Supporting Information for:

Synthesis, Characterization, and Binding Property of Isoelectronic Analogues of Nucleobases, B(6)-Substituted 5-Aza-6-borauracils and -thymines

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1. Materials and Instruments

Materials. Mesitylborane,[1] thexylborane,[2] and methylbiuret[3] were synthesized according to the reported methods. Starting materials and dehydrated solvents were obtained from Tokyo Chemical Industry (Tokyo, Japan) or Wako Pure Chemical Industries (Osaka, Japan). Tetrahydrofuran (THF) was distilled from sodium wire and benzophenone before use. Silica gel (SiO_2) for column chromatography was purchased from Merck (Darmstadt, Germany). Bio-beads SX-8 was purchased from Bio-Rad Laboratories (Richmond, USA).

Instruments. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Varian Mercury 300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. ^{11}B NMR spectra were recorded on a JEOL Alpha 500 spectrometer operating at 160 MHz. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) for ^{1}H, the deuterated solvent for ^{13}C, and external $BF_3{\cdot}OEt_2$ for ^{11}B, respectively. IR spectra were recorded on a JASCO FT/IR-480plus

spectrophotometer. Absorption and fluorescence spectra were measured using a 10-mm quartz cell on a JASCO V-550 spectrophotometer and a JASCO FP-6500 spectropolarimeter, respectively. X-Ray data were collected on a Rigaku/MSC Mercury diffractometer with graphite monochromated Mo Kα radiation. Melting points were measured on a Yamato melting point apparatus MP-21. Elemental analyses were performed at the Microanalytical Laboratory of Department of Chemistry, Graduate School of Science, The University of Tokyo.

2. Synthetic Procedures

$MesU_{BN}$. A solution of mesitylborane in dry THF (0.20 M, 10 mL, 2.0 mmol) was added dropwise to biuret (206 mg, 2.0 mmol) at rt. After the mixture was stirred at room temperature overnight, the solvent was evaporated to dryness. The residue was purified by recycling GPC (JAIGEL 1H+2H, $CHCl_3$) to afford **$MesU_{BN}$** (118 mg, 26% yield) as a white solid. Mp over 220 °C. IR (KBr, cm^{-1}) 3427, 3361, 3206, 3087, 2919, 2853, 1723, 1699, 1498, 1446, 1413, 1308, 1236, 1154, 1046, 933, 854, 517, 433. ^{1}H NMR (300 MHz, 10 mM, $CDCl_3$) δ 7.92 (br-s, 1H, NH), 6.87 (s, 2H, Ar-H), 6.60 (br-s, 2H, NH), 2.29 (s, 3H, Ar-CH_3), 2.28 (s, 6H, Ar-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.14, 140.31, 140.06, 127.89, 22.31, 21.34. ^{11}B NMR (160 MHz, $CDCl_3$) δ 35.10. Anal. Calcd for $C_{11}H_{14}BN_3O_2 \cdot 1/4CHCl_3$: C, 51.79; H, 5.51; N, 16.11. Found: C, 51.53; H, 5.58; N, 16.09.

$MesT_{BN}$. A solution of mesitylborane in dry THF (0.50 M, 48 mL, 24 mmol) was added dropwise to methylbiuret (2.68 g, 22.9 mmol) at 0 °C. After the mixture was stirred at room temperature overnight, the solvent was evaporated to dryness. The residue was purified by column chromatography (SiO_2, hexane/THF = 4/1 to 1/1 (v/v)), recycling GPC (JAIGEL 1H+2H, $CHCl_3$), and then recrystalization ($CHCl_3$/hexane) to afford **$MesT_{BN}$** (1.46 g, 26% yield) as a white

solid. Mp over 220 °C. IR (KBr, cm^{-1}) 3244, 3066, 2920, 2855, 1724, 1697, 1612, 1560, 1448, 1412, 1375, 1277, 1144, 1028, 847, 780, 651, 597, 527, 472, 418. ^{1}H NMR (300 MHz, 20 mM, $CDCl_3$) δ 8.48 (br-s, 1H, NH), 6.87 (s, 2H, Ar-H), 6.68 (br-s, 1H, NH), 2.90 (s, 3H, N-CH_3), 2.30 (s, 3H, Ar-CH_3), 2.22 (s, 6H, Ar-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.68, 152.97, 139.82, 139.28, 127.77, 31.13, 21.95, 21.36. ^{11}B NMR (160 MHz, $CDCl_3$) δ 35.61. Anal. Calcd for $C_{12}H_{16}BN_3O_2$: C, 58.81; H, 6.58; N, 17.15. Found: C, 58.59; H, 6.52; N, 17.15.

1,5-dimethylbiuret. A mixture of *N*-ethoxycarbonylurethane (2.87 g, 17.8 mmol) and aqueous methylamine (23 mL, 40%wt.) was stirred overnight at room temperature. The solvent was removed under reduced pressure. The residue was recystallized from $CHCl_3$/hexane to afford 1,5-dimethylbiuret (0.677 g, 29% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ 8.68 (br-s, 1H, NH), 7.18 (br-s, 2H, NH), 2.62 (d, J = 4.2 Hz, 6H, CH_3).

m^1MesT_{BN}. A solution of mesitylborane in dry THF (0.20 M, 10 mL, 2.0 mmol) was added dropwise to 1,5-dimethylbiuret (262 mg, 2.0 mmol) at rt. After the mixture was stirred at room temperature overnight, the solvent was evaporated to dryness. The residue was purified by recycling GPC (JAIGEL 1H+2H, $CHCl_3$) to afford **m^1MesT_{BN}** (225 mg, 43% yield) as a white solid. Mp 209–212 °C. IR (KBr, cm^{-1}) 3437, 3201, 3066, 2921, 1733, 1709, 1611, 1483, 1450, 1415, 1391, 1258, 1178, 1150, 1025, 769, 736, 655. ^{1}H NMR (300 MHz, 10 mM, $CDCl_3$) 7.77 (br-s, 1H, NH), 6.89 (s, 2H, Ar-H), 2.86 (s, 6H, N-CH_3), 2.31 (s, 3H, Ar-CH_3), 2.16 (s, 6H, Ar-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.40, 139.43, 139.41, 127.86, 31.43, 21.62, 21.38. ^{11}B NMR (160 MHz, $CDCl_3$) δ 36.53. Anal. Calcd for $C_{13}H_{18}BN_3O_2$: C, 60.26; H, 7.00; N, 16.22. Found: C, 60.15; H, 7.08; N, 16.14.

$ThxT_{BN}$. A solution of 2,3-dimethylbut-2-ene in THF (1.0 M, 10 mL, 10 mmol) was added dropwise to a solution of BH_3·THF in THF (1.0 M, 10 mL, 10 mmol) in a period of 10 min at 0 °C, and the mixture was stirred for 1 h at 0 °C. The resultant solution was added dropwise to methylbiuret (1.17 g, 10.0 mmol) at 0 °C. After the mixture was stirred at room temperature overnight, the solvent was evaporated to dryness. The residue was washed with dry hexane and purified by SEC (Bio-Beads S-X8, $CHCl_3$) and recycling GPC (JAIGEL 1H+2H, $CHCl_3$) to afford **$ThxT_{BN}$** (1.10 g, 52% yield) as a white solid. Mp 132-135 °C. IR (KBr, cm^{-1}) 3421, 3270, 3200, 3066, 2960, 2873, 1725, 1687, 1469, 1416, 1394, 1375, 1355, 1264, 1063, 1035, 997, 869, 786, 758, 658, 601, 547, 490, 479, 447. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.90 (br-s, 1H, NH), 6.67 (br-s, 1H, NH), 3.21 (s, 3H, N-CH_3), 2.04 (m, 1H, thexyl), 1.02 (s, 6H, thexyl), 0.85 (d, J = 6.8 Hz, 6H, thexyl). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ 155.94, 153.65, 33.03, 31.86, 21.61, 18.48. ^{11}B NMR (160 MHz, $CDCl_3$, 25 °C) δ 36.93. Anal. Calcd for $C_9H_{18}BN_3O_2$: C, 51.21; H, 8.60; N, 19.91. Found: C, 51.00; H, 8.71; N, 19.93.

3. X-ray Crystallography

The single crystals (**$MesU_{BN}$**, **$MesT_{BN}$** and **$MesT_{BN}$·DAP**) were prepared by a slow evaporation method from a chloroform–hexane solution at rt. Details of the crystal data and a summary of the intensity data collection parameters for **$MesU_{BN}$**, **$MesT_{BN}$** and **$MesT_{BN}$·DAP** are listed in Tables S1–3. In each case a suitable crystal was mounted with a mineral oil to the glass fiber and attached to the goniometer of a Rigaku Mercury CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). The structures were solved by direct methods with SIR-97[4] and refined by full-matrix least-squares techniques against F^2 (SHELXL-97).[5] The intensities were corrected for Lorentz and polarization effects. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically in the difference Fourier maps or placed using AFIX instructions.

References and Notes

(1) Matsumi, N.; Chujo, Y. *Polym. Bull.* **1997**, *38*, 531–536.

(2) Schwier, J. R.; Brown, H. C. *J Org. Chem.* **1993**, *58*, 1546–1552.

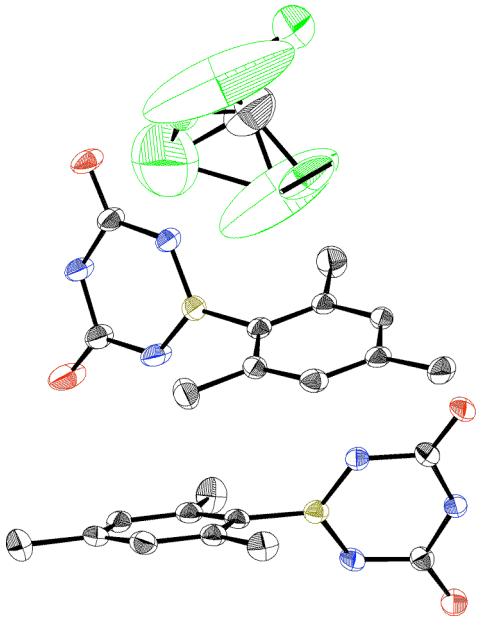
(3) Renis, H. E.; Skulnic, H. I. U.S. Patent 4239753, **1980**.

(4) A ltomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Cryst.* **1999**, *32*, 115–119.

(5) Sheldrick, G. M.; University of Göttingen: Göttingen, Germany, 1997.

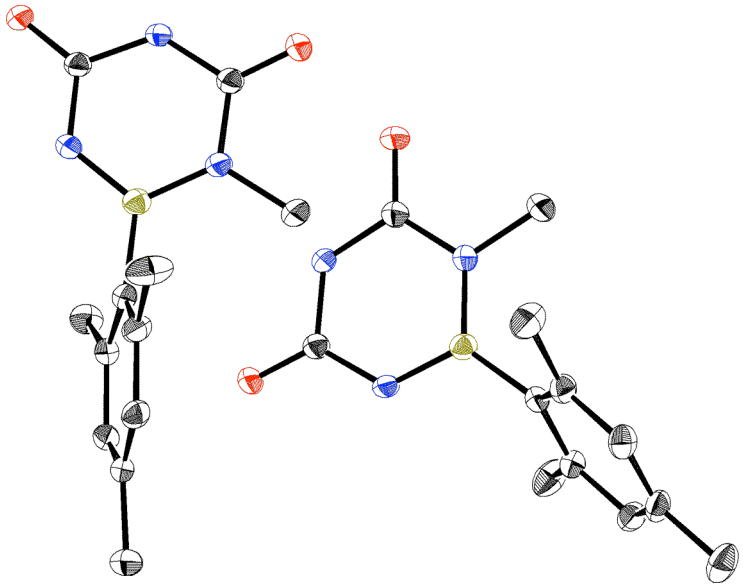
4. Crystal Structure of $MesU_{BN}$, and $MesT_{BN}$ and $MesT_{BN}s$·DAP

Table S1. Crystallographic data and structure refinement details for **$MesU_{BN}$**.



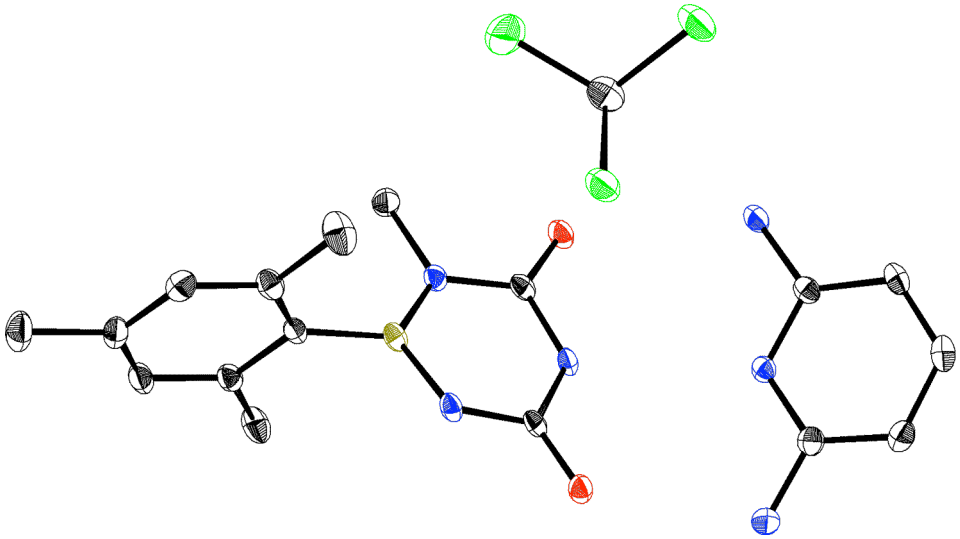
Empirical formula	C23 H29 B2 N6 O4 Cl3	
Formula weight	581.49	
Temperature	103(2) K	
Wavelength	0.71070 Å	
Crystal system	Triclinic	
Space group	P–1	
Unit cell dimensions	a = 8.673(4) Å	α = 100.899(5)°
	b = 10.421(5) Å	β = 94.817(5)°
	c = 17.737(8) Å	γ = 114.075(6)°
Volume	1413.9(12) $Å^3$	
Z	2	
Density (calculated)	1.366 Mg/m^3	
Absorption coefficient	0.365 mm^{-1}	
F(000)	604	
Crystal size	0.40 x 0.30 x 0.20 mm^3	
Theta range for data collection	2.75 to 25.00°.	
Index ranges	–10<=h<=10, –12<=k<=12, –14<=l<=21	
Reflections collected	9126	
Independent reflections	4836 [R(int) = 0.0306]	
Completeness to theta = 25.00°	96.7 %	
Max. and min. transmission	0.9307 and 0.8679	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	4836 / 0 / 377	
Goodness-of-fit on F2	1.080	
Final R indices [I>2sigma(I)]	R1 = 0.0854, wR2 = 0.2533	
R indices (all data)	R1 = 0.0988, wR2 = 0.2686	
Largest diff. peak and hole	1.956 and -0.824 $e.A^{-3}$	

Table S1 2. Crystallographic data and structure refinement details for **MesT$_{BN}$**.

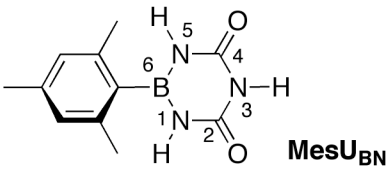


Empirical formula	C24 H32 B2 N6 O4	
Formula weight	490.18	
Temperature	103(2) K	
Wavelength	0.71069 Å	
Crystal system	Triclinic	
Space group	P–1	
Unit cell dimensions	a = 8.789(5) Å	α = 83.320(5)°
	b = 12.196(5) Å	β = 86.166(5)°
	c = 12.799(5) Å	γ = 71.603(5)°
Volume	1292.3(10) Å^3	
Z	2	
Density (calculated)	1.260 Mg/m^3	
Absorption coefficient	0.086 mm^{-1}	
F(000)	520	
Crystal size	0.80 x 0.35 x 0.15 mm^3	
Theta range for data collection	1.60 to 25.00°.	
Index ranges	–10<=h<=10, –14<=k<=13, –15<=l<=14	
Reflections collected	8069	
Independent reflections	4350 [R(int) = 0.0271]	
Completeness to theta = 25.00°	95.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9872 and 0.9343	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	4350 / 0 / 333	
Goodness-of-fit on F2	1.088	
Final R indices [I>2sigma(I)]	R1 = 0.0454, wR2 = 0.1320	
R indices (all data)	R1 = 0.0517, wR2 = 0.1384	
Largest diff. peak and hole	0.316 and –0.346 e.Å^{-3}	

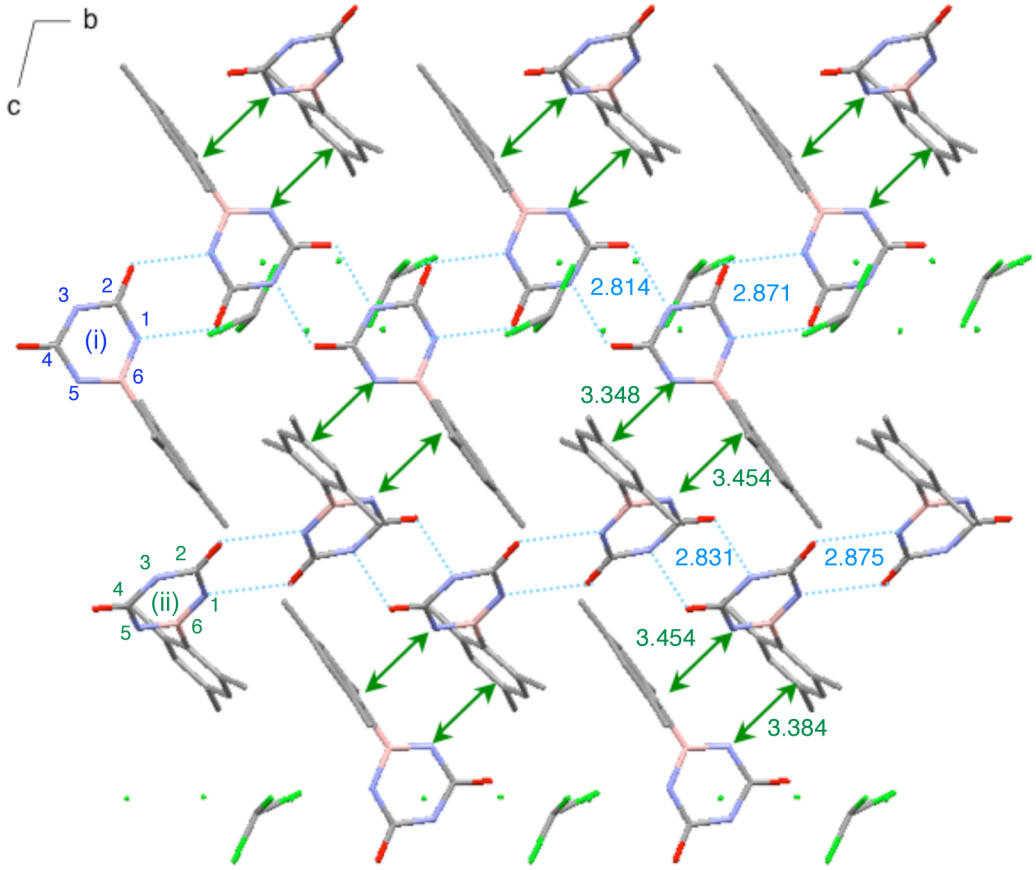
Table S2-3. Crystallographic data and structure refinement details for **MesT$_{BN}$•DAP** complex.



Empirical formula	C18 H24 B Cl3 N6 O2	
Formula weight	473.59	
Temperature	103(2) K	
Wavelength	0.71070 Å	
Crystal system	Triclinic	
Space group	P–1	
Unit cell dimensions	a = 8.135(4) Å	α = 68.629(18)°
	b = 11.106(5) Å	β = 74.897(19)°
	c = 14.144(6) Å	γ = 77.76(2)°
Volume	1139.1(9) Å^3	
Z	2	
Density (calculated)	1.381 Mg/m^3	
Absorption coefficient	0.429 mm^{-1}	
F(000)	492	
Crystal size	0.50 x 0.50 x 0.20 mm^3	
Theta range for data collection	2.62 to 25.00°.	
Index ranges	–9<=h<=9, –13<=k<=13, –16<=l<=16	
Reflections collected	7337	
Independent reflections	3904 [R(int) = 0.1014]	
Completeness to theta = 25.00°	97.3 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9191 and 0.8140	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	3904 / 0 / 307	
Goodness-of-fit on F2	0.801	
Final R indices [I>2sigma(I)]	R1 = 0.0669, wR2 = 0.1657	
R indices (all data)	R1 = 0.0835, wR2 = 0.1725	
Largest diff. peak and hole	0.452 and –0.479 e.Å^{-3}	



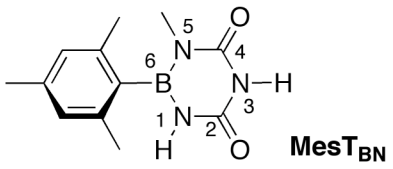
MesU$_{BN}$



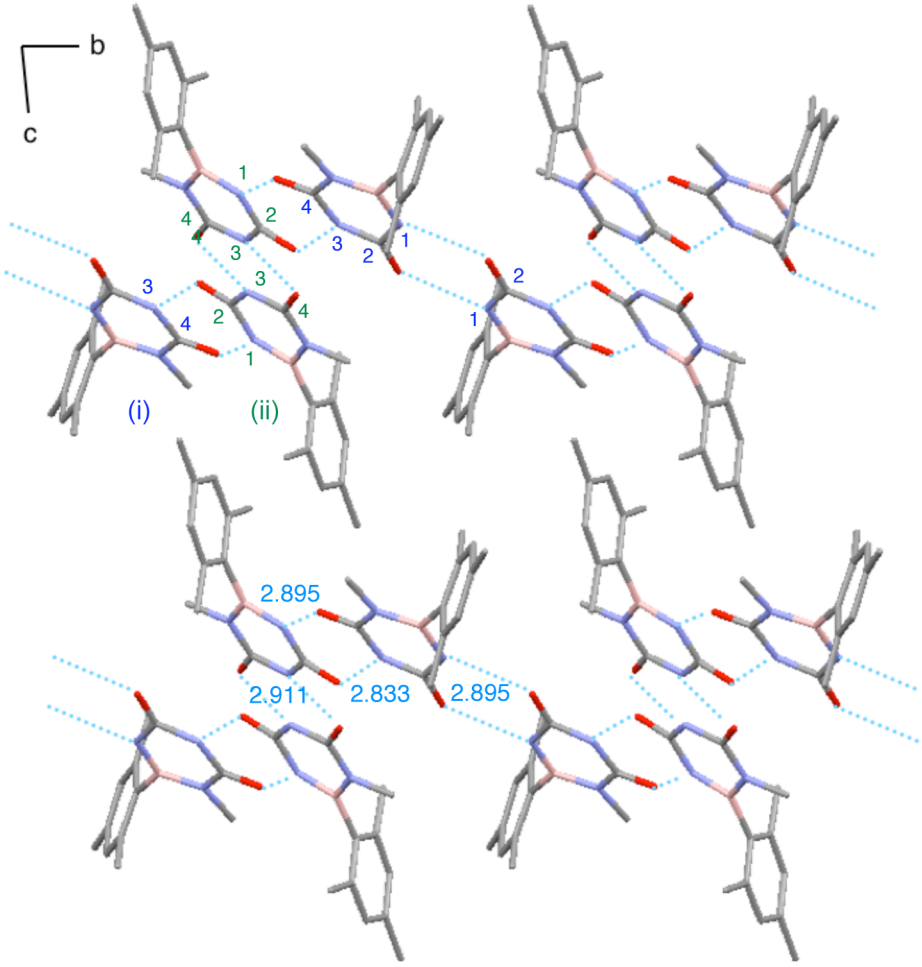
Bond Length / Å		
	(i)	(ii)
N_1–C_2	1.366(5)	1.365(4)
C_2–N_3	1.374(4)	1.375(4)
N_3–C_4	1.374(4)	1.374(4)
C_4–N_5	1.370(5)	1.375(4)
N_5–B_6	1.429(5)	1.427(5)
B_6–N_1	1.422(5)	1.418(5)
C_2=O	1.224(4)	1.230(4)
C_4=O	1.225(4)	1.221(4)

Bond Angle / °		
	(i)	(ii)
B_6–N_1–C_2	124.4(3)	124.0(3)
N_1–C_2–N_3	115.0(3)	115.1(3)
C_2–N_3–C_4	126.9(3)	127.4(3)
N_3–C_4–N_5	115.6(3)	114.7(3)
C_4–N_5–B_6	123.5(3)	123.8(3)
N_5–B_6–N_1	114.6(3)	114.9(3)

Figure S1. Crystal structure of **MesU$_{BN}$**.



MesT$_{BN}$



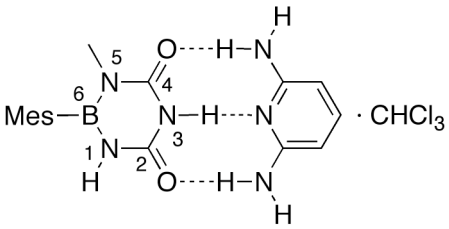
Bond Length / Å

	(i)	(ii)
N_1–C_2	1.361(2)	1.358(2)
C_2–N_3	1.380(2)	1.380(2)
N_3–C_4	1.382(2)	1.379(2)
C_4–N_5	1.375(2)	1.377(2)
N_5–B_6	1.442(2)	1.436(2)
B_6–N_1	1.422(2)	1.428(2)
C_2=O	1.2264(19)	1.2270(19)
C_4=O	1.223(2)	1.225(2)

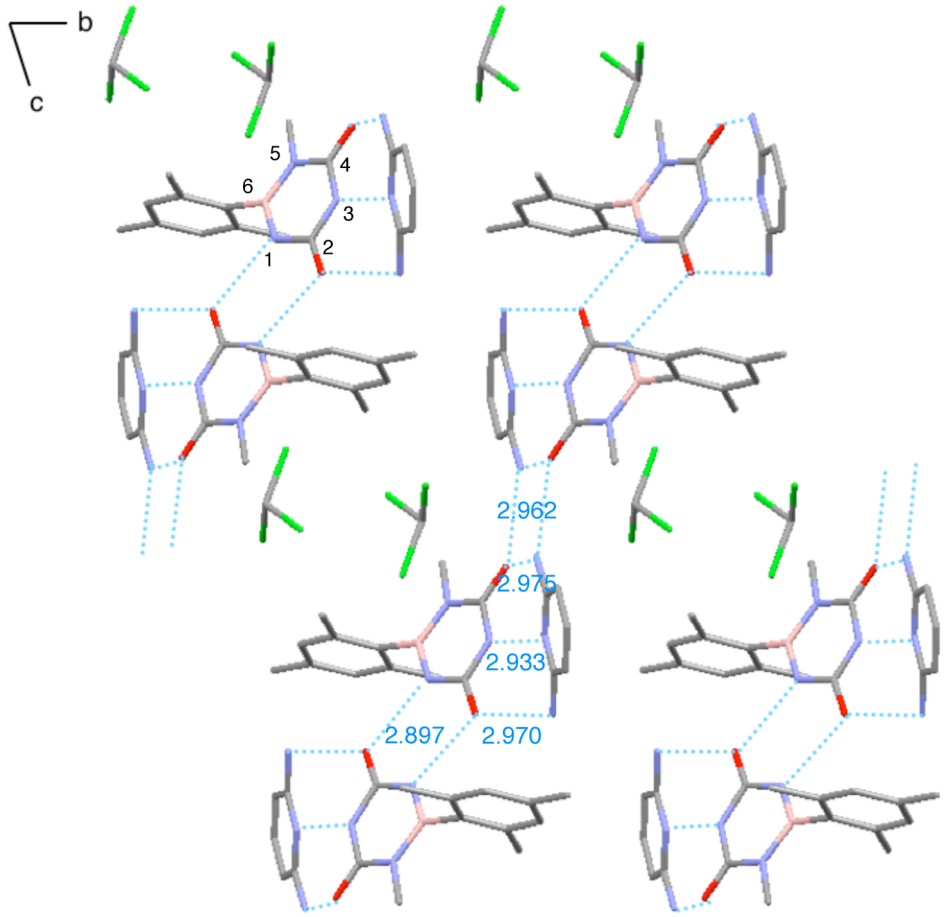
Bond Angle / °

	(i)	(ii)
B_6–N_1–C_2	124.02(14)	124.88(13)
N_1–C_2–N_3	115.03(13)	114.54(13)
C_2–N_3–C_4	127.01(13)	126.90(13)
N_3–C_4–N_5	115.91(13)	116.25(14)
C_4–N_5–B_6	121.96(13)	122.08(13)
N_5–B_6–N_1	115.81(15)	115.07(14)

Figure S2. Crystal structure of **MesT$_{BN}$**.



$MesT_{BN}$ **DAP**



Bond Length / Å		Bond Angle / °	
N_1–C_2	1.360(3)	B_6–N_1–C_2	123.84(19)
C_2–N_3	1.374(3)	N_1–C_2–N_3	115.5(2)
N_3–C_4	1.385(3)	C_2–N_3–C_4	126.26(19)
C_4–N_5	1.371(3)	N_3–C_4–N_5	116.47(19)
N_5–B_6	1.440(3)	C_4–N_5–B_6	121.7(2)
B_6–N_1	1.425(3)	N_5–B_6–N_1	115.7(2)
C_2=O	1.235(3)		
C_4=O	1.228(3)		

Figure S3. Crystal structure of **$MesT_{BN}$·DAP** complex.

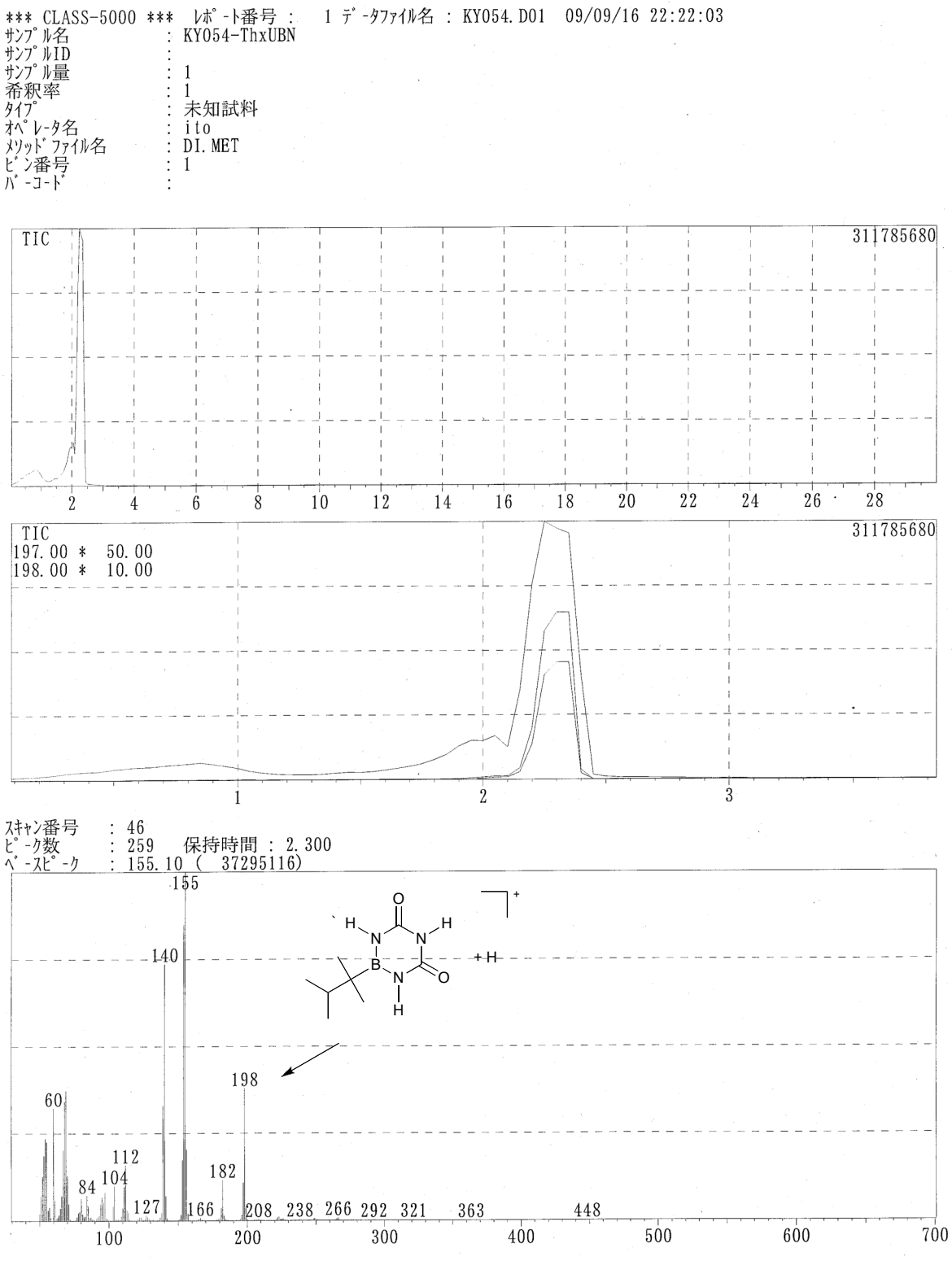


Figure S4. EI-MS spectrum of **ThxU$_{BN}$** recorded using a Shimadzu GCMS-QP5000 spectrometer with a direct inlet system.

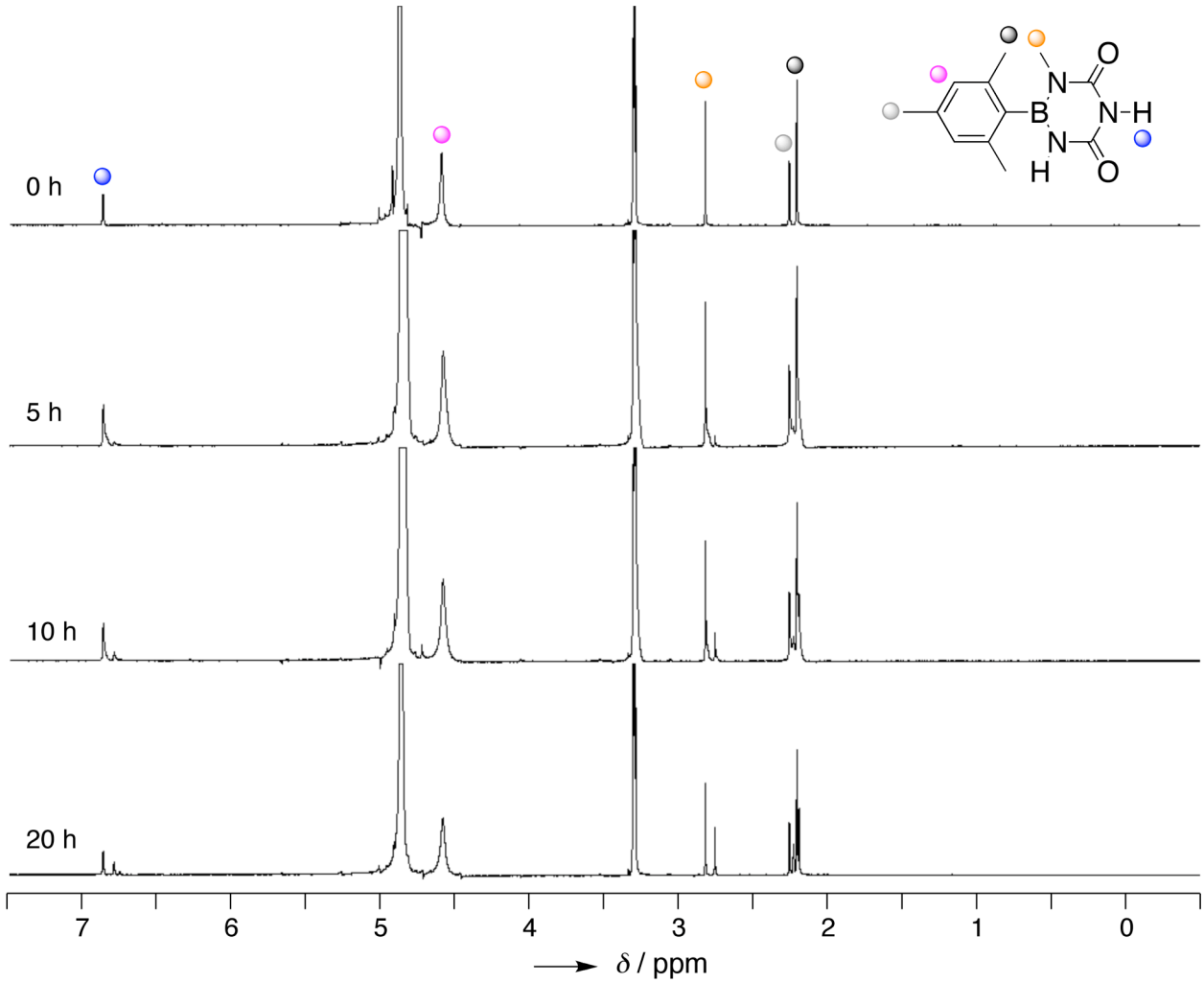


Figure S5. Time dependent ^{1}H NMR specta of $MesT_{BN}$ in CD_3OD (6.0 mM) at 60 °C (bath temperature).

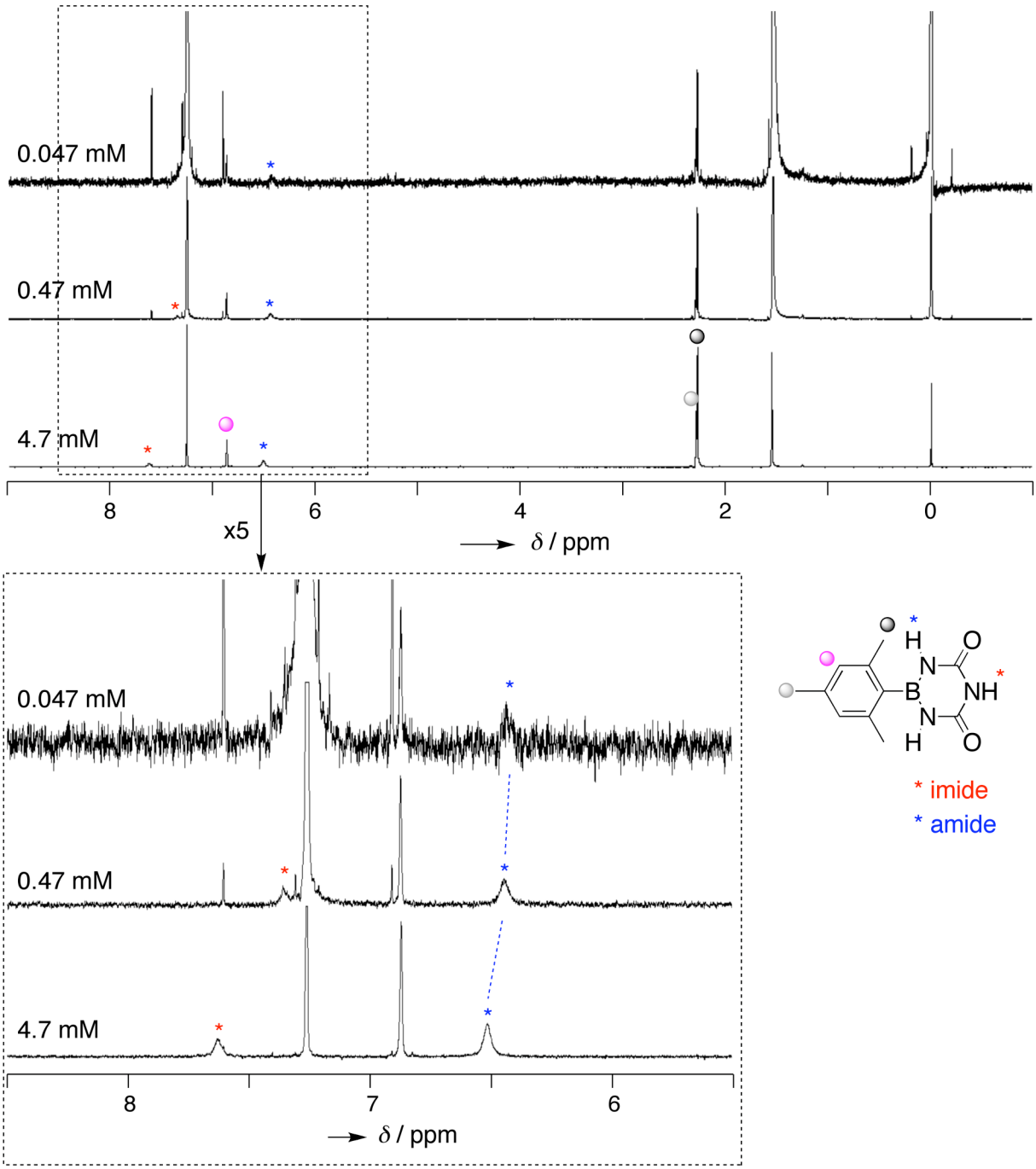


Figure S6. ^{1}H NMR spectra of **MesU$_{BN}$** at various concentrations in $CDCl_3$ at rt..

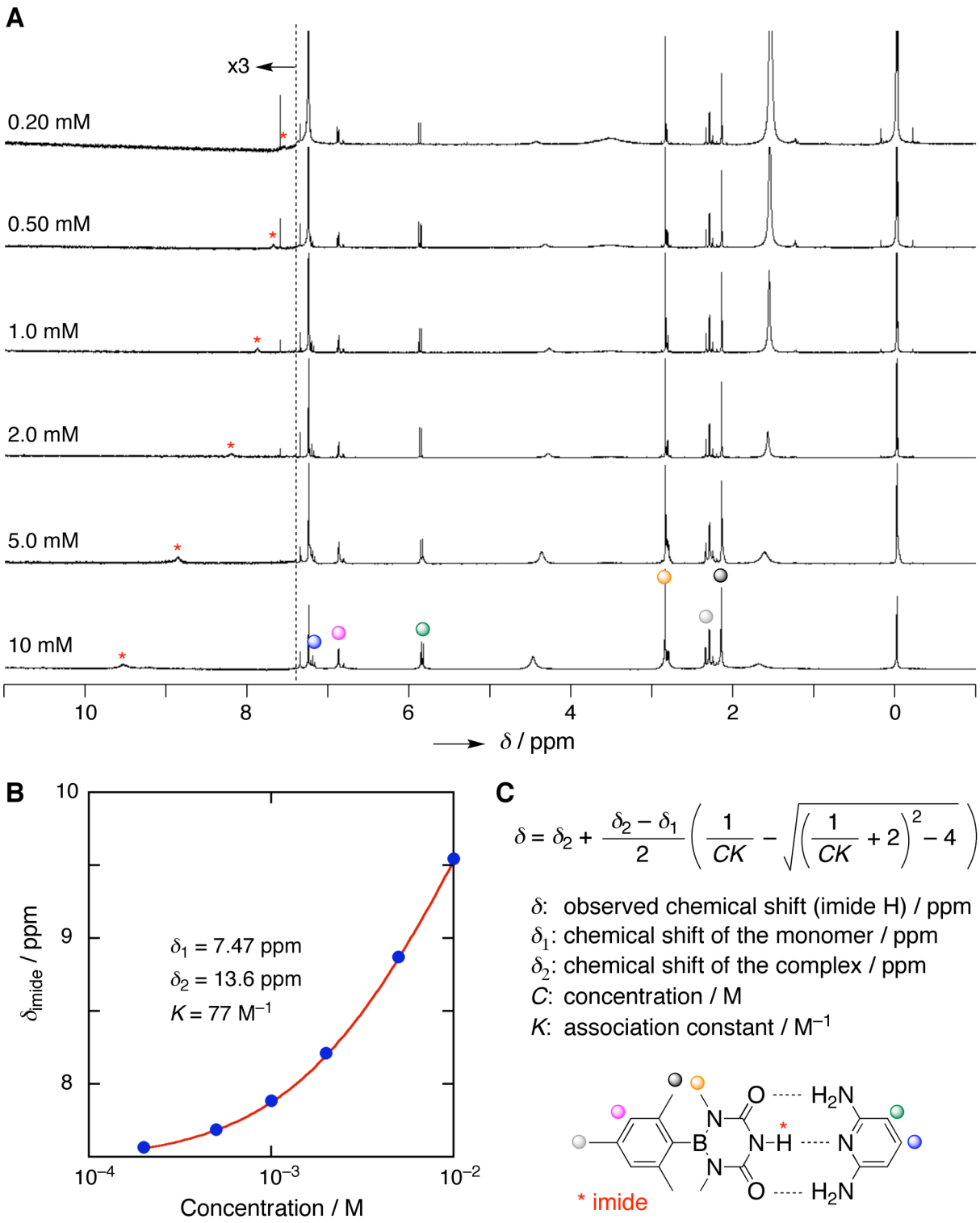


Figure S7. Association constant of **MesT$_{BN}$·DAP** complex in $CDCl_3$ at 25 °C; A, ^{1}H NMR spectra of **MesT$_{BN}$·DAP** complex at various concentrations. B, Chemical shift of the imide proton (asterisk). Curvefitting was performed by using KalidaGraph 4.0 by incorporating the equation C.

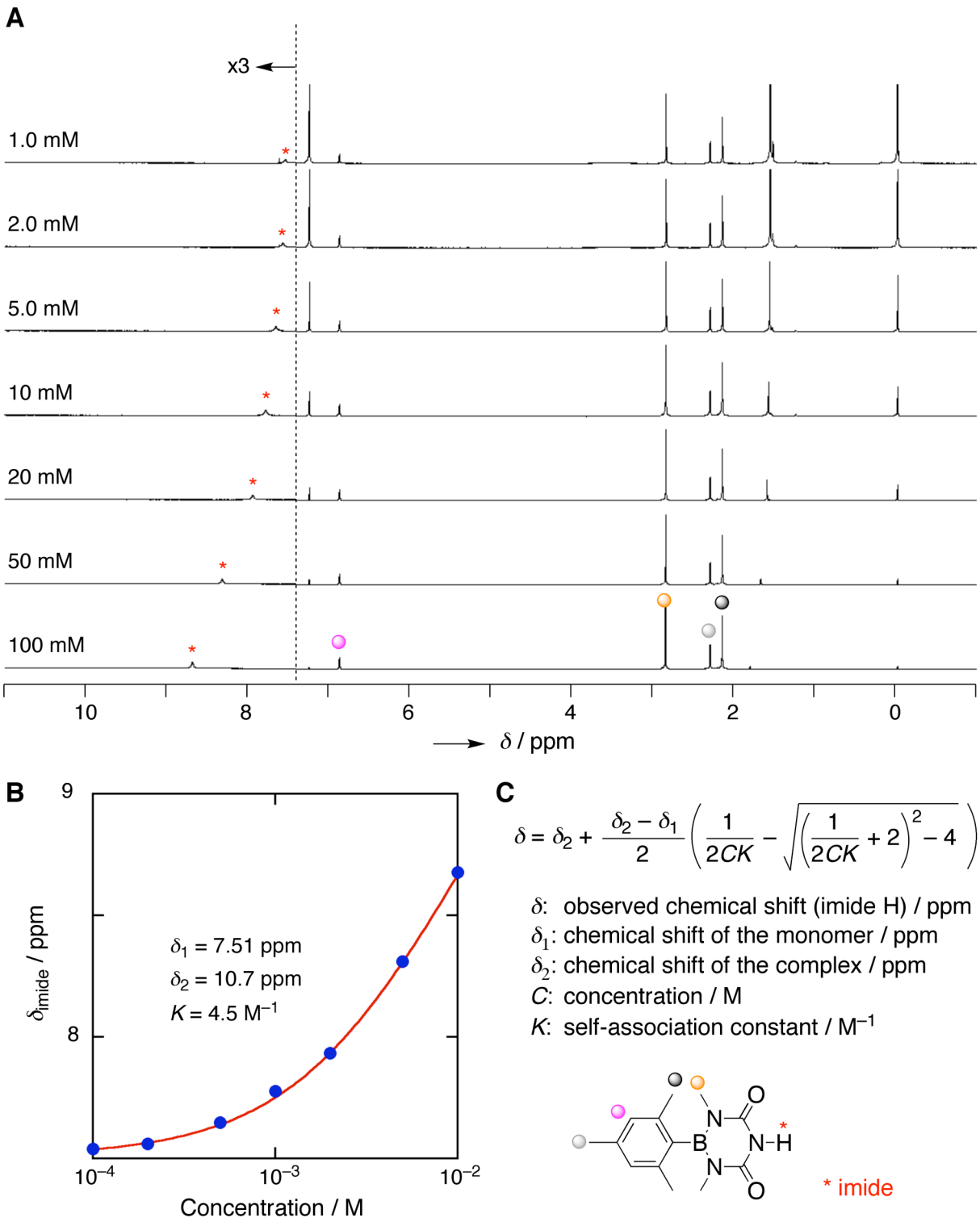


Figure S8. Self-association constant of **MesT$_{BN}$** in $CDCl_3$ at 25 °C; A, ^{1}H NMR spectra of **MesT$_{BN}$** at various concentrations. B, Chemical shift of the imide proton (asterisk). Curvefitting was performed by using KalidaGraph 4.0 by incorporating the equation C.

5. IR data of $MesU_{BN}$, T_{BN}s and thymine

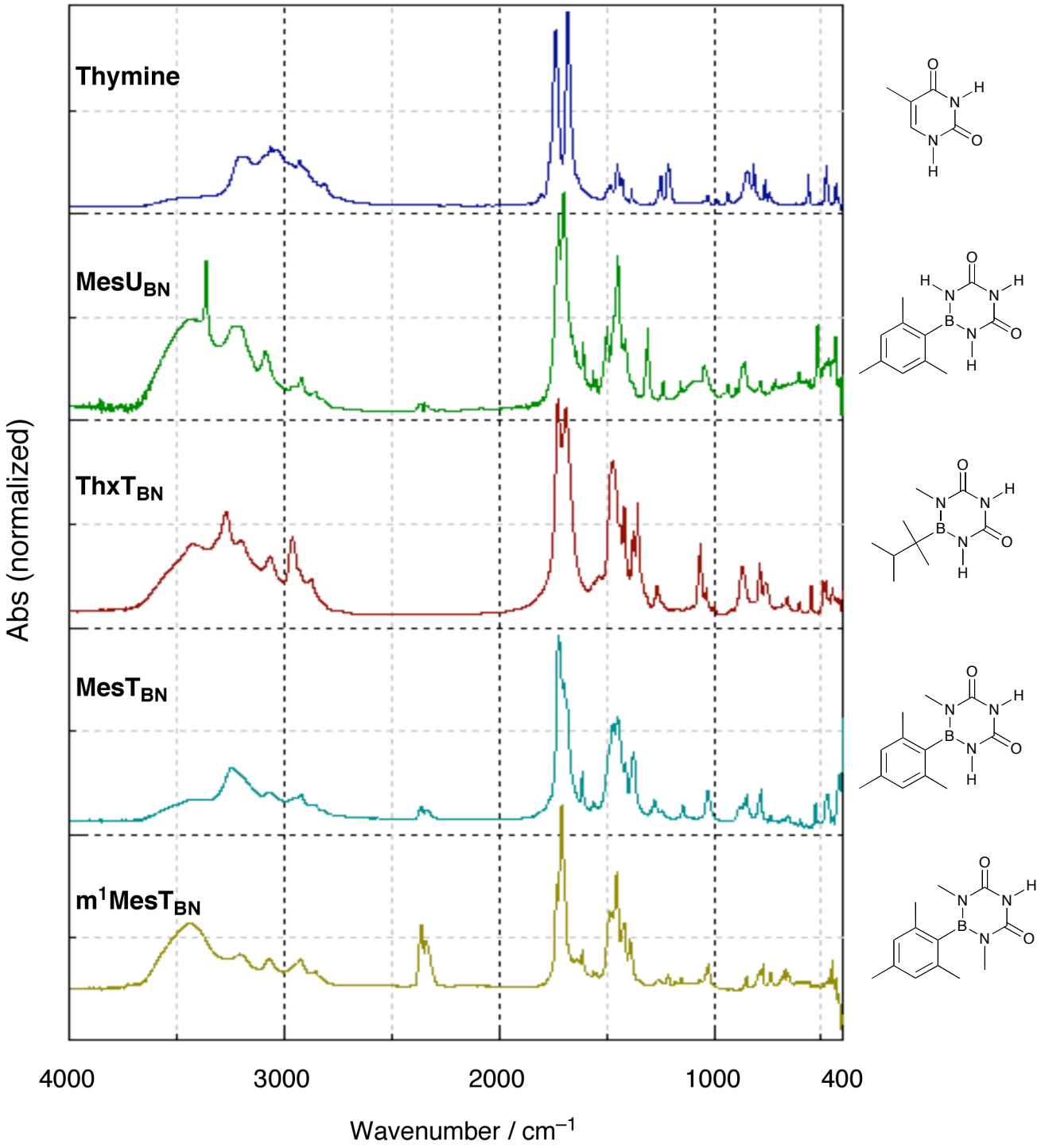
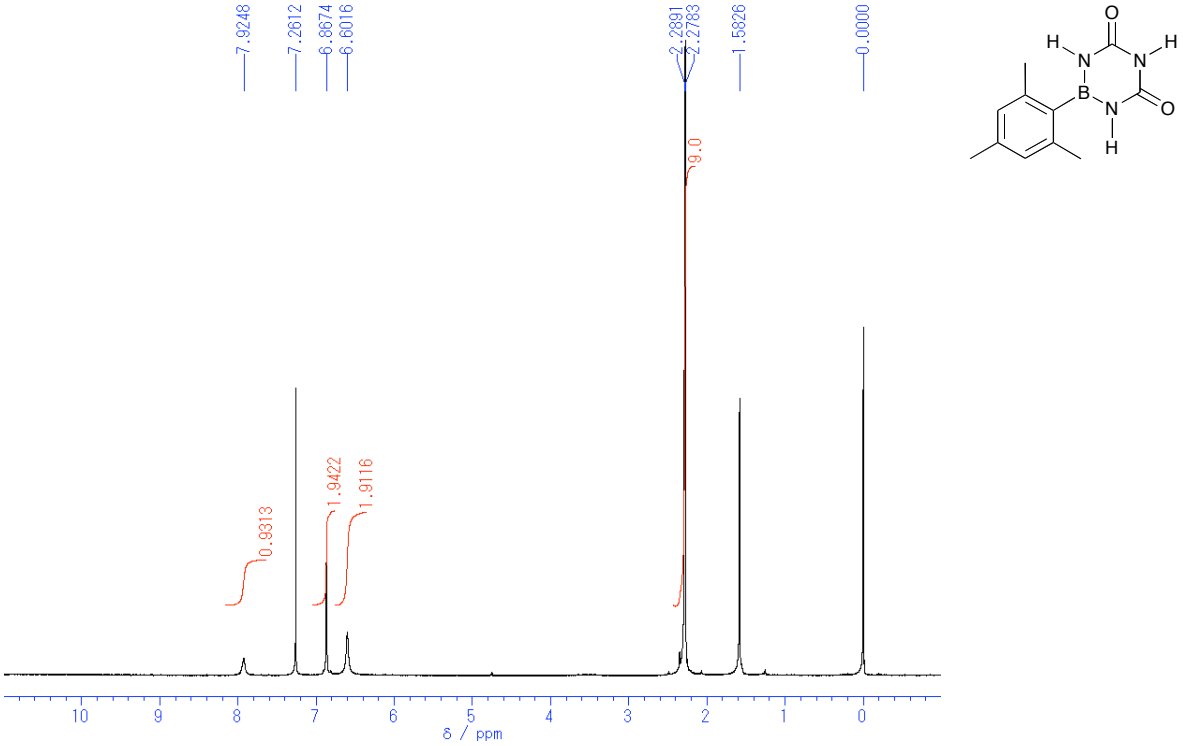


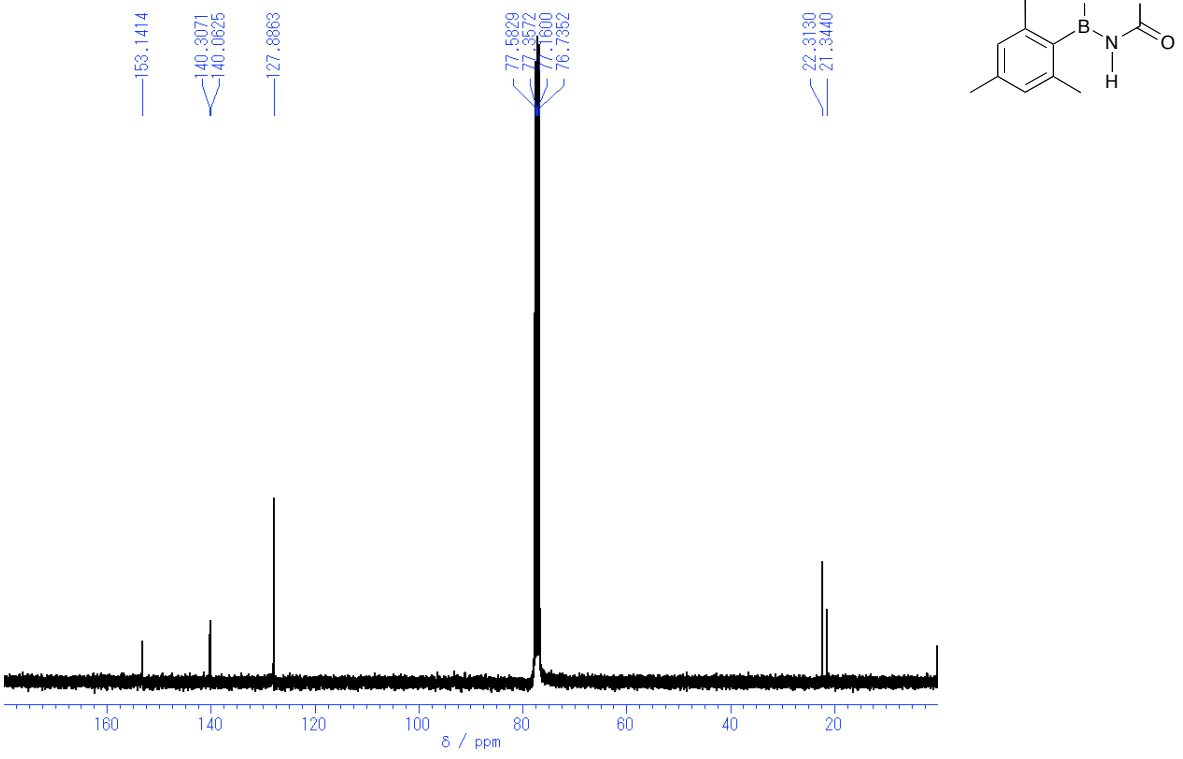
Figure S9. IR specta of **$MesU_{BN}$** and **T_{BN}**s, and thymine (KBr).

6. NMR data

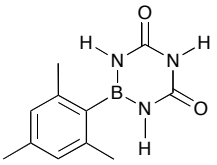
MesU$_{BN}$ ^{1}H NMR

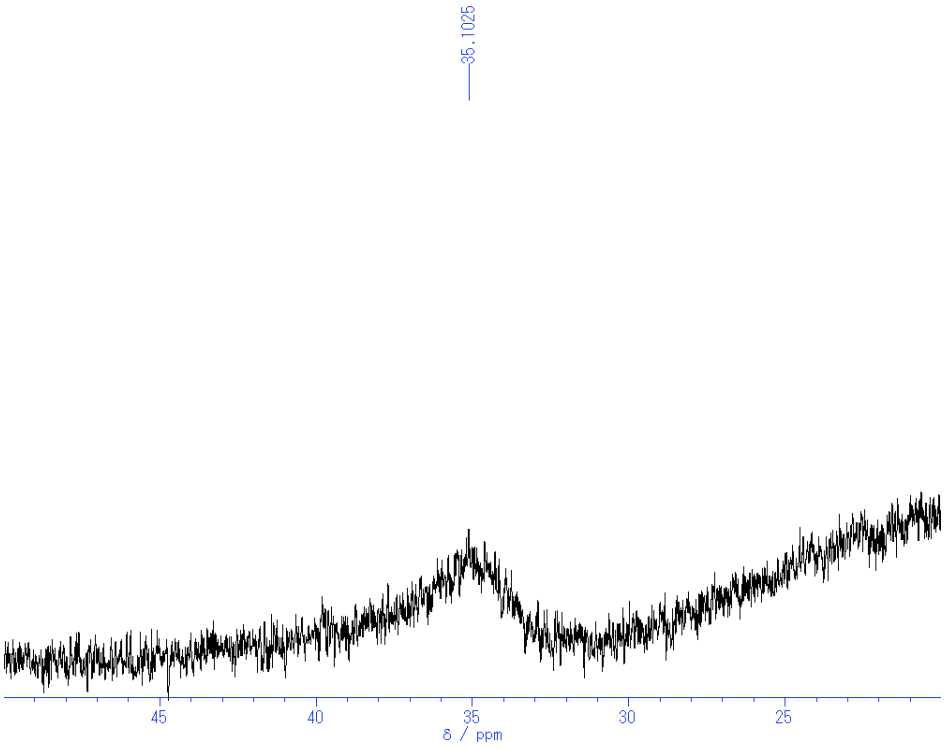


MesU$_{BN}$ ^{13}C NMR

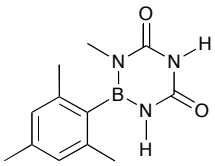


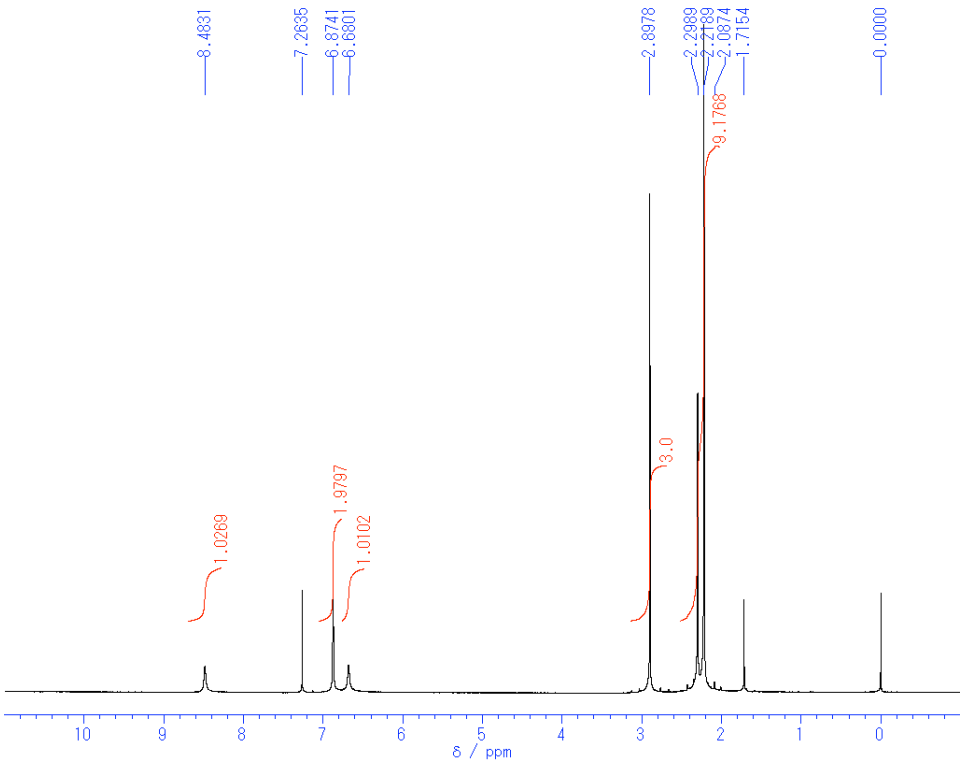
MesU$_{BN}$ ^{11}B NMR



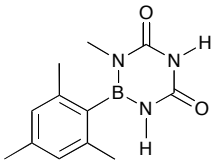


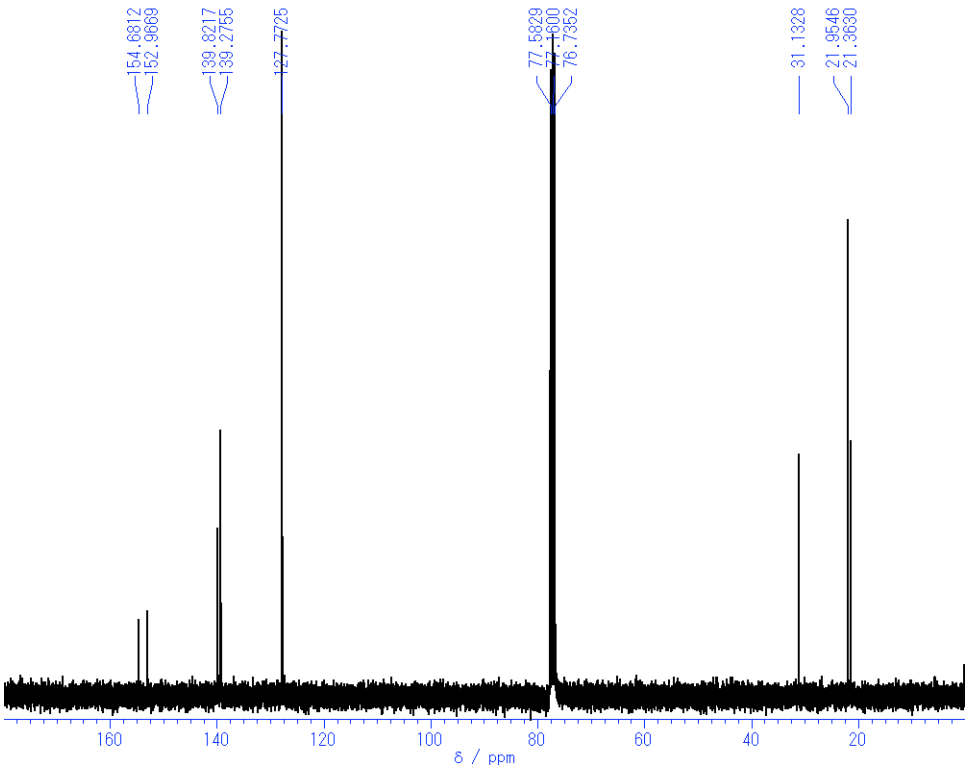
MesT$_{BN}$ ^{1}H NMR



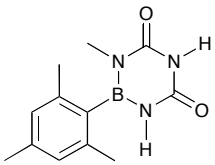


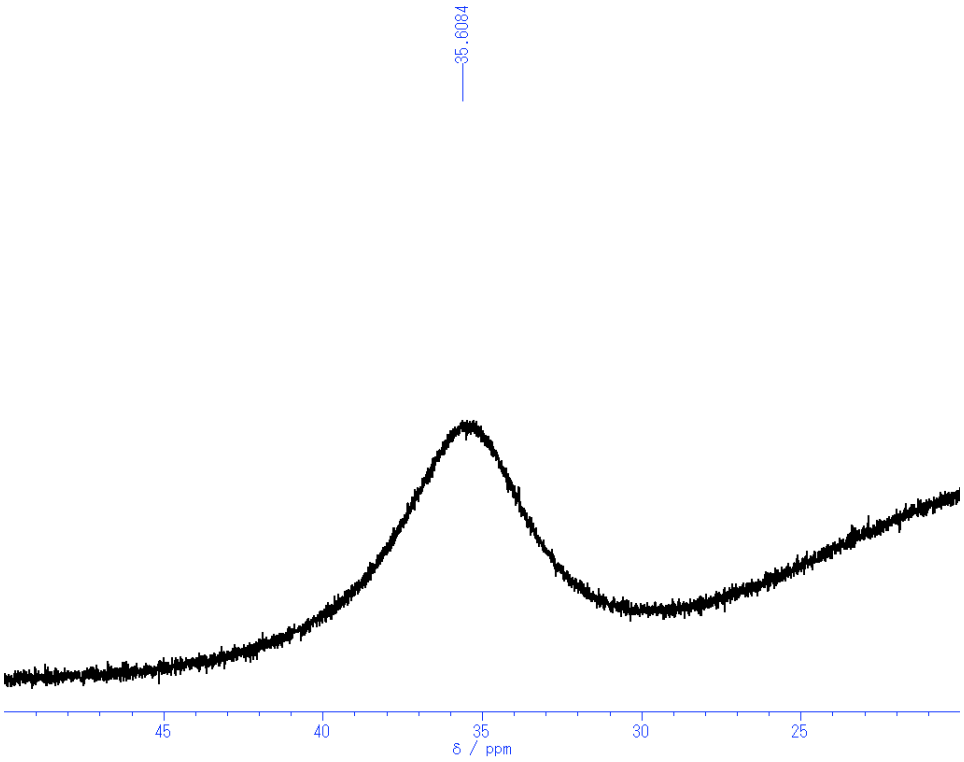
MesT$_{BN}$ ^{13}C NMR



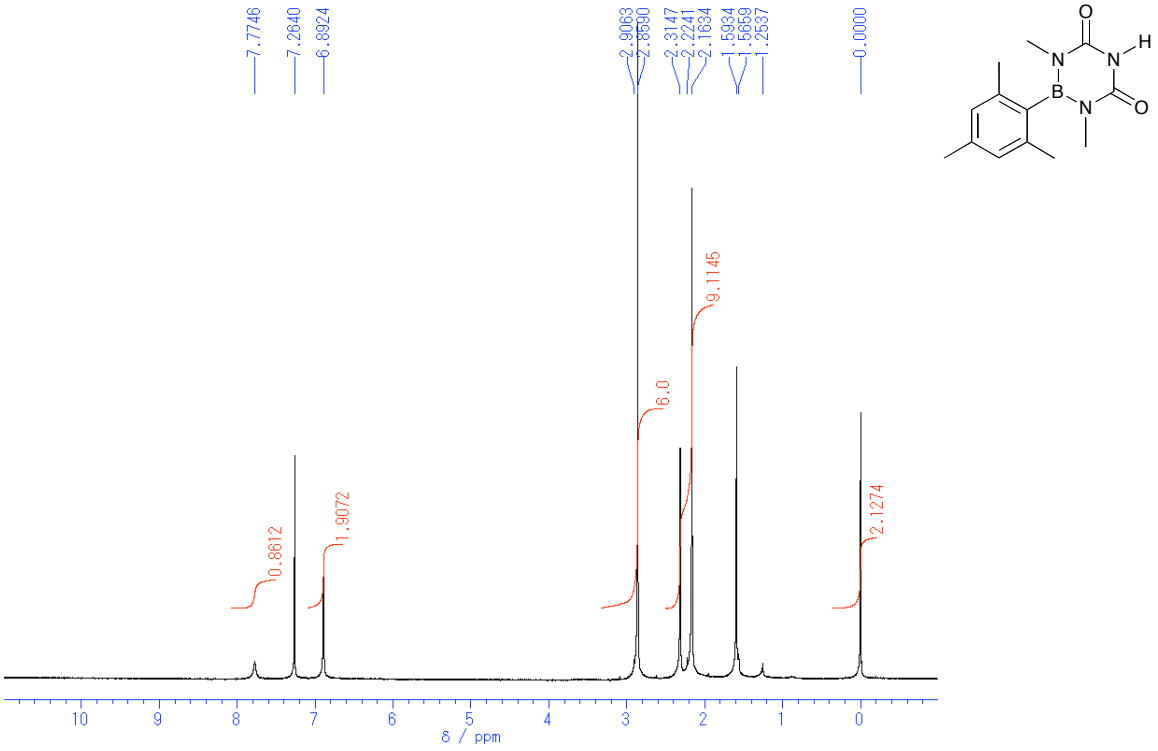


MesT$_{BN}$ ^{11}B NMR

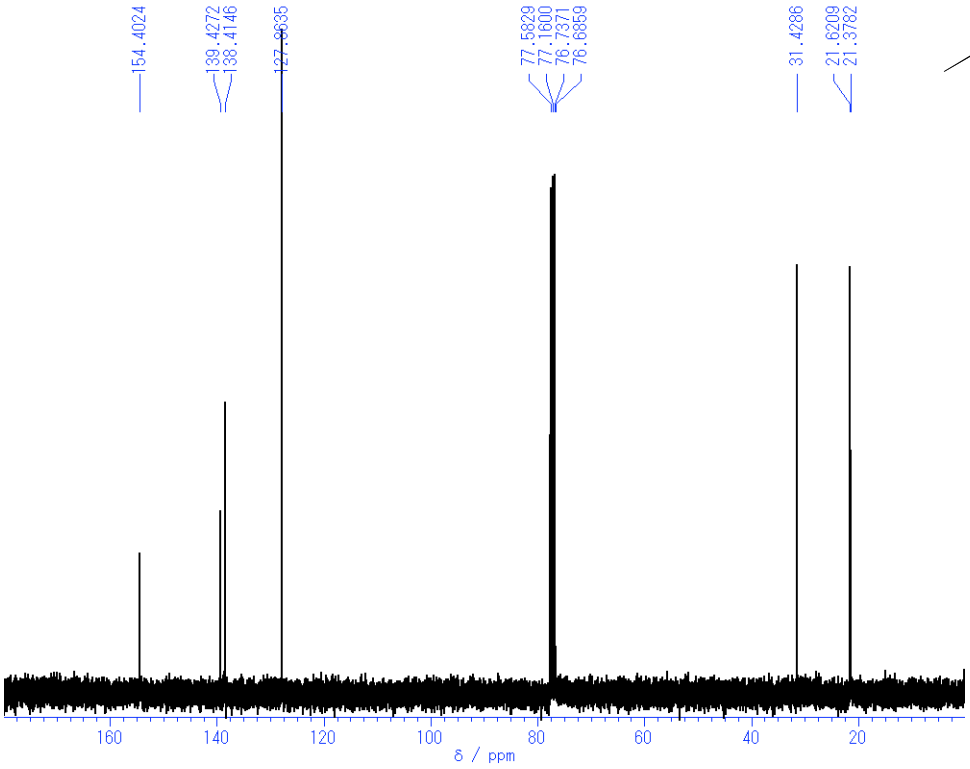




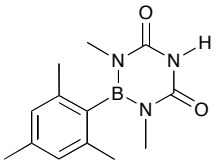
m^{1}MesT$_{BN}$ ^{1}H NMR

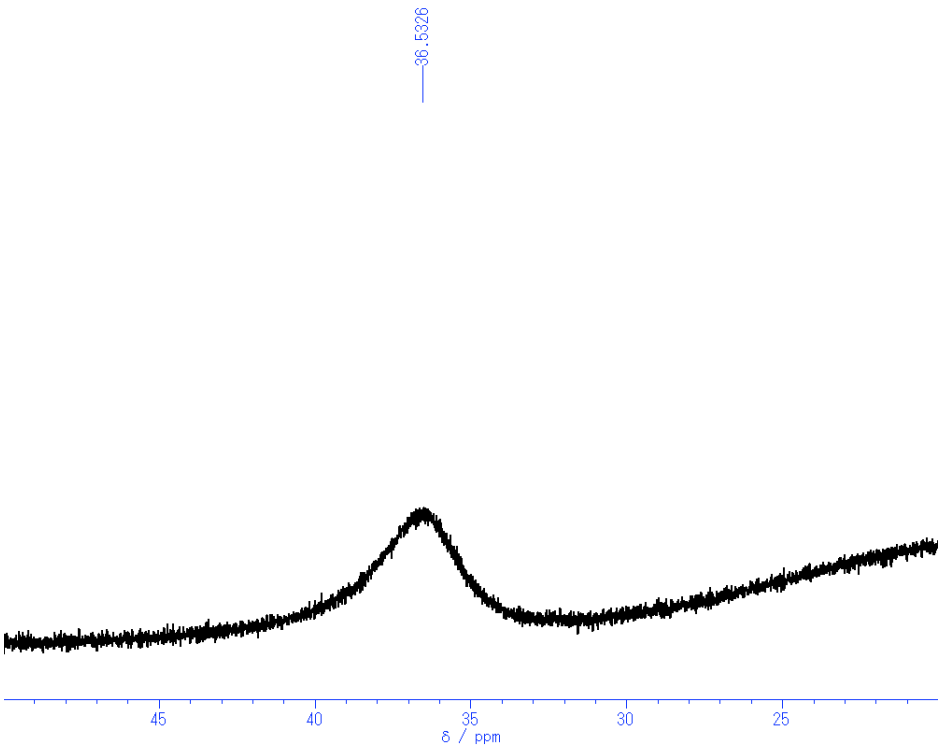


m^{1}MesT$_{BN}$ ^{13}C NMR

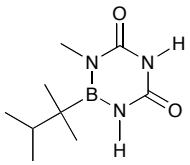


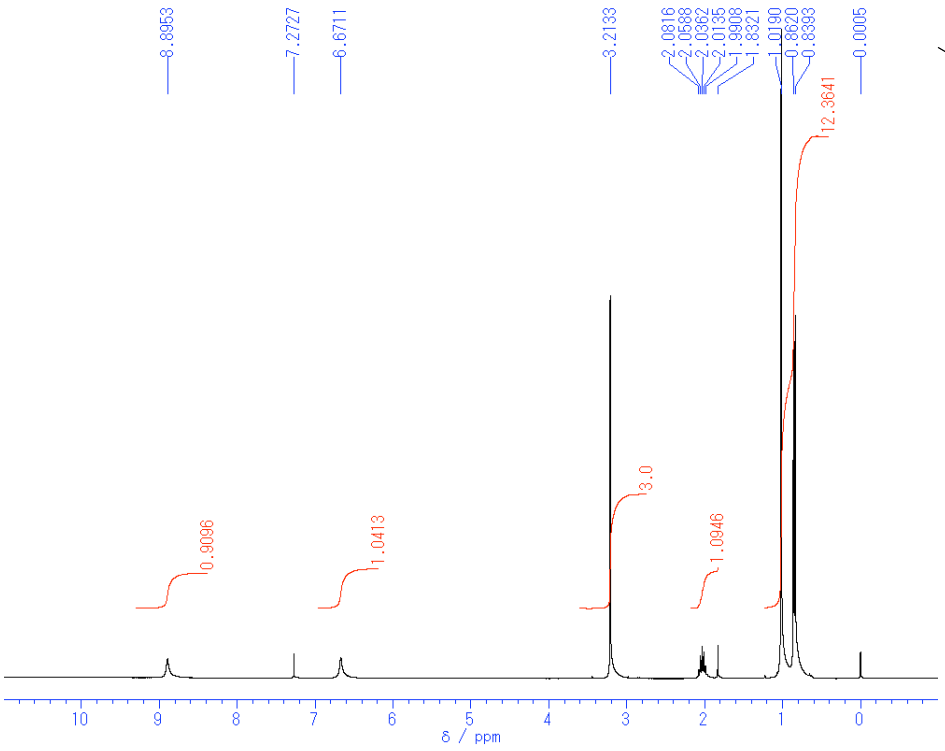
m^{1}MesT$_{BN}$ ^{11}B NMR



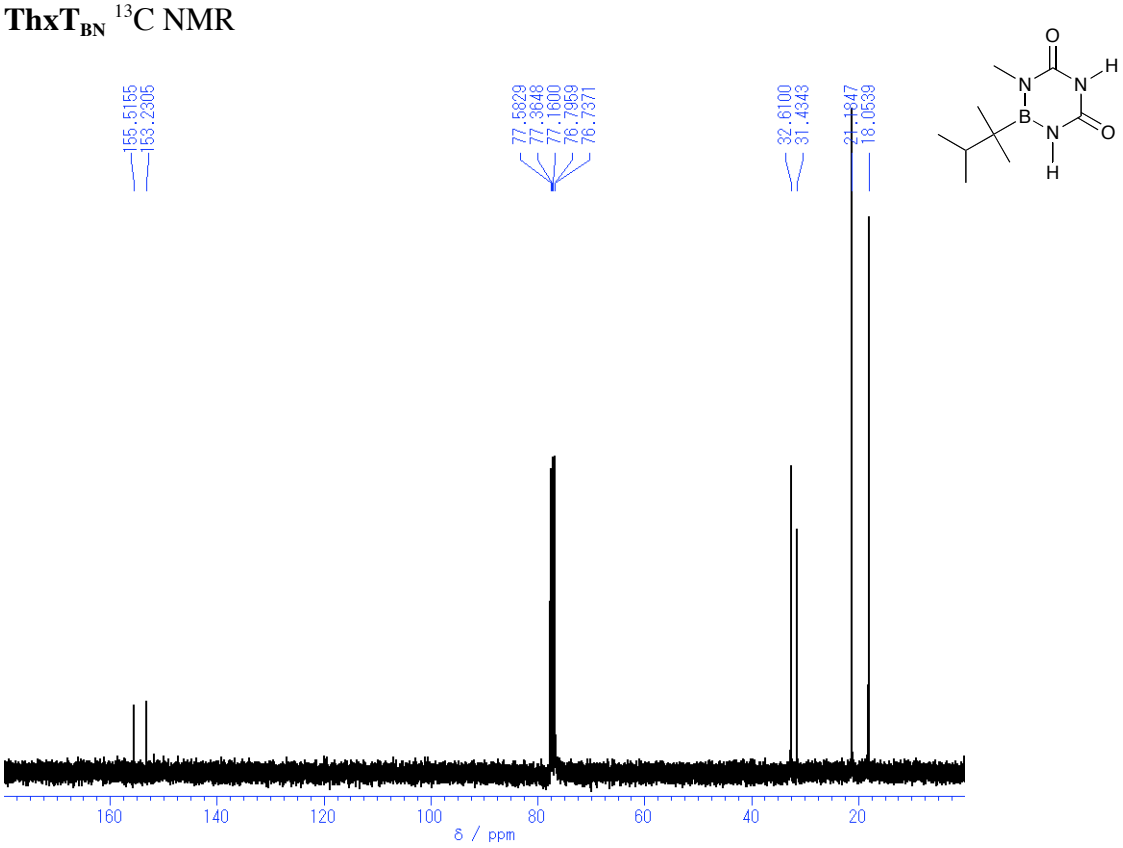


ThxT$_{BN}$ ^{1}H NMR





ThxT$_{BN}$ ^{13}C NMR



ThxT$_{BN}$ ^{11}B NMR