Supporting Information

# Synthetic Glycosphingolipids for Live-Cell Labeling

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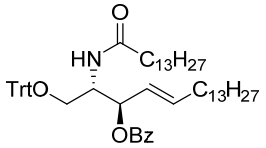
### Synthesis of Azidoglucosylceramide 3

#### *N*-[(1*S*,2*R*,3*E*)-2-(Benzoyloxy)-1-[(triphenylmethoxy)methyl]-3-heptadecen-1-yl]-tetradecanamide (25)



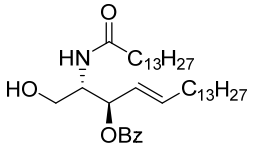

$C_{58}H_{81}NO_4$
856,27 g/mol

150 mg (0.29 mmol) ceramide **17** and 98 mg (0.35 mmol) trityl chloride were dissolved in 3 mL pyridine/DCM/THF 1:1:1. The reaction was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The remaining material was dissolved in a small amount of DCM and washed with saturated $NaHCO_3$ solution and water. The organic phase was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product (**21**) was dissolved in 3 mL toluene/pyridine 4:1. Benzoyl chloride (70 µL, 0.6 mmol) was added, and the mixture was stirred for 16 h at room temperature. A small amount of DCM was added, and the mixture was washed with saturated $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (eluent petroleum ether/ethyl acetate 9:1). **25** was obtained as a colorless solid (176 mg, 0.21 mmol, 72 % over two steps).

**TLC**: $R_f$ = 0.43 (eluent petroleum ether/ethyl acetate 4:1)

**$^1$H NMR** (250 MHz, $CDCl_3$): $\delta$ = 8.02 – 7.97 (m, 3 H, aromat.), 7.88 (d, $J$ = 7.1 Hz, 3 H, aromat.), 7.57 – 7.09 (m, 15 H, aromat.), 5.89 – 5.78 (m, 2 H, NH, CH=C<u>H</u>$CH_2$), 5.65 (‘t’, $J$ = 7.6 Hz, CHOBz), 5.38 (dd, $J$ = 15.5, 7.7 Hz, CH=C<u>H</u>$CH_2$), 4.45 – 4.37 (m, 1 H, C<u>H</u>NH), 3.38 (dd, $J$ = 9.6, 3.9 Hz, 1 H, $CH_2$OTrt), 3.19 – 3.08 (m, 1 H, $CH_2$OTrt), 2.08 (t, $J$ = 7.5 Hz, 2 H, C(O)$CH_2$), 1.96 – 1.89 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.61 – 1.45 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.32 – 1.10 (m, 42 H, 21x $CH_2$), 0.83 (t, $J$ = 6.5 Hz, 6 H, 2x $CH_3$).

#### *N*-[(1*S*,2*R*,3*E*)-2-(Benzoyloxy)-1-(hydroxymethyl)-3-heptadecen-1-yl]-tetradecanamide (29)




$C_{39}H_{67}NO_4$
613,95 g/mol

Ceramide **29** was synthesized as described for **23**, starting from 176 mg ceramide **25** (0.21

mmol). After purification by silica chromatography 94 mg **29** (0.15 mmol, 74 %) was obtained as colorless solid.

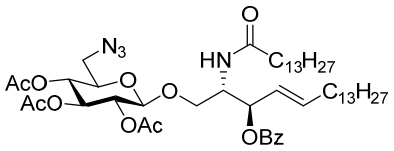
**TLC:** $R_f$ = 0.26 (eluent petroleum ether/ethyl acetate 1:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.05 – 8.02 (m, 2 H, aromat.), 7.58 – 7.55 (m, 1 H, aromat.), 7.45 – 7.42 (m, 2 H, aromat.), 6.22 (d, $J$ = 8.5 Hz, 1 H, NH), 5.86 (dt, $J$ = 14.5, 6.8 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.55 (dd, $J$ = 14.6, 7.4 Hz, 1 H, C<u>H</u>=CH$CH_2$), 5.51 (m, 1 H, CHOBz), 4.25 (m, 1 H, C<u>H</u>NH), 3.73 (dd, $J$ = 12.2, 3.5 Hz, 1 H, C<u>H$_2$</u>OH), 3.62 (dd, $J$ = 12.2, 3.3 Hz, 1 H, C<u>H$_2$</u>OH), 2.65 (b, 1 H, OH), 2.22 – 2.17 (m, 2 H, C(O)$CH_2$), 2.03 – 1.99 (m, 2 H, CH=CHC<u>H$_2$</u>), 1.67 – 1.53 (m, 2 H, C(O)$CH_2$C<u>H$_2$</u>), 1.37 – 1.16 (m, 42 H, 21x $CH_2$), 0.89 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**ESI-MS:** calculated $[M+H]^+ = 614.5$, $[M+Na]^+ = 636.5$

found $[M+H]^+ = 614.5$, $[M+Na]^+ = 636.6$

**(2*S*,3*S*,4*E*)-2-Tetradecanamido-3-(benzoyloxy)-4-octadecen-1-yl-2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (35)**




$C_{51}H_{82}N_4O_{11}$
927,22 g/mol

Glycolipid **35** was synthesized as described for **34**, starting from 94 mg (0.153 mmol) ceramide **29** and 87 mg (0.18 mmol) trichloroacetimidate **33**. After purification 28 mg (0.03 mmol, 20 %) of **35** was obtained as a colorless solid.

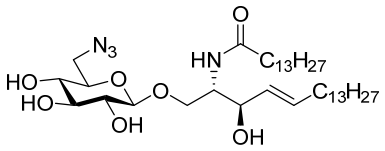
**TLC:** $R_f$ = 0.59 (eluent toluene/acetone 4:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.04 – 8.00 (m, 2 H, aromat.), 7.55 – 7.52 (m, 1 H, aromat.), 7.45 – 7.41 (m, 2 H, aromat.), 5.90 – 5.81 (m, 1 H, CH=C<u>H</u>$CH_2$), 5.76 (d, $J$ = 9.2 Hz, 1 H, NH), 5.53 (‘t’, $J$ = 6.9 Hz, 1 H, CHOBz), 5.45 (dd, $J$ = 15.2, 7.4 Hz, 1 H, C<u>H</u>=CH$CH_2$), 5.17 (‘t’, $J$ = 9.5 Hz, 1 H, H-3), 4.97 – 4.90 (m, 2 H, H-2, H-4), 4.50 (d, $J$ = 8.0 Hz, 1 H, H-1), 4.50 – 4.45 (m, 1 H, C<u>H</u>NH), 4.04 (dd, $J$ = 10.0, 4.3 Hz, 1 H, $CH_2$OGlc), 3.68 (dd, $J$ = 10.2, 4.3 Hz, 1 H, $CH_2$OGlc), 3.67 – 3.61 (m, 1 H, H-5), 3.23 (dd, $J$ = 13.4, 6.8 Hz, 1 H, H-6a), 3.15 (dd, $J$ = 13.4, 2.7 Hz, 1 H, H-6b), 2.18 – 2.11 (m, 2 H, C(O)$CH_2$), 2.05 – 1.95 (m, 12 H, CH=CHC<u>H$_2$</u>, 3x C(O)$CH_3$), 1.64 – 1.53 (m, 2 H, C(O)$CH_2$C<u>H$_2$</u>), 1.32 – 1.16 (m, 42 H, 21x $CH_2$), 0.86 (t, $J$ = 6.7 Hz, 6 H, 2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+ = 949.6$

found $[M+Na]^+ = 949.4$

**(2*S*,3*S*,4*E*)-2-Tetradecanamido-3-hydroxy-4-octadecen-1-yl-6-azido-6-deoxy-β-D-glucopyranoside (3)**



$C_{38}H_{72}N_4O_7$
697,00 g/mol

**3** was synthesized as described for **2,** starting from 128 mg (0.18 mmol) **35**. After purification with silica chromatography (eluent DCM/MeOH 96:4) 61 mg **3** (0.09 mmol, 48 %) was obtained as colorless solid.

**TLC:** $R_f$ = 0.26 (eluent DCM/MeOH 9:1)

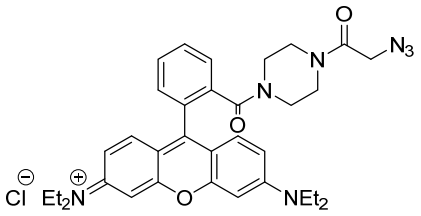
**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 6.74 (b, 1 H, NH), 5.75 (dt, $J$ = 15.2 Hz, 7.2 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.44 (dd, $J$ = 15.3 Hz, 6.1 Hz, C<u>H</u>=$CHCH_2$), 4.36 (d, $J$ = 7.3 Hz, 1 H, H-1), 4.20 – 4.12 (m, 2 H, C<u>H</u>(NHR)C<u>H</u>(OH), 4.03 – 3.97 (m, 1 H, $CH_2$OGlc), 3.78 – 3.72 (m, 1 H, $CH_2$OGlc), 3.59 – 3.33 (m, 6 H, H-2, H-3, H-4, H-5, H-6a/b), 2.25 (t, $J$ = 7.4 Hz, 2 H, C(O)$CH_2$), 2.05 – 2.00 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.63 – 1.56 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.38 – 1.17 (m, 42 H, 21x $CH_2$), 0.88 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 175.2 (C(O)), 135.1 (CH=<u>C</u>H$CH_2$), 128.2 (<u>C</u>H=$CHCH_2$), 103.0 (C1), 76.4, 75.7, 73.5, 73.1, 71.2 (C2, C3, C4, C5, sphingosine-CHOH), 69.3 (sphingosine-$CH_2$OH), 53.7 (CNH), 51.7 (C6), 36.9 (C(O)<u>C</u>$H_2$), 32.5 (CH=CH<u>C</u>$H_2$), 32.1, 29.9 – 29.4 (m), 26.0 ($CH_2$), 14.3 (2x $CH_3$).

**ESI-MS:** calculated $[M+H]^+$ = 697.5, $[M+Na]^+$ = 719.5
found $[M+H]^+$ = 697.4, $[M+Na]^+$ = 719.2

**HR-ESI-MS:** calculated $[M+H]^+$ = 697.54738
found $[M+H]^+$ = 697.54749

**Synthesis of Rhodamine Azide 5**



$C_{34}H_{40}ClN_7O_3$
630,18 g/mol

Rhodamine B-piperazinamide[1, 2] (150 mg, 0.32 mmol) was dissolved in 15 ml dry DCM. After addition of azidoacetic acid *N*-succinimidyl ester[3] (69 mg, 0.35 mmol) and DIPEA (216 µL, 1.26 mmol), the reaction was stirred for 3 days at room temperature. The solvent was removed under reduced pressure. The crude product was purified by silica column chromatography (eluent DCM/MeOH 9:1), and **5** was obtained as a dark purple solid (95 mg, 0.16 mmol, 50 %).

**TLC:** $R_f$ = 0.19 (eluent DCM/MeOH 9:1)

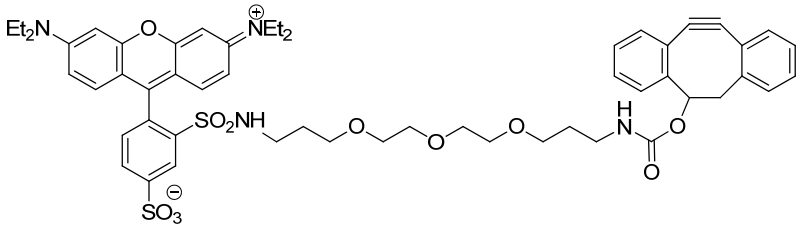
**$^1$H NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.80 – 7.75 (m, 2 H, aromat.), 7.73 – 7.69 (m, 1 H, aromat.), 7.55 – 7.51 (m, 1 H, aromat.), 7.28 (d, $J$ = 9.5 Hz, 2 H, aromat.), 7.08 (dd, $J$ = 9.6, 2.5 Hz, 2 H, aromat.), 6.97 (d, $J$ = 2.4 Hz, 2 H, aromat.), 3.69 (q, $J$ = 7.1 Hz, 8 H, 4x $C\underline{H}_2CH_3$), 3.43 (br, 8 H, 2x $NCH_2CH_2N$), 3.30 – 3.26 (obscured, 2 H, $CH_2N_3$), 1.31 (t, $J$ = 7.1 Hz, 12 H, 4x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CD_3OD$): $\delta$ = 159.3, 157.2 (2x C(O)), 157.0, 136.4, 133.24, 133.18, 132.4, 132.3, 131.8, 131.4, 129.9, 128.9, 115.4, 114.9, 97.4 (13x C aromat.), 49 (obscured, 2x $NCH_2CH_2N$), 46.9 (4x $N\underline{C}H_2CH_3$), 45.2 ($CH_2N_3$), 12.8 ($CH_3$).

**ESI-MS:** calculated $[M]^+ = 594.3$

found $[M]^+ = 594.3$

**Synthesis of DIBO-Lissamine 6**



$C_{54}H_{62}N_4O_{11}S_2$
1007,22 g/mol

Carbonic acid 7,8-didehydro-1,2:5,6-dibenzocyclooctene-3-yl ester 4-nitrophenyl ester[4] (44 mg, 0.114 mmol), *N*-(1-amino-4,7,10-trioxa-tetradec-13-yl)-sulforhodamin-B-sulfonamide-2,2,2-trifluoroacetate[5] (100 mg, 0.114 mmol), and DIPEA (39 µL, 0.228 mmol, 30 mg) were dissolved in 10 mL dry DMF. The reaction was stirred for 4 h at room temperature, followed by evaporation at reduced pressure. After purification by silica column chromatography (eluent DCM/MeOH 95:5), **6** was obtained as a purple solid (80 mg, 80 µmol, 70 %, mixture of isomers).

**TLC:** $R_f$ = 0.37 (eluent DCM/MeOH 9:1)

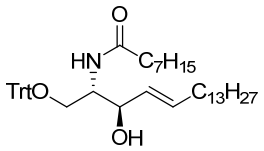
**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.74 – 8.69 (m, 1 H, aromat.), 8.00 – 7.96 (m, 1 H, aromat.), 7.49 – 7.47 (m, 1 H, aromat.), 7.34 – 7.14 (m, 10 H, aromat.), 6.80 – 6.73 (m, 2 H, aromat.), 6.66 – 6.63 (m, 2 H, aromat.), 5.41 (br, 1 H, CHO cyclooctyne), 3.72 – 3.47 (m, 20 H, 6x $OCH_2$, 4x $NC\underline{H}_2CH_3$), 3.26 – 3.23 (m, 2 H, $NCH_2$), 3.16 – 3.11 (m, 3 H, $NCH_2$, $CH_2$ cyclooctyne), 2.81 (dd, $J$ = 14.9, 3.6 Hz, 1 H, $CH_2$ cyclooctyne), 1.84 – 1.73 (m, 4 H, 2x $CH_2$), 1.28 – 1.23 (m, 12 H, $NCH_2C\underline{H}_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 158.4 (C(O)), 158.0 (2x C aromat.), 155.6 (2x C aromat.), 152.5, 151.3, 147.6 (3x C aromat.), 142.4 (C aromat.), 133.6 (C aromat.), 133.3 (2x C aromat.), 130.1 (2x C aromat.), 130.0 (C aromat.), 128.1 (2x C aromat.), 127.5 (C aromat.), 127.0 (2x C aromat.), 126.1, 125.90, 124.1, 123.9, 121.2 (5x C aromat.), 114.4 (2x C aromat.), 113.7 (2x C aromat.), 112.8 (C aromat.), 110.1 (2x C Alkin), 95.7 (2x C aromat.), 76.5 (CHOR cyclooctyne), 70.7, 70.6, 70.24, 70.21, 69.5, 69.2 (6x $OCH_2$), 46.3 ($CH_2$ cyclooctyne), 45.9 (4x $N\underline{C}H_2CH_3$), 41.4 ($NHCH_2$), 38.8 ($NHCH_2$), 29.5, 29.3 (2x $CH_2$), 12.6 (4x $NCH_2\underline{C}H_3$).

**MALDI-MS:** calculated $[M+H]^+ = 1007.4$, $[M+Na]^+ = 1029.4$
found $[M+H]^+ = 1007.3$, $[M+Na]^+ = 1029.3$

**Synthesis of Azidolactosylceramides 47, 48, and 49**

***N*-((2*S*,3*R*,*E*)-3-Hydroxy-1-(trityloxy)octadec-4-en-2-yl)octanamide (20)**




$C_{45}H_{65}NO_3$
668,00 g/mol

**20** was prepared as described for **19**, starting from 100 mg (0.24 mmol) ceramide **16**. After purification by silica chromatography (eluent petroleum ether/ethyl acetate 3:1) **20** was obtained as a colorless solid (76.2 mg, 0.114 mmol, 49 %).

**TLC:** $R_f$ = 0.19 (eluent petroleum ether/ethyl acetate 3:1)

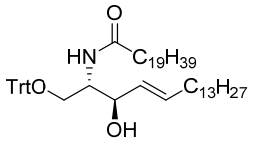
**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.40 – 7.37 (m, 6 H, aromat.), 7.31 – 7.20 (m, 9 H, aromat.), 6.03 (d, $J$ = 7.9 Hz, 1 H, NH), 5.66 – 5.59 (m, 1 H, CH=C<u>H</u>$CH_2$), 5.28 – 5.22 (m, 1 H, C<u>H</u>=$CHCH_2$), 4.18 – 4.15 (m, 1 H, C<u>H</u>OH), 4.07 – 4.01 (m, 1 H, C<u>H</u>NH), 3.37 (dd, $J$ = 9.7, 3.8 Hz, 1 H, $CH_2$OTrt), 3.29 (dd, $J$ = 9.7, 4.1 Hz, 1 H, $CH_2$OTrt), 2.22 – 2.15 (m, 2 H, C(O)$CH_2$), 1.95 – 1.85 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.67 – 1.57 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.34 – 1.19 (m, 30 H, 15x $CH_2$), 0.87 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$ ).

**$^{13}$C NMR** (101 MHz, $CDCl_3$) $\delta$ 173.5 (C(O)), 143.5 (C aromat.), 133.6 (CH=<u>C</u>H$CH_2$), 128.6 (<u>C</u>H=$CHCH_2$), 128.2, 128.08, 128.05, 127.5, 127.4, (5x C aromat.), 87.5 ($CPh_3$), 74.5 (COH), 63.2 (COTrt), 53.6 (CNH), 37.0 (C(O)<u>C</u>$H_2$), 32.1 (CH=CH<u>C</u>$H_2$), 31.8 ($CH_2$), 29.8 – 29.2 (m, $CH_2$), 22.8 ($CH_2$), 21.2 (C(O)$CH_2$<u>C</u>$H_2$), 14.24, 14.19 (2x $CH_3$).

**ESI-MS:** calculated $[M+H]^+$ = 668.5, $[M+Na]^+$ = 690.5

found $[M+H]^+$ = 668.7, $[M+Na]^+$ = 690.7

***N*-[(1*S*,2*R*,3*E*)-2-Hydroxy-1-[(triphenylmethoxy)methyl]-3-heptadecen-1-yl]-eicosanamide (22)**




$C_{57}H_{89}NO_3$
836,32 g/mol

**22** was synthesized as described for **19**, starting from 200 mg (0.34 mmol) ceramide **18**. After purification with silica chromatography (eluent petroleum ether/ethyl acetate 3:1) 273 mg **22** (0.33 mmol, quant.) was obtained as a colorless solid.

**TLC:** $R_f$ = 0.20 (eluent petroleum ether/ethyl acetate 3:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 7.39 – 7.36 (m, 6 H, aromat.), 7.30 – 7.20 (m, 9 H, aromat.), 6.06 (d, $J$ = 8.0 Hz, 1 H, NH), 5.65 – 5.58 (m, 1 H, CH=C<u>H</u>$CH_2$), 5.27 – 5.21 (m, 1 H, C<u>H</u>=$CHCH_2$), 4.18 – 4.14 (m, 1 H, C<u>H</u>OH), 4.06 – 4.01 (m, 1 H, C<u>H</u>NH), 3.36 (dd, $J$ =

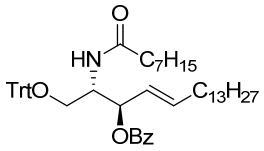
9.7, 3.8 Hz, 1 H, $CH_2OTrt$), 3.28 (dd, $J$ = 9.7, 4.1 Hz, 1 H, $CH_2OTrt$), 2.20 – 2.14 (m, 2 H, $C(O)CH_2$), 1.94 – 1.86 (m, 2 H, $CH=CHC\underline{H}_2$), 1.65 – 1.58 (m, 2 H, $C(O)CH_2C\underline{H}_2$), 1.34 – 1.17 (m, 54 H, 27x $CH_2$), 0.86 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 173.5 (C(O)), 143.5 (C aromat.), 133.5 ($CH=\underline{C}HCH_2$), 128.6 ($\underline{C}H=CHCH_2$), 128.12, 128.06, 128.00, 127.4, 127.3 (5x C aromat.), 87.5 ($CPh_3$), 74.4 (COH), 63.2 ($CH_2OTrt$), 53.5 (CHNH), 37.0 ($C(O)\underline{C}H_2$), 32.1 ($CH=CH\underline{C}H_2$), 29.8 – 29.2, 26.0, 22.9, 21.1 ($CH_2$), 14.3, 14.2 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+$ = 858.7, $[M-H]^-$ = 834.7

found $[M+Na]^+$ = 858.3, $[M-H]^-$ = 834.2

**(2*S*,3*R*,*E*)-2-Octanamido-1-(trityloxy)octadec-4-en-3-yl benzoate (24)**




$C_{52}H_{69}NO_4$
772,11 g/mol

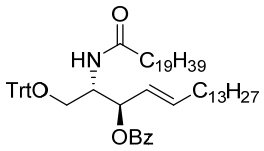
**24** was prepared as described for **23**, starting from 76 mg (0.114 mmol) ceramide **20**. After purification by silica chromatography (eluent petroleum ether/ethyl acetate 7:1) **24** was obtained as a colorless solid (48 mg, 0.063 mmol, 55 %).

**TLC:** $R_f$ = 0.44 (eluent petroleum ether/ethyl acetate 4:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.14 – 8.12 (m, 2 H, aromat.), 7.92 – 7.90 (m, 2 H, aromat.), 7.66 – 7.62 (m, 1 H, aromat.), 7.57 – 7.12 (m, 15 H, aromat.), 5.85 (dt, $J$ = 13.6, 6.8 Hz, 1 H, $CH=C\underline{H}CH_2$), 5.70 – 5.63 (m, 2 H, NH, CHOBz), 5.42 (dd, $J$ = 15.4, 7.5 Hz, 1 H, $C\underline{H}=CHCH_2$), 4.49 – 4.43 (m, 1 H, $C\underline{H}NH$), 3.42 (dd, $J$ = 9.5, 3.6 Hz, 1 H, $CH_2OTrt$), 3.17 (dd, $J$ = 9.5, 4.1 Hz, 1 H, $CH_2OTrt$), 2.07 (t, $J$ = 7.6 Hz, 2 H, $C(O)CH_2$), 2.01 – 1.94 (m, 2 H, $CH=CHC\underline{H}_2$), 1.60 – 1.51 (m, 2 H, $C(O)CH_2C\underline{H}_2$), 1.33 – 1.14 (m, 30 H, 15x $CH_2$), 0.87 – 0.83 (m, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 172.6, 165.5 (2x C(O)), 147.0, 143.6 (2x C aromat.), 137.3 ($CH=\underline{C}HCH_2$), 134.7, 133.0, 130.7, 129.0, 128.7, 128.4, 128.06, 128.03 , 128.0, 127.4 (10x C aromat.), 125.2 ($\underline{C}H=CHCH_2$), 87.0 ($CPh_3$), 74.5 (COBz), 61.8 (COTrt), 51.2 (CNH), 37.1 ($C(O)\underline{C}H_2$), 32.5 ($CH=CH\underline{C}H_2$), 32.1, 31.8, 29.8 – 29.0 (m), 25.9, 22.8, 22.8 ($CH_2$), 14.24, 14.19 (2x $CH_3$).

***N*-[(1*S*,2*R*,3*E*)-2-(Benzoyloxy)-1-[(triphenylmethoxy)methyl]-3-heptadecen-1-yl]-eicosanamide (26)**




$C_{64}H_{93}NO_4$
940,45 g/mol

**26** was synthesized as described for **24**, starting from 270 mg (0.33 mmol) ceramide **22**. After purification with silica chromatography (eluent petroleum ether/ethyl acetate 7:1) 144 mg **26** (0.153 mmol, 46 %) was obtained as colorless solid.

**TLC:** $R_f$ = 0.43 (eluent petroleum ether/ethyl acetate 4:1)

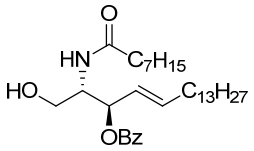
**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.15 – 8.12 (m, 2 H, aromat.), 7.93 – 7.90 (m, 2 H, aromat.), 7.67 – 7.62 (m, 2 H, aromat.), 7.55 – 7.48 (m, 6 H, aromat.) 7.40 – 7.12 (m, 8 H, aromat.), 5.89 – 5.82 (dt, $J$ = 15.8, 6.8 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.70 – 5.63 (m, 2 H, CHOBz, NH), 5.46 – 5.40 (m, 1 H, C<u>H</u>=$CHCH_2$), 4.50 – 4.43 (m, 1 H, C<u>H</u>NH), 3.42 (dd, $J$ = 9.5, 3.6 Hz, 1 H, $CH_2$OTrt), 3.17 (dd, $J$ = 9.5, 4.2 Hz, 1 H, $CH_2$OTrt), 2.07 (t, $J$ = 7.6 Hz, 2 H, C(O)$CH_2$), 2.00 – 1.94 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.59 – 1.51 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.36 – 1.16 (m, 54 H, 27x $CH_2$), 0.86 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 172.6, 162.5 (2x C(O)), 147.0, 143.6 (2x C aromat.), 137.3 (CH=<u>C</u>$HCH_2$), 134.7, 130.7, 129.9, 129.0, 128.7, 128.1, 128.04, 127.98, 127.4, 127.2 (10x C aromat.), 125.3 (<u>C</u>H=$CHCH_2$), 87.0 ($CPh_3$), 74.6 (COBz), 61.8 (COTrt), 51.2 (CNH), 37.1 (C(O)<u>C</u>$H_2$), 32.5 (CH=CH<u>C</u>$H_2$), 32.1, 29.9 – 29.4 (m), 29.1, 26.0, 22.8 ($CH_2$), 14.3 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+ = 962.7$

found $[M+Na]^+ = 962.1$

**(2*S*,3*R*,*E*)-2-Octanamido-1-hydroxyoctadec-4-en-3-yl benzoate (28)**




$C_{33}H_{55}NO_4$
529,79 g/mol

**28** was prepared as described for **27**, starting from 118 mg (0.153 mmol) ceramide **24**. After purification by silica chromatography (eluent petroleum ether/ethyl acetate 1:1, then 2:3) **28** was obtained as a colorless solid (64 mg, 0.120 mmol, 78 %).

**TLC:** $R_f$ = 0.18 (eluent petroleum ether/ethyl acetate 1:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.05 – 8.02 (m, 2 H, aromat.), 7.61 – 7.57 (m, 1 H, aromat.), 7.47 – 7.44 (m, 2 H, aromat.), 6.07 (d, $J$ = 8.6 Hz, 1 H, NH), 5.89 – 5.82 (dt, $J$ = 14.8, 6.8 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.63 – 5.52 (m, 2 H, C<u>H</u>=$CHCH_2$, CHOBz), 4.27 (ddt, $J$ =

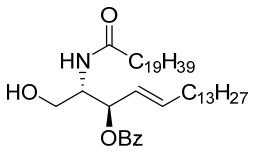
8.6, 7.0, 3.5 Hz, 1 H, CHNH), 3.75 (dd, *J* = 11.9, 3.8 Hz, 1 H, $CH_2OH$), 3.70 (dd, *J* = 12.0, 3.2 Hz, 1 H, $CH_2OH$), 2.22 – 2.17 (m, 2 H, $C(O)CH_2$), 2.07 – 2.02 (m, 2 H, $CH=CHCH_2$), 1.65 – 1.58 (m, 2 H, $C(O)CH_2CH_2$), 1.39 – 1.20 (m, 30 H, 15x $CH_2$), 0.88 (t, *J* = 6.9 Hz, 3 H, $CH_3$), 0.87 (t, *J* = 6.9 Hz, 3 H, $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 173.6, 166.7 (2x C(O)), 137.7 ($CH=CHCH_2$), 133.6 (C aromat.), 130.0 (2x C aromat.), 129.8 (C aromat.), 128.7 (2x C aromat), 125.0 ($CH=CHCH_2$), 74.9 (CHOBz), 62.1 ($CH_2OH$), 53.7 (CHNH), 37.0 ($C(O)CH_2$), 32.5 ($CH=CHCH_2$), 32.1, 31.8, 29.83 – 29.80 (m), 29.6, 29.5, 29.4, 29.2, 29.1, 25.9, 22.8 ($CH_2$), 14.24, 14.18 (2x $CH_3$).

**ESI-MS:** calculated $[M+H]^+ = 530.4$, $[M+Na]^+ = 552.4$

found $[M+H]^+ = 530.4$, $[M+Na]^+ = 552.4$

***N*-[(1*S*,2*R*,3*E*)-2-(Benzoyloxy)-1-(hydroxymethyl)-3-heptadecen-1-yl]-eicosanamide (30)**




$C_{45}H_{79}NO_4$
698,13 g/mol

**30** was synthesized as described for **27**, starting from 140 mg **26** (0.153 mmol). After purification with silica chromatography (eluent petroleum ether/ethyl acetate 2:1) 58 mg **30** (0.083 mmol, 54 %) was obtained as a colorless solid.

**TLC:** $R_f$ = 0.46 (eluent petroleum ether/ethyl acetate 1:1)

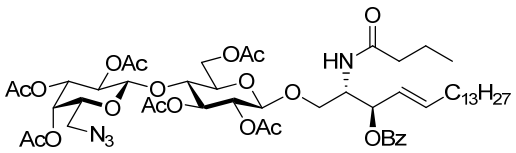
**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.03 (d, *J* = 7.5 Hz, 2 H, aromat.), 7.58 (t, *J* = 7.4 Hz, 1 H, aromat.), 7.45 (t, *J* = 7.7 Hz, 2 H, aromat.), 6.11 (d, *J* = 8.7 Hz, 1 H, NH), 5.89 – 5.82 (dt, *J* = 14.9, 6.7 Hz, 1 H, $CH=CHCH_2$), 5.63 – 5.52 (m, 2 H, $CH=CHCH_2$, CHOBz), 4.31 – 4.25 (m, 1 H, CHNH), 3.76 – 3.67 (m, 2 H, $CH_2OH$), 3.02 (b, 1 H, OH), 2.24 – 2.12 (m, 2 H, $C(O)CH_2$), 2.07 – 2.01 (m, 2 H, $CH=CHCH_2$), 1.64 – 1.57 (m, 2 H, $C(O)CH_2CH_2$), 1.40 – 1.13 (m, 54 H, 27x $CH_2$), 0.87 (t, *J* = 6.7 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 173.5, 166.6 (2x C(O)), 137.6 ($CH=CHCH_2$), 133.6 (C aromat.), 130.0 (2x C aromat.), 129.8 (C aromat.), 128.6 (2x C aromat.), 125.0 ($CH=CHCH_2$), 74.8 (COBz), 62.0 (COH), 53.6 (CNH), 37.0 ($C(O)CH_2$), 32.5 ($CH=CHCH_2$), 32.1, 29.8 – 29.5 (m), 29.4, 29.1, 26.0, 22.9 ($CH_2$), 14.3 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+ = 720.6$

found $[M+Na]^+ = 720.3$

**(2*S*,3*S*,*E*)-2-Butyramido-3-(benzoyloxy)-octadec-4-en-1-yl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (44)**




$C_{53}H_{78}N_4O_{19}$
1075,20 g/mol

Azidolactosylceramide **44** was prepared as described for compound **34**, starting from ceramide **27** (71 mg, 0.15 mmol) and trichloroacetimidate **43** (114 mg, 0.15 mmol). After silica column chromatography (eluent toluene/acetone 4:1) **44** was obtained as a colorless solid (66 mg, 62 μmol, 41 %).

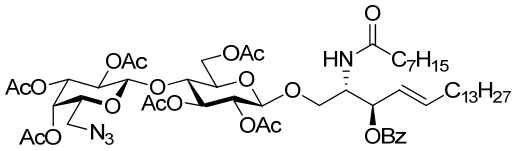
**TLC:** $R_f$ = 0.45 (eluent toluene/acetone 7:3)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.01 – 7.99 (m, 2 H, aromat.), 7.57 – 7.53 (m, 1 H, aromat.), 7.45 – 7.40 (m, 2 H, aromat.), 5.89 – 5.82 (m, 2 H, NH, CH=C<u>H</u>$CH_2$), 5.53 ('t', $J$ = 7.3 Hz, 1 H, CHOBz), 5.45 (dd, $J$ = 15.3, 7.5 Hz, 1 H, C<u>H</u>=$CHCH_2$), 5.34 – 5.31 (m, 1 H, H-4'), 5.17 ('t', $J$ = 9.3 Hz, 1 H, H-3), 5.06 (dd, $J$ = 10.3, 7.9 Hz, 1 H, H-2'), 4.95 (dd, $J$ = 10.3, 3.5 Hz, 1 H, H-3'), 4.87 (dd, $J$ = 9.3, 8.0 Hz, 1 H, H-2), 4.49 – 4.44 (m, 3 H, H-1, H-1', C<u>H</u>NH), 4.35 (dd, $J$ = 11.8, 1.6 Hz, 1 H, H-6a), 4.00 – 3.95 (m, 2 H, H-6b, $CH_2$OLac), 3.82 ('t', $J$ = 9.5 Hz, 1 H, H-4), 3.73 ('t', $J$ = 6.5 Hz, 1 H, H-5'), 3.63 (dd, $J$ = 10.1, 4.3 Hz, 1 H, $CH_2$OLac), 3.59 – 3.54 (m, 1 H, H-5), 3.44 (dd, $J$ = 12.8, 7.3 Hz, 1 H, H-6a'), 3.26 – 3.21 (m, 1 H, H-6b'), 2.16 – 2.12 (m, 5 H, C(O)$CH_3$, C(O)$CH_2$), 2.06 (s, 3 H, C(O)$CH_3$), 2.01 (s, 3 H, C(O)$CH_3$), 2.00 (s, 3 H, C(O)$CH_3$), 1.95 (s, 3 H, C(O)$CH_3$), 1.92 (s, 3 H, C(O)$CH_3$), 1.65 – 1.59 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.34 – 1.18 (m, 24 H, 12x $CH_2$), 0.92 (t, $J$ = 7.4 Hz, 3 H, $CH_3$), 0.87 (t, $J$ = 6.9 Hz, 3 H, $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 173.0 (C(O)NH), 170.5, 170.21, 170.15, 169.9, 169.8, 169.3 (6x <u>C</u>(O)$CH_3$), 165.4 (C(O)Ph), 136.0 (CH=<u>C</u>H$CH_2$), 129.7, 129.0 (2x), 128.7 (2x), 128.6 (C aromat.), 124.7 (<u>C</u>H=$CHCH_2$), 100.6 (C1), 100.5 (C1'), 75.4 (C4), 74.2 (CHOBz), 72.9 (C5), 72.6 (C3), 72.3 (C5'), 71.8 (C2), 71.0 (C3'), 69.2 (C2'), 67.6 (C4'), 67.5 ($CH_2$OLac), 62.0 (C6), 50.9 (CHNH), 50.3 (C6'), 32.4, 32.0, 29.8 – 29.7 (m), 29.7, 29.68, 29.55, 29.4, 29.0, 22.8 ($CH_2$), 21.1, 21.0, 20.8 (m), 20.7 (C(O)<u>C</u>$H_3$), 19.2 ($CH_2$), 14.2, 13.8 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+$ = 1097.5
found $[M+Na]^+$ = 1097.8

**(2*S*,3*S*,*E*)-2-Octanamido-3-(benzoyloxy)-octadec-4-en-1-yl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (45)**




$C_{57}H_{86}N_4O_{19}$
1131,31 g/mol

Azidolactosylceramide **45** was prepared as described for compound **34**, starting from ceramide **28** (63 mg, 0.12 mmol) and trichloroacetimidate **43** (100 mg, 0.13 mmol). After silica column chromatography (eluent toluene/acetone 4:1) lactosylceramide **45** was obtained as a colorless solid (91 mg, 0.08 mmol, 67 %).

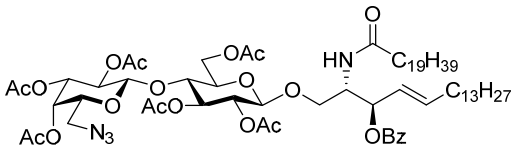
**TLC:** $R_f$ = 0.56 (eluent toluene/acetone 7:3)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.02 – 7.99 (m, 2 H, aromat.), 7.58 – 7.53 (m, 1 H, aromat.), 7.45 – 7.41 (m, 2 H, aromat.), 5.86 (dt, $J$ = 14.7, 6.7 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.74 (d, $J$ = 9.2 Hz, 1 H, NH), 5.55 – 5.51 (m, 1 H, CHOBz), 5.49 – 5.43 (m, 1 H, C<u>H</u>=$CHCH_2$), 5.34 (dd, $J$ = 3.3, 0.7 Hz, 1 H, H-4'), 5.18 ('t', $J$ = 9.3 Hz, 1 H, H-3), 5.07 (dd, $J$ = 10.3, 7.8 Hz, 1 H, H-2'), 4.95 (dd, $J$ = 10.4, 3.4 Hz, 1 H, H-3'), 4.88 (dd, $J$ = 9.4, 7.8 Hz, 1 H, H-2), 4.48 (d, $J$ = 7.8 Hz, 1 H, H-1'), 4.49 – 4.43 (m, 1 H, C<u>H</u>NH), 4.44 (d, $J$ = 7.7 Hz, 1 H, H-1), 4.38 (dd, $J$ = 11.9, 1.9 Hz, 1 H, H-6a), 4.01 – 3.95 (m, 2 H, H-6b, $CH_2$OLac), 3.82 ('t', $J$ = 9.5 Hz, 1 H, H-4), 3.73 – 3.70 (m, 1 H, H-5'), 3.63 (dd, $J$ = 10.1, 4.5 Hz, 1 H, $CH_2$OLac), 3.57 (ddd, $J$ = 9.8, 4.9, 2.0 Hz, 1 H, H-5), 3.48 – 3.43 (m, 1 H, H-6a'), 3.25 (dd, $J$ = 12.8, 5.7 Hz, 1 H, H-6b'), 2.17 – 2.11 (m, 5 H, C(O)$CH_3$, C(O)$CH_2$), 2.06 (s, 3 H, C(O)$CH_3$), 2.01 (2 s, 6 H, 2x C(O)$CH_3$), 1.96 (s, 3 H, C(O)$CH_3$), 1.95 (s, 3 H, C(O)$CH_3$), 1.63 – 1.56 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.37 – 1.20 (m, 32 H, 16x $CH_2$), 0.87 (t, $J$ = 6.9 Hz, 3 H, $CH_3$), 0.86 (t, $J$ = 6.9 Hz, 3 H, $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 172.8 (C(O)NH), 170.4, 170.2, 170.1, 169.8, 169.7, 169.2 (6x <u>C</u>(O)$CH_3$), 165.4 (C(O)Ph), 137.7 (CH=<u>C</u>H$CH_2$), 133.2, 130.4 (2x C aromat.), 129.8, 128.6 (4x C aromat.), 124.8 (<u>C</u>H=$CHCH_2$), 100.7 (C1'), 100.6 (C1), 75.5 (C4), 74.3 (CHBz), 72.9 (C5), 72.9 (C3), 72.4 (C5'), 71.9 (C2), 71.0 (C3'), 69.2 (C2'), 67.6 ($CH_2$OLac), 62.0 (C6), 50.8 (CNH), 50.3 (C6'), 37.0 (C(O)<u>C</u>$H_2$), 32.5 (CH=CH<u>C</u>$H_2$), 32.1, 31.8, 29.81 – 29.79 (m), 29.8, 29.5, 29.41, 29.37, 29.2, 29.1, 25.8, 22.8, 22.8 ($CH_2$), 21.0, 20.9, 20.75, 20.74, 20.73, 20.6 (6x C(O)<u>C</u>$H_3$), 14.24, 14.18 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+$ = 1053.6
found $[M+Na]^+$ = 1053.7

**(2*S*,3*S*,4*E*)-2-Eicosanamido-3-(benzoyloxy)-4-octadecen-1-yl-2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (46)**




$C_{69}H_{110}N_4O_{19}$
1299,65 g/mol

**46** was synthesized as described for **34**, starting from 58 mg (0.083 mmol) ceramide **30** and 76 mg (0.10 mmol) trichloroacetimidate **43**. After purification with silica chromatography 103 mg **46** (79 µmol) was obtained as colorless solid.

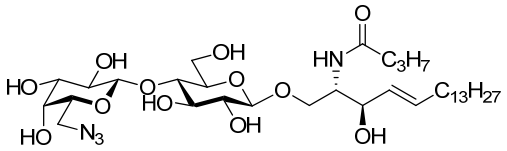
**TLC:** $R_f$ = 0.27 (eluent toluene/acetone 4:1)

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ = 8.05 – 7.00 (m, 2 H, aromat.), 7.60 – 7.53 (m, 1 H, aromat.), 7.47 – 7.41 (m, 2 H, aromat.), 5.89 – 5.82 (m, 1 H, CH=C<u>H</u>$CH_2$), 5.75 (d, $J$ = 9.2 Hz, 1 H, NH), 5.55 – 5.50 (m, 1 H, CHOBz), 5.48 – 5.42 (m, 1 H, C<u>H</u>=$CHCH_2$), 5.33 (m, 1 H, H-4'), 5.15 ('t', $J$ = 9.3 Hz, 1 H, H-3), 5.05 (dd, $J$ = 10.4, 7.9 Hz, 1 H, H-2'), 5.00 – 4.93 (m, 1 H, H-3'), 4.86 (dd, $J$ = 9.4, 7.8 Hz, 1 H, H-2), 4.48 – 4.43 (m, 3 H, H-1', C<u>H</u>NH, H-1), 4.35 (dd, $J$ = 11.9, 1.8 Hz, 1 H, H-6a), 4.00 – 3.94 (m, 2 H, $CH_2$OLac, H-6b), 3.82 ('t', $J$ = 9.5 Hz, 1 H, H-4), 3.72 – 3.69 (m, 1 H, H-5'), 3.62 (dd, $J$ = 10.1, 4.5 Hz, 1 H, $CH_2$OLac), 3.56 (ddd, $J$ = 9.8, 4.9, 1.9 Hz, 1 H, H-5), 3.45 (dd, $J$ = 12.7, 7.3 Hz, 1 H, H-6a'), 3.23 (dd, $J$ = 12.8, 5.7 Hz, 1 H, H-6b'), 2.16 – 2.14 (m, 5 H, C(O)$CH_3$, C(O)$CH_2$), 2.06 – 2.01 (m, 11 H, 3x C(O)$CH_3$, CH=CHC<u>H</u>$_2$), 1.96 – 1.95 (m, 6 H, 2x C(O)$CH_3$), 1.64 – 1.54 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.38 – 1.22 (m, 54 H, 27x $CH_2$), 0.85 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, $CDCl_3$): $\delta$ = 172.9, 170.4, 170.21, 170.15, 169.84, 169.79, 169.2, 165.4 (8x C(O)), 137.7 (CH=<u>C</u>H$CH_2$), 133.2 (C aromat.), 130.5 (C aromat.), 129.8 (2x C aromat.), 128.6 (2x C aromat.), 124.6 (<u>C</u>H=$CHCH_2$), 100.8, 100.6 (C1, C1'), 75.5 (C4), 74.3 (COBz), 72.9 (C5), 72.6 (C3), 72.4 (C5'), 71.9 (C2), 71.1 (C3'), 69.2 (C2'), 67.7 (COLac), 67.6 (C4'), 62.0 (C6), 50.8 (CNH), 50.4 (C6'), 37.0 (C(O)<u>C</u>$H_2$), 32.5 (CH=CH<u>C</u>$H_2$), 32.1, 29.9 – 29.8 (m), 29.73, 29.68, 29.6, 29.6 – 29.5 (m), 29.4, 29.1, 25.9, 22.8 ($CH_2$), 21.1, 20.9, 20.80, 20.79, 20.78, 20.7 (6x C(O)<u>C</u>$H_3$), 14.3 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+$ = 1321.8
found $[M+Na]^+$ = 1321.1

**(2*S*,3*S*,*E*)-2-Butyramido-3-hydroxy-octadec-4-en-1-yl 6-azido-6-deoxy-β-D-galac-topyranosyl-(1→4)-β-D-glucopyranoside (47)**



$C_{36}H_{64}N_4O_{13}$
719,44 g/mol

**47** was prepared as described for compound **2**. Starting from **44** (66.4 mg, 62 µmol), **47** was obtained as a colorless solid (27.6 mg, 38 µmol).

**TLC:** $R_f$ = 0.34 (eluent DCM/MeOH 4:1)

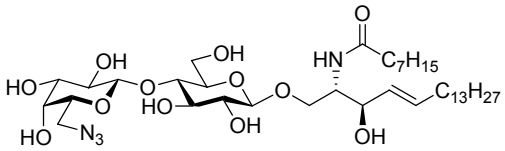
**$^1$H NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.82 (d, $J$ = 9.2 Hz, 1 H, NH), 5.70 (dt, $J$ = 13.7, 6.6 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.46 (m, 1 H, C<u>H</u>=CH$CH_2$), 4.39 (d, $J$ = 7.3 Hz, 1 H, H-1'), 4.30 (d, $J$ = 7.8 Hz, 1 H, H-1), 4.15 (dd, $J$ = 10.1, 4.8 Hz, 1 H, $CH_2$OLac), 4.11 – 4.07 (m, 1 H, sphingosine-C<u>H</u>OH), 4.04 – 3.96 (m, 1 H, C<u>H</u>NH), 3.91 (dd, $J$ = 12.1, 2.5 Hz, 1 H, H-6a), 3.87 – 3.81 (m, 1 H, H-6b), 3.79 – 3.77 (m, 1 H, H-4'), 3.73 – 3.67 (m, 1 H, H-5'), 3.62 – 3.48 (m, 7 H, $CH_2$OLac, H-3, H-4, H-2', H-3', H-6a/b'), 3.45 – 3.40 (m, 1 H, H-5), 3.30 – 3.27 (m, 1 H, H-2), 2.16 (t, $J$ = 7.4 Hz, 2 H, C(O)$CH_2$), 2.06 – 1.99 (m, 2 H (CH=CHC<u>H</u>$_2$), 1.67 – 1.56 (m, 2 H, $CH_2$), 1.43 – 1.23 (m, 22 H, 11x $CH_2$), 0.94 (t, $J$ = 7.4 Hz, 3 H, $CH_3$), 0.90 (t, $J$ = 6.9 Hz, 3 H, $CH_3$).

**$^{13}$C NMR** (101 MHz, $CD_3OD$): $\delta$ = 175.9 (C(O)), 135.0 (CH=<u>C</u>H$CH_2$), 131.2 (<u>C</u>H=CH$CH_2$), 105.0 (C1), 104.5 (C1'), 80.6, 76.4 (C5), 76.2, 74.9 (C5'), 74.8 (C2), 74.5, 73.1 (sphingosine-CHOH), 72.2, 70.4 (C4'), 69.9 ($CH_2$OLac), 61.8 (C6), 54.8 (CHNH), 52.4 (C6'), 39.2 (C(O)<u>C</u>$H_2$), 33.4 (CH=CH<u>C</u>$H_2$), 33.0, 30.78 – 30.73 (m), 30.71, 30.4, 30.34, 30.30, 23.7, 20.4 ($CH_2$), 14.4, 14.1 (2x $CH_3$).

**ESI-MS:** calculated $[M+Na]^+$ = 741.4, $[M-H]^-$ = 717.4
found $[M+Na]^+$ = 742.0, $[M-H]^-$ = 718.0

**HR-ESI-MS:** calculated $[M+H]^+$ = 719.44370
found $[M+H]^+$ = 719.44255

**(2*S*,3*S*,*E*)-2-Octanamido-3-hydroxy-octadec-4-en-1-yl 6-azido-6-deoxy-β-D-galac-topyranosyl-(1→4)-β-D-glucopyranoside (48)**



$C_{40}H_{72}N_4O_{13}$
774,98 g/mol

Deacylation of **45** (91 mg, 80 µmol) to yield **48** was carried out as described for compound **2**. After purification by silica column chromatography (eluent DCM/MeOH 9:1 to 87:13) **48** was obtained as a colorless solid (46 mg, 59 µmol, 74 %).

**TLC:** $R_f$ = 0.44 (eluent DCM/MeOH 4:1)

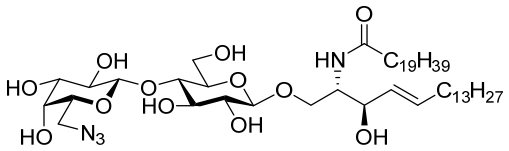
**$^1$H NMR** (400 MHz, $CD_3OD$): $\delta$ = 7.84 (d, $J$ = 9.1 Hz, 1 H, NH), 5.69 (dt, $J$ = 14.8, 6.7 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.45 (dd, $J$ = 15.3, 7.6 Hz, 1 H, C<u>H</u>=$CHCH_2$), 4.38 (d, $J$ = 7.3 Hz, 1 H, H-1'), 4.30 (d, $J$ = 7.8 Hz, 1 H, H-1), 4.17 (dd, $J$ = 10.0, 4.6 Hz, 1 H, $CH_2$OLac), 4.08 ('t', $J$ = 7.9 Hz, 1 H, sphingosine-C<u>H</u>OH), 4.01 – 3.95 (m, 1 H, C<u>H</u>NH), 3.90 (dd, $J$ = 12.0, 2.3 Hz, 1 H, H-6a), 3.84 (dd, $J$ = 12.1, 4.2 Hz, 1 H, H-6b), 3.78 (dd, $J$ = 2.8, 0.5 Hz, 1 H, H-4'), 3.72 – 3.69 (m, 1 H, H-5'), 3.62 – 3.52 (m, 6 H, H-3, H-4, H-2', H-6a/b', $CH_2$OLac), 3.50 (dd, $J$ = 9.7, 3.0 Hz, 1 H, H-3'), 3.45 – 3.40 (m, 1 H, H-5), 3.32 – 3.29 (obscured, 1 H, H-2), 2.17 (t, $J$ = 7.6 Hz, 2 H, C(O)$CH_2$), 2.06 – 2.00 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.62 – 1.55 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.42 – 1.25 (m, 30 H, 15x $CH_2$), 0.91 (t, $J$ = 6.8 Hz, 3 H, $CH_3$), 0.90 (t, $J$ = 6.8 Hz, $CH_3$).

**$^{13}$C NMR** (101 MHz, $CD_3OD$): $\delta$ = 176.0 C(O), 135.0 (CH=<u>C</u>H$CH_2$), 131.3 (<u>C</u>H=$CHCH_2$), 105.0 (C1'), 104.5 (C1), 80.6 (C4), 76.4 (C5), 76.2, 75.9 (C5'), 74.9 (C2), 74.5 (C3'), 73.0 (sphingosine-CHOH), 72.24, 70.4 (C4'), 49.9 ($CH_2$OLac), 61.8 (C6), 54.7 (CNH), 52.4 (C6'), 37.4 (C(O)<u>C</u>$H_2$), 33.4 (CH=CH<u>C</u>$H_2$), 33.4, 33.1 (2x $CH_2$), 33.0, 30.82 – 30.77 (m, $CH_2$), 30.72, 30.5, 30.41, 30.40, 30.28, 27.2, 23.75, 23.73 (8x $CH_2$), 14.47, 14.43 (2x $CH_3$).

**HR-ESI-MS:** calculated $[M+H]^+$ = 775.50630

found $[M+H]^+$ = 775.50412

**(2*S*,3*S*,4*E*)-2-Eicosanamido-3-hydroxy-4-octadecen-1-yl-6-azido-6-deoxy-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (49)**




$C_{50}H_{94}N_4O_{12}$
943,32 g/mol

Glycolipid **49** was synthesized as described for **2**, starting from 102 mg **46**. After purification with silica chromatography (eluent DCM/MeOH 8:1, then 7:1) 40 mg **49** (43 µmol, 52 % over two steps) was obtained as colorless solid.

**TLC:** $R_f$ = 0.26 (eluent DCM/MeOH 6:1)

**$^1$H NMR** (400 MHz, DMSO): $\delta$ = 7.47 (d, $J$ = 9.0 Hz, 1 H, NH), 5.53 (dt, $J$ = 15.0, 6.6 Hz, 1 H, CH=C<u>H</u>$CH_2$), 5.35 (dd, $J$ = 15.3, 7.1 Hz, 1 H, C<u>H</u>=$CHCH_2$), 5.18 – 5.13 (m, 2 H, 2-OH, 2'-OH), 4.89 – 4.85 (m, 2 H, 3'-OH, sphingosine-OH), 4.76 (d, $J$ = 4.7 Hz, 1 H, 3-OH), 4.56 (t, $J$ = 5.9 Hz, 1 H, 6-OH), 4.40 (s, 1 H, 4'-OH), 4.30 – 4.27 (m, 1 H, H-1), 4.16 (d, $J$ = 7.8 Hz, 1 H, H-1'), 3.97 (dd, $J$ = 10.0, 4.6 Hz, 1 H, $CH_2$OLac), 3.91 – 3.84 (m, 1 H, sphingosine-C<u>H</u>OH), 3.81 – 3.71 (m, 2 H, C<u>H</u>NH, H-6a), 3.66 – 3.56 (m, 3 H, H-3, H-6b, H-5'), 3.51 (dd, $J$ = 12.8, 4.2 Hz, 1 H, H-6a'), 3.46 – 3.40 (m, 2 H, H-6b', $CH_2$OLac), 3.38 – 3.28 (m, 5 H, H-2, H-4, H-5, H-3', H-4'), 3.08 – 3.01 (m, 1 H, H-2'), 2.02 (t, $J$ = 7.4 Hz, 2 H, C(O)$CH_2$), 1.97 – 1.89 (m, 2 H, CH=CHC<u>H</u>$_2$), 1.48 – 1.38 (m, 2 H, C(O)$CH_2$C<u>H</u>$_2$), 1.31 – 1.17 (m, 54 H, 27x $CH_2$), 0.85 (t, $J$ = 6.8 Hz, 6 H, 2x $CH_3$).

**$^{13}$C NMR** (101 MHz, DMSO): $\delta$ = 171.8 (C(O)), 131.4 (2x C, <u>C</u>H=<u>C</u>H$CH_2$), 103.6 (C1'), 103.3 (C1), 79.4 (C3'), 74.8 (C5), 74.34 (C4'), 73.28 (C2'), 73.1 (C5'), 70.73 (C2), 70.70 (sphingosine-COH), 70.2 (C4), 69.2 ($CH_2$OLac), 68.7 (C3), 60.2 (C6), 53.0 (CNH), 51.1 (C6'), 35.6 (C(O)<u>C</u>$H_2$), 31.8 (CH=CH<u>C</u>$H_2$), 31.31, 31.28, 29.2 – 29.0 (m), 28.8 – 28.7 (m), 25.4, 22.09 – 22.07 (m, $CH_2$), 13.9 (2x $CH_3$).

**MALDI-MS:** calculated $[M+Na]^+ = 965.7$, $[M+K]^+ = 981.7$
found $[M+Na]^+ = 965.8$, $[M+K]^+ = 981.8$

**HR-ESI-MS:** calculated $[M+H]^+ = 819.53251$
found $[M+H]^+ = 819.53168$

## Additional Structures

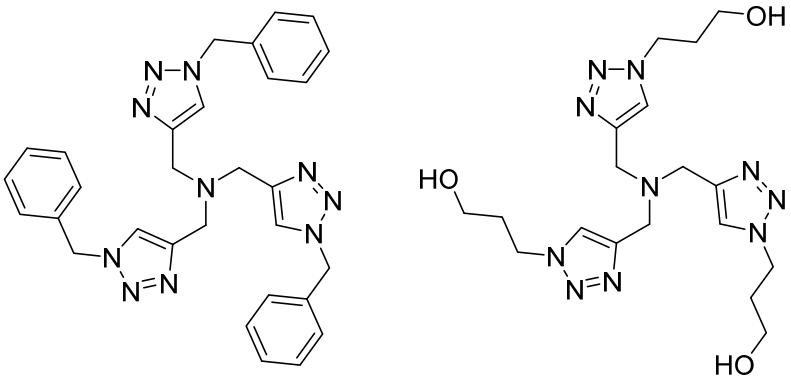



**Figure S1.** Structures of TBTA and THPTA.

## Incorporation of Azidolactosylceramides 47 and 48 at Different Temperatures

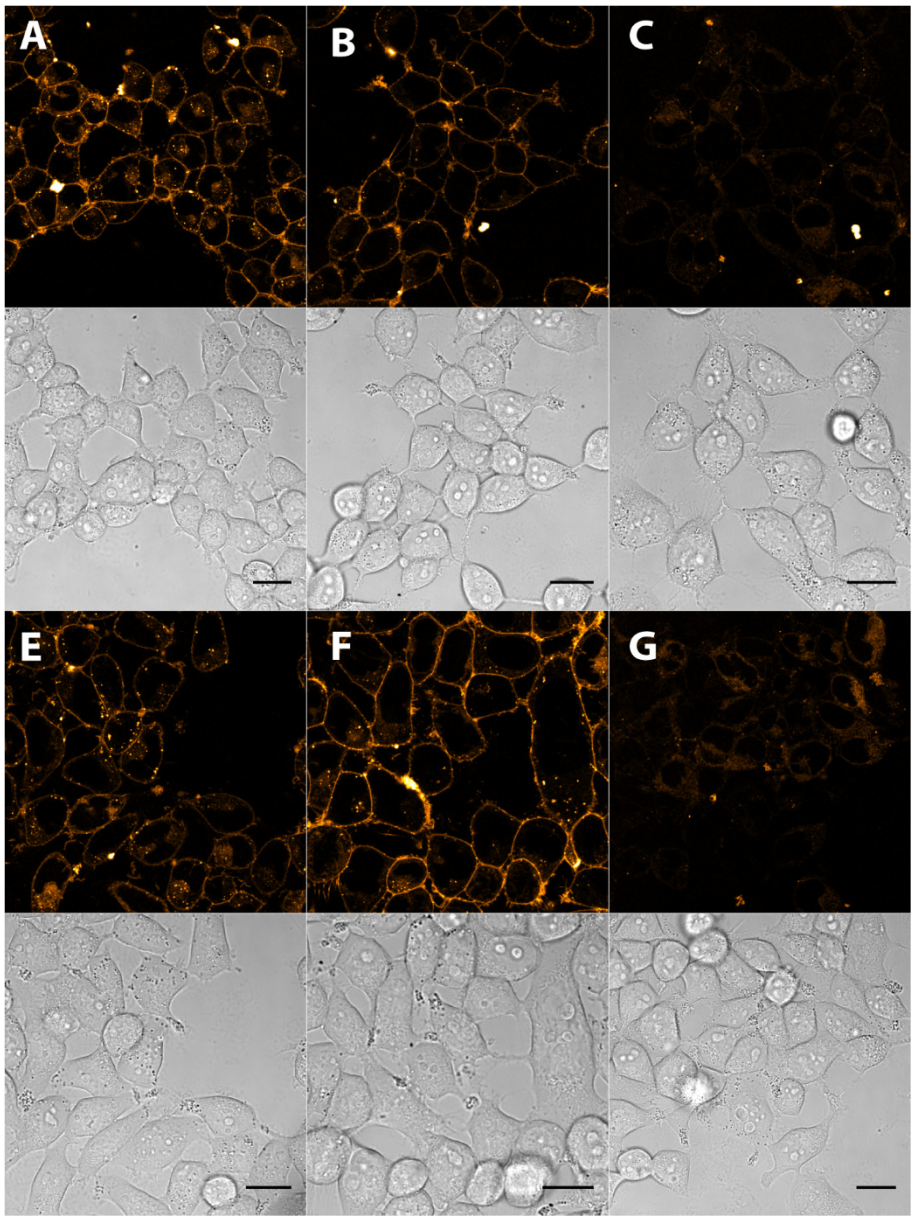



**Figure S2.** Cell experiments with azide-labeled lactosylceramides **47** and **48** at different temperatures. HEK 293T cells were treated with 10 µM **47** (A, E), 10 µM **48** (B, F) or without glycolipid (C, G) for 30 min at 4 °C (A-C) or at ambient temperature (E-G) followed by labeling with 2 µM DIBO-lissamine **6**. Scale bar: 20 µm.

## Analysis by Flow Cytometry

$10^6$ HEK 293T cells were seeded in 6-well plates that had been coated with 1 µg $mL^{-1}$ fibronectin and 10 µg $mL^{-1}$ poly-L-lysine in PBS for 1 h at 37 °C and grown for 16 h in DMEM + 10 % CS at 37 °C. Incubation with glycosphingolipids and labeling reactions were carried out as described in the experimental part of this publication. After fluorescence labeling, cells were washed twice with PBS and harvested by treatment with 1 mL trypsin/EDTA solution for 1 min at 37 °C. After addition of 1 mL DMEM + 10 % CS, cells were centrifuged for 3 min at 800 rpm and suspended in 1200 µL FACS buffer (PBS + 5 % heat inactivated fetal calf serum + 0.1 % $NaN_3$). Approximately $10^5$ cells of each sample were analyzed by flow cytometry (BD Biosciences LSRII with FACSDiva software, analysis with FlowJo software).

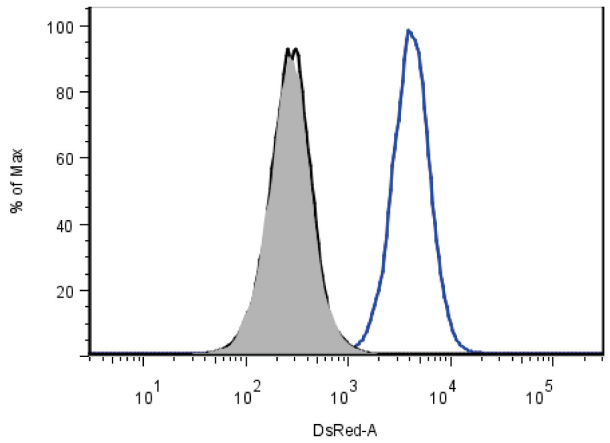



**Figure S3.** Flow cytometry with Streptavidin-AlexaFluor-647-labeled cells. Blue curve: HEK 293T cells were incubated with 10 µM **48** (30 min, 0 °C), 30 µM DIBO-biotin (**50**) (30 min, 0 °C) and streptavidin-AlexaFluor-647 (30 min, 0° C). Grey: untreated HEK 293T cells. Black: cells were treated with DIBO-biotin (**50**) and Streptavidin-AlexaFluor-647. The x-axis shows the fluorescence intensity of AlexaFluor-647, whereas the y-axis represents the relative number of cells.

## References


(1) Yang, P.-Y., Liu, K., Ngai, M. H., Lear, M. J., Wenk, M. R., and Yao, S. Q. (2010) Activity-Based Proteome Profiling of Potential Cellular Targets of Orlistat - An FDA-Approved Drug with Anti-Tumor Activities. *J. Am. Chem. Soc. 132*, 656-666.

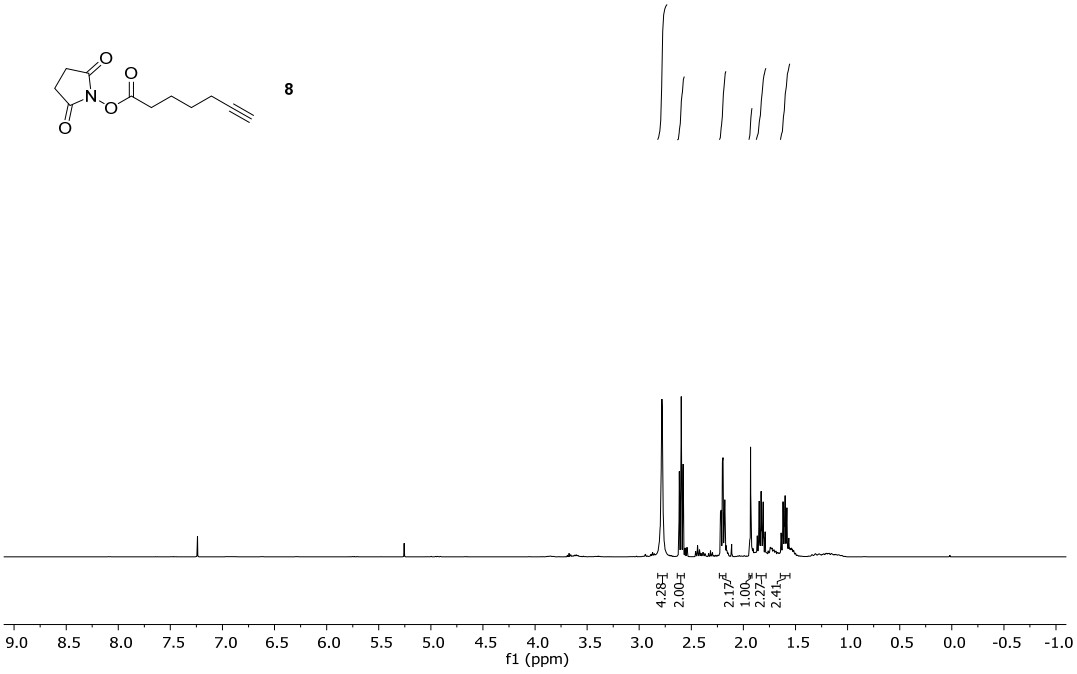
(2) Nguyen, T., and Francis, M. B. (2003) Practical Synthetic Route to Functionalized Rhodamine Dyes. *Org. Lett. 5*, 3245-3248.

(3) Loka, R. S., Sadek, C. M., Romaniuk, N. A., and Cairo, C. W. (2010) Conjugation of Synthetic N-Acetyl-Lactosamine to Azide-Containing Proteins Using the Staudinger Ligation. *Bioconjugate Chem. 21*, 1842-1849.

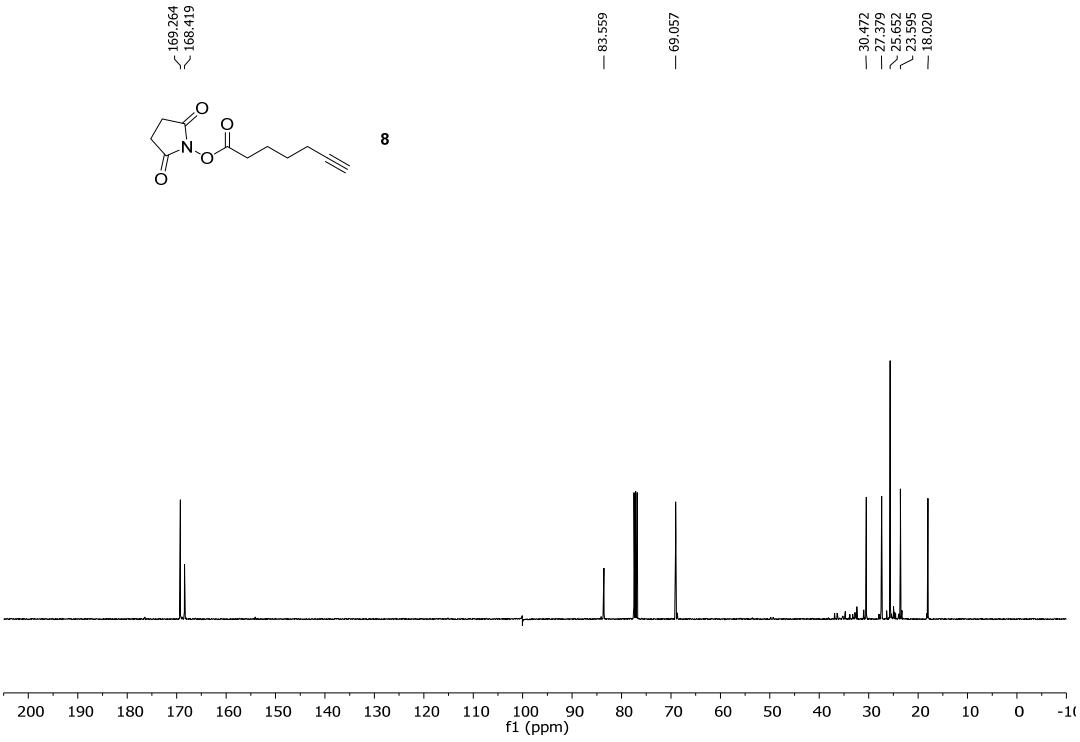
(4) Ning, X., Guo, J., Wolfert, Margreet A., and Boons, G.-J. (2008) Visualizing Metabolically Labeled Glycoconjugates of Living Cells by Copper-Free and Fast Huisgen Cycloadditions. *Angew. Chem., Int. Ed. 47*, 2253-2255.

(5) Anderson, S. (2008) Surfaces for Immobilization of N-Terminal Cysteine Derivatives via Native Chemical Ligation. *Langmuir 24*, 13962-13968.

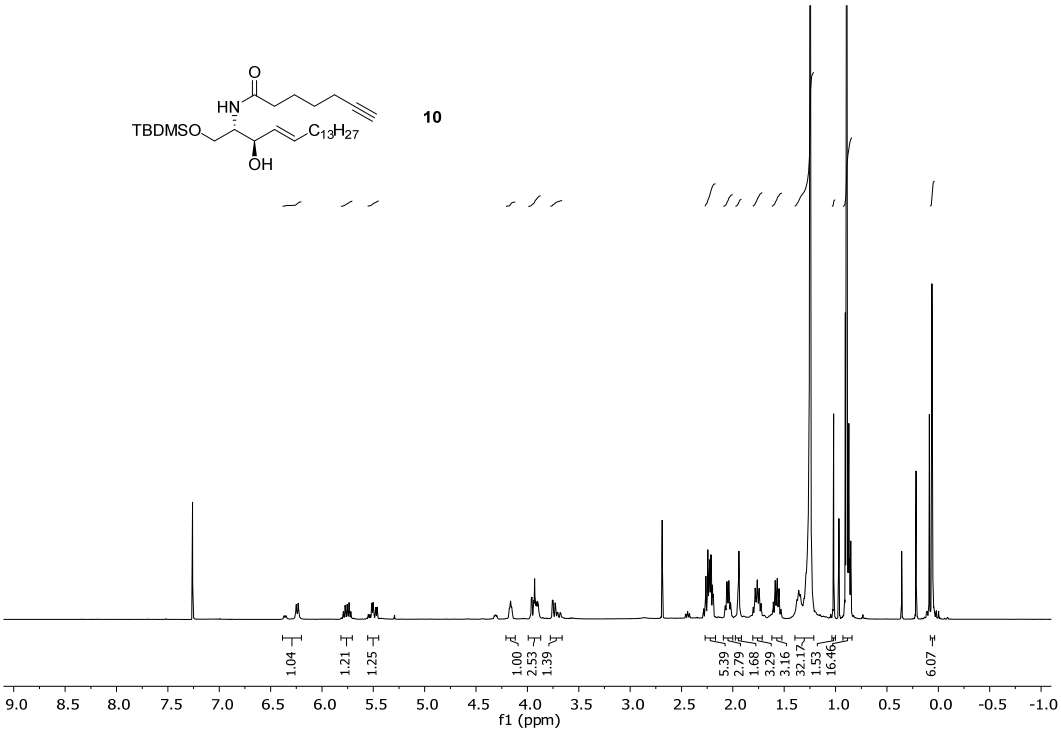
# NMR Spectra



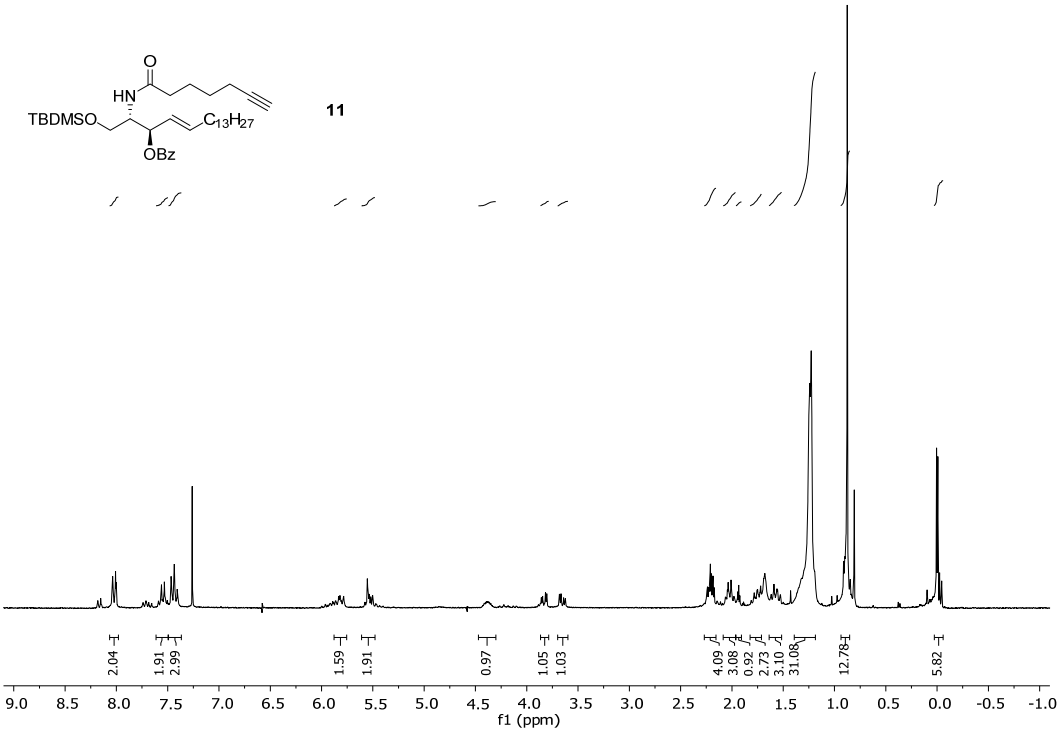

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **8**.



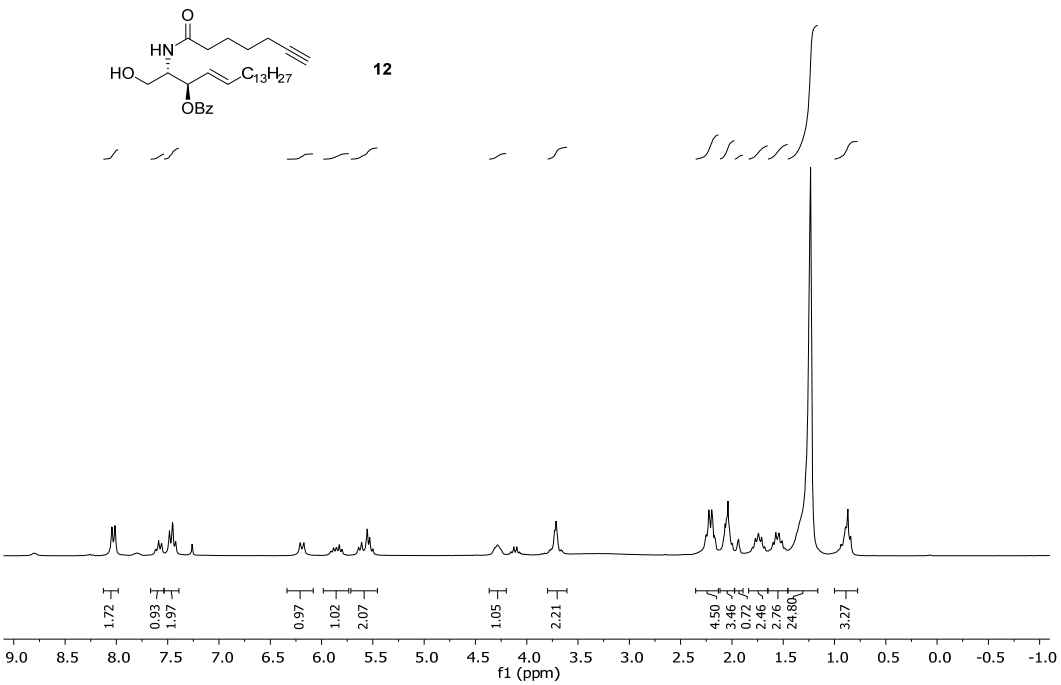

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **8**.



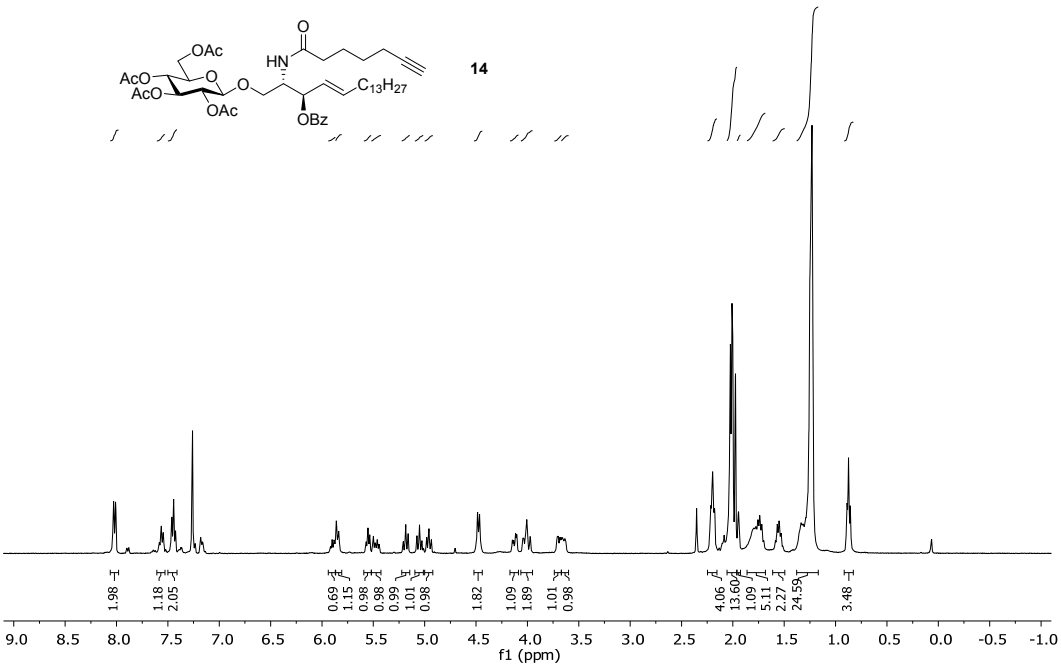

$^1$H NMR spectrum (250 MHz, $CDCl_3$) of compound **10**.



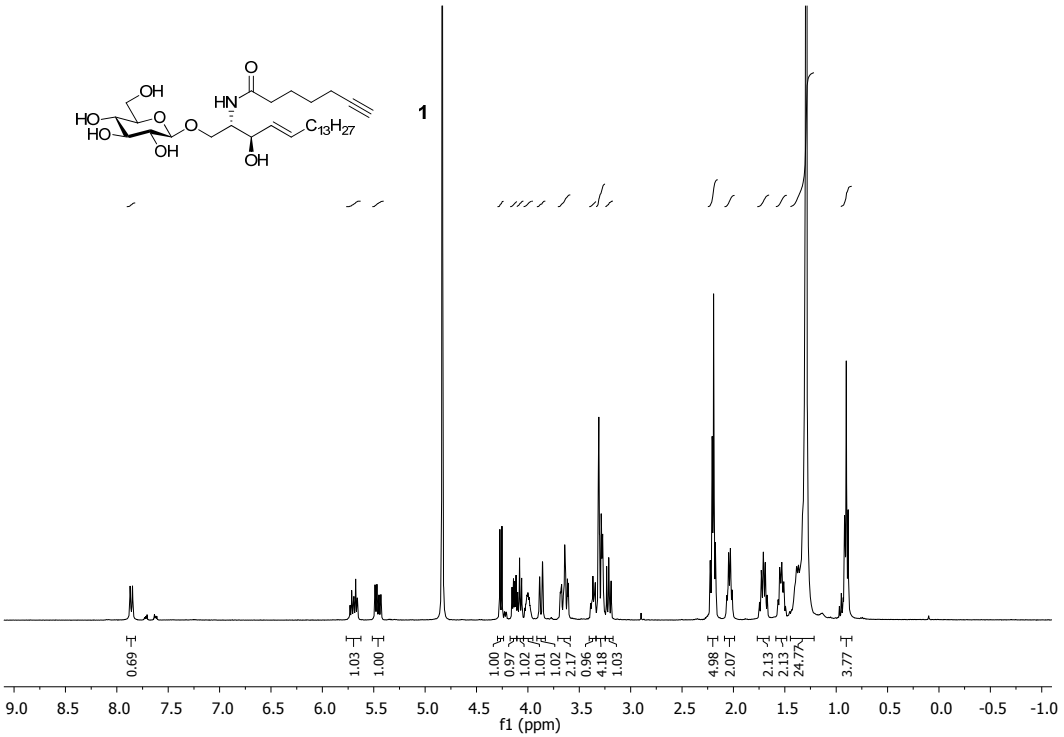

$^1$H NMR spectrum (250 MHz, $CDCl_3$) of compound **11**.



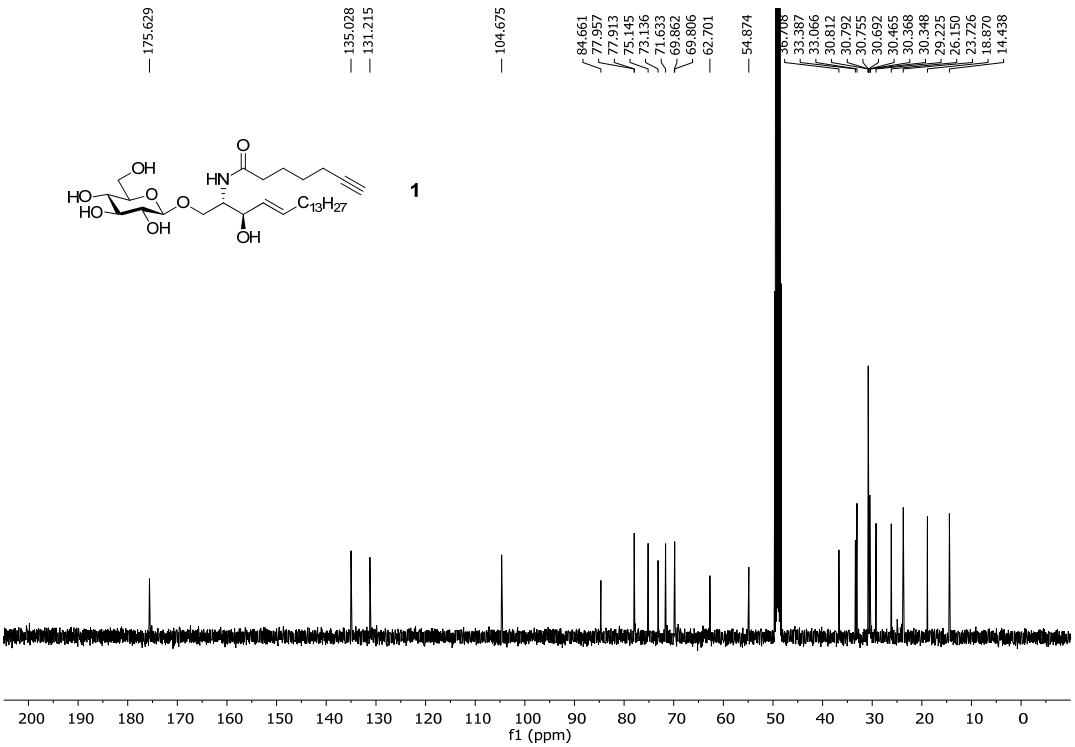

$^1$H NMR spectrum (250 MHz, $CDCl_3$) of compound **12**.



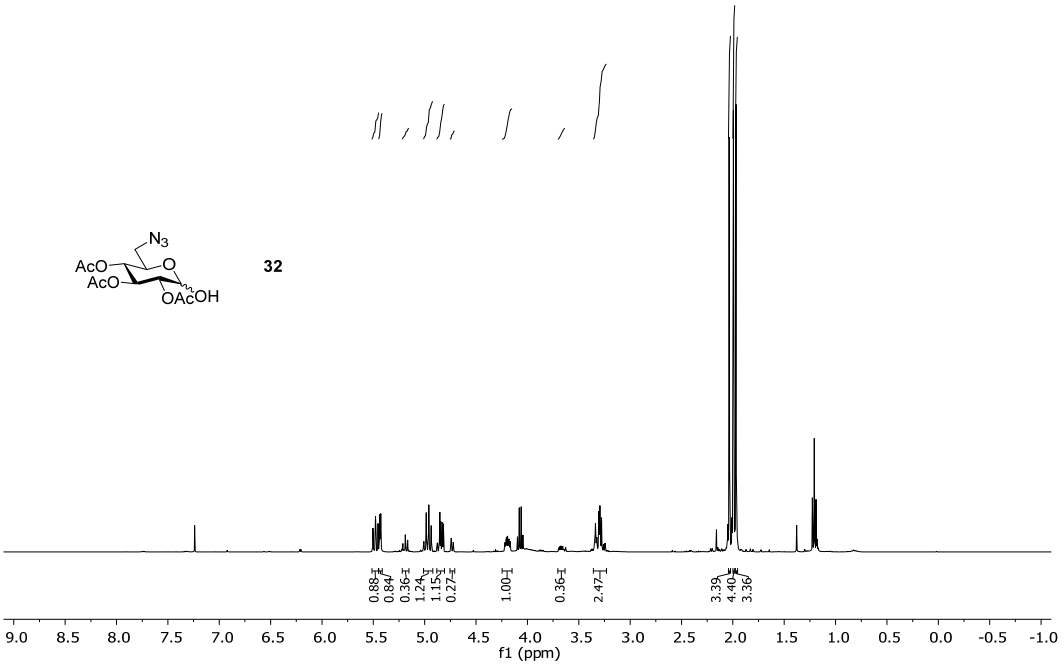

$^1$H NMR spectrum (250 MHz, $CDCl_3$) of compound **14**.



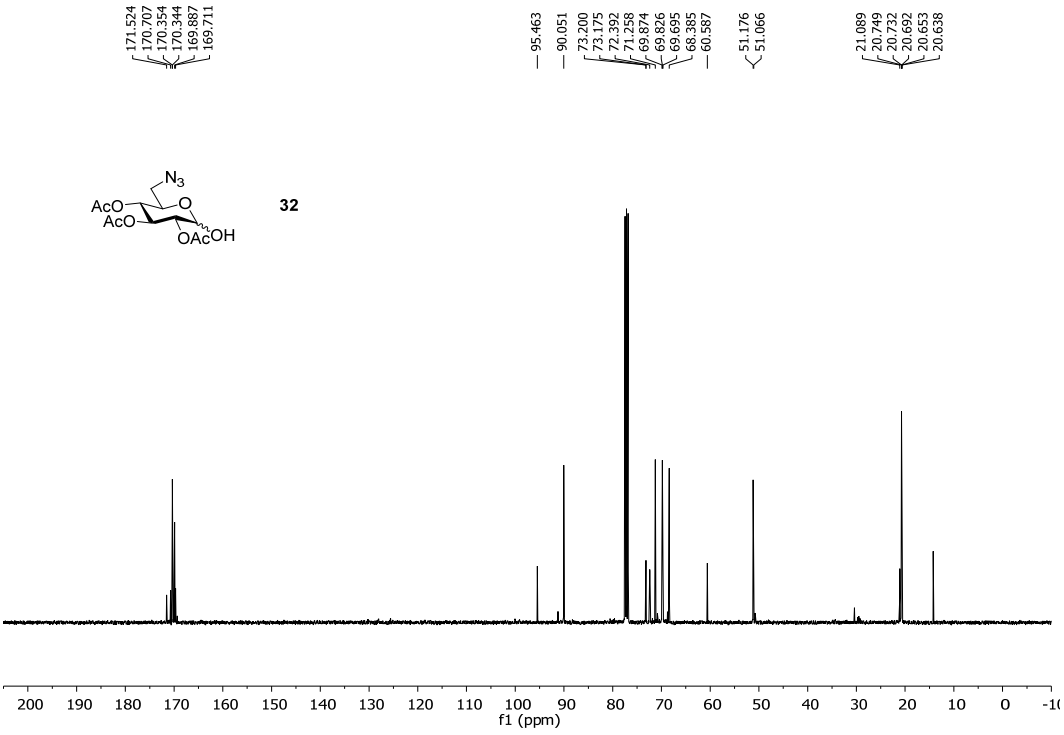

$^{1}$H NMR spectrum (400 MHz, $CD_3OD$) of compound **1**.



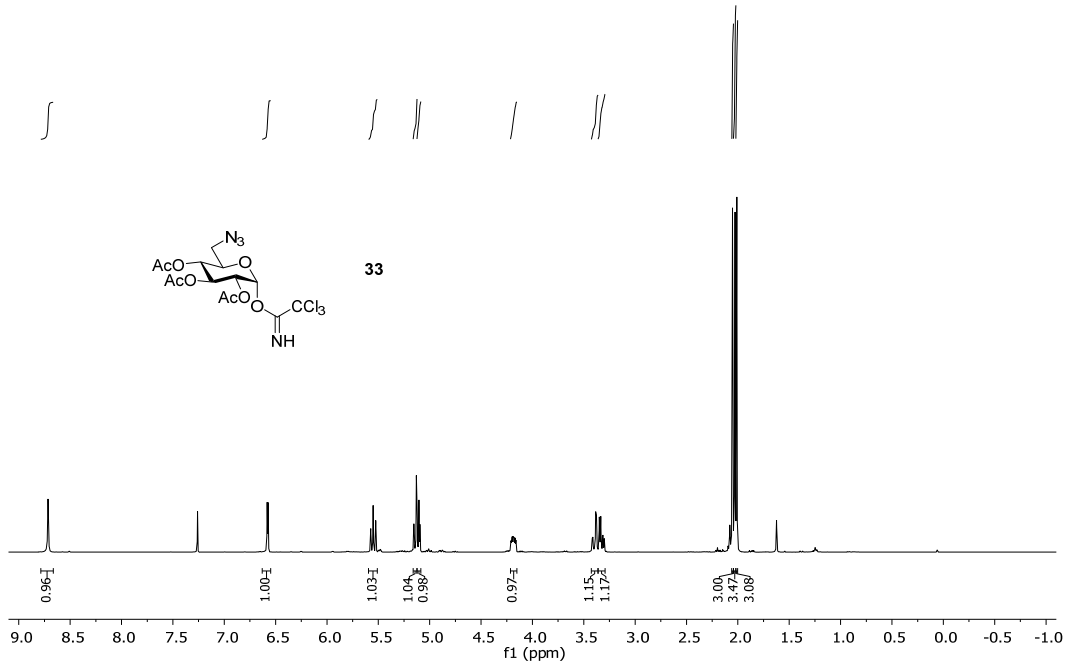

$^{13}$C NMR spectrum (100.6 MHz, $CD_3OD$) of compound **1**.



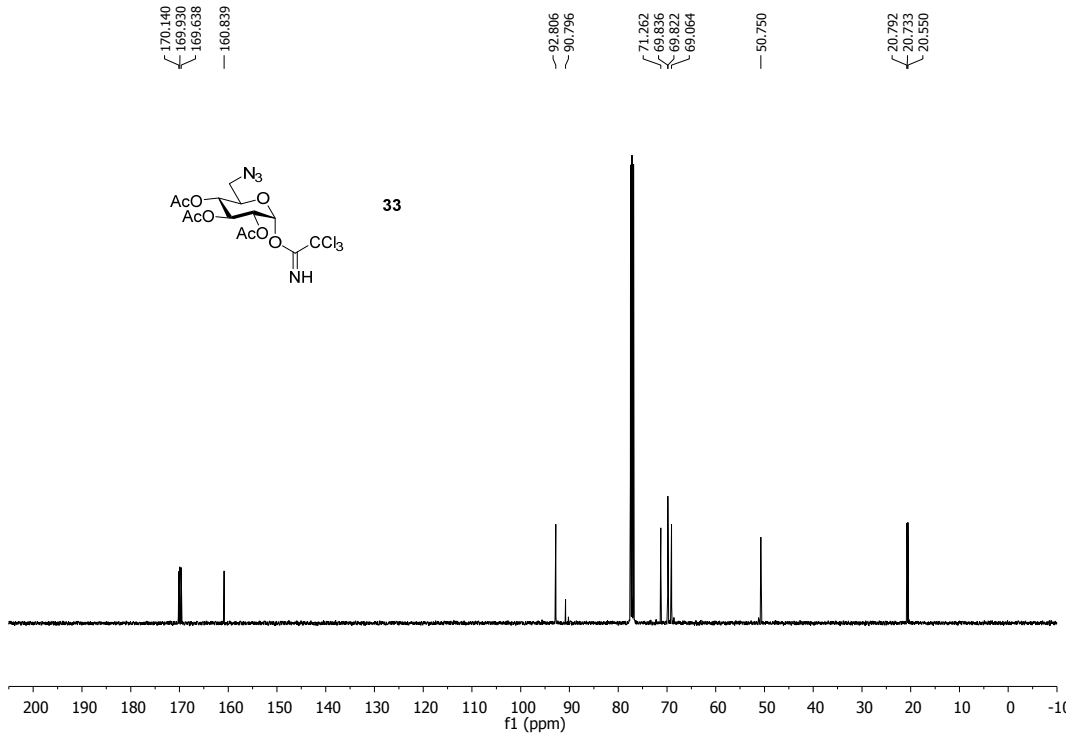

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **32**.



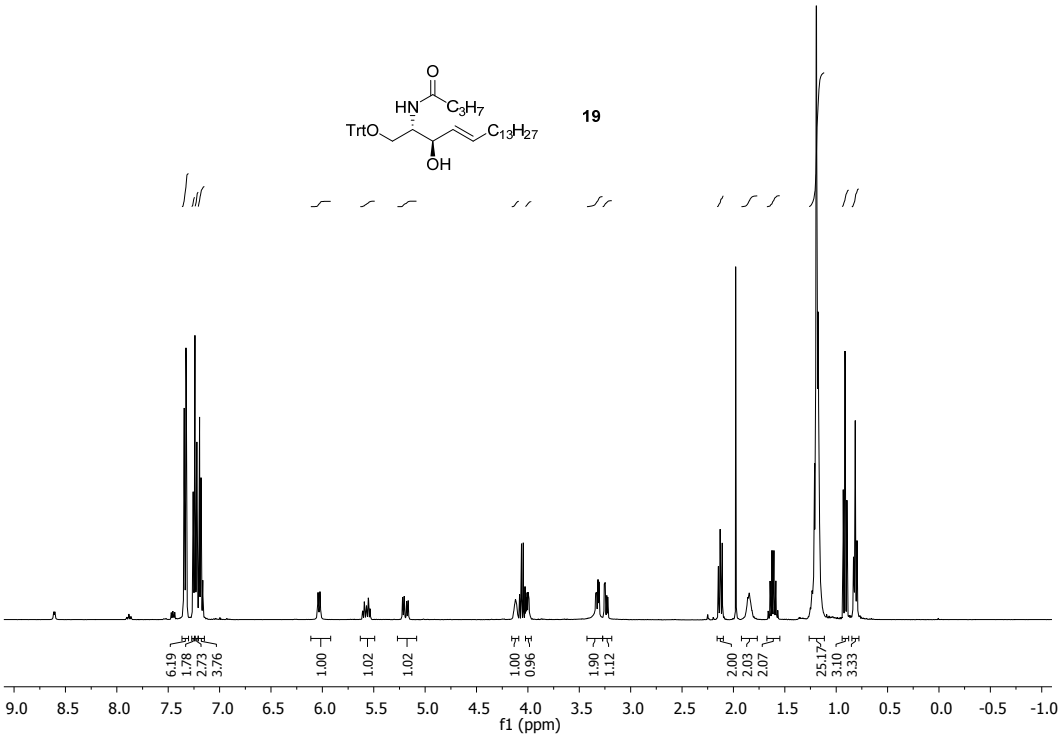

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **32**.



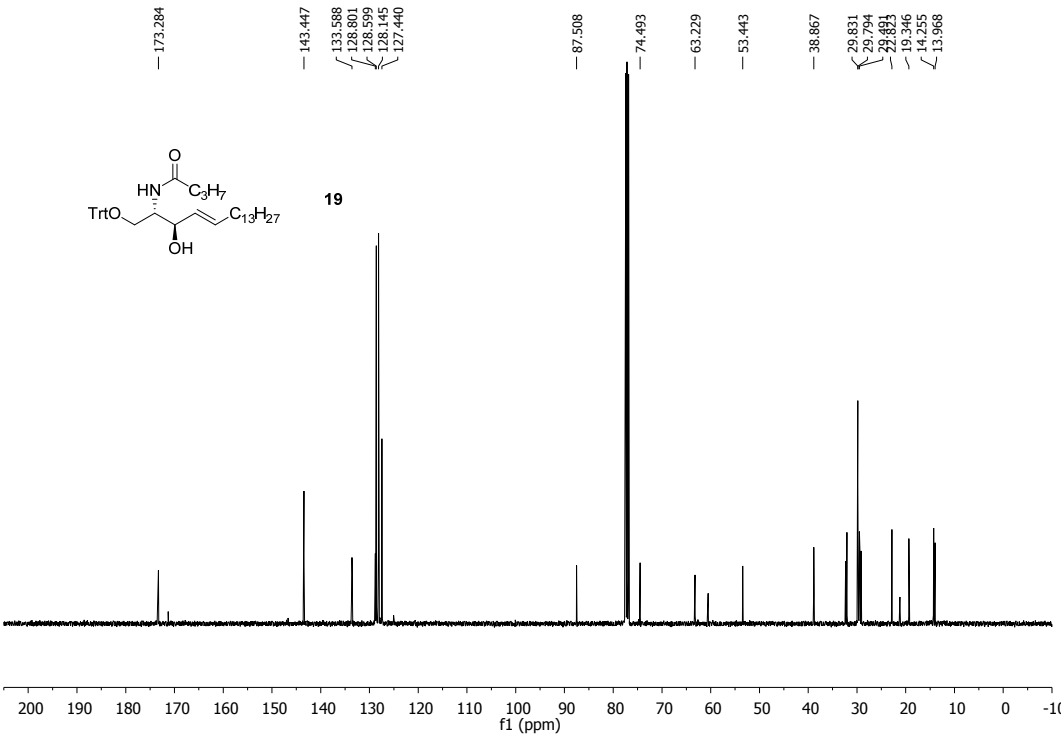

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **33**.



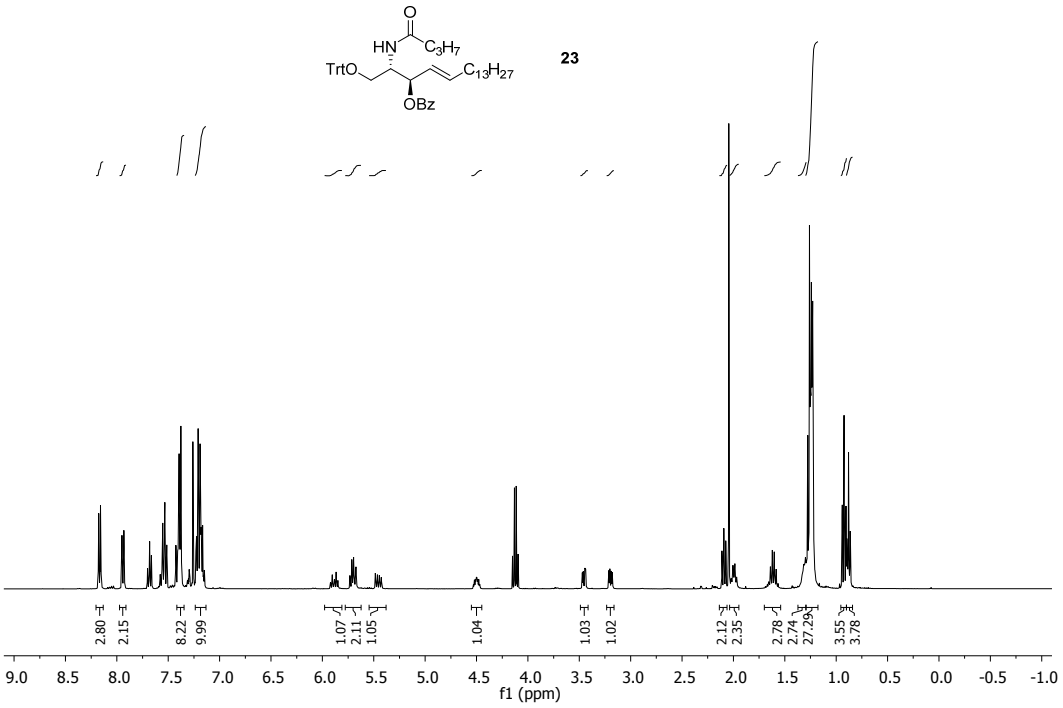

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **33**.



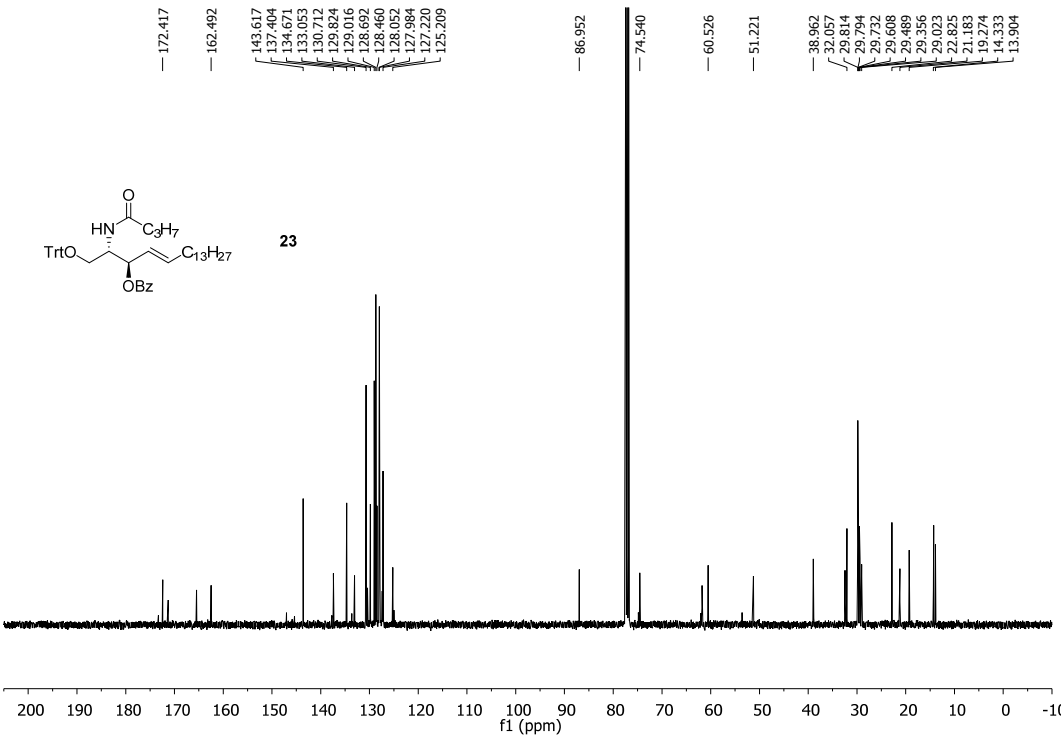

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **19** (containing traces of pyridine and ethyl acetate).



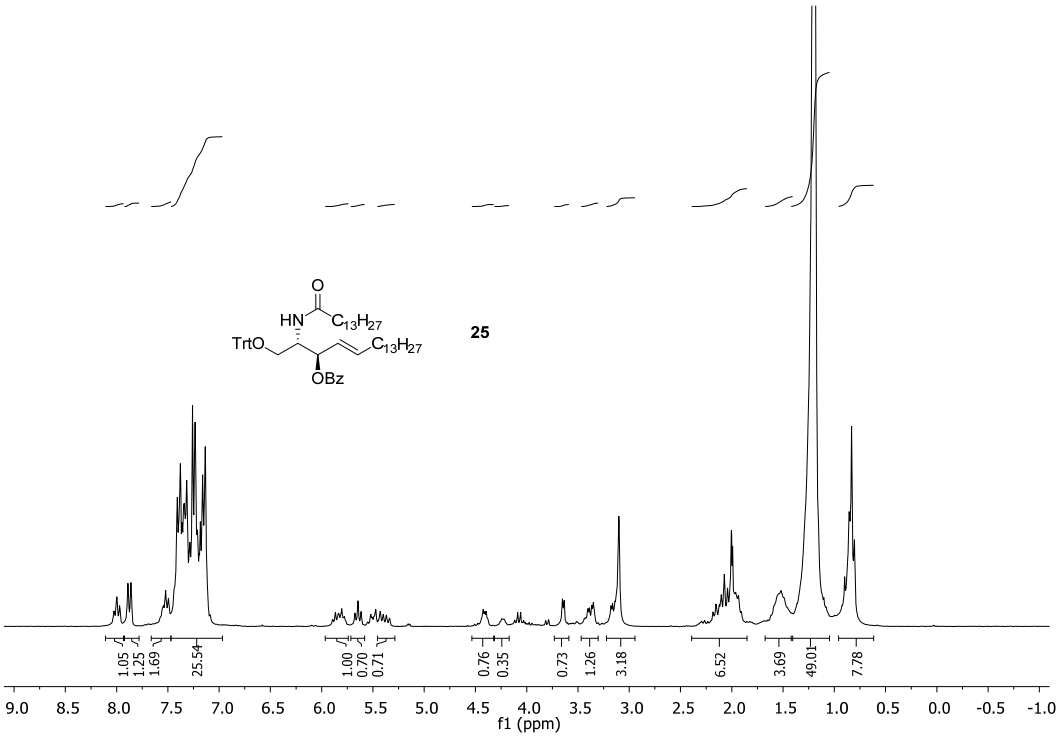

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **19** (containing traces of pyridine and ethyl acetate).



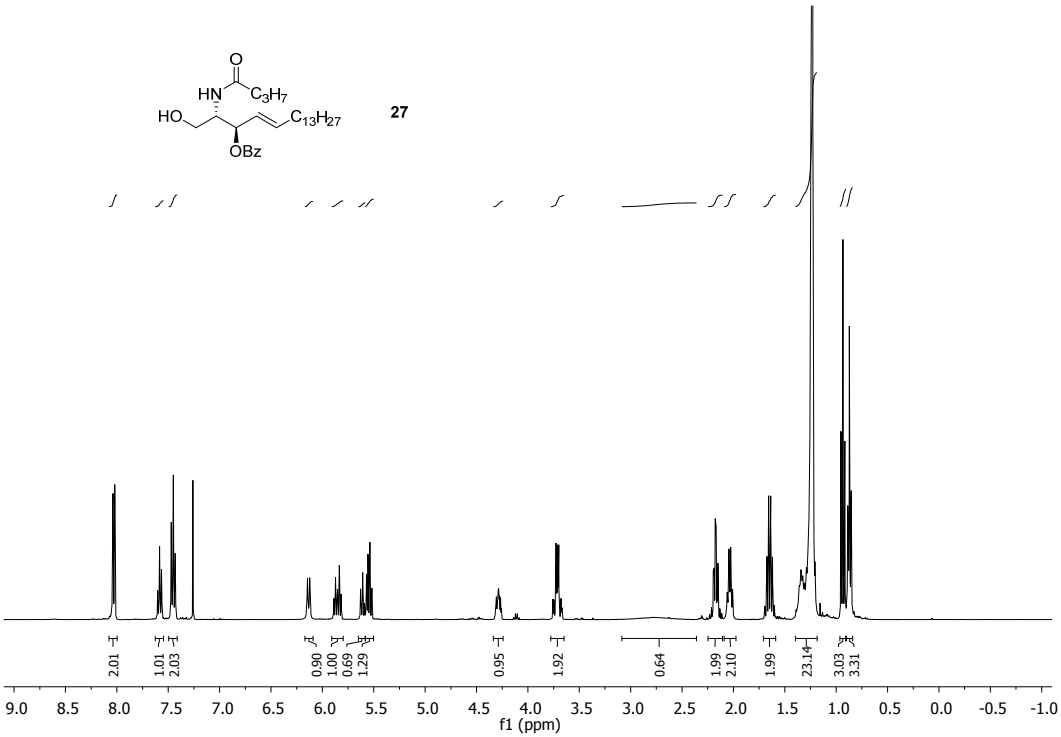

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **23** (containing residual ethyl acetate).



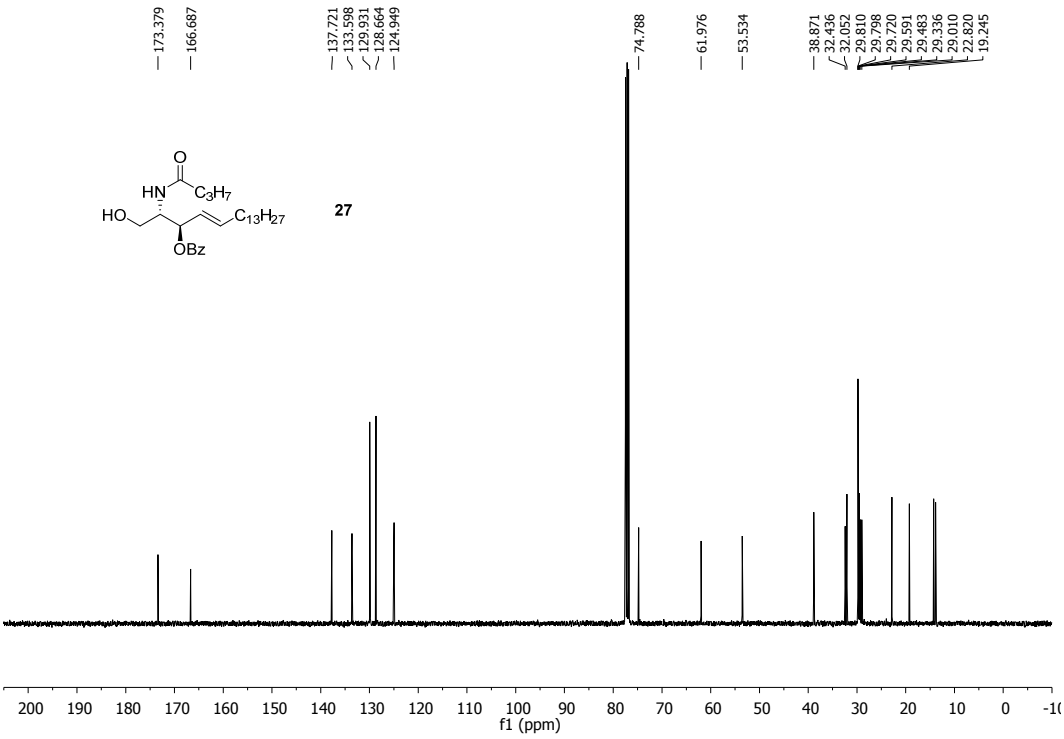

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **23** (containing residual ethyl acetate).



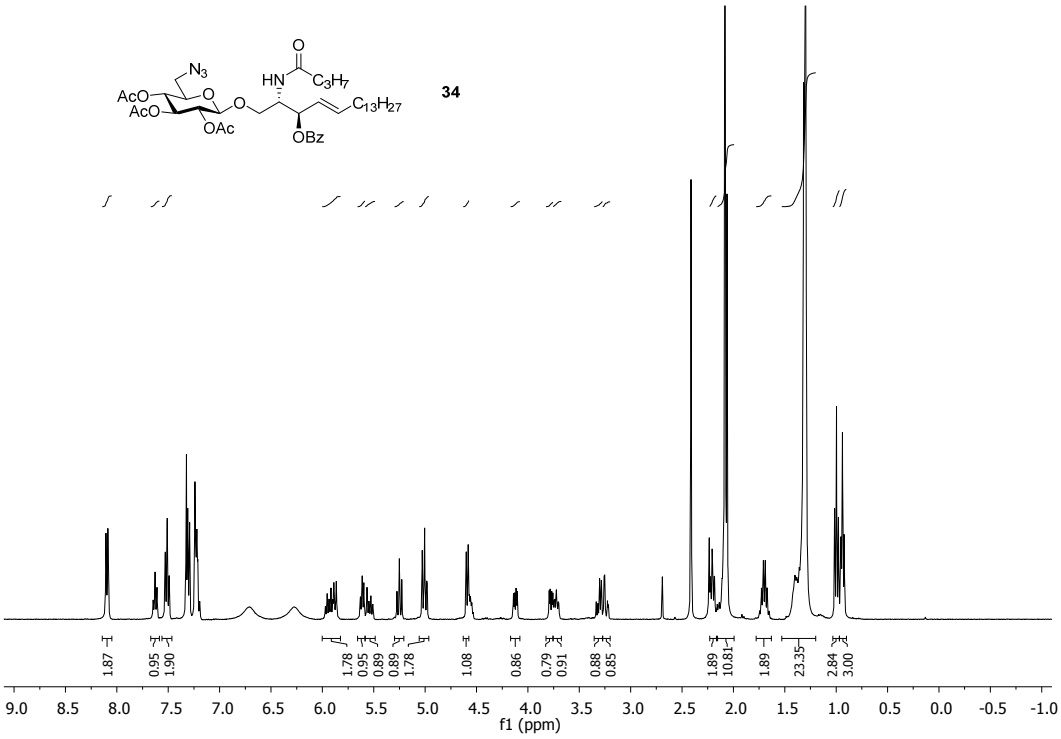

$^1$H NMR spectrum (400 MHz, $CDCl_3$) of compound **25**.



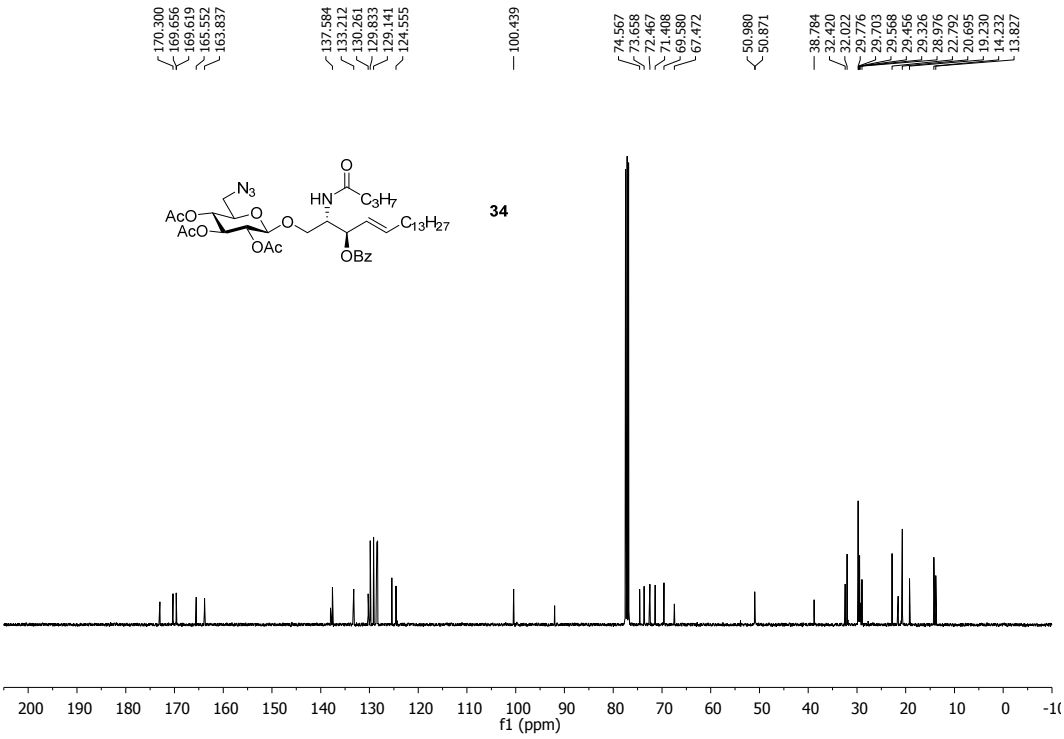

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **27.**



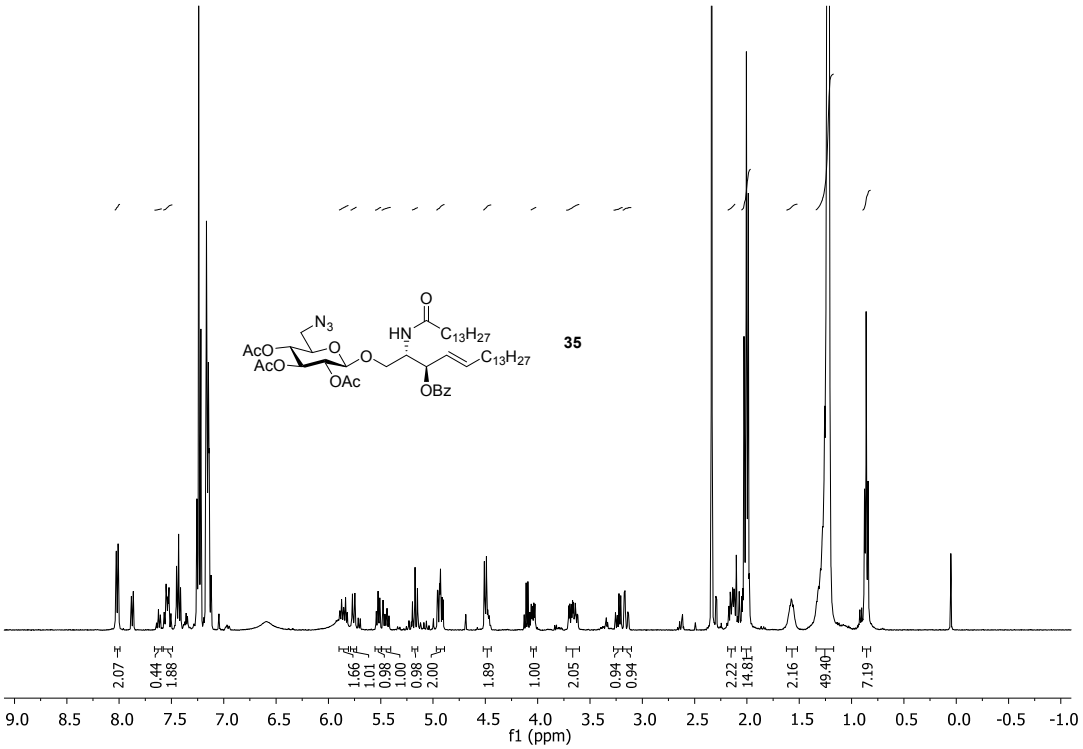

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **27**.



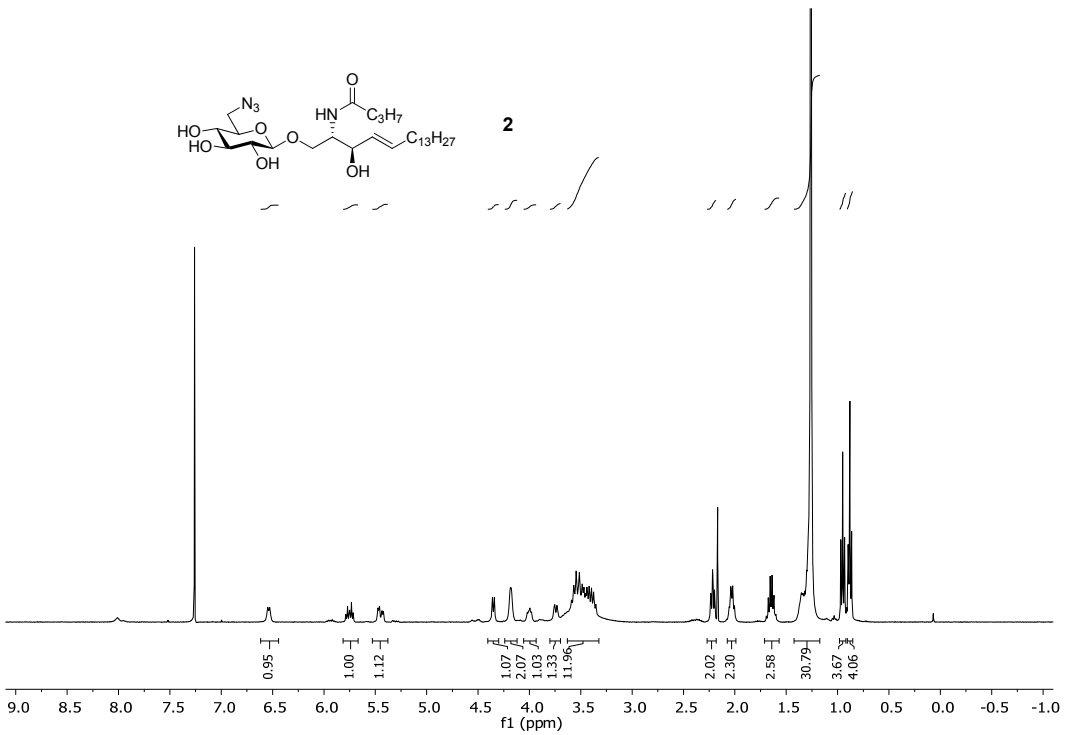

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **34** (containing residual toluene and trichloroacetamide).



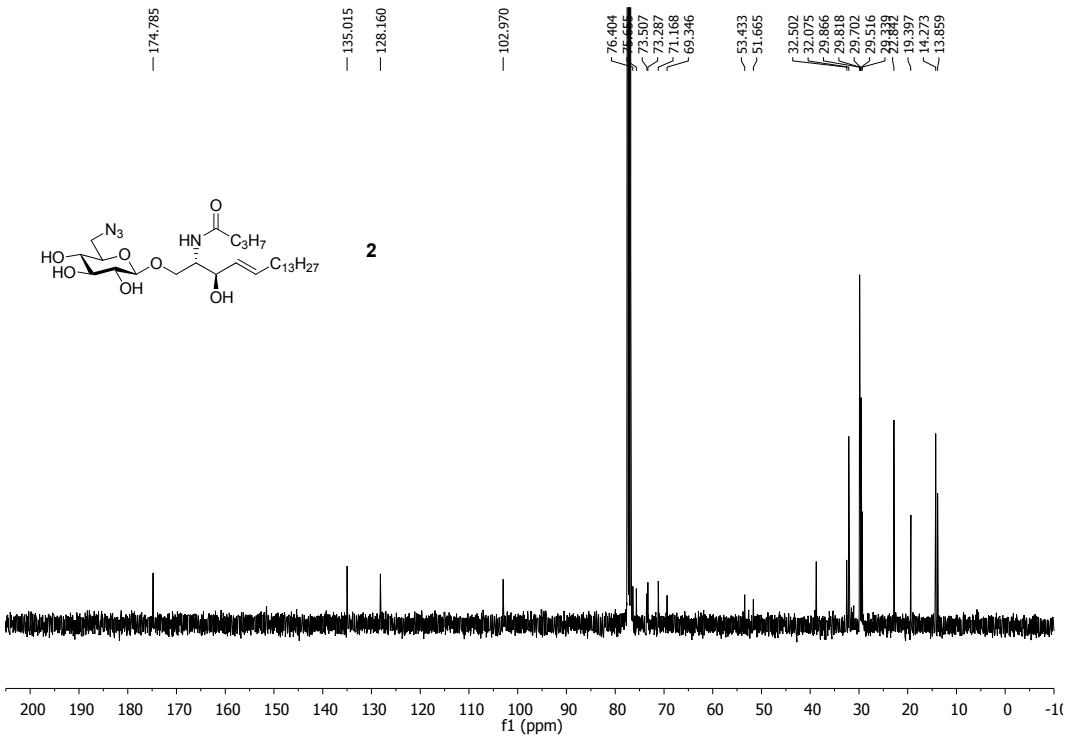

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **34** (containing residual toluene and trichloroacetamide).



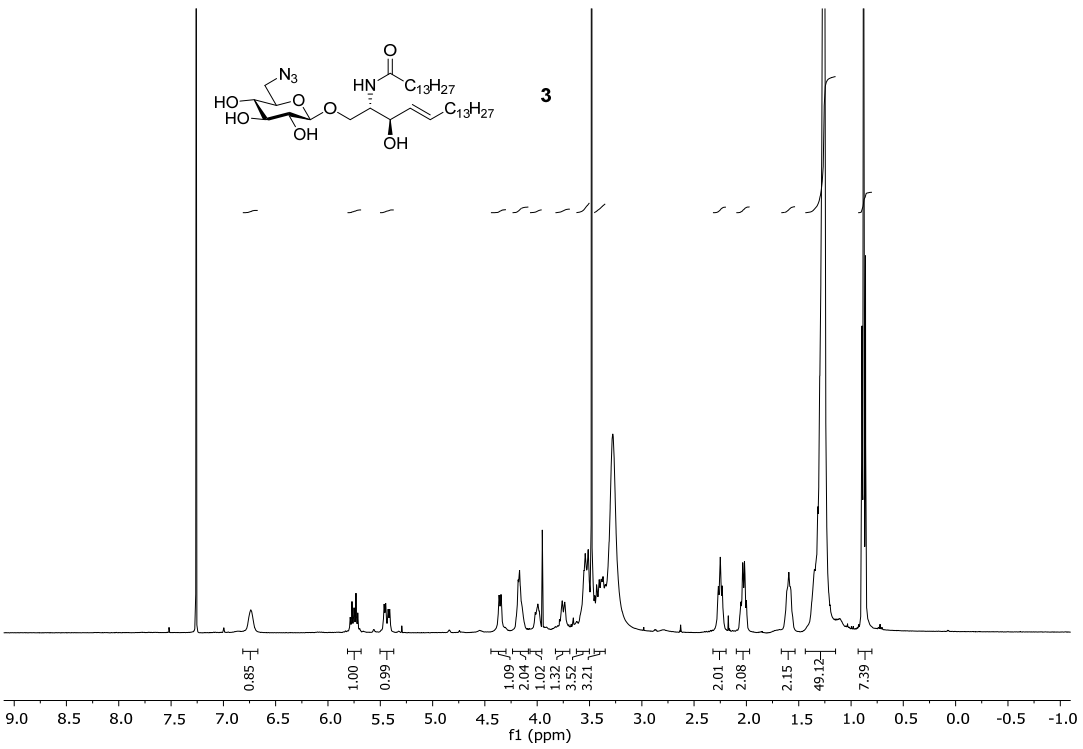

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **35** (containing residual toluene and trichloroacetamide).



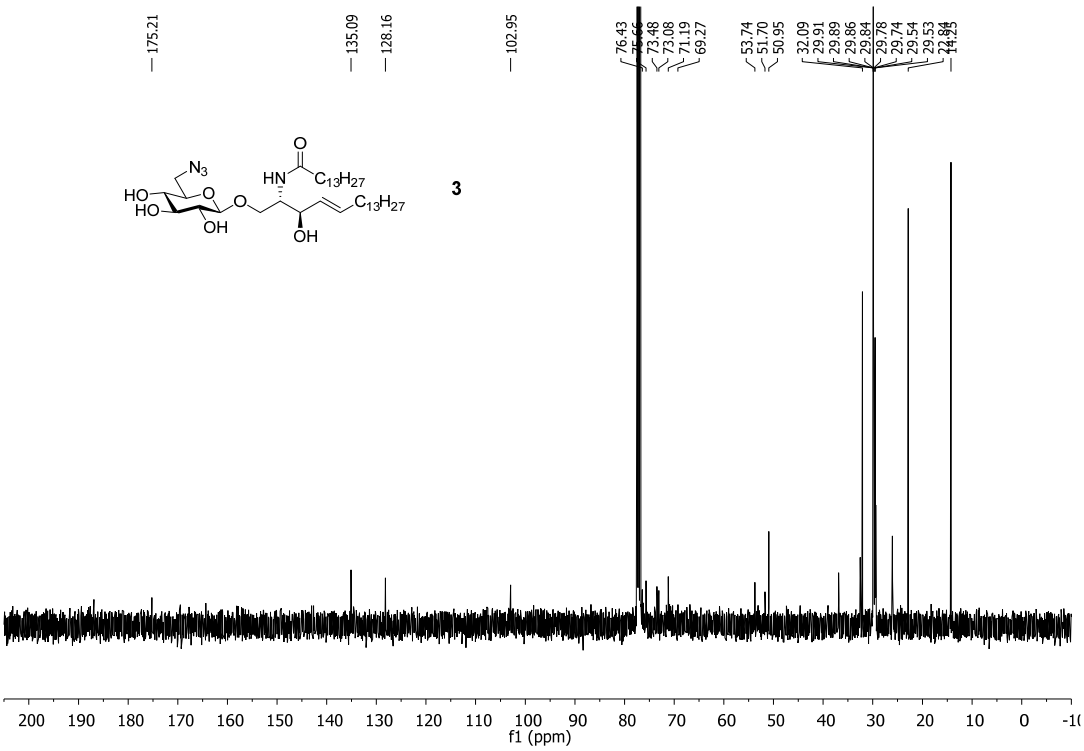

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **2**.



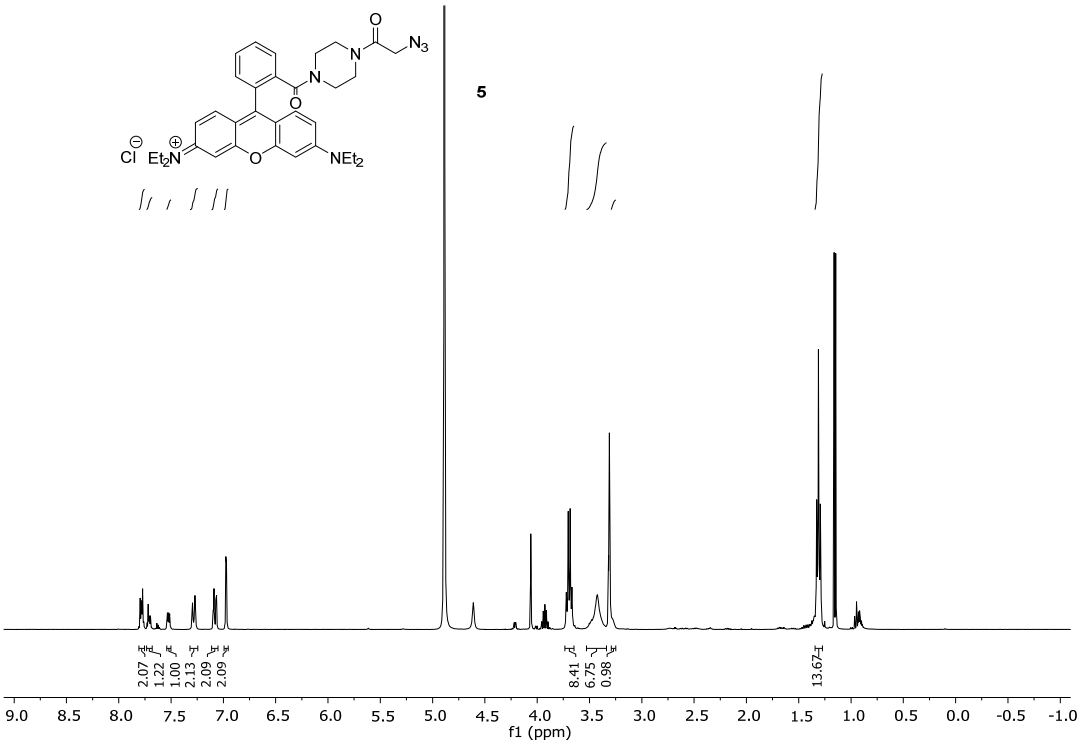

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **2**.



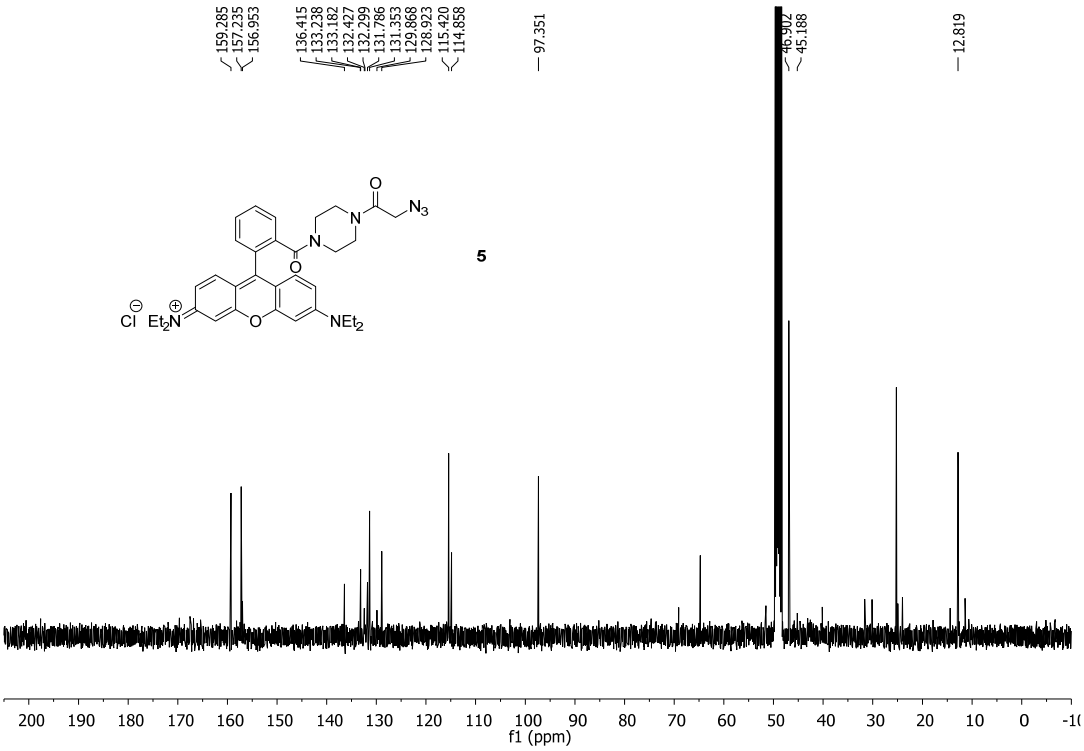

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **3**.



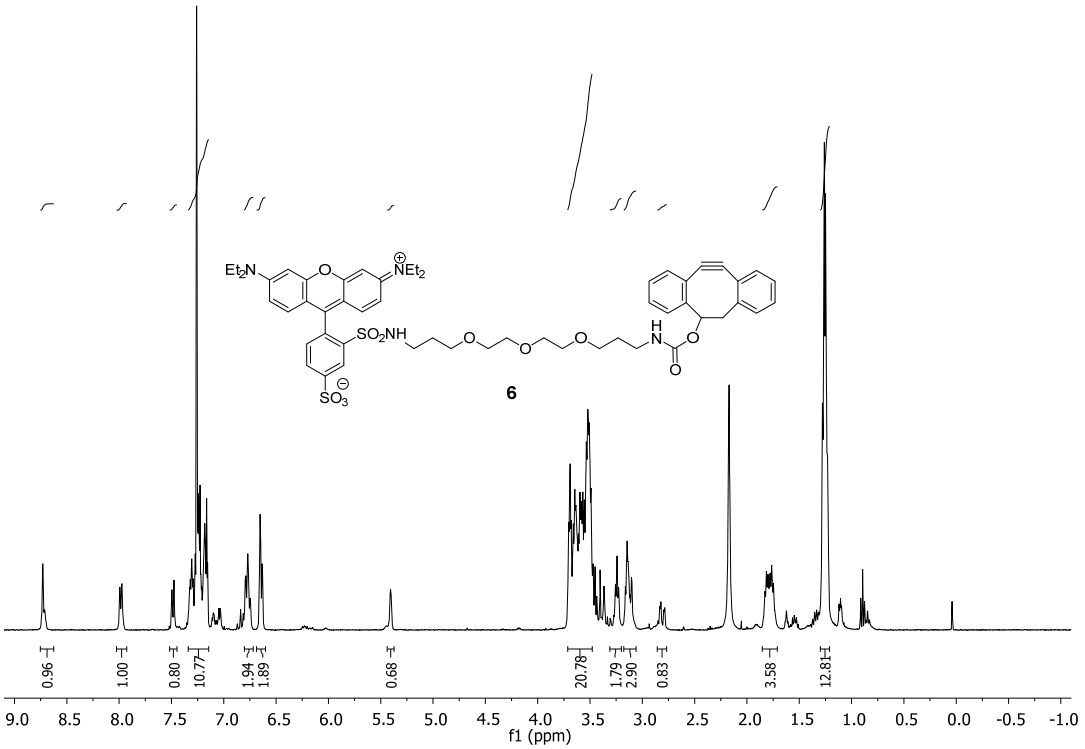

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **3**.



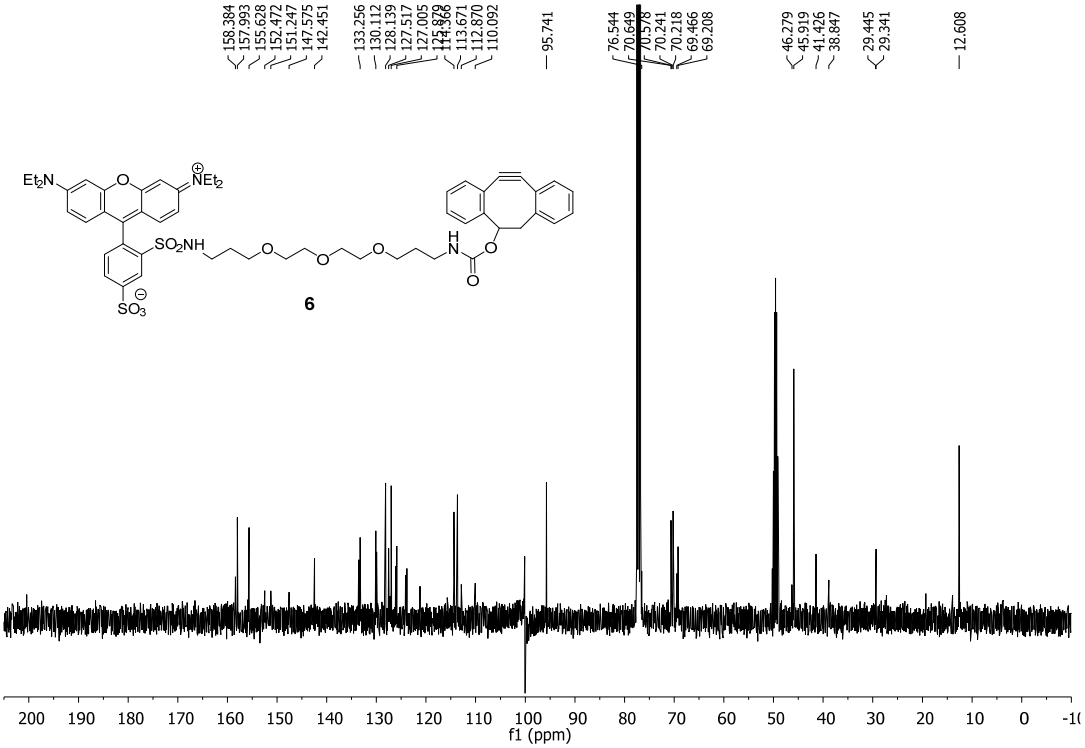

$^{1}$H NMR spectrum (400 MHz, $CD_3OD$) of compound **5**.



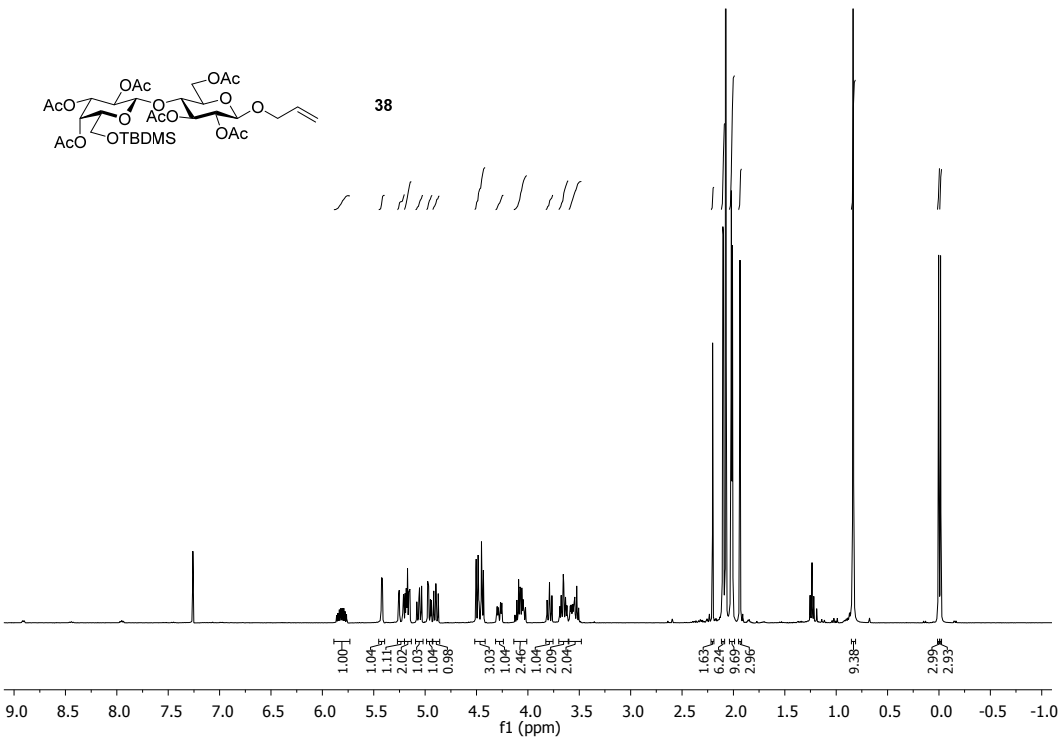

$^{13}$C NMR spectrum (100.6 MHz, $CD_3OD$) of compound **5**.



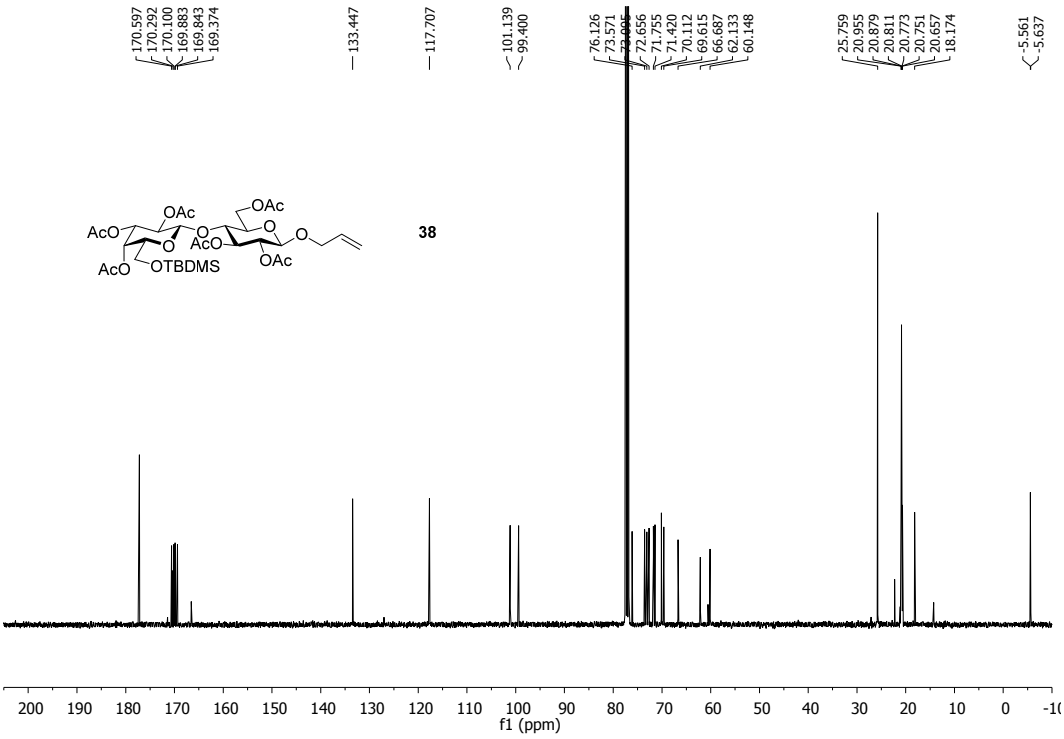

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **6**.



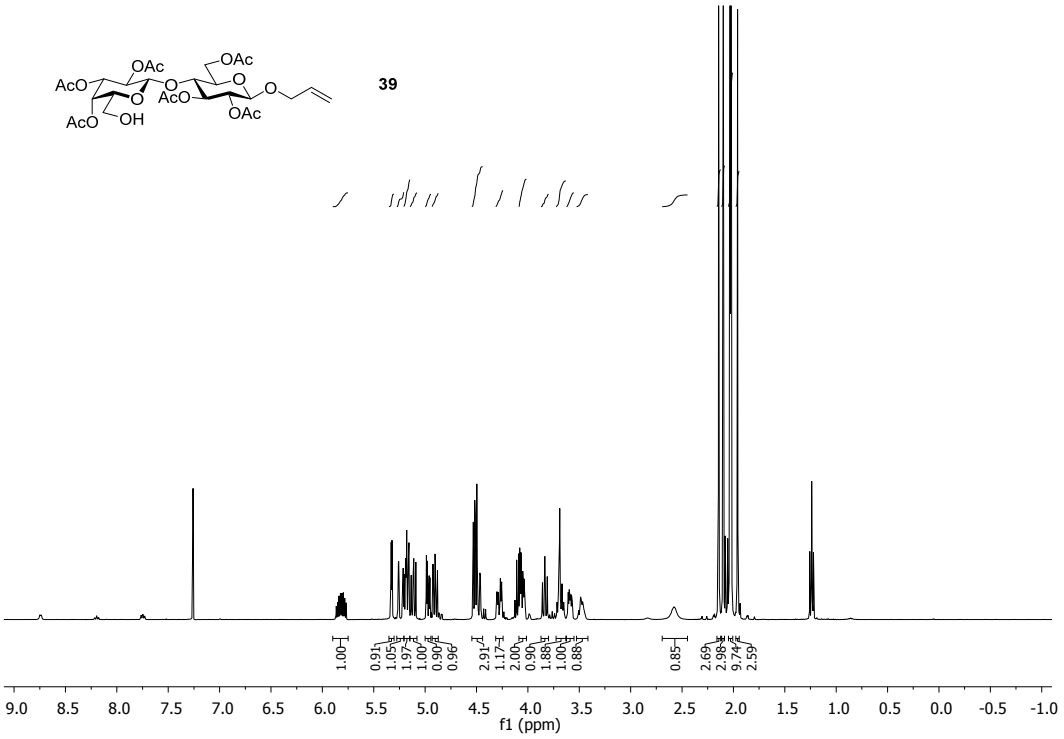

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **6**.



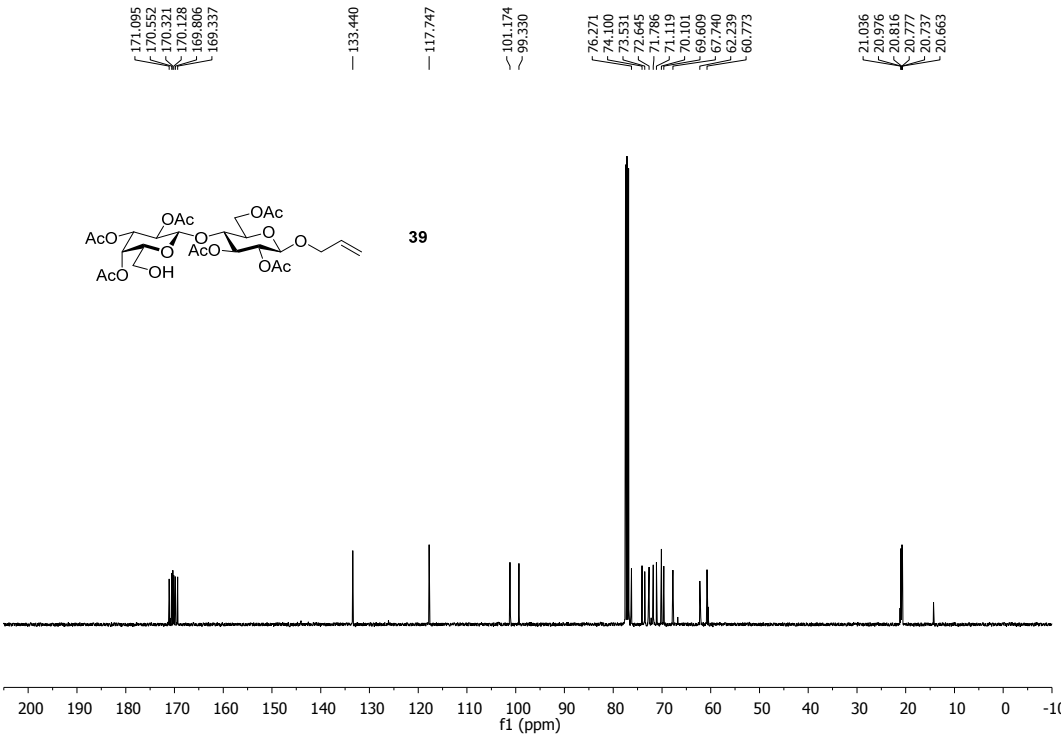

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **38** (containing traces of ethyl acetate).



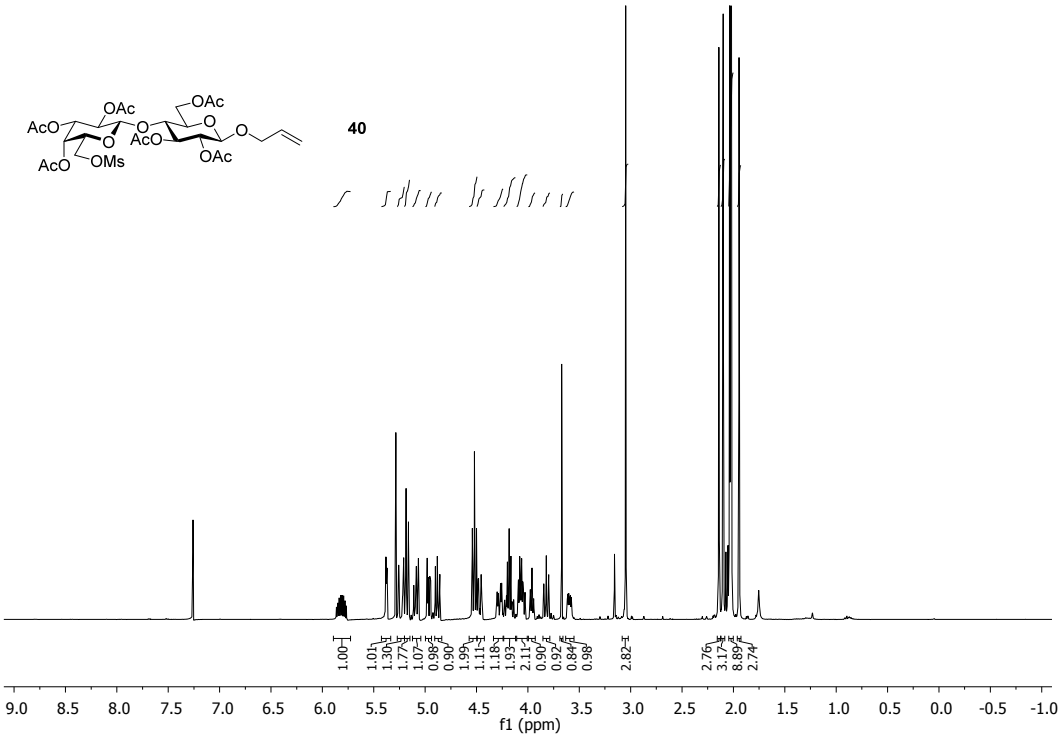

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **38** (containing traces of ethyl acetate).



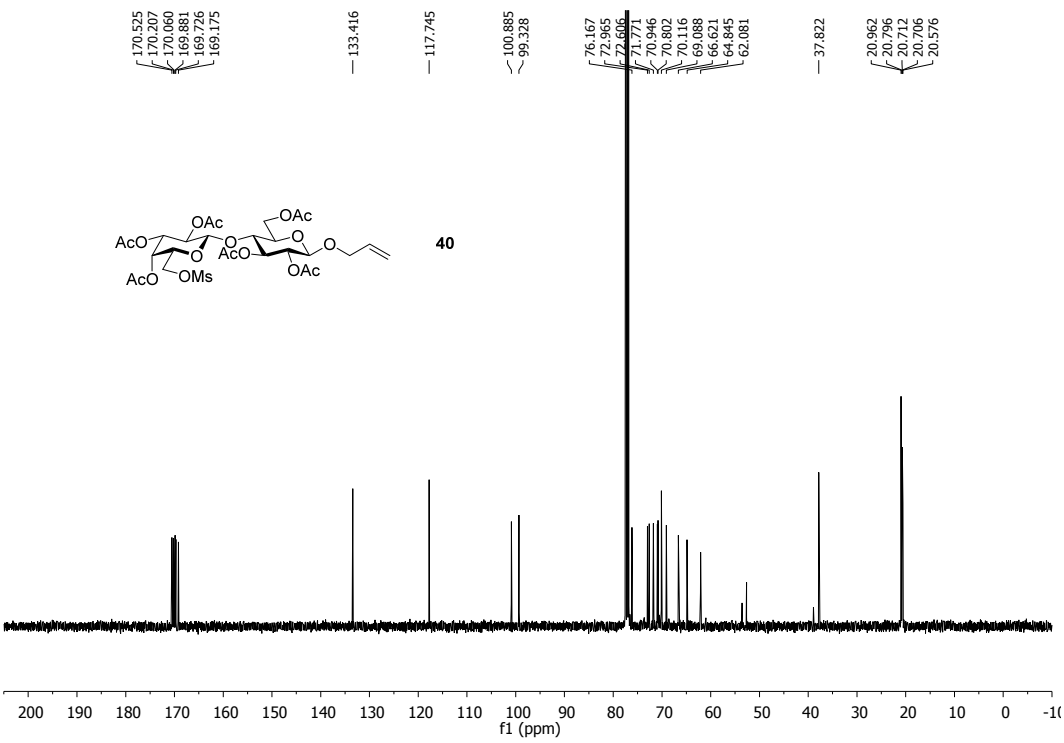

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **39** (containing traces of pyridine and ethyl acetate).



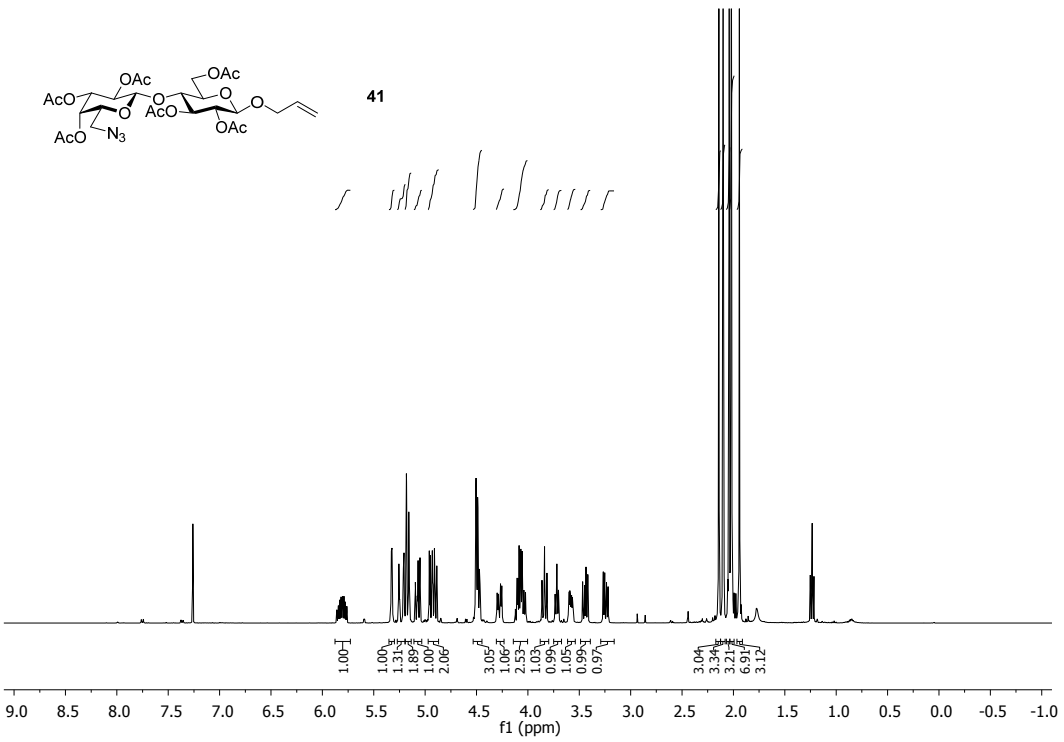

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **39** (containing traces of pyridine and ethyl acetate).



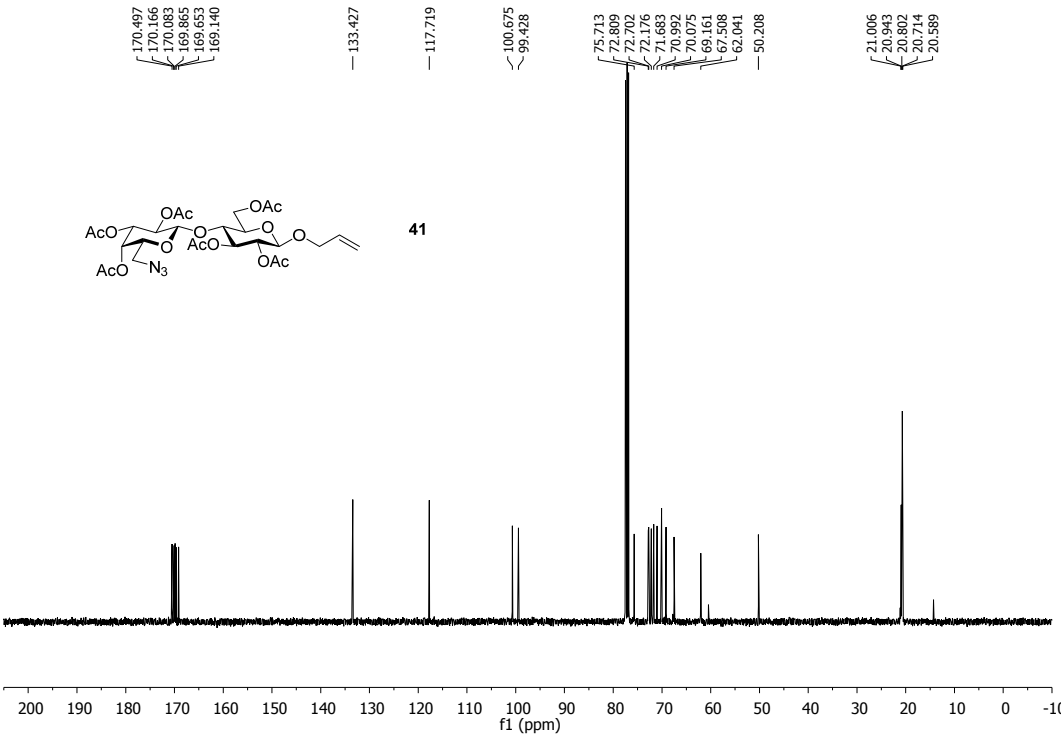

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **40**.



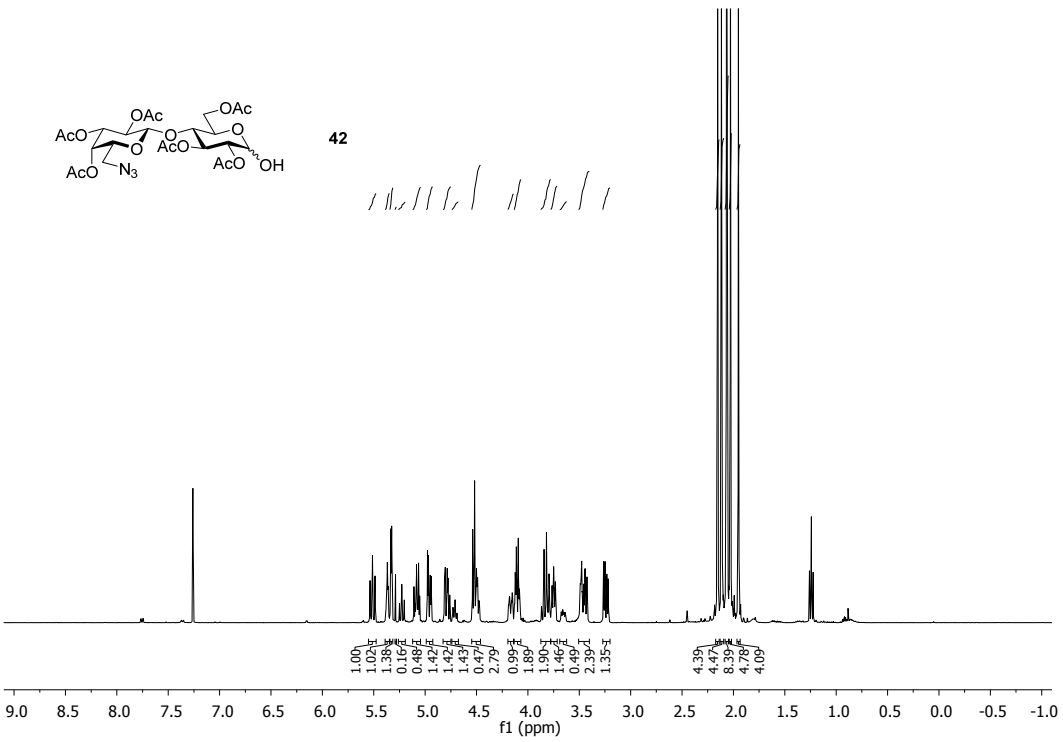

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **40**.



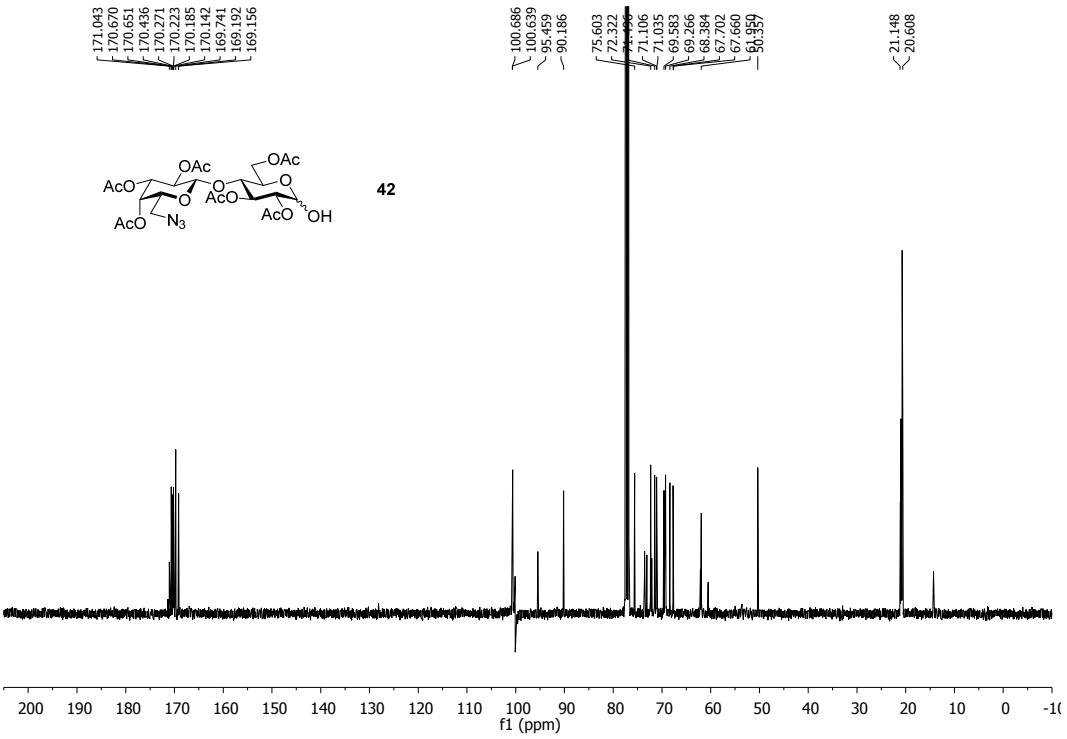

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **41** (containing residual ethyl acetate).



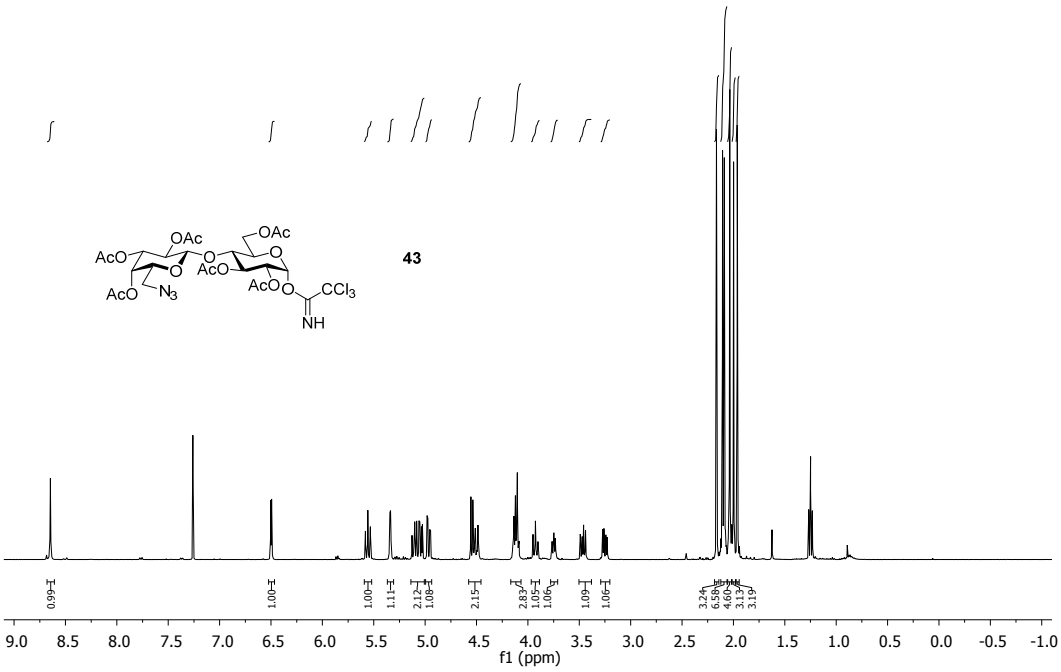

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **41** (containing residual ethyl acetate).



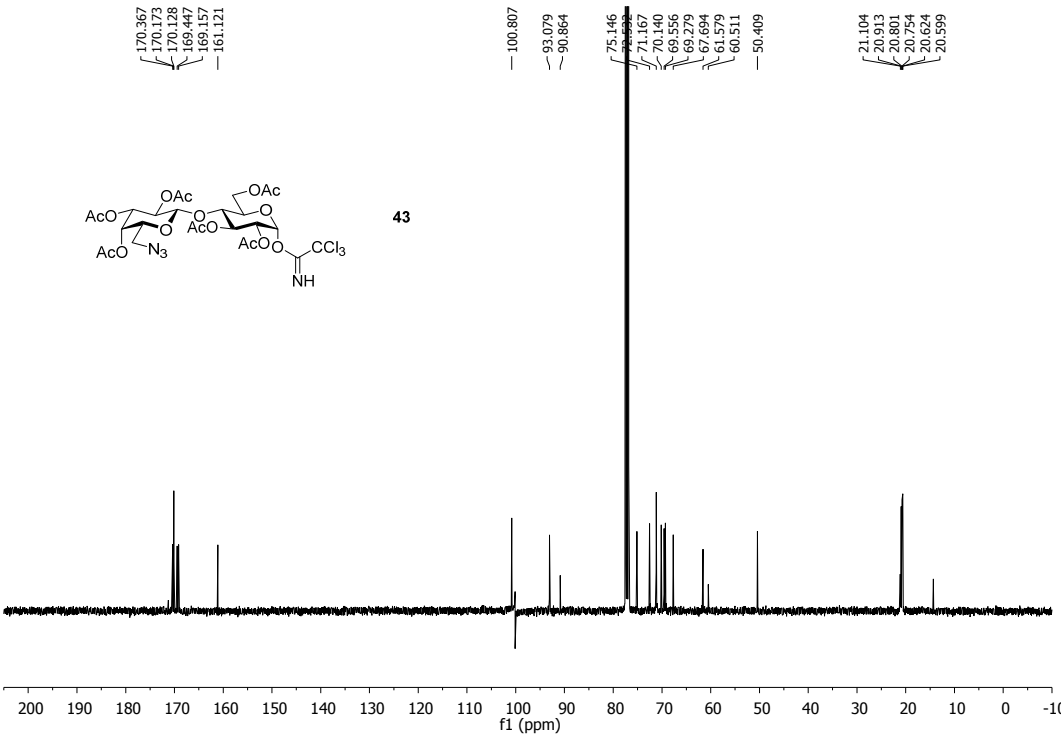

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **42** (containing residual ethyl acetate).



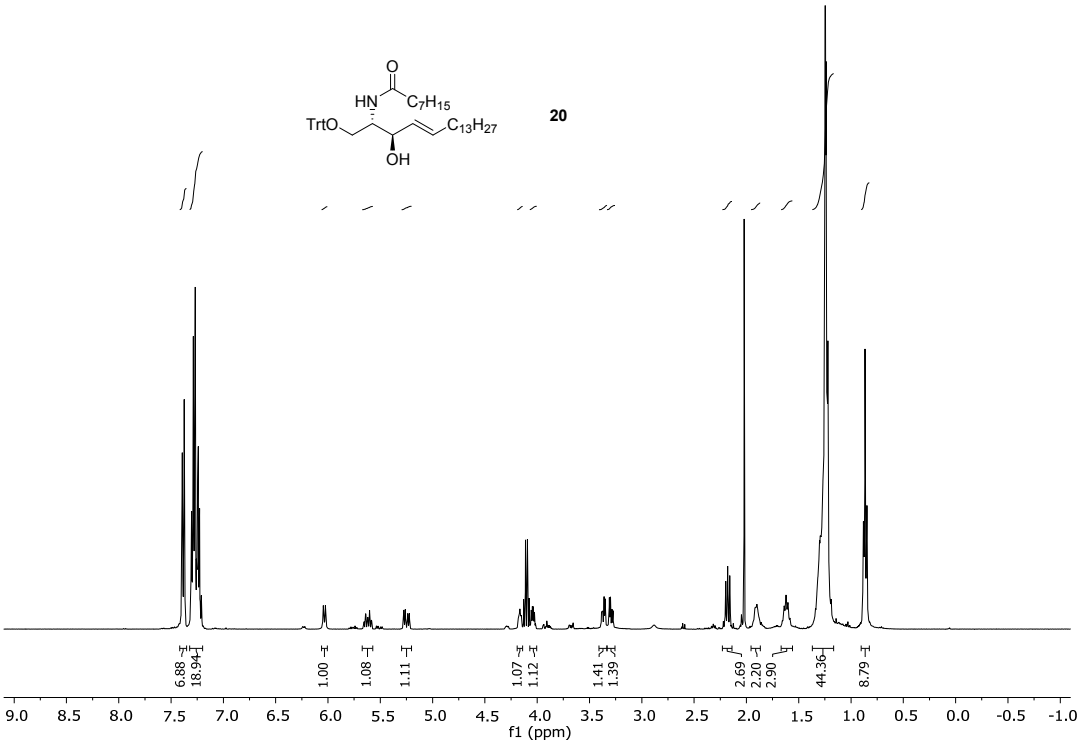

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **42** (containing residual ethyl acetate).



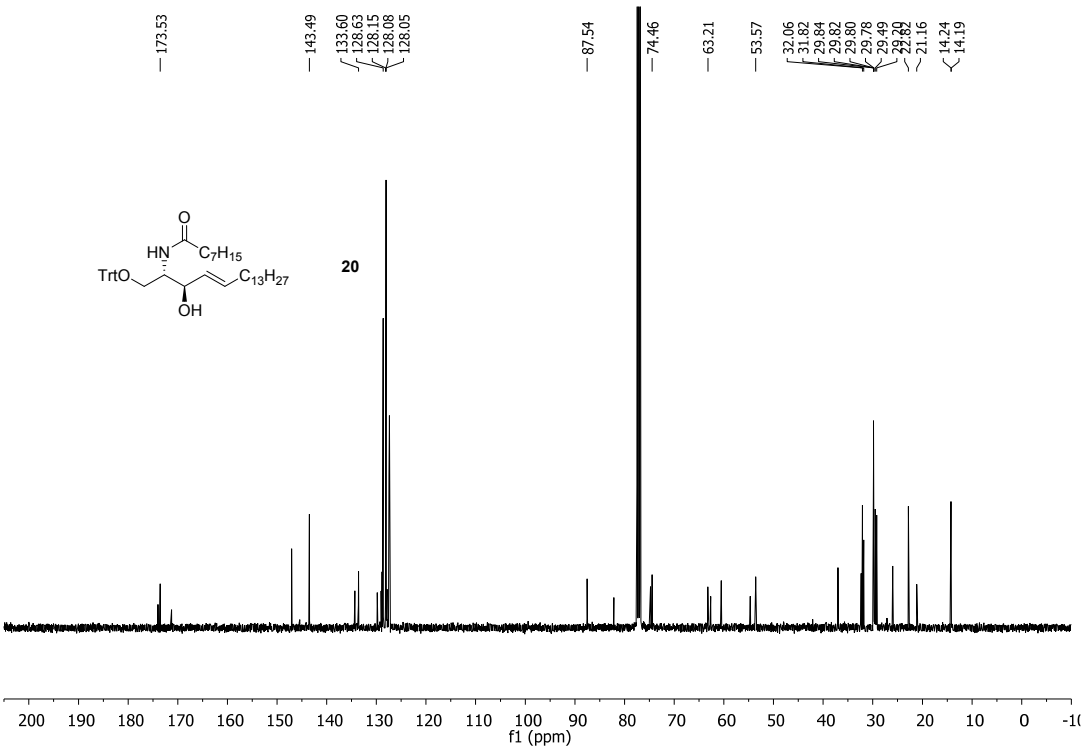

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **43** (containing residual ethyl acetate).



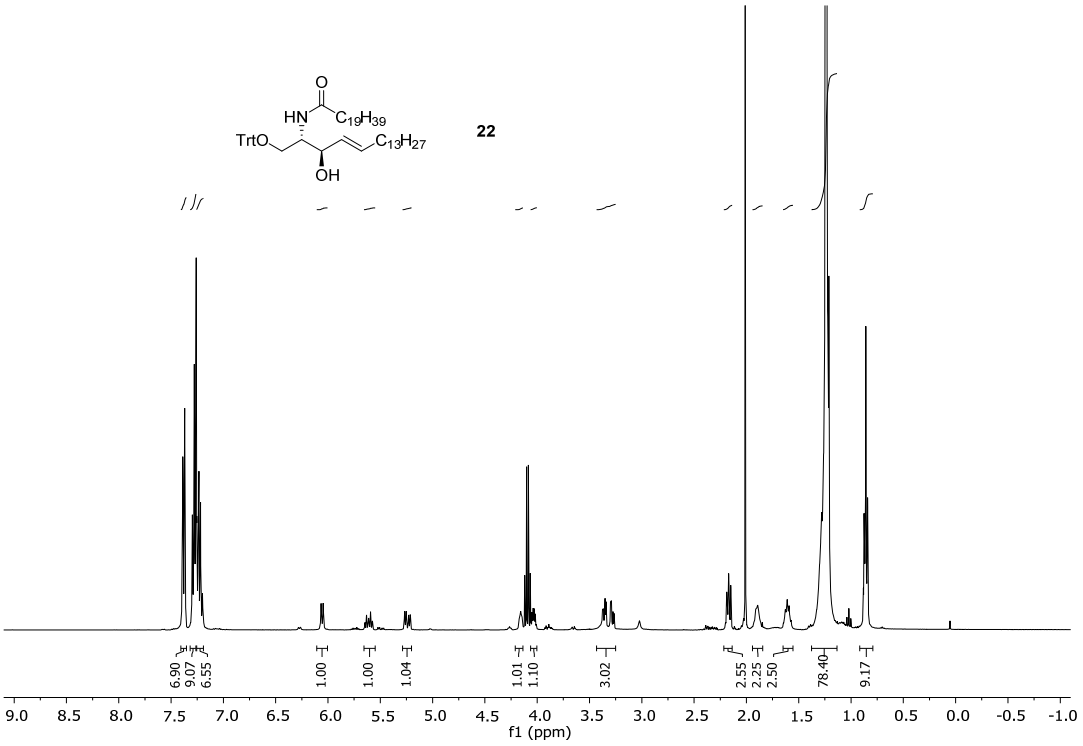

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **43** (containing residual ethyl acetate).



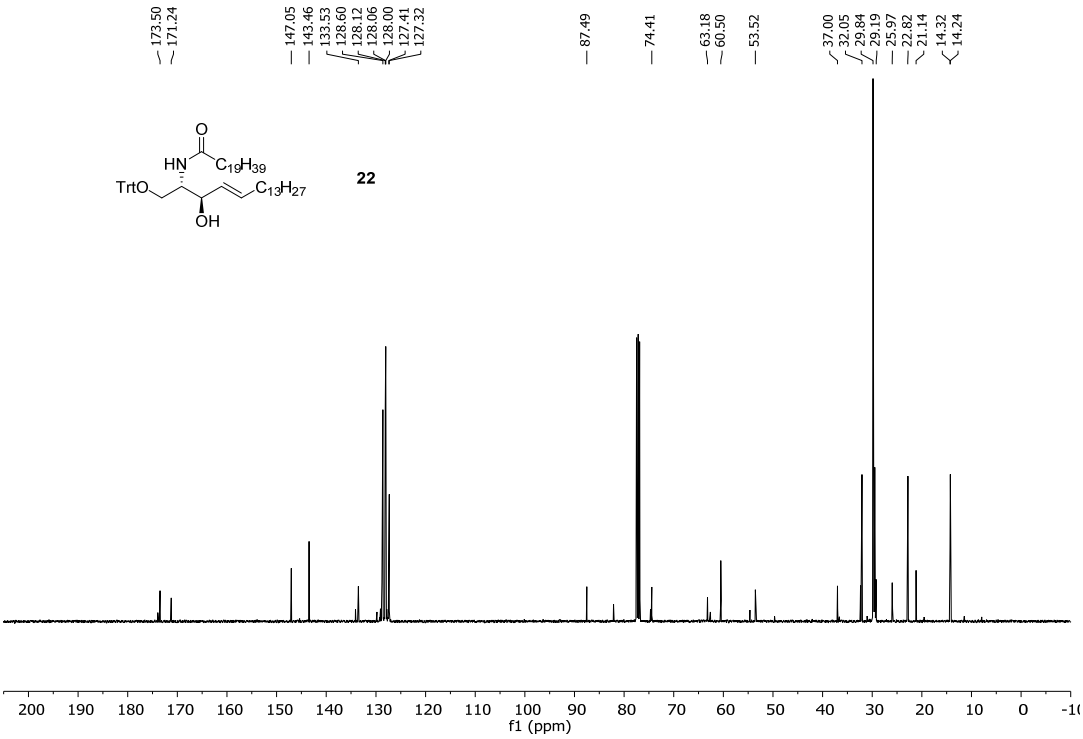

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **20** (containing residual ethyl acetate).



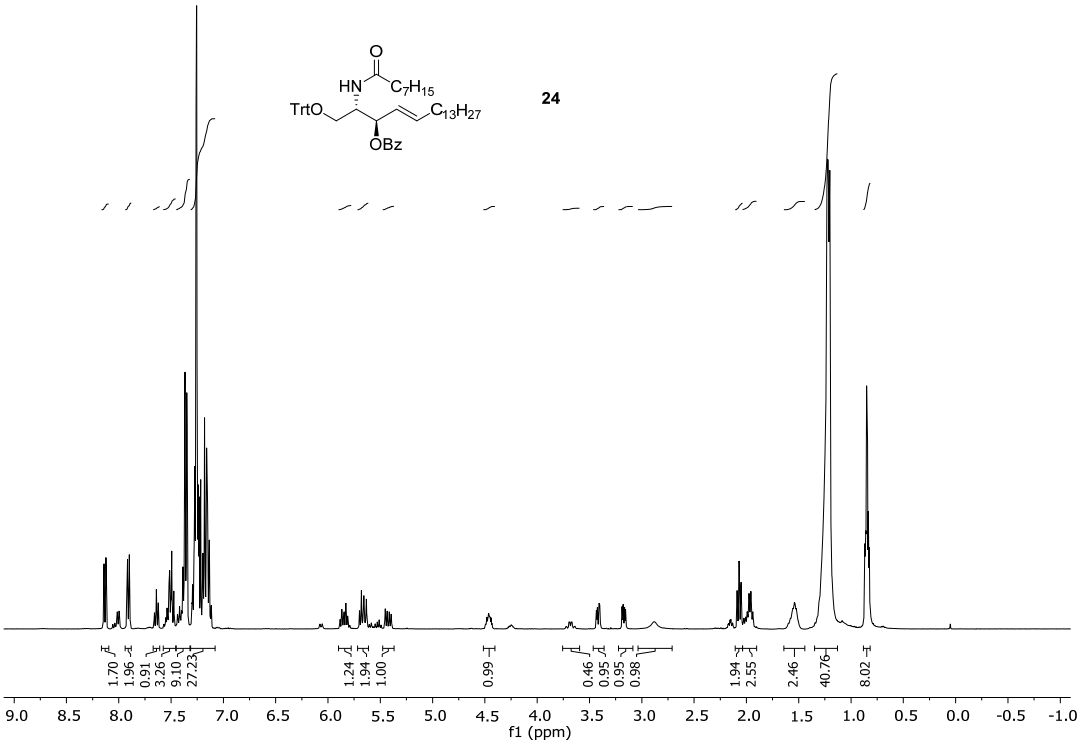

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **20** (containing residual ethyl acetate).



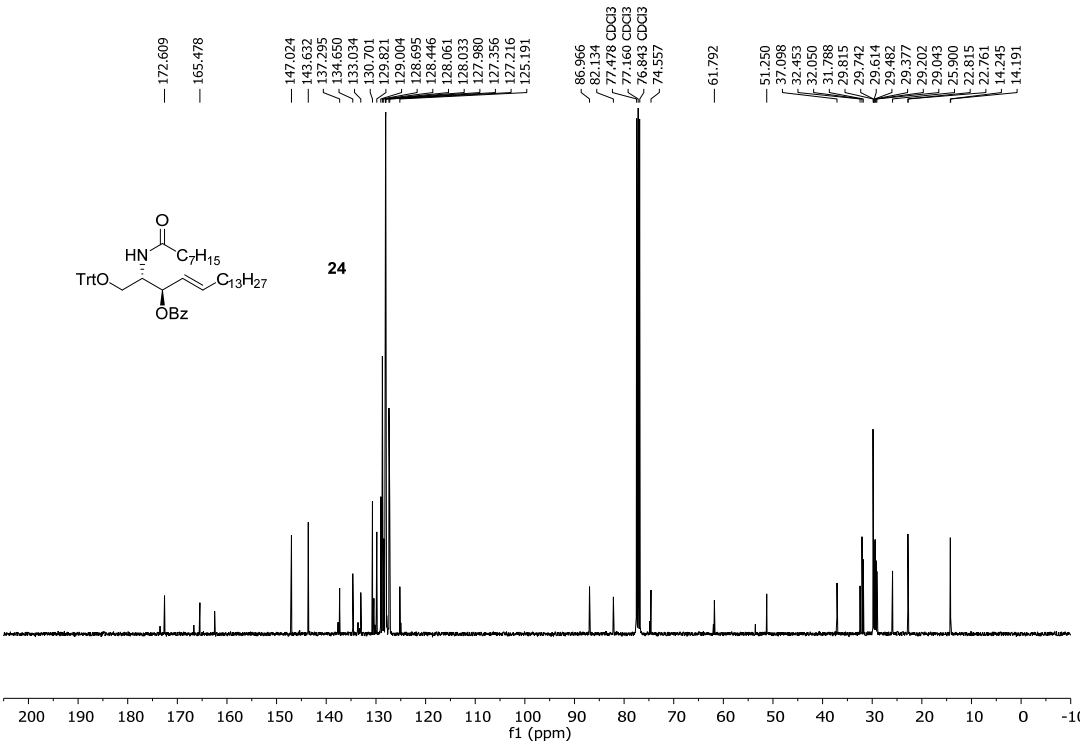

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **22** (containing residual ethyl acetate).



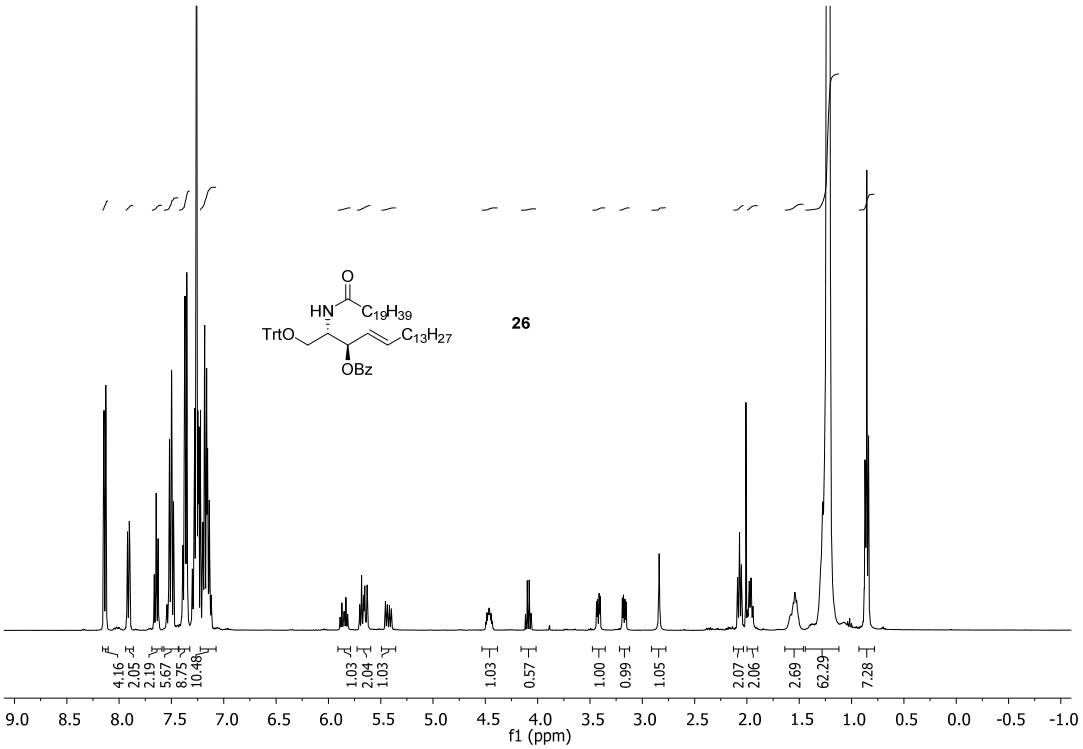

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **22** (containing residual ethyl acetate).



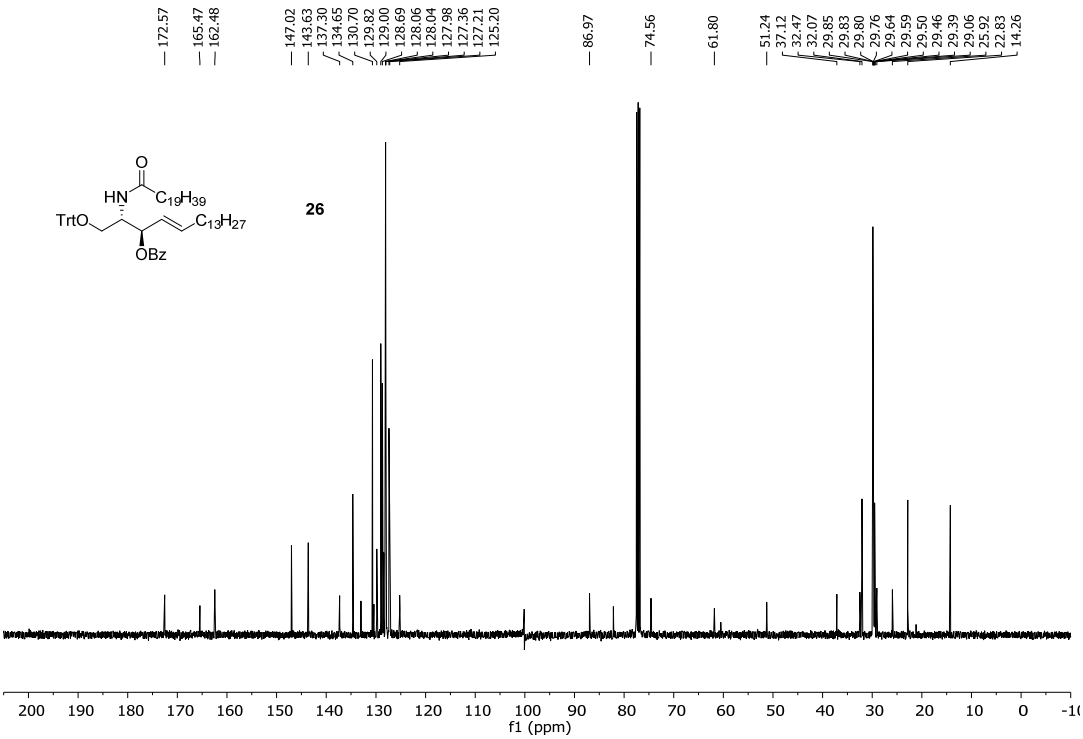

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **24**.



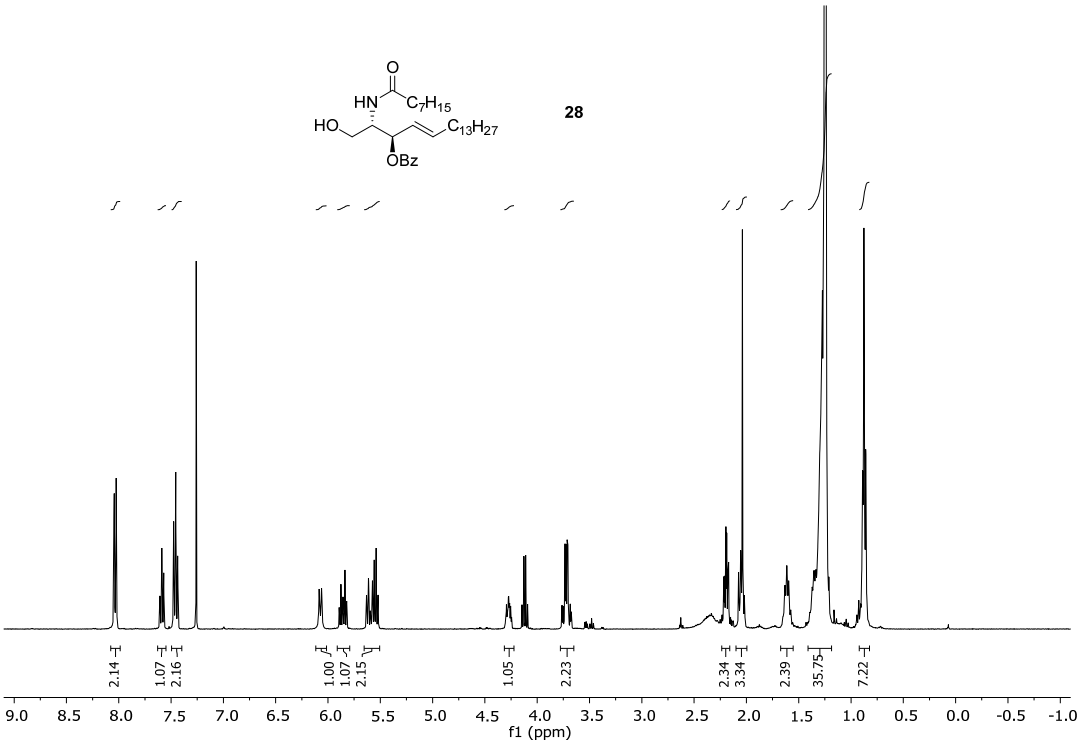

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **24**.



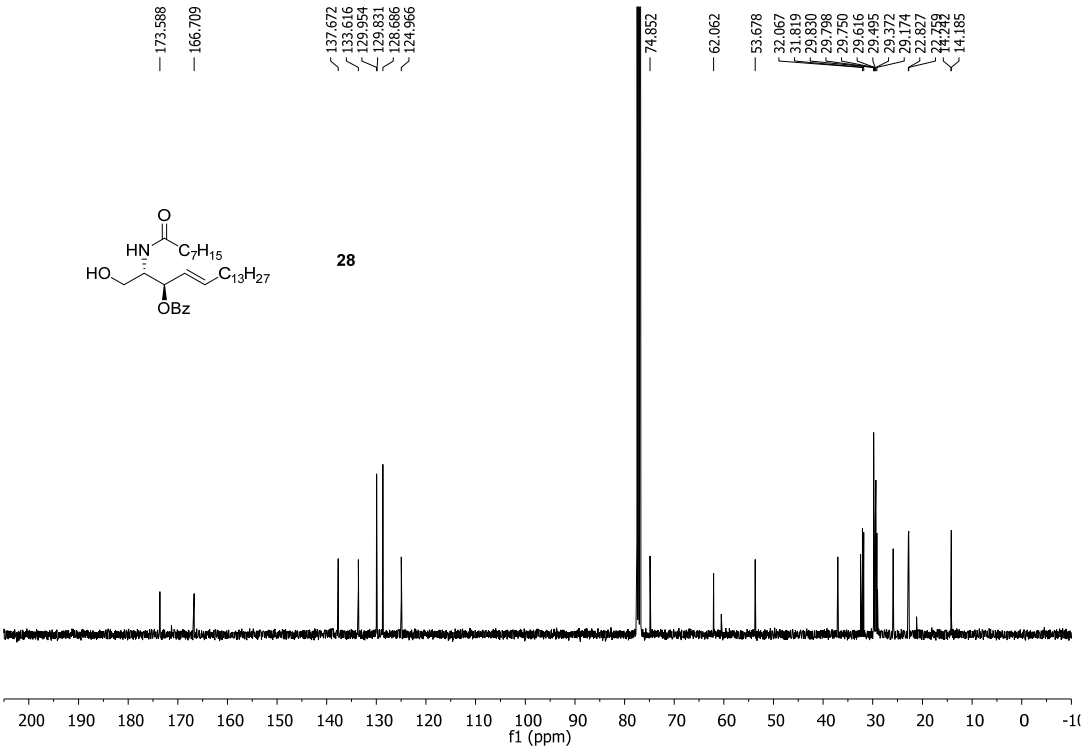

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **26** (containing residual ethyl acetate).



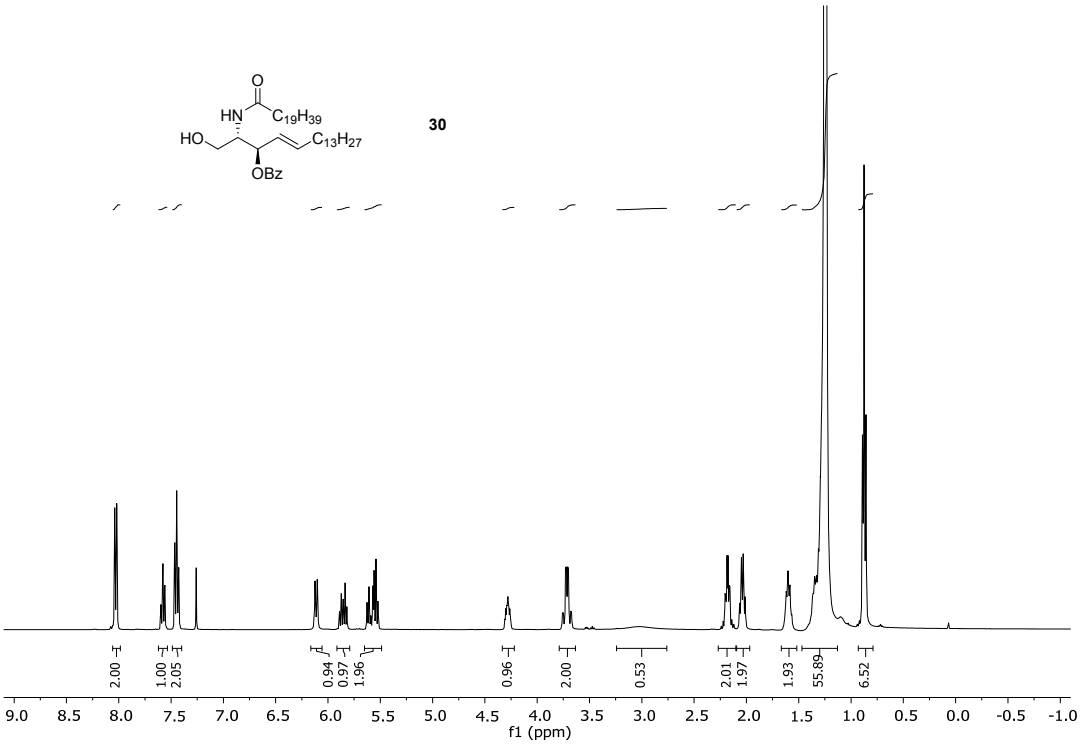

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **26**.



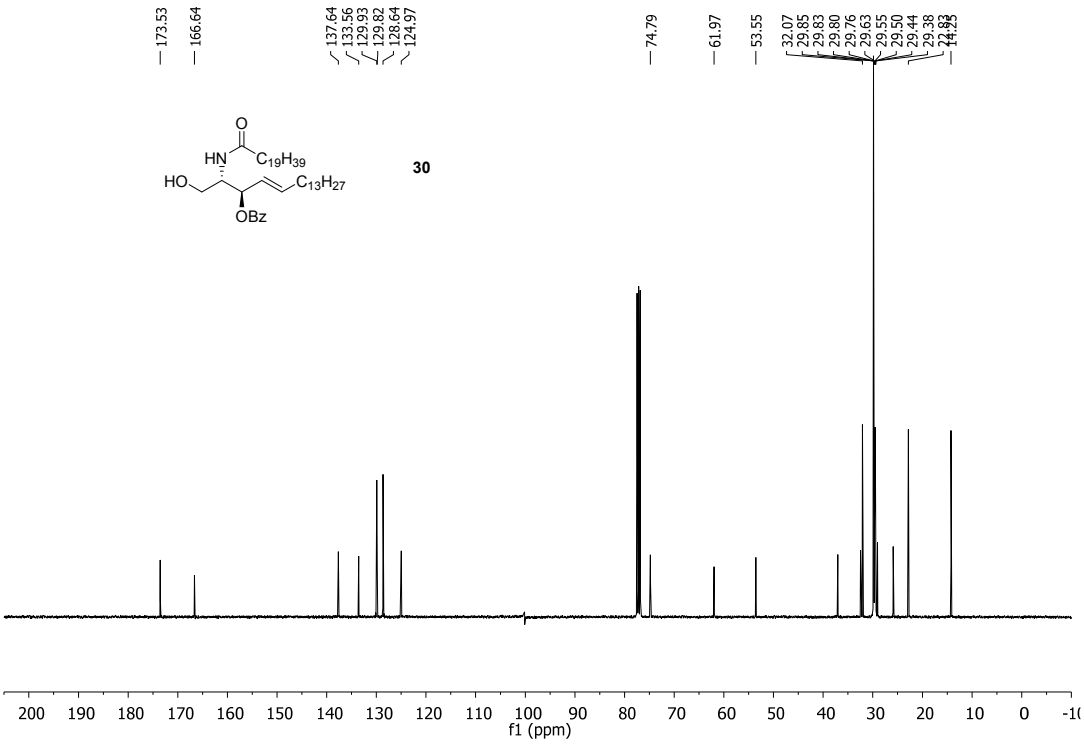

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **28** (containing residual ethyl acetate).



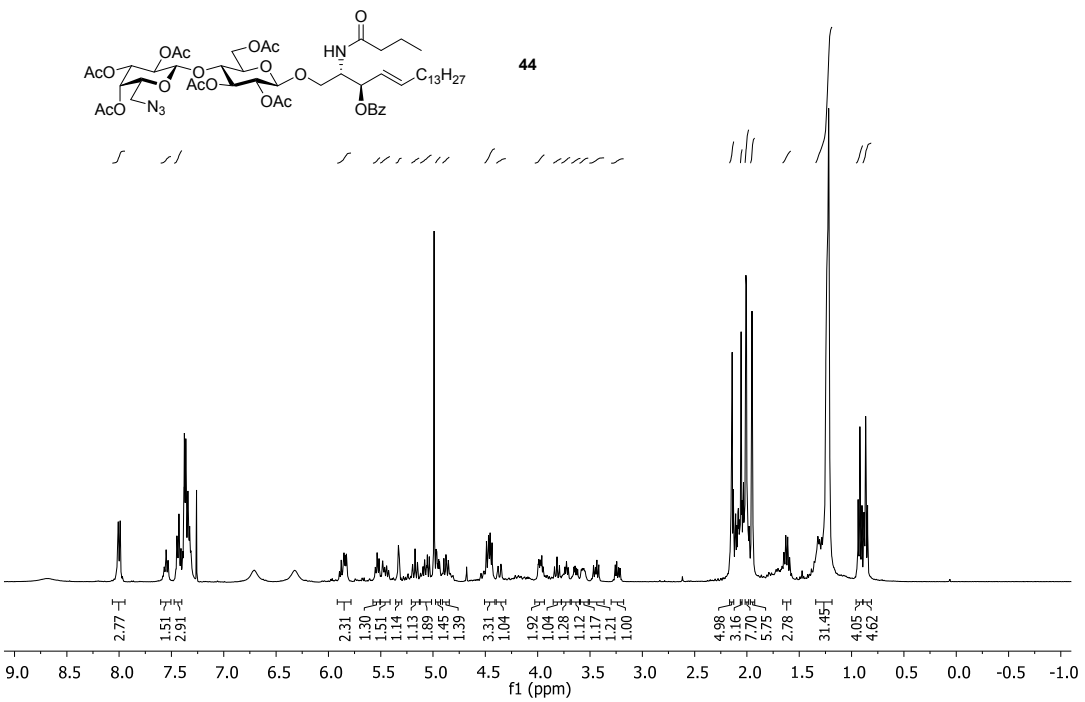

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **28** (containing residual ethyl acetate).



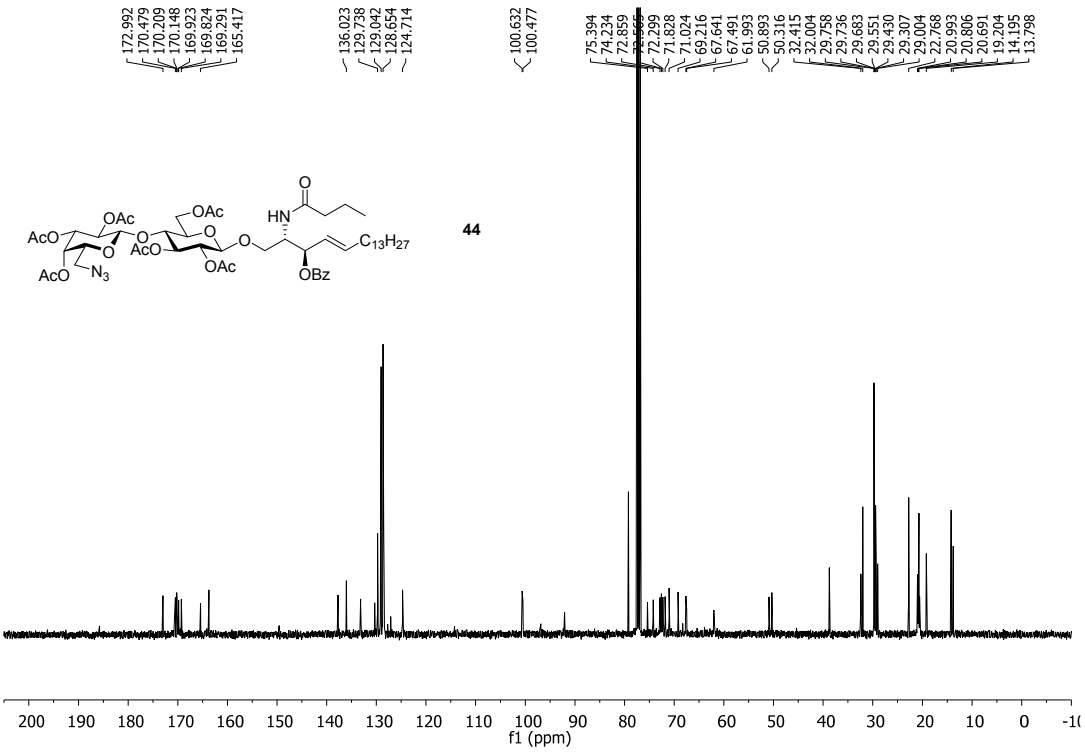

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **30**.



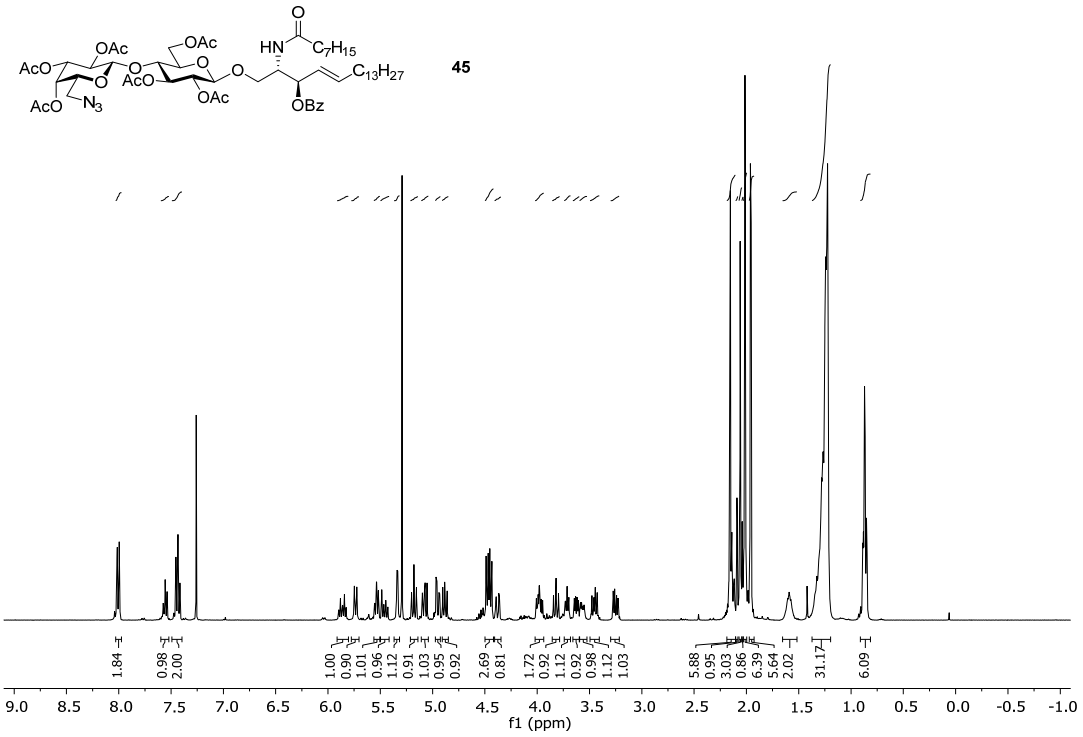

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **30**.



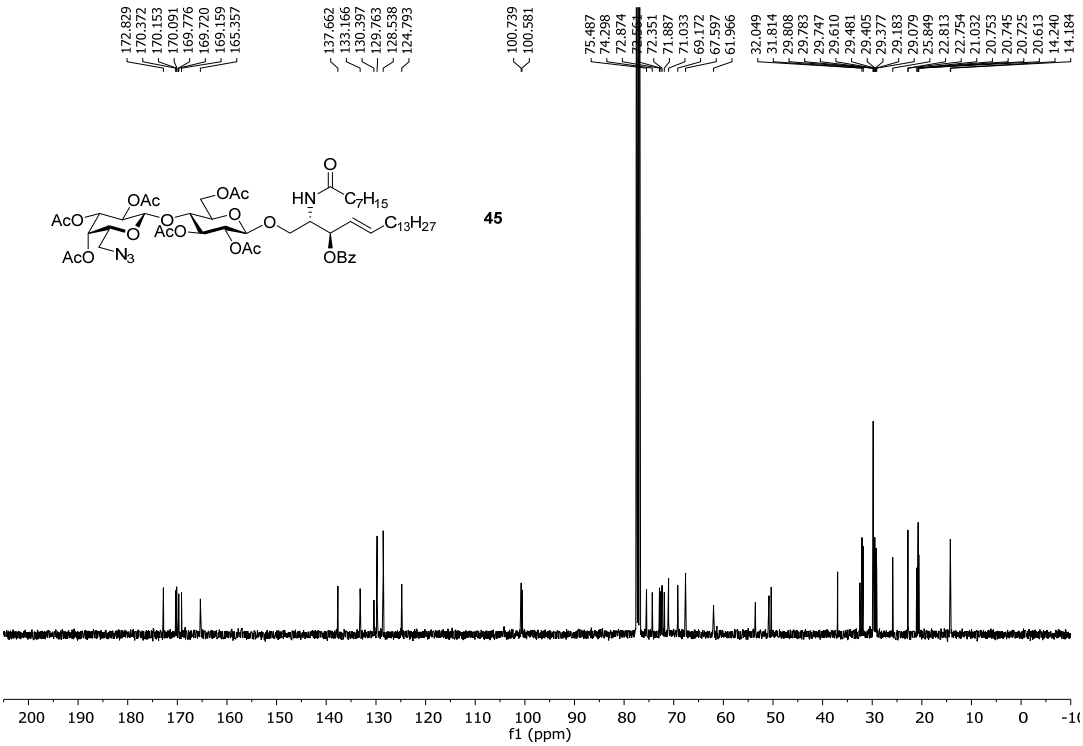

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **44** (containing residual trichloroacetamide).



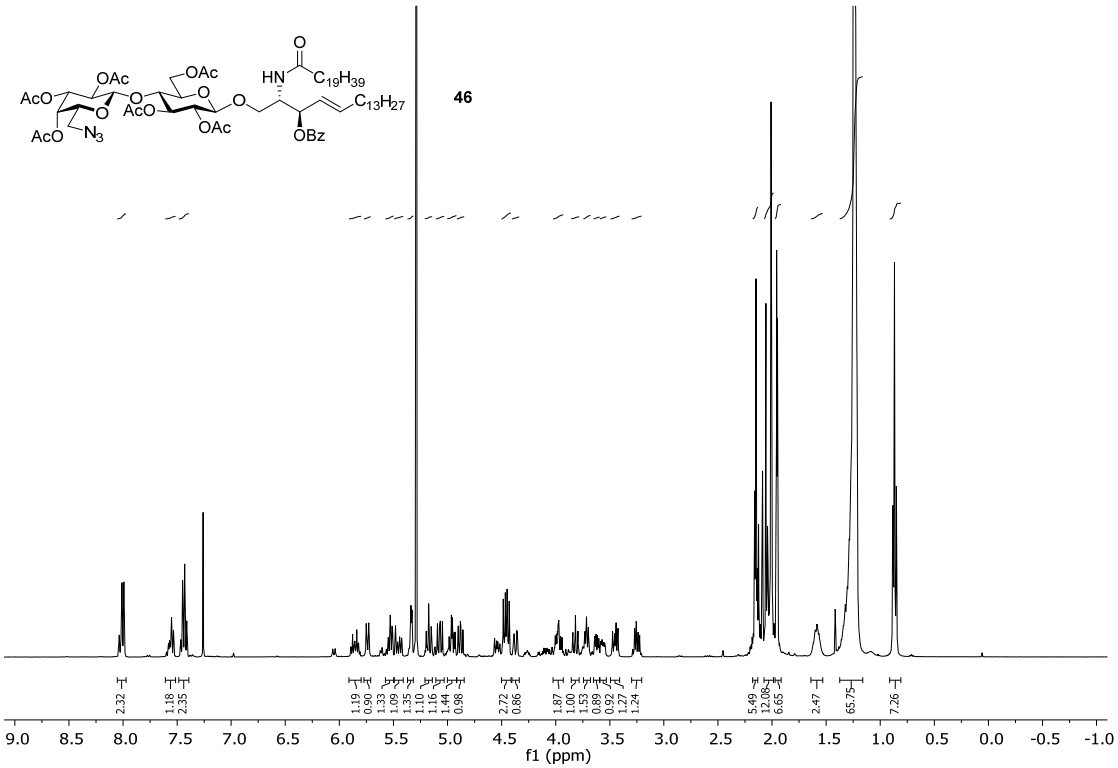

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **44** (containing residual trichloroacetamide).



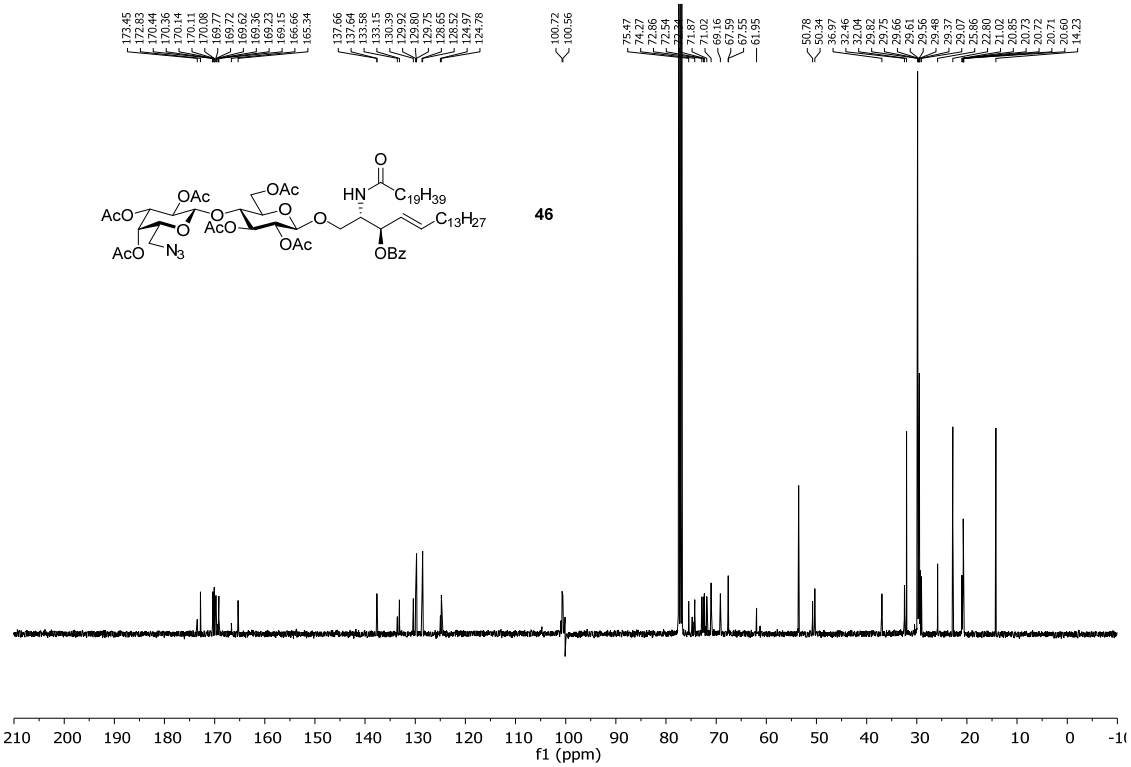

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **45**.



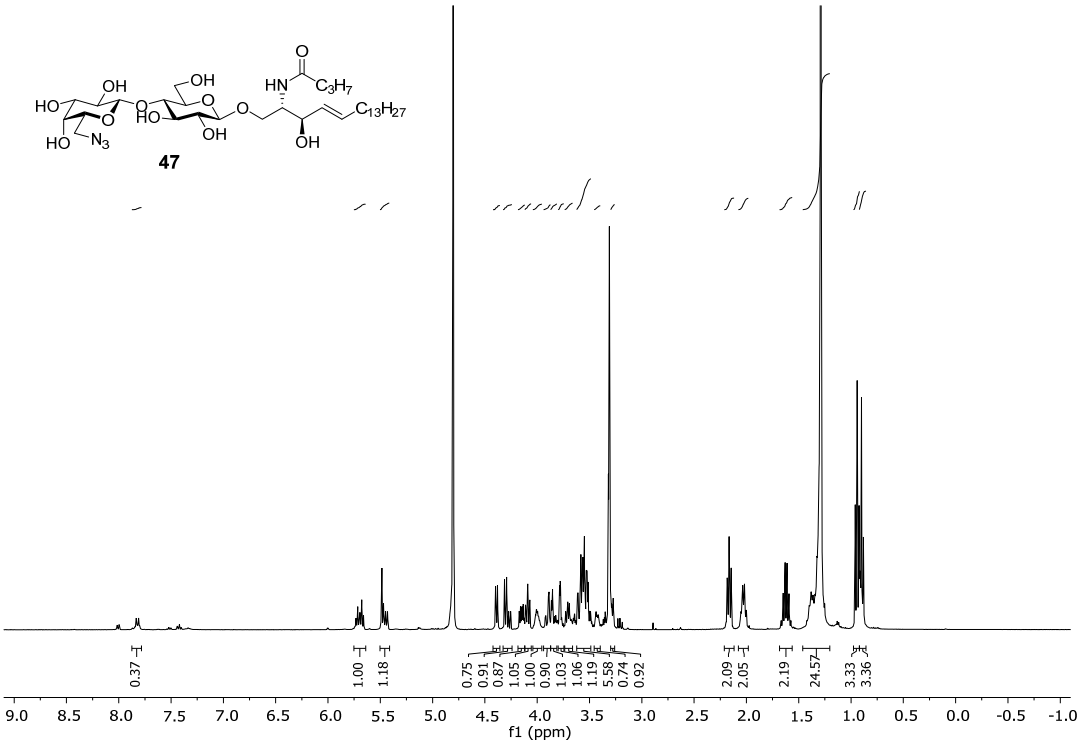

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **45**.



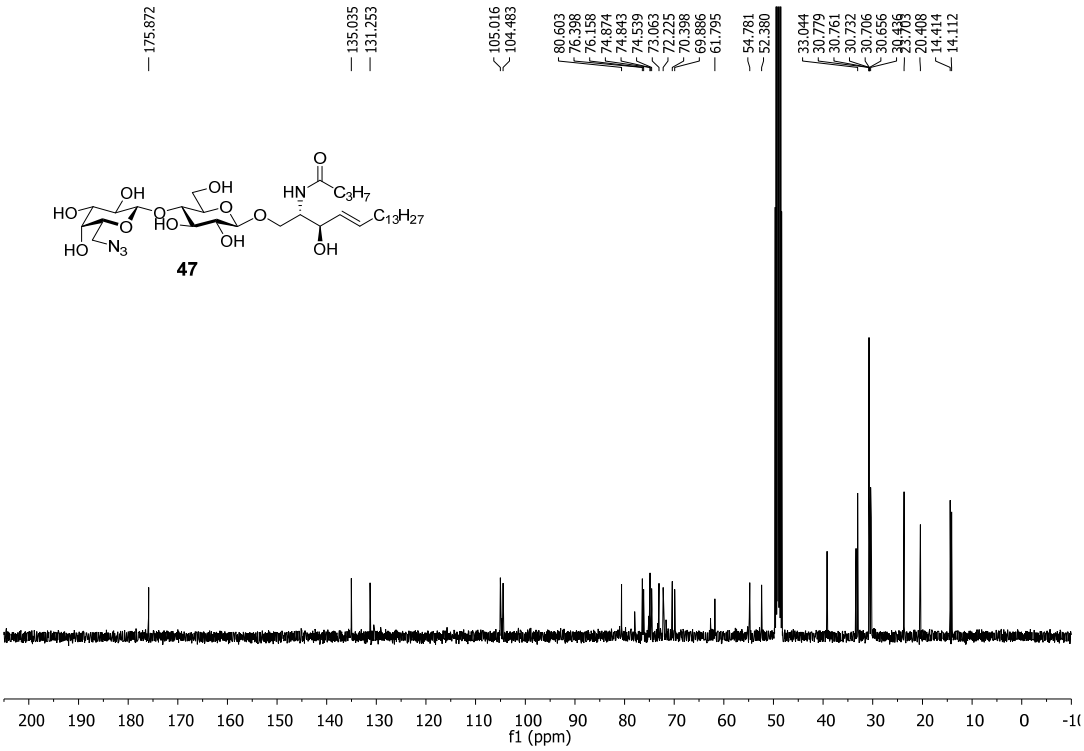

$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **46**.



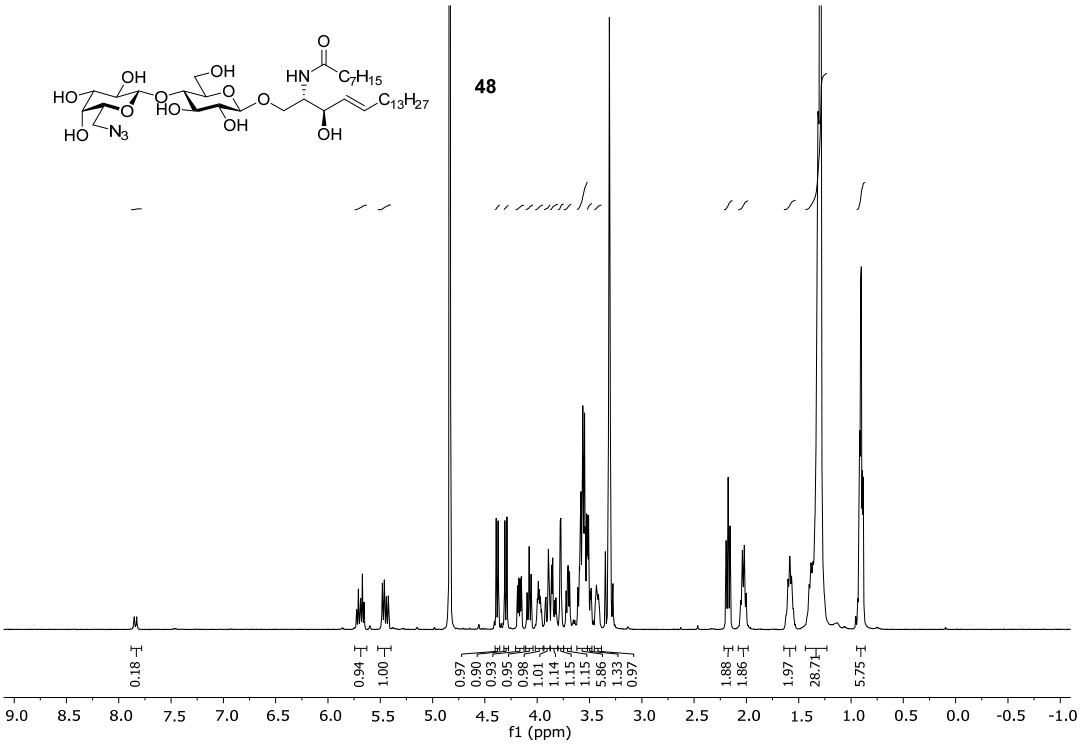

$^{13}$C NMR spectrum (100.6 MHz, $CDCl_3$) of compound **46**.



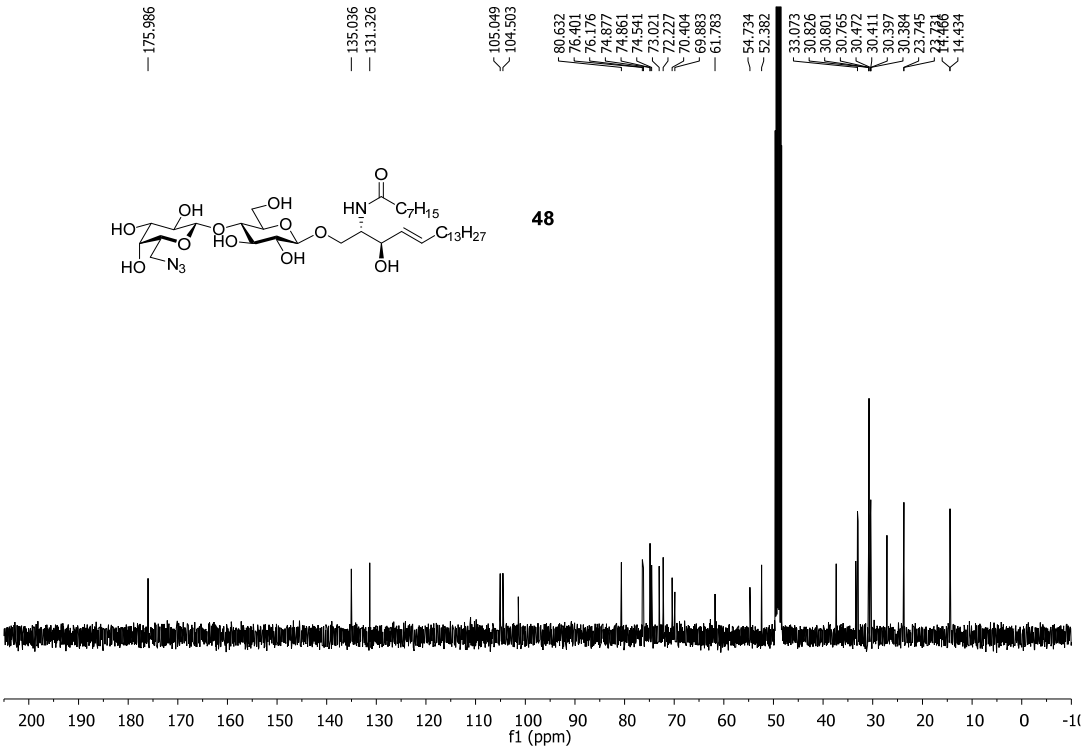

$^{1}$H NMR spectrum (400 MHz, $CD_3OD$) of compound **47**.



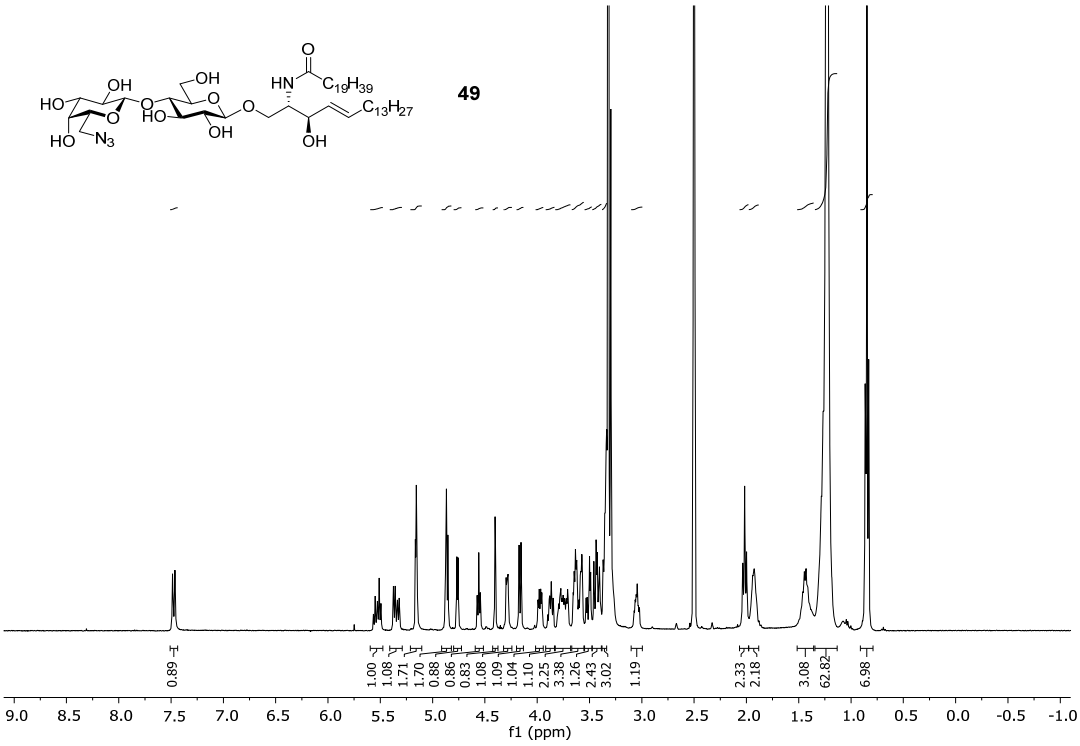

$^{13}$C NMR spectrum (100.6 MHz, $CD_3OD$) of compound **47**.



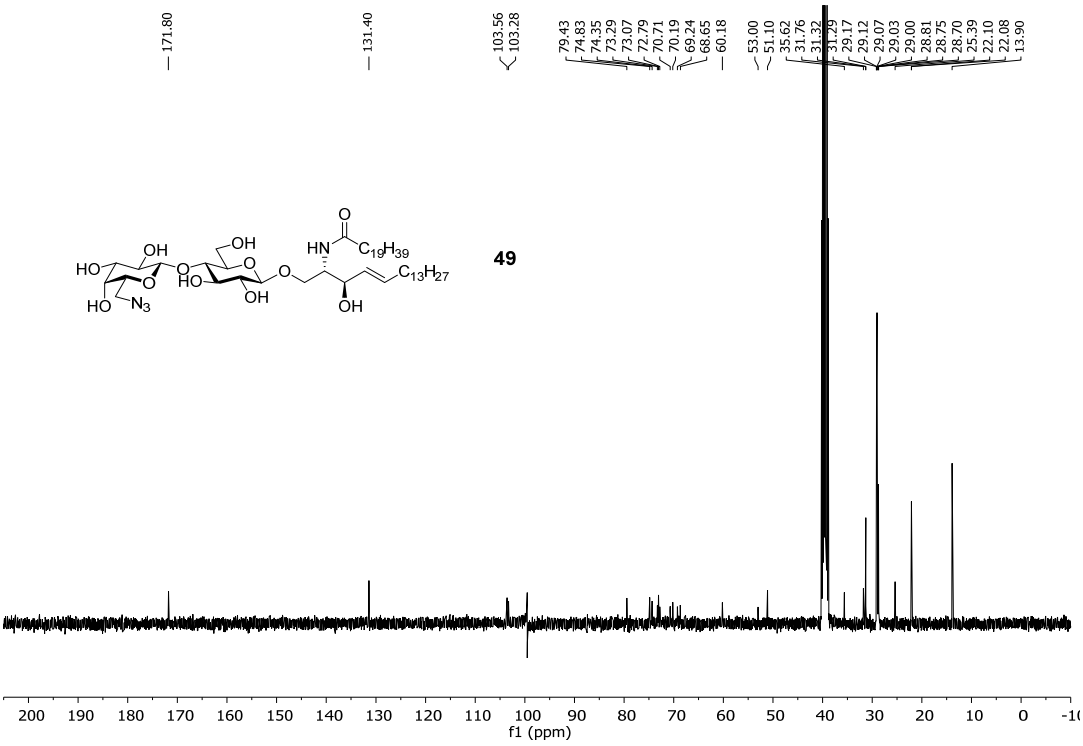

$^{1}$H NMR spectrum (400 MHz, $CD_3OD$) of compound **48**.




$^{13}$C NMR spectrum (100.6 MHz, $CD_3OD$) of compound **48**.




$^{1}$H NMR spectrum (400 MHz, DMSO) of compound **49**.




$^{13}$C NMR spectrum (100.6 MHz, DMSO) of compound **49**.