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SUPPORTING INFORMATION

Switchable Synthesis of Glycosyl Selenides or Diselenides With Direct Use of Selenium as Selenating Agent

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General remarks.

All reagents adopted in the one-pot protocols are commercially available and were used as supplied without any pre-treatment. Selenium, sodium borohydride, iodine and poly(methylhydrosiloxane) (PMHS) were purchased from Sigma Aldrich. The progress of reactions was monitored by TLC; after elution in the suitable eluent, the plates were soaked in 5% concentrated H_2SO_4 in ethanol and heated at 230 °C. NMR spectra were recorded in a 400 Bruker MHz device. Reactions were performed at 0.5–1 mmol scale adopting the stoichiometric ratios indicated in the pertinent entries of the tables and in the schemes.

General procedure for the synthesis of glycosyl iodides

To a solution of a peracetylated sugar (390 mg, 1.0 mmol) in anhydrous DCM (6 mL) was added I_2 (279 mg, 1.1 mmol), and poly(methylhydrosiloxane) (PMHS) (65 μL, 1.1 mmol) (caution: exothermic reaction). The system was refluxed until complete consumption of the starting material (5–10 min as monitored by TLC, eluent: hexane/ethyl acetate mixtures). The mixture was then diluted with DCM and the organic phase washed with aq. sodium carbonate containing sodium thiosulfate (this latter is added portionwise as a solid, until consumption of residual iodine indicated by discoloration of the organic phase upon shaking). The organic phase was then washed with water, dried, and concentrated. The crude residue was directly adopted for the subsequent selenoglycosidation steps.

General procedure for the synthesis of symmetrical diglycosyl selenides

A mixture of elementary selenium (95 mg, 1.2 mmol) and sodium borohydride (137 mg, 3.6 mmol) was suspended in DMF (3 mL) and the suspension was kept under stirring at rt. After 40 minutes triethyl phosphite (175 μL, 1.0 mmol) was added (with an instantaneous discoloration) and the mixture was poured to a vessel containing crude glycosyl iodide (or the GlcNAc chloride). The vessel adopted for the first step was washed with portionwise DMF (3 mL overall). The mixture was kept under stirring at room temperature until completion of the reaction (in all cases less than two hours). The reaction was quenched with acetic acid (0.25 mL) and the mixture was transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase re-extracted with DCM. Combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was treated with pyridine (1 mL) and acetic anhydride (0.5 mL) for one-hour at rt. The mixture was then treated with methanol (ca 0.5 mL) in a cold bath, transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase was then re-extracted with DCM. Combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was purified by silica-gel flash chromatography (hexane/ethyl acetate or

dichloromethane/methanol mixtures). When detectable, the selenide/diselenide ratio was determined by NMR analysis of chromatographed products.

General procedure for the synthesis of diglycosyl diselenides

A mixture of elementary selenium (1.1 or 1.5 mmol, see Scheme 2) and sodium borohydride (1.2 or 1.0 mmol, see Scheme 2) was suspended in DMF (3 - 4 mL) and the suspension was kept under stirring at rt. After 40 minutes the mixture was poured to a vessel containing the crude glycosyl iodide (or the GlcNAc chloride) (1.0 mmol) in DMF (3 mL). The vessel adopted for the first step was washed with portionwise DMF (3 mL overall). The mixture was kept under stirring at room temperature until completion of the reaction (in all cases less than two hours). The reaction was quenched with acetic acid (0.25 mL) and the mixture was transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase re-extracted with DCM and combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was treated with pyridine (1 mL) and acetic anhydride (0.5 mL) for one-hour at rt. The mixture was then treated with methanol (ca 0.5 mL) in a cold bath, transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase was then re-extracted with DCM and combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was purified by silica-gel flash chromatography (hexane/ethyl acetate or dichloromethane/methanol mixtures). When detectable, the selenide/diselenide ratio was determined by NMR analysis of chromatographed products.

Synthesis of unsymmetrical diglycosyl selenides from a diselenide precursor

To a solution of galactosyl diselenide (31 mg, 0.038 mmol) in DMF (0.45 mL) were added sequentially triethyl phosphite (165 μL of a 0.23 M solution in DMF, 1 equiv) and $NaBH_4$ (29 μL of a 1. 3 M solution in DMF, 2 equiv). After a few seconds the mixture was poured to a vessel containing the crude glycosyl iodide (or the GlcNAc chloride) (0.11 mmol). The vessel adopted for the first step was washed with portionwise DMF (0.5 mL overall). After the appropriate time (see Scheme 4 and 5), the reaction was quenched with acetic acid (five drops) and the mixture transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase was then re-extracted with DCM. Combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was treated with pyridine (0.5 mL) and acetic anhydride (0.25 mL) for one-hour at rt. The mixture was then treated with methanol (ca 0.3 mL) in a cold bath, transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase was then re-extracted with DCM, and combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was purified by silica-gel flash chromatography (hexane/ethyl acetate or ethyl acetate alone).

One-pot synthesis of unsymmetrical diglycosyl selenide 25

A mixture of elementary selenium (10 mg, 0,126 mmol) and sodium borohydride (5.2 mg, 0.137 mmol) was suspended in DMF (0.35 mL) and the suspension was kept under stirring at rt. After 40 minutes the mixture was poured to a vessel containing the crude galactosyl iodide **7** (prepared from 45 mg of the peracetylated precursor, 0.115 mmol). The vessel adopted for the first step was washed with portionwise DMF (0.5 mL overall). After stirring for 1 hour at rt, were added sequentially triethyl phosphite (10 μL, 0.057 mmol), $NaBH_4$ (90 μL of a 1.3 M solution in DMF, 0.115 mmol), and solid GlcNAc chloride **14** (62 mg, 0.169 mmol). The mixture was stirred for an additional hour and then the reaction was quenched with acetic acid (0.15 mL) and the mixture transferred into a separatory funnel and diluted with DCM. The organic phase was washed with aq sodium carbonate and the aqueous phase was then re-extracted with DCM and combined organic phases were dried with sodium sulfate, filtrated and concentrated. The residue was purified by silica-gel flash chromatography (eluent: ethyl acetate) to yield pure **25** (52 mg, 61 % yield).

Zemplen deacetylation: synthesis of galactosyl selenide 26 and galactosyl diselenide 27

To a solution of **9** or **11** in MeOH (20-30 mg/mL) was added a solution of sodium methoxide in methanol (0.1 eq), preliminarily prepared by adding a weighted amount of NaH (60 % suspension) in methanol. The mixture was left under stirring at rt until detection via TLC (eluent: ethyl acetate) of the reaction completion (generally 2-4 hours were needed). The mixture was treated with Amberlyst H^+ resin (preliminarily washed with methanol) until neutrality, and then a slightly differentiated procedure was applied to isolate either **26** or **27**. Owing to the poor solubility of **26** in methanol, multiple washings with methanol (ca 1 mL each) were needed to suspend this product (a white solid) in a pipette and transfer it to another vessel, where it was concentrated *in vacuo*.

When diselenide **27** had to be separated from minor amounts of **26** (in the case in which the peracetylated diselenide **11** precursor was partially contaminated by **9**, see Scheme 2), small amounts of methanol (ca 0.3 - 0-5 mL) were used in each washing, taking care of not transferring the insoluble solid (if present) in the pipette. The transferred methanol solution was concentrated under vacuum to afford purified diselenide **27**.

Characterization data of all synthesized compounds

Di(2,3,4,6-tetra-O-acetyl-β-D-galactosyl) selenide (9)[1]

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 3:7). Rf (hexane/ethyl acetate 2:3) 0.42. $[\alpha]_D$ -29.5 (c 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.41 (1H, bd, J = 2.8 Hz, H-4), 5.26 (1H, t, J = 10.0 Hz, H-2), 5.10 (1H, dd, J = 2.8 and 10.0 Hz, H-3), 5.10 (1H, d, J =

10.0 Hz, H-1), 4.15-4.00 (H_2-6, m, 2 H), 3.86 (1H, bt, J = 6.8 Hz, H-5), 2.13, 2.02, 2.01, 1.94 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 170.0, 169.8, 169.5, 76.9, 75.7, 67.9, 67.1, 61.3, 20.5. Anal. Calcd. for $C_{28}H_{38}O_{18}Se$: C, 45.35; H, 5.17. Found: C, 45.45; H, 5.10. MALDI HRMS m/z [M + Na]$^+$ calc. for ($C_{28}H_{38}O_{18}SeNa$) 765.1121, found 765.1115.

Di(2,3,4,6-tetra-O-acetyl-β-D-glucosyl) selenide (10)[2]

Purified as a white solid by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 3:7). Rf (hexane/ethyl acetate 2:3) 0.40. $[\alpha]_D$ -61.5 (c 1.10, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25–5.10 (3H, overlapped signals, H-2, H-3, and H-4), 5.03 (1H, d, J = 10.0 Hz, H-1), 4.25 (1H, dd, J = 4.8 and 12.4 Hz, H-6a), 4.16 (1H, dd, J = 2.4 and 12.4 Hz, H-6b), 3.67 (1H, m, H-5), 2.11, 2.03, 2.02, 1.99 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.8, 169.1 (x2), 76.9, 73.6, 70.8, 68.0, 61.9, 20.4. Anal. Calcd. for $C_{28}H_{38}O_{18}Se$: C, 45.35; H, 5.17. Found: C, 45.30; H, 5.20. MALDI HRMS m/z [M + Na]$^+$ calc. for ($C_{28}H_{38}O_{18}SeNa$) 765.1121, found 765.1128.

Di(2,3,4,6-tetra-O-acetyl-β-D-galactosyl) diselenide (11)[1]

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 1:4). Rf (hexane/ethyl acetate 1:1) 0.30. $[\alpha]_D$ -37.5 (c 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (1H, bd, J = 2.8 Hz, H-4), 5.35 (1H, t, J = 10.0 Hz, H-2), 5.06 (1H, dd, J = 2.8 and 10.0 Hz, H-3), 4.91 (1H, d, J = 10.0 Hz, H-1), 4.20 (1H, dd, J = 6.8 and 12.0 Hz, H-6a), 4.09 (1H, bt, J = 6.8 Hz, H-5), 4.03 (1H, dd, J = 6.8 and 12.0 Hz, H-6b), 2.16, 2.06, 2.03, 1.97 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (x2), 169.9, 169.6, 81.1, 75.4, 71.4, 69.3, 67.0, 60.7, 20.5. Anal. Calcd. for $C_{28}H_{38}O_{18}Se_2$: C, 40.99; H, 4.67. Found: C, 41.00; H, 4.65. MALDI HRMS m/z [M + Na]$^+$ calc. for ($C_{28}H_{38}O_{18}Se_2Na$) 845.0286, found 845.0281.

Di(2,3,4,6-tetra-O-acetyl-β-D-glucosyl) diselenide (12)[2]

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 1:4). Rf (hexane/ethyl acetate 1:1) 0.32. $[\alpha]_D$ -102.6 (c 1.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (1H, t, J = 9.6 Hz), 5.15 (1H, t, J = 9.6 Hz), 5.06 (1H, t, J = 9.6 Hz), 4.91 (1H, d, J = 9.6 Hz, H-1), 4.27 (1H, dd, J = 3.6 and 12.4 Hz, H-6a), 4.14 (1H, dd, J = 2.0 and 12.4 Hz, H-6b), 3.75 (1H, m, H-5), 2.08, 2.04, 1.99, 1.96 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 169.2 (x2), 79.6, 73.5, 71.5, 67.8, 61.5, 20.6-20.4. Anal. Calcd. for $C_{28}H_{38}O_{18}Se_2$: C, 40.99; H, 4.67. Found: C, 41.5 H, 4.60. MALDI HRMS m/z [M + Na]$^+$ calc. for ($C_{28}H_{38}O_{18}Se_2Na$) 845.0286, found 845.0293.

Di(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucosyl) selenide (15)

Purified as a foam by silica gel chromatography (eluent: dichlormethane/MeOH from 1:0 to 95:5). $[\alpha]_D$ -59.6 (c 0.40, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.10 (1H, d, J = 9.6 Hz, 2-NH), 5.15–5.10 (2H, overlapped signals, H-3 and H-4), 5.07 (1H, d, J = 10.4 Hz, H-1), 4.30–4.10 (3H, overlapped signals, H-2, H_2-6), 3.67 (1H, m, H-5), 2.10, 2.03 (x2), 1.93 (12H, 3 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.6, 170.5, 169.2, 77.2, 73.7, 68.1, 62.0, 53.4, 23.0, 20.7-20.5. Anal. Calcd. for $C_{28}H_{40}N_2O_{16}Se$: C, 45.47; H, 5.45. Found: C, 45.55 H, 5.40. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{40}N_2O_{16}SeNa$) 763.1441, found 763.1445.

Di(2,3,6,2',3',4',6'-hepta-O-acetyl-β-D-lactosyl) selenide (16)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 2:3 to 1:4). Rf (hexane/ethyl acetate 3:7) 0.40. $[\alpha]_D$ -20.9 (c 1.58, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.29 (1H, bd, J = 2.8 Hz, H-4'), 5.13 (1H, t, J = 8.8 Hz), 5.05 (1H, dd, J = 7.6 and 10.0 Hz), 5.00-4.95 (2H, overlapped signals), 4.92 (1H, dd, J = 3.2 and 10.4 Hz, H-3'), 4.44 (1H, d, J = 7.6 Hz, H-1'), 4.42 (1H, bd, J = 9.6 Hz, H-6a), 4.15-4.00 (3H, overlapped signals), 3.83 (1H, bt, J = 6.8 Hz, H-5'), 3.75 (1H, t, J = 9.6 Hz, H-4), 3.54 (1H, m, H-5), 2.09, 2.08, 2.00 (x3), 1.99, 1.91 (21H, 7 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2-168.9, 100.9, 77.8, 76.1, 76.0, 73.6, 71.2, 70.8, 70.5, 68.9, 66.4, 62.1, 60.6, 20.5. Anal. Calcd. for $C_{52}H_{70}O_{34}Se$: C, 47.39; H, 5.35. Found: C, 47.50 H, 5.30. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{52}H_{70}O_{34}SeNa$) 1341.2811, found 1341.2820.

Di(2,3,4,6-tetra-O-acetyl-α-D-mannosyl) selenide (17)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 2:3 to 1:4). Rf (hexane/ethyl acetate 3:7) 0.47. ^1H NMR (400 MHz, $CDCl_3$) δ 5.49 (1H, bd, J = 2.8 Hz, H-2), 5.30 (1H, bs, H-1), 5.25 (1H, t, J = 10.0 Hz, H-4), 5.06 (1H, dd, J = 2.8 and 10.0 Hz, H-3), 4.22 (1H, dd, J = 6.8 and 12.4 Hz, H-6a), 4.16 (1H, dd, J = 12.4 Hz, H-6a), 3.68 (1H, m, H-5), 2.16, 2.08, 2.03, 1.95 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5-169.2 ($COCH_3$), 77.7, 75.1, 71.5, 70.5, 65.5, 62.5, 20.4. Anal. Calcd. for $C_{28}H_{38}O_{18}Se$: C, 45.35; H, 5.17. Found: C, 45.30; H, 5.15. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{38}O_{18}SeNa$) 765.1121, found 765.1127.

Di(2,3,4-tri-O-acetyl-β-L-fucosyl) selenide (18)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 2:3). Rf (hexane/ethyl acetate 1:1) 0.45. $[\alpha]_D$ +7.7 (c 0.90, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.29 (1H, bd, J = 2.8 Hz, H-4), 5.27 (1H, t, J = 10.0 Hz, H-2), 5.05 (1H, dd, J = 2.8 and 10.0 Hz, H-3), 5.02 (1H, d, J = 10.0 Hz, H-1), 3.79 (1H, bq, J = 6.8 Hz, H-5), 2.17, 2.03, 1.93 (9H, 3 x s, 3 x -$COCH_3$), 1.21 (3H, d, J =

6.8 Hz, CH_3-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.9, 169.4, 76.9, 74.4, 71.9, 70.2, 68.1, 20.6-20.5, 16.2. Anal. Calcd. for $C_{24}H_{34}O_{14}Se$: C, 46.09; H, 5.48. Found: C, 46.20; H, 5.40. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{24}H_{34}O_{14}SeNa$) 649.1011, found 649.1003.

Di(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucosyl) diselenide (19)

Purified as a foam by silica gel chromatography (eluent: dichlormethane/MeOH from 1:0 to 95:5). $[\alpha]_D$ -11.3 (c 1.10, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.30 (1H, d, J = 9.6 Hz, NH-2), 5.30 (1H, t, J = 10.0 Hz, H-3), 5.12 (1H, d, H = 10.4 Hz, H-1), 5.02 (1H, t, J = 10.0 Hz, H-4), 4.36 (1H, dd, J = 5.2 and 12.4 Hz, H-6a), 4.15-4.00 (2H, overlapped signals, H-6b and H-2), 3.75 (1H, m, H-5), 2.09, 2.06 (x2), 1.99 (12H, 3 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.4 (x2), 169.3, 81.8, 76.9, 72.9, 68.3, 61.9, 55.0, 23.2, 20.8, 20.5 (x2). Anal. Calcd. for $C_{28}H_{40}N_2O_{16}Se_2$: C, 41.08; H, 4.93. Found: C, 41.00; H, 4.95. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{40}N_2O_{16}Se_2Na$) 843.0606, found 843.0615.

Di(2,3,6,2',3',4',6'-hepta-O-acetyl-β-D-lactosyl) diselenide (20)[3]

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 2:3 to 1:4). Rf (hexane/ethyl acetate 1:3) 0.54. $[\alpha]_D$ -48.4 (c 1.45, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.32 (1H, bd, J = 2.8 Hz, H-4'), 5.19 (1H, t, J = 9.2 Hz), 5.15-5.00 (3H, overlapped signals), 4.83 (1H, d, J = 10.0 Hz, H-1), 4.50 (1H, bd, J = 9.6 Hz, H-6a), 4.45 (1H, d, J = 7.6 Hz, H-1'), 4.15-3.95 (3H, overlapped signals), 3.88 (1H, bt, J = 6.8 Hz, H-5'), 3.85 (1H, t, J = 9.6 Hz, H-4), 3.70 (1H, m, H-5), 2.12 (x2), 2.02 (x4), 1.94 (21H, 7 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5-168.7, 100.8, 79.4, 77.6, 75.7, 73.5, 72.0, 70.8, 70.4, 68.9, 66.4, 61.5, 60.5, 20.6-20.5. Anal. Calcd. for $C_{52}H_{70}O_{34}Se_2$: C, 44.71; H, 5.05. Found: C, 44.80 H, 5.00. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{52}H_{70}O_{34}$ Se_2Na) 1421.1977, found 1421.1970.

Di(2,3,4,6-tetra-O-acetyl-α-D-mannosyl) diselenide (21)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 2:3 to 1:4). Rf (hexane/ethyl acetate 3:7) 0.47. $[\alpha]_D$ + 5.5 (c 1.15, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.60 (1H, bd, J = 2.4 Hz, H-2), 5.31 (1H, bs, H-1), 5.28 (1H, t, J = 10.0 Hz, H-4), 5.13 (1H, dd, J = 2.4 and 10.0 Hz, H-3), 4.22 (2H, d, J = 3.6 Hz, H_2-6), 3.75 (1H, m, H-5), 2.19, 2.10, 2.04, 1.98 (12H, 4 x s, 4 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.8 (x2), 169.4 ($COCH_3$), 81.2, 77.5, 71.3, 70.3, 65.3, 62.0, 20.5. Anal. Calcd. for $C_{28}H_{38}O_{18}Se_2$: C, 40.99; H, 4.67. Found: C, 41.90; H, 4.60. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{38}O_{18}Se_2Na$) 845.0286, found 845.0281.

Di(2,3,4-tri-O-acetyl-β-L-fucosyl) diselenide (22)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate from 1:1 to 2:3). Rf (hexane/ethyl acetate 1:1) 0.45. $[\alpha]_D$ + 2.5 (c 0.60, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.32 (1H, t, J

= 10.0 Hz, H-2), 5.25 (1H, bd, J = 3.2 Hz, H-4), 5.03 (1H, dd, J = 3.2 and 10.0 Hz, H-3), 4.95 (1H, d, J = 10.0 Hz, H-1), 3.87 (1H, bq, J = 6.4 Hz, H-5), 2.16, 2.05, 1.96 (9H, 3 x s, 3 x -$COCH_3$), 1.20 (3H, d, J = 6.4 Hz, CH_3-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 169.6, 82.3, 74.4, 71.9, 70.3, 69.3, 20.8, 20.5 (x2), 16.2. Anal. Calcd. for $C_{24}H_{34}O_{14}Se_2$: C, 40.92; H, 4.86. Found: C, 40.80; H, 4.90. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{24}H_{34}O_{14}Se_2Na$) 729.0177, found 729.0185.

(2,3,4,6-tetra-O-acetyl-β-D-galactosyl)-(2,3,4,6-tetra-O-acetyl-β-D-glucosyl) selenide (23)

Purified as a foam by silica gel chromatography (eluent: hexane/ethyl acetate 2:3). Rf (hexane/ethyl acetate 2:3) 0.50. $[\alpha]_D$ - 21.7 (c 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (1H, bd, J = 2.8 Hz, H-4 Gal), 5.29 (1H, t, J = 10.0 Hz, H-2 Gal), 5.18 (1H, t, J = 9.6 Hz, H-3 Glc), 5.20-5.00 (5H, overlapped signals; H-1, H-2 and H-4 Glc, H-1 and H-3 Gal), 4.24 (1H, dd, J = 5.2 and 11.6 Hz, H-6a Glc), 4.20-4.00 (3H, overlapped signals, H-6b Glc and H_2-6 Gal), 3.89 (1H, bt, J = 6.8 Hz, H-5 Gal), 3.67 (1H, m, H-5 Glc), 2.16, 2.09, 2.05, 2.04, 2.03 (x2), 2.02, 1.99 (24H, 8 x s, 8 x -$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (x2), 169.5, 169.2, 76.2, 75.7, 73.6, 71.5, 70.8, 68.1, 67.9, 67.1, 62.0, 61.4, 20.5. Anal. Calcd. for $C_{28}H_{38}O_{18}Se$: C, 45.35; H, 5.17. Found: C, 45.30; H, 5.15. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{38}O_{18}SeNa$) 765.1121, found 765.1127.

(2,3,4,6-tetra-O-acetyl-β-D-galactosyl)-(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucosyl) selenide (25)

Purified as a foam by silica gel chromatography (eluent: ethyl acetate). Rf (ethyl acetate) 0.60. $[\alpha]_D$ -32.8 (c 1.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (1H, d, J = 9.2 Hz, NH-2 GlcNAc), 5.44 (1H, bd, J = 2.8 Hz, H-4 Gal), 5.30 (1H, t, J = 10.0 Hz, H-2 Gal), 5.10-5.00 (5H, overlapped signals; H-1, H-3 and H-4 GlcNAc, H-1 and H-3 Gal), 4.24-4.05 (5H, overlapped signals; H-2, H_2-6 GlcNAc, H_2-6 Gal), 3.89 (1H, bt, J = 6.8 Hz, H-5 Gal), 3.62 (1H, m, H-5 Glc), 2.15, 2.07, 2.05, 2.03, 2.02, 2.00 (x2), 1.96 (24H, 7 x s, 8 x -$COCH_3$).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9-170.1, 169.1, 78.0, 77.3, 71.4, 68.2, 67.8, 67.3, 62.2, 61.5, 53.4, 22.9, 20.5. Anal. Calcd. for $C_{28}H_{39}NO_{17}Se$: C, 45.41; H, 5.31. Found: C, 45.50; H, 5.25. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{28}H_{39}NO_{17}SeNa$) 764.1281, found 764.1274.

Di(β-D-galactopyranosyl) selenide (26)[1]

Purified as a white solid after filtration (see procedure of the Zemplen deacetylation). ^{1}H NMR (400 MHz, D_2O) δ 4.96 (1H, d, J = 10.0 Hz, H-1), 3.90 (1H, bd, J = 3.2 Hz, H-4), 3.72-3.55 (5H, overlapped signals, H-2, H-3, H-5 and H_2-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 80,3, 80.2, 73.6, 70.2, 68.7, 61.1. Anal. Calcd.

for $C_{12}H_{22}O_{10}Se$: C, 35.56; H, 5.47. Found: C, 35.45; H, 5.50. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{12}H_{22}O_{10}SeNa$) 429.0276, found 429.0285.

Di(β-D-galactopyranosyl) diselenide (27)[1]

Purified as an oil after selective solubilization with methanol (see procedure of the Zemplen deacetylation). ^1H NMR (400 MHz, D_2O) δ 4.78 (1H, d, J = 10.0 Hz, H-1), 3.87 (1H, bd, J = 3.2 Hz, H-4), 3.75 (1H, t, J = 10.0 Hz, H-2), 3.70-3.50 (4H, overlapped signals, H-3, H-5 and H_2-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 83,5, 80.4, 73.5, 70.2, 68.7, 60.9. Anal. Calcd. for $C_{12}H_{22}O_{10}Se_2$: C, 29.76; H, 4.58. Found: C, 29.85; H, 4.55. MALDI HRMS m/z $[M + Na]^+$ calc. for ($C_{12}H_{22}O_{10}Se_2Na$) 508.9441, found 508.9433.

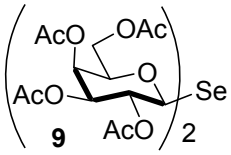
References

1) André, S.; Kövér, K. E.; Gabius, H.-J.; Szilágyi, L. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 931-935.

2) Wagner, G.; Nuhn, P. *Arch. Pharm.* **1964**, 297, 461-473

3) Saravanan, V.; Porhiel, E.; Chandrasekaran, S. *Tetrahedron Lett.* **2003**, *44*, 2257-2260

4) Kim, C.; Lee, J.; Park, M.-S. *Arch. Pharm. Res.* **2015**, *38*, 659–665.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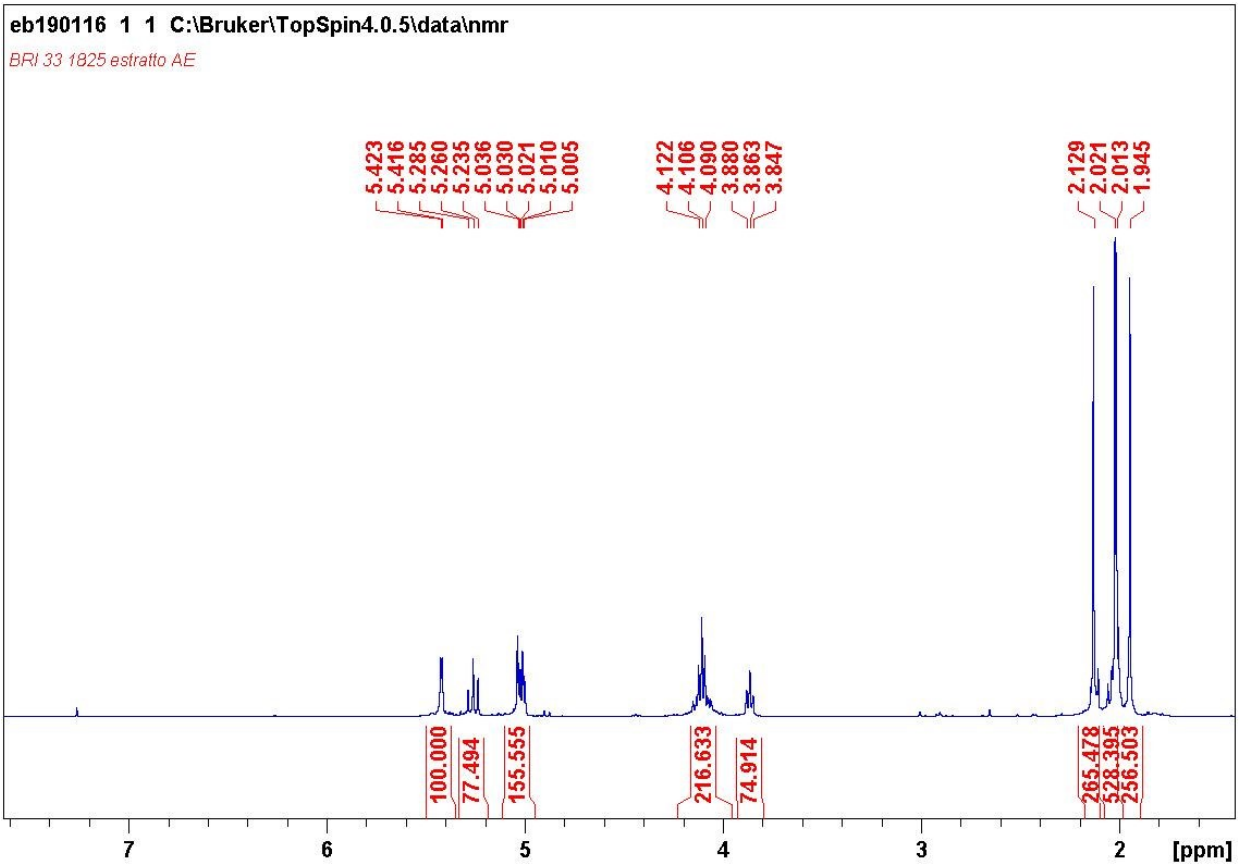
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5.030
5.021
5.010
5.005
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4.106
4.090
3.880
3.863
3.847
2.129
2.021
2.013
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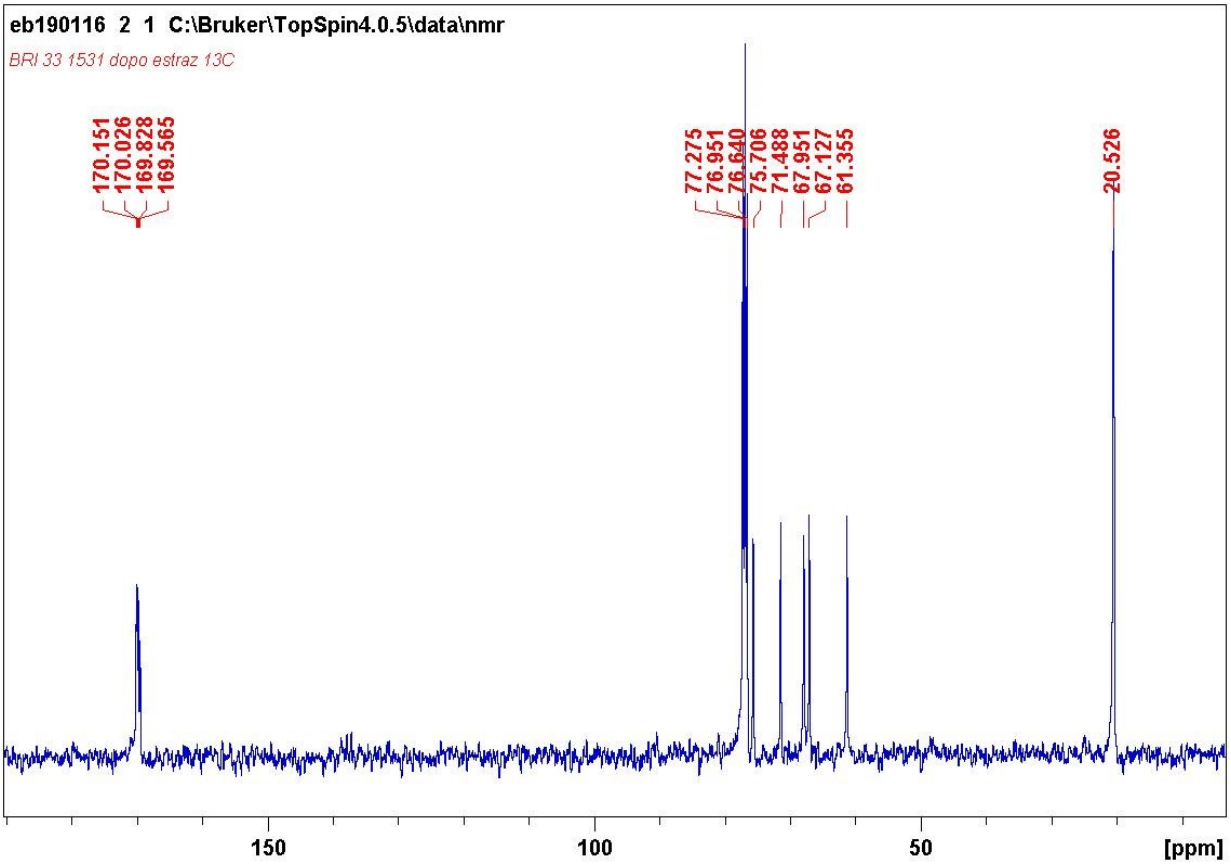
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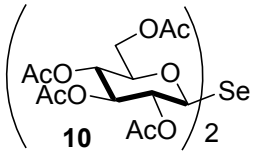
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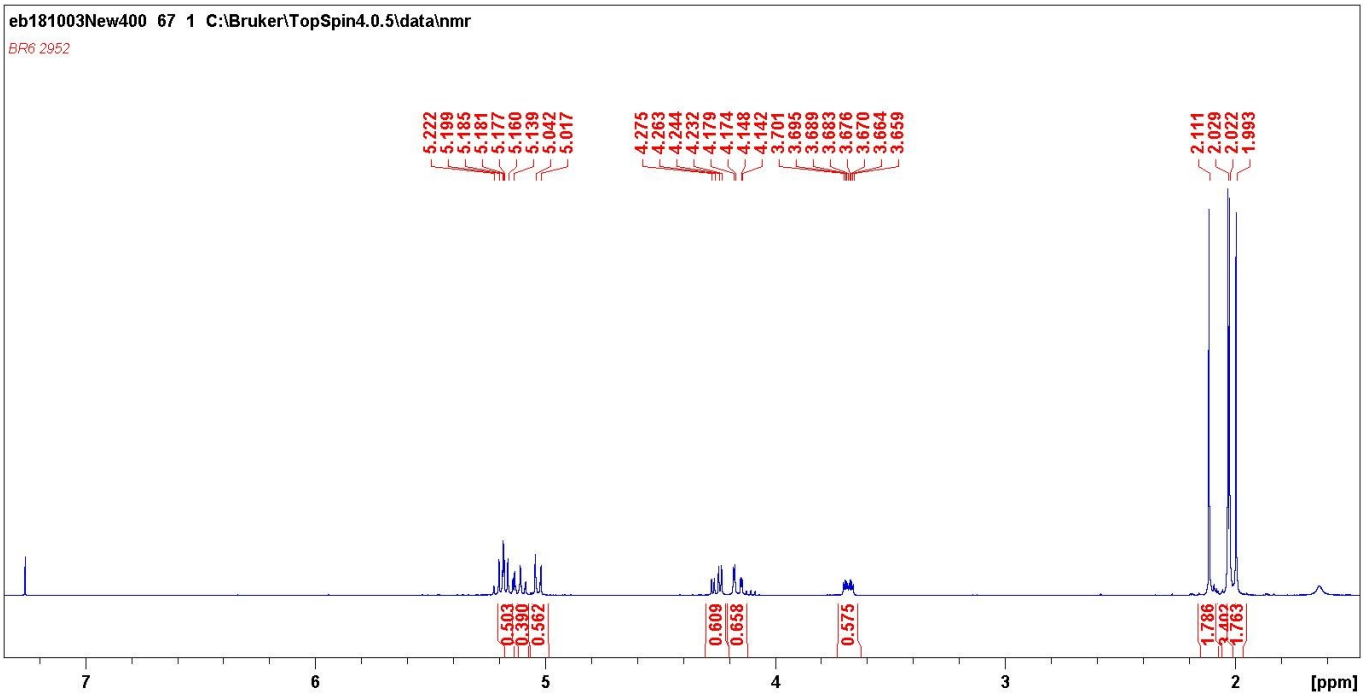
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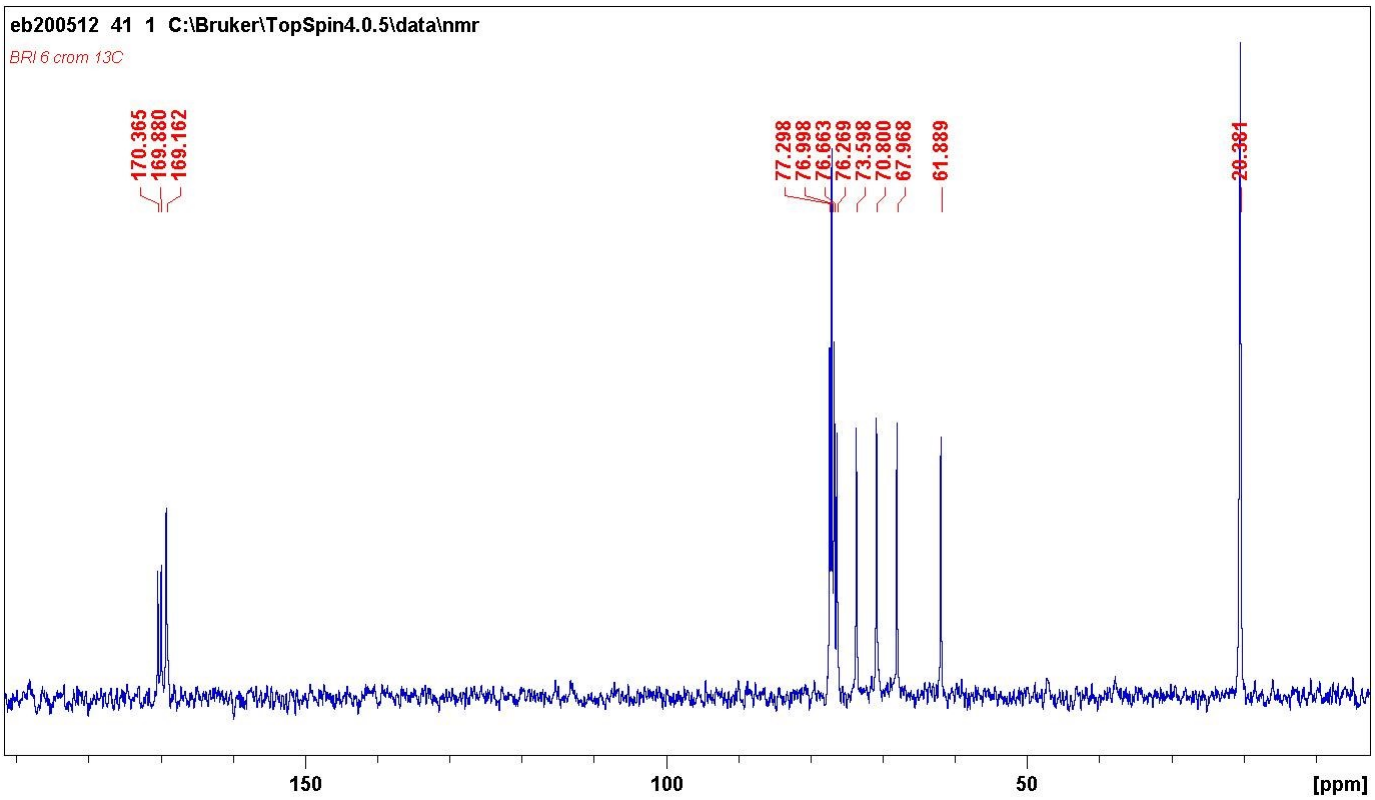
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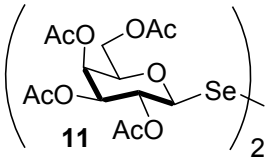
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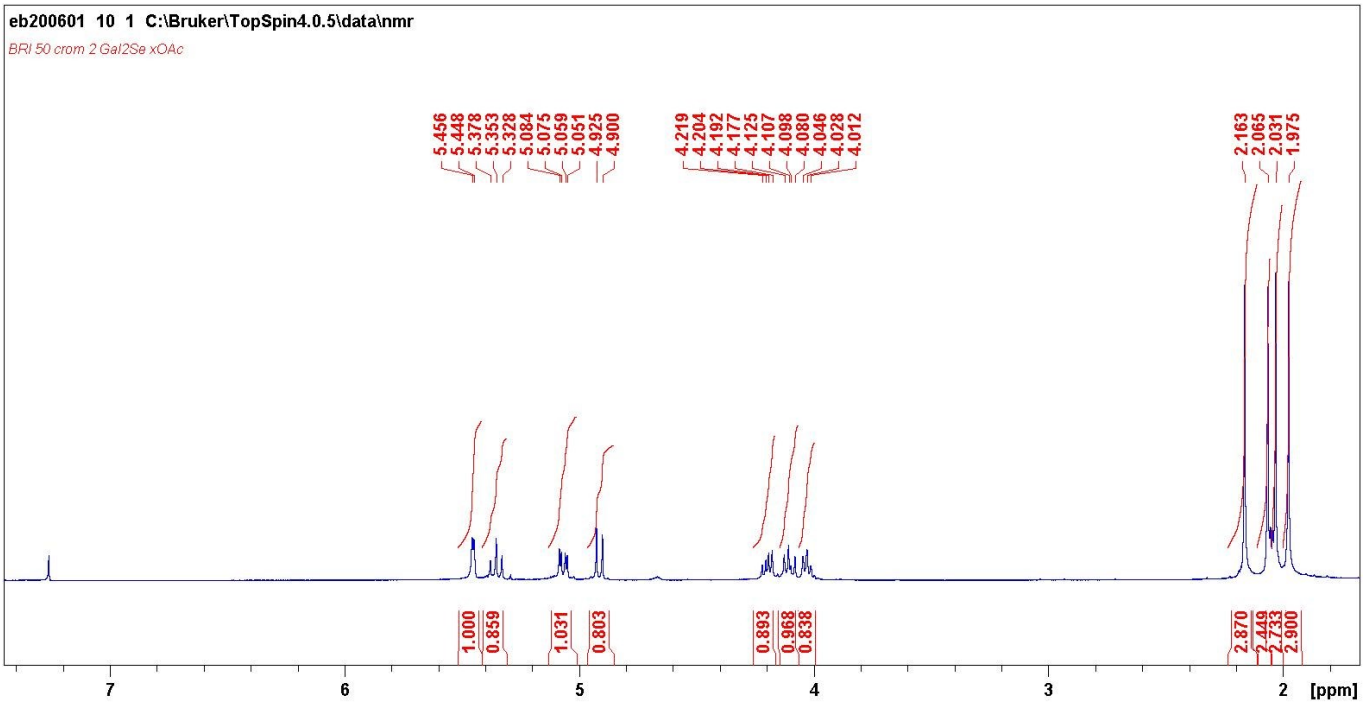
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4.219
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4.192
4.177
4.125
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4.028
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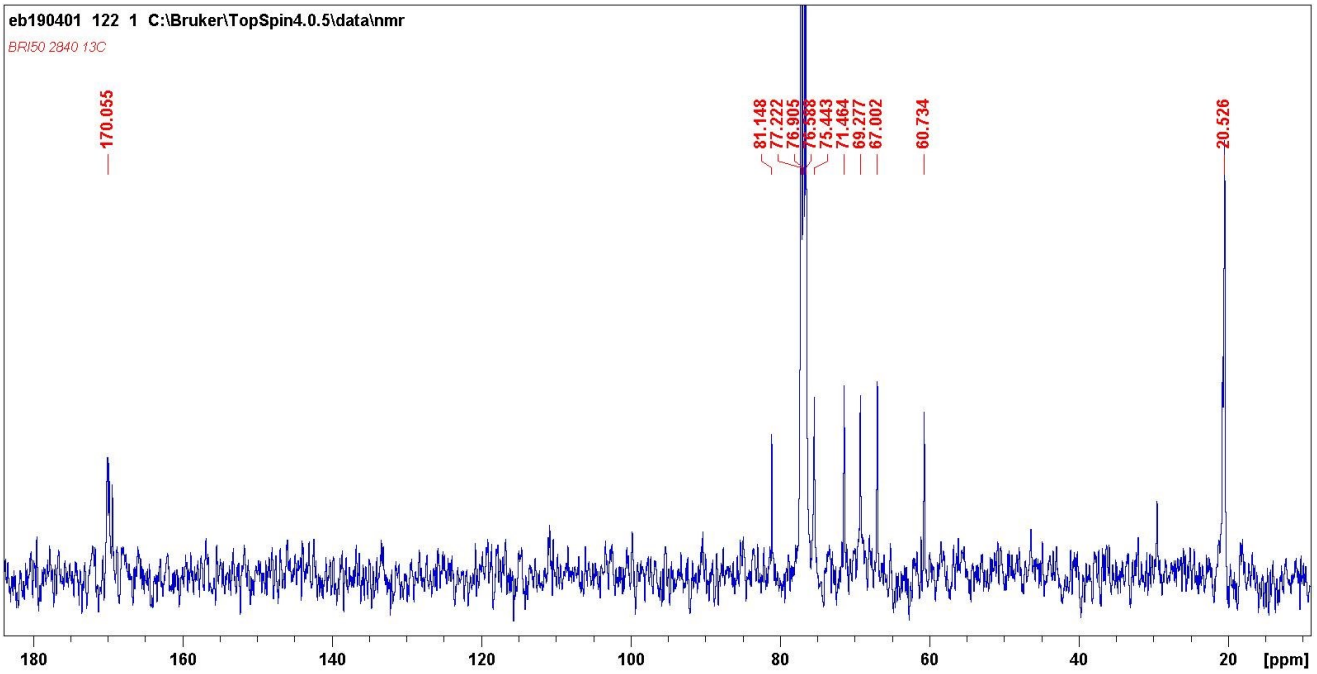
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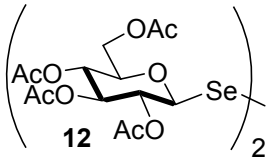
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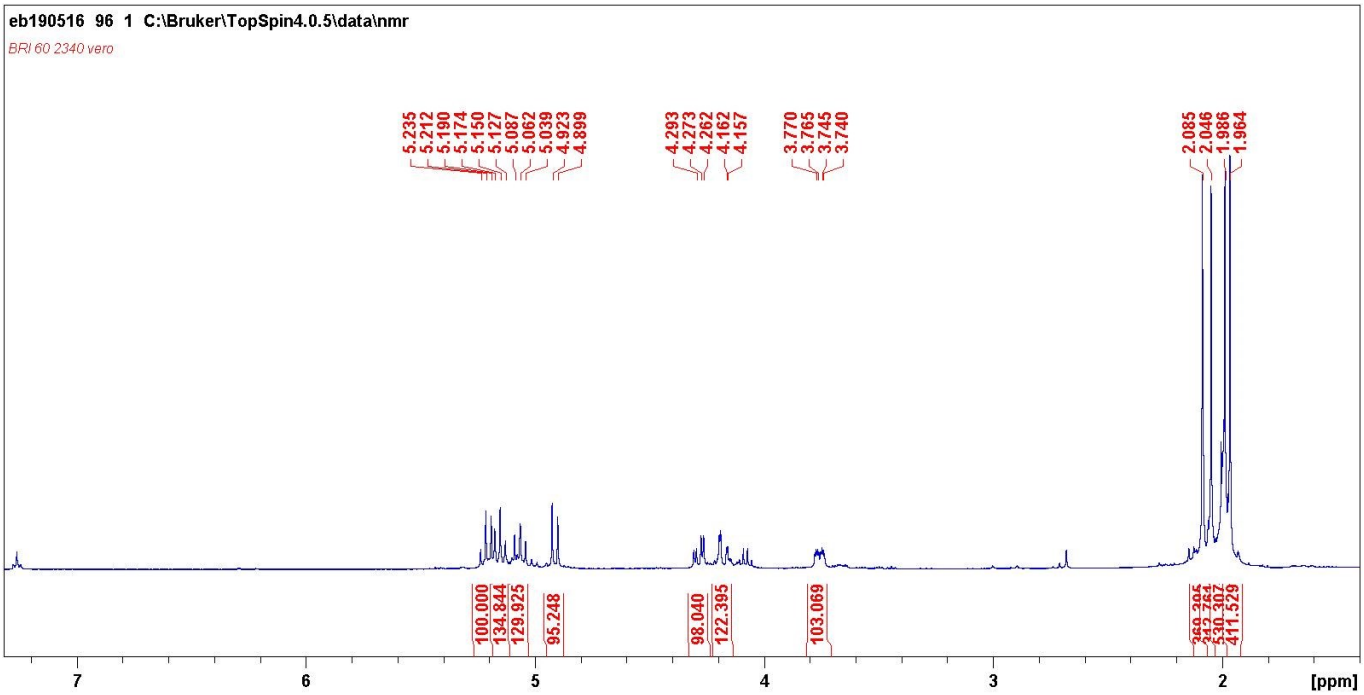
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5.039
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4.899
4.293
4.273
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3.765
3.745
3.740
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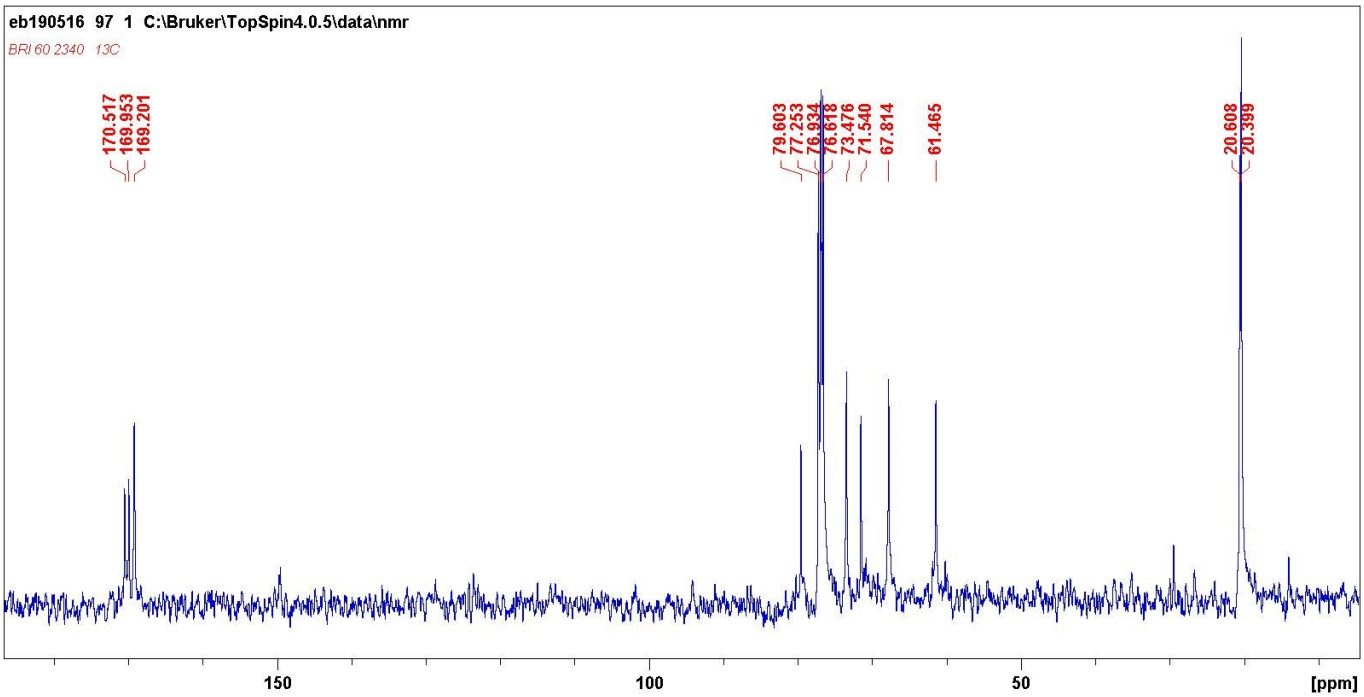
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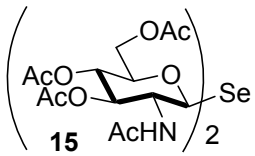
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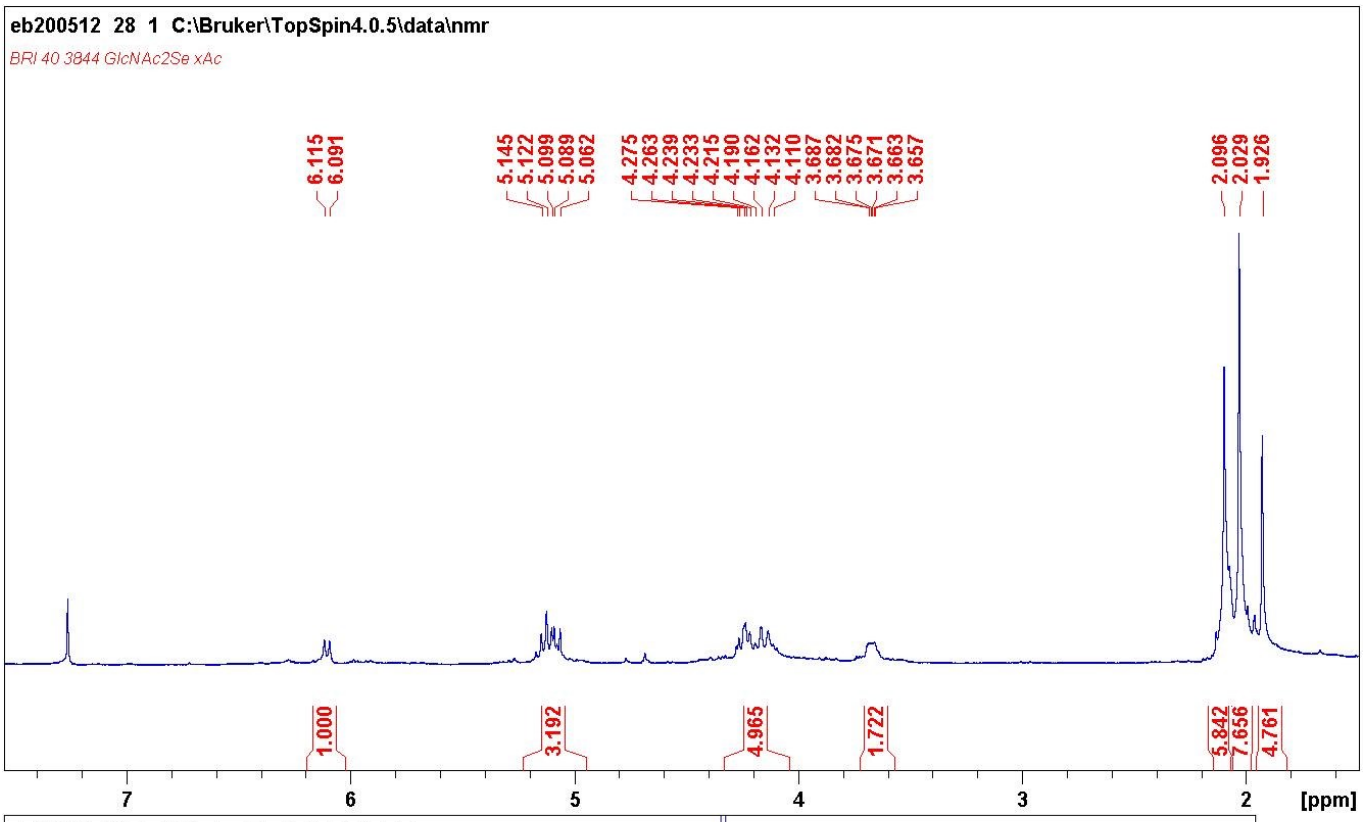
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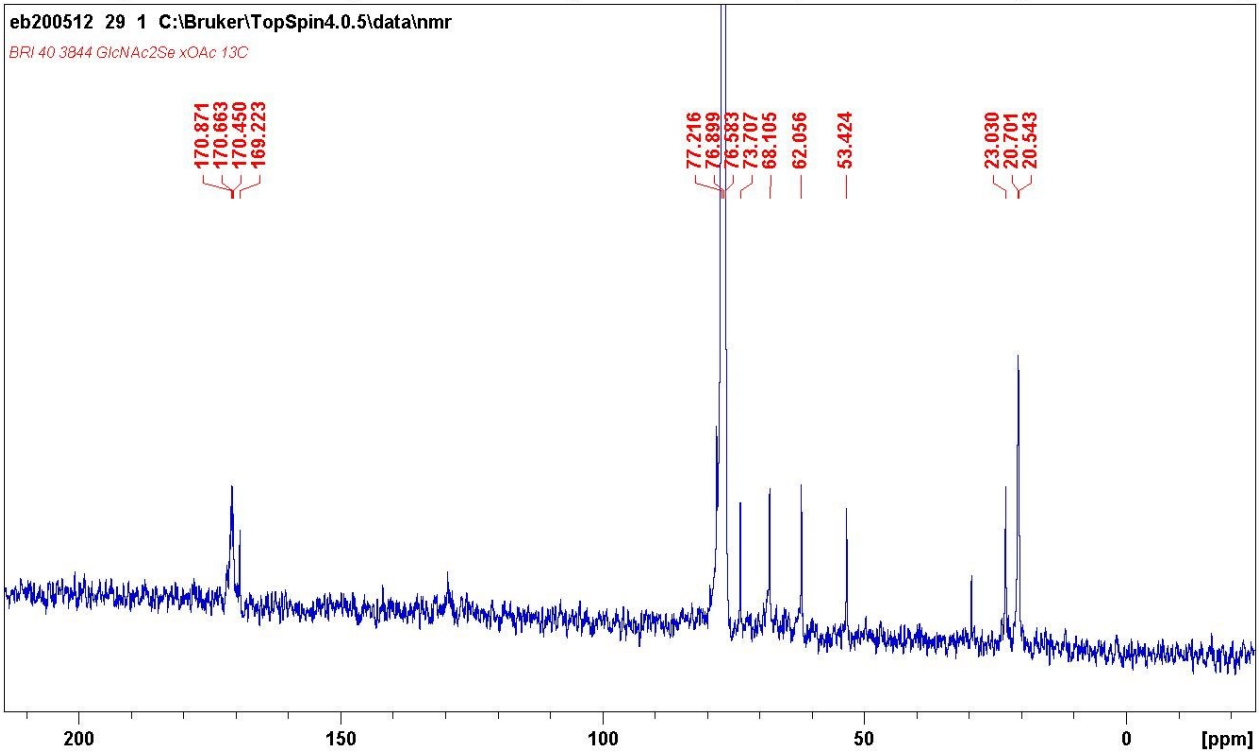
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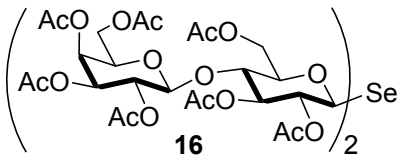
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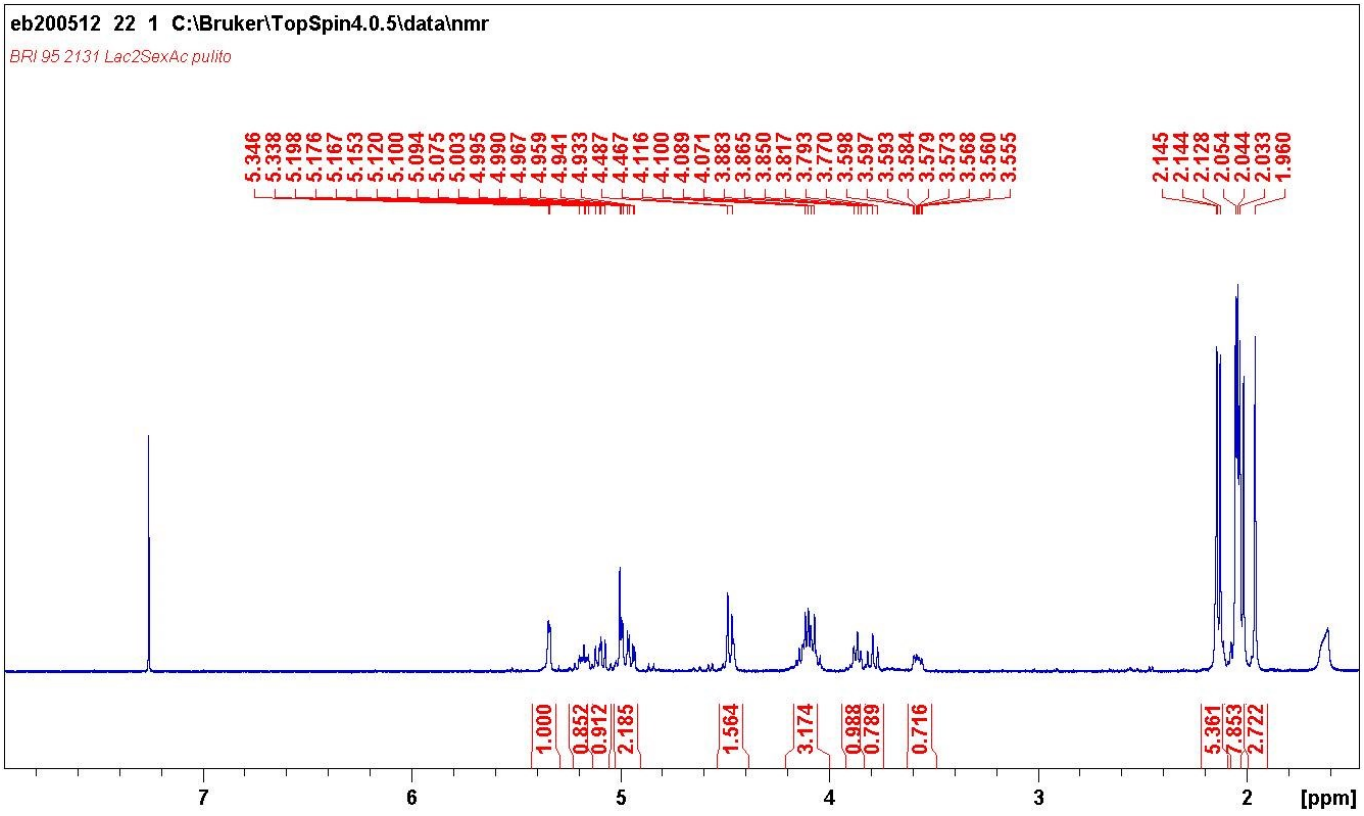
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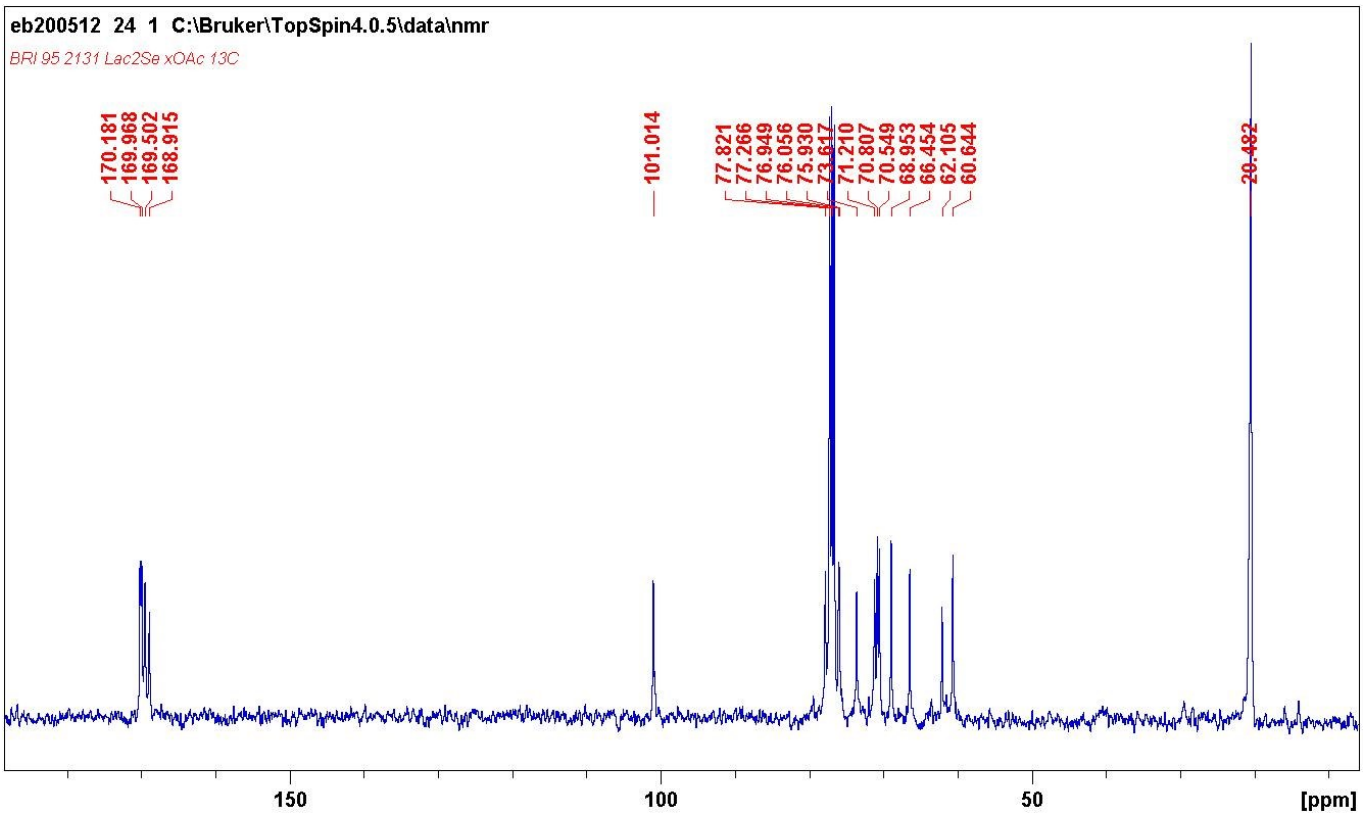
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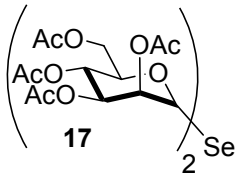
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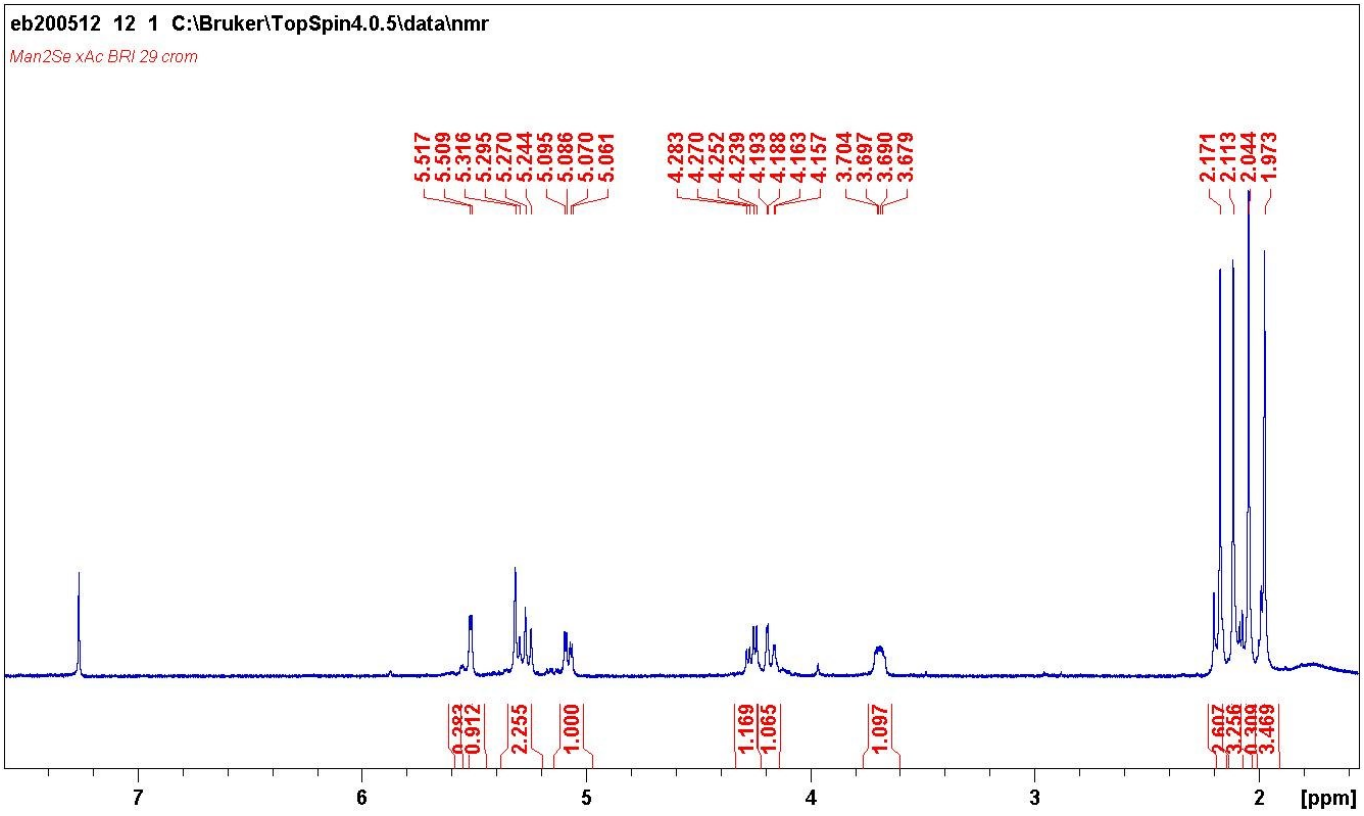
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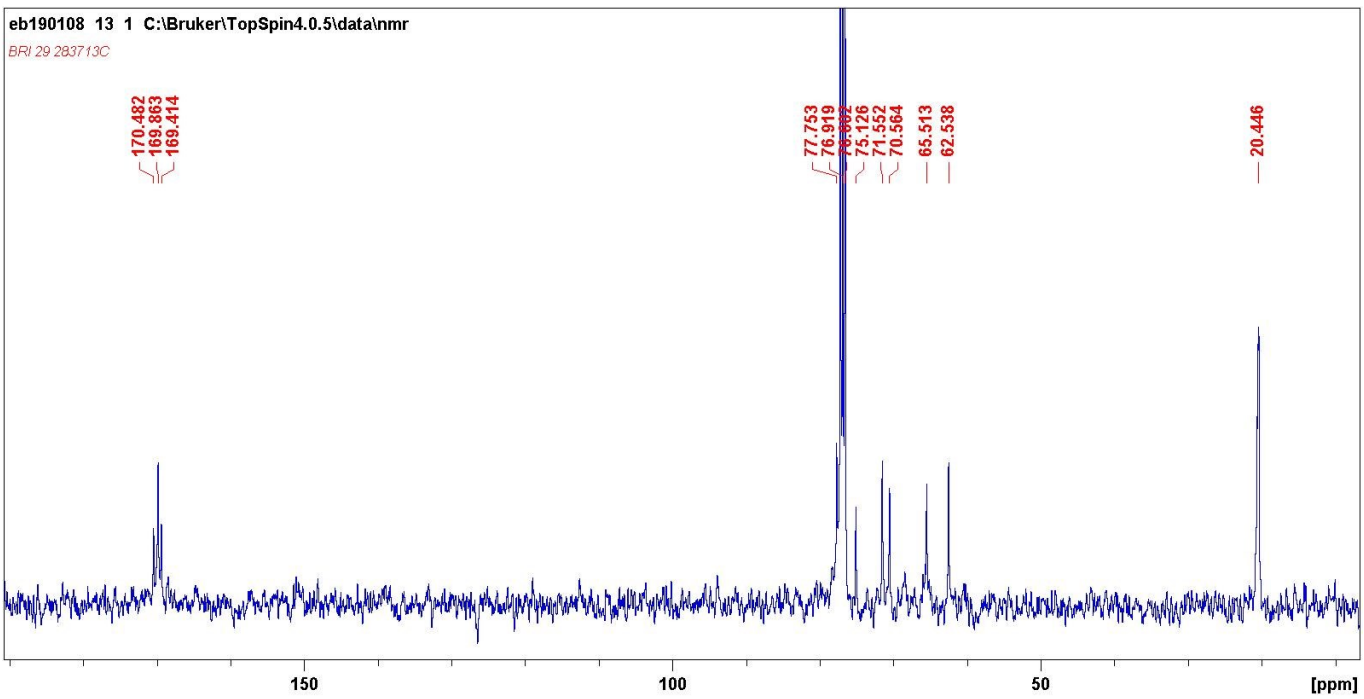
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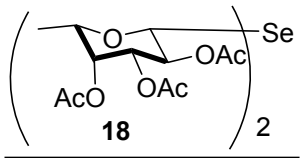
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OAc
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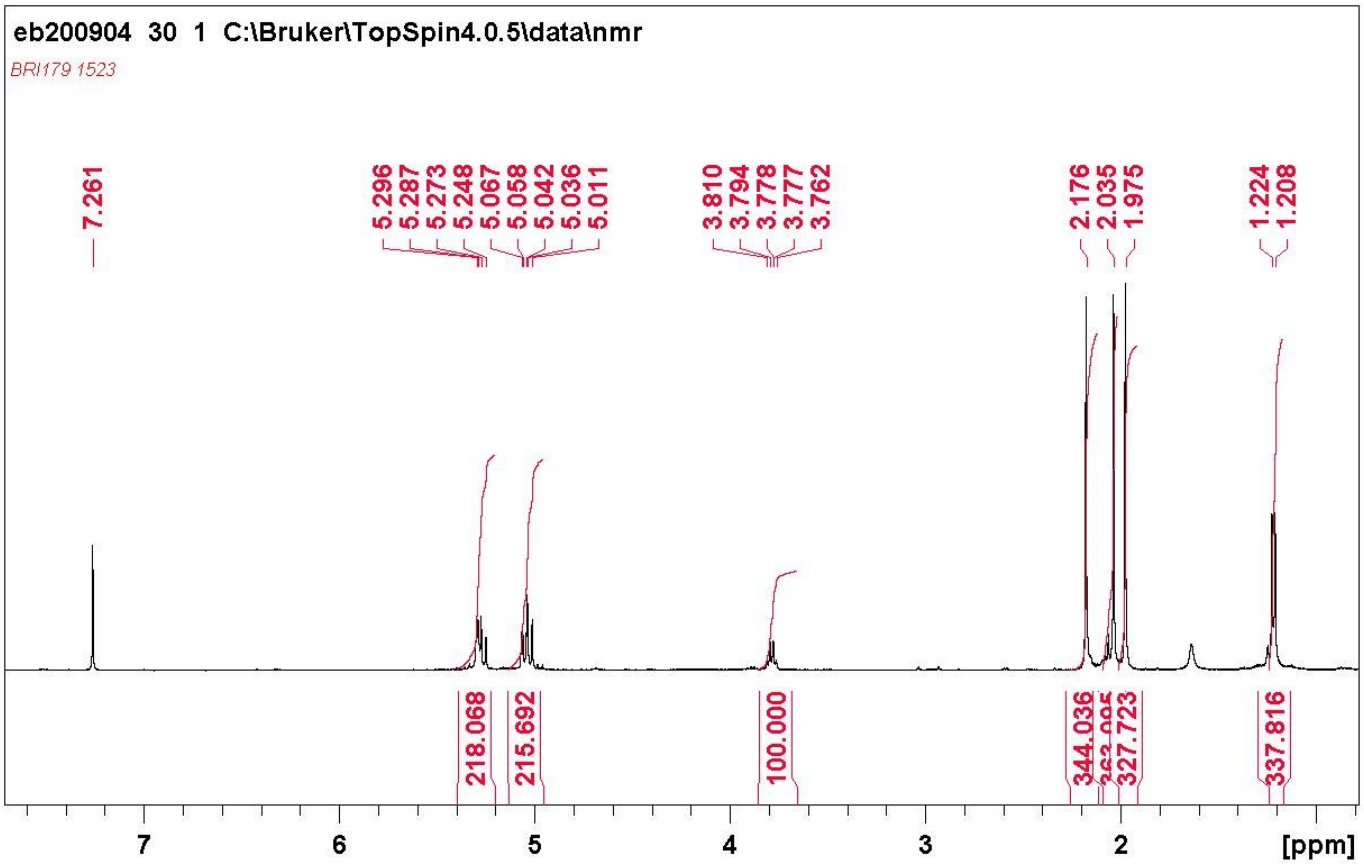
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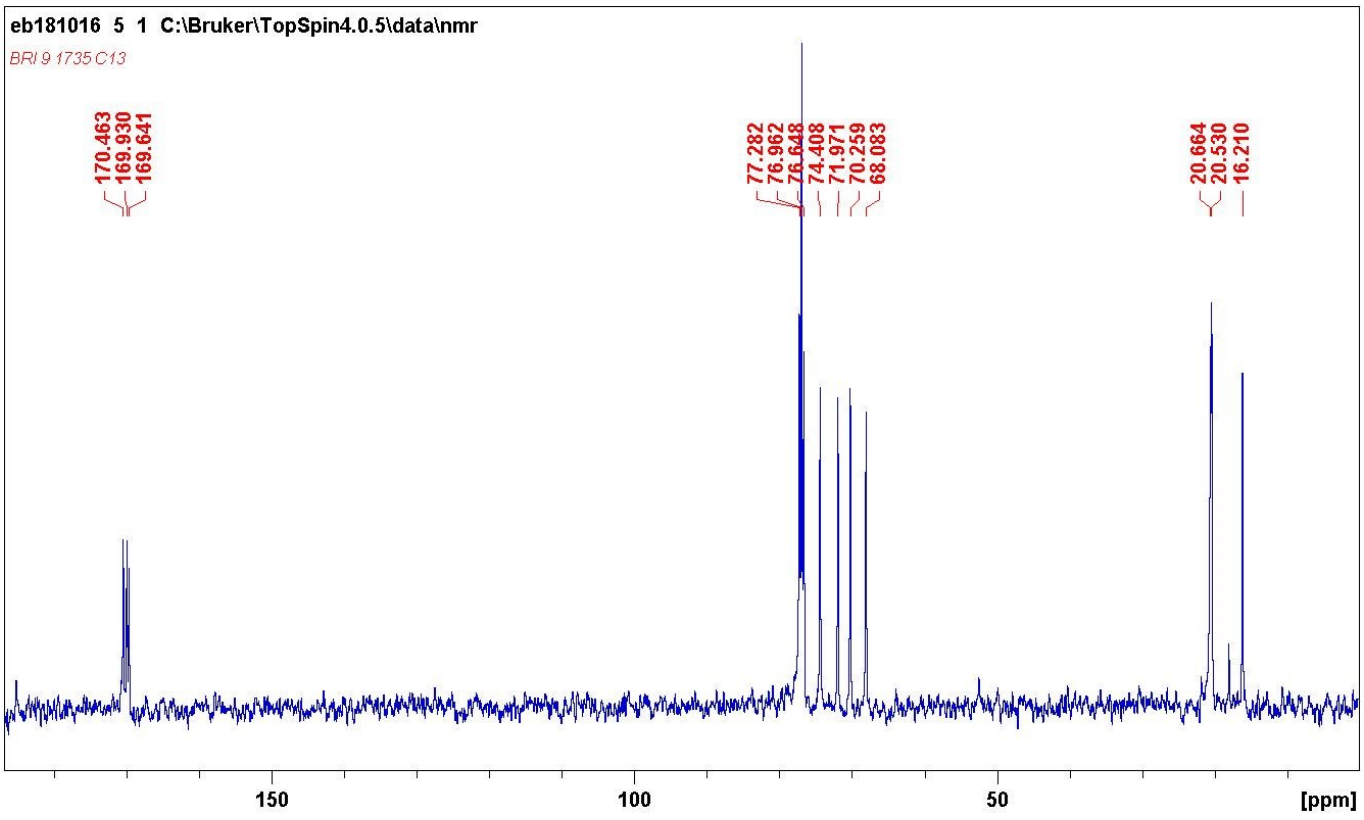
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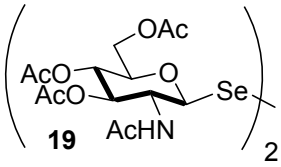
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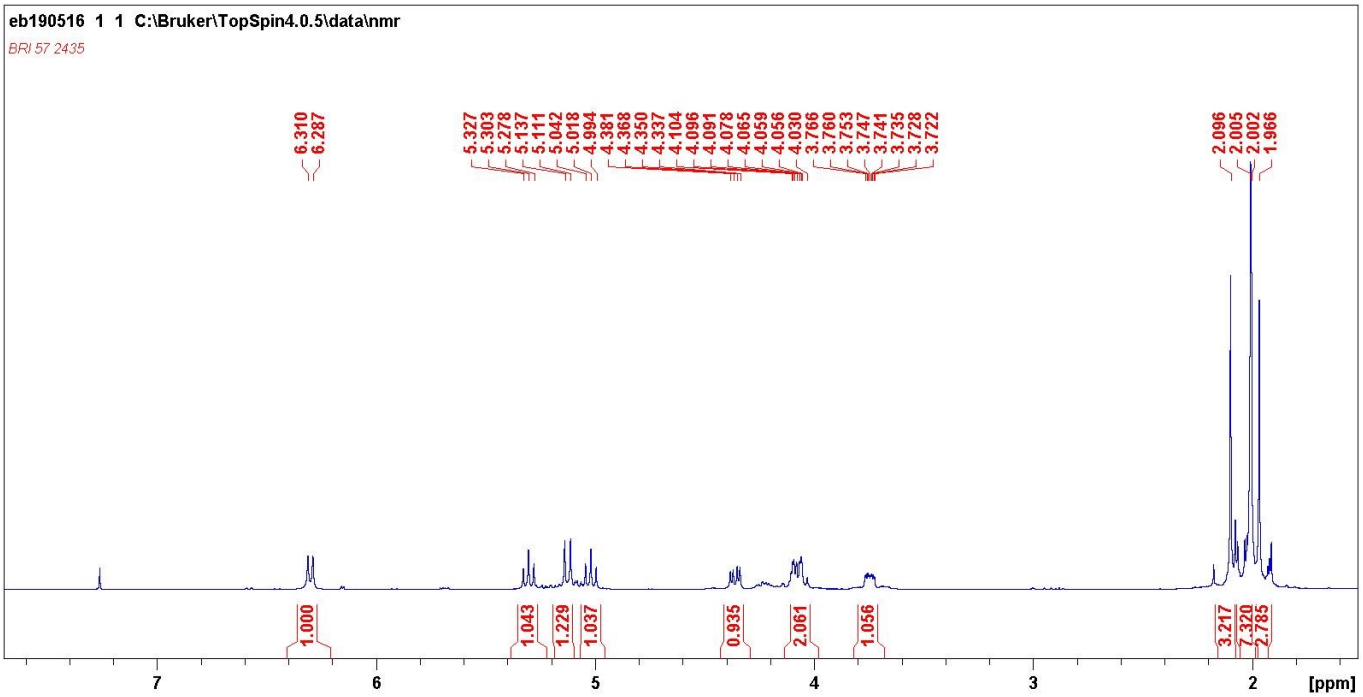
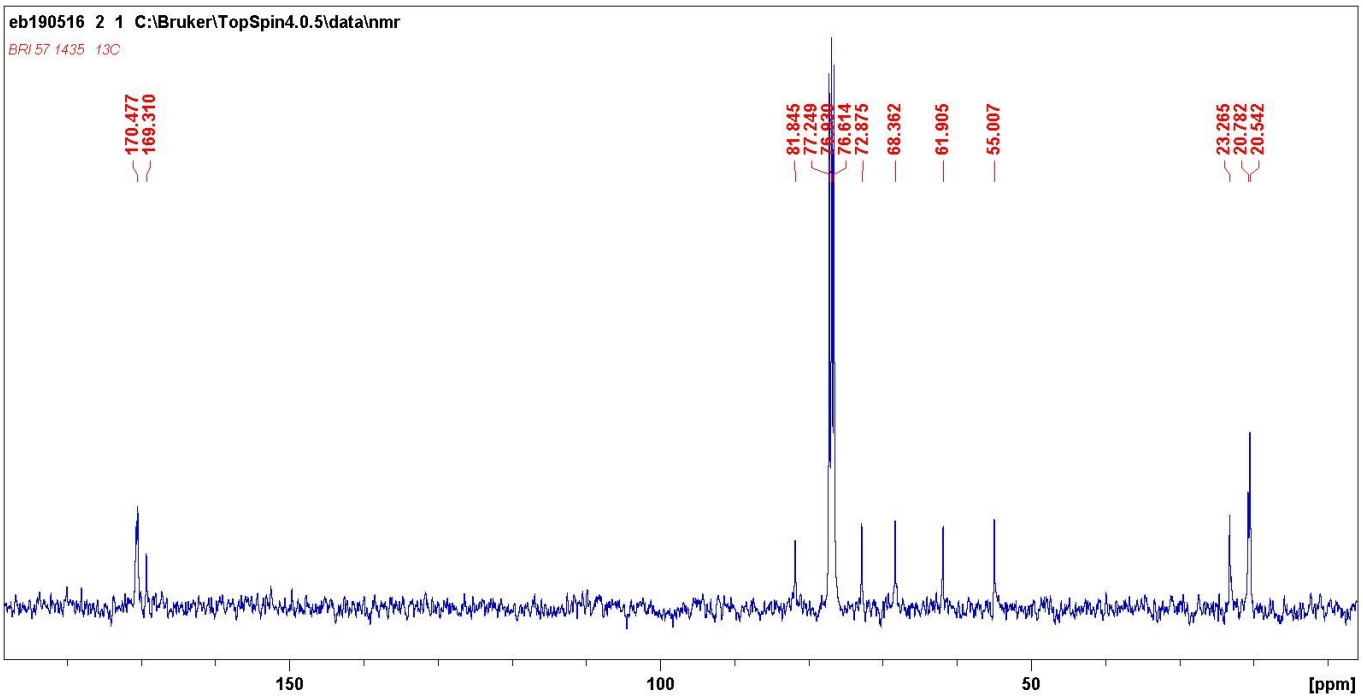
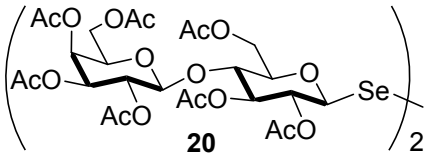
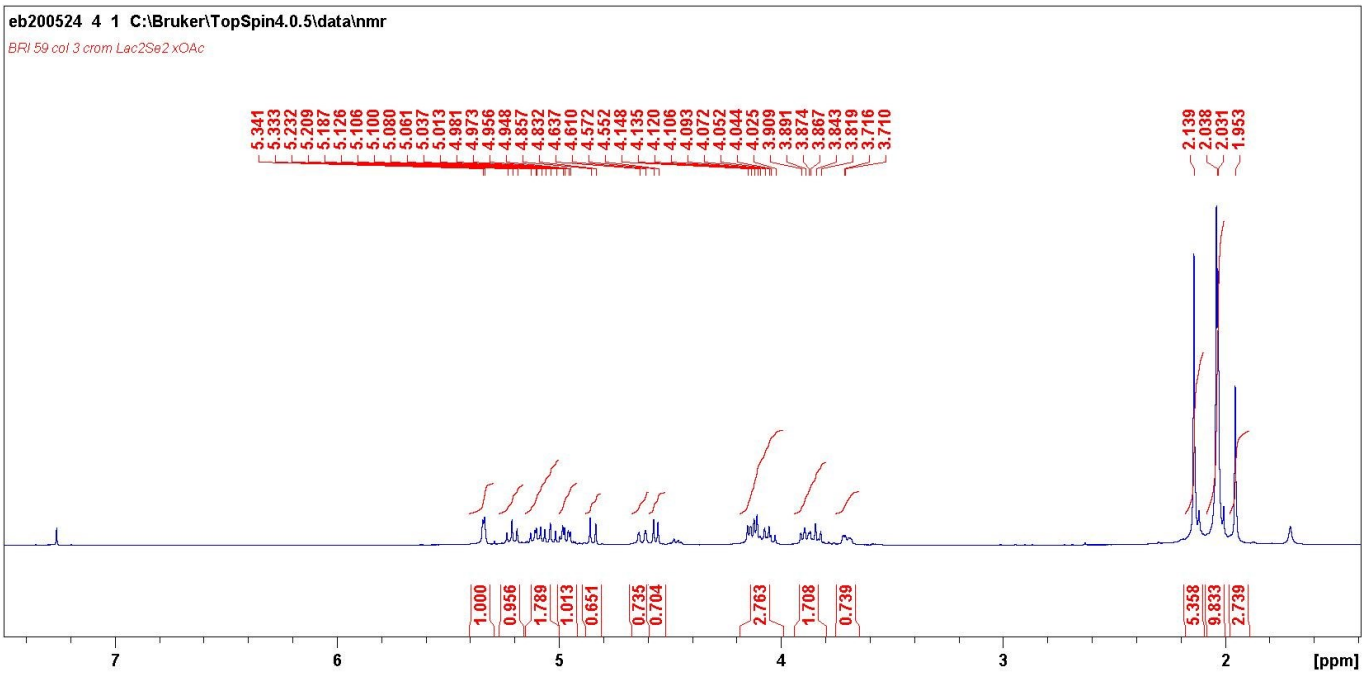
19
2

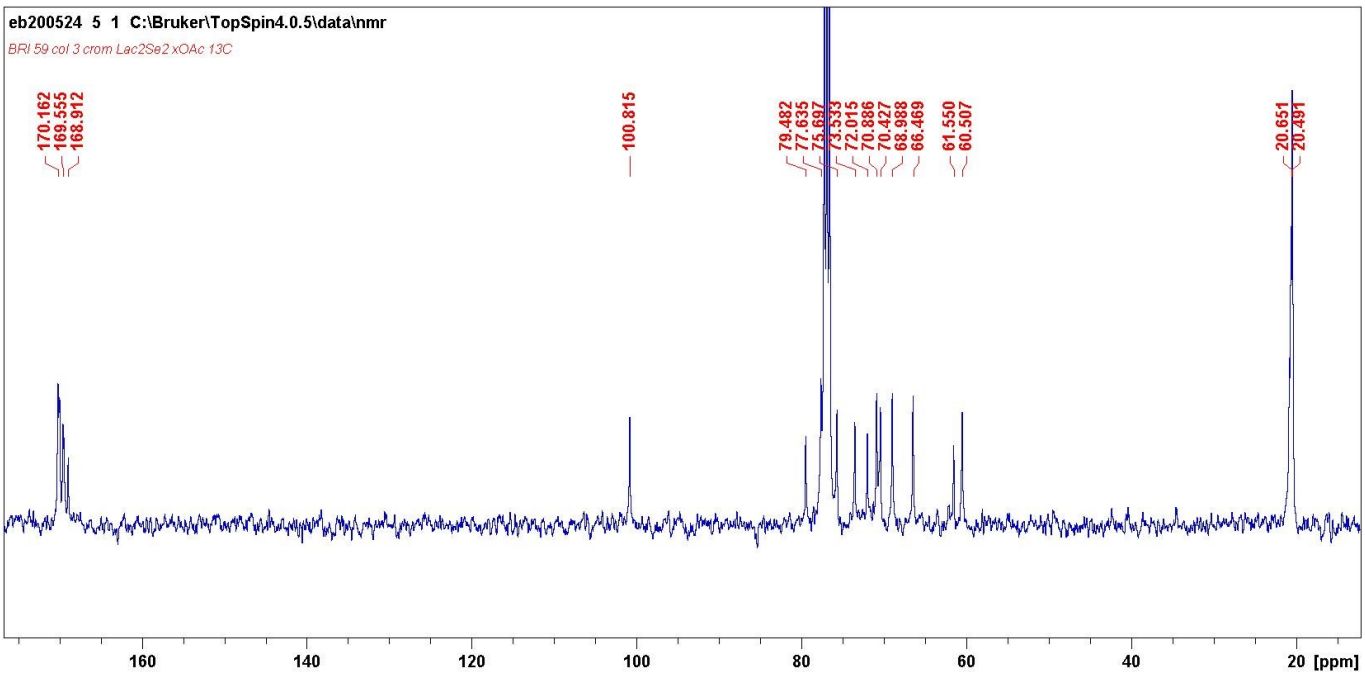

eb190516 1 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 57 2435
6.310
6.287
5.327
5.303
5.278
5.137
5.111
5.042
5.018
4.994
4.381
4.368
4.350
4.337
4.104
4.096
4.091
4.078
4.065
4.059
4.056
4.030
3.766
3.760
3.753
3.747
3.741
3.735
3.728
3.722
2.096
2.005
2.002
1.966
1.000
1.043
1.229
1.037
0.935
2.061
1.056
3.217
7.320
2.785
7
6
5
4
3
2
[ppm]

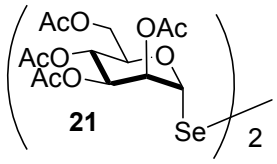

eb190516 2 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 57 1435 13C
170.477
169.310
81.845
77.249
76.614
72.875
68.362
61.905
55.007
23.265
20.782
20.542
150
100
50
[ppm]

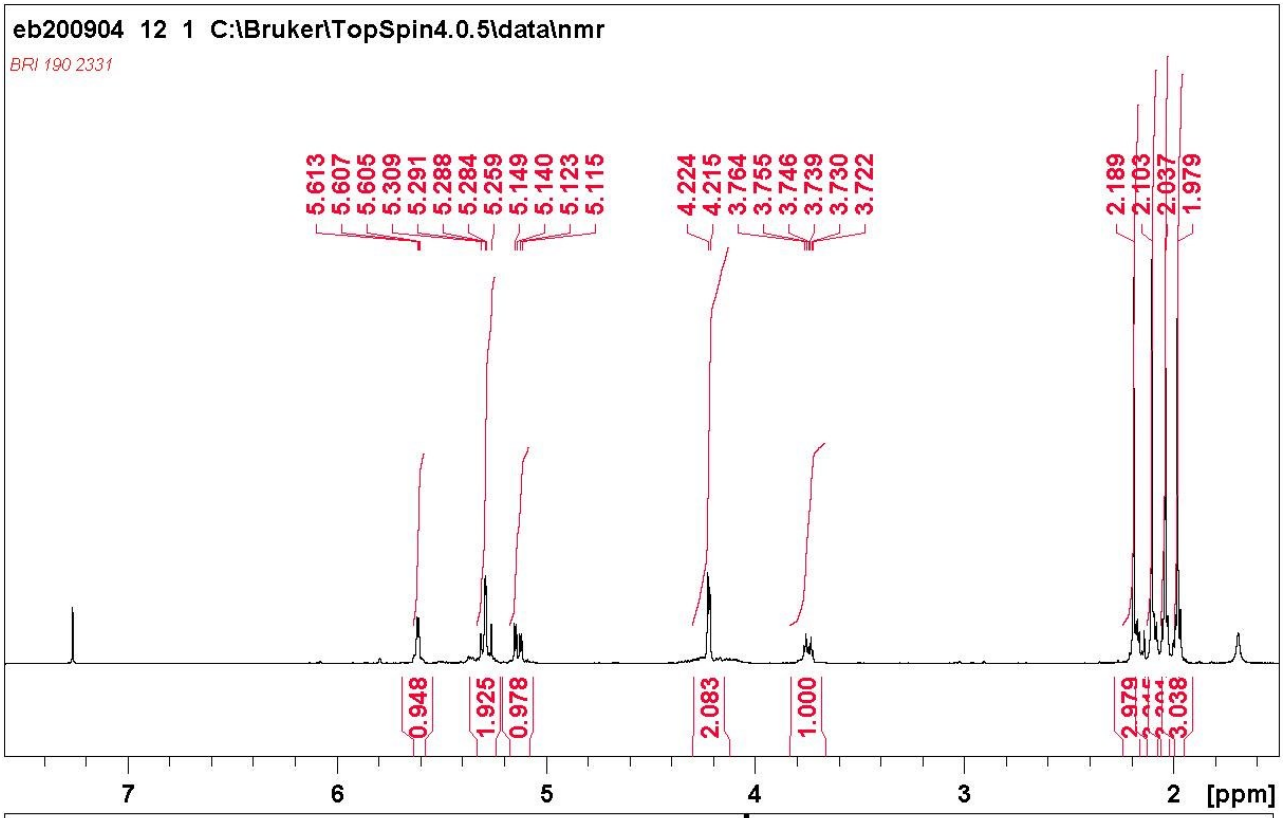

AcO
OAc
AcO
O
AcO
O
AcO
O
Se
AcO
AcO
AcO
2

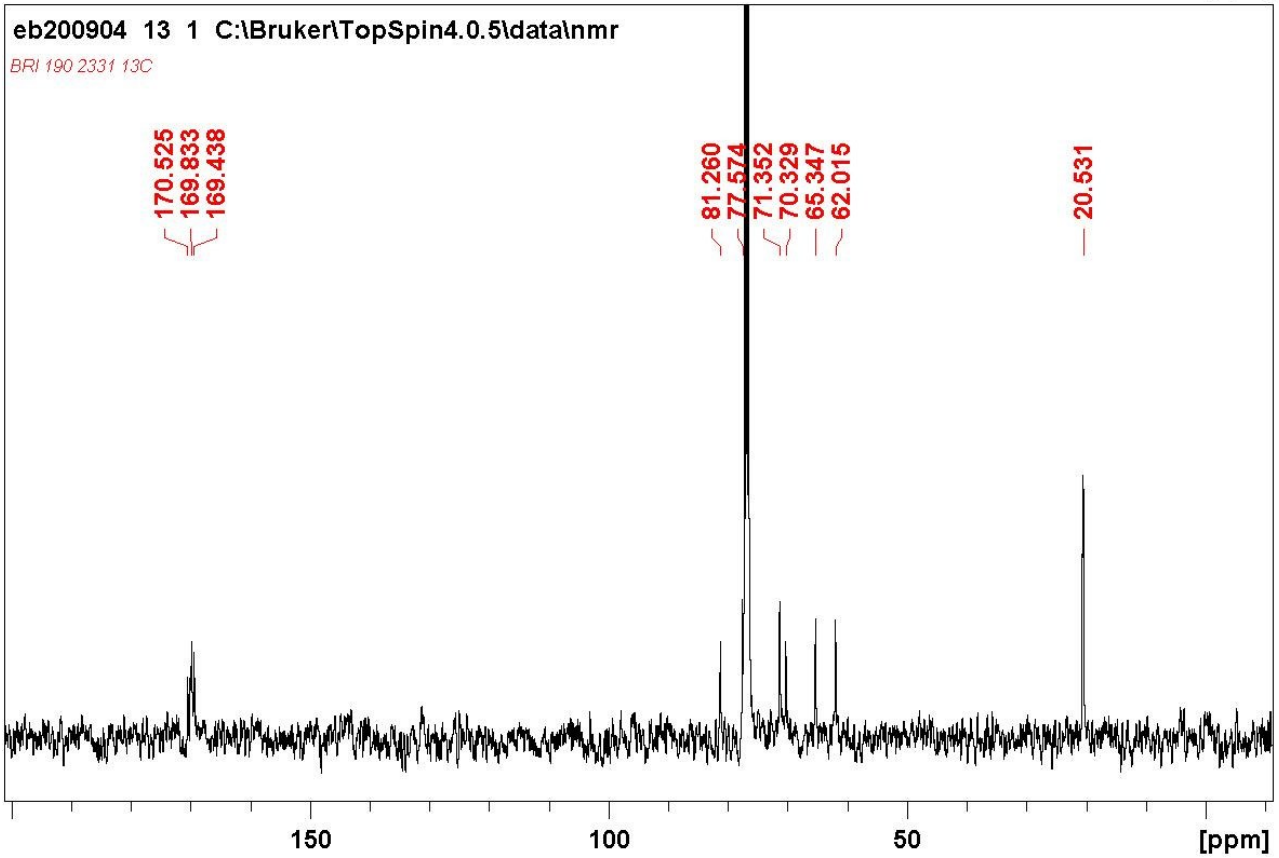
20

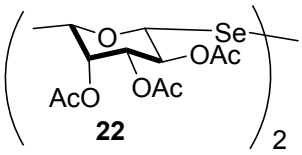

eb200524 4 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 59 col 3 crom Lac2Se2 xOAc
5.341 5.333 5.232 5.209 5.187 5.126 5.106 5.100 5.080 5.061 5.037 5.013 4.981 4.973 4.956 4.948 4.857 4.832 4.637 4.610 4.572 4.552 4.148 4.135 4.120 4.106 4.093 4.072 4.052 4.044 4.025 3.909 3.891 3.874 3.867 3.843 3.819 3.716 3.710
2.139 2.038 2.031 1.953
1.000 0.956 1.789 1.013 0.651 0.735 0.704 2.763 1.708 0.739 5.358 9.833 2.739
7 6 5 4 3 2 [ppm]

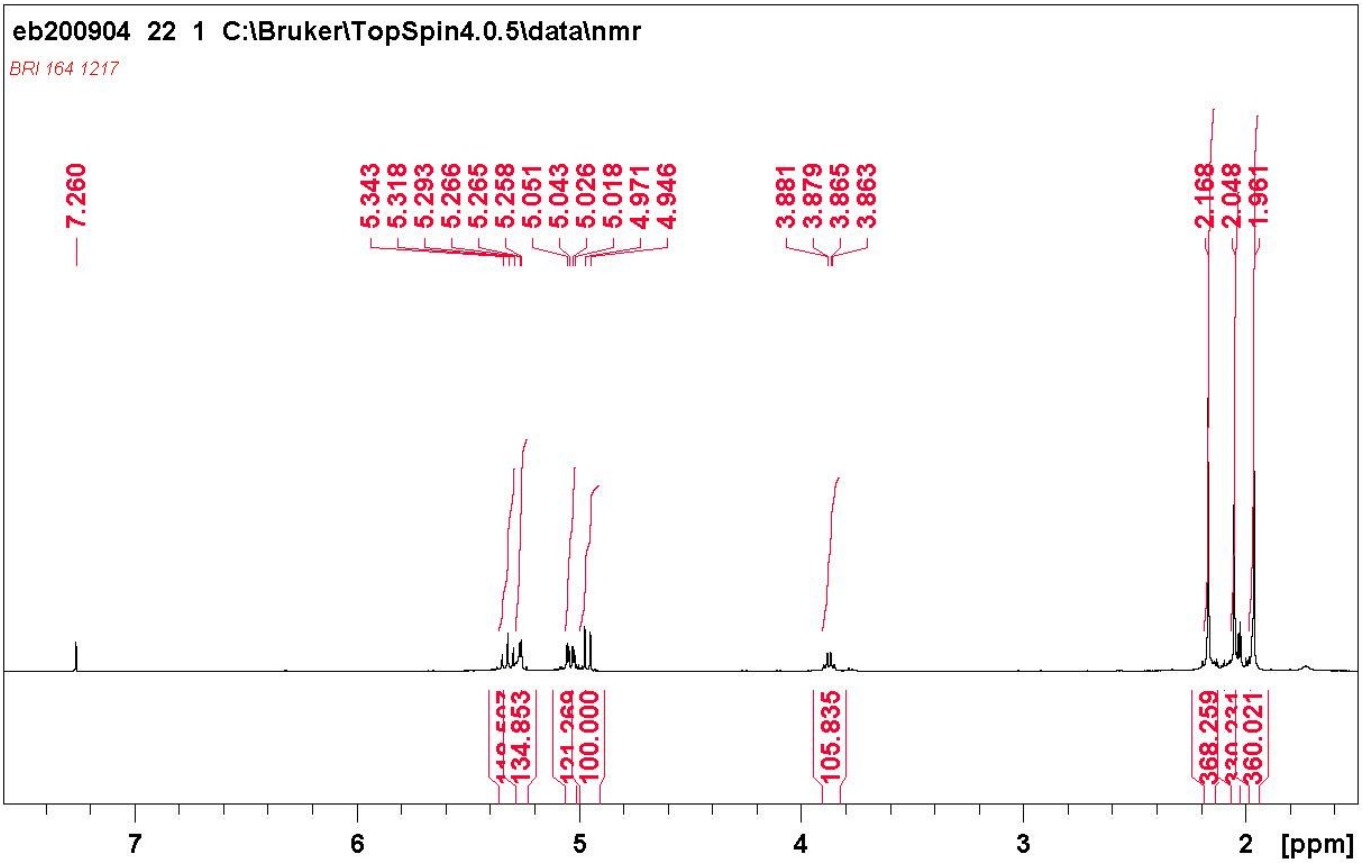

eb200524 5 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 59 col 3 crom Lac2Se2 xOAc 13C
170.162 169.555 168.912
100.815
79.482 77.635 75.697 73.533 72.015 70.886 70.427 68.988 66.469
61.550 60.507
20.651 20.491
160 140 120 100 80 60 40 20 [ppm]

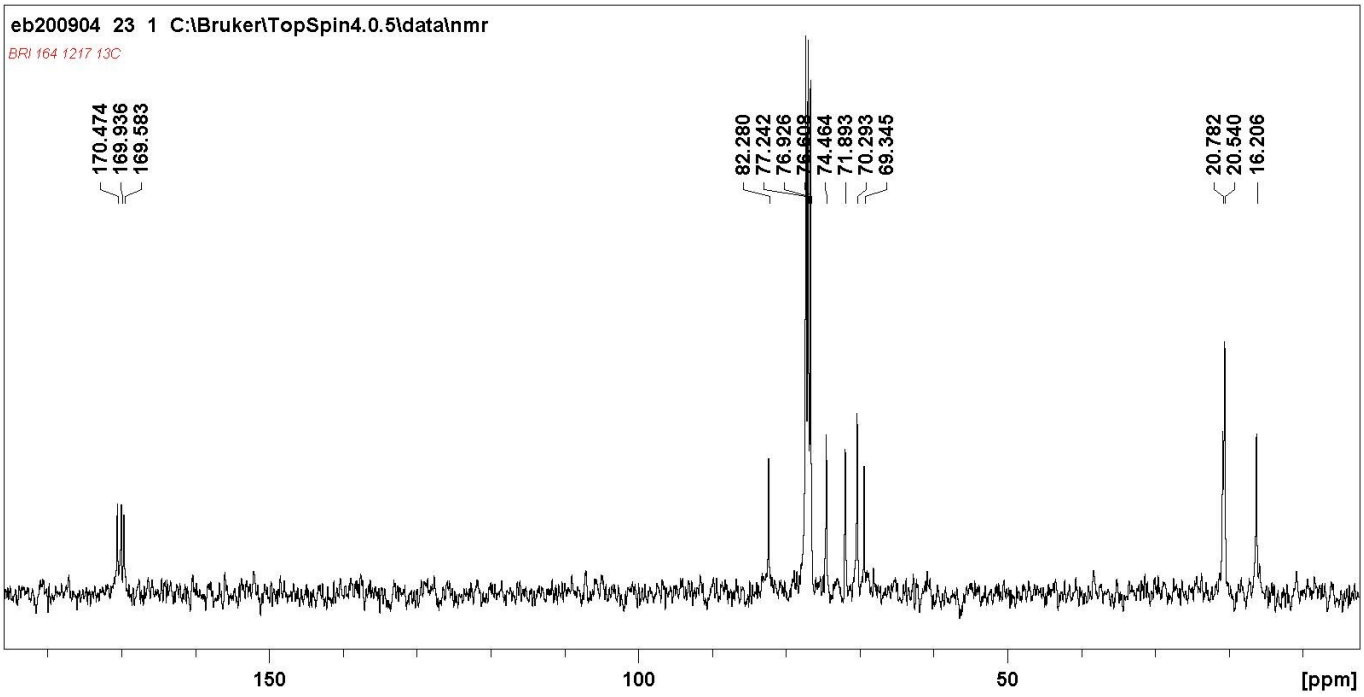

AcO
OAc
AcO
O
AcO
21
Se
2

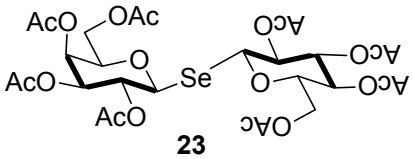

eb200904 12 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 190 2331
5.613
5.607
5.605
5.309
5.291
5.288
5.284
5.259
5.149
5.140
5.123
5.115
4.224
4.215
3.764
3.755
3.746
3.739
3.730
3.722
2.189
2.103
2.037
1.979
0.948
1.925
0.978
2.083
1.000
2.979
3.038
7
6
5
4
3
2
[ppm]


eb200904 13 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 190 2331 13C
170.525
169.833
169.438
81.260
77.574
71.352
70.329
65.347
62.015
20.531
150
100
50
[ppm]

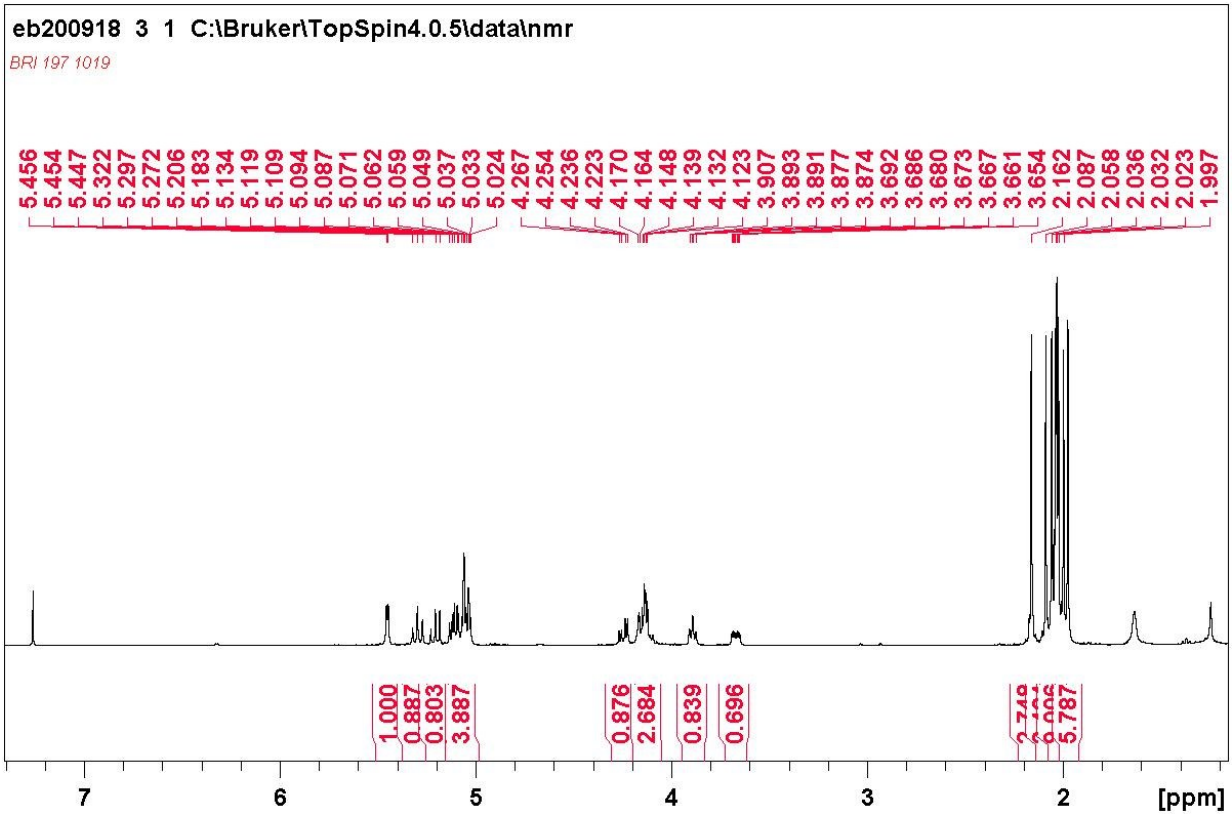

O
Se
OAc
AcO
OAc
22
2


eb200904 22 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 164 1217
7.260
5.343
5.318
5.293
5.266
5.265
5.258
5.051
5.043
5.026
5.018
4.971
4.946
3.881
3.879
3.865
3.863
2.168
2.048
1.961
134.853
121.269
100.000
105.835
368.259
230.231
360.021
7
6
5
4
3
2
[ppm]


eb200904 23 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 164 1217 13C
170.474
169.936
169.583
82.280
77.242
76.926
76.608
74.464
71.893
70.293
69.345
20.782
20.540
16.206
150
100
50
[ppm]


AcO
OAc
O
Se
AcO
AcO
AcO
O
AcO
AcO
OAc

23


eb200918 3 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 197 1019
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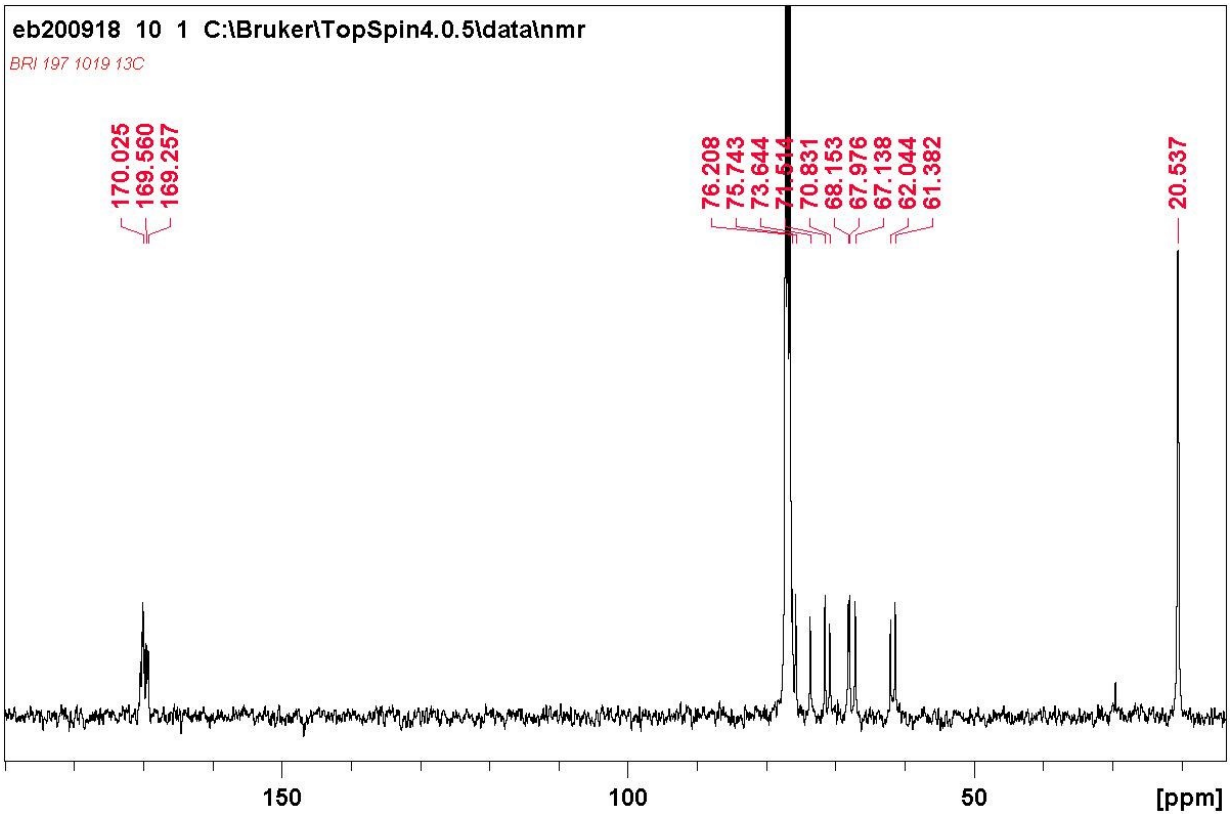
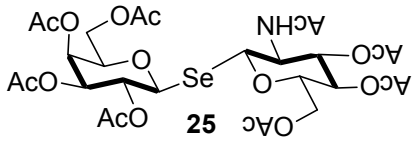
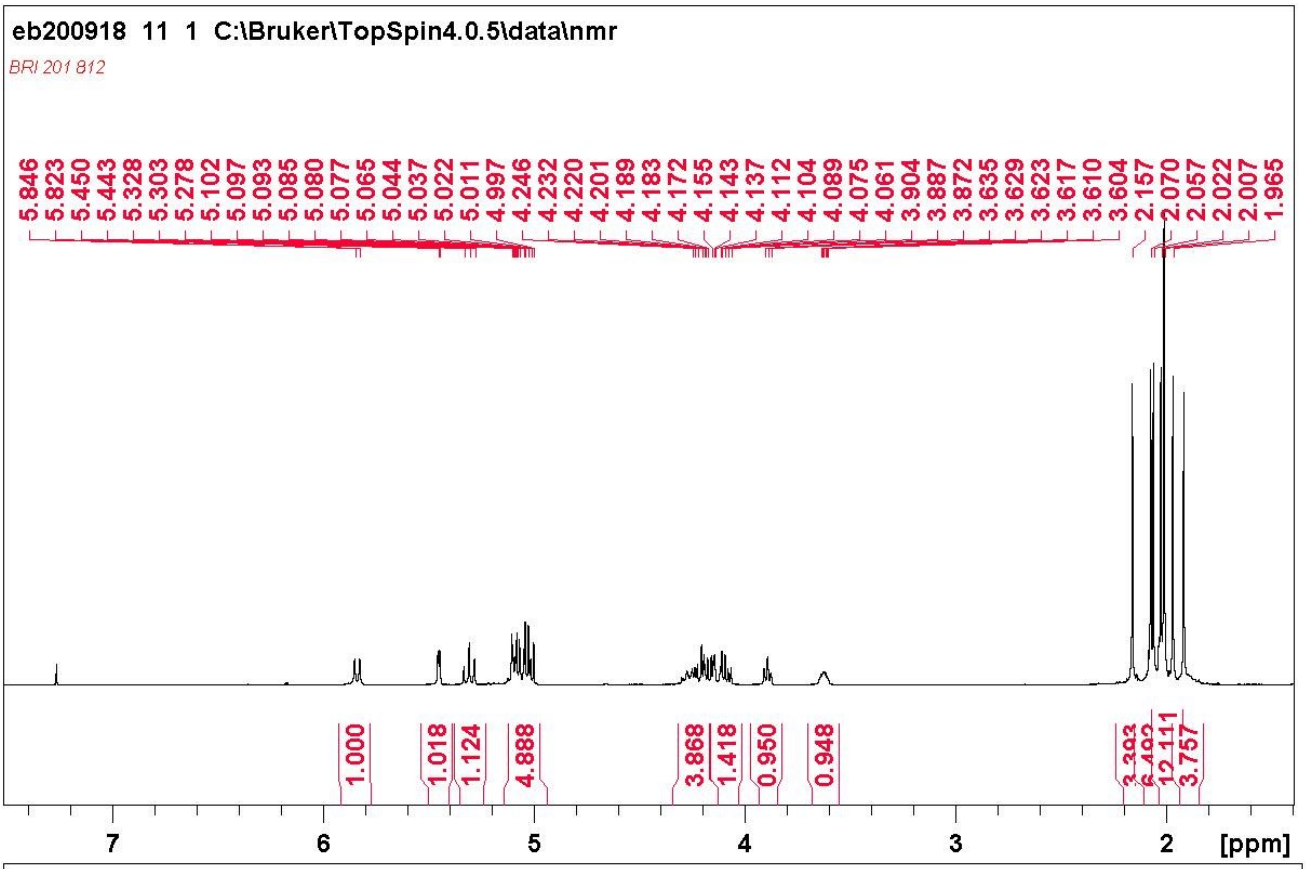
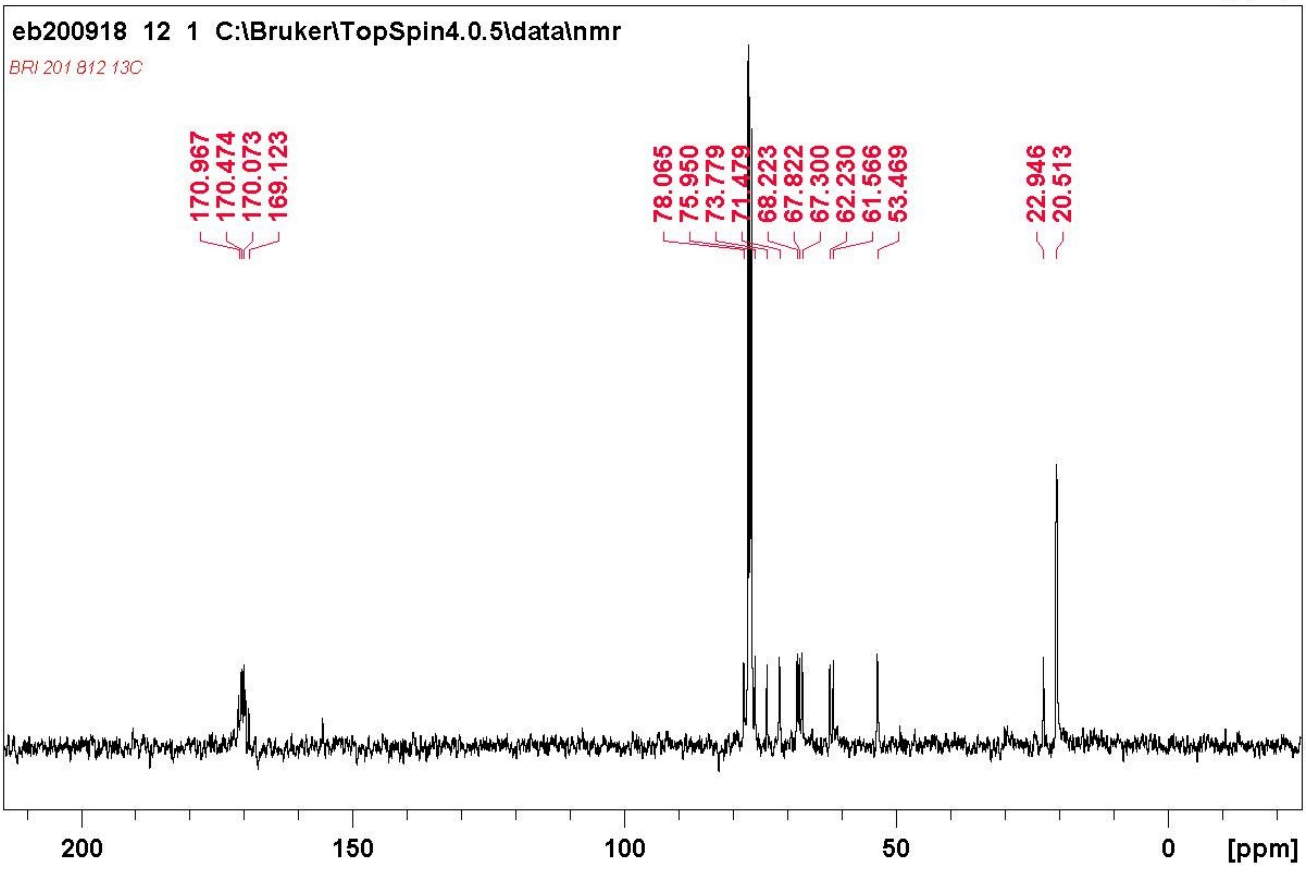
5.454
5.447
5.322
5.297
5.272
5.206
5.183
5.134
5.119
5.109
5.094
5.087
5.071
5.062
5.059
5.049
5.037
5.033
5.024
4.267
4.254
4.236
4.223
4.170
4.164
4.148
4.139
4.132
4.123
3.907
3.893
3.891
3.877
3.874
3.692
3.686
3.680
3.673
3.667
3.661
3.654
2.162
2.087
2.058
2.036
2.032
2.023
1.997
1.000
0.887
0.803
3.887
0.876
2.684
0.839
0.696
5.787
7
6
5
4
3
2
[ppm]

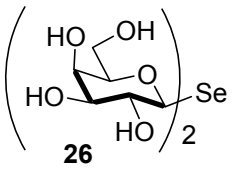

eb200918 10 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 197 1019 13C
170.025
169.560
169.257
76.208
75.743
73.644
71.514
70.831
68.153
67.976
67.138
62.044
61.382
20.537
150
100
50
[ppm]


AcO
OAc
AcO
O
Se
AcO
NHAc
O
AcO
AcO
OAc

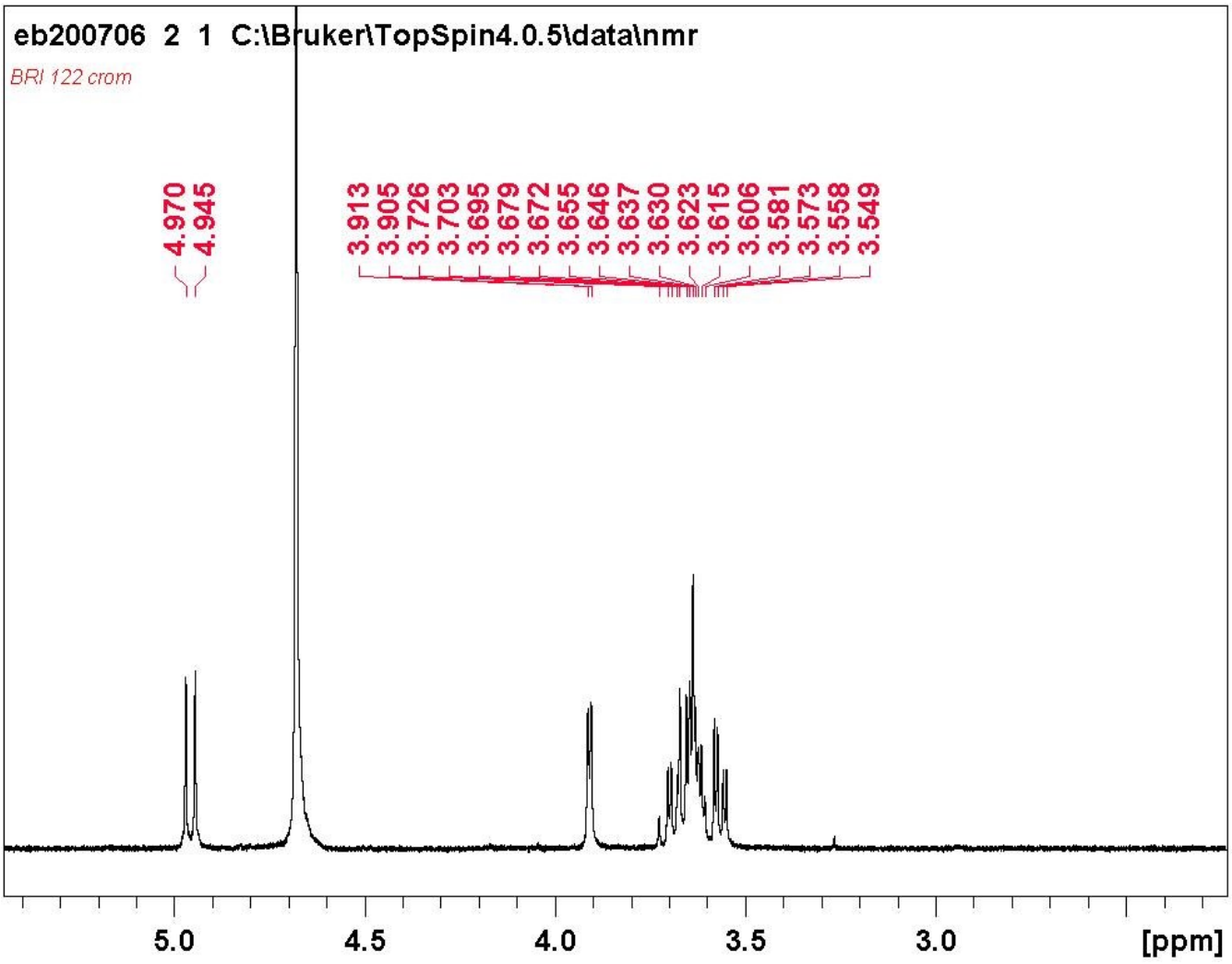
25

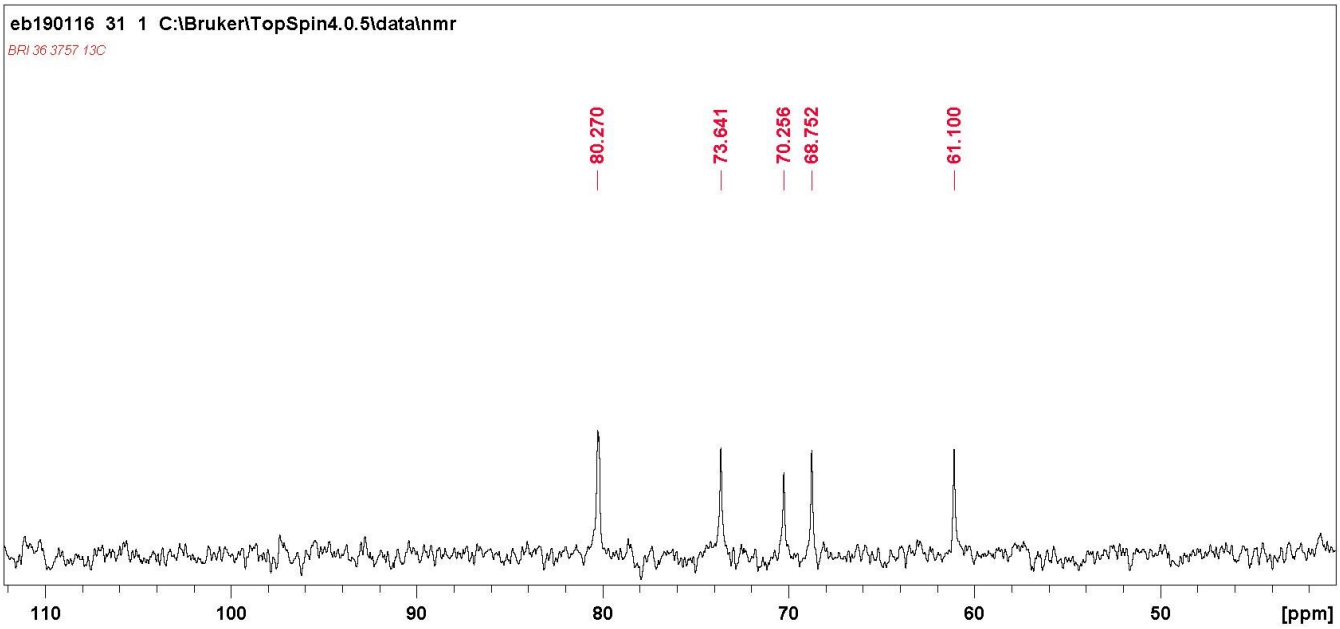

eb200918 11 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 201 812
5.846
5.823
5.450
5.443
5.328
5.303
5.278
5.102
5.097
5.093
5.085
5.080
5.077
5.065
5.044
5.037
5.022
5.011
4.997
4.246
4.232
4.220
4.201
4.189
4.183
4.172
4.155
4.143
4.137
4.112
4.104
4.089
4.075
4.061
3.904
3.887
3.872
3.635
3.629
3.623
3.617
3.610
3.604
2.157
2.070
2.057
2.022
2.007
1.965
1.000
1.018
1.124
4.888
3.868
1.418
0.950
0.948
12.111
3.757
7
6
5
4
3
2
[ppm]

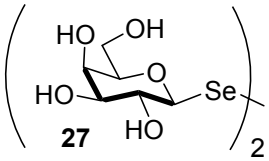

eb200918 12 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 201 812 13C
170.967
170.474
170.073
169.123
78.065
75.950
73.779
71.479
68.223
67.822
67.300
62.230
61.566
53.469
22.946
20.513
200
150
100
50
0
[ppm]

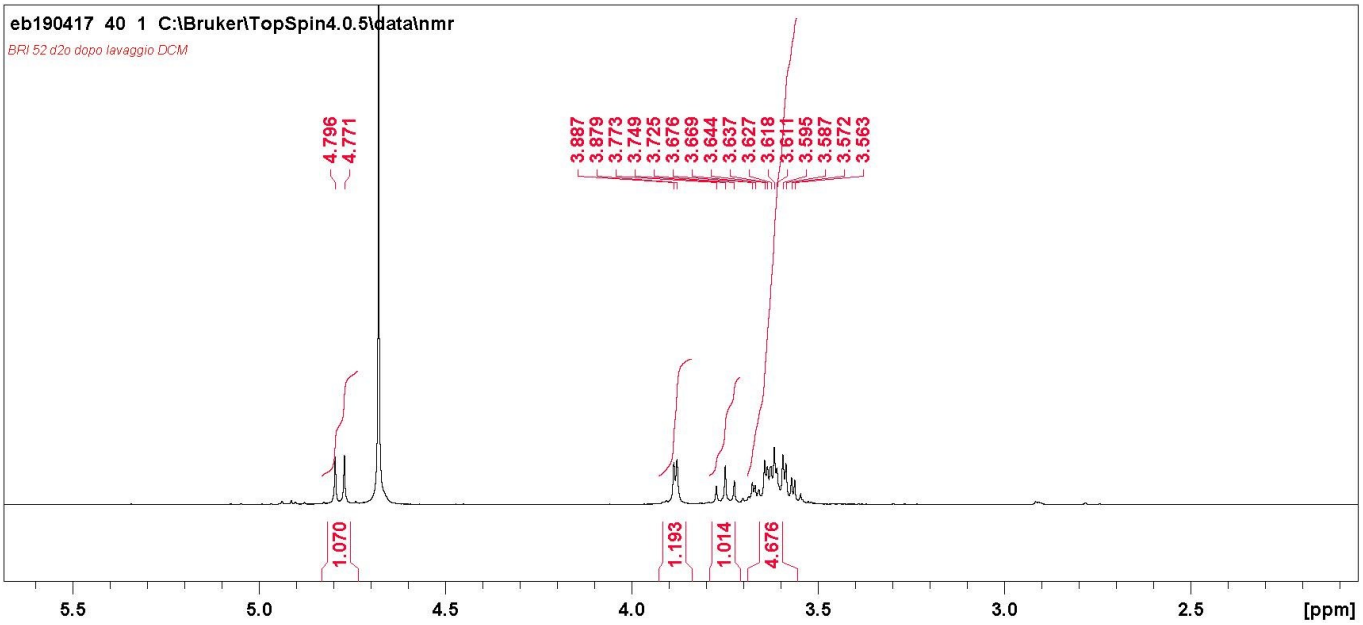

HO
OH
O
HO
Se
HO
2

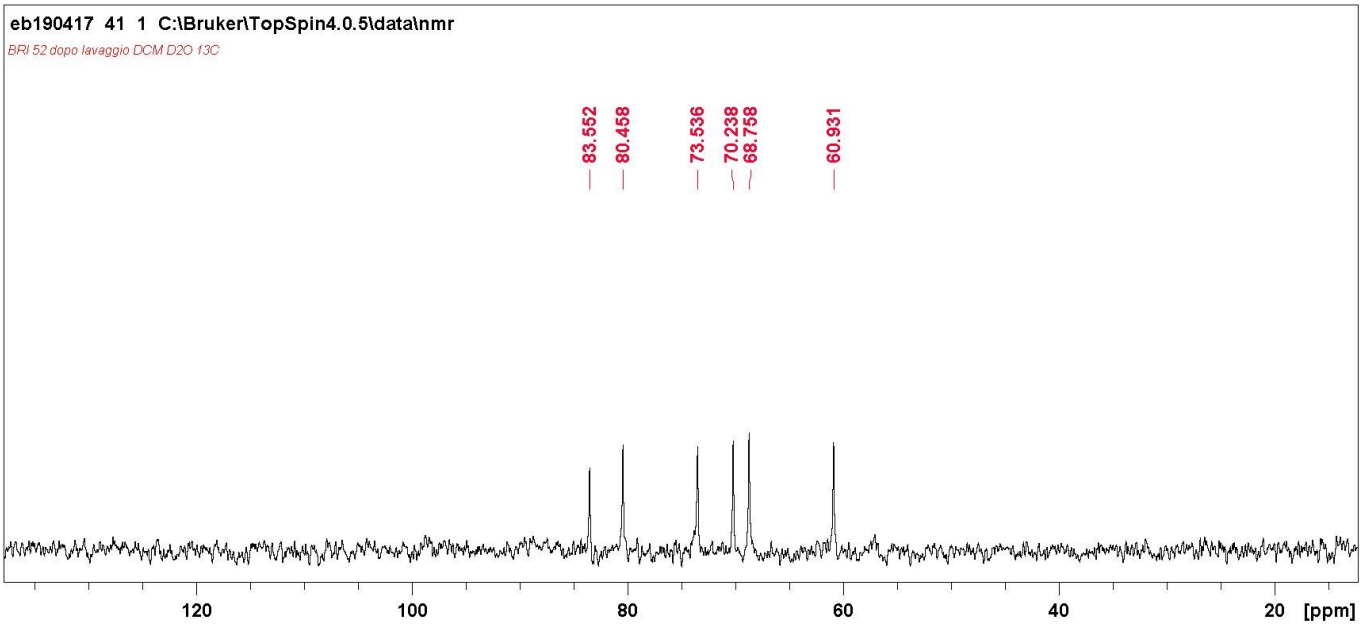
26


eb200706 2 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 122 crom
4.970
4.945
3.913
3.905
3.726
3.703
3.695
3.679
3.672
3.655
3.646
3.637
3.630
3.623
3.615
3.606
3.581
3.573
3.558
3.549
5.0
4.5
4.0
3.5
3.0
[ppm]


eb190116 31 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 36 3757 13C
80.270
73.641
70.256
68.752
61.100
110
100
90
80
70
60
50
[ppm]


HO
OH
O
HO
Se
HO
27
2


eb190417 40 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 52 d2o dopo lavaggio DCM
4.796
4.771
3.887
3.879
3.773
3.749
3.725
3.676
3.669
3.644
3.637
3.627
3.618
3.611
3.595
3.587
3.572
3.563
1.070
1.193
1.014
4.676
5.5
5.0
4.5
4.0
3.5
3.0
2.5
[ppm]


eb190417 41 1 C:\Bruker\TopSpin4.0.5\data\nmr
BRI 52 dopo lavaggio DCM D2O 13C
83.552
80.458
73.536
70.238
68.758
60.931
120
100
80
60
40
20
[ppm]

Experimental section for the biological activity

Culture conditions. Human T lymphoblastoid (Jurkat) and human melanoma WM266 cell lines were grown in RPMI medium supplemented with heat inactivated 10% fetal bovine serum (FBS), 2.5 mM glutamine, 100 U/ mL penicillin, and 100 μg/mL streptomycin (Euroclone). Human adenocarcinoma cell line (HeLa) was grown in DMEM supplemented with 10% fetal bovine serum (FBS), 1% glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Euroclone, Milano, Italy). Cells were maintained in humidified air containing 5% CO_2, at 37 °C.

Antiproliferative Activity. Cells were plated at density of 10000 cells/well for Jurkat, 2000/well for WM266 and 1200 cells/well for HeLa, in 96 well microplates (Corning). After 24h incubation, cells were treated with increasing concentration of synthetized compounds previously solubilized in H_2O at 10 mM concentration. Cell proliferation was determined by using (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (CCK-8 Sigma Aldrich) for Jurkat cells and the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide assay (MTT, Sigma Aldrich) for HeLa and WM266 cells, after 48h treatment. Plates were then analyzed by using a microplate reader (Enspire, Perkin Elmer, USA) at 450 (CCK-8) or 570 nm (MTT). The results are presented as the percentage of proliferating cells respect to the control (vehicle treated cells) and are expressed as means ± SE of, at least, two independent experiments performed in triplicate. The IC_{50} values were calculated by GraphPad Prism software.

Results

In vitro antiproliferative activity

The anti-proliferative effect of synthetized compounds was evaluated on three human tumor cell lines of disparate histological origin, leukemia (Jurkat), cervical adenocarcinoma (HeLa), metastatic melanoma cells (WM266). Cells were treated with the molecules at rising concentrations from 7 to 500 μM, for 48h. The digalactosyl selenide does not affect the proliferation of each cell line examined, also at concentration > 500 μM. Differently, digalactosyl diselenide shows a dose-dependent inhibition of proliferation of all tested cell lines. In particular, digalactosyl diselenide, displays a significant effect on WM266 and Jurkat cell proliferation, with IC50 values of 54.7 and 56.0 μM respectively, and a lower interference with the growth of HeLa cells (IC50=120μM).

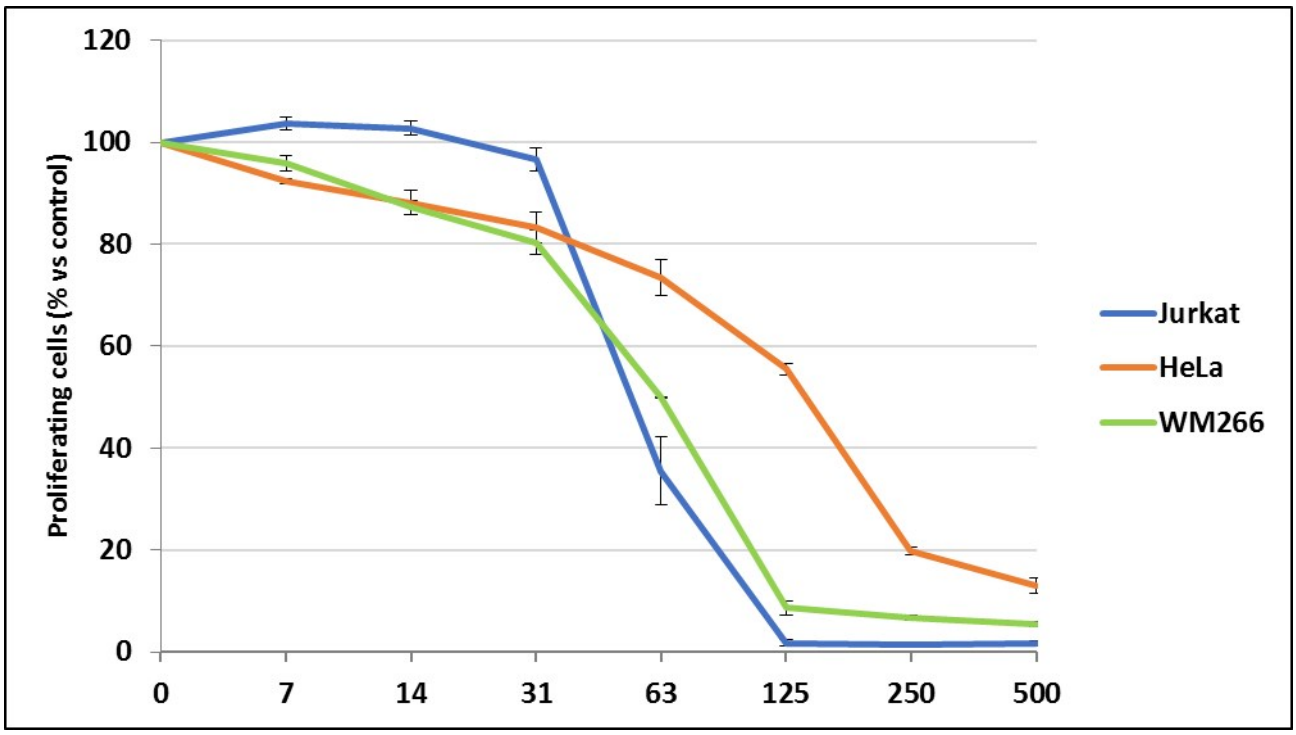


Figure. Anti–proliferative assay of digalactosyl diselenide **27** on Jurkat, WM266 and HeLa cell lines. The cells were incubated in the presence of compound at the indicated concentrations at 37 °C for 48h. The results are presented as the percentage of proliferating cells with respect to the control (vehicle treated cells) and are expressed as means ± SE.

Table: IC50 values

	IC50 (μM)		
Entry	WM266	Jurkat	HeLa
	54.7 ± 18	56.0 ± 24	120 ± 16