Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Development of a benzophenone and alkyne functionalised trehalose probe to study trehalose dimycolate binding proteins

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Supplementary Information

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Experimental

Material and methods for Biological Assays

C57BL/6 male mice were bred and housed in a conventional animal facility at the Malaghan Institute of Medical Research, Wellington, New Zealand. All animals used for the experiments were aged between 8-10 weeks. All experimental procedures were approved by the Victoria University Animal Ethics Committee in accordance with their guidelines for the care of animals. Synthesised TDEs were tested to be endotoxin-free (≤0.125 EU/mL) with an endotoxin kit (Pyrotell, Limulus Amebocyte Lysate).

Generation of bone marrow derived macrophages (BMMs): Bone marrow cells were collected from the tibia and femur of C57BL/6 mice and were cultured (250 000 cells/mL) in Iscove's Modified Dulbecco's Media (IMDM-Gibco) supplemented with 5% FBS (Gibco), 1% Penicillin streptomycin (Gibco) and 55 μM 2-mercaptoethanol (Invitrogen) containing 10 ng/mL GM-CSF (clone X63/GM-CSF murine cells). Cells were incubated at 37 °C (5% CO_2) and the media changed on days 2, 5 and 7. On day 10, the media was removed and the BMM were primed with 10ng/mL IFN-γ (Peprotech) for 3 h prior to the addition of the compounds.

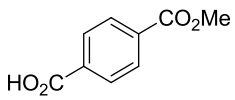
BMM Assay: TDB **2** and A*f*BP probe **3** stock solutions were prepared (2.5 mg/mL in PBS containing 2% DMSO), vortexed and warmed to 50 °C for 30 min (x 3) to ensure complete solubilisation of the compounds prior to administration to BMM cultures. BMM cultures were then treated with 20 or 40 μg/mL of TDB **2**, oA*f*BP probe **3** or with LPS (100 ng/mL) as a positive control and media only as a negative control for the times indicated. Supernatants were collected and tested immediately for NO levels.

NO analysis: NO levels in supernatants were determined using the Griess assay.[42]

Chemistry Experimental

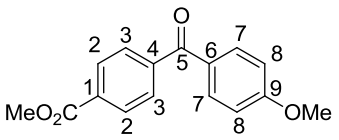
Unless stated otherwise, all reactions were performed under N_2. Prior to use, THF was distilled from Na wire and benzophenone, pyridine was dried and stored over 4 Å molecular sieves (4 Å MS), toluene was dried and stored over Na wire and CH_2Cl_2 was distilled from P_2O_5. Terephthalic acid (BDH), thionyl chloride (Acros), KOH (Science), 33% HCl (Panreac), H_2SO_4 (Panreac) $AlCl_3$ (Sigma-Aldrich), sodium acetylide (Aldrich, 18% in xylene), bromohexadecane (BDH), 1,4-butanediol (BDH), HBr (Acros), PPTS (Aldrich), dihydropyran (Pfaltz and Bauer), *n*-BuLi (Aldrich, 2M solution in hexanes), PTSA (Sigma-Aldrich), diaminopropane (Aldrich), tosyl chloride (Aldrich), 18-crown-6 ether (Sigma-Aldrich), CrO_3 (M&B), NaH (Avocado Research Chemicals, 60% dispersion in mineral oil), D-(+)-trehalose dihydrate (Sigma), TBAF (Aldrich), BSA (Fluka), Dowex-H^+ (Supelco), 1-docosanol (Aldrich), EDCI (Aldrich), DMAP (Merck), anhydrous DMF (Acros), anhydrous Et_2O (Biolab), EtOAc (Panreac), petroleum ether (Pure Science, b.p. 40–60 °C), $CHCl_3$ (Panreac), anhydrous MeOH (Panreac), isopropanol (Pure Science), $NaHCO_3$ (Pure Science), K_2CO_3 (Pure Science), $MgSO_4$ (Pure Science) and NaCl (Panreac) were used as received.

All solvents were removed by evaporation at reduced pressure. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by UV-absorption (short wave UV–254 nm; long wave UV–366 nm); coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating or by dipping in 10% H_2SO_4 in EtOH followed by charring at ~150 °C. Column chromatography was performed using Pure Science silica gel (40-63 μm). High resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter or Autopol II (Rudolph Research Analytical) at 589 nm (sodium D-line). Infrared spectra were recorded as thin films using a Brüker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 20 °C in $CDCl_3$ or C_5D_5N using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to residual solvent peak. NMR peak assignments were made using COSY, HSQC and HMBC 2D experiments.



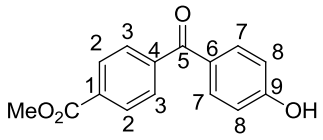
Monomethyl terephthalate (5). A solution of terephthalic acid (**4**) (10 g, 60.2 mmol) in MeOH (250 mL) was refluxed for 30 min after which time $SOCl_2$ (87.2 mL, 1.20 mol) was added and the reaction mixture was refluxed for a further 10 h. The reaction mixture was then cooled and the solvent removed *in vacuo*. The product was extracted with Et_2O (3 x 200 mL) and washed with saturated $NaHCO_3$ (3 x 60 mL) solution. The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo* to yield dimethyl terephthalate as white solid (*quant.*) and it was used in subsequent reaction without further purification.

Dimethyl terephthalate (9.77 g, 50.2 mmol) was dissolved in MeOH (165 mL) and the reaction mixture was refluxed for 30 min after which time it was cooled to r.t. followed by KOH addition (3.11 g, 55.4 mmol). The reaction was then heated at reflux overnight after which time the mixture was cooled and the solvent removed *in vacuo*. The resulting crude product was dissolved in water and washed with CH_2Cl_2 (3 x 100 mL). The aqueous layer was then neutralised with concentrated 1M HCl and the precipitate was extracted with Et_2O, dried with $MgSO_4$, and concentrated to yield the title compound as a white solid (7.86 g, 43.7 mmol, 87% over two steps). Characterisation data matched those reported in literature.[4]

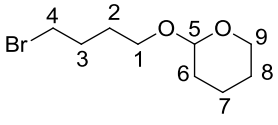


Methyl 4–(4-methoxybenzoyl)-benzoate (6). $SOCl_2$ (3.92 mL, 53.9 mmol) was added to acid **5** (2.44 g, 13.9 mmol) and the reaction mixture was refluxed until a clear solution had formed (*ca.* 4 h). The solvent was reduced *in vacuo* (under nitrogen), and the resulting acid chloride (2.76 g, 13.9 mmol) was dissolved in $CHCl_3$ (71 mL), and anisole (1.51 mL, 13.9 mmol) and $AlCl_3$ (1.85 g, 13.9 mmol) were added. The reaction mixture was then refluxed for 10 h, after which time another portion of $AlCl_3$ (1.85 g, 13.9 mmol) was added and the mixture refluxed for a further 10 h. Following this, the solution was cooled, quenched with H_2O (100 mL), and extracted with EtOAc (3 x 80 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, and the solvent removed *in vacuo* to yield the crude product. The title compound was obtained after silica gel flash chromatography (eluting in 20:1, Pet. Ether/EtOAc, v/v) as a white solid (2.60 g, 9.62 mmol, 70%); R_f = 0.76 (EtOAc); IR (film): 2944, 2832, 1448, 1022, 737 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, $J_{2,3}$ = 8.5 Hz, 2H, H-2), 7.83 (d, $J_{7,8}$ = 8.7 Hz, 2H, H-7), 7.79 (d, $J_{2,3}$ = 8.5 Hz, 2H, H-3), 6.98 (d, $J_{7,8}$ = 8.7 Hz, 2H, H-8), 3.98 (s, 3H, CO_2Me), 3.91 (s, 3H, OMe); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.8 (C-5), 166.4 (CO_2Me), 163.6 (C-9), 142.2 (C-4), 132.8 (C-1), 132.6 (C-7), 129.6 (C-6), 129.4

(C-2 and C-3), 113.8 (C-8), 55.6 (-OCH_3), 52.4 (CO_2*Me*); HRMS(ESI) *m/z* calcd. for $[C_{16}H_{14}O_4+Na]^+$: 293.0790, obsd.: 293.0790.

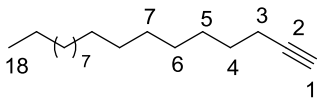


Methyl 4–(4-hydroxybenzoyl)-benzoate (7). $AlCl_3$ (2.92 g, 21.9 mmol) was added to a solution of benzophenone **6** (0.99 g, 3.66 mmol) in toluene (15 mL) and the reaction mixture was stirred at r.t. for 30 min followed by 30 min at reflux. The reaction was then cooled to r.t. followed by further addition of $AlCl_3$ ((1.46 g, 11.0 mmol) and the reaction mixture was refluxed for another 30 min. The reaction mixture was then poured into an ice/water mixture, stirred for a further 30 min and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The title compound was obtained, after silica gel flash chromatography (eluting in 10:1, Pet. Ether/EtOAc, v/v), as a white solid (0.80 g, 3.13 mmol, 85%). R_f = 0.24 (2/1, PE/EA, v/v); IR (film): 3317, 2944, 2832, 1448, 1022, 741 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, $J_{2,3}$ = 8.3 Hz, 2H, H-2), 7.79 (d, $J_{2,3}$ = 8.3 Hz, 2H, H-3), 7.79 (d, $J_{7,8}$ = 8.5 Hz, 2H, H-7), 6.93 (d, $J_{7,8}$ = 8.5 Hz, 2H, H-8), 3.98 (s, 3H, CO_2Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.8 (C-5), 166.4 (*C*O_2Me), 160.0 (C-9), 142.0 (C-4), 133.0 (C-7), 132.8 (C-1), 129.8 (C-6), 129.47 (C-2), 129.46 (C-3), 115.3 (C-8), 52.4 (CO_2Me); HRMS(ESI) *m/z* calcd. for $[C_{15}H_{12}O_4+Na]^+$: 279.0626, obsd.: 279.0633.

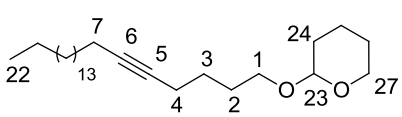


2-(4-Bromobutyloxyl)-tetrahydropyran (9). To a solution of 1,4-butanediol (**8**) (4.46 mL, 50.3 mmol) in toluene (70 mL) was added a 48% HBr solution in water (6.25 mL, 55.2 mmol) and the reaction mixture was refluxed using a Dean-Stark apparatus, until all the starting material had been consumed (as determined by TLC analysis). The solution was then cooled, extracted with CH_2Cl_2 (3 x 60 mL), washed with water, dried with $MgSO_4$, and the solvent removed *in vacuo* to yield 4-bromo-1-butanol (3.06 g, 20.1 mmol, 40%), which was used in the consecutive reaction without further purification. To a solution of 4-bromo-1-butanol (2.0 g, 13.0 mmol) in CH_2Cl_2 (30 mL) was added pyridinium *para*-toluenesulfonate (PPTS) (0.65 g, 2.61 mmol) and 3,4-dihydropyran (2.77 mL, 30.4 mmol) and the reaction was stirred at r.t.

overnight. The reaction mixture was then quenched with H_2O (20 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (30 mL), dried with $MgSO_4$, and the solvent reduced *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 50:1, Pet. Ether/EtOAc, v/v) as a white solid (3.03 g, 12.7 mmol, 97%). R_f = 0.83 (1/1, PE/EA, v/v); IR (film): 2940, 2860, 1133, 1075, 904, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.57 (t, $J_{5,6a} = J_{5,6b} = 2.5$ Hz, 1H, H-5), 3.84 (ddd, $J_{9a,9b} =$ 11.0 Hz, $J_{8a,9a} = J_{8b,9a} = 2.4$ Hz, 1H, H-9a), 3.76 (dt, $J_{1a,1b} = 9.8$ Hz, $J_{1a,2a} = J_{1a,2b} = 6.6$ Hz, H-1a), 3.52–3.48 (m, 1H, H-9b), 3.45 (t, $J_{3,4} = 7.1$ Hz, 2H, H-4), 3.41 (dt, $J_{1a,1b} = 9.8$ Hz, $J_{1b,2a} = J_{1b,2b} = 6.1$ Hz, 1H, H-1b), 1.97 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma} = 7.1$ Hz, 2H, H-3), 1.83–1.78 (m, 1H, H-7a), 1.76–1.71 (m, 1H, H-2), 1.71–1.68 (m, 1H, H-6a), 1.60–1.50 (m, 4H, H-6b, H-7b, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 98.8 (C-5), 66.5 (C-1), 62.3 (C-9), 33.8 (C-4), 30.7 (C-6), 29.8 (C-3), 28.4 (C-2), 25.4 (C-8), 19.6 (C-7); HRMS(ESI) *m/z* calcd. for $[C_9H_{17}O_2Br+Na]^+$: 259.0304, obsd.: 259.0318.

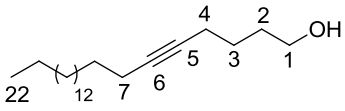


Octadecyne (11). A solution of bromo-hexadecane (**10**) (4.54 g, 14.9 mmol) in DMF (20 mL) was cooled to 0 °C and sodium acetylide (18% in xylene, 5.95 mL, 22.3 mmol) was added to it. The reaction mixture was stirred at r.t. overnight after which time it was diluted with Et_2O (100 mL), washed with water (50 mL), 1M HCl (50 mL), brine (25 mL), dried with $MgSO_4$, and the solvent reduced *in vacuo*. Octadecyne (**11**) was obtained after silica gel flash chromatography (eluting in Pet. Ether), as a white solid (3.62 g, 14.5 mmol, 98%). R_f = 0.50 (Pet. Ether); IR (film): 3314, 2922, 2853, 2341, 1236, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (dt, $J_{3a,3b} = 9.8$ Hz, $J_{3a,4} = 7.1$ Hz, 1H, H-3a), 2.18 (dt, $J_{3a,3b} = 9.8$ Hz, $J_{3b,4} = 7.1$ Hz, 1H, H-3b), 1.94 (t, $^4J_{1,3} = 2.6$ Hz, 1H, H-1), 1.55 (pentet, $J_{\alpha,\beta} = J_{\beta\gamma} = 7.1$ Hz, 2H, H-4), 1.44–1.36 (m, 2H, H-5) 1.32–1.26 (m, 24H, H-6–H-17), 0.89 (t, $J_{17,18} = 6.8$ Hz, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 84.8 (C-2), 68.0 (C-1), 31.9, 29.70, 29.68, 29.66, 29.62, 29.52, 29.37, 29.12, 28.77, 28.50, 22.70 (C-4–C-17), 18.4 (C-3), 14.1 (C-18); HRMS(ESI) *m/z* calcd. for $[C_{18}H_{34}+Na]^+$: 273.2558, obsd.: 273.2550.



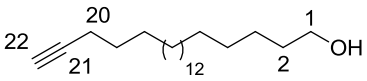
2-(5-Docosynyloxy)-tetrahydropyran (12). *n*-BuLi (8.28 mL, 2.0 M solution in hexanes, 16.6 mmol) was added to a

solution of octadecyne (**11**) (2.07 g, 8.28 mmol) in THF (30 mL) at 0 °C and the reaction mixture was refluxed for 10 h, after which time it was cooled to r.t. and THP ether **9** (4.97 g, 20.7 mmol) in THF (30 mL) was added. The reaction was refluxed for a further 24 h then quenched with H_2O (50 mL) and extracted with Pet. Ether (3 x 60 mL). The extracts were washed with brine (40 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 75:1, Pet. Ether/EtOAc, v/v) as a colourless oil (2.8 g, 6.84 mmol, 83%). R_f = 0.69 (2/1, PE/EA, v/v); IR (film): 2922, 2852, 2214, 1363, 1186, 738, 704 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.58 (t, $J_{23,24a} = J_{23,24b}$ = 2.9Hz, 1H, H-23), 3.86 (ddd, $J_{27a,27b}$ = 11.1 Hz, $J_{26a,27a} = J_{26b,27a}$ = 3.0 Hz, 1H, H-27a), 3.75 (dt, $J_{1a,1b}$ = 9.7 Hz, $J_{1a,2a} = J_{1a,2b}$ = 3.9 Hz, 1H, H-1a), 3.51–3.48 (m, 1H, H-27b), 3.40 (dt, $J_{1a,1b}$ = 9.7 Hz, $J_{1b,2a} = J_{1b,2b}$ = 3.1 Hz, 1H, H-1b), 2.19 (tt, $J_{3,4}$ = 7.1 Hz, $^5J_{4,7}$ = 2.2 Hz, 2H, H-4), 2.13 (tt, $J_{7,8}$ = 7.0 Hz, $^5J_{4,7}$ = 2.2 Hz, 2H, H-7), 1.86–1.77 (m, 1H, H-25a), 1.75–1.62 (m, 3H, H-24a and H-26), 1.61–1.50 (m, 6H, H-2, H-3, H-24b and H-25b), 1.47 (pentet, $J_{7,8} = J_{8,9}$ = 7.3 Hz, 2H, H-8), 1.37–1.31 (m, 2H, H-9), 1.30–1.25 (m, 24H, H-10–H-21), 0.88 (t, $J_{1,2}$ = 6.8 Hz, 2H, H-22); ^{13}C NMR (125 MHz, $CDCl_3$) δ 98.8 (C-23), 80.5 (C-6), 79.8 (C-5), 67.1 (C-1), 62.3 (C-27), 31.9, 30.7, 29.70, 29.68, 29.66, 29.65, 29.57, 29.4, 29.19, 29.16, 28.9, 28.9, 25.9, 25.5, 22.7 (C-2, C-3, C-8–C-21, C-24, C-26), 19.6 (C-25), 18.8 (C-7), 18.6 (C-4), 14.1 (C-22); HRMS(ESI) *m/z* calcd. for $[C_{27}H_{50}O_2+Na]^+$: 429.3709, obsd.: 429.3713.

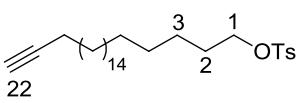


5-Docosyn-1-ol (13). To a solution of alkyne **12** (2.01 g, 4.91 mmol) in MeOH (20 mL) was added PTSA (0.28 g, 1.47 mmol) and the reaction mixture was stirred at r.t. overnight before being quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (30 mL), brine (30 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 20:1, Pet. Ether/EtOAc, v/v) as a white solid (1.40 g, 4.34 mmol, 89%); R_f = 0.33 (5/1, PE/EA, v/v); IR (film): 2984, 2361, 1447, 1234, 1044 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.68 (t, $J_{1,2}$ = 6.4 Hz, 2H, H-1), 2.20 (tt, $J_{3,4}$ = 7.1 Hz, $^5J_{4,7}$ = 2.2 Hz, 2H, H-4), 2.14 (tt, $J_{7,8}$ = 6.9 Hz, $^5J_{4,7}$ = 2.2 Hz, 2H, H-7), 1.69 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma}$ = 6.8 Hz, 2H, H-2), 1.57 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma}$= 6.9 Hz, 2H, H-3),1.47 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma}$ = 7.2 Hz, 2H, H-8), 1.40–1.32 (m, 2H, H-9), 1.29–1.22 (m, 24H, H-10–H-21), 0.89 (t, $J_{21,22}$ = 7.0 Hz, 2H, H-22); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 80.8 (C-6), 79.7 (C-5), 62.6 (C-1), 31.93 (C-2), 31.88, 29.71, 29.68, 29.66, 29.65, 29.57, 29.37, 29.18, 29.15, 28.90, 25.35, 22.70 (C-3, C-8–C-21), 18.7 (C-7), 18.5 (C-4), 14.1 (C-22); HRMS(ESI) *m/z* calcd. for $[C_{22}H_{42}O+Na]^+$: 345.3133, obsd.: 345.3134.

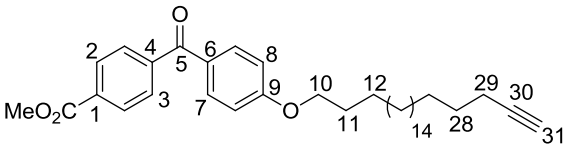


21-Docosyn-1-ol (14). Diaminopropane (10 mL) was added to NaH (0.88 g, 22.0 mmol, 60% dispersion in mineral oil) and the resulting mixture was stirred at 70 °C until gas evolution ceased (*ca.* 1 h), after which time the mixture was cooled, and alcohol **13** (0.36 g, 1.10 mmol) dissolved in diaminopropane (5 mL), was added dropwise. The reaction was stirred at 70 °C overnight after which time it was quenched with H_2O and extracted with EtOAc (3 x 35 mL). The combined organic layers were then washed with water (25 mL), brine (25 mL), dried ($MgSO_4$) and the solvent reduced *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 20:1, Pet. Ether/EtOAc, v/v) as a white solid (3.1 g, 0.74 mmol, 66%) R_f = 0.32 (5/1, PE/EA, v/v); IR (film): 3287, 2917, 2849, 2341, 1122 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.65 (t, $J_{1,2}$ = 6.6 Hz, 2H, H-1), 2.19 (dt, $J_{20a,20b}$ = 11.5 Hz, $J_{19,20a}$ = 7.1 Hz, 1H, H-20a), 2.18 (dt, $J_{20a,20b}$ = 11.5 Hz, $J_{19,20b}$ = 7.1 Hz, 1H, H-20b), 1.94 (t, $^4J_{20,22}$ = 2.6 Hz, 1H, H-22), 1.54 (pentet, $J_{\alpha,\beta}$ = $J_{\beta,\gamma}$ = 6.8 Hz, 2H, H-2), 1.51 (pentet, $J_{\alpha,\beta}$ = $J_{\beta,\gamma}$ = 7.3 Hz, 2H, H-19), 1.41–1.14 (m, 32H, H-3–H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 84.8 (C-21), 68.0 (C-22), 63.1 (C-1), 32.8 (C-2) 29.70, 29.69, 29.66, 29.62, 29.51, 29.44, 29.12, 28.77, 28.50 (C-3–C-19), 22.74 (C-20); HRMS(ESI) *m/z* calcd. for $[C_{22}H_{42}O+H]^+$: 323.3314, obsd.: 323.3309.

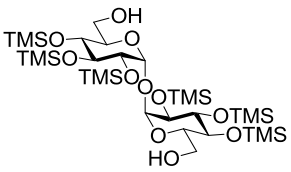


21-Docosynyl *p*-toluenesulfonate (17). To a solution of alcohol **14** (0.20 g, 0.63 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added pyridine (0.10 mL, 1.25 mmol) then TsCl (0.18 g, 0.94 mmol) and the reaction stirred at r.t. overnight. Et_2O (30 mL) and water (20 mL) were added to the reaction mixture and the organic layer was washed with 2M HCl (30 mL), followed by 5% $NaHCO_3$ (20 mL), water (30 mL), brine (30 mL) and then dried with $MgSO_4$. The solvent was removed *in vacuo* and the title compound obtained after silica gel flash chromatography (eluting in 50:1, Pet. Ether/EtOAc, v/v) as a white solid (0.28 g, 0.61 mmol, 97%). R_f = 0.46 (5/1, PE/EA, v/v); IR (film): 2917, 2850, 2359, 1472, 1173, 668 cm^{-1}; ^1H NMR (500 MHz,

$CDCl_3$) δ 7.80 (d, J = 8.3 Hz, 2H, H_{arom}), 7.35 (d, J = 8.1 Hz, 2H, H_{arom}), 4.03 (t, $J_{1,2}$ = 6.6 Hz, 2H, H-1), 2.46 (s, 3H, CH_3), 2.19 (dt, $J_{20a,20b}$ = 13.1 Hz, $J_{19,20a}$ = 7.1 Hz, 1H, H-20a), 2.18 (dt, $J_{20a,20b}$ = 13.1 Hz, $J_{19,20b}$ = 7.1 Hz, 1H, H-20b), 1.95 (t, $^4J_{20,22}$ = 2.6 Hz, 1H, H-22), 1.64 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma}$ = 6.8 Hz, 2H, H-2), 1.52 (pentet, $J_{\alpha,\beta} = J_{\beta,\gamma}$ = 6.6 Hz, 2H, H-19), 1.40–1.38 (m, 2H, H-3), 1.34–1.22 (m, 30H, H-4–H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6 (C_{ar}-CH_3), 129.8 (C_{ar}), 127.9 (C_{ar}), 84.8 (C-21), 70.7 (C-1), 68.0 (C-22), 29.70, 29.68, 29.65, 29.61, 29.50, 29.39, 29.11, 28.92, 28.81, 28.77, 28.50, 25.32 (C-2–C-19), 21.63 (CH_3), 18.4 (C-20); HRMS(ESI) *m/z* calcd. for $[C_{29}H_{48}O_3S+Na]^+$: 499.3222, obsd.: 499.3222.

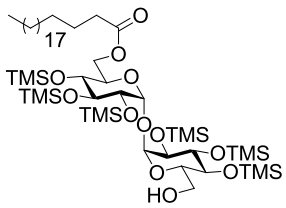


Methyl 4-[4-(21-docosynyloxy)benzoyl]-benzoate (16). To a solution of benzophenone **7** (30.5 mg, 0.12 mmol) in DMF (2 mL) was added Cs_2CO_3 (42.9 mg, 0.13 mmol), 18-crown-6-ether (37.8 mg, 0.14 mmol) and tosylate **17** (83.0 mg, 0.18 mmol) in toluene (2 mL) and the reaction was stirred at r.t. for 30 min and then heated to 50 °C over 2 nights. The reaction mixture was then cooled down, diluted with CH_2Cl_2 and water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL) and the combined organic layers were dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 30:1, Pet. Ether/EtOAc, v/v) as a white solid (43.0 mg, 0.08 mmol, 64 %); R_f = 0.63 (2/1, PE/EA, v/v); IR (film): 2916, 2848, 2360, 1638, 1604, 1256, 1107 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, $J_{2,3}$ = 8.0 Hz, 2H, H-2), 7.81 (d, $J_{7,8}$ = 8.8 Hz, 2H, H-7), 7.79 (d, $J_{2,3}$ = 8.0 Hz, 2H, H-3), 6.96 (d, $J_{7,8}$ = 8.8 Hz, 2H, H-8), 4.04 (t, $J_{10,11}$ = 6.6 Hz, 2H, H-10), 3.97 (s, 3H, CO_2Me), 2.19 (tt, $J_{28,29a}$ = 7.3 Hz, $^4J_{29,31}$ = 2.4 Hz, 1H, H-29a), 2.18 (tt, $J_{28,29b}$ = 7.1 Hz, $^4J_{29,31}$ = 2.4 Hz, 1H, H-29b), 1.94 (t, $^4J_{29,31}$ = 2.4 Hz, 1H, H-31), 1.82 (pentet, $J_{9,10} = J_{10,11}$ = 7.1 Hz, 2H, H-11), 1.58–1.50 (m, 2H, H-28), 1.50–1.42 (m, 2H, H-12), 1.40–1.22 (m, 30H, H-13–H-27); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.8 (C-5), 166.4 (CO_2Me), 163.6 (C-9), 142.2 (C-4), 132.7 (C-1), 132.6 (C-7), 129.4 (C-3), 129.4 (C-2), 129.3 (C-6), 114.2 (C-8), 84.8 (C-30), 68.4 (C-31), 68.0 (C-10), 52.4 (CO_2Me), 29.70, 29.68, 29.66, 29.62, 29.60, 29.57, 29.51, 29.36, 29.12, 29.09, 28.77, 28.50, 25.98 (C-11–C-28), 18.40 (C-29); HRMS(ESI) *m/z* calcd. for $[C_{37}H_{52}O_4+Na]^+$: 583.3763, obsd.: 583.3763.



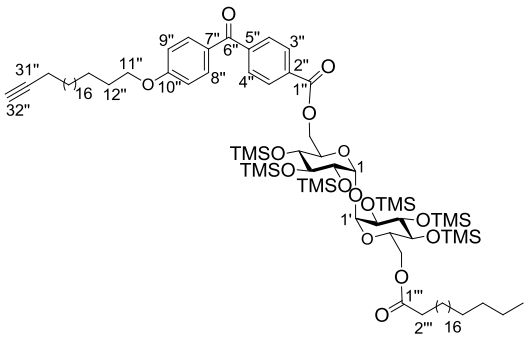
2,2',3,3',4,4'-Hexa-*O*-trimethylsilyl-α,α'-D-trehalose S10

Trehalose dihydrate (**18**) (0.25 g, 0.66 mmol) was co-with anhydrous DMF (3 x 5 mL) and then dissolved in DMF (1 mL). To this solution, *N*,*O*-bis(trimethylsilyl)acetamide (1.4 mL, 5.70 mmol) and TBAF (0.04 mL, 0.04 mmol) were added and the reaction stirred at room temperature for 1.5 h. The reaction was quenched with 0.25 mL isopropanol and the resulting solution was diluted with 15 mL MeOH and cooled to 0 °C. K_2CO_3 solution (0.091 g in 20 mL MeOH) was added to the reaction mixture and stirring at 0 °C was continued for 2 h. The reaction mixture was concentrated *in vacuo* and the resulting residue partitioned between ether and brine. The aqueous layer was extracted twice more with diethyl ether (30 mL x 2) and the combined organic layer dried ($MgSO_4$) and concentrated *in vacuo*. Silica gel flash chromatography of the crude product gave diol **19**, **(**eluting in 4:1 Pet. Ether/EtOAc, v/v) as a white solid (0.43 g, 0.56 mmol, 85%). R_f = 0.29 (PE/EA, 3/1, v/v); $[\alpha]^{18}_D$ = +109 (*c* = 1.0, $CHCl_3$), Lit[3] $[\alpha]^{25}_D$ = +103; IR (film): 3628, 3019, 2958, 1558, 1541, 1387, 1250, 1215, 1166, 1109, 1075, 1005 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.91 (d, $J_{1,2}$ = 3.1 Hz, 2H, H-1), 3.90 (t, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 2H, H-3), 3.88–3.84 (m, 2H, H-5), 3.72 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 2.7 Hz, 2H, H-6a), 3.70, (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 3.9 Hz 2H, H-6b), 3.49 (t, $J_{3,4} = J_{4,5}$ = 9.1 Hz, 2H, H-4), 3.42 (dd, $J_{2,3}$ = 9.0 Hz, $J_{1,2}$ = 3.1 Hz, 2H, H-2), 0.17, 0.15, 0.13 (3 s, 54H, CH_3, TMS); ^{13}C NMR (125 MHz, $CDCl_3$) δ 94.6 (C-1), 73.3 (C-3), 73.0 (C-2), 72.8 (C-5), 71.4 (C-4), 61.7 (C-6), 1.0, 0.8, 0.07 (TMS); HRMS(ESI) *m/z* calcd. for $[C_{30}H_{70}O_{11}Si_6+Na]^+$: 797.3431, obsd.:797.3424.



6-*O*-Docosanoyl-2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (20). To a solution of diol **19** (48.9 mg, 0.063 mmol) and behenic acid (28.0 mg, 0.082 mmol) in dry toluene (2 mL) was added EDCI (30.2 mg, 0.16 mmol,) and DMAP (1.1 mg, 0.01 mmol).The reaction was stirred at 70 °C until analysis by TLC showed no further product formation. The resulting precipitate was removed by filtration and washed thoroughly with EtOAc (2 x 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 20:1, Pet. Ether/EtOAc, v/v) as

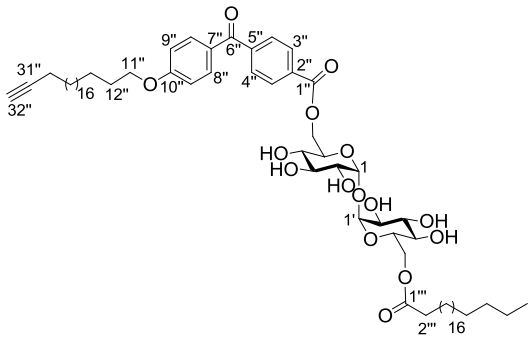
colourless oil (52.0 mg, 0.047 mmol, 75%). R_f = 0.55 (PE/EA, 5/1, v/v); $[\alpha]_D^{18}$ = +20.2 (*c* = 1.0, $CHCl_3$); IR (film): 3017, 2923, 2852, 1737, 1372, 1250, 1076, 1007, 965, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.94 (d, $J_{1,2/1',2'}$ = 3.5 Hz, 1H, H-1/1'), 4.92 (d, $J_{1,2'/1',2'}$ = 3.5 Hz, 1H, H-1/1'), 4.30 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 2.2 Hz, 1H, H-6a), 4.07 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 4.6 Hz, 1H, H-6b), 4.03–4.00 (m, 1H, H-5), 3.92 (t, $J_{2,3/2'/3'} = J_{3,4/3',4'}$ = 9.0 Hz, 1H, H-3/3'), 3.91 (t, $J_{2,3/2',3'} = J_{3,4/3',4'}$ = 9.0 Hz, 1H, H-3/3'), 3.85 (dt, $J_{4',5'}$ = 9.0 Hz, $J_{5',6a'} = J_{5',6b'}$ = 3.5 Hz, 1H, H-5'), 3.86–3.83 (m, 2H, H-6a' and H-6b'), 3.49 (t, $J_{3,4} = J_{4,5}$ = 9.0 Hz, 1H, H-4), 3.47 (t, $J_{3',4'} = J_{4',5'}$ = 9.0 Hz, 1H, H-4'), 3.44 (dd, $J_{2,3/2',3'}$ = 9.0 Hz, $J_{1,2/1',2'}$ = 3.5 Hz, 1H, H-2/2'), 3.43 (dd, $J_{2,3/2',3'}$ = 9.0 Hz, $J_{1,2/1',2'}$ = 3.5 Hz, 1H, H-2/2'), 2.35 (dt, $J_{8a,8b}$ = 13.2 Hz, $J_{8a,9}$ = 7.3 Hz, 1H, H-8a), 2.34 (dt, $J_{8a,8b}$ = 13.2 Hz, $J_{8b,9}$ = 7.4 Hz, 1H, H-8b), 1.66–1.57 (m, 2H, H-9), 1.34–1.26 (m, 36H, H-10–H-27), 0.89 (t, $J_{27,28}$ = 7.1 Hz, 3H, H-28), 0.17, 0.16, 0.15, 0.14, 0.13 (6 s, 54H, CH_3, TMS); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (C-7), 94.5 (C-1/1'), 94.4 (C-1/1'), 73.5 (C-3/3'), 73.3 (C-3/3'), 72.9 (C-2/2'), 72.8 (C-5'), 72.6 (C-2/2'), 71.9 (C-4), 71.4 (C-4'), 70.8 (C-5), 63.3 (C-6), 61.7 (C-6'), 34.2 (C-8), 31.9, 29.70, 29.66, 29.60, 29.5, 29.4, 29.3, 29.2, 24.8, 22.7 (C-9–C-27), 14.1 (C-28), 1.04, 1.00, 0.89, 0.85, 0.14, 0.10 (TMS) HRMS (ESI) *m/z* calcd. for $[C_{52}H_{112}O_{12}Si_6+Na]^+$: 1119.6662, obsd.: 1119.6667.



6'-*O*-Docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (22). $LiOH.H_2O$ (15 mg, 0.36 mmol) was added to a solution of **16** (20 mg, 0.04 mmol) in 0.5 mL $THF:H_2O:MeOH$ (3:1:1) and the reaction mixture was refluxed for 10 min after which it was neutralised with 0.1 M HCl (pH =3) and extracted with EtOAc (3x 10 mL). The combined organic layers were washed with water, brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield carboxylic acid **21** as a white solid (15 mg, 0.03 mmol, 80%) which was used without further purification.

To a solution of mono-trehalose ester **20** (8.5 mg, 0.008 mmol) and carboxylic acid **21** (14 mg, 0.03 mmol) in dry toluene (1 mL) was added EDCI (6.5 mg, 0.034 mmol) and DMAP (0.2 mg, 0.001 mmol) and the reaction was refluxed for 7 days after which time the resulting precipitate was removed by filtration and the precipitate washed thoroughly with EtOAc (3 x 10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL) dried

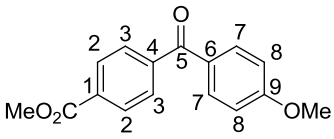
with $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was obtained after silica gel flash chromatography (eluting in 25:1, Pet. Ether/EtOAc, v/v) as colourless oil (3.5 mg, 0.002 mmol, 30%). R_f = 0.54 (PE/EA, 5/1, v/v); $[\alpha]^{22}_D$ = +0.06 (*c* = 0.1 $CHCl_3$); IR (film): 3628, 2920, 1684, 1457, 720, 677 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, $J_{3'',4''}$ = 8.5 Hz, 2H, H-3''), 7.81 (d, $J_{8'',9''}$ = 8.8 Hz, 2H, H-8''), 7.79 (d, $J_{3'',4''}$ = 8.5 Hz, 2H, H-4''), 6.96 (d, $J_{8'',9''}$ = 8.8 Hz, 2H, H-9''), 4.95 (d, $J_{1,2/1',2'}$ = 3.2 Hz, 1H, H-1/1'), 4.94 (d, $J_{1,2/1',2'}$ = 3.2 Hz, 1H, H-1/1'), 4.59 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 2.3 Hz, 1H, H-6a), 4.33 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 3.5 Hz, 1H, H-6b), 4.27 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{5',6a'}$ = 2.3 Hz, 1H, H-6a'), 4.13 (ddd, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 3.5 Hz, 1H, H-5), 4.09 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{5',6b'}$ = 3.5 Hz, 1H, H-6b'), 4.04 (t, $J_{11'',12''}$ = 6.4 Hz, 2H, H-11''), 4.02–3.97 (m, 1H, H-5'), 3.96 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H-3), 3.92 (dd, $J_{2',3'}$ =$J_{3',4'}$ = 9.0 Hz, 1H, H-3'), 3.64 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, 1H, H-4), 3.49 (dd, $J_{2,3/2',3'}$ = 9.0 Hz, $J_{1,2/1',2'}$ = 3.2 Hz, 1H, H-2/2') 3.48 (dd, $J_{3',4'}$ = $J_{4',5'}$ = 9.0 Hz, 1H, H-4'), 3.47 (dd, $J_{2,3/2',3'}$ = 9.0 Hz, $J_{1,2/1',2'}$ = 3.2 Hz, 1H, H-2/2'), 2.34 (dt, $J_{2a''',2b'''}$ = 11.4 Hz, $J_{2a''',3'''}$ = 7.3 Hz, 1H, H-2a'''), 2.33 (dt, $J_{2a''',2b'''}$ = 11.4 Hz, $J_{2b''',3'''}$ = 7.3 Hz, 1H, H-2b''') 2.19 (dtt, $J_{30a'',30b''}$ = 9.9 Hz, $J_{29'',30a''}$ = 7.3 Hz, $^4J_{30a'',32''}$ = 2.6 Hz, 1H, H-30a''), 2.17 (ddt, $J_{30a'',30b''}$ = 9.9 Hz, $J_{29'',30b''}$ = 7.3 Hz, $^4J_{30b'',32''}$ = 2.6 Hz, 1H, H-30b''), 1.94 (t, $^4J_{30'',32''}$ = 2.6 Hz, 1H, H-32), 1.82 (pentet, $J_{11'',12''}$ = $J_{12''13''}$ = 6.7 Hz, 2H, H-12''), 1.64–1.52 (m, 4H, H-3''' and H-13''), 1.50–1.22 (m, 68H, H-14''–H-29'' and H-4'''–H-21'''), 0.88 (t, $J_{21''',22'''}$ = 7.0 Hz, H-22'''), 0.17, 0.16, 0.15, 0.14, 0.13 (6 s, 54H, CH_3, TMS); ^{13}C NMR (125 MHz, $CDCl_3$) δ194.5 (C-6''), 173.8 (C-1'''), 165.5 (C-1''), 163.1 (C-10''), 142.2 (C-5''), 132.4 (C-4''), 132.3 (C-2''), 129.3 (C-3''), 129.2 (C-8''), 128.7 (C-7''), 114.0 (C-9''), 94.29 (C-1/1'), 94.25 (C-1/1'), 84.6 (C-31''), 73.4 (C-3), 73.2 (C-3'), 72.44 (C-2/2'), 72.40 (C-2/2'), 71.66 (C-4), 71.60 (C-4'), 70.53 (C-5'), 70.50 (C-5), 68.2 (C-11''), 67.8 (C-32''), 63.8 (C-6), 63.0 (C-6'), 33.9 (C-2'''), 31.7, 31.2, 29.9, 29.8, 29.5, 29.43, 29.38, 29.34, 29.35, 29.27, 29.24, 29.20, 29.14, 29.1, 28.9, 28.88, 28.54, 28.26, 25.76, 24.78, 24.54, 22.47, 21.85, (C-3'''–C21''' and C-12''–C-29''), 18.17 (C-30''), 13.9 (C-22'''), 0.86, 0.83, 0.70, 0.65, 0.01, 0.00 (TMS); HRMS (ESI) *m/z* calcd. for $[C_{88}H_{160}O_{15}Si_6+Na]^+$: 1648.0265, obsd.: 1648.0280.

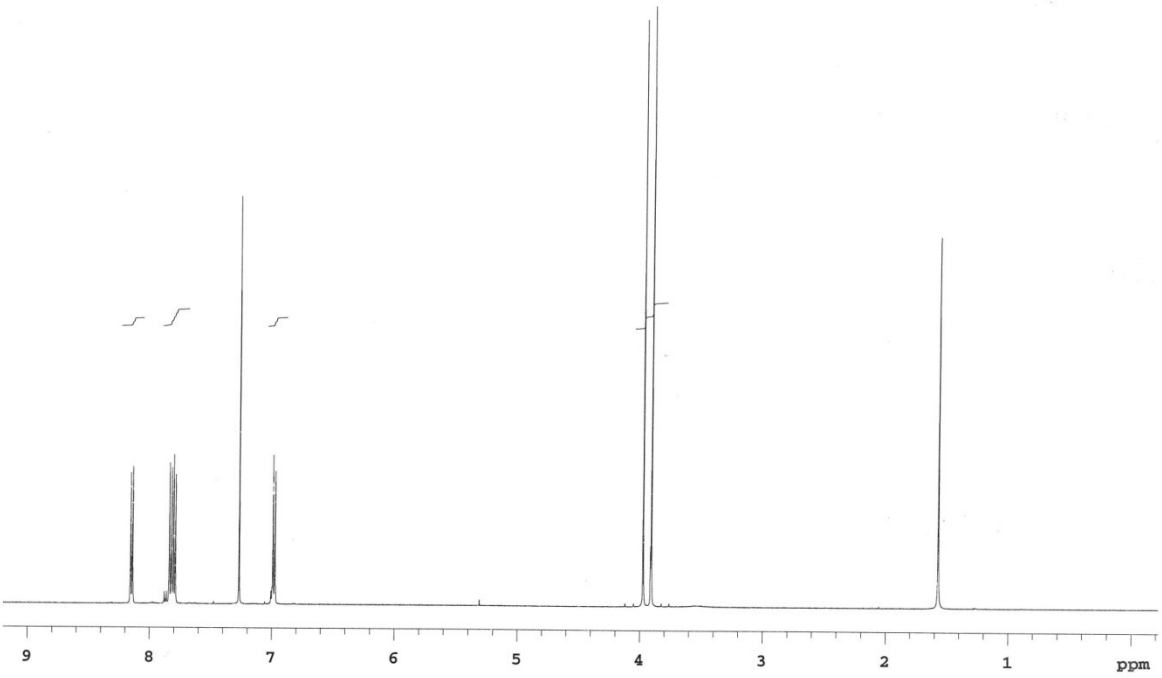


6'-*O*-Docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-α,α'-D-trehalose (3). To a solution of the protected probe **22** (1.7 mg, 0.001 mmol) in 0.4 mL

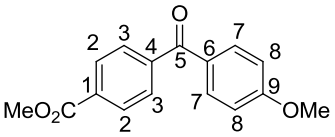
CH_2Cl_2:MeOH mixture (3/1, v/v) was added Dowex-H^+ (10% by weight) and the reaction stirred at room temperature. After 30 min, the reaction mixture was filtered and concentrated *in vacuo*. A*f*BP probe **3** was obtained after silica gel flash column chromatography (eluting in 97:3, EtOAc/MeOH, v/v) as a white solid (1.25 mg, 0.001 mmol, *quant.*). R_f = 0.31 (EtOAc); $[\alpha]^{18}_D$ = +0.6 (*c* = 0.1, C_5H_5N); IR (film); 3567, 2970, 1739, 1365, 1217, 651 cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 8.20 (d, $J_{3'',4''}$ = 8.2 Hz, 2H, H-3''), 7.86 (d, $J_{8'',9''}$ = 8.8 Hz, 2H, H-8''), 7.68 (d, $J_{3'',4''}$ = 8.2 Hz, 2H, H-4''), 7.06 (d, $J_{8'',9''}$ = 8.8 Hz, 2H, H-9''), 5.84 (d, $J_{1,2/1',2'}$ = 3.6 Hz, 1H, H-1/1'), 5.83 (d, $J_{1,2/1',2'}$ = 3.6 Hz, 1H, H-1/1'), 5.20–5.17 (m, 1H, H-5), 5.15–4.94 (m, 5H, H-5, H-6a,b and H-6'a,b), 4.70 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-3), 4.68 (dd, $J_{2',3'}$ =$J_{3',4'}$ = 9.6 Hz, 1H, H-3'), 4.28 (dd, $J_{2,3/2',3'}$ = 9.6 Hz, $J_{1,2/1',2'}$ = 3.6 Hz, 1H, H-2/2'), 4.24 (dd, $J_{2,3/2',3'}$ = 9.6 Hz, $J_{1,2/1',2'}$ = 3.6 Hz, 1H, H-2/2'), 4.09 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 4.09 (dd, $J_{3',4'}$ = $J_{4',5'}$ = 9.6 Hz, 1H, H-4'), 3.96 (t, $J_{11'',12''}$ = 6.3 Hz, 2H, H-11''), 2.68 (t, $^4J_{30'',32''}$ = 2.7 Hz, 1H, H-32''), 2.20 (dt, $J_{2a''',2b'''}$ = 12.2 Hz, $J_{2a''',3'''}$ = 7.9 Hz, 2H, H-2a'''), 2.19 (dt, $J_{2a''',2b'''}$ = 12.2 Hz, $J_{2b''',3'''}$ = 7.9 Hz, 1H, H-2b'''), 2.12 (dtt, $J_{30a'',30b''}$ = 9.9 Hz, $J_{29'',30a''}$ = 7.0 Hz, $^4J_{30a'',32''}$ = 2.7 Hz, 1H, H-30a''), 2.10 (dtt, $J_{30a'',30b''}$ = 9.9 Hz, $J_{29'',30b''}$ = 7.0 Hz, $^4J_{30b'',32''}$ = 2.7 Hz 1H, H-30b''), 1.69 (pentet, $J_{11'',12''}$ = $J_{12''13''}$ = 6.3 Hz, 2H, H-12''), 1.50 (pentet, $J_{2''',3'''}$ = $J_{3''',4'''}$ = 6.1 Hz, 2H, H-3'''), 1.39–1.35 (m, 2H, H-13''), 1.20–1.06 (m, 68H, H-14''–H-29'' and H-4'''–H-21'''), 0.77 (t, $J_{21''',22'''}$ = 6.6 Hz, H-22'''); ^{13}C NMR (125 MHz, C_5D_5N) δ193.0 (C-6''), 172.3 (C-1'''), 164.6 (C-1''), 162.2 (C-10''), 133.8 (C-5''), 131.7 (C-4''), 128.4 (C-2''), 128.3 (C-3''), 128.0 (C-8''), 127.3 (C-7''), 113.3 (C-9''), 94.6 (C-1 and C-1'), 83.7 (C-31''), 73.6 (C-3), 73.5 (C-3'), 72.0 (C-2/2'), 72.40 (C-2/2'), 70.70 (C-4), 70.60 (C-4'), 70.23 (C-5'), 70.16 (C-5), 68.7 (C-32'') 67.2 (C-11''), 64.3 (C-6), 62.9 (C-6'), 33.0 (C-2'''), 30.7, 28.62, 28.60, 28.54, 28.52, 28.51, 28.48, 28.38, 28.34, 28.25, 28.21, 28.16, 28.0, 27.96, 27.94, 27.58, 27.41, 24.87, 23.85, 21.54 (C-3'''–C21''' and C-12''–C-29''), 17.18 (C-30''), 12.9 (C-22'''); HRMS(ESI) *m/z* calcd. for $[C_{70}H_{112}O_{15}+Na]^+$:1215.7893, obsd.:1215.7897.

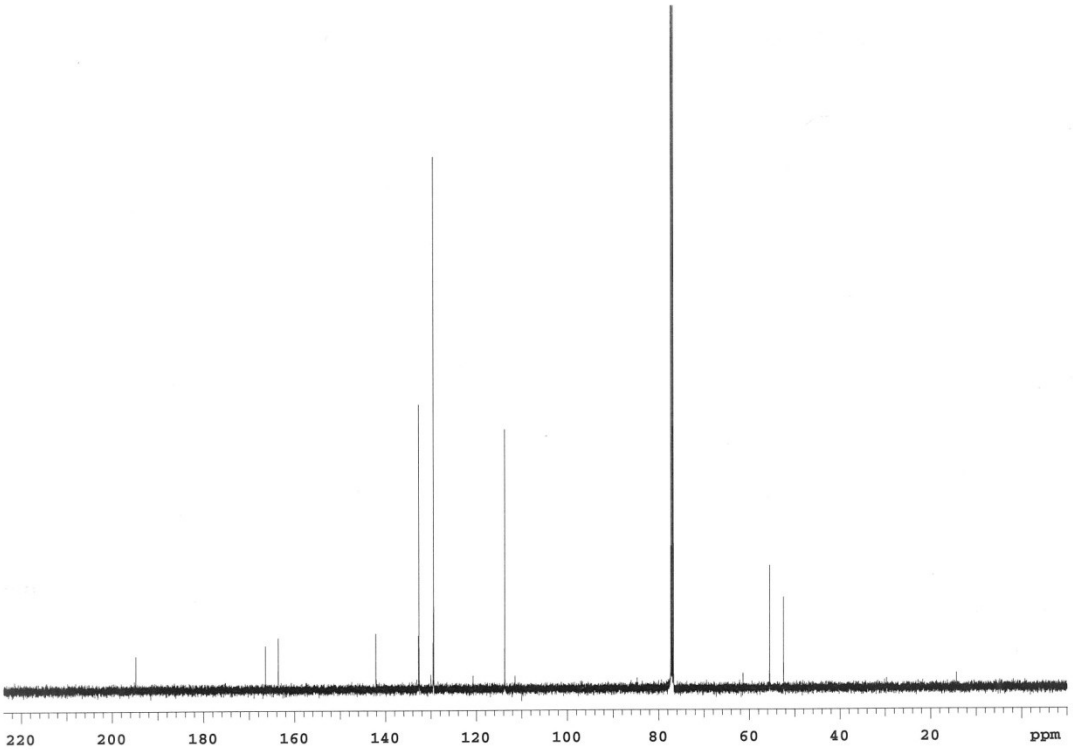
Methyl 4–(4-methoxybenzoyl)-benzoate (6)
^{1}H NMR (500 MHz, $CDCl_3$)



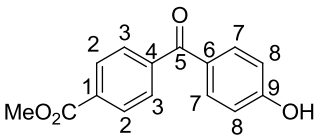


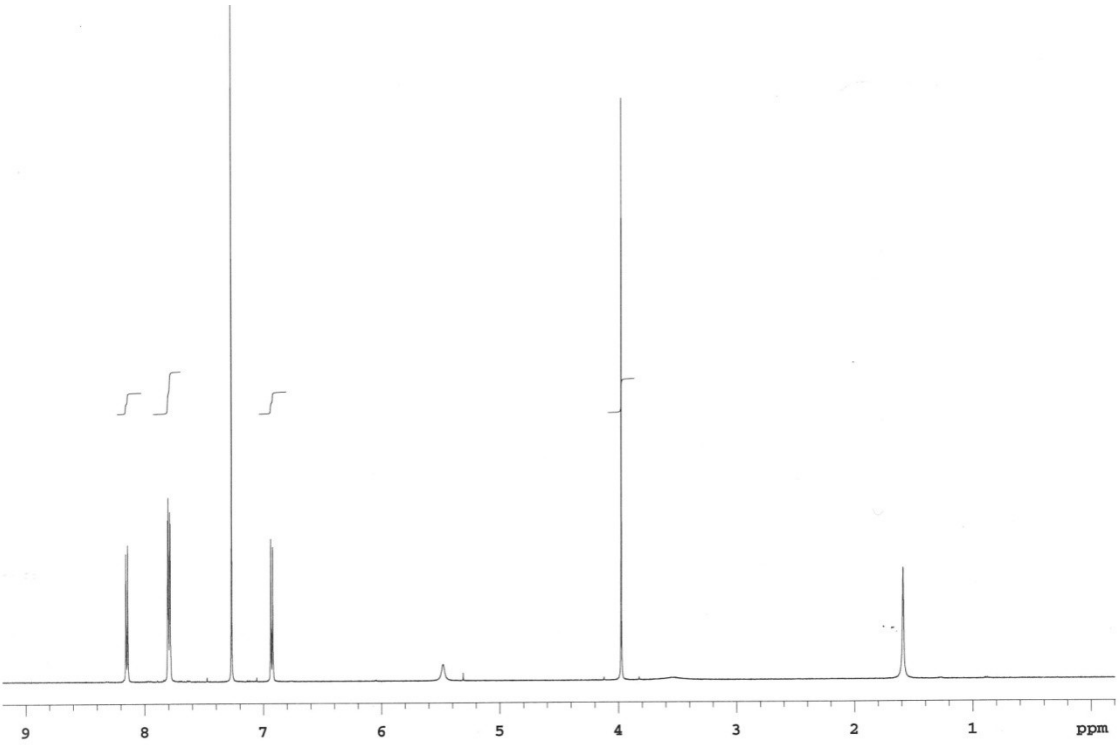
Methyl 4–(4-methoxybenzoyl)-benzoate (6)
^{13}C NMR (125 MHz, $CDCl_3$)



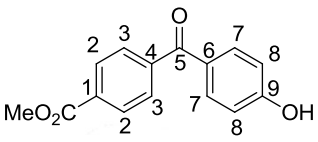


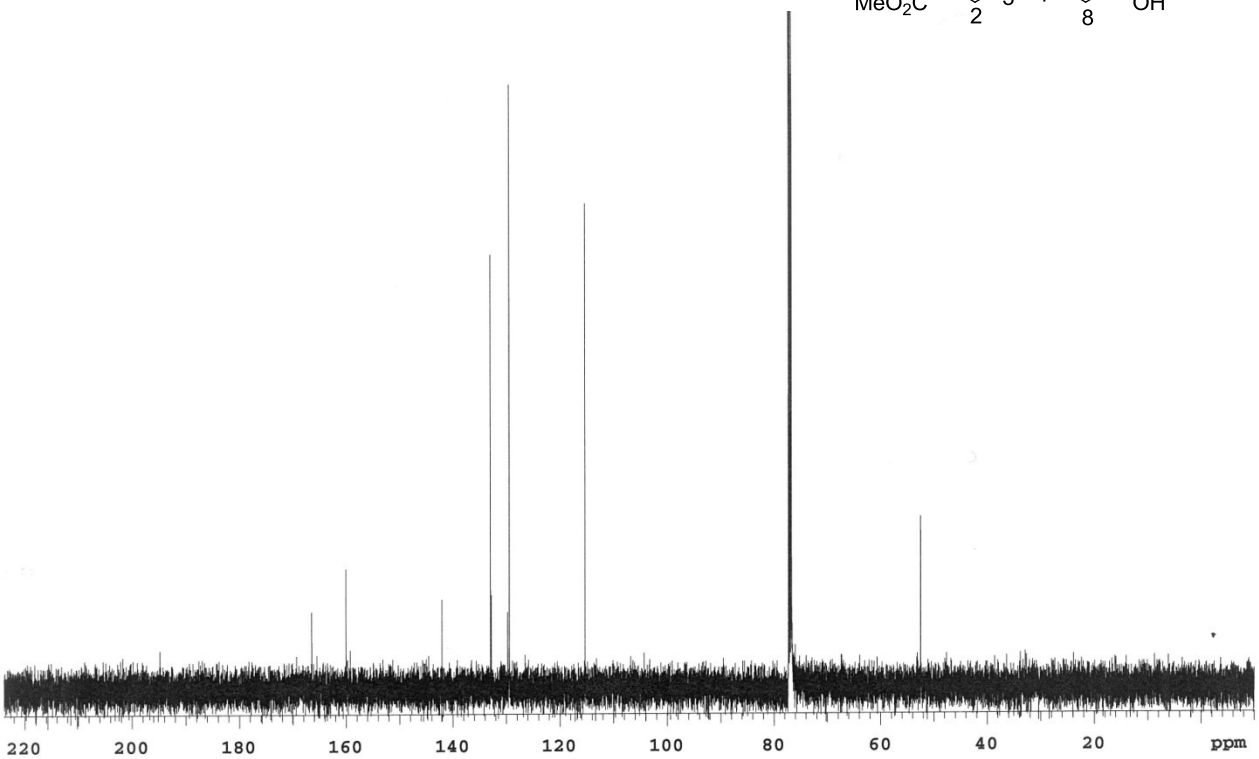
Methyl 4–(4-hydroxybenzoyl)-benzoate (7)
^{1}H NMR (500 MHz, $CDCl_3$)





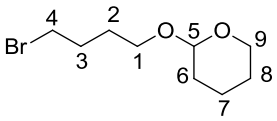
Methyl 4–(4-hydroxybenzoyl)-benzoate (7)
^{13}C NMR (125 MHz, $CDCl_3$)

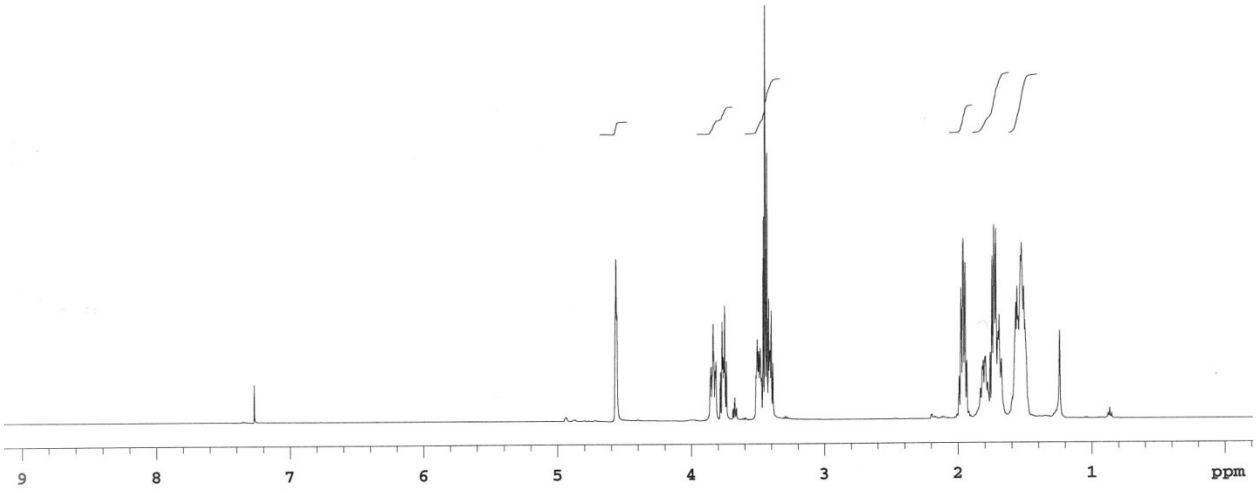




2-(4-Bromobutyloxyl)-tetrahydropyran (9)

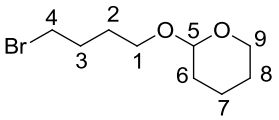
^{1}H NMR (500 MHz, $CDCl_3$)

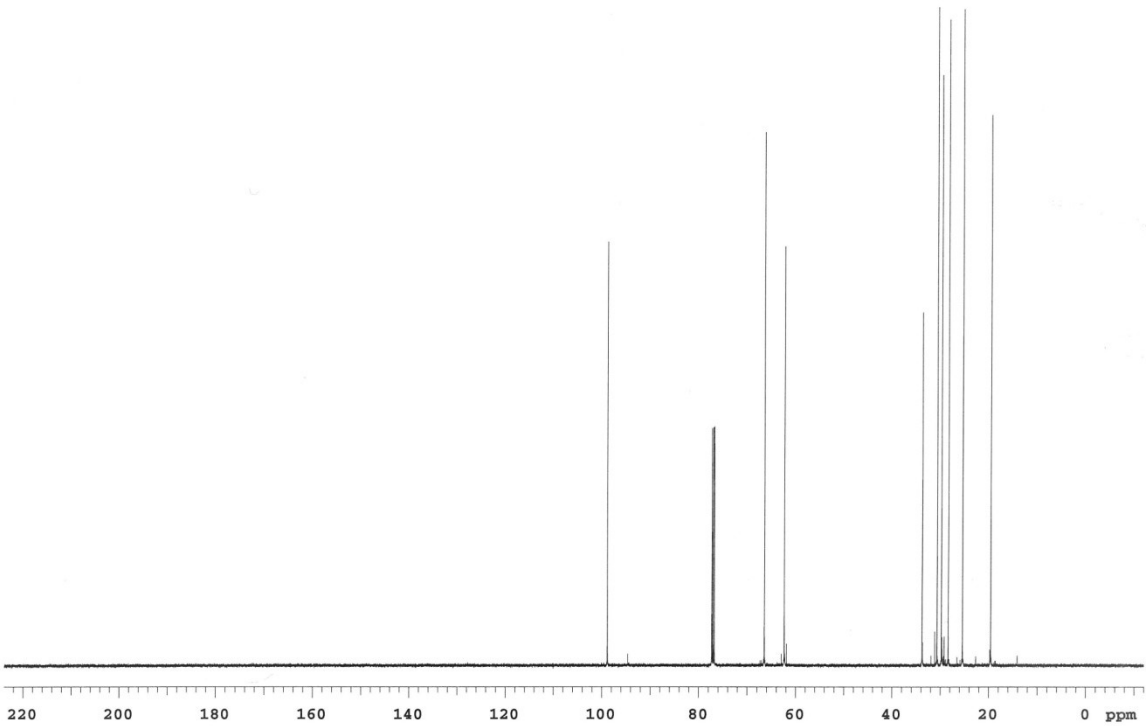




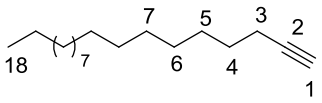
2-(4-Bromobutyloxyl)-tetrahydropyran (9)

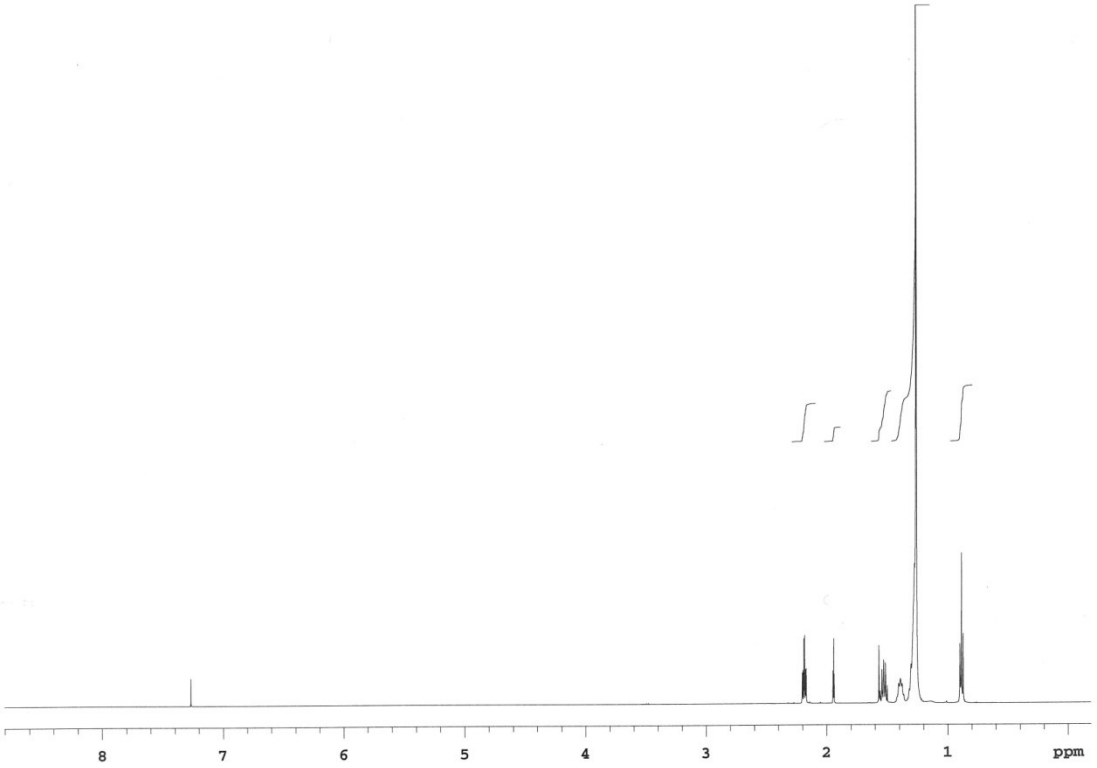
^{13}C NMR (125 MHz, $CDCl_3$)



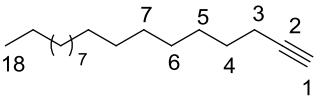


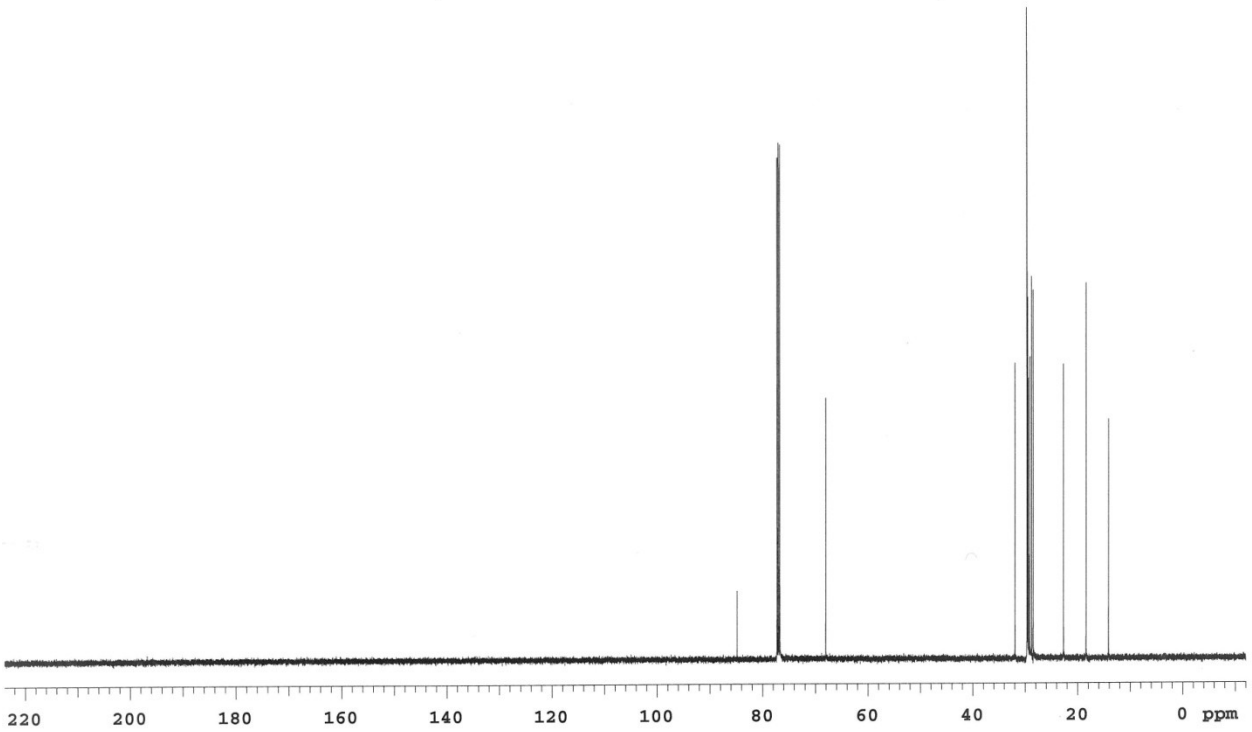
Octadecyne (11)
^{1}H NMR (500 MHz, $CDCl_3$)



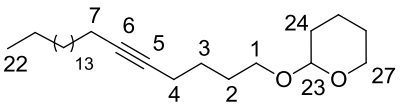


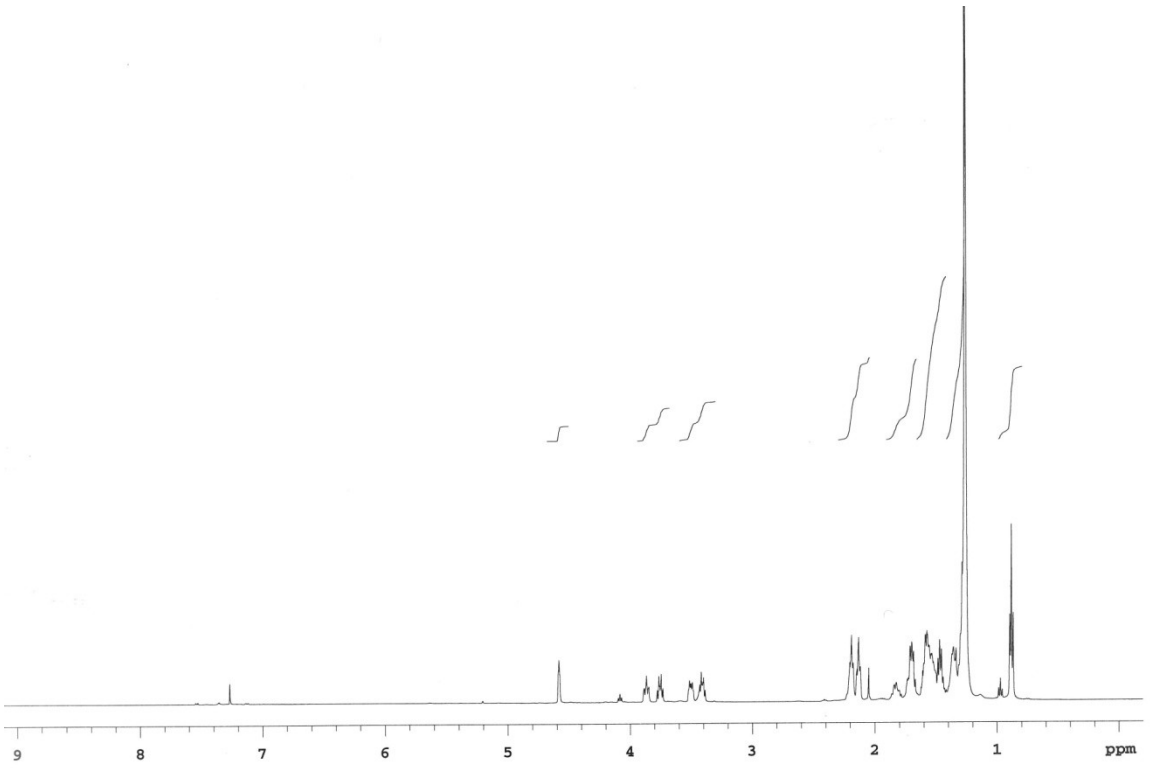
Octadecyne (11)
^{13}C NMR (125 MHz, $CDCl_3$)



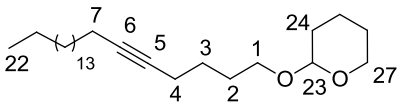


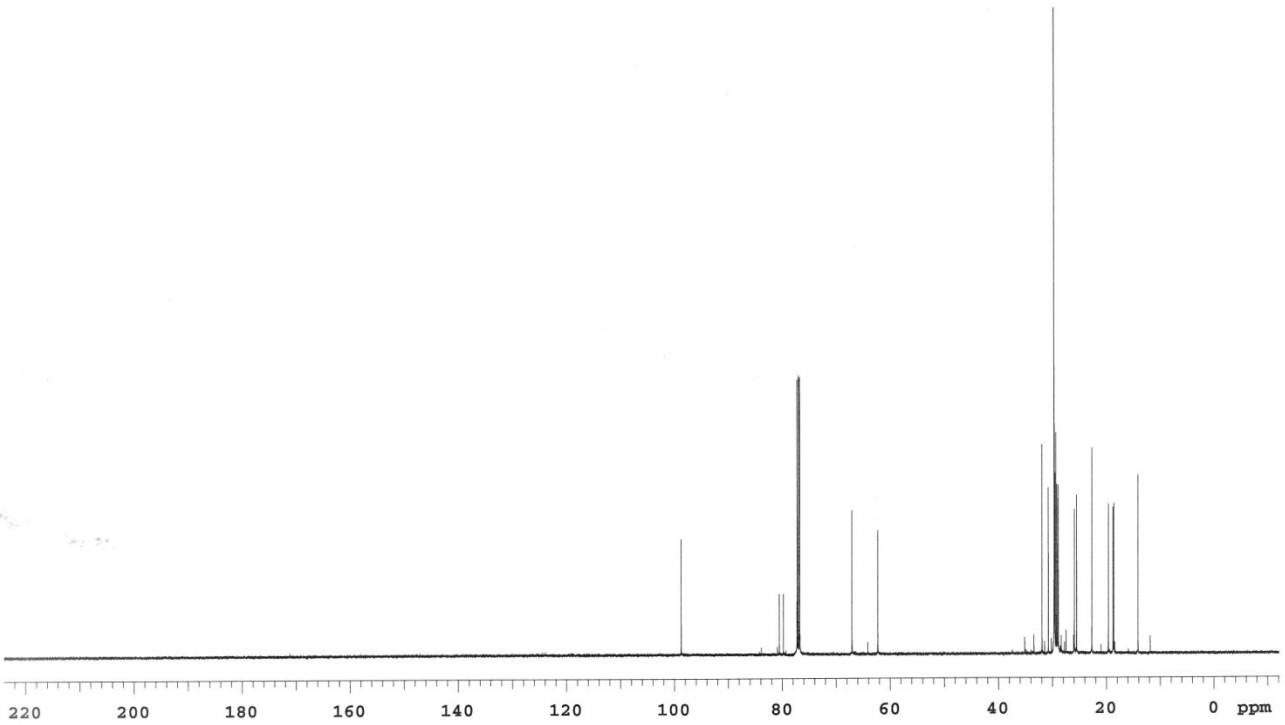
2-(5-Docosynyloxyl)-tetrahydropyran (12)
^{1}H NMR (500 MHz, $CDCl_3$)





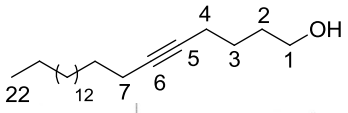
2-(5-Docosynyloxyl)-tetrahydropyran (12)
^{13}C NMR (125 MHz, $CDCl_3$)

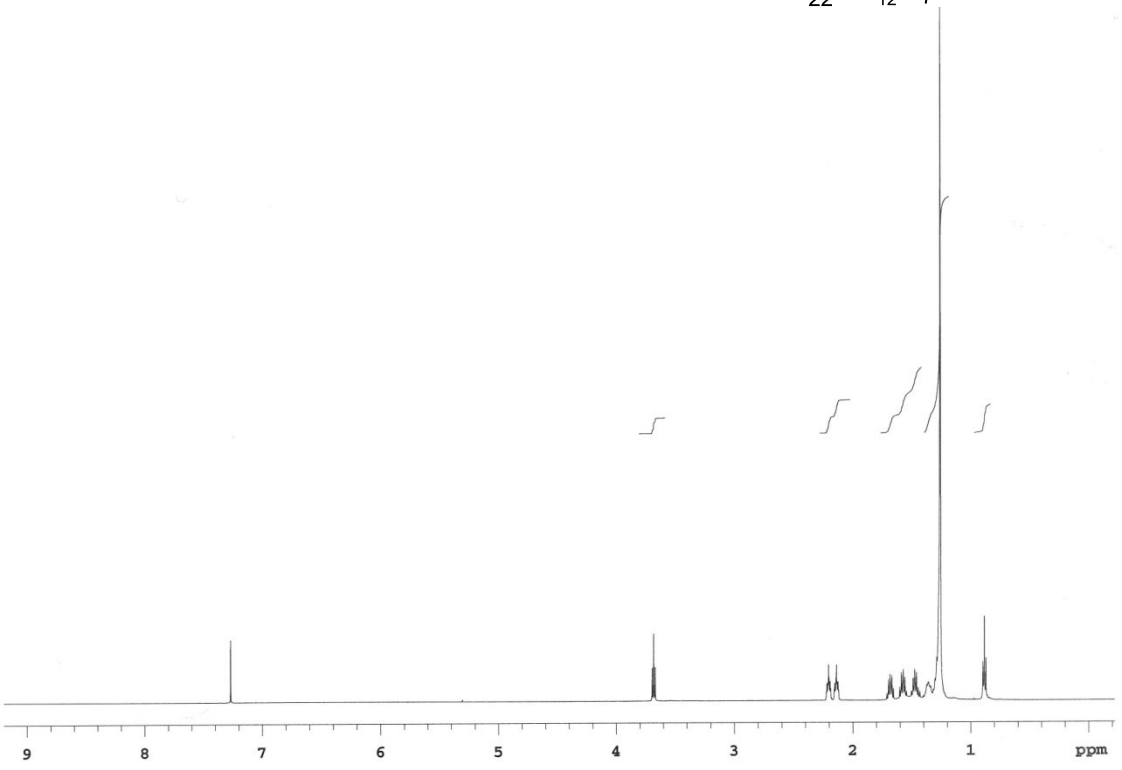




5-Docosyn-1-ol (13)

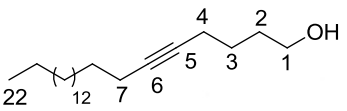
^{1}H NMR (500 MHz, $CDCl_3$)

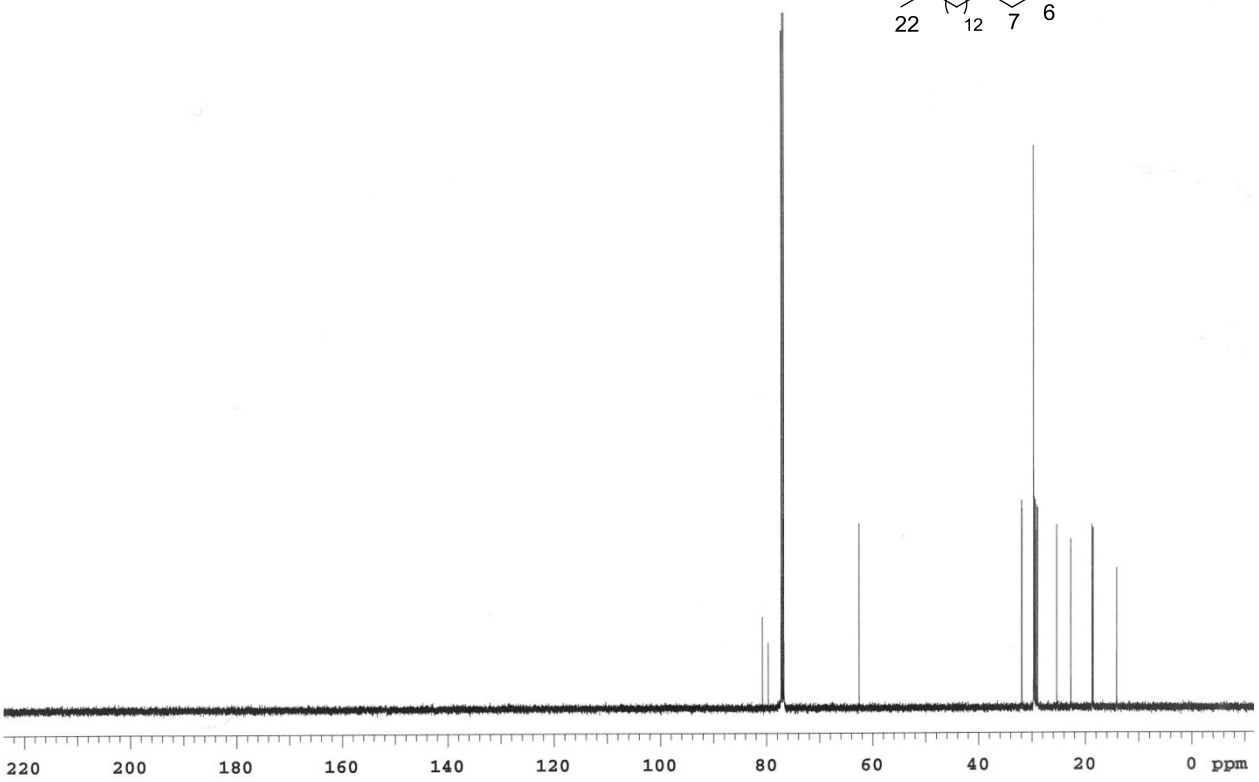




5-Docosyn-1-ol (13)

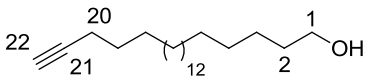
^{13}C NMR (125 MHz, $CDCl_3$)

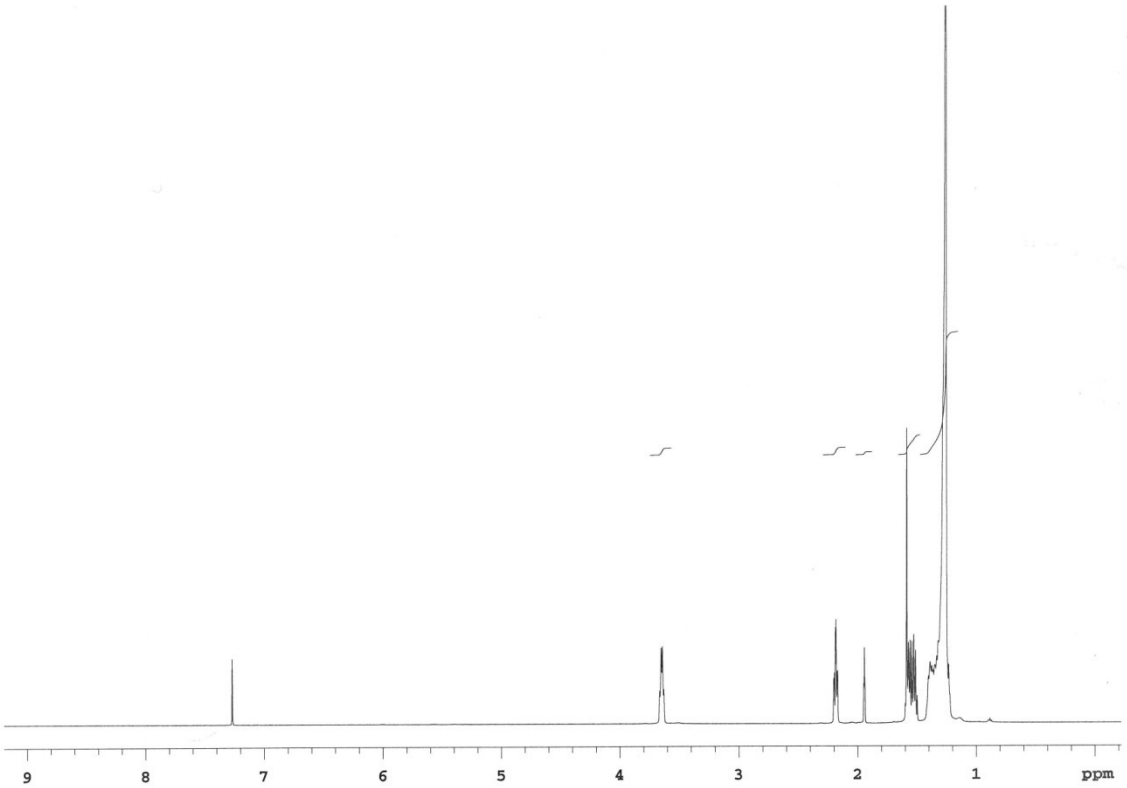




21-Docosyn-1-ol (14)

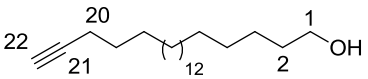
^{1}H NMR (500 MHz, $CDCl_3$)

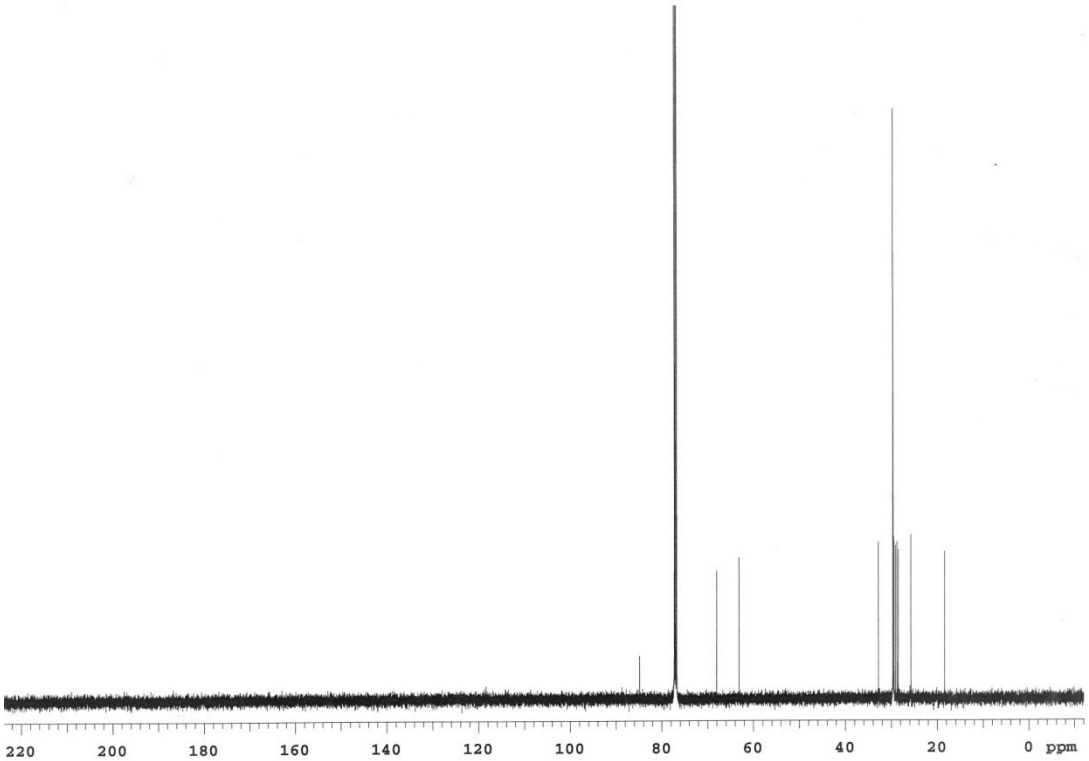




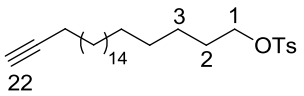
21-Docosyn-1-ol (14)

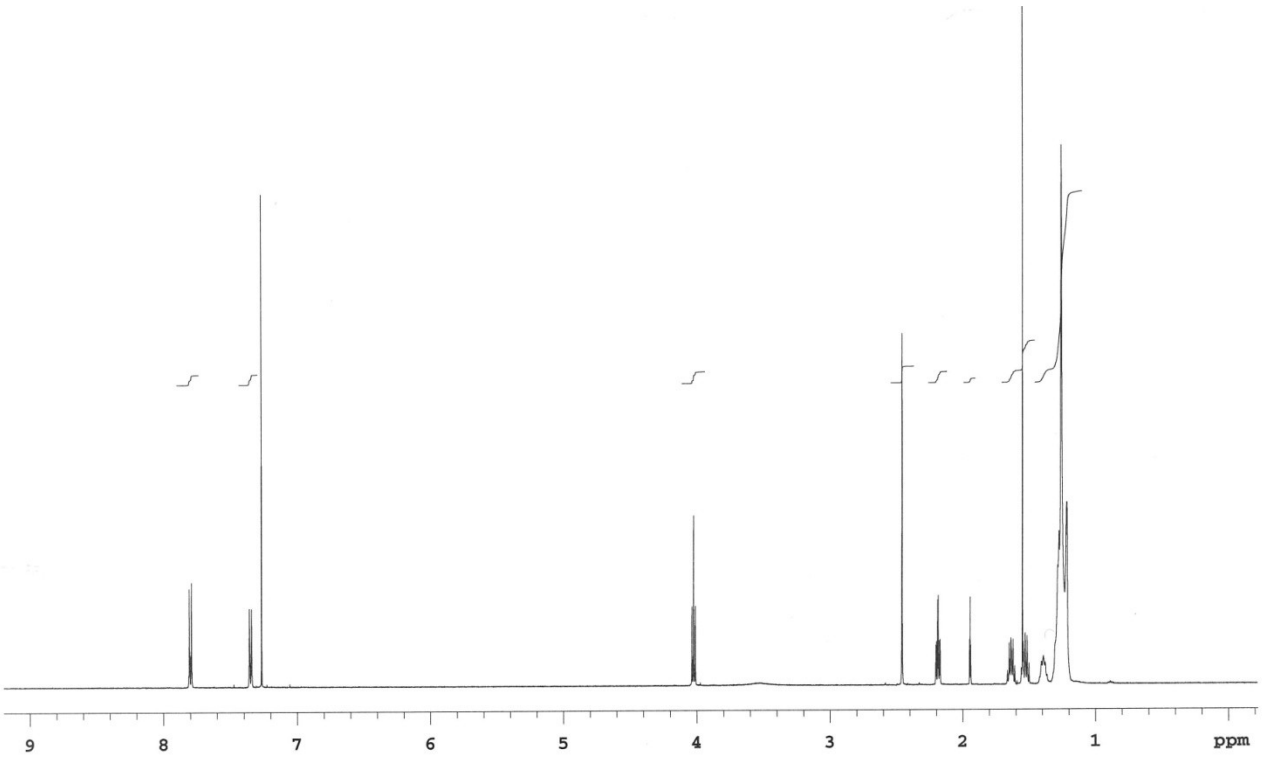
^{13}C NMR (125 MHz, $CDCl_3$)



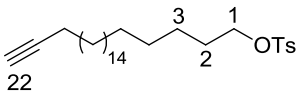


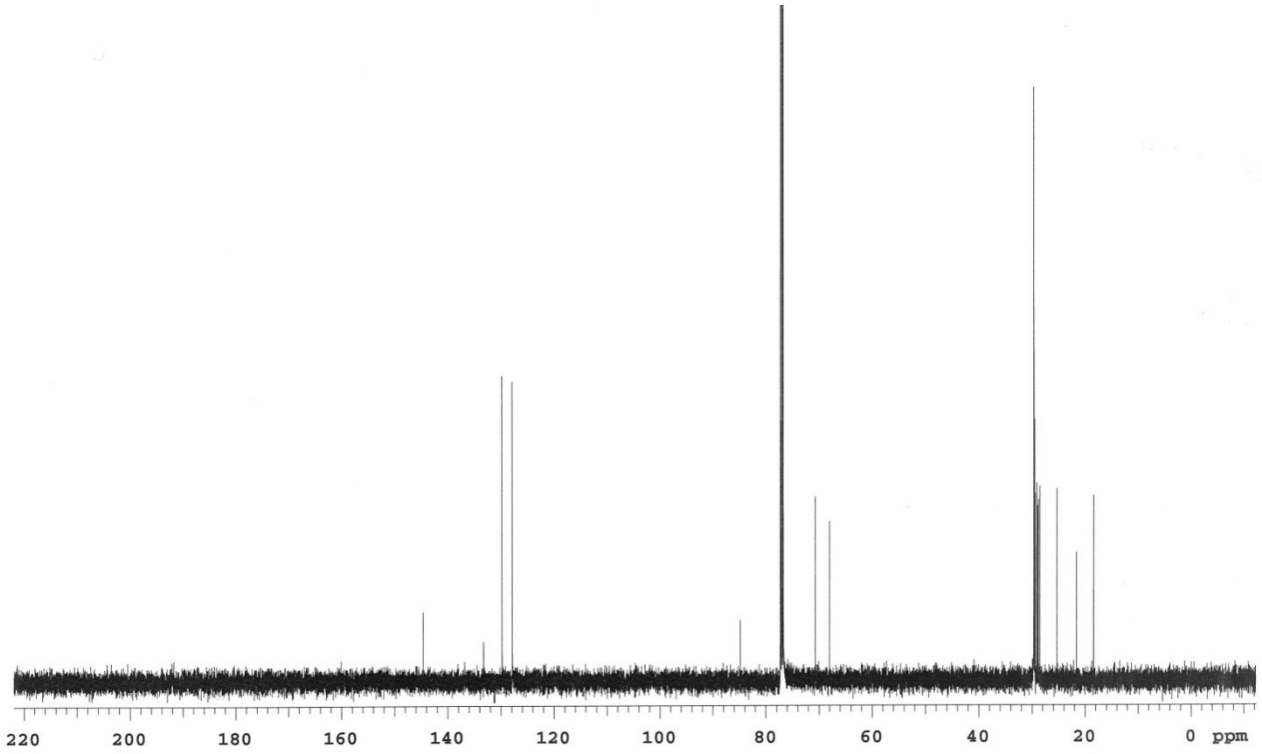
21-Docosynyl *p*-toluenesulfonate (17)
^{1}H NMR (500 MHz, $CDCl_3$)





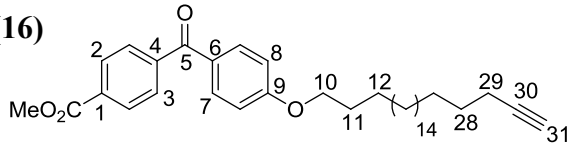
21-Docosynyl *p*-toluenesulfonate (17)
^{13}C NMR (125 MHz, $CDCl_3$)

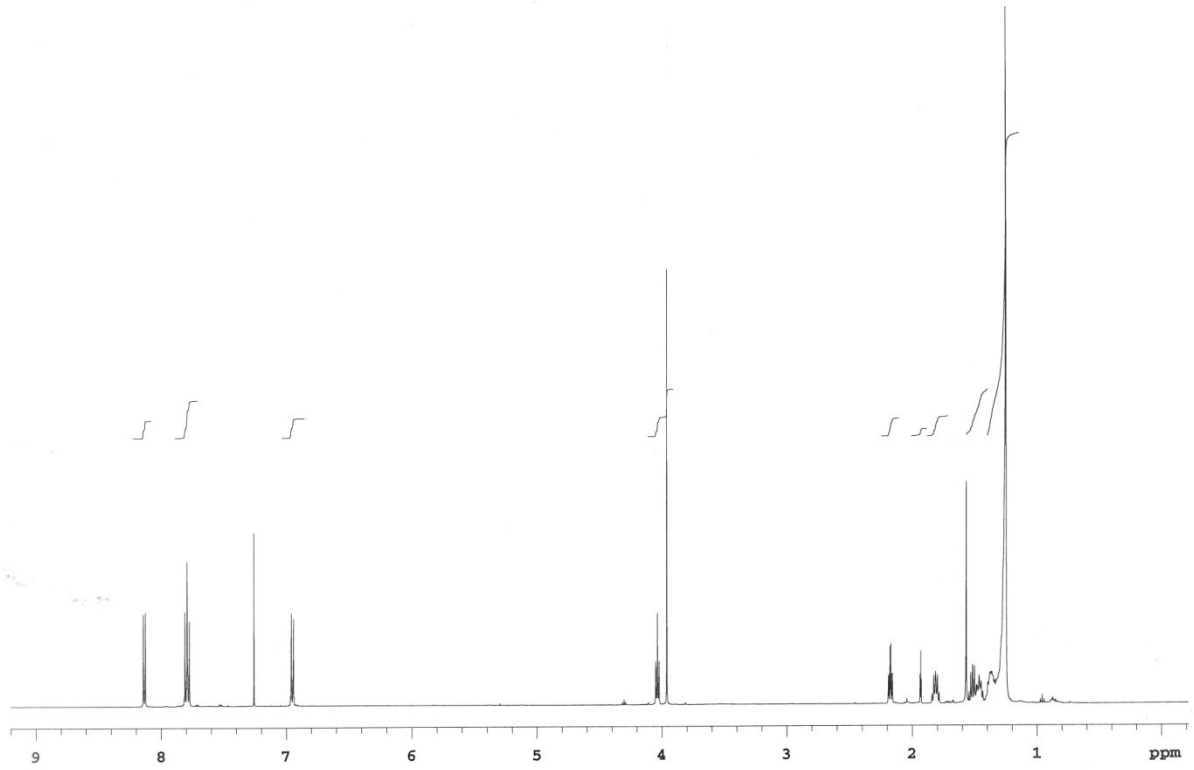




Methyl 4-[4-(21-docosynyloxy)benzoyl]-benzoate (16)

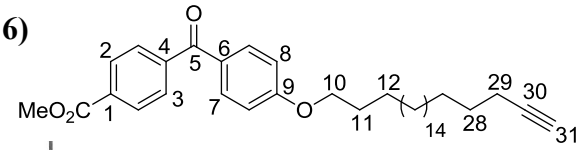
^{1}H NMR (500 MHz, $CDCl_3$)

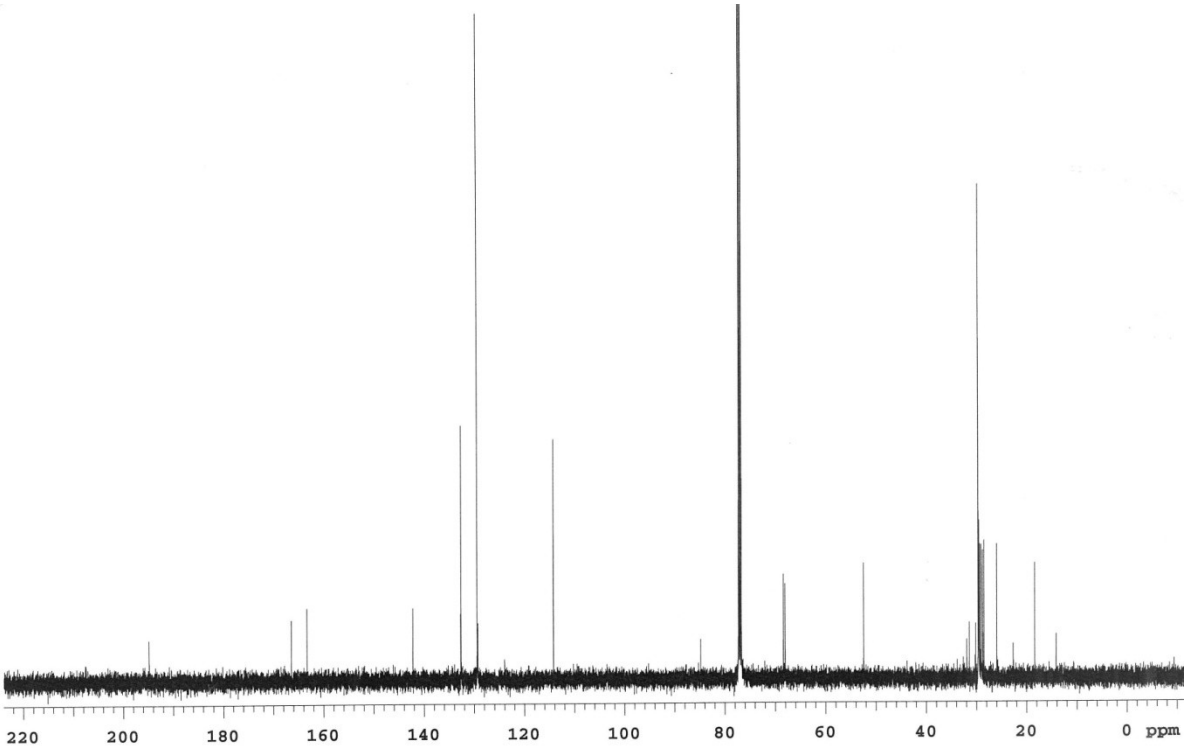




Methyl 4-[4-(21-docosynyloxy)benzoyl]-benzoate (16)

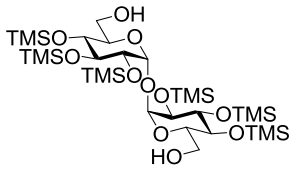
^{13}C NMR (125 MHz, $CDCl_3$)

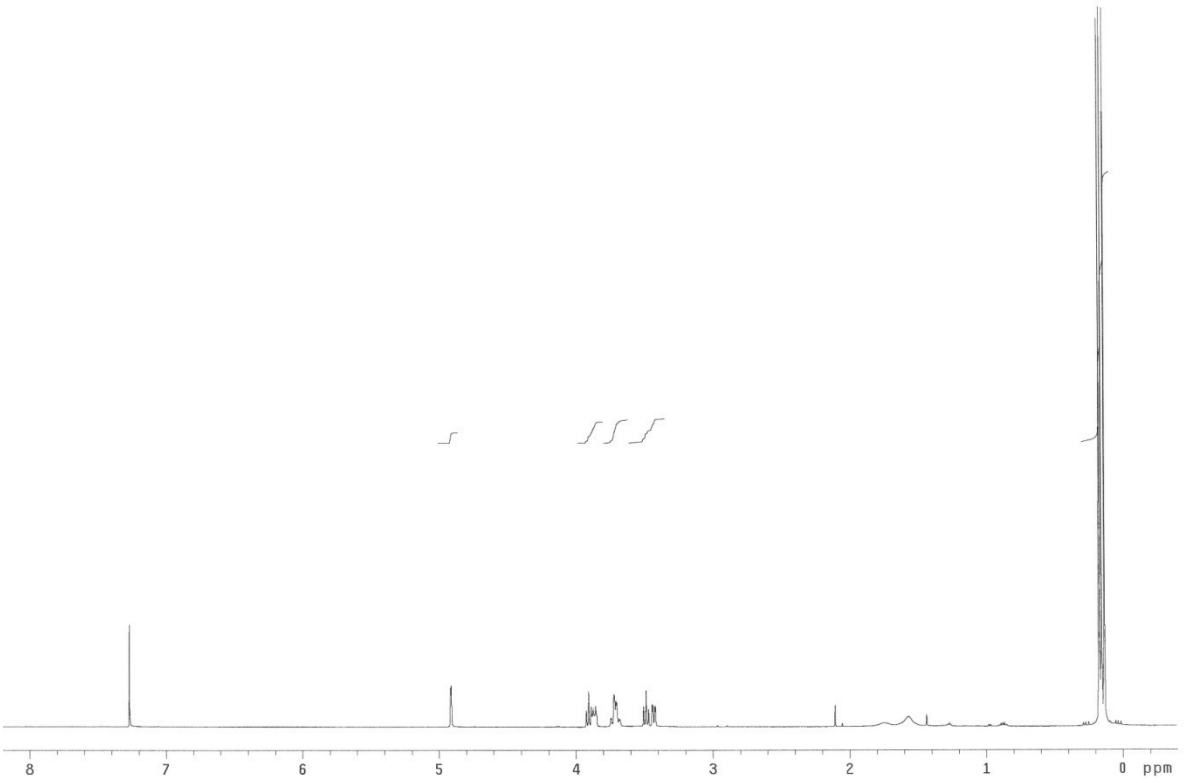




2,2',3,3',4,4'-Hexa-*O*-trimethylsilyl-α,α'-D-trehalose (19)

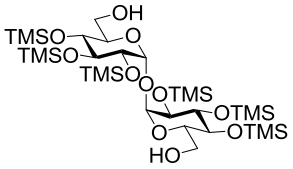
^{1}H NMR (500 MHz, CD_3OD)

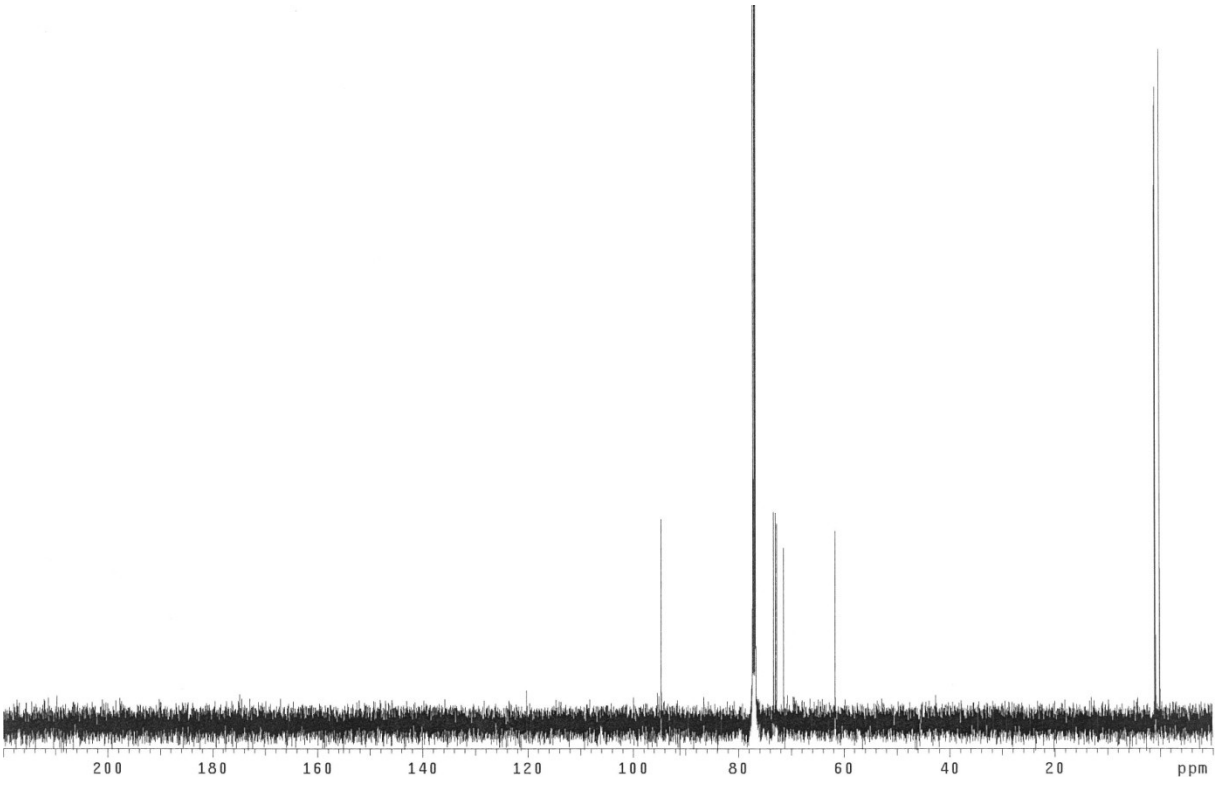




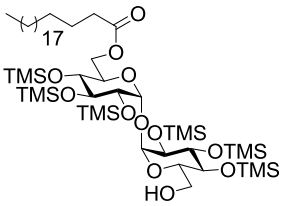
2,2',3,3',4,4'-Hexa-*O*-trimethylsilyl-α,α'-D-trehalose (19)

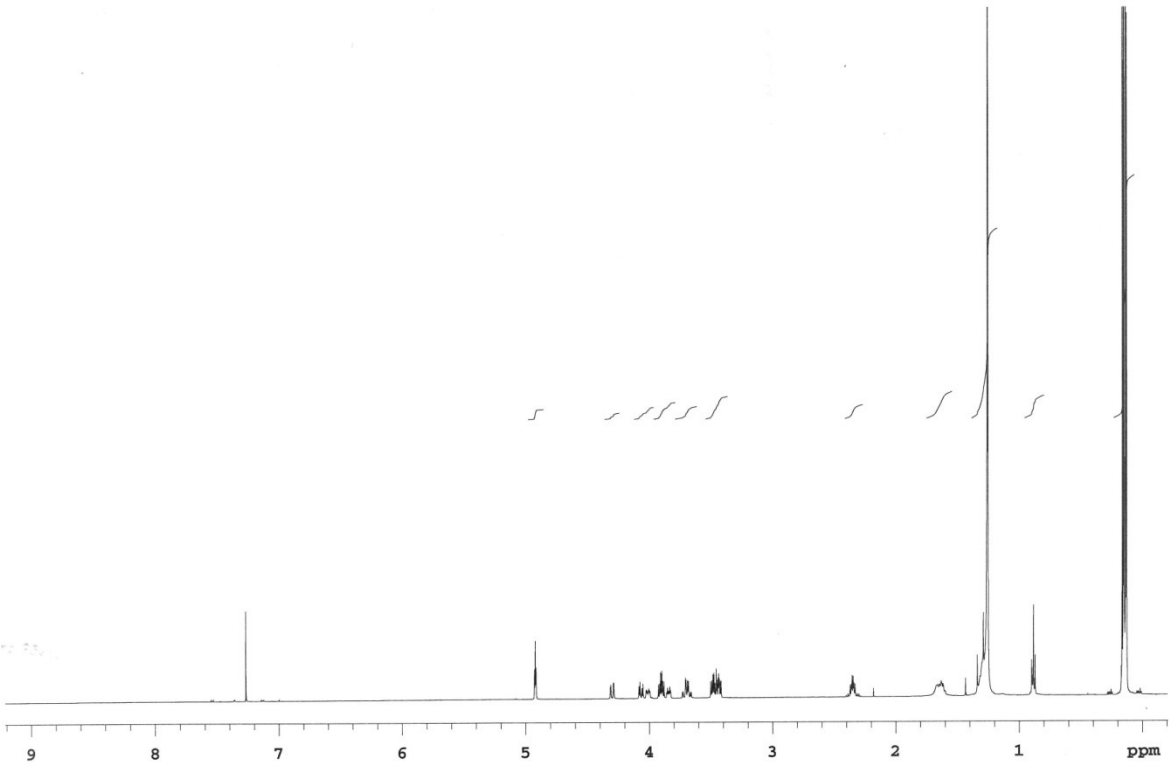
^{13}C NMR (125 MHz, CD_3OD)



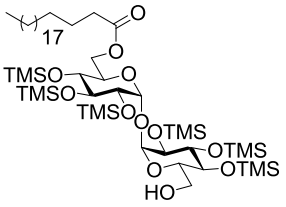


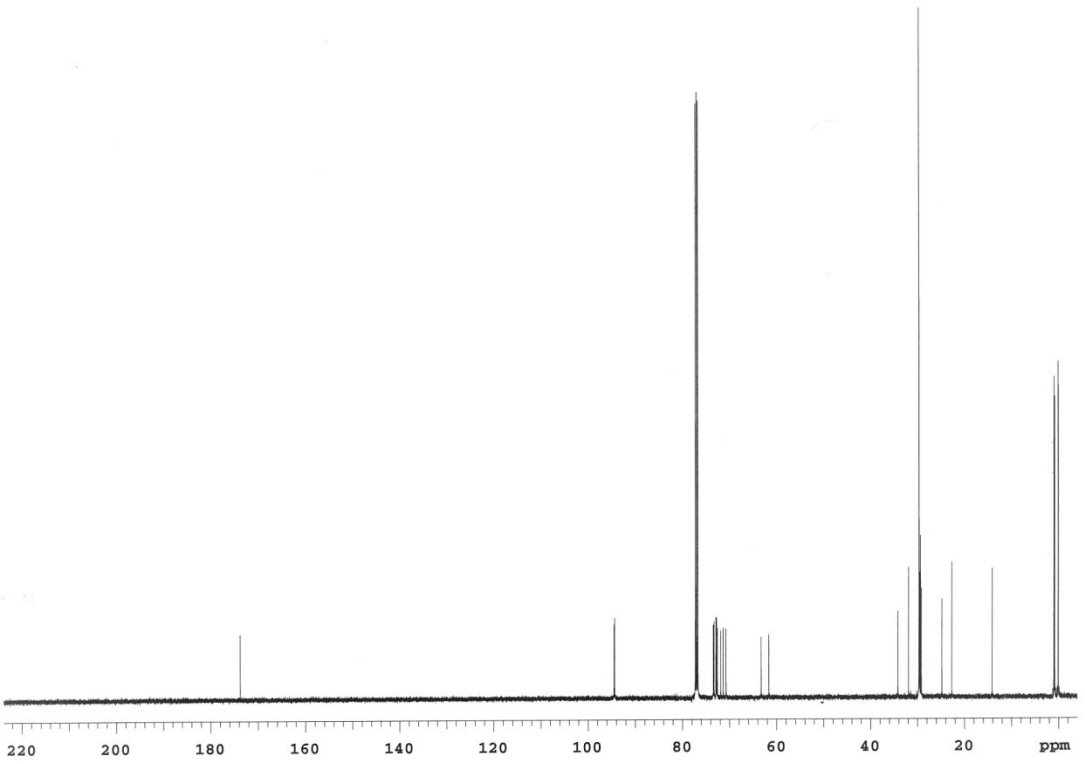
6-*O*-Docosanoyl -2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (20).
^{1}H NMR (500 MHz, $CDCl_3$)





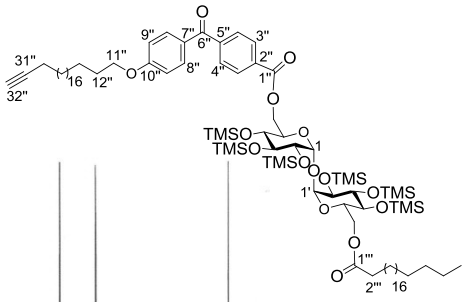
6-*O*-Docosanoyl -2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (20).
^{13}C NMR (125 MHz, CD_3OD)

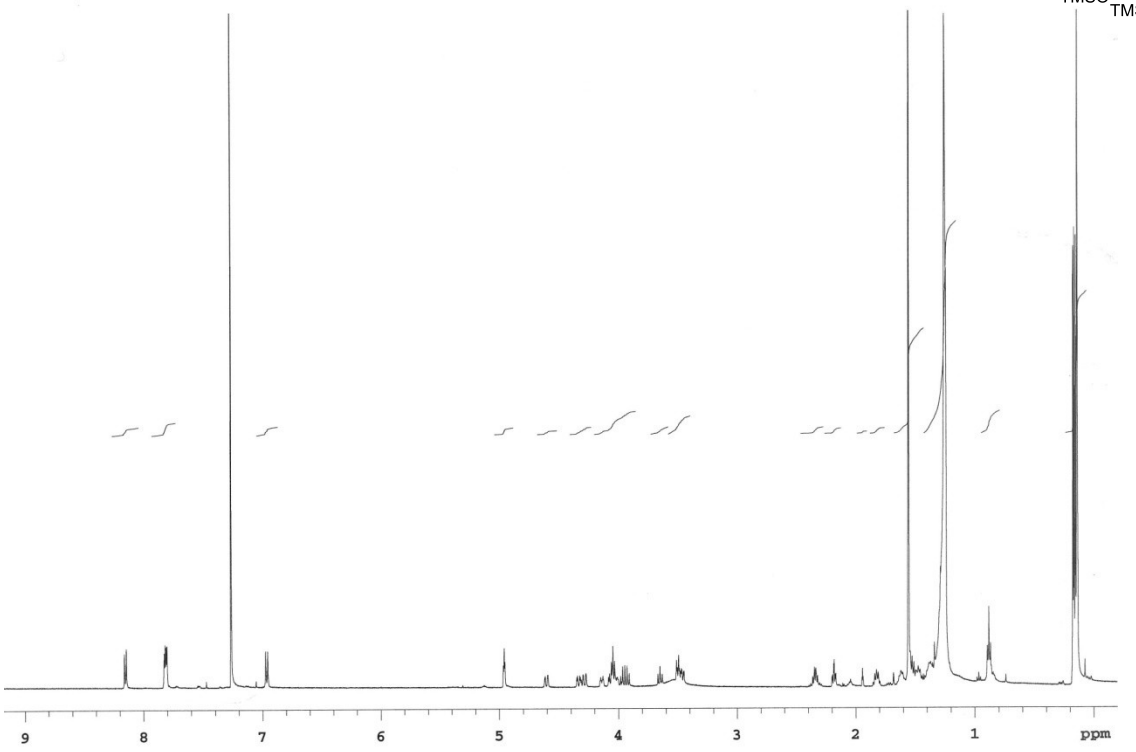




6'-*O*-docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (22).

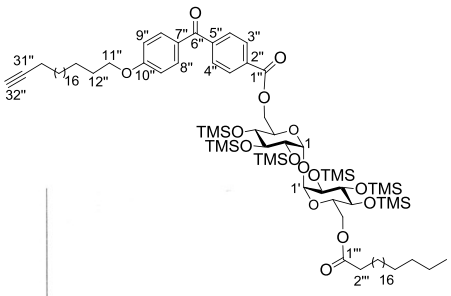
^{1}H NMR (500 MHz, $CDCl_3$)

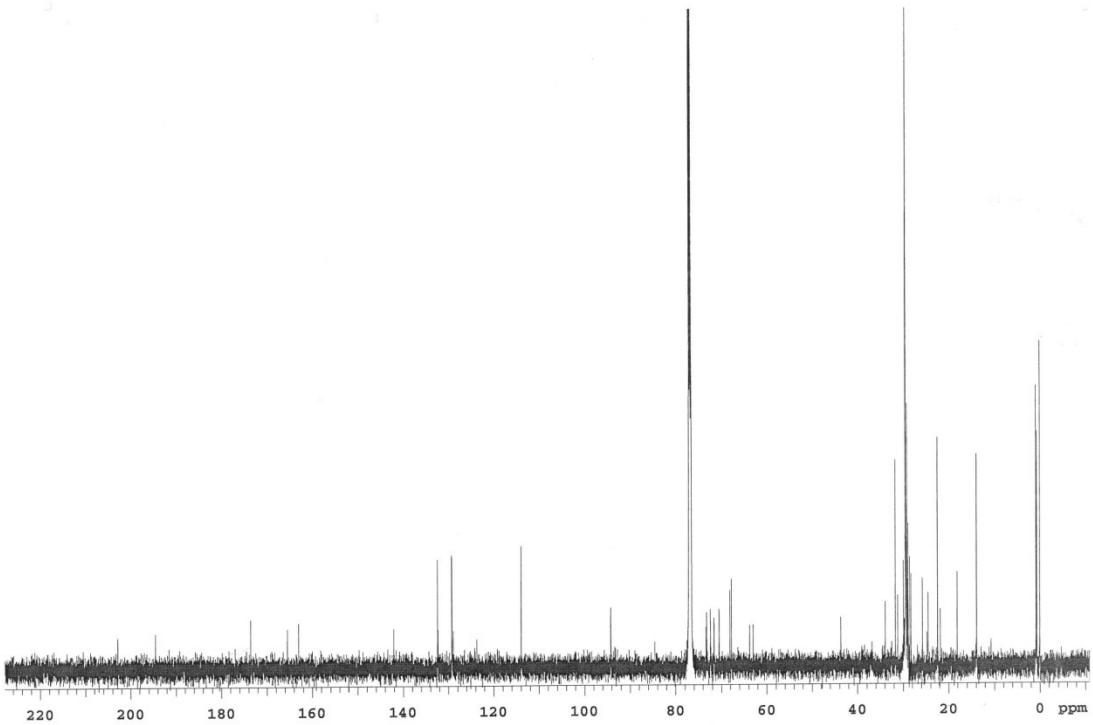




6'-*O*-docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α'-D-trehalose (22).

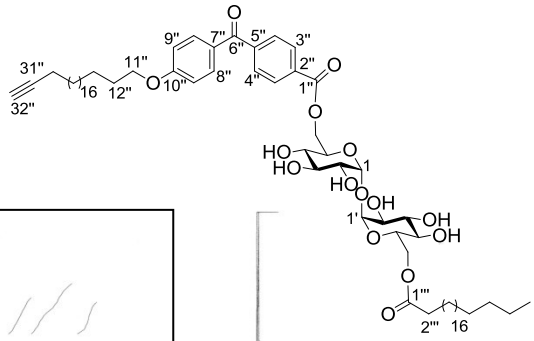
^{13}C NMR (125 MHz, $CDCl_3$)

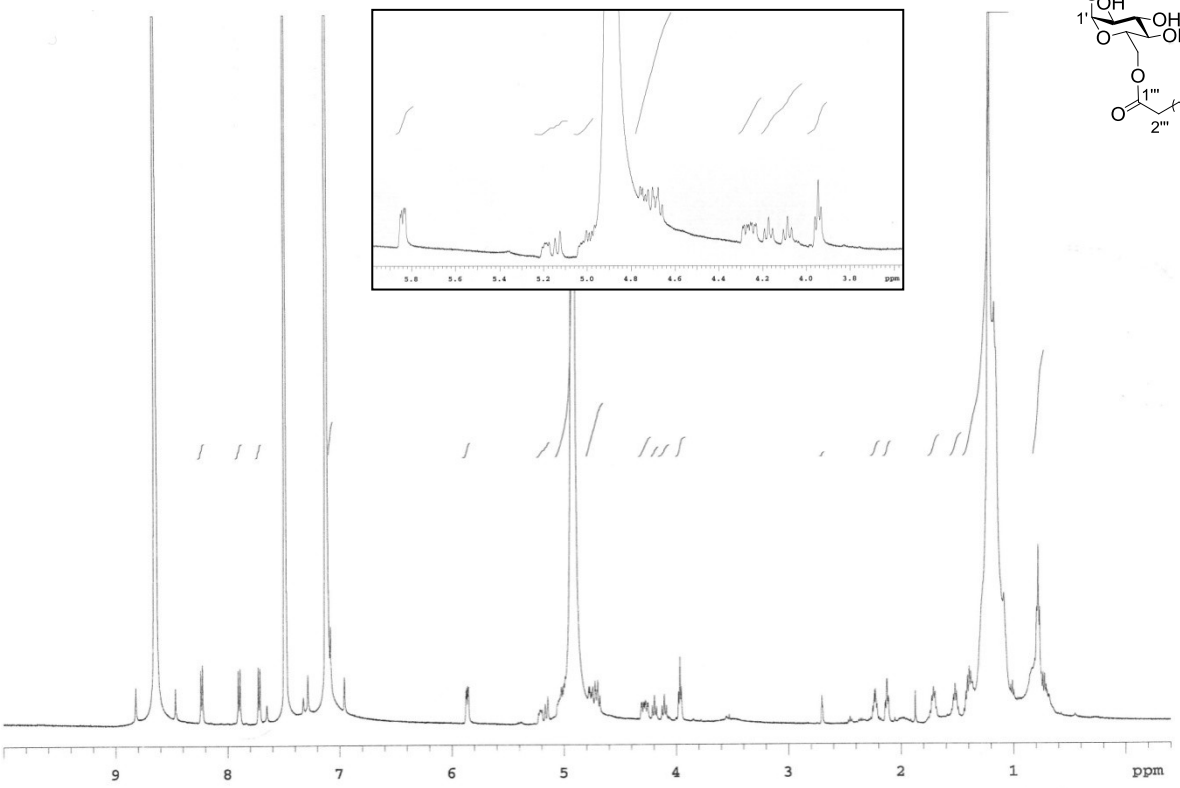




6'-*O*-Docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-α,α-D-trehalose (3)

^{1}H NMR (500 MHz, C_5D_5N)





6'-*O*-Docosanoyl-6-*O*-4-[4-(21-docosynyloxy)benzoyl]benzoyl-α,α-D-trehalose (3)

^{13}C NMR (125 MHz, C_5D_5N)