Thio-ether functionalized glycolipid amphiphilic compounds reveal a potent activator of SK3 channel with vasorelaxation effect.

C. M. Sevrain, D. Fontaine, A. Bauduin, M. Guéguinou, B.L. Zhang, A. Chantôme, K. Mahéo, C. Pasqualin, V. Maupoil, H. Couthon, C. Vandier, P.A. Jaffrès

1. Synthesis of compounds **1-4**.

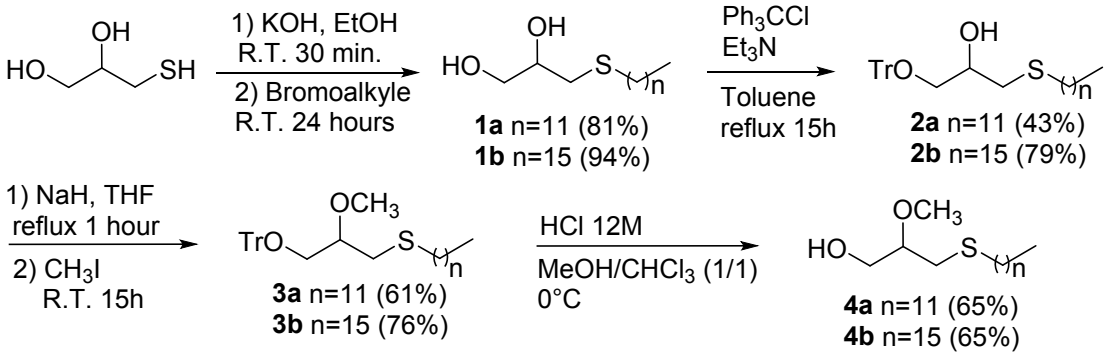

Figure ESI1-1 : synthesis of **4a** and **4b** starting from thioglycerol

1-S-dodecyl-rac-thioglycerol (**1a**).[1] To a stirred solution of KOH (3.1 g, 55.5 mmol, 1.8 eq.) in ethanol (40 mL) is added α-thioglycerol (4.9 mL, 55.5 mmol, 1.2 eq.) dropwise. The mixture is stirred 30 minutes at room temperature and bromododecane (11.1 mL, 46.2 mmol, 1.0 eq.) is slowly added. The reaction mixture is stirred 2 days at room temperature. The white solid is filtered and dried, the filtrated is concentrated. The oil is diluted in ethyl acetate (100 mL) and the organic layer is washed with water (30 mL), dried over $MgSO_4$, filtrated and concentrated to give the crude compound 1a as a white solid. Compound **1a** is purified by recrystallization in hexane (V = 60 mL) to give **1a** in 81 % overall yield (10.3 g). Rf (hexane/ethyl acetate: 20/80): 0.1; ^{1}H NMR ($CDCl_3$, 300.131): 3.81-3.74 (m, 2 H, CH_2 *sn*-3 + CH *sn*-2); 3.56 (dd, 1 H, J_{HH} = 6, J_{HH} = 12 Hz, CH_2 *sn*-3); 2.71 (dd, 1H, J_{HH} = 4 Hz, J_{HH} = 14 Hz, CH_2 *sn*-1 (Ha)) ; 2.60 (dd, 1 H, J_{HH} = 8 Hz, J_{HH} = 14 Hz, CH_2 *sn*-1 (Hb)); 2.53 (t, 2 H, J_{HH} = 7 Hz, CH_2 α fatty chain); 1.58 (qt, 2 H, J_{HH} = 7 Hz, CH_2 β fatty chain); 1.36 (qt, 2 H, J_{HH} = 7 Hz, CH_2 γ fatty chain); 1.26 (s, 16 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH} = 7 Hz, CH_3 fatty chain);

1-S-hexadecyl-rac-thioglycerol (**1b**)[2]. To a stirred solution of KOH (2.6 g, 46.2 mmol, 1.2 eq.) in ethanol (40 mL) is added α-thioglycerol (4.0 mL, 46.2 mmol, 1.2 eq.) dropwise. The mixture is stirred 30 minutes at room temperature and bromohexadecane (11.8 mL, 38.5 mmol, 1.0 eq.) is slowly added. The reaction mixture is stirred 24 hours at room temperature. The white solid is filtered and dried, the filtrated is concentrated. The oil is diluted in ethyl acetate (100 mL) and the organic layer is washed with water (30 mL), a saturated solution of NH_4Cl (30 mL) and a saturated solution of NaCl (30 mL), dried over $MgSO_4$, filtrated and concentrated to give **1b** as a white solid in 81% yield (10.4 g). Rf (petroleum ether/ethyl acetate: 1/10): 0.24 ; ^{1}H NMR ($CDCl_3$, 399.992): 3.81-3.74 (m, 2 H, CH_2 *sn*-3); 3.58 (q, 1 H, J_{HH} = 5.6 Hz, C*H sn*-2); 2.74-2.56 (m, 2 H, CH_2 *sn*-1) ; 2.53 (t, 2 H, J_{HH} = 7.4 Hz, CH_2 α fatty chain); 2.06 (brs, 2 H, 2 O*H*); 1.58 (qt, 2 H, J_{HH} = 7.3 Hz, CH_2 β fatty chain); 1.38-1.33 (m, 2 H, CH_2 γ fatty chain); 1.33-1.21 (m, 24 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH} = 6.6 Hz, CH_3 fatty chain) ; ^{13}C NMR ($CDCl_3$, 75.474): 71.0 (CH *sn*-2); 66.8 (CH_2 *sn*-3); 37.2 (CH_2 *sn*-1); 33.7 (CH_2 α fatty chain); 33.3 (CH_2 fatty chain); 31.0 (CH_2 fatty chain); 30.8 (CH_2 fatty chain); 30.7 (CH_2 fatty chain); 30.5 (CH_2 fatty chain); 30.2 (CH_2 fatty chain); 24.0 (CH_2 fatty chain); 15.4 (CH_3 fatty chain)

*1-S-dodecyl-3-O-trityl-rac-thioglycerol (***2a***)*[3]. To a stirred solution of **1a** (4.0 g, 14.5 mmol, 1.0 eq.) in dry toluene (60 mL) is added trityl chloride (6.1 g, 21.8 mmol, 1.5 eq.) and triethylamine (4.9 mL, 34.8 mmol, 2.4 eq.) dropwise. The mixture is stirred at reflux during 2 days, filtered upon Celite and concentrated. The oil is dissolved in petroleum ether (40 mL) and the mixture is filtered and concentrated to give the crude compound **2a**. This product is purified on chromatography on silica gel: Eluent (hexane/ethyl acetate (80/20) + 1% of trimethylamine) to give **2a** in 43 % overall yield (3.23 g). Rf (hexane/ethyl acetate: 80/20): 0.84 ; ^{1}H NMR (CH_3OD, 300.131): 7.19-7.46 (m, 15 H, C*H* Phenyl); 3.81 (qt, 1 H, J_{HH}= 6 Hz, C*H sn-2*); 3.17 (d, 2 H, J_{HH}= 6 Hz, CH_2 *sn*-3); 2.75 (dd, 1 H, J_{HH}= 15 Hz, J_{HH}= 6 Hz, CH_2 *sn*-1); 2.56 (dd, 1 H, J_{HH}= 12 Hz, J_{HH}= 6 Hz, CH_2 *sn*-1); 2.45 (t, 2 H, J_{HH}= 6 Hz, CH_2 α fatty chain); 1.51 (qt, 2 H, J_{HH} = 6 Hz, CH_2 β fatty chain); 1.25 (s, 18 H, CH_2 fatty chain); 0.89 (t, 3 H, J_{HH}= 6 Hz, CH_3 fatty chain) ; ^{13}C NMR ($CDCl_3$, 125.771): 143.8 (C quat. trityl); 128.9 (CH trityl); 127.8 (CH trityl); 127.1 (CH trityl); 86.8 (Cq_{ar});69.4 (CH *sn*-2) 66.4 (CH_2 *sn*-3); 34.2 (CH_2 *sn*-1); 36.4 (CH_2 α fatty chain); 32.5 (CH_2 fatty chain); 31.9 (CH_2 fatty chain); 29.7 (CH_2 fatty chain); 29.6 (CH_2 fatty chain); 29.4 (CH_2 fatty chain); 29.3 (CH_2 fatty chain); 22.7 (CH_2 fatty chain); 14.1 (CH_3 fatty chain);

[1] H.A. Van Doren, R. Van der Geest, R.M. Kellogg, and H. Wynberg, *Recl. Trav. Chim. Pays-B.* 1990, **109**, 197-203.
[2] S. Morris-Natschke, J. R. Surles, L. W. Danie, M. E. Berens, E. J. Modest and C. Piantadosi, *J. Med. Chem*., 1986, **29**, 2114-2117.
[3] L. S. Kucera, S. L. Morris-Natschke, K. S. Ishaq, J. Hes, N. Iyer, P.A. Furman and R. A. Fleming, *Nucleos. Nucleot. Nucl.*, 2004, **23**, 385-399.

*1-S-hexadecyl-3-O-trityl-rac-thioglycerol (***2b***)*[2]. To a stirred solution of **1b** (12.0 g, 36.1 mmol, 1.0 eq.) in dry toluene (40 mL) is added trityl chloride (15.1 g, 54.2 mmol, 1.5 eq.) and triethylamine (12.1 mL, 86.6 mmol, 2.4 eq.) dropwise. The mixture is stirred at reflux overnight, filtered upon Celite and concentrated. The oil is dissolved in petroleum ether (40 mL) and the mixture is filtered and concentrated to give **2b**, which was used without further purification (16.4 g; 79 % yield). Rf (petroleum ether/ethyl acetate: 3/10): 0.48 ; ^{1}H NMR ($CDCl_3$, 399.992): 7.32-7.17 (m, 15 H, C*H* Phenyl); 3.90-3.82 (m, 1 H, C*H sn-2*); 3.29 (dd, 1 H, J_{HH}= 18.4 Hz; J_{HH}= 9.6 Hz, CH_{2a} *sn*-3); 3.27 (dd, 1 H, J_{HH}= 17.6 Hz, J_{HH}= 9.2 Hz, CH_{2b} *sn*-3); 2.78 (dd, 1 H, J_{HH}= 13.6 Hz, J_{HH}= 4.8 Hz, CH_{2a} *sn*-1); 2.64 (dd, 1 H, J_{HH}= 13.8 Hz, J_{HH}= 7.6 Hz, CH_{2b} *sn*-1); 2.48 (t, 2 H, J_{HH}= 7.4 Hz, CH_2 α fatty chain); 1.56 (qt, 2 H, J_{HH}= 7.3 Hz, CH_2 β fatty chain); 1.36-1.28 (m, 26 H, CH_2 fatty chain); 0.90 (t, 3 H, J_{HH}= 6.8 Hz, CH_3 fatty chain).

*1-S-dodecyl-2-O-methyl-3-O-trityl-rac-thioglycerol (***3a***).* To a stirred solution of NaH (210 mg, 8.6 mmol, 1.4 eq.) in dry THF (40 mL) is added **2a** (3.2 g, 6.2 mmol, 1.0 eq.) in solution in dry THF (25 mL) dropwise. The mixture is stirred 1 hour and CH_3I (1.34 mL, 21.7 mmol, 1.5 eq.) in solution in dry THF (25 mL) is added dropwise. The reaction mixture is stirred at room temperature overnight. The reaction is quenched by addition of water (few mL) and the solvent is removed. The oil is dissolved in diethyl ether (100 mL). The organic layer is washed three times with water (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated to give the crude compound **3a** which is purified on chromatography on silica gel: Eluent (hexane / ethyl acetate (85 / 15)) to give **3a** in 61 % overall yield (2.15 g). Rf (hexane/ethyl acetate: 80/20): 0.93 ; ^{1}H NMR ($CDCl_3$, 500.253): 7.45-7.42 (m, 6 H, C*H* Phenyl); 7.27-7.19 (m, 9H, C*H* Phenyl); 3.40 (qt, 1 H, J_{HH}=6 Hz; C*H sn-2); 3.36 (*s, 3H, OCH_3); 3.26 (dd, 1 H, J_{HH}=6 Hz, J_{HH}=9 Hz, CH_2 *sn-3*); 3.20 (dd, 1 H, J_{HH}= 6 Hz, J_{HH}= 12 Hz, CH_2 *sn-3*); 2.71 (dd, 1 H, J_{HH}= 12 Hz, J_{HH}= 6 Hz, CH_2 *sn*-1); 2.62 (dd, 1 H, J_{HH}= 15 Hz, J_{HH}=6 Hz, CH_2 *sn*-1); 2.45 (t, 2 H, J_{HH}= 6 Hz, CH_2 α fatty chain); 1.50 (qt, 2 H, J_{HH} = 6 Hz, CH_2 β fatty chain); 1.27 (s, 18 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH}= 6 Hz, CH_3 fatty chain) ; ^{13}C NMR ($CDCl_3$, 125.803): 144.0 (C quat. trityl); 129 (CH trityl); 128 (CH trityl); 127 (CH trityl); 86.9 (C_q-O); 80.9 (CH *sn*-2); 64.2 (CH_2 *sn*-3); 58.1 (OCH_3); 34.3 (CH_2 *sn*-1); 33.0 (CH_2 α fatty chain); 32.0 (CH_2 fatty chain); 29.7 (CH_2 fatty chain); 29.6 (CH_2 fatty chain); 29.5 (CH_2 fatty chain); 29.4 (CH_2 fatty chain); 29.3 (CH_2 fatty chain); 28.9 (CH_2 fatty chain); 22.7 (CH_2 fatty chain); 14.1 (CH_3 fatty chain);

*1-S-hexadecyl-2-O-methyl-3-O-trityl-rac-thioglycerol (***3b***)*[2]. To a stirred solution of NaH (485 mg, 20.2 mmol, 1.4 eq.) in dry THF (50 mL) is added **2b** (8.5 g, 14.5 mmol, 1.0 eq.) in solution in dry THF (25 mL) dropwise. The mixture is stirred 1 hour and CH_3I (1.34 mL, 21.7 mmol, 1.5 eq.) in solution in dry THF (25 mL) is added dropwise. The reaction mixture is stirred at room temperature overnight. The reaction is quenched by addition of water (few mL) and the solvent is removed. The oil is dissolved in diethyl ether (100 mL). The organic layer is washed three times with an aqueous saturated NaCl solution (3 x 20 mL), dried over $MgSO_4$, filtered and concentrated to give **3b** which was used without further purification (6.71 g; 76 % yield). Rf (petroleum ether/ethyl acetate: 3/10): 0.75 ; ^{1}H NMR ($CDCl_3$, 399.992): 7.43-7.21 (m, 15 H, C*H* Phenyl); 3.45-3.40 (m, 4 H, C*H sn-2* + OCH_3); 3.24-3.22 (m, 2 H, CH_2 *sn-3*); 2.77 (dd, 1 H, J_{HH}= 13.8 Hz, J_{HH}= 5.2 Hz, CH_{2a} *sn*-1); 2.69 (dd, 1 H, J_{HH}= 13.6 Hz, J_{HH}= 6.4 Hz, CH_{2b} *sn*-1); 2.48 (t, 2 H, J_{HH}= 7.4 Hz, CH_2 α fatty chain); 1.54 (qt, 2 H, J_{HH} = 7.2 Hz, CH_2 β fatty chain); 1.36-1.25 (m, 26 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH}= 6.6 Hz, CH_3 fatty chain) ; ^{13}C NMR ($CDCl_3$, 75.474): 144.1 (C quat. trityl); 128.8 (CH trityl); 127.9 (CH trityl); 127.1 (CH trityl); 80.9 (CH *sn*-2); 64.4 (CH_2 *sn*-3); 58.3 (OCH_3); 34.2 (CH_2 *sn*-1); 33.2 (CH_2 α fatty chain); 32.0 (CH_2 fatty chain); 29.8 (CH_2 fatty chain); 29.7 (CH_2 fatty chain); 29.5 (CH_2 fatty chain); 29.4 (CH_2 fatty chain); 29.0 (CH_2 fatty chain); 22.8 (CH_2 fatty chain); 14.2 (CH_3 fatty chain).

1-S-dodecyl-2-O-methyl-rac-thioglycerol (**4a**). To a stirred solution of MeOH/$CHCl_3$ (1/1; 25 mL) is added HCl concentrated (320 µL, 3.9 mmol, 1.0 eq.). At 0°C **3a** (2.0g, 3.9 mmol, 1.0 eq.) in solution in MeOH/$CHCl_3$ (1/1; 25 mL) is added dropwise. The mixture is stirred 6 hours at 0°C. An aqueous saturated $NaHCO_3$ solution (30 mL) is added and the mixture is stirred 15 minutes at room temperature. The aqueous layer is extracted three times with $CHCl_3$ (3 x 70 mL) and the combined organic layers are washed three time with water (3 x 50 mL). The organic layer is dried upon $MgSO_4$, filtered and concentrated to give the crude compound **4a**. The product is purified on chromatography on silica gel (eluent: hexane / ethyl acetate (8:2 to 6:4)) to give **4a** in 65 % overall yield (730 mg). Rf (hexane/ethyl acetate: 8/2): 0.22; ^{1}H NMR ($CDCl_3$, 300.131): 3.76 dd, 1 H, J_{HH} = 6 Hz, J_{HH} = 3 Hz, CH_2 *sn-3*); 3.59 (dd, 1H, J_{HH} = 12 Hz, J_{HH} = 6 Hz, CH_2 *sn-3*); 3.42 (s, 3 H, OCH_3); 3.36 (m, 1 H, C*H sn-2*); 2.70 (dd, 1 H, J_{HH} =15 Hz, J_{HH} = 6 Hz, CH_2 *sn-1*); 2.58 (dd, 1 H, J_{HH}= 12 Hz, J_{HH}= 6 Hz, CH_2 *sn-1*); 2.52 (t, 2 H, J_{HH} = 6 Hz, CH_2 α fatty chain); 2.28 (s, 1 H, O*H*); 1.54 (qt, 2 H, J_{HH}= 6 Hz, CH_2 β fatty chain); 1.23 (s, 18 H, CH_2 fatty chain); 0.85 (t, 3 H, J_{HH}= 6 Hz, CH_3 fatty chain) ; ^{13}C NMR ($CDCl_3$, 125.771): 81.1 (*C*H *sn-2*); 63.0 (*C*H_2 *sn-3*); 57.3 (O*C*H_3); 33.3 (*C*H_2 *sn-1*); 31.9 (CH_2 α fatty chain); 31.7 (*C*H_2 fatty chain); 29.5 (*C*H_2 fatty chain); 29.4 (*C*H_2 fatty chain); 29.3 (*C*H_2 fatty chain); 29.2 (*C*H_2 fatty chain); 29.0 (*C*H_2 fatty chain); 28.7 (*C*H_2 fatty chain); 22.5 (*C*H_2 fatty chain); 14.0 (*C*H_3 fatty chain)

1-S-hexadecyl-2-O-methyl-rac-thioglycerol (**4b**)[2]. To a stirred solution of MeOH/$CHCl_3$ (1/1, 70 mL) is added HCl concentrated (560 µL, 10.0 mmol, 1.0 eq.). At 0°C 3b (5.90 g, 10.0 mmol, 1.0 eq.) in solution in MeOH/$CHCl_3$ (1/1; 70 mL) is added dropwise. The mixture is stirred 48 hours at 0°C. An aqueous saturated $NaHCO_3$ solution (30 mL) is added and the mixture is stirred 15 minutes at room temperature. The aqueous layer is extracted three times with $CHCl_3$ (3 x 70 mL) and the combined organic layers are washed three time with an aqueous saturated NaCl solution (3 x 50 mL). The organic layer is dried upon $MgSO_4$, filtered and

concentrated to give the crude compound **4b**. The product is purified on chromatography on silica gel (eluant: petroleum ether/ ethyl acetate (9:1 to 8:2)) to give **4b** as a solid in 65 % overall yield (2.2 g). Rf (petroleum ether/ethyl acetate: 9/1): 0.17; ^{1}H NMR ($CDCl_3$, 300.131): 3.80 (ABX, part A, dd, 1 H, J= 11.7 Hz, J_{HH}= 3.6 Hz, H_a C*H*$_2$ *sn-3*); 3.61 (ABX, part B, dd, 1 H, J_{HH}= 11.7 Hz, J_{HH}= 5.4 Hz, H_b C*H*$_2$ *sn-3*); 3.42 (s, 3 H, OC*H*$_3$); 3.41-3.35 (m, 1 H, C*H* *sn-2*); 2.80 (ABX, part B, dd, 1 H, J_{HH}= 13.5 Hz, J_{HH}= 5.1 Hz, H_a C*H*$_2$ *sn-1*); 2.62 (ABX, part B, dd, 1 H, J_{HH}= 13.5 Hz, J_{HH}= 7.5 Hz, H_b C*H*$_2$ *sn-1*); 2.55 (t, 2 H, J_{HH} = 7.4 Hz, C*H*$_2$ α fatty chain); 2.18 (brs, 1 H, O*H*); 1.59 (qt, 2 H, J_{HH}= 7.4 Hz, C*H*$_2$ β fatty chain); 1.40-1.23 (m, 26 H, C*H*$_2$ fatty chain); 0.88 (t, 3 H, J_{HH}= 6.6 Hz, C*H*$_3$ fatty chain) ; ^{13}C NMR ($CDCl_3$, 75.475): 81.3 (*C*H *sn-2*); 63.3 (*C*H$_2$ *sn-3*); 57.6 (O*C*H$_3$); 33.3 (*C*H$_2$ *sn-1*); 32.2 (CH$_2$ α fatty chain); 32.0 (*C*H$_2$ fatty chain); 29.8 (*C*H$_2$ fatty chain); 29.6 (*C*H$_2$ fatty chain); 29.5 (*C*H$_2$ fatty chain); 29.3 (*C*H$_2$ fatty chain); 29.0 (*C*H$_2$ fatty chain); 22.8 (*C*H$_2$ fatty chain); 14.2 (*C*H$_3$ fatty chain).

2. NMR Spectra
   When impurities are detected, we have noted them with a star on the spectra.

1-S-hexadecyl-2-O-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**6b**).

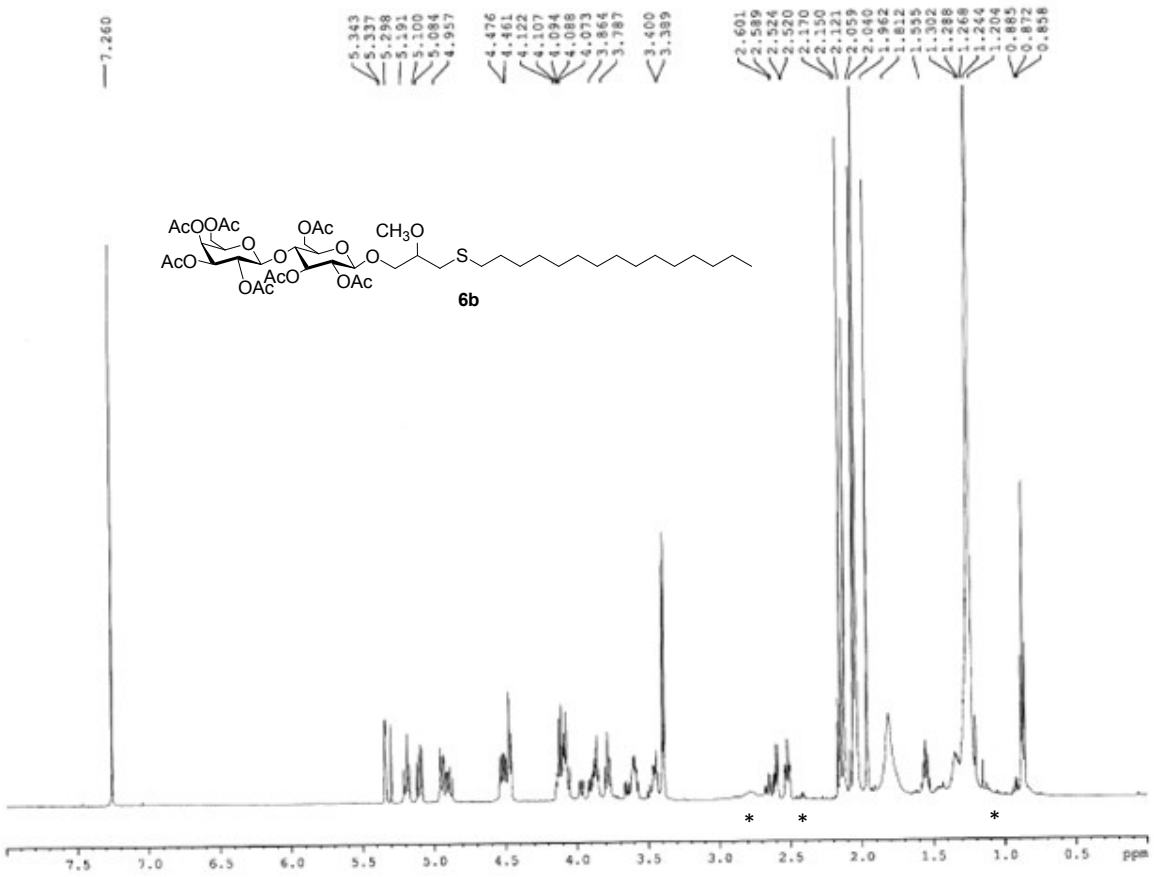

Figure ESI 2-1: ^{1}H NMR ($CDCl_3$) of compound **6b**

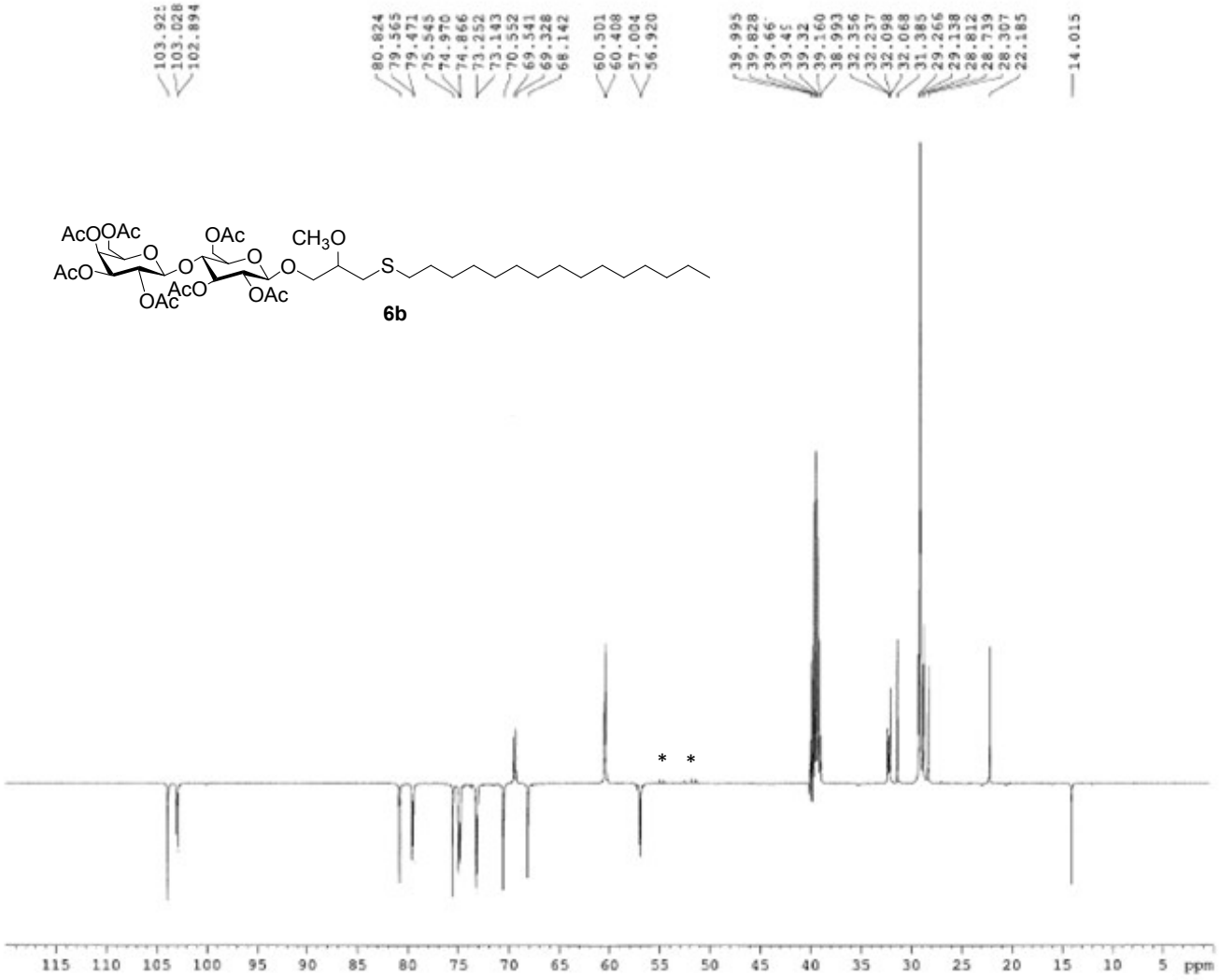

Figure ESI 2-2: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **6b**

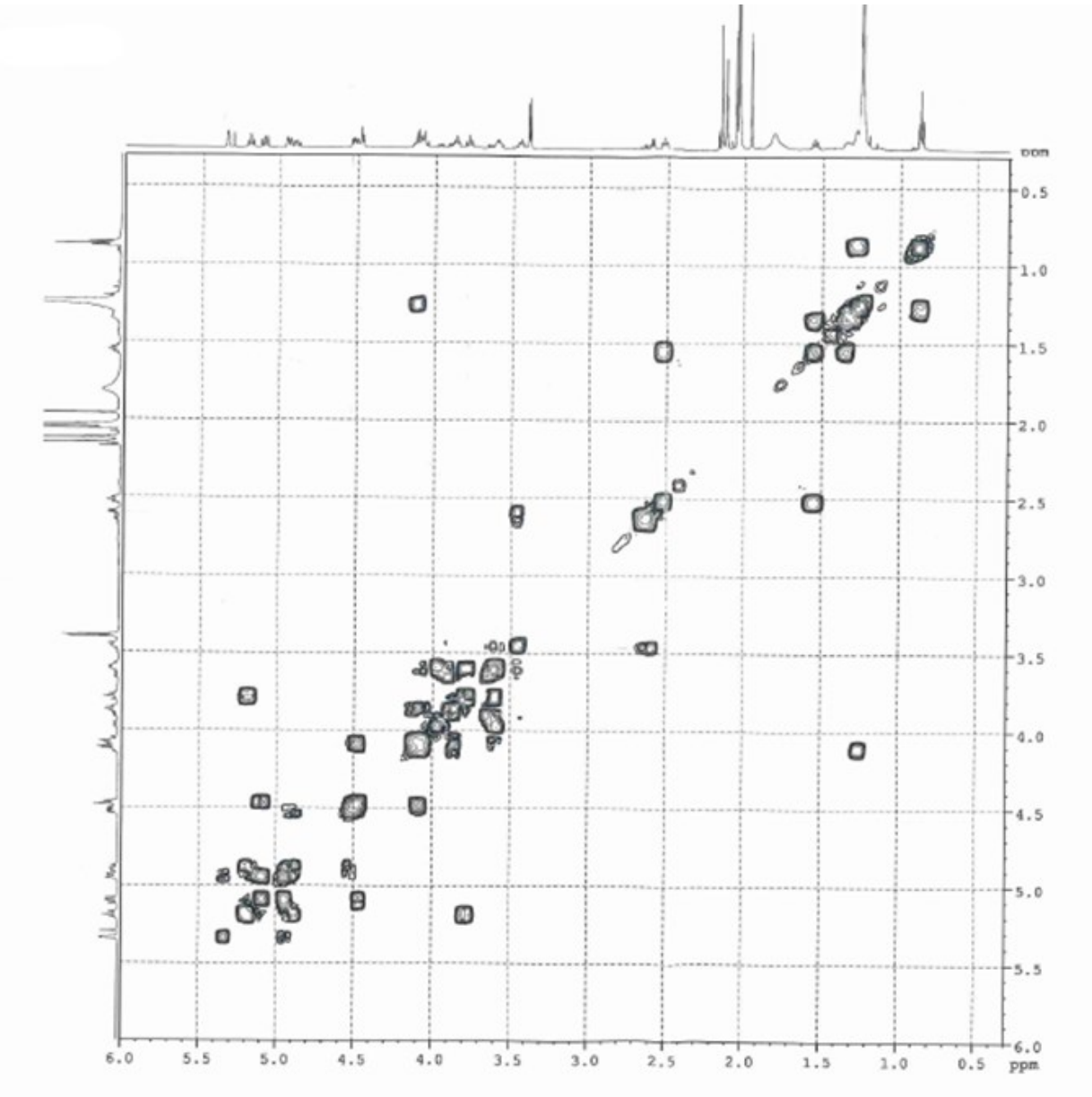

Figure ESI 2-3: ^{1}H COSY ($CDCl_3$) of compound **6b**

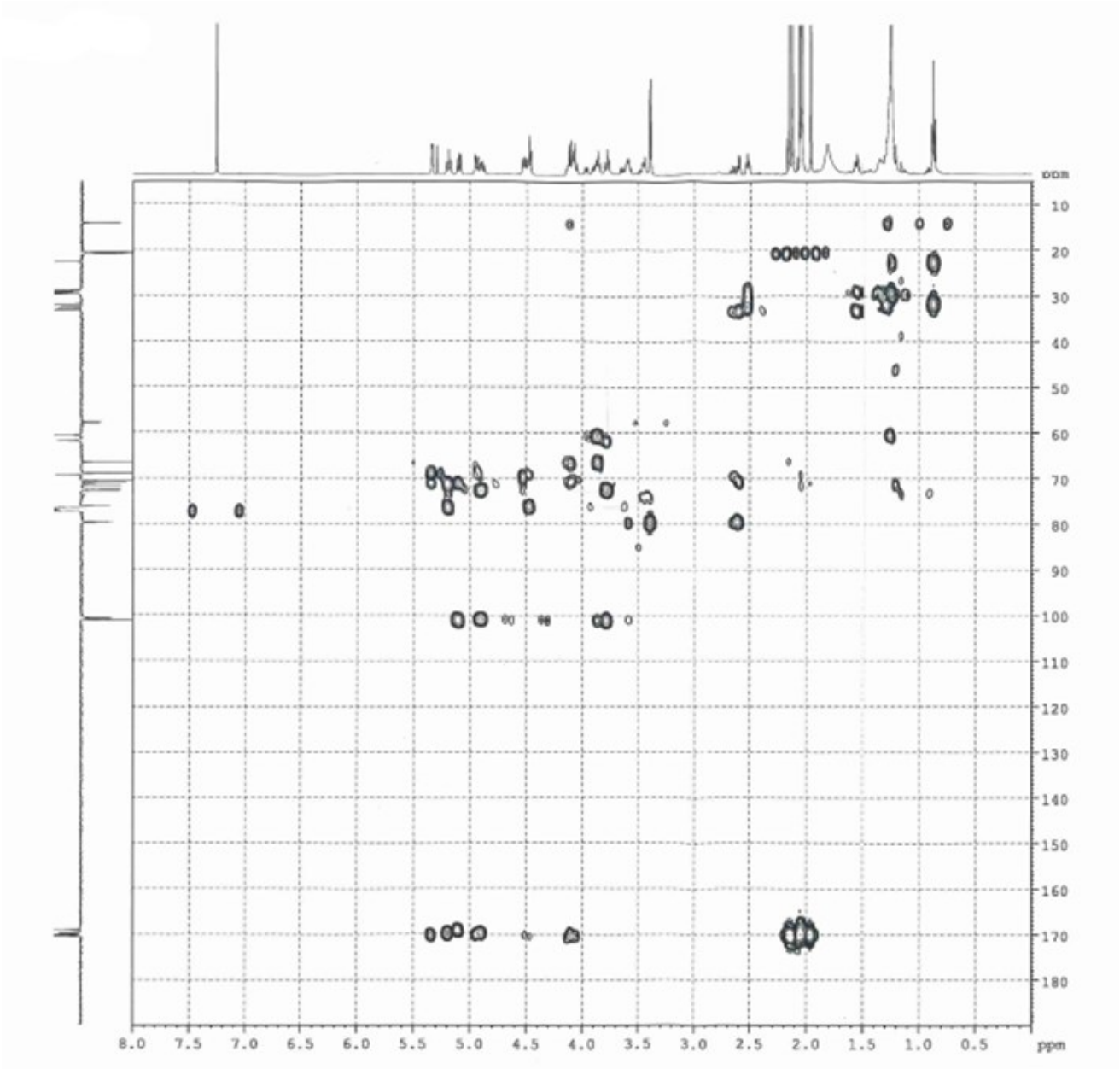

Figure ESI 2-4: HMQC ($CDCl_3$) of compound **6b**

1-S-dodecyl-2-O-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **7a** (**Ohmline-4S**)

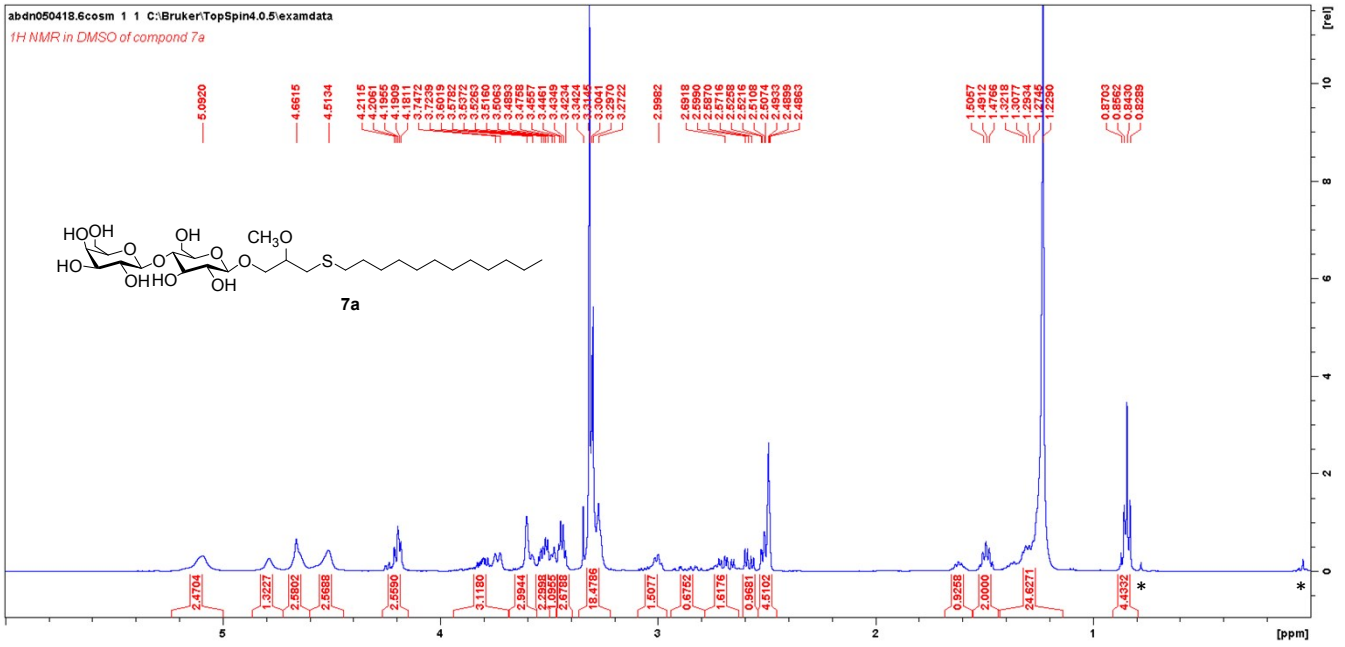

Figure ESI 2-5: ^{1}H NMR (DMSO-d_6) of compound **7a**

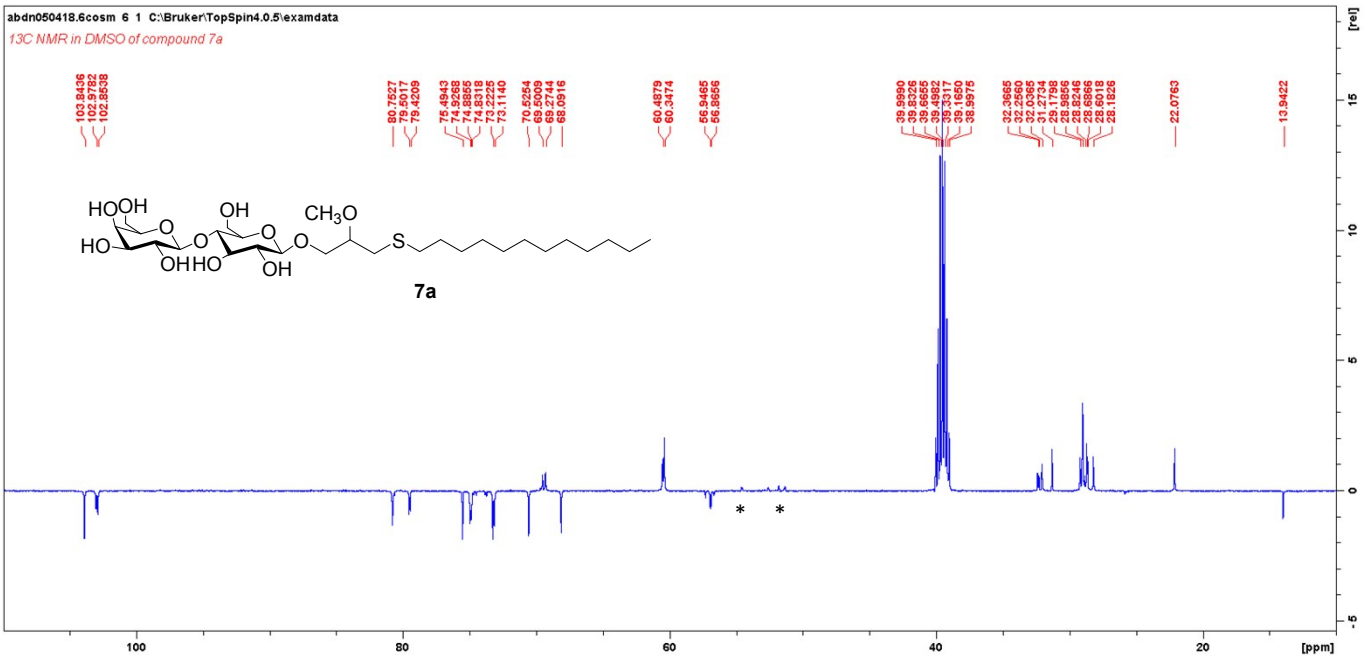

Figure ESI 2-6: ^{13}C{^{1}H} JMOD NMR (DMSO-d_6) of compound **7a**

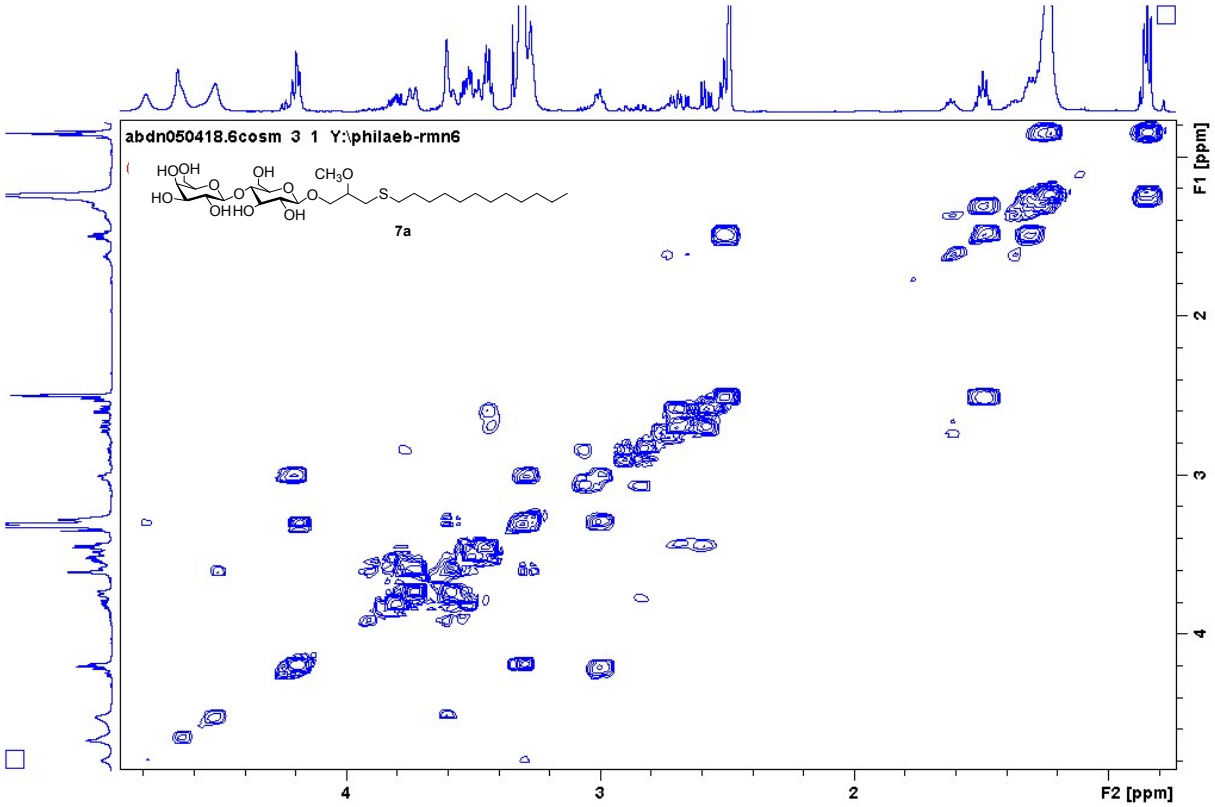

Figure ESI 2-7: COSY ^{1}H-^{1}H (DMSO-d_6) of compound **7a**

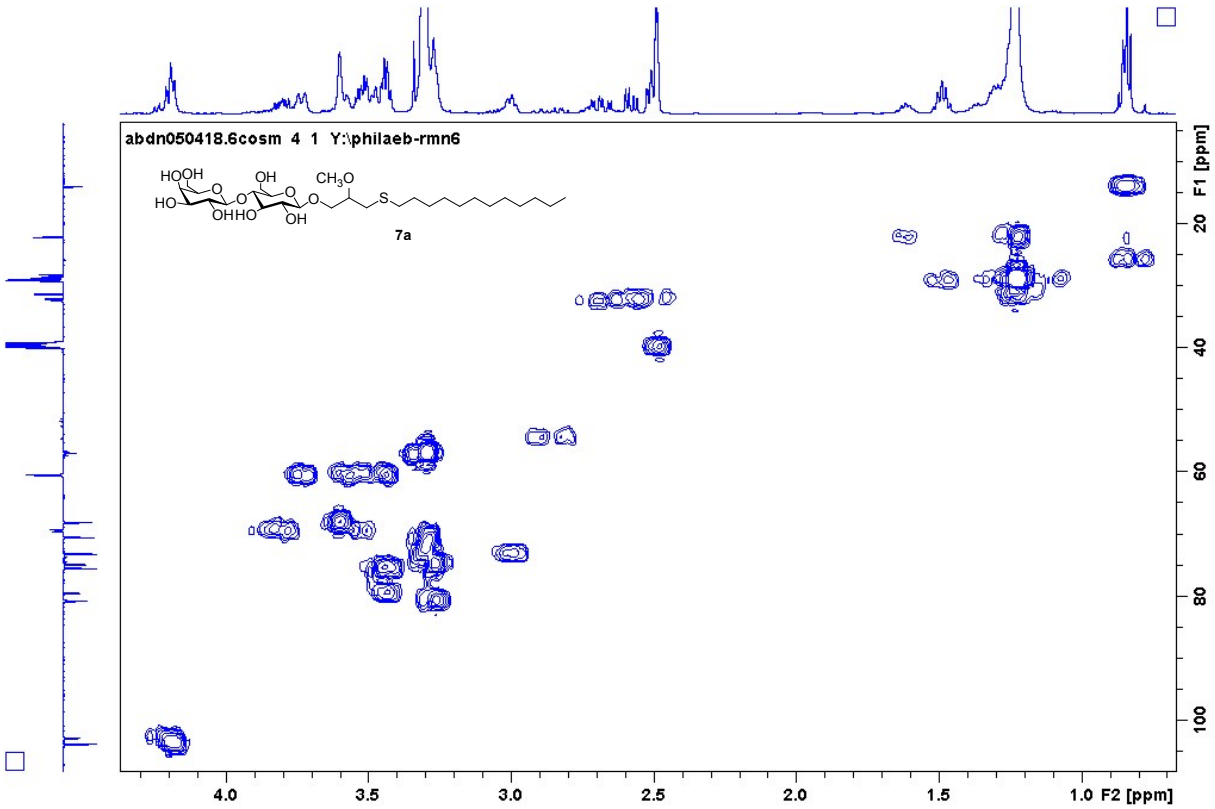

Figure ESI 2-8: HMQC (DMSO-d_6) of compound **7a**

1-S-hexadecyl-2-O-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **7b** (**Ohmline-4S**)

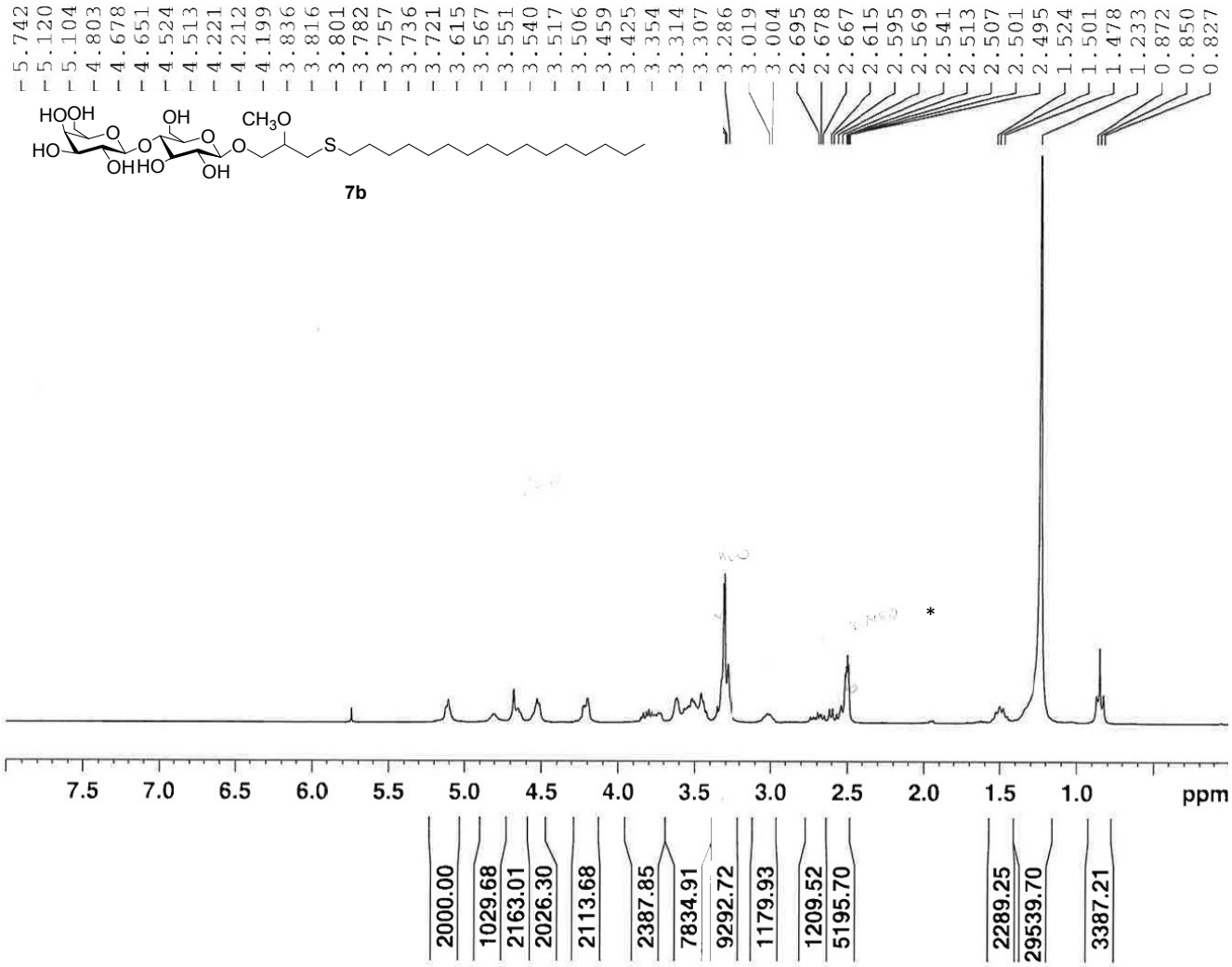

Figure ESI 2-9: ^{1}H NMR (DMSO-d_6) of compound **7b**

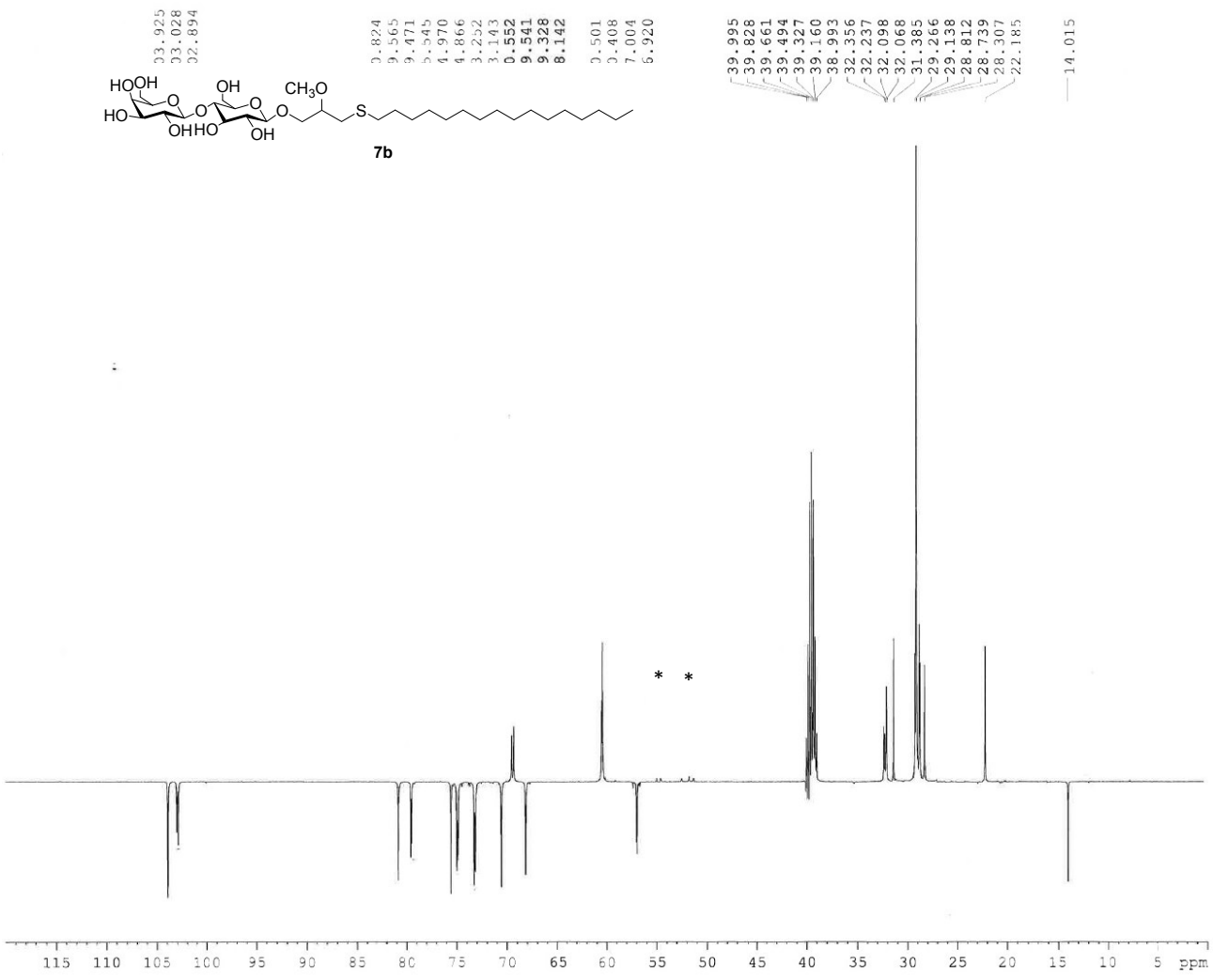

Figure ESI 2-10: $^{13}C\{^{1}H\}$ JMOD NMR (DMSO-d_6) of compound **7b**

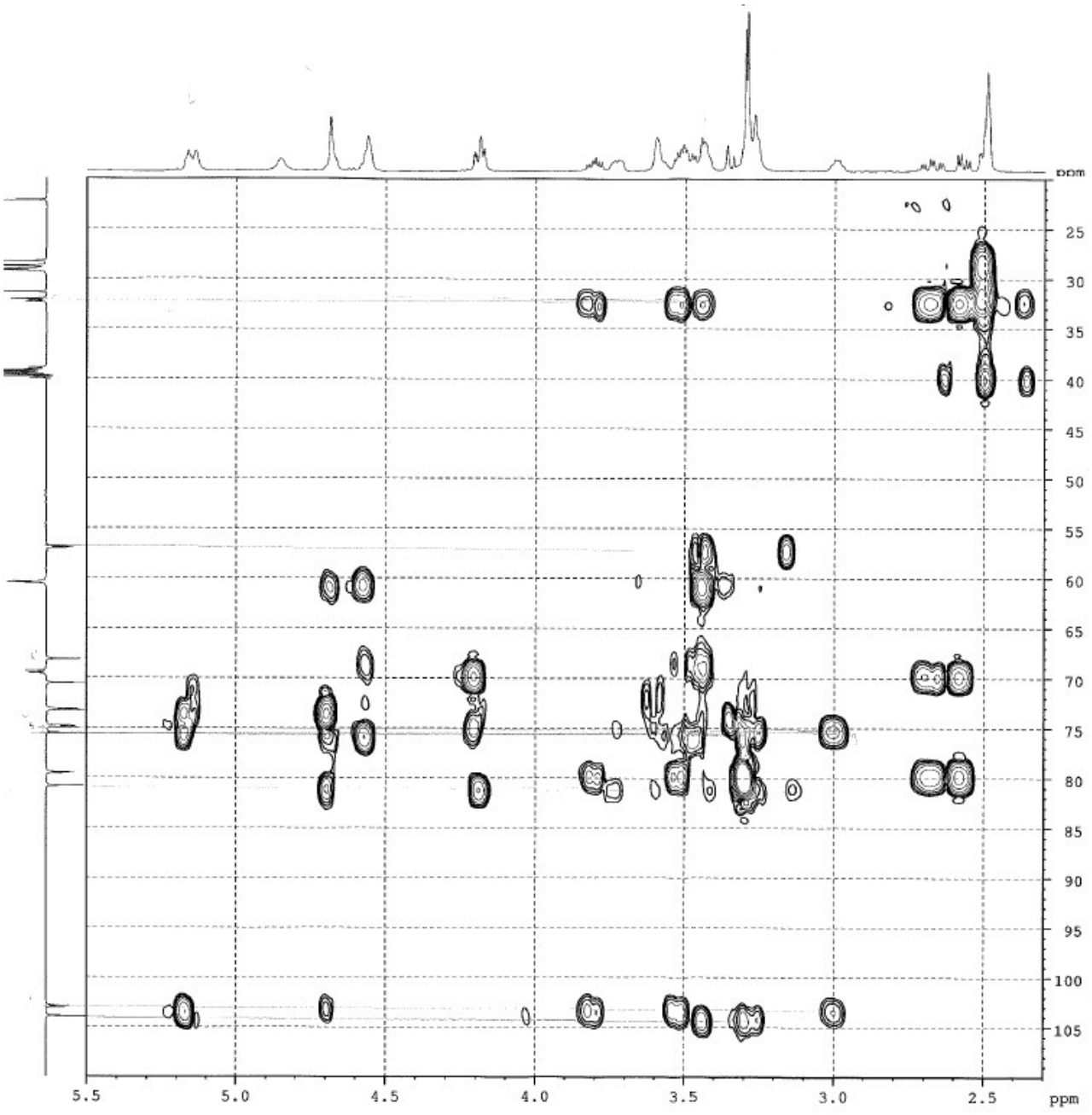

Figure ESI 2-11: HMBC (DMSO-d_6) of compound **7b**

3-(hexadecylsulfinyl)-2-methoxypropan-1-ol (**8**).

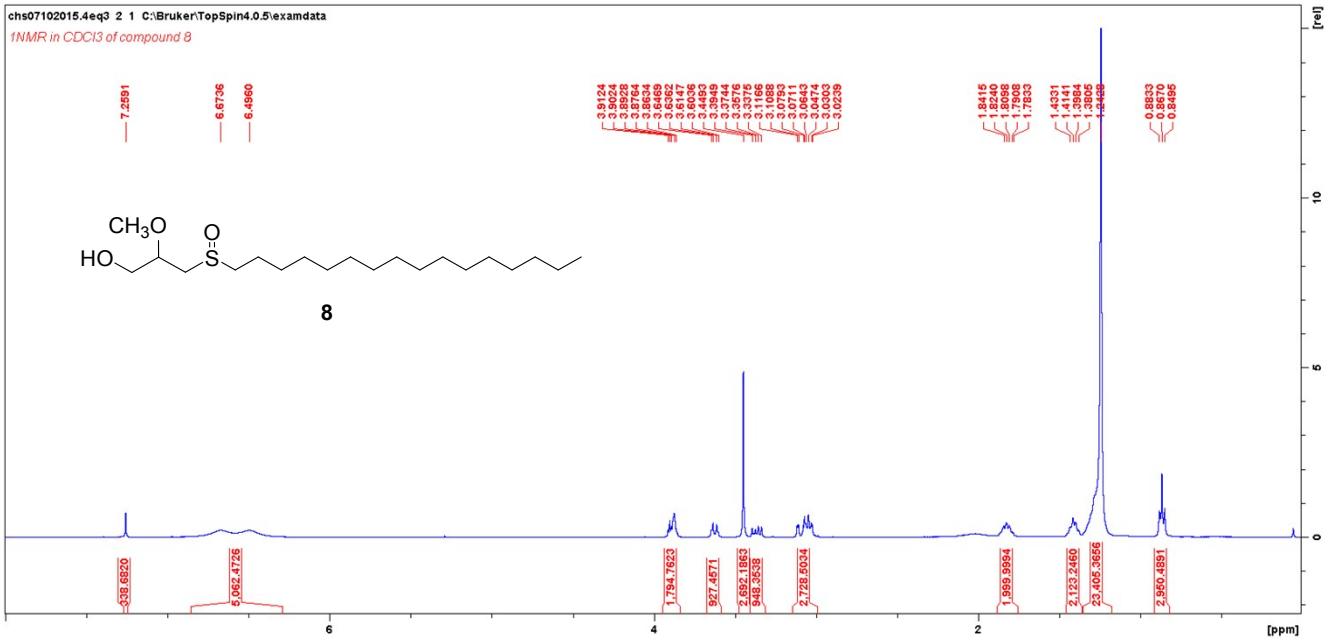

Figure ESI 2-12: ^{1}H NMR ($CDCl_3$) of compound **8**

1-S-hexadecylsulfinyl-2-O-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**9**)

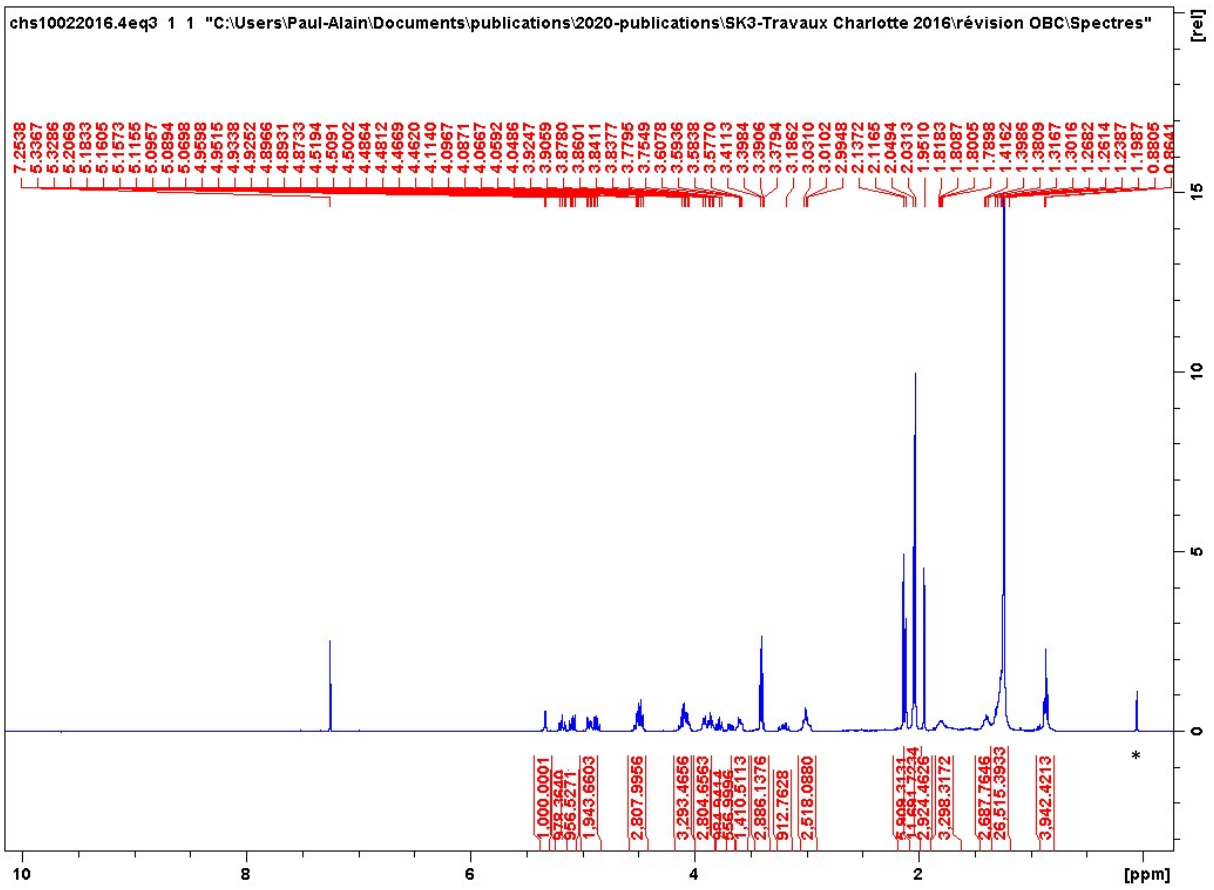

Figure ESI 2-13: ^{1}H NMR ($CDCl_3$) of compound **9**

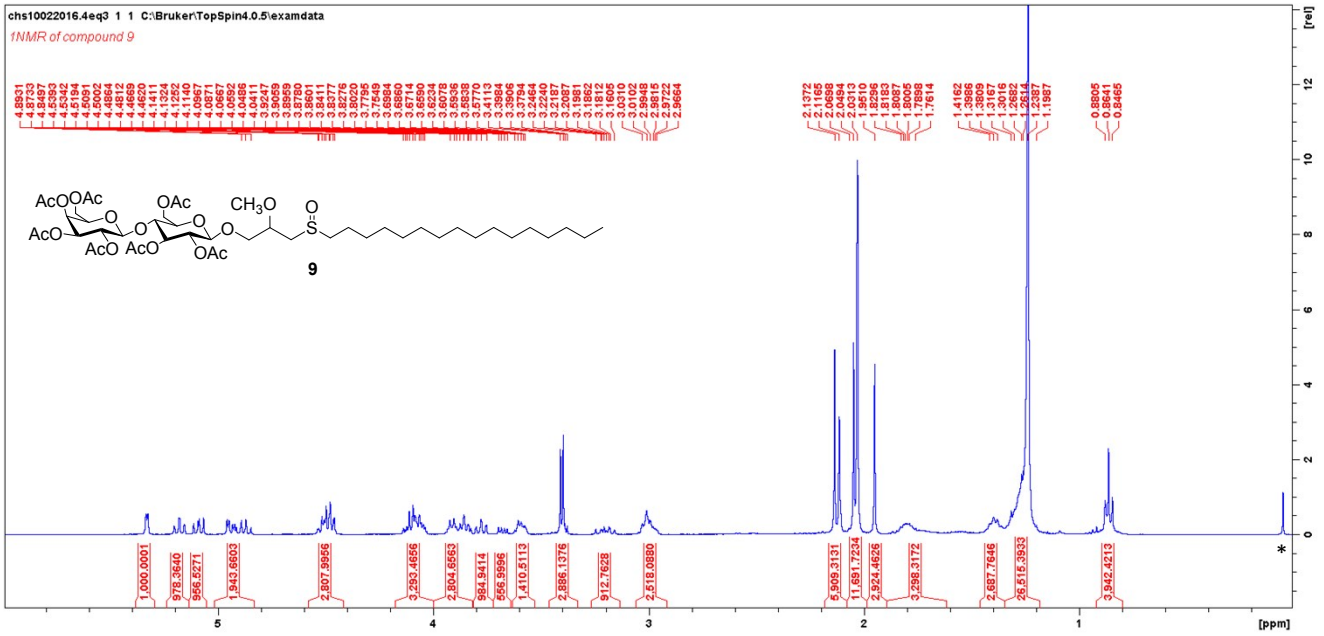

Figure ESI 2-14: ^{1}H NMR ($CDCl_3$) of compound **9** (enlargement)

1-S-hexadecylsulfinyl-2-O-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **10 (Ohmline-4-SO)**

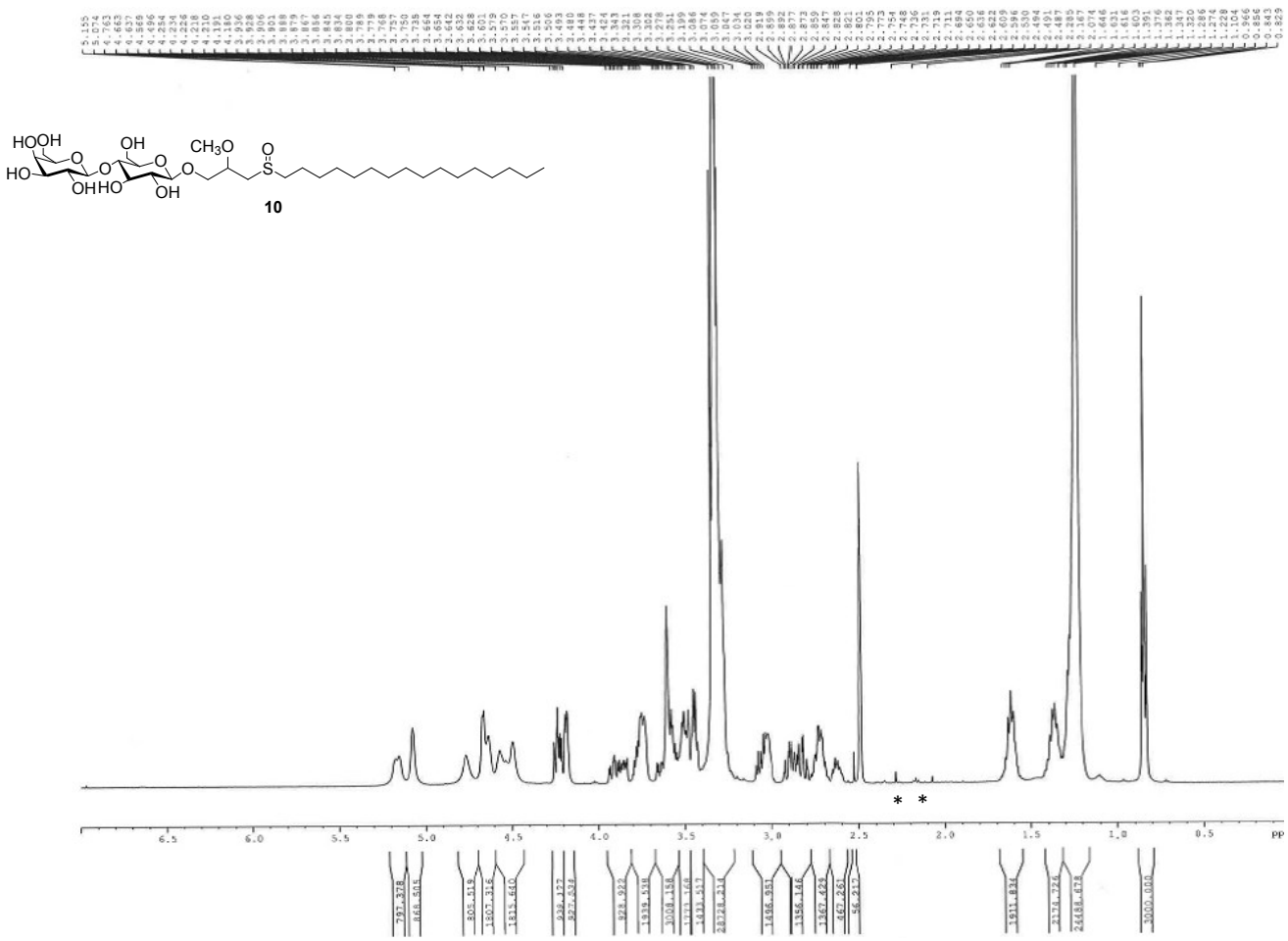

Figure ESI 2-15: ^{1}H NMR (DMSO-d_6) of compound **10**

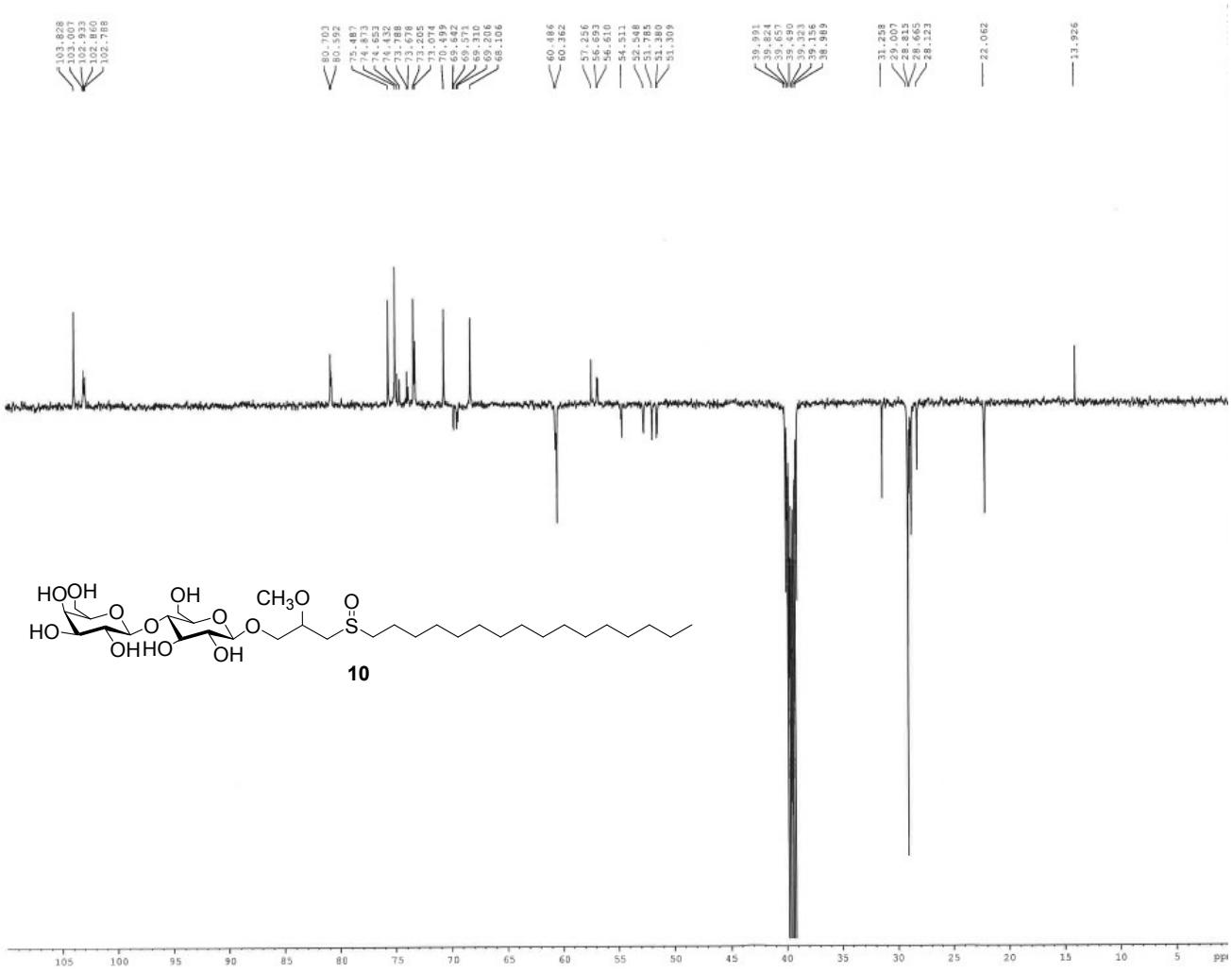

Figure ESI 2-16: $^{13}C\{^{1}H\}$ JMOD NMR (DMSO-d_6) of compound **10**

1-S-hexadecylsulfonyl-2-O-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside **12 (Ohmline- 4-SO_2)**

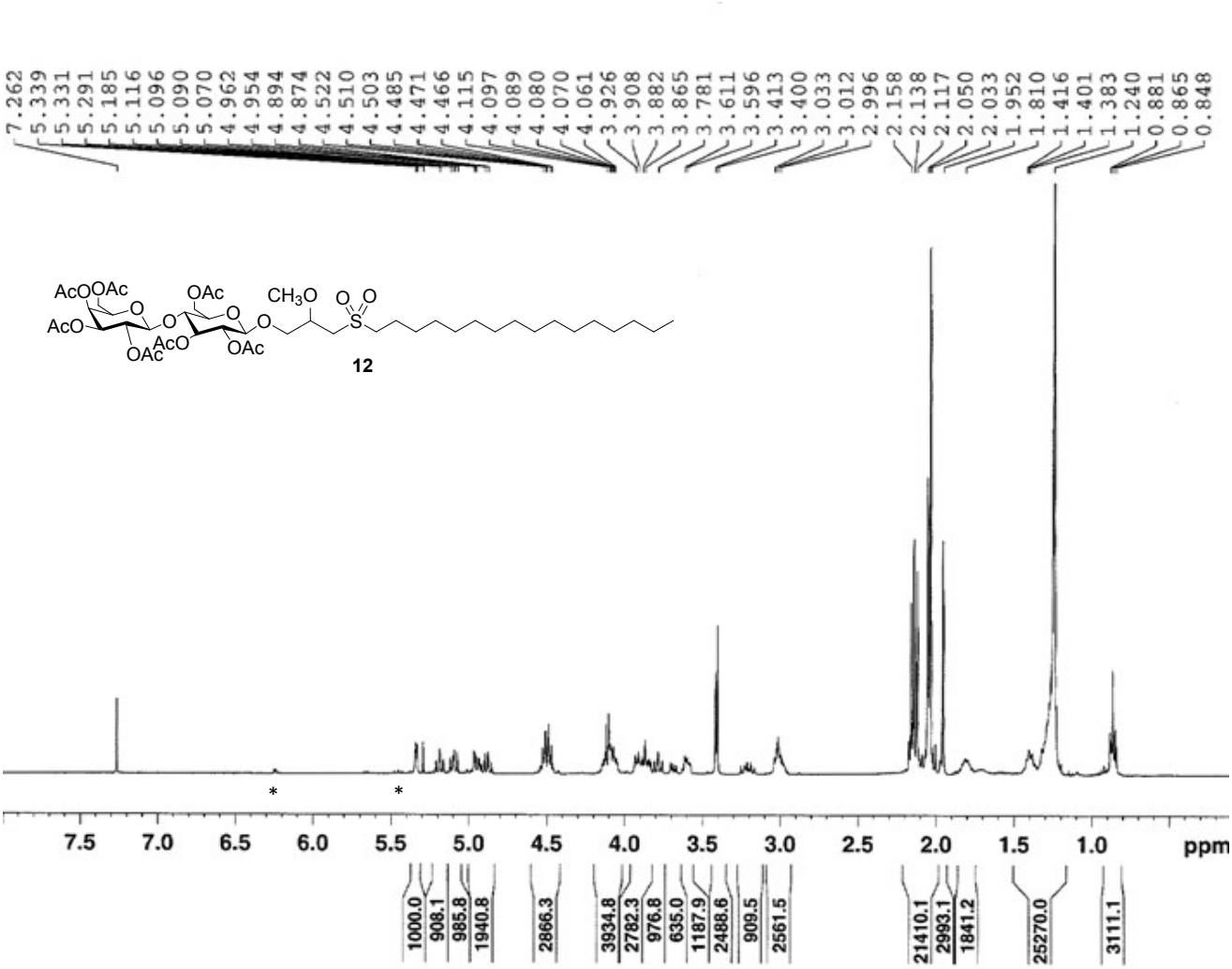

Figure ESI 2-17: ^{1}H NMR ($CDCl_3$) of compound **12**

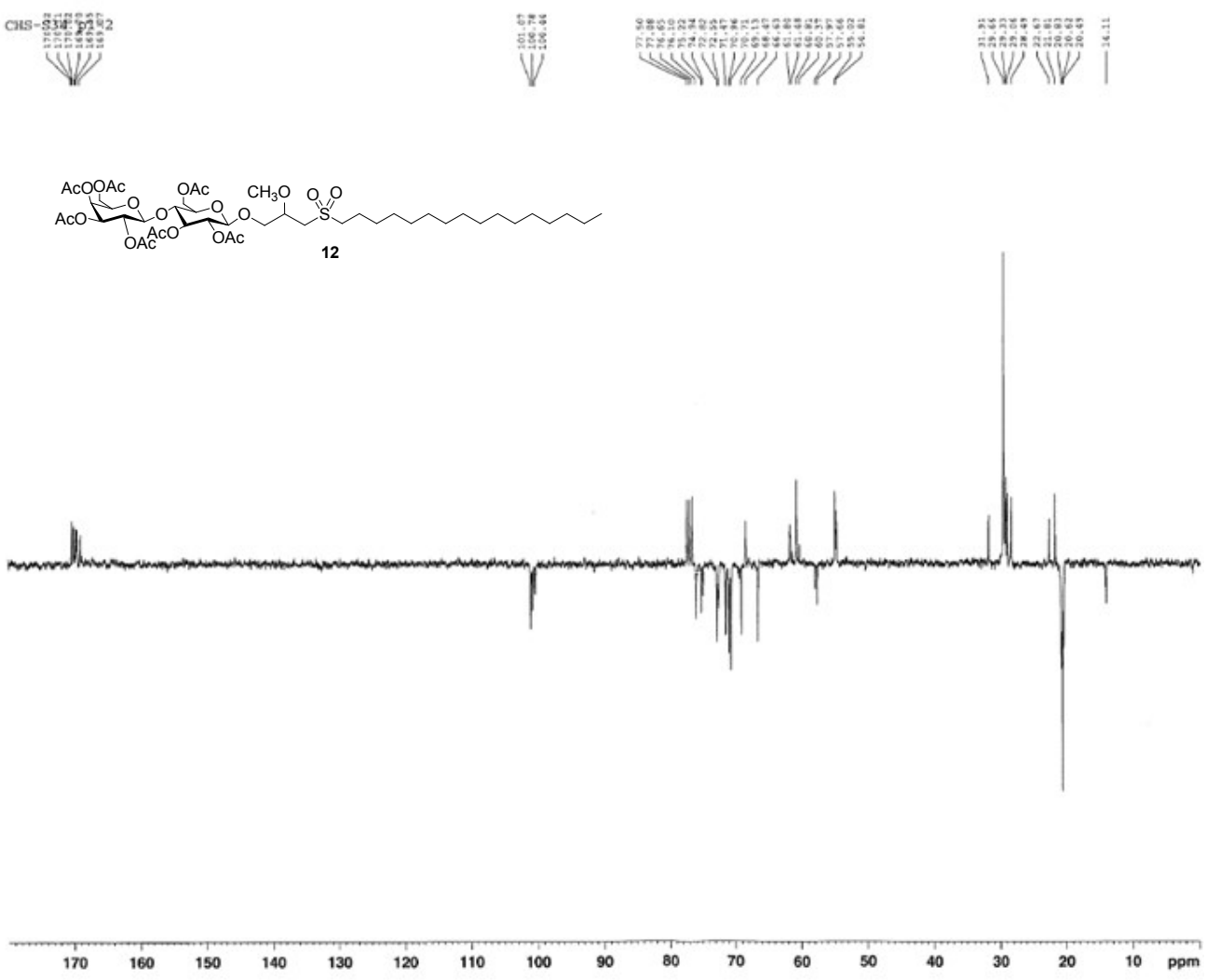

Figure ESI 2-18: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **12**

1-S-hexadecylsulfonyl-2-O-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **13 (Ohmline- 4-SO_2)**

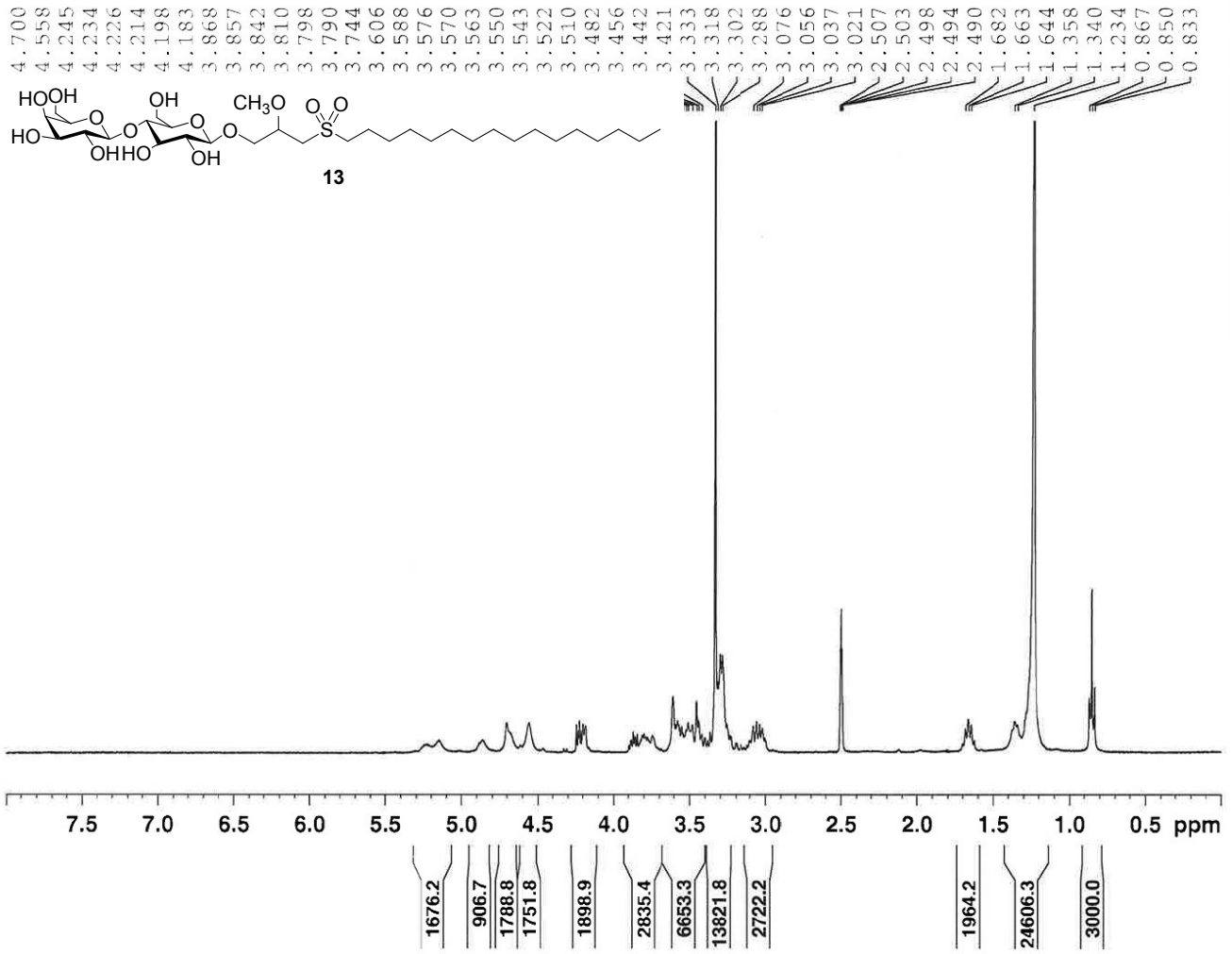

Figure ESI 2-19: ^{1}H NMR (DMSO-d_6) of compound **13**

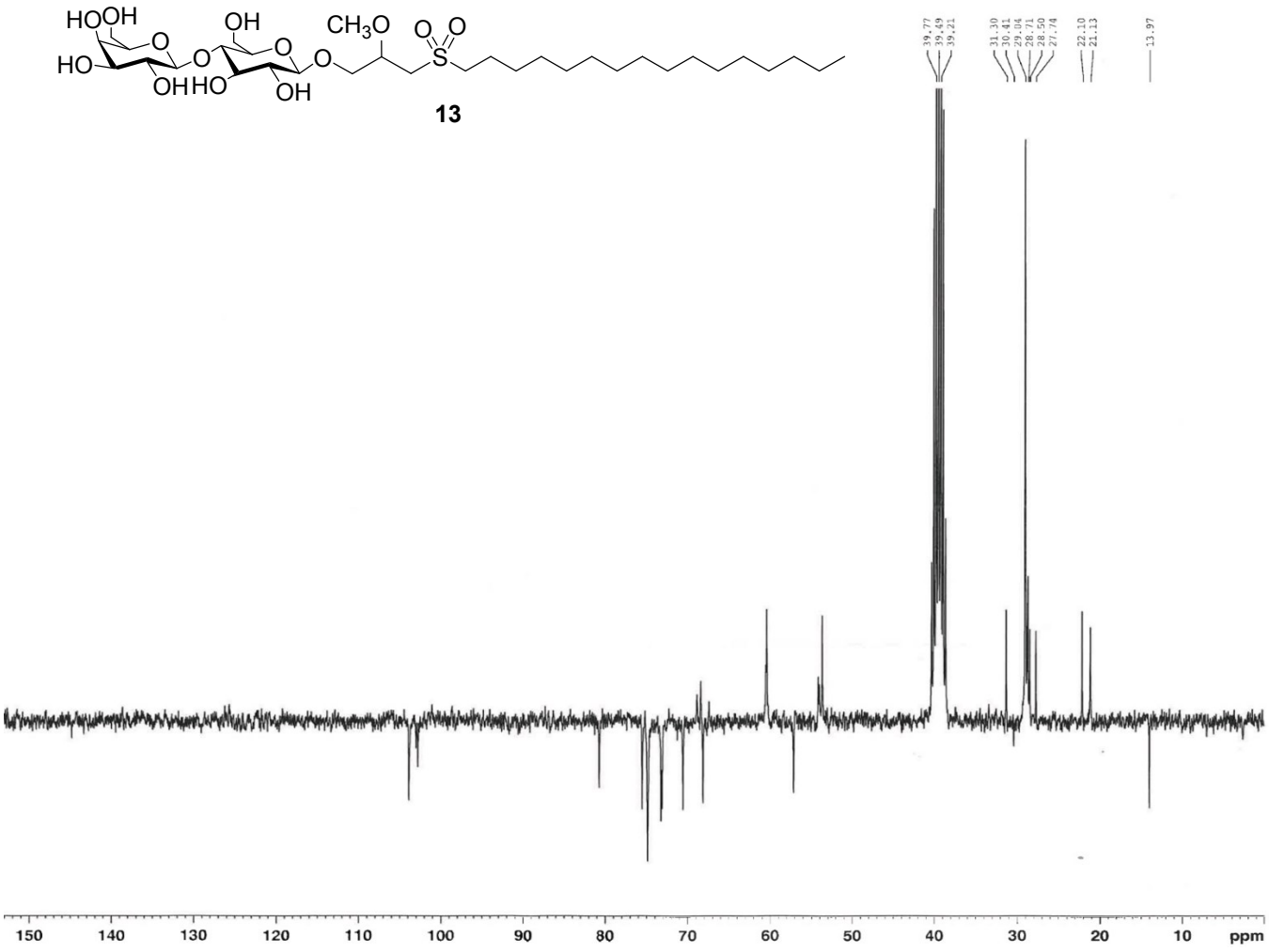

Figure ESI 2-20: $^{13}C\{^{1}H\}$ JMOD NMR (DMSO-d_6) of compound **13**

2-methoxy-but-3-en-1-ol (**14a**).

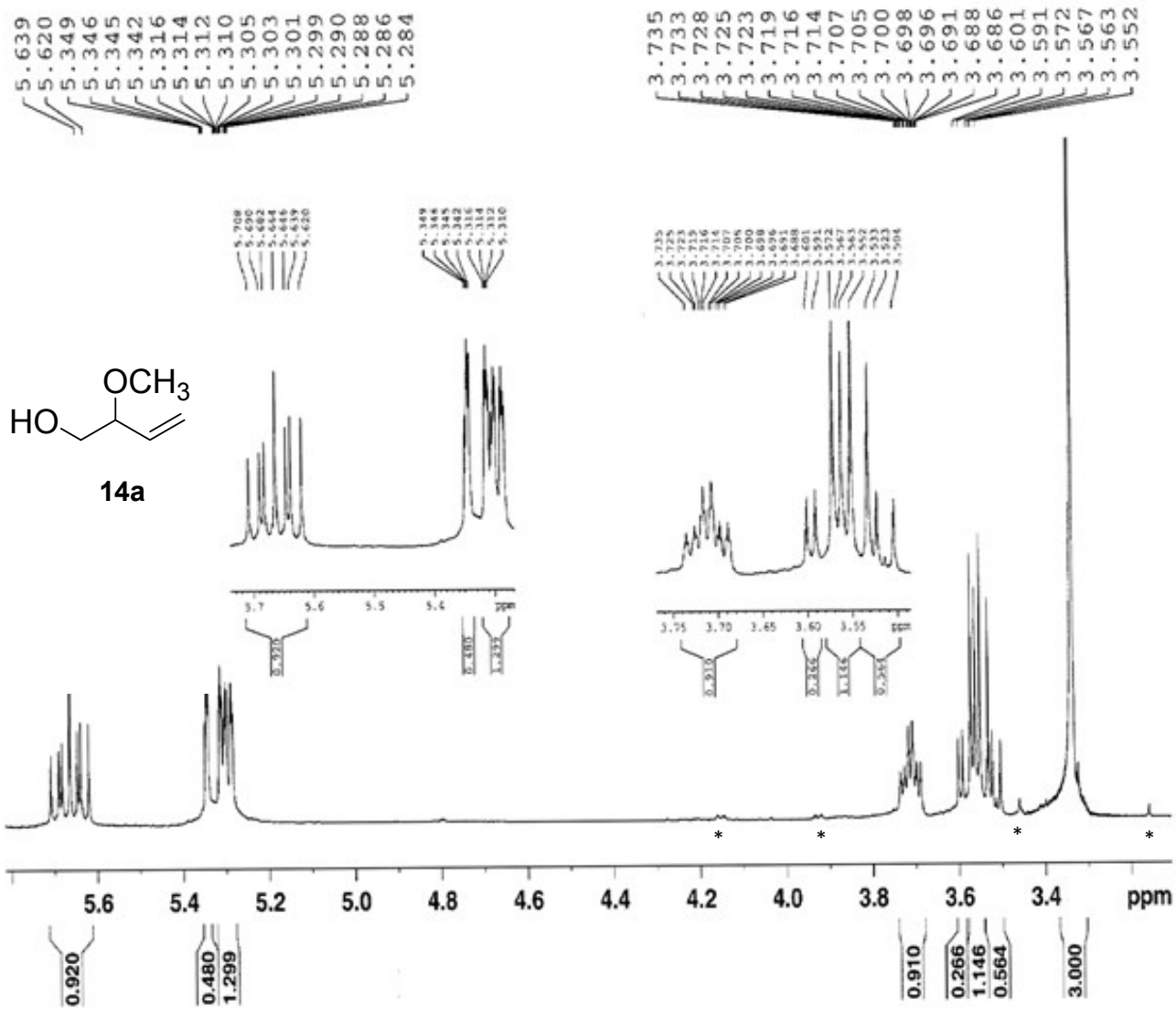

Figure ESI 2-21: ^{1}H NMR ($CDCl_3$) of compound **14a**

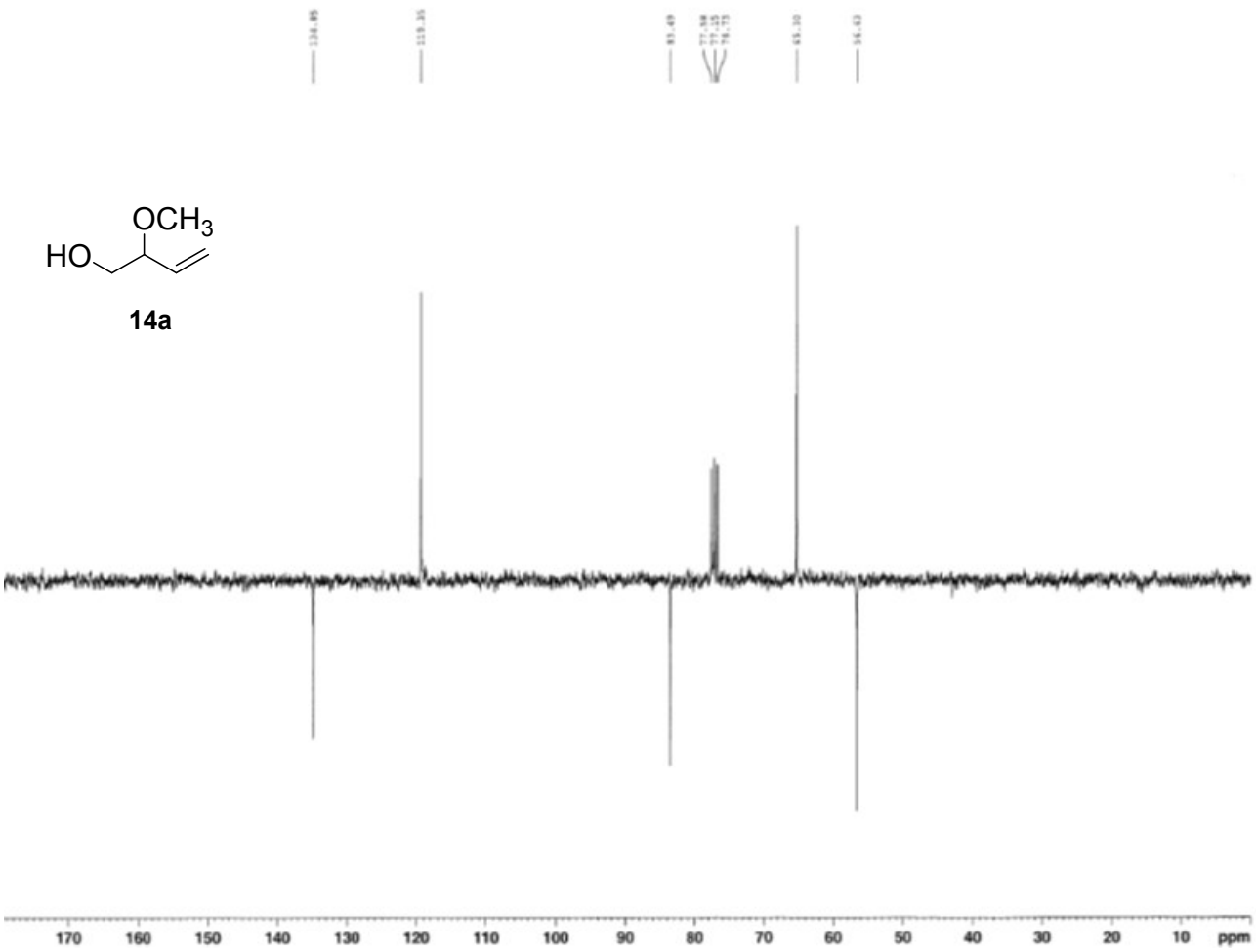

Figure ESI 2-22: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **14a**

2-ethoxy-but-3-en-1-ol (**14b**).

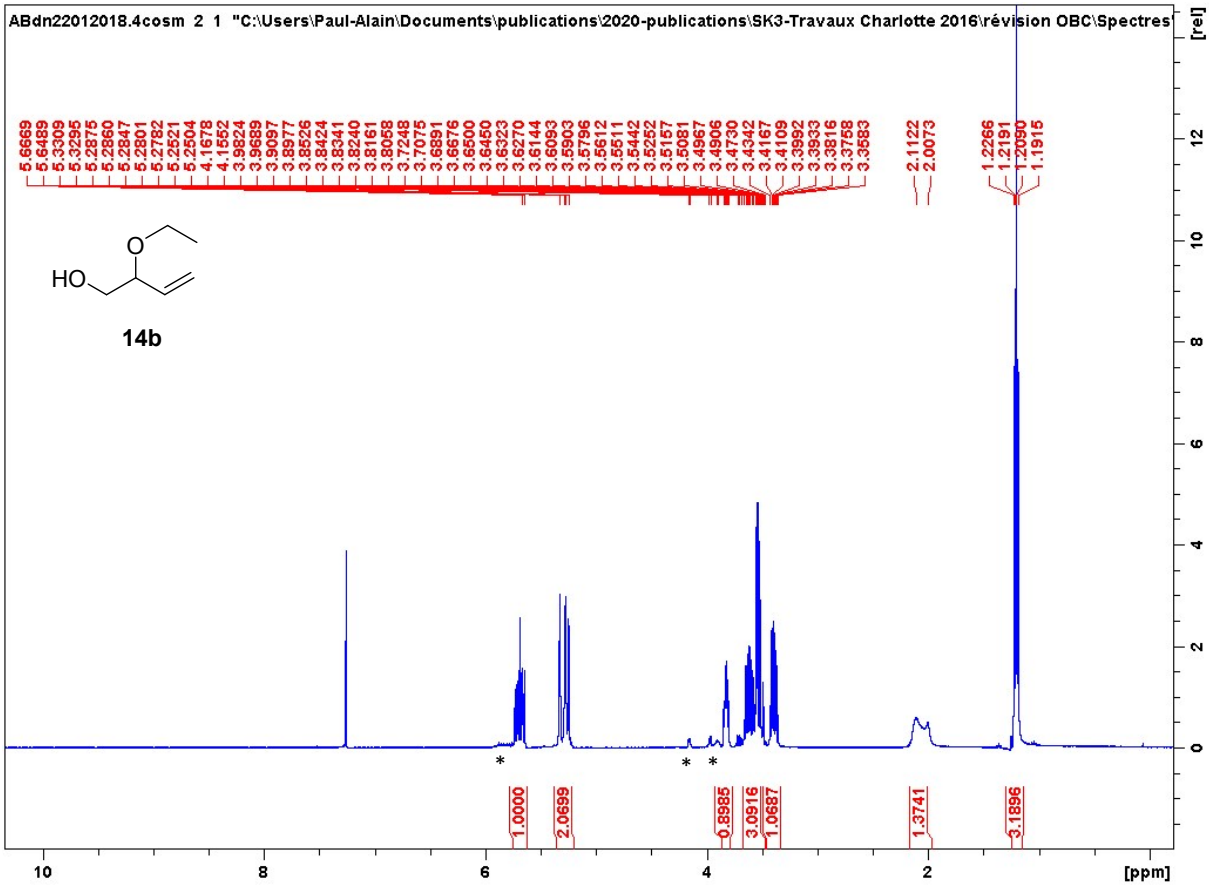

Figure ESI 2-23: ^{1}H NMR ($CDCl_3$) of compound **14b**

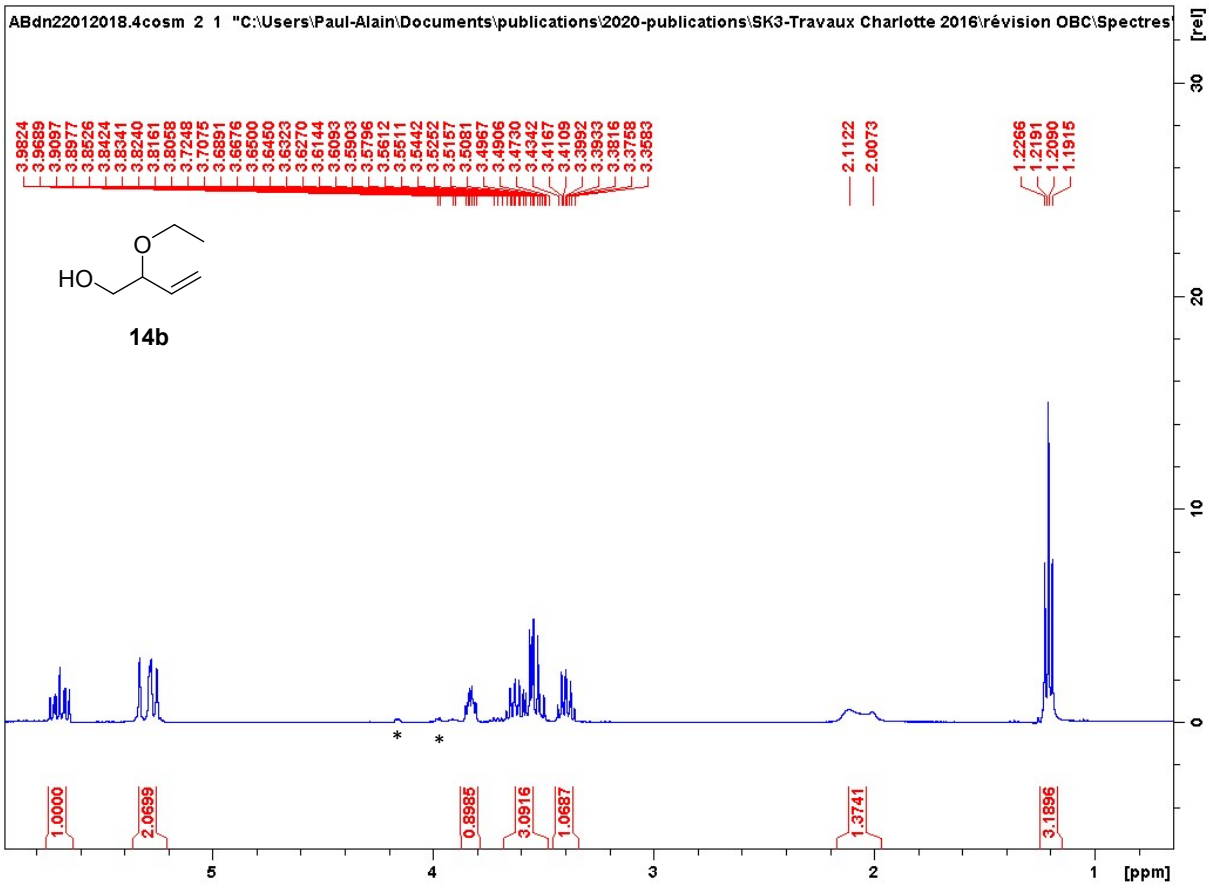

Figure ESI 2-24: ^{1}H NMR ($CDCl_3$) of compound **14b** (enlargement)

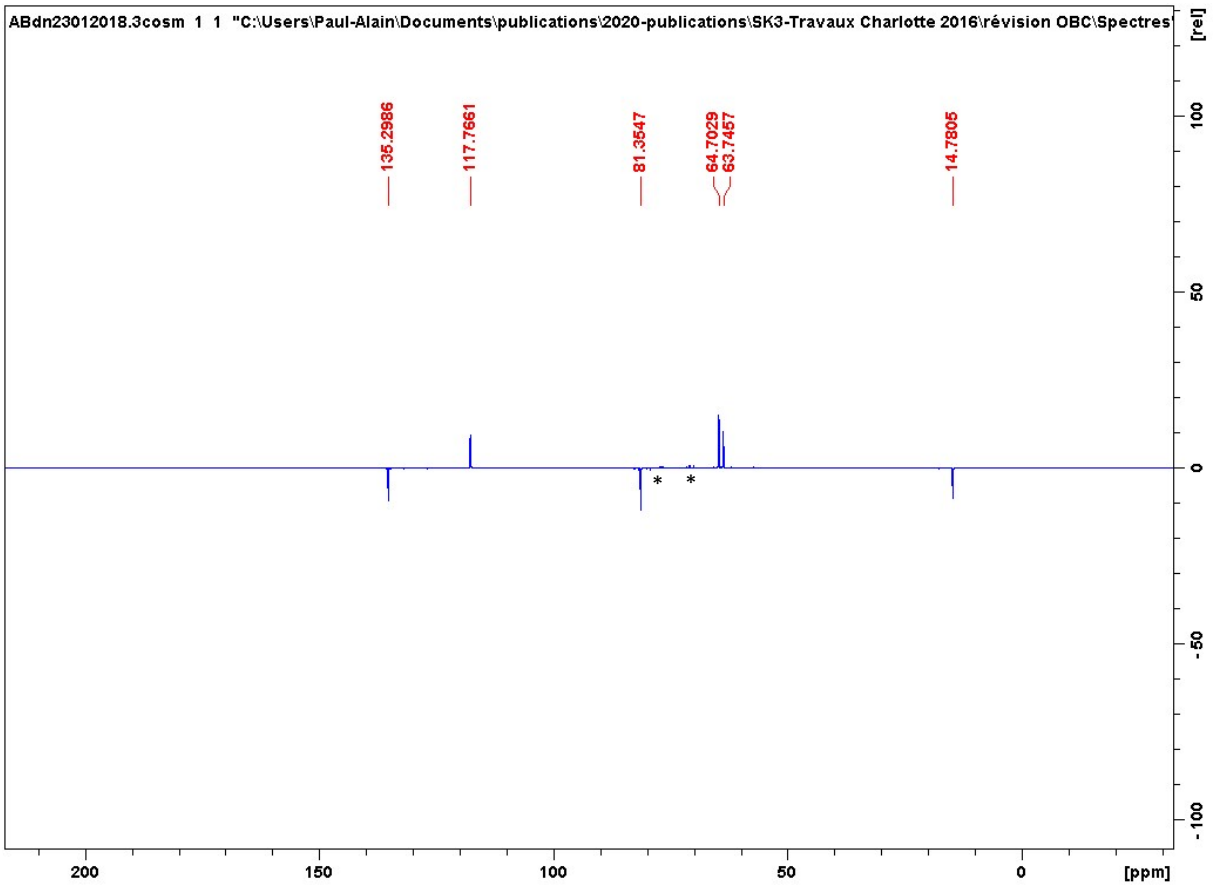

Figure ESI 2-25: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **14b**

4-(hexadecylthio)-2-methoxybutan-1-ol (**15a**).

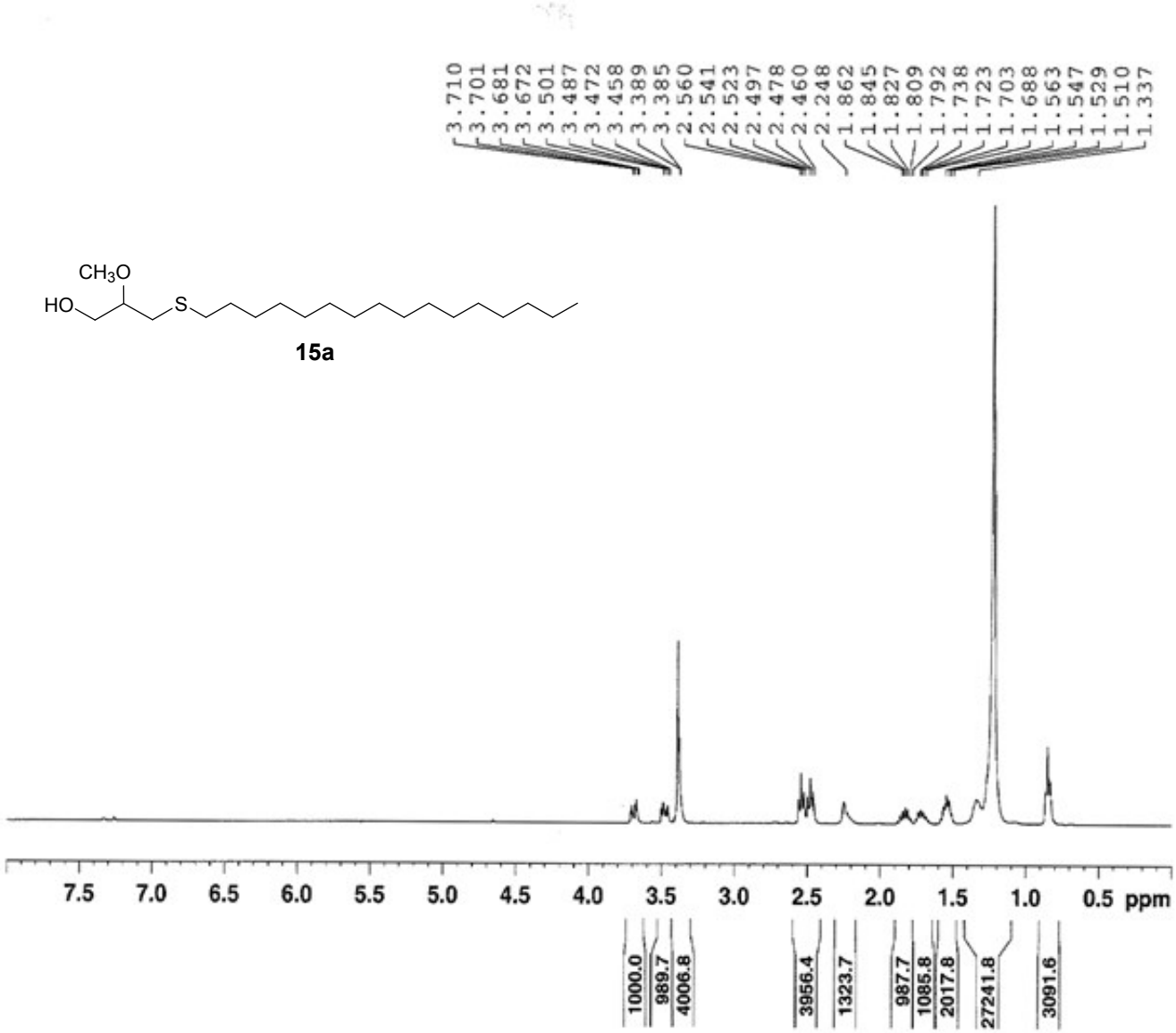

Figure ESI 2-26: ^{1}H NMR ($CDCl_3$) of compound **15a**

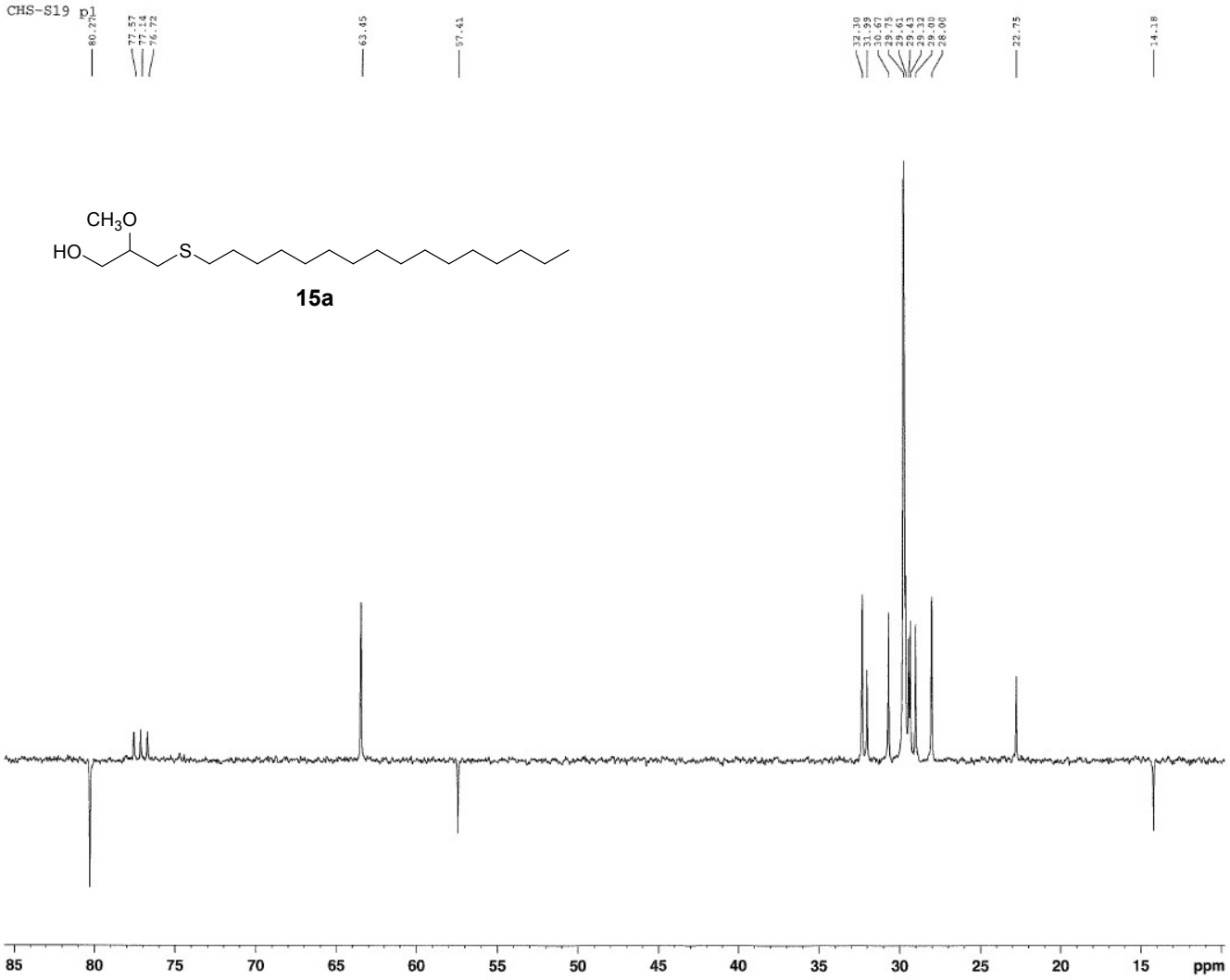

Figure ESI 2-27: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **15a**

4-(hexadecylthio)-2-ethoxybutan-1-ol (**15b**).

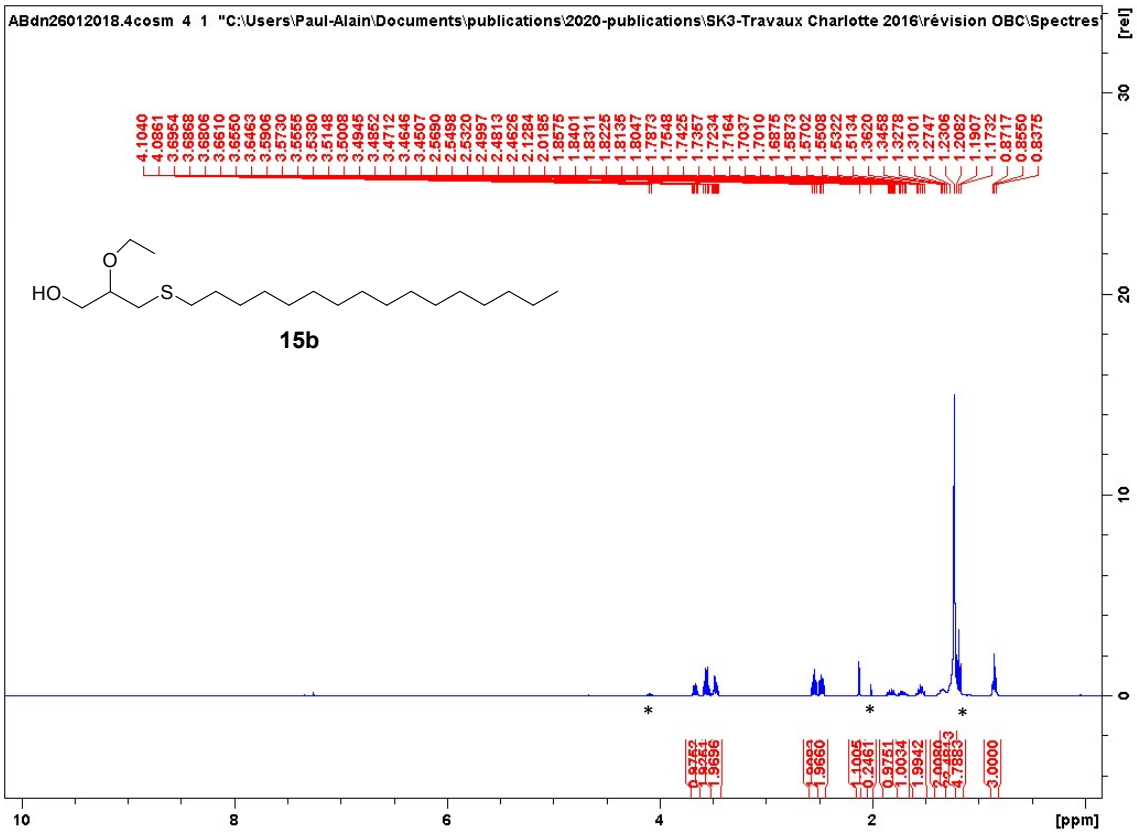

Figure ESI 2-28: ^{1}H NMR ($CDCl_3$) of compound **15b**

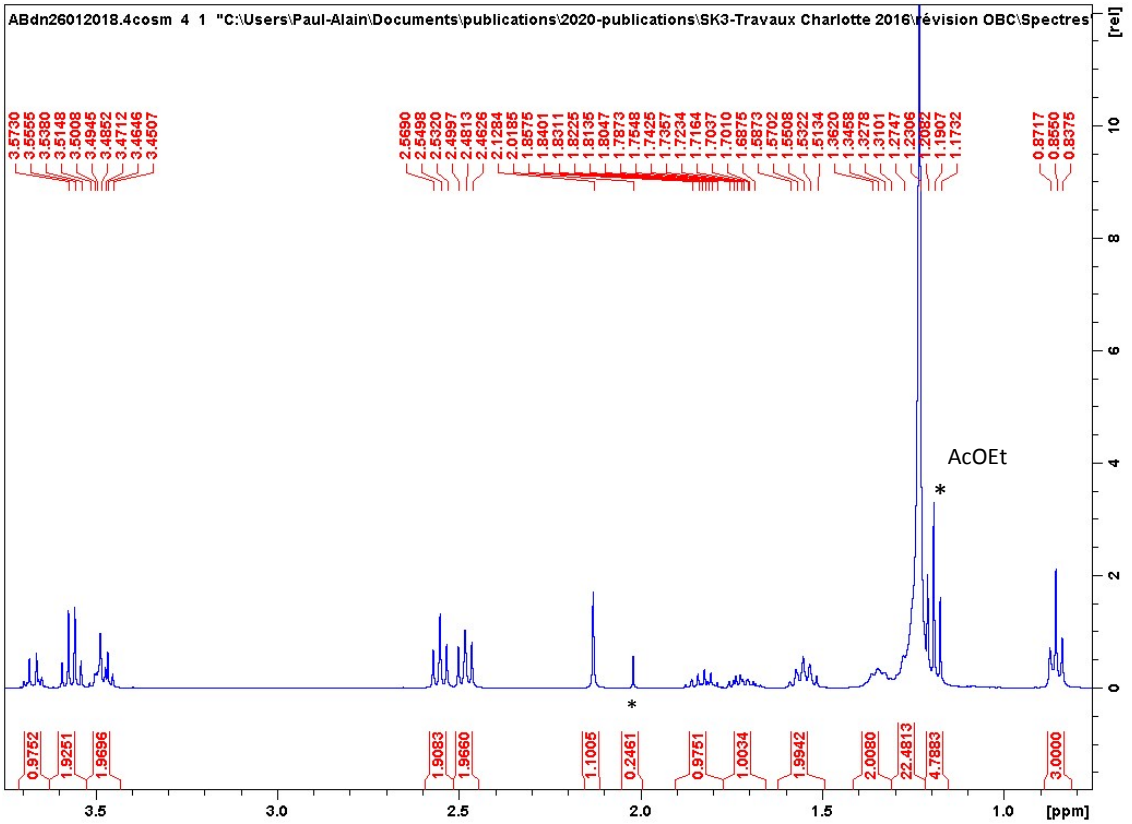

Figure ESI 2-29: ^{1}H NMR ($CDCl_3$) of compound **15b** (enlargement)

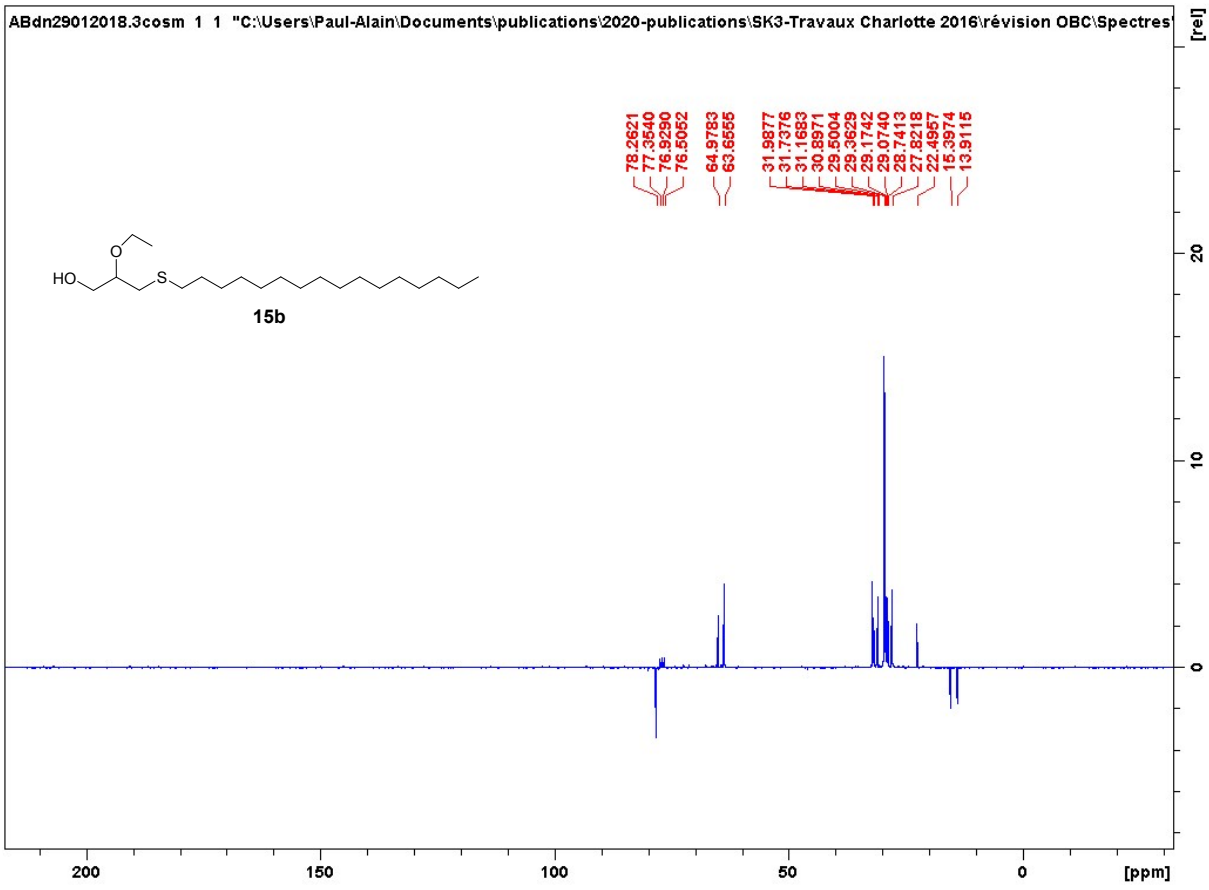

Figure ESI 2-30: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **15b**.

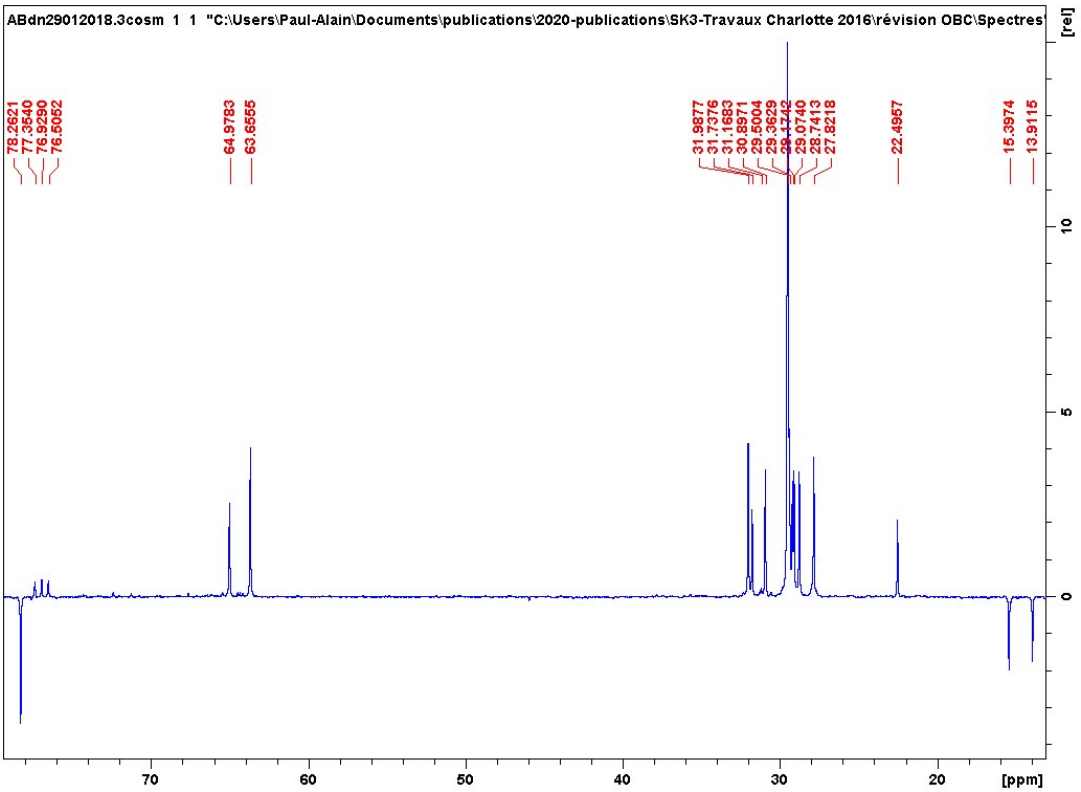

Figure ESI 2-31: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **15b** (enlargement)

1-O-[-4-(hexadecylthio)-2-methoxybutyl]-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside **16a (Ohmline-5-S)**

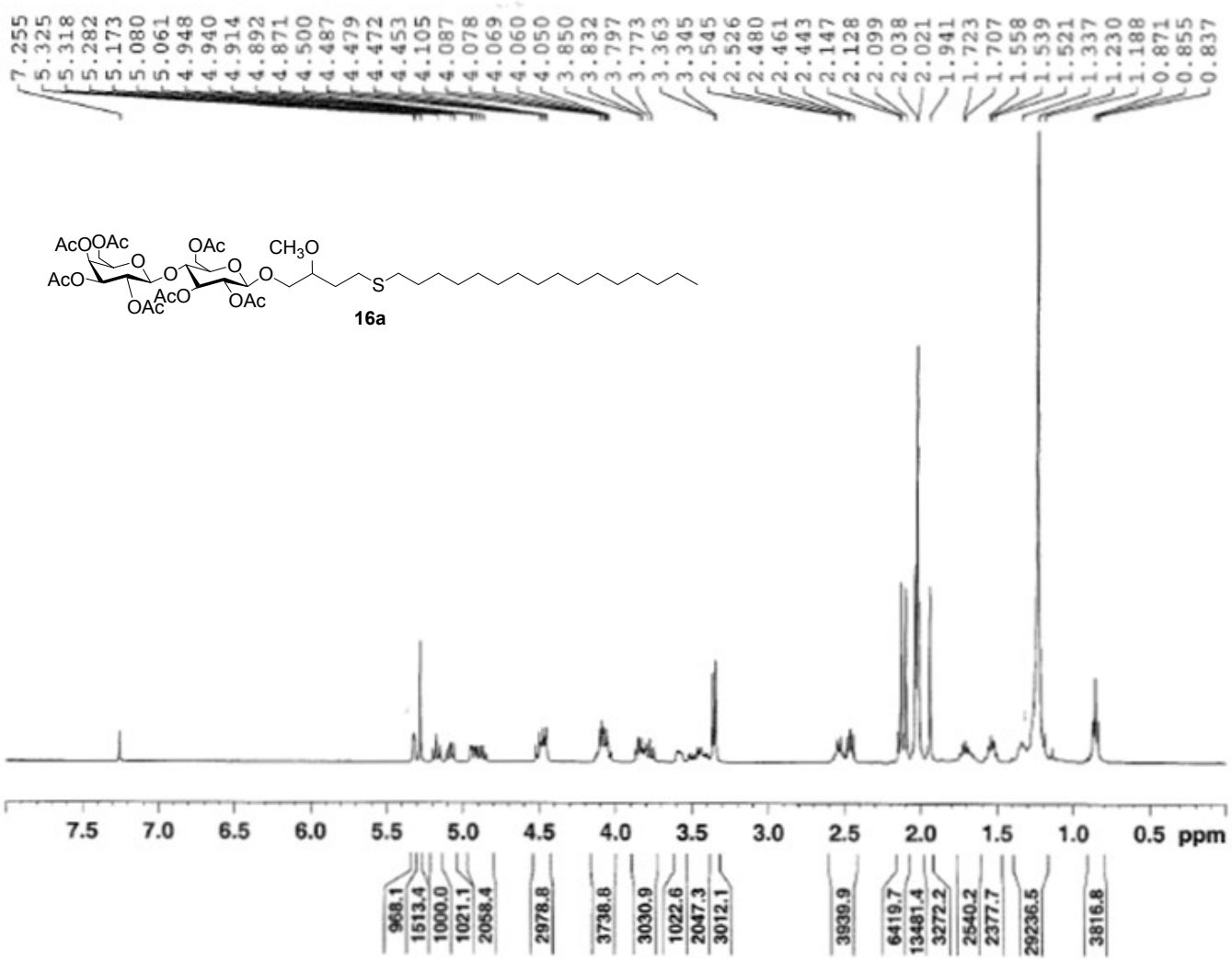

Figure ESI 2-32: ^{1}H NMR ($CDCl_3$) of compound **16a**

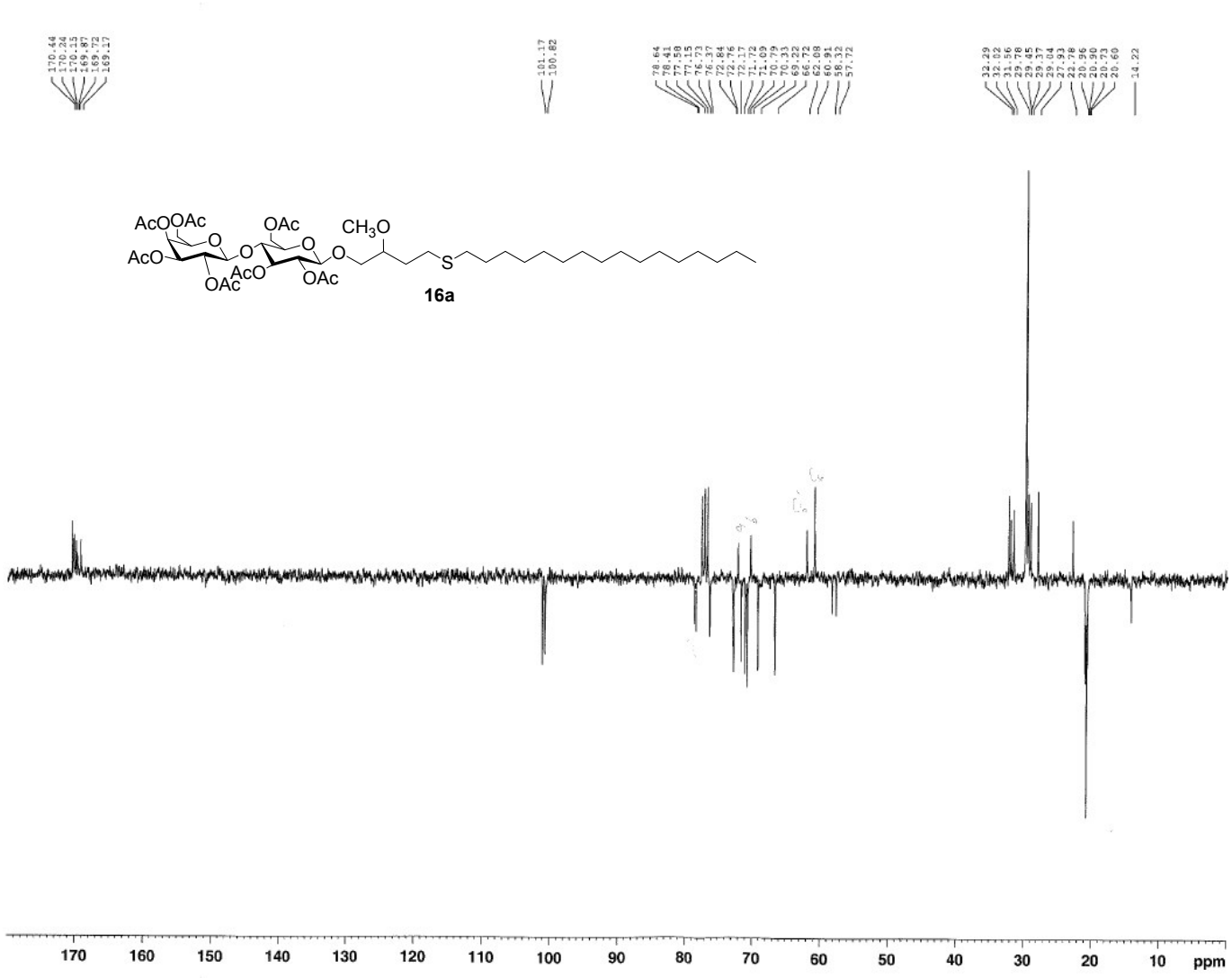

Figure ESI 2-33: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **16a**

1-O-[-4-(hexadecylthio)-2-ethoxybutyl]-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**16b**)

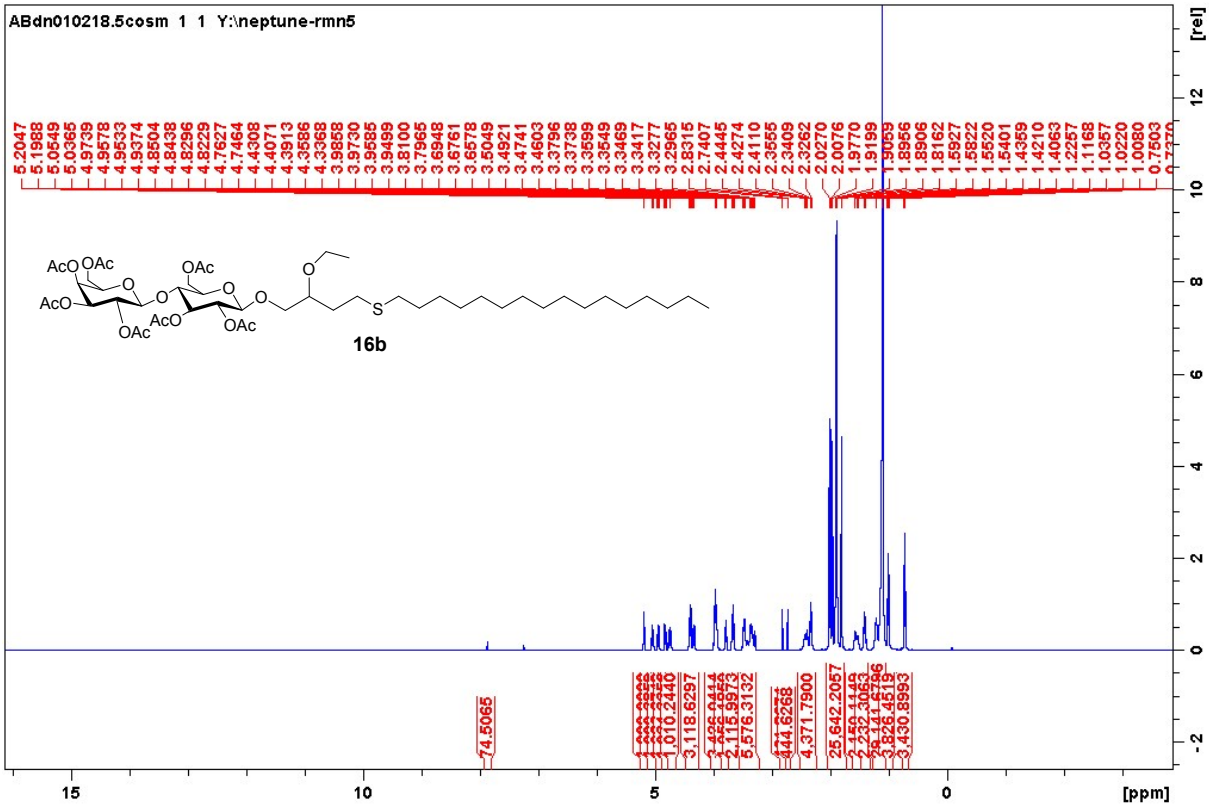

Figure ESI 2-34: ^{1}H NMR ($CDCl_3$) of compound **16b**

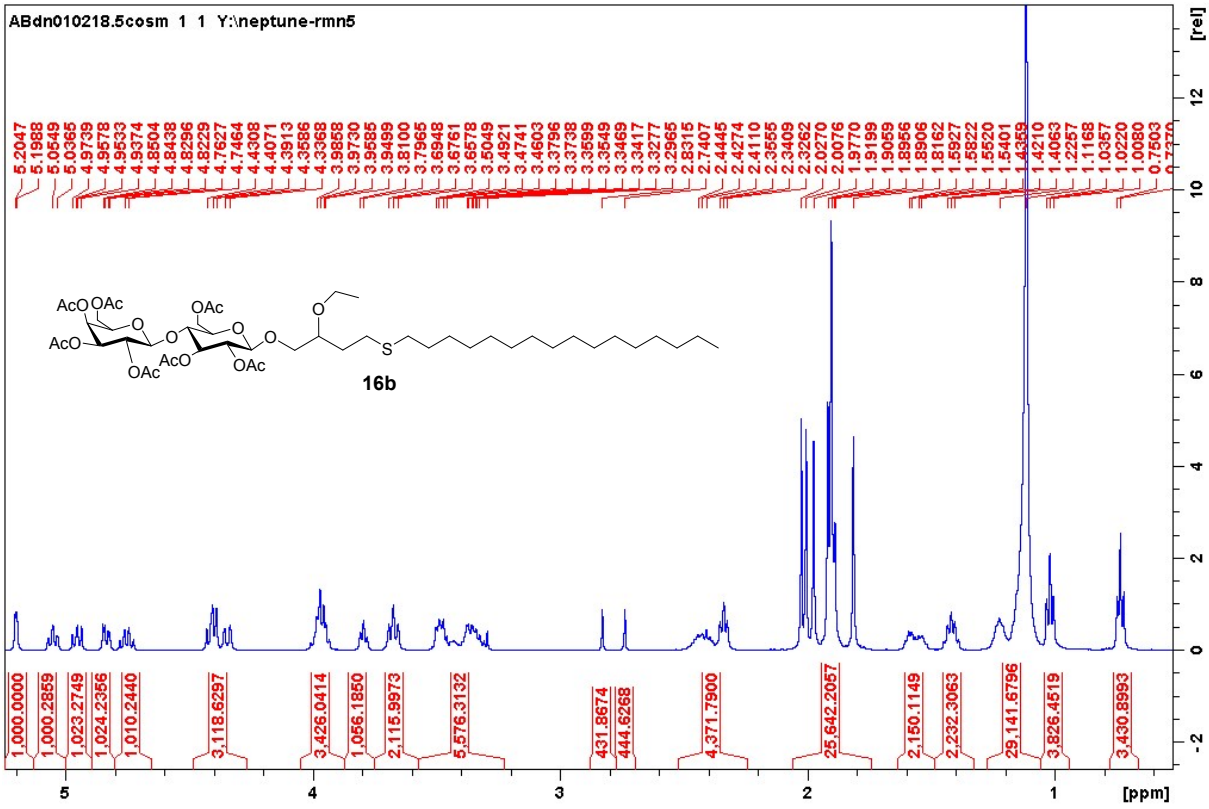

Figure ESI 2-35: ^{1}H NMR ($CDCl_3$) of compound **16b** (enlargement)

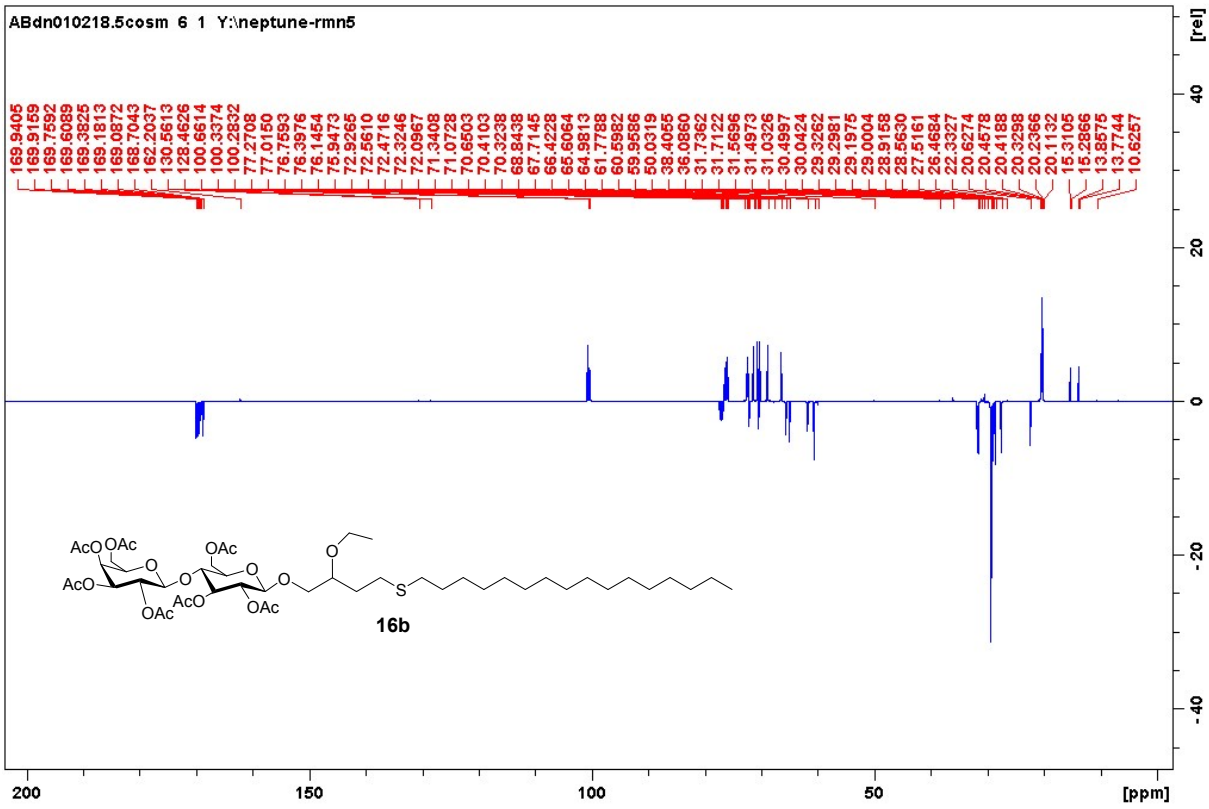

Figure ESI 2-36: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **16b**.

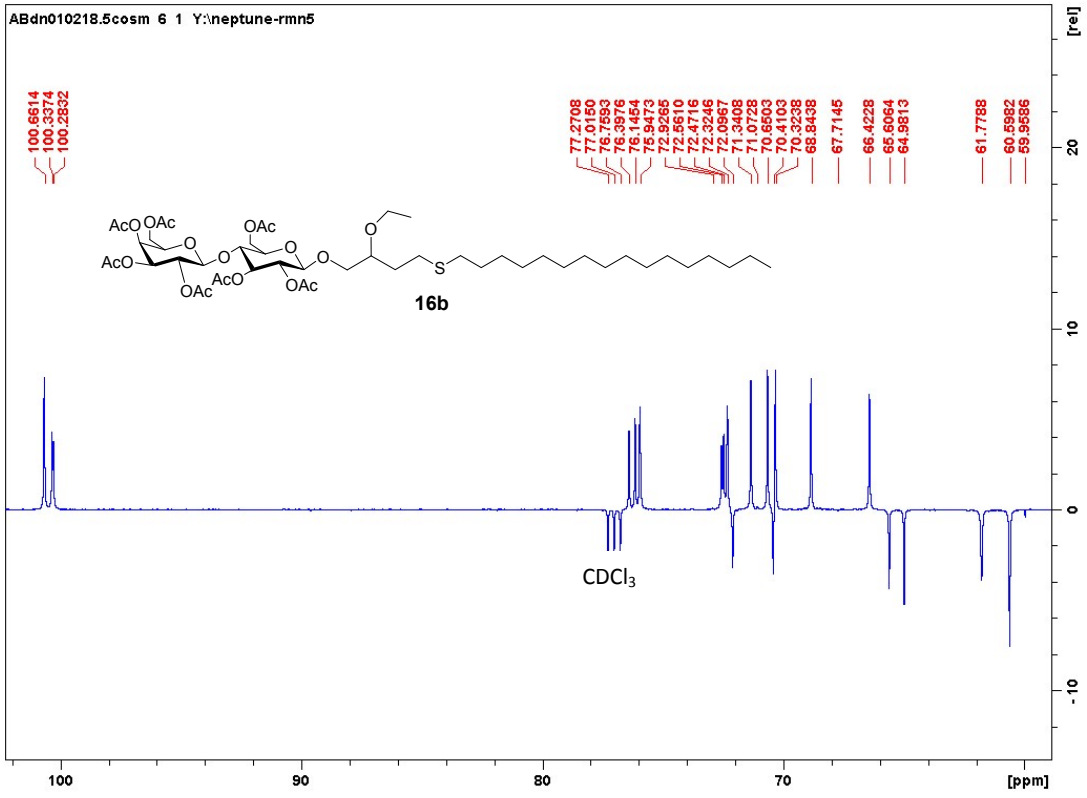

Figure ESI 2-37: $^{13}C\{^{1}H\}$ JMOD NMR ($CDCl_3$) of compound **16b**.

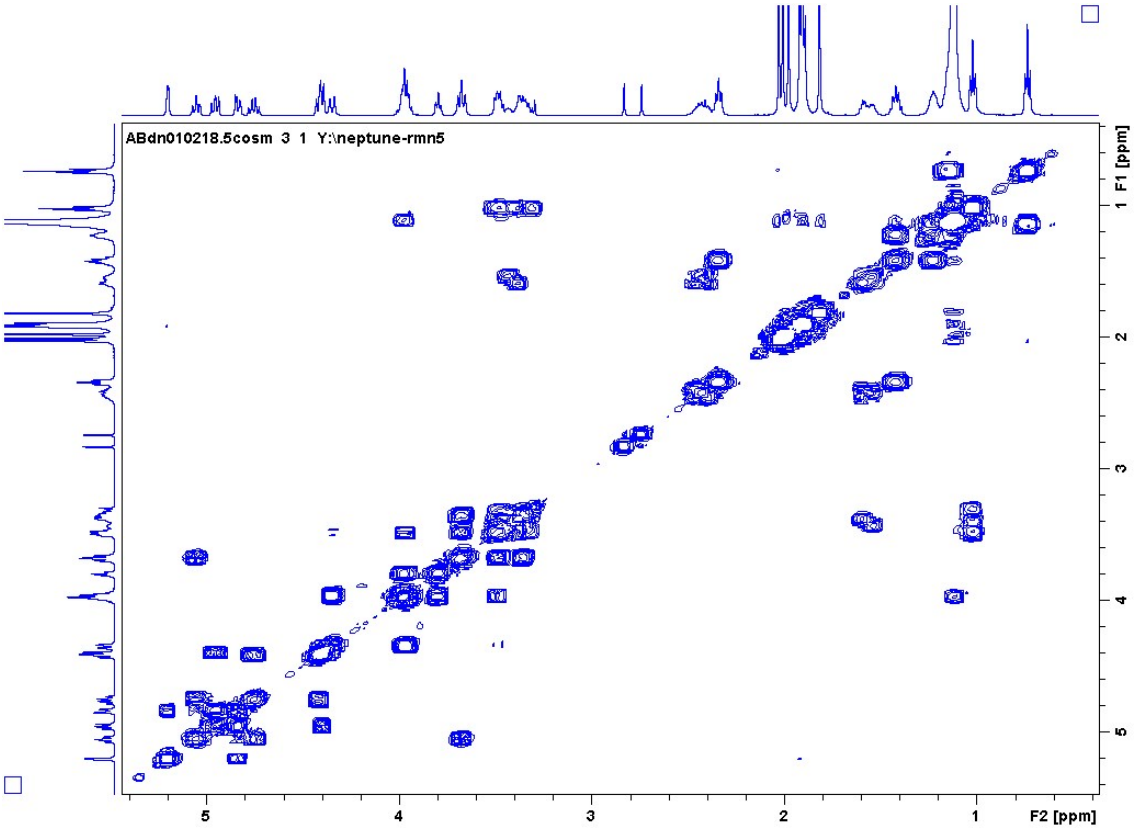

Figure ESI 2-38: ^{1}H COSY ($CDCl_3$) of compound **16b**

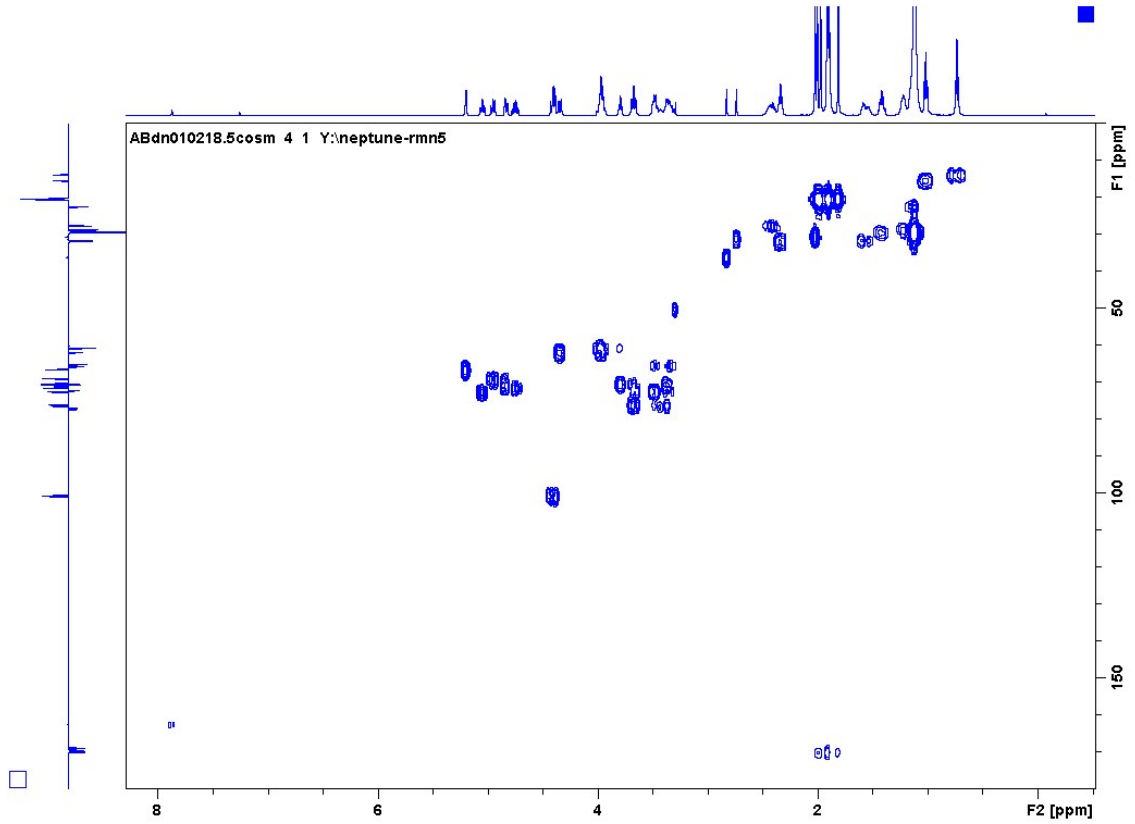

Figure ESI 2-39: HMQC ($CDCl_3$) of compound **16b**

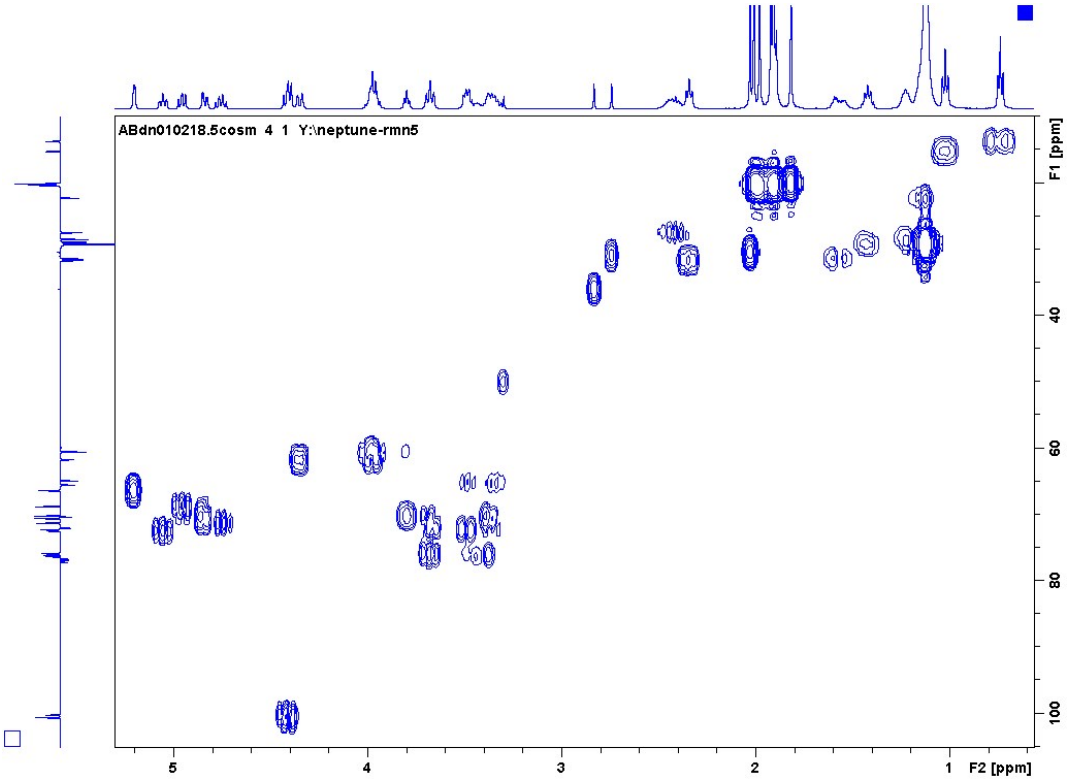

Figure ESI 2-40: HMQC ($CDCl_3$) of compound **16b** (enlargement)

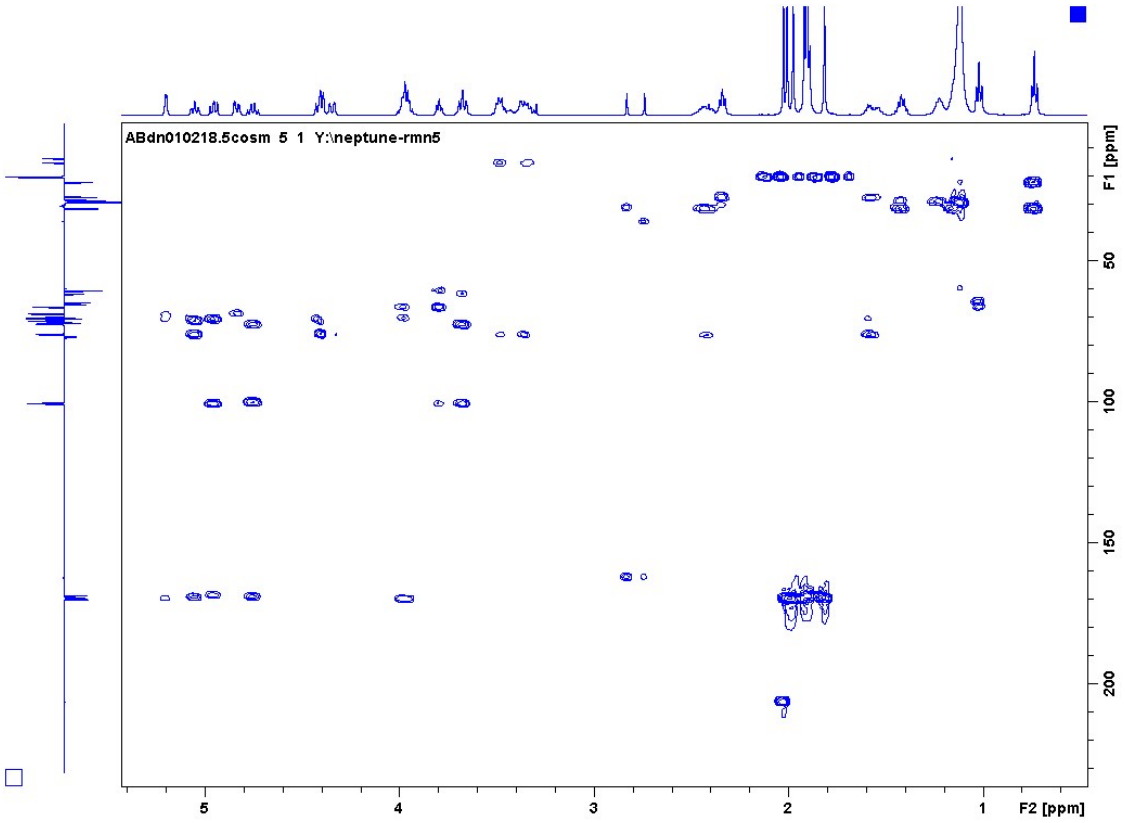

Figure ESI 2-41: HMBC ($CDCl_3$) of compound **16b**

1-O-[-4-(hexadecylthio)-2-methoxybutyl]-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **17a** **(Ohmline-5-S)**

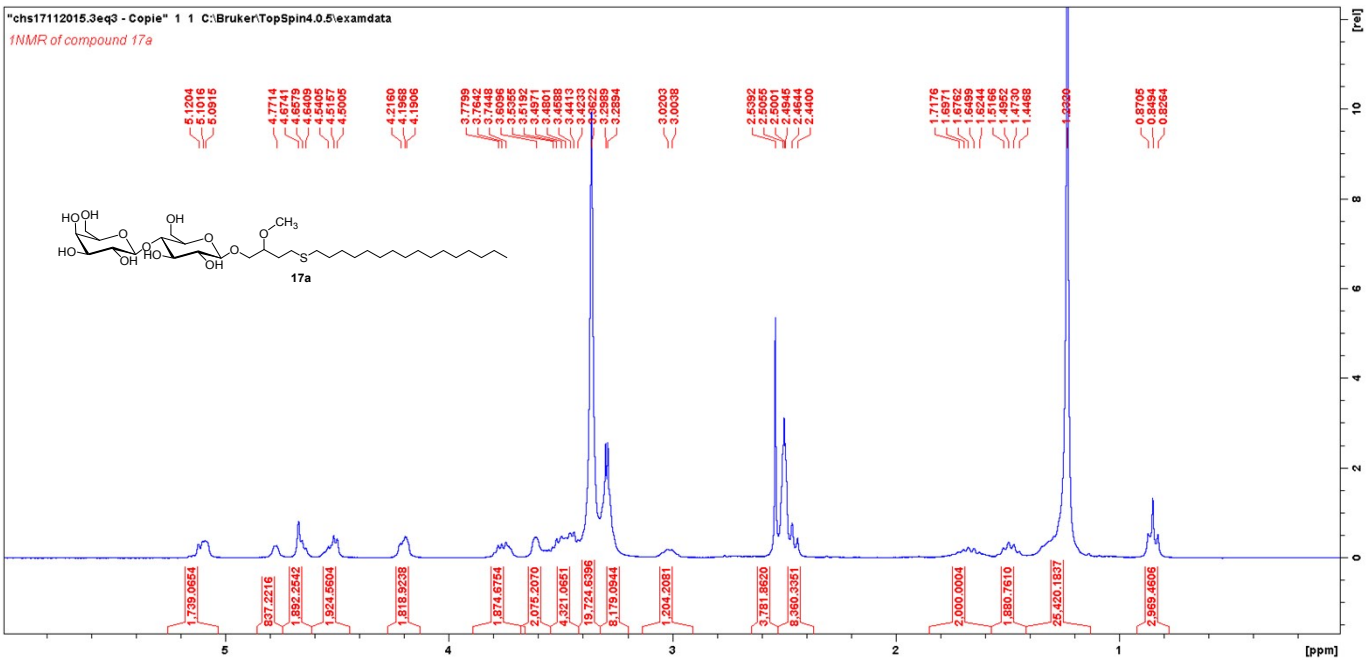

Figure ESI 2-42: ^{1}H NMR (DMSO-d_6) of compound **17a**

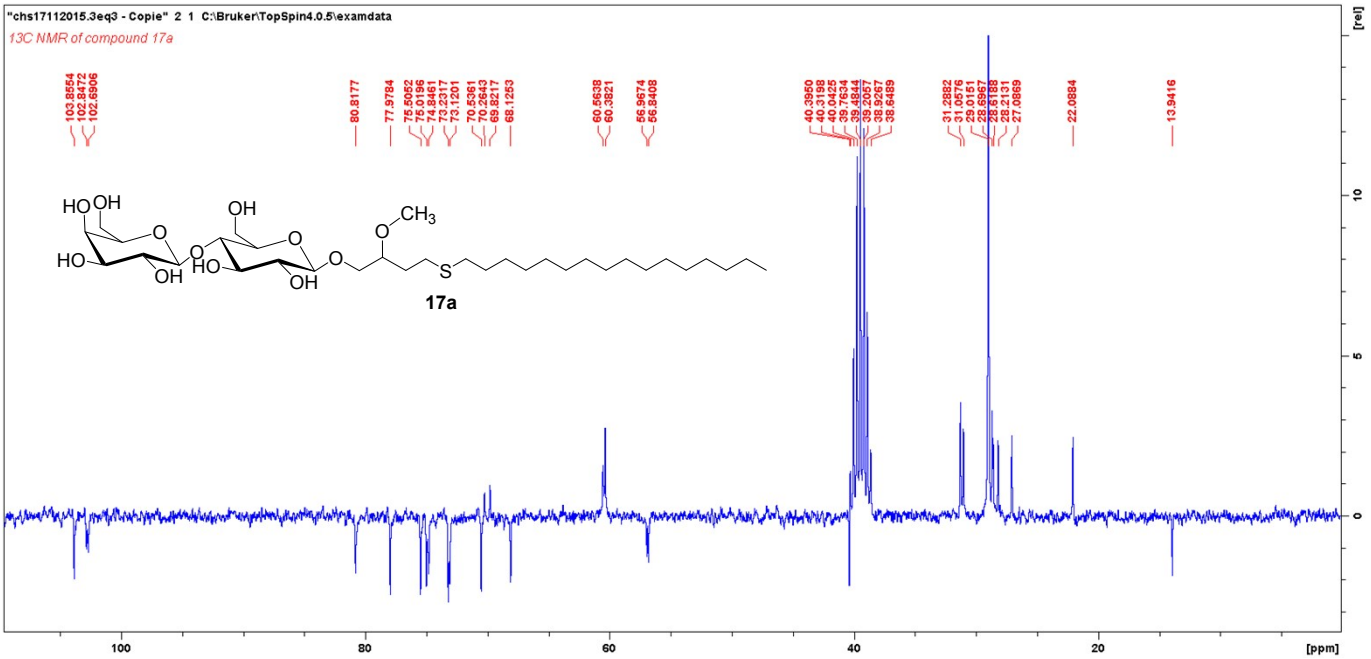

Figure ESI 2-43: ^{13}C{^{1}H} JMOD NMR (DMSO-d_6) of compound **17a**

1-O-[-4-(hexadecylthio)-2-ethoxybutyl]-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **17b** (Ohmline-2-OEt-5S)

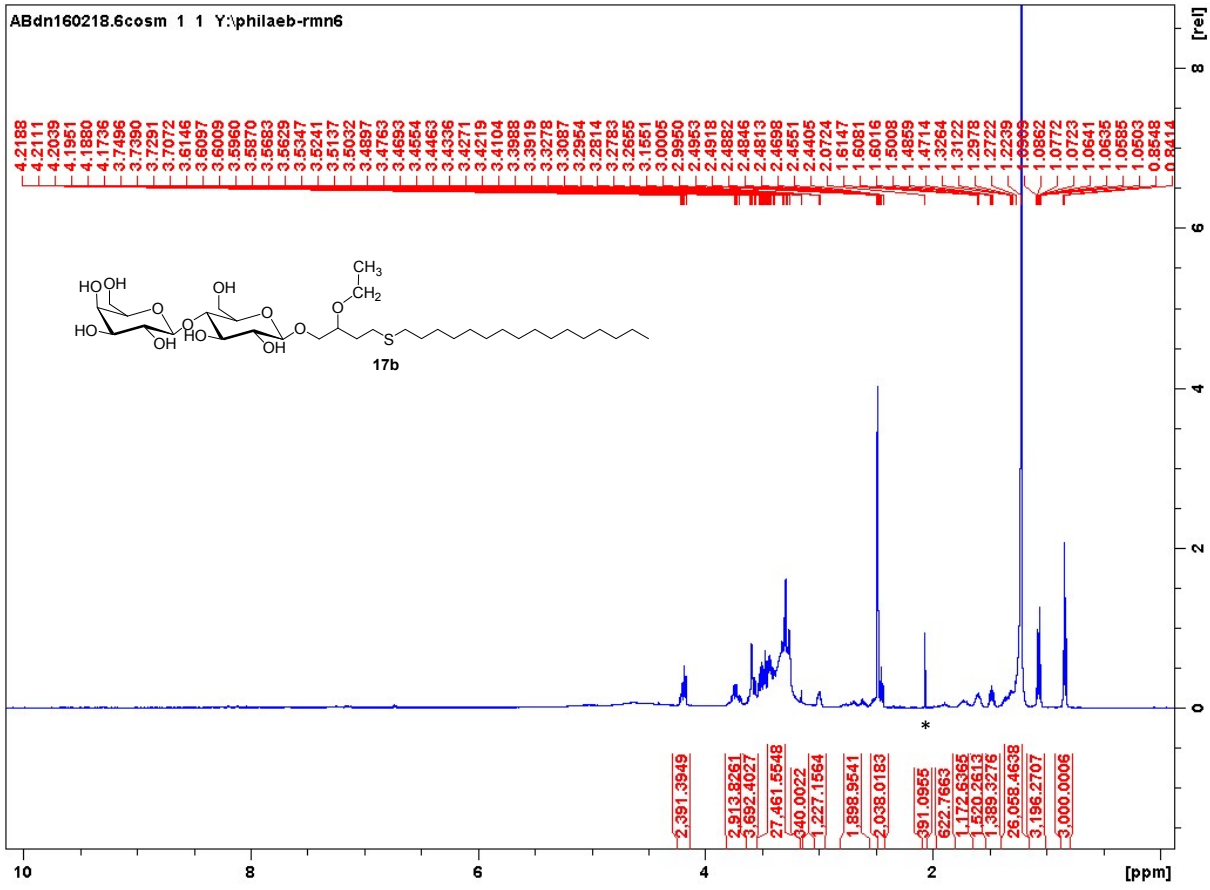

Figure ESI 2-44: ^{1}H NMR ($CDCl_3$) of compound **17b**

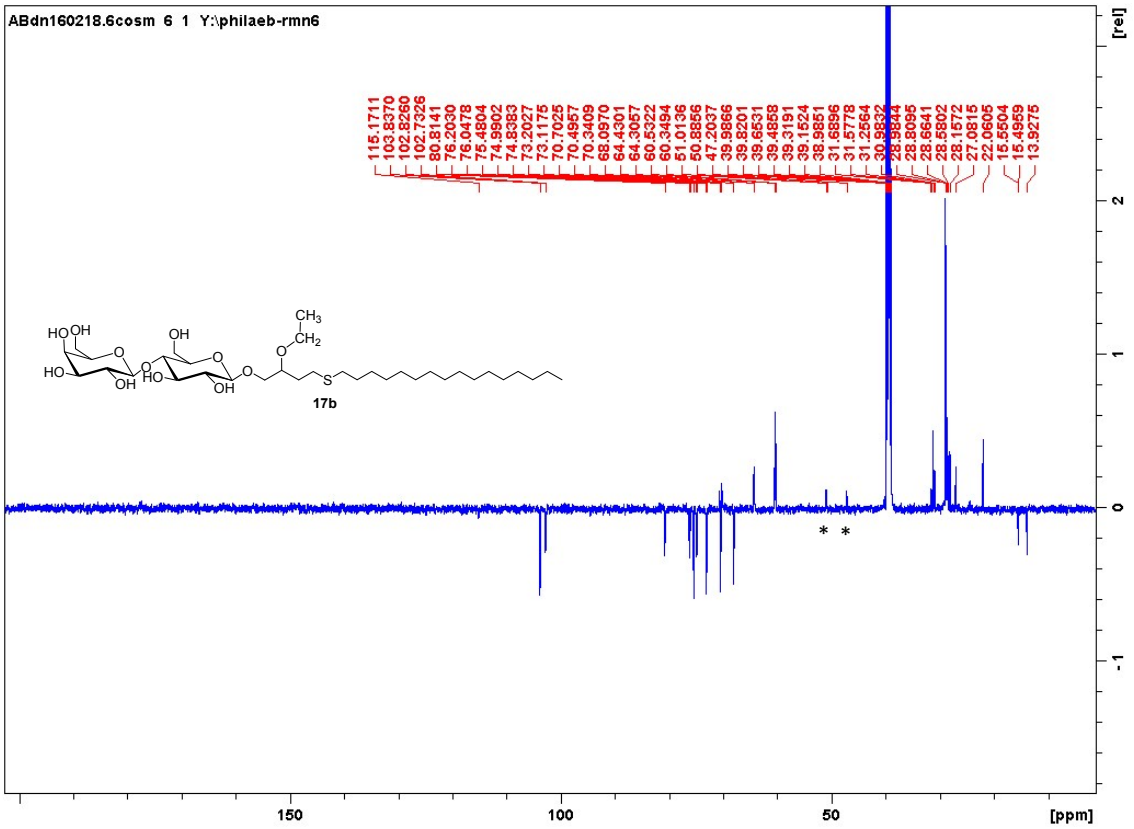

Figure ESI 2-45 ^{13}C NMR (DMSO-d_6) of compound **17b**

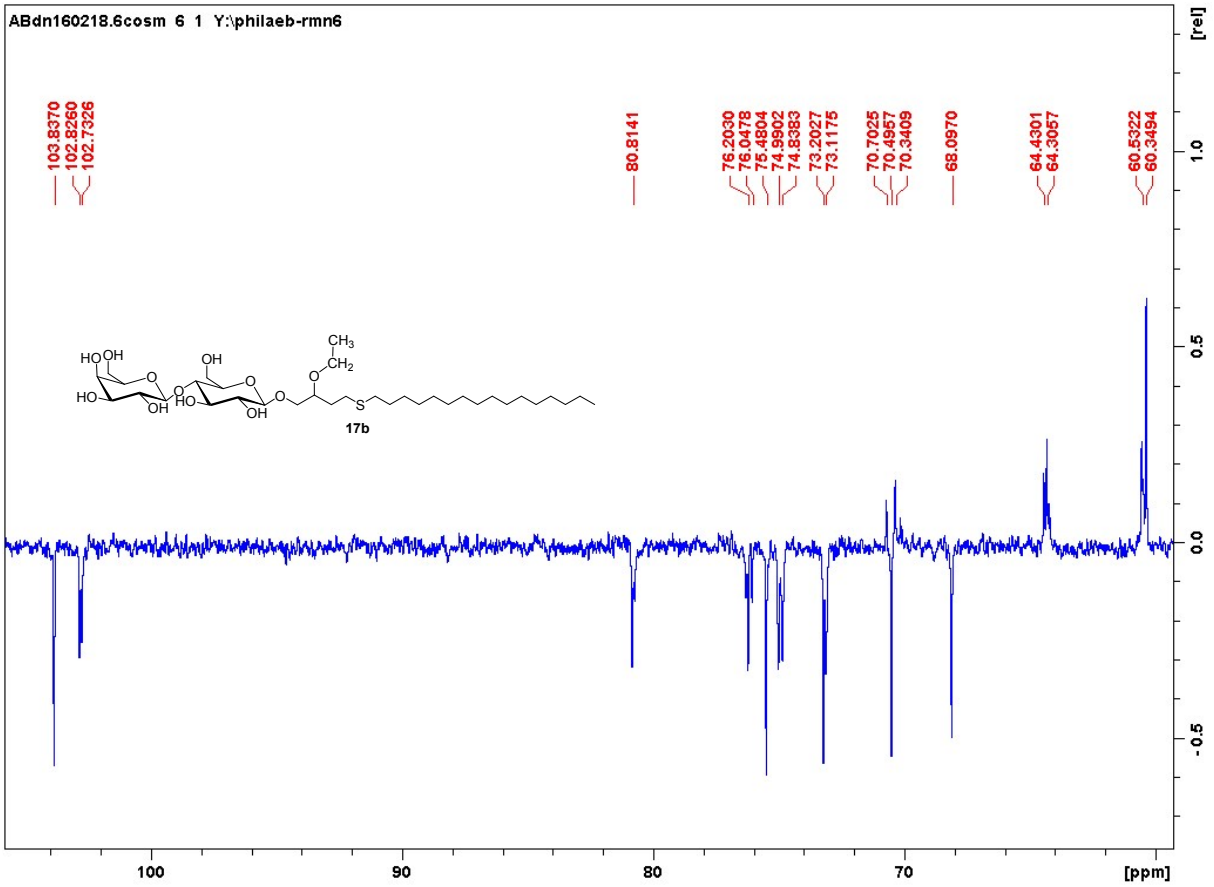

Figure ESI 2-46 ^{13}C NMR (DMSO-d_6) of compound **17b** (enlargement)

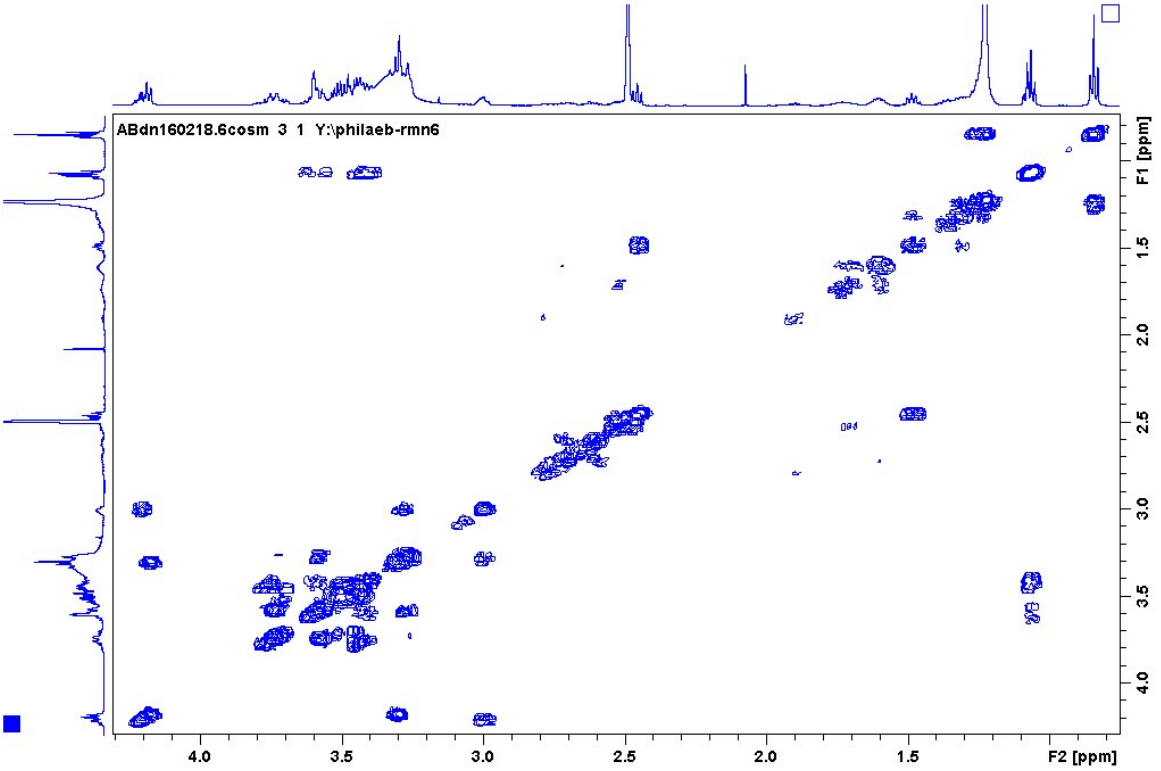

Figure ESI 2-47: ^{1}H COSY (DMSO-d_6) of compound **17b**

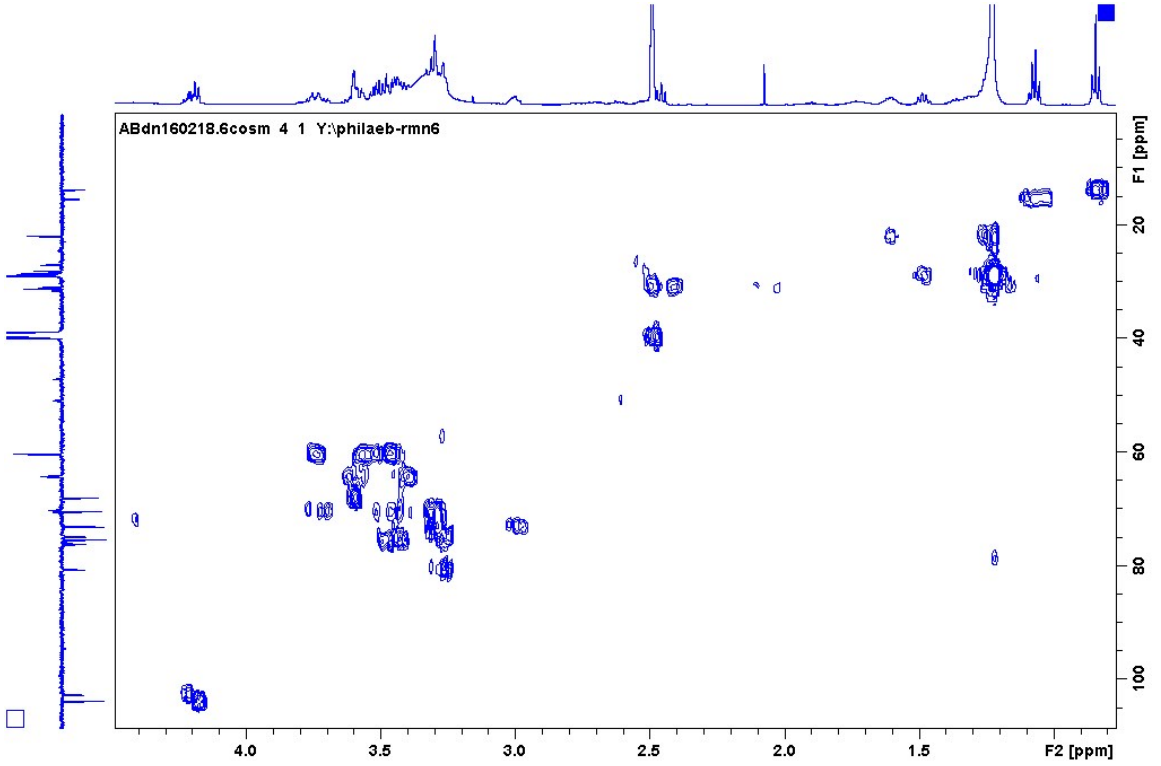

Figure ESI 2-48: HMQC (DMSO-d_6) of compound **17b**

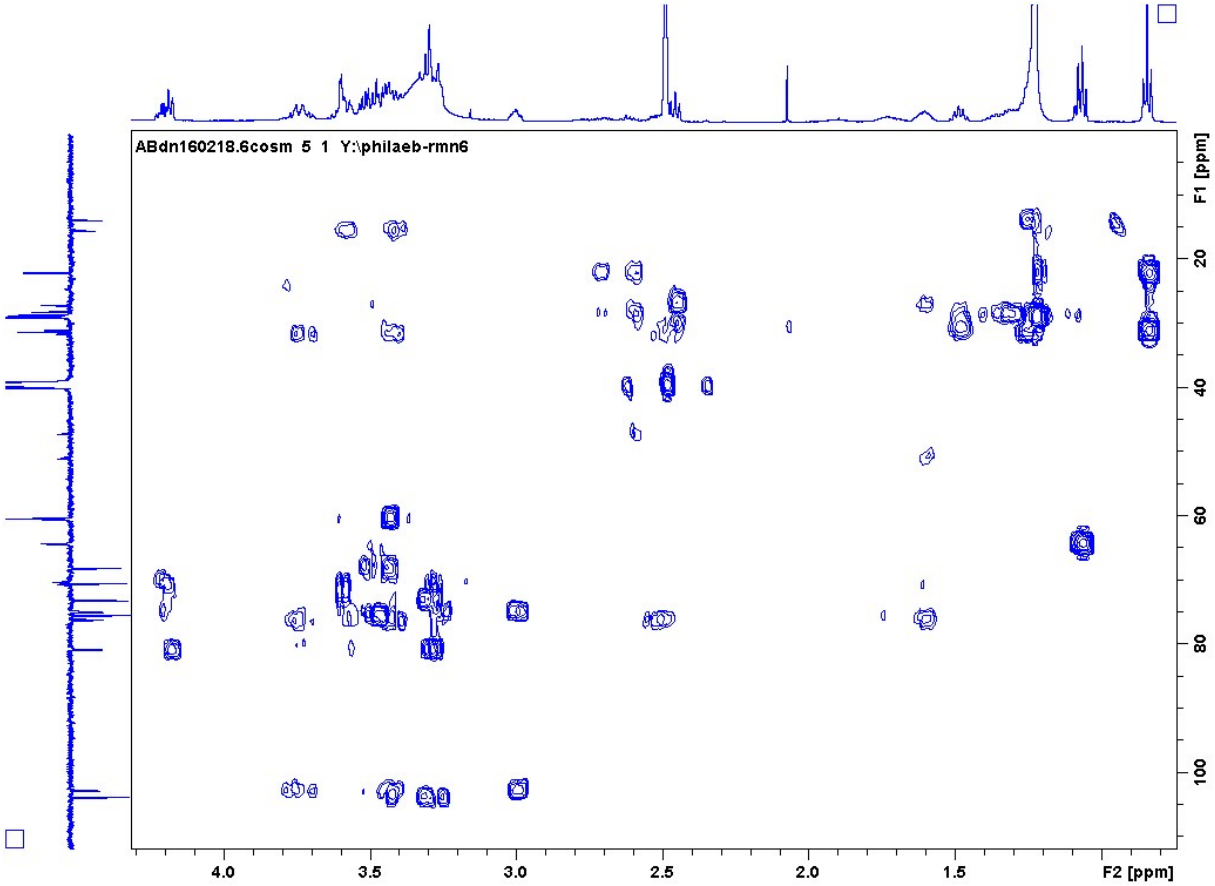

Figure ESI 2-49: HMBC (DMSO-d_6) of compound **17b**

3. Cell migration

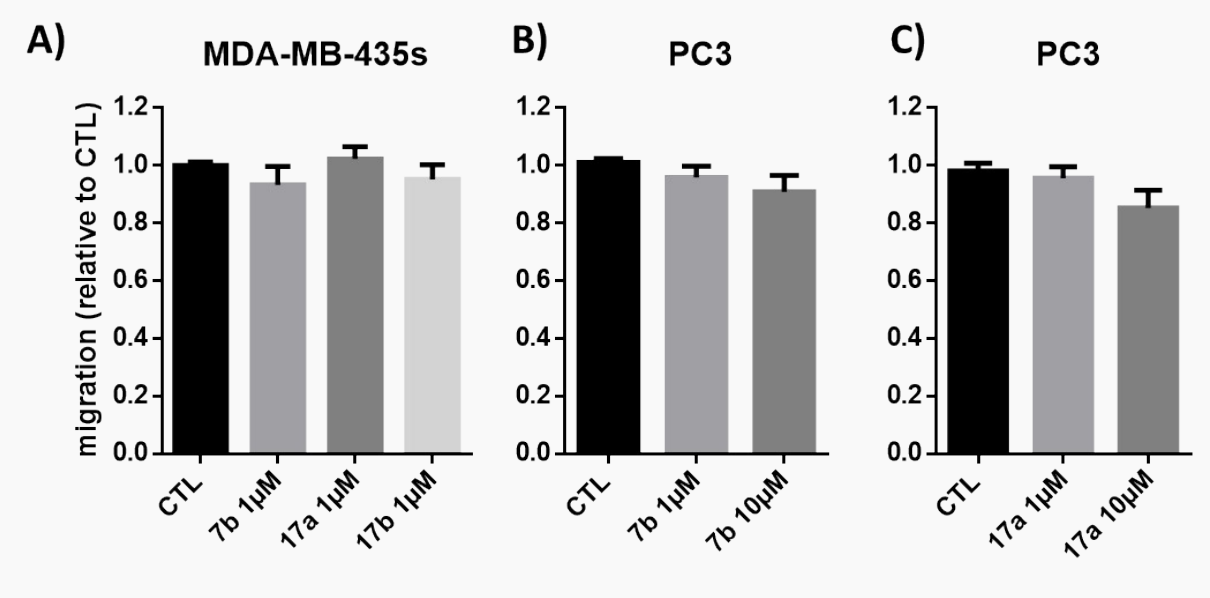

Figure ESI3-1 : Effect of **7b**, **17a**, **17b** compounds during 24 h on cell migration of MDA-MB-435s and PC3 cells. The relative cell number corresponds to the ratio of the number of migrating cells in the presence of compounds over the number of migrating cells in control conditions (means ± SEM, N=3, n=3). No significant effect was found ($p>0.05$, Kruskal-Wallis and post hoc tests).

4. Effect of **17b** on cytosolic calcium concentration

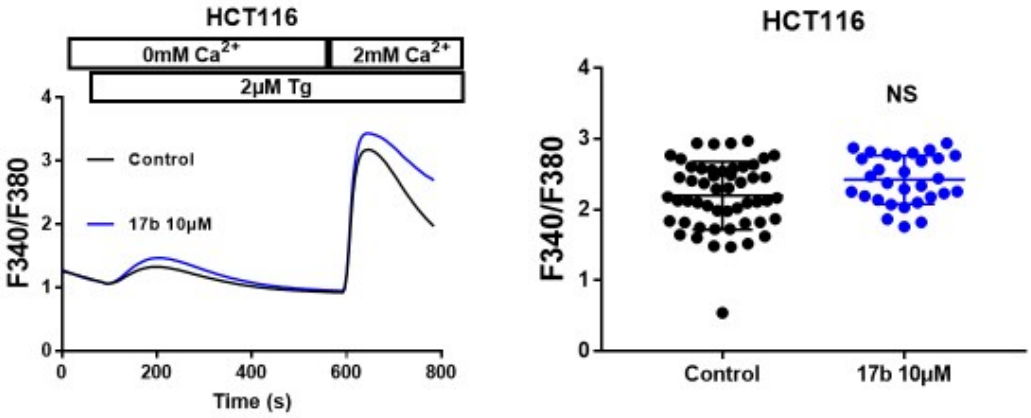

Figure ESI4-1: Representative traces showing the Tg-evoked SOCE in HCT116 cells after acute treatment (13 min) with 10µM **17b**. Quantification of peak SOCE values are expressed as mean ± SD, N = 3 (n 30-52 per condition); NS: non-significant

5. Effect of **13** on cytosolic calcium concentration

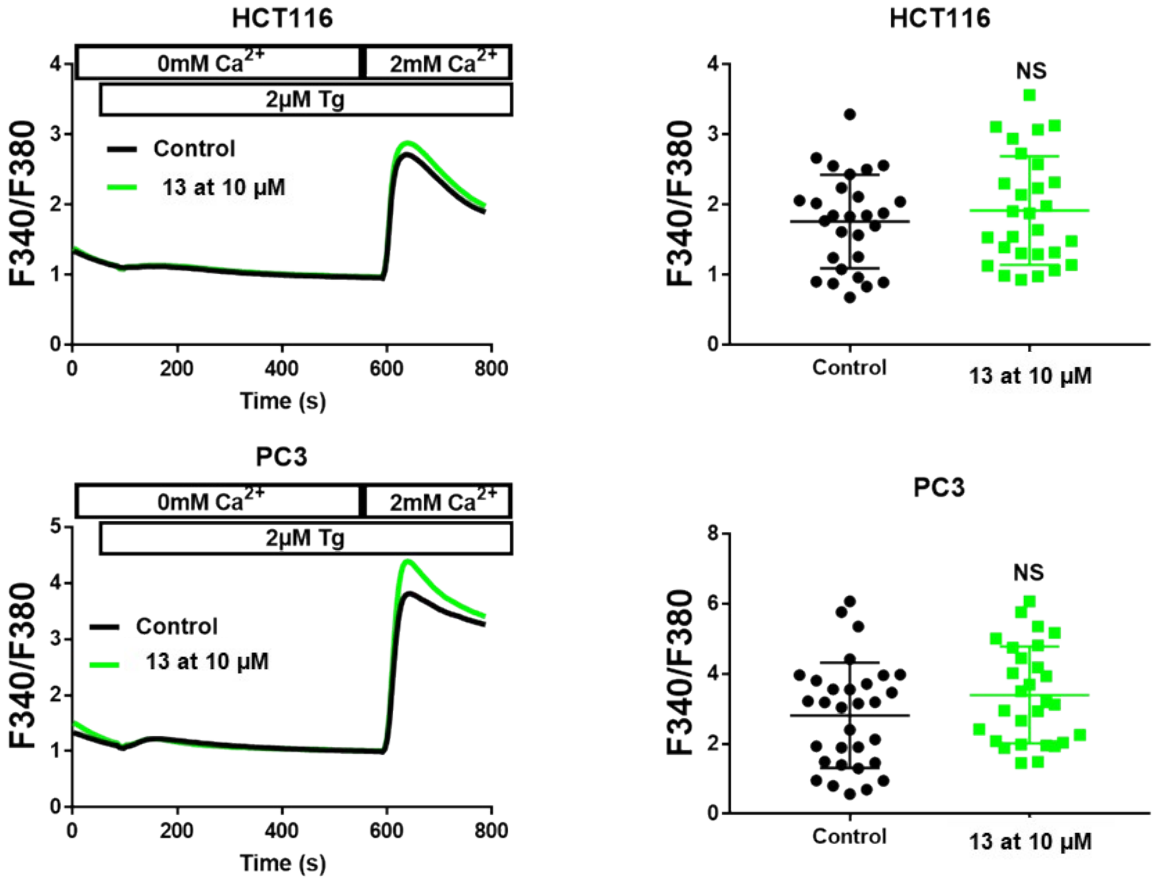

Figure ESI5-1 : Representative traces showing the Tg-evoked SOCE in HCT116 and PC3 cells after acute treatment (13 min) with 10µM compound **13**. Quantification of peak SOCE values are expressed as mean ± SD, N = 3 (n 27-32 per condition); NS.

6. Cytotoxicity

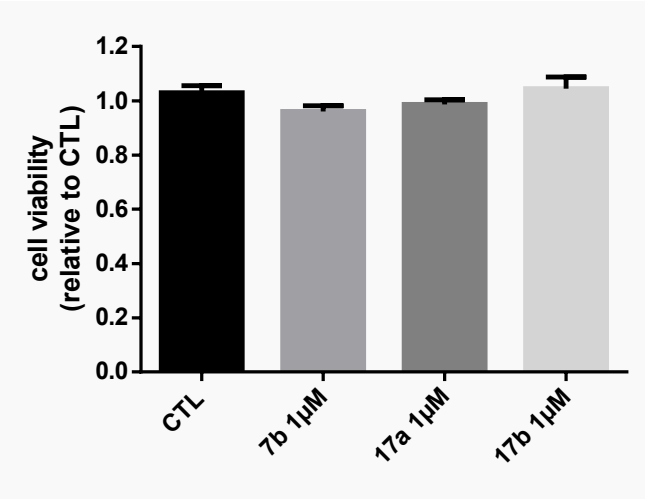

Figure ESI6-1 : Effect of **7b**, **17a**, **17b** compounds in cell viability of MDA-MB-435s. Cells were exposed to 1µM during 48h with each of the compounds (means ± SEM, N=3, n=3). No significant effect was found ($p>0.05$, Kruskal-Wallis and post hoc tests).