Discovery of deoxyceramide analogs as highly selective ACER3 inhibitors in live cells

N. Bielsa, M. Casasampere, M. Aseeri, J. Casas, A. Delgado, J. L. Abad and G. Fabriàs

SUPPLEMENTARY INFORMATION

SYNTHESIS OF COMPOUNDS

General Synthetic Methods

Unless otherwise stated, reactions were carried out under argon atmosphere. Dry solvents were obtained by passing through an activated alumina column on a Solvent Purification System (SPS). Commercially available reagents were used with no further purification. Benzoic acid, 4-etylbenzoic acid, 4-propylbenzoic acid, 4-pentylbenzoic acid, 4-hexylbenzoic acid 4-tert-butylbenzoic acid, 4-pentyloxybenzoic acid, 4-hexyloxybenzoic acid, 4-heptyloxybenzoic acid, methyl 4-hydroxybenzoate, bromomethyl methyl ether, bromoethyl methyl ether, and 1-bromo-2-(2-methoxyethoxy)ethane were obtained from commercial sources.

The following MOM bromides were prepared following reported protocols: 1-bromo-3-(methoxymethoxy)propane, 1-bromo-4-(methoxymethoxy)butane, 1-bromo-5-(methoxymethoxy)pentane, 1-bromo-7-(methoxymethoxy)heptane [1]. 1-Deoxysphingoid bases **1-4** and **21-24** were obtained according to described procedures [2].

All reactions were monitored by TLC analysis using Silica gel on precoated aluminum plates with fluorescent indicator at 254 nm (Sigma-Aldrich). UV light or a 5% (w/v) ethanolic solution of phosphomolybdic acid were used as the visualizing agents. Flash column chromatography was carried out with the indicated solvents using flash-grade silica gel (37-70 μm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. The purity of all synthesized (deoxy)ceramide analogs was >95% as estimated by ^{1}H NMR and TLC analysis. Specific rotations were recorded on a digital Perkin-Elmer 34 polarimeter at 25 ºC in a 1 mL cell. The lamp used was a sodium light lamp (589 nm). Results of specific optical rotation are reported in deg^{-1} cm^{3} g^{-1} ($[\alpha]^{20}_{D}$) and concentrations (c) are expressed in g/mL.

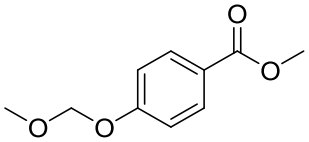
High Resolution Mass Spectrometry analyses were carried out on an Acquity UPLC system coupled to a LCT Premier orthogonal accelerated time-of-flight mass spectrometer (Waters) using electrospray ionization (ESI) technique.

Synthesis of *O*-alkylbenzoic acids: General method

Synthesis of polyether alkylbenzoic acids were carried out using two reaction steps.

Step A. Polyalkoxy chain coupling: This reaction was performed using the methodology described by Bost and Winstead with minor modifications [3]. In this case, cesium carbonate (720 mg, 2.21 mmol) was added portionwise over a solution of methyl *p*-hydroxybenzoate (150 mg, 0,98 mmol) in dry acetone (10 mL). A solution of the proper bromide in acetone (1.5 mmol/5 mL) was next added dropwise. After 8h reflux, the reaction mixture was cooled, the solids were removed by filtration and the recovered solution was evaporated to dryness to give an oil which was chromatographed using a gradient of hexanes/EtOAc (from 0 to 20%) to give the corresponding pure methyl O-alkyl benzoate esters in yields ranging between 75 and 90%.

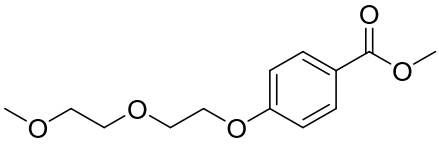
Methyl 4-(methoxymethoxy)benzoate (41) [4]



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (m, 2H), 7.05 (m, 2H), 5.22 (s, 2H), 3.89 (s, 3H), 3.48 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 161.0, 131.6, 123.7, 115.7, 94.1, 56.3, 51.9.

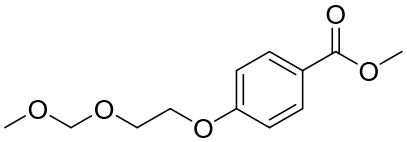
Methyl 4-(2-(2-methoxyethoxy)ethoxy)benzoate (42) [5]



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (m, 2H), 6.92 (m, 2H), 4.19 (m, 2H), 3.89 (s + m, 5H), 3.71 (m, 2H), 3.57 (m, 2H), 3.38 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 162.4, 131.4, 122.6, 114.1, 71.8, 70.6, 69.4, 67.4, 58.9, 51.6.

Methyl 4-(2-(methoxymethoxy)ethoxy)benzoate (43)

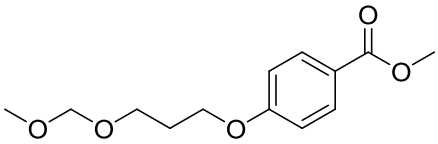


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (m, 2H), 6.94 (m, 2H), 4.72 (s, 2H), 4.20 (m, 2H), 3.92 (m, 2H), 3.88 (s, 3H), 3.40 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 162.5, 131.5, 122.8, 114.1, 96.6, 67.4, 65.7, 55.2, 51.8.

HRMS calcd. for $C_{12}H_{17}O_5^+$ ($[M+H]^+$): 241.1071, Found: 241.1074

Methyl 4-(3-(methoxymethoxy)propoxy)benzoate (44)

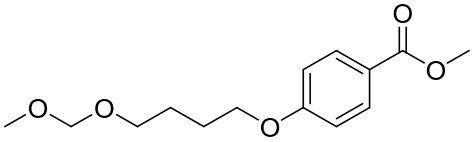


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (m, 2H), 6.92 (m, 2H), 4.63 (s, 2H), 4.13 (t, *J* = 6.5 Hz, 2H), 3.88 (s, 3H), 3.72 (t, *J* = 6.0 Hz, 2H), 3.34 (s, 3H), 2.09 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 162.8, 131.6, 122.5, 114.1, 96.5, 64.9, 63.9, 55.2, 51.8, 29.5.

HRMS calcd. for $C_{13}H_{19}O_5^+$ ($[M+H]^+$): 255.1227, Found: 255.1221

Methyl 4-(4-(methoxymethoxy)butoxy)benzoate (45)

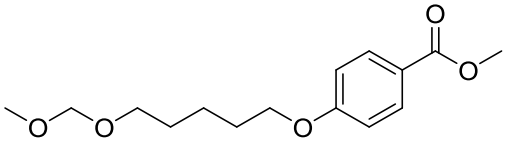


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (m, 2H), 6.90 (m, 2H), 4.64 (s, 2H), 4.05 (t, *J* = 6.0 Hz, 2H), 3.88 (s, 3H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.37 (s, 3H), 1.90 (m, 2H), 1.79 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 162.9, 131.6, 122.5, 114.1, 96.5, 67.9, 67.3, 55.2, 51.9, 26.4, 26.1.

HRMS calcd. for $C_{14}H_{21}O_5^+$ ($[M+H]^+$): 269.1384, Found: 269.1391

Methyl 4-(5-(methoxymethoxy)pentyloxy)benzoate (46)

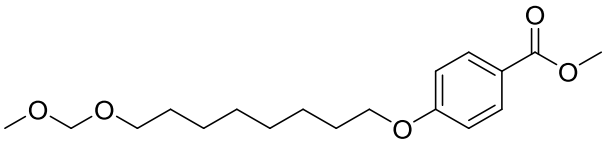


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 4.62 (s, 2H), 4.02 (t, *J* = 6.5 Hz, 2H), 3.88 (s, 3H), 3.56 (t, *J* = 6.5 Hz, 2H), 3.36 (s, 3H), 1.84 (m, 2H), 1.66 (m, 2H), 1.57 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 162.9, 131.6, 122.4, 114.1, 96.5, 68.0, 67.6, 55.2, 51.9, 29.5, 28.9, 22.9.

HRMS calcd. for $C_{15}H_{23}O_5^+$ ($[M+H]^+$): 283.1540, Found: 283.1540

Methyl 4-(8-(methoxymethoxy)octyloxy)benzoate (47)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 4.62 (s, 2H), 4.02 (t, *J* = 6.5 Hz, 2H), 3.88 (s, 3H), 3.56 (t, *J* = 6.5 Hz, 2H), 3.36 (s, 3H), 1.84 (m, 2H), 1.66 (m, 2H), 1.57 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 162.9, 131.6, 122.4, 114.1, 96.5, 68.0, 67.6, 55.2, 51.9, 29.5, 28.9, 22.9.

HRMS calcd. for $C_{18}H_{29}O_5^+$ ($[M+H]^+$): 325.2010, Found:325.2019

Step B: **Saponification of the benzoate ester and condensation with the sphingoid bases**.

Saponification: General method. A solution of the corresponding benzoate ester (0.5 mmol) in EtOH (5 mL) was treated with aqueous 2*N* NaOH (5 mL). After stirring for 12h at rt, the reaction mixture was evaporated to dryness and the residue taken up in aqueous 1*N* HCl, extracted with CH_2Cl_2 (3 x 5 mL), dried ($MgSO_4$) and evaporated to give an oil, which was purified by flash chromatography (CH_2Cl_2-/MeOH from o to 3%) to afford the required acid in yields ranging from 85 to 95%

Acylation of the sphingoid bases: General method

To a solution of the corresponding benzoic acid derivative (0.11 mmol) and HOBt (0.13 mmol) in anhydrous CH_2Cl_2 (5 mL) was added EDC (0.14 mmol). The resulting mixture was stirred at rt for 5 min, and next added dropwise to a solution of the corresponding sphingoid base (0.1 mmol) and Et_3N (0.3 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 2 h and controlled by TLC. The mixture was next diluted by addition of CH_2Cl_2 (10 mL), and washed successively with 1*M* $NaHCO_3$ solution and brine (5 mL each). The organic layer was dried over $MgSO_4$, and filtered. Concentration under reduced pressure afforded crude compounds. Final products were purified by flash chromatography (hexanes/EtOAc, 7/3) to give the corresponding amides in 70-85% yield.

Compounds' characterization

33 [RBM1-32 (2*S*,3*R*-stereoisomer)] /// 35 [RBM1-58 (2*R*,3*S*-stereoisomer)]

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (d, *J* = 8.0 Hz, 1H), 4.09-3.98 (m, 1H), 4.05 (s, 2H), 3.68 (br s, 1H), 1.58 (br, 2H), 1.44 (m, 2H), 1.19 (br, 26H), 1.15 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 8.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 73.9, 49.8, 42.8, 33.9, 32.1, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.5, 26.1, 22.8, 14.3, 13.7.

HRMS calcd. for $C_{20}H_{41}BrNO_2^+$ ($[M+H]^+$): 406.2315 (100.0%), 408.2295 (97.3%), Found: 406.2307, 408.2287

33 [RBM1-32] $[\alpha]_D$ -4.2 (c=1, $CHCl_3$); **35 [RBM1-58]** $[\alpha]_D$ +4.4 (c=1, $CHCl_3$).

25 [RBM1-33] (2*S*,3*R*-stereoisomer) /// 27 [RBM1-55] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (m, 1H), 4.01 (m, 1H), 3.62 (m, 1H), 2.16 (t, *J* = 8.0 Hz, 2H), 1.62 (m, 2H), 1.48 (br, 1H), 1.39 (m, 2H), 1.19 (br, 34H), 1.09 (d, *J* = 8.0 Hz, 3H), 0.87 (t, *J* = 8.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 74.5, 49.6, 37.1, 33.7, 32.1, 31.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 29.4, 29.2, 26.2, 26.0, 22.8, 22.7, 14.3, 14.2.

HRMS calcd. for $C_{26}H_{54}NO_2^+$ ($[M+H]^+$): 412.4149 Found: 412.4153

25 [RBM1-33] $[\alpha]_D$ -11.6 (c=1, $CHCl_3$); **27 [RBM1-55]** $[\alpha]_D$ +11.4 (c=1, $CHCl_3$).

13 [RBM1-34] (2*S*,3*R*-stereoisomer) /// 15 [RBM1-70] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8 Hz, 1H), 5.75 (dtd, *J* = 14.5, 8.0, 1.0 Hz, 1H), 5.45 (m, 1H), 4.17 (m, 1H), 4.10 (m, 1H), 4.05 (s, 2H), 2.06 (q, *J* = 7 Hz, 2H), 1.65 (broad, 1H), 1.37 (m, 2H), 1.19 (broad, 20H), 1.15 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 134.7, 128.1, 75.0, 50.3, 42.8, 32.5, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 14.9, 14.2.

HRMS calcd. for $C_{20}H_{39}BrNO_2^+$ ($[M+H]^+$): 404.2159 (100.0%), 406.2138 (97.3%), Found: 404.2169, 406.2148

13 [RBM1-34] $[\alpha]_D$ -13.6 (c=1, $CHCl_3$); **15 [RBM1-70]** $[\alpha]_D$ +12.2 (c=1, $CHCl_3$).

5 [RBM1-36] (2*S*,3*R*-stereoisomer) /// 7 [RBM1-67] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (m, 1H), 5.68 (d, *J* = 8 Hz, 1H), 5.41 (m, 1H), 4.11 (d, *J* = 6.5 Hz, 1H), 4.09 (m, 1H), 2.60 (br, 1H), 2.17 (t, *J* = 8.0 Hz, 2H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.62 (m, 2H), 1.36 (m, 2H), 1.19 (br, 34H), 1.09 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 134.2, 128.3, 75.8, 50.2, 37.0, 32.5, 32.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.2, 26.8, 22.8, 22.7, 15.4, 14.3, 14.2.

HRMS calcd. for $C_{26}H_{52}NO_2^+$ ([M+H]$^+$): 410.3993 Found: 410.3989

5 [RBM1-36] $[\alpha]_D$ -31.2 (c=1, $CHCl_3$); **7 [RBM1-67]** $[\alpha]_D$ +30.4 (c=1, $CHCl_3$).

9 [RBM1-37] (2*S*,3*R*-stereoisomer) /// 11 [RBM1-68] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78-5.64 (ca, 2H), 5.41 (m, 1H), 4.11 (d, *J* = 6.0 Hz, 1H), 4.09 (m, 1H), 2.54 (br, 1H), 2.04 (q, *J* = 7.0 Hz, 2H), 1.37 (m, 2H), 1.25 (broad s, 20H), 1.20 (s, 9H), 1.10 (d, *J* = 8 Hz, 3H), 0.87 (t, *J* = 7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.4, 134.2, 128.2, 76.0, 50.2, 38.8, 32.5, 32.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.4, 27.7, 22.8, 15.6, 14.2.

HRMS calcd. for $C_{23}H_{46}NO_2^+$ ([M+H]$^+$): 368.3523 Found: 368.3526

9 [RBM1-37] $[\alpha]_D$ -34.0 (c=1, $CHCl_3$); **11 [RBM1-68]** $[\alpha]_D$ +33.2 (c=1, $CHCl_3$).

17 [RBM1-38] (2*S*,3*R*-stereoisomer) /// 19 [RBM1-69] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8 Hz, 2H), 7.23 (d, *J* = 8 Hz, 2H), 6.25 (d, *J* = 8.0 Hz, 1H), 5.76 (dtd, *J* = 15.0, 7.0, 1.0 Hz, 1H), 5.49 (ddt, *J* = 15.5, 6.5, 1.5 Hz, 1H), 4.31 (ddt, *J* = 10.0, 8.0, 5.5 Hz, 1H), 4.24 (ddd, *J* = 6.5, 3.0, 1.0 Hz, 1H), 2.64 (t, *J* = 8 Hz, 2H), 2.06 (q, *J* = 7 Hz, 2H), 1.82 (Br, 1H), 1.61 (m, 2H), 1.32-1.09 (bs, 30H), 1.21 (d, *J* = 7 Hz, 3H), 0.88 (2x t, *J* = 6.5 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 147.1, 134.3, 131.9, 128.7, 128.5, 127.1, 75.7, 50.6, 36.0, 32.5, 32.1, 31.9, 31.4, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.3, 22.8, 22.7, 15.3, 14.3, 14.2.

HRMS calcd. for $C_{32}H_{56}NO_2^+$ ([M+H]$^+$): 486.4306, Found: 486.4299

17 [RBM1-38] $[\alpha]_D$ -9.2 (c=1, $CHCl_3$); **19 [RBM1-69]** $[\alpha]_D$ +9.4 (c=1, $CHCl_3$).

6 [RBM1-39] (2*S*,3*S*-stereoisomer) /// 8 [RBM1-63] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (m, 1H), 5.68 (d, *J* = 8 Hz, 1H), 5.41 (m, 1H), 4.11 (d, *J* = 6.5 Hz, 1H), 4.09 (m, 1H), 2.60 (br, 1H), 2.17 (t, *J* = 8.0 Hz, 2H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.62 (m, 2H), 1.36 (m, 2H), 1.19 (br, 34H), 1.09 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 134.2, 129.7, 76.2, 49.8, 37.1, 32.4, 32.1, 31.9, 29.8, 29.8, 29.8, 29.6, 29.7, 29.5, 29.4, 29.4, 29.3, 29.2, 26.0, 22.8, 22.8, 17.7, 14.3, 14.2.

HRMS calcd. for $C_{26}H_{52}NO_2^+$ ([M+H]$^+$): 410.3993 Found: 410.3999

6 [RBM1-39] $[\alpha]_D$ -22.4 (c=1, $CHCl_3$); **8 [RBM1-63]** $[\alpha]_D$ +20.2 (c=1, $CHCl_3$).

10 [RBM1-40] (2*S*,3*S*-stereoisomer) /// 12 [RBM1-64] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (d, *J* = 8.0 Hz, 1H), 5.68 (dt, *J* = 14.0, 8.0 Hz, 1H), 5.43 (ddt, *J* = 15.5, 6.5, 1.5 Hz, 1H), 3.98 (m, 1H), 3.95 (m, 1H),), 2.25 (br, 1H) 2.02 (q, *J* = 7 Hz, 2H), 1.34 (m, 2H), 1.30-1.20 (br, 22H), 1.18 (s, 9H), 1.16 (d, *J* = 6.5 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.9, 134.0, 129.7, 76.0, 49.5, 38.8, 32.4, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.7, 22.8, 17.5, 14.3.

HRMS calcd. for $C_{23}H_{46}NO_2^+$ ($[M+H]^+$): 368.3523 Found: 368.3532

10 [RBM1-40] $[\alpha]_D$ -26.4 (c=1, $CHCl_3$); **12 [RBM1-64]** $[\alpha]_D$ +25.2 (c=1, $CHCl_3$).

18 [RBM1-41] (2*S*,3*S*-stereoisomer) /// 20 [RBM1-65] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.37 (d, *J* = 8.0 Hz, 1H), 5.73 (dt, *J* = 15.0, 7.0 Hz, 1H), 5.52 (ddt, *J* = 15.5, 6.5, 1.5 Hz, 1H), 4.19 (m, 1H), 4.10 (m, 1H), 2.63 (t, *J* = 8 Hz, 2H), 2.34 (Br, 1H), 2.02 (q, *J* = 7 Hz, 2H), 1.61 (m, 2H), 1.36–1.18 (br, 32H), 0.88 (2x t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 147.0, 134.2, 132.0, 129.7, 128.6, 127.1, 75.9, 50.2, 36.0, 32.4, 32.1, 31.9, 31.4, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.3, 22.8, 22.8, 17.7, 14.2, 14.2.

HRM calcd. for $C_{32}H_{56}NO_2^+$ ($[M+H]^+$): 486.4306, Found: 486.4212

18 [RBM1-41] $[\alpha]_D$ +9.0 (c=1, $CHCl_3$); **20 [RBM1-65]** $[\alpha]_D$ -8.8 (c=1, $CHCl_3$).

14 [RBM1-42] (2*S*,3*S*-stereoisomer) /// 16 [RBM1-66] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8.0 Hz, 1H), 5.73 (dt, *J* = 14.5, 8.0 Hz, 1H), 5.46 (m, 1H), 4.06 (m, 1H), 4.05 (s, 2H), 4.01 (m, 1H), 2.20 (broad, 1H), 2.03 (q, *J* = 7 Hz, 2H), 1.36 (m, 2H), 1.19 (broad, 20H), 1.22 (d, *J* = 6.5 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 134.9, 129.1, 75.5, 50.0, 42.9, 32.4, 32.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 29.3, 29.3, 22.8, 17.4, 14.3.

HRMS calcd. for $C_{20}H_{39}BrNO_2^+$ ($[M+H]^+$): 404.2159 (100.0%), 406.2138 (97.3%), Found: 404.2166, 406.2145

14 [RBM1-42] $[\alpha]_D$ -6.2 (c=1, $CHCl_3$); **16 [RBM1-66]** $[\alpha]_D$ +5.4 (c=1, $CHCl_3$).

26 [RBM1-43] (2*S*,3*S*-stereoisomer) /// 28 [RBM1-59] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (m, 1H), 3.96 (m, 1H), 3.51 (m, 1H), 2.44 (br, 1H), 2.17 (t, *J* = 8.0 Hz, 2H), 1.62 (m, 2H), 1.42 (m, 2H), 1.39 (m, 4H), 1.19 (br, 34H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 8.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5, 74.8, 48.9, 37.2, 34.7, 32.1, 31.9, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 29.4, 29.2, 26.0, 25.8, 22.8, 22.7, 18.5, 14.2, 14.2.

HRMS calcd. for $C_{26}H_{54}NO_2^+$ ($[M+H]^+$): 412.4149 Found: 412.4150

26 [RBM1-43] $[\alpha]_D$ -11.0 (c=1, $CHCl_3$); **28 [RBM1-59]** $[\alpha]_D$ +9.6 (c=1, $CHCl_3$).

30 [RBM1-44] (2*S*,3*S*-stereoisomer) /// 32 [RBM1-60] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (d, *J* = 8.5 Hz, 1H), 3.94 (m, 1H), 3.53 (m, 1H), 2.23 (br, 1H), 1.48-1.33 (4H), 1.25 (broad s, 26H), 1.19 (s, 9H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.8, 74.9, 48.8, 38.9, 34.7, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 27.8, 25.8, 22.8, 18.4, 14.3.

HRMS calcd. for $C_{23}H_{48}NO_2^+$ ($[M+H]^+$): 370.3680, Found: 370.3688

30 [RBM1-44] $[\alpha]_D$ -14.0 (c=1, $CHCl_3$); **32 [RBM1-60]** $[\alpha]_D$ +13.0 (c=1, $CHCl_3$).

38 [RBM1-45] (2*S*,3*S*-stereoisomer) /// 40 [RBM1-61] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.49 (d, *J* = 8.5 Hz, 1H), 4.18 (m, 1H), 3.63 (m, 1H), 2.63 (t, *J* = 8.0 Hz, 3H), 1.60 (m, 2H), 1.55 – 1.38 (4H), 1.34-1.20 (broad, 36H), 1.29 (d, 3H), 0.88 (2 x t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 146.9, 132.1, 128.7, 127.1, 102.7, 74.9, 49.4, 35.9, 34.8, 32.1, 31.9, 31.4, 29.9, 29.8, 29.8, 29.8, 29.7, 29.5, 29.3, 29.3, 25.9, 22.8, 22.8, 18.6, 14.2, 14.2.

HRMS calcd. for $C_{32}H_{58}NO_2^+$ ($[M+H]^+$): 488.4462, Found: 488.4462

38 [RBM1-45] $[\alpha]_D$ +8.4 (c=1, $CHCl_3$); **40 [RBM1-61]** $[\alpha]_D$ -8.0 (c=1, $CHCl_3$).

34 [RBM1-46] (2*S*,3*S*-stereoisomer) /// 36 [RBM1-62] (2*R*,3*R*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (d, *J* = 8.5 Hz, 1H), 4.06 (s, 2H), 4.00 (m, 2H), 3.58 (m, 1H), 1.98 (broad, 1H), 1.52–1.34 (4H), 1.34-1.19 (broad, 27H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 74.5, 49.4, 42.9, 34.6, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.5, 25.8, 22.8, 18.3, 14.3.

HRMS calcd. for $C_{20}H_{41}BrNO_2^+$ ($[M+H]^+$): 406.2315 (100.0%), 408.2295 (97.3%), Found: 406.2314, 408.2294

34 [RBM1-46] $[\alpha]_D$ -4.8 (c=1, $CHCl_3$); **36 [RBM1-62]** $[\alpha]_D$ +4.0 (c=1, $CHCl_3$).

37 [RBM1-47] (2*S*,3*R*-stereoisomer) /// 39 [RBM1-57] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 6.50 (d, *J* = 8.0 Hz, 1H), 4.21 (m, 1H), 3.75 (m, 1H), 2.63 (t, *J* = 8.0 Hz, 2H), 1.61 (m, 2H), 1.56–1.36 (4H), 1.36–1.15 (broad, 34H), 1.20 (t, *J* = 7.0 Hz, 3H), 0.88 (2 x t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 147.0, 132.0, 128.7, 127.1, 74.5, 50.0, 36.0, 33.9, 32.1, 31.9, 31.4, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 29.3, 29.3, 26.2, 22.8, 22.8, 14.3, 14.2, 14.1.

HRMS calcd. for $C_{32}H_{58}NO_2^+$ ($[M+H]^+$): 488.4462, Found: 488.4471

37 [RBM1-47] $[\alpha]_D$ +5.0 (c=1, $CHCl_3$); **39 [RBM1-57]** $[\alpha]_D$ -4.8 (c=1, $CHCl_3$).

29 [RBM1-48] (2*S*,3*R*-stereoisomer) /// 31 [RBM1-56] (2*R*,3*S*-stereoisomer)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 (d, *J* = 8.0 Hz, 1H), 3.99 (m, 1H), 3.61 (m, 1H), 2.03 (br, 1H), 1.54-1.30 (4H), 1.30-1.20 (broad s, 24H), 1.20 (s, 9H), 1.09 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.6, 74.5, 49.5, 38.7, 33.8, 32.1, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 27.7, 26.1, 22.8, 14.2, 14.2.

HRMS calcd. for $C_{23}H_{48}NO_2^+$ ($[M+H]^+$): 370.3680, Found: 370.3686

29 [RBM1-48] $[\alpha]_D$ -15.0 (c=1, $CHCl_3$); **31 [RBM1-56]** $[\alpha]_D$ +14.6 (c=1, $CHCl_3$).

20a [RBM1-65a]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (m, 2H), 7.50 (m, 1H), 7.43 (m, 2H), 6.34 (d, *J* = 8 Hz, 1H), 5.75 (dt, *J* = 14.0, 6.5 Hz, 1H), 5.53 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.21 (m, 1H), 4.12 (m, 1H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.39-1.20 (broad, 24H), 1.30 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 134.7, 134.4, 131.6, 129.7, 128.6, 127.1, 75.9, 50.2, 32.4, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 17.8, 14.3.

HRMS calcd. for $C_{25}H_{42}NO_2^+$ ($[M+H]^+$): 388.3210, Found: 388.3219

$[\alpha]_D$ -3.8 (c=1, $CHCl_3$)

20b [RBM1-65b]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.29 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.5 Hz, 1H), 4.20 (m, 1H), 4.10 (m, 1H), 2.69 (q, *J* = 7.5 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.36-1.29 (m, 2H), 1.27 (d, 3H), 1.26-1.19 (br, 24H), 0.86 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 148.2, 134.3, 132.1, 129.7, 128.1, 127.2, 76.0, 50.2, 32.5, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 28.9, 22.8, 17.7, 15.5, 14.3.

HRMS calcd. for $C_{27}H_{46}NO_2^+$ ($[M+H]^+$): 416.3523, Found: 416.3515

$[\alpha]_D$ -8.2 (c=1, $CHCl_3$)

20c [RBM1-65c]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.29 (d, *J* = 8.5 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.20 (m, 1H), 4.11 (m, 1H), 2.63 (t, *J* = 8.0 Hz, 2H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.65 (m, 2H), 1.33 (m, 2H), 1.29 (d, *J* = 7.0 Hz, 3H), 1.31-1.21 (m, 20H), 0.94 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 146.7, 134.3, 132,1, 129.7, 128.7, 127,1, 76.0, 50.2, 38.0, 32.5, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 24.5, 22.8, 17.7, 14.3, 13.9.

HRMS calcd. for $C_{28}H_{48}NO_2^+$ ($[M+H]^+$): 430.3680, Found: 430.3674

$[\alpha]_D$ -8.4 (c=1, $CHCl_3$)

20d [RBM1-65d]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.30 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.20 (m, 1H), 4.10 (m, 1H), 2.64 (t, *J* = 8.0 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.68-1.54 (m, 4H), 1.39-1.19 (ca, 27H), 0.90 (t, *J* = 8.0 Hz, 3H), 0.88 (t, *J* = 8.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 147.0, 134.3, 132.1, 129.7, 128.7, 127.1, 76.0, 50,2, 35.9, 32.5, 32.1, 31,5, 31.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 22.6, 17.7, 14.2, 14.1.

HRMS calcd. for $C_{30}H_{52}NO_2^+$ ($[M+H]^+$): 458.3993, Found: 458.4004

$[\alpha]_D$ -8.6 (c=1, $CHCl_3$)

20e [RBM1-65e]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.33 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.19 (m, 1H), 4.10 (m, 1H), 2.64 (t, *J* = 8.0 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.61 (m, 2H), 1.39-1.19 (ca, 31H),0.88 (2xt, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 147.0, 134.3, 132.1, 129.7, 128.6, 127.1, 76.0, 50.2, 36.0, 32.5, 32.1, 31.80 31.3, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.0, 22.8, 22.7, 17.7, 14.2, 14.2.

HRMS calcd. for $C_{31}H_{54}NO_2^+$ ($[M+H]^+$): 472.4149, Found: 472.4139

$[\alpha]_D$ -8.6 (c=1, $CHCl_3$)

20f [RBM1-65f]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.5 Hz, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 6.40 (d, *J* = 8.0 Hz, 1H), 5.73 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.19 (m, 1H), 4.10 (m, 1H), 2.70 (broad, 1H), 2.01 (q, *J* = 7.0 Hz, 2H), 1.33 (s, 9H), 1.33-1.19 (broad, 24H), 0.87 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 155.1, 134.3, 131.8, 129.7, 126.9, 125.6, 76.0, 50.1, 35.0, 32.4, 32.1, 31.3, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 17.7, 14.3.

HRMS calcd. for $C_{29}H_{50}NO_2^+$ ($[M+H]^+$): 444.3836, Found: 444.3832

$[\alpha]_D$ -9.0 (c=1, $CHCl_3$)

20g [RBM1-65g]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 2H), 6.90 (m, 2H), 6.22 (d, *J* = 8.0 Hz, 1H), 5.73 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.18 (m, 1H), 4.09 (m, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.83-1.72 (broad, 1H), 1.80 (m, 2H), 1.47-1.35 (m, 4H), 1.35-1.28 (m, 2H), 1.28 (d, *J* = 7 Hz, 3H), 1.25-1.19 (broad, 20H), 0.94 (t, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 161.9, 134.2, 129.8, 128.9, 126.6, 114.3, 76.1, 68.3, 50.2, 32.4, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.0, 28.3, 22.8, 22.6, 17.8, 14.2, 14.1.

HRMS calcd. for $C_{30}H_{52}NO_3^+$ ($[M+H]^+$): 474.3942, Found: 474.3951

$[\alpha]_D$ -9.0 (c=1, $CHCl_3$)

20h [RBM1-65h]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 2H), 6.90 (m, 2H), 6.24 (br d, *J* = 8.0 Hz, 1H), 5.73 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.18 (m, 1H), 4.09 (m, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 2.20-1.85 (broad, 1H), 1.79 (m, 2H), 1.40-1.31 (m, 6H), 1.28 (d, *J* = 7 Hz, 3H), 1.25-1.19 (broad, 20H), 0.91 (t, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 161.9, 134.2, 129.8, 128.9, 126.6, 114.3, 76.1, 68.3, 50.2, 32.4, 32.1, 31.7, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 25.8, 22.8, 22.7, 17.8, 14.2, 14.1.

HRMS calcd. for $C_{31}H_{54}NO_3^+$ ($[M+H]^+$): 488.4098 , Found: 488.4086

$[\alpha]_D$ -8.8 (c=1, $CHCl_3$)

20i [RBM1-65i]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 2H), 6.90 (m, 2H), 6.21 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.18 (m, 1H), 4.09 (m, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.84-1.55 (broad, 1H), 1.79 (m, 2H), 1.40-1.31

(m, 6H), 1.28 (d, *J* = 7 Hz, 3H), 1.25-1.19 (broad, 22H), 0.90 (t, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 161.9, 134.2, 129.8, 128.9, 126.6, 114.3, 76.1, 68.3, 50.2, 32.4, 32.1, 31.7, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.3, 29.2, 26.1, 22.8, 22.7, 17.8, 14.2, 14.2.

HRMS calcd. for $C_{32}H_{56}NO_3^+$ ($[M+H]^+$): 502.4255, Found: 502.4246

$[\alpha]_D$ -7.4 (c=1, $CHCl_3$)

20j [RBM1-65j]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (m, 2H), 7.06 (m, 2H), 6.24 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 5.21 (s, 2H), 4.18 (m, 1H), 4.10 (m, 1H), 3.48 (s, 3H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.28 (d, *J* = 7 Hz, 3H), 1.24 (broad, 25H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 159.9, 134.2, 129.7, 128.8, 128.1, 115.9, 94.3, 76.0, 56.3, 50.2, 32.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 17.8, 14.2.

HRMS calcd. for $C_{27}H_{46}NO_4^+$ ($[M+H]^+$): 448.3421, Found: 448.3421

$[\alpha]_D$ -9.2 (c=1, $CHCl_3$)

20k [RBM1-65k]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.5 Hz, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.25 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.72 (s, 2H), 4.23-4.12 (m, 3H), 4.10 (m, 1H), 3.91 (m, 2H), 3.40 (s, 3H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.27 (d, *J* = 7 Hz, 3H), 1.39-1.15 (22H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 161.5, 134.3, 129.8, 128.9, 127.2, 114.4, 96.7, 76.1, 67.5, 65.9, 55.4, 50.2, 32.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 17.8, 14.2.

HRMS calcd. for $C_{29}H_{50}NO_5^+$ ($[M+H]^+$): 492.3684, Found: 492.3688

$[\alpha]_D$ -9.6 (c=1, $CHCl_3$)

20l [RBM1-65l]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.5 Hz, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.25 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.72 (s, 2H), 4.23-4.12 (m, 3H), 4.10 (m, 1H), 3.91 (m, 2H), 3.40 (s, 3H), 2.09 (m, 2H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.27 (d, *J* = 7 Hz, 3H), 1.39-1.15 (22H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 161.7, 134.3, 129.8, 128.9, 126.9, 114.3, 96.6, 76.1, 65.0, 64.1, 55.3, 50.2, 32.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 22.8, 17.8, 14.2.

HRMS calcd. for $C_{30}H_{52}NO_5^+$ ($[M+H]^+$): 506.3840, Found: 506.3829

$[\alpha]_D$ -9.0 (c=1, $CHCl_3$)

20m [RBM1-65m]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.22 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.64 (s, 2H), 4.18 (m, 1H), 4.09 (m, 1H), 4.03 (t, *J* = 6.0 Hz, 2H), 3.60 (t, *J* = 6.0 Hz, 2H), 3.37 (s, 3H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.90 (m, 2H), 1.79 (m, 2H), 1.28 (d, *J* = 7 Hz, 3H), 1.39-1.15 (22H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 161.8, 134.2, 129.8, 128.9, 126.8, 114.3, 96.5, 76.1, 67.9, 67.4, 55.3, 50.2, 32.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 26.5, 26.2, 22.8, 17.8, 14.2.

HRMS calcd. for $C_{31}H_{54}NO_5^+$ ($[M+H]^+$): 520.3997, Found: 520.3979

$[\alpha]_D$ -9.0 (c=1, $CHCl_3$)

20n [RBM1-65n]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.22 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.63 (s, 2H), 4.18 (m, 1H), 4.09 (m, 1H), 4.01 (t, *J* = 6.5 Hz, 2H), 3.56 (t, *J* = 6.5 Hz, 2H), 3.37 (s, 3H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.84 (m, 2H), 1.68 (m, 2H), 1.57 (m, 2H), 1.28 (d, *J* = 7 Hz, 3H), 1.39-1.15 (22H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 161.8, 134.2, 129.8, 128.8, 126.7, 114.3, 96.5, 76.1, 68.1, 67.7, 55.3, 50.2, 32.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 29.1, 22.9, 22.8, 17.8, 14.2.

HRMS calcd. for $C_{32}H_{56}NO_5^+$ ($[M+H]^+$): 534.4153, Found: 534.4166

$[\alpha]_D$ -8.8 (c=1, $CHCl_3$)

20o [RBM1-65o]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.22 (d, *J* = 8.0 Hz, 1H), 5.74 (dt, *J* = 14.0, 7.0 Hz, 1H), 5.52 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.62 (s, 2H), 4.17 (m, 1H), 4.09 (m, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 3.52 (t, *J* = 6.5 Hz, 2H), 3.36 (s, 3H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.79 (m, 2H), 1.68-1.52 (br, 6H), 1.46 (m, 2H), 1.42-1.20 (28H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 161.9, 134.3, 129.8, 128.8, 126.7, 114.3, 96.5, 76.2, 68.3, 68.0, 55.2, 50.2, 32.4, 32.0, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.3, 26.3, 26.1, 22.8, 17.8, 14.3.

HRMS calcd. for $C_{35}H_{62}NO_5^+$ ($[M+H]^+$): 576.4623, Found: 576.4630

$[\alpha]_D$ -8.7 (c=1, $CHCl_3$)

20p [RBM1-65p]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.26 (br s, 1H), 5.73 (dt, *J* = 14.0, 6.5 Hz, 1H), 5.51 (dd, *J* = 15.5, 7.0 Hz, 1H), 4.23-4.12 (3H), 4.08 (m, 1H), 3.87 (m, 2H), 3.72 (m, 2H), 3.58 (m, 2H), 3.39 (s, 3H), 2.10 (broad, 1H, OH), 2.02 (q, *J* = 7.0 Hz, 2H), 1.42-1.15 (broad, 26H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 161.4, 134.1, 129.8, 128.8, 127.1, 114.4, 76.0, 72.0, 70.9, 69.7, 67.6, 59.2, 50.2, 32.4, 32.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.3, 29.3, 22.8, 17.7, 14.2.

HRMS calcd. for $C_{30}H_{52}NO_5^+$ ($[M+H]^+$): 506.3840, Found: 506.3850

$[\alpha]_D$ -9.4 (c=1, $CHCl_3$)

Synthesis of (2*S*,3*R*)-*N*-[2-(1,3-dihydroxy-4*E*-octadecene)]-*N*-hexane-urea (C6-urea-Cer).

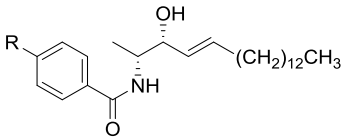
To a solution of (2*S*,3*R*,4*E*)-sphingosine (21 mg, 0.07 mmol) and NEt_3 (10 μL) in anhydrous CH_3CN (4 mL) and anhydrous methyl tert-butyl ether (2 mL), hexyl isocyante (17 μL, 0.181 mmol) was added, and the mixture was stirred at room temperature under N_2 for 4 h. After evaporation of the solvents under a reduced pressure, residue was dissolved in CH_2Cl_2 (10 mL) washed with H_2O (2 x 3 mL) and concentrated to dryness. Flash chromatography purification with silica gel using a stepwise solvent gradient (CH_2Cl_2/MeOH, 0-4%) gave the pure urea isoster of ceramide (27 mg, 86% yield); TLC (CH_2Cl_2-MeOH, 10:1, v/v; Rf 0.5)

^{1}H NMR ($CDCl_3$) 5.73 (dt, *J* =15.5, 7.0 Hz, 1H), 5.49 (dd, *J* = 15.5, 6.5 Hz, 1H), 4.22 (m, 1H), 3.78 (m, 1H), 3.72 (m, 1H), 3.67 (m, 1H), 3.11 (t, *J* = 7 Hz, 1H), 2.02 (m, 2H), 1.46 (m, 2H), 1.20 (m, 2H), 1.41-1.19 (brs, 28H), 0.88 (t, *J* =7.0 Hz, 3H), 0.87 (t, *J* =7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 133.9, 128.9, 77.5, 77.2, 76.8, 74.6, 63.0, 56.0, 40.8, 32.6, 32.1, 31.7, 30.3, 29.9, 29.8, 29.8, 29.7, 29.5, 29.4, 26.8, 22.8, 22.8, 14.3, 14.2.

SUPPLEMENTARY TABLE

Table S1. CLogP values of analogs of compound 20.[a]



Name	R	CLogP	Name	R	CLogP
20a	-H	7.03	**20i**	$-O(CH_2)_6CH_3$	9,39
20b	$-CH_2CH_3$	7.93	**20j**	$-OCH_2OCH_3$	7,02
20c	$-(CH_2)_2CH_3$	8.35	**20k**	$-O(CH_2)_2OCH_2OCH_3$	6,86
20d	$-(CH_2)_4CH_3$	9.18	**20l**	$-O(CH_2)_3OCH_2OCH_3$	6,97
20e	$-(CH_2)_5CH_3$	9.6	**20m**	$-O(CH_2)_4OCH_2OCH_3$	7,42
20	$-(CH_2)_6CH_3$	10.02	**20n**	$-O(CH_2)_5OCH_2OCH_3$	7,84
20f	$-C(CH_3)_3$	8.73	**20o**	$-O(CH_2)_8OCH_2OCH_3$	8,68
20g	$-O(CH_2)_4CH_3$	8.56	**20p**	$-O(CH_2)_2O(CH_2)_2OCH_3$	6,59
20h	$-O(CH_2)_5CH_3$	8.98	*N*-octanoylsphingosine		7.01

[a]CLogP were determined using the calculation algorithm included in ChemDraw Professional 15.0.

SUPPLEMENTARY FIGURES

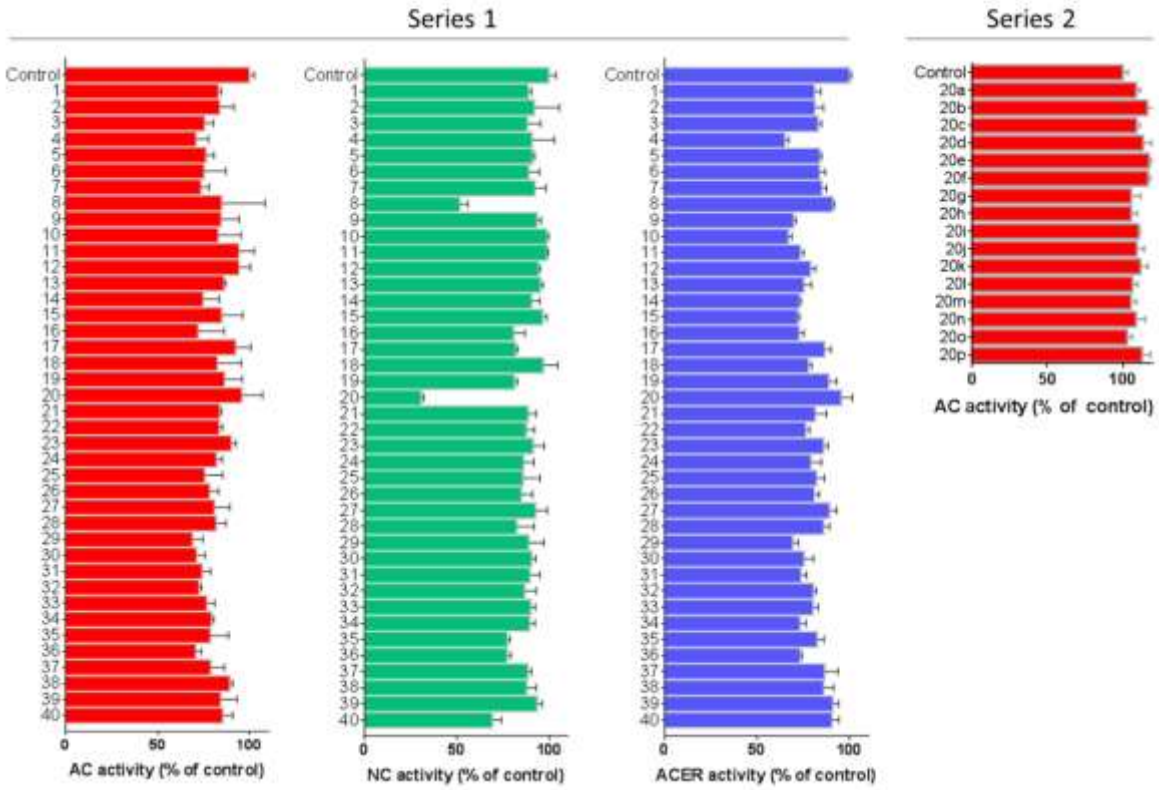


Figure S1. Activity of compounds on ceramidases. Experiments were carried out using recombinant NC, lysates of AC overexpressing Farber cells or lysates of MEF from ASAH2-null mice. The suitable buffers and pH were used for each specific activity. Concentration of substrate was 20 µM and those of test compounds were: 1 µM for NC and 20 µM for AC and ACER. Data (mean ± SD) were obtained from two experiments with triplicates.

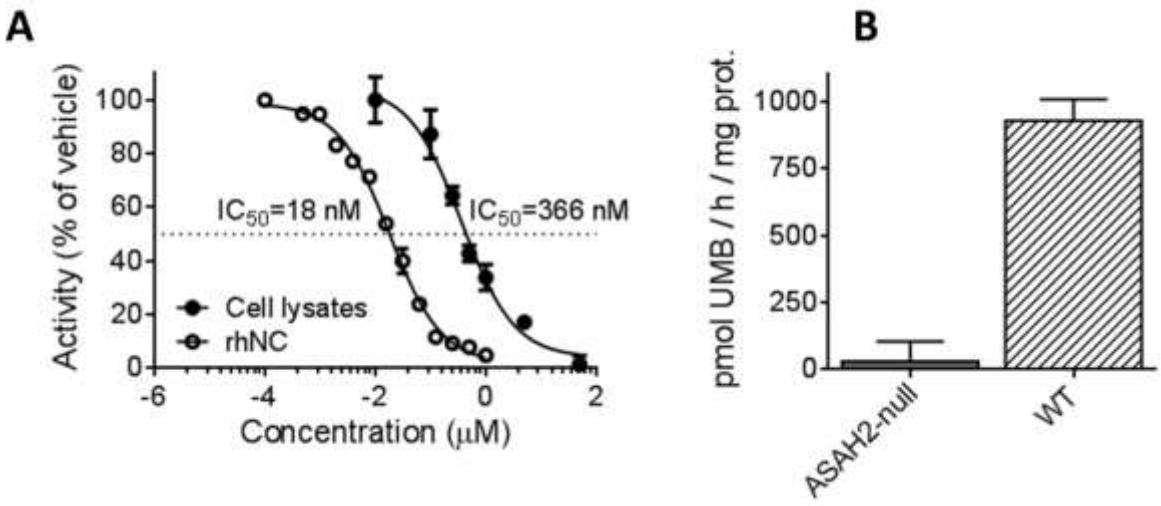


Figure S2. A. Activity of C6-urea-Cer on NC. Experiments were carried out using rhNC (5 ng) or lysates (25 µg) of WT-MEF using 25 mM phosphate buffer, 150 mM NaCl, 1% NaChol (sodium cholate), pH 7.4. and the specific NC substrate (RBM14C24:1, 10 µM) and 3h incubation time. Lysates were obtained from ASAH2-null (B) and wild type (WT) MEF (A and B) cells that had been growing for at least 14 days after thawing. Data (mean ± SD) were obtained from two experiments with triplicates. Data adjustment with the four parameter logistic equation (GraphPad Prism 6) afforded the indicated IC_{50} values. B. NC activity in ASAH2-null and wild type (WT) MEF (mean ± SD, n=6 (two experiments with triplicates)).

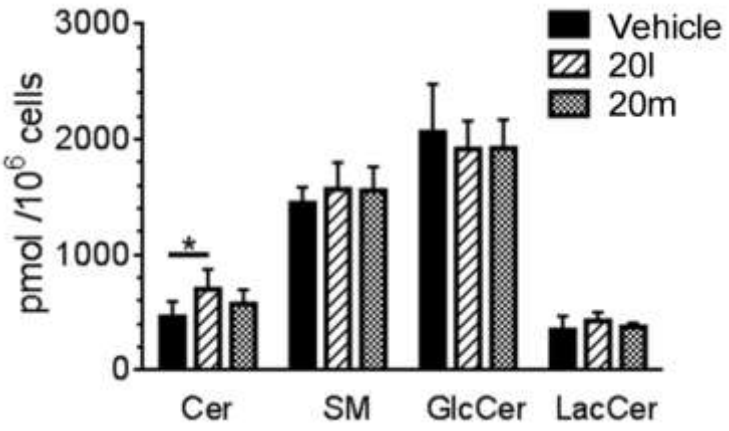


Figure S3. Effect of compounds 20l and 20m on sphingolipid levels in lysates of AC-overexpressing A375 cells. Cells were treated with 50 µM of compound and vehicle (control) in DMEM 10% FBS. After 3 h, lipids were extracted and analysed by LC/MS. Data (mean ± SD) were obtained from two experiments with triplicates. Asterisks indicate statistical significance at *, p = 0,052 (unpaired, two-tailed *t* test).

A

MSAITVALLSLLFITSGTIENHKDLGGHFFSTTQSPPATQGSTAAQRSTATQHSTATQSSTATQTSPVPLTPESPLF
QNFSGYHIGVGRADCTGQVADINLMGYGKSGQNAQGILTRLYSRAFIMAEPDGSNRTVFVSIDIGMVSQRLRLE
VLNRLQSKYGSLYRRDNVILSGTHTHSGPAGYFQYTVFVIASEGFSNQTFQHMVTGILKSIDIAHTNMKPGKIFIN
KGNVDGVQINRSPYSYLQNPQSERARYPSNTDKEMIVLKMVDLNGDDLGLISWFAIHPVSMNNSNHLVNSDN
VGYASYLLEQEKNKGYLPGQGPFVAAFASSNLGDVSPNILGPRCINTGESCDNANSTCPIGGPSMCIAKGPGQD
MFDSTQIIGRAMYQRAKELYASASQEVTGPLASAHQWVDMTDVTVWLNSTHASKTCKPALGYSFAAGTIDGV
GGLNFTQGKTEGDPFWDTIRDQILGKPSEEIKECHKPKPILLHTGELSKPHPWHPDIVDVQIITLGSLAITAIPGEFT
TMSGRRLREAVQAEFASHGMQNMTVVISGLCNVYTHYITTYEEYQAQRYEAASTIYGPHALSAYIQLFRNLAKAI
ATDTVANLSRGPEPPFFKQLIVPLIPSIVDRAPKGRTFGDVLQPAKPEYRVGEVAEVIFVGANPKNSVQNQTHQT
FLTVEKYEATSTSWQIVCNDASWETRFYWHKGLLGLSNATVEWHIPDTAQPGIYRIRYFGHNRKQDILKPAVILS
FEGTSPAFEVVTI

B

HHHHHHSGTIENHKDLGGHFFSTTQSPPATQGSTAAQRSTATQHSTATQSSTATQTSPVPLTPESPLFQNFSGY
HIGVGRADCTGQVADINLMGYGKSGQNAQGILTRLYSRAFIMAEPDGSNRTVFVSIDIGMVSQRLRLEVLNRLQ
SKYGSLYRRDNVILSGTHTHSGPAGYFQYTVFVIASEGFSNQTFQHMVTGILKSIDIAHTNMKPGKIFINKGNVDG
VQINRSPYSYLQNPQSERARYPSNTDKEMIVLKMVDLNGDDLGLISWFAIHPVSMNNSNHLVNSDNVGYASYL
LEQEKNKGYLPGQGPFVAAFASSNLGDVSPNILGPRCINTGESCDNANSTCPIGGPSMCIAKGPGQDMFDSTQII
GRAMYQRAKELYASASQEVTGPLASAHQWVDMTDVTVWLNSTHASKTCKPALGYSFAAGTIDGVGGLNFTQ
GKTEGDPFWDTIRDQILGKPSEEIKECHKPKPILLHTGELSKPHPWHPDIVDVQIITLGSLAITAIPGEFTTMSGRRL
REAVQAEFASHGMQNMTVVISGLCNVYTHYITTYEEYQAQRYEAASTIYGPHALSAYIQLFRNLAKAIATDTVAN
LSRGPEPPFFKQLIVPLIPSIVDRAPKGRTFGDVLQPAKPEYRVGEVAEVIFVGANPKNSVQNQTHQTFLTVEKYE
ATSTSWQIVCNDASWETRFYWHKGLLGLSNATVEWHIPDTAQPGIYRIRYFGHNRKQDILKPAVILSFEGTSPAF
EVVTI

C

FSGYHIGVGRADCTGQVADINLMGYGKSGQNAQGILTRLYSRAFIMAEPDGSNRTVFVSIDIGMVSQRLRLEVL
NRLQSKYGSLYRRDNVILSGTHTHSGPAGYFQYTVFVIASEGFSNQTFQHMVTGILKSIDIAHTNMKPGKIFINKG
NVDGVQINRSPYSYLQNPQSERARYSSNTDKEMIVLKMVDLNGDDLGLISWFAIHPVSMNNSNHLVNSDNVG
YASYLLEQEKNKGYLPGQGPFVAAFASSNLGDVSPNILGPRCINTGESCDNANSTCPIGGPSMCIAKGPGQDMF
DSTQIIGRAMYQRAKELYASASQEVTGPLASAHQWVDMTDVTVWLNSTHASKTCKPALGYSFAAGTIDGVGGL
NFTQGKTEGDPFWDTIRDQILGKPSEEIKECHKPKPILLHTGELSKPHPWHPDIVDVQIITLGSLAITAIPGEFTTMS
GRRLREATLSAYIQLFRNLAKAIATDTVANLSRGPEPPFFKQLIVPLIPSIVDRAPKGRTFGDVLQPAKPEYRVGEV
AEVIFVGANPKNSVQNQTHQTFLTVEKYEATSTSWQIVCNDASWETRFYWHKGLLGLSNATVEWHIPDTAQP
GIYRIRYFGHNRKQDILKPAVILSFEGTSPAFEVVTIHHHHHH

Figure S4. Comparison of amino acid sequences of human NC (>AAF86240.1 mitochondrial ceramidase [Homo sapiens]) (A), the commercial rhNC used in this study (B) and the crystalized NC (C) as reported by Airola et al. [6] (>pdb|4wgk|A B). The signal/anchor sequence is highlighted in blue and the mucin-like domain is

highlighted in green. The genetically introduced hexahistidine tags are in red. In the commercial rhNC, the MSAITVALLSLLFIT sequence from the natural protein (Accession # AAF86240) is replaced with a 6His tag. The crystalized protein lacks both anchor sequence and mucin-like domain sequences.

REFERENCES

[1] J.L. Abad, G. Fabriás, F. Camps, Synthesis of dideuterated and enantiomers of monodeuterated tridecanoic acids at C-9 and C-10 positions, J. Org. Chem. 65 (2000) 8582–8588. https://doi.org/10.1021/jo000957k.

[2] J.L. Abad, I. Nieves, P. Rayo, J. Casas, G. Fabrias, A. Delgado, Straightforward access to spisulosine and 4,5-dehydrospisulosine stereoisomers: probes for profiling ceramide synthase activities in intact cells., J. Org. Chem. 78 (2013) 5858–5866. https://doi.org/10.1021/jo400440z.

[3] R.W. Bost, M.B. Winstead, The Preparation of Some Polyalkoxy Ethers of the Isomeric Hydroxybenzoic Acids, J. Am. Chem. Soc. 74 (1952) 1821–1824. https://doi.org/10.1021/ja01127a058.

[4] Y. Xia, Z. Mo, L. Sun, L. Zou, W. Zhang, J. Zhang, L. Wang, First total synthesis of quiquesetinerviusin A, J. Chem. Res. 41 (2017) 296–300. https://doi.org/10.3184/174751917X14931195075599.

[5] C.P. Collier, J.O. Jeppesen, Y. Luo, J. Perkins, E.W. Wong, J.R. Heath, J.F. Stoddart, Molecular-based electronically switchable tunnel junction devices, J. Am. Chem. Soc. 123 (2001) 12632–12641. https://doi.org/10.1021/ja0114456.

[6] M. V. Airola, W.J. Allen, M.J. Pulkoski-Gross, L.M. Obeid, R.C. Rizzo, Y.A. Hannun, Structural Basis for Ceramide Recognition and Hydrolysis by Human Neutral Ceramidase, Structure. 23 (2015) 1482–1491. https://doi.org/10.1016/j.str.2015.06.013.