Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Monitoring of reversible boronic acid-diol interactions by fluorine NMR spectroscopy in aqueous media

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Supporting Information

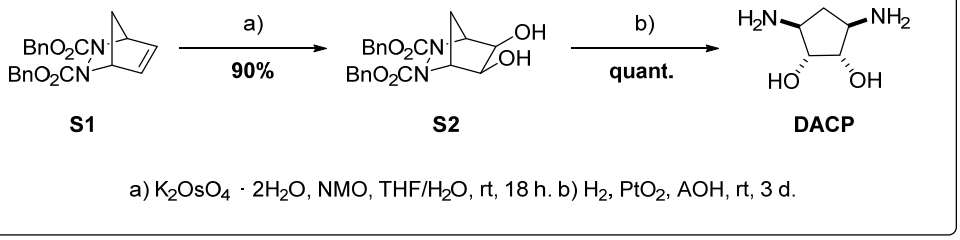
Table of Contents

General Remarks

All reactions were carried out under argon atmosphere in oven-dried glassware unless otherwise specified. All commercially available compounds were purchased from Aldrich Chemical Co., Acros Organics or Alfa Aesar and used as received. CA DNA dinucleotide was purchased from Eurogentec. Dichloromethane (DCM) was distilled from calcium hydride. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Merck $60F_{254}$) visualized either with a UV lamp (254 nm) or by using solutions of p-anisaldehyde/sulfuric acid/acetic acid (AcOH) in ethanol (EtOH) or $KMnO_4/K_2CO_3$/AcOH in water followed by heating. Flash chromatography was performed on silica gel (60-230 mesh) unless otherwise specified. Organic extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avancell 500 at 500 MHz or on a Bruker Avance 600 MHz Ultrashield at 600 MHz, in $CDCl_3$, DMSO-d_6, CD_3OD, D_2O or H_2O/D_2O (9/1), and the observed signals are reported as follows: chemical shift in parts per million (ppm) from tetramethylsilane with the solvent as an internal indicator, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet or overlap of nonequivalent resonances), integration. Coupling constants *J* are reported in Hz. ^1H-decoupled ^{19}F NMR spectra were recorded on a Bruker Avancell 500 at 470 MHz, in DMSO-d_6 or H_2O/D_2O (9/1). All NMR spectra were obtained at 300K unless otherwise specified.

Experimental Procedures and Spectral Data

Synthesis of 3,5-diaminocyclopentane-1,2-diol (DACP)



a) $K_2OsO_4 \cdot 2H_2O$, NMO, THF/H_2O, rt, 18 h. b) H_2, PtO_2, AOH, rt, 3 d.

The preparation of dibenzyl-2,3-diazabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (**S1**) followed the procedure reported by Moore and co-workers.[1]

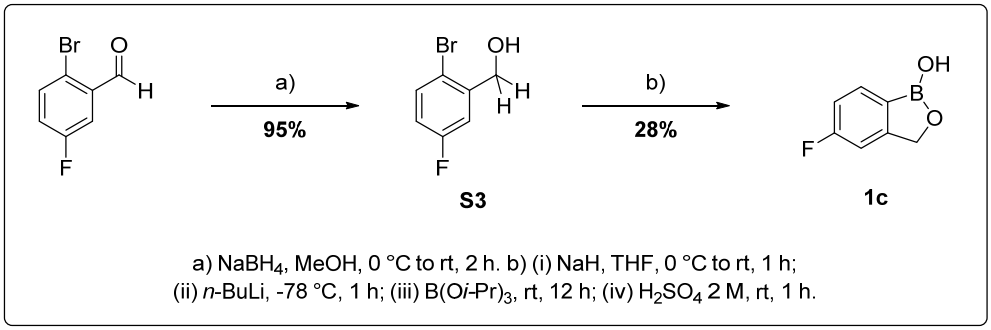
a) Synthesis of dibenzyl-5,6-dihydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (S2). Compound **S1** (5 g, 13.7 mmol) was dissolved in THF/H_2O (9/1, 160 mL), NMO (2 g, 16.8 mmol) and potassium osmate (40 mg, 0.11 mmol) were added, and the reaction was stirred rt for 18 h. HCl (6 N, 200 mL), and $NaHSO_3$ (15% in water, 100 mL) were added, and the mixture was stirred for an additional 2 h. EtOAc (200 mL) was then added, the phases were separated, and the aqueous layer was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried over $MgSO_4$, gravity filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using cyclohexane/EtOAc (6/4) as the eluent to afford compound **S2** as a colorless oil (5 g, 90% yield). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.31 (br s, 10H), 5.19-5.12 (br m, 4H), 4.36 (br, s, 2H), 3.97 (br, s, 2H), 3.27 (br, s, 2H), 2.02 (d, J = 11.0 Hz, 1H), 1.60 (d, J = 11.0 Hz, 1H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ 157.0 (2C), 135.6 (2C), 128.6 (4CH), 128.4 (2CH), 128.0 (4CH), 70.5 (2CH), 68.4 ($2CH_2$), 63.0 (2CH), 31.2 (CH_2) ppm. Spectroscopic data were consistent with the literature data for this compound.[2]

b) Synthesis of 3,5-diaminocyclopentane-1,2-diol (DACP). Compound **S2** (5 g) was dissolved in acetic acid (80 mL) under a hydrogen atmosphere, PtO_2 (750 mg, 3 mmol) was added, and the reaction was stirred at rt for 3 days. The mixture was then filtered through a Celite® pad and concentrated under reduced pressure. The crude product was purified on a DOWEX resin (50WX8-400) using a 1 M aqueous solution of ammonia as the eluent. DACP was isolated as a colorless oil (1.65 g, quantitative yield).**^{1}H NMR** (500 MHz, CD_3OD): δ 4.14-4.11 (m, 2H), 3.50-3.45 (m, 2H), 2.62 (dt, J = 13.3, 8.0 Hz, 1H), 1.73 (dt, J = 13.3, 9.7 Hz, 1H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ

[1] J. A. Moore, R. Muth and R. Sorace, *J. Org. Chem.*, 1974, **39**, 3799.
[2] G. Bégis, M. Bonin, C. Bournaud, F. Dardel, F. Maurice, L. Micouin, C. Tisné, and A. Pérez Luna, WO2006024784.

70.1 (2CH), 55.6 (2CH), 30.4 (CH_2) ppm. Spectroscopic data were consistent with the literature data for this compound.[3]

Synthesis of 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole ***1c***



a) $NaBH_4$, MeOH, 0 °C to rt, 2 h. b) (i) NaH, THF, 0 °C to rt, 1 h; (ii) *n*-BuLi, -78 °C, 1 h; (iii) $B(O\textit{i}\text{-}Pr)_3$, rt, 12 h; (iv) H_2SO_4 2 M, rt, 1 h.

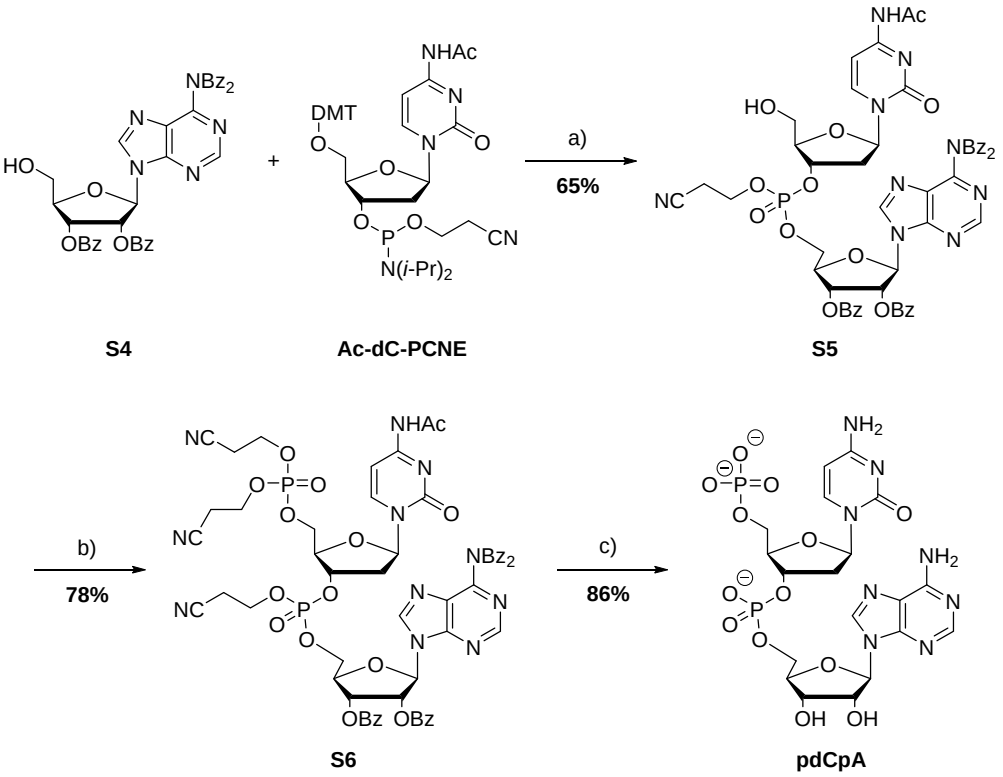
a) Synthesis of (2-bromo-5-fluorophenyl)methanol (S3). $NaBH_4$ (447 mg, 11.7 mmol) was added to a solution of 2-bromo-5-fluorobenzaldehyde (2 g, 9.8 mmol) in MeOH (20 mL) at 0 °C. The reaction was warmed to rt and stirred for 2 h. Water (40 mL) was then added, and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (40 mL) and brine (2 × 40 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using cyclohexane/EtOAc (8/2) as the eluent to afford compound **S3** as a white solid (1.91 g, 95%). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.47 (dd, J = 8.7, 5.2 Hz, 1H), 7.25 (dd, J = 9.3, 3.1 Hz, 1H), 6.87 (td, J = 8.1, 3.1 Hz, 1H), 4.71 (s, 2H), 2.1 (s, 1H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ 162.4 (d, $^1J_{C\text{-}F}$ = 245 Hz, C); 142.1 (d, $^3J_{C\text{-}F}$ = 7 Hz, C), 133.7 (d, $^3J_{C\text{-}F}$ = 7 Hz, CH), 115.9 (d, $^2J_{C\text{-}F}$ = 22 Hz, CH), 115.8 (C), 115.6 (d, $^2J_{C\text{-}F}$ = 24 Hz, CH), 64.5 (d, $^4J_{C\text{-}F}$ = 1 Hz, CH_2) ppm. Spectroscopic data were consistent with the literature data for this compound.[4]

b) Synthesis of 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (1c). Compound **S3** (1 g, 4.9 mmol) in THF (60 mL) was added to a cooled (0 °C) suspension of sodium hydride (60 % in mineral oil, 280 mg, 5.9 mmol) in THF (10 mL). The reaction was stirred for 1 h, then cooled to −78 °C and *n*-BuLi (1.8 M in hexanes, 3.25 mL, 5.9 mmol) was added dropwise. The resulting mixture was stirred for an additional 1 h. Tri-isopropylborate (1.35 mL, 5.9 mmol) was added at −78 °C and the reaction was stirred for 12 h while gradually warming to rt. The mixture was then acidified to pH = 1 using a 2 M aqueous solution of H_2SO_4 (20 mL) and stirred at rt for 1 h. EtOAc (40 mL) was added, the phases were separated and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with H_2O (2 × 30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using cyclohexane/EtOAc (7/3) as the eluent. A second purification over silica using DCM/MeOH (99/1) as eluent afforded compound **1c** as a white solid (207 mg, 28%). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 9.21

[3] M. Pasco, R. Moumné, T. Lecourt and L. Micouin, *J. Org. Chem.* 2011, **76**, 5137.
[4] C. Z. Ding. *et al*. *Bioorg. Med. Chem. Lett.* 2010, **20**, 7317.

(s, 1H), 7.74-7.76 (m, 1H), 7.23 (d, J = 9.6 Hz, 1H), 7.15 (dt, J = 9.6, 2.1 Hz, 1H), 4.97 (s, 2H) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ 162.4 (d, $^1J_{C\text{-}F}$ = 245 Hz, C), 156.7 (d, $^3J_{C\text{-}F}$ = 9 Hz, C), 132.6 (d, $^3J_{C\text{-}F}$ = 9 Hz, CH), 114.4 (d, $^2J_{C\text{-}F}$ = 22 Hz, CH), 108.4 (d, $^2J_{C\text{-}F}$ = 22 Hz, CH), 69.5 (d, $^4J_{C\text{-}F}$ = 3 Hz, CH_2) ppm. **^{19}F NMR** (470 MHz, DMSO-d_6): δ -110.3 (s, 1F) ppm. Spectroscopic data were consistent with the literature data for this compound.[5]

Synthesis of pdCpA



a) (i) Tetrazole, DCM, rt, 1 h.; (ii) I_2, H_2O/Py/THF, rt, 30 min; (iii) TCA, DCM, rt, 30 min.
b) (i) Bis(2-cyanoethyl)diisopropylphosphoramidite, tetrazole, DCM, rt, 3 h; (ii) I_2, H_2O/Py/THF, rt, 1 h
c) $MeNH_2$, EtOH/H_2O, rt, overnight.

The preparation of pdCpA followed a procedure previously reported by Zhu and Scott.[6]

a) Synthesis of dinucleotide S5. To a solution of Ac-dC-PCNE phosphoramidite (250 mg, 0.32 mmol) in DCM (350 µL) at rt were added tetrazole (0.45 M solution in DCM, 2.9 mL, 1.3 mmol) and **S4**[7] (88 mg, 0.13 mmol) in DCM (350 µL). The reaction was stirred at rt for 1 h, then iodine (81 mg, 0.32 mmol) in THF/H_2O/Pyridine (75/2/20, 3.2 mL) was added. The mixture was stirred at rt for an

[5] D. S. Gunasekera, D. J. Gerold, N. S. Aalderks, J. S. Chandra, C. A. Maanu, P. Kiprof, V. V. Zhdankin and M. V. R. Reddy *Tetrahedron* 2007, **63**, 9401.
[6] X.-F. Zhu and A. I. Scott, *Nucleos. Nucleot. Nucl.*, 2001, **20**, 197.
[7] X.-F. Zhu and A. I. Scott, *Synth. Commun.*, 2008, **38**, 1346.

additional 30 min, then diluted with EtOAc (30 mL). The resulting solution was successively washed with water (3 × 10 mL), saturated $Na_2S_2O_3$ aqueous solution (3 × 10 mL) and brine (2 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was then stirred with a 3% TCA solution in DCM (7.2 mL) at rt for 30 min. The mixture was diluted with DCM (20 mL), washed with a saturated $NaHCO_3$ aqueous solution (3 ×15 mL) and brine (2 × 10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash column chromatography using DCM/MeOH (96/4) as the eluent to afford dinucleotide **S5** as a white foam (89 mg, 65%).

b) Synthesis of dinucleotide S6. Bis(2-cyanoethyl)diisopropylphosphoramidite (117 mg, 0.43 mmol) and tetrazole (0.45 M solution in DCM, 3.8 mL, 1.17 mmol) were added to a solution of dinucleotide **S5** (184 mg, 0.17 mmol) in DCM (2 mL) at rt. The reaction was stirred at rt for 3 h, then iodine (109 mg, 0.43 mmol) in THF/H_2O/Pyridine (75/2/20, 4.3 mL) was added. The resulting mixture was stirred at rt for an additional 1 h, then diluted with EtOAc (30 mL), and successively washed with saturated $Na_2S_2O_3$aqueous solution (3 × 10 mL) and brine (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using DCM/MeOH (96/4) as the eluent to afford the desired phosphorylated dinucleotide **S6** as a white foam (168 mg, 78%).

c) Synthesis of pdCpA: $MeNH_2$ (5 M solution in EtOH/H_2O, 5 mL) was added to dinucleotide **S6** (168 mg, 0.13 mmol) and the resulting solution was stirred at rt overnight. The reaction was then concentrated under reduced pressure, and the crude residue was purified by HPLC (CH_3CN/NH_4OAc 0:100 to 63:37 in 40 min, retention time: 15 min). The appropriate fractions were collected and lyophilized to afford pdCpA (ammonium salt) as a white foam (80 mg, 86%). **^{1}H NMR** (500 MHz, D_2O): δ 8.51 (s, 1H, $H2^{Ad}$ or $H8^{Ad}$), 8.25 (s, 1H, $H2^{Ad}$ or $H8^{Ad}$), 7.80 (d, *J* = 7.6 Hz, 1H, $H6^{Cyt}$), 6.13 (dd, *J* = 8.0, 5.9 Hz, 1H, $H5^{Cyt}$), 6.11 (d, *J* = 5.8 Hz, 1H, $H1'^{Ad}$), 6.06 (d, *J* = 7.6 Hz, 1H, $H1'^{Cyt}$), 4.83 (t, *J* = 5.5 Hz, 1H, $H2'^{Ad}$), $H3'^{Cyt}$ and $H3'^{Ad}$ masked in residual H_2O peak of D_2O, 4.39 (m, 1H, $H4'^{Cyt}$), 4.29 (m, 1H, $H4'^{Ad}$), 4.16-4.16 (m, 2H, $H5'^{Ad}$), 4.04 (m, 2H, $H5'^{Cyt}$), 2.40 (ddd, *J* = 14.0, 5.8, 2.3 Hz, 1H, $H2'^{Cyt}$), 1.89-1.83 (m, 1H, $H2'^{Cyt}$) ppm. **^{13}C NMR** (125 MHz, D_2O): δ 179.9 ($C=O^{Cyt}$), 164.1 (Cq) 154.9 (Cq), 152.5 ($C2^{Ad}$ or $C8^{Ad}$), 149.1 (Cq), 141.6 ($C6^{Cyt}$), 139.7 ($C2^{Ad}$ or $C8^{Ad}$), 118.4 (Cq), 96.1 ($C1'^{Ad}$), 86.7 ($C1'^{Cyt}$), 85.7 ($C5^{Cyt}$), 84.6 ($C4'^{Ad}$), 83.6 ($C3'^{Cyt}$), 75.9 ($C2'^{Ad}$), 73.6 ($C4'^{Cyt}$), 70.1 ($C3'^{Ad}$), 64.7 ($C5'^{Cyt}$), 64.5 ($C5'^{Ad}$), 38.1 ($C2'^{Cyt}$) ppm. Spectroscopic data were consistent with the literature data for this compound.[6]

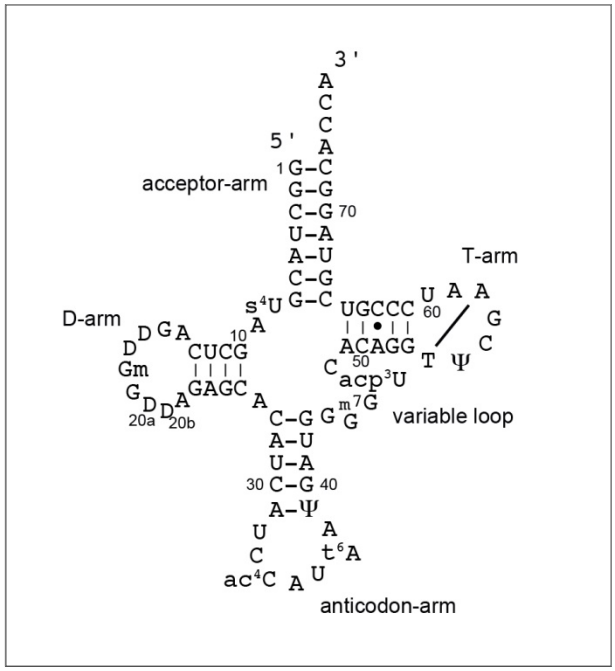
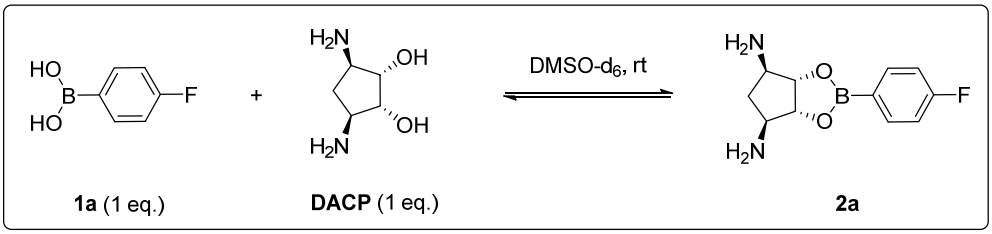


Figure 1: Secondary structure of tRNA$_m^{Met}$ used in this study.

tRNA$_m^{Met}$ was produced in *E. coli* and purified as previously described by Meinnel[8] and Wallis.[9]

[8] T. Meinnel, Y. Mechulam and G. Fayat, *Nucleic Acids Res.*, 1988, **16**, 8095.
[9] N. G. Wallis, F. Dardel and S. Blanquet, *Biochemistry*, 1995, **34**, 7668.

^{1}H NMR and ^{19}F NMR spectroscopic studies



Visualization of the interaction between the commercially available 4-fluorophenylboronic acid **1a** and DACP by NMR spectroscopy in DMSO-d_6. ^{1}H NMR and ^{19}F NMR spectra were recorded on a Bruker Avancell 500, at 500 MHz and 470 MHz respectively.

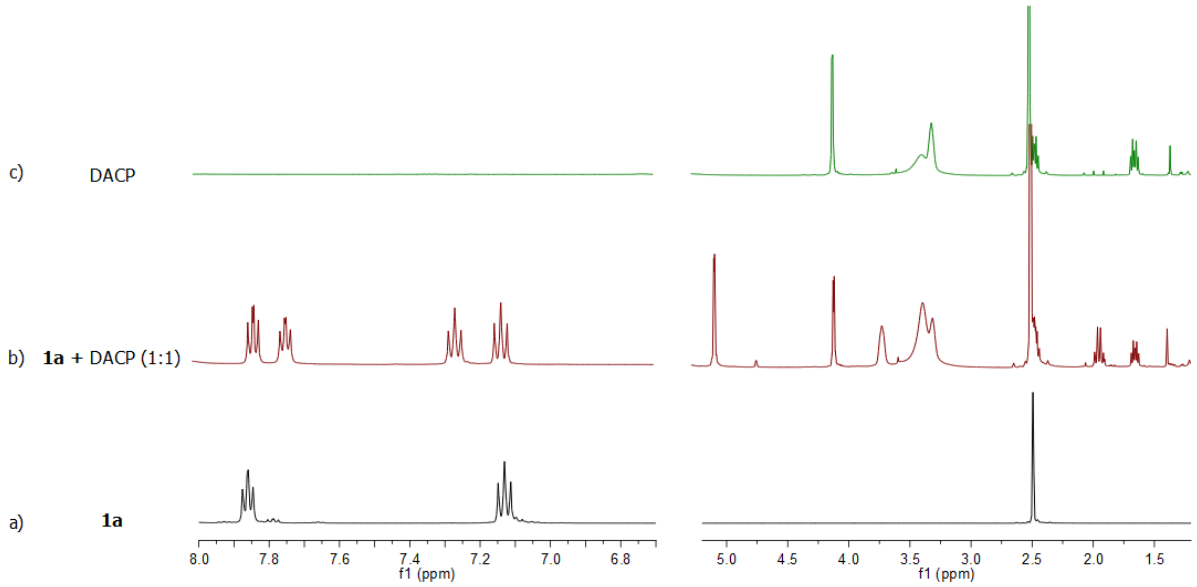


Figure 2: Selected areas of the ^{1}H NMR spectra of (a) 60 mM of **1a**, (b) 60 mM of **1a** and 60 mM of DACP, (c) 60 mM of DACP. The samples were dissolved in DMSO-d_6 (500 µL) and the spectra were acquired at 300K. The solvent residual peak was used as reference (2.49 ppm).

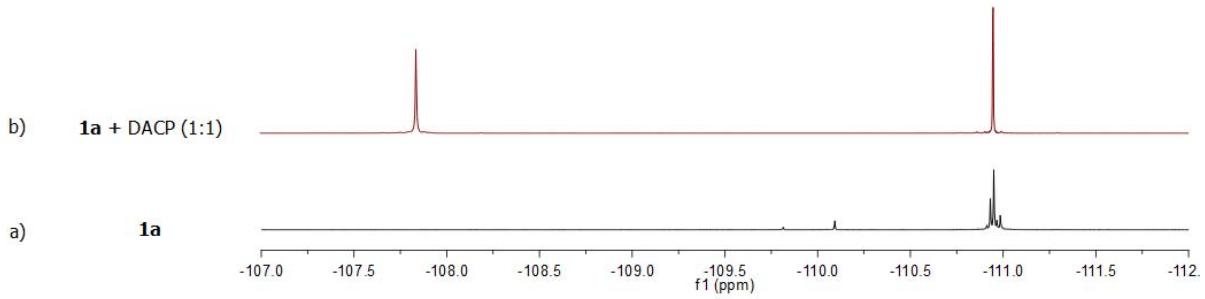
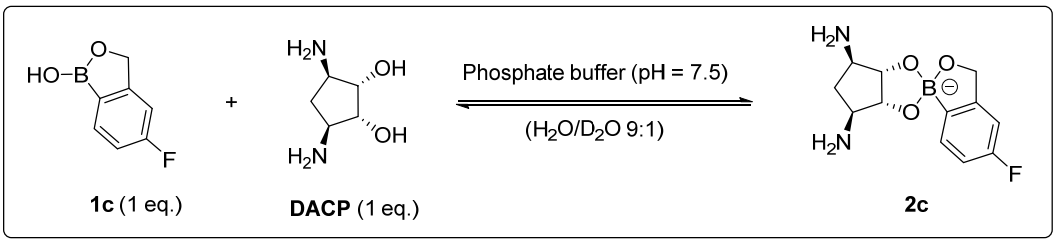


Figure 3: Selected areas of the ^{19}F NMR spectra of (a) 60 mM of **1a**, (b) 60 mM of **1a** and 60 mM of DACP. The samples were dissolved in DMSO-d_6 (500 µL) and the spectra were acquired at 300K.



Visualization of the interaction between 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole **1c** and DACP by NMR spectroscopy in a phosphate buffer solution at pH = 7.5 (10% of D_2O was added in order to lock the samples). ^1H NMR spectra were recorded on a Bruker Avancell 500, at 500 MHz.

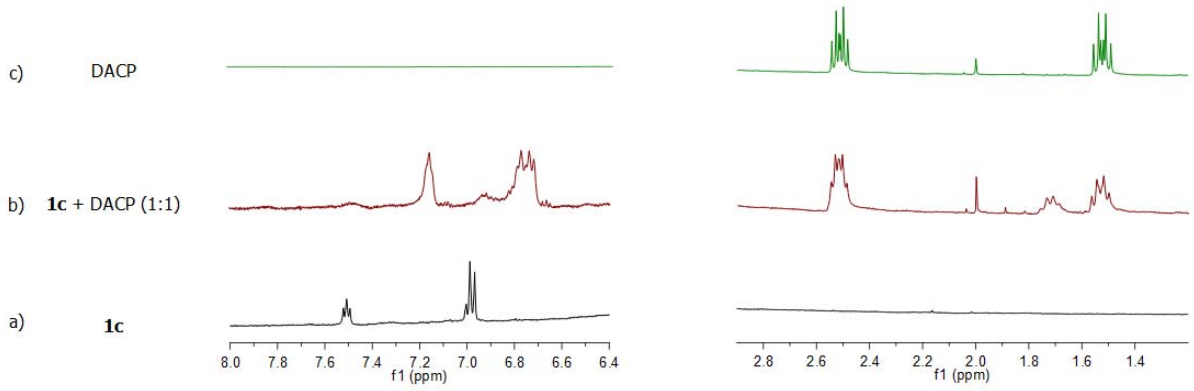
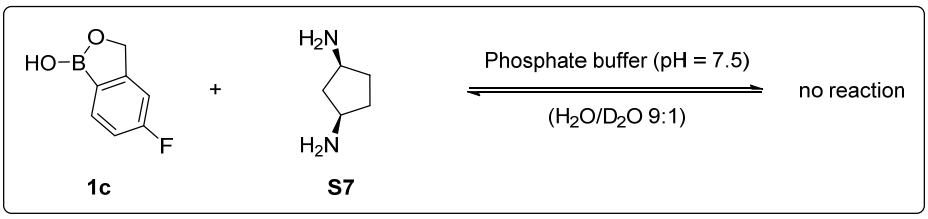


Figure 4: Selected areas of the ^1H NMR spectra of (a) 62 mM of **1c**, (b) 62 mM of **1c** and 62 mM of DACP, (c) 62 mM of DACP. The samples were dissolved in a phosphate buffer solution at pH = 7.5 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.



Control experiment: no reaction between 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole **1c** and cyclopentane-1,3-diamine **S7** could be observed by NMR spectroscopy in a phosphate buffer solution at pH = 7.5 (10% of D_2O was added in order to lock the samples). ^{19}F NMR spectra were recorded on a Bruker Avancell 500, at 470 MHz.

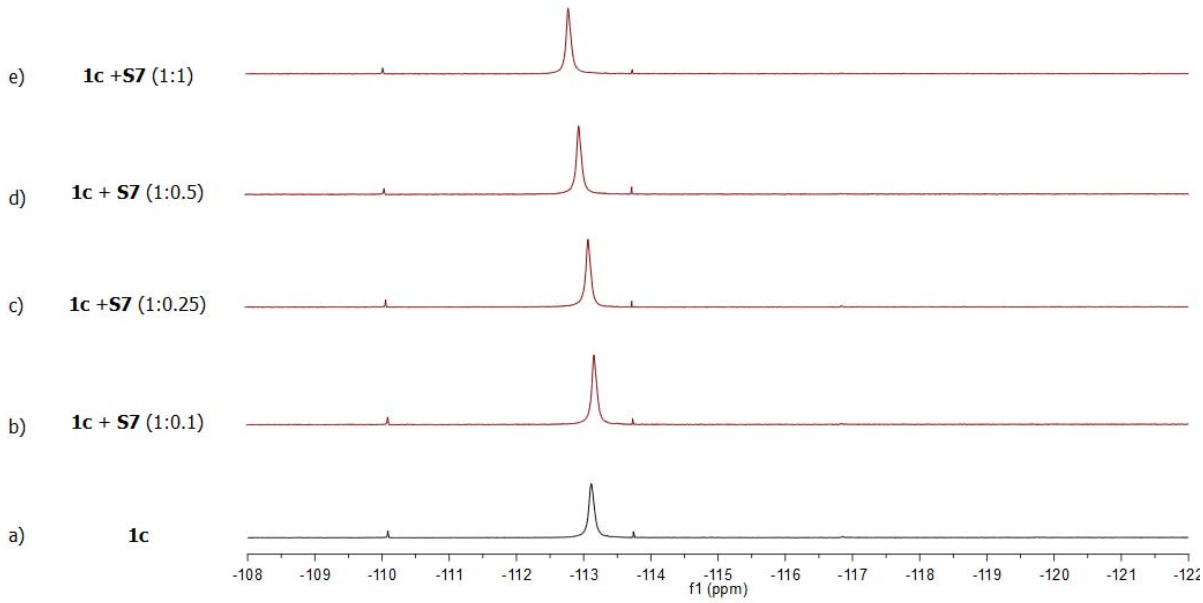
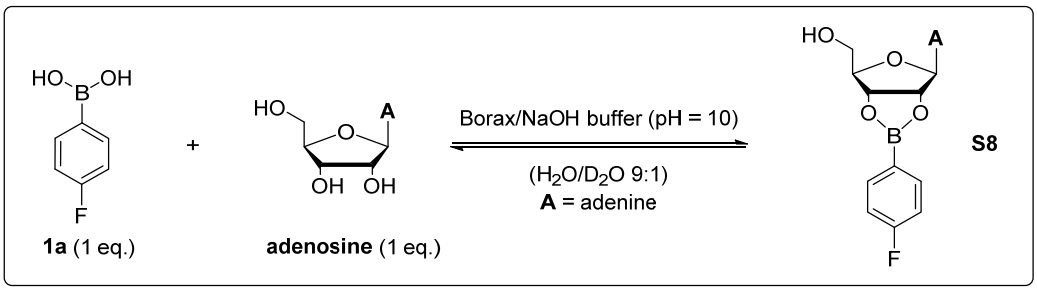


Figure 5: Selected areas of the ^{19}F NMR spectra of (a) 52 mM of **1c**, (b) 52 mM of **1c** and 5.2 mM of **S7**, (c) 52 mM of **1c** and 13 mM of **S7**, (d) 52 mM of **1c** and 26 mM of **S7**, (e) 52 mM of **1c** and 52 mM of **S7**. The samples were dissolved in a phosphate buffer solution at pH = 7.5 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.



Visualization of the interaction between the commercially available 4-fluorophenylboronic acid **1a** and adenosine by NMR spectroscopy in a borax/NaOH buffer solution at pH = 10 (10% of D_2O was added in order to lock the samples). ^{1}H NMR and ^{19}F NMR spectra were recorded on a Bruker Avancell 500, at 500 MHz and 470 MHz respectively.

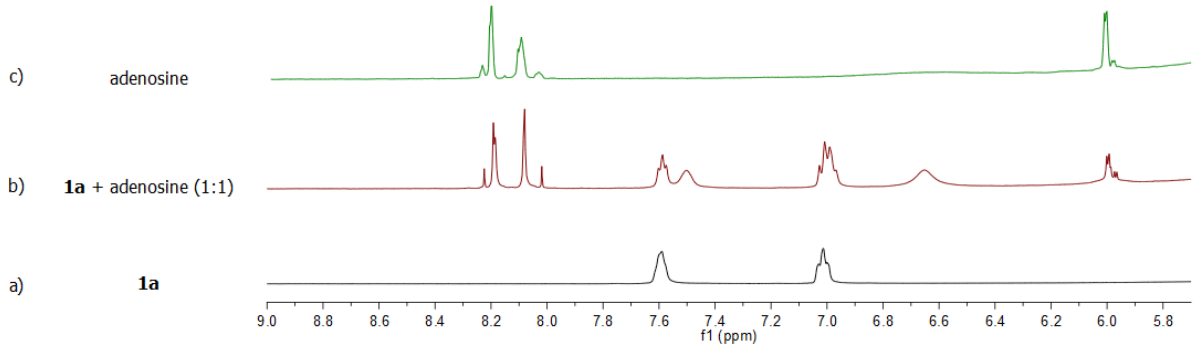


Figure 6: Selected areas of the ^{1}H NMR spectra of (a) 63 mM of **1a**, (b) 63 mM of **1a** and 63 mM of adenosine, (c) 63 mM of adenosine. The samples were dissolved in a borax/NaOH buffer solution at pH = 10 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.

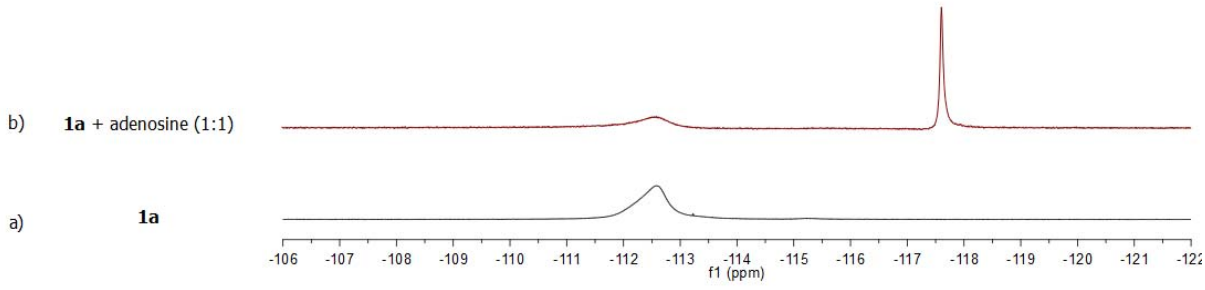
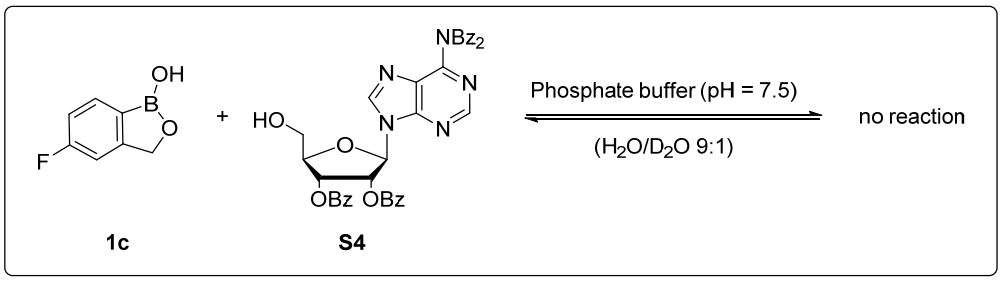


Figure 7: Selected areas of the ^{19}F NMR spectra of (a) 63 mM of **1a**, (b) 63 mM of **1a** and 63 mM of adenosine. The samples were dissolved in a borax/NaOH buffer solution at pH = 10 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.



Control experiment: no reaction between 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole **1c** and the 2',3'-diol-protected adenosine **S4** could be observed by NMR spectroscopy in a phosphate buffer solution at pH = 7.5 (10% of D_2O was added in order to lock the samples). ^{19}F NMR spectra were recorded on a Bruker Avancell 500, at 470 MHz.

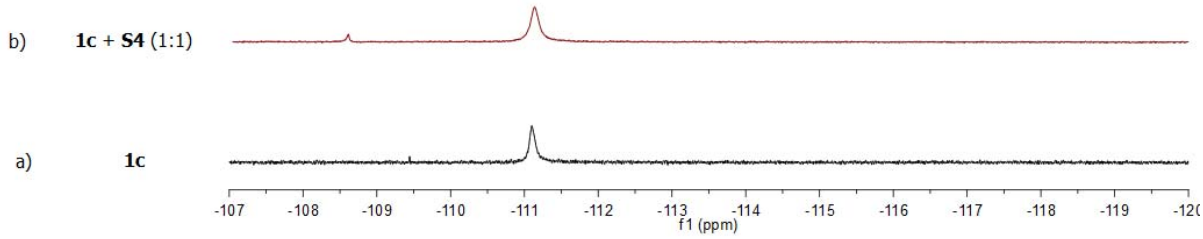
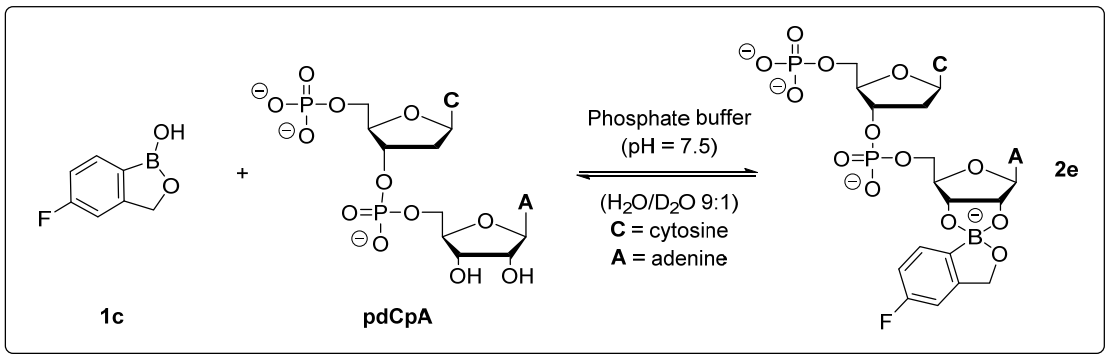


Figure 8: Selected areas of the ^{19}F NMR spectra of (a) 63 mM of **1c**, (b) 63 mM of **1c** and 63 mM of **S4**. The samples were dissolved in a phosphate buffer solution at pH = 7.5 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.



Visualization of the interaction between 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole **1c** and pdCpA by NMR spectroscopy in a phosphate buffer solution at pH = 7.5 (10% of D_2O was added in order to lock the samples). ^1H NMR spectra were recorded on a Bruker Avance 600 MHz Ultrashield, at 600 MHz.

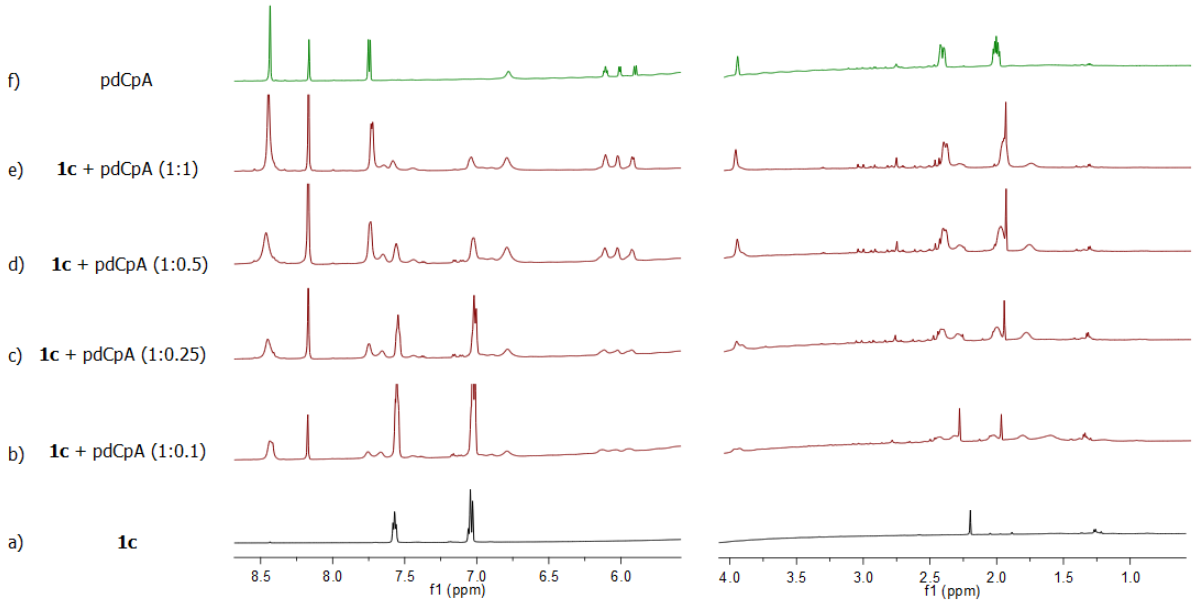
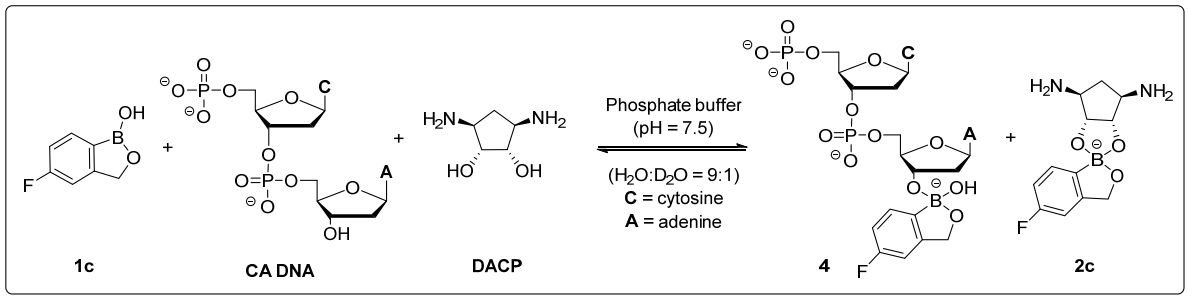


Figure 9: Selected areas of the ^1H NMR spectra of (a) 10 mM of **1c**, (b) 10 mM of **1c** and 1 mM of pdCpA, (c) 10 mM of **1c** and 2.5 mM of pdCpA, (d) 10 mM of **1c** and 5 mM of pdCpA, (e) 10 mM of **1c** and 10 mM of pdCpA, (f) 10 mM of pdCpA. The samples were dissolved in a phosphate buffer solution at pH = 7.5 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.



Visualization of the interaction between 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole **1c,** CA DNA and DACP by NMR spectroscopy in a phosphate buffer solution at pH = 7.5 (10% of D_2O was added in order to lock the samples). ^{19}F NMR spectra were recorded on a Bruker Avancell 500, at 470 MHz.

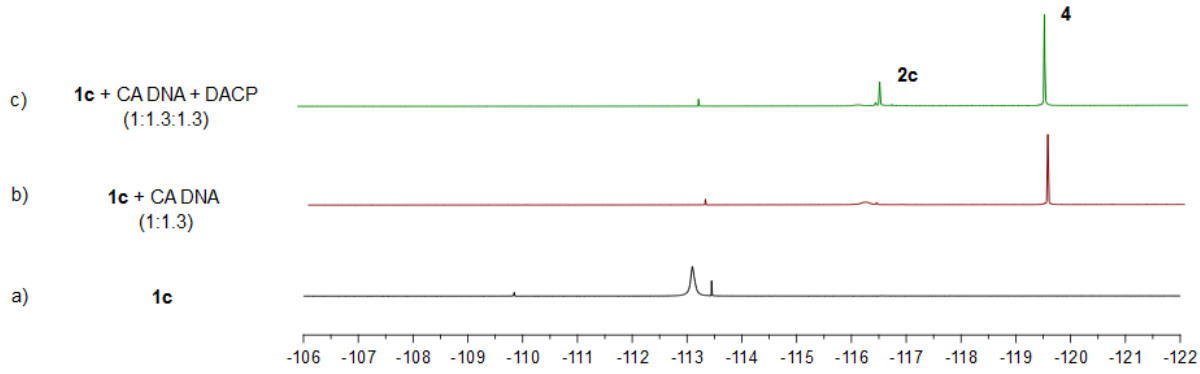
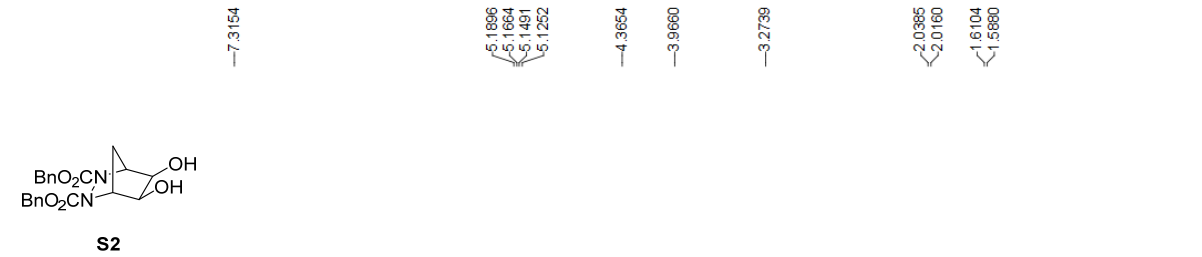


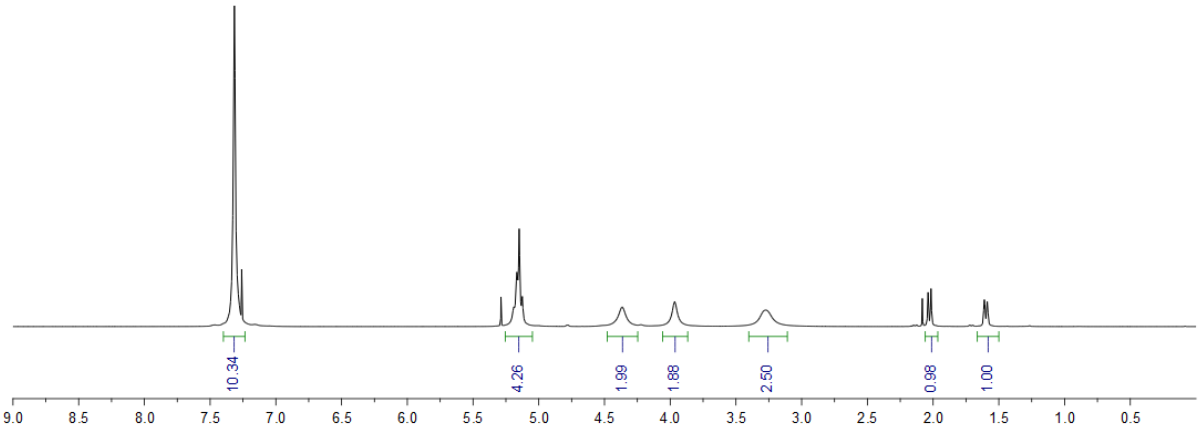
Figure 10: Selected areas of the ^1H NMR spectra of (a) 10 mM of **1c**, (b) 10 mM of **1c** and 13 mM of CA DNA, (c) 10 mM of **1c**, 13 mM of CA DNA and 13 mM of DACP. The samples were dissolved in a phosphate buffer solution at pH = 7.5 (450 µL), D_2O (50 µL) was added, and the spectra were acquired at 300K.

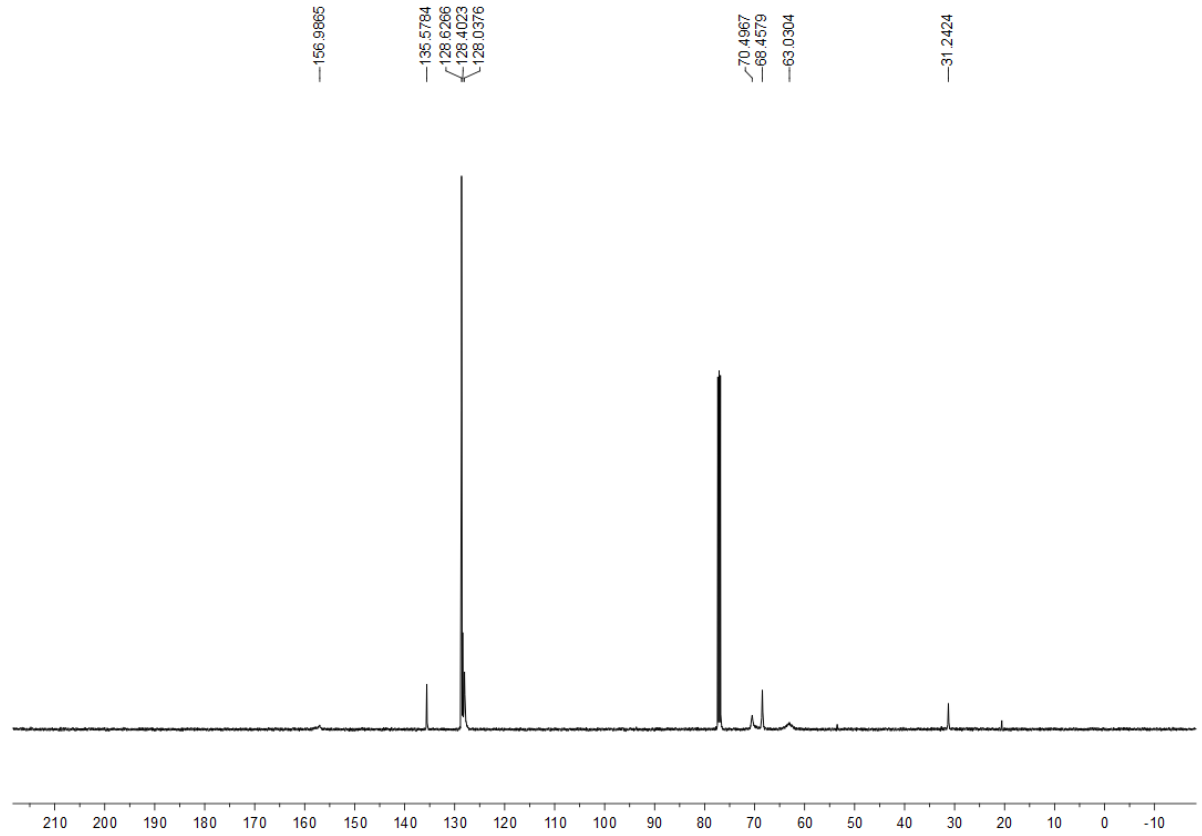
Copies of ^{1}H NMR and ^{13}C NMR

Dibenzyl-5,6-dihydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (S2).

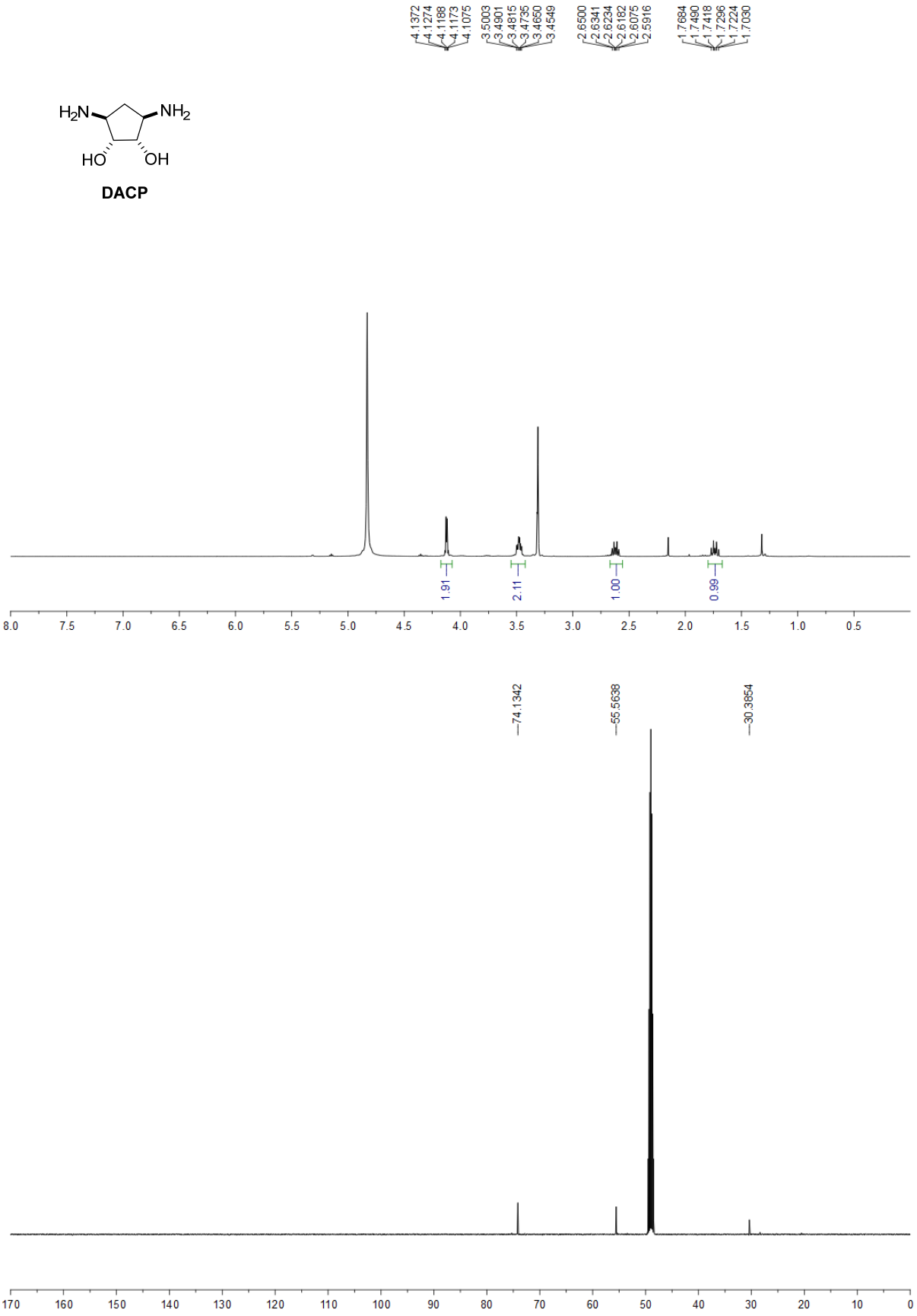


S2

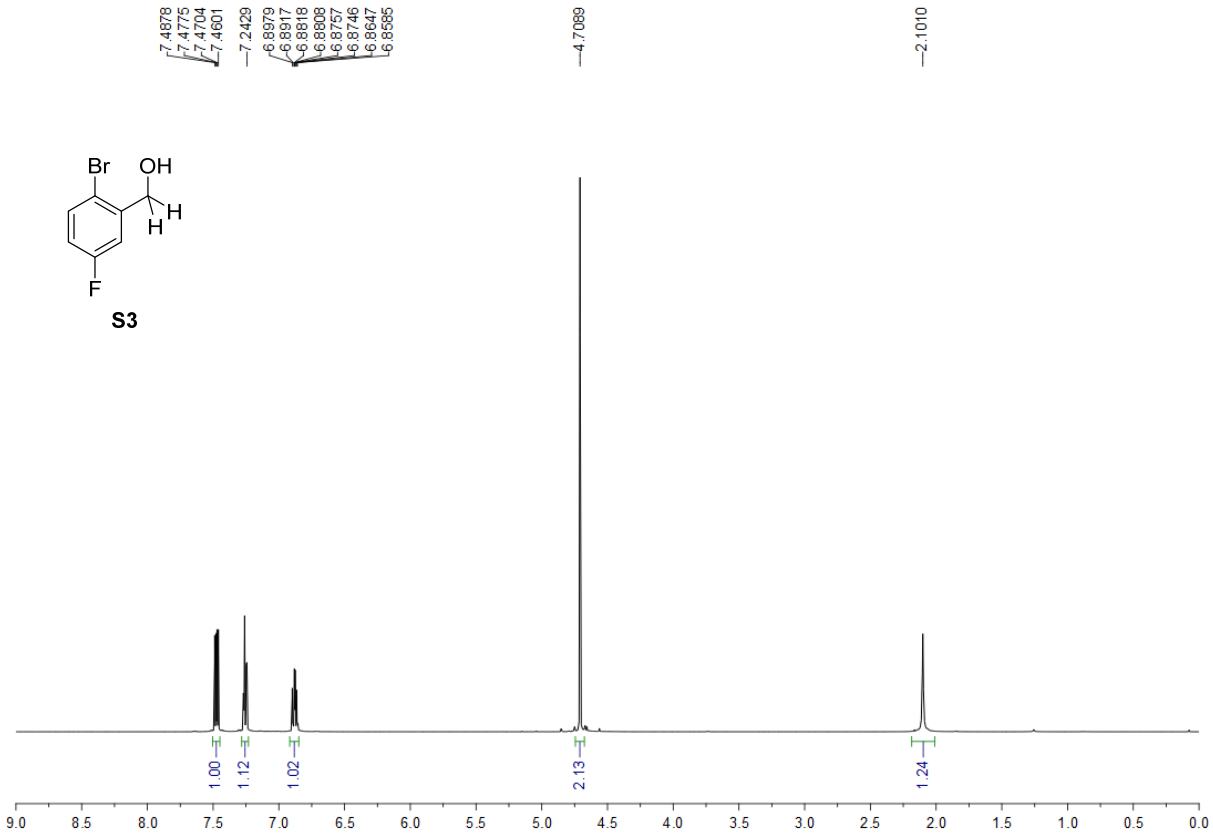


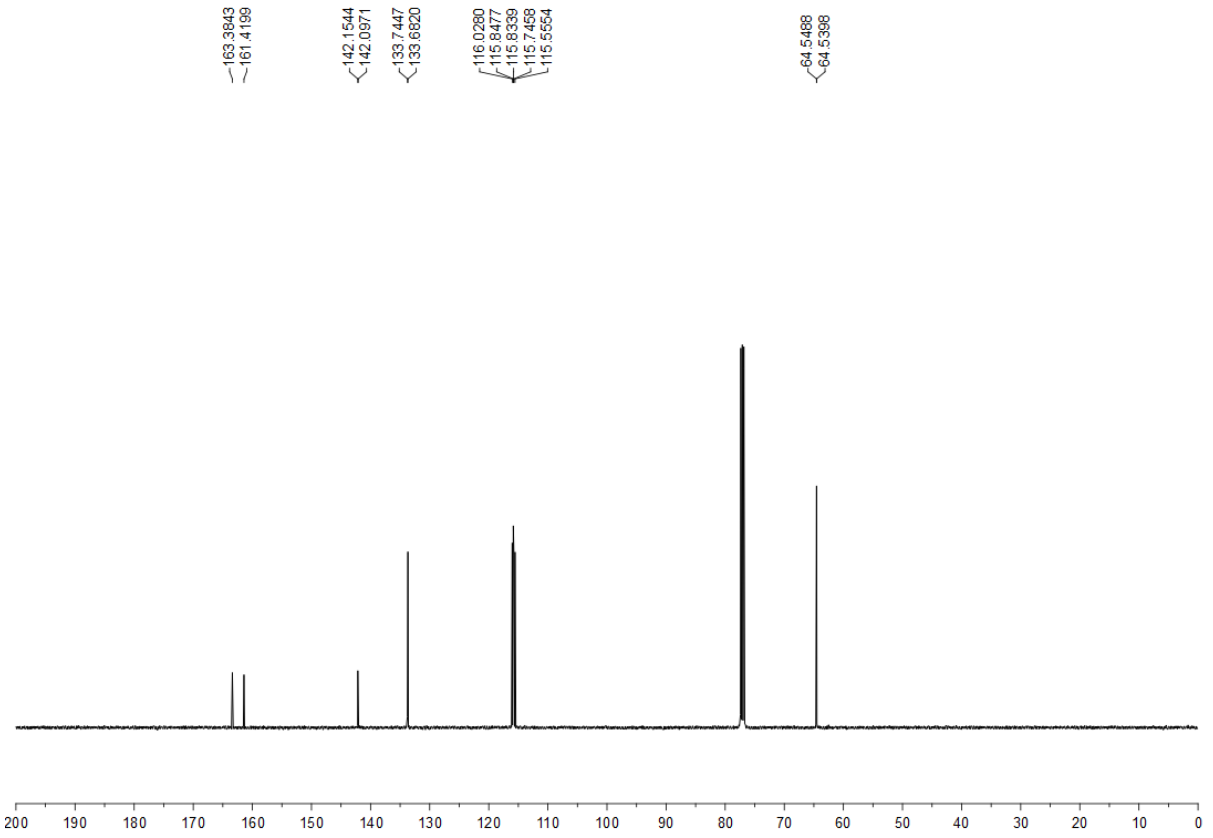


3,5-Diaminocyclopentane-1,2-diol (DACP).

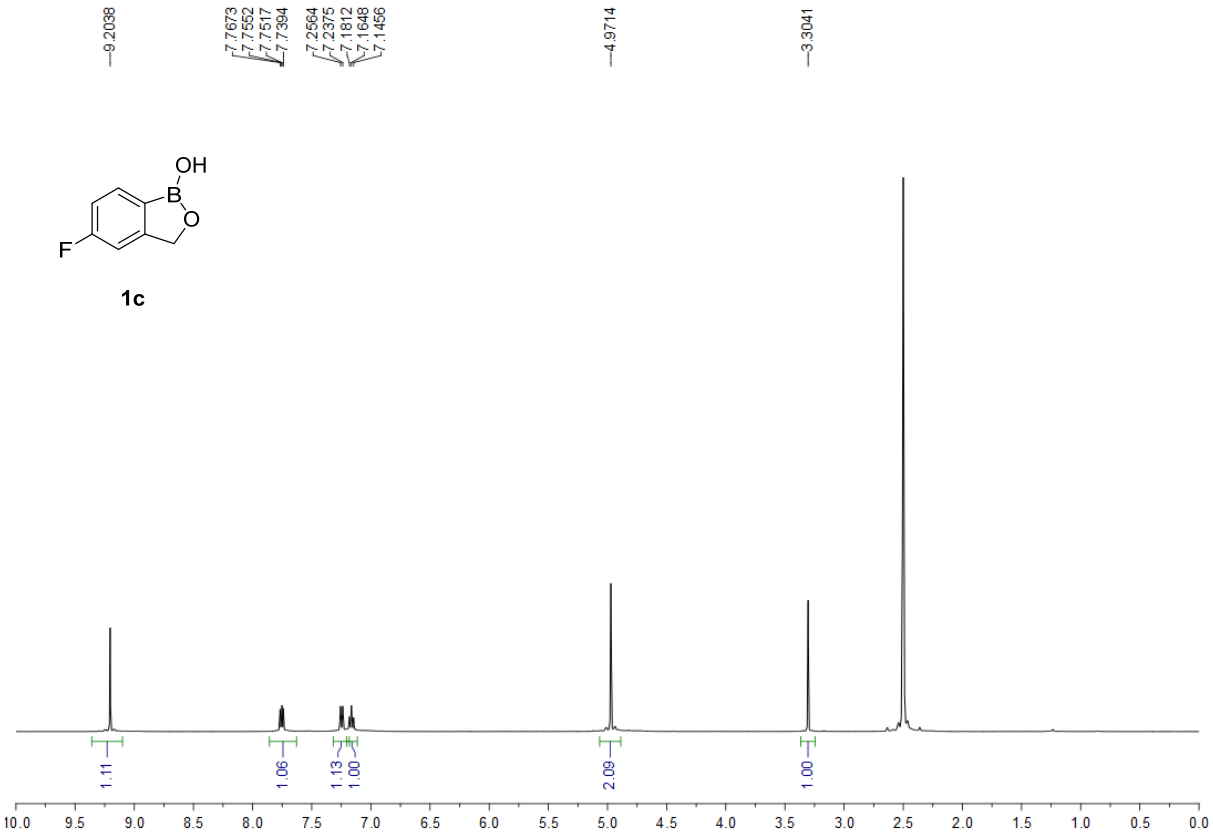


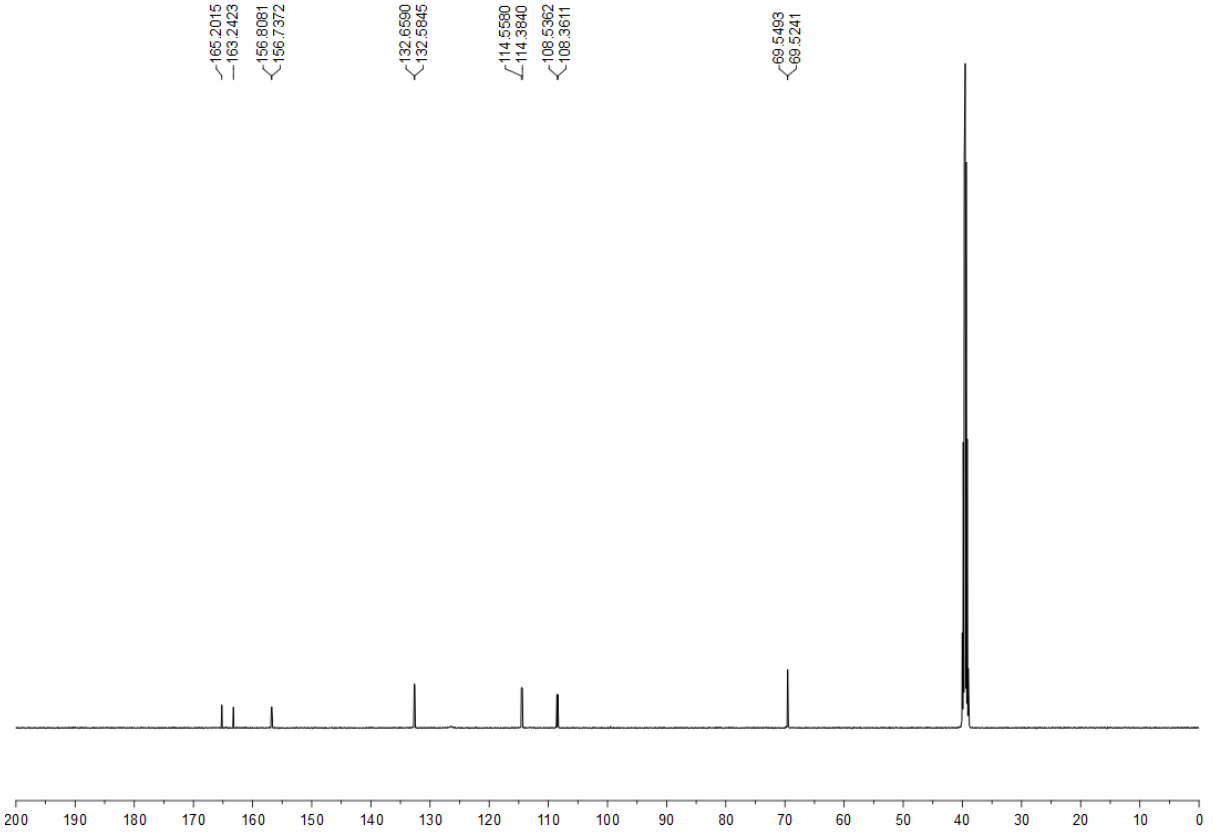
(2-Bromo-5-fluorophenyl)methanol (S3).



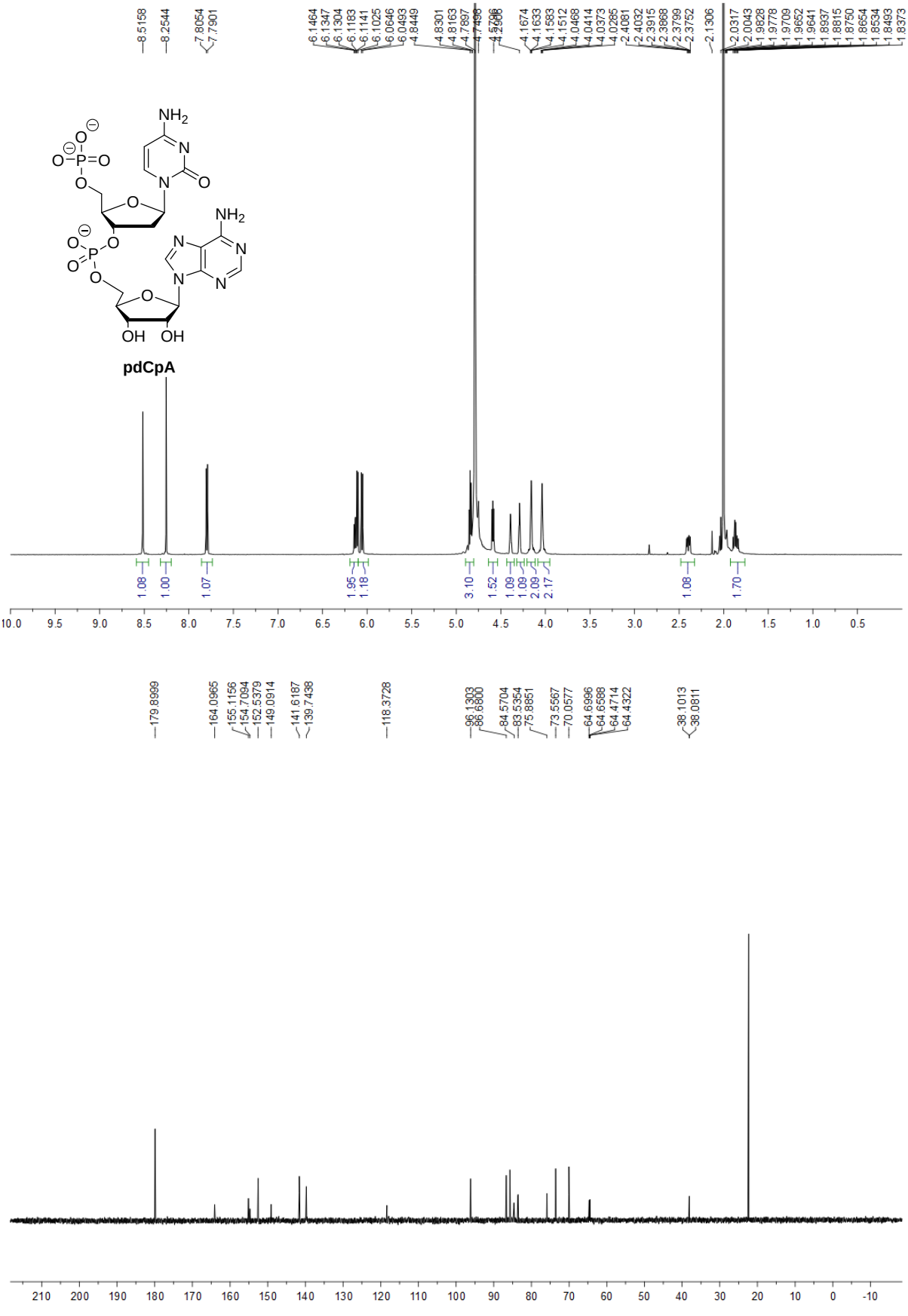


5-Fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (1c).



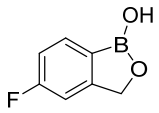


pdCpA.



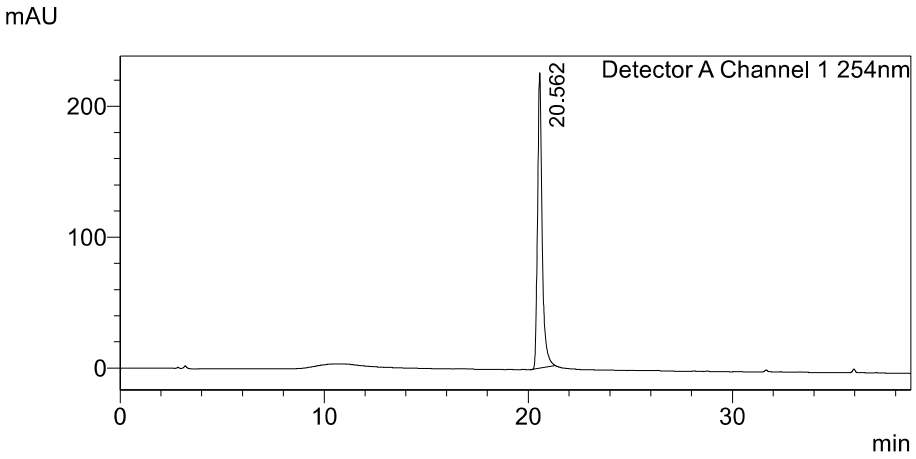
HPLC

5-Fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (1c).



1c

Chromatogram:



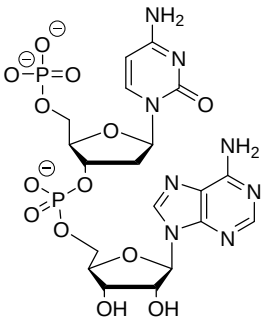
Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.
1	20.562	3488725	225365	100.000
Total		3488725	225365	

Conditions:

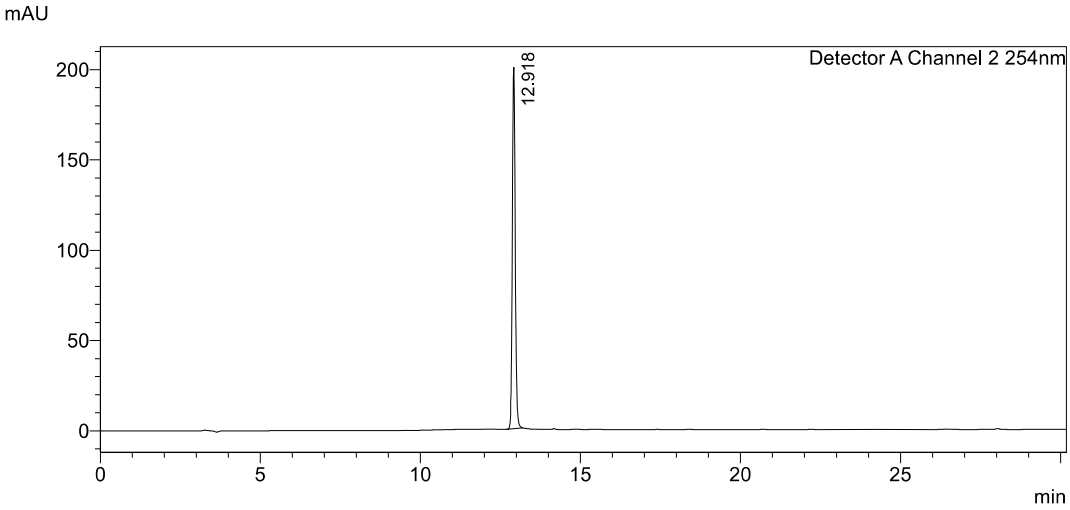
HPLC reversed-phase C-18 column (NUCLEOSIL), 1.3 mL/min
Gradient: Linear gradient of MeCN:H_2O (0.1 % TFA) from 0:100 to 100:0 over 30 min.

pdCpA.



pdCpA

Chromatogram:



Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Height	Conc.
1	12.918	1249883	200164	100.000
Total		1249883	200164	

Conditions:

HPLC reversed-phase C-18 column (NUCLEOSIL), 1.3 mL/min
Gradient: Linear gradient of MeCN:NH_4OAc from 0:100 to 63:37 over 30 min.