Electronic Supplementary Information

A Photocleavable Linker for the Chemoselective Functionalization of Biomaterials

Liz O'Donovan and Paul A. De Bank*

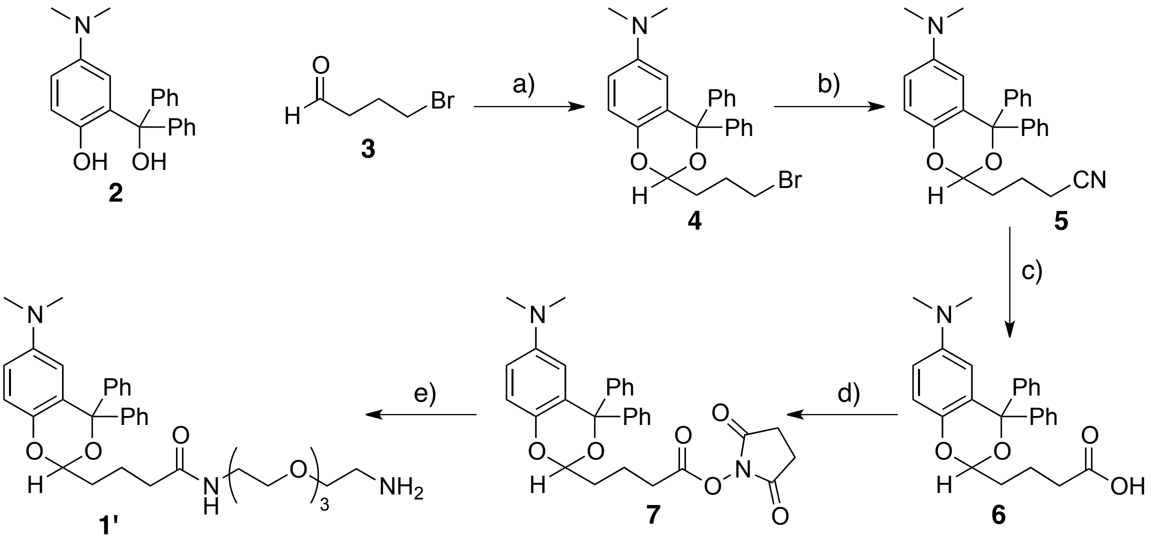
Department of Pharmacy and Pharmacology and Centre for Regenerative Medicine, University of Bath, Bath BA2 7AY, UK.

**Email: p.debank@bath.ac.uk; Fax: +44 (0) 1225 384017; Tel: +44 (0) 1225 384017*

General Experimental

All reagents were purchased from Sigma-Aldrich (Dorset, U.K.) and used without further purification unless otherwise specified. All reactions were carried out in freshly dried and distilled solvents under a dry nitrogen atmosphere apart from those involving aqueous solutions. NMR spectra were recorded on Bruker Avance III 400 MHz or 500 MHz NMR spectrometers. Data are expressed in parts per million downfield from $SiMe_4$ as an internal standard or relative to $CHCl_3$. NMR assignments were supported by ^{1}H-^{13}C and ^{1}H-^{1}H NMR 2D spectra and DEPT for compound **4-7** and caged aldehyde **1'**. J vales are given in Hz. IR spectra were measured on a Perkin Elmer Spectrum RXI FT-IR spectrophotometer and reported as cm^{-1}. Mass spectra were obtained using Bruker micrOTOF spectrometers in electrospray positive ion mode.

Reaction Scheme



a) $Bi(OTf)_3$, toluene, 80 °C; b) NEt_4CN, MeCN, reflux; c) KOH, 2-methoxyethanol, reflux; d) *N*-hydroxysuccinimide, *N*,*N'*-diisopropylcarbodiimide, CH_2Cl_2, rt; e) $H_2N(CH_2CH_2O)_3(CH_2)_2NH_2$ (**8**), CH_2Cl_2 rt.

Synthesis

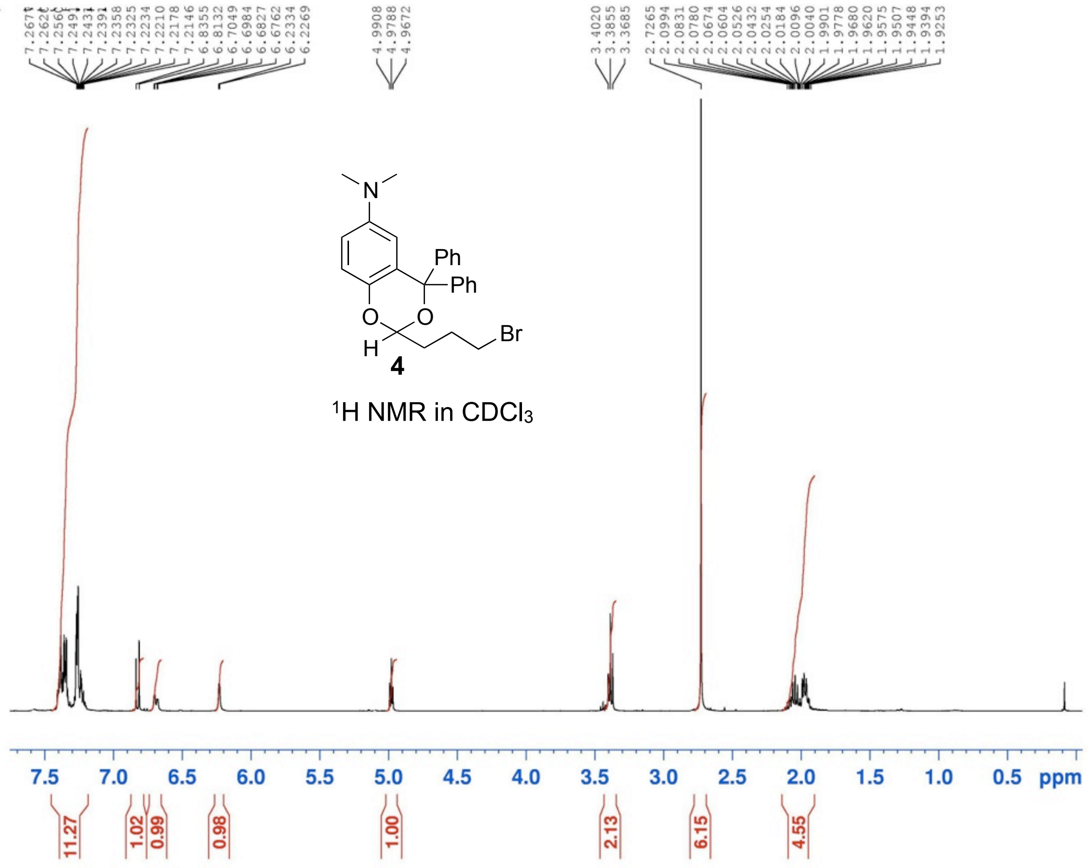
4-(Dimethylamino)-2-(hydroxydiphenylmethyl)phenol (**2**) was prepared according to the synthetic route described by Wang *et al.*[1]

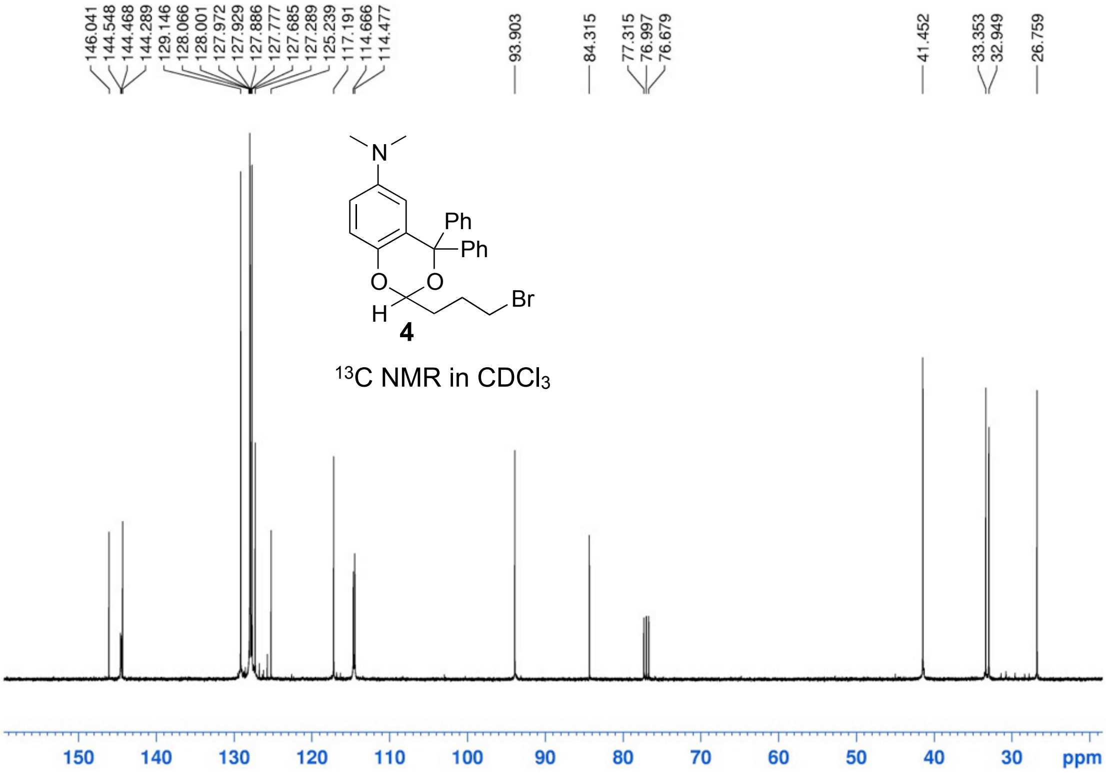
4-Bromobutanal (**3**)

DMSO (0.50 mL, 6.44 mmol) in dichloromethane (4 mL) was added to oxalyl chloride (0.41 mL, 4.57 mmol) in dichloromethane (4 mL) at -78 °C. After 5 minutes a solution of 4-bromo-1-butanol (0.68 g, 4.44 mmol) in dichloromethane (8 mL) was added to the mixture and after 10 minutes, *N,N*-diisopropylethylamine (2.50 mL, 14.33 mmol) was added. The solution was stirred at -65 °C for 15 minutes and for an additional 15 minutes at room temperature, poured into 10% citric acid and extracted with dichloromethane (3 x 30 mL). The organic layer was washed with saturated $NaHCO_3$ (30 mL) and dried over $MgSO_4$. The solution was evaporated to dryness and the residue purified by silica column chromatography (1:4 dichloromethane/hexane) to yield compound **3** (0.54 g, 80%) as a colourless oil; R_f 0.45 (1:1 dichloromethane/hexane); δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.15-2.22 (2 H, quintet, CH_2), 2.65-2.68 (2 H, m, CH_2), 3.44-3.47 (2 H, t, $J = 6.0$, CH_2), 9.81 (1 H, s, CHO). Spectroscopic data are consistent with those described in the literature.[2]

*2-(3-Bromopropyl)-*N,N*-dimethyl-4,4-diphenyl-*4H*-benzo*[d]*[1,3]dioxin-6-amine* (**4**)

Photolabile protecting group **2** (0.70 g, 2.20 mmol), 4-bromobutanal **3** (1.00 g, 6.60 mmol), Bismuth (III) trifluromethanesulfonate (0.015 g, 0.023 mmol), in toluene (8 mL) were heated at 80 ºC under nitrogen for 72 hr. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution (10 ml) and extracted with EtOAc (2 x 20 mL). The organic extracts were combined and dried ($MgSO_4$). The solution was evaporated to dryness and the residue purified by silica column chromatography (0.5:9.5 EtOAc/hexane) to yield compound **4** as a colourless oil (0.80 g, 81%); R_f 0.48 (0.5:9.5 EtOAc/hexane); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.93-2.10 (4 H, m, $(CH_2)_2$), 2.73 (6 H, s, 2 x CH_3), 3.37-3.40 (2 H, t, J_3 6.7, CH_2Br), 4.97-4.99 (1 H, t, J_3 4.7, CH), 6.22-6.23 (1 H, d, J_5 2.6, Ar-H), 6.68-6.70 (1 H, dd, J_3 8.9, J_5 2.6, Ar-H), 6.81-6.84 (1 H, d, J_3 8.9, Ar-H), 7.21-7.48 (10 H, m, Ph-H); δ_C (300 MHz; $CDCl_3$; Me_4Si) 26.8 (CH_2), 32.9 (CH_2), 33.4 (CH_2), 41.5 (2 x CH_3), 84.3 (C), 93.9 (CH), 114.5 (Ar-CH), 114.7 (Ar-CH), 117.2 (Ar-CH), 125.2, 127.3, 127.7, 127.8, 127.9, 128.0, 129.1 (all Ph-CH), 144.3, 144.5, 144.6, 146.0 (*ipso*-C); ν_{max}/cm^{-1} 3468, 3417, 1650, 1501, 1439; HRMS (ESI): found MH^+, 452.1208 ($C_{25}H_{27}BrNO_2$ requires 452.1225); m/z (ES1) 452 ($C_{25}H_{27}BrNO_2$, 45%), 302 (100), 289 (8).



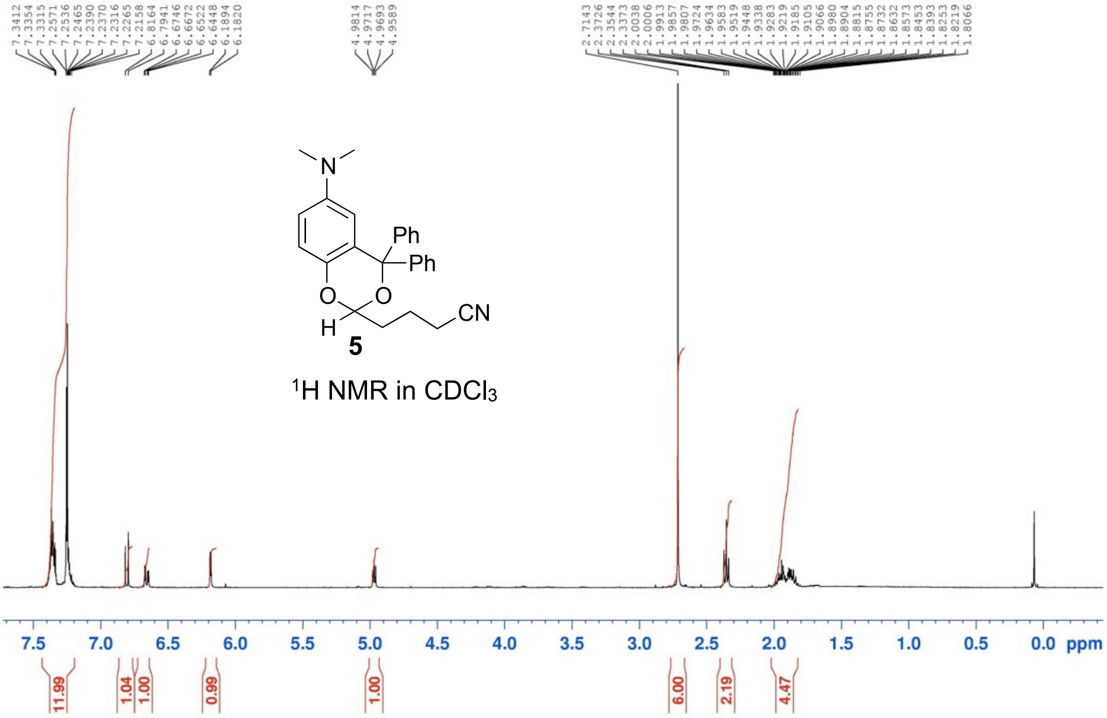


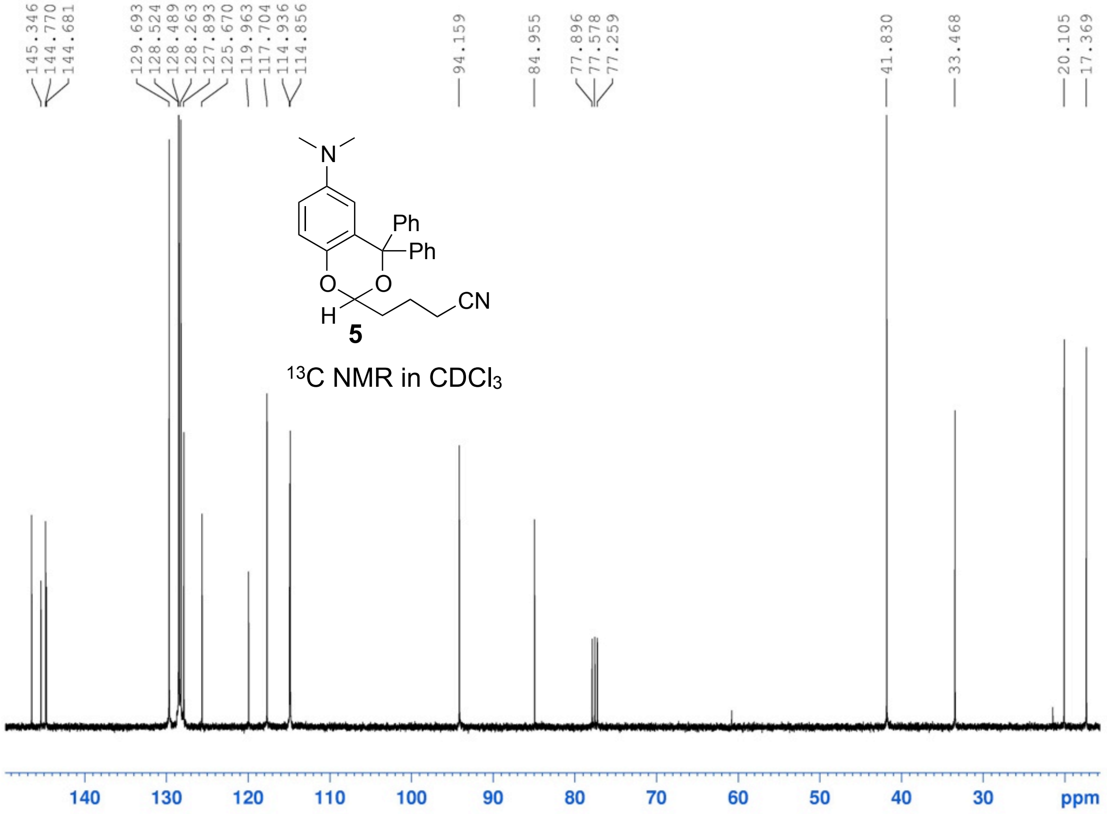
*4-(6-(Dimethylamino)-4,4-diphenyl-*4H*-benzo[*d*][1,3]dioxin-2-yl)butanenitrile* (**5**)

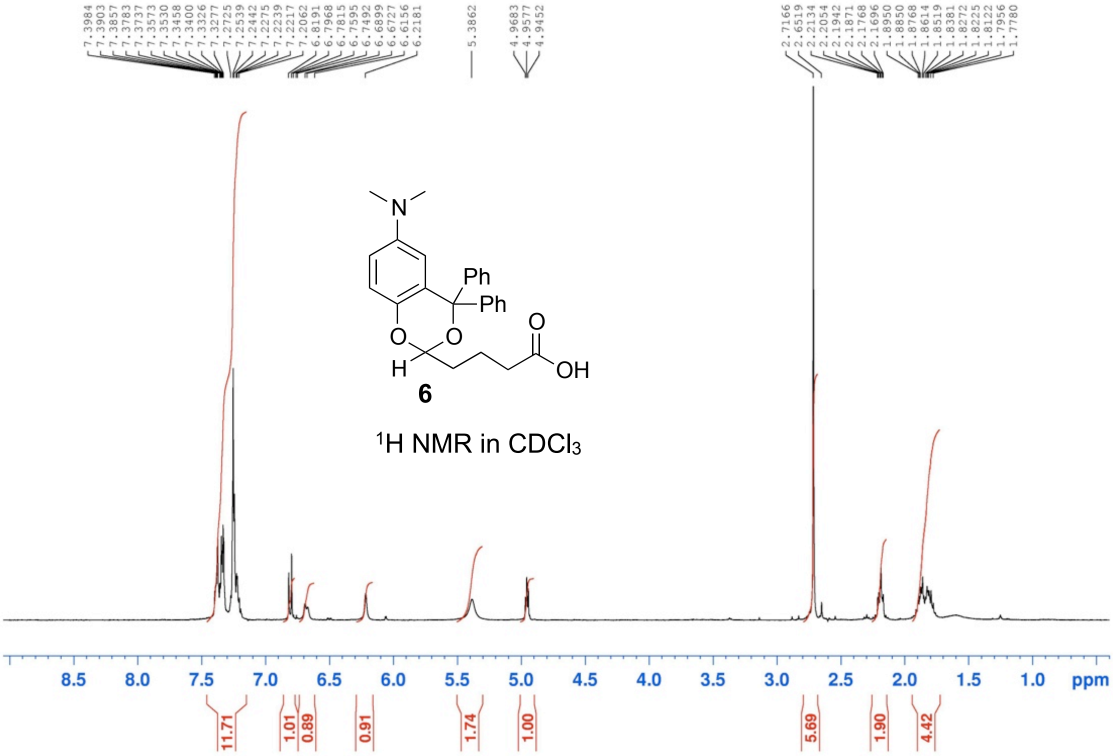
Bromide **4** (0.130 g, 0.29 mmol) and tetraethylammonium cyanide (NEt$_4$CN) (0.067 g, 0.426 mmol) were dissolved in MeCN (15 mL). The solution was heated at reflux for 5.5 hr, cooled and reduced to dryness. The resultant orange residue was purified by silica column chromatography (2:8 EtOAc/hexane) to yield compound **5** as a colourless oil (0.11 g, 96%) R_f 0.5 (2:3 EtOAc/hexane); δ_H (400 MHz; CDCl$_3$; Me$_4$Si) 1.81-2.00 (4 H, m (CH$_2$)$_2$), 2.34-2.37 (2 H, t, J_3 7.0, CH$_2$CN), 2.71 (6 H, s, 2 x CH$_3$), 4.96-4.98 (1 H, t, J_3 4.5, CH), 6.18-6.19 (1 H, d, J_5 3.0, Ph-H), 6.64-6.67 (1 H, dd, J_3 9.0, J_5 3.0, Ph-H), 6.79-6.82 (1 H, d, J_3 9.0, Ph-H), 7.22-7.44 (10 H, m Ph-H); δ_C (300 MHz; CDCl$_3$; Me$_4$Si) 17.4 (CH$_2$), 20.1 (CH$_2$), 33.5 (CH$_2$), 41.8 (2 x CH$_3$), 85.0 (CH), 94.2 (C), 114.9 (Ar-CH), 117.7 (Ar-CH), 119.0 (C), 125.7, 127.9, 128.3, 128.5, 128.5, 128.7, 144.7, 144.8, 145.3 (all Ar-CH); ν_{max}/cm^{-1} 2923, 1623, 1516, 1446, 1238; HRMS (ESI): found MH$^+$, 399.2076 ($C_{26}H_{27}N_2O_2$ requires 399.2073); m/z (ES1) 399 ($C_{26}H_{27}N_2O_2$, 73%), 302 (100), 288 (55).

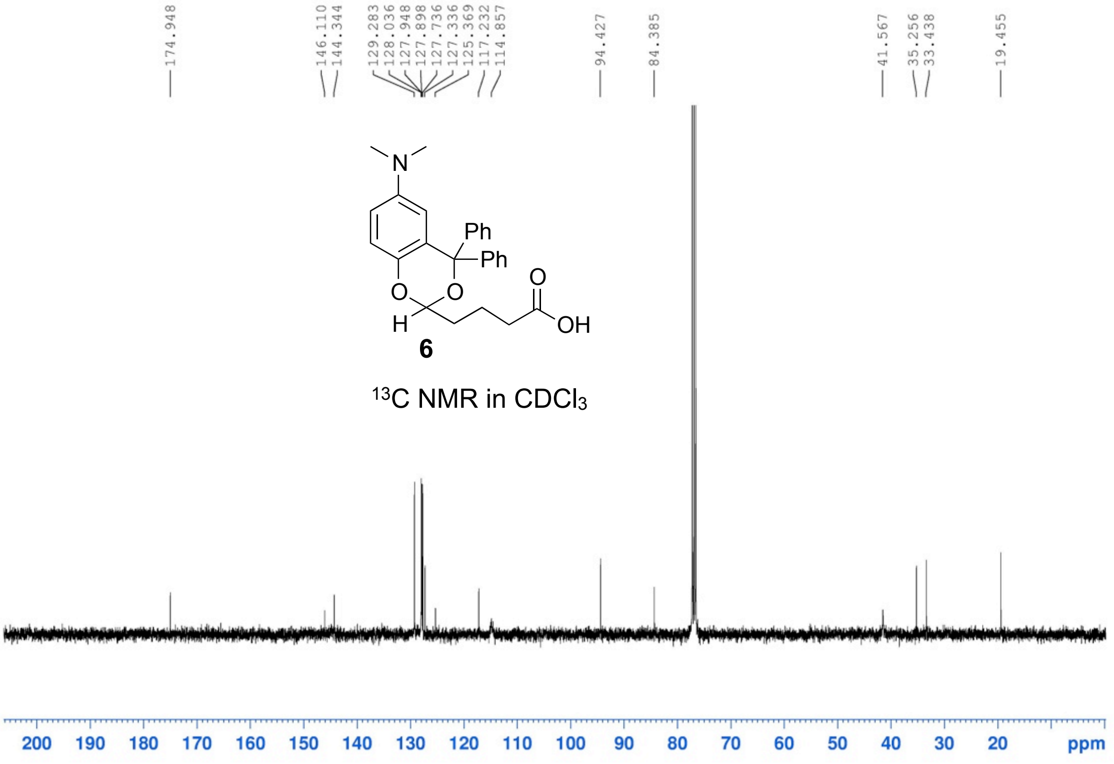
*4-(6-(Dimethylamino)-4,4-diphenyl-*4H*-benzo[*d*][1,3]dioxin-2-yl)butanoic acid* (**6**)

Nitrile **5** (0.30 g, 0.75 mmol), was dissolved in a saturated solution of KOH in 2-methoxy methanol (2 mL). The solution was heated at reflux for 18 hr, cooled and HCl (2 M) was added dropwise until the pH was adjusted to 2. The solution was extracted with CH$_2$Cl$_2$ (2 x 20 mL), the organic extracts were combined, dried over MgSO$_4$ and reduced to dryness to yield compound **6** as a brown oil (0.28 g, 89%); R_f 0.2 (1:1 EtOAc/hexane); δ_H (400 MHz; CDCl$_3$; Me$_4$Si) 1.78-1.86 (4 H, m (CH$_2$)$_2$), 2.17-2.21 (2 H, m, CH$_2$CO), 2.71 (6 H, s, 2 x CH$_3$), 4.95-4.97 (1 H, t, J_3 4.6, CH), 5.35-5.39 (1 H, br s, OH), 6.19-6.21 (1 H, br s, Ph-H), 6.61-6.68 (1 H, m, Ph-H), 6.75-6.82 (1 H, m, Ph-H), 7.20-7.40 (10 H, m Ph-H); δ_C (300 MHz; CDCl$_3$; Me$_4$Si) 19.5 (CH$_2$), 33.4 (CH$_2$), 35.3 (CH$_2$), 41.6 (2 x CH$_3$), 84.4 (C), 94.4 (CH), 114.9 (Ar-CH), 117.2 (Ar-CH), 125.4 (C), 127.3, 127.7, 127.8, 127.9, 128.0, 129.3, 144.3, 146.1, 174.9 (C=O); ν_{max}/cm^{-1} 3074, 1706, 1565, 1367; HRMS (ESI): found MH$^+$, 418.2013 ($C_{26}H_{28}NO_4$ requires 418.2018); m/z (ES1) 418 ($C_{26}H_{28}NO_4$, 100%), 302 (67.9), 217 (37).


7.3412
7.3354
7.3315
7.2571
7.2536
7.2465
7.2390
7.2370
7.2316
7.2265
7.2158
6.8164
6.7941
6.6746
6.6672
6.6522
6.6448
6.1894
6.1820
4.9814
4.9717
4.9693
4.9589
2.7143
2.3726
2.3544
2.3373
2.0038
2.0006
1.9913
1.9857
1.9807
1.9724
1.9634
1.9583
1.9519
1.9448
1.9338
1.9283
1.9219
1.9185
1.9105
1.9066
1.8980
1.8904
1.8815
1.8755
1.8732
1.8632
1.8573
1.8453
1.8393
1.8253
1.8219
1.8066
N
Ph
Ph
O
O
H
CN
5
¹H NMR in CDCl₃
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
11.99
1.04
1.00
0.99
1.00
6.00
2.19
4.47


145.346
144.770
144.681
129.693
128.524
128.489
128.263
127.893
125.670
119.963
117.704
114.936
114.856
94.159
84.955
77.896
77.578
77.259
41.830
33.468
20.105
17.369
N
Ph
Ph
O
O
H
CN
5
¹³C NMR in CDCl₃
140
130
120
110
100
90
80
70
60
50
40
30
ppm

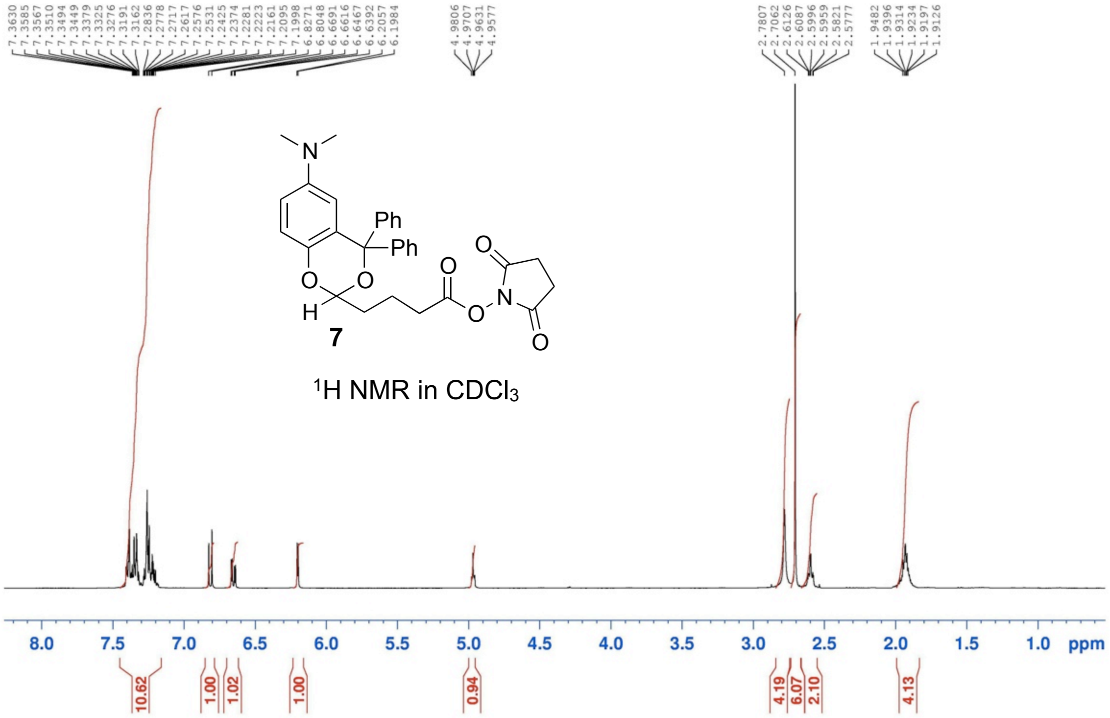


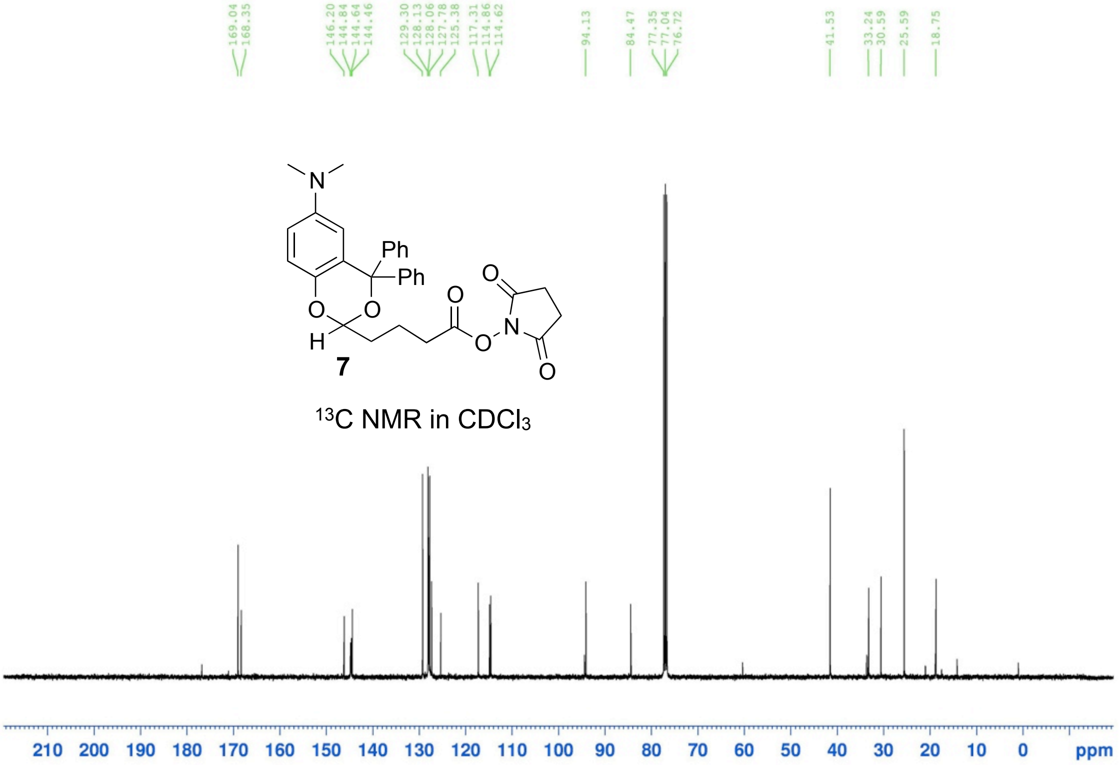


*2,5-Dioxopyrrolidin-1-yl 4-(6-(dimethylamino)-4,4-diphenyl-*4H-*benzo[*d*][1,3]dioxin-2-yl) butanoate* (**7**)

To a solution of acid **6** (0.20 g, 0.479 mmol) and *N*-hydroxysuccinimide (0.066 g 0.574 mmol) in anhydrous CH_2Cl_2 (2 mL) was added *N,N'*-diisopropylcarbodiimide (0.073 g, 0.575 mmol) in anhydrous CH_2Cl_2 (2 mL). The solution was stirred for 72 hr at room temperature and quenched with brine. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), the organic layers were combined and dried over $MgSO_4$. The solution was evaporated to dryness and the residue purified by silica column chromatography (1:1 EtOAc/hexane) to yield compound **7** as a yellow oil (0.234 g, 95%); R_f 0.60 (1:1 EtOAc/hexane); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.91-1.95 (4 H, m, $(CH_2)_2$), 2.58-2.61 (2 H, m, CH_2), 2.71 (6 H, s, 2 x CH_3), 2.78 (4 H, s, $(CH_2)_2$), 4.96-4.98

(1 H, t, J_3 3.1, CH), 6.20-6.21 (1 H, d, J_5 2.9, Ar-H), 6.65-6.67 (1 H, dd, J_3 8.9, J_5 2.9, Ar-H), 6.81-6.83 (1 H, d, J_3 8.9, Ar-H), 7.21-7.44 (10 H, m, Ph-H); δ_C (300 MHz; CDCl$_3$; Me$_4$Si) 18.8 (CH$_2$), 25.6 (2 x *C*H$_2$C=O), 30.6 (CH$_2$), 33.2 (CH$_2$), 41.5 (2 x CH$_3$), 84.5 (C), 94.1 (CH), 114.6, 114.9, 117.3, 125.4, 127.8, 128.0, 128.1, 129.3, 144.5, 144.6, 146.2 (all Ph-CH), 168.4 (C=O), 169.0 (2 x NC=O); ν_{max}/cm^{-1} 3010, 1711, 1652, 1426, 1135 HRMS (ESI): found MH$^+$, 515.2197 (C$_{30}$H$_{31}$N$_2$O$_6$ requires 515.2182); m/z (ES1) 537 (C$_{30}$H$_{30}$NaN$_2$O$_6$, 1%), 515 (C$_{30}$H$_{31}$N$_2$O$_6$, 83), 418 (37.5), 302 (100).

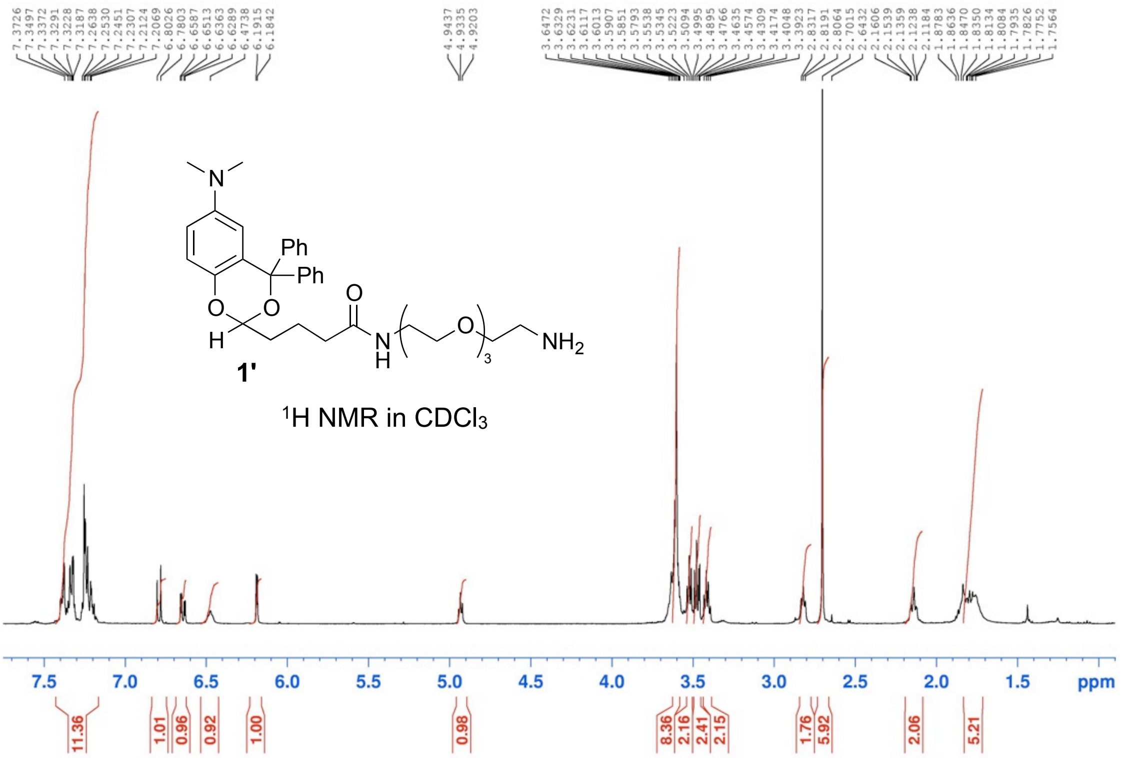


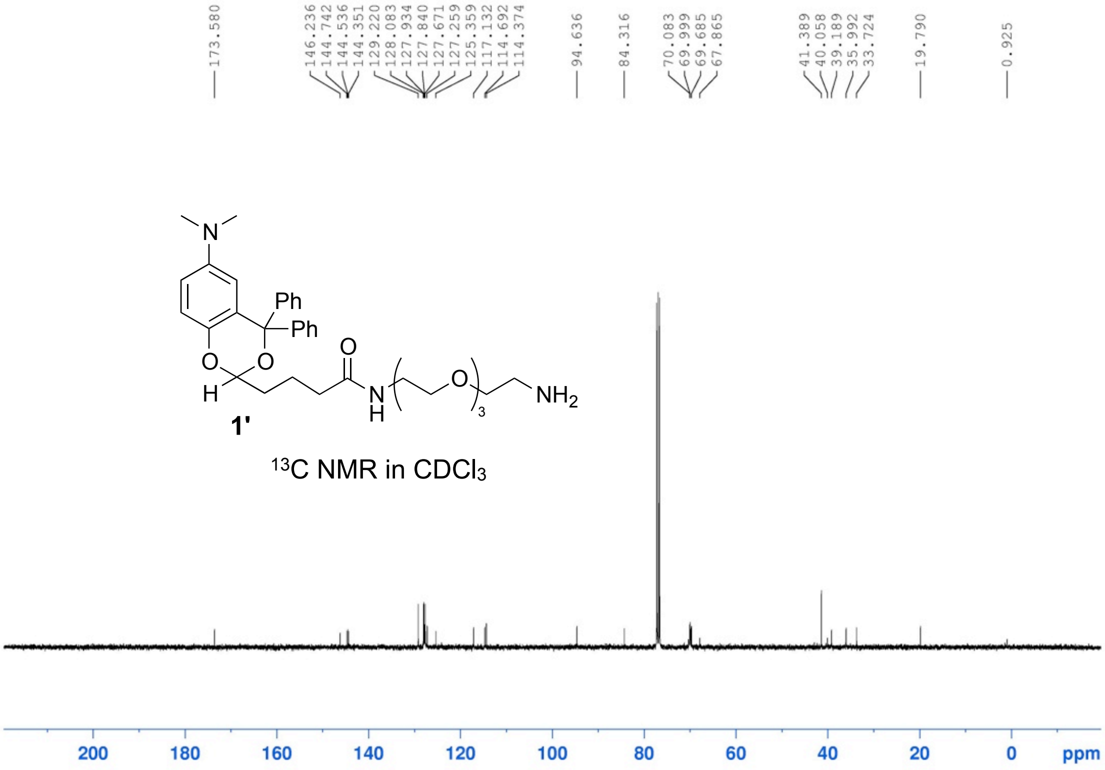


2,2'-((Oxybis(ethane-2,1-diyl))bis(oxy))diethanamine (**8**) was prepared according to the synthetic route described by Numata *et al*.[3]

N-*(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-4-(6-(dimethylamino)-4,4-diphenyl*-4H-*benzo*[d]*[1,3]dioxin-2-yl)butanamide* (**1'**)

NHS ester **7** (0.100 g, 0.195 mmol) in dichloromethane (1 mL) was added dropwise to a solution of tetraethyleneglycol diamine **8** (0.149 g, 0.777 mmol) in dichloromethane (1 mL) over 30 minutes. The solution was stirred for 48 hours at room temperature and quenched with brine (10 mL). The aqueous layer was extracted with dichloromethane (3 x 10 mL), the organic layers were combined and dried over $MgSO_4$. The solution was evaporated to dryness and the residue purified by silica column chromatography (2:8:0.1 $MeOH/CH_2Cl_2/TEA$) to yield compound **1** as a colourless oil (0.039 g, 40%); R_f 0.20 (3:7 $MeOH/CH_2Cl_2$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.76 -1.88 (4 H, m $(CH_2)_2$), 2.12-2.16 (2 H, m, CH_2), 2.70 (6 H, s, 2 x CH_3), 2.82-2.83 (2 H, t, J_3 = 5.1 Hz, CH_2), 3.39-3.43 (2 H, m, CH_2), 3.46-3.50 (2 H, m, CH_2), 3.52-3.55 (2 H, m, CH_2), 3.58-3.65 (8 H, m, $(CH_2)_4$), 4.92-4.94 (1 H, t, J_3 4.7, CH), 6.18-6.19 (1 H, d, J_5 3.0, Ar-H), 6.47-6.49 (1 H, bs, NH_2) 6.63-6.66 (1 H, dd, J_3 9.0 Hz, J_5 3.0 Hz, Ar-H), 6.78-6.80 (1 H, d, J_3 9.0 Hz, Ar-H), 7.20-7.41 (10 H, m, Ph-H); δ_C (300 MHz; $CDCl_3$; Me_4Si) 19.8 (CH_2), 33.7 ($\mathit{C}H_2CH$), 36.0 ($\mathit{C}H_2C{=}O$), 39.2 (CH_2NH) 40.1 (CH_2NH), 41.4 (2 x CH_3), 67.9 (CH_2), 69.7 (CH_2), 70.0 (CH_2) 70.1 (CH_2), 84.3 (C), 94.6 (CH), 114.4, 114.7, 117.1, 125.4, 127.3, 127.7, 127.8, 127.9, 128.1, 129.2, 129.3, 144.4, 144.5, 144.7, 146.2, (all Ph-CH), 173.6 (C=O); ν_{max}/cm^{-1} 2955, 2985, 1650, 1525, 1500, 1255; HRMS (ESI): found MNa^+, 614.3229 ($C_{34}H_{45}N_3O_6Na$ requires 614.3206); m/z (ES1) 592 ($C_{34}H_{46}N_3O_6$, 47%), 302 (45%), 242 (100).





References

1. P. Wang, Y. Wang, H. Hu, C. Spencer, X. Liang, and L. Pan, *J. Org. Chem.*, 2008, **73**, 6152–6157.
2. E. Vedejs, M. Arnost, and J. Hagen, *J. Org. Chem.*, 1979, **44**, 3230–3238.
3. M. Numata, K. Koumoto, M. Mizu, K. Sakurai, and S. Shinkai, *Org. Biomol. Chem.*, 2005, **3**, 2255–2261.