Supporting Information

Cell-cell interactions via non-covalent click chemistry

Chad Plumet,[a] Achmet Said Mohamed,[a] Tanguy Vendeuvre,[b] Brigitte Renoux,[a] Jonathan Clarhaut,[a, b] and Sébastien Papot*[a]

[a] *University of Poitiers, UMR-CNRS 7285, Institut de Chimie des milieux et des Matériaux de Poitiers (IC2MP), rue Michel Brunet, TSA 51106, 86073 Poitiers cedex 9, France.*

**E-mail: sebastien.papot@univ-poitiers.fr*

[b] *CHU de Poitiers ; F-86021, Poitiers, France.*

Table of Contents

I. Chemistry Section

I.1. General experimental methods

All reactions were performed under an argon atmosphere. Unless otherwise stated, solvents used were of HPLC quality. Chemicals were of analytical grade from commercial sources and were used without further purification. The reaction progress was monitored either by liquid chromatography, liquid chromatography coupled mass spectroscopy or on precoated silica gel TLC plates MACHEREY-NAGEL ALUGRAM® SIL G/UV254. (0.2 mm silica gel 60). Spots were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of phosphomolybdic acid (3 g) in ethanol (100 mL) followed by heating with a heat gun.

Automatic chromatographies were performed with a COMBIFLASH® RF 200I TELEDYNE ISCO instrument equipped with UV and ESLD detector and using flash cartridges Interchim® silica 15 or 50 μm for normal phase chromatography and HP C18 RediSep® GOLD 4g, 15.5g or 30g for reverse phase chromatography. ^{1}H, ^{13}C NMR spectra were respectively recorded at 500 MHz and 125 MHz on a Bruker 400 Avance III instrument, equipped with an ultra-shielded magnet and a BBFO 5 mm broadband probe. Chemical shifts (δ) are reported in parts per million (ppm) from low to high field and referenced to residual solvent. Coupling constants (*J*) are reported in hertz (Hz).

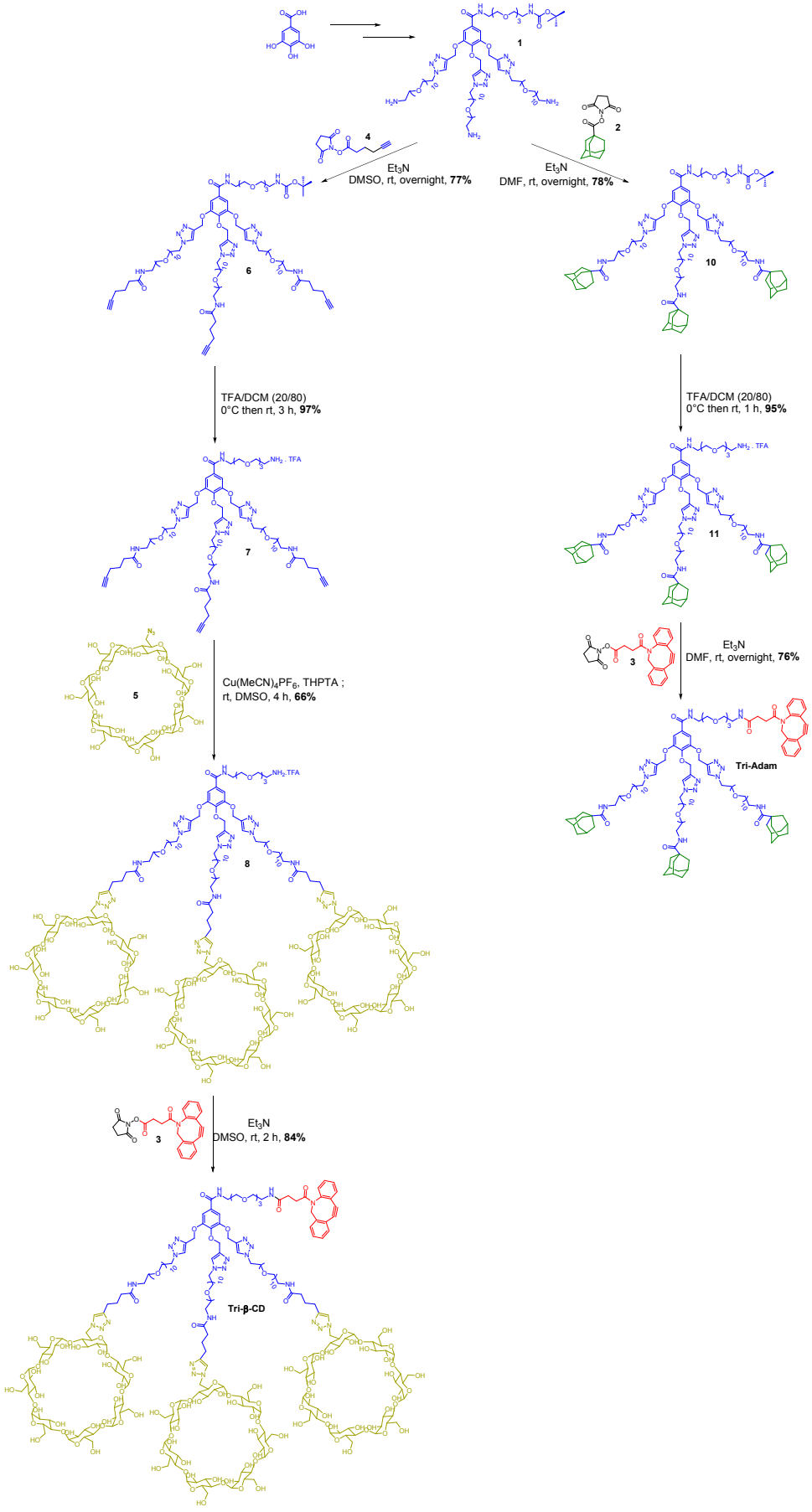
Accurate mass was determined for all derivatives through their infusion on high resolution ESI mass spectrometers in the CBM/ICOA FR2708, at the University of Orléans and in the Organic Analysis Center of IC2MP at University of Poitiers.

Analytical RP-HPLC was carried out on a Dionex Ultimate 3000 system equipped with a UV/Visible variable wavelength detector and with a reverse-phase column chromatography MACHEREY-NAGEL NUCLEOSHELL® (150/4.6, RP18, 5 μm) at 30°C and 1.2 mL.min-1. Method 1 used a linear gradient composed of A (0.2% TFA in water) and B (CH_3CN) beginning with 20% of B and reaching 100 % of B within 15 min. All chromatograms were recorded at 254 nm.

Analytical LC-MS was performed on a Shimadzu LCMS-2020. A reverse-phase column chromatography MACHEREY-NAGEL NUCLEOSHELL® (150/4.6, RP18, 5 μm) at 40°C was used for chromatographic separation at a flow rate of 1 mL.min-1. The column effluent was introduced into the electrospray ionisation source (ESI) of the mass spectrometer. Analyses were performed in positive and negative ion mode. The electrospray voltage was set at 4.5 kV. The capillary and heater temperatures were 250°C and 400°C respectively. The drying gas (nitrogen) and nebulizing gas (nitrogen) flow were set at 15 L.min-1 and 1.5

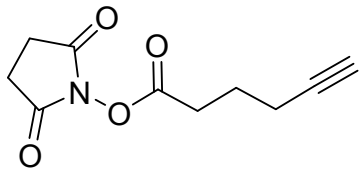
L.min-1 respectively. Analysis of data was performed with LabSolutions software. LC/MS experiments were performed using a linear gradient composed of A (0.1% formic acid in water) and B (0.1% formic acid in CH_3CN) starting from 10 or 20% of B and reaching 100% of B within 15 min.

I.2. Synthetic overviews of cell surface markers Tri-β-CD and Tri-Adam.



I.3. Synthetic procedures and characterization details.

Compound **4**



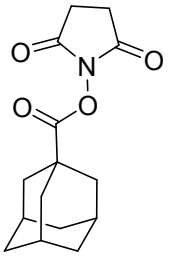
500 mg (4.462 mmol; 1 eq.) of 5-hexynoïc acid were dissolved in anhydrous CH_2Cl_2 (30 mL) under argon. 860 mg (4.462 mmol; 1 eq.) of EDC.HCl and 510 mg (4.462 mmol; 1 eq.) of N-hydroxysuccinimide were then added. The mixture was allowed to stir overnight at room temperature and monitored by TLC (PE/EtOAc: 70/30). After completion, the crude was diluted with CH_2Cl_2 (65 mL) and washed successively with water (65 mL) and brine (65 mL). The organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by chromatography on silica gel (gradient elution PE/EtOAc: 100/0 to 55/45) afforded compound **1** (798 mg; 86%) as a yellow oil.

RMN ^{1}H (500 MHz, $CDCl_3$, δ ppm): 2.84 (bs, 4H), 2.77 (t, 2H, *J* = 7.6 Hz), 2.35 (td, 2H, *J* = 7.2 Hz, *J* = 2.8 Hz), 2,01 (t, 1H, *J* = 2.8 Hz), 1.96 (quint, 2H, *J* = 7.2 Hz).

RMN ^{13}C (125 MHz, $CDCl_3$, δ ppm): 169.08, 168.18, 82.41, 69.84, 29.67, 25.59, 23.34, 17.60.

HRMS (ESI+): m/z 232.057881 (calcd. for $C_{10}H_{11}NaO_4$ 232.058029 $[M+Na]^+$); 248.031641 (calcd. for $C_{10}H_{11}KO_4$ 248.031966 $[M+K]^+$).

Compound **2**[1]



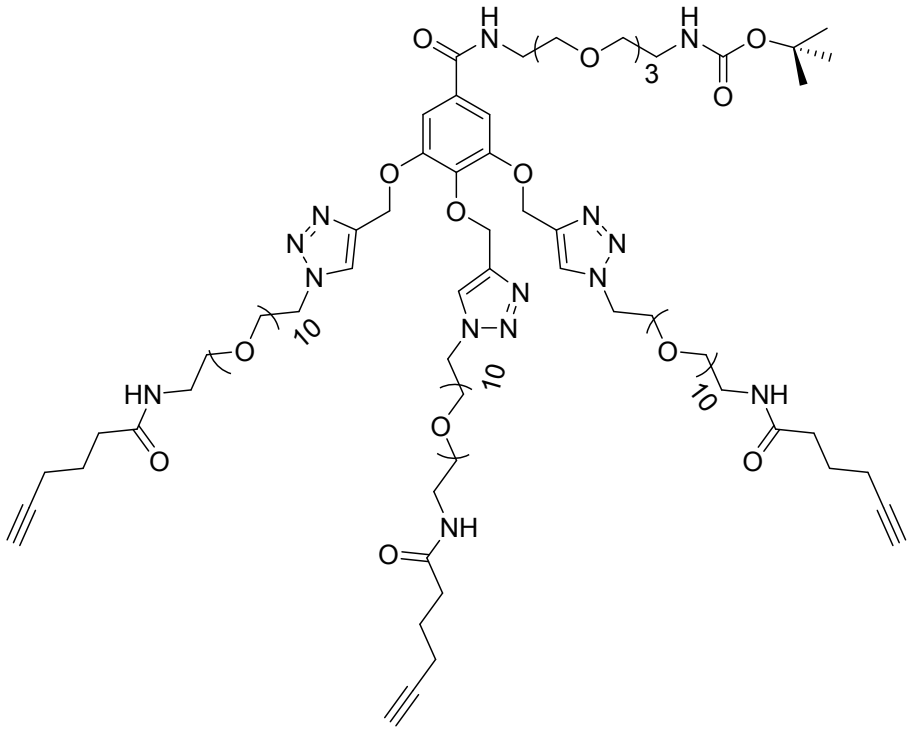
1.0 g (5.032 mmol; 1 eq.) of commercially available 1-adamantanecarbonyl chloride was dissolved in anhydrous CH_2Cl_2 and allowed to stir under argon. 579 mg (5.032 mmol; 1 eq.) of N-hydroxysuccinimide and 965 mg (5.032 mmol; 1 eq.) of EDC.HCl were then added. The mixture was allowed to stir overnight at room temperature and monitored by TLC (PE/EtOAc: 70/30). After completion, the crude was diluted with 100 mL of CH_2Cl_2 and washed successively with distilled water (100 mL) and brine (100 mL). The organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Precipitation of the product in petroleum ether followed by filtration afforded compound **2** (1.36 g; 97%) as a white solid.

RMN ^{1}H (500 MHz, $CDCl_3$, δ ppm): 2.82 (d, *J* = 6.0 Hz, 4H), 2.08 (s, 9H), 1.80 – 1.71 (m, 6H).

RMN ^{13}C (125 MHz, $CDCl_3$, δ ppm): 172.47, 169.46, 40.59, 38.50, 36.27, 31.12, 27.70, 25.77.

[1] Hiroaki, K.; Misa, J.; Naomi, H. *ACS Applied Bio Materials* **2020**, 3, 8, 4902-4911.

Compound **6**



To a solution of N-hydroxysuccinimide ester **4** (213 mg; 1.02 mmol; 3.5 eq.) in dry DMSO (3.5 mL) was added triamine **1**[2] (622 mg; 0.291 mmol; 1 eq.) and Et_3N (122 µL; 0.870 mmol; 3 eq.). The mixture was stirred at room temperature overnight under argon and monitored by LC/MS. The solvent was then removed and the crude mixture was purified over silica gel chromatography (gradient elution H_2O (0.05 % TFA)/MeCN: 90/10 to 50/50) to give trialkyne **6** (540 mg; 77%) as a brownish oil.

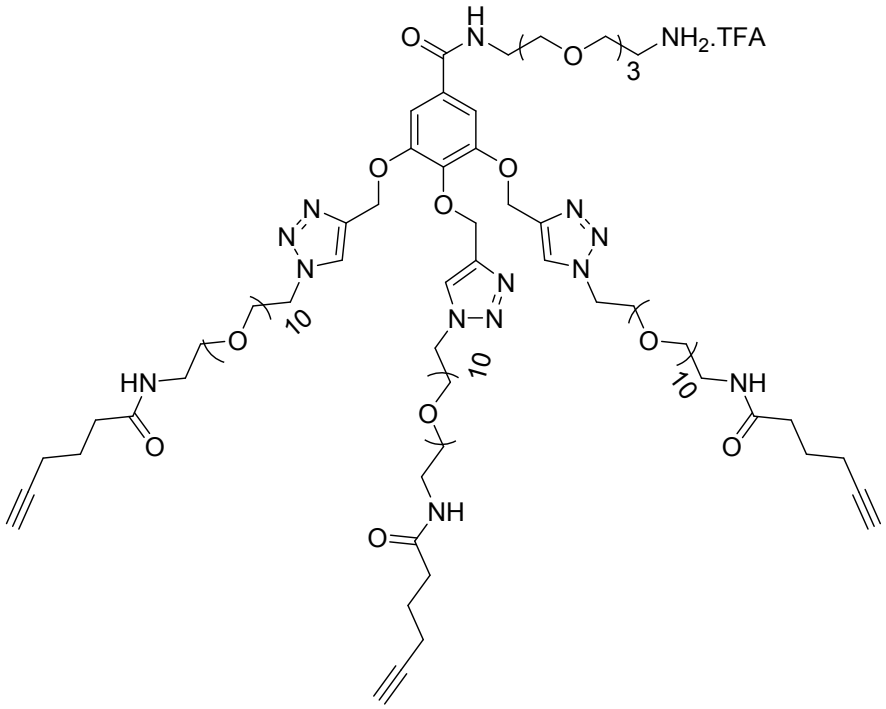
RMN ^{1}H (500 MHz, CD_2Cl_2, δ ppm): 7.96 (s, 2H), 7.83 (s, 1H), 7.28 (s, 2H), 7.04 (bs, 1H), 6.38 (bs, 3H), 5.21 (s, 4H), 5.12 (s, 2H), 4.54 (t, *J* = 5.1 Hz, 4H), 4.47 (t, *J* = 5.3 Hz, 2H), 3.87 (t, *J* = 5.1 Hz, 4H), 3.82 (t, *J* = 5.3 Hz, 2H), 3.61 – 3.50 (m, 127H), 3.46 (t, *J* = 5.3 Hz, 3H), 3.38 (dd, *J* = 10.6, 5.3 Hz, 6H), 3.20 (q, *J* = 5.4 Hz, 2H), 2.57 (bs, 1H), 2.24 (t, *J* = 7.4 Hz, 6H), 2.19 (td, *J* = 7.1 Hz, *J* = 2.4 Hz, 6H), 2.03 (t, *J* = 2.4 Hz, 3H), 1.77 (quint, *J* = 7.1 Hz, *J* = 7.4 Hz, 6H), 1.37 (s, 9H).

RMN ^{13}C (125 MHz, CD_2Cl_2, δ ppm): 172.23, 166.71, 156.26, 152.48, 144.31, 143.54, 140.39, 130.70, 125.00, 124.82, 107.31, 84.07, 70.81, 70.77, 70.74, 70.69, 70.65, 70.53, 70.39, 70.12, 69.7, 69.72, 69.16, 66.55, 63.30, 50.61, 50.38, 40.34, 39.49, 35.20, 29.98, 28.47, 24.69, 18.14.

[2] Châtre, R.; Lange, J.; Péraudeau, E.; Poinot, P.; Lerondel, S.; Le Pape, A.; Clarhaut, J.; Renoux, B.; Papot, S. *Journal of Controlled Release* **2020**, 327, 19-2.

HRMS (ESI+): m/z 1232.664308 (calcd. for $C_{113}H_{194}N_{14}Na_2O_{42}$ 1232.662974 $[M+2Na]^+$); m/z 1248.637450 (calcd. for $C_{113}H_{194}K_2N_{14}O_{42}$ 1232.636911 $[M+2K]^+$).

Compound **7**



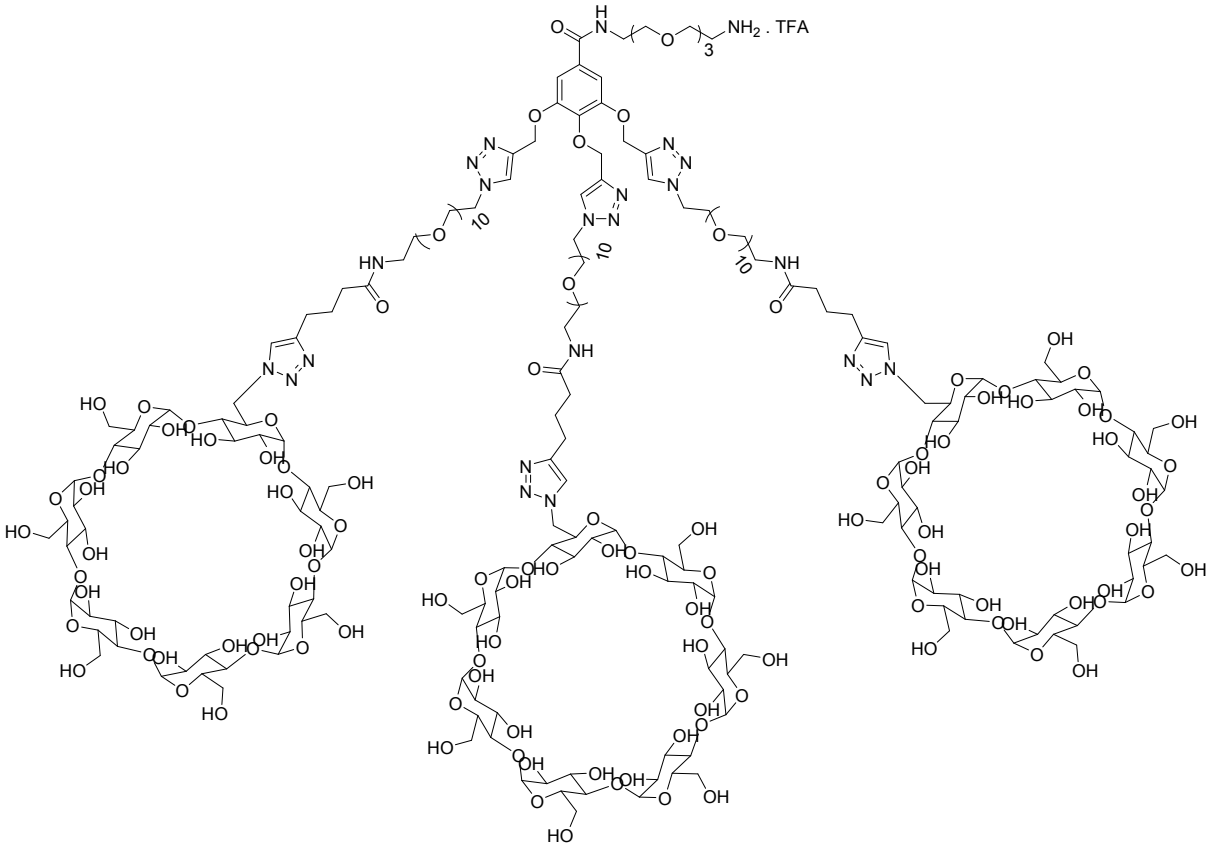
To a cooled solution (0°C) of compound **6** (200 mg; 0,0827 mmol; 1 eq.) in dry CH_2Cl_2 (2 mL) was added TFA (0.5 mL). The mixture was allowed to stir under argon during 3 hours and monitored by LC/MS. After completion, the solvent was removed under reduced pressure. The crude product was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 90/10 to 50/50) affording **7** (187 mg; 93%) as a colorless oil.

RMN ^{1}H (500 MHz, CD_2Cl_2, δ ppm): 8.01 (s, 2H), 7.91 (s, H), 7.32 (s, 2H), 7.76 (s, 1H), 7.67 (s, 2H), 6.56 (bs, 3H), 5.22 (s, 4H), 5.17 (s, 2H), 4.57 (t, *J* = 5.1 Hz), 4.52 (t, *J* = 5.3 Hz), 3.89 (t, *J* = 5.1 Hz, 4H), 3.85 (t, *J* = 5.3 Hz, 2H), 3.63 – 3.51 (m, 134H), 3.41 – 3.38 (t, 6H), 3.13 (bs, 2H), 2.29 (t, *J* = 7.4 Hz, 6H), 2.23 (td, *J* = 7.1 Hz, *J* = 2.4 Hz, 6H), 2.03 (t, *J* = 2.4 Hz, 3H), 1.80 (quint, *J* = 7.1 Hz, *J* = 7.4 Hz, 6H).

RMN ^{13}C (125 MHz, CD_2Cl_2, δ ppm): 172.51, 152.31, 144.11, 143.55, 139.87, 130.60, 125.37, 125.10, 107.41, 84.13, 70.81, 70.73, 70.63, 70.61, 70.53, 70.50, 70.25, 70.20, 69.66, 69.61, 69.14, 67.18, 66.01, 62.88, 50.69, 50.47, 40.31, 40.08, 39.48, 35.18, 24.73, 18.15.

HRMS (ESI+): m/z 2320.308699 (calcd. for $C_{108}H_{187}N_{14}NaO_{40}$ 2320.302353 $[M+H]^+$); m/z 2342.289855 (calcd. for $C_{108}H_{186}N_{14}O_{40}$ 2342.284298 $[M+Na]^+$); m/z 2358.268514 (calcd. for $C_{108}H_{186}KN_{14}O_{40}$ 2358.258235 $[M+K]^+$).

Compound **8**



Previously reported 6-monoazido-6-monodeoxy-β-cyclodextrin **5**[3] (192 mg; 0.166 mmol; 4 eq.) and deprotected compound **7** (101 mg; 0.0415 mmol; 1 eq.) were dissolved in dry DMSO (3 mL). Then the mixture was degassed and put under an argon atmosphere. THPTA (53.9 mg; 0.124 mmol; 3 eq.) followed by $Cu(MeCN)_4PF_6$ (46.4 mg; 0.124 mmol; 3 eq.) were added and the mixture was stirred for 5 hours at room temperature. The reaction was monitored by LC/MS. After completion, 700 mg of resin QuadraPure® IDA was added to the mixture which was allowed to stir for 2 additional hours. The solvent was then removed and the crude material was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 90/10 to 50/50) to give compound **8** (160.8 mg; 66%) as a white solid.

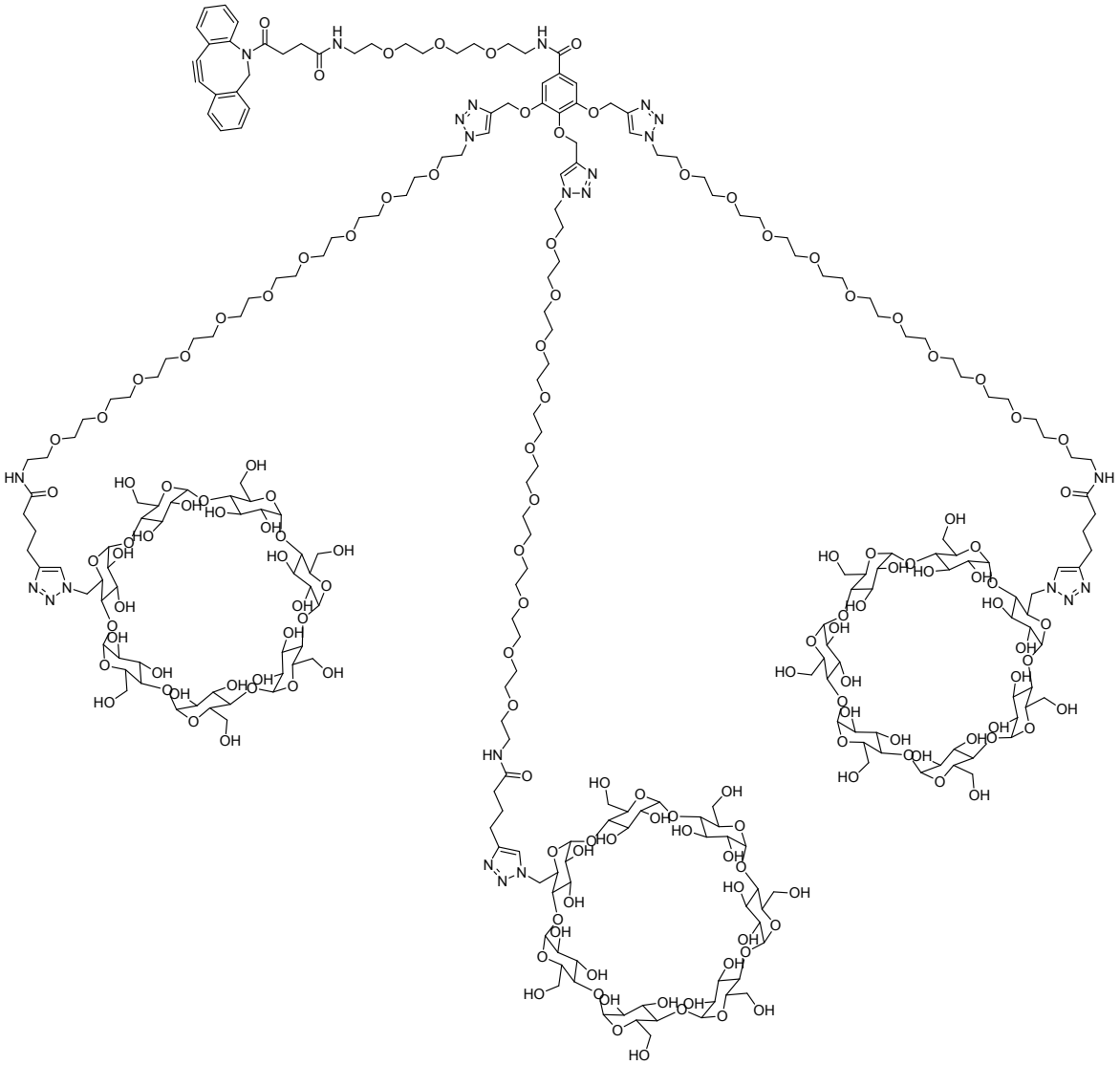
RMN ^{1}H (500 MHz, CD_3OD, δ ppm): 8.24 (s, 2H), 7.98 (s, 1H), 7.85 (s, 3H), 7.42 (s, 2H), 5.28 (s, 4H), 5.18 (s, 2H), 5.14 (d, *J* = 3.4 Hz, 4H), 5.00 – 4.84 (m), 4.66 (t, *J* = 5.1 Hz, 4H), 4.62 (t, *J* = 5.3 Hz, 2H), 4.57 (t, *J* = 5.1 Hz, 4H), 4.13 (t, *J* = 5.3 Hz, 2H), 4.05 – 3.41 (m, 292H), 3.38 (t, *J* = 5.1 Hz, 6H), 3.14-3.07 (m, *J* = 3.9 Hz, 4H), 2.76 (t, *J* = 7.1 Hz, 6H), 2.31 (t, *J* = 7.4 Hz, 6H), 2.00 (quint, *J* = 7.1 Hz, *J* = 7.4 Hz, 6H).

[3] Jicsinsky, L.; Ivànyi, R. Carbohydrate Polymers, **2001**, 45, 139-145.

RMN ^{13}C (125 MHz, CD_3OD, δ ppm): 174.24, 167.51, 158.93, 152.18, 146.84, 143.05, 140.03, 129.82, 125.32, 124.14, 107.14, 102.63, 102.47, 101.94, 83.70, 81.83, 81.62, 81.44, 81.21, 73.43, 73.36, 73.32, 73.11, 72.81, 72.66, 72.52, 72.29, 71.98, 70.67, 70.13, 70.07, 69.99, 69.93, 69.84, 69.16, 68.94, 66.58, 62.41, 60.42, 59.64, 50.96, 50.15, 39.45, 38.99, 34.97, 25.27, 24.38.

HRMS (ESI+): m/z 1160.4954 (calcd. for $C_{234}H_{397}N_{23}O_{142}$ 1160.4920 $[M+5H]^+$).

Tri-β-CD



To a solution of compound **8** (20 mg; 0.0034 mmol; 1 eq.) in dry DMSO (2 mL) was added commercially available Dibenzocyclooctyne-N-hydroxysuccinimidyl ester (2.8 mg; 0.0069 mmol; 2 eq.) and Et_3N (2.4 µL; 0.017 mmol; 5 eq). The mixture was stirred at room temperature under argon atmosphere and progress was followed by LC/MS. After 3 hours of reaction, the solvent was removed under reduced pressure and the crude material was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 90/10 to 50/50). **Tri-β-CD** (17.5 mg; 84%) was obtained as a white solid.

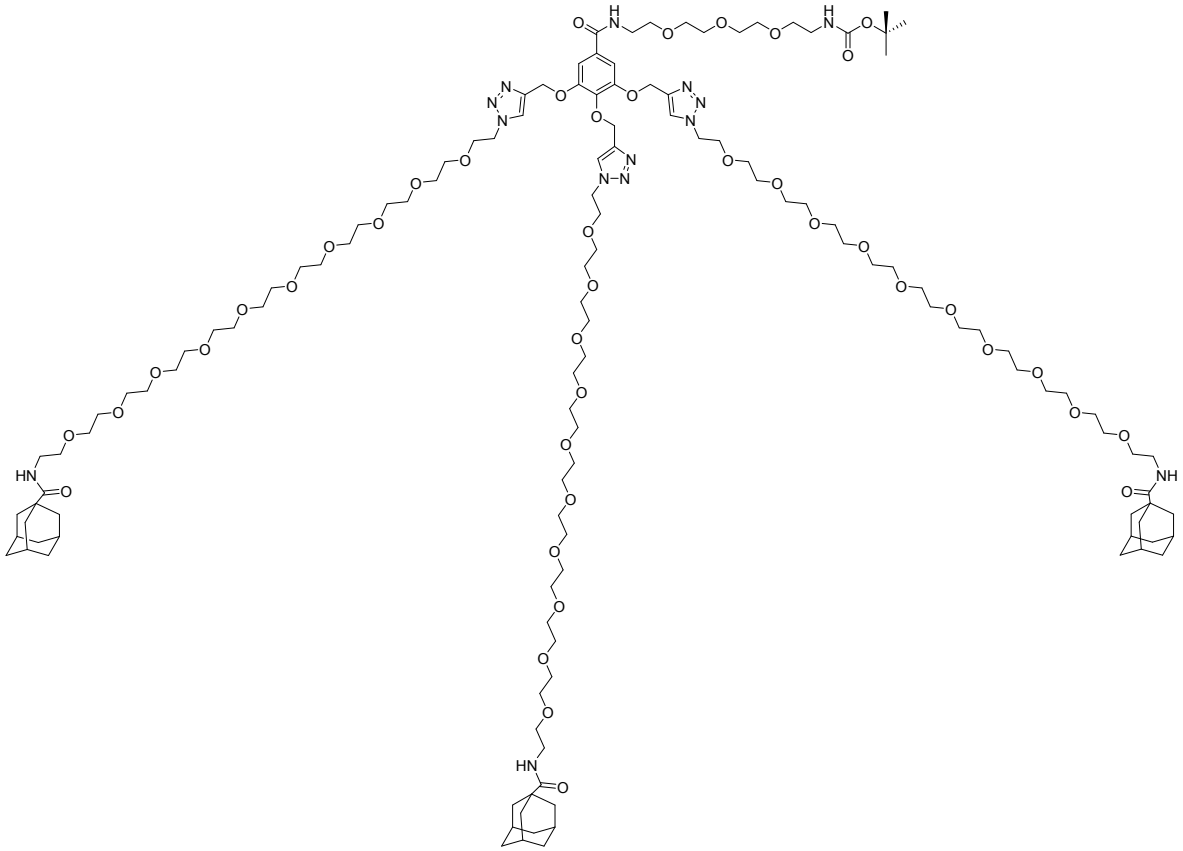
RMN ^{1}H (500 MHz, DMSO-d_6, δ ppm): 8.19 (s, 2H), 7.95 (s, 1H), 7.91 (s, 1H), 7.79 (s, 3H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.51 – 7,44 (m, 3H), 7.38 (s, 2H), 7.36 (td, *J* = 7.4, 1.7 Hz, 1H), 7.33 (td, *J* = 7.5, 1.3 Hz, 5H), 7.29 (dd, *J* = 7.4, 1.4 Hz, 4H), 5.18 (s, 4H), 5.04 (s, 2H), 5.03 (d, 4H), 5.00 (s, 1H), 4.82 – 4,86 (m, 14H), 4.81 – 4,79 (m, 6H), 4.76 –

4.74 (m, 4H), 4.55 (t, *J* = 5.0 Hz, 4H), 4.51 – 4,46 (m, 5H), 3.99 (t, *J* = 8.8 Hz, 2H), 3.83 (t, *J* = 5.1 Hz, 4H), 3.77 (t, *J* = 5.2 Hz, 2H), 3.75 – 3.22 (m, 254H), 3.19 (t, *J* = 5.8 Hz, 6H), 3.08 – 3.05 (m, 4H), 2.86 (d, *J* = 10.4 Hz, 2H), 2.61 – 2.53 (m, 7H), 2.26 – 2.20 (m, 1H), 2.14 (t, *J* = 7.4 Hz, 6H), 2.02 – 1.96 (m, 1H), 1.80 (quint, 6H), 1.73 (m, 1H).

RMN ^{13}C (125 MHz, DMSO-d$_6$, δ ppm): 172.50, 172.42, 171.51, 152.05, 148.90, 148.90, 146.79, 143.69, 143.69, 142.94, 139.55, 132.90, 132.90, 130.10, 129.40, 129.40, 128.61, 128.47, 128.16, 127.26, 127.26, 125.63, 125.29, 123.29, 122.99, 121.85, 121.85, 108.62, 107.15, 102.46, 102.32, 101.54, 83.97, 82.49, 81.94, 81.82, 81.12, 73.28, 72.67, 72.51, 70.21, 70.06, 70.01, 69.99, 69.57, 69.14, 65.99, 62.75, 60.21, 55.33, 50.70, 49.91, 49.76, 48.41, 48.24, 48.07, 47.91, 47.74, 40.52, 40.35, 40.18, 40.01, 39.85, 38.92, 38.80, 35.31, 30.14, 25.60, 25.05.

HRMS (ESI+): m/z 1522.136868 (calcd. for $C_{253}H_{410}N_{24}O_{144}$ 1522.136888 $[M+4H]^+$); m/z 1217.908396 (calcd. for $C_{253}H_{411}N_{24}O_{144}$ 1217.910965 $[M+5H]^+$)

Compound **10**



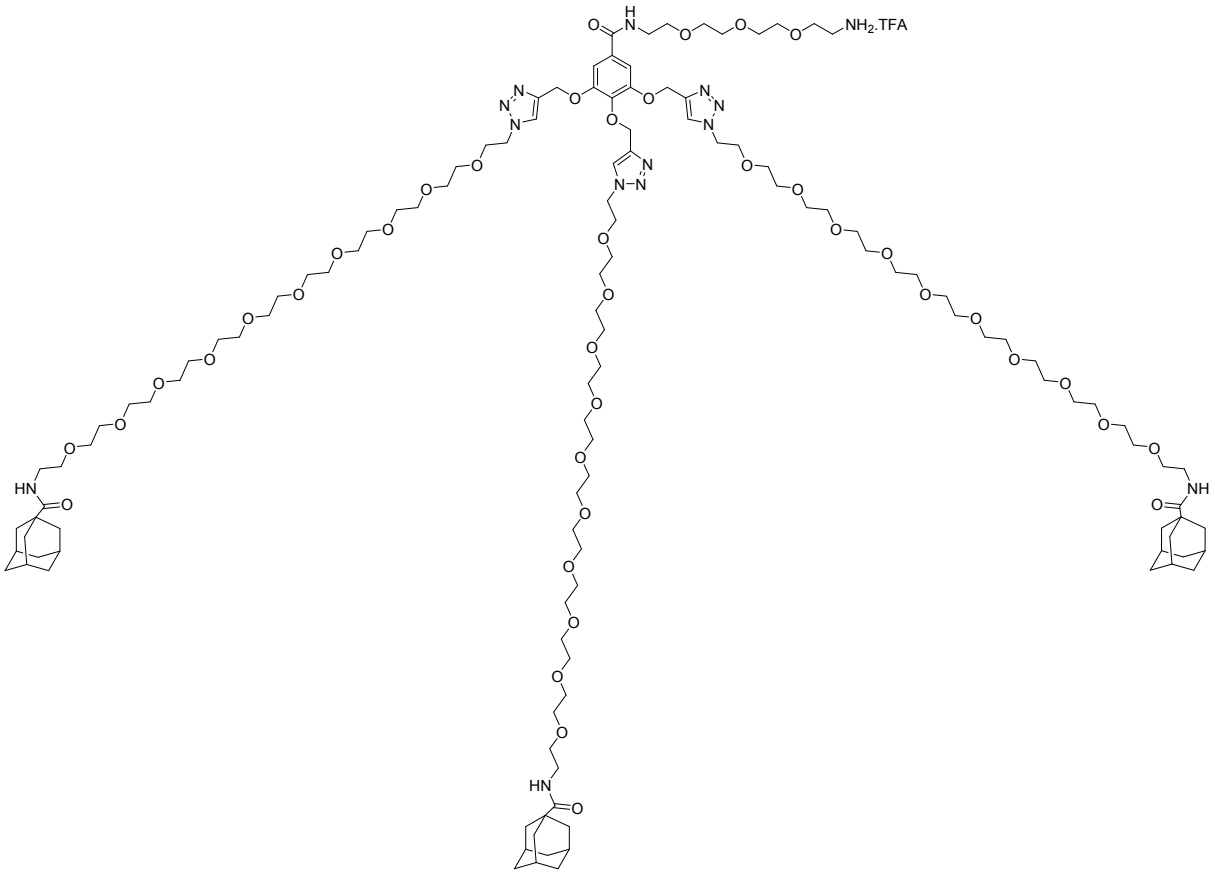
Triamine **1** (58 mg; 0.0271 mmol; 1 eq.) and Adamantane-N-hydrosuccinimidyl ester **2** (22.5 mg; 0.0812; 3 eq.) were dissolved in dry DMF (2 mL). Then Et_3N (18.9 µL; 0.135 mmol; 5 eq) was added. The solution was stirred overnight at room temperature under argon and monitored by LC/MS. After completion, the solvent was evaporated under reduced pressure and the crude was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 70/30 to 40/60) affording compound **10** (55.4 mg; 78%) as a colorless oil.

RMN ^{1}H (500 MHz, CD_2Cl_2, δ ppm): 7.98 (s, 2H), 7.86 (s, 1H), 7.28 (s, 2H), 7.17 (s, 1H), 6.14 (s, 3H), 5.22 (s, 4H), 5.19 (s, 1H), 5.14 (s, 2H), 4.56 (t, *J* = 5.2 Hz, 4H), 4.50 (t, *J* = 5.3 Hz, 2H), 3.89 (t, *J* = 5.1 Hz, 4H), 3.84 (t, *J* = 5.3 Hz, 2H), 3.81 – 3.52 (m, 131H), 3.51 (t, *J* = 5.2 Hz, 6H), 3.46 (t, *J* = 5.2 Hz, 2H), 3.37 (td, *J* = *J* = 10.0, 5.3 Hz, 6H), 3.21 (d, *J* = 3.7 Hz, 2H), 2.00 (bs, 9H), 1.81 (d, *J* = 2.6 Hz, 18H), 1.71 (q, *J* = 12.2 Hz, 18H), 1.40 (s, 9H).

RMN ^{13}C (125 MHz, CD_2Cl_2, δ ppm): 178.14, 166.79, 156.20, 152.43, 144.23, 143.48, 140.23, 130.65, 125.15, 124.94, 107.16, 79.11, 70.79, 70.72, 70.67, 70.65, 70.62, 70.58, 70.50, 70.39, 70.14, 69.69, 69.64, 66.42, 63.11, 50.66, 50.46, 40.81, 40.66, 40.36, 39.46, 39.36, 36.83, 36.70, 28.74, 28.63, 28.47.

HRMS (ESI+): m/z 2624.549345 (calcd. for $C_{128}H_{219}N_{14}O_{42}$ 2624.542584 $[M+H]^+$); m/z 2646.534596 (calcd. for $C_{128}H_{218}N_{14}NaO_{42}$ 2646.524528 $[M+Na]^+$); m/z 1312.774440 (calcd. for $C_{128}H_{220}N_{14}O_{42}$ 1312.774930 $[M+2H]^+$).

Compound **11**



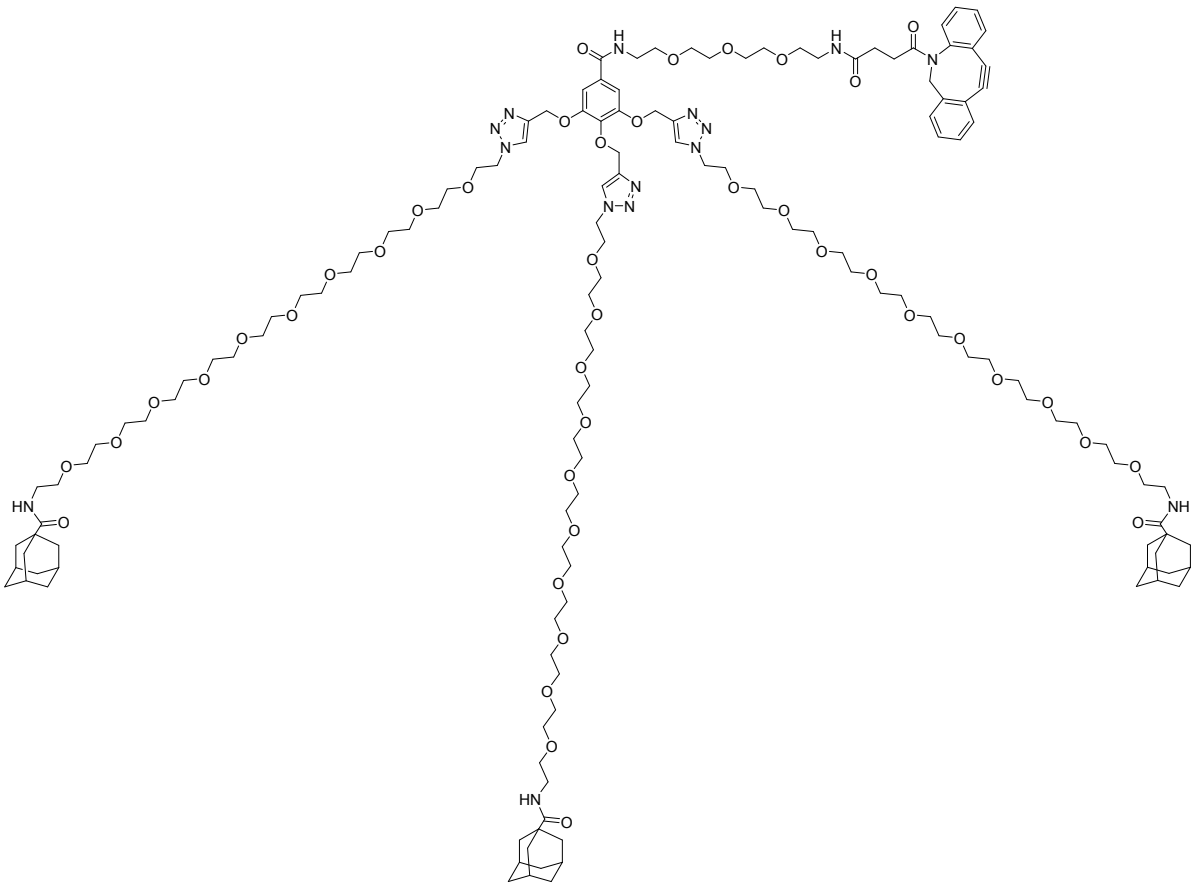
To a cooled solution (0°C) of compound **10** (57 mg; 0,0235 mmol; 1 eq.) in dry CH_2Cl_2 (2 mL) was added TFA (0.5 mL). The mixture was allowed to stir under argon during 1 hour and monitored by LC/MS. Then the solvent was removed under reduced pressure. The crude product was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 70/30 to 40/60) affording **11** (55.1 mg; 96%) as a colorless oil.

RMN ^{1}H (500 MHz, CD_2Cl_2, δ ppm): 8.00 (s, 2H), 7.89 (s, 1H), 7.77 (bs, 3H), 7.33 (s, 2H), 6.15 (bs, 3H), 5.22 (s, 4H), 5.16 (s, 2H), 4.56 (t, *J* = 5.2 Hz, 4H), 4.51 (t, *J* = 5.3 Hz, 2H), 3.89 (t, *J* = 5.1 Hz, 4H), 3.84 (t, *J* = 5.3 Hz, 2H), 3.74 – 3.52 (m, 122H), 3.50 (t, *J* = 5.1 Hz, 6H), 3.37 (td, *J* = 10.0, 4.9 Hz, 28H), 3.13 (s, 2H), 2.00 (bs, 9H), 1.81 (d, *J* = 2.0 Hz, 18H), 1.70 (q, *J* = 12.1 Hz, 18H).

RMN ^{13}C (125 MHz, CD_2Cl_2, δ ppm): 178.45, 152.00, 143.58, 142.99, 139.75, 130.01, 125.18, 124.86, 107.02, 70.40, 70.33, 70.30, 70.23, 70.18, 70.13, 69.99, 69.70, 69.17, 66.80, 65.55, 62.46, 50.43, 50.34, 40.51, 40.05, 39.14, 39.03, 38.47, 36.42, 36.18, 28.34, 28.22, 28.09, 27.83.

HRMS (ESI+): m/z 2524.490093 (calcd. for $C_{123}H_{211}N_{14}O_{40}$ 2524.490154 $[M+H]^+$); m/z 2546.477495 (calcd. for $C_{128}H_{210}N_{14}NaO_{40}$ 2546.472098 $[M+Na]^+$); m/z 1262.746573 (calcd. for $C_{128}H_{212}N_{14}O_{40}$ 1262.748715 $[M+2H]^+$).

Tri-Adam



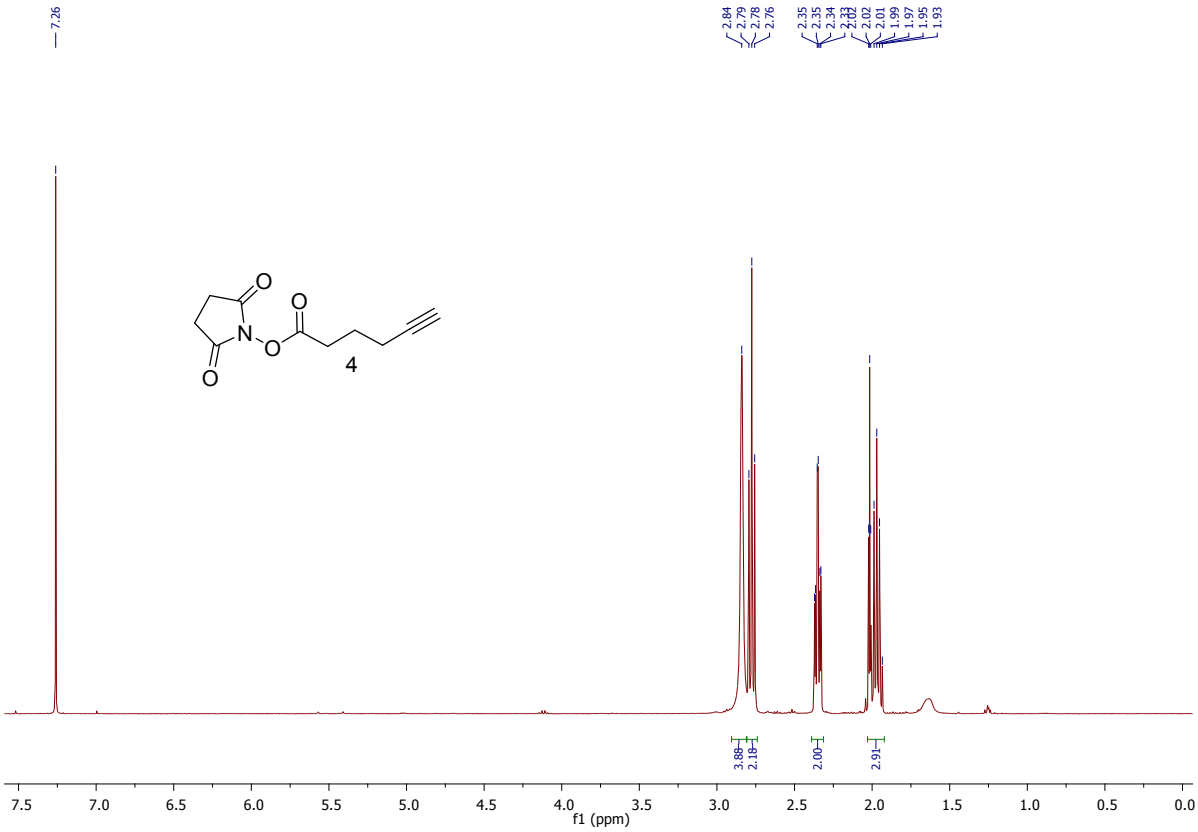
To a solution of compound **11** (51.1 mg; 0.0194 mmol; 1 eq.) in dry DMF (2 mL) was added commercially available Dibenzocyclooctyne-N-hydroxysuccinimidyl ester (7.8 mg; 0.0194 mmol; 1 eq.) and Et_3N (13.5 µL; 0.0968 mmol; 5 eq). The mixture was stirred at room temperature under argon atmosphere overnight and monitored by LC/MS. After completion, the solvent was removed under reduced pressure and the crude material was purified by reverse phase chromatography on C18 grafted silica (gradient elution H_2O (0.05 % TFA)/MeCN: 70/30 to 40/60) to give **Tri-Adam** (41.3 mg; 76%) as a colorless oil.

RMN ^{1}H (500 MHz, CD_2Cl_2, δ ppm): 7.98 (s, 2H), 7.86 (s, 1H), 7.61 – 7.56 (m, 1H), 7.52 – 7.49 (m, 1H), 7.42 – 7.37 (m, 4H), 7.32 (s, 2H), 7.30 – 7.27 (m, 2H), 7.26 – 7.22 (m, 1H), 6.43 (bs, 1H), 6.09 (bs, 3H), 5.21 (s, 4H), 5.14 (s, 2H), 5.10 (s, 0.5H), 5.07 (s, 0.5H), 4.56 (t, *J* = 5.1 Hz, 4H), 4.49 (t, *J* = 5.3 Hz, 2H), 3.89 (t, *J* = 5.1 Hz, 4H), 3.84 (t, *J* = 5.3 Hz, 2H), 3.66 – 3.49 (m, 128H), 3.37 (dd, *J* = 10.6, 5.3 Hz, 6H), 3.24 – 3.18 (m, 1H), 3.11 – 3.05 (m, 1H), 2.81 – 2.72 (m, 1H), 2.39 – 2.33 (m, 1H), 2.13 – 2.07 (m, 1H), 2.00 (s, 9H), 1.91– 1.86 (m, 1H), 1.81 (d, *J* = 2.6 Hz, 18H), 1.71 (q, *J* = 12.2 Hz, 18H).

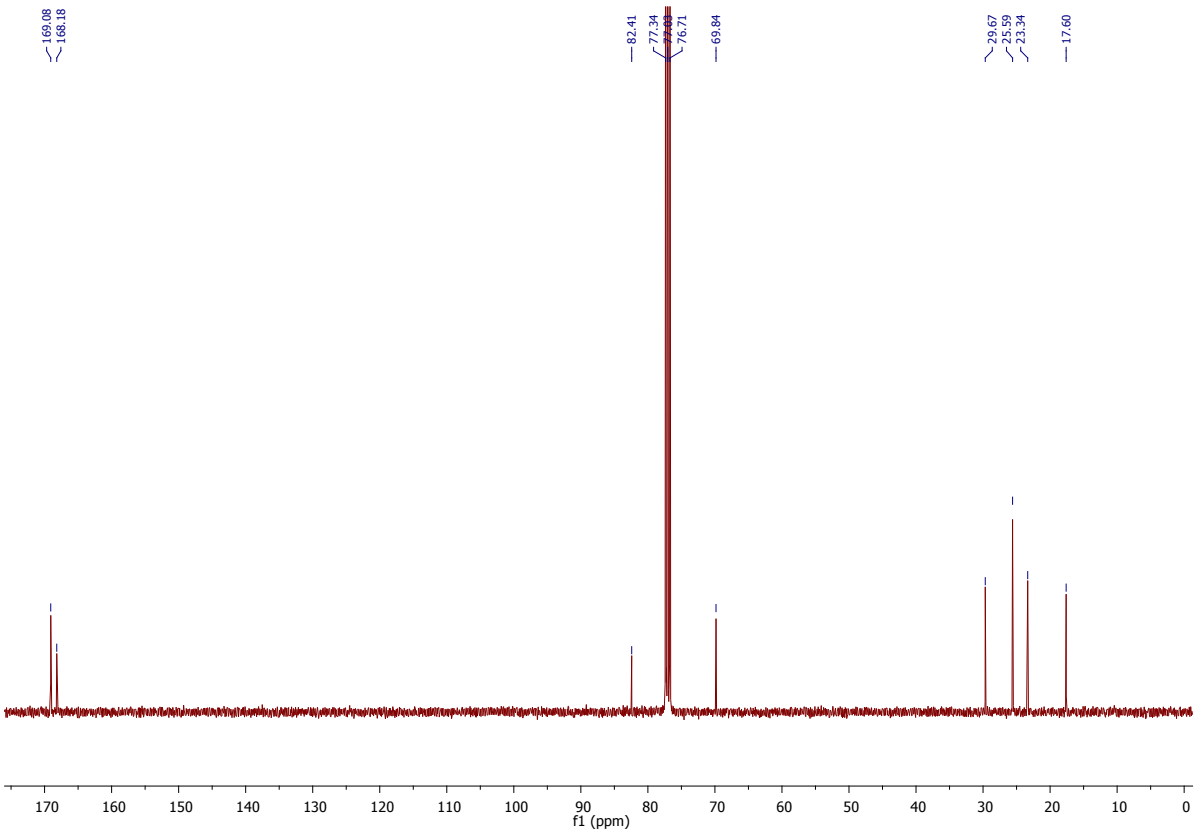
RMN ^{13}C (125 MHz, CD_2Cl_2, δ ppm): 177.77, 172.30, 172.00, 166.37, 152.06, 151.45, 148.44, 143.24, 139.73, 132.26, 130.22, 129.52, 128.66, 128.09, 127.93, 127.63, 126.96, 125.40, 124.81, 124.63, 123.18, 122.33, 114.39, 107.93, 106.77, 70.48, 70.43, 70.37, 70.32, 70.30, 70.22, 70.13, 70.03, 69.88, 69.77, 69.56, 69.37, 69.31, 66.10, 62.71, 55.51, 50.30, 50.07, 40.47, 39.94, 39.13, 39.07, 39.01, 36.50, 30.92, 30.11, 28.30.

HRMS (ESI+): m/z 1428.273027 (calcd. for $C_{142}H_{223}N_{15}Na_2O_{42}$ 1428.277974 $[M+H]^+$); m/z 1444.250642 (calcd. for $C_{142}H_{223}N_{15}Na_2O_{42}$ 1444.251911 $[M+Na]^+$).

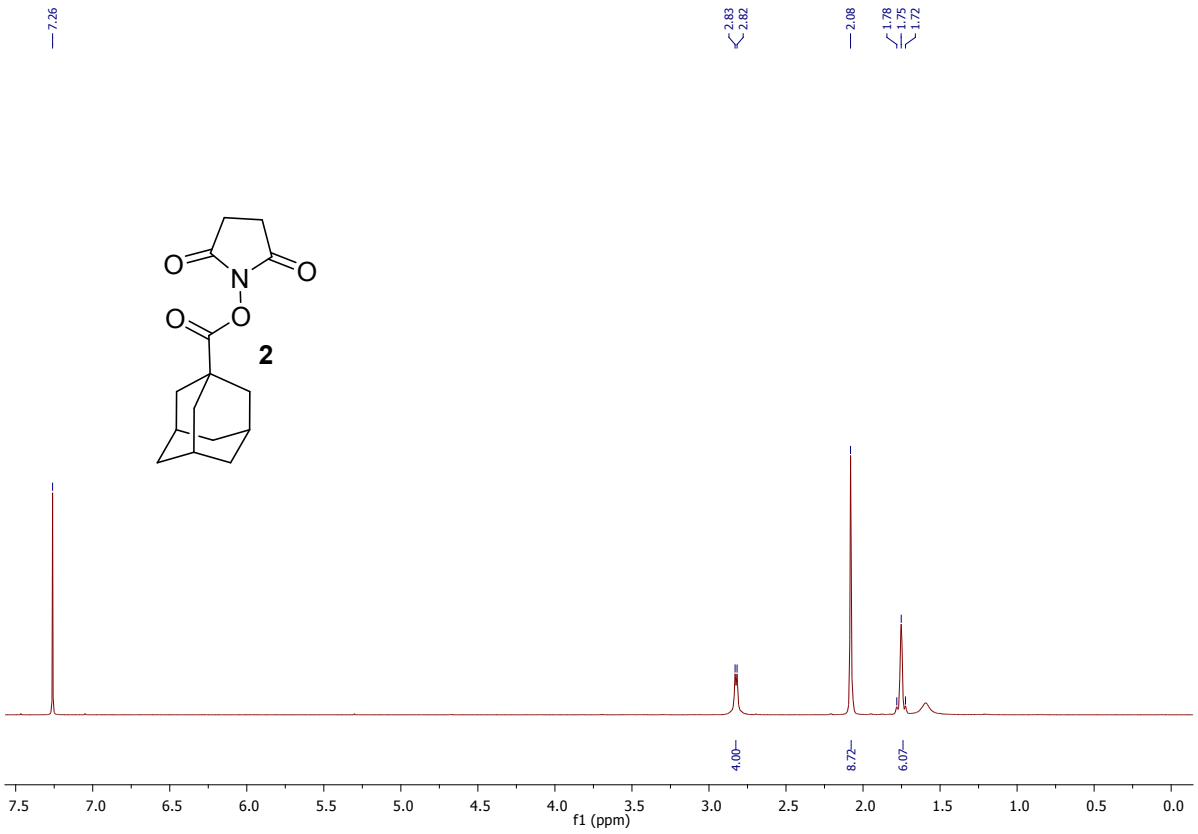
I.6. ^{1}H NMR and ^{13}C NMR plots



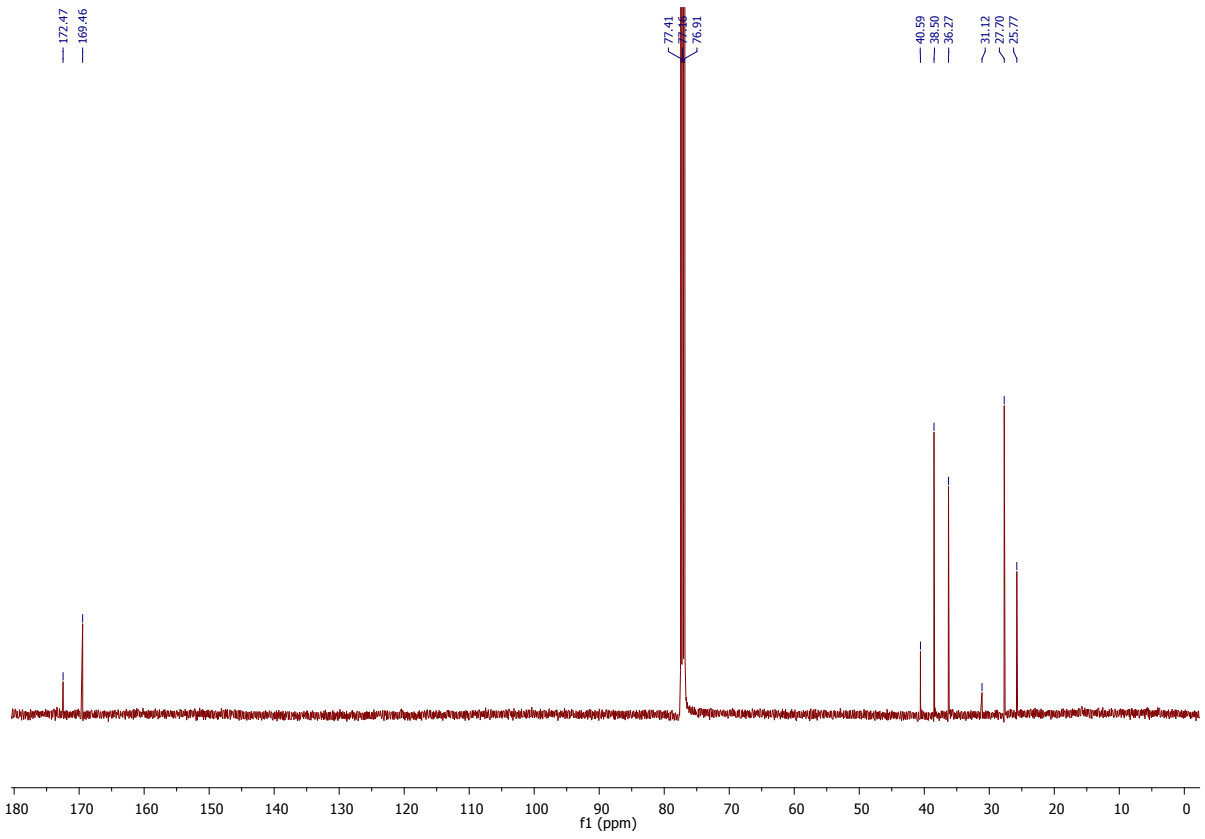
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 4



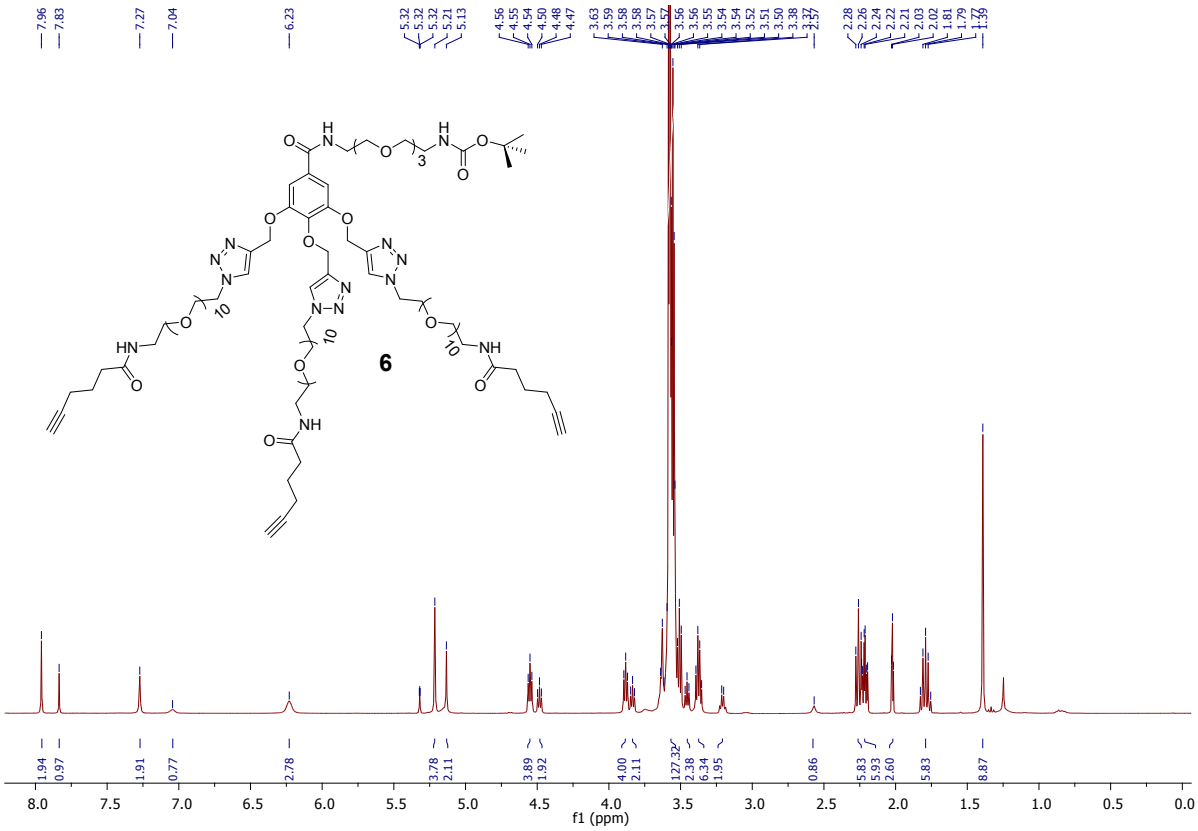
^{13}C NMR spectrum (125 MHz, $CDCl_3$) of 4



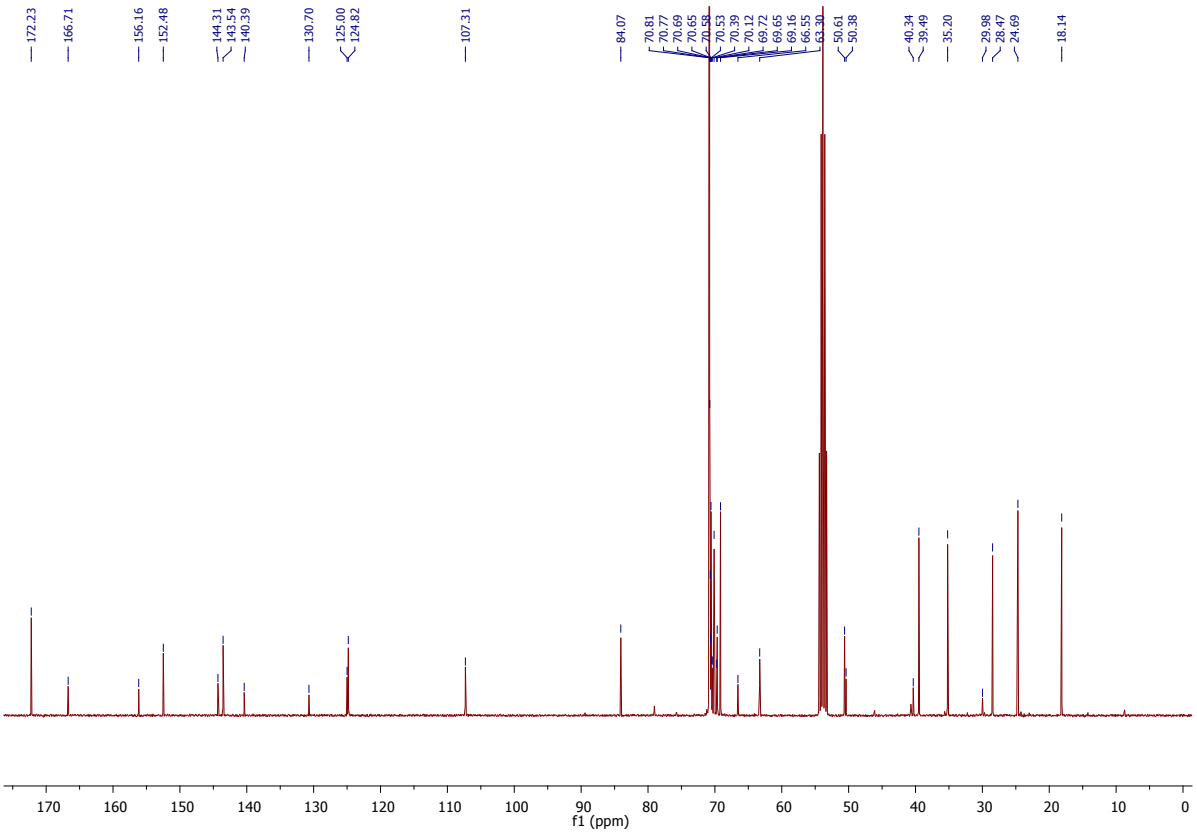
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of 2



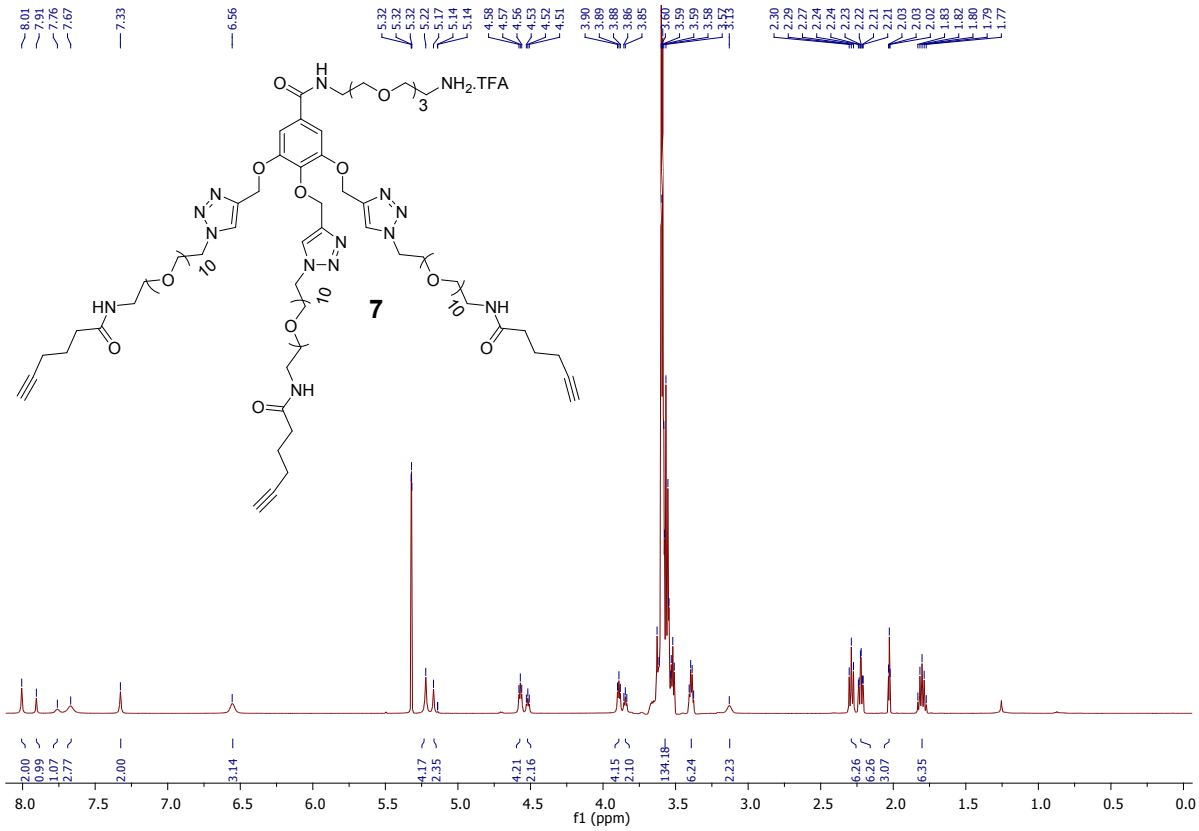
^{13}C NMR spectrum (500 MHz, $CDCl_3$) of 2



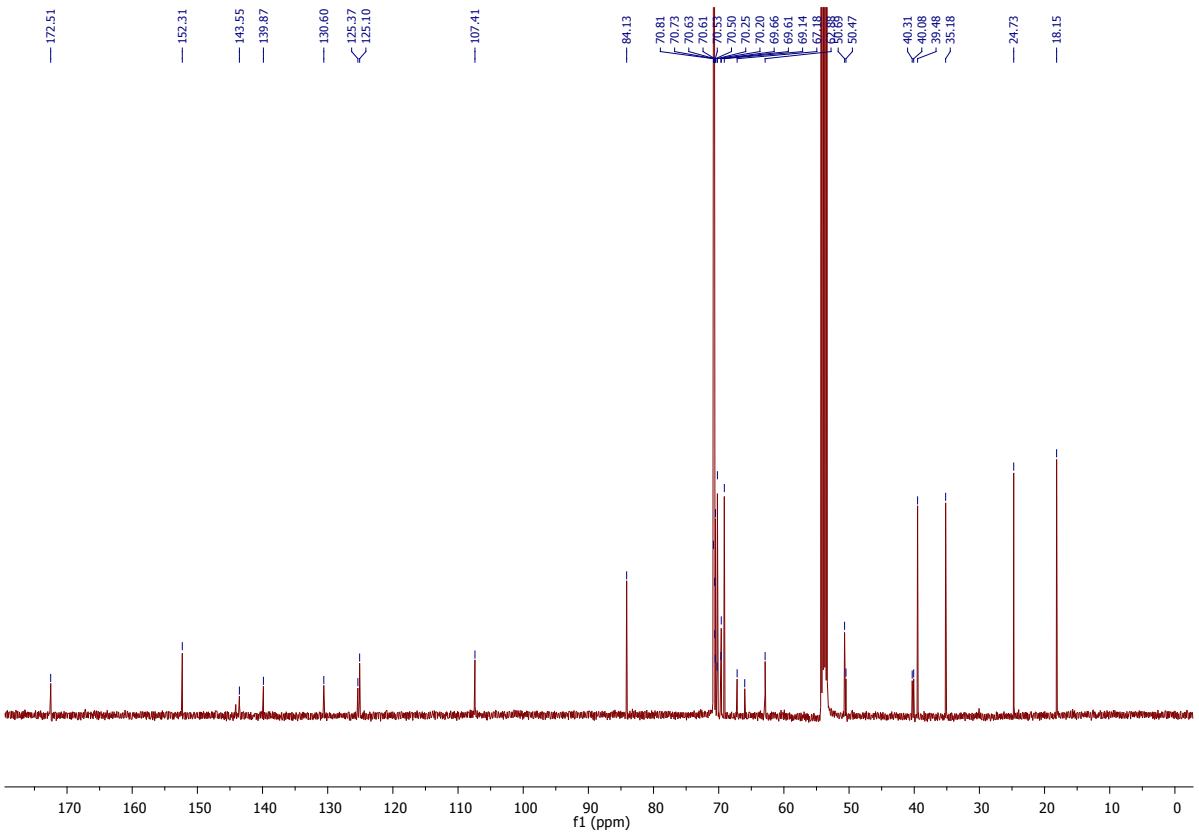
^{1}H NMR spectrum (500 MHz, CD_2Cl_2) of 6



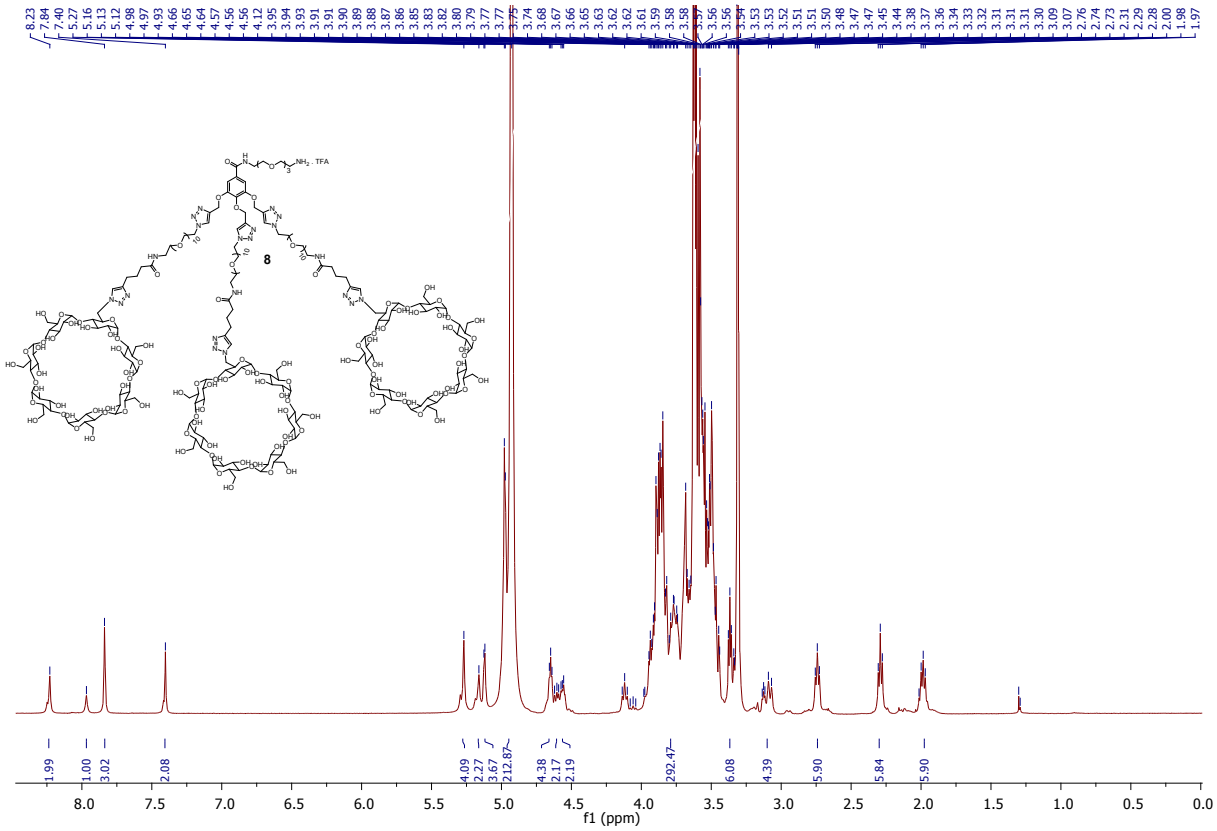
^{13}C NMR spectrum (125 MHz, CD_2Cl_2) of 6



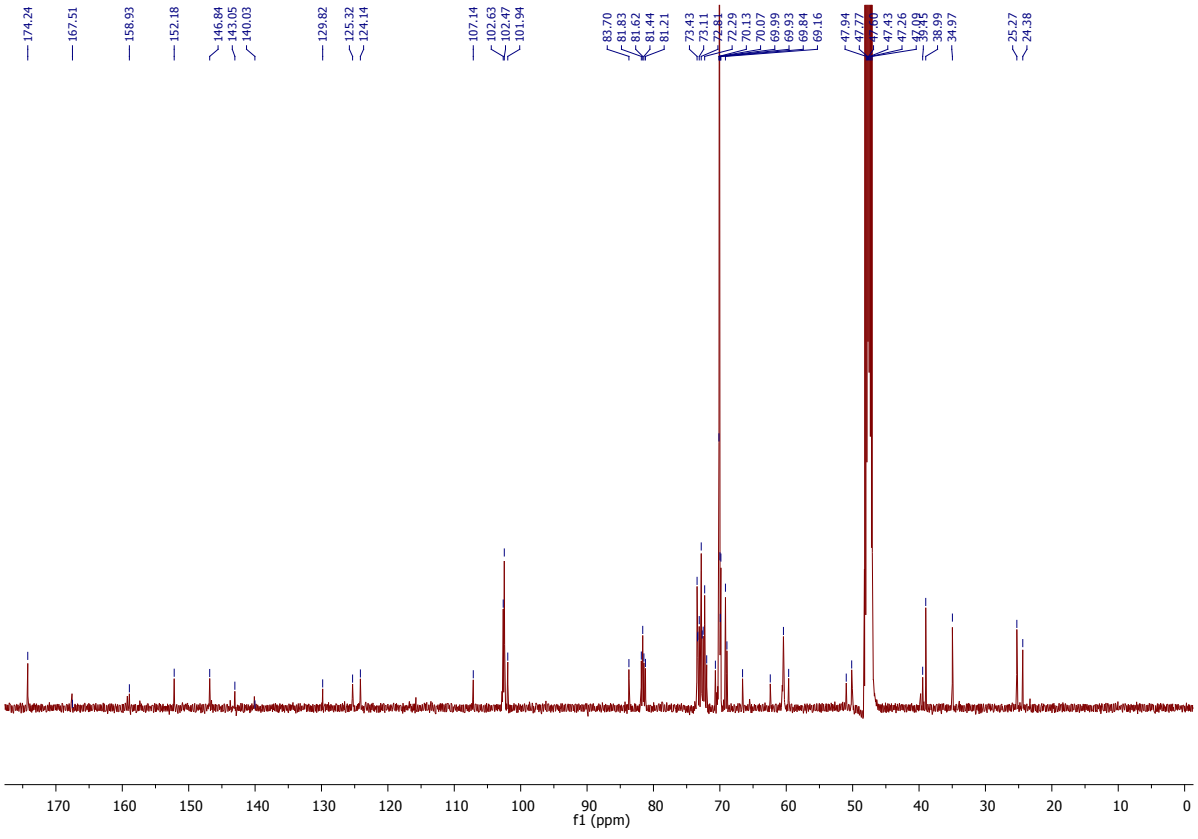
^{1}H NMR spectrum (500 MHz, CD_2Cl_2) of 7



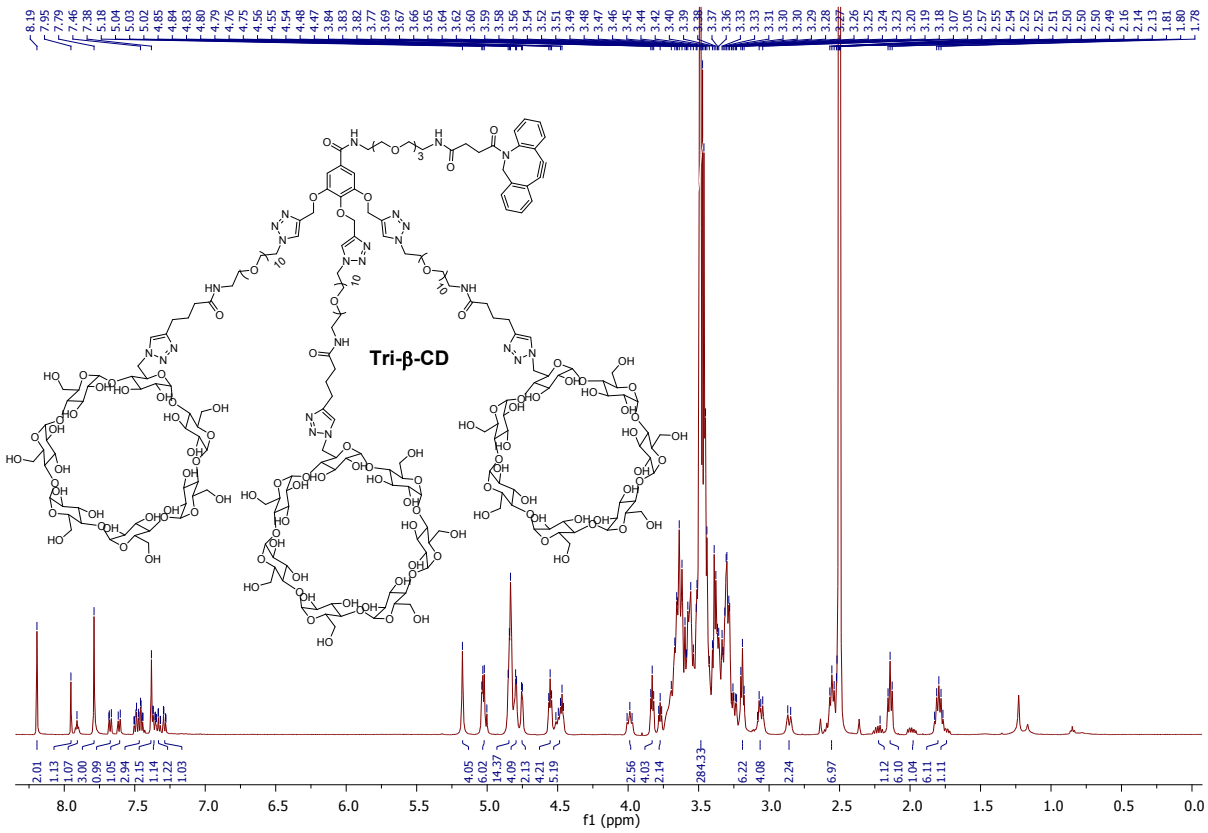
^{13}C NMR spectrum (125 MHz, CD_2Cl_2) of 7



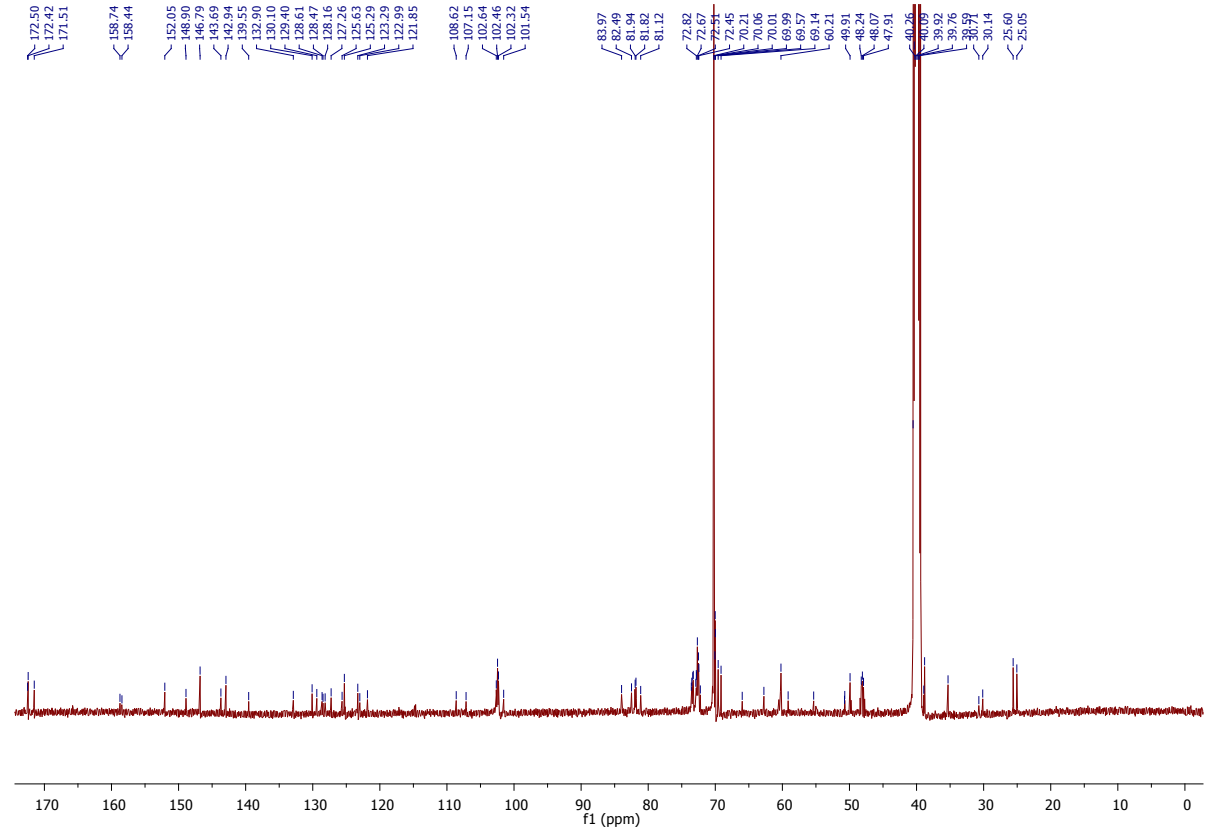
^{1}H NMR spectrum (500 MHz, CD_3OD) of 8



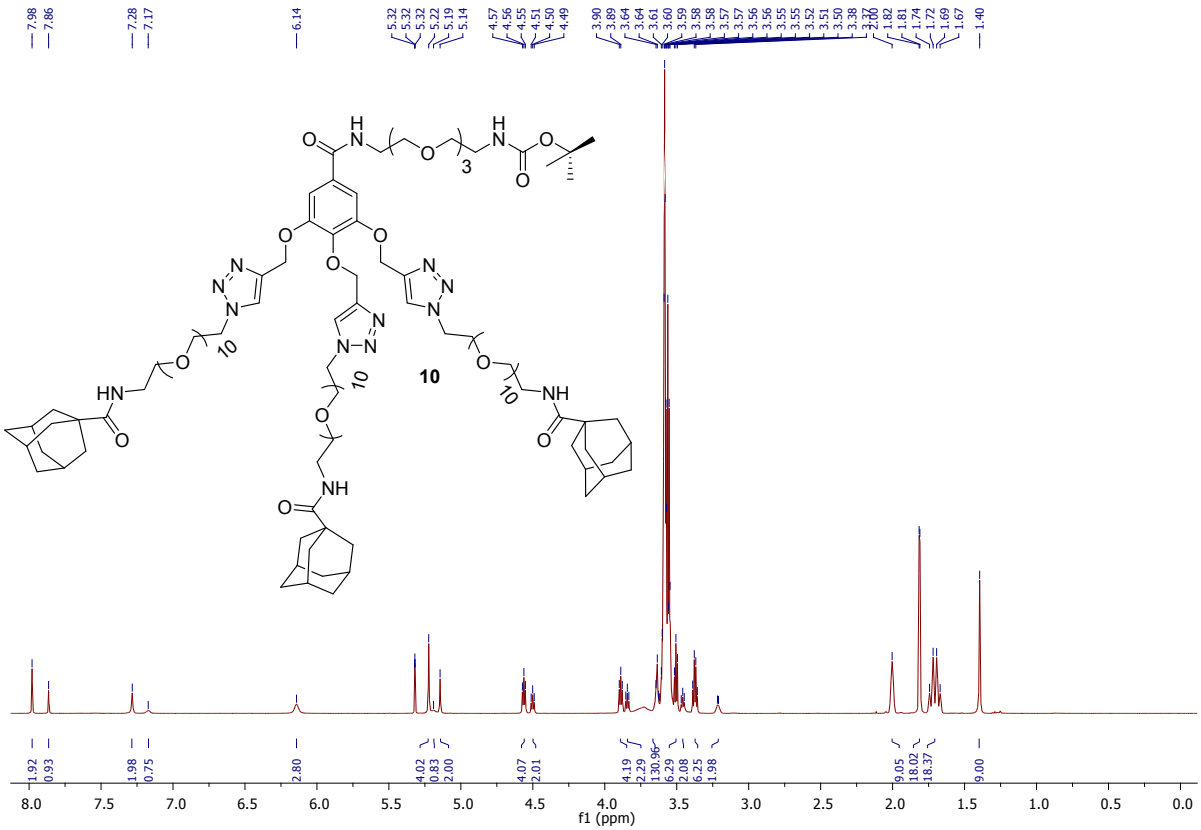
^{13}C NMR spectrum (125 MHz, CD_3OD) of 8



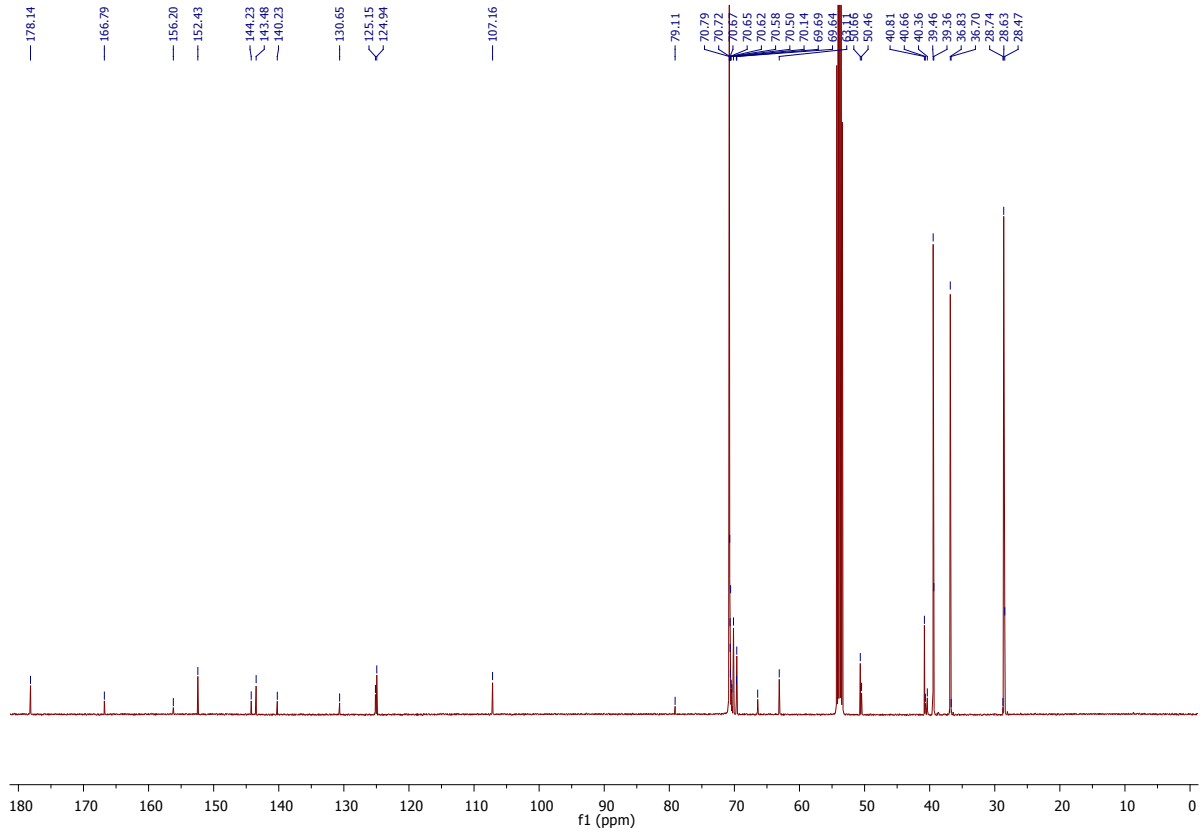
^{1}H NMR spectrum (500 MHz, DMSO-d$_6$) of Tri-β-CD



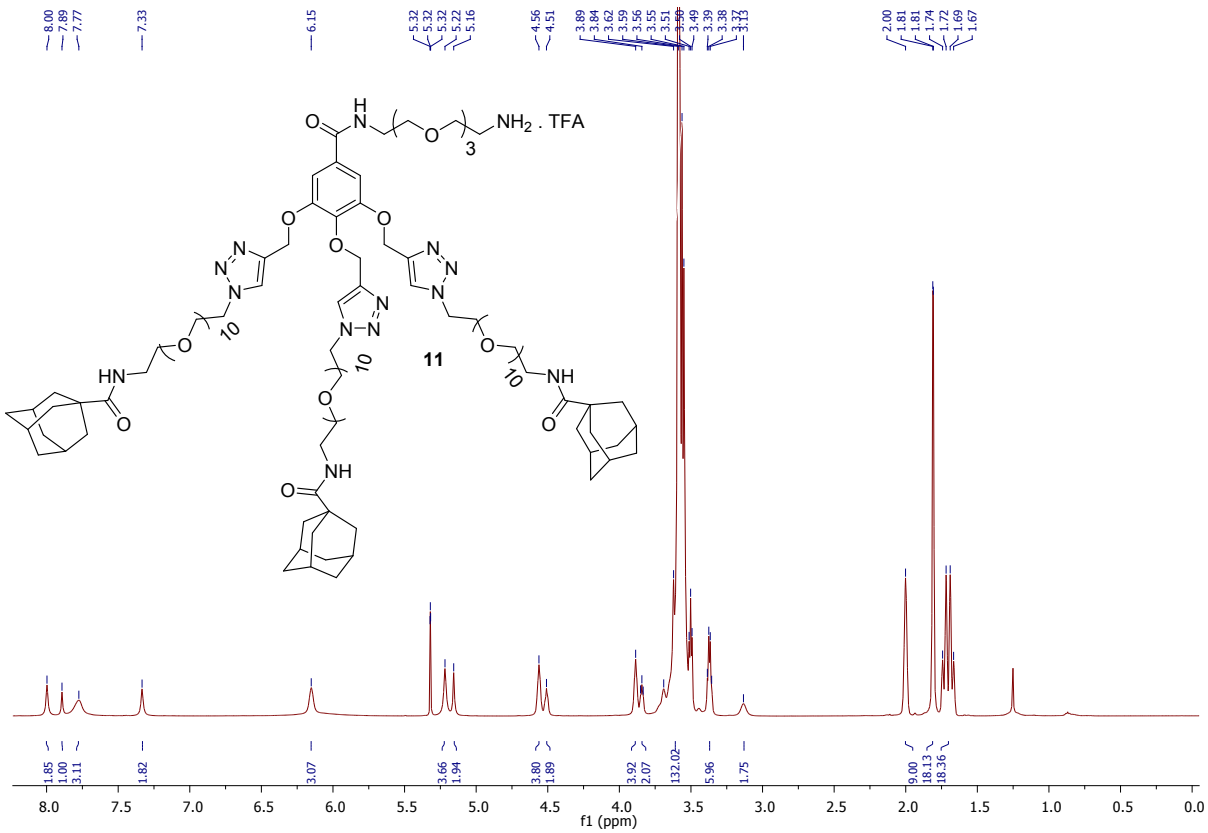
^{13}C NMR spectrum (125 MHz, DMSO-d$_6$) of Tri-β-CD



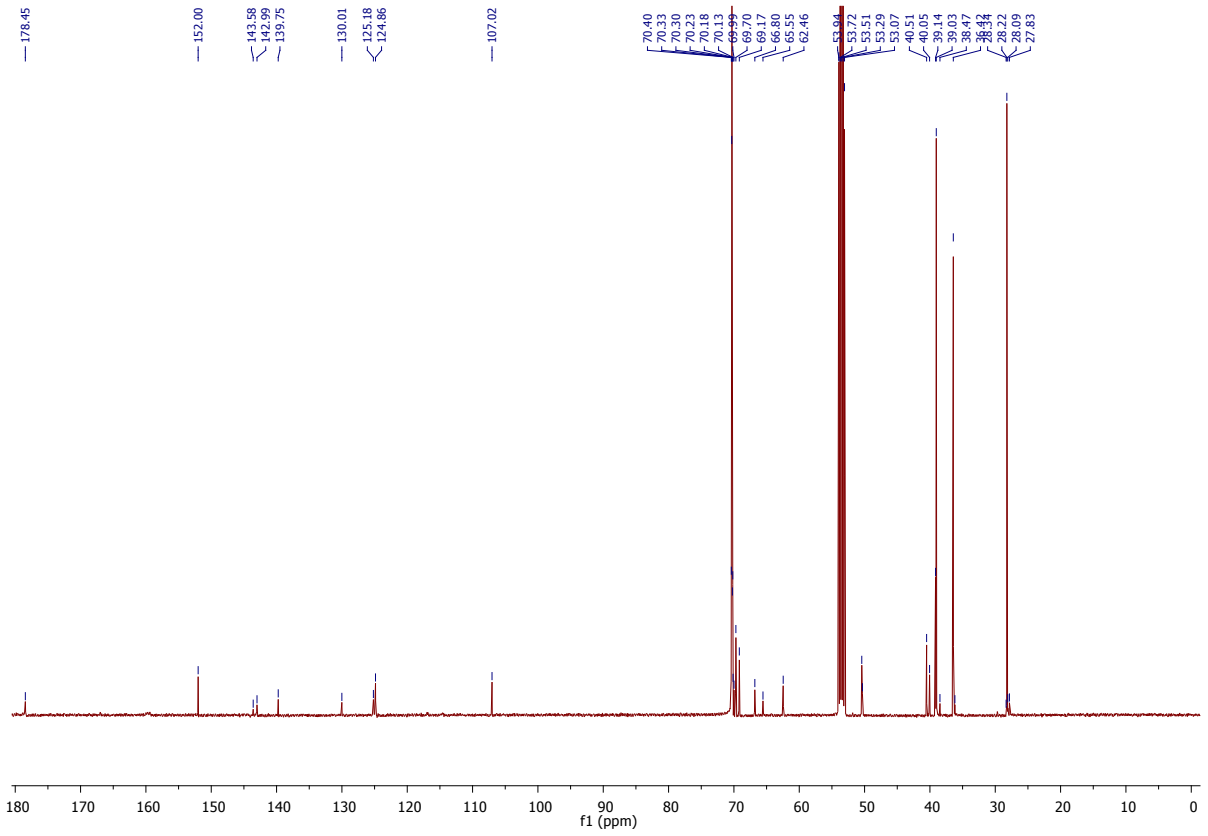
^{1}H NMR spectrum (500 MHz, CD_2Cl_2) of 10



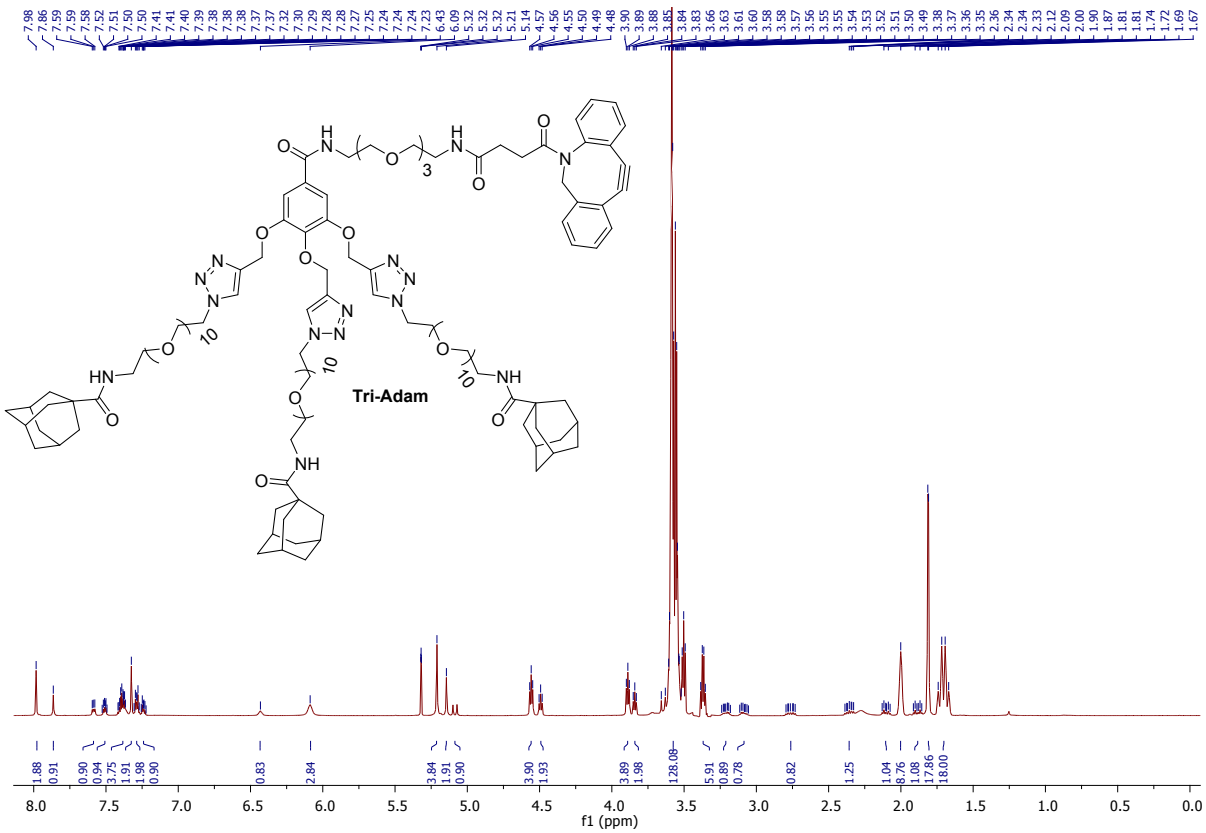
^{13}C NMR spectrum (125 MHz, CD_2Cl_2) of 10



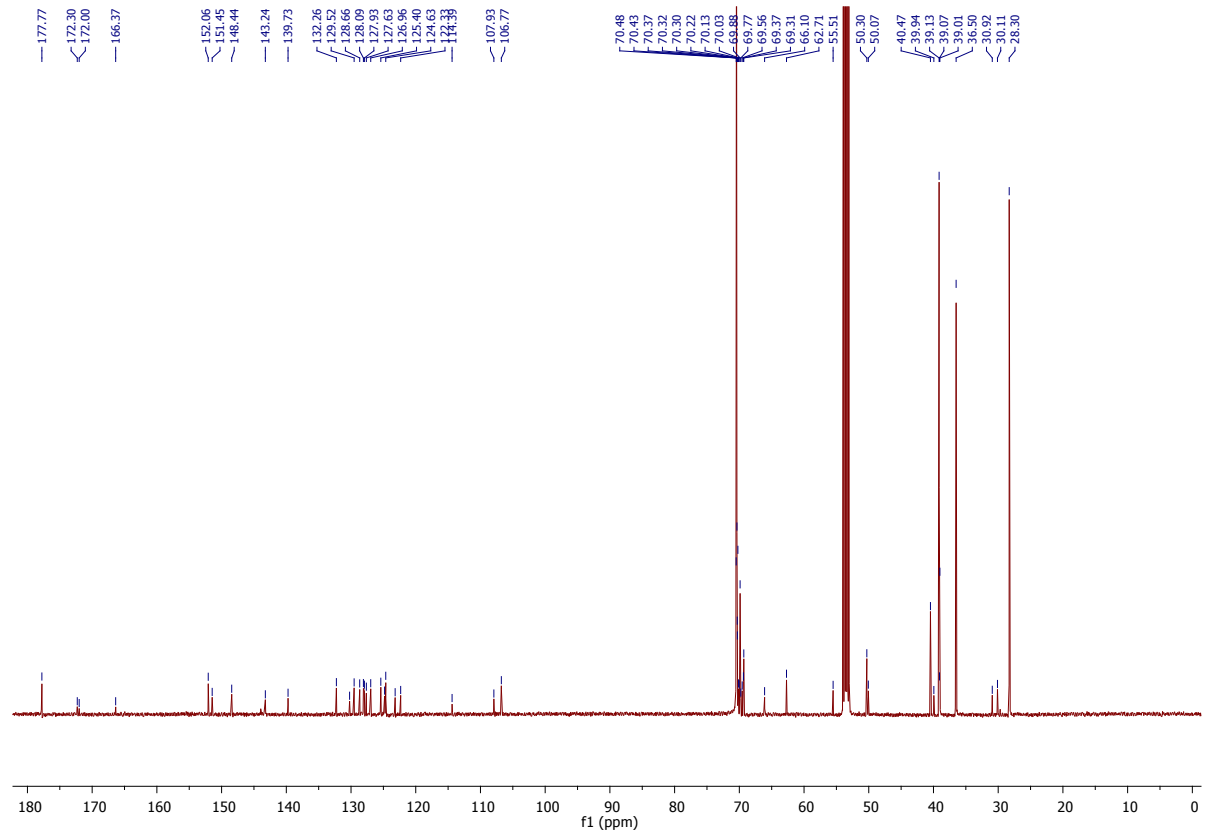
^{1}H NMR spectrum (500 MHz, CD_2Cl_2) of 11



^{13}C NMR spectrum (125 MHz, CD_2Cl_2) of 11



^{1}H NMR spectrum (500 MHz, CD_2Cl_2) of Tri-Adam



^{13}C NMR spectrum (125 MHz, CD_2Cl_2) of Tri-Adam

II. Biological Section

Cell Culture

A549 cells (human lung carcinoma CCL-185) and Jurkat (clone E6-1 human acute T-cell leukemia TIB-152) were purchased from the European Collection of Authenticated Cell Cultures (ECACC). Cells were grown in RPMI 1640-GlutaMax supplemented by 10% fetal bovine serum and 100 u/ml Penicillin/Streptomycin in a humidified incubator at 37 °C and 5% CO_2. Cells were used at early passages (<10).

Human primary CD56+ NK cells (PCS-800-019) were purchased from the American Type Culture Collection (ATCC). These cells were thawed just before use in RPMI1640-Glutamax containing 10% Human Serum Albumin in accordance to the supplier's recommendations.

Cell Viability Assay

To investigate their toxicities, each compound was tested alone or in combination. 4.10^3 A549 or 8.10^3 Jurkat cells were seeded on 96-well plates. Twenty-four hours later, cells were treated with 50 µM Ac_4ManAz for 3 days, then with 50 µM **Tri-β-CD** (for A549) or **Tri-Adam** (for Jurkat) for 30 minutes and finally with or without 1 ng/mL Phytohemaglutinin (PHA) and 1% Phorbol 12-myristate 13-acetate (PMA) for 24 or 48 additional hours. The Cell Proliferation Kit II (XTT) was used to assess cell viability. Briefly, 25 µL of the XTT labeling mixture were added per well and cells were further incubated for additional 4 hours at 37 °C before determination of the absorbance at 490 nm on a 96-well microplate reader Multiskan Go (Thermo Scientific). IC50 values were determined using Graphpad software.

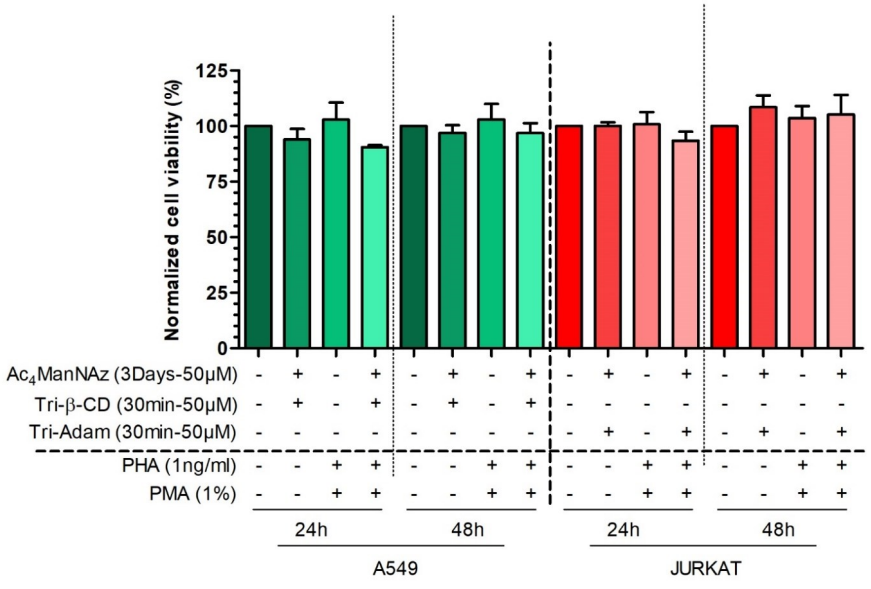


Figure S1: Cell toxicity. A549 and Jurkat cells were treated with the indicated compounds before cell viability measurement. Error bars: s.e.m.

Imaging of cell adhesion

Cells were grown in full growth media containing 50 µM Ac_4ManAz for 3 days. A549 cells were then stained with CellTracker green CMFDA for 30 min and treated with 50 µM **Tri-β-CD** whereas Jurkat cells were stained with CellTracker red CMTPX for 30 min and treated with 50 µM **Tri-Adam** for 30 minutes. After washing with PBS, Jurkat cells were collected and seeded on A549 cells (1:5 A549:Jurkat ratio). After a 10 minutes incubation, non-adherent Jurkat cells were washed whereas A549 and adherent Jurkat cells were fixed prior observation using a FV3000 confocal microscope (Olympus). 3D-analyses were performed using Imaris software.

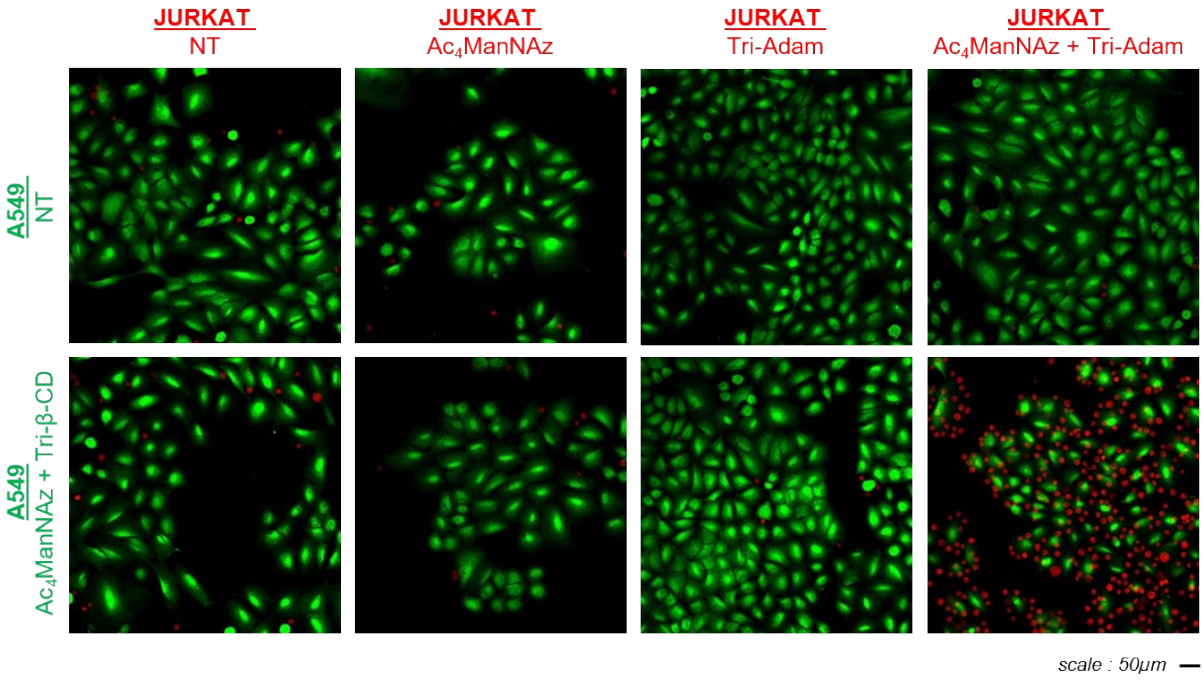


Figure S2: Cell adhesion. Jurkat (red) adhesion on A549 (green) cells is highly stronger when both cells are fully glyco-engineered.

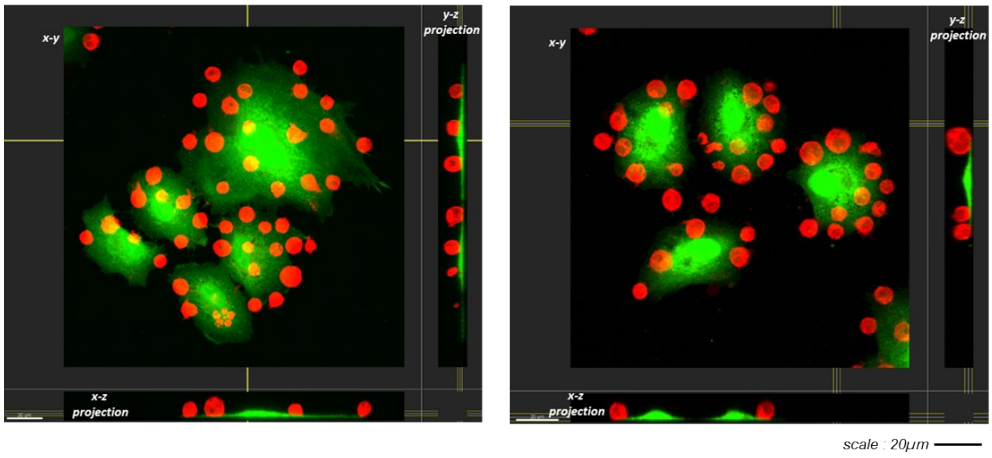


Figure S3: The x-z and y-z projections highlight a physical link between cells since Jurkat are literally laid on the surface of A549 cells

Interleukin-2 (IL-2) ELISA

A549 and Jurkat cells were seeded and, twenty-four hours later, treated with 50 µM Ac_4ManAz for 3 days, then with 50 µM Tri-β-CD (for A549) or Tri-Adam (for Jurkat) for 30 minutes. After washing with PBS, Jurkat cells were collected and seeded on A549 cells (1:5 A549:Jurkat ratio). After a 10 minutes incubation, cells were washed twice with PBS and finally, remaining cells, were treated with or without 1 ng/mL Phytohemagglutinin (PHA) and 1% Phorbol 12-myristate 13-acetate (PMA) for 24 or 48 additional hours. Supernatants were then harvested and amount of secreted IL-2 was analyzed by human IL-2 ELISA (R&D Systems)

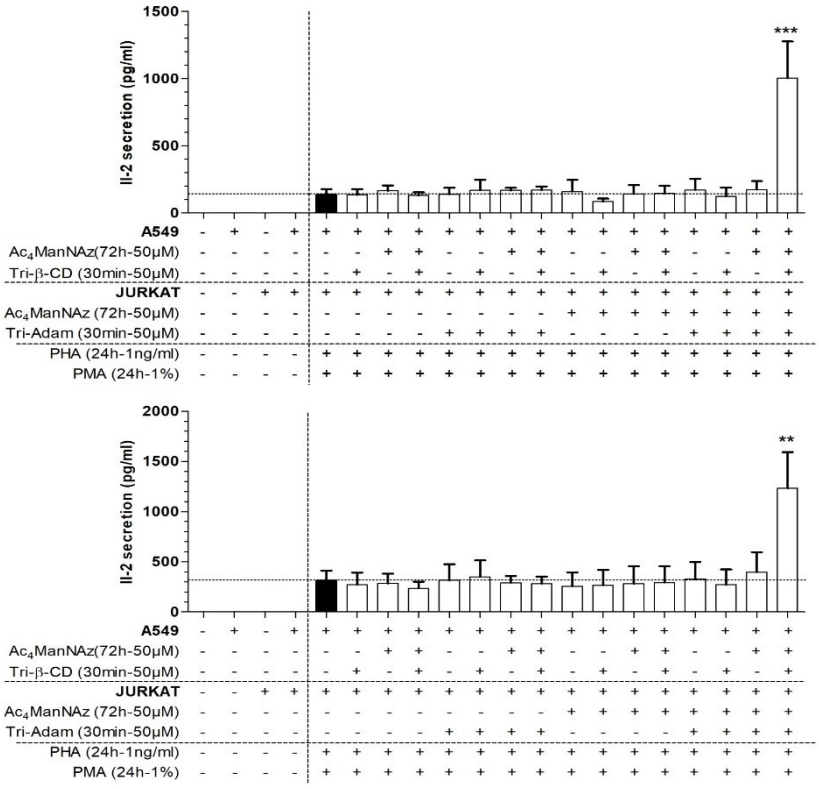


Figure S5: IL-2 secretion after 24 h (top) or 48 h (bottom) from adherent Jurkat cells. Error bars: s.e.m.

Calcein-AM release based NK cells toxicity assay

A549 or Jurkat cells were seeded and, twenty-four hours later, treated with 50 µM Ac_4ManAz for 3 days, then with 50 µM **Tri-β-CD** (for A549) or **Tri-Adam** (for Jurkat) for 30 minutes. After washing with PBS, Jurkat cells were collected and seeded on A549 cells respecting 1:5 A549:Jurkat ratio. After a 10 minutes incubation, non-adherent Jurkat cells were washed and A549 and adherent Jurkat cells were treated with or without 1 ng/mL Phytohemagglutinin (PHA) and 1% Phorbol 12-myristate 13-acetate (PMA) for 48 additional hours. Supernatants were individually collected and Jurkat cells eliminated by low-speed centrifugation. A549 cells were resuspended, incubated with 15 µM Calcein-AM for 30 minutes, washed and finally diluted at a final concentration of $2.5x10^6$ cells/mL in their original supernatant. $CD56^+$-Natural Killer cells were prepared as recommended by the manufacturer. 5.10^3 (50 µL) labelled A549 cells and 5.10^4 (100 µL) $CD56^+$-Natural Killer cells were then incubated in a V-Bottom 96-well microtiter plate (Nunc). After a 4 hours incubation, supernatants were harvested and transferred in a new plate for Calcein-AM detection using a FlexStation 3 Multi-Mode Microplate Reader (Molecular Device). The percentage of cytotoxicity was calculated according to the following formula:100x(experimental release-spontaneous release)/maximum release-spontaneous release). Maximum release was determined by the addition of 10% Triton X-100 and spontaneous release was determined by incubating A549 cells with complete media. Recombinant IL-2 treatment (10 ng/mL) of $CD56^+$-Natural Killer cells was used as a positive control of the cytotoxic assay.

III. References

[1] Hiroaki, K.; Misa, J.; Naomi, H. *ACS Applied Bio Materials* **2020**, 3, 8, 4902-4911.

[2] Châtre, R.; Lange, J.; Péraudeau, E.; Poinot, P.; Lerondel, S.; Le Pape, A.; Clarhaut, J.; Renoux, B.; Papot, S. *Journal of Controlled Release* **2020**, 327, 19-2.

[3] Jicsinsky, L.; Ivànyi, R. Carbohydrate Polymers, **2001**, 45, 139-145.