Supporting information

Incorporation and visualization of azido-functionalized *N*-oleoyl serinol in Jurkat cells, mouse brain astrocytes, 3T3 fibroblasts and human brain microvascular endothelial cells

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Experimental Section

Thionylchloride mediated esterification from L- and D-serine with methanol yielded methyl esters **4a** and **4b** respectively. Reaction of ethylbenzimidate hydrochloride **5** with **4** and 1.15 eq. of NEt_3 at room temperature in methylene chloride formed phenyloxazoline stereoisomers **6** in 84 % for **6a** and 83 % for **6b**. In the following step the methyl ester was converted into the primary alcohol **7** by treatment with 2 eq. DIBAL and transformed into the azide **8** via mesyl activation and substitution by NaN_3. The phenyloxazoline was cleaved at 60 °C in 4 N HCl within 5 h resulting in 1-azido-desoxy serinol **9**. However, due to its low stability even at -20 °C **9** needed to be directly converted in **3**. After formation of the amide bond with oleic acid, the resulting **3** proved to be stable. The enantiomeric purity was determined via esterification with *R*-Mosher's acid forming the esters **10** from **3a** and **11** from **3b**. In correlation with the specific rotation the ^{19}F-NMR indicated an enantiomer excess (*ee*) of 92 for **3a** and 95 for **3b**.

Synthesis

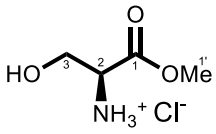
General: Commercially available reagents were used as received (Sigma, Acros, Alfa Aesar). All solvents but dmf were distilled before usage. Moisture-sensitive reactions were performed under a nitrogen atmosphere. Reactions were monitored by TLC analysis with the use of silica gel coated plates (0.2 mm thickness). The detection was achieved by 254 nm UV light or treatment following staining solutions: a) 0.60 g ninhydrin, 200 ml *n*-butanol, 6.00 ml glacial acid b) 15 g vanillin, 250 ml EtOH, 2.50 ml concentrated H_2SO_4, c) 1.50 g $KMnO_4$, 10.0 g K_2CO_3, 100 mg NaOH, 200 ml H_2O. Liquid column chromatography purification was performed using silica gel 60 (40–63 µm mesh). Optical rotations were determined on a Jasco P1020 polarimeter with a sodium lamp (l=589 nm, D-line). The NMR spectra were recorded with a BRUKER AVANCE 400 FT-NMR or a BRUKER AVANCE DMX 600 FT-NMR spectrometer at 25 °C. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. ^{19}F-NMR was used without a standard. Mass spectrometry (MS) was performed on a BRUKER Daltonics-Microflex MALDI TOF-MS (matrix-assisted laser desorption ionization, MALDI) or on a BRUKER Daltonics autoflex II (electrospray ionization, ESI) instrument.

General procedure for the preparation of β-Hydroxy Amides **2**, **3a** and **3b**

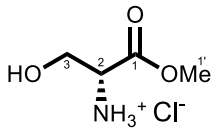
β-Hydroxy Amine (1.00 eq) and DIPEA (1.00 eq) were solved in dmf (5.50 ml per mmol Amine). The mixture was stirred at room temperature for 15 min, then oleic acid (1.10 eq), DCC (1.10 eq) and NHS (1.10 eq) were added. The mixture was stirred for additional 14 h, filtrated over a short plug of silica gel, washed with ethyl acetate (3.5 ml per mmol Amine) and the filtrate was washed with water (3.00 ml per mmol Amine). The phases were separated, the aqueous phase extracted with ethyl acetate (three times with 1.50 ml per mmol Amine), the organic phases combined, dried over Na_2SO_4 and the solvents were removed under reduced pressure. The crude Product was purified via column chromatography (cyclohexane/ ethyl acetate 1 : 1) to yield **3** as a colorless solid.

General procedure for the preparation of serine methyl ester hydrochloride **4**

$SOCl_2$ (1.10 eq) was added dropwise to an ice-cold emulsion of D- respectively L-serine (1.00 eq.) in methanol (2.50 ml per mmol serine). After the addition of $SOCl_2$, the Solution was allowed to warm to room temperature and was stirred for 24 h. The solvents were removed via water-jet vacuum and the resulting white solid was washed with cyclohexane (three times with 1.00 ml per mmol serine) resulting in a colorless solid that was used in the following reaction without any further purification.

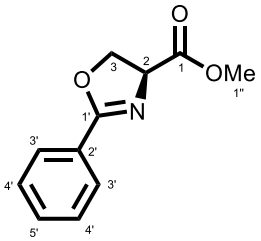


L-serine methyl ester hydrochloride **4a.** General procedure gave **4a** as colorless solid. Yield: quantitative. R_f: 0.62 (methanol +1 % formic acid). ^{1}H-NMR: (400 MHz, MeOD): δ = 4.13 (dd, ^3J = 4.4, 3.6 Hz, 1H, H-2), 4.01 (dd, ^2J = 11.8, ^3J = 4.5 Hz, 1H, H-3a), 3.92 (dd, ^2J = 11.8, ^3J = 3.5 Hz, 1H, H-3b), 3.85 (br, 3H, H-1') ppm. ^{13}C-NMR: (100 MHz, MeOD): δ = 169.4 (C-1), 60.7 (C-3), 56.1 (C-1'), 53.7 (C-2) ppm.

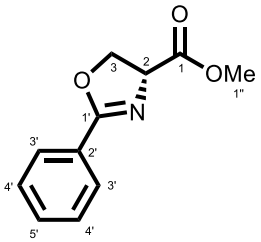


D-serine methyl ester hydrochloride **4b.** General procedure gave **4b** as colorless solid. Yield: quantitative. R_f: 0.62 (methanol +1 % formic acid). ^{1}H-NMR: (400 MHz, MeOD): δ = 4.13 (dd, ^3J = 4.6, 3.5 Hz, 1H, H-2), 4.01 (dd, ^2J = 11.8, ^3J = 4.6 Hz, 1H, H-3a), 3.92 (dd, ^2J = 11.8, ^3J = 3.5 Hz, 1H, H-3b), 3.85 (br, 3H, H-1') ppm. ^{13}C-NMR: (100 MHz, MeOD): δ = 169.4 (C-1), 60.7 (C-3), 56.1 (C-1'), 53.7 (C-2), ppm.

General procedure for the preparation of 2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **6.** Ethyl benzimidate hydrochloride **5** (1.00 eq.) was solved in CH_2Cl_2 (2.00 ml per mmol **4**). Triethylamine (1.15 eq.) was added dropwise and the solution was stirred at room temperature for 30 minutes. Now D-/L-serine methyl ester HCl salt **8** (1.25 eq.) was added by portion. The resulting mixture was stirred for 48 h at room temperature. The solvents were removed under reduced pressure and the residue was purified via column chromatography (cyclohexane/ethyl acetate 1:1) to provide 2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **6**.

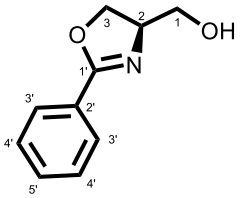


S-2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **6a.** General procedure gave **6a** as yellow oil. Yield: 84 %. R_f: 0.53 (ethyl acetate). ^{1}H-NMR: (400 MHz, $CDCl_3$): δ = 7.99-7.96 (m, 2H, H-3'), 7.51-7.46 (m, 1H, H-5'), 7.42-7.37 (m, 2H, H-4'), 4.95 (dd, ^2J = 10.6, ^3J = 7.9 Hz, 1H, H-3a), 4.68 (dd, ^3J = 8.7, 8.0 Hz, 1H, H-2), 4.58 (dd, ^2J = 10.6, ^3J = 8.6 Hz, 1H, H-3b), 3.80 (br, 3H, H-1") ppm. ^{13}C-NMR: (100 MHz, $CDCl_3$): δ = 171.7 (C-1), 166.4 (C-1'), 132.0 (C-5'), 128.7 (C-4'), 128.4 (C-3'), 127.0 (C-2'), 69.6 (C-3), 68.7 (C-2), 52.8 (C-1") ppm. MS-MALDI (+), *m/z*: 206.113 [M+H$^+$] calcd. for $C_{11}H_{12}NO_3^+$: 206.081. $[\alpha]_D^{22}$: 130.2 (c = 1, MeOH).

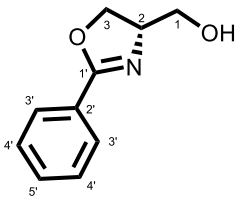


R-2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **6b.** General procedure gave **6b** as yellow oil. Yield: 83 %. R_f: 0.53 (ethyl acetate). ^{1}H-NMR: (400 MHz, $CDCl_3$): δ = 7.99-7.96 (m, 2H, H-3'), 7.51-7.46 (m, 1H, H-5'), 7.43-7.38 (m, 2H, H-4'), 4.94 (dd, ^2J = 10.6, ^3J = 8.0 Hz, 1H, H-3a), 4.69 (dd, ^3J = 8.7, 8.0 Hz, 1H, H-2), 4.58 (dd, ^2J = 10.6, ^3J = 8.7 Hz, 1H, H-3b), 3.80 (br, 3H, H-1") ppm. ^{13}C-NMR: (100 MHz, $CDCl_3$): δ = 171.7 (C-1), 166.4 (C-1'), 132.0 (C-5'), 128.7 (C-4'), 128.5 (C-3'), 127.0 (C-2'), 69.6 (C-3), 68.7 (C-2), 52.8 (C-1") ppm. MS-MALDI (+), *m/z*: 206.107 [M+H$^+$] calcd. for $C_{11}H_{12}NO_3^+$: 206.081. $[\alpha]_D^{22}$: -116.6 (c = 1, MeOH).

General procedure for the preparation of (2-Phenyl-4,5-dihydrooxazol-4-yl)methanol **7.** 2-Phenyl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **6** (1.00 eq) was solved in thf (2.00 mL per mmol **6**) and cooled to 0 °C. Now a 1.00 M solution of diisobutyl-aluminum hydride in cyclohexane were added dropwise over 20 min (2 eq). The resulting mixture was allowed to warm to room temperature and was stirred for 6 h. The reaction was cooled to 0 °C and was quenched with ethyl acetate and afterwards a half-saturated solution of Potassium sodium tartrate aq. (1 mL per mmol **6**) was added. The mixture was stirred vigorously overnight at room temperature. The phases were separated and the aqueous phase was extracted with ethyl acetate (three times with 2.00 ml per mmol **6**). The organic phases were combined dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified via column chromatography (ethyl acetate).



R-(2-Phenyl-4,5-dihydrooxazol-4-yl)methanol **7a.** General procedure gave **7a** as colorless solid. Yield: 74 %. R_f: 0.12 (ethyl acetate). ^{1}H-NMR: (400 MHz, MeOD): δ = 7.94-7.91 (m, 2H, H-3'), 7.55-7.51 (m, 1H, H-5'), 7.47-7.42 (m, 2H, H-4'), 4.54 (dd, ^2J = 7.8, ^3J = 9.4 Hz, 1H, H-3a), 4.41 (dd, ^2J = 7.8, ^3J = 7.1 Hz, 1H, H-3b), 4.40-4.34 (m, 1H, H-2), 3.73 (dd, ^2J = 11.3, ^3J = 3.9 Hz, 1H, H-1a), 3.68 (dd, ^2J = 11.3, ^3J = 5.0 Hz, 1H, H-1b) ppm. ^{13}C-NMR: (100 MHz, MeOD): δ = 167.2 (C-1'), 132.9 (C-5'), 129.5 (C-4'), 129.4 (C-3'), 128.5 (C-2'), 71.2 (C-3), 69.0 (C-2), 64.5 (C-1) ppm. HRMS-ESI (+), *m/z*: 178.08618 [M+H$^+$] calcd. for $C_{10}H_{12}NO_2^+$: 178.08625. $[\alpha]_D^{22}$: 44.7 (c = 1, MeOH).

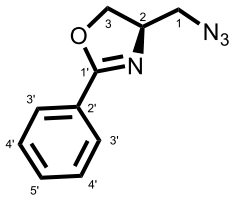


S-(2-Phenyl-4,5-dihydrooxazol-4-yl)methanol **7b.** General procedure gave **7b** as colorless solid. Yield: 94 %. R_f: 0.12 (ethyl acetate). ^{1}H-NMR: (400 MHz, MeOD): δ = 7.94-7.91 (m, 2H, H-3'), 7.55-7.51 (m, 1H, H-5'), 7.46-7.42 (m, 2H, H-4'), 4.54 (dd, ^2J = 7.9, ^3J = 9.4 Hz, 1H, H-3a), 4.41 (dd, ^2J = 7.8, ^3J = 7.1 Hz, 1H, H-3b), 4.40-4.34 (m, 1H, H-2), 3.73 (dd, ^2J = 11.3, ^3J = 3.9 Hz, 1H, H-1a), 3.68 (dd, ^2J = 11.3, ^3J = 5.0 Hz, 1H, H-1b) ppm. ^{13}C-NMR: (100 MHz, MeOD): δ = 167.2 (C-1'), 132.9 (C-5'), 129.5 (C-4'), 129.4 (C-3'), 128.5 (C-2'), 71.2 (C-3), 69.0 (C-2), 64.5 (C-1) ppm. HRMS-ESI (+), *m/z*: 178.08621 [M+H$^+$] calcd. for $C_{10}H_{12}NO_2^+$: 178.08625. $[\alpha]_D^{22}$: 48.5 (c = 1, MeOH).

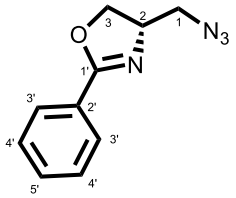
General procedure for the preparation of (2-Phenyl-4,5-dihydrooxazol-4-yl)methyl methane sulfonate **12.** (2-phenyl-4,5-dihydrooxazol-4-yl)methanol **11** (1.00 eq) and trimethylamine (1.20 eq) were solved in CH_2Cl_2 (7.00 mL per mmol **11**). The mixture was cooled to 0 °C and methane sulfonyl chloride was added dropwise over 10 min. the resulting mixture was allowed to warm to room temperature and was stirred for 2 h. The reaction was quenched with 0.10 M HCl aq. (2.00 ml per mmol **11**) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (4 times with 2.00 ml per mmol **11**). The organic phases were combined, washed with saturated $NaHCO_3$ (2.00 ml per mmol **11**), dried over Na_2SO_4 and filtrated. Evaporation of the solvents provided crude **12** that was used in the next reaction without any further purification.

General procedure for the preparation of 4-(azido methyl)-2-phenyl-4,5-dihydrooxazole **8.** Crude (2-phenyl-4,5-dihydrooxazol-4-yl)methyl methane sulfonate **12** was solved in dmf (3.50 ml per mmol **12**), NaN_3 (5.00 eq) was added and the mixture was stirred over night at 70 °C. The mixture was concentrated under reduced pressure and

diluted in H_2O (15 ml per mmol **12**). The aqueous phase was extracted with diethyl ether (four times with 10 ml per mmol **12**) the layers were separated, the organic phases combined, dried over Na_2SO_4, filtrated and the solvents removed under reduced pressure. The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2 : 1) to provide 4-(azido methyl)-2-phenyl-4,5-dihydrooxazole.

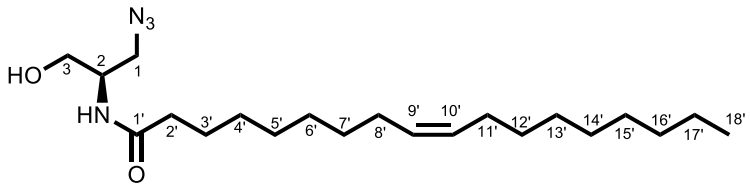


R-4-(azido methyl)-2-phenyl-4,5-dihydrooxazole **8a.** General procedure gave **8a** as colorless oil. Yield: 80 % over two steps. R_f : 0.66 (ethyl acetate) ^{1}H-NMR: (400 MHz, MeOD): δ = 7.94-7.92 (m, 2H, H-3'), 7.57-7.53 (m, 1H, H-5'), 7.47-7.44 (m, 2H, H-4'), 4.57-4.47 (m, 2H, H-3), 4.33-4.30 (m, 1H, H-2), 3.62 (dd, ^2J = 12.7.3, ^3J = 3.8 Hz, 1H, H-1a), 3.43 (dd, ^2J = 12.7, ^2J = 3.9 Hz, 1H, H-1b) ppm. ^{13}C-NMR: (100 MHz, MeOD): δ = 167.7 (C-1'), 133.2 (C-5'), 129.6 (C-4'), 129.4 (C-3'), 128.2 (C-2'), 71.2 (C-3), 67.2 (C-2), 55.2 (C-1) ppm. HRMS-ESI (+), *m/z*: 203.09276 [M+H$^+$] calcd. for $C_{10}H_{11}N_4O^+$: 203.09274.

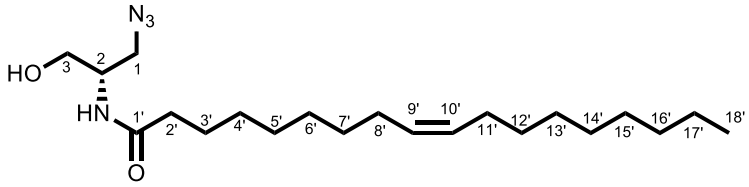


S-4-(azido methyl)-2-phenyl-4,5-dihydrooxazole **8b.** General procedure gave **8b** as colorless oil. Yield: 86 % over two steps. R_f: 0.66 (ethyl acetate). ^{1}H -NMR: (400 MHz, MeOD): δ = 7.95-7.92 (m, 2H, H-3'), 7.57-7.53 (m, 1H, H-5'), 7.48-7.43 (m, 2H, H-4'), 4.57-4.47 (m, 2H, H-3), 4.33-4.30 (m, 1H, H-2), 3.63 (dd, ^2J = 12.7, ^3J = 4.2 Hz, 1H, H-1a), 3.44 (dd, ^2J = 12.7, ^3J = 4.3 Hz, 1H, H-1b) ppm. ^{13}C -NMR: (100 MHz, MeOD): δ = 167.8 (C-1'), 133.2 (C-5'), 129.6 (C-4'), 129.5 (C-3'), 128.2 (C-2'), 71.4 (C-3), 67.2 (C-2), 55.2 (C-1) ppm. HRMS-ESI (+), *m/z*: 203.09270 [M+H$^+$] calcd. for $C_{10}H_{11}N_4O^+$: 203.09274.

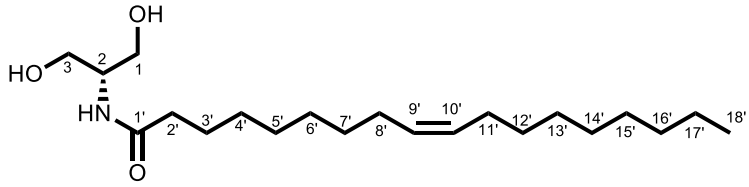
General procedure for the preparation of 2-amino-3-azidopropan-1-ol hydrochloride **9.** 4-(azido methyl)-2-phenyl-4,5-dihydrooxazole **8** was solved in 4 N HCl aq. (5.00 ml per mmol **8**) and stirred for 5 h at 60 °C. After cooling to room temperature, the aqueous layer was washed with methylene chloride (three times with 5.00 ml per mmol **8**). The phases were separated and the aqueous Layer was concentrated under reduced pressure. The brown oil **8** was directly used as crude product in the next step without any further purification.



S-2-amino-3-azidopropan-1-ol hydrochloride **3a.** General procedure gave **3a** as colorless wax. Yield 47 % over two steps. R_f: 0.40 (cyclohexane/ethyl acetate 1 : 2). ^{1}H-NMR: (600 MHz, MeOD): δ = 5.89 (d, ^3J = 7.6 Hz, 1H, N*H*), 5.37-5.31 (m, 2H, H9'/H10'), 4.09-4.05 (m, 1H, H-2), 3.80 (ddd, ^2J = 11.1 Hz, ^3J = 4.5, 4.5 Hz, 1H, H-3b), 3.69 (ddd, ^2J = 11.1 Hz, ^3J = 6.2, 4.8 Hz, 1H, H-3a), 3.58 (dd, ^2J = 12.5 Hz, ^3J = 5.2 Hz, 1H, H-1a), 3.55 (dd, ^2J = 12.5 Hz, ^3J = 5.6 Hz, 2H, H-1b), 2.32 (dd, ^3J = 6.0, 4.9 Hz, 1H, O*H*), 2.21 (dd, ^3J = 7.7, 7.7 Hz, 2H, H2'), 2.02-1.99 (m, 4H, H8'/H11'), 1.64 (dddd, ^3J = 7.7, 7.5, 7.5, 6.9 Hz, 2H, H3'), 1.36-1.23 (m, 20H, H4'-H7',H12'-17'), 0.88 (t, ^3J = 7.1 Hz, 1H, H18') ppm. ^{13}C-NMR: (150 MHz, MeOD): δ = 173.7 (C-1'), 130.2 (C-9'), 129.9 (C-10'), 62.9 (C-3), 51.8 (C-1), 50.3 (C-2), 36.9 (C-2'), 32.1 (C-alkyl), 29.9 (C-alkyl), 29.9 (C-alkyl), 29.7 (C-alkyl), 29.5 (C-alkyl), 29.5 (C-alkyl), 29.4 (C-alkyl), 29.4 (C-alkyl), 29.3 (C-alkyl), 27.4 (C-8'), 27.3 (C-11'), 25.8 (C-3'), 22.8 (C-17'), 14.3 (C-18') ppm. HRMS-ESI (+), *m/z*: 381.32262 [M+H$^+$] calcd. for $C_{21}H_{41}N_4O_2^+$: 381.32240. $[\alpha]_D^{22}$: -9.9 (c = 1, MeOH).



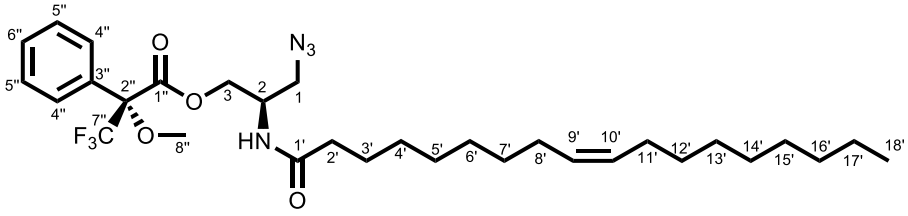
R-2-amino-3-azidopropan-1-ol hydrochlorid **3b.** General procedure gave **3b** as colorless wax. Yield: 73 % over two steps. R_f: 0.40 (cyclohexane/ethyl acetate 1 : 2). ^{1}H-NMR: (600 MHz, MeOD): δ = 5.89 (d, ^3J = 7.1 Hz, 1H, N*H*), 5.37-5.31 (m, 2H, H9'/H10'), 4.09-4.05 (m, 1H, H-2), 3.80 (ddd, ^2J = 11.1 Hz, ^3J = 4.0, 4.0 Hz, 1H, H-3b), 3.69 (ddd, ^2J = 10.8 Hz, ^3J = 5.3, 5.3 Hz, 1H, H-3a), 3.58 (dd, ^2J = 12.5 Hz, ^3J = 5.2 Hz, 1H, H-1a), 3.55 (dd, ^2J = 12.5 Hz, ^3J = 5.6 Hz, 2H, H-1b), 2.33 (dd, ^3J = 5.5, 4.9 Hz, 1H, O*H*), 2.21 (dd, ^3J = 7.8, 7.5 Hz, 2H, H2'), 2.02-1.99 (m, 4H, H8'/H11'), 1.64 (dddd, ^3J = 7.4, 7.4, 7.4, 7.4 Hz, 2H, H3'), 1.35-1.23 (m, 20H, H4'-H7',H12'-17'), 0.88 (t, ^3J = 7.1 Hz, 1H, H18') ppm. ^{13}C-NMR: (150 MHz, MeOD): δ = 173.7 (C-1'), 130.2 (C-9'), 129.9 (C-10'), 62.9 (C-3), 51.8 (C-1), 50.3 (C-2), 36.9 (C-2'), 32.1 (C-alkyl), 29.9 (C-alkyl), 29.9 (C-alkyl), 29.7 (C-alkyl), 29.5 (C-alkyl), 29.5 (C-alkyl), 29.4 (C-alkyl), 29.4 (C-alkyl), 29.3 (C-alkyl), 27.4 (C-8'), 27.3 (C-11'), 25.8 (C-3'), 22.8 (C-17'), 14.3 (C-18') ppm. . HRMS-ESI (+), *m/z*: 381.32284 [M+H$^+$] calcd. for $C_{21}H_{41}N_4O_2^+$: 381.32240. $[\alpha]_D^{22}$: 12.0 (c = 1, MeOH).



General procedure for the preparation of *N*-Oleoyl serinol **2**. General Procedure gave **2** as a colorless solid. Yield 70 % over two steps. R_f : 0.35 (methylene chloride/methanol 10 : 1). ^{1}H-NMR: (400 MHz, $CDCl_3$/MeOD 4:1): δ = 5.30-5.20 (m, 2H, H9'/H10'), 3.96-3.89 (m, 1H, H-2), 3.74 (tt, ^3J = 7.4, 5.0 Hz, 1H, H-2), 3.60 (dd, ^2J = 11.3 Hz, ^3J = 4.6 Hz, 2H, H-1a/H-3b), 3.50 (dd, ^2J = 11.3 Hz, ^3J = 5.3 Hz, 2H, H-1b/H-3a), 2.12 (t, ^3J = 7.7 Hz, 2H, H-2'), 1.94-1.88 (m, 4H, H8'/H11'), 1.54-1.50 (m, 2H, H3'), 1.27-1.12 (m, 20H, H4'-H7',H12'-17'), 0.78 (t, ^3J = 6.9 Hz, 1H, H-18') ppm. ^{13}C-NMR: (100 MHz, $CDCl_3$/MeOD 4:1): δ = 174.8 (C-1'), 123.0 (C-9'), 129.7 (C-10'), 61.4 (C-3), 52.4 (C-1), 36.5 (C-2'), 31.8 (C-alkyl), 29.7 (C-alkyl), 29.7 (C-alkyl), 29.5 (C-alkyl), 29.2 (C-alkyl), 29.2 (C-alkyl), 29.2 (C-alkyl), 29.1 (C-alkyl), 27.1 (C-8'/11'), 27.1 (C-8'/C-11'), 25.7 (C-3'), 22.6 (C-17'), 14.0 (C-18') ppm. HRMS-ESI (+), *m/z*: 342.33665 [M+H$^+$] calcd. for $C_{21}H_{44}NO_2^+$: 342.33665.

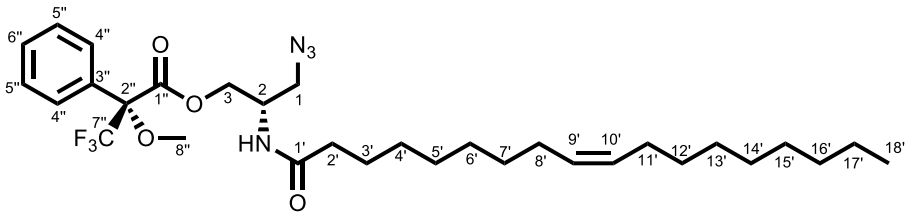
General procedure for the preparation of Mosher ester **21** and **22.** MTPA (2.50 eq) was solved in methylene chloride (15 ml per mmol **3**) and added dropwise to a solution of **3** (1.00 eq), DCC (2.50 eq) and DMAP (2.50 eq) in methylene chloride (15 ml per mmol **3**). The mixture was stirred for 14 h at room temperature. The mixture was filtered over a short plug of silica gel, washed with ethyl acetate (100 mL per mmol **3**), the solvents removed under reduced pressure and the crude product purified via column chromatography (cyclohexane/ethyl acetate 10 : 1) to yield **10** respectively **11**.

(*S*)-(*R*)-3-azido-2-oleamidopropyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **10.** General procedure gave **10a** as colorless oil. Yield: 56 %. R_f: 0.62 (cyclohexane/ethyl acetate 2 : 1).



^{1}H-NMR: (400 MHz, $CDCL_3$): δ = 7.52-7.46 (m, 2H, Ar), 7.46-7.38 (m, 2H, Ar), 5.50 (m, 1 H, NH), 5.38-5.30 (m, 2H, H9'/H10'), 4.43-4.31 (m, 3H, H-2, H-3a/b), 3.54-3.53 (m, 3H, H-8‘) 3.43 (dd, $_2J$ = 12.4 Hz, ^3J = 4.3 Hz, 1 H, H-1a) 3.38 (dd, ^2J = 12.4 Hz, ^3J = 5.5 Hz, 1 H, H-1b), 2.11 (dd, ^3J = 8.2, 7.1 Hz, 2 H, H-2'), 2.03-1.98 (m, 4 H, H8'/H11'), , 1.61-1.51 (m, 2H, H3'), 1.37-1.20 (m, 20H, H4'-H7',H12'-17'), 0.87 (t, ^3J = 6.9 Hz, 1H, H18') ppm. ^{13}C-NMR: (100 MHz, $CDCl_3$): δ = 173.0 (C-1'), 166.4 (C-1"), 132.0 (C-3"), 130.2 (C-9'/10'), 130.0 (C-6"), 129.8 (C-9'/10'), 128.8 (C-4"), 127.3 (C-5"), 124.8 (C-7"), 85.0 (C-2"), 64.5 (C-3), 55.6 (C-8"), 51.1 (C-1), 47.3

(C-2), 36.7 (C-2'), 32.0 (C-alkyl), 29.9 (C-alkyl), 29.8 (C-alkyl), 29.7 (C-alkyl), 29.5 (C-alkyl), 29.5 (C-alkyl), 29.3 (C-alkyl), 29.3 (C-alkyl), 29.2 (C-alkyl), 27.4 (C-8'), 27.3 (C-11'), 25.5 (C-3'), 22.8 (C-17‘), 14.3 (C-18') ppm. ^{19}F (375 MHz, $CDCL_3$) = -71.3 (3 F) ppm.



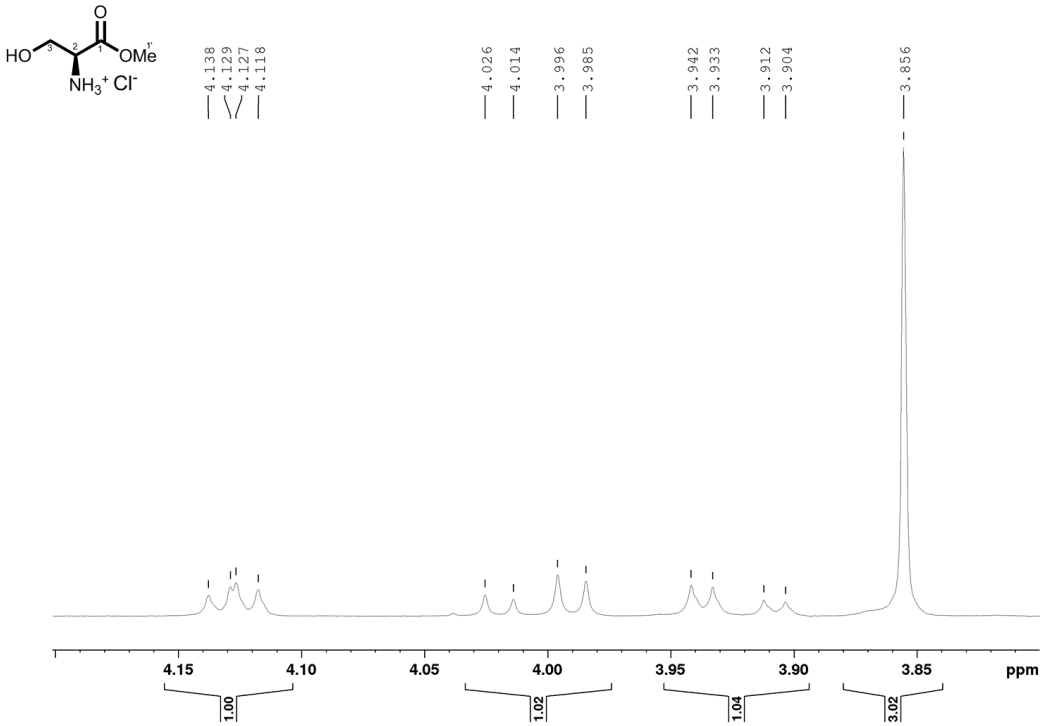
(*R*)-(*R*)-3-azido-2-oleamidopropyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **11.** General procedure gave **10b** as colorless oil. Yield: 93 %. R_f: 0.62 (cyclohexane/ethyl acetate 2 : 1).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ = 7.51-7.46 (m, 2H, Ar), 7.46-7.39 (m, 2H, Ar), 5.56 (d, ^3J = 8.4 Hz, 1 H, NH), 5.38-5.30 (m, 2H, H9'/H10'), 4.48 (dd, ^2J = 11.0 Hz, ^3J = 5.1 Hz, 1H, H-3b), 4.43-4.35 (m, 1H, H-2), 4.27 (dd, ^2J = 11.0 Hz, ^3J = 5.6 Hz, 1H, H-3a), 3.53-3.50 (m, 3H, H-8‘), 3.45 (dd, ^2J = 12.4 Hz, ^3J = 4.6 Hz, 1 H, H-1b), 3.38 (dd, ^2J = 12.5 Hz, ^3J = 5.6 Hz, 1 H, H-1a), 2.13 (dd, ^3J = 8.1, 7.1 Hz, 2 H, H-2'), 2.03-1.98 (m, 4 H, H8'/H11'), 1.61-1.51 (m, 2H, H3'), 1.37-1.20 (m, 20H, H4'-H7',H12'-17'), 0.87 (t, ^3J = 6.9 Hz, 1H, H18') ppm. ^{13}C-NMR: (100 MHz, $CDCl_3$): δ = 173.0 (C-1'), 166.4 (C-1"), 131.9 (C-3"), 130.2 (C-9'/10'), 130.1 (C-6"), 129.8 (C-9'/10'), 128.8 (C-4"), 127.5 (C-5"), 122.0 (C-7"), 84.7 (C-2"), 64.5 (C-3'), 55.6 (C-8"), 51.2 (C-1), 47.3 (C-2), 36.7 (C-2'), 32.0 (C-alkyl), 29.9 (C-alkyl), 29.8 (C-alkyl), 29.7 (C-alkyl), 29.5 (C-alkyl), 29.5 (C-alkyl), 29.3 (C-alkyl), 29.3 (C-alkyl), 29.2 (C-alkyl), 27.4 (C-8'), 27.3 (C-11'), 25.5 (C-3'), 22.8 (C-17‘), 14.3(C-18') ppm. ^{19}F (375 MHz, $CDCL_3$) = -71.4 (3 F) ppm.

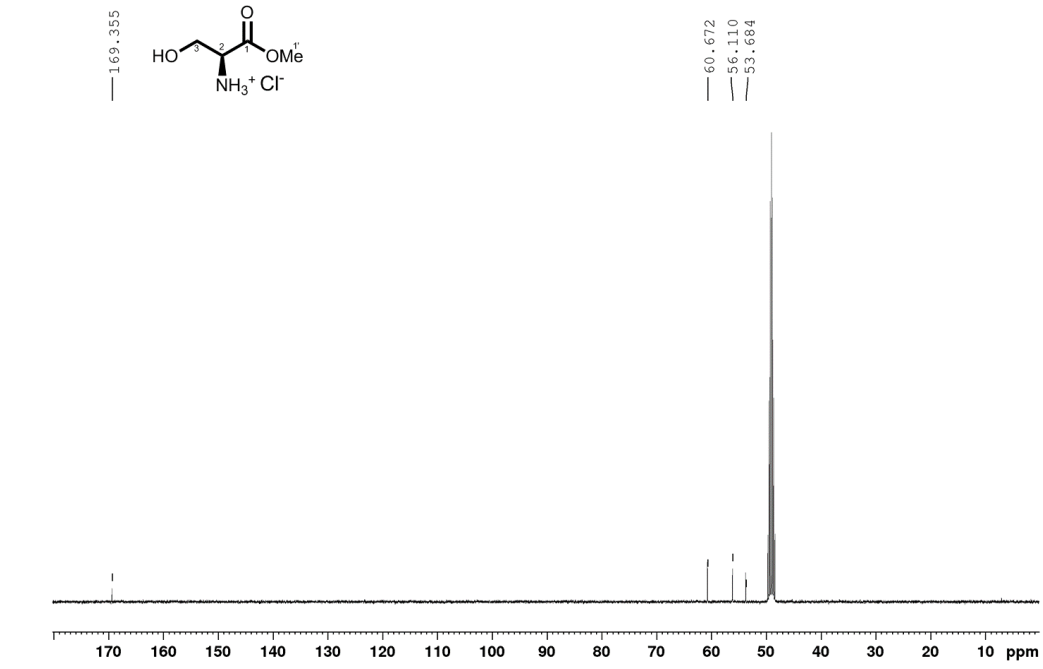
Spectral data

Compound **4a**

^{1}H-NMR: (400 MHz, MeOD)

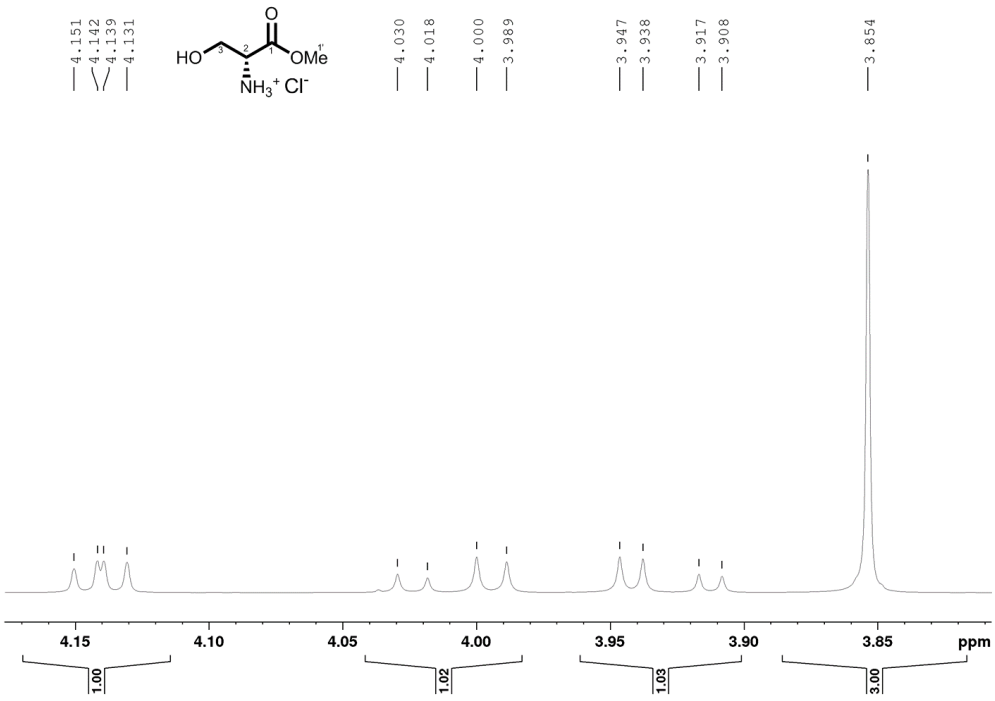


^{13}C-NMR: (100 MHz, MeOD):

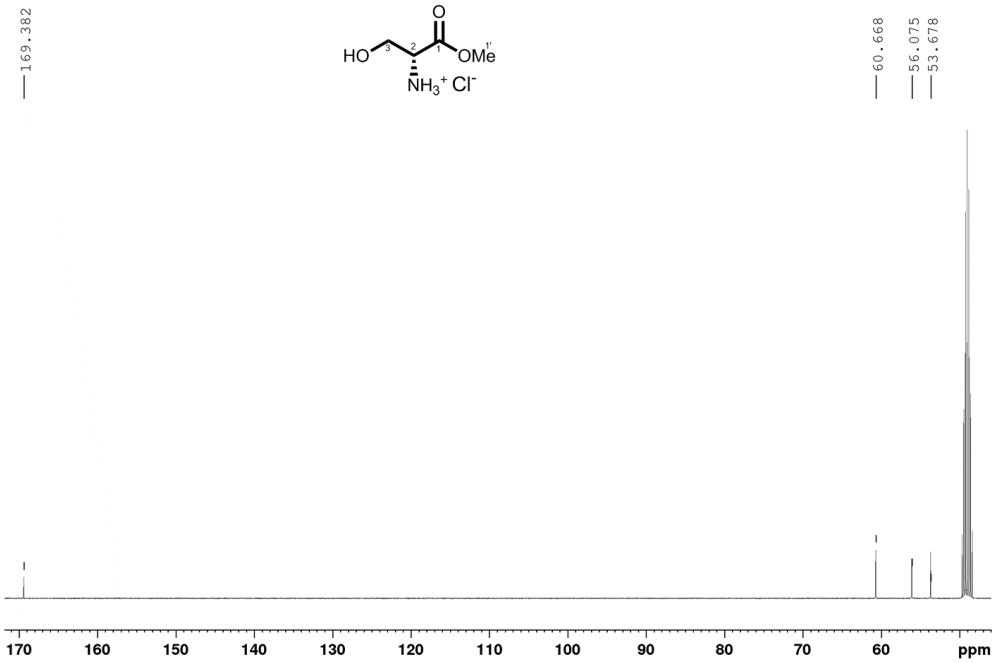


Compound **4b**

^{1}H-NMR: (400 MHz, MeOD)

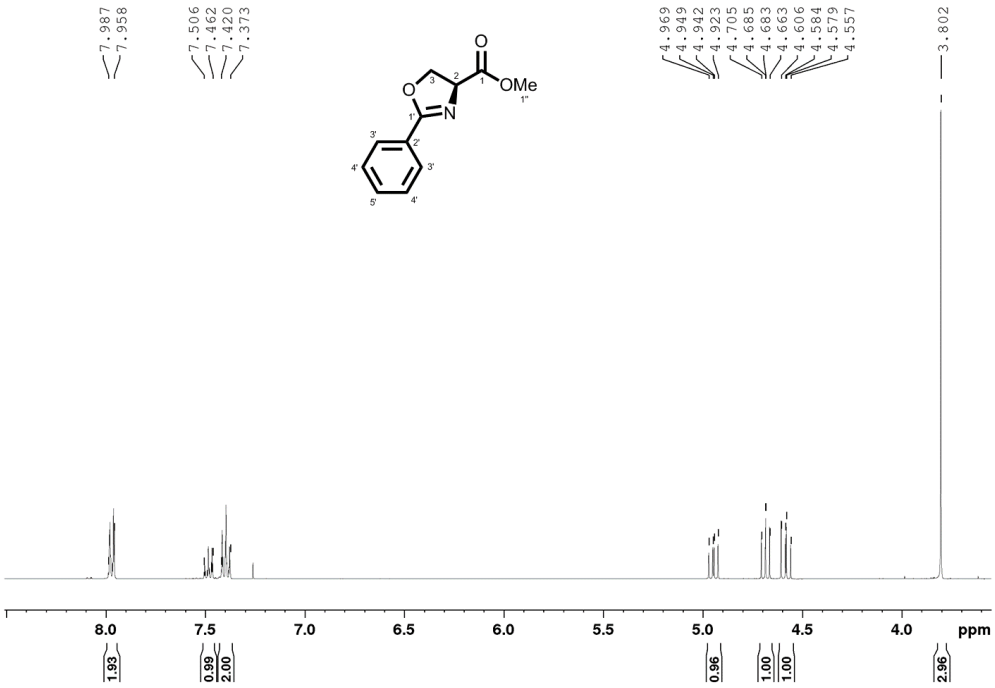


^{13}C-NMR: (100 MHz, MeOD):

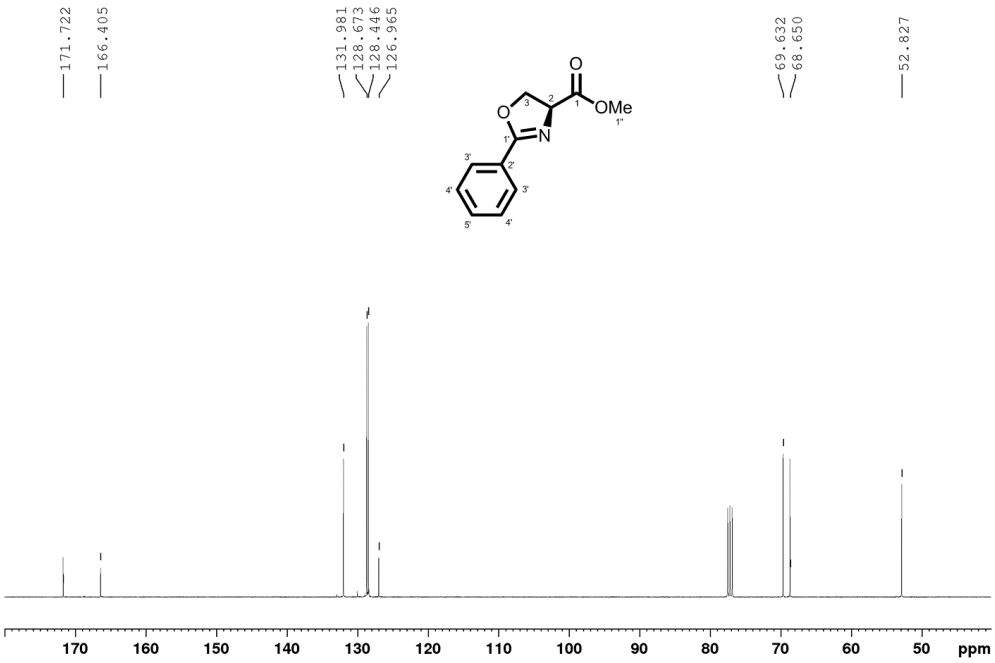


Compound **6a**

^{1}H-NMR: (400 MHz, $CDCl_3$)

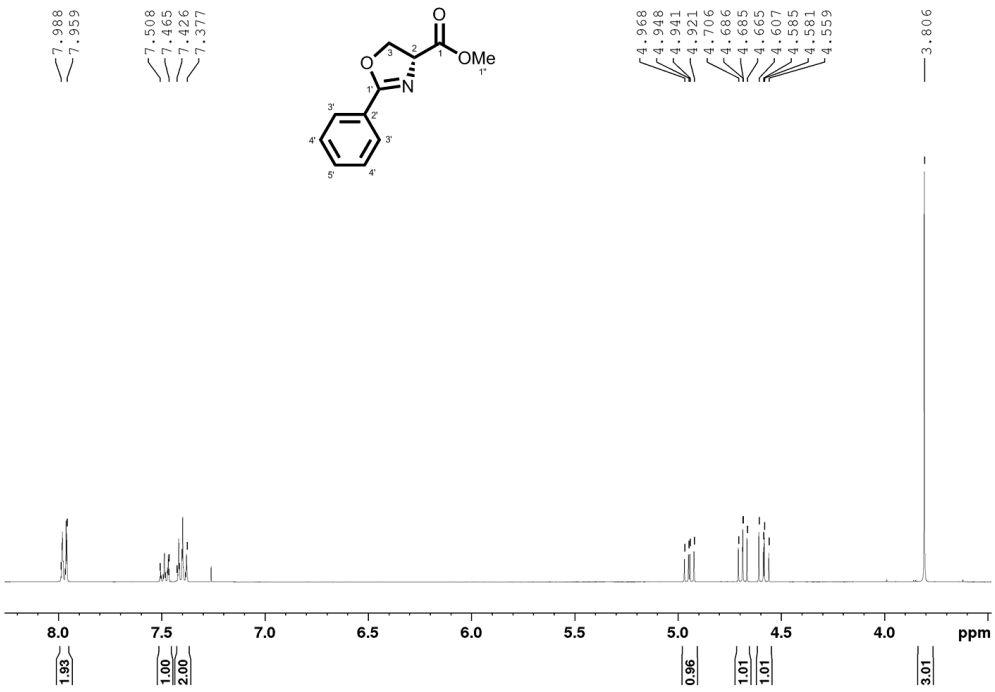


^{13}C-NMR: (100 MHz, $CDCl_3$)

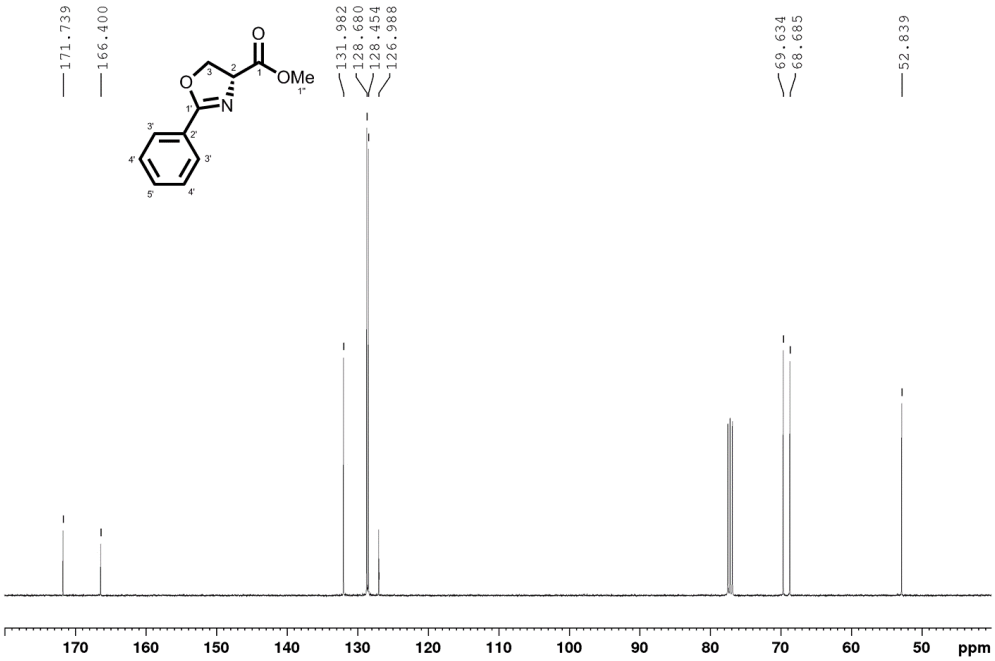


Compound **6b**

^{1}H-NMR: (400 MHz, $CDCl_3$)

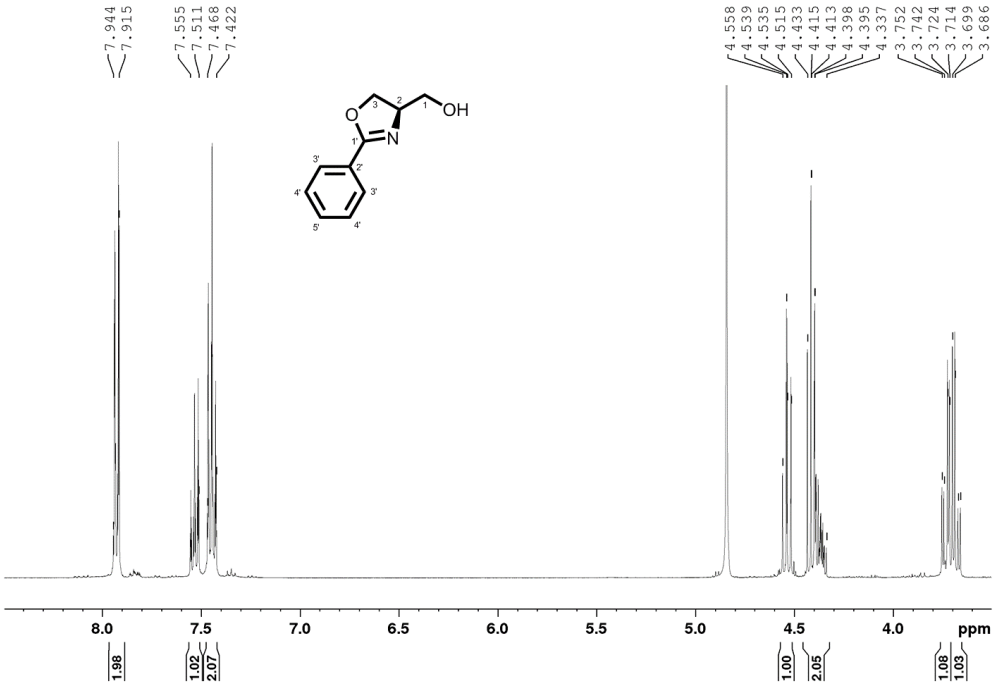


^{13}C-NMR: (100 MHz, $CDCl_3$)

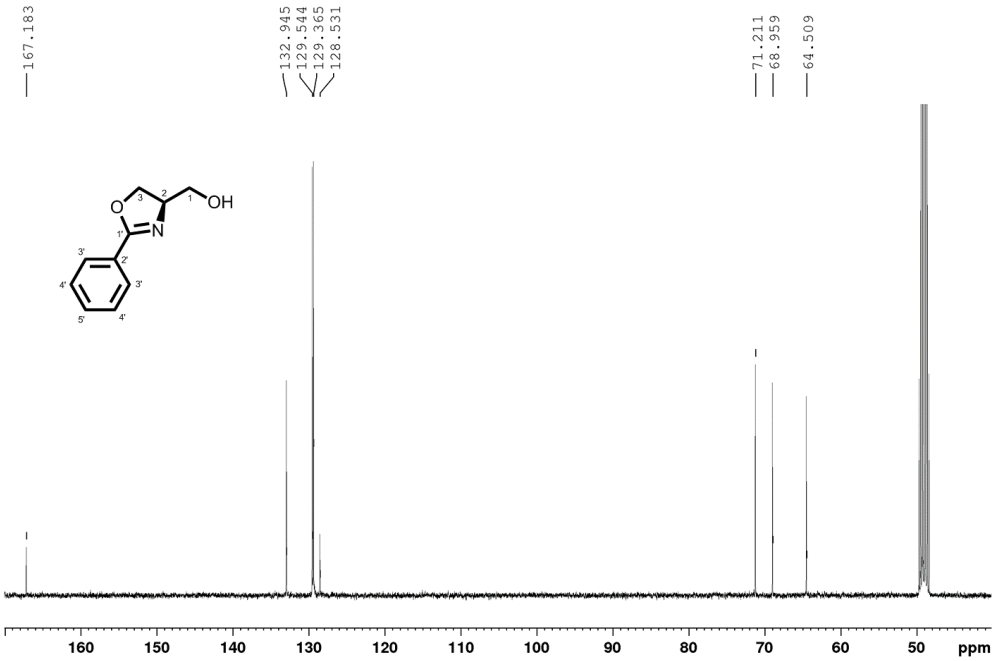


Compound **7a**

^{1}H-NMR: (400 MHz, MeOD)

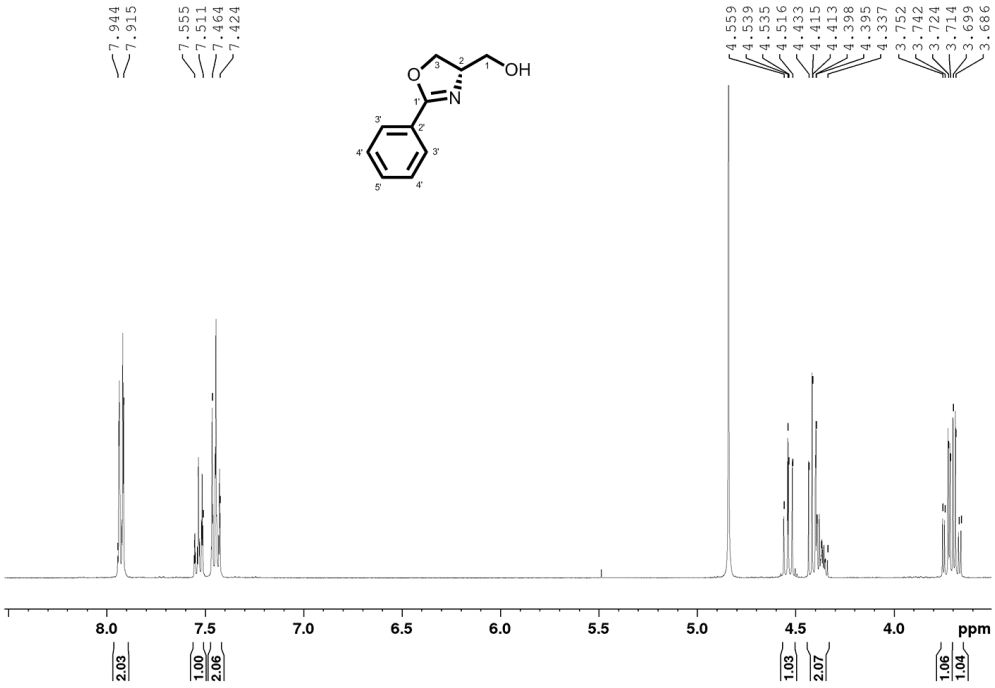


^{13}C-NMR: (100 MHz, MeOD)

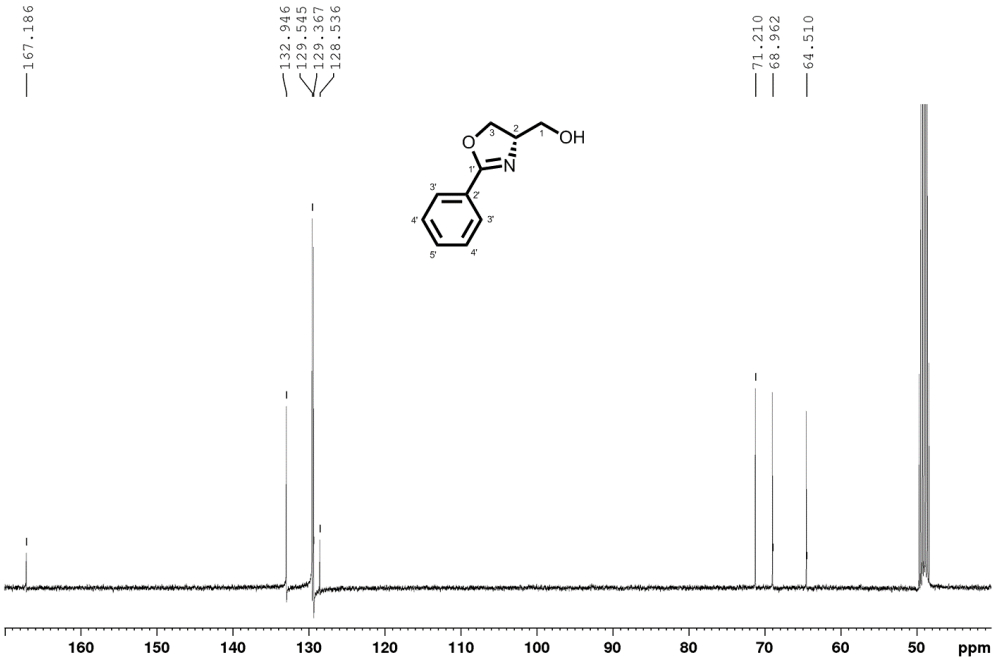


Compound **7b**

^{1}H-NMR: (400 MHz, MeOD)

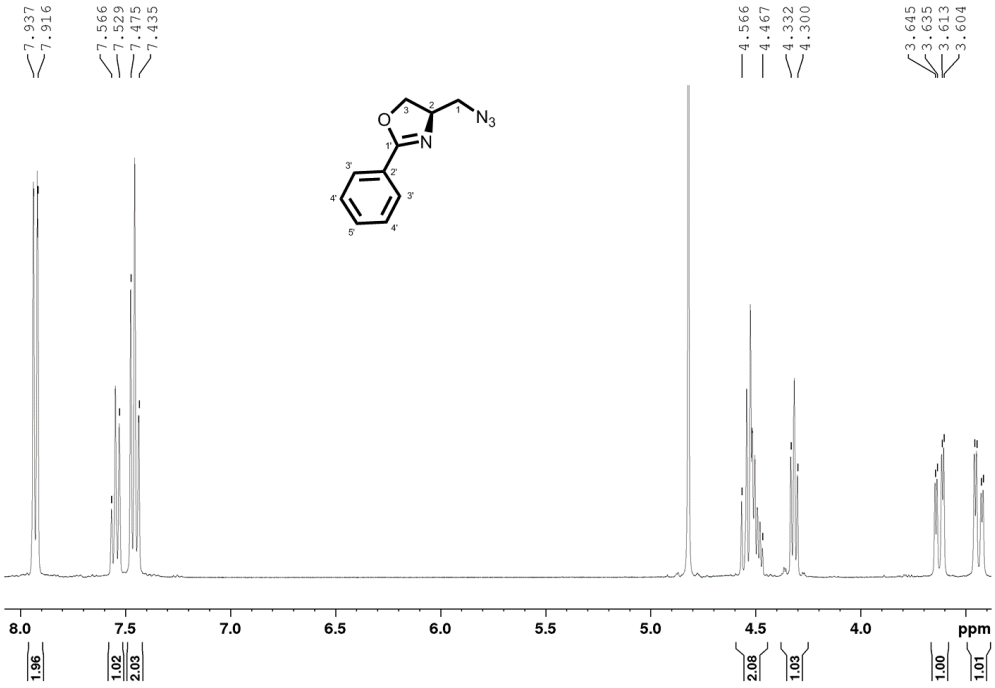


^{13}C-NMR: (100 MHz, MeOD)

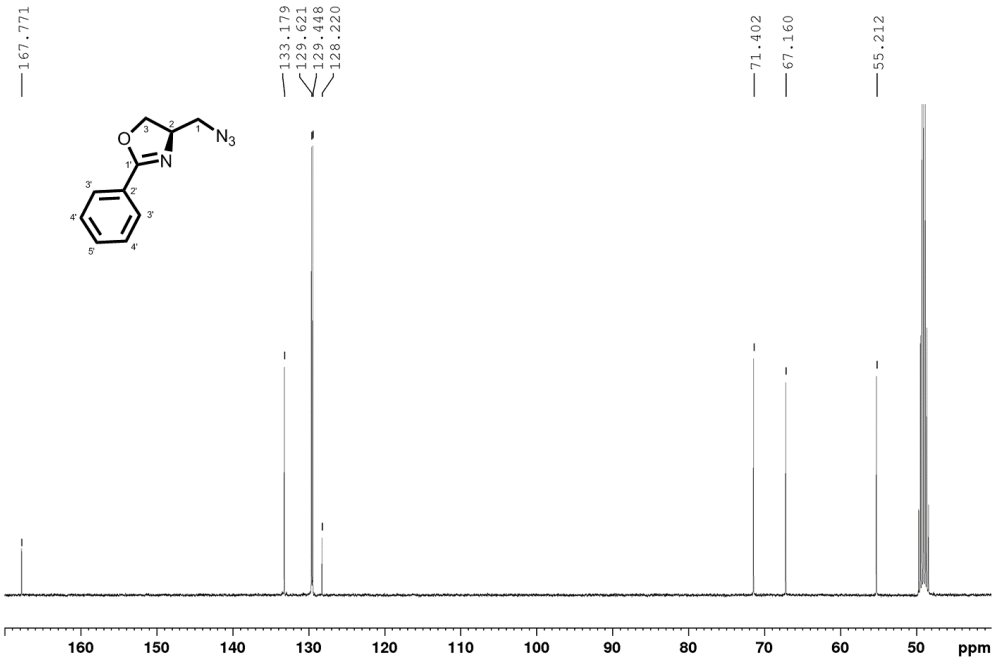


Compound **8a**

^{1}H-NMR: (400 MHz, MeOD)

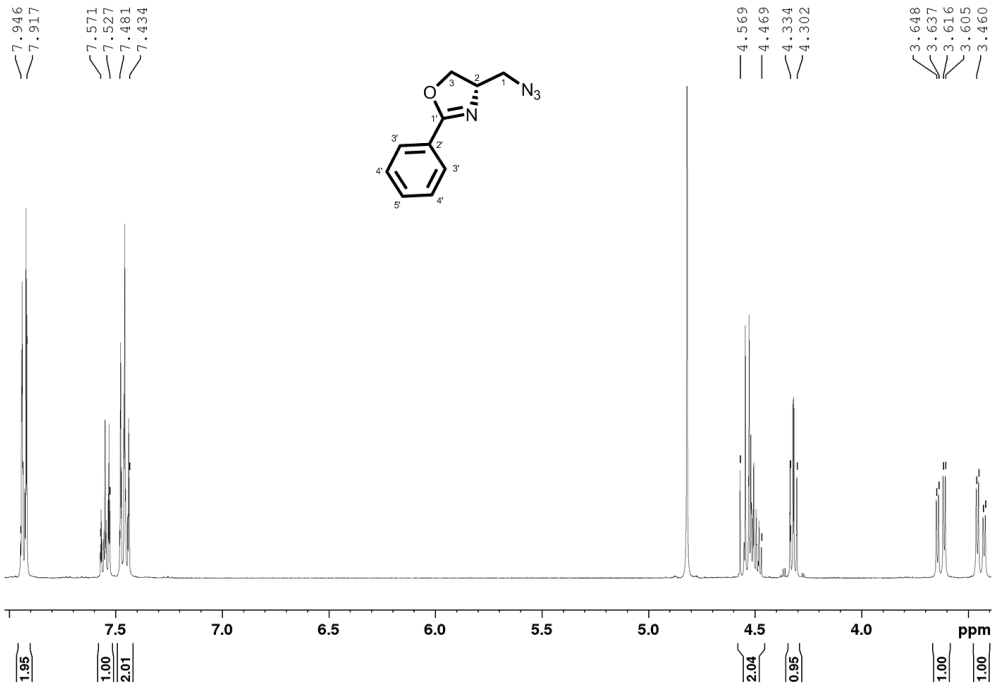


^{13}C-NMR: (100 MHz, MeOD):



Compound **8b**

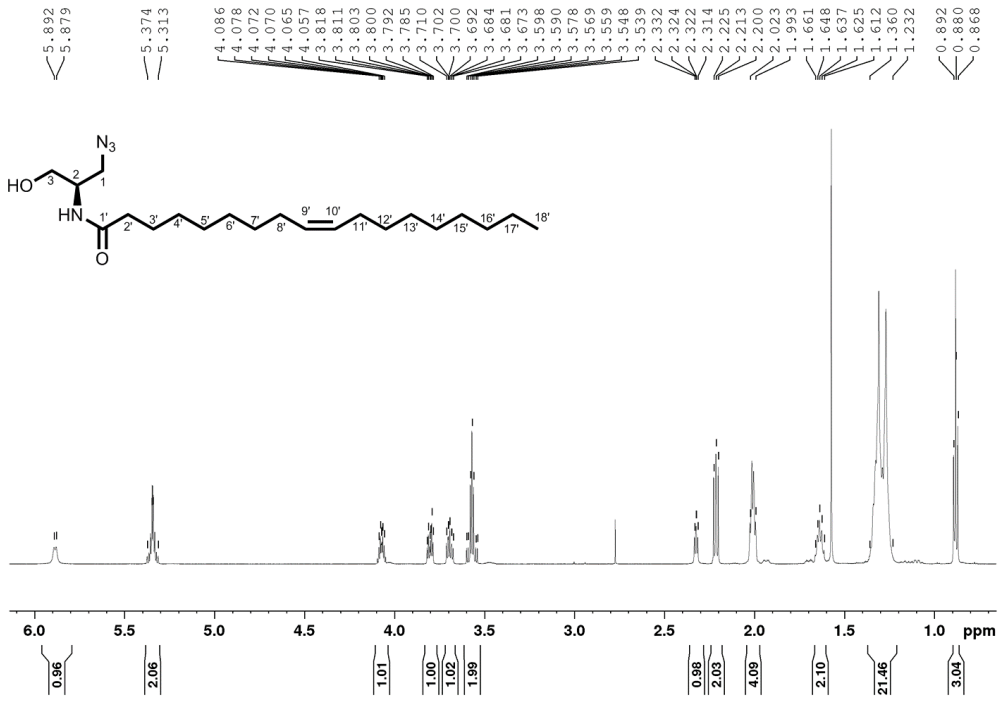
^{1}H-NMR: (400 MHz, MeOD)



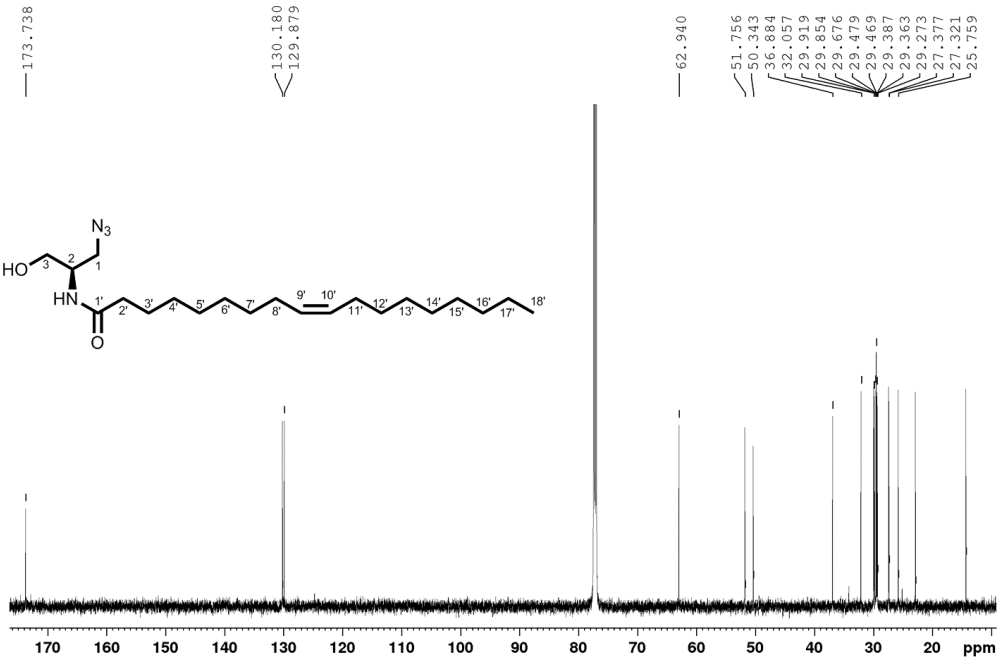
^{13}C-NMR: (100 MHz, MeOD):

Compound **3a**

^{1}H-NMR: (600 MHz, MeOD)

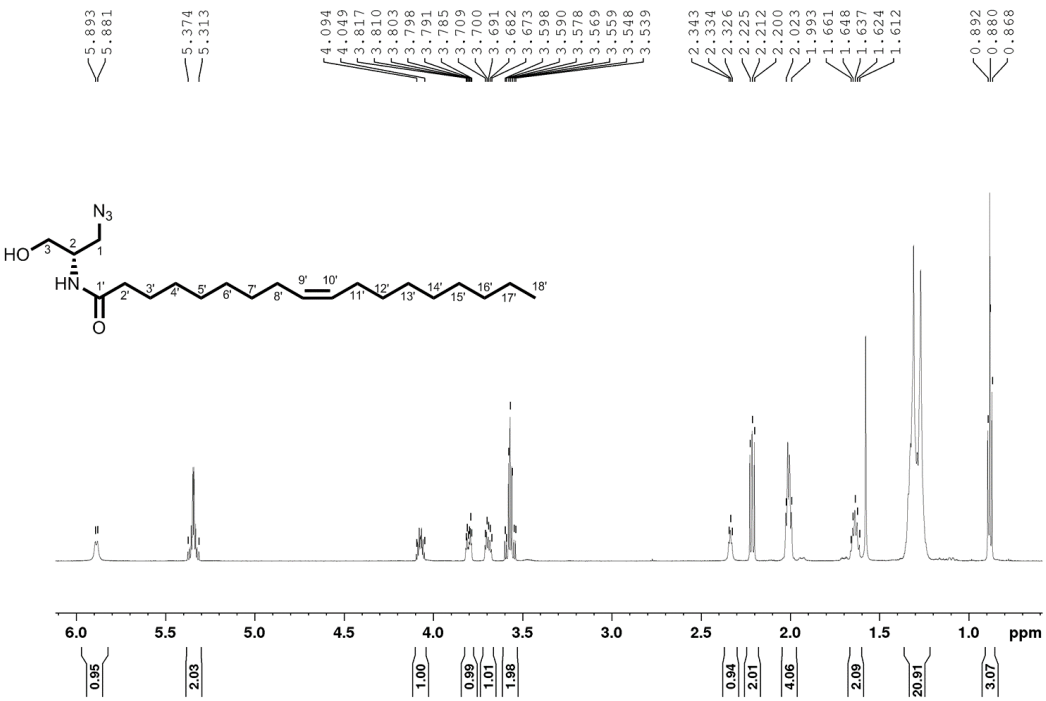


^{13}C-NMR: (150 MHz, MeOD)

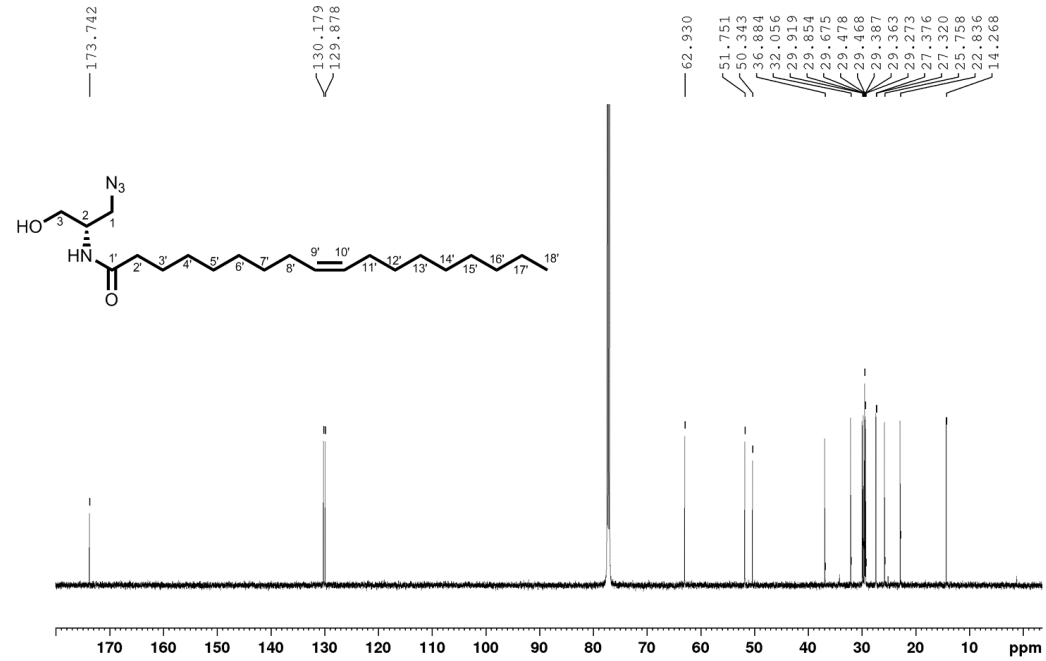


Compound **3b**

^{1}H-NMR: (600 MHz, MeOD)

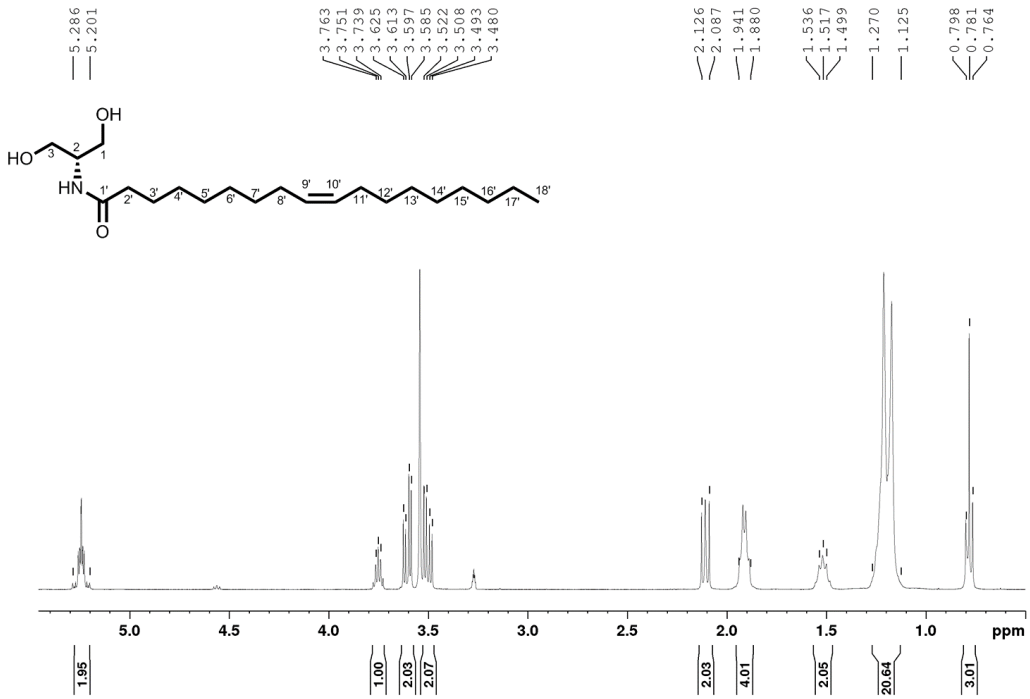


^{13}C-NMR: (150 MHz, MeOD)

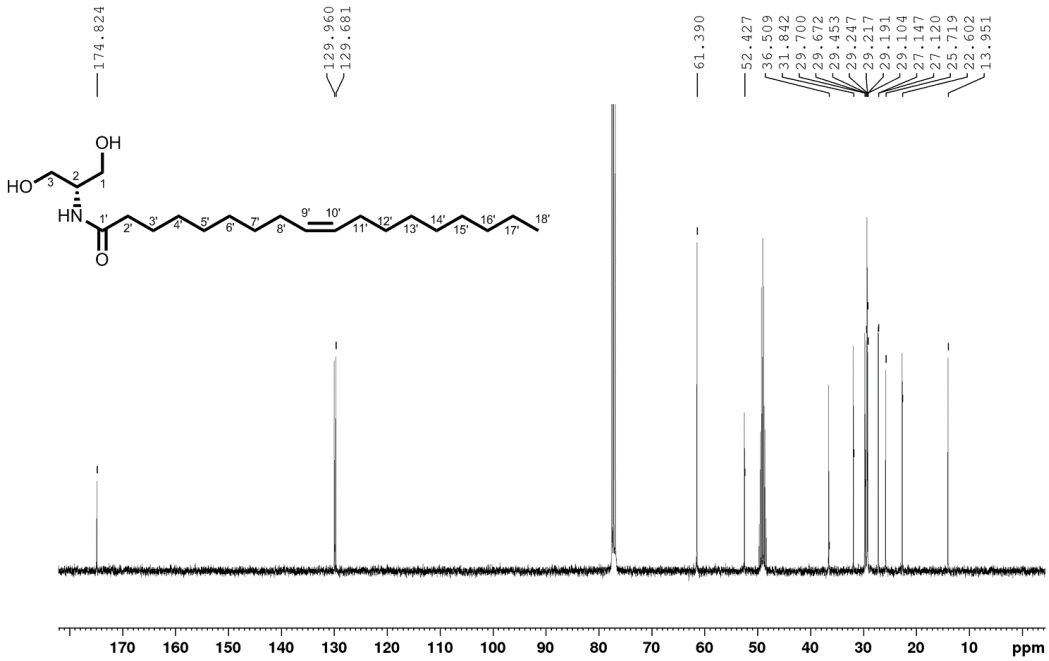


Compound **2**

^{1}H-NMR: (400 MHz, $CDCl_3$/MeOD 4:1)

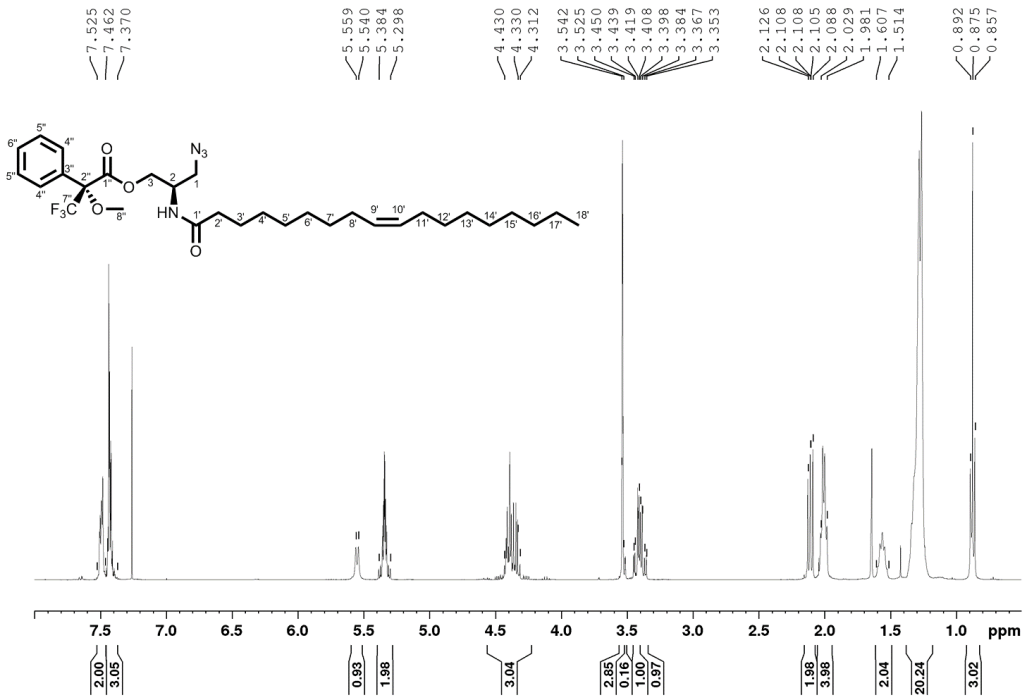


^{13}C-NMR: (100 MHz, $CDCl_3$/MeOD 4:1)

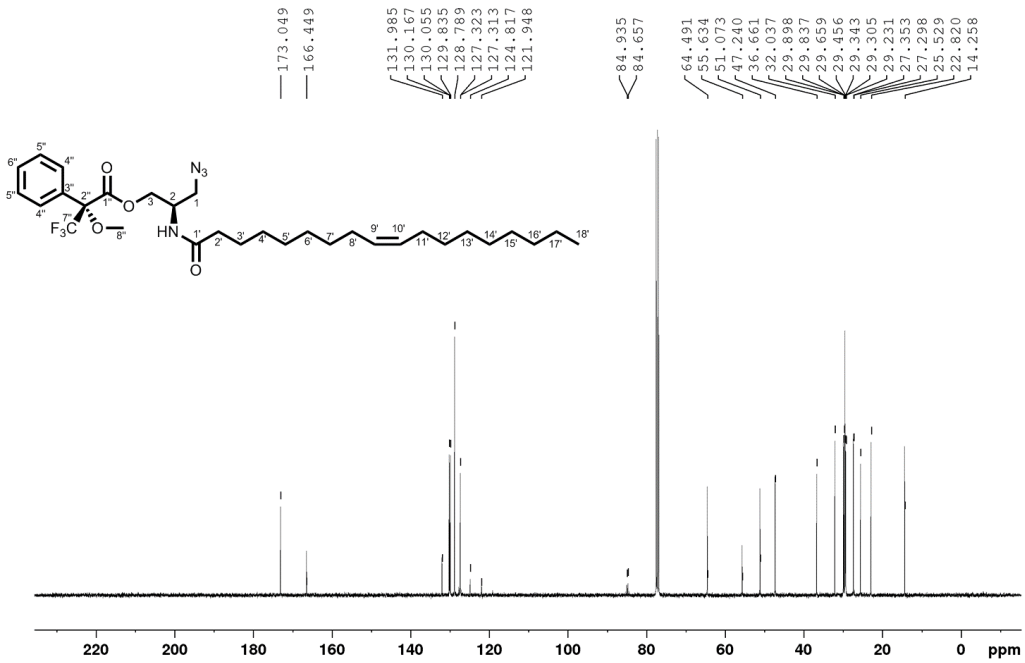


Compound **10a**

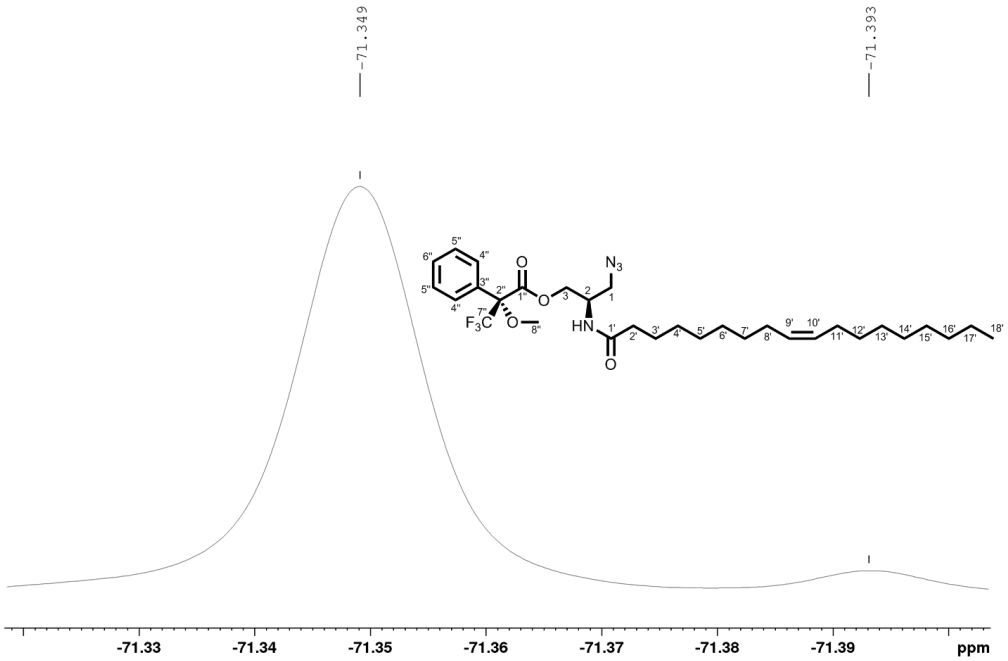
^{1}H-NMR: (400 MHz, $CDCl_3$)



^{13}C-NMR: (100 MHz, $CDCl_3$)

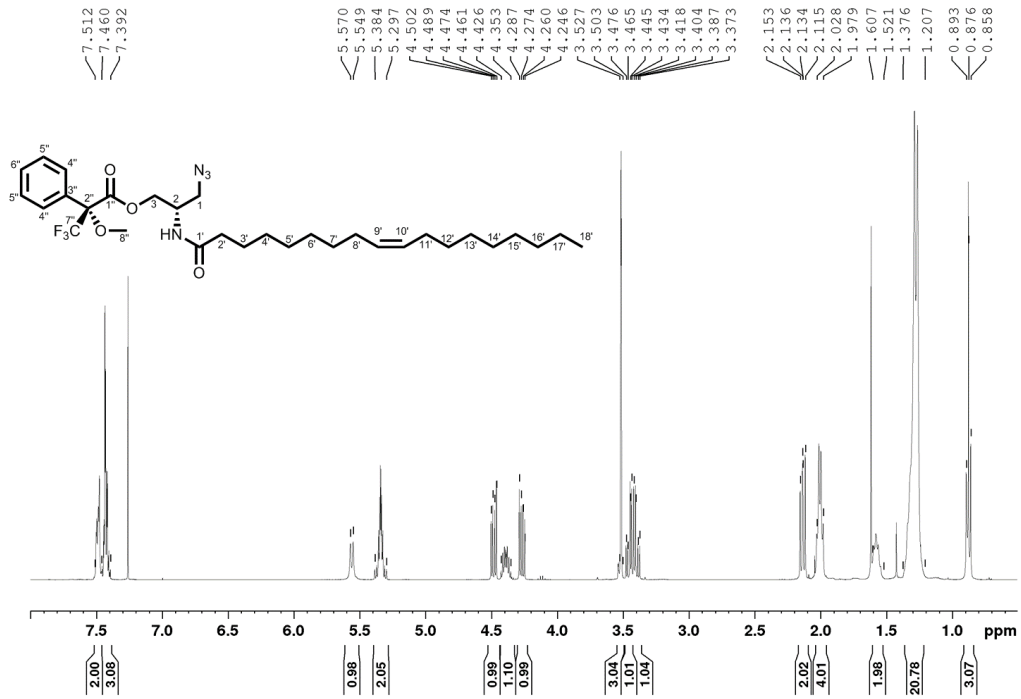


^{19}F-NMR: (375 MHz, $CDCl_3$)

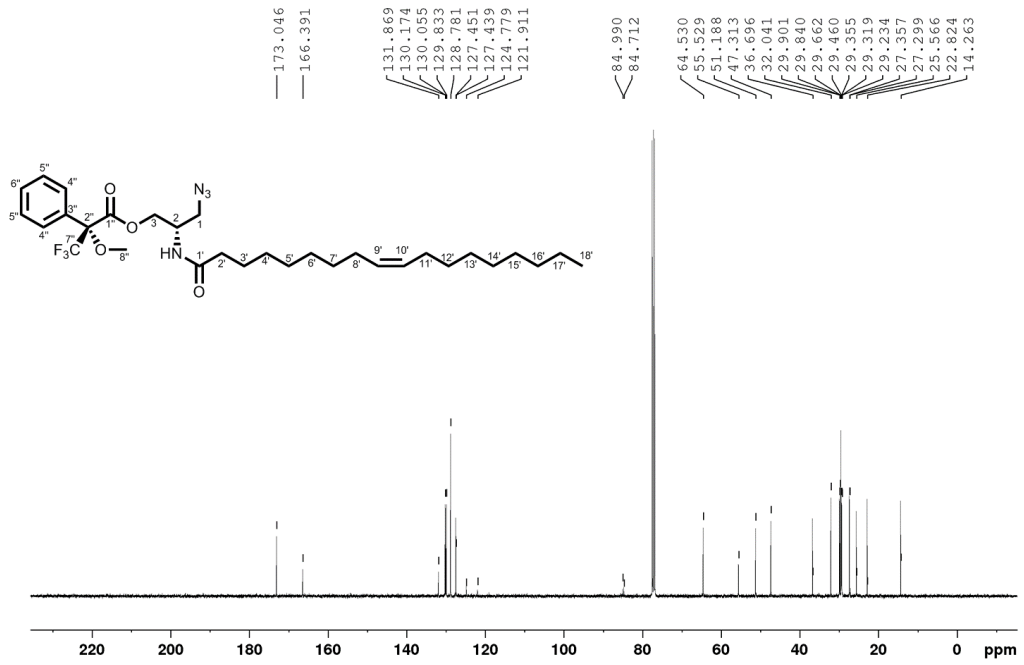


Compound **10b**

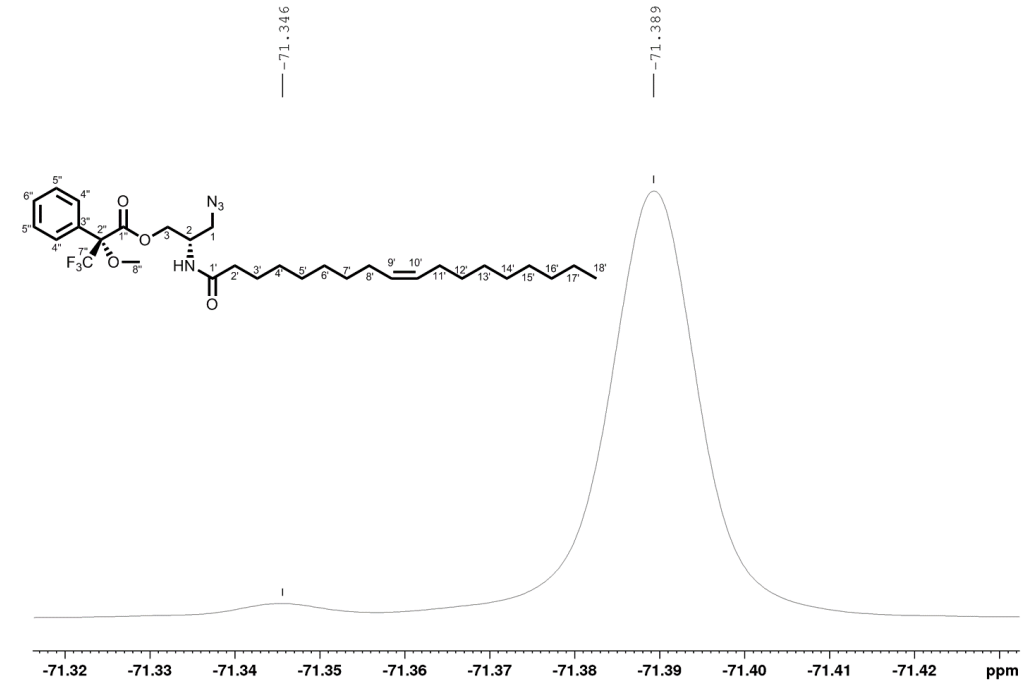
^{1}H-NMR: (400 MHz, $CDCl_3$)



^{13}C-NMR: (100 MHz, $CDCl_3$)

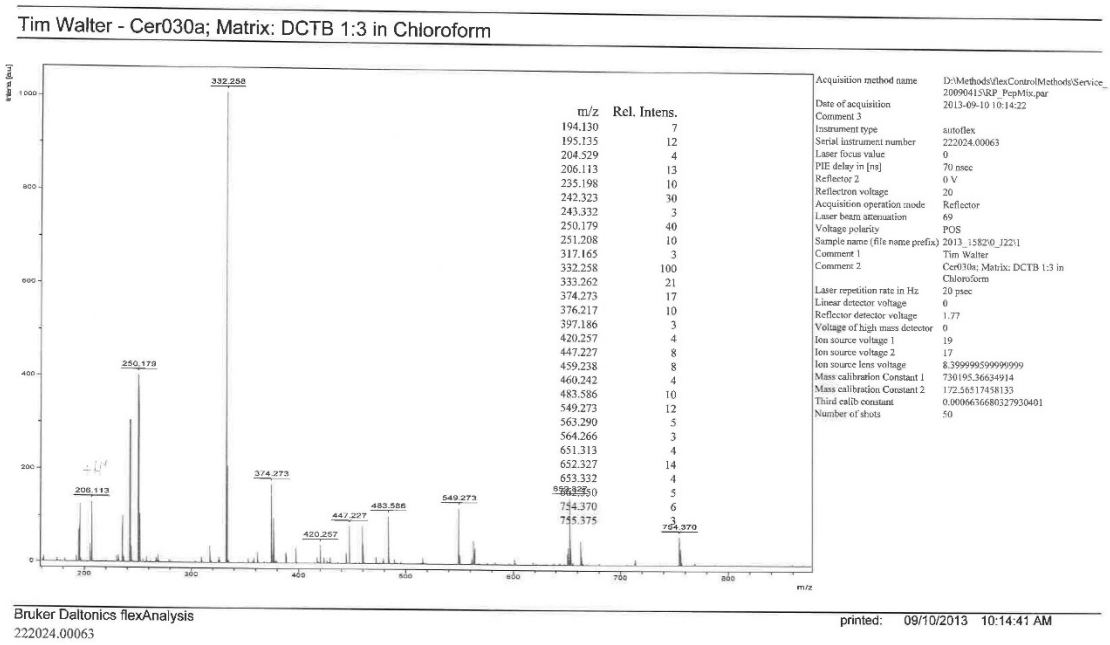


^{19}F-NMR: (375 MHz, $CDCl_3$)

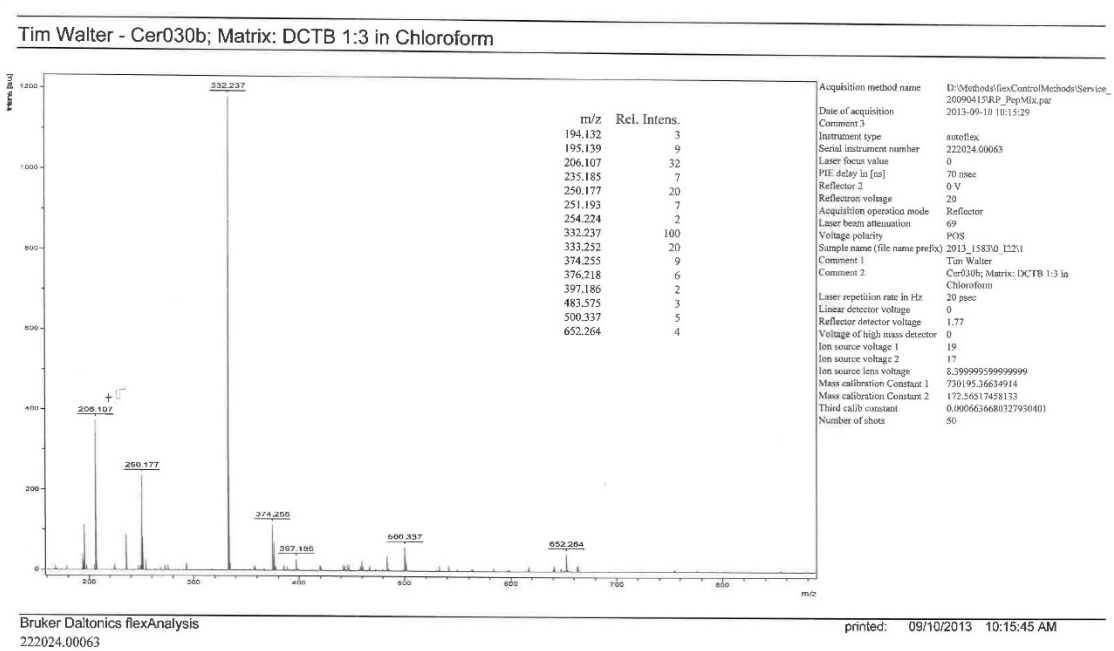


Mass Spectrometry

Compound **6a**



Compound **6b**



Compound **7a**

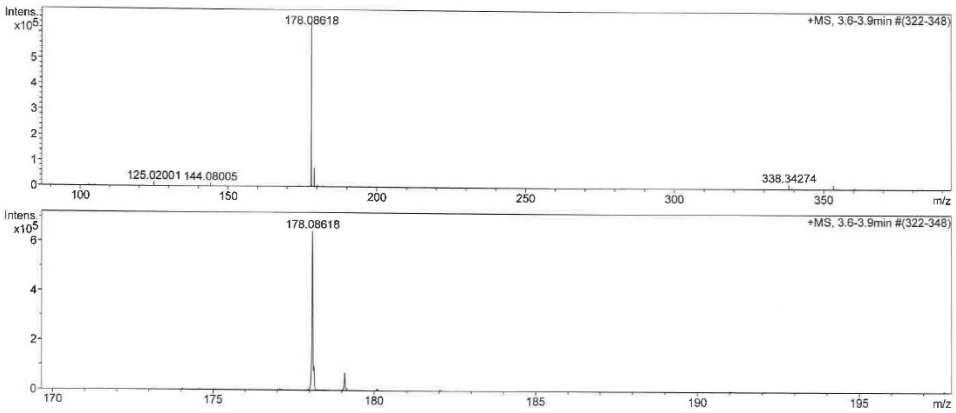
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Spektren2014\2014_0430_SEI.d	Acquisition Date	27.03.2014 08:07:16
Method	esi_tune_pos_wide.m		
Comment	Tim Walter	Operator	administrator
	Cer 031a	Instrument	micrOTOF 88
	1 pmol / µl in Methanol		

Acquisition Parameter

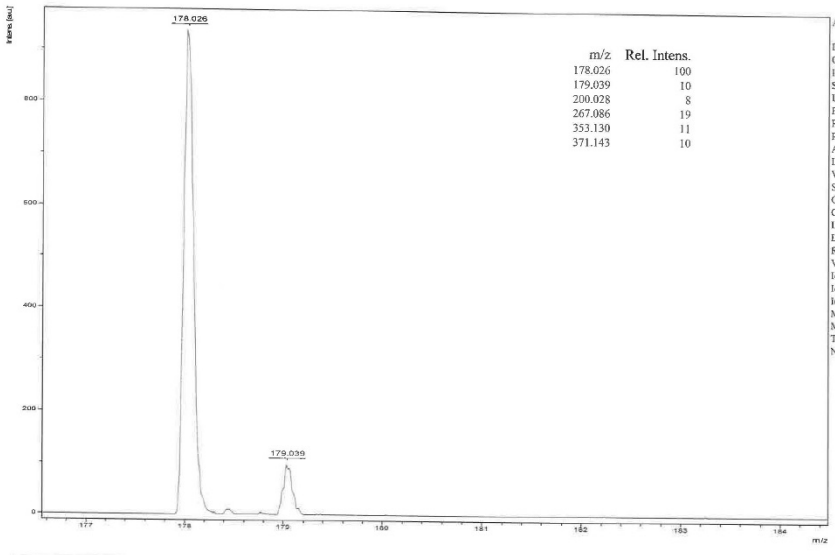
Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	42 V
Scan Range	n/a	Capillary Exit	180.0 V	Set Pulsar Pull	802 V
Scan Begin	50 m/z	Hexapole RF	280.0 V	Set Pulsar Push	804 V
Scan End	1650 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	23.0 V	Set Flight Tube	8600 V
				Set Detector TOF	2150 V



Bruker Daltonics DataAnalysis 3.3 printed: 27.03.2014 08:16:47 Page 1 of 2

Compound **7b**

Tim Walter - Cer031b; Matrix: SDHB 1:3 in Methanol



m/z	Rel. Intens.
178.026	100
179.039	10
200.028	8
267.086	19
353.130	11
371.143	10

Acquisition method name	D:\Methods\flexControlMethods\Service_20090415\RP_PepMix.par
Date of acquisition	2013-09-09 10:15:13
Comment 3	
Instrument type	autoflex
Serial instrument number	222024.00063
Laser focus value	0
PIE delay in [ns]	70 nsec
Reflector 2	0 V
Reflectron voltage	20
Acquisition operation mode	Reflector
Laser beam attenuation	59
Voltage polarity	POS
Sample name (file name prefix)	2013_15640_J171
Comment 1	Tim Walter
Comment 2	Cer031b; Matrix: SDHB 1:3 in Methanol
Laser repetition rate in Hz	20 psec
Linear detector voltage	0
Reflector detector voltage	1.77
Voltage of high mass detector	0
Ion source voltage 1	19
Ion source voltage 2	17
Ion source lens voltage	8.399999599999999
Mass calibration Constant 1	730695.5907435827
Mass calibration Constant 2	181.6829149008186
Third calib constant	0.018060335751864822
Number of shots	50

Bruker Daltonics flexAnalysis printed: 09/09/2013 10:15:33 AM
222024.00063

Compound **8a**

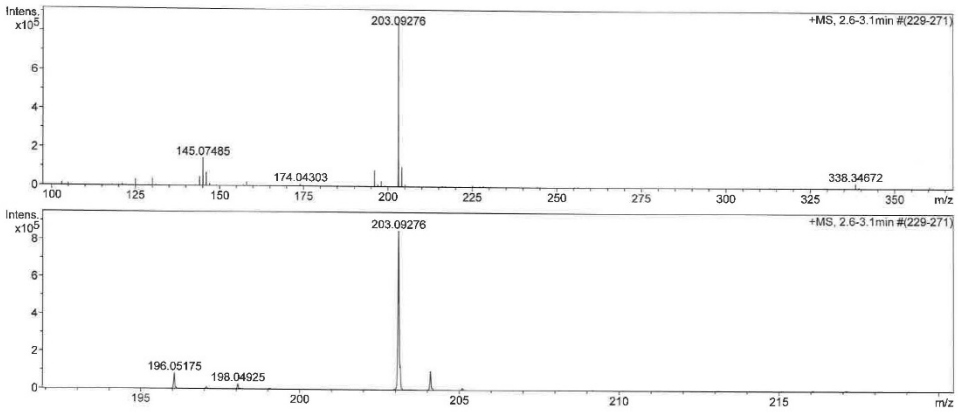
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Spektren2014\2014_0438_SEI.d	Acquisition Date	27.03.2014 12:43:29
Method	esi_tune_pos_wide.m		
Comment	Tim Walter Cer 033a 1 pmol / µl in Methanol	Operator Instrument	administrator micrOTOF 88

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	42 V
Scan Range	n/a	Capillary Exit	180.0 V	Set Pulsar Pull	802 V
Scan Begin	50 m/z	Hexapole RF	280.0 V	Set Pulsar Push	804 V
Scan End	1650 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	23.0 V	Set Flight Tube	8600 V
				Set Detector TOF	2150 V



Compound **8b**

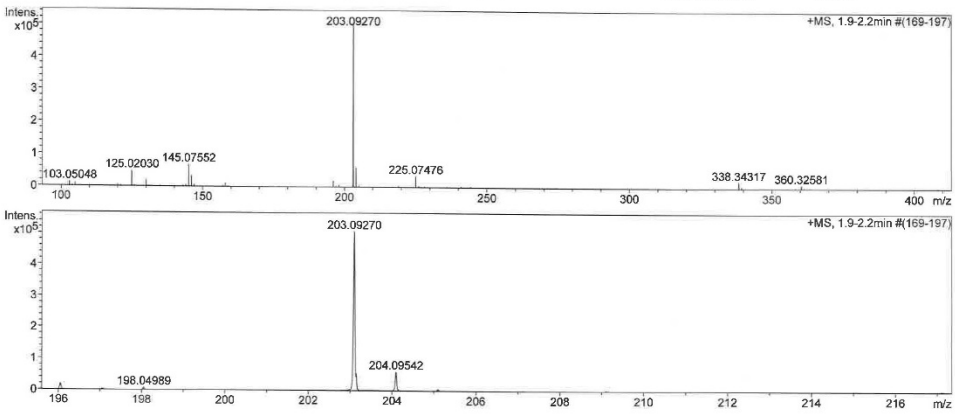
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Spektren2014\2014_0465_SEI.d	Acquisition Date	07.04.2014 10:04:12
Method	esi_tune_pos_wide.m		
Comment	Tim Walter Cer033b 1 pmol / µl in Methanol	Operator Instrument	administrator micrOTOF 88

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	44 V
Scan Range	n/a	Capillary Exit	180.0 V	Set Pulsar Pull	804 V
Scan Begin	50 m/z	Hexapole RF	280.0 V	Set Pulsar Push	804 V
Scan End	1650 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	23.0 V	Set Flight Tube	8600 V
				Set Detector TOF	2150 V



Compound **3a**

Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Spektren2014\2014_0432_SEI.d	Acquisition Date	27.03.2014 10:49:12
Method	esi_tune_pos_wide.m		
Comment	Tim Walter Cer 035a 1,6 pmol / µl in Methanol	Operator Instrument	administrator micrOTOF 88

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	42 V
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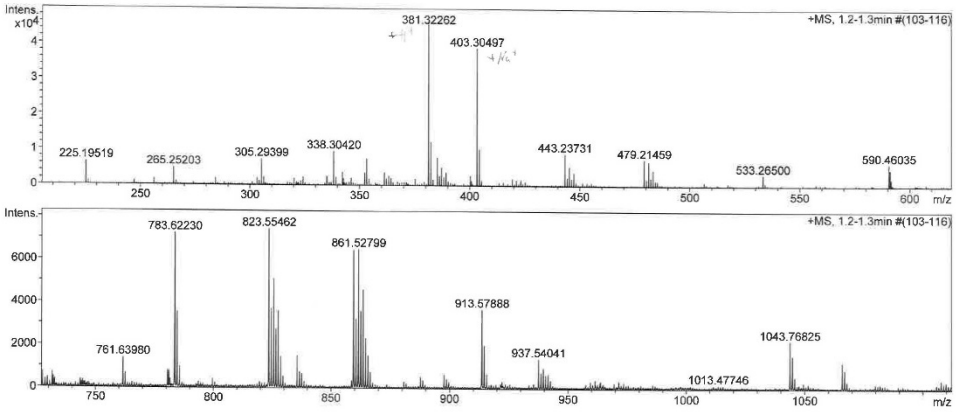
Scan End	1650 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	23.0 V	Set Flight Tube	8600 V
				Set Detector TOF	2150 V



Compound **3b**

Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Spektren2014\2014_0464_SEI.d	Acquisition Date	04.04.2014 11:53:59
Method	esi_tune_pos_wide.m		
Comment	Tim Walter Cer035b 1,7 pmol / µl in Methanol	Operator Instrument	administrator micrOTOF 88

Acquisition Parameter

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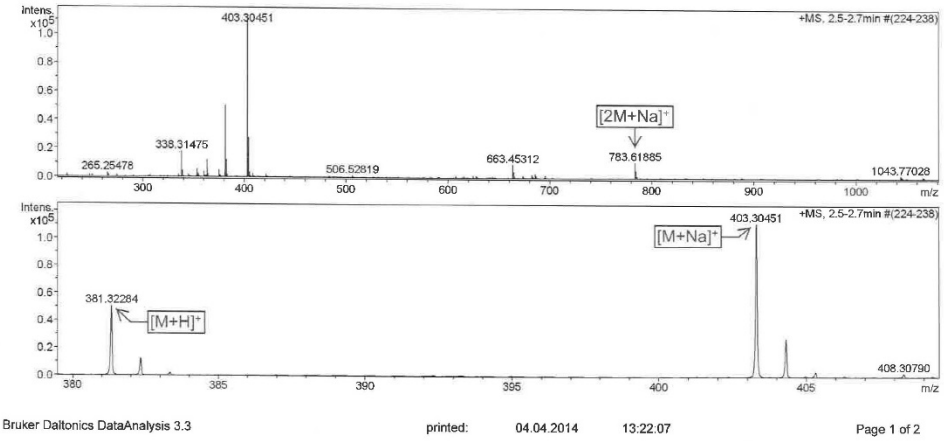
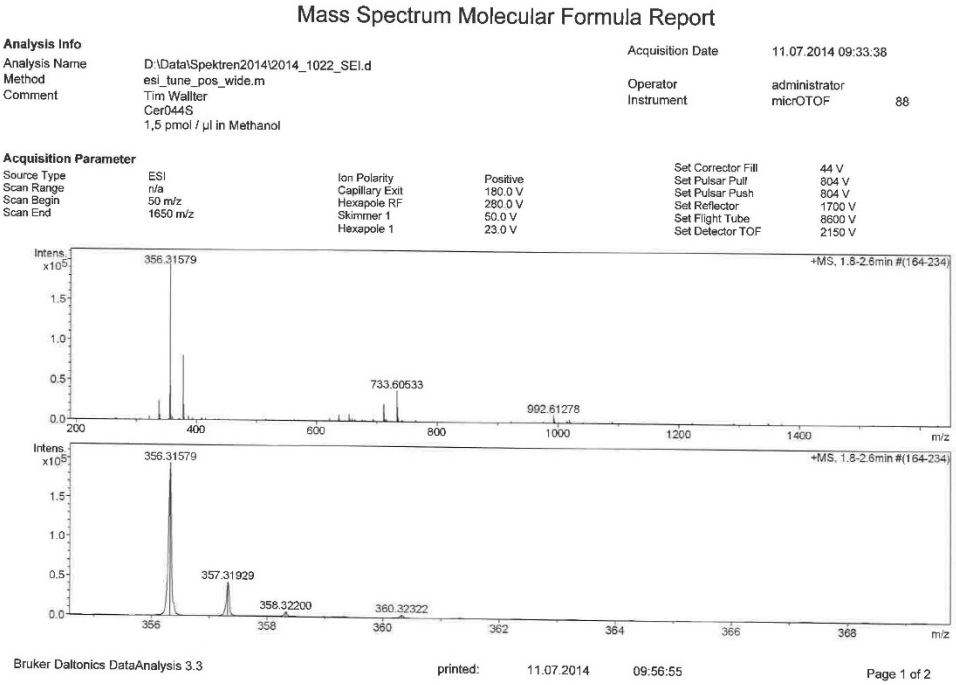
Scan Begin	50 m/z	Hexapole RF	280.0 V	Set Pulsar Push	804 V
Scan End	1650 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	23.0 V	Set Flight Tube	8600 V
				Set Detector TOF	2150 V



Compound **2**



Cell culture and reagents.

Peripheral blood monocytic cells from healthy donors were subjected to Ficoll gradient centrifugation and were used as source for primary human T cells (enriched by nylon wool columns). Primary human and Jurkat T cells were maintained in RPMI1640/10 % FCS.

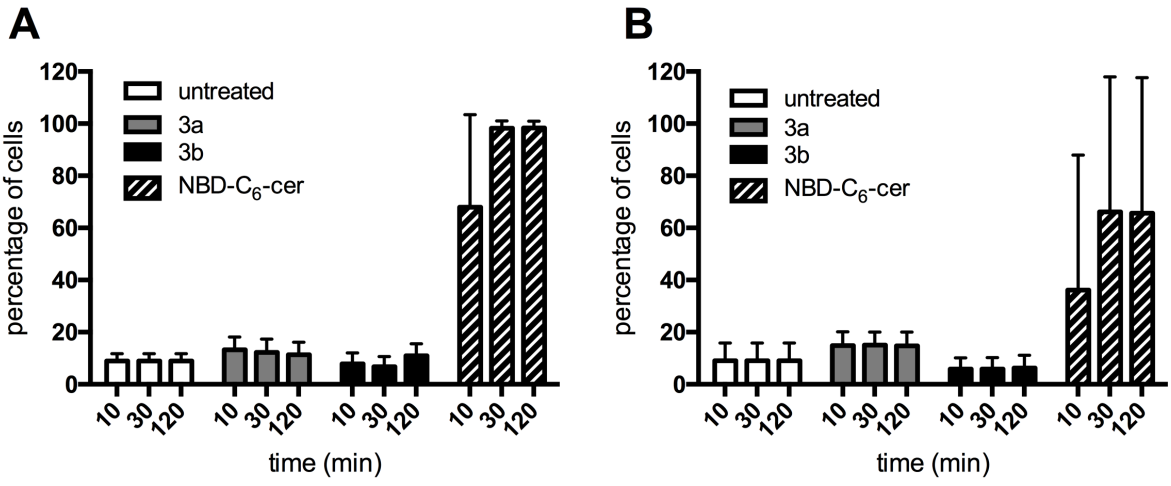


Figure S1 3b is non-toxic to T cells. Jurkat (A) or primary human T cells (B) were left untreated (upper and middle panels, white bars) or fed with each 25 µM **3b** (black bars) or NBD-C6-ceramide (hatched bars) for the time intervals indicated at RT, or after a 5 mins click reaction to DIBO488 for 120 min at RT and the frequencies of PI+ cells were determined by flow cytometry. Means of three independent experiments are shown.

Endothelial cell lines

The simian virus 40 large T antigen-transformed human brain microvascular endothelial cells (HBMEC) were cultured as previously described. Briefly, HBMECs were cultured in RPMI-1640 medium (Gibco Life Technologies) supplemented with FCS (10 %) (Gibco Life Technologies), Nu serum IV (10 %; Becton Dickinson), vitamins (1 %), non-essential amino acids (1 %), sodium pyruvate (1 mM), L-glutamine (2 mM), heparin (5 U ml^{-1}) (all reagents were from Biochrom) and endothelial cell growth supplement (30 mg ml^{-1}) (Cell Systems Clonetics). Cultures were incubated in a humid atmosphere at 37 °C with 5% CO_2. Cells between the 10th and 25th passages were used for infection assays. HBMEC were cultured in T25 flasks (Corning Costar Corporation, Cambridge, MA, USA) to a confluent monolayer. At 48 hours prior to treatment, HBMEC were split and seeded on gelatine-coated 24–well tissue culture plates (Sarstedt; Germany) at a density of 5×10^4 cells per well. Cells were grown to approximately 1×10^5 cells prior to treatment.

Primary astrocytes and 3T3 fibroblasts

Primary cultures of astrocytes were prepared from newborn mouse brain and cultivated in DMEM/10 % FCS as described in [ref5]. 3T3 fibroblasts were purchased from ATCC (CRL-1658) and also cultivated in DMEM/10 % FCS. To stop cell division, astrocytes and fibroblasts were grown in serum-free DMEM medium for 24 h prior to incubation with **3a/b** and click reaction.

Apoptosis Analysis

Apoptosis Analysis

HBMEC were seeded in 24-well tissue culture plates to a density of 2×10^6 cells/well. The medium was changed and cells were treated with the compounds for 1 h or 16 h, respectively. Cells were harvested and washed once with 1 x $PBS^{-/-}$ and once with Annexin V binding buffer (BD Biosciences). Cells were resuspended and transferred into a 500 µL siliconized polypropylene tube. Annexin V-Alexa Fluor 488 (Molecular Probes) was added at a 1:20 dilution and cells were incubated for 15 min at RT. Following this, propidium iodide (PI) was added at a final concentration of 1 µg/mL and cells were stained for a further 15 min at RT. After staining, cells were washed twice with $PBS^{-/-}$ and fixed with 2 % formaldehyde for 10 min on ice. Following fixation, cells were washed twice with $PBS^{-/-}$ and treated with 50 µg/mL DNase-free RNase (Sigma, R4642) for 15 min at 37 C. Cells were then washed once with $PBS^{-/-}$ and immediately analyzed using a BD FACSCalibur™ flow cytometer (BD Biosciences) and BD CellQuest™ Pro Software (BD Biosciences). For each measurement, at least 10,000 cells were counted. Cells that stained positive for annexin V represented cells with intact membranes and externalized phosphatidylserine (early apoptosis) and cells positive for annexinV/PI represent cells that had lost membrane integrity (late apoptosis/necrosis).

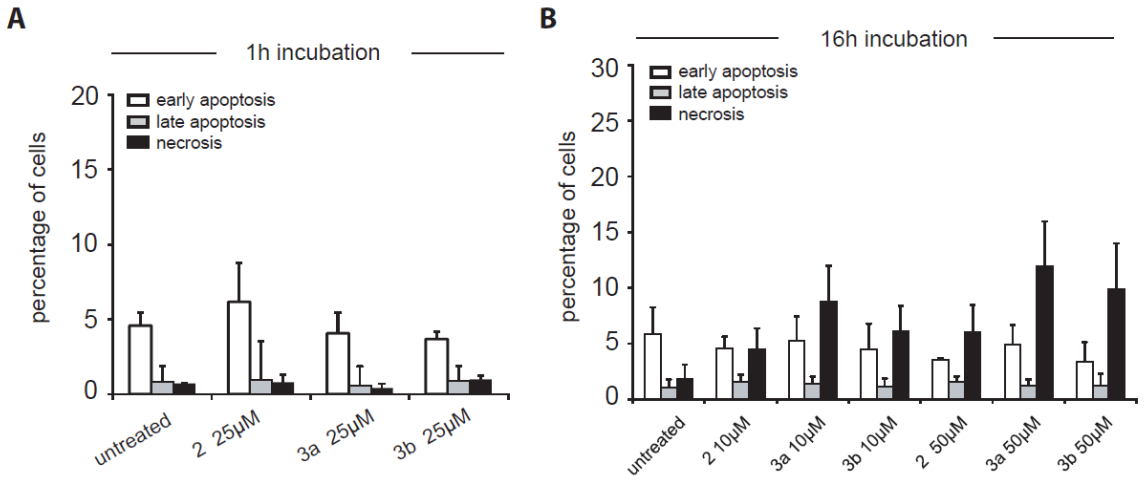


Fig S2 Effects of **2**, **3a** and **3b** on HBMEC cell apoptosis. HBMEC were left untreated or were treated with indicated concentrations of **2**, **3a**, **3b** for 1 h (A) or 16 hrs (B). Apoptosis was quantified using flow cytometry after staining with Annexin V (AnnV)/propidium iodide (PI). (B) Percentage of early (AnnV+/PI-), late apoptotic cells (AnnV+/PI+) and ÿŜŎǊŽƚŝĐ cells (AnnV-/PI+Ϳ are shown. Data are presented as the mean ± S.E. of triplicate experiments. * $P < 0.05$; vs. untreated cells. Further studies including metabolic pathway studies of **3** are under investigation.

Labeling, expression of PKCζ-GFP, and confocal microscopy

$2*10^5$ Jurkat T cells were extensively washed and resuspended in HBSS containing N_3-S_{18}-ser (stocks 10mM in DMSO, 25µM final) and incubated for 30 mins at RT and washed once with HBSS. For click reactions, Click-IT Alexa Fluor 488 DIBO Alkyne (20 µM, Life technologies, Germany) were added for 5 min if not stated otherwise, and cells were kept at RT until microscopical analysis. Primary T cells followed the same feeding and labelling protocol. Primary cultured astrocytes and transfected 3T3 fibroblasts were first incubated for 1 h with an equimolar mixture of **3a** and **b** (5 µM each), washed with PBS, and then subjected to the click reaction with Alexa Fluor 546 DIBO Alkyne (5 µM) for 20 min at RT. Cells were fixed with 4 % para-formaldehyde/0.5 % glutaraldehyde in PBS for 15 min at RT. Fixed cells were washed with PBS and then permeabilized with 0.2 % Triton X-100/PBS for 5 min at RT, a method previously shown to preserve ceramide distribution in a variety of cell types, but also allowing for colabeling with antibodies against other intracellular antigens Colabeling was performed with antibodies against ceramide (1:100, rabbit IgG), GM130 (1:100, mouse IgG), and PKCζ (C20, 1:100, rabbit IgG) as previously described [refs2-4]. Colabeling of PKCζ with **3a/b** was performed by first transfecting 3T3 fibroblasts with the cDNA for human PKCζ-GFP fusion protein as reported previously followed by labelling with **3a/b** and click reaction 24 h post-incubation to ensure sufficient expression of the protein. Expression of GFP and **3a/b** click reaction was used as negative control without showing any signs of colabeling (not shown).

Confocal Laser Scanning Microscopy (CLSM) imaging was performed using a LSM 780 (Zeiss, Germany), equipped with an incubation system and a 40x Plan-Apochromat oil objective (NA 1.4) and laser line 488. Images were processed using CLSM software ZEN2012.

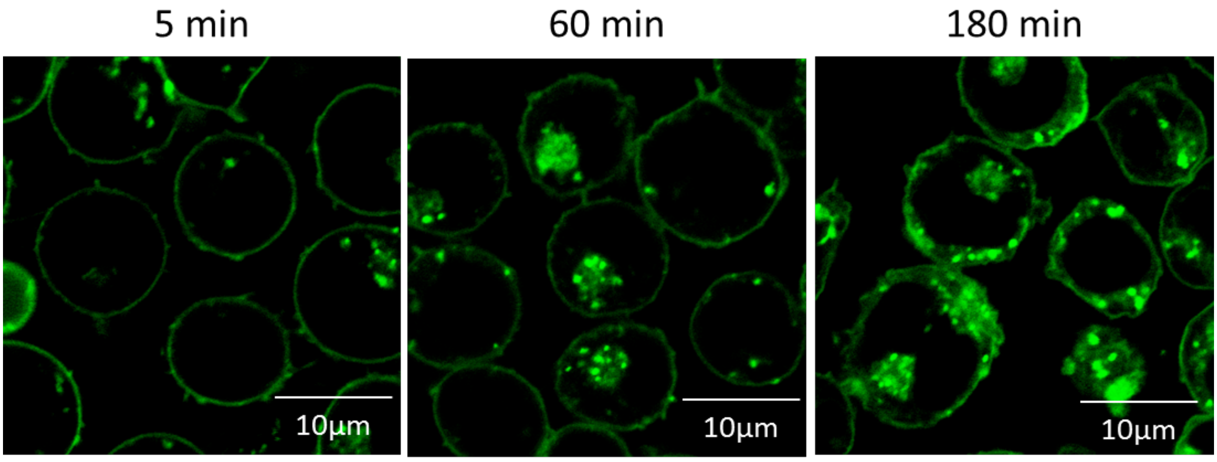


Fig S3 Jurkat T cells were incubated with 25µM **3b** in HBSS for 30min at rt. After washing, the cells were exposed to 20µM DIBO488 dye and the click reaction was performed for 5min at rt and then excess dye was washed away three times with HBSS. For following **3b** through cell compartments, cells were kept at 37 °C for 5, 60 and 180 min before microscopic analysis.

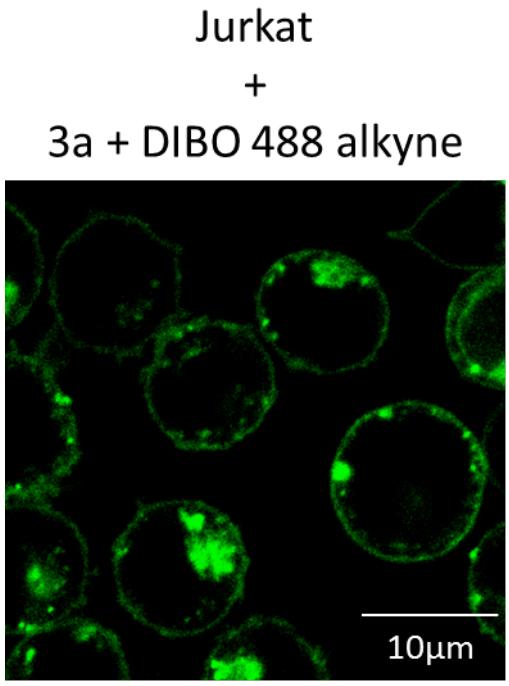


Fig S4 Jurkat T cells were incubated with 25 µM **3a** in HBSS for 30 min at rt. After washing, the cells were exposed to 20 µM DIBO488 dye and the click reaction was performed for 5 min at rt. Cells were washed three times with HBSS and kept at rt until microscopic analysis.

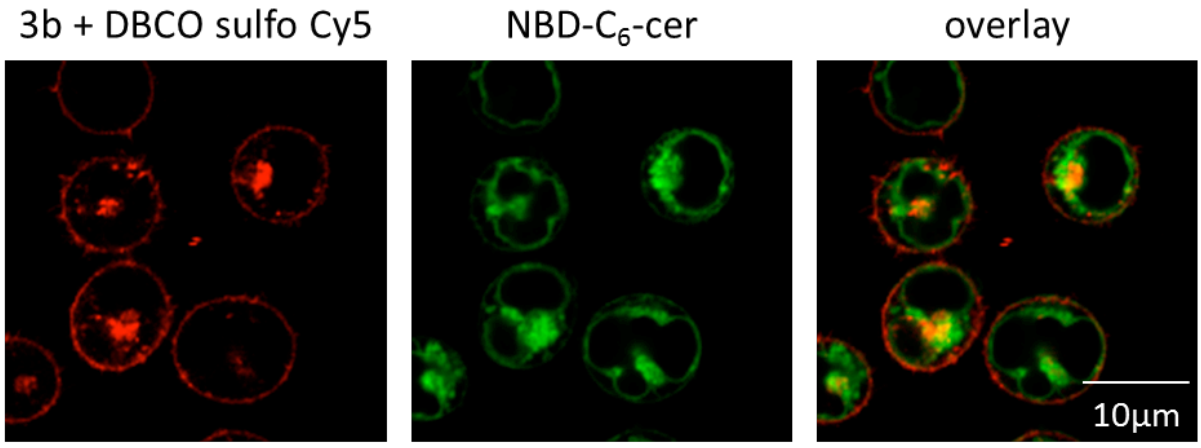


Fig S5 Jurkat T cells were incubated with 25 µM **3b** and 5 µM NBD-C6-cer in HBSS for 30 min at 37 °C. After washing, the cells were exposed to 20 µM DBCO sulfo Cy5 dye and the click reaction was performed for 5 min at rt. Cells were washed three times with HBSS and kept at rt until microscopic analysis.

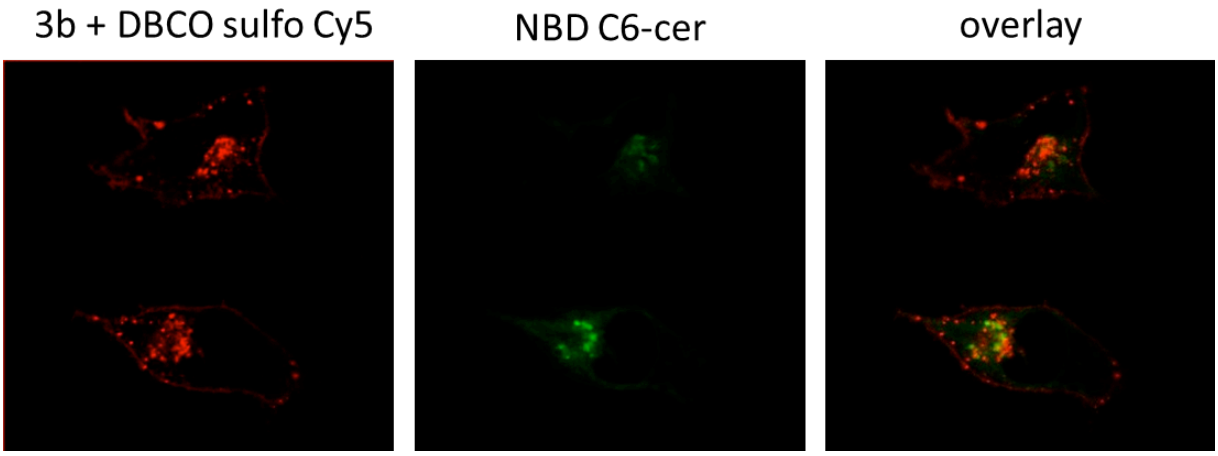


Fig S6 HBMEC were treated with 5 µM 3b in RPMI for 30 min. After washing, the cells were exposed to 5 µM DBCO-sulfo-Cy5 + NBD C6 for 30 min at 37 °C. Cells were washed three times with PBS and kept at RT until microscopic analysis.

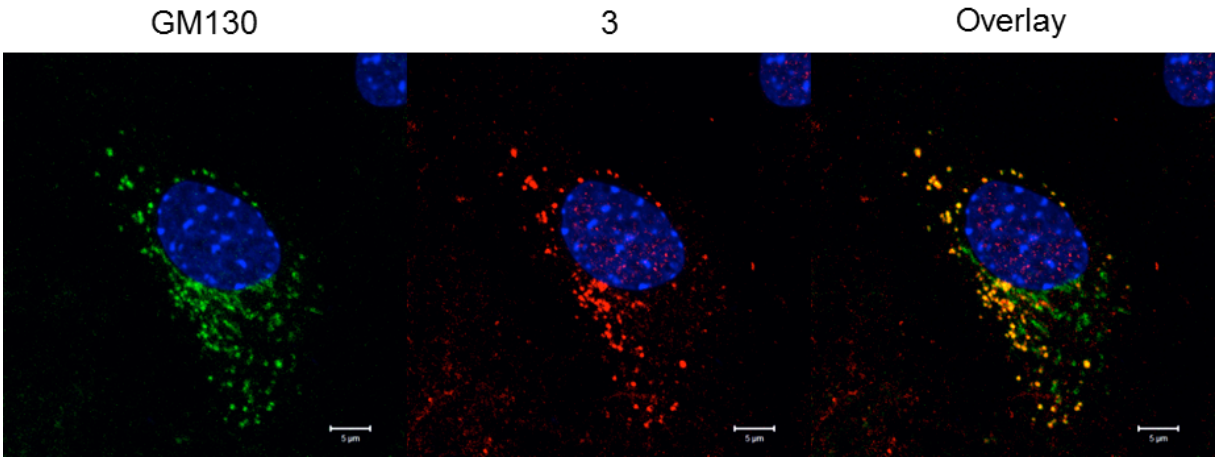


Fig S7 Primary cultured astrocytes were incubated with 5 µM each **3a** and **b** for 1 h, washed with PBS, and then click reaction performed with 5 µM Alexa 546 DIBO alkyne for 20 min at RT. After washing, cells were fixed with p-formaldehyde/glutaraldehyde and permeabilized under mild conditions (0.2 % Triton X-100/PBS, 5 min, RT). Immuncytochemistry and co-labeling was performed with anti-GM130 mouse IgG and Alexa 488 anti- mouse IgG.

Flow cytometry

Cell surface ceramides were detected using FACsCALIBURFor detection of cell death, cells were incubated with Propidium Iodide Staining Solution (5 µL/test, eBioscience, CA, USA) following treatment with functionalized lipids and immediately analysed by FACsCALIBUR.

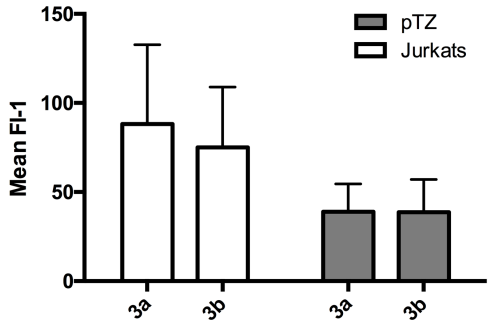


Fig S8 Primary (grey bars)and Jurkat T cells (white bars) were incubated with 25 µM **3a** or **3b** in HBSS for 30 min at 37 °C. After washing, the cells were exposed to 20 µM DIBO488 dye and the click reaction was performed for 5 min at rt. Cells were washed three times with HBSS and uptake of the serinols was measured by FACS analysis. All measurements were done with a sample size of n=3 and standard errors ar indicated as bars

Mass spectrometry

N_3-S_{18}-ser treated cells were collected and dissolved in 1 mL methanol by sonification on ice for 30 minutes. After centrifugation (15000g, 5 min) the organic phase was transferred into glass tube and evaporated using a vacuum system (Thermo Fisher Scientific, Waltham, MA, USA). The dried lipids were than resolved in methanol and sample analysis was carried out by rapid-resolution liquid chromatography (LC)-MS/MS using a Q-TOF 6530 mass spectrometer (Agilent Technologies, Waldbronn, Germany) operating in the positive ESI mode. The compounds were separated by reverse phase LC using a 2.1 x 150 mm Zobrax Eclipse Plus C8 column (Agilent Technologies) and a binary solvent system at a flow rate of 0.5 mL/min. Prior to injection of the sample, the column was equilibrated for 1 minute with a solvent mixture of 40% mobile phase A (H_2O/HCOOH, 99.9/0.1 v/v) and 60% mobile phase B (CH_3OH/C_2H_3N/HCOOH, 49.95/49.95/0.1, v/v/v), and after sample injection, the A/B ratio was maintained at 40/60 for 3 minutes, followed by a linear gradient to 95% B over 9 minutes, which was held at 95% B for 3 minutes, followed by a 1 minute gradient return to 40/60 A/B. **3a** was detected via accurate mass of m/z 381.3224 respectively and quantified by the use of an external calibration curve. Quantification was performed with Mass Hunter Software (Agilent Technologies).

Aquisition Parameter	
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Max Range(m/z)	1700
Scan Rate(spectra/sec)	4.00

Source Parameter	***Value***	***Scan Source Parameter***	***Value***
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Gas Flow(l/min)	10	Nozzle Voltage(V)	2000
Nebulizer(psig)	45	Fragmentor	155
SheathGasTemp	380	Skimmer1	65
SheathGasFlow	12	OctopoleRFPeak	750

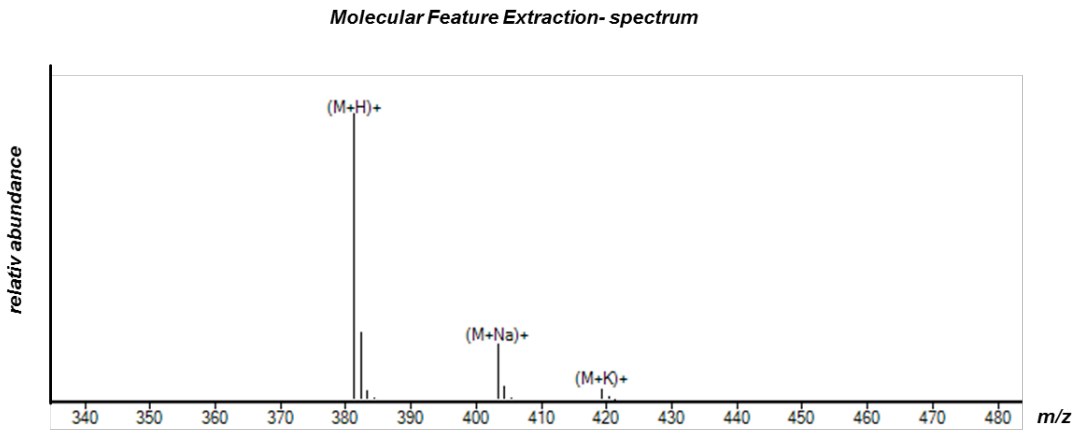

Fig S9 Molecular Feature Extraction-spectrum of **3b**