolerance of nucleophilic, coordinating functional groups, such as free alcohols, phenols and guanidino groups found in serine-, tyrosine- and arginine-containing dipeptides **20**–**24** leading to the desired products in good to excellent yields. In addition, serine-containing tripeptides **11i** and **11j** were chemoselectively functionalized in good yields, leading to the arylated peptide **25** and labeled peptide **26**. Furthermore, arylated decapeptide **27**, featuring unprotected arginine, tyrosine and serine, was obtained with good efficiency.

The mild and selective route for the formation of the arylthianthrenium salts led us to probe whether thianthrenium salts derived from drug scaffolds and natural products could likewise be employed to our C–H arylation manifold, resulting in unprecedented peptide/drug and peptide/natural product conjugates (Scheme 4). Thus, drugs featuring electron-rich phenol scaffolds, such as gemfibrozil, bezafibrate, clofibrate, and fenofibrate, were readily stitched to the indole moiety of the tryptophan derivative **1**. Furthermore, the highly decorated indole scaffold of indometacin was efficiently utilized leading to the formation of hybrid architecture **35**, featuring the indole/indole biaryl motif. Furthermore, the salicin-derivative, featuring glucose, was smoothly employed leading to the desired amino acid/sugar conjugate **36** in good yield. Encouraged by the efficiency of the stitching between amino acid derivative **1** and drug-derived thianthrenium salt **29** we advanced on the stitching of larger peptides to drug scaffolds. Gratifyingly, dipeptides featuring unprotected alcohols embedded in serine residues, were efficiently conjugated with various drug scaffolds. In addition, tetrapeptide **11k** was stitched to clofibrate leading to conjugate **40** in a chemoselective manner.

Peptide ligation represents a strategy towards large peptides without the need for lengthy de novo syntheses. Hence, we explored whether thianthrenium salts derived from amino acids and peptides could be readily accessed and whether they would be suitable partners for our palladium-catalyzed C–H arylation. Tyrosine and phenylalanine were selected as the most suitable residues, owing to their electron-rich arene moieties. Gratifyingly, both amino acid derivatives and peptides possessing such residues delivered the desired thianthrenium salts **42**[17c] suitable for further late-stage manipulation. Under our standard reactions conditions we established a convergent method for the assembly of complex peptides

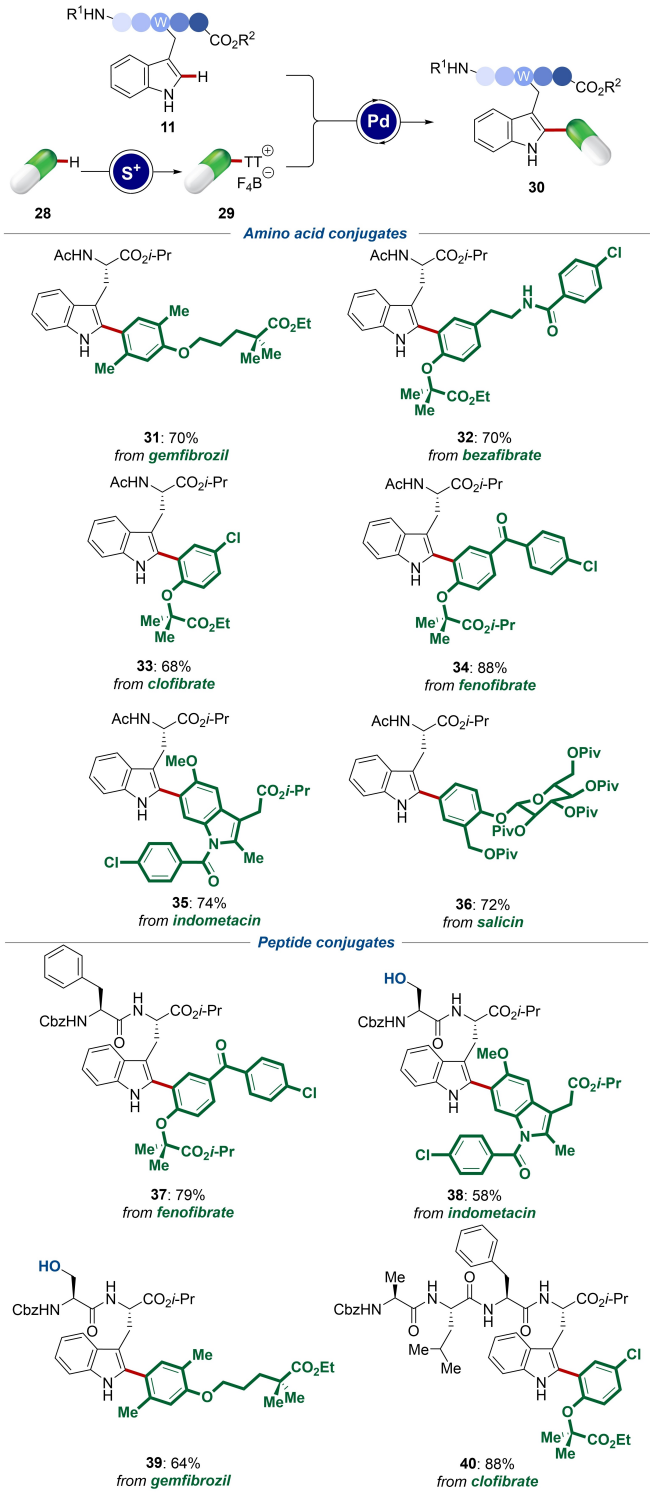


Scheme 3. Site- and chemoselective arylation of linear tryptophan-containing peptides with arylthianthrenium salts. Cbz: benzyloxycarbonyl, Fmoc: fluorenylmethoxycarbonyl, Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl. *In parentheses UPLC conversion.

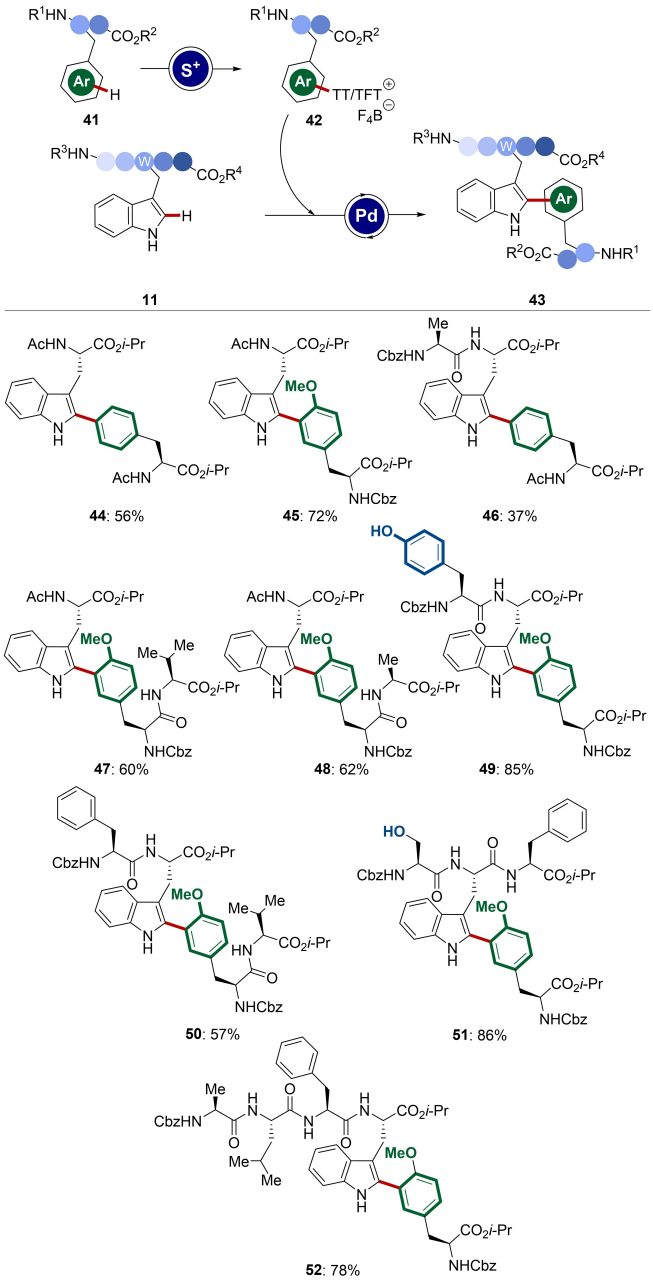
featuring unnatural linkages (Scheme 5). More precisely, unnatural dipeptides Trp-Phe **44** and Trp-Tyr **45** were obtained in moderate to good yields. Then, various tripeptides, featuring unprotected alcohols and phenols, were smoothly assembled in moderate to excellent yields through either a [2+1] or a [1+2] approach. Likewise, tetra- and pentapeptides **50**–**52** were obtained in a site- and chemoselective manner through our convergent approach.

Having established a robust protocol for the diversification, labeling, conjugation and ligation of linear tryptophan-containing peptides we examined the late-stage functionalization of 2,5-diketopiperazines and cyclic peptides (Scheme 6). Thus, the palladium-catalyzed C–H arylation of conformationally rigid tryptophan-containing 2,5-diketopiperazines was realized, enabling access to, among other products, brevianamide F (cyclo-[Trp-Pro]) analogue **58**. Furthermore, cyclo[Trp-Val] (**53c**) was efficiently stitched to a tyrosine residue, resulting in the Trp-Tyr unnatural linkage. Moreover, cyclic penta- and hexapeptides **53e** and **53f** were also site- and chemoselectively arylated, giving access to peptides **60** and **61**, featuring serine, threonine and unprotected arginine residues. In addition, lysine-containing cyclic pentapeptide **53g** was merged in a bio-orthogonal manner with the salicin moiety leading to the glycopeptide **62**.

In summary, we have developed an efficient palladium-catalyzed C–H arylation of structurally complex peptides with modular and readily available arylthianthrenium salts. The site- and chemoselective peptide arylation was characterized by excellent functional-group compatibility, tolerating a plethora of sensitive and coordinating groups, under mild and epimerization-free reaction conditions. The tunable nature of arylthianthrenium salts allowed the efficient assembly of peptide/drug conjugates and ligated peptides featuring unnatural biaryl motifs, without the need for lengthy and costly prefunctionalization. This robust method paves the way for the development of a bioorthogonal convergent approach to peptide synthesis.



Scheme 4. Palladium-catalyzed assembly of peptide/drug conjugates. Piv: pivaloyl.



Scheme 5. Palladium-catalyzed convergent assembly of complex peptides.

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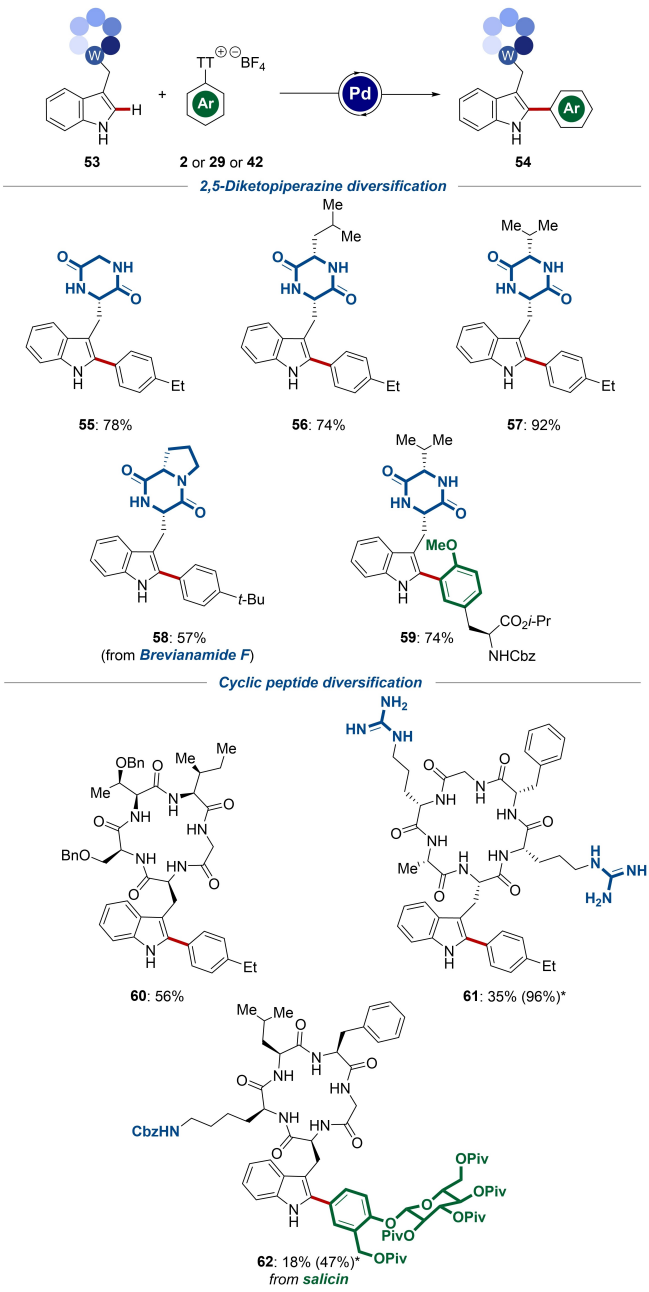
Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: C–H Activation · Late-Stage Functionalization · Ligation · Palladium · Peptides



Scheme 6. Late-stage functionalization of 2,5-diketopiperazines and cyclic peptides. *In parentheses UPLC conversions.

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SUMMARY AND CONCLUSIONS/RESUMEN Y CONCLUSIONES

The present PhD report is divided into three different chapters, dedicated to the synthesis of rigidified iminosugars and studies on their interaction with glycosidases, novel rearrangements of cyclopropene containing systems, and C-H activations in tryptophan, respectively.

Chapter I:

Glycosidases play an important role in nature. They catalyze different biochemical reactions that involve sugars such as degradation of carbohydrates and transglycosidation. These processes have a role in cell recognition, viral and bacterial infection, etc. A typical way to inhibit or modulate the activity of these enzymes is using compounds that mimic the natural sugars or the transition state during the enzymatic reaction. One of these kinds of compounds are iminosugars, which are generally piperidine and pyrrolidine based structures possessing numerous hydroxyl groups. Thus, with respect to natural carbohydrates, the endocyclic oxygen is switched into a nitrogen atom rendering more metabolic stability. Since many years, iminosugars are known to be good mimetics of carbohydrates although they sometimes lack selectivity towards specific enzymes which precludes their conversion into drugs. However, some iminosugars are actually comercially available for the treatment of different sugar-related diseases.

In the first chapter of this report, the aim has been to synthetize new piperidine-based iminosugars, rigidified by a fused cyclopropane potentially giving more selectivity against specific glycosidases. For the synthesis we have used natural aminoacids as chiral pools, but we have pursued the formation of as many diasteroisomers as possible, avoiding racemization reactions. Thus, we have obtained several enantiomerically pure products. We began the synthesis with L-serine as chiral pool, which after various reactions, was transformed into different diastereoisomers of the desired final products which bear up to 5 stereogenic centers. We also used L-alanine as starting material which gave final products related with L-fucose, a natural occurring carbohydrate present in some viral infections. All the final products were submitted to *in vitro* and NMR studies to study their ability to interact with different glycosidases. This was possible thanks to a short stay in Prof. Javier Cañada´s laboratory in CIB, CSIC.

The conclusions for this chapter are:

- We have prepared 11 final compounds, containing a piperidine fused to a cyclopropane and up to 5 stereogenic centers. The synthesis started from L-alanine or L-serine. Final products differed in stereochemistry and substitution pattern at the cyclopropane ring.

- The synthesis were based on ring closing metathesis for the construction of the piperidine ring, using the 2nd generation Grubb´s catalyst on an α,β-unsaturated ketone, and a cyclopropanation using sulfur ylides, as the key steps.

- The stereochemical outcome of the cyclopropanation can be modulated by changes on the reaction conditions. Using a base such as DBU to form *in situ* the ylide led to the *exo* isomer as sole product. Synthetizing the ylide prior to the cyclopropanation reaction led to the formation of the *endo* isomer. We observed that carrying the reaction at a concentration of 0.06 M at 0 ^{o}C we could isolate the *endo* isomer in up to 18% yield in one of the L-serine derivatives.

- Depending on the stereochemistry obtained in the cyclopropanation reaction, the reduction of the ketone was stereoselective, as it was seen in the case of the L-serine, due to steric hindrance.

- The products were submitted to an enzymatic assay against 8 different glycosidases. Most results were negative showing no activity against the enzimes. However, some compounds were inhibitors in the range of mM of only one enzyme each meaning that we had achieved the desired selectivity. In other cases we found activation of the enzymes.

- In order to explain the activation, we followed the enzymatic reaction by ^{1}H-NMR to see if any product related with a transglicosidation reaction was being formed. However, we could not observe any transglicosidated product.

Chapter II:

The main aim in the second chapter has been the study of the reactivity on ene-cyclopropene systems. Cyclopropenes are the smallest unsaturated cycles and have a high strain energy. Eventhough, they are stable since the main degradation pathways are pericyclic reactions and the activation energy for the ring opening is high. Using metal complexes or light the opening of the cyclopropene is facilitated giving rise to a rich reactivity, generally trough vinyl carbene intermediates, which can further react with double or triple bonds. Regarding ene-cyclopropenes, it had been described that gold catalysts react with the unsubstituted olefinic carbon of the cyclopropene giving a vinylcarbene which can cyclopropanate the pending olefine. With ruthenium catalysts ene-cyclopropenes mostly give ring closing metathesis (RCM). However, our group studied the reactivity against Cp*RuCl(cod), observing reverse regioselective during the cyclopropene cleavage compared with gold catalysts, giving rise to [3.1.0], [4.1.0] and [5.1.0]bicycles. The limitation of this reaction was the need to use terminal olefins. Moreover, we found that using ruthenium dimeric catalysts indanes and tetralines were formed following a similar mechanism.

Cyclopropenes are also known to be active in pericyclic reactions since they have low LUMO energies. Thermodynamically, the strain release is a powerful driving force for these reactions. The stereochemistry outcome is governed both by secondary orbital interaction (SOI), which favors the *endo* approach and by the steric hindrance made by the substituents. The first intramolecular Alder-ene reaction involving cyclopropenes was described in 1985. In addition, cyclopropenes are well known dienophiles since the work of Wiberg in 1960. In 2010, Patel and Boger described the first intramolecular Diels-Alder reaction involving a cyclopropene. This reaction has been used as a bioortogonal approach to label biomolecules.

The conclusions for this chapter are:

- We optimized the synthesis of functionalized indanes and tetralines from ene-cyclopropenes using a 5 mol% of ruthenium dimer catalyst under microwave irradiation at 180 ^{o}C in toluene during 30 minutes of reaction.

- When the aromatization was not possible, an α,β-unsaturated ketone was obtained. If the cyclopropene was disubstituted, a migration of the methyl that was impeding the rearomatization step was observed.

- We proposed a mechanism that involves the formation of a metal carbene in the inner carbon of the cyclopropene after the opening of the metalacyclobutene, rendering the double bond in *Z* configuration. This settles the carbonyl group near enough to react with ruthenium, forming a dihydrofuran, that evolves into a tricycle. In the presence of the catalyst, a deoxygenative aromatization takes place to form the final products.

- During the scope study, we found that when the ene-cyclopropene was disubstitued in the external carbon of the olefin, a spirocycle was formed. It was unnecessary to catalyze the reaction.

- We proposed a one pot reaction to form the spirocycles from the cyclopropene containing compound and the corresponding bromine derivative. We found that 2-MeTHF at reflux for 6 hours were the best conditions.

- When using α,β-unsaturated ketones cyclopropa[*b*]pyrans were obtained following a similar mechanism.

- We also studied the effect of the stereochemistry of the double bond. We saw that the *Z*-isomer needed 48h to be totally converted into a single isomer, while the *E* needed only 3h, forming two different isomers as final products.

- We found that viniylarenes reacted through a Diels-Alder mechanism with the cyclopropane, forming a polyfused system. The best conditions were *n*-BuOH as solvent at 140 ^{o}C for 15 minutes.

- The reaction tolerates the use of electrowithdrawing and electrodonating groups at the aromatic ring. These final compounds contain up to 5 stereocentres which are formed in a totally stereoselective fashion. When the vinylarene has substituents at the olefinic part, a non nucleofilic base such as DBU is required.

- X-Ray diffraction analysis was performed to confirm the stereochemistry of these final products. Computational studies were also carried out to understand the stereochemistry outcome of the reaction.

- Mechanistic studies using NMR were performed. We could se a nonaromatic compound with multiple olefinic signal that was constant in time until the total conversion of the starting material, suggesting a Diels-Alder cycloadduct was forming before the rearomatization step.

Chapter III

The last chapter summarizes the work done in Prof. Lutz Ackermann's lab, in Göttingen, Germany, during a four month stay. Late-stage functionalization methodologies for the modification of biomolecules need to be specific, robust and highly functional group tolerant reactions. Among them, C-H activations have found many applications. The group has been working on selective C-H activations of the indole ring, particularly in tryptophan. Among all the proteogenic aminoacids, tryptophan is the one with the most electron rich π-system. This makes this aminoacid to be present mostly in active sites. The previously reported methods for its modification involved the use of harsh condition, stoichiometric amounts of oxidant salts and different additives and the use of directing groups in tryptophan. Electrophilic charged salts have been prepared to try to avoid the use of these oxidants because they can make easier the oxidative addition to the catalyst. Diariliodonium salts and arildiazo salts did not need the use of oxidants and the conditions for the transformation were softer, but the preparation of these kind of compounds still needs harsh conditions. On the other hand, thiantrenium salts are electrophilic charged salts that do not need harsh conditions to be prepared. Furthermore, the preparation of this salts is totally regioselective and they are now becoming a powerful tool for C-H activation.

The conclusions for this chapter are:

- Thiantrenium salts have been prepared following previously reported protocols. We synthetized peptide, drugs and aromatic rings with different substitution patterns derivatives.

- Under our optimized conditions it was not necessary to use a directing group in the tryptophan moiety. We needed a 10 mol% of $Pd(OAc)_2$ in $^{i}PrOH$ as solvent at 70 ^{o}C for 16 hours.

- We performed a robustness test to evaluate the functional group tolerance of the reaction. We found that methionine and cysteine poisoned the catalyst.

- We could ligate tryptophan with different aromatic rings, including a fluorescent probe as xanthone. We could also ligate bigger peptides, even a decapeptide, with some drug derivatives or even other aminoacids such as phenylalanine and tyrosine.

- We modified 2,5-diketopiperazines and cyclic peptides.

La presente tesis está dividida en tres capítulos diferentes, dedicados a la síntesis de iminoazúcares rigidificados y el estudio de sus interacciones con glicosidasas, a nuevos reordenamientos de sistemas que contienen un ciclopropenos y, por ultimo, a la activación C-H en triptófano, respectivamente.

Capítulo I:

Las glicosidasas tienen un papel importante en la naturaleza. Catalizan diferentes reacciones bioquímicas que involucran azúcares como la degradación de carbohidratos y la transglicosidación. Estos procesos intervienen en reconocimiento celular, infección vírica y bacteriana, etc. Una manera típica de inhibir o modular la actividad de estas encimas es mediante el uso de compuestos que mimeticen a los azúcares naturales o al estado de transición durante la reacción enzimática. Uno de estos tipos de compuestos son los iminoazúcares, que generalmente son estructuras basadas en piperidina y pirrolidina que poseen numerosos grupos hidroxilo. Por lo tanto, haciendo referencia a los azúcares naturales, los iminoazúcares son análogos en los cuales el átomo de oxígeno endocíclico ha sido cambiado por uno de nitrógeno, lo que les da una mejorada estabilidad metabólica. Desde hace muchos años, se conoce que estos compuestos son buenos miméticos de carbohidratos aunque, a veces, la falta de selectividad entre enzimas les impide llegar a convertirse en fármacos. A pesar de ello, algunos iminoazúcares se comercializan como tratamiento para diferentes enfermedades relacionadas con los azúcares.

En el primer capítulo de esta memoria, el objetivo ha sido la síntesis de nuevos iminoazúcares basados en piperidina, rigidificados mediante la fusión a un anillo de ciclopropano, lo que debería conferirles una mayor selectividad frente a glicosidasas. Para la síntesis hemos utilizado aminoácidos naturales buscando la formación de la mayor cantidad posible de diastereoisómeros, evitando reacciones de racemización. De este modo conseguimos diferentes productos enantioméricamente puros. Comenzamos la síntesis con L-serina, la cual tras varias reacciones, se transformó en diferentes diastereoisómeros de los productos finales deseados. Estos compuestos poseían hasta 5 centros esterogénicos. También usamos la L-alanina como producto de partida, con lo cual obtuvimos productos relacionados con la L-fucosa, un carbohidrato natural presente en alguna infección vírica. Se estudió en todos los compuestos finales la capacidad para interaccionar con diferentes glicosidasas mediante ensayos *in vitro* y técnicas de RMN. Esto fue possible gracias a una pequeña estancia en el laboratorio del professor F. Javier Cañada en el CIB, CSIC.

Las conclusiones para este capítulo son:

- Hemos preparado 11 compuestos finales, basados en piperidina fusionados a un anillo de ciclopropano con hasta 5 centros estereogénicos. Para las síntesis se usaron L-serina y L-alanina como productos de partida. Los productos finales se diferencian en la estereoquímica y en la sustitución en el anillo de ciclopropano.

- Las reacciones principales en las rutas de síntesis eran una metátesis de cierre de anillo para la construcción del anillo de piperidina, catalizada por el carbeno de Grubbs de 2ª generación sobre una cetona α,β-insaturada, y una ciclopropanación usando iluros de azufre.

- La estereoquímica del producto de ciclopropanación puede ser modulada por cambios en las condiciones de reacción. El uso de una base como la DBU, para formar *in situ* el iluro, daba como único resultado el isómero *exo*. Sintetizando y aislando previamente el iluro, pudimos observar el isómero *endo*. Comprobamos que realizando la reacción a una concentración de 0,06

M a 0 ^{o}C se pudo aislar el isómero *endo* en un 18% de rendimiento para uno de los derivados de la L-serina.

- En función de la estereoquímica obtenida después de la ciclopropanación, la reducción de la cetona podia ser diastereoselectiva, como se vio en el caso de la L-serina, debido al impedimento estérico.

- Los productos fueron ensayados enzimáticamente contra 8 diferentes glicosidasas. La mayoría de los resultados no fueron satisfactorios mostrando que no había inhibición de las enzimas. Aún así, algunos compuestos eran inhibidores en el rango de mM de una sóla enzima, lo que significa que habíamos conseguido la deseada selectividad. En otros casos, encontramos activación de las enzimas.

- Para intentar explicar la activación de las enzimas, seguimos la reacción enzimática por ^{1}H-RMN para ver si podíamos identificar algún producto relacionado con una transglicosidación, pero los resultados no fueron concluyentes.

Capítulo II:

El objetivo principal en este segundo capítulo ha sido el estudio de la reactividad de estructuras ciclopropen-énicas. Los ciclopropenos son los ciclos insaturados más pequeños y tienen una elevada energía de tensión. Aun así, estos ciclos son estables ya que el principal mecanismo de degradación son las reacciones pericíclicas y la energía necesaria para la apertura del anillo es muy alta. El uso de complejos metálicos o luz facilita la apertura del anillo para dar lugar a una amplia reactividad, generalmente a través de vinilcarbenos, que pueden reaccionar con dobles o triples enlaces. En cuanto a los sistemas ciclopropen-énicos, ha sido descrito que reaccionan con catalizadores de oro por el carbono olefínico menos sustituido del ciclopropeno generando un vinilcarbeno que ciclopropana con la olefina. Estos sistemas, con rutenio, principalmente dan reacciones de metátesis de cierre de anillo. Nuestro grupo estudió la reactividad de estos sistemas utilizando Cp*RuCl(cod), observando una regioselectividad diferente durante la apertura del ciclopropeno a la observada con oro, obteniendo [3.1.0], [4.1.0] y [5.1.0]biciclos. La limitación de esta reacción era la necesidad de usar olefinas terminales. Encontramos que usando catalizadores dímeros de rutenio se formaban indanos y tetralinas siguiendo un mecanismo similar.

Los ciclopropenos también son conocidos por ser buenos sustratos en las reacciones pericíclicas ya que poseen un orbital LUMO de baja energía. Termodinámicamente, estas reacciones están favorecidas por la liberación de la tensión del anillo de ciclopropeno. La estereoquímica resultante se define tanto por la teoría del SOI, que favorece la aproximación *endo* y por el impedimento estérico de los sustituyentes. La primera reacción Alder-énica intramolecular de un ciclopropeno fue descrita en 1985. Además, son conocidos por ser buenos dienófilos desde el trabajo de Wiberg en 1960. En 2010, Patel y Boger describieron la primera reacción de Diels-Alder intramolecular entre un ciclopropeno y un dieno. Esta reacción ha sido usada como reacción bioortogonal en la señalización de biomoléculas.

Las conclusiones para este capítulo son:

- Hemos optimizado la síntesis de indanos y tetralinas funcionalizadas a partir de estructuras ciclopropen-énicas usando un 5 mol% del catalizador dímero de rutenio bajo radiación microondas a 180 ^{o}C durante 30 minutos.

- Cuando la aromatización no era possible se obtuvo una cetona α,β-insaturada. Cuando el ciclopropeno estaba disustituido, se observó una migración del grupo metilo que estaba impidiendo la etapa de aromatización.

- Propusimos un mecanismo que implica la formación de un carbeno metálico en el carbono interno del ciclopropeno después de la apertura del metalaciclobuteno, dando como resultado un doble enlace con configuración *Z*. Esto permite al carbonilo del éster acercarse lo suficiente al rutenio para reaccionar con él formando un dihidrofurano, que evoluciona hacia un triciclo. En presencia del metal, ocurría una aromatización desoxigenativa hacia los productos finales.

- Durante el estudio del alcance de la reacción, encontramos que cuando el ciclopropeneno estaba disustituido terminalmente en la parte olefínica, se formaba un espirociclo. No era necesaria la presencia de catalizador para esta reacción.

- Propusimos una reacción "*one pot"* para formar los espirociclos a partir del compuesto que contiene el ciclopropeno y el derivado bromado que era usado para introducir la cadena olefínica. Encontramos que las mejores condiciones eran usando 2-MeTHF como disolvente a reflujo durante 6 horas de reacción.

- Cuando utilizábamos cetonas α,β-insaturadas se obtenían ciclopropa[*b*]piranos siguiendo un mecanismo similar.

- También estudiamos el efecto en la estereoquímica del doble enlace. Observamos que el isómero *Z* necesitaba 48 horas para convertirse en un único isómero final, mientras que el isómero *E* sólo necesitaba 3 horas, dando lugar a una mezcla de isómeros.

- Observamos que los derivados de vinilarenos reaccionaban a través de una reacción de Diels-Alder con el ciclopropeno para formar sistemas policíclicos fusionados. Las mejores condiciones se daban con *n*-BuOH como disolvente a 140 ^{o}C durante 15 minutos.

- La reacción toleraba sustituyentes electrodonadores y electroaceptores en el anillo aromático. Estos compuestos finales poseían hasta 5 centros estereogénicos formados de manera totalmente diastereoselectiva. Cuando el vinilareno poseía sustituyentes en la parte olefínica era necesario el uso de una base no nucleófila como DBU.

- La difracción de Rayos-X de uno de los productos obtenidos confirmó la estereoquímica propuesta por RMN. Se realizaron estudios computacionales para entender el resultado estereoquímico .

- Llevamos a cabo estudios mecanísticos por RMN. Pudimos obervar la formación de un compuesto no aromático con multiples señales olefínicas que era constante en el tiempo hasta la conversión total del producto de partida. Esto sugiere la formación de un cicloaducto de Diels-Alder previo al paso de rearomatización.

Capítulo III:

El ultimo capítulo recoge el trabajo realizado en el laboratorio del professor Lutz Ackermann, en Göttingen, Alemania, durante mi estancia de 4 meses. Las metodologías para la funcionalización de última etapa en biomoléculas tienen que ser específicas, robustas y con alta tolerancia de grupos funcionales. Entre ellas, en las activaciones C-H se han encontrado muchas aplicaciones. El grupo ha estado trabajando en activaciones C-H selectivas del anillo de indol, particularmente en triptófano. De entre todos los aminoácidos, el triptófano es el que posee el sistema π más rico en electrones. Esto provoca que este aminoácido se encuentre sobre todo

en centros activos. Las metodologías previas para esta modificación requerían condiciones duras, cantidades estequiométricas de oxidantes y diferentes aditivos, además de necesitar de un grupo director. Se han sintetizado sales electrofílicas para intentar evitar el uso de oxidantes ya que pueden facilitar la adición oxidativa al catalizador. Las sales de diarilyodonio y arildiazonio no necesitaban el uso de oxidantes y las condiciones para la transformación eran más suaves, pero su preparación aún requería el uso de condiciones drásticas. Por otro lado, las sales de tiantreno son sales electrofílicas que no necesitan condiciones drásticas para su síntesis. Además, la preparación de estas sales es totalmente regioselectiva y se están convirtiendo en un poderosa herramienta en las activaciones C-H.

Las conclusiones para este capítulo son:

- Se han preparado diversas sales de tiantreno siguiendo protocolos ya publicados. Sintetizamos derivados de péptidos, fármacos y anillos aromáticos con diferente sustitución.

- Usando nuestras condiciones no era necesario el uso de un grupo director. Se necesitaba un 10 mol% de $Pd(OAc)_2$ en $^{i}PrOH$ como disolvente a 70 ^{o}C durante 16 horas.

- Realizamos un test para comprobar si nuestro método era robusto y evaluar la tolerancia a grupos funcionales. Observamos que en presencia de metionina y cisteina la reacción no tenía lugar.

- Pudimos ligar triptófano con muchos anillos aromáticos, incluyendo la xantona, que es una sonda fluorescente. Pudimos unir también péptidos más grandes, hasta un decapéptido, con derivados de fármacos y otros aminoácidos como fenilalanina y tirosina.

- Modificamos 2,5-dicetopiperacinas y péptidos cíclicos.