ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Novel cytotoxic 1,10-phenanthroline–triterpenoid amphiphiles with supramolecular characteristics capable of coordinating ^{64}Cu(II) labels

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1. Experimental part

1.1. General

The NMR measurements were performed either on a Bruker AVANCE 500 MHz or on a Bruker AVANCE II 600 MHz spectrometer equipped with a 5 mm TCI cryoprobe in a 5 mm tube in different solvents. The ^{1}H NMR and the ^{13}C NMR spectra were recorded at 600.13 MHz and 150.90 MHz (AVANCE II 600 MHz) in $CDCl_3$ or CD_3OD using tetramethylsilane (δ = 0.0 – $CDCl_3$) or signal of solvent (δ = 3.31 or 49.50 for $^{1}H/^{13}C$ – CD_3OD) as internal references. ^{1}H NMR data are presented in the following order: chemical shift (δ) expressed in ppm, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constants in Hertz. For unambiguous assignment of both ^{1}H and ^{13}C signals 2D NMR $^{1}H,^{13}C$ gHSQC and gHMBC spectra were measured using standard parameters sets and pulse programs delivered by producer of the spectrometer. Infrared spectra were measured with a Nicolet iS5 FT-IR spectrometer. Mass spectra (MS) were measured with a ZMD mass spectrometer (Waters, Eschborn, Germany) in a positive ESI mode (coin voltage, CV = 10 to 20 eV). MALDI-TOF mass spectra were measured with a Bruker Autoflex MALDI-TOF MS instrument. TLC was carried out on silica gel plates (Merck $60F_{254}$) and the visualization was performed by both, the UV detection and spraying with the methanolic solution of phosphomolybdic acid (5%) followed by heating. For column chromatography, silica gel 60 (0.063-0.200 mm) from Merck was used. All chemicals and solvents were purchased from regular commercial sources in analytical grade and the solvents were purified by general methods before use. Triterpenoids were purchased from Dr. Jan Šarek – Betulinines (www.betulinines.com).

Abbreviations of several chemicals and solvents used in the Experimental part:

CDI – 1,1'-carbonyldiimidazol;

DCM – dichloromethane;

DMAP – *N*,*N*-(4-dimethylamino)pyridine;

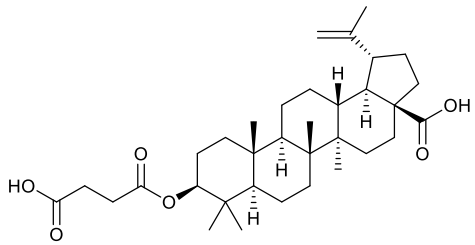
DMF – *N*,*N*-dimethylformamide;

TBTA – tris[(1-benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine;

THPTA – 3,3',3''-[4,4'4''-(nitrilotris(methylene)]tris(1*H*-1,2,3-triazole-4,1-diyl)tris(propan-1-ol);

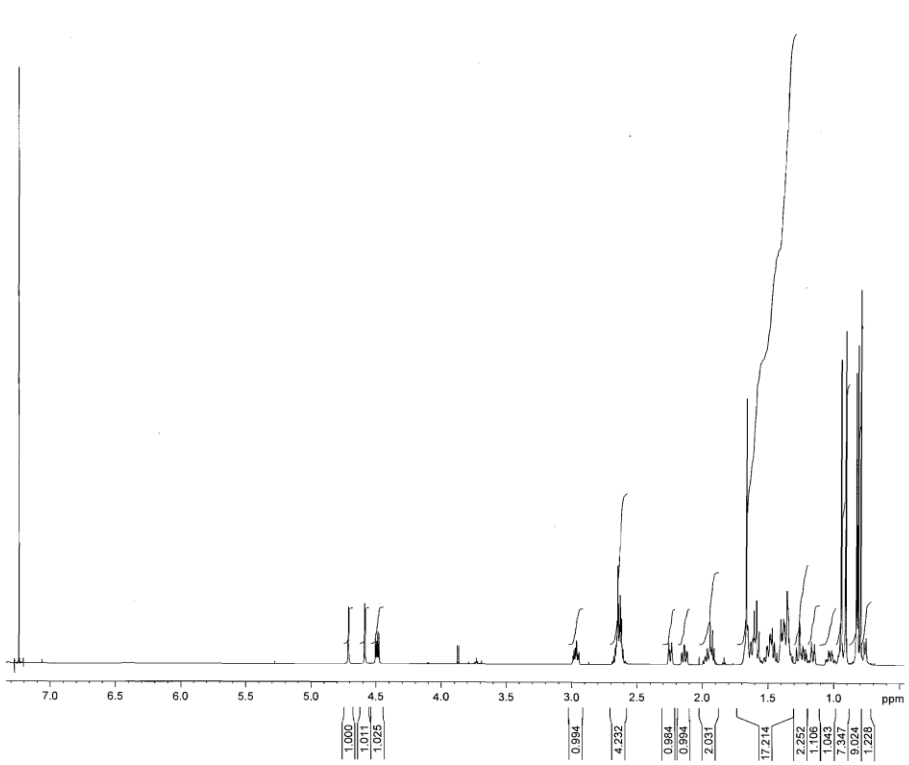
T3P – 1-propanephosphonic acid anhydride

1.2. (3β)-3-[(3-Carboxypropanoyl)oxy]lup-20(29)-en-28-oic acid (**2**)



Succinic anhydride (0.658 g, 6.58 mmol) and DMAP (0.054 g, 0.44 mmol) were added to a solution of betulinic acid (**1**; 1 g, 2.19 mmol) in dry pyridine (15 mL), and the reaction mixture was stirred at r.t. for 96 h. The mixture was then diluted with ice, pH was adjusted to pH = 7 by several drops of HCl, and the product was extracted by chloroform. The extract was washed with water and dried with sodium sulphate. After evaporation of the solvent, the residue was purified by column chromatography on silica gel using chloroform as mobile phase, yielding 1.199 g (98 %) of the product **2**.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.76 (dd, 1H, *J*=2.2; 11.4 Hz, H-5), 0.80 (s, 3H, H-23), 0.82 (s, 3H, H-25), 0.83 (s, 3H, H-24), 0.91 (s, 3H, H-26), 0.95 (s, 3H, H-27), 1.03 (dt, 2H, *J*=2.2; 11.4 Hz, H-11), 1.16 (dt, 2H, *J*=3.3; 3.3; 13.8 Hz, H-21), 1.67 (dd, 3H, *J*=0.7; 1.3 Hz, H-29), 2.14 (ddd, 1H, *J*=3.6; 11.7; 12.8 Hz, H-13), 2.25 (dt, 2H, *J*=3.6; 3.6; 13.1 Hz, H-16), 2.58-2.69 (m, 2H, H-2′, H-3′), 2.97 (dt, 1H, *J*=5.0; 10.9; 10.9 Hz, H-19), 4.49 (dd, 1H, *J*=5.4; 11.1 Hz, H-3), 4.59 (dq, 2H, *J*=1.3; 1.3; 1.3; 2.3 Hz, H-30), 4.71 (dt, 2H, *J*=0.7; 0.7; 0.7; 2.3 Hz, H-30). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 14.62 (q, C-27), 16.24 (q, C-26), 16.29 (q, C-25), 16.57 (q, C-24), 18.19 (t, C-6), 19.33 (q, C-29), 20.87 (t, C-11), 23.58 (t, C-2), 25.34 (t, C-12), 28.00 (q, C-23), 29.18 (t, C-2′), 29.45 (t, C-3′), 29.69 (t, C-21), 30.53 (t, C-15), 32.11 (t, C-16), 34.11 (t, C-22), 37.05 (s, C-10), 37.10 (t, C-7), 37.86 (t, C-1), 38.21 (s, C-4), 38.38 (d, C-13), 40.63 (s, C-8), 42.37 (s, C-14), 46.93 (s, C-19), 49.22 (d, C-18), 50.18 (d, C-9), 55.31 (d, C-5), 56.45 (s, C-17), 81.50 (d, C-3), 109.73 (t, C-30), 150.33 (s, C-20), 171.68 (s, C-1′), 178.12 (s, C-4′), 182.60 (s, C-28). MS: *m/z* = 557.3 Da ($[M+H]^+$). For $C_{34}H_{52}O_6$ calculated exact mass = 556.38 Da.



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in CDCl3, rfp=TMS
26.4.2018
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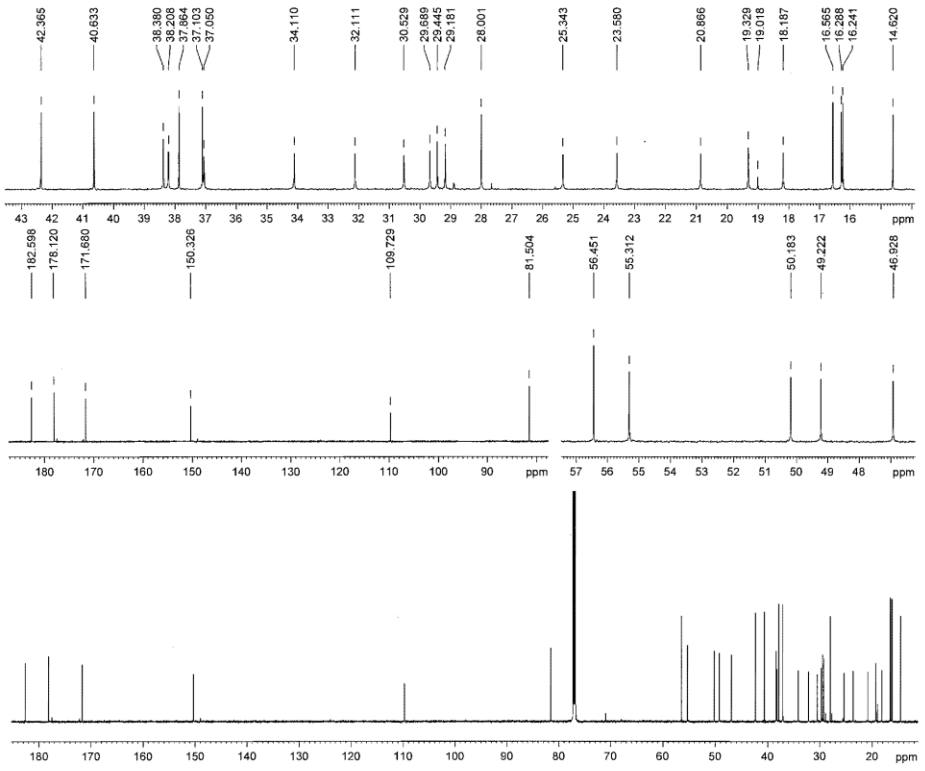
55829



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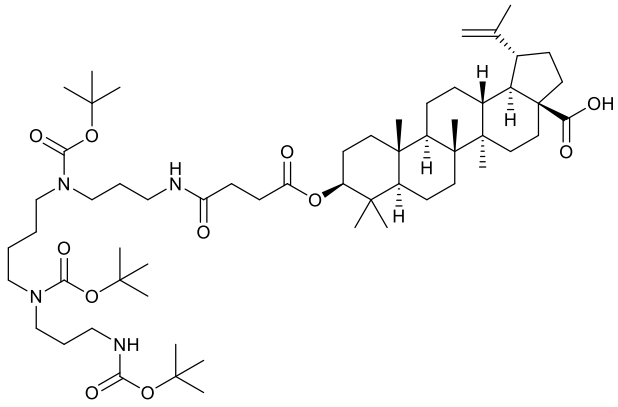
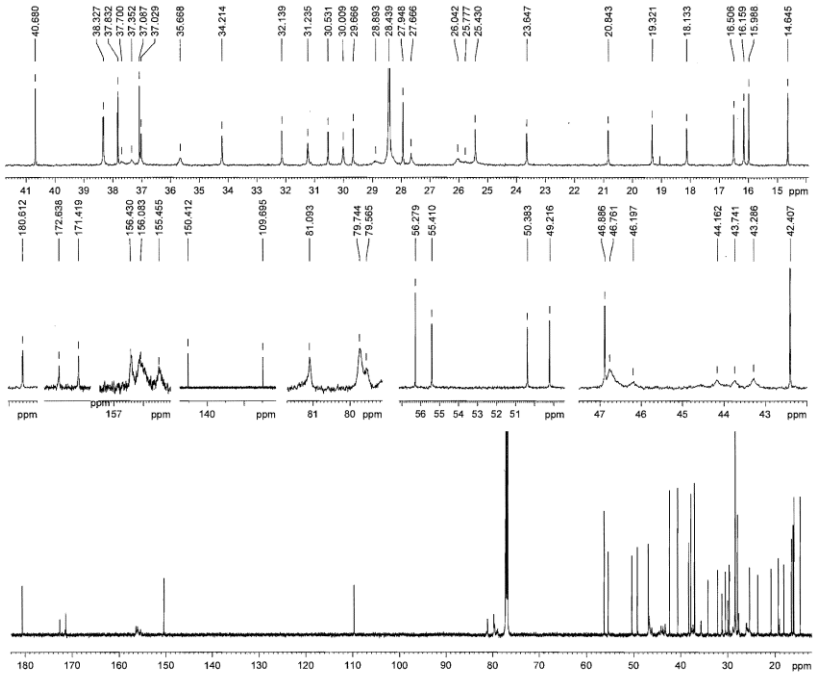
55829

1.3. (3β)-3-{[9,14-bis(*tert*-Butoxycarbonyl)-2,2-dimethyl-4,19,22-trioxo-3-oxa-5,9,14,18-tetraazadocosan-22-yl]oxy}lup-20(29)-en-28-oic acid (**3**)



tris-Boc-spermine (**17**; 1.14 g, 2.28 mmol) and T3P (6.2 mL, 20.66 mmol) were added to a solution of **2** (1.15 g, 2.06 mmol) in dry pyridine (14 mL) and stirred at r.t. for 40 h. The product was extracted by chloroform, and the extract was dried over sodium sulphate. After evaporation of the solvent, the residue was purified by column chromatography on silica gel using chloroform / ethanol as mobile phase, yielding 1.273 g (68 %) of the product **3**.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.75 (dd, 1H, *J*=1.8; 11.4 Hz, H-5), 0.79 (s, 3H, H-23), 0.81 (s, 3H, H-25), 0.82 (s, 3H, H-24), 0.90 (s, 3H, H-26), 0.94 (s, 3H, H-27), 1.02 (dt, 2H, *J*=4.9; 13.8; 13.8 Hz, H-11), 1.15 (dt, 2H, *J*=3.3; 3.3; 13.6 Hz, H-21), 1.67 (dd, 3H, *J*=0.7; 1.4 Hz, H-29), 2.17 (dt, 1H, *J*=3.7; 11.8; 11.8 Hz, H-13), 2.24 (dt, 2H, *J*=3.4; 3.4; 13.0 Hz, H-16), 2.44-2.50 (m, 2H, H-2′), 2.58-2.68 (m, 2H, H-3′), 2.98 (dt, 1H, *J*=4.7; 11.0; 11.0 Hz, H-19), 3.03-3.28 (m, 2H, H-5′, H-7′, H-8′, H-11′, H-12′, H-14′), 4,45 (dd, 1H, *J*=5.6; 11.1 Hz, H-3), 4.58 (dq, 2H, *J*=1.4; 1.4; 1.4; 2.3 Hz, H-30), 4.71 (dq, 2H, *J*=0.7; 0.7; 0.7; 2.3 Hz, H-30). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 14.65 (q, C-27), 15.99 (q, C-26), 16.16 (q, C-25), 16.51 (q, C-24), 18.13 (t, C-6), 19.32 (q, C-29), 20.84 (t, C-11), 23.65 (t, C-2), 25.43 (t, C-12), 25.78 (t, C-10′), 26.04 (t, C-9′), 27.67 (q, C-23), 27.67 (t, C-13′), 28.89 (t, C-6′), 29.67 (s, C-2′), 30.01 (t, C-3′), 30.53 (t, C-21), 31.24 (t, C-15), 32.14 (t, C-16), 34.21 (t, C-22), 35.67 (t, C-14′), 37.03 (s, C-10), 37.09 (t, C-7), 37.35 (t, C-5′), 37.83 (s, C-4), 37.93 (t, C-1), 38.33 (d, C-13), 40.68 (s, C-8), 42.41 (s, C-14), 43.29 (t, C-11′), 43.74 (t, C-12′), 44.16 (t, C-8′), 46.76 (t, C-7′), 46.89 (s, C-19), 49.22 (d, C-18), 50.38 (d, C-9), 55.41 (d, C-5), 56.28 (s, C-17), 81.09 (d, C-3), 109.70 (t, C-30), 150.41 (s, C-20), 171.42 (s, C-1′), 172.64 (s, C-4′), 180.61 (s, C-28), Boc-groups: 28.44 (q), 79.57 (s), 79.74 (s), 155.46 (s), 156.08 (s), 156.43 (s). MS: *m/z* = 1041.7 Da ($[M+H]^+$). For $C_{59}H_{100}N_4O_{11}$ calculated exact mass = 1040.74 Da.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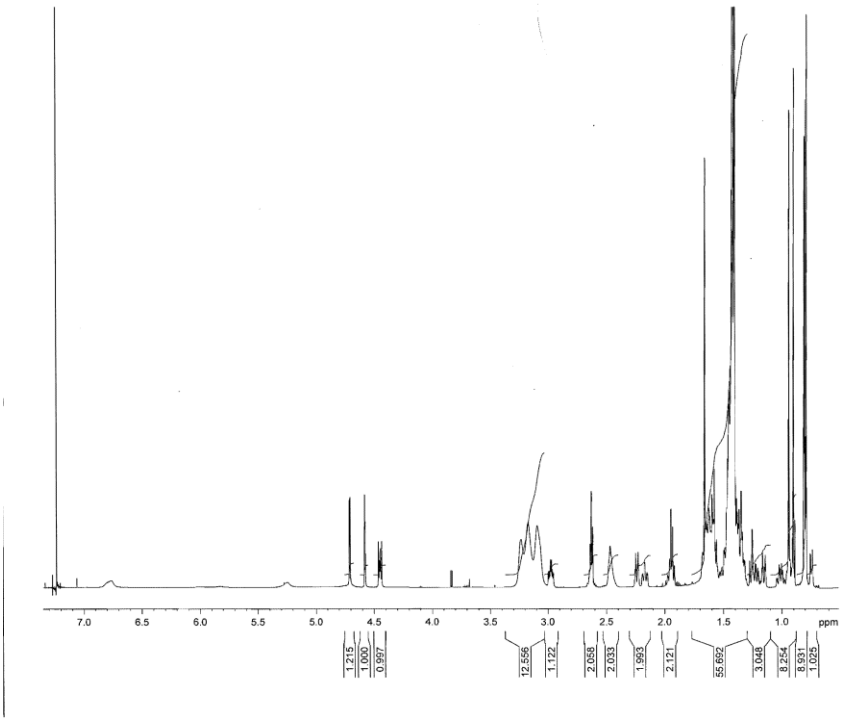
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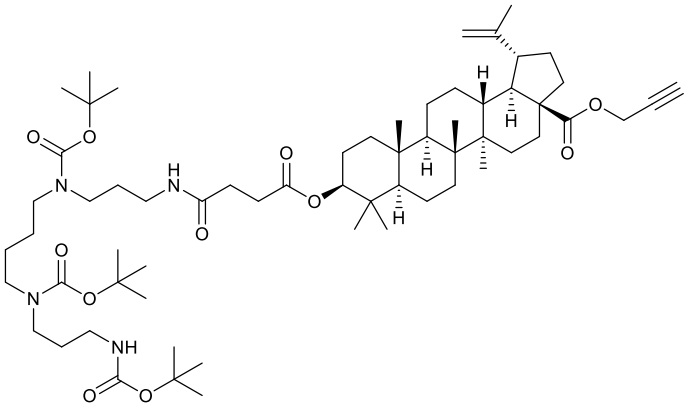
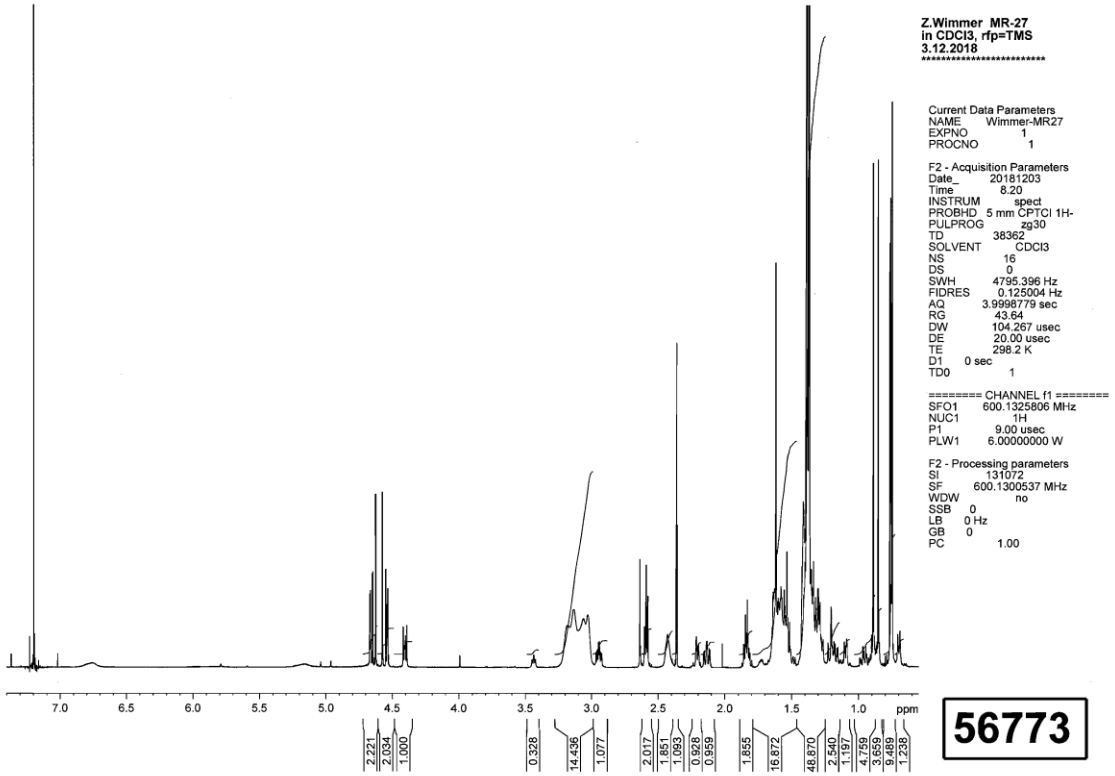
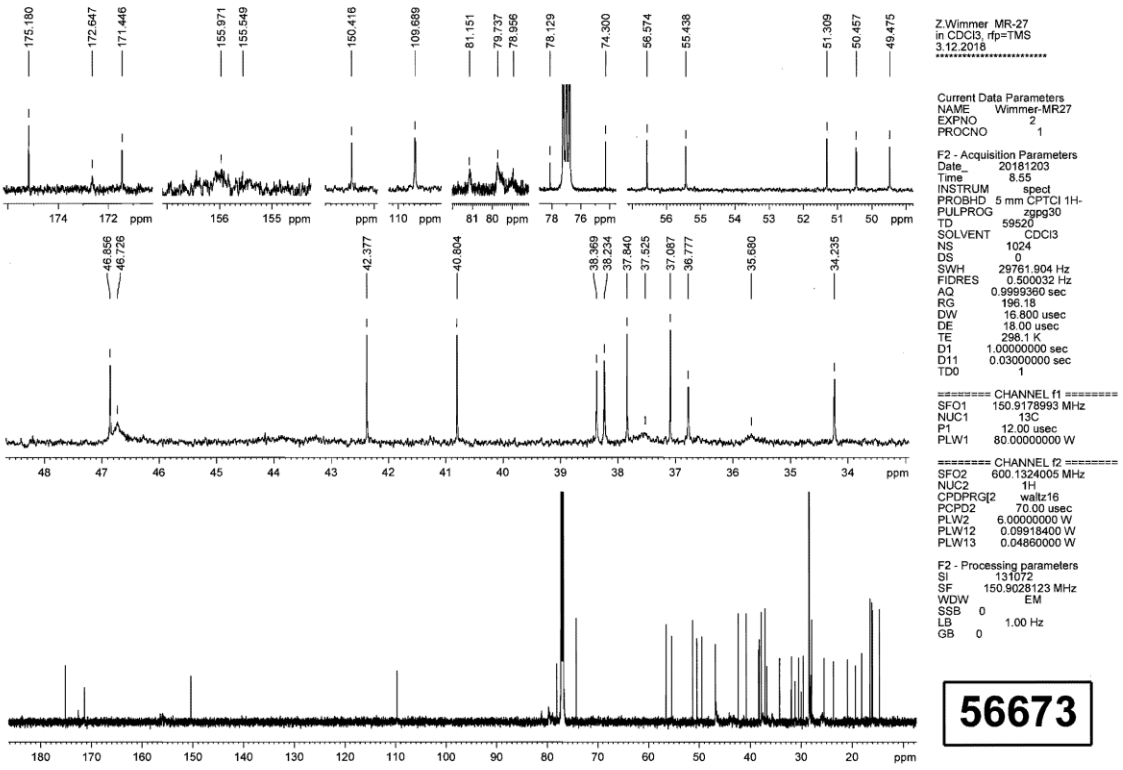
1.4. Prop-2-in-1-yl-(3β)-3-{[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4,19,22-trioxo-3-oxa-5,9,14,18-tetraazadocosan-22-yl]oxy}lup-20(29)-en-28-oate (**4**)



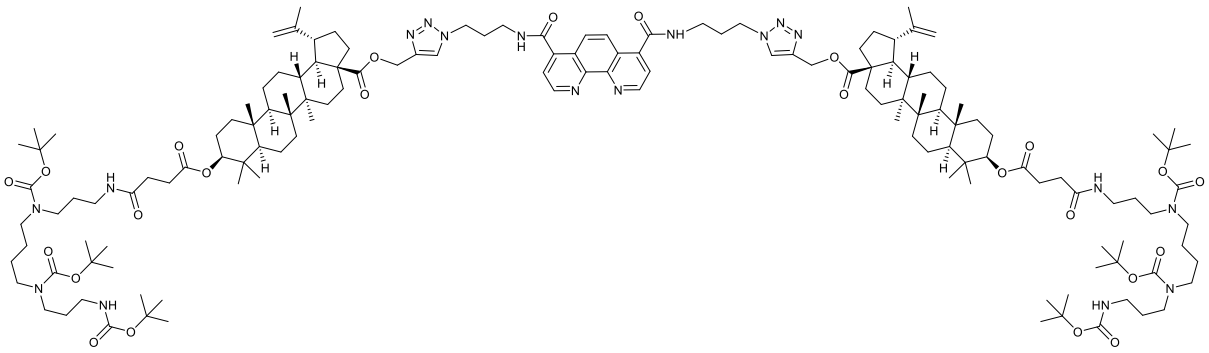
Potassium carbonate (0.784 g, 5.674 mmol) was added to a solution of **3** (1.15 g, 1.14 mmol) in DMF (10 mL), and the reaction mixture was stirred at r.t. for 15 min. Then propargyl bromide (506 µL, 5.674 mmol) was added and stirring continued for an additional 24 h. To work-up the reaction mixture, DMF was first evaporated and the residue was extracted with benzene. The extract was dried over sodium sulphate, and the crude residue was purified by column chromatography, using first petroleum ether as mobile phase to elute the rest of DMF, and then chloroform / ethanol as mobile phase to get the product **4** (0.944 g) in a 77 % yield.
NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.70 (dd, 1H, *J*=5.7; 10.8 Hz, H-5), 0.75 (s, 3H, H-24), 0.76 (s, 3H, H-23), 0.76 (d, 3H, *J*=0.6 Hz, H-25), 0.85 (s, 3H, H-26), 0.89 (s, 3H, H-27), 1.62 (dd, 3H, *J*=0.7; 1.4 Hz, H-29), 2.13 (ddd, 1H, *J*=3.6; 11.5; 12.9 Hz, H-13), 2.19-2.23 (m, 2H, H-16), 2.36 (t, 1H, *J*=2.5 Hz, H-3‘), 2.40-2.46 (m, 2H, H-5‘), 2.56-2.62 (m, 2H, H-6‘), 2.95 (dt, 1H, *J*=4.8; 10.9; 10.9 Hz, H-19), 3.00-3.25 (m, 2H, H-8‘, H-10‘, H-11‘, H-14‘, H-15‘, H-17‘), 4.40 (dd, 1H, *J*=5.7; 10.8 Hz, H-3), 4.53 (dq, 2H, *J*=1.4; 1.4; 1.4; 2.3 Hz, H-30), 4.56 (dd, 2H, *J*=2.5; 15.5 Hz, H-1‘), 4.64 (dd, 2H, *J*=2.5; 15.5 Hz, H-1’), 4.67 (dq, 2H, J=0.7; 0.7; 0.7; 2.3 Hz, H-30), BOC groups: 1,37 (s), 1,38 (s, 2x). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 14.67 (q, C-27), 15.99 (q, C-26), 16.17 (q, C-25), 16.51 (q, C-24), 18.14 (t, C-6), 19.34 (q, C-29), 20.88 (t, C-11), 23.66 (t, C-2), 25.48 (t, C-12), 25.74 (t, C-12‘), 26.00 (t, C-13‘), 27.94 (q, C-23), 29.61 (t, C-21), 30.02 (t, C-6‘), 30.48 (t, C-15), 31.21 (t, C-5‘), 31.92 (t, C-16), 34.24 (t, C-22), 35.68 (t, C-16‘), 36.78 (t, C-7), 37.09 (s, C-10), 37.53 (t, C-9‘), 37.84 (s, C-4), 38.23 (d, C-13), 38.37 (t, C-1), 40.80 (s, C-8), 42.38 (s, C-14), 42.9-44.5 (s, C-10‘, C-11‘, C-14‘, C-15‘), 46.63 (t, C-17‘), 46.73 (t, C-8‘), 46.79 (s, C-19), 49.48 (d, C-18), 50.46 (d, C-9), 51.31 (q, C-1‘), 55.44 (d, C-5), 56.57 (s, C-17), 74.30 (d, C-3‘), 78.13 (s, C-2‘), 81.15 (d, C-3), 109.69 (t, C-30), 150.42 (s, C-20), 171.45 (s, C-4‘), 172.65 (s, C-7‘), 175.18 (s, C-28).

Boc groups: 28.44 (q, 2x), 78.96 (s), 79.73 (s, 2x), 155.55 (s), 155.97 (s, 2x). MS: *m/z* = 1079.5 Da ($[M+H]^+$). For $C_{62}H_{102}N_4O_{11}$ calculated exact mass = 1078.75 Da.





1.5. 1,10-Phenanthrolin-4,7-diyl-bis(carbonyliminopropan-3,1-diyl-1*H*-1,2,3-triazol-1,4-diylmethandiyl)-(3β,3'β)bis[3-{[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4,19,22-trioxo-3-oxa-5,9,14,18-tetraazadocosan-22-yl]oxy}lup-20(29)-en-28-oate] (**5**)

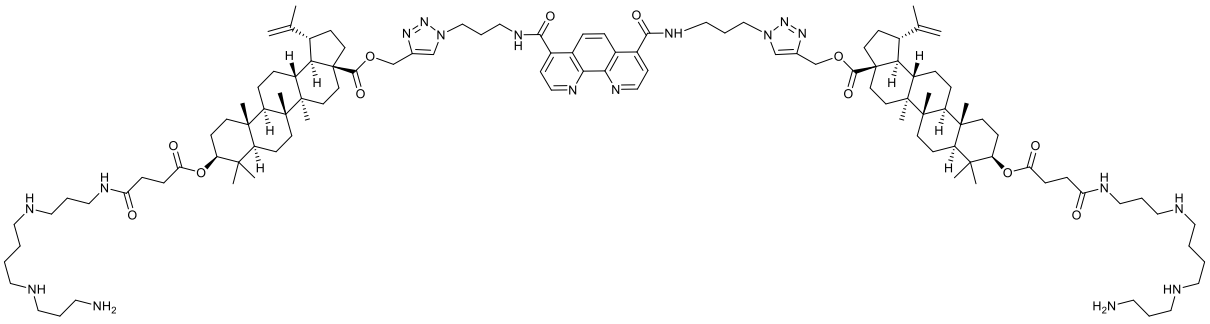


A solution of **4** (0.650 g, 0.618 mmol) in DCM (7.5 mL) was added to a solution of **8** (0.089 g, 0.206 mmol) in DCM (7.5 mL) under stirring. Then a solution of $CuSO_4 \cdot 5H_2O$ + THPTA (16.5 ml, 0.05 M) was added, stirred for 15 min, and finally, sodium ascorbate (0.326 g, 1.649 mmol) was added. Stirring continued for 24 h. Then the reaction mixture was quenched with water and extracted with chloroform. The extract was dried over sodium sulphate, and the solvent

was evaporated. The crude residue was purified by column chromatography on silica gel using chloroform / ethanol, and finally chloroform / ethanol / ammonia (6:4:1) as mobile phase, affording **5** (0.368 g) in a 69 % yield.

MALDI-TOF MS: *m/z* = 2612.9 Da ($[M+Na]^+$). For $C_{144}H_{224}N_{18}O_{24}$ calculated exact mass = 2589.69 Da.

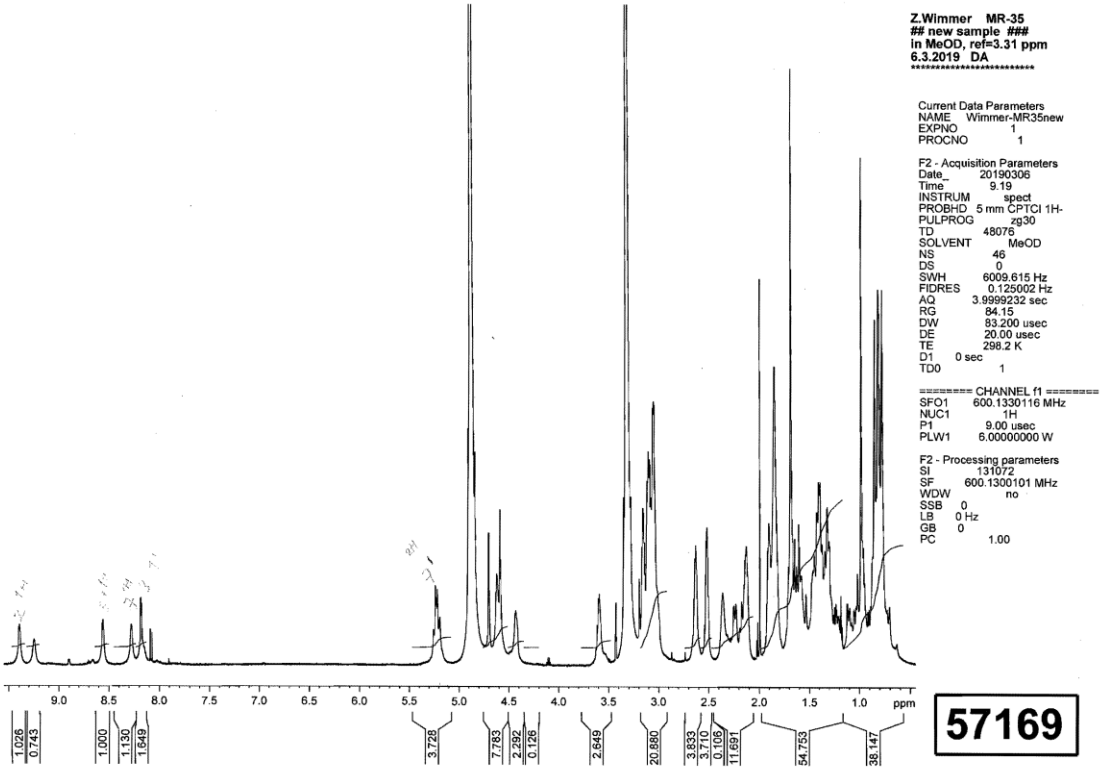
1.6. 1,10-Phenanthrolin-4,7-diyl-bis(carbonyliminopropan-3,1-diyl-1*H*-1,2,3-triazol-1,4-diylmethandiyl)-(3β,3'β)bis{3-[(4-{[3-({4-[(3-aminopropyl)amino]butyl}amino)propyl]-amino}-4-oxobutanoyl)oxy]lup-20(29)-en-28- oate} (**6**)

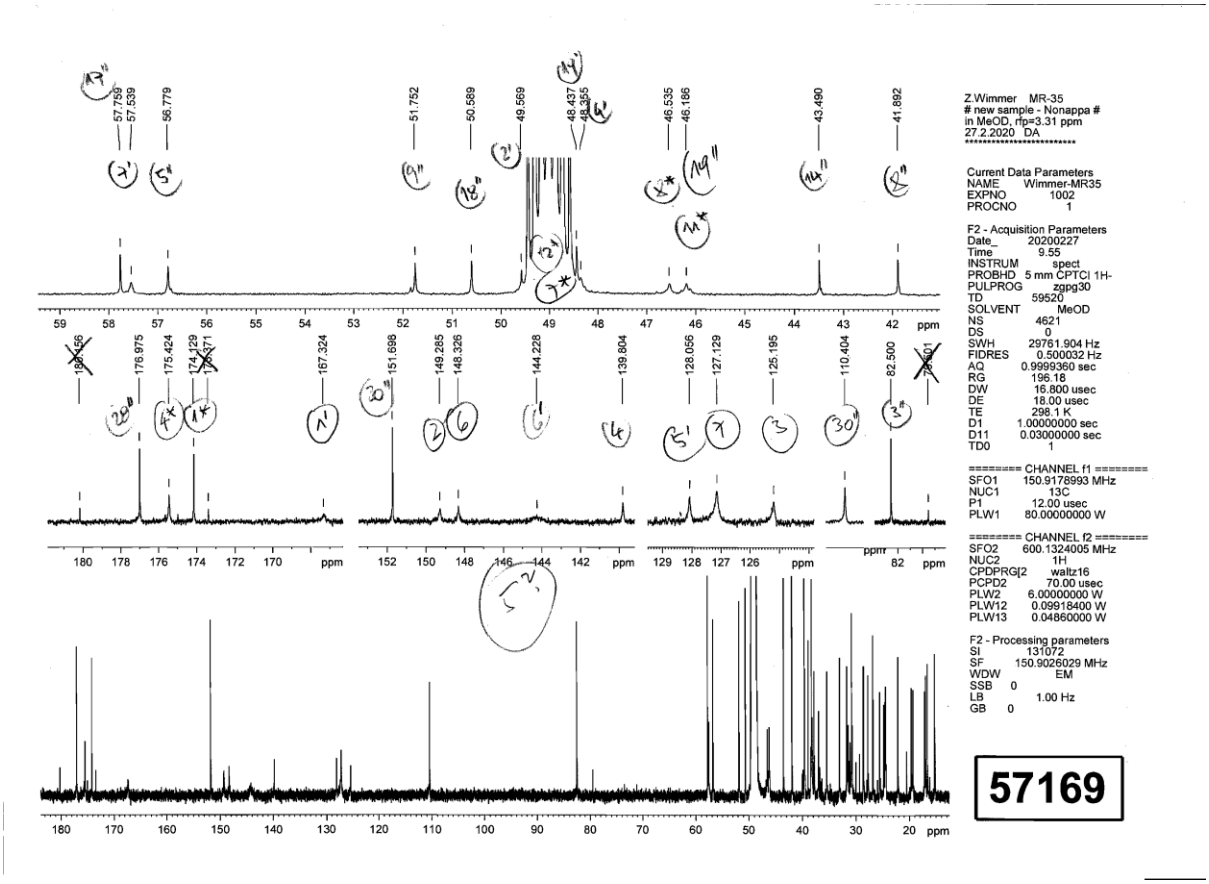


A solution of **5** (0.352 g, 0.135 mmol) in 1M HCl v EtOAc (8.15 ml, 8.15 mmol) was placed to an incubator and shaked at 25 ºC for 12 h. Then the appeared crystals were filtered off, and washed with ether, affording **6** (0.210 g) in a 77 % yield.

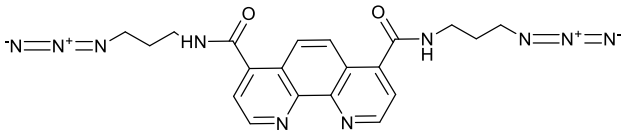
NMR: ^{1}H-NMR (600.13 MHz, CD_3OD): δ [ppm] 0.77 (bs, 3H, H-23''), 0.82 (bs, 3H, H-25''), 0.82 (bs, 3H, H-26''), 0.85 (bs, 3H, H-24''), 0.98 (bs, 3H, H-27''), 1.68 (bs, 3H, H-29''), 2.50-2.54 (m, 2H, H-2*), 2.63-2.70 (m, 2H, H-3*), 3.06-3.19 (m, 16H, H-7*, H-8*, H-9*, H10*, H-11*, H-12*, H-13*, H-14*), 3.56-3.62 (m, 2H, H-5*), 4.43 (bdd, 1H, *J*=4.8; 11.5 Hz, H-3''), 4.59 (bs, 1H, H-30''), 4.70 (bs, 1H, H-30''), 5.21 (bd, 1H, *J*=13.5 Hz, H-7'), 5.24 (bd, 1H, *J*=13.5 Hz, H-7'), 8.18 (bd, 1H, H-3), 8.28 (bs, 1H, H-7), 8.56 (s, 1H, H-5'), 9.39 (bs, 1H, H-2). ^{13}C-NMR (150.91 MHz, CD_3OD): δ [ppm] 15.16 (q, C-27''), 16.52 (q, C-24''), 16.84 (q, C-25''), 17.07 (q, C-26''), 19.24 (t, C-6''), 19.57 (q, C-29''), 22.06 (t, C-11''), 24.39 (t, C-9*), 24.48 (t, C-10*), 24.69 (t, C-12''), 25.47 (t, C-13*), 26.74 (t, C-2''), 27.71 (t, C-6*), 28.56 (q, C-23''), 30.66 (t, C-21''), 31.06 (t, C-3*), 31.40 (t, C-2*), 31.62 (t, C-15''), 32.95 (t, C-16''), 35.36 (t, C-22''), 36.94 (s, C-4''), 37.79 (t, C-1''), 38.12 (t, C-3'), 38.21 (t, C-5*), 38.39 (t, C-14*), 38.88 (s, C-10''), 39.52 (t, C-7''), 39.60 (d, C-13''), 41.89 (s, C-8''), 43.49 (s, C-14''), 46.15 (d, C-19''), 46.19 (t, C-11*), 46.54 (t, C-8*), 48.36 (t, C-4'), 48.85 (t, C-7*), 49.12 (t, C-12*), 49.57 (t, C-2'), 50.90 (d, C-18''), 51.75 (d, C-9''), 56.78 (d, C-5''), 57.54 (t, C-7'),

57.56 (s, C-17''), 82.50 (d, C-3''), 110.40 (t, C-30''), 125.20 (d, C-3), 127.13 (d, C-7), 128.06 (d, C-5'), 139.80 (s, C-4), 144.23 (s, C-6'), 148.33 (s, C-6), 149.29 (d, C-2), 151.70 (s, C-20''), 167.32 (d, C-1'), 174.13 (s, C-1*), 175.42 (s, C-4*), 176.98 (s, C-28''). MS: *m/2z* = 995.0 Da ($[M+2H]^{2+}$), *m/z* = 1990 Da ($[M+H]^{+}$). For $C_{114}H_{176}N_{18}O_{12}$ calculated exact mass = 1989.37 Da.





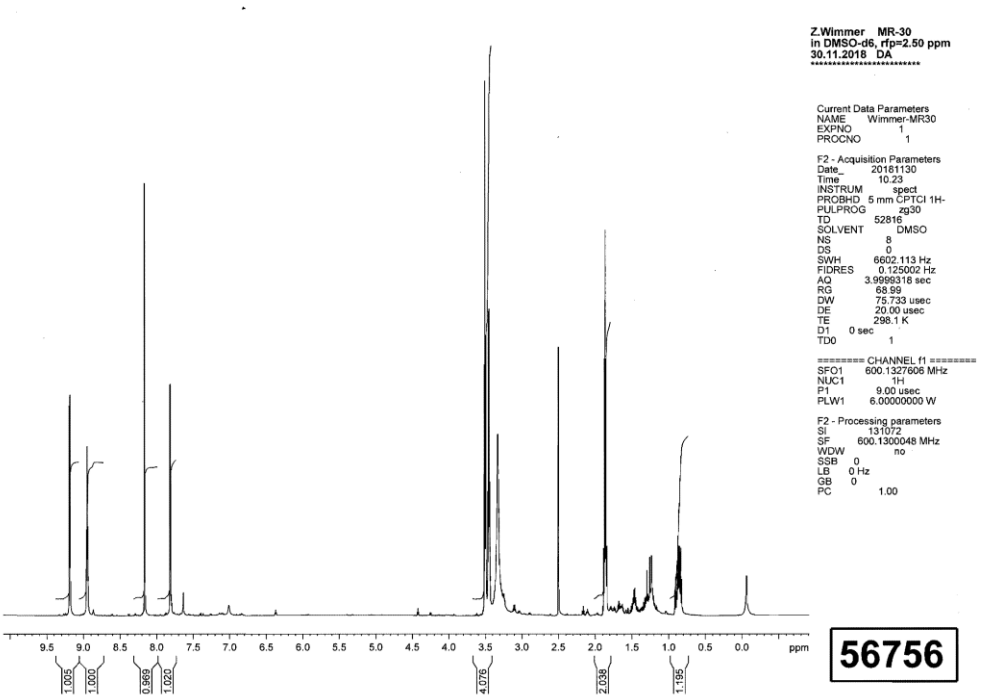
1.7. *N*,*N'*-bis(3-azidopropyl)-1,10-phenanthrolin-4,7-dicarboxamide (**8**)

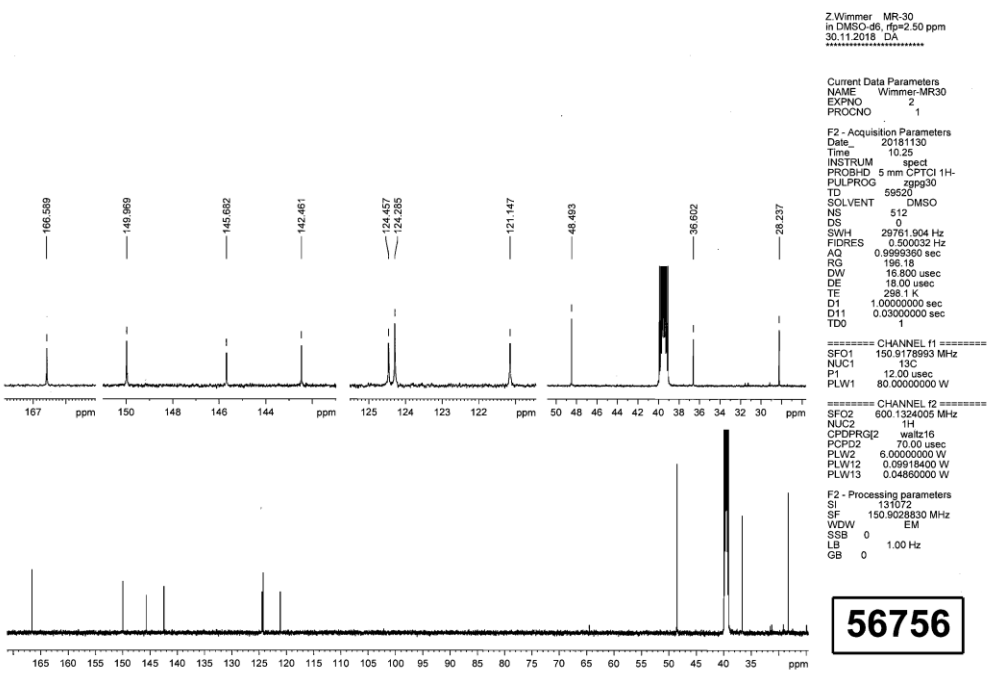


A solution of CDI (1.595 g, 7.381 mmol) in DMF (4 mL) was stepwise added to a solution of 1,10-phenanthrolin-4,7-dicarboxylic acid (**7**; 0.550 g, 2.050 mmol) in DMF (4 mL), and the mixture was stirred at r.t. for 3 h. Then a solution of 3-azidopropylamine (1.21 mL, 12.3 mmol) in DMF (8 mL) was added and stirring continued for an additional 12 h. To work-up, the mixture was first extracted by *tert*-butanol to remove the free amine, then by DCM. The extract was dried over sodium sulphate, the solvent was evaporated, and the crude residue was purified by column chromatography using chloroform / ethanol mixture as mobile phase. The product **8** (0.575 g) was obtained in a 65 % yield.

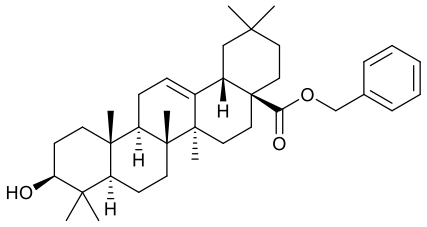
NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 1.86 (m, 2H, *J*=6.7; 6.7; 6.7; 6.7 Hz, H-10), 3.45 (dt, 2H, *J*=5.6; 6.7; 6.7 Hz, H-9), 3.50 (t, 2H. *J*=6.7 Hz, H-11), 7.81 (d, 1H, *J*=4.3 Hz, H-3), 8.16 (s, 1H, H-7), 8.95 (bt, 1H, *J*=5.6 Hz, NH-8), 9.18 (d, 1H, *J*=4.3 Hz, H-2). ^{13}C-NMR

(150.91 MHz, $CDCl_3$): δ [ppm] 28.24 (t, C-10), 36.60 (t, C-9), 48.49 (t, C-11), 121.15 (d, C-3), 124.29 (d, C-7), 124.46 (s, C-5), 142.46 (s, C-4), 145.68 (s, C-6), 149.97 (d, C-2), 166.59 (s, C-8). MS: *m/z* = 433 Da ($[M+H]^+$). For $C_{20}H_{20}N_{10}O_2$ calculated exact mass = 432.18 Da.



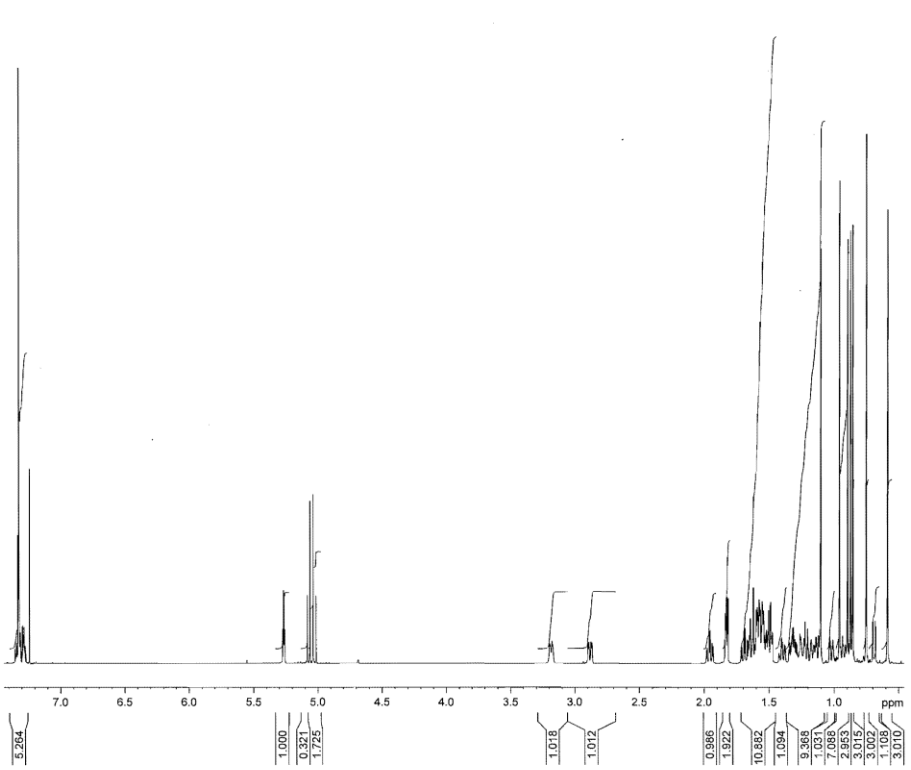


1.8. Benzyl-(3β)-3-hydroxyolean-12-en-28-oate (**10**)



Potassium carbonate (0.453 g, 3.284 mmol) was added to a solution of oleanolic acid (**9**; 1 g, 2.189 mmol) in DMF (15 mL) and the reaction mixture was stirred at r.t. for 15 min. Then benzyl bromide (391 μL, 3.284 mmol) was added and stirring continued for 4 days. To work-up the reaction mixture, DMF was first evaporated and the residue was extracted with benzene. The extract was dried over sodium sulphate, and the crude residue was purified by column chromatography, using chloroform as mobile phase to get the product **10** (0.988 g) in an 82 % yield.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.58 (s, 3H, H-26), 0.69 (dd, 1H, *J*=2.1; 11.8 Hz, H-5), 0.75 (s, 3H, H-25), 0.86 (s, 3H, H-24), 0.87 (s, 3H, H-29), 0.90 (s, 3H, H-23), 0.96 (s, 3H, H-30), 1.02 (ddd, 2H, *J*=2.8; 4.3; 13.8 Hz, H-16), 1.10 (d, 3H, *J*=0.5 Hz, H-27), 1.13 (ddd, 2H, *J*=2.4; 3.7; 13.6 Hz, H-19), 1.69 (dt, 2H, *J*=4.4; 13.8; 13.8 Hz, H-22), 1.80-1.87 (m, H-2), 1.96 (dt, 2H, *J*=4.2; 13.6; 13.6 Hz, H-11), 2.89 (bdd, 1H, *J*=4.2; 13.9 Hz, H-18), 3.19 (dd, 1H, *J*=4.1; 11.8 Hz, H-3), 5.03 (d, 2H, *J*=12.5 Hz, H-1′), 5.07 (d, 5H, *J*=12.5 Hz, H-1′), 5.27 (t, 1H, *J*=3.7 Hz, H-12), 7.26-7.35 (m, H-3′, H-5′). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.29 (q, C-24), 15.56 (q, C-25), 16.87 (q, C-26), 18.30 (t, C-6), 23.04 (t, C-11), 23.38 (t, C-2), 23.63 (q, C-30), 25.87 (q, C-27), 27.18 (t, C-15), 27.61 (t, C-16), 28.09 (q, C-23), 30.69 (s, C-20), 32.36 (t, C-22), 32.70 (t, C-7), 33.09 (q, C-29), 33.85 (t, C-21), 36.99 (t, C-1), 38.42 (s, C-10), 38.73 (s, C-4), 39.27 (s, C-8), 41.36 (d, C-18), 41.67 (s, C-14), 45.86 (t, C-19), 46.73 (s, C-17), 47.59 (d, C-9), 55.19 (d. C-5), 65.91(t, C-1′), 79.00 (d, C-3), 122.48 (d, C-12), 127.89 (d, C-5′), 127.96 (d, C-4′), 128.96 (d, C-3′), 136.43 (s, C-2′), 143.68 (s, C-13), 177.44 (s, C-28). MS: *m/z* = 547.4 Da ($[M+H]^+$). For $C_{37}H_{54}O_3$ calculated exact mass = 546.41 Da.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25.4.2018
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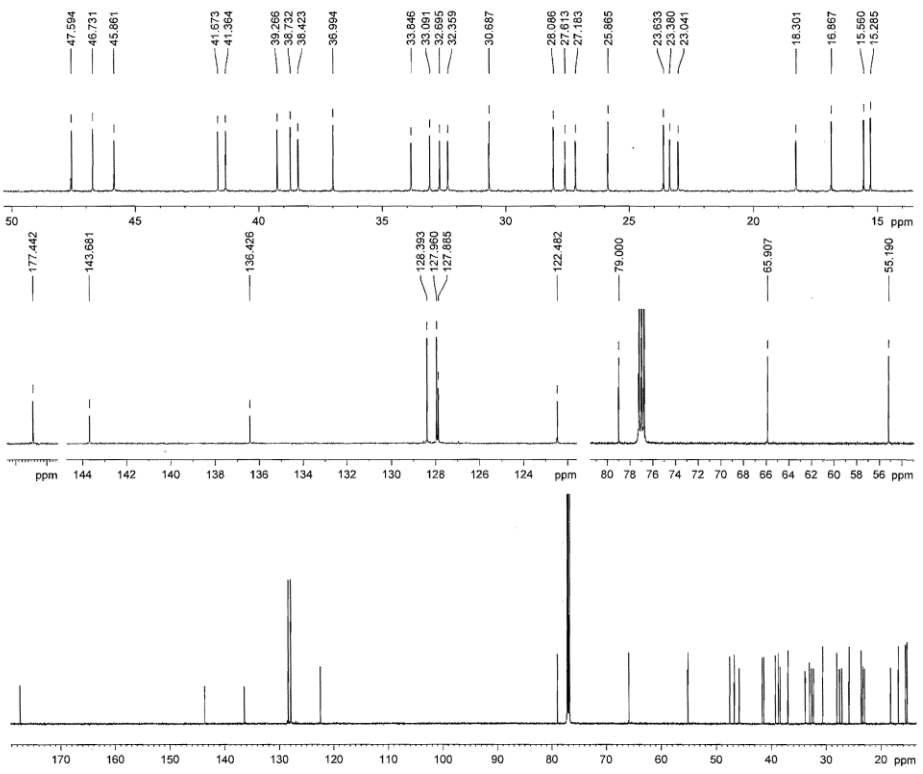
55827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50 45 40 35 30 25 20 15 ppm
177.442 143.681 136.426 128.393 127.960 127.885 122.482 79.000 65.907 55.190
ppm 144 142 140 138 136 134 132 130 128 126 124 ppm
80 78 76 74 72 70 68 66 64 62 60 58 56 ppm
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Z.Wimmer MR-11
in CDCl3, rfp=TMS
25.4.2018
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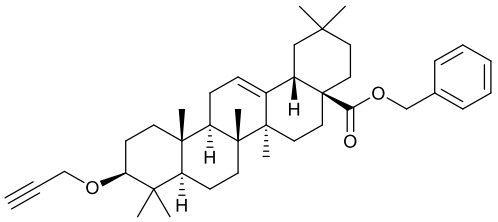
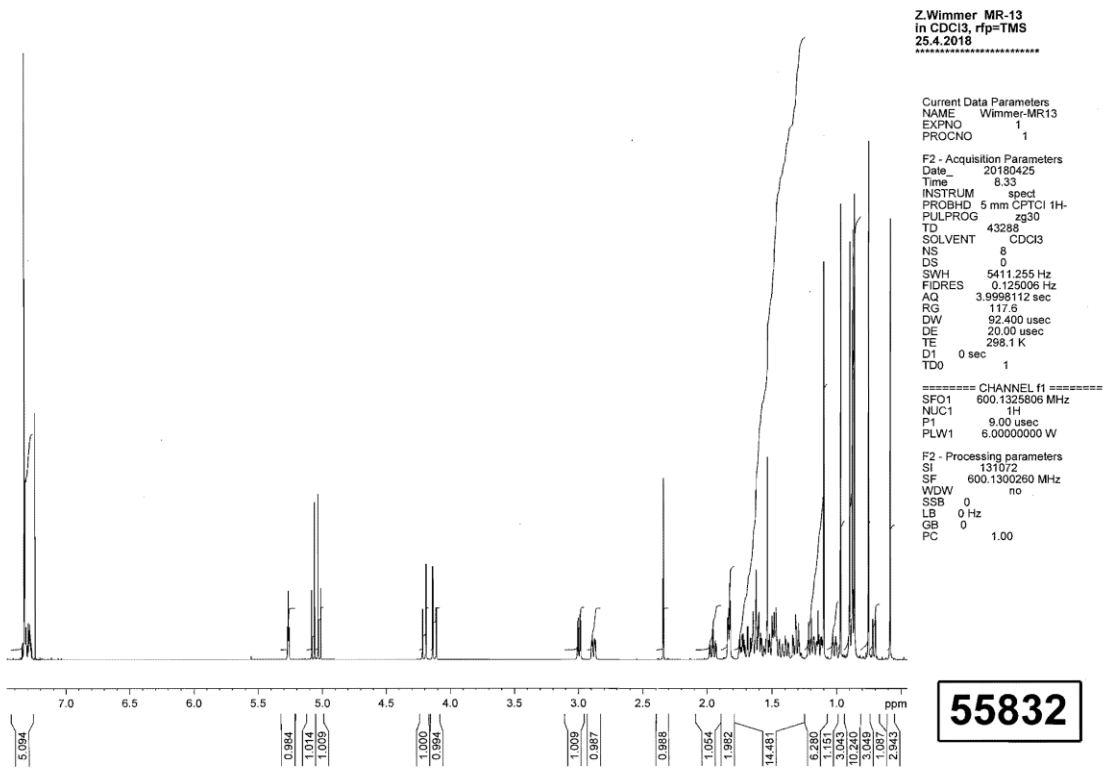
55827

1.9. Benzyl-(3β)-3-(prop-2-in-1-yloxy)olean-12-en-28-oate (**11**)



Sodium hydride (0.549 g, 13.728 mmol, 8 eq.) was added to a solution of **10** (0.938 g, 1.716 mmol) in THF (10 mL). The reaction mixture was stirred at r.t. for 1.5 h, then another portion of sodium hydride (0.549 g, 13.728 mmol, 8 eq.) was added, and stirring continued for an additional 1.5 h. Then propargyl bromide (390 μL, 5.418 mmol) was added, and stirring continued for 7 days. The mixture was worked-up by extracting it with chloroform, drying over sodium sulphate and evaporating the solvent. The crude residue was purified by column chromatography using petroleum ether / chloroform mixture as mobile phase, yielding 0.81 g (80 %) of product **11**.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.58 (s, 3H, H-26), 0.71 (dd, 1H, *J*=1.9; 11.7 Hz, H-5), 0.75 (s, 3H, H-25), 0.86 (s, 3H, H-24), 0.88 (s, 3H, H-23), 0.90 (s, 3H, H-29), 0.97 (s, 3H, H-30), 1.02 (ddd, 2H, *J*=2.6; 3.8; 13.8 Hz, H-16), 1.10 (d, 3H, *J*=0.7 Hz, H-27), 1.13 (ddd, 2H, *J*=2.2; 4.7; 13.6 Hz, H-19), 1.79-1.88 (m, 2H, H-2), 1.95 (dt, 2H, *J*=4.2; 13.7; 13.7 Hz, H-11), 2.34 (t, 1H, *J*=2.4 Hz, H-3′), 2.88 (bdd, 1H, *J*=4.4; 13.7 Hz, H-18), 3.00 (dd, 1H, *J*=4.3; 11.8 Hz, H-3), 4.13 (dd, 2H, *J*=2.4; 15.9 Hz, H-1′), 4.20 (dd, 2H, *J*=2.4; 15.6 Hz, H-1′), 5.03 (d, 2H, *J*=12.6 Hz, H-4′), 5.07 (d, 2H, *J*=12.6 Hz, H-4′), 5.27 (t, 1H, *J*=3.8 Hz, H-12), 7.26-7.35 (m, 1H, H-6′, H-8′). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.28 (q, C-24), 16.39 (q, C-25), 16.87 (q, C-26), 18.24 (t, C-6), 22.36 (t, C-11), 23.04 (q, C-30), 23.42 (t, C-2), 23.64 (t, C-15), 25.86 (q, C-27), 27.60 (t, C-16), 28.09 (q, C-23), 30.69 (s, C-20), 32.36 (t, C-22), 32.70 (t, C-7), 33.10 (q, C-29), 33.85 (t, C-21), 36.93 (t, C-1), 38.28 (s, C-10), 38.47 (s, C-4), 39.32 (s, C-8), 41.37 (d, C-18), 41.67 (s, C-14), 45.86 (t, C-19), 46.73 (s, C-17), 47.57 (d, C-9), 55.76 (d, C-5), 56.42 (t, C-1′), 65.91 (t, C-4′), 73.42 (s, C-3′), 80.96 (d, C-3), 85.88 (d, C-2′), 122.50 (d, C-12), 127.89 (d, C-8′), 127.97 (d, C-7′), 128.40 (d, C-6′), 136.42 (s, C-5′), 143.71 (s, C-13), 177.44 (s, C-28). MS: *m/z* = 584.4 Da ($[M+H]^+$). For $C_{40}H_{56}O_3$ calculated exact mass = 584.42 Da.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25.4.2018
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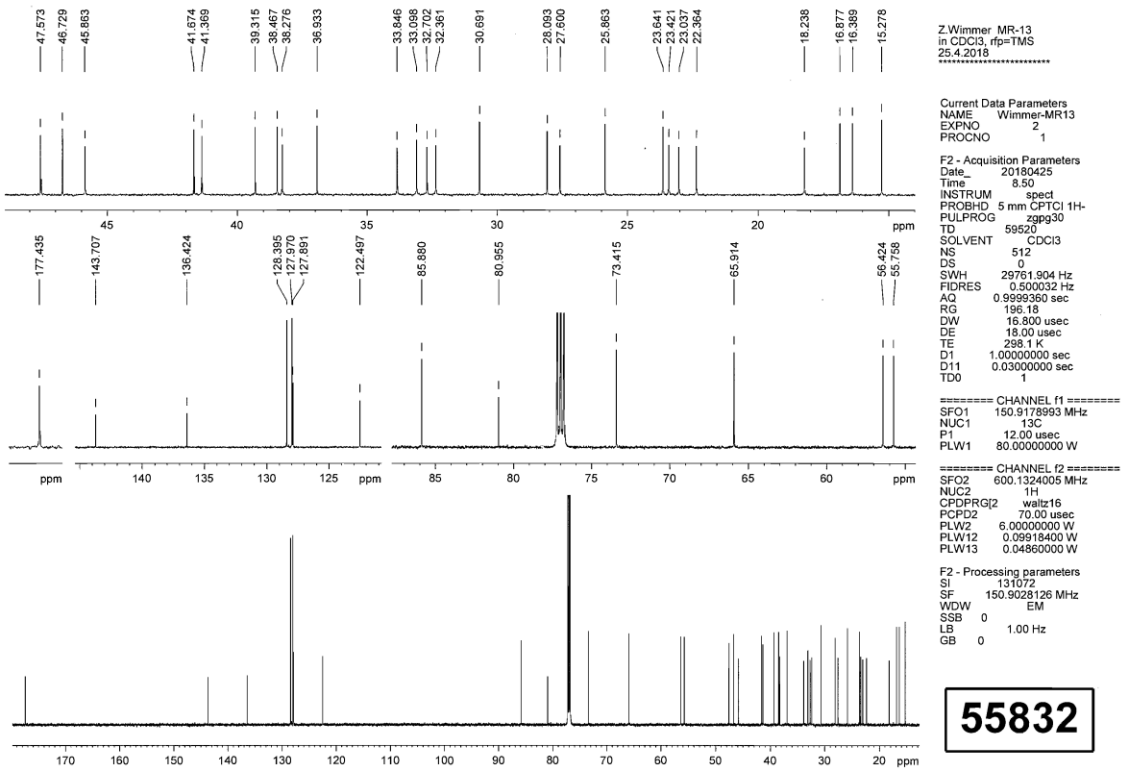
55832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25.4.2018
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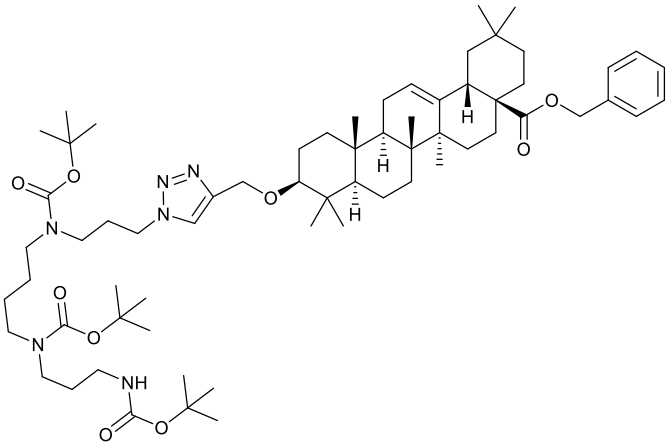
55832

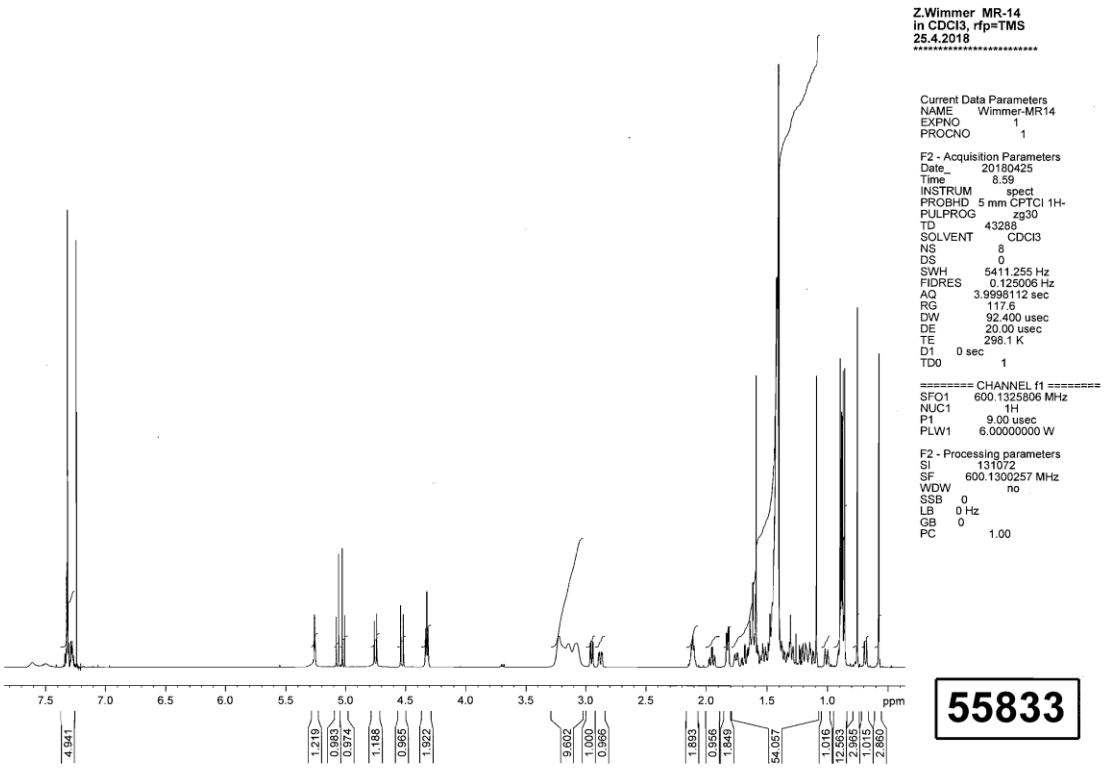
1.10. Benzyl-(3β)-3-({1-[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxo-3-oxa-5,9,14-triazaheptadecan-17-yl]-1*H*-1,2,3-triazol-4-yl}methoxy)olean-12-en-28-oate (**12**)

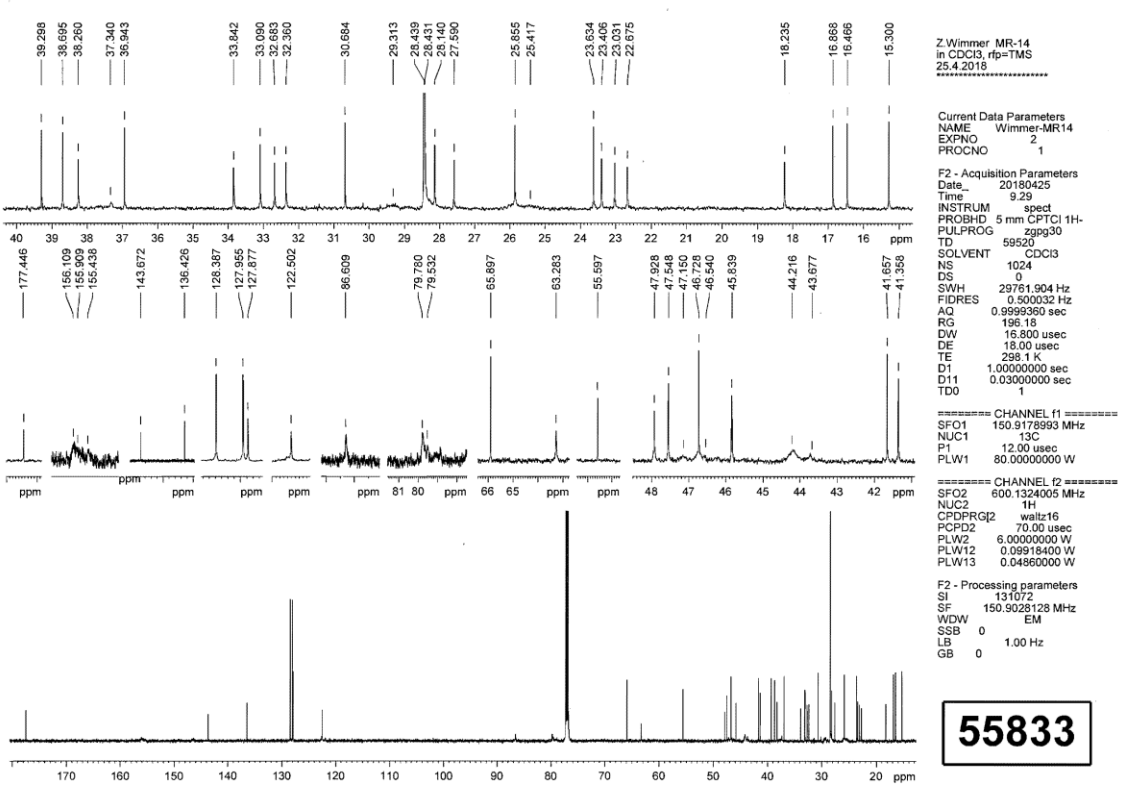


A solution of **18** (0.811 g, 1.534 mmol) in DCM (7 mL) was added to a solution of **11** (0.78 g, 1.334 mmol) in DCM (7 mL) under stirring. Then a solution of $CuSO_4 \cdot 5H_2O$ + TBTA (14 mL, 0.05 M) was added, followed by sodium ascorbate (0.265 g, 1.334 mmol). Reaction time was 12 h. Finally, the reaction mixture was worked-up by extracting it with chloroform, drying over sodium sulphate and evaporating the solvent. The crude residue was purified by column chromatography using chloroform / ethanol mixture as mobile phase, affording **12** (1.384 g) in a 91 % yield.

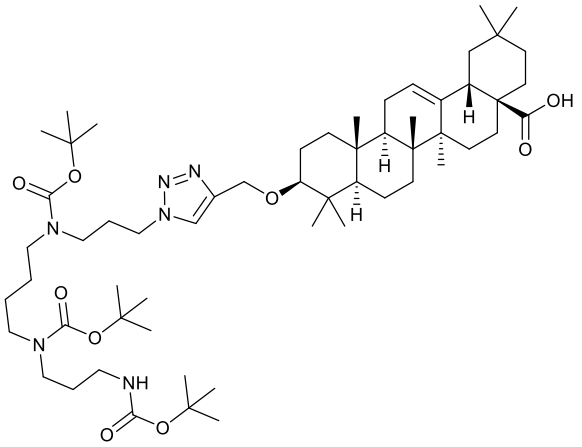
NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.58 (s, 3H, H-26), 0.69 (dd, 1H, *J*=1.8; 11.6 Hz, H-5), 0.75 (s, 3H, H-25), 0.86 (s, 3H, H-24), 0.87 (s, 3H, H-29), 0.88 (s, 3H, H-23), 0.90 (s, 3H, H-30), 1.01 (ddd, 2H, *J*=2.9; 3.8; 13.8 Hz, H-16), 1.09 (s, 3H, H-27), 1.33 (ddd, 2H, *J*=2.3; 4.8; 13.6 Hz, H-19), 1.76-1.87 (m, 2H, H-2), 1.95 (dt, 2H, *J*=4.1; 13.5; 13.5 Hz, H-11), 2.11 (m, 2H, *J*=7.0; 7.0; 7.0; 7.0 Hz, H-10′), 2.88 (bdd, 1H, *J*=4.8; 13.6 Hz, H-18), 2.95 (dd, 1H, *J*=4.3; 11.7 Hz, H-3), 3.03-3.27 (m, 2H, H-11′, H-12′, H-15′, H-16′, H-18′), 4.32 (t, 2H, *J*=7.1 Hz, H-9′), 4.53 (d, 2H, *J*=12.4 Hz, H-6′), 4.75 (d, 2H, *J*=12.4 Hz, H-6′), 5.02 (d, 2H, *J*=12.5 Hz, H-1′), 5.07 (d, 2H, *J*=12.5 Hz, H-1′), 5.26 (t, 1H, *J*=3.8 Hz, H-12), 7.50 (bs, 1H, H-7′), 7.61 (bs, 1H, H-8′), Boc groups: 1.41 (s), 1.42 (s, 2x). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.30 (q, C-24), 16.47 (q, C-25), 16.87 (q, C-26), 18.23 (t, C-6), 22.68 (t, C-11), 23.03 (t, C-17′), 23.41 (t, C-2), 23.63 (q, C-30), 25.42 (t, C-14′), 25.86 (q, C-27), 25.94 (t, C-13′), 27.59 (t, C-16), 28.14 (t, C-15), 28.44 (q, C-23), 29.31 (t, C-10′), 30.68 (s, C-20), 32.36 (t, C-22), 32.68 (t, C-7), 33.09 (q, C-29), 33.84 (t, C-21), 36.94 (t, C-1), 37.34 (t, C-18′), 38.26 (s, C-10), 38.70 (s, C-4), 39.30 (s, C-8), 41.36 (d, C-18), 41.66 (s, C-14), 43.68 (t, C-16′), 44.22 (t, C-15′), 45.84 (t, C-19), 46.54 (t, C-12′), 46.73 (s, C-17), 47.15 (t, C-11′), 47.55 (d, C-9), 47.93 (t, C-9′), 55.60 (d, C-5), 63.28 (t, C-6′), 65.90 (t, C-1′), 86.61 (d, C-3), 121.94 (d, C8′),

122.50 (d, C-12), 122.55 (d, C-7′), 127.88 (d, C-5′), 127.96 (d, C-4′), 128.39 (d, C-3′), 136.43 (s, C-2′), 143.67 (s, C-13), 177.45 (s, C-28). MS: *m/z* = 1114.8 Da ($[M+H]^+$). For $C_{65}H_{104}N_6O_9$ calculated exact mass = 1112.79 Da.





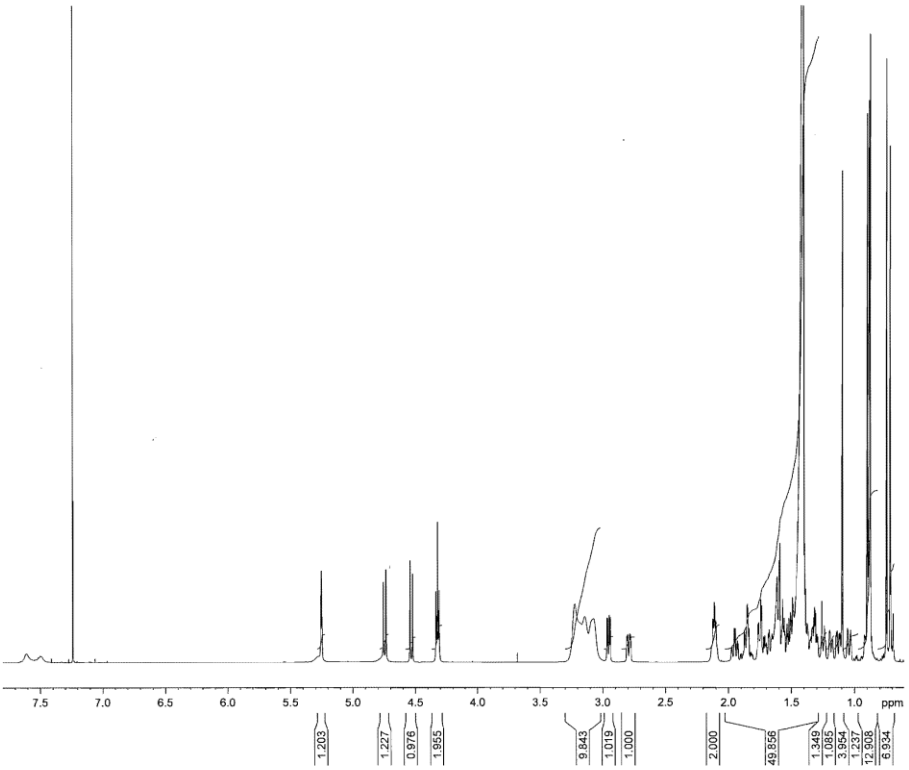
1.11. (3β)-3-({1-[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxo-3-oxa-5,9,14-triazaheptadecan-17-yl]-1*H*-1,2,3-triazol-4-yl}methoxy)olean-12-en-28-oic acid (**13**)



Palladium catalyst (Pd/C, 10 %, 0.849 g, 0.796 mmol) was added to a solution of **12** (1.324 g, 1.189 mmol) in a mixture of THF / ethanol (1:1, 10 mL), and the substrate **12** was subjected to a debenzylation by hydrogenation using H_2 (gas) at r.t. for 24 h. Then the solid phase was filtered off, solvent was evaporated and the crude residue was purified by column

chromatography on silica gel using chloroform / ethanol mixture as mobile phase. The product **13** (0.934 g) was obtained in a 76 % yield.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.70 (dd, 1H, *J*=1.7; 11.4 Hz, H-5), 0.72 (s, 3H, H-26), 0.75 (s, 3H, H-25), 0.88 (s, 3H, H-23), 0.88 (s, 3H, H-29), 0.89 (s, 3H, H-24), 0.91 (s, 3H, H-30), 1.05 (dt, 2H, *J*=3.6; 3.6; 13.8 Hz, H-16), 1.10 (s, 3H, H-27), 1.13 (ddd, 2H, *J*=2.6; 4.8; 13.8 Hz, H-19), 1.83 (ddd, 2H, *J*=3.7; 7.1; 18.8 Hz, H-2), 1.88 (ddd, 2H, *J*=3.6; 11.0; 18.8 Hz, H-2), 1.95 (dt, 2H, *J*=4.1; 13.7; 13.7 Hz, H-11), 2.80 (bdd, 1H, *J*=4.8; 13.9 Hz, H-18), 2.96 (dd, 1H, *J*=4.3; 11.4 Hz, H-3), 3.03-3.28 (m, 2H, H-6′, H-7′, H-10′, H-11′, H-12′), 4.32 (t, 2H, *J*=7.1 Hz, H-4′), 4.54 (d, 2H, *J*=12.5 Hz, H-1′), 4.75 (d, 2H, *J*=12.5 Hz, H-1′), 5.25 (t, 1H, *J*=3.8 Hz, H-12), 7.50 (bs, 1H, H-2′), 7.61 (bs, 1H, H-3′), Boc groups: 1.41 (s), 1.42 (s). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.32 (q, C-24), 16.44 (q, C-25), 17.09 (q, C-26), 18.21 (t, C-6), 22.67 (t, C11), 22.91 (t, C-12′), 23.40 (t, C-2), 23.56 (q, C-30), 25.47 (t, C-9′), 25.90 (q, C-27), 25.99 (t, C-8′), 27.64 (t, C-16), 28.13 (t, C-15), 28.13 (q, C-23), 29.29 (t, C-5′), 30.66 (s, C-20), 32.42 (t, C-22), 32.59 (t, C-7), 33.05 (q, C-29), 33.79 (t, C-21), 37.00 (t, C-1), 37.34 (t, C-13′), 38.22 (s, C-10), 38.70 (s, C-4), 39.28 (s, C-8), 41.00 (d, C-18), 41.58 (s, C-14), 43.74 (t, C-11′), 44.18 (t, C-10′), 45.86 (t, C-19), 46.24 (t, C-7′), 46.48 (s, C-17), 47.18 (t, C-6′), 47.56 (d, C-9), 47.94 (t, C-4′), 55.60 (d, C-5), 63.24 (t, C-1′), 86.56 (t, C-3), 120.78 (d, C-3′), 121.99 (d, C-2′), 122.60 (d, C-12), 143.59 (s, C-13), 182.82 (s, C-28), Boc groups: 28.40 (q), 79.55 (s), 79.80 (s), 155.40 (s), 156.11 (s). MS: *m/z* = 1023.6 Da ($[M+H]^+$). For $C_{58}H_{98}N_6O_9$ calculated exact mass = 1022.74 Da.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25.4.2018
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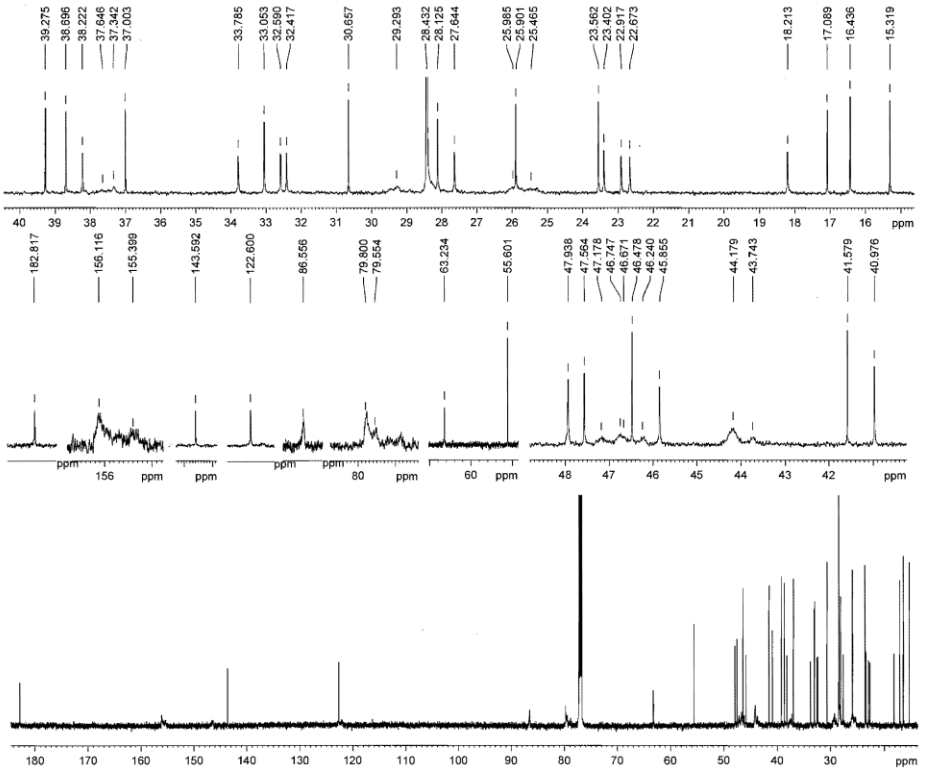
55834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182.817 156.116 155.399 143.592 122.600 86.556 79.800 79.554 63.234 55.601 47.938 47.584 47.178 46.747 46.671 46.478 46.240 45.855 44.179 43.743 41.579 40.976
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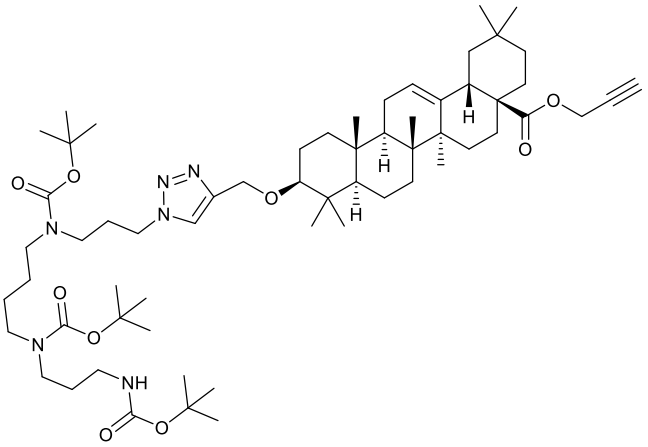
55834

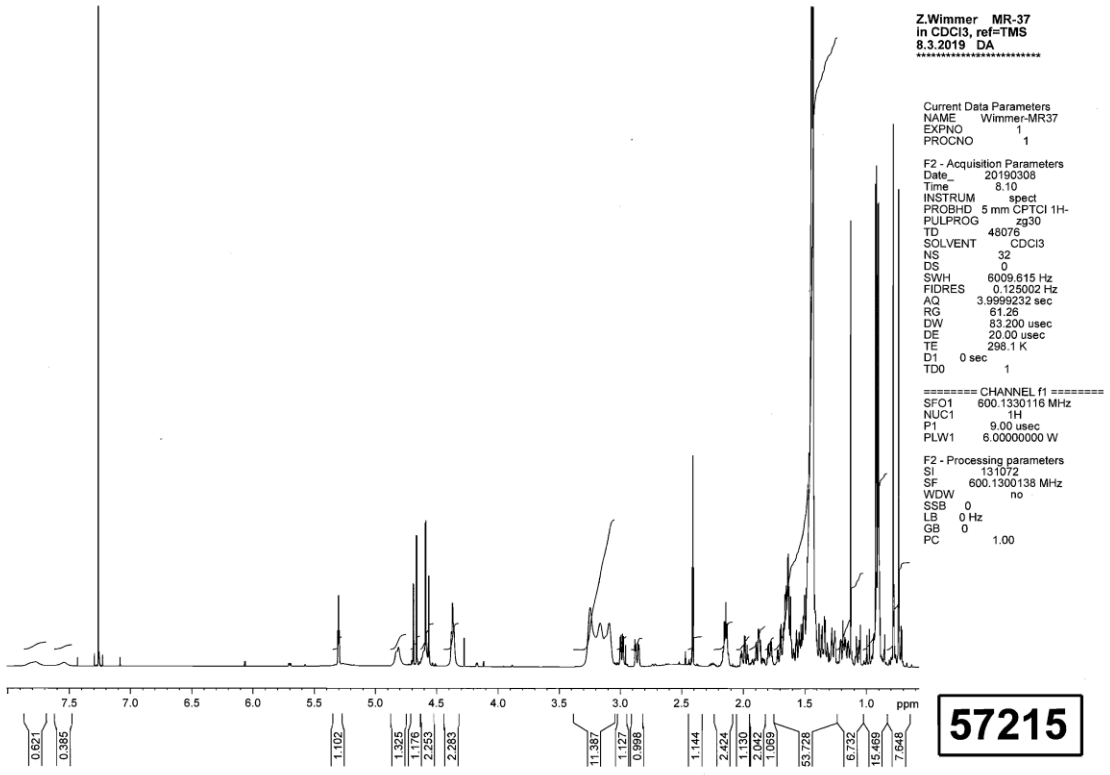
1.12. Prop-2-yn-1-yl-(3β)-3-({1-[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxo-3-oxa-5,9,14-triazaheptadecan-17-yl]-1*H*-1,2,3-triazol-4-yl}methoxy)olean-12-en-28-oate (**14**)

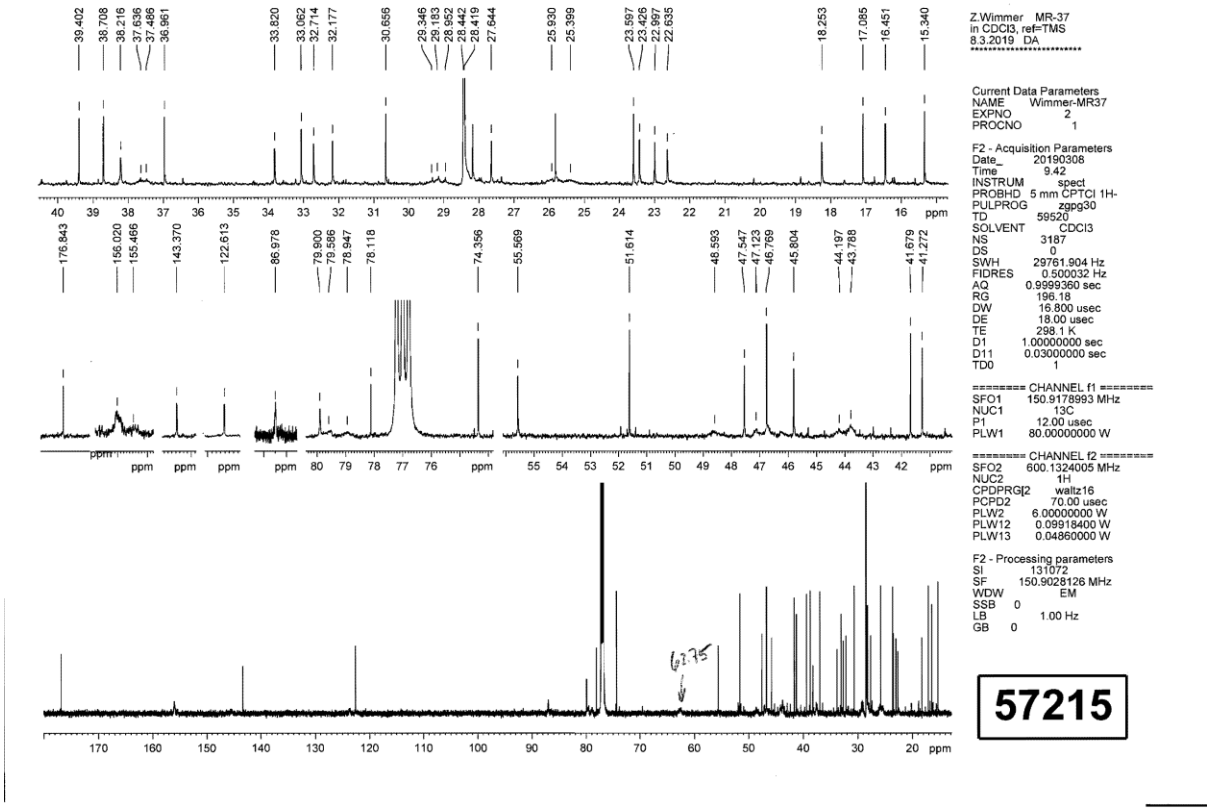


Potassium carbonate (0.506 g, 3.664 mmol) was added to a solution of **13** (0.750 g, 0.732 mmol) in DMF (10 mL), and the mixture was stirred at r.t. for 15 min. Then propargyl bromide (327 µL, 3.664 mmol) was added, and stirring continued overnight. To work-up the mixture, DMF was evaporated, and the residue was extracted with chloroform, the extract was dried over sodium sulphate and the solvent was evaporated. The crude residue was purified by column chromatography on silica gel, first using petroleum ether as mobile phase to remove the rest of DMF, then using chloroform / ethanol mixture as mobile phase affording **14** (0.426 g) in a 54 % yield.

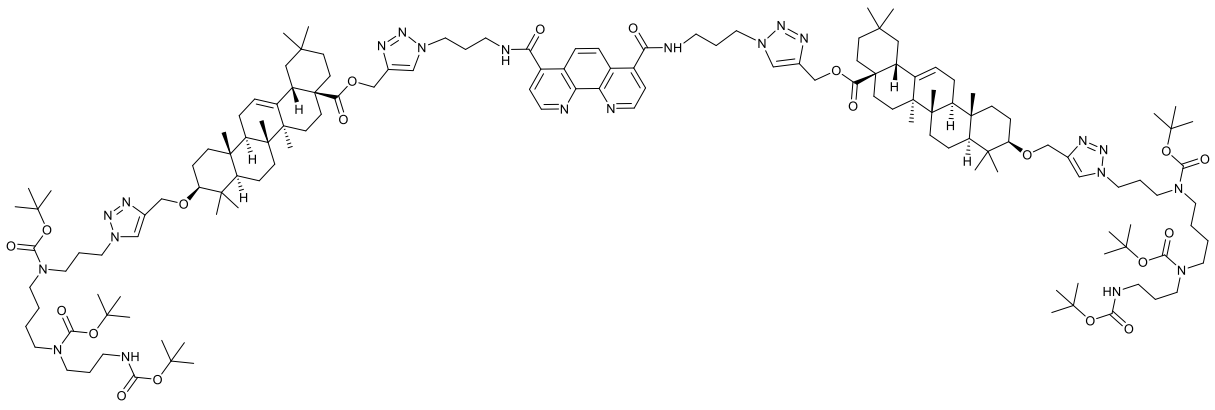
NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.72 (dd, 1H, *J*=1.8; 11.5 Hz, H-5), 0.74 (s, 3H, H-26), 0.78 (s, 3H, H-25), 0.90 (s, 3H, H-29), 0.91 (s, 3H, H-24), 0.92 (s, 3H, H-23), 0.93 (s, 3H, H-30), 1.07 (dt, 2H, *J*=3.9; 3.9; 14.0 Hz, H-16), 1.13 (s, 3H, H-27), 1.27 (dt, 2H, *J*=3,2; 3.2; 12.5 Hz, H-7), 1.56 (ddd, 2H, *J*=2.9; 4.2; 13.8 Hz, H-22), 1.70 (dt, 2H, *J*=4.4; 14.0; 14.0 Hz, H-22), 1.82-1.93 (m, 2H, H-2), 1.99 (dt, 2H, *J*=4.1; 13.5; 13.5 Hz, H-11), 2.11-2.18 (m, 2H, H-5‘), 2.41 (t, 1H, *J*=2.5 Hz, H-16‘), 2.86 (bdd, 1H, *J*=4.7; 14.0 Hz, H-18), 2.99 (dd, 1H, *J*=4.3; 11.8 Hz, H-3), 3.05-3.28 (m, 2H, H-4‘, H-6‘, H-7‘, H-10‘, H-11‘, H-13‘), 4.37 (t, 1H, *J*=5.9 Hz, H-13‘), 4.58 (dd, 2H, *J*=2.5; 12.6 Hz, H-14‘), 4.67 (dd, 2H, *J*=2.5; 12.6 Hz, H-14‘), 4.78-4.85 (m, 2H, H-1‘), 5.30 (t, 1H, *J*=3.8 Hz, H-12), Boc groups: 1.43 (s). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.34 (q, C-24), 16.45 (q, C-25), 17.09 (q, C-26), 18.25 (t, C-6), 22.64 (t, C-6‘), 22.96 (t, C-11), 23.00 (t, C-12‘), 23.43 (t, C-2), 23.60 (q, C-30), 25.40 (t, C-9‘), 25.82 (q, C-27), 25.93 (t, C-8‘), 27.64 (t, C-16), 28.18 (t, C-15), 28.42 (q, C-23), 29.18 (t, C-5‘), 30.66 (s, C-20), 32.18 (t, C-22), 32.71 (t, C-7), 33.06 (q, C-29), 33.82 (t, C-21), 36.96 (t, C-1), 37.64 (t, C-13‘), 38.22 (s, C-10), 38.71 (s, C-4), 39.40 (s, C-8), 41.27 (d, C-18), 41.68 (s, C-

14), 43.79 (t, C-11‘), 44.20 (t, C-10‘), 45.80 (t, C-19), 46.77 (s, C-17), 47.12 (t, C-7‘), 47.55 (d, C-9), 48.59 (t, C-4‘), 51.61 (t, C-14‘), 55.57 (d, C-5), 62.75 (t, C-1‘), 74.36 (d, C-16‘), 78.12 (s, C-15‘), 86.98 (d, C-3), 122.61 (d, C-12), 123.68 (d, C-3‘), 143.37 (s, C-13), 145.45 (s, C-2‘), 176.84 (s, C-28), Boc groups: 28.44 (q, 3C), 78.95 (s), 79.59 (s), 79.90 (s), 155.47 (s), 156.02 (s, 2C). MS: *m/z* = 1061.4 Da ($[M+H]^+$). For $C_{61}H_{100}N_6O_9$ calculated exact mass = 1060.76 Da.





1.13. 1,10-Phenanthrolin-4,7-diyl-bis(carbonyliminopropan-3,1-diyl-1*H*-1,2,3-triazol-1,4-diylmethandiyl)-(3β,3'β)bis[3-({1-[9,14-bis(*tert*-butoxycarbonyl)-2,2-dimethyl-4-oxo-3-oxa-5,9,14-triazaheptadekan-17-yl]-1*H*-1,2,3-triazol-4-yl}methoxy)olean-12-en-28-oate] (**15**)

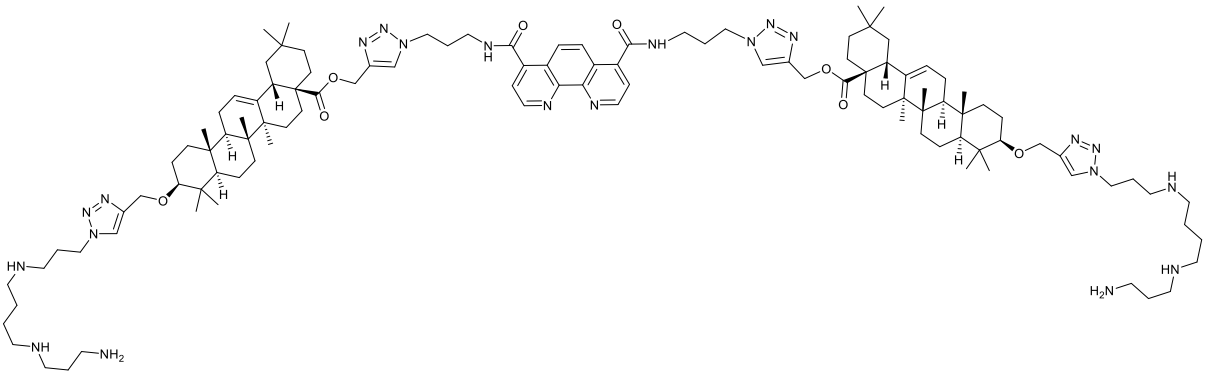


A solution of **14** (0.328 g, 0.297 mmol) in DCM (4 mL) was added to a solution of **8** (0.042 g, 0.099 mmol) in DCM (4 mL) under stirring. Then a solution of $CuSO_4 \cdot 5H_2O$ + THPTA (8 mL, 0.05 M) was added and stirring continued for an additional 15 min. Then sodium ascorbate (0.157 g, 0.079 mmol) was added, and stirring continued for 12 h. The mixture was then quenched with water and extracted with chloroform. The extract was dried over sodium

sulphate, solvent was evaporated, and the residue was purified by column chromatography on silica gel, using chloroform / ethanol mixture as mobile phase, affording **15** (0.169 g) in a 66 % yield.

MALDI-TOF MS: *m/z* = 2577 Da ($[M+Na]^+$). For $C_{142}H_{220}N_{22}O_{20}$ calculated exact mass = 2553.69 Da.

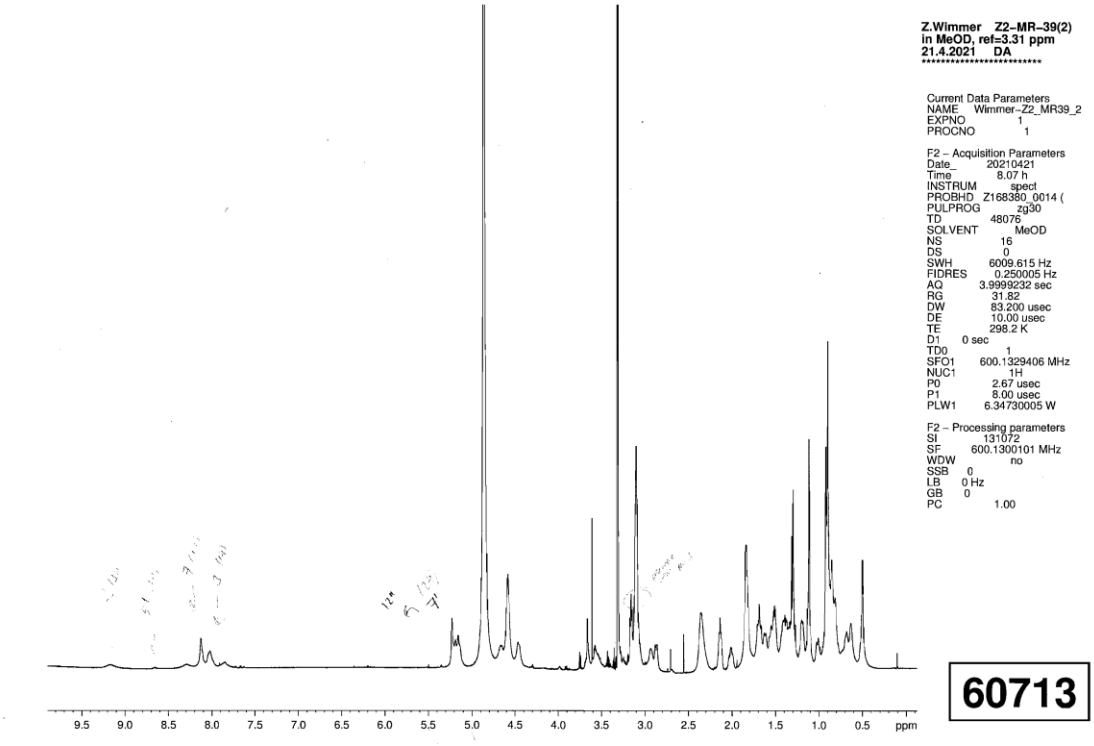
1.14. 1,10-Phenanthrolin-4,7-diyl-bis(carbonyliminopropan-3,1-diyl-1*H*-1,2,3-triazol-1,4-diylmethandiyl)-(3β,3'β)bis[3-({1-[3-({4-[(3-aminopropyl)amino]butyl}amino)propyl]-1*H*-1,2,3-triazol-4-yl}methoxy)olean-12-en-28-oate] (**16**)



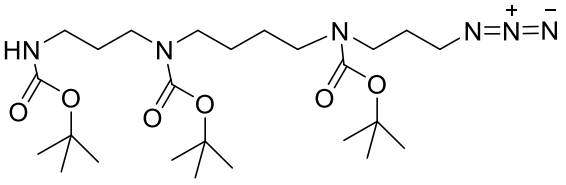
A solution of **15** (0.155 g, 0.068 mmol) in 1M HCl in ethyl acetate (4 mL, 3.91 mmol) was placed to an incubator and shaked at 25 ºC for 12 h. Then the appeared crystals were filtered off, and washed with ether, affording **16** (0.083 g) in a 62 % yield.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.72 (dd, 1H, *J*=1.8; 11.5 Hz, H-5), 0.74 (s, 3H, H-26), 0.78 (s, 3H, H-25), 0.90 (s, 3H, H-29), 0.91 (s, 3H, H-24), 0.92 (s, 3H, H-23), 0.93 (s, 3H, H-30), 1.07 (dt, 2H, *J*=3.9; 3.9; 14.0 Hz, H-16), 1.13 (s, 3H, H-27), 1.27 (dt, 2H, *J*=3,2; 3.2; 12.5 Hz, H-7), 1.56 (ddd, 2H, *J*=2.9; 4.2; 13.8 Hz, H-22), 1.70 (dt, 2H, *J*=4.4; 14.0; 14.0 Hz, H-22), 1.82-1.93 (m, 2H, H-2), 1.86 (m, 2H, *J*=6.7; 6.7; 6.7; 6.7 Hz, H-10''), 1.99 (dt, 2H, *J*=4.1; 13.5; 13.5 Hz, H-11), 2.11-2.18 (m, 2H, H-5'), 2.41 (t, 1H, *J*=2.5 Hz, H-16'), 2.86 (bdd, 1H, *J*=4.7; 14.0 Hz, H-18), 2.99 (dd, 1H, *J*=4.3; 11.8 Hz, H-3), 3.05-3.28 (m, 2H, H-4', H-6', H-7', H-10', H-11', H-13'), 3.45 (dt, 2H, *J*=5.6; 6.7; 6.7 Hz, H-9''), 3.50 (t, 2H. *J*=6.7 Hz, H-11''), 4.37 (t, 1H, *J*=5.9 Hz, H-13'), 4.58 (dd, 2H, *J*=2.5; 12.6 Hz, H-14'), 4.67 (dd, 2H, *J*=2.5; 12.6 Hz, H-14'), 4.78-4.85 (m, 2H, H-1'), 5.30 (t, 1H, *J*=3.8 Hz, H-12), 7.81 (d, 1H, *J*=4.3 Hz, H-3''), 8.16 (s, 1H, H-7''), 8.95 (bt, 1H, *J*=5.6 Hz, NH), 9.18 (d, 1H, *J*=4.3 Hz, H-2''). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 15.34 (q, C-24), 16.45 (q, C-25), 17.09 (q, C-26), 18.25 (t, C-6), 22.64 (t, C-6'), 22.96 (t, C-11), 23.00 (t, C-12'), 23.43 (t, C-2), 23.60 (q, C-30), 25.40 (t, C-9'), 25.82 (q, C-27), 25.93 (t, C-8'), 27.64 (t, C-16), 28.18 (t, C-15), 28.24 (t, C-10''),

28.42 (q, C-23), 29.18 (t, C-5‘), 30.66 (s, C-20), 32.18 (t, C-22), 32.71 (t, C-7), 33.06 (q, C-29), 33.82 (t, C-21), 36.60 (t, C-9’’), 36.96 (t, C-1), 37.64 (t, C-13‘), 38.22 (s, C-10), 38.71 (s, C-4), 39.40 (s, C-8), 41.27 (d, C-18), 41.68 (s, C-14), 43.79 (t, C-11‘), 44.20 (t, C-10‘), 45.80 (t, C-19), 46.77 (s, C-17), 47.12 (t, C-7‘), 47.55 (d, C-9), 48.49 (t, C-11’’), 48.59 (t, C-4‘), 51.61 (t, C-14‘), 55.57 (d, C-5), 62.75 (t, C-1‘), 74.36 (d, C-16‘), 78.12 (s, C-15‘), 86.98 (d, C-3), 121.15 (d, C-3’’), 122.61 (d, C-12), 123.68 (d, C-3‘), 124.29 (d, C-7’’), 124.46 (s, C-5’’), 142.46 (s, C-4’’), 143.37 (s, C-13), 145.45 (s, C-2‘), 145.68 (s, C-6’’), 149.97 (d, C-2’’), 166.59 (s, C-8’’), 176.84 (s, C-28). MS: *m/2z* = 977 ($[M+2H]^{2+}$), *m/z* = 1954 Da ($[M+H]^{+}$). For $C_{112}H_{172}N_{22}O_8$ calculated exact mass = 1953.37 Da.



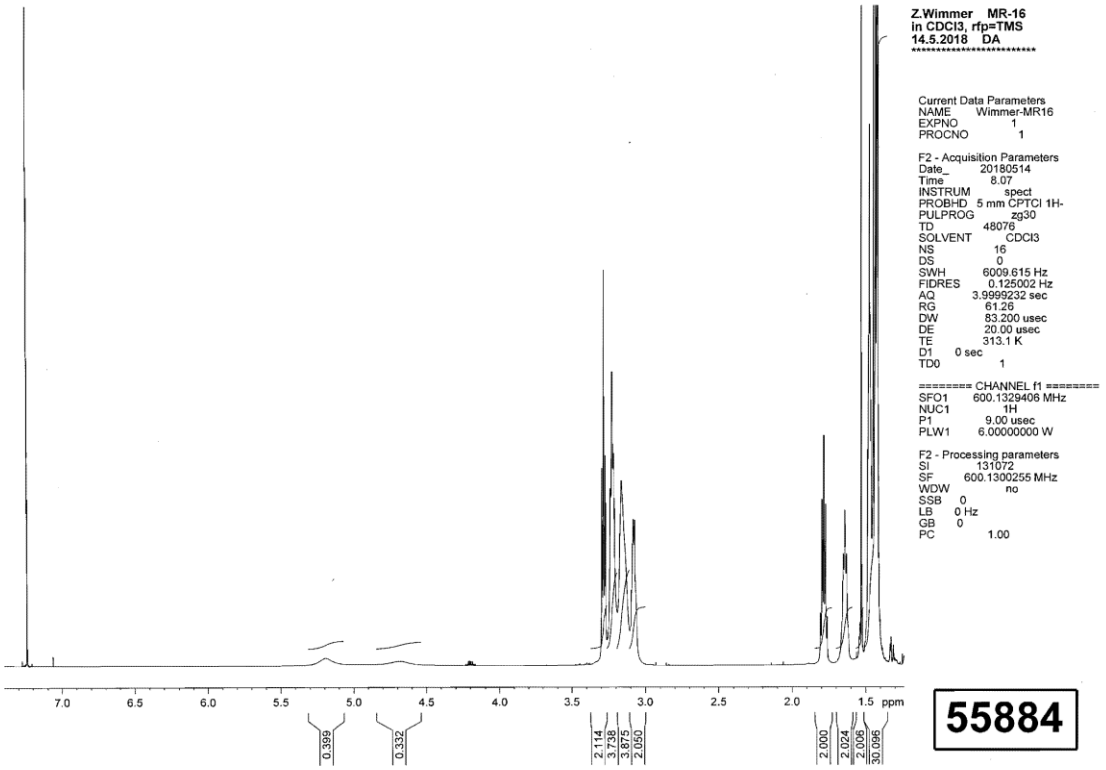
1.15. 1-Azido-4,9,13-tri(*tert*-butoxycarbonyl)-4,9,13-triazatridecane (**18**)

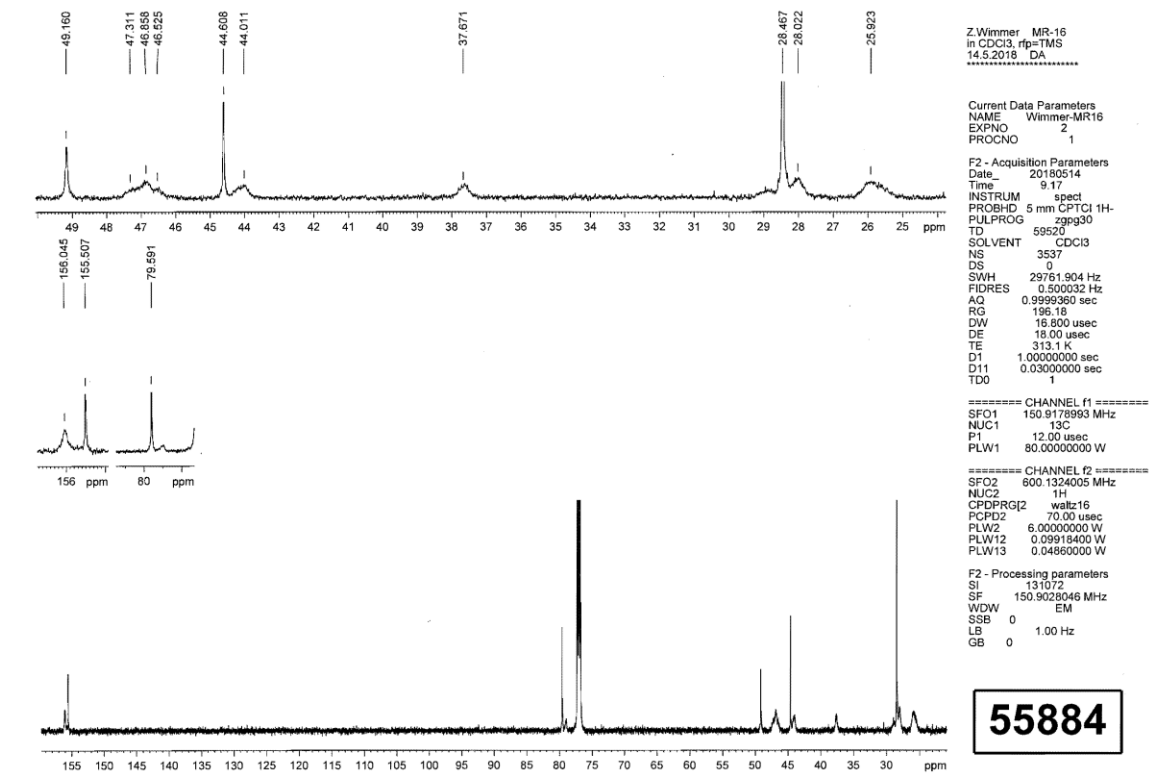


Potassium carbonate (3.844 g, 27.817 mmol) and $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.019 mmol) were added to a solution of *tris*-Boc-spermine (**17**; 1 g, 1.989 mmol) in methanol (7 mL). After dissolving all ingredients, 3-azidosulphonyl-3*H*-imidazol-1-ium hydrogen sulphate (0.647 g,

2.386 mmol) was added, and the reaction mixture was stirred overnight. Then the reaction mixture was extracted by ethyl acetate, and the crude residue obtained after drying a solvent evaporation was purified by column chromatography using chloroform / ethanol mixture as mobile phase, affording **18** (0.865 g) in an 82 % yield.

NMR: ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 1.46-1.49 (m, 2H, H-5, H-6), 1.62-1.67 (m, 2H, H-9), 1.78 (m, 2H, *J*=6.8; 6.8; 6.8; 6.8 Hz, H-2), 3.08 (bq, 2H, *J*=6.5 Hz. H-10), 3.12-3.19 (m, 2H, H-4, H-7), 3.23 (bt, 2H, *J*=7.0 Hz, H-1, H-8), 3.28 (t, 2H, *J*=6.7 Hz, H-3), Boc groups: 1.42 (s), 1.44 (s), 1.45 (s). ^{13}C-NMR (150.91 MHz, $CDCl_3$): δ [ppm] 25.92 (t, C-4), 26.20 (t, C-5), 28.02 (t, C-2), 29.20 (t, C-9), 37.67 (t, C-10), 44.01 (t, C-8), 44.61 (t, C-1), 46.86 (t, C-4), 47.31 (t, C-7), 49.16 (t, C-3), Boc groups: 28.47 (q, 3x), 79.59 (s, 3x), 155.51 (s), 156.05 (s, 2x). MS: *m/z* = 529.3 Da ($[M+H]^+$). For $C_{25}H_{48}N_6O_6$ calculated exact mass = 528.36 Da.





1.16. Transmission electron microscopy: TEM and cryo-TEM microscopy

Transmission electron microscopy (TEM) was performed with a Tecai G2 Spirit Twin 12 microscope (FEI, Czech Republic) equipped with a cryo-attachment (Gatan, CA, USA). The samples for TEM were prepared by fast drying method: The studied compounds (2 μL droplets of their water solution with concentration 5 $mg.mL^{-1}$) were dropped onto a standard carbon-coated copper TEM grid (300 mesh). After 2 min the rest of the solution was removed by touching the bottom of the grid with filter paper. Then the sample was left to dry completely at ambient temperature and observed in the TEM microscope at room temperature and accelerating voltage 120 kV. The samples for cryogenic TEM (cryo-TEM) were prepared by fast freezing method: The studied compounds (4 μL droplets of their water solution with concentration $5mg.mL^{-1}$) were dropped to an electron microscopy grid covered with a lacey carbon supporting film (Electron Microscopy Sciences, Hatfield, PA, USA), which was hydrophilized just before the experiment by means of glow discharge (Expanded Plasma Cleaner, Harrick Plasma, NY, USA). The excess of the solution was removed by blotting (Whatman no. 1 filter paper) for 1 s, and the grid was plunged into liquid ethane held at –181

°C. The frozen sample was immediately inserted in the cryo-holder, transferred into the TEM microscope and observed at –173 °C at the accelerating voltage of 120 kV (cf. Fig. 1).

1.17. UV spectrometry

UV spectra were measured on a Specord 210 spectrometer (Jena Analytical, Germany) in the wavelength range of 200–400 nm. The stock solutions of the studied compounds were prepared in water at a concentration of 1 mg mL^{-1}. A series of water/methanol mixtures were then prepared starting from water/methanol ratio 0/100 up to 100/0 in 20% steps. The stock solutions of the studied compounds (0.15 mL) were added separately to each vial containing water/methanol mixtures (3 mL), and the UV spectra recorded in the wavelength range of 200–400 nm. Spectra were recorded every 24 h for 4 days.

1.18. Metal chelation experiments

The stock solution of the ligand was prepared in a concentration of 2 mg mL^{-1}. For the metal chelation, an aliquot amount of 100 µL (1×10^{-7} mol L^{-1}) was used.

Copper(II) and nickel(II) salts ($c = 1 \times 10^{-9}$ mol L^{-1} of Cu(II) or Ni(II) ions) were dissolved in a distilled water (10 mL), and then mixed with the appropriate ligand. The resulting ligand / metal ratio was 100 / 1. After 1 h of stirring, UV and MS spectra were recorded.

For the experiments with the radioisotopic ^{64}Cu(II) salt, ligands (20 µL) were transferred into vials, physiological solution (100 µL) was added, and, finally, the ^{64}Cu(II) salt (15 µL; 22.3 MBq) was added. The mixture was allowed to stir for 1 h, and then subjected to the HPLC, UV and MS analysis.

1.19. Cytotoxicity tests

In the cytotoxicity screening tests, cancer cell lines of T-lymphoblastic leukemia (CEM), breast carcinoma (MCF7), cervical carcinoma (HeLa), and malignant melanoma (G-361) were used, all of which were obtained from the European Collection of Authenticated Cell Cultures (ECACC, London, UK). Human foreskin fibroblasts (BJ) were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Sigma, MO, USA). The media used were supplemented with 10% foetal bovine serum, 2 mM L-glutamine, and 1% penicillin-streptomycin. The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment. Cells were sub-cultured twice or three times a week using the standard trypsinization procedure. The experimental procedure has already published.[10]

2. Results

2.1. A formation of complexes ligand–metal salt

Salts of ^{64}Ni(II), Cu(II) and ^{64}Cu(II) were selected for this short investigation. The isotope ^{64}Cu(II) is important metal used in PET to monitor cancer. A formation of the complexes ligand–metal salt was analysed by HPLC-MS. A formation of a complex ligand–Ni(II) was not proven, however, the complexes ligand–Cu(II) and ligand–^{64}Cu(II) were observed. The respective MS of the complexes of **6** and **16** with Cu(II) and ^{64}Cu(II) are shown in Figs. S1-S4.

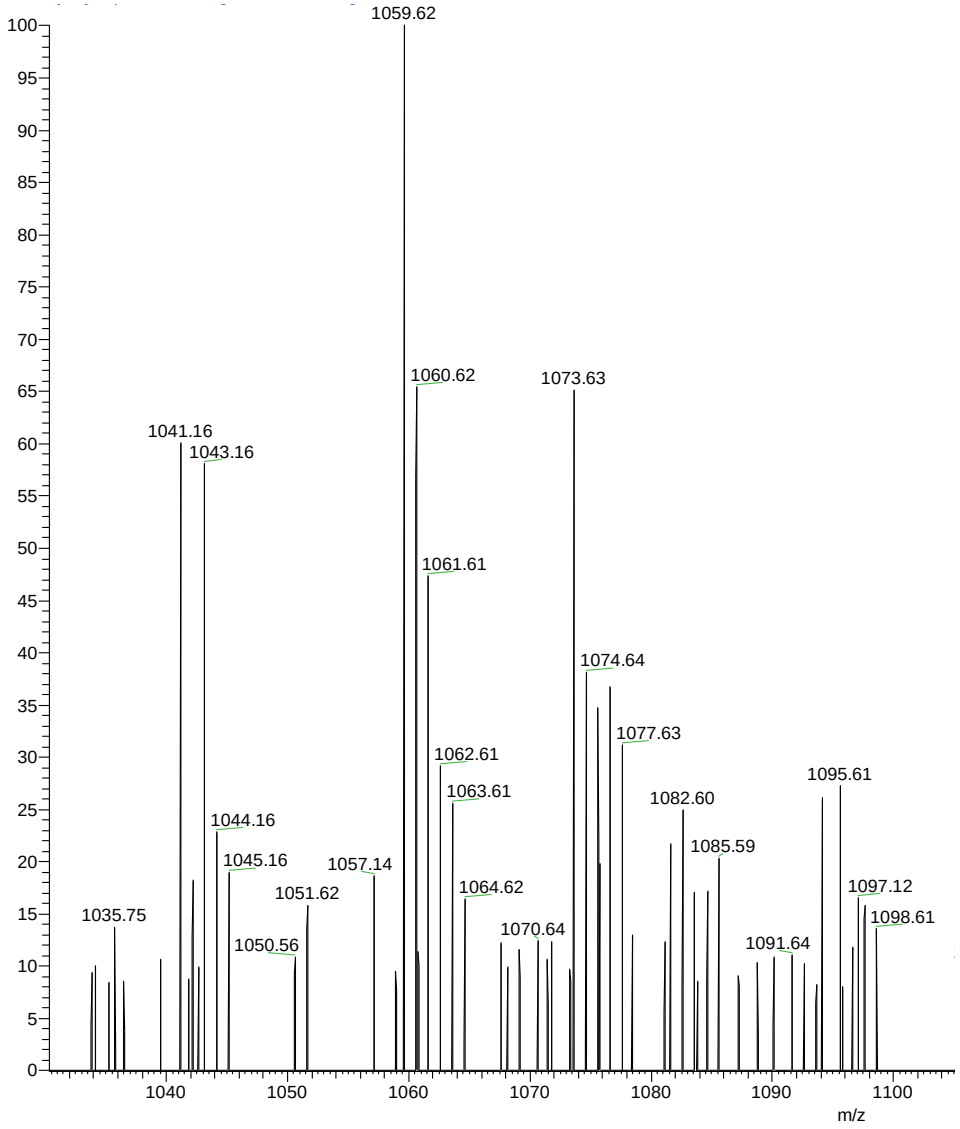


Fig. S1. MS of **6** with coordinated Cu(II) ions: *m/2z* = 1059 $[M+Cu]^{2+}$.

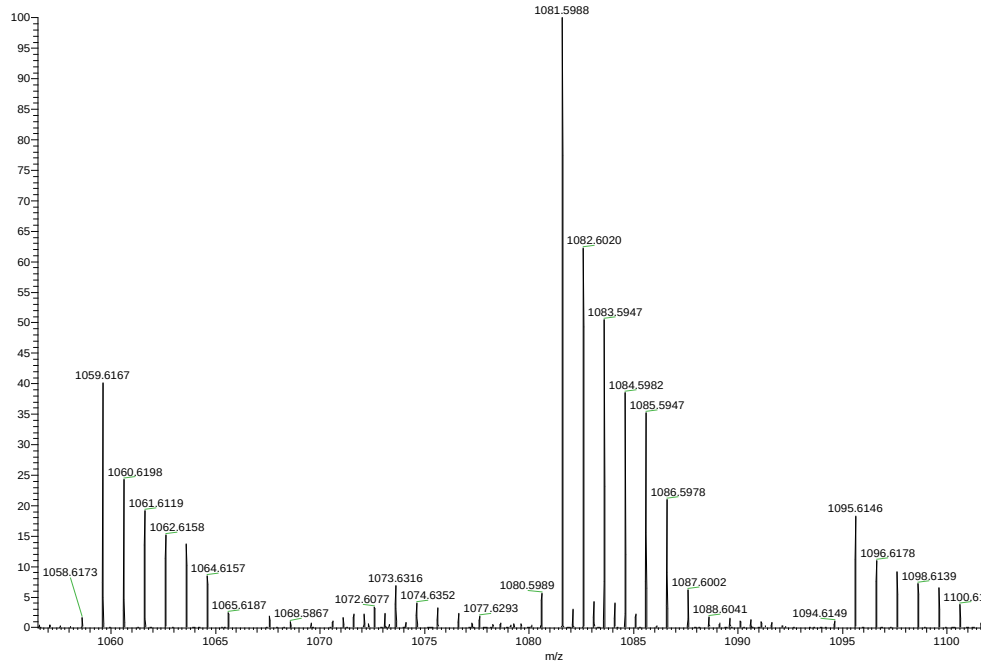


Fig. S2. MS of **6** with coordinated ^{64}Cu(II) ions: *m/2z* = 1082 $[M+^{64}Cu+Na]^{2+}$.

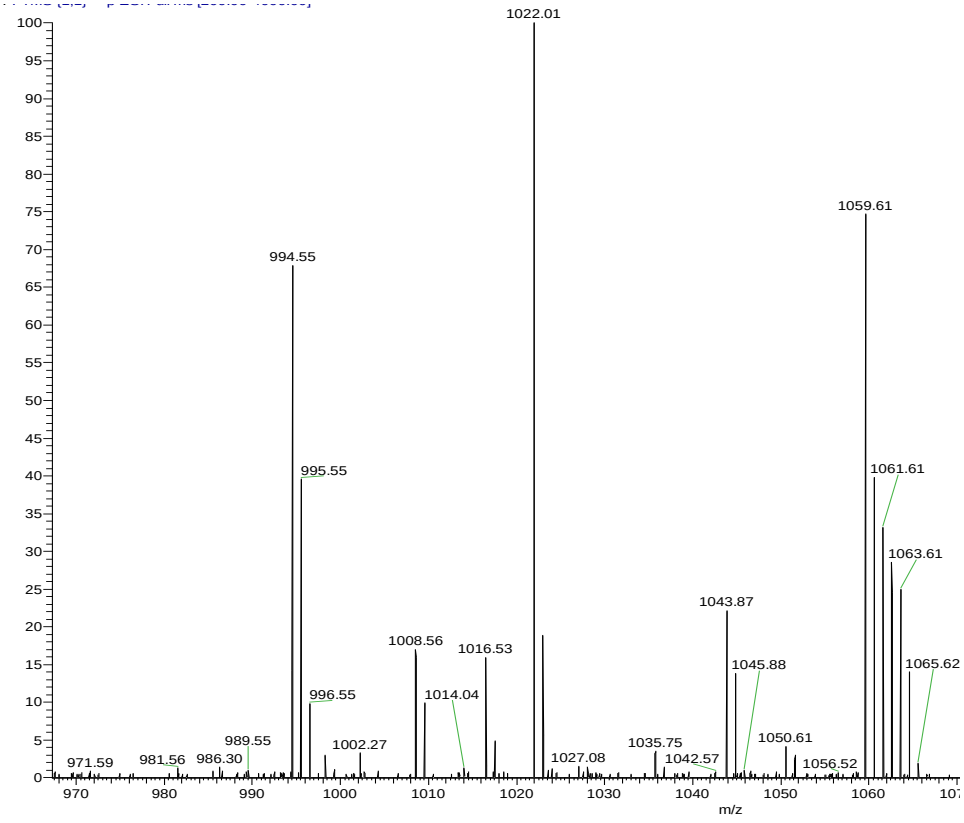


Fig. S3. MS of **16** with coordinated Cu(II) ions: *m/2z* = 994 $[M+H_2O]^{2+}$, 1022 $[M+2Na]^{2+}$, 1059 $[M+Cu+H_2O]^{2+}$.

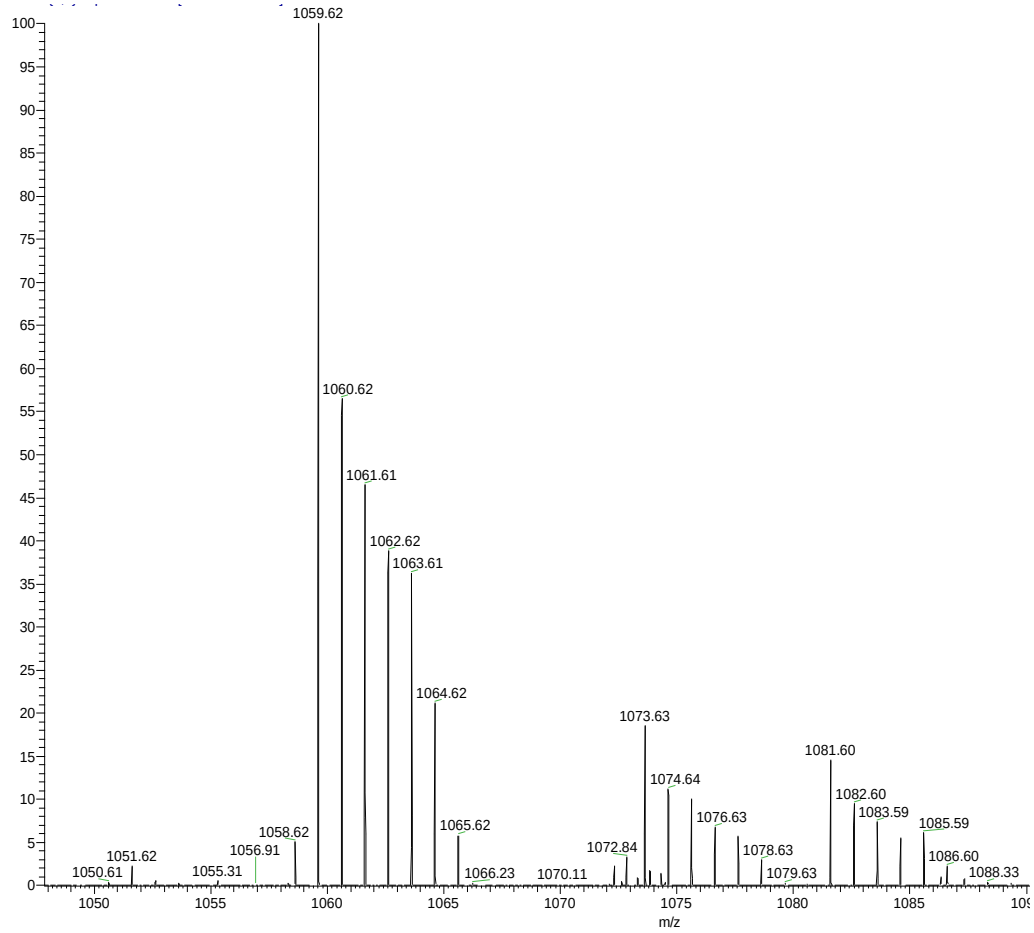


Fig. S4. MS of **16** with coordinated ^{64}Cu(II) ions: *m/2z* = 1059 $[M+^{64}Cu+H_2O]^{2+}$.

2.2. UV spectroscopy

UV spectroscopy was used to monitor a formation of supramolecular systems in the solutions of **6** and **16** in mixtures of methanol / water with changing ratio of both solvents and with constant concentration of the studied compound. The UV spectra of the compounds **7** and **8** were taken as reference UV spectra to detect UV maxima (Fig. S5). The resulting sets of spectra of **6** and **16** were evaluated, and the changes are shown in Figs. S6 and S7.

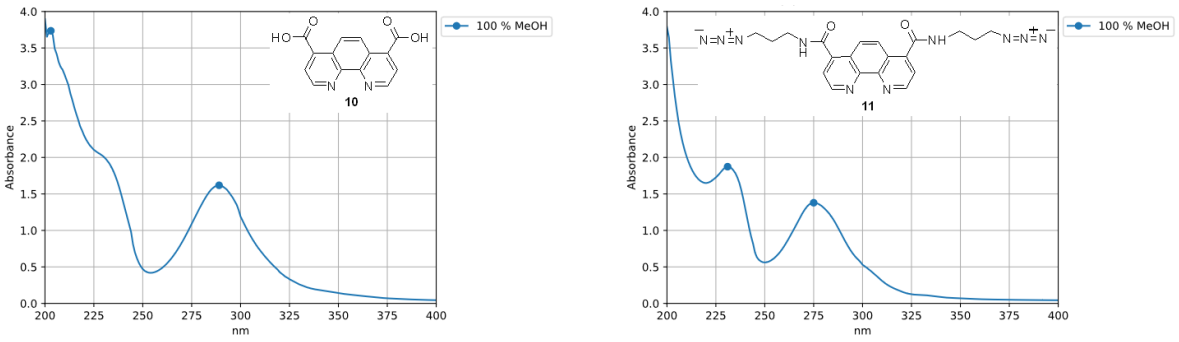


Fig. S5. UV spectrum of **7** (left) and **8** (right).

Fig. S6 shows a detail taken from the UV spectra of **6**. Irregularity is observed in the UV maxima at about 276 nm wavelength starting from 100 % methanol to 0 % methanol in methanol / water mixtures in 20 % steps. With no self-assembly observed, the maxima should decrease regularly in dependence of stepwise decrease of concentration of methanol in the mixtures. However, the presented Fig. S6 shows important irregularity showing very rapid formation of self-aggregates in time 0 h (top spectrum) and 52 h (bottom spectrum).

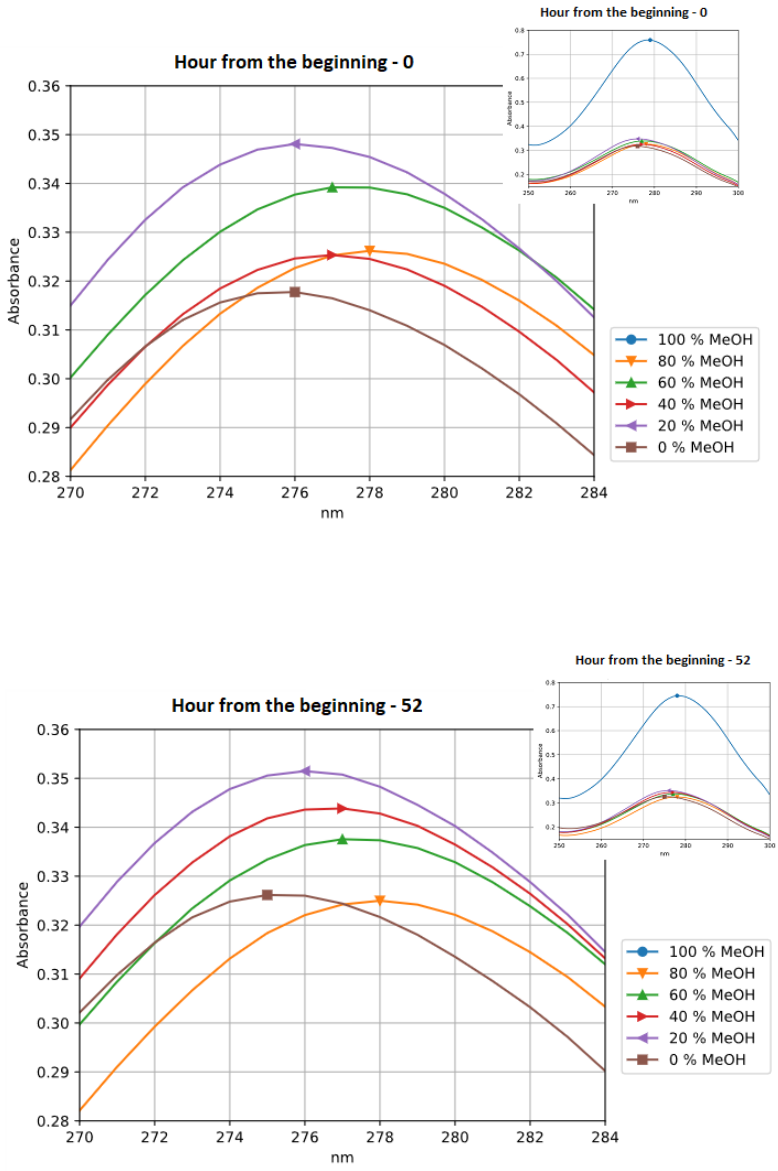


Fig. S6. A detail from the UV spectrum of **6** in the mixtures of methanol / water with the constant concentration of **6** in time 0 h and 52 h.

Fig. S7 shows analogous analysis of the UV spectra of **16**. Irregularity of the order of the UV maxima were also observed.

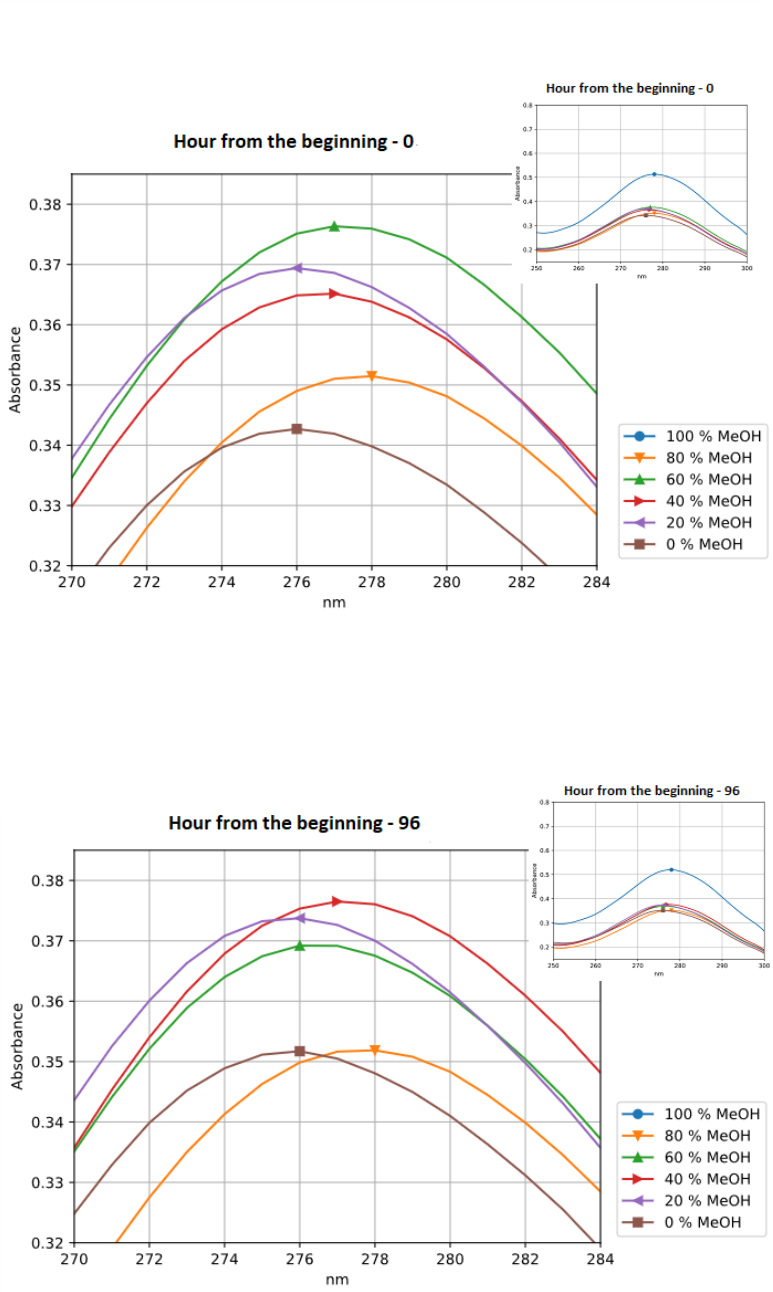


Fig. S7. A detail from the UV spectrum of **16** in the mixtures of methanol / water with the constant concentration of **16** in time 0 h and 96 h.

2.3. Cytotoxicity screening tests and a relation between cytotoxicity and self-assembly

As it was already stressed in the main text of this paper, cytotoxicity of **6** and **16** was always based on repeated experiments. The stock solutions of the studied compounds were prepared at the very beginning of the experiments, and kept in a freezer before used in the repeated study. Because the compounds **6** and **16** show self-assembly characteristics, self-aggregation occurs either in the stock solution or during the cytotoxicity screening test on a cell wall or inside the cell. This effect always resulted in different values of cytotoxicity calculated from different runs of the experiments, and those values are shown in Table S1 in italics. The molecules behave as nanoprodrugs,[12,34] liberating the cytotoxic molecule in the dependence on the structure of the self-aggregated system. With these two compounds, their behaviour partly supports the finding from cryo-TEM imaging: Compound **6**, forming a supramolecular system quickly, showed higher cytotoxicity in HeLa cancer cell line and in the reference cell line BJ during the first run of experiment. In turn, **16** formed a supramolecular system much slowly, and the higher values of cytotoxicity were observed in the second run of experiments in CEM and G-361 cancer cell lines. We have no exact proof of this hypothesis, and the cytotoxicity values are not so nicely pronounced as earlier with other but similar compounds,[12] however, results presented in a recently published paper support our hypothesis and our findings.[34]

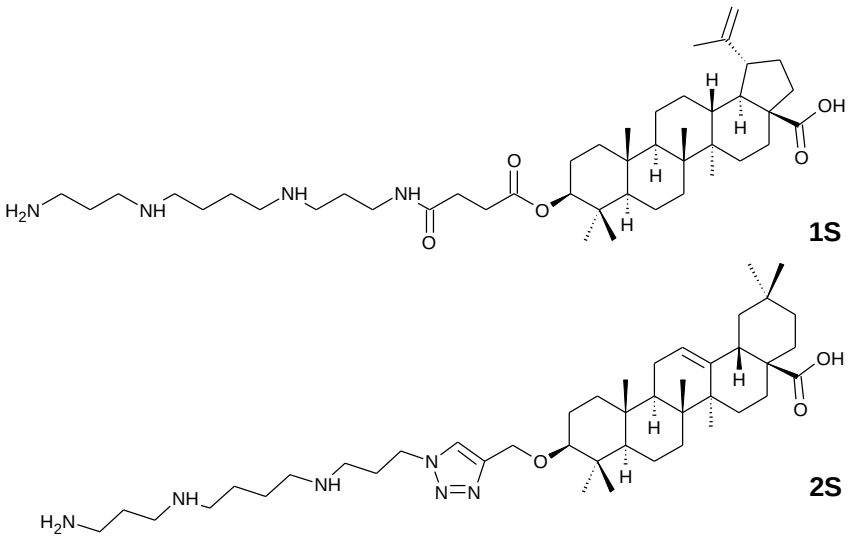


Fig. S8. The structures of compounds **1S** and **2S**, formal precursors of **6** and **16**.

In addition, cytotoxicity data of the compounds **1S** and **2S** (Fig. S8) are presented in Table S1 to support our statement on enhancing effect of **6** and **16** in comparison with their parent components.

Table S1. Cytotoxicity screening tests (IC_{50} values [μM], 72 h)

Compound	MW	Cytotoxicity (IC_{50} [μM]) [a]				
		CEM [b]	MCF7 [c]	HeLa [d]	G-361 [e]	BJ [f]
6	1990.73	6.5 ± 0.6	3.0 ± 0.0	5.7 ± 1.3	5.2 ± 0.6	4.9 ± 0.6
		6.9/6.0	*3.0/3.0*	*4.7/6.6*	*5.6/4.8*	*4.5/5.3*
16	1954.71	8.3 ± 1.7	4.1 ± 1.1	6.9 ± 0.1	5.0 ± 0.1	4.6 ± 0.1
		9.5/7.1	*3.3/4.9*	*6.9/6.8*	*5.0/4.9*	*4.5/4.7*
1S	743.07	28.0 ± 4.7	> 50	> 50	> 50	46.0 ± 0.8
2S	723.10	29.8 ± 3.3	28.0 ± 1.0	12.6 ± 2.8	27.8 ± 0.9	14.2 ± 0.4
CDDP [g]	300.05	0.8 ± 0.1	7.7 ± 1.7	11.4 ± 3.8	4.5 ± 0.6	6.9 ± 0.9

[a] IC_{50} > 50 μM values were measured for 1,10-phenanthroline, triterpenoids and spermine; [b] CEM, cells of human T-lymphoblastic leukemia; [c] MCF7, cells of human breast adenocarcinoma; [d] HeLa, cells of human cervical cancer; [e] G-361, cells of malignant melanoma; [f] BJ, normal human fibroblasts; [g] *cis*-diamminedichloridoplatinum(II) (cisplatin), a pharmacologically used agent for treating cancers, a positive reference compound.

2.4. Physico-chemical and ADME parameters: *in silico* calculations

Physico-chemical and ADME parameters of the studied compounds, calculated *in silico*,[S1] and based on the Lipinski[S2] and Ghose[S3] rules that were postulated for testing *in vivo*. Nevertheless, they are applicable in the screening tests in cancer cell lines, as we have proven several times in our previous papers.[11,S4,S5] However, deviations of experimental and calculated values have already been observed.[12] The rules describe molecular properties important for a small molecule drug pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (known as ADME parameters). However, the rules do not predict displaying of the pharmacological activity. The Lipinski rule of five[S2] and the Ghose rule[S3] consider partition coefficient (log *P*, range -0.4 to +5.6), molar refractivity (range 40 to 130), molecular weight (range 180 to 500), number of atoms in the molecule (20 to 70), and polar

surface area (up to 14 nm). The calculated physico-chemical and ADME parameters are summarized in Table S2. Recommended ranges of several of those parameters is known from the literature,[S6] and based on them, only several of the calculated parameters for the studied compounds appeared within the given range: log *D* and log *S* (both at pH = 7.4) as physico-chemical parameters, and log *PB* (the extent of brain penetration parameter, indicating if the drug might be CNS active or CNS inactive; range +2 (CNS active) to -2 (CNS inactive) and log *BB* (a hybrid parameter determined by permeability, plasma and brain tissue binding and active transport mechanism) that are connected with the activity on central nervous system, the ADME parameters. However, the calculated values of log *PB* and log *BB* indicate that the studied compounds are not considered as CNS active drugs.

Table S2. Physico-chemical and ADME parameters of the compounds **1**–**6** and **9**–**16** calculated using the ACD/iLabs software.[S1]

Compd. or ref. No.	MW	Physico-chemical and ADME parameters [a]									
		log *P*	log *D* (pH 7.4)	log *S* (pH 7.4)	bioav. [%]	log *PS* * $f_{u,\,brain}$	log *PS*	log *PB*	log *BB*	PPB [%]	H_{acc} / H_{don} / n.m.b.
1	456.70	6.26	3.77	-3.34	30-70	-4.4	-2.6	-0.35	-0.35	99.29	3/2/2
2	556.77	6.67	1.80	-2.52	< 30	-5.0	-3.2	-0.59	-0.59	99.59	6/2/7
3	1041.44	10.13	7.63	-4.93	< 30	-8.8	-7.0	-2.00	-2.00	99.44	15/3/26
4	1079.49	11.46	11.46	-8.55	< 30	-8.6	-6.3	-2.00	-2.00	99.77	15/2/29
5	2591.42	22.10	22.10	-0.41	< 30	-20.2	-17.9	-2.00	-2.00	100.00	42/6/68
6	1990.73	11.52	3.50	-0.30	< 30	-13.0	-10.8	-2.00	-2.00	99.99	30/12/54
9	456.70	7.84	5.35	-3.76	30-70	-5.5	-3.7	-0.41	-0.19	99.39	3/2/1
10	546.82	10.32	10.32	-8.30	< 30	-6.3	-4.0	-0.38	-0.38	99.79	3/1/4
11	584.87	11.03	11.03	-9.29	< 30	-6.6	-4.3	-1.10	-1.10	99.96	3/0/7
12	1113.55	14.5	14.50	-8.77	< 30	-10.7	-8.4	-2.00	-2.00	99.95	15/2/23
13	1023.43	12.28	9.79	-6.08	< 30	-10.2	-8.4	-2.00	-2.00	99.84	15/2/23
14	1061.48	13.18	13.18	-8.83	< 30	-9.7	-7.4	-2.00	-2.00	99.95	15/1/26
15	2555.39	25.37	25.37	-0.41	< 30	-22.3	-20.0	-2.00	-2.00	100.00	42/4/62
16	1954.71	14.65	6.63	-0.29	< 30	-15.0	-12.8	-2.00	-2.00	100.00	30/10/48
recom. range	180/500	-0.4/+5.6	-	-6.5/+0.5	-	-	-	-1.5/+1.5	-3.0/+1.2	-	10/5/-

[a] log *P* – partition coefficient; log *D* – distribution coefficient; log *S* – predicted aqueous solubility; bioav. = bioavailability – the degree of availability of a chemical by the target tissue; log *PS* * $f_{u,\,brain}$ – the brain/plasma equilibration rate, the parameter that is a mathematical modeling parameter based on time required for reaching brain equilibrium; log *PS* – logarithm of the permeability-surface area coefficient; log *PB* – the extent of brain penetration parameter; log *BB* – a hybrid parameter determined by permeability, plasma and brain tissue binding,

and active transport mechanism; PPB – plasma protein binding; H_{acc} / H_{don} / n.m.b. = number of H-bond acceptors / number of H-bond donors / number of movable bonds.

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[S1] Advanced Chemistry Development, Version 12.02; software ACD/iLabs; Advanced Chemistry Development: Toronto, ON, Canada, 2011.

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