Dynamic Amphiphile Libraries to Screen for the "Fragrant" Delivery of siRNA into HeLa Cells and Human Primary Fibroblasts

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Supporting Information

Table of Content

1. Materials and Methods

As in refs. S1-S4, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka, Sigma and Aldrich and used as they were received. Commercially available aldehydes, oleyl alcohol, 1-octadecanololeyl, 1-hexadecanol, linoleic alcohol, palmitoleic alcohol and petroselinyl alcohol were all from Sigma; *cis*-1,3-docosenol, ethylene glycol monododecyl ether and DIEA were respectively from Bachem, TCI Europe and Fluka. A PCC oxidation protocol[S5] was used to prepare non-commercial aliphatic aldehydes **T16**,[S6] **T17**,[S7] **T18**,[S8] **T19**,[S9] **T20**,[S10] **T21**,[S11] **T23**[S12] and **T24**[S13] starting from the corresponding commercial alcohol. Aldehydes **T35, T36**, **T40, T47** and **T48** were gifts from Firmenich, Geneva.

All reactions were performed under N_2 atmosphere. Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical thin layer chromatography (TLC) was performed in silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1000 μm), respectively. The filtration was performed using florisil (fluka, 200-300 mesh). $[\alpha]_D^{20}$ values were recorded on a Jasco P-1030 Polarimeter (reported for concentrations c in g / 100 ml solvent), melting points (Mp) on a heating table from Reichert (Austria). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, unless stated otherwise) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), b (broad). ^1H and ^{13}C NMR spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (*J*) given in Hz, or multiplet (m). ^1H

and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). ESI-MS for the characterization of new compounds was performed on a Finnigan MAT SSQ 7000 instrument or a ESI API 150EX and are reported as mass-per-charge ratio *m/z* (intensity in %, [assignment]). Accurate mass determinations using ESI (HR ESI-MS) were performed on a Sciex QSTAR Pulsar mass spectrometer. Fluorescence measurements were obtained using an Infinite 500 plate-reading fluorimeter (Tecan, Switzerland) from the Biomolecular Screening Facility (EPFL, Switzerland) and a SynergyMX plate-reading fluorimeter (BioTek, USA), usage courtesy of Prof. Thierry Soldati. Absorbance measurements were obtained using a Spectra Max Plus microplate reader (MolecularDevices, USA) at the Biomolecular Screening Facility (BSF, EPFL, Switzerland). The ζ potentials and complex sizes were measured with Zetasizer 2000 (Malervn, Ltd.).

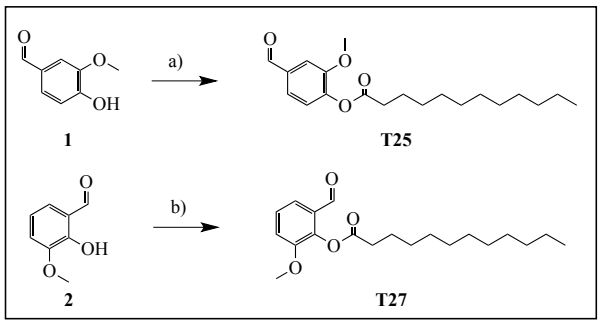
2. Abbreviations

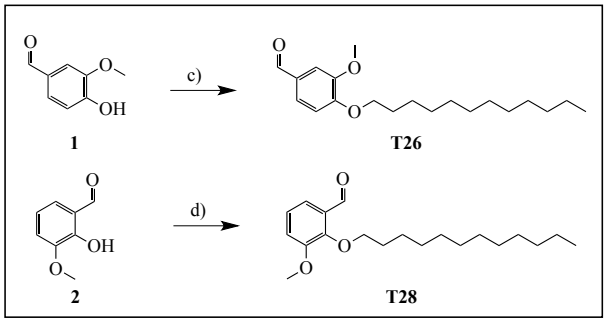
AcOEt: Ethyl Acetate; AcOH: Acetic Acid; Calc: Calculated; Cbz: (Benzyloxy)carbonyl; DCM: Dichloromethane; DIEA: *N*-Ethyl-*N*,*N*-diisopropyl amine; DMF: Dimethylformamide; DMSO: Dimethylsulfoxide; Et_3N: Triethylamine ; Et_2O: Diethyl Ether; h: hour; HCl: Hydrochloric acid; HR-MS (ESI): High resolution mass spectrometry (electrospray ionization); IR: Infrared; MeOH: Methanol; min: minutes; PCC: Pyridinium chlorochromate; rt: Room temperature; THF: Tetrahydrofuran, TsCl: 4-Toluenesulfonyl chloride.

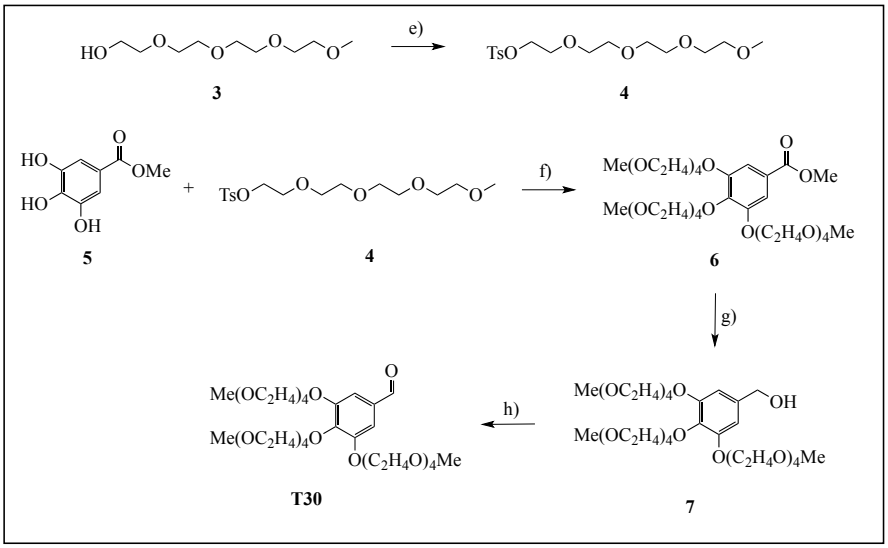
3. Synthesis

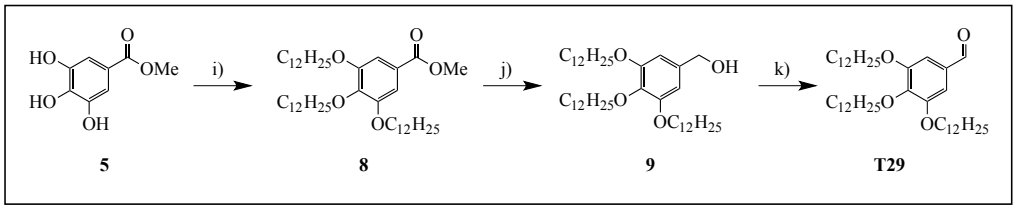
Synthesis of Reactive Counterions (Heads). Synthesis of reactive counterions (heads) was described elsewhere[S4]: For **G1H1**, **G1H2**, **G1H3**, **A1H1**, **A1H2** and **A1H3**, see ref S4a. For **G1H4** and **A1H4**, see ref. S4b. For **G1H6**, see ref. S4c. For **G1O1**, **G1O2**, **G1O3**, **G1O4**, **G2H4**, **A2H4**, **G1S2H2** and **G1S3H3**, see S4d.

T25.[S14] A solution of dodecanoyl chloride (719 mg, 3.29 mmol) in DCM (5 ml) was added to a solution of vanillin (**1**) (500 mg, 3.29 mmol) and Et_3N (458 µl, 3.29 mmol) in DCM (5 ml) at rt under Ar atmosphere. The reaction mixture was stirred at rt for 3 h and then quenched with water. The product was extracted with DCM (3x15 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (DCM/MeOH 99:1, R_f 0.82) to afford **T25** (923 mg, 84%) as a colorless solid. Mp: 46-47 ºC; IR (neat): 2872 (m), 1718 (m), 1586 (m), 1431 (m), 1333 (m), 1245 (m), 1214 (m), 1098 (s), 926 (m), 852 (m), 766 (m), 730 (m), 668 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 9.95 (s, 1H), 7.5-7.4 (m, 2H), 7.21 (d, ^3J(H,H) = 7.9 Hz, 1H), 3.90 (s, 3H), 2.61 (t, ^3J(H,H) = 7.5 Hz, 2H), 1.80-1.70 (m, 2H), 1.5-1.4 (m, 2H), 1.3-1.2 (m, 14H), 0.89 (t, ^3J(H,H) = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 191.3 (d), 171.5 (s), 152.2 (s), 145.3 (s), 135.4 (s), 125.0 (d), 123.7 (d), 111.0 (d), 56.3 (q), 34.3 (t), 32.1 (t), 29.8 (2x, t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 25.2 (t), 22.9 (t), 14.3 (q); MS (ESI, MeOH): 352 (100, $[M+NH_4]^+$), 335 (67, $[M+H]^+$); HR-MS (ESI): Calcd for $C_{20}H_{31}O_4$: 335.2216; found: 335.2226.









Scheme S1. Synthesis of tails **T25-T30**. a) dodecanoyl chloride, Et_3N, DCM, rt, 84%; b) dodecanoyl chloride, Et_3N, DCM, rt, 74%; c) 1-bromodecane, K_2CO_3, DMF, rt, 87%; d) 1-bromodecane, K_2CO_3, DMF, rt, 74%; e) NaOH, TsCl, THF/H_2O, 0 ^{o}C $\rightarrow$ rt, 94%; f) K_2CO_3, DMF, 90 ^{o}C, 59%; g) $LiAlH_4$, THF, rt, 96%; h) PCC, DCM, rt, 48%. i) 1-bromodecane, K_2CO_3, DMF, 70 ^{o}C, 87%; j) $LiAlH_4$, THF, rt, 63%; k) PCC, DMC, rt, 41%.

T26. K_2CO_3 (1045 mg, 7.59 mmol) was added to a solution of vanillin (**1**) (500 mg, 3.29 mmol) in DMF (5 ml) at rt under N_2 atmosphere. The reaction mixture was stirred at rt for 30 min and a solution of 1-bromodecane (1229 mg, 4.93 mmol) in DMF (5 ml) was added dropwise. The reaction mixture was stirred at rt for 3 h and then quenched with water. The product was extracted with DCM (3x15 ml) and washed with H_2O (10 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (DCM/MeOH 99:1, R_f 0.91) to afford **T26** (595 mg, 87%) as a colorless solid. Spectroscopic data match those reported in the literature.[S15]

T27. A solution of dodecanoyl chloride (719 mg, 3.29 mmol) in DCM (5 ml) was added to a solution of *o*-vanillin (**2**) (500 mg, 3.29 mmol) and Et_3N (458 μL, 3.29 mmol) in DCM (5 ml) at rt under N_2 atmosphere. The reaction mixture was stirred at rt for 4 h and then quenched with water. The product was extracted with DCM (3x15 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (DCM/MeOH 99:1, R_f 0.93) to afford **T27** (813 mg, 74%) as a colorless solid. Mp: 50-52 ºC; IR (neat): 2978 (w), 2916 (m), 2850 (m), 2758 (w), 1766 (s), 1698 (m), 1583 (m), 1381 (w), 1315 (w), 1274 (m), 1247 (m), 1198 (m), 1178 (m), 1125 (m), 1107 (m), 1082 (w), 1061 (m), 997 (w), 916 (m), 827 (w), 790 (m), 756 (w), 717 (m), 689 (w), 649 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 10.14 (s, 1H), 7.47 (dd, ^3J(H,H) = 7.8 Hz, ^4J(H,H) = 1.5 Hz, 1H), 7.33 (br t, ^3J(H,H) = 8.0 Hz, 1H), 7.21 (dd, ^3J(H,H) = 8.2 Hz, ^3J(H,H) = 1.5 Hz, 1H), 3.87 (s, 3H), 2.68 (t, ^3J(H,H) = 7.5 Hz, 2H), 1.81 (q, ^3J(H,H) = 7.6 Hz, 2H), 1.5-1.18 (m, 16H), 0.89 (t, ^3J(H,H) = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 188.9 (d), 171.8 (s), 152.0 (s), 142.2 (s), 129.5 (s), 126.9 (d), 121.1 (d), 118.0 (d), 56.5 (q),

34.1 (t), 32.1 (t), 29.8 (2x, t), 29.7 (t), 29.6 (t), 29.5 (t), 29.3 (t), 25.1 (t), 22.9 (t), 14.4 (q); MS (ESI, CH_3CN): 352 (100, $[M+NH_4]^+$), 335 (67, $[M+H]^+$); HR-MS (ESI): Calcd for $C_{20}H_{31}O_4$: 335.2216; found: 335.2215.

T28. K_2CO_3 (1045 mg, 7.59 mmol) was added to a solution of *o*-vanillin (**2**) (500 mg, 3.29 mmol) in DMF (5 ml) at rt under N_2 atmosphere. The reaction mixture was stirred at rt for 30 min and a solution of 1-bromodecane (1229 mg, 4.93 mmol) in DMF (5 ml) was added dropwise. The reaction mixture was stirred at rt for 3h and then quenched with water. The product was extracted with DCM (3x15 ml) and washed with H_2O (10 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (DCM/MeOH 99:1, R_f 0.91) to afford **T28** (781 mg, 74%) as a colorless solid. Mp: 45-46 ºC; IR (neat): 2916 (s), 2850 (m), 1689 (m), 1598 (w), 1583 (m), 1485 (m), 1468 (m), 1434 (w), 1381 (m), 1312 (w), 1264 (s), 1250 (m), 1223 (m), 1181 (w), 1129 (w), 1064 (m), 1016 (w), 991 (w), 973 (w), 911 (m), 822 (w), 778 (m), 766 (m), 741 (m), 717 (w), 662 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 10.44 (s, 1H), 7.4-7.3 (m, 1H), 7.1-7.0 (m, 2H), 4.10 (t, ^3J(H,H) = 6.6 Hz, 2H), 3.86 (s, 3H), 1.8-1.7 (m, 2H), 1.5-1.4 (m, 2H), 1.3-1.2 (m, 16H), 0.86 (t, ^3J(H,H) = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 190.3 (d), 153.2 (s), 152.3 (s), 130.1 (s), 123.9 (d), 119.1 (d), 118.1 (d), 75.1 (t), 56.1 (q), 32.1 (t), 30.3 (t), 29.8 (4x, t), 29.6 (2x, t), 26.1 (t), 22.9 (t), 14.3 (q); MS (ESI, MeOH): 321 (100, $[M+H]^+$); HR-MS (ESI): Calcd for $C_{20}H_{33}O_3$: 321.2424; found: 321.2424.

1-(4-Methylbenzenesulfonate)-3,6,9,12-tetraoxatridecan-1-ol (4).[S16] A solution of **3** (493 mg, 2.37 mmol) in THF (700 μl) was added to a solution of NaOH

(173 mg, 4.34 mmol) in distilled H_2O (600 μl) at 0 °C under N_2 atmosphere. After stirring for 15 min, a solution of TsCl (452 mg, 2.37 mmol) in THF (1 ml) was added dropwise into the reaction mixture at 0 °C. The reaction mixture was allowed to warm to rt for 22 h. Volatile products and solvent were removed under vacuum. The product was extracted with Et_2O (3 x 5 ml) and washed with H_2O (1 ml). The combined organic layers were dried over $NaSO_4$ and concentrated under vacuum (avoid heating) to afford **4** (810 mg, 94%) as a yellow oil. IR (neat): 2872 (m), 1718 (m), 1586 (w), 1431 (m), 1333 (m), 1245 (w), 1214 (w), 1098 (s), 926 (w), 852 (m), 766 (w), 730 (m), 668 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 7.8-7.7 (m, 2H), 7.4-7.3 (m, 2H), 4.2-4.1 (m, 2H), 3.70-3.67 (m, 2H), 3.64-3.61 (m, 6H), 3.58 (s, 4H), 3.55-3.51 (m, 2H), 3.37 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 145.0 (s), 133.2 (s), 130.0 (2x, d), 128.2 (2x, d), 72.1 (t), 70.9 (t), 70.8-70.7 (4x, t), 69.5 (t), 68.9 (t), 59.2 (q), 21.9 (q); MS (ESI, MeOH): 385 (33, $[M+Na]^+$), 380 (100, $[M+NH_4]^+$), 363 (48, $[M+H]^+$); HR-MS (ESI): Calcd for $C_{16}H_{27}O_7S$: 363.1472; found: 363.1474.

3,4,5-Tris(methyltetraethyleneoxy)benzoate (6).[S17] Methyl 3,4,5-trihydroxybenzoate (**5**) (70 mg, 0.38 mmol) was added to a suspension of K_2CO_3 (351 mg, 2.54 mmol) in DMF (4 ml) at rt under N_2 atmosphere. The reaction mixture was heated to 90 °C and a solution of **4** (1265 mg, 3.49 mmol) in DMF (1.5 ml) was added dropwise. The reaction mixture was stirred at 90 °C for 20 h. and then cooled to rt and poured into ice-water. The product was extracted with DCM (3 x 3 ml) and washed with brine and 0.5 M NaOH (5ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The yellow oil was purified by column chromatography (DCM/MeOH 95:5, R_f 0.78) to afford **6** (168 mg, 59%) as a yellow oil. IR (neat): 3485 (w,b), 2871 (m), 1718 (m), 1586 (w), 1431 (m), 1334 (m), 1247

(m), 1215 (m), 1098 (s), 942 (w), 851 (m), 766 (w), 570 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 7.22 (s, 2H), 4.2-4.1 (m, 6H), 3.8-3.7 (m, 7H), 3.75-3.70 (m, 2H), 3.7-3.6 (m, 6H), 3.6-3.5 (m, 24H), 3.5-3.4 (m, 6H), 3.29 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): 166.5 (s), 152.2 (2x, s), 142.5 (s), 124.9 (s), 108.9 (2x, d), 72.3 (t), 71.8 (3x, t), 70.7 (2x, t), 70.6-70.4 (14x, t), 69.5 (2x, t), 68.8 (2x, t), 58.9 (3x, q), 52.1 (q); MS (ESI, MeOH): 772 (100, $[M+NH_4]^+$); HR-MS (ESI): Calcd for $C_{35}H_{66}N_1O_{17}$: 772.4325; found: 772.4310.

3,4,5-Tris(3,6,9,12-tetraoxatridec-1-yloxy)benzenemethanol (7). A solution of **6** (332 mg, 0.44 mmol) in THF (1.5 ml) was added to a suspension of $LiAlH_4$ (33 mg, 0.88 mmol) in THF (1.5 ml) at rt under N_2 atmosphere. The reaction was stirred at rt for 4 h and then quenched by dropwise addition of 0.2 ml H_2O, 0.5 ml 1 M NaOH and 1 ml H_2O. The granular salts were filtered off, rinsed copiously with THF and the filtrate was concentrated under vacuum. The product was extracted with DCM (3 x 3 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (DCM/MeOH 95:5, R_f 0.69) to afford **7** (307 mg, 96%) as a colorless oil. Spectroscopic data match those reported in the literature.[S18]

T30. A solution of **7** (221 mg, 0.30 mmol) in DCM (2 ml) was added to a suspension of PCC (131 mg, 0.61 mmol) in DCM (4.8 ml) at 0 °C under N_2 atmosphere. The reaction mixture was stirred at rt for 3 h. Solvent was removed and the black residue was dissolved in water. The product was extracted with DCM (3 x 2 ml) and washed with brine (2 ml). The yellow residue was purified by column chromatography (DCM/MeOH 95:5, R_f 0.76) to afford **T30** (105 mg, 48%) as a yellow oil; IR (neat): 3512 (w,b), 2873 (m), 1690 (m), 1583 (m), 1495 (w), 1440 (m),

1327 (m), 1243 (w), 1199 (w), 1097 (s), 945 (m), 849 (m), 735 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 9.81 (s, 1H), 7.13 (s, 2H), 4.3-4.2 (m, 2H), 4.2-4.1 (m, 4H), 3.9-3.8 (m, 4H), 3.8-3.7 (m, 2H), 3.7-3.6 (m, 6H), 3.6-3.5 (m, 24H), 3.5-3.4 (m, 6H), 3.36 (t, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): 191.2 (d), 153.2 (2x, s), 144.2 (s), 131.8 (s), 109.1 (2x, d), 71.4 (t), 72.1 (3x, t), 71.0 (2x, t), 70.8 (12x, t), 70.5 (t) 69.8 (2x, t), 69.1 (2x, t), 61.9 (t), 59.2 (3x, q); MS (ESI, MeOH): 742 (100, $[M+NH_4]^+$); HR-MS (ESI): Calcd for $C_{34}H_{64}O_{16}N$: 742.4219; found: 742.4241.

Methyl 3,4,5-tris(dodecyloxy)benzoate (8). Methyl 3,4,5-trihydroxybenzoate (**5**) (1000 mg, 5.43 mmol) was added to a suspension of K_2CO_3 (5028 mg, 36.39 mmol) in DMF (15 ml) at rt under N_2 atmosphere. The reaction mixture was heated to 70 °C and a solution of 1-bromodecane (1250 mg, 50.07 mmol) in DMF (12 ml) was added dropwise. The reaction mixture was stirred at 70 °C for 20 h and then was cooled to rt and poured into ice-water. The precipitate was filtered off and washed with water. The product was extracted with DCM (2 x 15 ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The yellow residue was purified by column chromatography (hexane/AcOEt 95:5, R_f 0.82) to afford **8** (2994 mg, 87%) as a colorless solid. Spectroscopic data match those reported in the literature.[S20]

Methyl 3,4,5-tris(dodecyloxy)benzyl alcohol (9). A solution of **8** (2994 mg, 4.35 mmol) in THF (18 ml) was added to a suspension of $LiAlH_4$ (247mg, 6.52 mmol) in THF (15 ml) at rt under N_2 atmosphere. The reaction mixture was stirred at rt for 2 h and then quenched by dropwise addition of 2 ml H_2O, 5 ml 1 M NaOH and 10 ml H_2O. The granular salts were filtered off, rinsed copiously with THF and the filtrate was concentrated under vacuum. The product was extracted with DCM (2 x 20

ml). The organic layers were combined, dried over $NaSO_4$ and concentrated under vacuum. The residue was purified by column chromatography (Hexane/AcOEt 97:3, R_f 0.63) to afford **9** (1824 mg, 63%) as a colorless solid. Spectroscopic data match those reported in the literature.[S20]

T29. A solution of **9** (1824 mg, 2.76 mmol) in DCM (10 ml) was added to a suspension of PCC (595 mg, 2.76 mmol) in DCM (20 ml) at rt under N_2 atmosphere. The reaction mixture was stirred at rt for 4 h and then quenched by addition of 10 ml Et_2O. The brown crude was filtered through Florisil with DCM. The yellow residue was purified by column chromatography (hexane/AcOEt 95:5, R_f 0.83) to afford **T29** (738 mg, 41%) as a colorless solid. Spectroscopic data match those reported in the literature.[S21]

Synthesis of Dynamic Amphiphiles.[S4] 2.5 µl of a 100 mM DMSO stock solution of heads and 2.5 µl of 1 M DMSO stock solution of tails were mixed with 20 µl of dry DMSO (final total volume of DMSO 25 µl). The reactor vessel was shaken at 62.5 °C for 1 h affording a final amphiphile concentration of ~ 10 mM. Preparation of stock solution of heads: the required amount of head was weighted and dissolved in the corresponding volume of DMSO/AcOH 9:1. Preparation of tails stock solution was done in normal DMSO. When solution was not clear, a short pre-warming step (60 ^oC, 2 min) was required for pipetting the desired tail volume for amphiphile formation.

4. Cell Lines and Culture

HeLa cells stably expressing GPI-EGFP (Courtesy of Pr. Lucas Pelkmans, Zurich) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, High Glucose, GlutaMAX™, Pyruvate, Life Technologies™) supplemented with 10% (v/v) fetal bovine serum (FBS) from Hyclone (Thermo Scientific), and 500 µg/mL Geneticin (Life Technologies™). Transfections of HeLa GPI-EGFP were performed in the same medium, free of antibiotics. Human Primary Skin Fibroblasts (Courtesy of Dr. Charna Dibner, University of Geneva) were both maintained and transfected in DMEM, High Glucose, GlutaMAX™, Pyruvate (Life Technologies™) supplemented with 20% (v/v) FBS. Cell incubations were performed in a water-jacketed 37 °C/5% CO2 incubator.

5. *In Vitro* Screening for siRNA Delivery

Amphiphile stock solutions were prepared the day before the experiment in 100% (v/v) DMSO, at different concentrations, corresponding to different siRNA/amphiphiles molar and N/P ratios, as shown in Table S1. The solutions of siRNA/amphiphiles were freshly prepared prior to the transfection experiments and by the following procedure. 5 µl of the siRNA solution (1.35 µM in water) and 5 µl of the Amphiphile Stock solution in DMEM, High Glucose, GlutaMAX™, Pyruvate, 20% (v/v) DMSO, supplemented with 10% (v/v) FBS, were added to 210 µl DMEM, High Glucose, GlutaMAX™, Pyruvate, supplemented with 10% (v/v) FBS and the mixture was homogenized by pipetting. Then, cell medium was aspirated from the 96-well plate and 100 µl of the mixture was added in each well. The final concentration

of DMSO in each well was 0.25% (v/v). The screening experiment was performed with the Liquid Handler Biomek FX (Biomolecular Screening Facility, EPFL). All conditions were repeated three times.

For the best amphiphiles, at the most efficient and less toxic concentration, siRNA solutions were prepared at different concentrations, as shown in Table S2. Forward transfection with Lipofectamine™ RNAiMax was performed according to the supplier instructions.

Table S1. Concentration of reagents used for *in vitro* screening of dynamic amphiphiles for siRNA delivery and hit confirmation (The N/P ratio refers to the number of ammonium / guanidinium groups per oligonucleotide phosphate).

Molar Ratio (Amphiphiles / siRNAs)	N/P ratio		Concentration of Amphiphiles Stock solution (mM)	Final Concentration of Amphiphiles (μM)	Final Concentration of siRNAs (nM)
	G2H4Tx, A2H4Tx	G1HxTy, G1OxTy, G1SxHyTz			
100	5	2.5	0.14	3.4	33.8
200	10	5	0.27	6.8	
400	20	10	0.54	13.5	
600	30	15	0.81	20.3	
800	40	20	1.08	27.0	
1000	50	25	1.35	33.8	

Table S2. Final concentration of siRNA and dynamic amphiphiles in samples for transfection optimization experiments.

Amphiphiles	[Amphiphiles]
G2H4T12	30µM
G2H4T20	12µM
DMSO	0.25%

[siRNA]	siRNA
0nM	0 pmol
2nM	0.2 pmol
5nM	0.5 pmol
10nM	1 pmol
20nM	2 pmol
30nM	3 pmol

6. Transfection in HeLa GPI-EGFP

HeLa cells expressing GPI-EGFP were transfected either with Ambion® Silencer® GFP (eGFP) siRNA from Life Technologies™ (siEGFP) or scramble RNA (All Star Negative Control from Qiagen). 24 h, 48 h and/or 72 h post siRNA transfection, cell supernatant was removed, kept for cytotoxicity evaluation and replaced with PBS before measuring EGFP expression by fluorescence spectroscopy (λ_{ex} = 489 nm; λ_{em} = 509 nm). Percentage of EGFP knockdown was calculated as the percentage of fluorescence decrease observed in cells transfected with siEGFP compared to transfection with scrambled RNA with the same reagents under the same conditions. In the same way, the percentage of cell viability was calculated as the percentage of remaining fluorescence in samples transfected with scrambled RNA compared to non-transfected cells in DMEM, High Glucose, GlutaMAX™, and

Pyruvate, supplemented with 0.25% (v/v) DMSO. Cytotoxicity of **G1H3T12**, **G2H4T12**, and **G2H4T20**/siRNA complexes was calculated in parallel from cell supernatants of transfected cells using the Cytotoxicity Detection Kit^{PLUS} (LDH) from Roche™ according to supplier's instructions.

Lipofectamine™ RNAiMax and **G2H4T20** were used as positive control of siRNA transfection reagent in the *in vitro* screening of dynamic amphiphiles in HeLa stably expressing GPI-EGFP. Robotically assisted transfection experiments using **G2H4T20** show its ability to transfect siRNA similar to experiments performed with commercially available transfection reagent such as Lipofectamine™ RNAiMax (Figure S1). The quality of the transfection experiments with both transfection reagents was assessed calculating the z-factor with equation (S1).[S22]

$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}. \qquad \text{(S1)}$$

With the means and standard deviations of relative fluorescence units (RFU) of both the positive (p = cells transfected with mixture of siGFP and G2H4T20/Lipofectamine RNAiMax™) and negative (n = DMSO = non-transfected cells in medium supplemented with 0.25% (v/v) DMSO) controls (μ_p, σ_p, and μ_n, σ_n). A z-factor between 0.5 and 1.0 indicates an excellent assay, 0.5 is equivalent to a separation of 12 standard deviations between μ_p and μ_n.

Detailed results for transfection efficiency and cell viability in HeLa GPI-GFP from the *in vitro* screening of dynamic amphiphiles for siRNA delivery are shown in Figures S2 and S3. Comparison between amphiphiles **G1H3T12**, **G1H3T19**,

G2H4T12, **G2H4T20**, **G2H4T21**, **G2H4T22** and **G2H4T23** and their correspondence with amine heads, oxime or sulfide bonds shows that hydrazones are the most active and least toxic molecules able to deliver siRNAs in human cells.

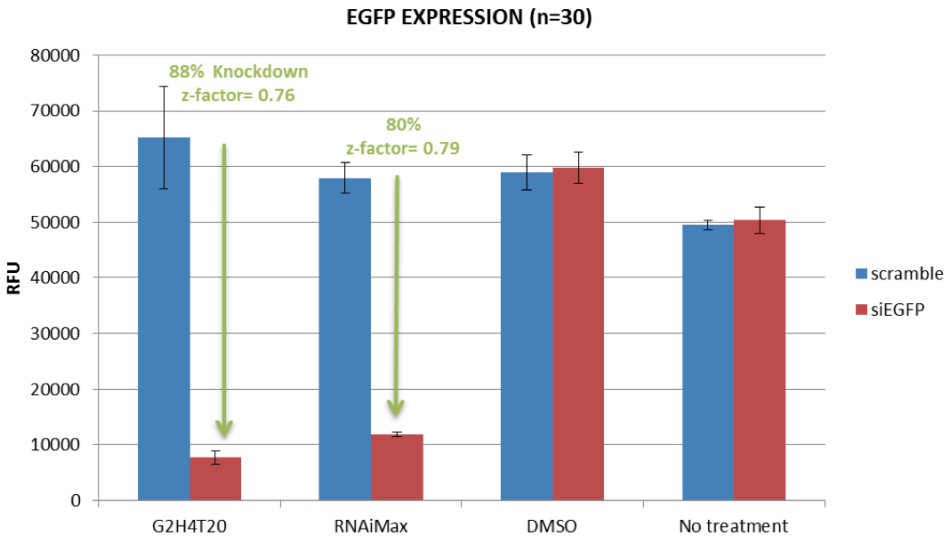


Figure S1. Determination of the z-factor for the EGFP suppression in HeLa cells by siRNA delivery with **G2H4T20** and Lipofectamine™ RNAiMax.

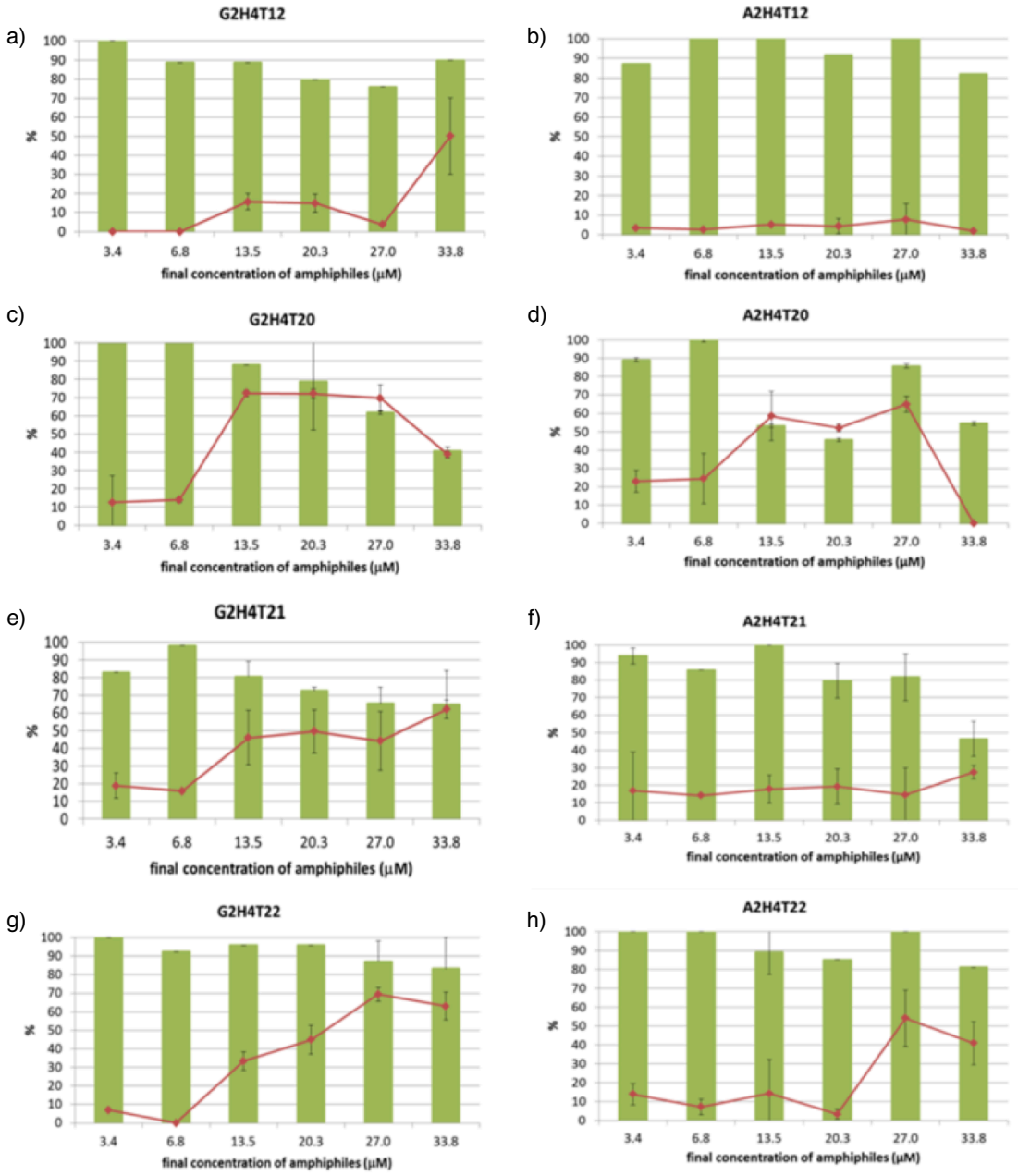


Figure S2. Snapshots from *in vitro* library screening for transfection efficiency (percent of GFP knockdown, red lines) and cell viability (red squares, green columns) in HeLa GPI-GFP at constant siRNA concentration (33.8 nM) with increasing concentration of amphiphiles (a) **G2H4T12**, (b) **A2H4T12**, (c) **G2H4T20**, (d) **A2H4T20**, (e) **G2H4T21**, (f) **A2H4T21**, (g) **G2H4T22** and (h) **A2H4T22**.

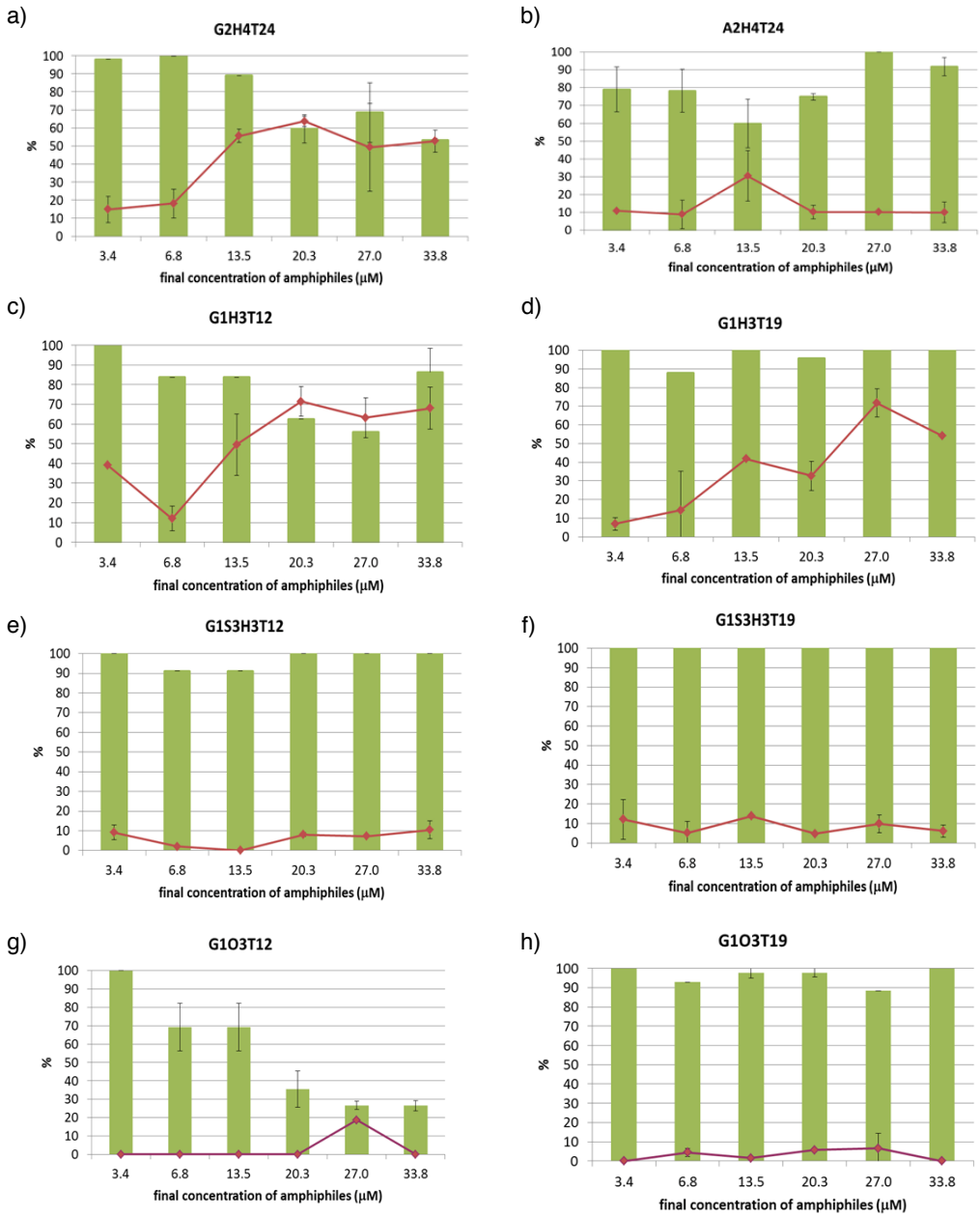


Figure S3. Snapshots from *in vitro* library screening for transfection efficiency (percent of GFP knockdown, red lines) and cell viability (red squares, green bars) in HeLa GPI-GFP at constant siRNA concentration (33.8 nM) with increasing concentration of amphiphiles (a) **G2H4T24**, (b) **A2H4T24**, (c) **G1H3T12**, (d) **G1H3T19**, (e) **G1S3H3T12**, (f) **G1S3H3T19**, (g) **G1O3T12** and (h) **G1O3T19**.

Transfection conditions were further validated for the most reproducible and most active amphiphiles **G2H4T12** and **G2H4T20**. To optimize the concentration of the amphiphiles, HeLa cells expressing GPI-EGFP were treated with EGFP-targeting siRNA-amphiphile complexes. The average percent reduction in EGFP expression after treatment with siRNA-amphiphile complexes was measured in triplicate for different final concentration of dynamic amphiphiles in medium: 3 μM, 6 μM, 12 μM, 18 μM, 25 μM or 30 μM (Figure S4).

Cell viability of HeLa GPI-EGFP cells was measured as the percentage of fluorescence decrease in samples transfected with amphiphiles/scramble RNA complexes compared to untreated cells in medium supplemented with 0.25% (v/v) DMSO (Figure S5a). Cell viability was confirmed in parallel from cell supernatant using Roche Cytotoxicity Detection kit™ (Figure S5b). All experiments were performed in triplicate.

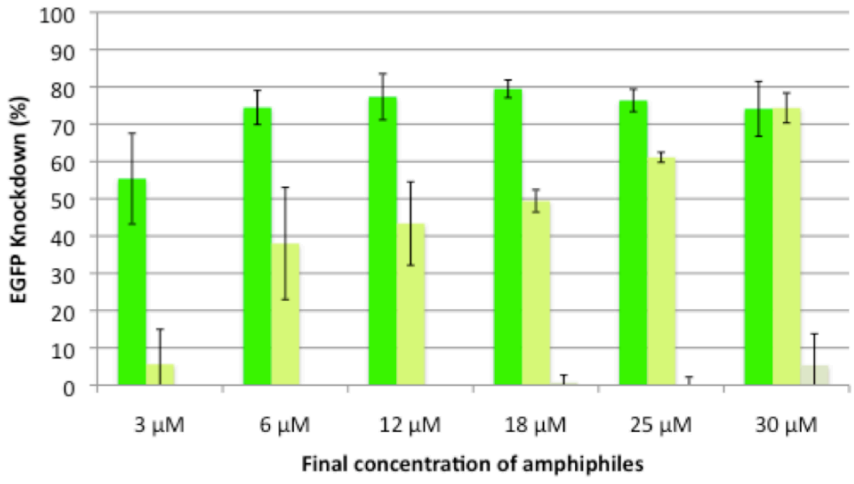


Figure S4. Validation of screening results: Transfection efficiency (percent of GFP knockdown) with HeLa GPI-GFP at constant siRNA concentration (30 nM) and increasing concentrations of amphiphiles **G2H4T12** (green), **G2H4T20** (light green) and DMSO (grey); measured in triplicates.

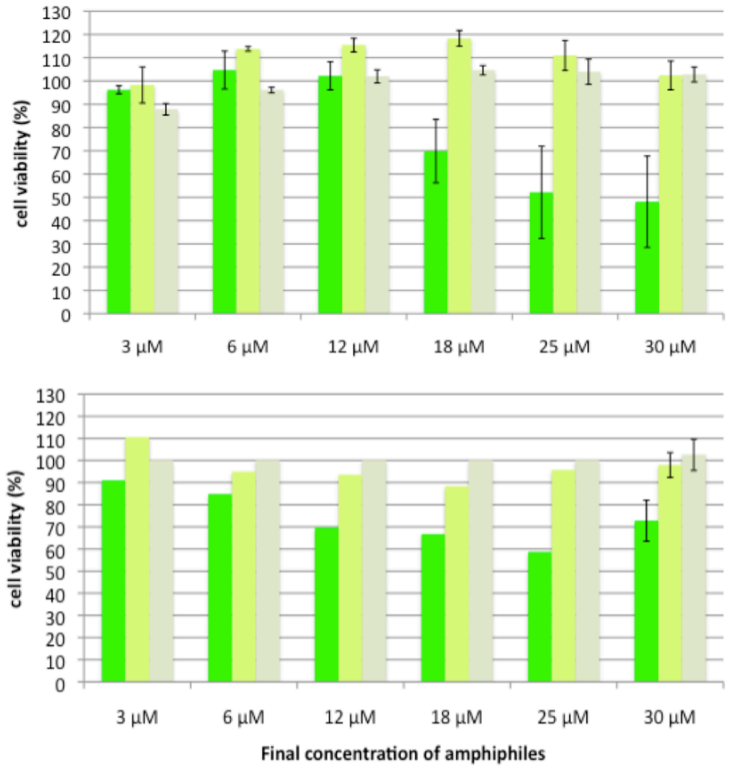


Figure S5. Validation of screening results: Cell viability from fluorescence decrease (top) and Roche cytotoxicity kit (bottom) with HeLa GPI-GFP at constant siRNA concentration (30 nM) and increasing concentrations of amphiphiles **G2H4T12** (green), **G2H4T20** (light green) and DMSO (grey); measured in triplicates.

To optimize the concentration of the siRNA, HeLa cells expressing GPI-EGFP were treated with EGFP-targeting siRNA-amphiphile complexes. The average percent reduction in EGFP expression after treatment with siRNA-amphiphile complexes at the optimized concentration of dynamic amphiphiles in medium: 12 µM and 30 µM of **G2H4T12** and **G2H4T20**, respectively (Figure S6). Cell viability of HeLa GPI-

EGFP cells was measured as the percentage of fluorescence decrease in samples transfected with amphiphiles/scramble RNA complexes compared to untreated cells in medium supplemented with 0.25% (v/v) DMSO. All experiments were performed in triplicate.

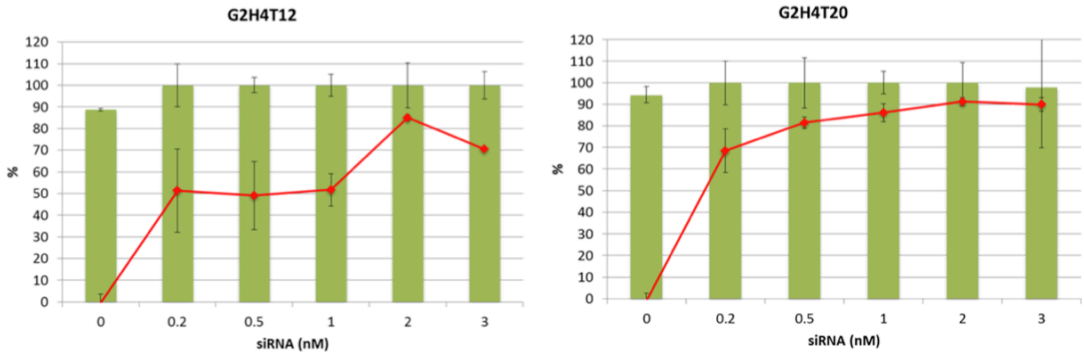


Figure S6. Transfection efficiency (percent of GFP knockdown, red lines) and cell viability (green bars) with HeLa GPI-GFP at constant concentration of amphiphiles **G2H4T12** (left, 12 μM), **G2H4T20** (right, 30 μM) and increasing concentrations of siRNA.

7. Transfection in Human Primary Skin Fibroblasts

Human primary skin fibroblasts were transfected either with siRNA against human GAPDH (Silencer GAPDH siRNA Hs,Mm, Applied Biosystem) or scrambled RNA (All Star Negative Control from Qiagen). 72 h post siRNA transfection, the cell medium was removed, cells were lysed and GAPDH activity was measured by fluorimeter (KDalert™ GAPDH Enzyme Assay). Percentage of GAPDH knockdown was calculated according to supplier's instructions. Cytotoxicity of amphiphiles/siRNA complexes was measured with Roche's Cytotoxicity Detection

Kit^{PLUS} (LDH) according to supplier's instructions.

8. Confocal Microscopy

To elaborate on the uptake mechanism with dynamic amphiphiles, the modes of cellular uptake of an FITC-labeled oligo DNA complex with **G1H3T12** was analyzed using confocal laser scanning microscopy. HeLa cells (2.0×10^5 cells/dish) were plated into 35-mm glass-bottomed dishes (Iwaki) and cultured for 48 h.

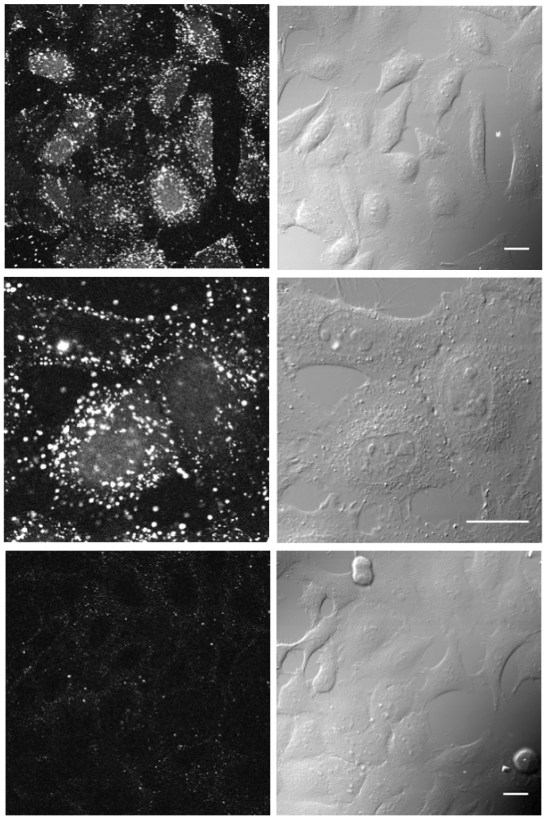


Figure S7. Fluorescence images of HeLa cells treated with FITC-labeled DNA (2 μM) in the presence of **G1H3T12** (40 μM) for 1 h at 37 ºC (top, middle) or at 4 ºC (bottom). Scale bars, 20 μm.

HeLa cells were washed twice with α-minimum essential medium (α-MEM)

containing 10% heat-inactivated fetal bovine serum [α-MEM(+)]. HeLa cells were then incubated in α-MEM(+) (200 μL) containing **G1H3T12** (40 μM) and 5'-FITC-labeled dsDNA (5'-FITC-CGCAGTGTAATCCCCTCGAC-3'/5'-GTCGAGGGGATTACACTGCG-3', purchased from Invitrogen) (2 μM) for 1 h at 37 ºC (Figure S7, top, middle) or at 4 ºC (Figure S7, bottom). The cells were observed immediately after incubation without fixation, using a confocal scanning laser system (FV1000; Olympus) consisting of an inverted microscope (IX81; Olympus).

9. ζ Potentials and Complex Sizes

The **G1H3T12** and **G2H4T20** (5 μl of 10 mM stock solution in DMSO) were freshly mixed with corresponding amounts of scrambled dsRNAs solution (995 μl in water) before measuring. For the measurement of amphiphile alone, 995 μl of siRNA solution was replaced by water. The bi-distilled water was used after filtering through the membrane filter (PTFE, 0.45 μm). The complex size was determined by dynamic light scattering. All the experiments were done at 25 °C, and the mean values and errors (standard deviation) from three experiments were plotted in Figure S8. As increasing the amounts of dsRNAs, the complexes tend to decrease in ζ potential and to increase in size. All the particles were smaller than 200 nm, which are appropriate for the clathrin-mediated endocytosis.[S23]

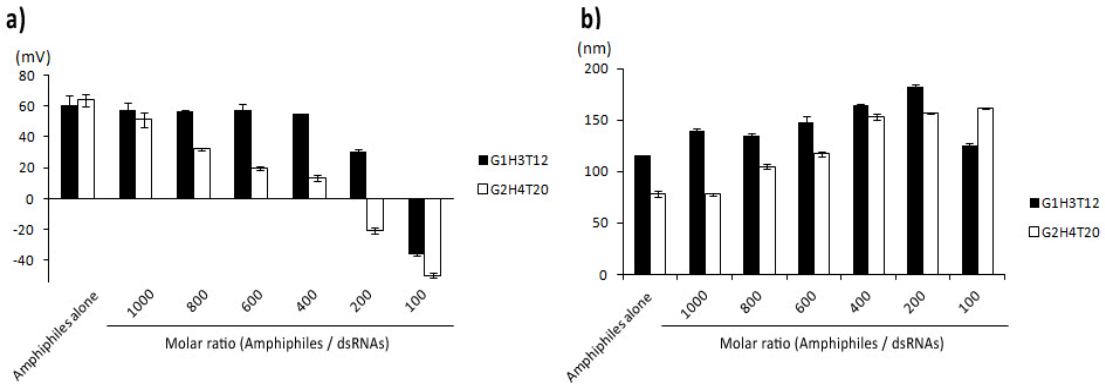


Figure S8. The ζ potentials (a) and sizes (b) of the complexes according to the described molar ratios.

10. Gel Retardation Assays

The pre-mixed complexes solution of scrambled dsRNAs (3 pmol) and corresponding amounts of amphiphile solution (10 mM in DMSO) was loaded to 2% agarose gel containing ethidium bromide. The bands of free dsRNAs disappeared by increasing the quantity of amphiphiles and the bands of complete complexes were observed in the each well.

Figure S9. Gel retardation assay with **G2H4T20**. The dsRNAs (lane 1) and complexes with molar ratios of 100 (lane 2), 200 (lane 3), 400 (lane 4), 600 (lane 5), 800 (lane 6), 1000 (lane 7) were loaded.

11. References

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12. NMR Spectra

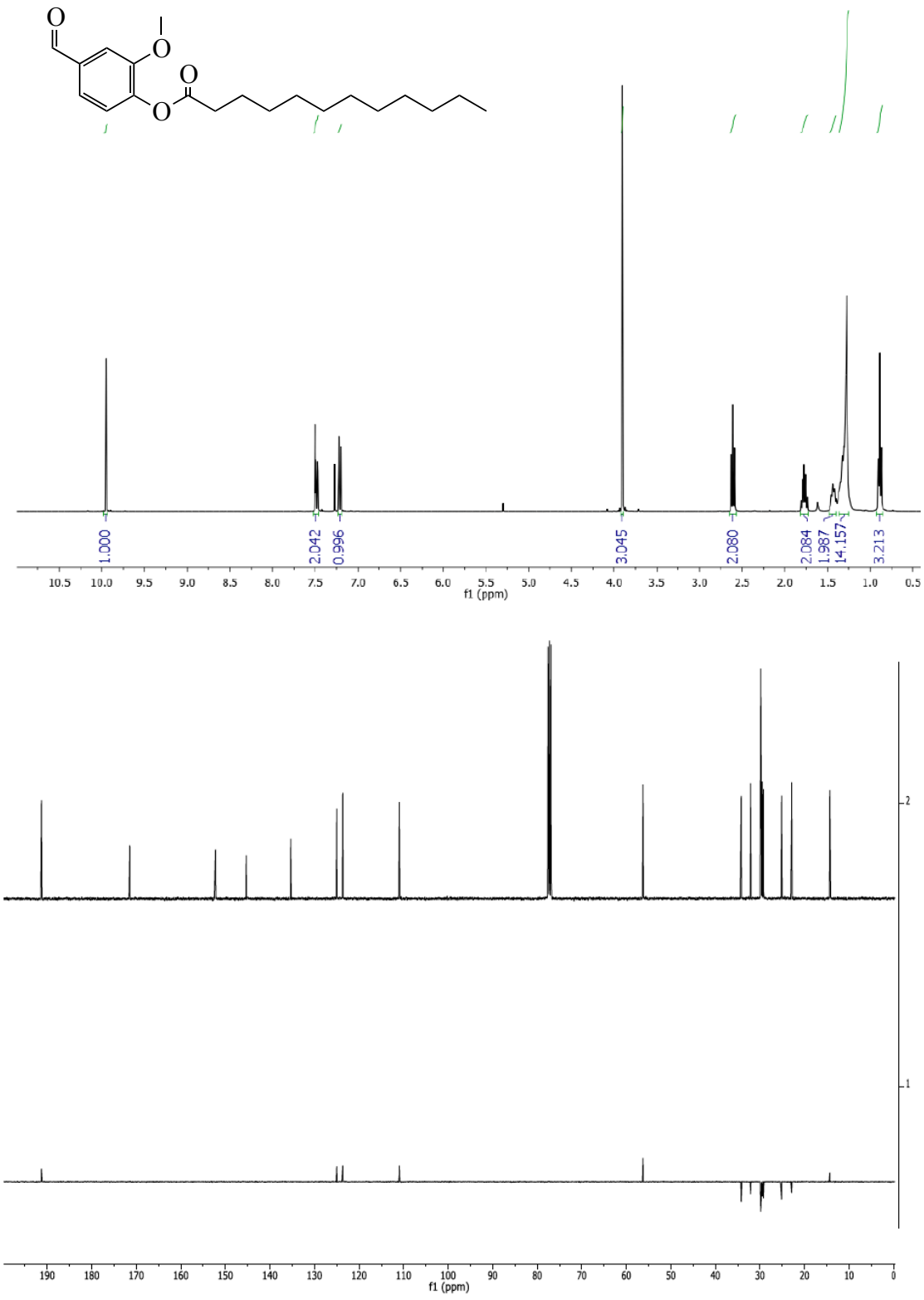

Figure S10. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T25**.

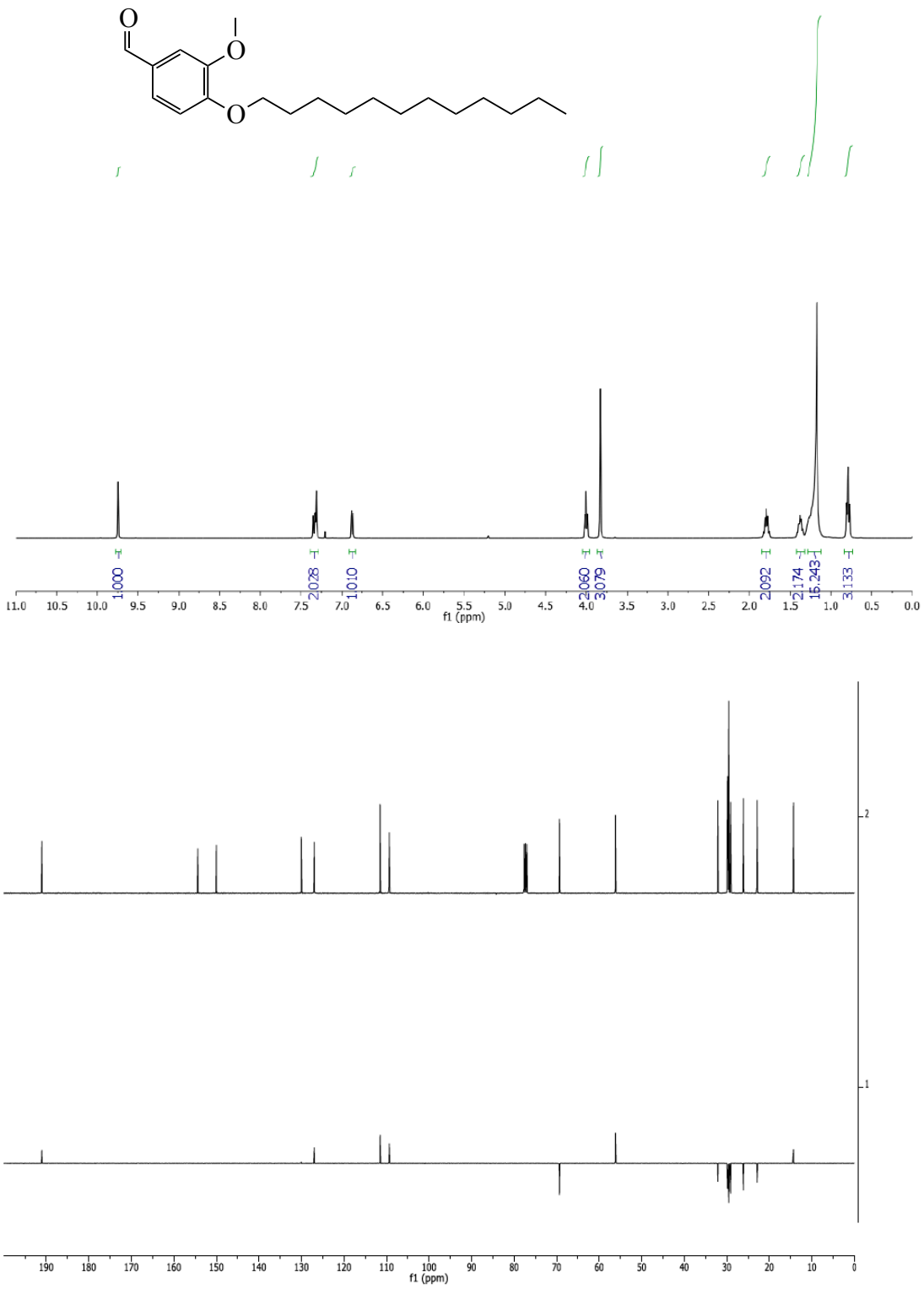

Figure S11. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T26**.

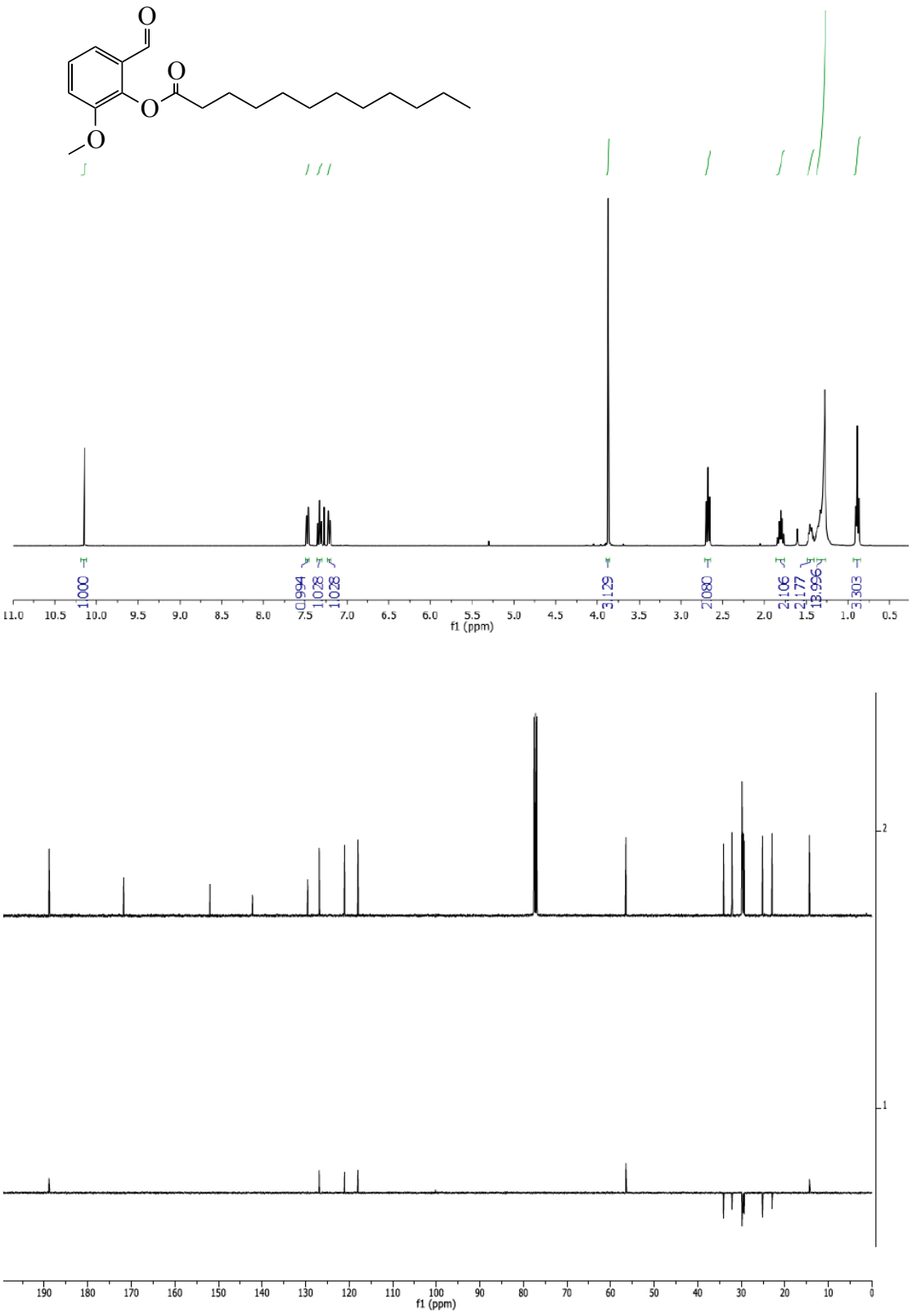

Figure S12. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T27**.

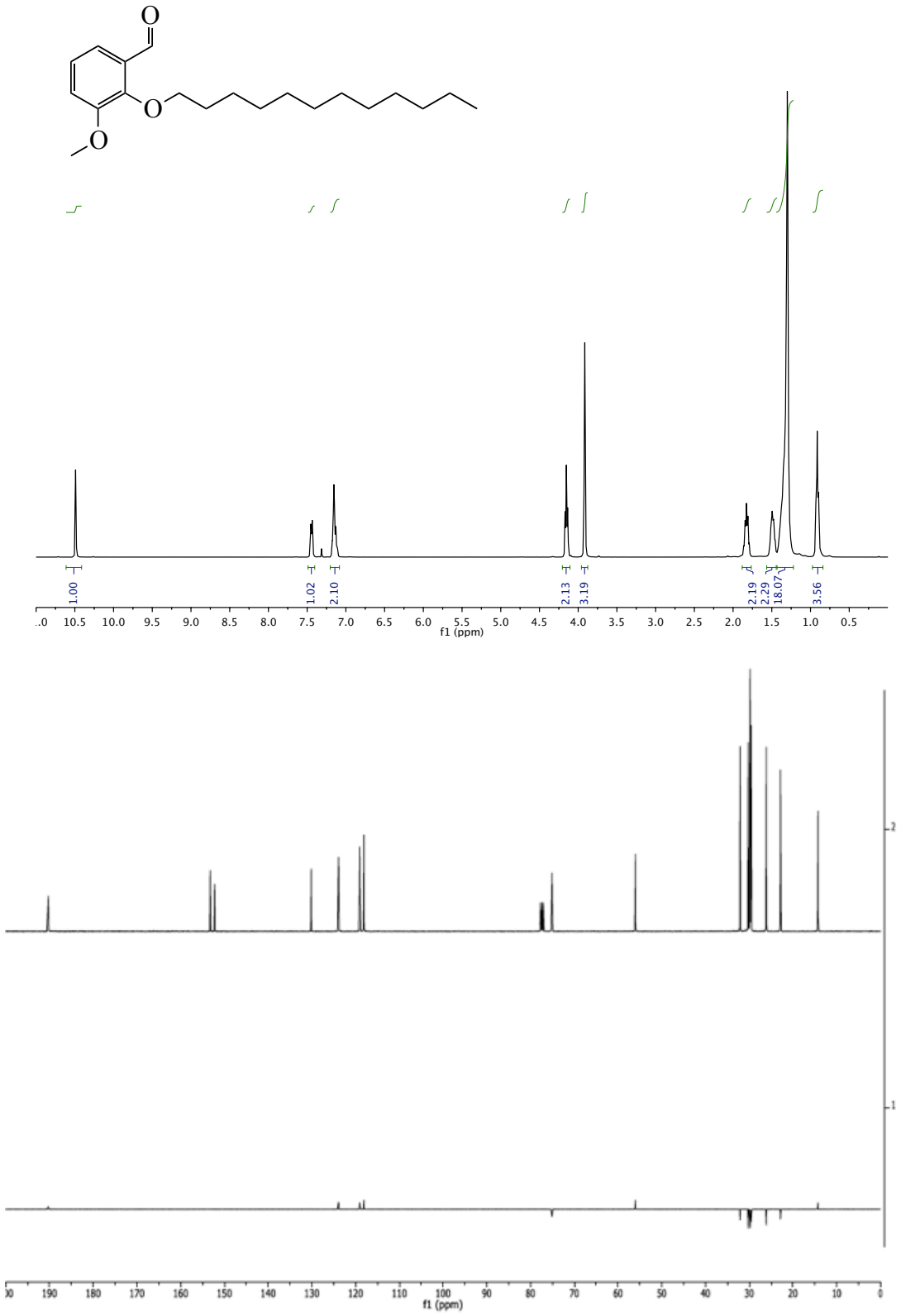

Figure S13. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T28**.

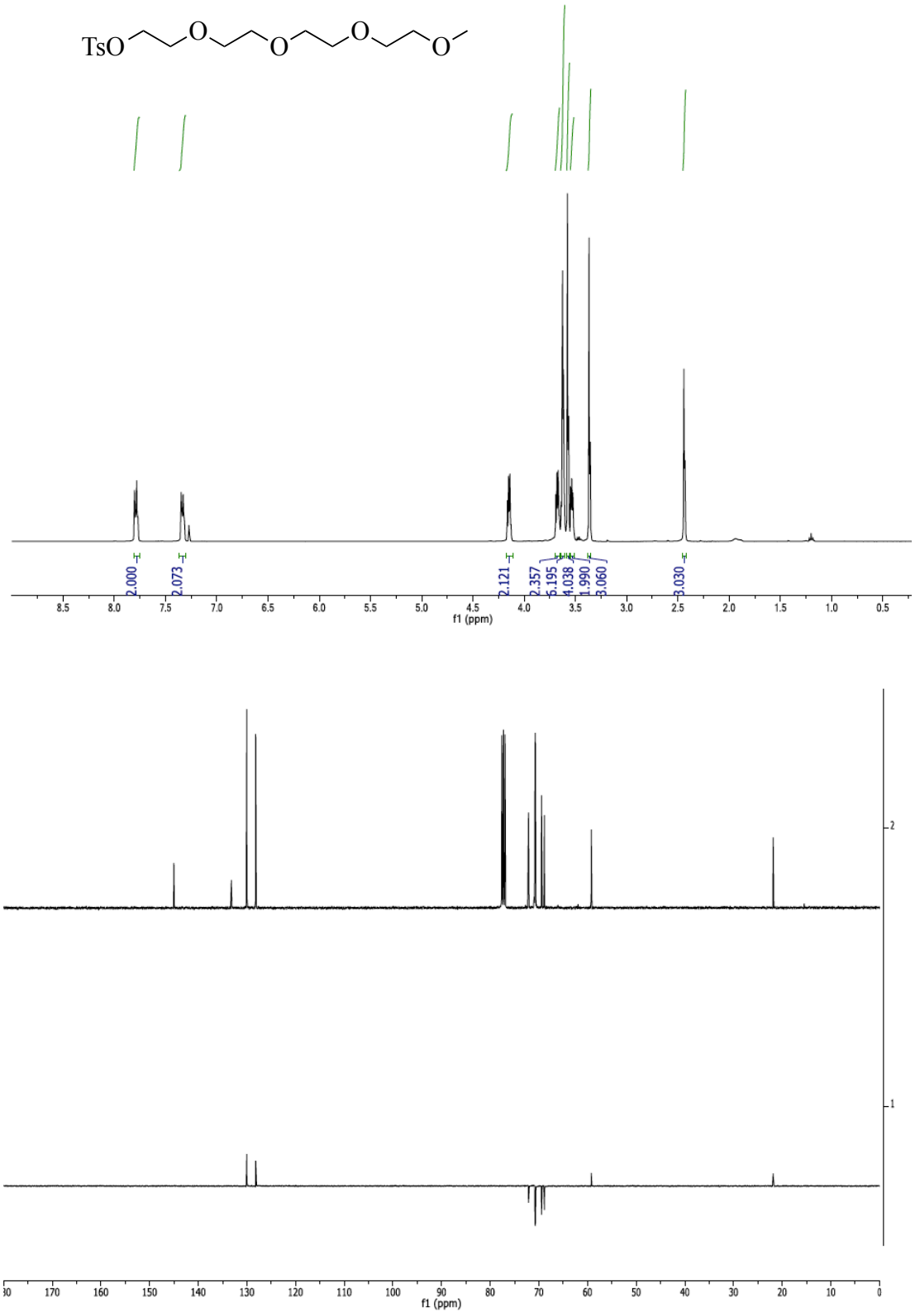

Figure S14. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **4**.

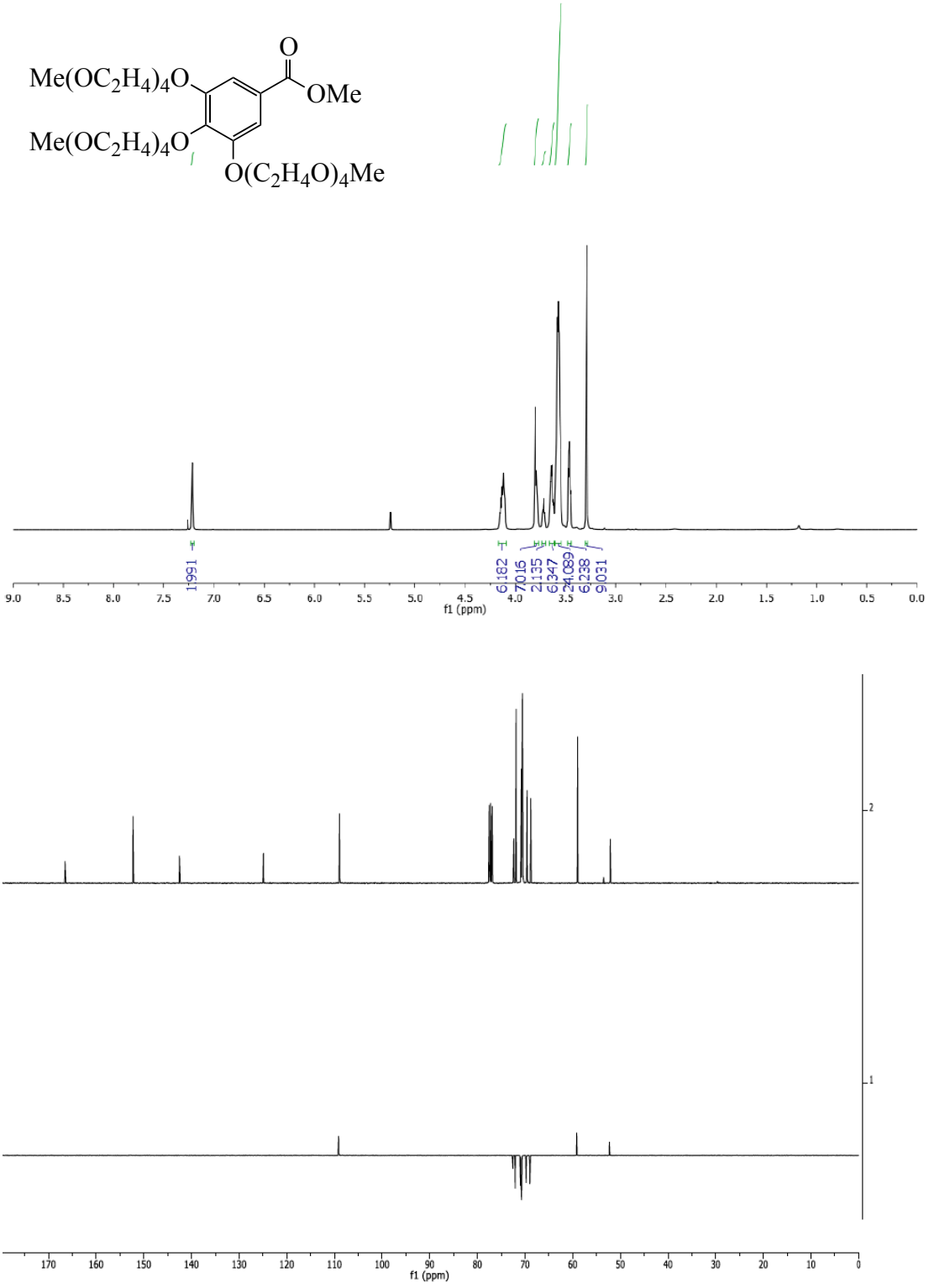

Figure S15. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **6**.

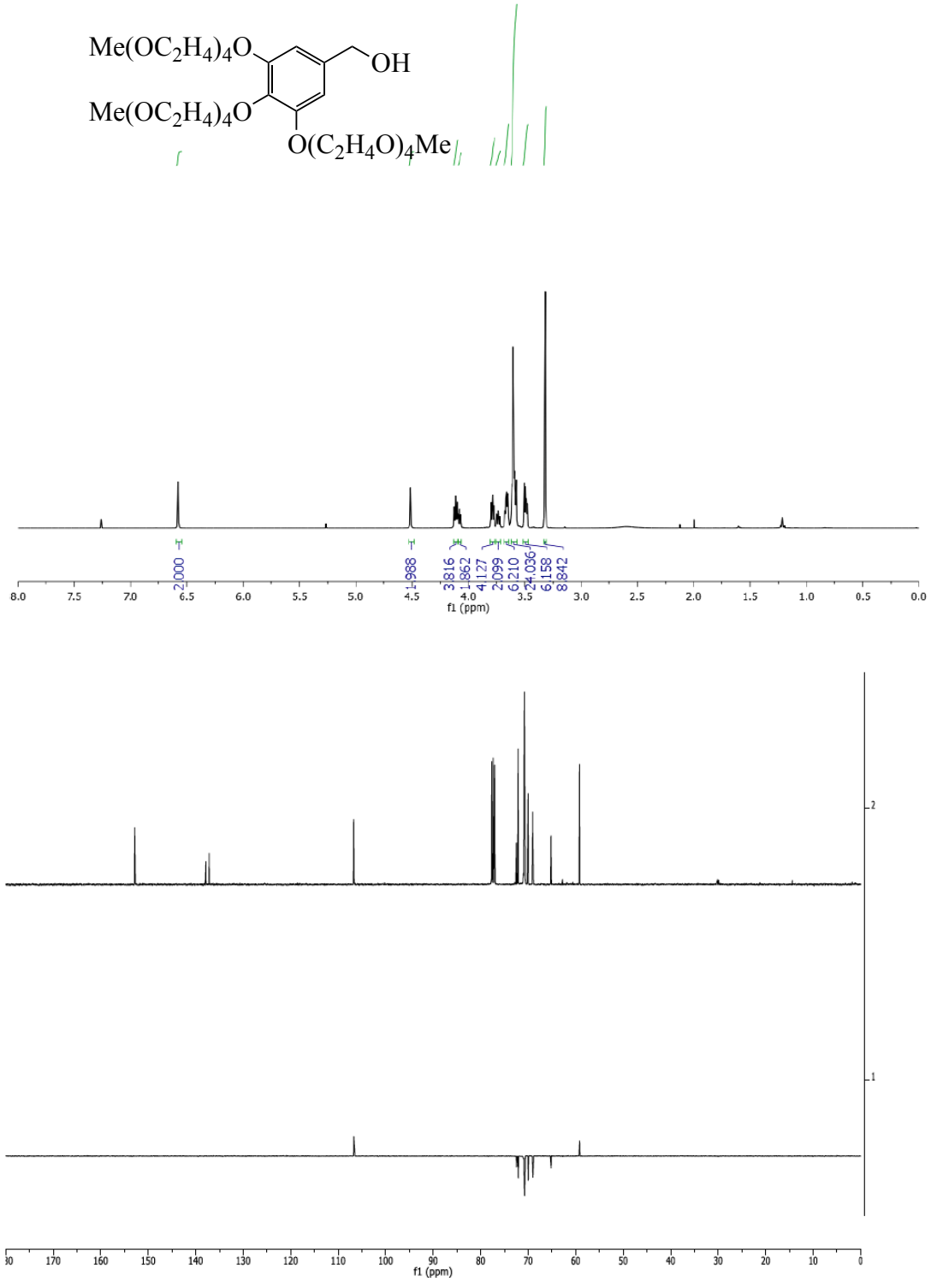

Figure S16. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **7**.

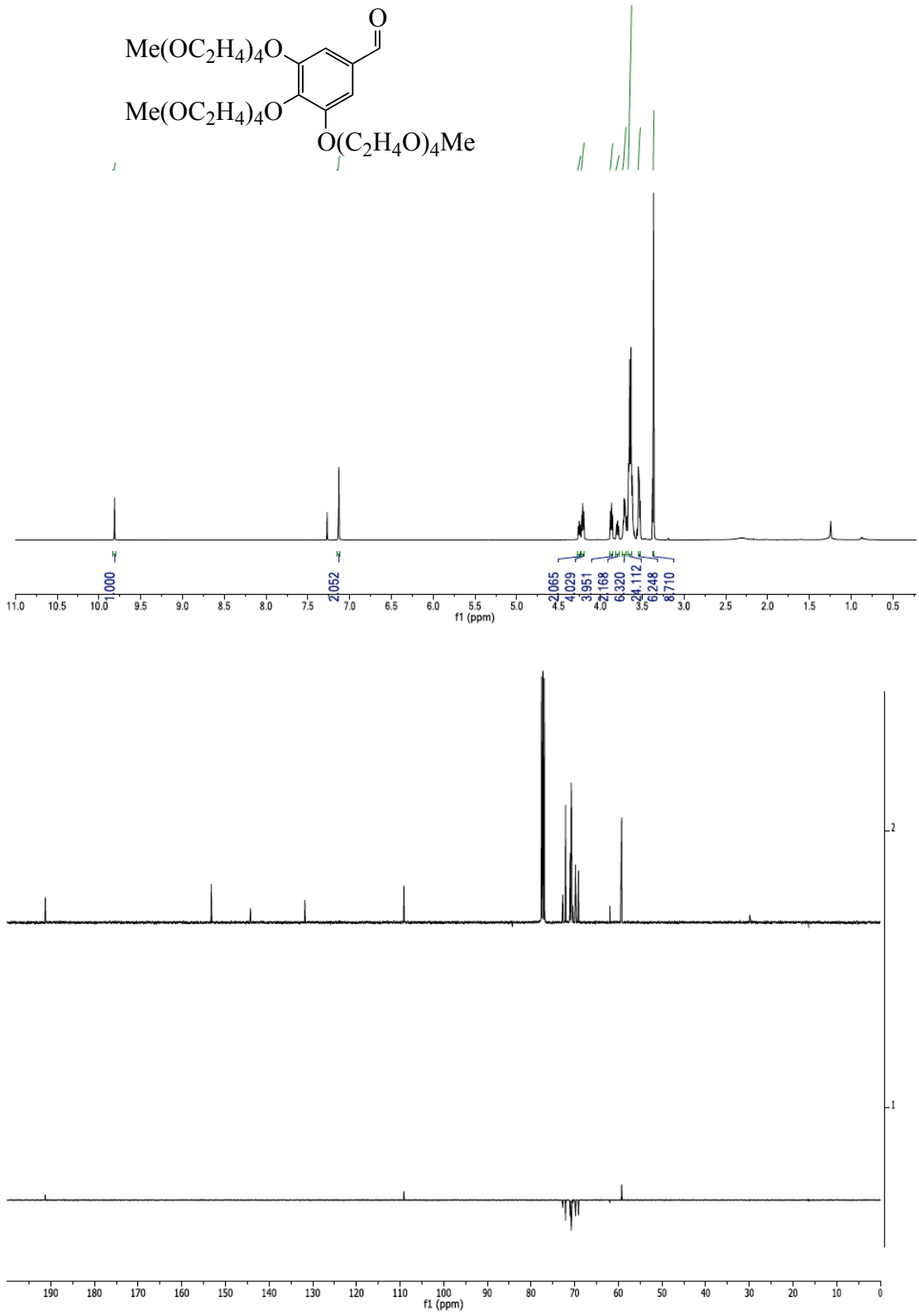

Figure S17. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T29**.

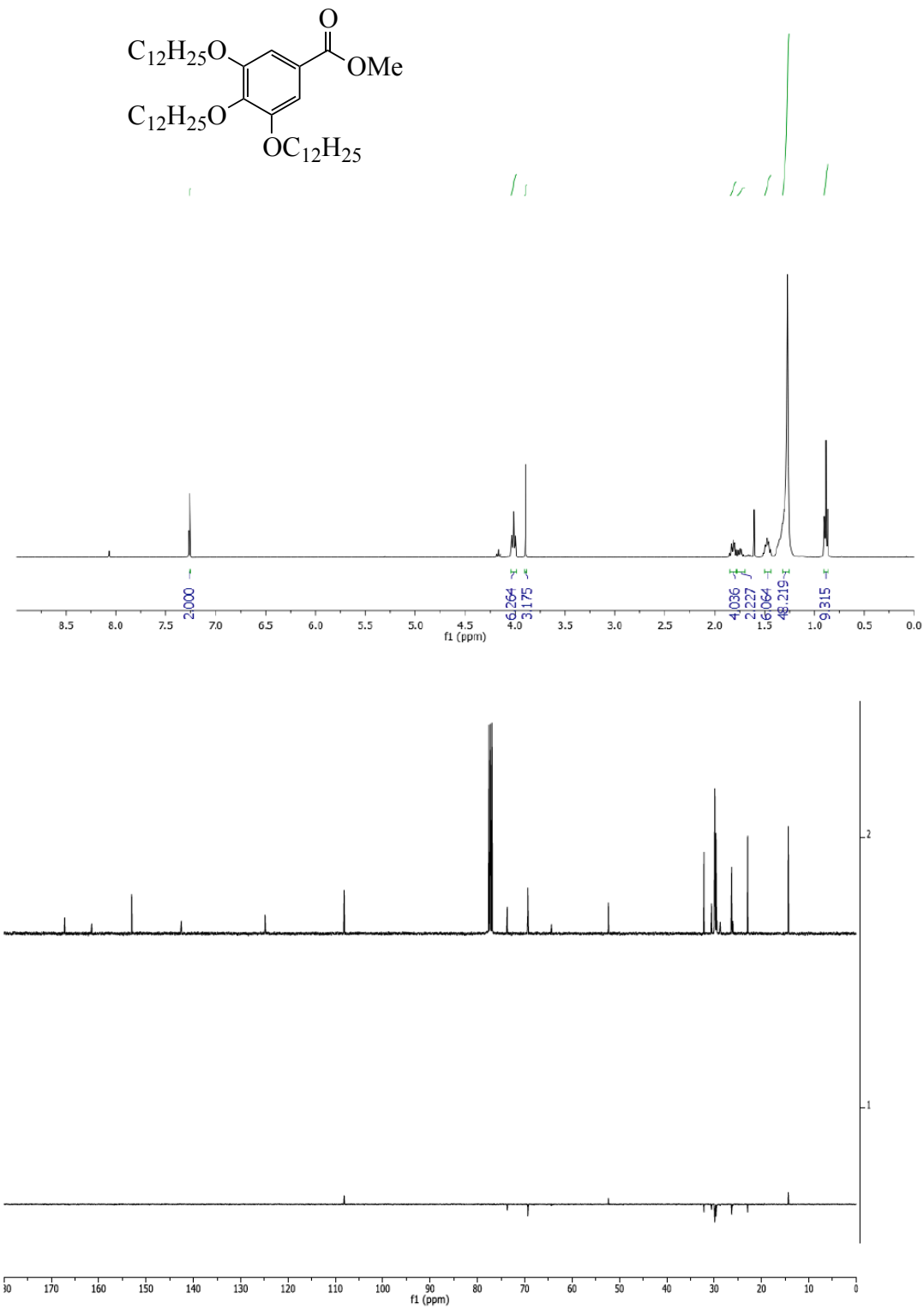

Figure S18. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T8**.

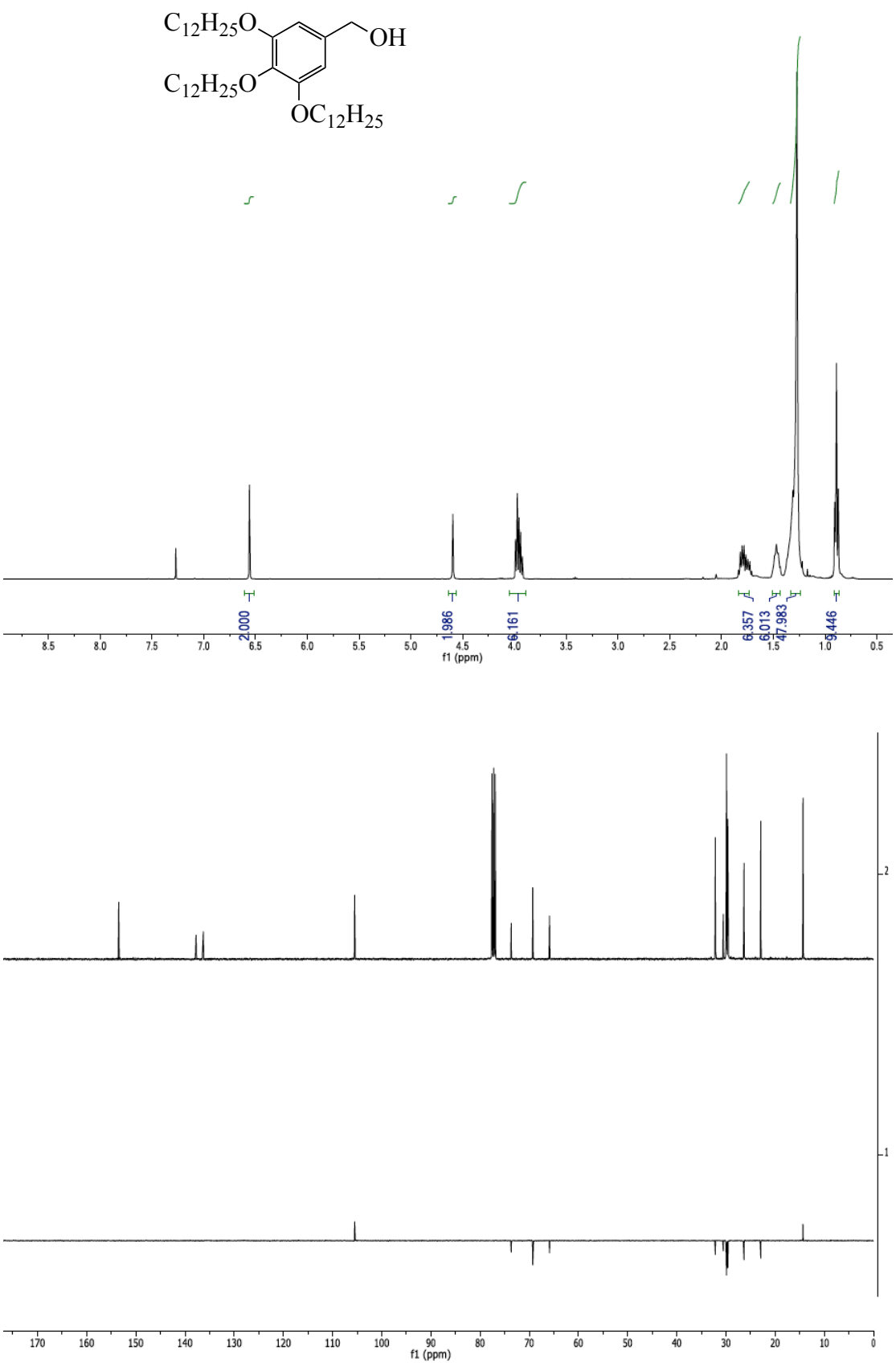

Figure S19. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T9**.

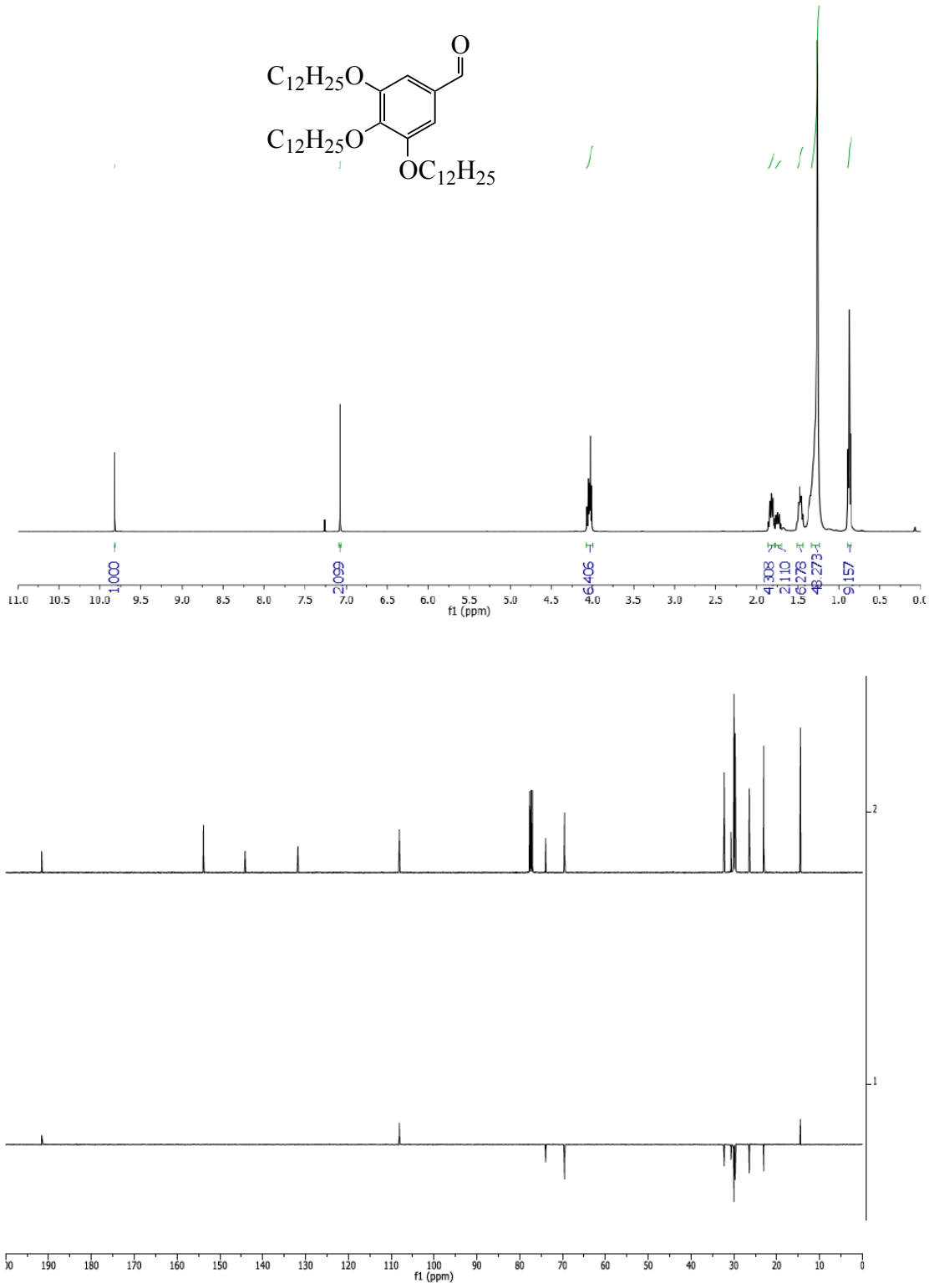

Figure S20. ^{1}H and ^{13}C NMR in $CDCl_3$ spectra for **T30**.