Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Total synthesis and biological evaluation of histone deacetylase inhibitor WF-3161

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Supporting Information

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General Information

All air and moisture sensitive reactions were carried out in dried glassware (> 100 °C) under nitrogen or argon atmosphere. THF was dried over sodium and distilled before use. The products were purified by automated column chromatography on silica columns (RediSep Rf, Teledyne Isco) or C18 columns (Telos/Büchi). Mixtures of ethyl acetate (EtOAc), petroleum ether (PE, 40–60 °C fraction), cyclohexane, dichloromethane (DCM), methanol (MeOH) or acetonitrile (MeCN) and water were generally used as eluents. Analytical TLC was performed on pre-coated silica gel plates (Macherey-Nagel, Polygram Sil G/UV_{254}). Detection was accomplished with UV light (254 nm), $KMnO_4$ solution, ninhydrin solution or ceric ammonium sulfate solution. Compounds were occasionally freeze-dried with a Christ lyophiliser. Melting points were detected with a MEL-TEMP II (Laboratory devices) apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV400 [400 MHz (^{1}H) and 100 MHz (^{13}C)] or a Bruker AV500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] spectrometer. For some rotamer containing samples the NMR spectra were measured at 373 K. Chemical shifts are reported in ppm relative to TMS or internal solvent signal. Mass spectra were recorded with a Finnigan MAT 95 spectrometer (quadrupole) using the CI technique or a Bruker maXis 4G UHR-TOF using the ESI technique. Optical rotations were measured with a Perkin-Elmer polarimeter (model 241 or 341) in a thermostated (20 °C ± 0.1 °C) cuvette, using a sodium vapour lamp (λ = 589 nm) as radiation source. The concentrations are given in g per 100 mL. LCMS analyses were accomplished on a Shimadzu (LC-10At, autoinjector SCL-6B, mass spectrometer LCMS-2020) with a Phenomenex Luna C18(2) column (50 x 4.6 mm, grain size 3 μm).

Synthesis of Compounds

[((4*S*,5*S*)-5-{(*Z*)-4-[(4-Methoxybenzyl)oxy]but-1-ene-1-yl}-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]triisopropylsilane (3)

Alcohol **1** was synthesized according to a modified literature procedure.[1]

Swern-oxidation:

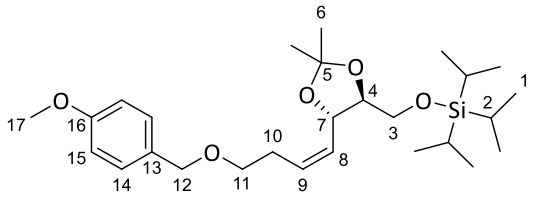
DMSO (2.4 mL, 2.67 g, 34.1 mmol, 3.0 equiv) was added to a solution of oxalyl chloride (1.6 mL, 2.31 g, 18.2 mmol, 1.6 equiv) in DCM (46 mL) keeping the reaction temperature between –71 °C and –66 °C. After stirring the reaction mixture for 5 minutes, a solution of alcohol **1** (3.63 g, 11.4 mmol, 1.0 equiv) in DCM (23 mL) was added dropwise keeping the temperature below –65 °C. After stirring the solution for 30 minutes, triethylamine (7.95 mL, 5.76 g, 56.9 mmol, 5.0 equiv) was added dropwise keeping the temperature below –60 °C before replacing the cooling bath with an ice bath. The reaction mixture was stirred for 60 minutes at 0 °C and thereafter hydrolyzed with distilled water. After phase separation, the organic phase was washed twice with water and thereafter dried with Na_2SO_4. The solvent was evaporated in vacuo and the obtained aldehyde was used in the next step without further purification.

Wittig-reaction:

A solution of KHMDS (25.0 mL, 12.5 mmol, 1.1 equiv) in toluene (0.5 M) was added dropwise to a mixture of Wittig salt **2**[2] (7.12 g, 13.7 mmol, 1.2 equiv) in THF (137 mL) at 0 °C, resulting in a red colored reaction mixture. After stirring the solution for 30 minutes, a mixture of the previously synthesized aldehyde in THF (2 mL) was added and the reaction mixture was stirred for 75 minutes at 0 °C before being hydrolyzed with water. After phase separation, the aqueous phase was extracted with diethyl

[1] A. Horn, U. Kazmaier, *Org. Lett.* 2019, **21**, 4595–4599.
[2] M. Brovetto, G. Seoane, *J. Org. Chem*. 2008, **73**, 5776–5785.

ether and the combined organics thereafter dried with Na_2SO_4. After solvent removal in vacuo, the crude product was purified by flash chromatography (SiO_2, PE:EtOAc 100:0 → 80:20) to afford alkene **3** (4.72 g, 9.86 mmol, 87 % over 2 steps) as a yellowish oil. **R_f (3)** = 0.47 (SiO_2, PE:EtOAc 80:20); $[\alpha]_D^{20}$ = – 0.6 [c = 1.0, $CHCl_3$].



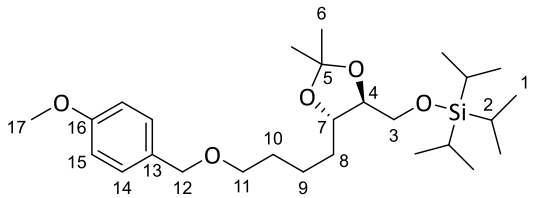
3

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.03 – 1.13 (m, 21 H, 1-H, 2-H), 1.41 (s, 3 H, 6-H), 1.43 (s, 3 H, 6-H'), 2.41 (ddtd, $^2J_{10a,10b}$ = 14.5 Hz, $^3J_{10a,9}$ = 7.1 Hz $^3J_{10a,11}$ = 6.9 Hz, $^4J_{10a,8}$ = 1.5 Hz, 1 H, 10-H_a), 2.53 (ddtd, $^3J_{10b,10a}$ = 14.5 Hz, $^3J_{10b,9}$ = 7.6 Hz $^3J_{10b,11}$ = 6.9 Hz, $^4J_{10b,8}$ = 1.4 Hz, 1 H, 10-H_b), 3.47 (t, $^3J_{11,10}$ = 6.9 Hz, 2 H, 11-H), 3.71 (dd, $^2J_{3a,3b}$ = 11.1 Hz, $^3J_{3a,4}$ = 3.6 Hz, 1 H, 3-H_a), 3.76 (ddd, $^3J_{4,7}$ = 8.2 Hz, $^3J_{4,3a}$ = 3.6 Hz, $^3J_{4,3b}$ = 3.4 Hz, 1 H, 4-H), 3.80 (s, 3 H, 17-H), 3.88 (dd, $^2J_{3b,3a}$ = 11.1 Hz, $^3J_{3b,4}$ = 3.4 Hz, 1 H, 3-H_b), 4.43 (s, 2 H, 12-H), 4.80 (ddd, $^3J_{7,8}$ = 8.9 Hz, $^3J_{7,4}$ = 8.2 Hz, $^4J_{7,9}$ = 0.5 Hz, 1 H, 7-H), 5.50 (dddd, $^3J_{8,9}$ = 10.9 Hz, $^3J_{8,7}$ = 8.9 Hz, $^4J_{8,10a}$ = 1.5 Hz, $^4J_{8,10b}$ = 1.4 Hz, 1 H, 8-H), 5.72 (dddd, $^3J_{9,8}$ = 10.9 Hz, $^3J_{9,10b}$ = 7.6 Hz, $^3J_{9,10a}$ = 7.1 Hz, $^4J_{9,7}$ = 0.5 Hz, 1 H, 9-H), 6.87 (m, 2 H, 15-H), 7.25 (m, 2 H, 14-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 11.9 (d, C-2), 17.9 (q, C-1), 18.0 (q, C-1'), 26.9 (q, C-6), 27.3 (q, C-6'), 28.4 (t, C-10), 55.3 (q, C-17), 62.1 (t, C-3), 69.4 (t, C-11), 72.5 (t, C-12), 73.1 (d, C-7), 81.8 (d, C-4), 108.8 (s, C-5), 113.7 (d, C-15), 128.3 (d, C-8), 129.2 (d, C-14), 130.5 (s, C-13), 131.8 (d, C-9), 159.1 (s, C-16).

HRMS (CI) calculated for $C_{27}H_{46}O_5Si$ $[M]^+$: 478.3109, found: 478.3125.

4-((4*S*,5*S*)-2,2-Dimethyl-5-{[(triisopropylsilyl)oxy]methyl}-1,3-dioxolan-4-yl)butan-1-ol (4)

Palladium on charcoal (417 mg, 10 wt% Pd) was added to a solution of alkene **3** (4.17 g, 8.71 mmol, 1.0 equiv) in methanol (44 mL) and the resulting suspension was stirred under hydrogen atmosphere (1 atm) for 16 hours. Subsequently, the reaction mixture was filtrated through a pad of Celite before removing the solvent in vacuo. The saturated product (4.13 g, 8.59 mmol, 99 % d. Th.) was obtained as a yellowish oil. **R_f** = 0.45 (Kieselgel, PE:EE 80:20); $[\alpha]_D^{20}$ = – 3.8 [c = 1.0, $CHCl_3$].

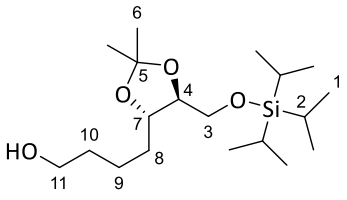


^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.03 – 1.16 (m, 21 H, 1-H, 2-H), 1.37 (s, 3 H, 6-H), 1.40 (s, 3 H, 6-H'), 1.45 – 1.74 (m, 6 H, 8-H, 9-H, 10-H), 3.45 (t, $^3J_{11,10}$ = 6.5 Hz, 2 H, 11-H), 3.69 (ddd, $^3J_{4,7}$ = 7.6 Hz, $^3J_{4,3a}$ = 5.9 Hz, $^3J_{4,3b}$ = 4.0 Hz, 1 H, 4-H), 3.75 (dd, $^2J_{3a,3b}$ = 10.2 Hz, $^3J_{3a,4}$ = 5.9 Hz, 1 H, 3-H_a), 3.80 (s, 3 H, 17-H), 3.87 (dd, $^2J_{3b,3a}$ = 10.2 Hz, $^3J_{3b,4}$ = 4.0 Hz, 1 H, 3-H_b), 3.94 (ddd, $^3J_{7,4}$ = $^3J_{7,8a}$ = 7.6 Hz, $^3J_{7,8b}$ = 3.7 Hz, 1 H, 7-H), 4.42 (s, 2 H, 12-H), 6.87 (m, 2 H, 15-H), 7.25 (m, 2 H, 14-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 11.9 (d, C-2), 18.0 (q, C-1), 22.8 (t, C-9), 27.0 (q, C-6), 27.4 (q, C-6'), 29.8 (t, C-8), 33.4 (t, C-10), 55.3 (q, C-17), 64.2 (t, C-3), 70.0 (t, C-11), 72.6 (t, C-12), 79.1 (d, C-7), 81.0 (d, C-4), 108.3 (s, C-5), 113.7 (d, C-15), 129.2 (d, C-14), 130.7 (s, C-13), 159.1 (s, C-16).

HRMS (CI) calculated for $C_{27}H_{47}O_5Si$ $[M-H]^+$: 479.3187, found: 479.3199.

DDQ (387 mg, 1.71 mmol, 1.1 equiv) was added to a solution of the previously synthesized saturated compound (746 mg, 1.55 mmol, 1.0 equiv) in DCM:H_2O (20:1, 15.5 mL) and the resulting solution was stirred for 2 hours at room temperature. Subsequently, the reaction mixture was treated with $NaHCO_3$-

solution (saturated) and the phases thereafter separated. The aqueous phase was extracted three times with DCM and the combined organics were dried with Na_2SO_4. After solvent removal in vacuo, the crude product was purified by flash chromatography (SiO_2, PE:EtOAc 100:0 → 50:50) to afford alcohol **4** (462 mg, 1.28 mmol, 83 %) as a colorless resin. **R_f (4)** = 0.19 (SiO_2, PE:EtOAc 80:20); $[\alpha]_D^{20}$ = – 3.6 [c = 1.0, $CHCl_3$].



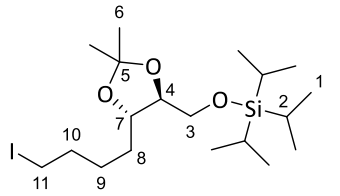
4

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.04 – 1.14 (m, 21 H, 1-H, 2-H), 1.37 (s, 3 H, 6-H), 1.40 (s, 3 H, 6-H'), 1.43 – 1.77 (m, 7 H, 8-H, 9-H, 10-H, O-H), 3.66 (t, $^3J_{11,10}$ = 6.3 Hz, 2 H, 11-H), 3.70 (ddd, $^3J_{4,7}$ = 7.7 Hz, $^3J_{4,3a}$ = 6.0 Hz, $^3J_{4,3b}$ = 3.8 Hz, 1 H, 4-H), 3.75 (dd, $^2J_{3a,3b}$ = 10.2 Hz, $^3J_{3a,4}$ = 6.0 Hz, 1 H, 3-H_a), 3.88 (dd, $^2J_{3b,3a}$ = 10.2 Hz, $^3J_{3b,4}$ = 3.8 Hz, 1 H, 3-H_b), 3.95 (ddd, $^3J_{7,8b}$ = $^3J_{7,4}$ = 7.7 Hz, $^3J_{7,8a}$ = 3.6 Hz, 1 H, 7-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 11.9 (d, C-2), 17.9 (q, C-1), 22.4 (t, C-9), 26.9 (q, C-6), 27.4 (q, C-6'), 32.7 (t, C-10), 33.1 (t, C-8), 62.8 (t, C-11), 64.2 (t, C-3), 79.2 (d, C-7), 80.9 (d, C-4), 108.4 (s, C-5).

HRMS (CI) calculated for $C_{19}H_{41}O_4Si$ $[M+H]^+$: 361.2769, found: 361.2774.

{[(4*S*,5*S*)-5-(4-Iodobutyl)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}triisopropylsilane (5)

Triphenylphosphine (1.90 g, 7.24 mmol, 1.25 equiv) and imidazole (493 mg, 7.24 mmol, 1.25 equiv) were dissolved in DCM (26 mL) and the resulting solution was cooled to 0 °C. Iodine (1.83 g, 7.21 mmol, 1.24 equiv) was added and the mixture was stirred at room temperature for 20 minutes. A solution of alcohol **4** (2.10 g, 5.82 mmol, 1.0 equiv) in DCM (13 mL) was added dropwise at 0 °C and the reaction mixture was stirred for 25 minutes at 0 °C followed by 3.5 hours at room temperature. Subsequently, the mixture was washed with Na_2SO_3 solution (1 M) and the aqueous phase thereafter extracted twice with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. After flash chromatography (SiO_2, PE:EtOAc 100:0 → 90:10) iodide **5** (2.55 g, 5.42 mmol, 93 %) was obtained as a colorless oil. **R_f (5)** = 0.63 (SiO_2, PE:EtOAc 80:20); $[\alpha]_D^{20}$ = – 3.8 [c = 1.0, $CHCl_3$].



5

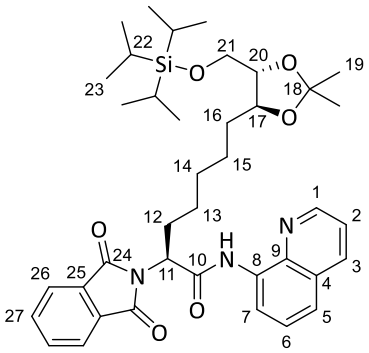
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.04 – 1.15 (m, 21 H, 1-H, 2-H), 1.37 (s, 3 H, 6-H), 1.40 (s, 3 H, 6-H'), 1.48 – 1.75 (m, 4 H, 8-H, 9-H), 1.87 (tt, $^3J_{10,9}$ = $^3J_{10,11}$ = 7.1 Hz, 2 H, 10-H), 3.19 (t, $^3J_{11,10}$ = 7.1 Hz, 2 H, 11-H), 3.69 (ddd, $^3J_{4,7}$ = 7.5 Hz, $^3J_{4,3a}$ = 6.1 Hz, $^3J_{4,3b}$ = 3.9 Hz, 1 H, 4-H), 3.75 (dd, $^2J_{3a,3b}$ = 10.0 Hz, $^3J_{3a,4}$ = 6.1 Hz, 1 H, 3-H_a), 3.88 (dd, $^2J_{3b,3a}$ = 10.0 Hz, $^3J_{3b,4}$ = 3.9 Hz, 1 H, 3-H_b), 3.94 (ddd, $^3J_{7,4}$ = $^3J_{7,8a}$ = 7.5 Hz, $^3J_{7,8b}$ = 3.4 Hz, 1 H, 7-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 6.5 (t, C-11), 11.9 (d, C-2), 18.0 (q, C-1), 27.0 (q, C-6), 27.2 (t, C-9), 27.4 (q, C-6'), 32.4 (t, C-8), 33.6 (t, C-10), 64.2 (t, C-3), 79.1 (d, C-7), 80.9 (d, C-4), 108.4 (s, C-5).

HRMS (CI) calculated for $C_{19}H_{40}IO_3Si$ $[M+H]^+$: 471.1786, found: 471.1774.

(2*S*)-7-(2,2-Dimethyl-5-{[(triisopropylsilyl)oxy]methyl}-1,3-dioxolan-4-yl)-2-(1,3-dioxoisoindolin-2-yl)-*N*-(quinolin-8-yl)heptanamide (7)

Alanine derivative **6** was synthesized according to a modified literature procedure.[3]

Silver carbonate (15.6 mg, 56.8 µmol, 80 mol%), palladium acetate (1.6 mg, 7.10 µmol, 10 mol%) and dibenzyl phosphate (5.9 mg, 21.3 µmol, 30 mol%) were added successively to a solution of iodide **5** (43.3 mg, 92.0 µmol, 1.3 equiv) and alanine derivative **6** (24.5 mg, 71.0 µmol, 1.0 equiv) in a *tert*-amyl alcohol/1,2-dichloroethane mixture (1:1, 532 µL) and the resulting suspension was stirred at 60 °C for 25 hours. Subsequently, the brown suspension was concentrated in vacuo and the crude product purified by flash chromatography (SiO_2, PE:EtOAc 100:0 → 70:30) to afford compound **7** (39.6 mg, 57.6 µmol, 81 %) as a colorless resin. **R_f (7)** = 0.43 (SiO_2, PE:EtOAc 60:40); $[\alpha]_D^{20}$ = – 3.4 [c = 1.0, $CHCl_3$].



7

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.01 – 1.10 (m, 21 H, 22-H, 23-H), 1.35 (s, 3 H, 19-H), 1.38 (s, 3 H, 19-H'), 1.40 – 1.69 (m, 8 H, 13-H, 14-H, 15-H, 16-H), 2.40 (m, 1 H, 12-H_a), 2.59 (m, 1 H, 12-H_b), 3.66 (ddd, $^3J_{20,17}$ = 7.7 Hz $^3J_{20,21a}$ = 6.0 Hz, $^3J_{20,21b}$ = 4.0 Hz, 1 H, 20-H), 3.73 (dd, $^2J_{21a,21b}$ = 10.2 Hz, $^3J_{21a,20}$ = 6.0 Hz, 1 H, 21-H_a), 3.85 (dd, $^2J_{21b,21a}$ = 10.2 Hz, $^3J_{21b,20}$ = 4.0 Hz, 1 H, 21-H_b), 3.91 (ddd, $^3J_{17,16b}$ = $^3J_{17,20}$ = 7.7 Hz, $^3J_{17,16a}$ = 3.7 Hz, 1 H, 17-H), 5.12 (dd, $^3J_{11,12a/b}$ = 10.9 Hz, $^3J_{11,12a/b}$ = 5.3 Hz, 1 H, 11-H), 7.42 (dd, $^3J_{2,3}$ = 8.2 Hz, $^3J_{2,1}$ = 4.2 Hz, 1 H, 2-H), 7.49 – 7.53 (m, 2 H, 5-H, 6-H), 7.76 (m, 2 H, 27-H), 7.90 (m, 2 H, 26-H), 8.14 (dd, $^3J_{3,2}$ = 8.2 Hz, $^4J_{3,1}$ = 1.5 Hz, 1 H, 3-H), 8.70 (dd, $^3J_{1,2}$ = 4.2 Hz, $^4J_{1,3}$ = 1.5 Hz, 1 H, 1-H), 8.72 (dd, $^3J_{7,6}$ = 5.3 Hz, $^4J_{7,5}$ = 3.8 Hz, 1 H, 7-H), 10.33 (s, 1 H, N-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 11.8 (d, C-22), 17.9 (q, C-23), 26.0 (t, C-15), 26.7 (t, C-13), 27.0 (q, C-19), 27.4 (q, C-19'), 28.7 (t, C-12), 29.2 (t, C-14), 33.4 (t, C-16), 55.3 (d, C-11), 64.2 (t, C-21), 79.1 (d, C-17), 81.0 (d, C-20), 108.3 (s, C-18), 116.8 (d, C-7), 121.6 (d, C-2), 121.9 (d, C-5), 123.6 (d, C-26), 127.3 (d, C-6), 127.9 (s, C-4), 131.8 (s, C-25), 133.9 (s, C-8), 134.2 (d, C-27), 136.4 (d, C-3), 138.4 (s, C-9), 148.2 (d, C-1), 167.0 (s, C-10), 168.1 (s, C-24).

HRMS (CI) calculated for $C_{39}H_{53}N_3O_6$ $[M]^+$: 687.3698, found: 687.3711.

(4*R*,5*R*)-2-[(*R*)-1-(Benzyloxy)-2-(trityloxy)ethyl]-4,5-dicyclohexyl-1,3,2-dioxaborolane (9)

LDA preparation:

n-Butyllithium (1.6 M in hexanes, 6.65 mL, 10.6 mmol, 1.35 equiv) was added dropwise to a cooled solution (–40 °C) of diisopropylamine (1.65 mL, 1.15 g, 11.4 mmol, 1.45 equiv) in THF (1.6 mL). The resulting solution was stirred for 10 minutes at –40 °C and for 20 minutes at room temperature.

Alcoholate preparation 1:

Benzyl alcohol (1.15 mL, 1.19 g, 11.0 mmol, 1.4 equiv) was added dropwise to a solution of sodium hydride (60 wt% in mineral oil, 409 mg, 10.2 mmol, 1.3 equiv) in a THF/DMSO mixture (5.0 mL THF, 15.3 mL DMSO) and the resulting mixture was stirred at room temperature for 3 hours.

[3] B. Wang, C. Lu, S. Zhang, G. He, W. A. Nack, G. Chen, *Org. Lett*. 2014, **16**, 6260–6263.

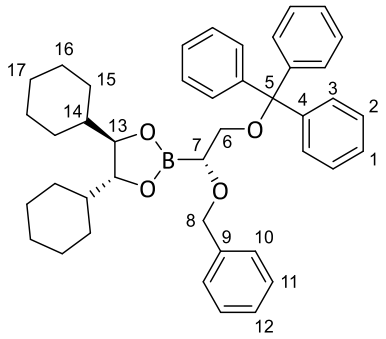
Alcoholate preparation 2:

Benzyl alcohol (716 µL, 745 mg, 6.89 mmol, 0.88 equiv) was added dropwise to a solution of sodium hydride (60 wt% in mineral oil, 236 mg, 5.90 mmol, 0.75 equiv) in a THF/DMSO mixture (2.9 mL THF, 8.8 mL DMSO) and the resulting mixture was stirred at room temperature for 2 hours.

Matteson homologation:

Boronic ester **8** was prepared according to a modified literature procedure.[4]

The freshly prepared LDA solution was added dropwise at –40 °C to a solution of boronic ester **8** (4.00 g, 7.87 mmol, 1.0 equiv) and DCM (1.65 mL, 2.17 g, 25.6 mmol, 3.25 equiv) in THF (12.0 mL) and the resulting mixture was stirred for 10 minutes at –40 °C. Subsequently, zinc chloride (3.22 g, 23.6 mmol, 3.0 equiv) in THF (14.2 mL) was added slowly to the solution which was thereafter stirred at room temperature for 3 hours. The chloroboronic ester solution was cooled to 0 °C and treated with the previously synthesized alcoholate solution 1. The reaction mixture was stirred at room temperature for 16 hours, which led to uncomplete conversion. The solution was cooled to 0 °C and treated with alcoholate solution 2. The reaction mixture was stirred at room temperature for 16 hours and was thereafter hydrolyzed with NH_4Cl solution (saturated). The mixture was extracted twice with PE and the combined organics were dried over Na_2SO_4. After solvent removal, the crude product was purified by flash chromatography (SiO_2, PE → PE:EtOAc 98:2 → 95:5) to afford boronic ester **9** (3.80 g, 6.04 mmol, 77 %) as a colorless resin. **R_f (9)** = 0.39 (SiO_2, PE:EtOAc 80:20); $[\alpha]_D^{20}$ = + 23.9 [c = 1.0, $CHCl_3$].



9

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 – 1.25 (m, 10 H, 15-H_{ax}, 15-H_{ax}', 16-H_{eq}, 16-H_{eq}', 17-H_{eq}), 1.34 (m, 2 H, 14-H), 1.53 – 1.80 (m, 10 H, 15-H_{eq}, 15-H_{eq}', 16-H_{ax}, 16-H_{ax}', 17-H_{ax}), 3.33 (dd, $^2J_{6a,6b}$ = 9.9 Hz, $^3J_{6a,7}$ = 5.5 Hz, 1 H, 6-H_a), 3.39 (d, $^2J_{6b,6a}$ = 9.9 Hz, $^3J_{6b,7}$ = 3.8 Hz, 1 H, 6-H_b), 3.54 (dd, $^3J_{6a,7}$ = 5.5 Hz, $^3J_{6b,7}$ = 3.8 Hz, 1 H, 7-H), 3.91 (m, 2 H, 13-H), 4.56 (d, $^2J_{8a,8b}$ = 12.2 Hz, 1 H, 8-H_a), 4.66 (d, $^2J_{8b,8a}$ = 12.2 Hz, 1 H, 8-H_b), 7.18 – 7.35 (m, 14 H, 1-H, 3-H, 10-H, 11-H, 12-H), 7.49 (m, 6 H, 2-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 25.9 (t, C-16), 26.0 (t, C-17), 26.4 (t, C-16'), 27.4 (t, C-15), 28.2 (t, C-15'), 42.8 (d, C-14), 64.5 (t, C-6), 67.5 (d, C-7), 72.3 (t, C-8), 83.8 (d, C-13), 86.4 (s, C-5), 126.7 (d, C-1), 127.2 (d, C-12), 127.6 (d, C-3), 127.7 (d, C-10), 128.1 (d, C-11), 128.8 (d, C-2), 139.1 (s, C-9), 144.2 (s, C-4).

HRMS (CI) calculated for $C_{23}H_{34}BO_4$ [M-Trityl]$^+$: 385.2545, found: 385.2560.

(2*S*,3*S*)-2-(Benzyloxy)-7-[(*tert*-butyldiphenylsilyl)oxy]-1-(trityloxy)heptan-3-ol (11)

LDA preparation:

n-Butyllithium (1.6 M in hexanes, 12.6 mL, 20.2 mmol, 1.35 equiv) was added dropwise to a cooled solution (–40 °C) of diisopropylamine (3.10 mL, 2.19 g, 21.7 mmol, 1.45 equiv) in THF (3.0 mL). The resulting solution was stirred for 10 minutes at –40 °C and for 20 minutes at room temperature.

[4] O. Ho, R. Soundararajan, J. Lu, D. Matteson, Z. Wang, X. Chen, M. Wei and R. Willett, *Organometallics* **1995**, *14*, 2855–2860.

Grignard preparation:

Chloride **10** was prepared according to a modified literature procedure.[5]

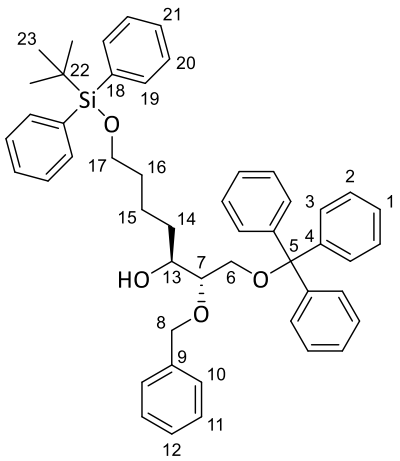
1,2-Dibromoethane (258 µL, 561 mg, 2.99 mmol, 20 mol%) was added to a suspension of magnesium (1.49 g, 61.3 mmol, 4.1 equiv) in THF (5.90 mL), which was thereafter heated repeatedly with a heat gun until the solvent started boiling. Subsequently, chloride **10** (7.78 g, 22.4 mmol, 1.5 equiv) in THF (16.2 mL) was added dropwise at 35 °C to the solution before it was refluxed for 2 hours.

Matteson homologation:

The freshly prepared LDA solution was added dropwise at –40 °C to a solution of boronic ester **9** (9.39 g, 14.9 mmol, 1.0 equiv) and DCM (2.90 mL, 3.81 g, 44.8 mmol, 3.0 equiv) in THF (21 mL) and the resulting mixture was stirred for 10 minutes at –40 °C. Subsequently, zinc chloride (8.14 g, 59.8 mmol, 4.0 equiv) in THF (36 mL) was added slowly to the solution which was thereafter stirred at room temperature for 2.5 hours. The chloroboronic ester solution was diluted with *n*-pentane and hydrolyzed with NH_4Cl solution (saturated). After phase separation, the aqueous phase was extracted three times with PE and the combined organic phases were dried over Na_2SO_4. After solvent removal, the crude α-chloroboronic ester was dissolved in THF (60 mL) and cooled to 0 °C. At this temperature the previously prepared Grignard solution was added dropwise and the reaction mixture was warmed to room temperature over 16 hours. After hydrolyzation with NH_4Cl solution (saturated), the mixture was extracted three times with PE and the combined organics were dried over Na_2SO_4. After solvent removal, the crude boronic ester was used in the next step without further purification.

Oxidation:

Hydrogen peroxide (30 wt% in water, 3.4 mL, 33.6 mmol, 2.25 equiv) and NaOH (1 M, 34 mL, 34.0 mmol, 2.25 equiv) were added at 0 °C to a solution of the previously synthesized boronic ester in THF (75 mL) and the resulting mixture was stirred at room temperature for 90 minutes. Remaining peroxide was reduced with a Na_2SO_3 solution (1 M) and the aqueous phase was extracted three times with diethyl ether. The combined organics were dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash chromatography (SiO_2, PE:EtOAc 90:10 → 85:15) to afford alcohol **11** (7.53 g, 10.2 mmol, 69 % over 3 steps) as a colorless resin. **R_f (11)** = 0.35 (SiO_2, PE:EtOAc 80:20); $[\alpha]_D^{20}$ = + 7.1 [c = 0.5, $CHCl_3$].



11

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.03 (s, 9 H, 23-H), 1.24 – 1.37 (m, 3 H, 14-H, 15-H_a), 1.41 – 1.55 (m, 3 H, 15-H_b, 16-H), 2.39 (d, $^3J_{OH,13}$ = 5.4 Hz, 1 H, O-H), 3.24 (dd, $^2J_{6a,6b}$ = 9.8 Hz, $^3J_{6a,7}$ = 4.7 Hz, 1 H, 6-H_a), 3.35 (ddd, $^3J_{7,6a}$ = 4.7 Hz, $^3J_{7,13}$ = 4.4 Hz $^3J_{7,6b}$ = 4.1 Hz, 1 H, 7-H), 3.38 (dd, $^2J_{6b,6a}$ = 9.8 Hz, $^3J_{6b,7}$ = 4.1 Hz, 1 H, 6-H_b), 3.61 (t, $^3J_{17,16}$ = 6.5 Hz, 2 H, 17-H), 3.67 (m, 1 H, 13-H), 4.46 (d, $^2J_{8a,8b}$ = 11.4 Hz, 1 H, 8-H_a), 4.76 (d,

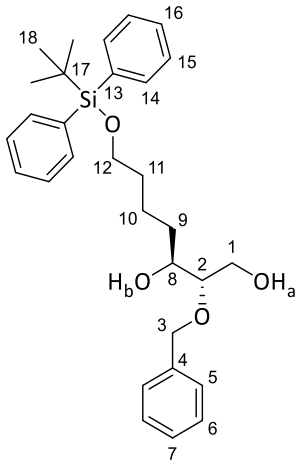
[5] I. Erdelmeier, G. Bülow, C. W. Woo, J. Decker, G. Raabe, H. J. Gais, *Chem. - A Eur. J.* **2019**, *25*, 8371–8386.

$^2J_{8b,8a}$ = 11.4 Hz, 1 H, 8-H_b), 7.20 – 7.42 (m, 20 H, 1-H, 3-H, 10-H, 11-H, 12-H, 20-H, 21-H), 7.46 (m, 6 H, 2-H), 7.65 (m, 4 H, 19-H). **^{13}C-NMR (125 MHz, $CDCl_3$):** δ = 19.2 (s, C-22), 21.9 (t, C-15), 26.9 (q, C-23), 32.6 (t, C-14), 32.9 (t, C-16), 63.4 (t, C-6), 63.9 (t, C-17), 71.6 (d, C-13), 72.9 (t, C-8), 80.9 (d, C-7), 86.9 (s, C-5), 127.1 (d, C-1), 127.6 (d, C-20), 127.8 (d, C-12), 127.9 (d, C-3), 128.0 (d, C-10), 128.4 (d, C-11), 128.6 (d, C-2), 129.5 (d, C-21), 134.1 (s, C-18), 135.5 (d, C-19), 138.2 (s, C-9), 143.8 (s, C-4).

HRMS (CI) calculated for $C_{30}H_{37}O_4Si$ [M-Trityl-2H]$^+$: 489.2456, found: 489.2466.

(2*S*,3*S*)-2-(Benzyloxy)-7-[(*tert*-butyldiphenylsilyl)oxy]heptan-1,3-diol (12)

A solution of alcohol **11** (7.53 g, 10.2 mmol, 1.0 equiv) in DCM (28.5 mL) was added at 0 °C to zinc bromide (dried with a heat gun, 9.22 g, 41.0 mmol, 4.0 equiv) and the resulting suspension was stirred at room temperature for 2.5 hours. Subsequently, 738 µL water (738 µL, 738 mg, 41.0 mmol, 4.0 equiv) was added and the resulting solution was stirred at room temperature for 50 minutes. The reaction mixture was hydrolyzed with $NaHCO_3$ solution (saturated), extracted three times with ethyl acetate and the combined organic phases were dried over Na_2SO_4. After solvent removal, the crude product was purified by flash chromatography (SiO_2, PE:EtOAc 80:20 → 60:40) to afford diol **12** (4.03 g, 8.18 mmol, 80 %) as a colorless resin. **R_f (12)** = 0.19 (SiO_2, PE:EtOAc 60:40); $[\alpha]_D^{20}$ = + 3.2 [c = 1.0, $CHCl_3$].



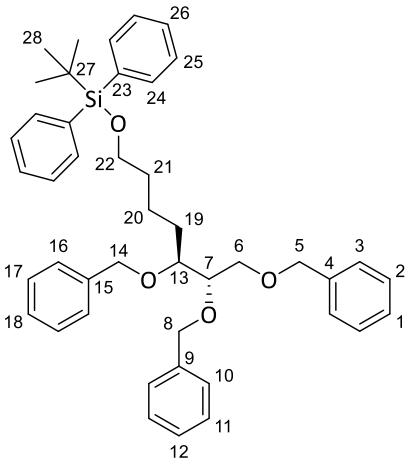
12

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 9 H, 18-H), 1.37 (m, 1 H, 10-H_a), 1.46 – 1.62 (m, 5 H, 9-H, 10-H_b, 11-H), 2.21 (bs, 1 H, O-H_a), 2.33 (bs, 1 H, O-H_b), 3.34 (ddd, $^3J_{2,1a}$ = $^3J_{2,8}$ = 4.4 Hz, $^3J_{2,1b}$ = 4.1 Hz, 1 H, 2-H), 3.65 (t, $^3J_{17,16}$ = 6.3 Hz, 2 H, 12-H), 3.66 – 3.72 (m, 2 H, 1-H_a, 8-H), 3.86 (dd, $^2J_{1b,1a}$ = 11.7 Hz $^3J_{1b,2}$ = 2.8 Hz, 1-H_b), 4.59 (d, $^2J_{3a,3b}$ = 11.4 Hz, 1 H, 3-H_a), 4.71 (d, $^2J_{3b,3a}$ = 11.4 Hz, 1 H, 3-H_b), 7.27 – 7.43 (m, 11 H, 5-H, 6-H, 7-H, 15-H, 16-H), 7.66 (m, 4 H, 14-H). **^{13}C-NMR (125 MHz, $CDCl_3$):** δ = 19.2 (s, C-17), 21.9 (t, C-10), 26.8 (q, C-18), 32.4 (t, C-9), 33.2 (t, C-11), 61.9 (t, C-1), 63.8 (t, C-12), 72.1 (d, C-8), 72.7 (t, C-3), 81.1 (d, C-2), 127.6 (d, C-15), 127.9 (d, C-5), 128.0 (d, C-7), 128.5 (d, C-6), 129.5 (d, C-16), 134.0 (s, C-13), 135.5 (d, C-14), 137.9 (s, C-4).

HRMS (CI) calculated for $C_{30}H_{41}O_4Si$ [M+H]$^+$: 493.2769, found: 493.2769.

tert-Butyldiphenyl{[(5*S*,6*S*)-5,6,7-tris(benzyloxy)heptyl]oxy}silane (13)

t-BuOK (758 mg, 6.76 mmol, 3.0 equiv) and benzyl bromide (1.05 mL, 1.54 g, 9.01 mmol, 4.0 equiv) were added successively to a solution of diol **12** (1.11 g, 2.25 mmol, 1.0 equiv) in THF (22.5 mL). The reaction mixture was stirred at 0 °C for 100 minutes and thereafter hydrolyzed with NH_4Cl solution (saturated). The mixture was extracted three times with diethyl ether and the combined organics were dried over Na_2SO_4. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 90:10) to afford compound **13** (1.35 g, 2.00 mmol, 89 %) as a colorless oil. **R_f (13)** = 0.26 (SiO_2, PE:EtOAc 90:10); $[\alpha]_D^{20}$ = + 0.7 [c = 1.0, $CHCl_3$].



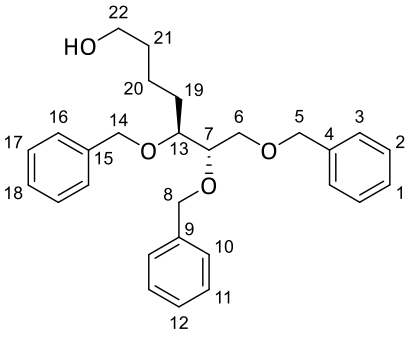
13

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.04 (s, 9 H, 28-H), 1.24 (m, 1 H, 20-H_a), 1.37 – 1.65 (m, 5 H, 19-H, 20-H_b, 21-H), 3.50 (dt, $^{3}J_{13,7}$ = 8.2 Hz, $^{3}J_{13,19}$ = 4.3 Hz, 1 H, 13-H), 3.57 – 3.63 (m, 3 H, 6-H_a, 22-H), 3.68 – 3.73 (m, 2 H, 6-H_b, 7-H), 4.49 (d, $^{2}J_{14a,14b}$ = 11.5 Hz, 1 H, 14-H_a), 4.50 (s, 2 H, 5-H), 4.56 (d, $^{2}J_{14b,14a}$ = 11.5 Hz, 1 H, 14-H_b), 4.61 (d, $^{2}J_{8a,8b}$ = 11.9 Hz, 1 H, 8-H_a), 4.75 (d, $^{2}J_{8b,8a}$ = 11.9 Hz, 1 H, 8-H_b), 7.21 – 7.43 (m, 21 H, 1-H, 2-H, 3-H, 10-H, 11-H, 12-H, 16-H, 17-H, 18-H, 25-H, 26-H), 7.66 (m, 4 H, 24-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 19.2 (s, C-27), 22.3 (t, C-20), 26.9 (q, C-28), 29.9 (t, C-19), 32.6 (t, C-21), 63.8 (t, C-22), 70.5 (t, C-6), 72.8 (t, C-14), 72.9 (t, C-8), 73.4 (t, C-5), 79.1 (d, C-13), 79.2 (d, C-7), 127.5, 127.5, 127.5 (3d, C-1, C-12, C-18), 127.6 (d, C-25), 127.6, 128.0, 128.0 (3d, C-3, C-10, C-16), 128.2, 128.3, 128.3 (3d, C-2, C-11, C-17), 129.5 (d, C-26), 134.1 (s, C-23), 135.5 (d, C-24), 138.3 (s, C-4), 138.6 (s, C-15), 138.8 (s, C-9).

HRMS (CI) calculated for $C_{44}H_{53}O_4Si$ $[M+H]^+$: 673.3708, found: 673.3731.

(5*S*,6*S*)-5,6,7-Tris(benzyloxy)heptan-1-ol (14)

A TBAF solution (1 M in THF, 2.50 mL, 2.50 mmol, 1.25 equiv) was added slowly at 0 °C to a solution of compound **13** (1.34 g, 1.99 mmol, 1.0 equiv) in THF (13.3 mL) and the reaction mixture was stirred at room temperature for 16 hours. Subsequently, the solution was hydrolyzed with NH_4Cl solution (saturated) and thereafter extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. After flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 ⟶ 60:40) alcohol **14** (827 mg, 1.90 mmol, 96 %) was obtained as a colorless oil. **R_f (14)** = 0.22 (SiO_2, PE:EtOAc 60:40); $[\alpha]_D^{20}$ = – 3.9 [c = 1.0, $CHCl_3$].



14

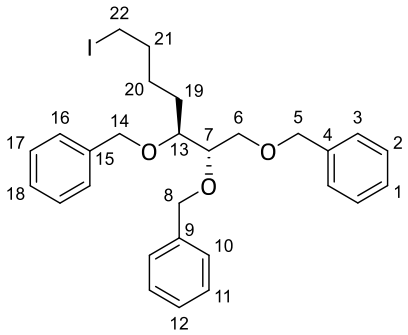
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.22 (m, 1 H, 20-H_a), 1.34 – 1.50 (m, 5 H, 19-H_a, 20-H_b, 21-H, O-H), 1.60 (m, 1 H, 19-H_b), 3.49 – 3.56 (m, 3 H, 13-H, 22-H), 3.62 (dd, $^{2}J_{6a,6b}$ = 11.0 Hz, $^{3}J_{6a,7}$ = 7.2 Hz, 1 H, 6-H_a), 3.69 – 3.74 (m, 2 H, 6-H_b, 7-H), 4.50 (d, $^{2}J_{14a,14b}$ = 11.8 Hz, 1 H, 14-H_a), 4.51 (s, 2 H, 5-H), 4.58 (d, $^{2}J_{14b,14a}$ = 11.8 Hz, 1 H, 14-H_b), 4.61 (d, $^{2}J_{8a,8b}$ = 11.9 Hz, 1 H, 8-H_a), 4.76 (d, $^{2}J_{8b,8a}$ = 11.9 Hz, 1 H, 8-H_b), 7.23 – 7.36 (m, 15 H, 1-H, 2-H, 3-H, 10-H, 11-H, 12-H, 16-H, 17-H, 18-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 22.0 (t, C-20), 29.8 (t, C-19), 32.6 (t, C-21), 62.7 (t, C-22), 70.4 (t, C-6), 72.7 (t, C-14), 72.9 (t, C-8), 73.4 (t, C-5),

79.0 (d, C-13), 79.0 (d, C-7), 127.5, 127.5, 127.6 (3d, C-1, C-12, C-18), 127.6, 128.0, 128.0 (3d, C-3, C-10, C-16), 128.2, 128.3, 128.3 (3d, C-2, C-11, C-17), 138.3 (s, C-4), 138.6 (s, C-15), 138.7 (s, C-9).

HRMS (CI) calculated for $C_{28}H_{35}O_4$ $[M+H]^+$: 435.2530, found: 435.2531**.**

({[(2*S*,3*S*)-7-Iodoheptane-1,2,3-triyl]tris(oxy)}tris(methylene))tribenzene (15)

Triphenylphosphine (2.33 g, 8.88 mmol, 1.25 equiv) and imidazole (604 mg, 8.88 mmol, 1.25 equiv) were dissolved in DCM (32 mL) and the resulting solution was cooled to 0 °C. Iodine (2.24 g, 8.84 mmol, 1.24 equiv) was added and the mixture was stirred at room temperature for 20 minutes. A solution of alcohol **14** (3.10 g, 7.13 mmol, 1.0 equiv) in DCM (16 mL) was added dropwise at 0 °C and the reaction mixture was stirred for 25 minutes at 0 °C followed by 3 hours at room temperature. Subsequently, the mixture was washed with Na_2SO_3 solution (1 M) and the aqueous phase thereafter extracted twice with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. After flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 ⟶ 80:20) iodide **15** (3.64 g, 6.69 mmol, 94 %) was obtained as a colorless oil. **R_f (15)** = 0.30 (SiO_2, PE:EtOAc 90:10); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 1.3 [c = 1.0, $CHCl_3$].



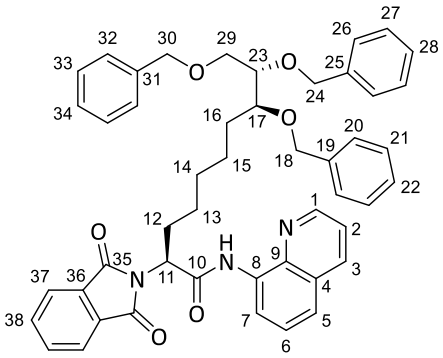
15

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.24 (m, 1 H, 20-H_a), 1.37 – 1.50 (m, 2 H, 19-H_a, 20-H_b), 1.57 (m, 1 H, 19-H_b), 1.71 (m, 2 H, 21-H), 3.08 (t, $^3J_{22,21}$ = 7.1 Hz, 2 H, 22-H), 3.50 (dt, $^3J_{13,7}$ = 8.1 Hz, $^3J_{13,19}$ = 4.4 Hz, 1 H, 13-H), 3.61 (dd, $^2J_{6a,6b}$ = 10.9 Hz, $^3J_{6a,7}$ = 7.1 Hz, 1 H, 6-H_a), 3.68 – 3.73 (m, 2 H, 6-H_b, 7-H), 4.49 (d, $^2J_{14a,14b}$ = 11.6 Hz, 1 H, 14-H_a), 4.51 (s, 2 H, 5-H), 4.59 (d, $^2J_{14b,14a}$ = 11.6 Hz, 1 H, 14-H_b), 4.62 (d, $^2J_{8a,8b}$ = 12.0 Hz, 1 H, 8-H_a), 4.77 (d, $^2J_{8b,8a}$ = 12.0 Hz, 1 H, 8-H_b), 7.24 – 7.39 (m, 15 H, 1-H, 2-H, 3-H, 10-H, 11-H, 12-H, 16-H, 17-H, 18-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 6.9 (t, C-22), 26.9 (t, C-20), 29.0 (t, C-19), 34.4 (t, C-21), 70.3 (t, C-6), 72.8 (t, C-14), 72.9 (t, C-8), 73.4 (t, C-5), 78.7 (d, C-13), 78.9 (d, C-7), 127.6, 127.6, 127.6 (3d, C-1, C-12, C-18), 127.7, 128.0, 128.1 (3d, C-3, C-10, C-16), 128.3, 128.3, 128.4 (3d, C-2, C-11, C-17), 138.2 (s, C-4), 138.5 (s, C-15), 138.7 (s, C-9).

HRMS (CI) calculated for $C_{28}H_{34}IO_3$ $[M+H]^+$: 435.2530, found: 435.2531.

(2*S*,8*S*,9*S*)-8,9,10-Tris(benzyloxy)-2-(1,3-dioxoisoindolin-2-yl)-*N*-(quinolin-8-yl)decanamide (16)

Silver carbonate (31.2 mg, 113 µmol, 80 mol%), palladium acetate (3.2 mg, 14.0 µmol, 10 mol%) and dibenzyl phosphate (11.8 mg, 42.0 µmol, 30 mol%) were added successively to a solution of iodide **15** (100 mg, 184 µmol, 1.3 equiv) and alanine derivative **6** (48.8 mg, 141 µmol, 1.0 equiv) in a *tert*-amyl alcohol/1,2-dichloroethane mixture (1:1, 1.06 mL) and the resulting suspension was stirred in a sealed tube at 60 °C for 22 hours. Subsequently, the brown suspension was concentrated in vacuo and the crude product purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 ⟶ 70:30) to afford compound **16** (83.1 mg, 109 µmol, 77 %) as a colorless resin. **R_f (16)** = 0.34 (SiO_2, PE:EtOAc 60:40); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 2.9 [c = 0.5, $CHCl_3$].



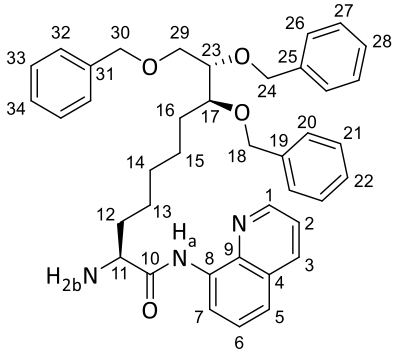
16

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.15 (m, 1 H, 15-H_a), 1.25 – 1.46 (m, 6 H, 13-H, 14-H, 15-H_b, 16-H_a), 1.55 (m, 1 H, 16-H_b), 2.33 (m, 1 H, 12-H_a), 2.55 (m, 1 H, 12-H_b), 3.48 (dt, $^3J_{17,23}$ = 8.0 Hz, $^3J_{17,16}$ = 4.4 Hz, 1 H, 17-H), 3.60 (dd, $^2J_{29a,29b}$ = 11.0 Hz, $^3J_{29a,23}$ = 7.3 Hz, 1 H, 29-H_a), 3.66 – 3.72 (m, 2 H, 23-H, 29-H_b), 4.48 (d, $^2J_{18a,18b}$ = 11.6 Hz, 1 H, 18-H_a), 4.49 (s, 2 H, 30-H), 4.55 (d, $^2J_{18b,18a}$ = 11.6 Hz, 1 H, 18-H_b), 4.60 (d, $^2J_{24a,24b}$ = 12.0 Hz, 1 H, 24-H_a), 4.74 (d, $^2J_{24b,24a}$ = 12.0 Hz, 1 H, 24-H_b), 5.10 (dd, $^3J_{11,12b}$ = 11.0 Hz, $^3J_{11,12a}$ = 5.3 Hz, 1 H, 11-H), 7.20 – 7.35 (m, 15 H, 20-H, 21-H, 22-H, 26-H, 27-H, 28-H, 32-H, 33-H, 34-H), 7.40 (dd, $^3J_{2,3}$ = 8.3 Hz, $^3J_{2,1}$ = 4.3 Hz, 1 H, 2-H), 7.48 – 7.54 (m, 2 H, 5-H, 6-H), 7.75 (m, 2 H, 38-H), 7.90 (m, 2 H, 37-H), 8.13 (dd, $^3J_{3,2}$ = 8.3 Hz, $^4J_{3,1}$ = 1.6 Hz, 1 H, 3-H), 8.69 (dd, $^3J_{1,2}$ = 4.3 Hz, $^4J_{1,3}$ = 1.6 Hz, 1 H, 1-H), 8.72 (m, 1 H, 7-H), 10.33 (s, 1 H, N-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 25.7 (t, C-15), 26.6 (t, C-13), 28.7 (t, C-12), 29.1 (t, C-14), 29.9 (t, C-16), 55.2 (d, C-11), 70.4 (t, C-29), 72.7 (t, C-18), 72.9 (t, C-24), 73.3 (t, C-30), 78.9 (d, C-17), 78.9 (d, C-23), 116.7 (d, C-7), 121.6 (d, C-2), 121.9 (d, C-5), 123.6 (d, C-37), 127.3 (d, C-6), 127.5, 127.6, 128.0, 128.0, 128.2, 128.3 (9d, C-20, C-21, C-22, C-26, C-27, C-28, C-32, C-33, C-34), 127.8 (s, C-4), 131.8 (s, C-36), 133.9 (s, C-8), 134.2 (d, C-38), 136.2 (d, C-3), 138.3 (s, C-31), 138.5 (s, C-9), 138.6 (s, C-19), 138.7 (s, C-25), 148.3 (d, C-1), 167.0 (s, C-10), 168.1 (s, C-35).

HRMS (CI) calculated for $C_{48}H_{48}N_3O_6$ $[M+H]^+$: 762.3538, found: 762.3579.

(2*S*,8*S*,9*S*)-2-Azido-8,9,10-tris(benzyloxy)-*N*-(quinolin-8-yl)decanamide (17)

DIPEA (312 µL, 231 mg, 1.79 mmol, 1.0 equiv) and hydrazine hydrate (105 µL, 107 mg, 2.15 mmol, 1.2 equiv) were added successively to a solution of compound **16** (1.36 g, 1.79 mmol, 1.0 equiv) in ethanol (12.3 mL) and the resulting mixture was stirred at 80 °C for 20 hours. Subsequently, the reaction mixture was concentrated and the crude product purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 50:50) to afford the free amine (1.06 g, 1.68 mmol, 94 %) as a colorless resin. $\mathbf{R_f}$ = 0.15 (SiO_2, PE:EtOAc 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = + 3.6 [c = 1.0, $CHCl_3$].



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.19 (m, 1 H, 15-H_a), 1.27 (m, 2 H, 14-H), 1.32 – 1.46 (m, 4 H, 13-H, 15-H_b, 16-H_a), 1.56 (m, 1 H, 16-H_b), 1.66 (m, 1 H, 12-H_a), 1.69 (bs, 2 H, N-H_b), 1.95 (m, 1 H, 12-H_b), 3.49 (dt, $^3J_{17,23}$ = 8.1 Hz, $^3J_{17,16}$ = 4.3 Hz, 1 H, 17-H), 3.57 – 3.64 (m, 2 H, 11-H, 29-H_a), 3.67 – 3.73 (m, 2 H, 23-H, 29-H_b), 4.48 (d, $^2J_{18a,18b}$ = 11.6 Hz, 1 H, 18-H_a), 4.50 (s, 2 H, 30-H), 4.56 (d, $^2J_{18b,18a}$ = 11.6 Hz, 1 H, 18-H_b), 4.61 (d, $^2J_{24a,24b}$ = 11.9 Hz, 1 H, 24-H_a), 4.75 (d, $^2J_{24b,24a}$ = 11.9 Hz, 1 H, 24-H_b), 7.21 – 7.36 (m, 15 H,

20-H, 21-H, 22-H, 26-H, 27-H, 28-H, 32-H, 33-H, 34-H), 7.44 (dd, $^3J_{2,3}$ = 8.2 Hz, $^3J_{2,1}$ = 4.2 Hz, 1 H, 2-H), 7.49 – 7.57 (m, 2 H, 5-H, 6-H), 8.15 (dd, $^3J_{3,2}$ = 8.3 Hz, $^4J_{3,1}$ = 1.7 Hz, 1 H, 3-H), 8.85 (dd, $^3J_{7,6}$ = 7.3 Hz, $^3J_{7,5}$ = 1.7 Hz, 1 H, 7-H), 8.87 (dd, $^3J_{1,2}$ = 4.2 Hz, $^4J_{1,3}$ = 1.7 Hz, 1 H, 1-H), 11.38 (s, 1 H, N-H$_a$). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ = 25.8 (t, C-15), 25.8 (t, C-13), 29.4 (t, C-14), 30.0 (t, C-16), 35.2 (t, C-12), 56.5 (d, C-11), 70.4 (t, C-29), 72.7 (t, C-18), 72.9 (t, C-24), 73.3 (t, C-30), 79.0 (d, C-17), 79.0 (d, C-23), 116.4 (d, C-7), 121.5 (d, C-2), 121.6 (d, C-5), 127.3 (d, C-6), 127.5, 127.5, 127.6, 128.0, 128.0, 128.2, 128.3 (9d, C-20, C-21, C-22, C-26, C-27, C-28, C-32, C-33, C-34), 128.1 (s, C-4), 134.4 (s, C-8), 136.2 (d, C-3), 138.3 (s, C-31), 138.6 (s, C-19), 138.7 (s, C-25), 139.0 (s, C-9), 148.5 (d, C-1), 174.1 (s, C-10).

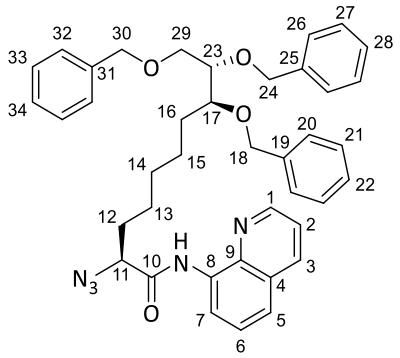
HRMS (CI) calculated for $C_{40}H_{46}N_3O_4$ $[M+H]^+$: 632.3483, found: 632.3502.

Preparation of triflyl azide:

Toluene (1.0 mL) was added to a solution of sodium azide (404 mg, 6.21 mmol, 1.6 equiv) in water (1.0 mL) and the biphasic mixture was cooled to 0 °C. Subsequently, Tf_2O (660 µL, 1.10 g, 3.91 mmol, 1.0 equiv) was added dropwise and the mixture was stirred for 30 minutes at 0 °C and for 2 hours at 10 °C. $NaHCO_3$ solution (saturated) was added to the triflyl azide mixture until the gas evolution was over followed by phase separation. The aqueous phase was extracted twice with toluene (2x 1 mL) and the organic phases were combined to yield the triflyl azide solution (ca. 1.3 M in toluene).

Diazotransfer reaction:

$NaHCO_3$ (182 mg, 2.17 mmol, 4.0 equiv), copper sulfate pentahydrate (5.8 mg, 23.0 µmol, 4.3 mol%), triflyl azide solution (1.30 mL, 1.66 mmol, 3.1 equiv) and methanol (4.7 mL) were added successively to a mixture of the free amine (339 mg, 537 µmol, 1.0 equiv) in water (637 µL) and the reaction mixture was stirred at room temperature for 2 hours. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 70:30, then C18, H_2O:MeCN 100:0 → 0:100) to afford azide **17** (338 mg, 513 µmol, 96 %) as a colorless resin. **R_f (17)** = 0.20 (SiO_2, PE:EtOAc 80:20); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 20.3 [c = 1.0, $CHCl_3$].



17

^{1}H-NMR (400 MHz, CDCl$_3$): δ = 1.16 (m, 1 H, 15-H$_a$), 1.27 (m, 2 H, 14-H), 1.33 – 1.51 (m, 4 H, 13-H, 15-H$_b$, 16-H$_a$), 1.56 (m, 1 H, 16-H$_b$), 1.99 (m, 2 H, 12-H), 3.48 (dt, $^3J_{17,23}$ = 8.1 Hz, $^3J_{17,16}$ = 4.4 Hz, 1 H, 17-H), 3.60 (dd, $^2J_{29a,29b}$ = 10.9 Hz, $^3J_{29a,23}$ = 7.1 Hz, 1 H, 29-H$_a$), 3.67 – 3.72 (m, 2 H, 23-H, 29-H$_b$), 4.19 (dd, $^3J_{11,12a}$ = 7.2 Hz, $^3J_{11,12b}$ = 4.9 Hz, 1 H, 11-H), 4.48 (d, $^2J_{18a,18b}$ = 11.5 Hz, 1 H, 18-H$_a$), 4.50 (s, 2 H, 30-H), 4.56 (d, $^2J_{18b,18a}$ = 11.5 Hz, 1 H, 18-H$_b$), 4.60 (d, $^2J_{24a,24b}$ = 11.9 Hz, 1 H, 24-H$_a$), 4.75 (d, $^2J_{24b,24a}$ = 11.9 Hz, 1 H, 24-H$_b$), 7.21 – 7.36 (m, 15 H, 20-H, 21-H, 22-H, 26-H, 27-H, 28-H, 32-H, 33-H, 34-H), 7.47 (dd, $^3J_{2,3}$ = 8.2 Hz, $^3J_{2,1}$ = 4.2 Hz, 1 H, 2-H), 7.52 – 7.57 (m, 2 H, 5-H, 6-H), 8.17 (dd, $^3J_{3,2}$ = 8.2 Hz, $^4J_{3,1}$ = 1.6 Hz, 1 H, 3-H), 8.78 (m, 1 H, 7-H), 8.87 (dd, $^3J_{1,2}$ = 4.2 Hz, $^4J_{1,3}$ = 1.6 Hz, 1 H, 1-H), 10.61 (s, 1 H, N-H). **^{13}C-NMR (100 MHz, CDCl$_3$):** δ = 25.4 (t, C-13), 25.7 (t, C-15), 29.3 (t, C-14), 29.9 (t, C-16), 32.4 (t, C-12), 65.1 (d, C-11), 70.4 (t, C-29), 72.7 (t, C-18), 72.9 (t, C-24), 73.3 (t, C-30), 78.9 (d, C-17), 78.9 (d, C-23), 116.7 (d, C-7), 121.7 (d, C-2), 122.3 (d, C-5), 127.2 (d, C-6), 127.5, 127.6, 128.0, 128.0, 128.2, 128.3 (9d, C-20, C-21, C-22, C-26, C-27, C-28, C-32, C-33, C-34), 128.0 (s, C-4), 133.7 (s, C-8), 136.3 (d, C-3), 138.3 (s, C-31), 138.6 (s, C-19), 138.7 (s, C-9), 138.7 (s, C-25), 148.6 (d, C-1), 168.0 (s, C-10).

HRMS (CI) calculated for $C_{40}H_{43}N_5O_4$ $[M]^+$: 657.3310, found: 657.3314.

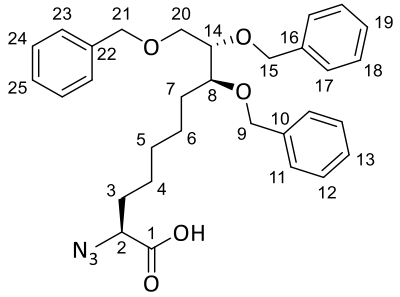
Methyl (2*S*,8*S*,9*S*)-2-azido-8,9,10-tris(benzyloxy)decanoate (18)

Boc-activation of the amide:

4-Dimethylaminopyridine (105 mg, 859 µmol, 64 mol%) and Boc_2O (1.18 g, 5.41 mmol, 4.0 equiv) were added successively to a solution of azide **17** (889 mg, 1.35 mmol, 1.0 equiv) in acetonitrile (13.5 mL) and the red colored reaction mixture was stirred at room temperature for 16 hours. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 70:30) to afford the Boc-protected derivative as a colorless resin.

Aminoquinoline-cleavage:

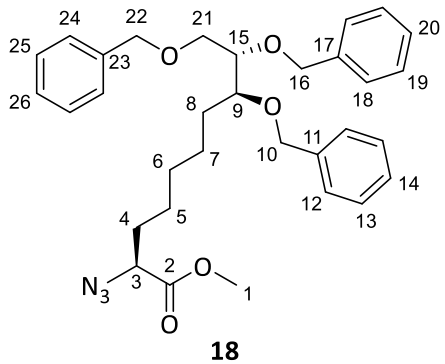
Lithium hydroxide monohydrate (114 mg, 2.70 mmol, 2.0 equiv) in water (6.8 mL) and hydrogen peroxide (30 wt% in water, 678 µL, 6.76 mmol, 5.0 equiv) were added dropwise at 0 °C to a solution of the previously synthesized Boc-protected derivative in THF (13.5 mL) and the reaction mixture was stirred at 0°C for 60 minutes. The remaining peroxide was reduced with Na_2SO_3 solution (1 M) and the mixture was acidified with aqueous HCl (1 M). After threefold extraction mit ethyl acetate, the combined organic phases were dried over Na_2SO_4. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc+1 % HOAc 100:0 → 0:100) to afford the carboxylic acid (678 mg, 1.28 mmol, 94 % over 2 steps) as a yellow resin. $\mathbf{R_f}$ = 0.27 (SiO_2, PE:EtOAc:HOAc 70:30:1); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = – 5.6 [c = 1.0, $CHCl_3$].



^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.08 – 1.25 (m, 3 H, 5-H, 6-H_a), 1.28 – 1.46 (m, 4 H, 4-H, 6-H_b, 7-H_a), 1.56 (m, 1 H, 7-H_b), 1.74 (m, 2 H, 3-H), 3.50 (dt, $^3J_{8,14}$ = 8.1 Hz, $^3J_{8,7}$ = 4.3 Hz, 1 H, 8-H), 3.62 (dd, $^2J_{20a,20b}$ = 10.8 Hz, $^3J_{20a,14}$ = 7.1 Hz, 1 H, 20-H_a), 3.68 – 3.74 (m, 2 H, 14-H, 20-H_b), 3.80 (dd, $^3J_{2,3a}$ = 8.3 Hz, $^3J_{2,3b}$ = 5.0 Hz, 1 H, 2-H), 4.49 (d, $^2J_{9a,9b}$ = 11.8 Hz, 1 H, 9-H_a), 4.51 (s, 2 H, 21-H), 4.58 (d, $^2J_{9b,9a}$ = 11.8 Hz, 1 H, 9-H_b), 4.61 (d, $^2J_{15a,15b}$ = 12.0 Hz, 1 H, 15-H_a), 4.76 (d, $^2J_{15b,15a}$ = 12.0 Hz, 1 H, 15-H_b), 7.23 – 7.35 (m, 15 H, 11-H, 12H, 13-H, 17-H, 18-H, 19-H, 23-H, 24-H, 25-H), 9.02 (bs, 1 H, COO-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 25.5 (t, C-6), 25.6 (t, C-4), 28.9 (t, C-5), 29.8 (t, C-7), 31.1 (t, C-3), 61.7 (d, C-2), 70.2 (t, C-20), 72.7 (t, C-9), 72.8 (t, C-15), 73.3 (t, C-21), 78.8 (d, C-8), 78.9 (d, C-14), 127.6, 127.6, 127.6, 127.7, 128.0, 128.1, 128.3, 128.3 (9d, C-11, C-12, C-13, C-17, C-18, C-19, C-23, C-24, C-25), 138.1 (s, C-22), 138.4 (s, C-10), 138.5 (s, C-16), 175.6 (s, C-1).

HRMS (CI) calculated for $C_{31}H_{38}N_3O_5$ $[M+H]^+$: 532.2806, found: 532.2818.

Potassium carbonate (255 mg, 1.85 mmol, 2.5 equiv) and methyl iodide (139 µL, 315 mg, 2.22 mmol, 3.0 equiv) were added at 0 °C to a solution of the carboxylic acid (393 mg, 739 µmol, 1.0 equiv) in DMF (7.4 mL) and the reaction mixture was warmed to room temperature over 3 hours. Subsequently, the solution was hydrolyzed with water and thereafter extracted three times with ethyl acetate. The combined organics were dried over Na_2SO_4 and the solvent was removed under reduced pressure. After flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 80:20) methyl ester **18** (364 mg, 667 µmol, 90 %) was obtained as a colorless oil. $\mathbf{R_f}$ **(18)** = 0.39 (SiO_2, PE:EtOAc 80:20); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = – 8.0 [c = 0.5, $CHCl_3$].



18

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.10 – 1.27 (m, 3 H, 6-H, 7-H_a), 1.28 – 1.46 (m, 4 H, 5-H, 7-H_b, 8-H_a), 1.56 (m, 1 H, 8-H_b), 1.73 (m, 2 H, 4-H), 3.50 (dt, $^3J_{9,15}$ = 8.2 Hz, $^3J_{9,8}$ = 4.3 Hz, 1 H, 9-H), 3.62 (dd, $^2J_{21a,21b}$ = 10.9 Hz, $^3J_{21a,15}$ = 7.2 Hz, 1 H, 21-H_a), 3.68 – 3.74 (m, 2 H, 15-H, 21-H_b), 3.75 – 3.80 (m, 4 H, 1-H, 3-H), 4.49 (d, $^2J_{10a,10b}$ = 11.6 Hz, 1 H, 10-H_a), 4.51 (s, 2 H, 22-H), 4.58 (d, $^2J_{10b,10a}$ = 11.6 Hz, 1 H, 10-H_b), 4.62 (d, $^2J_{16a,16b}$ = 12.0 Hz, 1 H, 16-H_a), 4.76 (d, $^2J_{16b,16a}$ = 12.0 Hz, 1 H, 16-H_b), 7.24 – 7.37 (m, 15 H, 12-H, 13-H, 14-H, 18-H, 19-H, 20-H, 24-H, 25-H, 26-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 25.6 (t, C-7), 25.6 (t, C-5), 29.0 (t, C-6), 29.9 (t, C-8), 31.2 (t, C-4), 52.5 (q, C-1), 61.9 (d, C-3), 70.4 (t, C-21), 72.7 (t, C-10), 72.9 (t, C-16), 73.4 (t, C-22), 78.9 (d, C-9), 78.9 (d, C-15), 127.5, 127.5, 127.6, 127.6, 128.0, 128.0, 128.2, 128.3, 128.3 (9d, C-12, C-13, C-14, C-18, C-19, C-20, C-24, C-25, C-26), 138.3 (s, C-23), 138.6 (s, C-11), 138.7 (s, C-17), 171.1 (s, C-2).

HRMS (CI) calculated for $C_{32}H_{40}NO_5$ $[M\text{-}N_2+H]^+$: 518.2901, found: 518.2910.

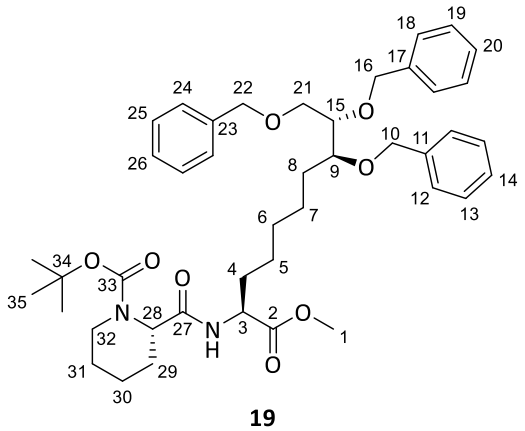
tert-Butyl (*S*)-2-{[(2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-1-methoxy-1-oxodecan-2-yl]carbamoyl}-piperidine-1-carboxylate (19)

Staudinger-Reduction:

Triphenylphosphine (338 mg, 1.29 mmol, 3.0 equiv) and water (166 µL) were added to a solution of azide **18** (235 mg, 430 µmol, 1.0 equiv) in THF (4.1 mL) and the resulting solution was stirred at 50 °C for 3 hours. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 0:100, then C18, H_2O:MeCN 100:0 → 0:100) to afford the free amine (210 mg, 404 µmol, 94 %) as a colorless resin.

Peptide coupling:

TBTU (143 mg, 444 µmol, 1.1 equiv) and DIPEA (85.0 µL, 62.6 mg, 484 µmol, 1.2 equiv) were added to a mixture of the previously synthesized amine (210 mg, 404 µmol, 1.0 equiv) and Boc-L-pipecolic acid (102 mg, 444 µmol, 1.1 equiv) in acetonitrile (4.0 mL) at 0 °C. The reaction mixture was warmed to room temperature over 16 hours before ethyl acetate was added. The mixture was washed with aqueous HCl solution (1 M) and the aqueous phase was extracted three times with ethyl acetate. The combined organics were washed with $NaHCO_3$ solution (saturated), dried over Na_2SO_4 and the solvent was removed in vacuo. After flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 75:25), dipeptide **19** (271 mg, 371 µmol, 92 % over 2 steps) was obtained as a colorless resin. **R_f (19)** = 0.28 (SiO_2, PE:EtOAc 70:30); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 31.3 [c = 0.5, $CHCl_3$].



19

Major rotamer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.10 – 1.30 (m, 5 H, 5-H_a, 6-H, 7-H), 1.30 – 1.45 (m, 3 H, 5-H_b, 8-H_a, 31-H_a), 1.48 (s, 9 H, 35-H), 1.50 – 1.66 (m, 6 H, 4-H_a, 8-H_b, 29-H_a, 30-H, 31-H_b), 1.78 (m, 1 H, 4-H_b), 2.27 (m, 1 H, 29-H_b), 2.75 (m, 1 H, 32-H_a), 3.49 (dt, $^{3}J_{9,15}$ = 8.2 Hz, $^{3}J_{9,8}$ = 4.4 Hz, 1 H, 9-H), 3.61 (dd, $^{2}J_{21a,21b}$ = 10.8 Hz, $^{3}J_{21a,15}$ = 7.2 Hz, 1 H, 21-H_a), 3.68 – 3.72 (m, 2 H, 15-H, 21-H_b), 3.72 (s, 3 H, 1-H), 3.99 (m, 1 H, 32-H_b), 4.49 (d, $^{2}J_{10a,10b}$ = 11.6 Hz, 1 H, 10-H_a), 4.51 (s, 2 H, 22-H), 4.56 (m, 1 H, 3-H), 4.57 (d, $^{2}J_{10b,10a}$ = 11.6 Hz, 1 H, 10-H_b), 4.61 (d, $^{2}J_{16a,16b}$ = 12.0 Hz, 1 H, 16-H_a), 4.74 (m, 1 H, 28-H), 4.75 (d, $^{2}J_{16b,16a}$ = 12.0 Hz, 1 H, 16-H_b), 6.62 (m, 1 H, N-H), 7.24 – 7.36 (m, 15 H, 12-H, 13-H, 14-H, 18-H, 19-H, 20-H, 24-H, 25-H, 26-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 20.5 (t, C-30), 24.8 (t, C-31), 25.3 (t, C-7), 25.4 (t, C-29), 25.7 (t, C-5), 28.3 (q, C-35), 29.2 (t, C-6), 30.0 (t, C-8), 32.5 (t, C-4), 42.2 (t, C-32), 52.0 (d, C-3), 52.2 (q, C-1), 53.7 (d, C-28), 70.4 (t, C-21), 72.7 (t, C-10), 72.9 (t, C-16), 73.3 (t, C-22), 79.0 (d, C-9), 79.0 (d, C-15), 80.6 (s, C-34), 127.5, 127.5, 127.6, 127.9, 128.0, 128.2, 128.3 (9d, C-12, C-13, C-14, C-18, C-19, C-20, C-24, C-25, C-26), 138.3 (s, C-23), 138.6 (s, C-11), 138.7 (s, C-17), 170.9 (s, C-27), 172.6 (s, C-2).

The signal of C-33 is located in the noise.

Minor rotamer (selected signals):

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.15 (m, 1 H, 32-H_b), 6.43 (m, 1 H, N-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 41.3 (t, C-32), 55.4 (d, C-28).

HRMS (CI) calculated for $C_{38}H_{51}N_2O_6$ $[M\text{-}t\text{BuOCO}+2H]^+$: 631.3742, found: 631.3755.

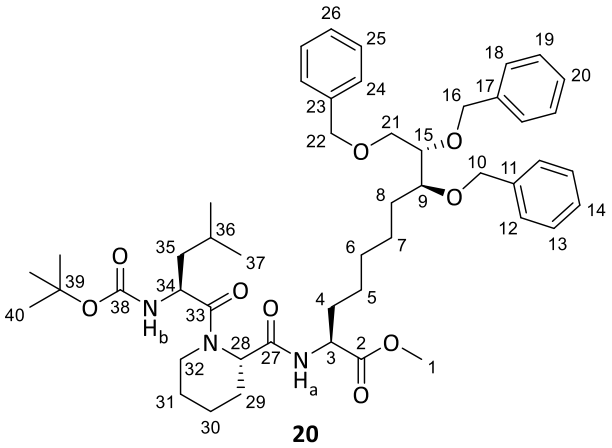
Methyl (2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-2-{(*S*)-1-[(*tert*-butoxycarbonyl)-L-leucyl]piperidine-2-carboxamido}decanoate (20)

Boc-cleavage:

Dipeptide **19** (265 mg, 363 µmol, 1.0 equiv) was dissolved in HCl solution (4 M in 1,4-dioxane, 908 µL, 3.63 mmol, 10.0 equiv) and the solution was stirred at room temperature for 60 minutes. The crude product was used in the next step without further purification.

Peptide coupling:

PyAOP (227 mg, 435 µmol, 1.2 equiv) and DIPEA (158 µL, 117 mg, 907 µmol, 2.5 eq) were added to a mixture of the previously synthesized amine hydrochloride and Boc-L-leucine (227 mg, 435 µmol, 1.2 equiv) in DMF (3.65 mL) at 0 °C. The reaction mixture was warmed to room temperature over 16 hours before ethyl acetate was added. The mixture was washed with $NaHCO_3$ solution (saturated), dried over Na_2SO_4 and the solvent was removed in vacuo. After flash chromatography (C18, H_2O:MeCN 100:0 → 0:100), tripeptide **20** (286 mg, 339 µmol, 93 % over 2 steps) was obtained as a colorless resin. **R_f (20)** = 0.34 (SiO_2, PE:EtOAc 60:40); $[\alpha]_D^{20}$ = – 43.8 [c = 0.5, $CHCl_3$].



20

Major rotamer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.91 (d, $^3J_{37,36}$ = 6.6 Hz, 3 H, 37-H), 0.99 (d, $^3J_{37',36}$ = 6.5 Hz, 3 H, 37-H'), 1.10 – 1.28 (m, 5 H, 5-H_a, 6-H, 7-H), 1.28 – 1.45 (m, 5 H, 5-H_b, 8-H_a, 31-H_a, 35-H), 1.44 (s, 9 H, 40-H), 1.50 – 1.65 (m, 3 H, 4-H_a, 8-H_b, 31-H_b), 1.65 – 1.80 (m, 5 H, 4-H_b, 29-H_a, 30-H, 36-H), 2.21 (m, 1 H, 29-H_b), 3.14 (ddd, $^2J_{32a,32b}$ = $^3J_{32a,31b}$ = 12.4 Hz, $^3J_{32a,31a}$ = 1.6 Hz, 1 H, 32-H_a), 3.49 (dt, $^3J_{9,15}$ = 8.2 Hz, $^3J_{9,8}$ = 4.2 Hz, 1 H, 9-H), 3.61 (dd, $^2J_{21a,21b}$ = 10.9 Hz, $^3J_{21a,15}$ = 7.2 Hz, 1 H, 21-H_a), 3.67 – 3.72 (m, 2 H, 15-H, 21-H_b), 3.72 (s, 3 H, 1-H), 3.82 (m, 1 H, 32-H_b), 4.48 (m, 1 H, 3-H), 4.48 (d, $^2J_{10a,10b}$ = 11.6 Hz, 1 H, 10-H_a), 4.51 (s, 2 H, 22-H), 4.57 (d, $^2J_{10b,10a}$ = 11.6 Hz, 1 H, 10-H_b), 4.61 (d, $^2J_{16a,16b}$ = 12.0 Hz, 1 H, 16-H_a), 4.73 (m, 1 H, 34-H), 4.75 (d, $^2J_{16b,16a}$ = 12.0 Hz, 1 H, 16-H_b), 5.21 (m, 1 H, 28-H), 5.29 (d, $^3J_{NHb,34}$ = 9.1 Hz, 1 H, N-H_b), 6.34 (d, $^3J_{NHa,3}$ = 7.7 Hz, 1 H, N-H_a), 7.24 – 7.36 (m, 15 H, 12-H, 13-H, 14-H, 18-H, 19-H, 20-H, 24-H, 25-H, 26-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 20.2 (t, C-30), 21.6 (q, C-37'), 23.4 (q, C-37), 24.6 (d, C-36), 25.3 (t, C-31), 25.3 (t, C-7), 25.5 (t, C-29), 25.7 (t, C-5), 28.3 (q, C-40), 29.3 (t, C-6), 30.0 (t, C-8), 32.3 (t, C-4), 42.4 (t, C-35), 43.6 (t, C-32), 48.7 (d, C-34), 52.0 (d, C-28), 52.1 (d, C-3), 52.3 (q, C-1), 70.4 (t, C-21), 72.7 (d, C-10), 72.9 (t, C-16), 73.4 (t, C-22), 79.0 (d, C-9), 79.0 (d, C-15), 79.6 (s, C-39), 127.5, 127.6, 127.6, 127.9, 128.0, 128.2, 128.3, 128.3 (9d, C-12, C-13, C-14, C-18, C-19, C-20, C-24, C-25, C-26), 138.3 (s, C-23), 138.6 (s, C-11), 138.7 (s, C-17), 155.5 (s, C-38), 170.4 (s, C-27), 171.9 (s, C-33), 172.6 (s, C-2).

Minor rotamer (selected signals):

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.96 (d, $^3J_{37,36}$ = 6.6 Hz, 3 H, 37-H), 0.96 (d, $^3J_{37',36}$ = 6.5 Hz, 3 H, 37-H'), 1.38 (s, 9 H, 40-H), 1.85 (m, 1 H, 4-H_b), 2.46 (ddd, $^2J_{32a,32b}$ = $^3J_{32a,31b}$ = 13.0 Hz, $^3J_{32a,31a}$ = 1.8 Hz, 1 H, 32-H_a), 2.55 (m, 1 H, 29-H_b), 3.69 (s, 3 H, 1-H), 4.38 – 4.45 (m, 2 H, 3-H, 34-H), 4.51 (m, 1 H, 28-H), 4.62 (m, 1 H, 32-H_b), 4.98 (d, $^3J_{NHb,34}$ = 7.3 Hz, 1 H, N-H_b), 7.97 (d, $^3J_{NHa,3}$ = 7.6 Hz, 1 H, N-H_a). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 20.7 (t, C-30), 23.4 (q, C-37), 24.7 (d, C-36), 25.8 (t, C-5), 26.1 (t, C-29), 26.1 (t, C-7), 28.2 (q, C-40), 29.2 (t, C-6), 30.1 (t, C-8), 30.7 (t, C-4), 40.0 (t, C-32), 40.8 (t, C-35), 49.2 (d, C-34), 53.2 (d, C-3), 56.2 (d, C-28), 70.5 (t, C-21), 79.0 (d, C-9), 79.1 (d, C-15), 80.3 (s, C-39), 138.3 (s, C-23), 138.6 (s, C-11), 155.6 (s, C-38), 169.8 (s, C-27), 172.7 (s, C-2), 173.2 (s, C-33).

HRMS (ESI) calculated for $C_{49}H_{70}N_3O_9$ $[M+H]^+$: 844.5107, found: 844.5119.

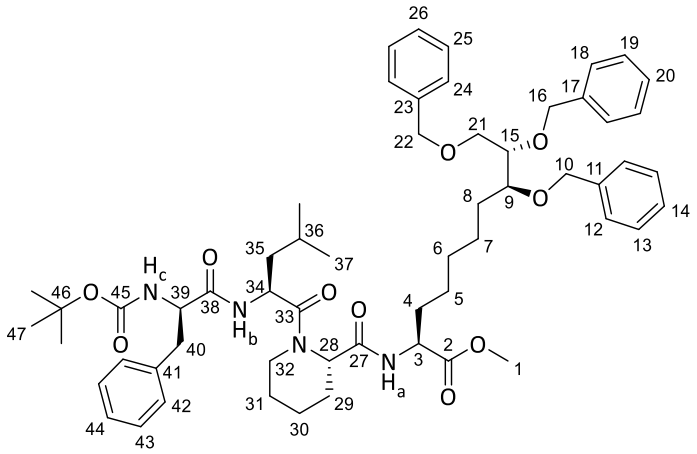
Methyl (2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-2-{(*S*)-1-[(*tert*-butoxycarbonyl)-D-phenylalanyl-L-leucyl]piperidine-2-carboxamido}decanoate (21)

Boc-cleavage:

Tripeptide **20** (274 mg, 324 µmol, 1.0 equiv) was dissolved in DCM (1.4 mL) and thereafter treated at 0 °C with a TFA:triisopropylsilane:water mixture (92.5:5:2.5, 1.4 mL). The resulting solution was stirred at 0 °C for 60 minutes before the solvent was removed under reduced pressure and the crude product was used in the next step without further purification.

Peptide coupling:

PyAOP (186 mg, 356 µmol, 1.1 equiv) and DIPEA (141 µL, 105 mg, 810 µmol, 2.5 equiv) were added to a mixture of the previously synthesized TFA salt and Boc-D-phenylalanine (101 mg, 356 µmol, 1.1 equiv) in DMF (3.24 mL) at 0 °C. The reaction mixture was warmed to room temperature over 16 hours before ethyl acetate was added. The mixture was washed with aqueous HCl solution (1 M) and the aqueous phase was extracted three times with ethyl acetate. The combined organics were washed with $NaHCO_3$ solution (saturated), dried over Na_2SO_4 and the solvent was removed in vacuo. After flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 60:40), tetrapeptide **21** (276 mg, 278 µmol, 86 % over 2 steps) was obtained as a colorless resin. **R_f (21)** = 0.39 (SiO_2, PE:EtOAc 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 37.9 [c = 1.0, $CHCl_3$].



21

Major rotamer:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.85 (d, $^3J_{37,36}$ = 6.4 Hz, 3 H, 37-H), 0.86 (d, $^3J_{37',36}$ = 6.3 Hz, 3 H, 37-H'), 1.20 – 1.67 (m, 17 H, 4-H, 5-H, 6-H, 7-H, 8-H, 29-H_a, 30-H, 31-H, 35-H), 1.32 (s, 9 H, 47-H), 1.71 (m, 1 H, 36-H), 2.16 (m, 1 H, 29-H_b), 2.80 (dd, $^2J_{40a,40b}$ = 13.9 Hz, $^3J_{40a,39}$ = 9.2 Hz, 1 H, 40-H_a), 2.99 (m, 1 H, 40-H_b), 3.33 (m, 1 H, 32-H_a), 3.55 (dt, $^3J_{9,15}$ = 7.8 Hz, $^3J_{9,8}$ = 4.4 Hz, 1 H, 9-H), 3.62 (dd, $^2J_{21a,21b}$ = 10.2 Hz, $^3J_{21a,15}$ = 6.0 Hz, 1 H, 21-H_a), 3.63 (s, 3 H, 1-H), 3.68 – 3.75 (m, 2 H, 15-H, 21-H_b), 3.88 (m, 1 H, 32-H_b), 4.23 – 4.29 (m, 2 H, 34-H, 39-H), 4.50 (d, $^2J_{10a,10b}$ = 11.8 Hz, 1 H, 10-H_a), 4.51 (s, 2 H, 22-H), 4.55 (d, $^2J_{10b,10a}$ = 11.8 Hz, 1 H, 10-H_b), 4.59 (d, $^2J_{16a,16b}$ = 11.9 Hz, 1 H, 16-H_a), 4.69 (d, $^2J_{16b,16a}$ = 11.9 Hz, 1 H, 16-H_b), 4.81 (m, 1 H, 3-H), 5.04 (m, 1 H, 28-H), 6.35 (m, 1 H, N-H_c), 7.15 – 7.65 (m, 20 H, 12-H, 13-H, 14-H, 18-H, 19-H, 20-H, 24-H, 25-H, 26-H, 42-H, 43-H, 44-H), 7.56 (m, 1 H, N-H_b), 7.75 (d, $^3J_{NHa,3}$ = 7.9 Hz, 1 H, N-H_a). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 20.1 (t, C-30), 21.7 (q, C-37'), 23.3 (q, C-37), 24.3 (d, C-36), 25.4 (t, C-31), 25.7 (t, C-7), 26.3 (t, C-5), 28.2 (q, C-47), 29.2 (t, C-29), 29.3 (t, C-4), 30.0 (t, C-6), 32.1 (t, C-8), 39.2 (t, C-40), 42.0 (t, C-35), 43.7 (t, C-32), 48.2 (d, C-34), 52.0 (d, C-39), 52.2 (d, C-3), 52.2 (q, C-1), 56.5 (d, C-28), 70.4 (t, C-21), 72.7 (t, C-10), 72.9 (t, C-16), 73.4 (t, C-22), 79.0 (d, C-15), 79.0 (d, C-9), 80.0 (s, C-46), 126.8, 127.5, 127.6, 127.6, 127.9, 127.9, 127.8, 128.2, 128.3, 128.6, 128.7, 129.2 (12d, C-12, C-13, C-14, C-18, C-19, C-20, C-24, C-25, C-26, C-42, C-43, C-44), 136.5 (s, C-41), 138.3 (s, C-23), 138.6 (s, C-11), 138.7 (s, C-17), 155.1 (s, C-45), 169.6 (s, C-38), 170.7 (s, C-27), 172.3 (s, C-33), 172.8 (s, C-2).

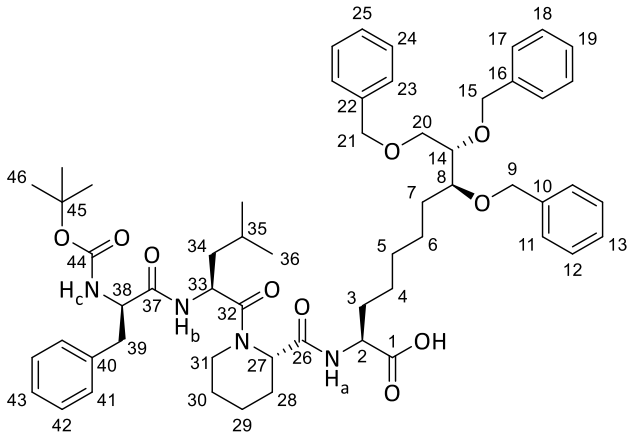
Minor rotamer (selected signals):

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 20.6 (t, C-30), 21.7 (q, C-37'), 23.2 (q, C-37), 24.6 (d, C-36), 24.8 (t, C-31), 25.8 (t, C-7), 26.1 (t, C-5), 30.9 (t, C-8), 40.1 (t, C-35), 40.7 (t, C-40), 46.8 (d, C-34), 53.2 (d, C-3), 55.7 (d, C-28), 70.5 (t, C-21), 79.0 (d, C-15), 79.1 (d, C-9), 136.7 (s, C-41), 138.3 (s, C-23), 138.6 (s, C-11), 138.8 (s, C-17), 170.9 (s, C-33), 172.8 (s, C-2).

HRMS (ESI) calculated for $C_{58}H_{79}N_4O_{10}$ $[M+H]^+$: 991.5791, found: 991.5743.

(3*S*,6*R*,9*S*,15a*S*)-6-Benzyl-9-isobutyl-3-[(6*S*,7*S*)-6,7,8-tris(benzyloxy)octyl]octahydro-2H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecyne-1,4,7,10(3H,12H)-tetraone (22)

A LiOH solution (1 M in water, 334 µL, 334 µmol, 1.2 equiv) was added dropwise at 0 °C to a solution of tetrapeptide **21** (276 mg, 278 µmol, 1.0 equiv) in 1,4-dioxane (2.3 mL) and the resulting solution was warmed to room temperature over 16 hours. Subsequently, the mixture was acidified with aqueous HCl solution (1 M) and extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo to afford the carboxylic acid (263 mg, 269 µmol, 97 %) as a white lyophilisate. $\mathbf{R_f}$ = 0.28 (SiO_2, PE:EtOAc 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = – 32.3 [c = 0.5, $CHCl_3$].



Major rotamer:

^{1}H-NMR (500 MHz, DMSO-d$_6$, 373 K): δ = 0.85 (d, $^3J_{36,35}$ = 6.3 Hz, 3 H, 36-H), 0.86 (d, $^3J_{36',35}$ = 6.3 Hz, 3 H, 36-H'), 1.20 – 1.66 (m, 17 H, 3-H, 4-H, 5-H, 6-H, 7-H, 28-H$_a$, 29-H, 30-H, 34-H), 1.32 (s, 9 H, 46-H), 1.73 (m, 1 H, 35-H), 2.18 (m, 1 H, 28-H$_b$), 2.80 (dd, $^2J_{39a,39b}$ = 13.8 Hz, $^3J_{39a,38}$ = 9.2 Hz, 1 H, 39-H$_a$), 2.99 (m, $^2J_{39b,39a}$ = 13.8 Hz, $^3J_{39b,38}$ = 5.3 Hz, 1 H, 39-H$_b$), 3.27 (m, 1 H, 31-H$_a$), 3.55 (dt, $^3J_{8,14}$ = 7.6 Hz, $^3J_{8,7}$ = 4.6 Hz, 1 H, 8-H), 3.62 (dd, $^2J_{20a,20b}$ = 10.1 Hz, $^3J_{20a,14}$ = 5.8 Hz, 1 H, 20-H$_a$), 3.68 – 3.75 (m, 2 H, 14-H, 20-H$_b$), 3.89 (m, 1 H, 31-H$_b$), 4.19 – 4.29 (m, 2 H, 33-H, 38-H), 4.50 (d, $^2J_{9a,9b}$ = 11.8 Hz, 1 H, 9-H$_a$), 4.50 (s, 2 H, 21-H), 4.55 (d, $^2J_{9b,9a}$ = 11.8 Hz, 1 H, 9-H$_b$), 4.59 (d, $^2J_{15a,15b}$ = 12.1 Hz, 1 H, 15-H$_a$), 4.68 (d, $^2J_{15b,15a}$ = 12.1 Hz, 1 H, 15-H$_b$), 4.81 (m, 1 H, 2-H), 5.05 (m, 1 H, 27-H), 6.36 (m, 1 H, N-H$_c$), 7.15 – 7.36 (m, 21 H, 11-H, 12-H, 13-H, 17-H, 18-H, 19-H, 23-H, 24-H, 25-H, 41-H, 42-H, 43-H, N-H$_b$), 7.75 (d, $^3J_{NHa,3}$ = 8.2 Hz, 1 H, N-H$_a$).
^{13}C-NMR (100 MHz, CDCl$_3$): δ = 20.0 (t, C-29), 21.6 (q, C-36'), 23.3 (q, C-36), 24.6 (d, C-35), 25.4 (t, C-30), 25.7 (t, C-6), 26.3 (t, C-4), 28.2 (q, C-46), 28.2 (t, C-28), 29.3 (t, C-3), 30.0 (t, C-5), 32.0 (t, C-7), 39.1 (t, C-39), 41.5 (t, C-34), 43.8 (t, C-31), 47.1 (d, C-33), 52.2 (d, C-38), 52.2 (d, C-2), 56.8 (d, C-27), 70.4 (t, C-20), 72.7 (t, C-9), 72.9 (t, C-15), 73.3 (t, C-21), 79.0 (d, C-14), 79.0 (d, C-8), 126.8, 127.5, 127.6, 127.6, 127.9, 128.0, 128.2, 128.3, 128.5, 128.6, 129.2 (12d, C-11, C-12, C-13, C-17, C-18, C-19, C-23, C-24, C-25, C-41, C-42, C-43), 138.2 (s, C-40), 138.3 (s, C-22), 138.5 (s, C-10), 138.7 (s, C-16), 155.3 (s, C-44), 170.8 (s, C-37), 172.6 (s, C-26), 174.3 (s, C-32), 174.8 (s, C-1).

The signal of C-45 is located in the noise.

Minor rotamer (selected signals):

^{13}C-NMR (100 MHz, CDCl$_3$): δ = 25.3 (t, C-30), 25.8 (t, C-6), 26.0 (t, C-4), 26.9 (t, C-7), 40.3 (t, C-34), 53.3 (d, C-2), 70.5 (t, C-20), 138.6 (s, C-10), 138.7 (s, C-16).

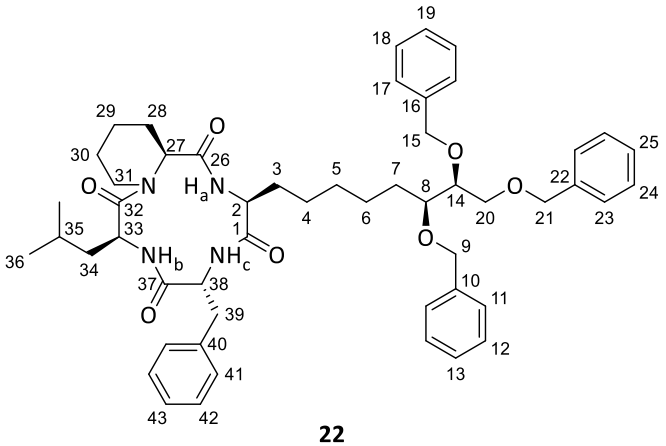
HRMS (ESI) calculated for $C_{57}H_{77}N_4O_{10}$ $[M+H]^+$: 977.5634, found: 977.5661

Boc-cleavage:

The carboxylic acid (263 mg, 269 µmol, 1.0 equiv) was dissolved in DCM (1.1 mL) and thereafter treated at 0 °C with a TFA:triisopropylsilane:water mixture (92.5:5:2.5, 1.1 mL). The resulting solution was stirred at 0 °C for 40 minutes before the solvent was removed under reduced pressure and the crude product was used in the next step without further purification.

Cyclization:

A solution of the previously synthesized TFA salt in DMF (20 mL) was added dropwise at 0 °C over 4 hours to a solution of PyAOP (1.40 g, 2.69 mmol, 10.0 equiv), HOAt (366 mg, 2.69 mmol, 10.0 equiv) and DIPEA (470 µL, 348 mg, 2.69 mmol, 10.0 equiv) in DMF (250 mL)and the reaction mixture was thereafter warmed to room temperature over 16 hours. After solvent removal, the residue was dissolved in ethyl acetate and was washed with $NaHCO_3$ solution (saturated). The aqueous phase was extracted with ethyl acetate ad the combined organic phases were dried over Na_2SO_4. After solvent removal, the crude product was purified by flash chromatography (SiO_2, cyclohexane:EtOAc 100:0 → 60:40) to afford cyclopeptide **22** (145 mg, 168 µmol, 63 % over 2 steps) as a white lyophilisate. **R_f (22)** = 0.43 (SiO_2, PE:EtOAc 50:50); $[\alpha]_D^{20}$ = – 51.1 [c = 0.5, $CHCl_3$].



22

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.80 (d, $^3J_{36,35}$ = 6.5 Hz, 3 H, 36-H), 0.81 (d, $^3J_{36',35}$ = 6.4 Hz, 3 H, 36-H'), 1.06 – 1.30 (m, 7 H, 4-H_a, 5-H, 6-H, 29-H_a, 35-H), 1.30 – 1.45 (m, 3 H, 4-H_b, 7-H_a, 30-H_a), 1.46 – 1.62 (m, 2 H, 3-H_a, 7-H_b), 1.62 – 1.82 (m, 4 H, 3-H_b, 28-H_a, 29-H_b, 30-H_b), 2.48 (m, 1 H, 28-H_b), 2.66 (m, 1 H, 31-H_a), 2.87 (dd, $^2J_{39a,39b}$ = 14.0 Hz, $^3J_{39a,38}$ = 6.7 Hz, 1 H, 39-H_a), 3.25 (dd, $^2J_{39b,39a}$ = 14.0 Hz, $^3J_{39b,38}$ = 8.8 Hz, 1 H, 39-H_b), 3.49 (dt, $^3J_{8,14}$ = 8.2 Hz, $^3J_{8,7}$ = 4.3 Hz, 1 H, 8-H), 3.61 (dd, $^2J_{20a,20b}$ = 10.9 Hz, $^3J_{20a,14}$ = 7.1 Hz, 1 H, 20-H_a), 3.67 – 3.73 (m, 2 H, 14-H, 20-H_b), 4.45 (m, 1 H, 2-H), 4.49 (d, $^2J_{9b,9a}$ = 11.6 Hz, 1 H, 9-H_a), 4.51 (s, 2 H, 21-H), 4.57 (d, $^2J_{9b,9a}$ = 11.6 Hz, 1 H, 9-H_b), 4.58 (m, 1 H, 31-H_b), 4.61 (d, $^2J_{15a,15b}$ = 12.0 Hz, 1 H, 15-H_a), 4.65 (m, 1 H, 38-H), 4.75 (d, $^2J_{15b,15a}$ = 12.0 Hz, 1 H, 15-H_b), 4.76 (m, 1 H, 27-H), 4.91 (m, 1 H, 33-H), 5.83 (m, 2 H, N-H_a, N-H_b), 6.15 (d, $^3J_{NHc,38}$ = 10.8 Hz, 1 H, N-H_c), 7.16 – 7.36 (m, 20 H, 11-H, 12-H, 13-H, 17-H, 18-H, 19-H, 23-H, 24-H, 25-H, 41-H, 42-H, 43-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 20.7 (t, C-29), 22.0 (q, C-36'), 23.1 (q, C-36), 23.9 (d, C-35), 24.8 (t, C-30), 25.6 (t, C-6), 25.8 (t, C-4), 26.8 (t, C-28), 27.7 (t, C-3), 29.3 (t, C-5), 29.9 (t, C-7), 34.8 (t, C-39), 40.5 (t, C-31), 41.1 (t, C-34), 47.0 (d, C-33), 54.1 (d, C-2), 55.5 (d, C-38), 57.1 (d, C-27), 70.4 (t, C-20), 72.7 (t, C-9), 72.9 (t, C-15), 73.3 (t, C-21), 79.0 (d, C-14), 79.0 (d, C-8), 126.6, 127.5, 127.5, 127.6, 127.9, 128.0, 128.2, 128.3, 128.3, 128.4, 129.2 (12d, C-11, C-12, C-13, C-17, C-18, C-19, C-23, C-24, C-25, C-41, C-42, C-43), 136.7 (s, C-40), 138.3 (s, C-22), 138.6 (s, C-10), 138.7 (s, C-16), 169.5 (s, C-37), 171.2 (s, C-32), 174.3 (s, C-1), 174.5 (s, C-26).

HRMS (ESI) calculated for $C_{52}H_{67}N_4O_7$ $[M+H]^+$: 859.5004, found: 859.5005.

(3*S*,6*R*,9*S*,15a*S*)-6-Benzyl-3-{(*S*)-6-hydroxy-6-[(*S*)-oxiran-2-yl]hexyl}-9-isobutyloctahydro-2H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecyne-1,4,7,10(3H,12H)-tetraone (23)

Hydrogenation:

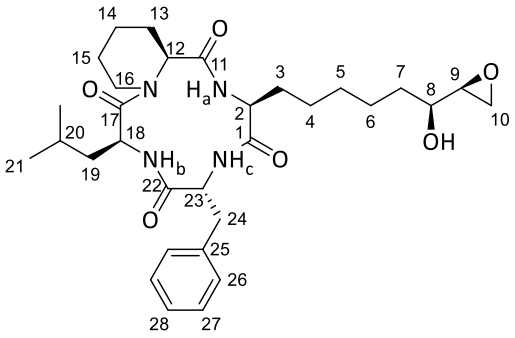
A solution of cyclopeptide **22** (145 mg, 168 µmol, 1.0 equiv) in methanol (3.4 mL) was treated with palladium on charcoal (10 wt% Pd, 30.0 mg) and the resulting suspension was stirred under hydrogen atmosphere (20 bar) at room temperature for 16 hours. The reaction mixture was filtered through a syringe filter and the filtrate was concentrated in vacuo. After flash chromatography (C18, H_2O:MeCN 100:0 → 0:100) the triol (87.8 mg, 149 µmol, 89 %) was obtained as a white lyophilisate.

Mesylation:

2,4,6-Collidine (97.0 µL, 89.0 mg, 732 µmol, 10.0 equiv) and mesyl chloride (6.9 µL, 10.1 mg, 88.6 µmol, 1.2 equiv) were added at 0 °C to a solution of the previously synthesized triol (43.1 mg, 73.0 µmol, 1.0 equiv) in DCM (732 µL) and the reaction mixture was stirred at 0 °C for 21 hours. Subsequently, the solution was diluted with methanol and the solvent was removed under reduced pressure. The crude product was used in the next step without further purification.

Ring closure:

The previously synthesized crude product was dissolved in methanol (14.6 mL) and the resulting solution was treated with DBU (55.0 µL, 55.6 mg, 365 µmol, 5.0 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 5.5 h before removing the solvent in vacuo. After flash chromatography (C18, H_2O:MeCN 100:0 → 0:100) and preparative HPLC (Phenomenex Luna, H_2O:MeCN 100:0 → 0:100) epoxide **23** (13.3 mg, 23.0 µmol, 32 %) was obtained as a white lyophilisate. A significant amount of the triol intermediate (6.2 mg, 10.5 µmol, 14 %) could be recovered. **R_f (23)** = 0.25 (SiO_2, DCM:MeOH 95:5); $[\alpha]_D^{20}$ = – 97.8 [c = 1.0, $CHCl_3$].



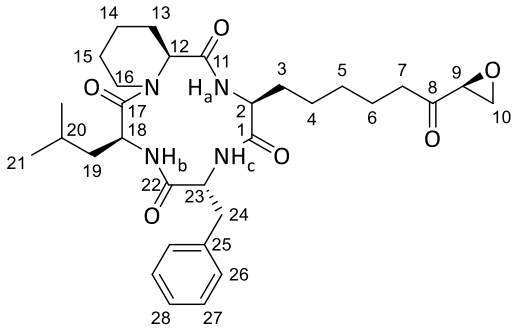
23

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.80 (d, $^3J_{21,20}$ = 6.6 Hz, 3 H, 21-H), 0.81 (d, $^3J_{21',20}$ = 6.6 Hz, 3 H, 21-H'), 1.16 (m, 1 H, 20-H), 1.20 – 1.45 (m, 7 H, 4-H, 5-H, 6-H_a, 14-H_a, 15-H_a), 1.45 – 1.69 (m, 7 H, 3-H_a, 6-H_b, 7-H, 13-H_a, 19-H), 1.70 – 1.79 (m, 2 H, 14-H_b, 15-H_b), 1.83 (m, 1 H, 3-H_b), 2.12 (bs, 1 H, O-H), 2.48 (m, 1 H, 13-H_b), 2.68 (ddd, $^2J_{16a,16b}$ = $^3J_{16a,15a}$ = 13.4 Hz, $^3J_{16a,15b}$ = 2.9 Hz, 1 H, 16-H_a), 2.71 (dd, $^2J_{10a,10b}$ = 4.9 Hz, $^3J_{10a,9}$ = 2.9 Hz, 1 H, 10-H_a), 2.82 (dd, $^2J_{10b,10a}$ = 4.9 Hz, $^3J_{10b,9}$ = 4.3 Hz, 1 H, 10-H_b), 2.88 (dd, $^2J_{24a,24b}$ = 13.9 Hz, $^3J_{24a,23}$ = 6.8 Hz, 1 H, 24-H_a), 2.97 (ddd, $^3J_{9,8}$ = 5.0 Hz, $^3J_{9,10b}$ = 4.3 Hz, $^3J_{9,10a}$ = 2.9 Hz, 1 H, 9-H), 3.25 (dd, $^2J_{24b,24a}$ = 13.9 Hz, $^3J_{24b,23}$ = 8.9 Hz, 1 H, 24-H_b), 3.42 (m, 1 H, 8-H), 4.52 (ddd, $^3J_{2,3b}$ = 10.2 Hz, $^3J_{2,3a}$ = $^3J_{2,NHa}$ = 7.7 Hz, 1 H, 2-H), 4.59 (m, 1 H, 16-H_b), 4.64 (ddd, $^3J_{23,NHc}$ = 10.8 Hz, $^3J_{23,24b}$ = 8.9 Hz, $^3J_{23,24a}$ = 6.8 Hz, 1 H, 23-H), 4.80 (m, 1 H, 12-H), 4.91 (td, $^3J_{18,19}$ = 8.9 Hz, $^3J_{18,NHb}$ = 6.1 Hz, 1 H, 18-H), 5.95 – 6.15 (m, 2 H, N-H_a, N-H_b), 6.29 (d, $^3J_{NHc,23}$ = 10.8 Hz, 1 H, N-H_c), 7.16 – 7.26 (m, 5 H, 26-H, 27-H, 28-H). **^{13}C-NMR (125 MHz, $CDCl_3$):** δ = 20.8 (t, C-14), 22.1 (q, C-21'), 23.1 (q, C-21), 24.0 (d, C-20), 24.8 (t, C-15), 25.0 (t, C-6), 25.5 (t, C-4), 26.8 (t, C-13), 27.6 (t, C-3), 29.1 (t, C-5), 34.2 (t, C-7), 34.8 (t, C-24), 40.5 (t, C-16), 41.1 (t, C-19), 45.2 (t, C-10), 47.0 (d, C-18), 54.2 (d, C-2), 55.4 (d, C-9), 55.5 (d, C-23), 57.2 (d, C-12), 71.5 (d, C-8), 126.7 (d, C-28), 128.4 (d, C-27), 129.2 (d, C-26), 136.7 (s, C-25), 169.9 (s, C-22), 171.3 (s, C-17), 174.3 (s, C-1), 174.6 (s, C-11).

HRMS (CI) calculated for $C_{31}H_{47}N_4O_6$ $[M+H]^+$: 571.3490, found: 571.3508.

WF-3161

DMP (10.1 mg, 23.8 µmol, 2.0 equiv) was added to a solution of epoxide **23** (6.8 mg, 11.9 µmol, 1.0 equiv) in DCM (1.2 mL) and the reaction mixture was stirred at room temperature for 2 hours. After solvent removal, the crude product was purified by flash chromatography (C18, H_2O:MeCN 100:0 → 0:100) and preparative HPLC (Phenomenex Luna, H_2O:MeCN 100:0 → 0:100) to afford **WF-3161** (3.9 mg, 6.86 µmol, 58 %) as a white lyophilisate. $[\alpha]_D^{20}$ = – 115.5 [c = 0.5, $CHCl_3$].



WF-3161

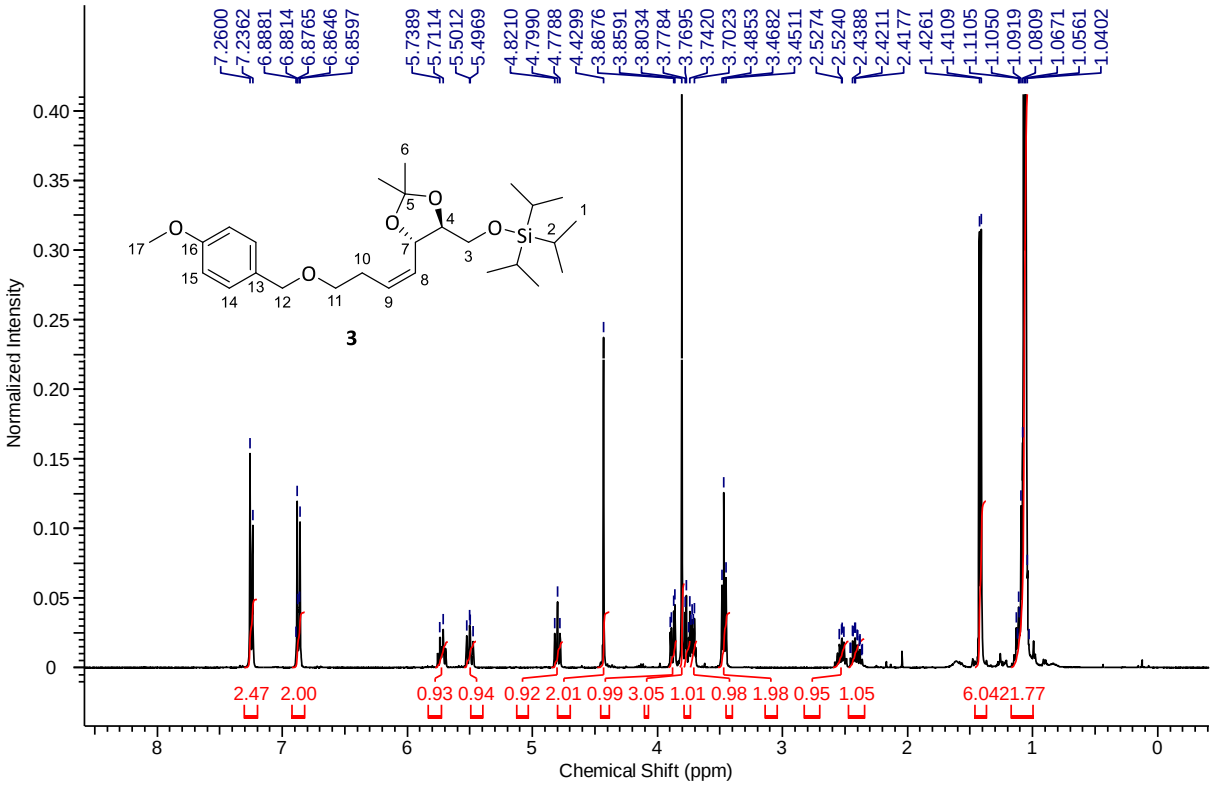
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.80 (d, $^{3}J_{21,20}$ = 6.7 Hz, 3 H, 21-H), 0.81 (d, $^{3}J_{21',20}$ = 6.7 Hz, 3 H, 21-H'), 1.17 (m, 1 H, 20-H), 1.23 – 1.35 (m, 5 H, 4-H, 5-H, 14-H_a), 1.41 (m, 1 H, 15-H_a), 1.48 – 1.69 (m, 6 H, 3-H_a, 6-H, 13-H_a, 19-H), 1.69 – 1.79 (m, 2 H, 14-H_b, 15-H_b), 1.82 (m, 1 H, 3-H_b), 2.27 (ddd, $^{2}J_{7a,7b}$ = 17.1 Hz, $^{3}J_{7a,6a}$ = 7.9 Hz, $^{3}J_{7a,6b}$ = 6.7 Hz, 1 H, 7-H_a), 2.42 (ddd, $^{2}J_{7b,7a}$ = 17.1 Hz, $^{3}J_{7b,6b}$ = 8.1 Hz, $^{3}J_{7b,6a}$ = 6.6 Hz, 1 H, 7-H_b), 2.47 (m, 1 H, 13-H_b), 2.69 (m, 1 H, 16-H_a), 2.85 (dd, $^{2}J_{10a,10b}$ = 5.7 Hz, $^{3}J_{10a,9}$ = 2.4 Hz, 1 H, 10-H_a), 2.88 (dd, $^{2}J_{24a,24b}$ = 13.9 Hz, $^{3}J_{24a,23}$ = 6.7 Hz, 1 H, 24-H_a), 2.99 (dd, $^{2}J_{10b,10a}$ = 5.7 Hz, $^{3}J_{10b,9}$ = 4.6 Hz, 1 H, 10-H_b), 3.25 (dd, $^{2}J_{24b,24a}$ = 13.9 Hz, $^{3}J_{24b,23}$ = 9.0 Hz, 1 H, 24-H_b), 3.42 (dd, $^{3}J_{9,10b}$ = 4.6 Hz, $^{3}J_{9,10a}$ = 2.4 Hz, 1 H, 9-H), 4.50 (m, 1 H, 2-H), 4.58 (m, 1 H, 16-H_b), 4.63 (m, 1 H, 23-H), 4.81 (m, 1 H, 12-H), 4.91 (m, 1 H, 18-H), 5.90 – 6.20 (m, 2 H, N-H_a, N-H_b), 6.28 (d, $^{3}J_{NHc,23}$ = 10.4 Hz, 1 H, N-H_c), 7.15 – 7.25 (m, 5 H, 26-H, 27-H, 28-H). **^{13}C-NMR (125 MHz, $CDCl_3$):** δ = 20.7 (t, C-14), 22.1 (q, C-21'), 22.7 (t, C-6), 23.1 (q, C-21), 24.0 (d, C-20), 24.8 (t, C-15), 25.4 (t, C-4), 26.8 (t, C-13), 27.5 (t, C-3), 28.6 (t, C-5), 34.8 (t, C-24), 36.3 (t, C-7), 40.5 (t, C-16), 41.1 (t, C-19), 46.1 (t, C-10), 47.0 (d, C-18), 53.4 (d, C-9), 54.1 (d, C-2), 55.6 (d, C-23), 57.1 (d, C-12), 126.7 (d, C-28), 128.4 (d, C-27), 129.2 (d, C-26), 136.7 (s, C-25), 169.9 (s, C-22), 171.2 (s, C-17), 174.2 (s, C-1), 174.6 (s, C-11), 207.5 (s, C-8).

HRMS (CI) calculated for $C_{31}H_{45}N_4O_6$ $[M+H]^+$: 569.3334, found: 569.3325.

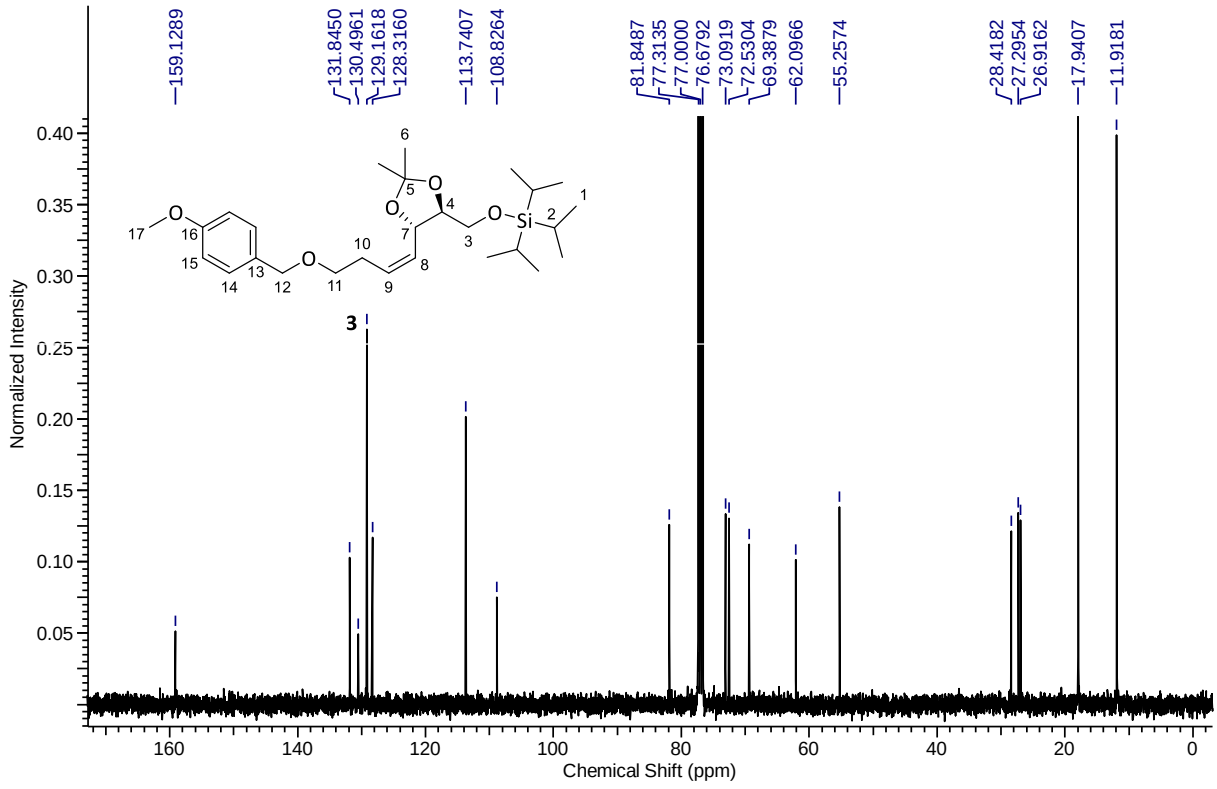
NMR spectra

[((4*S*,5*S*)-5-{(*Z*)-4-[(4-Methoxybenzyl)oxy]but-1-ene-1-yl}-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]triisopropylsilane (3)

400 MHz, $CDCl_3$

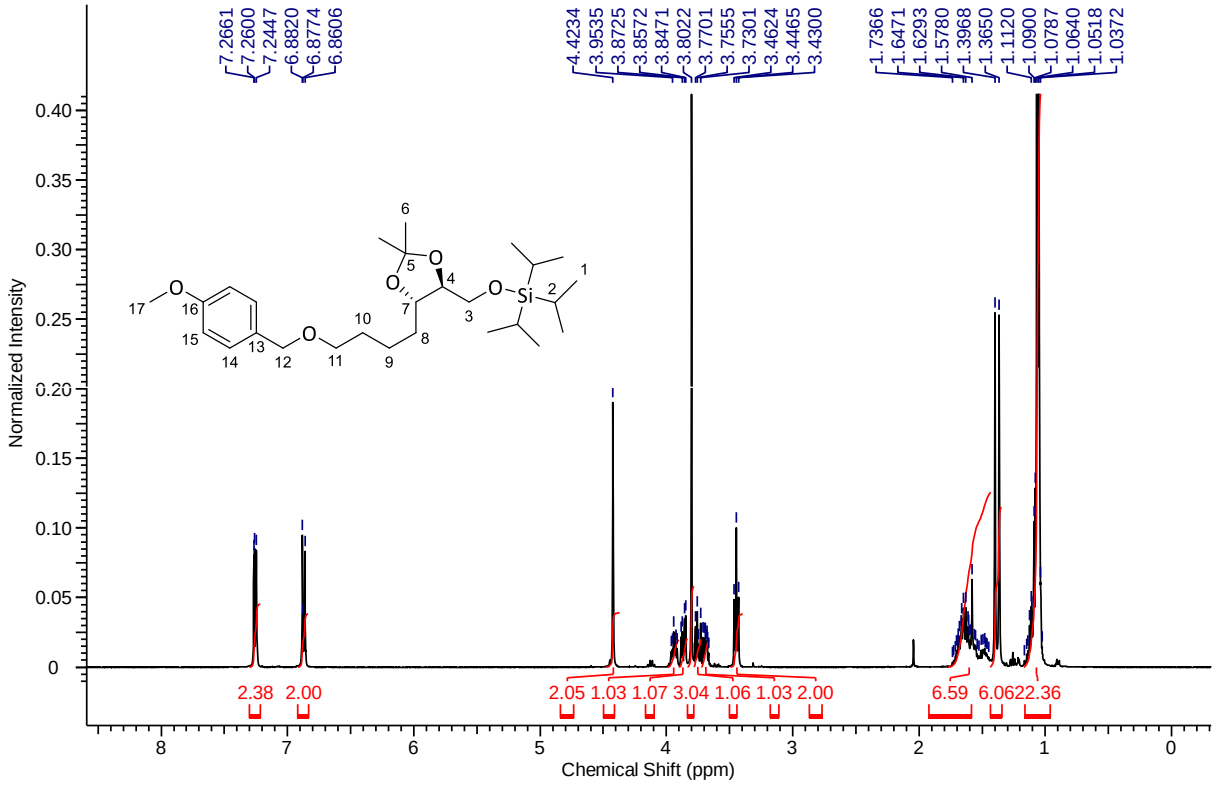


100 MHz, $CDCl_3$

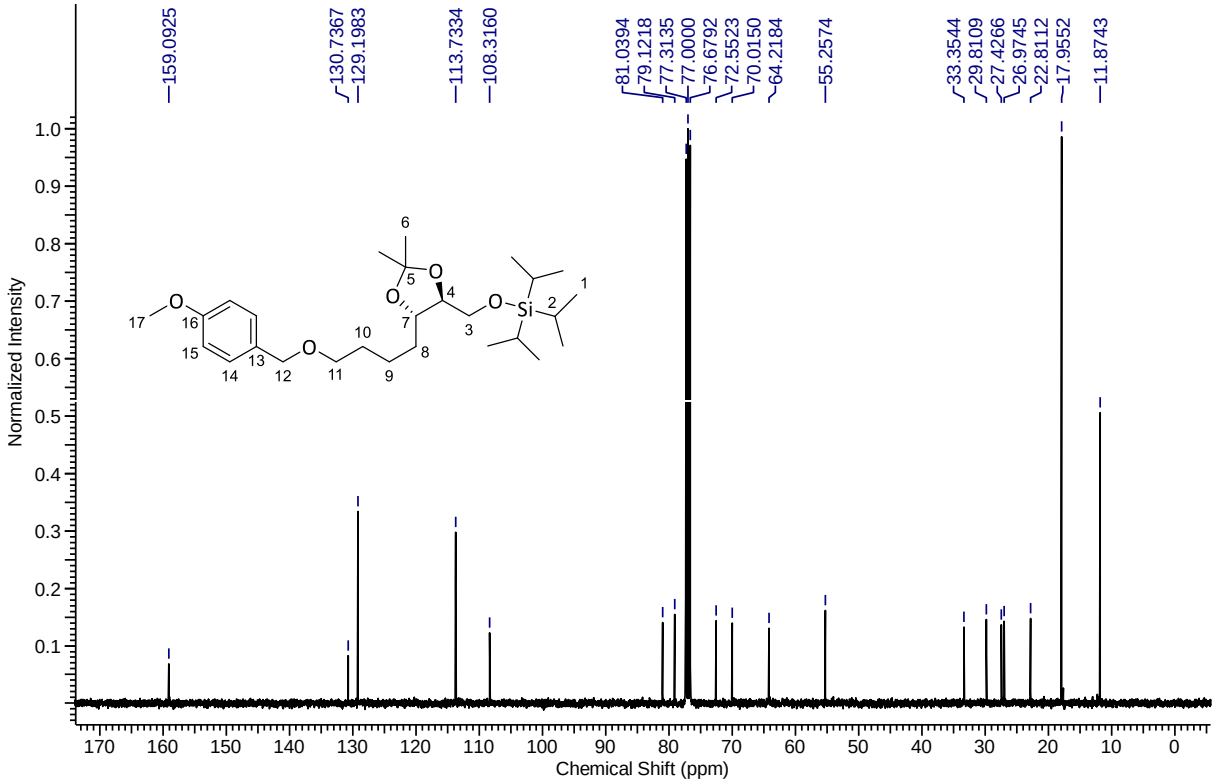


((4S,5S)-5-{4-[(4-Methoxybenzyl)oxy]butyl}-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]triisopropylsilane

400 MHz, $CDCl_3$

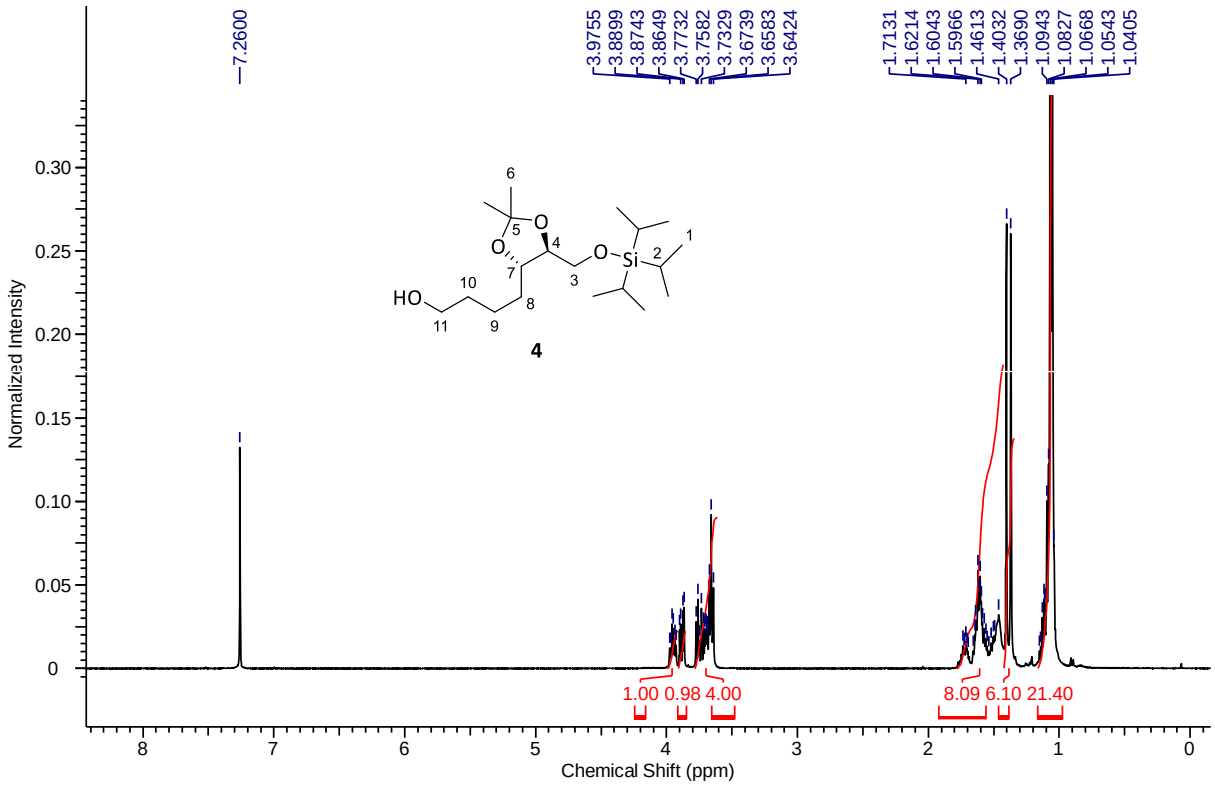


100 MHz, $CDCl_3$

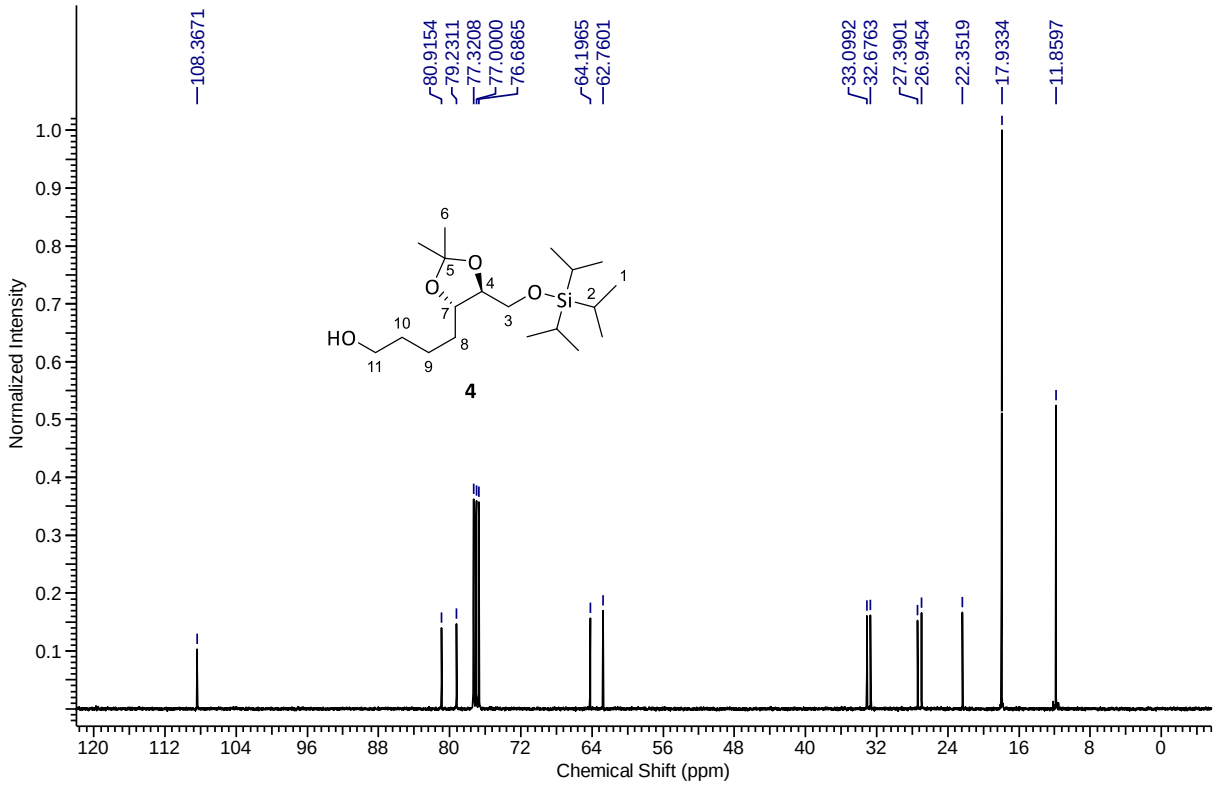


4-((4*S*,5*S*)-2,2-Dimethyl-5-{[(triisopropylsilyl)oxy]methyl}-1,3-dioxolan-4-yl)butan-1-ol (4)

400 MHz, $CDCl_3$

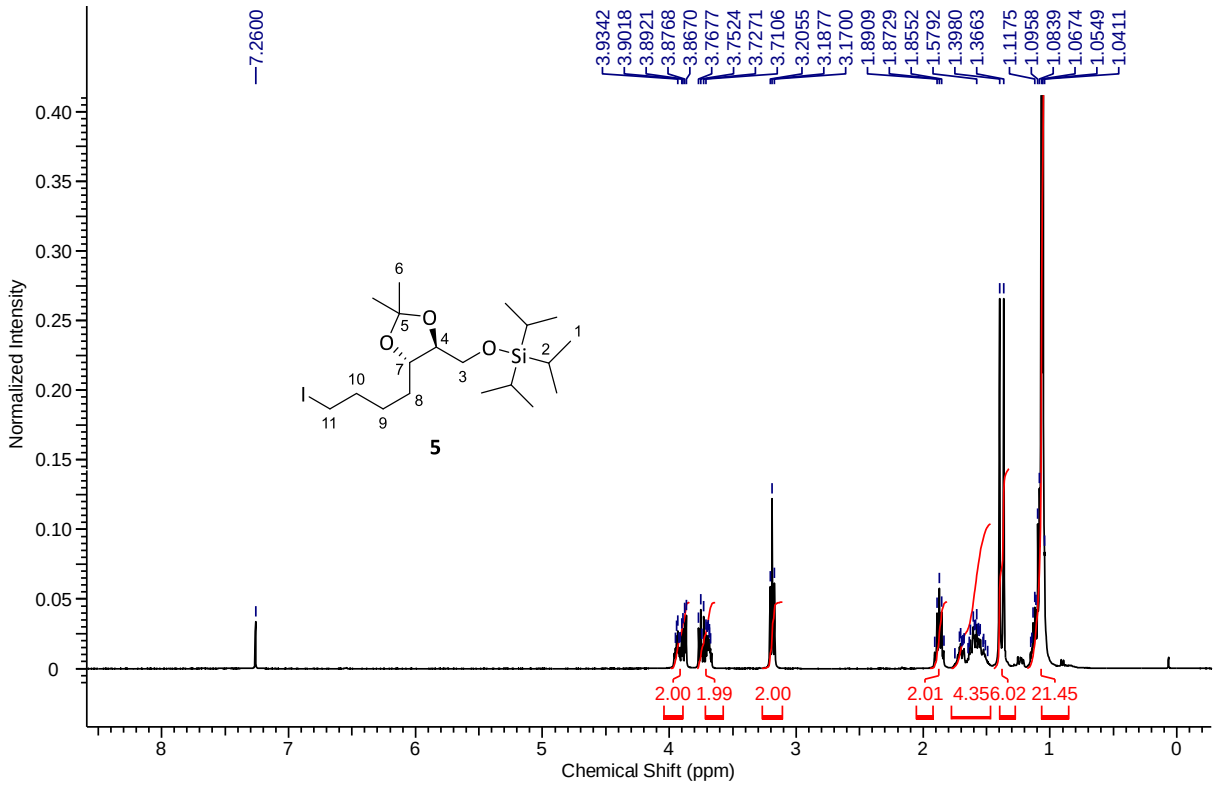


100 MHz, $CDCl_3$

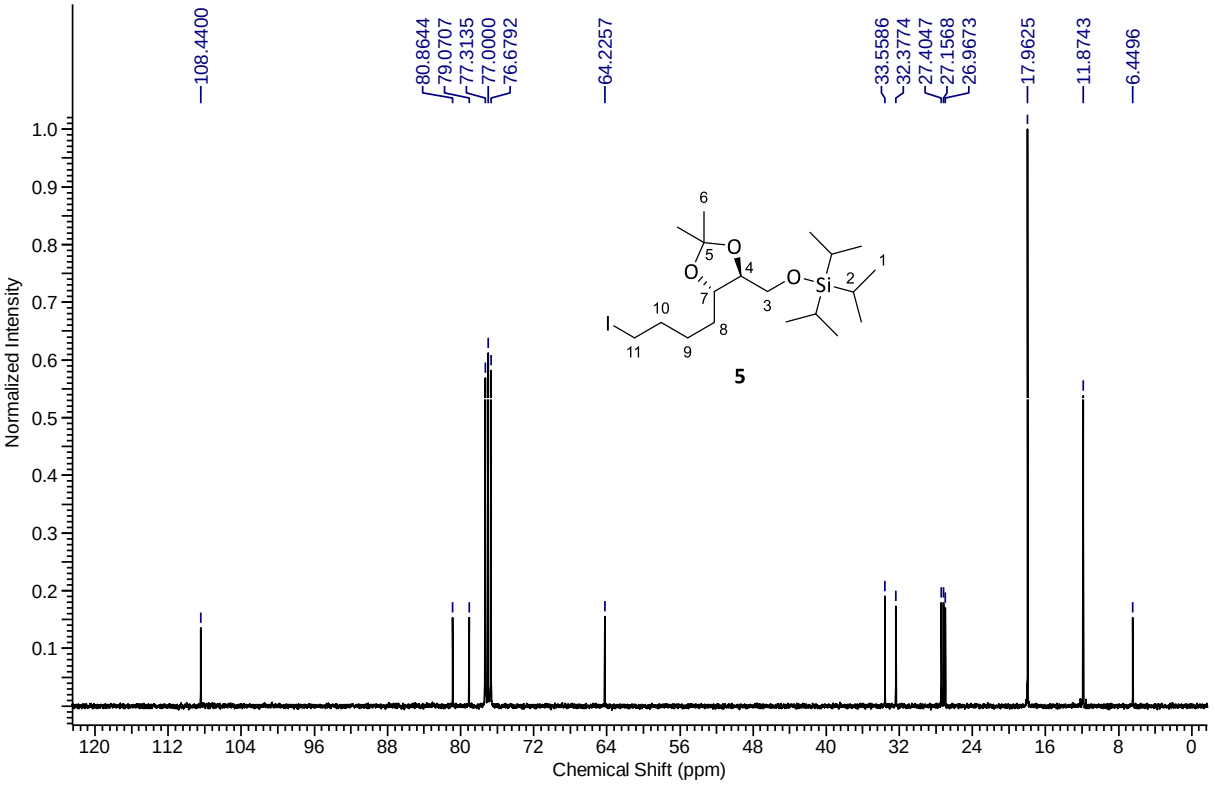


{[(4*S*,5*S*)-5-(4-Iodobutyl)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}triisopropylsilane (5)

400 MHz, $CDCl_3$

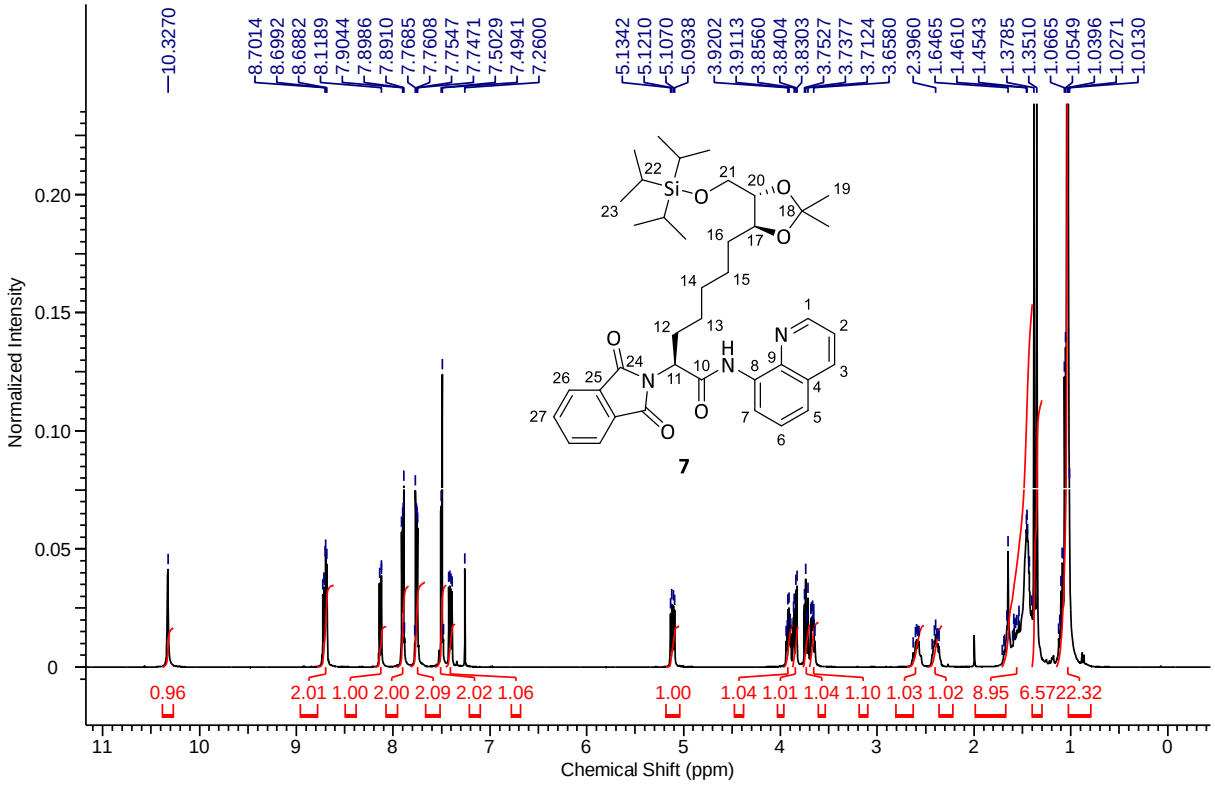


100 MHz, $CDCl_3$

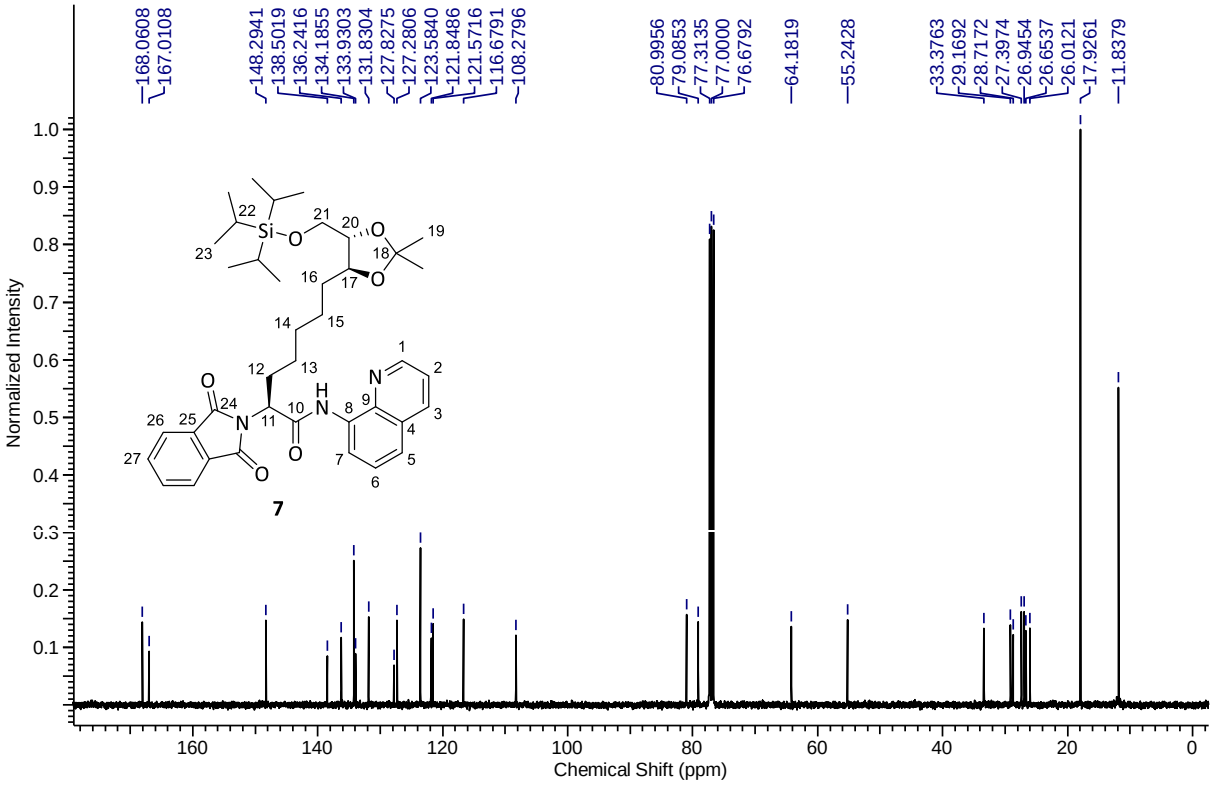


(2*S*)-7-(2,2-Dimethyl-5-{[(triisopropylsilyl)oxy]methyl}-1,3-dioxolan-4-yl)-2-(1,3-dioxoisoindolin-2-yl)-*N*-(quinolin-8-yl)heptanamide (7)

400 MHz, $CDCl_3$

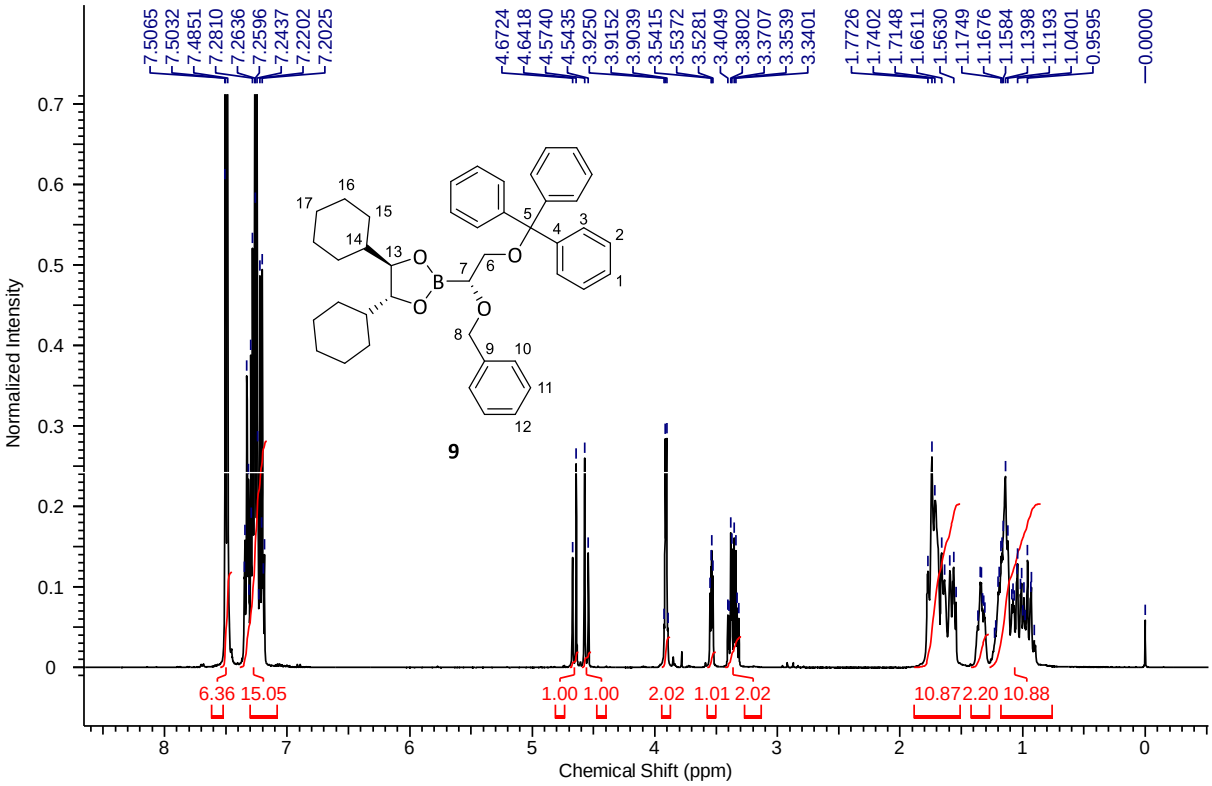


100 MHz, $CDCl_3$

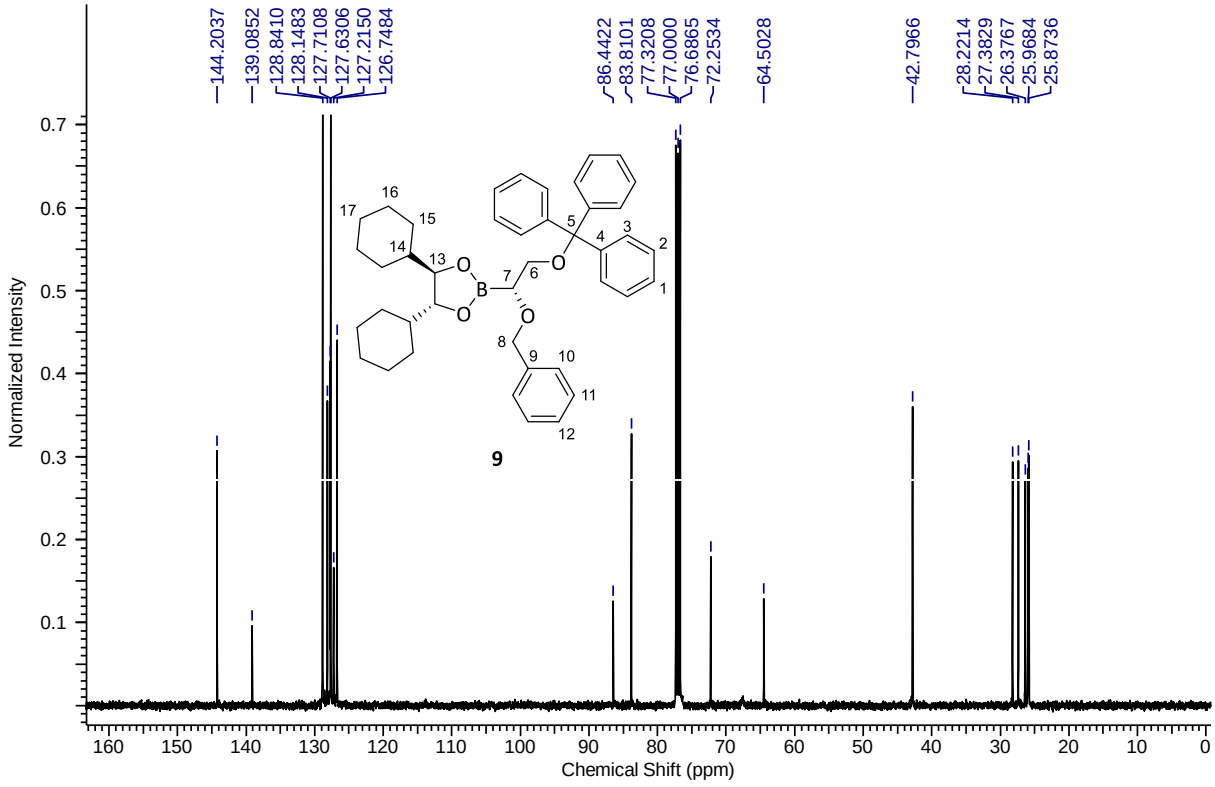


(4*R*,5*R*)-2-[(*R*)-1-(Benzyloxy)-2-(trityloxy)ethyl]-4,5-dicyclohexyl-1,3,2-dioxaborolane (9)

400 MHz, $CDCl_3$

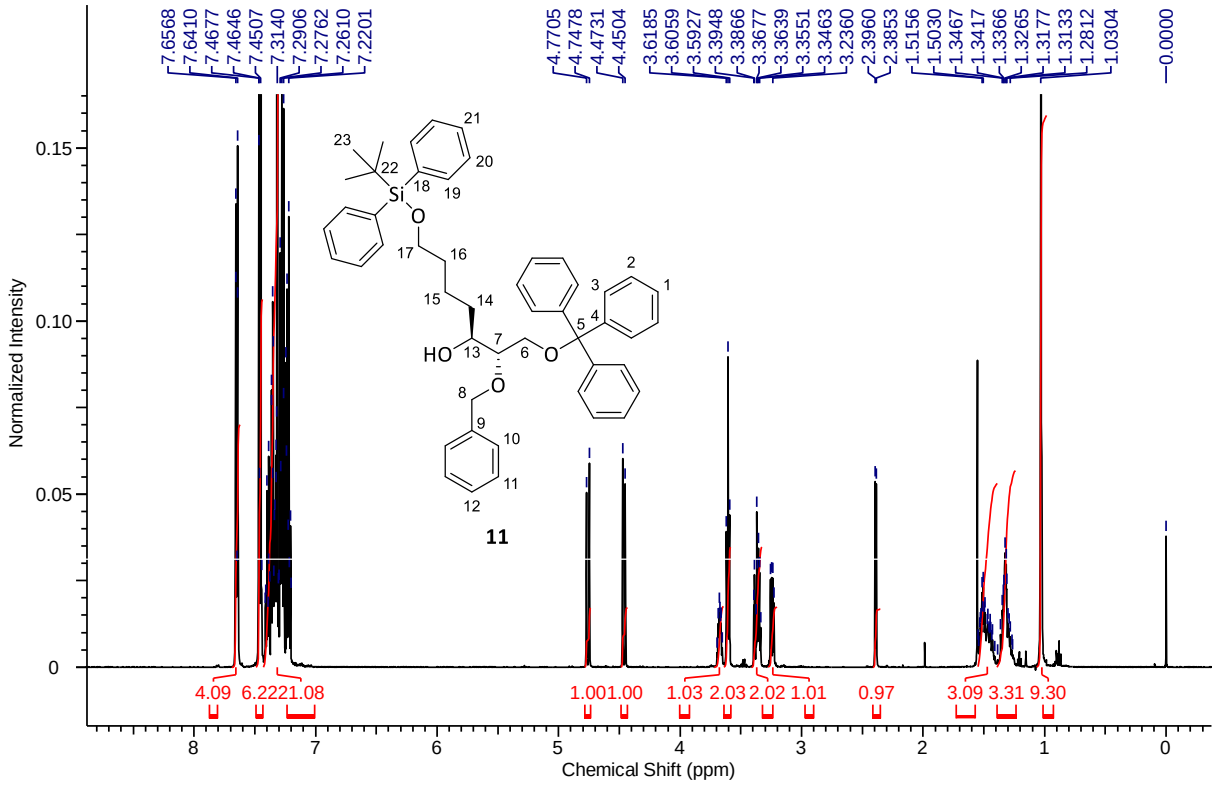


100 MHz, $CDCl_3$

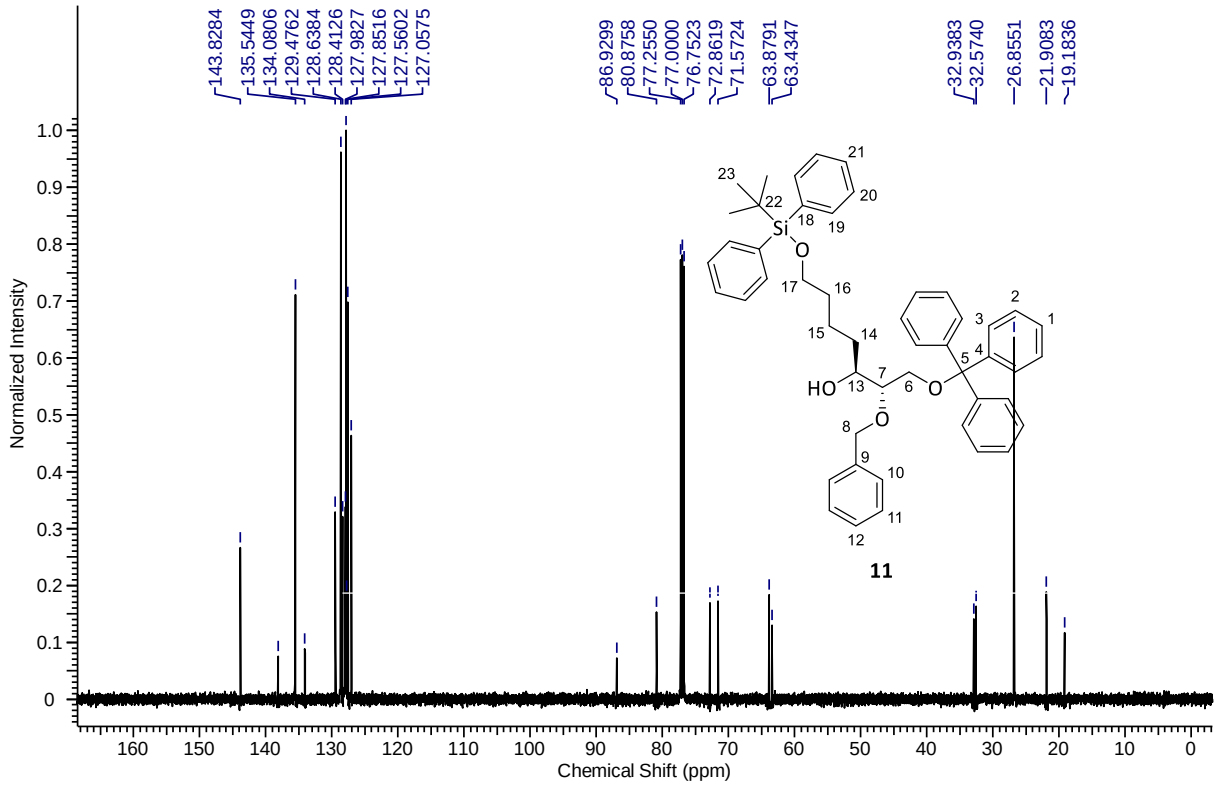


(2*S*,3*S*)-2-(Benzyloxy)-7-[(*tert*-butyldiphenylsilyl)oxy]-1-(trityloxy)heptan-3-ol (11)

500 MHz, $CDCl_3$

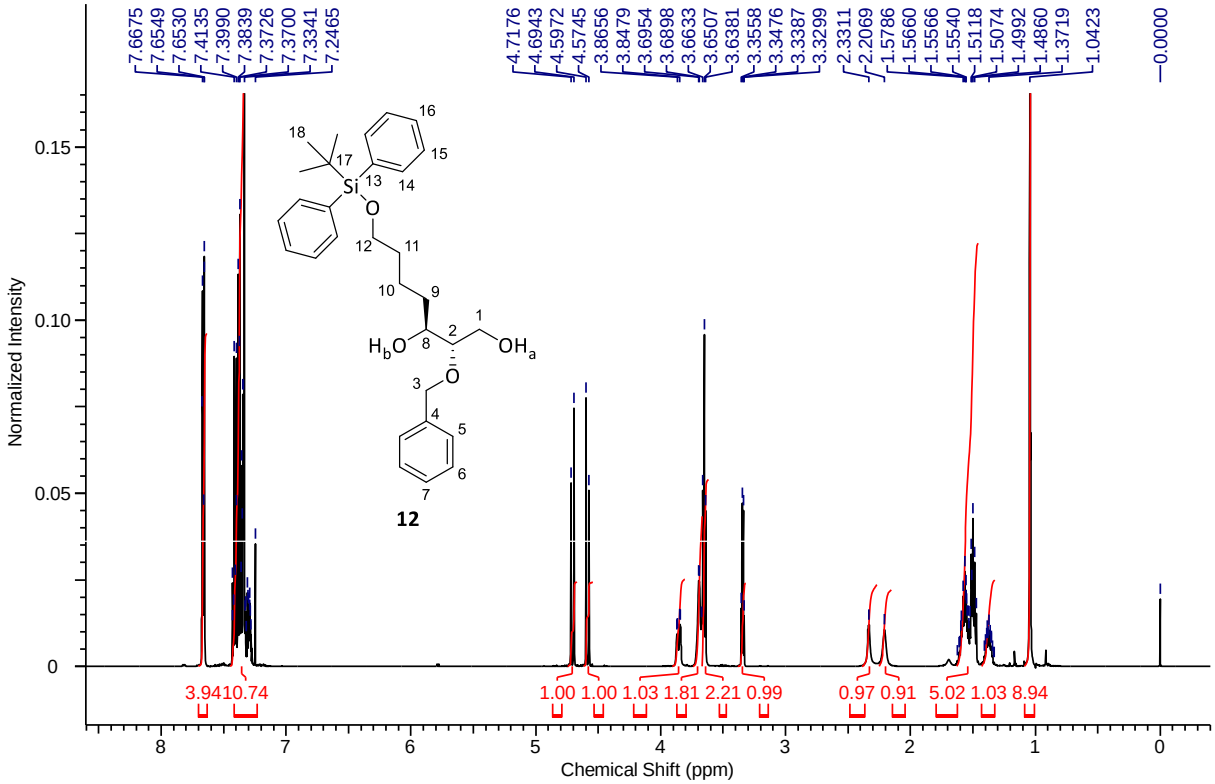


125 MHz, $CDCl_3$

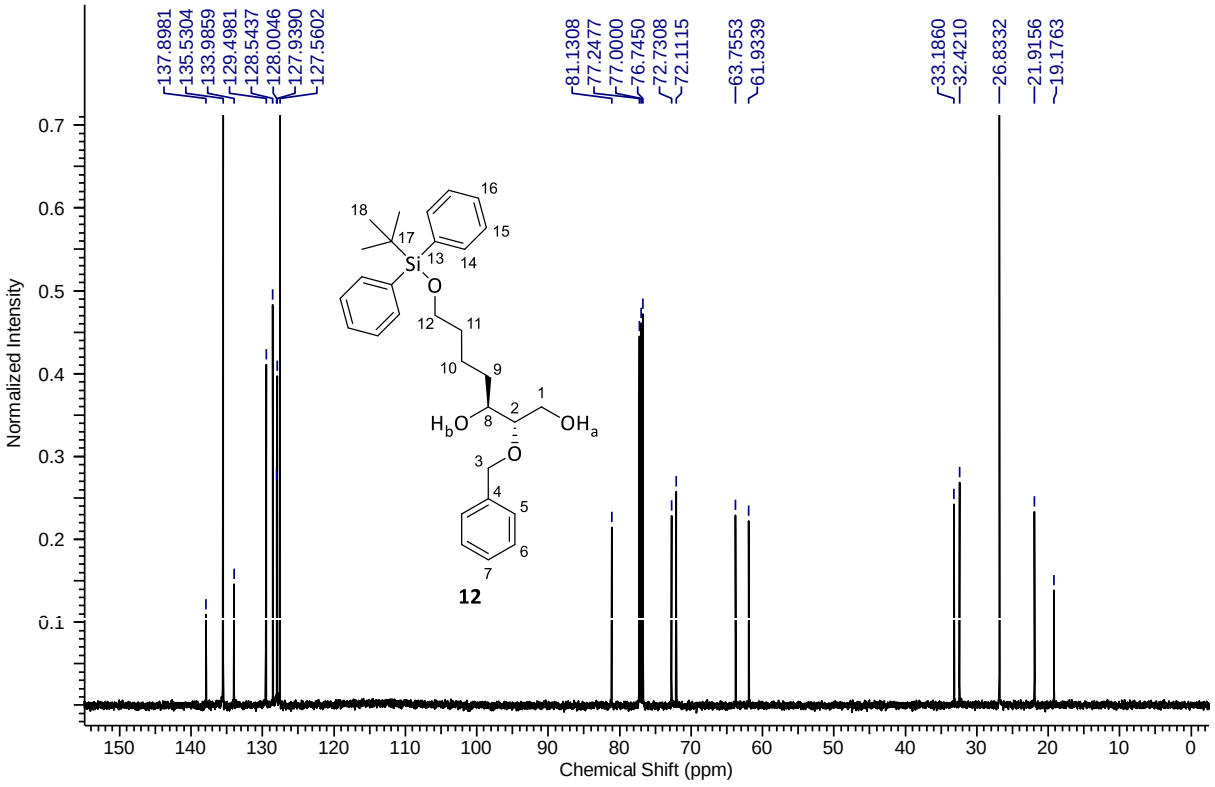


(2*S*,3*S*)-2-(Benzyloxy)-7-[(*tert*-butyldiphenylsilyl)oxy]heptan-1,3-diol (12)

500 MHz, $CDCl_3$

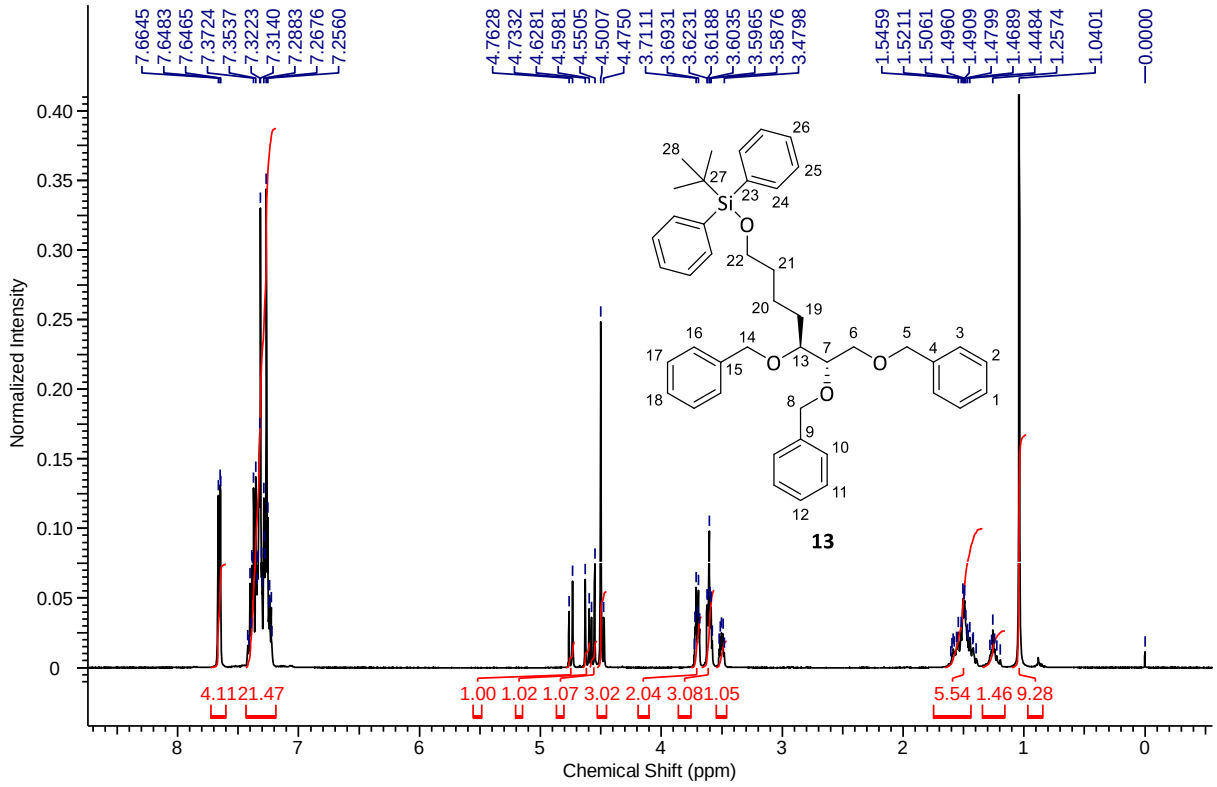


125 MHz, $CDCl_3$

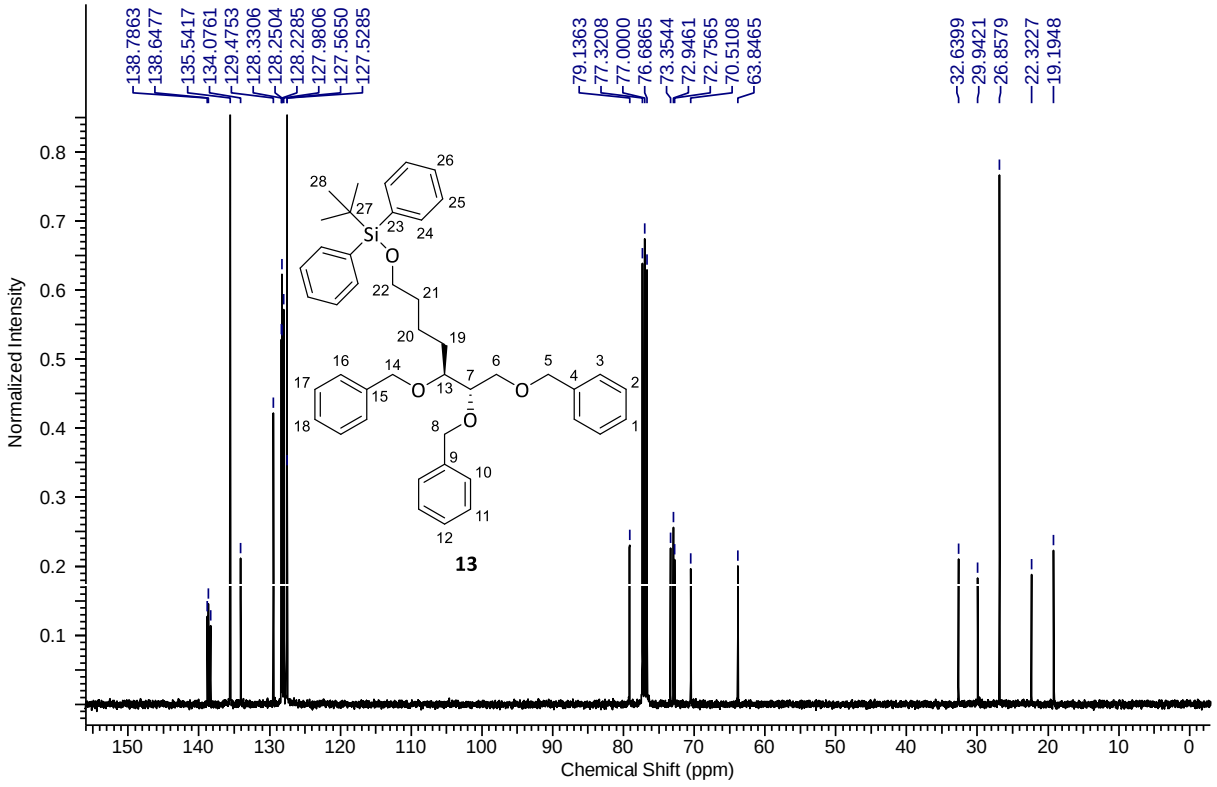


***tert*-Butyldiphenyl{[(5*S*,6*S*)-5,6,7-tris(benzyloxy)heptyl]oxy}silane (13)**

400 MHz, $CDCl_3$

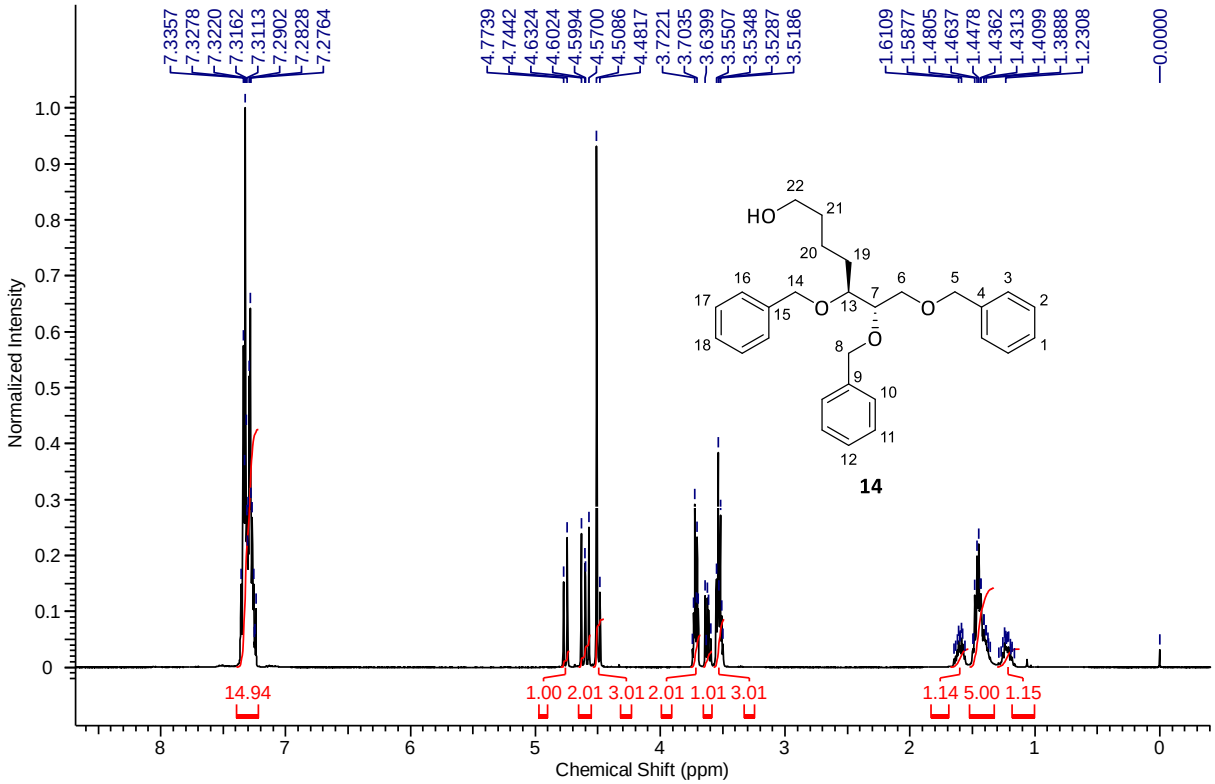


100 MHz, $CDCl_3$

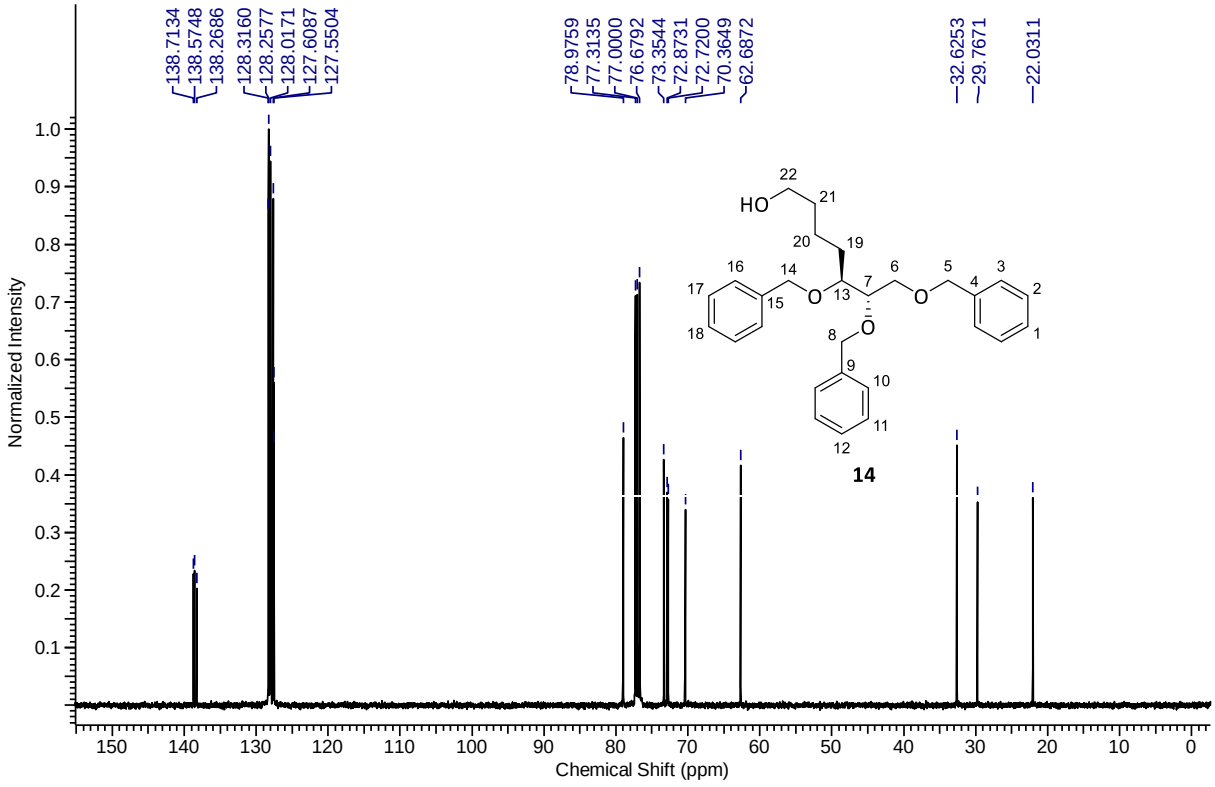


(5*S*,6*S*)-5,6,7-Tris(benzyloxy)heptan-1-ol (14)

400 MHz, $CDCl_3$

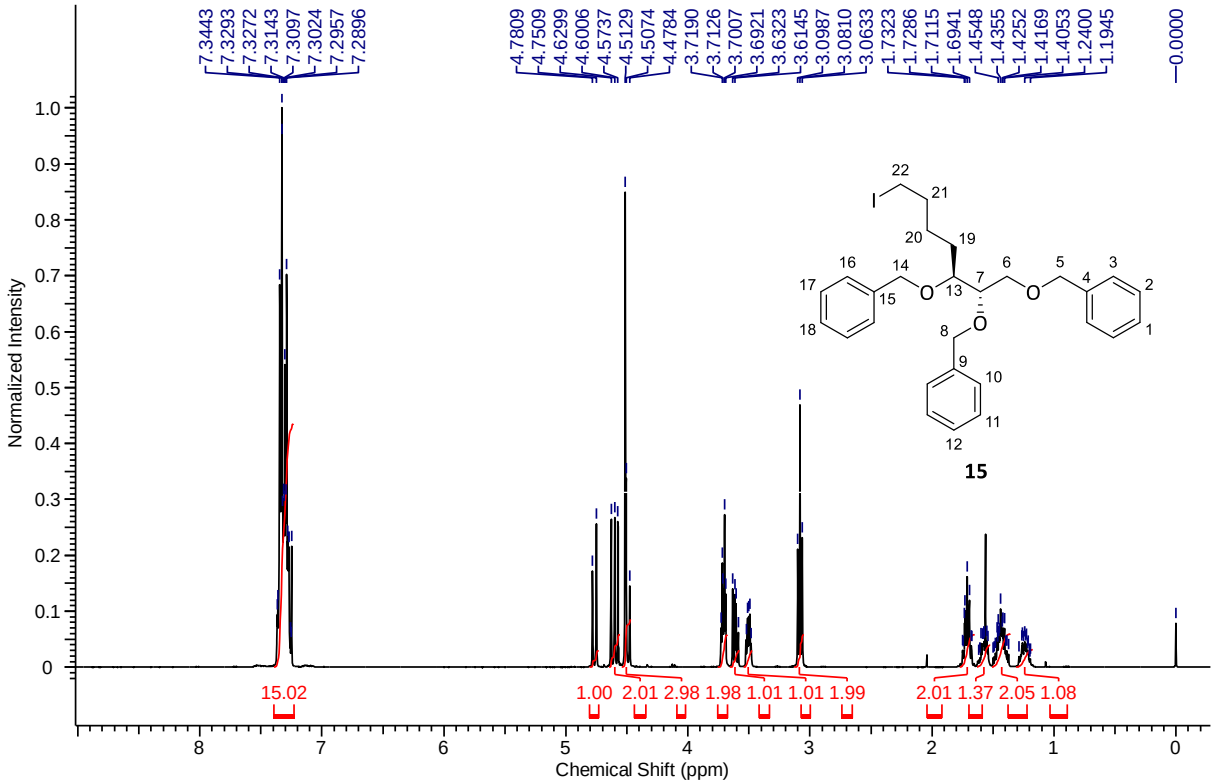


100 MHz, $CDCl_3$

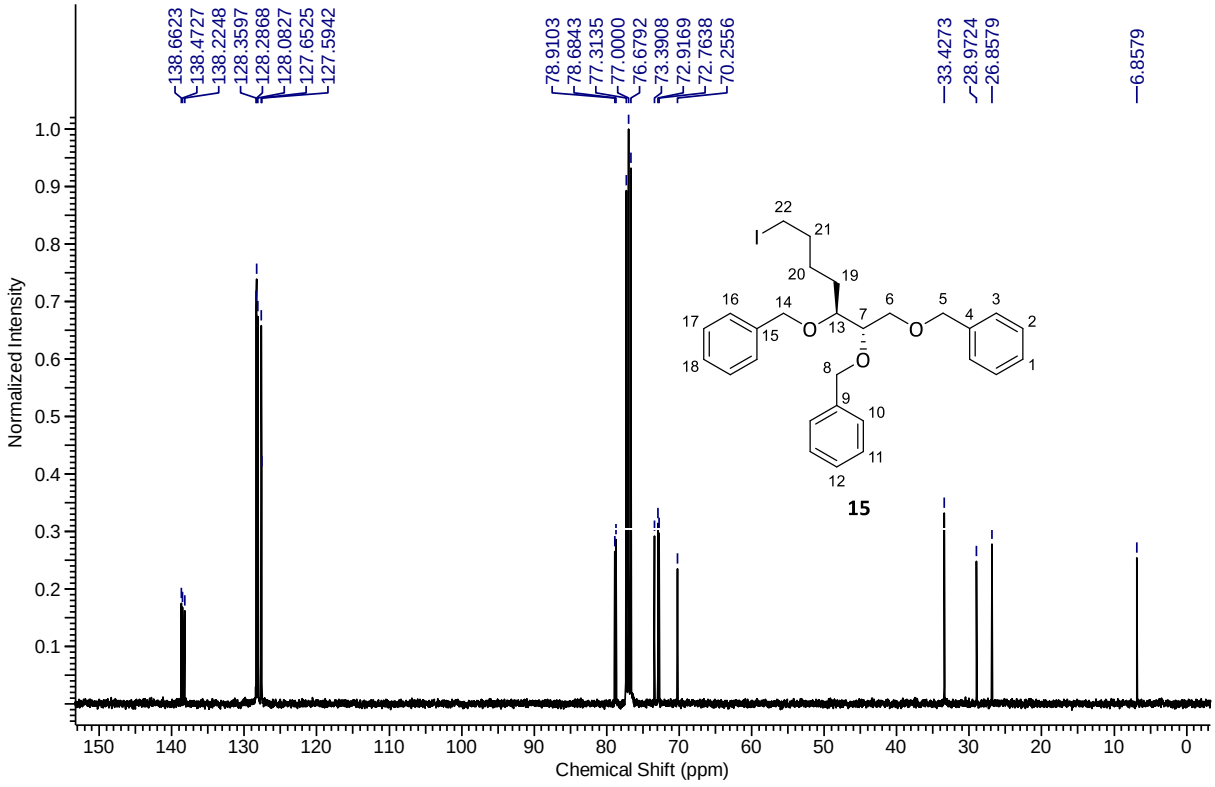


({[(2*S*,3*S*)-7-Iodoheptane-1,2,3-triyl]tris(oxy)}tris(methylene))tribenzene (15)

400 MHz, $CDCl_3$

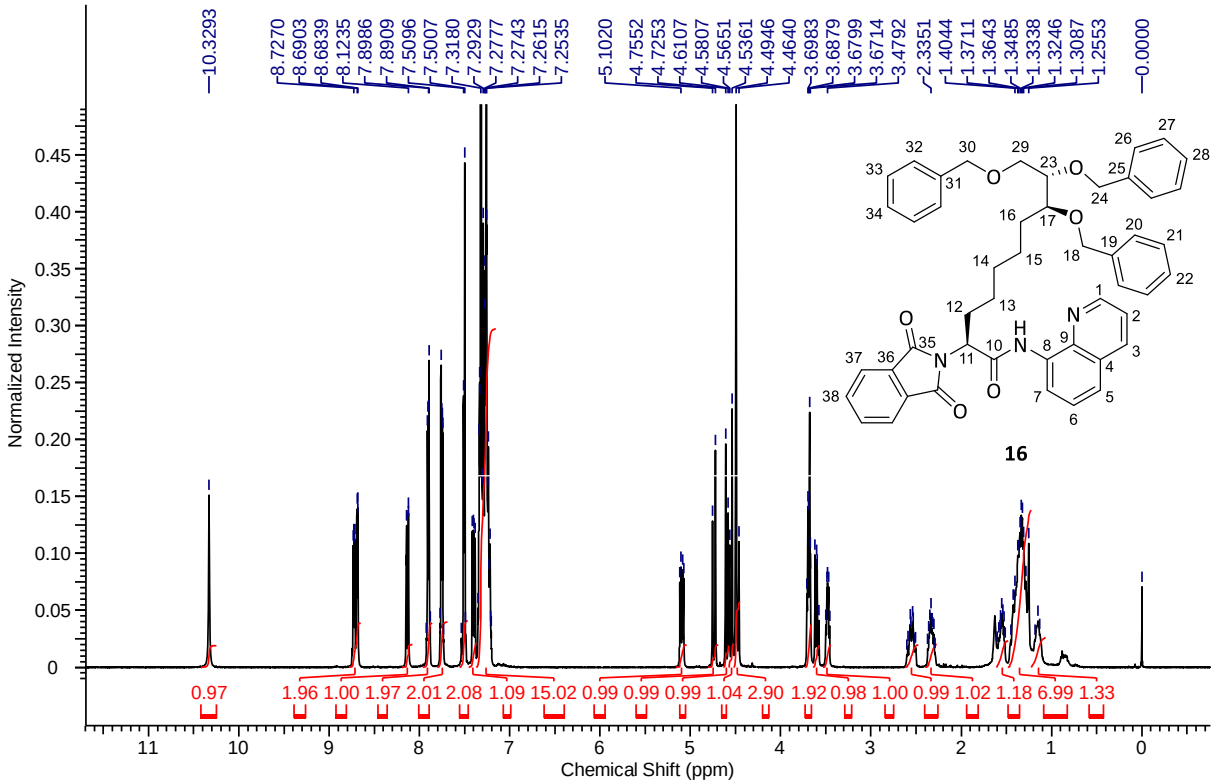


100 MHz, $CDCl_3$

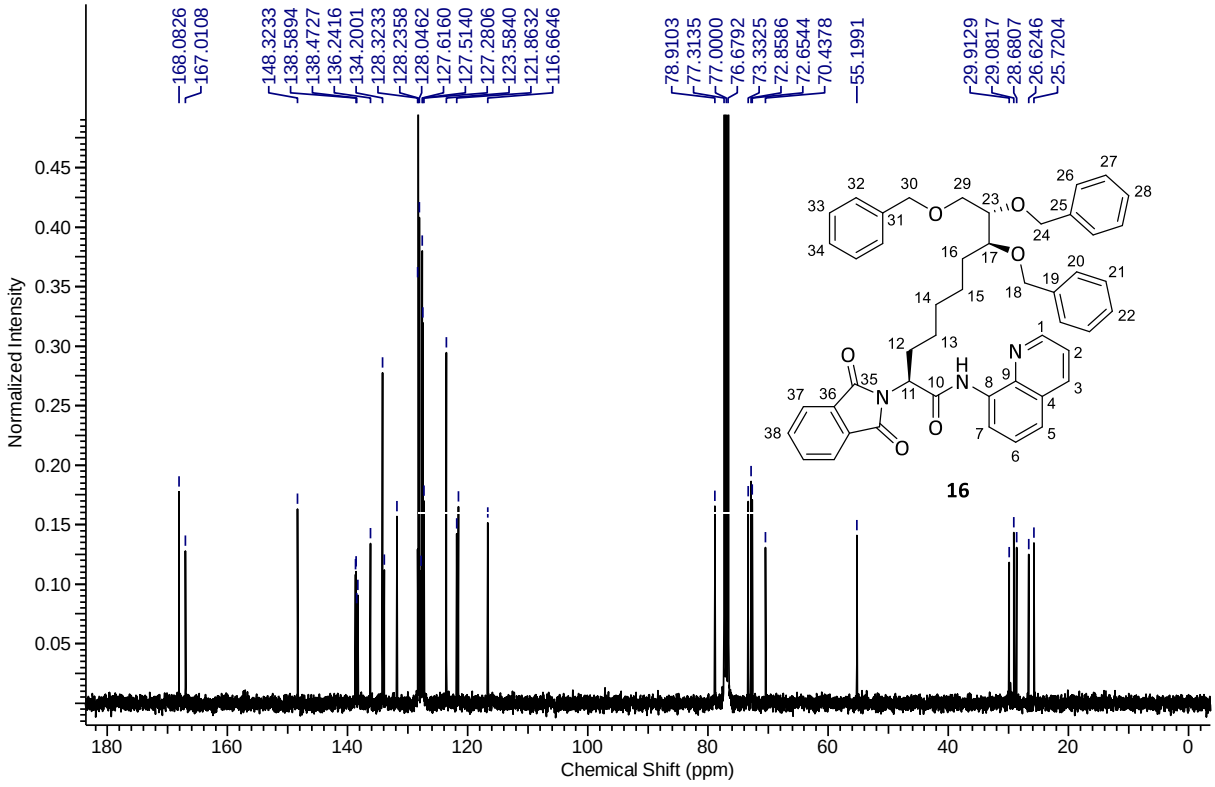


(2*S*,8*S*,9*S*)-8,9,10-Tris(benzyloxy)-2-(1,3-dioxoisoindolin-2-yl)-*N*-(quinolin-8-yl)decanamide (16)

400 MHz, $CDCl_3$

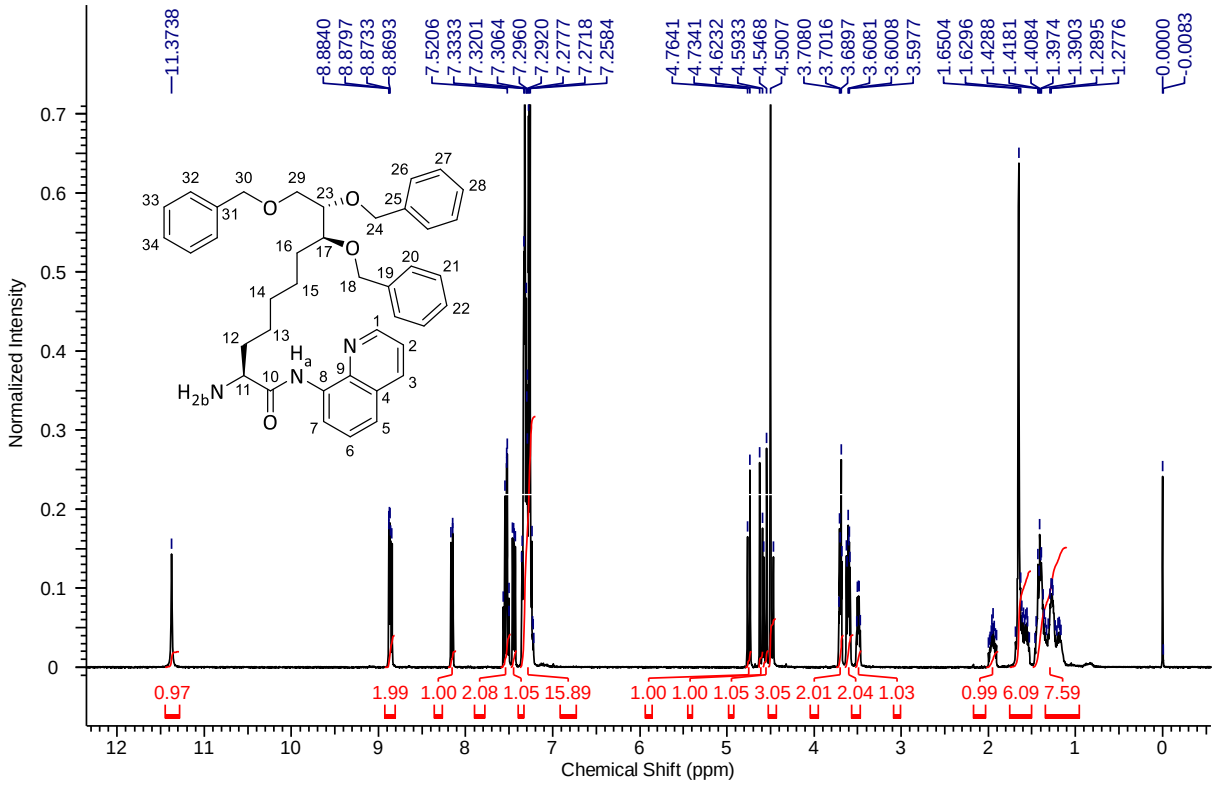


100 MHz, $CDCl_3$

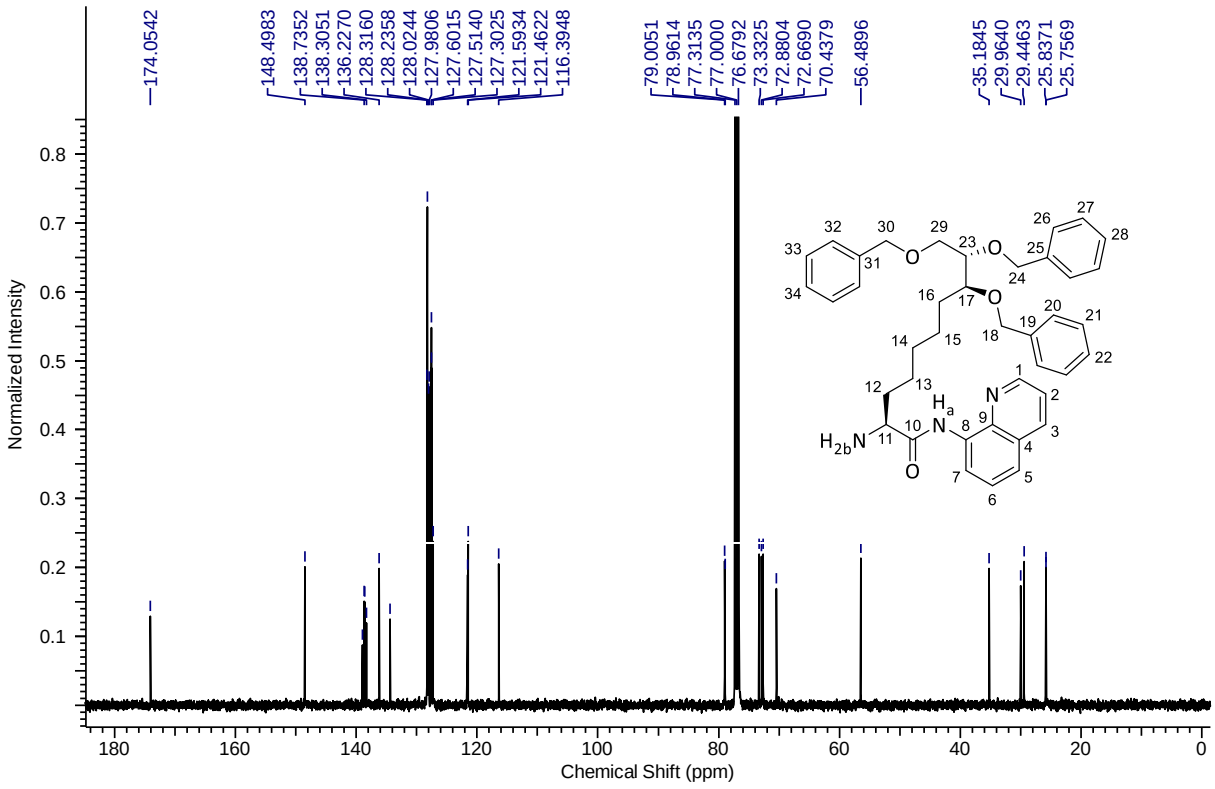


(2*S*,8*S*,9*S*)-2-Amino-8,9,10-tris(benzyloxy)-*N*-(quinolin-8-yl)decanamide

400 MHz, $CDCl_3$

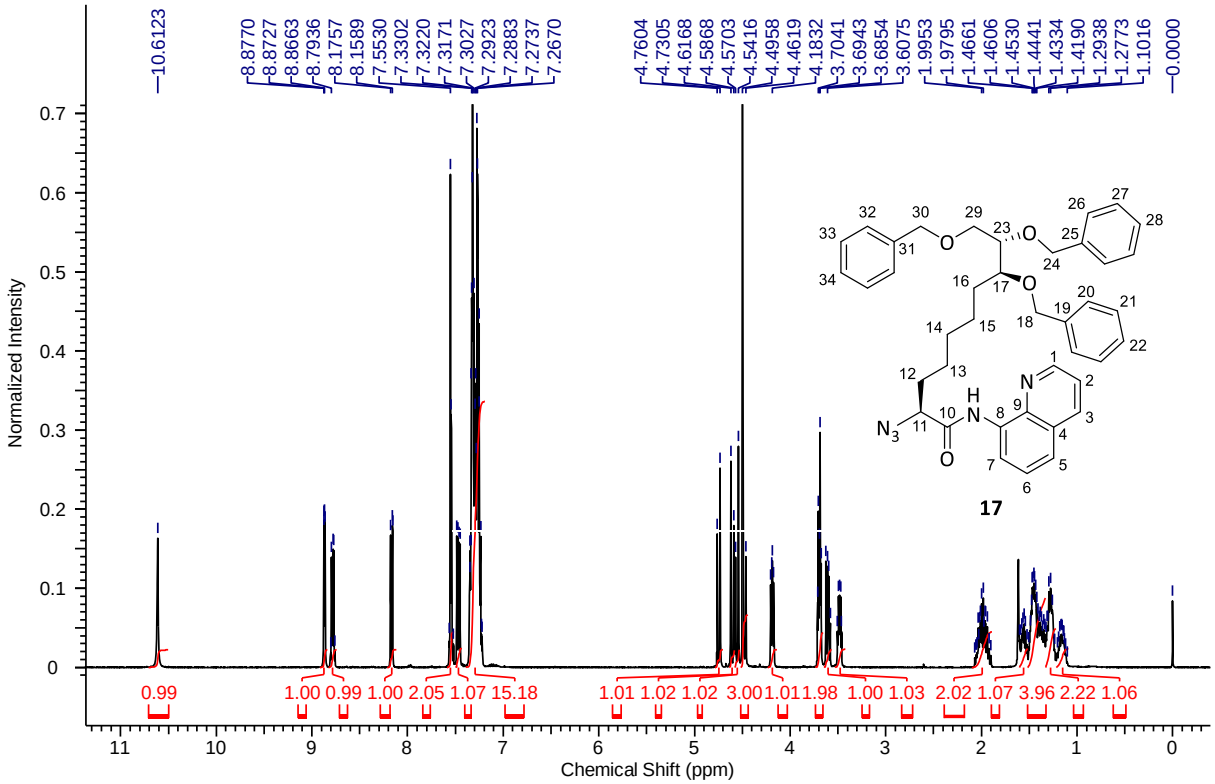


100 MHz, $CDCl_3$

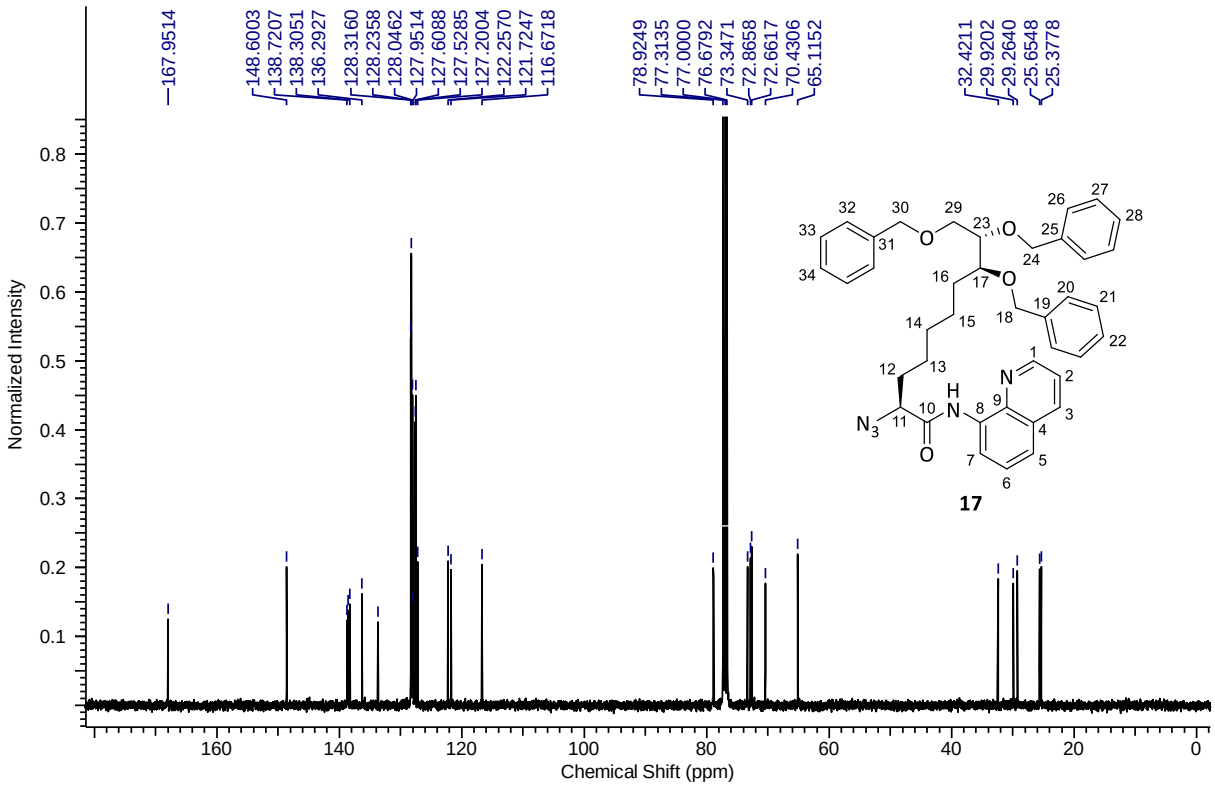


(2*S*,8*S*,9*S*)-2-Azido-8,9,10-tris(benzyloxy)-*N*-(quinolin-8-yl)decanamide (17)

400 MHz, $CDCl_3$

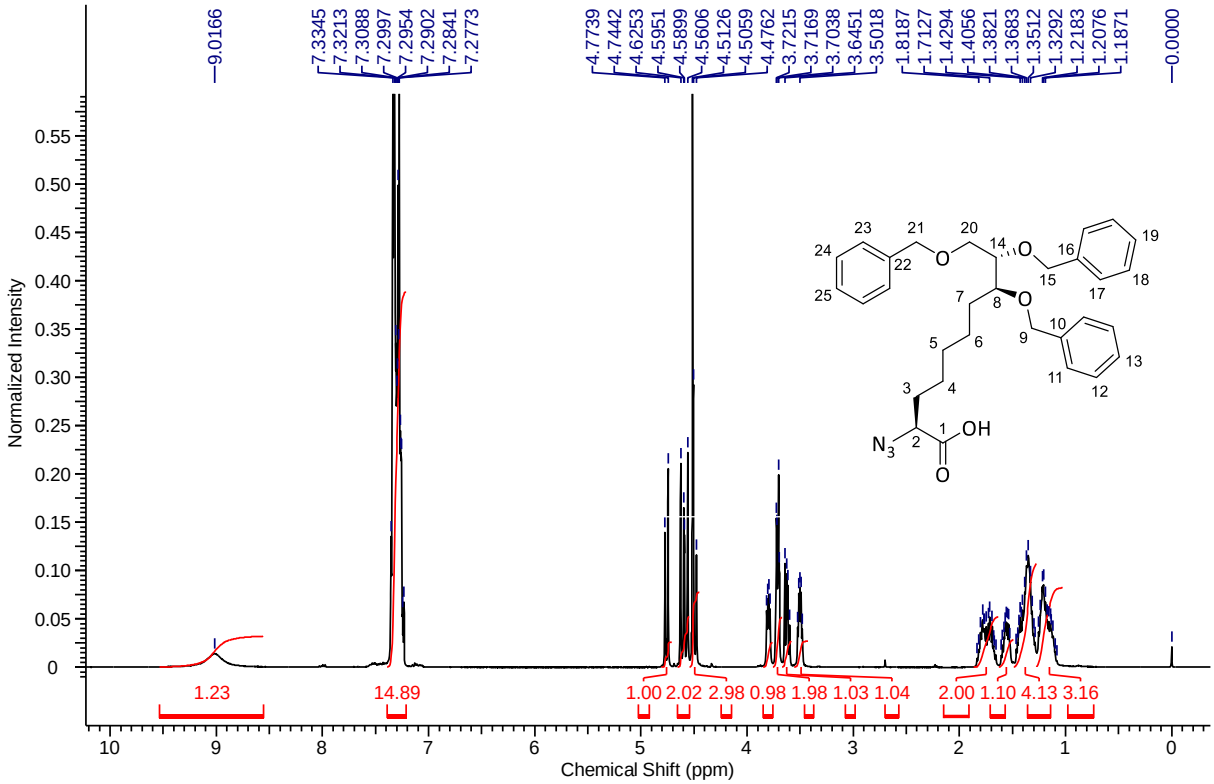


100 MHz, $CDCl_3$

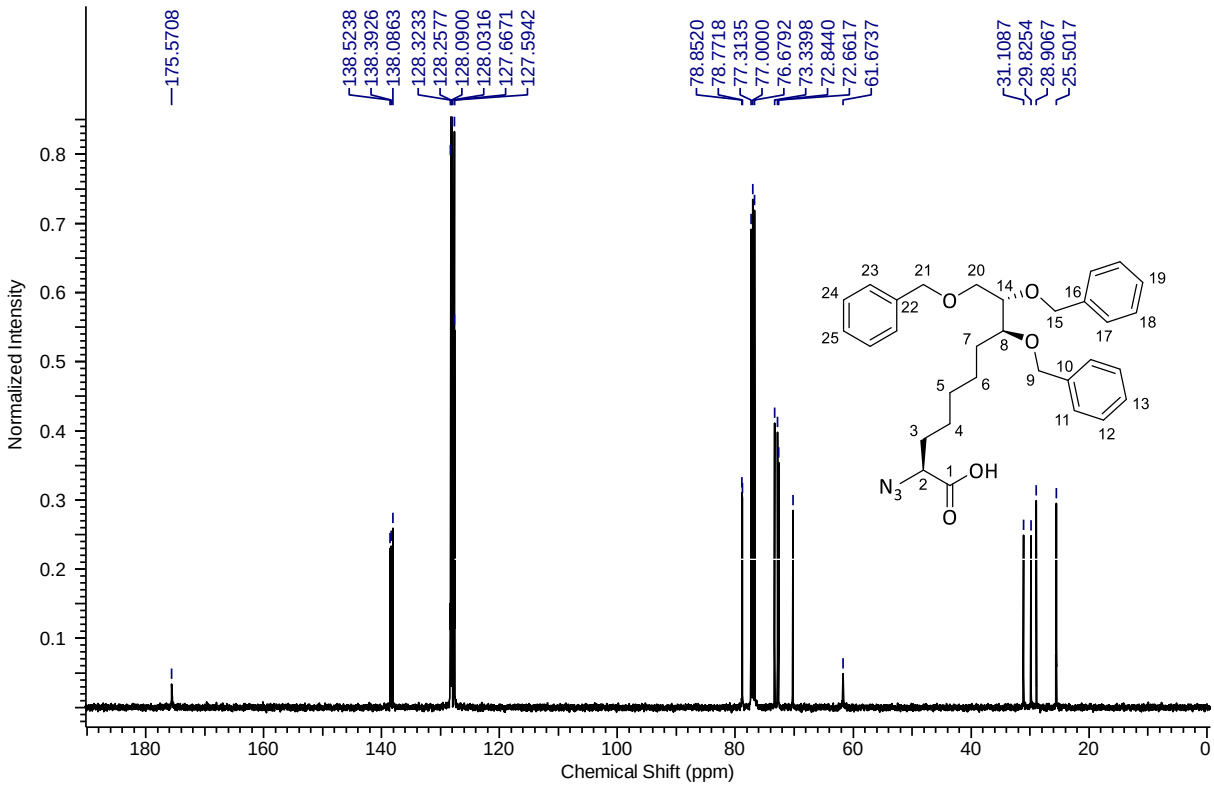


(2*S*,8*S*,9*S*)-2-Azido-8,9,10-tris(benzyloxy)decanoic acid

400 MHz, $CDCl_3$

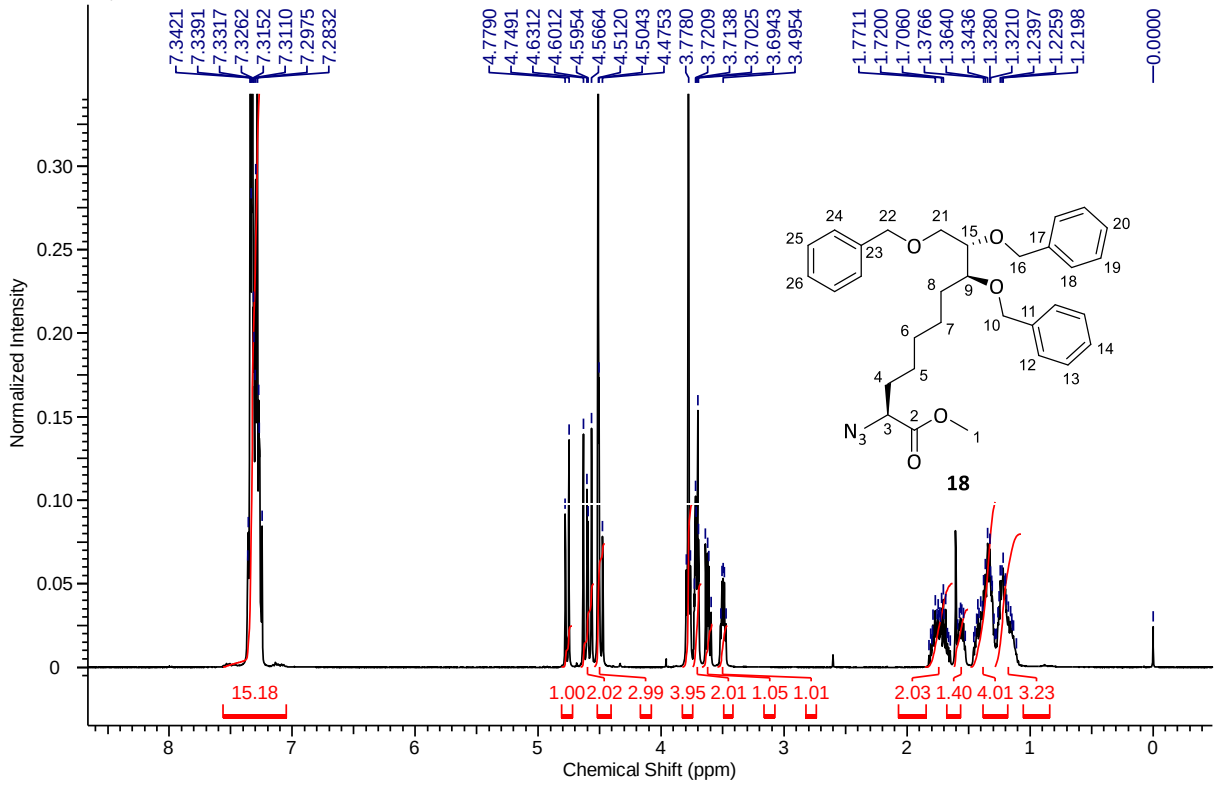


100 MHz, $CDCl_3$

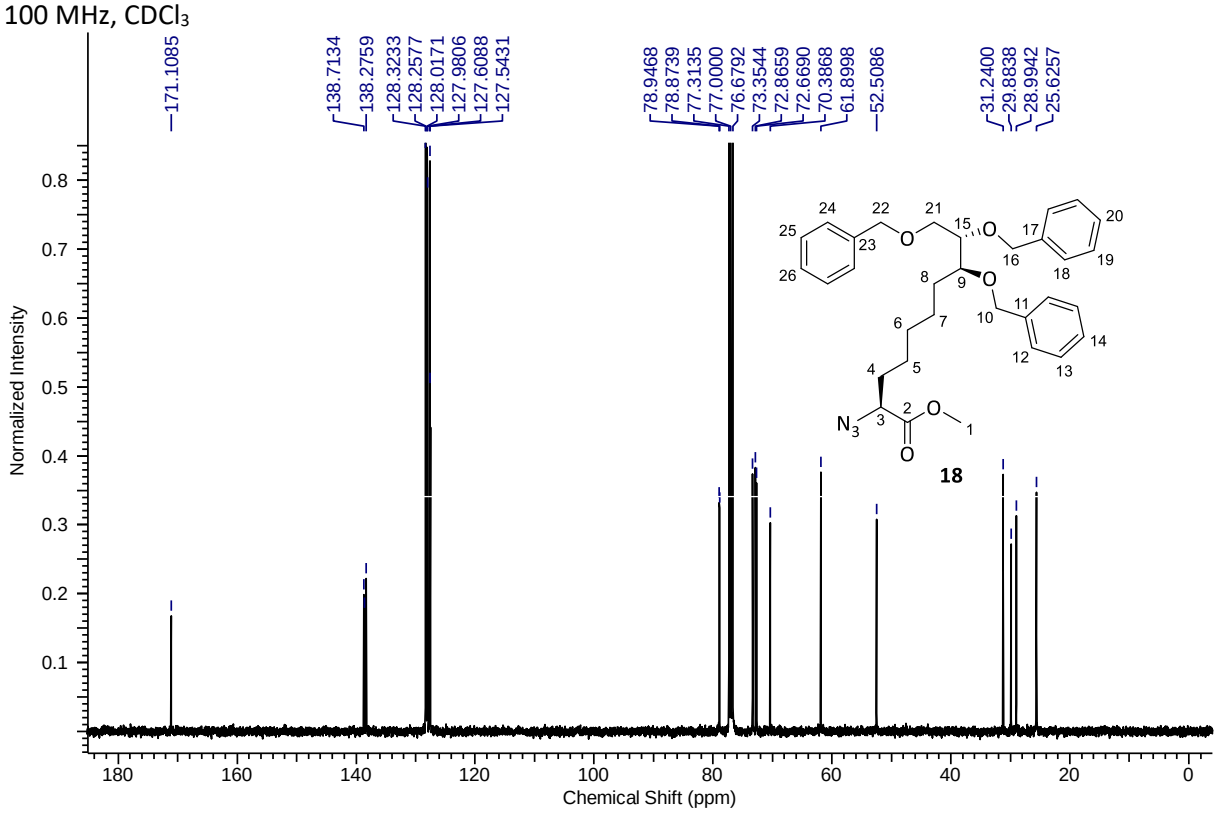


Methyl (2*S*,8*S*,9*S*)-2-azido-8,9,10-tris(benzyloxy)decanoate (18)

400 MHz, $CDCl_3$

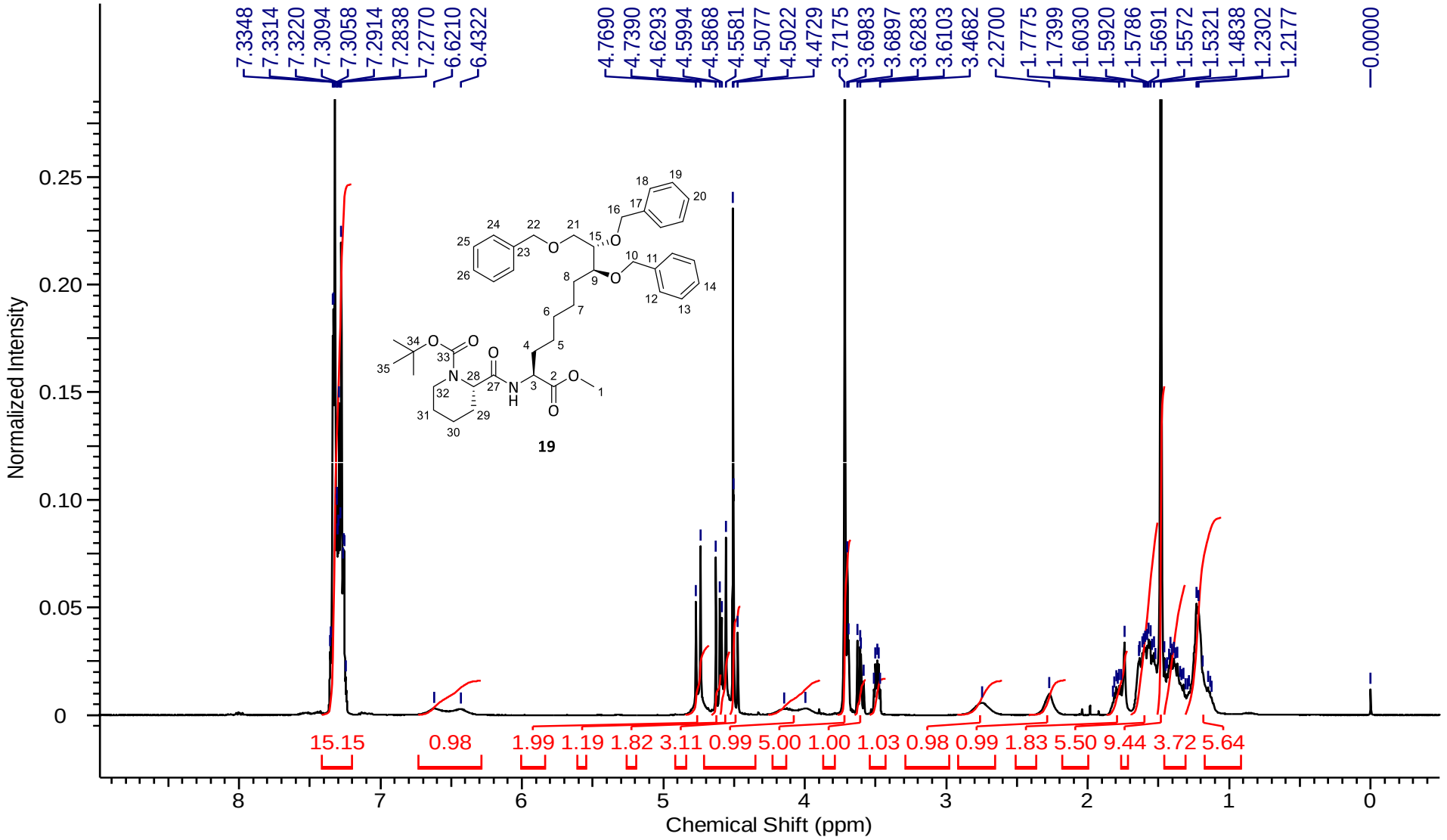


100 MHz, $CDCl_3$

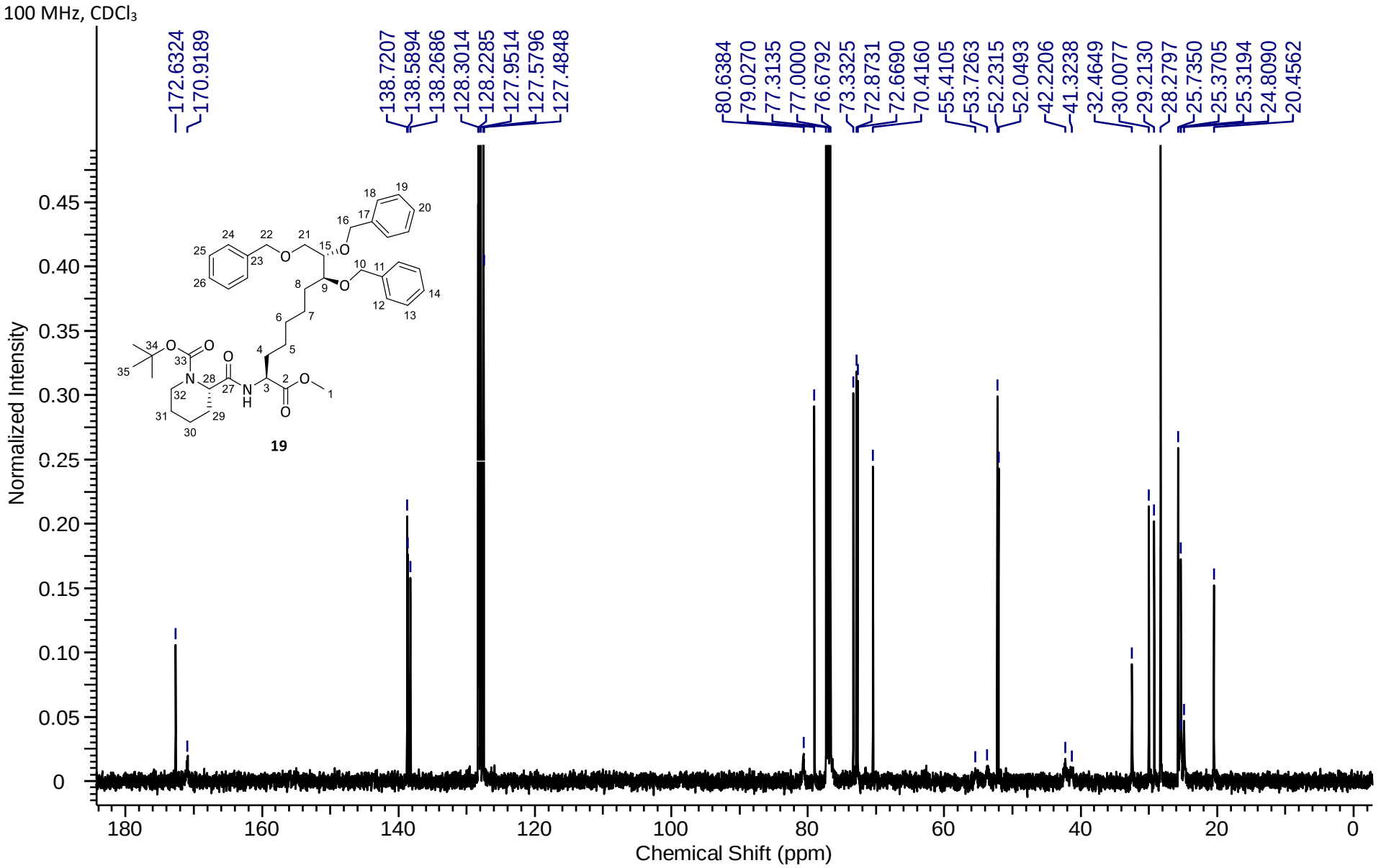


***tert*-Butyl (*S*)-2-{[(2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-1-methoxy-1-oxodecan-2-yl]carbamoyl}-piperidine-1-carboxylate (19)**

400 MHz, $CDCl_3$

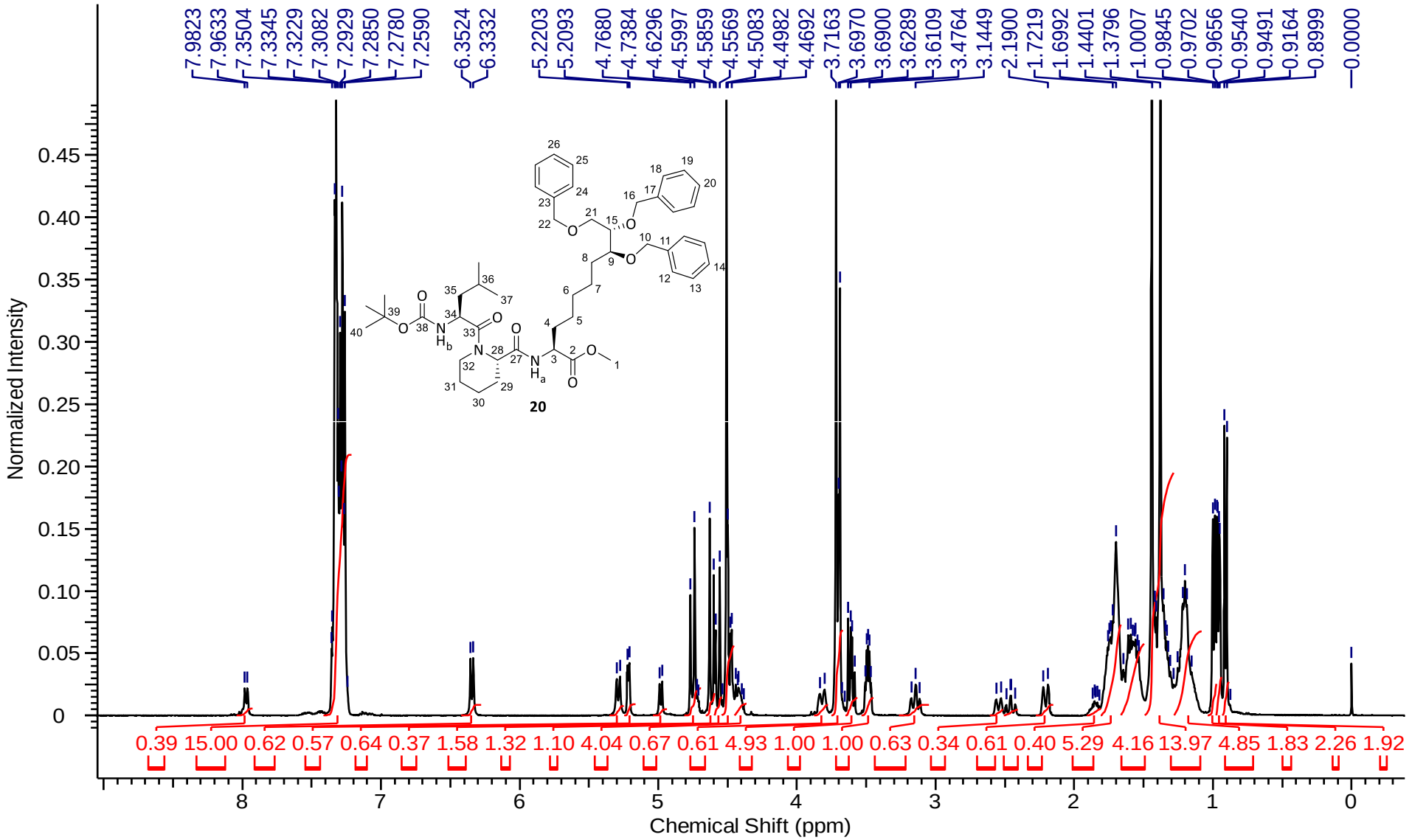


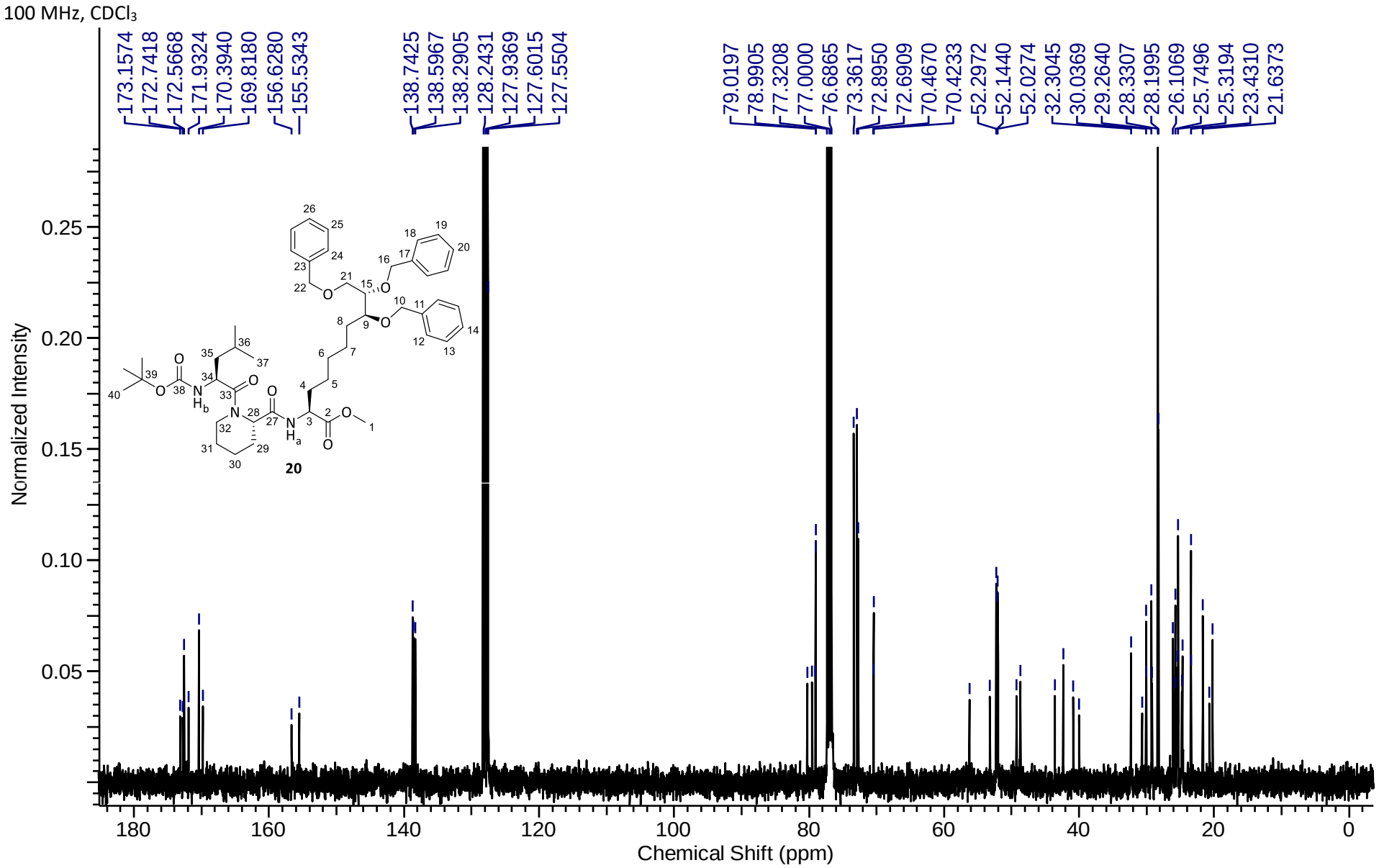
19


100 MHz, CDCl3
172.6324
170.9189
138.7207
138.5894
138.2686
128.3014
128.2285
127.9514
127.5796
127.4848
80.6384
79.0270
77.3135
77.0000
76.6792
73.3325
72.8731
72.6690
70.4160
55.4105
53.7263
52.2315
52.0493
42.2206
41.3238
32.4649
30.0077
29.2130
28.2797
25.7350
25.3705
25.3194
24.8090
20.4562
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)
19

Methyl (2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-2-{(*S*)-1-[(*tert*-butoxycarbonyl)-L-leucyl]piperidine-2-carboxamido}decanoate (20)

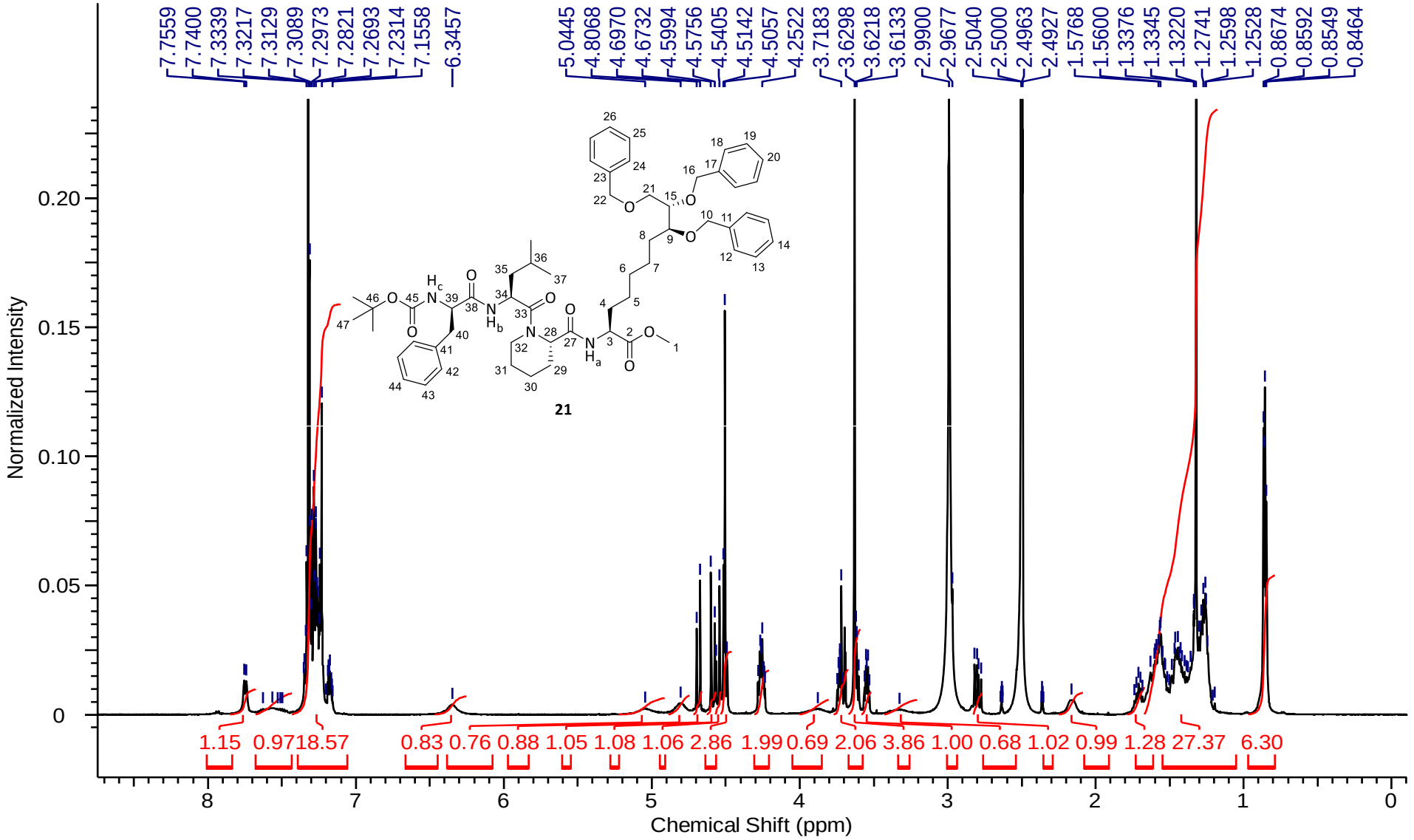
400 MHz, $CDCl_3$



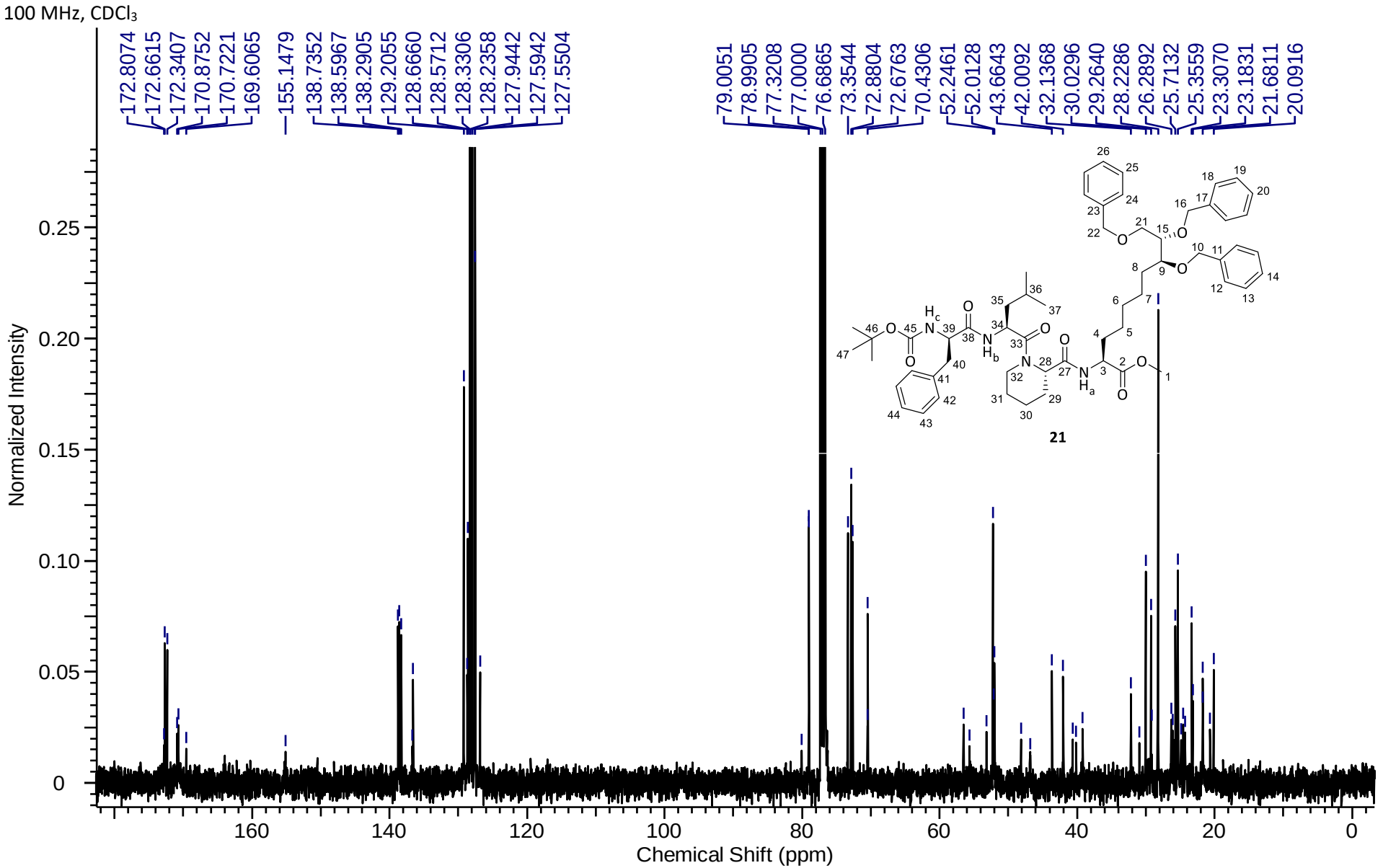

100 MHz, CDCl3
173.1574
172.7418
172.5668
171.9324
170.3940
169.8180
156.6280
155.5343
138.7425
138.5967
138.2905
128.2431
127.9369
127.6015
127.5504
79.0197
78.9905
77.3208
77.0000
76.6865
73.3617
72.8950
72.6909
70.4670
70.4233
52.2972
52.1440
52.0274
32.3045
30.0369
29.2640
28.3307
28.1995
26.1069
25.7496
25.3194
23.4310
21.6373
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)
20

Methyl (2*S*,8*S*,9*S*)-8,9,10-tris(benzyloxy)-2-{(*S*)-1-[(*tert*-butoxycarbonyl)-D-phenylalanyl-L-leucyl]piperidine-2-carboxamido}decanoate (21)

500 MHz, DMSO-d_6, 373 K

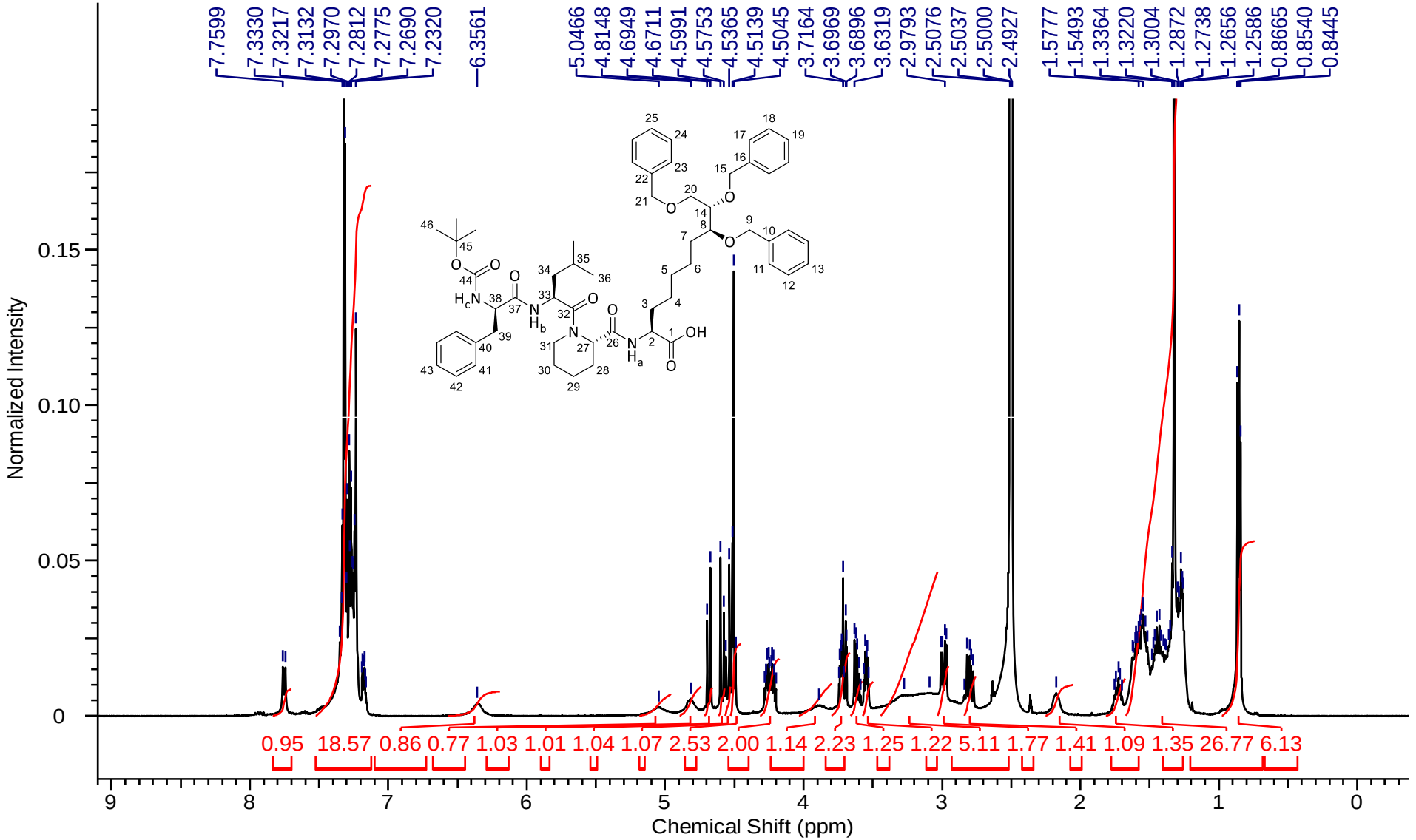


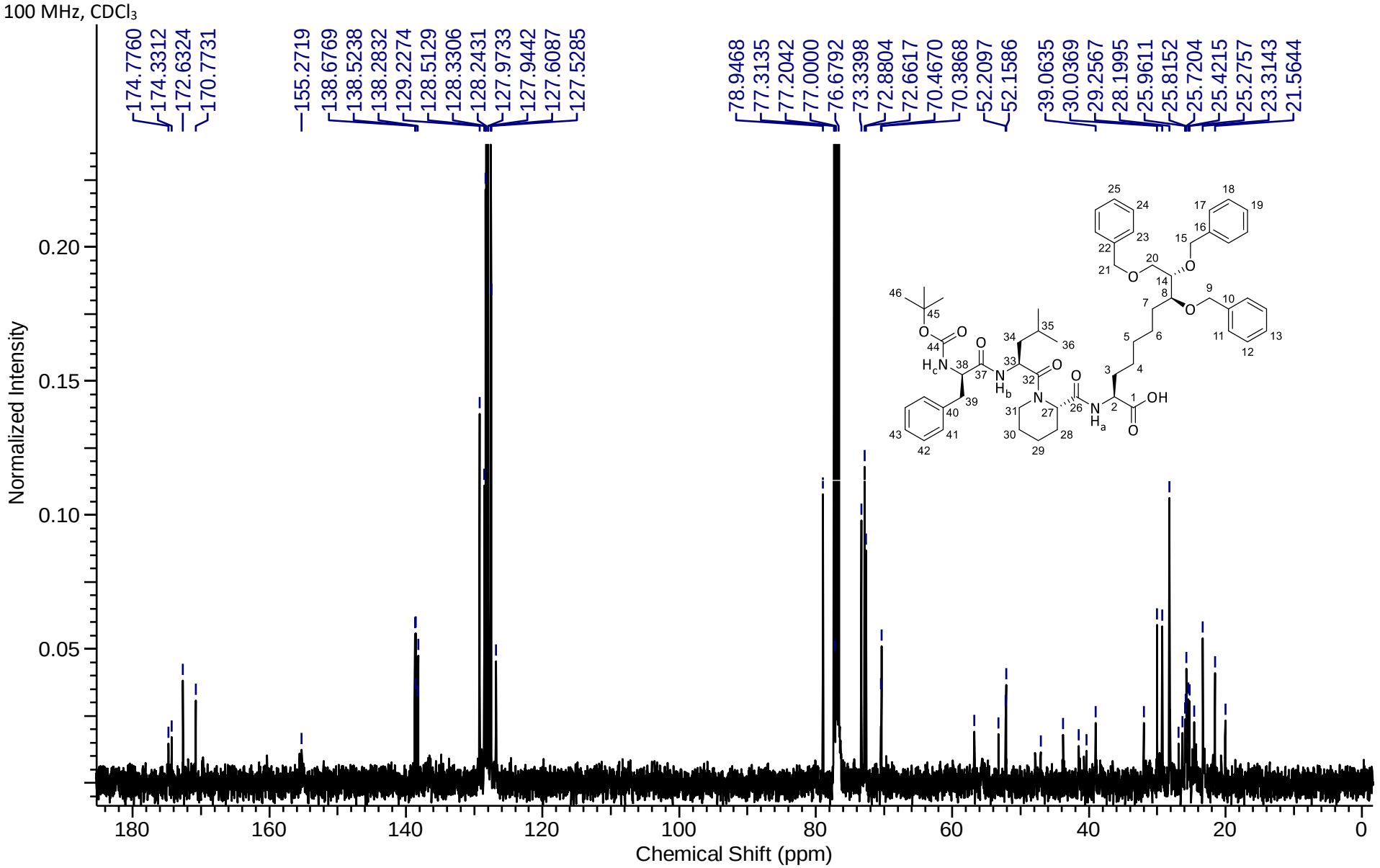
21


100 MHz, CDCl3
172.8074
172.6615
172.3407
170.8752
170.7221
169.6065
155.1479
138.7352
138.5967
138.2905
129.2055
128.6660
128.5712
128.3306
128.2358
127.9442
127.5942
127.5504
79.0051
78.9905
77.3208
77.0000
76.6865
73.3544
72.8804
72.6763
70.4306
52.2461
52.0128
43.6643
42.0092
32.1368
30.0296
29.2640
28.2286
26.2892
25.7132
25.3559
23.3070
23.1831
21.6811
20.0916
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
0
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)
21

(2*S*,8*S*,9*S*)-8,9,10-Tris(benzyloxy)-2-{(*S*)-1-[(*tert*-butoxycarbonyl)-D-phenylalanyl-L-leucyl]piperidine-2-carboxamido}decanoic acid

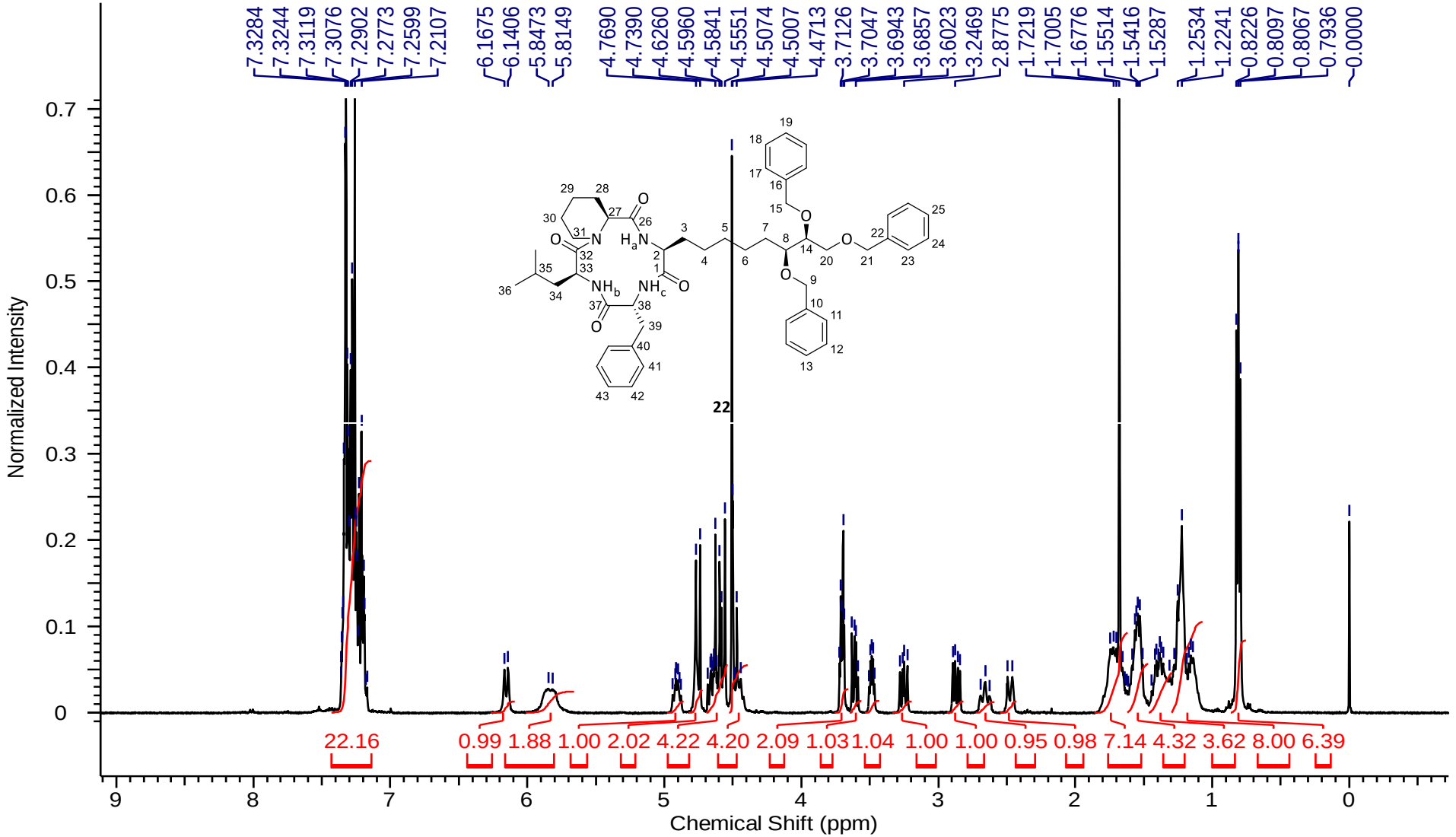
500 MHz, DMSO-d_6, 373 K

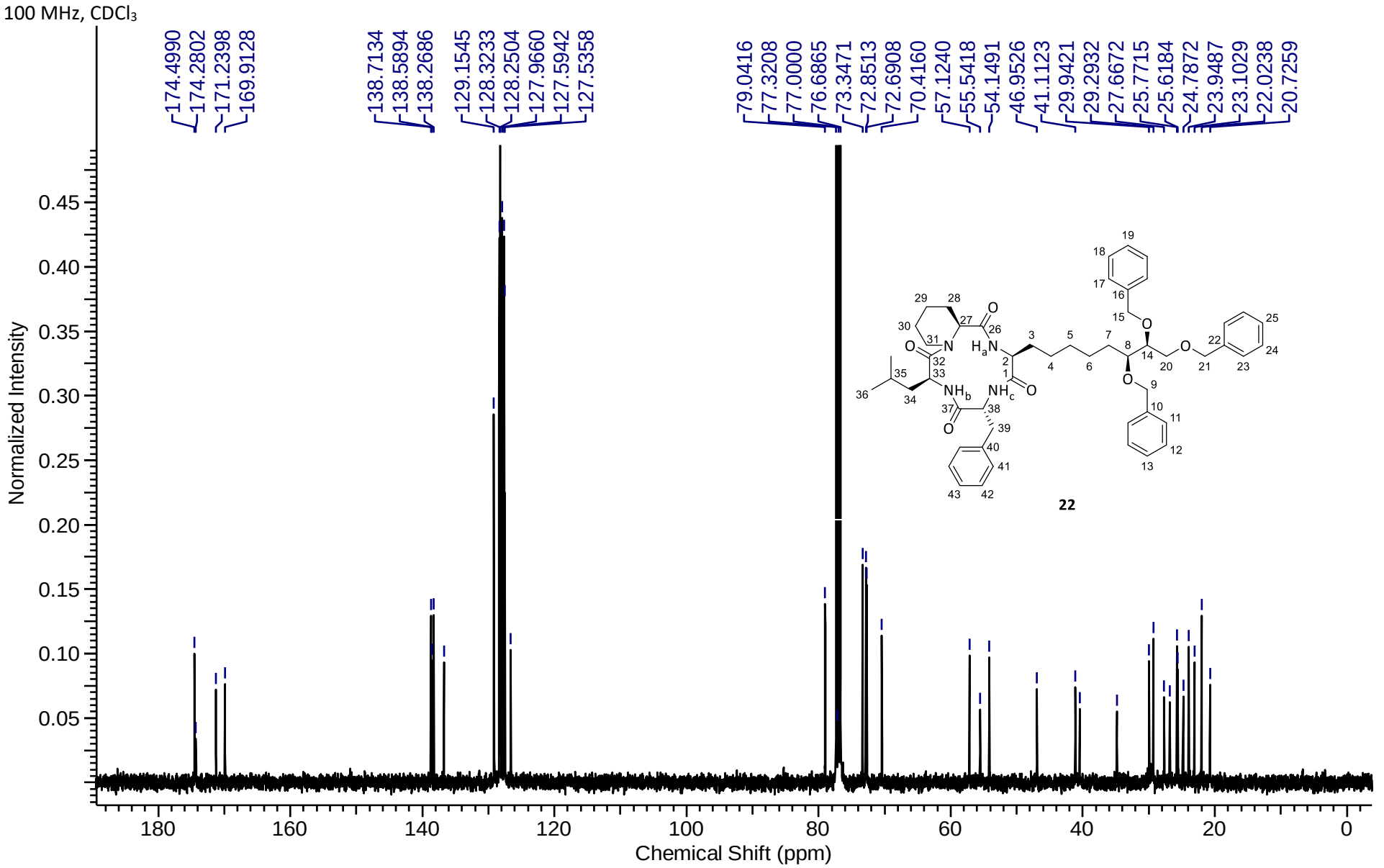



100 MHz, CDCl3
174.7760
174.3312
172.6324
170.7731
155.2719
138.6769
138.5238
138.2832
129.2274
128.5129
128.3306
128.2431
127.9733
127.9442
127.6087
127.5285
78.9468
77.3135
77.2042
77.0000
76.6792
73.3398
72.8804
72.6617
70.4670
70.3868
52.2097
52.1586
39.0635
30.0369
29.2567
28.1995
25.9611
25.8152
25.7204
25.4215
25.2757
23.3143
21.5644
Normalized Intensity
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

(3*S*,6*R*,9*S*,15a*S*)-6-Benzyl-9-isobutyl-3-[(6*S*,7*S*)-6,7,8-tris(benzyloxy)octyl]octahydro-2H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecyne-1,4,7,10(3H,12H)-tetraone (22)

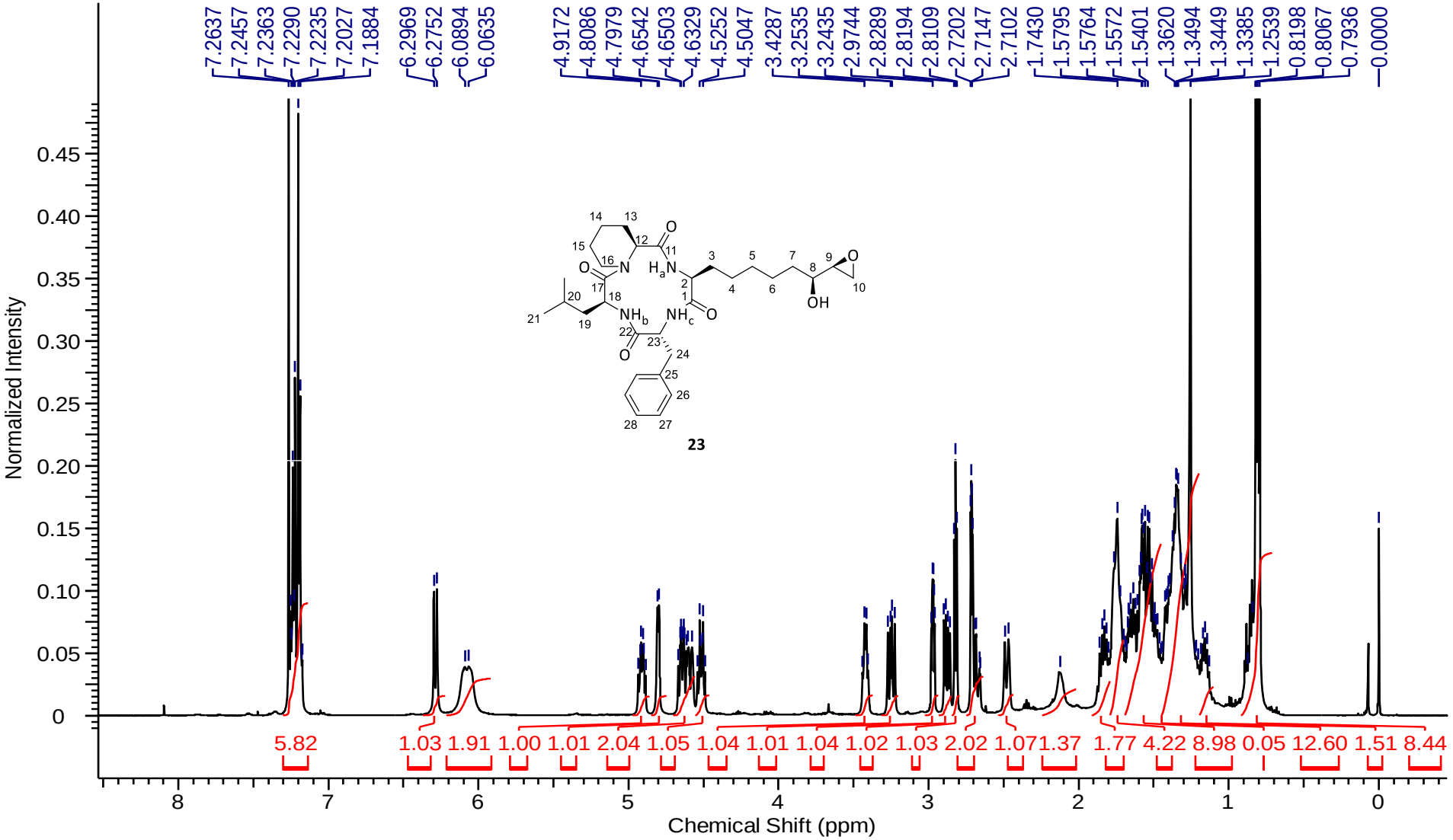
400 MHz, $CDCl_3$

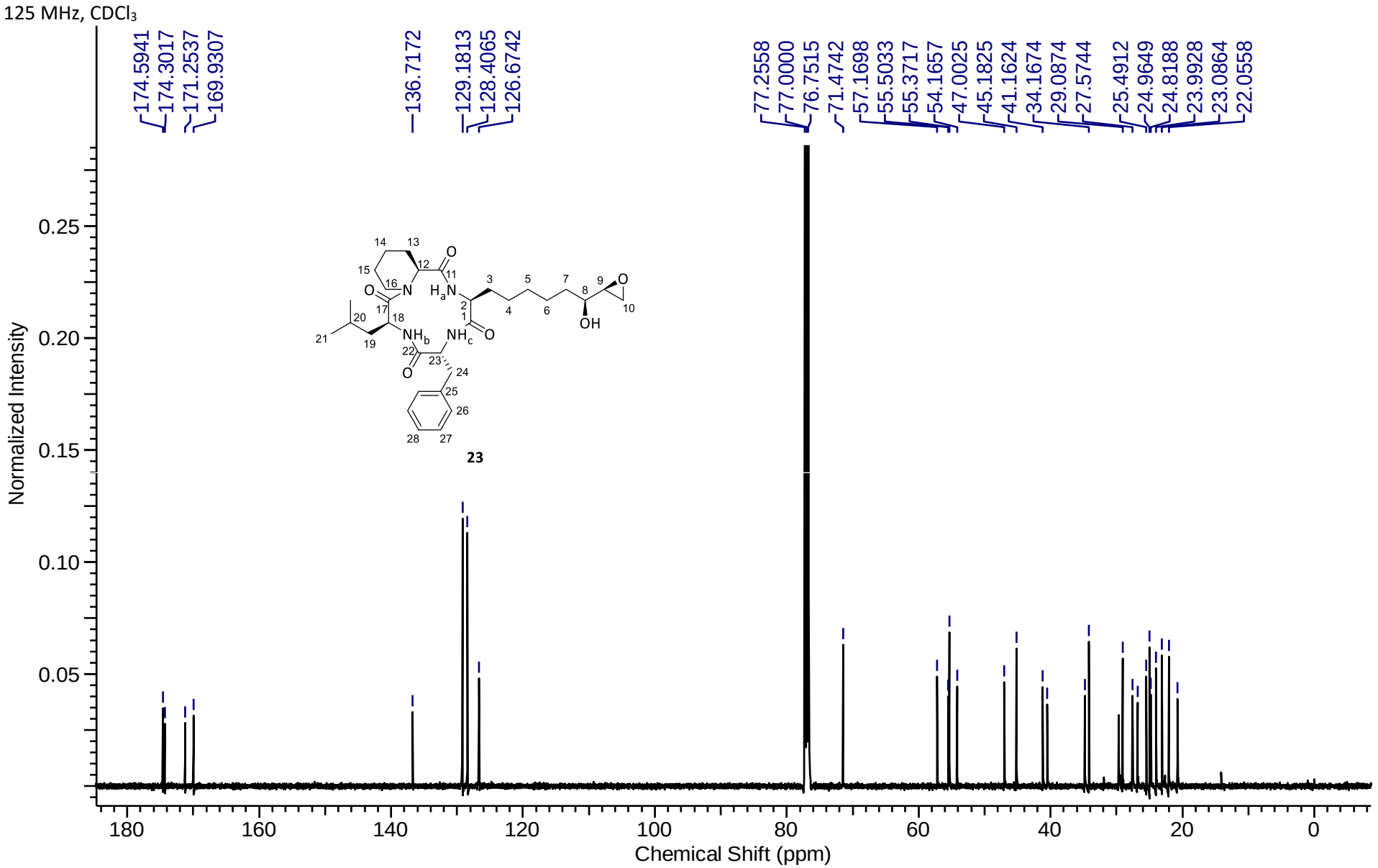



100 MHz, CDCl3
174.4990
174.2802
171.2398
169.9128
138.7134
138.5894
138.2686
129.1545
128.3233
128.2504
127.9660
127.5942
127.5358
79.0416
77.3208
77.0000
76.6865
73.3471
72.8513
72.6908
70.4160
57.1240
55.5418
54.1491
46.9526
41.1123
29.9421
29.2932
27.6672
25.7715
25.6184
24.7872
23.9487
23.1029
22.0238
20.7259
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)
22

(3*S*,6*R*,9*S*,15a*S*)-6-Benzyl-3-{(*S*)-6-hydroxy-6-[(*S*)-oxiran-2-yl]hexyl}-9-isobutyloctahydro-2H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecyne-1,4,7,10(3H,12H)-tetraone (23)

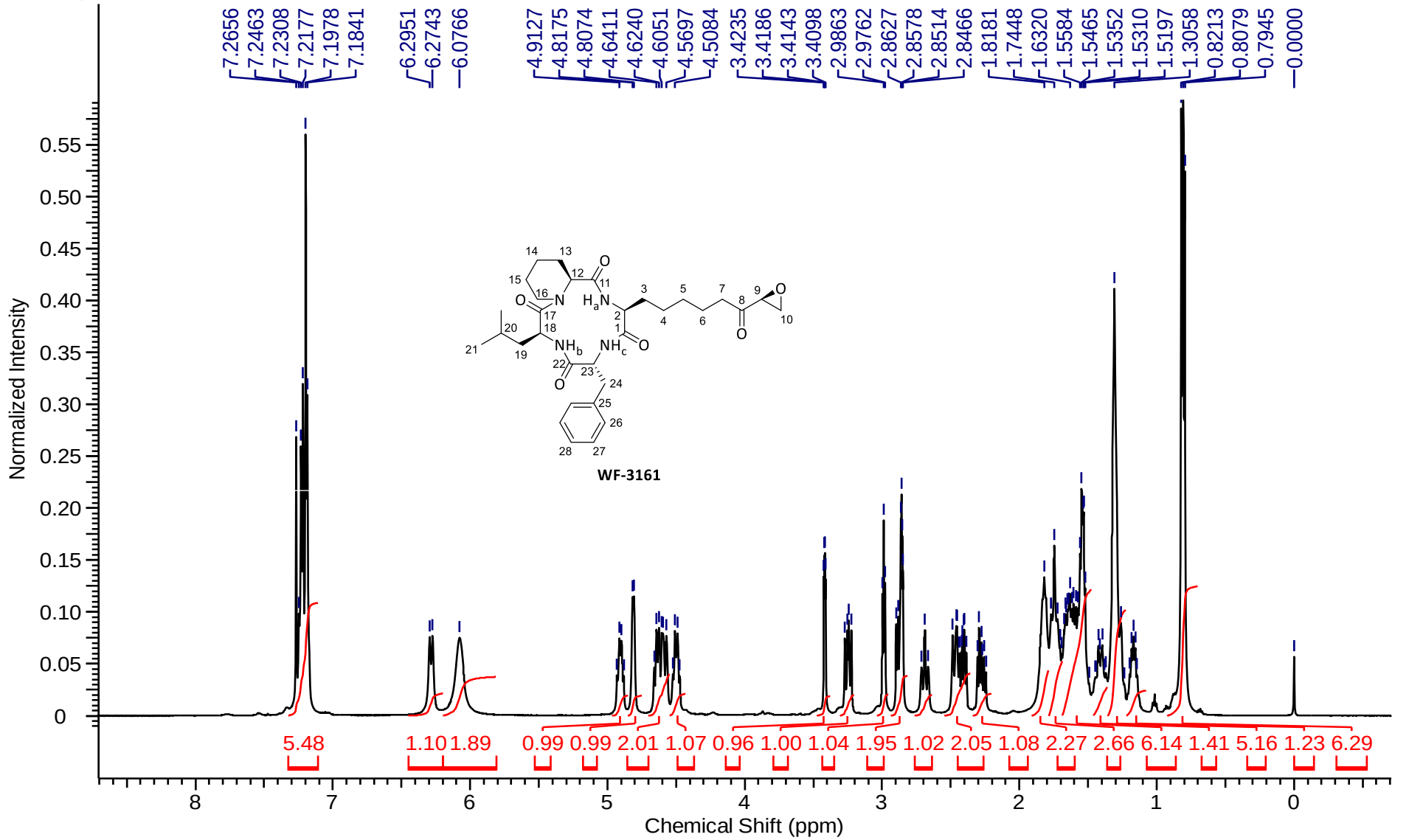
500 MHz, $CDCl_3$



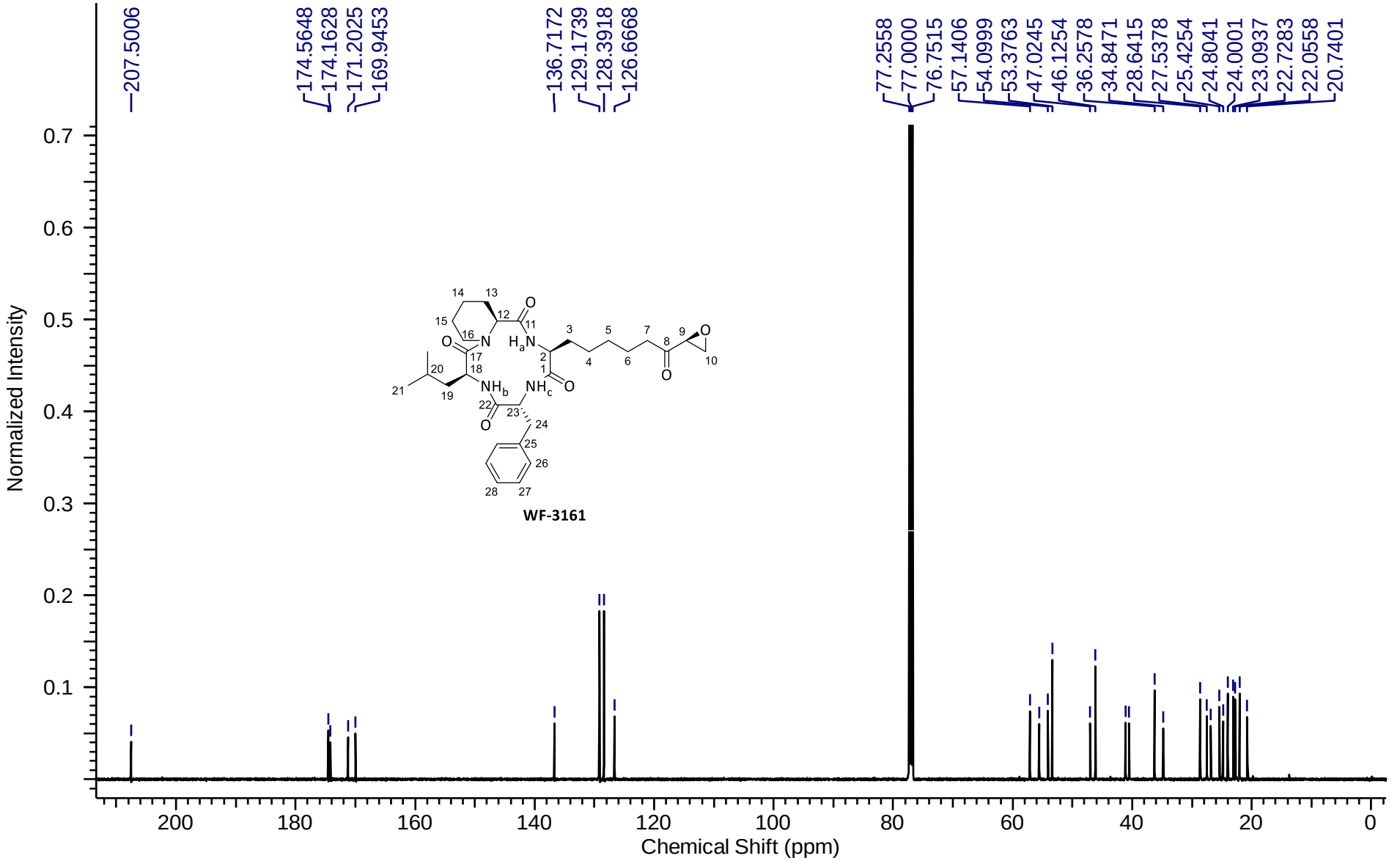

125 MHz, CDCl3
174.5941
174.3017
171.2537
169.9307
136.7172
129.1813
128.4065
126.6742
77.2558
77.0000
76.7515
71.4742
57.1698
55.5033
55.3717
54.1657
47.0025
45.1825
41.1624
34.1674
29.0874
27.5744
25.4912
24.9649
24.8188
23.9928
23.0864
22.0558
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)
23

WF-3161

500 MHz, $CDCl_3$



125 MHz, $CDCl_3$



WF-3161

Biological Assays

In Vitro Testing on HDAC1, HDAC6 and HDAC8

Enzyme inhibition was determined by using a homogenous fluorescence assay as previously described (Heltweg, 2005)[6]. For HDAC1 and 6 activity testing, OptiPlate-96 black microplates (PerkinElmer) were used. Assay volume was 60 µL, and 52 µL of human recombinant HDAC1 (BPS Bioscience, catalog no. 50051) or human recombinant HDAC6 (BPS Bioscience, catalog no. 50006) in incubation buffer (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, and 1 mg/mL bovine serum albumin) were incubated with increasing concentrations of inhibitors in DMSO and 5 µL of the fluorogenic substrate ZMAL (Z-(Ac)Lys-AMC) (126 µM) for 90 min at 37 °C. HDAC enzyme concentration was adjusted in order to have a final ZMAL conversion between 15 % and 30 %. After incubation time, an amount of 60 µL of the stop solution, comprising 5 µL TSA solution (33 µM in DMSO) and 10 µL trypsin (6 mg/mL) in trypsin buffer (Tris-HCl 50 mM, pH 8.0, NaCl 100 mM), was added. The plate was incubated again at 37 °C for 30 min, and fluorescence was measured on a BMG LABTECH POLARstar OPTIMA plate reader (BMG Labtechnologies, Germany) with an excitation wavelength of 390 nm and an emission wavelength of 460 nm. IC_{50} values were determined using OriginPro 9G (OriginLab, USA) by a non-linear regression to fit the dose response curves. Pre-test experiments were performed in duplicates, IC_{50} determination was carried out in triplicates.

Recombinant hHDAC8 was produced by Romier et al. in Strasbourg (M. Marek et al).[7] Inhibition of human HDAC8 was measured in 1/2 AREAPLATE- 96 F microplates (PerkinElmer) with an assay volume of 50 µL. The activity assay was performed using a commercial HDAC8 Fluorimetric Drug Discovery Kit [Fluor de Lys(R)-HDAC8, BML-KI178] according to the manufacturer's instructions. The enzyme was incubated for 90 min at 37 °C, with a substrate concentration of 50 µM and increasing concentrations of inhibitors. The stop-solution containing inhibitor, to stop the hHDAC8 activity, and Trypsin, to release AMC, was added. The solution was incubated for 20 min at 37 °C to develop the assay. Measurement was performed as described for HDAC1/6.

In Vitro Testing on Human Sirt1-3 deacetylation

Enzyme inhibition was determined by using a homogenous fluorescence assay as previously described (Heltweg, 2003)[8]. The assay was performed in black 96 well plates (OptiPlate TM 96-F, Perkin Elmer, USA) with a reaction volume of 60 µL per well. 47 µL hSirt1(134-747), hSirt2 (56-356), and hSirt3 (101-399) in assay buffer (see HDAC1 and HDAC6) were mixed with 5 µL of a substrate solution (10.5 µM final concentration) and 3 µL of inhibitor dissolved in DMSO at various concentrations or 3 µL of DMSO as control (final assay concentration 5 % (v/v)). 5 µL of NAD^+ solution (3.98 mg/mL in assay buffer, final assay concentration 500 µM) were added in order to start the reaction. The reaction was incubated for 4 h at 37 °C. hSirt assay concentration was adjusted in order to have a final ZMAL conversion between 15 % and 30 %. After the first incubation, 60 µL of stop solution (50 mM Tris, 100 mM NaCl, 6.7 % (v/v) DMSO, trypsin 1 mg/mL, 8 mM nicotinamide, pH=8.0) were added. The microplate was incubated further for 20 min at 37 °C and fluorescence intensity was measured as described for HDAC1/6. As blank control a solution containing ZMAL without the enzyme was used. IC_{50} values were determined using OriginPro 9G (OriginLab, USA) by a non-linear regression to fit the dose response curves. Pre-test experiments were performed in duplicates, IC_{50} determination was carried out in triplicates.

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[6] B. Heltweg, J. Trapp, M. Jung, *Methods*, 2005, **36**, 332–337.

[7] M. Marek, S. Kannan, A. T. Hauser, M. Moraes Mourão, S. Caby, V. Cura, D. A. Stolfa, K. Schmidtkunz, J. Lancelot, L. Andrade, J. P. Renaud, G. Oliveira, W. Sippl, M. Jung, J. Cavarelli; R. J. Pierce, C. Romier, *PLoS Pathog.* 2013, **9**, e1003645

[8] B. Heltweg, M. Jung, *SLAS Discovery*, 2003, **8**, 89–95.

Cell Viability Assay

Cell viability were assessed using the Celltiter 96 Aqueous nonradioactive Proliferation Assay (Promega). HL60 cells with a cell density of 5 x 10^3 cells per well, were immediately incubated in a 96-well tissue culture plate with inhibitor or DMSO vehicle to a total volume of 100 µL for 72 hours. As HL60 cells already differentiate with 1 % DMSO content, inhibitors were prepared as 200x stock solutions in DMSO. In all cases inhibitors were compared to DMSO vehicle only and three replicates per concentration were used. Cell viability was determined according to the manufacturer's instructions. Data was plotted as absorbance units against logarithm of compound concentration using OriginPro 9. 50% Growth inhibition (GI_{50}) was determined as compound concentration required to reduce the number of metabolically active cells by 50% compared to DMSO control.