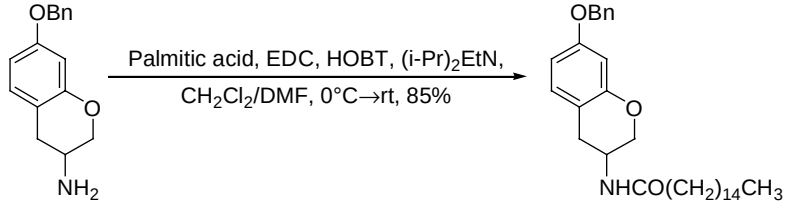
A Short and Efficient Route to the Fully Functionalized Polar Core of Scyphostatin

Emmanuel N. Pitsinos,* and Ana Cruz

SUPPORTING INFORMATION

General: All reactions were carried out under a dry argon atmosphere with anhydrous, freshly distilled solvents under anhydrous conditions unless otherwise noted. All reactions were magnetically stirred with Teflon stir bars, and temperatures were measured externally. Reactions requiring anhydrous conditions were carried out in oven dried (120°C, 24 h) or flame dried (vacuum < 0.5 Torr) glassware. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. All reagents were obtained from Aldrich Chemical Co. Inc. and used without further purification. All reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm E.Merck silica gel plates (60F-254). E.Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-250 or a Bruker Advance DRX-500 instrument as noted individually. Chemical shifts are measured in parts per million (δ) relative to the deuterated solvent used in the experiment. Multiplicities are designated as singlet (s), doublet (d), triplet (t), or multiplet (m). Broad or obscured peaks are indicated as “br” or “obs” respectively.

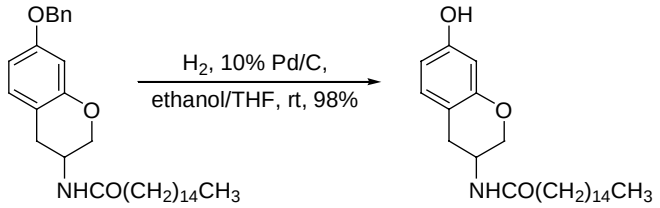
7-Benzyloxy-3-hexadecanoylamino-3,4-dihydro-2*H*-1-benzopyran (18).



8a **18**

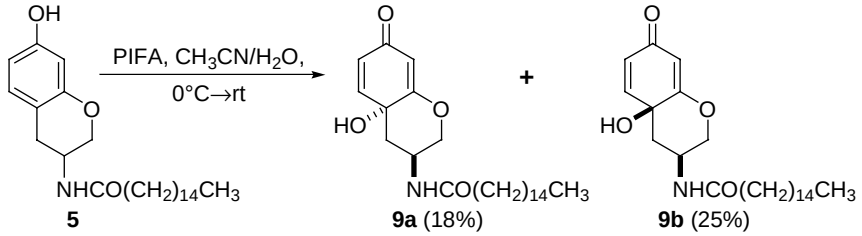
To a stirred solution of 7-benzyloxy-3-amino-3,4-dihydro-2*H*-1-benzopyran (**8a**) (0.51 g, 2.0 mmol) and palmitic acid (0.54 g, 2.1 mmol) in a mixture of dichloromethane (10 mL) and DMF (1.5 mL) were added at 0 °C HOBT (17 mg, 0.13 mmol), *N,N*-diisopropylethylamine (0.37 mL, 2.1 mmol) and EDC (0.40 g, 2.1 mmol). The mixture was stirred for 2 h at 0 °C and then for 20 h at ambient temperature. Upon reaction completion, the mixture was poured in a saturated aqueous solution of ammonium chloride (30 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic phases were washed sequentially with water (3 × 10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (8:2 hexane-ethyl acetate) to produce 7-benzyloxy-3-hexadecanoylamino-3,4-dihydro-2*H*-1-benzopyran (**18**) (0.84 g, 85%) as amorphous white solid: R_f 0.34 (8:2 hexane-ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.44–7.30 (m, 5 H, Ph*H*), 6.94 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 6.58 (dd, *J* = 8.4, 2.5 Hz, 1 H, Ar*H*), 6.48 (d, *J* = 2.5 Hz, 1 H, Ar*H*), 5.75 (brd, *J* = 7.7 Hz, 1 H, N*H*CO), 5.02 (s, 2 H, OCH_2Ph), 4.54–4.43 (m, 1 H, C*H*NHCO), 4.10 (ddd, *J* = 10.8, 2.9, 2.2 Hz, 1 H, C*H*HO), 4.06 (dd, *J* = 10.8, 1.1 Hz, 1 H, CH*H*O), 3.05 (dd, *J* = 16.5, 5.2 Hz, 1 H, C*H*HCH), 2.66 (brd *J* = 16.5 Hz, 1 H, CH*H*CH), 2.13 (t, *J* = 7.6 Hz, 2 H, COCH_2), 1.67–1.53 (m, 2 H, $COCH_2CH_2$), 1.25 (brs, 24 H, $(CH_2)_{12}$), 0.88 (t, *J* = 6.5 Hz, 3 H, CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 173.0, 158.5, 154.5, 136.9, 130.9, 128.6, 127.9, 127.4, 111.5, 108.9, 102.7, 70.0, 68.2, 42.1, 36.7, 31.9, 29.6, 29.4, 29.3, 25.6, 22.7, 14.1; HR-ESI: *m/z*: 494.3636, [*M*+H$^+$] for the compound $C_{32}H_{47}NO_3$ requires 494.3629.

7-Hydroxy-3-hexadecanoylamino-3,4-dihydro-2*H*-1-benzopyran (5).



A solution of 7-benzyloxy-3-hexadecanoylamino-3,4-dihydro-2*H*-1-benzopyran (**18**) (1.2 g, 2.4 mmol) in a mixture of ethanol (100 mL) and THF (200 mL) was subjected to hydrogenolysis over 10% Pd/C at ambient temperature. After 12 h, filtration of the reaction mixture through Celite® and removal of the solvent under reduced pressure provided phenol **5** (0.96 mg, 98%) as an amorphous white solid: R_f 0.14 (3:97 methanol-dichloromethane); ^{1}H NMR (250 MHz, 1:1 $CDCl_3$:DMSO-d_6) δ 8.85 (brs, 1 H, ArO*H*), 7.46 (d, *J* = 7.3 Hz, 1 H, N*H*CO), 6.73 (d, *J* = 8.2 Hz, 1 H, Ar*H*), 6.27 (dd, *J* = 8.2, 2.3 Hz, 1 H, Ar*H*), 6.17 (d, *J* = 2.3 Hz, 1 H, Ar*H*), 4.20–4.09 (m, 1 H, C*H*NHCO), 4.00 (dd, *J* = 10.4, 2.1 Hz, 1 H, C*H*HO), 3.76 (dd, *J* = 10.4, 7.0 Hz, 1 H, CH*H*O), 2.82 (dd, *J* = 15.9, 5.5 Hz, 1 H, C*H*HCH), 2.55 (dd *J* = 15.9, 7.1 Hz, 1 H, CH*H*CH), 2.07 (t, *J* = 7.5 Hz, 2 H, COCH_2), 1.54–1.44 (m, 2 H, $COCH_2CH_2$), 1.18 (brs, 24 H, $(CH_2)_{12}$), 0.81 (t, *J* = 6.5 Hz, 3 H, CH_3); ^{13}C NMR (62.5 MHz, 1:1 $CDCl_3$:DMSO-d_6) δ 172.5, 156.2, 153.9, 129.7, 109.9, 108.1, 102.4, 67.1, 41.8, 35.3, 31.1, 29.4, 28.8, 28.7, 28.6, 28.5, 25.0, 21.8, 13.5; HR-ESI: *m/*z: 426.2973, [*M*+Na^+] for the compound $C_{25}H_{41}NO_3$ requires 426.2979.

Preparation of *para*-quinols 9a and 9b.



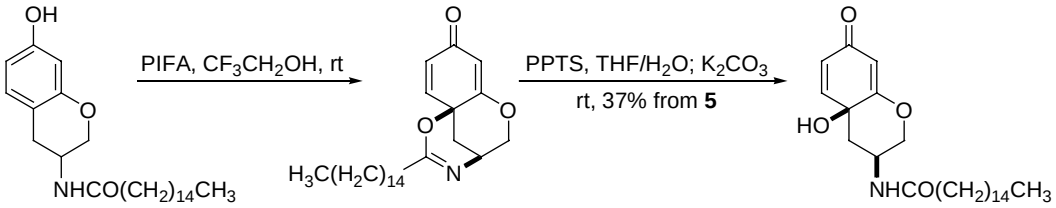
To a vigorously stirred fine suspension of phenol **5** (100 mg, 0.25 mmol) in a mixture of acetonitrile (20 mL) and water (1 mL) was added at 0 °C, [bis(trifluoroacetoxy)iodo]benzene (200 mg, 0.47 mmol). The mixture was

stirred for 1 h at 0 °C and then at ambient temperature for 6 h. The reaction mixture was then poured in water (40 mL) and extracted with dichloromethane (4×15 mL). The combined organic phases were washed sequentially with water (3×10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (3:97 methanol-dichloromethane) to produce, in order of elution,

compound **9b** as light yellow foam (25.7 mg, 25%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (brd, *J* = 8.0 Hz, 1 H, N*H*CO), 6.61 (d, *J* = 9.7 Hz, 1 H, C*H*=), 6.12 (d, *J* = 9.7 Hz, 1 H, C*H*=), 5.77 (s, 1 H, C*H*=), 4.46–4.39 (m, 2 H, C*H*NH+C*H*HO), 4.05 (dd, *J* = 11.2, 3.8 Hz, 1 H, CH*H*O), 3.61 (brs, 1 H, O*H*), 2.22 (t, *J* = 7.5 Hz, 2 H, COCH_2), 2.17 (dd, *J* = 12.9, 3.0 Hz, 1 H, C*H*HCHNH), 2.05 (dd, *J* = 12.9, 6.0 Hz, 1 H, CH*H*CHNH), 1.67–1.61 (m, 2 H, $COCH_2CH_2$), 1.35–1.21 (m, 24 H, $(CH_2)_{12}CH_3$), 0.88 (t, *J* = 6.7 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 187.8, 172.9, 172.2, 145.4, 127.9, 109.7, 73.1, 65.6, 42.4, 36.9, 35.9, 31.9, 29.6, 29.5, 29.3, 29.2, 25.6, 22.7, 14.1; HR-MALDI-FTMS: *m/*z: 420.3112, [*M*+H^+] for the compound $C_{25}H_{41}NO_4$ requires 420.3108.

and compound **9a** as light yellow foam (18.7 mg, 18%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (d, *J* = 9.7 Hz, 1 H, C*H*=), 6.04 (d, *J* = 9.7 Hz, 1 H, C*H*=), 5.90 (brd, *J* = 7.5 Hz, 1 H, N*H*CO), 5.66 (s, 1 H, C*H*=), 4.91 (dd, *J* = 10.9, 2.5 Hz, 1 H, C*H*HO), 4.62–4.56 (m, 1 H, C*H*NH), 4.01 (dd, *J* = 10.9, 2.7 Hz, 1 H, CH*H*O), 3.06 (brs, 1 H, O*H*), 2.67 (dd, *J* = 14.6, 8.3 Hz, 1 H, C*H*HCHNH), 2.15 (t, *J* = 7.4 Hz, 2 H, COCH_2), 1.70–1.55 (m, 3 H, $COCH_2CH_2$+CH*H*CHNH), 1.24 (brs, 24 H, $(CH_2)_{12}CH_3$), 0.87 (t, *J* = 6.6 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 187.6, 173.1, 171.7, 145.3, 127.2, 106.4, 69.9, 65.2, 41.4, 37.8, 36.6, 31.9, 29.6, 29.4, 29.3, 25.5, 22.7, 14.1; HR-ESI: *m/*z: 420.3111, [*M*+H^+] for the compound $C_{25}H_{41}NO_4$ requires 420.3114.

Diastereoselective preparation of *para*-quinol 9b.

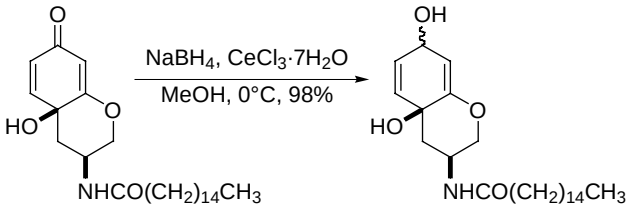


To a vigorously stirred fine suspension of phenol **5** (144.6 mg, 0.36 mmol) in 2,2,2-trifluoroethanol (10 mL) was added at ambient temperature [bis(trifluoro-acetoxy)iodo]benzene (240 mg, 0.56 mmol). The reaction mixture was stirred for 4 h and then it was concentrated under reduced pressure. The residue thus obtained was dissolved in ethyl acetate (20 mL), washed sequentially with a saturated aqueous solution of sodium bicarbonate (10 mL), water (2 × 10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained used without further purification in the next step. A small sample of it was subjected to flash column chromatography (3:97 methanol-dichloromethane) to produce compound **10** as light yellow oil: R_f 0.21 (3:97 methanol-dichloromethane); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.48 (d, *J* = 9.9 Hz, 1 H, C*H*=), 6.24 (dd, *J* = 9.9, 1.6 Hz, 1 H, C*H*=), 5.78 (d, *J* = 1.6 Hz, 1 H, C*H*=), 4.38 (brd, *J* = 10.8 Hz, 1 H, C*H*HO), 4.00 (dd, *J* = 10.8, 1.9 Hz, 1 H, CH*H*O), 3.88–3.82 (m, 1 H, C*H*N), 2.27 (t, *J* = 7.6 Hz, 2 H, CH_2C(O)N), 2.11–1.98 (m, 2 H, CH_2CHN), 1.65–1.54 (m, 2 H, N(O)CCH$_2$CH_2), 1.25 (brs, 24 H, (CH_2)$_{12}$CH$_3$), 0.88 (t, *J* = 6.4 Hz, 3 H, CH$_2$CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 161.8, 141.6, 130.3, 110.0, 74.4, 47.0, 34.7, 31.9, 29.7, 29.5, 29.3, 29.2, 28.9, 26.2, 22.7, 14.1; HR-ESI-TOF: *m/*z: 402.3008, [*M*+H$^+$] for the compound $C_{25}H_{39}NO_3$ requires 402.3003.

The above residue was dissolved in THF (5 mL) and water (0.5 mL) and PPTS (46 mg) were added to the solution. The mixture was stirred at ambient temperature for 18 h. Solid potassium carbonate (260 mg) was added and stirring was continued for 24 h. The reaction mixture was then poured in water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed sequentially with 0.01 N aqueous HCl

solution (10 mL), water (10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (3:97 methanol-dichloromethane) to produce compound **9b** as light yellow foam (55.5 mg, 37%).

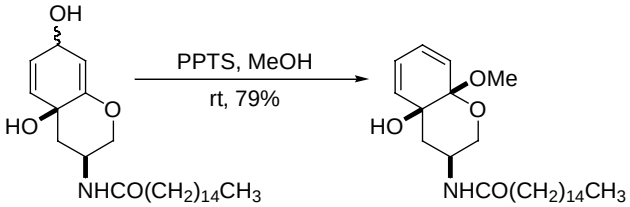
Preparation of diol 11.



9b **11**

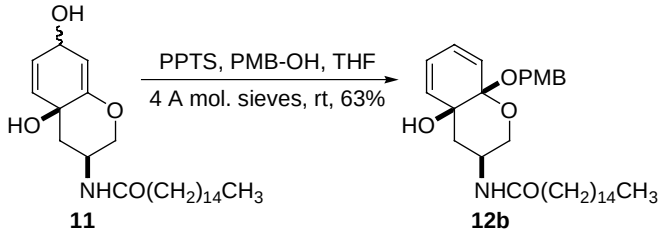
To a stirred solution of quinol **9b** (166 mg, 0.40 mmol) and $CeCl_3 \cdot 7H_2O$ (295 mg, 0.80 mmol) in methanol (10 mL) was added, at 0 °C and in small portions, sodium borohydride (23 mg, 0.6 mmol). Upon reaction completion, excess reagent was quenched by addition of acetone (2 mL). The mixture was stirred at ambient temperature for 30 min and was then poured in water (20 mL) and extracted with ethyl acetate (4 × 15 mL). The combined organic phases were washed sequentially with water (2 × 10 mL) and brine (2 × 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to produce diol **11** (163 mg, 98%) as a 1:2 mixture of diastereomers. Further purification of this sensitive intermediate is neither necessary nor advisable. Light yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.69, 7.53 (two brd in a ration 1:2, *J* = 7.5 Hz, 1 H, N*H*CO), 6.02–5.86 (m, 1 H, C*H*=), 5.82–5.68 (m, 1 H, C*H*=), 5.57–5.44 (m, 1 H, C*H*=), 4.69, 4.60 (two brs in a ratio 2:1, 1 H, C*H*OH), 4.34–3.95 (m, 2 H, C*H*NH+C*H*HO), 3.75–3.47 (m, 1 H, CH*H*O), 2.29–1.83 (m, 4 H, CH_2CHNH+COCH_2), 1.73–1.51 (m, 2 H, COCH$_2$CH_2), 1.48–1.11 (m, 24 H, (CH_2)$_{12}$CH$_3$), 0.87 (t, *J* = 6.5 Hz, 3 H, CH$_2$CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.8, 132.3, 131.6, 130.4, 111.3, 109.6, 73.7, 64.2, 43.7, 43.4, 37.4, 37.0, 31.9, 29.6, 29.5, 29.3, 25.6, 22.6, 14.1; HR-ESI-TOF: *m/*z: 422.3258, [*M*+H$^+$] for the compound $C_{25}H_{43}NO_4$ requires 422.3265.

Preparation of ketal 12a.



To a stirred solution of diol **11** (55 mg, 0.13 mmol) in methanol (15 mL) was added at ambient temperature, PPTS (16 mg, 0.07 mmol). The reaction mixture was stirred for 4 h. It was then poured in aqueous solution of sodium bicarbonate (20 mL) and extracted with ethyl acetate (4 $\times$ 15 mL). The combined organic phases were washed sequentially with water (2 $\times$ 10 mL) and brine (2 $\times$ 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (4:6 ethyl acetate-hexane) to produce ketal **12a** (44 mg, 79%) as colorless oil: R_f 0.25 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (brd, *J* = 8.0 Hz, 1 H, N*H*CO), 6.18 (ddd, *J* = 9.9, 4.7, 1.2 Hz, 1 H, C*H*=), 5.89 (d, *J* = 9.9 Hz, 1 H, C*H*=), 5.85–5.79 (m, 2 H, C*H*=), 4.07–4.02 (m, 1 H, C*H*NH), 3.90 (brd, *J* = 12.1 Hz, 1 H, C*H*HO), 3.72 (dd, *J* = 12.1, 1.9 Hz, 1 H, CH*H*O), 3.37 (brs, 1 H, O*H*), 3.34 (s, 3 H, OCH_3), 2.16 (t, *J* = 7.7 Hz, 2 H, COCH_2), 1.92 (dt, *J* = 14.7, 2.4 Hz, 1 H, C*H*HCHNH), 1.83 (dd, *J* = 14.7, 4.2 Hz, 1 H, CH*H*CHNH), 1.64–1.56 (m, 2 H, $COCH_2CH_2$), 1.33–1.19 (m, 24 H, $(CH_2)_{12}CH_3$), 0.87 (t, *J* = 6.7 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.7, 139.0, 129.7, 127.4, 121.2, 98.2, 72.8, 66.8, 50.1, 41.3, 36.9, 35.3, 31.9, 29.6, 29.5, 29.3, 25.6, 22.6, 14.1; HR-ESI-TOF: *m/z*: 458.3241, [*M*+Na^+] for the compound $C_{26}H_{45}NO_4$ requires 458.3241.

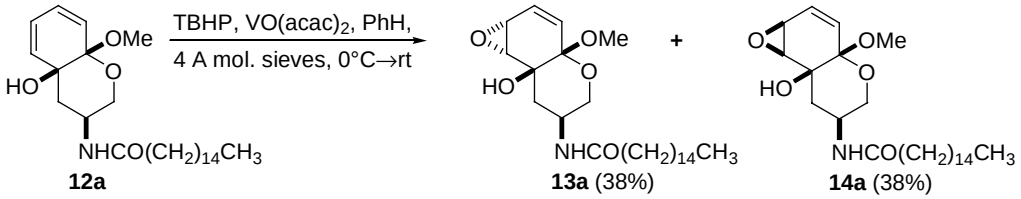
Preparation of ketal 12b.



To a stirred solution of diol **11** (163 mg, 0.39 mmol) in THF (7 mL) were added powdered molecular sieves 4 Å (160 mg), 4-methoxybenzyl alcohol

(280 mg, 2.0 mmol) and PPTS (60 mg, 0.2 mmol). The mixture was stirred at ambient temperature for 24 h. The reaction was quenched by addition of solid sodium bicarbonate (100 mg). The mixture was diluted with ethyl acetate (20 mL) and filtered with the aid of Celite®. The filtrate was poured in saturated aqueous solution of sodium bicarbonate (10 mL) and the mixture was extracted with ethyl acetate (2×15 mL). The combined organic phases were washed sequentially with water (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (3:7 ethyl acetate-hexane) to produce ketal **12b** (126 mg, 60%) as colorless oil: R_f 0.36 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, J = 8.4 Hz, 2 H, Ar*H*), 7.21 (obs 1 H, N*H*CO), 6.86 (d, J = 8.4 Hz, 2 H, Ar*H*), 6.20 (ddd, J = 9.9, 4.4, 1.4 Hz, 1 H, C*H*=), 6.01 (d, J = 9.9 Hz, 1 H, C*H*=), 5.87–5.82 (m, 2 H, C*H*=), 4.60 (AB_q, J = 10.8 Hz, $\Delta\nu$ = 82.9 Hz, 2 H, OCH_2Ar), 4.12–4.06 (m, 1 H, C*H*NH), 3.95 (brd, J = 12.0 Hz, 1 H, C*H*HO), 3.80 (s, 3 H, OCH_3), 3.79 (obs dd, J = 12.0, 1.9 Hz, 1 H, CH*H*O), 3.47 (brs, 1 H, O*H*), 2.19 (t, J = 7.6 Hz, 2 H, COCH_2), 1.95 (brd, J = 14.6 Hz, 1 H, C*H*HCHNH), 1.87 (dd, J = 14.6, 4.5 Hz, 1 H, CH*H*CHNH), 1.66–1.61 (m, 2 H, $COCH_2CH_2$), 1.36–1.22 (m, 24 H, $(CH_2)_{12}CH_3$), 0.89 (t, J = 6.8 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.6, 159.1, 139.1, 130.2, 129.5, 129.3, 127.7, 121.2, 113.6, 98.2, 72.7, 66.7, 64.1, 55.1, 41.3, 36.9, 35.2, 31.6, 29.6, 29.5, 29.3, 29.2, 25.6, 22.6, 14.1; HR-ESI-TOF: *m/z*: 542.3829, [M+H$^+$] for the compound $C_{33}H_{51}NO_5$ requires 542.3840.

Preparation of epoxides 13a and 14a.



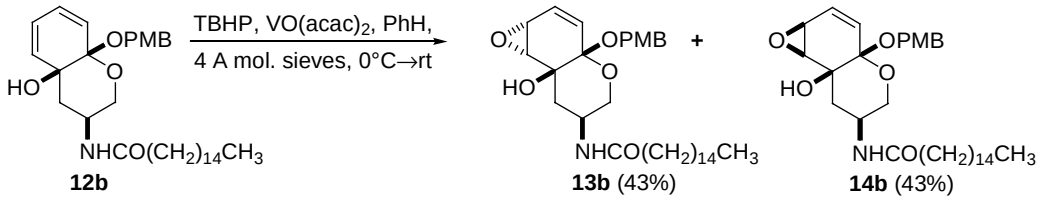
To a stirred solution of ketal **12a** (20 mg, 46 µmol) in benzene (3 mL) were added 0.02 M solution of $VO(acac)_2$ in benzene (0.3 mL, 6 µmol) and powdered molecular sieves 4 Å (30 mg). The mixture was cooled at 0 °C and then 5.5 M solution of TBHP in decane (20 µL, 0.1 mmol) was added. The

reaction mixture was stirred at 0 °C for one hour and then at ambient temperature for 24 h. Upon reaction completion, the molecular sieves were removed by filtration with the aid of Celite® and the filtrate was concentrated under reduced pressure. The residue thus obtained was purified by flash column chromatography (1:9 → 1:1 ethyl acetate-hexane) to produce, in order of elution:

compound **13a** as white wax: 7.1 mg (34%): R_f 0.13 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.13 (dd, *J* = 9.7, 4.0 Hz, 1 H, CHC*H*=CH), 6.00 (dd, *J* = 9.7, 1.7 Hz, 1 H, CHCH=C*H*), 5.22 (brd, *J* = 7.7 Hz, 1 H, N*H*CO), 4.05–3.92 (m, 1 H, C*H*NH), 3.79–3.75 (m, 2 H, C*H*HO + C*H*(O)CHCH=CH), 3.57 (s, 3 H, OCH_3), 3.47 (d, *J* = 7.3 Hz, 1 H, C*H*CH=CH), 3.18 (t, *J* = 10.6 Hz, 1 H, CH*H*O), 2.81 (brs, 1 H, O*H*), 2.14 (t, *J* = 7.6 Hz, 2 H, COCH_2), 1.92–1.84 (m, 2 H, CH_2CHNH), 1.63–1.58 (m, 2 H, $COCH_2CH_2$), 1.32–1.20 (m, 24 H, $(CH_2)_{12}CH_3$), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_2CH_3); HR-ESI-TOF: *m*/z: 474.3184, [*M*+Na^+] for the compound $C_{26}H_{45}NO_5$ requires 474.3190. and

compound **14a** as white wax: 7.1 mg (34%): R_f 0.07 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (brd, *J* = 8.5 Hz, 1 H, N*H*CO), 6.37 (dd, *J* = 10.1, 3.8 Hz, 1 H, CHC*H*=CH), 5.94 (d, *J* = 10.1 Hz, 1 H, CHCH=C*H*), 4.17–4.12 (m, 1 H, C*H*NH), 3.84 (dt, *J* = 12.1, 2.5 Hz, 1 H, C*H*HO), 3.74 (d, *J* = 2.4 Hz, 1 H, O*H*), 3.55 (dd, *J* = 12.1, 2.7 Hz, 1 H, CH*H*O), 3.41–3.38 (m, 1 H, C*H*CH=CH), 3.38 (s, 3 H, OCH_3), 3.28 (d, *J* = 4.0 Hz, 1 H, C*H*(O)CHCH=CH), 2.18 (t, *J* = 7.7 Hz, 2 H, COCH_2), 1.96 (dt, *J* = 14.6, 2.3 Hz, 1 H, C*H*HCHNH), 1.66–1.59 (m, 3 H, CH*H*CHNH + $COCH_2CH_2$), 1.32–1.24 (m, 24 H, $(CH_2)_{12}CH_3$), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.6, 133.2, 131.2, 96.2, 70.8, 66.5, 58.3, 50.9, 48.2, 41.4, 36.9, 33.2, 31.9, 29.6, 29.5, 29.3, 25.6, 22.7, 14.1; HR-ESI-TOF: *m*/z: 474.3186, [*M*+Na^+] for the compound $C_{26}H_{45}NO_5$ requires 474.3190.

Preparation of epoxides 13b and 14b.



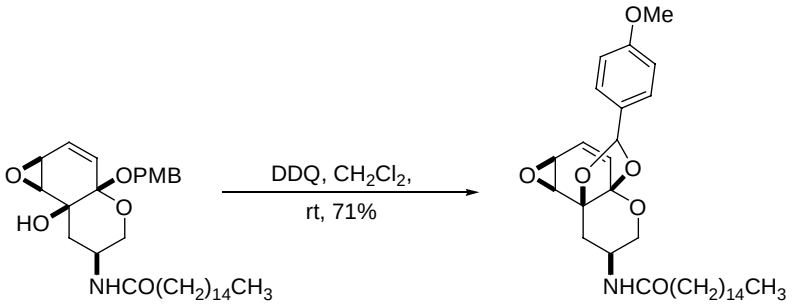
Oxidation of ketal **12b** (120 mg, 0.22 mmol), using the protocol described above, produces:

Compound **13b** as amorphous white solid: 52.8 mg (43%); R_f 0.26 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.9 Hz, 2 H, Ar*H*), 6.90 (d, *J* = 7.9 Hz, 2 H, Ar*H*), 6.12 (dd, *J* = 9.6, 2.7 Hz, 1 H, CHC*H*=CH), 6.00 (d, *J* = 9.6 Hz, 1 H, CHCH=C*H*), 5.29 (brd, *J* = 7.7 Hz, 1 H, N*H*CO), 4.82 (AB_q, *J* = 11.5 Hz, Δν = 67.8 Hz, 2 H, OCH_2Ar), 4.03–3.94 (m, 1 H, C*H*NH), 3.81 (s, 3 H, OCH_3), 3.81–3.74 (m, 2 H, C*H*HO + C*H*(O)CHCH=CH), 3.50 (brs, 1 H, C*H*CH=CH), 3.24 (dd, *J* = 10.7, 10.5 Hz, 1 H, CH*H*O), 2.88 (brs, 1 H, O*H*), 2.11 (t, *J* = 7.3 Hz, 2 H, COCH_2), 1.92 (dd, *J* = 12.2, 11.9 Hz, 1 H, C*H*HCHNH), 1.85 (brd, *J* = 11.9 hz, 1 H, CH*H*CHNH), 1.62–1.54 (m, 2 H, $COCH_2CH_2$), 1.33–1.19 (m, 24 H, $(CH_2)_{12}CH_3$), 0.87 (t, *J* = 6.5 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.7, 159.3, 140.2, 130.1, 129.5, 128.7, 125.4, 113.7, 96.3, 69.6, 63.5, 62.4, 55.3, 54.4, 50.2, 43.5, 37.6, 36.7, 31.9, 29.7, 29.5, 29.3, 29.2, 25.6, 22.7, 14.1; HR-ESI-TOF: *m/*z: 558.3785, [*M*+H^+] for the compound $C_{33}H_{51}NO_6$ requires 558.3789, and

Compound **14b** as amorphous white solid: 52.8 mg (43%); R_f 0.11 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.06 (brd, *J* = 8.5 Hz, 1 H, N*H*CO), 6.86 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 6.34 (dd, *J* = 10.1, 3.8 Hz, 1 H, CHC*H*=CH), 5.99 (d, *J* = 10.1 Hz, 1 H, CHCH=C*H*), 4.58 (AB_q, *J* = 10.4 Hz, Δν = 63.2 Hz, 2 H, OCH_2Ar), 4.19–4.14 (m, 1 H, C*H*NH), 3.85 (dt, *J* = 12.1, 2.0 Hz, 1 H, C*H*HO), 3.80 (d, *J* = 2.5 Hz, 1 H, O*H*), 3.79 (s, 3 H, OCH_3), 3.58 (dd, *J* = 12.1, 2.7 Hz, 1 H, CH*H*O), 3.41–3.38 (m, 1 H, C*H*CH=CH), 3.28 (d, *J* = 4.0 Hz, 1 H, C*H*(O)CHCH=CH), 2.17 (t, *J* = 7.6 Hz, 2 H, COCH_2), 1.95 (brd, *J* = 14.6 Hz, 1 H, C*H*HCHNH), 1.68–1.57 (m, 3 H, CH*H*CHNH + $COCH_2CH_2$), 1.34–1.21 (m, 24 H, $(CH_2)_{12}CH_3$), 0.87 (t, *J* = 6.8 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.5, 159.2, 133.6, 130.9, 130.0, 129.5, 113.6, 96.3, 70.8, 66.3, 65.1, 58.3, 55.2, 48.2, 41.5, 36.9, 33.1,

31.7, 29.7, 29.5, 29.3, 25.6, 22.7, 14.1; HR-ESI-TOF: *m/*z: 558.3780, [*M*+H^+] for the compound $C_{33}H_{51}NO_6$ requires 558.3789.

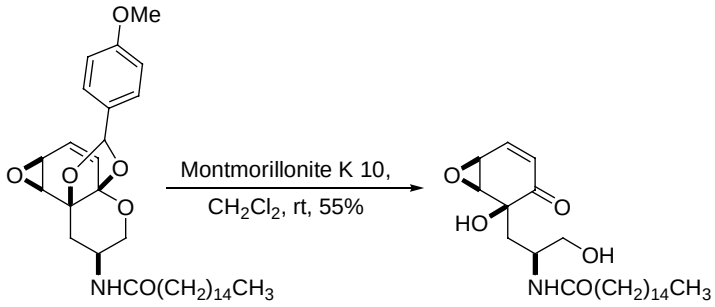
Preparation of acetal 15.



14b **15**

To a stirred solution of compound **14b** (35.5 mg, 64 μmol) in dichloromethane (5 mL) was added, under an atmosphere of argon, DDQ (20 mg, 88 μmol). The mixture was stirred at ambient temperature for 24 h and it was then poured in aqueous saturated solution of sodium bicarbonate (10 mL). The mixture was extracted with ethyl acetate (2 × 15 mL). The combined organic phases were washed sequentially with water (2 × 10 mL) and brine (2 × 10 mL) and were then dried over Na_2SO_4. Evaporation under reduced pressure and further chromatographic purification (1:1 ethyl acetate-hexane) of the residue produced compound **15** as amorphous white solid (25.2 mg, 71%): R_f 0.57 (4:1 ethyl acetate-acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 6.92 (d, *J* = 8.6 Hz, 2 H, Ar*H*), 6.60 (brd, *J* = 7.1 Hz, 1 H, CHN*H*CO), 6.54 (dd, *J* = 10.0, 3.8 Hz, 1 H, CHC*H*=CH), 6.27 (s, 1 H, OC*H*O), 6.04 (dd, *J* = 10.0, 1.1 Hz, CHCH=C*H*), 4.10–4.05 (m, 1 H, C*H*NH), 4.04 (dd, *J* = 15.2, 1.1 Hz, 1 H, C*H*HO), 3.81 (s, 3 H, OCH_3), 3.57–3.53 (m, 1 H, C*H*CH=CH), 3.47 (d, *J* = 15.2 Hz, 1 H, CH*H*O), 3.46 (s, 1 H, C*H*(O)CH), 2.37 (brd, *J* = 15.1 Hz, 1 H, C*H*HCHNH), 2.23 (t, *J* = 7.6 Hz, 2 H, NHCOCH_2), 1.75 (dd, *J* = 15.1, 4.9 Hz, CH*H*CHNH), 1.68–1.62 (m, 2 H, NHCOCH$_2$CH_2), 1.38–1.21 (m, 24 H, (CH_2)$_{12}$CH$_3$), 0.88 (t, *J* = 6.8 Hz, 3 H, CH$_2$CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.7, 160.8, 132.8, 131.4, 129.5, 129.0, 113.9, 101.8, 99.4, 66.0, 57.6, 55.3, 49.1, 41.8, 36.9, 31.9, 31.7, 29.7, 29.4, 25.7, 22.7, 14.1; HR-ESI-TOF: *m/*z: 578.3448, [*M*+Na^+] for compound $C_{33}H_{49}NO_6$ requires 578.3452.

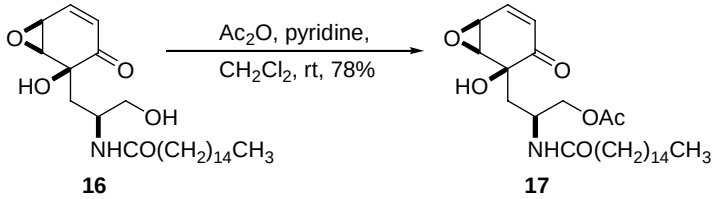
Preparation of palmitoyl analogue of scyphostatin 16.



15 **16**

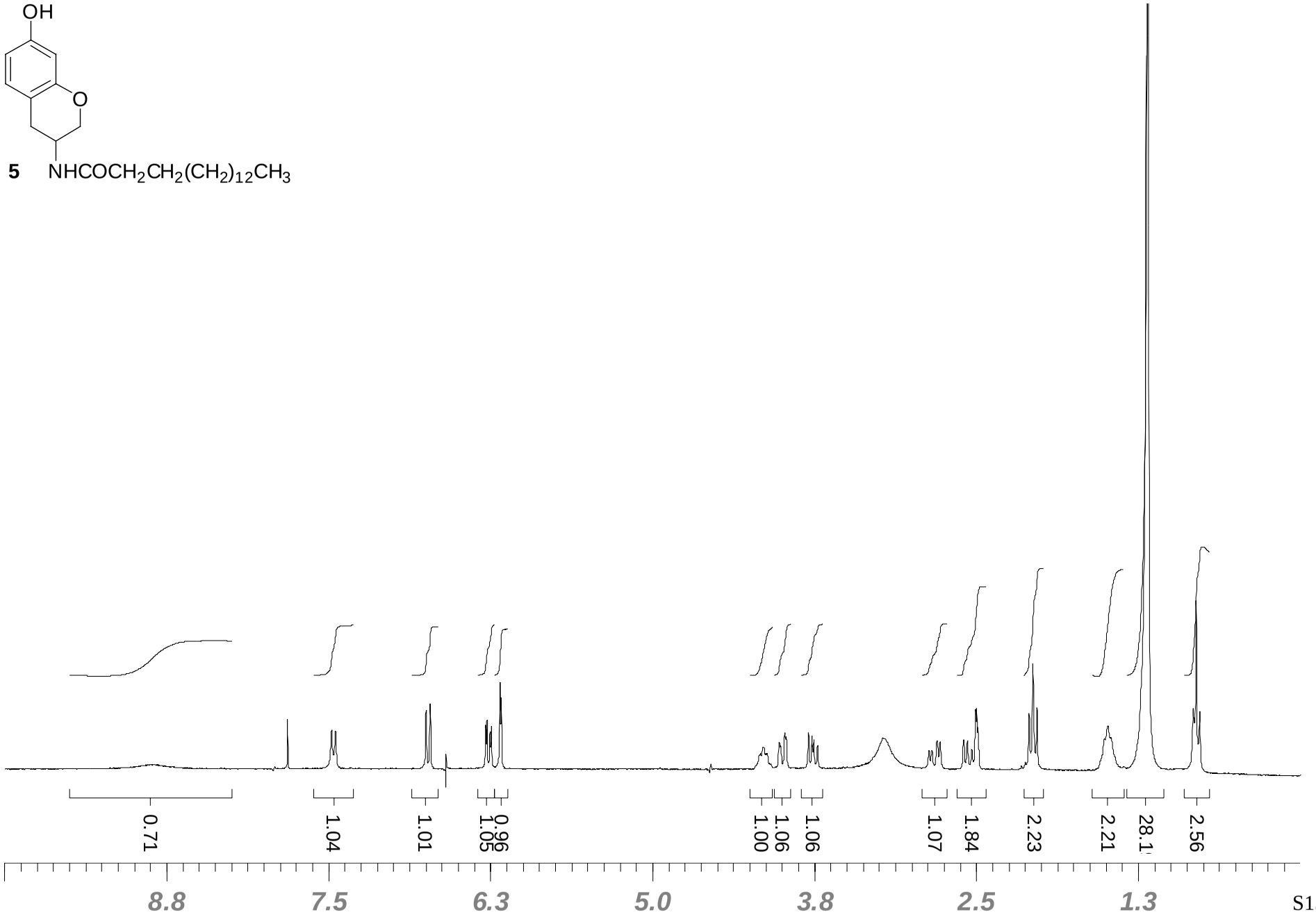
To a solution of 23 mg (41 μmol) of compound **15** in dichloromethane (3 mL) was added montmorillonite K 10 (150 mg). The mixture was stirred at ambient temperature for 1 h and was then filtered. The clay was washed on the filter with acetone (3 × 10 mL) and the combined filtrates were evaporated under reduced pressure. The residue thus obtained was purified by flash column chromatography (4:1 ethyl acetate-acetone) to produce, in order of elution, unreacted starting material (8.8 mg) and the palmitoyl analogue of scyphostatin **16** (6.1 mg, 55%) as amorphous white solid: R_f 0.32 (4:1 ethyl acetate-acetone); ^{1}H NMR (500 MHz, CD_3OD) δ 7.17 (dd, *J* = 9.9, 3.9 Hz, 1 H, CHC*H*=CH), 6.10 (dd, *J* = 9.9, 1.6 Hz, 1 H, CHCH=C*H*), 3.99–4.04 (m, 1 H, C*H*NH), 3.66 (d, *J* = 4.0 Hz, 1 H, C*H*(O)CHCH=CH), 3.61 (dt, *J* = 4.0, 1.6 Hz, 1 H, CH(O)C*H*CH=CH), 3.48 (dd, *J* = 10.9, 5.2 Hz, 1 H, C*H*HOH), 3.41 (dd, *J* = 10.9, 6.0 Hz, 1 H, CH*H*OH), 2.11 (dt, *J* = 7.6, 2.1 Hz, 2 H, COCH_2CH$_2$), 2.05 (dd, *J* = 14.7, 3.4 Hz, 1 H, C*H*HCHNH), 1.81 (dd, *J* = 14.7, 9.4 Hz, 1 H, CH*H*CHNH), 1.55–1.61 (m, 2 H, COCH$_2$CH_2), 1.25–1.31 (m, 24 H, CH$_2$(CH_2)$_{12}$CH$_3$), 0.90 (t, *J* = 6.9 Hz, 3 H, CH$_2$CH_3); ^{13}C NMR (62.5 MHz, CD_3OD) δ 199.5, 175.9, 145.8, 132.0, 77.5, 65.5, 58.2, 47.7, 39.5, 37.1, 33.1, 30.8, 30.7, 30.5, 30.4, 26.7, 23.8, 14.5; HR-ESI-TOF: *m/z*: 438.3219, [*M*+H$^+$] for the compound $C_{25}H_{43}NO_5$ requires 438.3214.

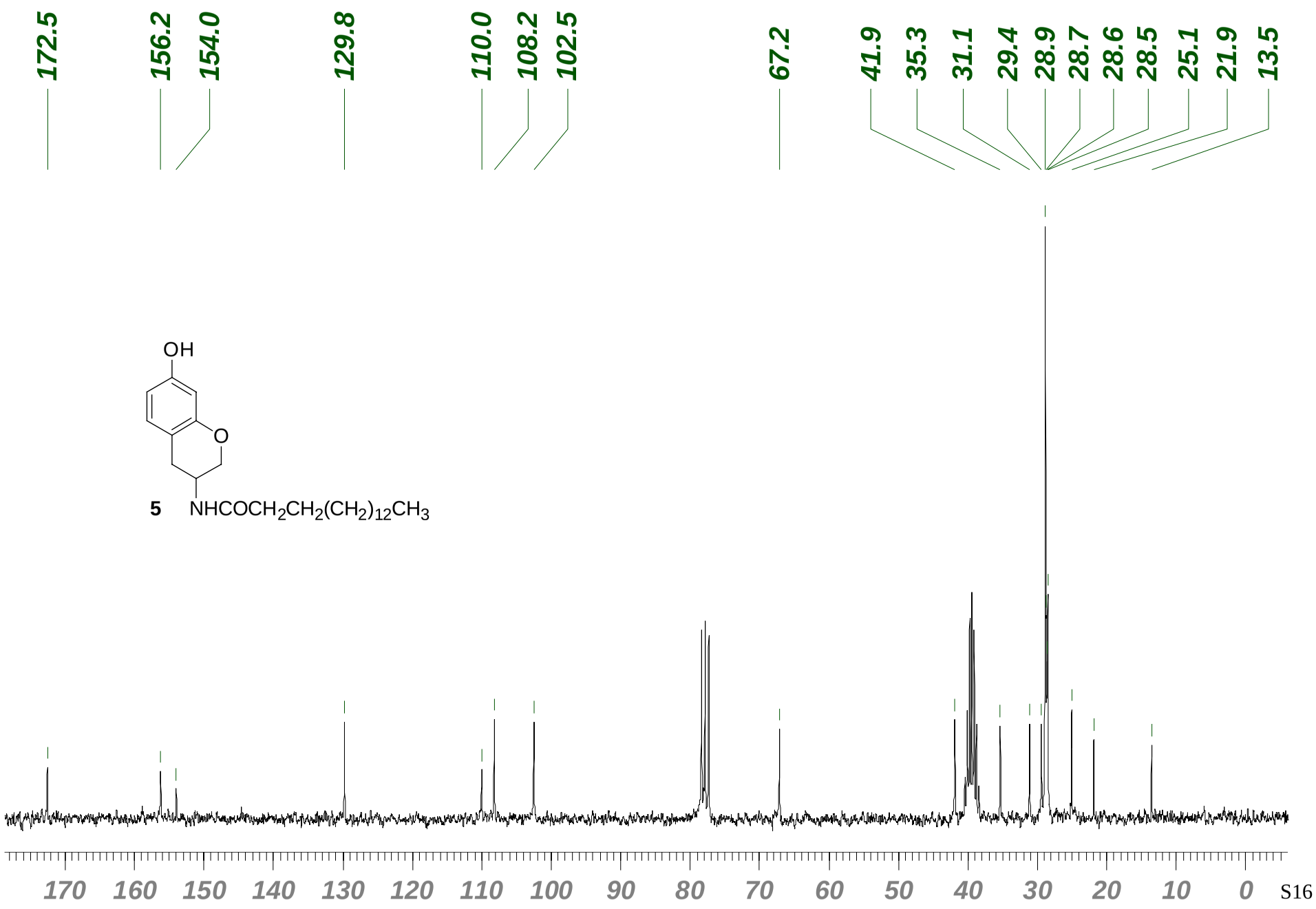
Preparation of acetate derivative 17.

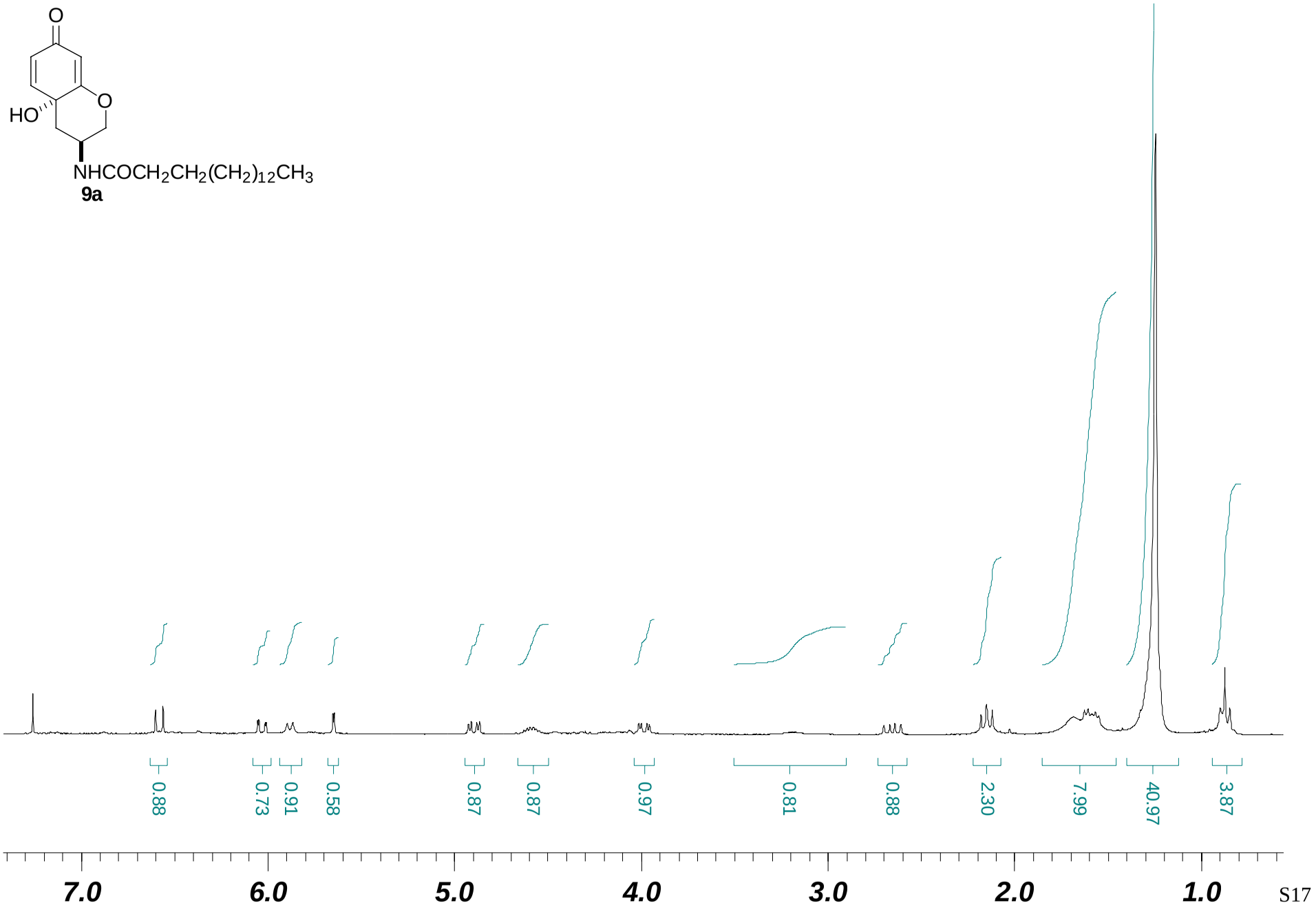


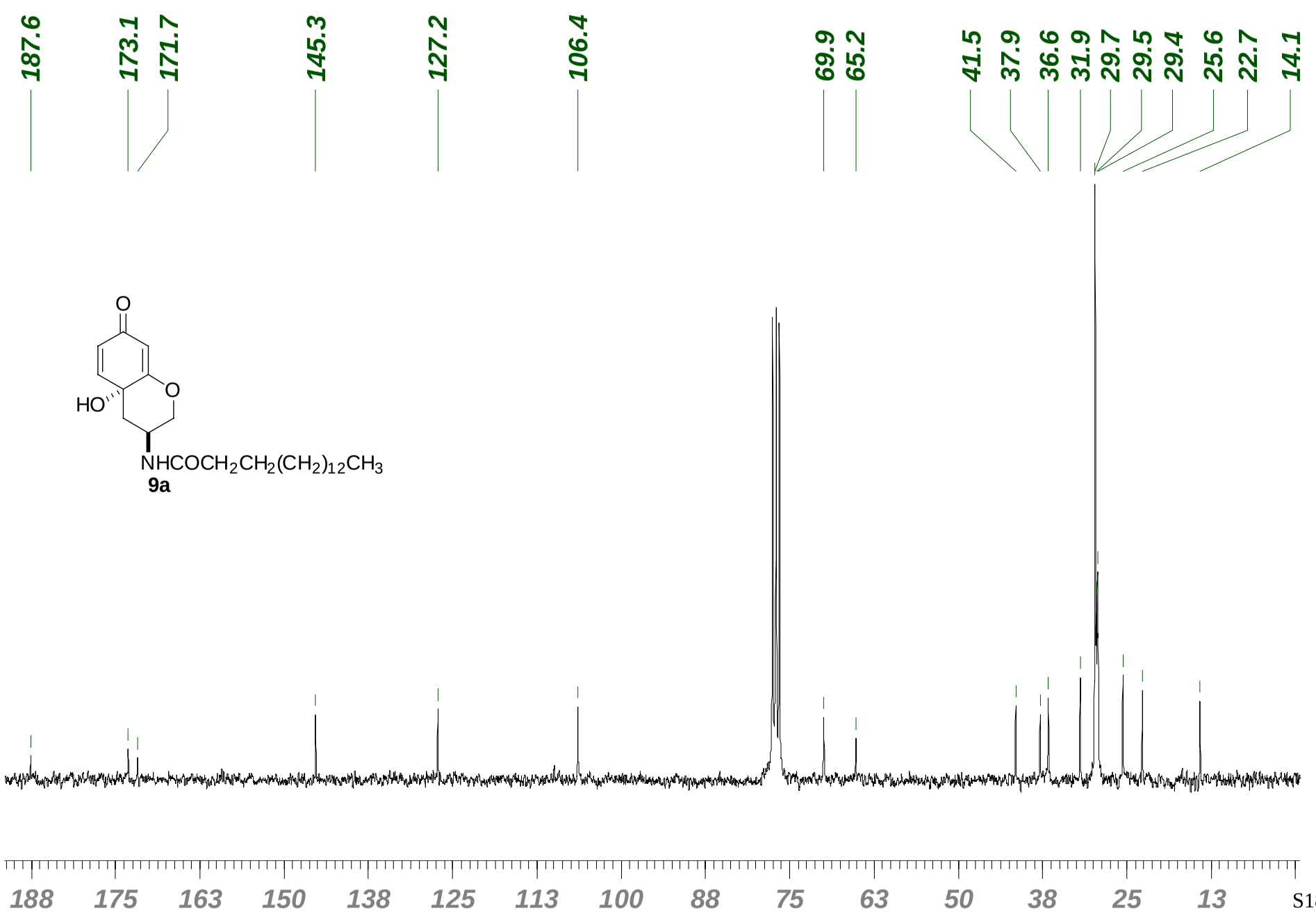
16 **17**

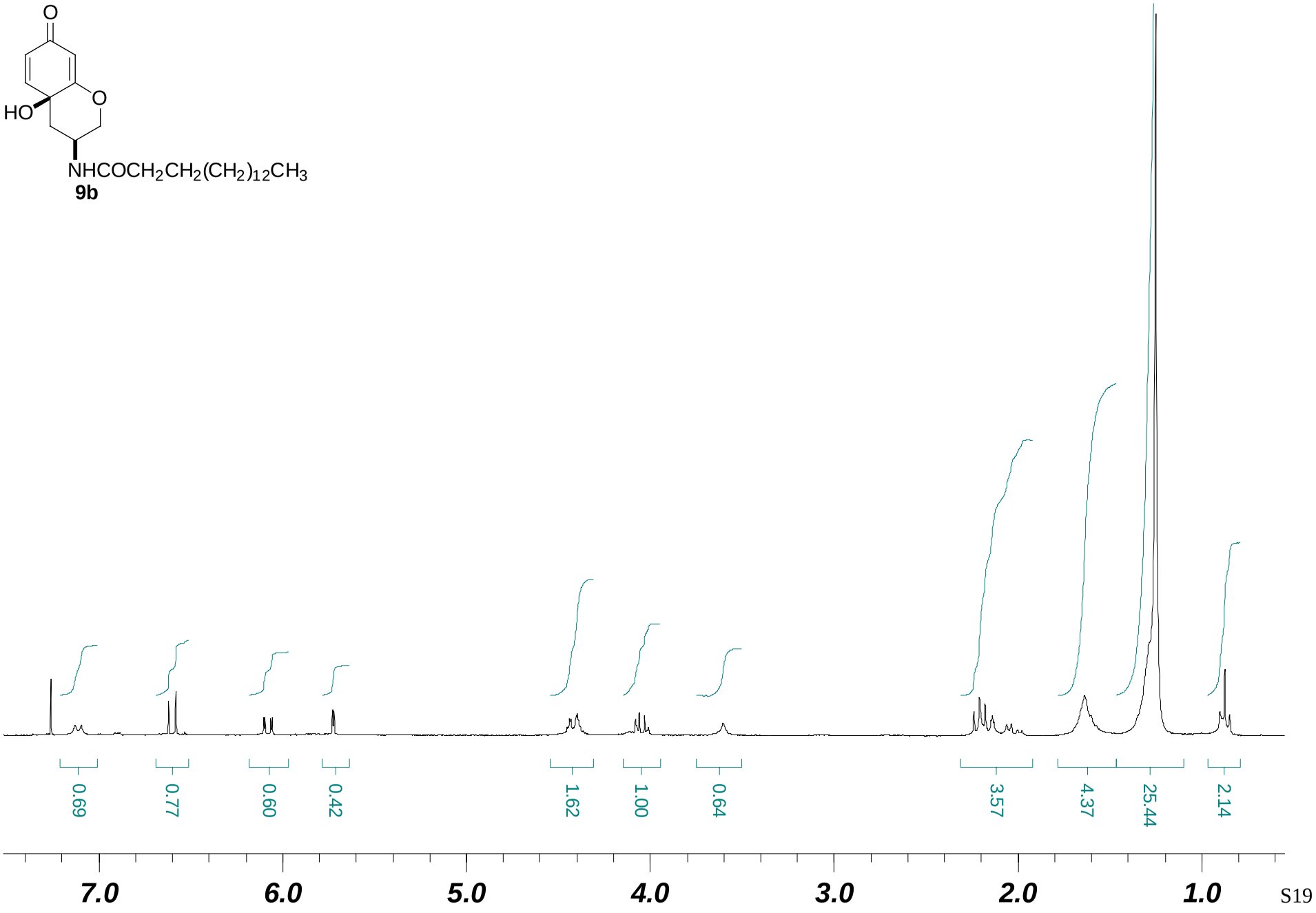
A mixture of compound **16** (2.4 mg, 5.5 μmol), acetic anhydride (50 μL, 0.53 mmol) and pyridine (50 μL, 0.63 mmol) in dichloromethane (0.5 mL) was stirred at ambient temperature and under an atmosphere of argon for 12 h. Upon reaction completion, a saturated aqueous solution of sodium bicarbonate (0.5 mL) was added to the mixture and stirring was continued for 1 h. The mixture was diluted with ethyl acetate (5 mL) and washed sequentially with water (5 ml), saturated aqueous solution of $CuSO_4$ (5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue thus obtained was purified by flash column chromatography (6:4 ethyl acetate-hexane) to produce acetate derivative **17** (2.0 mg, 78%) as white wax: R_f 0.07 (1:1 ethyl acetate-hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 9.9, 3.9 Hz, 1 H, CHC*H*=CH), 6.23 (dd, *J* = 9.9, 1.5 Hz, 1 H, CHCH=C*H*), 5.83 (brd, *J* = 7.0 Hz, N*H*CO), 4.19–4.27 (m, 2 H, C*H*HOAc + C*H*NH), 4.10 (dd, *J* = 10.7, 3.9 Hz, 1 H, CH*H*OAc), 3.98 (s, 1 H, CO*H*), 3.72 (d, *J* = 3.8 Hz, 1 H, C*H*(O)CHCH=CH), 3.58 (dt, *J* = 3.9, 1.6 Hz, 1 H, CH(O)C*H*CH=CH), 2.08–2.18 (m, 2 H, $COCH_2CH_2$), 2.07 (s, 3 H, $OCOCH_3$), 1.94 (dd, *J* = 14.8, 4.6 Hz, 1 H, C*H*HCHNH), 1.87 (dd, *J* = 14.7, 8.3 Hz, 1 H, CH*H*CHNH), 1.56–1.64 (m, 2 H, $COCH_2CH_2$), 1.22–1.30 (m, 24 H, $CH_2(CH_2)_{12}CH_3$), 0.88 (t, *J* = 6.9 Hz, 3 H, CH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 197.4, 172.9, 171.0, 144.6, 130.0, 76.5, 65.8, 55.7, 47.9, 44.9, 37.4, 36.5, 31.4, 29.5, 25.4, 22.6, 20.9, 14.2; HR-ESI-TOF: *m/z*: 480.3326, [M+H^+] for the compound $C_{27}H_{45}NO_6$ requires 480.3319.

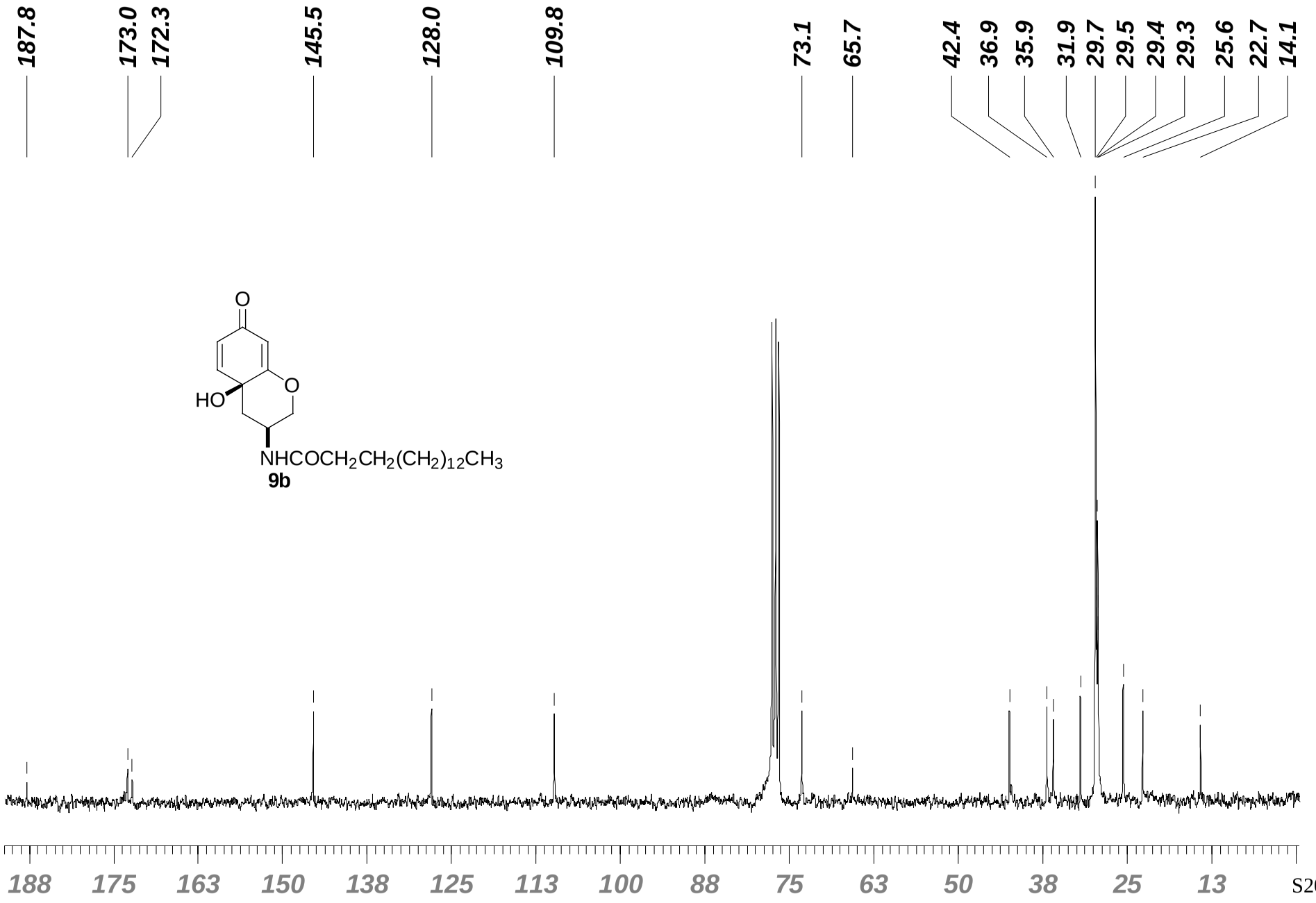

OH
O
5 NHCOCH2CH2(CH2)12CH3
0.71
1.04
1.01
0.96
1.05
1.00
1.06
1.06
1.07
1.84
2.23
2.21
28.1
2.56
8.8
7.5
6.3
5.0
3.8
2.5
1.3

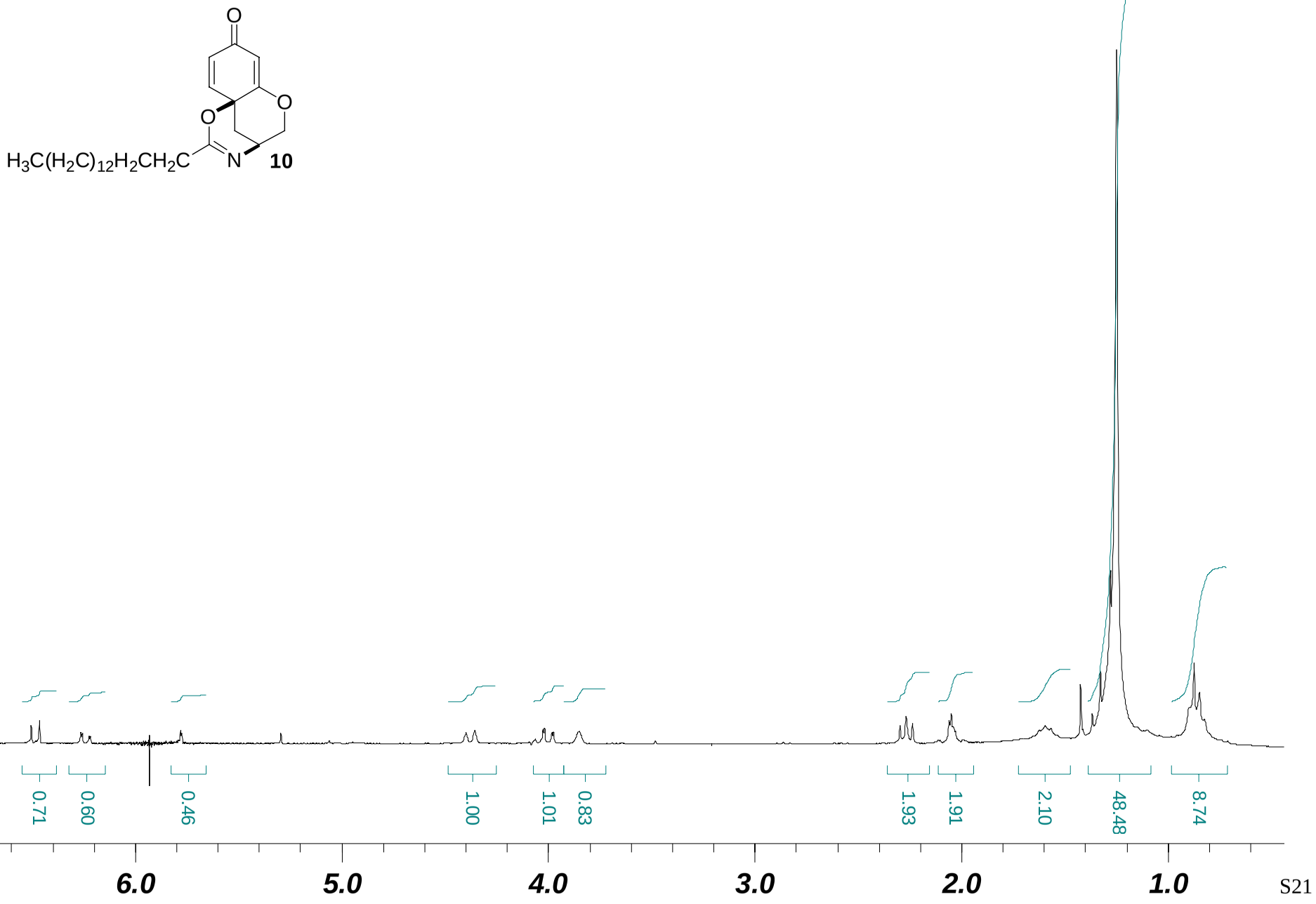

172.5
156.2
154.0
129.8
110.0
108.2
102.5
67.2
41.9
35.3
31.1
29.4
28.9
28.7
28.6
28.5
25.1
21.9
13.5
OH
O
5 NHCOCH2CH2(CH2)12CH3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

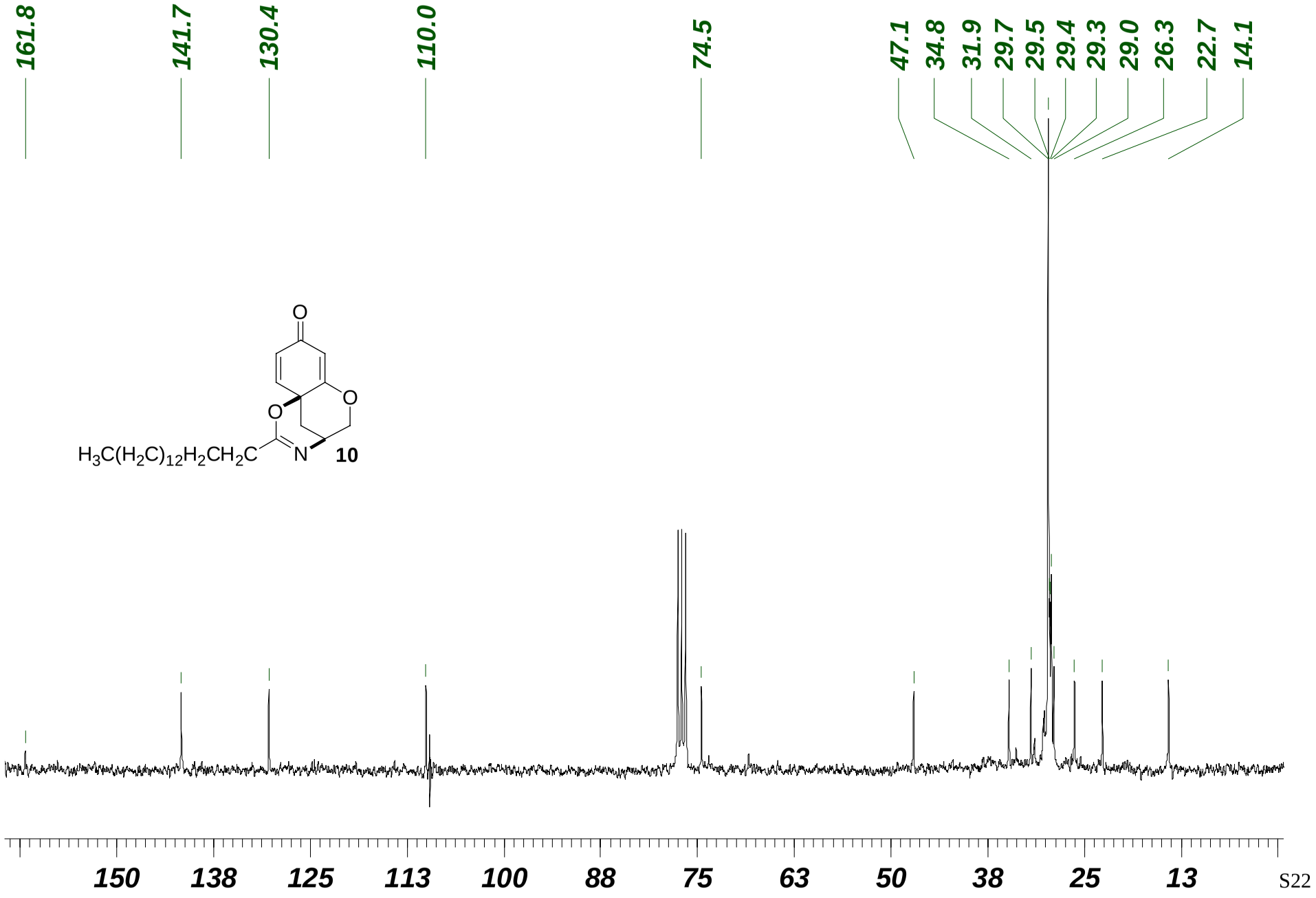

O
HO
O
NHCOCH2CH2(CH2)12CH3
9a
0.88
0.73
0.91
0.58
0.87
0.87
0.97
0.81
0.88
2.30
7.99
40.97
3.87
7.0
6.0
5.0
4.0
3.0
2.0
1.0

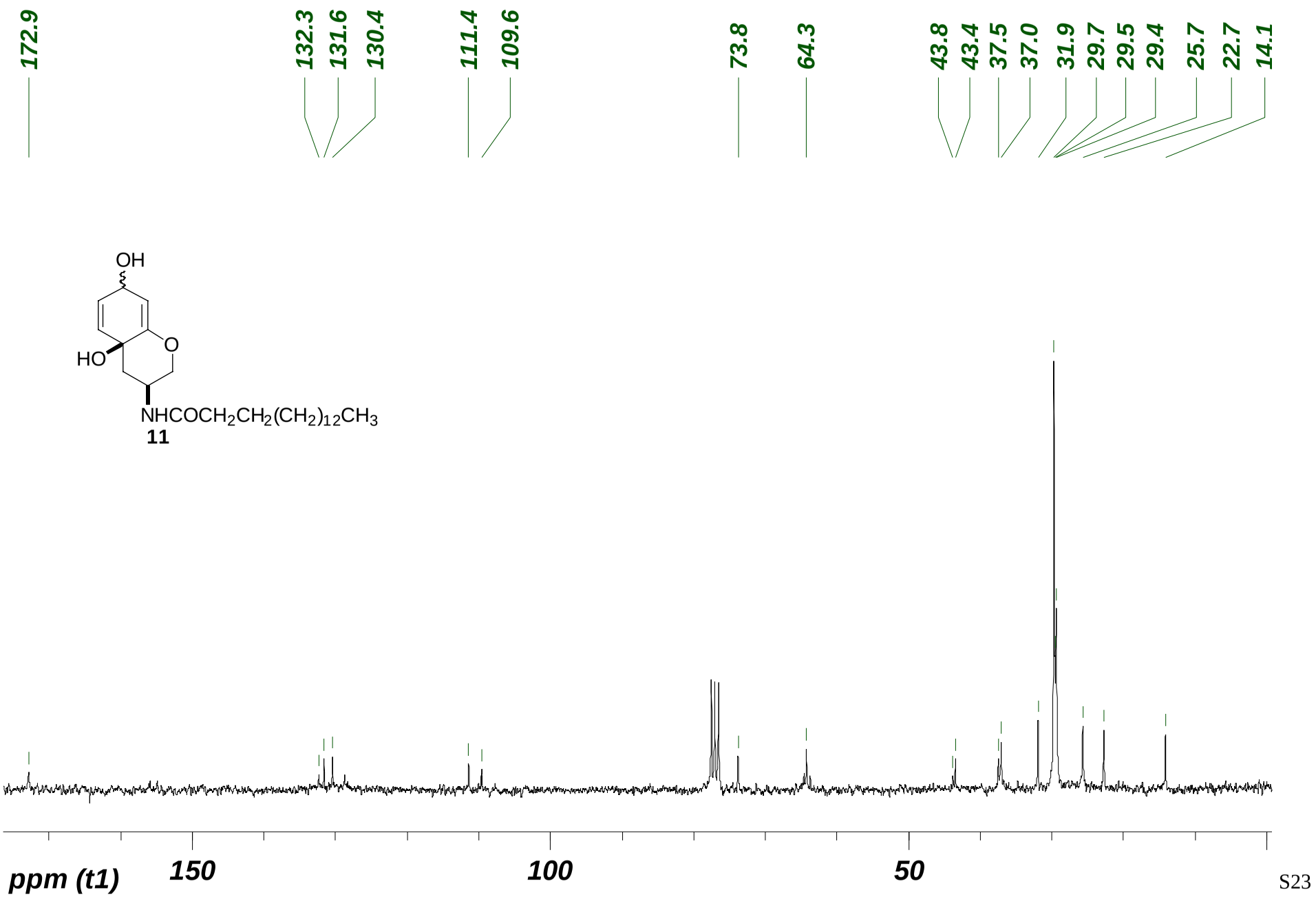

187.6
173.1
171.7
145.3
127.2
106.4
69.9
65.2
41.5
37.9
36.6
31.9
29.7
29.5
29.4
25.6
22.7
14.1
O
HO
O
NHCOCH2CH2(CH2)12CH3
9a
188
175
163
150
138
125
113
100
88
75
63
50
38
25
13

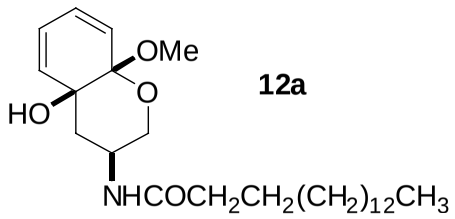

O
HO
O
NHCOCH2CH2(CH2)12CH3
9b
0.69
0.77
0.60
0.42
1.62
1.00
0.64
3.57
4.37
25.44
2.14
7.0
6.0
5.0
4.0
3.0
2.0
1.0


187.8
173.0
172.3
145.5
128.0
109.8
73.1
65.7
42.4
36.9
35.9
31.9
29.7
29.5
29.4
29.3
25.6
22.7
14.1
O
HO
O
NHCOCH2CH2(CH2)12CH3
9b
188
175
163
150
138
125
113
100
88
75
63
50
38
25
13


O
O
O
N
$H_3C(H_2C)_{12}H_2CH_2C$
10
0.71
0.60
0.46
1.00
1.01
0.83
1.93
1.91
2.10
48.48
8.74
6.0
5.0
4.0
3.0
2.0
1.0

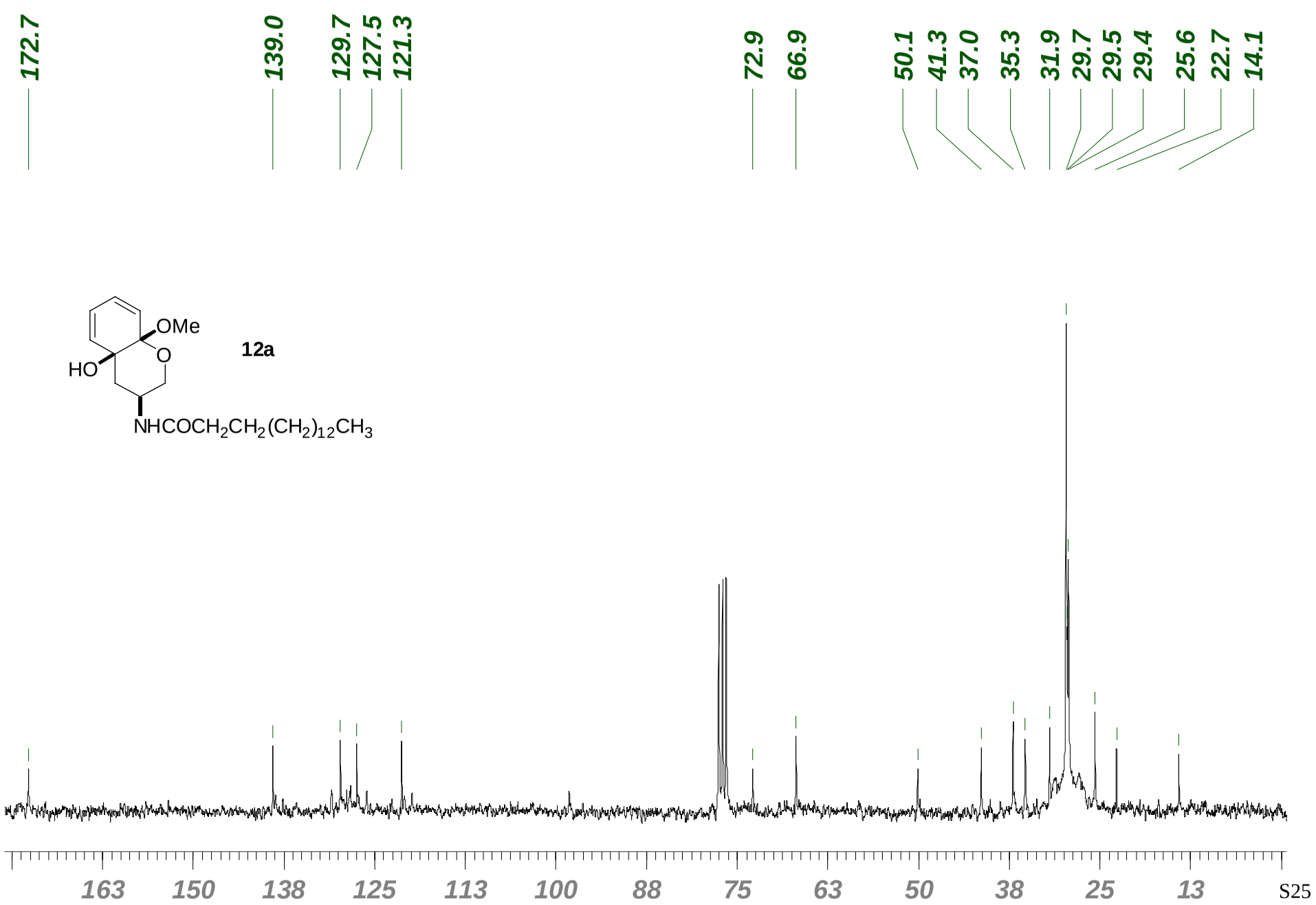

161.8
141.7
130.4
110.0
74.5
47.1
34.8
31.9
29.7
29.5
29.4
29.3
29.0
26.3
22.7
14.1
O
O
O
N
H3C(H2C)12H2CH2C
10
150
138
125
113
100
88
75
63
50
38
25
13

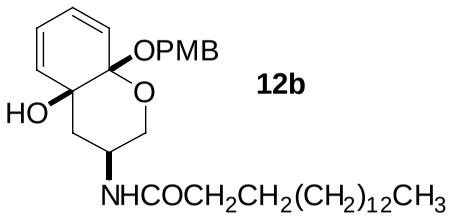

172.9
132.3
131.6
130.4
111.4
109.6
73.8
64.3
43.8
43.4
37.5
37.0
31.9
29.7
29.5
29.4
25.7
22.7
14.1
OH
HO
O
NHCOCH2CH2(CH2)12CH3
11
ppm (t1)
150
100
50


OMe
O
HO
NHCOCH2CH2(CH2)12CH3

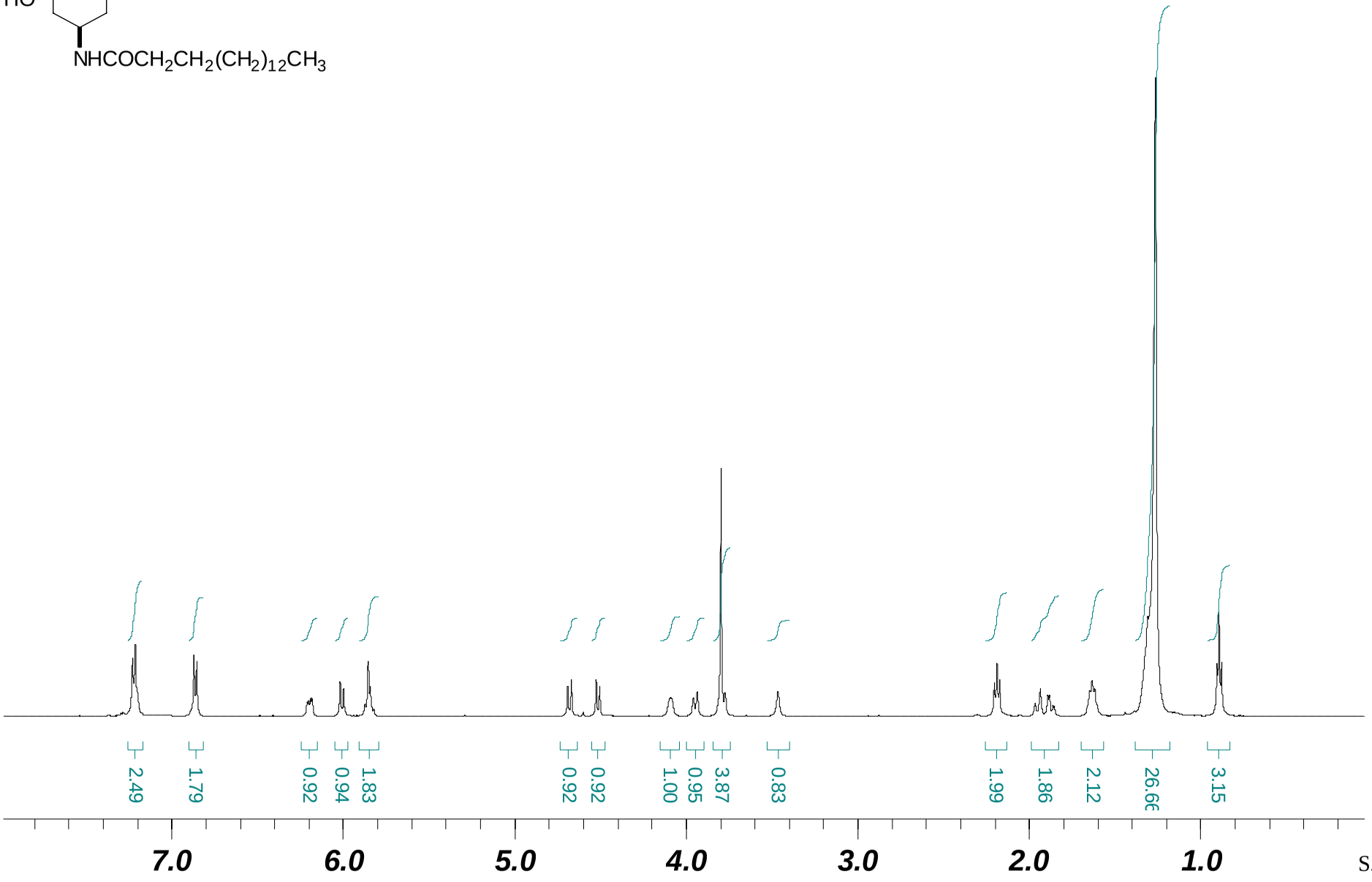
12a

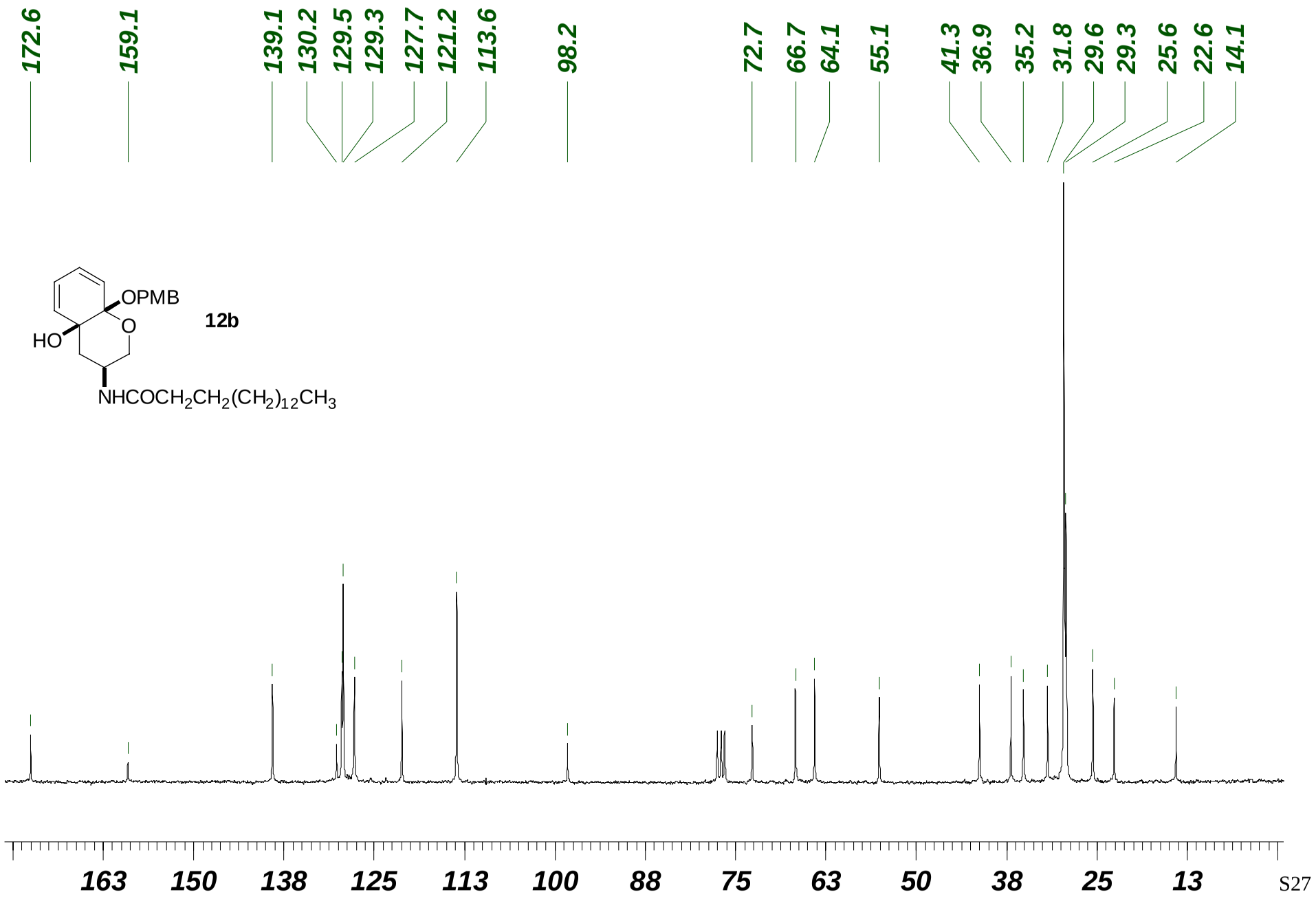
1.00
1.05
0.98
2.03
1.06
1.03
1.04
4.15
2.16
1.07
1.07
2.41
29.49
3.84
7.0
6.0
5.0
4.0
3.0
2.0
1.0

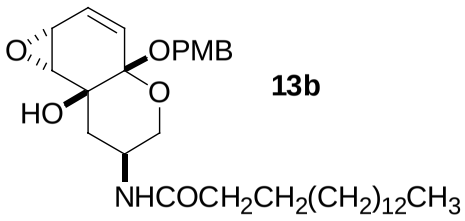

172.7
139.0
129.7
127.5
121.3
72.9
66.9
50.1
41.3
37.0
35.3
31.9
29.7
29.5
29.4
25.6
22.7
14.1
OMe
O
HO
NHCOCH2CH2(CH2)12CH3
12a
163
150
138
125
113
100
88
75
63
50
38
25
13


OPMB
O
HO
NHCOCH2CH2(CH2)12CH3

12b

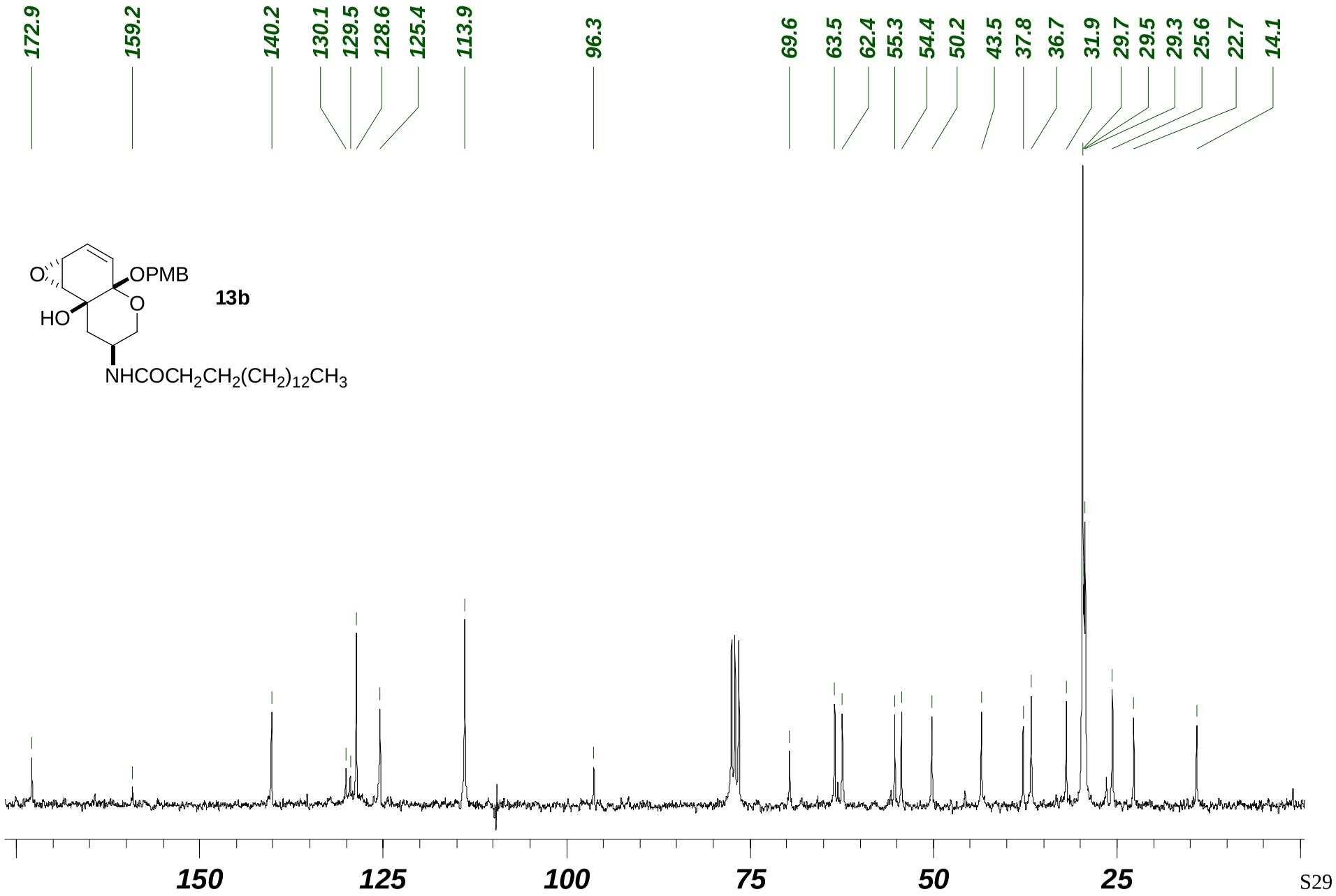

2.49
1.79
0.92
0.94
1.83
0.92
0.92
1.00
0.95
3.87
0.83
1.99
1.86
2.12
26.66
3.15
7.0
6.0
5.0
4.0
3.0
2.0
1.0

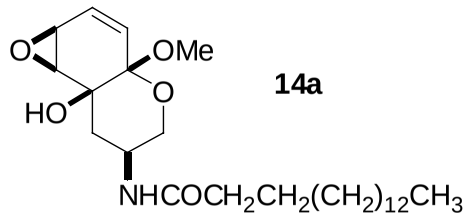

172.6
159.1
139.1
130.2
129.5
129.3
127.7
121.2
113.6
98.2
72.7
66.7
64.1
55.1
41.3
36.9
35.2
31.8
29.6
29.3
25.6
22.6
14.1
OPMB
O
HO
NHCOCH2CH2(CH2)12CH3
12b
163
150
138
125
113
100
88
75
63
50
38
25
13


O
OPMB
HO
O
NHCOCH2CH2(CH2)12CH3

13b

1.96
2.26
1.05
0.95
1.12
1.00
0.93
1.28
6.15
0.94
1.12
0.86
3.06
2.33
3.34
37.75
4.61
7.0
6.0
5.0
4.0
3.0
2.0
1.0

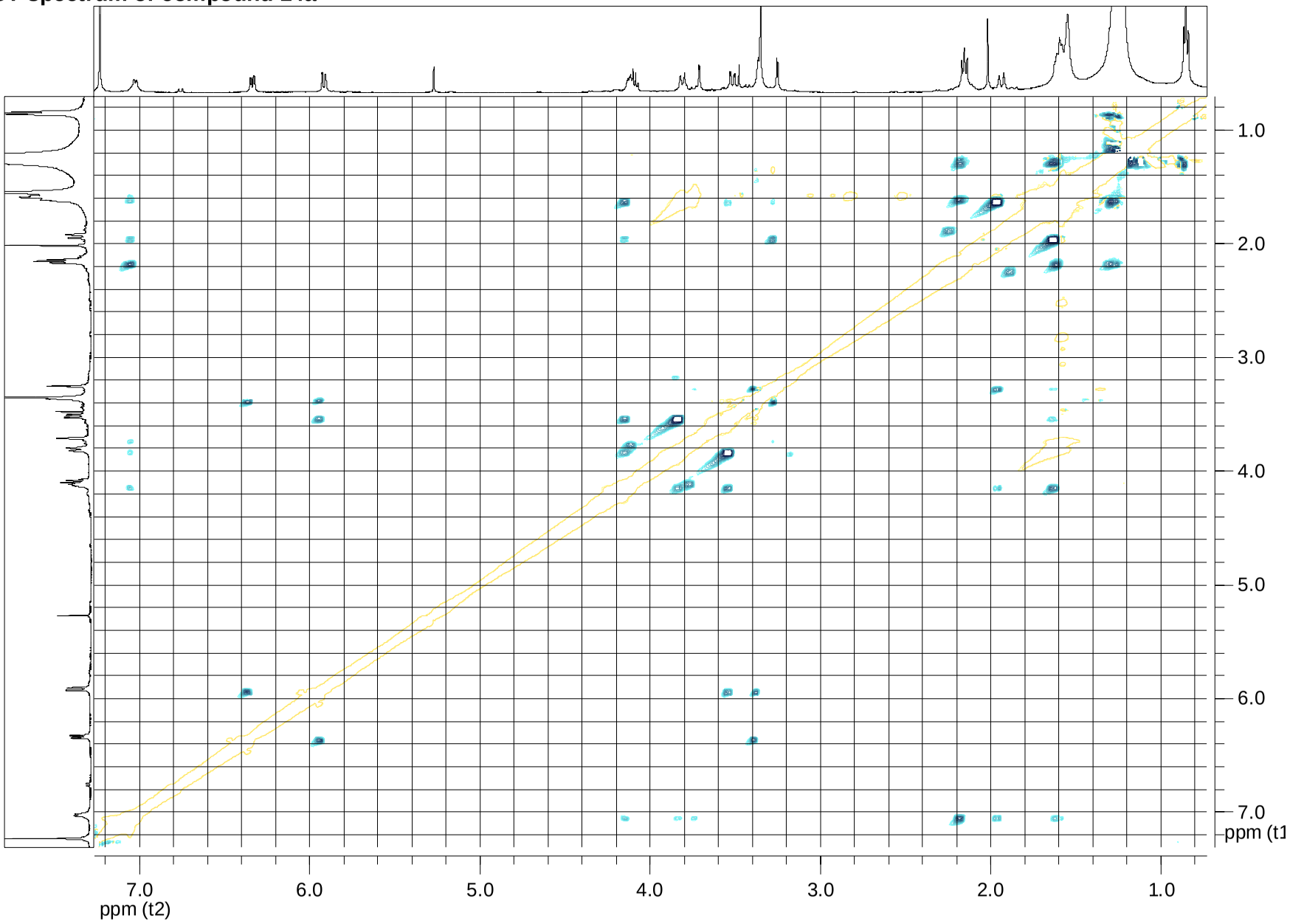

172.9
159.2
140.2
130.1
129.5
128.6
125.4
113.9
96.3
69.6
63.5
62.4
55.3
54.4
50.2
43.5
37.8
36.7
31.9
29.7
29.5
29.3
25.6
22.7
14.1
O
OPMB
HO
O
13b
NHCOCH2CH2(CH2)12CH3
150
125
100
75
50
25

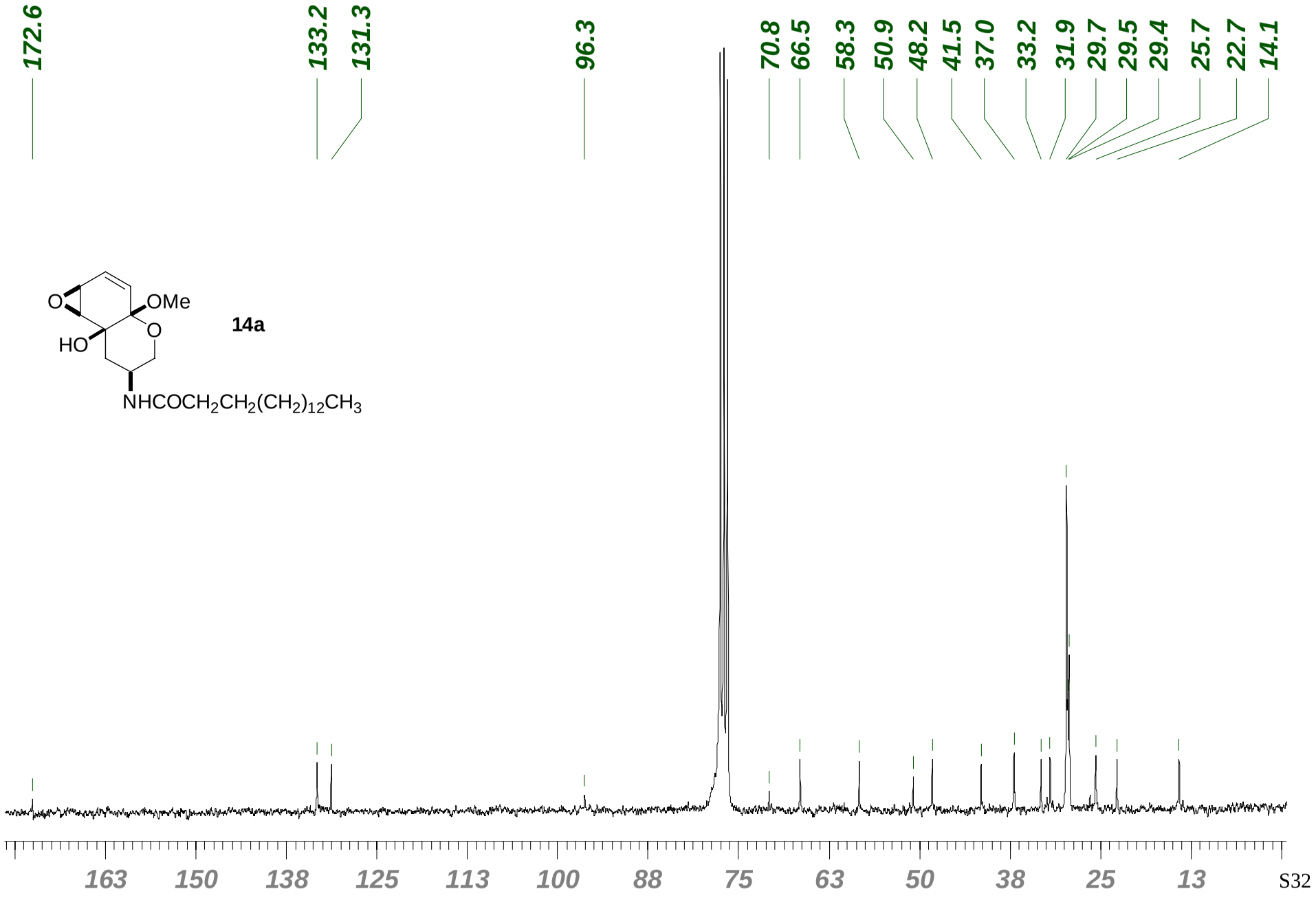

O
OMe
HO
O
NHCOCH2CH2(CH2)12CH3

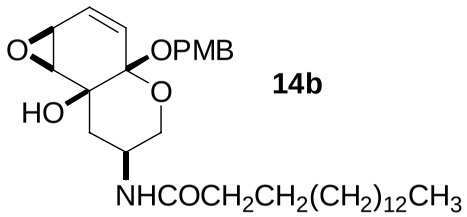
14a

1.08
0.95
1.00
1.11
1.20
0.79
1.15
4.72
1.04
2.69
1.23
4.43
34.65
3.59
7.0
6.0
5.0
4.0
3.0
2.0
1.0

NOESY spectrum of compound 14a



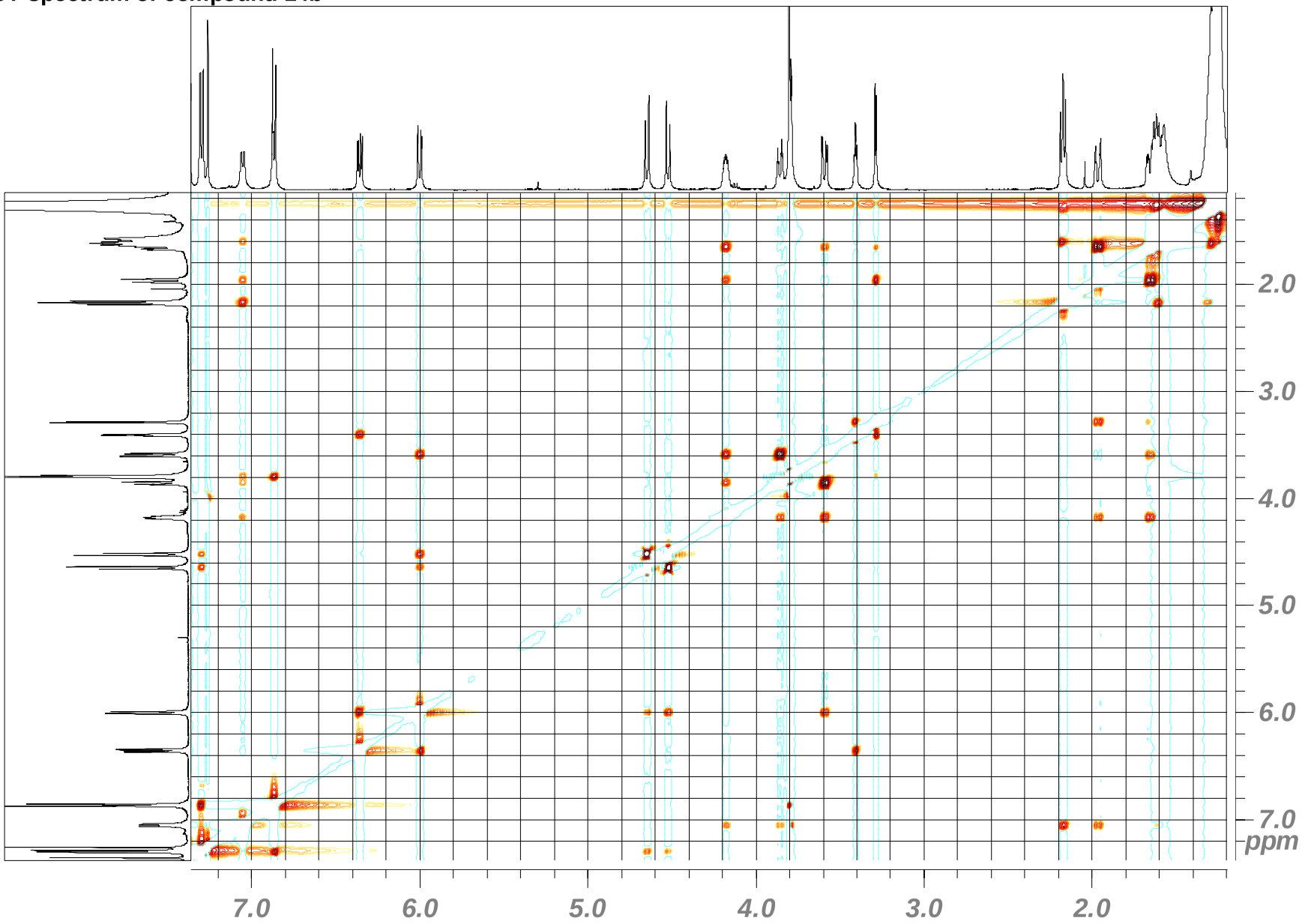

172.6
133.2
131.3
96.3
70.8
66.5
58.3
50.9
48.2
41.5
37.0
33.2
31.9
29.7
29.5
29.4
25.7
22.7
14.1
O
OMe
O
HO
NHCOCH2CH2(CH2)12CH3
14a
163
150
138
125
113
100
88
75
63
50
38
25
13

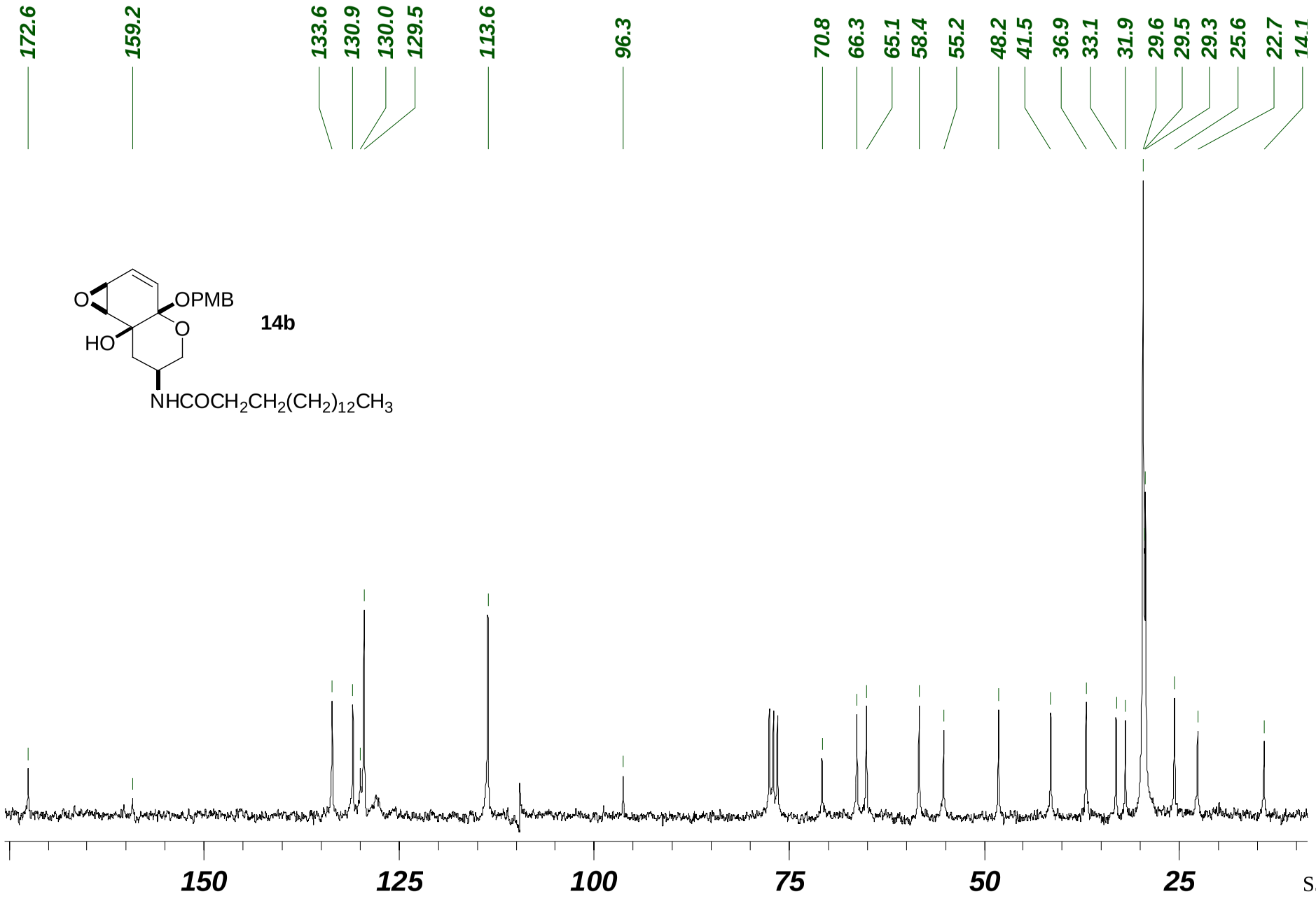

O
OPMB
HO
O
NHCOCH2CH2(CH2)12CH3

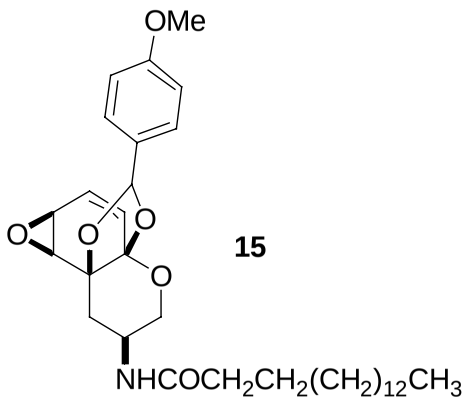
14b

3.51
0.97
1.72
0.88
0.93
1.00
0.96
1.41
4.65
0.96
0.90
0.93
2.09
1.04
2.76
29.66
4.66
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00

NOESY spectrum of compound 14b

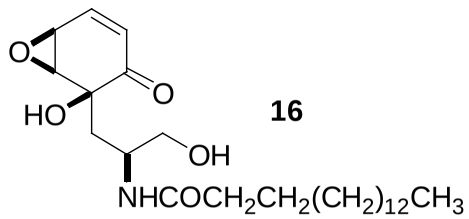



172.6
159.2
133.6
130.9
130.0
129.5
113.6
96.3
70.8
66.3
65.1
58.4
55.2
48.2
41.5
36.9
33.1
31.9
29.6
29.5
29.3
25.6
22.7
14.1
O
OPMB
HO
O
NHCOCH2CH2(CH2)12CH3
14b
150
125
100
75
50
25


OMe
O
O
O
O
NHCOCH2CH2(CH2)12CH3

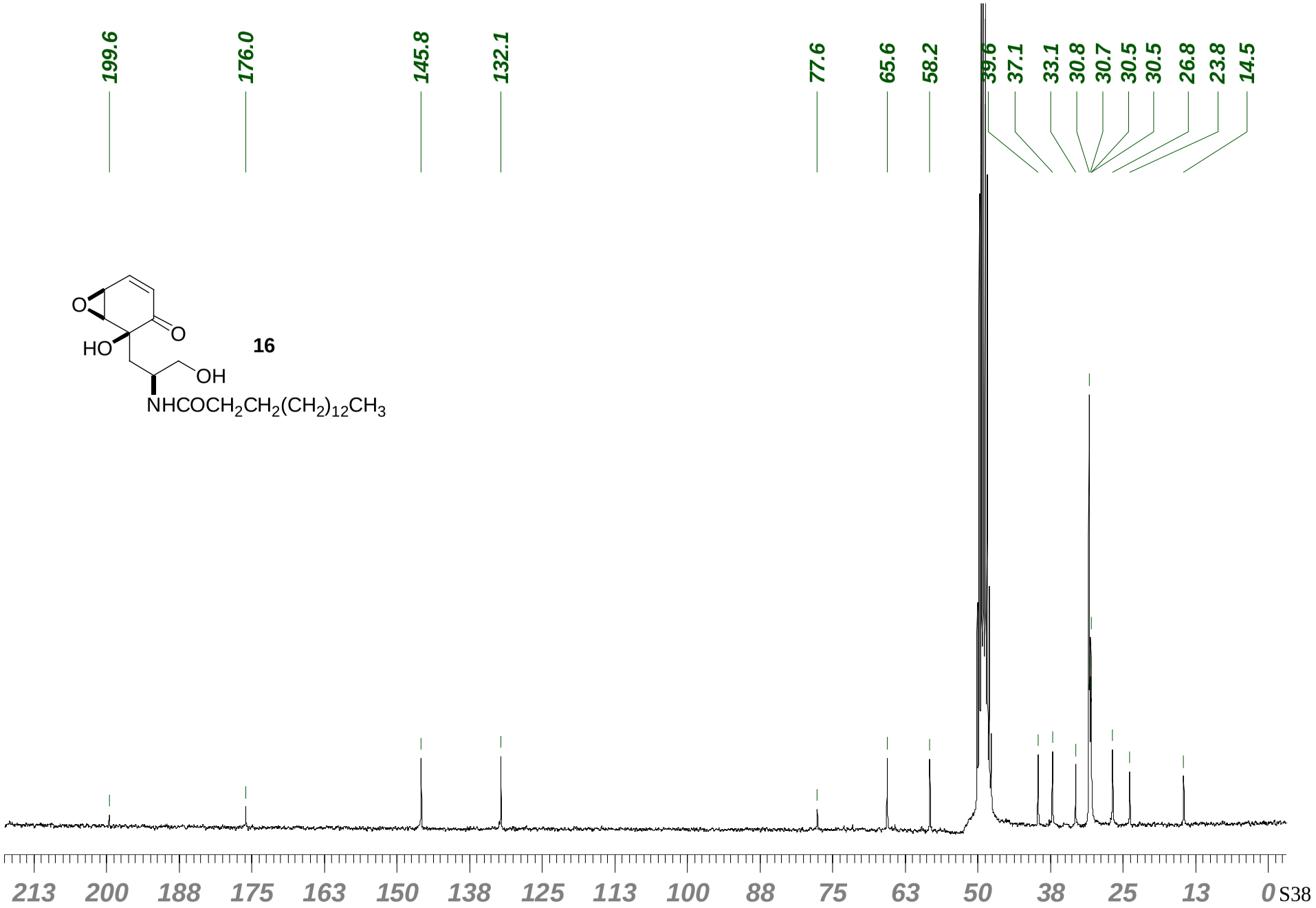
15

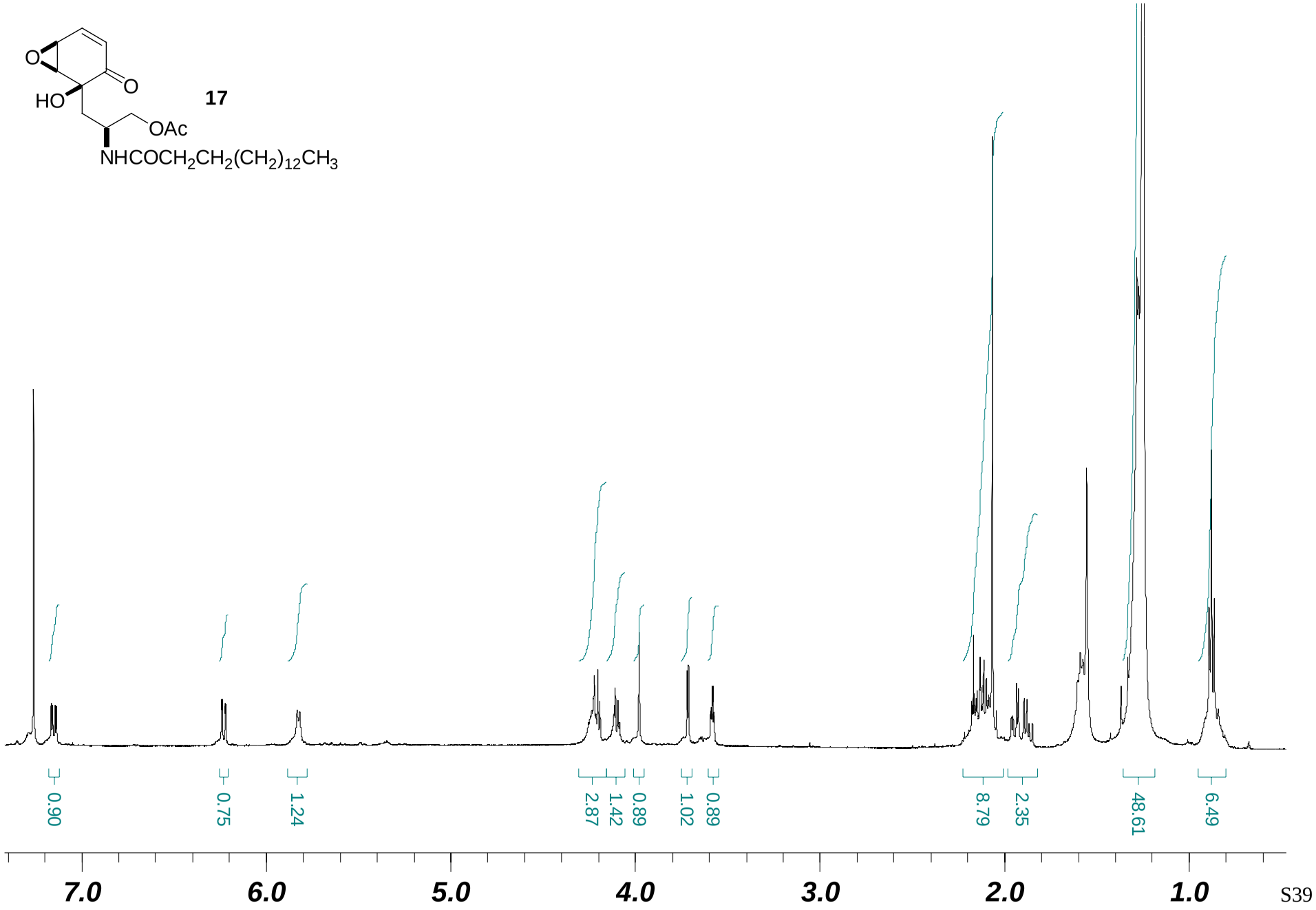
1.99
2.09
2.10
1.02
1.00
2.09
3.29
2.14
1.01
1.13
2.16
1.08
2.18
1.61
31.29
3.39
7.0
6.0
5.0
4.0
3.0
2.0
1.0


O
O
HO
OH
NHCOCH2CH2(CH2)12CH3

16

0.76
0.64
1.00
0.74
0.82
0.87
0.98
1.63
0.96
0.81
2.47
36.40
2.54
7.0
6.0
5.0
4.0
3.0
2.0
1.0


199.6
176.0
145.8
132.1
77.6
65.6
58.2
39.6
37.1
33.1
30.8
30.7
30.5
30.5
26.8
23.8
14.5
O
HO
O
OH
NHCOCH2CH2(CH2)12CH3
16
213
200
188
175
163
150
138
125
113
100
88
75
63
50
38
25
13
0


O
O
HO
OAc
NHCOCH2CH2(CH2)12CH3
17
0.90
0.75
1.24
2.87
1.42
0.89
1.02
0.89
8.79
2.35
48.61
6.49
7.0
6.0
5.0
4.0
3.0
2.0
1.0