Front. Chem. Sci. Eng.
https://doi.org/10.1007/s11705-019-1812-5
COMMUNICATION

A boronic acid-based fluorescent hydrogel for monosaccharide detection

Suying Xu[1,2], Adam C. Sedgwick[2,3], Souad A. Elfeky[2,4,5,6], Wenbo Chen[2,4,7], Ashley S. Jones[2], George T. Williams[2], A. Toby. A. Jenkins[2], Steven D. Bull[2], John S. Fossey (✉)[4], Tony D. James (✉)[2]

1 Department of Biochemistry, Faculty of Science, Beijing University of Chemical Technology, Beijing 100029, China

2 Department of Chemistry, University of Bath, Bath BA2 7AY, UK

3 Department of Chemistry, University of Texas at Austin, TX 78712-1224, USA

4 School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK

5 National Institute of Laser Enhanced Sciences, Cairo University, Giza 12613, Egypt

6 Higher Institute for Optics Technology, Sheraton, Cairo 17361, Egypt

7 Shanghai Key Laboratory of Materials Protection and Advanced Materials in Electric Power, Shanghai University of Electric Power, Shanghai 200090, China

E-mails: j.s.fossey@bham.ac.uk (Fossey J S); T.D.James@bath.ac.uk (James T D)

Electronic Supplementary Material

Contents

1. General information

Solvents and Reagents

Solvents and reagents were reagent grade unless stated otherwise and were purchased from Fisher Scientific UK, Fluorochem Ltd, TCI UK, Alfa Aesar and Sigma-Aldrich Company Ltd and were used without further purification.

Thin Layer Chromatography (TLC)

Thin layer chromatography was performed using commercially available Macherey-Nagel aluminium backed plates coated with a 0.20 mm layer of silica gel 60 Å with fluorescent indicator UV254. These plates were visualised using either ultraviolet light of 254 nm or 365 nm wavelength, or by staining the plates with vanillin or ninhydrin solution. Silica gel column chromatography was carried out using Fisher or Sigma- Aldrich 60 Å silica gel (35-70 μm).

Nuclear Magnetic Resonance (NMR) Spectra

Nuclear magnetic resonance (NMR) spectra were run in chloroform-D, methanol-D_4, and dimethyl sulfoxide-D_6. Where a Bruker AVANCE 300 was used, ^{1}H spectra were recorded at 300 MHz, ^{11}B spectra at 96 MHz and ^{13}C at 75 MHz.Chemical shifts (δ) are expressed in parts per million and are reported relative to the residual solvent peak as an internal standard in ^{1}H and ^{13}C NMR spectra. The multiplicities and general assignments of the spectroscopic data are denoted as: singlet (s), doublet (d), triplet (t), double of doublets (dd), unresolved multiplet (m), broad (br) and aryl (Ar). ^{13}C NMR spectra are proton decoupled.

Mass Spectrometry

High resolution mass spectrometry (HRMS) results were typically acquired on an externally calibrated Bruker Daltonics micrOTOF time-of-flight mass spectrometer coupled to an electrospray source (ESI-TOF). Calibration was achieved using a sodium formate solution. Samples were introduced either by syringe pump or flow injection using an autosampler in an Agilent 1100 LC system. Molecular ions were detected in positive mode as either the protonated or sodiated form and Bruker Daltonics software, DataAnalysis, was used to process the data. Alternatively, data was acquired externally at the EPSRC National Mass Spectrometry Facility, Swansea University.

Melting Points (MP)

Capillary melting points were determined using Stuart MDP10. Compounds were purified and dried before melting points were determined.

Fluorescence Measurements

Fluorescence measurements were performed on a Perkin-Elmer Luminescence Spectrophotometer LS 50B/ LS 55 B utilising Starna Silica (quartz) cuvette with 10 mm path length and four faces polished. Data were collected *via* the Perkin-Elmer FL Winlab software package. All solvents used in fluorescence measurements were HPLC or fluorescence grade and the water was de-ionised. Data was processed in Origin 2016

pH Measurement

All pH measurements taken during fluorescence/absorption experiments were recorded on a Hanna Instruments HI 9321 Microprocessor pH meter which was routinely calibrated using Fisher Chemicals standard buffer solutions (pH 4.0 - phthalate, 7.0 - phosphate, and 10.0 - borate).

UV-Vis and Fluorescence Measurements

UV-Vis measurements were performed on a Perkin-Elmer Lambda 20 Spectrophotometer, utilising Starna Silica (quartz) cuvette with 10 mm path lengths, two faces polished. Data was collected *via* the Perkin-Elmer UV Winlab software package. Data was processed in Origin 2016

The fluorescence titrations were carried out in a pH 8.21 aqueous methanolic buffer. The buffer was prepared following the literature method of Perrin and Dempsey [1] and contained:

> 52.1 *wt*% HPLC grade methanol in deionised water with KCl, 0.01000 M, KH_2PO_4, 0.002752 M and Na_2HPO_4, 0.002757 M.

For the fluorescence titrations, the spectrometer parameters were fixed as: slit, 5.0 nm / 3.0 nm, scan speed: 600 nm / min.

Data Analysis

Data was collected *via* the Perkin-Elmer Winlab software package. The observed stability constants (K_{obs}) with coefficient of determination (γ^2) were calculated by the fitting of emission intensity *versus* saccharide concentration using non-linear (Levenberg-Marquardt algorithm) curve fitting.

Hydrogel Fluorescence Measurements

The hydrogel fluorescence measurements were carried out using plate reader assay methodology from the fluoroSENS GILDEN Photonics instrument with excitation wavelength 370 nm and maximum emission 409 nm. The buffer solution consisted of 50 mL 0.1M KH_2PO_4 mixed with 46.1 mL 0.1 M NaOH and adding water to make up to 100 mL. Saccharide solutions consisted of the buffer solution to keep the pH value the same in the test system, since the total volume for gel titration is only 0.3 mL, of which 0.2 mL is hydrogel.

2. Procedure for incorporation of AM-5 into a hydrogel

Hydrogel containing **AM-5** (1 % w/w) was formed by co-polymerisation of acrylamide and bis-acrylamide in water through free radical polymerisation using ammonium persulfate (APS) to afford the free radicals under the catalysis of tetramethylethylenediamine (TMEDA).

Hydrogel formation procedure steps:

1) Ammonium persulfate (APS) solution (10% w/v) was freshly prepared and was kept below 4 °C until required.

2) Methylene bisacrylamide (0.040 g, 0.26 mmol, 1 % w/w) and acrylamide (1.52 g, 21.38 mmol, 38% w/w) were added to water (8 mL 60 % w/w) and stirred until dissolved as judged by visual inspection.

3) **AM-5** (2 mg, 3.38 mmol, 1 % w/w) was dissolved in methanol (0.2 mL) and then added to the solution obtained in step 2.

4) Tetramethylethylenediamine (TMEDA) (20 μL, 0.13 mmol) and 10 % APS solution (60 μL) were then added, and the solutions transferred to 3 mL plastic syringes to cast the hydrogels (~ 30 minutes for the gels to set).

Plate Reader Hydrogel Procedure:

APS/TMEDA/Methylene bisacrylamide and acrylamide mixture (0.2 mL) was added into each well and set using a buffer solution (pH = 8.00) - 50 mL 0.1M KH_2PO_4 mixed with 46.1 mL 0.1 M NaOH.

3. Hydrogel swelling

Hydrogels were placed into three different buffers to observe changes in volume: in pH 8.5 phosphate buffer the volume increased in size whereas the hydrogel in pH 8.21 phosphate buffer containing 52.1 *wt* % methanol, the volume shrunk. The hydrogel in neutral pH resulted in no change in volume size. The change in gel size for pH 8.5 phosphate buffer is due to the formation of the boronate anion which increased electrostatic repulsion within the hydrogel thus causing it to expand. For the methanolic buffer solution (pH = 8.21, 52.1% wt MeOH), it is known that organic solvents can partially dissolve hydrogels therefore causing it to shrink. As a result, buffer solutions containing organic solvents was avoided.

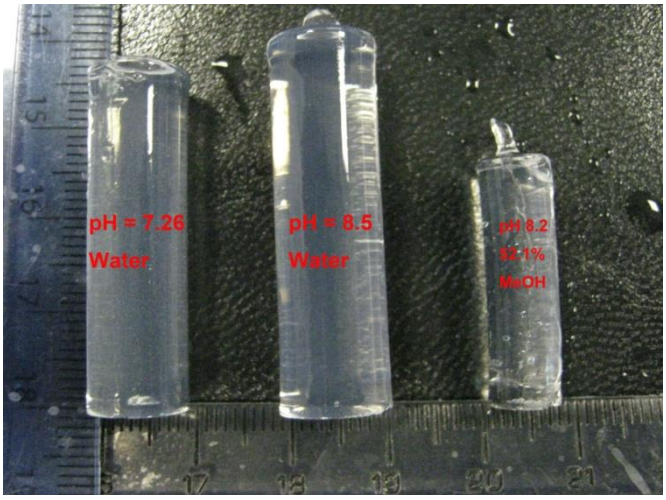
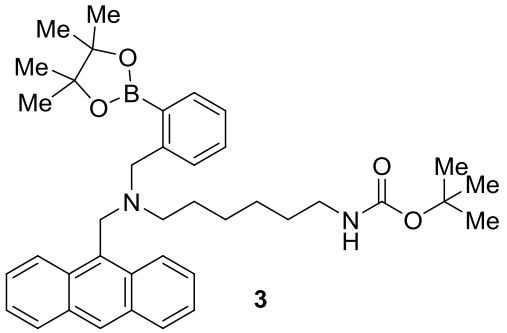


Figure S1 – Photograph of hydrogel made from **AM-5** after incubation (16 hrs) in different buffer solutions (left to right – PBS - pH = 7.26, PBS - pH = 8.5, PBS containing MeOH (52.1 %) - pH =8.2) and different pH values for 16 hours.

4. Binding constants and observed fluorescence enhancement with saccharide and pH

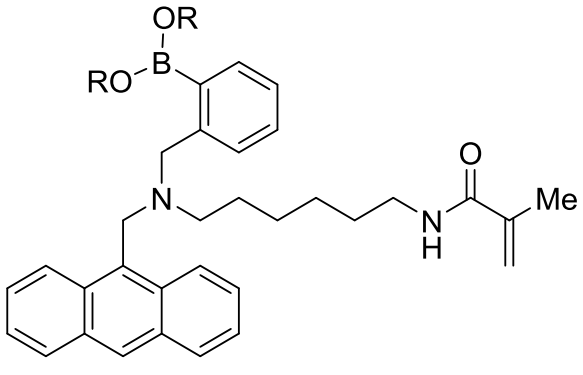
Table S1 - Observed stability constants (K_{obs}), coefficient of determination (R^2) and overall fluorescence enhancements of the boronic acid-based fluorescence probe **3** (0.6 µM) in pH 8.21 aqueous methanolic buffer solution [52.1 wt% methanol (KCl, 0.01000 M; KH_2PO_4, 0.002752 M and Na_2HPO_4, 0.002757 M)]



3

	K_{obs} / dm^3 mol^{-1}	Fluorescence enhancement	R^2
D-Fructose	1381.7 ±41.80	2.54 ± 0.00	0.999
D-Galactose	221.4 ±8.05	2.82 ± 0.02	0.999
D-Glucose	87.18 ±1.80	2.82 ± 0.01	1.00
D-Mannose	92.48 ±3.79	2.87 ± 0.02	0.998

Table S2 Observed stability constants (K_{obs}), fluorescence enhancements and coefficient of determination (R^2) of hydrogel incorporating monomer **AM-5**



AM-5

R = H or Pinacol

	K_{obs} / $dm^3\,mol^{-1}$	Fluorescence enhancement	R^2
D-Fructose	52.6 ± 5.3	10.3 ± 0.4	0.992
D-Galactose	10.8 ± 1.3	6.4 ± 0.4	0.993
D-Glucose	--	--	--
D-Mannose	17.8 ± 4.8	3.1 ± 0.4	0.975

Effect of PH

As the binding between boronic acid and saccharide is greatly affected by the pH of the medium,[3] the gel's fluorescence response to a pH sweep was investigated. A 0.05 M KH_2PO_4 buffer solution was adjusted by 2 N NaOH to afford the buffer solutions with different pH values, 6.30, 7.31, 7.91, respectively. Since the buffer range of KH_2PO_4 / NaOH system is 5.6 to 8.0, the buffer was then switched to a glycine / NaOH system, with a similar strategy to obtain the buffers with pH values of 8.60, 9.03, 9.49 and 10.17. D-Fructose was used as the model monosaccharide to evaluate the hydrogel responses with and without monosaccharides. It was found that there was slightly higher fluorescence response with the addition of fructose when pH > 8.

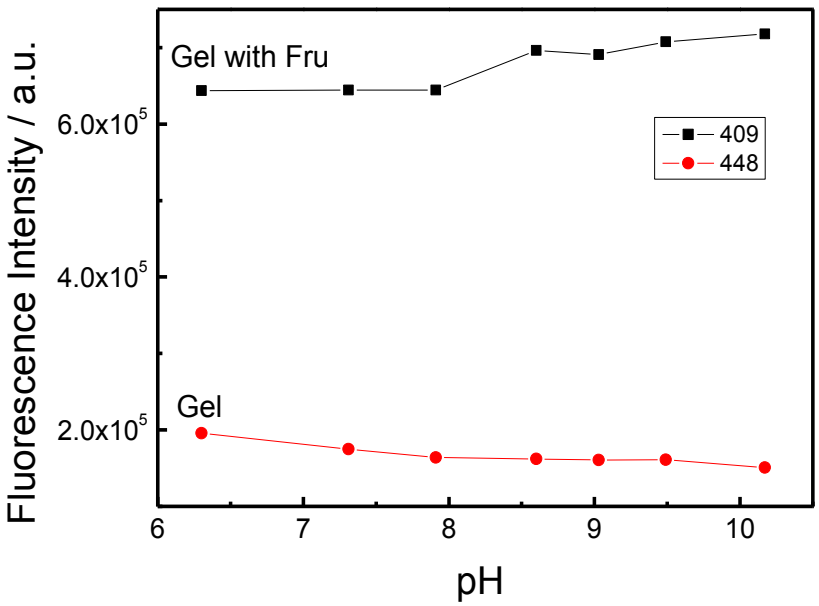


Figure S3 - Fluorescence intensity changes of the boronic acid **AM-5**-containing hydrogel in buffer solution over the range of different pH values

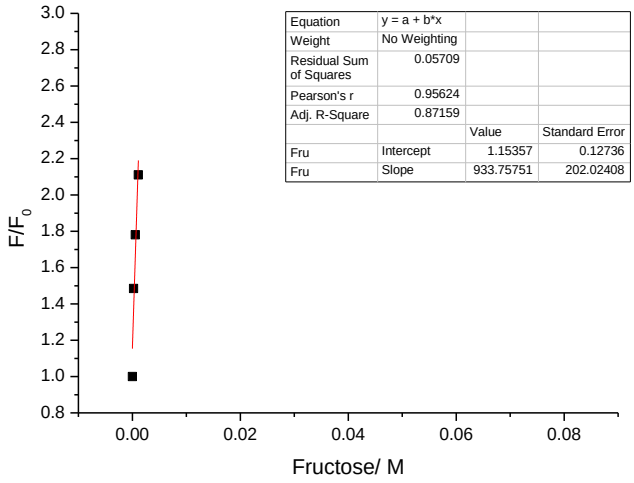
5. Limit of detection (LOD) for monosaccharides

The limit of detection was calculated using the formula shown below:

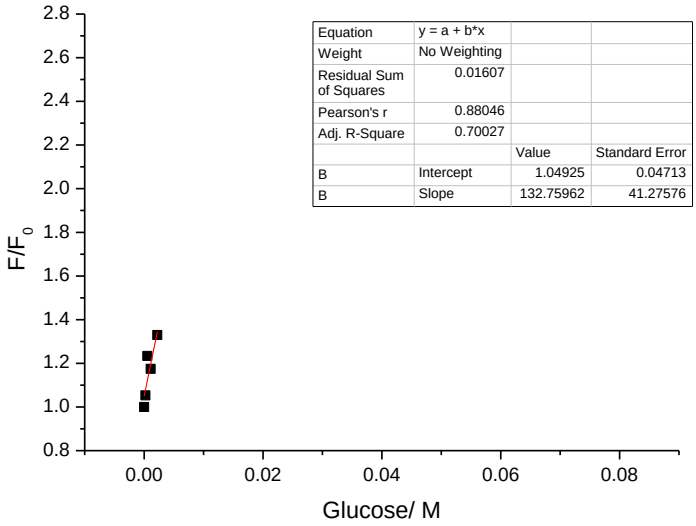
Limit of detection (LOD) = 3σ/slope

$$\sigma = \sqrt{RSS/(n-2)}$$

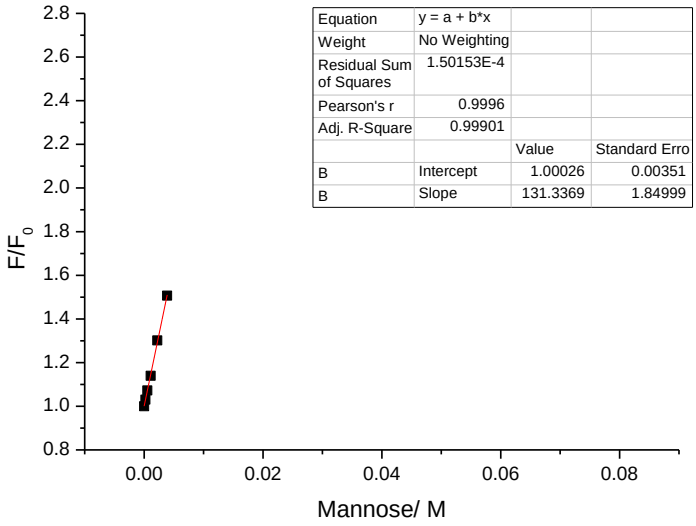
LOD of AM-5 In Solution:



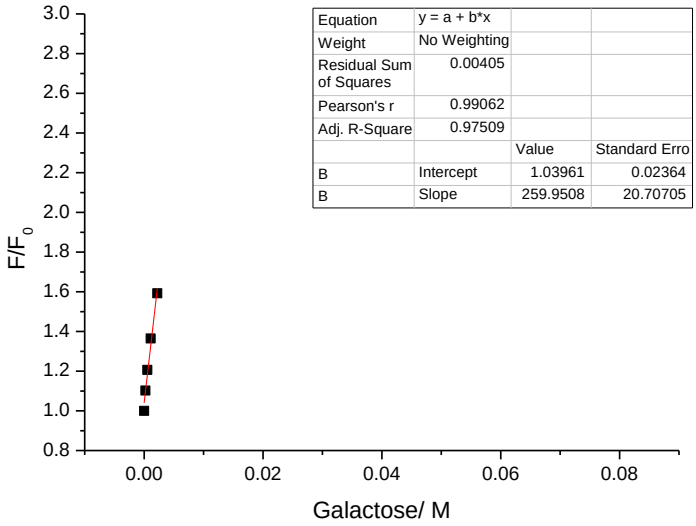
Fructose: LOD = (3x 0.169)/933.8 = 0.53 mM



Glucose: LOD = (3x 0.0567)/132.75962 = 1.28 mM

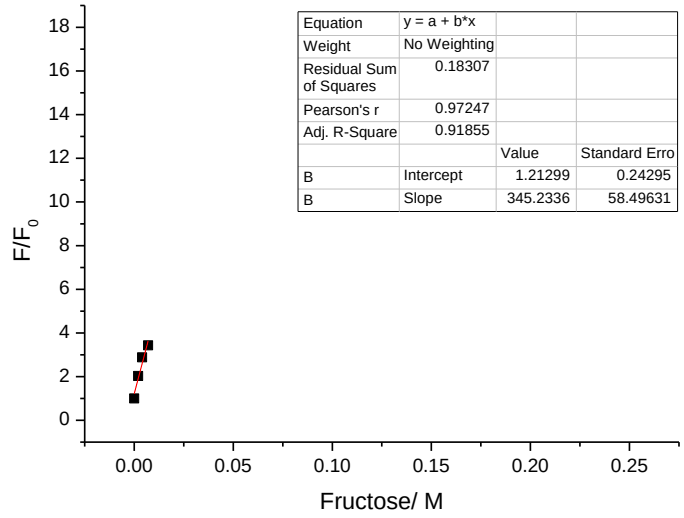


Mannose: LOD = (3x 6.12x10-3)/131.33699 = 0.14 mM



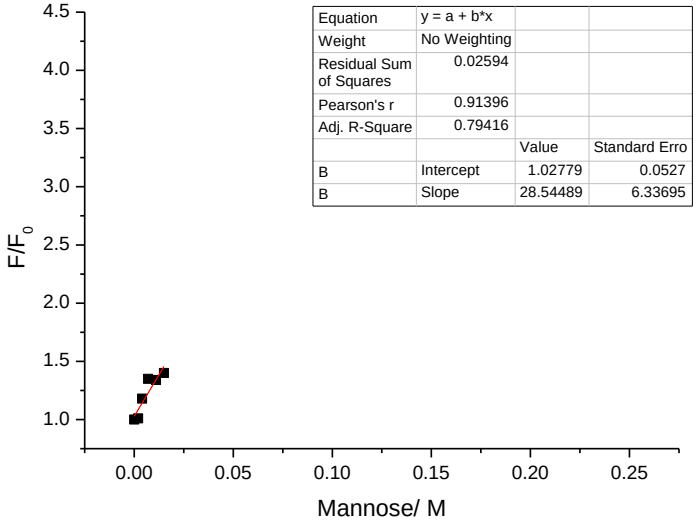
Galactose: LOD = (3x 0.037)/259.95081 = 0.42 mM

LOD of Hydrogel Bound AM-5:

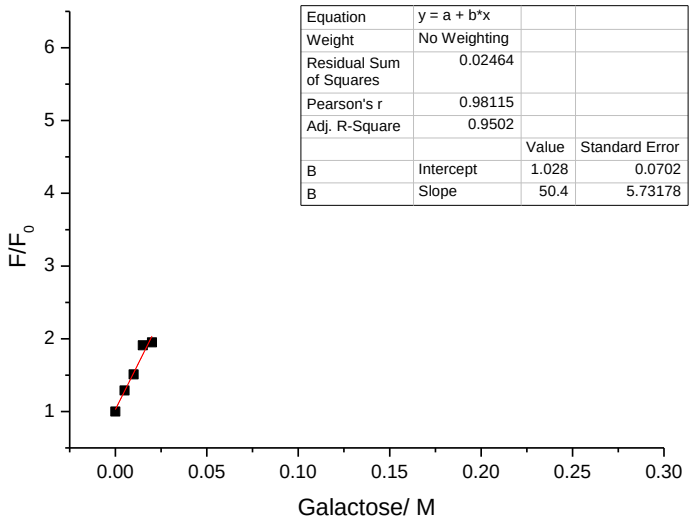


Fructose: LOD = (3x 0.30/345.23364 = 2.63 mM

Glucose: LOD = N/A



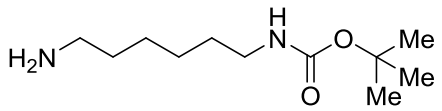
Mannose: LOD = (3x 0.081)/28.54489 = 8.46 mM



Galactose: LOD = (3x 0.091)/50.4 = 5.39 mM

6. Experimental

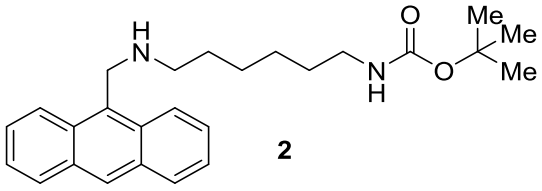
tert-Butyl (6-aminohexyl)carbamate (1)



1

Di-*tert*-butyl dicarbonate (1.09 g, 5 mmol) in 20 mL methanol was added dropwise into solution of 1,6-hexanediamine (2.95 g, 25 mmol) in 5 mL methanol. The progress of the reaction was monitored by TLC. Once complete, solvent was removed *in vacuo*. Dichloromethane (20 mL) and water (100 mL) were added and the organic layer was washed with water (3 x 100 mL), dried over anhydrous sodium sulphate, filtered and evapourated to dryness *in vacuo*. The material thus obtained was purified *via* flash chromatography (dichloromethane:hexane 9:1 to 20:1) to obtain the title compound as a clear oil (1.6 g, 7.40 mmol, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.54 (br. s., 1 H, N-*H*), 3.10 (appq, 2 H), 2.76 - 2.63 (appt, 2 H), 1.52 - 1.42 (m, 12 H), 1.38 - 1.28 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 50.6, 42.1, 40.6, 33.7, 30.1, 28.4, 26.6, 26.5; HRMS (ESI) $C_{11}H_{25}N_2O_2^+$ calculated for $[M+H]^+$ 217.1916, found 217.1993.

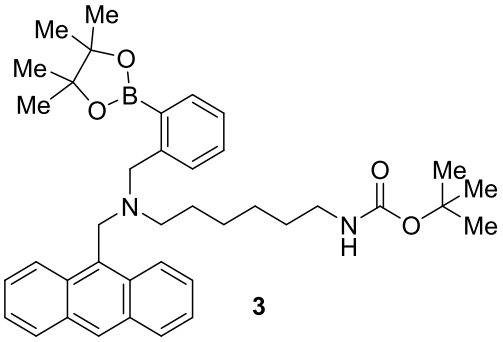
***tert*-Butyl (6-((anthracen-9-ylmethyl)amino)hexyl)carbamate (2)**



2

tert-Butyl (6-aminohexyl)carbamate (2.0 g, 9.2 mmol) and 9-anthracene aldehyde (1.7 g, 8.0 mmol) were dissolved in methanol (100 mL) and the reaction mixture was stirred at room temperature for 16 hr . $NaBH_4$ (0.35 g, 9.0 mmol) was then slowly added over the course of 5 minand the reaction was then stirred for 4 h. The reaction was then quenched by addition of water (3 mL) and concentrated *in vacuo*. Dichloromethane (100 mL) was added and the organic layer was washed with water (4 x 100mL), dried over anhydrous magnesium sulphate and concentrated *in vacuo* to afford the crude material, which was purified by flash chromatography (dichloromethane:hexane 10:1 to 100 % dichloromethane to dichloromethane:methanol 20:1) The title compound was isolated as a yellow solid (1.80 g, 4.33 mmol, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1 H,), 8.34 (d, *J* = 8.3 Hz, 2 H), 8.01 (d, *J* = 8.9 Hz, 2 H), 7.59 - 7.43 (m, 4 H), 4.73 (s, 2 H), 4.54 (br. s., 1 H), 3.10 (appq, 2 H), 2.87 (appt, 2 H), 1.82 (br. s., 1 H), 1.68 - 1.54 (m, 2 H), 1.50 - 1.41 (m, 11 H), 1.38 - 1.27 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 131.6, 130.3, 129.2, 127.2, 127.2, 126.2, 126.1, 125.0, 124.9, 124.1, 79.0, 50.4, 45.8, 40.6, 30.0, 28.5, 27.1, 26.7; HRMS (ESI) $C_{26}H_{34}N_2O_2Na^+$ calculated for [M + Na^+] 429.2517, found 429.2545.

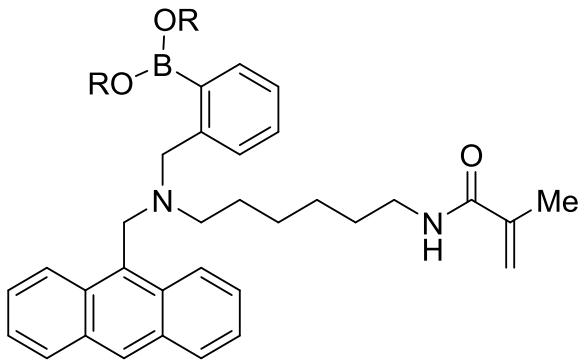
***tert*-Butyl(6-((anthracen-9-ylmethyl)(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)amino)hexyl)carbamate (3)**



3

A mixture of **2** (1.8 g, 4.4 mmol), 2-bromomethylphenylboronic acid pinacol ester (1.45 g, 4.6 mmol) and K_2CO_3 (1.82 g, 13.2 mmol) in MeCN (25 mL) was heated at reflux for 12 h. The reaction was then cooled to room temperature and the solvent was then removed *in vacuo.* Acetone (1.5 mL) was then added to the mixture to afford a clear solution, which was added dropwise into water (300 mL), which generated a precipitate. The precipitate thus formed was washed with water (100 mL) and dried under a flow of air (collected by vacuum filtration) to afford the title compound as a yellow solid. (2.46 g, 89 %). ^{1}H NMR (300 MHz, MeOD-d_4) δ 8.62 (s, 1 H), 8.20 (d, *J* = 8.5 Hz, 2 H), 8.14 - 8.01 (m, 2 H), 7.70 (d, *J* = 8.3 Hz, 1 H), 7.60 - 7.44 (m, 6 H), 7.40 - 7.24 (m, 2 H), 5.01 (br. s., 2 H), 4.45 (br. s., 2 H), 2.84 (appt, 2 H), 2.79 - 2.72 (m, 2 H), 1.57 - 1.38 (m, 15 H), 1.22 - 1.10 (m, 10 H), 1.04 - 0.84 (m, 4 H); ^{13}C NMR (75 MHz, MeOD-d_4) δ 170.7, 155.9, 136.2, 135.2, 133.4, ,131.1, 131.0, 129.87, 129.48, 128.0, 125.88, 123.48, 121.40, 77.70, 67.4, 65.28, 60.1, 59.3, 53.5, 48.9, 40.6, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 29.26, 28.6, 25.9, 25.7, 25.5, 23.8, 21.1, 15.5, 14.4; ^{11}B NMR (96 MHz, MeOD-d_4) δ 33.99; HRMS (ESI) $C_{39}H_{52}BN_2O_4^+$ calculated for [M + H^+] 623.4015, found 623.40

(2-(((Anthracen-9-ylmethyl)(6-methacrylamidohexyl)amino)methyl)phenyl)boronic acid (AM-5)



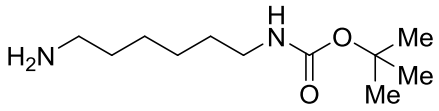
AM-5

R = H or Pinacol

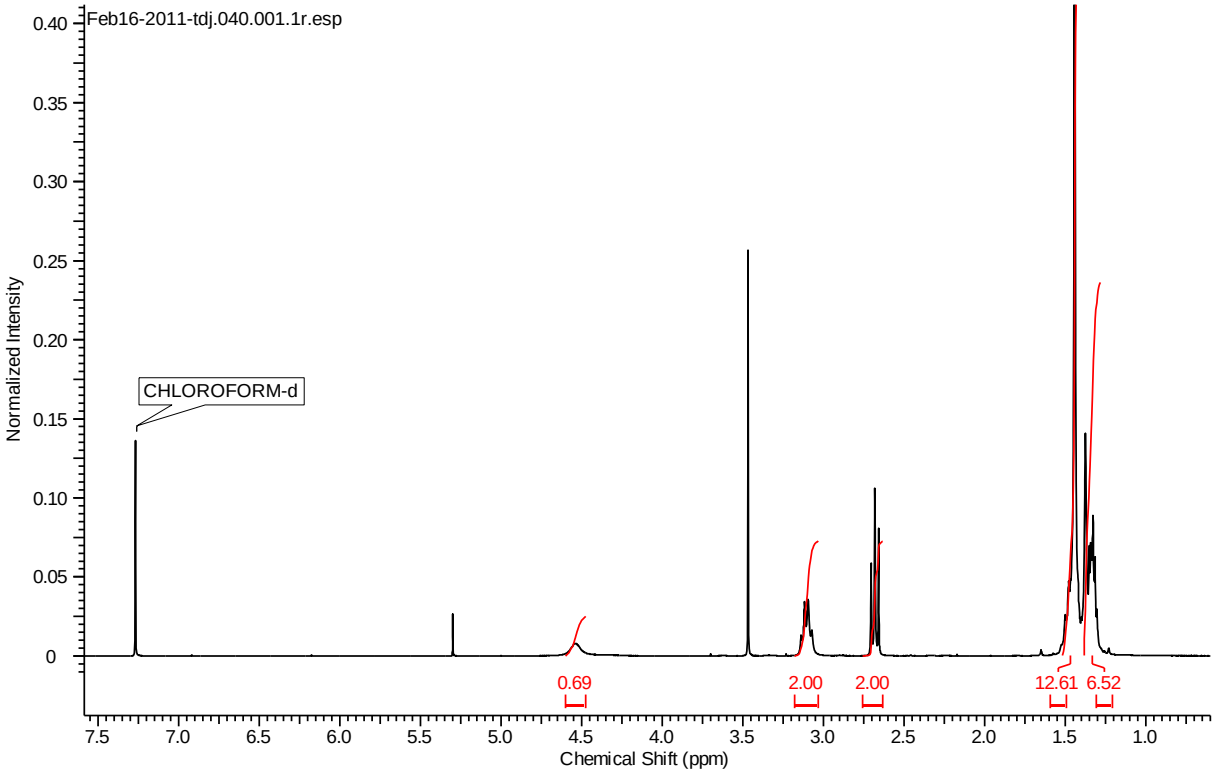
Trifluoroacetic acid (5 mL) was added slowly to a solution of **3** (1.8 g, 2.9 mmol) in dichloromethane (25 mL) and the reaction mixture was stirred for 4 h. The solvent was then removed under reduced pressure to afford **4** in quantitative yield. **4** was then dissolved in DCM (25 mL) and cooled in an ice bath to 0 ^{o}C. NEt_3 (1.62 mL, 11.6 mmol) was then added dropwise and the solution was stirred at 0 ^{o}C for 30 mins. Methacryloyl chloride (0.58 mL, 5.9 mmol) was then added dropwise and the reaction mixture was stirred for 5 h. The reaction mixture was then washed with saturated $NaHCO_3$ aqueous solution (2 x 100 mL), brine (3 x 100 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure. The title compound was afforded as a yellow solid. *Due to the* in situ *deprotection of the pinacol ester, a mixture of boronic anhydrides are formed which result in a complex NMR.*[4] ESI-MS: [M + H^+] $C_{38}H_{48}BN_2O_3^+$ calc. 591.3753, found: 547.3779; [M + Na^+] $C_{38}H_{47}BN_2O_3Na^+$, calculated 613.3572, found: 613.3038. For formula without pinacol group [M + H^+] $C_{32}H_{38}BN_2O_3^+$, calculated 509.2970 found: 509.2975; [M +Na^+] $C_{32}H_{37}BN_2O_3\,Na^+$, calculated 531.2789, found: 531.2814.

7. NMR spectra

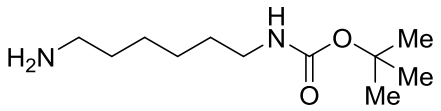
***tert*-Butyl (6-aminohexyl)carbamate (1) -** ^{1}H NMR (300 MHz, $CDCl_3$)



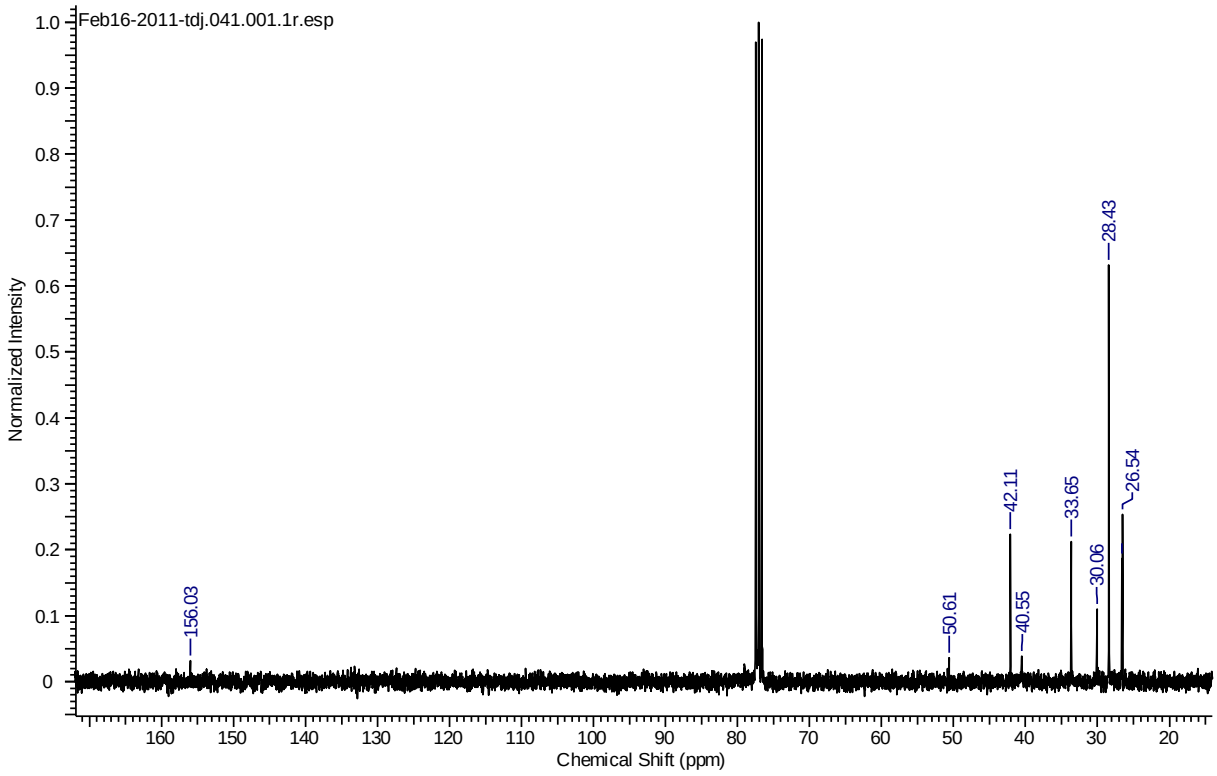
1



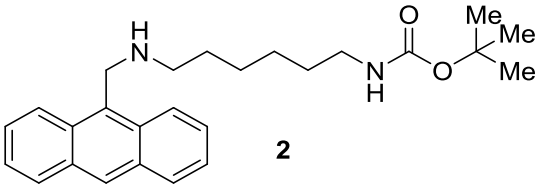
***tert*-Butyl (6-aminohexyl)carbamate (1)** ^{13}C NMR (75 MHz, $CDCl_3$)



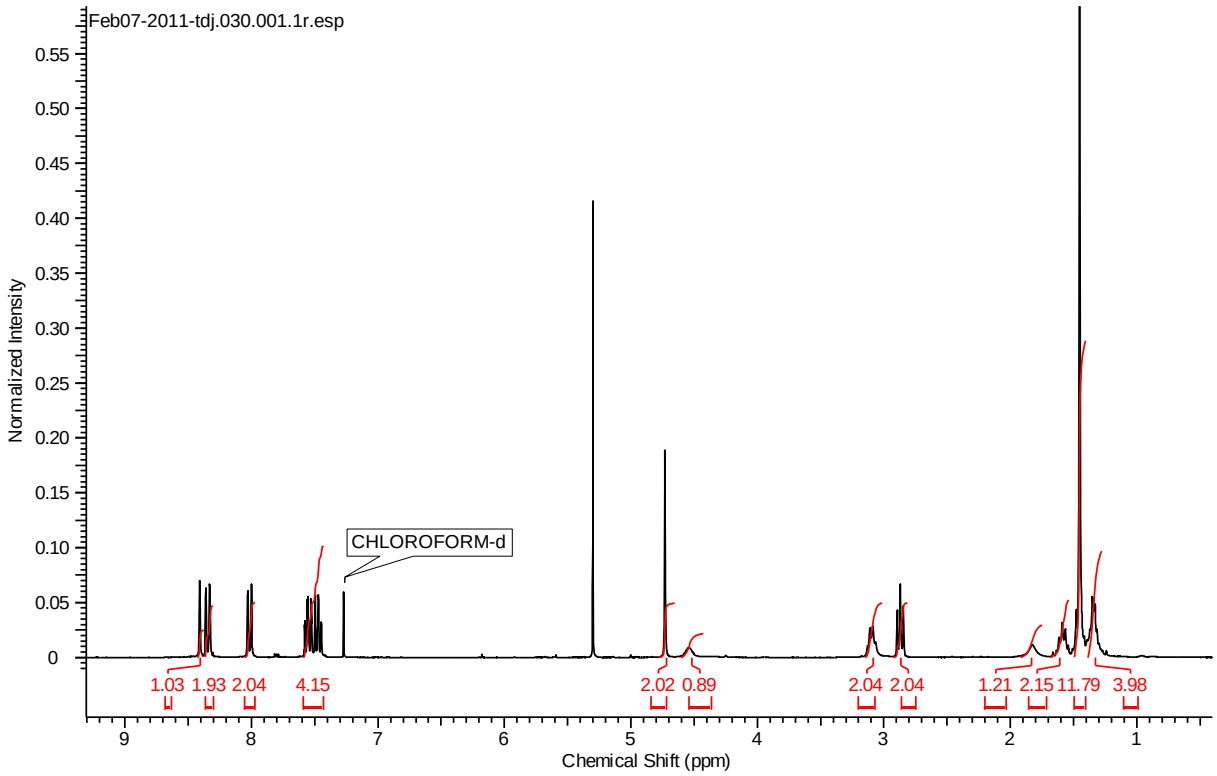
1



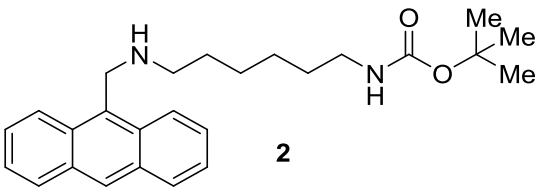
***tert*-Butyl (6-((anthracen-9-ylmethyl)amino)hexyl)carbamate (2)** ^{1}H NMR (300 MHz, $CDCl_3$)



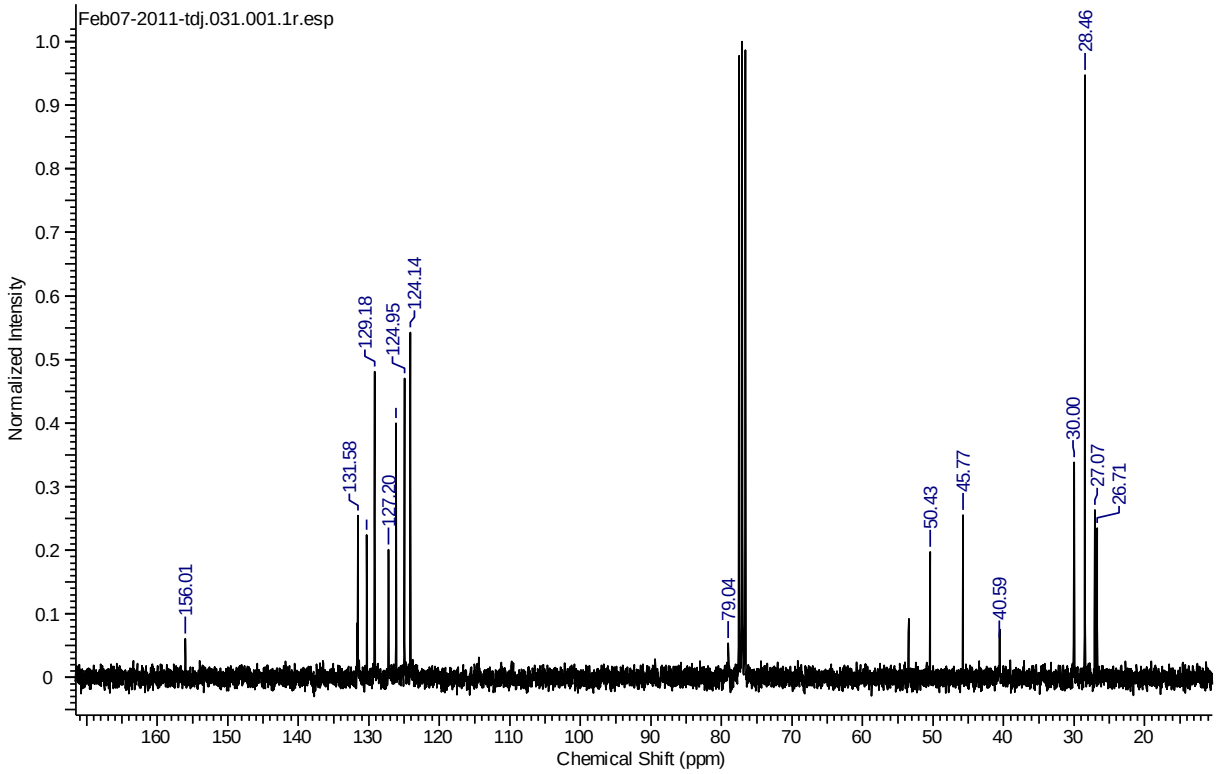
2



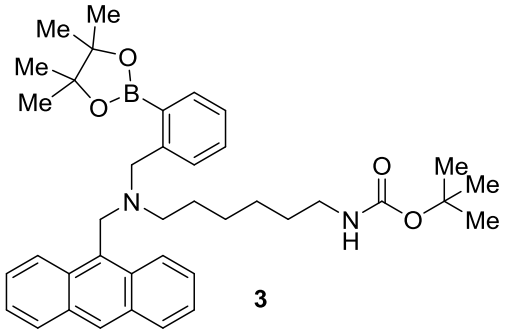
***tert*-Butyl (6-((anthracen-9-ylmethyl)amino)hexyl)carbamate (2) -** ^{13}C NMR (75 MHz, $CDCl_3$)



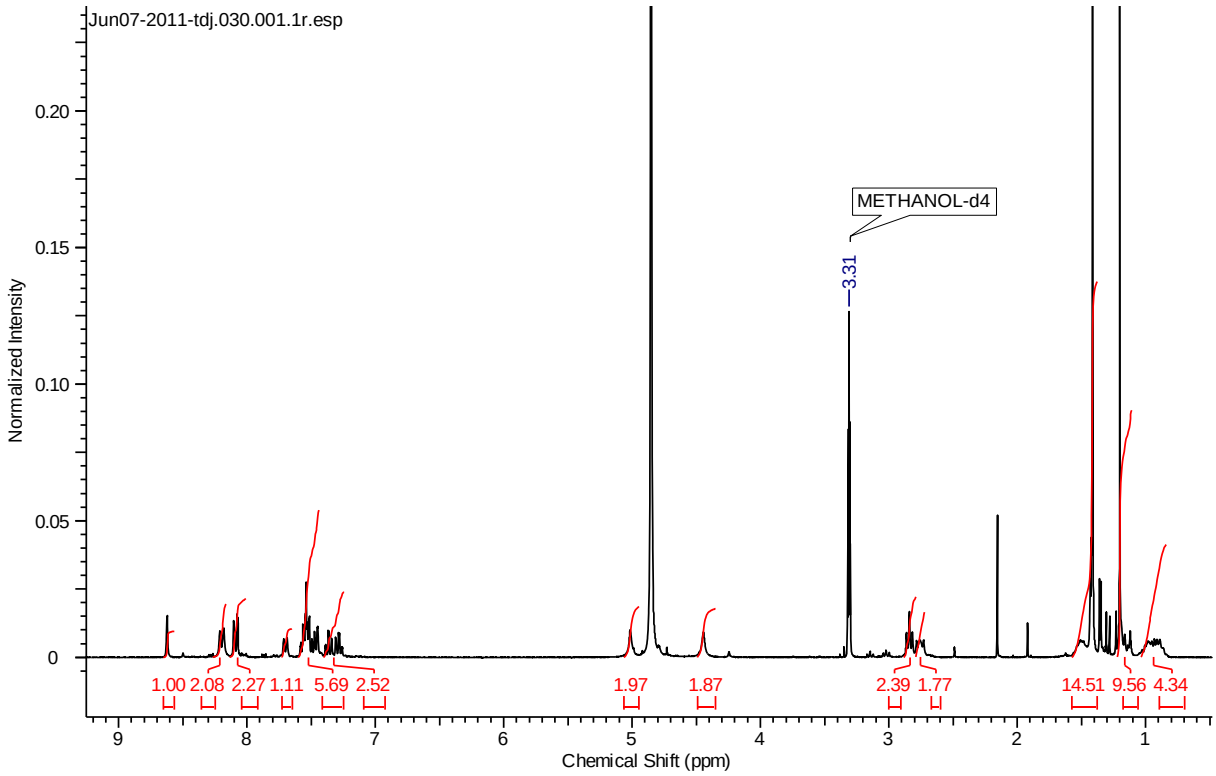
2



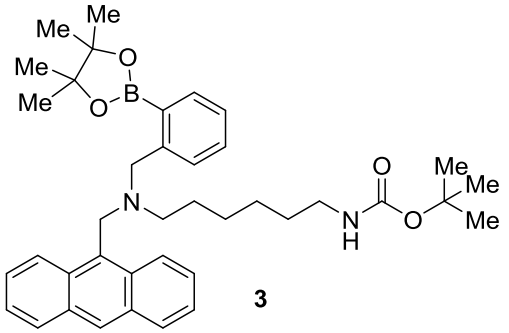
***tert*-Butyl(6-((anthracen-9-ylmethyl)(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)amino)hexyl)carbamate (3) -** ^{1}H NMR (300 MHz, MeOD-d_4)



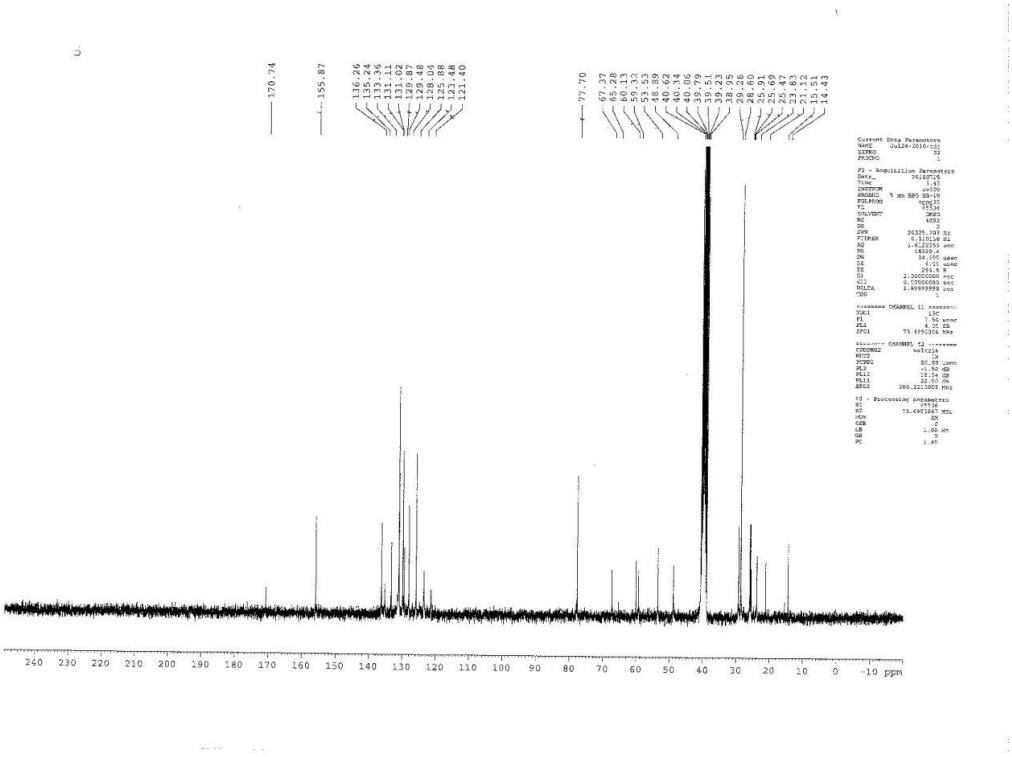
3



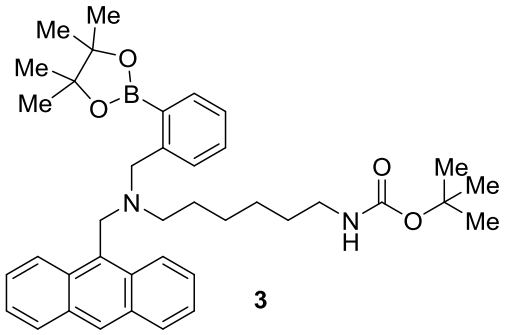
***tert*-Butyl(6-((anthracen-9-ylmethyl)(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)amino)hexyl)carbamate (3) -** ^{13}C NMR **(**75 MHz, DMSO-d_6)



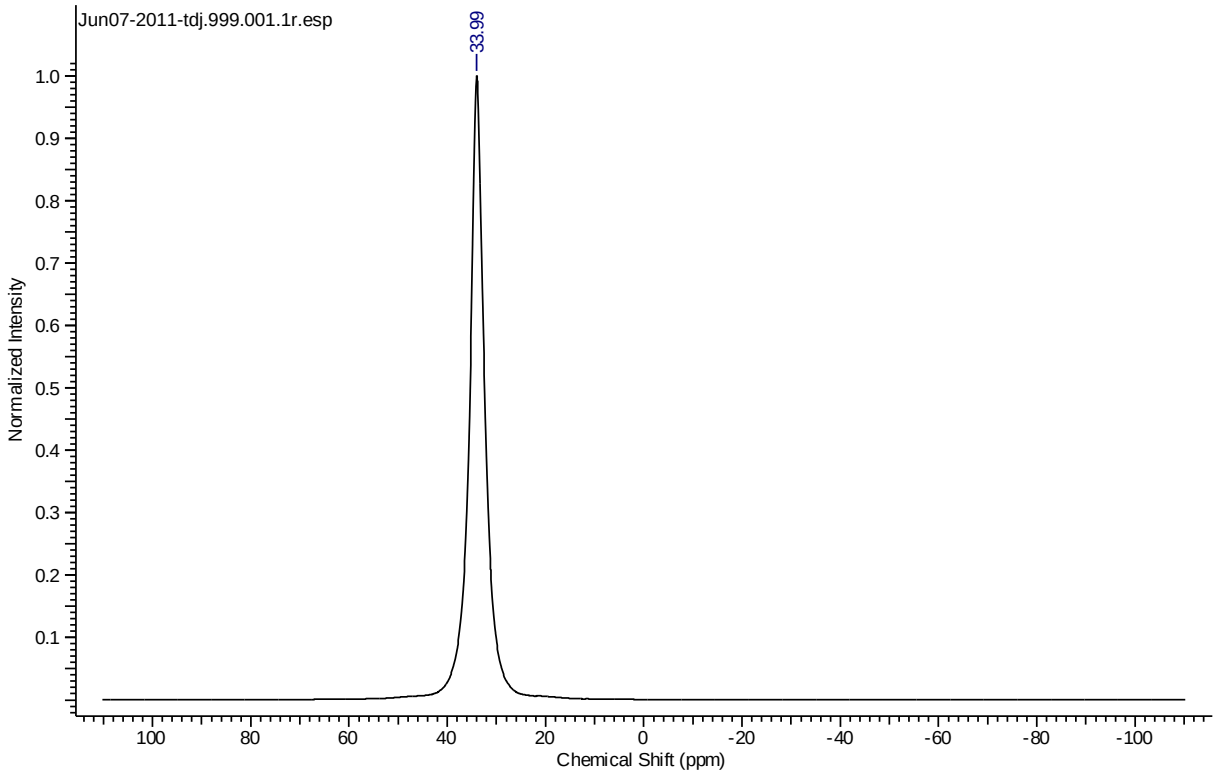
3



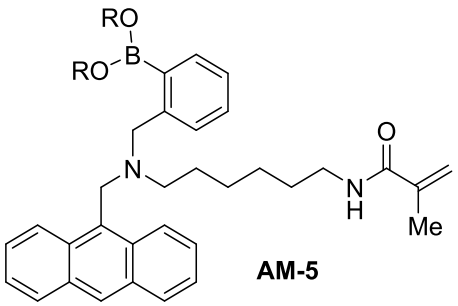
***tert*-Butyl(6-((anthracen-9-ylmethyl)(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)amino)hexyl)carbamate (3)** - ^{11}B NMR, (96 MHz $CDCl_3$)



3



(2-(((Anthracen-9-ylmethyl)(6-methacrylamidohexyl)amino)methyl)phenyl)boronic acid (AM-5)



AM-5

Due to the in situ *deprotection of the pinacol ester (when compared to compound **3**), a mixture of boronic anhydrides are formed which result in a complex ^{1}H NMR. This is a common problem with boronic acids.*[4]

8. References

1. D. D. Perrin and B. Dempsey, *Champan & Hall*, 1974.
2. C. R. Cooper and T. D. James, *J. Chem. Soc., Perkin Trans. 1*, 2000, **0**, 963-969.
3. J. Yan, G. Springsteen, S. Deeter and B. Wang, *Tetrahedron*, 2004, **60**, 11205-11209.
4. M. Li, Z. J. Liu, H. C. Wang, A. C. Sedgwick, J. E. Gardiner, S. D. Bull, H. N. Xiao and T. D. James, *Dyes Pigm.*, 2018, **149**, 669-675.