Electronic Supplementary Information

Simplified Immunosuppressive and Neuroprotective Agents Based on Gracilin A

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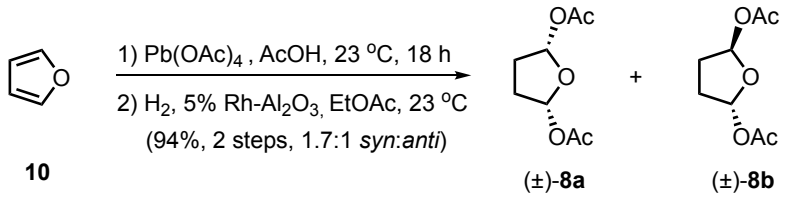
A. Synthetic Experimentals

General Procedures

All non-aqueous reactions were performed under a nitrogen atmosphere in oven-dried glassware. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), diethyl ether (Et_2O), acetonitrile (CH_3CN) and toluene (PhMe) were dried by filtration through activated alumina (solvent purification system). Diisopropylethylamine ($EtN(^iPr)_2$) and triethylamine (Et_3N) were distilled from calcium hydride prior to use. Other solvents and reagents were used as received from commercially available sources. Deuterated solvents were purchased from Cambridge Isotopes and used as received. ^1H NMR spectra were measured at 500 MHz and referenced relative to residual chloroform (7.26 ppm) or benzene (7.16 ppm) and were reported in parts per million. Coupling constants (*J*) were reported in Hertz (Hz), with multiplicity reported following usual convention: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; dq, doublet of quartets; td, triplet of doublets; tt, triplet of triplets; ddd, doublet of doublet of doublets; ddt, doublet of doublet of triplets; ddq, doublet of doublet of quartets; dddd, doublet of doublet of doublet of doublets; ddddt, doublet of doublet of doublet of doublet of triplets; ddquint, doublet of doublet of quintets; m, multiplet, br s, broad singlet. ^{13}C NMR spectra were measured at 125 MHz and referenced relative to chloroform-*d* signal (77.16 ppm) or benzene (128.06 ppm) and were reported in parts per million (ppm). Flash column chromatography was performed with 60Å Silica Gel (230-400 mesh) as stationary phase on an automated flash chromatography system (EtOAc/hexanes as eluent unless indicated otherwise). High-resolution mass spectra (ESI) were obtained through the Laboratory for Biological Mass Spectrometry (Texas A&M University). Thin Layer Chromatography (TLC) was performed using glass-backed silica gel F254 (Silicycle, 250 μm thickness). Visualization of developed plates was performed by fluorescence quenching or by treating with Seebach's staining solution. Fourier Transform Infrared (FTIR) spectra were recorded as thin films on NaCl plates. Optical rotations were recorded on a polarimeter at 589 nm employing a 25 mm cell. High Performance Liquid Chromatography (HPLC) was performed on a chromatographic system using various chiral columns (25 cm) as noted. X-ray diffraction was obtained by the X-ray Diffraction Laboratory at Texas A&M University. (S)-(–)-TM•HCl was purchased from TCI chemicals and used as received. All other chemicals were purchased from Sigma-Aldrich or Alfa Aesar and used as received.

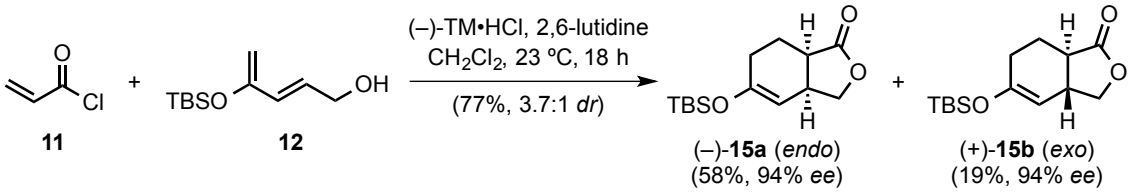
Abbreviation List

$EtN(^{i}Pr)_2$	*N,N*-Diisopropylethylamine
Et_3N	Triethylamine
$NaBH_4$	Sodium borohydride
(S)-(–)-TM·HCl	(S)-(–)-Tetramisole (levamisole) hydrochloride
$LiAlH_4$	Lithium aluminum hydride
TBAF	Tetrabutylammonium fluoride
Me_2S	Dimethyl sulfide
$(COCl)_2$	Oxalyl chloride
DMAP	4-(Dimethylamino)pyridine
Ac_2O	Acetic anhydride
DIBAl-H	Diisobutylaluminum hydride
DBU	1,8-Diazabicyclo(5.4.0)undec-7-ene



10 (±)-**8a** (±)-**8b**

***cis*- and *trans*-2,5-tetrahydrofuran-2,5-diyl diacetate ((±)-8a and (±)-8b):** To a suspension of $Pb(OAc)_4$ (2.00 g, 4.54 mmol, 1.10 equiv) in glacial AcOH (10.0 mL) was added furan **10** (0.30 mL, 4.13 mmol, 1.00 equiv) and the mixture was stirred at 23 ºC for 18 h. AcOH was evaporated and Et_2O was added to the residue. The precipitate was filtered, the filtrate was collected, evaporated and the residue was redissolved in anhydrous EtOAc (50 mL). 5% Rhodium on alumina (180 mg) was added and the hydrogenation was carried out under hydrogen atmosphere (1 atm, balloon) for 24 h. The solution was filtered through Celite, the filtrate was concentrated by rotary evaporation and purified by an automated flash chromatography system (5→50% EtOAc/hexanes) providing 721 mg (94% yield, 2 steps) of (±)-**8a** and (±)-**8b** as a clear colorless oil and as a mixture of two isomers (ratio 1.7:1 *cis:trans*, according to 500 MHz ^{1}H-NMR). IR (thin film): 2921, 2851, 1745 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_8H_{12}LiO_5$ $[M+Li]^+$: 195.0847, found: 195.0845. Spectral data matched that previously reported.[1]



11 **12** (–)-**15a** (*endo*) (58%, 94% *ee*) (+)-**15b** (*exo*) (19%, 94% *ee*)

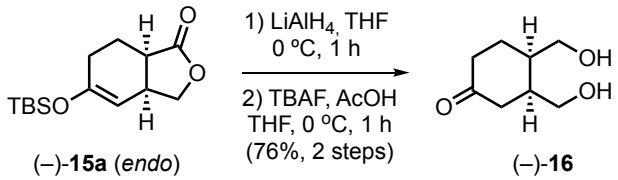
(3a*S*,7a*R*)-5-((*tert*-butyldimethylsilyl)oxy)-3a,6,7,7a-tetrahydroisobenzofuran-1(3*H*)-one ((–)-15a) and (3a*R*,7a*R*)-5-((*tert*-butyldimethylsilyl)oxy)-3a,6,7,7a-tetrahydroisobenzofuran-1(3*H*)-one ((+)-15b): *The following procedure, making use of commercially available and inexpensive levamisole·HCl as stoichiometric Lewis base promoter, was adopted for scale-up to obtain multi-gram quantities of the bicyclic lactone products and gave similar results to those obtained using catalytic benzotetramizole:* To an oven-dried, 250-mL pressure reaction vessel equipped with a magnetic stir bar was added silyloxydiene alcohol **12**[2] (4.00 g, 18.7 mmol, 1.00 equiv) and 150 mL of CH_2Cl_2, followed by (*S*)-(–)-TM·HCl (6.96 g, 28.9 mmol, 1.55 equiv), 2,6-lutidine (3.68 mL, 31.7

[1] Lee, S., Kaib, P. S. J. and List, B. *J. Am. Chem. Soc.*, **2017**, *139*, 2156-2159.
[2] Abbasov, M. E., Hudson, B. M., Tantillo, D. J. and Romo, D. *J. Am. Chem. Soc.*, **2014**, *136*, 4492-4495.

mmol, 1.70 equiv), and acryloyl chloride **11** (2.27 mL, 28.0 mmol, 1.50 equiv). The reaction vessel was then sealed and placed in a 40 °C oil bath and stirred for 48 h. The reaction mixture was then allowed to cool to ambient temperature (23 °C), volatiles were removed by rotary evaporation, and the residue was dissolved in a minimal amount of CH_2Cl_2 (~20 mL) and absorbed onto SiO_2, dried by rotary evaporation, and purified by automated flash column chromatography (0→50% EtOAc/hexanes) to afford bicyclic γ-lactones (–)-**15a** (2.90 g, 58% yield, 94% *ee*) and (+)-**15b** (954 mg, 19% yield, 94% *ee*).

(–)-**15a**: white crystalline solid; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.45; $[\alpha]_D^{20.1}$ = –32.77 (*c* = 0.82, $CHCl_3$); Enantiomeric excess was determined by chiral HPLC analysis in comparison with authentic racemic material using a Chiralcel OD-H column: hexanes:^{i}PrOH = 95:05, flow rate 0.5 mL/min, λ = 210 nm: t_{major} = 13.5 min, t_{minor} = 15.2 min, 94% *ee* (Supplementary Fig. 1); Absolute stereochemistry was assigned by analogy to bicyclic γ-lactone (+)-**3c'**;[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.76 – 4.73 (m, 1H), 4.32 (dd, *J* = 8.8, 5.9 Hz, 1H), 3.98 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.17 – 3.11 (m, 1H), 2.74 (dt, *J* = 7.8, 4.3 Hz, 1H), 2.19 – 2.12 (m, 1H), 2.12 – 2.06 (m, 1H), 1.96 – 1.89 (m, 1H), 1.86 – 1.78 (m, 1H), 0.89 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.48, 153.66, 103.01, 73.13, 37.65, 35.50, 26.12, 25.69 (3), 20.58, 18.10, -4.26, -4.44; IR (thin film): 2929, 2857, 1754, 1660 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{14}H_{24}NaO_3Si$ $[M+Na]^+$: 291.1392, found: 291.1387.

(+)-**15b**: clear colorless oil; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.65; $[\alpha]_D^{19.8}$ = +23.84 (*c* = 1.12, $CHCl_3$); Enantiomeric excess was determined by chiral HPLC analysis (see Supplementary Figure 4); Absolute stereochemistry was assigned by analogy to bicyclic γ-lactone (+)-**3c'**;[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.91 (d, *J* = 1.3 Hz, 1H), 4.38 (dd, *J* = 8.0, 6.6 Hz, 1H), 3.81 (dd, *J* = 11.4, 8.0 Hz, 1H), 2.91 – 2.82 (m, 1H), 2.28 – 2.17 (m, 3H), 1.70 – 1.58 (m, 2H), 0.91 (s, 9H), 0.14 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.35, 154.19, 100.93, 71.74, 43.76, 41.00, 30.47, 25.68 (3), 20.87, 18.11, -3.46, -4.39. IR (thin film): 2930, 2857, 1777, 1640 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{14}H_{24}NaO_3Si$ $[M+Na]^+$: 291.1392, found: 291.1394.

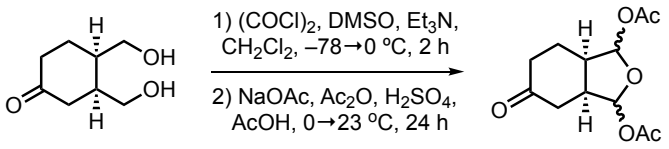


(–)-**15a** (*endo*) (–)-**16**

(3*S*,4*R*)-3,4-bis(hydroxymethyl)cyclohexan-1-one (16): To a 50 mL, single-necked, oven-dried, pear-shaped flask was added (–)-**15a** (493 mg, 1.85 mmol, 1.00 equiv) and 13.0 mL of THF. The resulting solution was cooled to 0 ºC with an ice bath and stirred for 10 min before adding $LiAlH_4$ as a solution in THF (2.40 *M*, 1.68 mL, 4.04 mmol, 2.20 equiv) dropwise via plastic syringe. After addition was complete, the mixture was allowed to stir for 1 h at 0 ºC before quenching by the method of Fieser[3]. After quenching at 0 °C the reaction mixture was allowed to warm to ambient temperature (23 ºC) with vigorous stirring over 3 h. $MgSO_4$ was then added to the mixture and the resulting slurry was filtered through Celite and the solvent removed by rotary evaporation, yielding an off-white oil which was used in the next step without purification.

To a 50 mL, single-necked, pear-shaped flask charged with the crude diol was added 5.0 mL of THF. The resulting solution was cooled to 0 ºC with an ice bath and AcOH was added (0.53 mL, 9.20 mmol, 5.00 equiv) followed by TBAF as a solution in THF (1.00 *M*, 9.20 mL, 9.20 mmol, 5.00 equiv). The reaction mixture was then stirred for 1 h at 0 ºC before removing the ice bath and allowing the mixture to warm to ambient temperature (23 ºC) over 1 h. The reaction mixture was then concentrated by rotary evaporation and the resulting crude oil was purified by automated flash column chromatography (0→20% MeOH/EtOAc) to afford keto diol **16** (220 mg, 76% yield, 2 steps) as a clear oil: TLC, R_f = 0.51 (MeOH/EtOAc, 1:4 *v/v*); $[\alpha]_D^{20.0}$ -0.40 (*c* = 1.00. $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.83-3.86 (m, 1H), 3.72-3.75 (m, 1H), 3.60-3.66 (m, 2H), 3.57 (br s, 1H), 3.48 (br s, 1H), 2.37-2.45 (m, 3H), 2.33 (t, *J* = 7.0, 2H), 2.21-2.25 (m, 1H), 1.90-1.96 (m, 1H), 1.83-1.89 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 211.8, 63.5, 63.0, 43.1, 41.5, 39.4, 39.4, 26.5; IR (thin film): 3382, 2934, 2883, 1698 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_8H_{14}O_3Na$ $[M+Na]^+$: 181.0835, found: 181.0836.

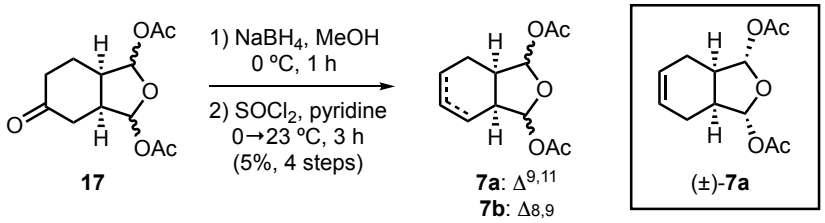
[3] Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis,* **1967**, 581-595.



(–)-**16** **17**

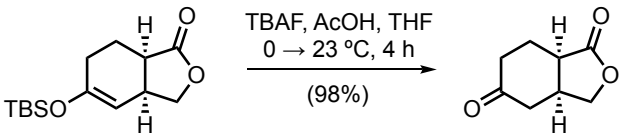
(1*S*,3*R*,3a*S*,7a*R*)-5-oxooctahydroisobenzofuran-1,3-diyl diacetate (17): To a 50 mL, single-necked, oven-dried, pear-shaped flask was added 10 mL of CH_2Cl_2 which was cooled to –78 °C and stirred for 10 min before adding $(COCl)_2$ (596 μL, 6.95 mmol, 5.00 equiv). The solution was then stirred for another 10 min before adding DMSO as a solution in anhydrous CH_2Cl_2 (2.80 *M*, 5.00 mL, 13.9 mmol, 10.0 equiv) slowly dropwise via plastic syringe. The resulting solution was stirred for 40 min at –78 °C. Then diol **16** was added as a solution in CH_2Cl_2 (0.15 *M*, 220 mg, 1.39 mmol, 1.00 equiv) slowly dropwise via plastic syringe and stirred for 1 h. Et_3N (3.87 mL, 27.8 mmol, 20.0 equiv) was then added to the reaction mixture quickly along the wall of the flask via plastic syringe. The reaction mixture was then stirred for 30 min at –78 °C before replacing cooling bath with a 0 ºC ice bath and stirring for 1 h. The reaction mixture was quenched with saturated, aqueous $NaHCO_3$, extracted with CH_2Cl_2 (3 x 15 mL), dried over $MgSO_4$, filtered through Celite, and concentrated by rotary evaporation to give a crude oil which was used in the next step without purification.

To a 50 mL, single-necked, pear-shaped flask charged with crude material was added 7.00 mL of AcOH and 7.00 mL of Ac_2O, followed by NaOAc (1.14 g, 13.9 mmol, 10.0 equiv) and concentrated H_2SO_4 (74.0 μL, 1.39 mmol, 1.00 equiv). The reaction mixture was stirred at ambient temperature (23 ºC) for 48 h, poured into saturated aqueous $NaHCO_3$ solution (50 mL), and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered through Celite, and concentrated under reduced pressure to give a crude oil which was filtered through a short column of silica gel to afford *bis*-acetoxy furanose **17** as a mixture of four diastereomers which was used in the following step without further purification: TLC, R_f = 0.39 (EtOAc/hexanes, 1:1 *v/v*). This bis-acetoxy furanose displayed some instability issues so was carried on directly to the following reduction/dehydration sequence without further characterization.



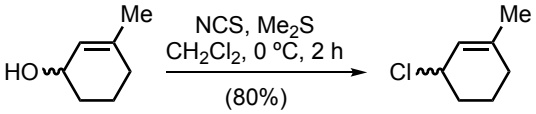
(3a*R*,7a*S*)-1,3,3a,4,5,7a-hexahydroisobenzofuran-1,3-diyl diacetate (7): To a 50 mL, single-necked, oven-dried, pear-shaped flask charged with **17** (105 mg, 0.41 mmol, 1.00 equiv) was added 12.0 mL of THF. The resulting solution was cooled to 0 ºC and stirred for 10 min before adding $NaBH_4$ (18.0 mg, 0.49 mmol, 1.20 equiv) in one portion. The resulting mixture was allowed to stir for 1 h at 0 ºC before quenching with AcOH (50.0 μL, 0.87 mmol, 2.10 equiv) and warming to ambient temperature (23 ºC). The mixture was concentrated by rotary evaporation to give a crude pale, yellow oil which was used in the next step without purification.

To a 10 mL, single-necked, oven-dried, pear-shaped flask charged with crude material was added 3.0 mL of pyridine. The resulting solution was cooled to 0 ºC and stirred for 10 min before adding $SOCl_2$ (87.0 μL, 1.20 mmol, 10.0 equiv). The cooling bath was removed and the mixture was allowed to warm to ambient temperature (23 ºC) and stir for 3 h. The reaction mixture was then concentrated by high-vacuum rotary evaporation and the crude residue was purified by flash column chromatography (0→100% EtOAc/hexanes) to afford alkene bicycle **7** (16.0 mg, 5% yield over 4 steps) as a clear oil and as an inseparable mixture of regioisomers and diastereomers (8 total). R_f = 0.39 (EtOAc/hexanes, 1:1 *v/v*). The spectral data for this mixture of 8 inseparable diastereomers and regioisomers was highly complex so full characterization is provided only for the major diastereomer, (±)-**7a**, which was prepared in racemic fashion through an alternative sequence: TLC (EtOAc/hexanes, 30% *v/v*), R_f = 0.58; ^{1}H NMR (600 MHz, C_6D_6) δ 6.19 (d, *J* = 3.1 Hz, 2H), 5.40 (m, 2H), 2.35 (m, 2H), 1.81-1.86 (m, 2H), 1.66-1.73 (m, 2H), 1.62 (s, 6H); ^{13}C NMR (150 MHz, C_6D_6) δ 169.36, 124.67, 102.45, 38.96, 22.30, 20.79; IR (thin film): 3028, 2922, 2848, 1744 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{12}H_{16}O_5Na$ $[M+Na]^+$: 263.0890, found: 263.0894.



(–)-**15a** (*endo*) (–)-**9**

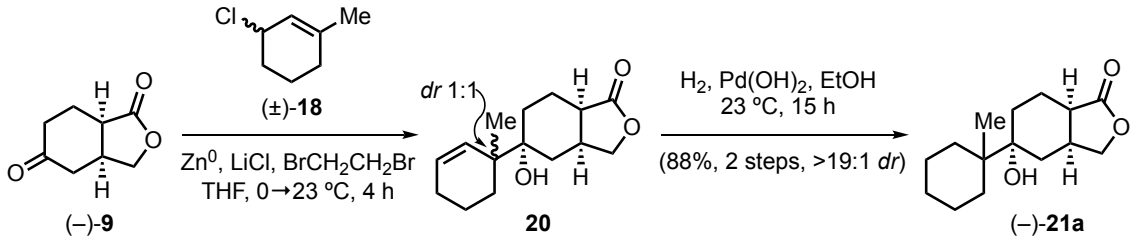
(3a*S*,7a*R*)-tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione ((–)-9): To a pre-cooled solution (0 °C) of silylenol ether (–)-**15a** (495 mg, 1.84 mmol, 1.00 equiv) and glacial AcOH (0.22 mL, 3.69 mmol, 2.00 equiv) in anhydrous THF (18.0 mL) was added TBAF (1.00 M in THF, 3.70 mL, 3.69 mmol, 2.00 equiv) dropwise. The reaction mixture was allowed to warm slowly up to ambient temperature (23 °C) over 4 h, and then concentrated by rotary evaporation. Purification by automated flash chromatography (5→80% EtOAc/hexanes) afforded keto lactone (–)-**9** (278 mg, 98% yield). (–)-**9**: clear colorless oil; TLC (EtOAc/hexanes, 1:1 *v/v*), R_f = 0.23; $[\alpha]_D^{20.0}$ –57.63 (*c* = 0.38. $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.39 (dd, *J* = 9.4, 6.1 Hz, 1H), 3.98 (dd, *J* = 9.4, 2.3 Hz, 1H), 3.08 – 2.98 (m, 1H), 2.94 – 2.86 (m, 1H), 2.53 (dd, *J* = 15.2, 6.4 Hz, 1H), 2.38 – 2.26 (m, 4H), 2.21 – 2.10 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.0, 177.6, 71.7, 41.2, 38.1, 37.3, 35.7, 22.7; IR (thin film): 2934, 2864, 1771, 1712 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_8H_{11}O_3$ $[M+H]^+$: 155.0708, found: 155.0711.



(±)-**18**

3-chloro-1-methylcyclohex-1-ene ((±)-18): *N*-chlorosuccinimide (2.48 g, 18.6 mmol, 1.10 equiv) was weighed into an oven-dried, round-bottomed flask and suspended in CH_2Cl_2 (33.0 mL). The slurry was cooled to 0 °C and Me_2S (1.61 mL, 21.9 mmol, 1.30 equiv) was added dropwise over ~5 min producing a white solution. 3-Methylcyclohex-2-en-1-ol (2.00 mL, 16.8 mmol, 1.00 equiv) was added dropwise with vigorous stirring to provide a clear solution. Within ~10 min, a precipitate formed. The reaction was continued at 0 °C for 2 h and concentrated under reduced pressure. Pentane (50 mL) was added leading to immediate formation a white precipitate. The flask was then placed in a freezer for 4 h and the supernatant was decanted. The remaining solid was washed with cold pentane (2 x 50 mL) and the combined organics were washed with brine (2 x 50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Allyl chloride (±)-**20a** was isolated as a clear colorless oil, (1.76 g, 80%) and this material was of sufficient purity (>95% as

judged by ^{1}H-NMR) to be carried directly to the next step without purification. TLC (EtOAc/hexanes, 3:7 *v/v*), R_f = 0.79. Spectral data matched that previously reported.[4]

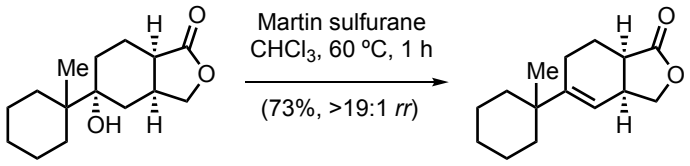


(3a*S*,5*R*,7a*R*)-5-hydroxy-5-(1-methylcyclohexyl)hexahydroisobenzofuran-1(3*H*)-one ((–)-21a): To an oven-dried round-bottomed flask was added zinc powder (2.30 g, 35.0 mmol, 25.0 equiv) and LiCl (300 mg, 7.00 mmol, 5.00 equiv) with a large bore vacuum adapter with Teflon lined seal for addition of liquids *via* syringe. The flask and solids were then carefully flame-dried under high vacuum (using a vacuum/N_2 double manifold) by repeated heating and cooling cycles with swirling of the solids to avoid the solids from clumping. After the final cycle of heating, the solids were allowed to cool for 3 min under N_2, and then anhydrous THF (20.0 mL) was slowly and carefully added with vigorous stirring. After addition of THF, 1,2-dibromoethane (0.12 mL, 1.40 mmol, 1.00 equiv) was added which sometimes led to an exotherm simultaneous with rapid gas evolution, which needs to be controlled. Pressure fluctuations generated upon addition of 1,2-dibromethane were controlled by momentarily switching the manifold from N_2 to vacuum. [Note: If gas evolution was not observed upon addition of 1,2-dibromoethane, a heat gun was employed to initiate gas evolution]. The solution was allowed to cool at ambient temperature over ~5 min before being further cooled to 0 ºC. A solution of (–)-**9** (215 mg, 1.40 mmol, 1.00 equiv) in THF (1.00 mL) was added dropwise by syringe followed by slow addition of (±)-**18** (1.65 g, 12.6 mmol, 9.00 equiv) in THF (4.0 mL) *via* syringe pump at a rate of 1.0 mL/h. During this time, the reaction mixture was allowed to slowly warm to ambient temperature (23 ºC). The reaction mixture was then quenched with saturated NH_4Cl solution (100 mL). The organic phase was separated and the aqueous phase was further extracted with EtOAc (3 x 100 mL). The organic layers were combined, washed with brine (200 mL), dried with $MgSO_4$, and concentrated *in vacuo* to afford cyclohexenol lactone **20** as a crude pale-yellow oil and as an inseparable mixture of two diastereomers (1:1 dr, based on ^{1}H-NMR analysis of the crude mixture) which was used in the next step without purification.

[4] Ren, H., Dunet, G., Mayer, P. and Knochel, P. *J. Am. Chem. Soc.*, **2007**, *129*, 5376-5377.

To a solution of the above crude cyclohexenol lactone **20** (267 mg, 1.07 mmol, 1.00 equiv) in absolute EtOH (25.0 mL) and under an atmosphere of N_2 was added $Pd(OH)_2$ on carbon (300 mg, 20 wt%, 2.14 mmol, 2.00 equiv). A H_2 balloon was attached to the flask and after three consecutive cycles of evacuation (under high vacuum) and H_2 purge, the mixture was stirred for 15 h. An aliquot was removed for ^{1}H-NMR analysis to ensure complete hydrogenation of the cyclohexene moiety. The reaction mixture was filtered through a pad of Celite, washed with EtOAc and concentrated *in vacuo*. Purification by automated flash chromatography (5→60% EtOAc/hexanes) afforded cyclohexanol lactone **21a** (307 mg, 88% yield over 2 steps) as a single diastereomer (>19:1 dr, based on ^{1}H-NMR analysis of the crude mixture).

21a : colorless crystalline solid; TLC (EtOAc/hexanes, 1:1 *v/v*), R_f = 0.43; $[\alpha]_D^{17.0}$ –34.15 (*c* = 0.41, $CHCl_3$); Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 (ddd, *J* = 8.8, 4.7, 0.7 Hz, 1H), 3.91 (d, *J* = 8.9 Hz, 1H), 2.75 (dq, *J* = 11.9, 6.2 Hz, 1H), 2.63 (t, *J* = 6.4 Hz, 1H), 2.10 – 1.92 (m, 2H), 1.84 (ddd, *J* = 13.8, 6.0, 2.9 Hz, 1H), 1.66 – 1.50 (m, 4H), 1.45 – 1.19 (m, 9H), 1.07 – 0.95 (m, 1H), 0.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.7, 75.5, 72.1, 40.1, 38.9, 33.2, 31.3, 30.4, 30.4, 26.4, 26.3, 22.0 (2), 18.7, 17.2; IR (thin film): 3509, 2966, 2929, 2862, 1747 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{15}H_{25}O_3$ $[M+H]^+$: 253.1804, found: 253.1793.

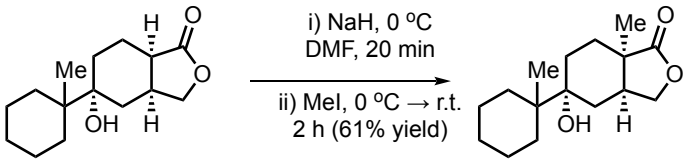


(–)-**21a** (–)-**26**

(3a*S*,7a*R*)-5-(1-methylcyclohexyl)-3a,6,7,7a-tetrahydroisobenzofuran-1(3*H*)-one ((–)-26): A glass tube was charged with a solution of (–)-**21a** (268 mg, 1.07 mmol, 1.00 equiv) in anhydrous $CHCl_3$ (35.0 mL) and Martin sulfurane dehydrating agent (1.44 g, 2.14 mmol, 2.00 equiv). The tube was sealed and placed in a 60 ºC oil bath for 1 h. The reaction mixture was cooled to ambient temperature (23 ºC), concentrated under reduced pressure and purified by automated flash chromatography (5→30% EtOAc/hexanes) to afford alkene lactone (–)-**26** (182 mg, 73% yield) as a single regioisomer (>19:1 *rr*, based on ^{1}H-NMR analysis of the crude mixture).

(–)-**26**: clear colorless oil; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.40; $[\alpha]_D^{16.9}$ –72.31 (*c* = 0.26, $CHCl_3$); Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (s, 1H), 4.38 (dd, *J* = 8.8, 6.7 Hz, 1H),

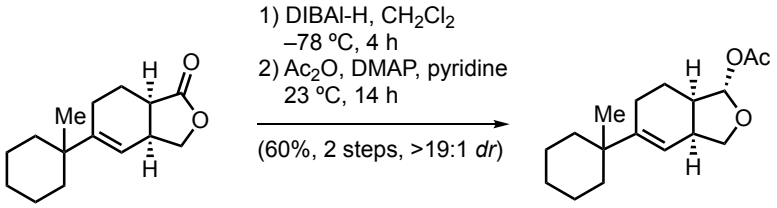
4.02 (dd, *J* = 8.8, 3.0 Hz, 1H), 3.10 (m, 1H), 2.76 (dt, *J* = 8.0, 4.9 Hz, 1H), 2.08 (dt, *J* = 13.3, 4.8 Hz, 1H), 2.02 – 1.88 (m, 2H), 1.73 (dddd, *J* = 13.1, 10.0, 5.4, 4.7 Hz, 1H), 1.69 – 1.58 (m, 2H), 1.48 – 1.17 (m, 8H), 0.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.1, 148.0, 117.8, 72.9, 38.9, 37.9, 37.9, 36.1, 36.0, 27.0, 26.4, 22.7, 22.5, 21.4, 20.8; IR (thin film): 2927, 2854, 1771 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{15}H_{22}LiO_2$ $[M+Li]^+$: 241.1780, found: 241.1793.



(–)-**21a** **30**

(3aS,5R,7aR)-5-hydroxy-7a-methyl-5-(1-methylcyclohexyl)hexahydroisobenzofuran-1(3H)-one (30): Alcohol (-)-**21a** (20.0 mg, 0.08 mmol, 1.00 equiv) was weighed out to a flame dried reaction vial and the atmosphere purged with N_2. The solid was dissolved in DMF (1.00 mL, 0.08 M) and cooled to 0 °C in an ice bath. NaH (60% w/w, 2.27 mg, 0.16 mmol, 2 equiv) was added in one portion and the reaction allowed to stir at 0 °C for 20 minutes. MeI (6.00 mL, 0.10 mmol, 1.20 equiv) was added and the cooling bath removed. The resulting solution was stirred for 2 hours at 23 °C before quenching with H_2O (1.5 mL). The layers were separated and the aqueous phase extracted with Et_2O (3 x 1.5 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude residue was purified directly by silica gel chromatography (20% EtOAc:Hexanes) to yield 12.8 mg (61% yield) of the title compound.

30: white solid, m.p. 102-104 ºC (recrystallized from pentane); TLC (EtOAc/hexanes, 1:1 v/v), Rf = 0.50; $[\alpha]_D^{23.5}$ –21.96 (*c* = 0.255, $CHCl_3$); Absolute stereochemistry was assigned based on X-ray analysis using anomalous dispersion (Supplementary Fig. 3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.43 (dd, J = 9.0, 4.7 Hz, 1H), 3.85 (d, J = 9.0 Hz, 1H), 2.39 (ddd, J = 11.5, 6.2, 4.7 Hz, 1H), 2.05 (ddd, J = 14.0, 4.7, 2.9 Hz, 1H), 1.88 (ddd, J = 13.9, 6.3, 3.1 Hz, 1H), 1.75 (app td, J = 13.7, 4.7 Hz, 1H), 1.70 – 1.63 (m, 1H), 1.62 – 1.52 (m, 4H), 1.49 – 1.26 (m, 7H), 1.25 (s, 3H), 1.11 – 0.99 (m, 2H), 0.91 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.1, 75.4, 71.0, 42.1, 39.9, 39.5, 32.8, 30.4, 30.3, 27.9, 27.3, 26.2, 23.5, 22.0, 21.9, 17.1; IR (thin film): 3517, 2924, 2854, 1758, 1452, 1381 cm^{-1}; HRMS (ESI+) m/z calcd for $C_{16}H_{26}O_3Na$ $[M+Na]^+$: 289.1780, found: 289.1776.

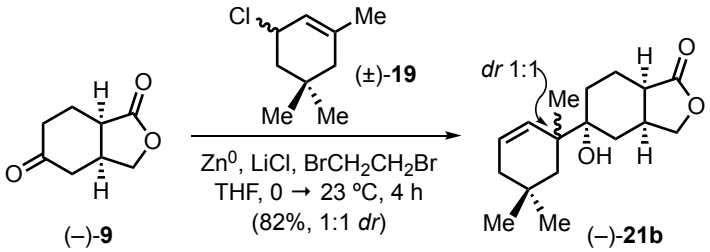


(–)-**26** (–)-**27a**

(1*S*,3a*S*,7a*R*)-5-(1-methylcyclohexyl)-1,3,3a,6,7,7a-hexahydroisobenzofuran-1-yl acetate ((–)-27): To a solution of (–)-**26** (30.0 mg, 0.128 mmol, 1.00 equiv) in CH_2Cl_2 (2.0 mL) was added DIBAl-H (1.00 M solution in CH_2Cl_2, 154 µL, 0.154 mmol, 1.20 equiv) dropwise at –78 ºC. The reaction mixture was stirred at –78 ºC for 4 h and carefully quenched in sequence with 6 µL H_2O, 11 µL 15% aqueous NaOH, and 16 µL H_2O. The dry-ice bath was removed and the mixture was allowed to warm slowly to ambient temperature (23 ºC). Subsequently, anhydrous $MgSO_4$ was added and the reaction mixture was vigorously stirred for 30 min, filtered through a pad of Celite, and concentrated by rotary evaporation. The crude lactol was of sufficient purity (>95% as judged by ^{1}H-NMR) to be carried on directly to the next step.

To a solution of the above crude lactol in anhydrous pyridine (1.00 mL) was added DMAP (2.00 mg, 12.0 µmol, 0.10 equiv) and Ac_2O (120 µL, 1.28 mmol, 10.0 equiv). The reaction mixture was stirred at ambient temperature (23 ºC) for 14 h, poured into saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by automated flash chromatography (0→30% EtOAc/hexanes) afforded acetyl lactol (–)-**27** (21.0 mg, 60% yield over 2 steps) as a single diastereomer (>19:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).

(–)-**27**: clear colorless oil; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.49; $[\alpha]_D^{19.1}$ –53.33 (*c* = 0.15, $CHCl_3$); Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.04 (s, 1H), 5.44 (dd, *J* = 4.3, 1.6 Hz, 1H), 4.28 (t, *J* = 8.2 Hz, 1H), 3.65 (t, *J* = 7.8 Hz, 1H), 3.00 (q, *J* = 7.5 Hz, 1H), 2.30 (ddd, *J* = 11.6, 7.3, 4.4 Hz, 1H), 2.13 – 2.08 (m, 1H), 2.07 (s, 3H), 1.96 – 1.87 (m, 1H), 1.84 (m, 1H), 1.71 – 1.62 (m, 3H), 1.52 – 1.22 (m, 8H), 0.95 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 145.9, 117.6, 103.9, 74.9, 43.1, 38.7, 36.6, 36.3, 36.0, 27.1, 26.5, 22.9, 22.8, 22.7, 22.5, 21.5; IR (thin film): 2931, 2857, 1746 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{17}H_{26}LiO_3$ $[M+Li]^+$: 285.2042, found: 285.2052.

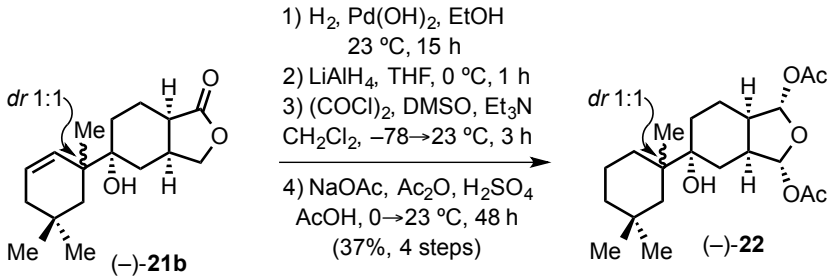


(3a*S*,5*R*,7a*R*)-5-hydroxy-5-(1,5,5-trimethylcyclohex-2-en-1-yl)hexahydroisobenzofuran-1(3*H*)-one ((–)-21b): To an oven-dried round-bottomed flask was added zinc powder (2.30 g, 35.0 mmol, 25 equiv) and LiCl (300 mg, 7.00 mmol, 5.0 equiv) with a large bore vacuum adapter with Teflon lined seal for addition of liquids *via* syringe. The flask and solids were then carefully flame-dried under high vacuum (using a vacuum/N_2 double manifold) by repeated heating and cooling cycles with swirling of the solids to avoid the solids from clumping. After the final cycle of heating, the solids were allowed to cool for 3 min under N_2, and then anhydrous THF (20.0 mL) was slowly and carefully added with vigorous stirring. After addition of THF, 1,2-dibromoethane (0.12 mL, 1.40 mmol, 1.00 equiv) was added which sometimes led to an exotherm simultaneous with rapid gas evolution, which needs to be controlled. Pressure fluctuations generated upon addition of 1,2-dibromoethane were controlled by momentarily switching the manifold from N_2 to vacuum. [Note: If gas evolution was not observed upon addition of 1,2-dibromoethane, a heat gun was employed to initiate gas evolution]. The solution was allowed to cool at ambient temperature over ~5 min before being further cooled to 0 ºC. A solution of keto lactone (–)-**9** (215 mg, 1.40 mmol, 1.00 equiv) in THF (1.00 mL) was added dropwise by syringe followed by slow addition of allyl chloride (±)-**19**[5] (2.22 g, 14.0 mmol, 10.0 equiv) in THF (4.0 mL) *via* syringe pump at a rate of 1.0 mL/h. During this time, the reaction mixture was allowed to slowly warm to ambient temperature (23 ºC). The reaction mixture was then quenched with saturated NH_4Cl solution (100 mL). The organic phase was separated and the aqueous phase was further extracted with EtOAc (3 x 100 mL). The organic layers were combined, washed with brine (200 mL), dried with $MgSO_4$, and concentrated under reduced pressure. Purification by automated flash chromatography (5→60% EtOAc/hexanes) afforded dimethyl cyclohexenol lactone (–)-**21b** (318 mg, 82% yield) as an inseparable mixture of two diastereomers (1:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).

(–)-**21b**: white crystalline solid, *m.p.* 161.2-163.7 ºC (recrystallized from pentane); TLC (EtOAc/hexanes, 1:1 *v/v*), R_f = 0.44; Absolute stereochemistry was assigned based on

[5] Harvey, N. L., Krysiak, J., Chamni, S., Cho, S. W., Sieber, S. A. and Romo, D. *Chem. Eur. J.*, **2015**, *21*, 1425-1428.

X-ray analysis using anomalous dispersion (Supplementary Fig. 3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.89 (ddd, *J* = 9.9, 6.3, 2.1 Hz, 1H), 5.52 (dd, *J* = 10.2, 1.8 Hz, 1H), 4.24 (dt, *J* = 9.0, 4.5 Hz, 1H), 3.93 (dd, *J* = 8.9, 4.3 Hz, 1H), 2.75 (tt, *J* = 12.0, 6.1 Hz, 1H), 2.64 (q, *J* = 5.5 Hz, 1H), 2.14 – 2.02 (m, 1H), 2.05 – 1.92 (m, 1H), 1.86 – 1.64 (m, 4H), 1.63 – 1.50 (m, 1H), 1.45 – 1.21 (m, 3H), 1.10 (dtd, *J* = 13.6, 2.7, 1.3 Hz, 1H), 1.02 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.67, 178.63, 130.28 (2), 130.23 (2), 75.46, 75.44, 72.09, 71.97, 43.47, 43.42, 42.90, 42.85, 39.04 (2), 38.25 (2), 33.23, 33.22, 32.34, 32.33, 30.91 (2), 30.59, 30.58, 27.25, 27.19, 25.98 (2), 22.16 (2), 18.79, 18.69; IR (thin film): 3518, 2951, 2867, 1766 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{17}H_{27}O_3$ $[M+H]^+$: 279.1960, found: 279.1963.



(1*S*,3*R*,3a*S*,5*R*,7a*R*)-5-hydroxy-5-(1,3,3-trimethylcyclohexyl)octahydroisobenzofuran-1,3-diyl diacetate ((–)-22): To a solution of cyclohexenol lactone (–)-**21b** (281 mg, 1.01 mmol, 1.00 equiv) in absolute EtOH (20.0 mL) and under an atmosphere of N_2 was added $Pd(OH)_2$ on carbon (284 mg, 20 wt%, 2.02 mmol, 2.00 equiv). A H_2 balloon was attached to the flask and after three consecutive cycles of evacuation (under high vacuum) and H_2 purge, the mixture was stirred for 15 h. An aliquot was removed for ^{1}H-NMR analysis to ensure complete hydrogenation of the cyclohexene moiety. The reaction mixture was filtered through a pad of Celite, washed with EtOAc, concentrated *in vacuo*, and taken on directly to the next step without purification.

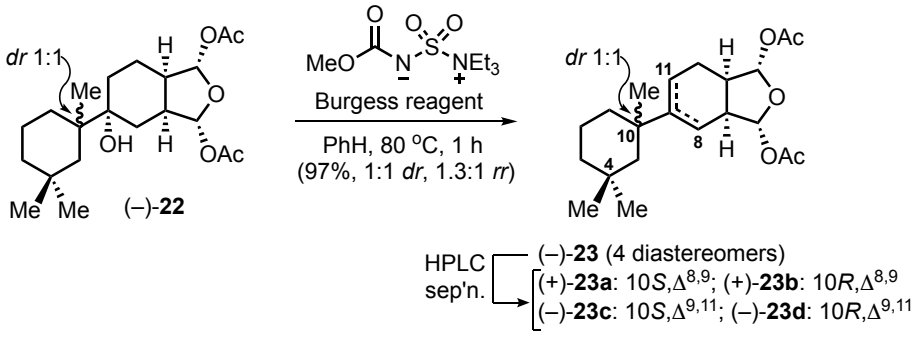
To a solution of the above crude lactone in anhydrous THF (9.00 mL) was added $LiAlH_4$ (2.00 M solution in THF, 0.60 mL, 1.20 mmol, 2.7 equiv) dropwise at 0 ºC. After stirring for 20 min, the ice bath was removed and the mixture was allowed to slowly warm to ambient temperature (23 ºC) over 40 min. Upon consumption of the starting material (as judged by TLC), the reaction mixture was cooled to 0 ºC and carefully quenched in sequence with 50 µL H_2O, 85 µL 15% aqueous NaOH, and 120 µL H_2O. The ice bath was removed and the mixture was allowed to slowly warm to ambient temperature (23 ºC). Subsequently, anhydrous $MgSO_4$ was added and the reaction mixture was vigorously stirred for 30 min, filtered through a pad of Celite and concentrated by rotary

evaporation. The crude diol was of sufficient purity (>95% as judged by ^{1}H-NMR) to be carried on directly to the next step.

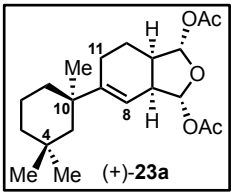
To a solution of $(COCl)_2$ (0.19 mL, 2.20 mmol, 10.0 equiv) in CH_2Cl_2 (15.0 mL) was added anhydrous DMSO dropwise as a solution in CH_2Cl_2 (2.50 M, 4.40 mmol, 20.0 equiv) followed by a solution of the above crude diol in CH_2Cl_2 (5.00 mL) *via* syringe pump over 1 h at –78 ºC. To the reaction mixture was added Et_3N (0.92 mL, 6.60 mmol, 30.0 equiv) quickly along the wall of the flask and the reaction mixture was stirred at –78 ºC for 1 h, and then at ambient temperature (23 ºC) for 1 h. The mixture was quenched with 2 N HCl (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude dialdehyde was taken on directly to the next step without purification.

To a solution of the above crude dialdehyde in AcOH/Ac_2O (1.5:1, 4.5/3.0 mL) was added NaOAc (180 mg, 2.20 mmol, 10.0 equiv) and concentrated H_2SO_4 (11.0 µL, 0.22 mmol, 1.00 equiv) successively at 0 ºC. The reaction mixture was stirred at ambient temperature (23 ºC) for 48 h, poured carefully into saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by automated flash chromatography (0→50% EtOAc/hexanes) afforded cyclohexanol diacetate (–)-**22** (142 mg, 37% yield over 4 steps) as an inseparable mixture of two diastereomers (1:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).

(–)-**22**: white solid; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.10; Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; Data provided for the mixture of diastereomers: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (d, *J* = 6.6 Hz, 1H), 5.94 (d, *J* = 2.1 Hz, 1H), 2.65 – 2.57 (m, 1H), 2.59 – 2.49 (m, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 2.02 – 1.77 (m, 2H), 1.70 – 1.47 (m, 5H), 1.42 – 1.21 (m, 7H), 1.15 – 1.09 (m, 1H), 1.01 (s, 3H), 1.00 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.93 (2), 170.12 (2), 103.25, 103.22, 100.82, 100.78, 75.73, 75.72, 43.38, 43.30, 41.50, 41.48, 40.91 (2), 39.15 (2), 38.92 (2), 36.16 (2), 30.79 (4), 30.16, 30.10, 28.88, 28.82, 27.82, 27.80, 25.45, 25.39, 21.45, 21.42, 20.51 (2), 18.98 (2), 18.54 (2); IR (thin film): 3527, 2948, 2865, 1748 cm^{-1}; HRMS (ESI–) *m/z* calcd for $C_{21}H_{34}ClO_6$ $[M+Cl]^-$: 417.2044, found: 417.2062.

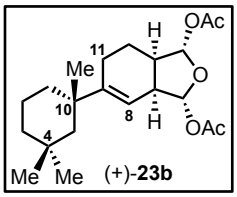


(1*S*,3*R*,3a*S*,7a*R*)-5-((*S*)-1,3,3-trimethylcyclohexyl)-1,3,3a,4,7,7a-hexahydroisobenzofuran-1,3-diyl diacetate ((+)-23a), (1*S*,3*R*,3a*S*,7a*R*)-5-((*R*)-1,3,3-trimethylcyclohexyl)-1,3,3a,4,7,7a-hexahydroisobenzofuran-1,3-diyl diacetate ((+)-23b), (1*R*,3*S*,3a*R*,7a*S*)-6-((*S*)-1,3,3-trimethylcyclohexyl)-1,3,3a,4,5,7a-hexahydroisobenzofuran-1,3-diyl diacetate ((–)-23c), and (1*R*,3*S*,3a*R*,7a*S*)-6-((*R*)-1,3,3-trimethyl-cyclohexyl)-1,3,3a,4,5,7a-hexahydroisobenzofuran-1,3-diyl diacetate ((–)-23d)): A glass tube was charged with cyclohexanol diacetate (–)-**22** (20.0 mg, 52.0 μmol, 1.00 equiv) and methyl *N*-(triethylammoniosulfonyl)carbamate (Burgess reagent, 25 mg, 0.10 mmol, 2.00 equiv) in PhH (2.00 mL). The tube was sealed and placed in an 80 ºC oil bath for 1 h. The reaction mixture was cooled to ambient temperature (23 ºC) and concentrated under reduced pressure. Purification by automated flash chromatography (0→20% EtOAc/hexanes) afforded cyclohexenyl diacetates **23a-d** (18.0 mg, 97% yield) as a clear colorless oil and as an inseparable mixture of four diastereomers (1:1 *dr*, 1.3:1 *rr*, based on ^{1}H-NMR analysis of the crude mixture). TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.32; Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; IR (thin film): 2924, 2864, 1750 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{21}H_{32}NaO_5$ $[M+Na]^+$: 387.2147, found: 387.2151. This diastereomeric mixture was then subjected to a chiral HPLC purification using a Chiralcel OJ-H column: hexanes:^{i}PrOH = 96:04, flow rate 0.4 mL/min, λ = 210 nm: $t_{(–)\text{-}\mathbf{23a}}$ = 11.4–13.5 min, $t_{(–)\text{-}\mathbf{23b}}$ = 13.6–15.4 min, $t_{(–)\text{-}\mathbf{23c}}$ = 15.5–17.5 min, $t_{(–)\text{-}\mathbf{23d}}$ = 17.6–20.8 min (Supplementary Fig. 2).

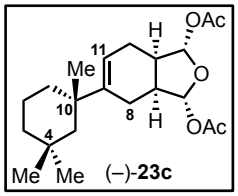


(+)-**23a**: $[\alpha]_D^{24.1}$ = +24.80 (*c* = 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.11 (d, *J* = 2.1 Hz, 1H), 6.03 (d, *J* = 3.0 Hz, 1H), 5.50 (d, *J* = 4.1 Hz, 1H), 2.96 – 2.92 (m, 1H), 2.52 (dddd, *J* = 9.4, 7.3, 4.5, 2.2 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 1.98 – 1.88 (m, 2H), 1.81 – 1.70 (m, 1H), 1.67 – 1.56 (m, 3H), 1.49 – 1.38 (m, 1H), 1.33 – 1.23 (m, 2H), 1.18 – 1.08 (m, 2H), 1.06 – 0.99 (m, 1H), 0.91 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.3, 147.9, 114.4, 103.7, 103.1, 48.2, 44.1, 41.7, 40.4, 39.0, 36.3, 33.3, 31.6,

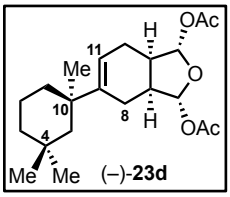
31.5, 26.7, 22.6, 21.8, 21.5, 21.5, 19.6; IR (thin film): 2947, 2864, 1752 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{21}H_{32}NaO_5$ $[M+Na]^+$: 387.2147, found: 387.2145.



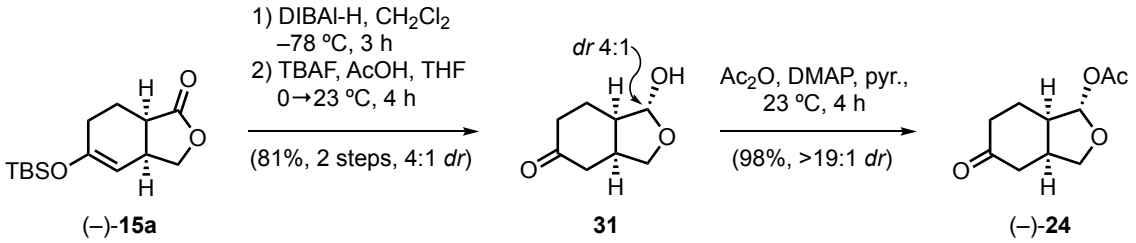
(+)-**23b**: $[\alpha]_D^{24.4}$ = +27.30 (*c* = 1.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.09 (d, *J* = 1.5 Hz, 1H), 6.00 (d, *J* = 3.6 Hz, 1H), 5.50 (d, *J* = 4.3 Hz, 1H), 3.01 – 2.94 (m, 1H), 2.47 (dddd, *J* = 9.7, 7.5, 4.6, 1.6 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.00 – 1.90 (m, 2H), 1.86 – 1.80 (m, 1H), 1.66 – 1.52 (m, 2H), 1.48 – 1.36 (m, 2H), 1.33 – 1.23 (m, 2H), 1.19 – 1.08 (m, 2H), 1.04 – 0.98 (m, 1H), 0.91 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.2, 147.8, 114.1, 104.0, 103.1, 47.7, 43.4, 42.0, 40.3, 38.9, 36.1, 35.1, 33.2, 31.4, 26.8, 24.6, 23.0, 22.5, 21.4, 19.4; IR (thin film): 2948, 2865, 1752 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{21}H_{32}NaO_5$ $[M+Na]^+$: 387.2147, found: 387.2145.



(–)-**23c**: $[\alpha]_D^{24.4}$ = -3.46 (*c* = 1.04, $CHCl_3$);^1H NMR (500 MHz, $CDCl_3$) δ 6.01 (d, *J* = 2.5 Hz, 1H), 5.97 (d, *J* = 2.7 Hz, 1H), 5.57 (t, *J* = 4.4 Hz, 1H), 2.65 – 2.53 (m, 2H), 2.36 – 2.22 (m, 2H), 2.10 (s, 3H), 2.09 (s, 3H), 2.07 – 1.95 (m, 2H), 1.93 – 1.87 (m, 1H), 1.63 – 1.53 (m, 2H), 1.48 – 1.38 (m, 1H), 1.31 – 1.23 (m, 1H), 1.18 – 1.10 (m, 2H), 1.03 (d, *J* = 14.0 Hz, 1H), 0.92 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3, 144.0, 116.8, 104.3, 104.1, 48.2, 42.1, 40.7, 40.4, 38.5, 36.2, 33.2, 31.4, 31.3, 27.1, 24.4, 23.7, 21.5 (2), 19.5; IR (thin film): 2949, 2865, 1752 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{21}H_{32}NaO_5$ $[M+Na]^+$: 387.2147, found: 387.2145.



(–)-**23d**: $[\alpha]_D^{24.7}$ = -10.71 (*c* = 0.93, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.06 (d, *J* = 1.8 Hz, 1H), 6.01 (d, *J* = 3.1 Hz, 1H), 5.57 (dt, *J* = 5.5, 2.8 Hz, 1H), 2.50 (ddt, *J* = 10.2, 7.3, 3.1 Hz, 1H), 2.46 – 2.35 (m, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.00 – 1.76 (m, 3H), 1.57 (d, *J* = 13.7 Hz, 1H), 1.49 – 1.38 (m, 1H), 1.31 – 1.22 (m, 2H), 1.14 (ddd, *J* = 13.8, 11.5, 3.6 Hz, 2H), 1.03 (d, *J* = 13.9 Hz, 1H), 0.91 (s, 3H), 0.86 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3, 144.9, 116.9, 104.1, 103.7, 48.3, 43.2, 41.1, 40.3, 38.7, 36.1, 33.0, 31.4, 30.9, 27.2, 24.6, 24.5, 21.5, 21.5, 19.5; IR (thin film): 2949, 2864, 1753 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{21}H_{32}NaO_5$ $[M+Na]^+$: 387.2147, found: 387.2144.



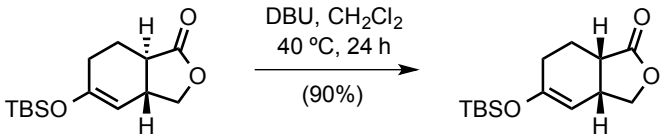
(1*S*,3a*S*,7a*R*)-5-oxooctahydroisobenzofuran-1-yl acetate ((–)-24): To a solution of lactone (–)-**15a** (72.0 mg, 0.27 mmol, 1.0 equiv) in CH_2Cl_2 (2.70 mL) was added DIBAl-H (1.00 M solution in CH_2Cl_2, 321 µL, 0.32 mmol, 1.2 equiv) dropwise at –78 ºC. The reaction mixture was stirred at –78 ºC for 4 h and carefully quenched in sequence with 12 µL of H_2O, 12 µL of 20% aqueous NaOH, and 32 µL of H_2O. The dry-ice bath was removed and the mixture was allowed to slowly warm to ambient temperature (23 ºC). Subsequently, anhydrous $MgSO_4$ was added and the reaction mixture was vigorously stirred for 30 min, filtered through a pad of Celite and concentrated under reduced pressure. The crude silyl enol ether lactol was of sufficient purity (>95% as judged by ^{1}H-NMR) to be carried on directly to the next step.

To a pre-cooled solution (0 ºC) of the above crude silyl enol ether lactol and glacial AcOH (31.0 µL, 0.54 mmol, 2.00 equiv) in anhydrous THF (2.70 mL) was added TBAF (1.00 M in THF, 0.54 mL, 0.54 mmol, 2.00 equiv) dropwise. The reaction mixture was allowed to slowly warm to ambient temperature (23 ºC) over 4 h, and then concentrated under reduced pressure. Purification by automated flash chromatography (5→80% EtOAc/hexanes) afforded ketolactol **31** (42.0 mg, 81% yield over 2 steps) as a mixture of two diastereomers (4:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).
31: clear colorless oil; TLC (EtOAc/hexanes, 4:1 *v/v*), R_f = 0.23; (NMR data is provided for the major diastereomer) ^{1}H NMR (500 MHz, C_6D_6) δ 5.08 (s, 1H), 3.94 (dd, *J* = 8.6, 7.4 Hz, 1H), 3.27 (dd, *J* = 8.6, 5.6 Hz, 1H), 2.40 (q, *J* = 7.0 Hz, 1H), 2.15 – 1.85 (m, 5H), 1.76 (ddd, *J* = 16.4, 11.2, 5.2 Hz, 1H), 1.50 – 1.38 (m, 1H), 1.36 – 1.23 (m, 1H); ^{13}C NMR (125 MHz, C_6D_6) δ 209.9, 103.4, 71.7, 44.1, 40.4, 37.8, 36.5, 23.8; IR (thin film): 3383, 2924, 1710 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_8H_{12}LiO_3$ $[M+Li]^+$: 163.0941, found: 163.0949.

To a solution of keto lactol **31** (20.0 mg, 0.12 mmol, 1.00 equiv) in anhydrous pyridine (1.00 mL) was added DMAP (2.00 mg, 12.0 µmol, 0.10 equiv) and Ac_2O (120 µL, 1.28 mmol, 10.0 equiv). The reaction mixture was stirred at ambient temperature (23 ºC) for 18 h, poured into saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

Purification by automated flash chromatography (0→40% EtOAc/hexanes) afforded acetyl keto lactol (–)-**24** (24.0 mg, 98% yield) as a single diastereomer (>19:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).

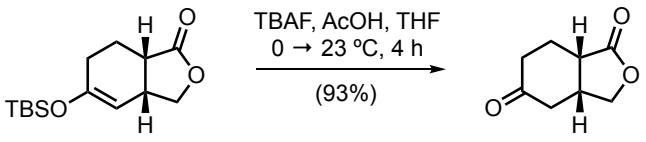
(–)-**24**: clear colorless oil; TLC (EtOAc/hexanes, 4:1 *v/v*), R_f = 0.49; $[\alpha]_D^{19.7}$ –21.08 (*c* = 0.13, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 6.05 (s, 1H), 3.76 (dd, *J* = 8.9, 7.7 Hz, 1H), 3.23 (dd, *J* = 8.8, 5.8 Hz, 1H), 2.19 – 2.09 (m, 1H), 1.96 – 1.83 (m, 3H), 1.70 – 1.60 (m, 4H), 1.46 – 1.33 (m, 2H), 1.26 – 1.10 (m, 1H); ^{13}C NMR (125 MHz, C_6D_6) δ 208.1, 169.4, 103.4, 73.2, 43.3, 39.9, 37.6, 35.9, 23.4, 20.9; IR (thin film): 2957, 2923, 2853, 1712 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{10}H_{14}LiO_4$ $[M+Li]^+$: 205.1047, found: 205.1052.



(+)-**15b** (+)-**15a**

(3a*R*,7a*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3a,6,7,7a-tetrahydroisobenzofuran-1(3H)-one ((+)-15a): To a glass tube containing a solution of *trans*-fused bicyclic γ-lactone (+)-**15b** (202 mg, 0.75 mmol, 1.0 equiv) in CH_2Cl_2 (7.50 mL, 0.10 *M*) was added DBU (0.34 mL, 2.25 mmol, 3.00 equiv). The glass tube was sealed and placed in a 40 ºC oil bath for 24 h. The reaction mixture was cooled to ambient temperature (23 ºC), filtered through a short pad of SiO_2 and the filtrate was concentrated by rotary evaporation. Purification by automated flash chromatography (5→50% EtOAc/hexanes) afforded *cis*-fused bicyclic γ-lactone (+)-**15a** (182 mg, 90% yield, 94% *ee*).

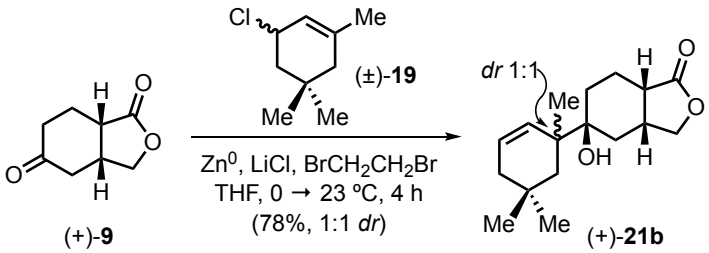
(+)-**15a**: white crystalline solid; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.45; $[\alpha]_D^{19.9}$ +52.18 (*c* = 0.77, $CHCl_3$); Enantiomeric excess was determined by chiral HPLC analysis in comparison with authentic racemic material using a Chiralcel OD-H column: hexanes:^{i}PrOH = 95:05, flow rate 0.5 mL/min, λ = 210 nm: t_{minor} = 13.5 min, t_{major} = 15.0 min, 94% *ee* (Supplementary Fig. 4); Absolute stereochemistry was assigned by analogy to bicyclic γ-lactone (+)-**3c'**;[2] All spectral data matched that reported for *cis*-fused bicyclic γ-lactone (–)-**15a**.



(+)-**15a** (+)-**9**

(3a*R*,7a*S*)-tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione ((+)-9): To a pre-cooled solution (0 ºC) of bicyclic γ-lactone (+)-**15a** (72.0 mg, 268 μmol, 1.00 equiv) and glacial AcOH (31.0 μL, 536 μmol, 2.0 equiv) in anhydrous THF (2.70 mL, 0.10 *M*) was added TBAF (1.00 *M* in THF, 0.54 mL, 536 μmol, 2.00 equiv) dropwise. The reaction mixture was allowed to slowly warm to ambient temperature (23 ºC) over 4 h, and then concentrated under reduced pressure. Purification by automated flash chromatography (5→80% EtOAc/hexanes) afforded ketolactone (+)-**9** (38 mg, 93% yield).

(+)-**19**: clear colorless oil; TLC (EtOAc/hexanes, 1:1 *v/v*), R_f = 0.23; $[\alpha]_D^{20.0}$ +24.35 (*c* = 0.11. $CHCl_3$); Absolute stereochemistry was assigned by analogy to bicyclic γ-lactone (+)-**3c'**;[2] All spectral data matched that reported for ketolactone (–)-**9**.

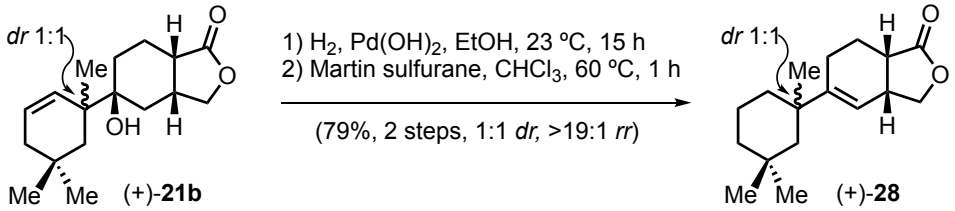


(+)-**9** (+)-**21b**

(3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1,5,5-trimethylcyclohex-2-en-1-yl)hexahydroisobenzofuran-1(3*H*)-one ((+)-21b): To an oven-dried round-bottomed flask was added zinc powder (766 mg, 11.6 mmol, 25.0 equiv) and LiCl (100 mg, 2.33 mmol, 5.00 equiv) with a large bore vacuum adapter with Teflon lined seal for addition of liquids *via* syringe. The flask and solids were then carefully flame-dried under high vacuum (using a vacuum/N_2 double manifold) by repeated heating and cooling cycles with swirling of the solids to avoid the solids from clumping. After the final cycle of heating, the solids were allowed to cool for 3 min under N_2, and then anhydrous THF (6.70 mL) was slowly and carefully added with vigorous stirring. After addition of THF, 1,2-dibromoethane (40.0 μL, 0.46 mmol, 1.00 equiv) was added which sometimes led to an exotherm simultaneous with rapid gas evolution, which needs to be controlled. Pressure fluctuations generated upon addition of 1,2-dibromoethane were controlled by momentarily switching the manifold from N_2 to vacuum. [Note: If gas evolution was not observed upon addition of 1,2-dibromoethane, a heat gun was employed to initiate gas evolution]. The solution was

allowed to cool at ambient temperature over ~5 min before being further cooled to 0 ºC. A solution of ketolactone (+)-**9** (71.0 mg, 0.46 mmol, 1.00 equiv) in THF (0.33 mL) was added dropwise by syringe followed by slow addition of allyl chloride (±)-**19**[5] (740 mg, 4.60 mmol, 10.0 equiv) in THF (1.30 mL) *via* syringe pump at a rate of 1.0 mL/h. During this time, the reaction mixture was allowed slowly warm to ambient temperature (23 ºC). The reaction mixture was then quenched with saturated NH_4Cl solution (30 mL). The organic phase was separated and the aqueous phase was further extracted with EtOAc (3 x 30 mL). The organic layers were combined, washed with brine (10 mL), dried with $MgSO_4$, and concentrated under reduced pressure. Purification by automated flash chromatography (5→60% EtOAc/hexanes) afforded dimethyl cyclohexenol lactone (+)-**21b** (98 mg, 78% yield) as an inseparable mixture of two diastereomers (1:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture).

(+)-**21b**: white crystalline solid; TLC (EtOAc/hexanes, 1:1 *v/v*), R_f = 0.44; Absolute stereochemistry was assigned by comparison to dimethyl cyclohexenol lactone (–)-**21b**; All spectral data matched that described for the enantiomeric dimethyl cyclohexenol lactone (–)-**21b** above.

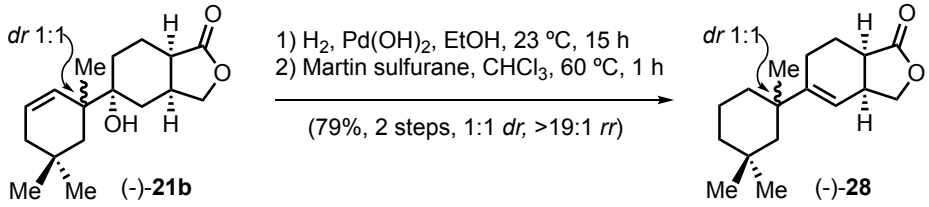


(3a*R*,7a*S*)-5-(1,3,3-trimethylcyclohexyl)-3a,6,7,7a-tetrahydroisobenzofuran-1(3*H*)-one ((+)-28): To a solution of dimethyl cyclohexenol lactone (+)-**21b** (93.0 mg, 0.33 mmol, 1.00 equiv) in absolute EtOH (6.60 mL) and under an atmosphere of N_2 was added $Pd(OH)_2$ on carbon (94.0 mg, 20 wt%, 0.67 mmol, 2.0 equiv). A H_2 balloon was attached to the flask and after three consecutive cycles of evacuation (under high vacuum) and H_2 purge, the mixture was stirred for 15 h. An aliquot was removed for ^{1}H-NMR analysis to ensure complete hydrogenation of the cyclohexene moiety. The reaction mixture was filtered through a pad of Celite, washed with EtOAc, concentrated under reduced pressure, and taken on directly to the next step without purification.

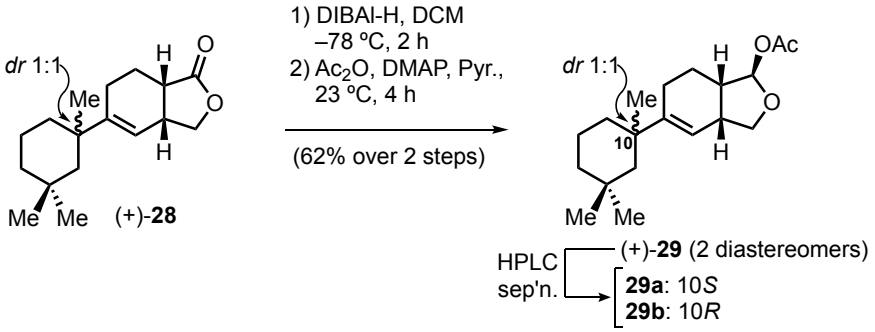
A glass tube was charged with the above crude alcohol (89.0 mg, 0.33 mmol, 1.00 equiv) in anhydrous $CHCl_3$ (11.6 mL, 0.02 *M*) and Martin Sulfurane dehydrating agent (478 mg, 0.67 mmol, 2.00 equiv). The tube was sealed and placed in a 60 ºC oil bath for 1 h. The reaction mixture was cooled to ambient temperature (23 ºC), concentrated under reduced pressure and purified by automated flash chromatography

(5→30% EtOAc/hexanes) to afford cyclohexene lactone (+)-**28** (69 mg, 79% yield over 2 steps) as a single regioisomer (>19:1 *rr*, based on ^{1}H-NMR analysis of the crude mixture).

(+)-**28**: clear colorless oil; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.41; Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; (NMR data is provided for two diastereomers) ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 (s, 2H), 4.43 – 4.33 (m, 2H), 4.08 – 3.96 (m, 2H), 3.15 – 3.02 (m, 2H), 2.82 – 2.69 (m, 2H), 2.16 – 1.85 (m, 9H), 1.80 – 1.49 (m, 7H), 1.47 – 1.38 (m, 2H), 1.31 – 1.23 (m, 2H), 1.20 – 1.05 (m, 4H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.24, 179.22, 148.22, 148.03, 116.76, 116.67, 72.81, 72.79, 48.02, 47.72, 40.46, 40.37, 39.00, 38.95, 37.83, 37.82, 36.26, 36.23, 35.97, 35.93, 33.38, 33.18, 31.66, 31.64, 31.46, 31.39, 21.58, 21.57, 21.18, 20.98, 19.61, 19.46; IR (thin film): 2932, 2860, 1769 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{17}H_{26}LiO_2$ $[M+Li]^+$: 269.2093, found: 269.2107.



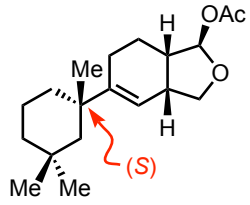
(3a*S*,7a*R*)-5-(1,3,3-trimethylcyclohexyl)-3a,6,7,7a-tetrahydroisobenzofuran-1(3*H*)-one ((-)-28): The title compound was prepared utilizing the same procedure as **(+)-28** (see above) starting from **(-)-21b**. Absolute configuration was assigned through analogy with **(-)-21b**. All spectral data was in agreement with **(+)-28** disclosed above.



(1*R*,3a*R*,7a*S*)-5-(1,3,3-trimethylcyclohexyl)-1,3,3a,6,7,7a-hexahydroisobenzofuran-1-yl acetate ((+)-29): To a solution of cyclohexene lactone (+)-**28** (20.0 mg, 76.0 μmol, 1.00 equiv) in CH_2Cl_2 (1.30 mL, 0.06 *M*) was added DIBAl-H (1.00 *M* solution in CH_2Cl_2, 92 μL, 92.0 μmol, 1.20 equiv) dropwise at –78 ºC. The reaction mixture was stirred at –

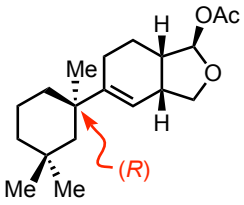
78 ºC for 4 h and carefully quenched in sequence with 92 µL MeOH and saturated aqueous Rochelle's salt (2.60 mL). The dry-ice bath was removed and the mixture was allowed to slowly warm to ambient temperature (23 ºC). Subsequently, the reaction mixture was filtered through a pad of Celite, extracted with CH_2Cl_2 (3 x 10 mL), washed with brine (10 mL), and concentrated under reduced pressure. The crude lactol was of sufficient purity (>95% as judged by ^{1}H-NMR) to be carried on directly to the next step.

To a solution of the above crude lactol in anhydrous pyridine (0.8 mL) was added DMAP (2.00 mg, 15.0 µmol, 0.20 equiv) and Ac_2O (36.0 µL, 0.38 mmol, 5.00 equiv). The reaction mixture was stirred at ambient temperature (23 ºC) for 14 h, poured into saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by automated flash chromatography (0→30% EtOAc/hexanes) afforded acetyl lactol (+)-**29** (14.0 mg, 62% yield over 2 steps) as a clear colorless oil and as a 1:1 mixture of diastereomers (based on ^{1}H-NMR analysis of the crude mixture). TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.51; $[\alpha]_D^{20.3}$ +50.91 (*c* = 0.11, $CHCl_3$); IR (thin film): 2939, 2861, 1744 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{19}H_{30}LiO_3$ $[M+Li]^+$: 313.2355, found: 313.2367. Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; The diastereomeric mixture was further separated by semi-preparative reverse-phase HPLC (100 x 21.20 mm, 5 µm; linear gradient, 65% CH_3CN/H_2O, 23 mL/min) to yield **29a** (T_R = 20.40 min) and **29b** (T_R = 22.18 min).



29a

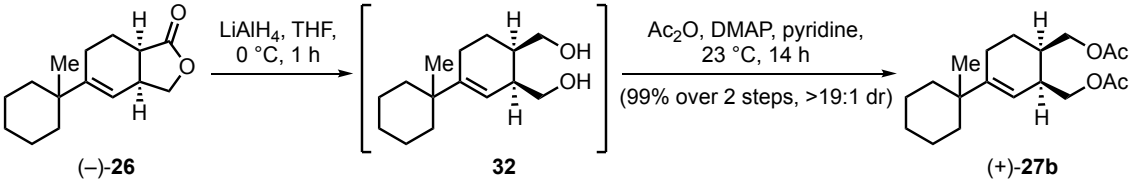
29a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.03 (s, 1H), 5.46 (dd, *J* = 4.6, 1.9 Hz, 1H), 4.27 (t, *J* = 8.2 Hz, 1H), 3.70 (t, *J* = 7.6 Hz, 1H), 2.96 (q, *J* = 7.3 Hz, 1H), 2.35 – 2.27 (m, 1H), 2.19 – 2.12 (m, 1H), 2.06 (s, 3H), 1.99 – 1.90 (m, 1H), 1.80 (dt, *J* = 12.9, 4.7 Hz, 1H), 1.73 – 1.67 (m, 1H), 1.66 – 1.24 (m, 5H), 1.21 – 1.07 (m, 1H), 1.03 (d, *J* = 14.0 Hz, 1H), 0.92 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 145.4, 117.4, 103.6, 74.6, 47.8, 40.2, 38.8, 37.6, 36.0, 35.7, 33.1, 31.4, 31.2, 26.8, 26.0, 21.3, 20.9, 19.4.



29b

29b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.01 (s, 1H), 5.43 (dd, *J* = 4.5, 2.0 Hz, 1H), 4.26 (t, *J* = 8.3 Hz, 1H), 3.61 (t, *J* = 7.9 Hz, 1H), 2.98 (dt, *J* = 13.4, 7.3 Hz, 1H), 2.26 (td, *J* = 7.4, 3.7 Hz, 1H), 2.09 (dt, *J* = 16.9, 4.5 Hz, 1H), 2.05 (s, 3H), 2.01 – 1.79 (m, 2H), 1.67 – 1.52 (m, 2H), 1.46 – 1.23 (m, 4H), 1.18 – 1.06 (m, 2H), 0.99 (d, *J* = 13.9

Hz, 1H), 0.91 (s, 3H), 0.86 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 145.4, 117.4, 103.8, 74.7, 4.5, 40.1, 38.7, 37.6, 36.0, 35.7, 32.9, 31.4, 31.1, 26.8, 26.0, 21.3, 20.7, 19.2.



((3*S*,4*R*)-1'-methyl-[1,1'-bi(cyclohexan)]-1-ene-3,4-diyl)bis(methylene) diacetate ((–)-27b): To a solution of the cyclohexene lactone (–)-**26** (27.0 mg, 0.12 mmol, 1.00 equiv) in anhydrous THF (1.50 mL, 0.08 M) was added $LiAlH_4$ (2.00 M solution in THF, 0.15 mL, 0.30 mmol, 2.50 equiv) dropwise at 0 ºC. After stirring for 1 h, the ice bath was removed and the mixture was allowed to warm slowly to ambient temperature (23 ºC) over 30 min. Upon consumption of starting material (as judged by TLC), the reaction mixture was cooled to 0 ºC and carefully quenched in sequence with 12 µL H_2O, 12 µL 25% aqueous NaOH, and 30 µL H_2O. The ice bath was removed and the mixture was allowed to warm slowly to ambient temperature (23 ºC). Subsequently, anhydrous $MgSO_4$ was added and the reaction mixture was vigorously stirred for 30 min, filtered through a pad of Celite and concentrated by rotary evaporation. The crude diol **32** was of sufficient purity (>95% as judged by ^{1}H-NMR) to be carried on directly to the next step.

To a solution of the above crude diol in anhydrous pyridine (1.00 mL, 0.10 M) was added DMAP (2.00 mg, 12.0 µmol, 0.10 equiv) and Ac_2O (120 µL, 1.28 mmol, 10.0 equiv). The reaction mixture was stirred at ambient temperature (23 ºC) for 14 h, poured into saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by automated flash chromatography (0→30% EtOAc/hexanes) afforded cyclohexene diacetate (+)-**27b** (37.0 mg, 99% yield over 2 steps) as a single diastereomer (>19:1 *dr*, based on ^{1}H-NMR analysis of the crude mixture). (+)-**27b**: clear colorless oil; TLC (EtOAc/hexanes, 1:4 *v/v*), R_f = 0.53; $[\alpha]_D^{18.5}$ +31.52 (*c* = 0.33, $CHCl_3$); Absolute stereochemistry was assigned by analogy to cyclohexenol lactone (–)-**21b**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (dt, J = 4.0, 1.6 Hz, 1H), 4.13 – 3.96 (m, 4H), 2.70 – 2.61 (m, 1H), 2.17 – 2.08 (m, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.00 – 1.92 (m, 1H), 1.70 – 1.62 (m, 4H), 1.63 – 1.52 (m, 1H), 1.49 – 1.38 (m, 3H), 1.38 – 1.29 (m, 3H), 1.28 – 1.18 (m, 2H), 0.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 171.2, 146.0, 118.6, 65.5, 65.1, 38.7, 36.6, 36.4, 36.3, 34.6, 26.5, 23.3, 23.0, 22.7, 21.1, 21.1; IR (thin film): 2930, 2856, 1742 cm^{-1}; HRMS (ESI+) *m/z* calcd for $C_{19}H_{31}O_4$ $[M+H]^+$: 323.2222, found: 323.2232.

B. Biological studies

Materials and methods

Chemicals and solutions

Carboxymethyl dextran (CM5) sensor chips, Hank´s Balance Solution Surfactant P20 (HBS-EP) buffer (pH 7.4, 0.01 M 4-(2-hydroxyethyl)-1-piperazinedsdethanesulfonic acid (HEPES), 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate), amine coupling kit (1-ethyl-3- (3-dimetylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were supplied by BiacoreAB (Uppsala, Sweden). Percoll was obtained from Pharmacia (Uppsala, Sweden). Plastic tissue-culture dishes were purchased from Falcon (Madrid, Spain). The Pan T cell Isolation Kit (human) was purchased from MiltenyiBiotec (Germany). Active human CypA full-length protein was from Abcam. Tetramethylrhodamine methyl ester (TMRM), ThiolTracker Violet, 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA) and Human IL-2 ELISA kit were purchased from Thermo Fisher Scientific (Madrid, Spain). Bovine Serum Albumin (BSA) and other chemicals were reagent grade and purchased from Sigma-Aldrich (Madrid, Spain). The composition of saline solution (PBS) used for human T lymphocytes purification was (mM): 137 NaCl, 8.2 Na_2HPO_4, 1.5 KH_2PO_4, 3.2 KCl and 2 EDTA. The composition of Umbreit saline solution was (mM): NaCl 119, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.85, KCl 5.94, $CaCl_2$ 1. Glucose (1 g/L) was added to the medium. The pH was equilibrated between 7.2-7.3. Stock solutions of drugs were done in dimethylsulphoxide (DMSO).

Cell culture

Human neuroblastoma SH-SY5Y cell line was purchased from American Type Culture Collection (ATCC), number CRL 2266. Cells were maintained in Dulbecco´s Modified Eagle´s medium: Nutrient Mix F-12 (DMEM/F-12) supplemented with 10% fetal bovine serum (FBS), glutamax, 100 U/mL penicillin and 100 µg/mL streptomycin at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air. Cells were dissociated weekly using 0.05% trypsin/EDTA. All reagents were provided by Thermo Fisher Scientific.

Human T lymphocyte isolation

Peripheral lymphocytes were isolated from human fresh heparinised blood from healthy volunteers as previously described.[6] The blood was diluted in the same proportion with PBS plus EDTA (2 mM) previously equilibrated at room temperature. 4 mL of diluted blood were placed over 3 mL of isotonic percoll (57.5%) carefully avoiding the mixture of

[6] Alfonso, A., Botana, M. A., Vieytes, M. R. and Botana, L. M. *Cell Signal,* **2001**, *13*, 819-826.

these two phases. Once the tubes were prepared they were centrifuged at 3000 rpm, 25 min at room temperature. After centrifugation, different phases were obtained and only the fraction that contained the population of lymphocytes was collected and washed three times with PBS-EDTA to removed percoll at 1500 rpm, 10 min, room temperature. Lymphocyte purity was always higher than 80%. T lymphocytes were purified from this population with a Pan T cell Isolation Kit. This is an indirect magnetic labelling system for the isolation of untouched T cells. T cell purity was always higher than 95%. Assessment of cell purity was performed by flow cytometry using a monoclonal antibody to human CD3 labelled with FITC. Viability (>95%) was determined by trypan blue exclusion. Pure T cells were maintained in RPMI (Roswell Park Memorial Institute) 1640 plus, 10% FBS, and plated in 24 plastic tissue-culture dishes in humidified 5% CO_2 and 95% air atmosphere at 37 ºC.

Note: The institutional and regional ethical board (Comité Autonómico de Ética da Investigación de Galicia, Comité Territorial de Ética da Investigación de Santiago-Lugo, Secretaria Xeral, Consellería de Sanidade, Xunta de Galicia) approved the study (Reference: 2016/508, Approved date: December 19, 2016, according to the principles outlined in the Declaration of Helsinki). Written informed consent was given to all the participants.

Cell viability assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) assay was used to determine the cytotoxicity of gracilin derivatives in T lymphocytes[7] and SH-SY5Y cells and the protective effect of these compounds against H_2O_2 insult in neuroblastoma cells as previously described.[8]

Human T lymphocytes were cultured in 24 well plates at the concentration of $2.5x10^6$ cells/mL and exposed to different compound concentrations (0.01, 0.1, 1 and 10 μM) added to the culture medium. Human T cells were maintained in the presence of the pure compound at 37 ºC in humidified 5% CO_2/95% air atmosphere for 48h. Saponin was used as cellular death control and its absorbance was subtracted from the other data. After the incubation period with compounds, assay was performed. First cells were centrifuged (1500 rpm, 5 min, 4 ºC). The pellets were resuspended in saline solution with MTT (250 μg/mL) and then incubated at 37 ºC during 30 min. After washing off excess MTT twice with saline solution, cells were disaggregated with H_2O and sonicated for 1

[7] Burton, J. D. *Methods Mol. Med.,* **2005**, *110*, 69-78.
[8]Leiros, M., Alonso, E., Sanchez, J. A., Rateb, M. E., Ebel, R., Houssen, W. E., Jaspars, M., Alfonso, A. and Botana, L. M. *ACS Chem. Neurosci.,* **2014**, *5*, 71-80.

min and the absorbance of the colored formazan salt was measured at 595 nm in a spectrophotometer plate reader. All experiments were performed three times.

SH-SY5Y cells were seeded in 96-well plates at a density of $2.5x10^5$ cells/mL and allowed to adhere for 24h. At first, cells were treated only with compounds at concentrations ranging from 10-0.01 μM for 24h to determine their possible cytotoxic effects. Experiments were carried out three times. For neuroprotective experiments, cells were treated with compounds at non-toxic concentrations (1 μM→1 nM) and 150 μM H_2O_2 for 6h. Briefly, cells were washed three times with saline solution and 200 μL of MTT (500 μg/mL) dissolved in saline buffer were added to each well. Following 1h of incubation at 37 ºC, the cells were disaggregated with sodium dodecyl sulphate at 5%. Absorbance of formazan crystals was measured at 595 nm with a spectrophotometer plate reader. Saponin was used as death control and its absorbance was subtracted from the other data. All experiments were performed fourtimes.

Interleukin 2 release

Human T lymphocytes at the concentration of $1x10^6$ cells/mL were plated in 24 well plates and pre-treated for 2 hours with gracilin derivatives (0.001-10 μM). Then, cells were stimulated with Concanavalin A (Con A) at 50μg/mL for 48 h to induce IL-2 release. The amount of IL-2 released to the culture medium was evaluated using Human IL-2 ELISA kit. The half-maximal inhibitory concentration (IC_{50}) was calculated by fitting the data with a log(inhibitor) *vs*. response model of GraphPad Prism 5.0 software. Experiments were carried out four times.

Mitochondrial membrane potential measurement

The mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using the tetramethylrhodamine methyl ester (TMRM) probe. SH-SY5Y cells were seeded in 96-well plates at $2.5x10^5$ cells/mL. After 24h, cells were treated with compounds at different concentrations (1 μM→1 nM) and 150 μM H_2O_2 for 6h.Then, cells were washed twice with saline solution and 1 μM TMRM was added to each well for 30 min at 37 ºC. After this time, cells were solubilized with DMSO and H_2O at 50%. The fluorescence was monitored with a spectrophotometer plate reader (535 nm excitation and 590 nm emission). All experiments were performed fourtimes.

Glutathione assay

Glutathione (GSH) levels were determined using ThiolTracker Violet dye, which reacts with reduced thiols in cells. Reduced glutathione represents the majority of intracellular free thiols, so this probe can be used to estimate its levels in cells. Measurements were

performed following manufacturer´s protocol. Cells were seeded in 96-well plates at $2.5x10^5$ cells/mL for 24h and treated with compounds at various concentrations (1 μM→1 nM) and 150 μM H_2O_2. After 6h, SH-SY5Y cells were washed twice with PBS and loaded with 100 μL of prewarmed ThiolTracker Violet dye (10 μM) for 30 min at 37 ºC. The fluorescence was measured at 404 nm excitation and emission at 526 nm. All experiments were performed four times.

CypA and D enzyme inhibition assay

The inhibition of the peptidyl-prolyl*cis-trans* isomerase (PPIase) activity of compounds was determined by following the rate of hydrolysis of *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide by chymotrypsin. CsA was used as positive control. The assay was performed as previously described[9] with small modifications. The assay buffer (20 mMTris-HCl, 50 mMNaCl, pH 7.8), CypD (1 nM) and the compounds at concentrations ranging from 0.001 to 10 μM were precooled at 4ºC for 1h. After that time, chymotrypsin at 0.4 mg/mL in 1 mMHCl was added to each well. The reaction was initiated by the addition of the peptide (0.1 mg/mL in 500 mMLiCl in THF). The absorbance at 380 nm was monitored every 30 sec for 5 min with a spectrophotometer plate reader. The blank rates of hydrolysis (in absence of CypD or A) were subtracted from the rates in the presence of the enzyme. The half-maximal inhibitory concentration (IC_{50}) was calculated by fitting the data with a log(inhibitor) *vs*. response model of GraphPad Prism 5.0 software. All experiments were performed four times.

Calcineurin phosphatase activity assay

The ability of the CypA-compounds complexes to inhibit the phosphatase activity of calcineurin was determined with the calcineurin Phosphatase Assay Kit (Enzo Life Sciences, Inc., Farmingdale, NY). CypA at 1 nM and the selected derivatives at 1 μM were dissolved in deionized water. The CypA-drug complex was allow to form for 30 min at room temperature. After this time, recombinant calmodulin (0.25 μM) and calcineurin (40 units per well) were added to the complex between CypA and the compounds for 30 min at 37 °C. Then, the reaction was started with the addition of the substrate at 0.15 mM during 1 h at 37 °C. Finally, the development reagent was added and incubated for 20 min at 37 °C. The absorbance was read at 620 nm in a microplate reader. The experiments were performed three times.

Statistical Analyses

[9] Yan, W., Qing, J., Mei, H., Nong, J., Huang, J., Zhu, J., Jiang, H., Liu, L., Zhang, L. and Li, J. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5682-5686.

Data are presented as mean ± SEM. Differences were evaluated by one way ANOVA with Dunnett's post hoc analysis. Statistical significance was considered at $p < 0.05$.

Discussion

CypA binding was analyzed by surface plasmon resonance (SPR).[10] Supplementary Fig. 1 shows association curves with different concentrations of gracilin A and five synthetic derivatives: **29a, 29b,** (–)-**23d,** (–)-**23c** and (+)-**23b**. The K_D association values were found to be similar between CsA, gracilinA and derivatives **29a**, **29b**, and (+)-**23b** (~2.8-6.8 μM, Supplementary Fig. 3). Compounds (+)-**23a** and (–)-**23d** displayed nanomolar K_D values (5.34 ± 1.68 nM and 7.57 ± 1.61 nM, respectively), hence high CypA affinity. The pH of immobilization buffer modifies the results of SPR experiments relative to enzymatic assays. The orientation of immobilized proteins (Cyps) changes with pH and therefore the binding pocket will have different accessibility. When Cyps are immobilized over the sensor surface at pH 4.5, CsA K_D is μM while at pH 6 the K_D value is in the nanomolar range (both for CypA and CypD).[10] Moreover, when an enzymatic fluorescence assay at pH 7.6 was used to calculate CsA K_D, the value was even lower.[11] However, for natural gracilins and synthetic compounds, the optimum pH is 4.5, since no binding was observed at pH 6.

CypA modulates interleukin-2 release (IL-2) through the calcineurinpathway.[12] We studied the cytotoxicity (Supplementary Fig. 1c) and the effect of these 5 compounds on IL-2 levels. After 48 hours, compounds **29b** and **23b-d** showed a 15-25% decrease in cell viability at 1 μM while **29a** showed this same decrease at 0.1 μM with no change at lower concentrations. Cells were pre-treated for 2 h with compounds at selected concentrations and then activated with concanavalin A. CsA was a positive control of IL-2 inhibition. Supplementary Fig. 1d shows gracilin A and compounds **29a, 23c, 23d and 23b** effect on IL-2 release inhibition. The highest inhibition was obtained by gracilin A (71%), followed by **23b** (44%) and **23d** (46%), and **29a** (26%).

Treatments for neurodegenerative diseases are a challenge for pharmaceutical development given the multiple and highly interconnected cellular processes implicated in neurodegeneration. For example, OS has been detected in the early stages of AD.[13] Neurons are highly sensitive to OS. The early role of neuronal OS in AD has been linked to amyloid-beta protein (Aβ) formation, mitochondrial dysfunction,[14] microglial activation

[10] (a) Alfonso, A., Pazos, M. J., Fernandez-Araujo, A., Tobio, A., Alfonso, C., Vieytes, M. R. and Botana, L. M. *Toxins***2014**, *6*, 96-107; (b) Sanchez, J. A., Alfonso, A., Leiros, M., Alonso, E., Rateb, M. E., Jaspars, M., Houssen, W. E., Ebel, R. and Botana, L. M. *Cell Physiol. Biochem.,***2015**, *37*, 779-792.

[11] Husi, H; Zurini, M.G.M. *Anal. Biochem.* **1994**, 222, ,251-255,

[12]Zydowsky, L. D., Etzkorn, F. A., Chang, H. Y., Ferguson, S. B., Stolz, L. A., Ho, S. I. and Walsh, C. T. *Protein Sci.,***1992**, *1*, 1092-1099.

[13] Nunomura, A., Castellani, R. J., Zhu, X., Moreira, P. I., Perry, G. & Smith, M. A. *J. Neuropathol. Exp. Neurol.* **2006**, *65***,** 631-641.

[14] Picone, P., Nuzzo, D., Caruana, L., Scafidi, V. & Di Carlo, M. Mitochondrial dysfunction: different routes to Alzheimer's disease therapy. *Oxid. Med. Cell Longev.* 780179 (2014).

and τ-hyperphosporylation,[15,16] and has led to the study of antioxidants now in Phase I or II clinical trials, such as *N*-acetyl-*L*-cysteine.[15]

For neuroprotection assays, the compound's cytotoxicity was first assessed toward SH-SY5Y cells (Supplementary Table 1). Compounds (–)-**27a**, (–)-**27b**, **29a, 29b, 22,** (+)-**23a,** (+)-**23b,** and (–)-**23c** led to cell death at the higher concentrations tested after 24h. Due to the toxicity displayed by derivative (–)-**23c** (IC_{50}: 4.2 μM), this gracilin derivative was assayed at lower concentration (0.001, 0.01 and 0.1 μM) in the neuroprotective tests, whereas the other compounds were tested at 0.01, 0.1 and 1 μM.

Four parameters were monitored to determine the antioxidant potential: cell viability, mitochondrial membrane potential ($\Delta\Psi_m$), ROS release and glutathione (GSH) levels in the presence of the pro-oxidant H_2O_2. The known antioxidant vitamin E (VitE) at 25 μM was used as positive control.

Neuroprotection against H_2O_2 toxicity was evaluated by co-treating cells with 150 μM H_2O_2 and the compounds for 6 h. H_2O_2 decreased viability by 44.4±4.4% (p= 0.00005). Eight gracilin A derivatives were protective (Fig. 4) at one or more concentrations. (–)-**27a**,(–)-**27b, 29a, 22** and (–)-**23c** protected cells against H_2O_2 insult at almost all concentrations (0.01, 0.1 and 1 μM; Supplementary Fig. 2a).

Tetramethylrodamine methyl ester (TMRM) was used to evaluate the $\Delta\Psi_m$. After H_2O_2 treatment, cells exhibit a TMRM signal decrease of 24.1±3% (p=0.0032). **29a,** (–)-**23c,** (–)-**27a,** (–)-**27b** and **22** recovered $\Delta\Psi_m$ compared to H_2O_2 control (Supplementary Fig. 2b).

To assess if compounds were acting as ROS scavengers, carboxy-H2DCFDA was used to determine ROS levels. H_2O_2-treated cells increased ROS levels by 141.4±4.6% (p=0.00002) versus untreated cells. A decrease was observed after co-treatment with eight compounds, achieving untreated cell levels (Fig. 4). Among them, 5 derivatives showed better results: (–)-**27a,** (–)-**27b, 22,** and (–)-**23c** diminishing ROS at two or three concentrations (Supplementary Fig. 2c).

For GSH, the main non-enzymatic antioxidant in cells, neuroblastoma co-treated with H_2O_2 and (–)-**27a, 22,** (–)-**27b** and **29a** recovered GSH levels (Supplementary Fig. 2d) at two or more concentrations versus H_2O_2 control (82.9 ±1.6%, p=0.027).

Since gracilin A's anti-OS effect is related with mPTP opening, we selected the effective concentration of each compound to determine their effect on mPTP.Derivatives

[15] Di Domenico, F., Barone, E., Perluigi, M. & Butterfield, D. A. Strategy to reduce free radical species in Alzheimer's disease: an update of selected antioxidants. *Expert Rev. Neurother.* **15**, 19-40 (2015).

[16] Yan, M. H., Wang, X. & Zhu, X. Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. *Free Radic Biol Med* **62**, 90-101 (2013).

29a, (–)-**27a,** (–)-**27b** and **22** prevent TBHP-induced opening (77.13 ± 2.3%, *p*=0.00002) (Fig. 4 and Supplementary Fig. 3a). CsA(0.2 μM) was used as a positive control.

CypD is involved in mPTP functioning. Its deficiency has been correlated to resistance to both Aβ and Ca^{2+}-induced mitochondrial swelling and permeability and enhanced cognition and memory in an AD mouse model.[17] CsA inhibits Cyp's activity but does not display the desired selectivity for CypD and this large macrocyclic peptide has not a desirable blood-brain barrier (BBB) permeability.[18] The induction of mPTP opening by CypD is related with its PPIase activity[19]. Therefore, we studied the modulation of this activity by gracilin A's derivatives.

Compounds **29a,** (–)-**27a,** (–)-**27b** and **22** inhibited CypD activity at low micromolar concentrations (IC_{50} < 0.5 μM) (Fig. 4 and Supplementary Fig. 3b-c). To analyze the specificity of these compounds for CypD, we also tested the PPIase inhibitory activity of these derivatives towardCypA (Fig. 4). Compound (–)-**27b** was inactive toward CypA (up to 10μM) showing complete selectivity for CypD with an IC_{50} = 0.48 (CI:0.09-2.3) μM. Moreover, (–)-**27b** in SPR experiments did not bind to CypA up to 10 μM and neither inhibits IL-2 release nor calcineurin activity (Supplementary Fig. 1). Derivatives (–)-**27a** and **29a** showed higher IC_{50} values for CypA than for CypD indicating some selectivity for CypD, ~3-fold and18-fold respectively. On the other hand, compound **22** showed greater activity towardCypAvsCypD (IC_{50} = 0.064 (CI: 0.022-0.19) vs 0.48 (CI:0.09-2.3) μM; ~4-fold selectivity for CypA).

[17] Du, H., Guo, L., Fang, F., Chen, D., Sosunov, A. A., McKhann, G. M., Yan, Y., Wang, C., Zhang, H., Molkentin, J. D., Gunn-Moore, F. J., Vonsattel, J. P., Arancio, O., Chen, J. X. & Yan, S. D. *Nat. Med.* **2008**, *14***,** 1097-1105.

[18] a) Tsuji, A; Tamai, I; Sakata, A; Tenda, Y; Terasaki, T. *Biochem Pharmacol.* **1993**, *46*, 1096–1099. b) Valasani, K. R.; Vangavaragu J. R.; Day, V. W.; Yan, S.S*. J. Chem. Inf. Model* **2014**, *54*, 902-912.

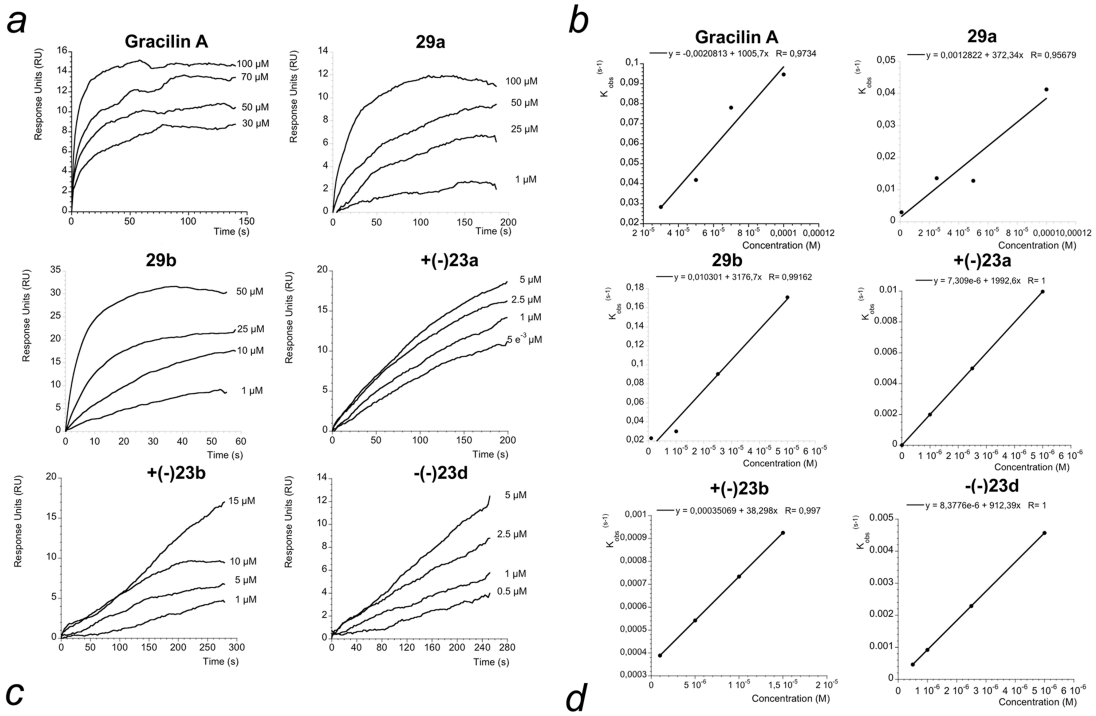
[19]Connern, C. P. and Halestrap, A. P. *Biochem. J.,***1994**, *302*, 321-324.

3.-Supplementary Table 1

Cells	Compound	IC_{50} (μM)	95% Confidence interval	R^2
SH-SY5Y	**29a**	7.1	1.9-26.1	0.98
	29b	7.9	0.85-74.3	0.98
	22	6.5	0.15-28	0.99
	(-)-23c	1.4	0.19-10.3	0.96
	(-)-23d	4.2	0.27-63.9	0.96
	(+)-23a	4.2	0.66-26.2	0.96
	(+)-23b	1.4	0.64-3.2	0.96
T-lymphocytes	**29a**	1.3	0.12-15.0	0.93
	29b	4.0	0.56-29.1	0.92
	(-)-23d	2.7	0.65-11.4	0.90
	(+)-23a	1.5	0.36-6.5	0.91
	(+)-23b	1.4	0.42-4.7	0.94

Supplementary Table 1. Gracilin A derivatives exhibiting cellular toxicity in SH-SY5Y cells and human T-lymphocytes. The table shows the results of the derivatives that displayed cytotoxicity up to 10 μM. IC_{50} obtained after 24 and 48 h incubation, respectively, and calculated with a log (inhibitor) *vs*. response fitting model of GraphPad Prism 5.0 software. Data of three independent experiments.

4. Biological Assay Figures



c

IL-2 release inhibition

Compound	IC_{50} (µM)	95% Confidence interval	R^2
29a	0.12	0.09-0.16	0.91
(+)-23a	0.15	0.014-1.5	0.93
(+)-23b	0.3	0.12-0.56	0.95
(-)-23d	0.36	0.12-1.03	0.98
(-)-27b	> 10.0	-	-

Supplementary Figure 1 |Activity of selected gracilin A derivatives in immunosuppressive assays. ***a***) Binding of gracilin A and selected derivatives to cyclophilin A (CypA) as measured by surface plasmon resonance (SPR). Association curves obtained by addition of compounds over immobilized CypA and subtraction of their respective solvent control. Graphs are from one experiment representative of 4 experiments. ***b***) Analysis of ligand binding. Kinetic plots of apparent association rate constant K_{obs} (s–1) obtained from Supplementary Fig. 1a. Graphs are from one experiment representative of 4 experiments. ***c***) Effect of selected derivatives on Interleukin–2 (IL–2) production in human T lymphocytes stimulated with Concanavalin A (ConA). Human T lymphocytes were pre–treated for 2h with compounds and with Con A (50 µg/mL) for 48h. IC_{50} was calculated by fitting the data with a log(inhibitor) vs. response model of GraphPad Prism 5.0 software (n=10). ***d***) Effect of selected derivatives on calcineurin phosphatase activity. CsA was used as positive control. Values are percentage of inhibition with respect to control, one-way ANOVA followed by post

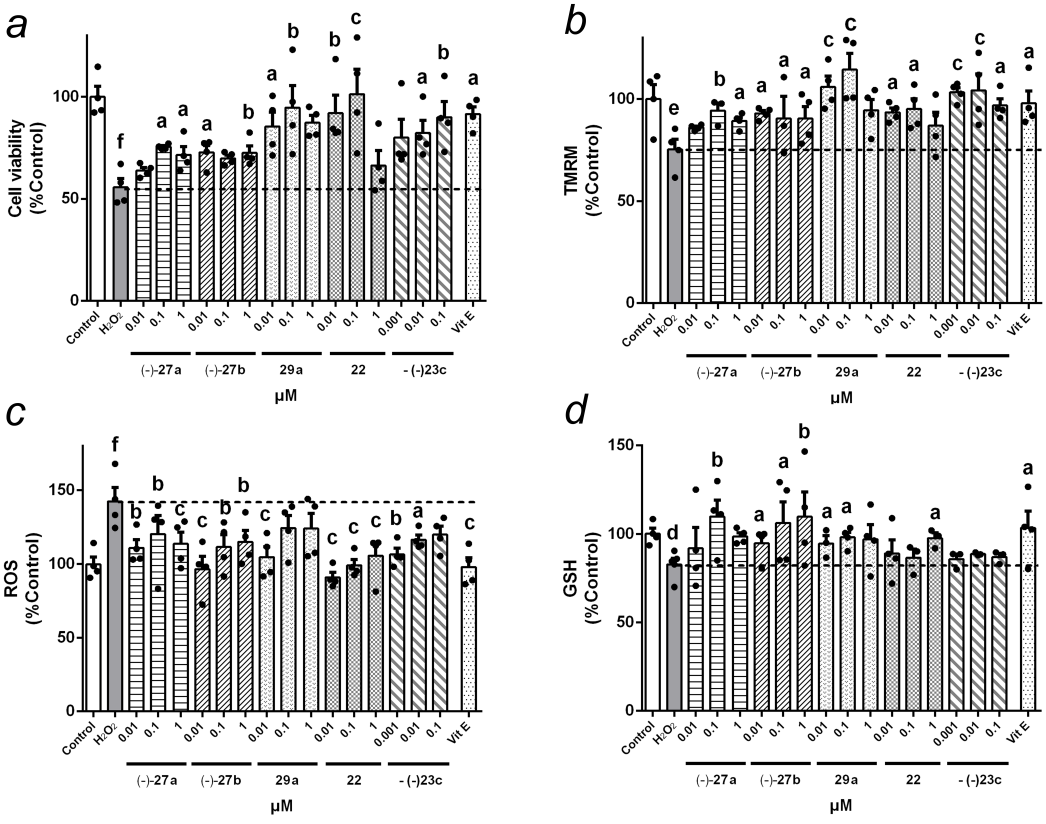
hoc Dunnett's test. [a]p<0.05, -[b]p<0.01 and [c]p<0.001. Data are mean ± SEM of four independent experiments (n = 4).

-p values for each condition are provided below for Supplementary Figure 1d.

-One Way ANOVA: F= 11.64; df= 21, CI: 95%, p<0.0001

-Dunnett's post hoc test p values:

Compound	Concentration	p value
CsA	2 µM	0.00002
	1 µM	0.0005
	0.2 µM	0.0100
29a	1 µM	0.0346
	0.1 µM	0.0013
	0.01 µM	0.0016
23a	10 µM	0.0002
	1 µM	0.0180
	0.1 µM	0.0941
	0.01 µM	0.1299
23b	10 µM	0.0002
	1 µM	0.0001
	0.1 µM	0.0020
	0.01 µM	0.0096
23d	1 µM	0.0001
	0.1 µM	0.00002
	0.01 µM	0.0023
	0.001 µM	0.0707
27b	10 µM	0.1466
	1 µM	0.1493
	0.1 µM	0.1339



Supplementary Figure 2 | Activity of selected gracilin A derivatives in neuroprotective assays. Dotted line indicates levels of cells treated only with 150 µM H_2O_2. Data are presented as percentage of untreated control cells and compared to cells treated only with 150µM H_2O_2 by one-way ANOVA and Dunnett's post hoc test. [a]p<0.05,[b]p<0.01 and [c]p<0.001. H_2O_2 control cells are compared to untreated control cells. [d]p< 0.05, [e]p<0.01 and [f]p<0.001. Values are mean ± SEM of four independent

experiments. ***a***) Effects of selected gracilin A derivatives on cell viability against H_2O_2 insult. Cell viability was measured by MTT assay. SH–SY5Y cells were treated with 150 μM H_2O_2 and derivatives for 6h. ***b***) Effects of selected gracilin A derivatives on mitochondrial membrane potential ($\Delta\Psi_m$) recovery. $\Delta\Psi_m$ measured by TMRM assay. Cells were co–treated with 150 μM H_2O_2 and derivatives for 6h. ***c***) Inhibition of reactive oxygen species (ROS) release by selected gracilin A derivatives. ROS levels were assessed with 5–(and–6)–carboxy–2',7'–dichlorodihydrofluorescein diacetate. SH–SY5Y cells were co–incubated with derivatives and 150 μM H_2O_2 for 6h. ***d***) Evaluation of glutathione (GSH) levels in cells treated with selected gracilin A derivatives. GSH levels were measured by Thiol Tracker Violet in neuroblastoma cells co–treated with 150 μM H_2O_2 and derivatives for 6h.

One Way ANOVA:

- (a) Cell viability: F= 4.12; df= 17, CI: 95%, $p<0.0001$

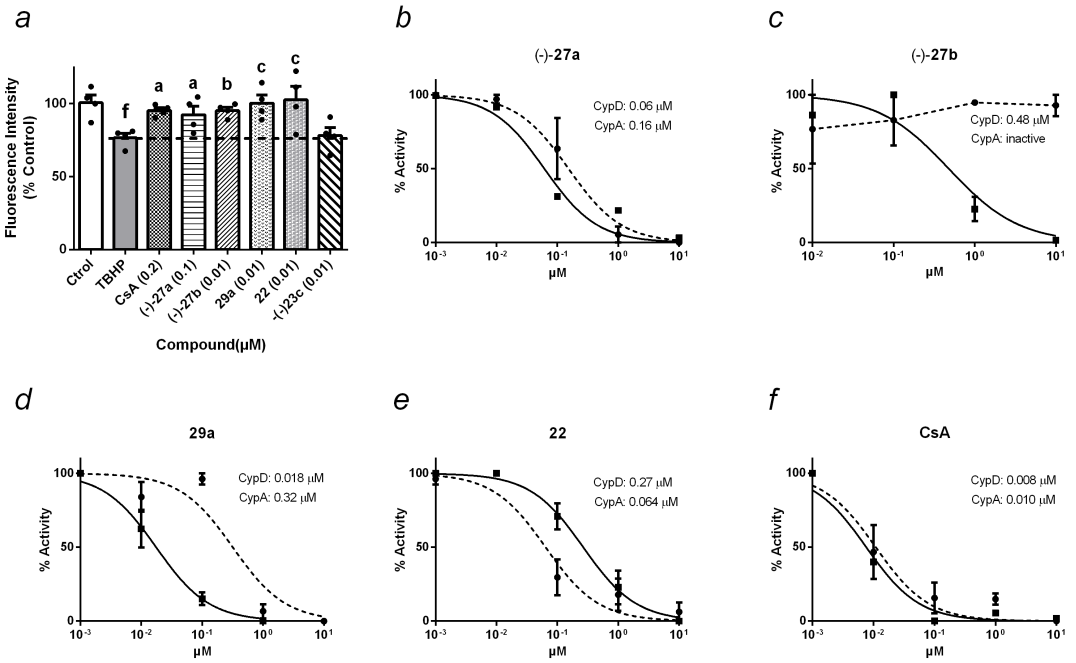
- (b) TMRM: F= 2.79; df= 17, CI: 95%, $p=0.0022$

- (c) ROS: F= 3.05; df= 17, CI: 95%, $p=0.0010$

- (d) GSH: F= 3.56; df= 17, CI: 95%, $p=0.0002$

Dunnett's post hoc test results: The *p* values for each condition are provided below:

Compound		*p* value (a) Cell Viability	*p* value (b) TMRM	*p* value (c) ROS	*p* value (d) GSH
H_2O_2		0.00005	0.0032	0.00002	0.0272
27a	0.01 μM	0.5032	0.2078	0.0021	0.1590
	0.1 μM	0.0116	0.0085	0.0028	0.0082
	1 μM	0.0223	0.0434	0.0009	0.1590
27b	0.01 μM	0.0165	0.0118	0.0001	0.0421
	0.1 μM	0.0594	0.0246	0.0026	0.0361
	1 μM	0.0100	0.0101	0.0035	0.0010
29a	0.01 μM	0.0421	0.0003	0.0001	0.0155
	0.1 μM	0.0012	0.0001	0.0539	0.0290
	1 μM	0.0994	0.0711	0.1480	0.3395
22	0.01 μM	0.0036	0.0245	0.0001	0.8688
	0.1 μM	0.0006	0.0317	0.0003	0.9963
	1 μM	0.9220	0.4141	0.0001	0.0492
23c	0.001 μM	0.2343	0.0002	0.0030	0.9993
	0.01 μM	0.0406	0.0004	0.0406	0.9767
	0.1 μM	0.0033	0.0174	0.1447	0.9924
Vit E		0.0280	0.0192	0.0001	0.0180



Supplementary Figure 3 | Effect of selected gracilin A derivatives on the mitochondrial membrane permeability transition pore (mPTP). ***a***) SH-SY5Y cells were treated with both 1 mM TBHP and gracilin derivatives and the extent of mPTP opening was evaluated by flow cytometry. Values are presented as percentage of untreated control cells. Statistical significance was determined with one-way ANOVA test, followed by Dunnett´s post hoc test. Treatments with compounds were compared to cells treated only with 1 mM TBHP. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. TBHP control cells were compared to untreated cells. $^{f}p<0.001$. Dotted line indicates the level of cells treated with 1 mM TBHP alone. Values are mean ± SEM of four independent experiments. ***b-e***) Inhibition of CypD and CypA PPIase activity by gracilin A derivatives. Solid line represents inhibition of CypDPPIase activity while dotted lines show inhibition of CypAPPIase activity. ***f***) CsA was used as a positive control. Data are presented as percentage of the maximal PPIase activity. Values are the mean ± SEM of four independent experiments.

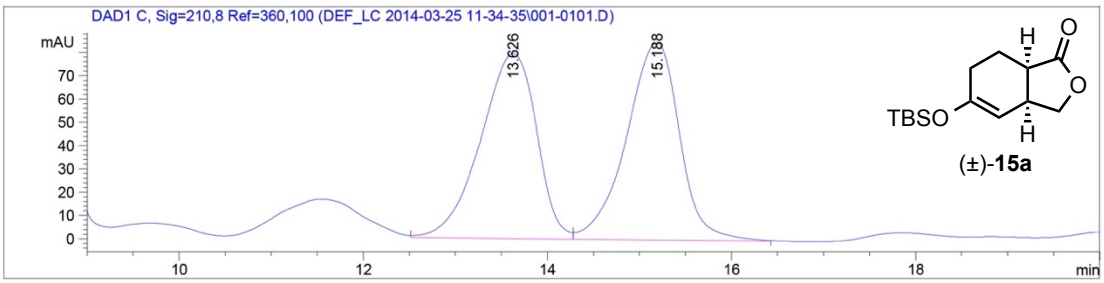
One Way ANOVA: F= 3.62; df= 7, CI: 95%, p= 0.0083
Dunnett's post hoc test: p values for each condition are provided below:

Compound	p value
TBHP	0.00002
0.2 μM CsA	0.0163
0.1 μM **27a**	0.0130
0.01 μM **27b**	0.0060
0.01 μM **29a**	0.0008
0.01 μM **22**	0.0002
0.01 μM **23c**	0.9997

C. Characterization Data

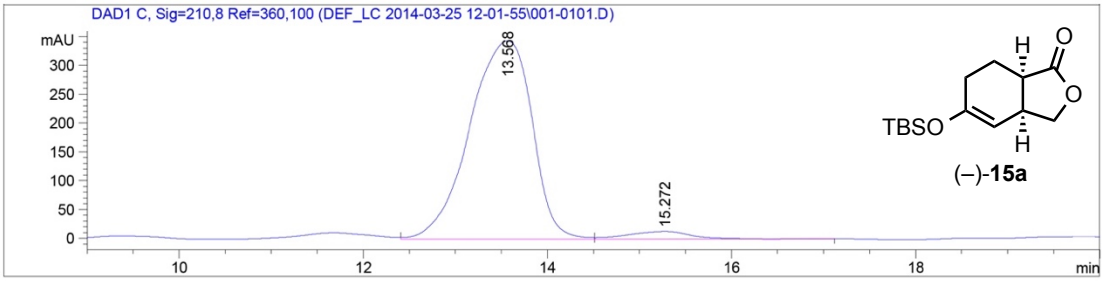
Supplementary Figure 4: Determination of enantiomeric excess of bicyclic γ-lactones (–)-15a and (+)-15a:

Chiral HPLC analysis of bicyclic γ-lactones (–)-15a and (+)-15a: Chiralcel OD-H column: hexanes:^{i}PrOH = 95:05, flow rate 0.5 mL/min, λ = 210 nm: t_{major} = 13.6 min, t_{minor} = 15.1 min; 94% ee.



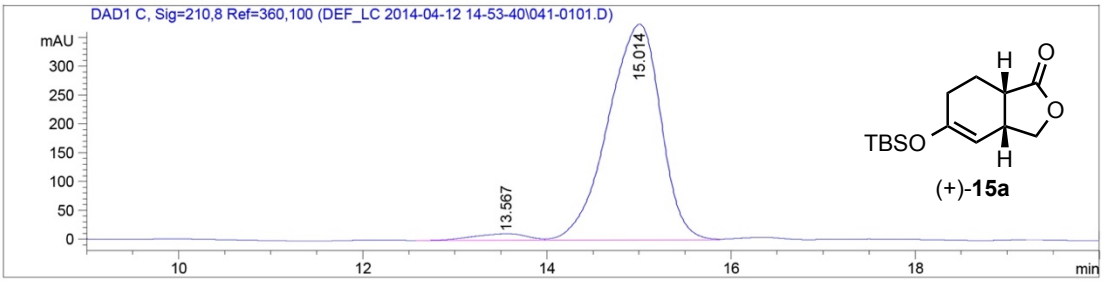
Signal 3: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.626	VV	0.6550	3356.05444	79.41861	49.7000
2	15.188	VB	0.6181	3396.56372	84.65210	50.3000
Totals :				6752.61816	164.07071	



Signal 3: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.568	VV	0.6929	1.61805e4	343.91486	96.4282
2	15.272	VB	0.6360	599.34845	12.90096	3.5718
Totals :				1.67798e4	356.81581	

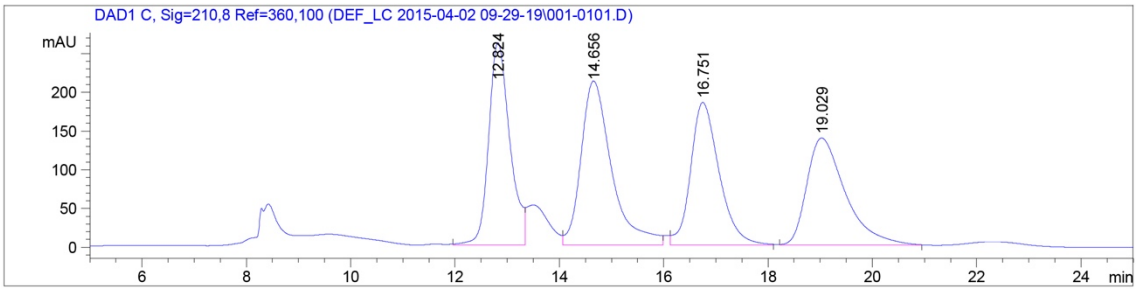


Signal 3: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.567	BV	0.5663	715.34674	17.66867	2.9723
2	15.014	VV	0.6327	2.33520e4	586.27814	97.0277
Totals :				2.40674e4	603.94681	

Supplementary Figure 5. Separation of diastereomeric/regioisomeric bis-acetoxy furanoses (–)-23a, (–)-23b, (–)-23c, and (–)-23d by chiral HPLC:

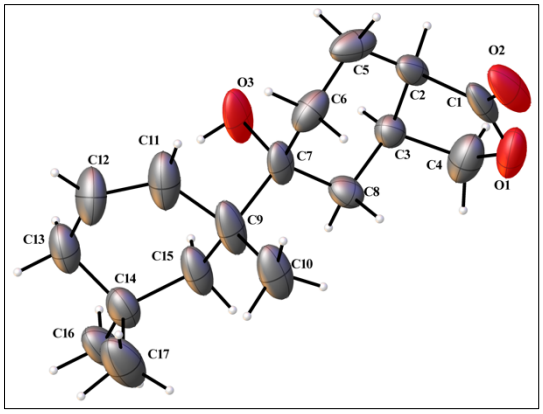
Chiral HPLC settings: Chiralcel OJ-H column: hexanes:^{i}PrOH = 96:04, flow rate 0.4 mL/min, λ = 210 nm: $t_{(-)\text{-}\mathbf{23a}}$ = 11.9 – 13.4 min, $t_{(-)\text{-}\mathbf{23b}}$ = 14.1 – 15.9 min, $t_{(-)\text{-}\mathbf{23c}}$ = 16.2 – 18.1 min, $t_{(-)\text{-}\mathbf{23d}}$ = 18.3 – 20.9 min.



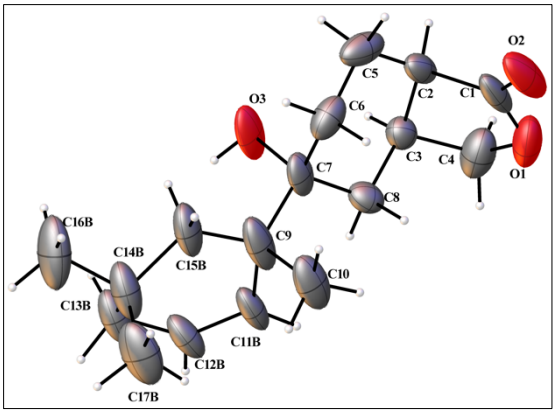
Single crystal X-ray structure and selected crystallographic data for (–)-21b

Supplementary Figure 6. Single crystal X-ray structure (ORTEP) of dimethyl cyclohexenol lactone (–)-21b as a mixture of two diastereomers at C9. The two diastereomers were crystallized together from a concentrated solution of (–)-**21b** in pentane (3.0 mL), using a slow evaporation method (probability ellipsoids are shown at the 50% level). The structures crystallized in chiral space group $P2_12_12_1$. The atoms C11 to C17 have a rotational disorder relative to the rest of the atoms signifying the presence of diastereomers. The Flack and Hooft parameters confirm the absolute structure of both diastereomers.

X-ray crystallographic data have been deposited in the Cambridge Crystallographic Data Centre database (http://www.ccdc.cam.ac.uk/) under accession code CCDC 1557733.



C2 (R), C3 (S), C7 (R), C9 (S).



C2 (R), C3 (S), C7 (R), C9 (R).

Alert level B:

THETM01_ALERT_3_B The value of sine(theta_max)/wavelength is less than 0.575 Calculated sin(theta_max)/wavelength = 0.5665. Author Response: Data was collected on a Bruker GADDS instrument with Cu-source and MWPC (multiwire proportional counter) detector. Under these experimental conditions the maximum angle that can be collected is 120 degrees two-theta.

PLAT089_ALERT_3_B Poor Data / Parameter Ratio (Zmax < 18) 5.54 Note. Author Response: Data was collected on a Bruker GADDS instrument with Cu-source and MWPC (multiwire proportional counter) detector which has geometrical restrictions.

PLAT414_ALERT_2_B Short Intra D-H H-X H3 H15B 1.80 Ang. Author Response: H3 attached to O3 was placed only to satisfy stoichiometry. No efforts were made to disorder this hydrogen atom to account for the disordered group (C11 to C17).

Supplementary Table 2. Crystal data and structure refinement for

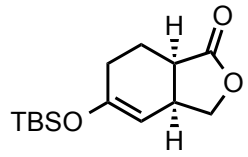
DRB_MA_150217_G_Zn.

Identification code	zincate	
Empirical formula	$C_{17}H_{26}O_3$	
Formula weight	278.38	
Temperature	110.15 K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P212121	
Unit cell dimensions	a = 6.5080(4) Å	α = 90°
	b = 8.1079(5) Å	β = 90°
	c = 28.8743(18) Å	γ = 90°
Volume	1523.59(16) $Å^3$	
Z	4	
Density (calculated)	1.214 Mg/m^3	
Absorption coefficient	0.645 mm^{-1}	
F(000)	608	
Crystal size	0.12 x 0.08 x 0.01 mm^3	
Theta range for data collection	3.061 to 60.857°	
Index ranges	-7≤h≤7, -9≤k≤9, -32≤l≤32	
Reflections collected	35266	
Independent reflections	2318 [R(int) = 0.0500]	
Completeness to theta = 67.679°	85.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7401 and 0.6483	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2318 / 318 / 251	
Goodness-of-fit on F^2	1.108	
Final R indices [I>2sigma(I)]	R_1 = 0.0486, wR_2 = 0.1123	
R indices (all data)	R_1 = 0.0553, wR_2 = 0.1175	
Absolute structure parameter	0.08(10)	
Extinction coefficient	N/A	
Largest diff. peak and hole	0.211 and -0.190 $e.Å^{-3}$	

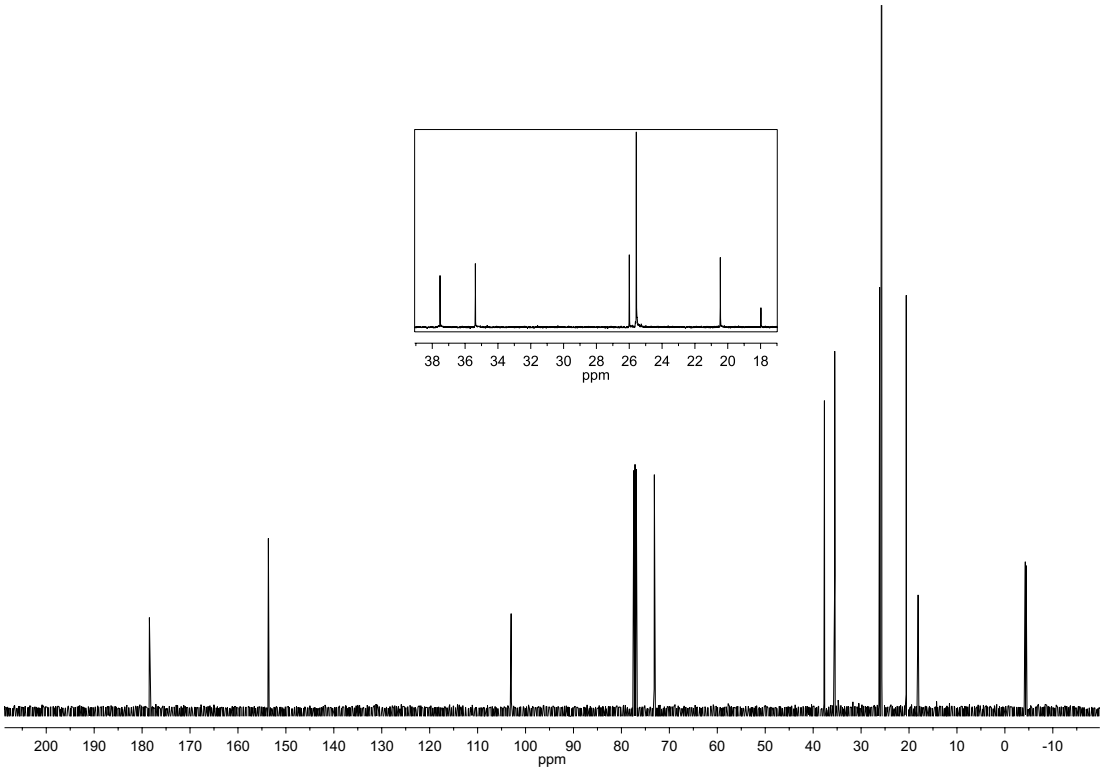
Supplementary Table 3. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for DRB_MA_150217_G_Zn. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	U(eq)
O(1)	8402(6)	7344(4)	2718(1)	72(1)
O(2)	5902(6)	8469(3)	3140(1)	81(1)
O(3)	5800(6)	1915(3)	3272(1)	71(1)
C(1)	6563(8)	7315(4)	2926(2)	57(1)
C(2)	5542(5)	5652(4)	2849(1)	43(1)
C(3)	7407(5)	4566(4)	2752(1)	39(1)
C(4)	8774(8)	5767(5)	2494(2)	69(1)
C(5)	4123(6)	5067(5)	3229(1)	58(1)
C(6)	5283(6)	4445(5)	3655(1)	53(1)
C(7)	6907(6)	3164(4)	3527(1)	46(1)
C(8)	8405(5)	3978(4)	3199(1)	41(1)
C(9)	8022(8)	2389(4)	3962(1)	65(1)
C(10)	8993(9)	3775(4)	4262(1)	69(1)
C(11)	6040(30)	1617(17)	4268(6)	75(4)
C(12)	6080(20)	5(10)	4413(3)	70(3)
C(13)	7741(18)	-1211(12)	4275(4)	62(2)
C(14)	9747(14)	-301(12)	4186(3)	54(2)
C(15)	9360(20)	1082(14)	3847(5)	60(3)
C(16)	11174(15)	-1516(10)	3942(3)	65(3)
C(17)	10660(20)	247(13)	4647(4)	89(4)
C(11B)	10130(20)	1397(14)	3753(5)	52(3)
C(12B)	10446(15)	-236(9)	3823(3)	55(2)
C(13B)	9080(20)	-1272(13)	4098(3)	64(3)
C(14B)	7900(20)	-238(12)	4464(3)	69(3)
C(15B)	6850(20)	1281(15)	4230(6)	63(3)
C(16B)	6130(20)	-1263(12)	4670(3)	99(4)
C(17B)	9210(20)	260(14)	4859(3)	89(4)

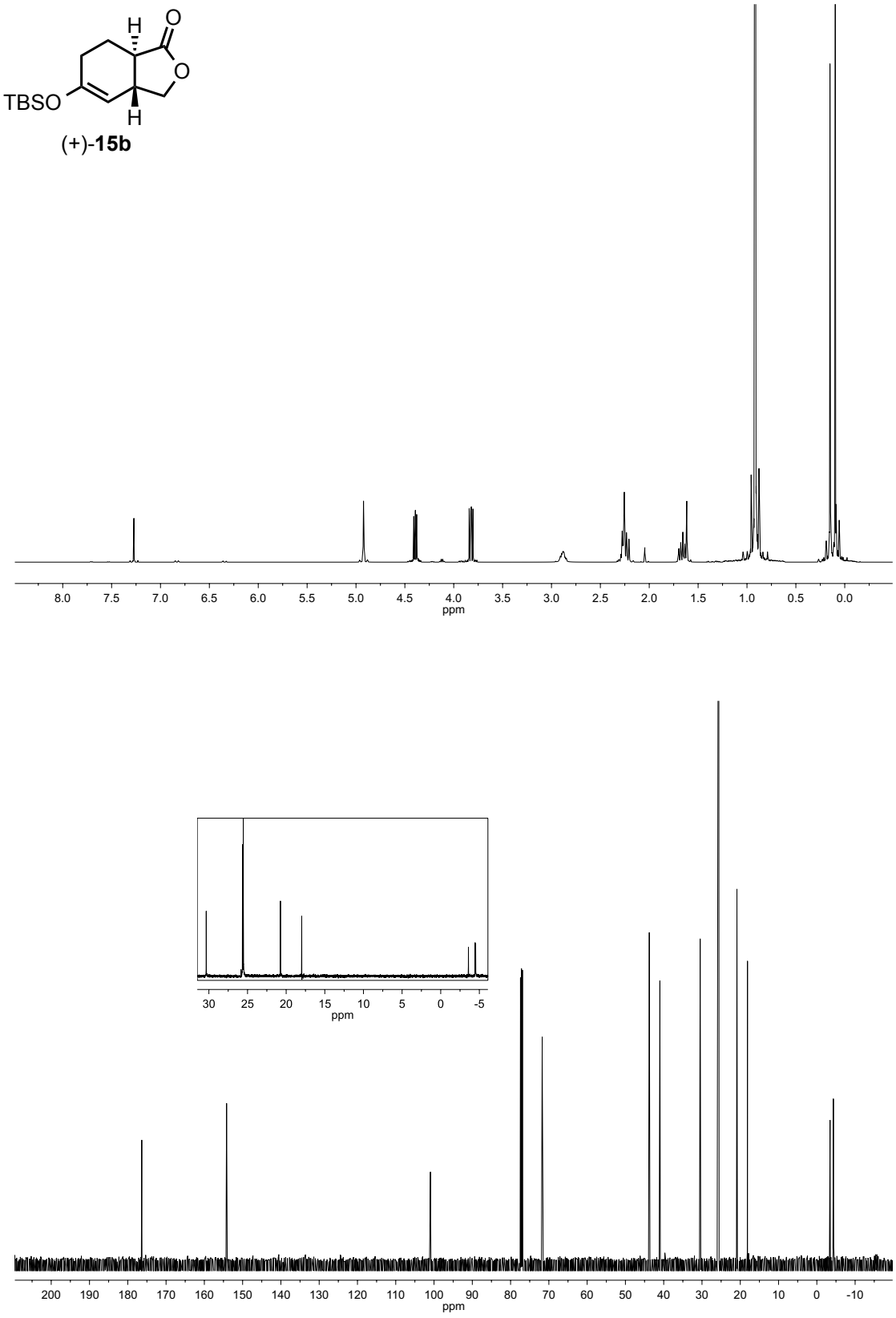
Supplementary Figure 7. ^{1}H and ^{13}C NMR spectra



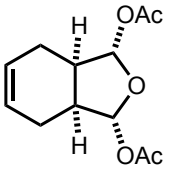
(–)-**15a**



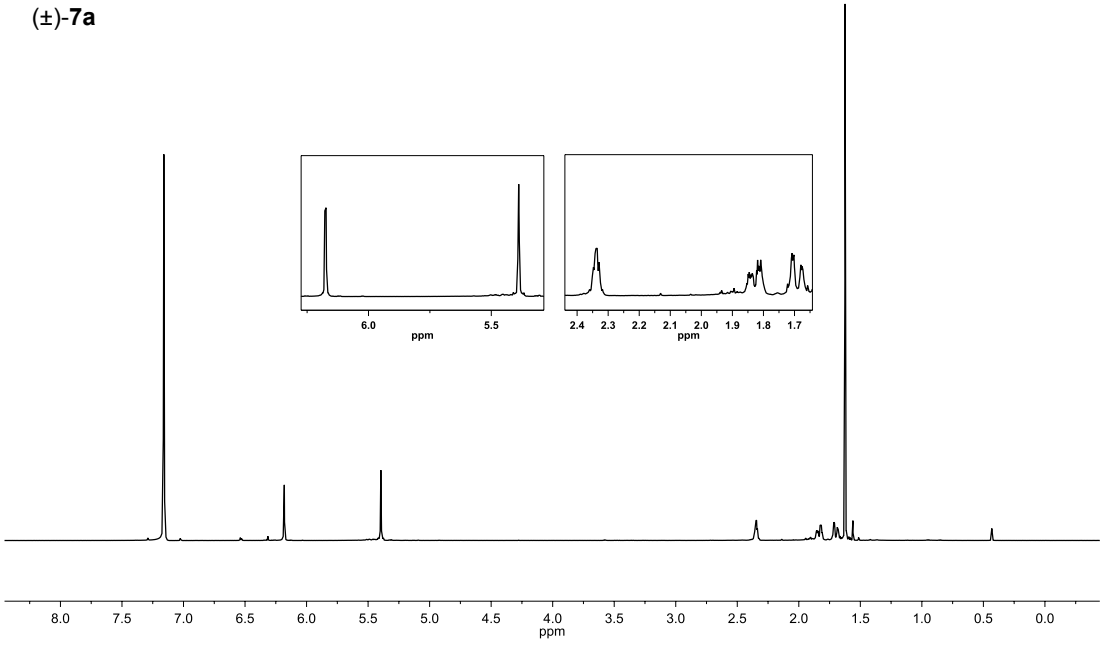
^{1}H (400 MHz) and ^{13}C NMR (125 MHz) spectra of lactone (–)-**15a** in $CDCl_3$

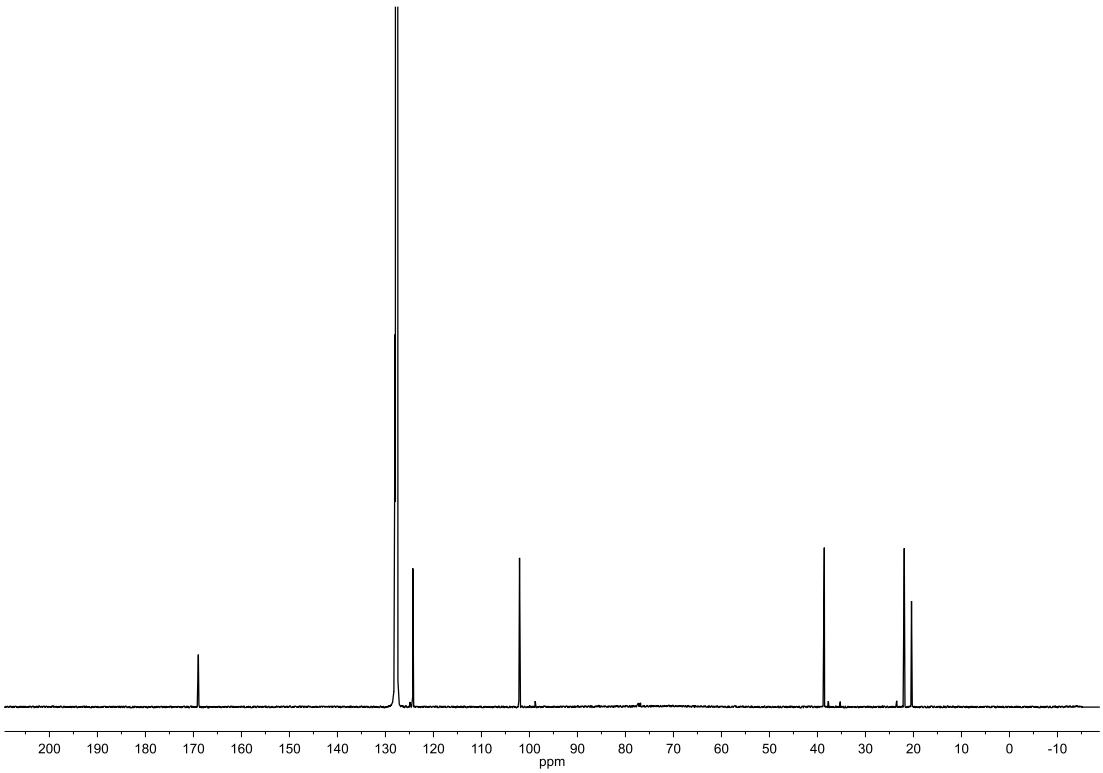


^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of lactone (+)-**15b** in $CDCl_3$

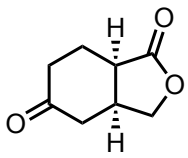


(±)-**7a**

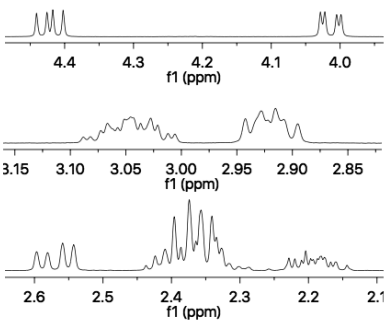


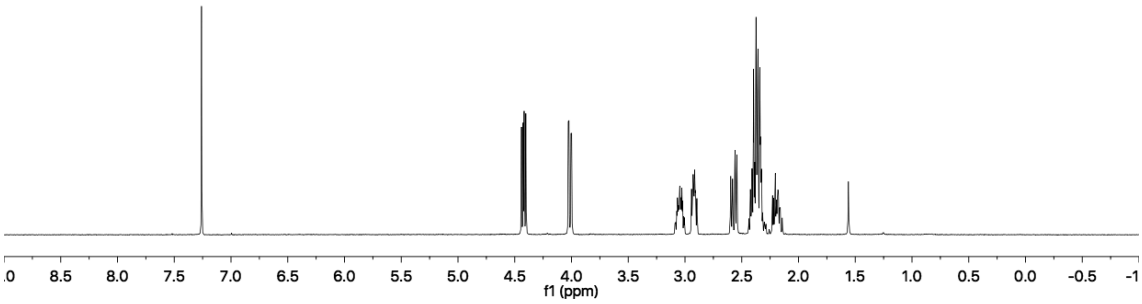


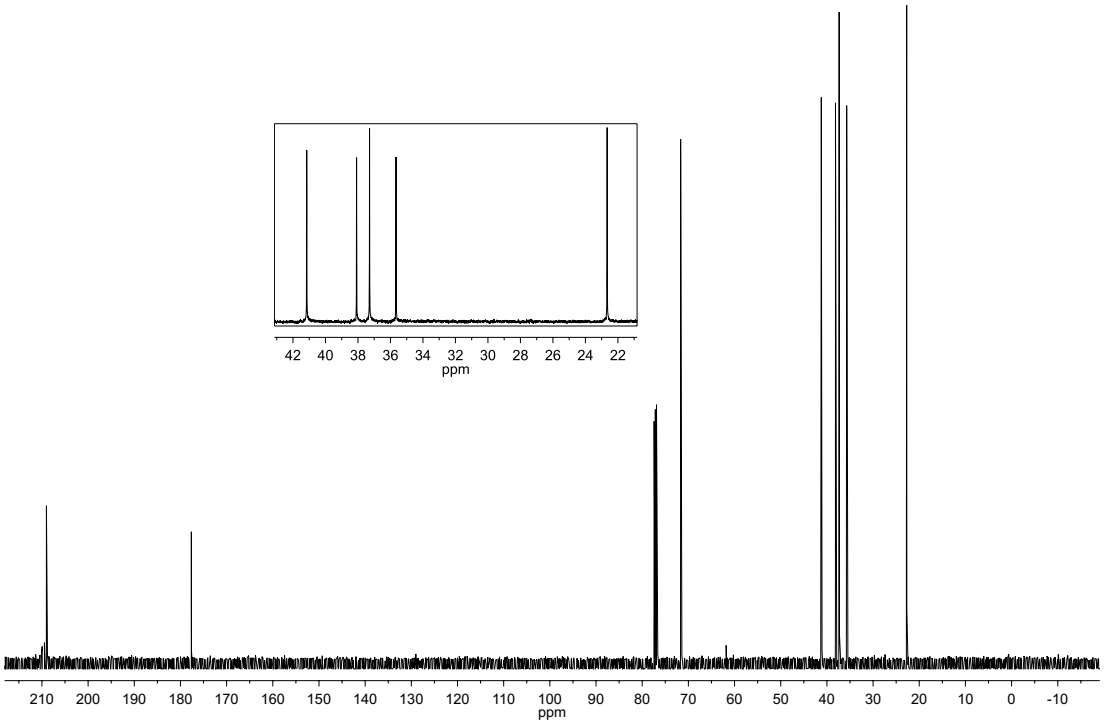
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of lactone (±)-**7a** in C_6D_6



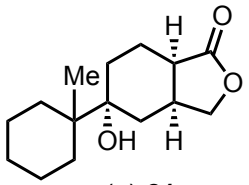
(–)-**9**





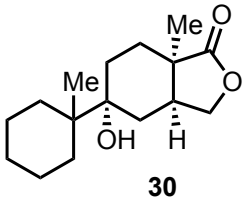


^{1}H (400 MHz) and ^{13}C NMR (125 MHz) spectra of ketolactone (–)-**9** in $CDCl_3$

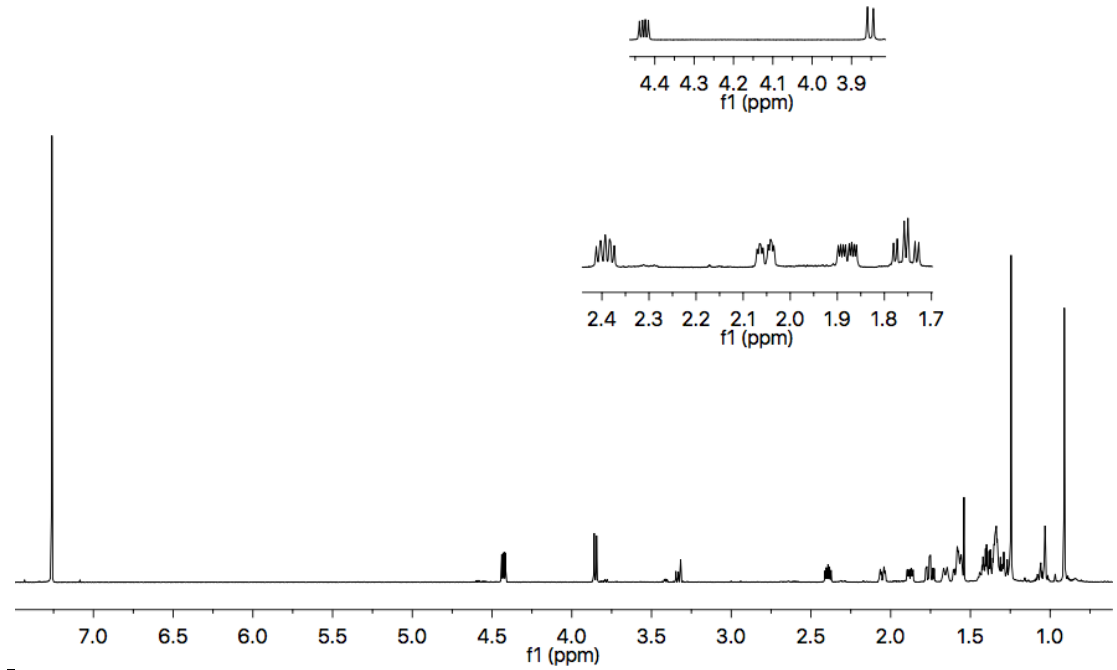


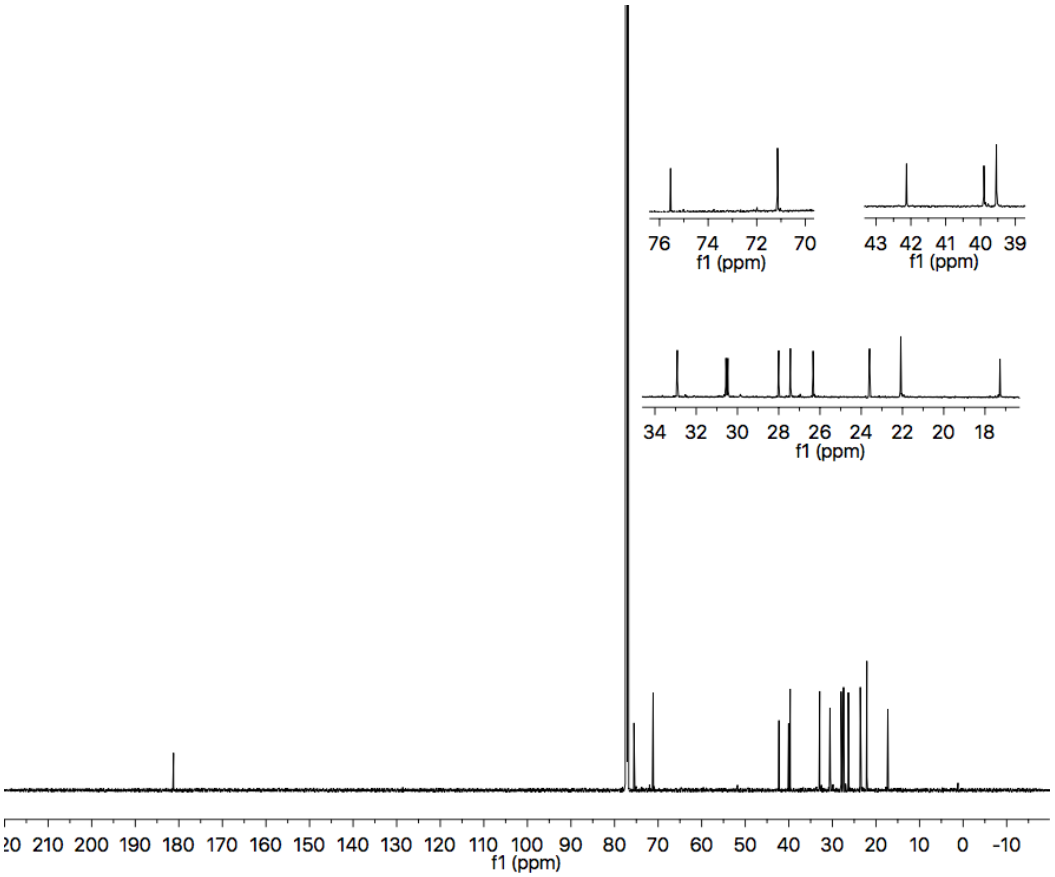
(–)-**21a**

^{1}H (500 MHz) and ^{13}C NMR (150 MHz) spectra of cyclohexanol lactone (-)-**21a** in $CDCl_3$

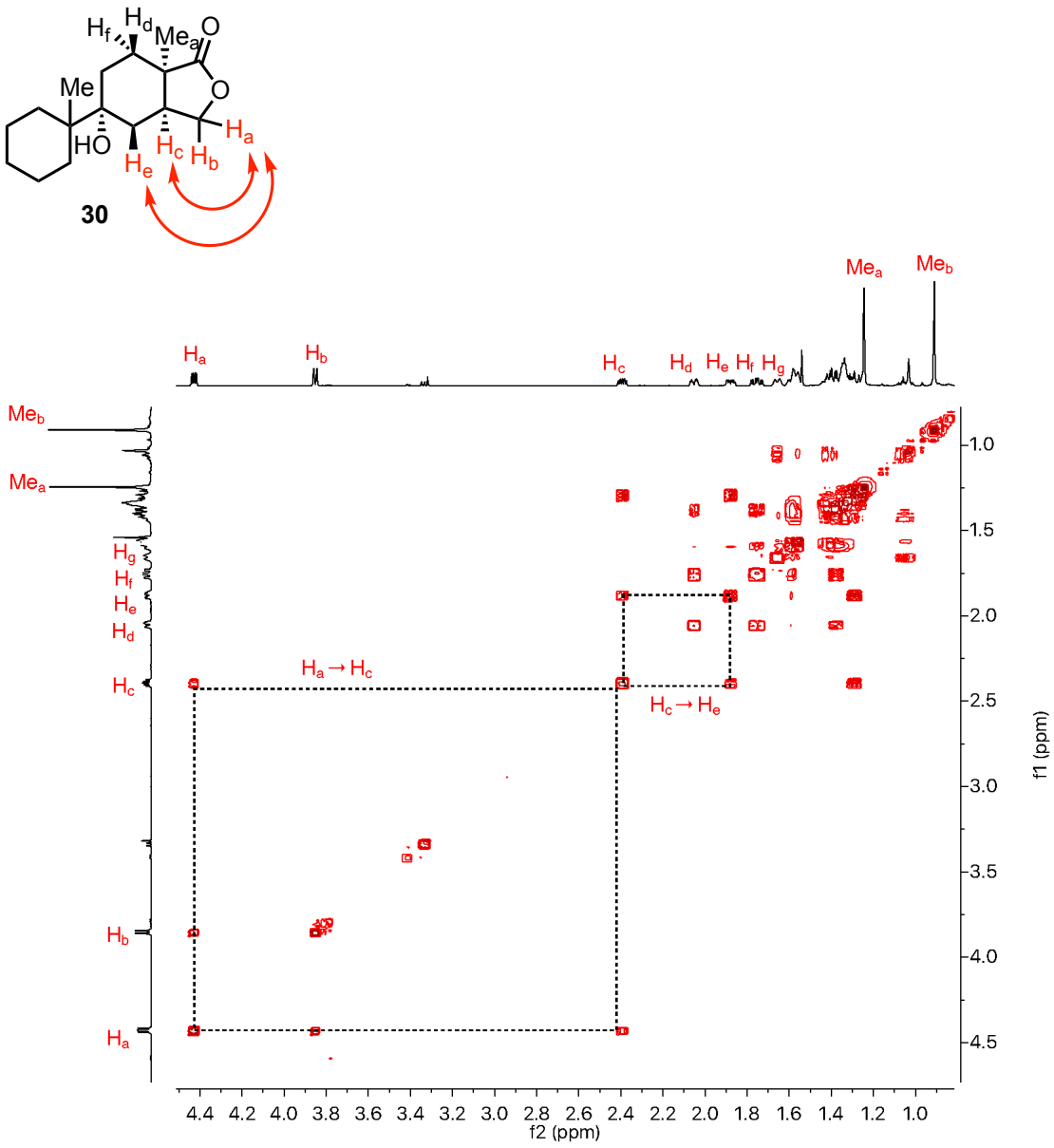


30

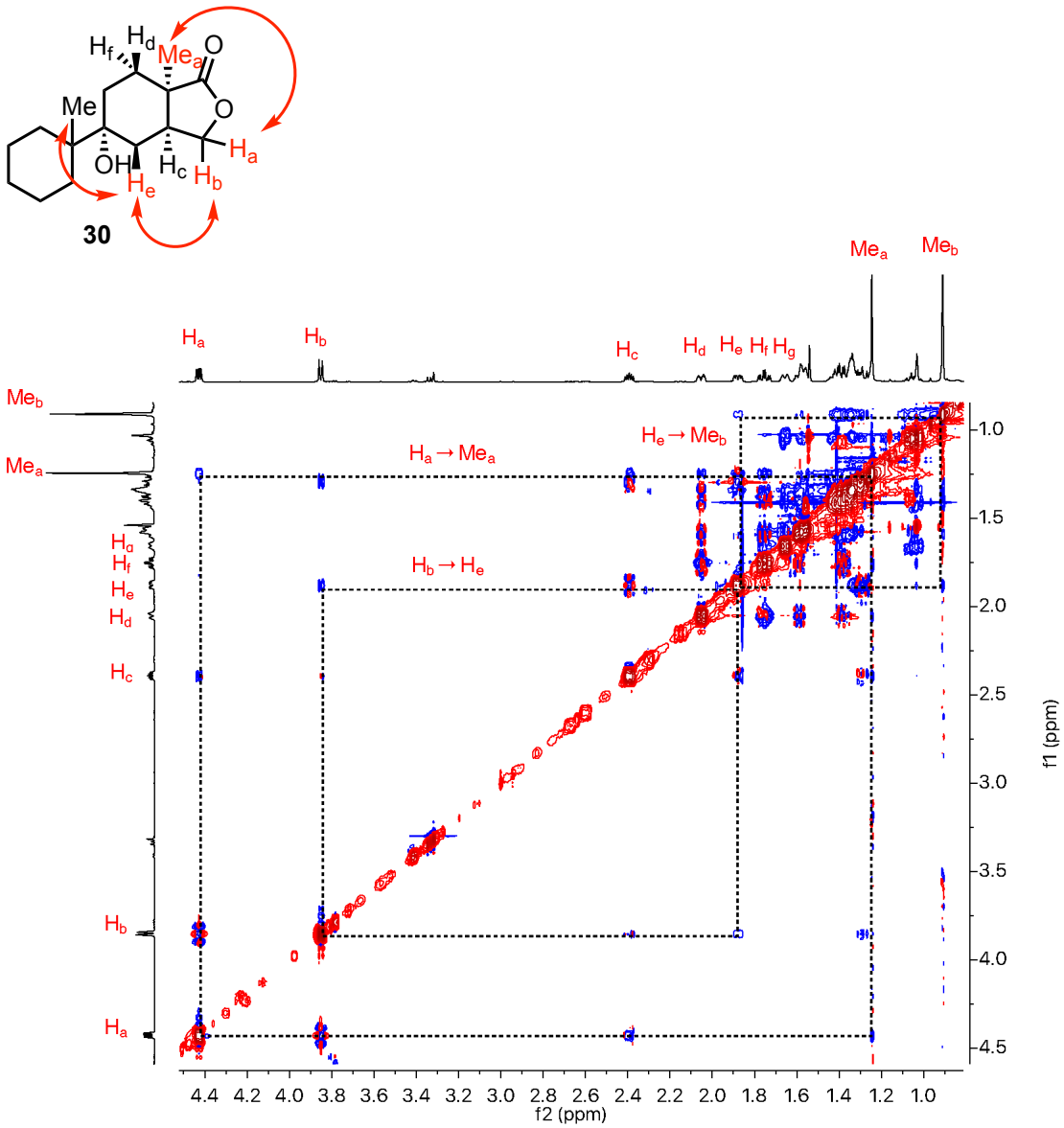




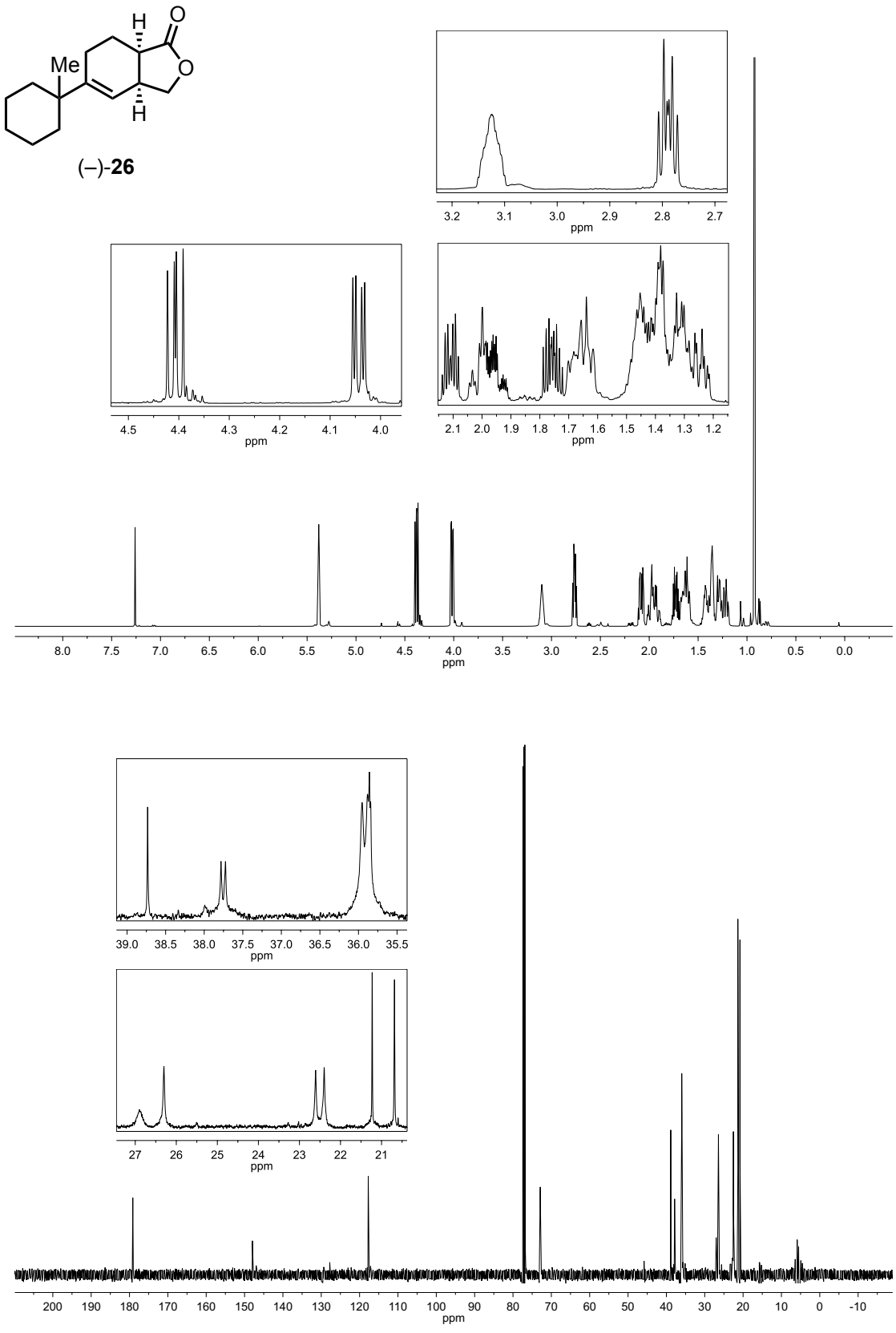
^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra of cyclohexanol lactone (–)-**30** in $CDCl_3$



2D ^{1}H–^{1}H gCOSY NMR spectrum (600 MHz) of cyclohexanol lactone (–)-**30** in $CDCl_3$

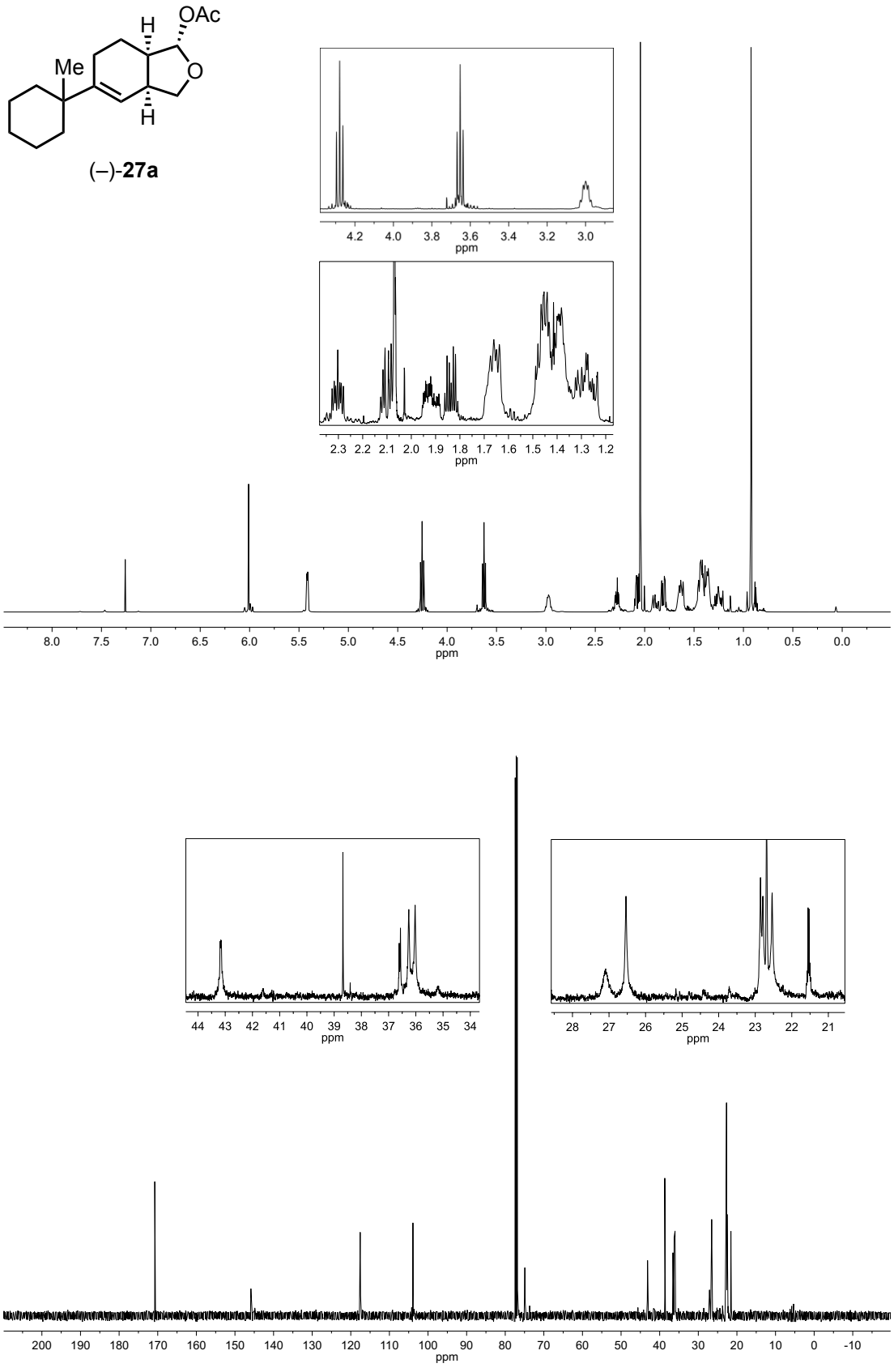


2D ^{1}H–^{1}H NOESY NMR spectrum (600 MHz) of cyclohexanol lactone (–)-**30** in $CDCl_3$

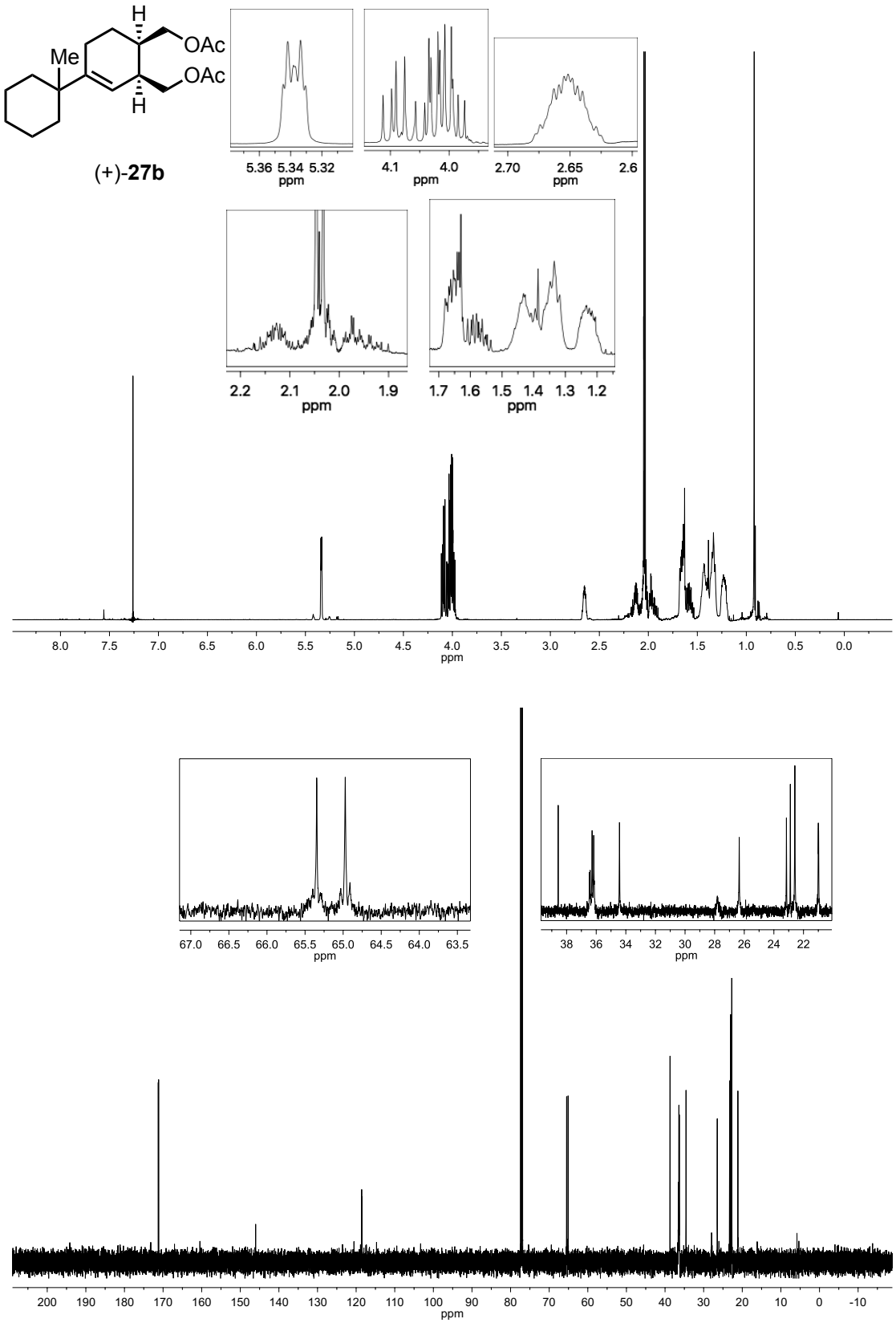


(–)-**26**

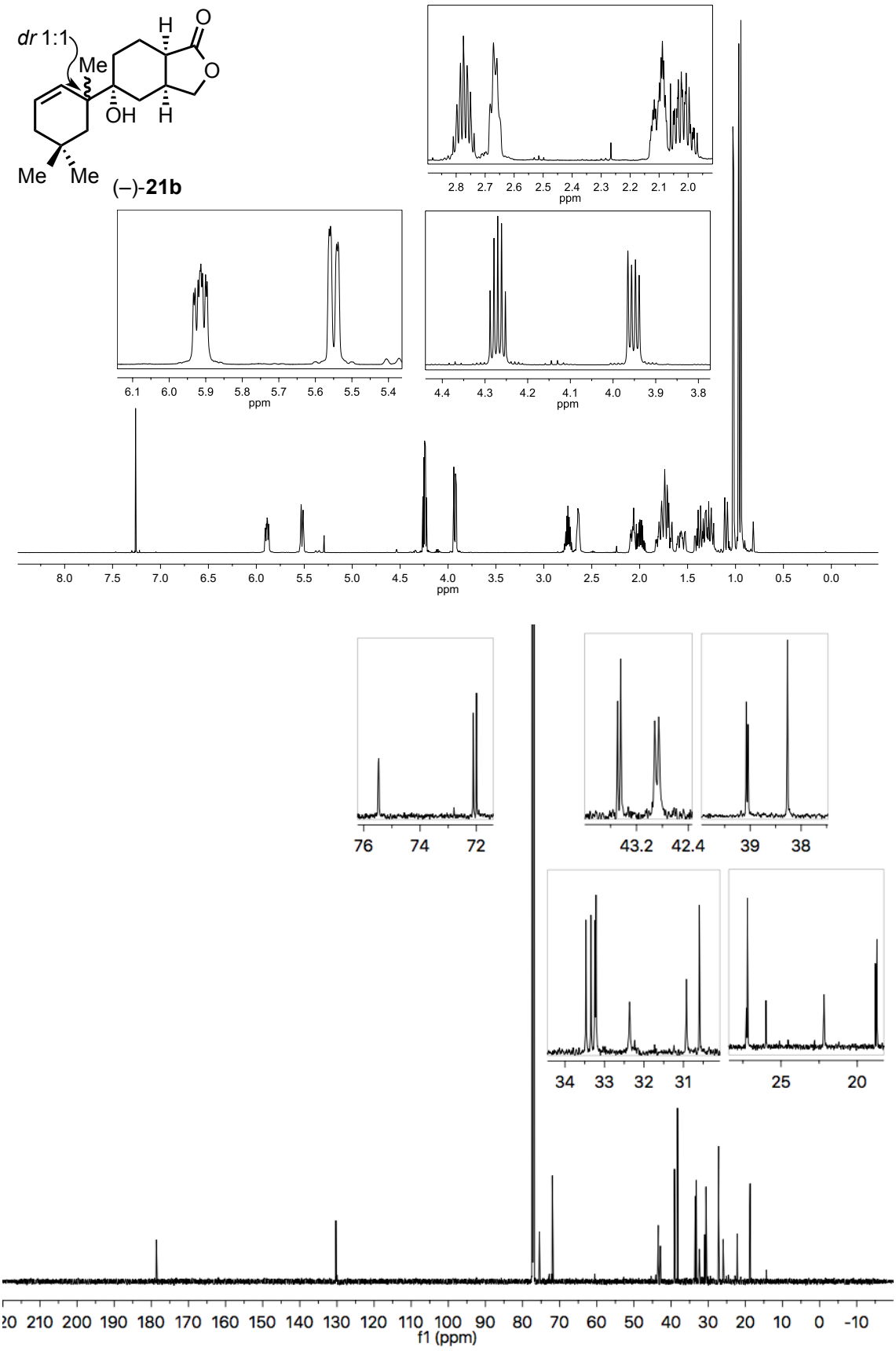
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexene lactone (–)-**26** in $CDCl_3$



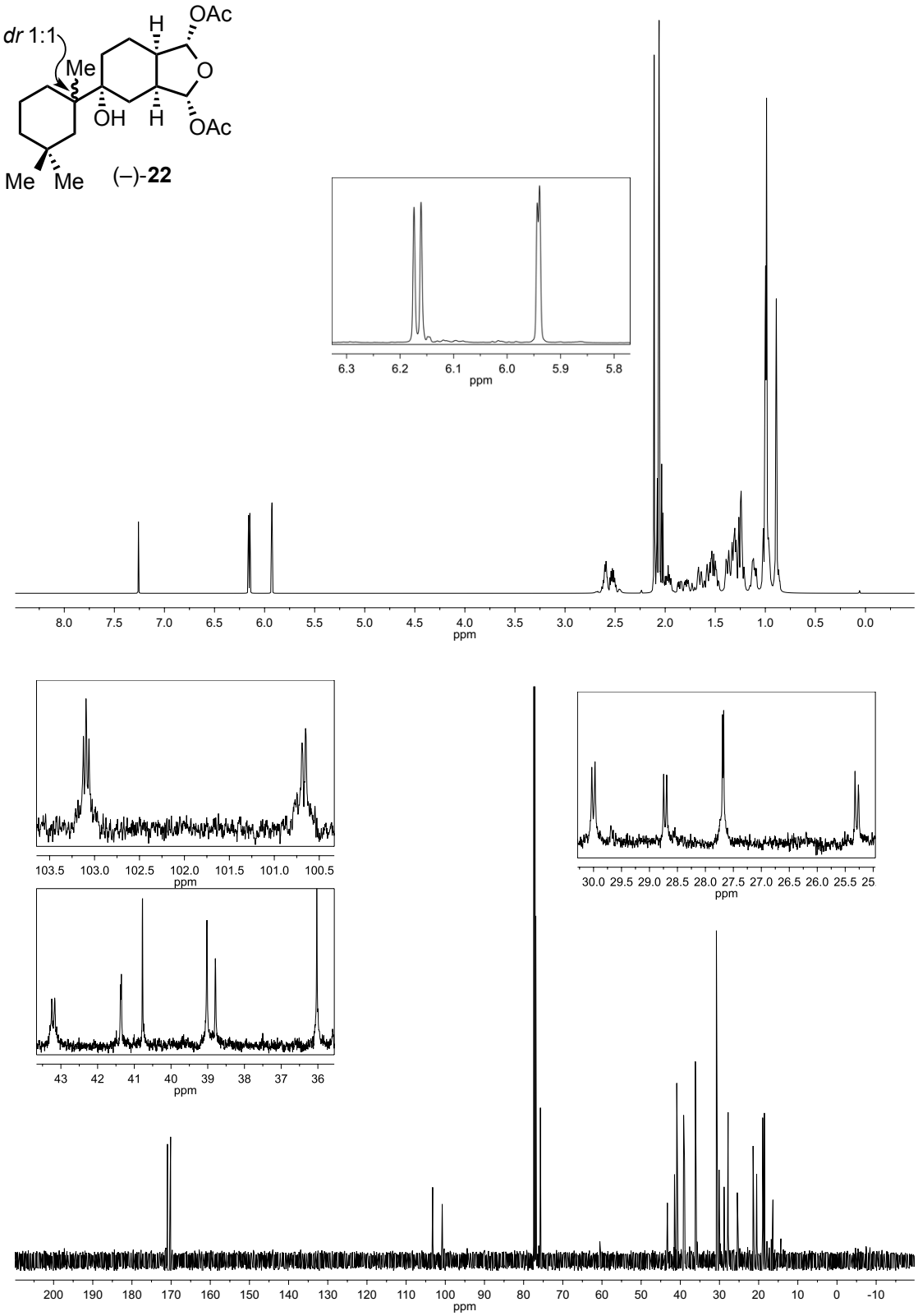
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of acetyl lactol (–)-**27a** in $CDCl_3$



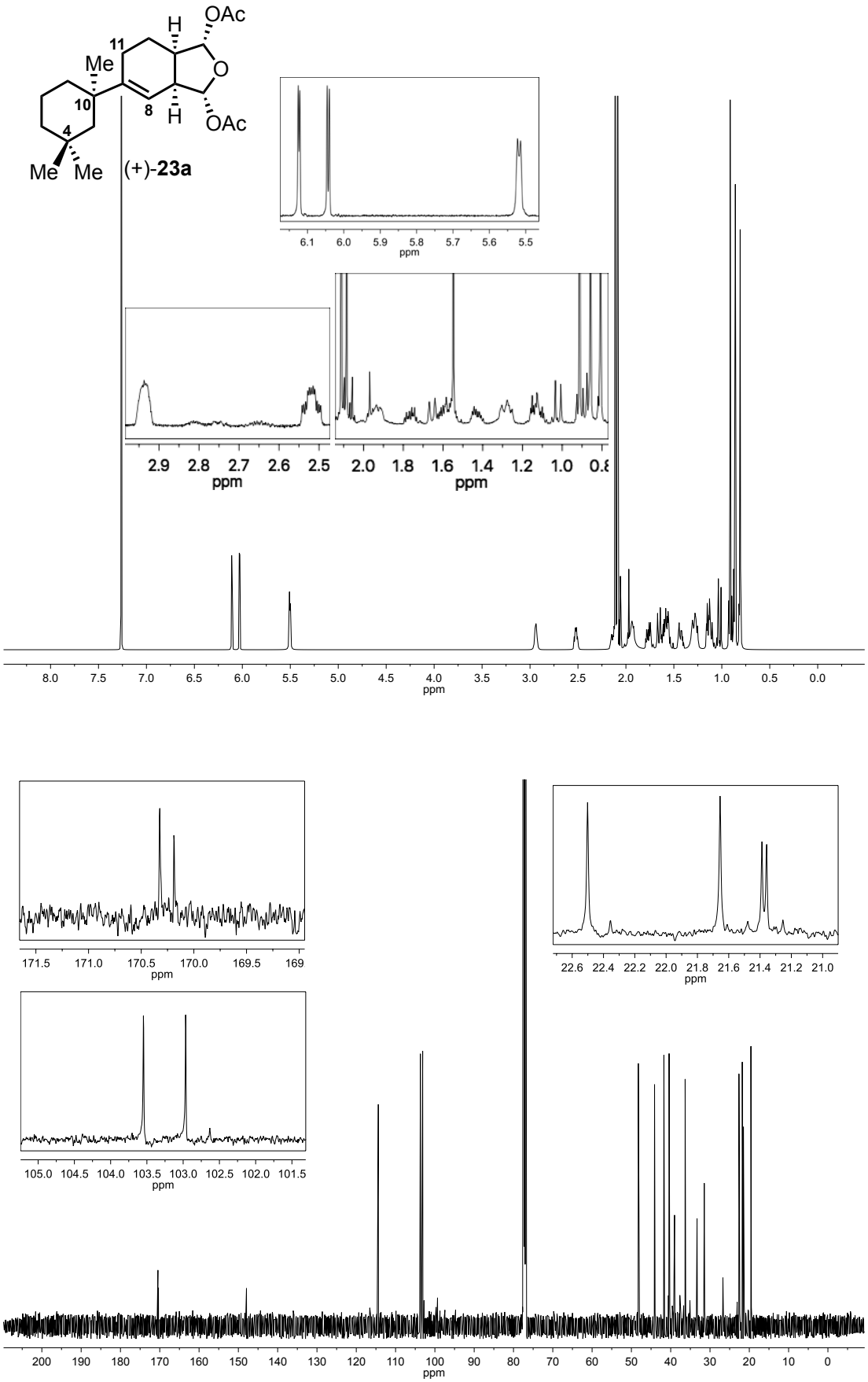
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexene diacetate (+)-**27b** in $CDCl_3$



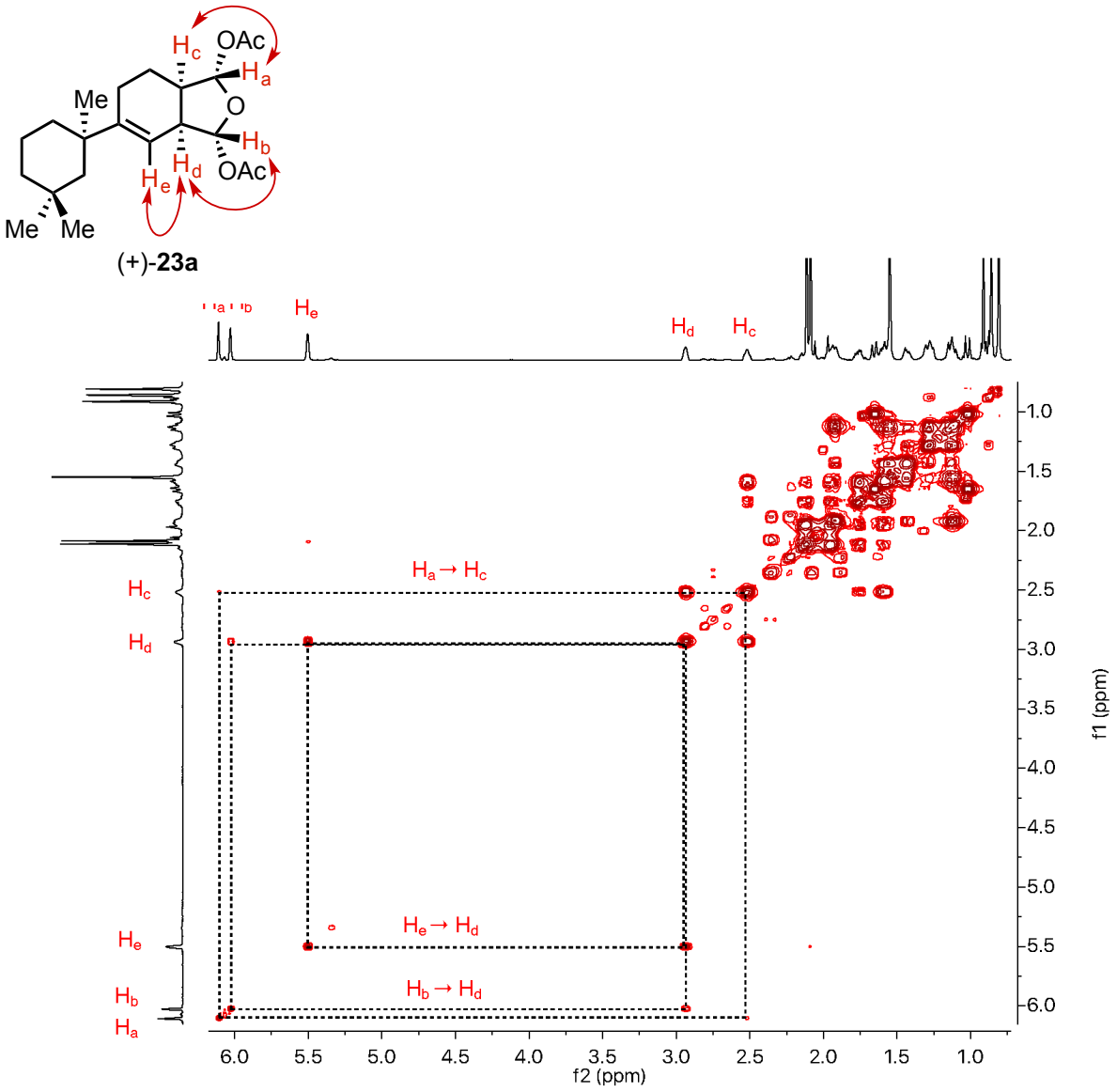
^{1}H (500 MHz) and ^{13}C NMR (150 MHz) spectra of acetyl lactol (–)-**21b** in $CDCl_3$



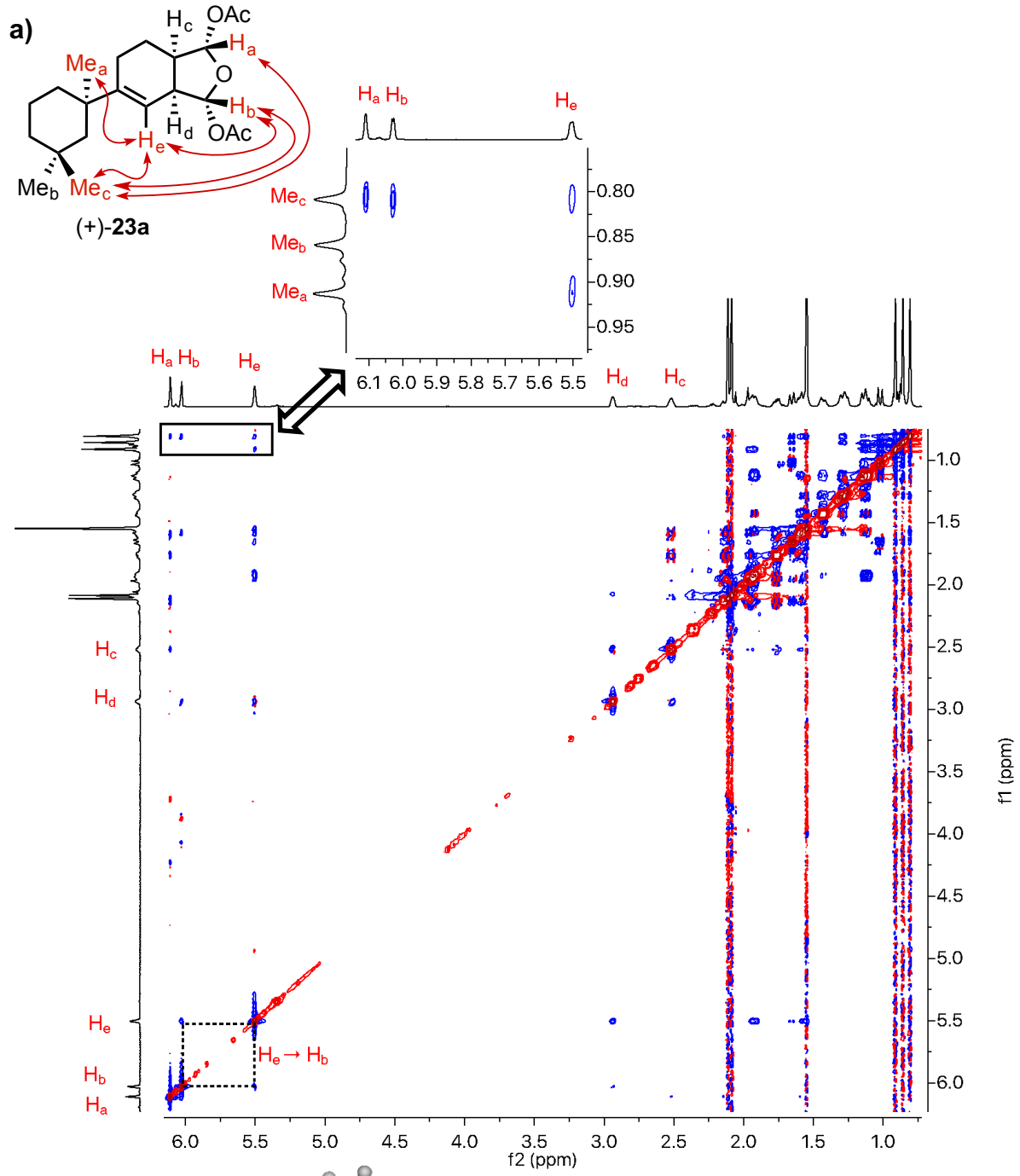
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexanol diacetate (–)-**22** in $CDCl_3$



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexenyl diacetate (+)-**23a** in $CDCl_3$

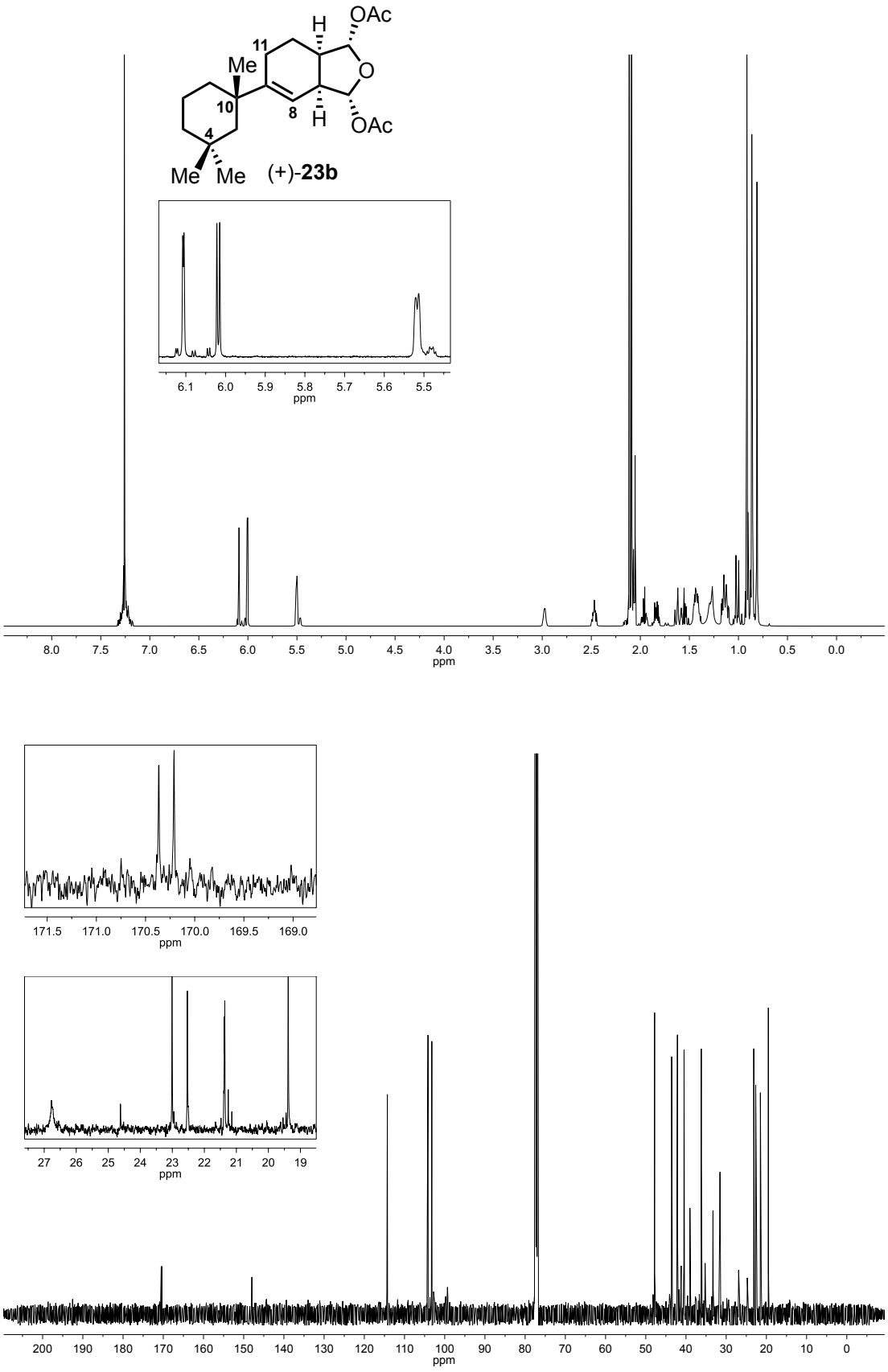


2D ^{1}H–^{1}H gCOSY NMR spectrum (500 MHz) of cyclohexenyl diacetate (+)-**23a** in $CDCl_3$

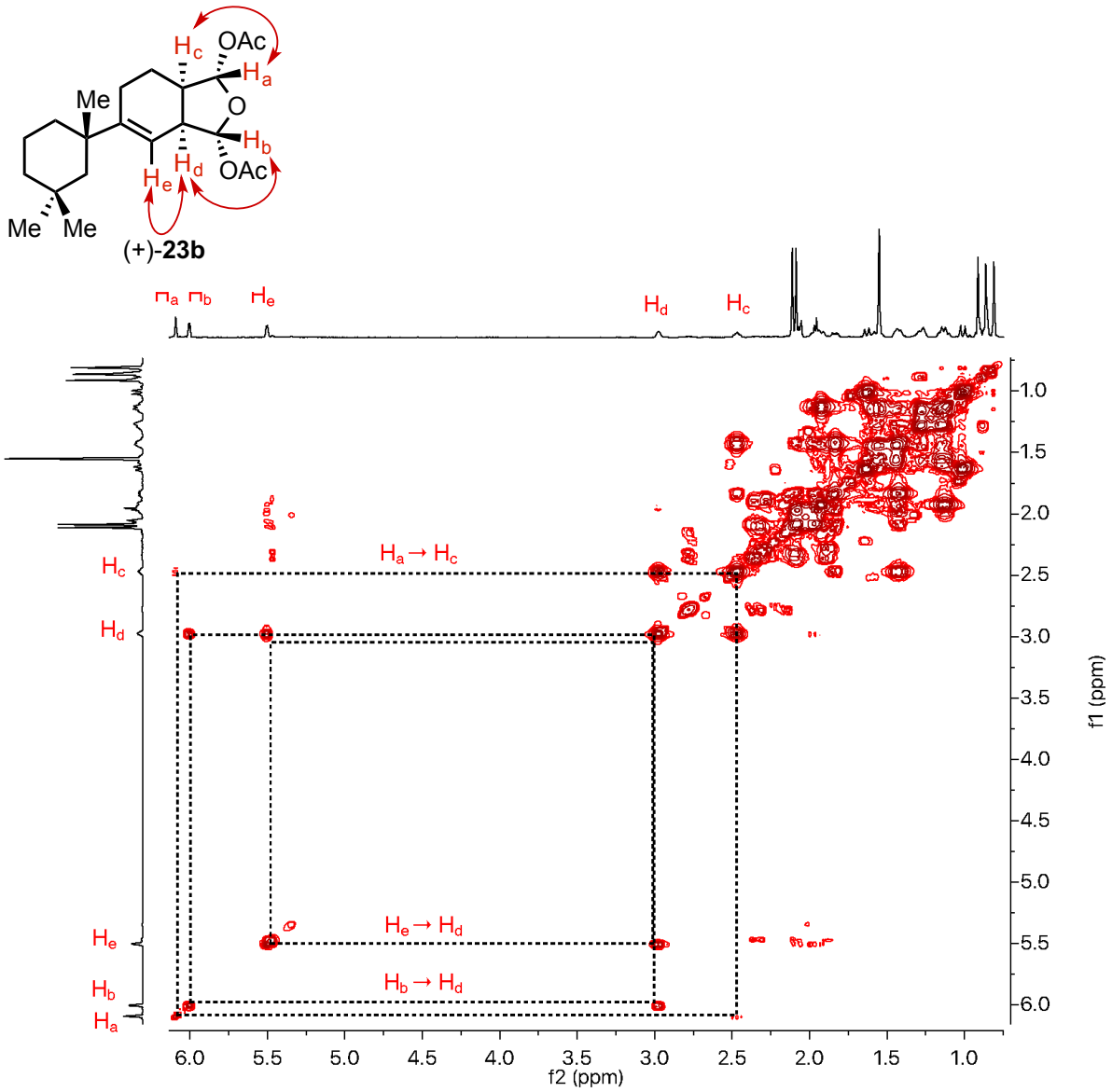


(a) 2D ^{1}H–^{1}H NOESY NMR spectrum (500 MHz) of cyclohexenyl diacetate (+)-**23a** in $CDCl_3$; The upper left panel shows the observed NOE interactions as red arrows; **(b)** Calculated global minimum conformation of (+)-**23a** using stochastic search methodology in the Molecular Operating Environment software.[1] Selected bond distances are shown in Angstroms (Å).

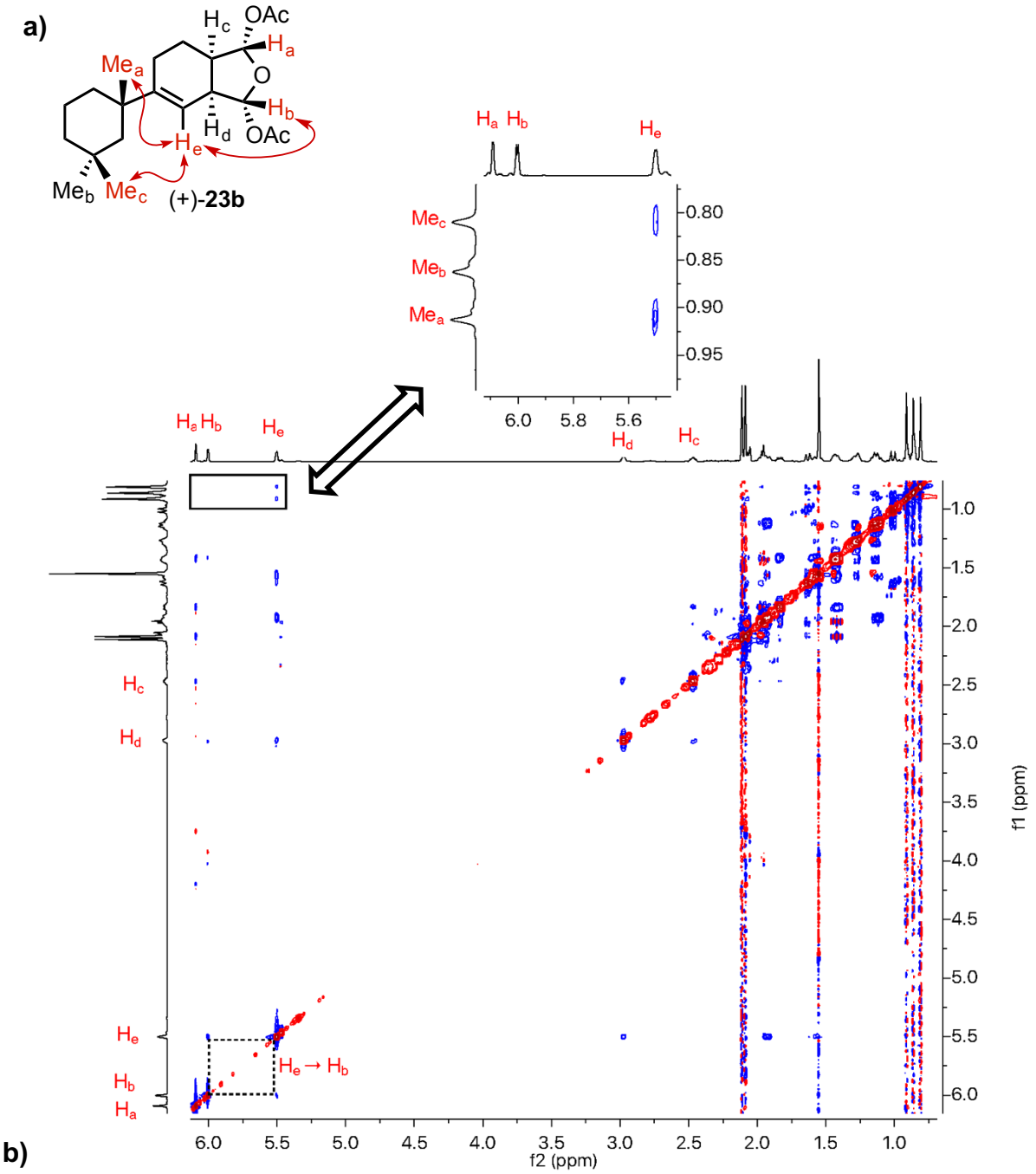
[1] *Molecular Operating Environment (MOE)* 2013.08; Chemical Computing Group, Inc., Montreal, QC, Canada, **2017**



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexenyl diacetate (+)-**23b** in $CDCl_3$

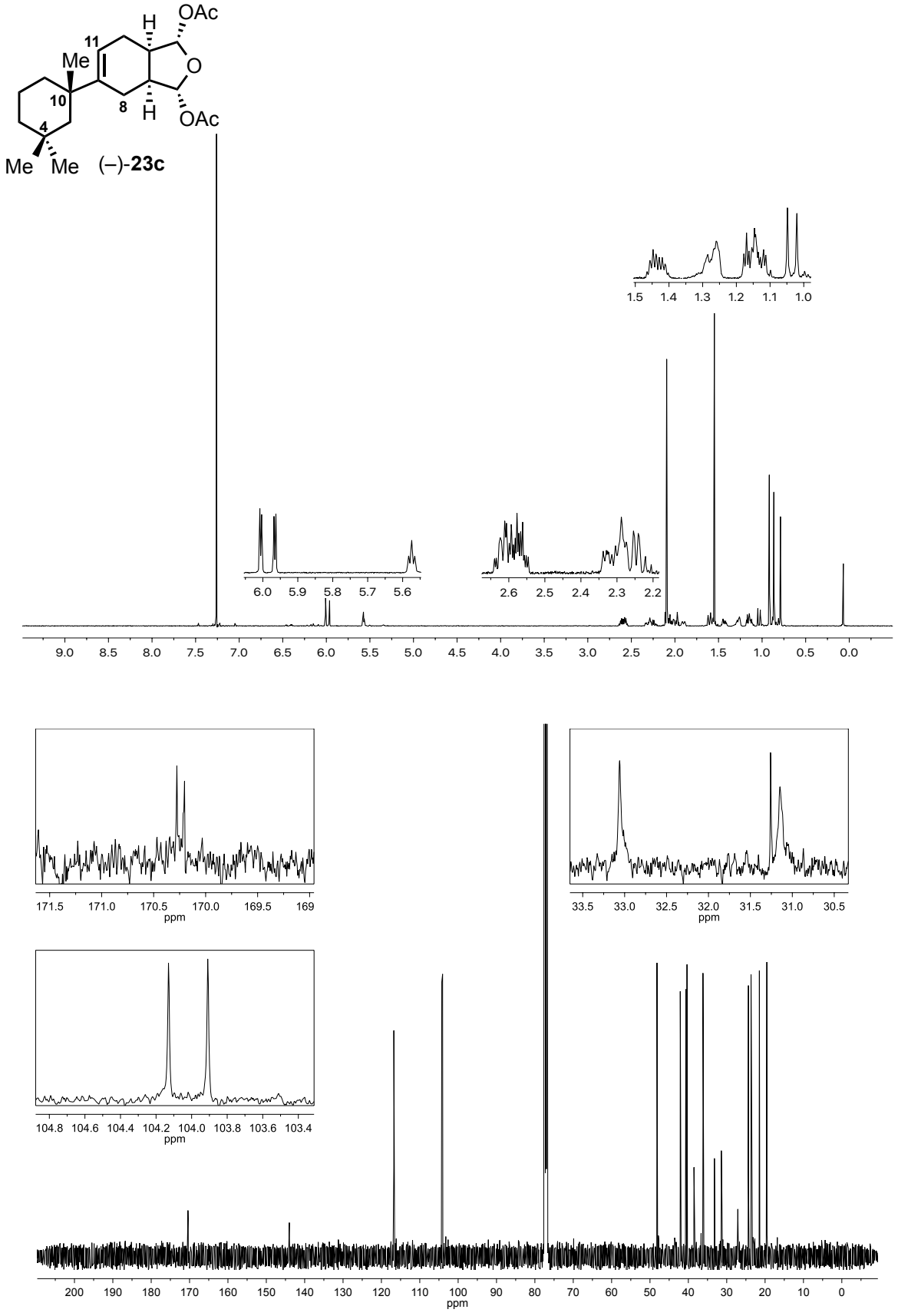


2D ^{1}H–^{1}H gCOSY NMR spectrum (500 MHz) of cyclohexenyl diacetate (+)-**23b** in $CDCl_3$

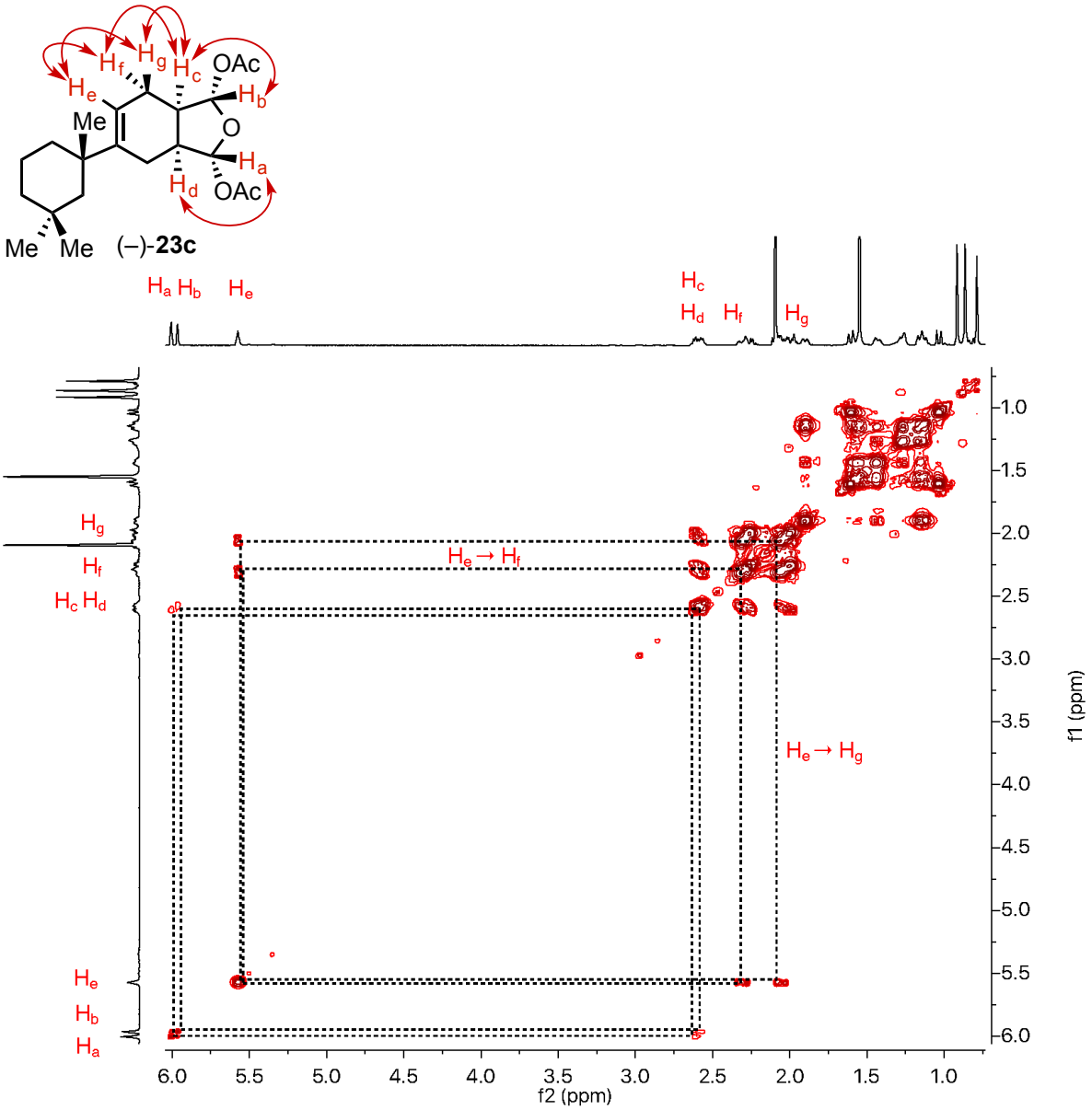


(a) 2D ^{1}H–^{1}H NOESY NMR spectrum (500 MHz) of cyclohexenyl diacetate (+)-**23b** in $CDCl_3$; The upper left panel shows the observed NOE interactions as red arrows; **(b)** Calculated global minimum conformation of (+)-**23b** using stochastic search methodology in the Molecular Operating Environment software.[1] Selected bond distances are shown in Angstroms (Å).

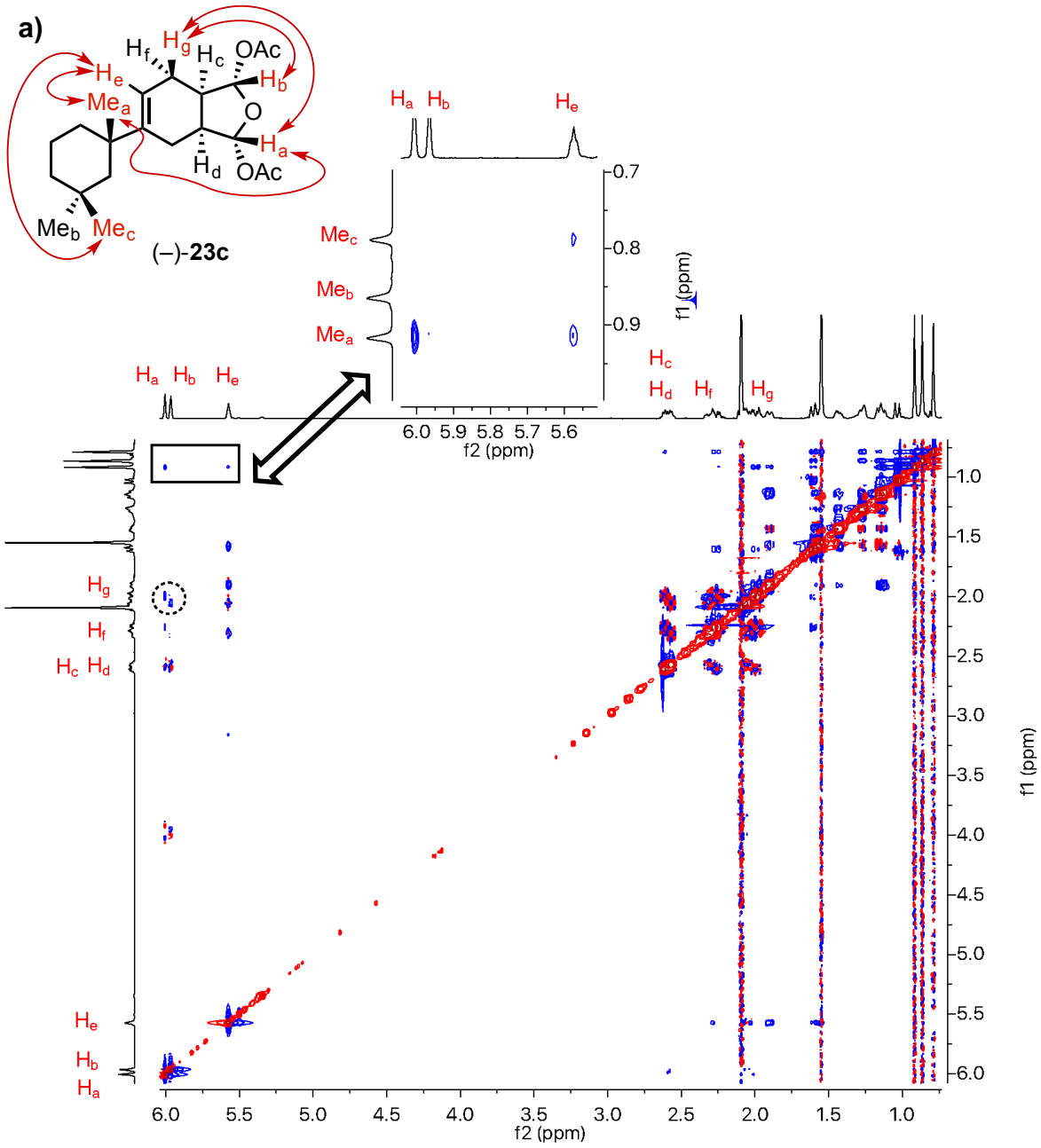
[1]*Molecular Operating Environment (MOE)* 2013.08; Chemical Computing Group, Inc., Montreal, QC, Canada, **2017**



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexenyl diacetate (–)-**23c** in $CDCl_3$

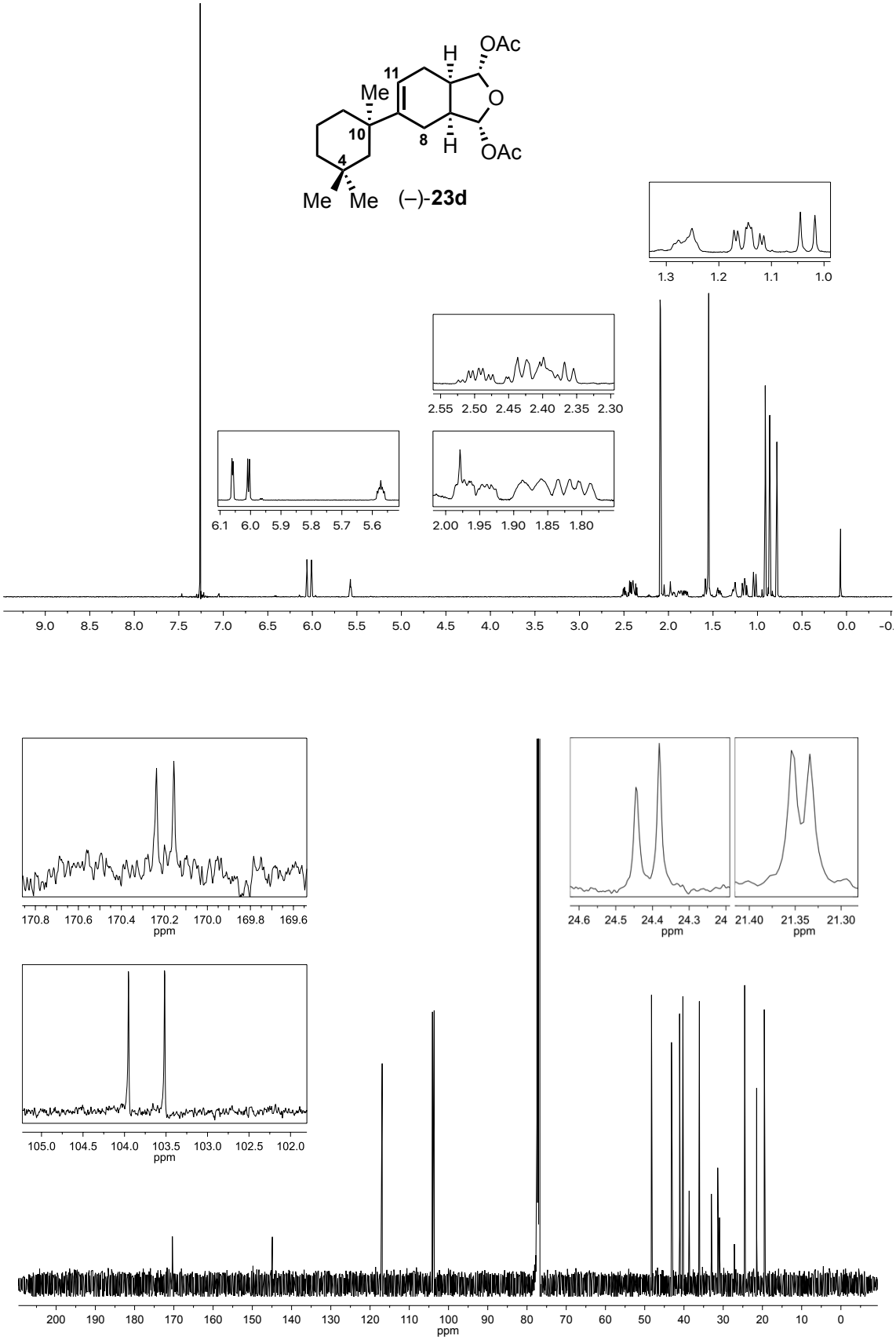


2D ^{1}H–^{1}H gCOSY NMR spectrum (500 MHz) of cyclohexenyl diacetate (–)-**23c** in $CDCl_3$

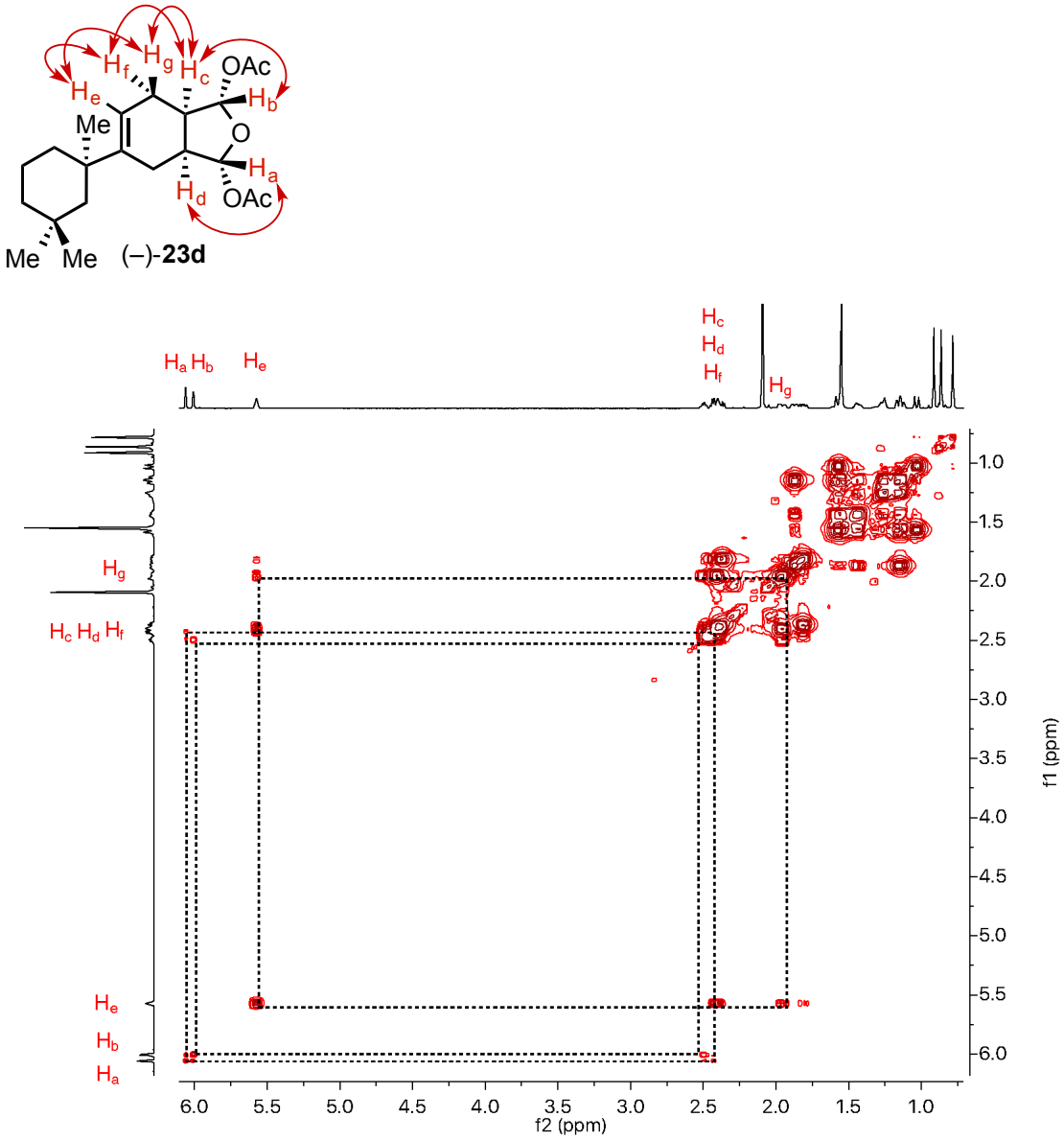


(a) 2D ^{1}H–^{1}H NOESY NMR spectrum (500 MHz) of cyclohexenyl diacetate (–)-**23c** in $CDCl_3$; The upper left panel shows the observed NOE interactions as red arrows; **(b)** Calculated global minimum conformation of (–)-**23c** using stochastic search methodology in the Molecular Operating Environment software.[1] Selected bond distances are shown in Angstroms (Å).

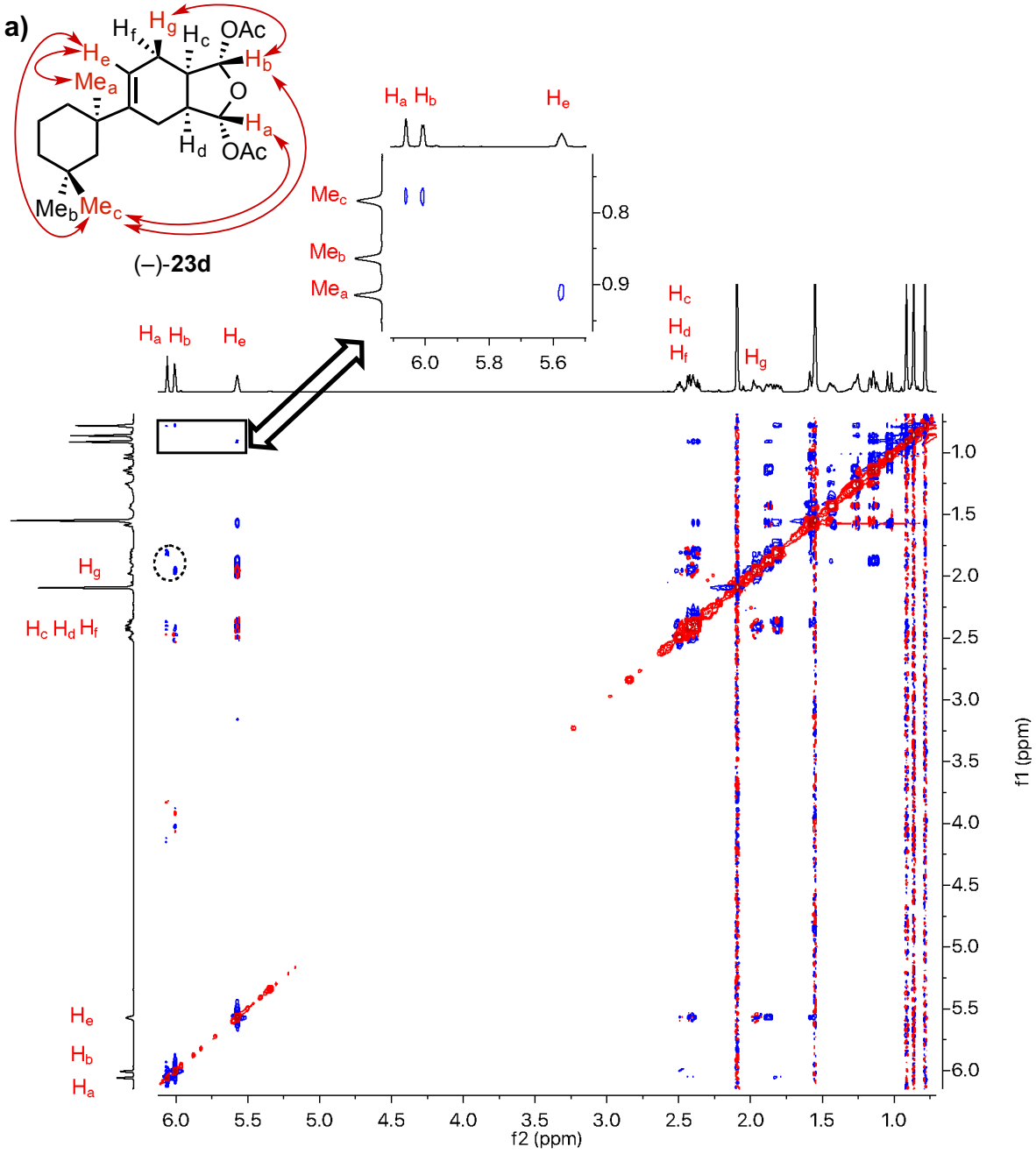
[1]*Molecular Operating Environment (MOE)* 2013.08; Chemical Computing Group, Inc., Montreal, QC, Canada, **2017**



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexenyl diacetate (–)-**23d** in $CDCl_3$

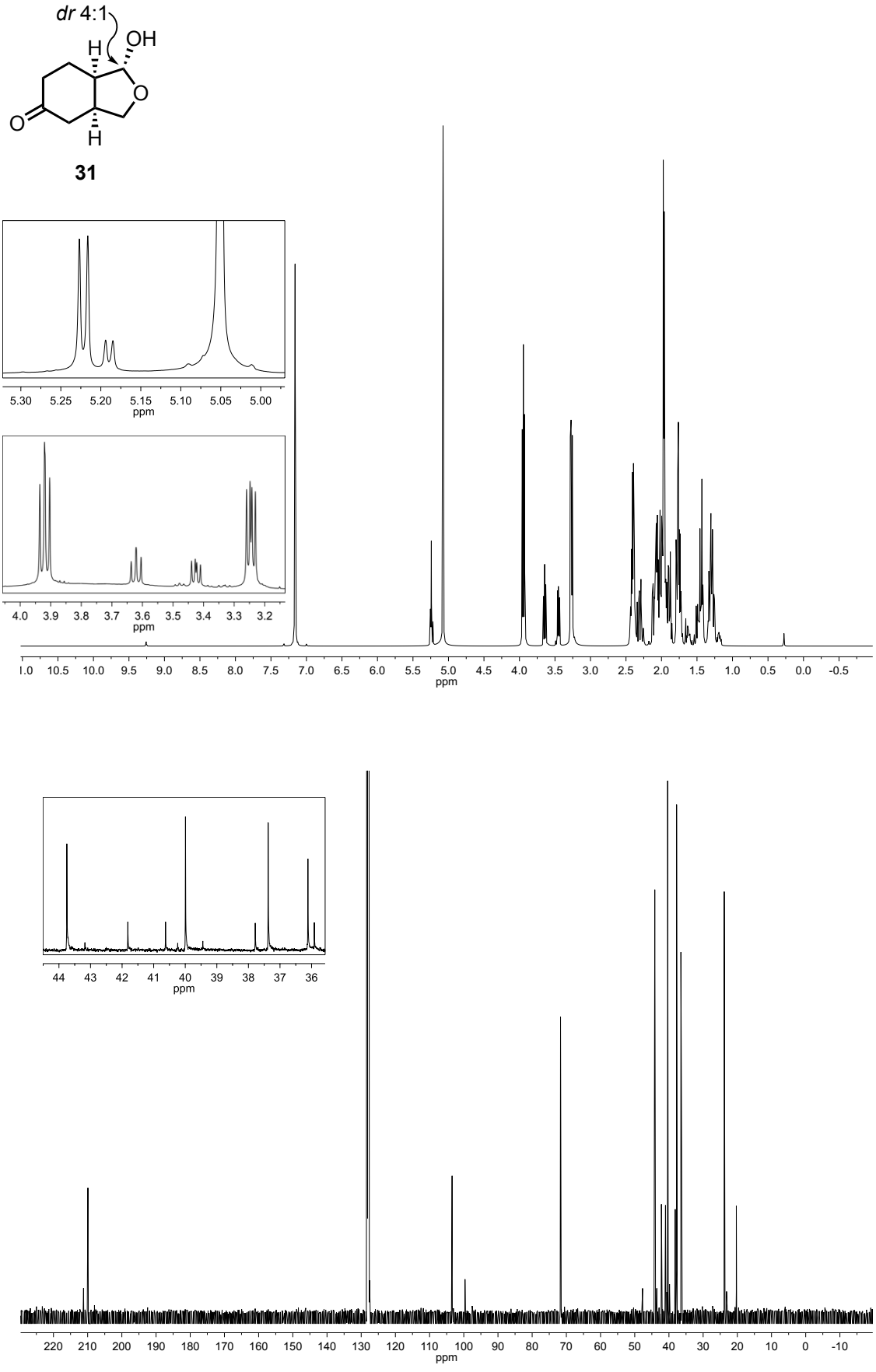


2D ^{1}H–^{1}H gCOSY NMR spectrum (500 MHz) of cyclohexenyl diacetate (–)-**23d** in $CDCl_3$

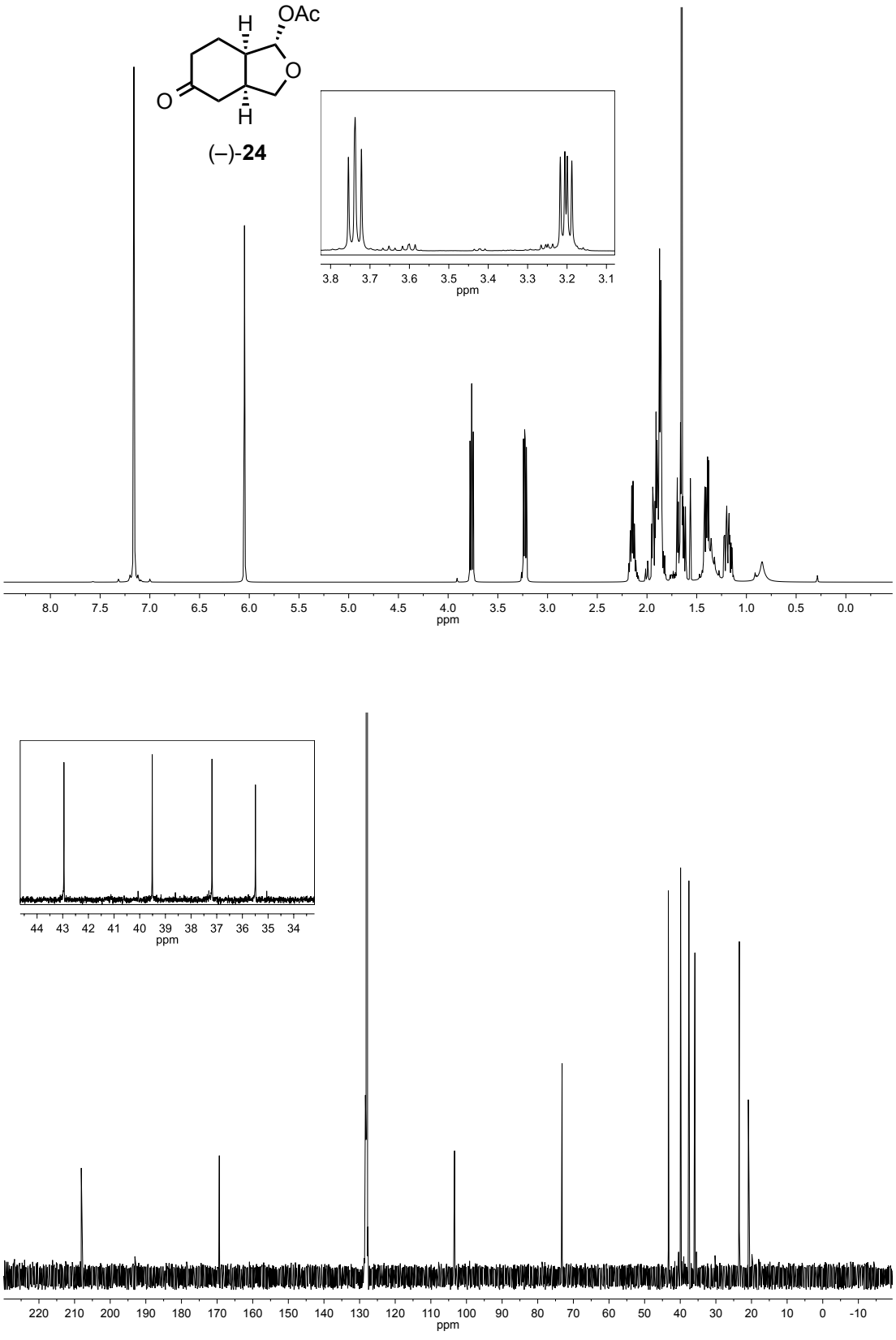


(a) 2D ^{1}H–^{1}H NOESY NMR spectrum (500 MHz) of cyclohexenyl diacetate (–)-**23d** in $CDCl_3$; The upper left panel shows the observed NOE interactions as red arrows; **(b)** Calculated global minimum conformation of (–)-**23d** using stochastic search methodology in the Molecular Operating Environment software.[1] Selected bond distances are shown in Angstroms (Å).

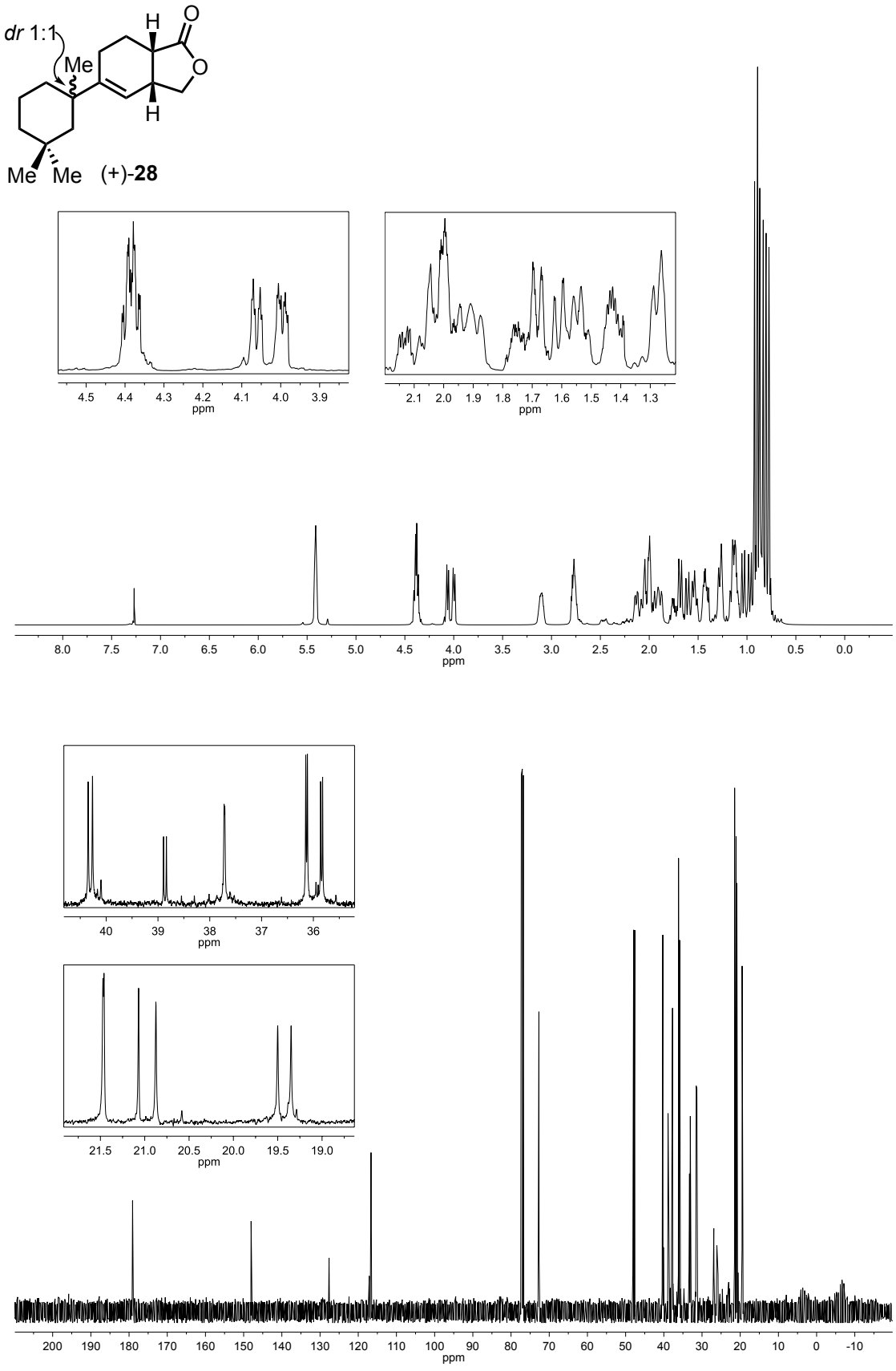
[1]*Molecular Operating Environment (MOE)* 2013.08; Chemical Computing Group, Inc., Montreal, QC, Canada, **2017**



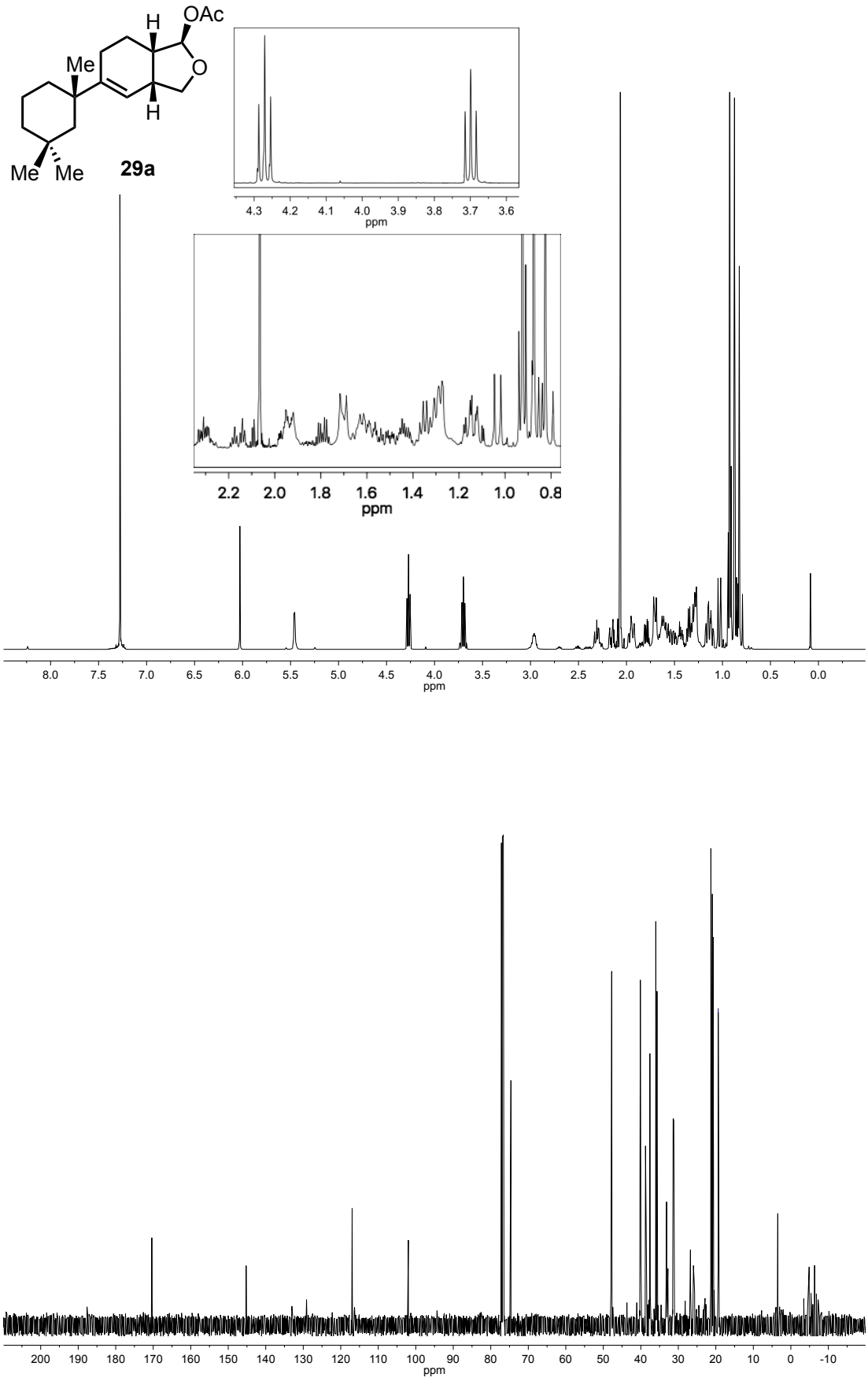
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of ketolactol **31** in C_6D_6



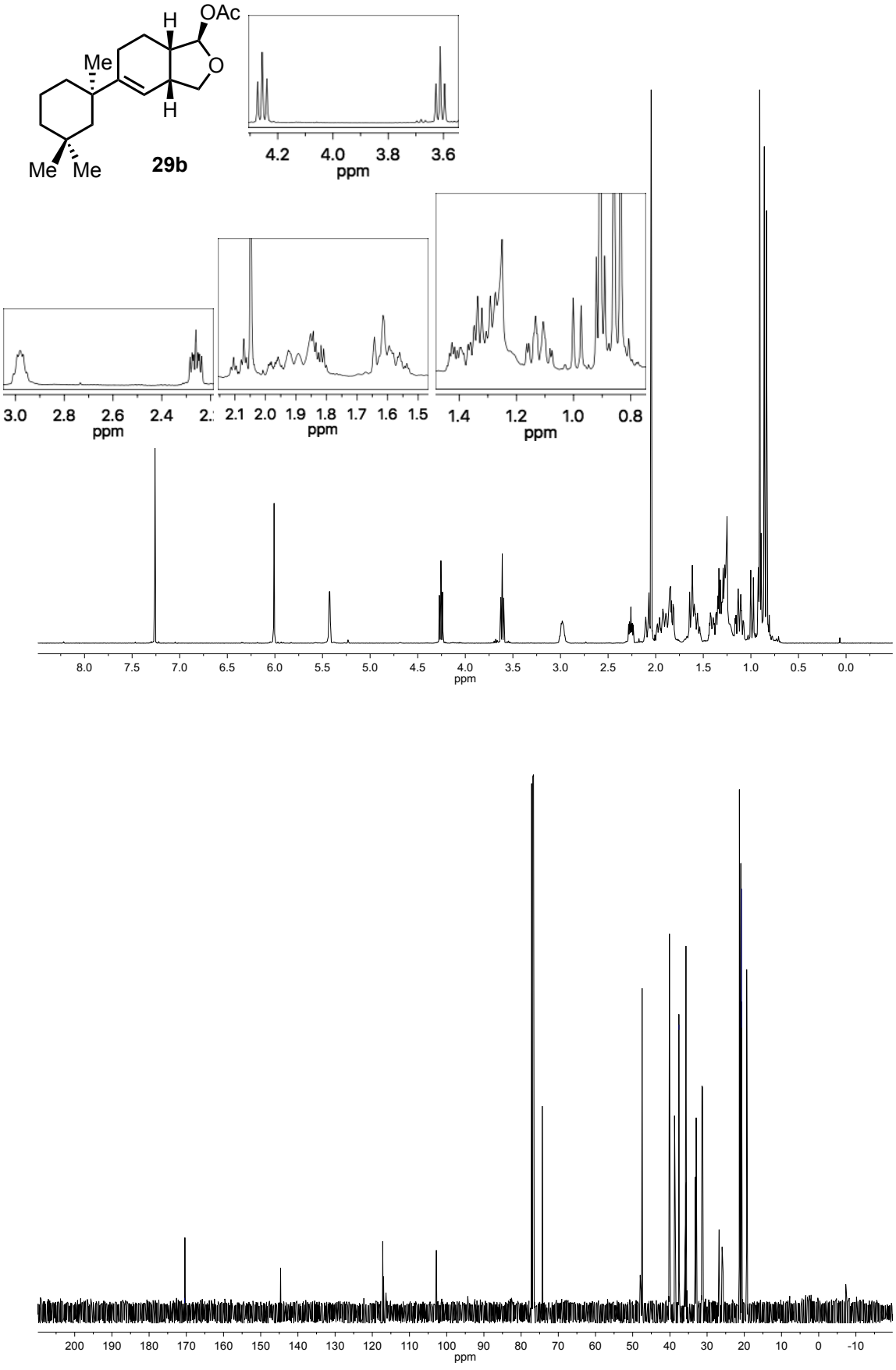
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of acetyl ketolactol (–)-**24** in C_6D_6



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of cyclohexene lactone (+)-**28** in $CDCl_3$



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of acetyl lactol **29a** in $CDCl_3$



^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra of acetyl lactol **29b** in $CDCl_3$