ELECTRONIC SUPPLEMENTARY INFORMATION

Nano-assembly and cytotoxicity of the L-valine–polyamine conjugates of betulinic acid

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1. Experimental part: Scanned ^{1}H and ^{13}C NMR spectra of the compounds and the calculated NMR spectra of the target compounds (Figures S1–S26), 3D-modelling of the selected target structures (Figures S27–S35) and the cytotoxicity data of the target compounds (Table S1)

1.1. General analytical methods used

The NMR measurements were performed 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 $CDCl_3$ (at 293 K) or in DMSO-d_6 (at 373 K) using tetramethylsilane (δ = 0.0 – for the measurements in $CDCl_3$) or the signal of the solvent (δ = 2.50 or 39.51 for $^{1}H/^{13}C$ – for the measurements in DMSO-d_6) as internal references. The ^{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 (IR) were measured with a Nicolet iS5 FT-IR spectrometer. Mass spectra (MS) were measured with a Waters ZMD mass spectrometer in a positive ESI mode (coin voltage, CV = 10 to 20 eV). Transmission electron microscopy (TEM) was performed with a Tecai G2 Spirit Twin 12 microscope (FEI, Brno, Czech Republic). 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). All analytical data, i.e., the evaluated ^{1}H and ^{13}C NMR spectra, IR and MS spectra, elemental analyses, the scanned NMR spectra of the prepared compounds, and the calculated ^{1}H and ^{13}C NMR spectra (ChemBioDraw Ultra, version 12.0) are presented in the ESI, Experimental part, Figures S1–S26.

1.2. (3β)-3-(Acetyloxy)lup-20(29)-en-28-oic acid (**2**).

^{1}H-NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.83 (s, 3H, 23-CH_3), 0.84 (s, 3H, 24-CH_3), 0.85 (d, 3H, *J*=0.6 Hz, 25-CH_3), 0.93 (s, 3H, 26-CH_3), 0.97 (d, 3H, *J*=0,6 Hz, 27-CH_3), 1.69 (dd, 3H, J_1=0.6 Hz, J_2=1.4 Hz, 30-CH_3), 2.04 (s, 3H, CH_3CO), 3.00 (dt, 1H, J_1=4.8 Hz, J_2=10.8 Hz, J_3=10.8 Hz, 19-CH), 4.46-4.49 (m, 1H, 3-CH), 4.60-4.62 (m, 1H, 29-CH), 4.73-4.75 (m, 1H, 29-CH). ^{13}C-NMR (150.92 MHz, $CDCl_3$): δ [ppm] 14.66 (C27), 16.05 (C26), 16.17 (C25), 16.46 (C24), 18.17(C6), 19.35 (C11), 20.87 (C29), 21.29 (CH_3CO), 23.70 (C2), 25.46 (C12), 27.95 (C23), 29.70 (C21), 30.58 (C15), 32.16 (C16), 37.04 (C22), 37.14 (C10), 37.26 (C7), 37.81 (C4), 38.40 (C13), 38.44 (C1), 40.72 (C8), 42.44 (C14), 46.95 (C18), 49.30 (C19), 50.42 (C9), 55.44 (C5), 56.40 (C17), 80.96 (C3), 109.73 (C30), 150.36 (C20), 171.03 (CH_3CO), 181.86 (C28). MS: *m/z* = 497.3 $[M–H]^-$ (ESI^-). For $C_{32}H_{50}O_4$ (498.74) calcd. C 77.06, H 10.10, found C 77.10, H 10.08.

Figure S1. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

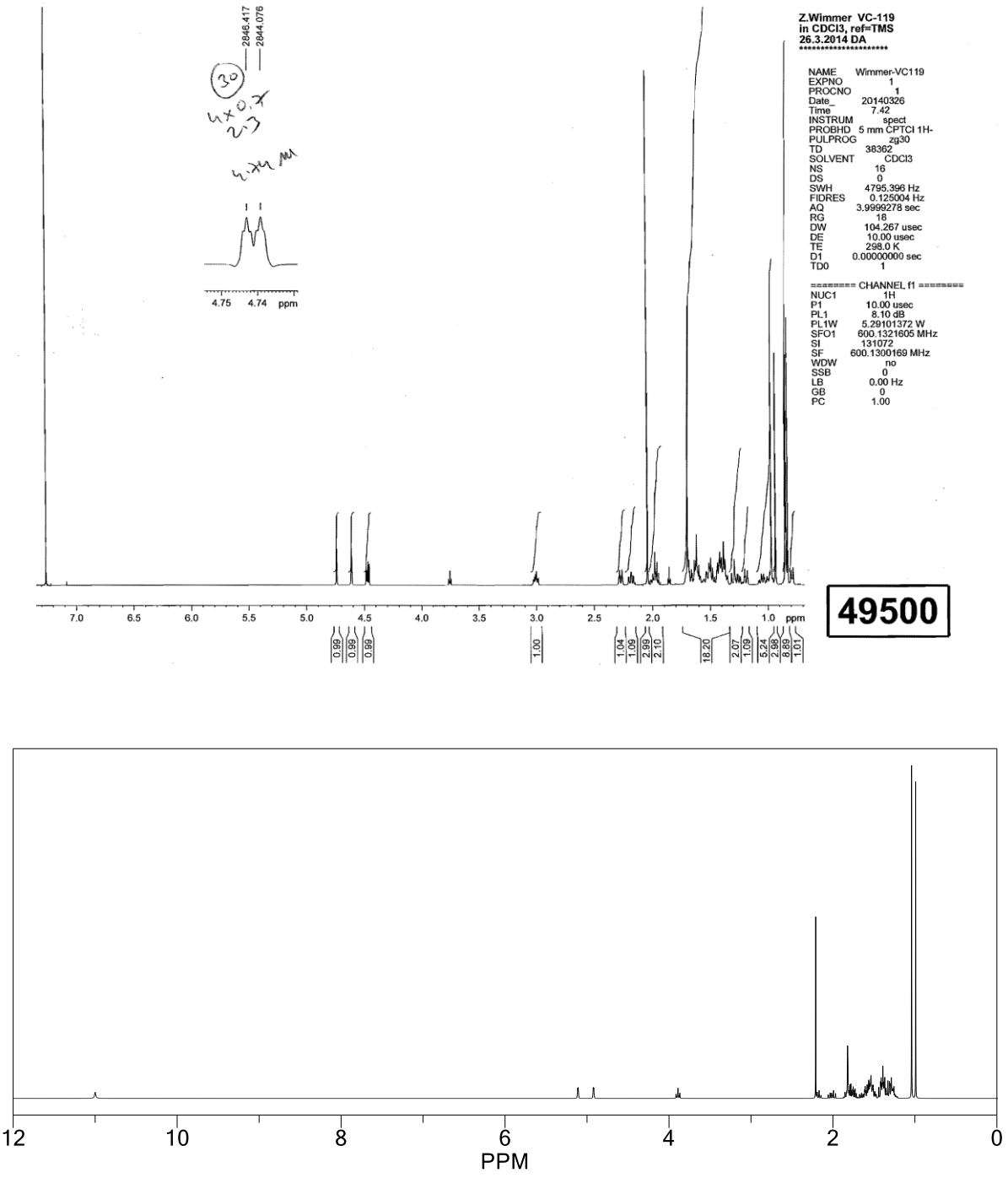
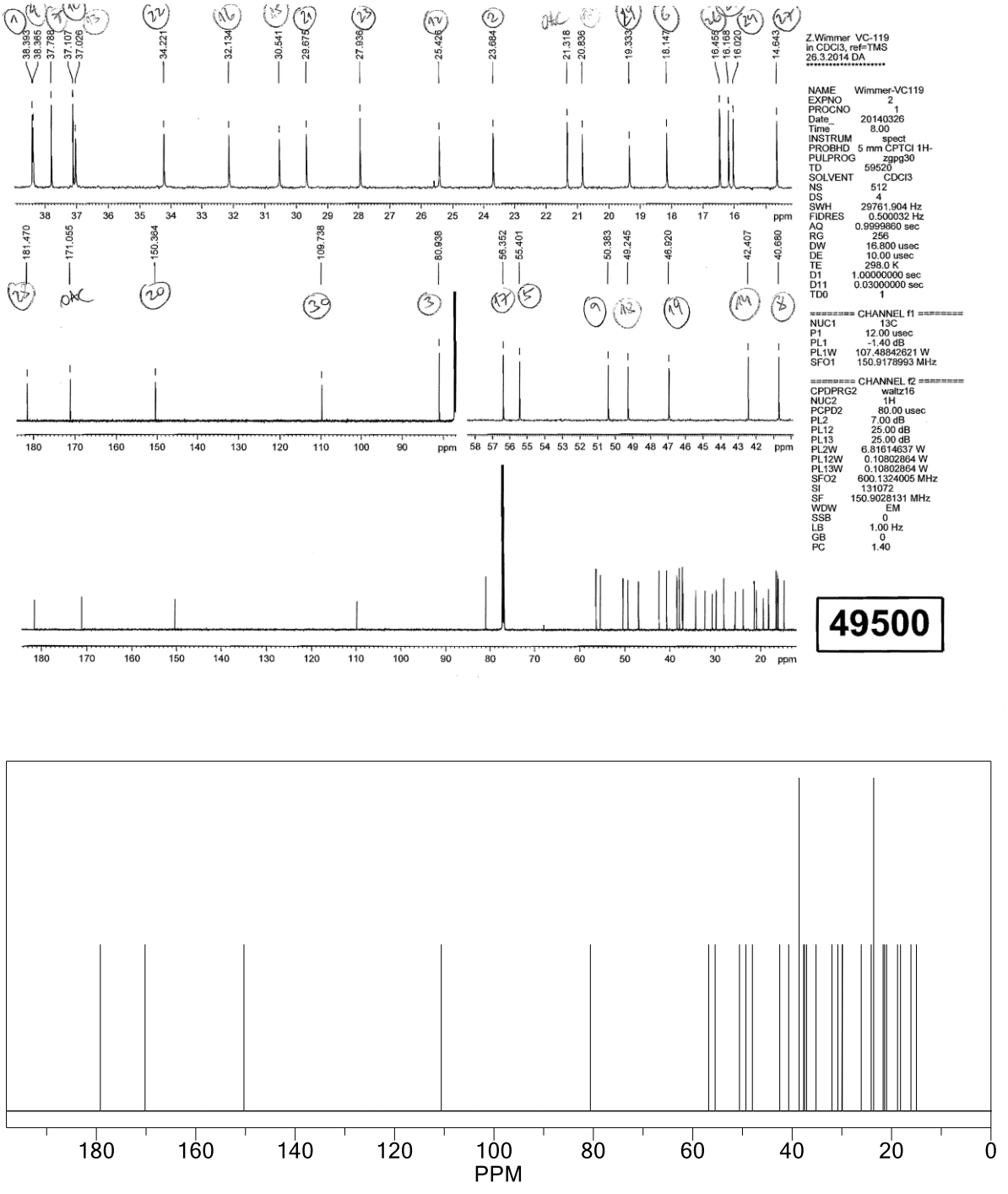


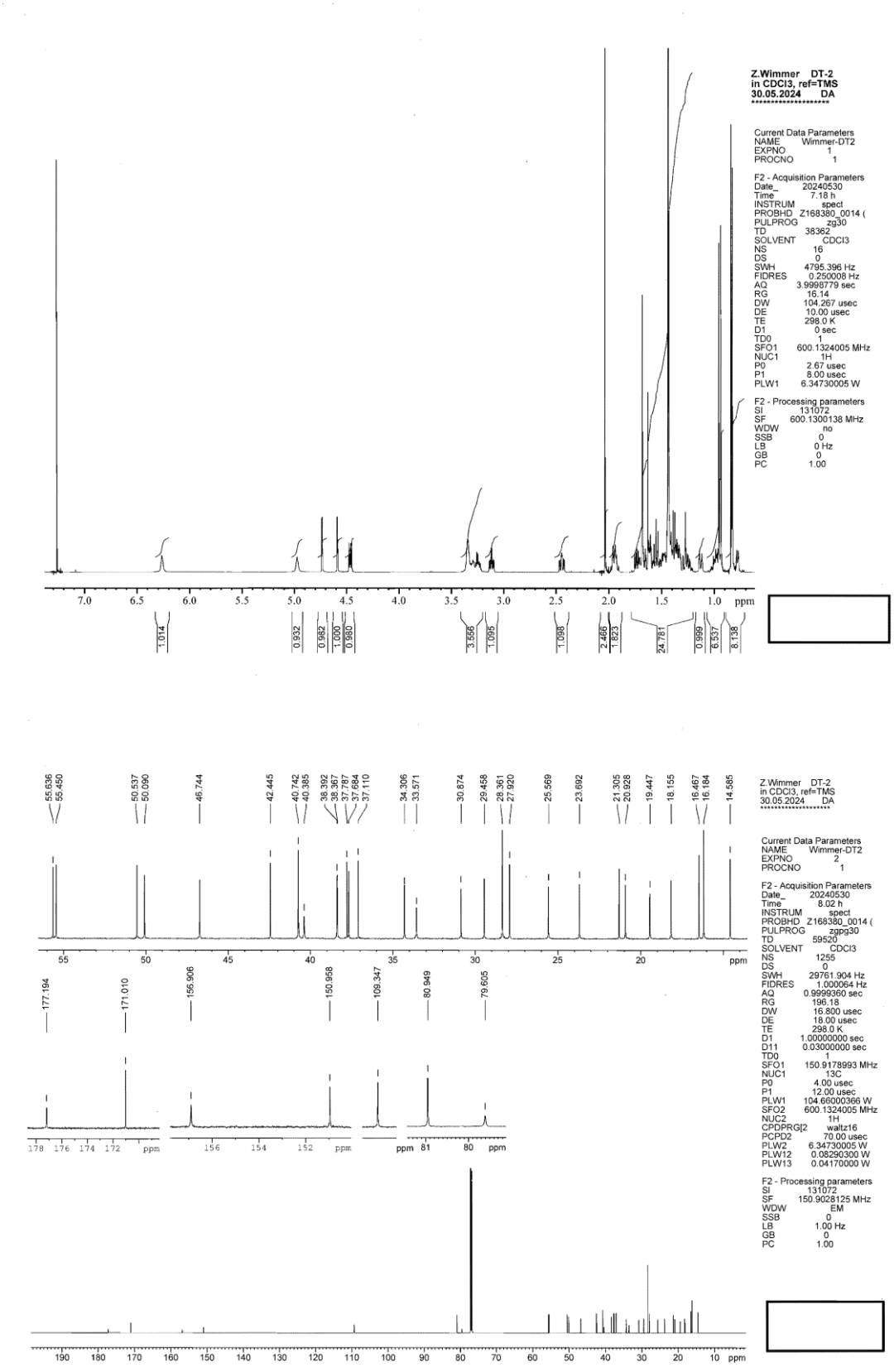
Figure S2. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



1.3. (3β)-28-({2-[(*tert*-Butoxycarbonyl)amino]ethyl}amino)-28-oxolup-20(29)-en-3-yl acetate (**3**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.82 (3H, s, H24), 0.84 (3H, s, H23), 0.84 (3H, s, H25), 0.87 (1H, dd, *J* = 2,2; 11.4 Hz, H5), 0.93 (3H, s, H26), 0.95 (3H, s, H27), 1.13 (2H, dt, *J* = 3.4; 3.4; 13.3 Hz, H21), 1.43 (9H, s, H7′), 1.55 (1H, t, *J* = 11.3 Hz, H18), 1.66 (2H, dt, *J* = 3.4; 3.4; 13.0 Hz, H7), 1.68 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.74 (2H, bdd, *J* = 7.8; 12.0 Hz, H1), 1.91–1.98 (4H, m, H15+H16), 2.04 (3H, s, H2′), 2.45 (1H, ddd, *J* = 3.7; 11.6; 13.0 Hz, H13), 3.12 (1H, dt, *J* = 4.4; 11.1; 11.1 Hz, H19), 3.22–3.38 (4H, m, H3′+H4′), 4.46 (1H, dd, *J* = 6.2; 10.4 Hz, H3), 4.59 (1H, dd, *J* = 3×1.4; 2.4 Hz, H30), 4.74 (1H, dd, *J* = 3×0.7; 2.4 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 16.2 (q, C24), 16.2 (q, C25), 16.5 (q, C26), 18.2 (t, C6), 19.4 (q, C29), 20.9 (t, C11), 21.3 (q, C2′), 23.7 (t, C2), 25.6 (t, C12), 27.9 (q, C23), 28.4 (q, C7′), 29.5 (t, C21), 30.9 (t, C15), 33.6 (t, C16), 34.3 (t, C22), 37.1 (s, C10), 37.7 (d, C13), 37.8 (s, C4), 38.4 (t, C1), 38.4 (t, C7), 40.4 (t, C3′), 40.7 (s, C8), 40.7 (t, C4′), 42.4 (s, C14), 46.7 (d, C19), 50.1 (d, C18), 50.5 (d, C9), 55.4 (s, C17), 55.6 (d, C5), 79.6 (s, C6′), 80.9 (d, C3), 109.4 (t, C30), 151.0 (s, C20), 156.9 (s, C5′), 171.0 (s, C1′), 177.2 (s, C28). MS (ESI^+, HCOOH): *m/z* = 641.4 $[M+H]^+$, 663,6 $[M+Na]^+$; (ESI^-, NH_4OH): *m/z* = 639.1 $[M-H]^-$.

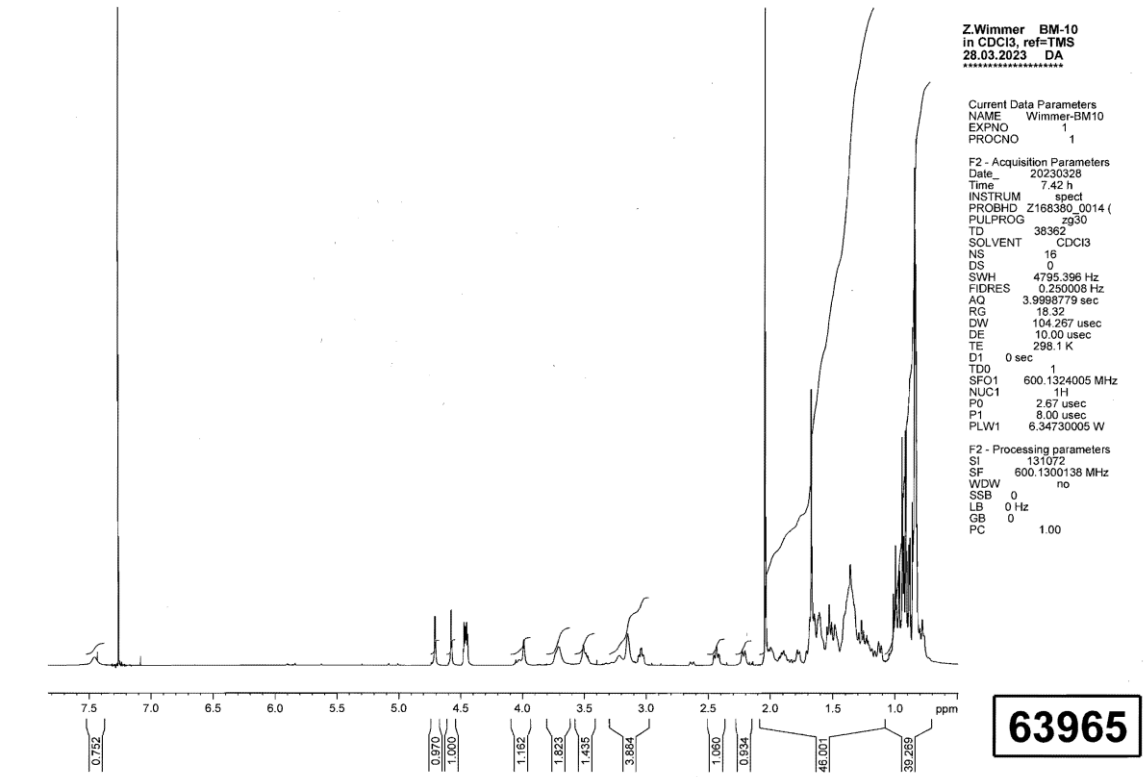
Figure S3. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.4. (3β)-28-[(2-Aminoethyl)amino}-28-oxolup-20(29)-en-3-yl acetate (**4**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.78 (1H, dd, J_1=1.9 Hz, J_2=11.5 Hz, H-5), 0.83 (3H, s, H-23), 0.84 (3H, s, H-24), 0.84 (3H, s, H-25), 0.92 (3H, s, H-26), 0.96 (3H, s, H-27), 1.68 (3H, dd, J_1=0.7 Hz, J_2=1.3 Hz, H-30), 2.04 (3H, s, H-4'), 2.10 (1H, dt, J_1=3.2 Hz, J_2=3.2 Hz, J_3=13.7 Hz, H-13), 2.43 (2H, ddd, J_1=3.7 Hz, J_2=11.7 Hz, J_3=12.8 Hz, H-16), 3.01 (2H, t, J=5.7 Hz, H-2'), 3.07 (2H, dt, J_1=4.5 Hz, J_2=11.0 Hz, J_3=11.0 Hz, H-19), 3.43 (1H, dq, J_1=5.6 Hz, J_2=5.6 Hz, J_3=5.6 Hz, J_4=14.0 Hz, H-1'), 3.51 (1H, dq, J_1=5.6 Hz, J_2=5.6 Hz, J_3=5.6 Hz, J_4=14.0 Hz, H-1'), 4.47 (2H, dd, J_1=5.7 Hz, J_2=10.7 Hz, H-3), 4.59 (1H, dq, J_1=1.3 Hz, J_2=1.3 Hz, J_3=1.3 Hz, J_4=2.4 Hz, H-29), 4.72 (1H, dq, J_1=0.7 Hz, J_2=0.7 Hz, J_3=0.7 Hz, J_4=2.4 Hz, H-29), 6.84 (2H, t, J=5.6 Hz, NH). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ [ppm] 14.58 (q, C27), 16.14 (q, C24), 16.19 (q, C25), 16.46 (q, C26), 18.17 (t, C6), 19.37 (q, C30), 20.96 (t, C11), 21.26 (q, C4'), 23.67 (t, C2), 25.54 (t, C12), 27.92 (q, C23), 29.46 (t, C21), 30.91 (t, C15), 33.45 (t, C16), 34.32 (t, C22), 37.10 (d, C13), 37.70 (s, C10), 37.76 (t, C7), 38.33 (s, C4), 38.38 (t, C1), 39.26 (t, C2'), 40.75 (s, C8), 41.00 (t, C1'), 42.42 (s, C14), 46.79 (t, C19), 50.15 (d, C18), 50.51 (d, C9), 55.44 (s, C17), 55.77 (d, C5), 80.90 (d, C3), 109.51 (t, C29), 150.72 (s, C20), 170.98 (s, C3'), 177.61 (s, C28). IR (cm^{-1}): 3350 (CO-NH, NH_2), 2939 (CH_2), 2850 (CH_3), 1730 (C=O), 1365 (CH_3), 1242 (C-O-C), 1025 (NH_2), 977 (CH), 751 (NH_2). MS (ESI^+, HCOOH): *m/z* = 541.6 $[M+H]^+$; (ESI^-, NH_4OH): *m/z* = 539.3 $[M-H]^-$. For $C_{34}H_{56}N_2O_3$ (540.82) calcd. C 75.51, H 10.44, N 5.18, found C 75.54, H 10.41, N 5.21.

Figure S4. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



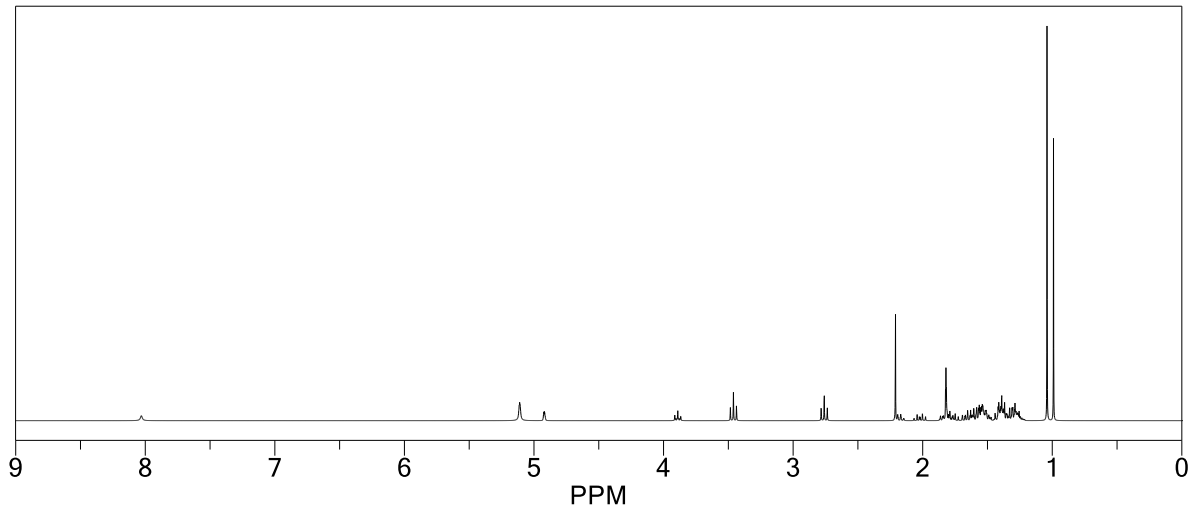
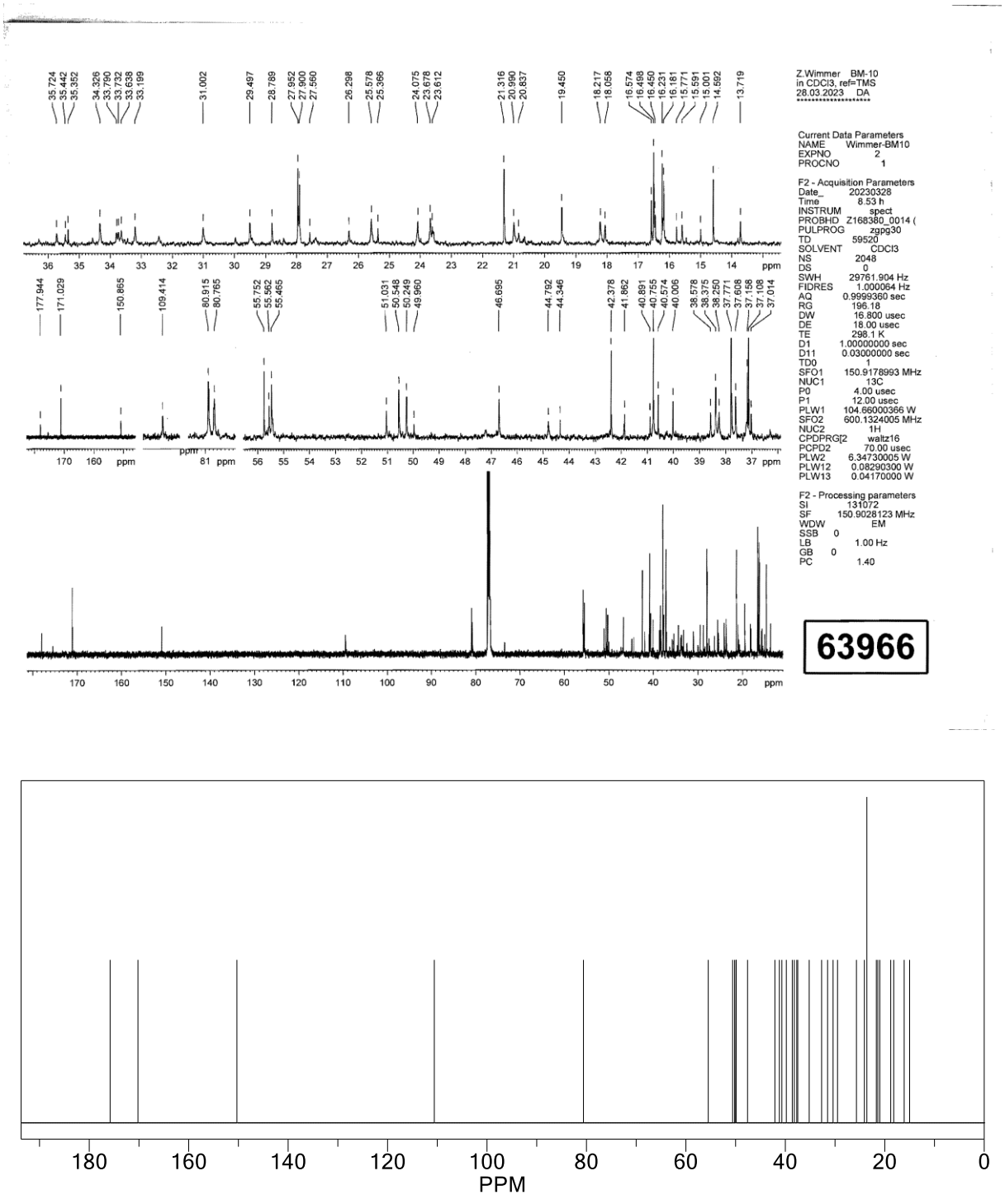


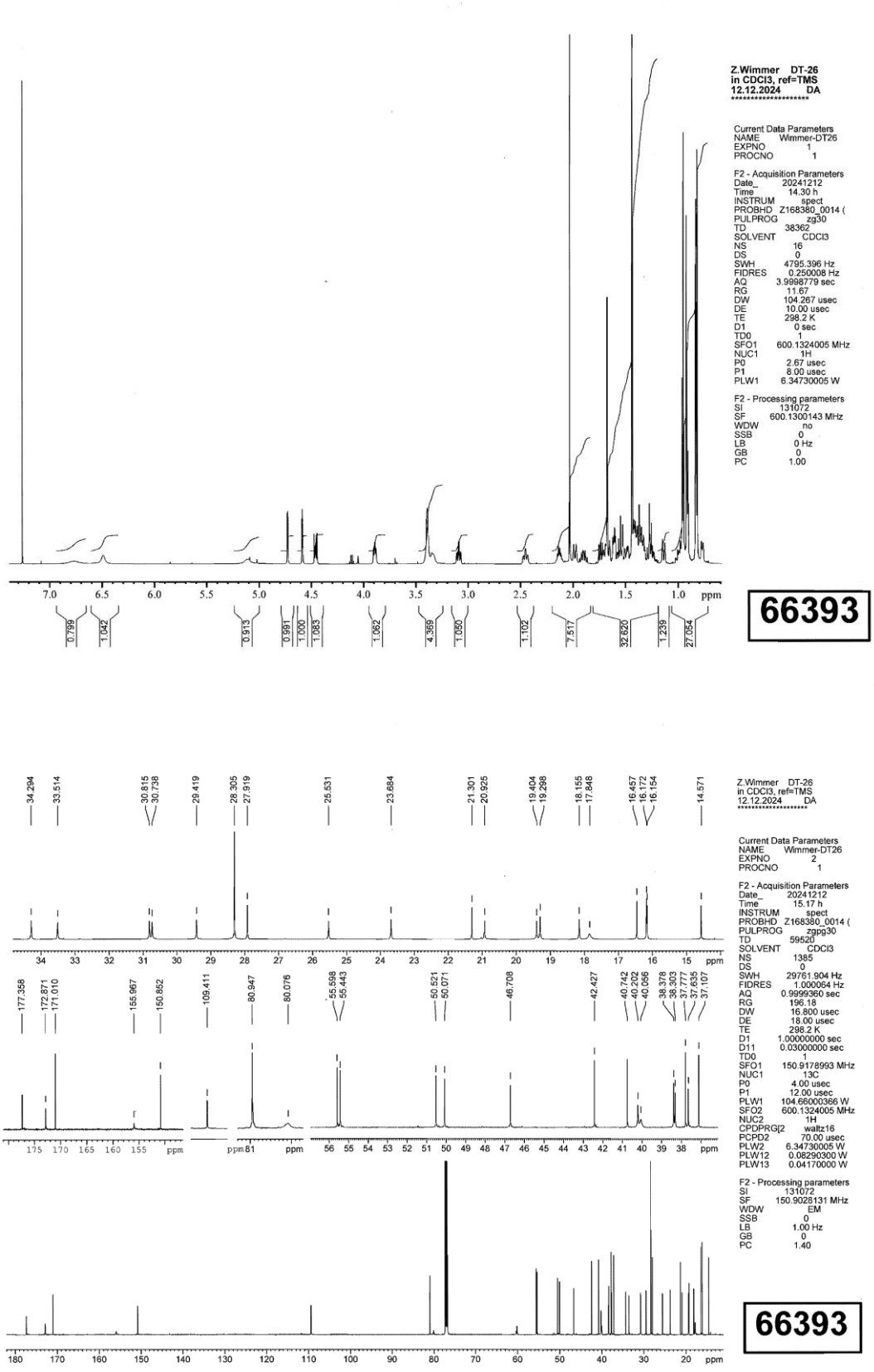
Figure S5. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



1.5. (3β)-28-[(2-{[((*N*-(*tert*-Butoxycarbonyl)-(2*S*)-2-amino-3-methylbutanoyl)amino)-ethyl]amino}-28-oxolup-20(29)-en-3-yl acetate (**5**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.77 (1H, dd, *J* = 1.9; 11.4 Hz, H5), 0.82 (3H, s, H26), 0.83 (3H, s, H23), 0.84 (3H, s, H25), 0.91 (3H, d, *J* = 6.7 Hz, H8′), 0.92 (3H, s, H24), 0.95 (3H, d, *J* = 6.7 Hz, H9′), 0.96 (3H, s, H27), 1.14 (2H, dt, *J* = 3.1; 3.1; 13.4 Hz, H21), 1.55 (1H, t, *J* = 11.3 Hz, H18), 1.67 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.98 (2H, dt, *J* = 3.2; 3.2; 13.6 Hz, H16), 2.03 (3H, s, H2′), 2.10–2.16 (1H, m, H7′), 2.45 (1H, bdt, *J* = 3.5; 11.5; 11.5 Hz, H13), 3.09 (1H, dt, *J* = 4.4; 11.1; 11.1 Hz, H19), 3.31–3.42 (4H, m, H3′, H4′), 3.89 (1H, dd, *J* = 6.2; 8.3 Hz, H6′), 4.46 (1H, dd, *J* = 6.1; 10.4 Hz, H3), 4.59 (1H, dq, *J* = 3×1.4; 2.3 Hz, H30), 4.73 (1H, dq, *J* = 3×0.7; 2.3 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 16.2 (q, C24), 16.2 (q, C25), 16.5 (q, C26), 17.8 (q, C8′), 18.2 (t, C6), 19.3 (q, C9′), 19.4 (q, C29), 20.9 (t, C11), 21.3 (q, C2′), 23.7 (t, C2), 25.5 (t, C12), 27.9 (q, C23), 28.3 (q, C12′), 29.4 (t, C21), 30.7 (d, C7′), 30.8 (t, C15), 33.5 (t, C16), 34.3 (t, C22), 37.1 (s, C10), 37.6 (s, C4), 37.6 (d, C13), 38.3 (t, C7), 38.4 (t, C1), 40.1 (t, C4′), 40.2 (t, C3′), 40.7 (s, C8), 42.4 (s, C14), 46.7 (d, C19), 50.1 (d, C18), 50.5 (d, C9), 55.4 (s, C17), 55.6 (d, C5), 60.2 (d, C6′), 80.1 (s, C11′), 80.9 (d, C3), 109.4 (t, C30), 137.4 (s, C28), 150.9 (s, C20). IR (ATR): $\tilde{\nu}$ = 3303 (br), 2941 (s), 2871 (m), 1717 (s), 1646 (s), 1506 (s), 1365 (m), 1242 (vs), 1103 (m) cm^{-1}. MS (ESI^+, HCOOH): *m/z* = 740.5 $[M+H]^+$; (ESI^-, NH_4OH): *m/z* = 738.3 $[M-H]^-$.

Figure S6. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.6. (3β)-28-Oxo-28-{[((2-(2*S*)-2-amino-3-methylbutanoyl)-amino)ethyl]amino}lup-20(29)-en-3-yl acetate (**6**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.79 (1H, bd, *J* = 11.5 Hz, H5), 0.83 (3H, s, H23), 0.83 (3H, s, H26), 0.84 (3H, s, H25), 0.93 (3H, s, H24), 0.96 (3H, s, H27), 0.97 (3H, bd, *J* = 6.5 Hz, H8′), 1.04 (3H, bd, *J* = 6.5 Hz, H9′), 1.14 (2H, dt, *J* = 3.2; 3.2; 13.2 Hz, H21), 1.56 (1H, t, *J* = 11.4 Hz, H18), 1.68 (3H, bs, H29), 2.03 (3H, s, H2′), 2.05 (2H, bd, *J* = 13.5 Hz, H16), 2.46 (1H, dt, *J* = 3.5; 12.0; 12.0 Hz, H13), 3.09 (1H, dt, *J* = 4.3; 11.2; 11.2 Hz, H19), 3.30–3.45 (4H, m, H3′, H4′), 3.55–3.60 (1H, bs, H6′), 4.47 (1H, dd, *J* = 5.3; 11.0 Hz, H3), 4.59 (1H, bs, H30), 4.72 (1H, bs, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 16.2 (q, C24), 16.3 (q, C25), 16.5 (q, C26), 17.2 (q, C8′), 18.2 (t, C6), 19.3 (q, C9′), 19.4 (q, C29), 21.0 (t, C11), 21.2 (q, C2′), 23.7 (t, C2), 25.6 (t, C12), 28.0 (q, C23), 29.6 (t, C21), 30.6 (d, C7′), 31.0 (t, C15), 33.6 (t, C16), 34.4 (t, C22), 37.2 (s, C10), 37.8 (s, C4), 37.9 (d, C13), 38.4 (t, C7), 38.5 (t, C1), 39.7 (t, C4′), 39.9 (t, C3′), 40.9 (s, C8), 42.5 (s, C14), 46.9 (d, C19), 50.2 (d, C18), 50.6 (d, C9), 55.6 (s, C17), 55.8 (d, C5), 57.8 (d, C6′), 81.0 (d, C3), 109.4 (t, C30), 150.9 (s, C20), 170.9 (s, C1′), 173.6 (s, C5′), 177.4 (s, C28). IR (ATR): $\tilde{\nu}$ = 3333 (br), 2936 (s), 2872 (m), 1718 (w), 1638 (m), 1517 (m), 1466 (w), 1370 (m), 1244 (s), 1029 (m), 978 (m), 881 (w), 750 (vs) cm^{-1}. MS (ESI$^+$, HCOOH): *m/z* = 640.4 [M+H]$^+$; (ESI$^-$, NH_4OH): *m/z* = 638.3 [M−H]$^-$. For $C_{39}H_{65}N_3O_4$ (639.97) calcd. C 73.20, H 10.24, N 6.57, found C 73.25, H 10.21, N 6.54.

Figure S7. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

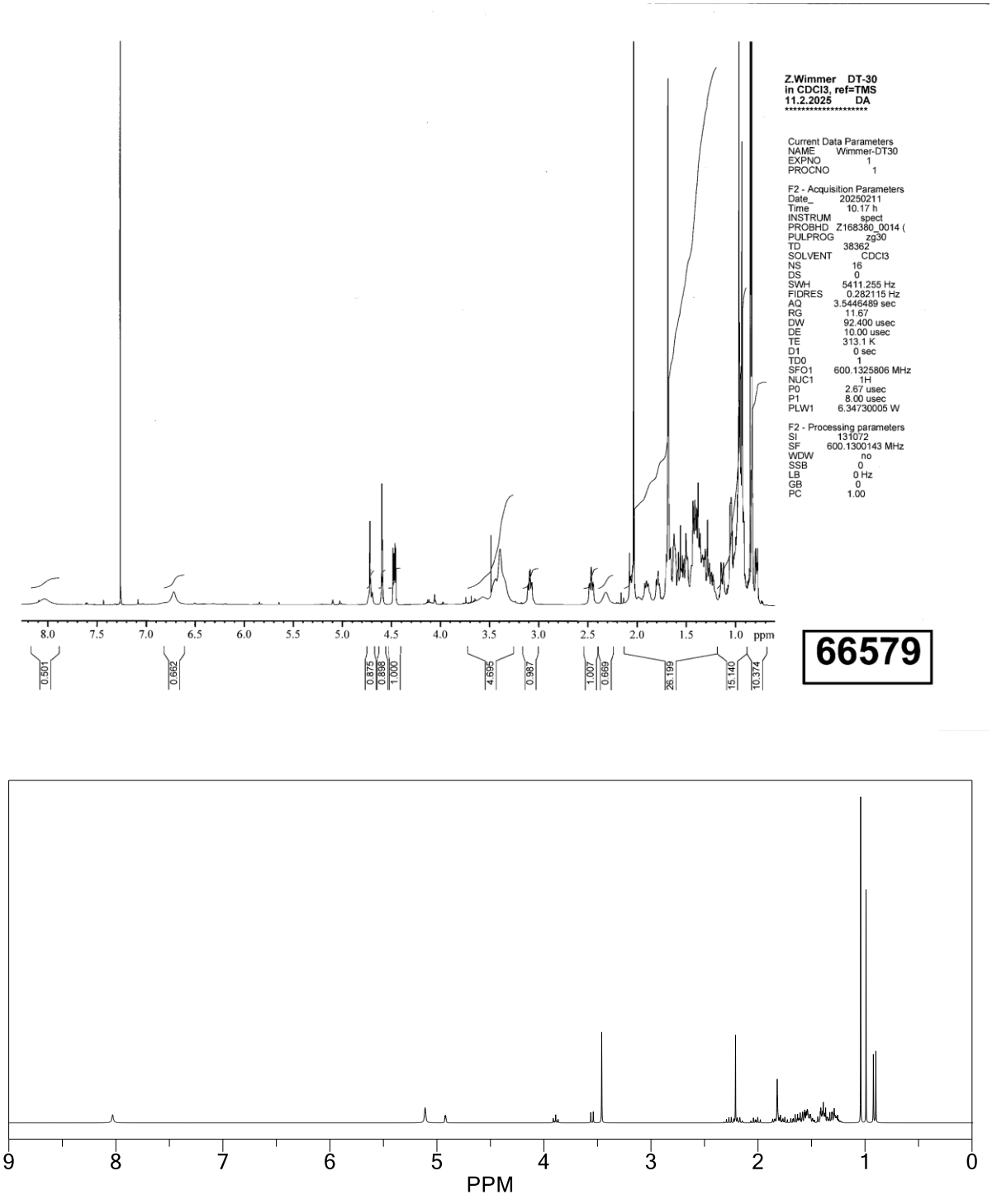
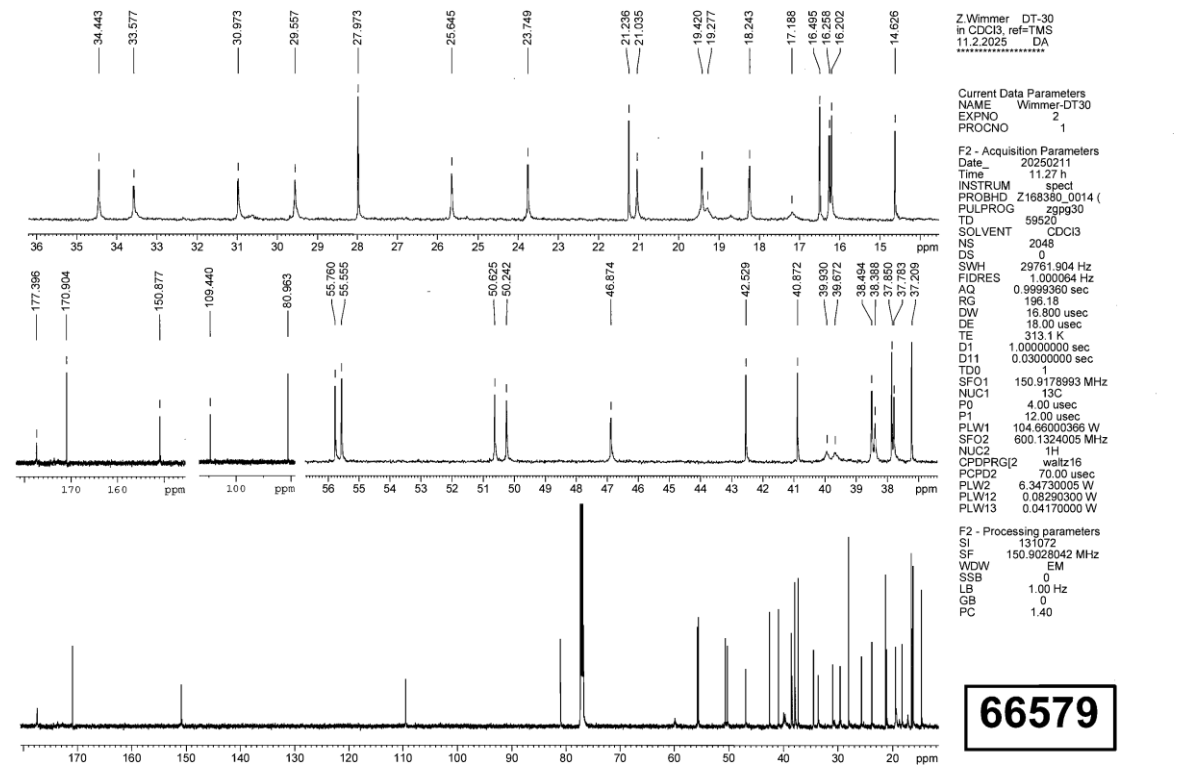
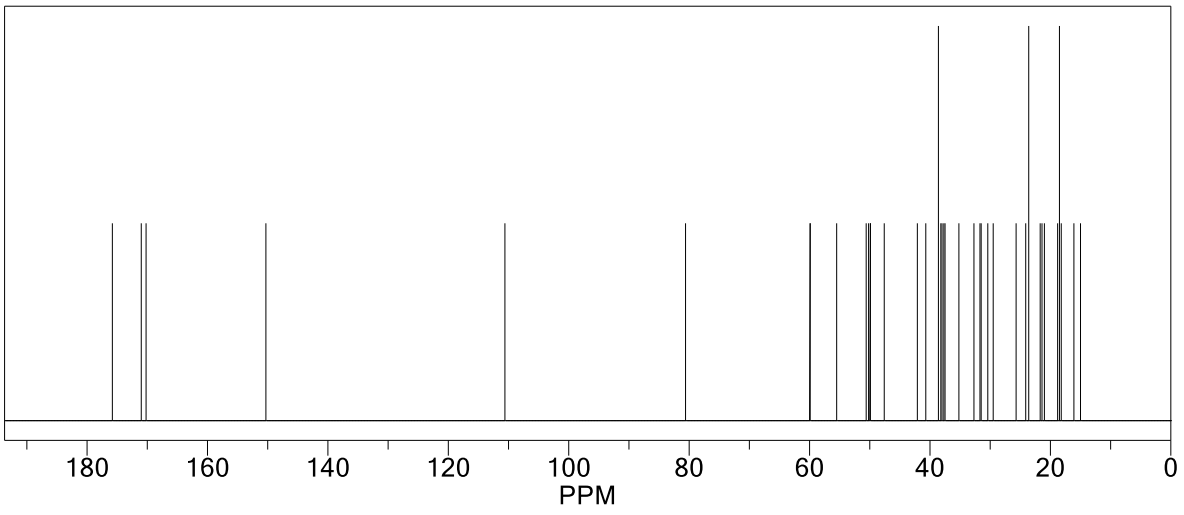


Figure S8. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

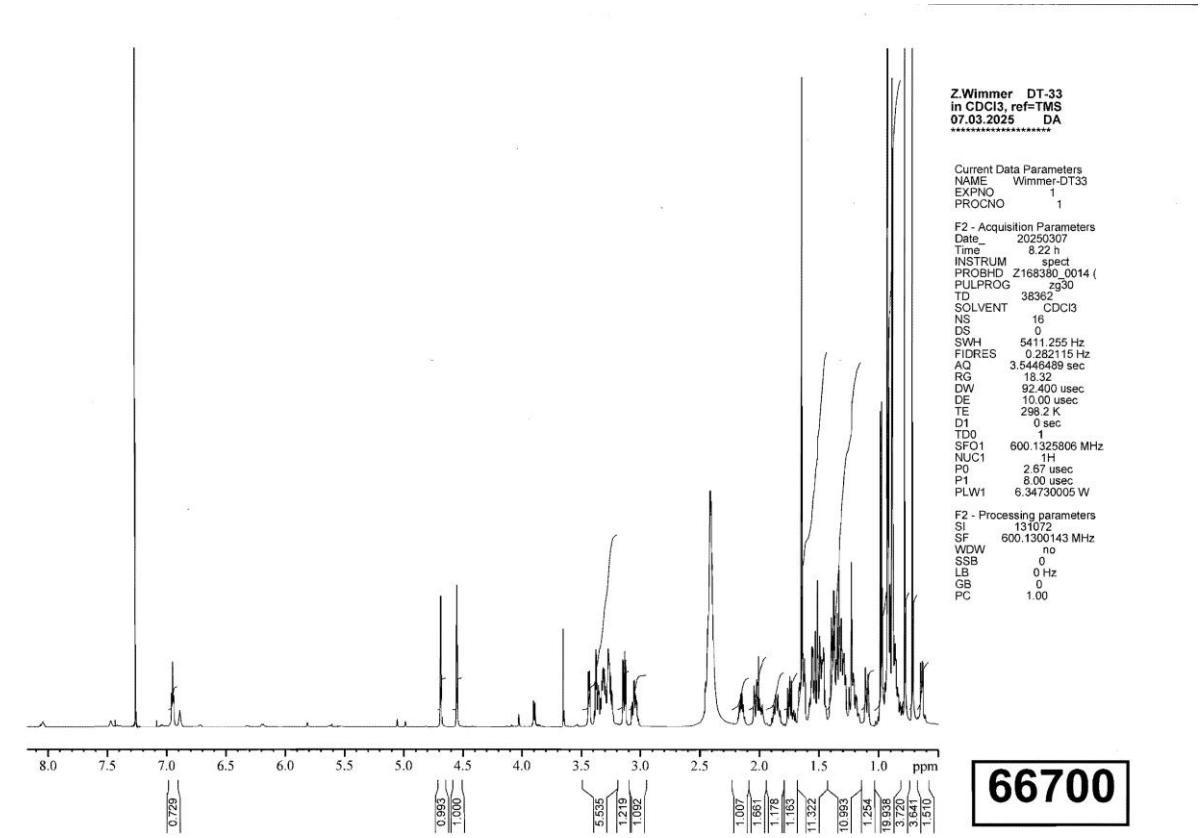




1.7. (3β)-3-Hydroxy-*N*-{[(2-(2*S*)-2-amino-3-methylbutanoyl)-amino]ethyl}lup-20(29)-en-28-amide (**7**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.64 (1H, dd, *J* = 1.5; 10.6 Hz, H5), 0.71 (3H, s, H24), 0.78 (3H, s, H25), 0.88 (3H, s, H26), 0.92 (3H, s, H23), 0.92 (3H, s, H27), 0.92 (3H, d, *J* = 6.9 Hz, H6′), 0.97 (3H, d, *J* = 6.9 Hz, H7′), 1.10 (2H, dt, *J* = 3.1; 3.1; 13.3 Hz, H21), 1.51 (1H, t, *J* = 11.2 Hz, H18), 1.63 (2H, dt, *J* = 3.5; 3.5; 13.3 Hz, H1), 1.64 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.75 (2H, dd, *J* = 8.0; 12.0 Hz, H7), 2.03 (2H, dt, *J* = 3.5; 3.5; 13.6 Hz, H16), 2.12–2.18 (1H, m, H5′), 2.43 (1H, dt, *J* = 3.4; 12.0; 12.0 Hz, H13), 3.05 (1H, dt, *J* = 4.4; 11.3; 11.3 Hz, H19), 3.13 (1H, dd, *J* = 5.0; 11.4 Hz, H3), 3.24–3.45 (4H, m, H1′, H2′), 4.55 (1H, dq, *J* = 3×1.4; 2.4 Hz, H30), 4.69 (1H, dq, *J* = 3×0.7; 2.4 Hz, H30), 6.95 (1H, bt, *J* = 5.4 Hz, H1′-NH). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.5 (q, C27), 15.3 (q, C24), 16.0 (q, C25), 16.0 (q, C26), 17.1 (q, C6′), 18.2 (t, C6), 18.9 (q, C7′), 19.3 (q, C29), 20.9 (t, C11), 25.5 (t, C12), 27.1 (t, C2), 27.8 (q, C23), 29.4 (t, C21), 30.5 (d, C5′), 30.8 (t, C15), 33.2 (t, C16), 34.3 (t, C22), 37.1 (s, C10), 37.6 (d, C13), 38.2 (t, C7), 38.6 (s, C4), 38.7 (t, C1), 39.3 (t, C2′), 39.5 (t, C1′), 40.6 (s, C8), 42.3 (s, C14), 46.7 (d, C19), 50.1 (d, C18), 50.6 (d, C9), 55.3 (s, C17), 55.6 (d, C5), 59.4 (d, C4′), 78.8 (d, C3), 109.2 (t, C30), 151.0 (s, C20), 172.3 (s, C3′), 177.6 (s, C28). IR (ATR): $\tilde{\nu}$ = 3327 (br), 2937 (s), 2867 (m), 1636 (s), 1519 (s), 1449 (m), 1373 (w), 752 (m) cm^{-1}. MS (ESI$^+$, HCOOH): *m/z* = 598.3 $[M+H]^+$; (ESI$^-$, NH_4OH): *m/z* = 596.8 $[M-H]^-$. For $C_{37}H_{63}N_3O_3$ (597.93) calcd. C 74.32, H 10.62, N 7.03, found C 74.35, H 10.60, N 7.00.

Figure S9. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



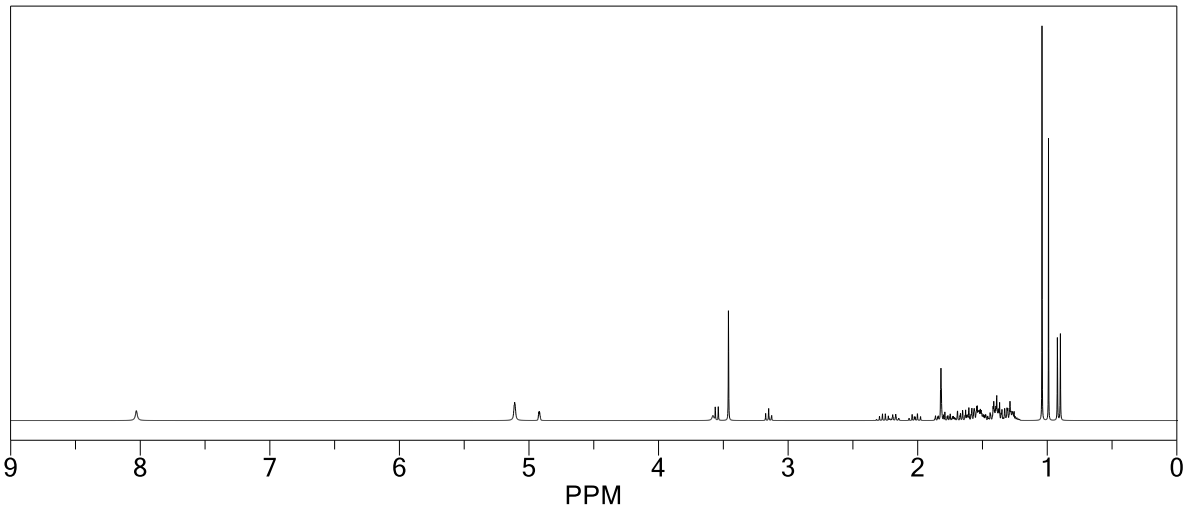
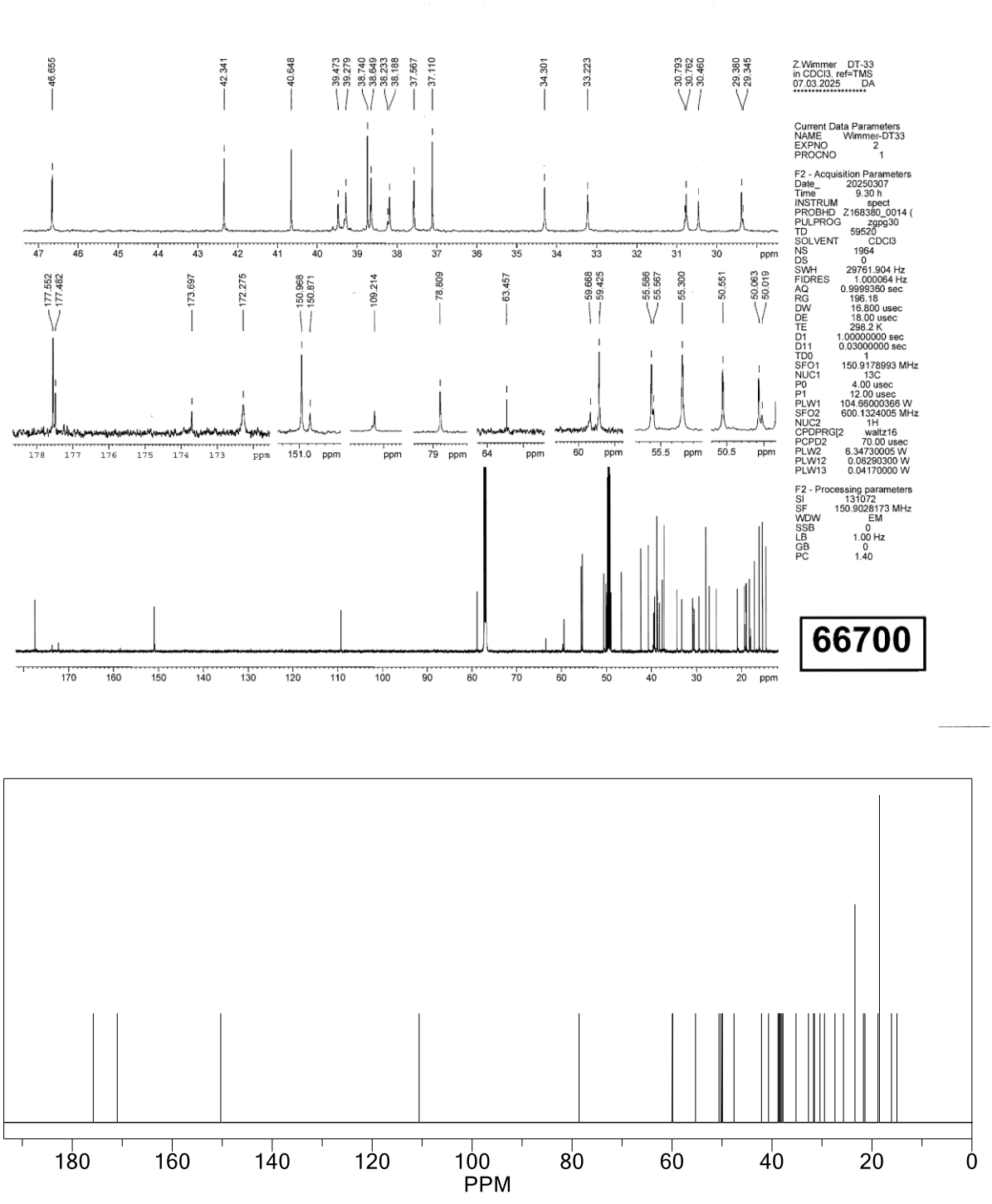


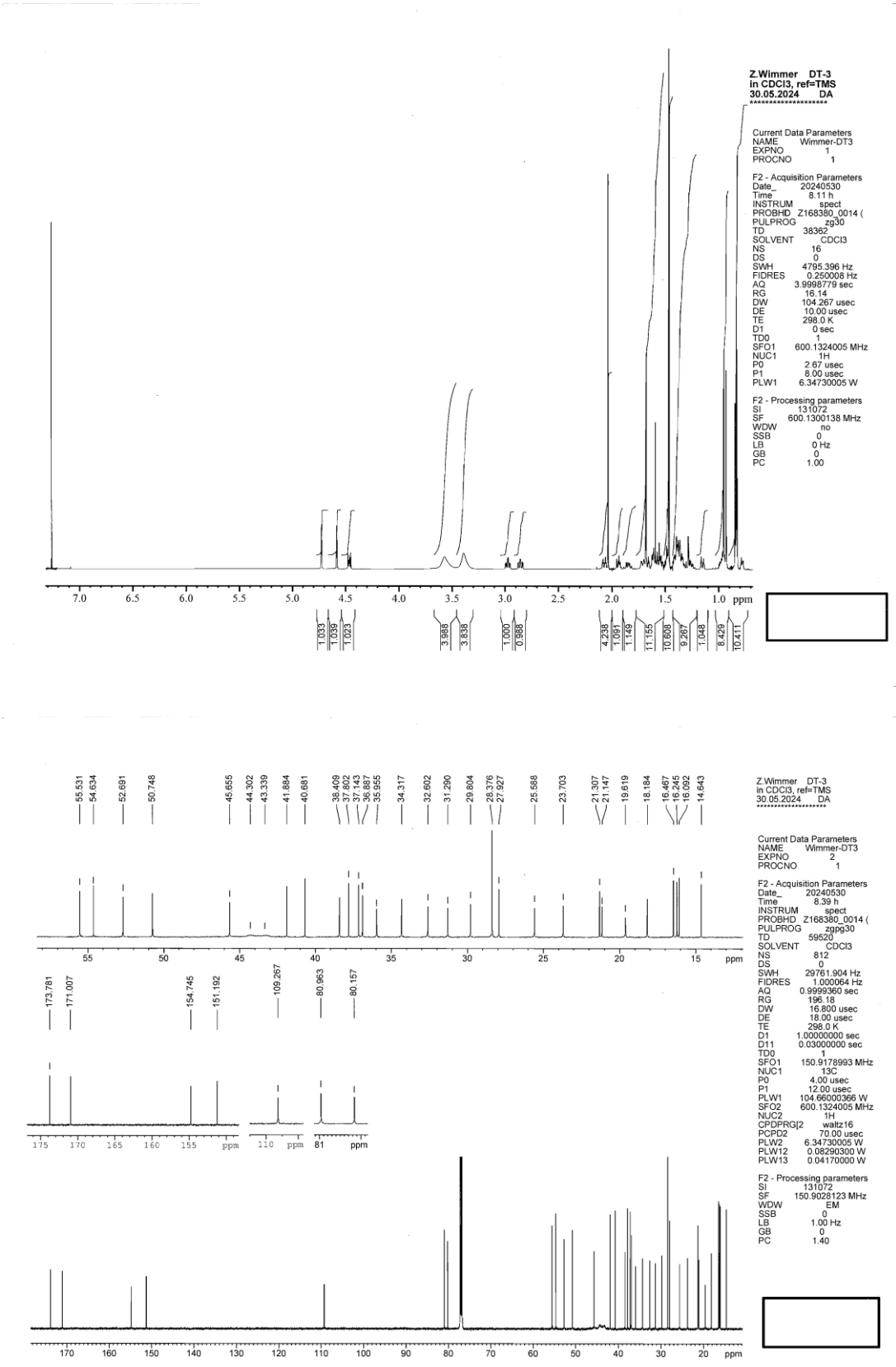
Figure S10. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



1.8. *tert*-Butyl 4-[(3β)-3-acetyloxy-28-oxolup-20(29)-en-28-yl]piperazin-1-carboxylate (**8**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0,78 (1H, dd, *J* = 2.1; 11.2 Hz, H5), 0.82 (3H, s, H25), 0.83 (3H, s, H26), 0.84 (3H, s, H24), 0.92 (3H, s, H23), 0.95 (3H, s, H27), 1.15 (2H, dt, *J* = 3.4; 4.4; 13.4 Hz, H21), 1.46 (9H, s, H7′), 1.68 (3H, dd, *J* = 0.7; 1.3 Hz, H29), 1.80–1.87 (2H, m, H15), 1.91–1.96 (2H, m, H7), 2.03 (3H, s, H2′), 2.07 (2H, dt, *J* = 3.2; 3.2; 13.3 Hz, H16), 2.85 (1H, ddd, *J* = 3.5; 11.4; 13.1 Hz, H13), 2.97 (1H, dt, *J* = 4.2; 11.2; 11.2 Hz, H19), 3.35–3.65 (8H, m, H3′–4′H), 4.46 (1H, dd, *J* = 5.6; 10.7 Hz, H3), 4.58 (1H, dq, *J* = 3×1.3; 2.4 Hz, H30), 4.72 (1H, dq, *J* = 3×0.7; 2.4 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 16.1 (q, C24), 16.2 (q, C25), 16.5 (q, C26), 18.2 (t, C6), 19.6 (q, C29), 21.1 (t, C11), 21.3 (q, C2′), 23.7 (t, C2), 25.6 (t, C12), 27.9 (q, C23), 28.4 (q, C7′), 29.8 (t, C21), 31.0 (d, C3), 31.3 (t, C15), 32.6 (t, C16), 34.3 (t, C22), 36.0 (s, C10), 36.9 (t, C7), 37.1 (d, C13), 37.8 (s, C4), 38.4 (t, C1), 40.7 (s, C8), 41.9 (s, C14), 43.4 (t, C3′), 44.2 (t, C4′), 45.7 (d, C19), 50.7 (d, C9), 52.7 (d, C18), 54.6 (s, C17), 55.5 (d, C5), 80.2 (s, C6′), 109.3 (t, C30), 151.5 (s, C20), 154.7 (s, C5′), 171.0 (s, C1′), 173.8 (s, C28). MS (ESI$^+$, HCOOH): *m/z* = 667.5 $[M+H]^+$.

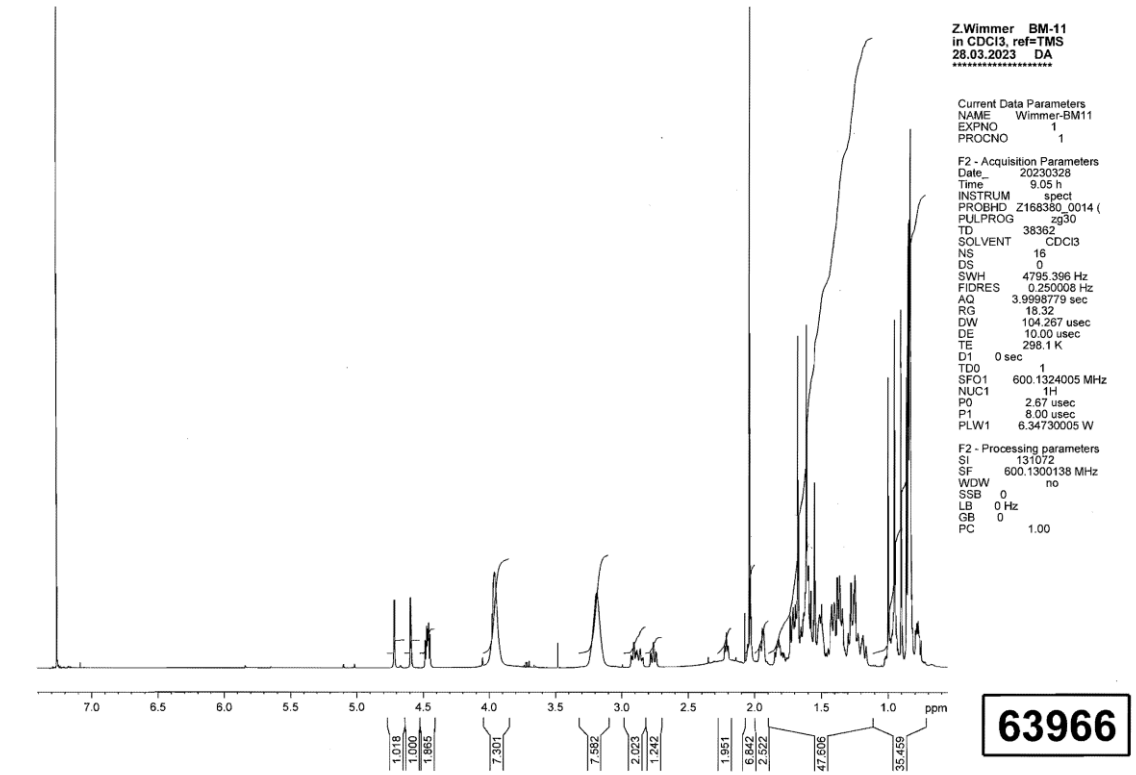
Figure S11. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.9. (3β)-28-Oxo-28-(piperazin-1-yl)lup-20(29)en-3-yl acetate (**9**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ [ppm] 0.78 (1H, dd, J_1=2.1 Hz, J_2=11.2 Hz, H-5), 0.82 (3H, s, H-25), 0.83 (3H, s, H-26), 0.84 (3H, s, H-24), 0.92 (3H, s, H-23), 0.95 (3H, s, H-27), 1.15 (2H, dt, J_1=3.4 Hz, J_2=3.4 Hz, J_3=13.4 Hz, H-21), 1.46 (9H, s, H-7'), 1.68 (3H, dd, J_1=0.7 Hz, J_2=1.3 Hz, H-30), 1.80 (2H, m, H-15), 1.91 (2H, m, H-7), 2.04 (3H, s, H-2'), 2.07 (2H, dt, J_1=3.2 Hz, J_2=3.2 Hz, J_3=13.3 Hz, H-16), 2.85 (1H, ddd, J_1=3.5 Hz, J_2=11.4 Hz, J_3=13.1 Hz, H-13), 2.97 (1H, dt, J_1=4.2 Hz, J_2=11.2 Hz, J_3=11.2 Hz, H-19), 3.35-3.65 (4H, m, H-3'), 3.35-3.66 (4H, m, H-4'), 4.46 (1H, dd, J_1=5.6 Hz, J_2=10.7 Hz, H-3), 4.58 (1H, dq, J_1=1.3 Hz, J_2=1.3 Hz, J_3=1.3 Hz, J_4=2.4 Hz, H-29), 4.72 (1H, dq, J_1=0.7 Hz, J_2=0.7 Hz, J_3=0.7 Hz, J_4=2.4 Hz, H-29). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ [ppm] 14.60 (q, C27), 16.10 (q, C24), 16.20 (q, C25), 16.50 (q, C26), 18.20 (t, C6), 19.60 (q, C30), 21.10 (t, C11), 21.30 (q, C2'), 23.70 (t, C2), 25.60 (t, C12), 27.90 (q, C23), 28.40 (q, C7'), 29.80 (t, C21), 31.00 (d, C3), 31.30 (t, C15), 32.60 (t, C16), 34.30 (t, C22), 36.00 (s, C10), 36.90 (t, C7), 37.10 (d, C13), 37.80 (s, C4), 38.40 (t, C1), 40.70 (s, C8), 41.90 (s, C14), 43.40 (t, C3'), 44.20 (t, C4'), 45.70 (d, C19), 50.70 (d, C9), 52.70 (d, C18), 54.60 (s, C17), 55.50 (d, C5), 80.20 (s, C6'), 109.30 (t, C29), 151.20 (s, C20), 154.70 (s, C5'), 171.00 (s, C1'), 173.80 (s, C28). IR (cm^{-1}): 3400 (CO-NH, NH_2), 2969 (CH_2), 2900 (CH_3), 1720 (C=O), 1630 (C=O), 1470 (CH_2), 1405 (CO-H), 1392 (CH_2), 1249 (C-O-C), 1075 (C-C) 1065 (C-O-C), 1045 (C-O-C), 1027 (C-O-C). MS: *m/z* = 567.6 $[M+H]^+$ (ESI^+). For $C_{36}H_{58}N_2O_3$ (566.44) calcd. C 76.28, H 10.31, N 4.94, found C 76.25, H 10.30, N 4.96.

Figure S12. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



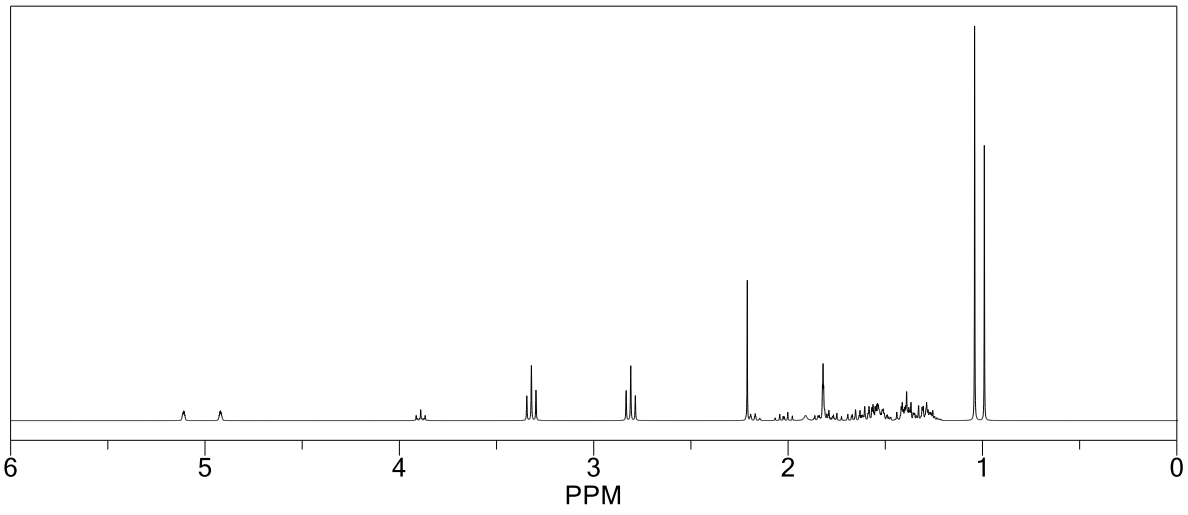
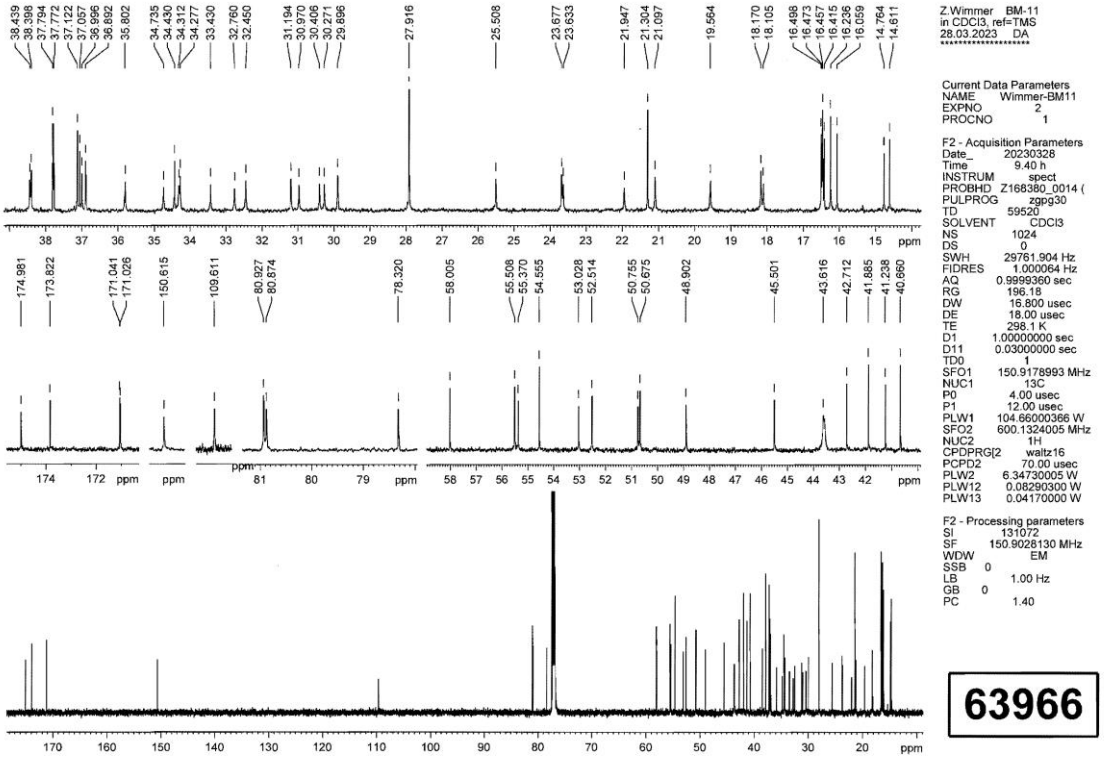
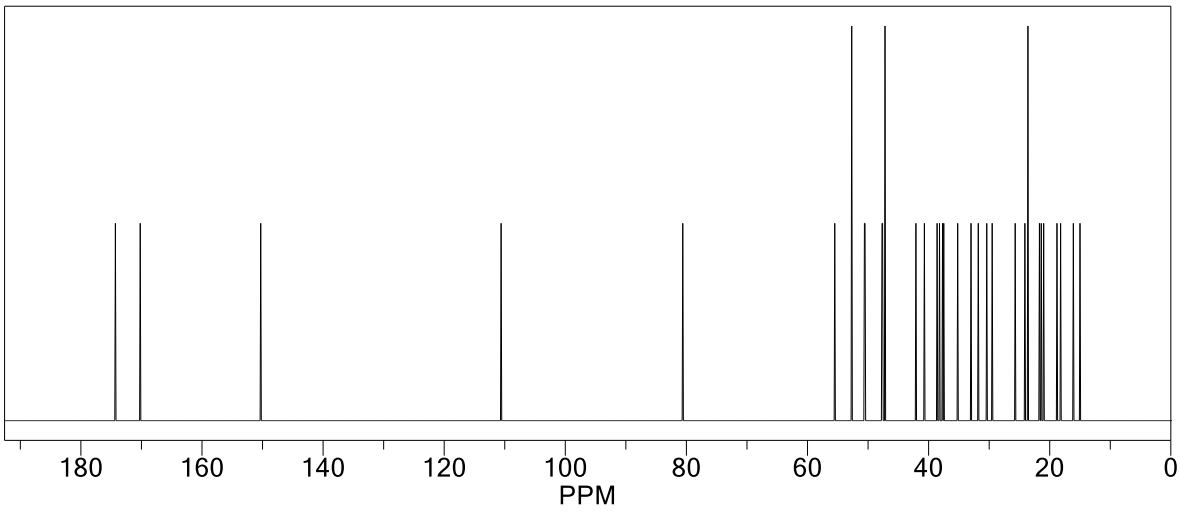


Figure S13. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

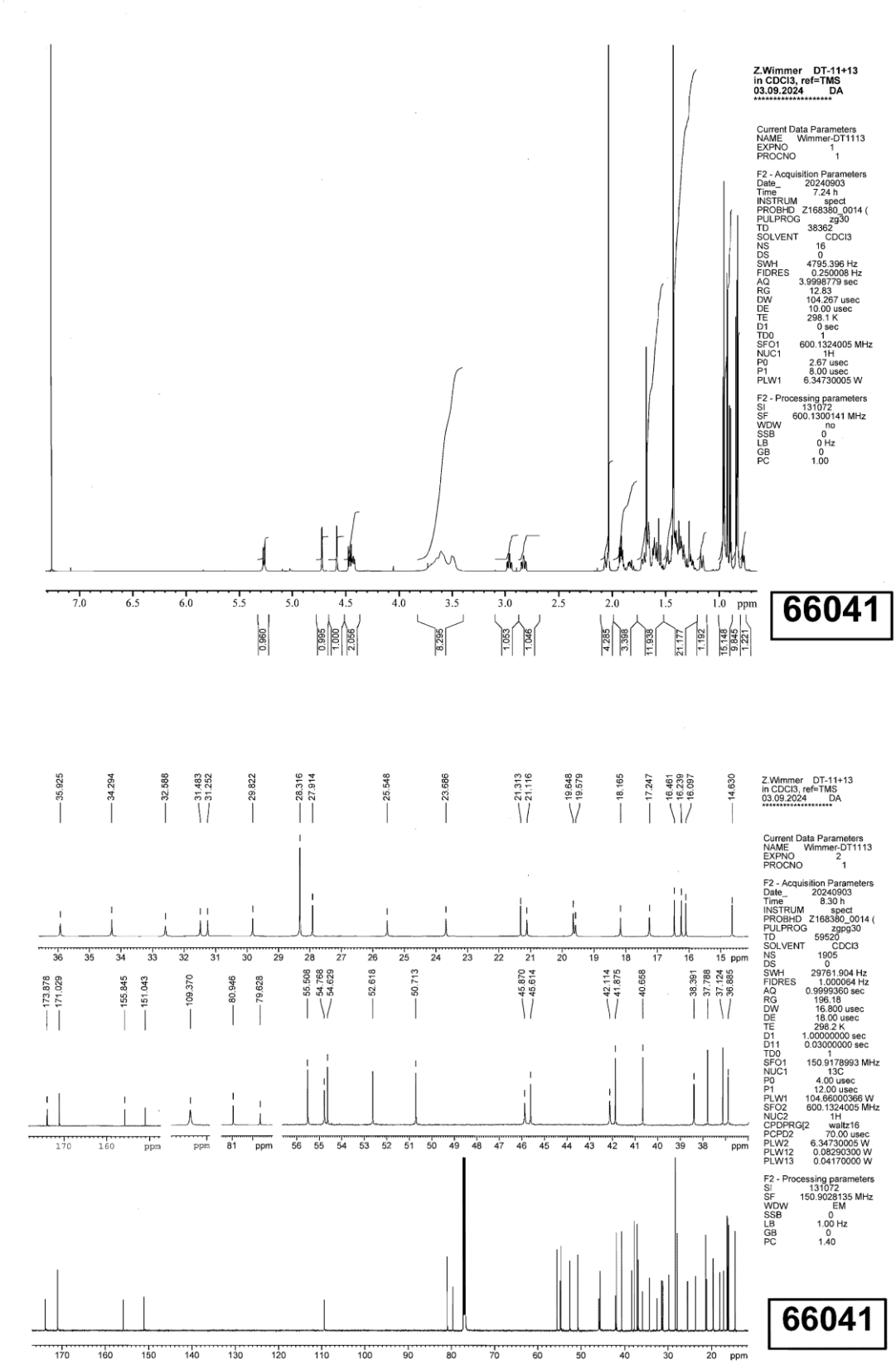




1.10. (3β)-28-{4-[(*N*-(*tert*-Butoxycarbonyl)-(2*S*)-2-amino-3-methylbutanoyl]piperazin-1-yl}-28-oxolup-20(29)-en-3-yl acetate (**10**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.78 (1H, dd, *J* = 2.3; 11.2 Hz, H5), 0.82 (3H, s, H26), 0.83 (3H, s, H23), 0.84 (3H, s, H25), 0.90 (3H, d, *J* = 6.7 Hz, H9′), 0.92 (3H, s, H24), 0.95 (3H, s, H27), 0.96 (3H, d, *J* = 6.8 Hz, H8′), 1.16 (2H, dt, *J* = 3.4; 3.4; 13.5 Hz, H21), 1.43 (9H, s, H12′), 1.56 (1H, t, *J* = 11.2 Hz, H18), 1.68 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.89–1.95 (3H, m, H7+H7′), 2.06 (2H, dt, *J* = 3.5; 3.5; 13.4 Hz, H16), 2.93 (1H, ddd, *J* = 3.6; 11.5; 13.0 Hz, H13), 2.96 (1H, dt, *J* = 3.7; 10.9; 10.9 Hz, H19), 3.44–3.72 (8H, m, H3′+H4′), 4.43 (1H, dd, *J* = 6.0; 9.2 Hz, H6′), 4.46 (1H, dd, *J* = 5.8; 10.5 Hz, H3), 4.72 (1H, dq, *J* = 3×0.7; 2.3 Hz, H30), 5.58 (1H, dq, *J* = 3×1.4; 2.3 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 16.1 (q, C24), 16.2 (q, C25), 16.5 (q, C26), 17.2 (q, C9′), 18.2 (t, C6), 19.6 (q, C29), 19.6 (q, C8′), 21.1 (t, C11), 21.3 (q, C2′), 23.7 (t, C2), 25.5 (t, C12), 27.9 (q, C23), 28.3 (q, C12′), 29.8 (t, C21), 31.3 (d, C7′), 31.5 (t, C15), 32.6 (t, C16), 34.3 (t, C22), 35.9 (t, C7), 36.9 (s, C10), 37.1 (d, C13), 37.8 (s, C4), 38.4 (t, C1), 40.7 (s, C8), 41.9 (s, C14), 42.1 (t, C4′), 45.6 (d, C19), 45.9 (t, C3′), 50.7 (d, C9), 52.6 (d, C18), 54.6 (d, C6′), 54.8 (s, C17), 55.5 (d, C5), 79.6 (s, C11′), 80.9 (d, C3), 109.4 (t, C30), 151.0 (s, C20), 155.8 (s, C10′), 171.0 (s, C1′), 171.0 (s, C5′), 173.9 (s, C28). MS (ESI$^+$, HCOOH): *m/z* = 766.8 $[M+H]^+$, 788.8 $[M+Na]^+$; (ESI$^-$, NH_4OH): *m/z* = 764.5 $[M–H]^-$.

Figure S14. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.11. (3β)-28-Oxo-28-{[(4-(2*S*)-2-Amino-3-methylbutanoyl)-piperazin-1-yl]lup-20(29)-en-3-yl} acetate (**11**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.78 (1H, dd, *J* = 2.3; 10.9 Hz, H5), 0.83 (3H, s, H23), 0.83 (3H, s, H26), 0.84 (3H, s, H25), 0.92 (3H, s, H24), 0.95 (3H, s, H27), 1.56 (1H, t, *J* = 11.5 Hz, H18), 1.68 (3H, bs, H29), 2.03 (3H, s, H2′), 2.83 (1H, bdt, *J* = 3.5; 12.2; 12.2 Hz, H13), 2.96 (1H, dt, *J* = 4.2; 11.0; 11.0 Hz, H19), 3.30–3.90 (8H, m, H3′+H4′), 4.46 (1H, dd, *J* = 6.0; 10.4 Hz, H3), 4.58 (1H, dq, *J* = 3×1.4; 2.2 Hz, H30), 4.72 (1H, bd, *J* = 2.2 Hz, H30). ^{13}C NMR (150.92 MHz, DMSO-*d*$_6$, 288 K): δ 14.4 (q, C27), 15.9 (q, C24), 16.0 (q, C25), 16.5 (q, C26), 17.8 (t, C6), 19.1 (q, C29), 20.7 (t, C11), 21.1 (q, C2′), 23.4 (t, C2), 25.1 (t, C12), 27.7 (q, C23), 29.4 (t, C21), 30.8 (t, C15), 31.6 (t, C16), 33.8 (t, C22), 35.3 (t, C7), 36.2 (s, C10), 36.7 (d, C13), 37.4 (s, C4), 37.8 (t, C1), 40.0 (s, C8), 40.0 (t, C4′), 40.2 (t, C3′), 41.5 (s, C14), 45.5 (q, C19), 49.9 (d, C9), 51.9 (d, C18), 54.0 (s, C17), 54.7 (d, C5), 79.9 (d, C3), 109.4 (t, C30), 151.0 (s, C20), 170.2 (s, C1′), 172.8 (s, C28). IR (ATR): $\tilde{\nu}$ = 2940 (m), 2860 (w), 1721 (m), 1634 (s), 1453 (m), 1371 (m), 1242 (vs), 1186 (m), 1011 (m), 978 (m), 883 (w), 755 (w) cm^{-1}. MS (ESI$^+$, CH_3COOH): *m/z* = 666.4 $[M+H]^+$. For $C_{41}H_{67}N_3O_4$ (665.51) calcd. C 73.94, H 10.14, N 6.31, found C 73.96, H 10.12, N 6.33.

Figure S15. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

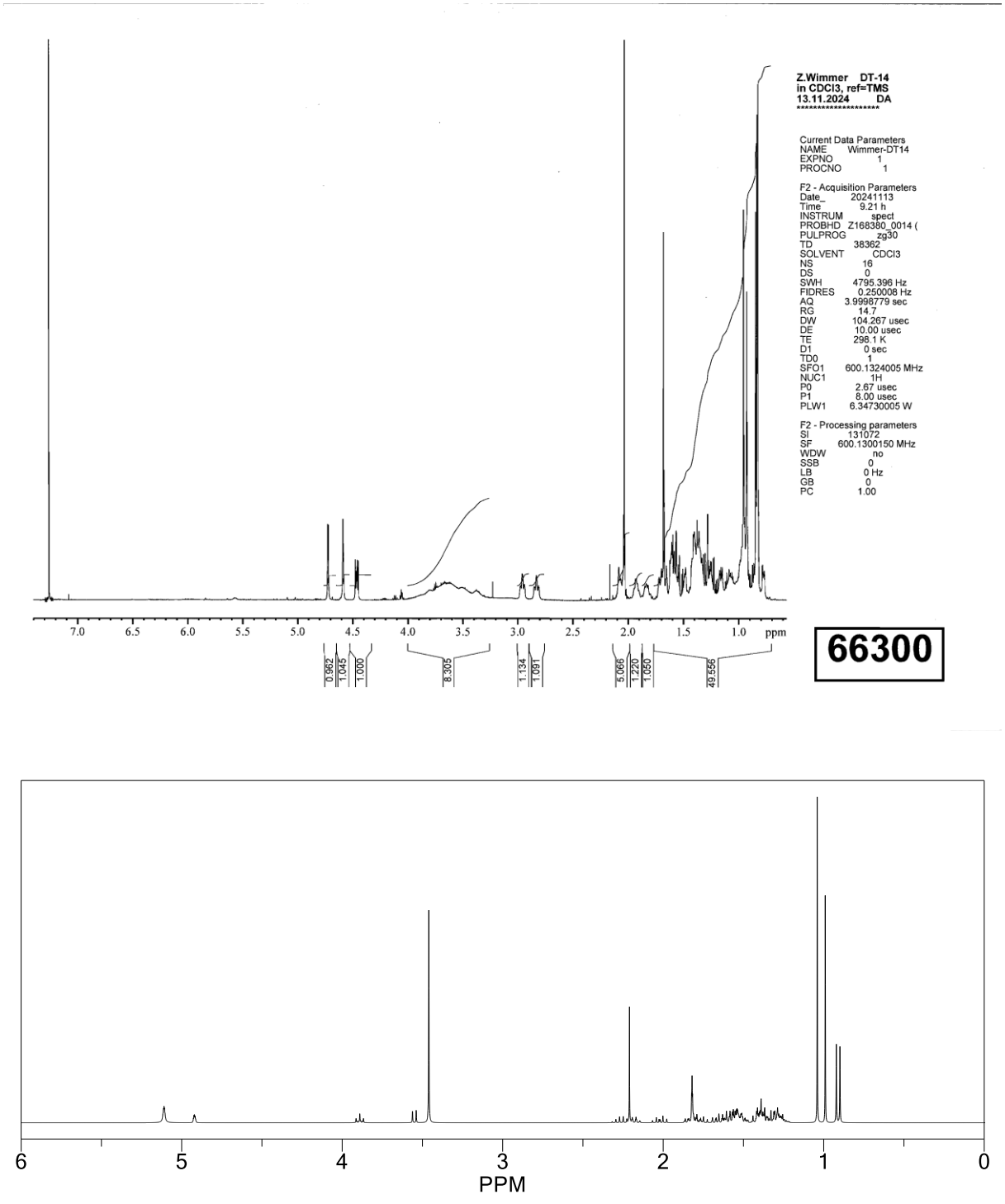
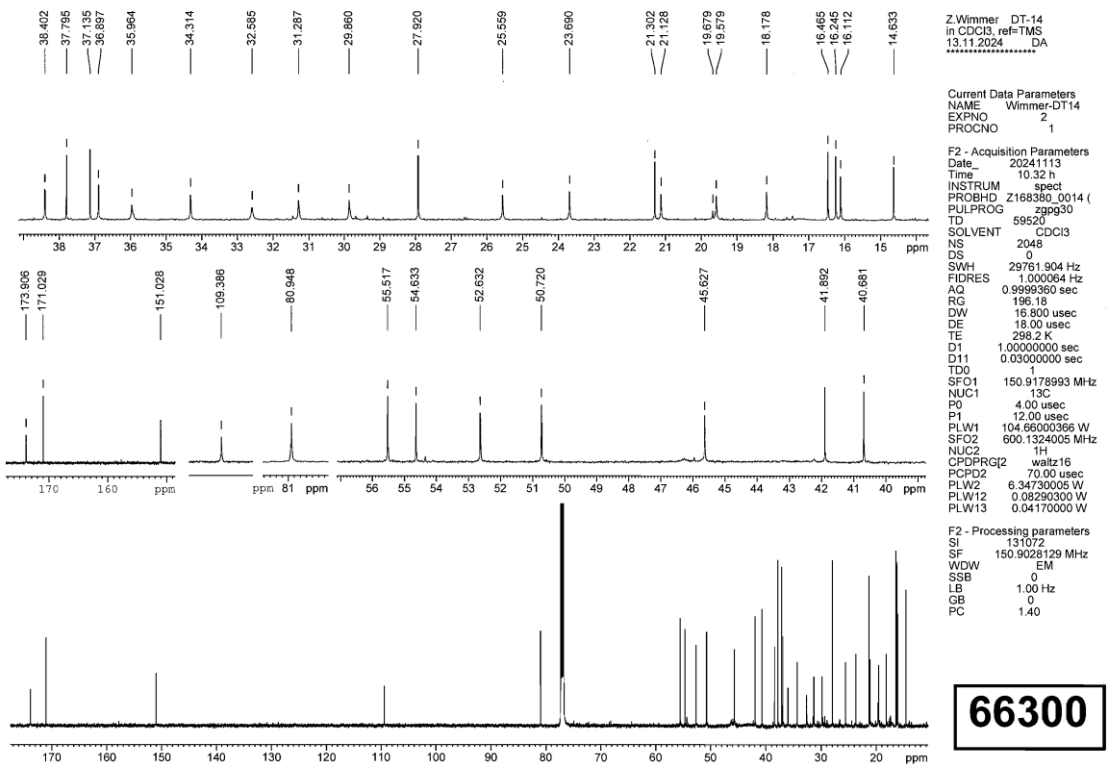
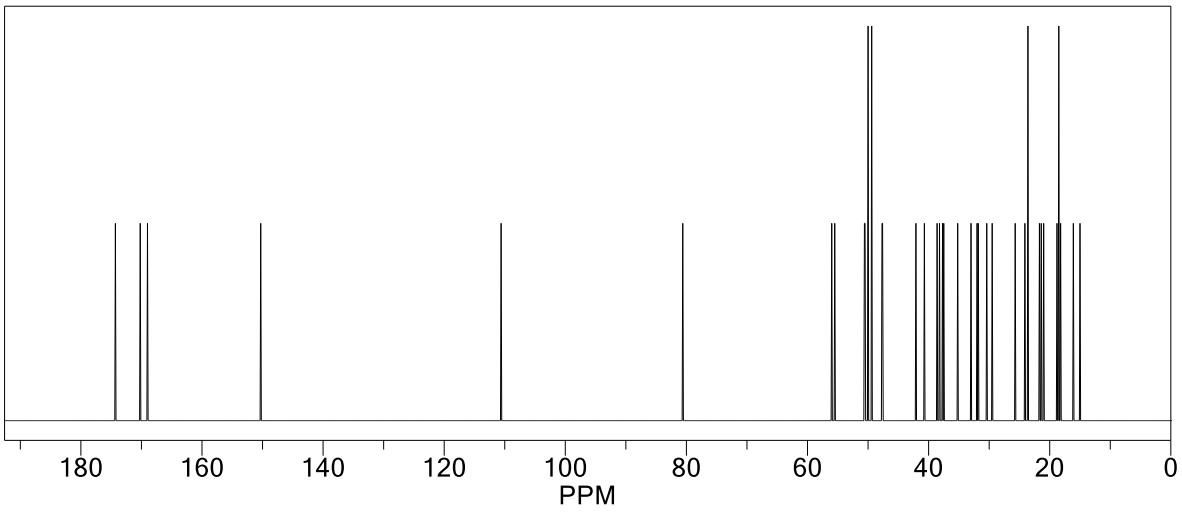


Figure S16. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)





1.12. (3β)-28-{4[(2*S*)-2-Amino-3-methylbutanoyl]piperazin-1-yl}-3-hydroxylup-20(29)-en-28-one (**12**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.66 (dd, *J* = 1.4; 10.8 Hz, H5), 0.74 (s, H25), 0.81 (s, H26), 0.91 (s, H24), 0.94 (s, H27), 0.95 (s, H23), 1.16 (bt, *J* = 12.8 Hz, H21), 1.67 (dd, *J* = 0.7; 1.4 Hz, H29), 2.83 (dt, *J* = 3.4; 12.5; 12.5 Hz, H13), 2.94 (dt, *J* = 4.2; 10.8; 10.8 Hz, H14), 3.16 (dd, *J* = 4.8; 11.3 Hz, H3), 3.38–3.86 (m, H1′+H2′), 4.57 (dq, *J* = 3×1.4; 2.2 Hz, H30), 4.71 (bd, *J* = 2.3 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.6 (q, C27), 15.3 (q, C24), 16.0 (q, C25), 16.1 (q, C26), 18.2 (t, C6), 19.5 (q, C29), 21.1 (t, C11), 25.6 (t, C12), 27.2 (t, C2), 27.9 (q, C23), 29.8 (t, C21), 31.2 (t, C15), 32.5 (t, C16), 34.3 (t, C22), 35.9 (t, C7), 36.9 (s, C10), 37.2 (d, C13), 38.7 (s, C4), 38.8 (t, C1), 40.6 (s, C8), 41.9 (s, C14), 42.6 (t, C2′), 45.6 (d, C19), 45.9 (t, C1′), 50.8 (d, C9), 52.6 (d, C18), 54.6 (s, C17), 55.4 (d, C5), 78.9 (d, C3), 109.3 (t, C30), 151.0 (s, C20), 174.0 (s, C28). MS (ESI$^+$, CH_3COOH): *m/z* = 624.7 $[M+H]^+$. For $C_{39}H_{65}N_3O_3$ (623.50) calcd. C 75.07, H 10.50, N 6.73, found C 75.10, H 10.48, N 6.72.

Figure S17. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

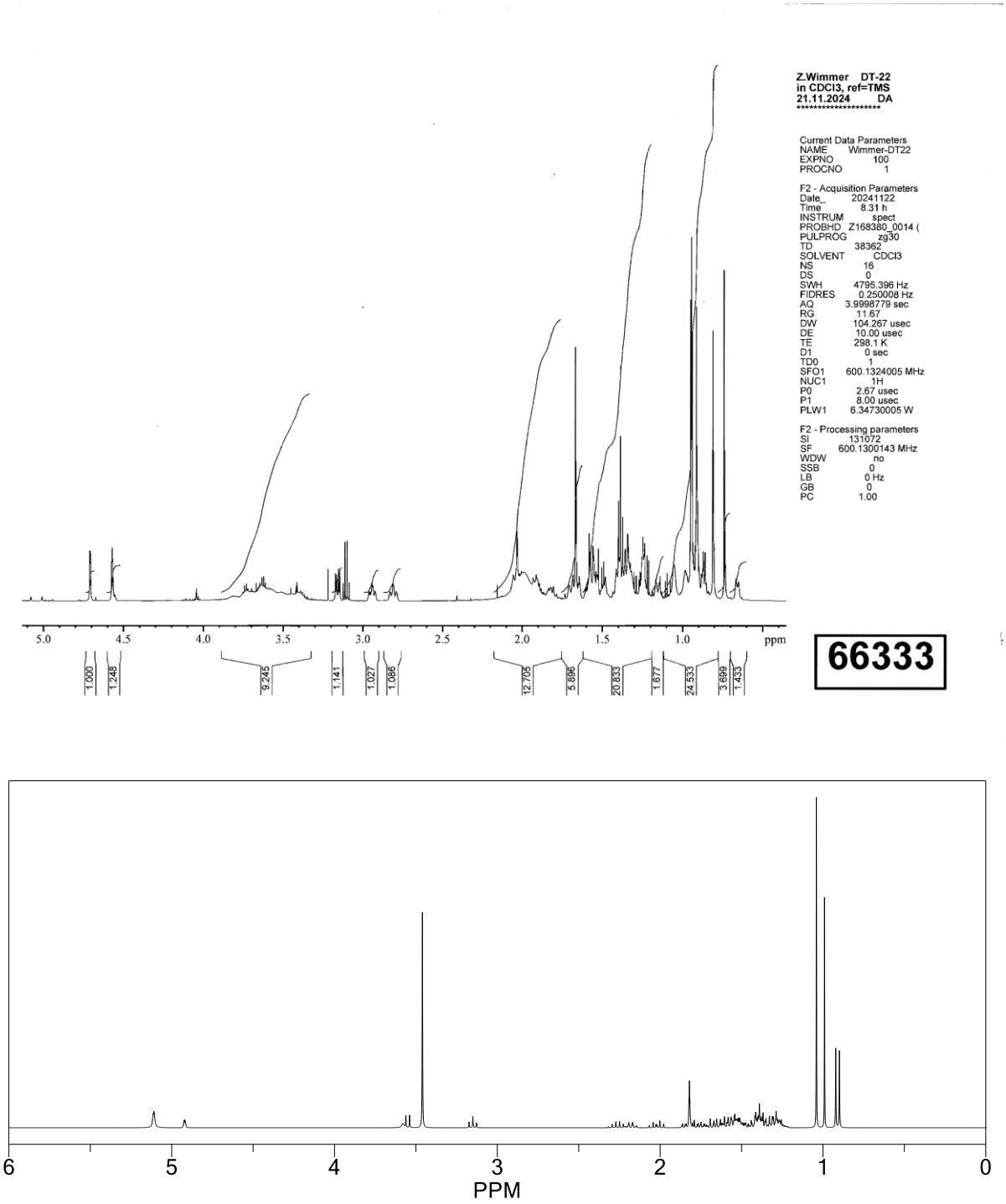
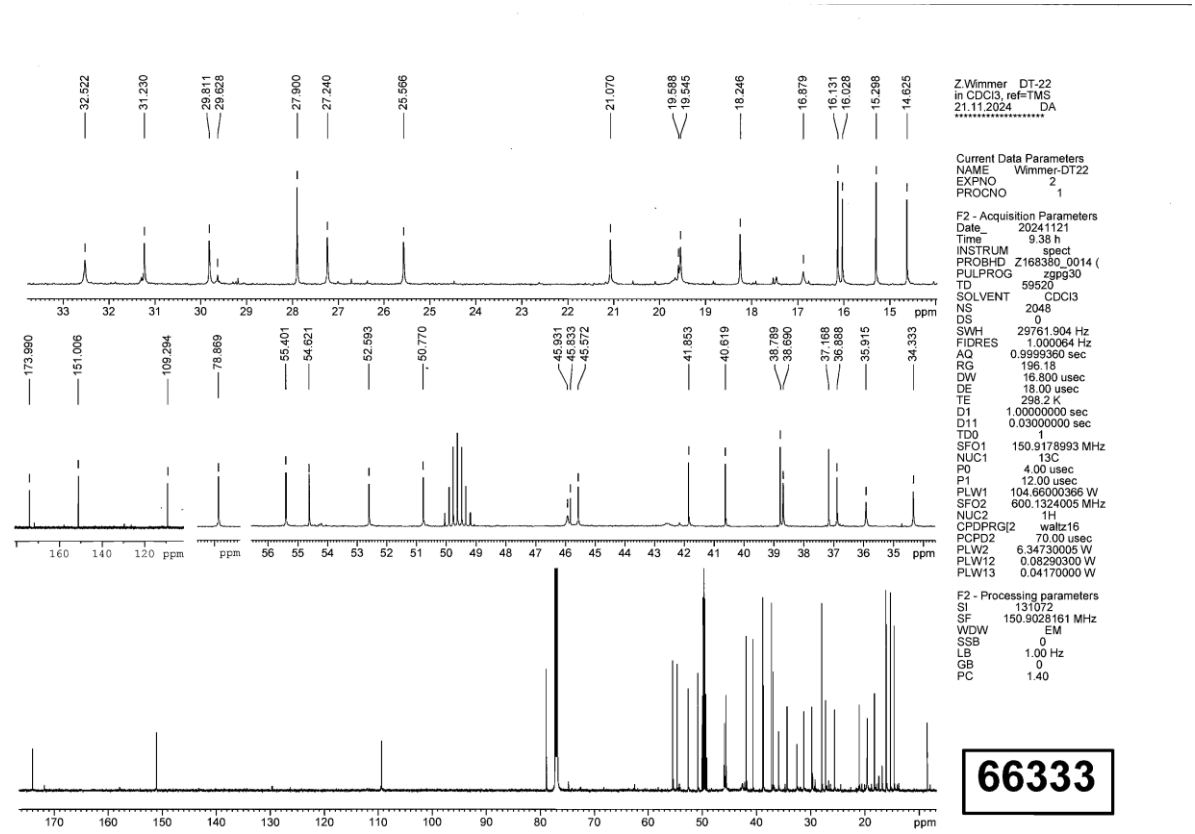
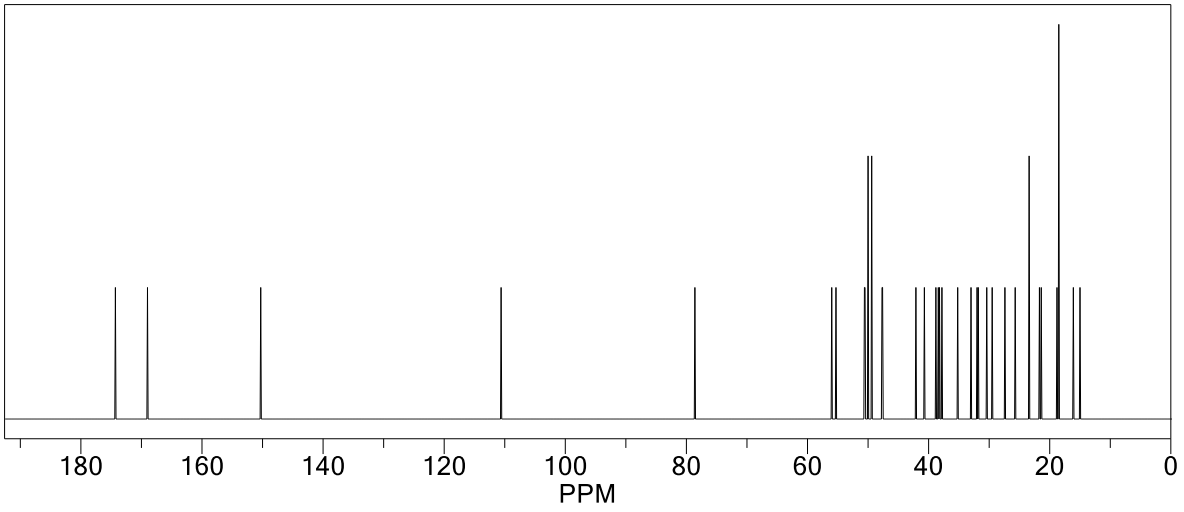


Figure S18. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

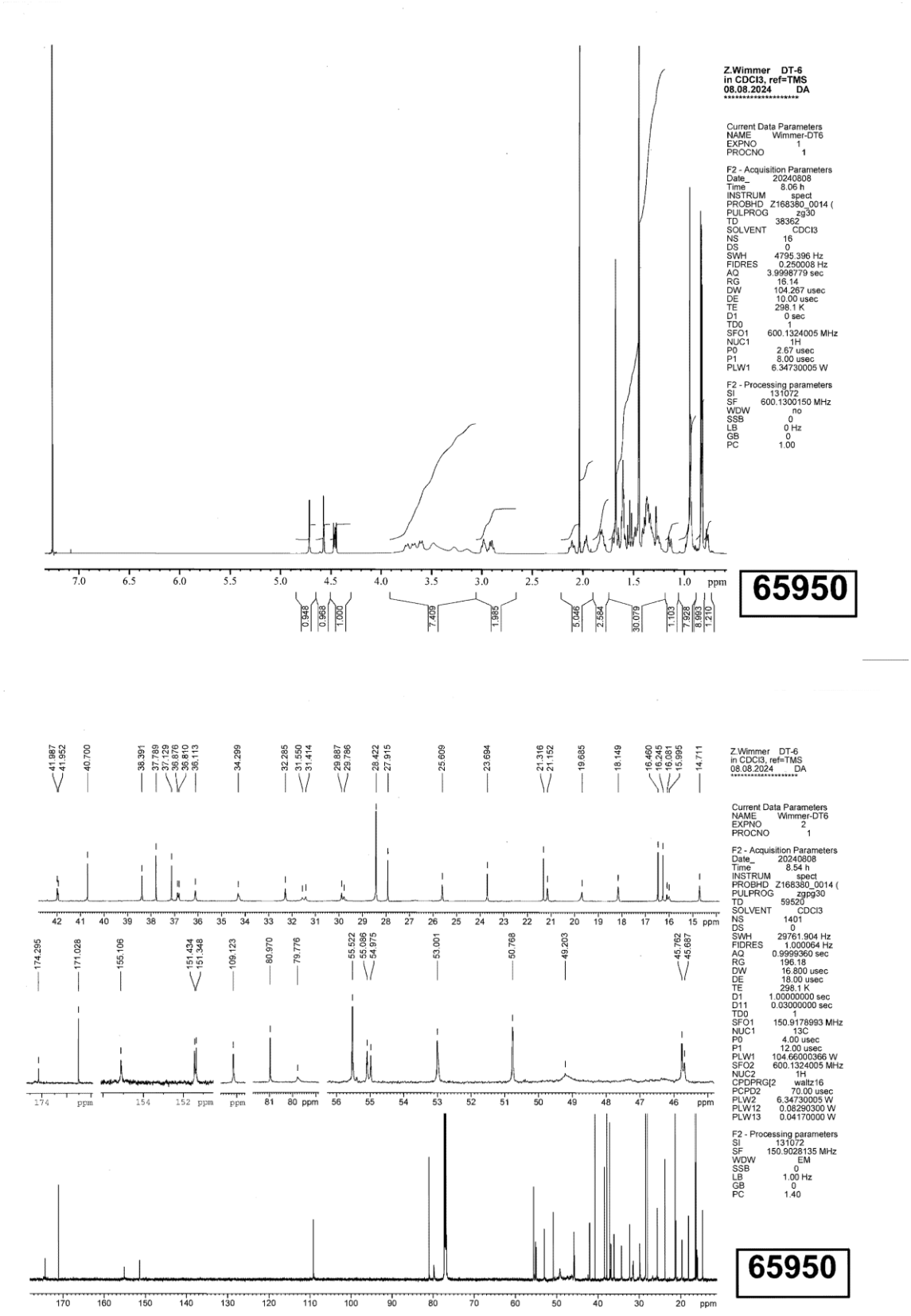




1.13. *tert*-Butyl 4-[(3β)-3-(acetyloxy)-28-oxolup-20(29)-en-28-yl]-1,4-diazepane-1-carboxylate (**13**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.78 (1H, dd, *J* = 2.4; 10.8 Hz, H5), 0.82 (3H, s, H25), 0.83 (3H, s, H26), 0.84 (3H, s, H24), 0.93 (3H, s, H23), 0.94 (3H, s, H27), 1.15 (2H, dt, *J* = 3.0; 3.0; 13.5 Hz, H21), 1.45 (9H, s, H10′), 1.54 (1H, t, *J* = 11.3 Hz, H18), 1.67 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.95–2.00 (2H, m, H7), 2.03 (3H, s, H2′), 2.08–2.13 (2H, m, H16), 2.88–3.01 (2H, m, H13+H19), 3.10–3.80 (10H, m, H3′–H7′), 4.46 (1H, dd, *J* = 5.8; 10.5 Hz, H3), 4.57 (1H, dq, *J* = 3×1.4; 2.3 Hz, H30), 4.72 (1H, dq, *J* = 3×0.7; 2.3 Hz, H30). ^{13}C NMR (150.92 MHz, $CDCl_3$): δ 14.7 (q, C27), 16.1 (q, C24), 16.2 (q, C25), 16.5 (q, C26), 18.1 (t, C6), 19.7 (q, C29), 21.2 (t, C11), 21.3 (q, C2′), 23.7 (t, C2), 25.6 (t, C12), 27.9 (q, C23), 28.4 (t, C6′), 28.4 (q, C10′), 29.9 (t, C15), 31.6 (t, C21), 32.3 (t, C16), 34.3 (t, C22), 36.1 (t, C7), 36.9 (s, C10), 37.1 (d, C13), 37.8 (s, C4), 38.4 (t, C1), 40.7 (s, C8), 42.0 (s, C14), 45.7 (t, C5′), 45.8 (d, C19), 46.3 (t, C4′), 47.4 (t, C7′), 49.2 (t, C3′), 50.8 (d, C9), 53.0 (d, C18), 55.0 (s, C17), 55.5 (d, C5), 79.8 (s, C9′), 81.0 (d, C3), 109.1 (t, C30), 151.4 (s, C20), 155.1 (s, C8′), 171.0 (s, C1′). MS (ESI^+, NH_4OH): *m/z* = 681.9 $[M+H]^+$.

Figure S19. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.14. (3β)-28-(1,4-Diazepan-1-yl)-28-oxolup-20(29)-en-3-yl acetate (**14**).

^{1}H NMR (600.13 MHz, $CDCl_3$): δ 0.77 (1H, dd, *J* = 2.3; 11.0 Hz, H5), 0.82 (3H, s, H26), 0.83 (3H, s, H23), 0.84 (3H, s, H25), 0.93 (3H, s, H24), 0.95 (3H, s, H27), 1.16 (2H, dt, *J* = 3.1; 3.1; 13.4 Hz, H21), 1.56 (1H, t, *J* = 11.2 Hz, H18), 1.66 (2H, dt, *J* = 3.5; 3.5; 13.1 Hz, H1), 1.68 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.78–1.87 (2H, m, H15), 1.90 (2H, m, H7), 2.03 (3H, s, H2′), 2.11 (2H, dt, *J* = 3.0; 3.0; 13.7 Hz, H16), 2.87 (1H, bt, *J* = 11.6; 11.6 Hz, H13), 2.96 (1H, dt, *J* = 3.8; 11.4; 11.4 Hz, H19), 3.60–3.78 (8H, m, H3′, H4′, H5′, H7′), 4.46 (1H, dd, *J* = 3.7; 10.6 Hz, H3), 4.58 (1H, dq, *J* = 3×1.4; 2.3 Hz, H30), 4.72 (1H, bd, *J* = 2.3 Hz, H30). ^{13}C NMR (150.92 MHz, DMSO-d_6, 288 K): δ 14.8 (q, C27), 16.1 (q, C24), 16.3 (q, C25), 16.8 (q, C26), 18.1 (t, C6), 19.5 (q, C29), 21.1 (t, C11), 21.4 (q, C2′), 23.7 (t, C2), 25.5 (t, C12), 27.1 (t, C6′), 28.0 (q, C23), 29.7 (t, C21), 31.1 (t, C15), 31.7 (t, C16), 34.1 (t, C22), 35.7 (t, C7), 36.5 (d, C13), 37.0 (s, C10), 37.7 (s, C4), 38.1 (t, C1), 40.6 (s, C8), 41.9 (s, C14), 45.7 (t, C7′), 45.9 (d, C19), 46.8 (t, C3′), 50.2 (d, C9), 52.4 (d, C18), 54.6 (s, C17), 55.5 (d, C5), 80.2 (d, C3), 109.7 (t, C30), 151.4 (s, C20), 171.5 (s, C1′), 174.0 (s, C28). MS (ESI^+): *m/z* = 581.4 $[M+H]^+$. For $C_{37}H_{60}N_2O_3$ (580.46) calcd. C 76.50, H 10.41, N 4.82, found C 76.53, H 10.39, N 4.80.

Figure S20. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

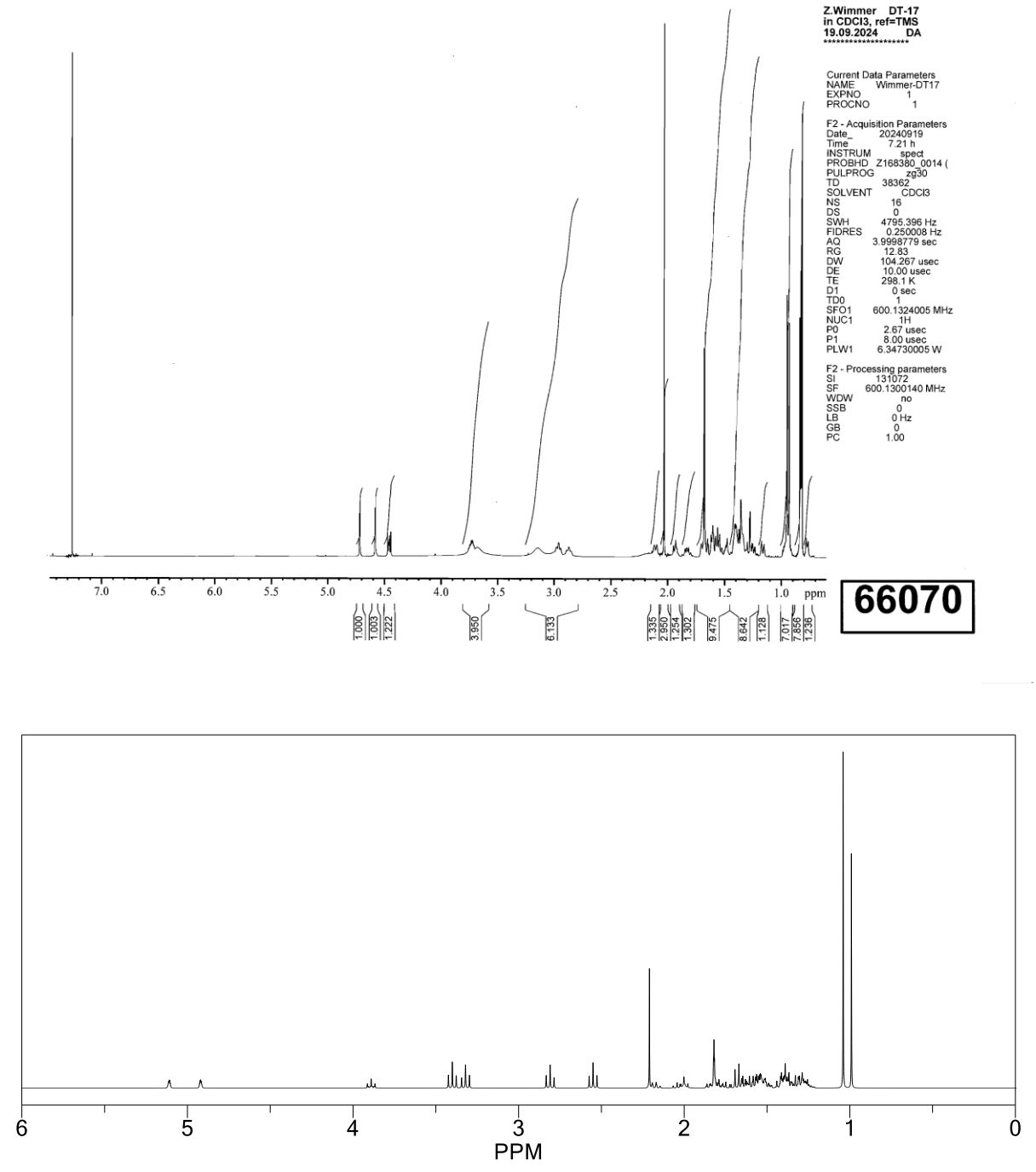
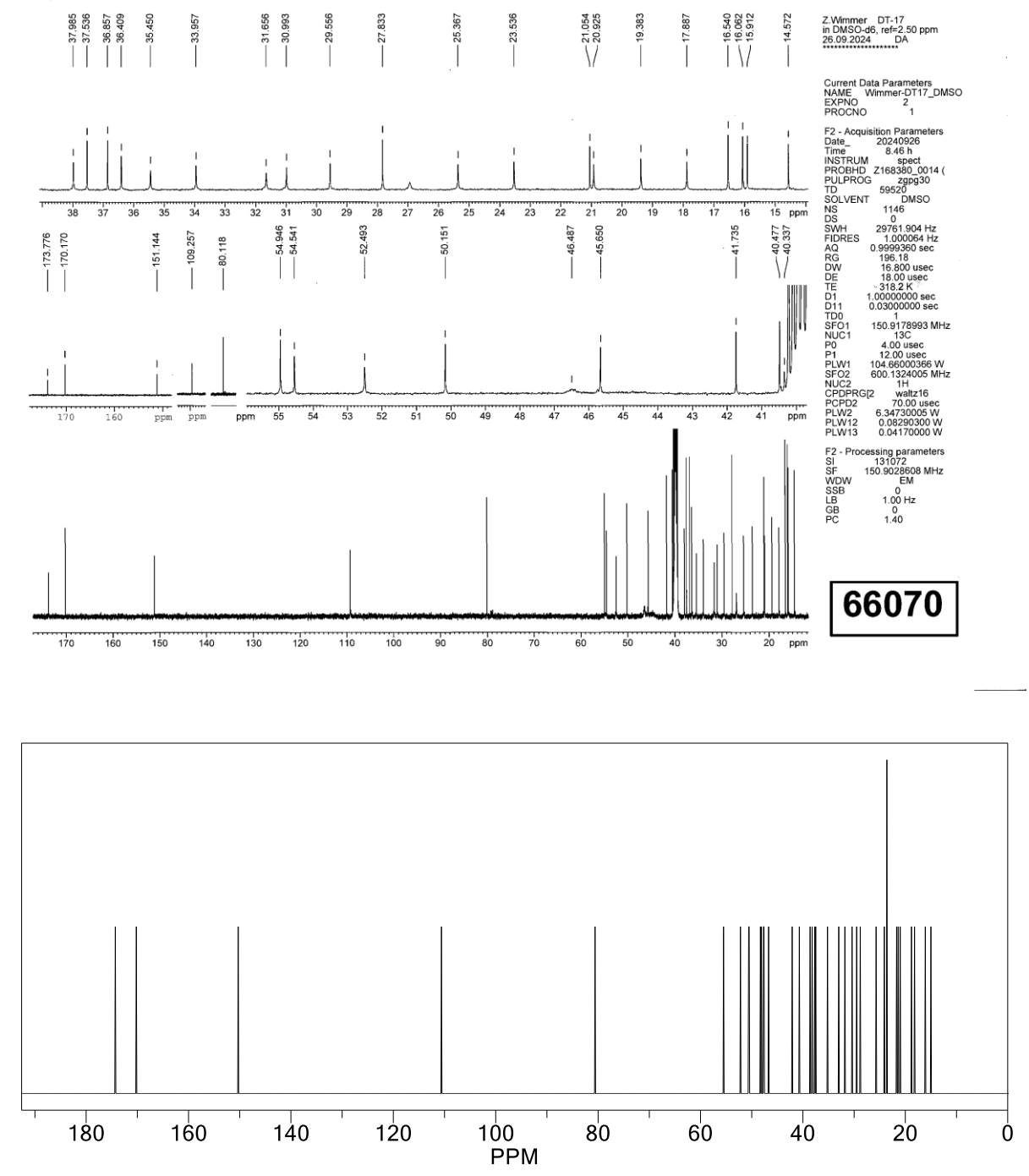


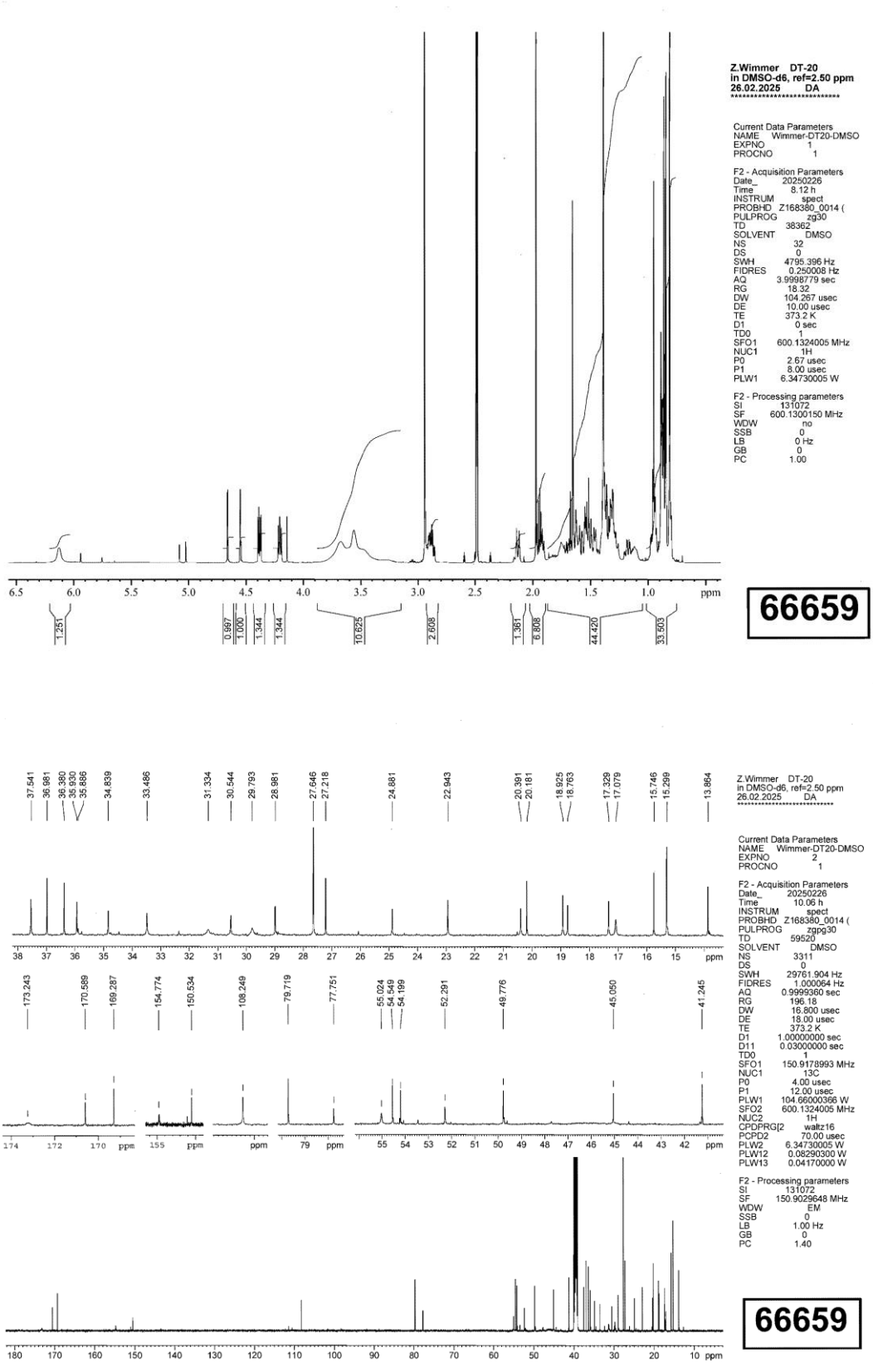
Figure S21. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)



1.15. (3β)-3-28-{4-[*N*-(*tert*-Butoxycarbonyl)-(2*S*)-2-amino-3-methylbutanoyl]-1,4-diazepan-1-yl}-28-oxolup-20(29)-en-3-yl acetate (**15**).

^{1}H NMR (600.13 MHz, DMSO-d_6, 373 K): δ 0.82 (1H, dd, *J* = 1.9; 11.6 Hz, H5), 0.82 (3H, s, H23), 0.82 (3H, s, H26), 0.86 (3H, s, H25), 0.87 (3H, d, *J* = 6.7 Hz, H11′), 0.89 (3H, d, *J* = 6.7 Hz, H12′), 0.90 (3H, s, H24), 0.96 (3H, s, H27), 1.40 (9H, s, H15′), 1.53 (1H, t, *J* = 11.2 Hz, H18), 1.67 (3H, dd, *J* = 0.7; 1.4 Hz, H29), 1.96 (2H, dt, *J* = 6.8; 6.8; 13.7 Hz, H7), 1.99 (3H, s, H2′), 2.15 (2H, dt, *J* = 3.4; 3.4; 13.7 Hz, H16), 2.89 (1H, dt, *J* = 4.5; 11.0; 11.0 Hz, H19), 2.92 (1H, bdt, *J* = 3.7; 11.4; 11.4 Hz, H13), 3.45–3.77 (8H, m, H3′, H4′, H5′, H7′), 4.22 (1H, dd, *J* = 7,0; 9,0 Hz, H9′), 4.40 (1H, dd, *J* = 5.0; 11.3 Hz, H3), 4.57 (1H, dq, *J* = 3×1.4; 2.4 Hz, H30), 4.68 (1H, dq, *J* = 3×0.7; 2.4 Hz, H30). ^{13}C NMR (150.92 MHz, DMSO-d_6, 373 K): δ 13.9 (q, C27), 15.3 (q, C24), 15.3 (q, C25), 15.7 (q, C26), 17.1 (q, C11′), 17.3 (t, C6), 18.8 (q, C29), 18.9 (q, C12′), 20.2 (q, C2′), 20.4 (t, C11), 22.9 (t, C2), 24.9 (t, C12), 27.2 (q, C23), 27.6 (q, C15′), 29.0 (t, C21), 29.8 (d, C10′), 30.5 (t, C15), 31.3 (t, C16), 33.5 (t, C22), 34.8 (t, C7), 35.9 (d, C13), 36.4 (s, C10), 37.0 (s, C4), 37.5 (t, C1), 41.2 (s, C8), 41.2 (s, C14), 45.1 (d, C19), 45.9 (t, C5′), 46.5 (t, C7′), 47.7 (t, C4′), 48.5 (t, C3′), 49.8 (d, C9), 52.3 (d, C18), 54.2 (s, C17), 54.5 (d, C5), 55.0 (d, C9′), 77.8 (s, C14′), 79.7 (d, C3), 108.2 (t, C30), 150.5 (s, C20), 154.8 (s, C13′), 169.3 (s, C1′), 170.6 (s, C8′), 173.2 (s, C28). IR (ATR): $\tilde{\nu}$ = 2969 (s), 2908 (s), 1714 (m), 1632 (m), 1449 (m), 1392 (m), 1366 (m), 1241 (s), 1166 (m), 1074 (s), 1066 (s), 1045 (s), 883 (w), 755 (w) cm^{-1}. MS (ESI^{+}, NH_4OH): *m/z* = 780.9 $[M+H]^{+}$; (ESI^{-}, NH_4OH): *m/z* = 778.5 $[M-H]^{-}$.

Figure S22. ^{1}H NMR spectrum measured (top) and ^{13}C NMR spectrum measured (bottom)



1.16. (3β)-28-Oxo-28-{[(2*S*)-2-amino-3-methylbutanoyl]-1,4-diazepan-1-yl}lup-20(29)-en-3-yl acetate (**16**).

^{1}H NMR (600.13 MHz, DMSO-d_6, 373 K): δ 0.81 (1H, dd, *J* = 2.0; 11.2 Hz, H5), 0.81 (3H, s, H23), 0.81 (3H, s, H26), 0.84 (3H, s, H25), 0.89 (3H, bs, H24), 0.95 (3H, s, H27), 0.95 (3H, d, *J* = 6.7 Hz, H11′), 1.01 (3H, d, *J* = 6.7 Hz, H12′), 1.53 (1H, t, *J* = 11.2 Hz, H18), 1.65 (3H, bdd, *J* = 0.7; 1.4 Hz, H29), 1.93 (2H, bdd, *J* = 7.1; 12.2 Hz, H7), 1.97 (3H, s, H2′), 2.86–2.93 (1H, m, H13), 2.86–2.93 (1H, m, H19), 3.20–3.95 (8H, m, H3′, H4′, H5′, H7′), 4.12 (1H, bm, H9′), 4.38 (1H, dd, *J* = 5.0; 11.4 Hz, H3), 4.55 (1H, dq, *J* = 3×1.4; 2.4 Hz, H30), 4.66 (1H, bd, *J* = 2.4 Hz, H30). ^{13}C NMR (150.92 MHz, DMSO-d_6, 373 K): δ 13.7 (q, C27), 15.3 (q, C24), 15.3 (q, C25), 15.8 (q, C26), 16.5 (q, C11′), 17.3 (t, C6), 18.2 (q, C12′), 18.8 (q, C29), 20.2 (t, C11), 20.4 (q, C2′), 23.0 (t, C2), 24.9 (t, C12), 27.2 (q, C23), 29.0 (t, C21), 29.0 (d, C10′), 30.6 (t, C15), 31.4 (t, C16), 33.5 (t, C22), 34.9 (t, C7), 36.0 (d, C13), 36.4 (s, C10), 37.0 (s, C4), 37.5 (t, C1), 41.3 (s, C8), 45.1 (d, C19), 45.4 (s, C14), 45.7 (t, C5′), 46.3 (t, C7′), 47.8 (t, C4′), 48.6 (t, C3′), 49.8 (d, C9), 52.3 (d, C18), 53.9 (d, C9′), 54.2 (s, C17), 54.5 (d, C5), 79.7 (d, C3), 108.4 (t, C30), 150.5 (s, C20), 169.3 (s, C1′). IR (ATR): $\tilde{\nu}$ = 2941 (s), 2872 (m), 1720 (m), 1647 (s), 1636 (s), 1617 (s), 1465 (s), 1374 (m), 1251 (s), 1194 (w) cm^{-1}. MS (ESI^{+}, CH_3COOH): *m/z* = 680.3 $[M+H]^{+}$. For $C_{42}H_{69}N_3O_4$ (679.53) calcd. C 74.18, H 10.23, N 6.18, found C 74.15, H 10.21, N 6.19.

Figure S23. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

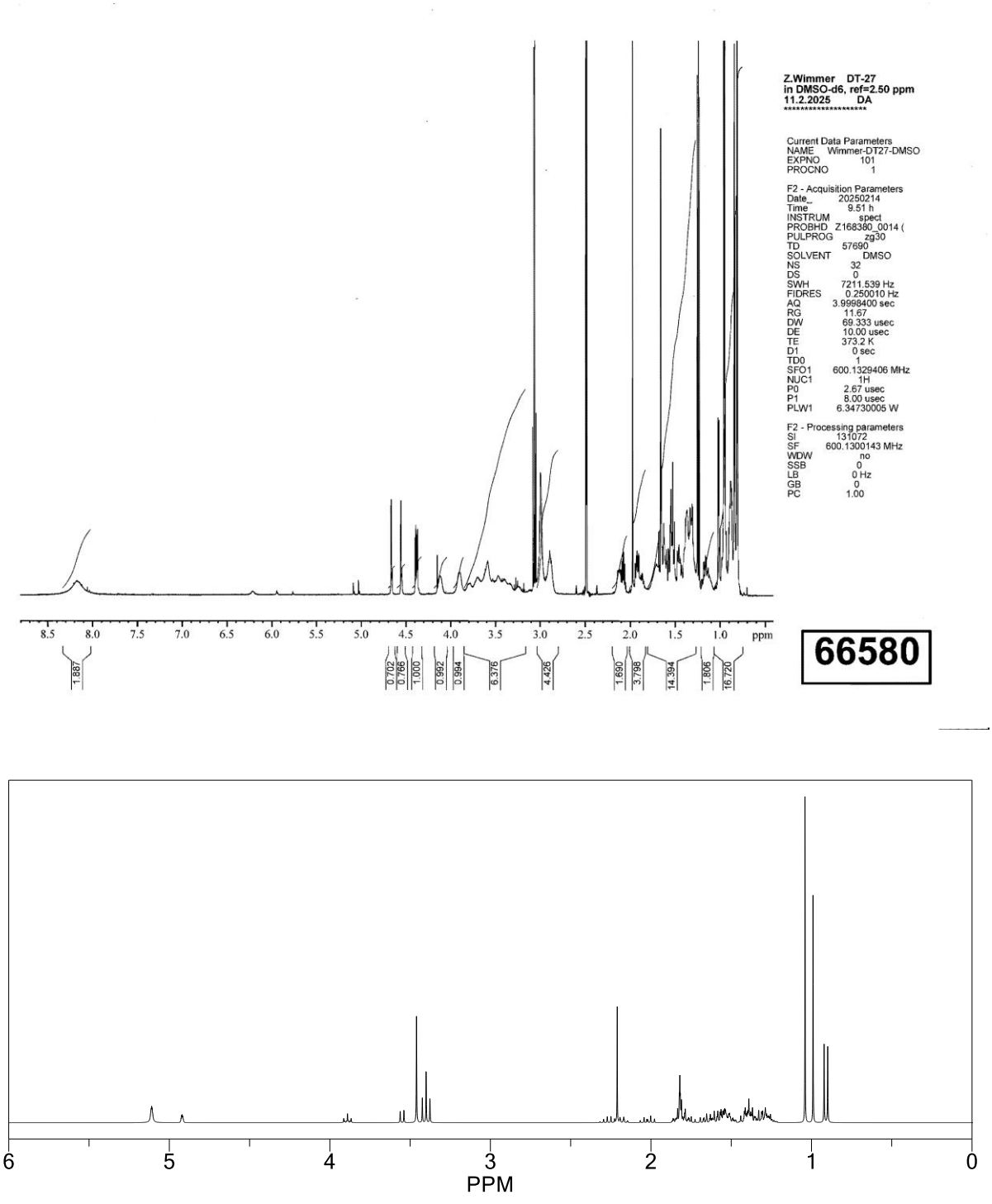
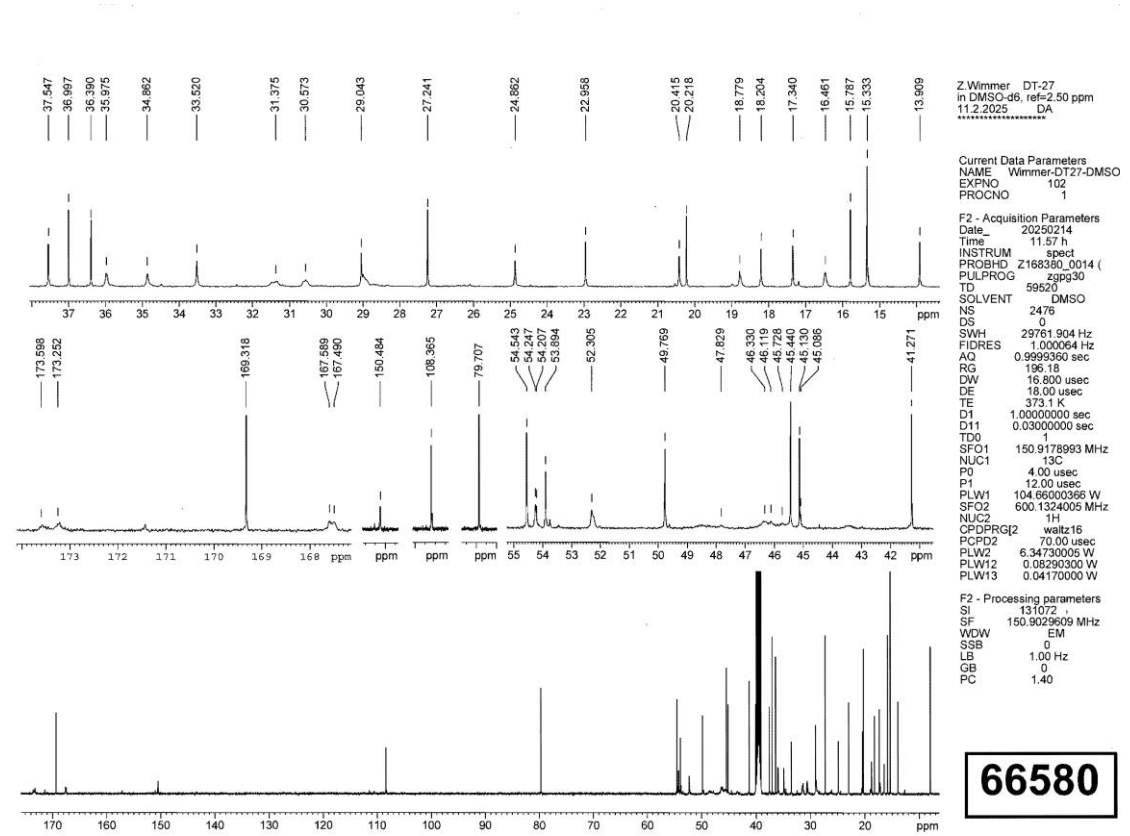
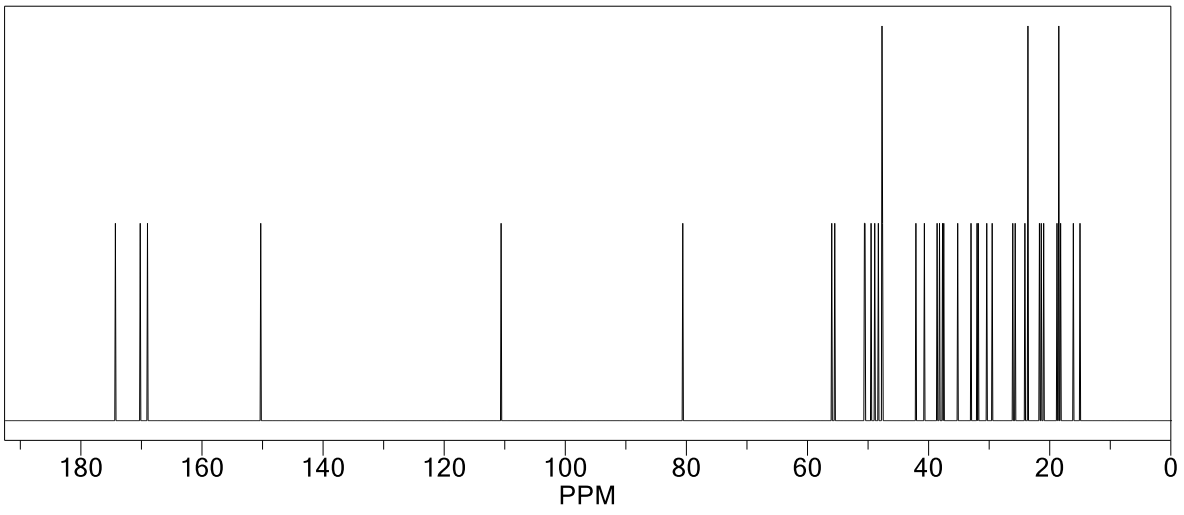


Figure S24. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)





1.17. (3β)-28-{4-[(2*S*)-2-Amino-3-methylbutanoyl]-1,4-diazepan-1-yl}-3-hydroxolup-20(29)-en-28-one (**17**).

^{1}H NMR (600.13 MHz, DMSO-d_6, 373K): δ 0.67 (1H, dd, *J* = 1.8, 11.2 Hz, H5), 0.70 (3H, s, H25), 0.81 (3H, s, H26), 0.84 (3H, s, H24), 0.85 (3H, d, *J* = 6.8 Hz, H9′), 0.91 (3H, s, H23), 0.96 (3H, s, H27), 0.96 (3H, d, *J* = 6.9 Hz, H10′), 1.54 (1H, t, *J* = 11.2 Hz, H18), 1.67 (3H, dd, *J* = 0.7, 1.4 Hz, H29), 1.94 (2H, bdd, *J* = 7.0, 12.7 Hz, H7), 2.87–2.94 (1H, m, H13), 2.87–2.94 (1H, m, H19), 3.02 (1H, dd, *J* = 7.2, 10.1 Hz, H3), 3.35–3.95 (8H, m, H1′,H2′,H3′,H5′), 4.12–4.15 (1H, m, H7′), 4.57 (1H, dq, *J* = 3×1.4, 2.4 Hz, H30), 4.68 (1H, bd, *J* = 2.4 Hz, H30). ^{13}C NMR (150.92 MHz, DMSO-d_6, 373K): δ,13.9 (q, C27), 15.0 (q, C25), 15.3 (q, C24), 15.3 (q, C26), 16.4 (q, C9′), 17.5 (t, C6), 18.2 (q, C10′), 18.8 (q, C29), 20.4 (t, C11), 24.9 (t, C12), 26.7 (t, C2), 27.6 (q, C23), 28.9 (d, C8′), 29.0 (t, C21), 30.6 (t, C15), 31.4 (t, C16), 33.7 (t, C22), 34.8 (t, C7), 36.0 (d, C13), 36.5 (s, C10), 38.0 (t, C1), 38.0 (s, C4), 41.2 (s, C8), 41.2 (s, C14), 45.1 (d, C19), 45.7 (t, C3′), 46.1 (t, C5′), 47.7 (t, C2′), 48.4 (t, C1′), 50.0 (d, C9), 52.3 (d, C18), 53.9 (s, C17), 54.2 (d, C7′), 54.8 (d, C5), 76.6 (d, C3), 108.3 (t, C30), 150.5 (s, C20), 173.2 (s, C6′), 173.5 (s, C28). IR (ATR): $\tilde{\nu}$ = 3383 (br), 2932 (m), 2865 (m), 1614 (s), 1455 (m), 1178 (w) cm^{-1}. MS (ESI^{+}, CH_3COOH): *m/z* = 638.6 $[M+H]^{+}$. For $C_{40}H_{67}N_3O_3$ (637.99) calcd. C 75.30, H 10.59, N 6.59, found C 75.32, H 10.57, N 6.58.

Figure S25. ^{1}H NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)

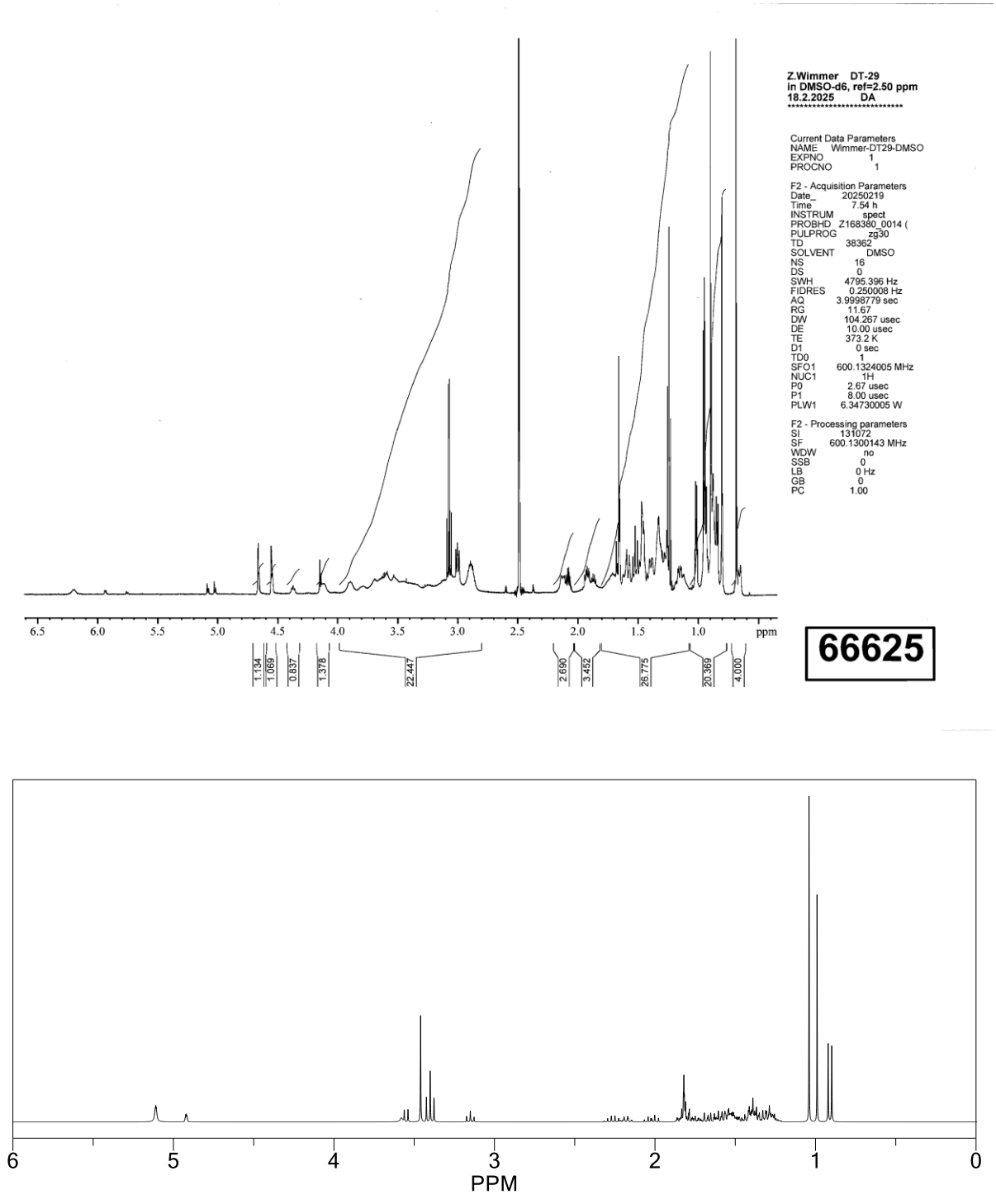
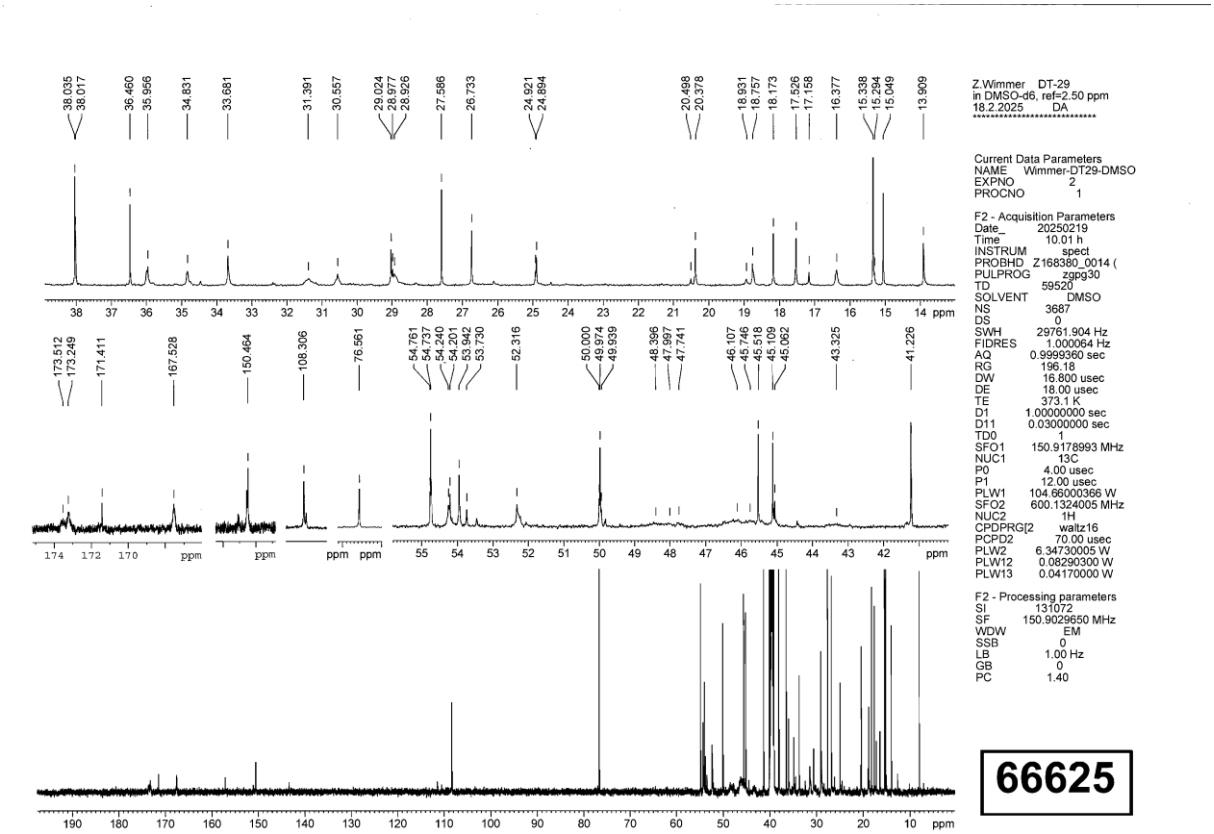
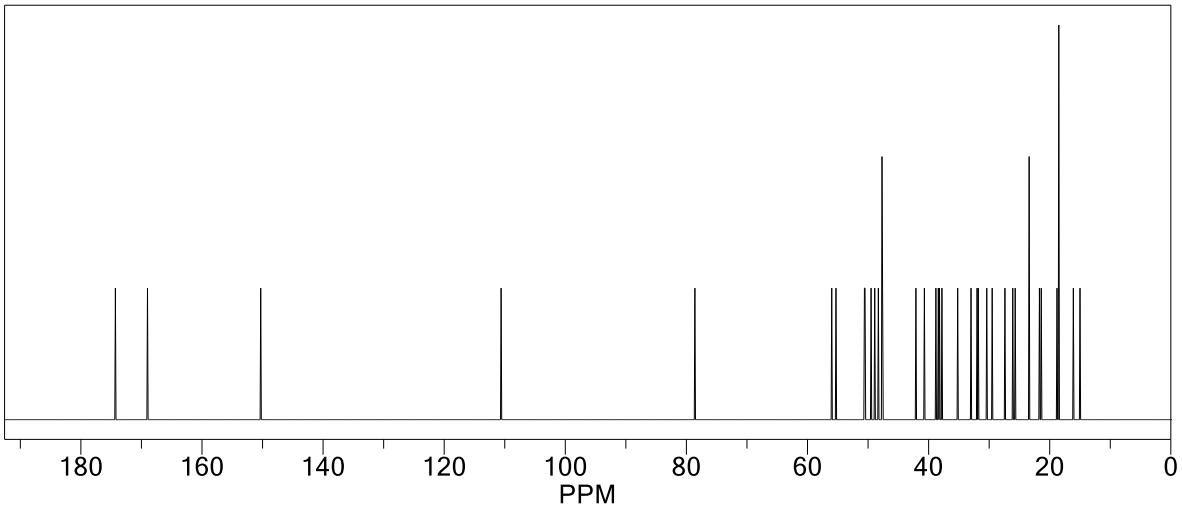


Figure S26. ^{13}C NMR spectrum measured (top) and calculated by ChemBioDraw Ultra, v. 12.0 (bottom)





1.18. The 3D-structures of the target compounds

Figure S27. The *in silico* 3D-modelling of **4**

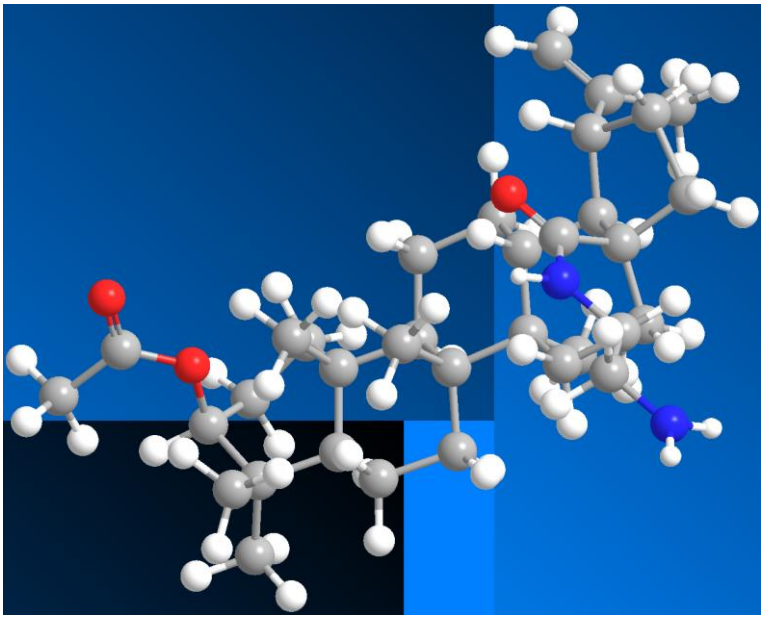


Figure S28. The *in silico* 3D-modelling of **6**

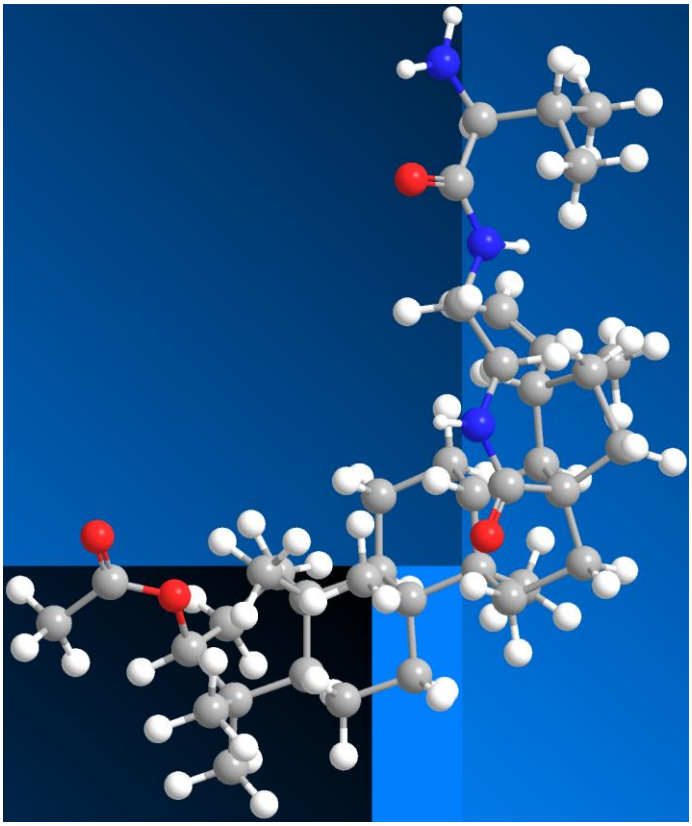


Figure S29. The *in silico* 3D-modelling of **7**

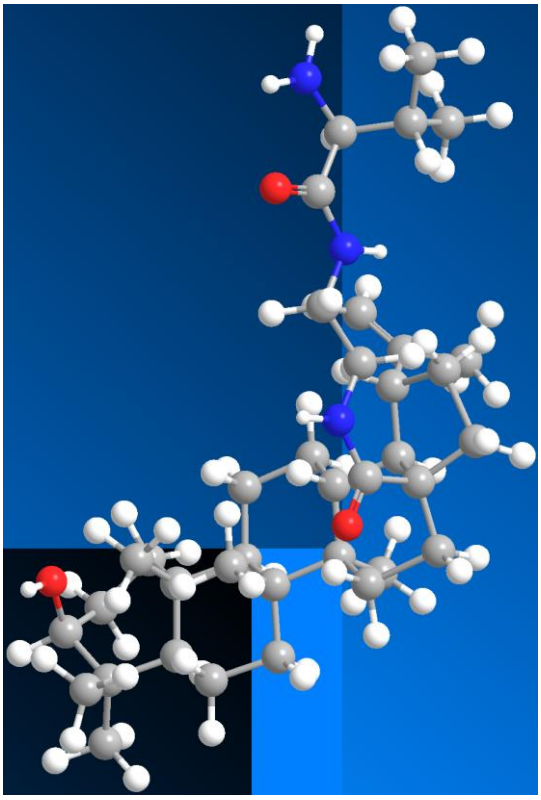


Figure S30. The *in silico* 3D-modelling of **9**

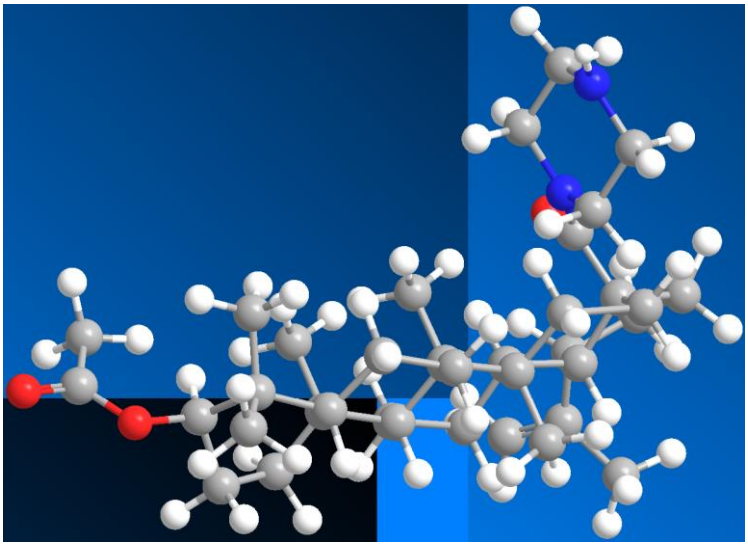


Figure S31. The *in silico* 3D-modelling of **11**

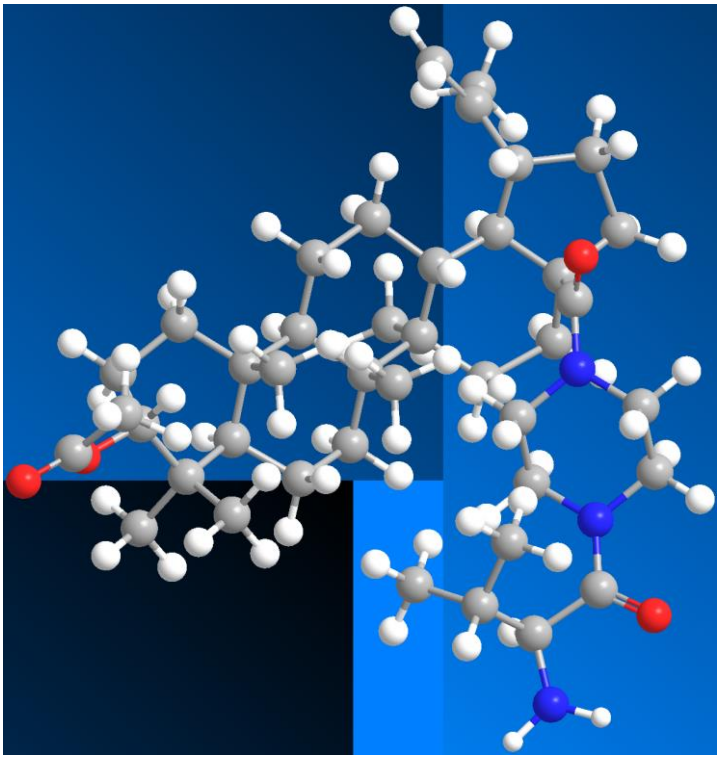


Figure S32. The *in silico* 3D-modelling of **12**

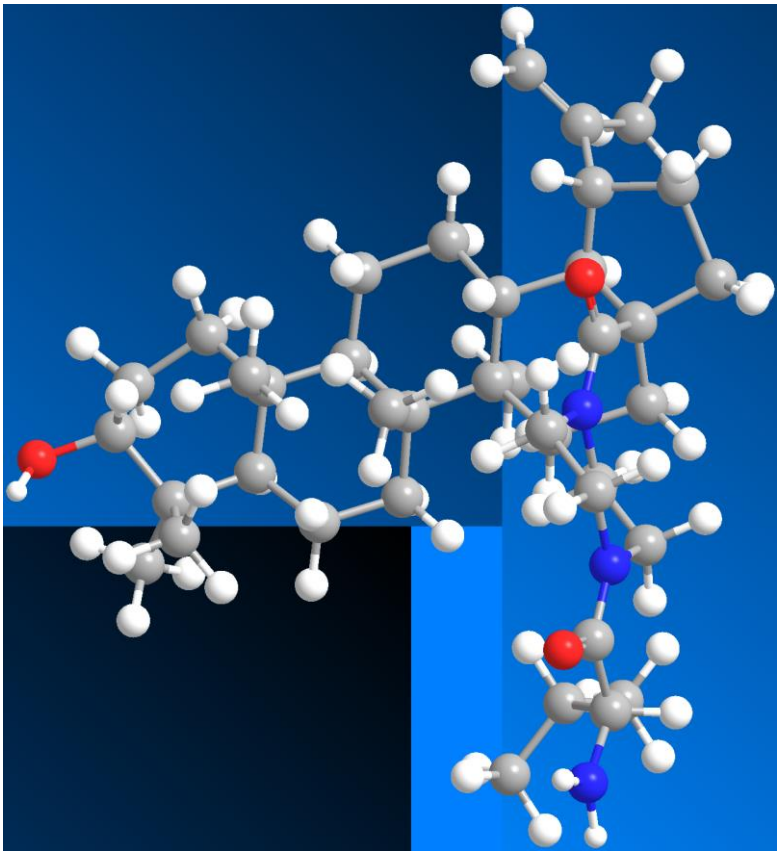


Figure S33. The *in silico* 3D-modelling of **14**

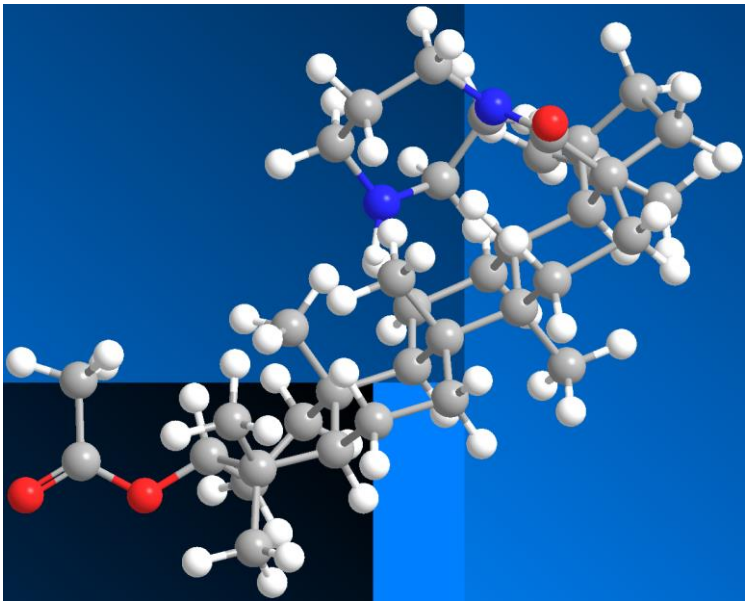


Figure S34. The *in silico* 3D-modelling of **16**

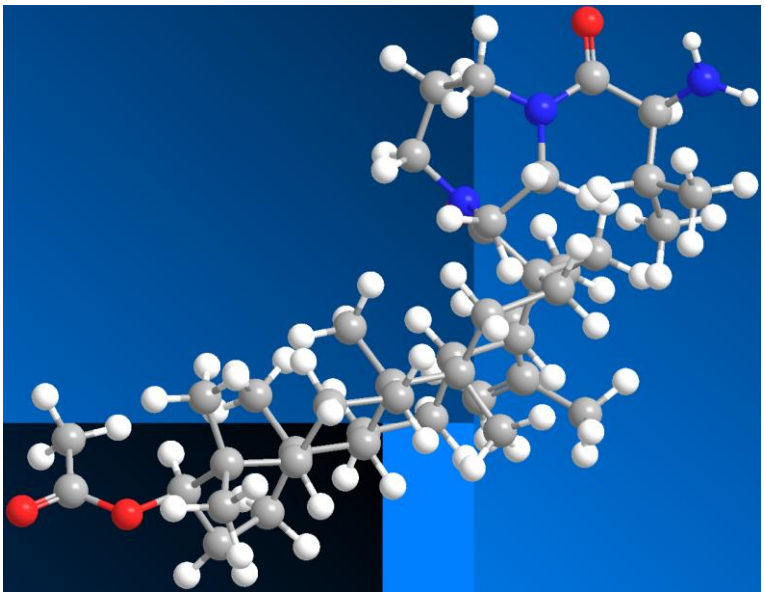
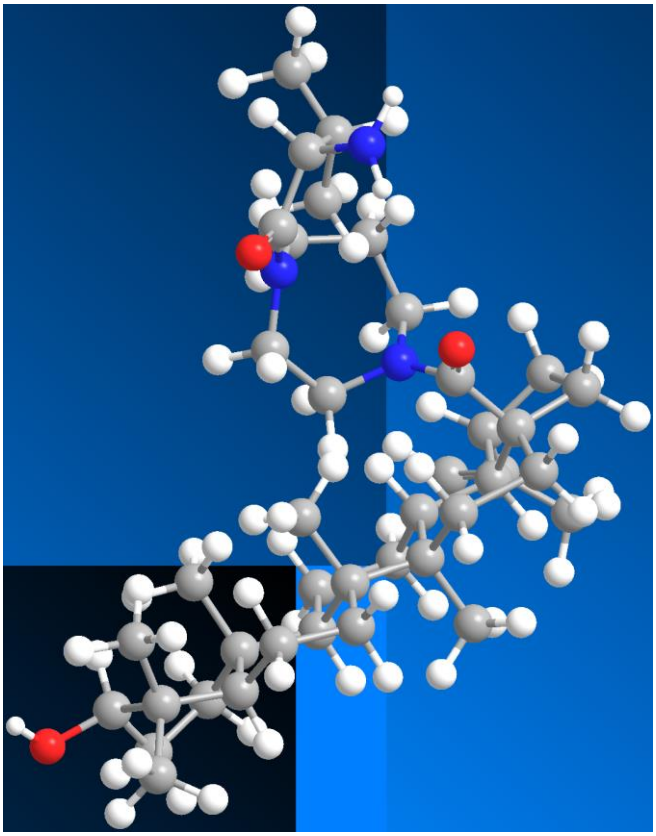


Figure S35. The *in silico* 3D-modelling of **17**



1.19. Cytotoxicity assays

All compounds subjected to the cytotoxicity assay displayed nano-assembly characteristics. Based on our results, these compounds are capable of forming nano-assemblies in the stock solutions of the studied compounds prepared for the cytotoxicity assays. Table S1 summarizes the calculated IC_{50} values obtained in different repetitions (4 to 6 for each compound) of the cytotoxicity assays. It shows that the cytotoxicity increased for most of the studied compounds with increasing time since the stock solution was prepared before being used in the cytotoxicity assays. The data indicate that a dynamic self-assembly process took place in the stock solutions of the studied compounds and affected their cytotoxicity. Because changes in the TEM images were also observed, and even if these two types of systems (the TEM imaging and the cytotoxicity assay) cannot be compared easily, a hypothesis can be presented on a formation of nano-assemblies during the cytotoxicity assays as well. This type of behaviour was observed in both tested cancer cell lines, and in normal human fibroblasts as well (Table S1). The hypothesis can assist in explaining the differences in the IC_{50} values of each studied compound in different repetitions of the biological experiments (Table S1).

Table S1. Cytotoxicity (IC_{50} [µM]) of the studied compounds in two cancer cell lines and normal cells after 72 h of exposure.

Compound	IC_{50} [µM] after 72 h					
	HeLa (repetition 1 to 6)					
6	> 50	12.45	8.16	8.37	9.00	-
7	> 50	19.64	14.08	4.92	7.72	-
11	18.95	7.83	7.42	6.74	-	-
12	> 50	6.99	4.66	3.33	3.05	-
16	3.36	8.38	7.99	6.94	7.31	-
17	> 50	12.64	7.55	3.47	3.83	-
	MCF7 (repetition 1 to 6)					
6	> 50	13.61	13.83	14.02	-	-
7	3.36	13.26	8.01	5.88	12.03	-
11	12.62	12.55	13.12	12.87	-	-
12	> 50	6.47	3.56	4.32	4.39	-
16	> 50	14.08	8.48	8.35	10.22	-
17	18.95	10.80	13.35	9.69	-	-
	BJ (repetition 1 to 6)					
6	> 50	28.81	-	7.65	8.10	-
7	> 50	23.25	-	7.22	13.57	11.02
11	> 50	30.14	-	11.00	11.54	-
12	> 50	8.46	4.18	5.55	6.73	-
16	5.76	18.53	12.93	9.37	11.01	10.67
17	> 50	13.51	3.79	5.64	13.03	4.40