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Supporting Information

69451 Weinheim, Germany

Uncharacterized 4,5-Dihydroxy-2,3-Pentanedione (DPD) Molecules Revealed Through NMR Spectroscopy: Implications for a Greater Signaling Diversity in Bacterial Species**

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Content

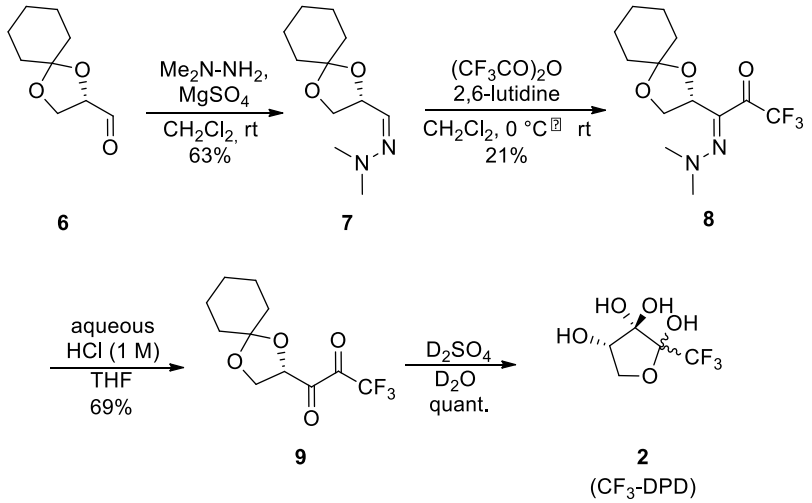
General procedure

Unless otherwise stated, all reactions were performed under an inert atmosphere with dry reagents, solvents, and flamed-dried glassware. All starting materials were purchased from Aldrich and Sigma, unless otherwise noted, and used as received. All column chromatography was performed with silica gel 60 (230–400 mesh). Analytical and preparative thin-layer chromatography (TLC) was performed with Merck Kieselgel 60 F254 silica gel plates (0.25, 0.5, 1.0 mm). ^{1}H and ^{13}C NMR spectra were measured on a Bruker DRX-600, Bruker DRX-500, or Varian 400 MHz spectrometer as indicated. ^{19}F NMR spectra were measured on a Varian 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. HRMS spectra were recorded on a VG ZAB-VSE or an ABI /SCIEX API-150 EX single quadrupole electrospray mass spectrometer.

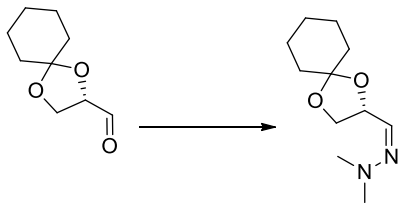
Synthesis of DPD derivatives

The synthesis of DPD (**1**), its C1-alkyl derivatives (**1a-d**) and the mono-^{13}C-labeled derivative **4** were performed as described.[1-2]

Synthesis of CF_3-DPD (2)



(2*R*)-2,3-(Cyclohexylidenedioxy)propyl-*N*,*N*-(dimethyl)-hydrazone, 7[3]

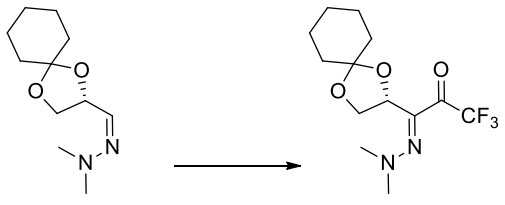


6 **7**

$MgSO_4$ (991.4 mg, 8.24 mmol) was added to a solution of aldehyde **6**[4] (1.48 g, 8.67 mmol) in CH_2Cl_2 (10 mL) followed by the dropwise addition of 1,1-dimethylhydrazine (691 µL, 9.10 mmol) under vigorous stirring at 0 °C. The reaction mixture was stirred for 6 h, before being filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 1:10 → 1:5) gave compound **7** as an orange oil (1.16 g, 5.46 mmol, 63%).

^{1}H NMR (600 MHz, $CDCl_3$) δ = 6.40 (d, ^3J=5.9, *H*C=NNMe$_2$, 1H), 4.64-4.57 (m, OCH, 1H), 4.13 (dd, ^2J=8.2, ^3J=6.4, OC*H*HCH, 1H), 3.73 (dd, ^2J=8.2, ^3J=7.4, OCH*H*CH, 1H), 2.82 (s, N(CH$_3$)$_2$, 6H), 1.64-1.52 (m, cyclohexyl, 8H), 1.44-1.30 (m, cyclohexyl, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 132.0, 109.9, 76.4, 67.7, 42.5, 36.3, 35.1, 25.1, 24.0, 23.8; HRMS (ESI+) calculated for $C_{11}H_{21}N_2O_2^+$ $(M+H)^+$: 213.1597, found: 213.1599.

1,1,1-Trifluoro-2-oxy-4,5-(cyclohexylidenedioxy)pentyl-3-[*N*,*N*-(dimethyl)-hydrazone], 8[5]

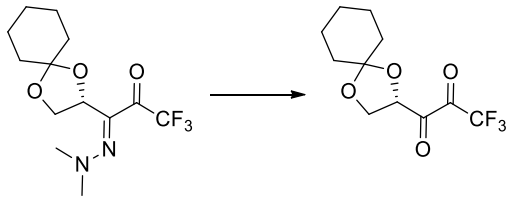


7 **8**

2,6-Lutidine (107 µL, 942 µmol) was added to a solution of hydrazone **7** (100 mg, 471 µmol) in CH_2Cl_2 (1 mL) at 0 °C, followed by dropwise addition of a solution of $(CF_3CO)_2O$ (200 µL, 1.41 mmol) in CH_2Cl_2 (1 mL) and stirred for 1 h at 0 °C. The reaction mixture was allowed to warm to rt and stirred for a further 14 h. Then the reaction mixture was poured onto a solution of 0.1 M HCl (40 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with H_2O (20 mL) and sat. aq. Na_2CO_3 (20 mL), then dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (1:10) gave compound **8** as a yellow oil (27 mg, 87.6 µmol, 21%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.07 (dd, ^{3}J=8.6, ^{3}J=6.6, OCH, 1H), 4.25-4.13 (m, OCH_2CH, 2H), 3.46 (s, N(CH_3)$_2$, 6H), 1.72-1.51 (m, cyclohexyl, 8H), 1.41-1.36 (m, cyclohexyl, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 177.5, 177.2, 125.5, 121.9, 119.0, 116.1, 109.7, 69.1, 66.4, 47.8, 35.5, 35.0, 25.0, 23.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -69.4.

(4*S*)-1,1,1-Trifluoro-4,5-(cyclohexylidenedioxy)-2,3-pentadione, 9[5]

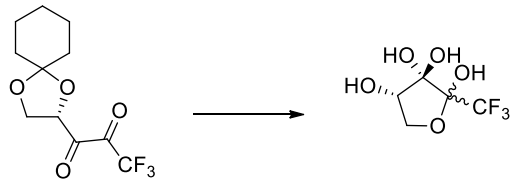


8 **9**

Aqueous HCl (1 M / 1.5 mL) was added to a solution of compound **8** (44.2 mg, 143.4 µmol) in THF (3 mL) and stirred for 3 h. H_2O (10 mL) was added to the reaction mixture and was then extracted with Et_2O (3 × 20 mL), the combined organic layers were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (1:5) gave compound **9** as a yellow oil (22.5 mg, 99.5 µmol, 69%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.05 (dd, ^{3}J=7.6, ^{3}J=5.5, OCH, 1H), 4.31 (dd, ^{2}J=9.0, ^{3}J=7.6, OC*H*HCH, 1H), 4.14 (dd, ^{2}J=9.1, ^{3}J=5.5, OCH*H*CH, 1H), 1.79-1.49 (m, cyclohexyl, 8H), 1.48-1.32 (m, cyclohexyl, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -82.3; HRMS (ESI-) calculated for $C_{11}H_{12}F_3O_4^-$ $(M-H)^-$: 265.0693, found: 265.0701.

(4*S*)-2-(Trifluoromethyl)dihydrofuran-2,3,3,4(2*H*)-tetraol (CF_3-DPD), 2



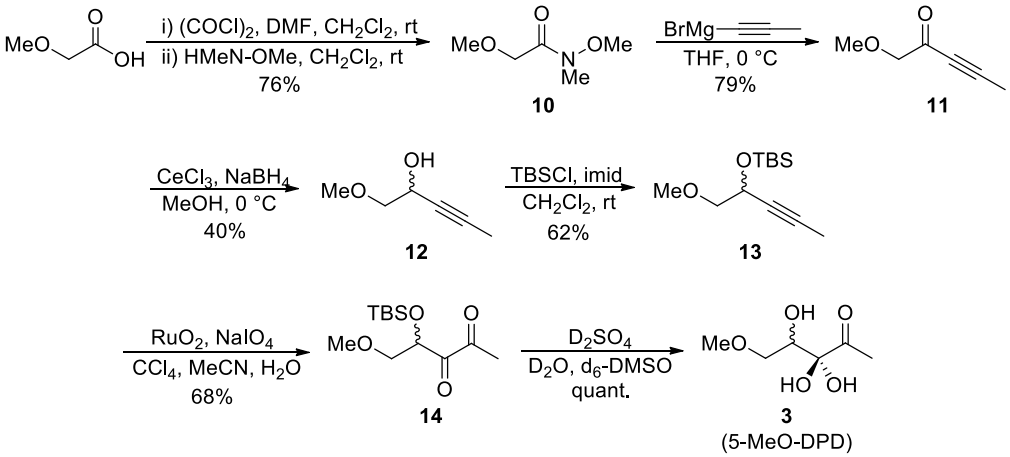
9 **2** (CF_3-DPD)

Diketone **9** (8.52 mg, 32.0 µmol) was dissolved in D_2O (600 µL) and D_2SO_4 (0.4 µL) was added. The reaction mixture was left at rt for 16 h to furnish CF_3-DPD (**2**). The solution was then extracted with $CDCl_3$ (400 µL).[6] Afterwards this solution was analyzed by NMR spectroscopy.

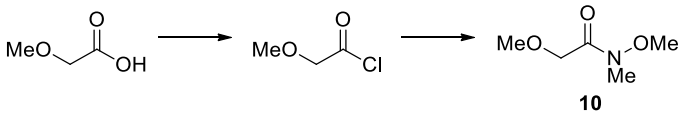
^{1}H NMR (600 MHz, D_2O) δ = 4.29 (t, ^{3}J=6.0, 1H), 4.26-4.24 (m, 2H), 4.17 (t, ^{3}J=5.5, 1H), 3.87 (dd, ^{2}J=9.4, ^{3}J=5.1, 1H), 3.75 (dd, ^{2}J=9.3, ^{3}J=5.7, 1H); ^{13}C NMR (151 MHz, D_2O)

δ = 123.7, 121.9, 100.4, 100.1, 75.6, 74.5, 73.9, 73.1, 71.9, 71.0; ^{19}F NMR (376 MHz, D_2O) δ = -79.3, -79.7. Data is in agreement with the literature.[6]

Synthesis of 5-MeO-DPD (3)



N-2-Dimethoxy-*N*-methylacetamide, 10[7]

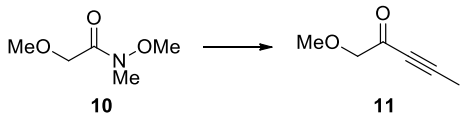


DMF (20 µL) and oxalyl chloride (2.20 mL, 26.0 mmol) were added to a solution of methoxyacetic acid (1.53 mL, 20.0 mmol) in CH_2Cl_2 (24 mL) at 0 °C and the solution stirred for 3 h. The solvent was removed *in vacuo* to give the corresponding acid chloride as a pale yellow oil, which was used without further purification.

HMeNOMe·HCl (2.15 g, 22.0 mmol) and K_2CO_3 (5.53 g, 40.0 mmol) were added to a solution of the crude acid chloride in CH_2Cl_2 (30 mL) and the solution stirred at rt for 18 h before the precipitate was removed by filtration and the solvent removed *in vacuo* to give compound **10** as a pale yellow oil (2.02 g, 15.2 mmol, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.20 (s, CH_2, 2H), 3.67 (s, $NOCH_3$, 3H), 3.45 (s, $CH_2OC\mathit{H}_3$, 3H), 3.18 (s, NCH_3, 3H). Data is in agreement with the literature.[7]

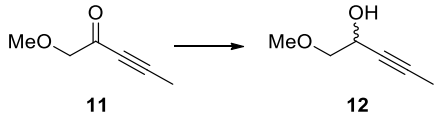
1-Methoxypent-3-yn-2-one, 11



1-Propynylmagnesium bromide (36 mL, 0.5 M in THF, 18.0 mmol) was added to a solution of Weinreb amide **10** (1.30 g, 12.0 mmol) in THF (115 mL) at 0 °C and the solution stirred at 0 °C for 4.5 h. The reaction was quenched with sat. aq. NH_4Cl (100 mL), the organic layer separated and the aqueous layer extracted with Et_2O (2 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to give compound **11** as a yellow oil (1.06 g, 9.45 mmol, 79%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.12 (s, CH_2, 2H), 3.44 (s, OCH_3, 3H), 2.05 (s, ≡-CH_3, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 185.1, 93.4, 78.5, 78.0, 59.6, 4.4; HRMS (ESI+) calculated for $C_6H_9O_2^+$ $(M+H)^+$: 113.0570, found: 113.0601.

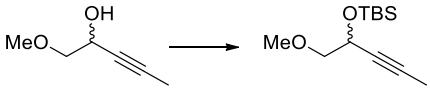
1-Methoxypent-3-yn-2-ol, 12



$CeCl_3{\cdot}7H_2O$ (3.52 g, 9.44 mmol) was added to a solution of ketone **11** (1.00 g, 8.58 mmol) in MeOH (375 mL) at 0 °C. After 2 min, $NaBH_4$ (389 mg, 10.3 mmol) was added portionwise over 2 min, whereupon the solution was allowed to warm to rt and stirred for 3.5 h. The reaction was quenched with sat. aq. NH_4Cl (300 mL), the solution extracted with CH_2Cl_2 (3 × 300 mL), the combined organic layers dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 2:3) gave compound **12** as a yellow oil (392 mg, 3.43 mmol, 40%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.45-4.41 (br m, C*H*OH, 1H), 3.44 (dd, ^2J=9.8, ^3J=3.7, C*H*HOMe, 1H), 3.39 (dd, ^2J=9.8, ^3J=7.4, CH*H*OMe, 1H), 3.35 (s, OCH_3, 3H), 2.96 (br s, OH, 1H), 1.77 (d, ^5J=2.2, ≡-CH_3, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 81.9, 77.1, 76.5, 61.4, 59.1, 3.5; HRMS (ESI+) calculated for $C_6H_{10}NaO_2^+$ $(M+Na)^+$: 137.0573, found: 137.0570.

***tert*-Butyl((1-methoxypent-3-yn-2-yl)oxy)dimethylsilane, 13**

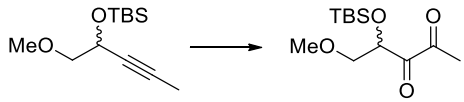


12 **13**

TBSCl (647 mg, 4.29 mmol) was added to a solution of alcohol **12** (196 mg, 1.72 mmol) and imidazole (585 mg, 8.59 mmol) in CH_2Cl_2 (17 mL) and the solution stirred at rt for 16 h. Sat. aq. NH_4Cl (20 mL) and CH_2Cl_2 (10 mL) were added and the aqueous layer extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:9) gave compound **13** as a pale yellow oil (244 mg, 1.07 mmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.48-4.43 (1H, m, CHOSi), 3.45 (dd, ^2J=10.2, ^3J=5.2, C*H*HOMe, 1H), 3.41 (dd, ^2J=10.0, ^3J=6.8, CH*H*OMe, 1H), 3.39 (s, OCH_3, 3H), 1.81 (d, ^5J=2.1, ≡-CH_3, 3H), 0.89 (s, $C(CH_3)_3$, 9H), 0.12 (s, $SiCH_3$, 3H), 0.10 (s, $SiCH_3$, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 81.2, 78.6, 77.4, 63.0, 59.5, 25.9, 18.4, 3.7, -4.5, -4.8; HRMS (ESI+) calculated for $C_{12}H_{25}O_2Si^+$ $(M+H)^+$: 229.1618, found: 229.1610.

4-((*tert*-Butyldimethylsilyl)oxy)-5-methoxypentane-2,3-dione, 14[1-2]

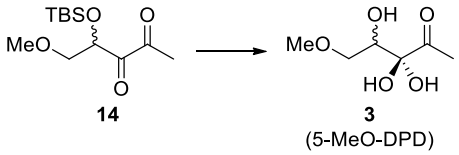


13 **14**

A solution of $NaIO_4$ (194 mg, 906 µmol) in H_2O (1.3 mL) was added to a solution of alkyne **13** (90 mg, 394 µmol) in CCl_4/MeCN (1:1, 1.8 mL) and the solution stirred vigorously at rt under air for 3 min before the addition of $RuO_2 \cdot H_2O$ (3.0 mg, 19.7 µmol). After a further 15 min, the solution was filtered through a silica pad, washing with CH_2Cl_2. Purification by column chromatography eluting with EtOAc/hexane (gradient, 0:1 → 1:9) gave diketone **14** as a yellow oil (70.0 mg, 267 µmol, 68%) with recovered alkyne **13** as a colorless oil (11.2 mg, 49.0 µmol, 12%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.02 (t, ^3J=4.7, CHOSi, 1H), 3.79 (dd, ^2J=10.0, ^3J=5.1, C*H*HOMe, 1H), 3.56 (dd, ^2J=10.1, ^3J=4.1, CH*H*OMe, 1H), 3.31 (s, OCH_3, 3H), 2.32 (s, $CCOCH_3$, 3H), 0.88 (s, $C(CH_3)_3$, 9H), 0.08 (s, $SiCH_3$, 3H), 0.05 (s, $SiCH_3$, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 199.7, 198.9, 74.8, 73.7, 59.6, 25.8, 24.6, 18.4, -4.8, -5.1; HRMS (ESI+) calculated for $C_{12}H_{25}O_4Si^+$ $(M+H)^+$: 261.1517, found 261.1517.

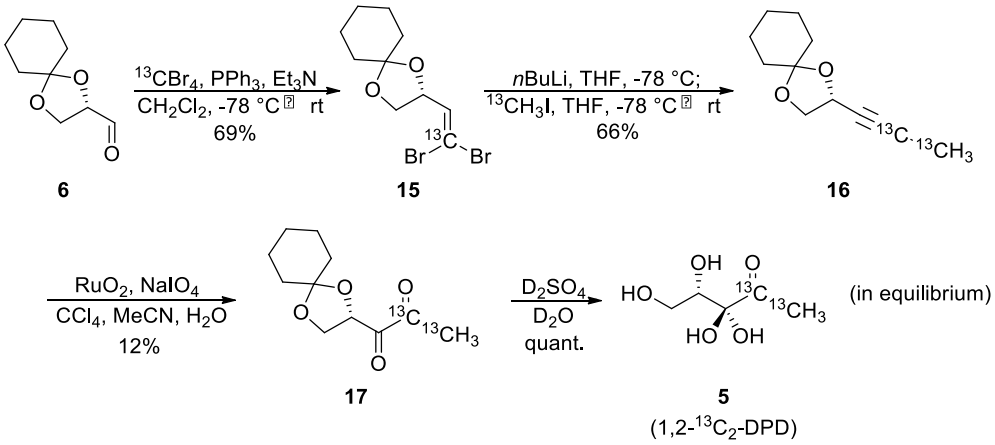
5-MeO-DPD, 3



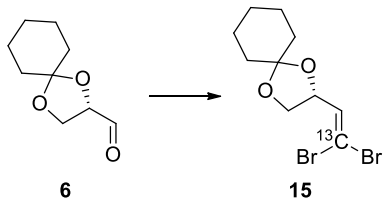
Diketone **14** (10.0 mg, 60.9 µmol) was dissolved in D_2O (600 µL) and D_2SO_4 (6 µL) was added. The reaction mixture was left at rt for 16 h to furnish 5-MeO-DPD (**3**). Afterwards the solution was separated from the solid by decantation and then analyzed by NMR spectroscopy.

^{1}H NMR (600 MHz, D_2O) δ = 4.06 (dd, ^3J=7.2, ^3J=3.6, C*H*OH, 1H), 3.67 (dd, ^2J=10.8, ^3J=3.6, C*H*HOMe, 1H), 3.54 (dd, ^2J=10.8, ^3J=7.2, CH*H*OMe, 1H), 3.36 (s, OCH_3, 3H), 2.35 (s, $CCOCH_3$, 3H); ^{13}C NMR (151 MHz, D_2O) δ = 210.6, 97.1, 72.6, 72.3, 59.1, 25.1.

Synthesis of 1,2-^{13}C-DPD (5)



1-^{13}C-(*R*)-1,1-Dibromo-3,4-(cyclohexylidenedioxy)but-1-ene, 15[2]

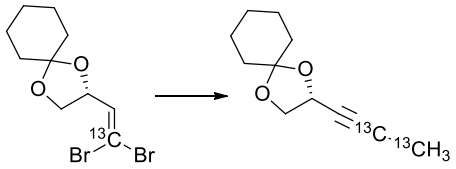


^{13}C-Tetrabromomethane (500 mg, 1.50 mmol) was added to a solution of PPh_3 (795 mg, 3.02 mmol) in CH_2Cl_2 (13 mL) at 0 °C and stirred for 30 min. Et_3N (153 µL, 1.10 mmol) was added and the solution further cooled to -78 °C, before a solution of aldehyde **6** (188 mg, 1.10 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The resulting solution was stirred at

-78 °C for 45 min, before being warmed to rt and stirred for a further 45 min. Sat. aq. $NaHCO_3$ (40 mL) was added to quench the reaction, the organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 × 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (1:50) gave compound **15** as a yellow oil (248 mg, 758 µmol, 69%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.55 (d, ^3J=7.6, O=CH, 1H), 4.74 (m, OCH, 1H), 4.21 (dd, ^2J=8.4, ^3J=6.3, OC*H*HCH, 1H), 3.70 (dd, ^2J=8.4, ^3J=6.5, OC*H*HCH, 1H), 1.70-1.52 (m, cyclohexyl, 8H), 1.46-1.33 (m, cyclohexyl, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 137.6 (d, ^1J=84.9), 110.9, 92.5, 76.1 (d, ^3J=2.4), 67.9 (d, ^2J=4.9), 36.4, 35.3, 25.2, 24.0; HRMS (ESI+) calculated for $C_9{}^{13}CH_{15}Br_2O_2^+$ $(M+H)^+$: 325.9467, found: 325.9468.

4,5-^{13}C-(*R*)-1,2-(Cyclohexylidenedioxy)pent-3-yne, 16[2]

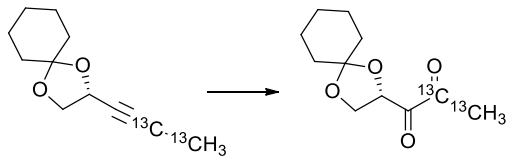


15 **16**

n-BuLi (760 µL, 1.90 mmol, 2.5 M in hexanes) was added dropwise to a solution of compound **15** (248 mg, 758 µmol) in THF (5 mL) at -78 °C and the solution stirred for 1 h, whereupon $^{13}CH_3I$ (284 µL, 4.55 mmol) was added. The solution was stirred at -78 °C for 1 h before being allowed to warm to rt and stirred for a further 2 h. The solvent was removed *in vacuo*, the residue was redissolved in CH_2Cl_2 (15 mL) and washed with H_2O (3 × 20 mL). The combined aqueous layers were extracted with CH_2Cl_2 (25 mL) and the combined organic layer dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by column chromatography eluting with EtOAc/hexane (1:20) gave compound **16** as a colorless oil (91 mg, 499 µmol, 66%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.74-4.57 (m, OCH, 1H), 4.09 (dd, ^2J=7.9, ^3J=6.1, OC*H*HCH, 1H), 3.79 (dd, ^2J=7.9, ^3J=7.0, OCH*H*CH, 1H), 1.83 (dd, $^1J_{CH}$=131.2, $^2J_{CH}$=10.3, $^{13}CH_3$, 3H), 1.66-1.47 (m, cyclohexyl, 8H), 1.46-1.27 (m, cyclohexyl, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 110.7, 82.5 (d, ^1J=67.7), 76.7 (d, ^2J=14.9), 69.9, 65.7, 36.0, 35.6, 25.2, 24.1, 3.9 (d, ^1J=67.8); HRMS (ESI+) calculated for $C_9(^{13}C)_2H_{17}O_2^+$ $(M+H)^+$: 183.1290, found:. 183.1286.

1,2-^{13}C-(*S*)-4,5-(Cyclohexylidenedioxy)-2,3-pentadione, 17[1-2]

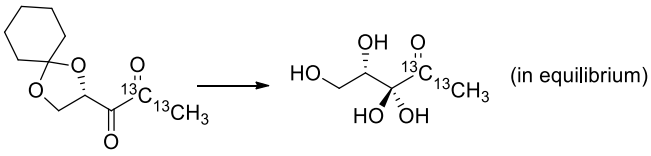


16 **17**

A solution of $NaIO_4$ (113 mg, 52.9 µmol) in H_2O (800 µL) was added to a solution of alkyne **16** (42.6 mg, 240 µmol) in CCl_4/MeCN (1:1, 1.1 mL) and the solution stirred vigorously at rt under air for 3 min before the addition of $RuO_2 \cdot H_2O$ (0.9 mg, 5.80 µmol). After a further 15 min, the solution was filtered through a silica pad, washing with CH_2Cl_2. Purification by column chromatography eluting with EtOAc/hexane (1:10) gave diketone **17** as a yellow oil (6.1 mg, 28.6 µmol, 12%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.13 (dd, ^3J=7.9, ^3J=5.3, OCH, 1H), 4.35 (dd, ^2J=8.9, ^3J=7.9, OC*H*HCH, 1H), 4.00 (dd, ^2J=8.9, ^3J=5.3, OCH*H*CH, 1H), 2.38 (dd, $^1J_{CH}$=130.5, $^2J_{CH}$=7.5, $^{13}CH_3$, 3H), 1.89 – 1.58 (m, cyclohexyl, 8H), 1.55-1.43 (m, cyclohexyl, 2H); ^{13}C NMR (126 MHz, d_6-DMSO) δ = 206.6 (d, ^1J=42.0; ^{13}CO of the minor monohydrated derivative of **17**), 198.4 (d, ^1J=42.5), 193.6, 112.1, 76.7 (d, ^3J=14.9), 65.8, 35.9, 35.1, 26.2, 25.4, 24.7 (d, ^1J=42.5), 24.3 (d, ^1J=42.2; $^{13}CH_3$ of the minor monohydrated derivative of **17**); HRMS (ESI+) calculated for $C_9(^{13}C)_2H_{17}O_4^+$ $(M+H)^+$: 215.1189, found: 215.1190.

1,2-^{13}C-DPD, 5



17 **5**
(1,2-^{13}C-DPD)

Diketone **17** (4.8 mg, 22.4 µmol) was dissolved in D_2O (0.6 mL) and D_2SO_4 (0.5 µL) was added. The reaction mixture was left at rt for 16 h to furnish $^{13}C_2$-DPD (**5**). Afterwards the solution was analyzed by NMR spectroscopy.

^{1}H NMR (600 MHz, DMSO) δ = 4.36 (t, ^3J=6.3, 1H), 4.20 – 4.12 (m, 2H), 4.04 (dt, ^2J=6.0, ^3J=3.0, 1H), 3.95 (dd, ^2J=7.0, ^3J=3.6, 1H), 3.81 (dd, ^2J=10.1, ^3J=3.3, 2H), 3.63 (dd, ^2J=11.8, ^3J=7.5, 1H), 3.55 (ddd, ^2J=9.6, ^3J=5.6, ^3J=4.2, 1H), 2.35 (dd, $^1J_{CH}$=129.1, $^2J_{CH}$=6.1, 1.5H), 1.42 (dd, $^1J_{CH}$=127.9, $^2J_{CH}$=5.0, 3H), 1.39 (dd, $^1J_{CH}$=127.9, $^2J_{CH}$=5.0, 3H); ^{13}C NMR (151 MHz, D_2O) δ = 210.8 (d, ^1J=41.5), 104.4 (d, ^1J=36.4), 104.1 (d, ^1J=37.0), 25.2 (d, ^1J=41.5), 20.5 (d, ^1J=47.2), 20.1 (d, ^1J=47.9). ^{1}H NMR peaks are in agreement with the literature.[2, 8] ^{13}C NMR peaks are only described for the major ^{13}C signals.

NMR titration

The final solutions of DPD (**1**), its derivatives **1a-d**, **2**, **4**, and **5** were extracted with $CDCl_3$ (400 µL) to remove the cyclohexanone byproduct to avoid overlapping ^{1}H NMR signals.[9] In the case of the 5-MeO-DPD derivative **3** we used the solution that still contains the byproduct TBSOH, because the signals are not overlapping with the DPD derivative.

The solutions were titrated stepwise using NaOD (0.1 M or 1 M) or adjusted to pH 7 (1 M NaD_2PO_4/Na_2DPO_4). The pH values were determined using a Micro pH electrode (Fisher Scientific) that can be used in the range of pH 0–14. We calibrated the electrode prior each analysis. Each adjusted step was analyzed by ^{1}H NMR, ^{13}C NMR and where noted we recorded COSY, NOESY, ROESY, HSQC, HMBC and ^{19}F NMR spectra.

NMR quantification

For precise ^{1}H NMR quantification, we determined the relaxation time of DPD (**1**) as 4.03 s using an inversion recovery pulse. Thus, the time between each pulse was increased to t = 20.16 s, which is five times the determined T_1 value. We determined this value for the natural DPD **1** as the molecule with the lowest molecular weight, which requires the highest full spin relaxation time as the described method for NMR quantification.[10-11] Quantification was performed for signals of the protons of C4 and C5 between 3.5–4.4 ppm. The peaks were assigned by 2D NMR (COSY) as it was described in previous reports for DPD (**1**).[2, 8] Important for analysis are the similar ^{1}H NMRs and 2D NMRs of the molecules **1a-d**, which facilitate analysis. In the case of DPD (**1**) and the Et-DPD **1a** we were able to integrate the methyl and ethyl group signals, which were in agreement with the integrated values of the C4 and C5 position.

Supporting Table 1. Quantitative ^{1}H NMR values determined in this study at pH 1.5 that are described and shown in Figure 4.

Derivative	Ratio of both cyclic/linear species
DPD **1**	4.3:1
CF_3-DPD **2**	Only closed
5-MeO-DPD **3**	0
Ethyl-DPD **1a**	1.2:1
Propyl-DPD **1b**	0.8:1
Butyl-DPD **1c**	0.9:1
Hexyl-DPD **1d**	0.8:1

For ^{13}C NMR of the mono-^{13}C-labeled DPD (**4**) and the bis-^{13}C-labeled DPD (**5**) we determined the relaxation time ($T_1 = 10.08$ s) to enable precise quantification using an inversion recovery pulse. We recorded each quantitative NMR spectrum with a delay time of 50.4 s between every scan to ensure total relaxation.

Supporting Table 2. Quantitative ^{13}C NMR values for the pH dependent ratios of both cyclic species **C_1/C_2** over the linear species **E**.

pH	Ratio of species **C_1/C_2** to **E** ^{13}C-DPD (**4**)	Ratio of species **C_1/C_2** to **E** $^{13}C_2$-DPD (**5**)
1	4.3:1	4.3:1
2	4.3:1	n.d.[a]
3	4.3:1	n.d.[a]
4	4.2:1	4.4:1
5	4.4:1	4.5:1
6	4.5:1	4.9:1
7	4.2:1	4.5:1

[a] n.d.: not determined

Supporting Figures

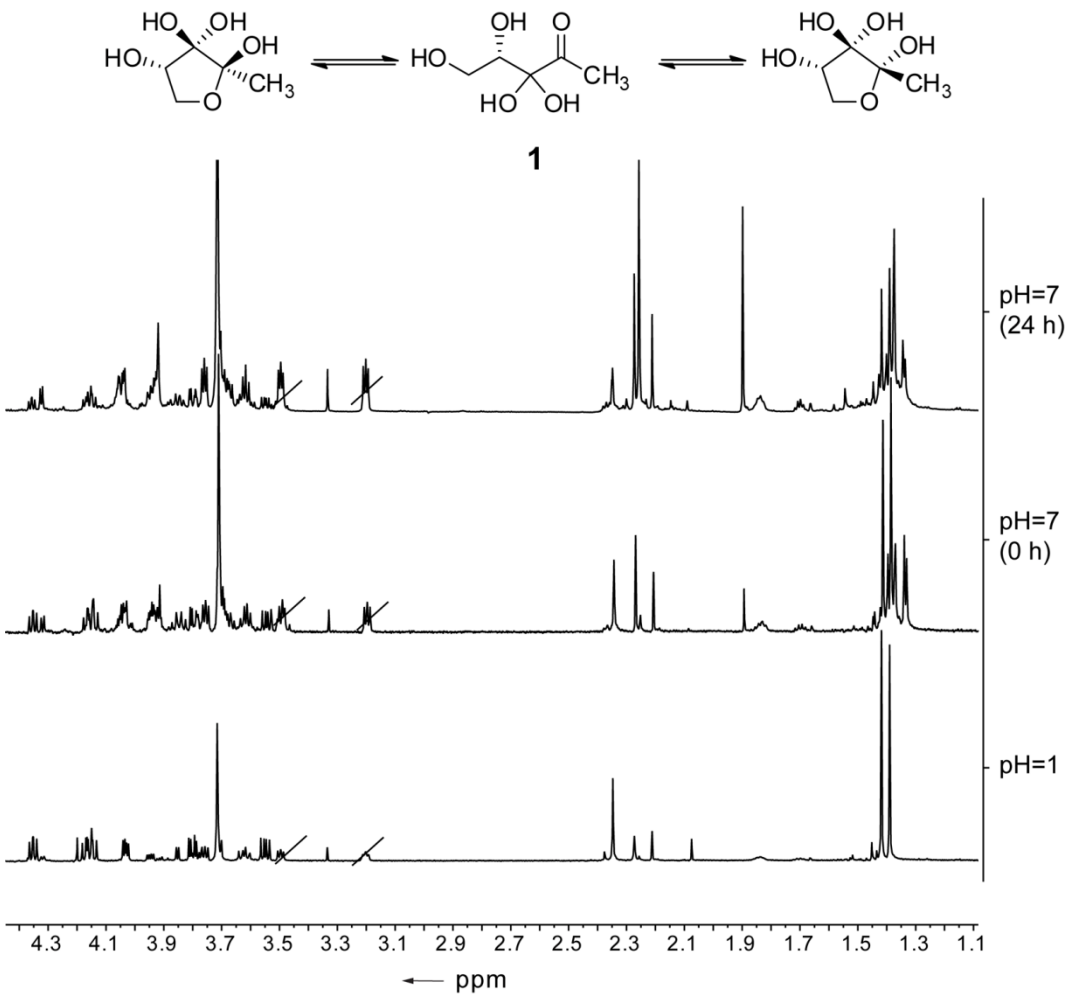


Figure S 1: pH dependent ^{1}H NMR spectra of DPD (**1**). Bottom NMR spectrum: DPD under acidic conditions. Middle NMR spectrum: DPD after buffering to pH 7 (0 h). Top NMR spectrum: DPD after buffering to pH 7 (24 h).

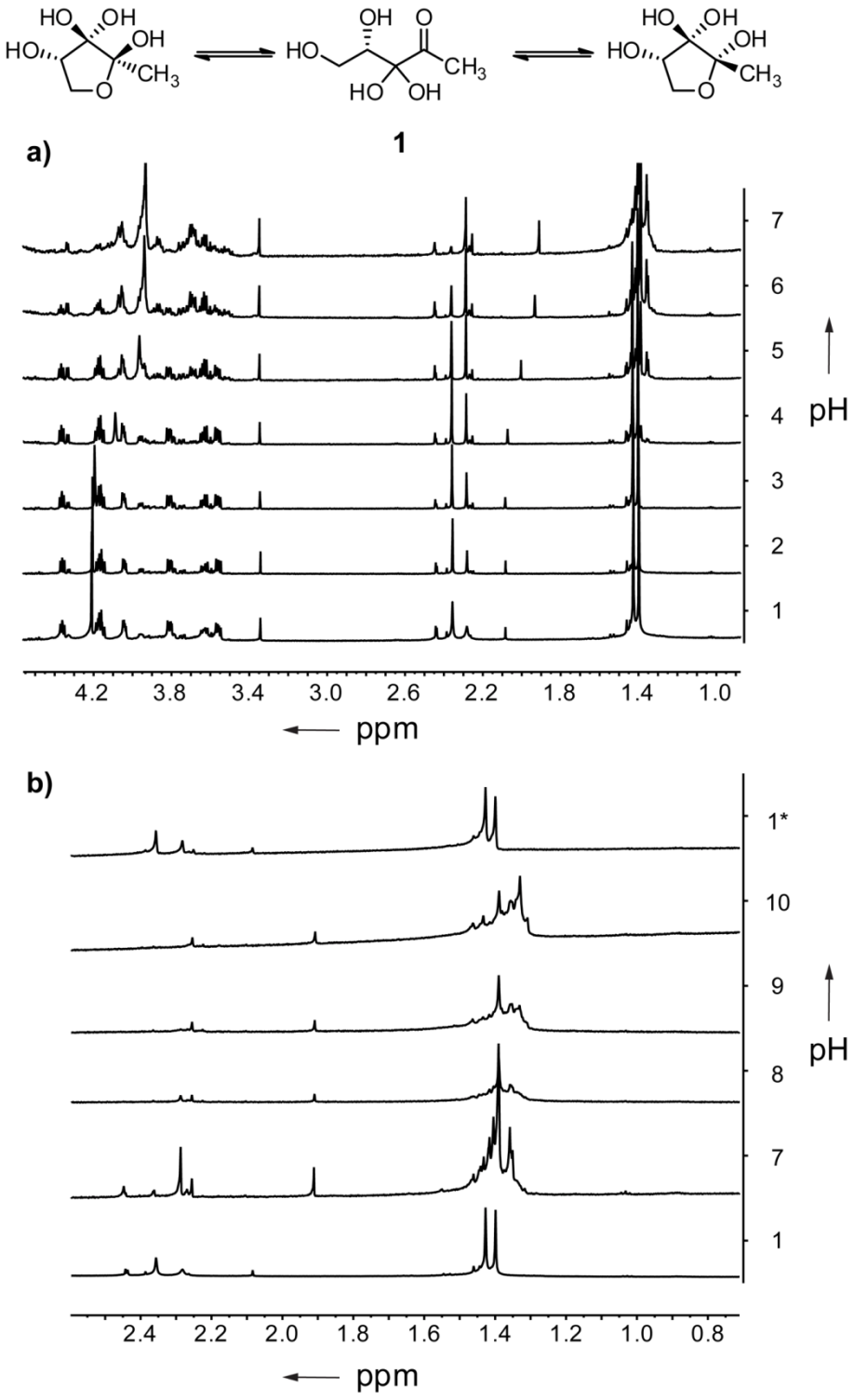


Figure S 2: ^{1}H NMR spectra illustrating the pH dependency of DPD (**1**). a) pH dependency of DPD during stepwise titration with NaOD (0.1 M) in the range of pH 1–7. b) Basic titration in the range of pH 7–10. Asterisk (*) indicates the spectrum after acidification of the spectrum at pH 10.

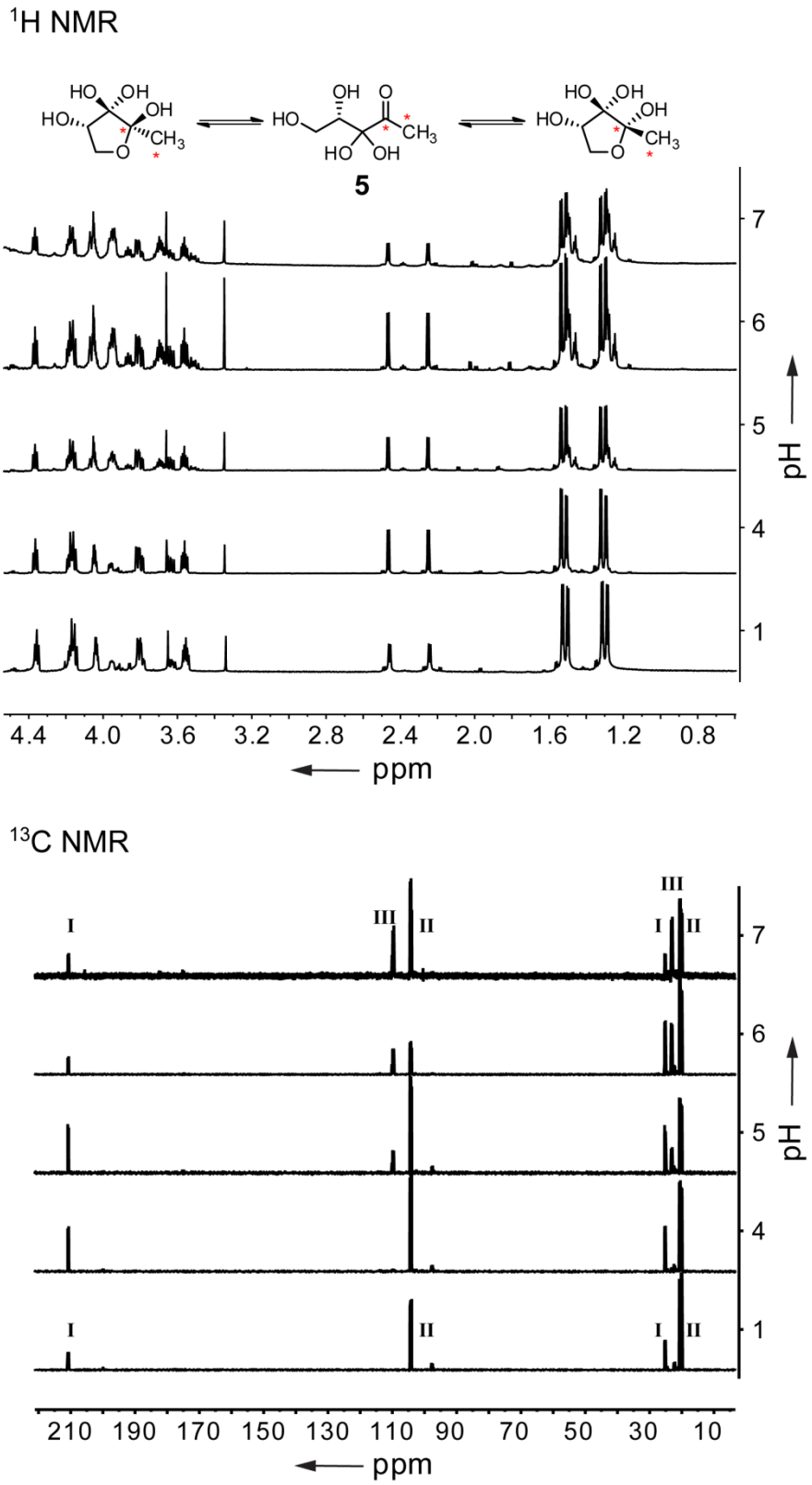


Figure S 3: ^{1}H NMR and full ^{13}C NMR spectra illustrating the pH dependency of the 1,2-bis-^{13}C-labeled DPD (**5**) in the range of pH 1–7.

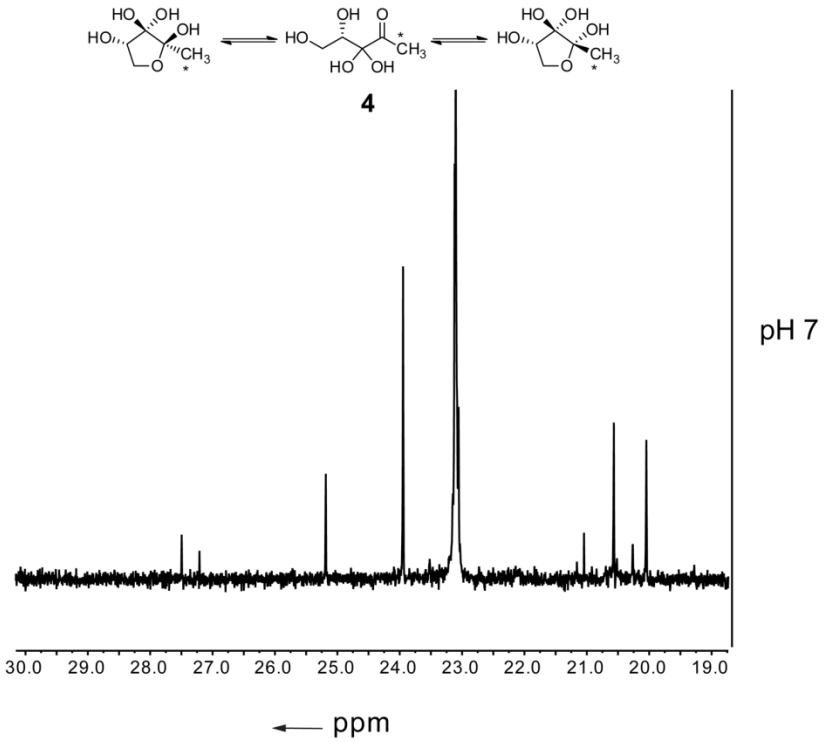


Figure S 4: ^{13}C NMR spectra demonstrating the species diversity of DPD at pH 7 of the mono-^{13}C-labeled DPD derivative **4**.

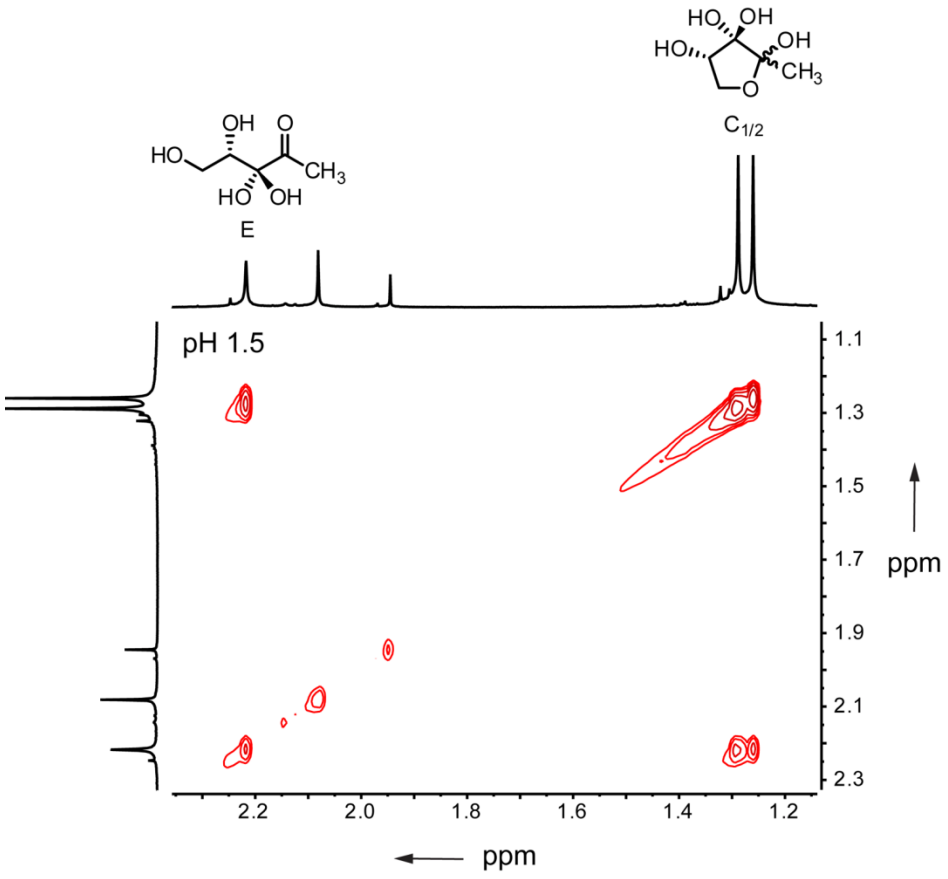


Figure S 5: ^{1}H-^{1}H-NOESY NMR spectrum of DPD (**1**) under acidic conditions illustrating the coupling of the three major DPD signals. This shows that species **E** is in equilibrium with $\mathbf{C_1}$ and $\mathbf{C_2}$.

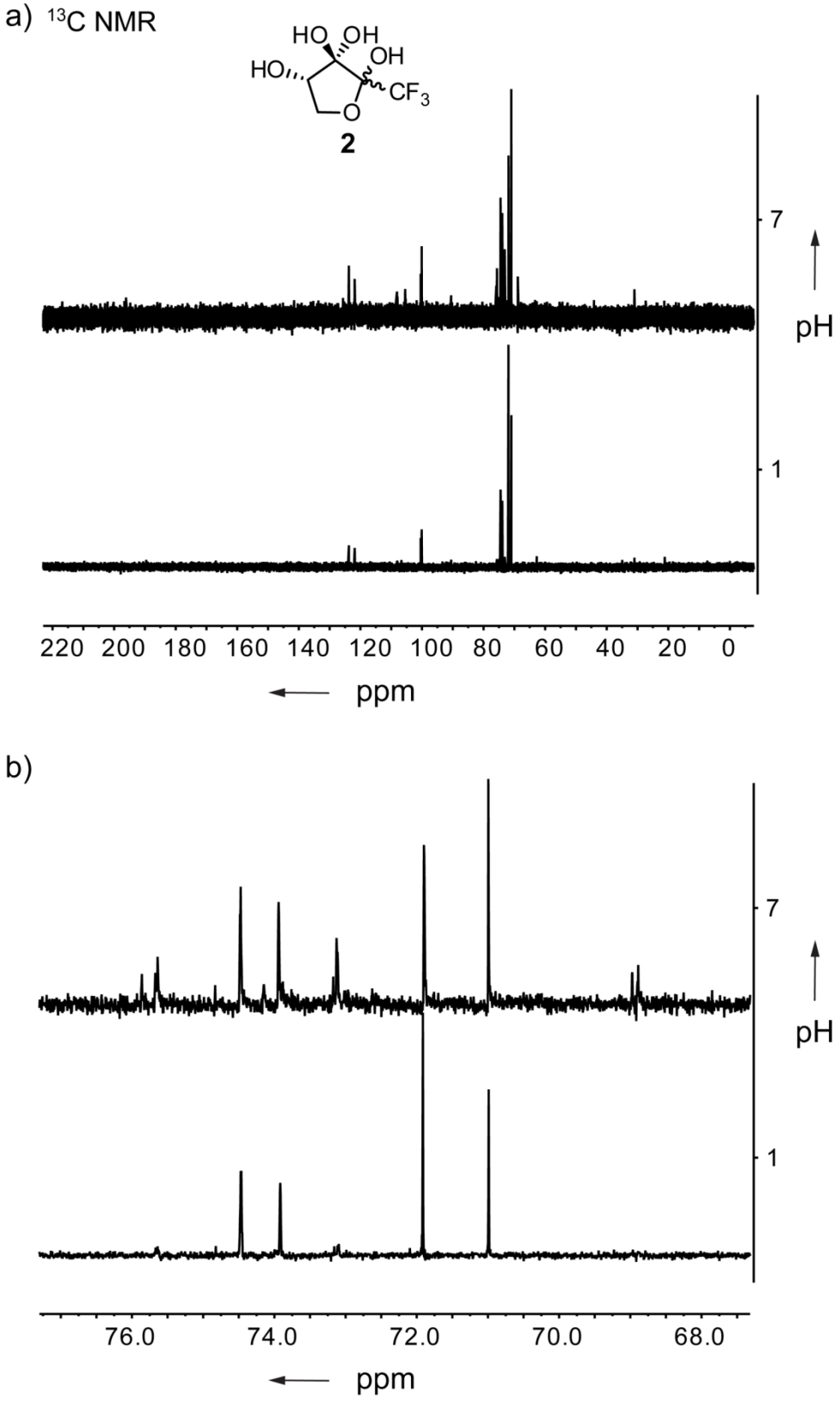


Figure S 6: ^{13}C NMR spectra showing the pH dependent changes of the cyclic DPD derivative CF_3-DPD (**2**) between pH 1 and 7, which are similar to the changes in natural DPD. a) Full spectrum indicating the absence of a ketone. b) Expansion of ^{13}C NMR spectra showing peaks between 68 and 76 ppm.

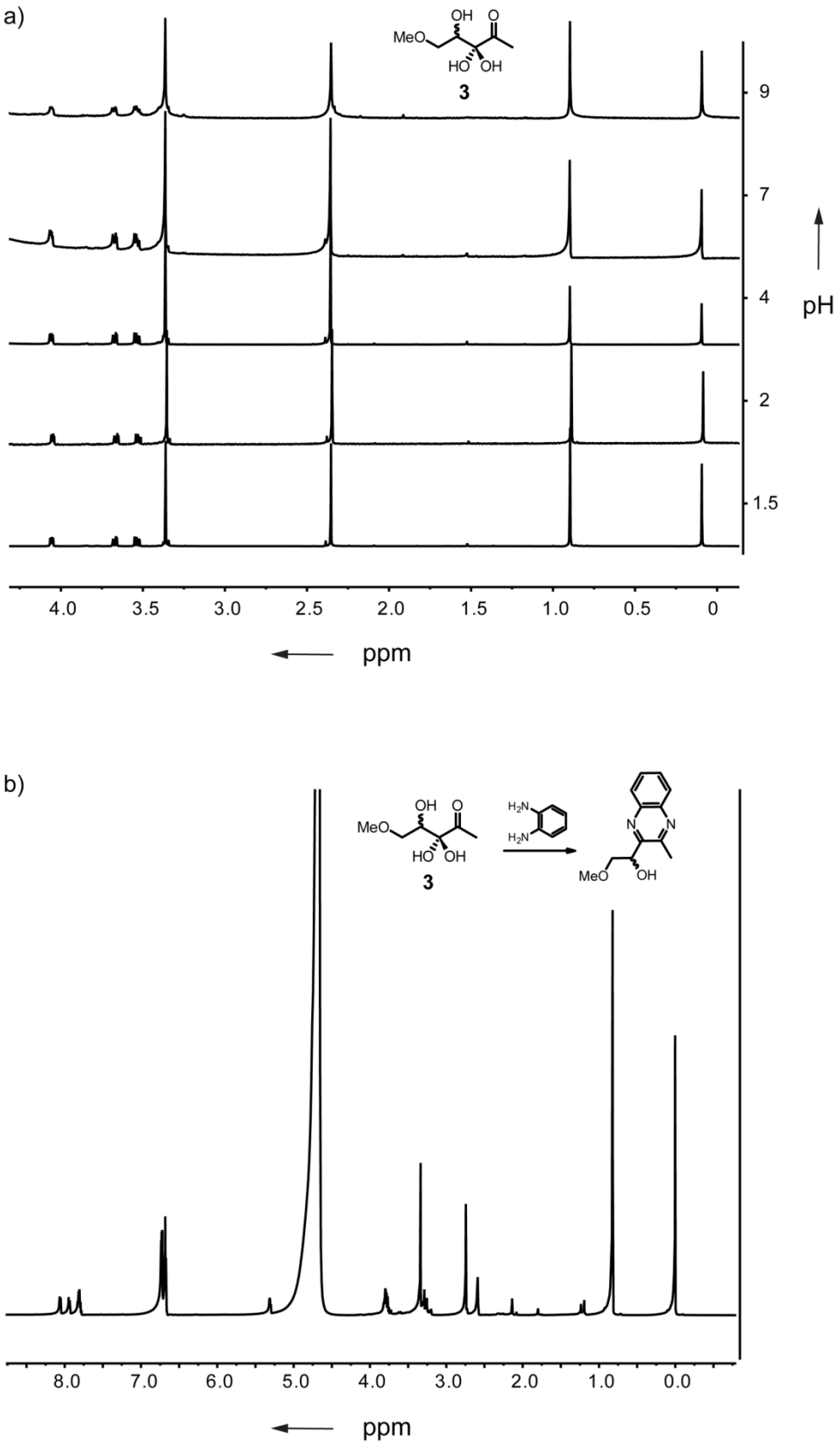
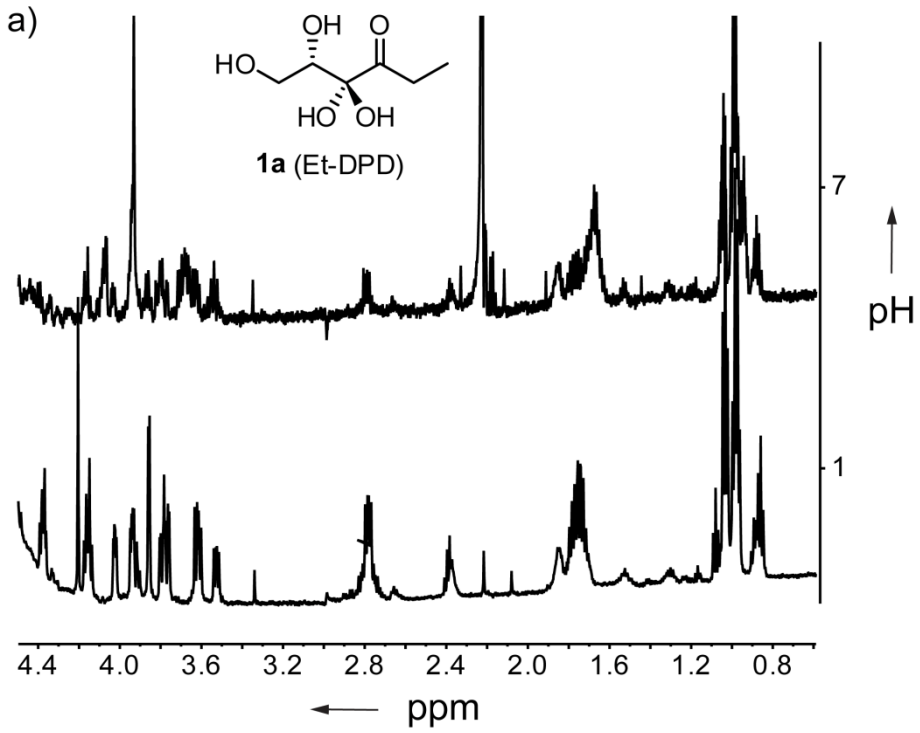
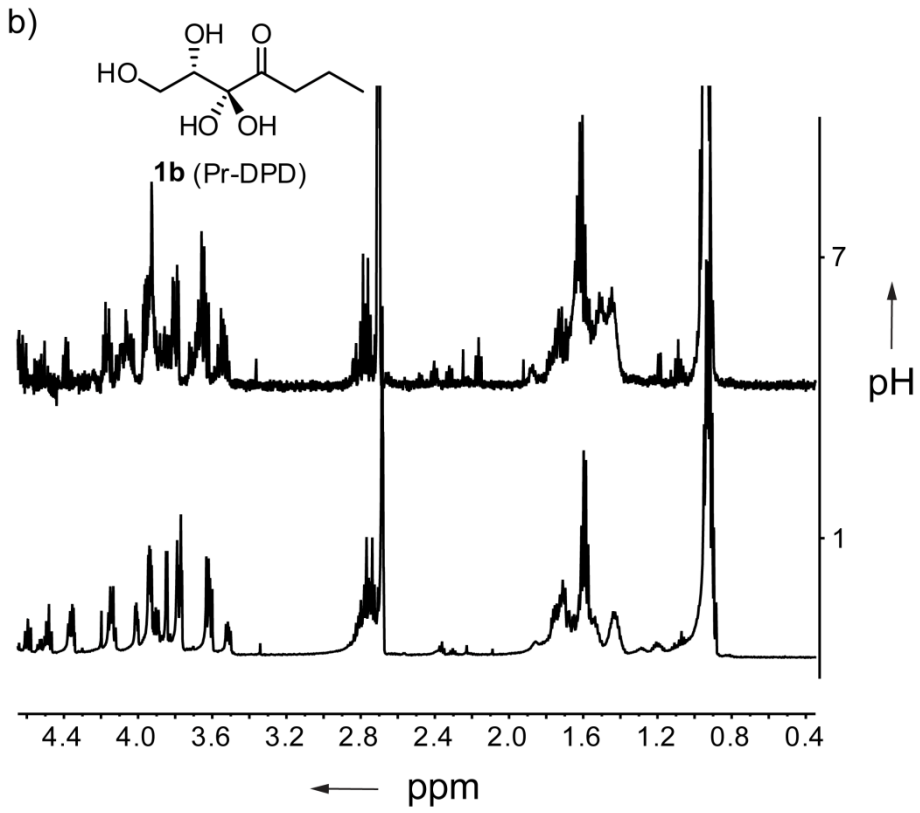


Figure S 7: a) ^{1}H NMR spectra illustrating the pH dependency of the linear model compound 5-MeO-DPD (**3**) in the range of pH 1–9. These spectra demonstrate that derivative **3** is not undergoing structural changes in this pH range. b) ^{1}H NMR spectrum of 5-MeO-DPD (**3**) after treatment with 1,2-phenylenediamine (1.5-fold excess) at pH 6.5.


a)
OH
O
HO
HO
OH
1a (Et-DPD)
7
1
pH
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
ppm


b)
OH
O
HO
HO
OH
1b (Pr-DPD)
7
1
pH
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
ppm

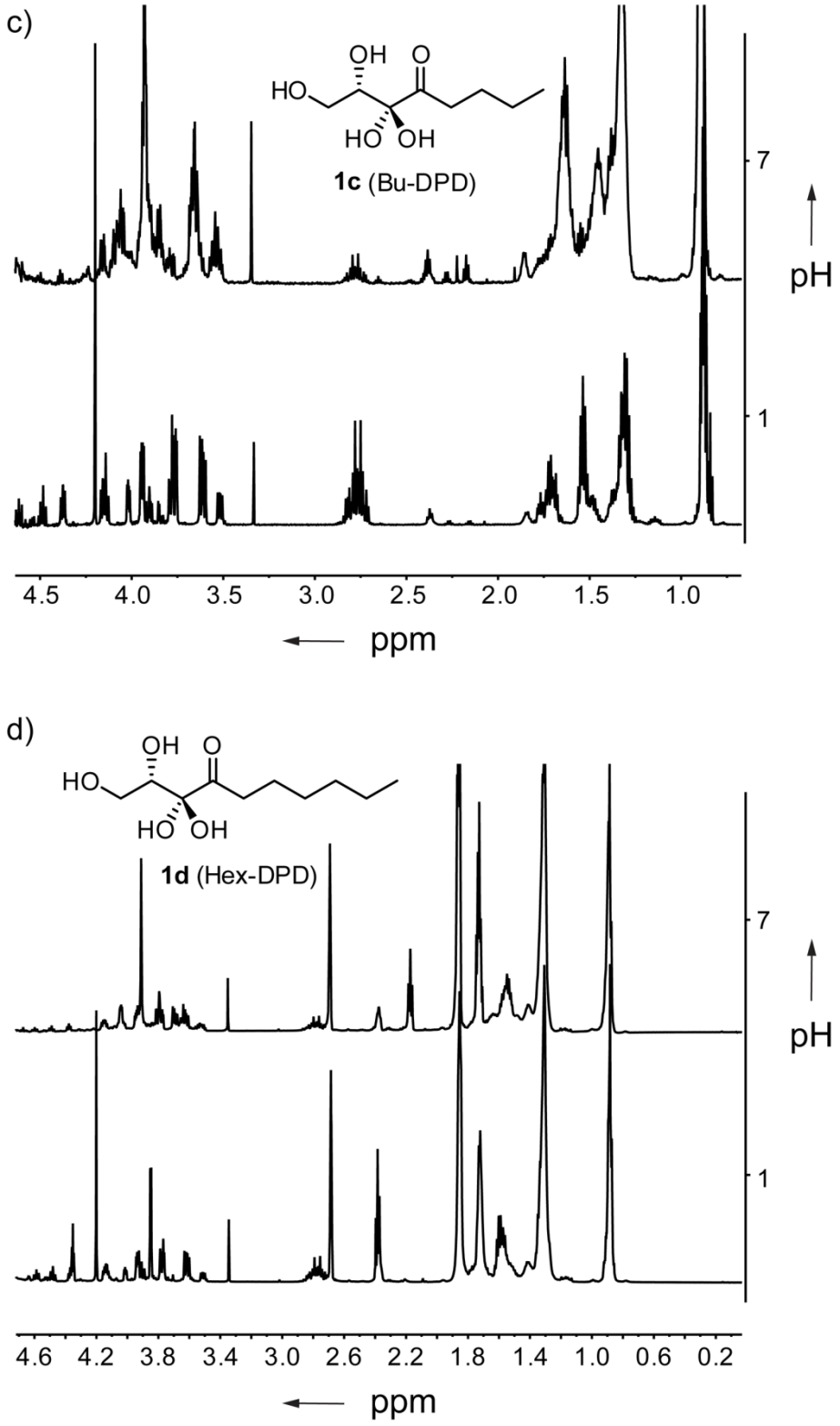


Figure S 8: ^{1}H NMR spectrum differences of C1-alkyl analogues of DPD (**1a**-**d**) at pH 1 and 7.

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