Chemoselective attachment of the water-soluble dark quencher hydrodabcyl to amino groups in peptides and preservation of its spectroscopic properties over a wide pH range

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Supporting Information

List of abbreviations

CH – cyclohexane

DMF – dimethylformamide

DMSO – dimethylsulfoxide

EDTA – thylendiaminetetraacetic acide

EtOAc – ethyl acetate

EtOH – ethanol

NEt_3 – triethylamine

r.t. – room temperature

–ESI – electron spray ionisation in negative mode

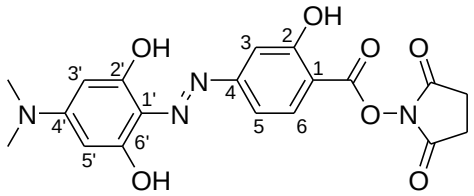
+ESI – electron spray ionisation in positive mode

Chemicals and analytical methods

All reactions were carried out under a nitrogen or argon atmosphere using dry solvents under anhydrous conditions, unless otherwise noted. N,N'-dimethylformamide (DMF), carbonyldiimidazole (CDI) and all other chemicals were purchased from Sigma-Aldrich and used without further purification, unless indicated otherwise. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) referenced with respect to residual solvent (DMSO = 2.50/39.52 ppm). The following abbreviations were used to indicate multiplicities: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br. s = broad singlet and qdd = double doublet of quartet. High resolution (HRMS) mass spectra were obtained by electrospray ionization and performed on a Q-exactive Orbitrap MS system, Thermo Fischer Scientific. For column chromatography, silica gel (40-63 μm, Merck) was used. The retention factors (R_f) were determined by thin layer chromatography on pre-coated silica plates (Merck TLC Silica gel 60 F254). The spots were visualized by UV light and stained with ceric ammonium molybdate (CAM) solution, followed by treatment with a heat gun.

Chemical Synthesis

Hydrodabcyl-ONSu ester (3)

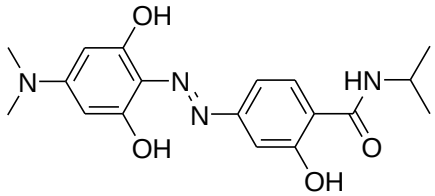


^{1}H-NMR (300 MHz, DMSO-d$_6$) δ ppm 2.88 (s, 4 H, C*H*$_2$ NSu), 3.09 (s, 6 H, N(C*H*$_3$)$_2$), 5.72 (s, 2 H, 2′-H, 6’-H), 7.27 (d, *J* = 1.7 Hz, 1 H, 3-H), 7.32 (dd, *J* = 8.8, 1.7 Hz, 1 H, 5-H), 7.89 (d, *J* = 8.8 Hz, 1 H, 6-H), 10.62 (br. s., 1 H, O*H*).

^{13}C-NMR (75 MHz, DMSO-d$_6$) δ 170.6 (2 × *C*O NSu), 170.6 (*C*OON), 161.3 (C2′, C6′), 160.9 (C2), 158.4 (C4′), 152.9 (C4), 132.6 (C6), 125.9 (C1′), 110.0 (C5), 106.8 (C1), 105.8 (C3), 91.8 (C3′, C5′), 40.1 (N(*C*H$_3$)$_2$), 25.5 (2 × *C*H$_2$ NSu).

HRMS (+ESI) *m/z*: [MOMe–H]$^-$ calculated for $C_{16}H_{16}N_3O_5^-$ 330.10954; found 330.10951.

Hydrodabcyl-isopropylamide (4)



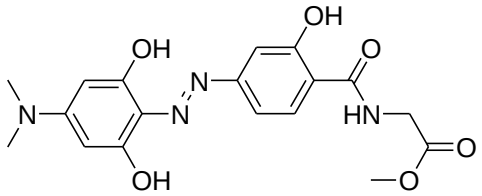
Isopropylamine (50 µL, 0.58 mmol) is added to a stirred solution of **3** (20 mg, 0.05 mmol) in DMF (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted with ethyl acetate (50 mL) and washed with aqueous citric acid (5 % w/w, 2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica (dry package, elution: CH/EtOAc 1:1) to afford a new compound **4** (18 mg, 99 % yeld) as a dark red solid (R*f* = 0.32, CH/EtOAc 1:1).

^{1}H-NMR (300 MHz, DMSO-*d*$_6$) *δ* ppm 1.17 - 1.22 (d, 6 H), 3.07 (s, 6 H), 4.16 (s, 1 H), 5.72 (s, 2 H), 7.20 (dd, *J*=8.5, 2.0 Hz, 1 H), 7.33 (d, *J*=2.0 Hz, 1 H), 7.94 (d, *J*=8.5 Hz, 1 H), 8.53 (d, *J*=7.8 Hz, 1 H), 12.90 - 13.22 (m, 1 H).

^{13}C NMR (75 MHz, DMSO-*d*$_6$) *δ* 168.2, 161.8, 157.7, 157.6, 151.2, 128.6, 128.6, 124.2, 112.5, 110.1, 105.8, 91.4, 91.4, 41.0, 40.0, 40.0, 22.1, 22.1.

HRMS (+ESI) *m/z*: $[M + H]^+$ calculated for $C_{18}H_{23}N_4O_4^+$ 359.17138; found 359.17065.

Hydrodabcyl-L-glycine methyl ester (7)



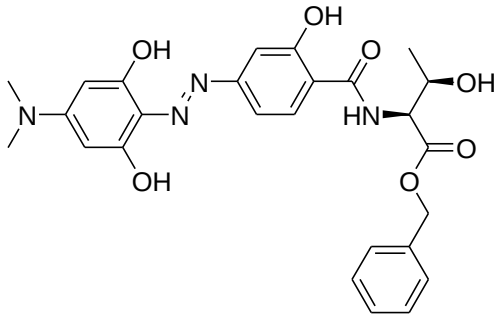
L-glycine methyl ester hydrochloride (7 mg, 0.055 mmol) and NEt_3 (8 µL, 0.055 mmol) were added to a stirred solution of **3** (19 mg, 0.05 mmol) in DMF (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted with ethyl acetate (100 mL) and washed with aqueous citric acid (5 % w/w, 2 x 50 mL) and brine (2 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica (dry package, elution: ethyl acetate) to afford a new compound **7** (19 mg, 99 % yeld) as a dark red solid (R*f* = 0.5, EtOAc).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.07 (s, 6 H), 3.68 (s, 3 H), 4.07 (d, *J*=5.7 Hz, 2 H), 5.72 (s, 2 H), 7.27 (dd, *J*=8.6, 1.9 Hz, 1 H), 7.32 (d, *J*=1.9 Hz, 1 H), 7.91 (d, *J*=8.6 Hz, 1 H), 9.20 (t, *J*=5.7 Hz, 1 H), 12.42 - 12.51 (m, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 170.1, 168.9, 161.1, 161.1, 157.8, 151.5, 129.2, 124.4, 112.4, 110.3, 106.0, 91.5, 91.5, 51.9, 41.0, 40.0, 40.0.

HRMS (-ESI) *m/z*: $[M - H]^-$ calculated for $C_{18}H_{19}N_4O_6^-$ 387.13101; found 387.13111.

Hydrodabcyl-L-threonine benzyl ester (8)



L-threonine benzyl ester hemioxalate (21 mg, 0.07 mmol) and NEt_3 (20 µL, 0.14 mmol) were added to a stirred solution of **3** (29 mg, 0.07 mmol) in DMF (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted withethyl acetate (100 mL) and washed with aqueous citric acid (5 % w/w, 2 x 50 mL) and brine (2 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude

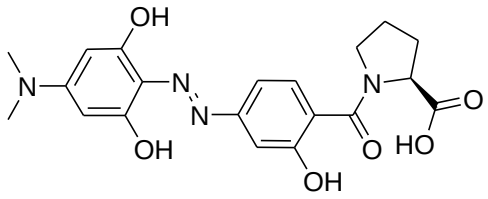
product was purified by column chromatography on silica (dry package, elution: ethyl acetate) to afford a new compound **8** (25 mg, 70 % yield) as a dark red solid (R*f* = 0.4, EtOAc).

^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, *J*=6.5 Hz, 3 H), 3.07 (s, 6 H), 4.27 (qdd, *J*=6.5, 6.5, 6.5, 5.6, 3.4 Hz, 1 H), 4.57 (dd, *J*=8.0, 3.4 Hz, 1 H), 5.18 (s, 2 H), 5.22 (d, *J*=5.6 Hz, 1 H), 5.73 (s, 2 H), 7.23 - 7.41 (m, 7 H), 8.00 (d, *J*=8.5 Hz, 1 H), 8.84 (d, *J*=7.9 Hz, 1 H), 11.75 - 11.97 (m, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 170.5, 166.8, 159.0, 157.6, 151.2, 136.0, 130.9, 128.4, 128.4, 128.0, 127.6, 127.6, 124.3, 114.6, 110.3, 106.1, 91.4, 91.4, 66.2, 66.0, 58.5, 40.0,40.0, 20.5

HRMS (-ESI) *m/z*: $[M - H]^-$ calculated for $C_{26}H_{27}N_4O_7^-$ 507.18743; found 507.18806.

Hydrodabcyl-L-proline (9)



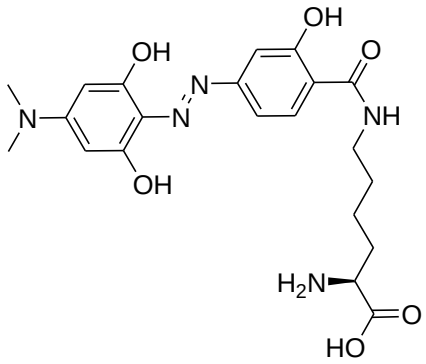
L-proline (23 mg, 0.2 mmol) and NEt_3 (28 µL, 0.2 mmol) were added to a stirred solution of **3** (42 mg, 0.1 mmol) in DMF (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted with ethyl acetate (100 mL) and washed with aqueous citric acid (5 % w/w, 2 x 50 mL) and brine (2 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica (dry package, elution: EtOAc / 0.1% HCOOH) to afford a new compound **9** (38 mg, 92 % yield) as a dark red solid (R*f* = 0.14, EtOAc / 0.1% HCOOH).

^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 1.71 - 1.97 (m, 3 H), 2.16 - 2.34 (m, 1 H), 3.01 - 3.11 (m, 6 H), 3.44 - 3.61 (m, 2 H), 4.30 - 4.45 (m, 1 H), 5.75 (s, 2 H), 7.04 - 7.38 (m, 3 H), 10.02 - 10.86 (m, 1 H), 12.26 - 12.81 (m, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 173.2, 167.5, 160.4, 160.2, 157.1, 156.4, 150.2, 129.2, 123.4, 120.7, 110.7, 106.2, 91.1, 91.1, 59.0, 48.4, 39.9, 39.9, 29.0, 24.7.

HRMS (+ESI) *m/z*: $[M + H]^+$ calculated for $C_{20}H_{23}N_4O_6^+$ 415.16121; found 415.16078.

Hydrodabcyl-ε-L-lysine (11)

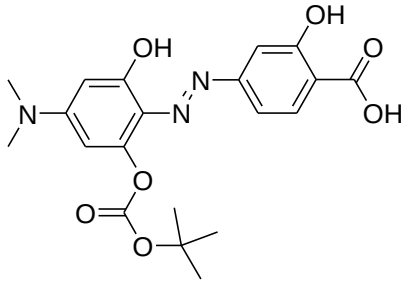


L-lysine copper (II) complex freshly prepared[1] (50 µL, 0.58 mmol) was added to a stirred solution of **3** (20 mg, 0.05 mmol) in DMSO (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. 0.1 M EDTA disodium salt (25 mL) was poured into the residue and stirred for 1 h at room temperature. The crude mixture was purified by puriFlash Interchim (column PF-30C18AQ-F0025, 15mL/min, 40% CH_3CN/60 % H_2O + 0.1% HCOOH isocratic) to afford a new compound **11** (5 mg, 30 % yield) as a dark red solid.

^{1}H NMR (300 MHz, methanol-d_4) δ ppm 1.45 - 1.60 (m, 2 H), 1.62 - 1.75 (m, 2 H), 1.93 - 2.09 (m, 2 H), 3.24 (s, 6 H), 3.38 (t, *J*=6.1 Hz, 2 H), 4.04 (t, *J*=6.6 Hz, 1 H), 4.93 - 5.05 under water signal (m, 2 H), 6.90 - 7.08 (m, 2 H), 7.73 (d, *J*=8.4 Hz, 1 H).

HRMS (+ESI) *m/z*: $[M + H]^+$ calculated for $C_{21}H_{28}N_5O_6^+$ 446.20341; found 446.20282.

Hydrodabcyl-mono-tert-bytylcarbonate (13)



NEt_3 (44 µL, 0.315 mmol) was added to a mixture of hydrodabcyl **2** (50 mg, 0.158 mmol) and di-tert-bytyldicarbonate (69 mg, 0.315 mmol) in DMF (5 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted with ethyl acetate (100 mL) and washed with brine (3 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica (dry package, elution: ethyl acetate / 10% EtOH)) to afford a new

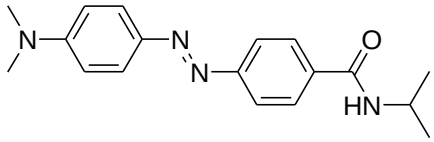
compound **13** (49 mg, 74 % yield) as a dark red solid (R*f* = 0.35, EtOAc : isopropanol : water 4 : 1 : 0.5).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.49 (s, 9 H), 3.10 (s, 6 H), 5.90 (d, *J*=2.5 Hz, 1 H), 6.53 (d, *J*=2.5 Hz, 1 H), 7.01 - 7.07 (m, 2 H), 7.82 (d, *J*=8.8 Hz, 1 H), 14.81 (br. s., 2 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 171.4, 163.0, 161.5, 155.2, 152.0, 151.3, 150.9, 131.2, 123.6, 115.9, 110.1, 105.8, 100.9, 95.5, 83.3, 40.1, 40.1, 27.3, 27.3, 27.3.

HRMS (-ESI) *m/z*: $[M - H]^-$ calculated for $C_{20}H_{22}N_3O_7^-$ 416.14632; found 416.14629.

Dabcyl-isopropylamide (14)



1 (269 mg, 1 mmol) was dissolved in DMF (5mL) and filled with dichloromethane (45 mL). To this reaction mixture isopropyl amide (172 µL, 2 mmol), NEt_3 (348 µL, 2.5 mmol) and T3P (1.15 mmol) were added. The reaction mixture was stirred overnight at room temperature. Thereafter, it was diluted with ethyl acetate (50 mL) and washed with brine (50 mL). The aqueous phase was extracted with dichloromethane (2 x 50 mL) The organic layer were combined and dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica (dry package, elution: CH/EtOAc 2:1 + 0.5 % NEt_3) to afford a new compound **14** (249 mg, 80 % yeld) as an orange solid (R*f* = 0.55, CH/EtOAc 1:1 + 0.5 % NEt_3).

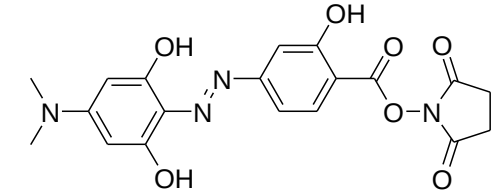
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.17 (br. s., 3 H), 1.19 - 1.22 (m, 3 H), 3.08 (s, 6 H), 4.12 (dq, *J*=13.4, 6.6 Hz, 1 H), 6.85 (d, *J*=8.8 Hz, 2 H), 7.81 (dd, *J*=8.4, 4.5 Hz, 4 H), 7.99 (d, *J*=8.2 Hz, 2 H), 8.32 (d, *J*=7.7 Hz, 2 H).

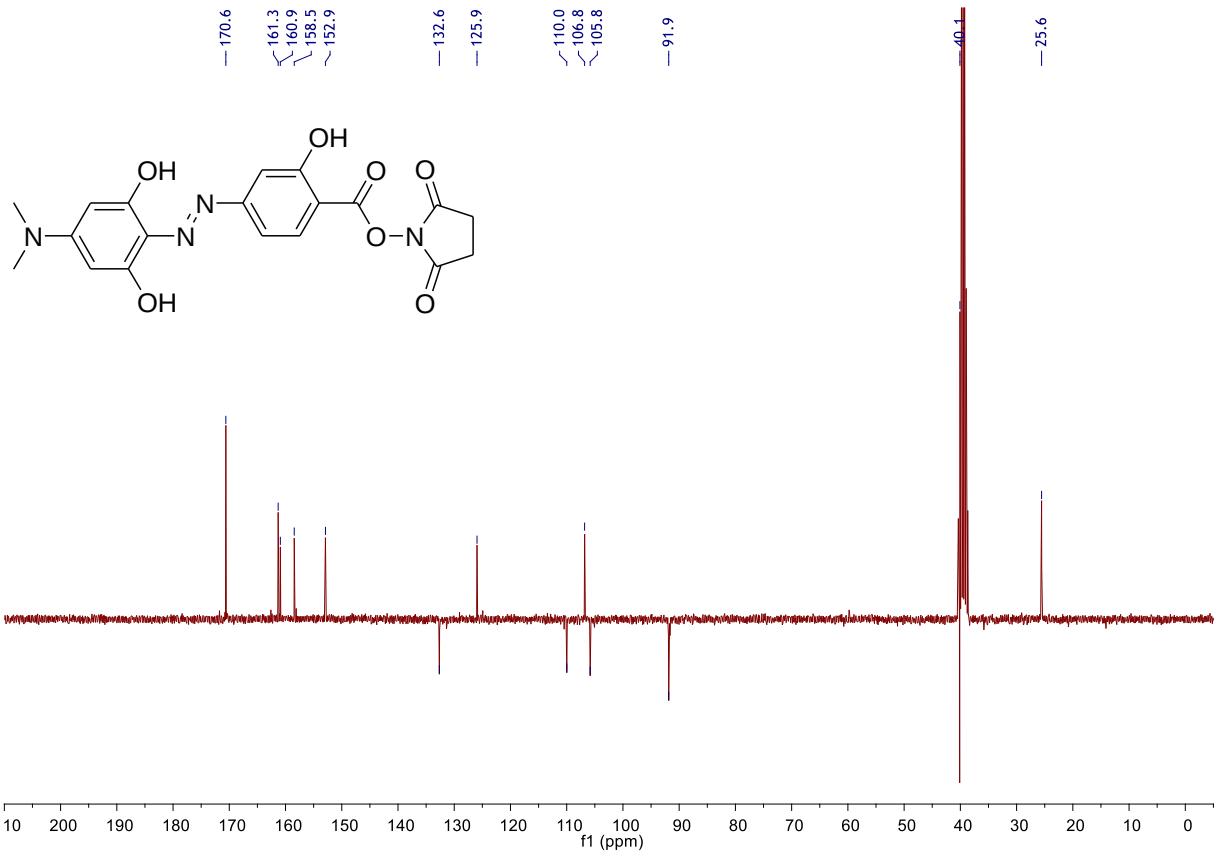
^{13}C NMR (75 MHz, DMSO-d_6) δ164.7, 153.8, 152.8, 142.6, 135.1, 128.4, 128.4, 125.1, 125.1, 121.4, 121.4, 111.6, 111.6, 41.1, 39.8, 39.8, 22.3, 22.3.

HRMS (+ESI) *m/z*: $[M + H]^+$ calculated for $C_{18}H_{23}N_4O^+$ 311.18664; found 311.18534.

NMR spectra

Hydrodabcyl-ONSu ester (3)





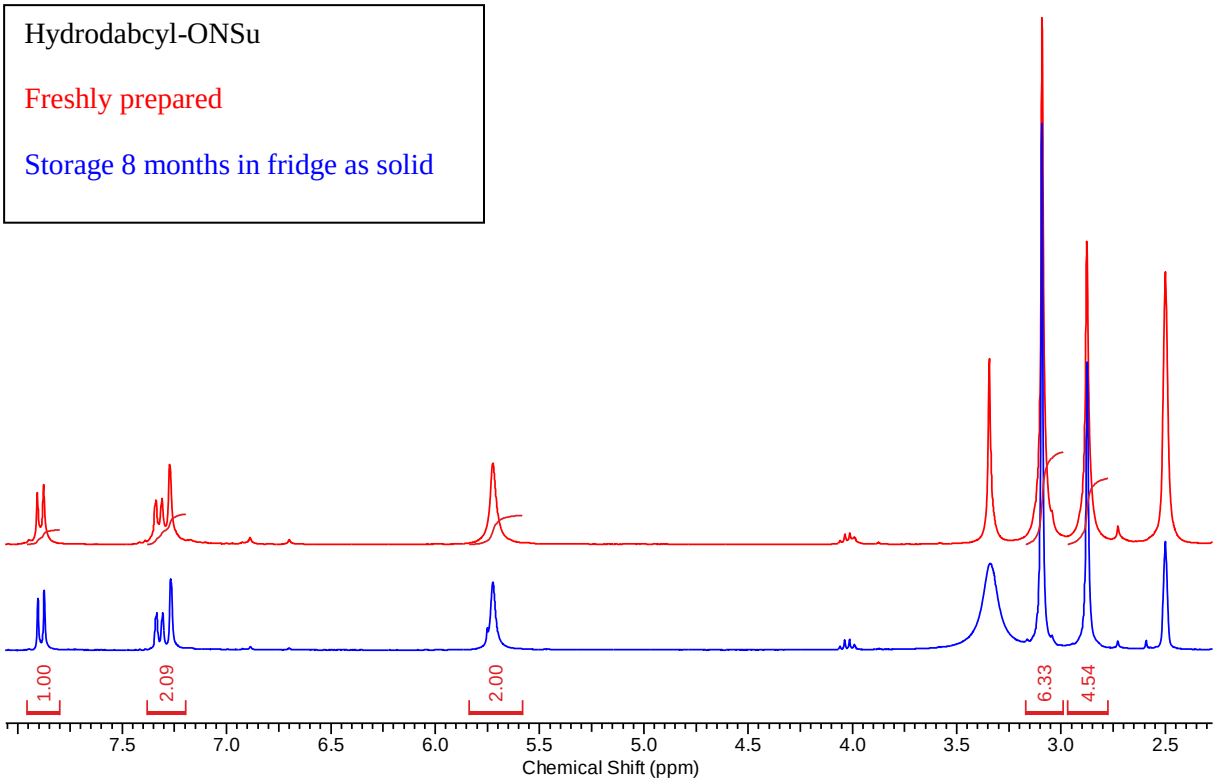


Figure S1. Stability test of hydrodabcyl-ONSu (**3**). ^{1}H-NMR spectra in DMSO-*d*$_6$ of **3** freshly prepared (in red) and after 8 months of storage at 4 °C as solid (in blue).

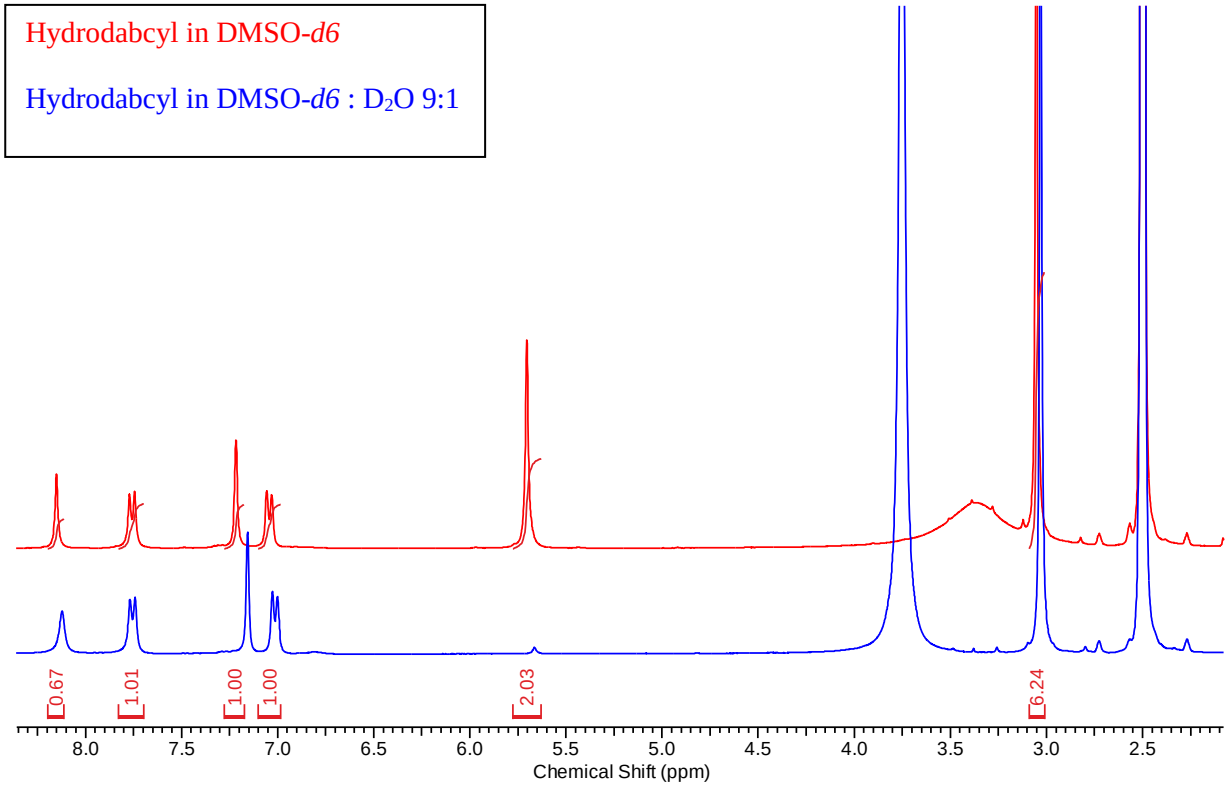
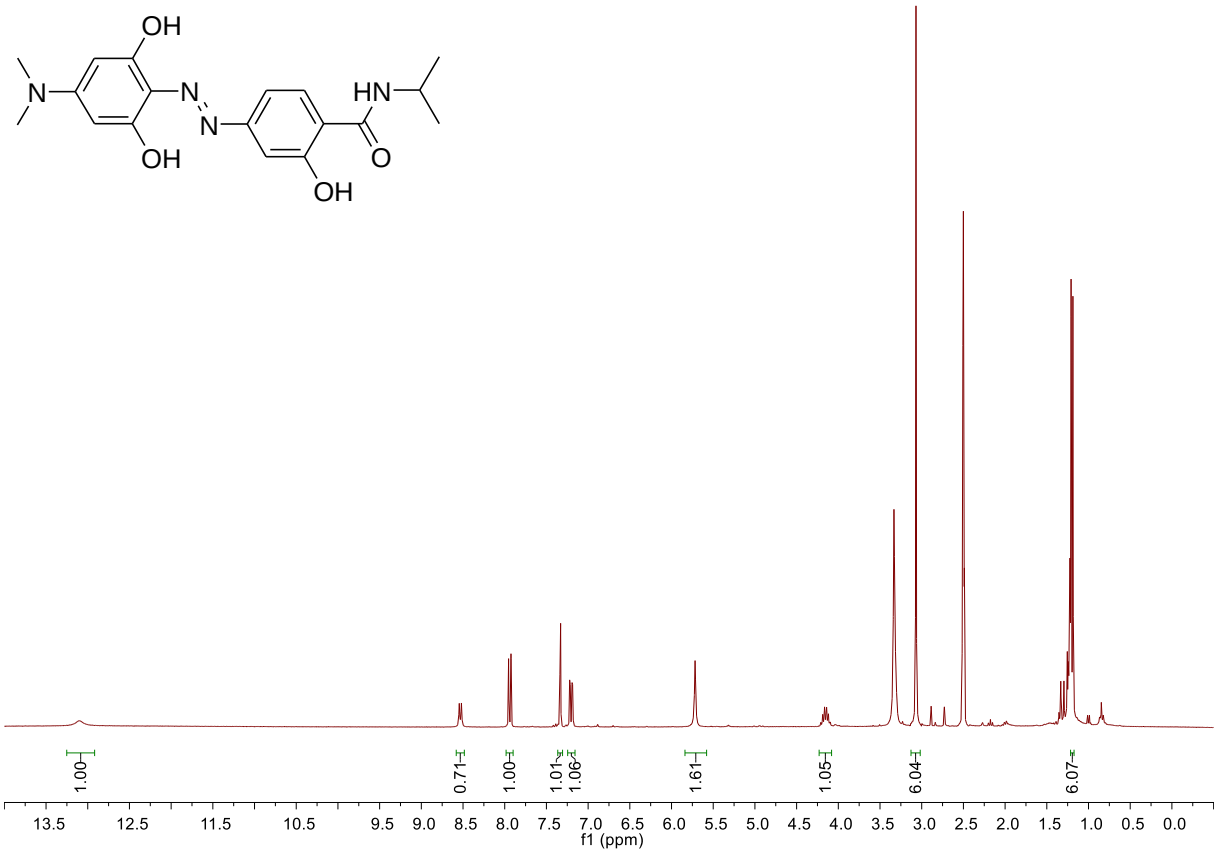
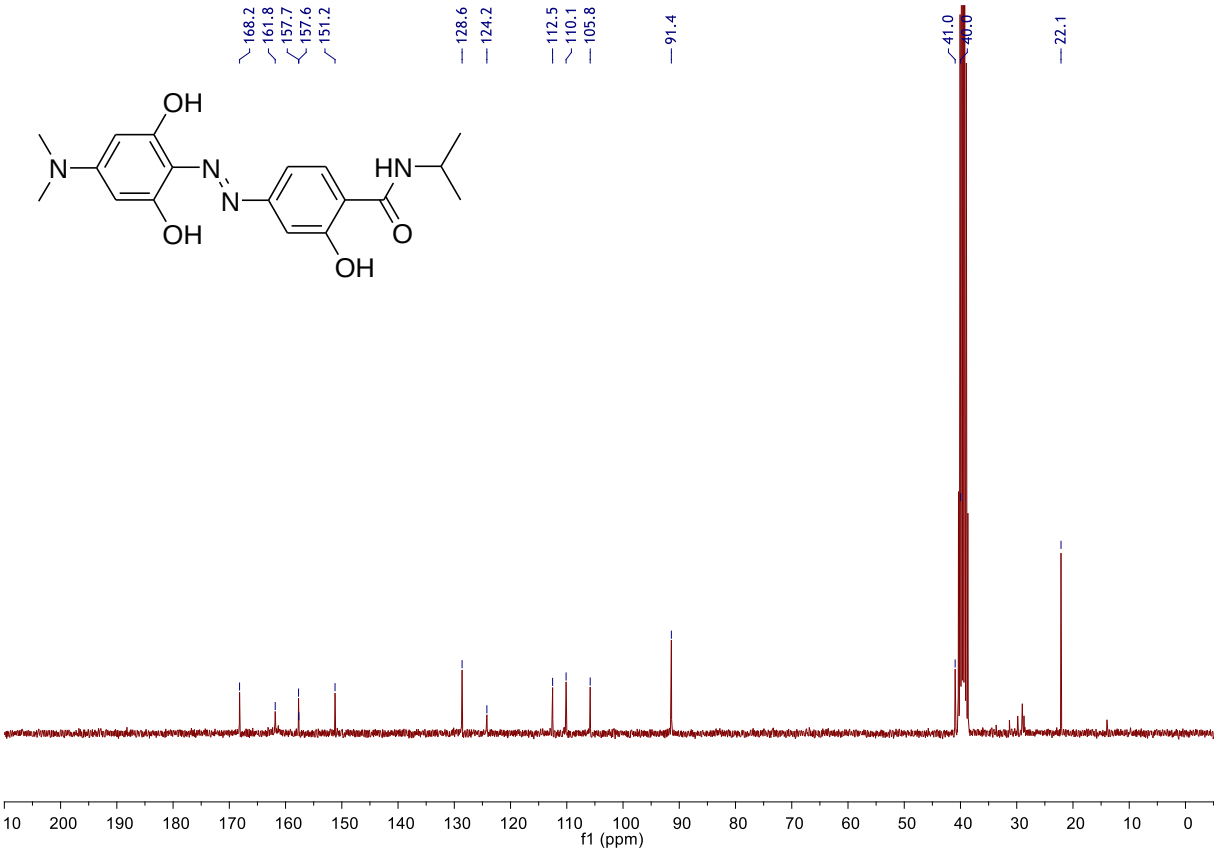


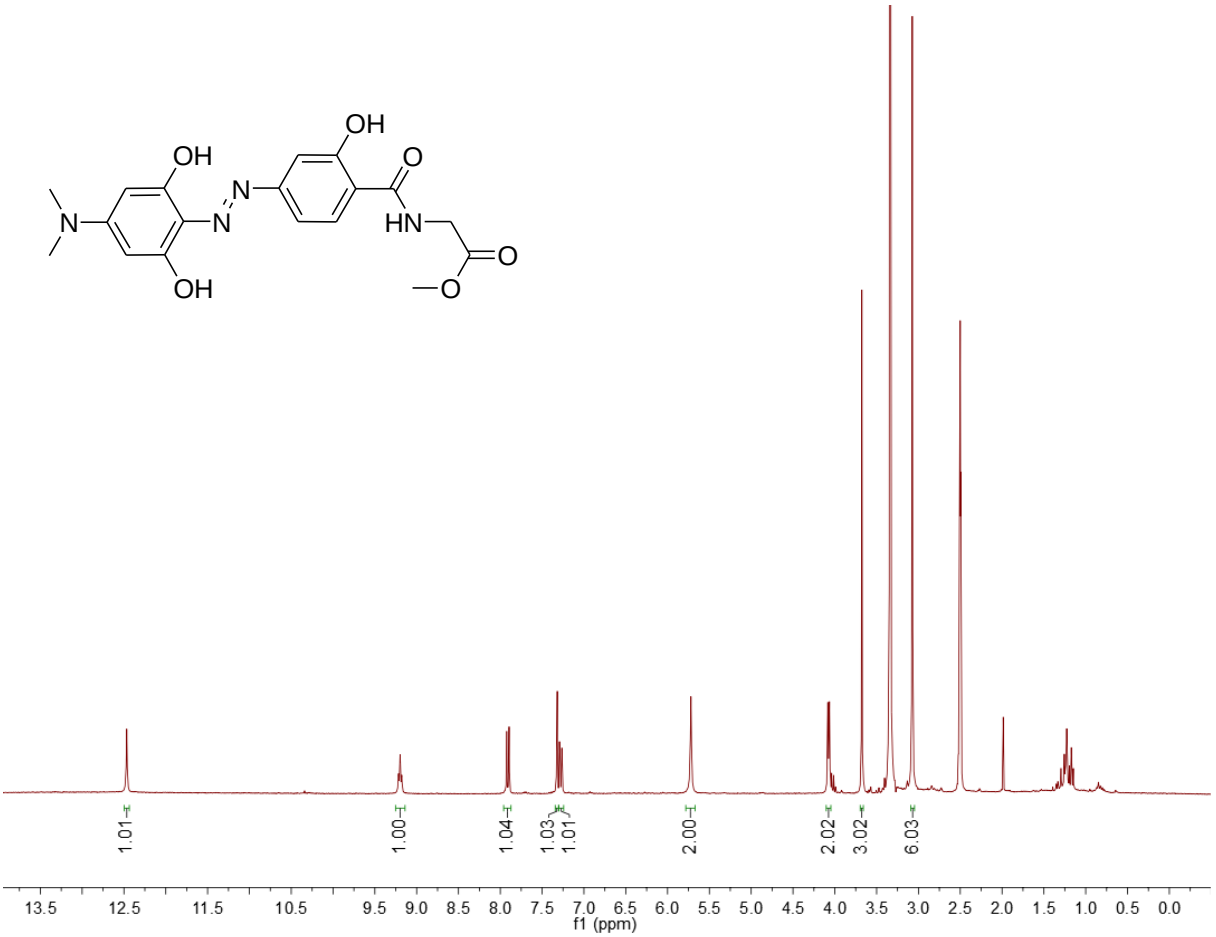
Figure S2. Exchange of the aromatic proton of hydrodabcyl (**2**) by deuterium from D_2O (in blue)

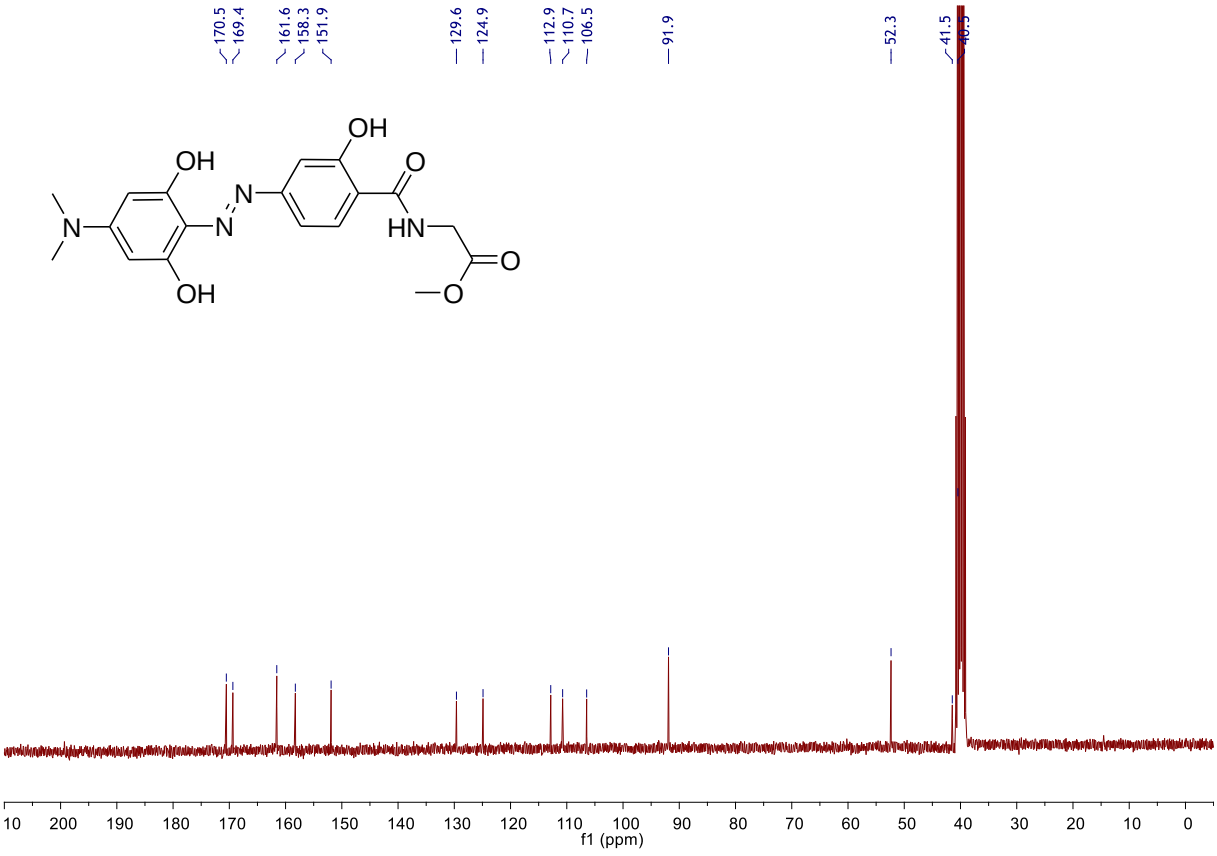
Hydrodabcyl-isopropylamide (4)



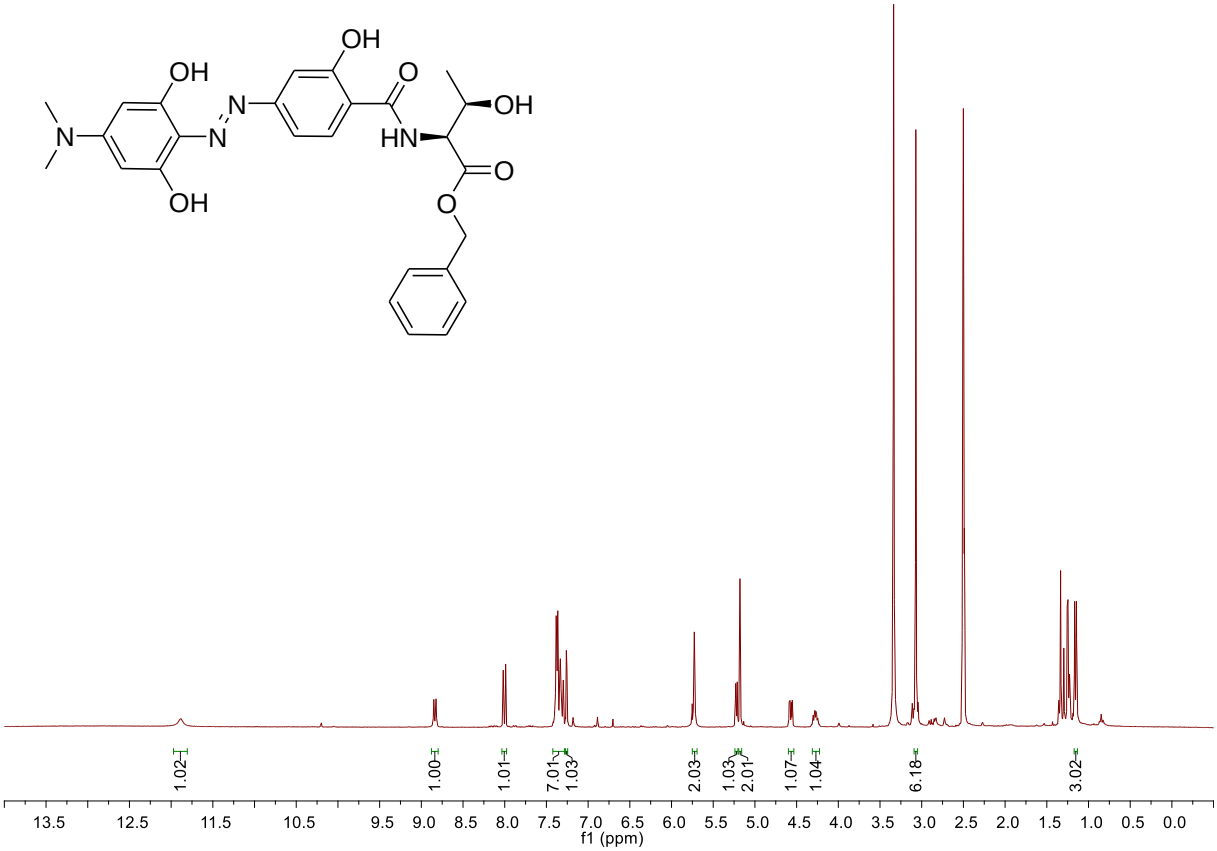


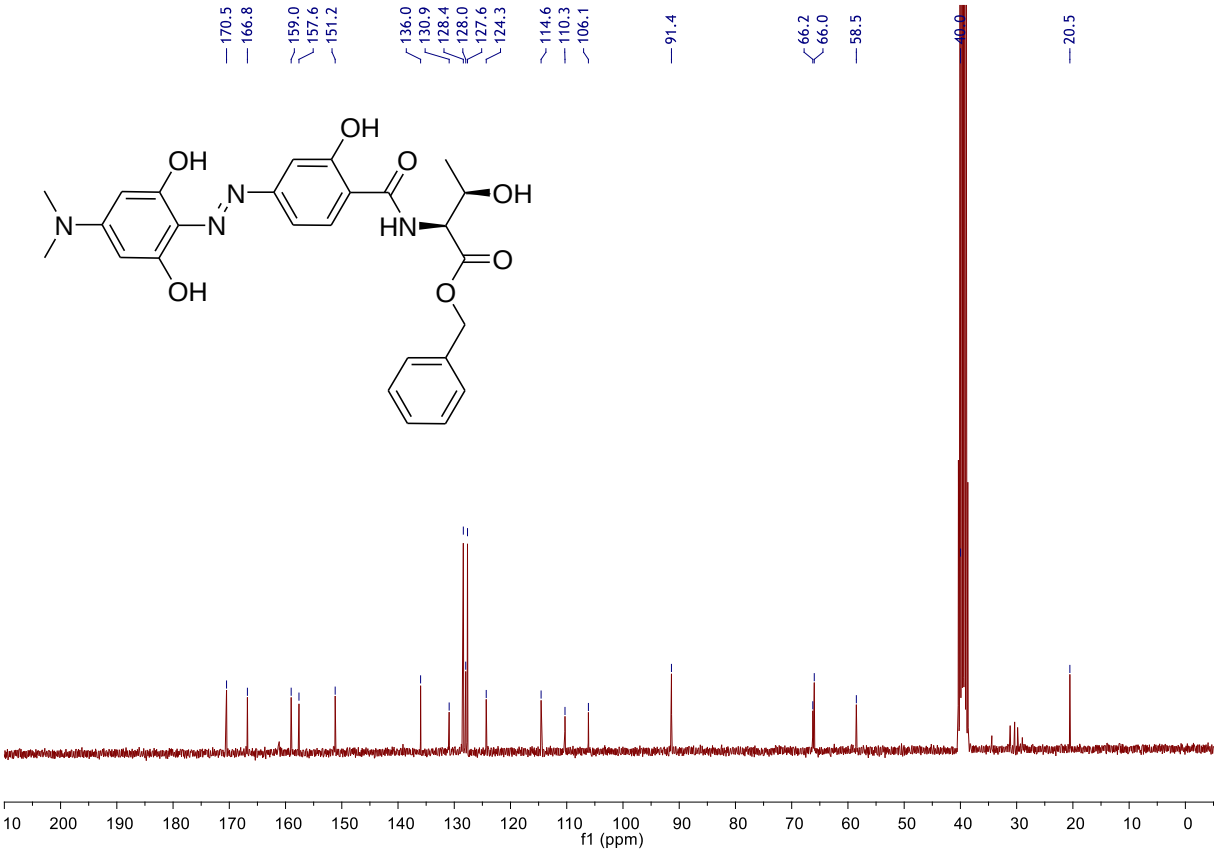
Hydrodabcyl-L-glycine methyl ester (7)



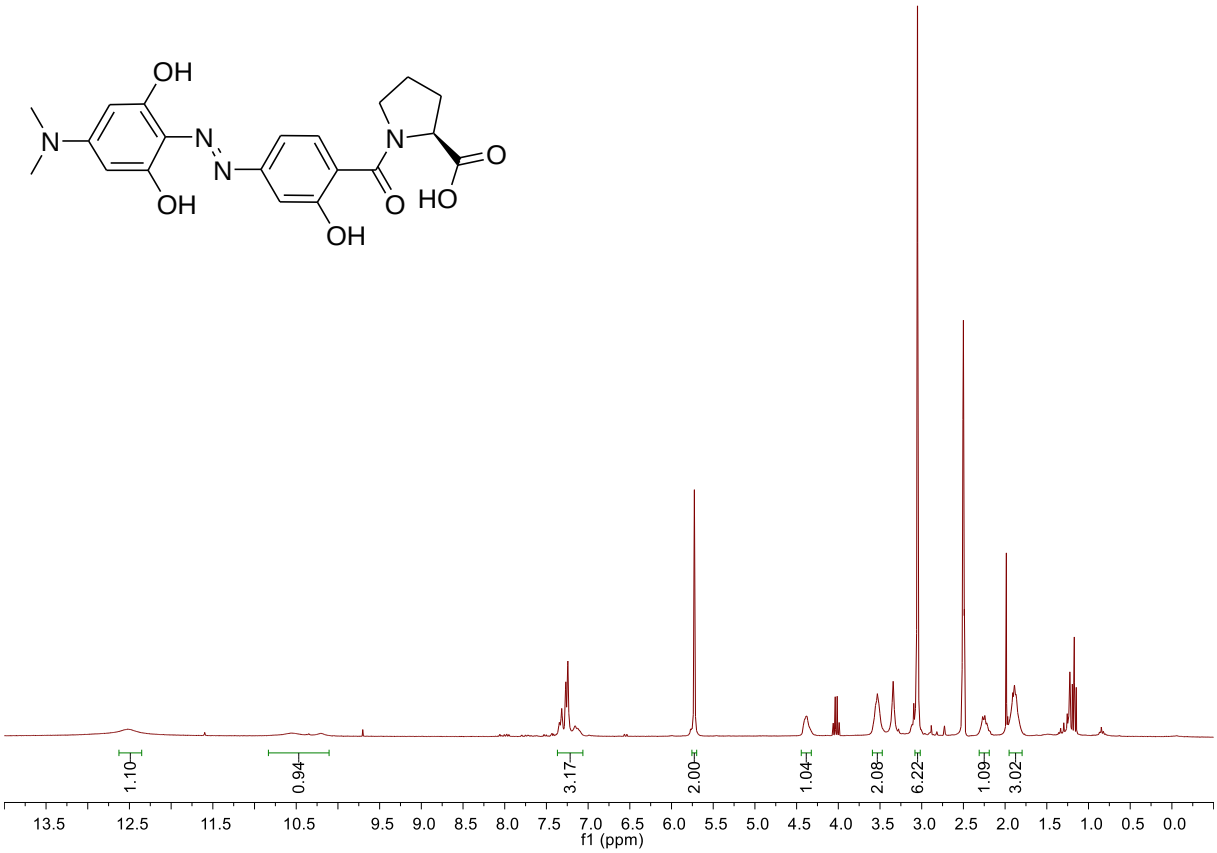


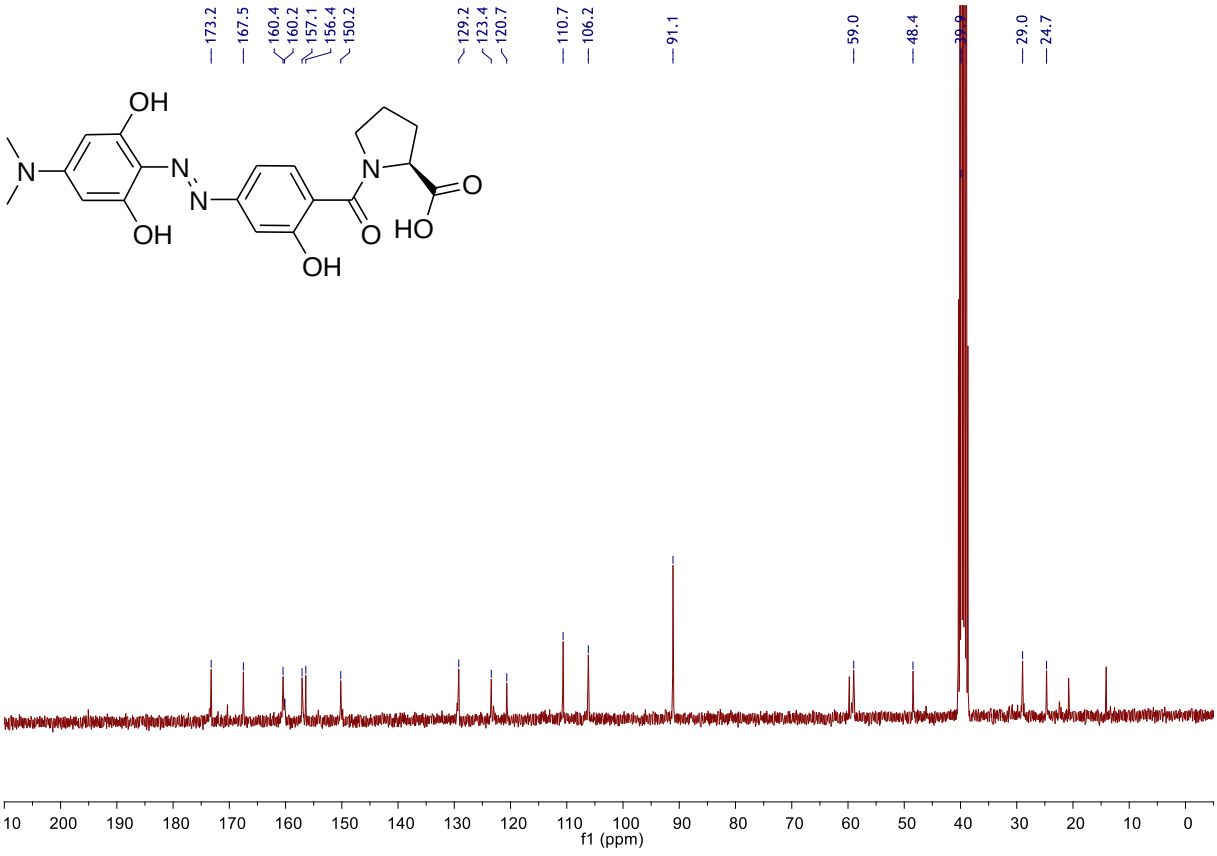
Hydrodabcyl-L-threonine benzyl ester (8)



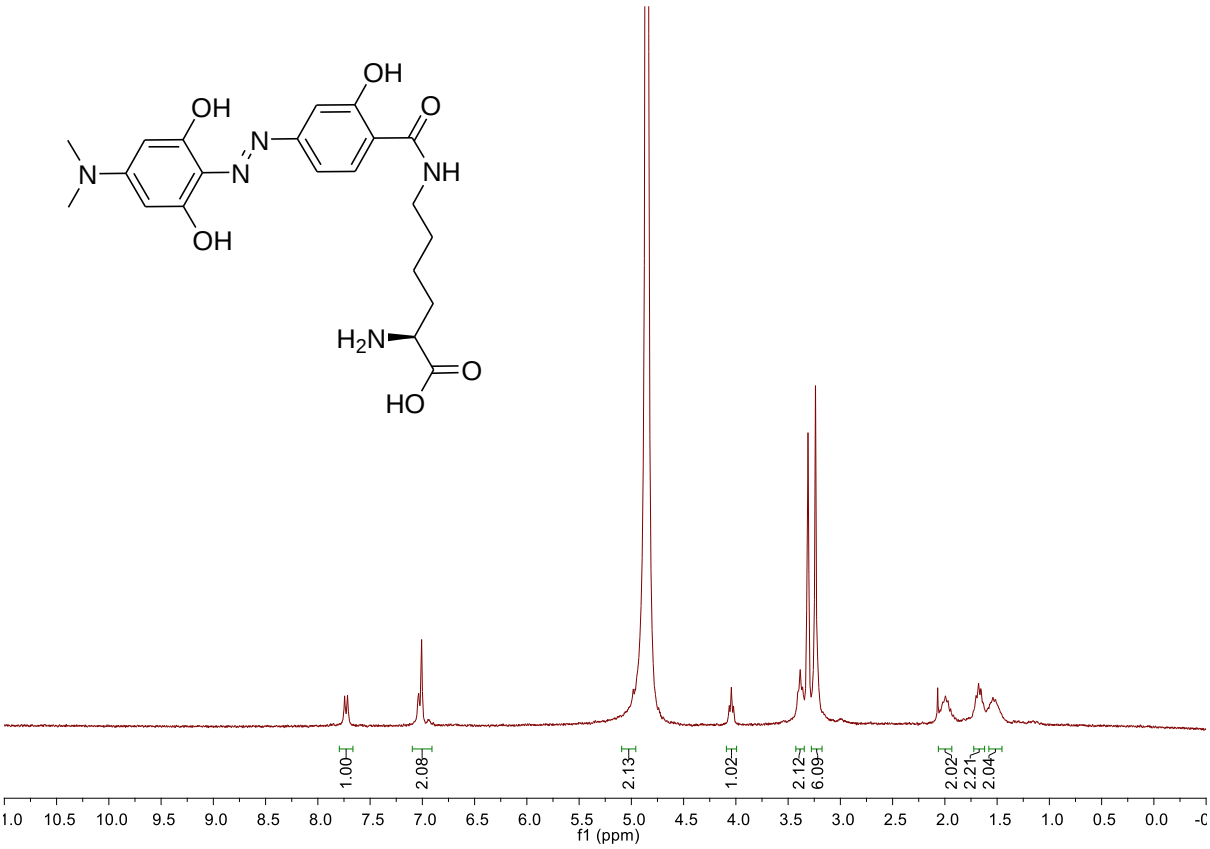


Hydrodabcyl-L-proline (9)

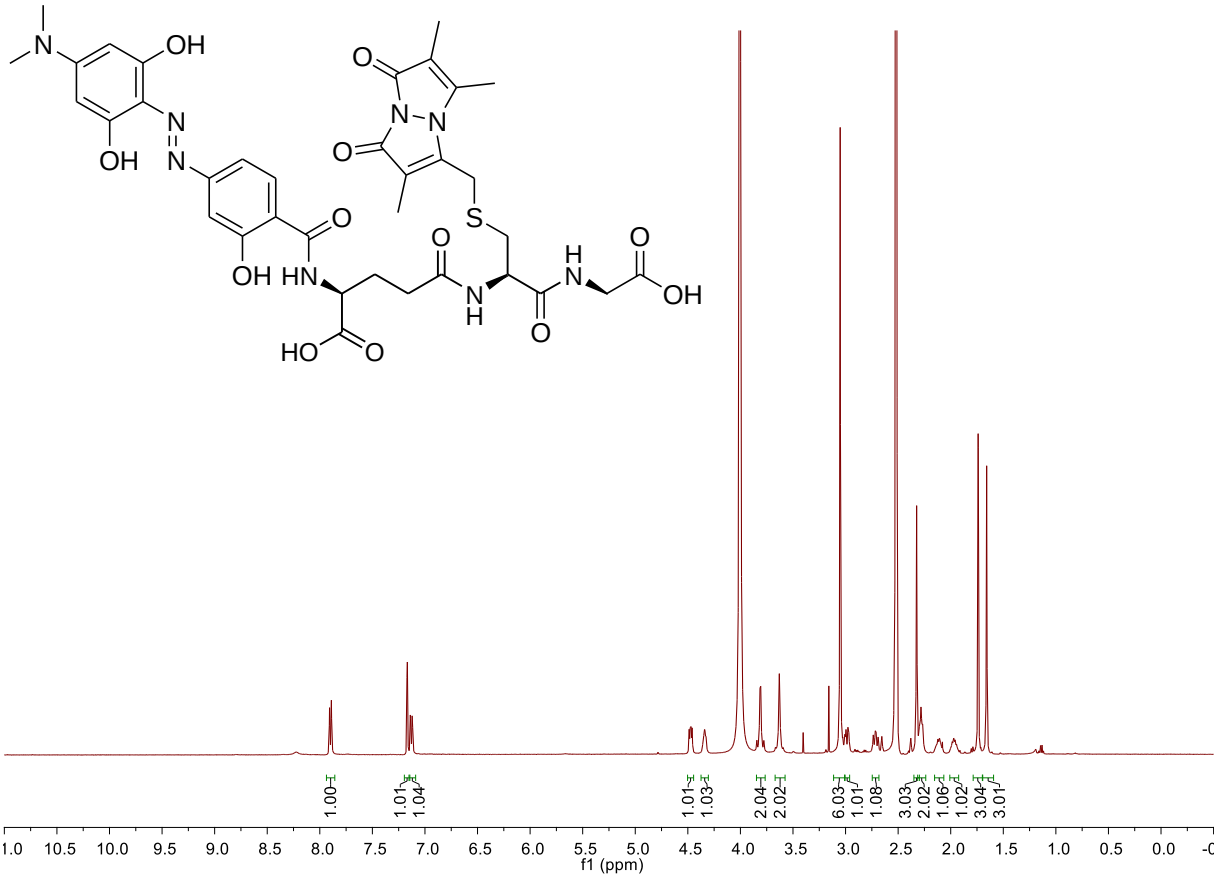


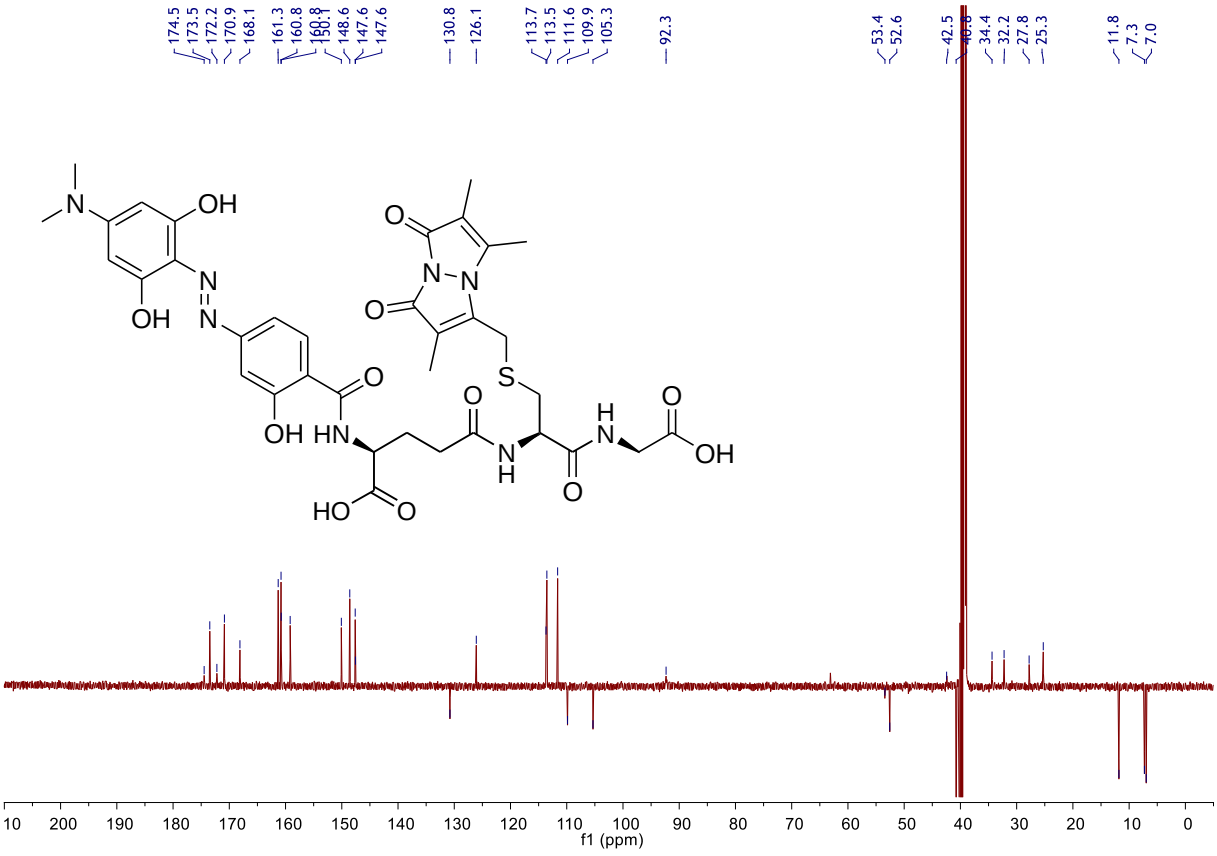


Hydrodabcyl-ε-L-lysine (11)



Hydrodabcyl-Gluthatione-Bimane (12)





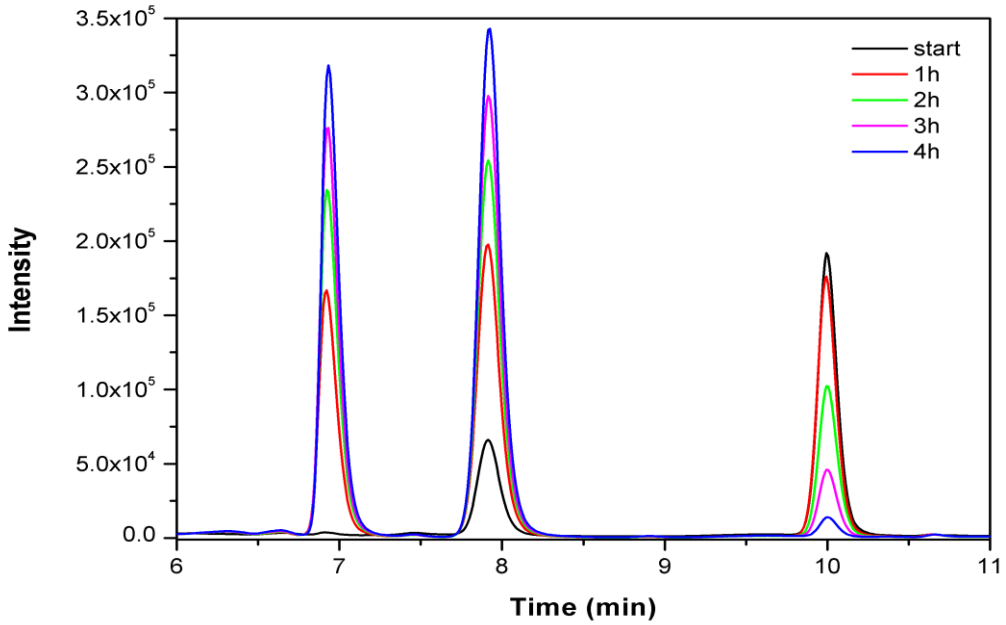


Figure S3. HPLC chromatogram of reaction monitoring of the one-pot double-functionalization of glutathione with hydrodabcyl-ONSu (**3**) and-bimane at 450 nm: start addition of **3** (in black), after 1h reaction (in red), 2h (in green), 3h (in magenta) and 4 h (in blue). The retention time: at 7 min is hydrodabcyl-glutathione-bimane **(12)**, at 8 min is hydrolysed hydrodabcyl acid **(2)**, at 10 min is hydrodabcyl-ONSu **(3)**.

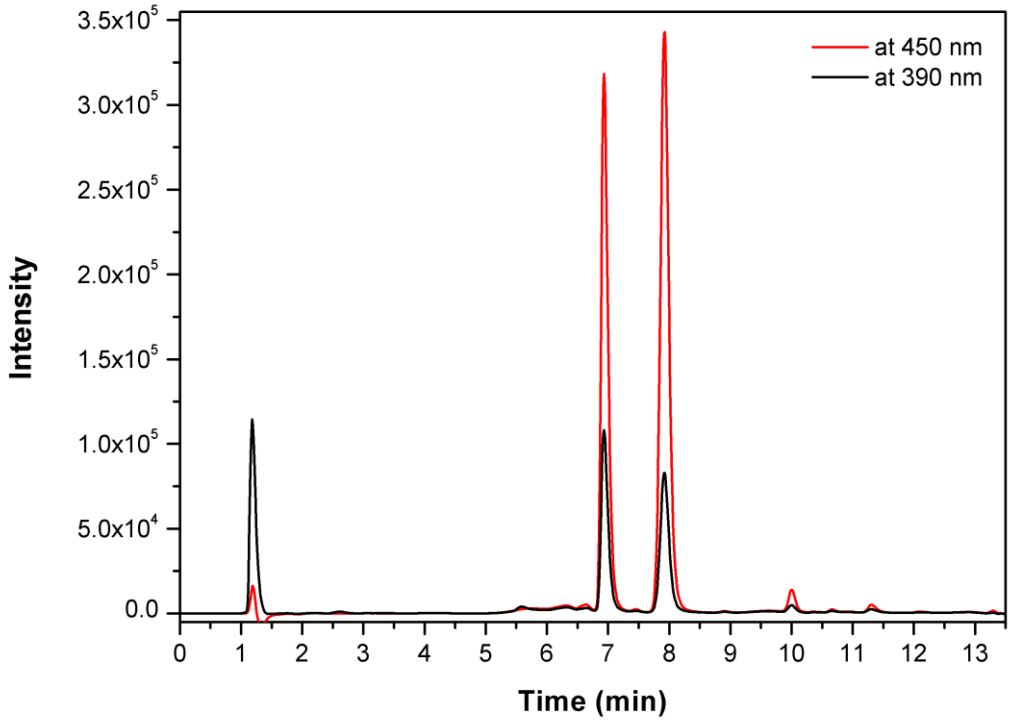
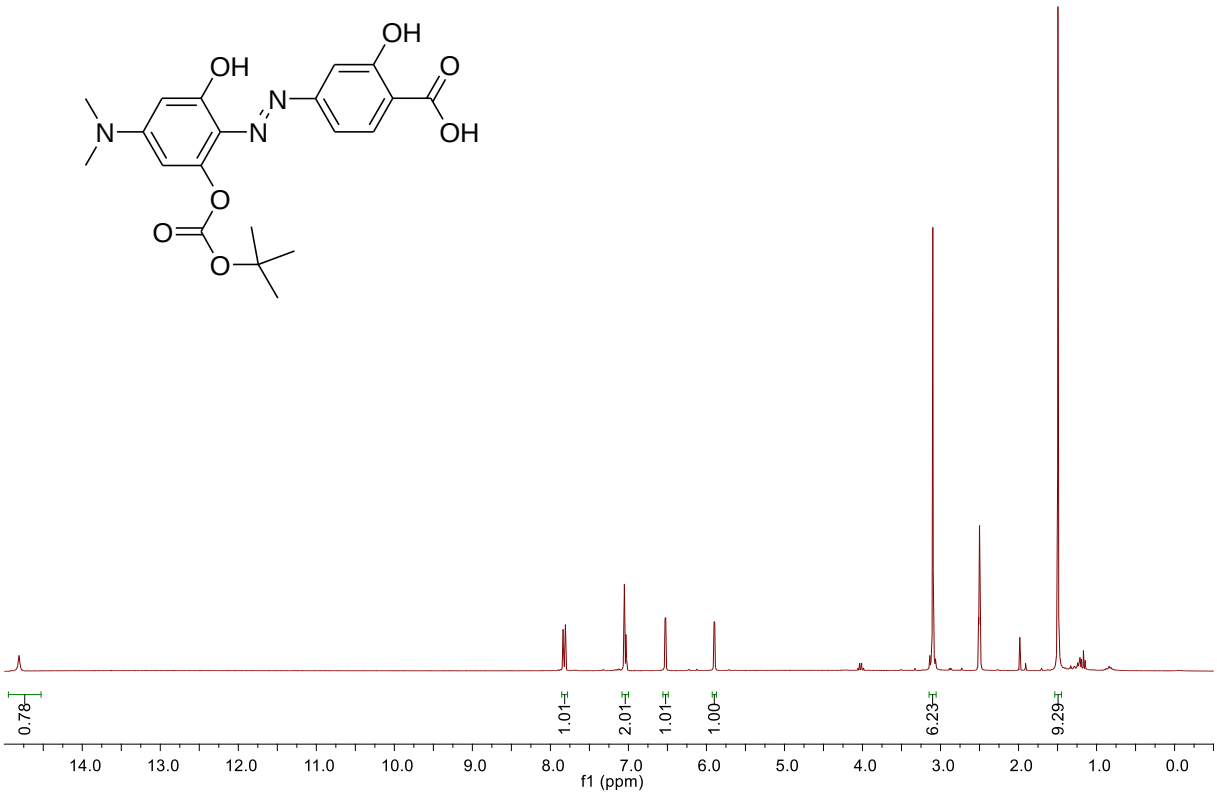
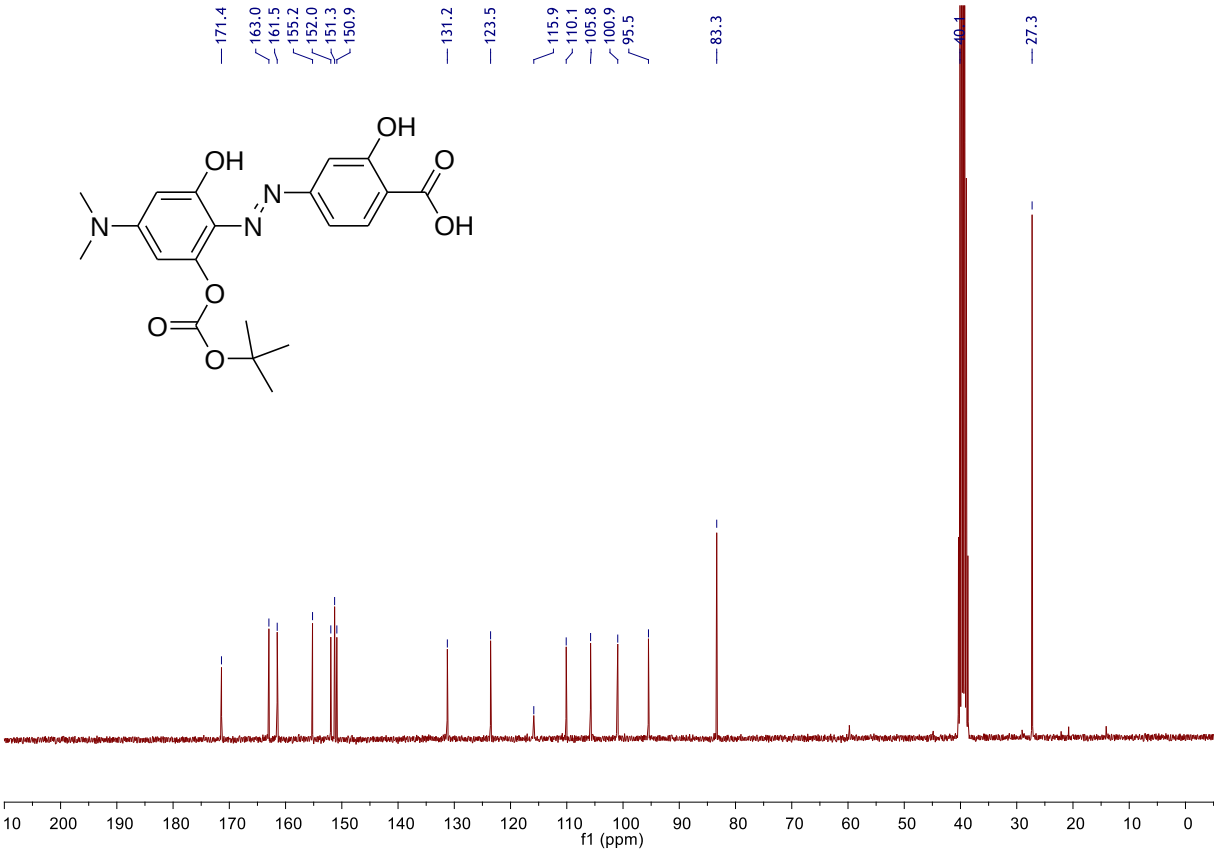


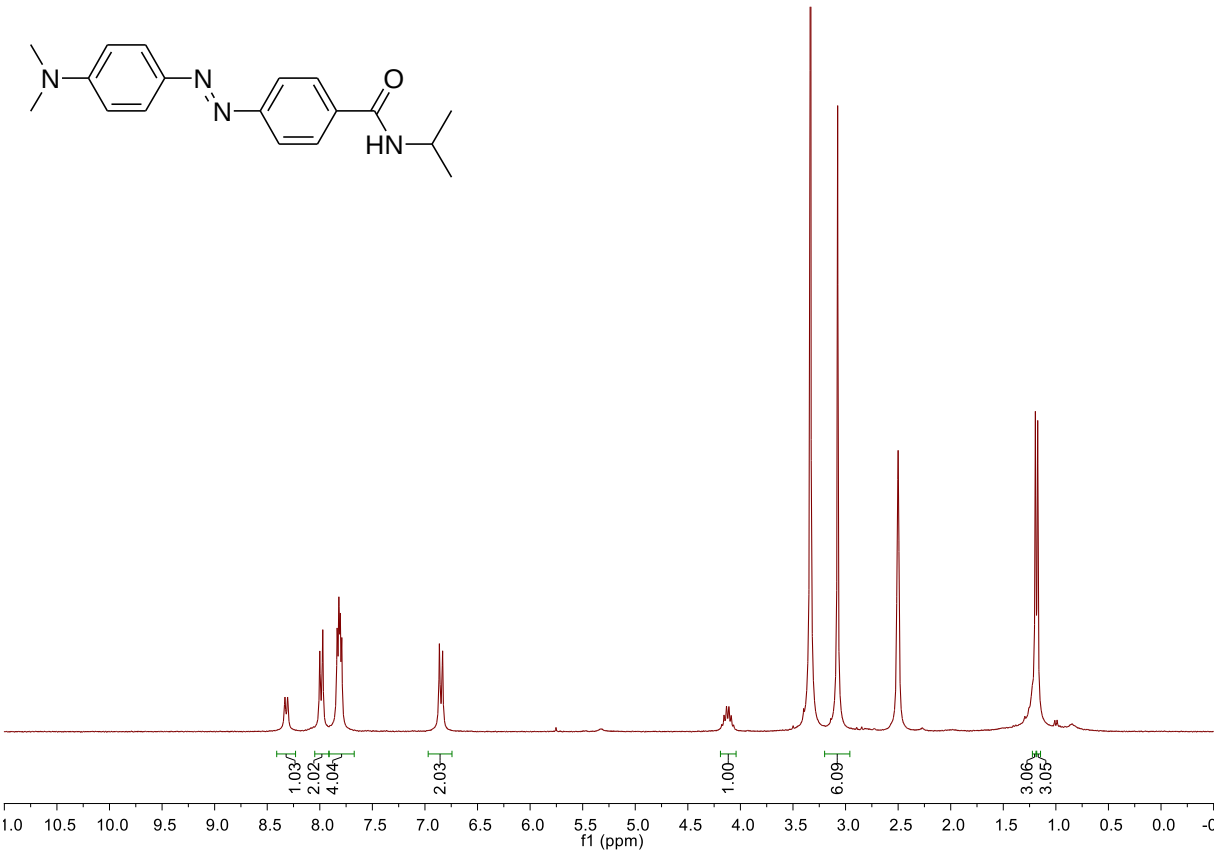
Figure S4. HPLC chromatogram of reaction monitoring after 4 h at 450 nm (in red) and at 390 nm (in black).

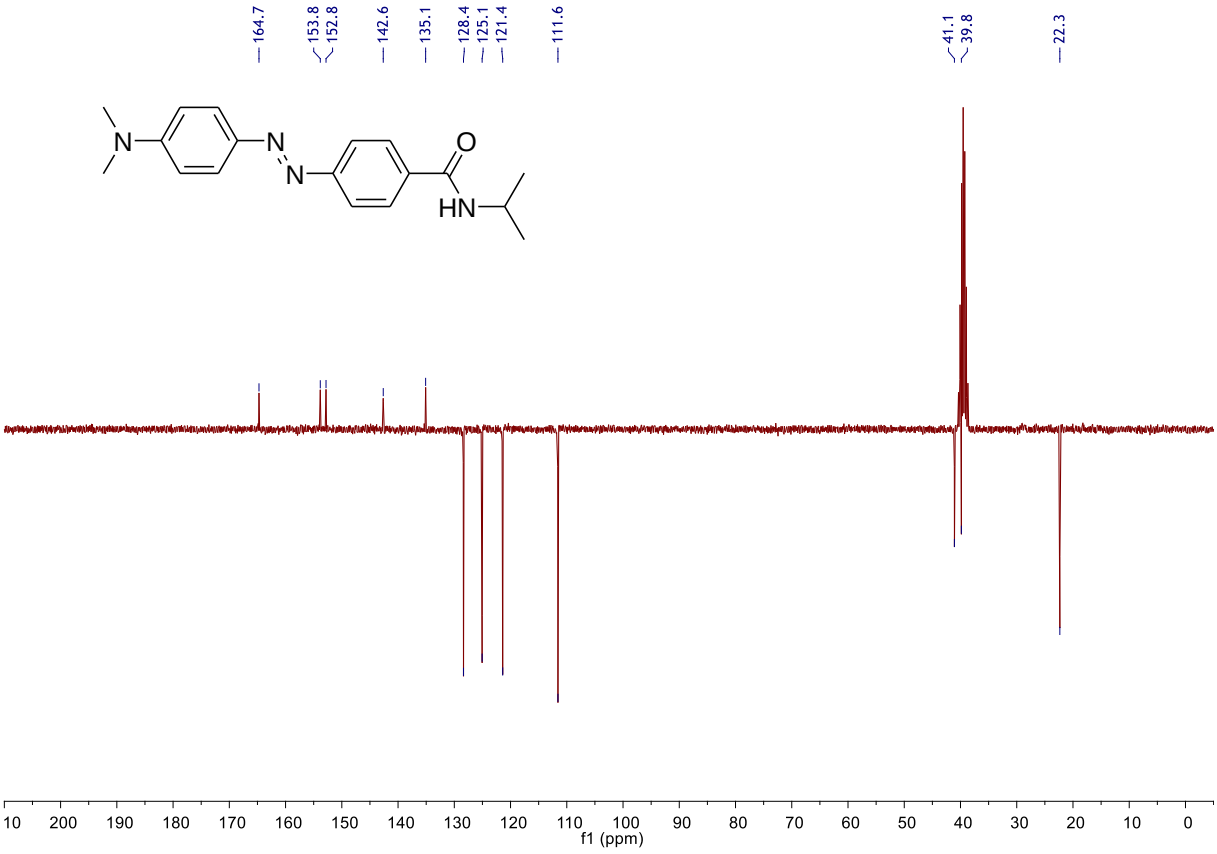
Hydrodabcyl-mono-tert-bytylcarbonate (13)





Dabcyl-isopropylamide (14)





[1] Wiejak, S; Masiukiewicz, E: Rzeszotarska, B. A Large Scale Synthesis of Mono- and Di-urethane Derivatives of Lysine. *Chem. Pharm. Bull.* **1999**, *47*, 1489−1490.