ChemBioChem

Supporting Information

Optochemical Control of Bacterial Gene Expression: Novel Photocaged Compounds for Different Promoter Systems

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S1 Bacterial strains and plasmids

All bacterial strains, plasmids and oligonucleotides used in this study are listed in Table S1.

Table S1: Bacterial strains, plasmids and oligonucleotides used in this study.

Strains, plasmids, oligonucleotides	Relevant features, description or sequences [a,b]	References
	Strains	
***E. coli* DH5α**	*F⁻ Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 phoA supE44 thi-1 gyrA96 relA1 deoR*	[1]
***E. coli* Tuner (DE3)**	*F⁻ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm lacY1(DE3)*	Novagen, Merck KGaA
***E. coli* LMG194**	*F– ΔlacX74 galE galK thi rpsL ΔphoA Δara714 leu::Tn10*	[2]
	Plasmids	
pRhotHi-2-lacI-eYFP	pBBR1-MCS-derivative, Km^R, Cm^R, pBBR22b-*lacI*, P$_{T7}$-lacO-MCS with *Nde*I *Xho*I inserted *eyfp*	[3]
pM117-R45T-GFPmut3	pMB1 replicon, *xylS* with R45T mutation, $P_{m\ M1\text{-}17}$ with inserted *gfpmut3*	[4]
pM-R45T-GFPmut3	pMB1 replicon, *xylS* with R45T mutation, P_m with inserted *gfpmut3*	[5] and this work
pBNTmcs(t)-Km	Km^R, *nagR*, vector for *PnagAa* and *tac* RBS controlled expression	[6]
pBNTmcs-mCherry-Km	pBNTmcs(t)-Km derivative with *EcoR*I/*Xba*I inserted *mcherry*	This work
pBTBX-2	pBBR1 replicon, Km^R, *araC*, araBAD promoter	[7]
pBTBX-2-mCherry	pBBR1 replicon, Km^R, *araC*, araBAD promoter with *tac* RBS and inserted *mcherry*	This work
	Oligonucleotides	
1) XylS_SalI_fw	Binds upstream of *Sal*I-site after *xylS*. Sequence: 5'-GAGACACAACGTGGCTTTCC-3'	[4]
2) XylS_SacI_rev	Binds upstream of *Sac*I-site in front of *xylS*. Sequence: 5'- ATCGACTTGGCGCCTTTCTAC-3'	[4]
3) XylS_R45T_rev	Binds within *xylS* and inserts R45T point mutation. Sequence: 5'- CAGGCA<u>C</u>GCTGCACCACAGAATC-3'	[4]
4) XlyS_R45T_fw	Binds within *xylS* and inserts R45T point mutation. Sequence: 5'- GATTCTGTGGTGCAGC<u>G</u>TGCCTG-3'	[4]
5) pBTBX_for	Binds at the 3' end of the pBTBX-2 plasmid. 5'- GTTCTAGAAAATTCGTCAACG -3'	This work
6) pBTBX_rev	Binds at the 5' end of the araBAD promoter on the pBTBX-2 plasmid. 5'- CATACCCGTTTTTTTGGGCTAG -3'	This work
7) mCherry_for	Binds at the 5' end of the *mcherry* gene, inserts overhangs for In-Fusion® cloning. Sequence: 5'- **GTTTTTTTGGGCTAG**CAGGAA ACAGGAGGTACC-3'	This work
8) mCherry_rev	Binds at the 3' end of *mcherry* gene, inserts overhangs for In-Fusion® cloning. Sequence: 5'-**GTTGACGAATTTTCTAGAAC**T TACTTGTACAGCTCG-3'	This work

[a] Underlined nucleotides indicate the point mutation used for XylS mutagenesis (AGG → ACG).

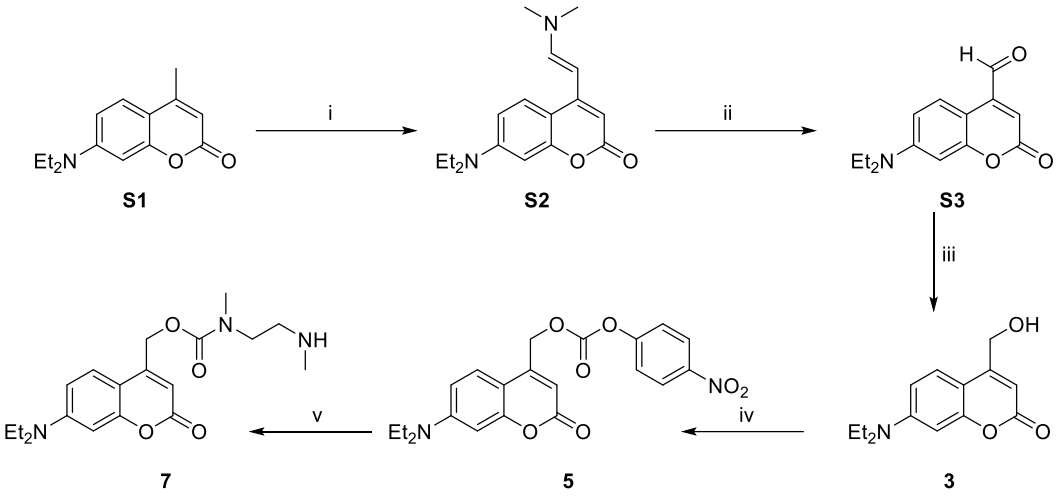
[b] Bold nucleotides indicate the inserted overhangs for In-Fusion® cloning.

S2 General methods for chemical synthesis procedures

All chemicals for synthesis were obtained from commercial suppliers and used without further purification unless stated otherwise. Solvents were reagent grade and were dried as well as purified by common methods. Thin-layer chromatography (TLC) was performed using pre-coated silica gel plates (Polygram® SIL G/UV, Macherey-Nagel) and components were visualized via oxidative staining or UV-light. Flash chromatography was performed on silica gel (Merck silica gel 60 (0.063−0.200 µm) and solvents for flash chromatography (petroleum ether/ethyl acetate/dichloromethane/*n*-pentane) were distilled prior to use. Optical rotation was determined at 20 °C on a Perkin Elmer Polarimeter 241 MC against sodium D-line and melting points were recorded using a Büchi melting point B-545 apparatus. The NMR spectra (^{1}H and ^{13}C) were measured at 20 °C on a Bruker Avance/DRX 600 spectrometer in deuterated solvents ($CDCl_3$, DMSO-d_6, acetone-d_6, D_2O). The chemical shifts are given in ppm relative to the solvent (^{1}H: $CDCl_3$ = 7.26 ppm, ^{1}H: DMSO-d_6 = 3.31 ppm, ^{1}H: acetone-d_6 = 2.05 ppm, ^{1}H: D_2O = 4.79 ppm / ^{13}C: $CDCl_3$ = 77.16 ppm, ^{13}C: DMSO-d_6 = 39.52 ppm, ^{13}C: acetone-d_6 = 29.84 ppm). Signals were assigned by means of H-COSY-, HSQC- and HMBC-experiments and splitting patterns are reported as singlet (s), doublet (d), triplet (t), multiplet (m), and broad singlet (brs). The IR spectra were recorded with a Perkin Elmer SpectrumOne IR-spectrometer ATR (Waltham, USA). HRMS (ESI) spectra were recorded by the centrum of analytics of the Heinrich Heine University. UV-Vis absorption spectra were recorded on a Genesys 10S UV/VIS Spectrophotometer (Thermo Scientific) and uncaging experiments were performed in a quartz cuvette with the LUMOS 43® from Atlas Photonics at 375 nm, 405 nm and 430 nm. Light intensity was quantified using a Thermal Power Sensor (S302C, Thorlabs Inc, USA) and the decay was detected by a Jasco HPLC system [column: Hyperclone 5 µ ODS (C18) 120 (Phenomenex)] combined with an UV/Vis-detector.

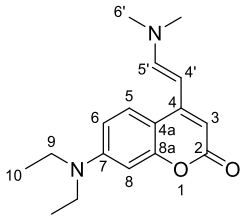
S3 Experimental procedures for the preparation of compounds

S3.1 Synthetic scheme for preparation of coumarin 7



Scheme S1: Synthetic scheme for preparation of coumarin **7**. Reagents and conditions: i) DMF-DMA, DMF, reflux, 23 h; ii) $NaIO_4$, THF/H_2O (1:1), RT, 2 h; iii) $NaBH_4$, EtOH, 0 °C → RT, 4 h; iv) 4-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, RT, 19 h; v) *N*,*N*′-dimethylethylenediamine, CH_2Cl_2, 0 °C, 30 min.

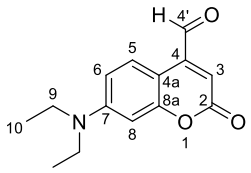
Synthesis of (*E*)-7-(Diethylamino)-4-[2-(dimethylamino)vinyl]-2*H*-chromen-2-one (S2)



Coumarin **S2** was synthesized using a procedure of Weinrich *et al.*[8] Coumarin **S1** (15.0 g, 64.9 mmol) was dissolved in DMF (150 mL). After the addition of DMF-DMA (17.2 mL, 130 mmol) the reaction mixture was heated to reflux for 23 h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 and saturated $NaHCO_3$ solution was added. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a brown solid (18.4 g, 64.3 mmol, 99%). The compound **S2** was used in the following reactions without further purification. The spectroscopic data are consistent with previously reported literature values.[8] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.18 (t, $^3J_{10,9}$ = 7.2 Hz, 6 H, 10-H), 2.98 (s, 6 H, 6′-H), 3.38 (q, $^3J_{9,10}$ = 7.2 Hz, 4 H, 9-H), 5.21 (d, $^3J_{4',5'}$ = 13 Hz, 1 H, 4′-H), 5.84 (s, 1 H, 3-H), 6.47 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.54 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.20 (d, $^3J_{5',4'}$ = 13.0 Hz, 1 H, 5′-H), 7.51 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.6 (C-10), 41.0 (C-6′), 44.7 (C-9), 87.5 (C-4′), 93.5 (C-3), 98.2 (C-8), 108.0 (C-6), 108.2 (C-4a), 124.9 (C-5), 146.7 (C-5′), 150.2 (C-7), 152.4 (C-4), 156.5 (C-8a), 163.5

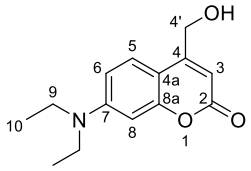
(C-2); R_f = 0.33 (CH_2Cl_2/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 1676, 1606, 1566, 1375, 1234, 1114, 1054, 974, 772, 625; MS (ESI, positive ion): m/z (%) = 287.3 (100) $[M+H]^+$; m.p.: 183 °C.

Synthesis of 7-(Diethylamino)-2-oxo-2*H*-chromene-4-carbaldehyde (S3)



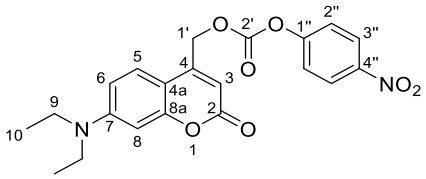
Coumarin **S3** was synthesized using a procedure of Weinrich *et al.*[8] Coumarin **S2** (18.4 g, 64.3 mmol) was dissolved in THF/H_2O (1:1, 110 mL). After the addition of $NaIO_4$ (41.3 g, 193 mmol) the reaction mixture was stirred for 2 h at room temperature. The precipitate was filtered off, washed with ethyl acetate and volatile solvents were removed under reduced pressure and washed with saturated $NaHCO_3$ solution. The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a brown solid (15.7 g, 64.1 mmol, quant.). The compound **S3** was used in the following reactions without further purification. The spectroscopic data are consistent with previously reported literature values.[8] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 3.43 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 6.45 (s, 1 H, 3-H), 6.53 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.63 (dd, $^3J_{6,5}$ = 9.2 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 8.31 (d, $^3J_{5,6}$ = 9.2 Hz, 1 H, 5-H), 10.03 (s, 1 H, 4′-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 45.0 (C-9), 97.9 (C-8), 104.0 (C-4a), 109.8 (C-6), 117.6 (C-3), 127.2 (C-5), 144.0 (C-8a), 151.0 (C-7), 157.5 (C-4), 162.0 (C-2), 192.6 (C-4′); R_f = 0.29 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2972, 1703, 1607, 1582, 1518, 1424, 1376, 1354, 1267, 1228, 1196, 1142, 1111, 1077, 1053, 901, 822, 780, 732, 640, 475; MS (ESI, positive ion): m/z (%) = 278.3 (100) $[M+CH_3OH+H]^+$; m.p.: 77.7 °C.

Synthesis of 7-(Diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-one (3)



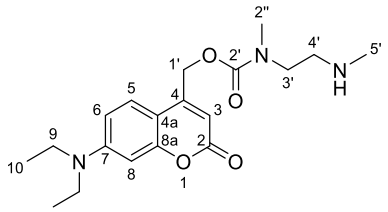
Coumarin **3** was synthesized using a modified procedure of Weinrich *et al.*[8] Coumarin **S3** (5.00 g, 20.4 mmol) was dissolved in ethanol (405 mL) and cooled to 0 °C. After the addition of $NaBH_4$ at 0 °C the reaction mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of 1 M HCl (150 mL) and diluted with H_2O (90.0 mL). The solution was extracted with CH_2Cl_2 (3×100 mL) and the combined organic phase was washed with H_2O (90 mL). Subsequently it was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 50:50) to yield a yellow solid (2.07 g, 8.37 mmol, 41%). The spectroscopic data are consistent with previously reported literature values.[8] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.93 (t, $^3J_{OH,4'}$ = 5.8 Hz, 1 H, 4′-OH), 3.41 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 4.83 (dd, $^3J_{4',OH}$ = 5.8 Hz, $^4J_{4',3}$ = 1.3 Hz, 2 H, 4′-H), 6.25 (t, $^4J_{3,4'}$ = 1.3 Hz, 1 H, 3-H), 6.51 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.56 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.32 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 44.8 (C-9), 60.8 (C-4′), 97.7 (C-8), 105.2 (C-3), 106.4 (C-4a), 108.7 (C-6), 124.5 (C-5), 150.6 (C-7), 155.6 (C-4), 156.1 (C-8a), 163.2 (C-2); R_f = 0.36 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2013, 2001, 1000; MS (ESI, positive ion): m/z (%) = 254.3 (100) $[M+Li]^+$; m.p.: 139.9 °C.

Synthesis of [7-(Diethylamino)-2-oxo-2*H*-chromen-4-yl]methyl (4-nitrophenyl) carbonate (5)



Coumarin **5** was synthesized using modified procedures of Gao *et al.*[9] and Fomina *et al.*[10] Coumarin **3** (2.00 g, 8.09 mmol) was dissolved in dry CH_2Cl_2 (10.0 mL) under nitrogen atmosphere. *N*,*N*-Diisopropylethylamine (DIPEA) (2.82 mL, 16.2 mmol) was added and the reaction mixture was stirred for 15 min before 4-nitrophenyl chloroformate (3.26 g, 16.2 mmol) dissolved in dry CH_2Cl_2 (10 mL) was added over 2 h via a syringe pump. The reaction mixture was stirred for 19 h and diluted with CH_2Cl_2. It was washed with 1 M HCl (20 mL) and saturated $NaHCO_3$ solution (3×20 mL). The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (CH_2Cl_2/*n*-pentane 98:2) to yield a yellow solid (1.48 g, 3.60 mmol, 44%). The spectroscopic data are consistent with previously reported literature values.[9] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 3.43 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 5.40 (s, 2 H, 1′-H) 6.22 (s, 1 H, 3-H), 6.54 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.61 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.31 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H), 7.42 (d, $^3J_{2'',3''}$ = 8.6 Hz, 2 H, 2″-H), 8.30 (d, $^3J_{3'',2''}$ = 8.6 Hz, 2 H, 3″-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 44.9 (C-9), 65.9 (C-1′), 98.0 (C-8), 105.7 (C-4a), 107.0 (C-3), 108.9 (C-6), 121.8 (C-2″), 124.4 (C-5), 125.5 (C-3″), 145.7 (C-4″), 147.9 (C-4), 151.0 (C-7), 152.3 (C-2′), 155.3 (C-1″), 156.5 (C-8a), 161.7 (C-2); R_f = 0.10 (CH_2Cl_2/*n*-pentane 98:2); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2968, 1772, 1708, 1591, 1520, 1489, 1446, 1423, 1335, 1268, 1218, 1194, 1142, 1109, 1090, 1040, 986, 956, 856, 838, 816, 792, 750, 704, 679, 567, 528, 493, 467; HRMS (ESI): m/z calculated for $C_{21}H_{21}N_2O_7^+$ $[M+H]^+$: 413.1343; found: 413.1340; m.p.: 159.9 °C.

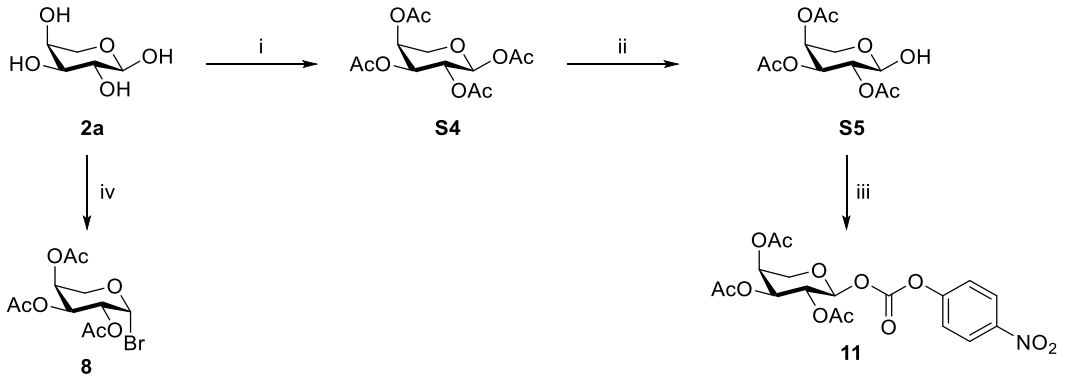
Synthesis of [7-(Diethylamino)-2-oxo-2*H*-chromen-4-yl]methyl methyl[2-(methylamino)ethyl]carbamate (7)



Coumarin **7** was synthesized using a modified procedure of Fomina *et al.*[10] Coumarin **5** (50.0 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (1.30 mL). It was added dropwise over 30 min to a solution of *N*,*N*′-dimethylethylenediamine (0.14 mL, 1.21 mmol) in CH_2Cl_2 (2.6 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. The solvent was removed under reduced pressure, the residue was

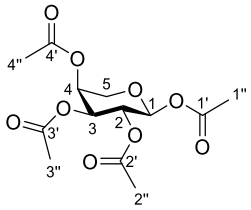
dissolved in ethyl acetate and washed subsequently with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (ethyl acetate/methanol/triethylamine 87:10:3) to yield a viscous dark yellow oil (27.0 mg, 74.7 µmol, 62%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.42–2.51 (m, 4 H, 5′-H, NH), 2.80 (m_c, 2 H, 4′-H), 2.95–3.04 (m, 3 H, 2″-H), 3.39 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.44–3.49 (m, 2 H, 3′-H), 5.23 (s, 2 H, 1′-H), 6.10 (s, 1 H, 3-H), 6.49 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.55 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.27–7.31 (m, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 34.8, 35.5 (C-2″), 36.1, 36.4 (C-5′), 44.8 (C-9), 48.6, 48.9 (C-3′), 49.3, 49.7 (C-4′), 62.5, 62.6 (C-1′), 97.9 (C-8), 105.8 (C-4a), 106.1, 106.2 (C-3), 108.7 (C-6), 124.4, 124.5 (C-5), 150.6, 150.7 (C-4, C-7), 155.6, 155.9 (C-2′), 156.3 (C-8a), 162.2 (C-2); R_f = 0.15 (EtOAc/MeOH/NEt_3 87:10:3); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2960, 2926, 2859, 1709, 1605, 1526, 1422, 1358, 1275, 1197, 1143, 1078, 821, 767; HRMS (ESI): m/z calculated for $C_{19}H_{28}N_3O_4^+$ $[M+H]^+$: 362.2074; found: 362.2077.

S3.2 Synthetic scheme for preparation of carbohydrates 8 and 11



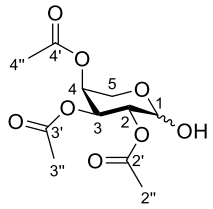
Scheme S2: Synthetic scheme for preparation of carbohydrates **8** and **11**. Reagents and conditions: i) Ac_2O, DMAP, pyridine, 0 °C → RT, 18 h; ii) AcOH, ethylenediamine, THF, RT, 24 h; iii) 4-nitrophenyl chloroformate, 2,6-lutidine, MeCN, RT, 18 h; iv) Ac_2O, HBr (33 wt%) in AcOH, RT, 3 h.

Synthesis of L-Arabinopyranose tetraacetate (S4)



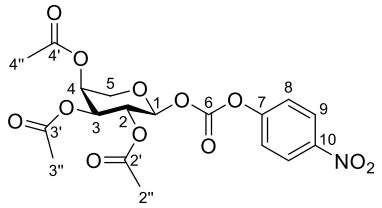
α-L-Arabinopyranose tetraacetate (**S4**) was synthesized using a procedure of Wahler *et al.*[11] α-L-Arabinopyranose (**2a**) (1.00 g, 6.66 mmol) was dissolved in dry pyridine (5.00 mL) and cooled to 0 °C. After the addition of acetic anhydride (5.04 mL, 53.3 mmol) and 4-dimethylaminopyridine (DMAP) (325 mg, 2.66 mmol) the reaction mixture was stirred for 18 h with the temperature slowly rising to room temperature. The reaction was quenched and diluted by addition of water and ethyl acetate. The organic phase was separated and washed with water as well as saturated NaCl solution. Subsequently it was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. Repeated coevaporation with toluene under reduced pressure yielded a colourless solid (1.77 g, 5.58 mmol, 84%). The spectroscopic data are consistent with previously reported literature values.[11] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.01, 2.01, 2.13, 2.14 (s, 12 H, 1″-H, 2″-H, 3″-H, 4″-H), 3.81 (dd, $^2J_{5a,5b}$ = 13.2 Hz, $^3J_{5a,4}$ = 2.0 Hz, 1 H, 5-H_a), 4.05 (dd, $^2J_{5b,5a}$ = 13.2 Hz, $^3J_{5a,4}$ = 1.5 Hz, 1 H, 5-H_b), 5.28–5.39 (m, 3 H, 2-H, 3-H, 4-H), 6.33 (d, $^3J_{1,2}$ = 3.1 Hz, 1 H, 1-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 20.7, 20.8, 21.0, 21.0 (C-1″, C-2″, C-3″, C-4″), 62.9 (C-5), 66.8 (C-3 or C-4), 67.1 (C-2), 68.5 (C-3 or C-4), 90.3 (C-1), 169.2 (C-1′), 170.0, 170.2, 170.4 (C-2′, C-3′, C-4′); R_f = 0.63 (PE/EtOAc 1:1); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 1736, 1372, 1212, 1137, 1113, 1065, 1010, 942, 894, 755, 602, 552, 471; MS (ESI, positive ion): m/z (%) = 341.0 (100) $[M+Na]^+$, 357.0 (15) $[M+K]^+$; m.p.: 94.4 °C; $[\alpha]_D^{20}$ = 151.8 (c = 0.5, $CHCl_3$).

Synthesis of 2,3,4-Tri-*O*-acetyl-α,β-L-arabinopyranose (S5)



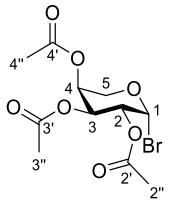
(2,3,4-tri-*O*-acetyl)-α,β-L-arabinopyranose (**S5**) was synthesized using a procedure of Duléry *et al*.[12] Glacial acetic acid (1.01 mL, 17.6 mmol) was added dropwise to a solution of ethylenediamine (1.01 mL, 15.1 mmol) in THF (250 mL), which immediately lead to the formation of a precipitate. L-arabinopyranose tetraacetate (**S4**) (4.00 g, 12.6 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. After the addition of water, the reaction mixture was extracted with CH_2Cl_2. The organic phase was subsequently washed with 1 M HCl, saturated $NaHCO_3$ solution and water. Following this, the organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 55:45) to yield (2,3,4-tri-*O*-acetyl)-α,β-L-arabinopyranose (**S5**) as a colorless oil (2.28 g, 8.26 mmol, 66%) in α:β ratio of 1:3. The spectroscopic data are consistent with previously reported literature values.[13] α/β-Anomers: R_f = 0.18 (PE/EtOAc 1:1); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3455, 1739, 1371, 1216, 1139, 1057, 1007, 936, 889, 765, 736, 603, 465; MS (ESI, positive ion): m/z (%) = 299.0 (100) $[M+Na]^+$; α-Anomer: ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.00 (s, 3 H, CH_3), 2.08 (s, 3 H, CH_3), 2.13 (s, 3 H, CH_3), 3.66 (dd, $^2J_{5a,5b}$ = 13.4 Hz, $^3J_{5a,4}$ = 1.1 Hz, 1 H, 5-H_a), 4.01 (dd, $^2J_{5b,5a}$ = 13.4 Hz, $^3J_{5a,4}$ = 2.5 Hz, 1 H, 5-H_b), 4.60 (d, $^3J_{1,2}$ = 6.7 Hz, 1 H, 1-H), 5.03–5.09 (m, 2 H, 2-H and 3-H), 5.24–5.27 (m, 1 H, 4-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 20.7, 20.9, 21.0 (C-2″, C-3″, C-4″), 64.2 (C-5), 68.1 (C-4), 70.2 (C-2), 71.3 (C-3), 96.2 (C-1), 170.2, 170.5, 171.2 (C-2′, C-3′, C-4′); β-Anomer: ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.00 (s, 3 H, 4″-H), 2.08 (s, 3 H, 2″-H or 3″-H), 2.12 (s, 3 H, 2″-H or 3″-H), 3.59 (brs, 1 H, 1-OH), 3.68 (dd, $^2J_{5a,5b}$ = 13.1 Hz, $^3J_{5a,4}$ = 2.3 Hz, 1 H, 5-H_a), 4.18 (dd, $^2J_{5b,5a}$ = 13.1 Hz, $^3J_{5a,4}$ = 1.5 Hz, 1 H, 5-H_b), 5.16 (dd, $^3J_{3,2}$ = 10.5 Hz, $^3J_{3,4}$ = 3.4 Hz, 1 H, 3-H), 5.33–5.36 (m, 1 H, 4-H), 5.38 (dd, $^3J_{2,3}$ = 10.5 Hz, $^3J_{2,1}$ = 3.5 Hz, 1 H, 2-H), 5.45 (d, $^3J_{1,2}$ = 3.5 Hz, 1 H, 1-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 20.8 (C-4″), 20.9 (C-2″ or C-3″), 21.0 (C-2″ or C-3″), 60.4 (C-5), 67.0 (C-2), 68.8 (C-3), 69.2 (C-4), 91.0 (C-1), 170.3 (C-4′), 170.6 (C-2′), 170.6 (C-3′).

Synthesis of 2,3,4-Tri-*O*-acetyl-1-*O*-(4-nitrophenyloxycarbonyl)-α-L-arabinopyranoside (11)



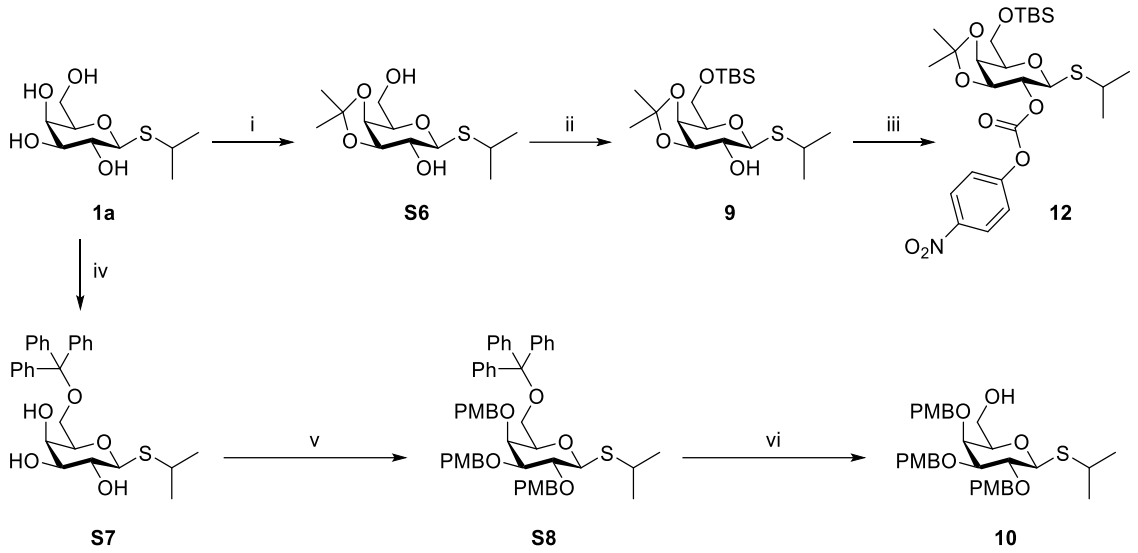
Carbohydrate **11** was synthesized using a modified procedure of André *et al*.[14] The anomeric mixture of carbohydrate **S5** (408 mg, 1.48 mmol) was dissolved in MeCN (49.0 mL) and cooled to 0 °C. After the addition of 4-nitrophenyl chloroformate (316 mg, 1.57 mmol) and 2,6-lutidine (0.18 mL, 1.57 mmol) the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was quenched by addition of water and diluted with CH_2Cl_2. The organic phase was washed with water and saturated NaCl solution. Following this, the organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 60:40) to mainly yield the α-anomer as a colorless solid (133 mg, 302 µmol, 20%). Unreacted substrate was reisolated as an anomeric mixture with α:β ratio of 1:1.8. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.10 (s, 3 H, 3″-H), 2.14 (s, 3 H, 4″-H), 2.14 (s, 3 H, 2″-H), 3.83 (dd, $^2J_{5a,5b}$ = 12.5 Hz, $^3J_{5a,4}$ = 2.8 Hz, 1 H, 5-H$_a$), 4.18 (dd, $^2J_{5b,5a}$ = 12.5 Hz, $^3J_{5a,4}$ = 5.1 Hz, 1 H, 5-H$_b$), 5.19 (dd, $^3J_{3,2}$ = 7.8 Hz, $^3J_{3.4}$ = 3.4 Hz, 1 H, 3-H), 5.31–5.36 (m, 2 H, 2-H, 4-H), 5.69 (d, $^3J_{1,2}$ = 5.6 Hz, 1 H, 1-H), 7.41 (d, $^3J_{8,9}$ = 9.1 Hz, 2 H, 8-H), 8.29 (d, $^3J_{9,8}$ = 9.1 Hz, 2 H, 9-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 20.8, 20.9, 21.0 (C-2″, C-3″, C-4″), 62.8 (C-5), 66.3 (C-4), 67.9 (C-2), 69.0 (C-3), 96.1 (C-1), 121.8 (C-8), 125.5 (C-9), 145.8 (C-10), 151.1 (C-6), 155.1 (C-7), 169.3 (C-2′), 170.0 (C-3′), 170.1 (C-4′); R_f = 0.28 (PE/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 1746, 1594, 1527, 1492, 1370, 1346, 1217, 1173, 1087, 1055, 965, 913, 860, 729, 599, 504; HRMS (ESI): m/z calculated for $C_{18}H_{23}N_2O_{12}^+$ $[M+NH_4]^+$: 459.1246; found: 459.1247; m.p.: 52–58 °C; $[\alpha]_D^{20}$ = -3 (c = 1.0, $CHCl_3$).

Synthesis of 2,3,4-Tri-*O*-acetyl-β-L-arabinopyranosyl bromide (8)



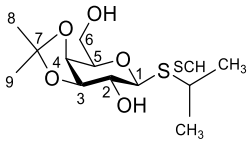
The carbohydrate **8** was synthesized using a procedure of Kartha *et al*.[15] α-L-Arabinopyranose (**2a**) (1.00 g, 6.66 mmol) was dissolved in acetic anhydride (5.04 mL, 53.3 mmol) and stirred at room temperature. HBr solution 33 wt% in AcOH (1.50 mL, 8.57 mmol) was added to the suspension. After the solid was completely dissolved (1 h) additional HBr solution 33 wt% in AcOH (7.5 mL, 42.9 mmol) was added and the reaction mixture was stirred for additional 2 h. Subsequently the reaction mixture was concentrated under reduced pressure. Toluene (3×20 ml) was added and removed under reduced pressure. The crude product was recrystallized from Et_2O to yield a colorless solid (926 mg, 2.73 mmol, 41%). The spectroscopic data are consistent with previously reported literature values.[16] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.03 (s, 3 H, CH_3), 2.11 (s, 3 H, CH_3), 2.15 (s, 3 H, CH_3), 3.93 (dd, $^2J_{5a,5b}$ = 13.3 Hz, $^3J_{5a,4}$ = 1.7 Hz, 1 H, 5-H_a), 4.21 (d, $^2J_{5b,5a}$ = 13.3 Hz, 1 H, 5-H_b), 5.09 (ddd, $^3J_{2,3}$ = 11.8 Hz, $^3J_{2,1}$ = 3.9 Hz, $^3J_{2,4}$ = 1.6 Hz, 1 H, 2-H), 5.37–5.43 (m, 2 H, 3-H, 4-H), 6.70 (d, $^3J_{1,2}$ = 3.9 Hz, 1 H, 1-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 20.8, 20.9, 21.0 (C-2″, C-3″, C-4″), 64.9 (C-5), 67.8, 68.0 (C-3, C-4), 68.1 (C-2), 89.8 (C-1), 169.9, 170.2, 170.2 (C-2′, C-3′, C-4′).; R_f = 0.70 (PE/EtOAc 1:1); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 1734, 1375, 1211, 1098, 1069, 1043, 992, 929, 891, 685, 577, 601, 539, 473; m.p.: 115.6 °C; $[\alpha]_D^{20}$ = 153.3 (c = 1.0, $CHCl_3$)

S3.3 Synthetic scheme for preparation of carbohydrates 10 and 12



Scheme S3: Synthetic scheme for preparation of carbohydrates **10** and **12**. Reagents and conditions: i) 2,2-dimethoxypropane, CSA, acetone, RT, 8 h; ii) TBS-Cl, pyridine, RT, 20 h; iii) 4-nitrophenyl chloroformate, pyridine, RT, 20 h; iv) TrCl, DMAP, pyridine, RT, 18 h; v) PMB-Cl, NaH, DMF, RT, 18 h; vi) CSA, MeOH, CH_2Cl_2, 0 °C → RT, 48 h.

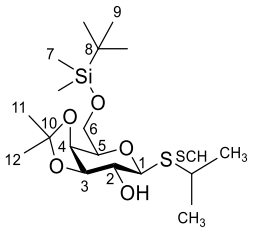
Synthesis of Isopropyl 3,4-*O*-(1-methylethylidene)-1-thio-β-D-galactopyranoside (S6)



Carbohydrate **S6** was synthesized using a modified procedure of Du *et al.*[17] Isopropyl β-D-1-thiogalactopyranoside (**1a**) (5.00 g, 19.9 mmol) was dissolved in acetone (147 mL). After the addition of camphorsulfonic acid (CSA) (945 mg, 3.99 mmol) and 2,2-dimethoxypropane (3.66 mL, 29.9 mmol) the reaction mixture was stirred for 8 h at room temperature. The reaction mixture was quenched by addition of $NaHCO_3$ and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and subsequently washed with $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×) and the combined organic phases were washed with saturated NaCl solution. Following this, the organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 50:50) to yield a white solid (3.90 g, 14.0 mmol, 70%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.22 (d, $^3J_{CH3,SCH}$ = 6.9 Hz, 3 H, CH_3), 1.23 (d, $^3J_{CH3,SCH}$ = 6.9 Hz, 3 H, CH_3), 1.25 (s, 3 H, 7-H or 8-H), 1.38 (s, 3 H, 7-H or 8-H), 3.14 (septet, $^3J_{SCH,CH3}$ = 6.9 Hz, 1 H, SCH), 3.23 (ddd, $^3J_{2,1}$ = 9.9 Hz, $^3J_{2,3}$ = 6.6 Hz, $^3J_{2,2\text{-OH}}$ = 6.1 Hz, 1 H, 2-H), 3.51 (m, 2 H, 6-H), 3.73 (td, $^3J_{5,6}$ = 6.3 Hz, $^3J_{5,4}$ = 2.0 Hz, 1 H, 5-H), 3.92 (dd, $^3J_{3,2}$ = 6.6 Hz, $^3J_{3,4}$ = 5.6 Hz, 1 H, 3-H), 4.14 (dd, $^3J_{4,3}$ = 5.6 Hz, $^3J_{4,5}$ = 2.0 Hz, 1 H, 4-H), 4.35 (d, $^3J_{1,2}$ = 9.9 Hz, 1 H, 1-H), 4.73 (t, $^3J_{6\text{-OH},6}$ = 5.6 Hz, 1 H, 6-OH), 5.29 (d, $^3J_{2\text{-OH},2}$ = 6.1 Hz, 1 H, 2-OH);

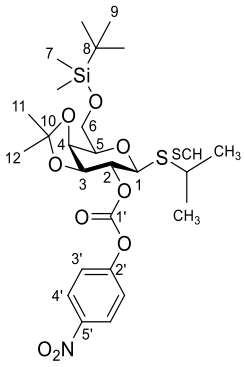
^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 23.7 (CH_3), 23.8 (CH_3), 26.4, 28.2 (C-7, C-8), 33.4 (SCH), 60.6 (C-6), 71.8 (C-2), 73.4 (C-4), 76.5 (C-5), 79.5 (C-3), 83.9 (C-1), 108.4 (C-7); R_f = 0.23 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3301, 2990, 2924, 2864, 1460, 1369, 1239, 1215, 1141, 1073, 1020, 962, 872, 840, 725, 641, 570, 536, 504; HRMS (ESI): m/z calculated for $C_{12}H_{26}NO_5S^+$ $[M+NH_4]^+$: 296.1526; found: 296.1527; m.p.: 89.8 °C; $[\alpha]_D^{20}$ = 3.4 (c = 1.0, $CHCl_3$)

Synthesis of Isopropyl 6-*O*-(*tert*-butyldimethylsilyl)-3,4-*O*-(1-methylethylidene)-1-thio-β-D-galactopyranoside (9)



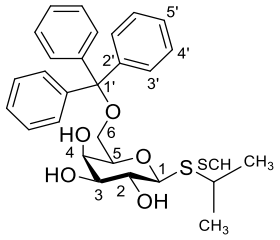
Carbohydrate **9** was synthesized using a modified procedure of Du *et al*.[17] Carbohydrate **S6** (1.00 g, 3.59 mmol) was dissolved in dry pyridine (20.0 mL) under nitrogen atmosphere. *Tert*-butyldimethylsilyl chloride (TBS-Cl) (1.14 g, 7.54 mmol) was added portion wise and the reaction mixture was stirred for 20 h at room temperature. After completion, the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and subsequently washed with water. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 85:15) to yield a white solid (1.05 g, 2.67 mmol, 74%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 0.04 (s, 3 H, 7-H), 0.05 (s, 3 H, 7-H), 0.86 (s, 9 H, 9-H), 1.22 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.23 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.24 (s, 3 H, 11-H or 12-H), 1.38 (s, 3 H, 11-H or 12-H), 3.13 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.23 (dd, $^3J_{2,1}$ = 9.9 Hz, $^3J_{2,3}$ = 7.0 Hz, 1 H, 2-H), 3.66 (dd, $^2J_{6a,6b}$ = 10.4 Hz, $^3J_{6a,5}$ = 7.2 Hz, 1 H, 6-H$_a$), 3.71 (dd, $^2J_{6b,6a}$ = 10.4 Hz, $^3J_{6b,5}$ = 5.4 Hz, 1 H, 6-H$_b$), 3.81 (ddd, $^3J_{5,6a}$ = 7.2 Hz, $^3J_{5,6b}$ = 5.4 Hz, $^3J_{5,4}$ = 2.0 Hz, 1 H, 5-H), 3.93 (dd, $^3J_{3,2}$ = 7.0 Hz, $^3J_{3,4}$ = 5.4 Hz, 1 H, 3-H), 4.14 (dd, $^3J_{4,3}$ = 5.4 Hz, $^3J_{4,5}$ = 2.0 Hz, 1 H, 4-H), 4.37 (d, $^3J_{1,2}$ = 9.9 Hz, 1 H, 1-H), 5.28 (brs, 1 H, 2-OH); ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = –5.6 (C-7), –5.4 (C-7), 17.9 (C-8), 23.7 (CH_3), 23.8 (CH_3), 25.6 (C-9), 26.3, 28.1 (C-11, C-12), 33.5 (SCH), 62.3 (C-6), 71.7 (C-2), 73.2 (C-4), 76.0 (C-5), 79.5 (C-3), 83.8 (C-1), 108.5 (C-10); R_f = 0.62 (PE/EtOAc 60:40); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3407, 2954, 2929, 2857, 1472, 1385, 1358, 1223, 1241, 1141, 1110, 1075, 1025, 962, 876, 836, 772, 714, 582, 532, 477; HRMS (ESI): m/z calculated for $C_{18}H_{37}O_5SSi^+$ $[M+H]^+$: 393.2125; found: 393.2121; m.p.: 45.3 °C; $[\alpha]_D^{20}$ = –15.5 (c = 1.0, $CHCl_3$)

Synthesis of Isopropyl 6-*O*-(*tert*-butyldimethylsilyl)-3,4-*O*-(1-methylethylidene)-2-*O*-(4-nitrophenyloxycarbonyl)-1-thio-β-D-galactopyranoside (12)



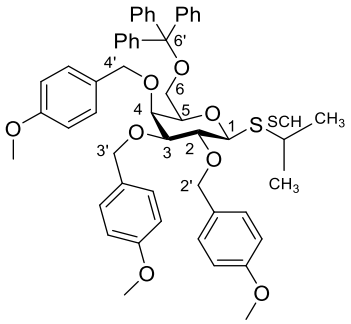
Carbohydrate **9** (1.00 g, 2.55 mmol) was dissolved in dry pyridine (20.0 mL) under nitrogen atmosphere. 4-nitrophenyl chloroformate (2.26 g, 11.2 mmol) was added portion wise and the reaction mixture was stirred for 20 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 85:15) to yield a white solid (1.23 g, 2.21 mmol, 87%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 0.08 (s, 6 H, 7-H), 0.89 (s, 9 H, 9-H), 1.33 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.33 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.36 (s, 3 H, 11-H or 12-H), 1.56 (s, 3 H, 11-H or 12-H), 3.21 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.81–3.93 (m, 3 H, 5-H, 6-H), 4.27 (dd, $^3J_{3,2}$ = 7.3 Hz, $^3J_{3,4}$ = 5.2 Hz, 1 H, 3-H), 4.32 (dd, $^3J_{4,3}$ = 5.2 Hz, $^3J_{4,5}$ = 1.4 Hz, 1 H, 4-H), 4.53 (d, $^3J_{1,2}$ = 10.4 Hz, 1 H, 1-H), 4.85 (dd, $^3J_{2,1}$ = 10.4 Hz, $^3J_{2,3}$ = 7.3 Hz, 1 H, 2-H), 7.37–7.45 (m, 2 H, 2′-H), 8.21–8.31 (m, 2 H, 3′-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = –5.4 (C-7), –5.2 (C-7), 18.4 (C-8), 24.1 (CH_3), 24.2 (CH_3), 25.9 (C-9), 26.5, 28.0 (C-11, C-12), 35.8 (SCH), 62.1 (C-6), 73.6 (C-4), 76.8 (C-3), 77.3 (C-5), 77.8 (C-2), 82.4 (C-1), 110.8 (C-10), 122.0 (C-2′), 125.4 (C-3′), 145.6 (C-4′), 152.1 (C-2′), 155.7 (C-1′); R_f = 0.73 (PE/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2954, 2929, 2853, 1768, 1617, 1594, 1519, 1492, 1464, 1374, 1345, 1307, 1247, 1215, 1164, 1117, 1072, 989, 957, 878, 836, 774, 751, 707, 674, 641, 573, 536, 499; HRMS (ESI): m/z calculated for $C_{25}H_{40}NO_9SSi^+$ $[M+H]^+$: 558.2188; found: 558.2186; m.p.: 135.0 °C; $[\alpha]_D^{20}$ = 48.8 (c = 1.0, $CHCl_3$)

Synthesis of Isopropyl 6-*O*-trityl-1-thio-β-D-galactopyranoside (S7)



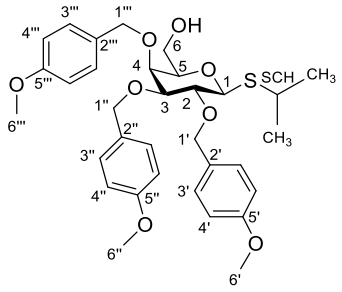
Carbohydrate **S7** was synthesized using a modified procedure of Du *et al.*[18] Isopropyl β-D-1-thiogalactopyranoside (**1a**) (5.00 g, 21.0 mmol) was dissolved in dry pyridine (5 mL) under nitrogen atmosphere. Trityl chloride (TrCl) (11.7 g, 42.0 mmol) and 4-dimethylaminopyridine (DMAP) (256 mg, 2.10 mmol) were added and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with ethyl acetate and subsequently washed with water (3×), saturated $NaHCO_3$ solution (1×) and saturated NaCl solution (1×). The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 50:50) to yield a white solid (7.68 g, 16.0 mmol, 76%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.28 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.31 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 2.93 (dd, $^2J_{6a,6b}$ = 9.7 Hz, $^3J_{6a,5}$ = 3.7 Hz, 1 H, 6-H_a), 3.20–3.28 (m, 2 H, SCH, 6-H_b), 3.28–3.32 (m, 2 H, 2-H, 3-H), 3.56 (brs, 1 H, 4-H), 3.61 (dd, ^3J = 7.7 Hz, $^3J_{5,6a}$ = 3.7 Hz, 1 H, 5-H), 4.36–4.39 (m_c, 1 H, 1-H), 4.42 (d, $^3J_{4\text{-}OH,4}$ = 4.5 Hz, 1 H, 4-OH), 4.82 (d, $^3J_{3\text{-}OH,3}$ = 4.6 Hz, 1 H, 3-OH), 4.92 (d, $^3J_{2\text{-}OH,2}$ = 5.2 Hz, 1 H, 2-OH), 7.25 (t, $^3J_{5',4'}$ = 7.2 Hz, 3 H, 5′-H), 7.32 (t, $^3J_{4',5'}$ = 7.6 Hz, 6 H, 4′-H), 7.41 (d, $^3J_{3',4'}$ = 7.4 Hz, 6 H, 3′-H); ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 23.7 (CH_3), 23.8 (CH_3), 33.6 (SCH), 64.0 (C-6), 69.3 (C-4), 69.8 (C-2), 74.5 (C-3), 77.6 (C-5), 84.8 (C-1), 85.7 (C-1′), 126.9 (C-5′), 127.8 (C-4′), 128.3 (C-3′), 143.9 (C-2′); R_f = 0.11 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3400, 3062, 2925, 2870, 1736, 1594, 1490, 1448, 1368, 1240, 1152, 1060, 1030, 899, 872, 833, 766, 746, 701, 650, 632, 584; HRMS (ESI): m/z calculated for $C_{28}H_{36}NO_5S^+$ $[M+NH_4]^+$: 498.2309; found: 498.2311; m.p.: 68.8 °C; $[\alpha]_D^{20}$ = –23.5 (c = 1.0, $CHCl_3$)

Synthesis of Isopropyl 2,3,4-tri-*O*-(4-methoxybenzyl)-6-*O*-trityl-1-thio-β-D-galactopyranoside (S8)



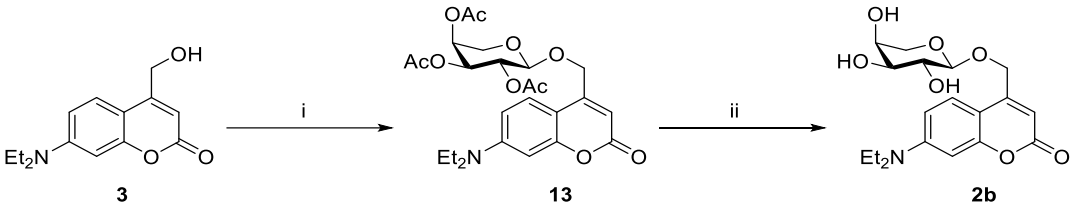
Carbohydrate **S8** was synthesized using modified procedures of Ruda *et al*.[19] Carbohydrate **S7** (100 mg, 208 µmol) and 4-methoxybenzyl chloride (PMB-Cl) (126 µL, 936 mmol) was dissolved in dry DMF (2.00 mL) under nitrogen atmosphere. The mixture was added dropwise over 30 min via a syringe pump to NaH (60%, 37.5 mg, 936 µmol) in dry DMF (2.00 mL) and was stirred for 18 h at room temperature. After completion, the reaction mixture was cooled to 0 °C and quenched by the addition of MeOH. Ethyl acetate was added, and the mixture was washed with water (3×) and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 90:10 to ethyl acetate/methanol 90:10) to yield a white solid (129 mg, 153 µmol, 74%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.27 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.30 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 2.82 (dd, $^2J_{6a,6b}$ = 9.5 Hz, $^3J_{6a,5}$ = 4.5 Hz, 1 H, 6-H_a), 3.17–3.28 (m, 2 H, 6-H_b, SCH), 3.40 (t, $^3J_{2,3}$ = 9.4 Hz, 1 H, 2-H), 3.63 (dd, $^3J_{3,2}$ = 9.4 Hz, $^3J_{3,4}$ = 2.9 Hz, 1 H, 3-H), 3.70–3.74 (m, 1 H, 5-H), 3.72 (s, 3 H, OCH_3), 3.73 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 3.88 (d, $^3J_{4,3}$ = 2.9 Hz, 1 H, 4-H), 4.23 (d, $^2J_{4'a,4'b}$ = 11.0 Hz, 1 H, 4-H_a), 4.53–4.62 (m, 5 H, 1-H, 2′-H, 3′-H_a, 4′-H_b), 4.67 (d, $^2J_{3'b,3'a}$ = 11.4 Hz, 1 H, 3′-H_b), 6.72–6.79 (m, 2 H, arom. H), 6.85–6.92 (m, 6 H, arom. H), 7.20–7.25 (m, 2 H, arom. H), 7.25–7.41 (m, 17 H, arom. H); ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 23.7 (CH_3), 23.7 (CH_3), 34.3 (SCH), 55.0 (OCH_3), 63.6 (C-6), 71.2 (C-3′), 73.3 (C-4′), 73.9 (C-4), 74.1 (C-2′), 76.7 (C-5), 77.8 (C-2), 82.8 (C-3), 83.3 (C-1), 85.9 (C-1′), 113.4 (arom. C), 113.6 (arom. C), 127.0 (arom. C), 127.9 (arom. C), 128.2 (arom. C), 129.3 (arom. C), 129.3 (arom. C), 129.4 (arom. C), 130.4 (arom. C), 130.5 (arom. C), 130.6 (arom. C), 143.7 (arom. C), 158.6 (arom. C), 158.7 (arom. C), 158.7 (arom. C); R_f = 0.05 (PE/EtOAc 90:10); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3038, 2931, 2835, 1612, 1586, 1512, 1449, 1360, 1302, 1245, 1173, 1152, 1078, 1031, 899, 820, 747, 705, 650, 632, 600, 515; HRMS (ESI): m/z calculated for $C_{52}H_{60}NO_8S^+$ $[M+NH_4]^+$: 858.4034; found: 858.4031; m.p.: 53.4 °C; $[\alpha]_D^{20}$ = 6.4 (c = 1.0, $CHCl_3$)

Synthesis of Isopropyl 2,3,4-tri-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (10)



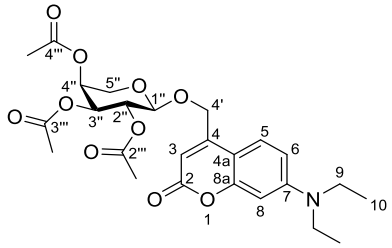
Carbohydrate **10** was synthesized using modified procedures of Ruda *et al*.[19] Carbohydrate **S8** (1.28 g, 1.52 mmol) was dissolved in dry CH_2Cl_2 (63 mL) under nitrogen atmosphere and cooled to 0 °C. Camphorsulfonic acid (CSA) (37.6 mg, 167 µmol) in methanol (6.3 mL) was added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred for 48 h. After completion, the solvent was evaporated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 70:30 to petroleum ether/ethyl acetate 30:70) to yield a white solid (662 mg, 1.11 mmol, 73%). The spectroscopic data are consistent with previously reported literature values.[20] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.32 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.33 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 3.21 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.34–3.38 (m, 1 H, 5-H), 3.40 (ddd, $^2J_{6a,6b}$ = 11.1 Hz, $^3J_{6a,5}$ = 8.2 Hz, $^3J_{6a,6\text{-}OH}$ = 5.1 Hz, 1 H, 6-H_a), 3.53 (dd, $^3J_{3,2}$ = 9.3 Hz, $^3J_{3,4}$ = 2.8 Hz, 1 H, 3-H), 3.70–3.79 (m, 3 H, 2-H, 4-H, 6-H_b), 3.80 (s, 6 H, OCH_3), 3.82 (s, 3 H, OCH_3), 4.46 (d, $^3J_{1,2}$ = 9.7 Hz, 1 H, 1-H), 4.59 (d, $^2J_{1‴a,1‴b}$ = 11.7 Hz, 1 H, 1‴-H_a), 4.68 (d, $^2J_{1″a,1″b}$ = 11.4 Hz, 1 H, 1″-H_a), 4.71 (d, $^2J_{1′a,1′b}$ = 9.8 Hz, 1 H, 1′-H_a), 4.72 (d, $^2J_{1″b,1″a}$ = 11.4 Hz, 1 H, 1″-H_b), 4.82 (d, $^2J_{1′b,1′a}$ = 9.8 Hz, 1 H, 1′-H_b), 4.87 (d, $^2J_{1‴b,1‴a}$ = 11.7 Hz, 1 H, 1‴-H_b), 6.86 (d, $^3J_{4′,3′}$ = 8.5 Hz, 2 H, 4′-H), 6.86 (d, $^3J_{4‴,3‴}$ = 8.5 Hz, 2 H, 4‴-H), 6.89 (d, $^3J_{4″,3″}$ = 8.5 Hz, 2 H, 4″-H), 7.25 (d, $^3J_{3‴,4‴}$ = 8.5 Hz, 2 H, 3‴-H), 7.31 (d, $^3J_{3″,4″}$ = 8.5 Hz, 2 H, 3″-H), 7.33 (d, $^3J_{3′,4′}$ = 8.5 Hz, 2 H, 3′-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 24.1 (CH_3), 35.4 (CH_3), 55.4 (SCH), 55.4, 55.4 (C-6′, C-6″, C-6‴), 62.4 (C-6), 72.6 (C-4), 73.0 (C-1″), 73.6 (C-1‴), 75.6 (C-1′), 78.6 (C-2), 78.7 (C-5), 84.1 (C-3), 85.2 (C-1), 113.8, 113.9, 114.0 (C-4′, C-4″, C-4‴), 129.4 (C-3″), 130.1 (C-3‴), 130.2 (C-3′), 130.5, 130.6, 130.7 (C-2′, C-2″, C-2‴), 159.4, 159.4, 159.5 (C-5′, C-5″, C-5‴).; R_f = 0.09 (PE/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3355, 2948, 2910, 2864, 2841, 1614, 1585, 1513, 1462, 1360, 1302, 1249, 1169, 1136, 1097, 1081, 1050, 1028, 996, 875, 819, 776, 700, 636, 603, 568, 515; HRMS (ESI): m/z calculated for $C_{33}H_{46}NO_8S^+$ $[M+NH_4]^+$: 616.2939; found: 616.2945; m.p.: 137.2 °C; $[\alpha]_D^{20}$ = –12.7 (c = 1.0, $CHCl_3$).

S3.4 Synthetic scheme for preparation of photocaged arabinose 2b



Scheme S4: Synthetic scheme for preparation of photocaged arabinose **2b**. Reagents and conditions: i) **8**, AgOTf, CH_2Cl_2, RT, 22 h; ii) NH_3 in MeOH (7 M), MeOH, RT.

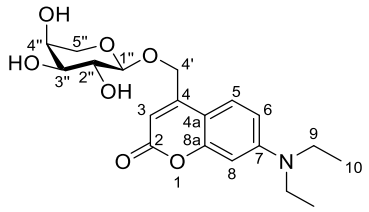
Synthesis of 2,3,4-Tri-*O*-acetyl-1-*O*-{[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methyl}-α-L-arabinopyranose (13)



Coumarin **13** was synthesized using a modified procedure of Binder *et al.*[16] A Schlenk tube was charged with 500 mg molecular sieve (5 Å) and carbohydrate **8** (200 mg, 0.59 mmol) dissolved in dry CH_2Cl_2 (10 mL) under nitrogen atmosphere. A second Schlenk tube was charged with 500 mg molecular sieve (5 Å) and coumarin **3** (438 mg, 1.77 mmol) dissolved in dry CH_2Cl_2 (10 mL) under nitrogen atmosphere. After stirring for 1 h the dissolved carbohydrate **8** was added to the coumarin solution. Silver triflate (182 mg, 708 µmol) was added and the reaction was stirred for 21 h at room temperature in the dark. The reaction mixture was filtered over celite to remove the molecular sieve. The solvent was removed under reduced pressure and the residue was purified by flash-column chromatography on aluminium oxide (neutral) (petroleum ether/ethyl acetate 50:50) to yield a yellow solid (176 mg, 348 µmol, 59%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.18 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.00 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 2.13 (s, 3 H, CH_3), 3.39 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.65 (dd, $^2J_{5''a,5''b}$ = 12.8 Hz, $^3J_{5''a,4''}$ = 2.1 Hz, 1 H, 5″-H_a), 4.04 (dd, $^2J_{5''b,5''a}$ = 12.8 Hz, $^3J_{5''b,4''}$ = 4.0 Hz, 1 H, 5″-H_b), 4.58 (d, $^3J_{1'',2''}$ = 6.4 Hz, 1 H, 1″-H), 4.67 (dd, $^2J_{4'a,4'b}$ = 14.7 Hz, $^4J_{4'a,3}$ = 1.3 Hz, 1 H, 4′-H_a), 4.93 (dd, $^2J_{4'b,4'a}$ = 14.7 Hz, $^4J_{4'b,3}$ = 1.3 Hz, 1 H, 4′-H_b), 5.07 (dd, $^3J_{3'',2''}$ = 8.8 Hz, $^3J_{3'',4''}$ = 3.5 Hz, 1 H, 3″-H), 5.24 (dd, $^3J_{2'',3''}$ = 8.8 Hz, $^3J_{2'',1''}$ = 6.4 Hz, 1 H, 2″-H), 5.27 (ddd, $^3J_{4'',5''b}$ = 4.0 Hz, $^3J_{4'',3''}$ = 3.5 Hz, $^3J_{4'',5''a}$ = 2.1 Hz, 1 H, 4″-H), 6.17 (t, $^4J_{3,4'}$ = 1.3 Hz, 1 H, 3-H), 6.51 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.56 (dd, $^3J_{6,5}$ = 8.9 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.27 (d, $^3J_{5,6}$ = 8.9 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 20.8, 20.8, 21.0 (CH_3), 45.0 (C-9), 62.8 (C-5″), 65.9 (C-4′), 67.3 (C-4″), 69.1 (C-2″), 69.8 (C-3″), 98.1 (C-8), 99.6 (C-1″), 106.5 (C-4a), 107.1 (C-3), 108.8 (C-6), 124.8 (C-5), 150.5 (C-4), 150.6 (C-7), 156.4 (C-8a), 162.0 (C-2), 169.5 (C-2‴), 170.2 (C-3‴), 170.4 (C-4‴); R_f = 0.51 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm$^-$

[1]] = 2972, 1744, 1710, 1602, 1527, 1419, 1358, 1217, 1138, 1062, 1022, 601, 530; HRMS (ESI): m/z calculated for $C_{25}H_{32}NO_{10}^+$ $[M+NH_4]^+$: 506.2021; found: 506.2028; m.p.: 113.6 °C; $[\alpha]_D^{20}$ = 36.4 (c = 0.25, $CHCl_3$); UV-Vis (MeOH): λ_{max} (ε) = 246 nm (11206 dm^3 mol^{-1} cm^{-1}), 379 (16536).

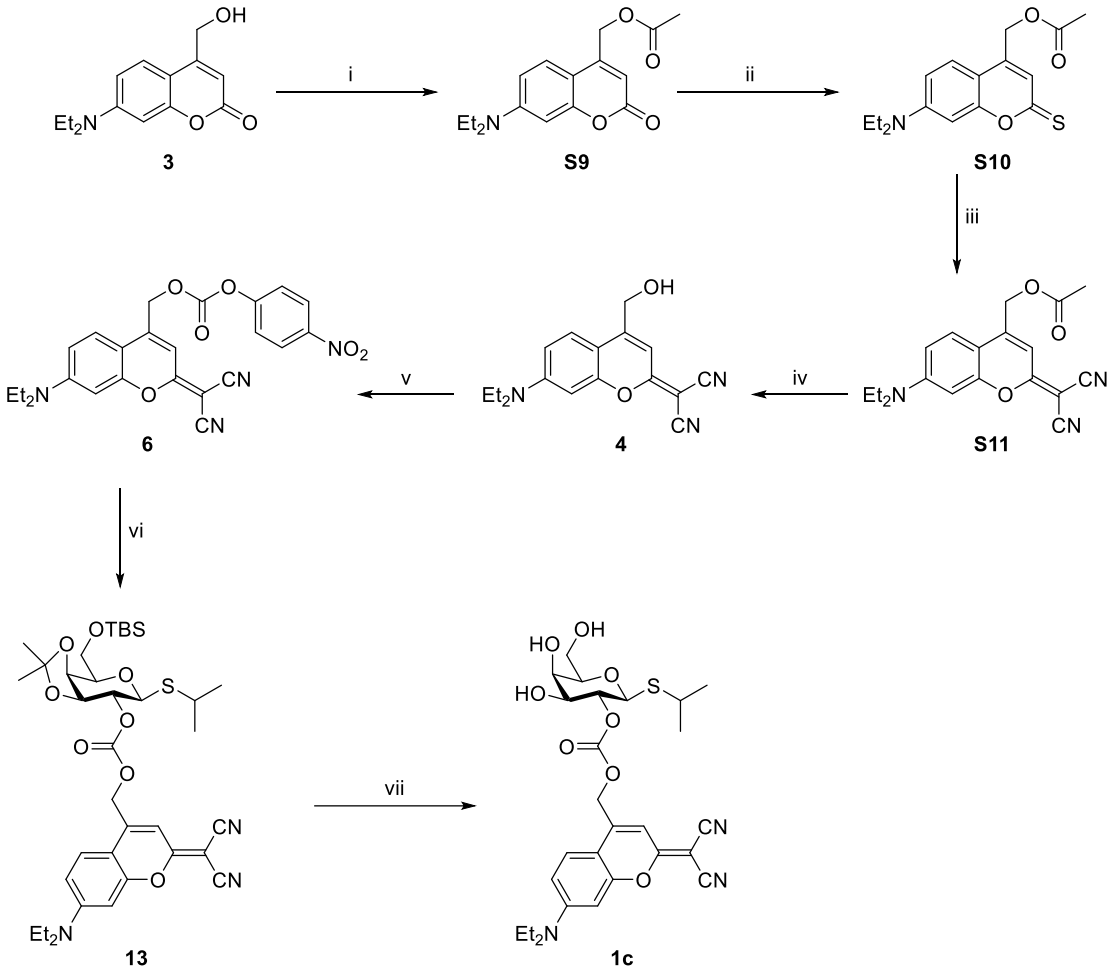
Synthesis of {[7-(Diethylamino)-2-oxo-2*H*-chromen-4-yl]methyl}-α-L-arabinopyranose (2b)



Photocaged arabinose **2b** was synthesized using modified procedures of Bier *et al*.[21] Coumarin **13** (135 mg, 267 µmol) was dissolved in MeOH (0.60 mL) and stirred at room temperature in the dark. Ammonia in MeOH (7 M, 230 µL, 1.61 mmol) was added and the reaction mixture was stirred until complete conversion. The solvent was evaporated under reduced pressure to yield a yellow solid (100 mg, 264 µmol, quant.). ^{1}H-NMR (600 MHz, Aceton-d_6): δ [ppm] = 1.20 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 3.50 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.59 (dd, $^2J_{5''a,5''b}$ = 12.4 Hz, $^3J_{5''a,4''}$ = 1.9 Hz, 1 H, 5″-H_a), 3.59–3.64 (m, 1 H, 3″-H), 3.68 (d, $^3J_{4''\text{-OH},4''}$ = 4.3 Hz, 1 H, 4″-OH), 3.71 (ddd, $^3J_{2'',3''}$ = 8.4 Hz, $^3J_{2'',1''}$ = 6.7 Hz, $^3J_{2'',2''\text{-OH}}$ = 4.1 Hz, 1 H, 2″-H), 3.82–3.86 (m, 1 H, 4″-H), 3.91 (dd, $^2J_{5''b,5''a}$ = 12.4 Hz, $^3J_{5''b,4''}$ = 3.3 Hz, 1 H, 5″-H_b), 3.98 (d, $^3J_{3''\text{-OH},3''}$ = 5.5 Hz, 1 H, 3″-OH), 4.41 (d, $^3J_{1'',2''}$ = 6.7 Hz, 1 H, 1″-H), 4.47 (d, $^3J_{2''\text{-OH},2''}$ = 4.1 Hz, 1 H, 2″-OH), 4.76 (dd, $^2J_{4'a,4'b}$ = 15.2 Hz, $^4J_{4'a,3}$ = 1.4 Hz, 1 H, 4′-H_a), 4.98 (dd, $^2J_{4'b,4'a}$ = 15.2 Hz, $^4J_{4'b,3}$ = 1.4 Hz, 1 H, 4′-H_b), 6.26 (t, $^4J_{3,4'}$ = 1.4 Hz, 1 H, 3-H), 6.49 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.69 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.47 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, Aceton-d_6): δ [ppm] = 12.7 (C-10), 45.1 (C-9), 66.3 (C-5″), 66.4 (C-4′), 68.9 (C-4″), 72.3 (C-2″), 73.9 (C-3″), 98.0 (C-8), 104.1 (C-1″), 106.9 (C-3), 107.1 (C-4a), 109.4 (C-6), 126.0 (C-5), 151.5 (C-7), 152.9 (C-4), 157.2 (C-8a), 161.8 (C-2); R_f = 0.33 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3443, 2954, 2921, 2847, 1706, 1623, 1527, 1441, 1360, 1328, 1138, 1088, 1011, 855, 824, 794, 763, 610, 511; HRMS (ESI): m/z calculated for $C_{19}H_{26}NO_7^+$ $[M+H]^+$: 380.1704; found: 380.1697; m.p.: 129–130 °C; $[\alpha]_D^{20}$ = –18.6 (c = 0.5, MeOH); UV-Vis (MeOH): λ_{max} (ε) = 272 nm (60914 dm^3 mol^{-1} cm^{-1}), 388 (17236).

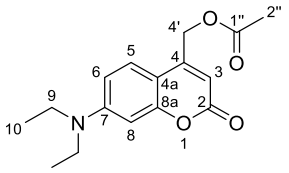
reversed-phase HPLC: t_R = 4.5 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 388 nm; eluent: H_2O/MeOH 40:60; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: H_2O.

S3.5 Synthetic scheme for preparation of photocaged IPTG 1c



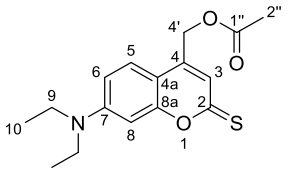
Scheme S5: Synthetic scheme for preparation of photocaged IPTG **1c**. Reagents and conditions: i) AcOH, DCC, DMAP, 0 °C → RT, 20 h; ii) Lawesson's reagent, toluene, reflux, 12 h; iii) malononitrile, NEt_3, $AgNO_3$, RT, 4 h; iv) HCl in EtOH (1.25 M), EtOH, reflux, 15 h; v) 4-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, 22 h; vi) **9**, DMAP, CH_2Cl_2, RT, 20 h; vii) TFA, H_2O, CH_2Cl_2, 0 °C, 10 min.

Synthesis of [7-(Diethylamino)-2-oxo-2*H*-chromen-4-yl]methyl acetate (S9)



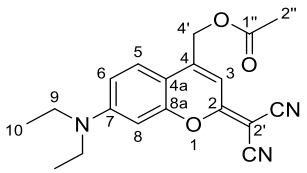
Coumarin **S9** was synthesized using a modified procedure of Gandioso *et al.*[22] Coumarin **3** (500 mg, 2.02 mmol) was dissolved in dry CH_2Cl_2 (35 mL) under nitrogen atmosphere. 4-Dimethylaminopyridine (DMAP) (299 mg, 2.43 mmol) and acetic acid (139 µL, 2.43 mmol) were added, and the reaction mixture was cooled to 0 °C. *N*,*N*′-Dicyclohexylcarbodiimide (DCC) (501 mg, 2.43 mmol) was added at 0 °C and the reaction mixture was stirred for 20 h in the dark. After filtration, the organic filtrate was washed with 1 M HCl and saturated $NaHCO_3$ solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (CH_2Cl_2) to yield a red solid (518 mg, 1.79 mmol, 89%). The spectroscopic data are consistent with previously reported literature values.[22] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.18 (s, 3 H, 2″-H), 3.40 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 5.20 (d, $^4J_{4',3}$ = 1.3 Hz, 2 H, 4′-H) 6.11 (t, $^4J_{3,4'}$ = 1.3 Hz, 1 H, 3-H), 6.50 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.56 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.27 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 20.9 (C-2″), 44.9 (C-9), 61.4 (C-4′), 97.9 (C-8), 106.1 (C-4a), 106.5 (C-3), 108.8 (C-6), 124.5 (C-5), 149.5 (C-4), 150.8 (C-7), 156.4 (C-8a), 162.0 (C-2), 170.3 (C-1″); R_f = 0.57 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2974, 1748, 1706, 1597, 1527, 1440, 1415, 1376, 1337, 1272, 1240, 1196, 1140, 1074, 1013, 933, 841, 823, 811, 666, 598, 560; HRMS (ESI): m/z calculated for $C_{16}H_{20}NO_4^+$ $[M+H]^+$: 290.1387; found: 290.1389; m.p.: 108.3 °C.

Synthesis of [7-(Diethylamino)-2-thioxo-2*H*-chromen-4-yl]methyl acetate (S10)



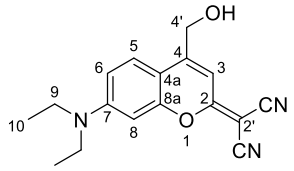
Coumarin **S10** was synthesized using a modified procedure of Gandioso *et al.*[22] Coumarin **S9** (2.00 g, 6.91 mmol) was dissolved in dry toluene (237 mL) under nitrogen atmosphere. Lawesson's reagent (1.82 g, 4.49 mmol) was added, and the reaction mixture was heated to reflux for 12 h in the dark. The solution was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (CH_2Cl_2) to yield an orange solid (1.78 g, 5.83 mmol, 84%). The spectroscopic data are consistent with previously reported literature values.[22] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.22 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.19 (s, 3 H, 2″-H), 3.43 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 5.18 (d, $^4J_{4',3}$ = 1.3 Hz, 2 H, 4′-H), 6.66 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 6.68 (d, $^3J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 7.06 (t, $^4J_{3,4'}$ = 1.3 Hz, 1 H, 3-H), 7.34 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 20.9 (C-2″) 45.1 (C-9), 61.1 (C-4′), 97.6 (C-8), 108.3 (C-4a), 110.4 (C-6), 120.7 (C-3), 124.6 (C-5), 142.0 (C-4), 151.2 (C-7), 159.1 (C-8a), 170.4 (C-1″), 197.3 (C-2); R_f = 0.60 (CH_2Cl_2); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2971, 1743, 1625, 1574, 1516, 1432, 1399, 1376, 1354, 1290, 1250, 1217, 1197, 1180, 1147, 1071, 1029, 967, 855, 822, 795, 652; HRMS (ESI): m/z calculated for $C_{16}H_{20}NO_3S^+$ $[M+H]^+$: 306.1158; found: 306.1160; m.p.: 137.9 °C

Synthesis of [2-(Dicyanomethylene)-7-(diethylamino)-2*H*-chromen-4-yl]methyl acetate (S11)



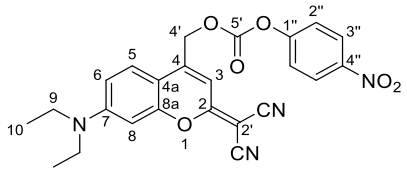
Coumarin **S11** was synthesized using a procedure of Gandioso *et al.*[22] Coumarin **S10** (1.00 g, 3.27 mmol) was dissolved in dry MeCN (100 mL) under nitrogen atmosphere. After addition of malononitrile (1.09 g, 16.5 mmol) and triethylamine (NEt_3) (9.13 mL, 65.5 mmol), the reaction mixture was stirred for 20 min at room temperature in the dark. The reaction mixture became intensely red. Silver nitrate (1.12 g, 6.58 mmol) was added and the reaction mixture was stirred for additional 4 h at room temperature in the dark. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (CH_2Cl_2) to yield an orange-red solid (616 mg, 1.83 mmol, 56%). The spectroscopic data are consistent with previously reported literature values.[22] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.24 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.21 (s, 3 H, 2″-H), 3.45 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 5.24 (d, $^4J_{4',3}$ = 1.2 Hz, 2 H, 4′-H), 6.62 (d, $^3J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.70 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 6.75 (t, $^4J_{3,4'}$ = 1.2 Hz, 1 H, 3-H), 7.34 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 20.9 (C-2″), 45.3 (C-9), 55.9 (C-2′), 61.2 (C-4′), 97.9 (C-8), 106.2 (C-3), 107.4 (C-4a), 111.1 (C-6), 113.9 (CN), 114.6 (CN), 125.0 (C-5), 146.1 (C-4), 151.6 (C-7), 155.1 (C-8a), 170.3 (C-1″), 171.9 (C-2); R_f = 0.48 (PE/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2969, 2924, 2215, 1750, 1638, 1586, 1524, 1430, 1358, 1320, 1259, 1224, 1148, 1079, 816, 687; HRMS (ESI): m/z calculated for $C_{19}H_{20}N_3O_3^+$ $[M+H]^+$: 338.1499; found: 338.1495; m.p.: 202–203 °C.

Synthesis of 2-(Dicyanomethylene)-7-(diethylamino)-4-(hydroxymethyl)-2*H*-chromen (4)



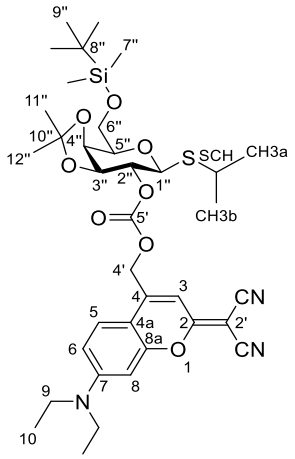
Coumarin **4** was synthesized using a procedure of Fournier *et al.*[23] Coumarin **S11** (500 mg, 1.48 mmol) was dissolved in dry EtOH (316 mL) under nitrogen atmosphere. After addition of HCl in EtOH (1.25 M, 2.96 mL, 3.71 mmol), the reaction mixture was heated to reflux for 15 h in the dark. Then, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (dichloromethane/acetone 95:5) to yield an orange-red solid (403 mg, 1.36 mmol, 92%). The spectroscopic data are consistent with previously reported literature values.[23] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.24 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 3.45 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 4.89 (s, 2 H, 4′-H), 6.63 (d, $^3J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.71 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 6.98 (s, 1 H, 3-H), 7.36 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.6 (C-10), 45.4 (C-9), 54.9 (C-2′), 60.8 (C-4′), 97.8 (C-8), 105.4 (C-3), 107.7 (C-4a), 111.2 (C-6), 114.2 (CN), 115.0 (CN), 125.0 (C-5), 151.4 (C-4), 151.6 (C-7), 154.9 (C-8a), 172.3 (C-2); R_f = 0.44 (CH_2Cl_2/acetone 95:5); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3431, 2972, 2918, 2199, 1741, 1634, 1575, 1504, 1418, 1355, 1319, 1254, 1189, 1144, 1085, 821, 686, 508; MS (ESI, positive ion): m/z (%) = 296.3 (100) $[M+H]^+$; m.p.: 179–180 °C

Synthesis of [2-(Dicyanomethylene)-7-(diethylamino)-2*H*-chromen-4-yl]methyl (4-nitrophenyl) carbonate (6)



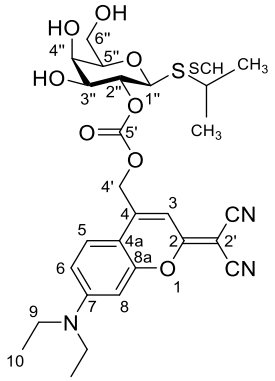
Coumarin **6** was synthesized using modified procedures of Gao *et al.*[9] and Fomina *et al.*[10] Coumarin **4** (250 mg, 846 µmol) was dissolved in dry CH_2Cl_2 (15.0 mL) under nitrogen atmosphere. *N*,*N*-Diisopropylethylamine (DIPEA) (295 µL, 1.69 mmol) was added and the reaction mixture was stirred for 15 min before 4-nitrophenyl chloroformate (341 mg, 1.69 mmol) was added portion wise. The reaction mixture was stirred for 22 h and diluted with CH_2Cl_2. It was washed with 1 M HCl (1×) and saturated $NaHCO_3$ solution (3×). The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 70:30) to yield a red solid (179 mg, 389 µmol, 46%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 3.46 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 5.42 (s, 2 H, 4′-H), 6.61 (d, $^3J_{8,6}$ = 2.5 Hz, 1 H, 8-H), 6.69 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.5 Hz, 1 H, 6-H), 6.80 (s, 1 H, 3-H), 7.35 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H), 7.43 (m_c, 2 H, 2″-H), 8.31 (m_c, 2 H, 3″-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.6 (C-10), 45.2 (C-9), 56.5 (C-2′), 65.5 (C-4′), 97.6 (C-8), 106.3 (C-3), 106.7 (C-4a), 110.9 (C-6), 113.7 (CN), 114.5 (CN), 121.9 (C-2″), 124.9 (C-5), 125.6 (C-3″), 144.2 (C-4), 145.9 (C-4″), 152.0 (C-7), 152.2 (C-5′), 155.2, 155.2 (C-8a, C-1″), 171.7 (C-2); R_f = 0.28 (PE/EtOAc 60:40); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2215, 1774, 1638, 1584, 1548, 1523, 1489, 1432, 1350, 1321, 1259, 1216, 1148, 1083, 860; HRMS (ESI): m/z calculated for $C_{24}H_{21}N_4O_6^+$ $[M+H]^+$: 461.1456; found: 461.1457; m.p.: 209–211 °C

Synthesis of Isopropyl 6-*O*-(*tert*-butyldimethylsilyl)-2-*O*-{[(dicyanomethylene)-7-(diethylamino)-2*H*-chromen-4-yl]methyloxycarbonyl}-3,4-*O*-(1-methylethylidene)-1-thio-β-D-galactopyranoside (13)



Coumarin **13** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **6** (0.12 g, 0.25 mmol) was dissolved in dry CH_2Cl_2 (4.0 mL) under nitrogen atmosphere. After the addition of 4-dimethylaminopyridine (DMAP) (31 mg, 0.26 mmol) and carbohydrate **9** (90 mg, 0.23 mmol) the reaction mixture was stirred for 20 h at room temperature in the dark. Then, it was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 80:20) to yield a red solid (0.15 g, 0.22 mmol, 96%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 0.07 (s, 3 H, 7″-H), 0.07 (s, 3 H, 7″-H), 0.89 (s, 9 H, 9″-H), 1.23 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.29 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.30 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.35 (s, 3 H, 11″-H or 12″-H), 1.55 (s, 3 H, 11″-H or 12″-H), 3.19 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.44 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.80–3.89 (m, $^3J_{5″,4″}$ = 5.6 Hz, 3 H, 5″-H, 6″-H), 4.23 (dd, $^3J_{3″,2″}$ = 7.3 Hz, $^3J_{3″,4″}$ = 5.3 Hz, 1 H, 3″-H), 4.29 (dd, $^3J_{4″,3″}$ = 5.3 Hz, $^3J_{4″,5″}$ = 1.9 Hz, 1 H, 4″-H), 4.49 (d, $^3J_{1″,2″}$ = 10.4 Hz, 1 H, 1″-H), 4.79 (dd, $^3J_{2″,1″}$ = 10.4 Hz, $^3J_{2″,3″}$ = 7.3 Hz, 1 H, 2″-H), 5.25 (dd, $^3J_{4′a,\ 4′b}$ = 15.5 Hz, $^4J_{4′a,3}$ = 1.2 Hz, 1 H, 4′-H_a), 5.39 (dd, $^3J_{4′b,\ 4′a}$ = 15.5 Hz, $^4J_{4′b,3}$ = 1.2 Hz, 1 H, 4′-H_b), 6.58 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.64 (dd, $^3J_{6,5}$ = 9.1 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 6.81 (m_c, 1 H, 3-H), 7.29 (d, $^3J_{5,6}$ = 9.1 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = –5.4 (C-7″), –5.3 (C-7″), 12.6 (C-10), 18.3 (C-8″), 24.0 (CH_3), 24.1 (CH_3), 25.9 (C-9″), 26.5, 28.0 (C-11″, C-12″), 35.8 (SCH), 45.1 (C-9), 55.9 (C-2′), 62.1 (C-6″), 64.7 (C-4′), 73.6 (C-4″), 77.0 (C-3″), 77.2 (C-5″), 77.3 (C-2″), 82.4 (C-1″), 97.5 (C-8), 106.0 (C-3), 106.8 (C-4a), 110.6 (C-10″), 110.8 (C-6), 114.0 (CN), 114.5 (CN), 124.9 (C-5), 145.4 (C-4), 151.8 (C-7), 154.2 (C-5′), 155.0 (C-8a), 171.9 (C-2); R_f = 0.24 (PE/EtOAc 80:20); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2955, 2929, 2859, 2216, 1756, 1639, 1586, 1548, 1525, 1432, 1383, 1356, 1319, 1257, 1223, 1196, 1148, 1111, 1078, 1047, 983, 871, 839, 760; HRMS (ESI): m/z calculated for $C_{36}H_{52}N_3O_8SSi^+$ $[M+H]^+$: 714.3239; found: 714.3237; m.p.: 79–81 °C; $[\alpha]_D^{20}$ = –18.0 (c = 0.1, $CHCl_3$).

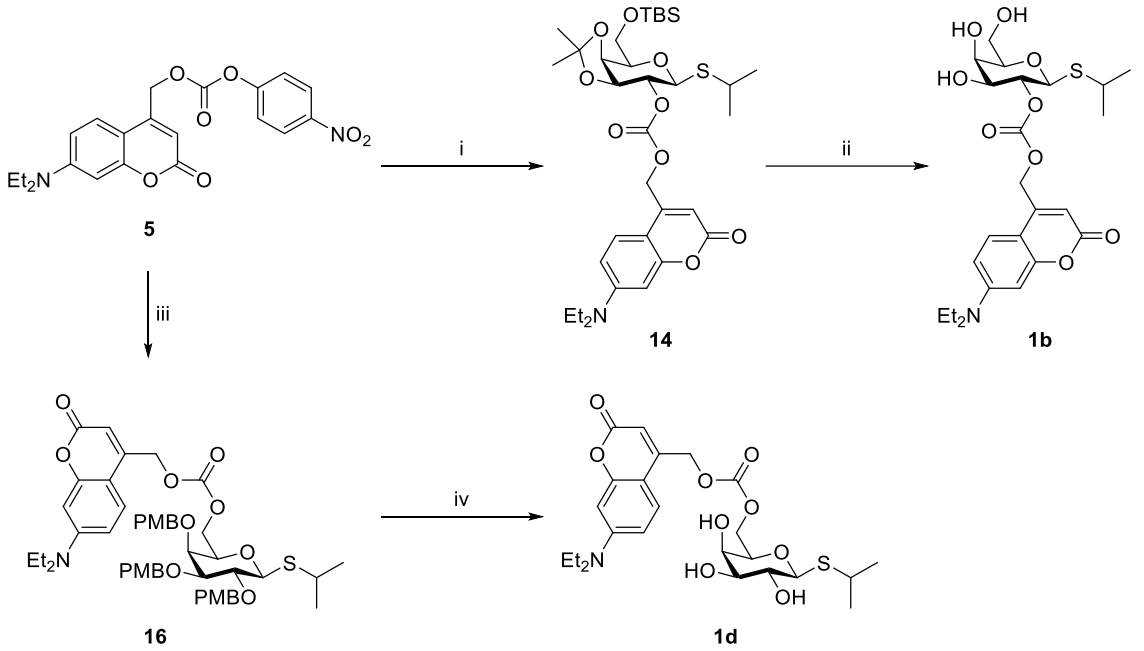
Synthesis of Isopropyl 2-*O*-{[(dicyanomethylene)-7-(diethylamino)-2*H*-chromen-4-yl]methyloxycarbonyl}-1-thio-β-D-galactopyranoside (1c)



Photocaged IPTG **1c** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **13** (0.10 g, 0.14 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and cooled to 0 °C. After the addition of trifluoroacetic acid (TFA) (1.0 mL, 13 mmol) and water (40 µL, 2.2 mmol), the reaction mixture was stirred for 10 min at 0 °C in the dark. Then the reaction mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (ethyl acetate) to yield a red solid (72 mg, 0.13 mmol, 92%). ^{1}H-NMR (600 MHz, Aceton-d_6): δ [ppm] = 1.18–1.28 (m, 12 H, 10-H, CH_3), 3.20 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.59 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.67 (t, $^3J_{5'',4''}$ = 5.6 Hz, 1 H, 5″-H), 3.77 (m, 2 H, 6″-H), 3.86 (dd, $^3J_{3'',2''}$ = 9.3 Hz, $^3J_{3'',4''}$ = 3.4 Hz, 1 H, 3″-H), 4.10 (d, $^3J_{4'',3''}$ = 3.4 Hz, 1 H, 4″-H), 4.65 (d, $^3J_{1'',2''}$ = 10.1 Hz, 1 H, 1″-H), 4.88 (dd, $^3J_{2'',1''}$ = 10.1 Hz, $^3J_{2'',3''}$ = 9.3 Hz, 1 H, 2″-H), 5.43 (d, $^3J_{4'a,\,4'b}$ = 15.5 Hz, 1 H, 4′-H_a), 5.53 (d, $^3J_{4'b,\,4'a}$ = 15.5 Hz, 1 H, 4′-H_b), 6.74–6.79 (m, 2 H, 3-H, 8-H), 6.91 (dd, $^3J_{6,5}$ = 9.1 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.63 (d, $^3J_{5,6}$ = 9.1 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, Aceton-d_6): δ [ppm] = 12.7 (C-10), 24.2 (CH_3), 24.6 (CH_3), 35.5 (SCH), 45.5 (C-9), 55.1 (C-2′), 62.3 (C-6″), 65.4 (C-4′), 70.4 (C-4″), 73.4 (C-3″), 77.3 (C-2″), 79.8 (C-5″), 83.5 (C-1″), 97.6 (C-8), 106.0 (C-3), 107.7 (C-4a), 111.9 (C-6), 114.3 (CN), 115.2 (CN), 126.8 (C-5), 147.9 (C-4), 153.0 (C-7), 155.1 (C-5′), 155.9 (C-8a), 172.6 (C-2); R_f = 0.23 (EtOAc); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3359, 2954, 2924, 2855, 2216, 1748, 1672, 1638, 1584, 1522, 1433, 1381, 1320, 1258, 1193, 1139, 1076, 1053, 984, 800, 725; HRMS (ESI): m/z calculated for $C_{27}H_{34}N_3O_8S^+$ $[M+NH_4]^+$: 560.2061; found: 560.2055; m.p.: 153.8 °C; $[\alpha]_D^{20}$ = 74 (c = 0.1, $CHCl_3$); UV-Vis [Tris buffer (20 mM, pH 7.5)/MeCN 1:1]: λ_{max} (ε) = 252 nm (18618 dm^3 mol^{-1} cm^{-1}), 276 (14338), 488 (18938).

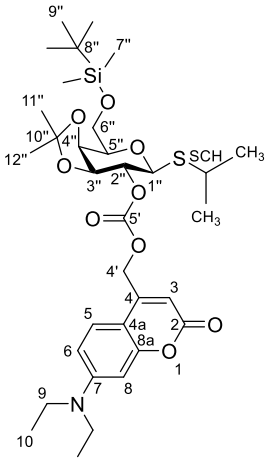
reversed-phase HPLC: t_R = 14.6 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 488 nm; eluent: H_2O/MeOH 40:60; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1.

S3.6 Synthetic scheme for preparation of photocaged IPTG 1b and 1d



Scheme S6: Synthetic scheme for preparation of photocaged IPTG **1b** and **1d**. Reagents and conditions: i) **9**, DMAP, CH_2Cl_2, RT, 20 h; ii) TFA, H_2O, CH_2Cl_2, 0 °C, 10 min; iii) **10**, DMAP, CH_2Cl_2, RT, 20 h; iv) TFA, H_2O, CH_2Cl_2, 0 °C → RT, 1 h.

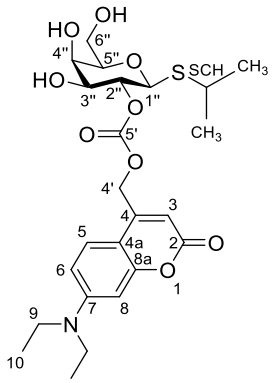
Synthesis of Isopropyl 6-*O*-(*tert*-butyldimethylsilyl)-2-*O*-{[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methyloxycarbonyl}-3,4-*O*-(1-methylethylidene)-1-thio-β-D-galactopyranoside (14)



Coumarin **14** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **5** (98 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (2 mL) under nitrogen atmosphere. After the addition of 4-dimethylaminopyridine (DMAP) (30 mg, 0.24 mmol) and carbohydrate **9** (85 mg, 0.22 mmol) the reaction mixture was stirred for 20 h at room temperature in the dark. Then, it was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column

chromatography on SiO_2 (petroleum ether/ethyl acetate 80:20) to yield a yellow solid (0.11 g, 0.17 mmol, 77%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 0.06 (s, 3 H, 7″-H), 0.07 (s, 3 H, 7″-H), 0.89 (s, 9 H, 9″-H), 1.19 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.29 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.30 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.35 (s, 3 H, 11″-H or 12″-H), 1.56 (s, 3 H, 11″-H or 12″-H), 3.17 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.40 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.79–3.91 (m, 3 H, 5″-H, 6″-H), 4.22 (dd, $^3J_{3″,2″}$ = 7.3 Hz, $^3J_{3″,4″}$ = 5.3 Hz, 1 H, 3″-H), 4.29 (d, $^3J_{4″,3″}$ = 5.2 Hz, $^3J_{4″,5″}$ = 1.9 Hz, 1 H, 4″-H), 4.48 (d, $^3J_{1″,2″}$ = 10.4 Hz, 1 H, 1″-H), 4.80 (dd, $^3J_{2″,1″}$ = 10.4 Hz, $^3J_{2″,3″}$ = 7.3 Hz, 1 H, 2″-H), 5.23 (d, $^3J_{4′a,\,4′b}$ = 14.9 Hz, 1 H, 4′-H_a), 5.38 (d, $^3J_{4′b,\,4′a}$ = 14.9 Hz, 1 H, 4′-H_b), 6.21 (s, 1 H, 3-H), 6.50 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.56 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.25 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = –5.4 (C-7″), –5.3 (C-7″), 12.5 (C-10), 18.3 (C-8″), 24.0 (CH_3), 24.1 (CH_3), 25.9 (C-9″), 26.5, 28.0 (C-11″, C-12″), 35.7 (SCH), 44.9 (C-9), 62.1 (C-6″), 65.0 (C-4′), 73.5 (C-4″), 76.9 (C-3″), 77.1 (C-5″), 77.1 (C-2″), 82.5 (C-1″), 98.0 (C-8), 106.0 (C-4a), 106.7 (C-3), 108.8 (C-6), 110.6 (C-10″), 124.4 (C-5), 148.7 (C-4), 150.7 (C-7), 154.3 (C-5′), 156.4 (C-8a), 162.0 (C-2); R_f = 0.24 (PE/EtOAc 80:20); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2969, 2931, 2870, 1745, 1713, 1603, 1527, 1420, 1356, 1336, 1217, 1139, 1094, 1064, 1023, 841, 751, 667, 601, 510; HRMS (ESI): m/z calculated for $C_{33}H_{52}NO_9SSi^+$ $[M+H]^+$: 666.3127; found: 666.3124; m.p.: 69 °C; $[\alpha]_D^{20}$ = 16 (c = 0.1, $CHCl_3$).

Synthesis of Isopropyl 2-*O*-{[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methyloxycarbonyl}-1-thio-β-D-galactopyranoside (1b)

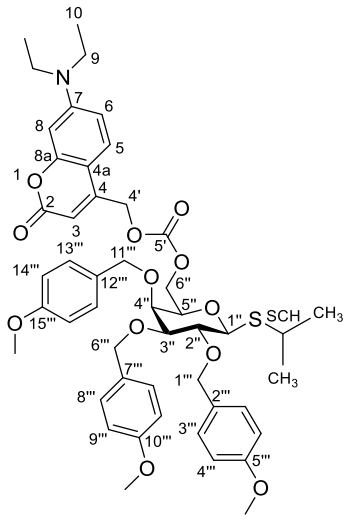


Photocaged IPTG **1b** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **14** (159 mg, 238 µmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 °C. After the addition of trifluoroacetic acid (TFA) (1.70 mL, 22.2 mmol) and water (68.8 µL, 3.82 mmol), the reaction mixture was stirred for 10 min at 0 °C in the dark. Then the reaction mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (ethyl acetate) to yield a yellow solid (117 mg, 229 µmol, 96%). ^{1}H-NMR (600 MHz, Aceton-d_6): δ [ppm] = 1.21 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.25 (d, $^3J_{CH3,SCH}$ = 6.7 Hz, 3 H, CH_3), 1.29 (d, $^3J_{CH3,SCH}$ = 6.7 Hz, 3 H, CH_3), 3.21 (septet, $^3J_{SCH,CH3}$ = 6.7 Hz, 1 H, SCH), 3.51 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.67 (t, $^3J_{5″,4″}$ = 5.6 Hz, 1 H, 5″-H), 3.77 (m, 2 H, 6″-H), 3.84 (dd, $^3J_{3″,2″}$ = 9.3 Hz, $^3J_{3″,4″}$ = 3.4 Hz, 1 H, 3″-H), 4.08 (d, $^3J_{4″,3″}$ = 3.4 Hz,

1 H, 4″-H), 4.65 (d, $^3J_{1'',2''}$ = 10.1 Hz, 1 H, 1″-H), 4.87 (dd, $^3J_{2'',1''}$ = 10.1 Hz, $^3J_{2'',3''}$ = 9.3 Hz, 1 H, 2″-H), 5.33 (d, $^3J_{4'a,4'b}$ = 15.2 Hz, 1 H, 4′-H_a), 5.45 (d, $^3J_{4'b,4'a}$ = 15.2 Hz, 1 H, 4′-H_b), 6.09 (s, 1 H, 3-H), 6.52 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.72 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.48 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, Aceton-d_6): δ [ppm] = 12.7 (C-10), 24.2 (CH_3), 24.6 (CH_3), 35.6 (SCH), 45.2 (C-9), 62.3 (C-6″), 65.4 (C-4′), 70.4 (C-4″), 73.5 (C-3″), 77.0 (C-2″), 79.9 (C-5″), 83.8 (C-1″), 98.1 (C-8), 106.5 (C-4a), 106.7 (C-3), 109.5 (C-6), 125.9 (C-5), 150.5 (C-4), 151.8 (C-7), 155.4 (C-5′), 157.3 (C-8a), 161.4 (C-2); R_f = 0.24 (EtOAc); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3405, 2962, 2925, 2862, 1755, 1679, 1603, 1528, 1424, 1357, 1255, 1200, 1139, 1054, 984, 800, 725; HRMS (ESI): m/z calculated for $C_{24}H_{34}NO_9S^+$ $[M+H]^+$: 512.1949; found: 512.1952; m.p.: 96.0 °C; $[\alpha]_D^{20}$ = 28 (c = 0.1, $CHCl_3$); UV-Vis [Tris buffer (20 mM, pH 7.5)/MeCN 1:1]: λ_{max} (ε) = 246 nm (21496 dm^3 mol^{-1} cm^{-1}), 386 (24984).

reversed-phase HPLC: t_R = 9.8 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 392 nm; eluent: H_2O/MeOH 40:60; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1.

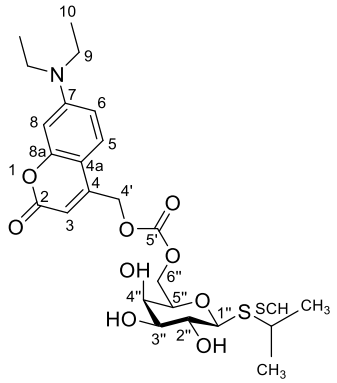
Synthesis of Isopropyl 6-*O*-{[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methyloxycarbonyl}-2,3,4-tri-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (16)



Coumarin **16** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **5** (60 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (2.0 mL) under nitrogen atmosphere. After the addition of 4-dimethylaminopyridine (DMAP) (20 mg, 0.16 mmol) and carbohydrate **10** (96 mg, 0.16 mmol) the reaction mixture was stirred for 20 h at room temperature in the dark. Then, it was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 60:40) to yield a yellow solid (84 mg, 0.10 mmol, 66%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.31 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 6 H, CH_3), 3.20 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.41 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H,

9-H), 3.52–3.58 (m, 2 H, 3″-H, 5″-H), 3.74–3.84 (m, 11 H, 2″-H, 4″-H, OCH_3), 4.03 (dd, $^2J_{6''a,6''b}$ = 10.9 Hz, $^3J_{6''a,5''}$ = 5.9 Hz, 1 H, 6″-H_a), 4.29 (dd, $^2J_{6''b,6''a}$ = 10.9 Hz, $^3J_{6''b,5''}$ = 6.6 Hz, 1 H, 6″-H_b), 4.47 (d, $^3J_{1,2}$ = 9.7 Hz, 1 H, 1-H), 4.58 (d, $^2J_{11'''a,11'''b}$ = 11.5 Hz, 1 H, 11‴-H_a), 4.67–4.74 (m, 3 H, 1‴-H_a, 6‴-H), 4.81 (d, $^2J_{1'''b,1'''a}$ = 9.8 Hz, 1 H, 1‴-H_b), 4.90 (d, $^2J_{11'''b,11'''a}$ = 11.5 Hz, 1 H, 11‴-H_b), 5.21 (m_c, 2 H, 4′-H), 6.12 (t, $^4J_{3,4'}$ = 1.3 Hz, 1 H, 3-H), 6.51 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.57 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 6.84 (d, $^3J_{14''',13'''}$ = 8.5 Hz, 2 H, 14‴-H), 6.86 (d, $^3J_{4''',3'''}$ = 8.5 Hz, 2 H, 4‴-H), 6.89 (d, $^3J_{9''',8'''}$ = 8.5 Hz, 2 H, 9‴-H), 7.24 (d, $^3J_{13''',14'''}$ = 8.5 Hz, 2 H, 13‴-H), 7.26 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H), 7.31 (d, $^3J_{8''',9'''}$ = 8.5 Hz, 2 H, 8‴-H), 7.32 (d, $^3J_{3''',4'''}$ = 8.5 Hz, 2 H, 3‴-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.6 (C-10), 24.0 (CH_3), 35.6 (SCH), 44.9 (C-9), 55.4, 55.4, 55.4 (OCH_3), 64.8 (C-4′), 67.2 (C-6″), 72.3 (C-4″), 73.0 (C-6‴), 73.8 (C-11‴), 75.6 (C-1‴), 75.6 (C-5″), 78.3 (C-2″), 83.9 (C-3″), 85.2 (C-1″), 98.0 (C-8), 105.9 (C-4a), 106.7 (C-3), 108.8 (C-6), 113.8, 113.9, 114.0 (C-4‴, C-9‴, C-14‴), 124.4 (C-5), 129.4 (C-8‴), 130.1 (C-3‴), 130.2 (C-13‴), 130.4, 130.5, 130.7 (C-2‴, C-7‴, C-12‴), 148.7 (C-4), 150.9 (C-7), 154.3 (C-5′), 156.4 (C-8a), 159.4, 159.4, 159.4 (C-5‴, C-10‴, C-15‴), 161.8 (C-2); R_f = 0.31 (PE/EtOAc 60:40); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2966, 2930, 2906, 2864, 2835, 1753, 1715, 1604, 1513, 1422, 1357, 1244, 1173, 1078, 1032, 822, 570, 519; HRMS (ESI): m/z calculated for $C_{48}H_{61}N_2O_{12}S^+$ $[M+NH_4]^+$: 889.3940; found: 889.3929; m.p.: 93.6 °C; $[\alpha]_D^{20}$ = –18.8 (c = 1.0, $CHCl_3$).

Synthesis of Isopropyl 6-*O*-{[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methyloxycarbonyl}-1-thio-β-D-galactopyranoside (1d)

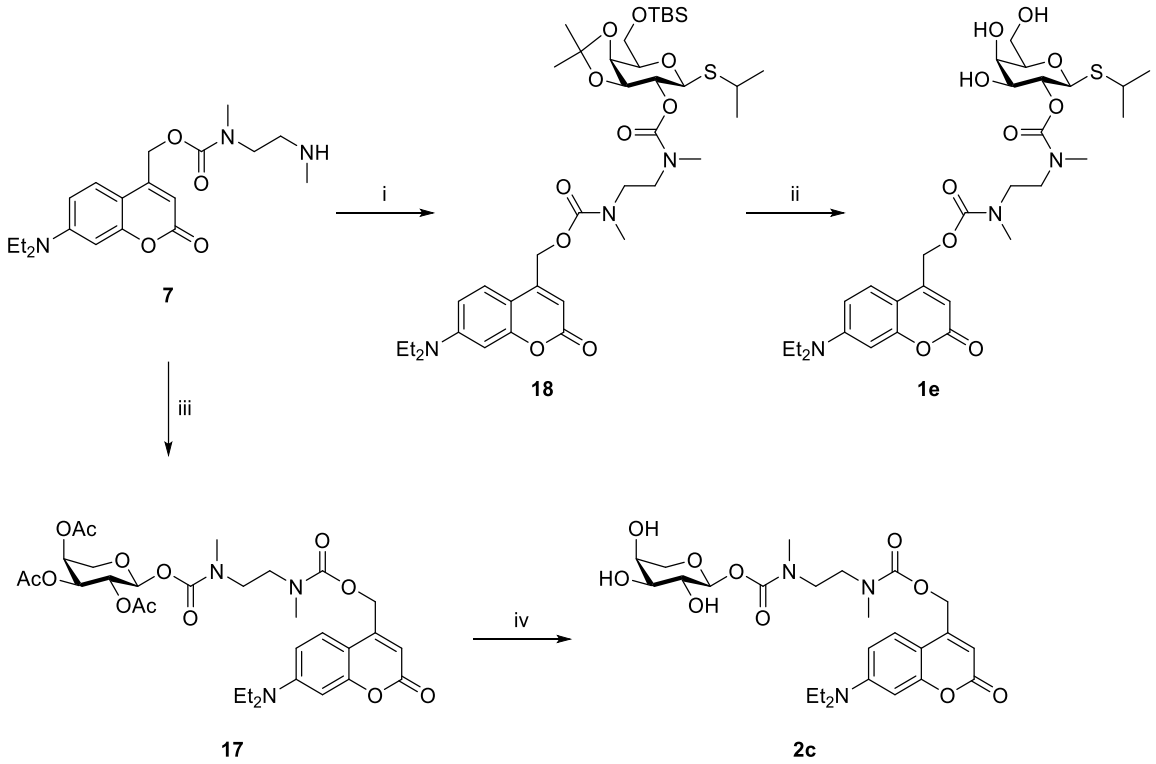


Photocaged IPTG **1d** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **16** (40 mg, 46 µmol) was dissolved in CH_2Cl_2 (1.0 mL) and cooled to 0 °C. After the addition of trifluoroacetic acid (TFA) (0.33 mL, 4.3 mmol) and water (13 µL, 0.73 mmol), the reaction mixture was stirred in the dark for 10 min at 0 °C and 1 h at room temperature. Then the reaction mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 50:50 to 10:90) to yield a yellow solid (20 mg, 39 µmol, 78%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.31 (d, $^3J_{CH3,SCH}$ = 6.7 Hz, 3 H, CH_3), 1.32 (d, $^3J_{CH3,SCH}$ = 6.7 Hz, 3 H, CH_3), 3.15–3.25 (m, 2 H, SCH, 2″-OH), 3.31 (s, 1 H, 4″-OH),

3.40 (q, $^{3}J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.62–3.71 (m, 2 H, 2″-H, 3″-H), 3.74 (s, 1 H, 3″-OH), 3.80 (m_c, 1 H, 5″-H), 4.04 (brs, 1 H, 4″-H), 4.40 (dd, $^{3}J_{6a'',6b''}$ = 11.4 Hz, $^{3}J_{6a'',5''}$ = 5.0 Hz, 1 H, 6″-H_a), 4.42 (m_c, 1 H, 1″-H), 4.48 (dd, $^{3}J_{6b'',6a''}$ = 11.4 Hz, $^{3}J_{6b'',5''}$ = 7.2 Hz, 1 H, 6″-H_b), 5.23 (dd, $^{3}J_{4'a,4'b}$ = 15.2 Hz, $^{4}J_{4'a,3}$ = 1.3 Hz, 1 H, 4′-H_a), 5.27 (dd, $^{3}J_{4'b,4'a}$ = 15.2 Hz, $^{4}J_{4'b,3}$ = 1.3 Hz, 1 H, 4′-H_b), 6.12 (dd, $^{4}J_{3,4'a}$ = 1.3 Hz, $^{4}J_{3,4'b}$ = 1.3 Hz, 1 H, 3-H), 6.49 (d, $^{4}J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.56 (dd, $^{3}J_{6,5}$ = 9.0 Hz, $^{4}J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.26 (d, $^{3}J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.6 (C-10), 24.1 (CH_3), 24.3 (CH_3), 36.1 (SCH), 44.9 (C-9), 64.9 (C-4′), 67.4 (C-6″), 68.8 (C-4″), 70.4 (C-2″), 74.6 (C-3″), 75.9 (C-5″), 86.1 (C-1″), 97.9 (C-8), 105.9 (C-4a), 106.5 (C-3), 108.9 (C-6), 124.5 (C-5), 148.9 (C-4), 150.9 (C-7), 154.7 (C-5′), 156.4 (C-8a), 162.0 (C-2); R_f = 0.35 (EtOAc); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3424, 2971, 2923, 2870, 1751, 1720, 1605, 1528, 1423, 1356, 1266, 1196, 1143, 1100, 1080, 1052, 1031, 967, 871, 828, 791, 743; HRMS (ESI): m/z calculated for $C_{24}H_{34}NO_9S^+$ $[M+H]^+$: 512.1949; found: 512.1953; m.p.: 85–87 °C; $[\alpha]_D^{20}$ = 22.0 (c = 0.1, $CHCl_3$); UV-Vis [Tris buffer (20 mM, pH 7.5)/MeCN 1:1]: λ_{max} (ε) = 273 nm (28210 dm^3 mol^{-1} cm^{-1}), 386 (12004).

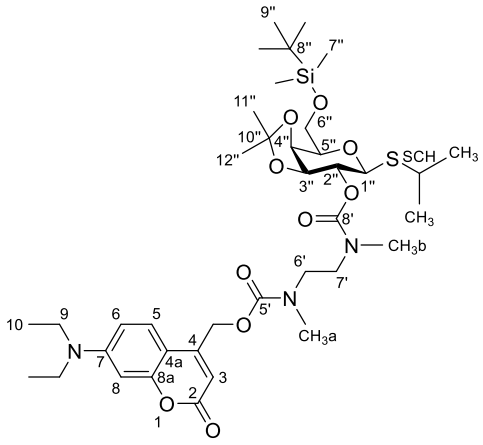
reversed-phase HPLC: t_R = 8.0 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 386 nm; eluent: H_2O/MeOH 40:60; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1.

S3.7 Synthetic scheme for preparation of photocaged IPTG 1e and photocaged arabinose 2c



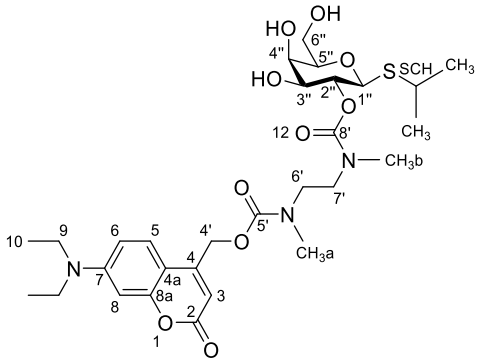
Scheme S7: Synthetic scheme for preparation of photocaged IPTG **1e** and photocaged arabinose **2c**. Reagents and conditions: i) **12**, DIPEA, DMAP, CH_2Cl_2, RT, 24 h; ii) TFA, H_2O, 0 °C, 10 min; iii) **11**, DIPEA, DMAP, CH_2Cl_2, RT, 24 h; iv) NH_3 in MeOH (7 M), MeOH, RT.

Synthesis of Isopropyl-6-*O*-(*tert*-butyldimethylsilyl)-2-*O*-{[2-({[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methoxycarbonyl}{methyl}amino)ethyl][methyl]carbamoyl}-3,4-*O*-(1-methylethylidene)-1-thio-β-D-galactopyranoside (18)



Coumarin **18** was synthesized using modified procedures of Wang *et al.*[25] Coumarin **7** (227 mg, 628 µmol) was dissolved in dry CH_2Cl_2 (11 mL) under nitrogen atmosphere. After the addition of 4-dimethylaminopyridine (DMAP) (6 mg, 0.06 mmol), carbohydrate **12** (200 mg, 359 µmol) and *N*,*N*-diisopropylethylamine (DIPEA) (1.10 mL, 6.29 mmol) the reaction mixture was stirred for 24 h at room temperature in the dark. Then, it was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 50:50) to yield a slightly yellow foam (270 mg, 346 µmol, 97%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 0.06 (s, 6 H, 7″-H), 0.88 (s, 9 H, 9″-H), 1.20 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.23–1.33 (m, 9 H, CH_3, 11-H or 12-H), 1.55 (s, 3 H, 11-H or 12-H), 2.86–3.07 (m, 6 H, CH_3a, CH_3b), 3.17 (septet, $^3J_{SCH,CH3}$ = 6.8 Hz, 1 H, SCH), 3.28–3.62 (m, 4 H, 6′-H, 7′-H), 3.41 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.75–3.90 (m, 3 H, 5″-H, 6″-H), 4.13–4.22 (m, 1 H, 3″-H), 4.22–4.28 (m, 1 H, 4″-H), 4.44–4.55 (m, 1 H, 1″-H), 4.79–4.91 (m, 1 H, 2″-H), 5.20–5.30 (m, 2 H, 4′-H), 6.03–6.13 (m, 1 H, 3-H), 6.50 (d, $^3J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.57 (d, $^3J_{6,5}$ = 9.0 Hz, 1 H, 6-H), 7.27–7.36 (m, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = –5.4 (C-7″), –5.3 (C-7″), 12.6 (C-10), 18.4 (C-8″), 23.9 (CH_3), 24.0 (CH_3), 25.9 (C-9″), 26.5, 28.0 (C-11″, C-12″), 34.9 (SCH), 35.0 (CH_3a/b), 35.3 (CH_3a/b), 35.6 (CH_3a/b), 35.9 (CH_3a/b), 36.0 (CH_3a/b), 36.1 (CH_3a/b), 44.9 (C-9), 46.7, 46.8, 47.2, 47.3, 47.5, 48.0, 48.1 (C-6′, C-7′), 62.2 (C-4′), 62.5 (C-6″), 62.7 (C-6″), 73.6, 73.6, 73.7 (C-2″, C-4″), 77.1 (C-5″), 77.5 (C-3″), 77.6 (C-3″), 77.6 (C-3″), 82.6 (C-1″), 82.7 (C-1″), 82.8 (C-1″), 82.9 (C-1″), 97.9 (C-8), 105.9, 106.1, 106.4 (C-3, C-4a), 108.8 (C-6), 110.4 (C-10″), 124.4 (C-5), 124.5 (C-5), 124.6 (C-5), 124.7 (C-5), 150.5, 150.6, 150.8 (C-4, C-7), 155.1, 155.3, 155.5, 155.6 (C-5′, C-8′), 156.3 (C-8a), 156.4 (C-8a), 162.1 (C-2); R_f = 0.31 (PE/EtOAc 50:50); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2960, 2930, 2859, 1710, 1606, 1529, 1466, 1422, 1358, 1219, 1125, 1081, 874, 839, 779; HRMS (ESI): m/z calculated for $C_{38}H_{62}N_3O_{10}SSi^+$ $[M+H]^+$: 780.3920; found: 780.3935; m.p.: 68.0 °C; $[\alpha]_D^{20}$ = 0.4 (c = 1.0, $CHCl_3$).

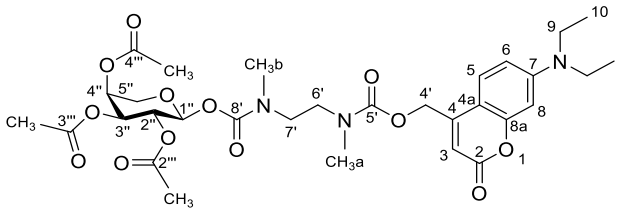
Synthesis of Isopropyl 2-*O*-{[2-({[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methoxycarbonyl}{methyl}amino)ethyl][methyl]carbamoyl}-1-thio-β-D-galactopyranoside (1e)



Photocaged IPTG **1e** was synthesized using modified procedures of Suzuki *et al.*[24] Coumarin **18** (0.11 mg, 0.14 mmol) was dissolved in trifluoroacetic acid (TFA) (1.0 mL) and cooled to 0 °C. After the addition of water (41 µL, 2.3 mmol) the reaction mixture was stirred for 10 min at 0 °C in the dark. Then the reaction mixture was concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (ethyl acetate/methanol 95:5) to yield a yellow solid (89 mg, 0.14 mmol, quant.). The product was a mixture of cis and trans isomers on carbamate bonds. ^{1}H-NMR (600 MHz, DMSO-d_6, 60 °C): δ [ppm] = 1.15 (t, $^3J_{10,9}$ = 7.0 Hz, 6 H, 10-H), 1.19 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 1.22 (d, $^3J_{CH3,SCH}$ = 6.8 Hz, 3 H, CH_3), 2.85 (s, 3 H, CH_3a or CH_3b), 2.95 (brs, 3 H, CH_3a or CH_3b), 3.07–3.12 (m, 1 H, SCH), 3.34–3.47 (m, 9 H, 9-H, 6′-H, 7′-H, 5″-H), 3.50 (dd, $^2J_{6''a,6''b}$ = 10.9 Hz, $^3J_{6''a,5''}$ = 6.0 Hz, 1 H, 6″-H$_a$), 3.54 (dd, $^2J_{6''b,6''a}$ = 10.9 Hz, $^3J_{6''b,5''}$ = 6.0 Hz, 1 H, 6″-H$_b$), 3.56 (brs, 1 H, 3″-H), 3.78 (d, $^3J_{4'',3''}$ = 3.3 Hz, 1 H, 4″-H), 4.48 (brs, 1 H, 1″-H), 4.72 (dd, $^3J_{2'',1''}$ = 10.1 Hz, $^3J_{2'',3''}$ = 9.3 Hz, 1 H, 2″-H), 5.25 (s, 2 H, 4′-H), 5.95 (s, 1 H, 3-H), 6.53 (d, $^4J_{8,6}$ = 2.6 Hz, 1 H, 8-H), 6.71 (dd, $^3J_{6,5}$ = 9.0 Hz, $^4J_{6,8}$ = 2.6 Hz, 1 H, 6-H), 7.48 (d, $^3J_{5,6}$ = 9.0 Hz, 1 H, 5-H); ^{13}C-NMR (151 MHz, DMSO-d_6, 60 °C): δ [ppm] = 12.0 (C-10), 23.4 (CH_3), 23.8 (CH_3), 33.7 (SCH), 34.7, 34.9 (CH_3a/b), 43.7 (C-9), 45.9, 46.1 (C-6′, C-7′), 60.3 (C-6″), 61.9 (C-4′), 68.4 (C-4″), 72.1 (C-2″), 72.6 (C-3″), 78.9 (C-5″), 82.7 (C-1″), 96.8 (C-8), 104.6 (C-3), 105.3 (C-4a), 108.7 (C-6), 125.0 (C-5), 150.4 (C-7), 151.0 (C-4), 154.5 (C-5′), 155.1 (C-8′), 155.6 (C-8a), 160.3 (C-2); R_f = 0.15 (EtOAc/MeOH 95:5); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3406, 2966, 2928, 2870, 1697, 1603, 1526, 1484, 1423, 1357, 1274, 1200, 1133, 1078, 862, 825, 803, 759; HRMS (ESI): m/z calculated for $C_{29}H_{44}N_3O_{10}S^+$ $[M+H]^+$: 626.2742; found: 626.2753; m.p.: 95.5 °C; $[\alpha]_D^{20}$ = –22.6 (c = 1.0, $CHCl_3$); UV-Vis [Tris buffer (20 mM, pH 7.5)/MeCN 1:1]: λ_{max} (ε) = 272 nm (17662 dm^3 mol^{-1} cm^{-1}), 305 (4142), 386 (17184).

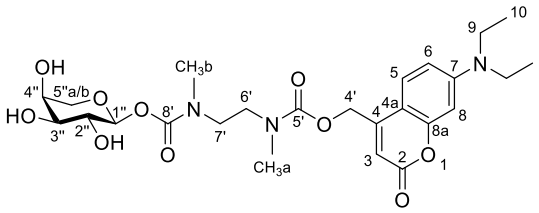
reversed-phase HPLC: t_R = 8.2 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 386 nm; eluent: H_2O/MeOH 40:60; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1.

Synthesis of 2,3,4-Tri-*O*-acetyl-1-*O*-{[2-({[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methoxycarbonyl}{methyl}amino)ethyl][methyl]carbamoyl}-α-L-arabinopyranoside (17)



Coumarin **17** was synthesized using modified procedures of Wang *et al.*[25] Coumarin **7** (57 mg, 0.16 mmol) was dissolved in dry CH_2Cl_2 (2.9 mL) under nitrogen atmosphere. After the addition of 4-dimethylaminopyridine (DMAP) (2.0 mg, 16 µmol), carbohydrate **11** (40 mg, 91 µmol) and *N*,*N*-diisopropylethylamine (DIPEA) (0.28 mL, 1.6 mmol) the reaction mixture was stirred for 24 h at room temperature in the dark. Then, it was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 30:70) to yield a slightly yellow foam (51 mg, 77 µmol, 85%). The product was a mixture of cis and trans isomers on carbamate bonds. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, $^3J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 1.99–2.07 (m, 6 H, CH_3), 2.09–2.16 (m, 3 H, CH_3), 2.88–2.98 (m, 3 H, CH_3b), 2.98–3.07 (m, 3 H, CH_3a), 3.18–3.68 (m, 4 H, 6′-H, 7′-H), 3.40 (q, $^3J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.77 (m_c, 1 H, 5″-H_a), 3.98 (dd, $^2J_{5b,5a}$ = 12.9 Hz, $^3J_{5b,4}$ = 3.7 Hz, 1 H, 5″-H_b), 5.06–5.33 (m, 5 H, 2″-H, 3″-H, 4″-H, 4′-H), 5.52–5.61 (m, 1 H, 1″-H), 6.01–6.13 (m, 1 H, 3-H), 6.50 (s, 1 H, 8-H), 6.57 (d, $^3J_{6,5}$ = 9.0 Hz, 1 H, 6-H), 7.26–7.34 (m, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 20.7 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 20.9 (CH_3), 21.0 (CH_3), 34.7, 34.7, 34.8, 35.2, 35.5, 35.5, 35.6, 35.8 (CH_3a, CH_3b), 44.9 (C-9), 46.3, 46.4, 46.9, 46.9, 47.1, 47.3, 47.6 (C-6′, C-7′), 62.4 (C-4′), 62.5 (C-4′), 62.7 (C-4′), 63.5 (C-5″), 63.6 (C-5″), 64.4 (C-5″), 64.5 (C-5″), 67.2, 67.6, 67.7, 68.2, 68.3 (C-2″, C-4″), 69.7 (C-3″), 69.8 (C-3″), 70.3 (C-3″), 70.4 (C-3″), 93.5 (C-1″), 93.6 (C-1″), 93.9 (C-1″), 94.1 (C-1″), 98.0 (C-8), 105.8, 105.9, 106.2, 106.4 (C-3, C-4a), 108.8 (C-6), 124.4 (C-5), 124.5 (C-5), 124.6 (C-5), 150.3, 150.4, 150.5, 150.7 (C-4, C-7), 153.9 (C-8′), 154.1 (C-8′), 154.4 (C-8′), 155.2 (C-5′), 155.3 (C-5′), 155.6 (C-5′), 155.7 (C-5′), 156.3 (C-8a), 162.0 (C-2), 162.1 (C-2), 169.5, 169.7, 169.8, 169.9, 169.9, 169.9, 170.0, 170.2, 170.3, 170.3 (C-2‴, C-3‴, C-4‴); R_f = 0.25 (PE/EtOAc 30:70); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2972, 2930, 1746, 1713, 1605, 1528, 1422, 1371, 1221, 1137, 1088, 1055, 760; m.p.: 82.7 °C; $[\alpha]_D^{20}$ = 10.6 (c = 1.0, $CHCl_3$)

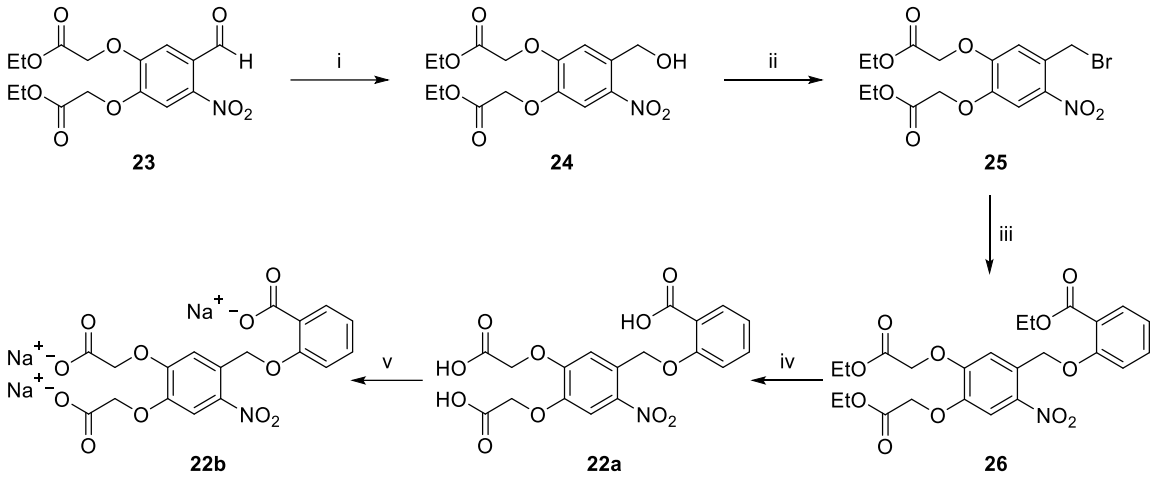
Synthesis of 1-*O*-{[2-({[7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]methoxycarbonyl}{methyl}amino)ethyl][methyl]carbamoyl}-α-L-arabinopyranoside (2c)



Photocaged arabinose **2c** was synthesized using modified procedures of Binder *et al.*[16] Coumarin **17** (40 mg, 60 µmol) was dissolved in MeOH (0.20 mL) and stirred at room temperature in the dark. Ammonia in MeOH (7 M, 54 µL, 0.38 mmol) was added and the reaction mixture was stirred until complete conversion. The solvent was evaporated under reduced pressure to yield a yellow solid (28 mg, 52 µmol, 86%). The product was a mixture of cis and trans isomers on carbamate bonds. ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, $^{3}J_{10,9}$ = 7.1 Hz, 6 H, 10-H), 2.92–2.98 (m, 3 H, CH_3b), 2.99–3.05 (m, 3 H, CH_3a), 3.05–3.99 (m, 4 H, 6′-H, 7′-H), 3.41 (q, $^{3}J_{9,10}$ = 7.1 Hz, 4 H, 9-H), 3.63–3.70 (m, 1 H, 5″-H_a), 3.70–3.78 (m, 1 H, 3″-H), 3.78–3.92 (m, 1 H, 2″-H), 3.92–3.99 (m, 1 H, 4″-H), 3.99–4.09 (m, 1 H, 5″-H_b), 5.14–5.32 (m, 2 H, 4′-H), 5.32–5.46 (m, 1 H, 1″-H), 6.03–6.17 (m, 1 H, 3-H), 6.52 (s, 1 H, 8-H), 6.57–6.68 (m, 1 H, 6-H), 7.27–7.36 (m, 1 H, 5-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 12.5 (C-10), 34.5, 34.6, 34.9, 35.4, 35.8, 36.2 (CH_3a, CH_3b), 45.0 (C-9), 46.5, 46.7, 47.1, 47.4, 47.5, 47.5 (C-6′, C-7′), 62.4 (C-4′), 62.5 (C-4′), 62.9 (C-4′), 63.1 (C-4′), 66.6 (C-5″), 66.8 (C-5″), 66.8 (C-5″), 68.2 (C-4″), 70.6 (C-2″), 70.8 (C-2″), 71.0 (C-2″), 73.3 (C-3″), 73.4 (C-3″), 73.6 (C-3″), 96.4 (C-1″), 96.5 (C-1″), 96.6 (C-1″), 98.0 (C-8), 105.9, 106.0 (C-3, C-4a), 109.1 (C-6), 124.5 (C-5), 124.6 (C-5), 150.4, 150.5, 151.4 (C-4, C-7), 154.6 (C-8′), 154.7 (C-8′), 156.0, 156.0, 156.2, 156.3, 156.6 (C-5′, C-8a), 162.6 (C-2), 162.7 (C-2); R_f = 0.08 (EtOAc/MeOH 95:5); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3423, 2966, 2927, 1703, 1603, 1528, 1490, 1423, 1357, 1275, 1216, 1131, 1080, 827, 761; HRMS (ESI): m/z calculated for $C_{25}H_{36}N_3O_{10}^+$ $[M+H]^+$: 538.2395; found: 538.2397; m.p.: 67–73 °C; $[\alpha]_D^{20}$ = 5.0 (c = 1.0, $CHCl_3$); UV-Vis [Tris buffer (20 mM, pH 7.5)/MeCN 1:1]: λ_{max} (ε) = 273 nm (28656 dm^3 mol^{-1} cm^{-1}), 385 (14070).

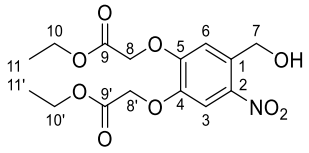
reversed-phase HPLC: t_R = 18.3 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 385 nm; eluent: H_2O/MeOH 55:45; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1.

S3.8 Synthetic scheme for preparation of photocaged salicylic acid 22a and the corresponding sodium form 22b



Scheme S8: Synthetic scheme for preparation of photocaged salicylic acid **22a** and the corresponding sodium form **22b**. Reagents and conditions: i) $NaBH_4$, CH_2Cl_2, EtOH, AcOH, 0 °C, 3 h; ii) CBr_4, PPh_3, CH_2Cl_2, 0 °C → RT, 6 h; iii) ethyl salicylate, K_2CO_3, acetone, RT, 2 d; iv) KOH (0.2 M), MeOH, 60 °C, 4 h; v) NaOH (0.2 M), MeOH, RT, 5 min.

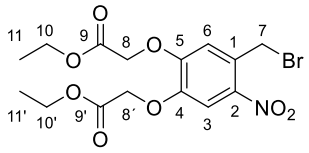
Synthesis of 4,5-Bis(ethoxycarbonylmethoxy)-2-nitrobenzylalcohol (24)



Alcohol **24** was synthesized using a procedure of Ni *et al.*[26] 4,5-Bis(ethoxycarbonylmethoxy)-2-nitrobenzaldehyde (**23**) (881 mg, 2.48 mmol) was dissolved in a mixture of CH_2Cl_2, EtOH and acetic acid (35:5:1, 10.0 mL). After the solution was cooled to 0 °C $NaBH_4$ (188 mg, 4.96 mmol, 2.00 Äq.) was added and the reaction mixture was stirred for 3 h at 0 °C. The reaction was quenched by addition of 1 M HCl (2 mL), diluted with ethyl acetate and washed with saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (toluene/ethyl acetate 80:20) to yield a yellow solid (649 mg, 1.82 mmol, 73%). The spectroscopic data are consistent with previously reported literature values.[26] ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.30 (t, $^3J_{11,10 \text{ or } 11',10'}$ = 7.2 Hz, 3 H, 11-H or 11′-H), 1.31 (t, $^3J_{11,10 \text{ or } 11',10'}$ = 7.2 Hz, 3 H, 11-H or 11′-H), 2.13 (br, 1 H, OH), 4.28 (q, $^3J_{10,11 \text{ or } 10',11'}$ = 7.2 Hz, 2 H, 10-H or 10′-H), 4.28 (q, $^3J_{10,11 \text{ or } 10',11'}$ = 7.2 Hz, 2 H, 10-H or 10′-H), 4.77 (s, 2 H, 8-H or 8′-H), 4.83 (s, 2 H, 8-H or 8′-H), 4.94 (s, 2 H, 7-H), 7.17 (s, 1 H, 3-H or 6-H), 7.70 (s, 1 H, 3-H or 6-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 14.3, 14.3 (C-11, C-11′), 61.8, 61.9 (C-10, C-10′), 62.5 (C-7), 66.2 (C-8′), 66.6 (C-8), 112.0, 113.5 (C-3, C-6), 133.6, 140.4 (C-1, C-2), 146.5 (C-5), 152.7 (C-4), 167.8 (C-9′), 168.1 (C-9); R_f = 0.32 (Toluol/EtOAc 80:20); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2992, 1742, 1580, 1507, 1282,

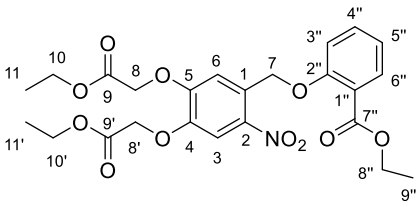
1193, 1072, 1019, 792; MS (ESI, positive ion): m/z (%) = 380.2 (100) $[M+Na]^+$, 737.3 (5) $[2M+Na]^+$; m.p.: 76 °C.

Synthesis of 4,5-Bis(ethoxycarbonylmethoxy)-2-nitrobenzyl bromide (25)



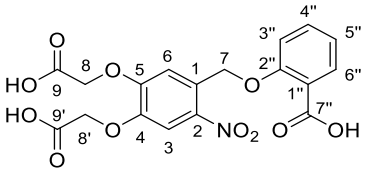
Bromide **25** was synthesized using a procedure of Tietze *et al*.[27] A Schlenk tube was charged with Alcohol **24** (1.00 g, 2.80 mmol) and tetrabromomethane (CBr_4) (1.16 g, 3.50 mmol) dissolved in dry CH_2Cl_2 (14.0 mL) under nitrogen atmosphere in the dark. A second Schlenk tube was charged with triphenylphosphane (PPh_3) (918 mg, 3.50 mmol) dissolved in dry CH_2Cl_2 under nitrogen atmosphere. Both solutions were cooled to 0 °C and the cooled PPh_3 solution was added dropwise to the dissolved alcohol **24**. The reaction mixture was stirred for 10 min at 0 °C and 6 h at room temperature. SiO_2 was added to the reaction mixture and the solvent was removed under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 65:35) to yield a white solid (1.13 g, 2.69 mmol, 96%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.31 (t, $^3J_{11,10}$ = 7.1 Hz, 3 H, 11-H), 1.31 (t, $^3J_{11',10'}$ = 7.1 Hz, 3 H, 11′-H), 4.29 (q,$^3J_{10,11;10',11'}$ = 7.1 Hz, 4 H, 10-H, 10′-H), 4.78 (2, 2 H, 8′-H), 4.81 (s, 2 H, 7-H), 4.83 (s, 2 H, 8-H), 6.94 (s, 1 H, 6-H), 7.65 (s, 1 H, 3-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 14.3 (C-11), 14.3 (C-11′), 29.7 (C-7), 61.9 (C-10), 62.0 (C-10′), 66.5 (C-8), 66.5 (C-8′), 112.1 (C-3), 117.2 (C-6), 128.5 (C-2), 141.2 (C-1), 147.6 (C-5), 151.9 (C-4), 167.7 (C-9), 167.8 (C-9′); R_f = 0.62 (PE/EtOAc 60:40); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2992, 1739, 1616, 1581, 1522, 1479, 1449, 1409, 1380, 1356, 1339, 1289, 1264, 1202, 1184, 1120, 1080, 1043, 1025, 930, 881, 798, 758, 732, 672; HRMS (ESI): m/z calculated for $C_{15}H_{18}O_8NBrNa^+$ $[M+Na]^+$: 442.0108; found: 442.0108; m.p.: 118°C.

Synthesis of Ethyl 2-*O*-[4,5-bis(ethoxycarbonylmethoxy)-2-nitrobenzyl]salicylate (26)



Ethyl salicylate (0.11 mL, 0.71 mmol) was dissolved in dry acetone (1.0 mL) and dry K_2CO_3 (56 mg, 0.40 mmol) was added. After stirring for 10 min bromide **25** (0.10 g, 0.24 mmol) was added and the reaction mixture was stirred for 2 d at room temperature in the dark. After completion, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and subsequently washed with water and saturated $NaHCO_3$ solution. The organic phase was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash-column chromatography on SiO_2 (petroleum ether/ethyl acetate 80:20) to yield a white solid (0.11 g, 0.22 mmol, 92%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, $^3J_{11,10}$ = 7.1 Hz, 3 H, 11-H), 1.31 (t, $^3J_{11',10'}$ = 7.1 Hz, 3 H, 11′-H), 1.40 (t, $^3J_{9'',8''}$ = 7.1 Hz, 3 H, 9″-H), 4.23 (q, $^3J_{10,11}$ = 7.1 Hz, 2 H, 10-H), 4.29 (q, $^3J_{10',11'}$ = 7.1 Hz, 2 H, 10′-H), 4.38 (q, $^3J_{8'',9''}$ = 7.1 Hz, 2 H, 8″-H), 4.81 (s, 2 H, 8′-H), 5.05 (s, 2 H, 8-H), 5.52 (s, 2 H, 7-H), 7.03 (dd, $^3J_{5'',4''}$ = 7.5 Hz, $^3J_{5'',6''}$ = 7.5 Hz, 1 H, 5″-H), 7.14 (d, $^3J_{3'',4''}$ = 8.4 Hz, 1 H, 3″-H), 7.53 (dd, $^3J_{4'',3''}$ = 8.4 Hz, $^3J_{4'',5''}$ = 7.5 Hz, 1 H, 4″-H), 7.82 (s, 1 H, 3-H), 7.93 (d, $^3J_{6'',5''}$ = 7.5 Hz, 1 H, 6″-H), 8.23 (s, 1 H, 6-H); ^{13}C-NMR (151 MHz, $CDCl_3$): δ [ppm] = 14.2 (C-11), 14.3 (C-11′), 14.5 (C-9″), 60.7 (C-8″), 61.6 (C-10), 61.7 (C-10′), 65.8 (C-8), 66.8 (C-8′), 67.3 (C-7), 111.9 (C-3), 112.8 (C-6), 113.2 (C-3″), 119.8 (C-1″), 120.8 (C-5″), 130.8 (C-1), 132.1 (C-6″), 134.2 (C-4″), 138.9 (C-2), 146.1 (C-4), 153.4 (C-5), 158.0 (C-2″), 165.2 (C-7″), 168.0 (C-9), 168.3 (C-9′); R_f = 0.23 (PE/EtOAc 70:30); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3104, 2992, 1771, 1743, 1713, 1582, 1524, 1488, 1449, 1425, 1377, 1329, 1291, 1242, 1195, 1112, 1080, 1020, 895, 859, 826, 803, 753, 700, 683, 661; HRMS (ESI): m/z calculated for $C_{24}H_{27}O_{11}NNa^+$ $[M+Na]^+$: 528.1476; found: 528.1476; m.p.: 133 °C

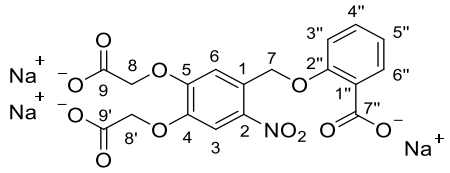
Synthesis of 2-*O*-[4,5-Bis(carboxymethoxy)-2-nitrobenzyl]salicylic acid (BC-cSal) (22a)



To a solution of salicylate **26** (200 mg, 396 µmol) in MeOH (11.9 mL) a 0.2 M solution of KOH (11.9 mL, 2.37 mmol) was added. The reaction mixture was heated to 60 °C and stirred until complete conversion (4 h). After the reaction was completed as indicated by TLC, 1 M HCl was added and the precipitate was filtered off and washed. The precipitate was dried to yield a white solid (153 mg, 363 µmol, 92%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 4.89 (s, 2 H, 8′-H), 4.91 (s, 2 H, 8-H), 5.49 (s, 2 H, 7-H), 7.06 (dd, $^{3}J_{5'',6''}$ = 7.0 Hz, $^{3}J_{5'',4''}$ = 6.9 Hz 1 H, 5″-H), 7.22 (d, $^{3}J_{3'',4''}$ = 7.9 Hz, 1 H, 3″-H), 7.56 (dd, $^{3}J_{4'',3''}$ = 7.9 Hz, $^{3}J_{4'',5''}$ = 6.9 Hz, 1 H, 4″-H), 7.72 (s, 1 H, 3-H), 7.79 (d, $^{3}J_{6'',5''}$ = 7.0 Hz, 1 H, 6″-H), 7.80 (s, 1 H, 6-H); ^{13}C-NMR (151 MHz, DMSO-d_6): δ [ppm] = 65.0 (C-8), 65.5 (C-8′), 66.7 (C-7), 110.4 (C-3), 112.2 (C-6), 113.7 (C-3″), 120.7 (C-1″), 120.7 (C-5″), 128.7 (C-1), 131.6 (C-6″), 133.7 (C-4″), 139.0 (C-2), 145.9 (C-4), 152.2 (C-5), 157.1 (C-2″), 166.9 (C-7″), 169.1 (C-9), 169.8 (C-9′); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 2923, 1708, 1801, 1585, 1525, 1490, 1428, 1380, 1331, 1285, 1245, 1214, 1079, 1027, 900, 849, 821, 750, 698, 671; HRMS (ESI): m/z calculated for $C_{18}H_{15}O_{11}NK^{+}$ [M+K]$^{+}$: 460.0276; found: 460.0276; m.p.: 264 °C (decay); UV-Vis [sodium phosphate buffer (100 mM, pH 7.4)]: λ_{max} (ε) = 290 nm (5027 dm^3 mol^{-1} cm^{-1}), 346 (5852).

reversed-phase HPLC: t_R = 9.6 min; column: *HyperClone* 5 µ ODS (C18) 120 (*Phenomenex*); detection (UV): 346 nm; eluent: sodium phosphate buffer (100 mM, pH 7.4)/MeOH 15:85; flowrate: 0.5 mL/min; column temperature: 25 °C; sample solvent: sodium phosphate buffer (100 mM, pH 7.4).

Synthesis of Trisodium 2-*O*-[4,5-bis(carboxymethoxy)-2-nitrobenzyl]salicylate (22b)



To a solution of salicylic acid **22a** (60 mg, 0.14 mmol) in MeOH (2.5 mL) a 0.2 M solution of NaOH (2.1 mL, 0.43 mmol) was added. The reaction mixture was stirred for 5 min before it was lyophilised overnight to yield a solid (69 mg, 0.14 mmol, quant.). ^{1}H-NMR (600 MHz, D_2O): δ [ppm] = 4.56 (s, 2 H, 8′-H), 4.62 (s, 2 H, 8-H), 5.43 (s, 2 H, 7-H), 7.03 (d, $^{3}J_{3'',4''}$ = 8.2 Hz, 1 H, 3″-H), 7.06 (ddd, $^{3}J_{5'',6''}$ = 7.5 Hz, $^{3}J_{5'',4''}$ = 7.4 Hz, $^{4}J_{5'',3''}$ = 1.0 Hz, 1 H, 5″-H), 7.36 (ddd, $^{3}J_{4'',3''}$ = 8.2 Hz, $^{3}J_{4'',5''}$ = 7.4 Hz, $^{4}J_{4'',6''}$ = 1.8 Hz, 1 H, 4″-H), 7.41 (s, 1 H, 3-H), 7.46 (dd, $^{3}J_{6'',5''}$ = 7.5 Hz, $^{3}J_{6'',4''}$ = 1.8 Hz, 1 H, 6″-H), 7.74 (s, 1 H, 6-H); ^{13}C-NMR (151 MHz, D_2O): δ [ppm] = 67.3, 67.3 (C-8, C-8′), 67.6 (C-7), 109.4 (C-3), 111.4 (C-6), 113.8 (C-3″), 121.4 (C-5″), 128.3 (C-6″), 129.5 (C-1), 129.7 (C-4″), 130.2 (C-1″), 139.0 (C-2), 145.7 (C-4), 152.1 (C-5), 154.1 (C-2″), 174.9 (C-9), 175.4 (C-9′), 176.0 (C-7″); IR (atr-film): $\tilde{\nu}$ [cm^{-1}] = 3216, 1603, 1553, 1521, 1425, 1382, 1329, 1273, 1214, 1100, 1070, 1020, 855, 818, 753, 695, 663; m.p.: 245 °C (decay).

S4 Supporting data

S4.1 UV-Vis spectra of compounds

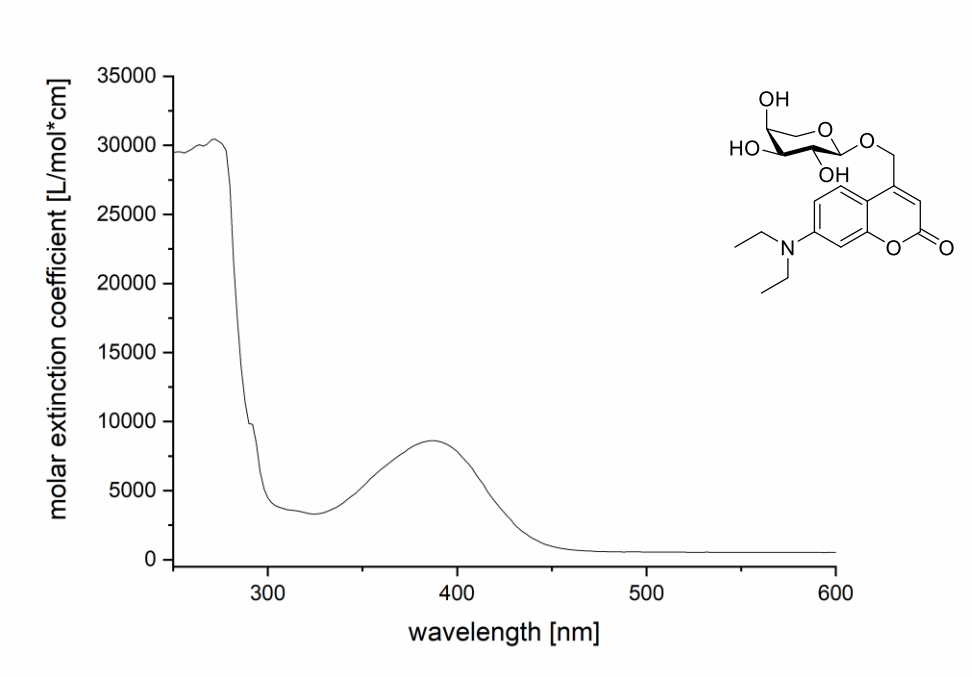


Figure S1: UV-Vis spectrum of compound **2b** (0.10 mM in MeOH, 25 °C).

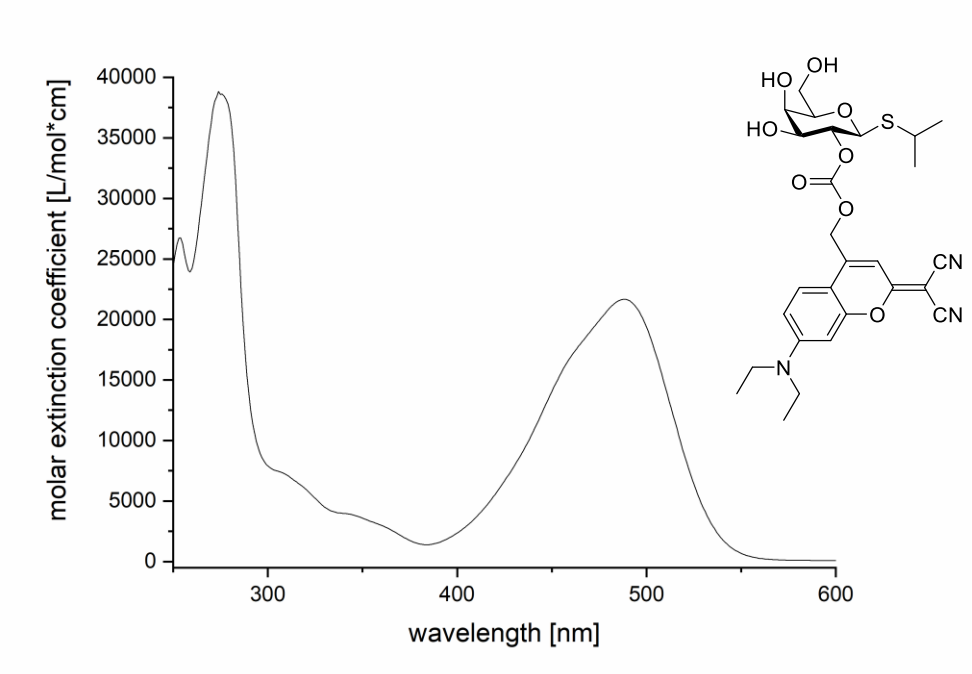


Figure S2: UV-Vis spectrum of compound **1c** [0.05 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50, 25 °C].

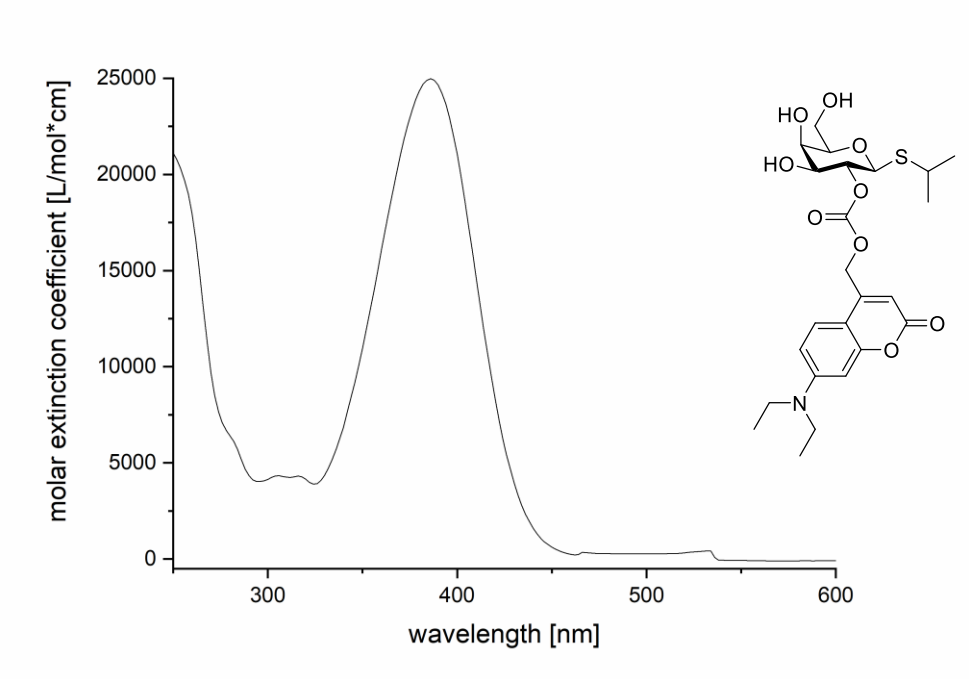


Figure S3: UV-Vis spectrum of compound **1b** [25.0 μM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50, 25 °C].

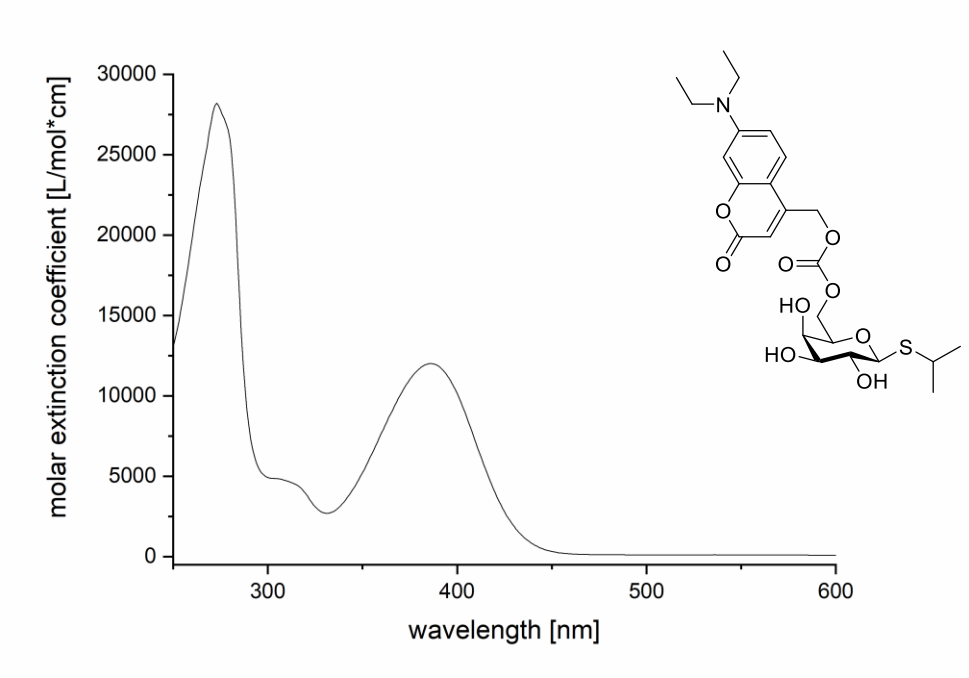


Figure S4: UV-Vis spectrum of compound **1d** [0.05 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50, 25 °C].

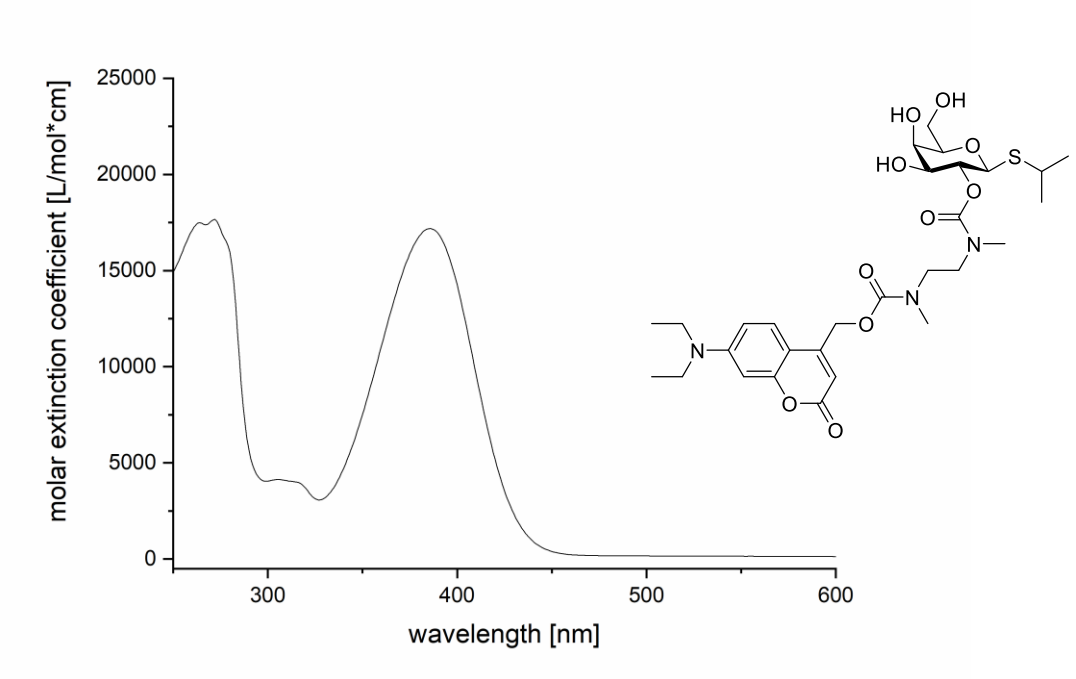


Figure S5: UV-Vis spectrum of compound **1e** [0.05 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50, 25 °C].

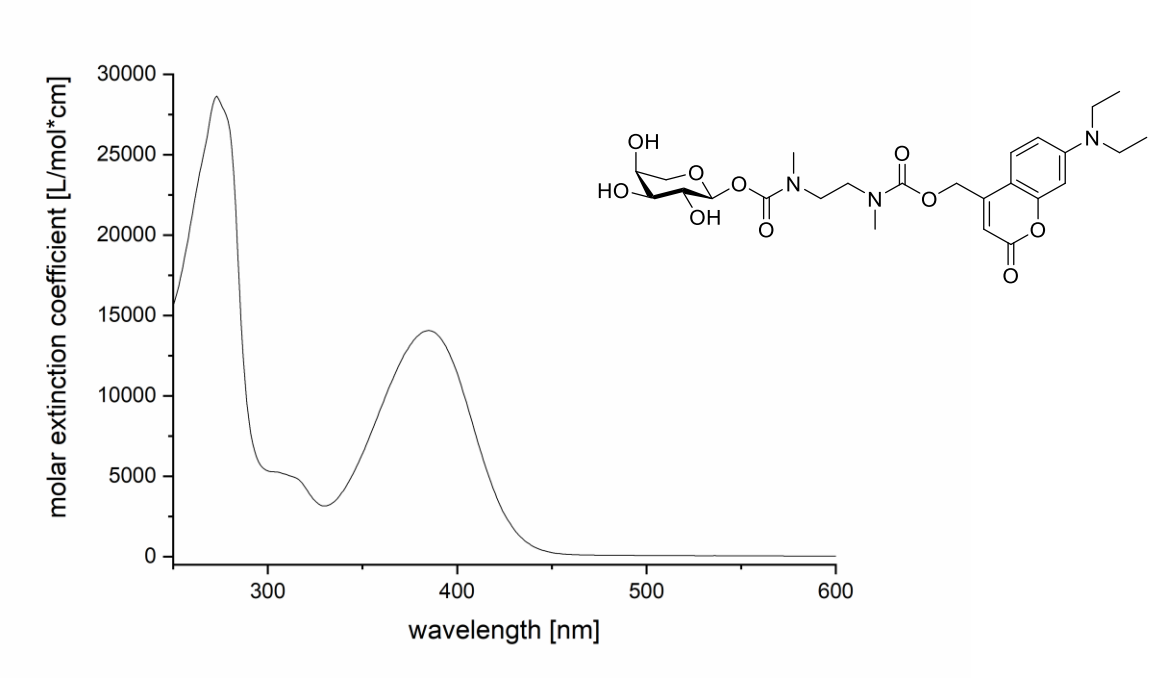


Figure S6: UV-Vis spectrum of compound **2c** [0.05 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50, 25 °C].

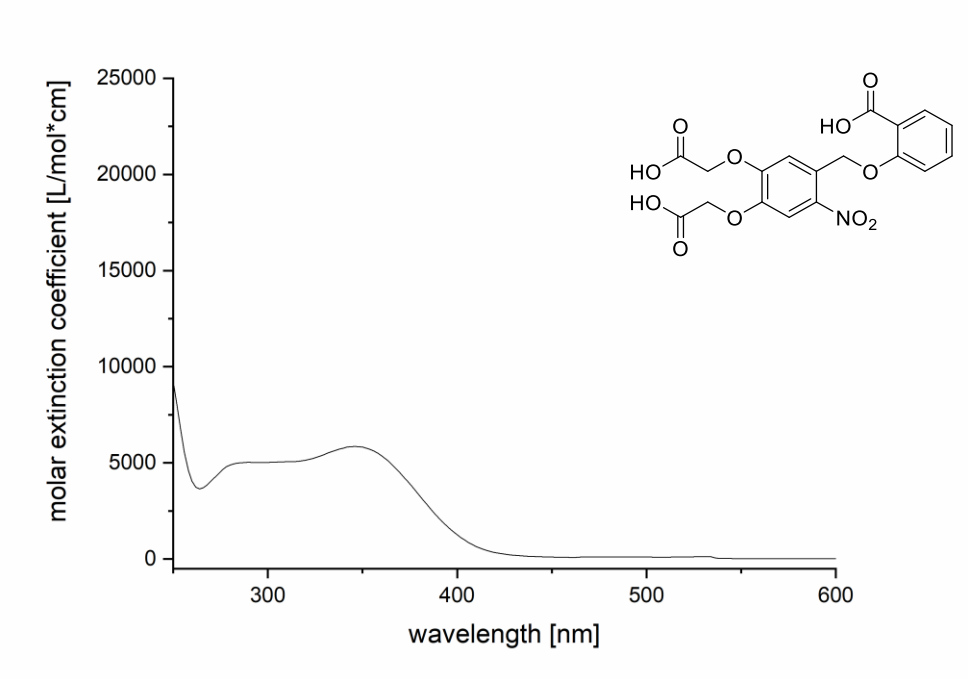


Figure S7: UV-Vis spectrum of compound **22a** [125 μM in sodium phosphate buffer (100 mM, pH 7.4), 25 °C].

S4.2 Photon flux densities of light sources

Table S3: Determined photon flux densities ($q_{n,p}$)

wavelength	$q_{n,p}$ (mol s^{-1})
365 nm	6.49×10^{-8}
405 nm	1.91×10^{-7}
430 nm	1.22×10^{-7}

S4.3 Irradiation experiments

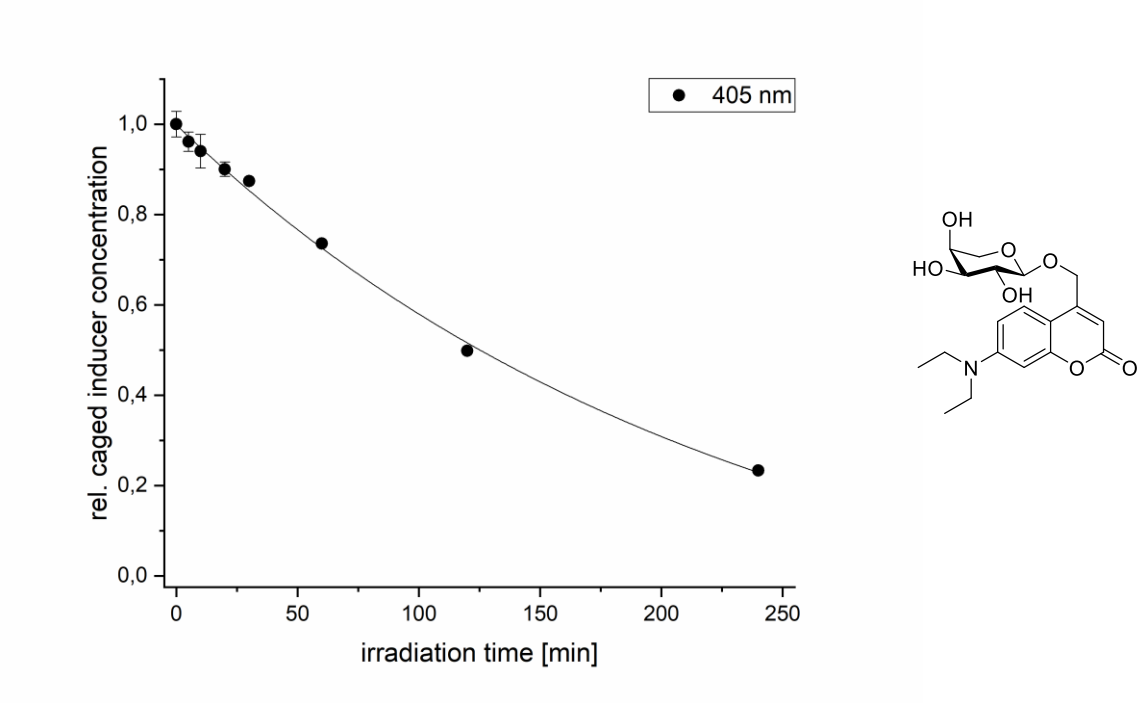


Figure S8: *In vitro* decay of **2b** (1 mM in H_2O/DMSO 99:1) via reversed-phase HPLC after irradiation with 405 nm (44.6 mW cm^{-2}, room temperature).

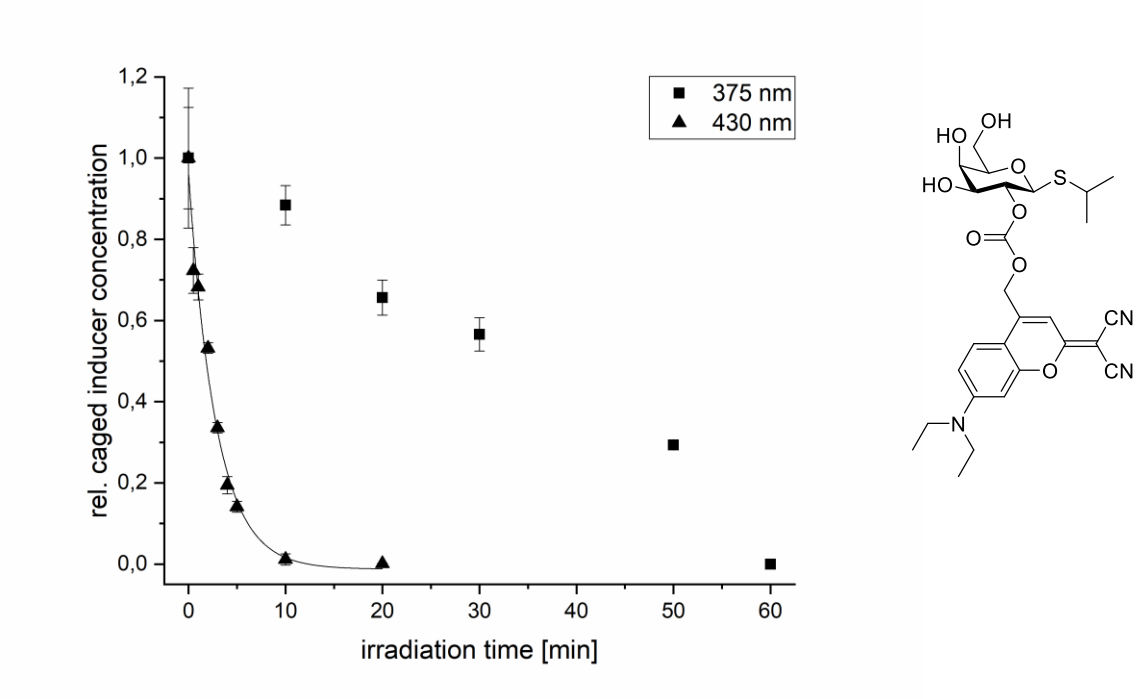


Figure S9: *In vitro* decay of **1c** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 1:1] via reversed-phase HPLC after irradiation with 375 nm (6.4 mW cm^{-2}, room temperature) and 430 nm (45.6 mW cm^{-2}, room temperature).

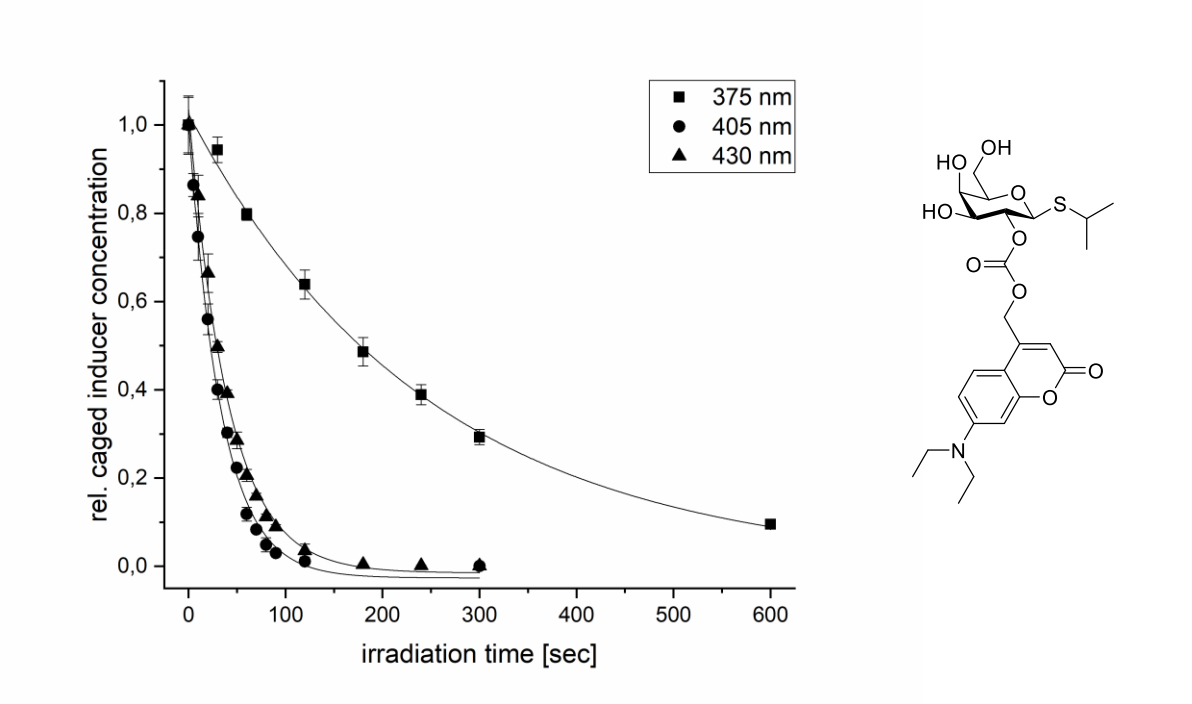


Figure S10: *In vitro* decay of **1b** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 1:1] via reversed-phase HPLC after irradiation with 375 nm (6.4 mW cm^{-2}, room temperature), 405 nm (44.6 mW cm^{-2}, room temperature) and 430 nm (45.6 mW cm^{-2}, room temperature).

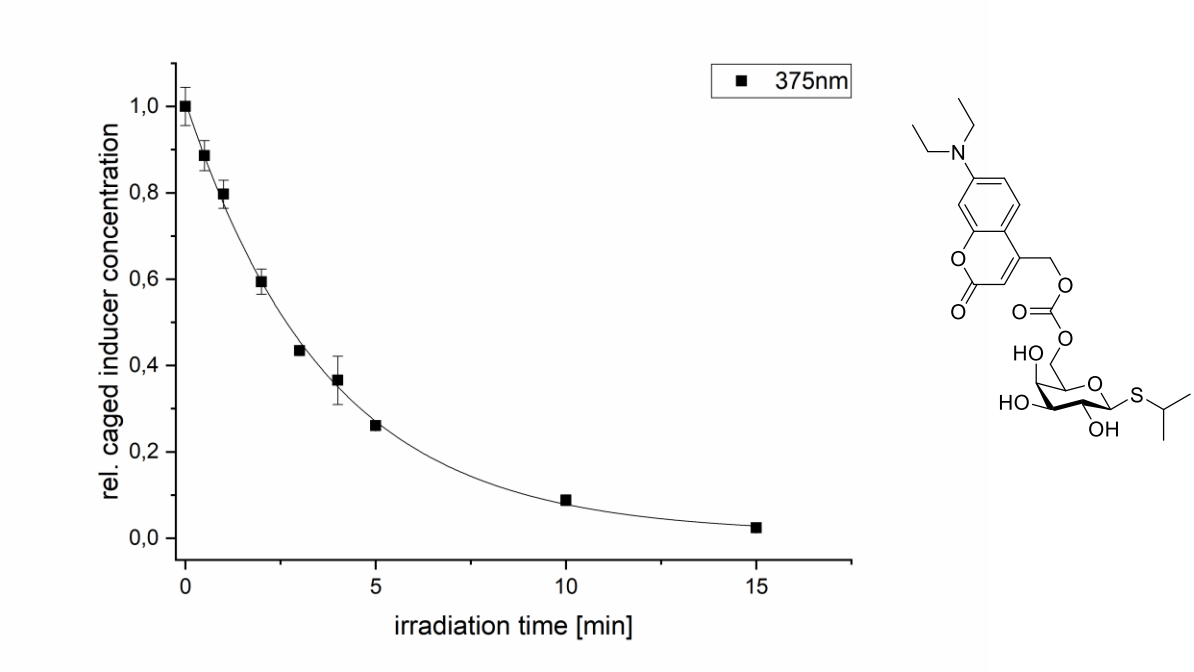


Figure S11: *In vitro* decay of **1d** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 1:1] via reversed-phase HPLC after irradiation with 375 nm (6.4 mW cm^{-2}, room temperature).

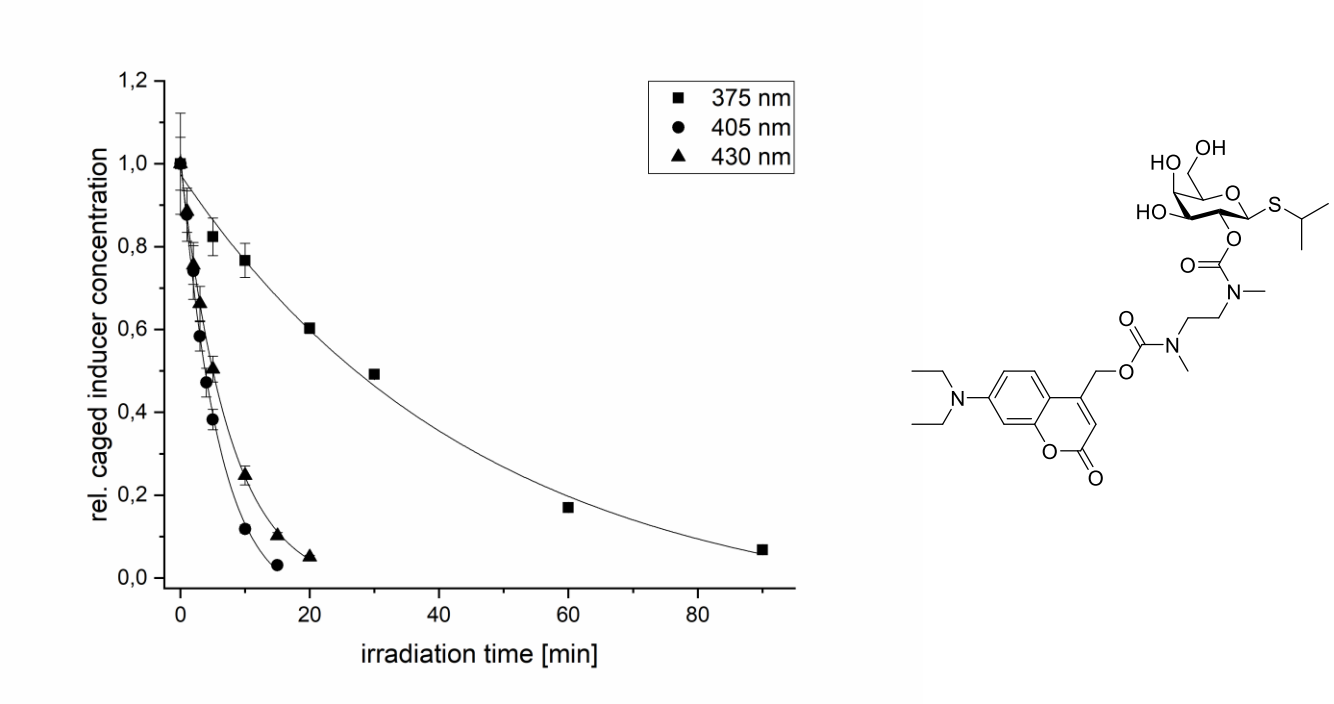


Figure S12: *In vitro* decay of **1e** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 1:1] via reversed-phase HPLC after irradiation with 375 nm (6.4 mW cm^{-2}, room temperature), 405 nm (44.6 mW cm^{-2}, room temperature) and 430 nm (45.6 mW cm^{-2}, room temperature).

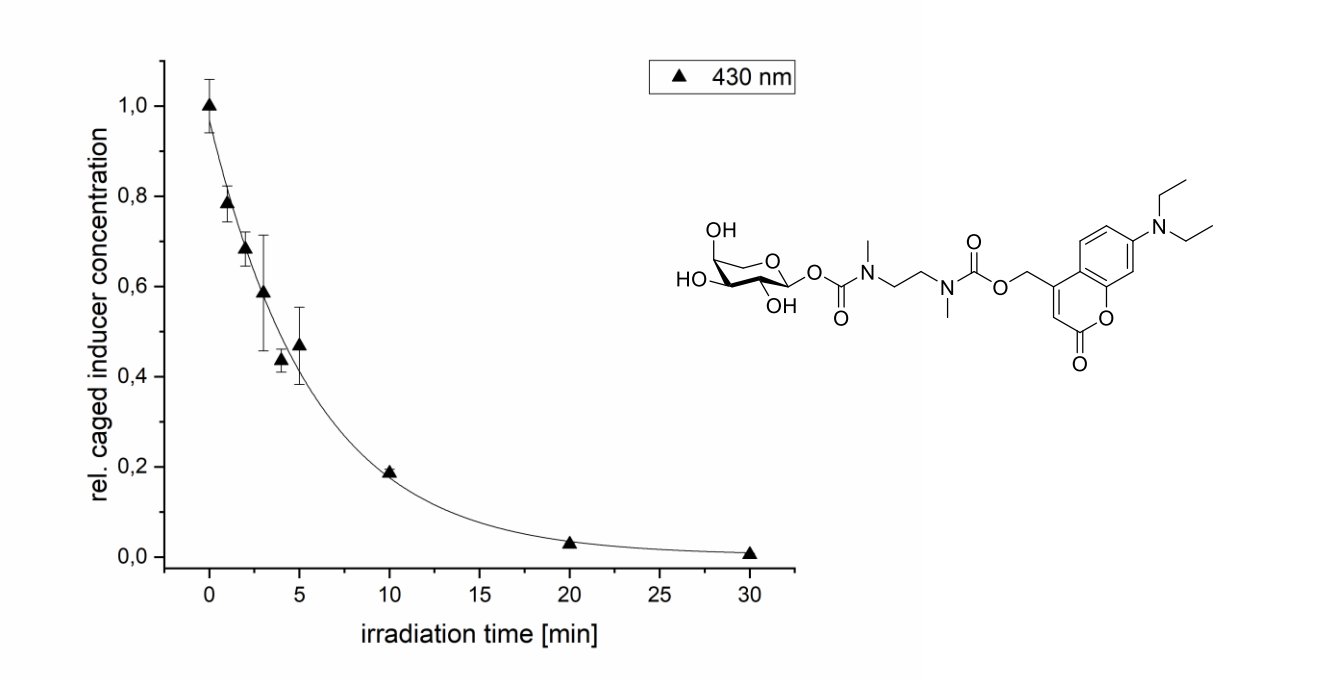


Figure S13: *In vitro* decay of **2c** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 1:1] via reversed-phase HPLC after irradiation with 430 nm (45.6 mW cm^{-2}, room temperature).

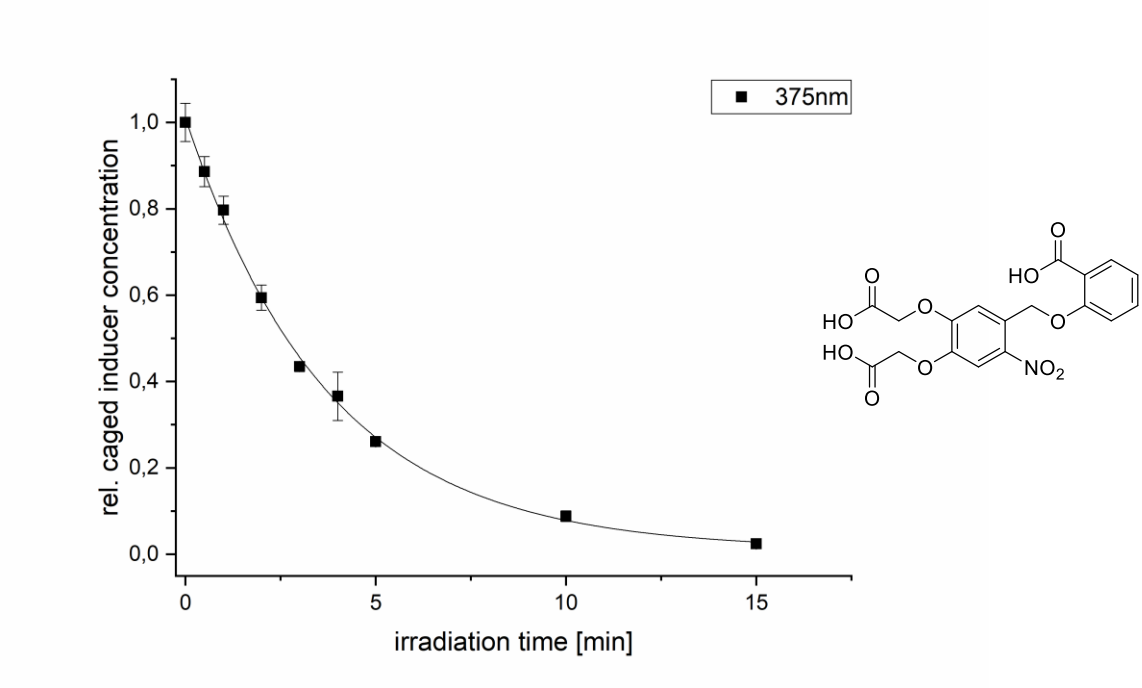


Figure S14: *In vitro* decay of **22a** [0.5 mM in sodium phosphate buffer (100 mM, pH 7.4)] via reversed-phase HPLC after irradiation with 375 nm (6.4 mW cm^{-2}, room temperature).

Table S3: Fitting parameters for **1b**–**e**, **2b**–**c** and **22a** at different wavelength.

photocaged inducer	λ [nm]	y_0	A_1	t_1	k	*r*
2b	375	-0,19104	1,18966	230,51535	0,00434	159,78106
1c	430	-0,01254	0,97483	2,83993	0,35212	1,96849
1b	375	0,0004873	1,02624	246,92187	0,00405	171,1532
1b	405	-0,02632	1,03705	33,42057	0,02992	23,16538
1b	430	-0,01504	1,05356	40,75615	0,02454	28,25001
1d	375	0,01084	1,00002	3,7118	0,26941	2,57283
1e	375	-0,09535	1,06979	46,27316	0,02161	32,07411
1e	405	-0,06395	1,09129	5,79684	0,17251	4,01806
1e	430	-0,02656	1,03055	7,48242	0,13365	5,18642
2c	430	0,00417	0,96575	5,79819	0,17247	4,019
22a	375	-0,00435	0,9935	3,52342	0,28382	2,44225

S4.4 HPLC-Traces

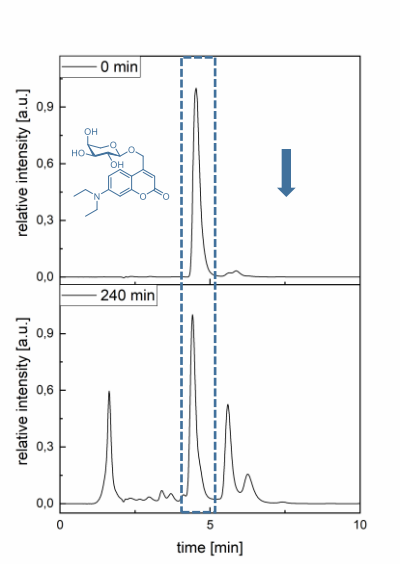


Figure S15: UV traces at 388 nm of the reversed-phase HPLC analysis of **2b** (1 mM in H_2O/DMSO 99:1) before irradiation and after 240 min of irradiation at 405 nm (44.6 mW cm^{-2}, room temperature).

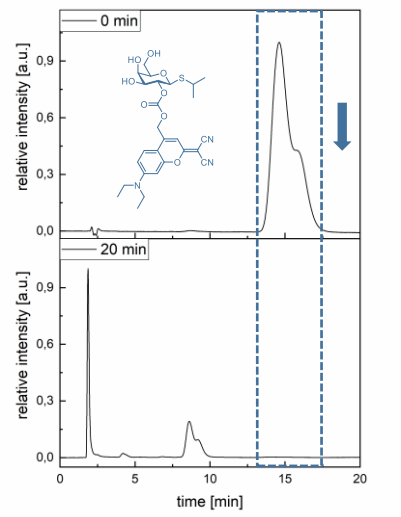


Figure S16: UV traces at 488 nm of the reversed-phase HPLC analysis of **1c** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 20 min of irradiation at 430 nm (45.6 mW cm^{-2}, room temperature).

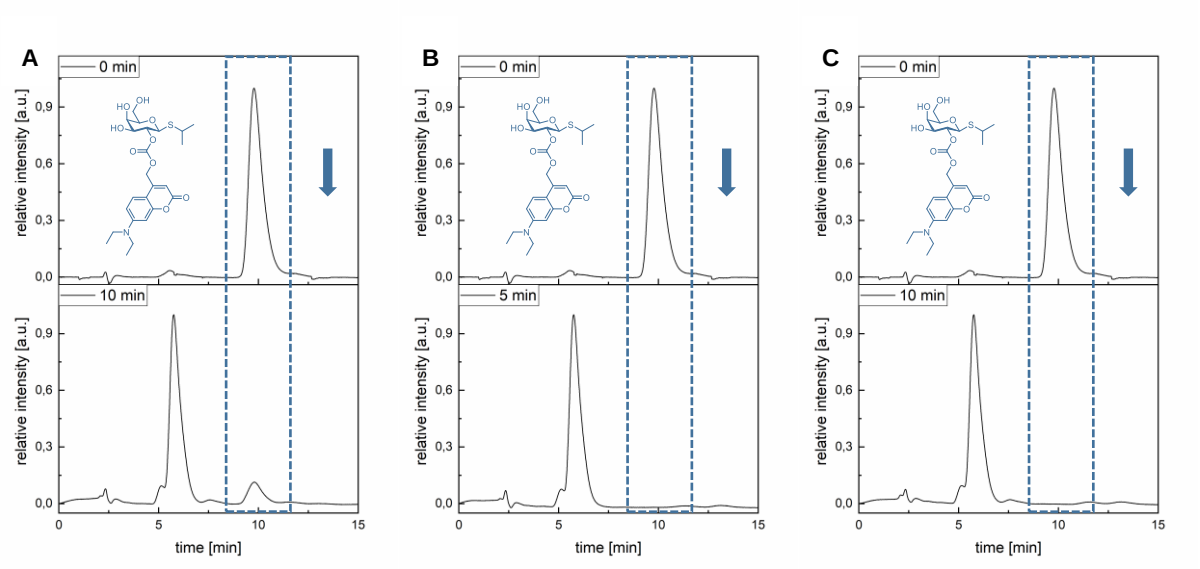


Figure S17: **A**) UV traces at 392 nm of the reversed-phase HPLC analysis of **1b** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 10 min of irradiation at 375 nm (6.4 mW cm^{-2}, room temperature); **B**) UV traces at 392 nm of the reversed-phase HPLC analysis of **1b** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 5 min of irradiation at 405 nm (44.6 mW cm^{-2}, room temperature); **C**) UV traces at 392 nm of the reversed-phase HPLC analysis of **1b** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 10 min of irradiation at 430 nm (45.6 mW cm^{-2}, room temperature).

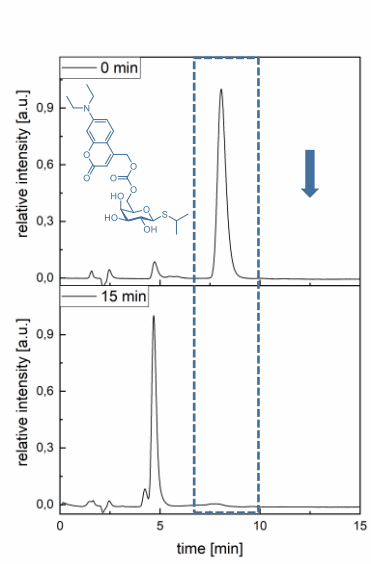


Figure S18: UV traces at 386 nm of the reversed-phase HPLC analysis of **1d** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 15 min of irradiation at 375 nm (6.4 mW cm^{-2}, room temperature).

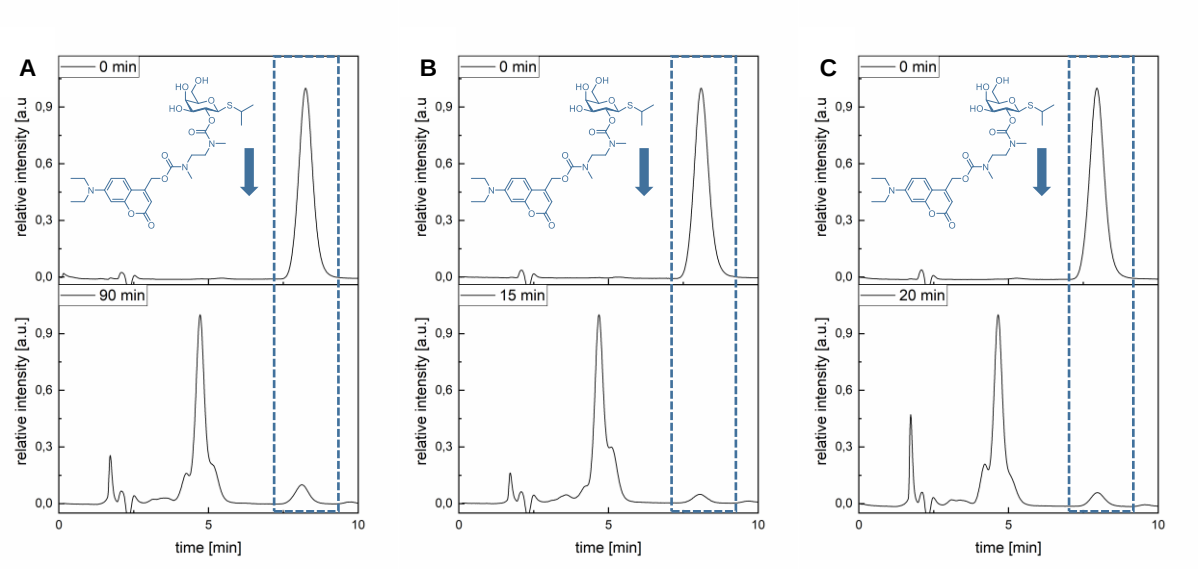


Figure S19: **A**) UV traces at 386 nm of the reversed-phase HPLC analysis of **1e** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 10 min of irradiation at 375 nm (6.4 mW cm^{-2}, room temperature); **B**) UV traces at 386 nm of the reversed-phase HPLC analysis of **1e** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 5 min of irradiation at 405 nm (44.6 mW cm^{-2}, room temperature); **C**) UV traces at 386 nm of the reversed-phase HPLC analysis of **1e** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 10 min of irradiation at 430 nm (45.6 mW cm^{-2}, room temperature).

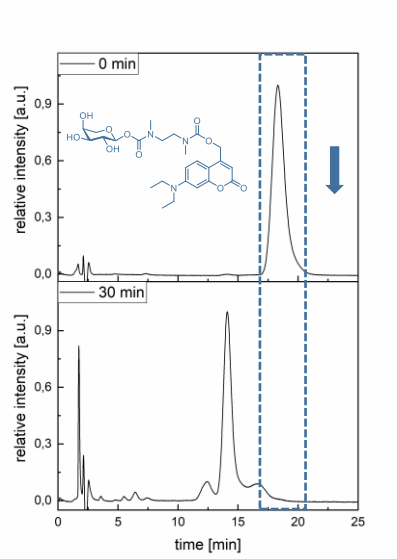


Figure S20: UV traces at 385 nm of the reversed-phase HPLC analysis of **2c** [0.5 mM in Tris buffer (20 mM, pH 7.5)/MeCN 50:50] before irradiation and after 10 min of irradiation at 430 nm (45.6 mW cm^{-2}, room temperature).

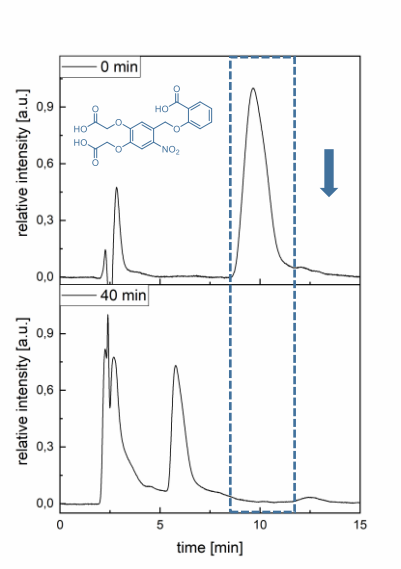


Figure S21: UV traces at 346 nm of the reversed-phase HPLC analysis of **22a** [0.5 mM in sodium phosphate buffer (100 mM, pH 7.4)] before irradiation and after 15 min of irradiation at 375 nm (6.4 mW cm^{-2}, room temperature).

S4.5 Stability measurements

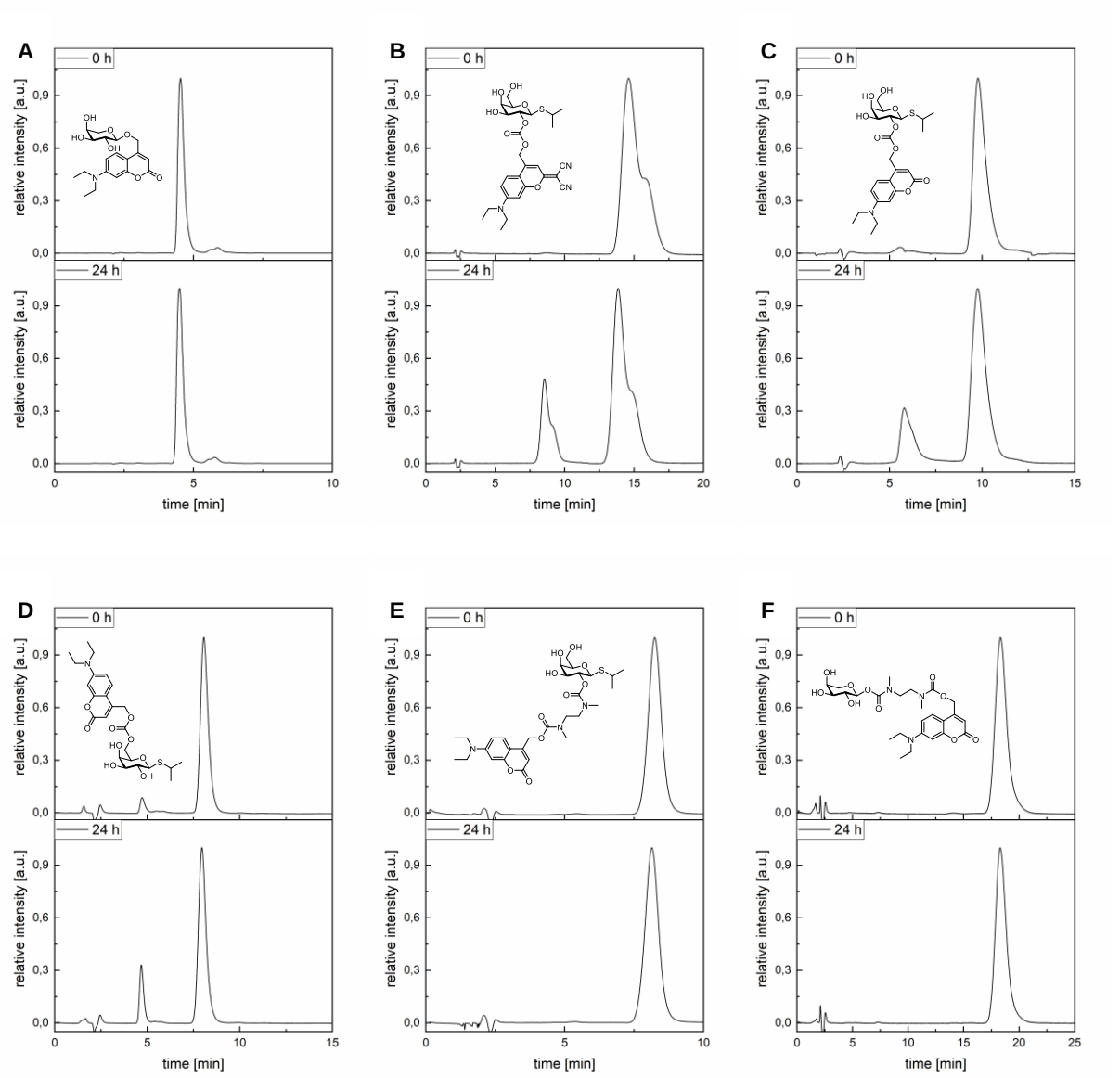


Figure S22: UV traces at absorption maxima of the reversed-phase HPLC analysis of **2b** (**A**), **1c** (**B**), **1b** (**C**), **1d** (**D**), **1e** (**E**) and **2c** (**F**) for determination of stability. The reported values in Table **1** are means of triplicate measurements.

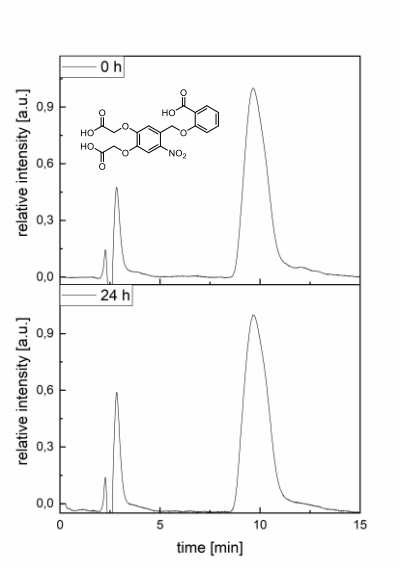


Figure S23: UV traces at absorption maxima of the reversed-phase HPLC analysis of **22a** for determination of stability. The reported value is the mean of triplicate measurements.

S4.6 ESI measurements

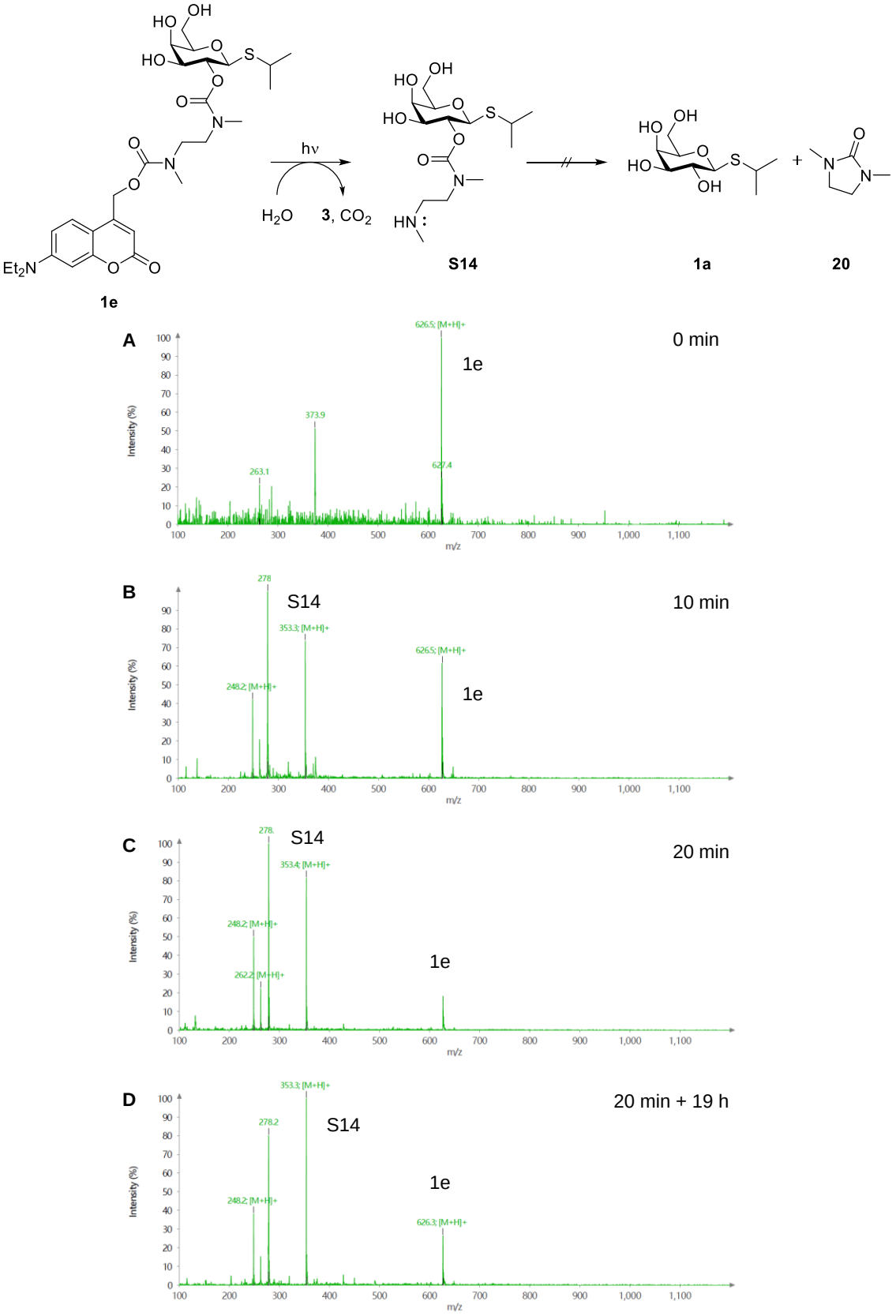


Figure S24: ESI-MS measurements of **1e** (1 mM in MeOH/H_2O 1:1) for detection of the intermediate **S14** after irradiation with 430 nm (45.6 mW cm^{-2}, room temperature) for **A**) 0 min; **B**) 10 min; **C**) 20 min; **D**) 20 min plus allowing it to stand for 19 h at room temperature.

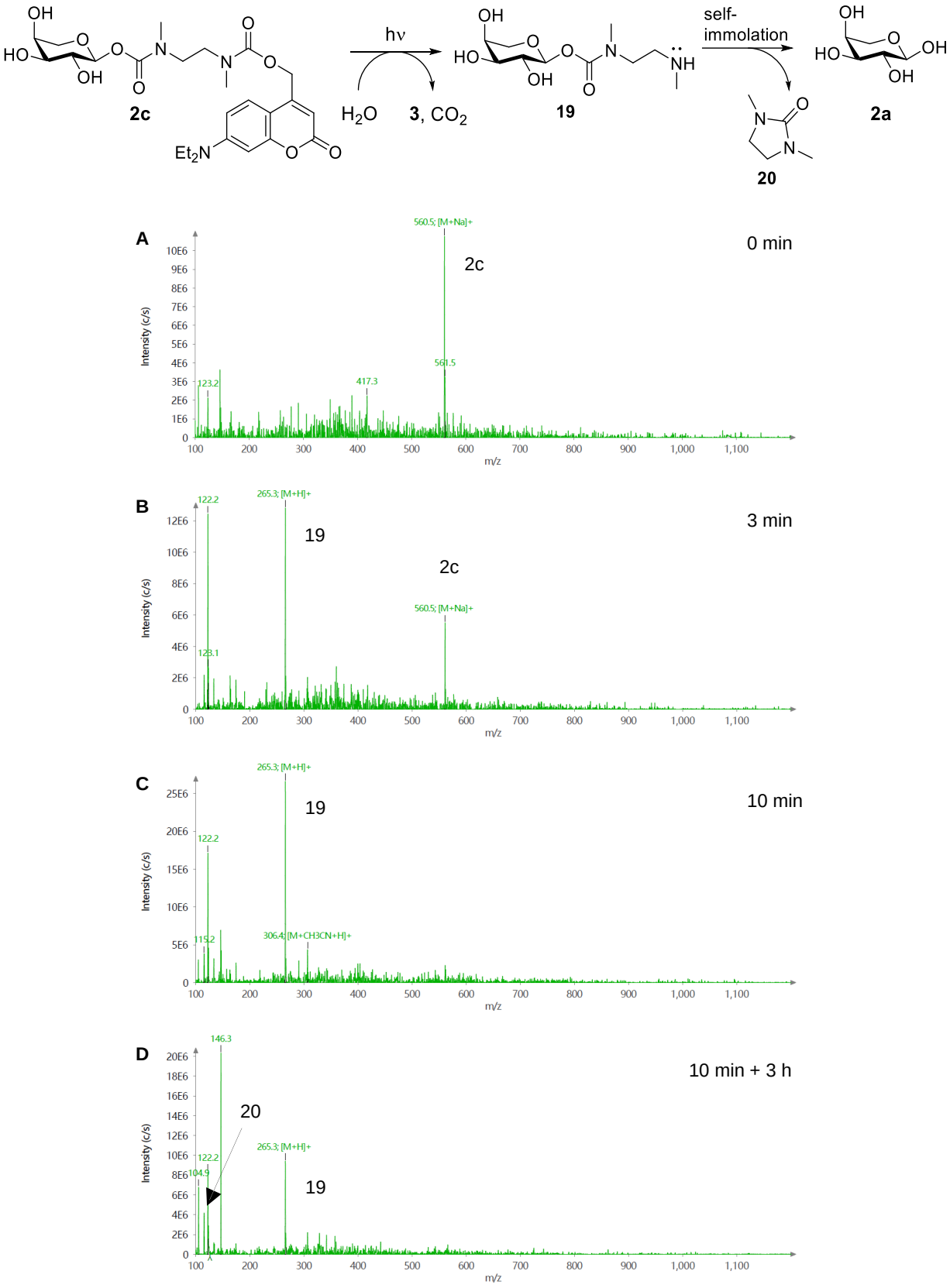


Figure S25: ESI-MS measurements of **2c** (1 mM in MeOH/H_2O 1:1) for monitoring of the intermediate **19** after irradiation with 430 nm (45.6 mW cm^{-2}, room temperature) for **A**) 0 min; **B**) 3 min; **C**) 10 min; **D**) 10 min plus allowing it to stand for 3 h at room temperature.

S4.7 Toxicity of both the novel photocaged inducer variants and the light exposure

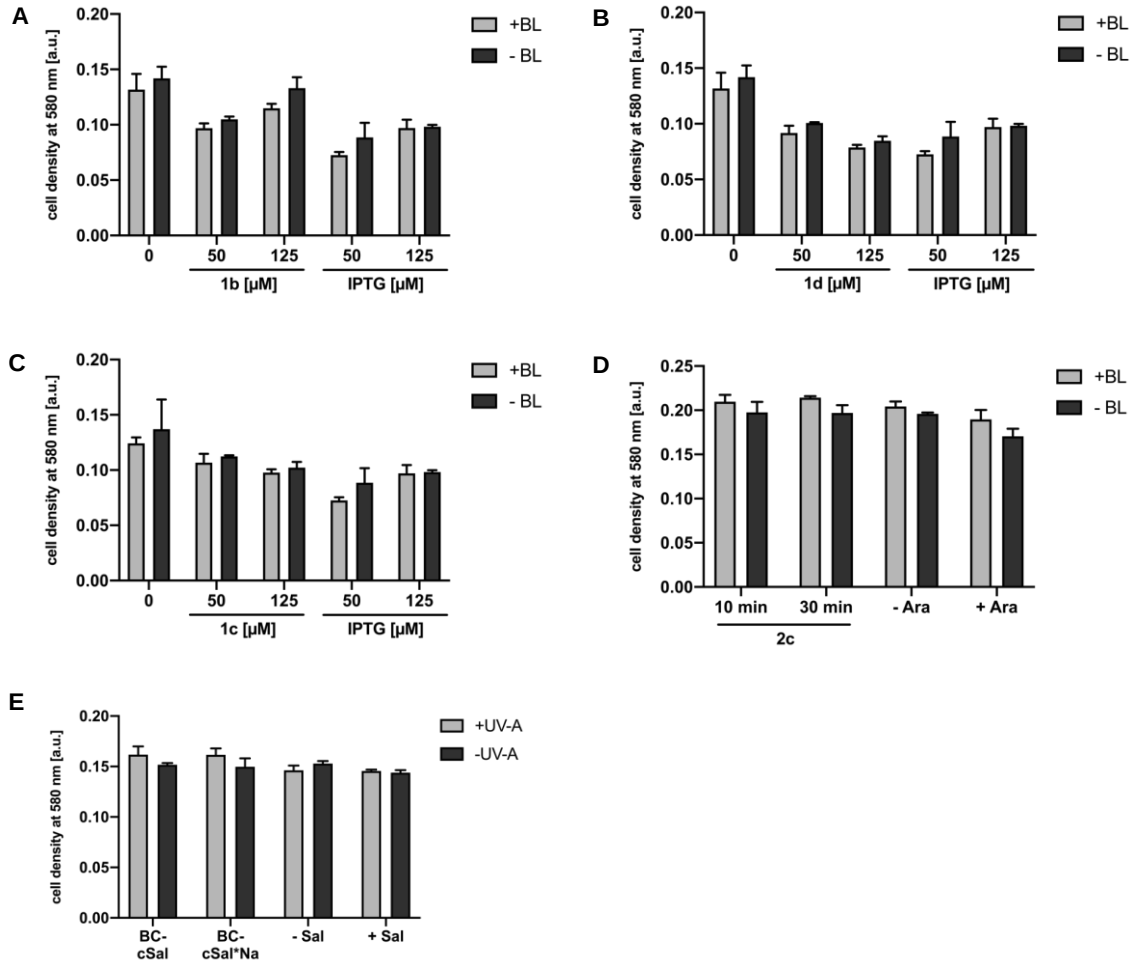


Figure S26: A–C: Growth of *E. coli* Tuner (DE3)/pRhotHi-2-lacI-eYFP expression cultures in the presence of the novel photocaged IPTG variants **1b** (**A**), **1d** (**B**) or **1c** (**C**) compared to uninduced (0 µM) and induced (50 or 125 µM IPTG) cultures. Cells were grown in LB medium at 30 °C and 1200 rpm over 20 h using a ThermoMixer C (Eppendorf, Germany). Cell growth was analysed by determining the optical density at 580 nm. After 2.5 h, formation of photoproducts was induced in cultures *via* blue light exposure at 447 nm for 10 min (BL; ~10 mW cm^{-2}) and conventional IPTG (**1a**) was added manually. **D**: Growth of *E. coli* LMG194/pBTBX-2-mCherry expression cultures in the presence of the novel photocaged arabinose variant **2c** (50 µM) compared to uninduced (-Ara; 0 µM) and induced (+Ara; 50 µM) cultures. Cells were grown in LB medium at 37 °C and 1200 rpm over 20 h using a ThermoMixer C (Eppendorf, Germany). Cell growth was analysed by determining the optical density at 580 nm. After 2.5 h, formation of photoproducts was induced in cultures *via* blue light exposure at 447 nm for 10 min or 30 min (BL; ~10 mW cm^{-2}) and conventional arabinose (**2a**) was added manually. **E**: Growth of *E. coli* Tuner(DE3)/pBNTmcs-mCherry expression cultures in the presence of the novel photocaged salicylic acid (Sal) variants BC-cSal (**22a**) and BC-cSal*Na (**22b**) compared to uninduced (-Sal; 0 µM) and induced (+ Sal; 1000 µM Sal) cultures. Cells were grown in LB medium at 30 °C and 1200 rpm over 20 h using a ThermoMixer C (Eppendorf, Germany). Cell growth was analysed by determining the optical density at 580 nm. After 2.5 h formation of photoproducts was induced in cultures *via* light exposure at 365 nm for 30 min (~1 mW cm^{-2}) and conventional Sal was added manually. Values are means of biological triplicate measurements and error bars indicate the respective standard deviation.

S4.8 *In vivo* results of photocaged IPTG 1e

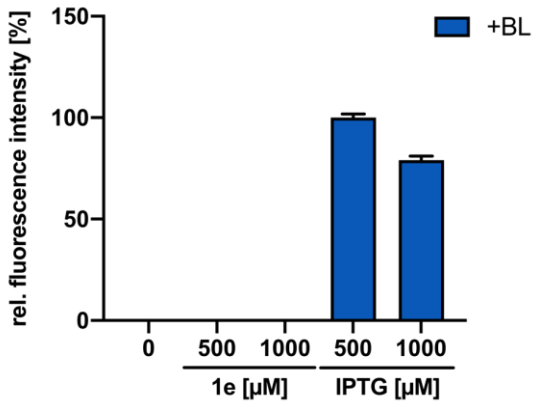


Figure S27: Normalized *in vivo* eYFP fluorescence intensity of *E. coli* Tuner (DE3)/pRhotHi-2-lacI-eYFP expression cultures supplemented with 500 µM or 1000 µM of the photocaged compound **1e**. All cultures were incubated in the dark for 20 h in LB medium at 30 °C and light-mediated induction of reporter gene expression was performed after 2.5 h by blue light exposure at 447 nm (BL; ~90 mW cm^2) for 10 min or the addition of respective amounts of conventional IPTG (**1a**). *In vivo* fluorescence intensities were determined by using a BioLector system (λ_{ex} = 508 nm, λ_{em} = 532 nm), normalized to cell densities and are shown in relation to the respective fluorescence intensities of a culture induced with conventional IPTG (**1a**). Values are means of triplicate measurements. Error bars indicate the respective standard deviations.

S4.9 NMR spectra of compounds

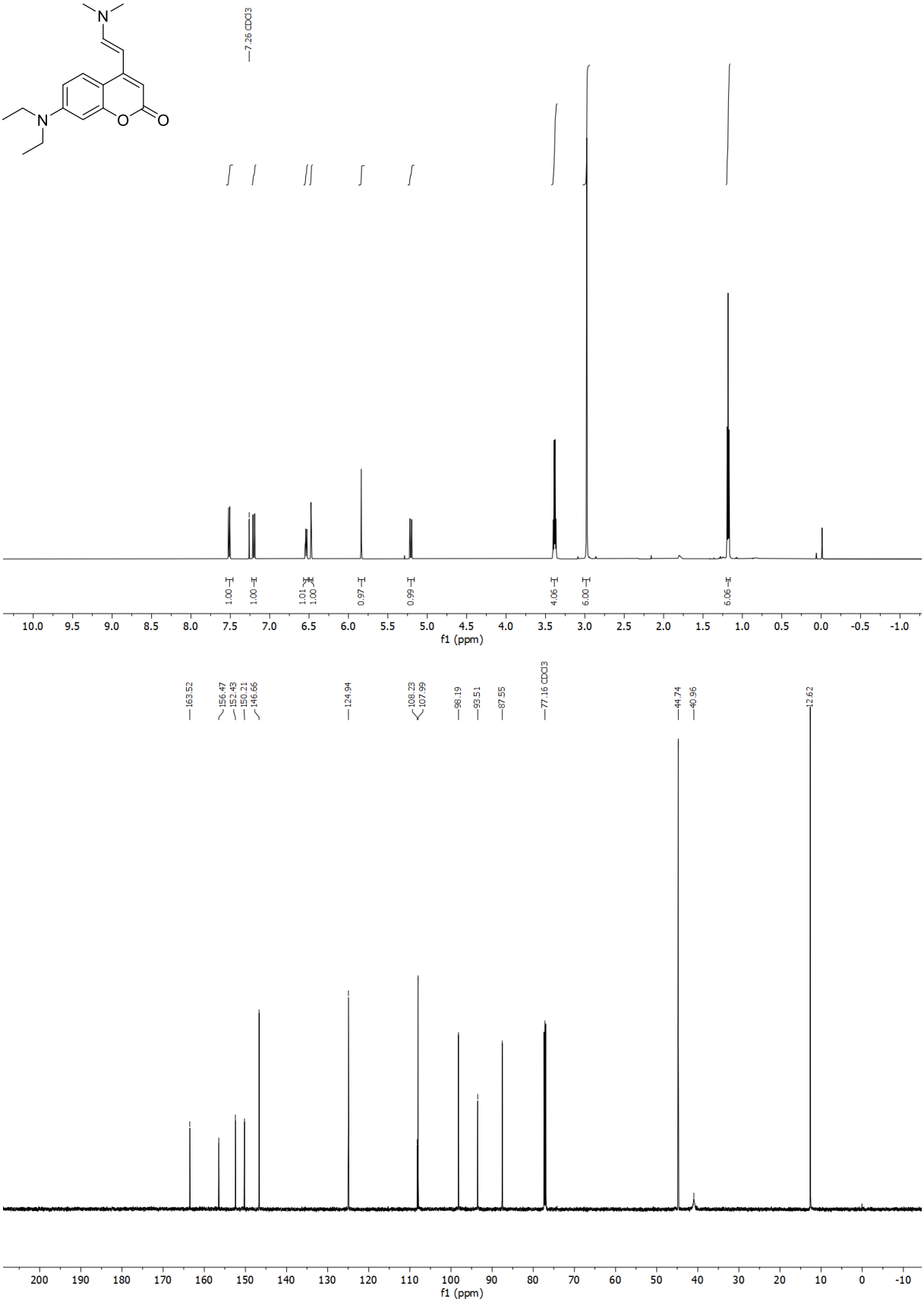


Figure S29: ^{1}H- and ^{13}C-NMR spectra of **S2** in $CDCl_3$ (600 MHz/151 MHz).

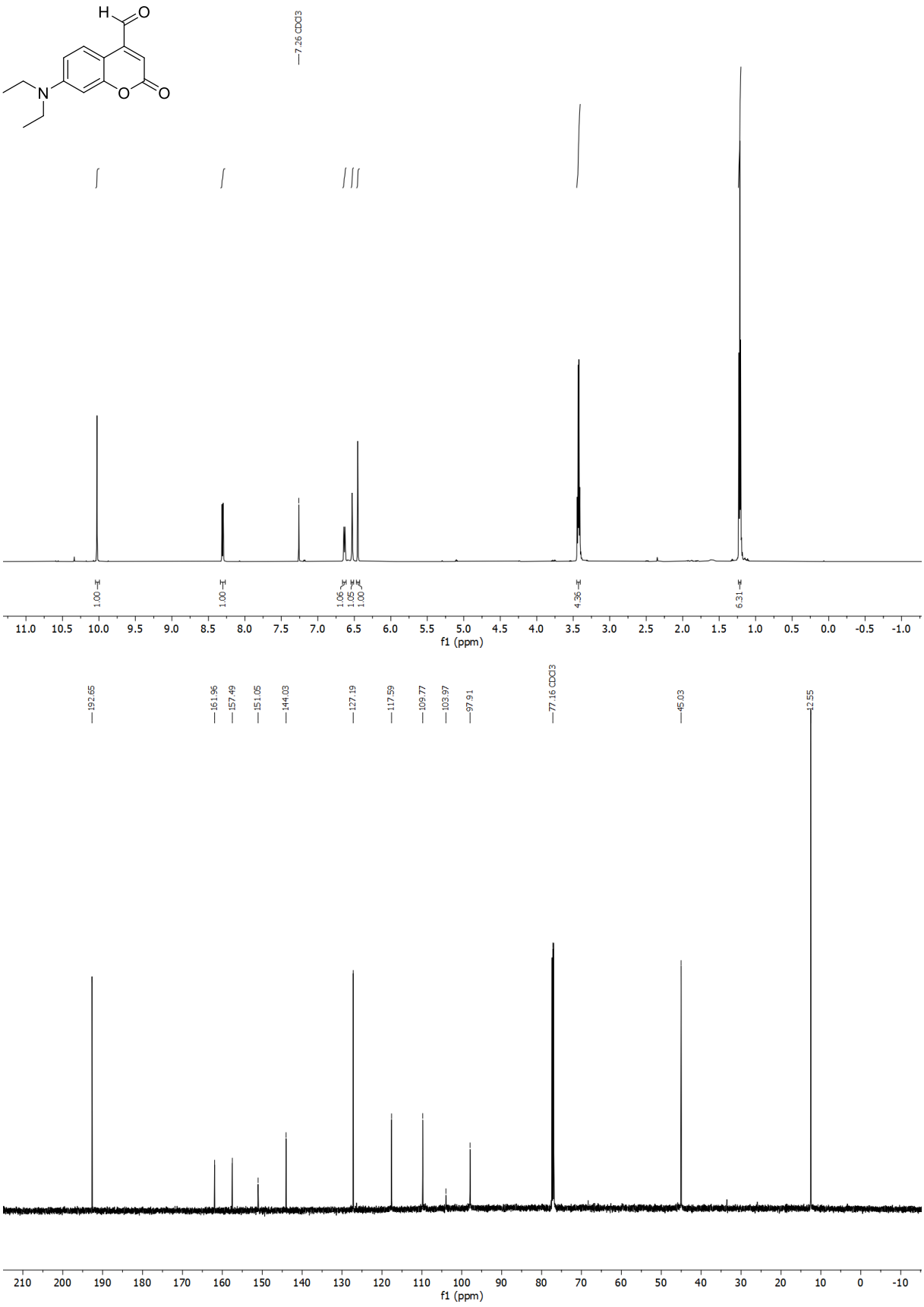


Figure S30: ^{1}H- and ^{13}C-NMR spectra of **S3** in $CDCl_3$ (600 MHz/151 MHz).

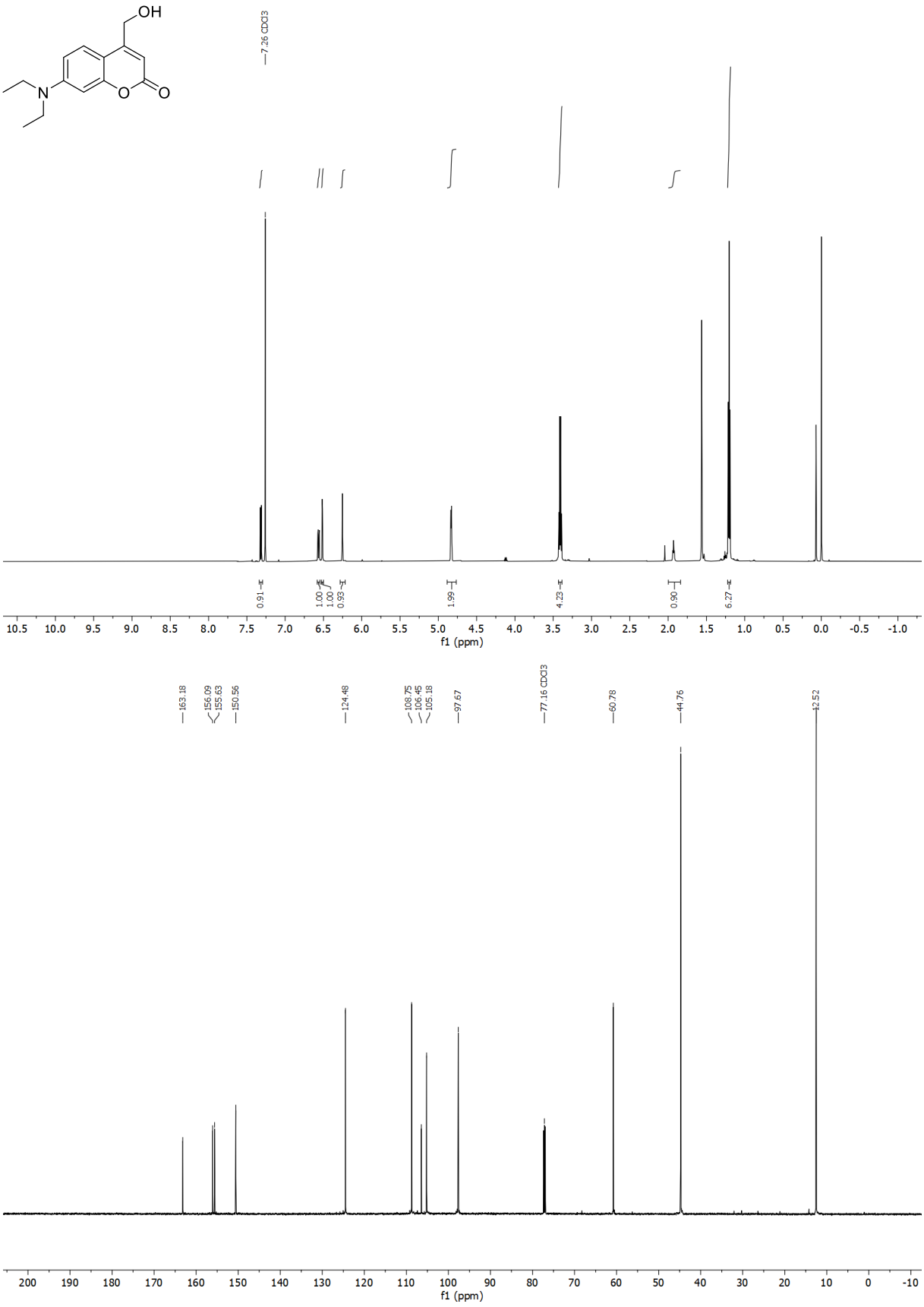


Figure S31: ^{1}H- and ^{13}C-NMR spectra of **3** in $CDCl_3$ (600 MHz/151 MHz).

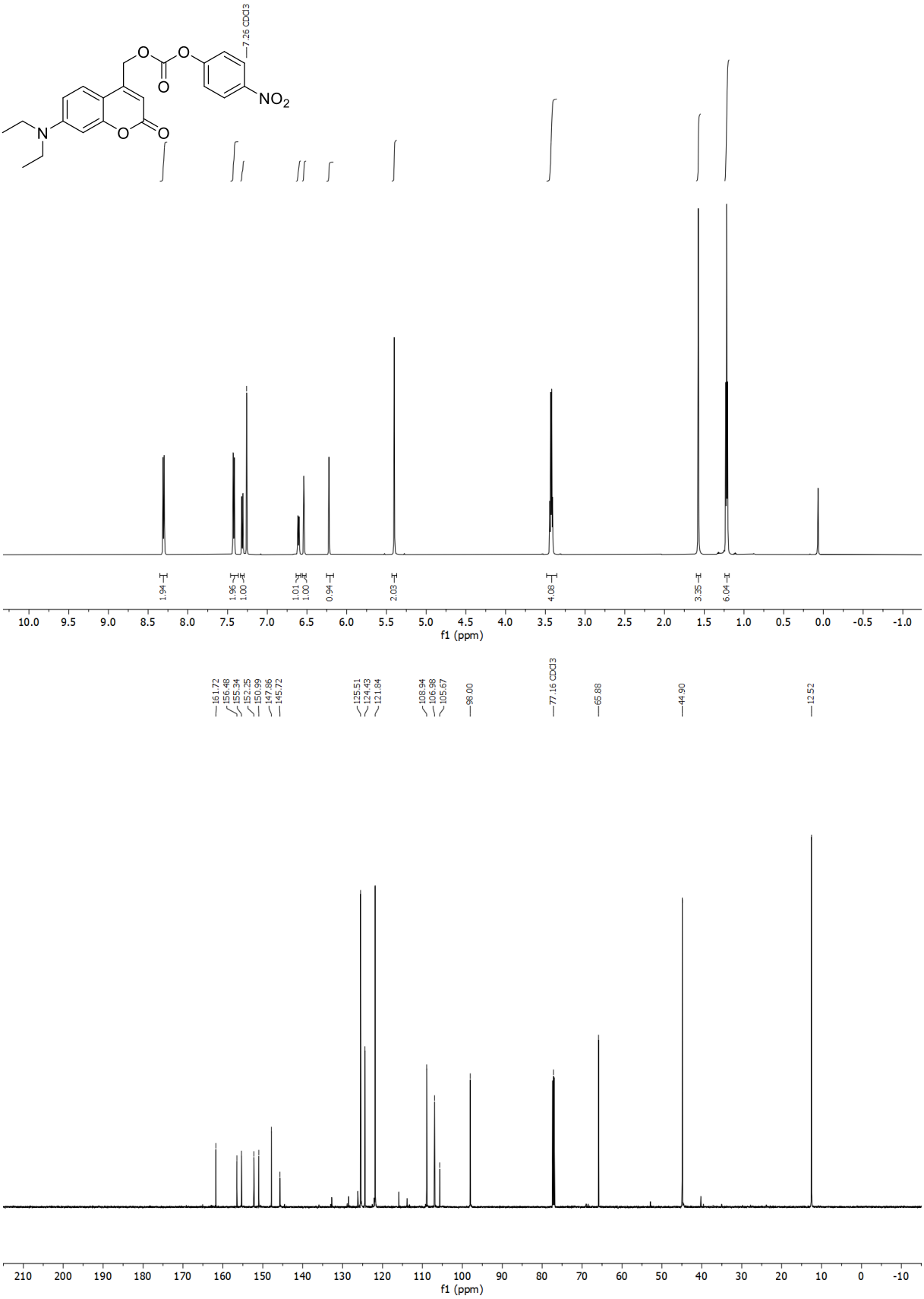


Figure S32: ^{1}H- and ^{13}C-NMR spectra of **5** in $CDCl_3$ (600 MHz/151 MHz).

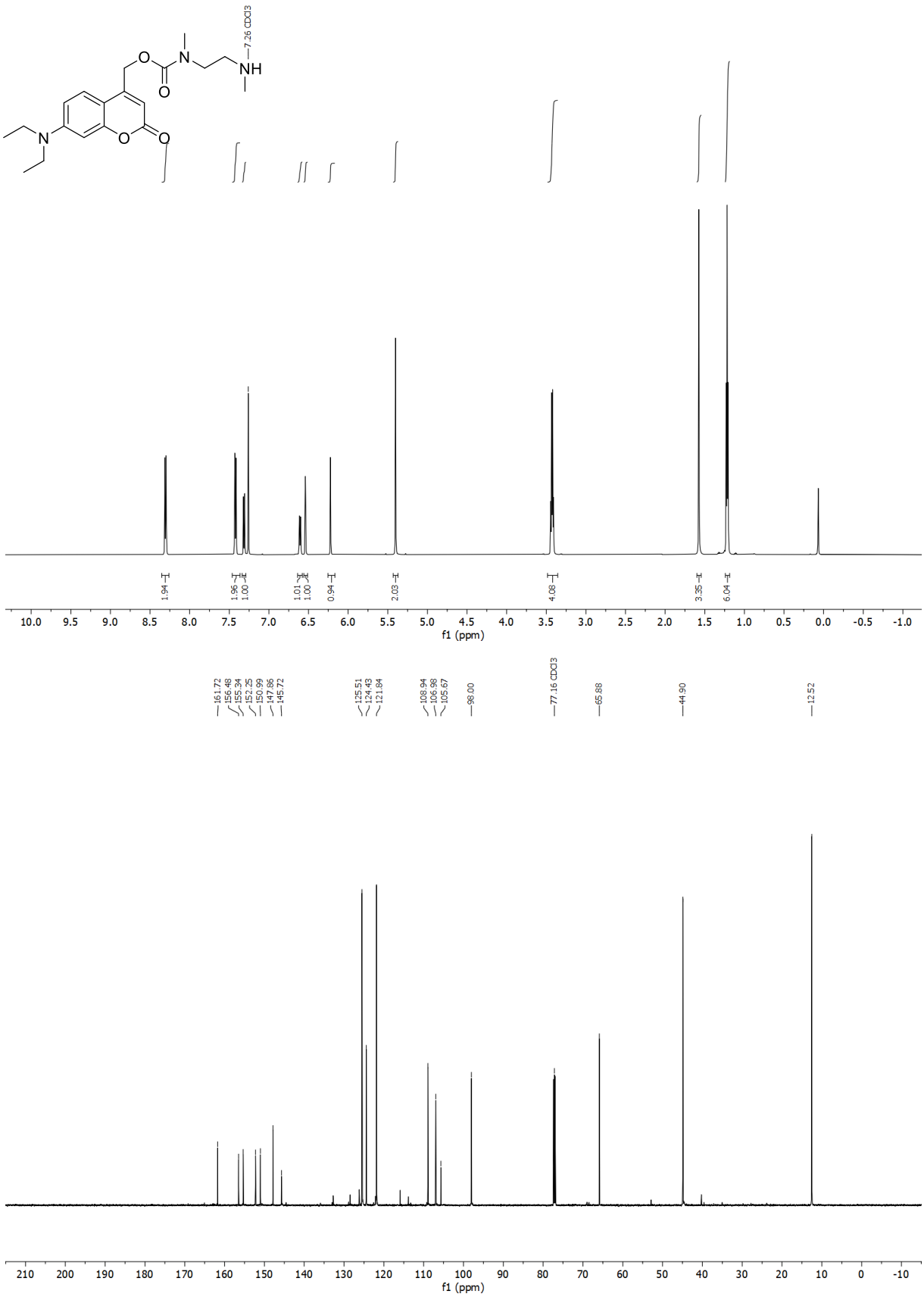


Figure S33: ^{1}H- and ^{13}C-NMR spectra of **7** in $CDCl_3$ (600 MHz/151 MHz).

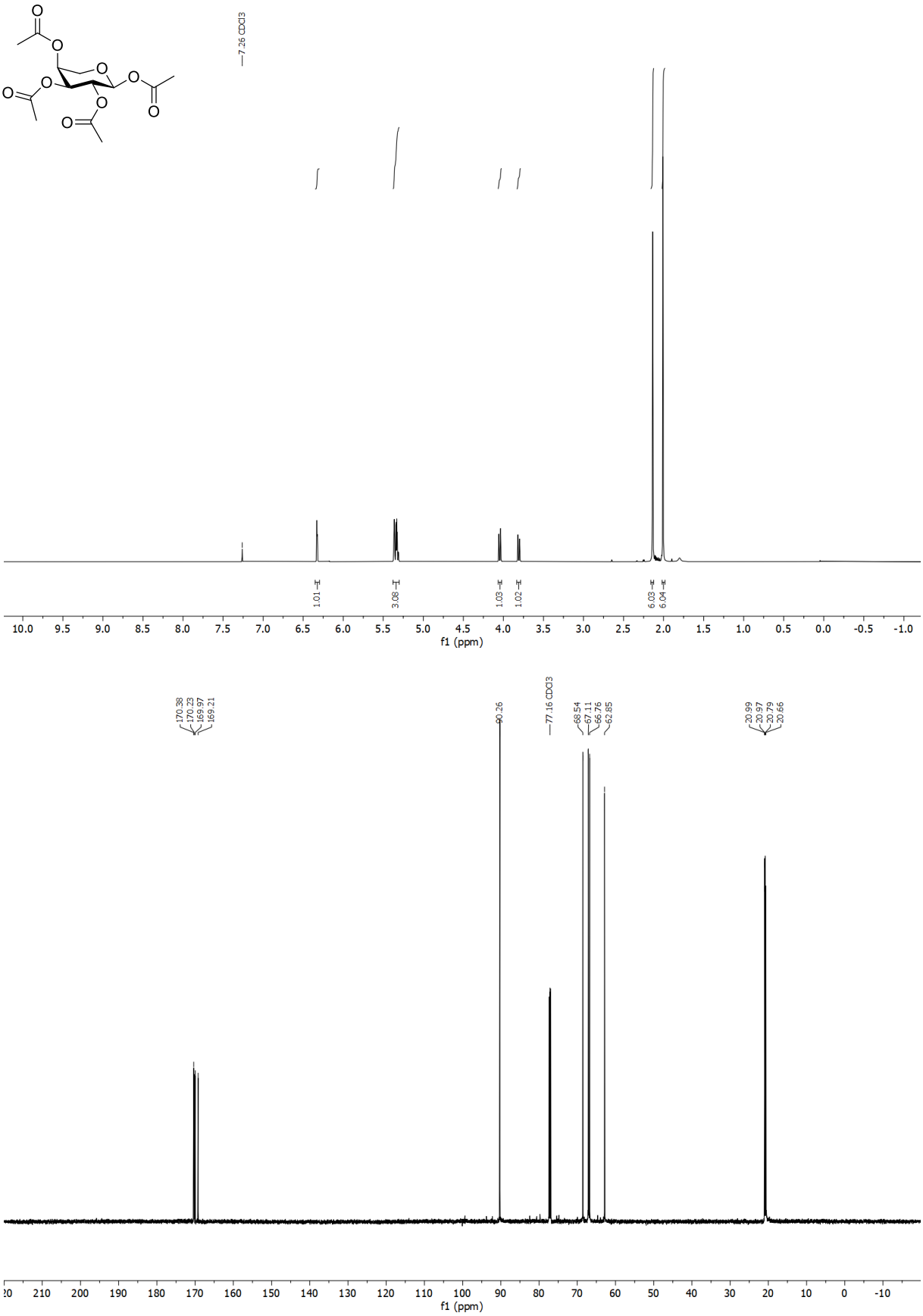


Figure S34: ^{1}H- and ^{13}C-NMR spectra of **S4** in $CDCl_3$ (600 MHz/151 MHz).

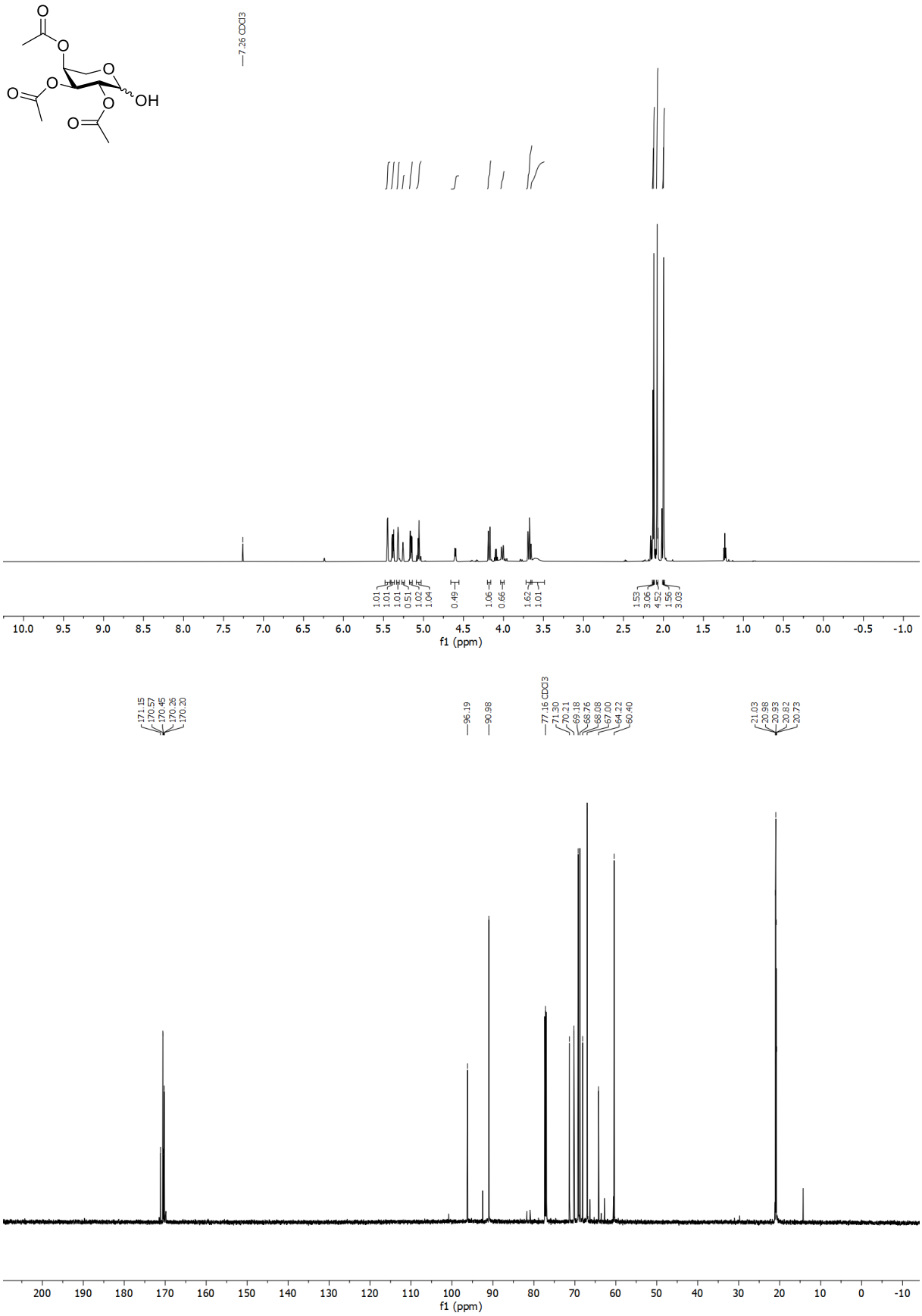


Figure S35: ^{1}H- and ^{13}C-NMR spectra of **S5** in $CDCl_3$ (600 MHz/151 MHz).

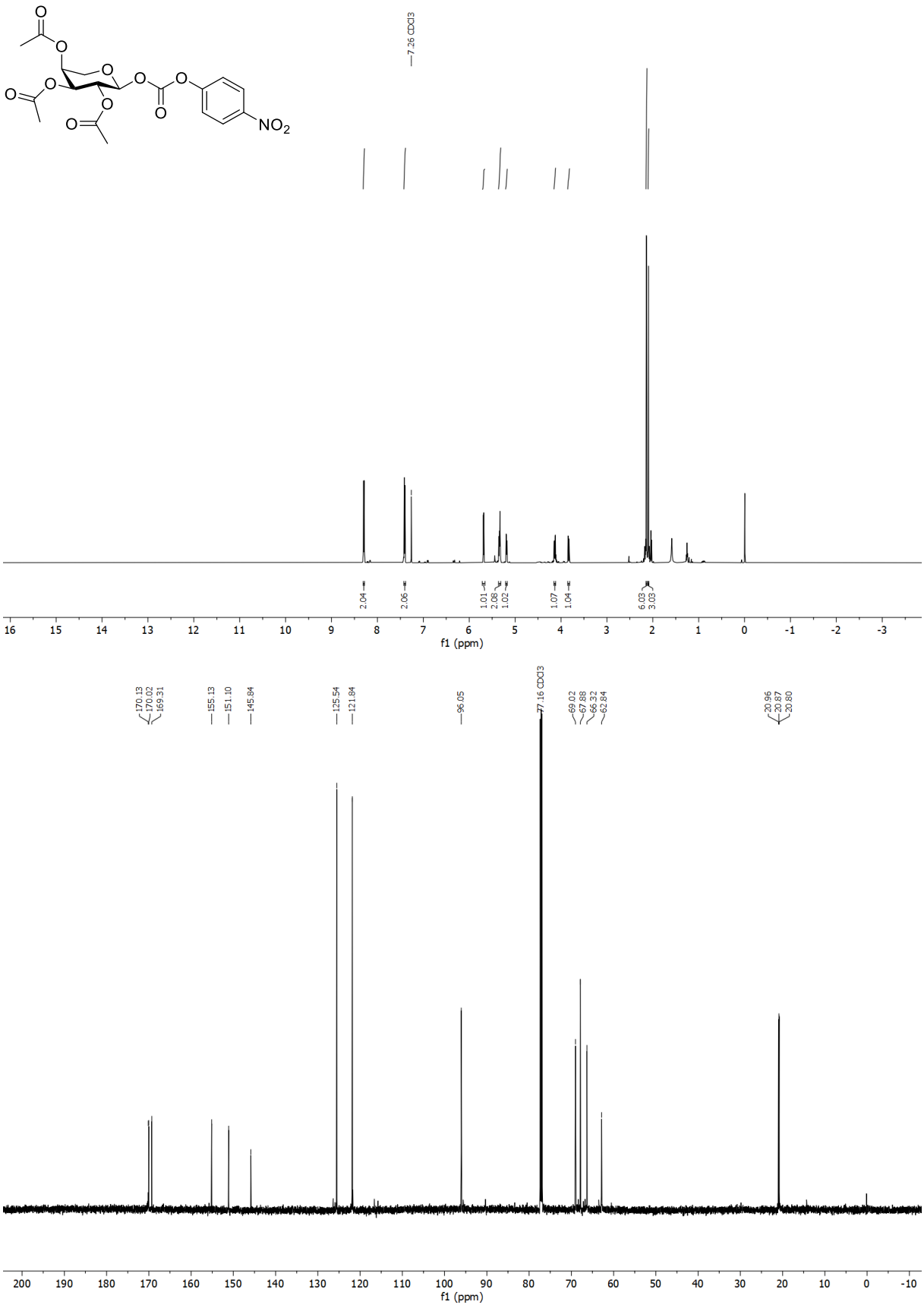


Figure S36: ^{1}H- and ^{13}C-NMR spectra of **11** in $CDCl_3$ (600 MHz/151 MHz).

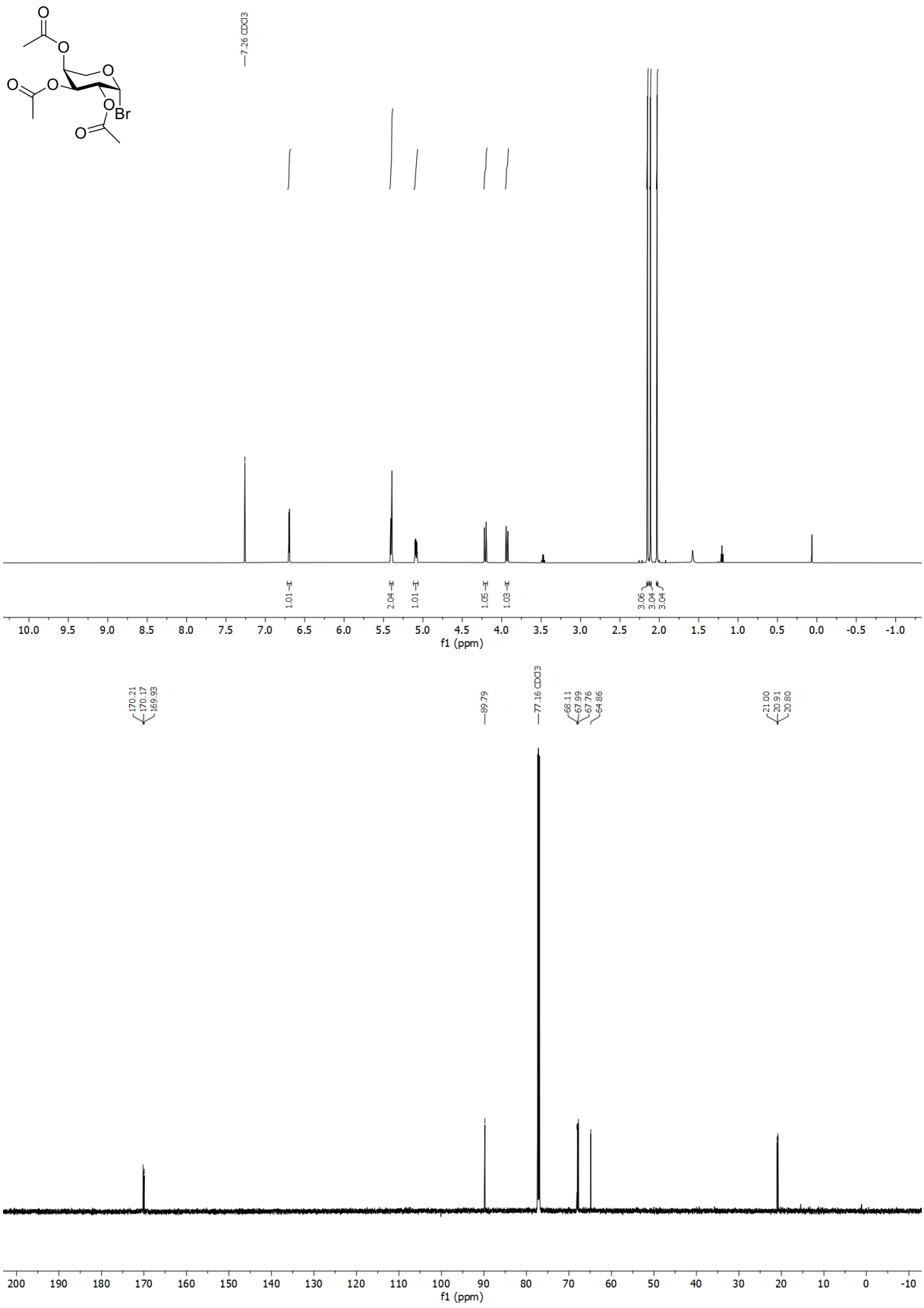


Figure S37: ^{1}H- and ^{13}C-NMR spectra of **8** in $CDCl_3$ (600 MHz/151 MHz).

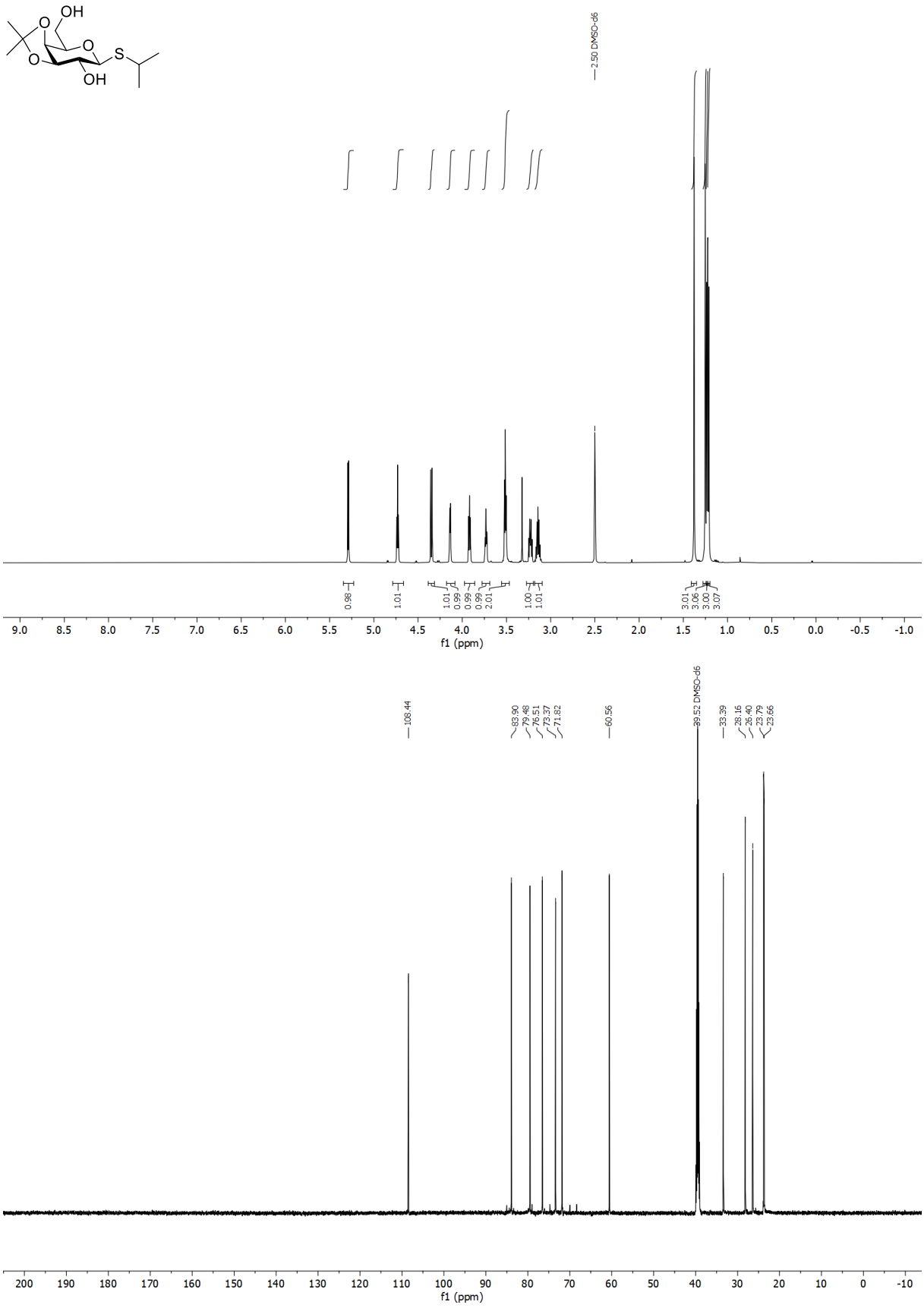


Figure S38: ^{1}H- and ^{13}C-NMR spectra of **S6** in DMSO-d_6 (600 MHz/151 MHz).

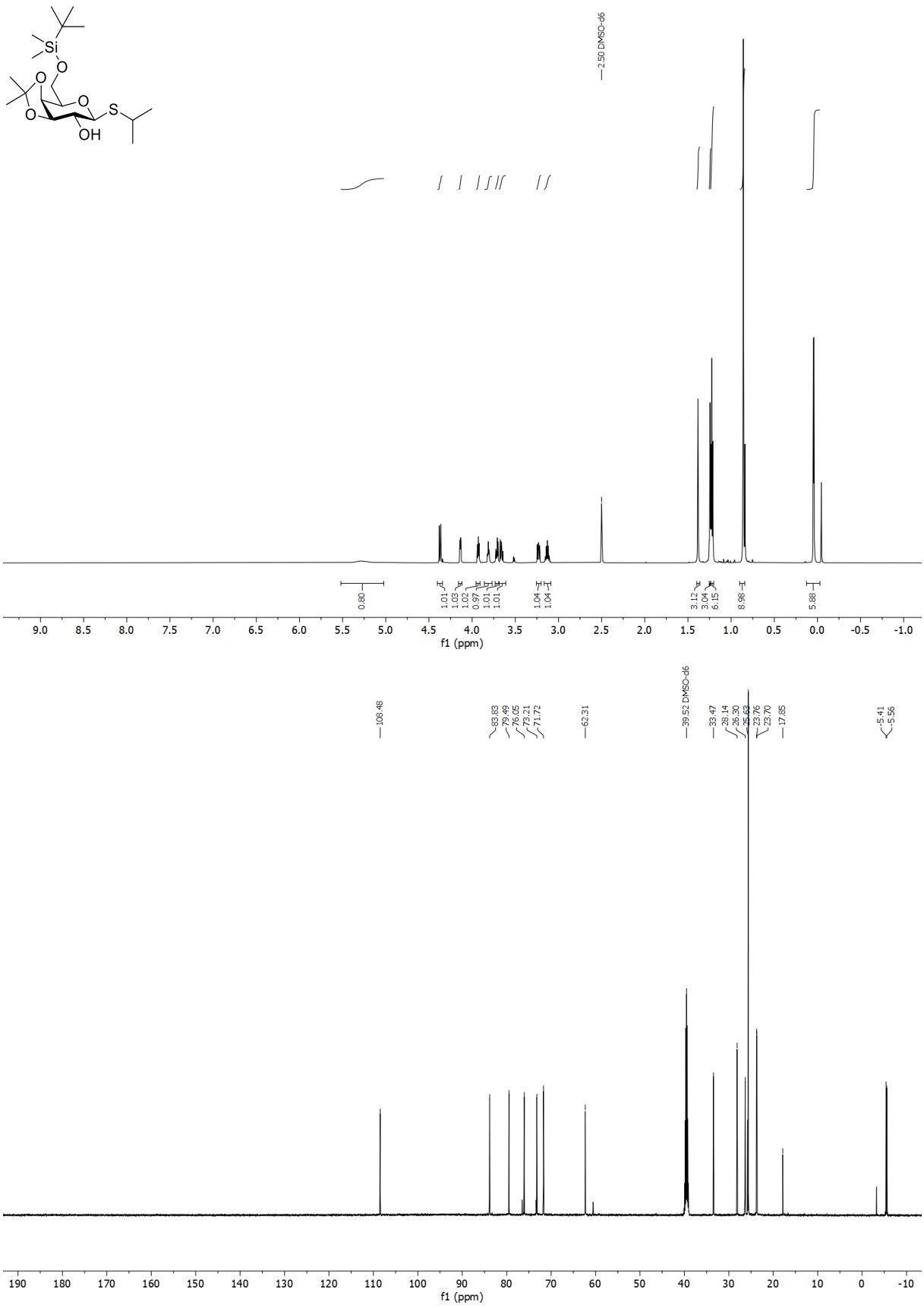


Figure S39: ^{1}H- and ^{13}C-NMR spectra of **9** in DMSO-d_6 (600 MHz/151 MHz).

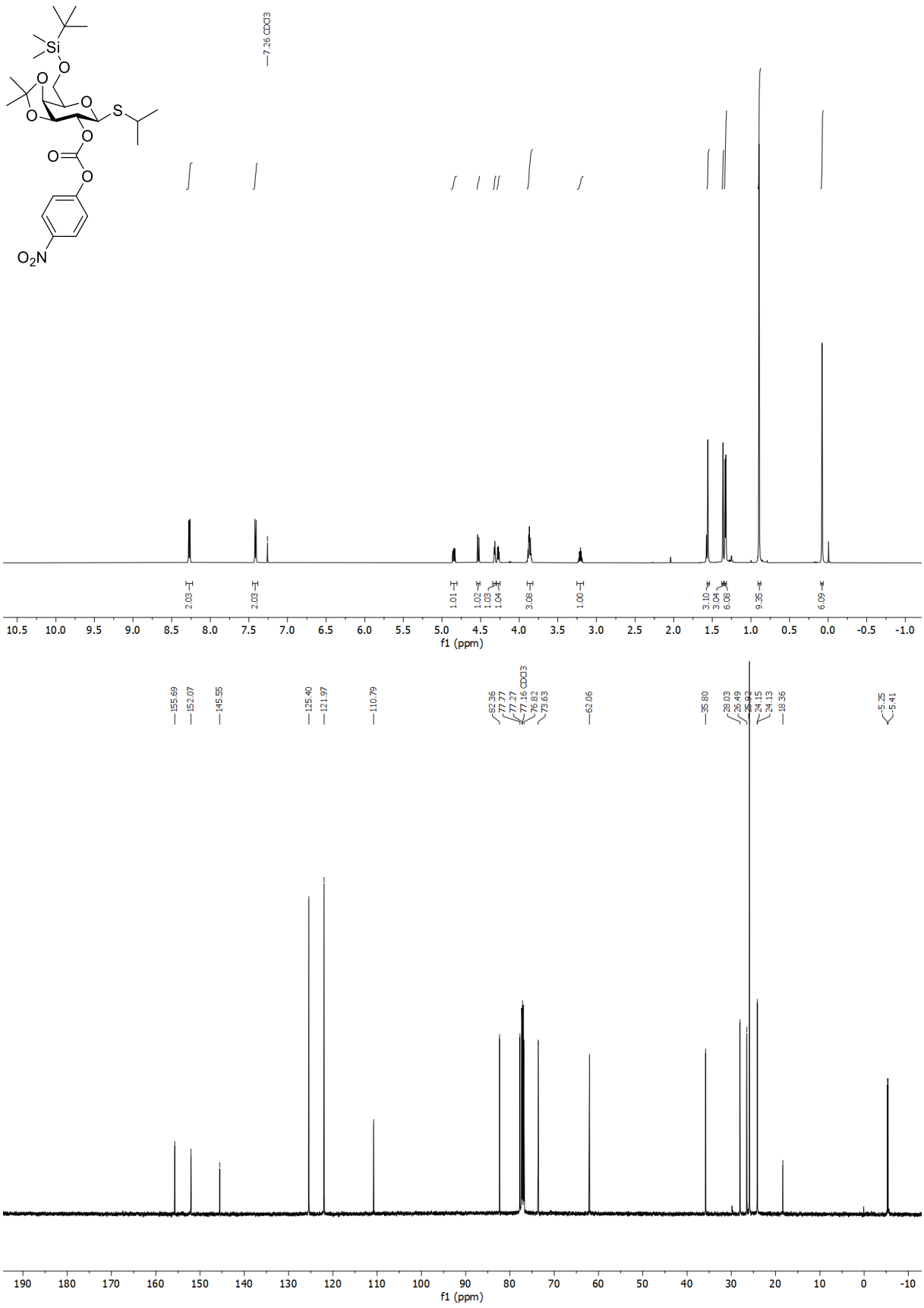


Figure S40: ^{1}H- and ^{13}C-NMR spectra of **12** in $CDCl_3$ (600 MHz/151 MHz).

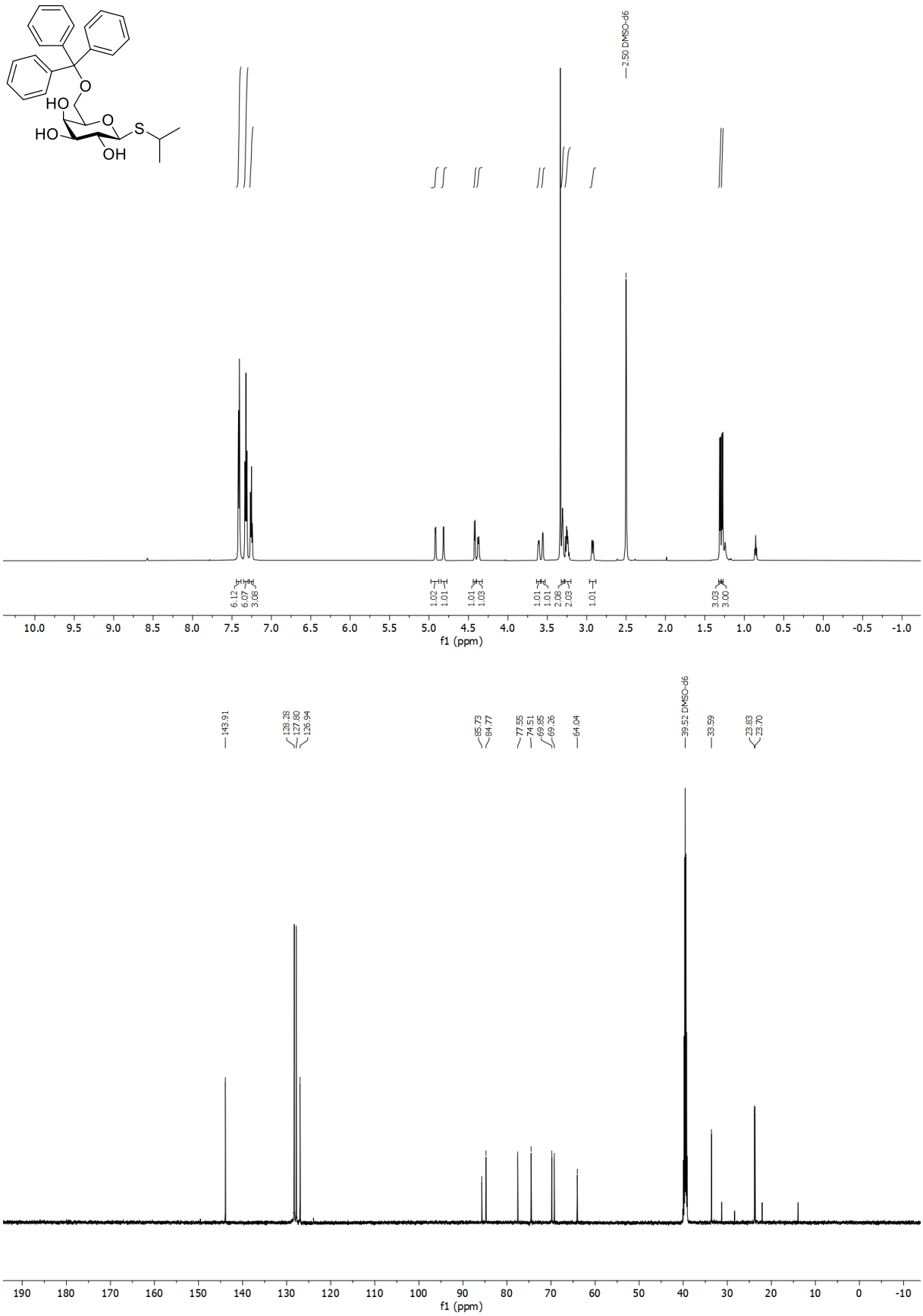


Figure S41: ^{1}H- and ^{13}C-NMR spectra of **S7** in DMSO-d_6 (600 MHz/151 MHz).

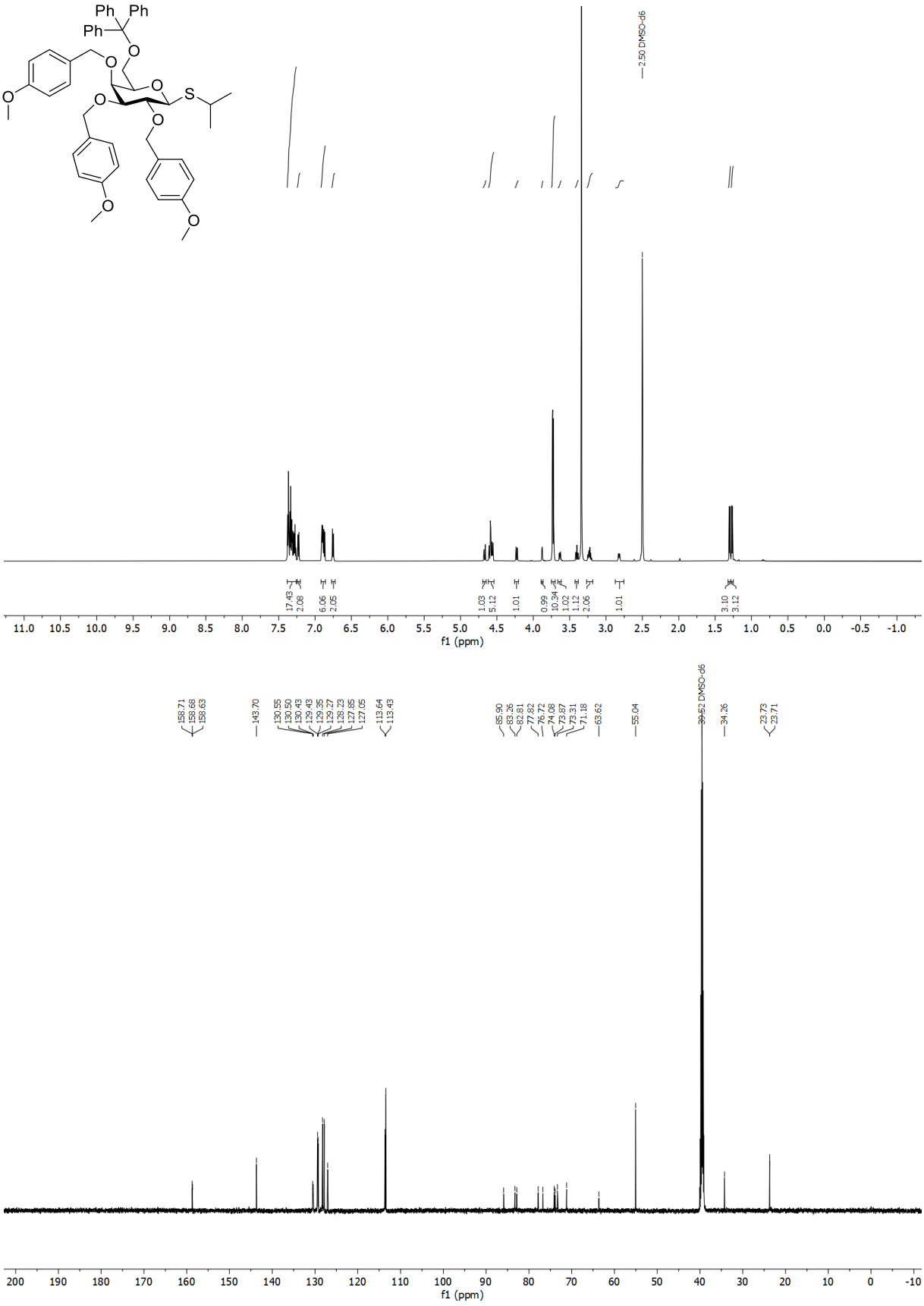


Figure S42: ^{1}H- and ^{13}C-NMR spectra of **S8** in DMSO-d_6 (600 MHz/151 MHz).

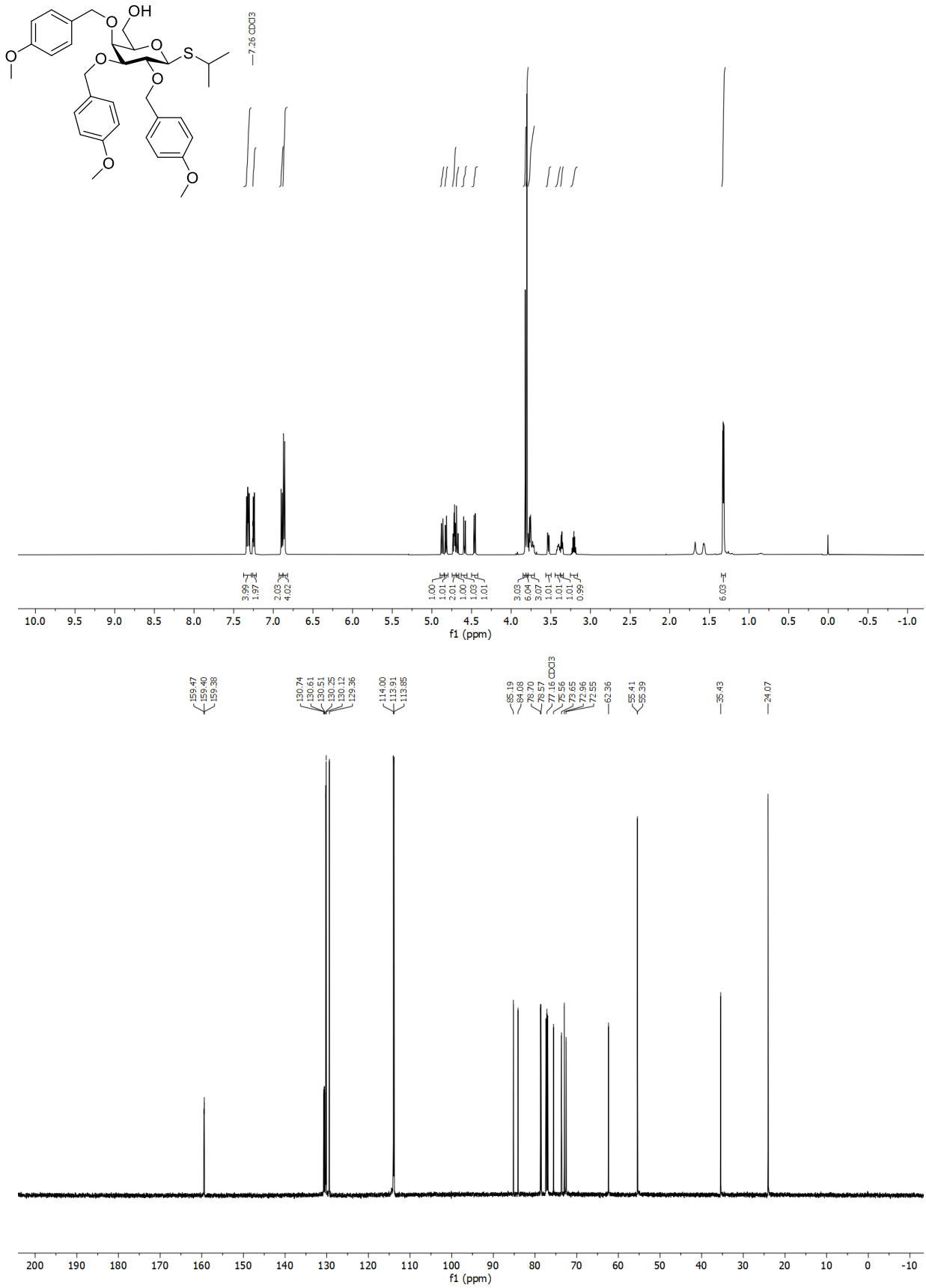


Figure S43: ^{1}H- and ^{13}C-NMR spectra of **10** in $CDCl_3$ (600 MHz/151 MHz).

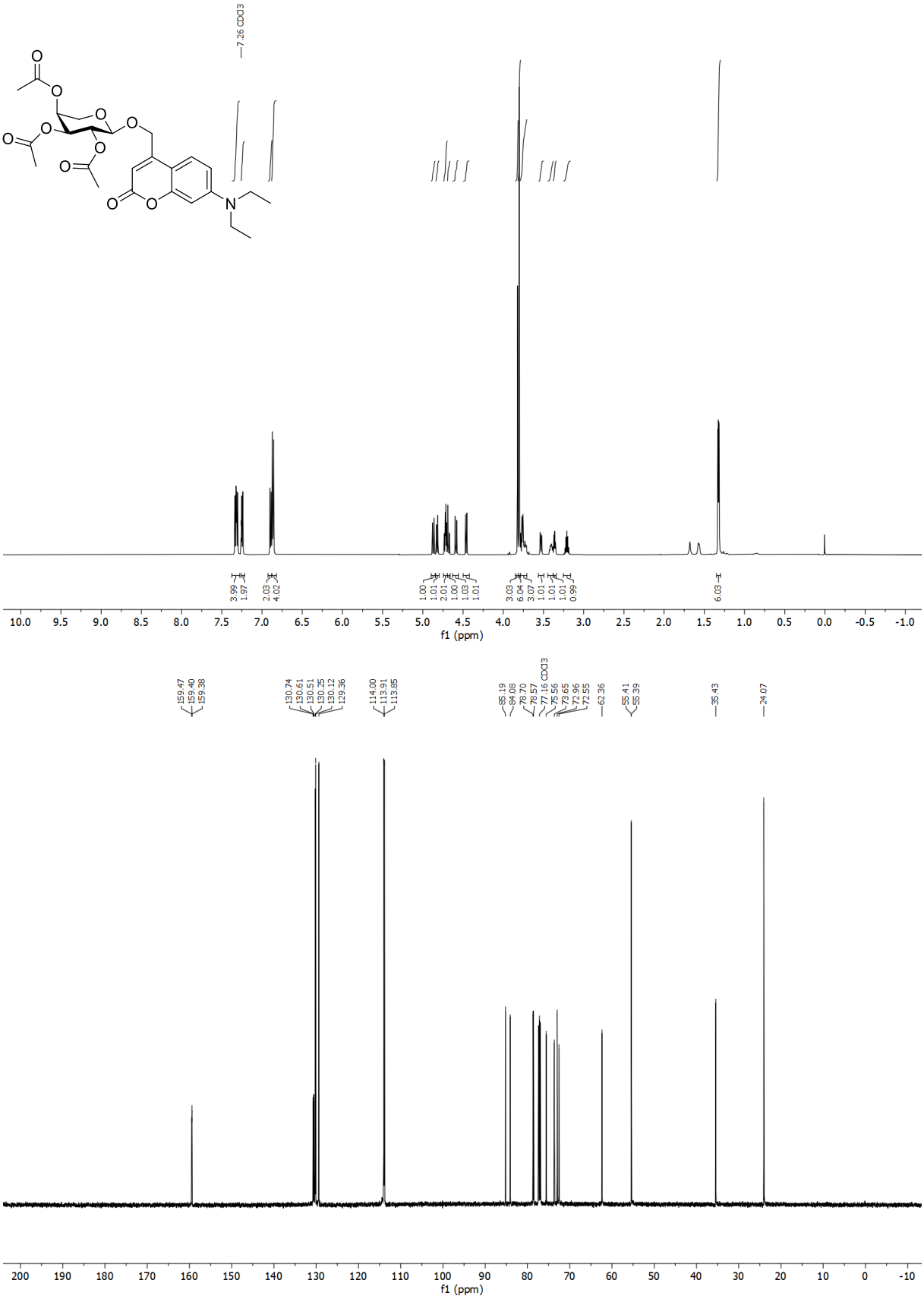


Figure S44: ^{1}H- and ^{13}C-NMR spectra of **13** in $CDCl_3$ (600 MHz/151 MHz).

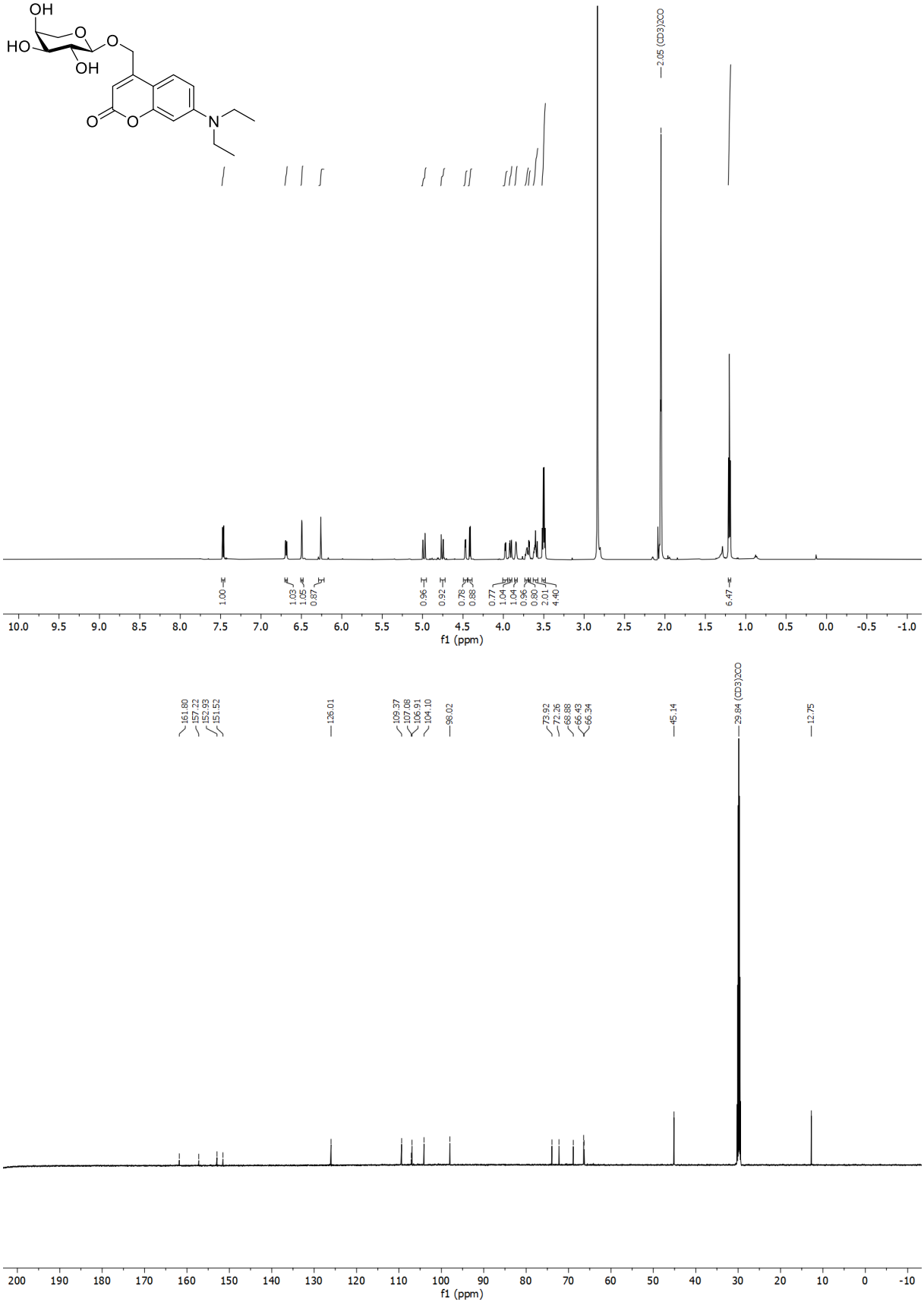


Figure S45: ^{1}H- and ^{13}C-NMR spectra of **2b** in acetone-d_6 (600 MHz/151 MHz).

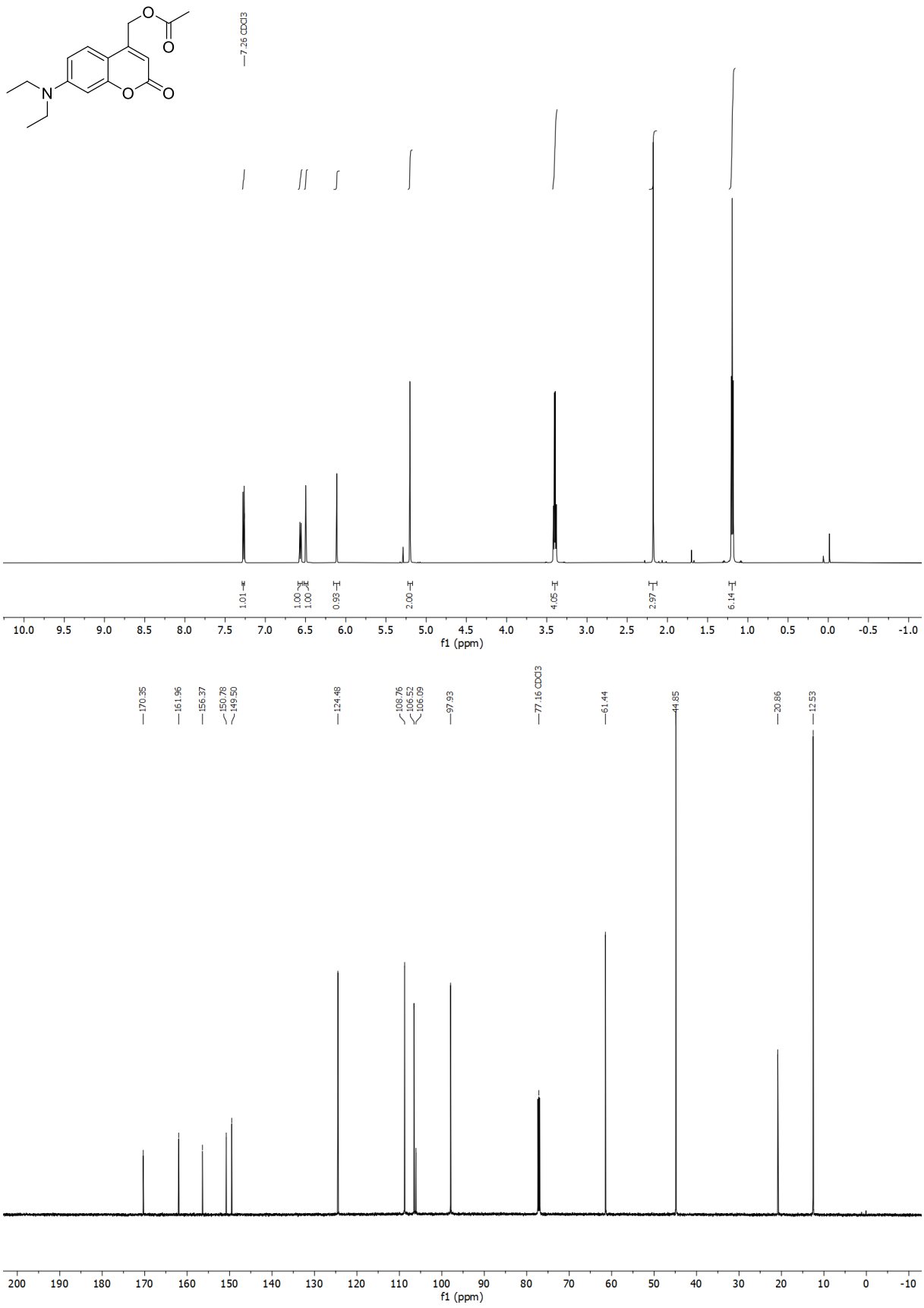


Figure S46: ^{1}H- and ^{13}C-NMR spectra of **S9** in $CDCl_3$ (600 MHz/151 MHz).

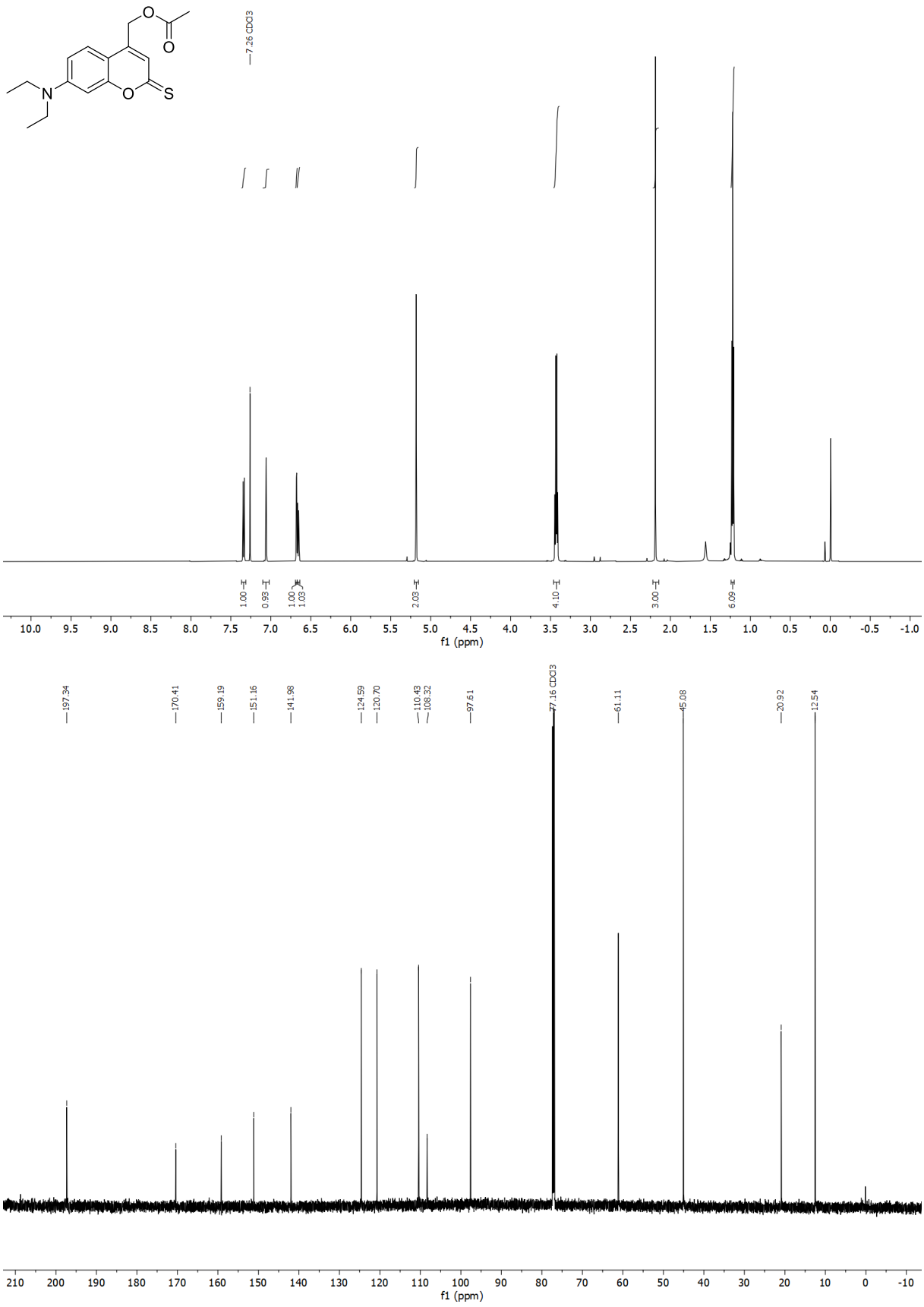


Figure S47: ^{1}H- and ^{13}C-NMR spectra of **S10** in $CDCl_3$ (600 MHz/151 MHz).

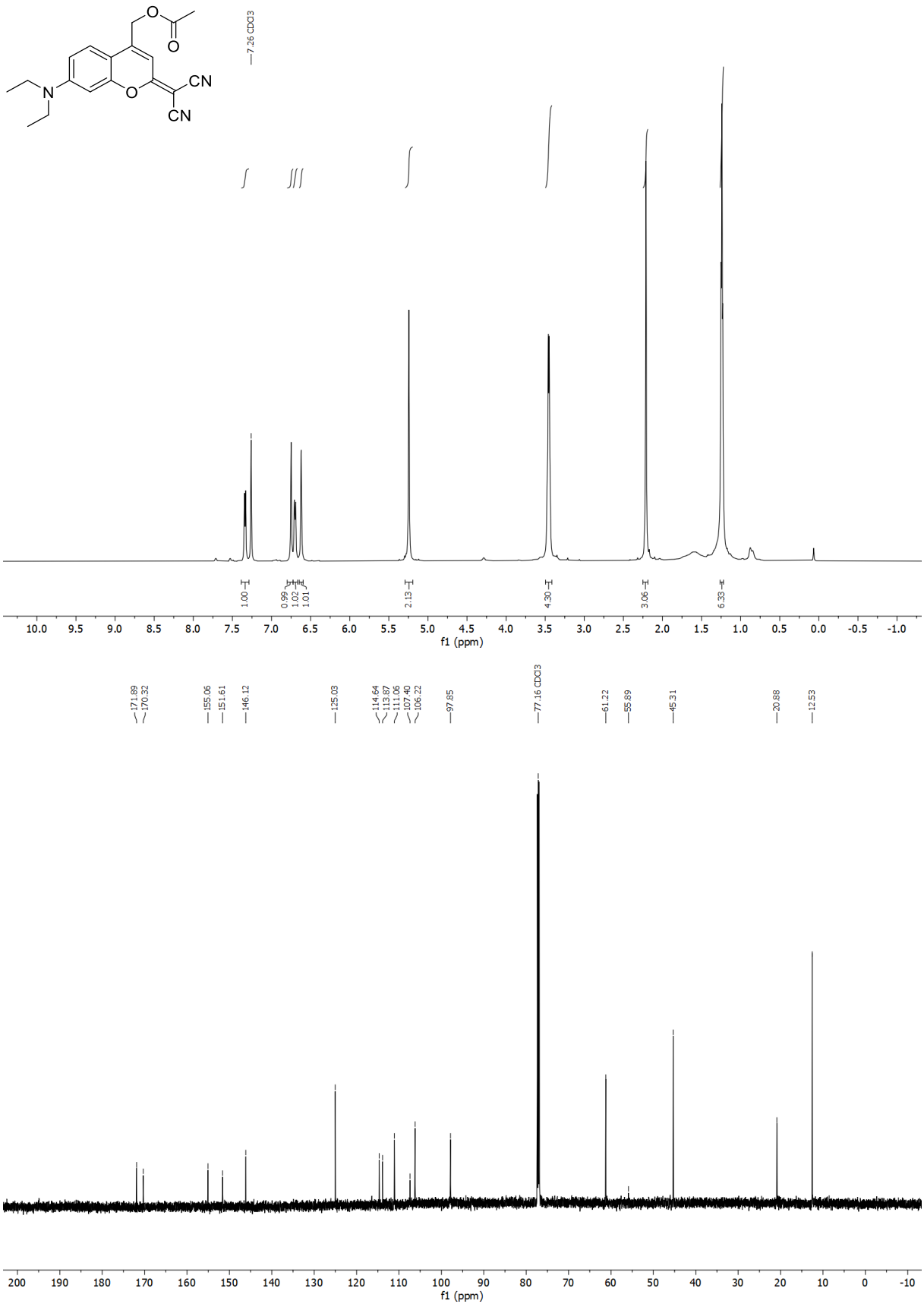


Figure S48: ^{1}H- and ^{13}C-NMR spectra of **S11** in $CDCl_3$ (600 MHz/151 MHz).

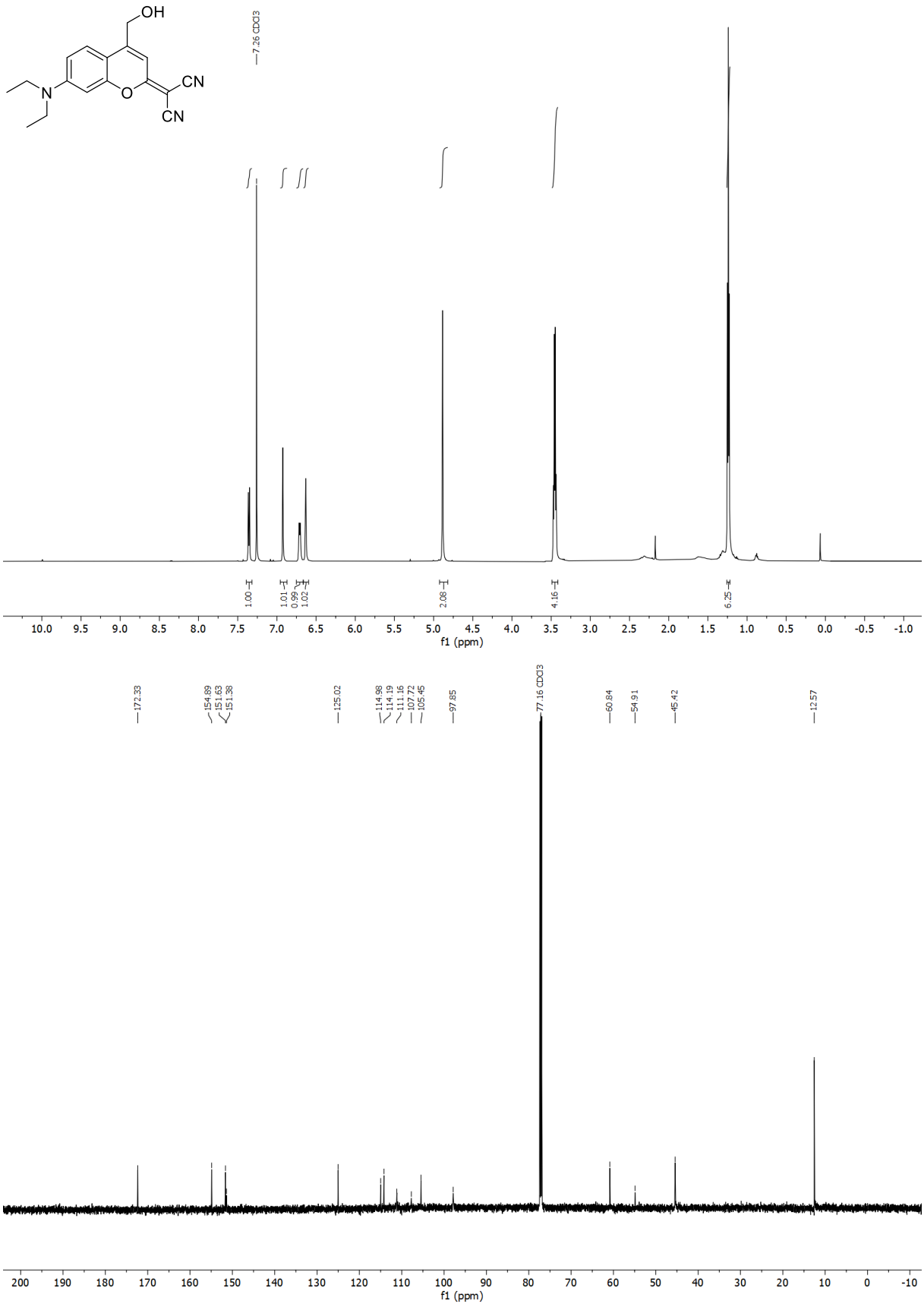


Figure S49: ^{1}H- and ^{13}C-NMR spectra of **4** in $CDCl_3$ (600 MHz/151 MHz).

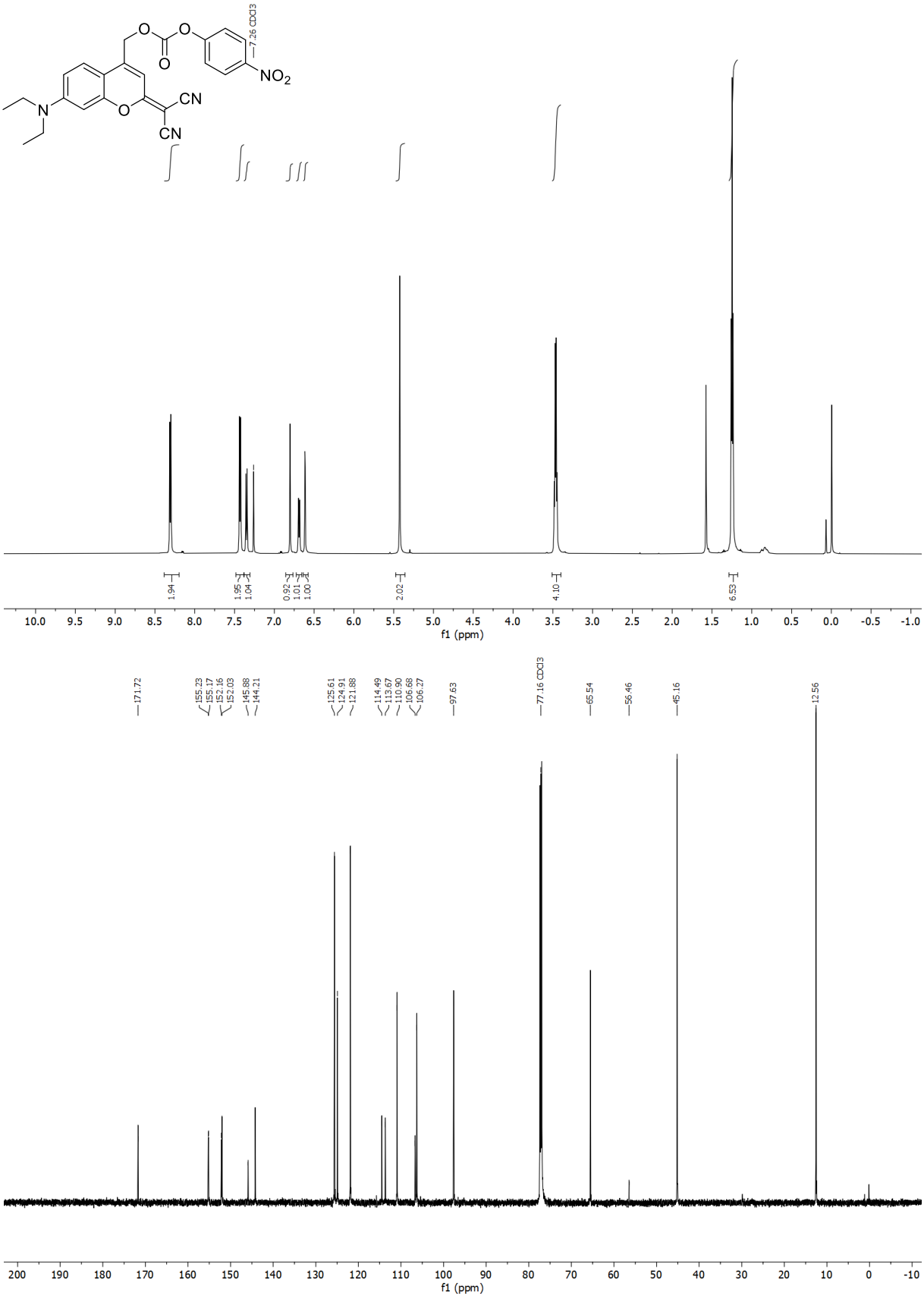


Figure S50: ^{1}H- and ^{13}C-NMR spectra of **6** in $CDCl_3$ (600 MHz/151 MHz).

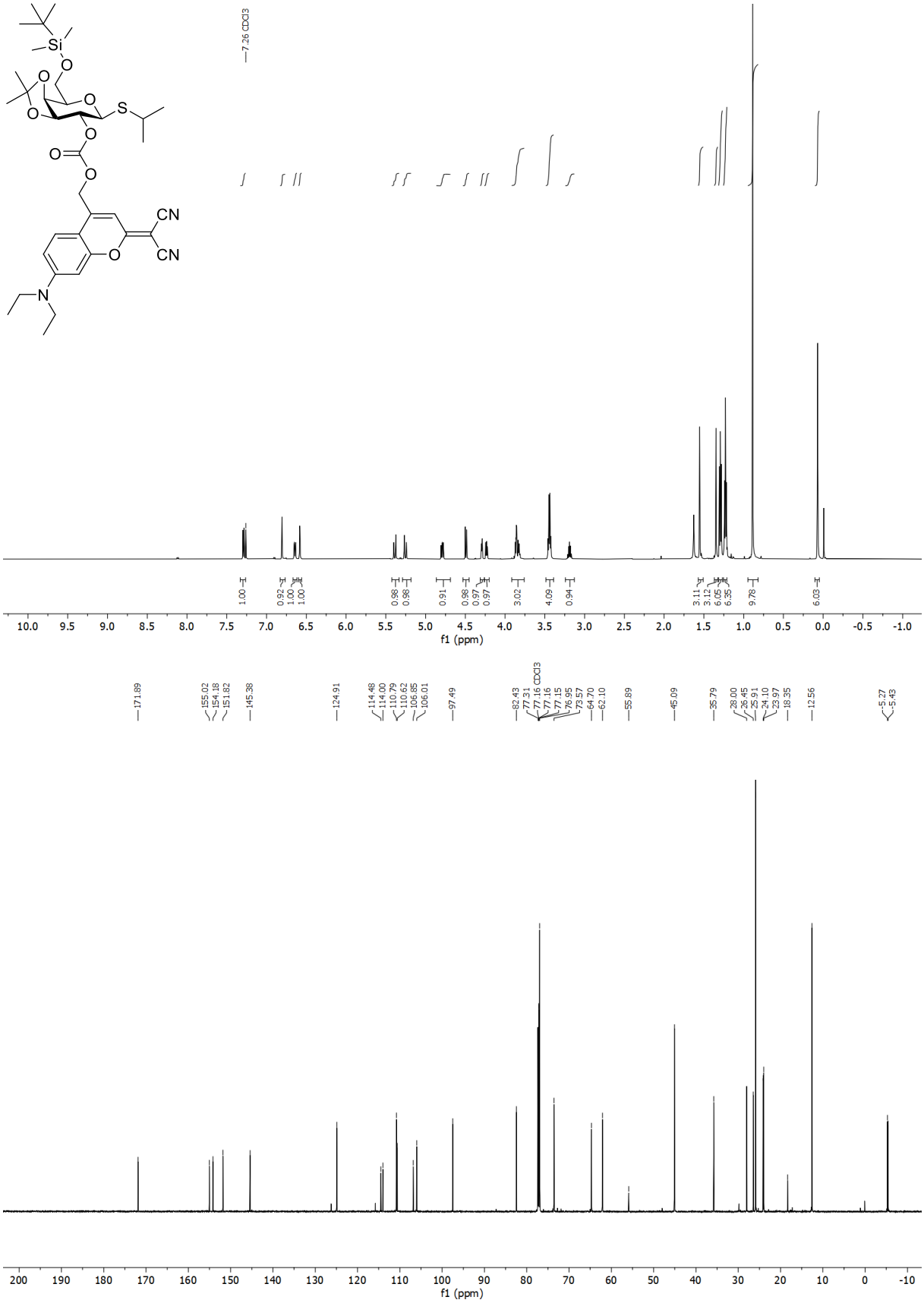


Figure S51: ^{1}H- and ^{13}C-NMR spectra of **13** in $CDCl_3$ (600 MHz/151 MHz).

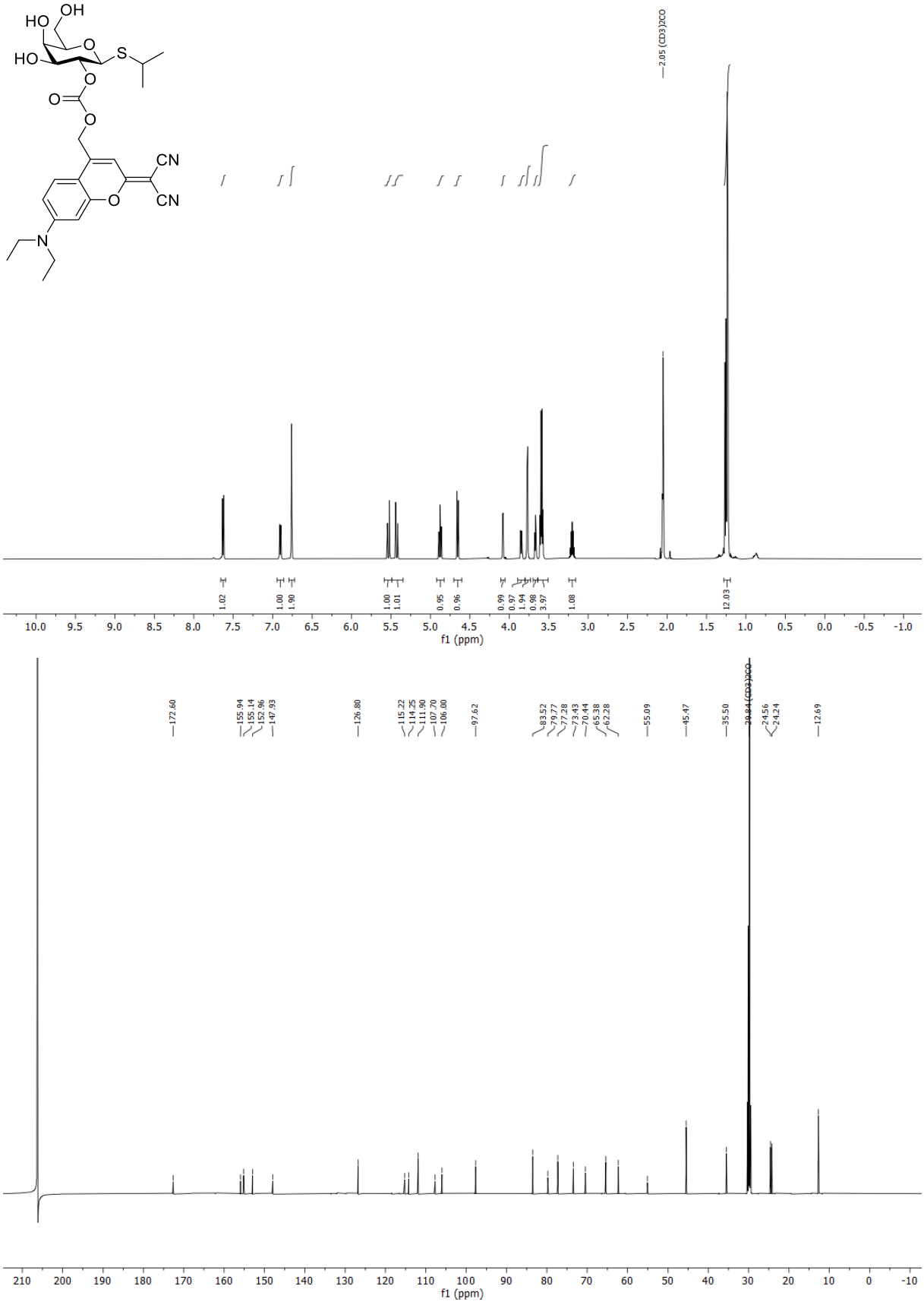


Figure S52: ^{1}H- and ^{13}C-NMR spectra of **1c** in acetone-d_6 (600 MHz/151 MHz).

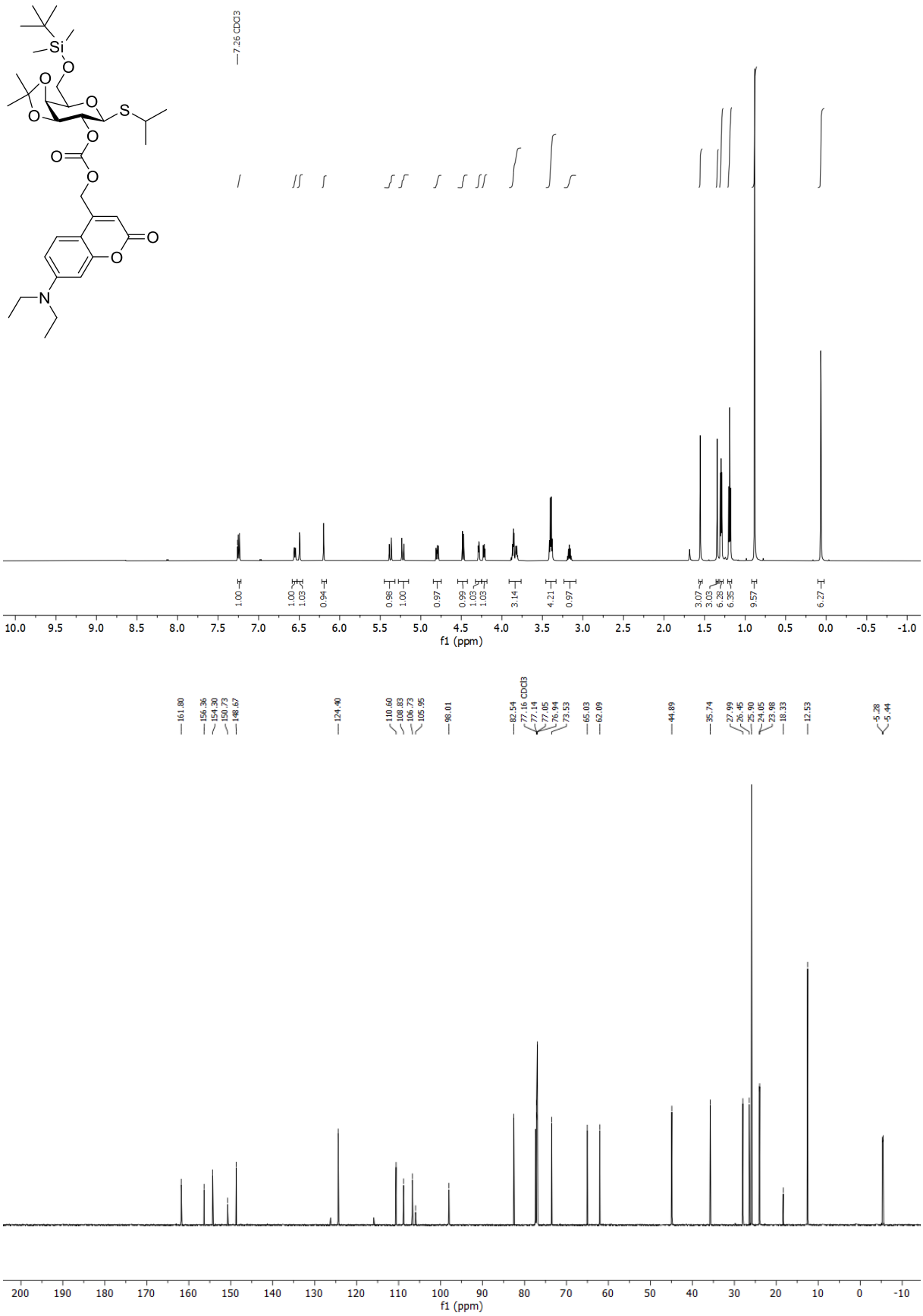


Figure S53: ^{1}H- and ^{13}C-NMR spectra of **14** in $CDCl_3$ (600 MHz/151 MHz).

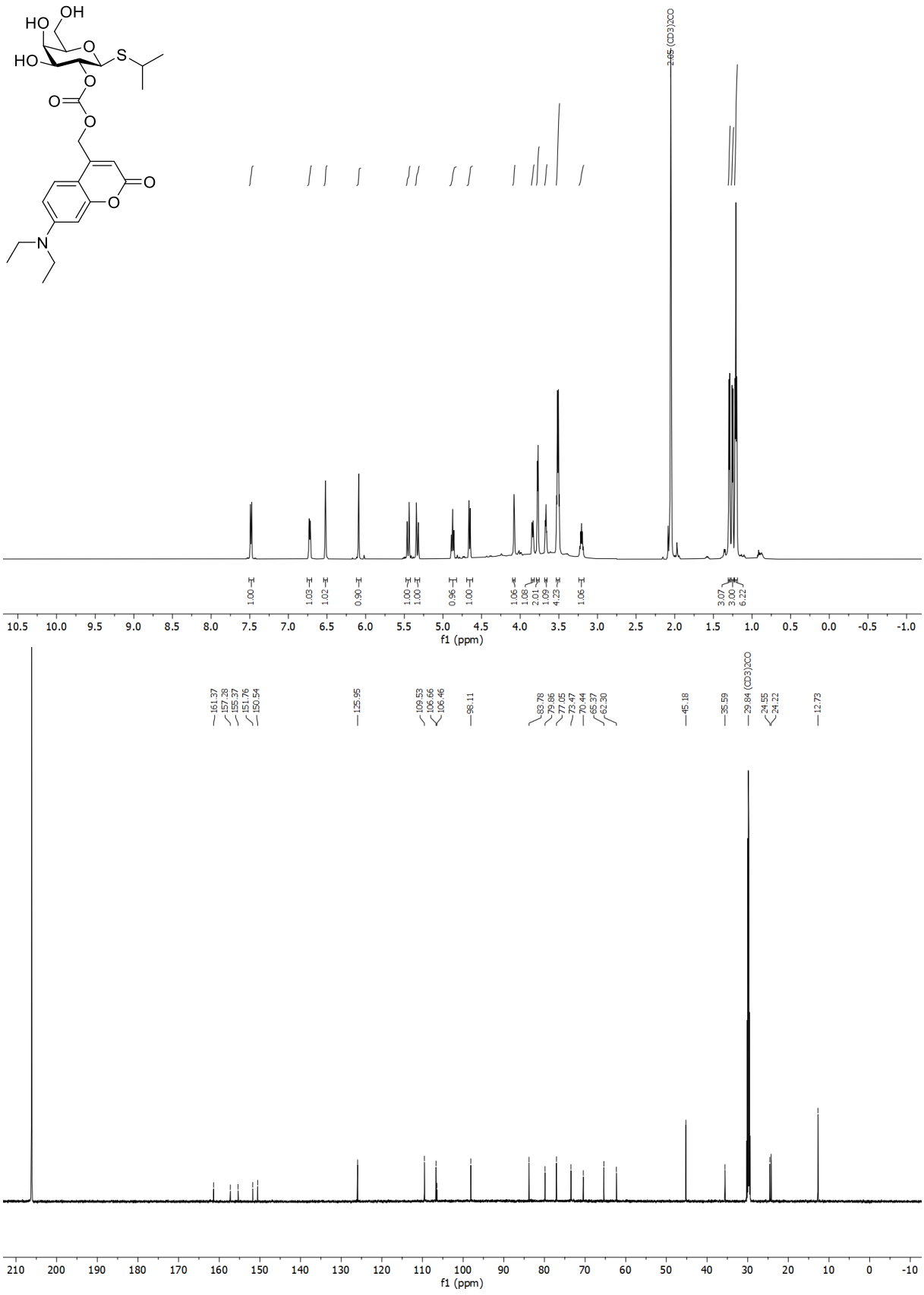


Figure S54: ^{1}H- and ^{13}C-NMR spectra of **1b** in acetone-d_6 (600 MHz/151 MHz).

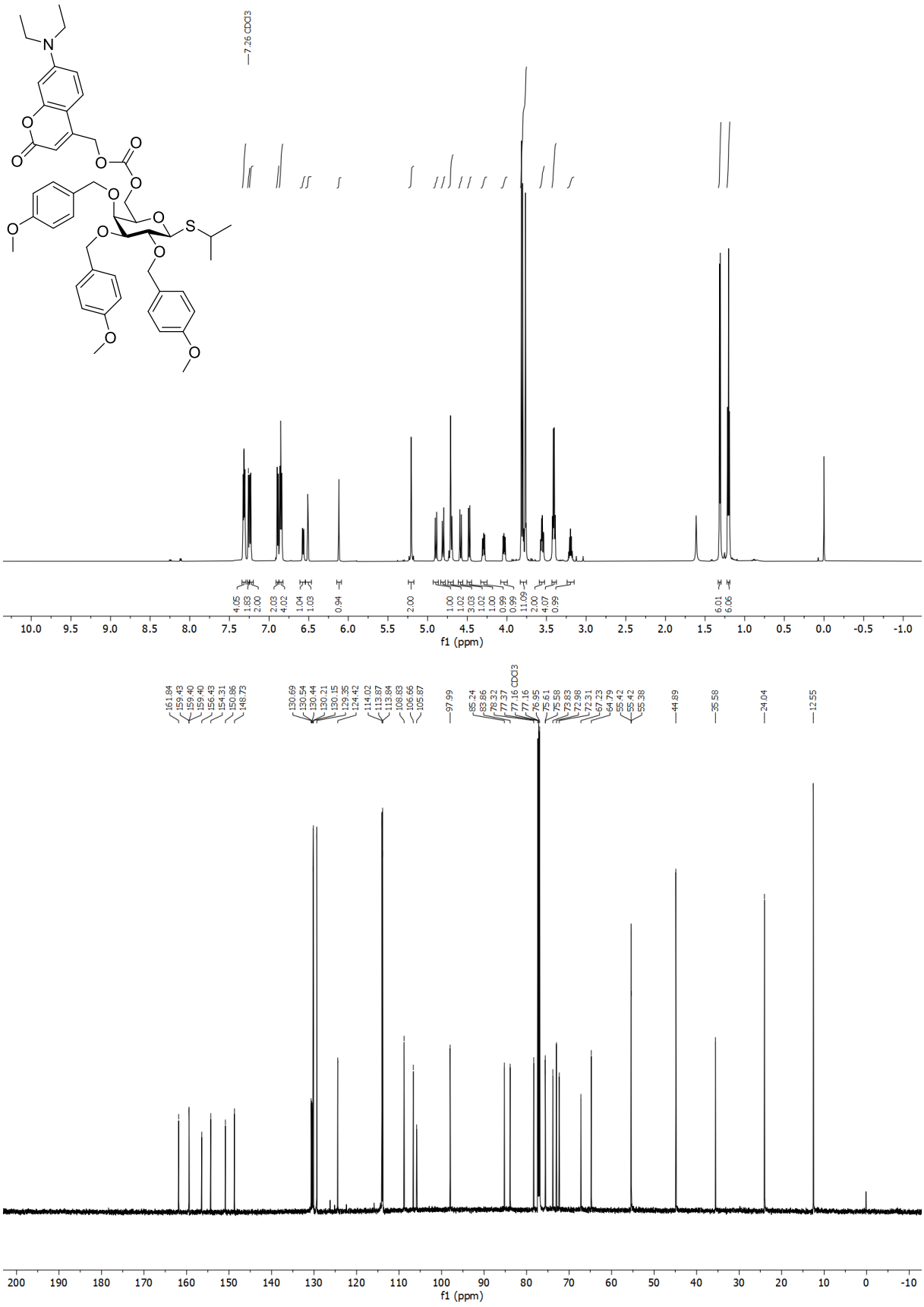


Figure S55: ^{1}H- and ^{13}C-NMR spectra of **16** in $CDCl_3$ (600 MHz/151 MHz).

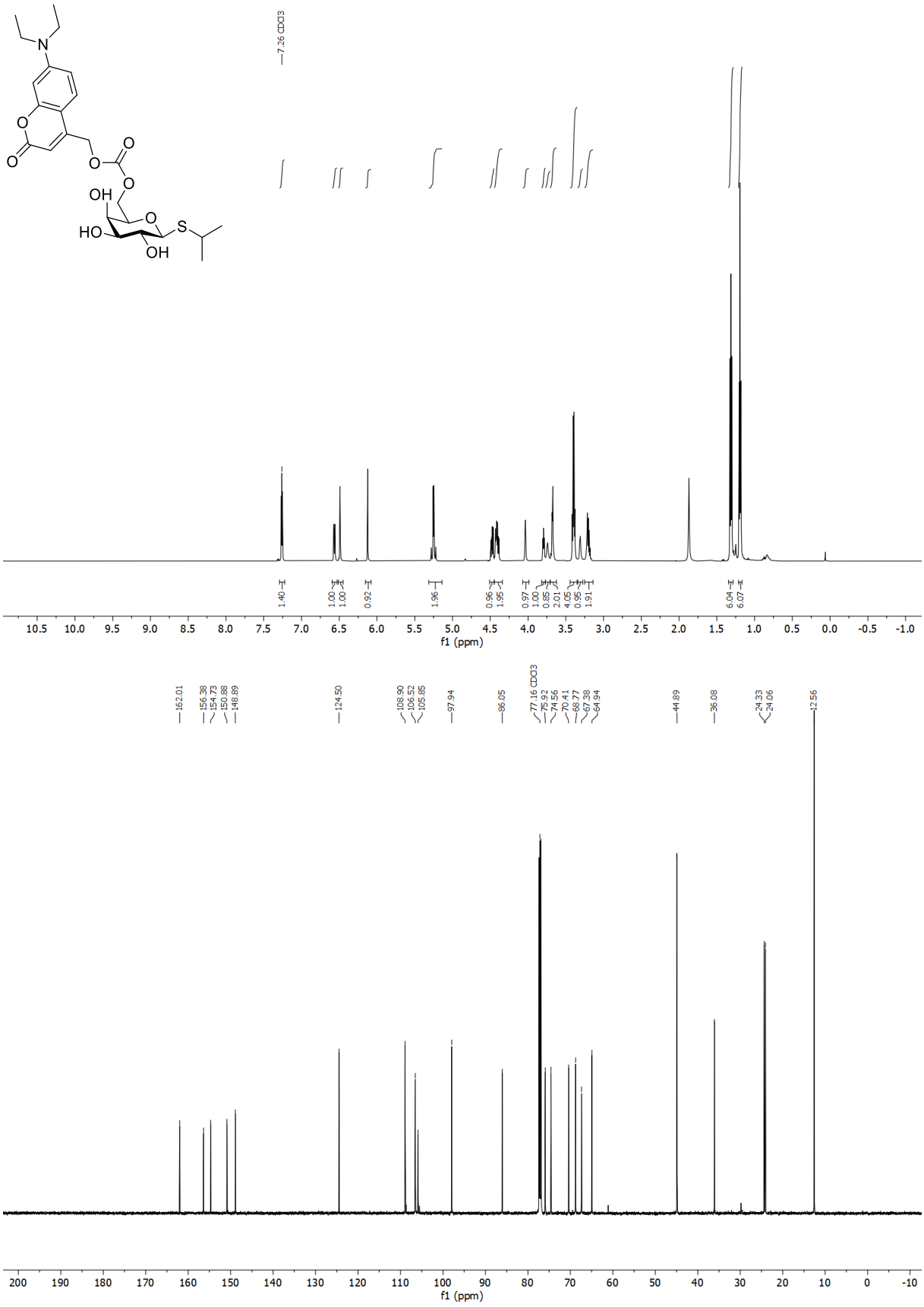


Figure S56: ^{1}H- and ^{13}C-NMR spectra of **1d** in $CDCl_3$ (600 MHz/151 MHz).

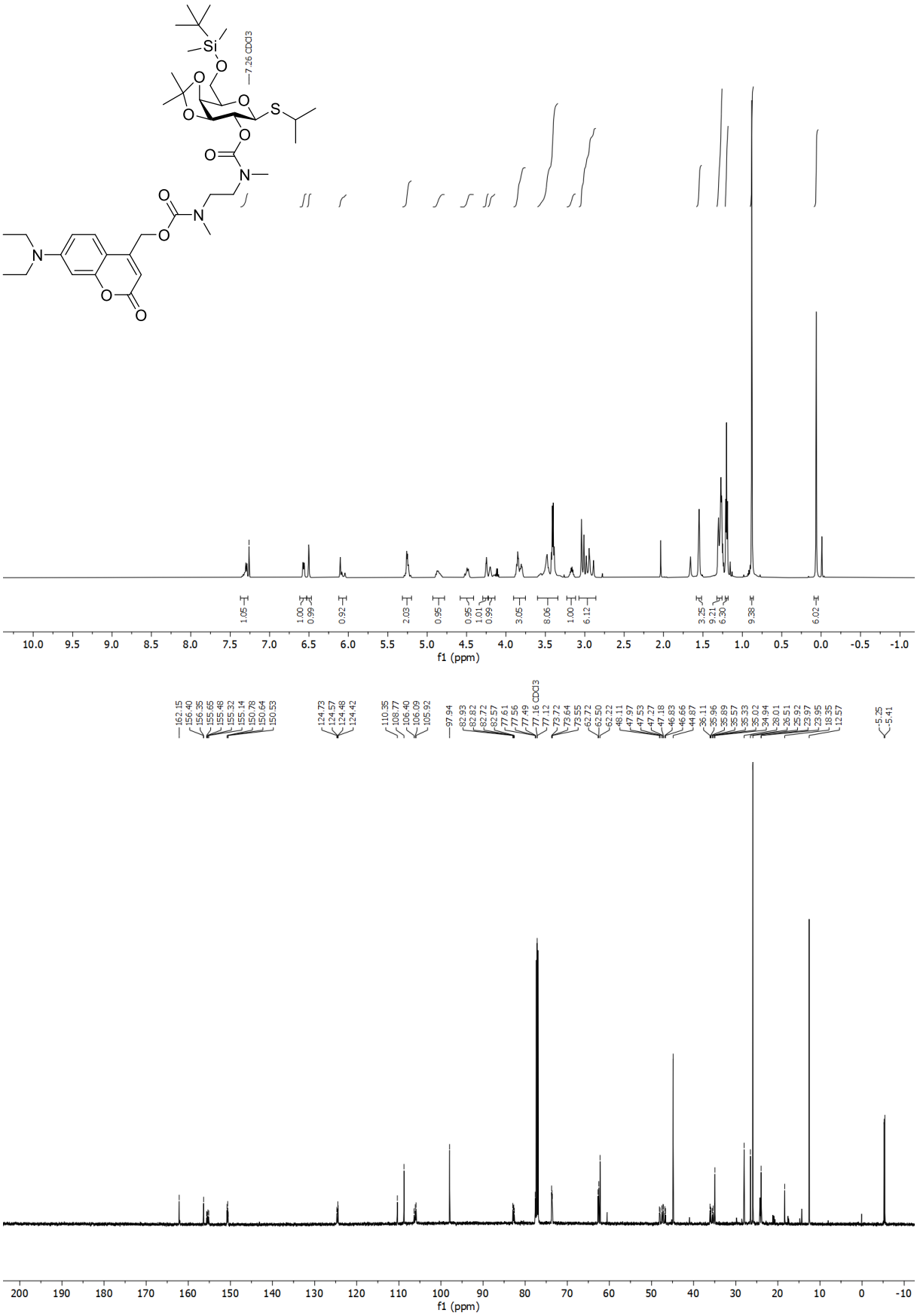


Figure S57: ^{1}H- and ^{13}C-NMR spectra of **18** in $CDCl_3$ (600 MHz/151 MHz).

Figure S58: ^{1}H- and ^{13}C-NMR spectra of **1e** in DMSO-d_6 (600 MHz/151 MHz) at 60 °C.

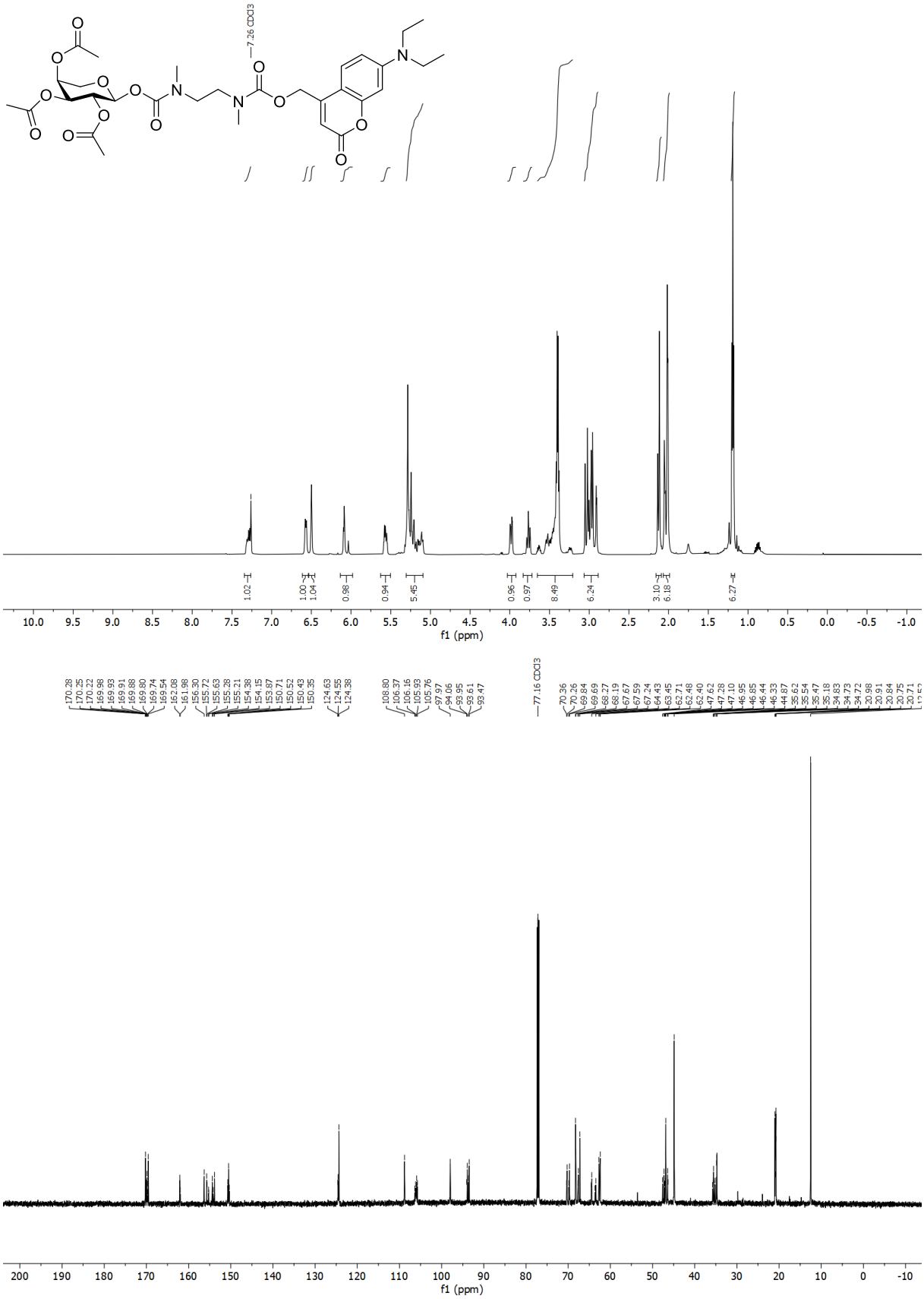


Figure S59: ^{1}H- and ^{13}C-NMR spectra of **17** in $CDCl_3$ (600 MHz/151 MHz).

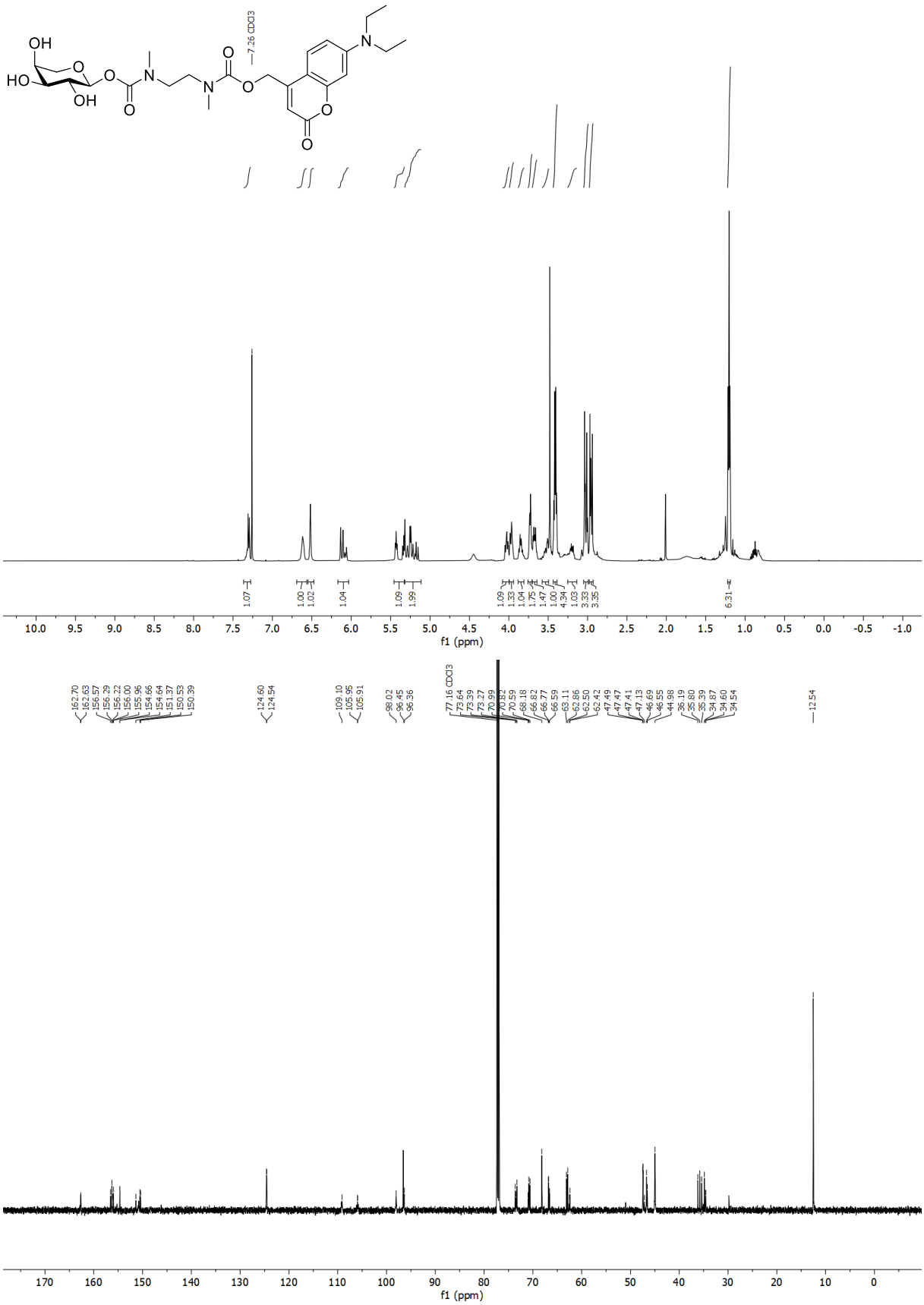


Figure S60: ^{1}H- and ^{13}C-NMR spectra of **2c** in $CDCl_3$ (600 MHz/151 MHz).

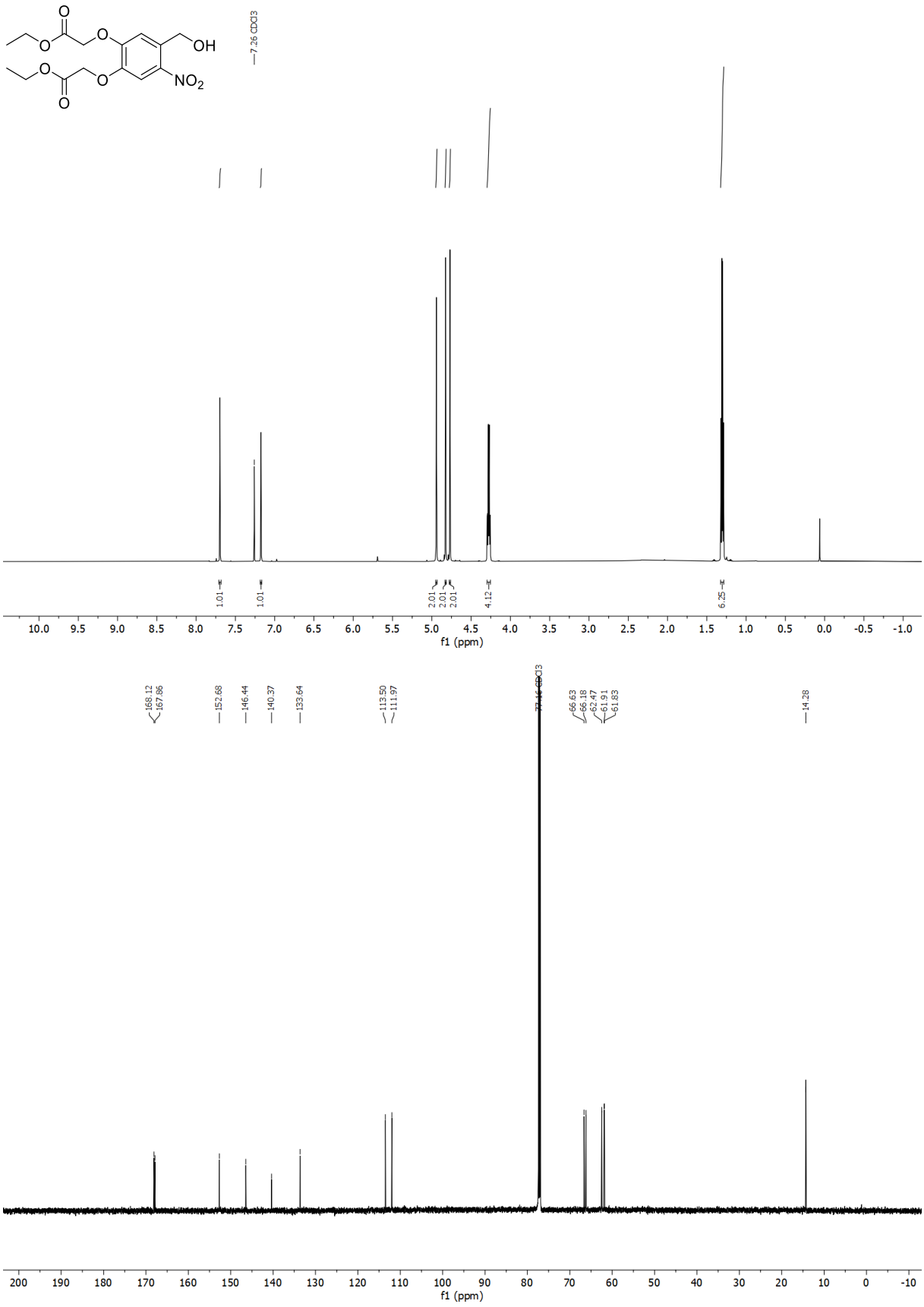


Figure S61: ^{1}H- and ^{13}C-NMR spectra of **22** in $CDCl_3$ (600 MHz/151 MHz).

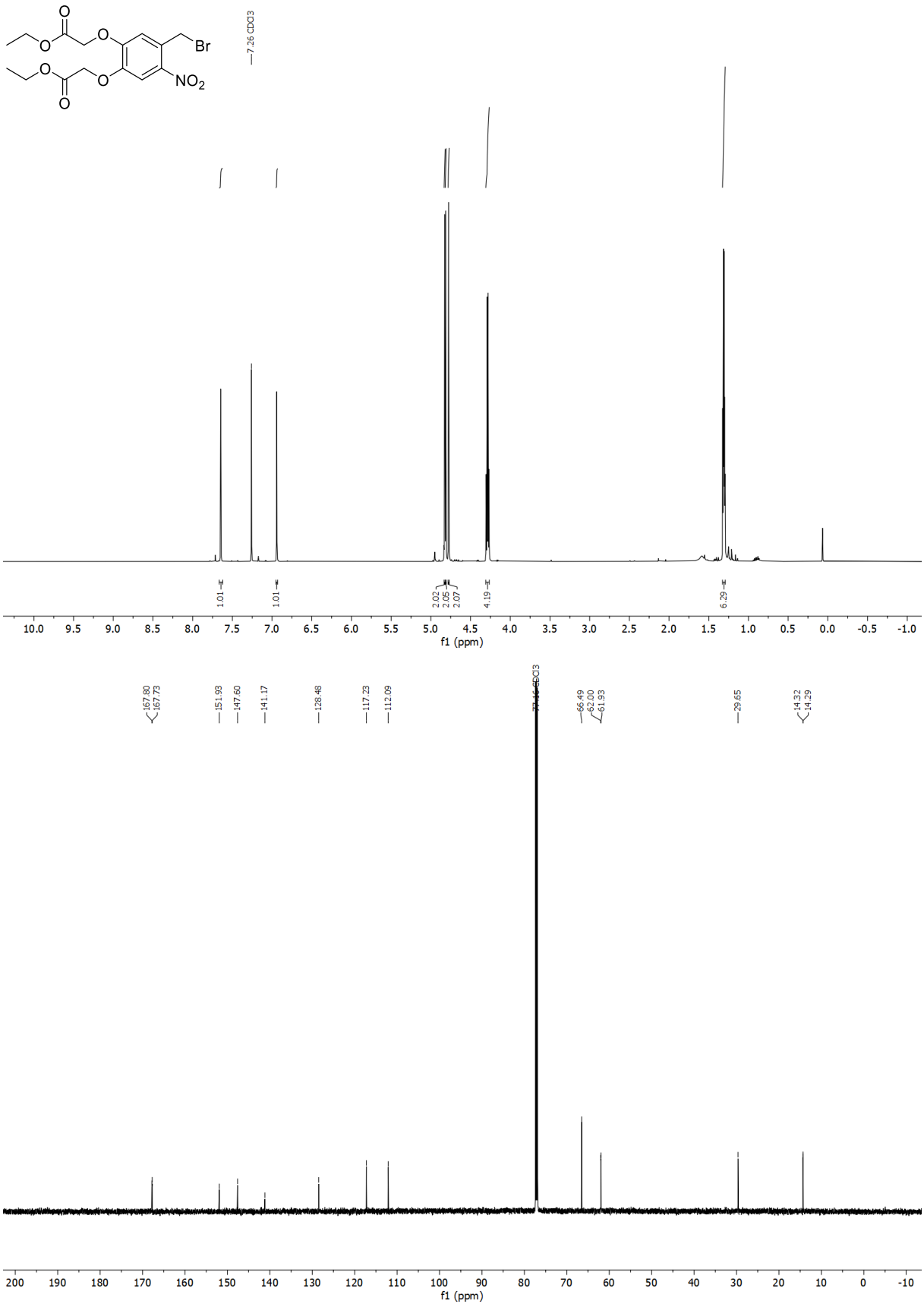


Figure 62: ^{1}H- and ^{13}C-NMR spectra of **23** in $CDCl_3$ (600 MHz/151 MHz).

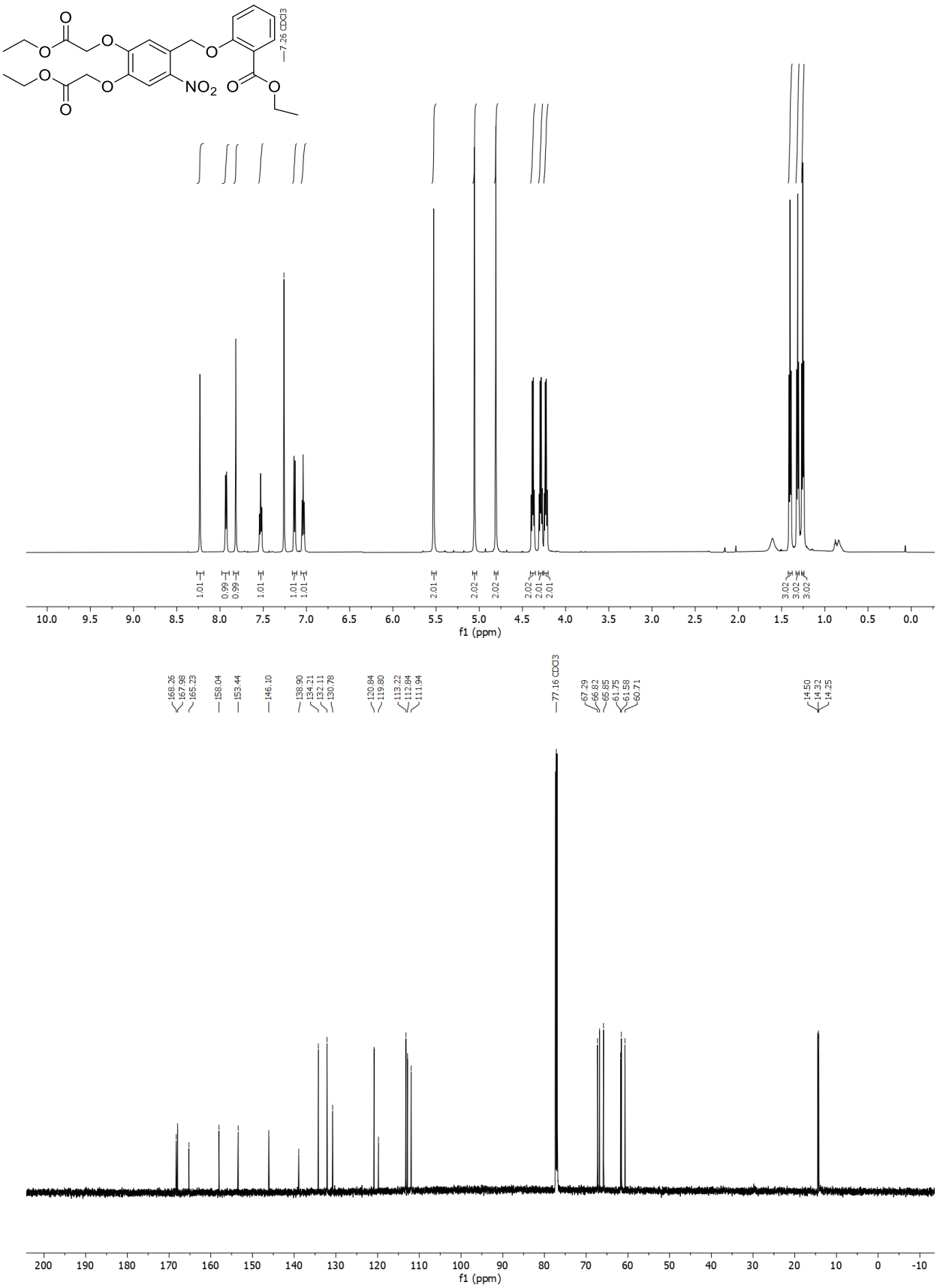


Figure S63: ^{1}H- and ^{13}C-NMR spectra of **24** in $CDCl_3$ (600 MHz/151 MHz).

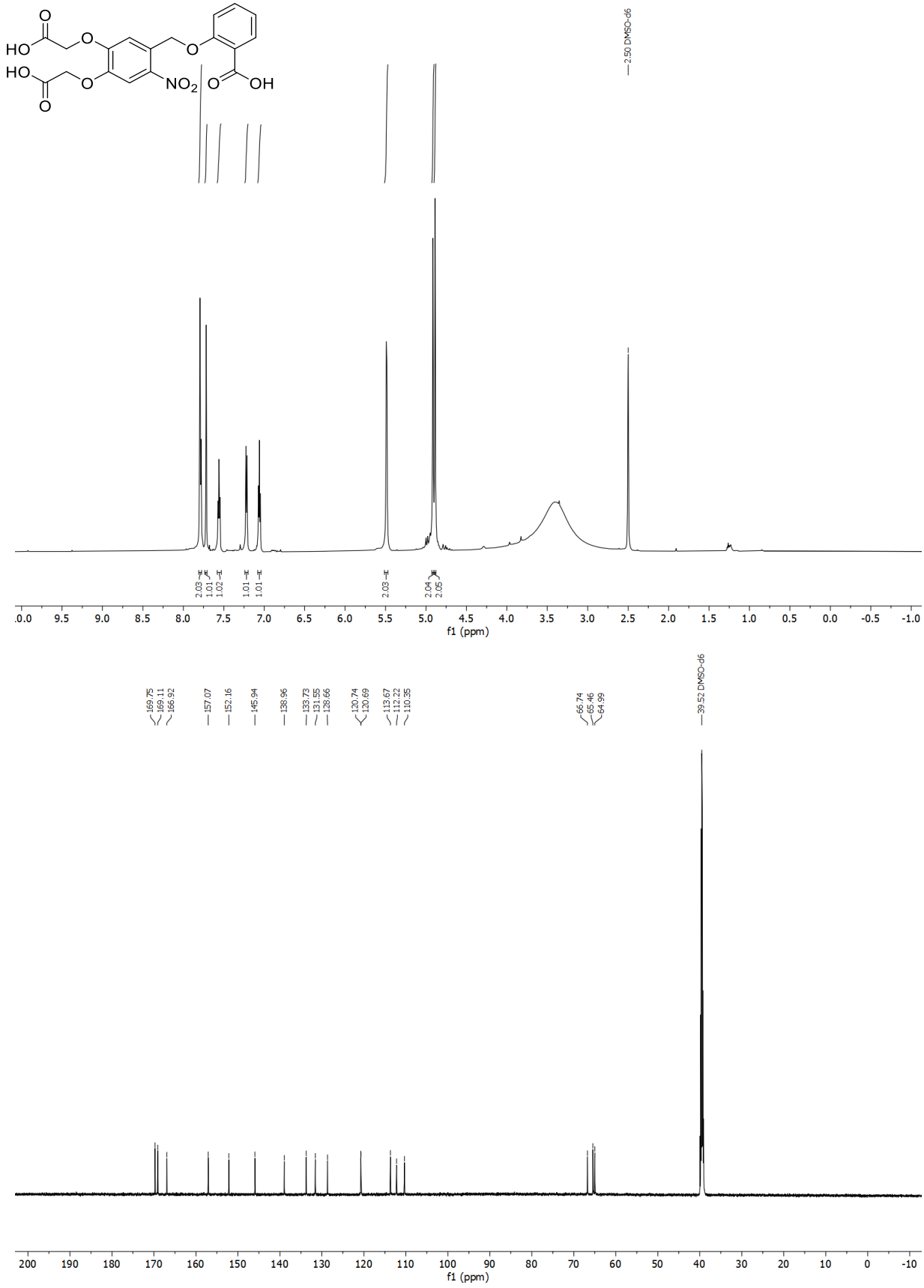


Figure S64: ^{1}H- and ^{13}C-NMR spectra of **22a** in DMSO-d_6 (600 MHz/151 MHz).

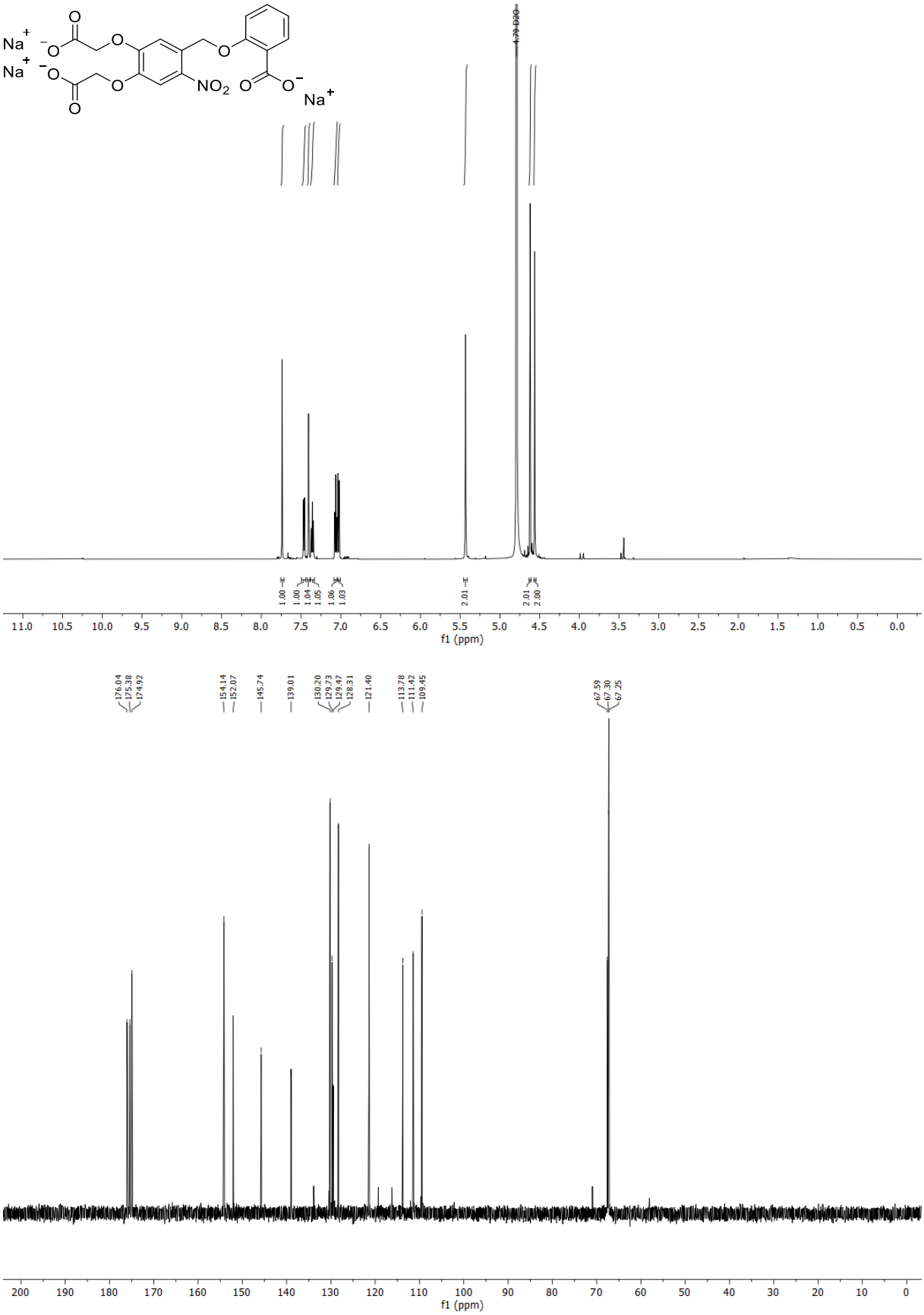


Figure S65: ^{1}H- and ^{13}C-NMR spectra of **22b** in D_2O (600 MHz/151 MHz).

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