Diseño y síntesis de bibliotecas combinatorias dinámicas: estudio de nuevas reacciones reversibles y su aplicación en la búsqueda de sustancias bioactivas.

Tesis presentada por la Q.F. Cecilia Saiz para aspirar al título de Doctor en Química

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A mi familia

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- Saiz, C.; Wipf, P.; Mahler, S. G. Synthesis and Ring-Chain-Ring Tautomerism of Bisoxazolidines, Thiazolidinyloxazolidines, and Spirothiazolidines. *J. Org. Chem.* **2011**, *76*, 5738 – 5746.

- Pizzo, C.; Saiz, C.; Talevi, A.; Gavernet, L.; Palestra, P.; Bellera, C.; Bruno-Blanch, L.; Benitez, D.; Wipf, P.; Mahler, S. G. Synthesis of 2-hydrazolyl-4-thiazolidinones based on multicomponent reactions and biological evaluation against *T. Cruzi*. *Chem. Biol. Drug Design* **2010**, *77*, 166 – 172.

- Saiz, C.; Wipf, P.; Manta, E.; Mahler, S. G. Reversible Thiazolidine Exchange: A New Reaction Suitable for Dynamic Combinatorial Chemistry. *Org. Lett.* **2009**, *11*, 3170 – 3173.

- Saiz, C.; Pizzo, C.; Manta, E.; Wipf, P.; Mahler, S. G. Microwave-assisted tandem reactions for the synthesis of 2-hydrazolyl-4-thiazolidinones. *Tetrahedron Lett.* **2009**, *50*, 901 – 904.

- Liang, M.; Saiz, C.; Pizzo, C.; Wipf, P. Synthesis of pyrrolo[1,3]diazepines by a dipolar cycloaddition-retro- Mannich domino reaction. *Tetrahedron Lett.* **2009**, *50*, 6810 – 6813.

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Trabajos presentados en congresos:

- 14th Brazilian Meeting on Organic Synthesis, Brasilia, Brasil **2011**. Poster: Synthesis of new scaffolds suitable for Dynamic Combinatorial Chemistry. Saiz, C.; Manta, E.; Wipf, P.; Mahler, G.

- Sao Paulo Advanced school of Chemistry, SP/Campinas, Brasil **2011**. Poster: Poster: Synthesis of new scaffolds suitable for Dynamic Combinatorial Chemistry. Saiz, C.; Manta, E.; Wipf, P.; Mahler, G.

- Segundo Encuentro Nacional de Ciencias Químicas (ENAQUI), Montevideo **2011**. Presentación oral: Nuevas reacciones reversibles y su aplicación en Química Combinatoria Dinámica. C. Saiz.

- XVII Simposio Nacional de Química Orgánica, Mendoza, Argentina, **2009.** Poster: Nueva reacción reversible útil para aplicar en Química Combinatoria Dinámica: aminotioles y carbonilos. Saiz, C.; Pizzo, C.; Manta, E.; Mahler, G.

- Primer Encuentro Nacional de Ciencias Químicas (ENAQUI), Montevideo, **2009**. Poster: Reacciones reversibles útiles para su utilización en química combinatoria dinámica: Aminotioles y Carbonilos. Saiz, C.; Pizzo, C.; Manta, E.; Mahler, G.

- 238th ACS National Meeting and Exposition, Washington DC, USA, **2009**. Poster: Novel Synthesis of Pyrrolodiazepine Scaffolds by a Spontaneous Retro-Mannich Domino Reaction. Liang, M.; Saiz, C.; Pizzo, C.; Wipf, P.

- 6th ACS Summer School on Sustainability and Green Chemistry, Golden, Colorado, USA **2008**. Poster: Synthesis of new sccafolds to generate dynamic combinatorial libraries (DCLs). Saiz, C.; Pizzo, C.; Manta, E.; Mahler, G.

- XV Simposio Nacional de Química Orgánica I Simposio Iberoamericano de Química Orgánica, Mar del Plata, Argentina **2007**. Poster: Síntesis de bibliotecas combinatorias dinámicas en la búsqueda de actividad enzimática. Saiz, C.; Pizzo, C.; Manta, E.; Mahler, G.

- XVII Jornadas de Jóvenes Investigadores, Montevideo, **2007.** Presentación oral: Síntesis en tándem y asistida por microondas de 2-hidrazolil-4-tiazolidinonas. C. Saiz.

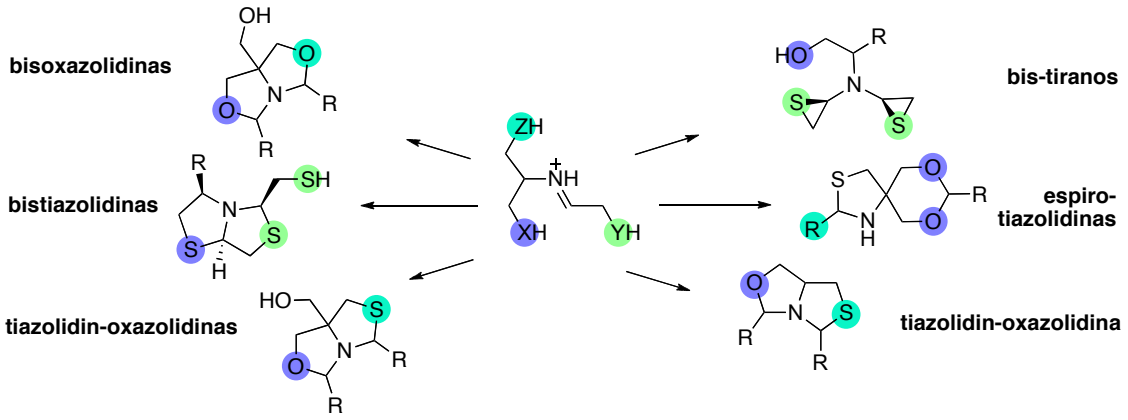
RESUMEN

Desarrollada a principio de los 90, la Química Combinatoria Dinámica (QCD) es una estrategia sintética que une la brecha existente entre la síntesis química de potenciales ligandos y el posterior ensayo biológico: combina ambos procesos en ensayo único.

En este contexto, la investigación realizada se puede dividir en dos grandes capítulos: por un lado el estudio de nuevas reacciones reversibles junto con la preparación de bloques de construcción, y por el otro la aplicación de la QCD en la búsqueda de inhibidores enzimáticos.

En primer lugar, se estudió el intercambio acetálico entre aminotioles y carbonilos como posible reacción reversible capaz de generar bibliotecas dinámicas. Se describió la generación de bibliotecas dinámicas a partir de dicha reacción reversible y se demostró que cumple con los requisitos necesarios para este tipo de sistemas.

Se trabajó en la preparación de diferentes bloques de construcción conteniendo tiazolidinas. Se estudió la reactividad de distintos cationes iminio β-sustituídos, dando lugar a la generación de una variada serie de heterociclos no conocidos hasta el momento, ver esquema general:



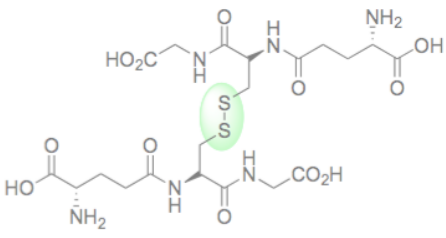
Finalmente, se generó una biblioteca dinámica de intercambio tiol/disulfuro y utilizando la enzima Tiorredoxina Glutatión Reductasa como molde. Esta enzima redox es esencial para el parásito responsable de la hidatidosis quística (*Echinococcus granulosus*). Dentro de una BCD de 21 posibles compuestos se observó la amplificación de un compuesto que contiene un disulfuro mixto. Posterior evaluación lo confirmó como inhibidor de esta enzima.

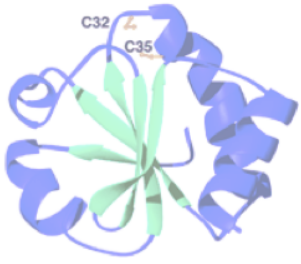
INDICE GENERAL

3. RESULTADOS Y DISCUSION

3.1 New reactions and building blocks for Dynamic Combinatorial Chemistry (DCC)

1. INTRODUCCION





1.1 Química Combinatoria Dinámica (QCD)

1.1 Química Combinatoria Dinámica

1.1.1 Definición

En las últimas décadas, el estudio de nuevas estrategias para el descubrimiento de drogas ha permitido el desarrollo de herramientas alternativas a las clásicas. Para ello, los químicos sintéticos se han inspirado en los procesos naturales guiados por un molde como, por ejemplo, la replicación del ADN o el ensamblado de proteínas.

En el marco de dichos procesos, la búsqueda de nuevos ligandos utilizando síntesis guiada por un blanco (*TGS: target guided synthesis*) se ha desarrollado recientemente a través de nuevas metodologías.[1] Dentro de la TGS, una de las principales aplicaciones es la química combinatoria dinámica (QCD).[2] Esta herramienta ha surgido como una promisoria estrategia de TGS ya que utiliza el reconocimiento molecular para dirigir la síntesis del mejor ligando.

La química combinatoria tradicional es una metodología que permite la generación de diversidad molecular a través de las posibles combinaciones de sus bloques de construcción. Basada en esta estrategia, la QCD tiene como ventaja adicional que el sistema está controlado termodinámicamente. Una condición necesaria para el control termodinámico es la reversibilidad, es decir que existan equilibrios dinámicos entre los distintos componentes de la biblioteca. De esta forma, en estos sistemas el producto termodinámico siempre estará favorecido con respecto a los otros posibles productos.

La biblioteca combinatoria dinámica (BCD) se implementa a partir de bloques de construcción en solución que se interconectan mediante enlaces reversibles. Los enlaces pueden ser covalentes, no covalentes o iónicos y se forman y rompen constantemente hasta alcanzar la distribución de menor energía, es decir el mínimo termodinámico. Esto significa que la composición del sistema está determinada por la estabilidad termodinámica relativa de cada uno de los miembros que la componen para las condiciones de reacción dadas.[3]

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[1] Hu, X.; Manetsch, R. Kinetic target-guided synthesis. *Chem. Soc. Rev.* **2010**, *39*, 1316–1324.
[2] Corbett, P.; Leclaire, J.; Vial, L.; West, K.; Wietor, J.; Sanders, J. K. M.; Otto, S. Dynamic combinatorial Chemistry. *Chem. Rev.* **2006**, *106*, 3652–3711.
[3] Otto, S.; Furlán, R.; Sanders, J. K. M. Recent developments in dynamic combinatorial chemistry. *Curr. Opin. Chem. Biol.* **2002**, *6*, 321-327.

El concepto de dinamismo implica la generación de sistemas que responden frente a un cambio, evolucionando hacia un nuevo sistema de mínima energía. Se puede decir que cualquier cambio en la estabilidad de un componente va a afectar a sus vecinos. Se llaman bibliotecas "vivientes", ya que responden a los cambios en el sistema y dirigen su distribución hacia el mínimo energético del sistema,[10] ver figura 1.

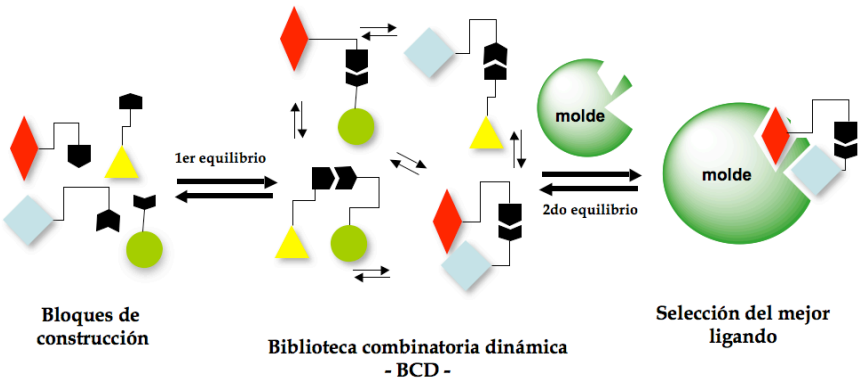


Figura 1. Equilibrios reversibles en Química Combinatoria Dinámica

Por ejemplo, un cambio en las condiciones del experimento puede ser variación del pH, de la temperatura, presión o introducción de un molde (ion, proteína, molécula). Si esta perturbación afecta a los componentes del sistema, puede inducir cambios en la composición de la biblioteca: una BCD responde a las influencias externas que dirigen la evolución del equilibrio hacia una nueva distribución más estable. Esto se refleja en un aumento en la cantidad de uno o varios componentes del sistema a expensas de los otros, fenómeno denominado *amplificación.*

Diversos estudios se han llevado acabo acerca de la respuesta de los componentes de la biblioteca en presencia de un molde. Si bien en la mayoría de los casos se observa que el o los compuestos amplificados son los más estabilizados por el molde, no siempre ocurre así. El grupo de Severin y col. demostró que no siempre hay una correlación directa entre la amplificación y la estabilidad termodinámica de los miembros de la biblioteca.[4,5] En determinadas situaciones, por ejemplo, con ligandos

[4] Saur, I.; Severin, K. Selection experiments with dynamic combinatorial libraries: the importance of the target concentration. *Chem. Commun.* **2005**, 1471-1473.
[5] Grote, Z.; Scopelliti, R.; Severin, K. Adaptive Behavior of Dynamic Combinatorial Libraries Generated by Assembly of Different Building Blocks. *Angew. Chem. Int. Ed.* **2003**, *42*, 3821–3825.

de afinidades similares o si se utiliza grandes cantidades de molde, puede ocurrir que la distribución final de la biblioteca no refleje el aumento de aquellos compuestos con mayor afinidad. Este problema se puede solucionar ajustando las condiciones experimentales, por ejemplo utilizando cantidades estequiométricas de molde con respecto a los componentes de la BCD se puede revertir este falso resultado.[6]

El uso de QCD se ha implementado como herramienta en la búsqueda de potenciales ligandos. Se ha aplicado en el descubrimiento de nuevos receptores, ligandos para iones y moléculas pequeñas,[7] ligandos de biomoléculas,[8] inhibidores enzimáticos,[9] catalizadores[10] y sensores,[11] entre otros.

Se han desarrollado bibliotecas dinámicas en presencia de diferentes blancos moleculares. En la figura 2 se muestran ejemplos de moldes que generaron respuestas de amplificación en sistemas dinámicos.

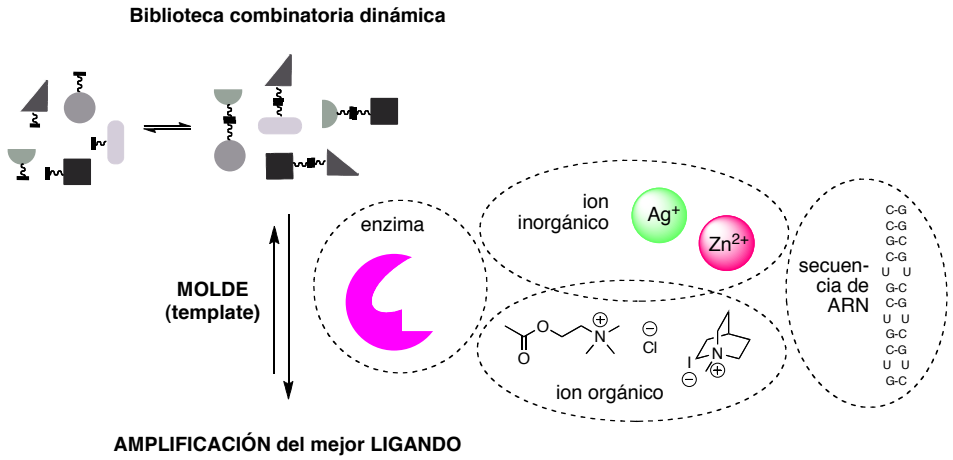
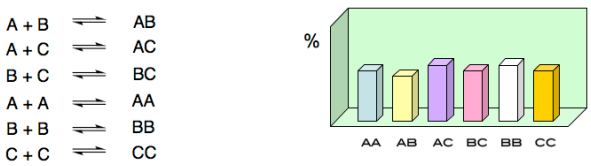


Figura 2. Ejemplos de moldes utilizados con BCDs en busca de ligandos.

[6] Corbett, P.; Sanders, J. K. M.; Otto, S. Competition between Receptors in Dynamic Combinatorial Libraries: Amplification of the Fittest? *J. Am. Chem. Soc.* 2005, *127*, 9390-9392.
[7] Furlán, R.; Ng, Y.; Otto, S.; Sanders, J. K. M. A New Cyclic Pseudopeptide Receptor for Li^+ from a Dynamic Combinatorial Library. *J. Am. Chem. Soc.* **2001**, *123*, 8876-8877.
[8] Zameo, S.; Vauzeilles, B.; Beau J-M. Dynamic Combinatorial Chemistry: Lysozyme Selects an Aromatic Motif that Mimics a Carbohydrate Residue. *Angew. Chem. Int. Ed.* **2005**, *44*, 965-969.
[9] Cancilla, M.; He, M.; Viswanathan, N.; Simmons, R.; Talylor, M.; Fung, A.; Cao, K.; Erlanson, D. Discovery of an Aurora kinase inhibitor through site-specific dynamic combinatorial chemistry. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3978-3981.
[10] Brisig, B.; Sanders, J.; Otto, S. Selection and amplification of a catalyst from a dynamic combinatorial library. *Angew. Chem. Int. Ed.*, **2003**, *42*, 1270-1273.
[11] Buryac, A.; Severin, K. Dynamic Combinatorial Libraries of Dye Complexes as Sensors. *Angew. Chem. Int. Ed.* **2005**, *44*, 7935-7938.

Para observar si el sistema responde al cambio introducido, primero se “congela” el intercambio: se detiene el equilibrio variando condiciones de modo que ya no ocurra intercambio de subunidades. Se separa el molde y se analiza la composición de la mezcla, comparando distribuciones de compuestos. La figura 3 es un ejemplo de las posibles distribuciones observadas en una BCD. En el equilibrio 1 se observa la distribución inicial de la mezcla. En el equilibrio 2 se observa el aumento del compuesto BC luego de introducir el molde, a expensas de los otros miembros que disminuyen su proporción. Esto ocurre porque el molde estabiliza al compuesto BC minimizando su energía y favoreciendo el desplazamiento del equilibrio hacia su formación.

1) **Distribución inicial (equilibrio 1)**



2) **Distribución en presencia de un molde T (equilibrio 2)**
Amplificación del mejor ligando BC

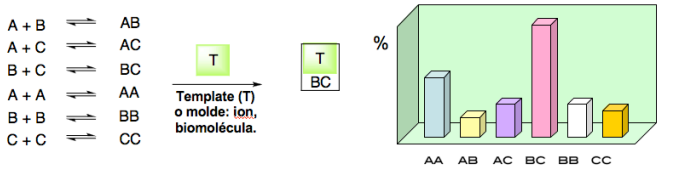


Figura 3. Distribución diferencial de los componentes de la biblioteca.

El análisis de la nueva distribución de la biblioteca se puede realizar mediante diferentes técnicas (HPLC, HPLC-MS, GC, fluorescencia). Estos sistemas permiten identificar y caracterizar a los ligandos amplificados, obteniendo información cuantitativa y cualitativa de la respuesta del sistema a la introducción del cambio.

En la búsqueda de inhibidores enzimáticos, la QCD tiene la ventaja de unir la brecha que existe entre la síntesis química de potenciales inhibidores y el posterior ensayo biológico. Esta metodología combina ambos procesos en un único ensayo en el cual la

estructura del blanco dirige la unión de su mejor ligando *in situ* mediante reconocimiento molecular.

Básicamente, esta disciplina aprovecha la gran cantidad y diversidad de componentes que maneja la química combinatoria tradicional y a su vez tiene la ventaja de que los sistemas son dinámicos y evolucionan hacia el mínimo termodinámico. Un número reducido de moléculas de partida permite la construcción de un pool intercambiable de componentes en equilibrio, cuyo objetivo final es encontrar una o varias moléculas que se vean estabilizadas por el blanco introducido.

1.1.2 Historia

Los primeros trabajos realizados en el estudio de sistemas en equilibrio termodinámico que se adaptan datan de mediados de los años 90. Uno de los primeros ejemplos es el del grupo de Sanders en 1996, en el cual describen una transesterificación en condiciones de equilibrio termodinámico de derivados del ácido cólico. Los bloques de construcción contienen un éster metílico en un extremo y un alcohol en el otro, formando distintos macrociclos en presencia de un complejo éter corona-MeOK, ver figura 4.[12]

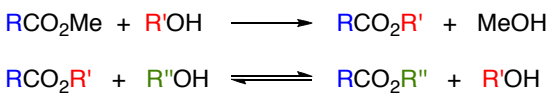


Figura 4. Transesterificación controlada termodinámicamente.

En este trabajo se demuestra que la reacción está controlada termodinámicamente partiendo de distintos macrociclos obteniendo siempre la misma distribución. La reversibilidad se estudia mediante las reacciones inversas y observando la misma distribución. Concluyen que están en condiciones de equilibrio adaptativo, por eso la llaman macrolactonización "vivente".

[12] Brady, P.; Bonar-Law, R.; Rowan, S.; Suckling, C.; Sanders, J. K. M. 'Living' macrolactonisation: thermodynamically-controlled cyclisation and interconversion of oligocholates. *Chem. Commun.* **1996**, 319-320.

Otro pionero en el desarrollo de este herramienta fue el grupo de Lehn, que comenzó con la búsqueda de inhibidores enzimáticos (molde: anhidrasa carbónica, CA) mediante intercambio de iminas, utilizando como bloques de construcción aldehídos y aminas. Se observó que la presencia del molde desplaza el equilibrio favoreciendo la formación de los compuestos con mayor afinidad por el sitio activo de la enzima.[13]

Por otro lado, una reacción reversible muy aplicada en sistemas que utilizan moldes biológicos es la de intercambio tiol-disulfuro. Esto se debe a que esta reacción se da en condiciones suaves, a pH cercano al neutro (levemente básico), y a temperatura ambiente, por lo que la mayoría de los sistemas biológicos la toleran.

Este equilibrio reversible fue propuesto para su aplicación en combinatoria dinámica inicialmente por Hioki y Still en 1998.[14] Se plantea un equilibrio entre tioles en medio básico (Et_3N en CH_2Cl_2), utilizando como molde un tripéptido. Estudios de las K_{eq} mostraron que la unión al sustrato peptídico logra desplazar el equilibrio de los receptores hacia el aumento del receptor más estabilizado por el sustrato. Más tarde, Sanders y col. describieron el primer ejemplo de intercambio de disulfuros a partir de ditioles alifáticos como bloques de construcción en medio acuoso (pH 7.5) para formar macrociclos.[15]

1.1.3 Reacciones reversibles en QCD.

a. Generalidades

Las reacciones útiles para construir bibliotecas reversibles deben cumplir con ciertos requisitos: a) deben ser reversibles, b) las condiciones de reacción deben ser compatibles con el proceso de selección y a su vez deben ser suaves para no interferir en las interacciones no covalentes implicadas en el reconocimiento molecular, c) todos los componentes deben ser solubles en el medio de reacción, d) debe ser posible detener el equilibrio (por un cambio en el pH por ejemplo). El éxito y la aplicabilidad de

[13] Huc, I.; Lehn, J. M. Virtual combinatorial libraries: Dynamic generation of molecular and supramolecular diversity by self-assembly. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110.
[14] Hioki, H.; Still, W. C. Chemical evolution: a model system that selects and amplifies a receptor for the tripeptide (D)Pro(L)Val(D)Val. *J. Org. Chem.* **1998**, *63*, 904-905.
[15] Otto, S.; Furlán, R.; Sanders, J. K. M. Dynamic Combinatorial Libraries of Macrocyclic Disulfides in Water. *J. Am. Chem. Soc.* **2000**, *122*, 12063-12064.

la metodología se basa en el cumplimiento de los requisitos necesarios para generar sistemas reversibles, de esto dependerá la validez de los resultados obtenidos. Debido a los requisitos enumerados para implementar estos sistemas, son acotadas las reacciones disponibles para generar BCDs y es entonces necesario el estudio de nuevas reacciones que permitan ampliar el abanico de posibilidades.

Existen tres tipos principales de enlaces utilizados para generar estos sistemas: covalentes, no covalentes y de coordinación. Dentro de los enlaces covalentes podríamos clasificarlos de acuerdo al grupo funcional involucrado: reacciones de intercambio que involucran grupos carbonilo, intercambio de disulfuros,[15] metátesis de alquenos,[16] reacciones de Diels-Alder,[17] entre otros, ver figura 5.

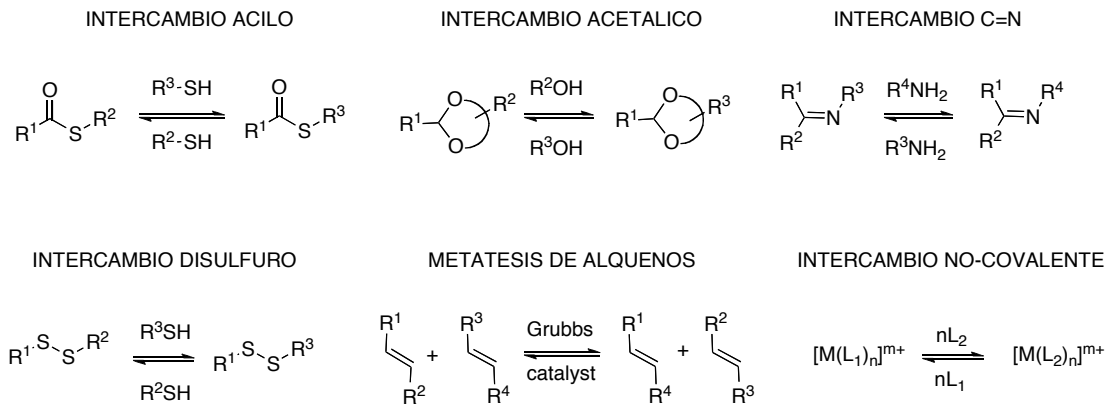


Figura 5. Ejemplos de reacciones de intercambio para QCD.

En general, los intercambios que involucran enlaces no covalentes alcanzan el equilibrio más rápido que los covalentes pero tienen la desventaja de que son productos inestables, lo que dificulta el análisis de la biblioteca.

Los intercambios que ocurren mediante formación y ruptura de enlaces covalentes tienen cinéticas mas lentas. El agregado de un catalizador o variaciones en pH, T son opciones para acelerar estos sistemas. Tienen la ventaja de que los productos son estables y es posible el análisis de la mezcla sin pérdida de información.

[16] (a) van Gerven, P.; Elemans, J.; Gerritsen, J.; Speller, S.; Nolte, R.; Rowan, A. Dynamic combinatorial olefin metathesis: templated synthesis of porphyrin boxes. *Chem Commun* **2005**, 3535-3537; (b) Poulsen, S.; Bornaghi, L. Fragment-based drug discovery of carbonic anhydrase II inhibitors by dynamic combinatorial chemistry utilizing alkene cross metathesis. *Bioorg. Med. Chem.* **2006**, *14*, 3275-3284.
[17] Boul, P.; Reutenauer, P.; Lehn, J-M. Reversible Diels-Alder Reactions for the Generation of Dynamic Combinatorial Libraries. *Org. Lett.* **2005**, *7*, 15-18.

b. Intercambios que involucran carbonilos

Para generar bibliotecas a partir de compuestos carbonílicos, existen tres grupos principales de reacciones reversibles: intercambio acilo,[18] intercambio imino C=N,[19] e intercambio acetálico, ver figura 6.

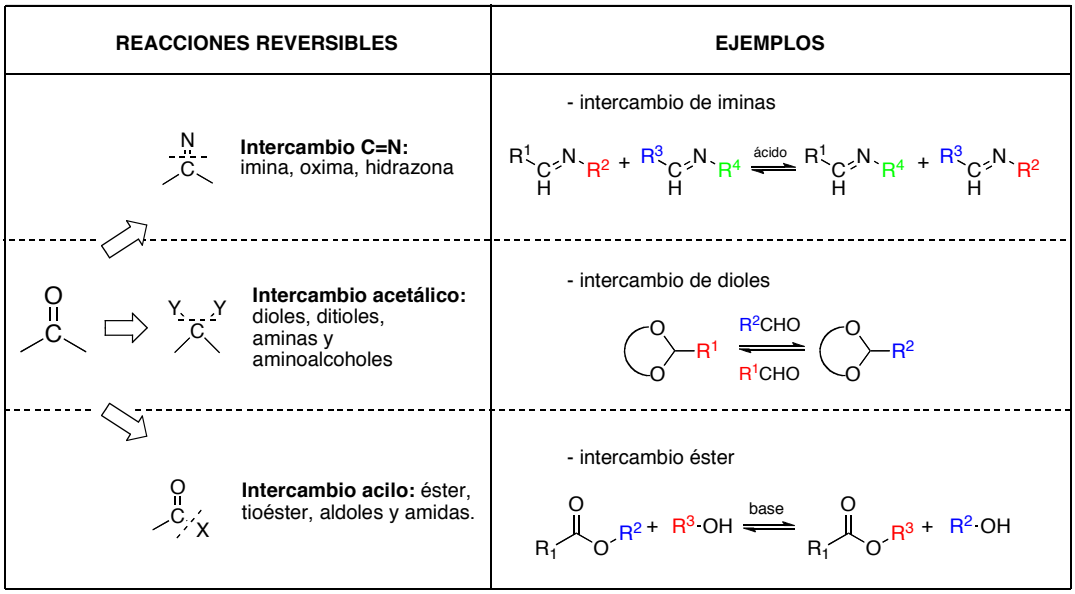


Figura 6. Reacciones de intercambio covalentes que involucran grupos carbonilo.

Dentro de los intercambios acetálicos, existen aquellos que involucran carbonilos en presencia de: dioles,[20] aminoalcoholes,[21] diaminas,[22] y tioles.[23]

[18] Lins, R.; Flitsch, S.; Turner, N.; Irving, E.; Brown, S. Enzymatic generation and in situ screening of a dynamic combinatorial library of sialic acid analogues. *Angew. Chem. Int. Ed.* **2002**, *41*, 3405-3407.

[19] (a) Giuseppone, N.; Schmitt, J.; Schwartz, E.; Lehn J-M. Scandium (III) catalysis of transimination reactions. Independent and constitutionally coupled reversible processes. *J. Am. Chem. Soc.* **2005**, *127*, 5528-5539; (b) Godoy-Alcántar, C.; Yatsimirsky, A.; Lehn, J-M. Structure-stability correlations for imine formation in aqueous solution. *J. Phys. Org. Chem.* **2005**, *18*, 979-985.

[20] (a) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. Metathesis Reaction of Formaldehyde Acetals: An Easy Entry
into the Dynamic Covalent Chemistry of Cyclophane Formation. *J. Am. Chem. Soc.* **2005**, *127*, 13666-13671; (b) Fuchs, B.; Nelson, A.; Star, A.; Stoddart, J.; Vidal S. Amplification of dynamic chiral crown ether complexes during cyclic acetal formation. *Angew. Chem. Int. Ed.* **2003**, *42*, 4220-4224; (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L.; Mencarelli, P.; Ugozzoli, F. Metathesis Reactions of Formaldehyde Acetals – Experimental and Computational Investigation of Isomeric Families of Cyclophanes under Dynamic Conditions. *Eur. J. Org. Chem.* **2008**, 186-195; (d) Berkovich-Berger, D.; Lemcoff, N. Facile Acetal Dynamic Combinatorial Library. *Chem. Commun.* **2008**, 1686-1688.

[21] (a) Star, A.; Goldberg, I.; Fuchs, B. Dioxadiazadecalin/salen tautomeric macrocycles and complexes: prototypal dynamic combinatorial virtual libraries. *Angew. Chem. Int. Ed.* **2000**, *39*, 2685-2689; (b) Star, A.; Goldberg, I.; Fuchs B. Dioxadiazadecaline and Salen Podands and Macrocycles within Dynamic Combinatorial Virtual Libraries: Structure, Prototropy, Complexation, and Enantioselective Catalysis. *J. Organomet. Chem.* **2001**, *630*, 67-77.

[22] Wipf, P.; Mahler, S.; Okumura, K. Metathesis Reactions of Pyrazolotriazinones Generate Dynamic Combinatorial Libraries. *Org. Lett.* **2005**, *7*, 4483-86.

Dado el número limitado de reacciones reversibles disponibles para construir BCDs, se ha estudiado la combinación de dos o más reacciones reversibles aplicadas a un único sistema dinámico, con el objetivo de aumentar la diversidad molecular.

Desarrollados por los grupos de Otto y Furlán,[24] estos intercambios permiten aumentar el número de combinaciones posibles a partir de un número dado de bloques de construcción. Se describió dos tipos de bibliotecas combinadas: ortogonal (los procesos reversibles son independientes) o simultánea (ambos procesos operan a la vez).

En un proceso ortogonal, los procesos deben ser compatibles para poder coexistir. Por ejemplo, el intercambio disulfuro (medio básico) ocurre a pH distinto respecto al de hidrazonas (medio ácido), por lo que se puede activar cada uno de ellos en forma independiente controlando el pH. Esto permite realizar los intercambios en dos etapas independientes.

Un ejemplo de este proceso es el de Otto y colaboradores en el que describe la combinación de dos reacciones en un único sistema.[25] A partir de bloques de construcción conteniendo unidades hidrazida/disulfuro, aldehídos y tioles se generó un sistema de intercambio tiol/disulfuro y el hidrazida/hidrazona controlado por variación del pH, ver figura 7.

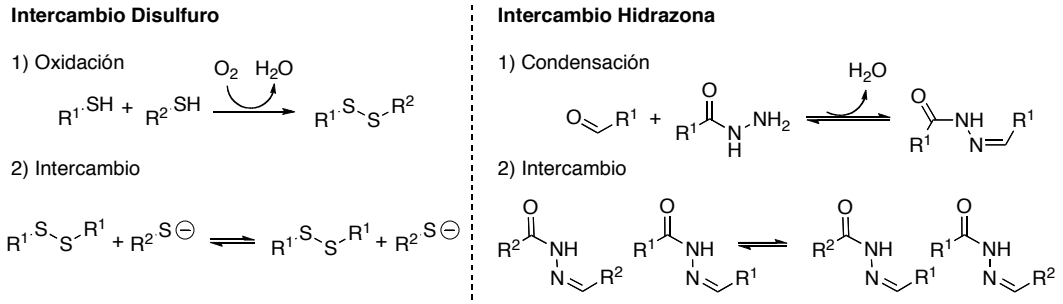


Figura 7. Reacciones reversibles: intercambio disulfuro e hidrazona.

[23] (a) Sutton, L.; Donaubauer, W.; Hampel, F.; Hirsch, A. Tris(thioacetals) from benezene hexathiol: towards covalent self-assembly. *Chem. Commun.* **2004**, 1758-1759; (b) Wu, Y.; Zhu, J. Hafnium Trifluoromethanesulfonate (Hafnium Triflate) as a Highly Efficient Catalyst for Chemoselective Thioacetalization and Transthioacetalization of Carbonyl Compounds. *J. Org. Chem.* **2008**, *73*, 9522-9524.

[24] Orrillo, A.; Escalante, A.; Furlán R. Covalent double level dynamic combinatorial libraries: selectively addressable exchange processes. *Chem. Commun.* **2008**, 5298-5300.

[25] Rodriguez-Docampo, Z.; Otto, S. Orthogonal or simultaneous use of disulfide chemistry and hydrazone exchange in dynamic covalent chemistry. *Chem. Commun.* **2008**, 5301-5303.

Ambas reacciones de intercambio se pueden dar en forma independiente o simultánea en el mismo sistema: al pasar de pH ácido a levemente básico se puede pasar de intercambio hidrazona a disulfuro, mientras que a un pH intermedio ambas reacciones ocurren simultáneamente. La diversidad de la biblioteca es notoria: a partir de los 4 bloques de construcción se puede generar 12 nuevos compuestos intercambiables. En conclusión, los intercambios disulfuro e hidrazona pueden ser activados o desactivados selectivamente en función del pH.

1.1.4 Ejemplos de aplicaciones con biomoléculas.

a) Inhibidores de GST de *Schistosoma japonicum*

Actualmente, la QCD es una poderosa herramienta para explorar sistemas basados en el reconocimiento molecular en el terreno de la ciencia de los materiales, la catálisis o el descubrimiento de fármacos.

La QCD dirigida por proteínas (protein-directed DCC) se basa en reacciones reversibles que ocurren en condiciones cercanas a las fisiológicas, requeridas por el blanco proteico. Dentro de la búsqueda de ligandos para biomoléculas, pocos grupos de reacciones han sido efectivos para generar BCDs debido a la compatibilidad necesaria entre los sistemas dinámicos y los moldes biológicos. La mayoría de los ejemplos en literatura utilizan el intercambio tiol/disulfuro e intercambios que involucran C=N para generar sistemas compatibles con los blancos biológicos.

Un ejemplo de la aplicación de la QCD para el descubrimiento de inhibidores es el trabajo de Greaney y colaboradores.[26] Se plantea la búsqueda de inhibidores de la Glutatión-S-Transferasa (GST) de *Schistosoma japonicum* mediante la generación de bibliotecas interconectadas por la adición conjugada de tioles a cetonas 2,3-insaturadas, ver Figura 8. La GST es una de las enzimas responsables de la detoxificación celular, blanco potencial de fármacos antiparasitarios contra la malaria y schistosomiasis entre otras.

[26] Shi, B.; Stevenson, R.; Campopiano, D.; Greaney M. Discovery of Glutathione S-Transferase Inhibitors Using Dynamic Combinatorial Chemistry. *J. Am. Chem. Soc.* **2006**, *128*, 8459-67.

Los mismos autores describieron la adición conjugada de tioles a enonas como una nueva reacción reversible útil para generar BCDs. Esta reacción es útil para aplicarla utilizando modelos biológicos ya que es rápida, reversible, responde a cambios de pH, ocurre en medio acuoso en condiciones suaves y no necesita de reactivos externos.[27]

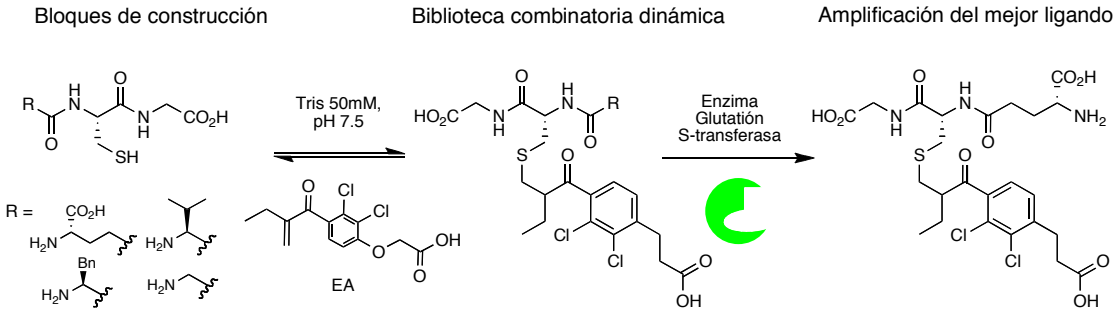


Figura 8. Generación de BCD, introducción de molde GST y amplificación.

En primer instancia, se deja que la biblioteca alcance el mínimo termodinámico a pH 7.5 durante 1h de intercambio. Luego se introduce la enzima como molde, y se analiza la nueva distribución. Se observa amplificación de un compuesto, el cual se sintetiza en forma independiente obteniendo un IC_{50} de 0.32 μM. Se continua estudiando otra región de la enzima encontrando nuevos inhibidores. La reacción ocurre en medio levemente básico y se logra el equilibro en forma rápida. Este trabajo es un ejemplo de la utilidad de estos sistemas en la búsqueda de inhibidores enzimáticos.

[27] Shi, B.; Greaney, M. F. Reversible Michael Addition of Thiols as a New Tool for Dynamic Combinatorial Chemistry. *Chem. Commun.* **2005**, 886-888.

b) Ligandos para lisozima HEWL.

En el siguiente ejemplo se presenta el trabajo realizado por el grupo de Beau,[8] en el cual los candidatos se sintetizan a partir de bloques de construcción conteniendo aldehídos y aminas. Para realizar el análisis de la biblioteca, se realiza una alquilación reductiva en presencia de $NaBH_3CN$ y se obtiene las correspondientes aminas. Primero se analiza la composición de la mezcla de los 12 compuestos en ausencia de enzima y luego se repite el ensayo en presencia de enzima. Se observa un aumento selectivo de uno de los compuestos, ver fig. 9.

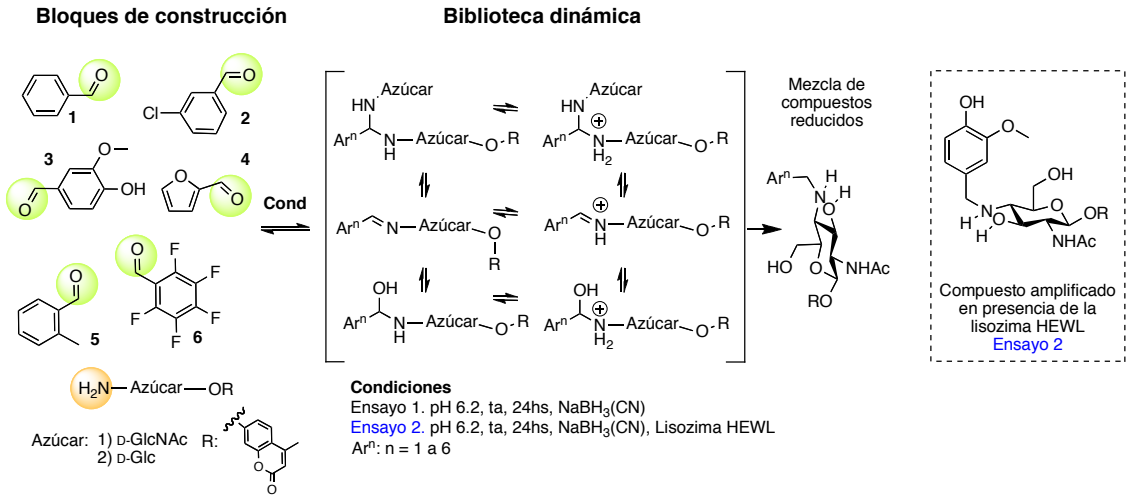


Figura 9. Síntesis de BCD y ensayo enzimático: ambos procesos en un único ensayo.

Podemos ver como se realiza al mismo tiempo y en el mismo sistema la síntesis dirigida de los ligandos en presencia de la enzima que actúa como molde y la consecutiva amplificación de aquellos más estabilizados.

1.1.5 Nuevas alternativas de análisis

a) Espectrometría de masa (Dynamic Combinatorial Mass Spectrometry, DCMS)

Inhibidores de BcII metallo-β-lactamasa

Recientemente se ha desarrollado un método de combinatoria dinámica que permite el complejo análisis de la composición de las mezclas vía espectrometría de masas (Dynamic Combinatorial Mass Spectrometry, DCMS),[28] y que tiene como ventaja un rápido análisis, permitiendo identificar en forma inmediata a los mejores ligandos. El uso de esta metodología ESI en condiciones no-desnaturalizantes permitió analizar la BCD de tioles y disulfuros en presencia de la enzima BcII metallo-β-lactamasa e identificar a los compuestos unidos a la misma. Se logró la rápida identificación de un inhibidor con una $K_i < 1$ μM. Este estudio ejemplifica la utilidad de MS de proteínas para hacer tamizado de mezclas dinámicas de potenciales inhibidores.

b) QCD en fase sólida (*resin-bound dynamic combinatorial chemistry*, RBDCC)

Poco después, Miller y colaboradores describieron un método de tamizado aplicando química combinatoria dinámica en fase sólida (RBDCC).[29] El mismo se basa en la síntesis de una BCD partiendo de la misma cantidad de monómeros unidos a resinas y en solución. En el ejemplo, son más de 11.000 miembros virtuales generados que están en equilibrio mediante intercambio de puentes disulfuro. En presencia de una secuencia de RNA marcado fluorescente como molde, se logra identificar a los mejores ligandos por microscopía de fluorescencia, ver figura 10.

[28] Liénard, B.; Huting, R.; Lassaux, P.; Galleni, M., Frère, J.; Schofield, C. Dynamic Combinatorial Mass Spectrometry Leads to Metallo-β-lactamase Inhibitors. *J. Med. Chem.* **2008,** *51,* 684-688.

[29] Gareiss, P.; Sobczak, K.; McNaughton, B.; Palde, P.; Miller, B. Dynamic Combinatorial Selection of Molecules Capable of Inhibiting the (CUG) Repeat RNA–MBNL1 Interaction In Vitro: Discovery of Lead Compounds Targeting Myotonic Dystrophy (DM1). *J. Am. Chem. Soc.* **2008**, *130*, 16254-61.

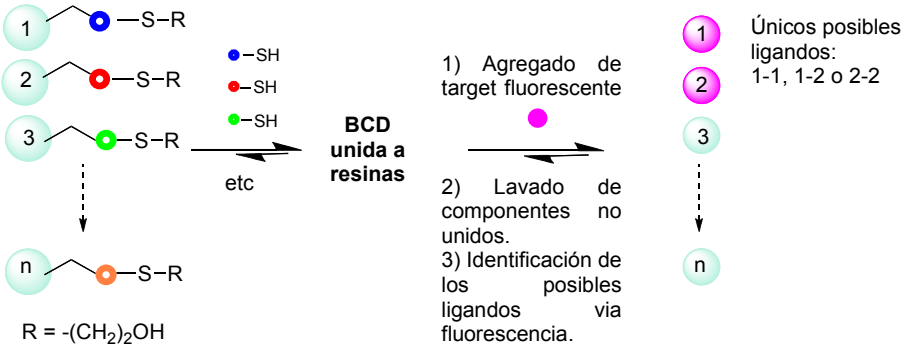


Figura 10. Generación de BCD unida a resina con introducción de molde fluorescente.

La ventaja del uso de QCD en fase sólida frente a la QCD clásica se basa en la posibilidad de generar bibliotecas con un número muy amplio de componentes y facilitar el posterior análisis.

Si bien recientemente Otto y colaboradores han demostrado que es posible el análisis de bibliotecas dinámicas de varios miles de componentes,[30] la mayoría de las BCDs descriptas en literatura no exceden los 100 componentes. Esto se debe al difícil análisis de resultados ya que requiere la utilización de cantidades muy diluidas de los componentes. Esta metodología (*resin-bound* DCC) logra sortear este problema, trabajando con un gran número de componentes en solución. En este caso, los compuestos seleccionados por fluorescencia se pueden identificar realizando una segregación espacial. Esto permite separar los compuestos seleccionados para realizar la posterior identificación de los mismos. La metodología logró identificar nuevos ligandos del segmento $(CUG)_n$ del RNA causante de la distrofia miotónica tipo 1 (DM1) y se encontraron 4 potenciales inhibidores con valores de K_i del orden μM.

Otro ejemplo reciente de *resind-bound* DCC es el presentado por Sherrington y col., donde se establece un intercambio disulfuro que utiliza una amina derivada de la amantadina unida a un polímero como molde.[31] Esta metodología permite un rápido análisis de los resultados mediante elución selectiva por afinidad. En primer lugar, la filtración de la resina separa aquellos componentes que no se unieron, luego un lavado con agua separa los compuestos de afinidad intermedia y por último un lavado con

[30] Ludlow, R.; Otto, S. Two-Vial, LC-MS Identification of Ephedrine Receptors from a Solution-Phase Dynamic Combinatorial Library of over 9000 Components. *J. Am. Chem. Soc.* **2008**, *130*, 12218–12219.
[31] Besenius, P.; Cormack, P.; Ludlow, R.; Otto, S.; Sherrington, D. Affinity chromatography in dynamic combinatorial libraries: one-pot amplification and isolation of a strongly binding receptor. *Org. Biomol. Chem.* **2010**, *8*, 2414–2418.

EtOH permite la elución del compuesto con mayor afinidad por el receptor. Se demuestra entonces la identificación y aislamiento de un receptor de alta afinidad a partir de una biblioteca dinámica de intercambio disulfuro en agua.

Como conclusión general, la QCD es una estrategia reciente que se ha desarrollado y evolucionado en las últimas décadas, siendo una elegante y eficiente herramienta para la generación de diversidad molecular y supramolecular. Si bien ofrece un amplio panorama de potenciales aplicaciones, aún son necesarios el desarrollo de nuevos sistemas capaces de comportarse reversiblemente y técnicas alternativas al HPLC para el análisis de grandes bibliotecas de compuestos.

1.1 Tiorredoxin Glutatión Reductasa de *Echinococcus granulosus*.

1.2 Tiorredoxina Glutatión Reductasa de *Echinococcus granulosus* como molde de BCDs.

1.2.1 Parásitos platelmintos. Generalidades.

Las helmintiasis, clasificadas dentro del grupo de enfermedades tropicales desatendidas (neglected tropical diseases, NTDs),[32] generan infecciones crónicas en mamíferos, afectando a gran parte de la población pobre de los países en desarrollo y al ganado ovino y bovino. Si bien tienen un bajo índice de mortalidad, generan debilidad y discapacidad en los pacientes afectados, causando graves problemas de salud. Muchas helmintiasis son zoonosis que no sólo afectan al ser humano, sino también al ganado, impactando en la producción pecuaria y ocasionando grandes pérdidas económicas.

Existen dos grandes grupos de helmintos: los nemátodos (gusanos redondos) y los platelmintos (gusanos planos: céstodos y tremátodos). Dentro de los platelmintos tremátodos, la schistosomiasis es la infección más frecuente e importante en humanos, afectando a más de 200 millones de personas por año en África, América del sur y Asia.[33] Las infecciones causadas por céstodes no son tan comunes, si bien también pueden causar serios problemas de salud. Por ejemplo, la hidatidosis (causada por el cestode *Echinococcus granulosus*) es una infección crónica, endémica en Uruguay, que afecta a los mamíferos (rumiantes y también humanos), generando problemas de salud en la población y grandes perdidas económicas en lo que respecta al ganado. Si bien los porcentajes de hidatidosis en Uruguay han ido disminuyendo según indican las estadísticas para el ganado ovino y bovino pasando de 54% en 1990 a un 8% en el 2002,[34] el problema de la resistencia ya se ha instalado en la región.

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[32] Hotez, P.; Molyneux, D.; Fenwick, A.; Kumaresan, J.; Sachs, S.; Sachs, J.; Savioli, L. Control of Neglected Tropical Diseases. *New. Eng. J. Med.* **2007**, *357*, 1018-1027.

[33] (a) Hotez, P. J.; Brindley, P. J.; Bethony, J. M.; King, C. H.; Pearce, E. J.; Jacobson, J. Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **2008**, *118*, 1311-1321; (b) Engels, D.; Chitsulo, L.; Montresor, A.; Savioli, L. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta. Trop.* **2002**, *82*, 139-146; (c) King, C. H.; Dickman, K.; Tisch, D. J. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **2005**, *365*, 1561-1569.

[34] Informe del proyecto sub-regional cono sur de control y vigilancia de la hidatidosis. Argentina, Brasil, Chile y Uruguay. I Reunión Constitutiva Montevideo, Uruguay, **2004**, OPS, OMS.

Por otra parte, la fasciolosis causada por el platelminto *Fasciola hepática*, conocido como saguaypé, en tanto es la principal helmintiasis que afecta a ovinos y bovinos en Uruguay. Este platelminto causa importantes pérdidas económicas a nivel de la producción pecuaria por la disminución en i) ganancia de peso, ii) producción de leche y lana y iii) fertilidad de los animales infectados, sumado al decomiso de los hígados parasitados.

Debido al frecuente uso de drogas antihelmínticas en las últimas décadas ha aumentado el fenómeno de resistencia como indicarían los trabajos recientes publicados por diferentes autores tanto en el ganado vacuno,[35] como en ovejas.[36] Además ha aparecido resistencia a los tratamientos multi-droga,[37] teniendo esto graves consecuencias. La resistencia a los antihelmínticos se ha vuelto un fenómeno global para parásitos helmintos gastrointestinales de animales de granja, convirtiéndose en un serio problema económico.[38] La búsqueda de nuevos fármacos y blancos de acción contra los parásitos helmintos es entonces de gran importancia debido a la escasez de drogas disponibles para dichas infecciones.

[35] (a) Coles, G. C.; Watson, C. L.; Anziani, O.S. Ivermectin resistance Cooperia in cattle. *Vet. Rec.* **2001**, *148*, 283-284; (b) Mejia, M. E.; Fernandez, B. M.; Schmidt, E. E.; Cabaret, J. Multispecies and multiple anthelmintic resistance on cattle nematodes in a farm in Argentina: the beginning of high resistance. *Vet. Research* **2003**, *34,* 461-467; (c) Waghorn, T. S.; Leathwick, D. M.; Rhodes, A. P.; Lawrence, K. E.; Jackson, R.; Pomroy, W. E.; West, D. M.; Moffat, J. R Prevalence of anthelmintic resistance on 62 beef cattle farms in the North Island of New Zealand. *NZ Vet. J.* **2006**, *54*, 278-282.

[36] (a) Kaplan R.M. Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitol.* **2004**, *20*, 477-481; (b) Love, S.C.J.; Neilson, F.J.A.; Biddle, A.J.; McKinnon, R. Moxidectin-resistant *Haemonchus contortus* in sheep in northern New South Wales. *Aust. Vet. J.* **2003**, *81*, 359-360; (c) Waghorn, T. S.; Leathwick, D.M.; Rhodes, A.P.; Lawrence, K.E.; Jackson, R.; Pomroy, W.E.; Moffat, J. R. Prevalence of anthelmintic resistance on sheep farms in New Zealand. *N. Z. Vet. J.* **2006**, *54,* 271-277.

[37] Traversa, D.; Paoletti, B.; Otranto, D.; Miller, J. First report of multiple drug resistance in trichostrongyles affecting sheep under field conditions in Italy. *Parasitol. Res.* **2007**, *101*, 1713-1736.

[38] Grover, J. K.; Vats, V.; Uppal, G.; Yadav, S. Anthelmintics: a review. *Trop. Gastroenterol.* **2001**, *22*, 180.

1.2.2 Sistemas redox: tiorredoxina y glutatión.

En la mayoría de los organismos, existen dos sistemas enzimáticos responsables de generar equivalentes de reducción para las células y reacciones esenciales: el de la tiorredoxina (Trx), una proteína de 12 kDa que contiene un sitio activo ditiol-disulfuro, y el del glutatión (GSH), un tripéptido (L-cisteína, ácido L-glutámico y glicina) que se encuentra como tiol libre si está reducido y en forma de dímero cuando está oxidado, ver figura 11.

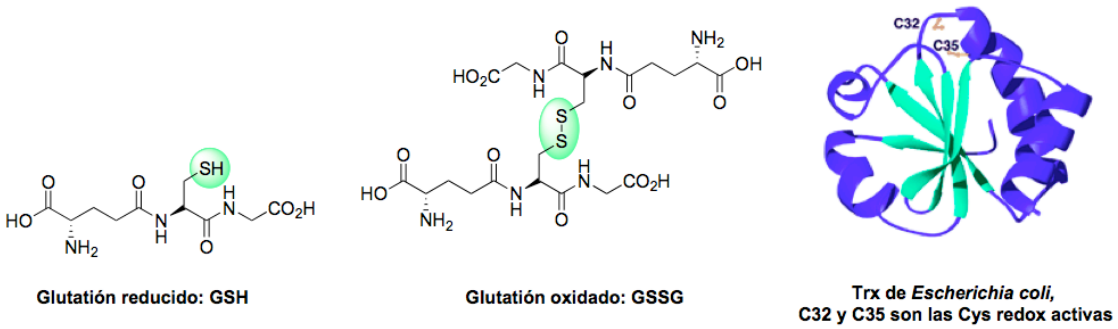

Figura 11. GSH, GSSG y Trx.[39]

Estos sistemas encargados de mantener la homeostasis redox celular, cediendo equivalentes de reducción a sustratos oxidados, deben regenerarse, es decir reducirse nuevamente. Este paso se lleva a cabo mediante distintas vías que utilizan óxido-reductasas: la glutatión reductasa (GR) y la tiorredoxina reductasa (TR), utilizando NADPH como dador de electrones.[40]

El sistema tiorredoxina está compuesto por TR y Trx, mientras que el del glutatión está formado por GR, GSH y glutarredoxina (Grx, una proteína redox de 11 kDa que es reducida únicamente por GSH). Ambos sistemas utilizan el intercambio tiol-disulfuro como mecanismo general redox. Estos sistemas actúan en un amplio abanico de procesos celulares. Si bien coinciden en ciertas rutas, en la mayoría de las reacciones son independientes entre si actuando en forma paralela.[41]

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[39] Trx tomada de: http://www.pnas.org/content/101/11/3759.long

[40] Holmgren, A. Glutathione-dependent synthesis of deoxyribonucleotides. Characterization of the enzymatic mechanism of *Escherichia coli* glutaredoxin. *J. Biol. Chem.* **1979**, *254*, 3672-3678.

[41] Gladyshev, V.; Jeang, K.; Stadtman, T. Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. *Proc. Natl. Acad. Sci.* **1996**, *93*, 6146-6151.

1.2.3 Sistema TGR

Estudios bioquímicos en parásitos platelmintos demostraron que tanto la TR y la GR convencionales están ausentes en estos organismos. Se observó sin embargo que los sistemas de la tiorredoxina (Trx) y del glutatión (GSH) se encuentran ligados mediante una enzima multifuncional, la tiorredoxin glutatión reductasa (TGR).

Esta enzima fue primero caracterizada en mamíferos y fue luego encontrada en varios platelmintos: *Schistosoma mansoni, Echinococcus granulosus, Taenia crassiceps* y *Fasciola hepática*. Todos estos parásitos carecen de los sistemas TR y GR y poseen el sistema ligado de las TGR, ver figura 12.

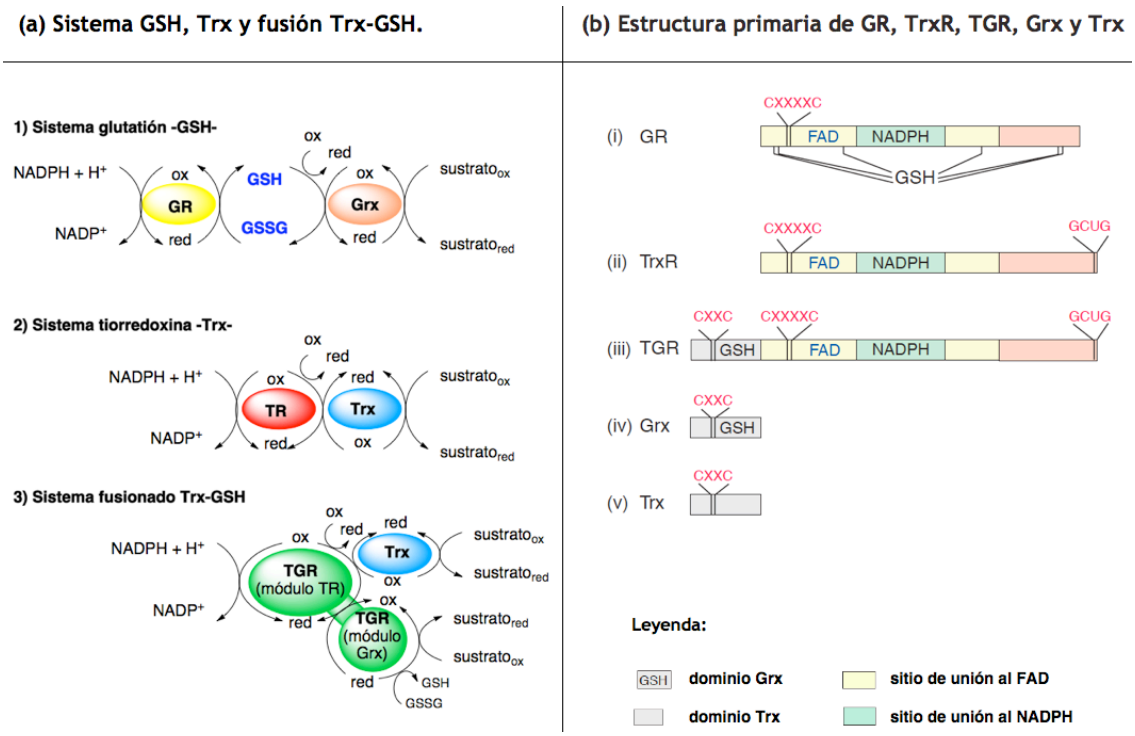

Figura 12. a) GSH, Trx y sistema ligado Trx-GSH. b) Estructuras primarias de GR, TR, TGR, Trx y Grx.[42]

En la parte a) de la Figura 12 se observa el flujo de electrones en los distintos sistemas. NADPH es el dador de electrones inicial en todos los casos. La TGR reemplaza la TR, Gr y Grx y provee equivalentes de reducción a sustratos de ambas

[42] Por una reciente y completa revisión ver: Functions, catalytic mechanism and regulation of *Echinococcus granulosus* Thioredoxin-Glutathione Reductase, Mariana Bonilla, Tesis de Doctorado en Ciencias Biológicas, Cátedra de Inmunología, Facultad de Química-Facultad de Ciencias, UdelaR, PEDECIBA, diciembre 2011.

vías. Ejemplos de sustratos pueden ser: glutatión peroxidasa, ribonucleótido reductasa, dímeros glutatión-proteína, peroxirredoxina y metionina sulfóxido reductasa.

En la parte b) se observan las estructuras primarias de GR, TR, TGR, Trx y Grx. Los centros redox de estas enzimas se indican en rojo. TR y TGR tienen una extensión en C-terminal ausente en GR, y contienen el centro activo redox GCUG (U= selenocysteina). GR, TR y TGR son homodímeros con sus monómeros orientados en forma cola-cabeza.

La TGR tiene un papel central en el control de las defensas antioxidantes de estos organismos, ya que es la única enzima capaz de transferir equivalentes de reducción al conjunto de ambas rutas. Evidencias recientes indican que existe en estos parásitos una forma citosólica y una mitocondrial de esta enzima así como de sus blancos,[43] razón por la cual resulta altamente probable que estos sistemas Trx-GSH ligados se hallen presentes en ambos compartimentos. Los hallazgos bioquímicos iniciales que indicaban que la única enzima encargada de reciclar la Trx y el GSH en estos organismos era la TGR y han sido recientemente confirmados por la información de los genomas de platelmintos parásitos: éstos carecen de los genes que codifican para Tioredoxina Reductasa (TR) y Glutatión Reductasa (GR) convencionales.

1.2.4 TGR como blanco de acción de fármacos.

La búsqueda de nuevos fármacos y nuevos blancos de acción contra los parásitos platelmintos es de gran importancia dada la escasez de drogas disponibles para dichas infecciones y la ausencia de vacunas.

En particular, el platelminto *Echinococcus granulosus*, responsable de la hidatidosis quística, produce infecciones crónicas en mamíferos. Al infectar al mamífero, estos parásitos se hallan expuestos a las especies oxidantes derivadas de su propio metabolismo (respiración, rutas metabólicas), y además deben soportar el estrés

[43] Bonilla, M.; Denicola, A.; Novoselov, S.; Turanov, A.; Salinas, G. Platyhelminth Mitochondrial and Cytosolic Redox Homeostasis Is Controlled by a Single Thioredoxin Glutathione Reductase and Dependent on Selenium and Glutathione. *J. Biol. Chem.* **2008**, *283*, 17898-17907.

oxidativo generado como parte de la respuesta inmune de su hospedador.[44] Macrófagos y leucocitos son reclutados en torno a la infección y se activan para generar especies oxidantes, junto con neutrófilos que también participan en el aporte de especies reactivas de oxígeno (ROS). Estos oxidantes causan daños importantes a nivel de las células de los parásitos a través de distintos mecanismos (daño de ácidos nucleicos, interferencia en funciones proteicas).

Como consecuencia de esta exposición, es de esperar que estos organismos estén equipados con sistemas antioxidantes adecuados para lograr sobrevivir en el ambiente que le presenta su hospedador. Por el contrario, estudios realizados muestran un precario y particular sistema antioxidante de defensa, dependiente básicamente de la TGR. Es por esta razón que se plantea dicha enzima como blanco de acción para tratar estas infecciones, ya que bloqueando esta enzima se bloquean todas las vías redox del parásito.

La enzima TGR ha sido recientemente caracterizada en los sistemas redox de *E. granulosus*[45] y *Schistosoma mansoni*,[46] evidenciando la presencia de un escenario bioquímico único. En el análisis de los genes de *S. mansoni* y *E. granulosus* encontraron un único gen para TGR, demostrando que estos parásitos dependen exclusivamente de la TGR para la reducción del glutatión y la tiorredoxina oxidados.[47]

Además, estos parásitos carecen de catalasa; la detoxificación de peróxido de hidrógeno es llevada a cabo por peroxirredoxinas y glutatión peroxidasas, enzimas ambas dependientes de los sistemas Trx-GSH ligados. Se dice entonces que la TGR es el cuello de botella para el metabolismo de estos parásitos ya que todas las otras vías dependen de ella.[43]

Esta situación es bien diferente de la que se ha descrito en mamíferos, hospedadores de estos parásitos, donde existe redundancia de enzimas TR y GR convencionales,

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[44] Callahan, H.; Crouch, R.; James, E. *Parasitol. Today* **1998**, *4*, 218-225.
[45] Salinas, G.; Selkirk, M.; Chalar, C.; Maizels, R.; Fernández, C. Linked thioredoxin-glutathione systems in platyhelminths. *Trends Parasitol*. **2004**, *20,* 340-346.
[46] Kuntz, A.; Davioud-Charvet, E.; Sabed, A.; Califa, L.; Dessolin, J.; Williams D. Thioredoxin-glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *Plos Medicine* **2007**, e6, 1-15.
[47] Otero, L.; Bonilla, M.; Protasio, A.; Fernandez, C.; Gladyshev, V.; Salinas, G. Thioredoxin and glutathione systems differ in parasitic and free-living platyhelminths. *BMC Genomics* **2010**, *11237*, 1-13.

además de la TGR que se encuentra en mayoritariamente en testículos de mamíferos, ver figura 13.

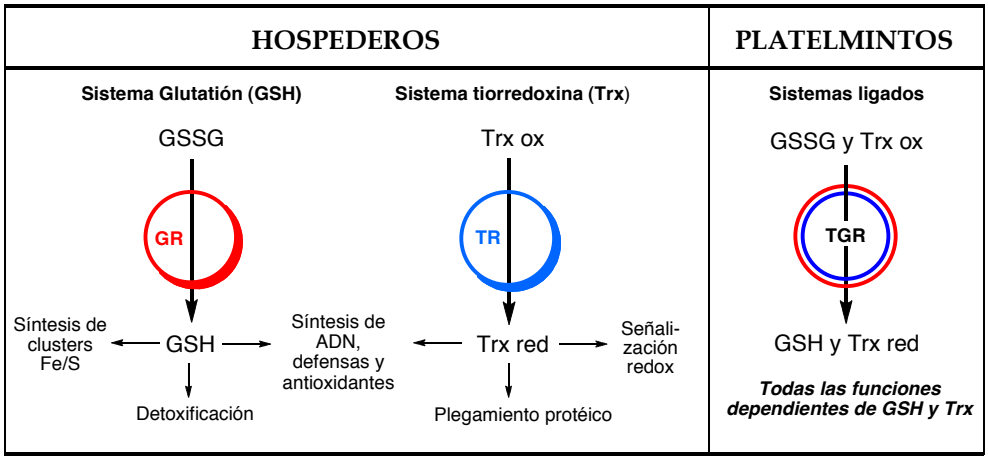


Figura 13. Comparación simplificada de las vías de Trx y GSH en platelmintos y sus hospederos. Adaptado de figura de Capítulo de libro.[48]

Estudios recientes han demostrado que el silenciamiento de la expresión de TGR en *S. mansoni* por interferencia de ARN conduce a la muerte de los parásitos in vitro.[38] A su vez, la auranofina, un excelente inhibidor de la TGR, mata a las larvas de *E. granulosus*[42] y a los parásitos de *S. mansoni* en cultivo in vitro, y produce una cura parcial en ratones infectados de forma experimental con *S. mansoni*.[38]

En conclusión, se ha demostrado que la TGR es un blanco sumamente promisorio para la quimioprofilaxis de estas infecciones ya que es esencial en las vías redox de platelmintos pero no en las de sus hospederos. La falta de vías metabólicas de respaldo en el control del daño oxidativo y el estrés oxidativo al que están expuestos los platelmintos frente a la respuesta inmune del hospedero hacen que esta enzima sea vital para la supervivencia de los parásitos. El bloqueo de la misma genera un daño en el sistema redox de los parásitos y por consecuencia de todas las cadenas dependientes lo que lleva finalmente a la muerte de los parásitos y cura de la infección.

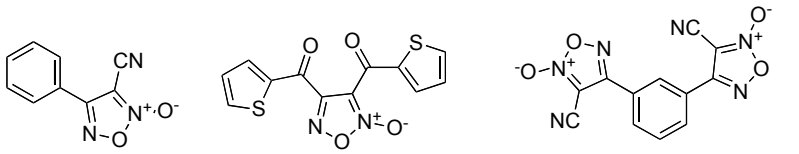
[48] Salinas, G.; Bonilla, M.; Otero, L.; Lobanov, A. V.; Gladyshev, V. N. Selenoproteins in Parasites. In: Hatfield, D. L., Berry, M. J., and Gladyshev, V. N. (eds). *Selenium: its molecular biology and role in human health*, 3rd Ed., Springer-Verlag Inc., New York, **2011**.

Por último, el uso de esta enzima como blanco de acción de drogas permite además enfocar el problema hacia nuevos objetivos, evitando así la resistencia generada hasta entonces. Se trabajará con el objetivo final de avanzar hacia la solución de un problema de salud importante con considerables pérdidas económicas en muchos países en vías de desarrollo.

1.2.5 Inhibidores conocidos de TGR

Existen diversos grupos de inhibidores de TGR, siendo los más representativos:

a) Los oxadiazoles, recientemente publicados,[49] presentan como farmacóforo un 1-ciano furoxano, sustituyente que se relaciona con la capacidad de liberar un radical NO^{*} e inhibir la enzima. Los compuestos de esta clase poseen una actividad inhibitoria sobre la enzima IC_{50} = 6.3 μM y una capacidad para matar al parásito (*S. mansoni*) del 100% luego de 72h. Se demostró que la incubación de TGR con este tipo de compuestos produce S-nitrosilación de los residuos tiol o selenol de la enzima, volviéndola inactiva. Se postula que se da un ataque nucleofílico de la Cisteína (Cys) o Selenocisteína (Sec) al carbono 3 del heterociclo, para producir una ruptura homolítica del enlace C-N, formándose un radical libre que es estabilizado por el grupo ciano liberándose el radical NO^{*} y regenerando el tiol o selenol, ver estructuras en figura 14.



FUROXAN (1) FUROXAN (2) BIS-OXADIAZOL-2-OXIDO (3)

Figura 14. Inhibidores de TGR conteniendo furoxanos

[49] Rai, G.; Sayed, A.; Lea, W.; Luecke, H; Chakrapani, H.; Prast-Nielsen, S.; Jadhav, A.; Leister, W.; Shen, M.; Inglese, J.; Austin, C.; Keefer, L.; Arner, E.; Simeonov, A.; Maloney, D.; Williams, D.; Thomas, C. Structure Mechanism Insights and the Role of Nitric Oxide Donation Guide the Development of Oxadiazole-2-Oxides as Therapeutic Agents against Schistosomiasis. *J. Med. Chem.* **2009**, *52*, 6474-6483.

b) Los complejos con metales son otro grupo de drogas con potente actividad, por ejemplo la Auranofina (Ki = 10 nM),[50] y otros organometálicos de platino, ver figura 15.[51]

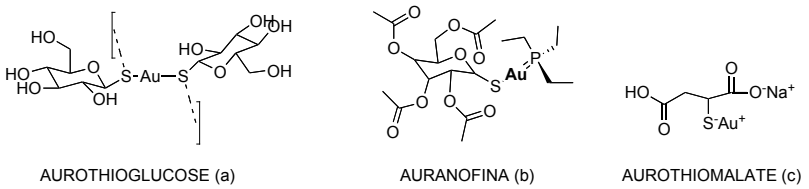


Figura 15. Inhibidores de TGR, complejos con Oro.

c) Los complejos de oro con carbenos de nitroimidazoles (NHC) actúan selectivamente sobre la enzima TrxR de células cancerosas de mama.[52]

1.2.6 TGR como molde enzimático en la búsqueda de inhibidores.

Debido al rol esencial que juega la tiorredoxin glutatión reductasa (TGR) en la biología redox de los platelmintos, detallado en secciones anteriores, esta enzima es un potencial blanco de acción para el desarrollo de nuevas drogas contra infecciones parasitarias. La TGR es una enzima redox que trabaja básicamente transfiriendo equivalentes de reducción desde el NADPH hacia sustratos oxidados, reduciendo los enlaces disulfuro a los correspondientes tioles. Si bien se han descrito distintos inhibidores de TGR, no se ha logrado identificar compuestos con perfiles de ADME adecuados para su uso en clínica, siendo la mayoría de ellos tóxicos.[53]

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[50] Angelucci, F.; Sayed, A.; Williams, D.; Boumis, G.; Bellelli, A. Inhibition of *Schistosoma mansoni* Thioredoxin-glutathione Reductase by Auranofin. *J. Biol. Chem.* **2009**, *284*, 28977-28985.

[51] Urig, S.; Fritz-Wolf, K.; Reau, R.; Herold-Mende, C.; Becker, K. Undressing of phosphine gold(I) complexes as irreversible inhibitors of human disulfide reductases. *Angew. Chem. Int. Ed.* **2006**, *45*, 1881-1886.

[52] Hickey, J.; Ruhayel, R.; Barnard, P.; Baker, M.; Filipovska, A. Mitochondria-Targeted Chemotherapeutics: The Rational Design of Gold(I) *N*-Heterocyclic Carbene Complexes That Are Selectively Toxic to Cancer Cells and Target Protein Selenols in Preference to Thiols. *J. Am. Chem. Soc.* **2008**, *130*, 12570-12571.

[53] Prast-Nielsen, S.; Huang, H.H.; Williams, D. Thioredoxin glutathione reductase: Its role in redox biology and potential as a target for drugs against neglected diseases. *Biochim. Biophys. Acta* **2011**, *1810*, 1262-1271.

Como se ha mencionado anteriormente, la QCD es una herramienta con gran potencial para el descubrimiento de ligandos para biomoléculas, con el objetivo final de desarrollar nuevas drogas. Se ha aplicado en sistemas conteniendo moldes protéicos y ácidos nucléicos, facilitando el estudio de la unión ligando-proteína. Dentro del número de reacciones reversibles disponibles en QCD, solo algunas son aplicables para su uso con biomoléculas. El intercambio disulfuro es una de las reacciones mas utilizadas en presencia de biomoléculas debido a que ocurre en condiciones cercanas a las fisiológicas, lo que permite un intercambio en presencia de biomoléculas activas.

Se decidió realizar la búsqueda de inhibidores de TGR aplicando como metodología la generación de una BCD mediada por intercambios reversibles tiol-disulfuro. Esta enzima demostró ser robusta y compatible con el sistema, ya que el intercambio ocurre a pH levemente básico (anteriormente, se realizaron estudios de estabilidad y actividad enzimática a distintos pHs).[40]

2. OBJETIVOS

OBJETIVOS

2.1 Objetivo general

El objetivo general de esta tesis es la búsqueda de nuevas reacciones reversibles útiles para generar sistemas dinámicos y la preparación de bloques de construcción útiles para implementar bibliotecas combinatorias dinámicas. Además se busca el desarrollo de BCDs utilizando como molde enzimas de importancia biológica.

2.2 Objetivos específicos

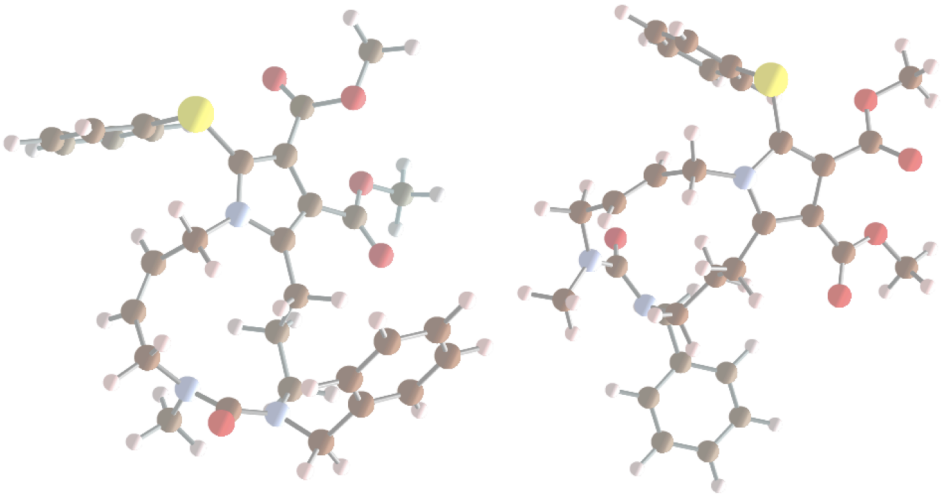
2.1.1 Desarrollo de nuevas reacciones útiles para la generación de BCDs. Estudio de intercambios acetálicos. Modelos de estudio: diaminas y carbonilos, aminotioles y carbonilos.

2.1.2 Síntesis de bloques de construcción: nuevas estructuras capaces de generar diversidad molecular. Estudio de ciclaciones involucrando iones iminio β-sustituídos.

2.1.3 Generar BCDs utilizando TGR como molde en búsqueda de inhibidores enzimáticos.

2.1.4 Screening primario de las colecciones de compuestos preparadas en busca de inhibidores de cruzipaína.

2. RESULTADOS Y DISCUSION



3.1 New reactions and building blocks for Dynamic Combinatorial Chemistry

3.1.1 New reactions for DCC: Thiazolidine exchange

Paper I (Saiz, C.; Wipf, P.; Manta, E.; Mahler G. *Org. Lett.* **2009**, *11*, 3170-3173)

Reversible Thiazolidine Exchange: A New Reaction Suitable for Dynamic Combinatorial Chemistry

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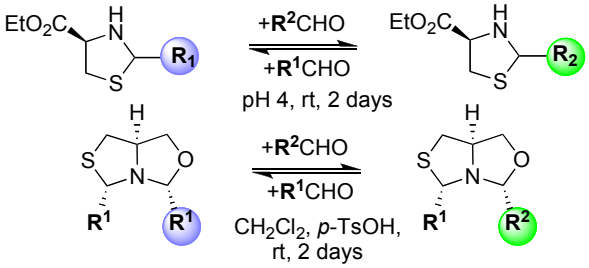
Received March 16, 2009.

Abstract



New dynamic combinatorial libraries (DCLs) were generated using the reversible aminothiol exchange reaction of thiazolidines and aromatic aldehydes. The reaction proceeded in aqueous buffered media at pH 4 and room temperature to generate thermodynamically controlled mixtures of heterocycles. The synthesis of an enantiomerically pure thiazolidinyloxazolidine is also reported. The oxazolidine moiety could be exchanged in CH_2Cl_2 in the presence of catalytic *p*-TsOH ac.

Introduction

Dynamic combinatorial chemistry (DCC) has considerable potential in the discovery of small molecule ligands for artificial receptors and large biomolecules. DCC is largely based on the use of reversible reactions to generate compound mixtures - Dynamic Combinatorial Libraries (DCLs) - that are in thermodynamic equilibrium. The composition of the library is determined by the properties of each of the library members under the particular conditions of the experiment; this equilibrium is likely to respond to the presence of a template or another change in the environment.[1] The covalent reversible reactions usable in DCC are relatively rare compared to the irreversible processes favored in traditional synthetic chemistry, and most of the former involve carbonyl compounds, imines and acetals.

Four different types of substrates have previously been used to generate DCLs from carbonyl compounds by acetal exchange (Figure 1): a) diols in the presence of catalytic TfOH,[2] H_2SO_4,[3] or *p*-TsOH ac.;[4] b) aminoalcohols, leading to mixtures of imines, oxazinanes and oxazolines;[5] c) dithiols in the presence of catalytic $Zn(OTf)_2$,[6a] or

[1]For reviews, see: (a) Ladame, S. Dynamic combinatorial chemistry: on the road to fulfilling the promise. *Org. Biomol. Chem.* **2008**, *6*, 219–226; (b) Ludlow, R. F.; Otto, S. Systems Chemistry. *Chem. Soc. Rev.* **2008**, *37*, 101–108; (c) Lehn, J.-M. From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. *Chem. Soc. Rev.* **2007**, *36*, 151–160; (d) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. Dynamic Combinatorial Chemistry. *Chem. Rev.* **2006**, *106*, 3652-3711.

[2] (a) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. Metathesis Reaction of Formaldehyde Acetals: An Easy Entry into the Dynamic Covalent Chemistry of Cyclophane Formation. *J. Am. Chem. Soc.* **2005**, *127*, 13666-13671; (b) Fuchs, B.; Nelson, A.; Star, A.; Stoddart, J. F.; Vidal, S. Amplification of dynamic chiral crown ether complexes during cyclic acetal formation. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 4220–4224; (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L.; Mencarelli, P.; Ugozzoli, F. Metathesis Reactions of Formaldehyde Acetals. Experimental and Computational Investigation of Isomeric Families of Cyclophanes under Dynamic Conditions. *Eur. J. Org. Chem.* **2008**, 186–195.

[3] Berkovich-Berger, D.; Lemcoff, N. G. Facile Acetal DCL. *Chem. Commun.* **2008**, 1686–1688.

[4] Lemcoff, N. G.; Fuchs, B. Toward Novel Polyacetals by Transacetalation Techniques: Dendrimeric Diacetals. *Org. Lett.* **2002**, *4*, 731-734.

[5] (a) Star, A.; Goldberg, I.; Fuchs, B. Dioxadiazadecalin/salen tautomeric macrocycles and complexes: prototypal dynamic combinatorial virtual libraries. *Angew. Chem., Int. Ed.* **2000**, *39*, 2685-2689; (b) Star, A.; Goldberg, I.; Fuchs, B. Dioxadiazadecaline and Salen Podands and Macrocycles within Dynamic Combinatorial Virtual Libraries: Structure, Prototropy, Complexation, and Enantioselective Catalysis. *J. Organomet. Chem.* **2001**, *630,* 67-77.

[6] (a) Sutton, L. R.; Donaubauer, W. A.; Hampel, F.; Hirsch, A. Tris(thioacetals) from benezene hexathiol: towards covalent self-assembly. *Chem. Commun.* **2004**, 1758-1759; (b) Wu, Y-C; Zhu J. Hafnium Trifluoromethanesulfonate (Hafnium Triflate) as a Highly Efficient Catalyst for Chemoselective Thioacetalization and Transthioacetalization of Carbonyl Compounds. *J. Org. Chem.* **2008**, *73*, 9522–9524.

$Hf(OTf)_4$,[6b] and d) diamines in aqueous buffered media at pH 4, conditions described by our group for the formation of pyrazolotriazinone heterocycles.[7]

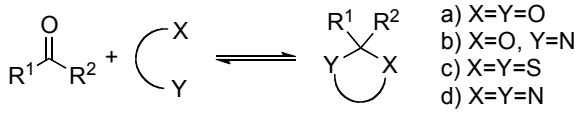
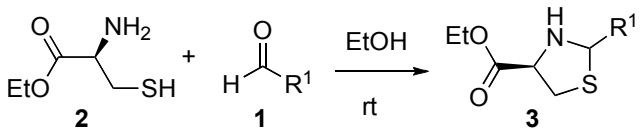


Figure 1. Cyclic acetal formations suitable for DCL.

The acid-catalyzed transacetylation of thiazolidines and related compounds attracted our closer attention as a possible extension of the latter heterocycle formation process.

Results and discussion

Thiazolidines **3** were selected as simple models in order to identify equilibration conditions for DCL formation. In spite of a literature report on the reversible formation of thiazolidines under basic conditions,[8] this transformation has not been previously reported as useful for DCC. 4-Carboxyl ethyl-2-arylthiazolidines can be readily obtained by condensation of aldehydes (**1**) and cysteine ethyl ester (**2**) in EtOH at room temperature (Scheme 1).

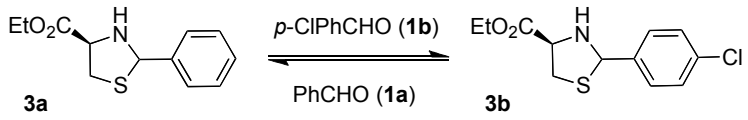


Scheme 1. Thiazolidine formation from cysteine ethyl ester.

The discovery of new reversible reactions compatible with an aqueous enviroment is an important objective for the use of biomolecules as templates in DCLs. We screened different aqueous conditions, with variations in pH and reaction time, in order to establish optimal thermodynamic exchange conditions for the thiazolidine exchange. The reaction of **3a** with equimolar amounts of *p*-Cl-benzaldehyde (**1b**) at room temperature was used as a reference (Table 1).

[7] Wipf, P.; Mahler, S. G.; Okumura, K. Metathesis Reactions of Pyrazolotriazinones Generate Dynamic Combinatorial Libraries. *Org. Lett.* **2005**, *7*, 4483-4486.
[8] (a) Woodward, G. E.; Schroeder, E. F. The Reaction of Cysteine with Acetone. A Note on the Titration of Cysteine by the Acetone—Hydrochloric Acid Method of Linderstrom-Lang. *J. Am. Chem. Soc.* **1937**, *59*, 1690–1694; (b) Szilagyi, L.; Gyorgydeak, Z. Comments on the putative stereoselectivity in cysteine-

Table 1. Optimization of thiazolidine exchange reaction of **3a**.



entry	reaction conditions[a]	time (h)	3a/3b ratio[b]	Compound 2 (%)[c]
1	pH 4, rt	24	45/55	3
2	pH 4, rt	48	44/56	2
3	pH 4, rt	72	46/54	10
4	pH 5, rt	24	71/29	5
5	pH 5, rt	48	68/32	10
6	pH 5, rt	72	52/48	9
7	pH 6, rt	24	96/4	0
8	pH 6, rt	48	97/3	0
9	pH 6, rt	72	90/10	0
10	pH 7, rt	72	98/2	0
11	pH 4, 35 ^{o}C	24	45/55	18 [d]
12	pH 4, 35 ^{o}C	48	35/65	16 [e]

[a]The starting concentration of each component was 1 mM; the reaction mixture was stirred in a buffered acetate solution at pH 4 and pH 5, and in a phosphate solution at pH 6 and pH 7.[b] The ratio was determined by ^{1}H NMR.[c] The mass balance was quantitative;[d] Total yield **3a** + **3b** (64%).[e] Total yield **3a + 3b** (49%).

Thermodynamic equilibration of a mixture of **3a** and **1b** occurred at pH 4 over 24 to 48 h at room temperature.[9] After 3 d, heterocycles **3a** and **3b** were stable in the aqueous environment and thiazolidines (90-98%) and ester **2** (2-10%) were recovered (entries 1, 2 and 3). Equilibration at pH 5 was slower, but after 3 d, the ratio indicated that equilibrium was reached (entries 4, 5 and 6). Equilibration at pH 6 was not complete after 3 d at rt (entries 7, 8 and 9). Equilibration at pH 7 did not proceed during 3 d at rt (entry 10) and the presence of ester **2** was not detected. The method of choice for blocking further equilibration is a simple raise of pH to 7; since we did not observe any further equilibration at this pH, the yields of recovered products were quantitative.

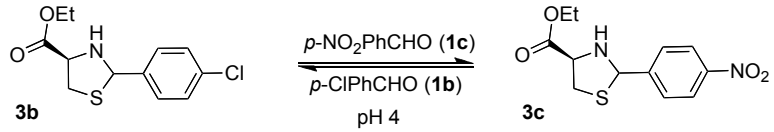
When the temperature was increased to 35 ^{o}C at pH 4, the equilibration ocurred faster, but significant amounts of cysteine ester **2** were observed. The total recovered yield for thiazolidines was 64% and 49% after 1 d and 2 d, respectively (Table 1, entries 11 and 12). The mass balance was decreasing, probably due to ester hydrolysis in **2**.

aldehyde reactions. Selective C(2) inversion and C(4) epimerization in thiazolidine-4-carboxylic acids. *J. Am. Chem. Soc.* **1979**, *101*, 427-432.
[9] See typical procedure fot thiazolidine exchange in the experimental section (5).

Thiazolidines **3a** and **3b** have different stabilities depending on pH; at pH 4-5 thiazolidine hydrolysis occurs to an acceptable extent (2-10%) at rt over 3 d. Temperature seems to play an important role in these systems; higher temperatures accelerate the exchange process but concomitant hydrolysis of the thiazolidine and the cysteine ethyl ester takes place.

We also examined the reversibility of the system by starting from products **3b** and **3c** at pH 4 (Table 2). If equilibration was reached, the distribution pattern should be identical. At pH 4, equilibration required 48 h at rt, providing a comparable product ratio as observed for the inverse process (entries 1 and 2, Table 2).

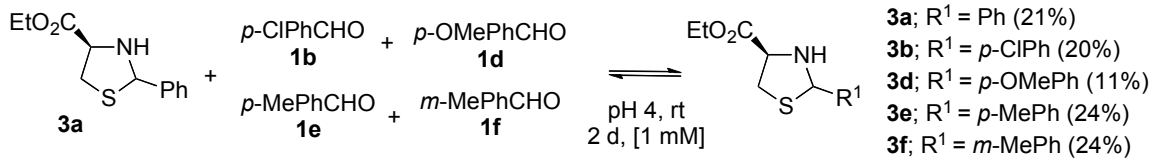
Table 2. Thiazolidine exchange processes starting with **3b** or **3c.**



Entry	Reaction conditions[a]	time (h)	3b:3c Ratio[b]	Compound 2 (%)
1	3b, 1c	48	25/75	4
2	3c, 1b	48	24/76	3

[a]The starting concentration of each component was 1 mM, the reaction mixture was stirred at rt in a buffered acetate solution at pH 4. [b]The ratio was determined by ^{1}H NMR and confirmed by preparative isolation.

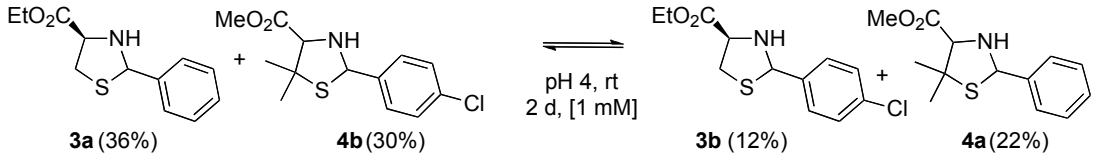
Thiazolidine **3a** with aldehydes **1b** and **1d-f** were allowed to equilibrate, the starting concentrations of the mixture components were kept at 1 mM each. The distribution of the corresponding heterocycles varied slightly for thiazolidines **3a**, **b**, **e** and **f** (20-24%) except for **3d** (11%) after 2d. Thiazolidine **3d** bearing an electron donating group (EDG) on the benzene ring has the lowest stability in the acidic medium. The mass balance indicated a 93% yield of thiazolidines and 7% of ester **2** after 4d, and the distribution remained unchanged. Even though some formation of compound **2** was observed, thiazolidines are stable in the acid media (pH 4) for 4d. This stability enables this DCL for the observation of template effects.



Scheme 2. Exchange reaction between thiazolidines **3a** and aldehydes **1b, 1d-f.**[a]

[a] The yields in parentheses reflect the equilibrium distribution. The ratio was determined by ^{1}H NMR and confirmed by preparative isolation.

We also studied the possibility for direct side-chain metathesis of thiazolidines. An equimolecular mixture of **3a** and **4b**, which differ by their substitution at 2, 4 and 5-positions of the heterocycles, was equilibrated during 2 d at pH 4 and rt (Scheme 3). As expected, a mixture of four products (the original starting materials and two crossover derivatives) was formed. Small amounts of penicillamine methyl ester (3%) and cysteine ethyl ester (7%) were also detected, but 90% of the thiazolidine products was recovered after 3 d.

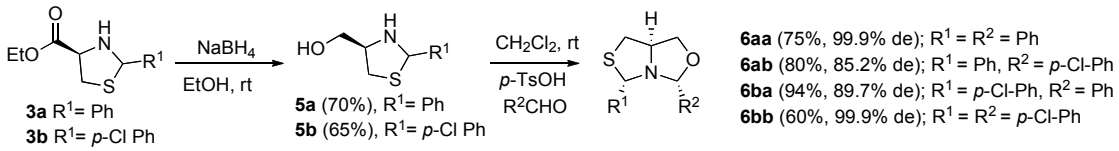


Scheme 3. Metathesis of thiazolidines.

The yields in parentheses reflect the equilibrium distribution. The ratio was determined by ^{1}H NMR and confirmed by preparative isolation.

As a means to increase diversity in the side chains at the 4-position of the heterocycles, the ester moiety of thiazolidines **3a-b** was converted to the alcohol by reduction with $NaBH_4$. When the aminoalcohols **5a-b** were treated with aldehydes **1a-b** in acid media (*p*-TsOH ac.), the fused thiazolidine-oxazolidines **6** were formed. This class of compounds represents a new example of a bicyclic DCC scaffold (Scheme 4).[10]

[10] Related fused thiazolidine-oxazolidinones and thiazolidine-oxazolidines were obtained when aminoalcohol **5a** was treated with phosgene: González, A.; Lavilla, R.; Piniella, J. F.; Alvarez-Larena, A. Protected Derivatives of (R)-Cysteine and (R)-Cysteinol. *Tetrahedron* **1995**, *51*, 3015- 3024.



Scheme 4. Synthesis of fused thiazolidine-oxazolidine heterocycles.

We hypothesize that this reaction proceeds by a thermodynamic equilibration, with *syn*-**6** being the more stable fused heterocycle.

Even though aminoalcohols **5** were used as a 1:1 mixture of diastereomers, only *syn*-**6** was formed. The relative configuration was confirmed by NOESY experiments (see Figure 2). This result is not unexpected due to the fact that thiazolidines undergo facile ring opening and closure reactions.[8b,11]

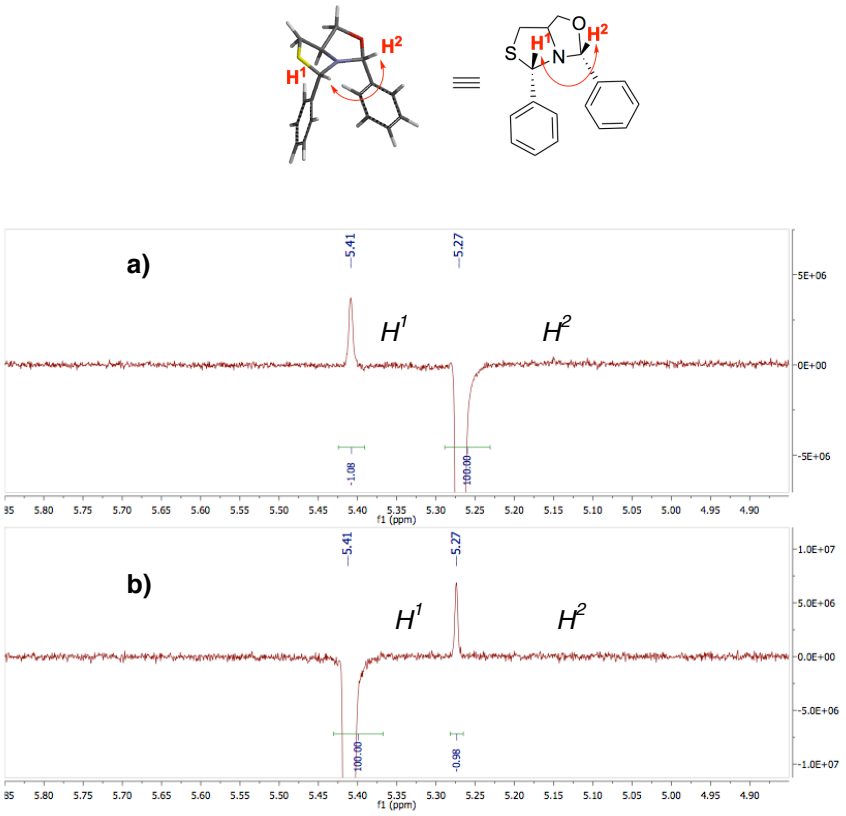
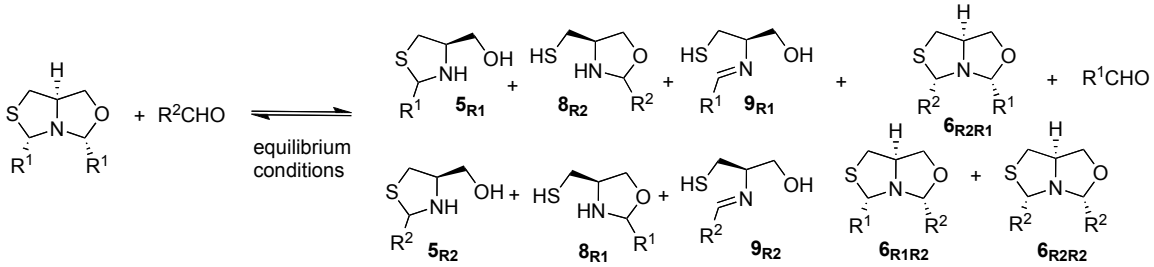


Figure 2. NOESY correlation of *syn*-**6aa**. Irradiation at 5.27 (a) and 5.41 ppm (b).

In this figure is shown the NOE between acetalic protons H^1 and H^2 of bicycle **6aa**. When either proton H^1 or H^2 are irradiated at the corresponding frequences, an enhancement of the H^2 resonance or the H^1 resonance is observed (1.08 and 0.98%,

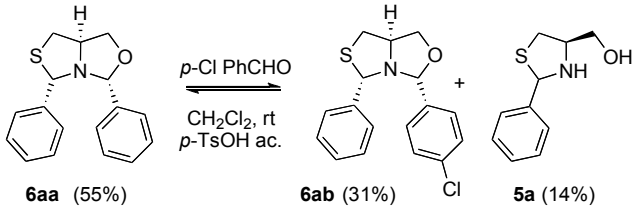
respectively, Figure 2). This suggests that the two protons are in a *syn* arrangement. We also performed *ab initio* calculations to confirm the stereochemistry, and the results indicated that the internal energy for the *syn* conformer was 2 kcal/mol lower than for the *anti*.

Thiazolidinyloxazolidine heterocycles present interesting opportunities for exchange processes: products include thiazolidine $\mathbf{5_R}$, oxazolidine $\mathbf{8_R}$, the fused heterocycles at the thiazolidine or the oxazolidine site $\mathbf{6_{RR}}$ and also the imine isomers $\mathbf{9_R}$,[12] as shown in Scheme 5.



Scheme 5. Potential library of thiazolidine-oxazolidine heterocycles.

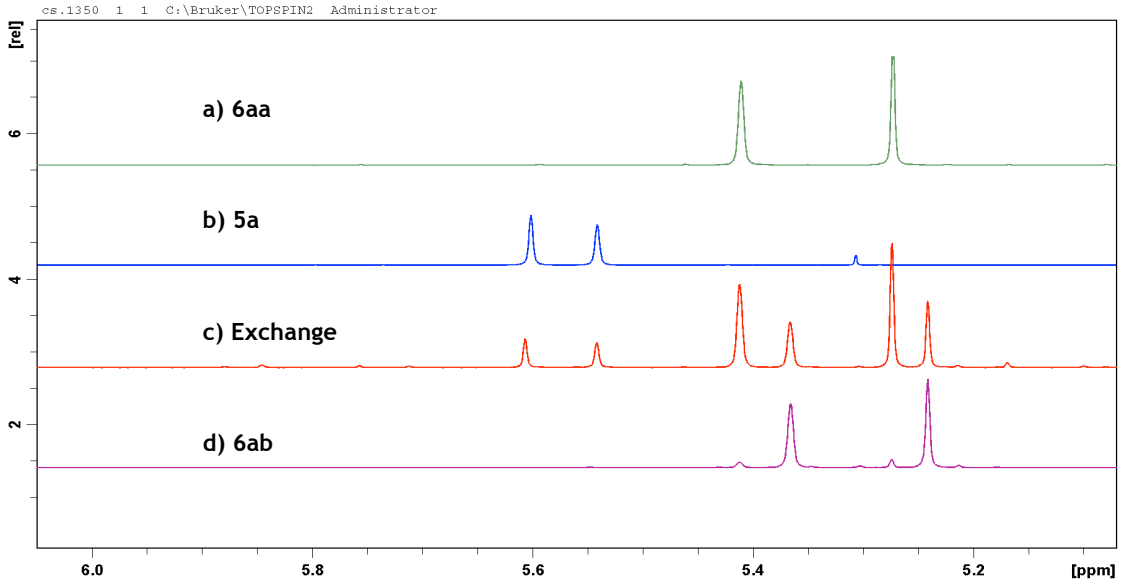
When bicycle **6aa** [10 mM] in CH_2Cl_2 was equilibrated at rt with an equimolar amount of aldehyde **1b** in the presence of catalytic *p*-TsOH ac., only the mixture of the exchanged products **6aa** and **6ab** and free aminoalcohol **5a** was obtained (Scheme 6).[13]



Scheme 6. Exchange reaction of bicycle **6aa.** The ratio was established by ^{1}H NMR.

[11] (a) Deroose, F. D.; De Clercq, P. J. Novel Enantioselective Syntheses of (+)-Biotin. *J. Org. Chem.* **1995**, *60*, 321-330.
[12] In ref 5a, Fuchs and coworkers report traces of imine in a DCL constructed from aminoalcohols.
[13] See typical procedure for preparation of **6aa** in the experimental section.

The mixtures were analyzed first by ^{1}H-NMR because they have different acetalic signals (see Figure 3). But the possible exchange products **6ba** and **6ab** resulted to have almost identical signals in the ^{1}H and ^{13}C NMR spectra so we could not conclude about the distribution.



a) ^{1}NMR acetalic signals for bicycle **6aa**; b) ^{1}H NMR acetal signals for aminoalcohol **5a**; c) ^{1}H NMR acetal signals for the exchange reaction of **6aa** and **1b** at 10 mM in CH_2Cl_2 in the presence of p-TsOH; d) ^{1}H NMR signals for compound **6ab**.

Figure 3. Comparison of ^{1}H NMR signals of **6aa**, **5b**, **1b**, **6ab** and the exchange reaction.

In order to identify the products, it was necessary to perform a chiral-HPLC (Chiralcel-OD) analysis of the possible products **6ba** and **6ab**. The chromatographic analysis indicated that the exchange was limited to the N-C-O linkage, forming **6ab** (Figure 4). Alternative species like imines **9a**, **9b** or oxazolidines **8a**, **8b** were not detected in the ^{1}H NMR spectra or the HPLC traces.

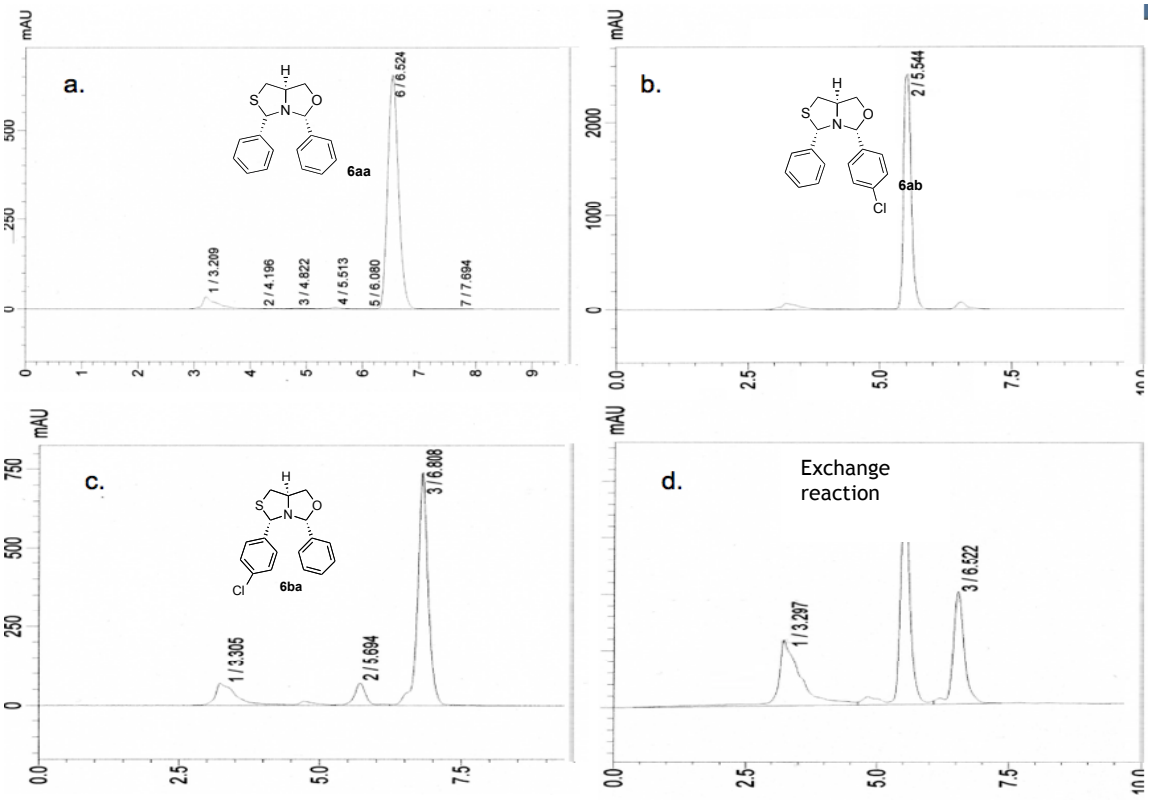
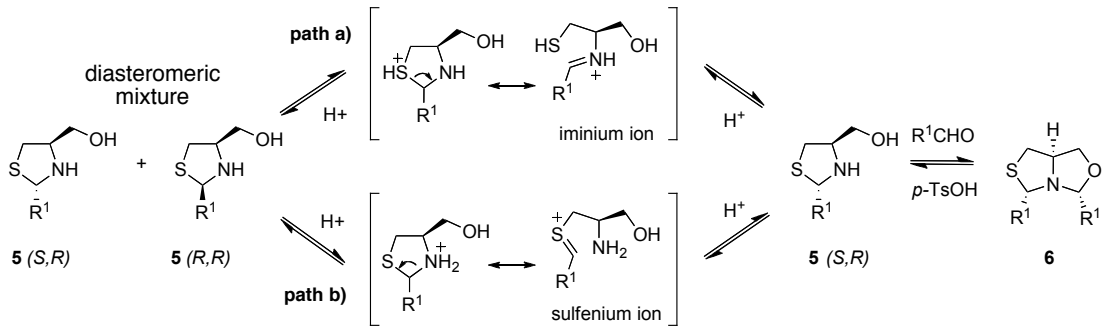


Figure 4. HPLC chromatograms of **6aa** (rt = 6.52 min), **6ab** (rt = 5.54 min), **6ba** (rt = 6.81 min) and the exchange reaction, λ = 210 nm.

We also performed an experiment in absence of *p*-TsOH ac. in $CDCl_3$ with compound **6aa** and aldehyde **1b** at 10 mM concentration, and we found no evidence of exchange or decomposition after 3 d. This observation is indicative that the products are stable and that the exchange requires acid catalysis.

It is important to point out that conditions for the synthesis and exchange of these bicycles are quite similar, i.e. CH_2Cl_2 and *p*-TsOH ac. In the acidic media, the diasteromeric mixture of alcohol **5** is in a fast equilibration by ring opening and closing. During this equilibration there are two alternatives of ring opening: path a) and path b), see scheme 7.



Scheme 7. Two proposed mechanisms for the dynamic diasteromeric resolution.

In *path a)* the ring opening would occurr with the formation of an iminium ion as a consequence of the S-C bond rupture in acidic media. The R^1 side chain remains then linked to the nitrogen. In *path b)* the acetal N-C bond of thiazolidine would be broken and the R^1 side chain would remain linked to the sulfur atom, probably via a sulfenium cation. After a re-equilibration, iminium and sulfenium intermediates would evolve to the preferred thiazolidine **5** *(S,R).* Both mechanisms should give the same rearrangement to afford the preferred product **5**, able to cyclize to form the fused *syn* thiazolidine-oxazolidine **6**.

Due to the major stability of iminium cation we propose *path a)* as the likely route for re-equilibration. Even if it could allow the formation of $\mathbf{6_{R2R1}}$ in the synthesis of $\mathbf{6_{R1R2}}$, this is not observed because it may occurr through a fast open-close equilibrium of the thiazolidine ring. This would not allow the interconversion to the oxazolidine product. *Path b)* via a sulfenium cation could also occurr and would therefore contribute to explain the exclusive exchange observed at the oxazolidine level, without formation of compound $\mathbf{6_{R2R1}}$.

Partial Conclusions

In summary, we explored a new exchange reaction between thiazolidines and carbonyl compounds. The thermodynamic exchange proceeds in an acidic aqueous environment (pH 4) and represents a new reversible reaction useful for DCC methodologies. A structural diversification of the core scaffolds can be accomplished by modifications at the 5- and 4-positions of the heterocycles. The thiazolidines **3a-f** are stable in buffered media over 4 d, and these conditions are suitable for the generation of DCLs as well as for the direct screening of these libraries. Moreover, the exchange reaction can be stopped by raising the pH to 7, thus providing a convenient way to analyze the compound distribution patterns.

As an important extension of this work, we also present the synthesis of fused thiazolidine-oxazolidine heterocycles such as *syn*-**6**, representing a new compound class. These bicycles are stable under neutral or basic conditions but can be equilibrated at the oxazolidine moiety in CH_2Cl_2 in the presence of catalytic *p*-TsOH ac.

3.1.2 Synthesis of new scaffolds: Thiazolidinyl-oxazolidines.

Paper II (Saiz, C.; Wipf, P.; Mahler, G. *J. Org. Chem.* **2011**, *76*, 5738–5746)

Synthesis and ring-chain-ring tautomerism of bisoxazolidines, thiazolidinyloxazolidines and spirothiazolidines.

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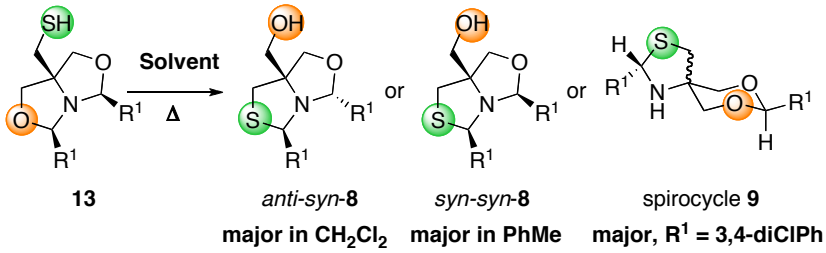
Departamento de Química Orgánica, Cátedra de Química Farmacéutica, (UdelaR), Gral Flores 2124, CC1157, Montevideo, Uruguay and Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA.

Abstract



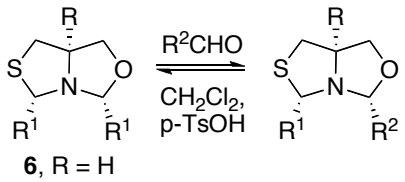
13 | *anti-syn*-**8** **major in CH_2Cl_2** | *syn-syn*-**8** **major in PhMe** | spirocycle **9** **major, R^1 = 3,4-diClPh**

The synthesis of fused heterocycles such as thiazolidinyl-oxazolidine **8** is described starting from Tris·HCl. The mercaptomethyl bisoxazolidine **13** was found to convert to the corresponding thiazolidinyloxazolidine **8** and the spiro-heterocycle **9** by a ring-chain-ring tautomerism, depending on the electronic nature of the ring substituents as well as the reaction conditions. This equilibration pathway is absent in the hydroxymethyl bisoxazolidines **7**. Computational studies confirm that both kinetic and thermodynamic control features play a role in the product distribution.

Introduction

Ring-chain tautomerism is a process that involves the reversible movement of a proton accompanied by a change from an open structure to a ring, often the result of an addition of a heteroatom to a heteropolar double bond to form a heterocycle. The reaction is usually acid catalyzed and represents a key step in the synthesis of 5- and 6-membered 1,3-heterocycles containing oxygen, nitrogen, or sulfur atoms.[14] This process can be exploited in the construction of dynamic combinatorial libraries,[1] and it has been applied to the discovery of new materials and pharmacologically active compounds.[15] The transformation has been studied thoroughly in the past decade, mainly in the context of ring-chain tautomeric equilibria involving 1,3-*X,N*-heterocyclic systems (X = S, O, N).[16]

As a continuation of our investigations on new reactions and scaffolds suitable to Dynamic Combinatorial Chemistry, we have explored readily available building blocks suitable for the exchange of carbonyl units in a dynamic library pool. Recently, we have reported the synthesis of thiazolidinyloxazolidine **6**, which is capable of exchanging carbonyl units in the oxazolidine moiety, retaining the thiazolidine heterocycle, in the presence of *p*-TsOH ac. in CH_2Cl_2 (Scheme 8).[17]



6, R = H

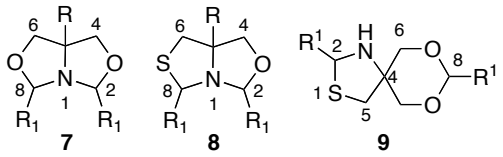
Scheme 8. Dynamic equilibrium: exchange reaction of thiazolidinyloxazolidine **6.**

[14] Lambert, J.B.; Wang, G. T.; Huseland, D. E.; Takiff, L. C. Acid-catalyzed ring-chain tautomerism in 1,3-diazolidines. *J. Org. Chem.* **1987**, *52*, 68-71.

[15] (a) Lazar, L.; Fulöp, F. Application of ring-chain tautomerism for the development of prodrugs. *Act. Pharm. Hung.* **1999**, *69*, 202-207; (b) Botti, P.; Pallin, T. D.; Tam, J. P. Cyclic Peptides from Linear Unprotected Peptide Precursors through Thiazolidine Formation. *J. Am. Chem. Soc.* **1996,** *118,* 10018-10024.

[16] (a) Talancón, D.; Bosque, R.; López, C. Study of the Effect Induced by the Substituents on the Ring–Chain Tautomerism of Schiff Bases Derived from Norephedrine. *J. Org. Chem.* **2010**, 75, 3294–3300; (b) Keiko, N. A.; Vchislo, N. V.; Stepanova, L. G.; Larina, L. I.; Chuvashev, Y. A.; Funtikova, E. A. Condensation of 2-Alkoxypropenals with N,N- and N,O-1,2-Binucleophiles. A Route to 2-(1′-Alkoxyvinyl)imidazolidines and oxazolidines. *Chem. Heterocycl. Compd.* **2008**, *44*, 1466–1471; (c) Lazar, L.; Fulöp, F. Recent Developments in the Ring-Chain Tautomerism of 1,3-Heterocycles. *Eur. J. Org. Chem.* **2003**, 3025-3042.

A logical extension of these studies was to investigate the bisoxazolidine **7**, a compound that had been reported in the literature a number of years ago.[18] We decided to prepare an analogous heterocycle, *i.e.* the fused thiazolidinyloxazolidine **8** (Figure 5). Bisoxazolidines **7** have been intensely studied during the last decades,[19] and some derivatives show interesting chemical and biological properties as chiral catalysts,[20] anticancer,[21] and neuroprotective agents.[22]



R = CH_2OH, R^1 = Ar

Figure 5. Structures of bisoxazolidine **7**, thiazolidinyloxazolidine **8** and spirothiazolidine **9**.

It is important to note that the replacement of the oxygen by a sulfur atom leads to a significant increase in the complexity of the system due to a break in the symmetry, allowing the potential formation of 2^3 fused bicycles upon variation of the substituents R and R^1.

The present paper describes our findings in the synthesis of the thiazolidinyloxazolidine **8**, a new bicyclic fused scaffold, and its structural isomer, the spirothiazolidine **9** (see Figure 5).

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[17] Saiz, C.; Wipf, P.; Manta, E.; Mahler G. Reversible Thiazolidine Exchange: A New Reaction Suitable for Dynamic Combinatorial Chemistry. *Org. Lett.* **2009**, *11*, 3170-3173.

[18] Senkus, M. Some New Derivatives of Amino Hydroxy Compounds. *J. Am. Chem. Soc.* **1945**, *67*, 1515-1519.

[19] Darabantu, M.; Maiereanu, C.; Silaghi-Dumitrescu, I.; Toupet, L.; Condamine, E.; Ramondenc, Y.; Berghian, C.; Plé, G.; Plé, N. 3,7-Dioxa-1-azabicyclo[3.3.0]octanes Substituted at C-5 position. From Local to Global Stereochemistry. *Eur. J. Org. Chem.* **2004**, *12*, 2644-2661.

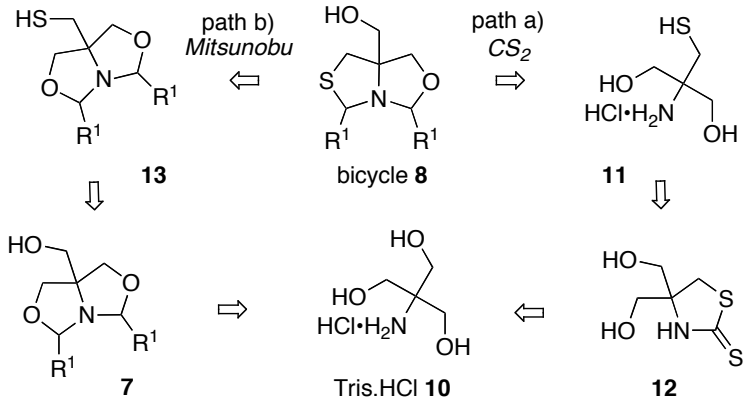
[20] Shi, S-L.; Xu, L-W.; Oisaki, K.; Kanai, M.; Shibasaki, M. Identification of Modular Chiral Bisphosphines Effective for Cu(I)-Catalyzed Asymmetric Allylation and Propargylation of Ketones. *J. Am. Chem. Soc.* **2010**, *132*, 6638–6639

[21] Shintani, Y.; Tanaka, T.; Nozaki, Y. GS-164, a small synthetic compound, stimulates tubulin polymerization by a similar mechanism to that of Taxol. *Cancer Chemother. Pharmacol.* **1997**, *40*, 513-520.

[22] Desino, K. E.; Ansar, S.; Georg, G. I.; Himes, R. H.; Michaelis, M. L.; Powell, D.; Reiff, E. A.; Telikepalli, H.; Audus K. L. (3*R*,5*S*,7a*s*) - (3,5-Bis(4-fluorophenyl)tetrahydro-1*H*-oxazolo[3,4-*c*]oxazol-7a-yl) methanol, a Novel Neuroprotective Agent. *J. Med. Chem.* **2009**, *52*, 7537–7543.

Results and discussion

Our first objective was the synthesis of the fused thiazolidinyloxazolidine **8**, the sulfur analogue of bicycle **7**. Since this was a hitherto unknown building block, we had to develop a new synthetic strategy to access it. Tris·HCl reagent **10** was used as a starting material; we explored two alternatives for its use in the synthesis of bicycle **8**. The key step was the replacement of a hydroxyl group with a thiol in either the Tris·HCl building block or the fused bicycle (Scheme 9).



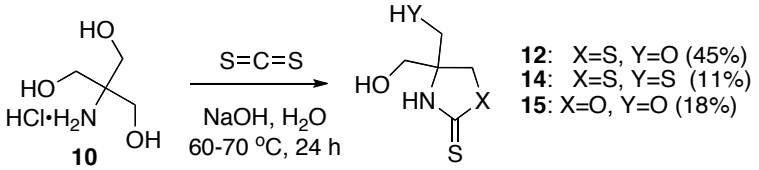
Scheme 9. Retrosynthetic analysis of bicycle **8**.

Path a) applied an early thiolation strategy involving as the key step the synthesis of thiazolidinone-2-thione **7** starting from Tris·HCl and carbon disulfide.[23] Subsequently a hydrolysis followed by a cyclization led to the target compound **8**. Path b) represents an alternative methodology that began with the protection of a diol unit in Tris·HCl, followed by the substitution of the free hydroxyl group in **7** with thioacetic acid under Mitsunobu's conditions. Thioester hydrolysis and an oxazolidine-thiazolidine interconversion in acidic media lead to compound **8** (Scheme 9).

Recently, Darabantu *et al.* reported a synthesis of thiazolidinyloxazolidine **8** (R^1 = H), similarly starting from Tris **10**, but otherwise using a different sequence.[24] A more detailed discussion of these routes is provided below.

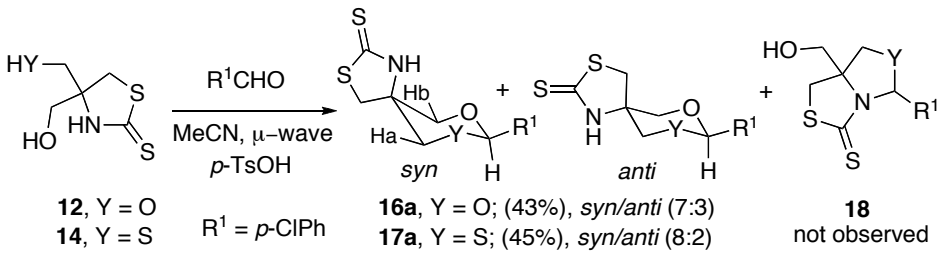
[23] Handrick, G. R.; Atkinson, E. R. Potential Antiradiation Drugs. III. 2-Amino-2-alkyl-1,3-propanedithiols and 3-Amino-4-mercapto-1-butanol. *J. Med. Chem.* **1966**, *9*, 558–562.
[24] But, A.; Lameiras, P.; Silaghi-Dumitrescu, I.; Batiu, C.; Guillard, S.; Ramondenc, Y.; Darabantu, M. Synthesis and Conformational Analysis of the First 3-Oxa-7-thia-1-r-azabicyclo[3.3.0]-c-5-octane Single Functionalized at the C-5 Position. *Lett. Org. Chem.* **2010**, *7,* 283-290.

Path a) The thiazolidine-2-thione **12** was prepared in moderate yields by heating a mixture of 5 M NaOH, Tris·HCl and carbon disulfide at reflux for several hours.[25] Various conditions were attempted in order to improve the selectivity and yield, but our best result was achieved by simply heating the reaction mixture for one day at 60-70 °C. A mixture of two thiazolidin-2-thiones (**12** and **14**) and the oxazolidin-2-thione **15** were obtained (Scheme 10).



Scheme 10. Preparation of thiazolidin-2-thiones (**12** and **14**) and oxazolidin-2-one **15.**

Thiazolidin-2-thiones **12** and **14** were subjected to a monocyclization protocol in MeCN in the presence of *p*-TsOH ac. and R^1CHO under microwave irradiation (90 °C, 12 min). Under these conditions, these substrates yielded exclusively the corresponding spirocycles **16a** and **17a**, instead of the expected fused bicycle **18a**, in analogy to the results obtained under conventional heating (Scheme 11).



Scheme 11. Cyclization and product distribution for thiazolidin-2-thiones **12** and **14.**

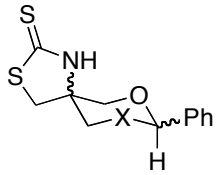
The spiro product *syn*-**17a** shows long-range couplings (^4J), due to the characteristic W-arrangement of the connecting bonds between H_a and H_b ("W-coupling"). The hydrogen atoms H_a and H_b are close to coplanar equatorial positions (Scheme 11). The formation of the spiro products corresponding to a 6-*endo-trig* cyclization is a favored process according to Baldwin's rules. The corresponding 5-*endo-trig* process leading to bicyclic **18** could not be observed; this cyclization process is disfavored by Baldwin's rules.

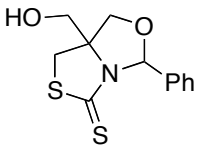
[25] For a discussion of S vs O nucleophilic attack, see: Baiget, J.; Cosp, A.; Gálvez, E.; Gómez-Pinal, L.; Romea, P.; Urpí, F. On the influence of chiral auxiliaries in the stereoselective cross-coupling reactions of titanium enolates and acetals. *Tetrahedron* **2008**, *64*, 5637-5644.

In order to gain further insight into these results, we undertook a theoretical calculation using a geometry optimization with Spartan 10 (DFT/B3LYP/6-311G*). The relative energy (E) for selected compounds is shown in Table 3. In order to simplify the calculations, we used R^1 = Ph.

Table 3. Relative Energies (Rel. E) for compounds *syn*/*anti*-**16b**, **17b,** and **18b**, R^1 = Ph.

syn/anti-**16b**, X = O
syn/anti-**17b**, X = S





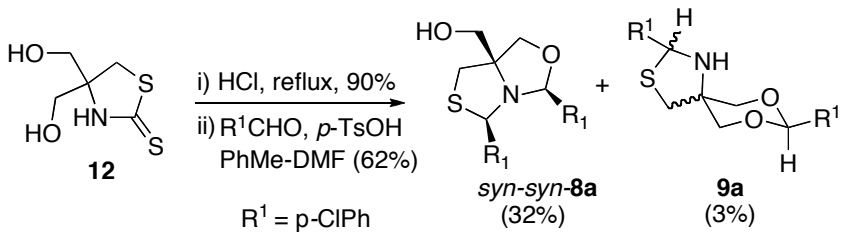
syn/anti-**18b**

Entry	Compounds	Rel. E [kcal/mol]
1	*syn*-**16b** vs *anti*-**16b**	-0.6
2	*syn*-**17b** vs *anti*-**17b**	-0.7
3	*syn*-**18b** vs *anti*-**18b**	-3.0
4	*syn*-**16b** vs *syn*-**18b**	-8.9

The spirocycles **16a** and **17a** were obtained as mixtures of *syn*- and *anti*-diastereomers. The theoretical calculations of the relative energies of the analogous products **16b** and **17b** predicted the *syn*-configurations to have a slightly lower energy in accordance with the experimental data and the configurations of the major diastereomers assigned by NOESY experiments. The differences in energy were -0.6 and -0.7 kcal/mol for spirocycles **16b** and **17b**, respectively (see Table 3, entries 1 and 2).

The calculations were also able to provide a rationale why the bicycle **13a** is not observed: the difference in energy between spirocycle *syn*-**11b** *vs* fused bicycle *syn*-**18b**, the more stable of the two stereoisomers, was about -9 kcal/mol (Table 3, entries 3 and 4), in preference for the formation of the spirocycle as the thermodynamically favored isomer.

Hydrolysis of thiazolidin-2-thione **12** in conc. HCl at reflux led to formation of Tris-SH·HCl **11** in 90% yield (Scheme 12). Cyclization in PhMe/DMF (9:1) with *p*-ClPhCHO and *p*-TsOH ac. for 3 h using a Dean-Stark trap led to a mixture of the fused bicycle *syn*-**8a** (32%) and a small amount of spiro compound **9a** (3%).



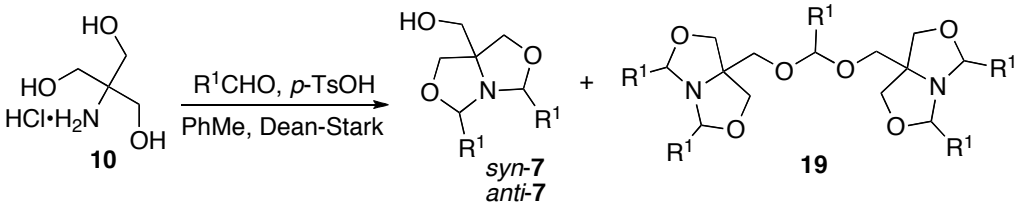
Scheme 12. Synthesis of fused bicycle **8a**.

Spirocycle **9a** was unexpected because it had not been described for the oxygen analogues **7**. Attempts to synthesize other fused heterocycles, using aldehydes such as *p*-FPhCHO and *p*-CF_3PhCHO, led to complex reaction mixtures with low recovery of the desired products, *syn*-**8b** (R^1 = *p*-FPh, 8%), spiro-**9b** (R^1 = *p*-FPh, 3%), and *syn*-**8c** (R^1 = *p*-CF_3Ph, 2%).

Our methodology for the synthesis of **11** has a 40% global yield in a 2-step sequence. Darabantu *et al.* reported the synthesis of Tris-SH in a four step sequence with 48% global and they only report the synthesis of bicycle **8** derived from formaldehyde.[24] Due to the difficulties we encountered in *path a)*, i.e. the low yield and limited scope for the formation of **12** and **8**, the tedious purification steps, the instability of Tris-SH **11** and the low solubility of the intermediate **11** under several reaction conditions, we decided to explore *path b)* as a new alternative.

For this purpose, we studied the direct substitution of the alcohol moiety in bisoxazolidine **7**, using Mitsunobu's conditions. Oxabicycles **7a-d** were synthesized as described previously by heating Tris·HCl, *p*-TsOH ac. and aldehydes in PhMe at reflux, using a Dean-Stark trap.[16]

Table 4. Product yields and diastereomeric ratios for compounds **7a-e**, **19a** and **19c-e**.



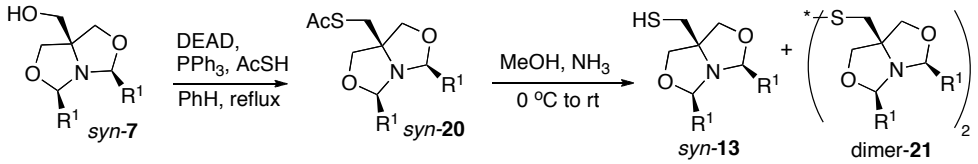
Entry	R^1	**Compound 7**, Yield % (*syn:anti*)[a]	**Compound 19**, Yield %
1	*p*-ClPh	**7a**, 77, (98:2)	-
2	*p*-FPh	**7b**, 34, (98:2)	-
3	*p*-CF_3Ph	**7c**, 32, (81:19)	**19c**, 13
4	*m*-BrPh	**7d**, 30, (80:20)	**19d**, 25
5	*p,m*-diClPh	**7e**, 20, (83:17)	**19e**, 35

a. Ratio based on integration of separated ^{1}H-NMR signals.

Using the previously mentioned conditions, we obtained the desired product *syn*-**7a-e** in moderate yields, mainly due to the formation of the dimer **19** (Table 4). The proportion of the side product **19** seemed to increase with the electron-withdrawing character of the aldehyde side chain R^1.

For the continuation of the synthesis of target molecule **8**, the substitution of the hydroxyl group present in the bicycles **7a-d** with thioacetic acid was achieved under Mitsunobu's conditions to give bicycles *syn*-**20a-d** in good yield, as shown in Table 5.

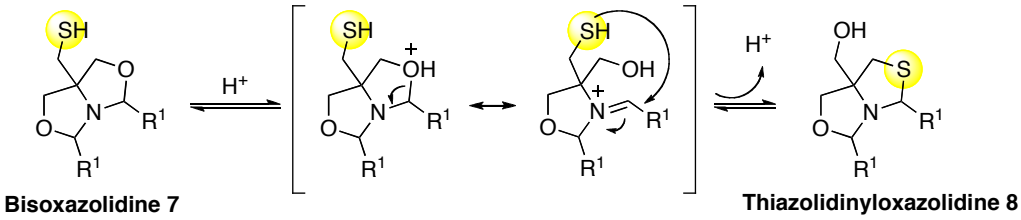
Table 5. Product yields and diastereomeric ratios for compounds **20a-e** and **13a-e.**



Entry	R^1	*syn*-**20**, Yield (%)	*syn*-**13,** Yield (%)
1	*p*-ClPh	**15a** (75)	**13a** (66)
2	*p*-FPh	**15b** (54)	**13b** (62)
3	*p*-CF_3Ph	**15c** (71)	**13c** (65)
4	*m*-BrPh	**15d** (84)	**13d** (44) + **21d** (43)
5	*p,m*-diClPh	**15e** (78)	**13e** (25) + **21e** (53)

Smooth hydrolysis of esters **20** in MeOH/NH_3 at room temperature led to the 5-thiomethyl-bisoxazolidines **13**, in good yields, excepting when R^1 = *m*-BrPh and *m,p*-diClPh, where the dimeric compound **21** was obtained as a byproduct. This methodology allowed us to prepare the new bisoxazolidines *syn*-**13a-e**, substituted at C_5 with a thiomethyl group (Table 5). Disulfides **21d** and **21e** could be recycled to the corresponding thiols **13d** (70%) and **13e** (60%) by treatment with $NaBH_4$ in MeOH at room temperature.

According to our previous work, is relevant the ability of this class of bicycles to exchange carbonyl units at the oxazolidine site.[16] Thus, we decided to explore such an interconversion in these new heterocycles. When using CH_2Cl_2 at reflux for 6 h, in the presence of *p*-TsOH ac. (cat.) and aldehyde (1 eq.) for the re-equilibration of *syn*-**13a-d**, we obtained thiazolidinyloxazolidines *anti-syn*-**8a-d** as main products (Table 6, entries 1, 3-5). These results were in accordance with our previous work with regard to the ability of these compounds to establish equilibrium in favor of the most stable bicyclic thiazolidinyloxazolidine **8** *vs* the bisoxazolidine **7**, see scheme 13.



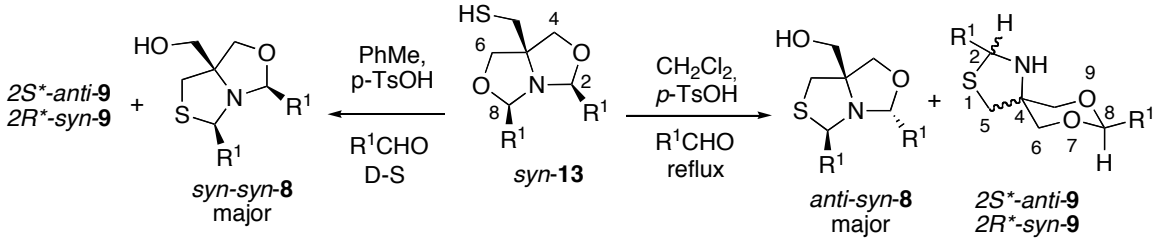
Bisoxazolidine 7 **Thiazolidinyloxazolidine 8**

Scheme 13. Acidic interconversion from oxazolidine to thiazolidine.

Based on our results, we can propose an interconversion of one oxazolidine ring into a more stable thiazolidine in presence of the corresponding methyl-thiol. The tautomeric equilibrium may occur through an iminium ion intermediate, as we proposed for the thiazolidinyloxazolidines **6** in 3.1. The iminium Csp^2 would be attacked by a thiol to form the corresponding *5*-membered ring fused heterocycle **8**.

This is an efficient and controlled route to synthesize compound **8**, allowing an easy and clean introduction of the S atom and consequent thiazolidine formation. As we can see on table 6, the product distribution will depend on the reaction conditions and the substituents in R^1.

Table 6. Product distribution for compounds **8** and **9.**



Entry	R^1	Starting Material	Reaction Conditions	Product Distribution, Yield **8** [%], (*anti-syn:syn-syn*)[c]	**9** [%]
1	*p*-ClPh	**13a**	A[a]	62, (99:1)	-
2	*p*-ClPh	**13a**	B[b]	41, (10:90)	46
3	*p*-FPh	**13b**	A[a]	44, (90:10)	-
4	*p*-CF_3Ph	**13c**	A[a]	64, (81:19)	-
5	*m*-BrPh	**13d**	A[a]	64, (95:5)	20
6	*m*-BrPh	**13d**	B[b]	66, (7:93)	20
7	*m,p*-di-ClPh	**13e**	A[a]	-	80

[a]A: R^1CHO, *p*-TsOH ac., CH_2Cl_2, reflux 6 h; [b]B: R^1CHO, *p*-TsOH ac., PhMe, Dean-Stark, reflux 4 h; [c] ratio based on integration of distinct ^{1}H-NMR signals.

For the thiols **13d** and **13e**, the situation was different under the same re-equilibration conditions. Starting from **13d** (R^1 = *m*-BrPh derivative), we observed the formation of a mixture of fused **8d** and the corresponding spiro compound **9d**. Starting from **13e** (R^1 = *m*,*p*-diClPh), we observed the exclusive formation of spirocycle **9e** instead of the bicycle **8e**, (Table 6, entry 7). In contrast, when using PhMe/*p*-TsOH ac. at reflux, we observed a different product distribution. For thiols **13a**, and **13d**, we obtained a mixture of *syn-syn*-fused-**8** and spiro-**9** (Table 6, entries 2 and 6).

It is noteworthy that the product distribution was strongly dependent on the electronic character of the substituents R^1, the solvent and the temperature. Bicycle **13e**, bearing the most electron-withdrawing group, only equilibrated to the spiro compound **9e.**

The new spiro-**9a, d** and **e** compounds were fully characterized in order to confirm their structure. Important evidence was the long-range coupling constant ^4J indicative of a typical W arrangement for H_a, and H_b in the ^{1}H-NMR spectra (see Figure 6).

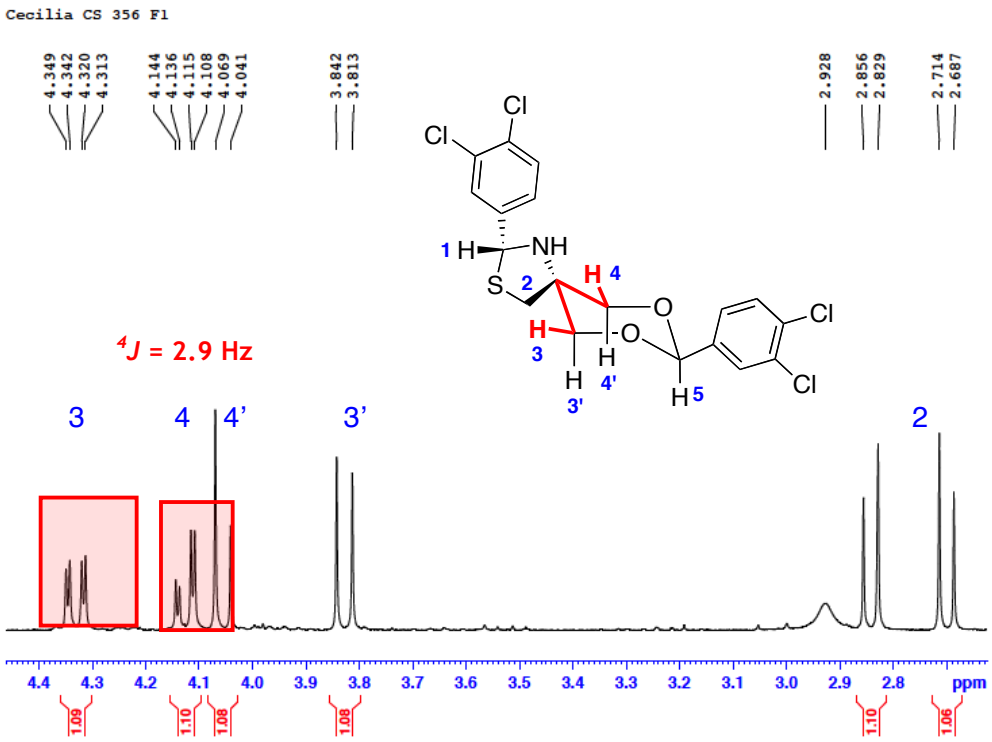
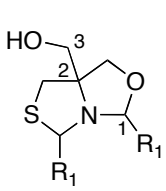


Figure 6. 1H-NMR signals of **9e**. Long-range coupling constant ^{4}J indicative of a typical W arrangement.

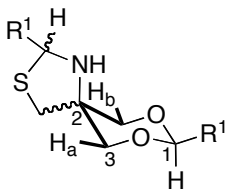
In addition, a significant shift in some carbon signals, such as the quaternary carbon C_2, the CH_2OR C_3, and the acetalic carbon of the oxazolidine or oxazine C_1, indicated the presence of compound **9** (see Table 7). The absence of the hydroxyl-stretching band in the IR spectra confirmed the spiro structure **9**.

Table 7. Characteristic ^{13}C-NMR δ signals for fused-**8** and spiro-**9** heterocycles.

Compound	^{13}C-NMR δ (ppm)		
	C_1	C_2	C_3
fused *anti-syn*-**8a**	94.6	79.8	65.8
fused *syn-syn*-**8a**	98.4	79.7	66.9
spiro *anti*-**9a**	101.1	64.1	73.4
fused *anti-syn*-**8d**	94.1	79.6	65.9
fused *syn-syn*-**8d**	98.3	79.8	66.9
spiro *anti*-**9d**	100.8	64.1	73.4



anti-syn-**8**
syn-syn-**8**



2S-anti*-**9**
2R-syn*-**9**

Intrigued by the formation of spirocycle **9**, we calculated the difference in energy for the fused bicycle **8f** and the spirocycle **9f** (R^1= Ph). According to the computation, the spirocycle **9f** is more stable than the thiazolidinyloxazolidine **8f** by about 5 kcal/mol (fig 7).

In agreement with the computational results for fused bicycles **8**, the diastereomer *syn-syn*-**8f** is 1.9 kcal more stable than the *anti-syn*-**8f**. This result was also experimentally confirmed since the apparent kinetic product *anti-syn*-**8** was obtained at a lower temperature in CH_2Cl_2 while higher temperatures in toluene yielded the thermodynamically preferred *syn-syn*-**8**.

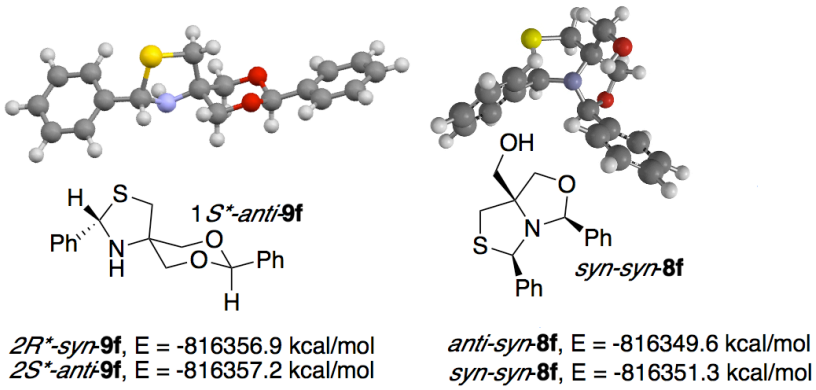
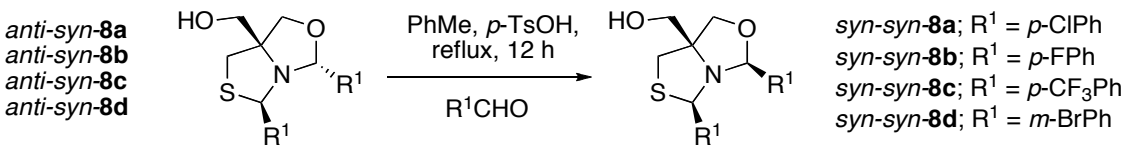


Figure 7. Calculated energies for spiro **9f** and fused **8f**.

However, attempts to obtain the spiro compound by further heating the fused *anti-syn*-**8a** at reflux using different solvents (MeCN, PhMe) and acidic conditions (*p*-TsOH ac. or $HClO_4/SiO_2$[26]) led only to the isomerization of the starting material into the thermodynamic *syn-syn*-**8** bicycle, without an apparent formation of the spiro product (Scheme 14).



Scheme 14. Isomerization of *anti-syn*-**8** into *syn-syn*-**8**.

The proposed mechanism for bicycle and spirocycle formation is depicted in Figure 8. In acidic media, fused bicycle **8** can be opened by ring-chain tautomerism, leading to the formation of two possible intermediates: oxonium ion I_1 and the probably more stable iminium ion I_2. Under kinetic conditions, intermediate I_2 is preferentially formed and reacts faster than I_1, leading again to the 5-*endo-trig* cyclization product **8**. In contrast, under thermodynamic conditions, the more stable spiro-thiazolidine **9** is formed. The selectivity of this product distribution can be explained on the basis of the ring-chain tautomeric equilibration of the intermediates, with a kinetic preference for the formation of the bicyclic product **8** under mild conditions, or a complete equilibration towards the spiro product **9** under more forcing conditions.

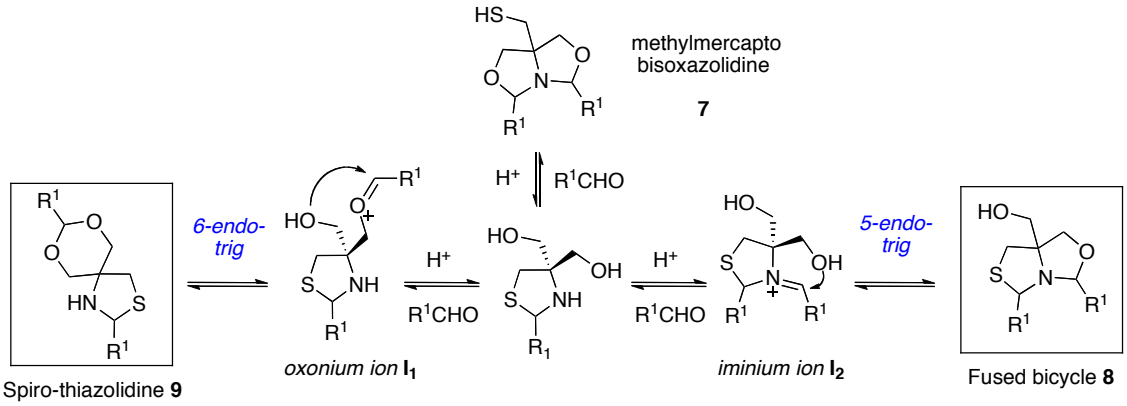


Figure 8. Mechanistic proposal for the formation of fused bicycles **8** and spirocycle **9**.

The reaction conditions are critical to the product distribution. There is a correlation with lower temperatures favoring the kinetic products and higher temperatures establishing a thermodynamic equilibrium.

There is no clear correlation between the electronic effect of substituents at R^1 and the product distribution bicycle versus spirocycle. The values of the Hammet coefficients are σ = 0.60 (*m,p*-diCl) > 0.54 (*p*-CF_3) > 0.39 (*m*-Br) > 0.23 (*p*-Cl) > 0.06 (*p*-F).[27] Electron-withdrawing groups at R^1 destabilize cationic intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$, but probably more so in the iminium ion $\mathbf{I_2}$ than the alkoxycarbenium ion $\mathbf{I_1}$, thus for example favoring, in CH_2Cl_2, the formation of spirocycles **9d** (*m,p*-diCl) and **9e** (*m*-Br).

[26] Kumar, R.; Kumar, D.; Chakraborti, A. K. Perchloric acid adsorbed on silica gel ($HClO_4$-SiO_2) as an inexpensive, extremely efficient, and Reusable Dual Catalyst System for Acetal/Ketal Formation and Their Deprotection to Aldehydes/Ketones. *Synthesis* **2007**, *2*, 299-303.
[27] Hansch, C.; Leo, A.; Taft, R. W. A survey of Hammett substituent constants and resonance and field parameters. *Chem. Rev.* **1991**, *91*, 165–195.

Otherwise compound **13c** (*p*-CF_3) having also a high σ value did not evolve to the spiro and led exclusively to bicycle **8c** formation (Table 6).

Partial Conclusions

We were able to synthesize the fused thiobicycles **8a-e** for the first time. As described in the literature, the oxygen-containing heterocycles **7b** and **7c** have attractive biological properties as neuroprotectives, which is likely shared or further enhanced by the sulfur analogues **8b** and **8c**. The methodology developed in path b) allowed the selective preparation of both diastereomers of these heterocycles, the *syn-syn*-**8** in PhMe as well as the *anti-syn*-**8**, in CH_2Cl_2. The replacement of oxygen by a sulfur atom in the structure of Tris (**10**) to form Tris-SH (**11**) has a remarkable impact in the cyclization process in the presence of aldehydes compared to Tris.

The spirocyclic compound **9** represents a novel molecular scaffold. It was obtained in a ring-chain-ring tautomeric equilibration of mercaptomethyl bisoxazolidine **13**. These compounds can be synthesized in acidic media by tuning the reaction conditions (temperature, solvent), and their formation is strongly dependent on the electronic properties of the substituents R^1. Thiazolidines are known to be more stable than oxazolidines,[28] this property is likely the reason why spirocyclic **9** is formed but the corresponding spirocyclic oxygen analogues are still unknown. These findings represent a significant extension of the number of small but densely functionalized scaffolds that can be obtained from relatively simple and readily available buildings blocks. We anticipate that this information can be utilized for the construction of new dynamic combinatorial libraries.

[28] Fulop, F.; Mattinen, J.; Pihlaja, K. Ring-chain tautomerism in 1,3-thiazolidines. *Tetrahedron* **1990**, *46*, 6545-6552.

3.1.3 Synthesis of new scaffolds: Bisthiazolidines

Paper III (Saiz, C.; Castillo, V.; Mahler, G. S. *Synlett.* **2012**, *ST-2011-0311-L in press*)

Imine Domino Reactions Generate Novel Scaffolds: Fused bis-Thiazolidines or bis-Thiiranes

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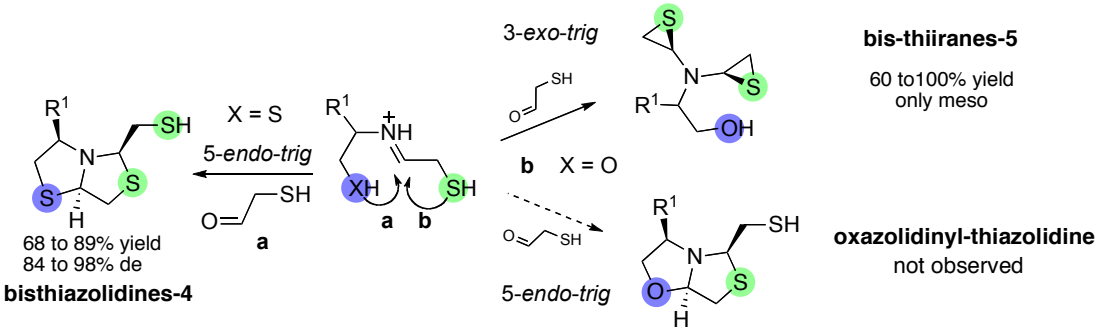
Abstract



Novel domino processes that involve sequential reactions via iminium ions derived from β-amino thiols or β-amino alcohols were developed to form bis-thiazolidines or bis-thiiranes respectively. The heterocycles were synthesized from readily available starting materials, forming four new chemical bonds in one process without the use of metals. The regioselectivity of the transformation could be explained on the basis of calculations of the coefficients of the frontier orbitals.

Key words: *bicycles, fused thiazolidines, thiiranes, cycloadditions, iminium ions.*

Introduction

The medicinal chemistry community has become increasingly receptive to the discovery of new chemotypes for novel synthetic protein ligands.[29] The chemical space to be explored requires much synthetic effort but the rate of publication of unknown

[29] (a) Marson C. M. New and unusual scaffolds in medicinal chemistry. *Chem. Soc. Rev.* **2011**, *40*, 5514–5533; (b) Walters W. P.; Green, J.; Weiss, J. R.; Murcko, M. A. What Do Medicinal Chemists Actually Make? A 50-Year Retrospective. *J. Med. Chem.* **2011**, *54*, 6405–6416.

heterocyclic molecules has decreased the last two decades to 5-10 per year.[30] Recently the work presented by Lovering and co-workers hypothesized that both structural complexity and the presence of stereogenic centers correlate with compound success as they transition from discovery, through clinical testing, into marketable drugs. In addition, more highly complex molecules, as measured by carbon bond saturation, have the ability to access a greater chemical space.[31]

Domino or cascade reactions are a combination of multiple transformations in a single process. They are of high potential in the synthesis of complex, even chiral target molecules and constitute a powerful tool for organic chemists.[32] Iminium ions are particularly useful as versatile intermediates for such kind of reactions as they may serve as electrophiles in a diverse range of bond forming posibilities.[33]

In this context 5,5-fused heterocycles containing a bridgehead nitrogen are complex and highly functionalized scaffolds. Bisoxazolidines **A**,[34] as an example, were described as anticancer agents[22] and neuroprotectives,[23] see Figure 9. Recently we focused our efforts on the possibility of expanding the diversity of heterocyles **A** and described the synthetic methodology for the preparation of thiazolidinyl-oxazolidine **B** analogs (Figure 9).[35,18]

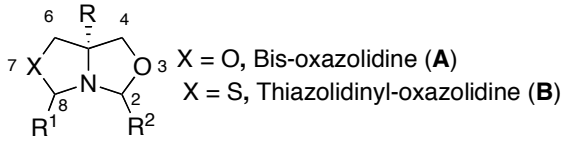


Figure 9. 5,5-fused bis-oxazolidine **A** and thiazolidinyl-oxazolidine **B**

The formation of heterocycles **A** and **B** occurs by a double imine cyclization to give 1,3-(*N,X*)-heterocycles. As an extension of our previous work, we focused on the

[30] Pitt, W.; Parry, D.; Perry, B.; Groom, C. Heteroaromatic Rings of the Future. *J. Med. Chem.* **2009**, *52*, 2952–2963.
[31] Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* **2009**, *52*, 6752–6756.
[32] (a) Tietze, L. F. Domino reactions in organic synthesis. *Chem. Rev.* **1996**, *96*, 115-136; (b) Tietze, L. F.; Beifuss, U. Sequential transformations in organic chemistry: a synthetic strategy with a future. *Angew. Chem.* **1993**, *105*, 137-170.
[33] Royer, J.; Bonin, M.; Micouin, L. Chiral Heterocyles by Iminium Ions Cyclization. *Chem. Rev.* **2004**, *104*, 2311–2352.
[34] Bergmann E. D. The oxazolidines. *Chem. Rev.* **1953**, *53*, 309–352.
[35] Saiz, C.; Wipf, P.; Mahler, G. Synthesis and ring-chain-ring tautomerism of bisoxazolidines, thiazolidinyloxazolidines and spirothiazolidines. *J. Org. Chem.* **2011**, *76*, 5738–5746.

preparation of novel 5,5-fused bicycles with a bridgehead nitrogen atom but with different atom connectivity.

We envisioned that iminium ions of general structure **I** (Figure 10), bearing a nucleophilic tether with a suitably located sulfur or oxygen atom, could be useful building blocks for the synthesis of biologically and synthetically relevant heterocycles.

Results and discussion

Herein, we report an efficient domino procedure for the synthesis of enantioenriched fused bis-thiazolidines (**22**) or meso bis-thiiranes (**23**) by the generation of an iminium ion followed by double cyclization (Figure 10).

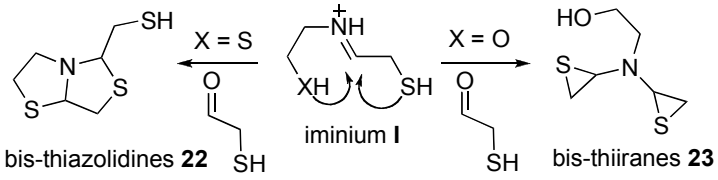
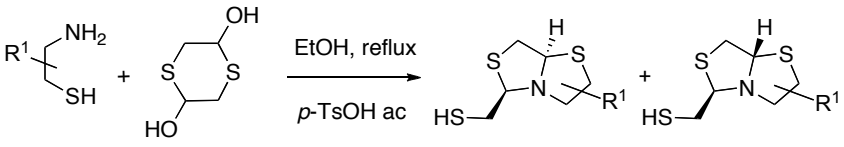


Figure 10. Iminium ions **I** generate: X = S, bis-thiazolidines **22** or X = O, 2-amino bis-thiiranes **23**.

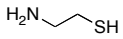
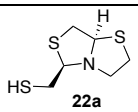
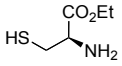
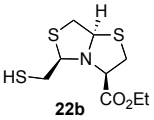
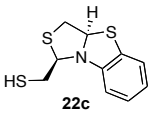
There are few examples in the literature about the preparation of fused bis-thiazolidines **22**, all of them requiring a multistep sequence.[36] Bicycles **22a-d** were obtained by heating readily available starting materials like: aminothiols **24a-c** in the presence of 1,4-dithiane-2,5-diol **25** in acidic media, see Table 8. Smooth depolymerization of **25** in EtOH at reflux led to the formation of 2-mercaptoacetaldehyde. The reaction of two molecules of aldehyde in the presence of **24a-c**, led to the formation of fused bis-thiazolidines **22a-c** in good yields (78 to 89%) and high diastereomeric excess (84 to 98%).

Table 8. Synthesis of bis-thiazolidines **22a-c.**

[36] (a) Stojanović, M.; Marković, R.; Kleinpeter, E.; Baranac-Stojanović, M. endo-Mode cyclizations of vinylogous N-acyliminium ions as a route to the synthesis of condensed thiazolidines. *Tetrahedron* **2011**, *67*, 9541-9554; (b) Stojanović, M.; Marković, R. Synthesis of the first thiazolidine-condensed five-, six-, and seven-membered heterocycles via cyclization of vinylogous N-acyliminium ions. *Synlett* **2009**, *12*, 1997-2001; (c) Koepper, S.; Lindner, K.; Martens, J. Die anlagerung von mercaptocarbonsäuren an 3-thiazoline und anschließende lactamisierung. *Tetrahedron* **1992**, *48*, 10277-10292.



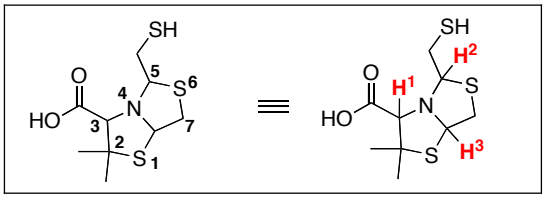
24 **25** *anti*-**22** *syn*-**22**

Entry	Starting material	Product	Yield (%), (anti:syn)[a]
1	 **24a**	 **22a**	78, (92:08)
2	 **24b**	 **22b**	86, (95:05)
3	 **24c**	 **22c**	89, (99:01)

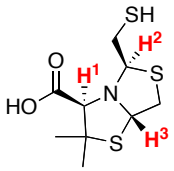
[a.] The diasteromeric ratio was determined by ^{1}H NMR.

The products derived from cysteamine (**24a**) or L-cysteine ethyl ester (**24b**) conducted to the *anti*-**22a** and *anti-anti*-**22b** in high yields and diasteromeric excess (entries 1, 2, Table 8). The aromatic aminothiol **24c** led to the formation of fused tricycle *anti*-**22c** as the only diastereomer (entry 3, Table 8). This efficient process leads to the generation of four new chemical bonds and high diasteroselectivity.

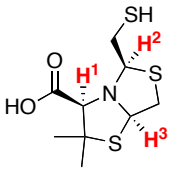
The relative configuration of the diastereomers **22a-c** was elucidated by NOESY correlations, ^{1}H and ^{13}C-NMR. In figure 11 irradiations at H^1, H^2 and H^3 frequences are shown. We observe that H^1 and H^2 are *syn* but H^3 is not seen by neither of them. If we analyze Figure 12, we can see that both H^2 and H^3 are syn with H^1. According to these results we can conclude that compound **22j** is *syn-syn* (fig 11) and **22j** *syn-anti* (fig 12).



22 (general structure)



syn-syn **22j**



syn-anti **22j**

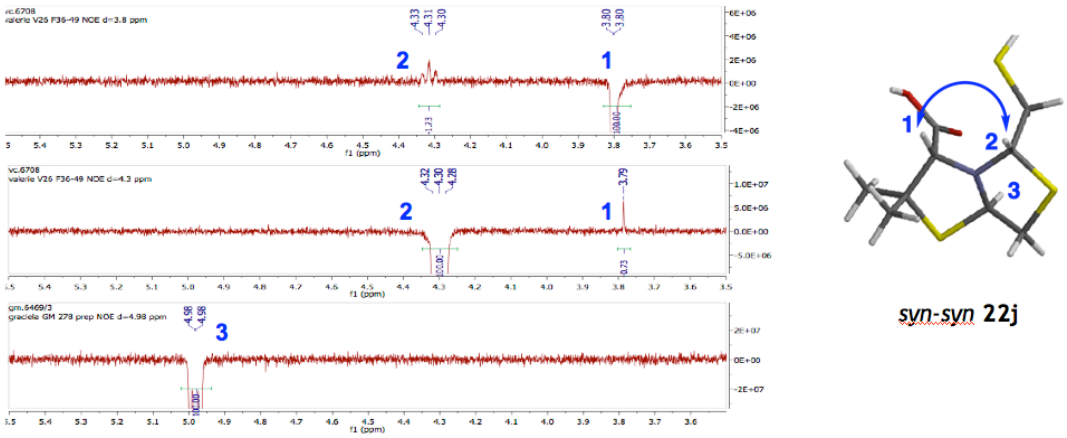


Figure 11. NOESY correlation of *syn-syn* **22j**. Irradiation at 3.8, 4.3 and 4.98 ppm.

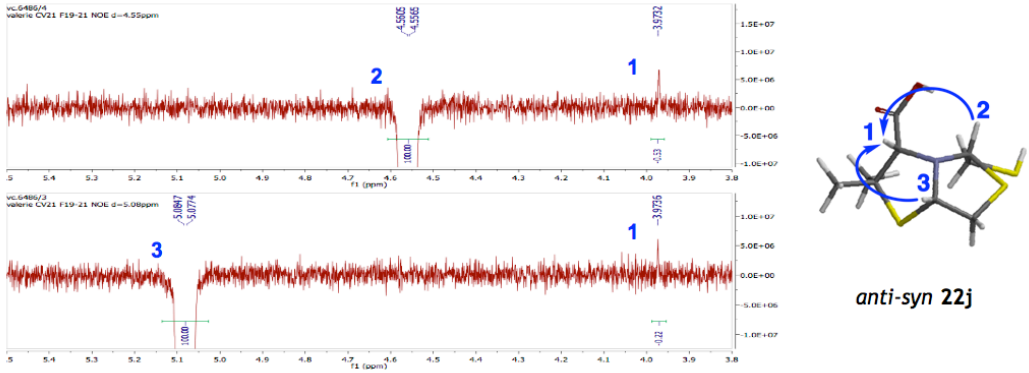


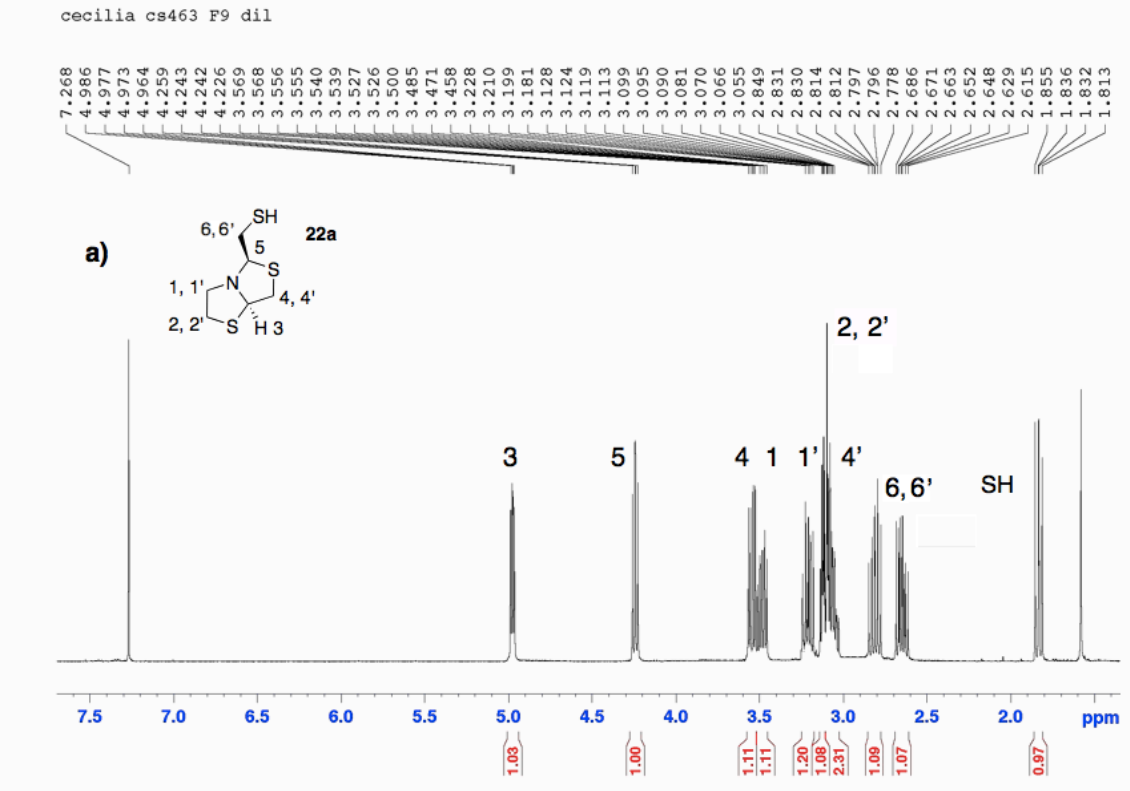
Figure 12. NOESY correlation of *anti-syn* **22j**. Irradiation at 4.55 and 5.08 ppm.

To study the scope of the reaction we evaluated if β-amino alcohols **26a-c** were able to form the 5-5-fused oxazolidinyl-thiazolidines via an iminium ion. Therefore we submitted **26a-c** to the same cyclization process: reaction with dithiane **25** in EtOH at reflux in acidic media. We obtained the unexpected bis-episulfides **23a-c** in good to very good yields (64-100%) see entries 1, 2, 3, Table 9. The reaction was clean with the exclusively formation of the bis-thiiranes (see Figure 13).

This methodology can be used with primary alkyl amines; compounds **23d** and **23e** were formed in similar fashion and very good yields starting from amines **26d** and **26e** (91 and 89%), (entries 4, 5, Table 9). Primary aromatic and secondary alkyl amines instead led to complex reaction mixtures, see entries 7, 8. Finally the use of

ethylendiamine led to the tetra-thiirane **23f** in good yield (51%), in a domino process where eight new chemical bonds were formed, see entry 6. When we used non-chiral starting aminoalcohols (**26a**, **26d**, **26e**) we obtained exclusively the meso-bis-thiiranes, based on the ^{1}H-NMR symmetry and ^{13}C-NMR spectrum. On the other hand, racemic aminoalcohols (**26b-c**) led to diasteromeric mixtures of two thiiranes.

Comparative ^{1}H-NMR spectra of bis-thiazolidine **22a** and bis-thiirane **23a** derived from analogous reagents - aminoalcohol and aminothiol - and mercaptoacetaldehyde are shown in Figure 13.



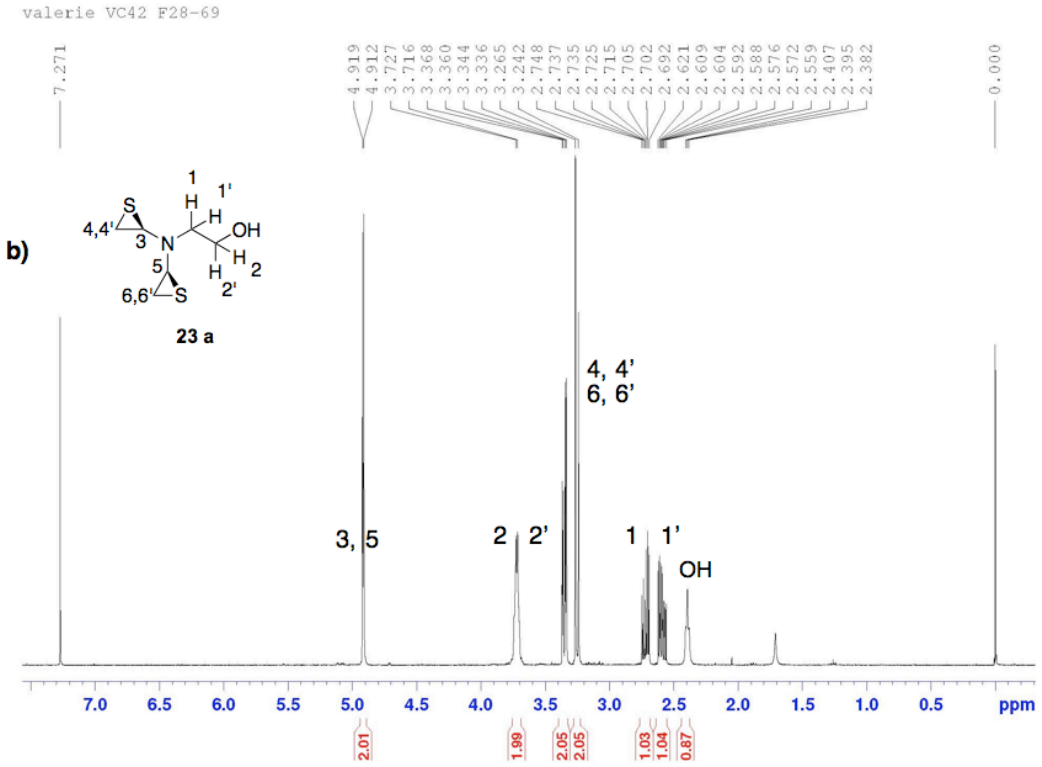


Figure 13. [1]H NMR spectra of a) bisthiazolidine **22a** and b) bis-episulfide **23a.**

We concluded that we obtained the meso-bisthiiranes **23** due to the symmetry of [1]H, and [13]C-NMR spectra: we observed identical chemical shifts for the acetalic protons and carbon signals for H^3 and H^5 (see Figure 13). Due to our experience on related compounds,[35] we could say that signals for two acetalic protons and carbons that are unique in the [1]H and [13]C-NMR spectrum could be attributed only to meso compounds. For compound **23a**, we observed both acetalic protons (H^3 and H^5) as a unique doublet at 4.86 ppm and both carbons (C^3 and C^5) as a single signal at 69.9 ppm. This shows that these protons and carbons are magnetically and chemically equivalents.

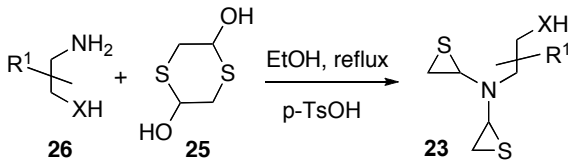
This is, to the best of our knowledge, the first report of 2-amino-bis-thiiranes preparation (Table 9). One important aspect of this finding is that thiiranes are useful molecules that have been used advantageously in pharmaceutical, polymer and herbicide industries.[37] They also display interesting biological activities as gelatinases,[38] and 17r-Hydroxylase/C17-20-Lyase inhibitors.[39] The most common

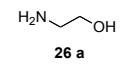
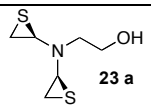
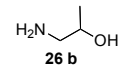
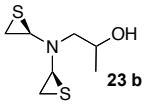
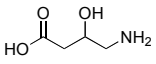
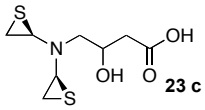
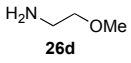
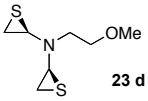
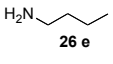
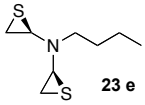
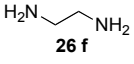
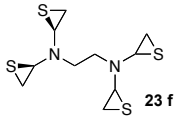
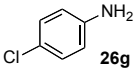
[37] Nakano, K.; Tatsumi, G.; Nozaki, K. Synthesis of Sulfur-Rich Polymers: Copolymerization of Episulfide with Carbon Disulfide by Using [PPN]Cl/(salph)Cr(III)Cl System. *J. Am. Chem. Soc.* **2007**, *129*, 15116-15117.

[38] (a) Testero, S. A.; Lee, M.; Staran, R. T.; Espahbodi, M.; Llarrull, L. I.; Toth, M.; Mobashery, S.; Chang, M. Sulfonate-Containing Thiiranes as Selective Gelatinase Inhibitors. *ACS Med. Chem. Lett.* **2011**, *2*, 177–181; (b) Brown, S.; Bernardo, M.; Li, Z-H.; Kotra, L. P.; Tanaka, Y.; Fridman, R.;

method to prepare these compounds is by the transformation of oxiranes into thiiranes using an adecuated source of sulfur like thiourea, thioamides, etc.[40]

Table 9. Synthesis of bis-thiiranes **23a-c.**



Entry	Starting material	Product	Yield (%)[a]
1	 **26 a**	 **23 a**	**23a:** 66
2	 **26 b**	 **23 b**	**23b:** 100
3	 **26 c**	 **23 c**	**23c:** 64
4	 **26d**	 **23 d**	**23d**: 91
5	 **26 e**	 **23 e**	**23e**: 89
6	 **26 f**	 **23 f**	**23f**: 51
7	 **26g**	b	b
8	Et_2NH, **26h**	b	b

a. Isolated yield, b. Complex mixture.

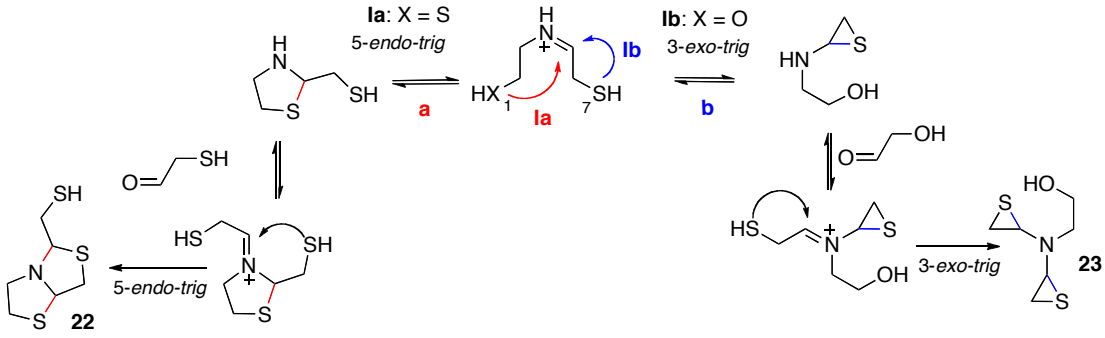
Mobashery S. Potent and Selective Mechanism-Based Inhibition of Gelatinases. *J. Am. Chem. Soc.* **2000**, *122*, 6799-6800.

[39] Hartmann, R. W.; Hector, M.; Wachall, B. G.; Palusczak, A.; Palzer, M.; Huch,V.; Veith, M. Synthesis and Evaluation of 17-Aliphatic Heterocycle-Substituted Steroidal Inhibitors of 17α-Hydroxylase/C17–20-Lyase (P450 17). *J. Med. Chem.* **2000**, *43*, 4437-4445.

[40] (a) Vedejs, E.; Krat, G. A. Cyclic sulfides in organic synthesis. *Tetrahedron* **1982**, *38*, 2857-2881; (b) Sander, M. Thiiranes. *Chem. Rev.* **1966**, *66*, 297-339; (c) Yadav, J. S.; Reddy, B. V. S.; Srinivas Reddy, C.; Rajasekhar, K. $[Bmim]PF_6$: A Novel and Recyclable Ionic Liquid for Conversion of Oxiranes to Thiiranes in Aqueous Media. *J. Org. Chem.* **2003**, *68*, 2525–2527.

The present methodology extends current synthetic methods for the synthesis of thiiranes and provides a facile route to highly functionalized building blocks.

We hypothesize that both reactions proceed via iminium ion formation, see scheme 15. In this sense the reaction of β-aminothiols with mercaptoacetaldehyde leads to the formation of iminium ion $\mathbf{I_a}$ (X = S), followed by the 5-*endo-trig* cyclization of ^{1}S atom to form the first thiazolidine ring. A second incorporation of mercaptoacetaldehyde followed by another cyclization leads to the fused bisthiazolidine **22**. Even if the 5-*endo-trig* cyclization is thermodynamically disfavored according to Baldwin rules,[41] frequently sulfur atoms can disobey it to give thiazolidines.[16c]



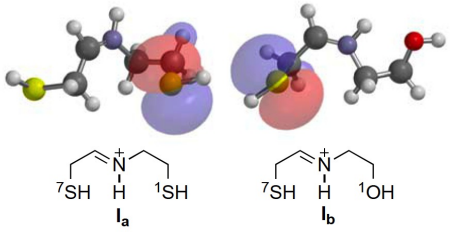
Scheme 15. Mechanistic proposal for the formation of bis-thiazolidines **22** or bis-thiiranes **23**.

The condensation of β-aminoalcohols with mercaptoacetaldehyde led to the formation of iminium ion $\mathbf{I_b}$ (X = O) followed by thiol -^{7}SH cyclization in a 3-*exo-trig* process to give the first thiirane ring, favored according to Baldwing rules. A similar sequence with the imine formation and a 3-*exo-trig* cyclization led to the second thiirane ring. The iminium ion $\mathbf{I_b}$ tethered with a β-hydroxyl group -^{1}OH could be able to generate the 5-*endo-trig* product oxazolidinyl-thiazolidine,[35] but in the reaction conditions it was not observed.

The reactivity differences of iminium ion derived from aminothiols ($\mathbf{I_a}$) and aminoalcohols ($\mathbf{I_b}$) were interpreted based on frontier molecular orbital analysis. We used DFT/B3LYP/6-311G* functions (Spartan 10) for theoretical calculations. Table 10 shows the differences in HOMO/LUMO energies; HOMO eigenvectors coefficients (Py direction) and charge distributions calculated for ^{1}O, ^{1}S and ^{7}S atoms of the intermediates $\mathbf{I_a}$ and $\mathbf{I_b}$.

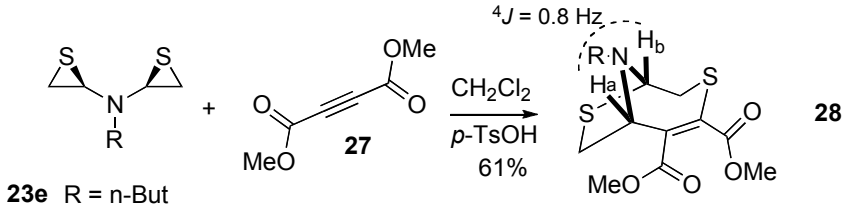
The results showed that HOMO/LUMO energy gap is small and both reactions seem to be kinetically favored. The iminium ion **I$_a$** (X = S) shows the highest HOMO coefficient located under ^{1}S atom, see Table 10. The results can explain the observed product regiochemistry where the 5-*endo-trig* instead of 3-*exo-trig* cyclization process takes place. The iminium ion **I$_b$** (X = O), showed the highest HOMO coefficient located under ^{7}S atom, this would explain the formation of the thiirane instead of the oxazolidine ring, see Table 10. The regioselectivity observed in these reactions correlates well with the highest HOMO coefficients for **I$_a$** and **I$_b$**, see picture above Table 10. Furthermore charges alone could not explain the observed regioselectivity.

Table 10. Electronic parameters calculated by DFT/B3LYP/6-311G* for iminium ions **Ia** and **Ib** and HOMO orbital surface plot.



Iminium ion	ΔE = E_{HOMO}-E_{LUMO} (eV)	HOMO eigenvector, Py direction	Charges
I$_a$	-11.21	^{1}S = 0.1511 ^{7}S = 0.0638	^{1}S = -0.025 ^{7}S = -0.363
I$_b$	-11,99	^{1}O = 0.0157 ^{7}S = 0.1886	^{1}O = -0.545 ^{7}S = -0.218

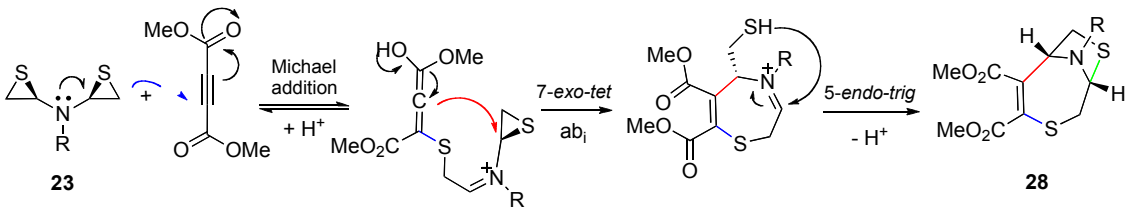
In order to explore the reactivity of the amino-thiiranes, **23e** was treated with dimethyl acetylidendicarboxylate (DMAD) **27**, a good Michael acceptor. The reaction took place smoothly at room temperature in CH_2Cl_2 and *p*-TsOH ac. to give the fused thiazolidinyl-thiazepine **28** in good yield (61%), see Scheme 16. The relative stereochemistry was confirmed by the long-range coupling constant between H_a and H_b protons, ^4J = 0.8 Hz.



Scheme 16. Synthesis of fused thiazolidinyl-thiazepine heterocyles **28**.

[41] Baldwin, J. E. Rules for Ring Closure. *J. Chem. Soc., Chem. Commun.* **1976**, *18*, 734-736.

The formation of product **28** can be rationalized as shown in scheme 17. The iminium ion promotes the thia-Michael addition to the triple bond, followed by a 7-*exo-tet* cyclization to open the next thiirane ring. The produced thiol attacks the iminium ion, generated in the first step, and cyclisize to form the bicycle **28**. This is a new domino process with the generation of two new chemical bonds.



Scheme 17. Mechanistic proposal for the formation of fused thiazolidinyl-thiazepine **28**.

Partial Conclusions

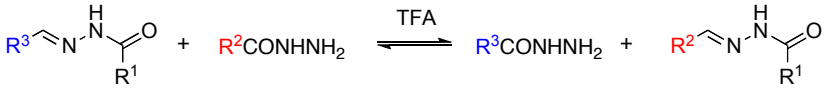
We were able to synthesize new fused bis-thiazolidines **22a-c** derived from β-aminothiols in high yield and diasteroselectively. This new scaffold was prepared using a metal free domino process, creating four new chemical bonds *via* the simple loss of water.

When using the iminium ion generated with primary alkyl amines and 2-mercaptoacetadehyde we obtained 2-amino-bis-thiiranes **23a-f** in a clean and high yielding fashion. It is noteworthy that a facile access to this new class of episulfides could have direct application on new materials and polymers. As proof of its utility we studied the reaction of amino-bisthiirane **23e** with DMAD **27** and were able to generate novel fused thiazolidinyl-thiazepine **28** in a domino process.

In summary, we explored the utility of iminium ions generated from aminothiols and amines with 2-mercaptoacetadehyde and were able to prepare three different unknown heterocyclic scaffolds.

3.1.4 Synthesis of new scaffolds: Hydrazolylthiazolidinones.

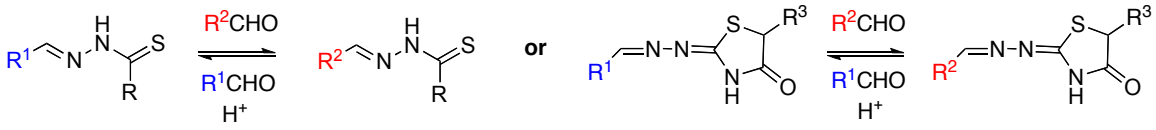
Imine generation starting from amines and aldehydes can exchange units providing a useful reaction suitable to generate dynamic libraries, described by Lehn in 1997.[42] An extension of the C=N reversible reactions was the transimination of hydrazones, reported by Sanders *et al.*[43] The interconversion occurs at room temperature in diluted TFA in CH_2Cl_2 and can be stopped with the addition of Et_3N, see scheme 18.[44]



Scheme 18. Reversible hydrazone transimination.

Later, Huc *et al.* optimized the reversibility of hydrazone formation for DCC, using hydrazines bearing electron-withdrawing groups and aromatic or aliphatic aldehydes. The exchange occurs at neutral pH and rt, and they postulate that it could be adapted to biological templates.[45]

In order to explore this type of exchange reaction and enlarge it to new building blocks, we decided to investigate the carbonyl exchange between thiosemicarbazones or hydrazolylthiazolidinones, see scheme 19. With this purpose, we synthesized different scaffolds and studied the exchange reaction.



Scheme 19. Exploring new exchange reactions: thiosemicarbazones and aldehydes.

[42] Huc, I.; Lehn, J. M. Virtual combinatorial libraries: Dynamic generation of molecular and supramolecular diversity by self-assembly. *Proc. Natl. Acad. Sci.* **1997**, *94*, 2106–2110.
[43] Cousins, G.; Poulsen, S. A.; Sanders, J. K. M. Dynamic combinatorial libraries of pseudo-peptide hydrazone macrocycles. *Chem. Commun.* **1999**, 1575-1576.
[44] Ramatrom, O.; Lohmann, S.; Bunyapaiboonsri, T.; Lehn, J. M. Dynamic combinatorial carbohydrate libraries: Probing the binding site of the concanavalin A lectin. *Chem. Eur. J.* **2004**, *10*, 1711-1715.
[45] Nguyen, R.; Huc, I. Optimizing the reversibility of hydrazone formation for dynamic combinatorial chemistry. *Chem. Commun.* **2003**, 942–943.

The interconversion was explored for thiosemicarbazones in several conditions: different acidic pH, temperature, solvent and time were used but no reversible exchange could be established. [46]

In this work we describe a new efficient preparation of a collection of hydrazolylthiazolidinones and derivatives thought as possible building blocks for exchange reactions, see *paper IV*.

[46] Trabajo realizado en la cátedra de Química Farmacéutica por el Q.F. Juan Estol en su proyecto de practicantado.

Paper IV (Saiz, C.; Pizzo, C.; Manta, E.; Wipf, P.; Mahler, S.G. *Tetrahedron Lett.* **2009**, *50*, 901-904)

Microwave assisted tandem reactions for the synthesis of 2-hydrazolyl-4-thiazolidinones.

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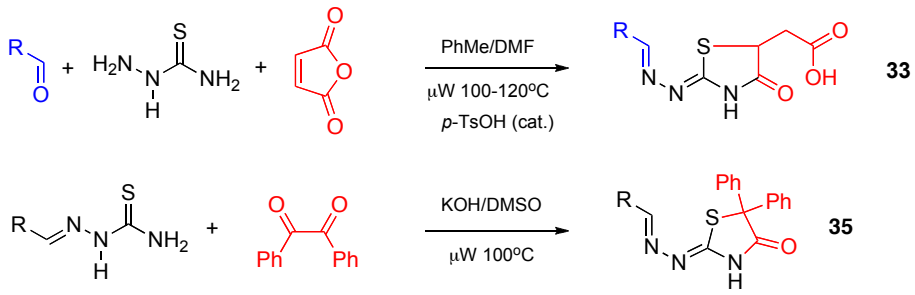
Abstract



A tandem method for the synthesis of 2-hydrazolyl-4-thiazolidinones (**5**) from commercially available materials in a 3-component reaction has been developed. The reaction connects aldehydes, thiosemicarbazides and maleic anhydride, effectively assisted by microwave irradiation. The synthesis of a new type of compound, 2-hydrazolyl-5,5-diphenyl-4-thiazolidinone (**7**), obtained by treatment of thiosemicarbazone with benzil in basic media is also reported. HOMO/LUMO energies, orbital coefficients and charge distribution were used to explain the proposed reaction mechanism.

Keywords: *2-hydrazolyl-4-thiazolidinones, 2-hydrazolyl-5,5-diphenyl-4-thiazolidinones, microwave, tandem reactions.*

Introduction

4-Thiazolidinones are an important group of heterocycles found in numerous natural products and pharmaceuticals.[47] In particular, 2-hydrazolyl-4-thiazolidinones (**33**) are a class of compounds that combine thiosemicarbazones with 4-thiazolidinones, two

[47] Verma, A.; Saraf, S. K. 4-thiazolidinone -a biologically active scaffold. *Eur. J. Med. Chem.* **2008**, *43*, 897-905.

building blocks with interesting biological activities. For example, *Trypanosoma cruzi*,[48] *Plasmodium falciparum*[49] and antitumor[50] activities have been described for thiosemicarbazones, and COX-2 inhibition,[51] anti-HIV,[52] and antibacterial[53] effects as well as human chondrocyte antidegenerative[54] properties have been found for 4-thiazolidinones. In addition, the combination of these two pharmacophores has been used to exhibit anti-*Toxoplasma Gondii*,[55a] antimicrobial,[55b] antiviral,[56] and antifungal properties.[57]

Among the reported methods for 2-hydrazolyl-4-thiazolidinone synthesis, there is a 2 step sequence involving: firstly a reaction between aldehydes (**29**) and thiosemicarbazides (**30**) to give thiosemicarbazones (**31**) and secondly a thia-Michael addition of thiosemicarbazones (**31**) to maleic anhydride (**32**) in dry PhMe and DMF at reflux to give the hydrazolyl-4-thiazolidinone (**33**) (Scheme 20).[56]

[48] (a) Du, X.; Guo, C.; Hansell, E.; Doyle, P.; Caffrey, C.; Holler, T.; McKerrow, J.; Cohen, F. Synthesis and Structure−Activity Relationship Study of Potent Trypanocidal Thio Semicarbazone Inhibitors of the Trypanosomal Cysteine Protease Cruzain. *J. Med. Chem.* **2002**, *45*, 2695-2703; (b) Cohen, F. E.; Du, X.; Guo, C.; McKerrow, J. H. U.S. Patent 6 897 240, **2004**.

[49] Walcourt, A.; Loyevsky, M.; Lovejoy, D. B.; Gordeuk, V. R.; Richardson, D. R. Novel aroylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine-resistant and -sensitive parasites. *Biochem. Cell. Biol.* **2004**, *36*, 401-407.

[50] Senaratne, D.; Kalinowski, D. S.; Islam, M.; Bernhardt, P. V. Dipyridyl Thiosemicarbazone Chelators with Potent and Selective Antitumor Activity Form Iron Complexes with Redox Activity. *J. Med. Chem.* **2006**, *49*, 6510-6521.

[51] Vigorita, M. G.; Ottanà, R.; Monforte, F.; Maccari, R.; Monforte, M. T.; Trovato, A.; Taviano, M. F.; Miceli, N.; De Luca, G.; Alcaro, S.; Ortuso, F. Chiral 3,3'-(1,2-ethanediyl)-bis[2-(3,4-dimethoxyphenyl)-4-thiazolidinones] with anti-inflammatory activity. Part 11: evaluation of COX-2 selectivity and modelling. *Bioorg. Med. Chem.* **2003**, *11*, 999-1006.

[52] Rawal, R. K.; Tripathi, R.; Katti, S.B.; Pannecouque, C.; De Clerq, E. Design, synthesis, and evaluation of 2-aryl-3-heteroaryl-1,3-thiazolidin-4-ones as anti-HIV agents. B*ioorg. Med. Chem.* **2007**, *15*, 1725-1731.

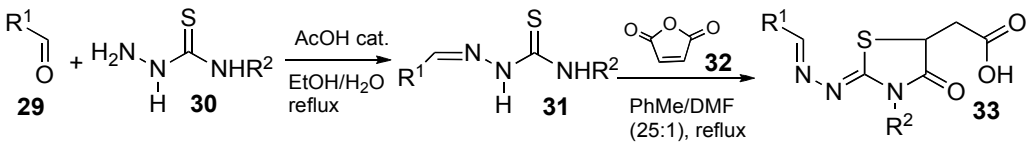
[53] Bonde, C. G.; Gaikwad, N. J. Synthesis and preliminary evaluation of some pyrazine containing thiazolines and thiazolidinones as antimicrobial agents. *Bioorg. Med. Chem.* **2004**, *12*, 2151-2161.

[54] Ottanà, R.; Maccari, R.; Ciurleo, R.; Vigorita, M. G.; Panico, A. M.; Cardile, V.; Garufi, F., Ronsisvalle, S. Synthesis and in vitro Evaluation of 5-Arylidene-3-hydroxyalkyl-2-phenylimino-4-thiazolidinones (V) with Antidegenerative Activity on Human Chondrocyte Cultures. *Bioorg. Med. Chem.* **2007***, 15*, 7618-7625.

[55] (a) Tenorio, R.; Carvalho, C.; Pessanha, C.; de Lima, J.; de Faria, A.; Alves, A.; de Melob, E. J.; Goes A. Synthesis of thiosemicarbazone and 4-thiazolidinone derivatives and their in vitro anti-*Toxoplasma gondii* activity. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2575–2578; (b) de Aquino, T.; Liesen, A.; da Silva, R.; Lima, V.; Carvalho, C.; de Faria, A.; de Araujo, J.M.; Lima, J.G.; Alves, A.J.; de Melo, E. J. T.; Goesa, A. J. S. Synthesis, anti-Toxoplasma gondii and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids. *Bioorg. Med. Chem.* **2008**, *16*, 446–456.

[56] Plut, M.; Pollak, A.; Tisler, M.; Likar, M.; Schauer, P. S. Derivatives of 5-Carboxymethylthiazolidine-2,4-dione, a New Group of Antiviral Compounds. *J. Med Chem.* **1966**, *9*, 430-431.

[57] Krbavcic, A.; Badawy, M. A.; Abdel-Hady, S. A.; Kadry, A. M.; Ibrahim, Y. A. Reinvestigation of the reactions of thiosemicarbazones with maleic anhydride. *Sulfur Lett.* **1989**, *9*, 149-57.



Scheme 20. Stepwise 2-hydrazolyl-4-thiazolidinone synthesis under conventional conditions.

As a part of our search for new biologically active heterocyclic compounds, we focused on the possibility of optimizing this procedure by developing a tandem microwave-assisted reaction sequence.

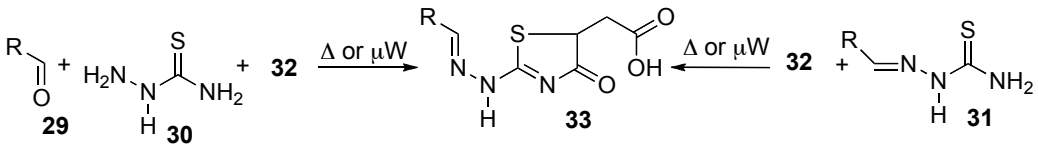
Multi-step or cascade reactions can be defined as the combination of two or more reactions in a specific order that occur in one pot.[58] They are very attractive due to their ease of setup. In traditional single-step processes, the reaction and product isolation are carried out independently and repeatedly to synthesize the target compounds. The former process allows a minimization of waste, and, compared to stepwise reactions, the amount of solvent, reagents, adsorbents, and energy is decreased.[59]

The use of microwave ovens to perform organic synthesis has received a great deal of attention over the last 10 years. Several publications have shown that microwave irradiation can circumvent the need for prolonged heating,[60] and it is generally accepted that this source of energy minimizes side reactions and accelerates the rate of chemical reactions.[61]

[58] Ho, T. *Tandem Organic Reactions*; Wiley-Interscience; New York, **1992.**
[59] (a) Tietze L. F.; Beifuss, U. Sequential transformations in organic chemistry: a synthesis strategy with a future. *Angew. Chem.* **1993**, *105*, 137-170; (b) Tietze L. F. Domino Reactions in Organic Synthesis. *Chem. Rev.* **1996**, *96*, 115-136.
[60] Wipf, P.; Fletcher, J. M.; Scarone, L. Microwave promoted oxazole synthesis: cyclocondensation cascade of oximes and acyl chlorides. *Tetrahedron Lett.* **2005**, *46*, 5463–5466.
[61] Hayes, B. L. *Microwave Synthesis, Chemistry at the Speed of Light*; CEM Publishing: Matthews, North Carolina, **2002**; Chapter 1, p 16.

Results and discussion

Herein, we wish to report an efficient tandem procedure for the synthesis of 2-hydrazolyl-4-thiazolidinones under microwave conditions. Different solvents and various reaction equivalents were explored until we obtained good isolated yields of thiazolidinones (Scheme 21, Table 11). Microwave heating for the synthesis of thiazolidinone **33a** resulted in a significantly better yield compared to thermal conditions (75% vs 40%, entries 2 and 1, Table 11). Microwave irradiation also allowed a faster conversion.



Scheme 21. Tandem and stepwise reactions for the synthesis of 2-hydrazolyl-4-thiazolidinone.

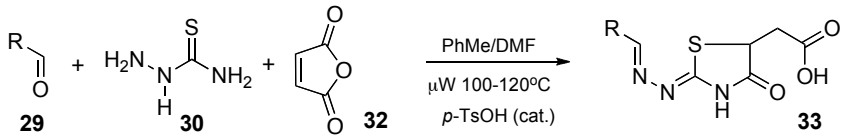
For tandem reactions, the best yields were obtained when a solvent mixture of PhMe/DMF (1:1) was used. Thiazolidinone **33a** was prepared in 68% yield (54% considering both reactions) using a stepwise sequence, and in 82% yield under tandem conditions (entries 3 and 4, Table 11). We found that a tandem sequence was more efficient than a stepwise conversion under microwave irradiation.

Table 11. Comparison of stepwise and tandem reactions using regular thermal and microwave heating.

Entry	**Reagents**	**Conditions**	**Solvent**	**Additive**	**Product, Yield** (%)[a]
1	**31a** (1 eq.), **32** (1.2 eq.)	Δ, reflux, 12 h.	PhMe	*p*-TsOH (0.1 eq.)	**33a** (40)
2	**31a** (1 eq.), **32** (1.2 eq.)	*μ*W, 120 °C, 6 min	PhMe	*p*-TsOH (0.1 eq.)	**33a** (72)
3	**31a** (1eq.), **32** (5 eq.)	*μ*W, 120 °C, 6 min	PhMe/DMF	*p*-TsOH (0.1 eq.)	**33a** (68)
4	**29a** (1eq.), **30** (1.2eq.), **32** (5eq.)	*μ*W, 120 °C, 6 min	PhMe/DMF	*p*-TsOH (0.1 eq.)	**33a** (82)

[a] Isolated yields after purification.

The optimal conditions for the microwave assisted tandem sequence were determined to be a mixture of PhMe/DMF (1:1) as a solvent, with catalytic *p*-TsOH ac. and an excess of maleic anhydride (5 eq.) at 100-120 °C (Scheme 22).[62]



Scheme 22. Optimized tandem reaction for 2-hydrazolyl-4-thiazolidinone synthesis.

Under optimized microwave conditions, a range of aromatic and some aliphatic aldehydes were converted to the desired heterocycles (Table 12). Aromatic aldehydes provided good yields from 45 to 82%, at 120°C after a 6-12 min reaction time, except for 2-thiophene-carboxaldehyde where the yield dropped to 33%, probably due to the formation of polymeric materials derived from the starting material (entries 1 to 8, Table 12). The reaction seems to be independent of electron withdrawing or donating substitutions in the aldehydes (entries 1 and 3, Table 12).

Table 12. Optimized tandem reaction for 2-hydrazolyl-4-thiazolidinone under μW irradiation conditions.

Entry	Compound RCHO	Temperature, time	Product (Yield) [a]	MP (°C)
1	**29a** *p*-N(Me)$_2$-Ph	120 ºC, 6 min	**33a** (82%)	278-279 dec.
2	**29b** *p*-OBut-Ph	120 ºC, 6 min	**33b** (63%)	249-250
3	**29c** *p*-NO$_2$-Ph	120 ºC, 12 min	**33c** (61%)	270-271 dec.
4	**29d** *o*-F-Ph	120 ºC, 12 min	**33d** (57%)	272-273
5	**29e** *p*-Cl-Ph	120 ºC, 12 min	**33e** (70%)	273-274
6	**29f** Ph	120 ºC, 6 min	**33f** (45%)	242-243
7	**29g** *p*-OMePh	120 ºC, 9 min	**33g** (61%)	262-263
8	**29h** 2-thiophenyl	120 ºC, 5 min	**33h** (33%)	255-256
9	**29i** CH(Me)$_2$	100 ºC, 12 min	**33i** (34%)	229-230
10	**29j** CH$_2$CH$_2$Ph	100 ºC, 6 min	**33j** (64%)	199-200

[a] Isolated yields after purification.

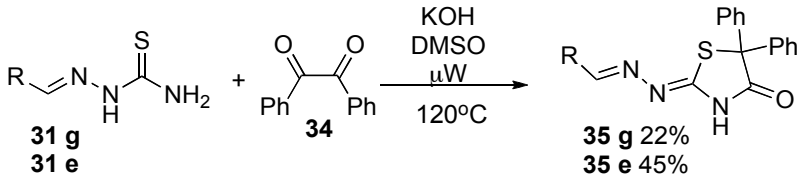
For aliphatic aldehydes, we found that the optimum temperature was 100 °C; otherwise polymerization products were obtained. Compounds **33i** and **33j** were thus isolated in

[62] See the typical procedure for thiazolidinone preparation in the experimental section (5).

34% and 64% yield, respectively (entries 9 and 10, Table 12). The reactivity of thiosemicarbazone **31g** with different Michael acceptors was also investigated. The reaction of thiosemicarbazone **31g** with methyl acrylate[63] or methyl cinnamate[64] did not produce the expected 6-membered 1,3-thiazin-4-one.

Furthermore, we explored the reaction of thiosemicarbazones **31e** and **31g** with benzil **34** as the electrophile. It is well known that ureas and thioureas react with benzil **34** to give 4-imidazolidinones through a benzilic acid rearrangement.[65] Braibante and co-workers reported recently the reaction of thiosemicarbazide (**30**) with benzil to give 1,2,4-triazin-3-thione. The formation of the 6-membered ring can be explained by the nucleophilic attack of both N_1 and N_4 in compound **30** to the benzil carbonyl groups.[66]

Our results indicate that the reaction of thiosemicarbazone **31** with benzil **34** in KOH/DMSO under microwave conditions led to 2-hydrazolyl-5,5-diphenyl-4-thiazolidinones **35e**, and **35g** in 45% and 22% yield, respectively (Scheme 23).[67] This heterocycle was previously unknown and was fully characterized. The HMBC experiment revealed a cross peak between the N-H proton and the carbonyl carbon at C_4, thus confirming the regiochemistry of the reaction.



Scheme 23. Synthesis of 2-hydrazolyl-5,5-diphenyl-4-thiazolidinone.

With the goal of rationalize how this reaction proceeds, we undertook a frontier orbital analysis using the semi-empirical parametrization PM3.[68] Table 13 shows HOMO/LUMO energies, coefficients and charge distributions calculated for model

---

[63] Under the following reaction conditions: methyl acrylate acid, **31h**, EtOH, μW, K_2CO_3, 120 ºC, 15 min or EtOH, KOH, μW 120 ºC, 15 min, no product was observed.
[64] Under the following reaction conditions: methyl cinnamate, **31h**, PhMe, μW 120 ºC, *p*-TsOH ac., 19 min, no product was observed.
[65] Muccioli, G. G.; Poupaert, J. H.; Wouters, J.; Norberg, B.; Poppitz, W.; Scriba, G.; Lambert D. M. A rapid and efficient microwave-assisted synthesis of hydantoins and thiohydantoins. *Tetrahedron* **2003**, *59*, 1301-1307.
[66] Braibante, M; Braibante, H.; Uliana, M. P.; Costa C. C.; Spenazzatto, M. The Use of Benzil to Obtain Functionalized *N*-Heterocycles. *J. Braz. Chem. Soc*. **2008**, *19*, 909-913.
[67] See the typical procedure for preparation of **35g** in the experimental section (5).
[68] Available in the commercial computer program package HyperChem Pro Molecular.

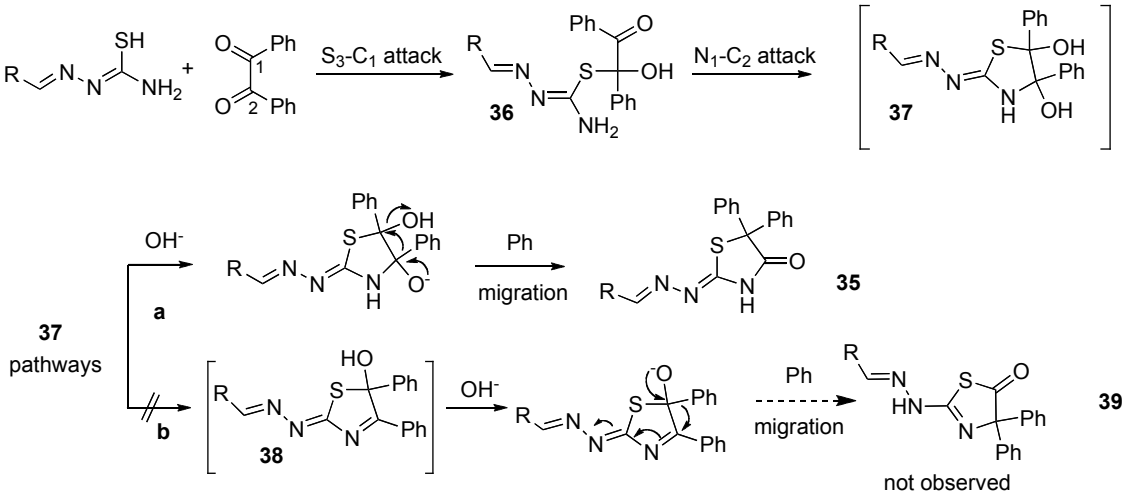
compounds. The HOMO/LUMO energy gap is small and it would seem that the reaction with thiosemicarbazone and benzil is kinetically favored.

Table 13. Electronic parameters for selected model compounds and intermediates.

Entry	Compound	Energy (eV)		Coefficients (%)[a]		Charge
		HOMO	LUMO	HOMO	LUMO	
1	**31 g**	-8.77	-1.05	S 44.3 N 13.6	- -	S -0.32 N 0.09
2	**34**	-10.01	-0.61		C_1 25.5 C_2 25.5	C_1 0.29 C_2 0.29
3	**37**	-8.69	-0.38		C_2 17.0 C_1 0.2	C_2 0.09 C_1 0.12

[a]Coefficients were calculated as ($\sum c_i^2$)x 100.

The process should be governed by frontier orbital control and not by charge control. The proposed mechanism for the formation of 2-hydrazolyl-5,5-diphenyl-4-thiazolidinone **35** is depicted in Scheme 24. Based on HOMO/LUMO energies and orbital coefficients, the first step should be the nucleophilic attack of S to C_1, one of the two carbonyl groups present in benzil, to form the tetrahedral intermediate **36**. This intermediate proceeds by nucleophilic attack of N_1 to C_2 to give intermediate **37**, in similar fashion to imidazoline formation.[69]



Scheme 24. Proposed mechanism for 2-hydrazolyl-5,5-diphenyl-4-thiazolidinone (**35**) formation.

[69] Butler, A. R.; Leitch, E. Mechanistic studies in the chemistry of urea. Part 2. Reaction with benzil, 4,4-dimethylbenzil, and 4,4-dimethoxybenzil. *J. Chem. Soc. Perkin Trans.* 2 **1977**, *14*, 1972–1976.

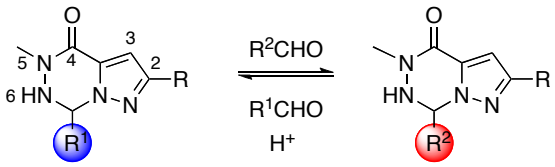
According to our results, the diol **37** undergoes a phenyl group migration, from C_1 to C_2, where the largest LUMO coefficient is located (entry 3, Table 13), which is in agreement with the observed regiochemistry, pathway a. Even though intermediate **38** was proposed by Butler and co-workers as the most favourable for phenyl group migration in the synthesis of imidazolines, in our case intermediate **38** would led to 5-thiazolidinone **39**, which was never isolated.

Partial Conclusions

In summary, in this investigation we explored the microwave-mediated tandem reactions of aldehydes, thiosemicarbazones and maleic anhydrides to produce 2-hydrazolyl-4-thiazolidinones, with yields ranging from 33 to 82%. The advantages in the use of this methodology are shorter time reactions, higher yields, and a minimization of synthetic operations, solvent use, and waste generation. When we investigated the scope of the tandem synthesis for hydrazolyl-4-thiazolidinones **33**, we were able to demonstrate that the process is general for aromatic and aliphatic aldehydes; however, the use of different types of Michael acceptors has not yet been accomplished. As an important part of this work, we also present the synthesis of 2-hydrazoyl-5,5-diphenyl-4-thiazolidinone **35**, a new class of 4-thiazolidinones. We propose a mechanism for the heterocycle formation based on a benzilic acid rearrangement promoted by thiosemicarbazones.

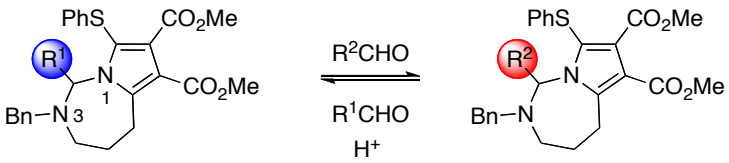
3.1.5 Synthesis of new scaffolds: Diazepines

As a second scaffold, we envisioned the synthesis of new diamine systems that could exchange carbonyl subunits, following the reversible cyclocondensation reaction between aldehydes and pyrazolotriazinones described by Wipf and co-workers,[7] see scheme 24.



Scheme 24. Reversible metathesis reactions of pyrazolotriazinones and aliphatic aldehydes or ketones.

In this context we synthesized the pyrrolo[1,3]diazepines shown in scheme 25 and explored several exchange conditions at the N-acetalic position.



Scheme 25. Diamine scaffold as a possible building block in the exchange of carbonyl units.

The acetalic metathesis was studied at the diamine moiety in presence of aldehydes, in acidic media. No exchange was observed under mild conditions. This is in accordance with the hypothesis proposed by Wipf, where substitution at *N* (6) of the pyrazolotriazinone scaffold prevent reversible ring opening by stabilizing the ring system. Analogous to this, our molecule presents the corresponding *N* (3) substituted with a bencil group. This can stabilize the aminal bond and avoid the reversible exchange.

These compounds are of synthetic interest, formed via a retro-Mannich reaction. Further derivations were accomplished to finally afford the azamacrocyle **54** as a new scaffold, see *paper V*.

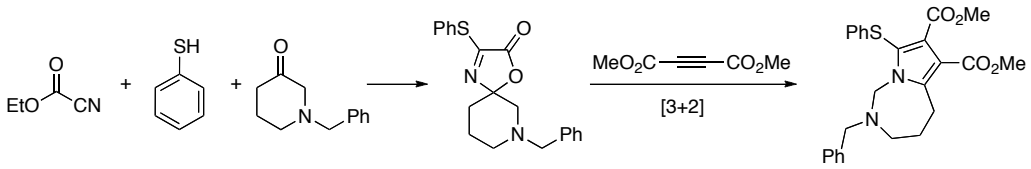
Paper V (Liang, M.; Saiz, C.; Pizzo, C.; Wipf, P. *Tetrahedron Lett.* **2009**, *50*, 6810-6813)

Novel synthesis of pyrrolo[1,3]diazepines by a dipolar cycloaddition – retro-Mannich domino reaction

Mary Liang, Cecilia Saiz, Chiara Pizzo, Peter Wipf.

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA.

Abstract



Microwave irradiation facilitated the synthesis of 4-arylthio-3-oxazolin-5-ones from ethyl cyanoformate, thiophenol, and cyclic ketones. Subsequent decarboxylation and *in situ* [3+2] cycloaddition provided novel 2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*c*][1,3]diazepine scaffolds after a spontaneous *retro*-Mannich domino reaction.

Introduction

The utility of 1,4-benzodiazepines in drug discovery has been very significant due to the extensive therapeutic value of this class of azaheterocyclic compounds.[70] Benzodiazepine derivatives have been found to possess anticonvulsant to anxiolytic activities, and strong anti-tumor properties. Among other pharmacological functions, 1,3-diazepine derivatives have been of interest due to their inhibitory effects on HIV-1 protease, adenosine deaminase and guanase, as well as NK1 receptor binding properties (Fig. 14).[71,72] However, there is still a considerable need for new synthetic methods to access arene-fused 1,3-diazepine scaffolds, in particular novel

[70] See, for example: Costantino, L.; Barlocco, D. Privileged structures as leads in medicinal chemistry. *Curr. Med. Chem.* **2006**, *13*, 65-85.

[71] Dieltiens, N.; Claeys, D. D.; Allaert, B.; Verpoort, F.; Stevens, C. V. Synthesis of 1,3-Dioxo-hexahydropyrido[1,2-c][1,3]diazepine carboxylates, a new bicyclic skeleton formed by ring-expansion RCM methodology. *Chem. Commun.* **2005**, 4477-4478.

[72] (a) Wang, L.; Hosmane, R. S. A Unique Ring-Expanded Acyclic Nucleoside Analogue That Inhibits Both Adenosine Deaminase (ADA) and Guanine Deaminase (GDA; Guanase): Synthesis and Enzyme Inhibition Studies of 4,6-Diamino-8-*H*-1-hydroxyethoxymethyl-8-iminoimidazo[4,5-*e*][1,3]diazepine. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2893-2896. (b) Boks, G. J.; Tollenaere, J. P.; Kroon, *J. Bioorg. Med. Chem.* **1997**, *5*, 535-547.

pyrrolo[1,3]diazepines.[73] We have discovered an elegant retro-Mannich domino reaction which readily converts 1,3-oxazolin-5-ones into pyrrolo[1,3]diazepines through a mechanistically intriguing cycloreversion, 1,3-dipolar cycloaddition, retro-Mannich reaction, and iminium ion addition sequence.

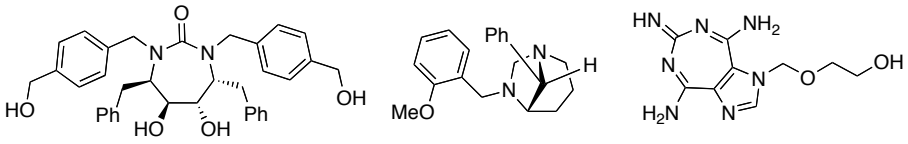


Figure 14. Pharmaceutically relevant examples of 1,3-diazepine derivatives.

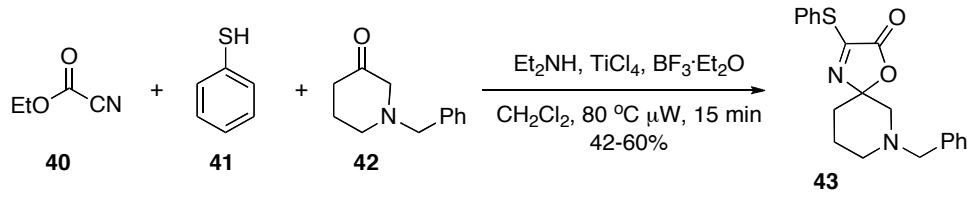
In this Letter, we describe a microwave accelerated synthesis of 4-arylthio-3-oxazolin-5-ones as well their in situ conversion to pyrrolo[1,3]diazepines and the further functionalization of these heterocyclic scaffolds.

Results and discussion

In an earlier approach, one of us had synthesized 4-arylthio-3-oxazolin-5-ones in a 'one-pot reaction' from ethyl cyanoformate, thiophenol, and carbonyl compounds in the presence of diethylamine and titanium tetrachloride.[74] In an analogous sequence, but using microwave heating in a monomode reactor, we synthesized the piperidyl-3-oxazolin-5-one **43** from 1-benzylpiperidin-3-one **42** (Scheme 26). Significantly, the oxazolinone formation was thus expedited from a 12–36 h reaction time[75] to 15 min at 80 °C under microwave irradiation, and the yield was increased to 60%.

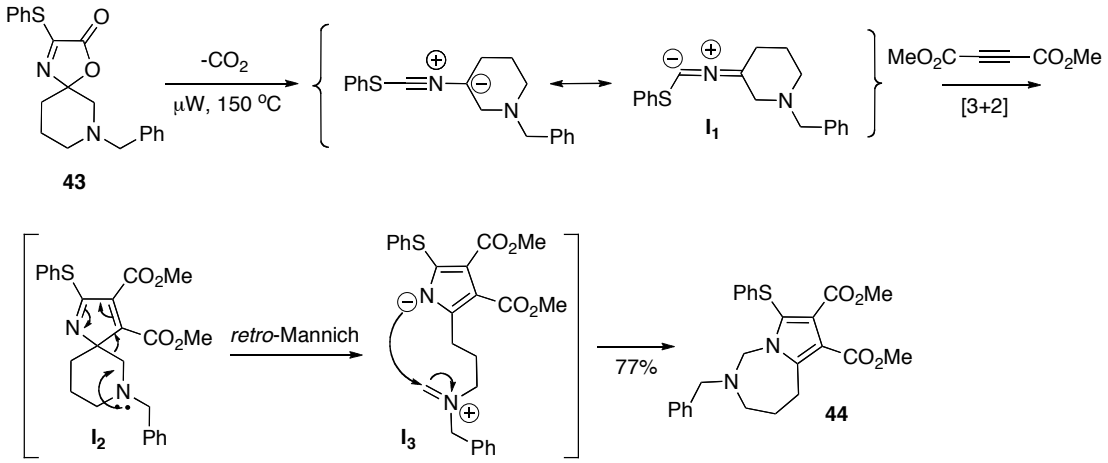
[73] The synthesis and biology of pyrrolo[1,3]diazepines remain essentially unexplored, in spite of some previous preparative work: (a) Daich, A.; Ohier, P.; Decroix, B. Synthesis of a New Pyrrolo(1,3)diazepine: 4H-Pyrrolo(1,2-a)thieno(2,3- e) (1,3)diazepine. *J. Heterocyclic Chem.* **1995**, *32*, 1731-1734; (b) El-Kashef, H.; Rathelot, P.; Vanelle, P.; Rault, S. On the Synthesis of Pyrrolobenzo[*b*]thieno[1,4]diazepines. *Monatshefte Chem.* **2007**, *138*, 469-476; (c) Mahaffey, R. L.; Atwood, J. L.; Humphrey, M. B.; Paudler, W. W. N-(p-Bromophenyl) [2.2] (2,5)pyrrolophane. Synthesis and self-condensation. *J. Org. Chem.* **1976**, *41*, 2963-2965.

[74] (a) Wipf, P.; Heimgartner, H. Synthese von 4-arylthio-3-oxazolin-5-onen. *Tetrahedron Lett.* **1984**, *25*, 5127-5128. (b) Wipf, P.; Prewo, R.; Bieri, J. H.; Heimgartner, H.; Nastopoulos, V.; Germain, G. Synthese von 4-Benzylthio- und 4-(Arylthio)-1,3-oxazol-5(2H)-onen. *Helv. Chim. Acta* **1987**, *70*, 1380-1388.



Scheme 26. Microwave-mediated synthesis of piperidyl 4-arylthio-3-oxazolin-5-one 4 in the presence of diethylamine and Lewis acid catalysts.

Heating 0.5 M solution of **43** in chlorobenzene at 150 °C for 10 min in the microwave reactor released carbon dioxide to generate the intermediate nitrile ylide $\mathbf{I_1}$,[75] which was spontaneously trapped by the powerful dipolarophile dimethyl acetylenedicarboxylate (DMAD) in a 1,3-dipolar cycloaddition reaction (Scheme 27).[76]



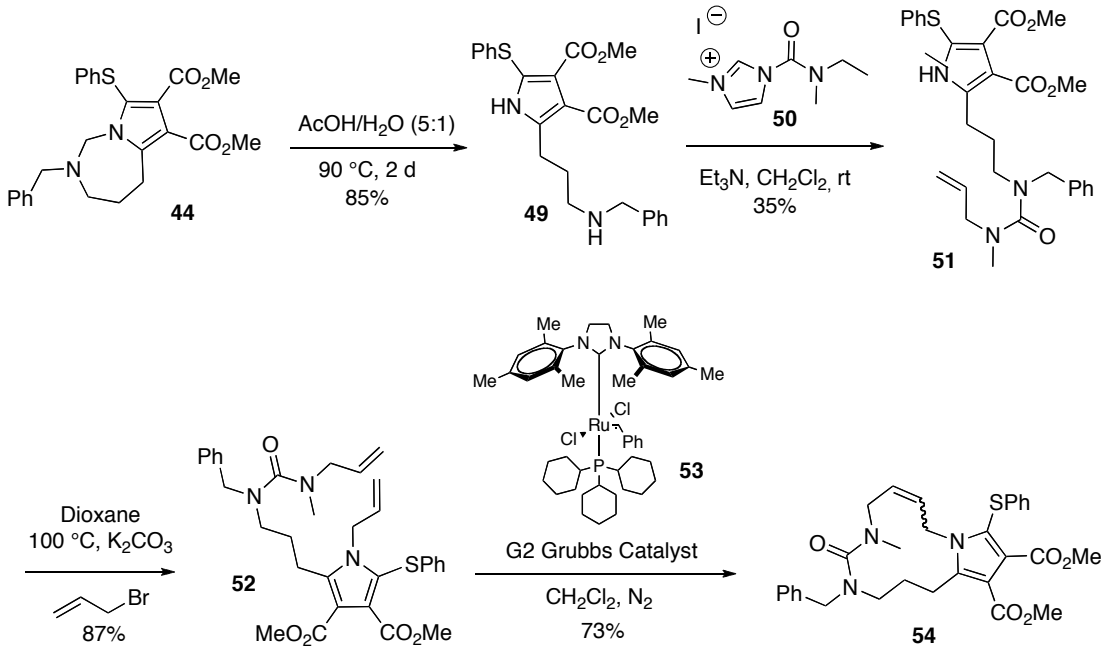
Scheme 27. Suggested mechanism for the domino process –1,3-dipolar cycloaddition of the nitrile ylide derived from **43** to DMAD, retro-Michael reaction and imine addition– generating the pyrrolo[1,3]-diazepine **44**.

However, rather than the expected spirocycle $\mathbf{I_2}$, the pyrrolo[1,3]diazepine **44** was isolated in good yield as the end product of a spontaneous retro-Mannich-aminal formation pathway which likely includes the zwitterionic $\mathbf{I_3}$ as yet another step along a fascinating and unprecedented cascade process. We were also able to showcase the

[75] (a) Wipf, P.; Heimgartner, H. Arylthio-Substituted Nitrile Ylids by Thermolysis of 4-Arylthio-3-oxazoline-5-ones. *Chimia* **1984**, *38*, 357-359. (b) Wipf, P.; Prewo, R.; Bieri, J. H.; Germain, G.; Heimgartner, H. Thermal Generation and Reactions of (Benzylthio)- and (Arylthio)-Substituted Nitrile Ylides. *Helv. Chim. Acta* **1988**, *71*, 1177-1190.

[76] Sharp, J. T., Nitrile ylides and nitrile imines. In "*Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*"; Padwa, A.; Pearson, W. H., Eds. **2002**, 473-538.

possibility for further ring conversions of pyrrolodiazepine **44** and generate an azamacrocyclic scaffold (Scheme 28).[77]



Scheme 28. Conversion of pyrrolodiazepine **44** to the free amine **49** serves as an entry point to further ring transformations to access the fused macrocycle **54.**

While the aminal function in the pyrrolo[1,3]diazepine **44** is quite resistant to hydrolysis, cleavage can be accomplished in a 5:1 mixture of AcOH and H_2O for 2d at 90 °C. The benzylic amine **49** was isolated in 85% yield. Subsequent selective N-amidation was affected by acyl transfer with in situ prepared imidazolium salt 50.[78] The resulting urea **51** was doubly N-allylated in the presence of a five-fold excess of allyl bromide and K_2CO_3 in dioxane at reflux to give diene **52**.[79] Ring closing metathesis with ruthenium catalyst **53**[80] under a nitrogen atmosphere gave the 12-membered macrocycle **54** in an acceptable combined yield of 73% as a 3:1 mixture of (Z/E)-isomers.

[77] For the use of azamacrocycles in diversity-oriented synthesis, see, for example: (a) Wipf, P.; Stephenson, C. R. J.; Walczak, M. A. A. Diversity-Oriented Synthesis of Azaspirocycles. *Org. Lett.* **2004**, *6*, 3009-3012. (b) Wessjohann, L. A.; Ruijter, E. Strategies for Total and Diversity-Oriented Synthesis of Natural Product(-Like) Macrocycles. *Top. Curr. Chem.* **2005**, *243*, 137-184.

[78] Grzyb, J. A.; Shen, M.; Yoshina-Ishii, C.; Chi, W.; Brown, S.; Batey, R. A. Carbamoylimidazolium and thiocarbamoylimidazolium salts: novel reagents for the synthesis of ureas, thioureas, carbamates, thiocarbamates and amides. *Tetrahedron* **2005**, 7153-7175.

[79] Albano, V.; Gualandi, A.; Monari, M.; Savoia, D. Asymmetric Synthesis of 8-Aminoindolizidine from Chiral 2-Pyrroleimines. *J. Org. Chem.* **2008**, 73, 8376-8381.

[80] Lee, C. W.; Grubbs, R. H. Formation of Macrocycles *via* Ring-Closing Olefin Metathesis. *J. Org. Chem.* **2001**, *66*, 7155-7158.

The formation of a mixture of cis/trans-isomers in the RCM of a macrocycle is not unexpected.[82,81] We performed conformational minimizations of the structures of *cis*- and *trans*-**54** using Spartan 08 with the MMFF parametrization and found them to have essentially identical steric energies. Cis-**54** displayed a half-boat-conformation of the 12-membered ring whereas trans-**54** showed a crown-like, more narrow conformation (Figure 15). In both cases, the macrocycle minimized the nonbonding interactions of the urea substituents.

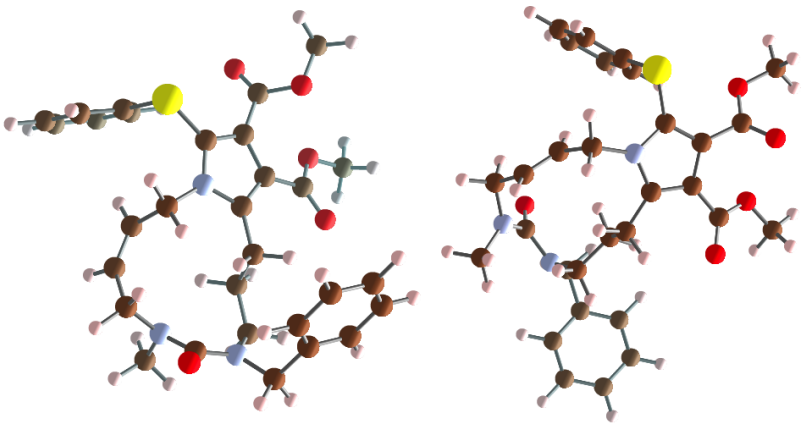


Figure 15. Ball-and-stick models of force-field minimized conformational minima for cis-**54** (left) and trans-**54** (right).

[81] (a) Hansen, E. C.; Lee, D. Ring Closing Enyne Metathesis: Control over Mode Selectivity and Stereoselectivity. *J. Am. Chem. Soc.* **2004**, *126*, 15074-15080. (b) Kaul, R.; Surprenant, S.; Lubell, W. D. Systematic Study of the Synthesis of Macrocyclic Dipeptide β-Turn Mimics Possessing 8-, 9-, and 10-Membered Rings by Ring-Closing Metathesis. *J. Org. Chem.* **2005**, *70*, 3838-3844.

Partial Conclusions

In conclusion, we were able to apply new microwave heating conditions to the synthesis of 4-arylthio-3-oxazolin-5-one **43**. Thermolysis of this spirocycle revealed a mechanistically unique domino reaction, whereby expulsion of CO_2 led to a nitrile ylide that underwent in situ trapping with a 1,3-dipolarophile followed by a retro-Mannich reaction and iminium ion cyclization, terminating in the preparation of a pyrrolo[1,3]diazepine. Hydrolysis of the aminal function, and a sequence of N-carbamoylation, N-allylation, and RCM, provided access to 12-membered pyrrole-fused urea **54**. Pyrrole-containing natural products are known for attractive biological properties,[82] and this work provides a new access to a diverse set of fused pyrrole derivatives.

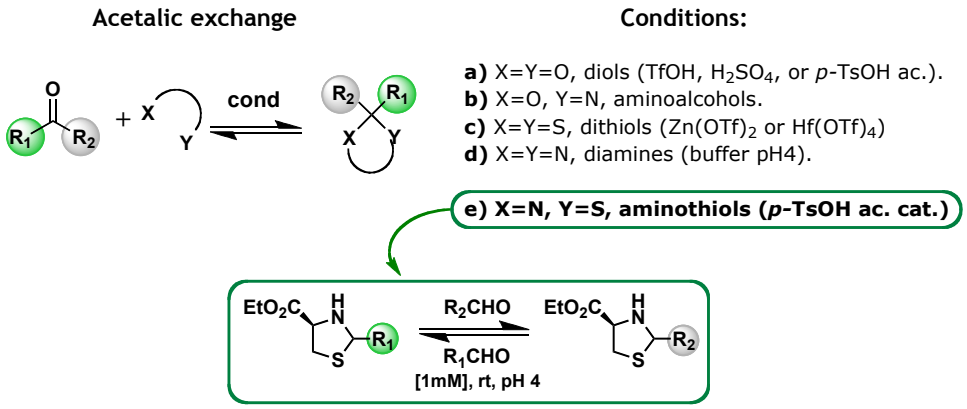
[82] Jin, Z. Muscarine, imidazole, oxazole and thiazole alkaloids. *Nat. Prod. Rep.* **2009**, 26, 382-445.

3.1.6 General discussion of New Reactions and Building Blocks for DCC

a) New reactions useful for DCC

The study and discovery of new reversible reactions suitable for DCC has become a challenge in the last decades. There are a small number of reversible reactions compared to the irreversible processes favored in traditional synthetic chemistry. Most of the reversible exchanges involve carbonyl compounds, imines and acetals.

In this context, a new acetalic reversible reaction useful for DCC methodologies is described. This reversible reaction belongs to the acetalic exchange group. The study was performed using thiazolidines as exchange models, see picture 1.



Picture 1. A new reversible acetalic exchange: aminothiol-carbonyl.

The aminothiol-carbonyl exchange can be used to generate dynamic libraries in aqueous acidic media after 48 h. The template used to amplify the stabilized compounds of the library must tolerate the acidic media needed to have a rapid dynamic exchange (pH 4).

Most of the biological systems are stable under near physiological conditions. The conditions found for aminothiols exchange are limited for the acidic environment not compatible with many biological targets. In order to develop a DCL using this reversible reaction, we could use serine proteases (as template) resistant to the acidic environment. Another application could be the generation of reversible systems using organic or inorganic ions as templates, looking for new stabilized structures, materials and catalysts.

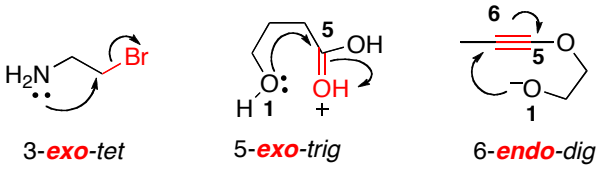
b) Synthesis of potential building blocks useful for DCC

We have explored the preparation of new building blocks potentially useful to generate dynamic libraries.

We studied the cyclization possibilities of iminium ions derived from β-amino thiols, alcohols and amines. The ionic intermediate gave arise to a large number of synthetic useful scaffolds, see picture 3. The prepared products include: bisthiazolidines-**22**, thiazolidinyl-oxazolidines-**6** and **8**, spiro-thiazolidines-**9** and **16**, bisoxazolidines-**7** and bis-thiiranes-**23**.

The relationships between ring size and facility of ring closure have been established after many examples of intramolecular reactions. The analysis between enthalpy and entropy of the energy of activation of typical ring-closing reactions shows a preference for 5 and 6-membered rings (higher ΔH for small rings and more negative ΔS for large rings). There are also geometric constraints on the transition state determining the ring closure.

Baldwin and co-workers[83] established a correlation between the stereoelectronic requirements of the transition state and the ring-closure feasibility, based on empirical results. There is a major classification respective to three factors: a) ring size (3, 4, 5, 6 or 7 members), b) hybridization of the carbon at the reaction site (*tet* = sp^3, *trig* = sp^2, *dig* = sp) and c) the relationship at the reacting bond to the forming ring (depending whether the bond broken during the ring closure is inside (*endo*) or outside (*exo*) the ring which is formed).



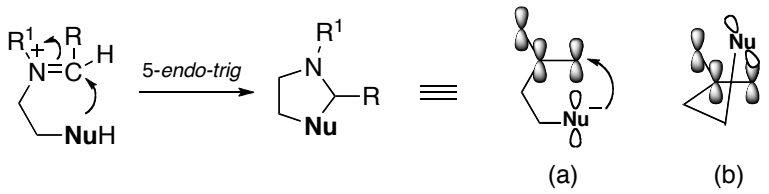
Picture 2. Examples of cyclization types favored by Baldwin's rules.

The following table shows the rules for ring-closure depending on the three variables described above.

[83] Baldwin, J. E. Rules for Ring Closure. *J. C. S. Chem. Commun.* **1976**, 734-736.

Ring size	Exocyclic bonds (exo)			Endocyclic bonds (endo)	
	sp (dig)	sp^2 (trig)	sp^3 (tet)	sp (dig)	sp^2 (trig)
3	unfav	fav	fav	fav	unfav
4	unfav	fav	fav	fav	unfav
5	fav	fav	fav	fav	unfav
6	fav	fav	fav	fav	fav
7	fav	fav	fav	fav	fav

As we can observe, there are unfavorable ring closure processes (unfav) and favorable ones (fav). For example, the formation of a five-membered ring through a 5-*endo-trig* cyclization is proposed as an unfavorable cyclization. The nucleophile cannot approach in the nodal plane of the π–system, see Picture 2 (a). Due to this, the nucleophile must attack from above or below, part (b).[84]



Picture 2. Stereoelectronic requirements for a 5-*endo-trig* cyclization.

In general, the 5-*endo-trig* ring-closure is unfavorable due to the stereoelectronic requirements. A large distortion of the molecule is required and the cyclization introduces strain.

It is important to notice that Baldwin's rules for favored and disfavored ring closures show the stereoelectronic requirements that may be imposed in the transition state. However, other factors will contribute to the ease of the cyclization process. There are some examples in literature of compounds that disobey the rules, like thiazolidines and sometimes oxazolidines.[29]

[84] Carey, F. A.; Sundberg, R. J. Advanced Organic Chemistry. Part A: Structure and Mechanism. 3rd Ed, Plenum Press, New York.

Sulfur, Oxygen and Nitrogen

Sulfur is a soft nucleophile, less electronegative and much more polarizable than oxygen. S is a *p*-block element and belongs to the VI group, immediately below oxygen. It has *d* orbitals and can expand its octet, forming hypervalent species. Thiolates can be classified as soft bases, with a higher HOMO (highest occupied molecular orbital) than strong bases as alkoxides. This makes the thiol a better nucleophile than the alcohol and directs the preferential attack of the thiol.[85]

The reactivity differences of iminium ion derived from aminothiols ($\mathbf{I_a}$) and aminoalcohols ($\mathbf{I_b}$), were interpreted based on frontier molecular orbital analysis. Calculations of HOMO coefficients of different iminium ions always showed a higher value for thiols compared to alcohols.

When comparing all the compounds prepared from iminium ions derived from alcohols, thiols or amines (see Picture 3), we observe different ring-closure patterns leading to 3, 5, 6 and 7 membered rings.

a) When **X = S**, we observed the exclusive formation of thiazolidines, corresponding to a 5-*endo*-trig cyclization. If the molecule has two thiols in β-position, a double 5-*endo-trig* imine cyclization occurs, leading to enantioenriched fused bis-thiazolidines. Even if this cyclization is thermodynamically disfavored according to Baldwing rules, frequently sulfur atoms can disobey it to give thiazolidines. As cited before, S has 3*d* orbital that can contribute to the initial interactions to form the C-C bond π orbital. In this case, the 5-*endo-trig* (unfav) is preferred to the 3-*exo-trig* (fav), leading to the thiazolidines **6** and **22**, see path (a) and (b), Picture 3.

In path (c), the 5-*endo-trig* cyclization promoted by a thiol was always preferred to the 6-*endo-trig* ring-closure, even if we are in presence of different nucleophilic groups at the β-position. In two examples, the formation of spiro-thiazolidines **9** and **16** was observed through a 6-*endo-trig* cyclization occurring via oxonium ion, see path (d).

b) When **X = O**, the situation is variable, depending on the heteroatoms present in the intermediates. If we analyze path (e), the iminium ion bearing two alcohols at the

β-position gave the fused bisoxazolidine **7** as the main product, cyclization occurring via a 5-*endo-trig* process. This ring-closure is unfavored according to Baldwin rules and undergoes through a double cascade cyclization with the attack the alcohols to the corresponding iminium ions.

The spiro-oxazolidines **Z**, expected as a possible product of the 6-*endo-trig* cyclization via an oxonium ion, were not observed, contrary to the sulfur analogs found in path (d).

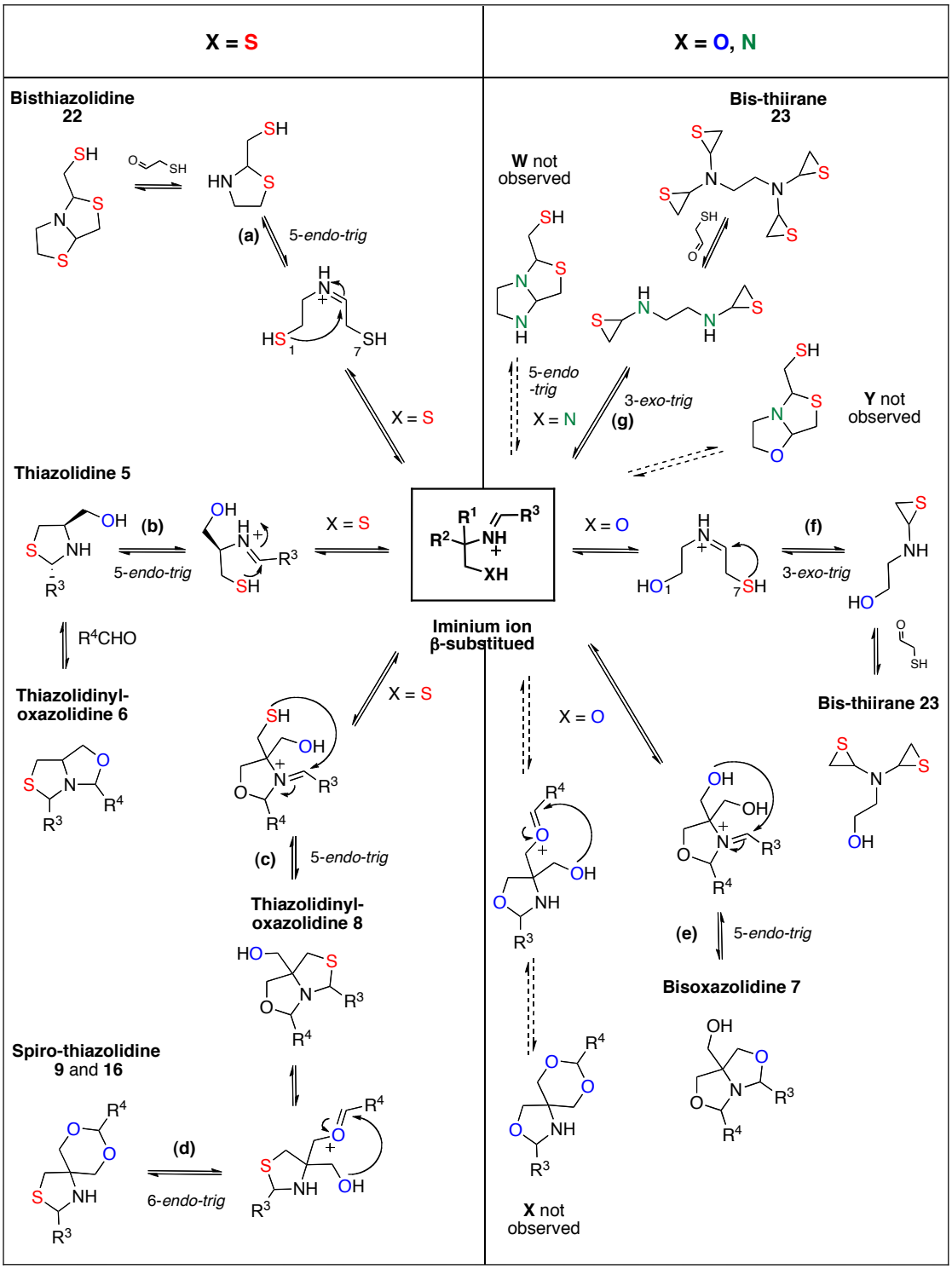
We expected the formation of a fused oxazolidinyl-thiazolidine **Y**, when working with iminium ion bearing both a thiol and an alcohol at the β-amino position. The cyclization would occur through a 5-*endo-trig* mechanism via a nucleophilic attack of the oxygen to the iminium ion. The fused heterocycle **Y** was not observed and the unpredicted bis-thiiranes **23** were formed as the result of a 3-*exo-trig* ring-closure promoted by the sulfur nucleophilic attack to the C=N moiety, see path (f). The alcohol group does not participate in the reaction.

The results observed when X = S and when X = O are a consequence of the different nucleophiles involved. Thiols have a HOMO of higher energy than alcohols. This explains the selected participation of thiols as nucleophiles instead of alcohols.

c) When **X = N**, the possible fused imidazoline-thiazolidine **W** through a 5-*endo-trig* cyclization was not observed. This result was expected due to the lack of examples showing N disobeying Baldwin's rules. We observed the exclusive formation of bis-thiiranes **23** through a double 3-*exo-trig* cyclization, see path (g).

The different iminium ions presented can evolve to a diverse arrange of tautomers that can interconvert each other via ring-chain-ring equilibration, depending on the selected media. This property can be further developed for application in DCC.

[85] Clayden, Greeves, Warren, Wothers. *Organic Chemistry*. Giunti Industrie Grafiche: Florence; pp 1247-1250, **2001**.



Picture 3. Iminium double cyclizations derived from β-amino thiols, alcohols or amines.

3.2 DCC application: Targeting *Echinococcus granulosus* Thiorredoxin Glutation Reductase.

Paper VI (in preparation)

Discovering *Echinococcus granulosus* Thioredoxin Glutathione Reductase inhibitors using Dynamic Combinatorial Chemistry

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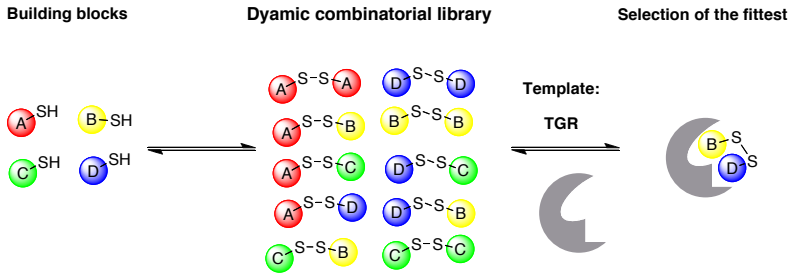
a. Cat. de Química Farmacéutica, Facultad de Química, Universidad de la República, Montevideo, Uruguay.
b. Lab. de Inmunología, Facultad de Química, Universidad de la República, Montevideo, Uruguay.
c. Área Análisis Químicos, Polo Tecnológico, Facultad de Química, UdelaR, Pando, Canelones, Uruguay.

Abstract



Dynamic combinatorial chemistry (DCC) is a potentially useful method for the identification of new biological ligands. Herein we report the first use of TGR as a template for a DCL of thiol-disulfide aqueous exchange. A bisthiazolidine-TNB disulfide 57di was identified by HPLC-MS, and subsequent analysis of the enzymatic activity shows an IC_{50} of 13 μM. Further exploration of the structural requirements of the binding site was made by the synthesis of new analogs.

Introduction

Parasites of the phylum *Platyhelminthes* (flatworms) causing mammalian infections are a major health problem in many developing countries. There are few drugs available for treatment, Praziquantel being the first choice. The emergent drug resistance is a health threat that requires the development of new antiparasitic drugs.

Parasite flatworms have a unique redox pathway based on thiol-disulfide reversible exchanges. In most organisms (including their mammalian hosts) two major enzymatic systems are responsible for providing reducing equivalents through thiol-disulfide exchange: the thioredoxin (Trx) and the glutathione (GSH) pathways. In contrast, platyhelminth parasites lack typical Trx and GSH systems. Conventional thioredoxin reductase (TR) and glutathione reductase (GR) enzymes are absent in flatworms and the thiol-redox homeostasis is carried out by the selenoenzyme thioredoxin glutathione reductase (TGR). By providing electrons to downstream targets, this homodimeric enzyme is responsible for redox-based antioxidant and detoxification pathways, and desoxyribonucleotide synthesis. This unique and dependent redox system lacking of alternative routes makes TGR a key drug target for flatworm infections.[86] Recent studies support this idea: inhibition of TGR expression by RNA interference caused death of the platyhelminth parasite *Schistosoma mansoni*,[87] and Auranofin, a potent inhibitor of TGR caused a partial cure in experimental Schistosoma infection.

Exploring for new anthelmintic drugs, we focused on *Echinococcus granulosus* TGR enzyme as target. *E. granulosus* is a flatworm parasite responsible for cystic echinococcosis or cystic hydatid disease. Many tools are available for the identification of small molecules that binds selectively to proteins, including combinatorial chemistry and HTS; this latter has been successfully used to identify molecules that inhibit *S. mansoni* TGR and resulted in the identification of oxadiazoles as drug hits for flatworm infections.

In the last decade, a new methodology based on molecular recognition has emerged combining thermodynamic control with combinatorial libraries: Dynamic Combinatorial Chemistry (DCC).[1] It merges the chemical synthesis with the biological assay in one single pot. The target protein functions as a template: it binds and stabilizes the best compound (hit) from a discrete library. Through library redistribution *via* reversible bonds this stabilization can result on an amplification of the best binder.

[86] Agorio, A.; Chalar, C.; Cardozo, S.; Salinas, G. Alternative mRNAs arising from trans-splicing code for mitochondrial and cytosolic variants of *Echinococcus granulosus* thioredoxin-glutathione reductase. *J. Biol. Chem.* **2003**, *278*, 12920-12928.

[87] Kuntz, A. N.; Davioud-Charvet, E.; Sayed, A.; Califf, L.; Dessolin, J.; Arnér, E. S.; Williams D. L. Thioredoxin-glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *PLoS Med.* **2007**, 4(6), e206.

The use of proteins as templates in dynamic libraries has been reported for ligand identification. Recent advances include the discovery of Glutathione-S transferase inhibitors,[88] the study of adenosine-binding to the *M. tuberculosis* pantothenate synthetase,[89] the discovery of an Aurora kinase inhibitor through site-specific DCC,[90] and the identification of a selective small-molecule ligand for a vital RNA regulator of the HIV-1 life cycle.[91] In this context the use of TGR as a template for a thiol-disulfide DCL seems to be a good election due to the compatibility of the enzyme with the experimental conditions (buffer pH = 8.8 and room temperature).

In this work we describe the generation of a thiol-disulfide DCL,[92] using TGR as a template finding a new class of disulfide inhibitors.

Results and discussion

DCL design

We selected 2-nitro-5-thiobenzoic acid (TNB) and GSH as anchor building blocks to construct the library. It is well established that Ellman's reagent [5,5′-dithiobis (2-nitrobenzoic acid) (DTNB)] **55** is a synthetic substrate of TGR. It is reduced to TNB in presence of NADPH, see figure 16.[93] The DTNB assay is used to determine the TrxR activity of the TGR enzyme. Another building block was oxidized glutathione GSSG, a substrate for the GR site of the enzyme. Our goal was to use these substrates as binding recognition structures and to explore further structural motifs in order to find new inhibitors.

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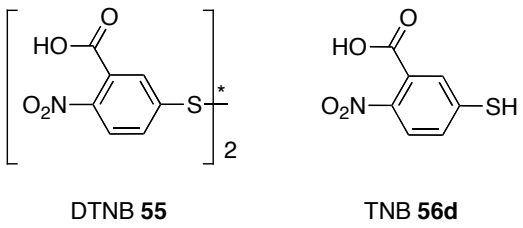
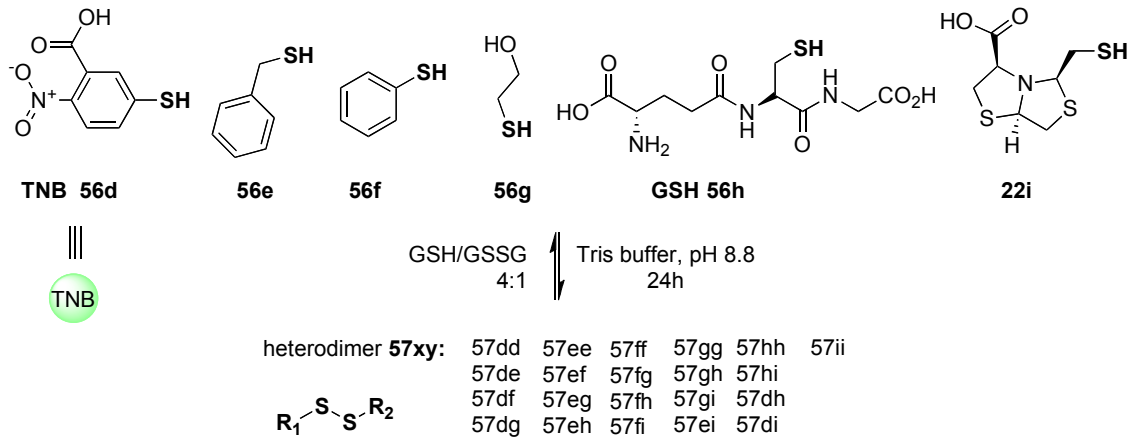
[88] Shi, B.; Stevenson, R.; Campopiano, D.; Greaney, M.F. Discovery of Glutathione S-Transferase Inhibitors Using Dynamic Combinatorial Chemistry. *J. Am. Chem. Soc.* **2006**, *128*, 8459–8467.
[89] Scout, D.; Dawes, G.; Ando, M.; Abell, C.; Ciulli, A. A fragment-based approach to probing adenosine recognition sites by using dynamic combinatorial chemistry. *ChemBioChem* **2009**, *10*, 2772-2779.
[90] Cancilla, M.T.; He, M.M.; Viswanathan, N.; Simmons, R.L.; Erlanson, D.A. Discovery of an Aurora kinase inhibitor through site-specific dynamic combinatorial chemistry. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3978-3981.
[91] McNaughton, B.; Gareiss, P.; Miller, B.L. Identification of a Selective Small-Molecule Ligand for HIV-1 Frameshift-Inducing Stem-Loop RNA from an 11,325 Member Resin Bound Dynamic Combinatorial Library. *J. Am. Chem. Soc.* **2007**, *129*, 11307-11308.
[92] Otto, S.; Furlán, R.; Sanders, J. K.M. Dynamic Combinatorial Libraries of Macrocyclic Disulfides in Water. *J. Am. Chem. Soc.* **2000**, *122*, 12063-12064.
[93] Arnér, E. S.; Zhong, L.; Holmgren, A. Preparation and assay of mammalian thioredoxin and thioredoxin reductase. *Methods Enzymol.* **1999**, *300*, 226-239.



DTNB **55** TNB **56d**

Figure 16. Disulfide DTNB **55** and the reduced thiol **56d**.

We prepared the library using TNB **56d**, commercially available thiols **56e-g,** glutathione GSH **56h** and the synthetic thiol **22i**. The bisthiazolidine building block **22i** is a L-cysteine derivative, so we envisioned a possible interaction with the binding sites of the Grx or TrxR domains due to its structural similarity with cystine or glutathione. The thiols can exchange disulfide bonds and can theoretically produce a total of 21 different disulfide compounds **57xy** under mild conditions, see scheme 29.



Scheme 29. Virtual dynamic combinatorial library of thiols and disulfides.

DCL synthesis and analysis by HPLC-MS

The dynamic library was constructed in aqueous buffer and pH 8.8, which increases the thiolate/thiol ratio since the thiolate is the reactive species. We also used the redox buffer GSH/GSSG (4:1) to favor the thiol disulfide exchange as other authors notice.[88,94] The interconversion can be stopped by acidifying the solution, with a consequent denaturalization of the enzyme, precipitation and separation.[92]

All the thiols were added in the mentioned conditions. After 24hs, the mixture was split into two vials: the template (TGR) was added to one vial and a buffer solution was added to the other as a blank. The libraries were re-equilibrated in absence of O_2 for other 24 hs.

In order to compare the libraries both systems were analyzed by HPLC-MS. As it is shown in Figure 17a, in absence of protein a mixture of thiol-glutathione disulfides were observed as the main species formed. In the presence of the enzyme the chromatogram showed a different distribution pattern (Figure 17b). A new peak corresponding to the compound **57di** was found, identified as the mixed disulfide of TNB **56d** and the bisthiazolidine **22i**.

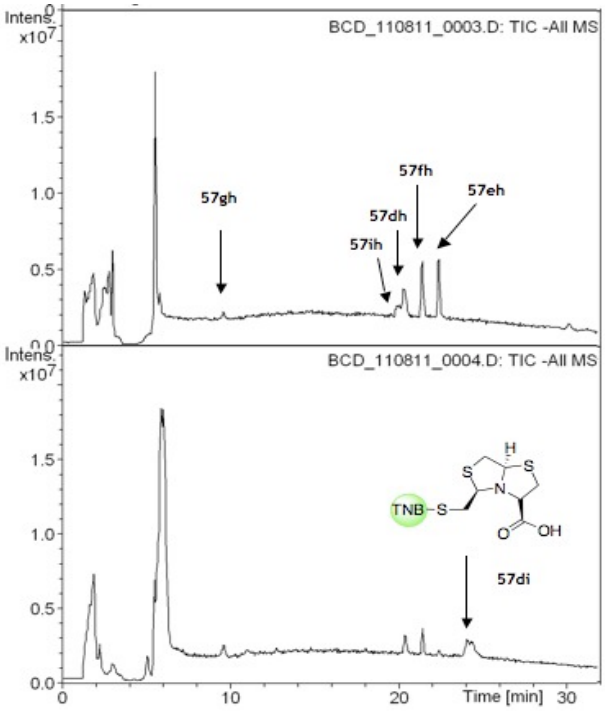


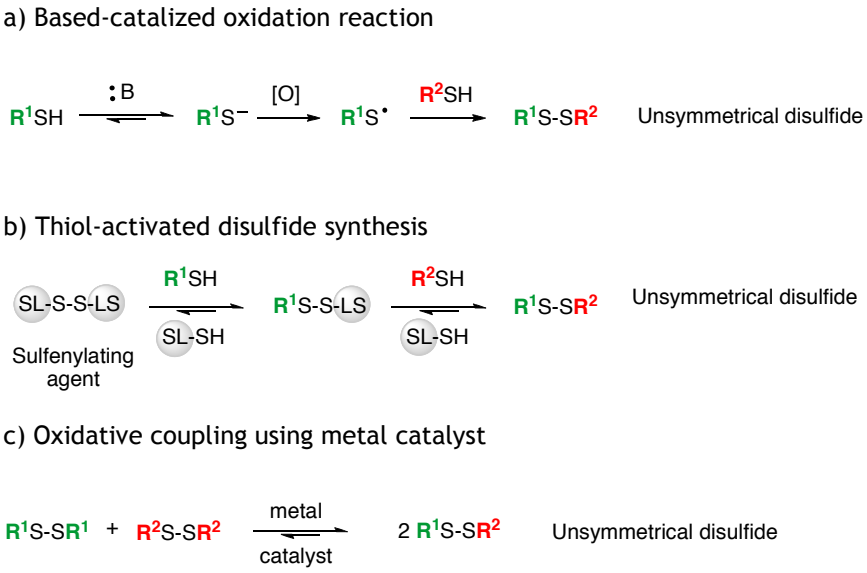
Figure 17. LC-MS analysis of the libraries (TIC^- all MS): (a) Library distribution in absence of TGR template; b) Library distribution in presence of TGR template.

[94] Ladame, S.; Whitney, A. M.; Balasubramanian, S. Targeting nucleic acid secondary structures with polyamides using an optimized dynamic combinatorial approach. *Angew. Chem. Int. Ed.* **2005**, *44*, 5736-5739.

The amplification of compound **57di** was observed with the correlative disappearance of adduct **57ih**. This shows a shift in the equilibrium towards the new stabilized specie.

Unsymmetrical disulfides preparation

The synthesis of unsymmetrical disulfides has been widely studied due to its importance in chemistry, natural products and biology. A large variety of methods are described in literature. Symmetric disulfides can be prepared by the classic direct transformation of thiols,[95] through oxidizing treatments (O_2, H_2O_2, I_2, Br_2, chromates or sulfuryl chlorides), see Scheme 30 a). Other efficient synthetic approaches were reported in literature over the past three decades: thiolysis of reactive sulfenylating agents (e.g 2,2'-dithiobisbenzothiazole),[96] see Scheme 30 b). Syntheses using metal complex catalysts,[97,98] see Scheme 30c).

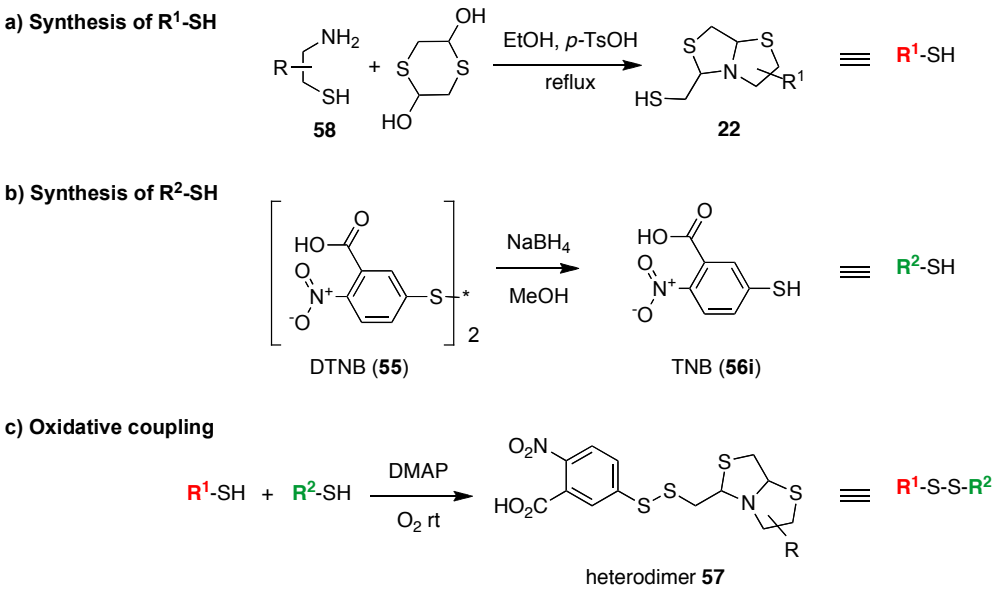


Scheme 30. Examples of synthetic routes to prepare unsymmetrical disulfides.

[95] Oae, S. Organic Sulfur Chemistry: Structure and Mechanisms; CRC Press: Boca Raton, USA; Chapter 6, **1992**.
[96] (a) Brzezinska, E.; Ternay Jr, A. L. Disulfides. 1. Syntheses Using 2,2'-Dithiobis(benzothiazole). *J. Org. Chem.* **1994**, *59*, 8239–8244; (b) Stellenboom, N.; Hunter, R.; Caira, M. One-pot synthesis of unsymmetrical disulfides using 1-chlorobenzotriazole as oxidant: Interception of the sulfenyl chloride intermediate. *Tetrahedron* **2010**, *66*, 3228–3241.
[97] Dhakshinamoorthy, A.; Alvaro, M.; Garcia, H. Aerobic oxidation of thiols to disulfides using iron metal–organic frameworks as solid redox catalysts. *Chem. Commun.* **2010**, *46*, 6476–6478.
[98] Han, M.; Lee, J. T.; Hahn, H.-G. A traceless, one-pot preparation of unsymmetric disulfides from symmetric disulfides through a repeated process involving sulfenic acid and thiosulfinate intermediates. *Tetrahedron Lett.* **2011**, *52*, 236–239.

Synthesis and inhibition studies of compound 57di

According to the library amplification observed, we started with the independent synthesis of compound **57di**. We decided to carry out an easy synthesis for the unsymmetrical disulfide **57di**, using O_2 as the oxidizing agent. Compound **57di** was prepared by simple exposure to air of thiols R^1-SH and R^2-SH in presence of DMAP as the catalytic base, see Scheme 31.



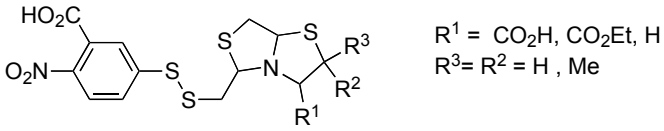
Scheme 31. General procedure for preparation of heterodimer 57.

The TR activity of **57di** was evaluated against TGR using the DTNB assay, showing an IC_{50} of 13 μM. In order to further explore the inhibition, activity of compound **57di** as substrate was, showing no enzymatic activity. Furthermore, the thiol-bisthiazolidine **22i** was evaluated as inhibitor, showing no inhibition (IC_{50} > 50 μM). This suggests that the presence of the mixed disulfide TNB-bisthiazolidine is needed to afford enzyme inhibition.

This was a promising result so we decided to prepare analogs with variations at different position of the bis-thiazolidine moiety in order to increase the inhibition activity.

Synthesis and biological studies of TNB-bisthiazolidine analogs

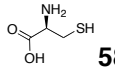
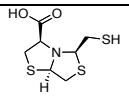
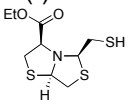
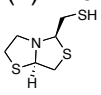
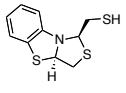
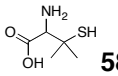
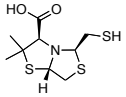
The analogs at the R^1, R^2 and R^3 positions (see Scheme 32) were prepared according to the general route described for **57di**.



Scheme 32. General structure of heterodimer analogs 57.

Compounds **57** were prepared by simple air oxidation of thiols in presence of DMAP as the catalytic base (see Scheme 31). Firstly, the thiols units containing bisthiazolidines **22a-c** and **22i-j** were prepared by double cyclization of aminothiols and mercaptoacetaldehyde following the methodology developed recently by our group.[99] DTNB **55** reduction with $NaBH_4$ gave TNB **56d** as the second thiol. The oxidative coupling between bisthiazolidine moieties (**22**) and TNB (**56a**) afforded heterodisulfides **57** in non-optimized reactions, yields ranged from 38-62%, Table 14.

Table 14. Synthesis of bisthiazolidines **22** and heterodimers **57**.

Entry	Thiol 58	Bisthiazolidine 22	**22** yield (%) (*anti:syn*)	**57** yield (%)
1	 **58i**	 (-)- **22i**	86, (10:90)	**57di:** 62
2	 **58b**	 (+)-**22b**	89, (95:05)	**57db:** 48
3	 **58a**	 (±)-**22a**	68, (92:08)	**57da**: 41
4	 **58c**	 (±)-**22c**	96, (99:01)	**57dc**: 43
5	 **58j**	 (±)-**22j**	76, (05:95)	**57dj**: 38

The prepared analogs (**57**) were evaluated as TGR inhibitors, as shown in Figure 19.

[99] Saiz, C.; Castillo, V.; Mahler, G. Imine Domino Reactions Generate Novel Scaffolds: Fused bis-Thiazolidines or bis-Thiiranes. *Synlett* **2012** ST-2011-12-0311-L online.

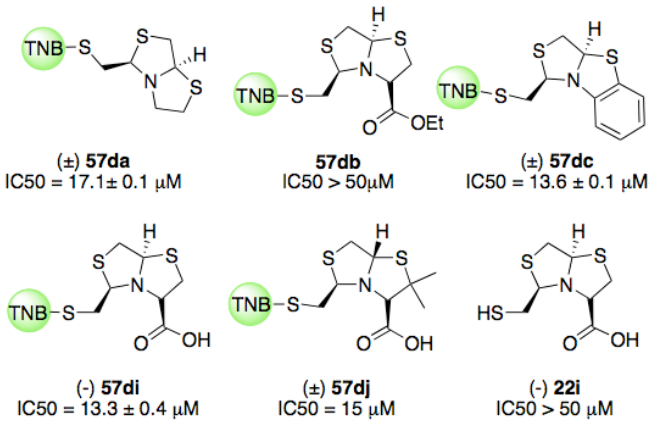


Figure 19. IC_{50} values of heterodimer Inhibitors **57da, 57db, 57dc, 57di** and thiol **22i.**

The lowest activity was observed for compound **57db**, bearing an ethyl ester moiety at R^1 (IC_{50} > 50 μM). This could be explained by the steric bulk of the substituent compared with the carboxylic acid.

Racemic compounds ±**57da**, ±**57dc**, ±**57dj** present similar IC_{50} values (17, 13 and 15 μM respectively) than the optically pure compound (-)-**57di** (IC_{50} = 13 μM)**.** Interestingly, these compounds have different substitution patterns at R^1, R^2 and R^3. The substituents R^1 can tolerate a carboxylic acid, H, or be part of a benzothiazoline. The R^2 = R^3 can be a proton, a methyl group or part of the benzothiazoline moiety, always maintaining the activity. This could mean that either a lipophilic center or a carboxylic acid may contribute in different ways to the inhibition activity. Further studies are needed in order to compare optically pure compounds.

About the diasterochemistry *vs* activity relationship for compounds *syn-anti*-**5di** and *syn-syn*-**5dj** further studies are being carried out in order to conclude.

Preliminary mechanistic inhibition studies

In order to investigate the inhibition mechanism, we carried out preliminary studies of the inhibition mechanism using three substrate concentrations ([DTNB] = 50, 250 and 1000 μM) and screening different inhibitor **57di** concentrations ranging from 5 to 250 μM. The results are shown in Figure 18.

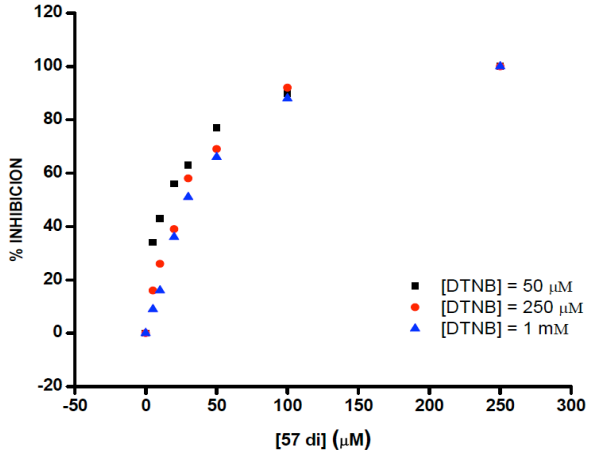


Figure 18. Inhibition studies of **57di** at different [DTNB] concentration.

We observe that the percentage of inhibition is higher when the DTNB concentration is lower. When the inhibitor concentration rises to 250 μM, the enzyme is fully inhibited at all DTNB concentrations. The assays were performed after 5 min preincubation, using standard conditions, but enzyme-inhibitor preincubation time optimization is needed to assess whether we are in presence of slow-binding inhibitors. This type of inhibitors usually correlates with irreversible inhibitors. In addition, experiments to test whether inhibition persists or not after dialysis should be performed to test if the inhibitors are irreversible or not. These experiments could help to elucidate if a covalent pathway is taking place.

Another approach to assess whether the inhibitors are competitive or not could be to determine the kinetic parameters. The corresponding plots of velocity *vs.* [DTNB] should be compared at different inhibitor concentrations. This would lead us to a type of mechanism depending on the results (Km and Vmax comparison).

Molecular Mechanistic discussion

Even if the type of inhibition is not elucidated yet, we decided to discuss about the possible molecular mechanism involving these inhibitors.

a) Reversible binding inhibitors

Most drugs bind to their enzyme target through reversible interactions. There are different reversible modes of inhibitor interaction with enzymes: competitive inhibitor (binds directly to the active site of the free form of the enzyme), uncompetitive inhibitor (binds to the enzyme-substrate complex *ES*) or noncompetitive inhibitor (binds to both situations).[100]

The binary complex *EI* is stabilized through a variety of non-covalent interactions, some of the most important include electrostatic interactions, H-bonds, hydrophobic forces and van der Waals forces.[101] In order to assign the mode of inhibition, kinetic studies must be performed. The plot of the velocity as a function of substrate at varying concentrations of inhibitor and the corresponding double reciprocal plot would show the inhibitor characteristics. Based on these results we will be able to classify the inhibitor.

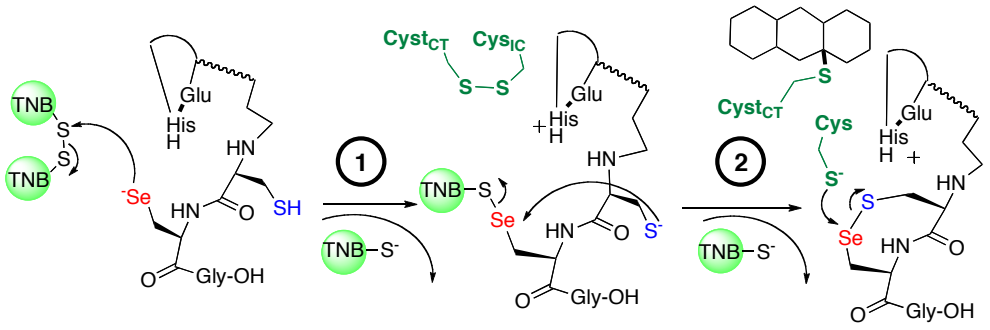
Different inhibition types can be proposed for compound **57**. It could be a reversible competitive inhibition. This would occur as a competition between the substrate and the inhibitor for the free enzyme. In this case, the inhibition process would take place at the substrate-binding site, directed by non-covalent interactions, before the nucleophilic attack of the selenol. The inhibitor would therefore bind to the enzyme through non-covalent interactions, with consequent exclusion of the substrate.

b) Covalently binding enzyme inhibitors

In some cases, enzymes are inactivated by formation of covalent complexes with the inhibitors. This occurs with a permanent interaction between the enzyme and the inhibitor, with consequent inactivation of the enzyme.

[100] Copeland, R. A. Evaluation of enzyme inhibitors in drug discovery. A guide for medicinal chemists and pharmacologists. John Wiley & sons, Inc. Hoboken, New Jersey, USA, **2005**.
[101] Copeland, R.A. Enzymes: A practical introduction to structure, mechanism and data analysis. 2nd. Ed., Wiley, New York; **2000**.

In this context, we will first discuss the covalent enzymatic mechanism using DTNB as substrate, in analogy to the macromolecular substrates reduction proposed by Hondal et al.[102] The first step (1) involves the nucleophilic attack of the selenolate onto a sulfur atom at the substrate disulfide bond. The second step (2) follows with a cysteine thiolate attack from the N-terminal redox center to the Se and generates an 8-member ring, which will be finally reduced by a third cysteine attack, see Scheme 33.



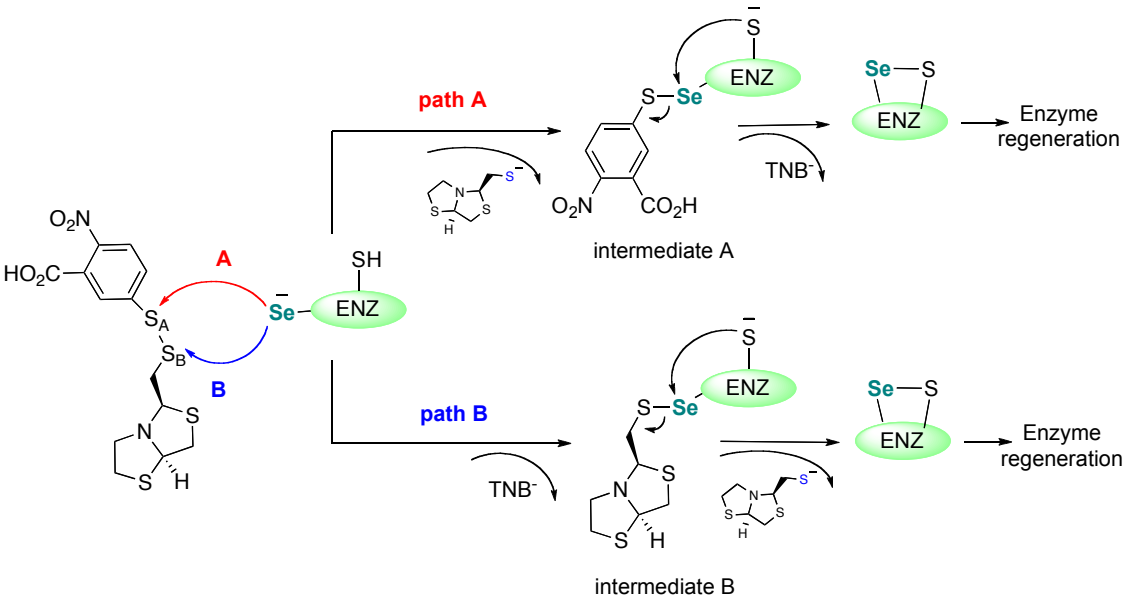
Scheme 33. Three step reduction proposed mechanism of TR with DTNB as substrate.[102]

They studied three main characteristics of the selenolate (nucleophile strength, electrophile strength and the leaving group stability) in order to determinate the rate-determining step in this mechanism.

If we compare DTNB substrate to compound **57di** we can observe some similarities. The heterodimer **57da** contains two subunits, the TNB and the bisthiazolidine moieties, linked by a disulfide bridge. Based on the DTNB mechanism, we postulate that the TNB subunit might act as the anchor moiety, responsible for the recognition at the enzyme active site. Therefore, the bisthiazolidine subunit could be the moiety responsible of the inhibition activity.

There could be two possible paths for the molecular mechanism (scheme 34) of **57di** inhibition, depending mainly on the first step involving the Se^- nucleophilic attack.

[102] Hondal, R. J.; Ruggles, E. L. Differing views of the role of selenium in thioredoxin reductase. *Amino Acids* **2010**, *41*, 73–89.



Scheme 34. Two possible covalent inhibition mechanisms proposed for **57da**: path A and path B.

In *path A*, the first S-S bond cleavage would occur with the attack of the selenolate to the S_A (TNB moiety) and contiguous release of the bisthiazolidine as leaving group. In *path B*, the first attack would occur to the S_B, to release the TNB moiety as leaving group.

We propose that the preferred route (*path A* or *B*) would mainly depend on the leaving group involved. It is known that S_A is a good leaving group due to the possibility of resonance of the negative charge into the ring, stabilizing the released group. Otherwise the pka of TNB thiol is 4.3,[102] a low value characteristic of a good leaving group.

In order to explore the bisthiazolidine moiety, we established a comparative relation between cysteine and inhibitor **57da**. We could observe the structural similarities between both compounds, derived from cysteine, both bearing R-CH_2SH thiols. Cysteine thiol pka is 8.5,[103] we assume a similar value for the bisthiazolidine thiol **22.** This pka (8.5) is not lower enough as the one observed for TNB (4.7),[104] so we can

[103] Ozawa, T.; Hanaki, A. A kinetic study of the thiol-disulfide exchange reaction between aminothiols and 5,5'-dithiobis(2-nitrobenzoic acid). *Chem. Pharm. Bull.* **1981**, *29*, 1101-1105.
[104] Danehy, J. P.; Elia, V. J.; Lavelle, C. J. Alkaline decomposition of organic disulfides. IV. Limitation on the use of Ellman's reagent, 2,2′-dinitro-5,5′-dithiodibenzoic acid. *J. Org. Chem.* **1971**, *36*, 1003–1005.

expect the bisthiazolidine to be the poorest leaving group. This idea is also supported in previous studies reported by Hondal et al, showing that cystine (the cysteine disulfide) is an inactivated disulfide with no activity as substrate for TR.[105] It is also worth considering that if route B takes place, the intermediate B may not be reduced by the enzyme, and thus leading to irreversible covalent inhibition.

Partial Conclusions

In this work we describe the successful use of TGR enzyme as a template in dynamic combinatorial library for the first time, using thiol-disulfide exchange as the reaction. The best binding component was identified as the TNB-bisthiazolidine disulfide **57di**.

We also prepared new TGR inhibitors (**57a-c, 57j**) combining 2-nitro-5-thiobenzoic acid as an anchor building block, with different bisthiazolidine derivatives.

The synthesis of **57da-c, 57di** and **57dj** was performed and the enzymatic activity was measured, in order to establish a primary structure-activity relationship by introducing variation at R^1, R^2 and R^3 of the bisthiazolidine moiety. We found for R^1 that the carboxylic acid is not essential for the activity but the introduction of an ethyl ester decrease TGR inhibition.

Future work

To assess the answer about the molecular mechanism we will continue to study the type of enzyme inhibition. Kinetic studies will be performed to better understanding the inhibitor behavior. Plots of velocity *vs.* substrate concentration at different inhibitor concentrations will be performed to conclude about the possible inhibition mechanism.

We will prepare new analogs with modifications at the TNB-ring level; e.g. elimination of the carboxylic acid and/or nitro group and introduction of a CF_3 group. The assays

[105] Lothrop, A. P.; Ruggles, E. L.; Hondal, R. J. No Selenium Required: Reactions Catalyzed by Mammalian Thioredoxin Reductase That Are Independent of a Selenocysteine Residue. *Biochemistry* **2009**, *48*, 6213–6223.

will be performed to find more potent inhibitors and to explore deeper the chemical space involve in the substrate recognition.

As a better understanding of the process we also plan to determinate pKa of the thiols. These experiments will include absorbance measures of the corresponding thiols at different pHs.[106]

Another important aspect for the design of new inhibitors could be measure of the redox potential of the inhibitors. In this sense we are planing to measure it in order to correlate it with the biological activity. These experiments would be carried out following the methodology described by Raines et al, concerning the measurement of equilibrium constants of the redox reaction between thiols and DTT. All these results will guide us to the future design of new inhibitors.

Appendix

Expression and purification of *E. granulosus* wildtype TGR.

Before the DCLs construction and evaluation of amplified compounds, the expression and purification of the *Eg*TGR was performed in order to generate considerable amounts of protein.

Expression of recombinant proteins was carried out following the protocol described previously,[107] which has been optimized in the laboratory for expression of selenoproteins.[43] Essentially, induction of recombinant proteins was carried out with 100 μM IPTG at late exponential phase (A_{600} = 2.4), during 24 h at 24 °C. Recombinant clones were grown in modified LB,[108] supplemented with 0.1 g/L cysteine and 0.37 g/L methionine in the presence of kanamycin (50 μg/mL) and chloramphenicol (33 μg/mL); the latter was used only in the case of bacterial cultures harboring constructs encoding selenoproteins. At the time of induction, the culture was supplemented with 5μM

[106] Lukesh, J.; Palte, M.; Raines, R. A Potent, Versatile Disulfide-Reducing Agent from Aspartic Acid. *J. Am. Chem. Soc.* **2012**, *134*, 4057–4059.
[107] Rengby, O.; Johansson, L.; Carlson, L. A.; Serini, E.; Vlamis-Gardikas, A.; Kårsnas, P.; Arner, E. S. Assessment of Production Conditions for Efficient Use of Escherichia coli in High-Yield Heterologous Recombinant Selenoprotein Synthesis. *Appl. Environ. Microbiol.* **2004**, *70*, 5159–5167.

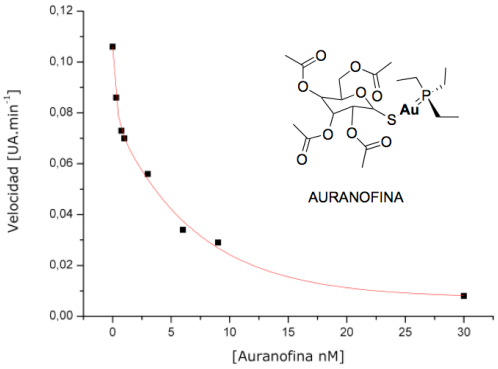
sodium selenite, 20μg/ml riboflavin, 20 μg/ml pyridoxine, and 20 μg/ml niacin.[10]
The bacterial cultures were centrifuged, and the pellets were resuspended in modified nickel-nitrilotriacetic acid lysis buffer (300 mM NaCl, 50 mM sodium phosphate, 20 mM imidazole, pH 7.2) containing 1 mM PMSF and 1 mg/ml lysozyme and sonicated (10 pulses of 1 min with 1-min pauses). The lysates were centrifuged for 1 h at 30.000 x *g* and supernatants were applied to a nickel-nitrilotriacetic acid column (Qiagen), washed with 300 mM NaCl, 50 mM sodium phosphate, 30 mM imidazole, pH 7.2, and eluted with 250 mM imidazole. The protein-containing fractions were applied to PD10 desalting columns (GE Healthcare) using phosphate-buffered saline (PBS), 150 mM potassium chloride, 50 mM sodium phosphate and pH 7.2.

Total protein concentration and FAD content were determined spectrophotometrically at 280 (ε = 54.2 $mM^{-1}.cm^{-1}$) and 460 nm (ε = 11.3 $mM^{-1}.cm^{-1}$), respectively. The selenium content was determined by atomic absorption using a plasma emission spectrometer (Jarrell-Ash 965 ICP), University of Georgia. Active selenoenzyme concentrations were calculated considering their selenium contents (selenium contents were close to 10% for all selenoproteins). The purity of the recombinant proteins was analyzed by running 10% SDS-polyacrylamide gels, under reducing conditions, and by size exclusion chromatography on a Superose 12 column (GE Healthcare).[109]

Optimizing DTNB reduction assay for TR Activity on TGR

Auranofin, an efficient inhibitor of TGR (K_i = 10nM), was used as a model.[87] After purifying the enzyme, inhibition assays were performed in order to optimize the experiment. Experimental conditions were improved: time and temperature for preincubation were studied and different NADPH concentrations were evaluated. We were able to confirm the inhibition characteristics of Auranofin, showing a high affinity union to the enzyme with a 90% of inhibition when using stoichiometric amounts (TGR/Auranofin 1:1), Scheme 35.

[108] Bar-Noy, S.; Gorlatov, S.; Stadtman, T. Overexpression of wild type and SeCys/Cys mutant of human thioredoxin reductase in E. coli: the role of selenocysteine in the catalytic activity. *Free Rad Biol Med 3*, **2001**, 51-61.
[109] Bonilla, M.; Denicola, A.; Marino, S. M.; Gladyshev, V. N.; Salinas, G. Linked thioredoxin-glutathione systems in platyhelminth parasites: alternative pathways for glutathione reduction and deglutathionylation. *J. Biol. Chem.* **2011,** 286, 4959–4967.



Scheme 35. Enzimatic activity vs. [Auranofin, nM]

In conlcusion, we carried out the expression and purification of *E. granulosus* Wildtype TGR in *E. coli*. This allowed us to have enough protein to use as template in the DCLs and for further inhibition assays. The DTNB assay was optimized to screen the prepared potential inhibitors.

3.3 Biological screening against cruzipain, a *T.cruzi* cistein-protease.

Cruzipain inhibition

Chagas disease represents a major public health challenge in Latin America: an estimated of 11 million people are currently infected. Because of population movements, an increasing number of Chagas disease cases have been detected in non-endemic areas, such as North America and some European countries. Unfortunately, only benznidazole and nifurtimox are clinically available for treatment of the disease, and both demonstrate only limited efficacy during the acute phase of the disease and show severe side effects.[110]

The growing knowledge of the basic biology of *Trypanosoma cruzi*, the ethiological agent causative of Chagas disease, empowers the development of new approaches to specific chemotherapy. The cathepsin L-like cysteine protease termed cruzipain or cruzain is responsible for the major proteolytic activity of all stages of the parasite life cycle and represents an interesting target for the development of potential therapeutics for the treatment of the disease.[111]

Cruzipain is differentially expressed in the four main stages of the parasite's biological cycle; it is located in different cellular compartments and is essential for the survival of *T. cruzi* within host cells.[112] While its exact function is unknown, it is likely involved in the degradation of proteins scavenged from the blood meal of the insect vector.[104]

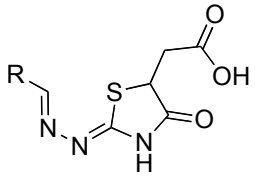
A part of the collection of synthesized compounds was evaluated against cruzipain: hydrazylthiazolidinones, fused heterocycles and spiro-compounds.

[110] McKerrow, J.H.; Doyle, P.S.; Engel, J.C.; Podust, L.M.; Crack, C.S. Two approaches to discovering and developing new drugs for Chagas disease. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 263–269.
[111] Cazzulo, J.; Stoka, V.; Turk, V. The major cysteine proteinase of *Trypanosoma cruzi*: A valid target for chemotherapy of Chagas disease. *Curr. Pharm. Design*. **2001**, *7*, 1143–1156.
[112] Franke de Cazzulo, B.; Martinez, J.; North, M.J.; Coombs, G.H.; Cazzulo, J. J. Effects of proteinase inhibitors on the growth and differentiation of *Trypanosoma cruzi*. *FEMS Microbiol. Lett*. **1994,** *124*,

a) Hydrazylthiazolidinones

In order to study the enzymatic activity of the hydrazylthiazolidinones prepared previously, we performed a preliminary screening using 100 μM concentration of inhibitor and a colorimetric assay measured at 412 nm, see table 15.

Table 15. Biological activities of synthetic thiazolidinones **33a-k** against cruzipain.



33

Entry	**Compound** [100 μM]	Yield	PCI [a]
1	**29a** *o*-OBut-Ph	**33a** (63%)	7
2	**29b** *p*-NO_2-Ph	**33b** (61%)	3
3	**29c** *p*-$N(Me)_2$-Ph	**33c** (82%)	0
4	**29d** *o*-F-Ph	**33d** (57%)	3
5	**29e** *p*-Cl-Ph	**33e** (70%)	28
6	**29f** Ph	**33f** (45%)	0
7	**29g** *p*-OMePh	**33e** (70%)	0
8	**29h** 2-thiophenyl	**33i** (33%)	17
9	**29i** $CH(Me)_2$	**33e** (70%)	7
10	**29j** CH_2CH_2Ph	**33k** (64%)	0

a. PCI = % of cruzipain inhibition at (Inhibitor) = 100 μM, (Cruzipain) = 0.140 μM, values are means of two values. E64: trans-Epoxysuccinyl-L-leucyl-amido(4-guanidino)butane was used as reference compound.

A virtual screening looking for cruzipain inhibitors was achieved and the thiazolidinones **33** were predicted as possible inhibitors. Even if most of the compounds have no activity against the enzyme, we found a 28% of cruzipain inhibition for compound **33e**. This result could be improved by further modifications at the R substituents and at the thiazolidine moiety in position 5.

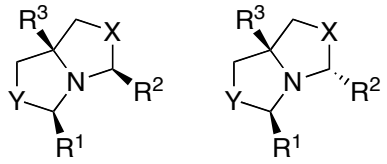
The inhibition values can be useful to refine the synthesis of new compounds. These preliminary assays are a guide to follow with the synthesis of new collections of compounds with an improved inhibitory activity.[113]

81–86.
[113] Pizzo, C.; Saiz, C.; Talevi, A.; Gavernet, L.; Palestro, P.; Bellera, C.; Blanch, L. B.; Benítez, D.;

b) Thiazolidinyloxazolidine heterocycles.

We evaluated the cruzipain inhibitory activity of fused heterocycles prepared before with the methodologies exposed in paper I and II. There was no evidence of similar compounds with this activity so we decided to explore this field, results shown in table 16.

Table 16. Biological activities of synthetic fused heterocycles **6-8** against cruzipain.



Entry	Compound	R1	R2	R3	X	Y	PCI [a]
1	*syn*-**6aa**	Ph	Ph	H	S	O	0
2	*syn*-**6bb**	*p*-ClPh	*p*-ClPh	H	S	O	32
3	*syn*-**6cc**	*p*-NO_2Ph	*p*-NO_2Ph	H	S	O	0
4	*syn*-**6ba**	*p*-ClPh	Ph	H	S	O	0
5	*syn*-**6ad**	*p*-ClPh	*p*-OMePh	H	S	O	0
6	*syn*-**7aa**	*p*-ClPh	*p*-ClPh	CH_2OH	O	O	23
7	*anti*-**8a**	*p*-ClPh	*p*-ClPh	CH_2OH	S	O	60
8	*syn*-**8a**	*p*-ClPh	*p*-ClPh	CH_2OH	S	O	70
9	anti-**8b**	*p*-FPh	*p*-FPh	CH_2OH	S	O	63
10	syn-**8b**	*p*-FPh	*p*-FPh	CH_2OH	S	O	nd
11	syn-**8d**	*m*-BrPh	*m*-BrPh	CH_2OH	S	O	nd
12	*anti*-**8c**	*p*-CF_3Ph	*p*-CF_3Ph	CH_2OH	S	O	5

a. PCI = % of cruzipain inhibition at [Inhibitor] = 100 μM, [Cruzipain] = 0.140 μM, values are means of two values. E64 = trans-Epoxysuccinyl-L-leucyl-amido(4-guanidino) butane was used as reference compound.

The best inhibition values were obtained for thiazolidin-oxazolidine *syn*-**8a** (entry 8, table 16), *anti*-**8a** (entry 7, table 16) and *anti*-**8b** (entry 9, table 16). It is noteworthy that

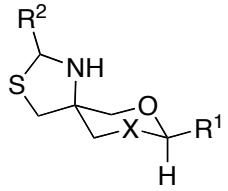
Cazzulo, J. J.; Chidichimo, A.; Wipf, P.; Mahler, S. G. Synthesis of 2-Hydrazolyl-4-Thiazolidinones Based on Multicomponent Reactions and Biological Evaluation Against Trypanosoma Cruzi. *Chem. Biol. Drug. Des.* **2011**, *77*, 166-172.

compounds bearing *p*-Cl phenyl substituents have the better inhibitory activity. We can also notice that the relative stereochemistry is not decisive for the inhibition. Both *anti* and *syn* fused heterocycles have very similar percentage of cruzipain inhibition.

If we compare inhibition values for *syn*-**7aa** (*p*-Cl bisoxazolidine) and *syn*-**8a** (*p*-Cl thiazolidinyloxazolidine), we can conclude that the introduction of a thiazolidine instead of an oxazolidine raises the inhibition percentage from 23% to 70%.

c) Spiro compounds

Table 16. Biological activities of synthetic spiro-compounds against cruzipain.



Entry	Compound	R^1	R^2	X	PCI [a]
1	dm **16a** [b]	*p*-ClPh	C=S	O	0
2	dm **17** [c]	*p*-ClPh	C=S	S	0
3	*syn*-**9d**	*m*-BrPh	*m*-BrPh	O	83
4	*syn*-**9e**	*m,p*-diClPh	*m,p*-diClPh	O	23

a. PCI = % of cruzipain inhibition at [Inhibitor] = 100 μM, [Cruzipain] = 0.140 μM.
b. dm = diasteromeric mixture *syn*/*anti* (8:2)
c. dm = diasteromeric mixture *syn*/*anti* (7:3)

The spiro-compounds were evaluated against cruzipain at 100μM concentration as a preliminary screening. Only *syn*-**9d** and *syn*-**9e** compounds and diasteromeric mixtures of **16** and **17** were evaluated. As shown in entry 3 (table 16), compound *syn*-**9d** shows the best result with a cruzipain inhibition of 83%. This value could be incremented with the *anti*-**9d** diasteromer.

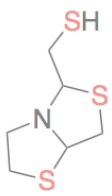
Further assays are needed to conclude about these compounds. The *anti* products could have better inhibition values so we will work to prepare the corresponding analogs and then finish with the corresponding enzymatic assays to be able conclude about the best inhibitor.

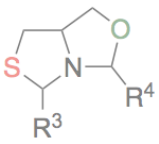
Partial Conclusions

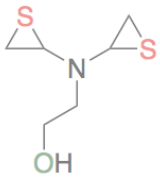
As a general conclusion, we can observe that hydrazilthiazolidinones **33** have no relevant inhibitory activity against cruzipain. When we study the inhibition values of fused heterocycles we observe that compound *syn*-**8a** (R^1 = R^2 = *p*-ClPh) shows a 70% of enzyme inhibition using a 100μM concentration. This is an encouraging value to keep forward with the synthesis of analogous heterocycles and consecutive inhibition studies.

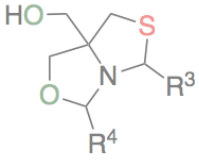
Finally, concerning the spiro compounds, the best inhibitor was found to be the *syn*-**9d**, with 83% of inhibition at 100μM concentration. Further studies will be accomplished to synthesize new analogs in order to increase the inhibitory activity.

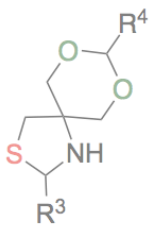
4. CONCLUSIONES











CONCLUSIONES GENERALES

a) Se estudió y desarrolló una nueva reacción útil para generar BCDs: intercambio aminotiol-carbonilo, utilizando como modelo de estudio tiazolidinas. El equilibrio termodinámico se alcanza en buffer acuoso a pH 4 luego de 48 hs y se puede detener variando el pH a 7 para un posterior análisis.

b) Se desarrolló una metodología general para la síntesis de biciclos fusionados de tiazolidina-oxazolidina, que comprenden los heterociclos 1-aza-3-oxa-7-tiabiciclo[3.3.0]octano **6** y los 5-hydroxymethyl-1-aza-3-oxa-7-tiabiciclo [3.3.0] octano **8**.

c) Los heterociclos **6**, preparados a partir de L-cisteína, fueron obtenidos con alta pureza enantiomérica mediante una resolución diasterómerica que involucra el comportamiento reversible de las tiazolidinas.

d) Los heterociclos **8** fueron preparados a partir de TRIS mediante una metodología selectiva que permite obtener los diasterómeros *syn-syn* o *anti-syn* controlando las condiciones de reacción.

e) Los compuestos espirocíclicos **9** fueron obtenidos mediante equilibrios tautoméricos, dependientes de las características electrónicas de los aldehídos empleados. Estos compuestos representan nuevas estructuras capaces generar diversidad molecular.

f) La preparación de nuevas bis-tiazolidinas **22** fue llevada a cabo a partir de β-aminotioles y mercaptoacetaldehído con muy buenos rendimientos y alta diastereoselectividad. Estas nuevas moléculas se prepararon mediante una reacción en cascada, generando 4 enlaces nuevos con pérdida de dos moléculas de agua y sin la necesidad de utilizar catalizadores metálicos.

g) La preparación de 2-amino tiranos **23** fue llevada a cabo a partir de aminas y mercaptoacetaldehído. Estas estructuras representan una nueva clase de episulfuros con posible aplicación en el desarrollo de nuevos materiales y polímeros. Como muestra de dicho potencial se estudió la reactividad de dichos

tiranos en presencia de un buen electrófilo como es el DMAD, obteniéndose una nueva tiazolidin-tiazepina **28** no reportada hasta el momento.

h) Se desarrolló una nueva metodología en tándem para la preparación de 2-hidrazil-tiazolidinonas **33** a partir de tiosemicarbazonas, aldehídos y anhídrido maléico. La reacción se da en un solo paso, utilizando como fuente de energía las microondas con rendimientos buenos a muy buenos.

i) Utilizando tiosemicarbazonas en presencia de una α,β-dicetona se preparó un nuevo tipo de tiazolidinonas, las 2-hidrazil-5,5-difenil-4-tiazolidinonas **35**. Se propone un mecanismo mediante un rearreglo bencílico.

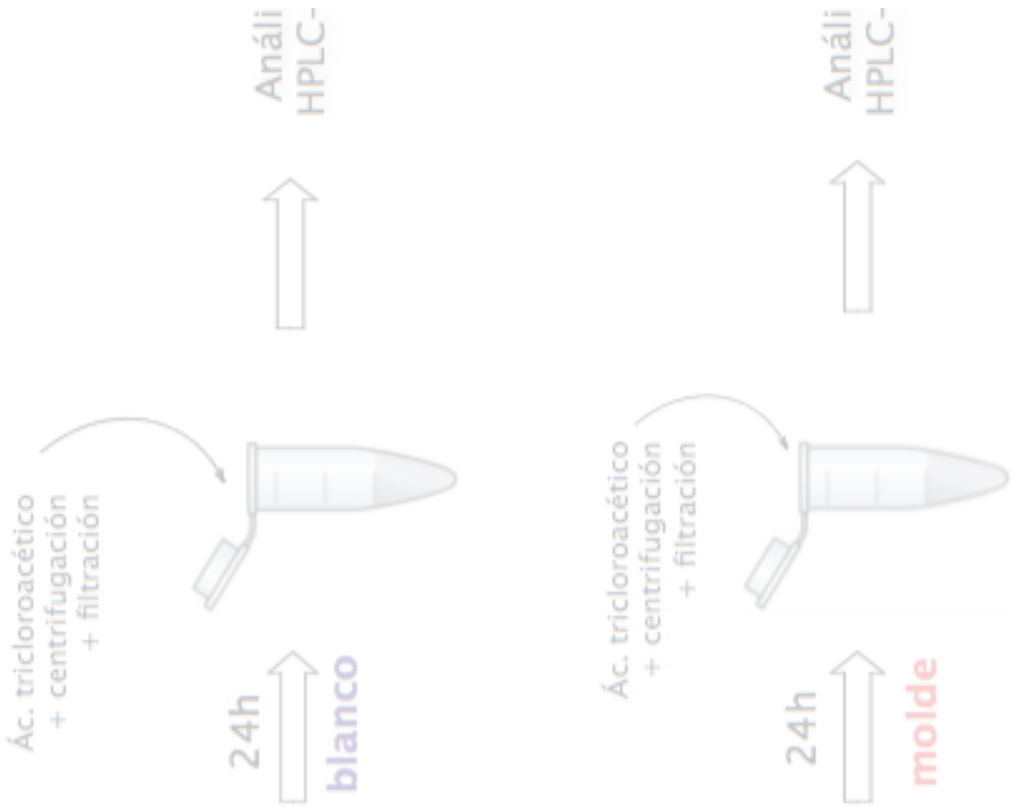
j) Se describió una nueva metodología para la preparación de pirrolo[1,3]diazepinas **44** utilizando microondas. Posteriores funcionalizaciones de estas estructuras permitieron la preparación de el azamacrociclo de 12 miembros **54** utilizando una reacción de ring closing metathesis (RCM) en presencia del catalizador de Grubbs.

k) En su conjunto, las estructuras **6, 8, 9, 22, 23, 28, 33, 44** y **54** amplían el número de moléculas pequeñas altamente funcionalizadas que pueden obtenerse a partir de materiales de partida sencillos y ser utilizados como bloques de construcción de estructuras altamente complejas.

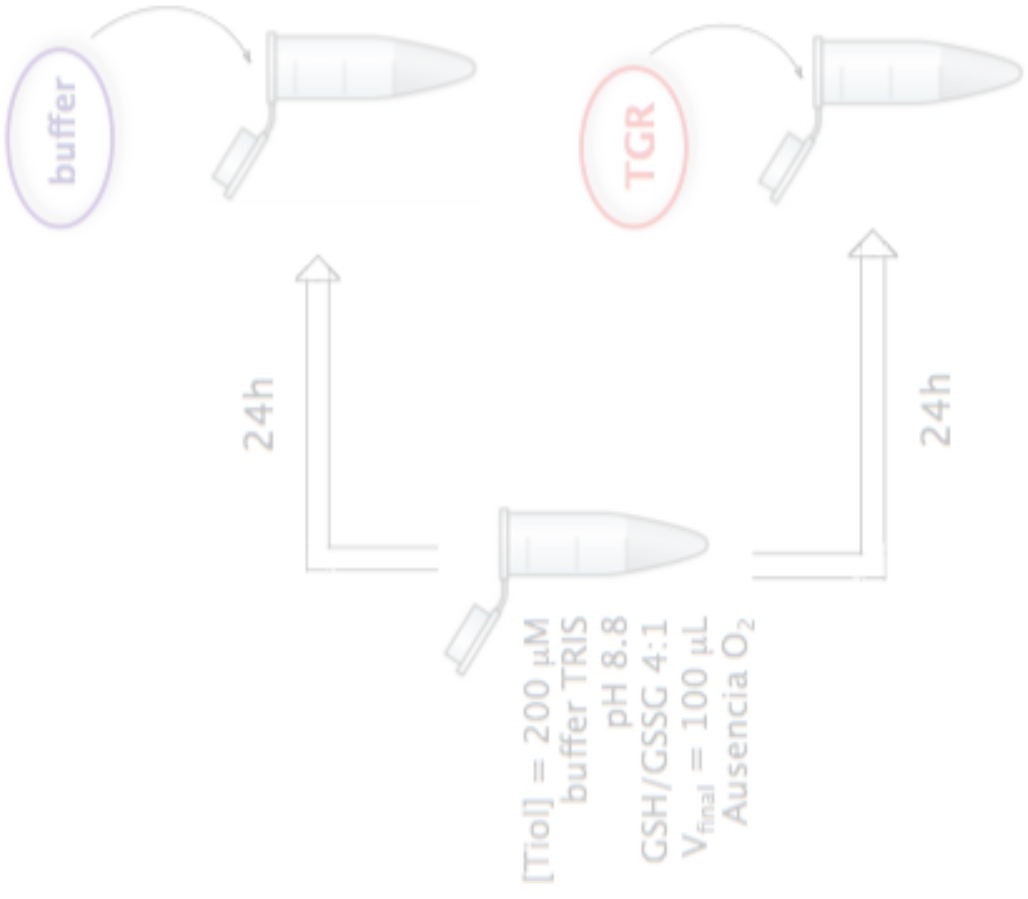
k) En lo que respecta a la aplicación de la QCD en la búsqueda de ligandos de biomoléculas, se sintetizó una biblioteca dinámica de intercambio tiol-disulfuro. Se describió el uso de la enzima redox TGR como molde para dicho sistema, encontrando un heterodímero **57di** como resultado de la amplificación. Este compuestos fue preparado en forma independiente y se evaluó su actividad inhibitoria, observando un IC_{50} = 13μM.

l) Se prepararon los análogos **57a-c, 57j** del disulfuro mixto **57di**. Estos compuestos fueron evaluados en TGR encontrando valores de inhibición similares al compuesto de partida.

m) Las colecciones de hidraziltiazolidinonas **33** y biciclos **6** y **8** fueron evaluados en cruzipaína encontrando valores intermedios de inhibición.



5. PARTE EXPERIMENTAL



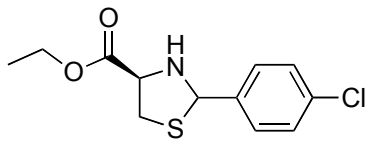
PARTE EXPERIMENTAL

General Methods.

Reactions were monitored by analytical thin layer chromatography (TLC) on 0.25 mm silica gel coated plastic sheets (Macherey-Nagel, Polygram® SIL G/UV 254). Flash chromatography on Silica gel 60 (J. T. Baker, 40 μm average particle diameter) was used to purify the crude reaction mixtures. NMR spectra were recorded at 400 MHz/100 MHz (^{1}H-NMR, ^{13}C-NMR) using a Bruker AVANCE at 21°C. Chemical shifts (δ) are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constant and integration. IR spectra were obtained on a Perkin Elmer 1310 and FTIR 8101A Shimadzu spectrometer, units cm^{-1}. Melting points were determined using a Laboratory Devices STUART SMP10 without correction or with a Laboratory Devices Gallenkamp apparatus. All reactions were carried out in dry, freshly distilled solvents under anhydrous conditions unless otherwise stated. All solvents were purified according to literature procedures. Yields are reported for chromatographic and spectroscopic pure compounds (^{1}H and ^{13}C-NMR) unless otherwise stated. Diasteromeric excess was calculated based on ^{1}H RMN spectra. High-resolution mass spectra were obtained on an ESI Q-Tof spectrometer, at the Polo Tecnológico de Pando, Facultad de Química, Universidad de la República, on a double focusing mass spectrometer (EI) at the University of Pittsburgh Mass Spectrometry facility and on a Thermo Scientific Exactive Orbitrap mass spectrometer equipped with an atmospheric solids analysis probe (ASAP), Philadelphia. Optical rotations were measured using a Kruss Optronic GmbH P8000 polarimeter with a 0.5-mL cell (concentration c given as g/100 mL). Liquid chromatography was performed with a Shimadzu LC 20 AT provided with UV (SPD-M20A) detection. Microwave-assisted reactions were carried out on a CEM Discover microwave reactor equipped with 10 mL vials. Assignments of ^{1}H and ^{13}C NMR peaks were made based on a combination of COSY, HSQC, and HMBC spectra. Relative stereochemistry was determined by NOESY experiments, using the pulse sequence selnogp, D8 at 300 and 800 ms. DFT calculations, geometry optimizations, and conformational searches were carried out in Spartan 10 (B3LYP/6-311G*), obtained from Wavefunction, Inc., Irvine, CA. HPLC-MS analysis were carried out on a HPLC Agilent 1200 equipped with a DIAD, binary pump and thermostated column at 40°C, coupled to a Mass Spectrometer with an Ion trap Esquire 6000 (Bruker Daltonik GmbH).

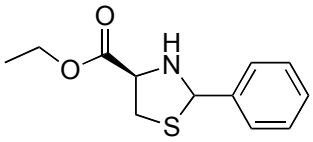
Typical procedure for the synthesis of thiazolidines 3.

3b



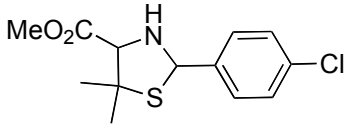
(2*RS*, 4*R*) Ethyl-2-(*p*-chlorophenyl)thiazolidine-4-carboxylate (3b). To a stirred solution of L-cysteine ethyl ester hydrochloride (880 mg, 4.7 mmol) in dry EtOH (10 ml) was added Et_3N (464 mg, 4.7 mmol) and *p*-chlorobenzaldehyde (1 g, 10.3 mmol). The reaction mixture was stirred for 3 hours and the solvent was evaporated. The residue was purified by chromatography on SiO_2 (EtOAc/Hexanes, 3:1) to afford compound **3b** (1.1g, 86%) as colorless oil: ^{1}H NMR ($CDCl_3$), δ 1.29 (m, 9 H), 2.60 (t, *J* = 12.2 Hz, $1H_{NH}$), 2.89 (t, *J* = 7.8 Hz, $1H_{NH}$), 3.11 (dd, *J* = 8.9, 10.3 Hz, 2 H), 3.16 (dd, *J* = 6.2, 10.6 Hz, 1 H), 3.38 (dd, *J* = 7.1, 10.6 Hz, 1 H), 3.47 (dd, *J* = 7.1, 10.3 Hz, 2 H), 3.93-4.00 (m, 2 H), 4.11 (m, 2 H), 4.26 (m, 6 H), 5.52 (d, *J* = 12.2 Hz, 2 H), 5.80 (d, *J* = 7.8 Hz, 1 H), 7.32 (m, 6 H), 7.45 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 37.4, 38.2, 39.3, 61.7, 61.8, 65.6, 69.9, 71.6, 128.3, 128.5, 128.9, 134.4, 136.7, 140.2, 171.0, 171.6.

3a



(*2RS, 4R*) Ethyl-2-phenylthiazolidine-4-carboxylate (3a). Prepared in 85% yield as colourless oil analogous to the route described for **3b**. ^{1}H NMR ($CDCl_3$), δ 1.31 (m, 9 H), 3.11 (dd, *J* = 8.9, 10.2 Hz, 2 H), 3.19 (dd, *J* = 6.0, 10.6 Hz, 1 H), 3.40 (dd, *J* = 7.1, 10.6 Hz, 1 H) 3.47 (*J* = 7.1, 10.2 Hz, 2 H), 3.98 (dd, *J* = 7.2, 8.9 Hz, 2 H), 4.19 (dd, *J* = 7.2, 8.9 Hz, 1 H), 4.26 (m, 6 H), 5.57 (s, 2 H), 5.83 (s, 1 H), 7.51(m, 9 H), 7.51 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 30.1, 38.2, 39.2, 61.6, 61.7, 64.3, 65.6, 70.8, 72.2, 72.6, 126.9, 127.4, 127.8, 128.4, 128.6, 128.7, 138.1, 141.3, 171.1, 171.7.

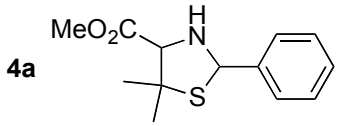
4b



Methyl (2*RS*,4*RS*)-2-(4-chlorophenyl)-5,5-dimethylthiazolidine-4-carboxylate (4b). To a stirred solution of L/D-penicillamine methyl ester hydrochloride[1] (200 mg, 1.0 mmol) in dry EtOH (6 ml) was added Et_3N (101 mg, 1.0 mmol) and *p*-chlorobenzaldehyde (280mg, 10.3 mmol). The reaction mixture was stirred overnight and the solvent was evaporated. The residue was purified by chromatography on SiO_2 (EtOAc/Hexanes, 1:9) to afford compound **4b** (120 mg, 43%) as colorless oil: the ^1H NMR spectrum showed the presence of two diastereoisomers (ratio 74/26).

Major component: ^1H NMR ($CDCl_3$), δ 1.35 (s, 3H), 1.72 (s, 3H), 3.09 (t, *J* = 12.3 Hz, $1H_{NH}$), 3.70 (m, 1H), 3.79 (s, 3H), 5.64 (d, *J* = 12.3 Hz, 1H), 7.31(m, 2H), 7.43 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.1, 29.1, 52.2, 59.8, 69.3, 74.6, 128.0, 128.4, 128.8, 128.9, 134.3, 137.2, 169.6.

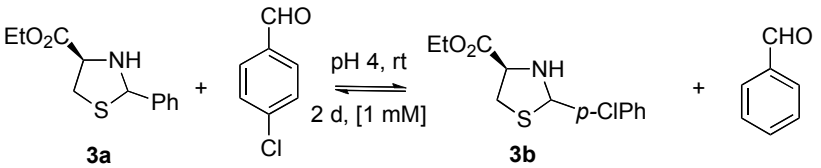
Minor component: ^1H NMR ($CDCl_3$), δ 1.30 (s, 3H), 1.60 (s, 3H), 3.09 (bs, 1 H_{NH}), 3.78 (s, 1 H), 3.79 (s, 3 H), 5.81 (s, 1H), 7.31 (m, 2H), 7.43 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.4, 52.2, 60.3, 67.5, 72.7, 128.0, 128.4, 128.8, 128.9, 133.1, 141.7, 170.1.



4a

Methyl (2*RS*,4*RS*)-5,5-dimethyl-2-phenylthiazolidine-4-carboxylate (4a).[2] Prepared in 79% yield as oil analogous to the route described for **4b**, the ^1H NMR spectrum showed the presence of two diastereoisomers (ratio 78/22). Major component: ^1H NMR ($CDCl_3$), δ 1.39 (s, 3 H), 1.75 (s, 3 H), 3.19 (bs, 1 H_{NH}), 3.76 (m, 1 H), 4.11 (s, 3 H), 5.73 (bs, 1 H), 7.38 (m, 5 H). Minor component: ^1H NMR ($CDCl_3$), δ 1.34 (s, 3 H), 1.65 (s, 3 H), 3.19 (bs, 1 H_{NH}), 3.76 (m, 1 H), 3.81 (s, 3 H), 5.90 (s, 1 H), 7.38 (m, 5H).

Typical procedure for thiazolidine exchange.

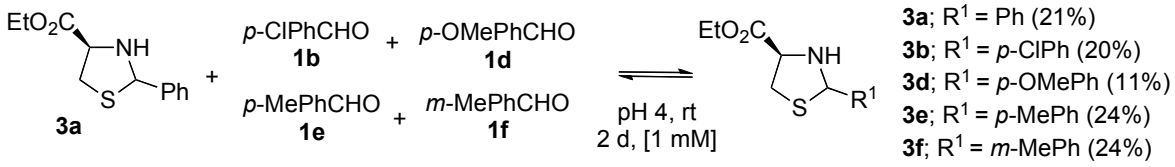


3a **3b**

[1] Wondrak, G. T.; Jacobson, M. K.; Jacobson, E. L. *J. Pharm. Expt. Ther*. **2005**, *316*, 805-814.
[2] Pinho e Melo, T.; Soares, M. I. L.; Nunes, C. M. *Tetrahedron* **2007**, *63*, 1833-1841.

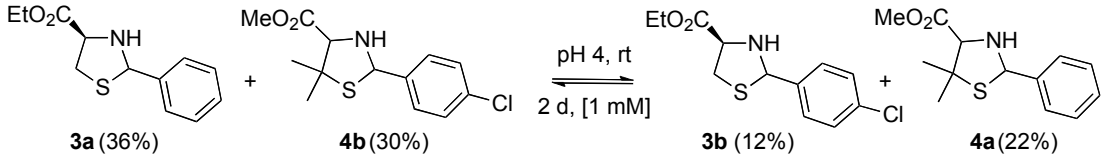
A solution of compound **3a** (21 mg, 0.09 mmol) and *p*-Cl-benzaldehyde (12.3 mg, 0.09 mmol) in a mixture of acetate buffer at pH 4 (62 ml) and MeOH (28 ml) was stirred at rt for 2 d. The pH was raised to 7 with a saturated solution of $NaHCO_3$ and the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo (temperature should never exceed 22°C). The residue was analyzed by ^1H NMR to determine the product ratio, and purified on SiO_2 (AcOEt/*n*-hexanes 1:7) to give a mixture of **3a + 3b** (23 mg, 98% yield).

Typical procedure for thiazolidine library exchange.



A solution of compound **3a** (51 mg, 0.215 mmol), *p*-Cl-benzaldehyde (30.3 mg, 0.215 mmol), *p*-MeO-benzaldehyde (29.3 mg, 0.43 mmol), *p*-Me-benzaldehyde (26 mg, 0.215 mmol) and *m*-Me-benzaldehyde (26 mg, 0.215 mmol) in a mixture of acetate buffer at pH 4 (150 ml) and MeOH (65 ml) was stirred at rt for 2 and 3 days. The pH was raised to 7 with a saturated solution of $NaHCO_3$ and the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo (temperature should never exceed 22 °C). The residue was analyzed by ^1H NMR to determine the product ratio **3a** (21%), **3b** (21%), **3c** (18%), **3d** (20%) and **3e** (20%) and purified on SiO_2 (AcOEt/*n*-hexanes 1:9) to give a mixture of **3a**, **3b**, **3c**, **3d** and **3e** (53 mg, 97% yield for 72 h).

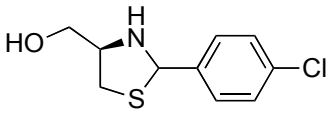
Typical procedure for thiazolidine metathesis.



A solution of compound **3a** (13 mg, 0.053 mmol) and **4b** (15 mg, 0.053 mmol) in a mixture of acetate buffer at pH 4 (40 mL) and MeOH (13 mL) was stirred at rt for 2 d. The pH was raised to 7 with a saturated solution of $NaHCO_3$ and the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo (temperature should never exceed 22 °C). The residue was analyzed by ^1H NMR to determine the product ratio as: **3a** (36%), **4b** (30%), **3b** (12%), **4a** (22%).

Typical procedure for reduction of carboxylic acids.

5b

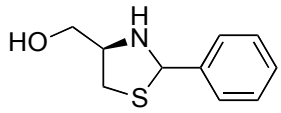


(2*RS*,4*R*)-4-(Hydroxymethyl)-2-(*p*-chlorophenylthiazolidine) (5b).[3] To a stirred solution of **3b** (2 g, 7.4 mmol) in MeOH (30 ml) was added $NaBH_4$ (1.7g, 44 mmol) portion wise at 0ºC. After the addition was complete, the mixture was allowed to reach room temperature and stirred for 4 hours. The solvent was removed under reduced pressure. The residue was poured into water and the pH was adjusted to 7 with HCl (1M) and extracted with EtOAc (5 x 30 ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude was purified by chromatography to afford compound **5b** (1.5 g, 88% yield, 50:50 check) as colorless oil. IR (KBr) ν = 3364, 2930, 1491, 1092, 1015, 820. **(2*R*,4*R*)-4-(Hydroxymethyl)-2-(*p*-chlorophenylthiazolidine) (2*R*-5b)**: ^1H NMR ($CDCl_3$), δ 2.85 (dd, *J* = 4.8, 10.6 Hz, 1 H), 3.22 (dd, *J* = 7.0, 10.6 Hz, 1 H), 3.60 (dd, *J* = 8.2, 11.2 Hz, 1 H), 3.65 (dd, *J* = 5.0, 11.2 Hz, 1 H), 3.85 (dddd, *J* = 4.8, 5.0, 7.0, 8.2 Hz, 1 H), 5.52 (s, 1 H), 7.31 (m, 2 H), 7.43 (m, 2 H).

[3] Mahler, S. G.; Serra G. L.; Manta, E. *Synth. Commun*. **2005**, *35,* 1481–1492.

(2*S*,4*R*)-4-(Hydroxymethyl)-2-(*p*-chlorophenylthiazolidine) (2*S*-5b): ^{1}H NMR ($CDCl_3$), δ 2.96 (t, *J* = 9.8, 1 H), 3.13 (dd, *J* = 6.2, 9.8 Hz, 1 H), 3.45 (dddd, *J* = 3.8, 4.8, 6.2, 9.8 Hz, 1 H), 3.75 (dd, *J* = 4.8, 11.2 Hz, 1 H), 3.97 (dd, *J* = 3.8, 11.2 Hz, 1 H), 5.54 (s, 1 H), 7.31 (m, 2 H), 7.43 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 36.8, 37.0, 62.1, 62.5, 65.0, 66.6, 69.7, 71.5, 128.6, 128.7, 128.7, 129.4, 133.9, 134.1, 137.8, 138.4; HRMS calculated for $C_{10}H_{12}ClNOS$ [M^+-H]: 229.0328, found: 229.0334.

5a

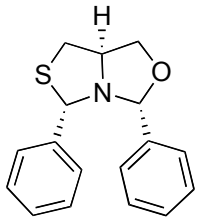


(2*RS*, 4*R*)-4-(Hydroxymethyl)-2-phenylthiazolidine (**5a).** Prepared in 82 % yield as a white solid analogous to the route described for **5b**. **(2*R*,4*R*)-4-(Hydroxymethyl)-2-phenylthiazolidine**: ^{1}H NMR ($CDCl_3$), δ 2.86 (dd, *J* = 4.8, 10.6 Hz, 1 H), 3.24 (dd, *J* = 6.9, 10.6 Hz, 1 H), 3.62 (dd, *J* = 8.2, 11.0 Hz, 1 H), 3.66 (dd, *J* = 5.0, 11.0, 1 H), 3.88 (dddd, *J* = 4.8, 5.0, 6.9, 8.2, 1 H), 5.53 (s, 1 H), 7.32 (m, 2 H), 7.45 (m, 2 H). **(2*S*,4*R*)-4-(Hydroxymethyl)-2-phenylthiazolidine**: ^{1}H NMR ($CDCl_3$), δ 2.98 (t, *J* = 9.8 Hz, 1 H), 3.15 (dd, *J* = 6.3, 9.8 Hz, 1 H), 3.48 (dddd, *J* = 3.7, 4.8, 6.3, 9.8 Hz, 1 H), 3.78 (dd, *J* = 4.8, 11.2 Hz, 1 H), 3.99 (dd, *J* = 3.7, 11.2 Hz, 1 H), 5.56 (s, 1 H), 7.32 (m, 2 H), 7.45 (m, 2 H).

(2*RS*, 4*R*)-4-(Hydroxymethyl)-2-phenylthiazolidine: ^{13}C NMR ($CDCl_3$) δ 36.7, 36.9, 62.1, 62.4, 65.0, 66.6, 70.2, 72.0, 127.1, 127.2, 128.3, 128.5, 128.6, 128.6, 138.9, 139.4.

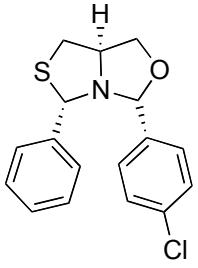
Typical procedure for preparation of fused thiazolidinyl-oxazolidines.

6aa



(2*R,5*R*,8*S**)-2,8-diphenyl-1-aza-3-oxa-7-thiabicyclo[3.3.0]octane (6aa).** To a stirred solution of (2*RS*, 4*R*)-4-(Hydroxymethyl)-2-phenylthiazolidine (550 mg, 2.82 mmol) in CH_2Cl_2 (30 ml) were added benzaldehyde (358 mg, 3.38 mmol) and *p*-TsOH ac. (30 mg, 0.37 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified by chromatography (4:1, *n*-hexanes/EtOAc) to give **6aa** (450 mg, 99.9%de, 75 % yield) as a white solid: mp 85.2-85.4 ºC. IR (KBr) ν = 3085, 2857, 1491, 1381, 1092, 821; ^{1}H NMR ($CDCl_3$), δ 2.91 (dd, *J* = 3.5, 11.4 Hz, 1 H), 3.12 (dd, *J* = 6.9, 11.4 Hz, 1 H), 3.80 (dd, *J* = 6.0, 8.3 Hz, 1 H), 4.21 (m, 1 H), 4.42 (dd, *J* = 6.5, 8.3Hz, 1 H), 5.27 (s, 1 H), 5.41 (s, 1 H), 7.28 (m, 6 H), 7.45 (m, 2 H), 7.56 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 36.5, 67.2, 71.8, 73.5, 96.7, 126.8, 127.2, 127.7, 128.1, 128.4, 129.0, 139.2, 142.1; HRMS calculated for $C_{17}H_{17}NOS$ $[M-H]^-$: 283.1031, found: 283.1035, $[\alpha]_D$ -98.7º (22ºC, CH_2Cl_2, c=1.02).

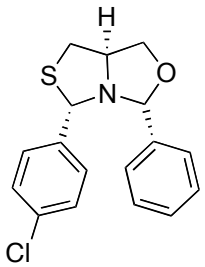
6ab



(2*R,5*R*,8S***)-2-*p*-chlorophenyl-8-phenyl-1-aza-3-oxa-7-thiabicyclo[3.3.0]octane (6ab)**. Prepared in 85.2% de, 80% yield, as a colourless oil analogous to the route described for **6aa**: IR (KBr) ν = 3085, 2932, 1599, 1491, 1105, 822 cm^{-1}; ^{1}H NMR ($CDCl_3$), δ 2.90 (dd, *J* = 3.5, 11.5 Hz, 1 H), 3.12 (dd, *J* = 6.9, 11.5 Hz, 1 H), 3.78 (dd, *J* = 6.0, 8.3 Hz, 1 H), 4.20 (m, 1 H), 4.40 (dd, *J* = 6.5, 8.3 Hz, 1 H), 5.24 (s, 1H), 5.37 (s, 1H), 7.20-7.34 (m, 5 H), 7.42 (m, 2 H), 7.49 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 36.4, 67.1, 71.9, 73.4, 96.0, 126.7, 127.4, 128.2, 128.6, 129.1, 134.8, 137.8, 141.9; HRMS

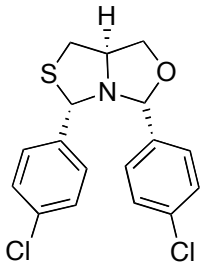
calculated for $C_{17}H_{16}ClNOS$ [M^+-H]: 317.0641, found: 317.0644, $[\alpha]_D$ -67.8º (22ºC, CH_2Cl_2, c=0.7).

6ba



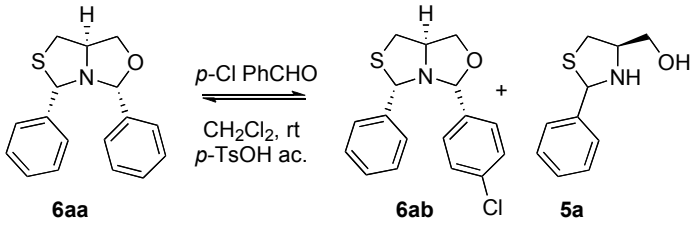
(2*R,5*R*,8*R**)-2-phenyl-8-*p*-chlorophenyl-1-aza-3-oxa-7-thiabicyclo[3.3.0]octane (6ba)** Prepared in 98.7% de, 95% yield, as a colourless oil analogous to the route described for **6aa**: IR (KBr) ν = 3087, 2859, 1489, 1383, 1090, 824 cm^{-1}; ^1H NMR ($CDCl_3$), δ 2.91 (dd, *J* = 3.5, 11.5 Hz, 2 H), 3.09 (dd, *J* = 6.6, 11.5 Hz, 2 H), 3.78 (dd, *J* = 6.2, 8.4 Hz, 2 H), 4.18 (dddd, *J* = 3.5, 6.2, 6.5, 6.6 Hz, 1 H), 4.40 (dd, *J* = 6.5, 8.4 Hz, 2 H), 5.25 (s, 1H), 5.35 (s, 1H), 7.23-7.26 (m, 1 H), 7.33-7.39 (m, 6 H), 7.53-7.55 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 36.48, 67.11, 71.82, 72.87, 96.69, 127.68, 128.19, 128.45, 129.14, 132.99, 138.97, 140.64; HRMS calculated for $C_{17}H_{16}ClNOS$ [M-H]$^+$: 317.0641, found: 317.0644; $[\alpha]_D$ -122.6º (22ºC, CH_2Cl_2, c=1.05).

6bb



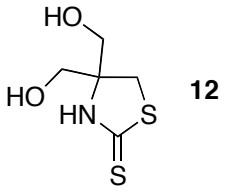
(2*R,5*R*,8S***)-2,8-di(-*p*-chlorophenyl)-1-aza-3-oxa-7-thiabicyclo[3.3.0]octane (6bb)** Prepared in 99.9%de, 60% yield, as a white solid analogous to the route described for **6aa**: mp 83.2 - 83.5 ºC; IR (KBr) ν = 3054, 2934, 1489, 1375, 1088, 828 cm^{-1}; ^1H NMR ($CDCl_3$), δ 2.90 (dd, *J* = 3.4, 11.5 Hz, 1 H), 3.09 (dd, *J* = 6.8, 11.5 Hz, 1 H), 3.77 (dd, *J* = 6.1, 8.4 Hz, 1 H), 4.16 (m, 1 H), 4.40 (dd, *J* = 6.5, 8.4 Hz, 1 H), 5.22 (s, 1 H), 5.31 (s, 1H), 7.25 (m, 2 H), 7.34 (m, 4 H), 7.47 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 36.4, 71.8, 72.8, 96.0, 128.1, 128.3, 128.7, 129.1, 133.1, 134.9, 137.6, 140.4; HRMS calculated for $C_{17}H_{15}Cl_2NOS$ [M-H]$^+$: 351.0251, found: 351.0236. $[\alpha]_D$ -122.7º (22ºC, CH_2Cl_2, c=1.00).

Procedure for thiazolidine-oxazolidine exchange



A mixture of compound **6aa** (100 mg, 0.36 mmol) and *p*-Cl-benzaldehyde (50 mg, 0.36 mmol) in CH_2Cl_2 (35.5 mL) and *p*-TsOH ac. (10 mg, 0.13 mmol,) was stirred at rt for 2 d. The reaction mixture was poured into water, the pH adjusted to pH 7 and extracted with CH_2Cl_2 (3x30 mL). The combined organic layers were dried, filtered and the solvent was removed under reduced pressure. The residue was analyzed by ^{1}H NMR to determine the product ratio: **6aa** (55 %), **6ab** (31 %), and **5a** (14%) and purified by chromatography on SiO_2 (AcOEt/*n*-hexanes 1:9) to give a mixture of **6aa + 6ab** (190 mg, 85 %) and **5a** (12 %) yields. Compounds were identified by running pure samples of compounds **6aa**, **6ab**, **6ba** and **5b** by HPLC in a CHIRALCEL OD column (0.46cm ID x 25 cm) using an isocratic system isopropanol/*n*-Hexanes (10:90), l = 210 or 190 nm. The compounds were identified at the following times: **5b**: *t* = 3.3 min, **6ab**: *t* = 5.5 min; **6aa**: *t* = 6.5 min; **6ba**: *t* = 6.8 min.

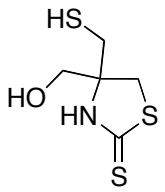
Path a)



12

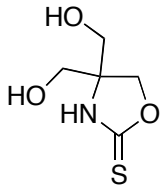
4,4-Bis(hydroxymethyl)thiazolidine-2-thione (12). To an ice cooled solution of NaOH (5.0 g, 0.12 mol) in H_2O (9 ml) was added with stirring Tris·HCl (5.0 g, 31.8 mmol), CS_2 (4.8 ml, 79.2 mmol) and PEG 400 (2 drops). The mixture was heated at 60 ºC for 24 h, then cooled down, poured into H_2O (100 ml) and extracted with EtOAc (3 x 100 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure. The yellow oil crude was purified by chromatography on SiO_2 (1:2, EtOAc:hexanes) to afford **12** (2.57 g, 45%), **14** (0.65 g, 11%) and **15** (0.91 g, 18%).

Thiazolidinethione 12: white solid: mp 90-91°C; ^{1}H NMR (CD_3OD) δ 3.45 (s, 2 H), 3.63 (d, *J* = 11.5 Hz, 2 H), 3.70 (d, *J* = 11.5 Hz, 2 H), 4.88 (bs, 2H); ^{13}C NMR (CD_3OD) δ 36.5, 63.3, 76.4, 202.0; HRMS calculated for $C_5H_9NO_2S_2$, $[M]^+$ 179.0075, found 179.0035.



14

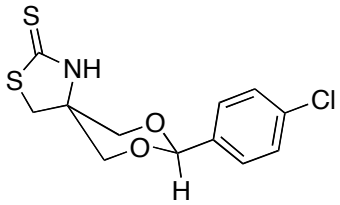
4-Hydroxymethyl-4-thiomethyl-thiazolidine-2-thione (14): oil; IR (NaCl) ν = 3300-3200 bs, 2926, 2513, 1732, 1458, 1007; ^{1}H NMR (CD_3CN) δ 1.78 (dd, *J* = 9.7, 8.4 Hz, 1 H_{SH}), 2.79 (dd, *J* = 14.3, 9.7 Hz, 1 H), 2.93 (dd, *J* = 14.3, 8.4 Hz, 1 H), 3.41 (d, *J* = 11.6 Hz, 1 H), 3.44 (t, *J* = 5.9 Hz, 1 H_{OH}), 3.53 (d, *J* = 11.6 Hz, 1 H), 3.62 (dd, *J* = 11.4, 5.9 Hz, 1 H), 3.68 (dd, *J* = 11.4, 5.9 Hz, 1 H) 7.96 (s, 1 H_{NH}); ^{13}C NMR (CD_3CN) δ 30.0, 38.4, 64.8, 75.5, 201.4 (C=S); HRMS calculated for $C_5H_{10}NOS_3$, $[M+H]^+$ 195.9925, found 195.9957.



15

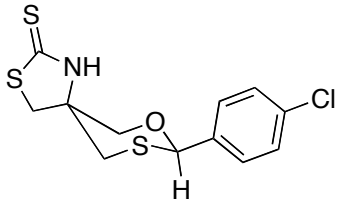
4,4-Bis(hydroxymethyl)oxazolidine-2-thione (15): solid: mp 111-112 °C; ^{1}H NMR (DMSO-d_6) δ 3.37 (d, *J* = 5.6 Hz, 4 H), 4.36 (s, 2 H), 5.16 (t, *J* = 5.6 Hz, 1 H), 5.17 (t, *J* = 5.6 Hz, 1 H), 9.84 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 62.1, 67.9, 73.1, 188.0; HRMS calculated for $C_5H_{10}NO_3S$, $[M+H]^+$ 164.0381, found 164.0389.

Typical procedure for preparation of spiro-thiazolidinthiones.



16a

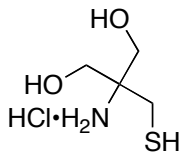
(***syn/anti*)-8-(4-Chlorophenyl)-7,9-dioxa-3-thia-1-azaspiro[4.5]decane-2-thione (16a)**. To a stirred solution of **12** (300 mg, 1.68 mmol) in MeCN (1ml) was added *p*-Cl-benzaldehyde (0.47 g, 3.4 mmol) and *p*-TsOH ac. (30 mg, 0.17 mmol). The reaction mixture was heated with stirring under microwave irradiation for 12 min at 90 ºC. Then it was cooled and poured into H_2O, neutralized with $NaHCO_3$ (sat solution) and extracted with EtOAc (3x30 ml). The solvent was removed under reduced pressure and the crude was purified by chromatography on SiO_2 (1:4, EtOAc:hexanes) to afford compound **16a** (206 mg, 43%, diasteromeric ratio = 7:3). Major diastereomer: white solid; mp 208-209 °C; IR (NaCl) ν = 3098, 2914, 1489, 1317, 1010, 829, 750; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 2 H), 3.87 (d, *J* = 11.0 Hz, 2 H), 4.20 (d, *J* = 11.0 Hz, 2 H), 5.56 (s, 1 H), 7.46 (m, 4 H), 10.29 (bs, 1 H_{NH}); ^{13}C NMR (DMSO-d_6) δ 39.7, 64.8, 71.0, 100.3, 128.6, 128.7, 134.1, 136.9, 199.8; HRMS calculated for $C_{12}H_{12}ClNO_2S_2$ $[M-H]^-$ 299.9925, found 299.9932.



17a

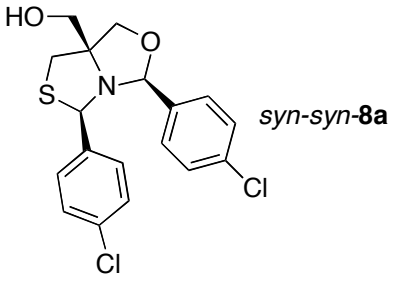
(**5*RS*,*syn/anti*)-8-(4-Chlorophenyl)-7-oxa-3,9-dithia-1-azaspiro[4.5]decane-2-thione (17a)**. Prepared in an analogous route as described for **16a** to give compound **17a** (225 mg, 45%, diastereomeric ratio = 8:2). Major diastereomer: solid: mp 75-76 °C; IR (NaCl) ν = 3209, 2993, 2853, 1489, 1190, 1032, 817; ^{1}H NMR (DMSO-d_6) δ 3.11 (dd, *J* = 12.9, 2.5 Hz, 1 H), 3.38 (d, *J* = 12.9 Hz, 1 H), 3.65 (d, *J* = 11.5 Hz, 1 H), 3.77 (d, *J* = 11.6 Hz, 1 H), 3.90 (d, *J* = 11.6 Hz, 1 H), 4.11 (dd, *J* = 11.5, 2.5 Hz, 1 H), 5.99 (s, 1 H), 7.45 (m, 4 H), 10.33 (s, 1 H_{NH}); ^{13}C NMR (DMSO-d_6) δ 35.6, 39.7, 64.4, 71.7, 81.9, 128.1, 128.4, 133.2, 137.0, 198.7 (C=S); HRMS calculated for $C_{12}H_{12}ClNOS_3$ [M-H] = 315.9697, found: 315.9704.

11



2-Amino-2-(thiomethyl)propane-1,3-diol Tris-SH·HCl (11). Diol **12** (400 mg, 2.24 mmol) was dissolved with stirring in conc. HCl (3 ml) and heated at reflux for 24h. Then, water (15 ml) was added and the mixture was washed with EtOAc (30 ml). The aqueous layer was evaporated at reduced pressure to afford compound **11** (350 mg, 90%). This residue was used without further purification. The ^{1}H NMR signals are in agreement with the reported spectroscopic data.[12] ^{1}H NMR (DMSO-d_6) δ 2.70 (d, J = 9.0 Hz, 2 H), 2.86 (t, J = 9.0 Hz, 1 H_{SH}), 3.48 (d, J = 11.5 Hz, 2 H) 3.54 (d, J = 11.5 Hz, 2 H), 5.44 (bs, 2 H), 7.95 (s, 2 H).

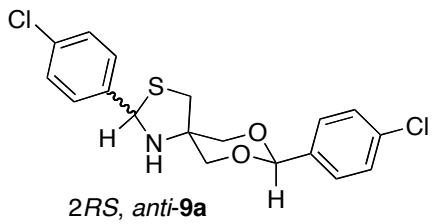
Path a) Representative procedure for preparation of thiazolidinyl-oxazolidines.



syn-syn-**8a**

(2*S*,5*S*,8*R*) and (2*R*,5*R*,8*S*)-2,8-di(-*p*-chlorophenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo [3.3.0]octane (*syn-syn*-8a). To a suspension of Tris-SH (**11**) (200 mg, 1.16 mmol) in PhMe (5 ml), and DMF (0.5 ml) were added *p*-Cl-benzaldehyde (404 mg, 2.87 mmol) and catalytic *p*-TsOH ac. (2 mg, 0.01 mmol). The mixture was heated at reflux in a Dean-Stark trap for 3 h. Then, it was cooled and poured into a saturated solution of $NaHCO_3$, extracted with EtOAc (3x50 ml), dried (Na_2SO_4) and filtered. The residue was purified by chromatography on SiO_2 (1:5, EtOAc:hexanes) to give *syn-syn*-**8a** (140 mg, 32%), and spiro-*anti*-**9a** (15 mg, 3%). *syn-syn*-**8a**: oil; IR (NaCl) ν = 3450-3350 bs, 2924, 1599, 1487, 1275, 1088, 1015, 825, 750; ^{1}H NMR ($CDCl_3$) δ 1.65 (t, J = 5.3 Hz, 1 H), 2.93 (d, J = 12.0 Hz, 1 H), 2.98 (d, J = 12.0 Hz, 1 H), 3.49 (dd, J = 10.5, 5.3 Hz, 1 H), 3.56 (dd, J = 10.5, 5.3 Hz, 1 H), 3.86 (d, J = 8.8 Hz, 1 H), 4.29 (d, J = 8.8 Hz, 1 H), 5.19 (s, 1 H), 5.31 (s, 1 H), 7.24 (d, J = 8.4 Hz, 2 H), 7.37 (m, 4 H), 7.46 (d, J = 8.4 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.2, 66.9, 73.4, 74.2, 79.7, 98.4, 128.5, 128.7,

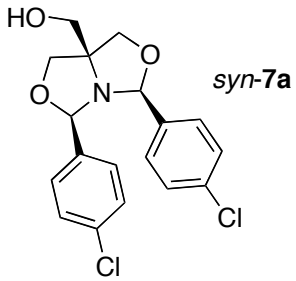
128.9, 129.0, 133.6, 135.2, 137.3, 139.2; HRMS calculated for $C_{18}H_{17}Cl_2NO_2SNa$ $[M+Na]^+$ 404.0249, found 404.0239.



2*RS*, *anti*-**9a**

(2*RS,anti*)-2,8-Bis(3-chlorophenyl)-7,9-dioxa-3-thia-1-azaspiro[4.5]decane (9a): white solid: mp 164-165 °C; IR (NaCl) ν = 2868, 1603, 1493, 1385, 1094, 1015, 824; ^{1}H NMR ($CDCl_3$) δ 3.25 (dd, *J* = 11.3 Hz, ^4J = 1.1 Hz, 1 H), 3.58 (d, *J* = 11.3 Hz, 1 H), 3.82 (dd, *J* = 10.8 Hz, ^4J = 1.1 Hz, 1 H), 4.01 (m, 2 H), 4.25 (dd, *J* = 10.8 Hz, ^4J = 2.2 Hz, 1H), 5.46 (s, 1 H), 5.48 (s, 1 H), 7.34 (m, 4 H), 7.44 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 42.1, 64.1, 69.3, 73.4, 73.7, 101.1, 127.5, 128.5, 128.6, 128.8, 134.3, 135.0, 136.0; HRMS calcd for $C_{18}H_{18}Cl_2NO_2S$ $[M+H]^+$ 382.0430, found 382.0443.

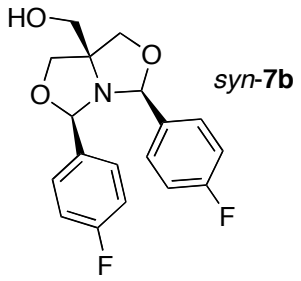
Path b) Representative procedure for the synthesis of bisoxazolidines *syn*-7a-e.



syn-**7a**

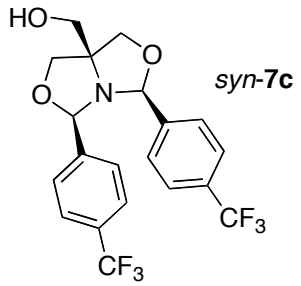
(*2R,8S*)-2,8-di(-*p*-Chlorophenyl)-5-hydroxymethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-7a). To a stirred suspension of Tris·HCl (**10**) (1.0 g, 6.4 mmol) in toluene (25 ml), were added *p*-Cl-benzaldehyde (2.0 g, 14.0 mmol) and catalytic *p*-TsOH ac. (0.1 g, 0.6 mmol). The mixture was heated at reflux in a Dean-Stark trap for 12 h. Then it was cooled and poured into a saturated solution of $NaHCO_3$, extracted with EtOAc (3x100 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:9, EtOAc:hexanes) to afford **7a** (1.8 g, 77%, *syn/anti*: 98:2) as a yellow oil; IR (NaCl) ν = 3500-3400 bs, 2872, 1597, 1489, 1379, 1207, 1088, 1014, 812; ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 2 H), 3.91 (d, *J* = 9.0 Hz, 2 H), 4.02 (d, *J* = 9.0 Hz, 2 H), 5.53 (s, 2 H), 7.35

(m, 8H); ^{13}C NMR ($CDCl_3$) δ 65.5, 72.6, 74.9, 96.6, 128.1, 128.8, 134.6, 137.9; HRMS calculated for $C_{18}H_{18}Cl_2NO_3$ $[M+H]^+$ 366.0664, found 366.0632.



syn-**7b**

(*2R,8S*)-2,8-di(*p*-Fluorophenyl)-5-hydroxymethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-7b). Prepared in an analogous route as described for *syn*-**7a** to give compound **7b** (34%, *syn/anti*: 98:2). *syn*-**7b**: the spectroscopic data for *syn*-**7b** is in agreement with the literature data:[4] IR (NaCl) ν = 3500-3400 b, 2935, 2874, 2367, 2345, 1607, 1508, 1225, 1078, 1007, 837; ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 2 H), 3.92 (d, *J* = 9.2 Hz, 2 H), 4.03 (d, *J* = 9.2 Hz, 2 H), 5.54 (s, 2 H), 7.04 (m, 4 H), 7.41 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 65.6, 72.6, 74.9, 96.6, 115.4 (d, J_{CF} = 21.4 Hz), 115.5 (d, J_{CF} = 21.2 Hz), 128.5 (d, J_{CF} = 7.7 Hz), 128.6 (d, J_{CF} = 7.5 Hz), 135.2 (d, J_{CF} = 3.0 Hz), 162.9 (d, J_{CF} = 245.8 Hz).

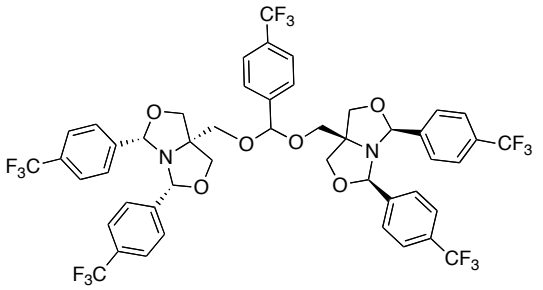


syn-**7c**

(*2R,8S*)-2,8-di(-*p*-Trifluoromethylphenyl)-5-hydroxymethyl-1-aza-3,7-dioxabicyclo [3.3.0] octane (***syn*-7c**). Prepared in an analogous route as described for *syn*-**7a** to give compounds **7c** (32%, *syn/anti*: 81:19) and dimer **19c** (13%). *syn*-**7c**: the sample had spectroscopic data in agreement with the literature data:[10] white solid: mp 100-101 °C; IR (NaCl) ν = 2936, 2874, 1618, 1412, 1327, 1165, 1124, 1018, 827; ^{1}H NMR ($CDCl_3$) δ 1.58 (t, *J* = 5.6 Hz, 1 H_{OH}), 3.49 (d, *J* = 5.6 Hz, 2 H), 3.97 (d, *J* = 9.0 Hz, 2 H), 4.06 (d, *J* = 9.0 Hz, 2 H), 5.63 (s, 2 H), 7.56 (d, *J* = 8.3 Hz, 4 H), 7.64 (d, *J* = 8.3 Hz, 4

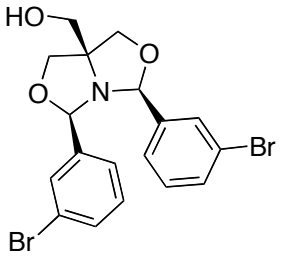
[4] Desino, K. E.; Ansar, S.; Georg, G. I.; Himes, R. H.; Michaelis, M. L.; Powell, D.; Reiff, E. A.; Telikepalli, H.; Audus, K. L. *J. Med. Chem.* **2009**, *52*, 7537–7543.

H); ^{13}C NMR ($CDCl_3$) δ 65.6, 72.7, 75.1, 96.8, 123.9 (q, J_{CF} = 270.5 Hz), 125.6 (q, J_{CF} = 3.6 Hz), 127.12, 131.0 (q, J_{CF} = 32.5 Hz), 143.4.



19c

(*2R,8S*)-{Bis(2,8-di(-*p*-trifluoromethylphenyl)-1-aza-3,7-dioxabicyclo[3.3.0] octane 5-methoxy) methyl)}-4-trifluoromethylbenzene (19c): pale yellow oil; IR (NaCl) ν = 3067, 2879, 1620, 1412, 1327, 1124, 1067, 827; ^{1}H NMR ($CDCl_3$) δ 3.23 (d, *J* = 8.9 Hz, 2 H), 3.30 (d, *J* = 8.9 Hz, 2 H), 3.76 (d, *J* = 9.0 Hz, 2 H), 3.82 (d, *J* = 9.0 Hz, 2 H), 3.84 (s, 4 H), 5.36 (s, 1 H), 5.55 (s, 2 H), 5.56 (s, 2 H), 7.23 (d, *J* = 8.1 Hz, 2 H), 7.53 (m, 18 H); ^{13}C NMR ($CDCl_3$) δ 69.4, 73.0, 73.2, 73.3, 96.4, 96.5, 96.5, 100.7, 123.9 (q, J_{CF} = 271.8 Hz), 125.3 (q, J_{CF} = 3.6 Hz), 126.4, 126.5, 126.7, 127.2, 127.3, 130.9 (q, J_{CF} = 32.0 Hz), 140.3, 143.1; HRMS calculated for $C_{48}H_{38}F_{15}N_2O_6$ $[M+H]^+$ 1023.2490, found 1023.2466.

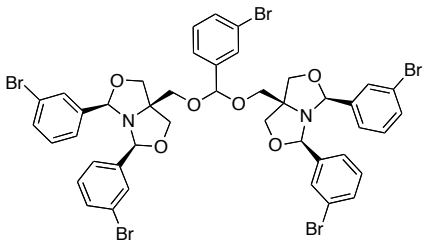


7d

(*2R,8S*)-2,8-di(-*m*-Bromophenyl)-5-hydroxymethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-**7d**). Prepared in analogous route as described for **7a** to give compounds **7d** (30% yield, *syn/anti*: 80:20) and dimer **19d** (25% yield). *syn*-**7d**: white solid: mp 103-104 °C; IR (NaCl) ν = 3500-3400, 2934, 2874, 1570, 1202, 1082, 1007, 773; ^{1}H NMR ($CDCl_3$) δ 1.64 (bs, 1 H_{OH}), 3.49 (s, 2 H), 3.93 (d, *J* = 9.0 Hz, 2 H), 4.04 (d, *J* = 9.0 Hz, 2 H), 5.52 (s, 2 H), 7.24 (t, *J* = 7.8 Hz, 2 H), 7.35 (d, *J* = 7.8 Hz, 2 H), 7.47 (m, 2 H), 7.61 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 65.6, 72.6, 74.7, 95.9, 126.1, 128.7, 130.6, 132.7, 132.8, 139.6; HRMS calculated for $C_{18}H_{17}Br_2NO_3Na$ $[M+Na]^+$ 475.9467, found 475.9448.

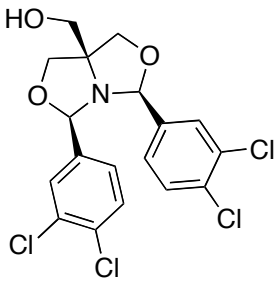
anti-**7d**: pale yellow oil; IR (NaCl) ν = 3500-3400, 2870, 1572, 1475, 1433, 1377, 1207, 1070, 758; ^{1}H-NMR ($CDCl_3$) δ 2.36 (bs, 1 H_{OH}), 3.70 (d, *J* = 11.1 Hz, 1 H), 3.75 (d, *J* = 11.1 Hz, 1 H), 3.82 (d, *J* = 8.8 Hz, 1 H), 3.86 (d, *J* = 8.9 Hz, 1 H), 4.08 (d, *J* = 8.9 Hz, 1 H), 4.19 (d, *J* = 8.8 Hz, 1 H), 5.08 (s, 1 H), 5.46 (s, 1 H), 6.86 (d, *J* = 7.6 Hz, 1 H), 7.03 (d, *J* = 7.6 Hz, 1 H), 7.06 (s, 1 H), 7.08 (d, *J* = 7.6 Hz, 1 H), 7.20 (d, *J* = 7.6 Hz, 1 H), 7.34 (m, 1 H), 7.40 (m, 1 H), 7.45 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 65.1, 71.7, 74.4, 74.5, 92.5, 93.1, 122.0, 122.4, 125.6, 125.7, 129.5, 129.6, 130.2, 130.3, 131.6, 131.8, 136.0, 141.7; HRMS calculated for $C_{18}H_{18}NO_3Br_2$ $[M+H]^+$ 453.9653, found 453.9665.

19d



(*2R,8S*)-{Bis(2,8-di(-*m*-bromophenyl)-1-aza-3,7-dioxabicyclo[3.3.0] octane 5-methoxy) methyl)}-3-bromobenzene (19d): pale yellow oil; IR (NaCl) ν = 3061, 2870, 1572, 1469, 1377, 1199, 1086, 779; ^{1}H NMR ($CDCl_3$) δ 3.25 (d, *J* = 9.1 Hz, 2 H), 3.28 (d, *J* = 9.1 Hz, 2 H), 3.77 (d, *J* = 9.0 Hz, 2 H), 3.81 (s, 2 H), 3.84 (s, 2H), 3.89 (d, *J* = 9.0 Hz, 2 H), 5.31 (s, 1 H), 5.44 (s, 2 H), 5.48 (s, 2 H), 7.18 (m, 5 H), 7.31 (m, 5 H), 7.44 (m, 5 H), 7.58 (bs, 5 H); ^{13}C NMR ($CDCl_3$) δ 69.4, 72.9, 73.2, 73.5, 96.0, 96.4, 100.7, 122.4, 122.5, 125.6, 125.7, 129.4, 129.9, 130.0, 130.1, 131.7, 131.8, 132.0, 139.1, 141.5, 141.6; HRMS calculated for $C_{43}H_{37}N_2O_6Br_5Na$ $[M+Na]^+$ 1098.8425, found 1098.8402.

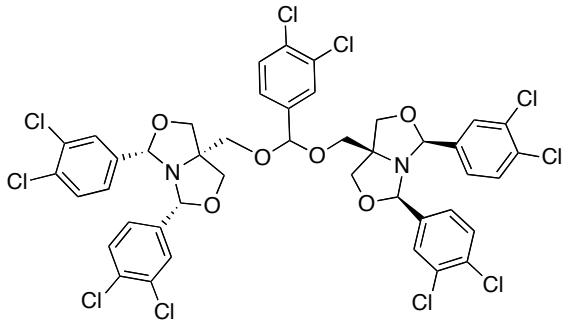
7e



(*2R,8S*)-2,8-Di(-3,4-dichlorophenyl)-5-hydroxymethyl-1-aza-3,7 dioxabicyclo [3.3.0] octane (***syn*-7e**). Prepared in an analogous route as described for **7a** to give **7e** (20% yield, *syn/anti*: 83:17) and dimer **19e** (35% yield). *syn*-**7e**: oil; IR (NaCl) ν = 3500 bs, 2936, 2874, 1468, 1204, 1086, 1032, 828, 756; ^{1}H NMR ($CDCl_3$) δ 3.50 (s, 2 H), 3.92 (d, *J* = 9.0 Hz, 2 H), 4.03 (d, *J* = 9.0 Hz, 2 H), 5.49 (s, 2 H), 7.23 (m, 2 H), 7.45 (d,

J = 8.3 Hz, 2 H), 7.54 (d, *J* = 2.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 65.7, 72.7, 74.9, 96.1, 126.1, 128.8, 130.7, 132.8, 132.9, 139.7; HRMS calculated for $C_{18}H_{15}Cl_4NO_3Na$ $[M+Na]^+$ 455.9698, found 455.9683.

anti-**7e**: white solid: mp 122-124°C; IR (NaCl) ν = 3500-3400 bs, 2870, 1472, 1412, 1366, 1213, 1082, 1032, 827; ^{1}H NMR ($CDCl_3$) δ 3.71 (d, *J* = 11.1 Hz, 1 H), 3.76 (d, *J* = 11.1 Hz, 1 H), 3.83 (*J* = 8.9 Hz, 1 H), 3.88 (d, *J* = 9.0 Hz, 1 H), 4.09 (d, *J* = 9.0 Hz, 1 H), 4.19 (d, *J* = 8.9 Hz, 1 H), 5.04 (s, 1 H), 5.45 (s, 1 H), 6.82 (dd, *J* = 8.3, 2.0 Hz, 1 H), 7.03 (d, *J* = 2.0 Hz, 1 H), 7.12 (dd, *J* = 8.3, 1.7 Hz, 1 H), 7.27 (d, *J* = 8.3 Hz, 1 H), 7.31 (d, *J* = 8.3 Hz, 1 H), 7.38 (d, *J* = 1.7 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 65.1, 72.0, 74.4, 74.5, 92.0, 92.6, 126.3, 126.4, 129.2, 129.3, 130.1, 130.2, 132.3, 132.6, 132.7, 133.1, 134.0, 139.7; HRMS calculated for $C_{18}H_{16}NO_3Cl_4$ $[M+H]^+$ 433.9884, found 433.9862.

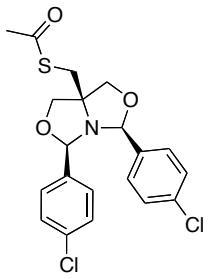


19e

(*2R,8S*)-{Bis(2,8-di(-*m*-*p*-dichlorophenyl)-1-aza-3,7-dioxabicyclo[3.3.0] octane 5-methoxy) methyl)}-3,4-dichlorobenzene (19e): pale yellow solid: mp 79-90 °C; IR (NaCl) ν = 3065, 2872, 1566, 1469, 1367, 1204, 1032, 825, 737; ^{1}H NMR ($CDCl_3$) δ 3.22 (d, *J* = 9.1 Hz, 2 H), 3.26 (d, *J* = 9.1 Hz, 2 H), 3.75 (d, *J* = 9.1 Hz, 2 H), 3.82 (d, *J* = 9.1 Hz, 2 H), 3.83 (d, J = 9.2 Hz, 2 H), 3.86 (d, *J* = 9.2 Hz, 2 H), 5.30 (s, 1H), 5.41 (s, 2 H), 5.44 (s, 2 H), 6.95 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.19 (m, 4 H), 7.25 (d, *J* = 2.0 Hz, 1H), 7.37 (t, *J* = 8.3 Hz, 5 H), 7.49 (d, *J* = 1.9 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 69.1, 73.0, 73.2, 95.7, 95.9, 126.2, 126.3, 128.9, 129.0, 130.5, 132.6, 132.8, 132.9, 139.4; HRMS calculated for $C_{43}H_{32}Cl_{10}N_2O_6Na$ $[M+Na]^+$ 1044.9038, found 1044.9040.

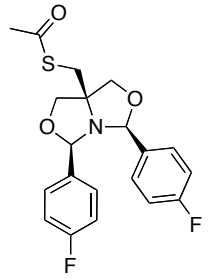
Representative procedure for the synthesis of 5-acetylhythiomethyl-bisoxazolidines 20a-e.

syn-**20a**



(*2R,8S*)-2,8-Di(4-chlorophenyl)-5-acetylthiomethyl-1-aza-3,7-dioxabicyclo [3.3.0]octane (*syn*-20a). Triphenylphosphine (1.4 g, 5.4 mmol) was dissolved in benzene (3 ml) with stirring at rt. Molecular sieves 4Å were added and the mixture was cooled down to 0 ºC. Then diethyl azodicarboxylate (0.86 ml, 5.4 mmol) was added over 5 min. After stirring for 30 min, a solution of compound *syn*-**7a** (0.9 g, 2.46 mmol) in 1 ml of benzene was added, and stirring was continued for 10 min. To the resulting suspension was added drop wise thioacetic acid (0.38 ml, 5.4 mmol) and stirring was continued for another hour at rt. The mixture was heated for 5 h to reflux and stirred at rt overnight. The solvent was removed under reduced pressure and the crude residue was purified by chromatography on SiO_2 (1:4, EtOAc:hexanes) to afford compound *syn*-**20a** (0.78 g, 75%) as a light yellow oil: IR (NaCl) ν = 2868, 1693, 1489, 1267, 1121, 1089, 1015, 812, 627; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3 H), 3.04 (s, 2 H), 3.85 (d, *J* = 9.0 Hz, 2 H), 3.90 (d, *J* = 9.0 Hz, 2 H), 5.48 (s, 2 H), 7.34 (d, *J* = 8.4 Hz, 4 H), 7.44 (d, *J* = 8.4 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 36.2, 73.7, 74.3, 96.8, 128.5, 128.6, 132.2, 132.3, 134.6, 137.6, 194.9; HRMS calculated for $C_{20}H_{19}Cl_2NO_3SNa$ $[M+Na]^+$ 446.0355, found 446.0349.

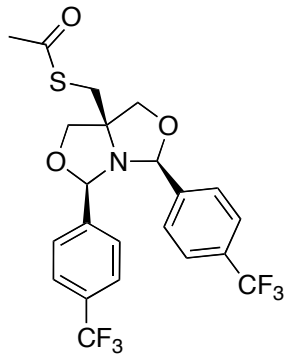
syn-**20b**



(*2R,8S*)-2,8-di(-*p*-Fluorophenyl)-5-acetylthiomethyl-1-aza-3,7-dioxabicyclo [3.3.0]octane (*syn*-20b). Prepared in an analogous route as described for **20a** to afford compound **20b** in 54% yield, as a white solid: mp 78-79 °C; IR (NaCl) ν = 2932, 2868, 1701, 1605, 1508, 1225, 1078, 1015, 837; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H), 3.05 (s, 2 H), 3.86 (d, *J* = 9.0 Hz, 2 H), 3.90 (d, *J* = 9.0 Hz, 2 H), 5.49 (s, 2 H), 7.05 (t, *J* =

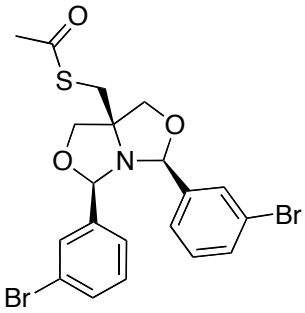
8.6 Hz, 4 H), 7.48 (d, *J* = 8.6 Hz, 2 H), 7.50 (d, *J* = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 36.4, 73.6, 74.4, 96.8, 115.3 (d, J_{CF} = 21.6 Hz), 129.0 (d, J_{CF} = 8.3 Hz), 134.8 (d, J_{CF} = 2.9 Hz), 163.0 (d, J_{CF} = 245.7 Hz), 195.0; HRMS calculated for $C_{20}H_{19}F_2NO_3SNa$ $[M+Na]^+$ 414.0946, found 414.0927.

syn-**20c**

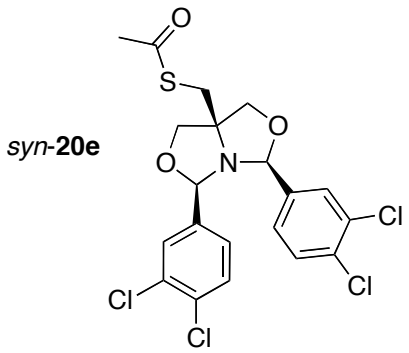


(*2R,8S*)-2,8-di(-*p*-Trifluoromethylphenyl)-5-acetylthiomethyl-1-aza-3,7-dioxabicyclo [3.3.0] octane (***syn*-20c**). Prepared in an analogous route as described for *syn*-**20a**, to afford compound **20c** in 71% yield as a white solid: mp 71-72 °C; IR (NaCl) ν = 3022, 2872, 1695, 1327, 1126, 1018, 827, 762; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3 H), 3.05 (s, 2 H), 3.87 (d, *J* = 9.1 Hz, 2 H), 3.94 (d, *J* = 9.1 Hz, 2 H), 5.58 (s, 2 H), 7.64 (m, 8 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 36.0, 73.9, 74.3, 97.0, 124.0 (q, J_{CF} = 271.0 Hz), 125.5 (q, J_{CF} = 3.7 Hz), 127.5, 130.8 (q, J_{CF} = 32.3 Hz), 130.9 (q, J_{CF} = 32.3 Hz), 143.0, 194.8; HRMS calculated for $C_{22}H_{19}F_6NO_3SNa$ $[M+Na]^+$ 514.0882, found 514.0909.

syn-**20d**



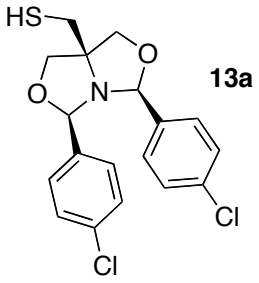
(*2R,8S*)-2,8-di(-*m*-Bromophenyl)-5-acetylthiomethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-20d). Prepared in an analogous route as described for **20a**, to afford compound **20d** in 84% yield as white solid: mp 101 °C (dec); IR (NaCl) ν = 2934, 2868, 1701, 1686, 1560, 1123, 1070, 773; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H), 3.06 (s, 2 H), 3.86 (d, *J* = 9.0 Hz, 2 H), 3.90 (d, *J* = 9.0 Hz, 2 H), 5.48 (s, 2 H), 7.25 (m, 2 H), 7.46 (m, 4 H), 7.67 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 36.1, 73.8, 74.3, 96.8, 122.6, 125.8, 130.1, 130.3, 131.8, 141.4, 194.8; HRMS calculated for $C_{20}H_{19}Br_2NO_3SNa$ $[M+Na]^+$ 533.9345, found 533.9347.



syn-**20e**

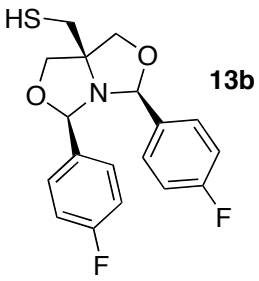
(*2R,8S*)-2,8-di(-*m,p*-Dichlorophenyl)-5-acetylthiomethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-20e). Prepared in an analogous route as described for **20a** to afford compound **20e** in 78% yield as white solid: mp 134-135 °C; IR (NaCl) ν = 2932, 2868, 1757, 1701, 1466, 1259, 1105, 1030; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H), 3.05 (s, 2 H), 3.85 (d, *J* = 9.0 Hz, 2 H), 3.90 (d, *J* = 9.0 Hz, 2 H), 5.45 (s, 2 H), 7.32 (dd, *J* = 8.2, 2.0 Hz, 2 H), 7.46 (d, *J* = 8.2 Hz, 2 H), 7.59 (d, *J* = 2.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.5, 36.0, 73.9, 74.3, 96.3, 126.5, 129.1, 130.6, 132.2, 132.3, 132.7, 132.9, 139.3, 194.7; HRMS calculated for $C_{20}H_{17}Cl_4NO_3SNa$ $[M+Na]^+$ 513.9575, found 513.9584.

Representative Procedure for the preparation of thiols *syn*-13a-e.



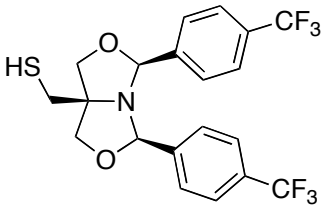
13a

(*2R,8S*)- and (*2S,8R*)-2,8-di(-4-Chlorophenyl)-5-thiomethyl-1-aza-3,7-dioxabicyclo [3.3.0] octane (*syn*-13a). To an ice cooled solution of MeOH saturated with NH_3 was added compound *syn*-**20a** (100 mg, 0.27 mmol). The reaction mixture was stirred at rt overnight under N_2. The solvent was removed under reduced pressure and the residue purified by chromatography (1:6 EtOAc/hexanes) to afford *syn*-**13a** (55 mg, 66% yield) as an oil: IR (NaCl) ν = 2868, 2804, 1701, 1605, 1508, 1225, 1078, 837; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, *J* = 8.8 Hz, 1 H_{SH}), 2.61 (d, *J* = 8.8 Hz, 2 H), 3.94 (d, *J* = 9.0 Hz, 2 H), 4.01 (d, *J* = 9.0 Hz, 2 H), 5.52 (s, 2 H), 7.33 (d, *J* = 8.2 Hz, 4 H), 7.42 (d, *J* = 8.2 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 32.0, 73.8, 75.2, 96.9, 128.4, 129.6, 134.5, 137.8; HRMS calculated for $C_{18}H_{17}Cl_2NO_2SNa$ $[M+Na]^+$ 404.0249, found, 404.0230.



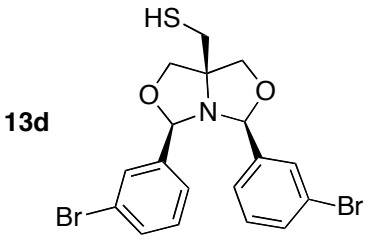
13b

(*2R,8S*)- and (*2S,8R*)-2,8-di(-4-Fluorophenyl)-5-thiomethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-13b). Prepared in an analogous route as described for **13a**, to afford **13b** (62%) as an oil: IR (NaCl) ν = 3073, 2934, 2868, 1605, 1506, 1225, 1153, 1076, 1015, 839; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, J = 8.6 Hz, 1 H_{SH}), 2.63 (d, J = 8.6 Hz, 2 H), 3.94 (d, J = 9.0 Hz, 2 H), 4.02 (d, J = 9.0 Hz, 2 H), 5.53 (s, 2 H), 7.04 (m, 4 H), 7.47 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 32.1, 73.9, 75.2, 97.0, 115.3 (d, J_{CF} = 21.7 Hz), 128.8 (d, J_{CF} = 8.3 Hz), 135.0 (d, J_{CF} = 3.0 Hz), 162.9 (d, J_{CF} = 245.7 Hz); HRMS calculated for $C_{18}H_{18}F_2NO_2S$ $[M+H]^+$ 350.1021, found 350.1025.



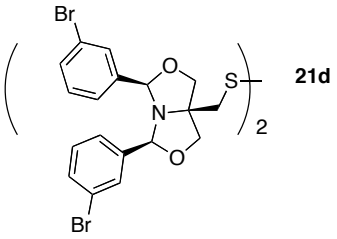
13c

(*2R,8S*)- and (*2S,8R*)-2,8-di(-4-Trifluoromethylphenyl)-5-thiomethyl-1-aza-3,7-dioxabicyclo [3.3.0] octane (*syn*-13c). Prepared in an analogous route as described for **13a**, to afford **13c** (65%) as a white solid: mp 63-64 °C; IR (NaCl) ν = 2938, 2870, 1412, 1327, 1124, 1067, 827; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, J = 8.6 Hz, 1 H_{SH}), 2.63 (d, J = 8.6 Hz, 2 H), 3.99 (d, J = 9.1 Hz, 2 H), 4.03 (d, J = 9.1 Hz, 2 H), 5.62 (s, 2 H), 7.62 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 31.8, 73.9, 75.5, 97.1, 123.9 (q, J_{CF} = 270.6 Hz), 125.5 (q, J_{CF} = 3.7 Hz), 127.4, 131.0 (q, J_{CF} = 32.0 Hz), 143.2; HRMS calculated for $C_{20}H_{17}F_6NO_2SNa$ $[M+Na]^+$ 472.0776, found 472.0767.



13d

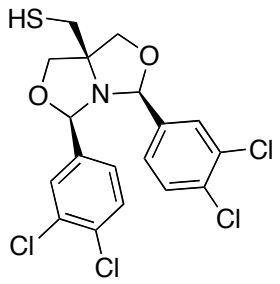
(*2R,8S*)- and (*2S,8R*)-2,8-di(-3-Bromophenyl)-5-thiomethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-13d). Prepared in an analogous route as described for **13a**, except that the mixture was stirred for 3h, to give a mixture of **13d** (44%), disulfide **21d** (43%) and recovered starting material **20d** (10%). *syn*-**13d**: white solid: mp 79-80 °C; IR (NaCl) ν = 3065, 2932, 2868, 1570, 1202, 1121, 997, 773; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, *J* = 8.6 Hz, 1 H_{SH}), 2.64 (d, *J* = 8.6 Hz, 2 H), 3.95 (d, *J* = 9.1 Hz, 2 H), 4.02 (d, *J* = 9.1 Hz, 2 H), 5.52 (s, 2 H), 7.24 (t, *J* = 7.8 Hz, 2 H), 7.40 (d, *J* = 7.8 Hz, 2 H), 7.46 (m, 2 H), 7.65 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 31.9, 73.8, 75.4, 96.9, 122.6, 125.7, 130.1, 131.8, 141.6; HRMS calculated for $C_{18}H_{16}Br_2NO_2S$ $[M-H]^+$ 467.9274, found 467.9294.



21d

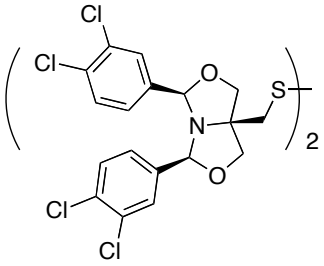
(*2R,8S*)- and (*2S,8R*)-Bis(2,8-di(-3-bromophenyl)-1-aza-3,7-dioxabicyclo[3.3.0] octane 5-methyldisulfide (*syn*-21d): oil; IR (NaCl) ν = 2868, 1572, 1469, 1435, 1199, 1119, 1069, 1009, 775; ^{1}H NMR ($CDCl_3$) δ 2.86 (s, 4 H), 3.87 (d, *J* = 9.1 Hz, 4 H), 4.01 (d, *J* = 9.1 Hz, 4 H), 5.47 (s, 4 H), 7.23 (t, *J* = 7.8 Hz, 4 H), 7.41 (d, *J* = 7.8 Hz, 4 H), 7.47 (d, *J* = 7.8 Hz, 4 H), 7.65 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ 47.5, 73.9, 74.4, 96.5, 122.5, 125.7, 130.0, 130.1, 131.8, 141.3. HRMS calculated for $C_{36}H_{33}Br_4N_2O_4S_2$ $[M+H]^+$ 936.8610, found 936.8592.

13e



(*2R,8S*)- and (*2S,8R*)-2,8-Di(-3,4-dichlorophenyl)-5-thiomethyl-1-aza-3,7-dioxabicyclo[3.3.0] octane (*syn*-13e). Prepared in an analogous route as described for **13a**, except that the mixture was stirred for 3h, to give a mixture of *syn*-**13e** (25%), disulfide **21e** (53%) and recovered starting material **20e** (10%). *syn*-**13e**: oil; IR (NaCl) ν = 2932, 2868, 1701, 1686, 1466, 1130, 1030, 827, 625; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, *J* = 8.6 Hz, 1 H_{SH}), 2.62 (d, *J* = 8.6 Hz, 2 H), 3.95 (d, *J* = 9.1 Hz, 2 H), 4.01 (d, *J* = 9.1 Hz, 2 H), 5.49 (s, 2 H), 7.30 (dd, *J* = 8.2, 2.1 Hz, 2 H), 7.44 (d, *J* = 8.2 Hz, 2 H), 7.57 (d, *J* = 2.1 Hz, 2 H) ; ^{13}C NMR ($CDCl_3$) δ 31.9, 73.8, 75.4, 96.4, 126.3, 129.0, 130.6, 132.7, 132.9, 139.5; HRMS calculated for $C_{18}H_{16}Cl_4NO_2S$ $[M+H]^+$ 449.9656, found 449.9647.

21e



(*2R,8S*)- and (*2S,8R*)-Bis(2,8-di(-3,4-dichlorophenyl)-1-aza-3,7-dioxabicyclo[3.3.0] octane 5-methyldisulfide (*syn*-21e): white solid: mp 147-148 °C; IR (NaCl) ν = 2932, 2870, 1468, 1367, 1204, 1113, 1030, 737; ^{1}H NMR ($CDCl_3$) δ 2.84 (s, 4 H), 3.87 (d, *J* = 9.2 Hz, 4 H), 3.99 (d, *J* = 9.2 Hz, 4 H), 5.43 (s, 4 H), 7.30 (dd, *J* = 8.3, 2.0 Hz, 4 H), 7.43 (d, *J* = 8.3 Hz, 4 H), 7.57 (d, *J* = 2.0 Hz, 4 H); ^{13}C NMR ($CDCl_3$) δ 47.6, 73.9, 74.4, 96.1, 126.4, 129.1, 130.6, 132.7, 133.0, 139.2.

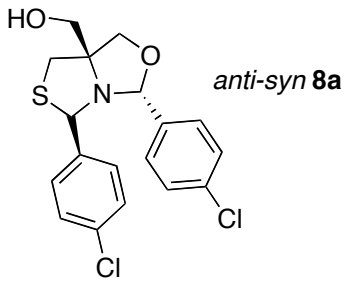
Reduction of disulfide 21d

To a stirred solution of dimer **21d** (90 mg, 0.10 mmol) in MeOH (3 ml) and CH_2Cl_2 (1 ml) was added portionwise $NaBH_4$ (21 mg, 0.60 mmol). The mixture was stirred at rt for 4h. Then, the solvent was removed under reduced pressure. The crude residue was poured into H_2O (15 ml), the pH was adjusted to 6 with HCl 5% and the mixture was extracted with CH_2Cl_2 (3x50 ml). The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:4, EtOAc:hexanes) to

afford *syn*-**13d** (20 mg, 25% yield, 60% yield based on recovered starting material **21d**).

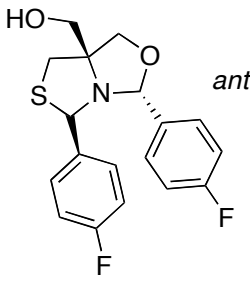
Reduction of disulfide 21e. Prepared in an analogous route as described for the reduction of disulfide **21d**, to give **13e** (40%, 70% yield based on recovered starting material **21e**).

Representative procedure for the synthesis of *anti-syn*-thiazolidinyl-oxazolidines 8a-d, conditions A.



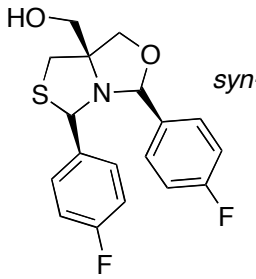
anti-syn **8a**

(2*R*,5*S*,8*R*)- and (2*S*,5*R*,8*S*)-2,8-di(-*p*-chlorophenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo [3.3.0] octane (*anti-syn*-**8a**). To a stirred solution of thiol *syn*-**13a** (40 mg, 0.10 mmol) in CH_2Cl_2 (8ml) was added *p*-TsOH ac. (4 mg, 0.02 mmol) and *p*-Cl benzaldehyde (0.10 mmol). The mixture was heated to reflux for 6 h and left overnight at rt. The solvent was removed under reduced pressure and the crude residue was poured into a saturated solution of $NaHCO_3$, extracted with EtOAc (3x50 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the crude residue was purified by chromatography on SiO_2 (1:4 EtOAc/hexanes) to afford compound **8a** as an oil (25 mg, 62%, *anti-syn/syn-syn* 99:1). ***anti-syn*-8a**: white solid: mp 106-107°C; IR (NaCl) ν = 3500-3400 bs, 2932, 2872, 1560, 1491, 1089, 1045, 1014, 827; ^{1}H NMR ($CDCl_3$) δ 2.33 (bs, 1 H_{OH}), 3.07 (d, *J* = 11.8 Hz, 1 H), 3.24 (d, *J* = 11.8 Hz, 1 H), 3.76 (d, *J* = 10.8 Hz, 1 H), 3.86 (d, *J* = 10.8 Hz, 1 H), 3.88 (d, *J* = 8.7 Hz, 1 H), 4.02 (d, *J* = 8.7 Hz, 1 H), 5.13 (s, 1 H), 5.54 (s, 1 H), 6.96 (d, *J* = 8.5 Hz, 2 H), 7.10 (d, *J* = 8.5 Hz, 2 H), 7.19 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 39.8, 65.8, 69.4, 72.8, 79.8, 94.6, 128.1, 128.2, 128.3, 128.7, 132.6, 133.1, 134.7, 140.3; HRMS calculated for $C_{18}H_{17}Cl_2NO_2SNa$ $[M+Na]^+$ 404.0249, found 404.0235.



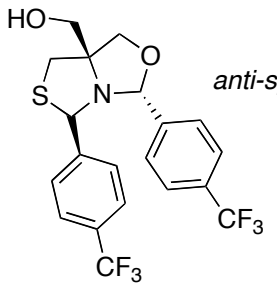
anti-syn **8b**

(2*R*,5*S*,8*R*)- and (2*S*,5*R*,8*S*)-2,8-Di(-4-fluorophenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo [3.3.0] octane (*anti-syn*-**8b**). Prepared under conditions A, in an analogous route as described for *anti-syn*-**8a,** to give **8b** (44% yield, *anti-syn/syn-syn* 90:10). *anti-syn*-**8b**: white solid: mp 90-91 °C; IR (NaCl) ν = 3500-3400 bs, 2929, 2872, 1605, 1510, 1385, 1227, 1155, 1047, 839; ^{1}H NMR ($CDCl_3$) δ 2.37 (bs, 1 H_{OH}), 3.09 (d, *J* = 11.8 Hz, 1 H), 3.23 (d, *J* = 11.8 Hz, 1 H), 3.77 (d, *J* = 10.9 Hz, 1 H), 3.86 (d, *J* = 10.9 Hz, 1 H), 3.87 (d, *J* = 8.8 Hz, 1 H), 4.02 (d, *J* = 8.8 Hz, 1 H), 5.18 (s, 1 H), 5.53 (s, 1 H), 6.80 (t, *J* = 8.8 Hz, 2 H), 6.87 (t, *J* = 8.8 Hz, 2 H), 6.97 (dd, J_{HH} = 8.5 Hz, J_{HF} = 5.5 Hz, 2 H), 7.23 (dd, J_{HH} = 8.5 Hz, J_{HF} = 5.5 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.9, 65.8, 69.4, 72.7, 79.6, 94.6, 114.8 (d, J_{CF} = 21.5 Hz), 114.9 (d, J_{CF} = 21.4 Hz), 128.4 (d, J_{CF} = 8.0 Hz), 129.2 (d, J_{CF} = 8.2 Hz), 129.8 (d, J_{CF} = 3.3 Hz), 137.1 (d, J_{CF} = 2.8 Hz), 162.0 (d, J_{CF} = 244.7 Hz), 162.8 (d, J_{CF} = 246.5 Hz); HRMS calculated for $C_{18}H_{18}F_2NO_2S$ $[M+H]^+$ 350.1021, found 350.1022.



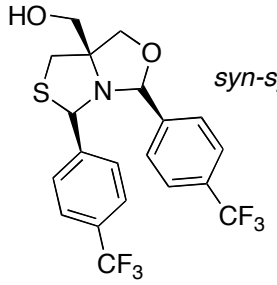
syn-syn **8b**

syn-syn-**8b**: pale yellow oil; IR (NaCl) ν = 3350-3450 bs, 2924, 2855, 1604, 1508, 1226, 1045, 837; ^{1}H NMR ($CDCl_3$) δ 2.93 (d, *J* = 12.0 Hz, 1 H), 3.00 (d, J = 12.0 Hz, 1 H), 3.50 (d, *J* = 10.0 Hz, 1 H), 3.55 (d, *J* = 10.0 Hz, 1 H), 3.86 (d, *J* = 8.8 Hz, 1 H), 4.28 (d, *J* = 8.8 Hz, 1 H), 5.21 (s, 1 H), 5.32 (s, 1 H), 6.95 (t, *J* = 8.6 Hz, 2 H), 7.10 (t, *J* = 8.6 Hz, 2 H), 7.41 (dd, J_{HH} = 8.6 Hz, J_{HF} = 5.2 Hz, 2 H), 7.51 (dd, J_{HH} = 8.6 Hz, J_{HF} = 5.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.3, 67.0, 73.4, 74.3, 79.7, 98.4, 115.2 (d, J_{CF} = 21.3 Hz), 115.6 (d, J_{CF} = 21.8 Hz), 129.1 (d, J_{CF} = 8.2 Hz), 129.5 (d, J_{CF} = 8.7 Hz) 134.7 (d, J_{CF} = 2.8 Hz), 136.3 (d, J_{CF} = 3.1 Hz), 162.3 (d, J_{CF} = 247.0 Hz), 163.3 (d, J_{CF} = 247.6 Hz); HRMS calculated for $C_{18}H_{17}F_2NO_2S$ $[M+H]^+$ 350.1021, found 350.1024.



anti-syn **8c**

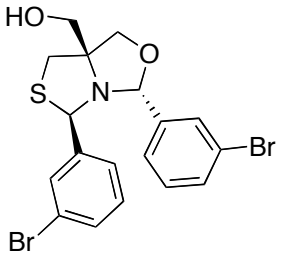
(2*R*,5*S*,8*R*) and (2*S*,5*R*,8*S*) 2,8-Di(-4-trifluoromethylphenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo [3.3.0]octane (*anti-syn*-8c). Prepared in an analogous route as described for *anti-syn*-**8a**, to give **8c** (64%, *anti-syn/syn-syn* 81:19). *anti-syn*-**8c**: white solid: mp 106-107 °C; IR (NaCl) ν = 3309, 2932, 2870, 1738, 1697, 1327, 1163, 1126, 1069, 837; ^{1}H NMR ($CDCl_3$) δ 2.30 (t, *J* = 5.6 Hz, 1 H), 3.12 (d, *J* = 11.9 Hz, 1 H), 3.30 (d, *J* = 11.9 Hz, 1 H), 3.81 (dd, *J* = 10.9, 5.6 Hz, 1 H), 3.91 (d, *J* = 8.8 Hz, 1 H), 3.93 (dd, *J* = 10.9, 5.6 Hz, 1 H), 4.06 (d, *J* = 8.8 Hz, 1 H), 5.15 (s, 1 H), 5.61 (s, 1 H), 7.10 (d, *J* = 8.2 Hz, 2 H), 7.36 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 39.7, 66.0, 69.5, 73.0, 79.7, 94.2, 124.9 (q, J_{CF} = 3.5 Hz), 126.4 (q, J_{CF} = 276.0 Hz), 127.14, 127.72, 129.7 (q, J_{CF} = 32.0 Hz), 130.9 (q, J_{CF} = 32.1 Hz), 137.78, 145.2; HRMS calculated for $C_{20}H_{17}F_6NO_2SNa$ $[M+Na]^+$ 472.0776, found 472.0773.



syn-syn **8c**

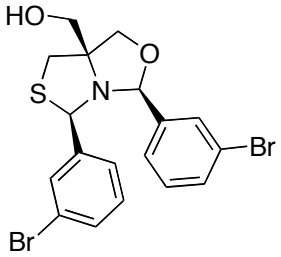
syn-syn-**8c**: oil; IR (NaCl) ν = 3500-3400 bs, 2934, 2876, 1419, 1327, 1124, 1069, 839; ^{1}H NMR ($CDCl_3$) δ 1.64 (t, *J* = 5.0 Hz, 1 H), 2.98 (s, 2 H), 3.52 (dd, *J* = 10.6, 5.0 Hz, 1 H), 3.63 (dd, *J* = 10.6, 5.0 Hz, 1 H), 3.90 (d, *J* = 8.8 Hz, 1 H), 4.34 (d, *J* = 8.8 Hz, 1 H), 5.28 (s, 1 H), 5.41 (s, 1 H), 7.53 (d, *J* = 8.7 Hz, 2 H), 7.57 (d, *J* = 8.7 Hz, 2 H), 7.64 (d, *J* = 8.6 Hz, 2 H), 7.68 (d, *J* = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.2, 67.0 , 73.7, 74.3, 80.0, 98.4, 123.9 (q, J_{CF} = 270.8 Hz), 125.3 (q, J_{CF} = 3.4 Hz), 125.7 (q, J_{CF} = 3.7 Hz), 127.7, 127.9, 130.1 (q, J_{CF} = 32.4 Hz), 131.5 (q, J_{CF} = 32.1 Hz) 142.8, 144.7 (q, J_{CF} = 1.0 Hz); HRMS calculated for $C_{20}H_{18}F_6NO_2S$ $[M+H]^+$ 450.0957, found 450.0953.

anti-syn **8d**

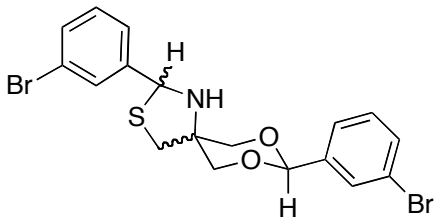


(2*R*,5*S*,8*R*) and (2*S*,5*R*,8*S*) 2,8-Di(-3-bromophenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo[3.3.0] octane (*anti-syn*-8d). Prepared in an analogous route as described for *anti-syn*-**8a**, to give a mixture of **8d** (64%, *anti-syn/syn-syn* (95:5) and spiro-**9d** (20%, diastereomeric ratio = 7:3). *anti-syn*-**8d**: oil; IR (NaCl) ν = 3500-3400 bs, 3067, 2928, 2872, 1570, 1213, 1045, 997, 787, 727; ^{1}H NMR ($CDCl_3$) δ 2.46 (bs, 1 H_{OH}), 3.08 (d, *J* = 11.9 Hz, 1 H), 3.26 (d, *J* = 11.9 Hz, 1 H), 3.77 (d, *J* = 10.9 Hz, 1 H), 3.86 (d, *J* = 8.7 Hz, 1 H), 3.87 (d, *J* = 10.9 Hz, 1 H), 4.01 (d, *J* = 8.7 Hz, 1 H), 5.11 (s, 1 H), 5.49 (s, 1 H), 6.99 (m, 3 H), 7.10 (t, *J* = 1.8 Hz, 1 H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.25 (m, 1 H), 7.37 (d, *J* = 7.8 Hz, 1 H), 7.42 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 39.7, 65.9, 69.5, 72.7, 79.6, 94.1, 121.9, 122.3, 125.5, 125.9, 129.4, 129.5, 130.0, 130.6, 130.7, 131.9, 136.0, 143.5; HRMS calculated for $C_{18}H_{18}Br_2NO_2S$ $[M+H]^+$ 469.9420, found 469.9432.

syn-syn **8d**

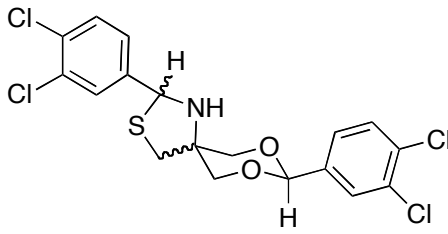


syn-syn-**8d**: oil; IR (NaCl) ν = 3500-3400 bs, 2928, 2870, 1574, 1469, 1433, 1367, 1209, 1068, 997, 785; ^{1}H NMR ($CDCl_3$) δ 1.65 (bs, 1 H_{OH}), 2.94 (d, *J* = 12.1 Hz, 1 H), 3.00 (d, *J* = 12.1 Hz, 1 H), 3.51 (d, *J* = 10.6 Hz, 1 H), 3.60 (d, *J* = 10.6 Hz, 1 H), 3.86 (d, *J* = 8.8 Hz, 1 H), 4.30 (d, *J* = 8.8 Hz, 1 H), 5.20 (s, 1 H), 5.29 (s, 1 H), 7.15 (t, *J* = 7.9 Hz, 1 H), 7.29 (t, *J* = 7.9 Hz, 1 H), 7.35 (m, 2 H), 7.44 (d, *J* = 7.9 Hz, 1 H), 7.52 (d, *J* = 7.9 Hz, 1 H), 7.66 (d, *J* = 7.9 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 39.2, 66.9, 73.4, 74.3, 79.8, 98.3, 122.6, 122.7, 126.0, 126.3, 129.9, 130.3, 130.5, 130.8, 130.9, 132.5, 141.1, 143.1; HRMS calculated for $C_{18}H_{17}Br_2NO_2SNa$ $[M+Na]^+$ 491.9239, found 491.9215.



Spiro **9d**

(2*SR, syn/anti*)-2,8-Bis(3-bromophenyl)-7,9-dioxa-3-thia-1-azaspiro[4.5]decane (spiro-9d). Major product: pale yellow oil; IR (NaCl) ν = 3065, 2857, 1570, 1474, 1425, 1379, 1207, 1107, 783; ^{1}H NMR ($CDCl_3$) δ 2.71 (d, *J* = 10.9 Hz, 1 H), 2.85 (d, *J* = 10.9 Hz, 1 H), 3.83 (d, *J* = 11.7 Hz, 1 H), 4.07 (d, *J* = 11.4 Hz, 1 H), 4.15 (dd, *J* = 11.4 Hz, ^4J = 2.9 Hz, 1 H), 4.35 (dd, *J* = 11.7, ^4J = 2.9 Hz, 1 H), 5.53 (s, 1 H), 5.58 (s, 1 H), 7.22 (m, 2 H), 7.42 (m, 4 H), 7.67 (m, 1 H), 7.74 (m, 1 H); HRMS calculated for $C_{18}H_{16}Br_2NO_2S$ $[M-H]^+$ 467.9274, found 467.9292.



Spiro **9e**

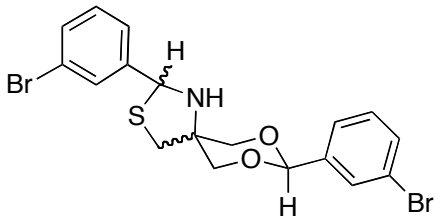
(2*SR,syn/anti*) 2,8-Bis(3,4-dichlorophenyl)-7,9-dioxa-3-thia-1-azaspiro[4.5]decane (spiro-9e). Prepared in an analogous route as described for *anti-syn*-**8a**, to give spiro-**9e** (80%, *syn/anti* 8:2).

syn-**9e**: solid: mp 108-109 °C; IR (NaCl) ν = 3323.35, 2926, 2855, 1472, 1375, 1101, 1031, 822; ^{1}H NMR ($CDCl_3$) δ 2.70 (d, *J* = 10.9 Hz, 1 H), 2.84 (d, *J* = 10.9 Hz, 1 H), 2.93 (bs, 1H), 3.83 (d, *J* = 11.5 Hz, 1 H), 4.05 (d, *J* = 11.5 Hz, 1 H), 4.13 (dd, *J* = 11.5, 2.9 Hz, 1 H), 4.33 (dd, *J* = 11.5, 2.9 Hz, 1 H), 5.51 (s, 1 H), 5.55 (s, 1 H), 7.33 (td, *J* = 8.4, 2.0 Hz, 2 H), 7.40 (d, *J* = 8.3 Hz, 1 H), 7.44 (d, *J* = 8.3 Hz, 1 H), 7.61 (d, *J* = 2.0 Hz, 1 H), 7.68 (d, *J* = 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 36.4, 63.6, 66.4, 71.3, 74.8, 100.1, 125.4, 126.8, 128.2, 129.4, 130.2, 130.3, 132.2, 132.5, 132.6, 133.1, 137.4, 140.8; HRMS calculated for $C_{18}H_{16}Cl_4NO_2S$ $[M+H]^+$ 449.9650, found 449.9634.

Representative procedure for the synthesis of *syn-syn*-8a and 8d, conditions B.

(2*S*,5*S*,8*R*) and (2*R*,5*R*,8*S*)-2,8-Di(-*p*-chlorophenyl)-5-hydroxymethyl-1-aza-3-oxa-7-thiabicyclo [3.3.0] octane (***syn-syn*-8a).** To a stirred solution of thiol *syn*-**13a** (100 mg, 0.28 mmol) in PhMe (10 ml) was added *p*-TsOH ac. (10 mg, 0.058 mmol) and *p*-Cl benzaldehyde (40 mg, 0.28 mmol). The mixture was heated at reflux in a Dean-Stark trap for 5h. The solvent was removed under reduced pressure and the crude residue was poured into a saturated solution of $NaHCO_3$, extracted with EtOAc (3x50 ml), dried and filtered. The solvent was removed under reduced pressure and the crude residue was purified by chromatography on SiO_2 (1:5, EtOAc:hexanes) to afford a mixture of **8a** (41 mg, 41%, *anti-syn/syn-syn* 10:90) and spiro-*anti*-**9a** (46 mg, 46% yield).

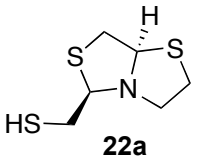
Synthesis of *syn-syn*-8d, conditions B. Prepared in analogous route as described for *syn-syn*-**8a** (method B), except the reaction mixture was heated for 2h, to give a mixture of *syn-syn*-**8d** (66% yield, *anti-syn/syn-syn* 7:93) and spiro-**9d** (20% yield, *anti*/*syn* 8:2).



Spiro **9d**

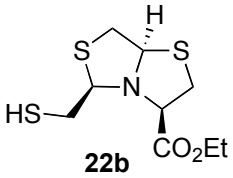
(2*RS*, *anti*)-9d: light yellow oil, ^{1}H NMR ($CDCl_3$) δ 3.25 (dd, *J* = 11.2 Hz, ^4J = 1.4 Hz, 1 H), 3.58 (d, *J* = 11.2 Hz, 1 H), 3.82 (dd, *J* = 10.8 Hz, ^4J = 1.4 Hz, 1 H), 4.01 (s, 1 H), 4.02 (d, ^4J = 2.4 Hz, 1 H), 4.25 (d, *J* = 10.8 Hz, ^4J = 2.4 Hz, 1 H), 5.46 (s, 1 H), 5.47 (s, 1 H), 7.24 (m, 2 H), 7.45 (m, 4 H), 7.67 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 42.1, 64.1, 69.2, 73.4, 73.8, 100.8, 122.4, 122.7, 124.8, 126.0, 129.3, 129.9, 130.2, 130.3, 131.6, 132.2, 139.5, 142.1. HRMS calculated for $C_{18}H_{17}Br_2NO_2SNa$ $[M+Na]^+$ 491.9239, found 491.9215.

Representative procedure for the preparation of bis-thiazolidines



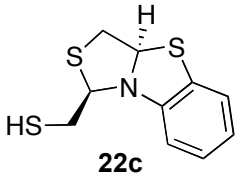
22a

(5*RS*,7a*RS*)-Tetrahydro-2*H*-thiazolo[4,3-*b*]thiazol-5-yl)methanethiol (*anti*-22a). To a stirred suspension of cysteamine·HCl (**24a**) (0.32 g, 2.8 mmol) in EtOH (15 ml), was added Et_3N (0.39 ml, 2.8 mmol), 1,4-dithiane-2,5-dithiol **25** (0.51 g, 3.4 mmol) and *p*-TsOH ac.(0.025 g, 0.12 mmol). The mixture was heated at reflux for 2 h. Then it was cooled and poured into a saturated solution of NaCl (15ml), extracted with CH_2Cl_2 (5x30 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:6 EtOAc:hexanes) to afford **22a** (0.42 g, 78%, *anti/syn* 92:08) as an oil: *anti*-**22a**; ^{1}H NMR ($CDCl_3$) δ 1.84 (dd, *J* = 9.1, 7.6 Hz, 1H), 2.65 (ddd, *J* = 13.6, 9.1, 6.0 Hz, 1H), 2.82 (ddd, 13.6, 7.6, 7.0 Hz, 1H), 3.08 (m, 2H), 3.12 (m, 1H), 3.21 (ddd, 11.5, 7.2, 6.6 Hz, 1H), 3.48 (ddd, 11.5, 5.7, 4.8 Hz, 1H), 3.54 (ddd, *J* = 11.6, 5.2, 0.5 Hz, 1H), 4.24 (dd, *J* = 7.0, 6.0 Hz, 1H), 4.97 (dd, *J* = 5.2, 3.7 Hz, 1H), ^{13}C NMR ($CDCl_3$) δ 31.8, 33.5, 38.4, 57.1, 73.2, 74.5; HRMS calculated for $C_6H_{12}NS_3$, $[M]^+$ 194.0132, found: 194.0156.



22b

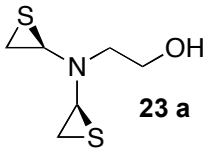
(3*S*,5*R*,7a*R*)- Ethyl 5-(thiomethyl)tetrahydro-2*H*-thiazolo[4,3-*b*]thiazole-3-carboxylate (*anti-anti*-22b). Prepared in an analogous route as described for *anti*-**22a**, starting from L-Cysteine ethyl ester·HCl (**24b**). Purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) led to compound **22b** (86%, *anti-anti/syn-syn* 95:05) as an oil: *anti-anti*-**22b**: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, *J* = 7.1 Hz, 3H), 1.98 (dd, *J* = 9.5, 7.5 Hz, 1H), 2.64 (ddd, *J* = 13.6, 9.5, 6.1 Hz, 1H), 2.89 (ddd, *J* = 13.6, 7.5, 7.5 Hz, 1H), 3.09 (dd, *J* = 11.8, 3.9 Hz, 1H), 3.28 (dd, *J* = 10.9, 6.6 Hz, 1H), 3.32 (dd, *J* = 10.9, 5.1 Hz, 1H), 3.54 (dd, *J* = 11.8, 5.4 Hz, 1H), 4.21 (dd, *J* = 6.6, 5.1 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 4.30 (dd, *J* = 7.5, 6.1 Hz, 1H), 5.09 (dd, *J* = 5.4, 3.9 Hz, 1H); ^{13}C NMR (CD_3Cl) δ 14.1, 33.7, 34.2, 39.1, 61.7, 70.3, 73.3, 74.9, 170.4; HRMS calculated for $C_9H_{16}NO_2S_3$, $[M+H]^+$ 266.0343, found 266.0329; $[\alpha]_D$ = -256 º (20 ºC, MeOH, c = 0.6).



22c

(2*RS*, 5*RS*) 1-Thiomethyl-3,3a-dihydro-benzo[*d*]thiazolo[4,3-b]thiazole (*anti*-22c). To a stirred suspension of **24c** (0.20 g, 1.6 mmol) in EtOH (7 ml), was added 1,4-dithiane-2,5-dithiol **25** (0.51 g, 3.4 mmol) and *p*-TsOH ac. (0.025 g, 0.12 mmol). The mixture was heated at reflux for 2 h. It was then cooled down and poured into brine (15 ml), extracted with CH_2Cl_2 (5x30 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:3, EtOAc:hexanes) to give compound **22c** (0.34 g, 89%, *anti/syn*: 99:01) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.91 (dd, *J* = 10.8, 6.3 Hz, 1H), 2.72 (ddd, *J* = 13.8, 10.8, 4.8 Hz, 1H), 2.93 (dd, *J* = 11.8, 8.7 Hz, 1H), 2.99 (ddd, *J* = 13.8, 9.0, 6.3 Hz, 1H), 3.23 (dd, *J* = 11.8, 5.3 Hz, 1H), 5.11 (dd, *J* = 8.7, 5.3 Hz, 1H), 5.19 (dd, *J* = 9.0, 4.8 Hz, 1H), 6.88 (m, 2H), 7.11 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 33.5, 40.0, 70.2, 70.5, 110.9, 122.2, 123.0, 124.5, 126.2, 145.2; HRMS calculated for $C_{10}H_{12}NS_3$ $[M+H]^+$ 242,0132, found 242.0119.

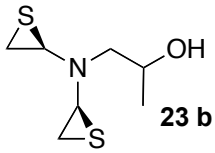
Representative procedure for the preparation of bis-thiiranes



23 a

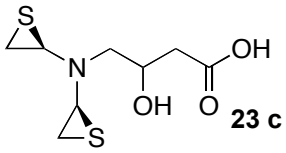
meso-2-(Di(thiiran-2-yl)amino)etanol (23a). To a stirred solution of ethanolamine **26a** (0.30 g, 4.1 mmol) in EtOH (8 ml), was added 1,4-dithiane-2,5-dithiol **25** (0.70 g, 4.9 mmol) and *p*-TsOH ac. (3 mg, 0.17 mmol). The mixture was heated at reflux for 1.30 h, and stirred overnight at rt under N_2. Then it was cooled down and poured into brine (15ml), extracted with EtOAc (5x30 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:1 EtOAc:hexanes) to afford **23a** (0.48 g, 66%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.39 (t, *J* = 9.9 Hz, $1H_{OH}$), 2.59 (m, 1H), 2.72 (m, 1H), 3.25 (d, *J* = 9.5 Hz, 2H), 3.35 (dd, *J* =

9.5, 3.0 Hz, 2H), 3.72 (m, 2H), 4.91 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 43.9, 50.7, 60.5, 70.0; HRMS calculated for $C_6H_{12}NOS_2$ $[M+H]^+$ 178.0360, found 178.0350.



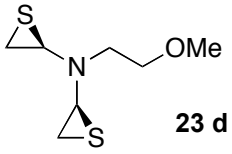
23 b

Diasteromeric mixture (2*RS*) 1-(Di(thiiran-2-yl)amino)propan-2-ol (23b). Prepared in an analogous route as described for **23a**, using 2-propanolamine **26b** as starting material. Purification on SiO_2 (1:2 EtOAc:*n*-hexanes) led to **23b** (0.21 g, 100%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.19 (t, *J* = 6.4 Hz, 6H), 2.30 (m, 2H), 2.53 (m, 2H), 2.68 (bs, H_{OH}), 2.93 (bs, H_{OH}), 3.24 (d, *J* = 9.5 Hz, 2H), 3.26 (d, *J* = 9.5 Hz, 2H), 3.33 (dd, *J* = 9.5, 3.0 Hz, 2H), 3.38 (dd, *J* = 9.5, 3.1 Hz, 2H), 3.87 (m, 2H), 4.86 (d, *J* = 3.0 Hz, 2H), 4.91 (d, *J* = 3.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 19.9, 20.4, 43.5, 44.3, 56.3, 56.5, 65.4, 65.5, 70.2, 70.3; HRMS calculated for $C_7H_{14}NOS_2$ $[M+H]^+$ 192.0517, found 192.0505.



23 c

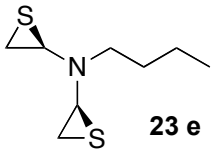
Diasteromeric mixture (3*RS*) 3-Hydroxy-4-(di-thiiran-2-yl-amino)butanoic acid (23c). Prepared in an analogous route as described for **23a**, using **26c** as starting material. Purification by chromatography on SiO_2 (1:1:0.05 EtOAc:hexanes:AcOH) led to **23c** (0.10 g, 64%) as a solid; ^{1}H NMR (CD_3OD) δ 2.55 (m, 4H), 3.09 (d, *J* = 9.3 Hz, 1H), 3.10 (d, *J* = 9.3 Hz, 1H), 3.32 (m, 2H), 4.19 (m, 1H), 5.00 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR (CD_3OD) δ 41.3, 44.4, 44.5, 54.9, 55.0, 68.4, 68.5, 71.9, 72.0, 175.3, 175.4; HRMS calculated for $C_8H_{14}NO_3S_2$ $[M+H]^+$ 236.0415, found 236.0404.



23 d

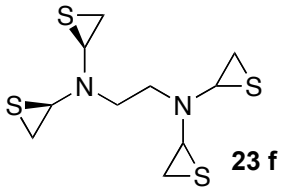
Meso *N*-(2-methoxyethyl)-*N*-(thiiran-2-yl)thiiran-2-amine (23d). Prepared in an analogous route as described for **23a**, using **26d** as starting material. Purification by chromatography on SiO_2 (1:4 EtOAc:hexanes) led to **23d** (0.19 g, 91%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.57 (m, 1H), 2.73 (m, 1H), 3.20 (d, *J* = 9.5 Hz, 2H), 3.35 (dd, *J* = 9.5,

3.0 Hz, 2H), 3.37 (s, 3H), 3.57 (apt, *J* = 5.2 Hz, 2H), 4.99 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 43.6, 48.2, 58.8, 70.6, 71.8; HRMS calculated for $C_7H_{14}NOS_2$ $[M+H]^+$ 192.0517, found 192.0506.



23 e

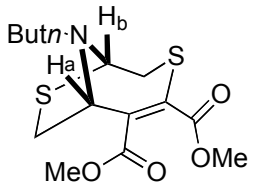
Meso *N*-butyl-*N*-(thiiran-2-yl)thiiran-2-amine (23e). Prepared in an analogous route as described for **23a**, using butylamine **26e** as starting material. Purification by chromatography on SiO_2 (1:10 EtOAc:hexanes) led to **23e** (0.23 g, 89%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.3 Hz, 3H), 1.38 (m, 2H), 1.54 (m, 2H), 2.37 (m, 1H), 2.48 (m, 1H), 3.21 (d, *J* = 9.4 Hz, 2H), 3.33 (dd, *J* = 9.4, 3.0 Hz, 2H), 4.86 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 13.9, 20.5, 30.8, 43.9, 48.4, 69.9; HRMS calculated for $C_8H_{16}NOS_2$ $[M+H]^+$ 190.0724, found 190.0713.



23 f

Diasteromeric mixture *N*^{1}-((*RS*)-thiiran-2-yl)-*N*^{1}-((*SR*)-thiiran-2-yl)-*N*^{2},*N*^{2}-di(thiiran-2-yl)ethane-1,2-diamine (23f). To a stirred suspension of etilendiamine·2HCl (**26f**) (0.30 g, 2.3 mmol) in EtOH (8 ml), was added 1,4-dithiane-2,5-dithiol **25** (0.70 g, 4.5 mmol), Et_3N (0.5 ml, 4.5 mmol) and *p*-TsOH ac. (3 mg, 0.17 mmol). The mixture was heated at reflux for 1h30. Then it was cooled down and poured into a saturated solution of NaCl, extracted with EtOAc (5x30 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:6 EtOAc:hexanes) to afford **23f** (0.33 g, 51%) as a oil: ^{1}H NMR ($CDCl_3$) δ 2.67 (m, 4H), 3.22 (d, *J* = 9.5 Hz, 2H), 3.23 (d, *J* = 9.5 Hz, 2H), 3.32 (dd, *J* = 3.0, 9.5 Hz, 2H), 3.34 (dd, *J* = 3.0, 9.5 Hz, 2H), 5.00 (d, *J* = 3.0 Hz, 2H), 5.01 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 43.6, 43.8, 47.7, 70.2, 70.3; HRMS calculated for $C_{10}H_{17}N_2S_4$ $[M+H]^+$ 293,0275, found 293.0260.

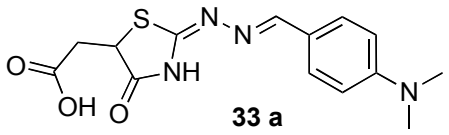
Synthesis of thiazolidinyl-thiazepine 28:



28

(1*SR*, 6*SR*)-dimethyl 9-butyl-3,8-dithia-9-azabicyclo[4.2.1]non-4-ene-4,5-dicarboxylate (28): To a stirred solution of bis-thiirane **23e** (52 mg, 0.27 mmol) in CH_2Cl_2 (3 mL), was added DMAD **27** (0.07 mL, 0.28 mmol) and *p*-TsOH ac. (5 mg, 0.017 mmol). The mixture was stirred overnight at rt under N_2. Then, it was poured into a saturated solution of NaCl, extracted with EtOAc (5x30 ml), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:6 EtOAc:hexanes) to afford **28** (53 mg, 61%) as a oil. ^{1}H NMR ($CDCl_3$) δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.36 (m, 2H), 1.59 (m, 2H), 2.49 (m, 1H), 2.62 (m, 1H), 2.99 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.24 (dd, *J* = 11.0, 3.3 Hz, 1H), 3.39 (dd, *J* = 14.0, 3.1 Hz, 1H), 3.49 (dd, *J* = 11.0, 9.2 Hz, 1H), 3.77 (s, 3H), 3.80 (s, 3H), 4.73 (ddd, ^2J = 9.2, 3.3, ^4J = 0.8 Hz, 1H), 4.97 (ddd, ^2J = 6.0, 3.1, ^4J = 0.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 13.90, 20.2, 31.1, 38.4, 45.0, 52.7, 53.0, 57.0, 68.6, 76.0, 138.6, 142.7, 166.0, 167.0; HRMS calculated for $C_{14}H_{21}NO_4S_2$ $[M+H]^+$ 332.0990, found 332.0975.

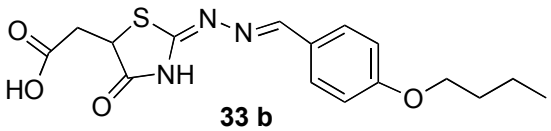
Typical procedure for thiazolidinone preparation



33 a

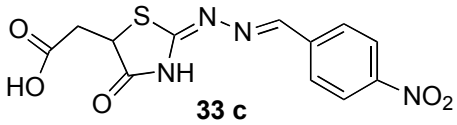
(*R,S*) 2-(4-dimethylaminebenzylidene)hydrazolyl-5-acetic acid-4-thiazolidinone (33a) To a stirred solution of *p*-*N*,*N*-dimethylamine benzaldehyde (300 mg, 2.0 mmol) in toluene (1 mL) and DMF (1 mL), were added thiosemicarbazide (220 mg, 2.4 mmol), *p*-TsOH ac. (30 mg, 0.2 mmol) and maleic anhydride (987 mg, 10.0 mmol). The reaction mixture was heated in a microwave for 9 min at 120 ºC (200 W) with stirring, poured into water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) filtered and concentrated. The residue was finally recrystalized from methanol to give **33c** (527 mg, 82 % yield) as a yellow solid mp 278-279 ºC: ^{1}H NMR (D_2O/K_2CO_3) δ 2.42 (dd, J_2 = 11.7, J_3 = 16.2 Hz, 1H), 2.93 (s, 6 H),

3.06 (dd, J_2 = 4.1, J_3= 16.2 Hz, 1H), 4.22 (dd, J_1 = 4.1, J_2 = 11.7 Hz, 1H), 6.88 (d, J = 9.0 Hz, 2H), 7.61 (d, J = 9.0 Hz, 2H), 8.20 (s, 1H); ^{13}C NMR (D_2O/K_2CO_3) δ 40.0, 41.9 (C_{endo}), 42.0 (C_{exo}), 49.9, 113.4, 122.8, 129.2, 152.8, 155.9, 161.0, 178.9 (C_{exo}), 179.4 (C_{endo}), 190.8 (COOH); HRMS calculated for $C_{14}H_{16}N_4O_3S$ [M^+-H]: 319.0865, found: 319.0859.



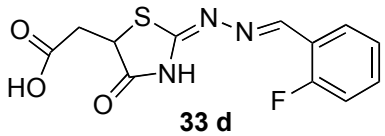
33 b

(*R,S*) 2-(4-butoxybenzylidene)hydrazolyl-5-acetic acid-4-thiazolidinone (33b). Typical procedure for thiazolidinone preparation was followed using aldehyde **29b** to give thiazolidinone **33b** (63% yield) as a white solid mp 249-250 ºC. ^{1}H NMR (D_2O/K_2CO_3), δ 0.68 (t, J_3 = 7.4 Hz, 3H), 1.14 (m, 2H), 1.42 (m, 2H), 2.30 (dd, J_3 = 11.0, J_2 = 16.3, 1H), 2.86 (dd, J_2 = 16.3, J_3 = 3.7 Hz, 1H), 3.64 (t, J_3 = 6.6 Hz, 2H), 4.04 (dd, J_3 = 3.7 Hz, J_3 = 11.0, 1H), 6.63 (d, J_3 = 8.6 Hz, 2H), 7.40 (d, J_3 = 8.6, 2H), 8.04 (s, 1H). ^{13}C NMR (D_2O/K_2CO_3), δ 13.6, 18.9, 30.8, 42.1, 49.9, 68.3, 100.1, 115.0, 127.2, 129.5, 160.6, 167.0, 179.2 ($C_{4\text{-exo}}$), 180.8 ($C_{4\text{-endo}}$), 191.9.



33 c

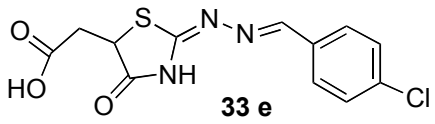
(*R,S*) 2-(4-nitrobenzylidene)hydrazolyl-5-acetic acid-4-thiazolidinone (33c). Typical procedure for thiazolidinone preparation was followed using aldehyde **29c** to give thiazolidinone **33c** (61% yield) as a white solid mp 270-271 ºC. IR (KBr) ν = 2946, 2779, 1716, 1641, 1527, 1344, 1263, 846. ^{1}H NMR (DMSO-d_6) δ 2.34 (dd, J_3 = 11.5, J_2 = 16.3 Hz, 1H), 2.93 (dd, J_2 = 3.7, J_3= 16.3 Hz, 1H), 4.09 (dd, J_3 = 3.7, J_3 = 11.5 Hz, 1H), 7.73 (d, J_3 = 8.8 Hz, 2H), 8,12 (d, J_3 = 8.8 Hz, 2H), 8.16 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 37.0, 44.1, 124.5, 129.0, 140.8, 148.7, 154.7, 167.2, 172.1, 175.9.



33 d

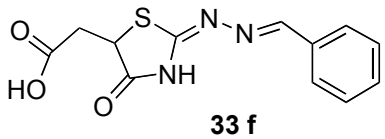
(*R,S*) 2-(2-fluorobenzylidene)hydrazolyl-5-acetic acid-4-thiazolidinone (33d). Typical procedure for thiazolidinone preparation was followed using aldehyde **29d** to give thiazolidinone **33d** (57% yield) as a white solid mp 272-273 ºC. IR (KBr) ν = 3079, 2775, 2467, 1731, 1683, 1595, 1407, 1231, 947, 759. ^{1}H NMR (DMSO-d_6) δ 2.91 (dd,

J_1 = 8.8, J_2 = 17.4 Hz, 1H), 3.03 (dd, J_1 = 3.6, J_2 = 17.4 Hz, 1H), 4.39 (dd, J_1 = 3.6, J_2 = 8.8 Hz, 1H), 7.29-7.33 (m, 2H), 7.53 (dd, J_1 = 6.8, J_2 = 14.0 Hz, 1H), 7.92 (t, J_1 = 7.5, 1H), 8.51 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 37.0, 44.0, 116.6, 122.1, 125.4, 127.7, 133.1, 149.4, 160.1, 162.6, 172.2, 176.0.



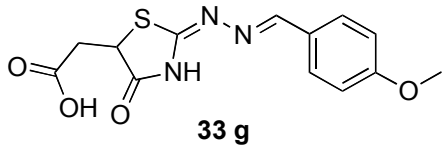
33 e

(*R,S*) 2-(4-chlorobenzylidene)hydrazolyl-5-acetic acid-4-thiazolidinone (33e). Typical procedure for thiazolidinone preparation was followed using aldehyde **29e** to give thiazolidinone **33e** (70% yield) as a white solid mp 273-274 ºC. IR (KBr) ν = 3089, 2964, 2784, 1734, 1689, 1629, 1404, 1294, 1089, 824. ^{1}H NMR (D_2O/K_2CO_3) δ 2.31 (dd, J_2 = 11.5, J_3 = 16.2 Hz, 1H), 2.92 (dd, J_2 = 3.6, J_3 = 16.2 Hz, 1H), 4.07 (dd, J_3 = 3.6, J_3 = 11.5 Hz, 1H), 7.28 (d, J_3 = 8.5Hz, 2H), 7.50 (d, J_3 = 8.5Hz, 2H), 8.09 (s, 1H). ^{13}C NMR (D_2O/K_2CO_3) δ 41.9, 50.0, 128.9, 132.7, 135.7, 153.7, 166.6, 179.3 ($C_{5\text{-exo}}$), 182.1 ($C_{5\text{-endo}}$), 191.7.



33 f

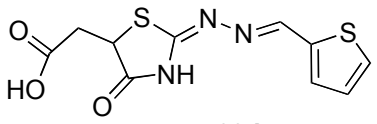
(*R,S*) 2-(benzylidene hydrazolyl)-5-acetic acid-4-thiazolidinone (33f). Typical procedure for thiazolidinone preparation was followed using aldehyde **29f** to give thiazolidinone **33f** (45% yield) as a white solid mp 242-243 ºC. ^{1}H NMR (D_2O/K_2CO_3) δ 2.31 (dd, J_3 = 11.5, J_3 = 16.2 Hz, 1H), 2.92 (dd, J_2 = 3.6, J_3 = 16.2 Hz, 1H), 4.08 (dd, J_2 = 3.6, J_3 = 11.5 Hz, 1H), 7.34 (m, 3H), 7.62 (dd, J_1 = 2.6, J_2 = 6.3 Hz, 2H), 8.2 (s, 1H). ^{13}C NMR (D_2O/K_2CO_3) δ 41.9, 48.9 ($C_{5\text{-endo}}$), 50.0 ($C_{5\text{-exo}}$), 127.6, 129.0, 130.8, 134.1, 155.2, 166.4, 179.4 ($C_{5\text{-exo}}$), 181.8 ($C_{5\text{-endo}}$), 191.7.



33 g

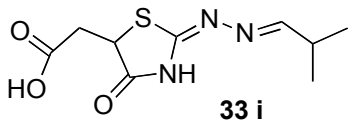
(*R,S*) 2-(4-metoxybenzylidene hydrazolyl)-5-acetic acid-4-thiazolidinone (33g). Typical procedure for thiazolidinone preparation was followed using aldehyde **29h** to give thiazolidinone **33g** (70% yield) as a white solid mp 262-263 ºC. IR (KBr) ν = 3079,

2952, 1729, 1690, 1605, 1513, 1255, 1169, 1007, 831. ^{1}H NMR (DMSO-d_6) δ 2.88 (dd, J_2 = 17.6, J_3 = 8.8 Hz, 1H), 3.01 (dd, J_2= 17.6, J_3= 3.8 Hz, 1H), 3.80 (s, 3H), 4.34 (dd, J_3 = 3.8, J_3 = 8.8 Hz, 1H) 7.01 (d, J_3 = 8.7 Hz, 2H), 7.70 (d, J_3 = 8.7 Hz, 2H), 8.33 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 37.1, 43.9, 55.8, 79.6, 114.8, 127.2, 129.8, 156.3, 161.7, 163.4, 172.2, 175.9.



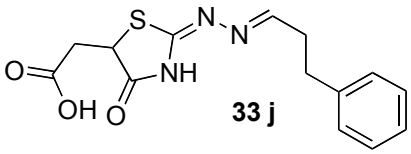
33 h

(*R,S*) 2-((thiophen-2-ylmethylene)hydrazolyl)-5-acetic acid-4-thiazolidinone (33h). Typical procedure for thiazolidinone preparation was followed using aldehyde **29h** to give thiazolidinone **33h** (33% yield) as a white solid mp 255-256 ºC. IR (KBr) ν = 3032, 2964, 2770, 1696, 1639, 1418, 1345, 1229, 1043, 712. ^{1}H NMR (DMSO-d_6) δ 2.90 (dd, J_2 = 17.6 Hz, J_3 = 8.7 Hz, 1 H), 3.01 (dd, J_2= 3.8, J_3= 17.6 Hz, 1 H), 4.35 (dd, J_3= 3.8, J_3 = 8.7 Hz, 1 H), 7.16 (dd, J_3 = 3.2 Hz, J_3 = 5.0 Hz, 1H), 7.50 (d, J_3 = 3.2Hz, 1 H), 7.70 (d, J_3 = 5.0Hz, 1 H), 8.56 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 37.0, 43.9, 128.5, 130.3, 132.4, 139.4, 151.1, 163.8, 172.2, 172.2, 175.7.



33 i

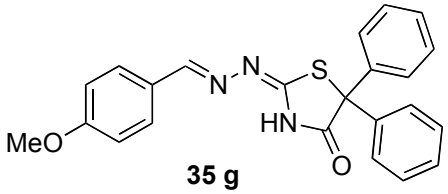
(*R,S*) 2-((2-methylpropylidene)hydrazolyl)-5-acetic acid-4-thiazolidinone (33i). Typical procedure for thiazolidinone preparation was followed using aldehyde **29i** to give thiazolidinone **33i** (34% yield) as a white solid mp 229-230 ºC. IR (KBr) ν = 3077, 2965, 2723, 1717, 1645, 1591, 1423, 1339, 1263, 1221, 761, 693. ^{1}H NMR (D_2O/K_2CO_3) δ 0.95 (d, J_3 = 6.8, 6H), 2.27 (J_2 = 16.2, J_3 = 11.7 Hz, 1H), 2.41 (qd, J_3 = 6.8, J_3 = 6.7, 1H), 2.91 (dd, J_2 = 16.2, J_3= 3.6, 1H), 4.07 (dd, J_3 = 3.6, J_3 = 11.7 Hz, 1H) 7.45 (d, J_3 = 6.7 Hz, 1H). ^{13}C NMR (D_2O/K_2CO_3) δ 19.0, 31.2, 42.0, 50.1, 162.3, 166.6, 178.9, 179.4, 191.1.



33 j

(*R,S*) 2-((3-phenylpropylidene))hydrazolyl)-5-acetic acid-4-thiazolidinone (33j). Typical procedure for thiazolidinone preparation was followed using aldehyde **29j** to give thiazolidinone **33j** (64% yield) as a white solid mp 199-200 °C. ^{1}H NMR (DMSO-d_6) δ 2.65 (qd, J_3 = 5.28 Hz, 2H), 2.86 (m, 2H), 2.88 (dd, J_2 = 17.5, J_3 = 9.2 Hz, 1H), 3.14 (dd, J_2 = 3.7 Hz, J_3 = 17.5 Hz, 1H), 4.32 (dd, J_3 = 3.7 Hz, J_3 = 9.2 Hz, 1H), 7.19 (m, 1H), 7.27 (m, 4H), 7.74 (t, J_3 = 5.2 Hz, 1H), 7.92 (s, 1H_{NH}); ^{13}C NMR (DMSO-d_6) δ 31.9, 34.0, 36.7, 43.9, 78.4, 125.7, 128.1, 128.1, 141.1, 160.6, 172.2, 177.1.

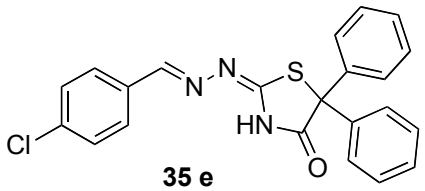
Typical procedure for the synthesis of diphenylthiazolidinones 35.



35 g

2-(4-metoxybenzylidenehydrazolyl)-5,5-diphenyl-4-thiazolidinone (**35g**). To a stirred solution of 4-methoxybenzylidene thiosemicarbazone (177 mg, 0.91 mmol) in DMSO (1.5 mL), were added benzil (150mg, 0.60 mmol) and KOH 1.2M (0.3mL, 0.36mmol). The reaction mixture was heated in a microwave for 10 min at 120º C (200 W) with stirring, poured into water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was purified by chromatography (1:4, AcOEt/Hexanes) to afford **35** (22 %) as a white solid. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 6.94 (d, J = 8.8 Hz, 2H), 7.39 (m, 10H), 7.81 (d, J = 8.8 Hz, 2H), 9.08 (s, 1H), 9.10 (s, 1H_{NH}). ^{13}C NMR ($CDCl_3$) δ 55.4, 71.4, 114.2, 125.3, 127.0, 127.6, 128.7, 129.0, 130.6, 137.6, 162.9, 169.7, 180.1. HRMS calculated for $C_{23}H_{19}N_3O_2SNa$ [M+Na]: 424.1096, found: 424.1090.

2-(4-Chlorobenzylidenehydrazolyl)-5,5-diphenyl-4-thiazolidinone (**35e**):

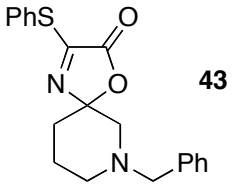


35 e

To a stirred solution of 4-chlorobenzylidene thiosemicarbazone (256 mg, 1.2 mmol) in DMSO (1.5 mL), were added benzyl (150mg, 0.60 mmol) and KOH 1.2M (0.5 mL, 0.6 mmol). The reaction mixture was heated in a microwave for 45 min at 120º C (200 W) with stirring, poured into water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) filtered and concentrated. The residue was purified by chromatography (1:9, EtOAc/Hexanes) to afford **35 g** (23% yield, 47% based on starting material recovery) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 12H), 7.81 (d, J = 8.0 Hz, 2H), 8.50 (s, 1H_{NH}), 9.27 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 71.3, 127.0, 129.2, 129.8, 131.3, 137.4, 138.3, 160.4, 169.8, 179.9.

Experimental protocol and spectral data of 43:

1-Benzyl-3-piperidone. A solution of K_2CO_3 (0.546 g, 2.63 mmol) in deionized water (5.0 ml) was added at rt to 1-benzyl-3-piperidone hydrochloride hydrate (0.6 g, 2.63 mmol). The reaction mixture was stirred for 90 min and extracted into ethyl acetate. The organic layer was dried ($MgSO_4$), filtered, and concentrated to obtain 1-benzyl-3-piperidone (free-base) as a viscous brown-oil (0.5 g, 2.60 mmol, 99%) upon drying under high vacuum for 1h.

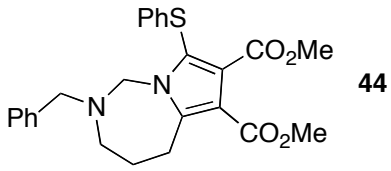


43

7-Benzyl-3-(phenylthio)-1-oxa-4,7-diazaspiro[4.5]dec-3-en-2-one (43). To a microwave vial containing a solution of thiophenol (0.0689 g, 0.625 mmol) in dry dichloromethane (0.98 mL) was added ethyl cyanoformate (0.05g, 0.507mmol) and

Et_2NH (1 drop) at 0 °C. The reaction mixture was stirred under an atmosphere of N_2 at rt for 2 h. At 0 °C, 0.5 mL of a freshly-prepared catalyst stock solution ($TiCl_4$ (20 drops) and $BF_3.Et_2O$ (10 drops) in dichloromethane (3.0 ml) were mixed under N_2 at rt., followed by addition of 1-benzyl-3-piperidone (0.0935 g, 0.516 mmol). The reaction mixture was heated in the microwave at 80 °C for 15 min and diluted with EtOAc and 5M NaOH (1.0 ml). The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The crude residue was purified by chromatography on SiO_2 (88% hexanes/EtOAc) to yield **43** (0.106 g, 0.301 mmol, 60%) as light yellow-orange viscous oil. Upon refrigeration, **43** turned into an amorphous wax after several days. IR ν = 2946, 2928, 2805, 2788, 2764, 1770, 1577, 1560, 1439, 1299, 1251, 1114, 1053, 1027, 975, 917, 900, 738, 701, 684 cm^{-1}; ^1H NMR ($CDCl_3$, 300 K) δ 1.79 (m, 4H), 2.20 (app t, *J* = 10.5 Hz, 1H), 2.51 (s, 2H), 2.82 (dt, *J* = 11.1, 3.6 Hz, 1H), 3.51 (AB, *J* = 13.5 Hz, 2H), 3.60 (s, 1H), 7.23 (m, 5H), 7.40 (m,3H), 7.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 300 K) δ 22.1, 34.3, 52.1, 59.8, 62.0, 106.8, 126.4, 127.2, 128.4, 128.8, 129.5, 129.9, 133.9, 137.7, 161.5, 162.7; TOFMS *m/z* 375 ([M+Na]+, 10), 365 (30), 353 ($[M+H]^+$, 100); HRMS (ES) *m/z* calculated for $C_{20}H_{20}N_2O_2SNa$ (M+Na) 375.1143, found 375.1114.

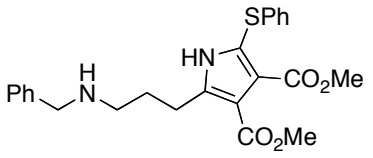
Experimental protocol and spectral data of dimethyl 2-benzyl-8-(phenylthio)-2,3,4,5-tetrahydro-1*H*pyrrolo[1,2c][1,3]diazepine-6,7-dicarboxylate (44).



44

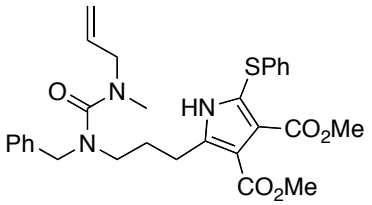
A solution of acetylenecarboxylic acid dimethyl ester (0.0745 g, 0.504 mmol) in chlorobenzene (0.5mL) was treated with **43** (0.0888 g, 0.252 mmol). The reaction mixture was stirred at 150 °C for 10 min under microwave irradiation (200 W). Without removal of the chlorobenzene, the crude reaction mixture was purified by chromatography on SiO_2 (eluting with 100% to 80% hexanes/EtOAc). The residue was further purified by Kugelrohr distillation (0.1 Torr, 100 °C) to yield **44** (0.0879 g, 0.195 mmol, 77%) as light yellow oil which turned into a yellow-orange glass upon further drying under high-vacuum and extended refrigeration. IR ν = 2945, 1705, 1495, 1439, 1273, 1204, 1178, 1128, 1049, 1027, 738, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 295 K) δ 1.67 (m, 2H), 3.01 (dd, *J* = 5.1, 4.8 Hz, 2H), 3.25 (bm, 2H), 3.39 (s, 2H), 3.83 (s, 3H), 3.85 (s,

3H), 5.13 (bs, 2H), 7.09 (m, 2H), 7.25 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 22.2, 25.6, 51.6, 52.4, 52.8, 54.6, 65.6, 111.5, 120.0, 125.4, 126.4, 127.4, 128.5, 129.3, 136.4, 138.0, 143.7, 164.7, 166.2; TOFMS *m/z* 474 (3), 473 ([M+Na]$^+$, 100), 451 ([M+H]$^+$, 3); HRMS (ES) *m/z* calculated for $C_{25}H_{26}N_2O_4SNa$ (M+Na) 473.1502, found 473.1511.



49

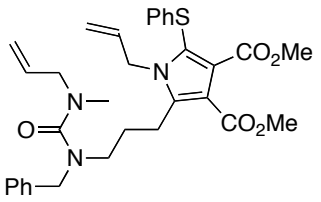
Dimethyl 2-(3-(benzylamino)propyl)-5-(phenylthio)-1H-pyrrole-3,4-dicarboxylate (49): In a one bottle flask, compound **44** (0.146 g, 0.322 mmol) was added to a solution of acetic acid (7.5 mL) and H_2O (1.5 mL), at 45°C with stirring for 48h. Then the reaction was heated at 80°C for 6 more hours. H_2O was distilled at 50°C under reduced pressure. The residue was then poured into a $NaHCO_3$ saturated aqueous solution and extracted with CH_2Cl_2 (m crude = 0.177 g). The organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude was purified by chromatography on SiO_2 (CH_2Cl_2, MeOH 2 to 5%) to obtain compound **49** (m = 0.120 g, 85% yield). ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 2H), 2.65 (m, 2H), 3.05 (m, 2H), 3.55 (d, *J* = 4.0 Hz, 2H), 3.80 (s, 3H), 3.84 (s, 3H), 7.21 (m, 10H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 23.7, 25.5, 44.9, 51.7, 52.0, 111.6, 118.4, 124.5, 125.9, 127.9, 130.7, 140.2, 164.0, 165.8; MS (EI) *m/z* 438 (M$^+$, 15), 91 (100), 286 (15), 134 (15), 120 (18), 106 (16).



51

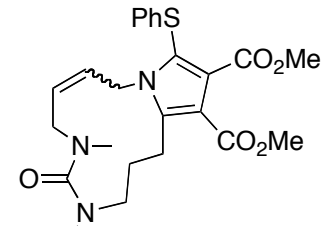
Dimethyl 2-(3-(3-allyl-1-benzyl-3-methylureido)propyl)-5-(phenylthio)-1H-pyrrole-3,4-dicarboxylate (51). To a solution of **49** (0.393 mmol) in CH_2Cl_2 was added 5 drops of Et_3N and the carbamoylimidazolium salt (0.589 mmol). The mixture was stirred for 24h at rt. Then 0.75 equivalents of the carbamoylimidazolium salt were added to the mixture with stirring for 3 more hours. A saturated solution of $NaHCO_3$ was added. The aqueous layer was extracted (3x5mL) and the combined organic layers were dried, filtered and concentrated in vacuo. The crude was purified by chromatography on SiO_2 (EtOAc/Hex, 2:1) to obtain **51** as yellow oil (m = 113 mg, 56.7 % yield). ^{1}H NMR

($CDCl_3$) δ 1.74 (m, 2H), 2.81 (m, 2H), 3.14 (m, 2H), 3.50 (s, 3H), 3.71 (d, *J* = 7.2 Hz, 2H), 3.77 (s, 3H), 3.85 (s, 3H), 4.39 (s, 2H), 5.14 (m, 2H), 5.73 (m, 1H), 7.27 (m, 10H).



52

Dimethyl 1-allyl-2-(3-(3-allyl-1-benzyl-3-methylureido)propyl)-5-(phenylthio)-1H-pyrrole-3,4-dicarboxylate (52). To a solution of **51** (0.030 g, 0.056 mmol) in dry dioxane (1 mL) were added K_2CO_3 (0.027 g) and allyl bromide (0.015 mL, 0.168 mmol). The mixture was heated to reflux for 4h (100°C). Then 3 more equivalents of allyl bromide (14 uL) were added and the mixture was heated to reflux for 12h. Then the mixture was cooled down and cyclohexane was added (1 mL). The solid was filtered off through a pad of celite, and the organic phase was evaporated under reduced pressure. Flash column chromatography on SiO_2 (EtOAc/Hexanes 3:1) gave **52** (30 mg, 87.2% yield) as white-transparent oil. ^1H NMR ($CDCl_3$) δ 2.80 (m, 5H), 3.14 (m, 2H), 3.78 (m, 5H), 3.85 (s, 3H), 4.40 (s, 2H), 4.55 (m, 2H), 4.99 (d, *J* = 14.0 Hz, 1H), 5.18 (m, 2H), 5.59 (m, 1H), 5.80 (m, 1H), 7.24 (m, 10H).



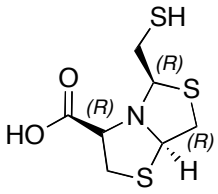
Z-**54**

Experimental protocol for RCM and spectral data of dimethyl 4-benzyl-6-methyl-5-oxo-12-(phenylthio)-1,2,3,4,5,6,7,10-octahydropyrrolo[2,1-g][1,3,8] triazacyclododecine-13,14-dicarboxylate ((*Z*)-**54**). A solution of diene **52** (0.0180 g, 0.0313 mmol) in dry dichloromethane (7.0 mL) was treated with 2nd generation Grubbs catalyst **53** (0.00531 g, 0.00625 mmol), heated at reflux for 5 h, cooled to rt, filtered through a pad of celite/florisil (1:1), and washed with EtOAc. The eluent was concentrated, and the residue was purified by chromatography on SiO_2 (80% hexanes/EtOAc) to yield **54** as colorless oil (0.0125 g, 0.0228 mmol, 73%) as a mixture of (*Z*/*E*)-isomers (3:1).

Major (*Z*)-isomer: IR ν = 2946, 1705, 1636, 1495, 1446, 1210, 1075, 1027, 732, 701 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 K) δ 1.65 (m, 2H), 2.85 (s, 3H), 2.90 (m, 2H), 3.24 (bs, 2H), 3.78 (s, 3H), 3.86 (s, 3H), 3.94 (bs, 2H), 4.53 (bs, 2H), 4.74 (d, *J* = 6.0 Hz, 2H), 5.15 (dt, *J* = 10.8, 6.6 Hz, 1H), 5.76 (dt, *J* = 10.8, 7.8 Hz, 1H), 7.25 (m, 10H), ^{13}C NMR ($CDCl_3$, 300 K) δ 22.9, 28.0, 29.7, 35.6, 42.1, 45.7, 47.0, 51.4, 52.4, 54.8, 111.5, 118.9, 125.4, 126.5, 127.3, 127.5, 127.7, 128.7, 129.1, 129.4, 133.8, 135.9, 137.8, 143.1, 163.8, 165.0, 166.2; MS (EI) *m/z* 547 (M+, 3.5), 424 (9), 91 (100); HRMS (EI) *m/z* calculated for $C_{30}H_{33}N_3O_5S$ 547.2122, found 547.2141.

Minor (*E*)-isomer: ^{1}H NMR ($CDCl_3$, 300 K) δ 1.64 (m, 2H), 2.82 (s, 3H), 2.87 (m, 2H), 3.12 (bs, 2H), 3.66 (m, 2H), 3.77 (s, 3H), 3.86 (s, 3H), 4.38 (s, 2H), 4.76 (d, *J* = 5.7 Hz, 2H), 5.38 (dt, *J* = 15.5, 6.3 Hz, 1H), 5.85 (dt, *J* = 15.5, 5.7, Hz, 1H), 7.23 (m, 10H).

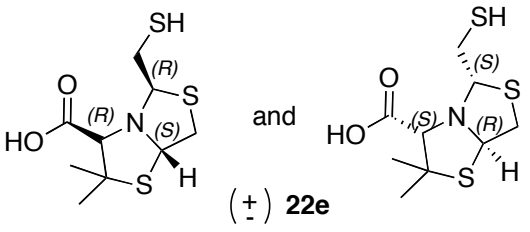
General procedure for the synthesis of bisthiazolidines 22



(*2R,3R,8R*)-8-carboxylate-2-mercaptomethyl-1-aza-3,6-dithiobicyclo[3.3.0] octane (*anti-syn-2*2d). To a stirred suspension of L-cysteine **5d** (0.5 g, 4.1 mmol) in EtOH (16 mL), was added 1,4-dithiane-2,5-dithiol **58** (0.8 g, 5.0 mmol) and *p*-TsOH ac. (0.030 g, 0.17 mmol). The mixture was heated to reflux for 2 h. Then it was cooled down and poured into brine, extracted with CH_2Cl_2 (5x30 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:3:0.1 EtOAc/hexanes/AcOH) to afford **22d** (0.830g, 86%, *syn-syn/anti-syn* 10:90) as a white solid.

anti-syn-**22d**: white solid, mp 103-104 ºC; ^{1}H NMR ($(CD_3)_2CO$) δ 2.13 (t, *J* = 8.3 Hz, $1H_{SH}$), 2.68 (m, 1H), 2.91 (m, 1H), 3.09 (dd, *J* = 11.9, 3.5 Hz, 1H), 3.31 (m, 2H), 3.59 (dd, *J* = 11.9, 5.3 Hz, 1H), 4.43 (dd, *J* = 7.2, 6.5 Hz, 1H), 4.45 (dd, *J* = 5.9, 4.5 Hz, 1H), 5.14 (dd, *J* = 5.3, 3.5 Hz, 1H). ^{13}C NMR ($(CD_3)_2CO$) δ 34.0, 34.3, 39.2, 71.5, 74.5, 75.6, 172.1. HRMS calculated for $C_7H_{11}NO_2S_3$, $[M+H]^+$ 238.0025, found: 238.0033.

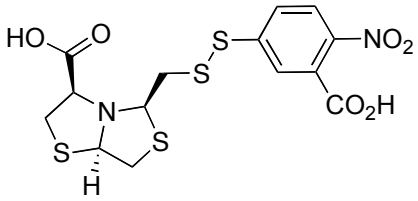
Compounds 22a, 22b and 22c are described in page 43 and 44 in this chapter.



(*2R,5S,8R*)-2-mercaptomethyl-7-dimethyl-8-carboxylate-1-aza-3,6-dithiobicyclo [3.3.0]octane *syn-syn*-22e. Prepared in an analogous route as described for **22d**, purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) to afford compound **22e** (89%, *syn-syn/anti-syn*: 95:05). ***syn-syn*-2e**: white solid mp 89-97 ºC; ^{1}H NMR ($CDCl_3$) δ 1.51 (s, 3H), 1.62 (s, 3H), 1.89 (m, $1H_{SH}$), 2.82 (m, 2H), 3.06 (dd, *J* = 11.7, 5.3 Hz, 1H), 3.43 (dd, *J*= 11.7, 6.6 Hz, 1H), 3.79 (s, 1H), 4.30 (dd, *J* = 8.5, 6.3 Hz, 1H), 4.98

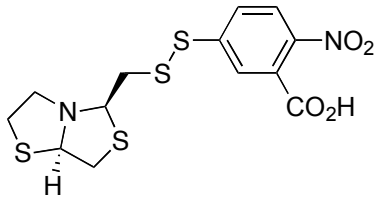
(dd, *J* = 6.6, 5.3 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.0, 28.1, 32.0, 40.5, 55.1, 68.7, 75.7, 78.5, 170.4. HRMS calculated for $C_9H_{16}NO_2S_3$, $[M+H]^+$ 266.0343, found 266.0330.

General procedure for the synthesis of disulfides 57



57di

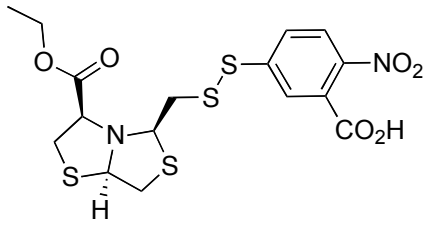
(***3R,5R,8R***) **5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo [4,3-b] thiazole-3-carboxylic acid (57di).** A stirred solution of DTNB (**1**) (0.2 g, 0.5 mmol) in MeOH (10 mL) was cooled down to 0°C and $NaBH_4$ (0.04 g, 1 mmol) was added in small quantities. After 3hs, a solution of bicycle **22d** (0.24mg, 1.0 mmol) in MeOH (3 mL) and DMAP cat. (0.010g) were added with stirring. The mixture was stirred at room temperature overnight opened to air. The solvent was then removed under reduced pressure and the crude was poured into water and an aqueous solution of HCl 5% was added to afford pH 4-5. The aqueous layer was extracted with EtOAc (5 x 50 mL), dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on SiO_2 (1:2 EtOAc/hexanes and 0.5% AcOH) to afford **57di** (0.270g, 62%) as yellow oil. ^{1}H NMR ($(CD_3)_2CO$) δ 3.13 (dd, *J* =11.9, 3.9 Hz, 1H), 3.17 (dd, *J* = 13.5, 6.5 Hz, 1H), 3.32 (m, 2H), 3.40 (dd, *J* = 13.5, 6.5 Hz, 1H), 3.62 (dd, *J* = 11.9, 5.4 Hz, 1H), 4.40 (dd, *J* = 6.6, 4.4 Hz, 1H), 4.72 (t, *J* = 6.5 Hz, 1H), 5.19 (dd, *J* = 5.4, 3.9 Hz, 1H), 8.00 (m, 3H), ^{13}C NMR ($(CD_3)_2CO$) δ 34.31, 39.5, 48.6, 70.6, 71.7, 71.8, 74.4, 125.6, 127.2, 129.3, 130.0, 142.8, 145.6, 166.0. HRMS calculated for $C_{14}H_{15}N_2O_6S_4$ $[M+H]^+$ 434.9813, found 434.9800.



57da

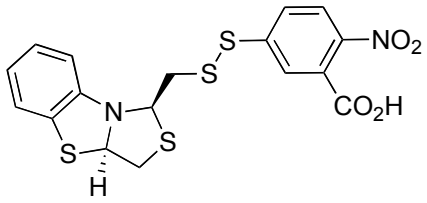
±5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole (**57da).** Prepared in an analogous route as described for **57di**, purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5% AcOH) to afford compound

57da (41% yield) as a pale yellow oil. ^{1}H NMR ($(CD_3)_2CO$) δ 3.06 (m, 5H), 3.32 (dd, *J* = 13.6, 7.0 Hz, 1H), 3.41 (dd, *J* = 10.9, 5.3 Hz, 1H), 3.56 (dd, *J* = 11.4, 5.5 Hz, 1H), 4.55 (dd, *J* = 7.0, 5.3 Hz, 1H), 5.00 (dd, *J* = 5.5, 4.0 Hz, 1H), 7.95 (dd, *J* = 8.5, 2.2 Hz, 1H), 8.02 (d, *J* = 2.2 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 31.9, 39.1, 49.1, 57.0, 70.3, 74.9, 125.6, 165.5, 126.0, 128.1, 130.1, 142.8, 146.0. HRMS calculated for $C_{13}H_{14}N_2O_4S_4$ $[M+H]^+$ 390.99092, found 390.99257.



57db

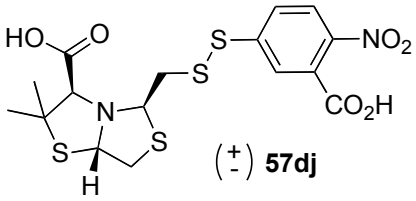
(***3R,5R,8R***) **5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b]thiazole-3-ethyl ester (57db).** Prepared in an analogous route as described for **57di**, purification by chromatography on SiO_2 (1:3, EtOAc:hexanes) to afford compound **57b** (48% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (t, *J* = 7.1 Hz, 3H), 3.09 (dd, *J* = 11.7, 4.2, 1H), 3.15 (dd, *J* = 13.4, 6.4 Hz, 1H), 3.24 (dd, *J* = 10.9, 5.2 Hz, 1H), 3.32 (dd, *J* = 10.9, 6.8 Hz, 1H), 3.35 (dd, *J* = 13.1, 7.2 Hz, 1H), 3.57 (dd, *J* = 11.7, 5.5 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.27 (t, *J* = 6.0 Hz, 1H), 4.68 (t, *J* = 6.6 Hz, 1H), 5.14 (t, *J* = 4.8 Hz, 1H), 7.99 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 14.5, 34.2, 39.4, 48.8, 61.9, 70.2, 71.7, 74.2, 125.7, 127.2, 129.3, 145.7, 147.3, 166.0, 170.9. HRMS calculated for $C_{16}H_{18}N_2O_6S_4$ $[M+Na]^+$ 484.9940, found 484.9964. $[\alpha]_D$ = +202 º (20 ºC, MeOH, c = 0.1).



57dc

±5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl) (2*RS*, 5*RS*) -3,3a-dihydro-benzo[*d*]thiazolo[4,3-b]thiazole (57dc). Prepared in analogous route as described for **57di**, purification by chromatography on SiO_2 (1:3, EtOAc:hexanes, 0.5% AcOH) to afford compound **57dc** (43% yield) as a pale yellow oil. ^{1}H NMR ($(CD_3)_2CO$) δ 2.78 (dd, *J* = 10.3, 9.5 Hz, 1H), 3.29 (dd, *J* = 14.0, 4.6 Hz, 1H), 3.37 (dd, *J* = 10.3, 5.7 Hz, 1H), 3.44 (dd, *J* = 14.0, 9.7 Hz, 1H), 5.34 (dd, *J* = 9.5, 5.7 Hz, 1H), 5.54 (dd, *J* = 9.7, 4.6 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.86 (td, *J* = 7.6, 1.1 Hz, 1H), 7.07 (td, *J* = 8.0, 1.2 Hz, 1H), 7.14 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.91 (dd, *J* = 8.5, 2.1 Hz,

1H), 7.97 (d, *J* = 2.1 Hz, 1H). HRMS calculated for $C_{17}H_{13}N_2O_4S_4$ $[M-H]^-$ 436.97636, found 436.97422.



57dj

±5-(((3-carboxy-4-nitrophenyl)disulfanyl)methyl)tetrahydro-2H-thiazolo[4,3-b] thiazole-2-dimethyl-3-carboxylic acid (57dj). Prepared in an analogous route as described for **57di**, purification by chromatography on SiO_2 (1:2, EtOAc:hexanes, 0.5% AcOH) to afford compound **57dj** (38% yield) as yellow oil. ^1H NMR ($(CD_3)_2CO$) δ 7.98 (m, 3H), 4.97 (dd, *J* = 7.5, 6.3 Hz, 1H), 4.75 (dd, *J* = 8.0, 6.4 HZ, 1H), 3.69 (s, 1H), 3.44 (dd, *J* = 13.4, 6.3 Hz, 1H), 3.41 (dd, *J* = 11.1, 6.4 Hz, 1H), 3.13 (dd, *J* = 13.4, 8.0 Hz, 1H), 3.06 (dd, *J* = 11.1, 7.5 Hz, 1H), 1.60 (s, 3H), 1.44 (s, 3H). ^{13}C NMR ($(CD_3)_2CO$) δ 28.5, 29.1, 41.5, 48.0, 55.8, 69.2, 72.3, 76.6, 125.7, 127.5, 129.5, 129.8, 130.1, 145.7, 166.1, 170.5. HRMS calculated for $C_{16}H_{17}N_2O_6S_4$ $[M-H]^-$ 460.99749, found 460.99570.

Expression and Purification of Recombinant TGR

Expression and purification of *E. granulosus* TGR was carried out as previously described.[5] Total protein concentration and FAD content of the recombinant protein were determined by spectrophotometry at 280 (ε=54.24 mM-1 cm-1) and 460 nm (ε=11.3 $mM^{-1}.cm^{-1}$), respectively. The selenium content of selenoproteins was determined by atomic absorption using a Plasma Emission Spectrometer (Jarrell-Ash 965 ICP) in Chemical Analysis Laboratory, University of Georgia. Active selenoenzyme concentrations were calculated considering their selenium contents. The purity of recombinant proteins was analyzed by running 10% SDS-PAGE gels, under reducing conditions.

Dynamic Combinatorial Chemistry

All buffered stock solutions were prepared in Tris buffer (50mM, pH 8.8). The enzyme activity was checked at pH 8.8 and was found to be 80%, so we can expect to have the enzyme active during the template process. Stock solutions of reduced and oxidized glutathione were freshly prepared in buffer and used at 1.5 mM and 375 μM, respectively. Stock solutions of thiols were prepared in DMSO. Each thiol was diluted then into the library buffer to give a final concentration of 200 uM. *E. granulosus* thioredoxin glutathione reductase was used at a final concentration of 5 uM. The final volume of the DCL was 100 uL and was prepared in eppendorf tubes placed in a round-bottomed flask under vacuum and refilled with N_2. Oxygen was removed by repeated cycles of vacuum and refilled with N_2. The exchange was quenched by the addition of an aqueous solution of trichloroacetic acid 50% (20 uL). Denatured protein was removed by centrifugation, spinning at 14.000 rpm 3 x 5 min.

HPLC-MS analysis were performed on a HPLC Agilent 1200 equipped with a diode array detector (DAD), binary pump and a thermostated column at 40 ºC, coupled to a ion-trap Mass spectrometer Esquire 6000 (Bruker Daltonik GmbH). The samples are analyzed with a reverse 5 μm Luna-C18 column (Phenomenex) 150mm x 4.6mm. The mobile phases were formic acid (10mM in ultra-pure H_2O) (A) and acetonitrile (B).

[5] Bonilla M., Denicola A., Gladyshev V. and Salinas G. Platyhelminth Mitochondrial and Cytosolic Redox Homeostasis Is Controlled by a Single Thioredoxin Glutathione Reductase and Dependent on Selenium and Glutathione *J. Biol. Chem.,* **2008**, 283, 17898-17907.

Injection volume: 20 μL, the samples are in the buffer solution used to prepare the DCLs. Eluent B was held at 2% for 5 min, increased to 85 % over 45 min. Then it was decreased to 2 % over 5 min and finally with an isocratic period of 2% of B over 5 min. The total run time is 60 min and the flow is 0.8mL/min. the flow is splitted in 2 before going into de MS. The analysis uses a trap-ion with electrospray ionization, alternating positive-negative ions. Electrospray conditions: endplate off set, voltage -500V, capillary voltage -4000V, N_2 nebulizer (40 psi), N_2 drying flow of 9.0 L/min and a temperature of 350°C.

DTNB Reduction Assay for TR Activity

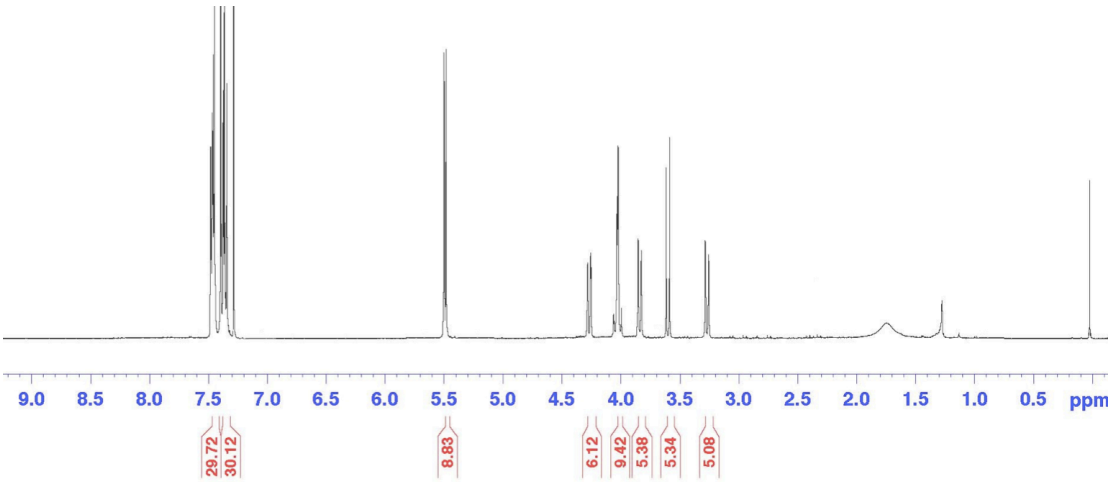
The TR activity was measured using the DTNB reduction assay[6]. The NADPH-dependent reduction of 5,5'-dithiobis 2-dinitrobenzoic acid) (DTNB) was followed by the increase in absorbance at 412 nm due to the formation of 5-thionitrobenzoic acid at 25 ºC (ε = 13.6 $mM^{-1}.cm^{-1}$). The absorbance was recorded using a PG-T70+ spectrophotometer (PG Instruments, UK) connected to a temperature control device. The reaction mixtures (1mL) contained 100 μM NADPH, 5 mM DTNB, 1 mM EDTA and 1 nM *Eg*TGRWT, in 50 mM potassium phosphate buffer. The reaction was initiated by the addition of DTNB. All experiments were performed in triplicate.

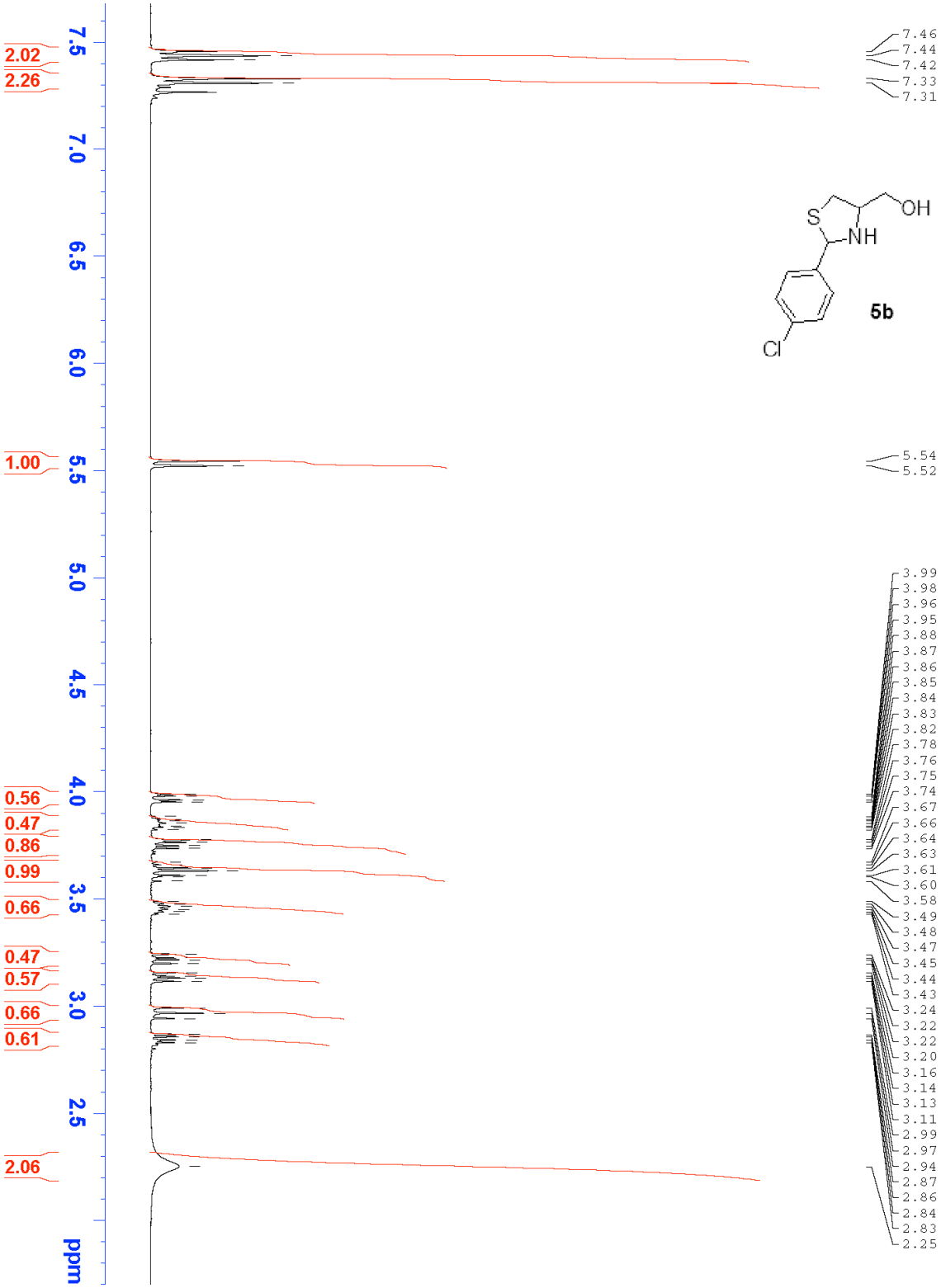
Cruzipain inhibitory activity

The activity was assayed in a reaction mixture (100 μL) containing in final concentration: Tris–HCl buffer, pH 7.6 (50 mM), Bz-Pro-Phe-Arg-pNA (0.150 mM), β-mercaptoethanol (10 mM) and cruzipain (0.140 μM). Absorbance at 410 nm was monitored at 30 ºC on a Beckman Model 25 spectrophotometer. The potential inhibitors were added as solutions in DMSO, and the control inhibitor was E64 (100% inhibition at 10 μM) added at same solvent concentration. All inhibitors were assayed by duplicate. The percentage of cruzipain inhibition (PCI) was calculated as follows: PCI (%) = (Ai / Ao) x 100, where Ai and Ao are the absorbance with and without inhibitor respectively.

Arner, E. S., Zhong, L., and Holmgren, A. Preparation and assay of mammalian thioredoxin and thioredoxin reductase. *Methods Enzymol.* **1999**, 300, 226–239.

6. APENDICE (FIGURAS)

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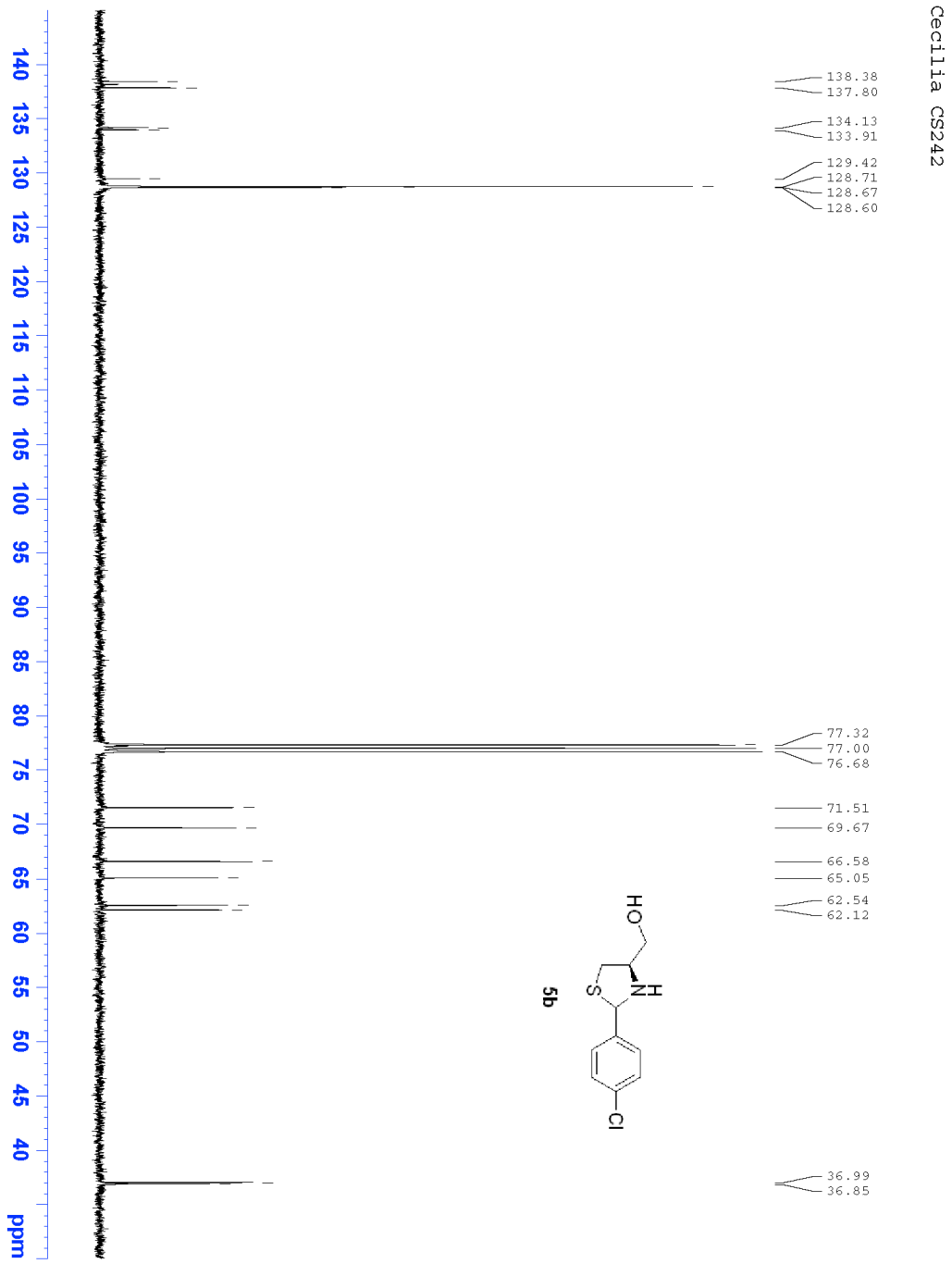
7.44
7.42
7.33
7.31
OH
S
NH
Cl
5b
5.54
5.52
3.99
3.98
3.96
3.95
3.88
3.87
3.86
3.85
3.84
3.83
3.82
3.78
3.76
3.75
3.74
3.67
3.66
3.64
3.63
3.61
3.60
3.58
3.49
3.48
3.47
3.45
3.44
3.43
3.24
3.22
3.22
3.20
3.16
3.14
3.13
3.11
2.99
2.97
2.94
2.87
2.86
2.84
2.83
2.25
2.02
2.26
1.00
0.56
0.47
0.86
0.99
0.66
0.47
0.57
0.66
0.61
2.06
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm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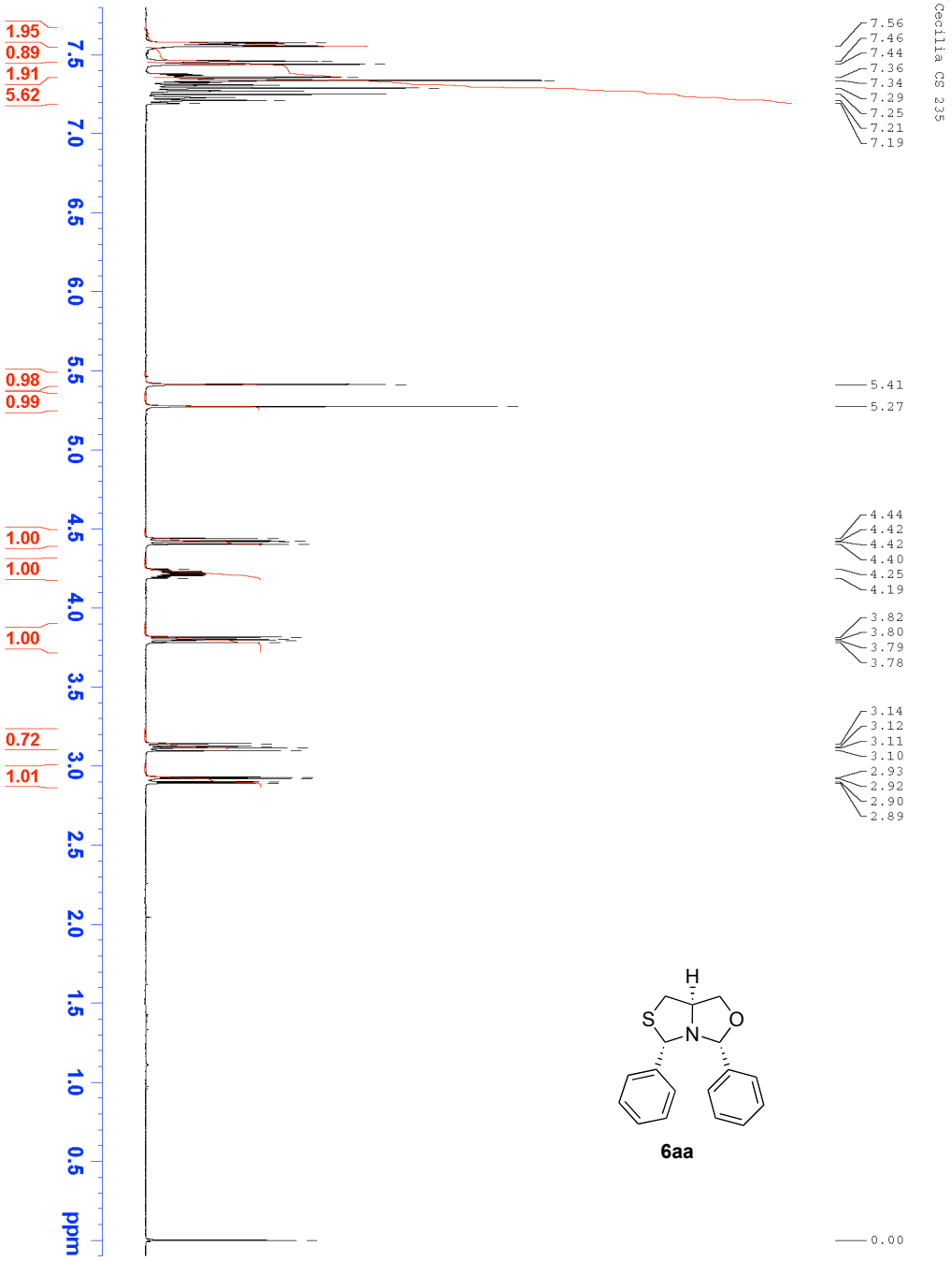
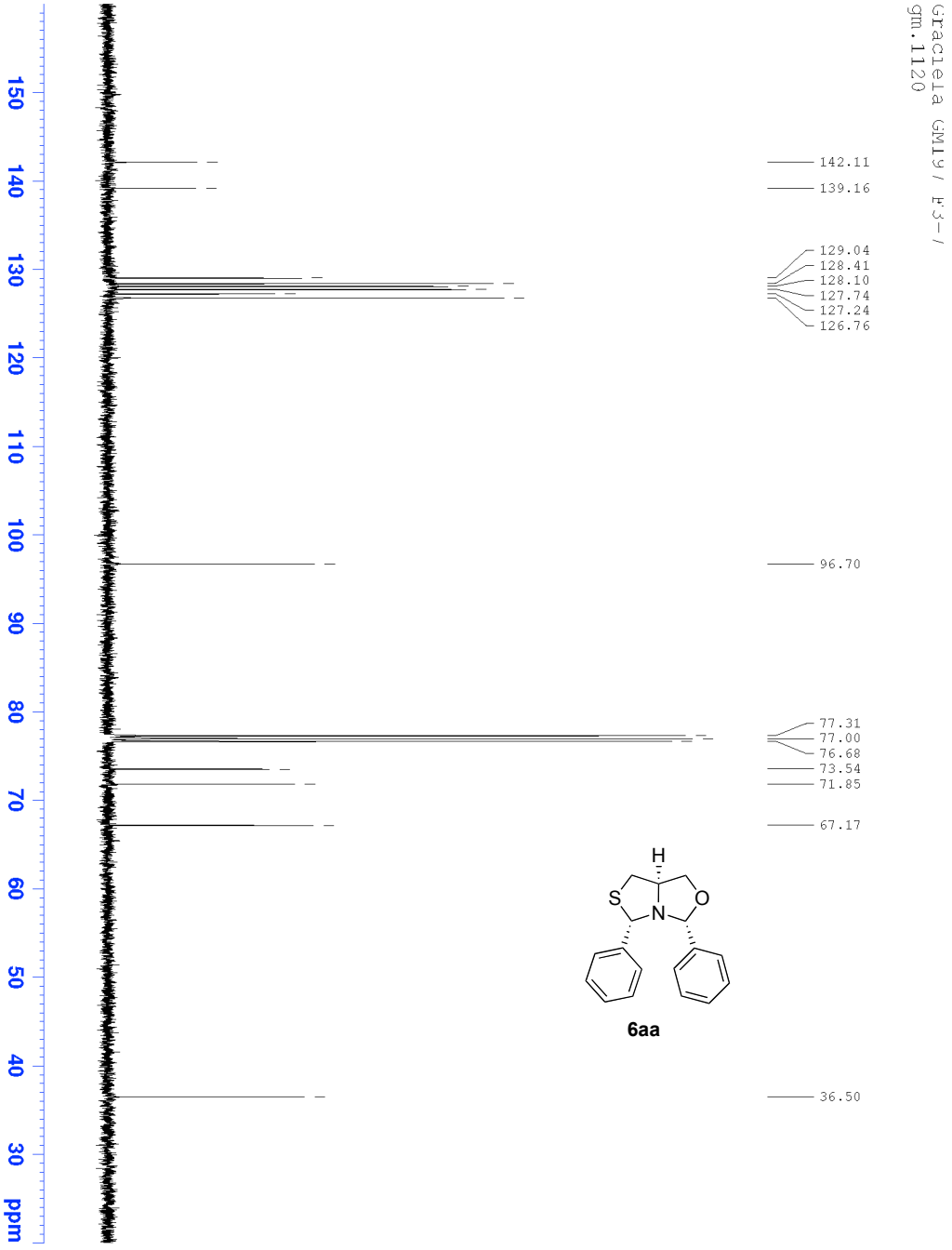
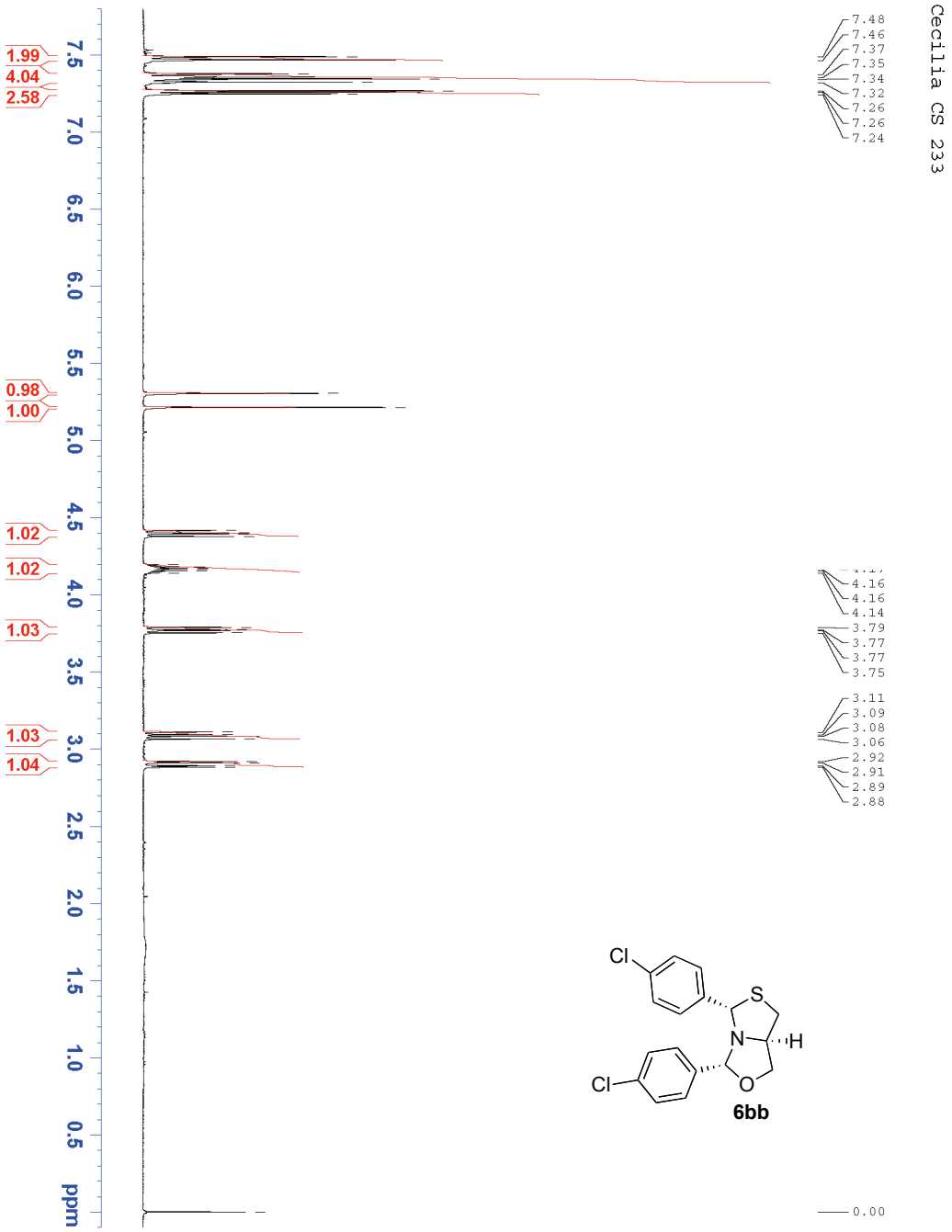
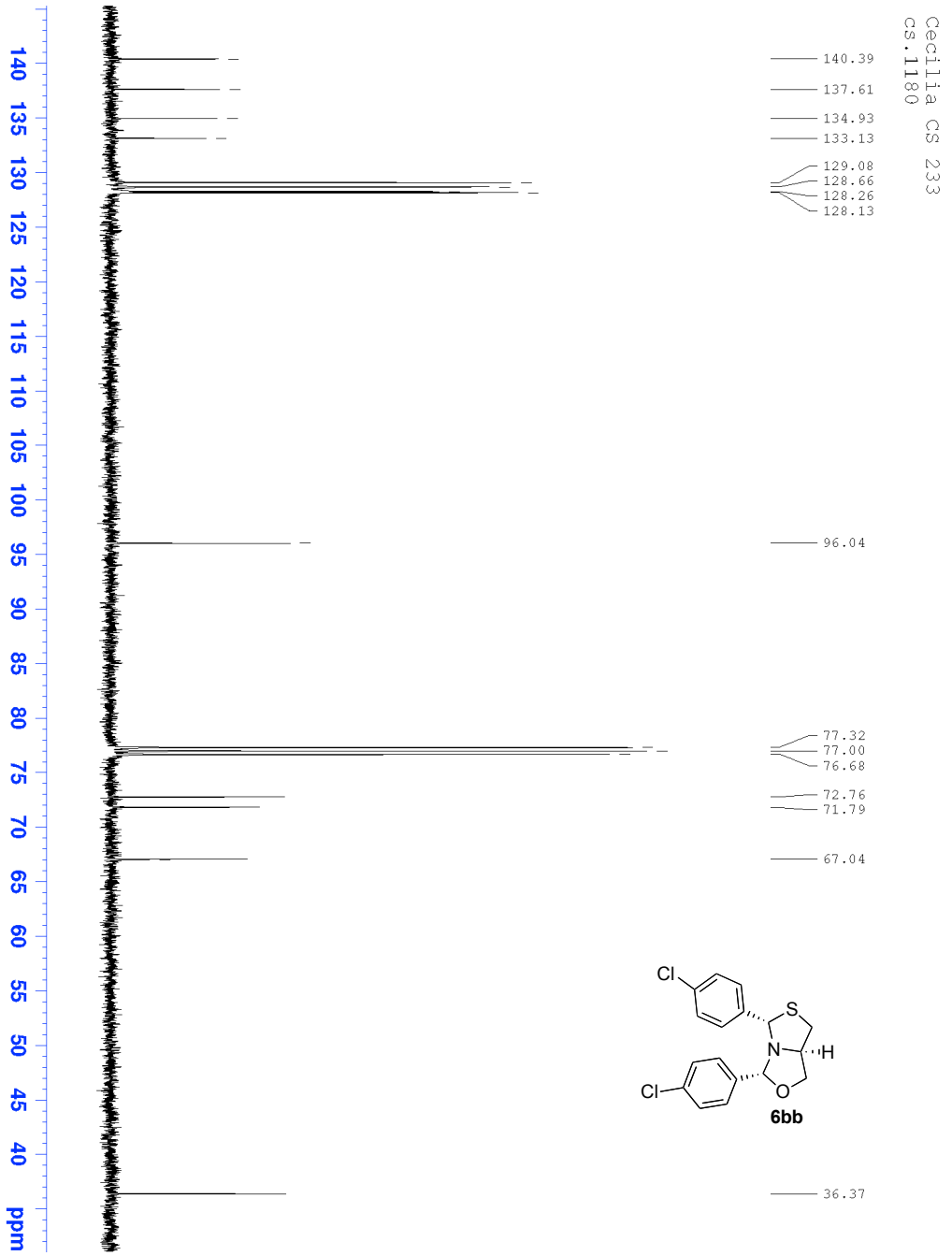
138.38
137.80
134.13
133.91
129.42
128.71
128.67
128.60
77.32
77.00
76.68
71.51
69.67
66.58
65.05
62.54
62.12
36.99
36.85
HO
H
N
S
Cl
5b
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 ppm

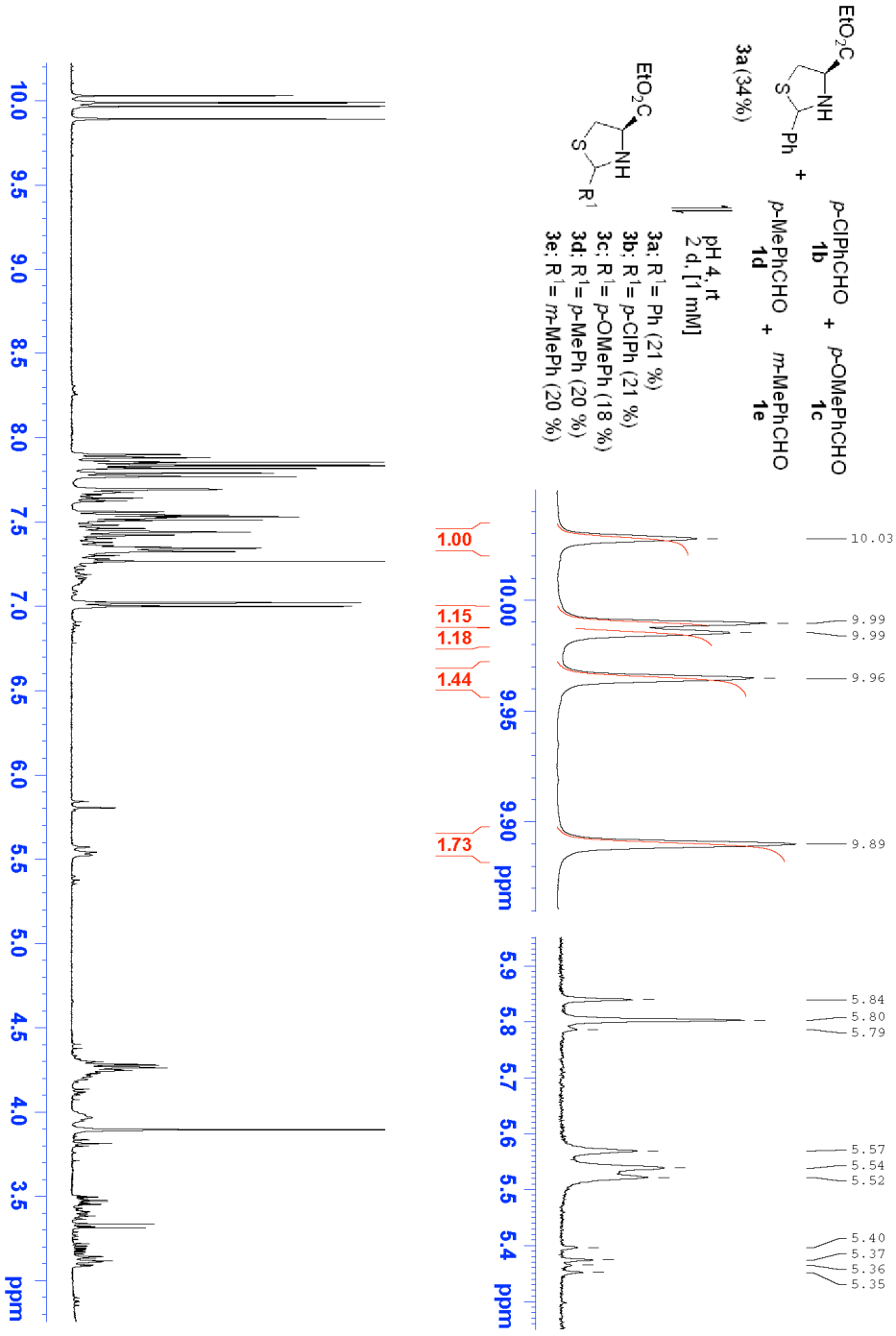

Cecilia cg 235
7.56
7.46
7.44
7.36
7.34
7.29
7.25
7.21
7.19
5.41
5.27
4.44
4.42
4.42
4.40
4.25
4.19
3.82
3.80
3.79
3.78
3.14
3.12
3.11
3.10
2.93
2.92
2.90
2.89
0.00
1.95
0.89
1.91
5.62
0.98
0.99
1.00
1.00
1.00
0.72
1.01
ppm
H
S
N
O
6aa

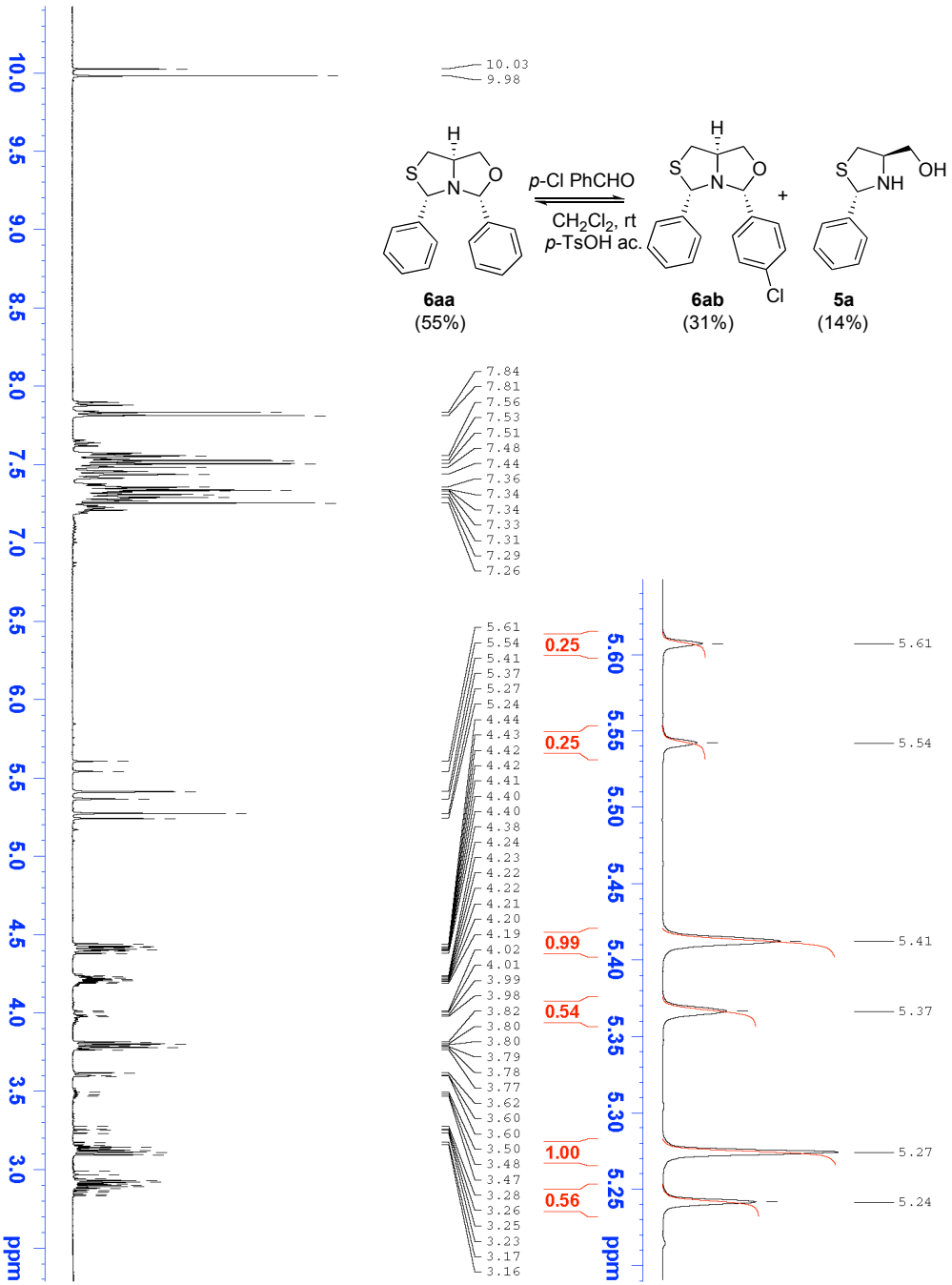

Graciela GM19 / F3-1
gm.1120
142.11
139.16
129.04
128.41
128.10
127.74
127.24
126.76
96.70
77.31
77.00
76.68
73.54
71.85
67.17
36.50
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm
H
S
N
O

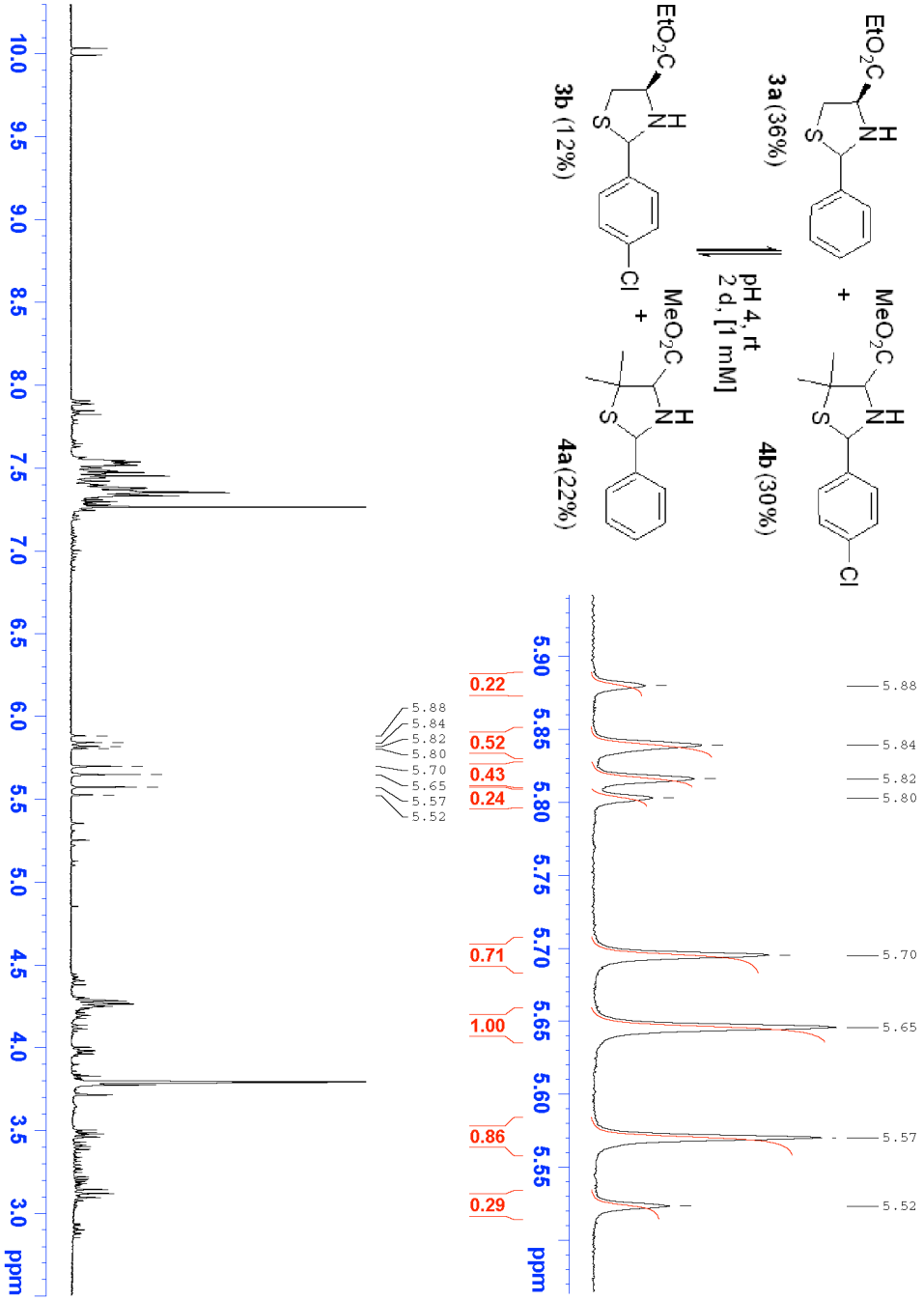
6aa

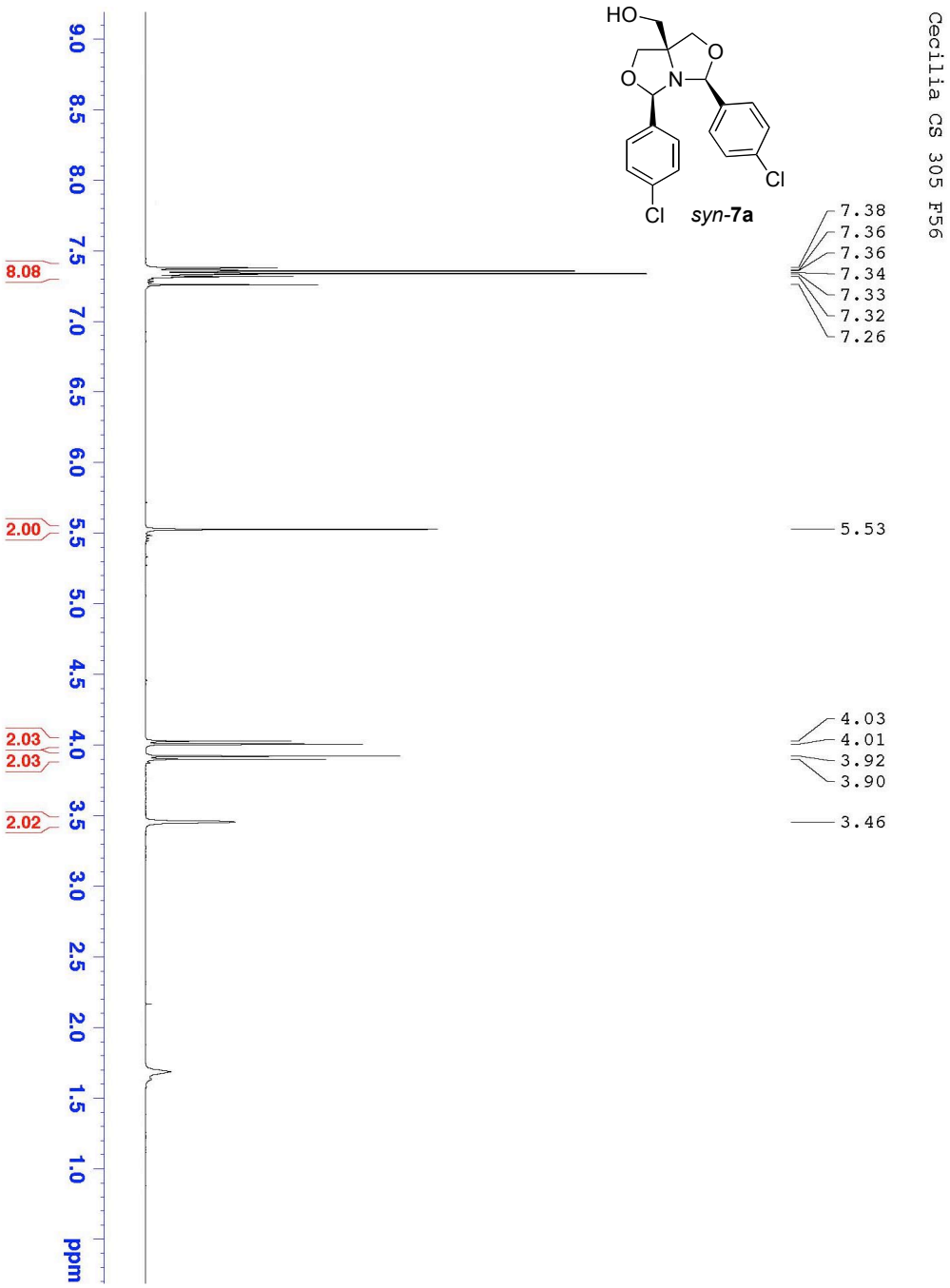

Cecilia CS 233
1.99
4.04
2.58
0.98
1.00
1.02
1.02
1.03
1.03
1.04
7.48
7.46
7.37
7.35
7.34
7.32
7.26
7.26
7.24
4.16
4.16
4.14
3.79
3.77
3.77
3.75
3.11
3.09
3.08
3.06
2.92
2.91
2.89
2.88
0.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
Cl
S
N
H
Cl
O
6bb

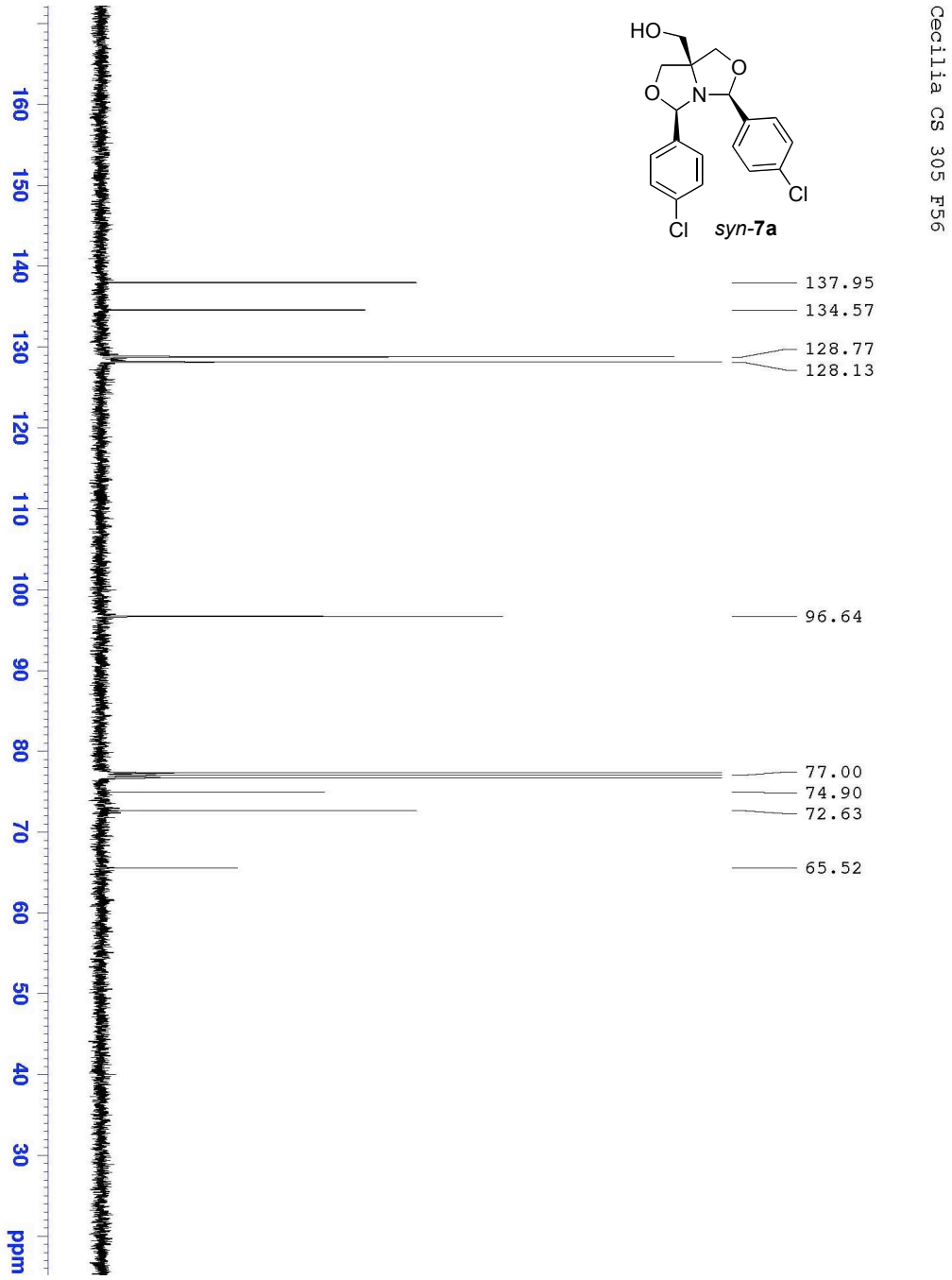

Cecilia CS 233
cs.1180
140.39
137.61
134.93
133.13
129.08
128.66
128.26
128.13
96.04
77.32
77.00
76.68
72.76
71.79
67.04
36.37
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 ppm
Cl
S
N
H
Cl
O
6bb


EtO2C
NH
S
Ph
3a (34 %)
+
p-ClPhCHO
1b
+
p-OMePhCHO
1c
p-MePhCHO
1d
+
m-MePhCHO
1e
pH 4, rt
2 d, [1 mM]
EtO2C
NH
S
R1
3a: R1 = Ph (21 %)
3b: R1 = p-ClPh (21 %)
3c: R1 = p-OMePh (18 %)
3d: R1 = p-MePh (20 %)
3e: R1 = m-MePh (20 %)
10.03
9.99
9.99
9.96
9.89
5.84
5.80
5.79
5.57
5.54
5.52
5.40
5.37
5.36
5.35
1.00
1.15
1.18
1.44
1.73
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
10.00
9.95
9.90
ppm
5.9
5.8
5.7
5.6
5.5
5.4
ppm


10.03
9.98
7.84
7.81
7.56
7.53
7.51
7.48
7.44
7.36
7.34
7.34
7.33
7.31
7.29
7.26
5.61
5.54
5.41
5.37
5.27
5.24
4.44
4.43
4.42
4.42
4.41
4.40
4.40
4.38
4.24
4.23
4.22
4.22
4.21
4.20
4.19
4.02
4.01
3.99
3.98
3.82
3.80
3.80
3.79
3.78
3.77
3.62
3.60
3.60
3.50
3.48
3.47
3.28
3.26
3.25
3.23
3.17
3.16
0.25
0.25
0.99
0.54
1.00
0.56
ppm
p-Cl PhCHO
CH2Cl2, rt
p-TsOH ac.
6aa
(55%)
6ab
(31%)
5a
(14%)


EtO2C
N
H
S
Cl
3a (36%)
3b (12%)
pH 4, rt
2 d, [1 mM]
MeO2C
4a (22%)
4b (30%)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 ppm
5.88
5.84
5.82
5.80
5.70
5.65
5.57
5.52
0.22
0.52
0.43
0.24
0.71
1.00
0.86
0.29
5.90 5.85 5.80 5.75 5.70 5.65 5.60 5.55 ppm


HO
O
O
N
Cl
Cl
syn-7a
Cecilia CS 305 F56
7.38
7.36
7.36
7.34
7.33
7.32
7.26
5.53
4.03
4.01
3.92
3.90
3.46
8.08
2.00
2.03
2.03
2.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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O
N
O
Cl
Cl
syn-7a
Cecilia CS 305 F56
137.95
134.57
128.77
128.13
96.64
77.00
74.90
72.63
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160
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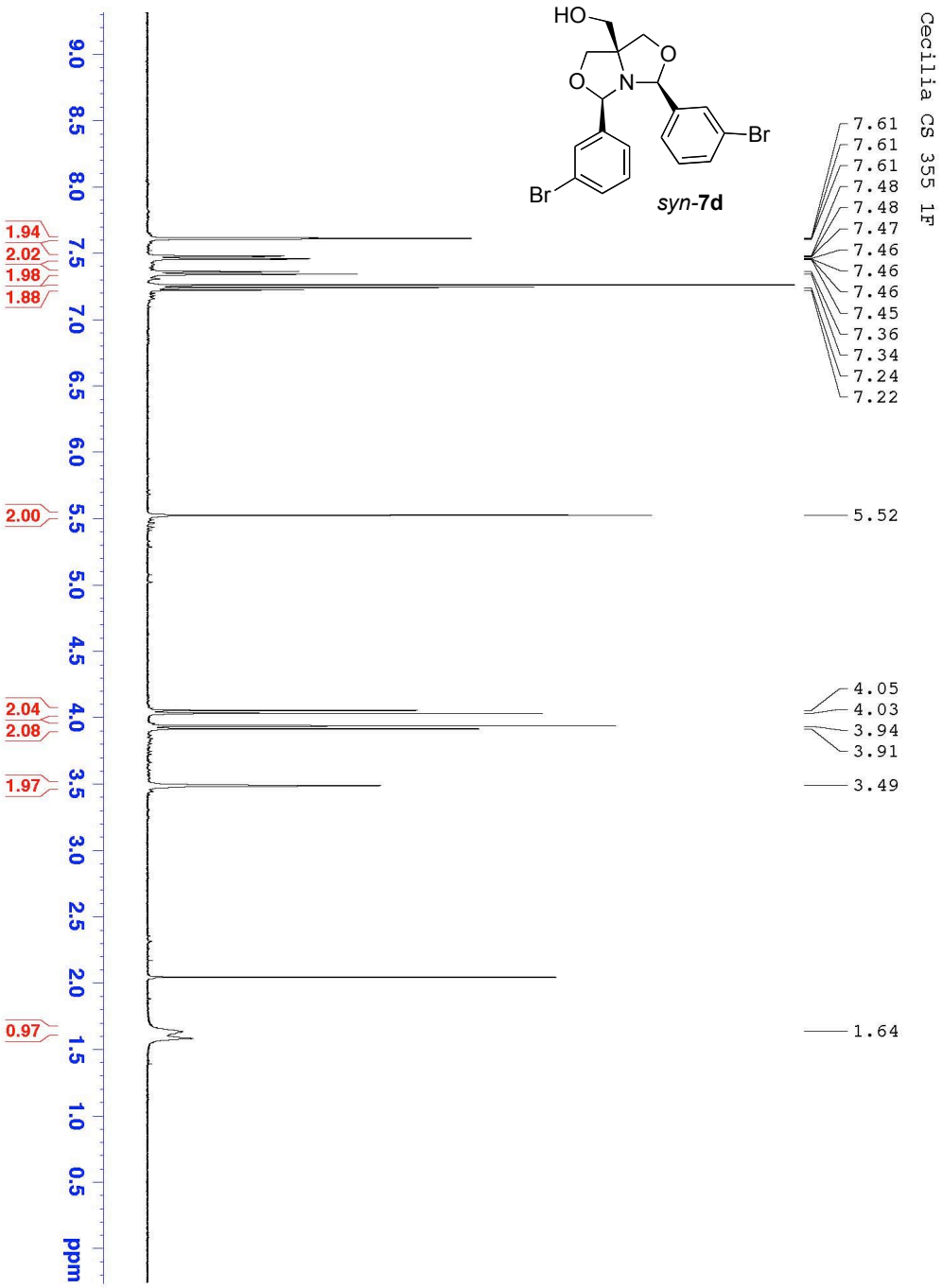
140
130
120
110
100
90
80
70
60
50
40
30
ppm


HO
O
O
N
Br
Br
syn-7d
Cecilia CS 355 1F
7.61
7.61
7.61
7.48
7.48
7.47
7.46
7.46
7.46
7.45
7.36
7.34
7.24
7.22
5.52
4.05
4.03
3.94
3.91
3.49
1.64
1.94
2.02
1.98
1.88
2.00
2.04
2.08
1.97
0.97
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
4.0
3.5
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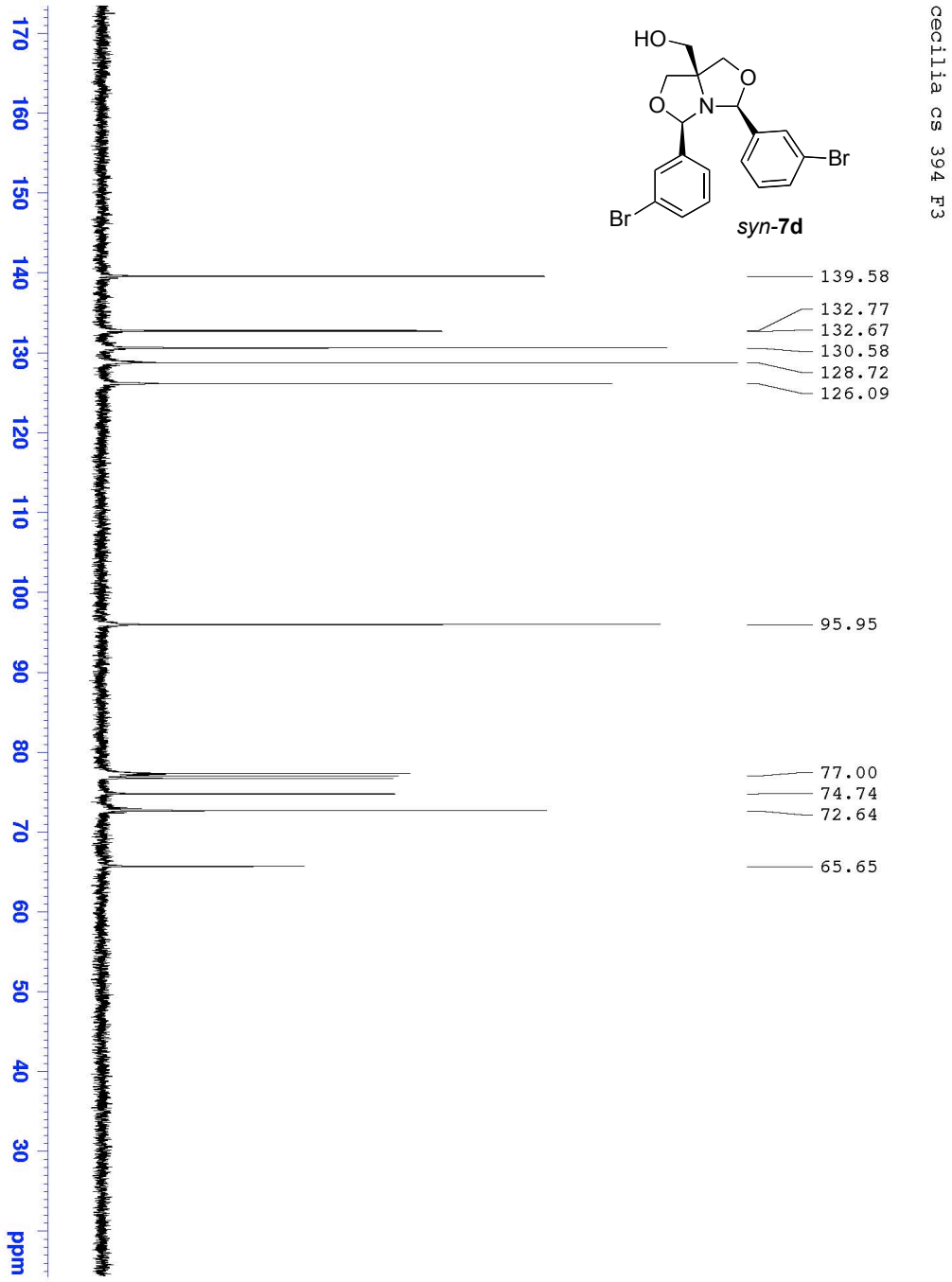
0.5
ppm


cecilia cs 394 F3
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O
O
N
Br
Br
syn-7d
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132.77
132.67
130.58
128.72
126.09
95.95
77.00
74.74
72.64
65.65
170
160
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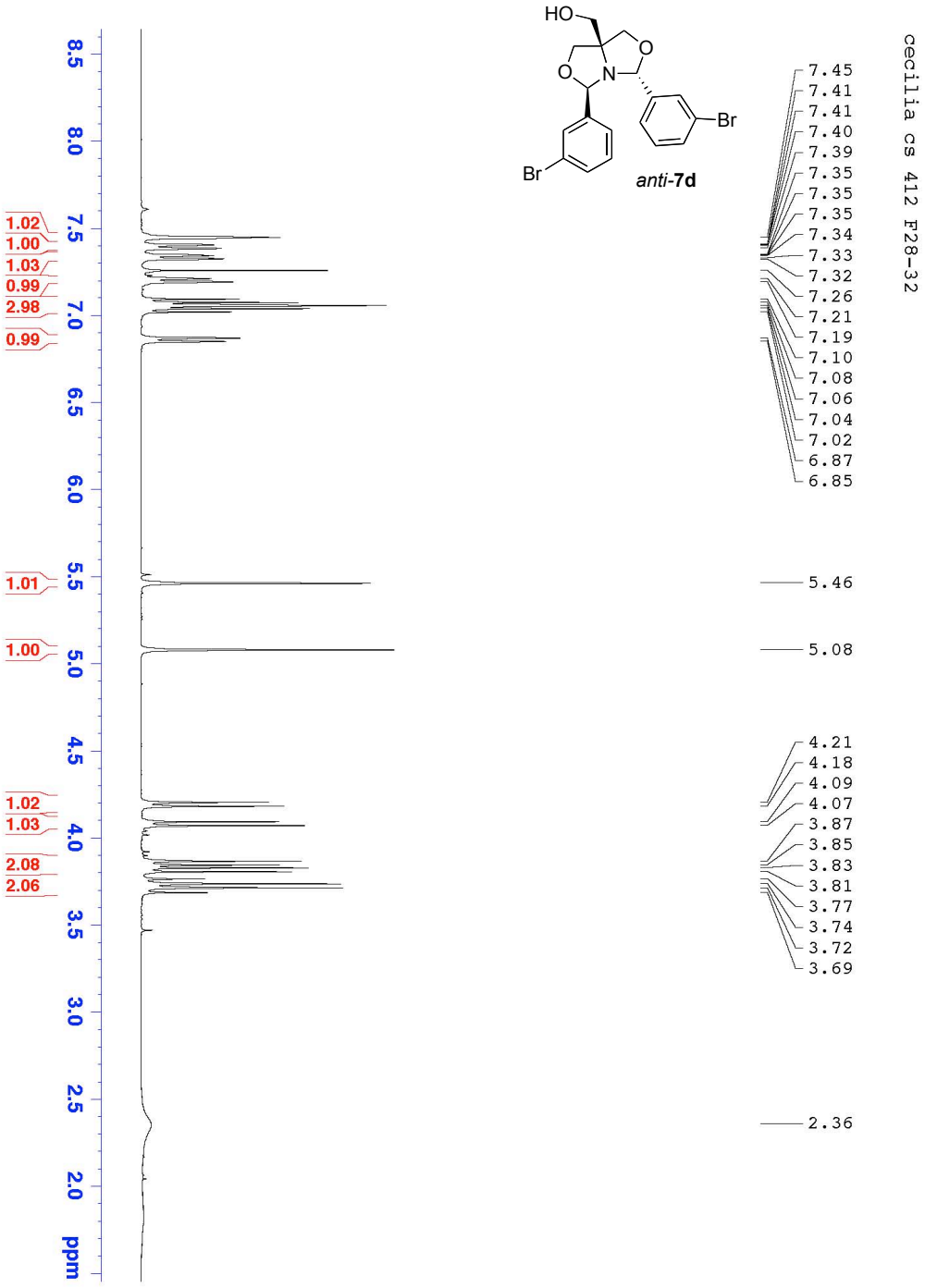
140
130
120
110
100
90
80
70
60
50
40
30
ppm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O
N
Br
Br
anti-7d
cecilia cs 412 F28-32
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7.35
7.35
7.35
7.34
7.33
7.32
7.26
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7.04
7.02
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3.87
3.85
3.83
3.81
3.77
3.74
3.72
3.69
2.36
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1.00
1.03
0.99
2.98
0.99
1.01
1.00
1.02
1.03
2.08
2.06
8.5
8.0
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3.5
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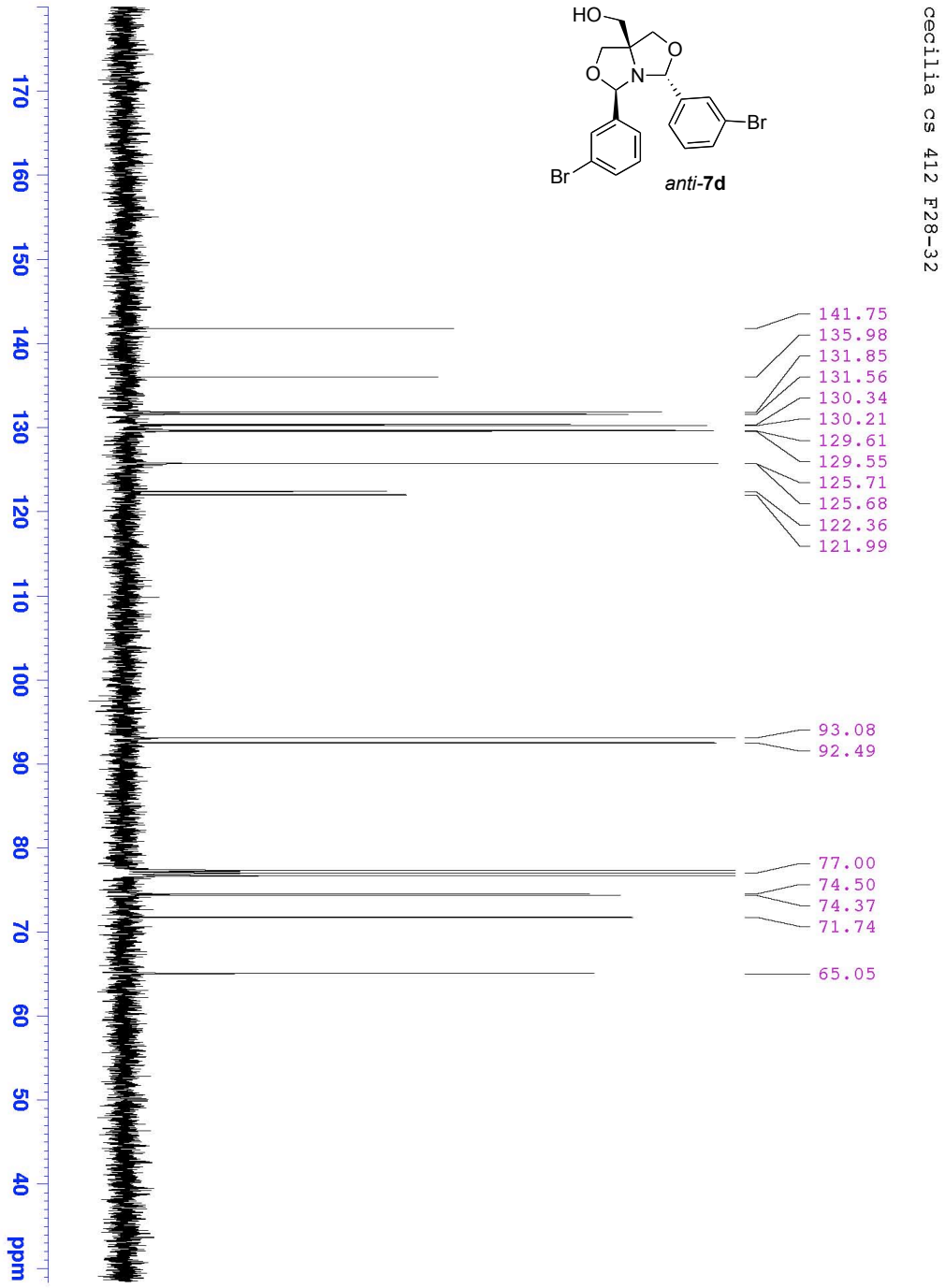
2.5
2.0
ppm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O
N
Br
Br
anti-7d
cecilia cs 412 F28-32
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130.21
129.61
129.55
125.71
125.68
122.36
121.99
93.08
92.49
77.00
74.50
74.37
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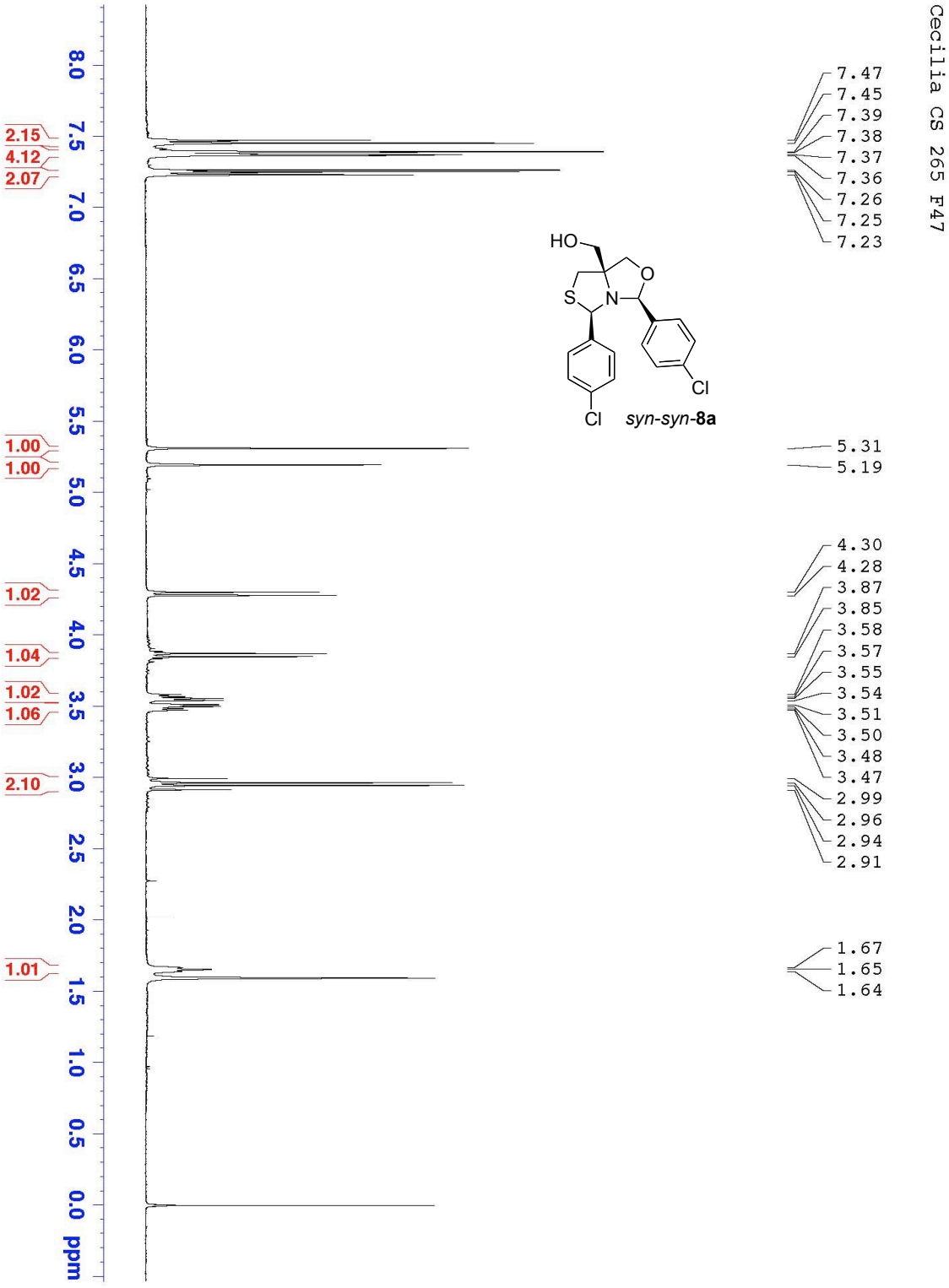
120
110
100
90
80
70
60
50
40
ppm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4.28
3.87
3.85
3.58
3.57
3.55
3.54
3.51
3.50
3.48
3.47
2.99
2.96
2.94
2.91
1.67
1.65
1.64
2.15
4.12
2.07
1.00
1.00
1.02
1.04
1.02
1.06
2.10
1.01
HO
O
S
N
Cl
Cl
syn-syn-8a
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
3.5
3.0
2.5
2.0
1.5
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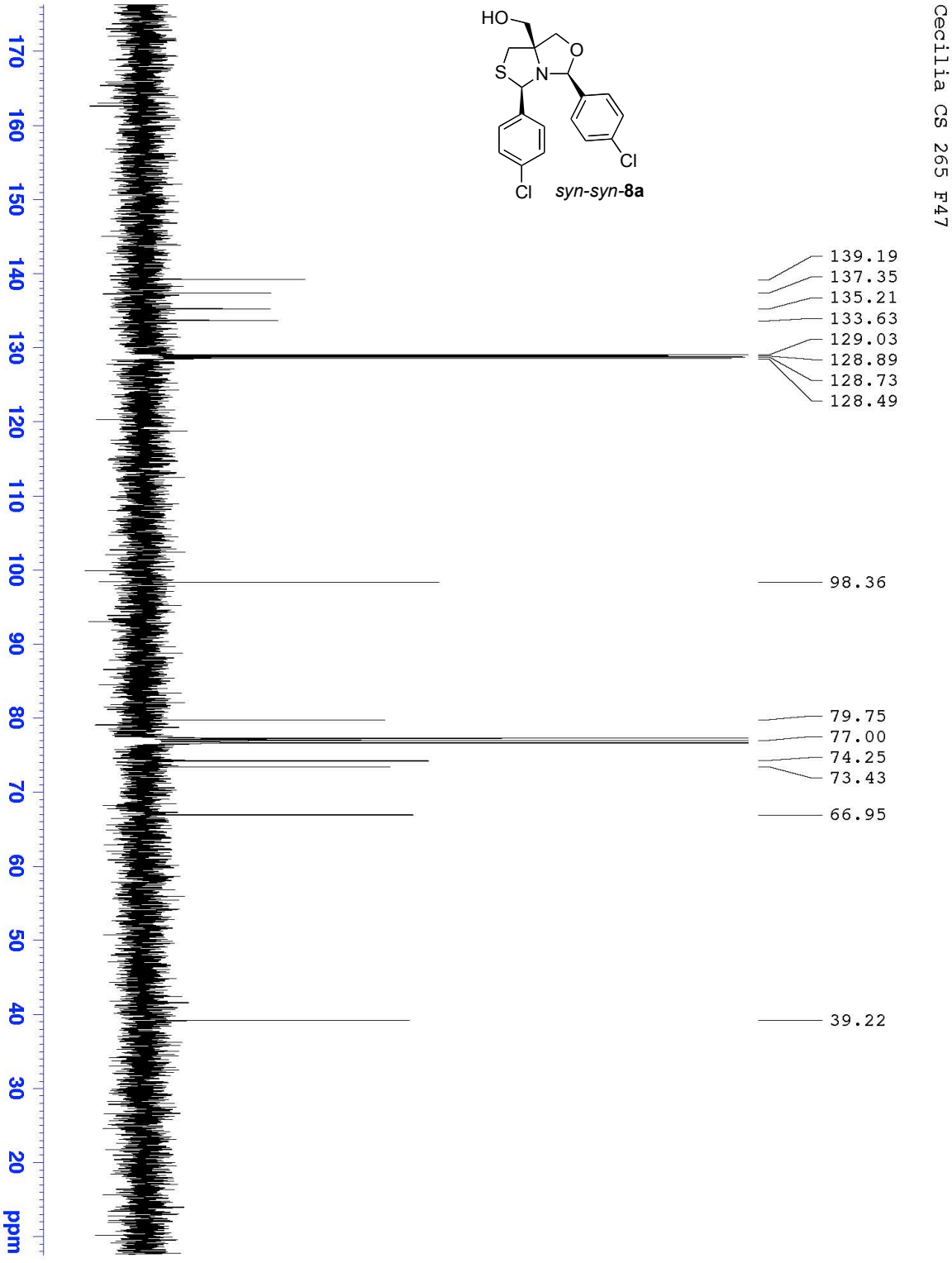
0.5
0.0
ppm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S
N
O
Cl
Cl
syn-syn-8a
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137.35
135.21
133.63
129.03
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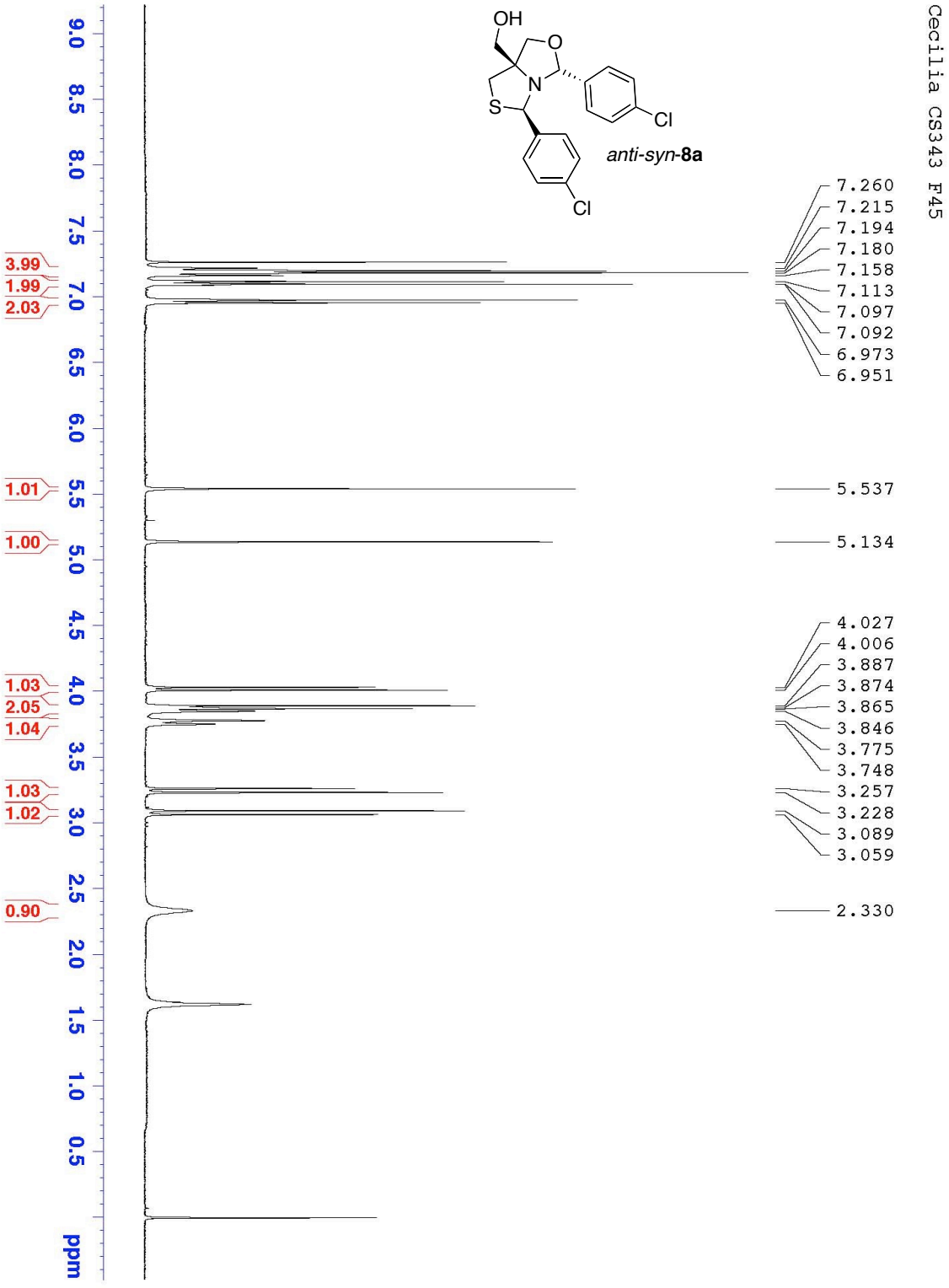
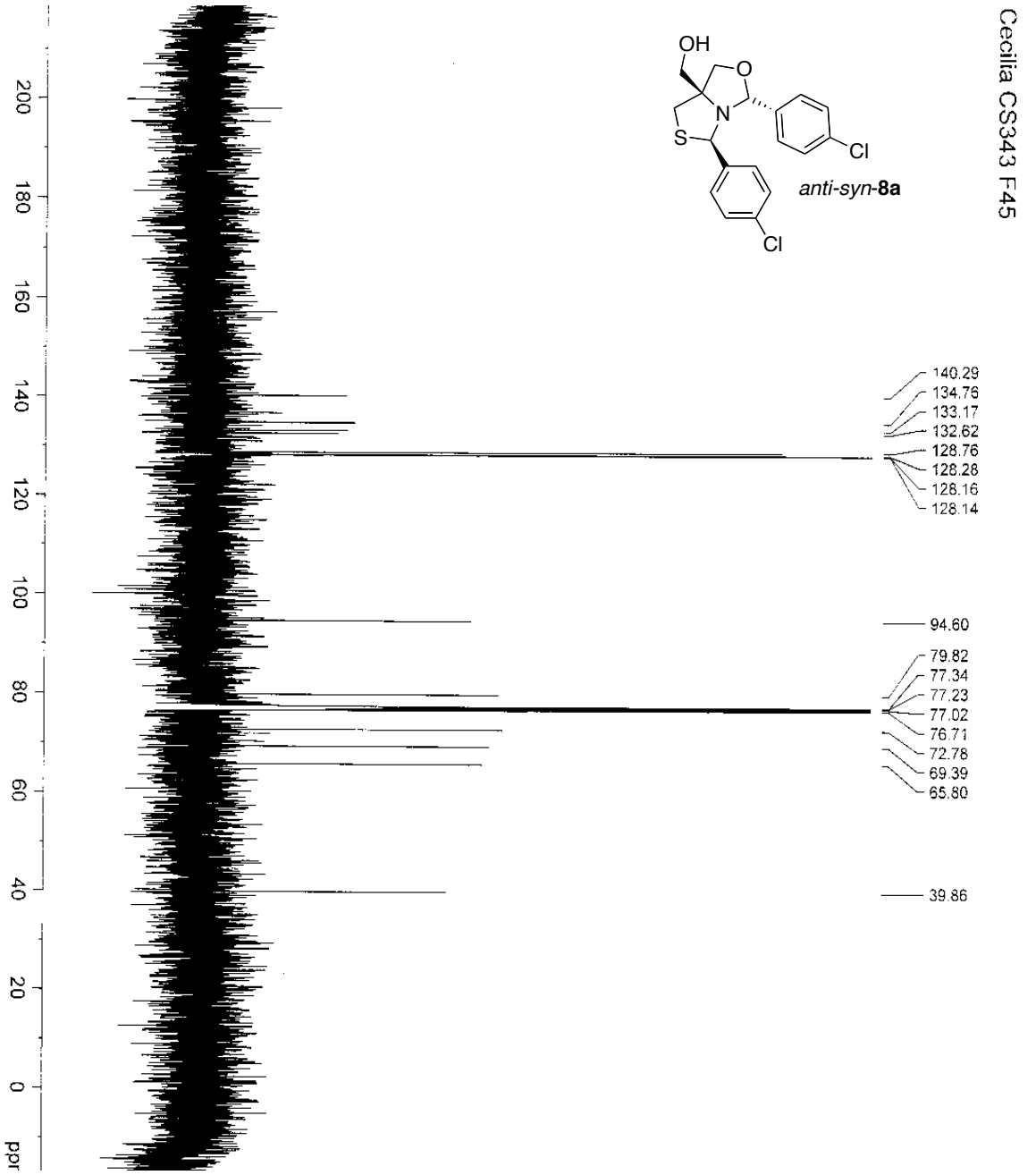
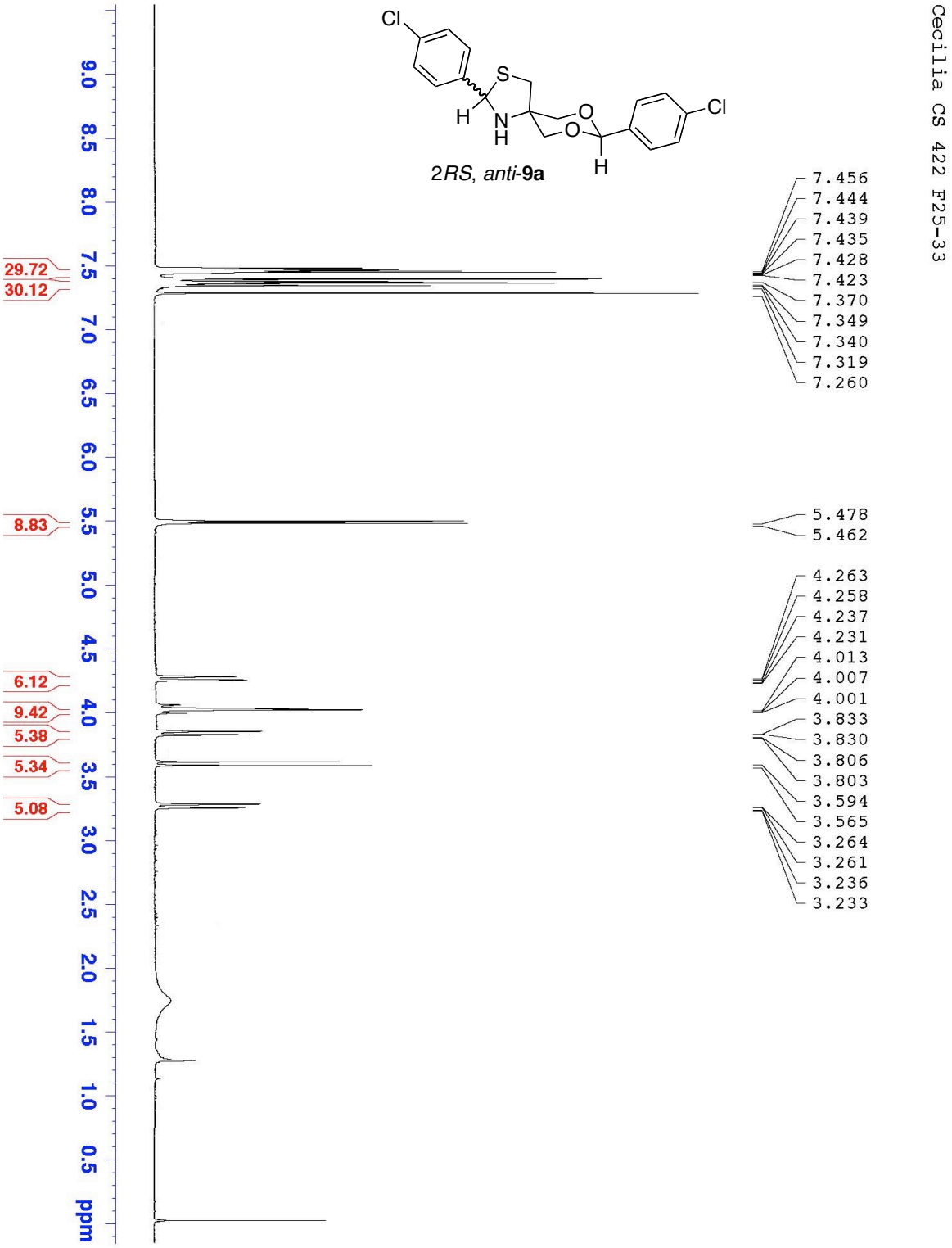
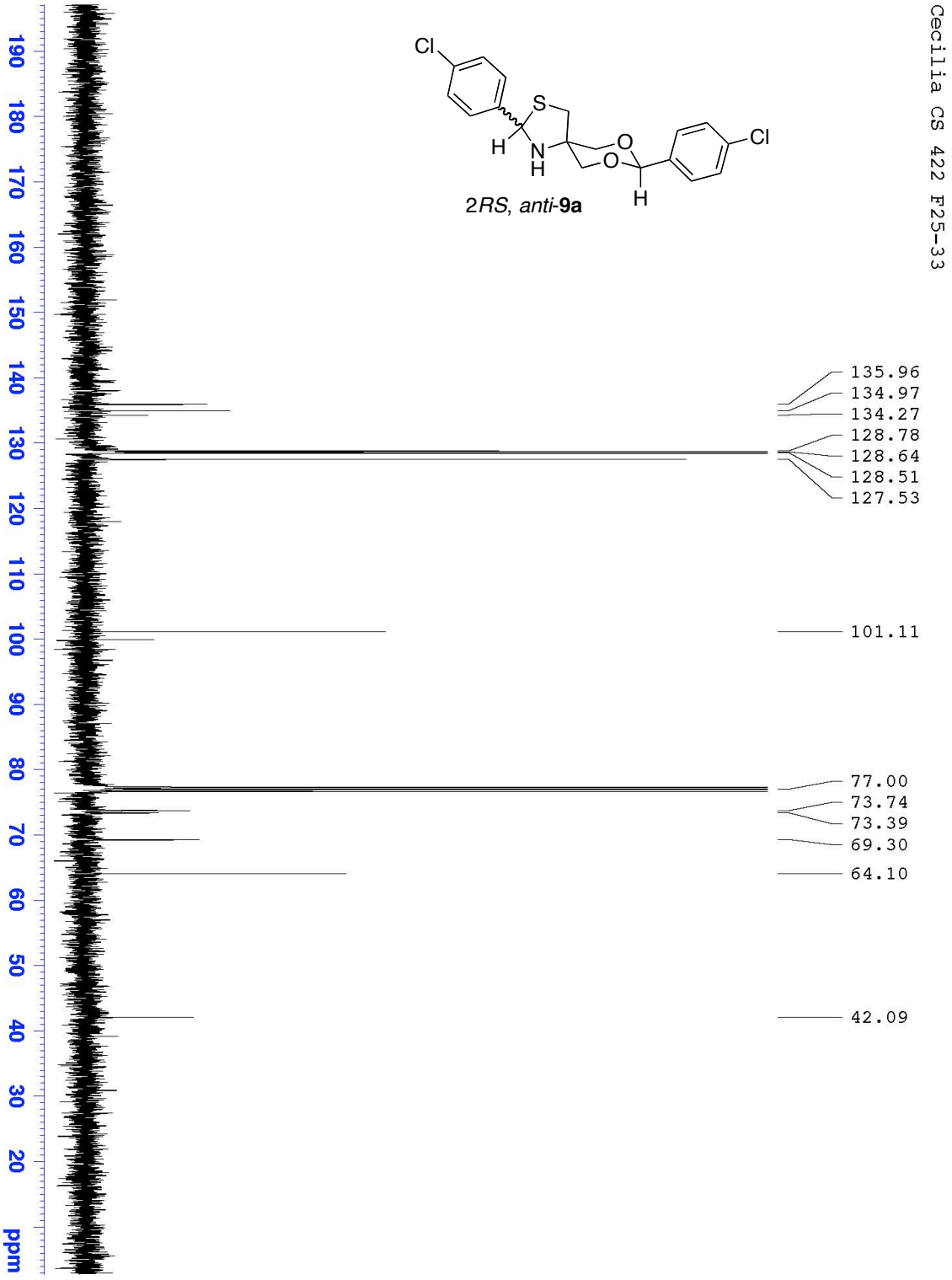
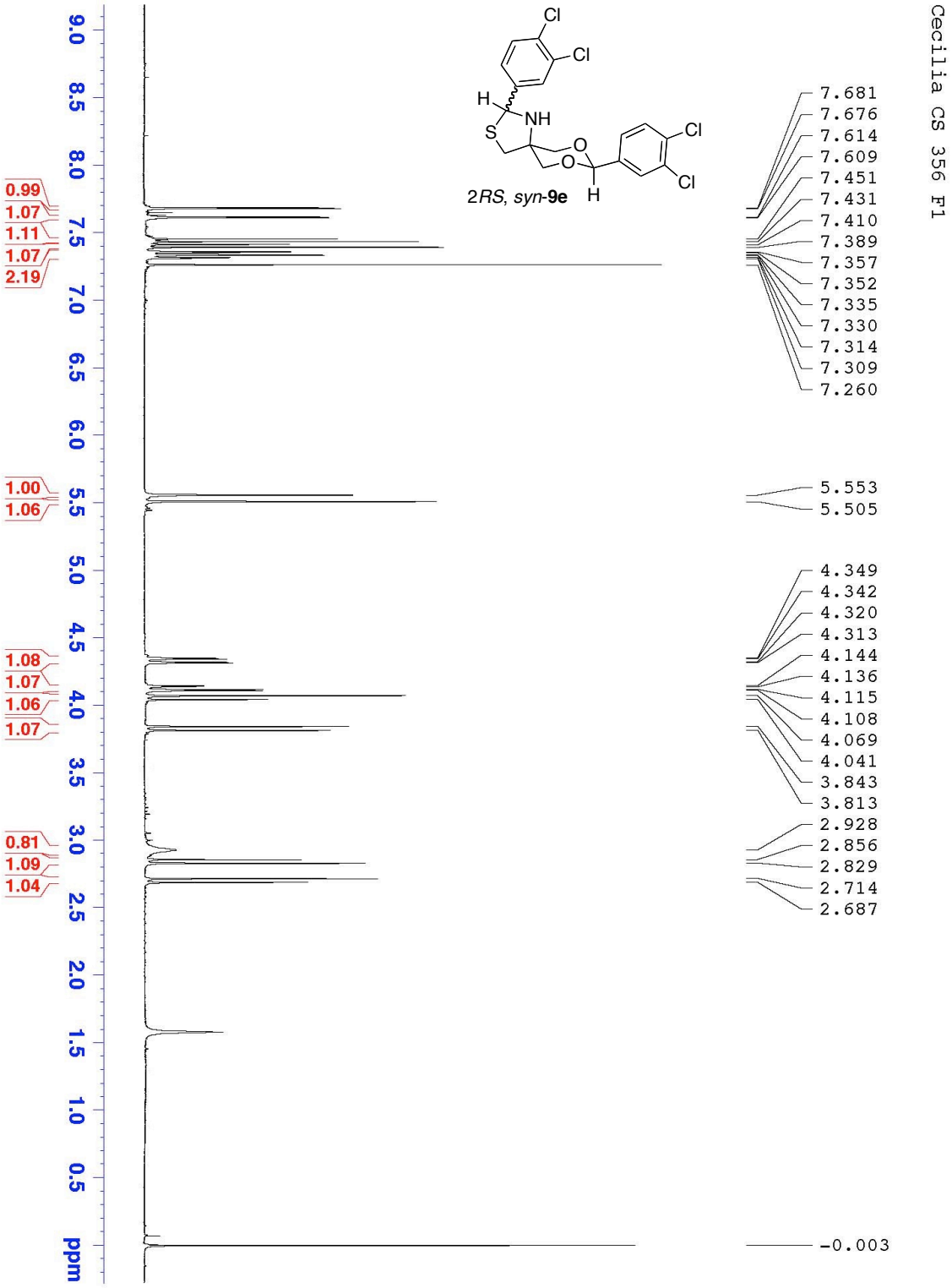
128.73
128.49
98.36
79.75
77.00
74.25
73.43
66.95
39.22
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

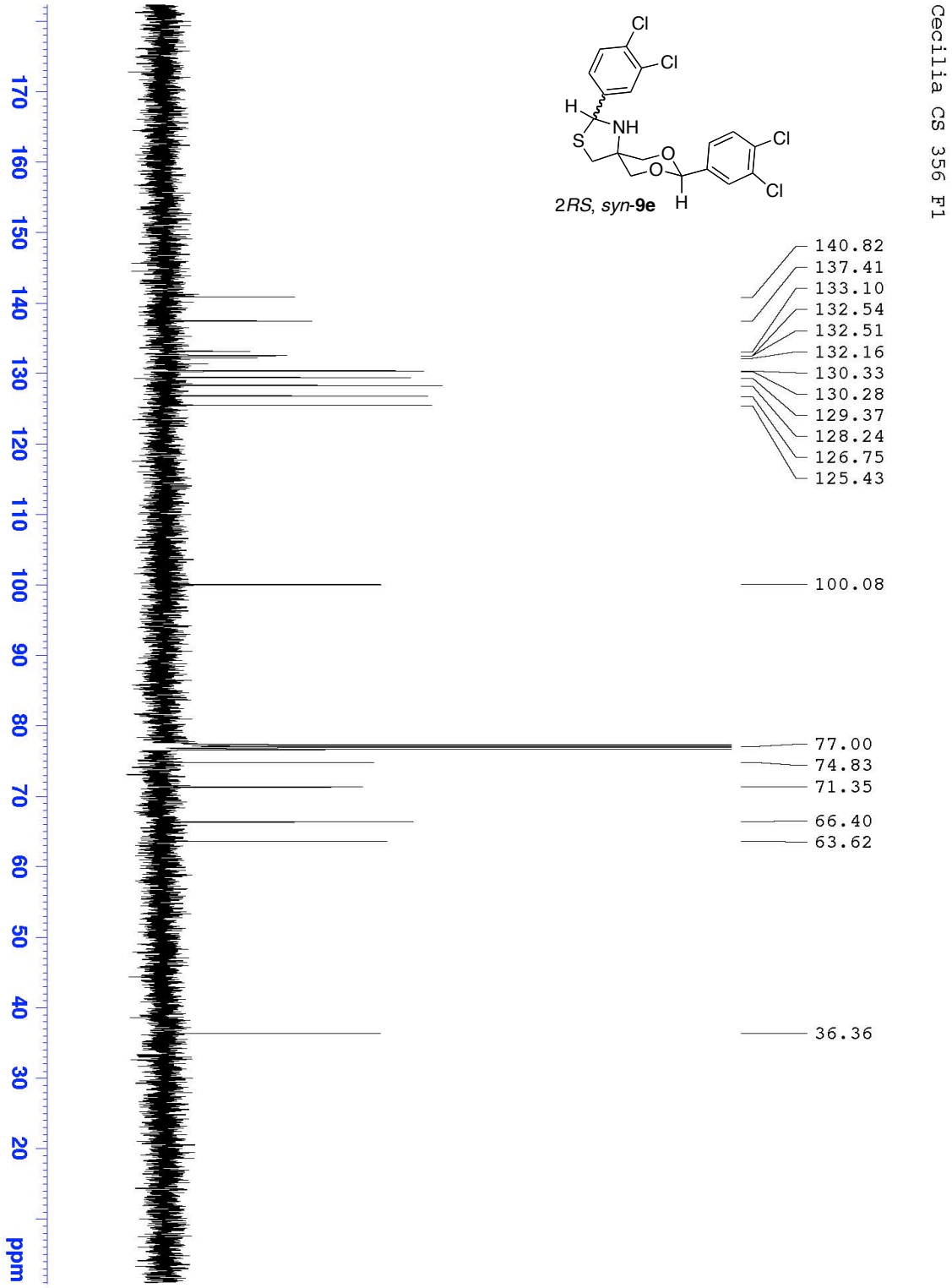

Cecilia CS343 F45
OH
O
N
S
Cl
Cl
anti-syn-8a
7.260
7.215
7.194
7.180
7.158
7.113
7.097
7.092
6.973
6.951
5.537
5.134
4.027
4.006
3.887
3.874
3.865
3.846
3.775
3.748
3.257
3.228
3.089
3.059
2.330
3.99
1.99
2.03
1.01
1.00
1.03
2.05
1.04
1.03
1.02
0.90
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

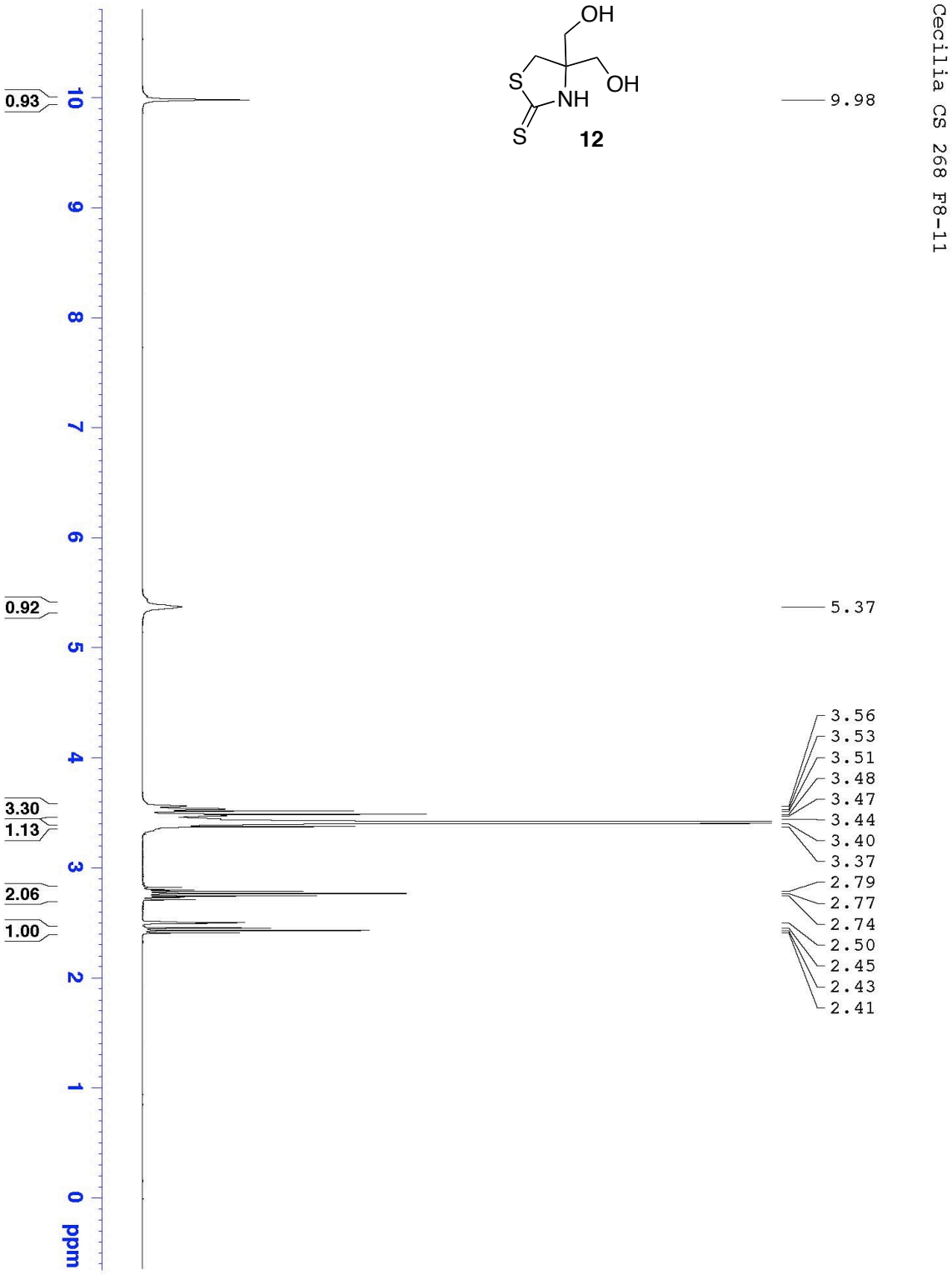

Cecilia CS343 F45
OH
O
N
S
Cl
Cl
anti-syn-8a
140.29
134.76
133.17
132.62
128.76
128.28
128.16
128.14
94.60
79.82
77.34
77.23
77.02
76.71
72.78
69.39
65.80
39.86
200
180
160
140
120
100
80
60
40
20
0
ppm

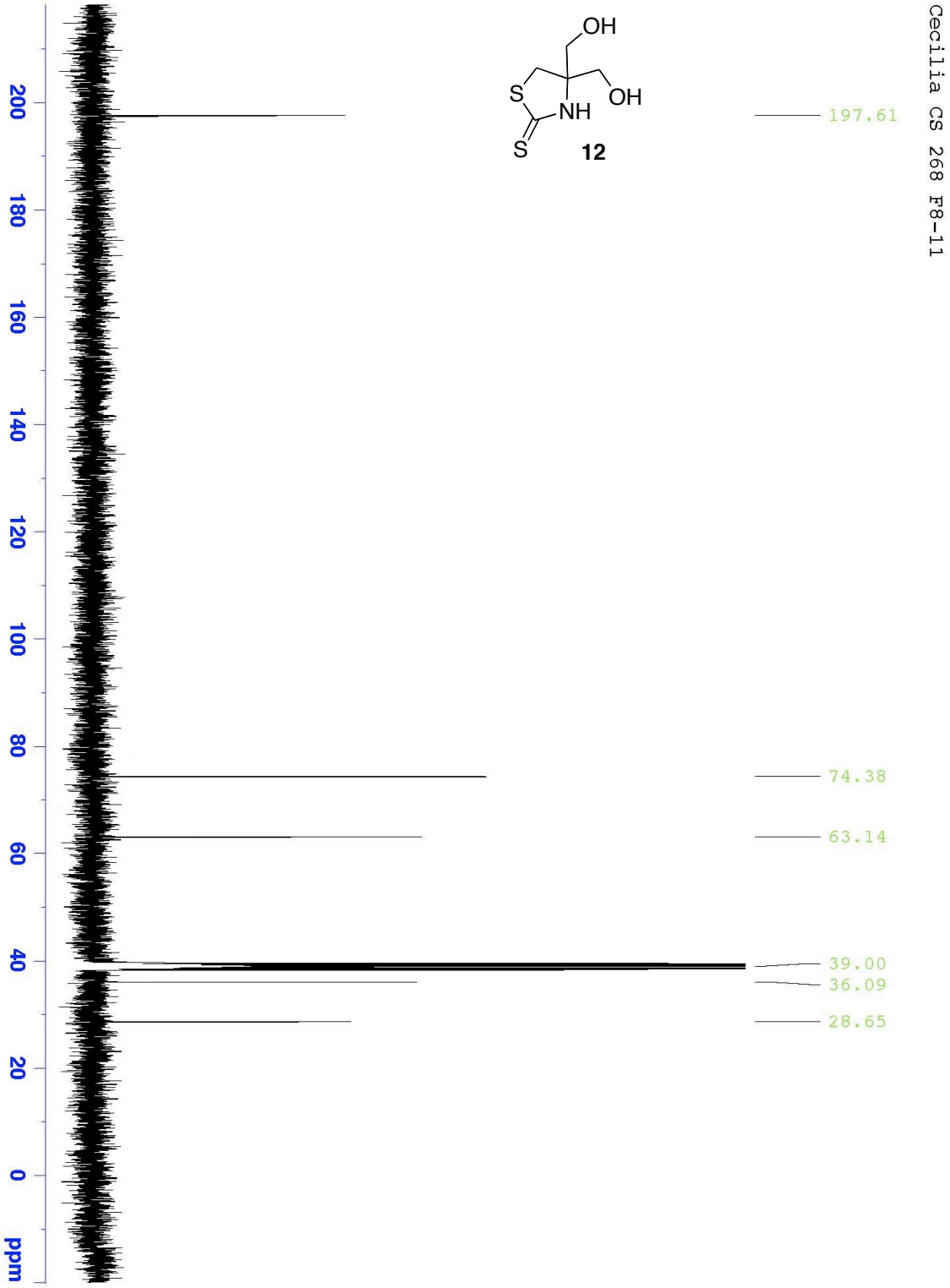

Cecilia CS 422 F25-33
Cl
S
H
N
H
O
O
Cl
H
2RS, anti-9a
7.456
7.444
7.439
7.435
7.428
7.423
7.370
7.349
7.340
7.319
7.260
5.478
5.462
4.263
4.258
4.237
4.231
4.013
4.007
4.001
3.833
3.830
3.806
3.803
3.594
3.565
3.264
3.261
3.236
3.233
29.72
30.12
8.83
6.12
9.42
5.38
5.34
5.08
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

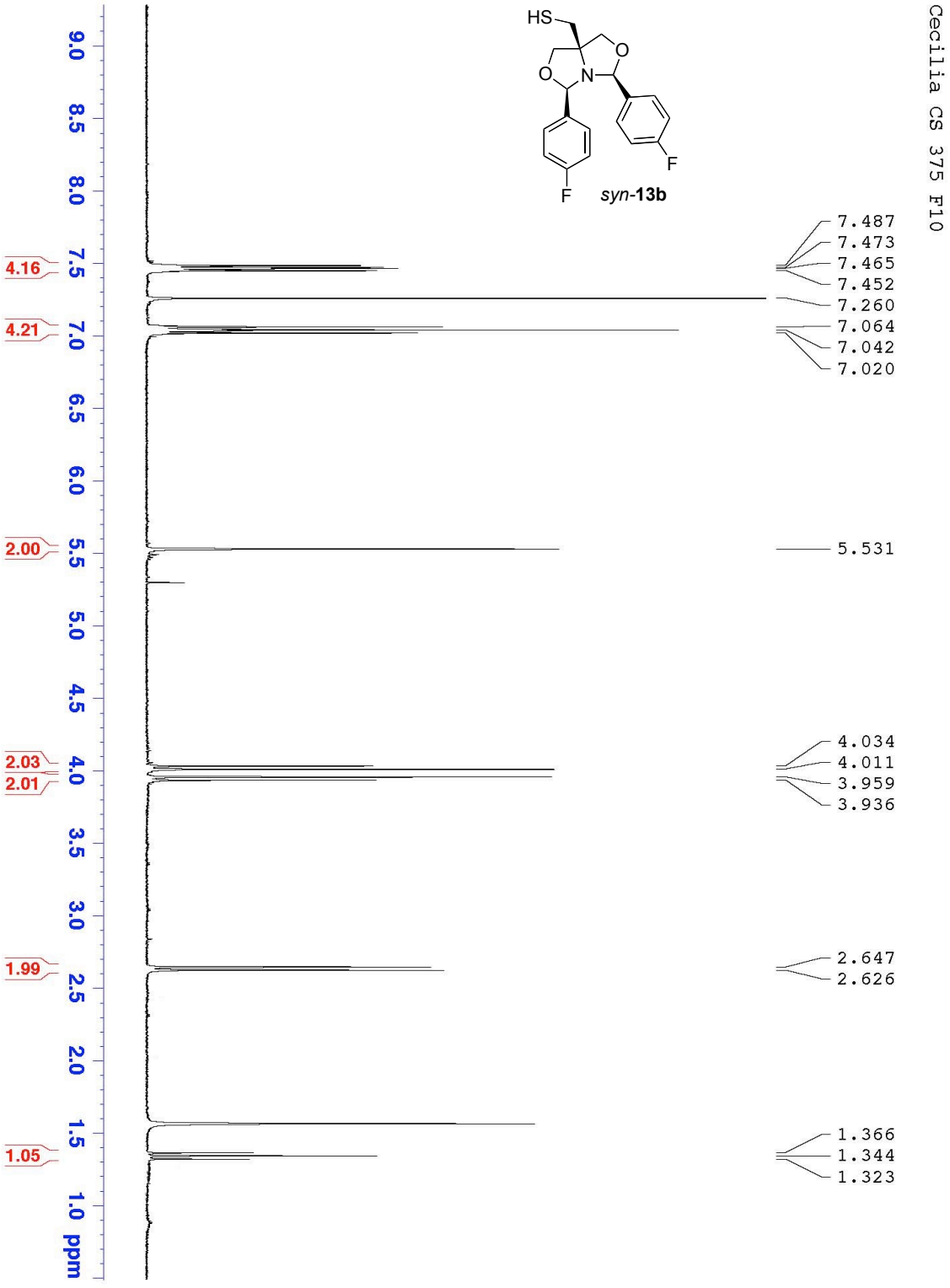

Cecilia CS 422 F25-33
Cl
S
H
N
H
O
O
H
Cl
2RS, anti-9a
135.96
134.97
134.27
128.78
128.64
128.51
127.53
101.11
77.00
73.74
73.39
69.30
64.10
42.09
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

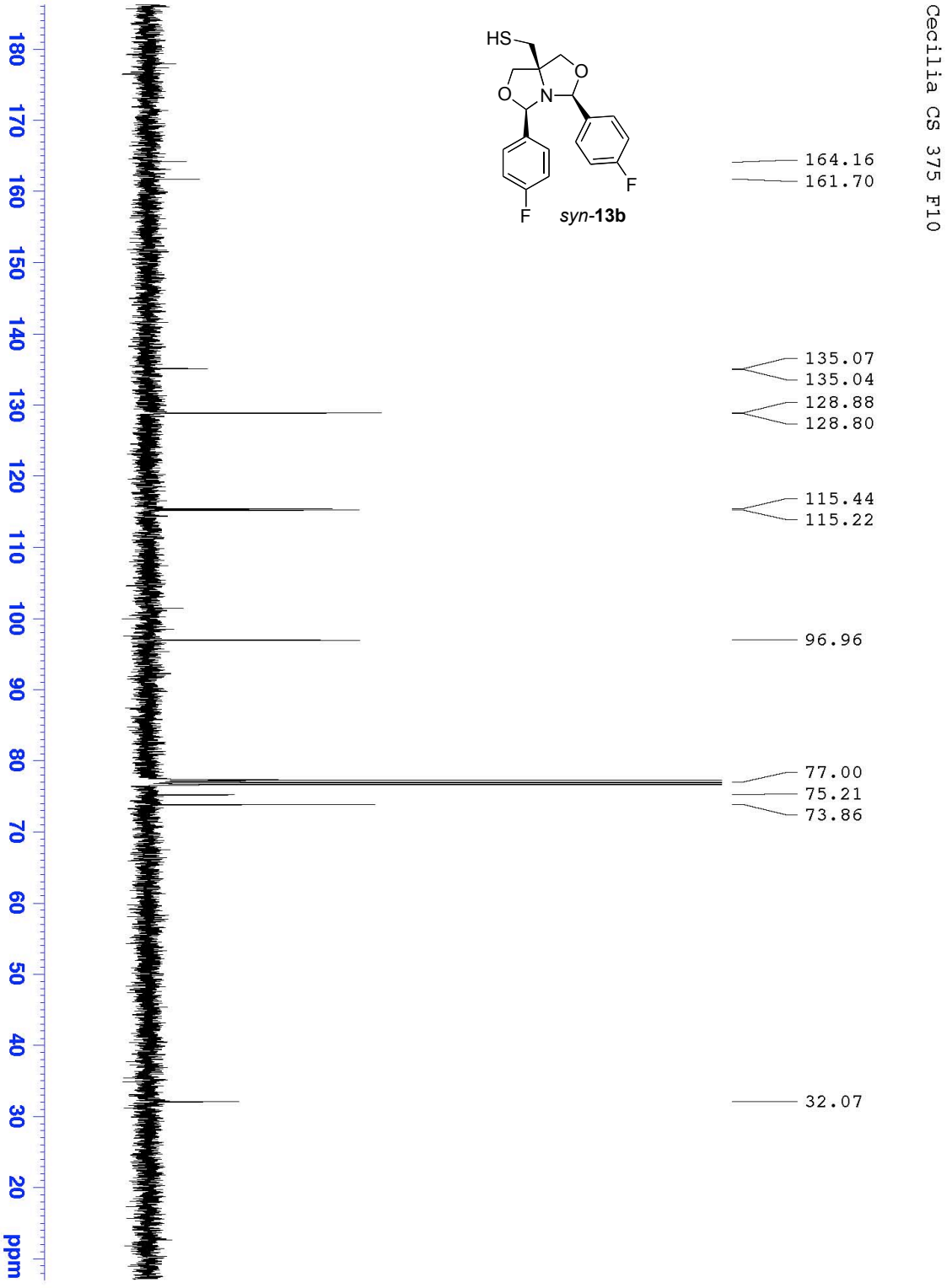

Cecilia CS 356 F1
2RS, syn-9e
7.681
7.676
7.614
7.609
7.451
7.431
7.410
7.389
7.357
7.352
7.335
7.330
7.314
7.309
7.260
5.553
5.505
4.349
4.342
4.320
4.313
4.144
4.136
4.115
4.108
4.069
4.041
3.843
3.813
2.928
2.856
2.829
2.714
2.687
-0.003
0.99
1.07
1.11
1.07
2.19
1.00
1.06
1.08
1.07
1.06
1.07
0.81
1.09
1.04
ppm

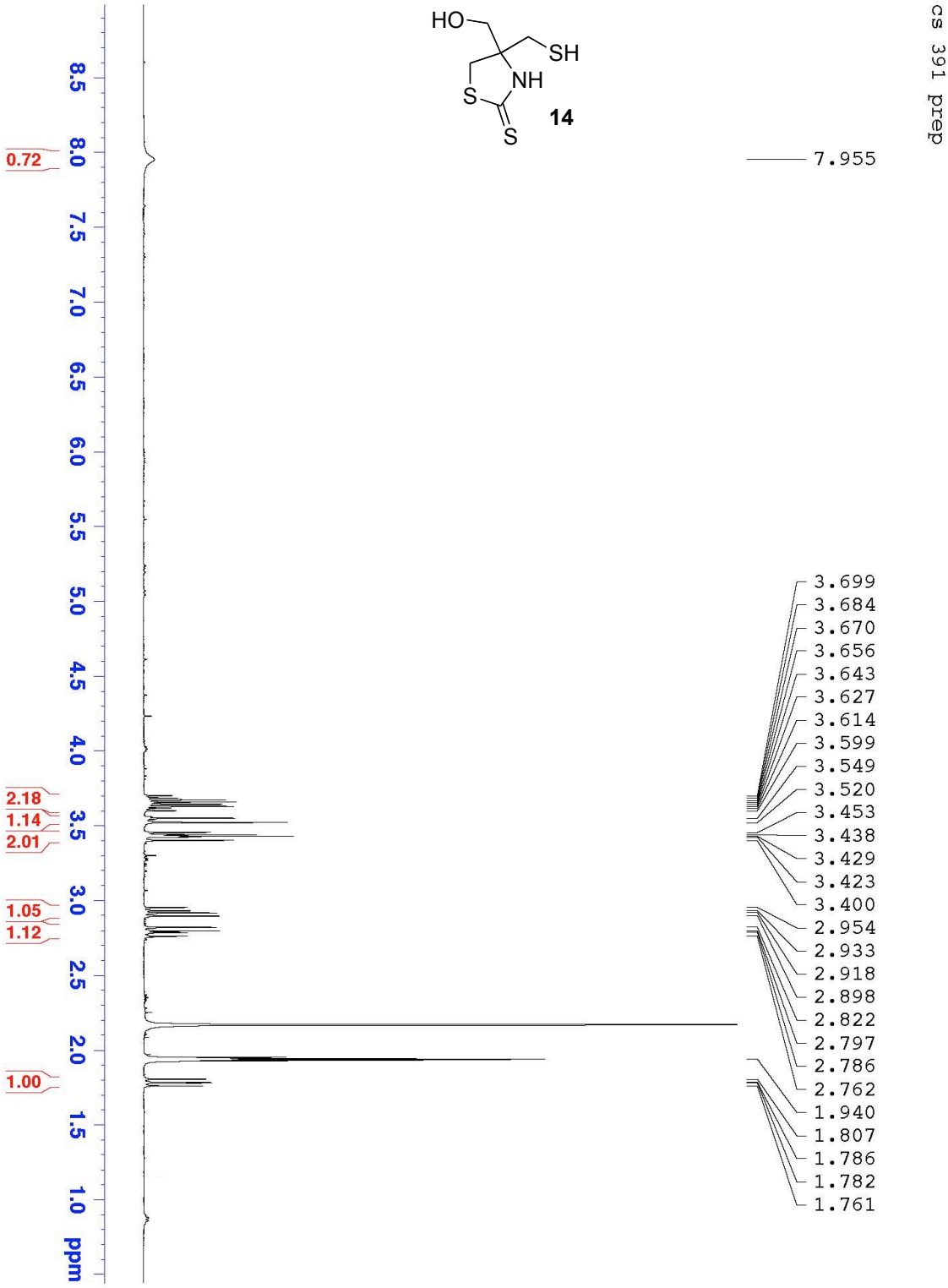

Cecilia CS 356 F1
Cl
Cl
H
NH
S
O
O
Cl
Cl
H
2RS, syn-9e
140.82
137.41
133.10
132.54
132.51
132.16
130.33
130.28
129.37
128.24
126.75
125.43
100.08
77.00
74.83
71.35
66.40
63.62
36.36
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

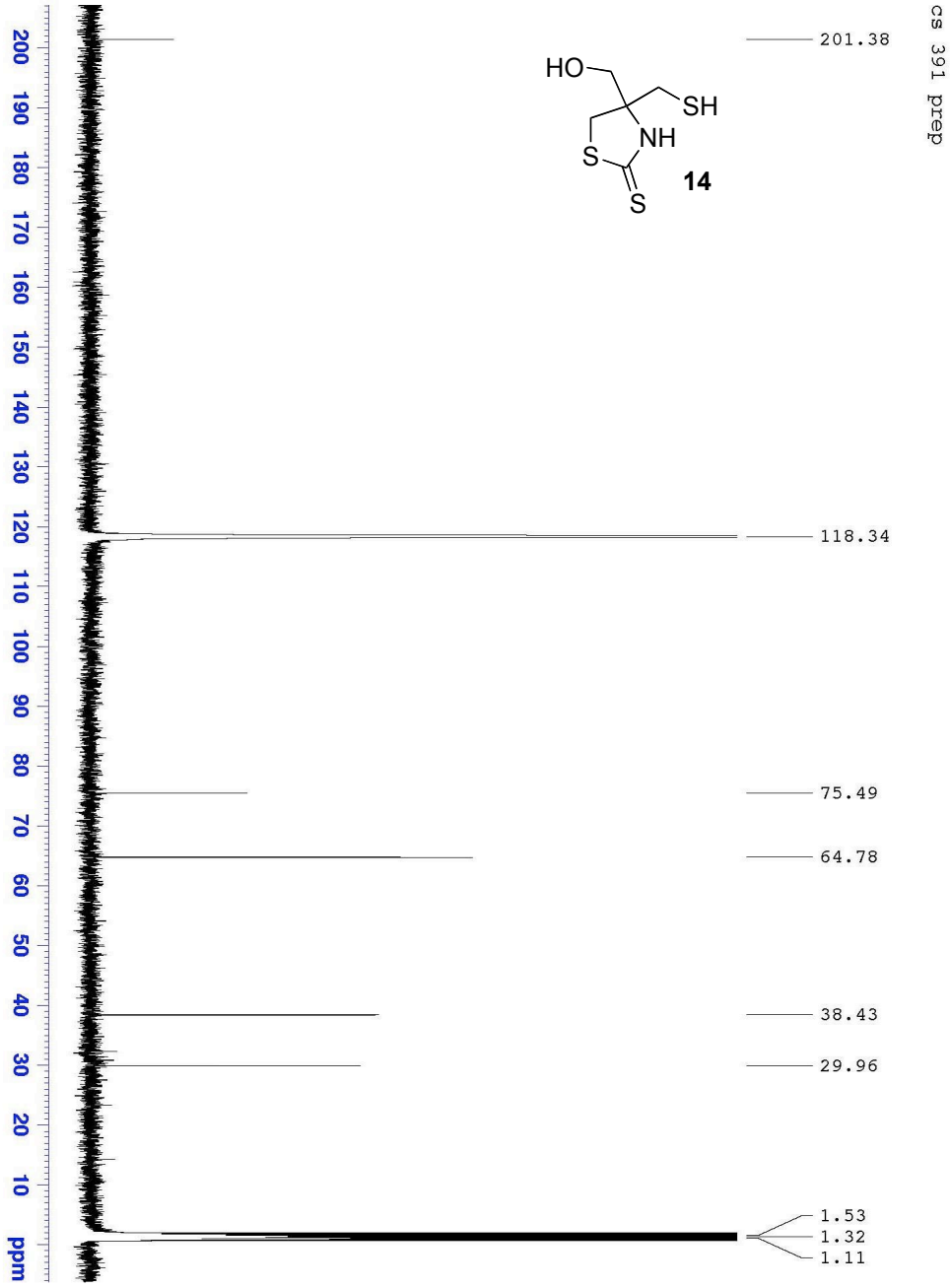

Cecilia CS 268 F8-11
OH
OH
S
NH
S
12
9.98
5.37
3.56
3.53
3.51
3.48
3.47
3.44
3.40
3.37
2.79
2.77
2.74
2.50
2.45
2.43
2.41
0.93
0.92
3.30
1.13
2.06
1.00
10
9
8
7
6
5
4
3
2
1
0
ppm

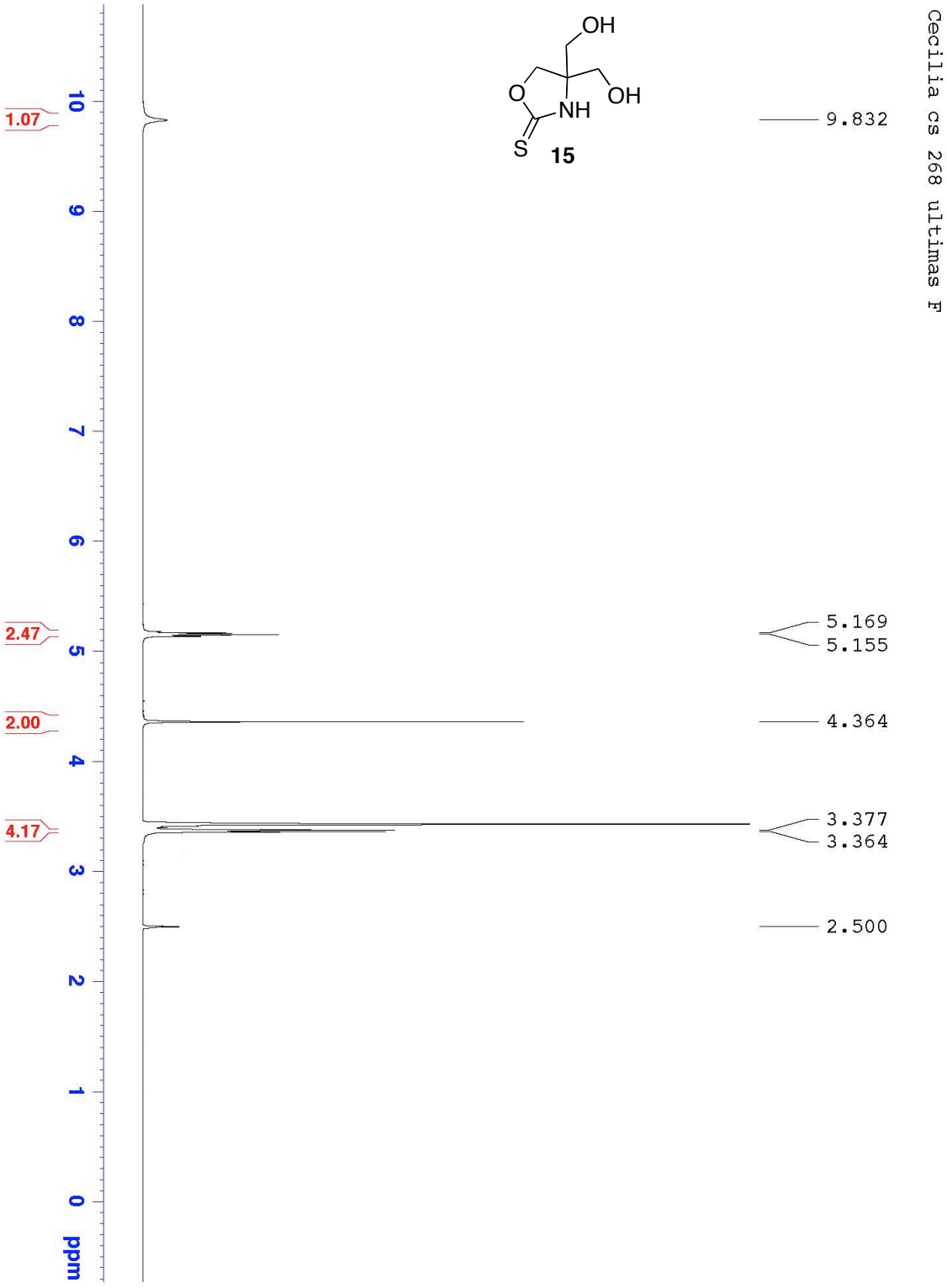

OH
S
NH
OH
S
12
197.61
74.38
63.14
39.00
36.09
28.65
200
180
160
140
120
100
80
60
40
20
0
ppm
Cecilia CS 268 F8-11

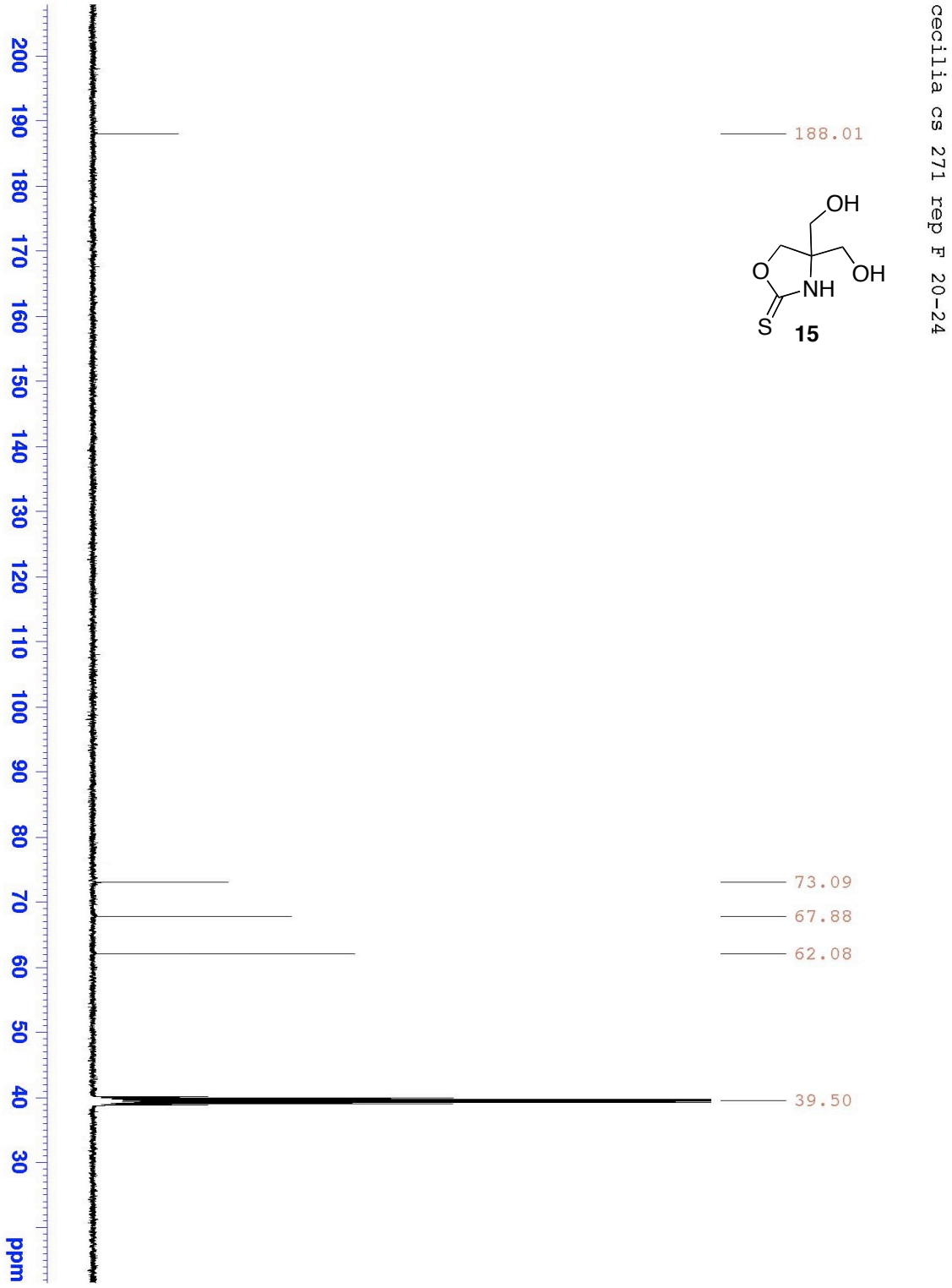

HS
O
O
N
F
F
syn-13b
Cecilia CS 375 F10
7.487
7.473
7.465
7.452
7.260
7.064
7.042
7.020
5.531
4.034
4.011
3.959
3.936
2.647
2.626
1.366
1.344
1.323
4.16
4.21
2.00
2.03
2.01
1.99
1.05
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

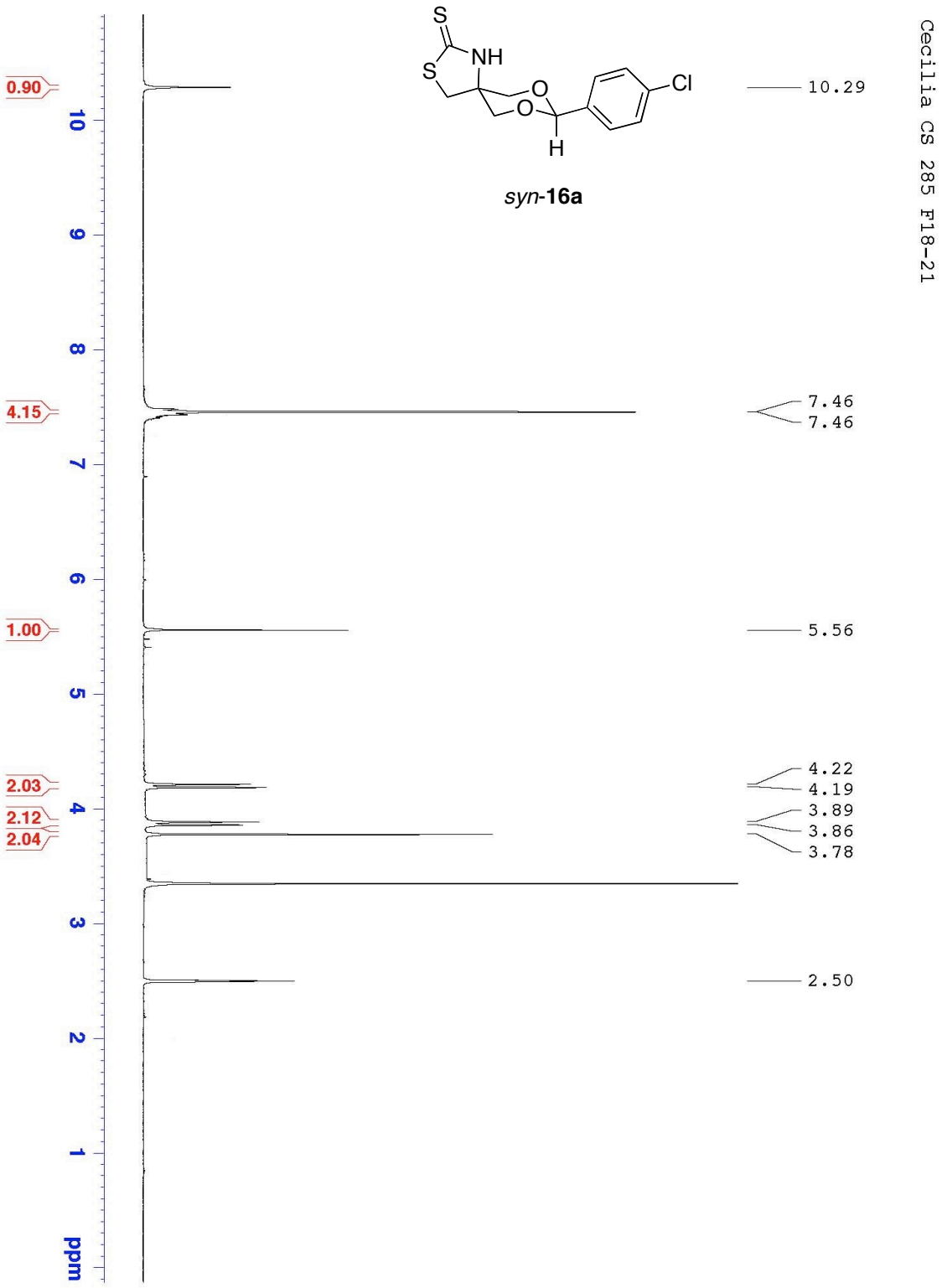

Cecilia CS 375 F10
HS
O
O
N
F
F
syn-13b
164.16
161.70
135.07
135.04
128.88
128.80
115.44
115.22
96.96
77.00
75.21
73.86
32.07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

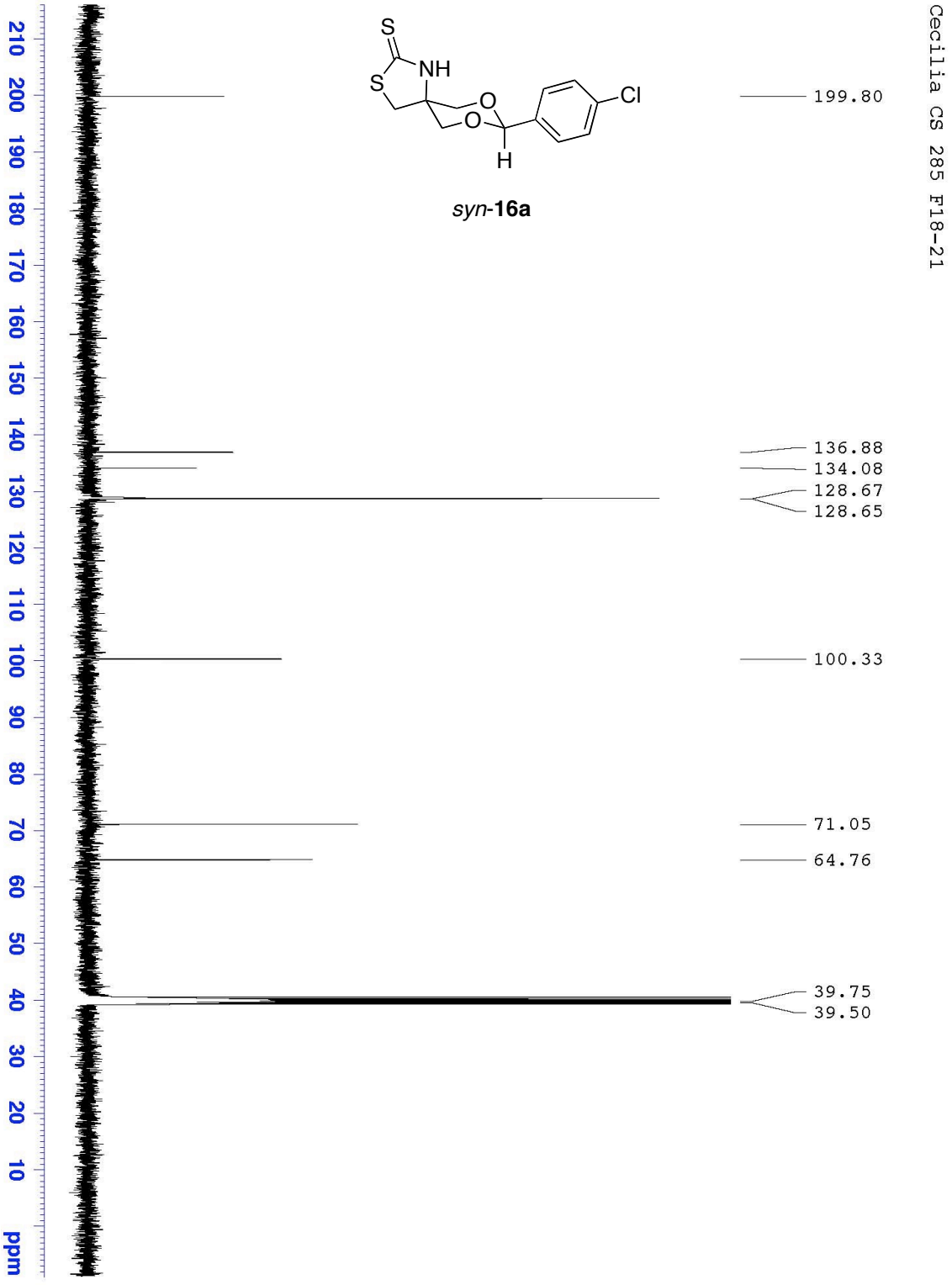

HO
SH
NH
S
S
14
cs 391 prep
7.955
3.699
3.684
3.670
3.656
3.643
3.627
3.614
3.599
3.549
3.520
3.453
3.438
3.429
3.423
3.400
2.954
2.933
2.918
2.898
2.822
2.797
2.786
2.762
1.940
1.807
1.786
1.782
1.761
0.72
2.18
1.14
2.01
1.05
1.12
1.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

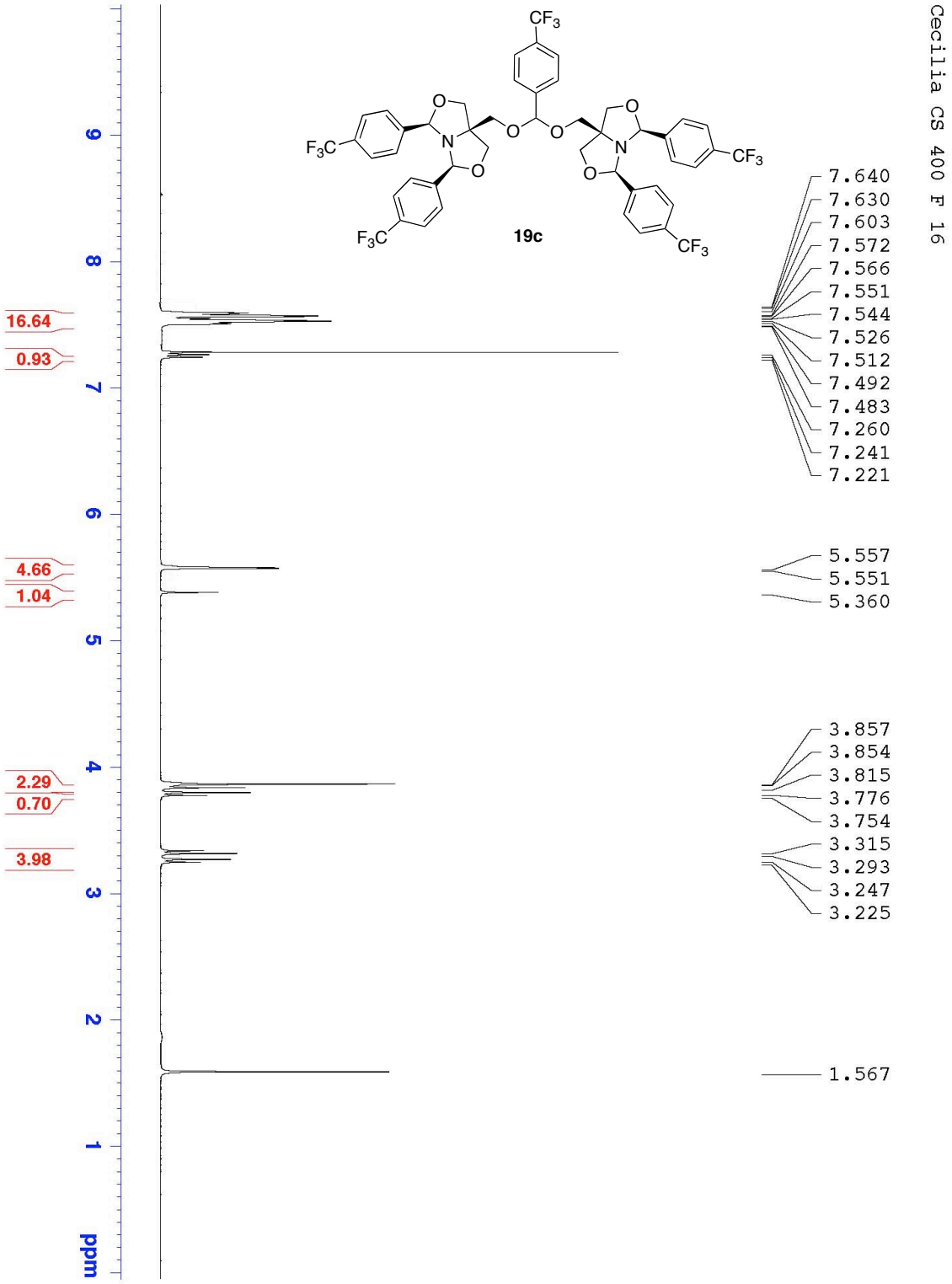

cs 391 prep
201.38
118.34
75.49
64.78
38.43
29.96
1.53
1.32
1.11
HO
SH
NH
S
S
14
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

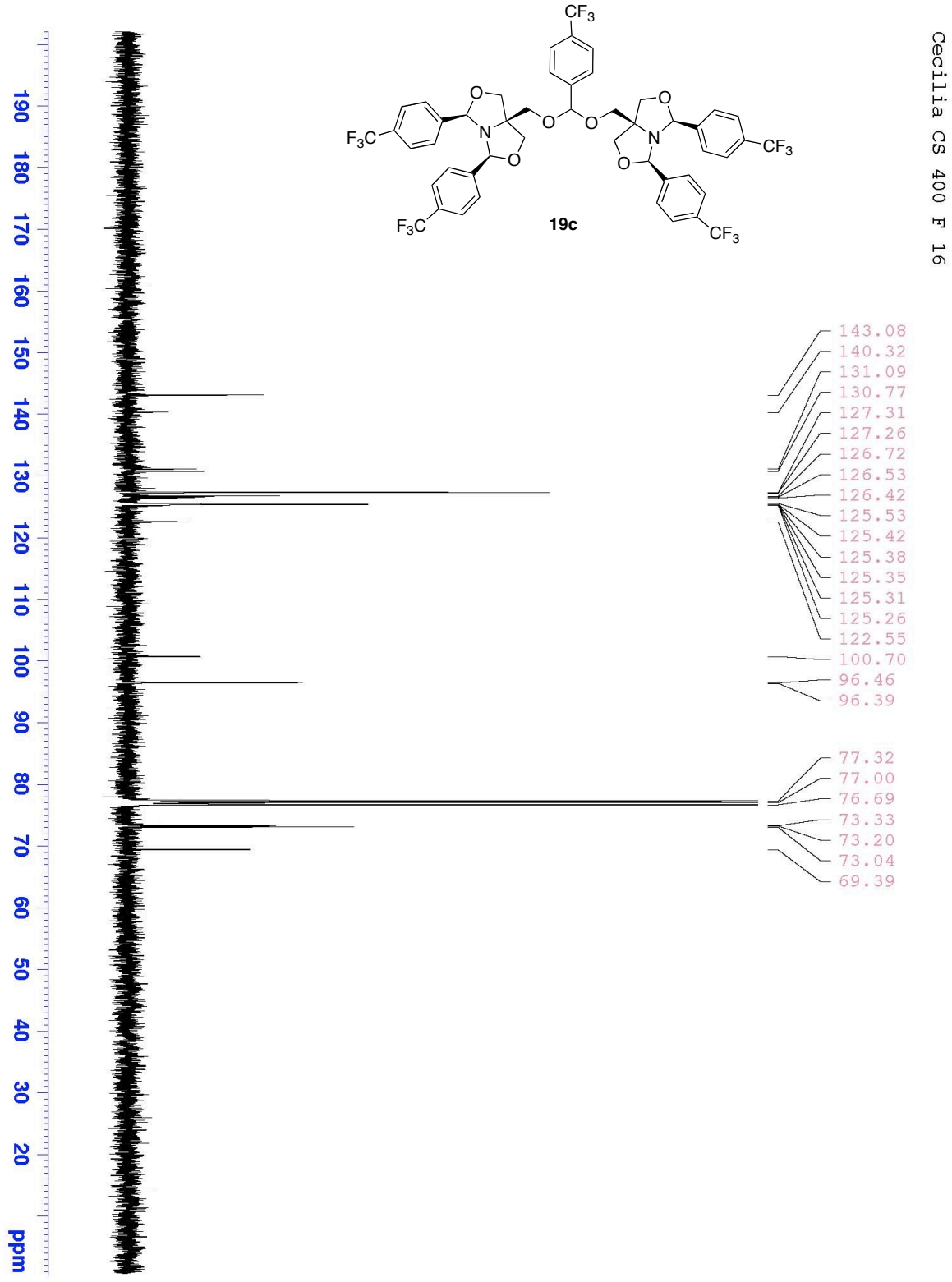

OH
O
NH
OH
S
15
Cecilia cs 268 ultimas F
9.832
5.169
5.155
4.364
3.377
3.364
2.500
1.07
2.47
2.00
4.17
10
9
8
7
6
5
4
3
2
1
0
ppm

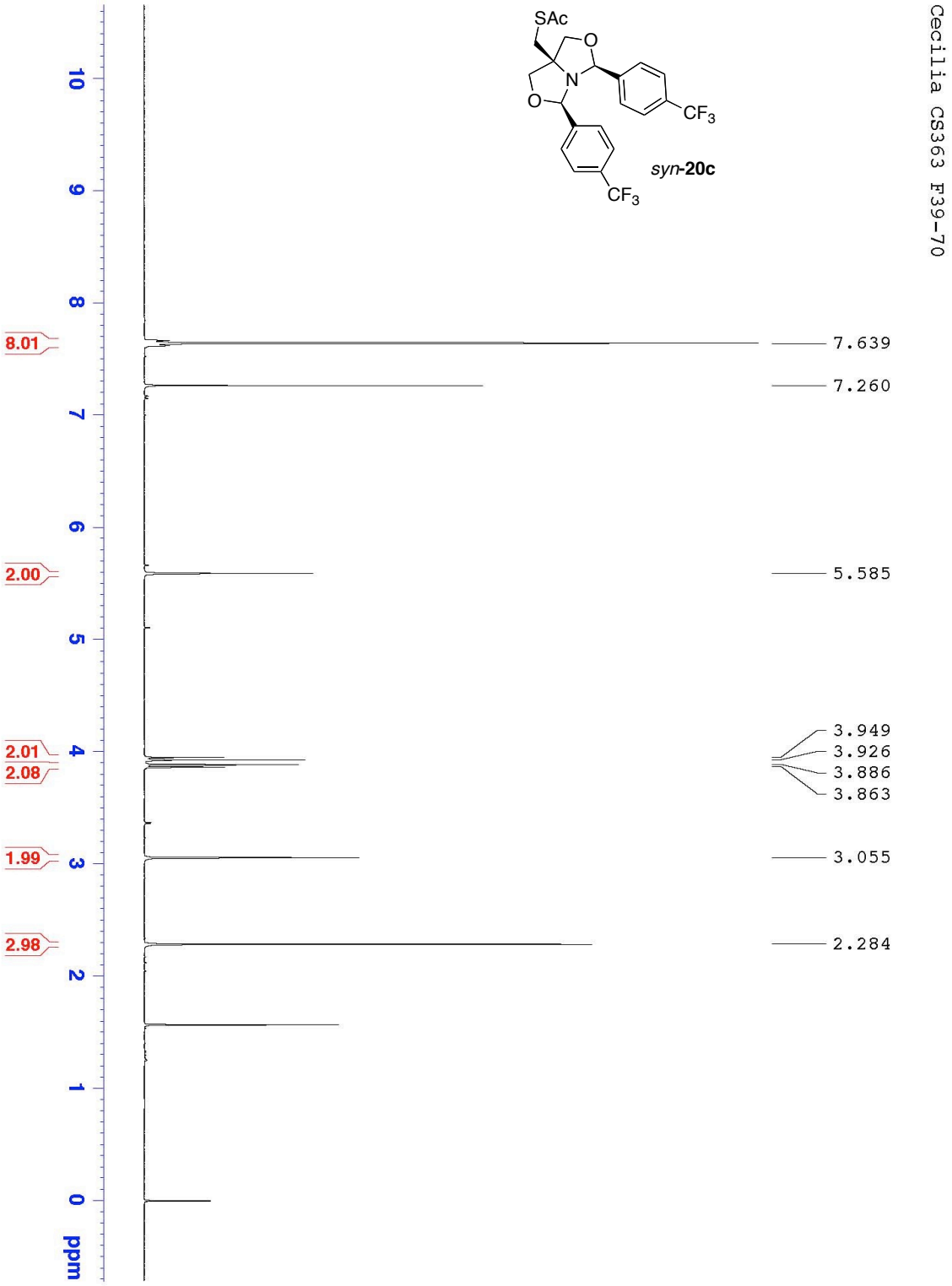

cecilia cs 271 rep F 20-24
188.01
73.09
67.88
62.08
39.50
OH
OH
O
NH
S
15
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

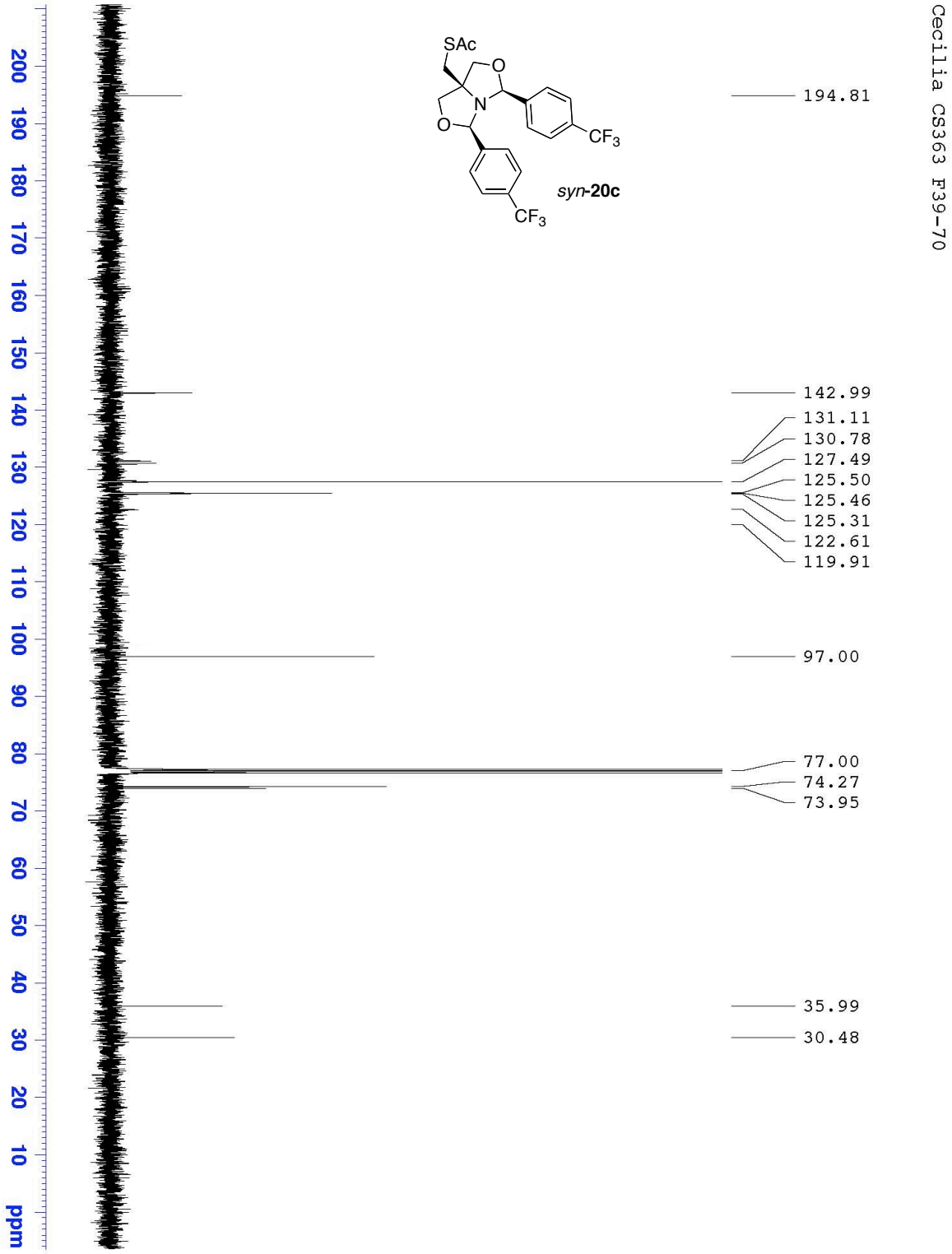

S
NH
S
O
O
Cl
H
syn-16a
Cecilia CS 285 F18-21
0.90
4.15
1.00
2.03
2.12
2.04
10.29
7.46
7.46
5.56
4.22
4.19
3.89
3.86
3.78
2.50
ppm

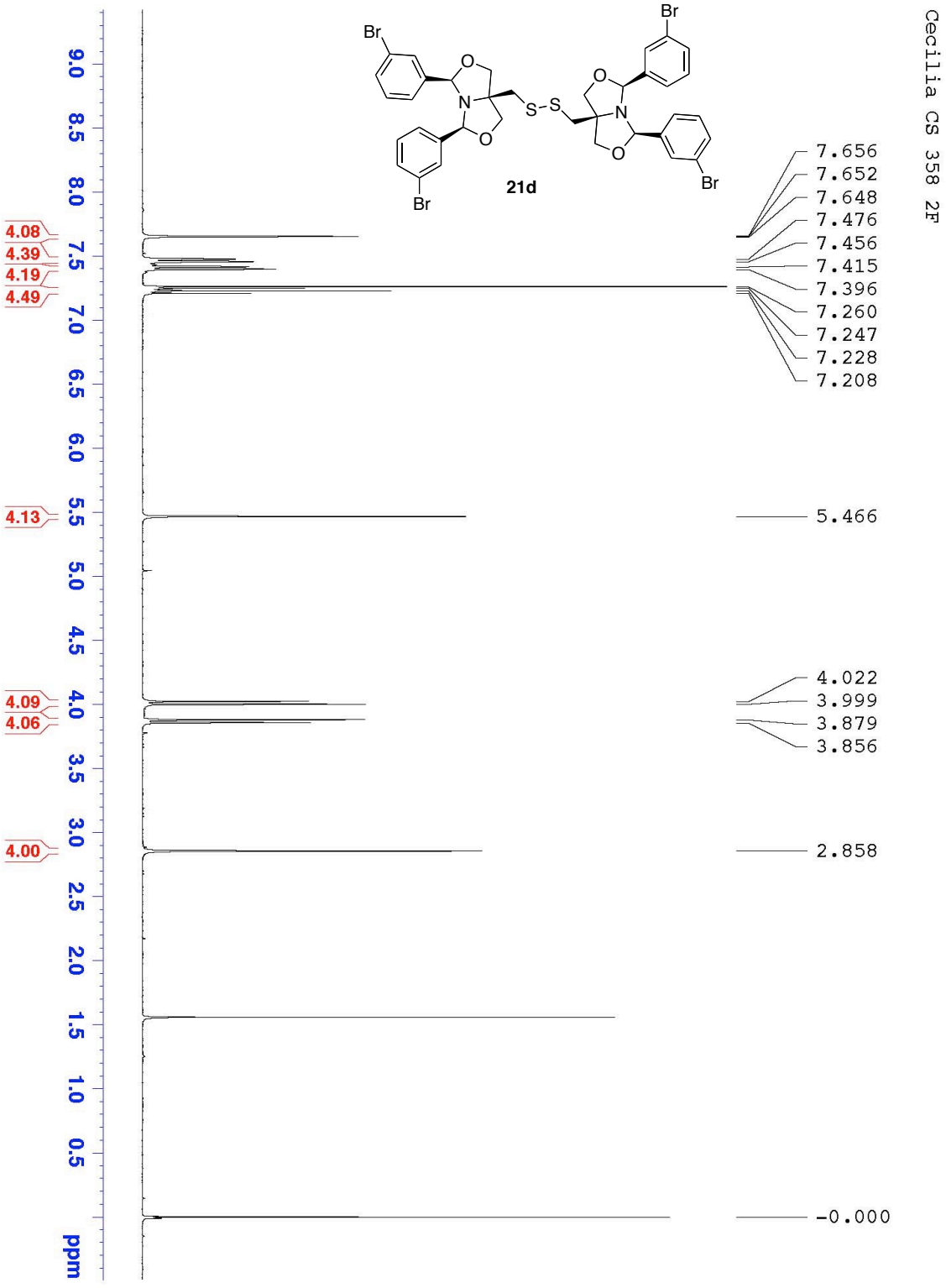

Cecilia CS 285 F18-21
S
NH
S
O
O
H
Cl
syn-16a
199.80
136.88
134.08
128.67
128.65
100.33
71.05
64.76
39.75
39.50
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

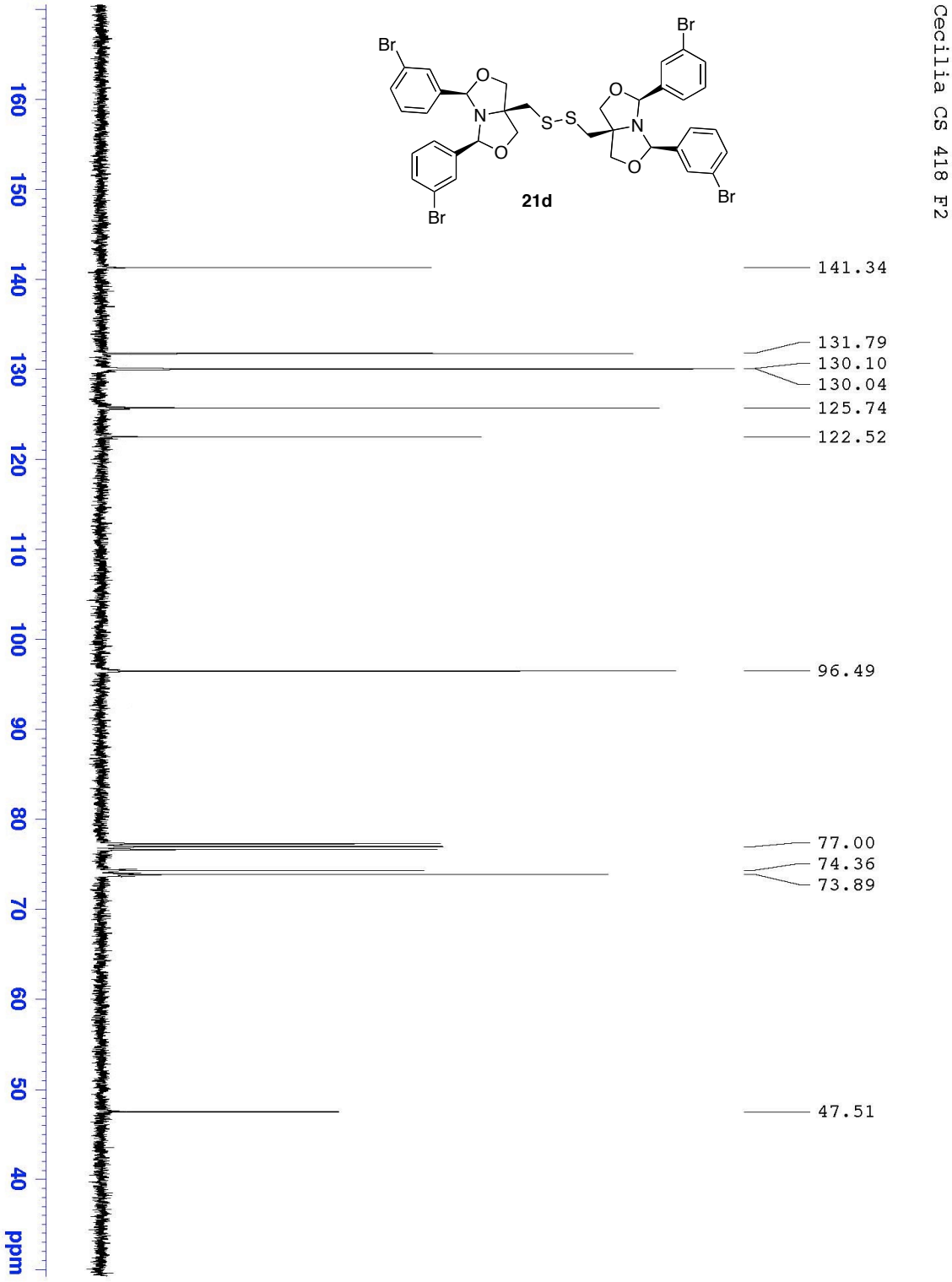

Cecilia CS 400 F 16
19c
7.640
7.630
7.603
7.572
7.566
7.551
7.544
7.526
7.512
7.492
7.483
7.260
7.241
7.221
5.557
5.551
5.360
3.857
3.854
3.815
3.776
3.754
3.315
3.293
3.247
3.225
1.567
16.64
0.93
4.66
1.04
2.29
0.70
3.98
ppm

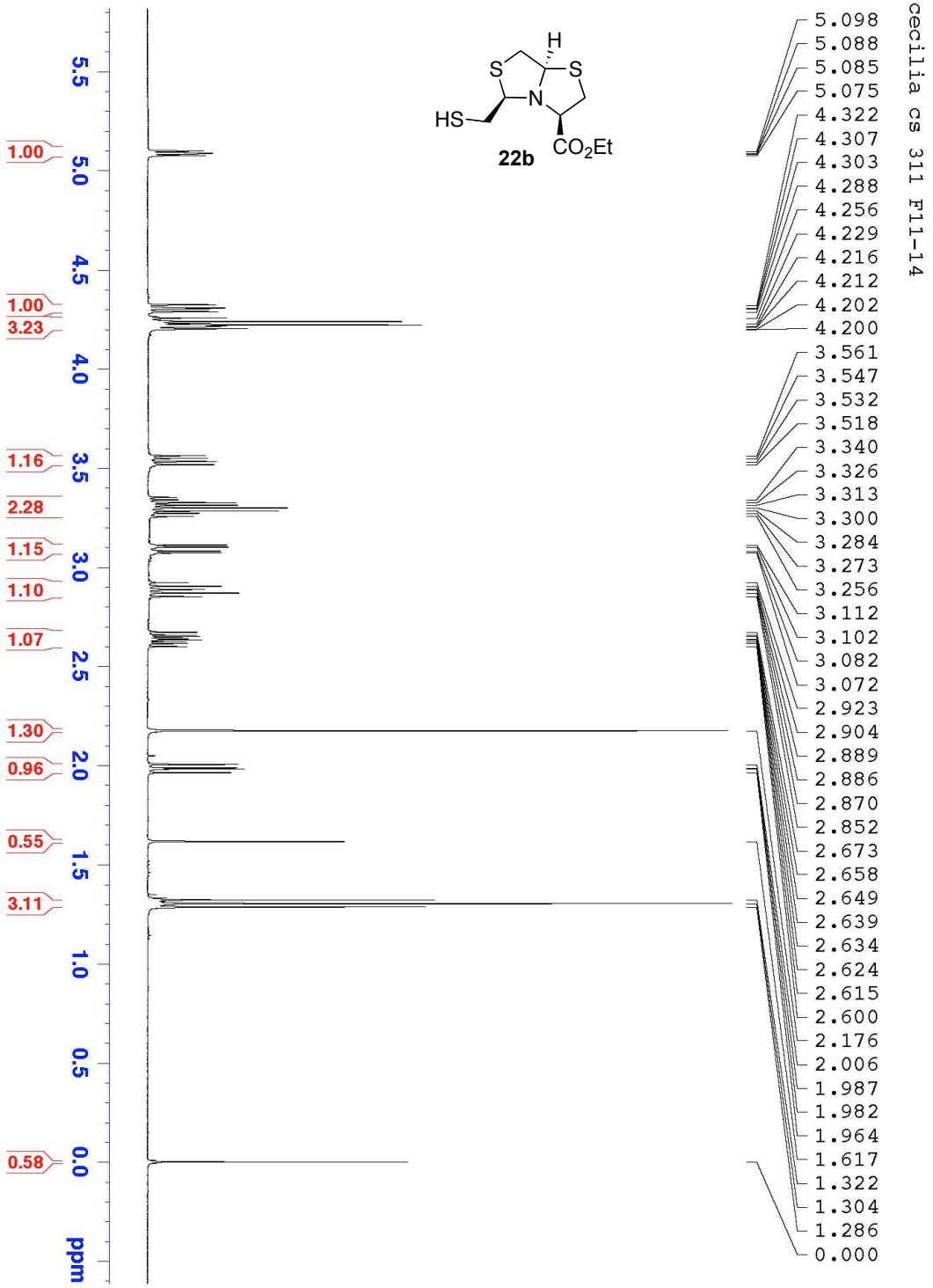

Cecilia CS 400 F 16
19c
143.08
140.32
131.09
130.77
127.31
127.26
126.72
126.53
126.42
125.53
125.42
125.38
125.35
125.31
125.26
122.55
100.70
96.46
96.39
77.32
77.00
76.69
73.33
73.20
73.04
69.39
ppm

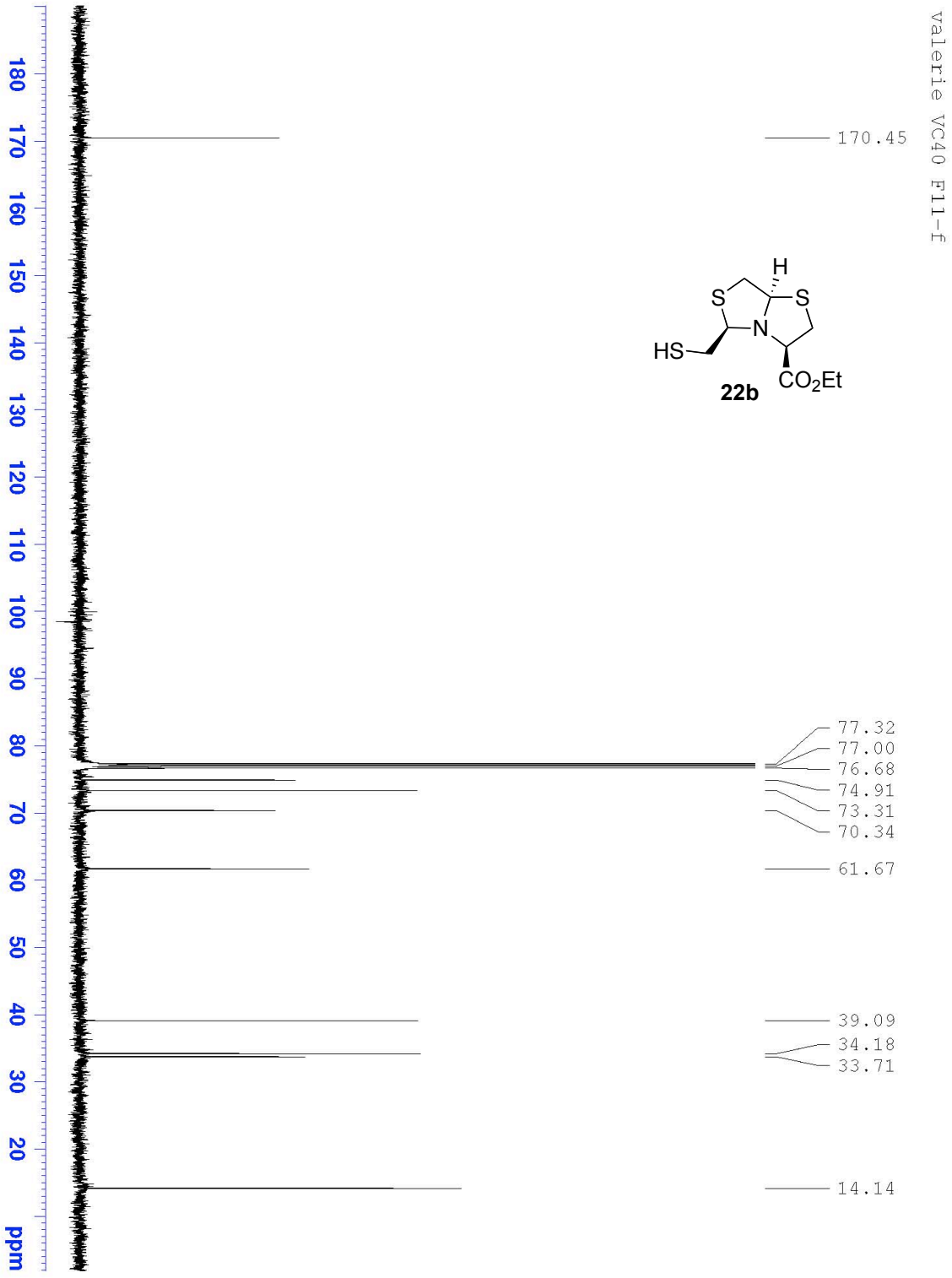

SAc
O
N
O
CF3
CF3
syn-20c
Cecilia CS363 F39-70
8.01
2.00
2.01
2.08
1.99
2.98
7.639
7.260
5.585
3.949
3.926
3.886
3.863
3.055
2.284
10
9
8
7
6
5
4
3
2
1
0
ppm

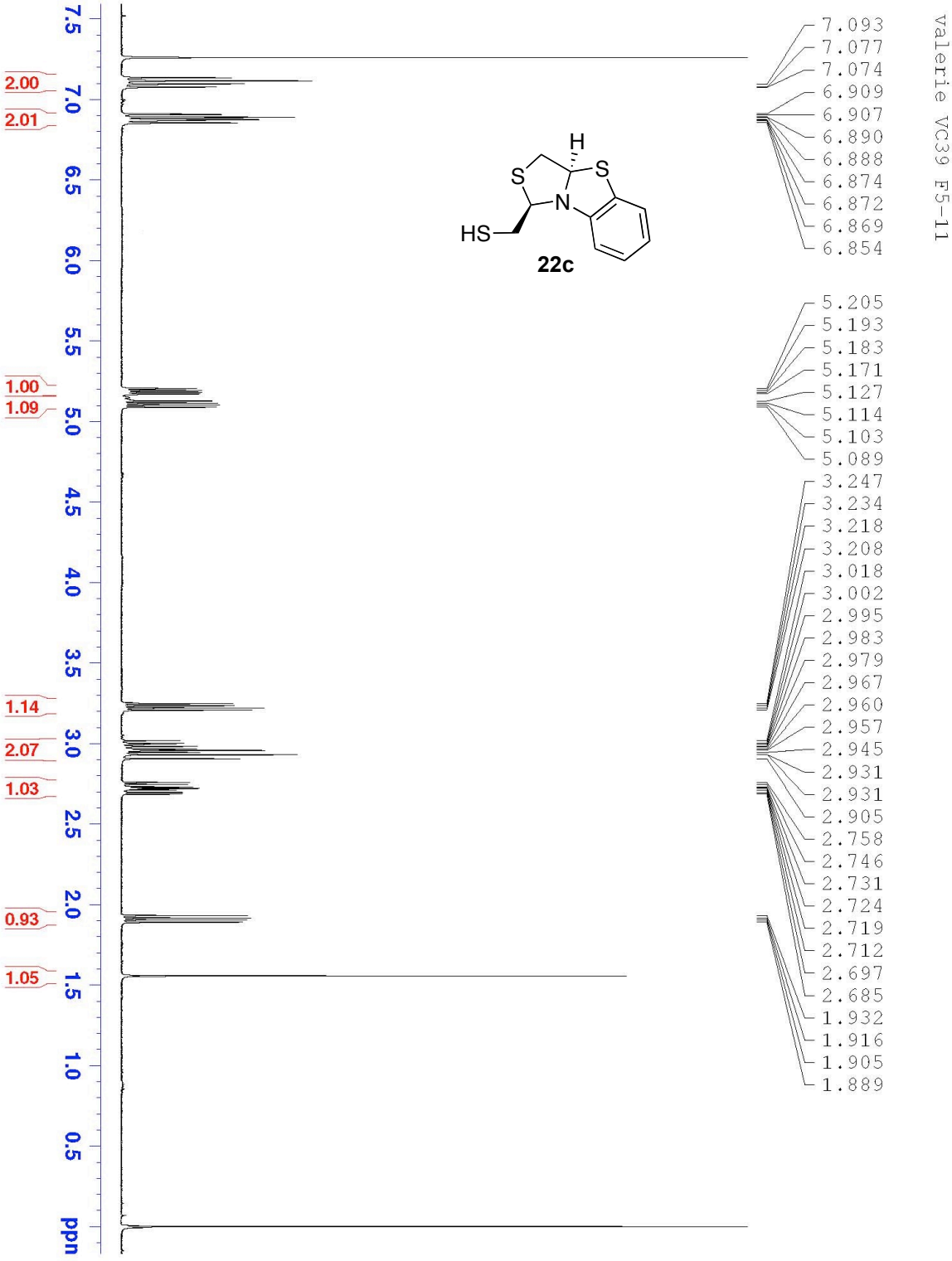

Cecilia CS363 F39-70
SAc
O
N
O
CF3
CF3
syn-20c
194.81
142.99
131.11
130.78
127.49
125.50
125.46
125.31
122.61
119.91
97.00
77.00
74.27
73.95
35.99
30.48
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

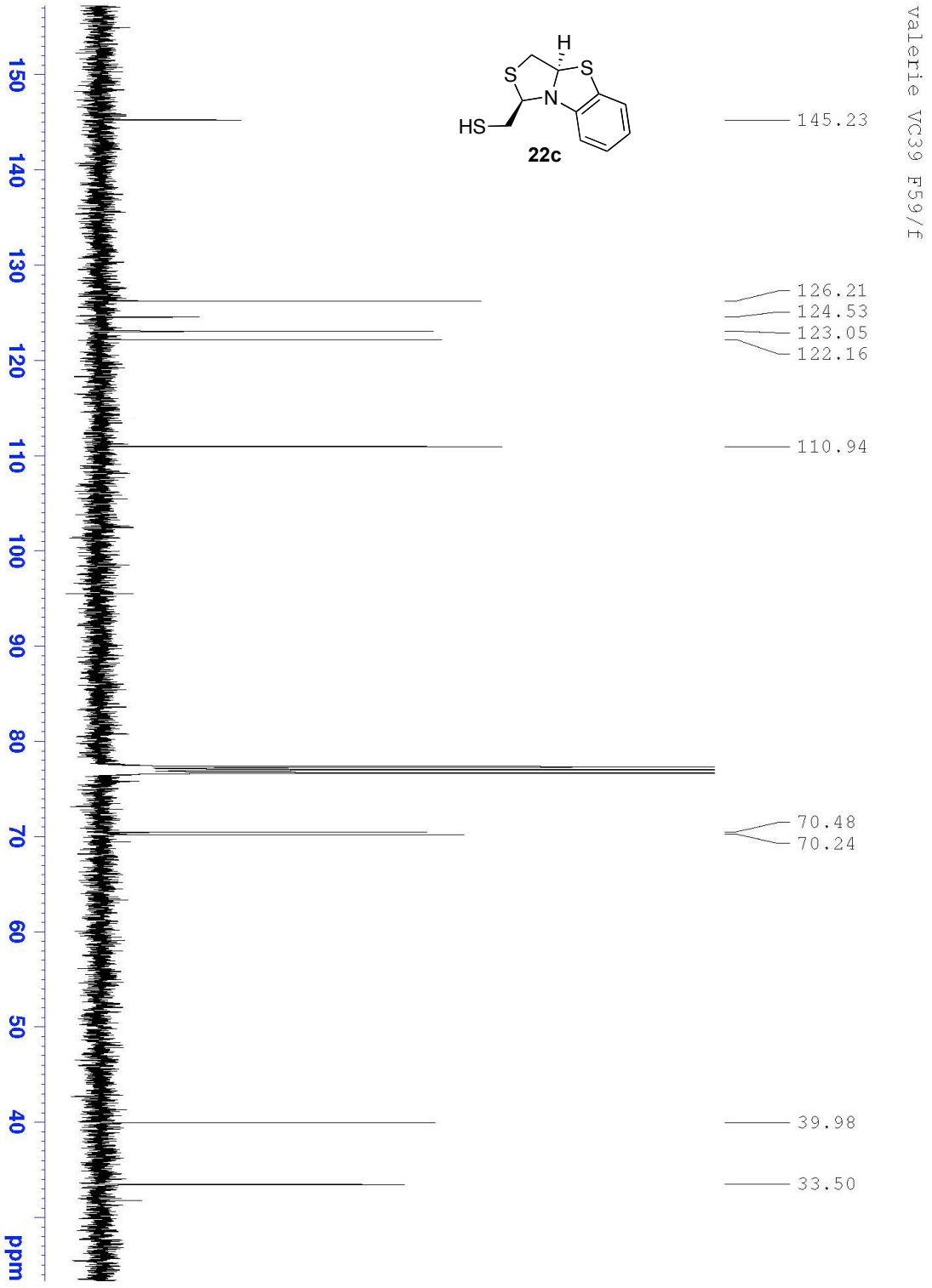

Cecilia CS 358 2F
21d
Br
Br
Br
Br
O
N
S-S
7.656
7.652
7.648
7.476
7.456
7.415
7.396
7.260
7.247
7.228
7.208
5.466
4.022
3.999
3.879
3.856
2.858
-0.000
4.08
4.39
4.19
4.49
4.13
4.09
4.06
4.00
ppm

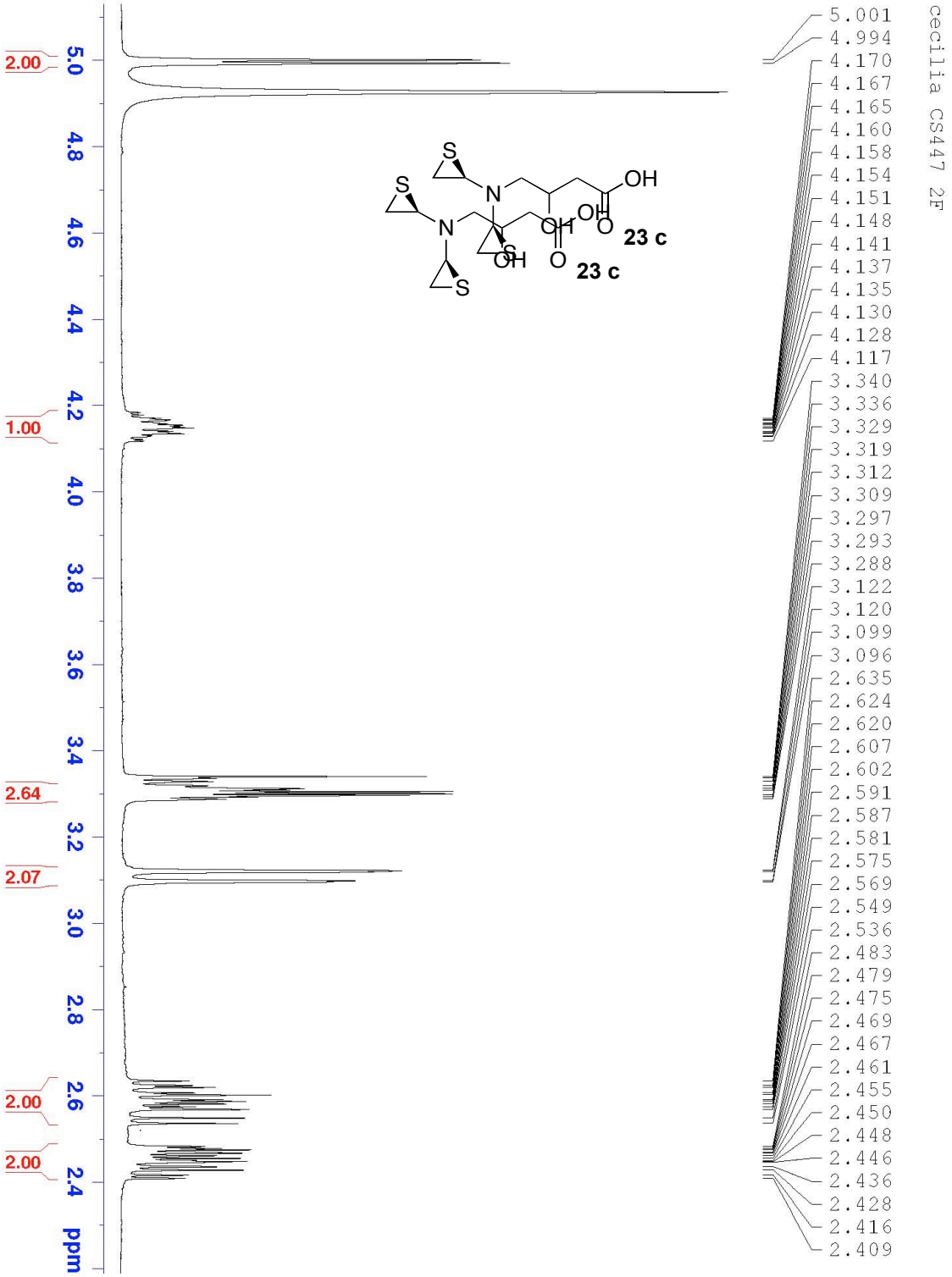

Cecilia CS 418 F2
Br
Br
O
N
O
S-S
O
N
O
Br
Br
21d
141.34
131.79
130.10
130.04
125.74
122.52
96.49
77.00
74.36
73.89
47.51
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm

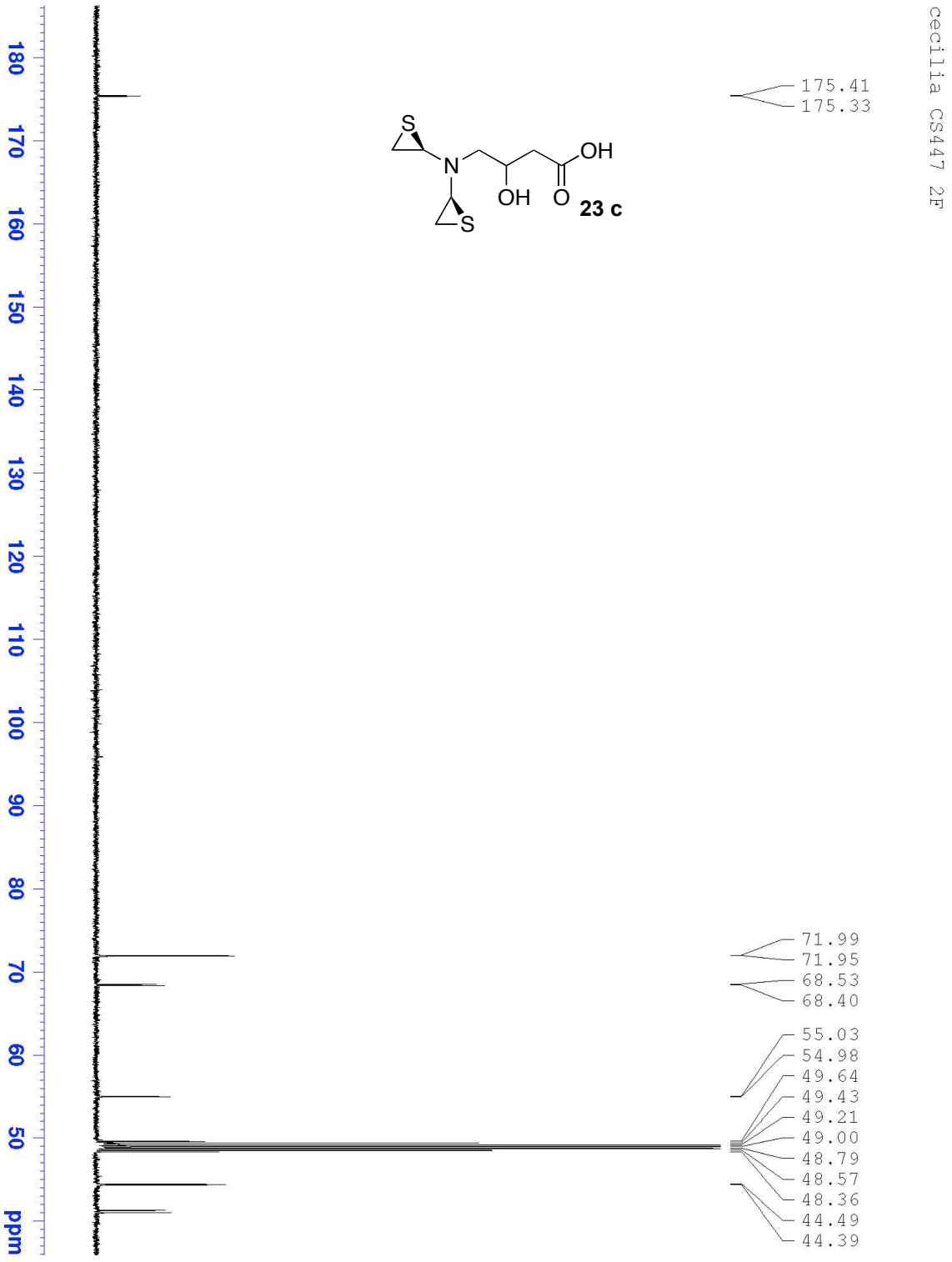

cecilia cs 311 F11-14
22b
5.098
5.088
5.085
5.075
4.322
4.307
4.303
4.288
4.256
4.229
4.216
4.212
4.202
4.200
3.561
3.547
3.532
3.518
3.340
3.326
3.313
3.300
3.284
3.273
3.256
3.112
3.102
3.082
3.072
2.923
2.904
2.889
2.886
2.870
2.852
2.673
2.658
2.649
2.639
2.634
2.624
2.615
2.600
2.176
2.006
1.987
1.982
1.964
1.617
1.322
1.304
1.286
0.000
1.00
1.00
3.23
1.16
2.28
1.15
1.10
1.07
1.30
0.96
0.55
3.11
0.58
ppm

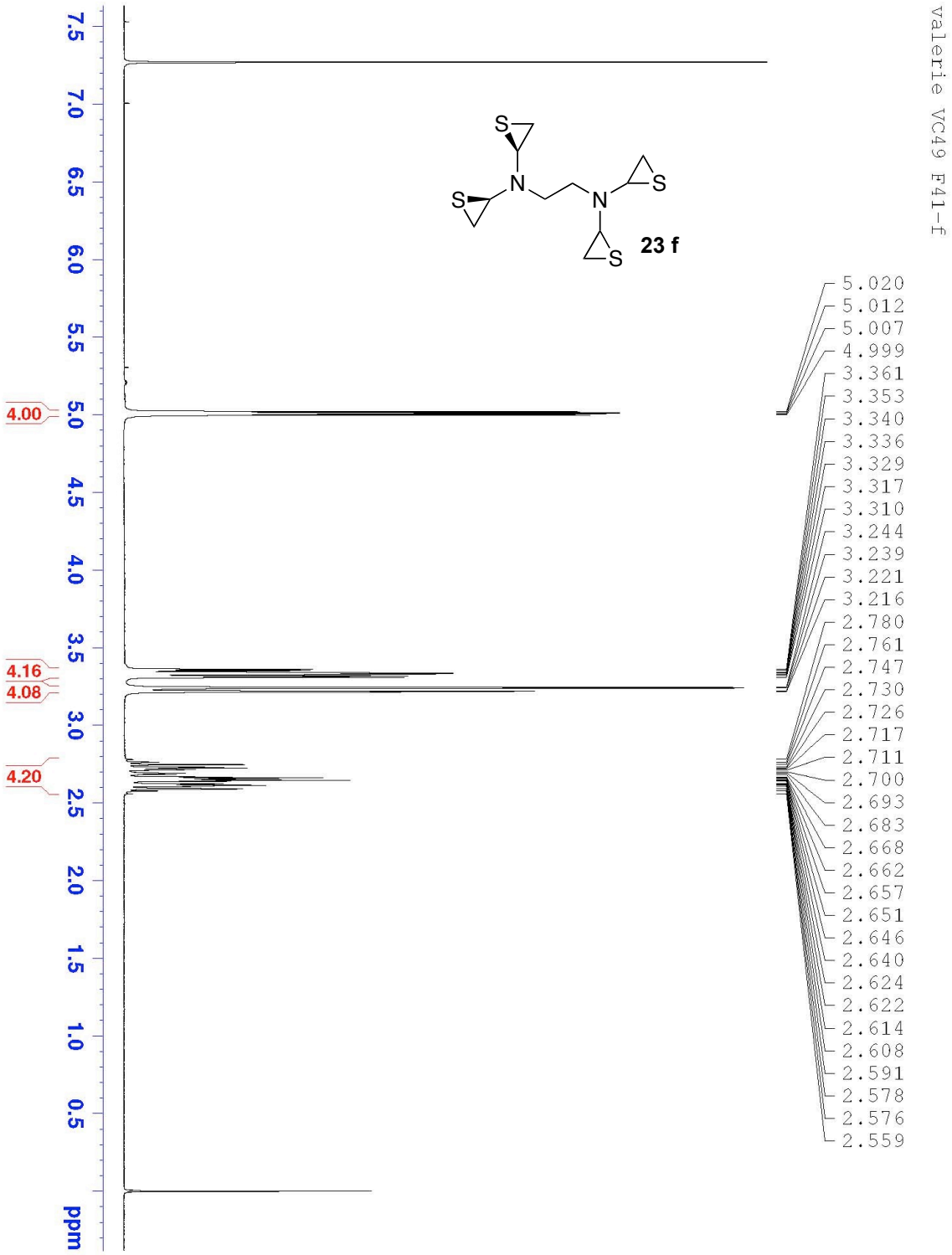

valerie VC40 F11-f
170.45
77.32
77.00
76.68
74.91
73.31
70.34
61.67
39.09
34.18
33.71
14.14
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm
H
S
S
N
HS
CO2Et
22b

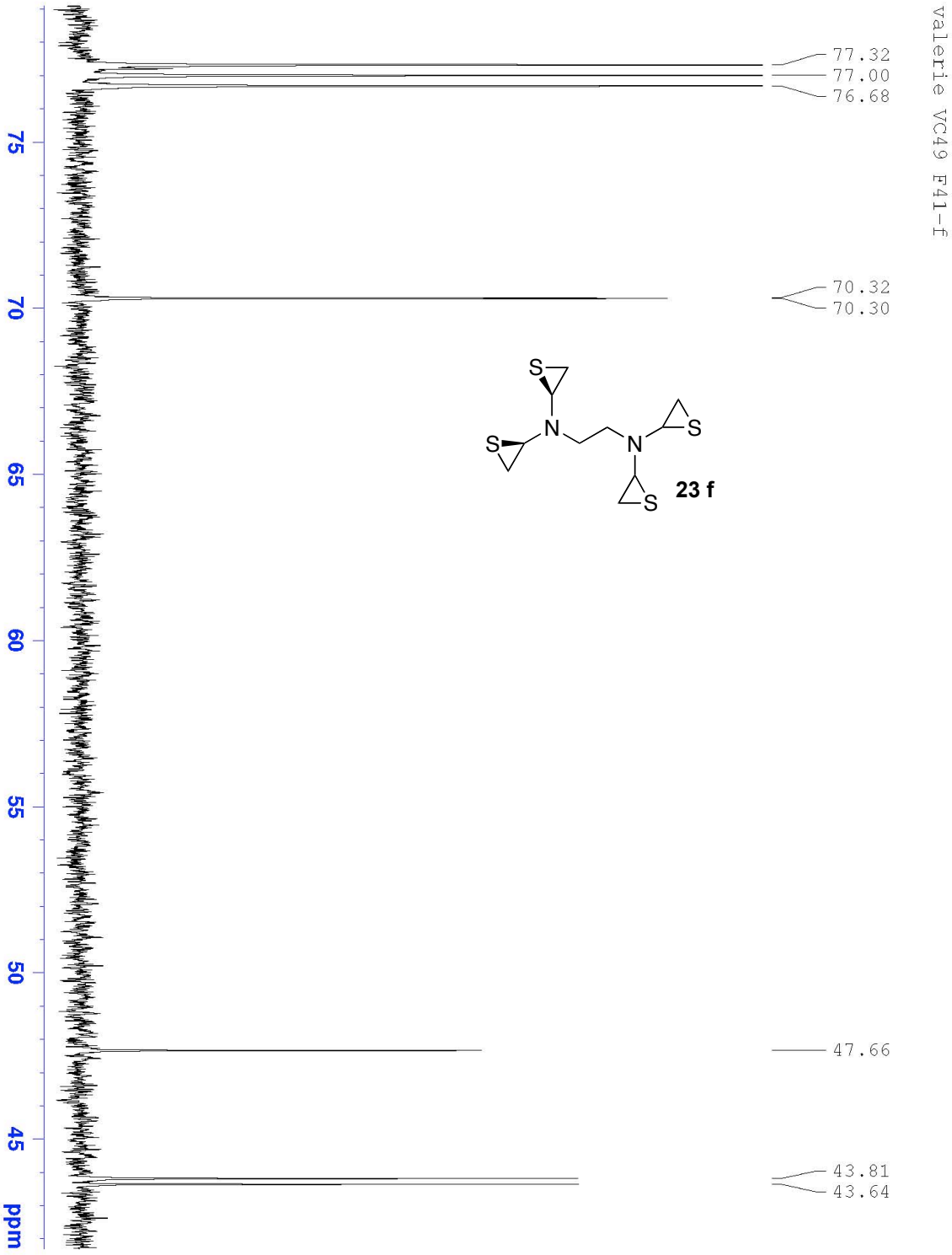

valerie VC39 F5-11
7.093
7.077
7.074
6.909
6.907
6.890
6.888
6.874
6.872
6.869
6.854
5.205
5.193
5.183
5.171
5.127
5.114
5.103
5.089
3.247
3.234
3.218
3.208
3.018
3.002
2.995
2.983
2.979
2.967
2.960
2.957
2.945
2.931
2.931
2.905
2.758
2.746
2.731
2.724
2.719
2.712
2.697
2.685
1.932
1.916
1.905
1.889
2.00
2.01
1.00
1.09
1.14
2.07
1.03
0.93
1.05
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
H
S
S
N
HS
22c

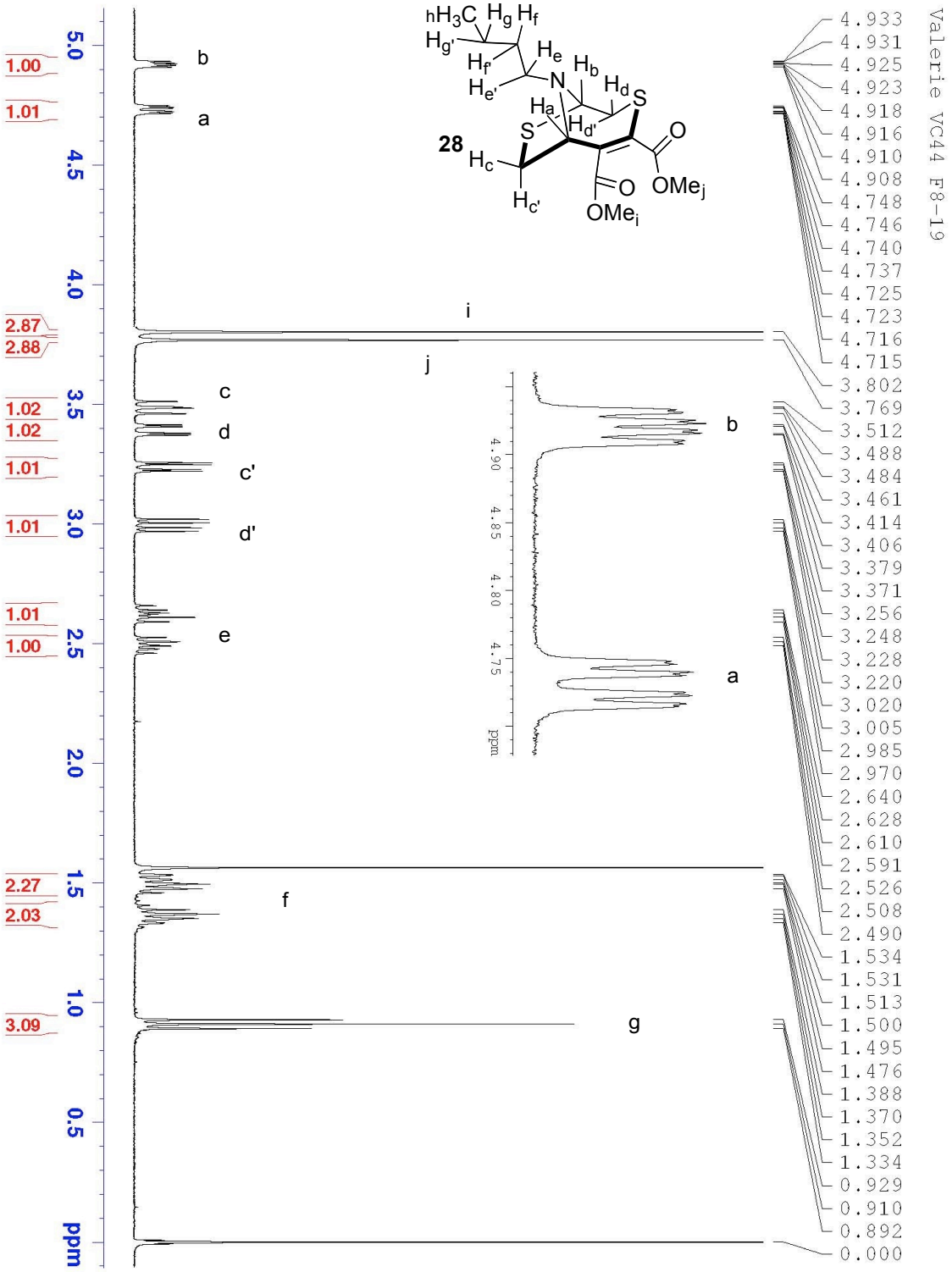

valerie VC39 F59/f
22c
145.23
126.21
124.53
123.05
122.16
110.94
70.48
70.24
39.98
33.50
ppm

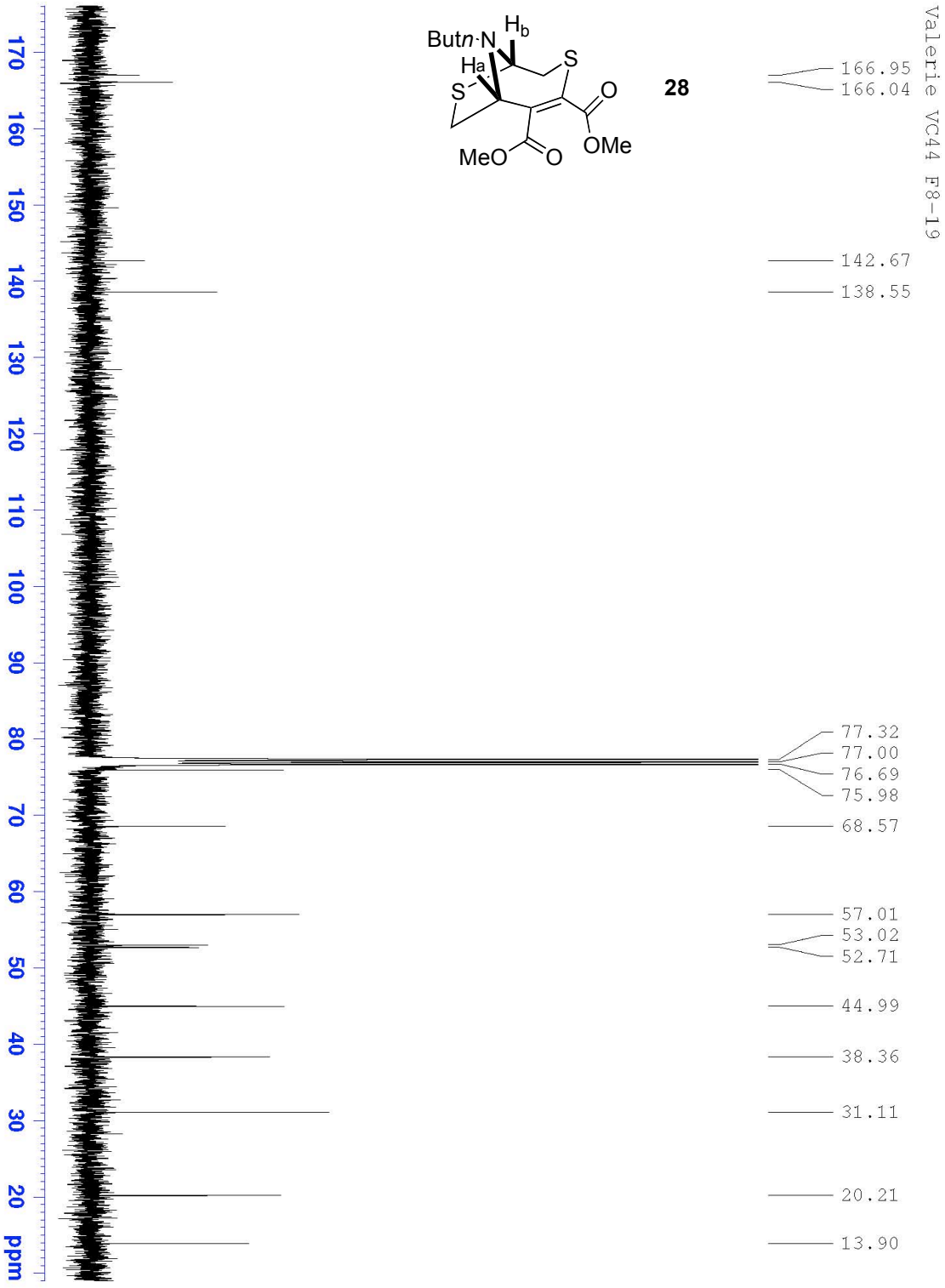

2.00
1.00
2.64
2.07
2.00
2.00
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
ppm
S
N
S
N
S
OH
OH
OH
O
OH
O
23 c
23 c
cecilia CS447 2F
5.001
4.994
4.170
4.167
4.165
4.160
4.158
4.154
4.151
4.148
4.141
4.137
4.135
4.130
4.128
4.117
3.340
3.336
3.329
3.319
3.312
3.309
3.297
3.293
3.288
3.122
3.120
3.099
3.096
2.635
2.624
2.620
2.607
2.602
2.591
2.587
2.581
2.575
2.569
2.549
2.536
2.483
2.479
2.475
2.469
2.467
2.461
2.455
2.450
2.448
2.446
2.436
2.428
2.416
2.409

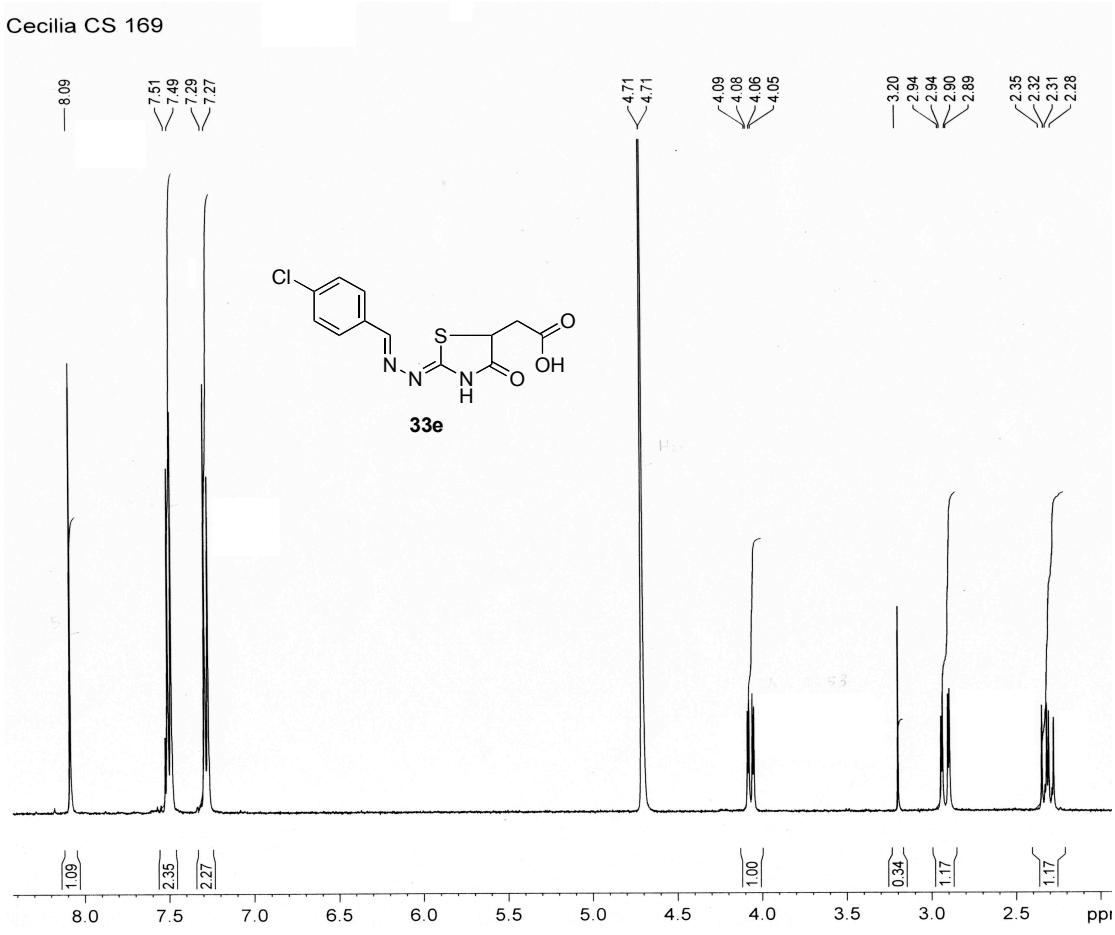

cecilia CS447 2F
175.41
175.33
71.99
71.95
68.53
68.40
55.03
54.98
49.64
49.43
49.21
49.00
48.79
48.57
48.36
44.49
44.39
ppm
180
170
160
150
140
130
120
110
100
90
80
70
60
50
S
N
S
OH
OH
O
23 c

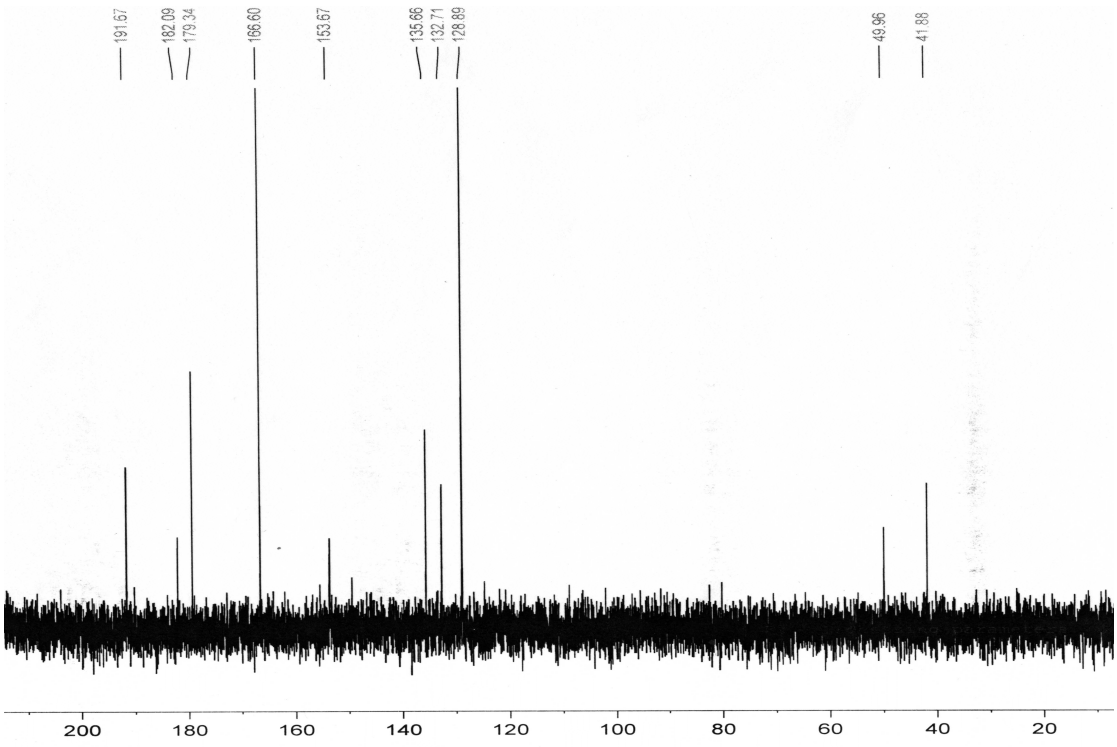

valerie VC49 F41-f
23 f
4.00
4.16
4.08
4.20
ppm
5.020
5.012
5.007
4.999
3.361
3.353
3.340
3.336
3.329
3.317
3.310
3.244
3.239
3.221
3.216
2.780
2.761
2.747
2.730
2.726
2.717
2.711
2.700
2.693
2.683
2.668
2.662
2.657
2.651
2.646
2.640
2.624
2.622
2.614
2.608
2.591
2.578
2.576
2.559

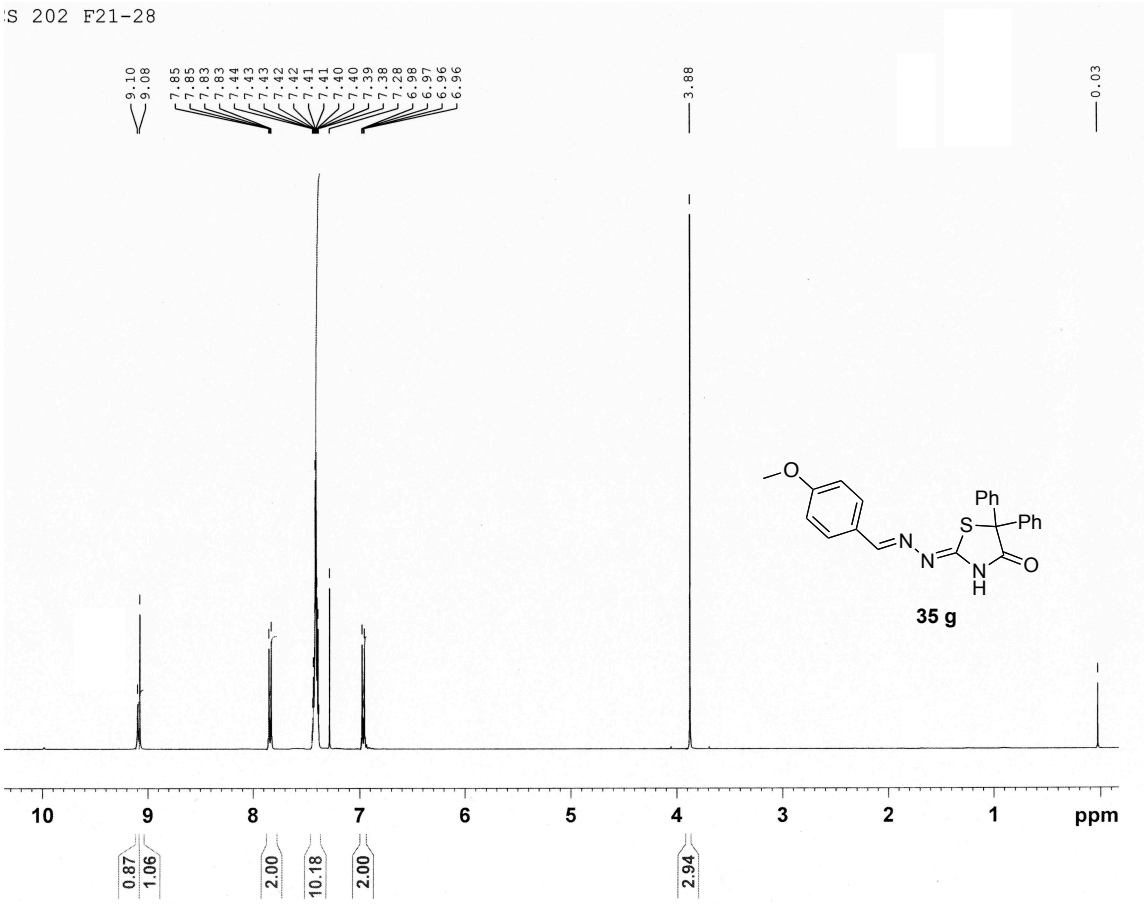

valerie VC49 F41-f
77.32
77.00
76.68
70.32
70.30
47.66
43.81
43.64
75
70
65
60
55
50
45
ppm
S
N
S
N
S
S
23 f

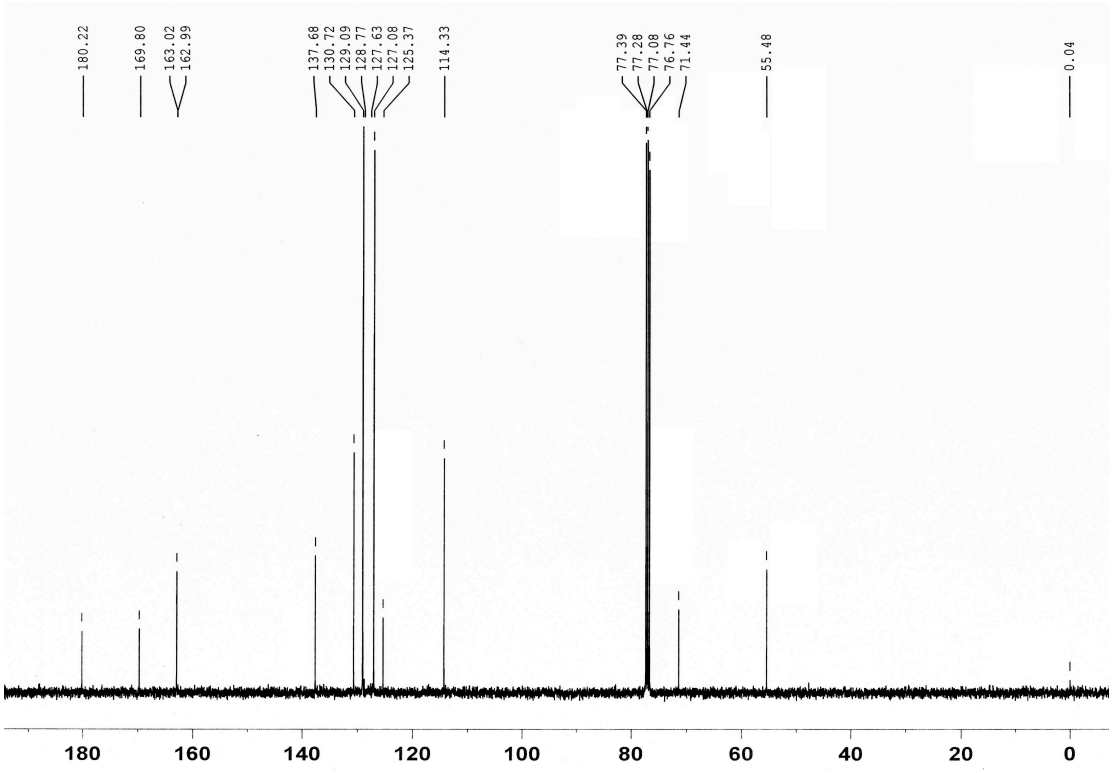

Valerie VC44 F8-19
28
1.00
1.01
2.87
2.88
1.02
1.02
1.01
1.01
1.01
1.00
2.27
2.03
3.09
a
b
c
d
c'
d'
e
f
g
i
j
ppm

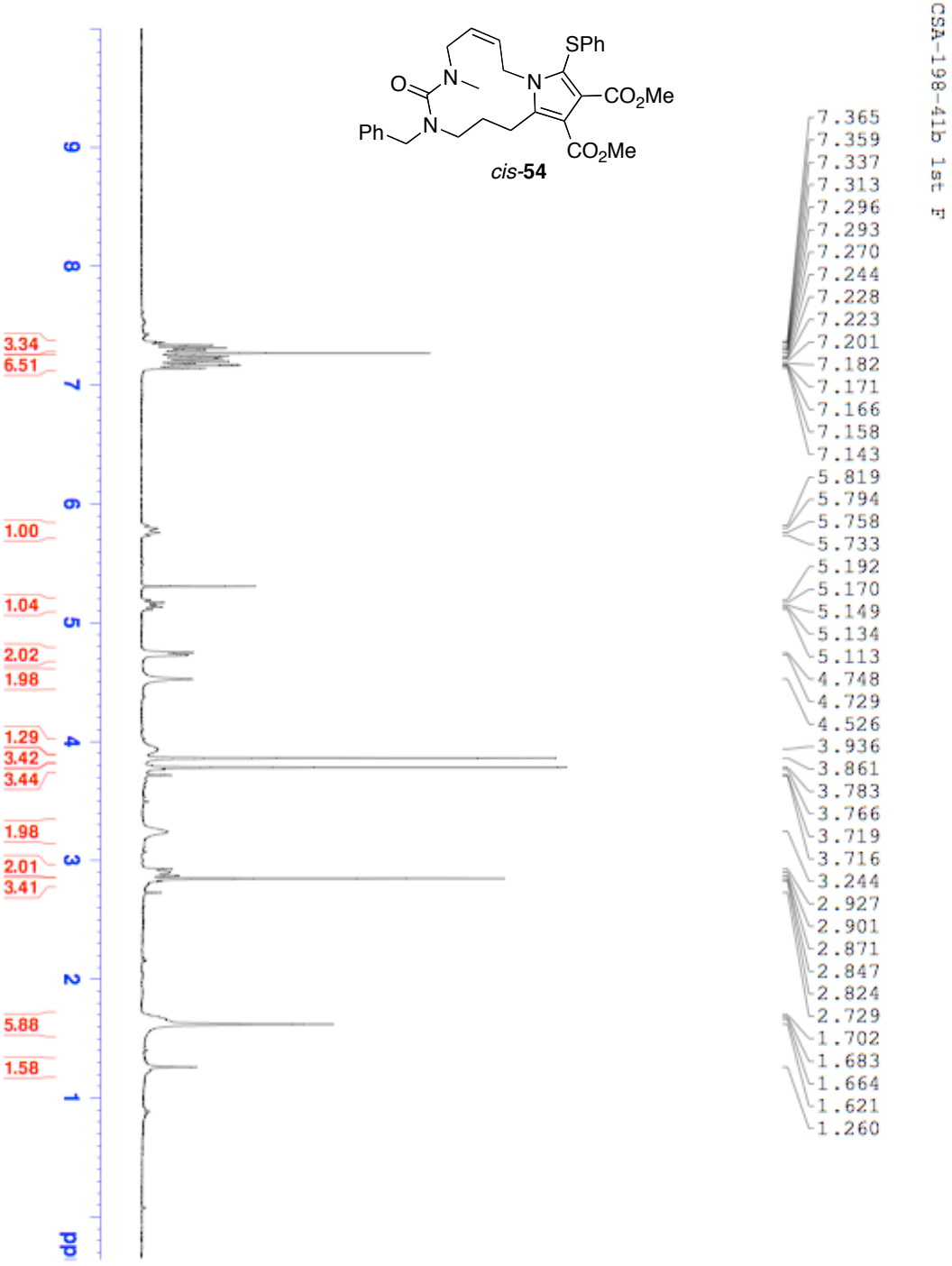

Valerie VC44 F8-19
Butn·N
Hb
Ha
S
S
O
OMe
MeO
O
28
166.95
166.04
142.67
138.55
77.32
77.00
76.69
75.98
68.57
57.01
53.02
52.71
44.99
38.36
31.11
20.21
13.90
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

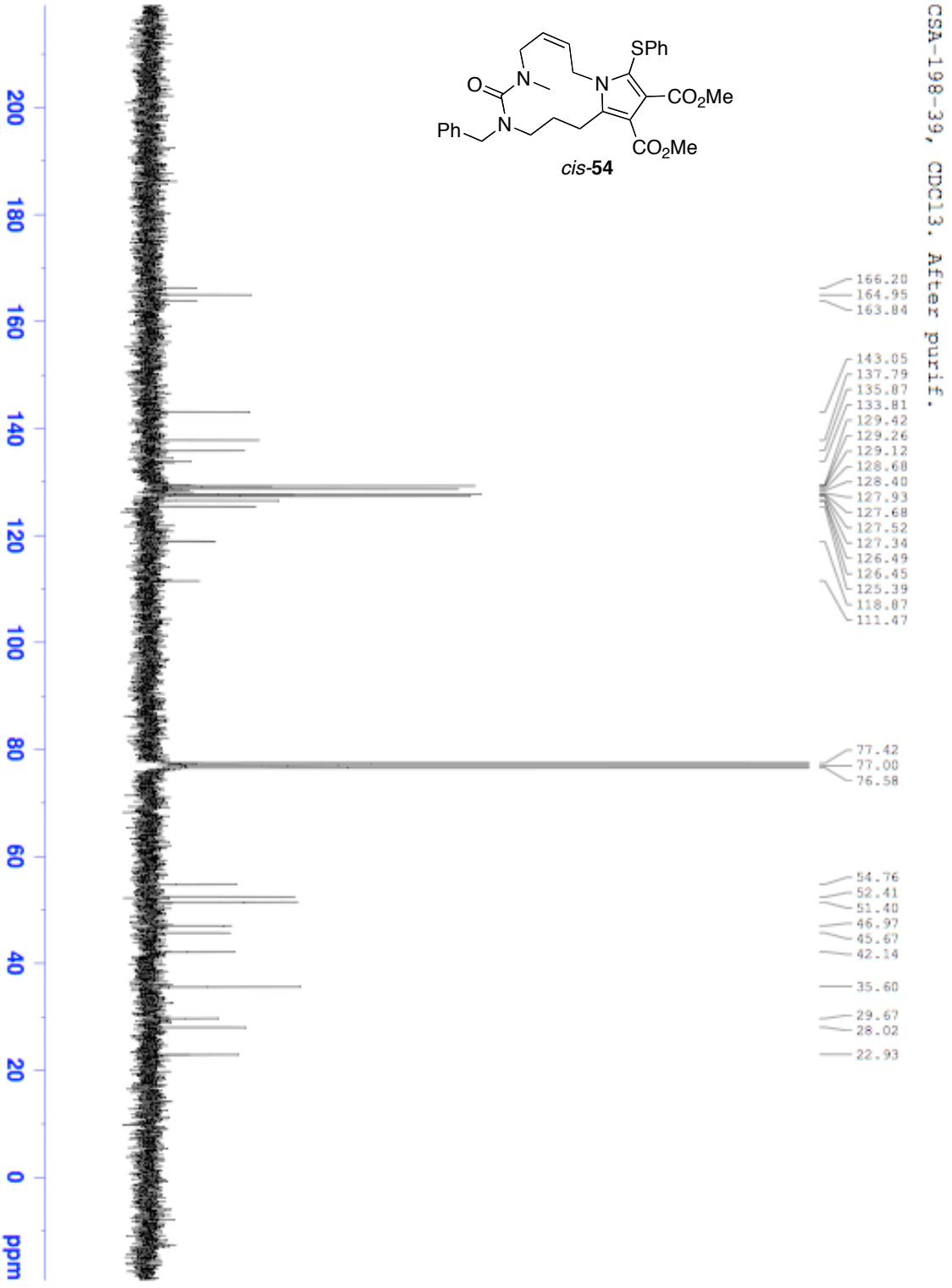

Cecilia CS 169
8.09
7.51
7.49
7.29
7.27
4.71
4.71
4.09
4.08
4.06
4.05
3.20
2.94
2.90
2.89
2.35
2.32
2.31
2.28
Cl
S
N
N
N
H
O
O
OH
33e
1.09
2.35
2.27
1.00
0.34
1.17
1.17
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppı


191.67
182.09
179.34
166.60
153.67
135.66
132.71
128.89
49.96
41.88
200
180
160
140
120
100
80
60
40
20

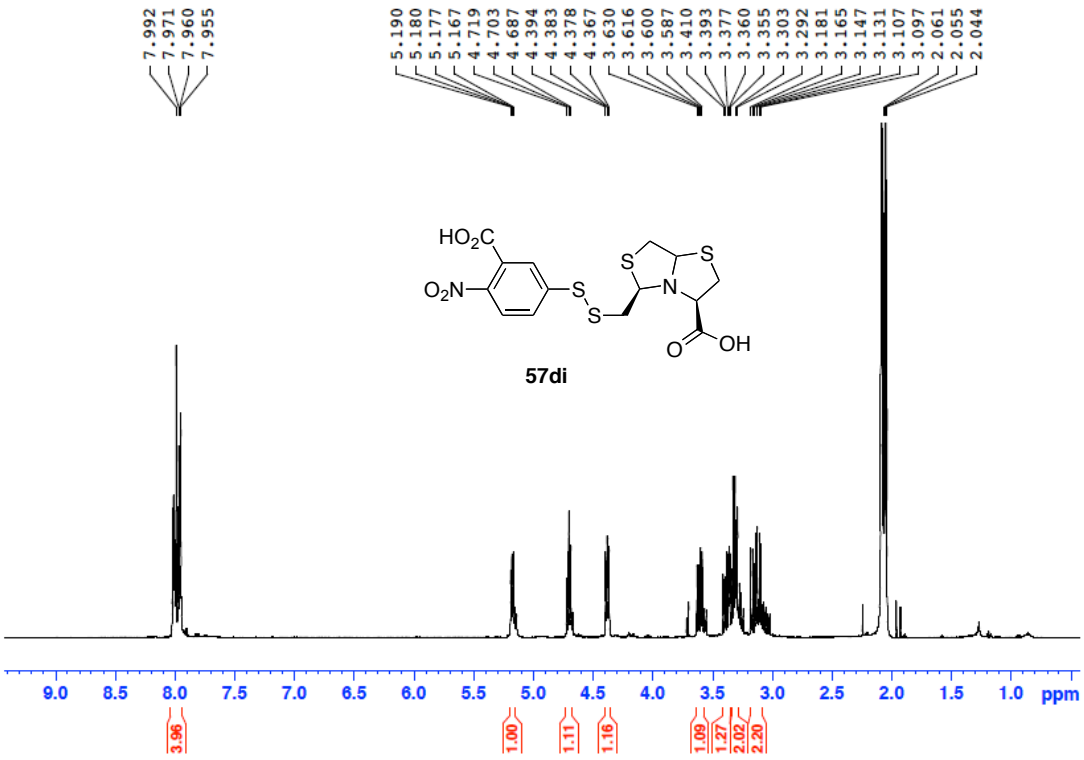

S 202 F21-28
9.10
9.08
7.85
7.85
7.83
7.83
7.44
7.43
7.43
7.42
7.42
7.41
7.41
7.40
7.40
7.39
7.38
7.28
6.98
6.97
6.96
6.96
3.88
0.03
O
Ph
S
Ph
N
N
N
H
O
35 g
10
9
8
7
6
5
4
3
2
1
ppm
0.87
1.06
2.00
10.18
2.00
2.94


180.22
169.80
163.02
162.99
137.68
130.72
129.09
128.77
127.63
127.08
125.37
114.33
77.39
77.28
77.08
76.76
71.44
55.48
0.04
180
160
140
120
100
80
60
40
20
0


SPh
O
N
N
CO2Me
Ph
N
CO2Me
cis-54
CSA-198-41b 1st F
7.365
7.359
7.337
7.313
7.296
7.293
7.270
7.244
7.228
7.223
7.201
7.182
7.171
7.166
7.158
7.143
5.819
5.794
5.758
5.733
5.192
5.170
5.149
5.134
5.113
4.748
4.729
4.526
3.936
3.861
3.783
3.766
3.719
3.716
3.244
2.927
2.901
2.871
2.847
2.824
2.729
1.702
1.683
1.664
1.621
1.260
3.34
6.51
1.00
1.04
2.02
1.98
1.29
3.42
3.44
1.98
2.01
3.41
5.88
1.58
ppm


CSA-198-39, CDCl3, After purif.
SPh
O
N
N
CO2Me
Ph
N
CO2Me
cis-54
166.20
164.95
163.84
143.05
137.79
135.87
133.81
129.42
129.26
129.12
128.68
128.40
127.93
127.68
127.52
127.34
126.49
126.45
125.39
118.87
111.47
77.42
77.00
76.58
54.76
52.41
51.40
46.97
45.67
42.14
35.60
29.67
28.02
22.93
200
180
160
140
120
100
80
60
40
20
0
ppm

cecilia cs 445 2F seco



57di

cecilia cs 445 2F seco