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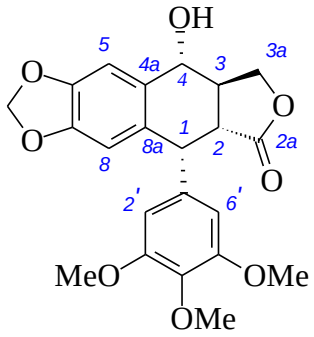
Novel bridged and caged C^4-podophyllotoxin derivatives as microtubule disruptors: synthesis, cytotoxic evaluation and structure–activity relationship

Nikolay A. Zefirov, Ann Kruth, Birgit Wobith, Evgenia V. Nurieva, Syed Riyaz, Ch. Venkata Ramana Reddy, Sergei A. Kuznetsov and Olga N. Zefirova

1. Chemistry

All reaction temperatures correspond to internal temperatures unless otherwise noted. Solvents for extraction and chromatography were technical grade and were purified by standard procedures prior to use. Flash and column chromatography were performed on silica gel Acros (40-60 μm). Reaction control was carried out by thin-layer chromatography on Silufol silica gel plates. Electron impact mass spectra were obtained with typical voltage of 70 eV. Elemental analysis was performed on CNH analyser "Carlo-Erba" ER-20. ^{1}H and ^{13}C NMR spectra were recorded on spectrometer Agilent 400-MR (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts were referenced to the solvent ($CDCl_3$, δ_H=7.24 ppm, δ_C=77.0 ppm). Chemical shifts (δ) are given in ppm, spin-spin coupling constants (*J*) are reported in Hz; multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet). Signals of atoms in podophyllotoxin fragment in NMR spectra were assigned according to [S1] using the following numbering of atoms in initial molecule [S2]:



General procedure A for the preparation of podophyllotoxin esters 3a-c, 4–7

To a solution of carboxylic acid in CH_2Cl_2 (10 ml) was added podophyllotoxin, DCC (N,N'-dicyclohexylcarbodiimide) and catalytic amount (0.01 g) of 4-dimethylaminopyridine (DMAP). The mixture was stirred at room temperature for 12 h, then acetic acid (5–10 μl) was added, and after 15 minutes the solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (5–10 ml) and maintained at 0–4°C for 2–3 h. The precipitated (crystalline or amorphous) precipitate of N,N'-dicyclohexylurea was filtered off, washed with ethyl acetate (2×10 ml). The filtrate was washed with brine (10 ml) and water (10 ml), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (ethyl acetate/petroleum ether (40–70°C) in a gradient mixture 1:8 – 1:5, unless indicated otherwise).

4-*O*-(1-Adamantylcarbonyl)-L-podophyllotoxin (3a) was synthesized by general procedure A from podophyllotoxin 0.050 g (0.12 mmol), 1-adamantanecarboxylic acid 0.027 g (0.15 mmol) and DCC 0.042 g (0.204 mmol). Yield 54% (0.037 g), white solid, m.p. 118–120°C.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.69–1.71 (3H, m, Ad), 1.76–1.79 (3H, m, Ad), 1.94 (6H, m, Ad), 2.05 (3H, m, Ad), 2.74–2.85 (1H, m, H-3), 2.94 (1H, dd, *J* = 14.5, 4.4, H-2), 3.77 (6H, s, 3',5'-OMe), 3.82 (3H, s, 4'-OMe), 4.20 (1H, dd, *J* = 10.4, 9.2, H-3a), 4.33 (1H, dd, *J*= 9.2, 7.0, H-3a), 4.61 (1H, d, *J* = 4.4, H-1), 5.86 (1H, d, *J* = 9.1, H-4), 5.99 (1H, d, *J* = 1.1, OCH_2O), 6.01 (1H, d, *J* = 1.1, OCH_2O), 6.40 (2H, s, H-2',6'), 6.55 (1H, s, H-8), 6.73 (1H, s, H-5).

^{13}C NMR ($CDCl_3$, δ): 27.78 (C-3,5,7 Ad), 36.35 (C-4,6,10 Ad), 38.83 (C-3), 38.85 (C-2,9,8 Ad), 40.83 (C-1 Ad), 43.76 (C-1), 45.52 (C-2), 56.04 (3',5'-OMe), 60.76 (4'-OMe), 71.44 (C-3a), 72.89 (C-4), 101.54 (OCH_2O), 106.87 (C-5), 107.96 (C-2',6'), 109.68 (C-8), 128.59 (C-8a), 132.31 (C-4a), 134.82 (C-1'), 137.02 (C-4'), 147.58 (C-7), 148.06 (C-6), 152.61 (C-3',5'), 173.68 (C-2a), 177.96 (Ad$\underline{C}O_2$).

MS (MALDI-TOF), *m/z*: 576 $[M]^+$, 599 $[M+Na]^+$, 615 $[M+K]^+$.

Anal. calcd. for, %: $C_{33}H_{36}O_9$, %: C 68.74; H 6.29. Found, %: C 68.70; H 6.35.

4-*O*-(3-Bromo-1-adamantylcarbonyl)-L-podophyllotoxin (3b) was synthesized by general procedure A from podophyllotoxin 0.080 g (0.193 mmol), 3-bromoadamantane-1-carboxylic acid 0.070 g (0.27 mmol) and DCC 0.68 g (0.330 mmol). Yield 33% (0.042 g), yellowish solid, m.p. 115–116°C.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.71 (1H, m, *Jgem* = 13.7, H-6Ad), 1.75 (1H, m, *Jgem* = 13.7, H-6Ad), 1.89–1.98 (4H, m, Ad), 2.25–2.37 (6H, m, Ad), 2.48 (1H, m, *Jgem* = 12.7, H-2Ad), 2.51 (1H, m, *Jgem* = 12.7, H-2Ad), 2.76–2.86 (1H, m, H-3), 2.94 (1H, dd, *J* = 14.7, 4.3, H-2), 3.78 (6H, s, 3',5'-OMe), 3.82 (3H, s, 4'-OMe), 4.19 (1H, dd, *J* = 10.2, 9.2, H-3a), 4.33 (1H, dd, *J*= 9.2, 7.0, H-3a), 4.61 (1H, d, *J* = 4.3, H-1), 5.88 (1H, d, *J* = 9.0, H-4), 6.00 (1H, d, *J* = 1.2, OCH_2O), 6.02 (1H, d, *J* = 1.2, OCH_2O), 6.40 (2H, s, H-2',6'), 6.56 (1H, s, H-8), 6.69 (1H, s, H-5).

^{13}C NMR ($CDCl_3$, δ): 31.47 (C-5Ad), 31.51 (C-7Ad), 34.32 (C-6Ad), 37.11 (C-9Ad), 37.25 (C-8Ad), 38.75 (C-3), 43.73 (C-1), 44.91 (C-1Ad), 45.48 (C-2), 47.87 (C-4Ad), 47.89 (C-10Ad), 49.44 (C-2Ad), 56.12 (3',5'-OMe), 60.75 (4'-OMe), 62.54 (C-3Ad), 71.25 (C-3a), 73.51 (C-4), 101.60 (OCH_2O), 106.73 (C-5), 107.94 (C-2',6'), 109.75 (C-8), 128.09 (C-8a), 132.41 (C-4a), 134.63 (C-1'), 137.09 (C-4'), 147.66 (C-7), 148.20 (C-6), 152.65 (C-3',5'), 173.48 (C-2a), 175.73 (Ad$\underline{C}O_2$).

MS (MALDI-TOF), m/z: 655 (M^+), 678 (M^+ + Na), 694 (M^+ + K).

Anal. calcd. for, %: $C_{33}H_{35}BrO_9$, %: C 60.46; H 5.38. Found, %: C 60.42; H 5.39.

4-*O*-(3-Noradamantylcarbonyl)-L-podophyllotoxin (3c) was synthesized by general procedure A from podophyllotoxin 0.080 g (0.19 mmol), 3-noradamantane acid 0.040 g (0.24 mmol) and DCC 0.68 g (0.330 mmol). Yield 70% (0.074 g), white solid, m.p. 128–130°C.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.61–1.69 (4H, m, norad), 1.78–1.87 (4H, m, norad), 2.08–2.11 (2H, m, norad), 2.34 (2H, m, norad), 2.69 (1H, m, *J* = 6.6, H-7norad), 2.78–2.88 (1H, m, H-3), 2.94 (1H, dd, *J* = 14.5, 4.4, H-2), 3.75 (6H, s, 3',5'-OMe), 3.82 (3H, s, 4'-OMe), 4.23 (1H, dd, *J* = 10.4, 9.2, H-3a), 4.38 (1H, dd, *J*= 9.2, 7.1, H-3a), 4.61 (1H, d, *J* = 4.4, H-1), 5.89 (1H, d, *J* = 9.1, H-4),

5.99 (1H, d, *J* = 1.2, OCH_2O), 6.01 (1H, d, *J* = 1.2, OCH_2O), 6.39 (2H, s, H-2',6'), 6.55 (1H, s, H-8), 6.76 (1H, s, H-5).

^{13}C NMR ($CDCl_3$, δ): 34.46 (norad), 37.33 (norad), 38.79 (C-3), 43.54 (norad), 43.70 (C-1), 44.33 (C-7 norad), 45.46 (C-2), 46.80 (norad), 53.76 (C-3 norad), 55.92 (3',5'-OMe), 60.69 (4'-OMe), 71.49 (C-3a), 73.11 (C-4), 101.50 (OCH_2O), 106.80 (C-5), 107.86 (C-2',6'), 109.61 (C-8), 128.64 (C-8a), 132.24 (C-4a), 134.81 (C-1'), 136.93 (C-4'), 147.56 (C-7), 148.01 (C-6), 152.55 (C-3',5'), 173.68 (C-2a), 177.84 (CO_2).

MS (MALDI-TOF), m/z: 562 (M^+), 585 (M^+ + Na), 601 (M^+ + K)

Anal. calcd. for, %: $C_{32}H_{34}O_9$, %: C 68.31; H 6.09. Found, %: C 68.27; H 6.13.

4-*O*-(Bicyclo[3.3.1]non-3-*endo*-ylcarbonyl)-L-podophyllotoxin (4) was synthesized by general procedure A from 0.040 g podophyllotoxin (0.097 mmol), bicyclo[3.3.1]nonan-3-*endo*-carboxylic acid [S3–S5] 0.020 g (0.120 mmol) and DCC 0.34 g (0.330 mmol). Yield 46% (0.025 g) waxy solid, m.p. 200–201ºC.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.20 (1H, m, *J* = 12.7, H-7bicycle), 1.41-1.74 (9H, m, bicycle), 2.10-2.44 (4H, m, bicycle), 2.64 (1H, tt, *J* = 12.4, 5.5, H-3 bicycle), 2.77-2.87 (1H, m, H-3), 2.94 (1H, dd, *J* = 14.6, 4.4, H-2), 3.78 (6H, s, 3',5'-OMe), 3.83 (3H, s, 4'-OMe), 4.22 (1H, dd, *J* = 10.4, 9.4, H-3a), 4.36 (1H, dd, *J*= 9.4, 7.3, H-3a), 4.61 (1H, d, *J* = 4.4, H-1), 5.89 (1H, d, *J* = 9.2, H-4), 5.99 (1H, d, *J* = 1.0, OCH_2O), 6.01 (1H, d, *J* = 1.0, OCH_2O), 6.41 (2H, s, H-2',6'), 6.55 (1H, s, H-8), 6.77 (1H, s, H-5).

^{13}C NMR ($CDCl_3$, δ): 15.89 (C-7 bicycle), 24.74 (C-1 bicycle), 24.79 (C-5 bicycle), 29.00 (C-9 bicycle), 29.08 (C-2 bicycle), 29.13 (C-4 bicycle), 32.77 (C-6,8 bicycle), 36.21 (C-3 bicycle), 38.78 (C-3), 43.72 (C-1), 45.49 (C-2), 56.08 (3',5'-OMe), 60.71 (4'-OMe), 71.38 (C-3a), 73.15 (C-4), 101.52 (OCH_2O), 106.87 (C-5), 108.04 (C-2',6'), 109.67 (C-8), 128.52 (C-8a), 132.25 (C-4a), 134.82 (S-1'), 137.07 (C-4'), 147.54 (C-7), 148.03 (C-6), 152.58 (C-3',5'), 173.64 (C-2a), 177.37 (CO_2).

MS (MALDI-TOF), m/z: 564 (M^+), 587 (M^+ + Na), 603 (M^+ + K).

Anal. calcd. for, %: $C_{32}H_{36}O_9$, %: C 68.07; H 6.43. Found, %: C 68.03; H 6.47.

4-*O*-{(*1RS,3SR,5SR*)-Bicyclo[3.3.1]non-6-en-3-ylcarbonyl}-L-podophyllotoxin (5) was synthesized by general procedure A from 0.080 g podophyllotoxin (0.193 mmol), *exo*-bicyclo[3.3.1]non-6-en-3-carboxylic acid [S6] 0.040 g (0.241 mmol) and DCC 0.68 g (0.330 mmol). Yield 52% (0.057 g), white crystals, m.p. 195ºC.

Spectral data are presented for a mixture of two diastereomers in the ratio ~ 1: 1. In ^{1}H and ^{13}C NMR spectra, the distinguished signals of the second diastereomer are shown in square brackets (Δδ for peaks, which are close in chemical shift, is given in Hz).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.57–1.94 (7H, m, bicycle), 2.19 (1H, m, bicycle), 2.38-2.45 (2H, m, bicycle), 2.74-2.88 (2H, m, H-3bicycle + H-3), 2.92 (1H, dd, *J* = 14.6, 4.3, H-2), 3.77 (6H, s, 3',5'-OMe), 3.82 (3H, s, 4'-OMe), 4.19 [4.19] (1H, two dd, Δδ = 1.5, *J* = 10.4, 9.3, H-3a), 4.34 [4.34] (1H, two dd, Δδ = 3.4, *J*= 9.3, 7.1, H-3a), 4.61 (1H, d, *J* = 4.3, H-1), 5.70-5.76 (1H, m, H-7bicycle), 5.84–5.88 (2H, m, H-6bicycle + H-4), 5.99 (1H, d, *J* = 1.2, OCH_2O), 6.00 (1H, d, *J* = 1.2, OCH_2O), 6.40 (2H, s, H-2',6'), 6.54 (1H, s, H-8), 6.73 [6.74] (1H, two s, Δδ = 4.8, H-5).

^{13}C NMR ($CDCl_3$, δ): 26.58 (bicycle), 28.73 [28.75] (bicycle), 30.74 (bicycle), 32.11 [32.12] (bicycle), 32.13 [32.22] (bicycle), 36.04 [36.06] (bicycle), 36.20 [36.29] (bicycle), 38.77 (C-3), 43.72 (C-1), 45.54 [45.55] (C-2), 56.09 [56.09], (Δδ = 0.7, 3',5'-OMe), 60.72 (4'-OMe), 71.34 [71.36] (C-3a), 73.00 [73.02] (C-4), 101.53 (OCH_2O), 106.97 [106.98] (C-5), 108.04 (C-2',6'), 109.65 (C-8), 128.50 [128.51] (C-8a), 129.67 [129.68] (C-7bicycle), 130.09 [130.10] (C-6bicycle), 132.29 (C-4a), 134.78 (C-1'), 137.07 (C-4'), 147.50 (C-7), 148.02 (C-6), 152.57 (C-3',5'), 173.64 [173.65] (C-2a), 177.23 (CO_2).
MS (MALDI-TOF), m/z: 562 (M^+), 585 (M^+ + Na), 601 (M^+ + K)
Anal. calcd. for, %: $C_{32}H_{34}O_9$, %: C 68.31; H 6.09. Found, %: C 68.25; H 6.05.

4-*O*-{(*2RS*)-Bicyclo[2.2.2]octan-2-ylcarbonyl}-L-podophyllotoxin (6) was synthesized by general procedure A from podophyllotoxin 0.080 g (0.193 mmol), bicyclo[2.2.2]octane-2-carboxylic acid 0.037 g (0.220 mmol) and DCC 0.68 g (0.330 mmol). Yield 70% (0.074 g), white solid, m.p. 129–130°C.
Spectral data are presented for a mixture of two diastereomers in the ratio ~ 1:1.
^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.47-1.77 (10H, m, bicycle), 1.88-2.07 (2H, m, bicycle), 2.65-2.87 (2H, m, H-2 bicycle + H-3), 2.92 (1H, dd, *J* = 14.7, 4.4, H-2), 3.75 [3.76] (6H, two s, Δδ = 3.7, 3',5'-OMe), 3.81 (3H, s, 4'-OMe), 4.23 [4.23] (1H, two dd, Δδ = 3.7, *J* = 10.4, 9.3, H-3a), 4.34 [4.37] (1H, two dd, Δδ = 14.9, *J*= 9.3, 7.1, H-3a), 4.61 (1H, d, *J* = 4.4, H-1), 5.87 [5.91] (1H, two d, Δδ = 12.7, *J* = 9.0, H-4), 5.98 (1H, d, *J* = 1.3, OCH_2O), 5.99 (1H, d, *J* = 1.3, OCH_2O), 6.39 [6.40] (2H, two s, Δδ = 3.7, H-2',6'), 6.54 [6.54] (1H, two s, Δδ = 2.4, H-8), 6.71 [6.77] (1H, two s, H-5).
^{13}C NMR ($CDCl_3$, δ): 21.96 (C-6 bicycle), 23.46 [23.47] (C-4 bicycle), 24.82 [24.83] (C-8 bicycle), 25.03 [25.10] (C-5 bicycle), 26.02 [26.11] (C-7bicycle), 27.57 [27.79] (C-1bicycle), 27.98 [28.19] (C-3bicycle), 38.83 [38.87] (C-3), 41.84 [41.98] (H-2 bicycle), 43.72 (C-1), 45.51 [45.56] (C-2), 56.03 [56.08] (3',5'-OMe), 60.71 (4'-OMe), 71.43 [71.50] (C-3a), 73.28 [73.35] (C-4), 101.53 (OCH_2O), 106.88 [107.06] (C-5), 107.99 [108.04] (C-2',6'), 109.61 [109.67] (C-8), 128.51 [128.60] (C-8a), 132.24 [132.29] (C-4a), 134.79 [134.81] (C-1'), 137.03 [137.06] (C-4'), 147.52 (C-7), 148.00 (C-6), 152.58 (C-3',5'), 173.61 [173.68] (C-2a), 176.57 [176.60] (CO_2).
MS (MALDI-TOF), m/z: 550 (M^+), 573 (M^+ + Na), 589 (M^+ + K).
Anal. calcd. for, %: $C_{31}H_{34}O_9$, %: C 67.62; H 6.22. Found, %: C 67.56; H 6.26.

4-*O*-{(*1RS,2SR,4RS*)-Bicyclo[2.2.1]hept-5-en-2-ylcarbonyl}-L-podophyllotoxin (7) was synthesized by general procedure A from podophyllotoxin 0.080 g (0.193 mmol), *exo*-5-norbornene-2-carboxylic 0.033 g (0.239 mmol) acid and DCC 0.68 g (0.330 mmol). Yield 52% (0.054 g), white solid, m.p. 110–112ºC.
Spectral data are presented for a mixture of two diastereomers in the ratio ~ 1:1.
^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 1.41–1.57 (3H, m, H-3*endo*,7*endo*,7*exo*-norb), 1.91 [1.99] (1H, two ddd, *J* = 11.6, 4.3, 3.8, H-3*exo*-norb), 2.33 [2.36] (1H, two ddd, *J* = 8.7, 4.3, 1.3, H-2norb), 2.78-2.89 (1H, m, H-3), 2.94 [2.94] (1H, two dd, Δδ = 1.7, *J* = 14.6, 4.3, H-2), 2.97–2.99 (1H, m, H-4norb), 3.03 [3.14] (1H, two m, H-1norb), 3.77 [3.78] (6H, two s, Δδ = 4.0, OMe), 3.82 [3.83] (3H, two s, Δδ = 2.5, OMe), 4.22 (1H, dd, *J* = 10.4, 9.3, H-3a), 4.36 [4.37] (1H, two dd, Δδ = 3.9, *J*= 9.3,

7.0, H-3a), 4.61 (1H, d, *J* = 4.3, H-1), 5.91 (1H, d, *J* = 9.0, H-4), 5.99 [6.00] (1H, two d, Δδ = 3.3, *J* = 1.3, OCH_2O), 6.00 [6.01] (1H, two d, Δδ = 3.6, *J* = 1.3, OCH_2O), 6.12 [6.15] (1H, two dd, Δδ = 12.5, *J*= 5.7, 3.0, H-5norb), 6.19 (1H, dd, *J*= 5.7, 2.8, H-6norb), 6.41 [6.41] (2H, two s, Δδ = 2.8, H-2',6'), 6.55 [6.55] (1H, two s, Δδ = 1.3, H-8), 6.78 [6.79] (1H, two s, Δδ = 5.8, H-5).

^{13}C NMR ($CDCl_3$, δ): 30.43 [30.65] (S-3norb), 38.79 [38.84] (C-3), 41.64 (S-4norb), 43.23 (S-2norb), 43.71 (C-1), 45.51 (C-2), 46.35 (S-7norb), 46.68 [46.75] (C-1norb), 56.07 (3',5'-OMe), 60.70 (4'-OMe), 71.34 [71.39] (C-3a), 73.41 [73.35] (C-4), 101.54 (OCH_2O), 106.95 (C-5), 108.02 (C-2',6'), 109.67 (C-8), 128.44 (C-8a), 132.19 [132.32] (C-4a), 134.78(S-1'), 135.37 [134.47] (S-6norb), 137.07 (C-4'), 138.23 (S-5norb), 147.55 (C-7), 148.07 (C-6), 152.59 (C-3',5'), 173.62 (C-2a), 176.68 (CO_2).

MS (MALDI-TOF), m/z: 534 (M^+), 557 (M^+ + Na), 573 (M^+ + K).

Anal. calcd. for, %: $C_{30}H_{30}O_9$, %: C 67.41; H 5.66. Found, %: C 67.37; H 5.70.

4-*O*-(Adamantan-1-ylmethyl)-L-podophyllotoxin (α-8) and 4-*O*-(adamant-1-ylmethyl)-L-epipodophyllotoxin (β-8). To a stirred and cooled to 0°C solution of 0.070 g (0.169 mmol) podophyllotoxin in CH_2Cl_2 (4 ml) was added BF_3•Et_2O (31 μl, 0.252 mmol). In 5 min (adamantan-1-yl)methanol (0.042 g, 0.253 mmol) was added, and the mixture was stirred at room temperature for 6 h. The reaction mass was diluted with water (20 ml) and extracted with CH_2Cl_2 (3×20 ml). The organic layers were combined, dried over Na_2SO_4 and evaporated. The residue was subjected to column chromatography [ethyl acetate/petroleum ether (40–70°C) in a gradient mixture 1:6 – 1:4] to give sequentially **α-8** (0.022 g, yield 23%), **β-8** (0.010 g, yield 11%) and **8 α+β** 3:2 mixture (according to ^{1}H NMR data, 0.025 g, yield 28%); transparent oily liquids.

α-8: ^{1}H NMR ($CDCl_3$, δ): 1.58 (6H, m, Ad), 1.65–1.68 (3H, m, Ad), 1.74-1.77 (3H, m, Ad), 2.00 (3H, m, Ad), 2.83 (1H, dd, *J* = 14.1, 4.6, H-2), 2.90-3.00 (1H, m, H-3), 3.04 (1H, d, *J* = 8.8, AdCH_2O), 3.05 (1H, d, *J* = 8.8, AdCH_2O), 3.75 (6H, s, 3',5'-OMe), 3.83 (3H, s, 4'-OMe), 4.10 (1H, dd, *J* = 9.7, 8.2, H-3a), 4.57 (1H, d, *J* = 4.6, H-1), 4.58 (1H, dd, *J*= 8.2, 7.6, H-3a), 4.62 (1H, d, *J* = 9.2, H-4), 5.97 (1H, d, *J* = 1.0, OCH_2O), 6.00 (1H, d, *J* = 1.0, OCH_2O), 6.38 (2H, s, H-2',6'), 6.51 (1H, s, H-8), 7.05 (1H, s, H-5).

β-8: ^{1}H NMR ($CDCl_3$, δ): 1.52 (6H, m, Ad), 1.62-1.65 (3H, m, Ad), 1.68-1.71 (3H, m, Ad), 1.96 (3H, m, Ad), 2.83-2.92 (1H, m, H-3), 3.03 (1H, d, *J* = 8.3, AdCH_2O), 3.24 (1H, d, *J* = 8.8, AdCH_2O), 3.43 (1H, dd, *J* = 13.9, 5.3, H-2), 3.74 (6H, s, 3',5'-OMe), 3.81 (3H, s, 4'-OMe), 4.34 (1H, dd, *J* = 8.1, 7.8, H-3a), 4.35 (1H, d, *J* = 4.9, H-4), 4.41 (1H, dd, *J*= 8.1, 10.8, H-3a), 4.61 (1H, d, *J* = 5.3, H-1), 5.99 (1H, d, *J* = 1.1, OCH_2O), 6.00 (1H, d, *J* = 1.1, OCH_2O), 6.26 (2H, s, H-2',6'), 6.55 (1H, s, H-8), 6.79 (1H, s, H-5).

^{13}C NMR ($CDCl_3$, δ, chemical shifts for isomer **β-8** are shown in brackets): 28.17 [28.15] (C-3,5,7Ad), 34.17 [34.46] (C-1Ad), 37.09 [37.10] (C-4,6,10Ad), 38.64 [38.30] (C-3), 39.79 [39.85] (C-2,8,9Ad), 44.02 [43.98] (C-1), 45.51 [41.27] (C-2), 56.02 [56.24] (3',5'-OMe), 60.74 (4'-OMe), 71.60 [67.52] (C-3a), 77.98 [79.40] (AdCH_2O), 81.12 [74.36] (C-4), 101.31 [101.38] (OCH_2O), 107.16 [110.76] (C-5), 107.94 [108.25] (C-2',6'), 109.53 [109.56] (C-8), 131.07 [129.70] (C-4a), 131.77 [132.16] (C-8a), 135.42 [135.59] (C-1'), 137.85 [138.33] (C-4'), 147.46 [146.46] (C-7), 147.61 [148.12] (C-6), 152.53 [152.48] (C-3',5'), 174.20 [175.26] (C=O).

MS (MALDI-TOF), m/z: 562 (M^+), 585 (M^+ + Na), 601 (M^+ + K).

Found, %: C 70.39; H 6.86. Anal. calcd. for, %: $C_{33}H_{38}O_8$, %: C 70.44; H 6.81.

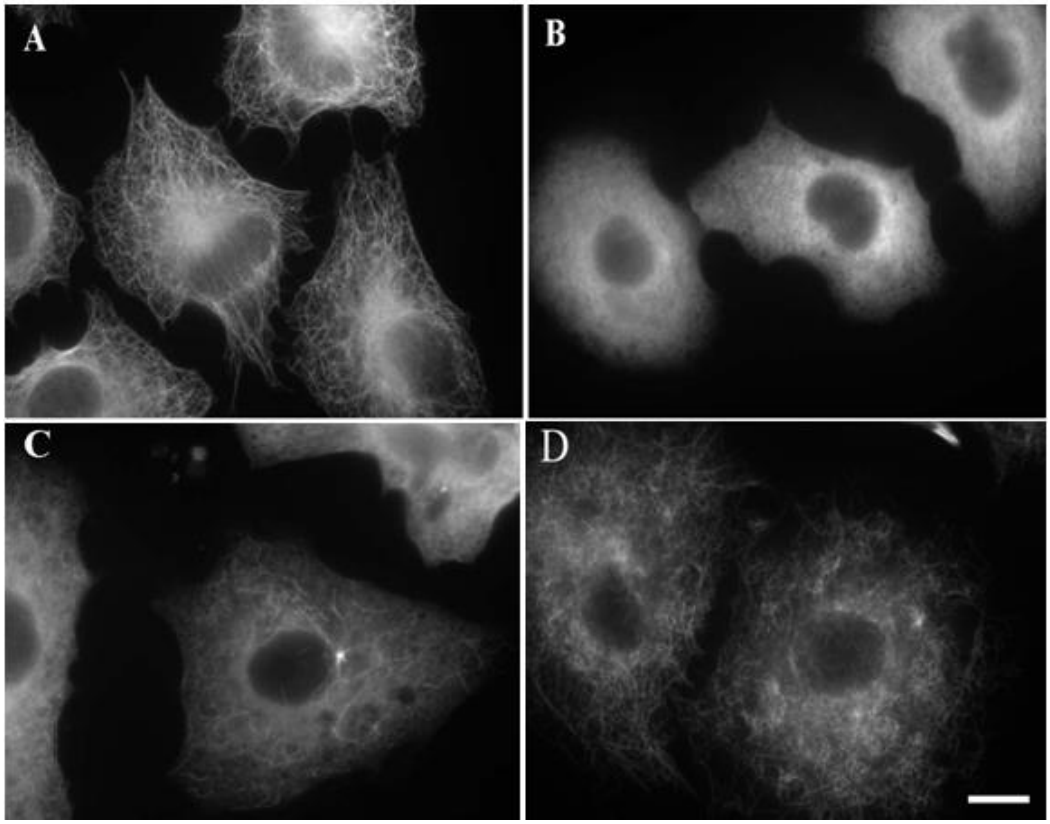
2. Biological tests

Cell culture. A549 human lung epithelial carcinoma cells (CCL-185™) were cultured with Dulbecco´s Modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic penicillin/streptomycin at 37 °C under a 5% CO_2 humidified atmosphere.

MTT Cytotoxicity Assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide, Roth GmbH, Karlsruhe, Germany) quantitative colorimetric assay was used to measure the cytotoxicity, viability and metabolic activity [S7]. The A549 cells were seeded in 96-well plates at a density of 8000 cells per well. Stock solutions of test compounds were prepared in DMSO at concentration 20 mM. Cells were treated with selected compounds at 1–50000 nM (8 wells for each concentration) for 24 h. DMSO (0.5%) was used as a negative control. Optical density was measured at 550 nm with 690 nm reference filter using a EL808 Ultra Microplate Reader (BioTek Instruments, Winooski, USA). Experiments for all compounds were repeated 3–6 times and EC_{50} values were determined by sigmoid curve fitting using Excel-based software.

Proliferation assay. A549 cells were incubated with 1, 10 and 100 μM of each tested compound during 24 and 48 hours. 0.5% DMSO was used as a control. After culturing the cells were resuspened in PBS and counted directly by phase-contract microscopy using hemocytometer.

Immunofluorescence staining of cellular microtubules and nuclei. A549 cells were cultured in 12-well plates on small glass coverslips (11 mm diameter) at a density of 20000 cells per coverslip. Cells were incubated with tested compounds at concentrations of 1, 10 and 100 μM for 24 and 48 hours. 0.5 % DMSO served as a negative control. The cells were fixed and stained as described in [S8]. Fixed cells were labelled for tubulin with mouse monoclonal antibody against α-tubulin at a dilution of 1:300 (Sigma, St. Louis. USA), followed by incubation of Alexa Fluor488 labelled goat anti-mouse IgG at a dilution of 1:300 (Invitrogen, Germany). In order to analyze the compound effect on the apoptosis induction effect, the cell nuclei were stained with the hoechst Nr. 33258 (Sigma, St. Louis. USA) at concentration 5 μg/ml. Images of all samples were acquired with a Nikon Diaphot 300 inverted microscope (Nikon GmbH, Düsseldorf, Germany) equipped with a cooled charge-couple device camera system (SenSys; Photometrics, Munich, Germany).



Immunofluorescence microscopy images of the microtubules in human epithelial lung carcinoma cells A549 treated with: A) 0.5% DMSO (negative control, intact microtubules); B) 1 μM of podophyllotoxin (positive control; complete depolymerization of microtubule cytoskeleton); C) 100μM of compound **3c**. Shortening and weak "curling" of microtubules; the rests of microtubules around centrosomes are seen as star-like structures. D) 100μM of compound **3a**. "Curling" of microtubules. Scale 20 μm.

Apoptotic index. Apoptotic index was defined as a percentage that refers to the indicated cell deaths in comparison to all cells [S9]. A549 cells were incubated with 10 or 100 μM of each tested compound during 24 and 48 hours. 0.5% DMSO was used as a control. After incubation the cells were fixed and stained with Hoechst. Since a main feature of apoptotic cell is a fragmentation of the nucleus [S10], the number of cells containing micronuclei related the total 200 cells was counted using fluorescent microscopy.

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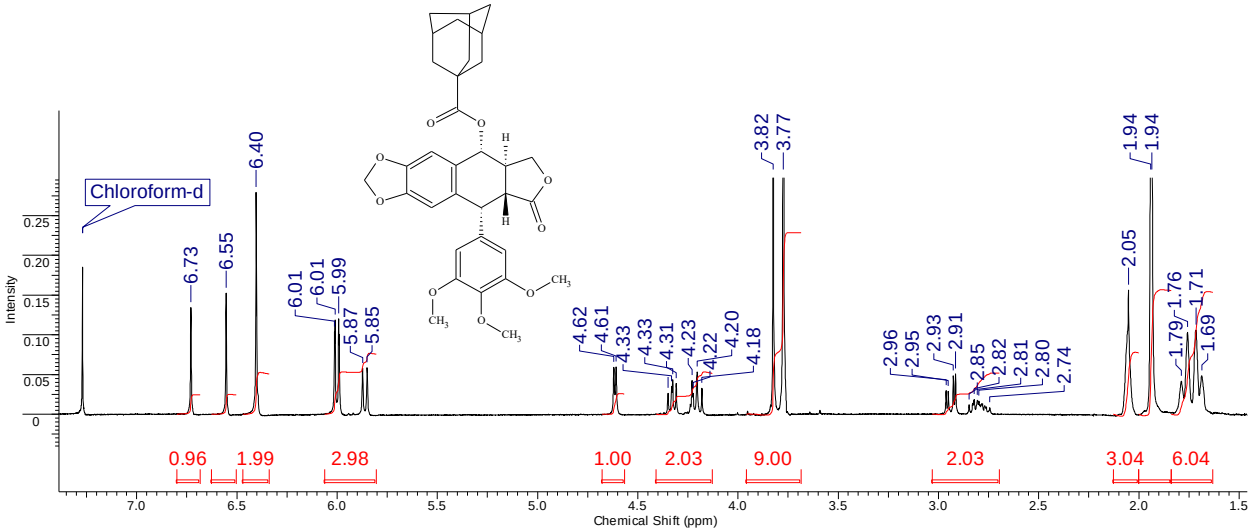
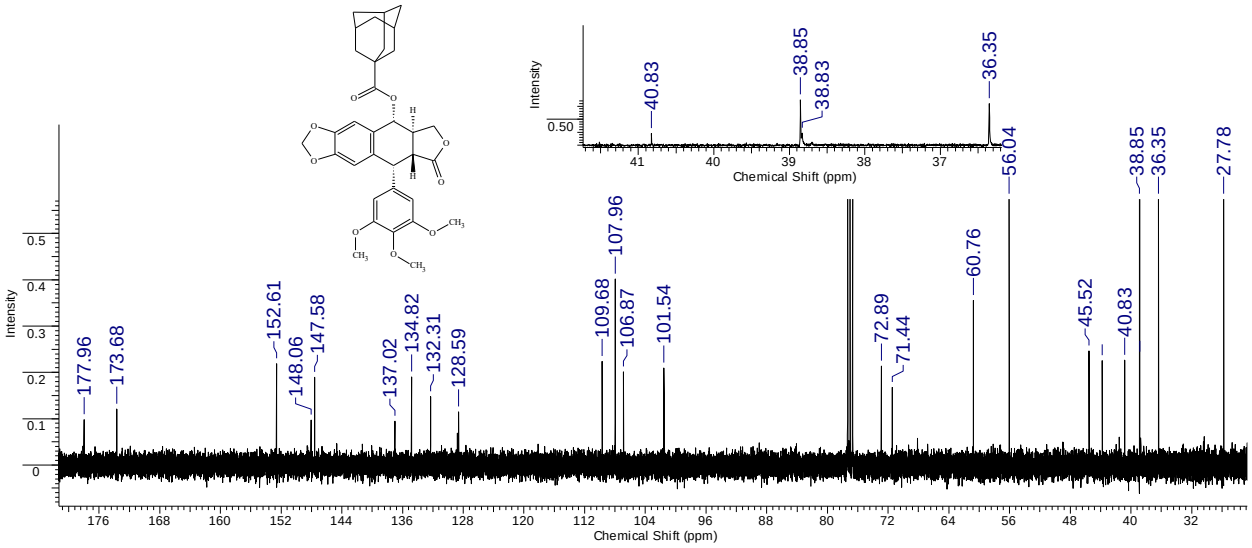
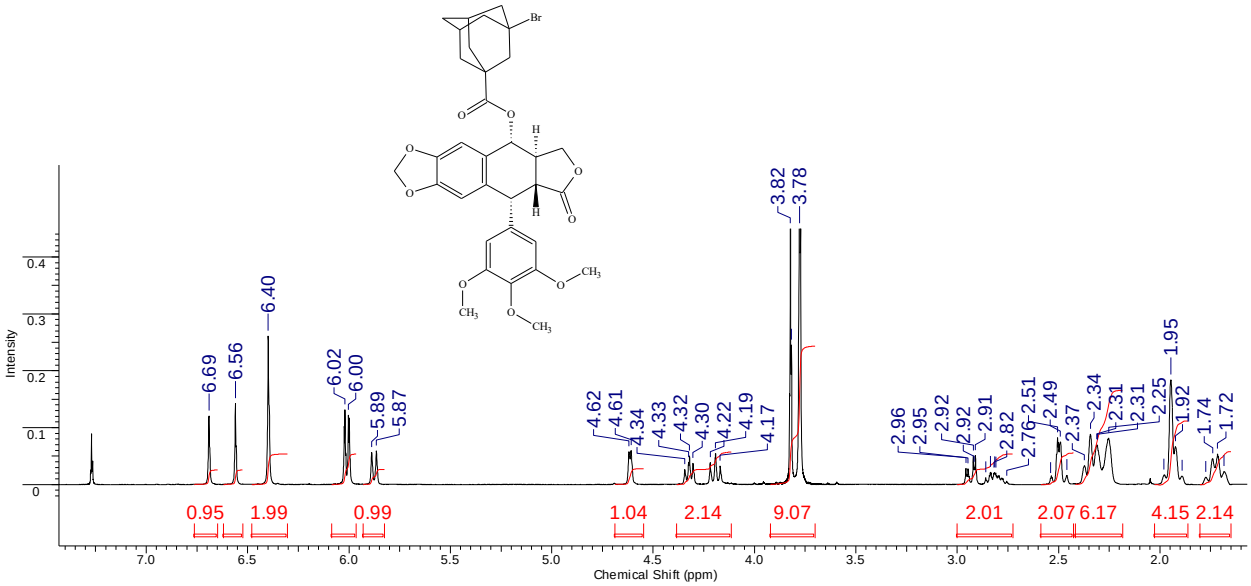
^{1}H NMR spectrum of 4-O-(1-adamantylcarbonyl)-L-podophyllotoxin (3a)



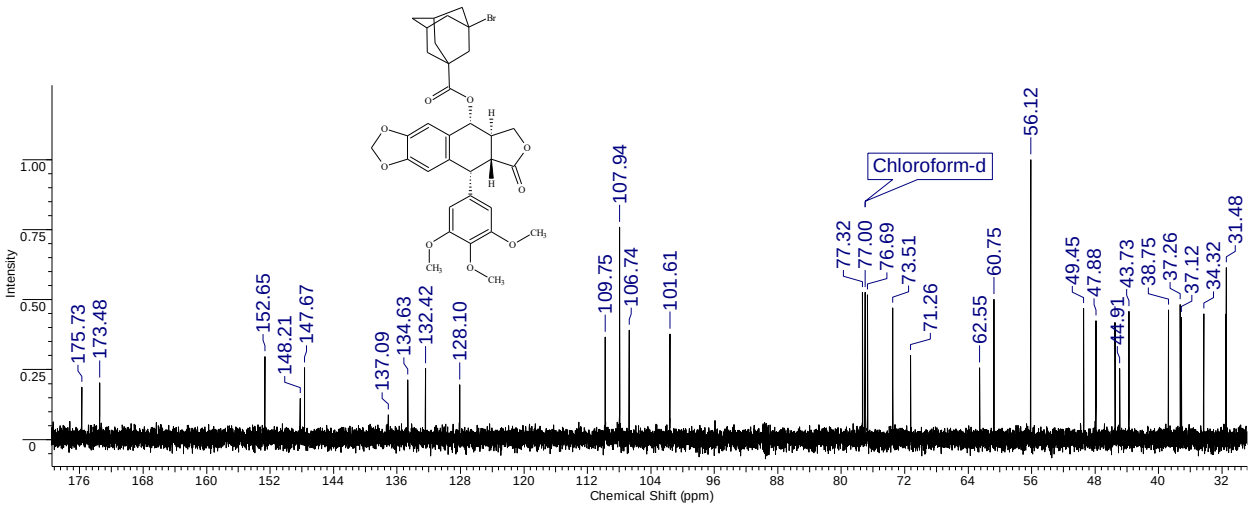
^{13}C NMR spectrum of 4-O-(1-adamantylcarbonyl)-L-podophyllotoxin (3a)



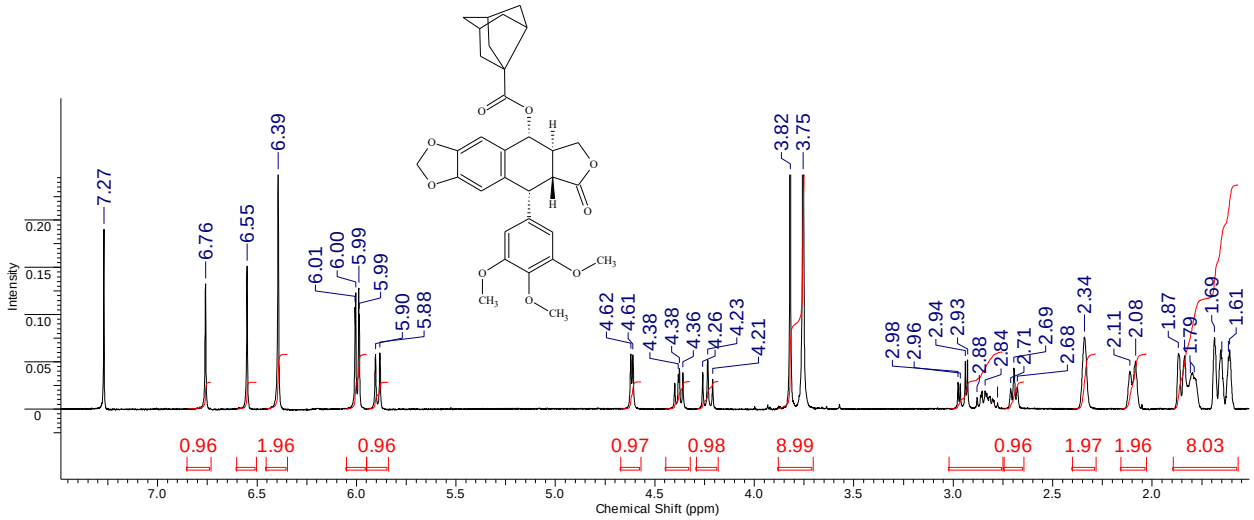
^{1}H NMR spectrum of 4-O-(3-bromo-1-adamantylcarbonyl)-L-podophyllotoxin (3b)



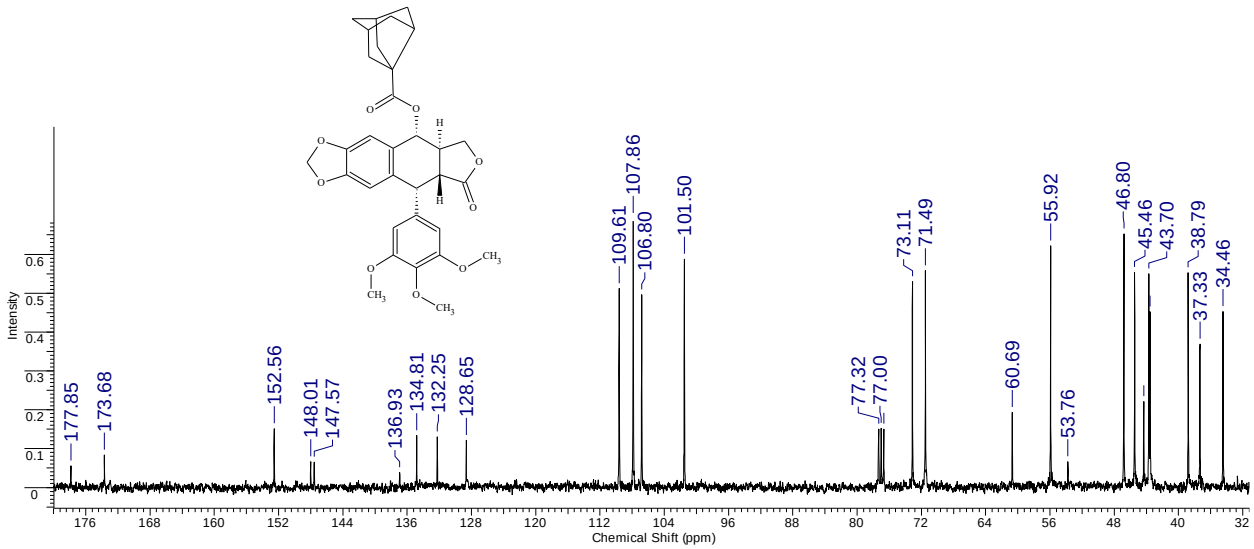
^{13}C NMR spectrum of 4-O-(3-bromo-1-adamantylcarbonyl)-L-podophyllotoxin (3b)



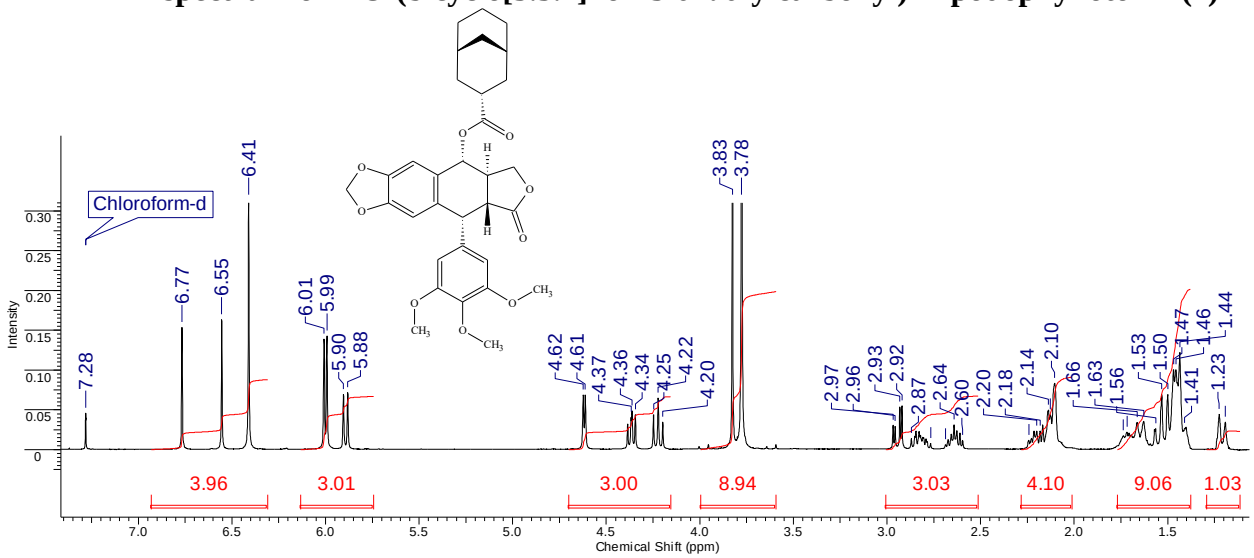
^{1}H NMR spectrum of 4-O-(3-noradamantylcarbonyl)-L-podophyllotoxin (3c)



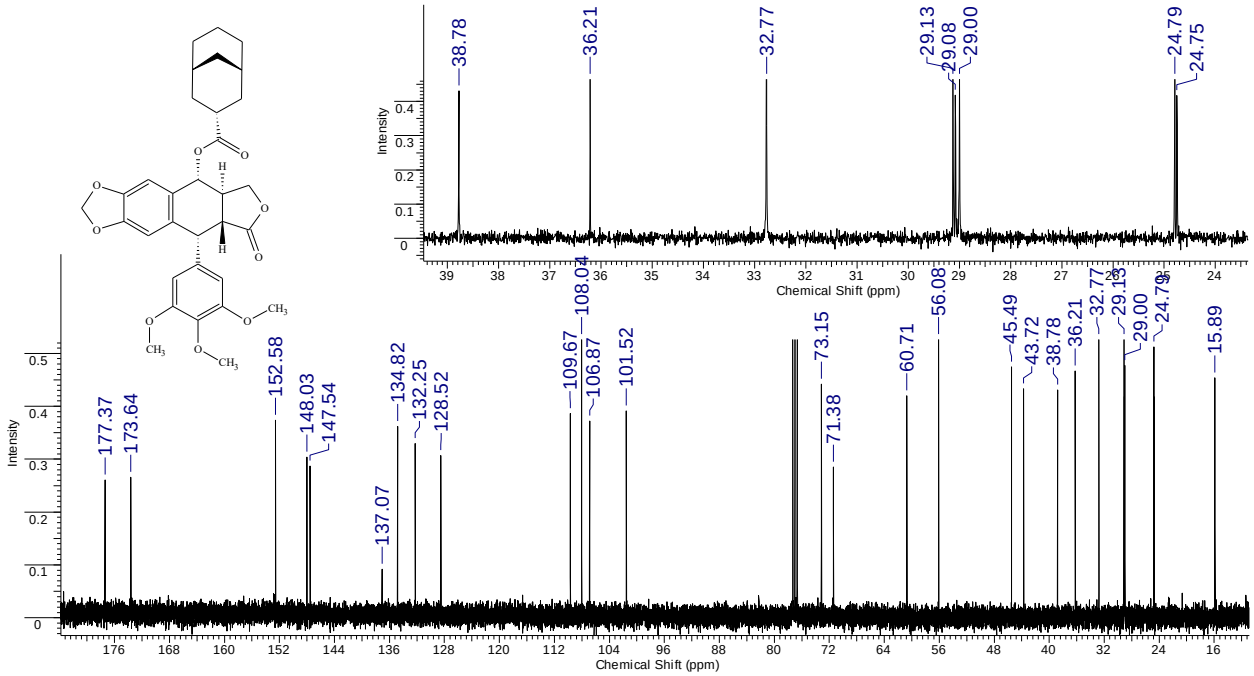
^{13}C NMR spectrum of 4-O-(3-noradamantylcarbonyl)-L-podophyllotoxin (3c)



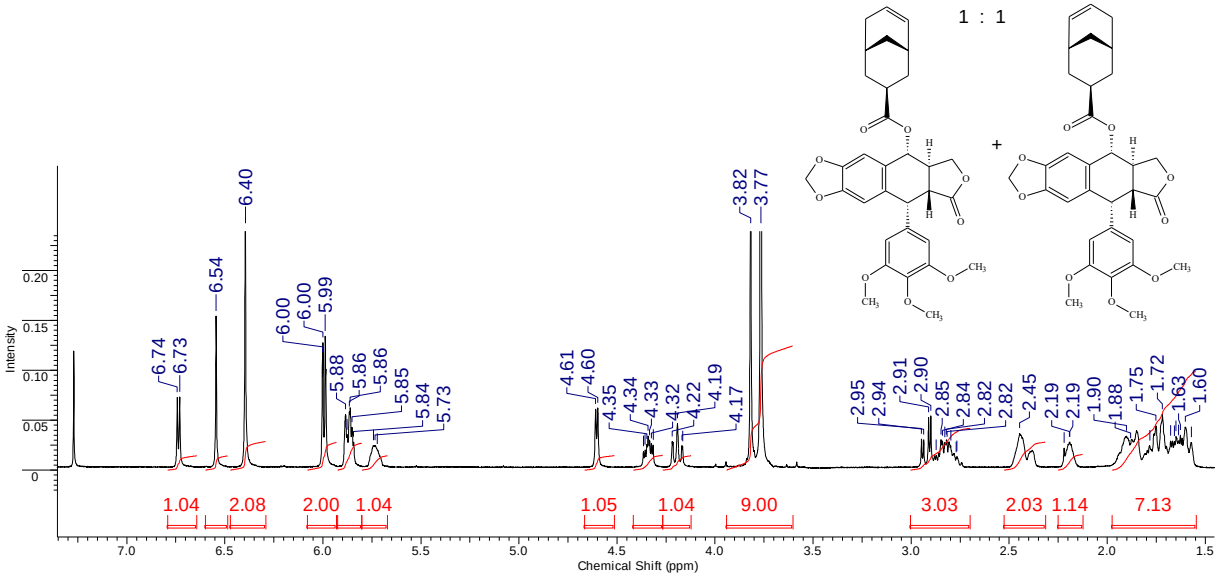
^{1}H NMR spectrum of 4-O-(bicyclo[3.3.1]non-3-*endo*-ylcarbonyl)-L-podophyllotoxin (4)



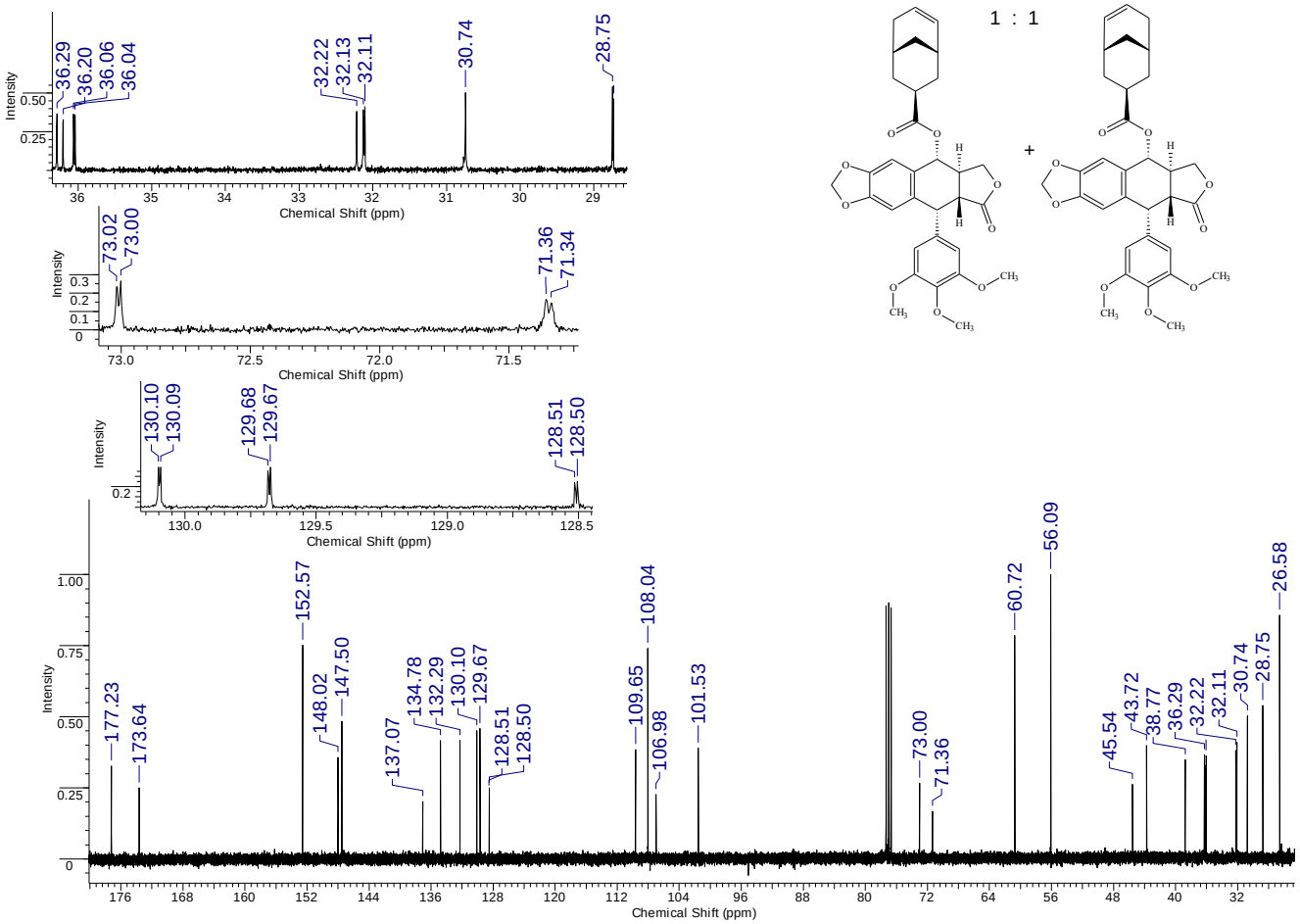
^{13}C NMR spectrum of 4-O-(bicyclo[3.3.1]non-3-*endo*-ylcarbonyl)-L-podophyllotoxin (4)



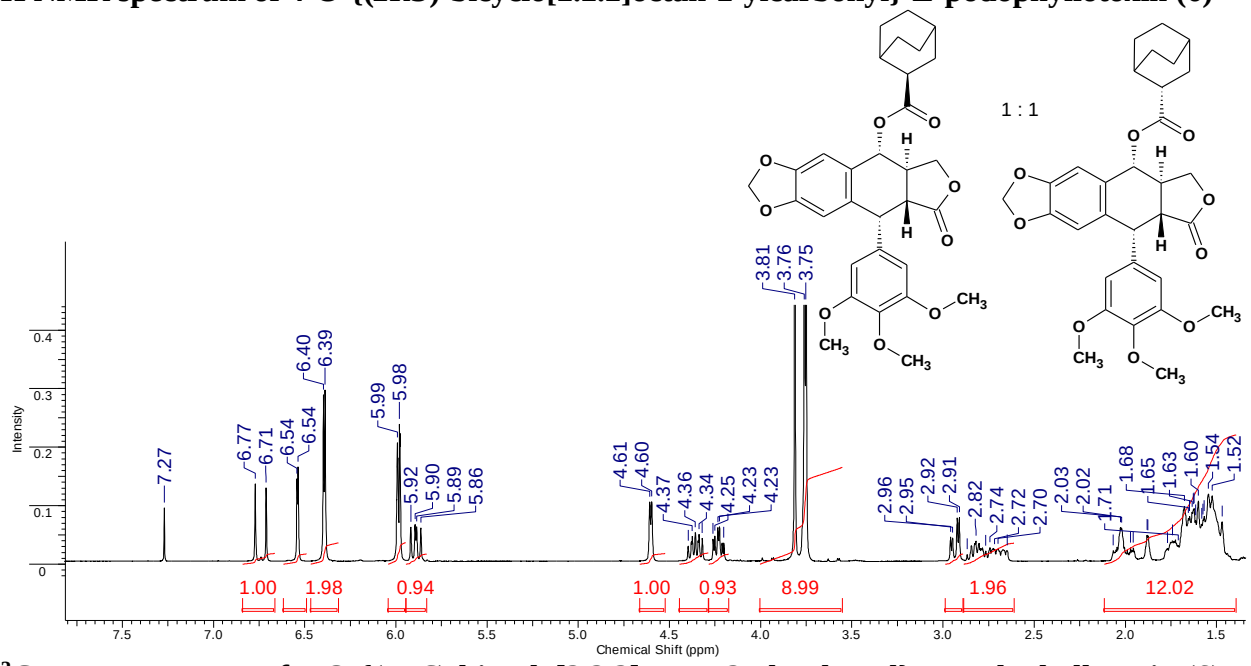
^{1}H NMR spectrum of 4-O-{(*1RS,3SR,5SR*)-bicyclo[3.3.1]non-6-en-3-ylcarbonyl}-L-podophyllotoxin (5)



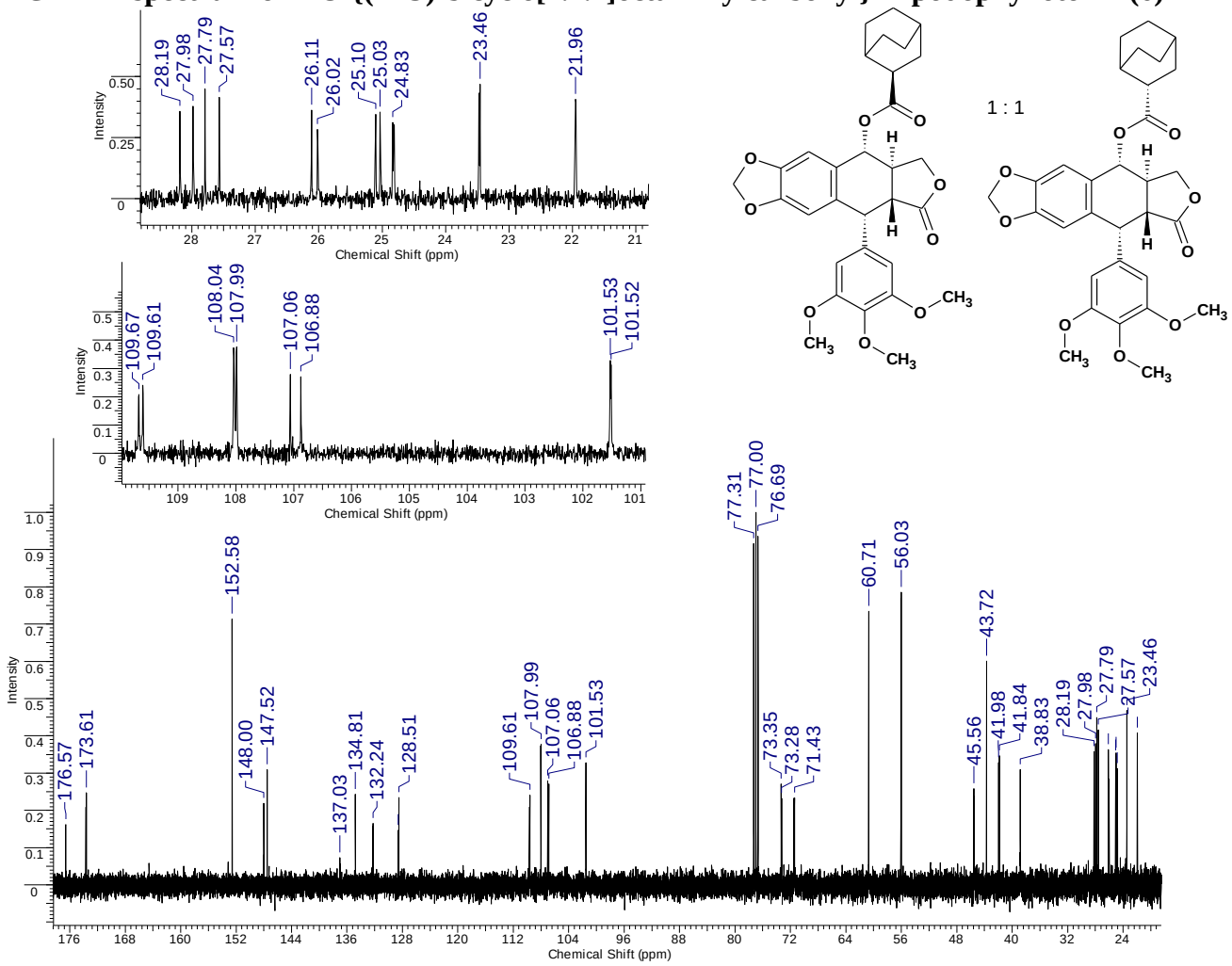
^{13}C NMR spectrum of 4-O-{(*1RS,3SR,5SR*)-bicyclo[3.3.1]non-6-en-3-ylcarbonyl}-L-podophyllotoxin (5)



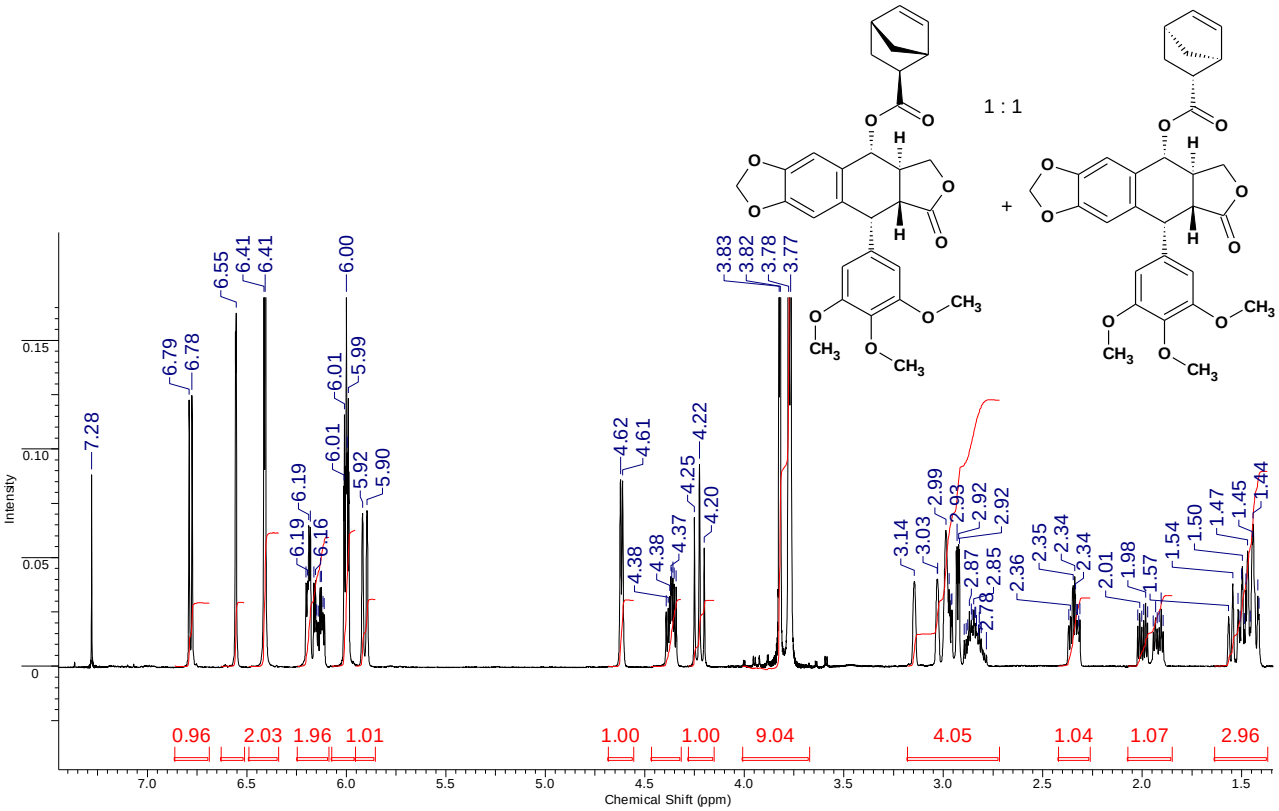
^{1}H NMR spectrum of 4-O-{(*2RS*)-bicyclo[2.2.2]octan-2-ylcarbonyl}-L-podophyllotoxin (6)



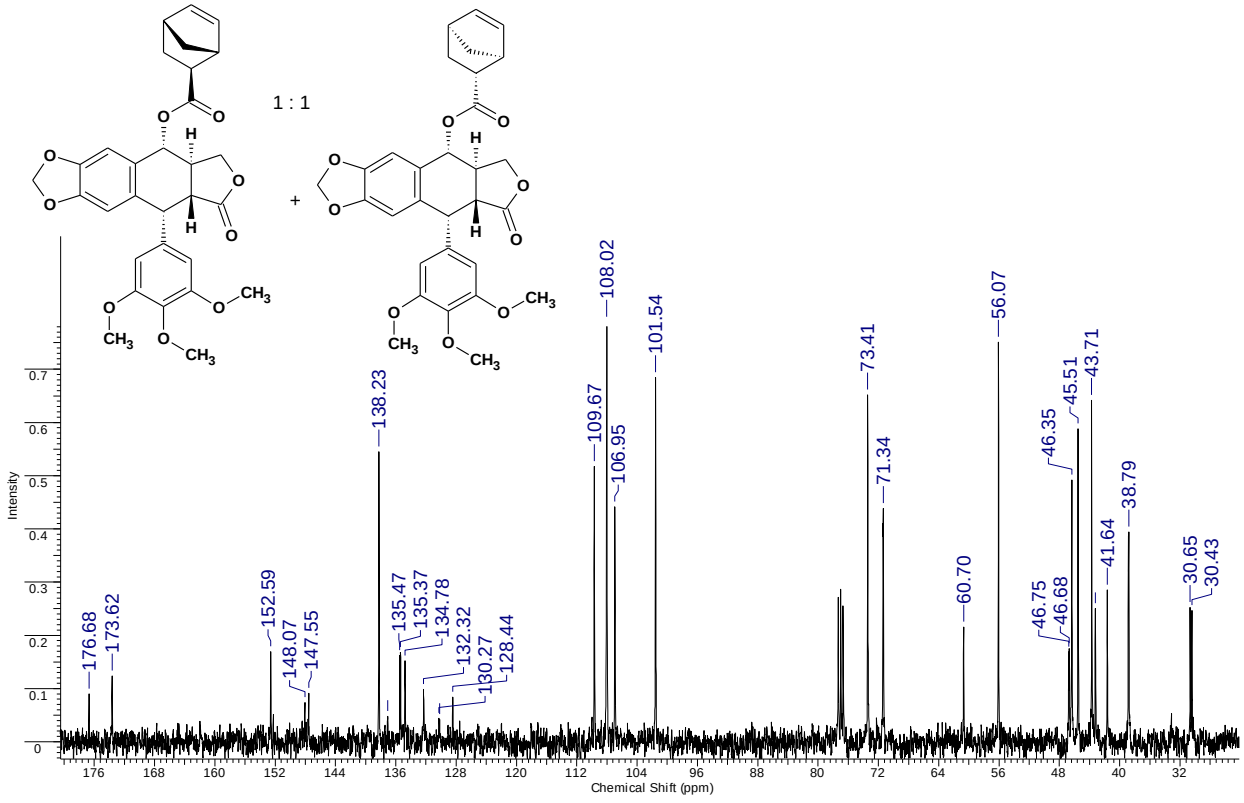
^{13}C NMR spectrum of 4-O-{(*2RS*)-bicyclo[2.2.2]octan-2-ylcarbonyl}-L-podophyllotoxin (6)



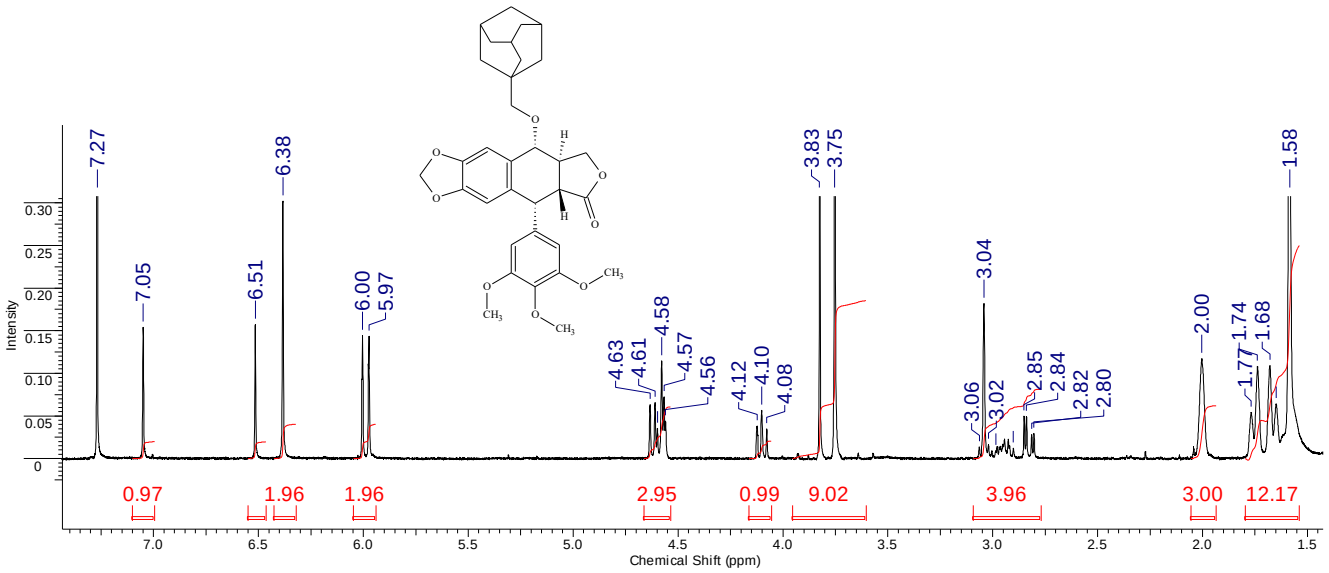
^{1}H NMR spectrum of 4-O-{(*1RS,2SR,4RS*)-bicyclo[2.2.1]hept-5-en-2-ylcarbonyl}-L-podophyllotoxin (7)



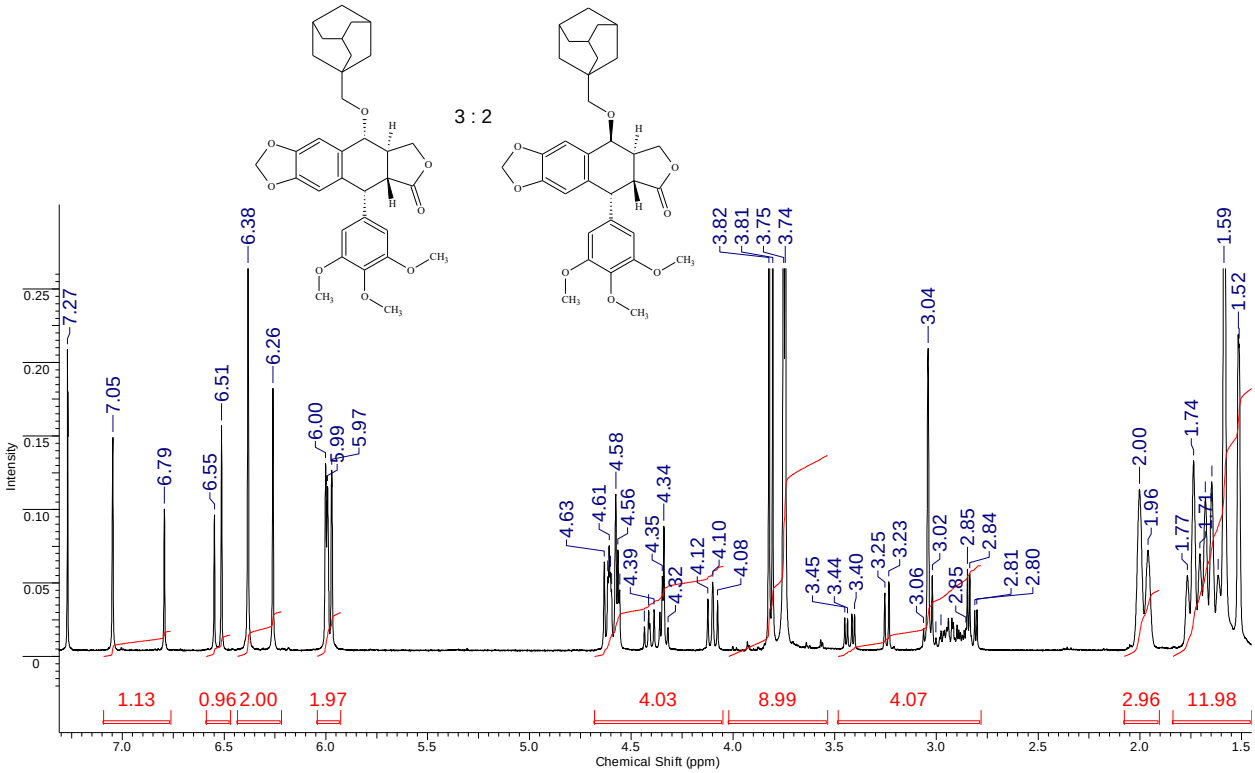
^{13}C NMR spectrum of 4-O-{(*1RS,2SR,4RS*)-bicyclo[2.2.1]hept-5-en-2-ylcarbonyl}-L-podophyllotoxin (7)



^{1}H NMR spectrum of 4-O-(adamant-1-ylmethyl)-L-podophyllotoxin (α-8)



^{1}H NMR spectrum of diastereomeric mixture of 4-O-(adamant-1-ylmethyl)-L-podophyllotoxin (8α) and 4-O-(adamant-1-ylmethyl)-L-epipodophyllotoxin (β-8)



^{13}C NMR spectrum of diastereomeric mixture of 4-O-(adamant-1-ylmethyl)-L-podophyllotoxin (8α) and 4-O-(adamant-1-ylmethyl)-L-epipodophyllotoxin (β-8)