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Syntheses, spectral and chiral properties and DNA interactions of multi-heterocyclic di- and trinuclear boron complexes†

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Tetrahedrally coordinated multi-heterocyclic boron complexes are stable, but much less investigated inorganic ring systems. In the present study, a series of dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes were synthesized from SalenH$_2$ type symmetrical bulky ligands [HOArCH═N–R–N═CHArOH; R = $(CH_2)_n$, *n* = 2 (**1a**), 3 (**1b**) and 4 (**1c**)], arylboronic acids (phenylboronic acid and 4-formylphenylboronic acid), and boric acid for the investigation of their spectral, stereogenic and DNA cleavage activities. The Salen-boron complexes have two equivalent chiral B-centers, giving rise to diastereoisomers. The stereogenic properties of these complexes were investigated by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopies. The stereoisomers were compared with each other for two different architectural types and a total of 12 Salen-boron complexes with seven- to nine-membered [(B–O–B)–(N–R–N)] heterocycles. The combination of NMR and CD spectra of the complexes shows that the boron complexes (**2bI** and **3bII**) and (**2bII** and **3bI**) from the $(CH_2)_3$ precursor give only a *cis–meso* (*RS*/*SR*) isomer and only one *trans*-enantiomer (*RR* or *SS*), respectively. The complexes (**2cI**, **3cI** and **3cII**) from the $(CH_2)_4$ precursor give only one enantiomer (*RR* or *SS*), whereas the boron complexes (**2aI**, **2aII**, **3aI** and **3aII**) from the $(CH_2)_2$ precursor and (**2cII**) from the $(CH_2)_4$ precursor form two diastereoisomers as one enantiomer (*RR* or *SS*) and one *meso* (*RS*/*SR*). Furthermore, the DNA cleavage activities of the adducts were determined using agarose gel electrophoresis and UV absorption in order to compare the cleavage efficiency of the boron complexes depending on the type of complexes (dinuclear or trinuclear) and the number of members in the heterocyclic frameworks. In this assay, the seven-membered trinuclear boron complex (**3bII**) showed the highest cleavage efficiency.

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1. Introduction

Stereoisomerism is of well-recognized importance in many realms such as in the life sciences due to the different activity of individual stereoisomers in biological systems.[1] Although considerable research has been devoted to investigating enantiomerism in compounds with tetrahedrally-coordinated main group elements other than carbon, such as stereogenic nitrogen,[2] phosphorus,[3] silicon,[4] and sulfur[5] atoms, rather less attention has been paid to studying the features of compounds with tetrahedrally-coordinated boron. This is not only primarily because the tetrahedrally-coordinated boron atom with a labile nature is configurationally stable only under certain conditions,[6] but also partly because tetrahedrally-coordinated boron is incorporated in a chiral environment created by enantiomerically pure ligands[7] or counterions.[8] The reactions for the synthesis of some complexes occur in a diastereoselective manner[9] or a manner producing a mixture of diastereomers.[10] Boron turns out to be a stable stereogenic center in complexes obtained from Schiff base ligands and aryl boronic acids. However, the Schiff base boron complexes either contain fixed carbon stereocenters in chiral Schiff base ligands or are racemic.[11] Diastereomerically and enantiomerically pure complexes were synthesized by taking advantage of the interactions between chiral Schiff base ligands derived from aromatic aldehydes and chiral aminoethanols, and arylboronic acids.[12]

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† Electronic supplementary information (ESI) available: Schematic presentation of the syntheses of dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes, IR and ^{11}B NMR spectra for all the complexes, ^{1}H and ^{13}C NMR and HSQC spectra of the complexes except **2cI**. See DOI: 10.1039/d0nj04474a

On the other hand, in the studies of boron complexes with Schiff base ligands, stereogenism only at the boron atom is much less common. The first example of one such optically pure stable Schiff base boron complex was reported by Hutton and coworkers.[13] The absolute configuration of stereogenic boron in enantiomeric Schiff base boron complexes was assigned by the evident accordance of measured and calculated circular dichroism (CD) for the first time by Braun and coworkers.[14] As part of the research on Schiff base boron complexes we have described the preparation and structural characterization of a series of dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) Salen-boron complexes (Table 1) that contain two equivalent stereogenic boron centers, and which give rise to diastereoisomers (Schemes 1 and 2). The Salen-boron complexes are the only examples of compounds in which the chirality is imparted exclusively by two boron stereocenters, and there is no paper reporting the CD spectrum of an enantiomer and a diastereoisomer of a boron complex with two equivalent stereogenic boron centers. We therefore have reason for studying dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes.

Additionally, boron-based compounds possess various and useful biological activities, including hypolipidemic, antiosteoporosis, antineoplastic,[15] antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory,[16] anticancer,[17,18] and DNA binding and cleavage[19] activities. Research along these lines is under development in laboratories, and some of these compounds are even used as FDA-approved drugs.[20,21] We therefore decided to examine the DNA cleavage activities of the novel tetrahedrally coordinated multi-heterocyclic dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes.

2. Experimental

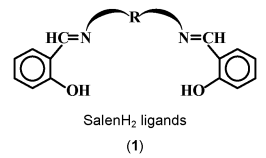
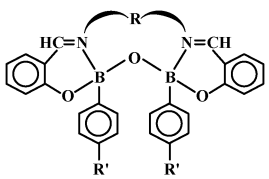
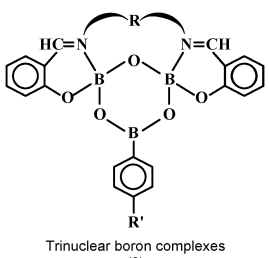
2.1. Materials

Commercial-grade reagents were used without further purification, and solvents were dried and distilled by standard methods. The solvents and salicylaldehyde were purchased from Merck, while aliphatic amines, boric acid, phenylboronic acid and 4-formylphenyl boronic acid were purchased from Fluka. All reactions were conducted under an argon atmosphere and monitored using thin-layer chromatography (TLC) on Merck DC Alufolien Kieselgel 60 B_{254} sheets.

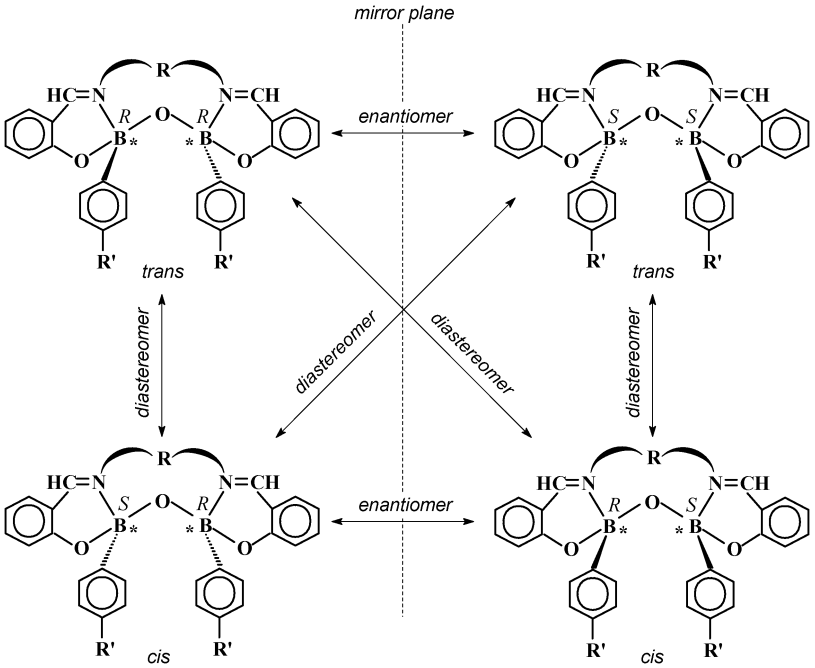
2.2. Physical methods

Melting points were measured with a Gallenkamp apparatus using a capillary tube. Microanalyses (C, H, N) were performed using a Leco CHNS-932 elemental analyzer at the Central Instrumental Analysis Laboratory in the Faculty of Pharmacy at Ankara University. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR, and HSQC spectra were recorded on a Varian Mercury 400 MHz FT spectrometer. ^{11}B NMR spectra were obtained using an Agilent 600 MHz PremiumCOMPACT NMR spectrometer at the Çankırı Karatekin University Research Center. FTIR spectra of the compounds were recorded on a Shimadzu Infinity FTIR spectrometer. ESI-MS mass spectra were obtained on an Agilent

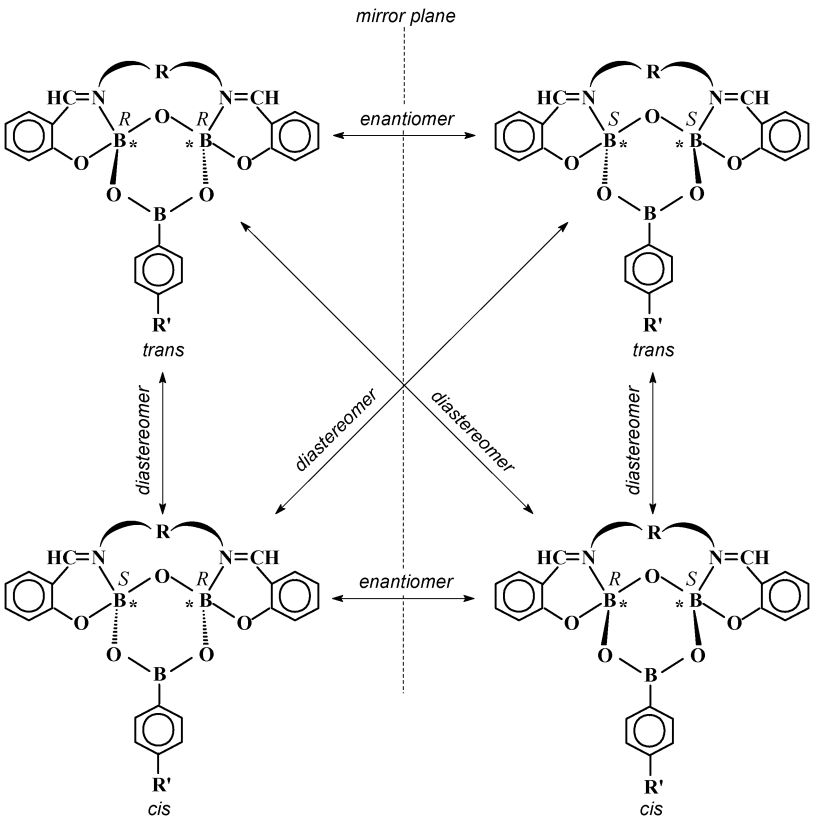
Table 1 Formulae of the dinuclear [(**2aI–2cI**) and (**2aII–2cII**)] and trinuclear [(**3aI–3cI**) and (**3aII–3cII**)] boron complexes

	Compound	R	R′	Diastereomeric ratio[a]
 SalenH$_2$ ligands (1)	(**1a**)		—	—
	(**1b**)		—	—
	(**1c**)		—	—
 Dinuclear boron complexes (2)	(**2aI**)		H	65 : 35
	(**2aII**)		CHO	96 : 4
	(**2bI**)		H	—
	(**2bII**)		CHO	—
	(**2cI**)		H	—
	(**2cII**)		CHO	86 : 14
 Trinuclear boron complexes (3)	(**3aI**)		H	53 : 47
	(**3aII**)		CHO	52 : 48
	(**3bI**)		H	—
	(**3bII**)		CHO	—
	(**3cI**)		H	—
	(**3cII**)		CHO	—

[a] Determined by ^{1}H NMR (400 MHz) examination of the crude reaction mixtures.



Scheme 1 Possible stereoisomers for the dinuclear [(**2aI–2cI**) and (**2aII–2cII**)] boron complexes.



Scheme 2 Possible stereoisomers for the trinuclear [(**3aI–3cI**) and (**3aII–3cII**)] boron complexes.

1100 MSD spectrometer. The ultra-violet (UV) absorption and CD spectra of the boron complexes were obtained with a Jasco J720 spectropolarimeter, flushed with N_2, and scanned at 10 nm min^{-1} with a spectral bandwidth of 1 nm and data resolution of 0.2 nm. The solvent was DMSO and pathlengths of the cells were 1 cm and 0.2 cm. CD spectra are presented with

respect to the normalized absorbance, A(265.6 nm) = 1, in a 1 cm cell.

2.3. Syntheses

2.3.1. Syntheses of the compounds (1–3 and 2aI, 2bI, 3aI and 3bI). The SalenH_2 ligands {*N*,*N*′-bis(salicylidene)-1,3-propanediamine (**1a**), *N*,*N*′-bis(salicylidene)-1,3-propanediamine (**1b**) and *N*,*N*′-bis(salicylidene)-1,4-butanediamine (**1c**)} were prepared according to the published procedure in which salicylaldehyde was reacted with 1,2-diaminoetane, 1,3-diaminopropane and 1,4-diaminobutane in dry MeOH, respectively.[22] The dinuclear boron complexes (**2aI** and **2bI**) were prepared according to a published procedure in which one equimolar amount of SalenH_2 ligands (**1a** and **1b**) was reacted with two equimolar amounts of phenylboronic acid in toluene, respectively, using a Dean–Stark trap.[23] The trinuclear boron complexes (**3aI** and **3bI**) were synthesized using a method in which one equimolar amount of SalenH_2 ligands (**1a** and **1b**), two equimolar amounts of boric acid and two equimolar amounts of phenylboronic acid were refluxed in acetonitrile, respectively, using a Dean–Stark trap according to the published procedure.[24] The spectroscopic data of the dinuclear (**2aI** and **2bI**) and trinuclear (**3aI** and **3bI**) boron complexes were reported,[23,24] but the stereochemistry and DNA interaction studies of **2aI**, **2bI**, **3aI** and **3bI** were discussed in this paper for comparison purposes.

2aI. Two diastereomers were obtained in a ratio of approximately 65 : 35. IR (KBr, cm^{-1}): ν 3051 (C–H arom.), 1638 (C═N), 1609 (C═C), 1557 (N–B), 1317 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 459 {$[MH]^+$, 100}.

2bI. IR (KBr, cm^{-1}): ν 3065; 3048 (C–H arom.), 1643 (C═N), 1608 (C═C), 1559 (N–B), 1319 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 473 {$[MH]^+$, 100}.

3aI. Two diastereomers were obtained in a ratio of approximately 53 : 47. IR (KBr, cm^{-1}): ν 3060; 3030 (C−H arom.), 1641 (C═N), 1611 (C═C), 1559 (N–B), 1302 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 269 {[OHArCH═N–$(CH_2)_2$–N═CHArOH*H*]$^+$, 100}.

3bI. IR (KBr, cm^{-1}): ν 3045; 3025 (C–H arom.), 1641 (C═N), 1611 (C═C), 1560 (N–B), 1300 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 283 {[OHArCH═N–$(CH_2)_3$–N═CHArOHH]$^+$, 100}.

2.3.2. Synthesis of the new dinuclear boron complexes (2cI, 2aII, 2bII and 2cII). The dinuclear boron complexes (**2cI**, **2aII**, **2bII** and **2cII**) were prepared by similar methods; therefore, the experimental procedure of the preparation was only described in detail for the first case.

2.3.2.1. ***2cI.*** SalenH_2 (**1c**) (0.3 g, 1.03 mmol) in toluene (50 mL) was slowly added during 0.5 h to a solution of phenylboronic acid (0.25 g, 2.05 mmol) in boiling toluene (100 mL) with argon being passed over the reaction mixture. The mixture was refluxed for 6 h and then cooled to room temperature. After 1 h of reflux, the solution was concentrated with a Dean–Stark trap. The yellow precipitate was filtered, washed with hot toluene, and dried in air. Yield: 0.17 g (35%). mp: 225 °C. Anal. calc. for $C_{30}H_{28}B_2N_2O_3$ (%): C, 74.11; H, 5.80; N, 5.76. Found; C, 74.01; H, 5.90; N, 5.84%. IR (KBr, cm^{-1}): ν 3063; 3040 (C–H arom.), 1649 (C═N), 1609 (C═C), 1560 (N–B), 1300 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 297 {[OHArCH═N–$(CH_2)_4$–N═CHArOHH]$^+$, 48}.

2.3.2.2. ***2aII.*** Complex **2aII** was prepared from SalenH_2 (**1a**) (0.22 g, 0.84 mmol) and 4-formylphenylboronic acid (0.25 g, 1.67 mmol) (6 h). Two diastereomers were obtained in a ratio of approximately 96 : 4. Yield: 0.34 g (79%). mp: 263 °C. Anal. calc. for $C_{30}H_{24}B_2N_2O_5$ (%): C, 70.08; H, 4.71; N, 5.45. Found; C, 68.96; H, 4.87; N, 5.36%. IR (KBr, cm^{-1}): ν 3055; 3024 (C–H arom.), 2822; 2724 (H–CO), 1703 (HC═O), 1645 (C═N), 1599 (C═C), 1557 (N–B), 1308 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 515 {$[MH]^+$, 100}.

2.3.2.3. ***2bII.*** Complex **2bII** was prepared from SalenH_2 (**1b**) (0.24 g, 0.84 mmol) and 4-formylphenylboronic acid (0.25 g, 1.67 mmol) (6 h). Yield: 0.30 g (75%). mp: 230 °C. Anal. calc. for $C_{31}H_{26}B_2N_2O_5$ (%): C, 70.83; H, 4.98; N, 5.33. Found; C, 69.64; H, 5.16; N, 5.01%. IR (KBr, cm^{-1}): ν 3067; 3030 (C–H arom.), 2820; 2724 (H–CO), 1699 (HC═O), 1637 (C═N), 1595 (C═C), 1559 (N–B), 1300 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 527 {$[MH]^+$, 100}.

2.3.2.4. ***2cII.*** Complex **2cII** was prepared from SalenH_2 (**1c**) (0.25 g, 0.84 mmol) and 4-formylphenylboronic acid (0.25 g, 1.67 mmol) (6 h). Two diastereomers were obtained in a ratio of approximately 86 : 14. Yield: 0.38 g (80%). mp: 220 °C. Anal. calc. for $C_{32}H_{28}B_2N_2O_5$ (%): C, 70.89; H, 5.20; N, 5.17. Found; C, 69.86; H, 5.55; N, 4.24%. IR (KBr, cm^{-1}): ν 3071; 3034 (C–H arom.), 2830; 2733 (H–CO), 1692 (HC═O), 1641 (C═N), 1597 (C═C), 1559 (N–B), 1304 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 297 {[OHArCH═N–$(CH_2)_4$–N═CHArOHH]$^+$, 28}.

2.3.3. Synthesis of the new trinuclear boron complexes (3cI, 3aII, 3bII and 3cII). The trinuclear boron complexes (**3cI**, **3aII**, **3bII** and **3cII**) were prepared by similar methods; therefore, the experimental procedure of the preparation was only described in detail for the first case.

2.3.3.1. ***3cI.*** A mixture of SalenH_2 (**1c**) (0.61 g, 2.05 mmol) and boric acid (0.25 g, 4.10 mmol) in acetonitrile (15 mL) was refluxed until a yellow precipitate formed. Then a solution of phenylboronic acid (0.25 g, 2.05 mmol) in acetonitrile (20 mL) was added without isolation of the dinuclear intermediate (Fig. S1, ESI†) and the mixture was refluxed for 16 h using a Dean–Stark trap with argon being passed over the reaction mixture. The yellow precipitate was filtered, washed with hot acetonitrile, and dried in air. Yield: 0.33 g (36%). mp: 245 °C. Anal. calc. for $C_{24}H_{23}B_3N_2O_5$ (%): C, 63.79; H, 5.13; N, 6.20. Found; C, 63.75; H, 5.08; N, 6.18%. IR (KBr, cm^{-1}): ν 3055; 3030 (C–H arom.), 1641 (C═N), 1609 (C═C), 1559 (N–B), 1300 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 297 {[OHArCH═N–$(CH_2)_4$–N═CHArOHH]$^+$, 100}.

2.3.3.2. ***3aII.*** Complex **3aII** was prepared from SalenH_2 (**1a**) (1.00 g, 3.70 mmol), boric acid (0.46 g, 7.50 mmol) and 4-formylphenylboronic acid (0.56 g, 3.70 mmol) (14 h). Two diastereomers

were obtained in a ratio of approximately 52 : 48. Yield: 1.24 g (74%). mp: >300 °C. Anal. calc. for $C_{23}H_{19}B_3N_2O_6$ (%): C, 61.14; H, 4.24; N, 6.30. Found; C, 61.11; H, 4.26; N, 6.16%. IR (KBr, cm^{-1}): ν 3050; 3028 (C–H arom.), 2820; 2730 (H–CO), 1701 (HC═O), 1641 (C═N), 1611 (C═C), 1560 (N–B), 1302 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 269 {[OHArCH═N–$(CH_2)_2$–N═CHArOHH]$^+$, 100}.

*2.3.3.3. **3bII**.* Complex **3bII** was prepared from SalenH$_2$ (**1b**) (0.66 g, 2.30 mmol), boric acid (0.28 g, 4.60 mmol) and 4-formylphenylboronic acid (0.35 g, 2.30 mmol) (14 h). Two diastereomers were obtained in a ratio of approximately 52 : 48. Yield: 0.52 g (49%). mp: >300 °C. Anal. calc. for $C_{24}H_{21}B_3N_2O_6$ (%): C, 61.88; H, 4.54; N, 6.01. Found; C, 61.85; H, 4.60; N, 6.04%. IR (KBr, cm^{-1}): ν 3060; 3030 (C–H arom.), 2849; 2725 (H–CO), 1694 (HC═O), 1641 (C═N), 1609 (C═C), 1560 (N–B), 1300 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 283 {[OHArCH═N–$(CH_2)_3$–N═CHArOHH]$^+$, 100}.

*2.3.3.4. **3cII**.* Complex **3cII** was prepared from SalenH$_2$ (**1c**) (0.97 g, 3.30 mmol), boric acid (0.48 g, 6.60 mmol) and 4-formylphenylboronic acid (0.50 g, 3.30 mmol) (12 h). Yield: 0.58 g (37%). mp: >300 °C. Anal. calc. for $C_{25}H_{23}B_3N_2O_6$ (%): C, 62.57; H, 4.83; N, 5.84. Found; C, 62.50; H, 4.79; N, 5.80%. IR (KBr, cm^{-1}): ν 3060; 3030 (C–H arom.), 2880; 2820 (H–CO), 1705 (HC═O), 1641 (C═N), 1609 (C═C), 1560 (N–B), 1302 (O–B). ESI-MS (fragments were based on ^{11}B, I_r %): *m*/*z* 297 {[OHArCH═N–$(CH_2)_4$–N═CHArOH*H*]$^+$, 49}.

2.4. DNA cleavage activity

Plasmid DNA (Thermo Scientific™ pBR322 Plasmid DNA SD0041, 0.5 μg μL^{-1}) was used in the gel electrophoresis experiments and diluted 25 times before use. It was run on 1% agarose gel in a tris(hydroxymethyl)aminomethane-borate-EDTA (TAE) running buffer solution. DMSO was often used to prepare solutions of compounds with low water solubility for investigating DNA interactions.[25] In several studies, it has been shown that using minimum amounts of DMSO for the preparation of the stock solutions has no effect on nucleic acids.[26] The reaction mixtures were prepared (20 μL) in 50 mM ammonium acetate buffer, pH 7.4 at 0 °C, containing 10 μL of plasmid DNA and 10 μL of the complex at two different concentrations by preparing [DNA]/[complex] ratios of 0.8 and 8. They were incubated at 37 °C for 24 h in the dark. From the reaction mixtures, 10 μL aliquots of complex-DNA mixtures were loaded onto 1% agarose gel with a loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol), and electrophoresis was performed on a Thermo Midi-cell Primo horizontal electrophoresis system in 0.05 M tris base, 0.05 M glacial acetic acid and 1 mM EDTA (TAE buffer, pH = 8.0) for 5 h at 35 V with a Thermo EC250-90 power supply. The gel was stained with ethidium bromide solution (0.5 μg mL^{-1}) for 30 min. It was rinsed with water for 20 min and then visualized under UV light. The experiments were repeated three times. Untreated DNA was used as a control.

2.5. UV titrations

Solutions of calf thymus DNA (CT-DNA; purchased from Sigma) in 50 mM ammonium acetate (pH = 7.4) had a UV-vis absorbance ratio A_{260}/A_{280}= 1.9, indicating that the DNA was sufficiently free of protein. The concentration of DNA was determined spectrophotometrically using a molar absorptivity of 6600 M^{-1} cm^{-1} (260 nm).[27] Double-distilled water was used to prepare buffers. Stock solutions of CT-DNA were stored at 4 °C and not used after 4 days. UV-vis spectra were recorded on a Carry WinUV 100 Bio Varian spectrophotometer. The complexes were dissolved in minimum amounts of DMSO before preparing stock solutions. The absorption titrations of the dinuclear (**2bI** and **2bII**) and trinuclear (**3bI** and **3bII**) boron complexes were performed in the buffer. The concentration of the boron complexes was fixed (20 μM). The increments of the 3 mM DNA stock solution were added to the complex solutions. The complex solutions were thoroughly mixed after every addition and incubated for 10 min before the absorption spectra were recorded.

3. Results and discussion

3.1. Chemistry

SalenH$_2$ type symmetrical bulky ligands are useful for the synthesis of boron complexes because the boron atoms form strong covalent bonds with the phenolic oxygens and coordinate covalent bonds with the azomethine nitrogens. Arylboronic acids (phenylboronic acid and 4-hydroxyphenylboronic acid) and SalenH$_2$ ligands [HOArCH═N–R–N═CHArOH; R = $(CH_2)_n$, *n* = 2 (**1a**), *n* = 3 (**1b**) and *n* = 4 (**1c**)] underwent condensation reactions in a 2 : 1 stoichiometry in toluene to obtain dinuclear boron complexes with a boraxane group (PhB–O–BPh) (**2aI–2cI** and **2aII–2cII**). Trinuclear boron complexes with a boroxine group (B–O–B)–(O_2BPh) (**3aI–3cI** and **3aII–3cII**) were synthesized through the combination of boric acid/arylboronic acid (phenylboronic acid and 4-hydroxyphenylboronic acid) and SalenH$_2$ ligands (**1a–1c**) in acetonitrile without isolation of the dinuclear intermediates (Fig. S1, ESI†). A Dean Stark trap was used to separate the water formed during the condensation. The dinuclear boron complexes contain two tetrasubstituted boron atoms, while the trinuclear boron complexes contain two tetrasubstituted and additionally a trisubstituted boron atom. The boron complexes contain central seven- (**2aI**, **2aII**, **3aI** and **3aII**), eight- (**2bI**, **2bII**, **3bI** and **3bII**) and nine- (**2cI**, **2cII**, **3cI** and **3cII**) membered [(B–O–B)–(N–R–N)] heterocyclic ring motifs with an oxygen atom bridging two boron atoms and two azomethine nitrogens forming N–B bonds with the boron atoms.

On the other hand, the choice of the SalenH$_2$ type symmetrical bulky ligands (**1a–1c**) is important because stereochemically controlled reactions may be achieved. Moreover, it is crucial to arrange the configurations of tetrahedrally-coordinated boron atoms to form controlled stereogenic centers. The condensation reaction between the SalenH$_2$ ligands (**1a–1c**) and arylboronic acids (phenylboronic acid and 4-formylphenylboronic acid) and boric acid/arylboronic acids provides two tetrasubstituted boron atoms, [B(N_{imine})(O_B)(O_{Ph})(Ph)], which are centers of chirality.

Therefore, a notable feature of all the boron complexes is the presence of two equivalent stereogenic B-centers and each is potentially capable of existing as two pairs of diastereoisomers of *RS*/*SR* (*meso*) and *RR*/*SS* (racemic) configurations. The stereoisomer distributions and the expected and the isolated isomers of the complexes are presented in Schemes 1 and 2. As is known, four optical isomers (*RR*, *SS*, *RS* and *SR*) are expected from two stereogenic centers. According to Schemes 1 and 2, only one racemic (*RR*/*SS*) and one *meso* (*RS* or *SR*) mixture must form since two phenyl groups are bonded to B-atoms in the *trans* and *cis* fashion, respectively, for the dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes. The *SS*/*RR* forms with a *trans*-configuration of the B-phenyl groups have C2 symmetry while the *RS*/*SR meso* form with a *cis*-configuration of the B-phenyl groups has a molecular plane.

It is noteworthy that the most important result is obtained from the CD and NMR spectra of the boron complexes. The CD spectra of the complexes are depicted in Fig. 1–3 together with the UV spectra. In the UV spectra of the complexes, two bands are observed; namely the first band (270–280 nm) is attributed to the $\pi \rightarrow \pi^*$ transitions of aromatic rings. The second band between 350 and 370 nm involves $n \rightarrow \pi^*$ transitions of the imine groups, which disappear completely in the CD spectra. The CD spectra of two dinuclear (**2aI** and **2aII**) and two trinuclear (**3aI** and **3aII**) boron complexes from the $(CH_2)_2$ precursor, one dinuclear (**2bII**) and one trinuclear (**3bI**) boron complex from the $(CH_2)_3$ precursor and two dinuclear (**2cI** and **2cII**) and two trinuclear (**3cI** and **3cII**) boron complexes from the $(CH_2)_4$ precursor show negative and positive Cotton effects, which is good evidence that the complexes consist of only one enantiomer, *RR* or *SS*. However, one dinuclear (**2bI**) and one trinuclear (**3bII**) boron complex from the $(CH_2)_3$ precursor do not display negative and positive Cotton effects, which indicates that the complexes have a *cis–meso* (*RS*/*SR*) isomer. When we consider all of the reactions, according to the NMR results, the dinuclear (**2bI** and **2bII**) and trinuclear (**3bI** and **3bII**) boron complexes with the $(CH_2)_3$ precursor have been found to be only one diastereomer. On the other hand, the dinuclear (**2aI** and **2aII**) and trinuclear (**3aI** and **3aII**) boron complexes with the $(CH_2)_3$ precursor and the dinuclear boron complex (**2cII**) with the $(CH_2)_4$ precursor were diastereomeric mixtures, and we have been unable to separate the diastereoisomers from any of the reactions. Contrary to this observation, Sánchez *et al.*[23] and Vargas *et al.*[24] conclude that only one diastereomer has been formed for dinuclear **2aI** and trinuclear **3aI**. The findings from the evaluation of NMR (Tables 2 and 3) and CD spectra (Fig. 1–3) together show that the boron complexes with seven- (**2aI**, **2aII**, **3aI** and **3aII**) and nine- (**2cII**) membered heterocycles form two diastereoisomers as one enantiomer (*RR* or *SS*) and one *meso* (*RS*/*SR*), whereas the boron complexes (**2bI** and **3bII**) and (**2bII** and **3bI**) with an eight-membered heterocycle give only a *meso* (*RS*/*SR*) isomer and only one enantiomer (*RR* or *SS*), respectively, and the boron complexes (**3cI** and **3cII**) with a nine-membered heterocycle give only one enantiomer (*RR* or *SS*). Therefore, the number of members in the heterocycles seems to have an effect on the formation of stereoisomers. When we compared the stereoisomers of all seven-membered boron complexes (**2aI**, **2aII**, **3aI** and **3aII**) with the eight-membered counterparts (**2bI**, **2bII**, **3bI** and **3bII**) regardless of whether the complexes are dinuclear or trinuclear, we indeed find the seven-membered complexes to be a mixture of the two diastereoisomers, but the eight-membered ones to be one of the two diastereoisomers. That situation could be significantly attributed to the fact that eight-membered heterocycles are highly flexible structures compared to seven-membered ones. In other words, the seven-membered heterocycles may possess considerably locked-conformations with respect to the eight-membered heterocycles. But, perhaps more significantly, it is not always easy to determine which of the two possible diastereomers are likely to form with higher yields than the other ones, due to the molecular symmetry of the diastereomers (*cis* or *trans* with respect to the B-phenyl groups) (Schemes 1 and 2). To determine which diastereomer is preferred for the boron complexes, an X-ray crystallographic study should be undertaken. Unfortunately, in the present study suitable crystals of the new boron complexes could not be grown in order to understand their preferred configuration.

Despite the preference for either *cis*- or *trans*-configurations being possible, the formation of *cis*-isomers (*RS*/*SR meso* stereoisomer) appears to be much more likely than the formation of *trans*-isomers (*RR* or *SS* enantiomers) during the reaction. However, in the *cis*-configured seven-membered $C_2B_2N_2O$ heterocycles, the two salicylidene groups are oriented parallel to each other, and the proximity between them would be repulsive due to π–π and and steric interactions. Hence, the *trans*-configuration is likely to emerge as a consequence of the twisting of the seven-membered ring. Thus, the yields of the *trans*-isomers ought to be larger than those of the *cis*-isomers. In the case of eight membered heterocycles with a propylene bridge between the two imino groups, as the number of members in the (B–O–B)–(N–R–N) heterocyclic ring increases, π–π interactions between the two salicyclidene groups decrease and only one configuration (*cis* or *trans*) becomes preferred (*meso* for **2bI** and **3bII**, enantiomeric for **2bII** and **3bI**). The molecular structure of eight-membered dinuclear boron complex **2bI** synthesized diastereoselectively was determined in the literature.[23] The X-ray study demonstrated that the *cis*-configuration was preferred over the *trans*-configuration and in this case a boat conformation was formed, with the two imino-nitrogen atoms at the bow/stern positions for **2bI**. Taken together, this result is consistent with our findings from the CD spectrum of **2bI** in solution.

According to our result, trinuclear complexes **3aI** and **3aII** contain an approximately equimolar mixture (50/50) of the two diastereomers (*meso* and enantiomeric forms). It shows that the probabilities for the B–O bond to be the *cis*- or *trans*-configurations are equal and the conformation of the seven-membered $C_2B_2N_2O$ heterocycle is stable for both configurations. These two configurations must form according to the effect on B during the reaction with H_3BO_3. The seven-membered (B–O–B)–(N–R–N) heterocyclic ring is more flexible than the six-membered BOPhCN ring. Even if the six-membered BOPhCN ring having a planar C=N bond and a phenyl group is rigid and there is electron delocalization here, the seven-membered (B–O–B)–(N–R–N)

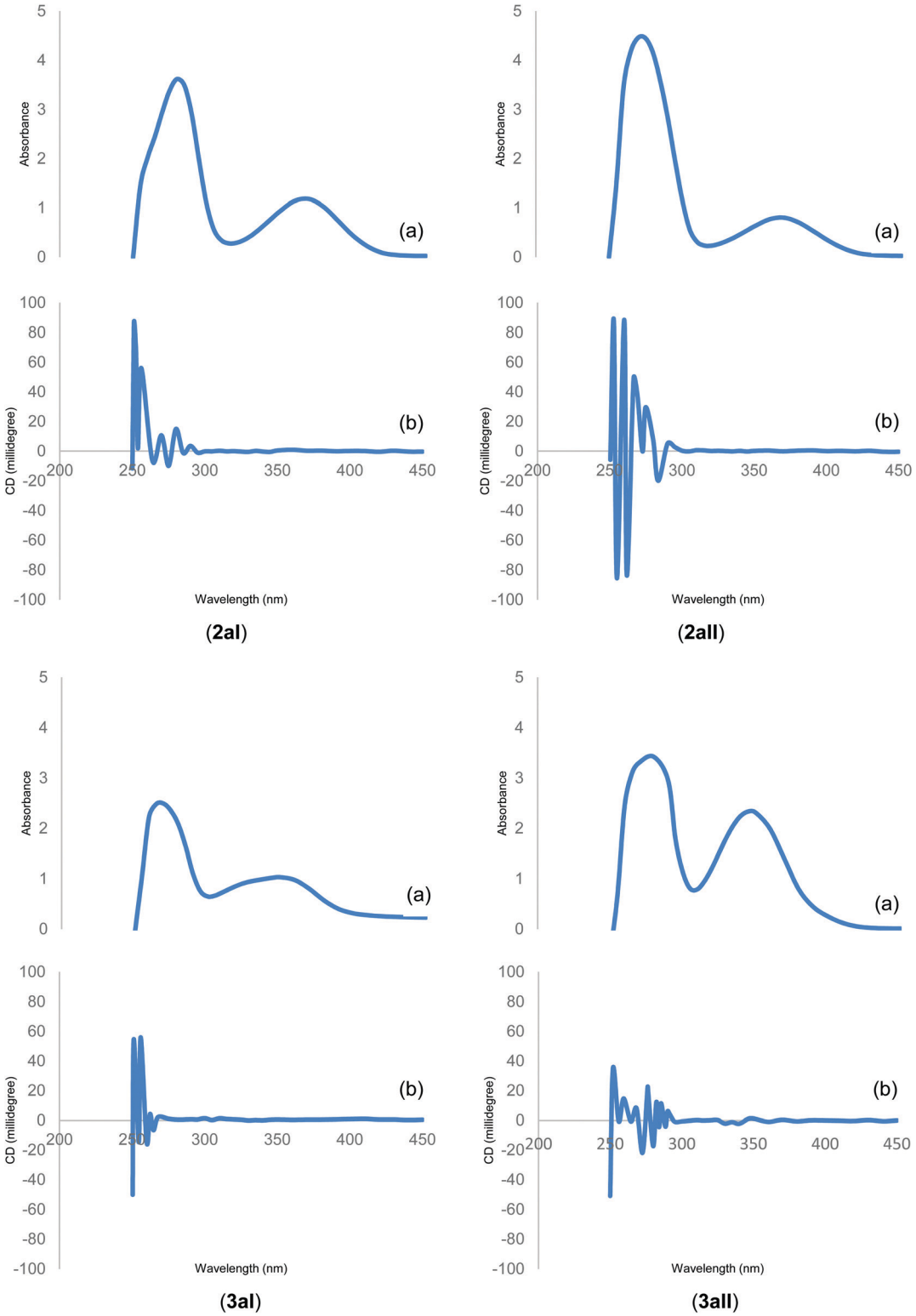


Fig. 1 (a) UV and (b) CD spectra of seven-membered dinuclear (**2aI** and **2aII**) and trinuclear (**3aI** and **3aII**) boron complexes. The spectra in the 400 to 250 nm region are from 1 cm path length cells, and for those in the 400 to 250 nm region the path lengths are 0.2 cm.

heterocyclic ring may be twisted. Therefore, accommodation of the B–N bonds in the seven-membered ring can regulate the conformation, and the seven-membered $B_2C_2N_2O$ heterocyclic ring may have different conformations. According to these conformations, the probability of being both *cis*- and *trans*-configurations seems to be 50%. Considering the dinuclear intermediate given in Fig. S1 (ESI†), it may be possible to be both *cis*- and *trans*-configurations depending on the state of OH. On the other

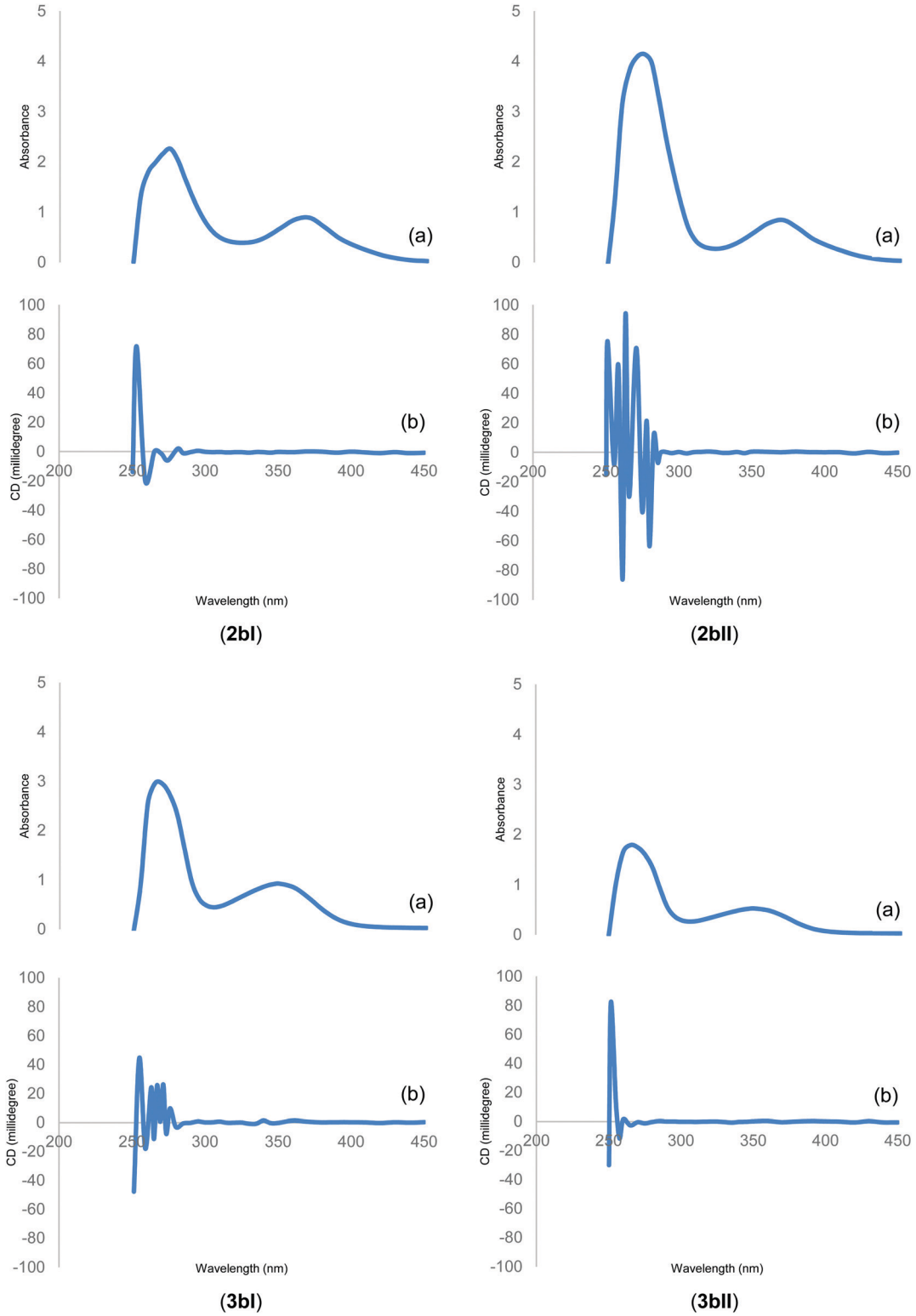


Fig. 2 (a) UV and (b) CD spectra of eight-membered dinuclear (**2bI** and **2aII**) and trinuclear (**3bI** and **3bII**) boron complexes. The spectra in the 400 to 250 nm region are from 1 cm path length cells, and for those in the 400 to 250 nm region the path lengths are 0.2 cm.

hand, the eight-membered (B–O–B)–(N–R–N) ring is not rigid and a single conformation is adopted by the ring. Thus, the conformation of the ring remained twisted, not rotated for trinuclear complexes **3bI** (enatiomeric) and **3bII** (*meso*).

Vargas *et al.*[24] conclude that the seven- and eight-membered (B–O–B)–(N–R–N) heterocyclic rings have different conformations based on X-ray crystallography and theoretical calculations. For example, the seven-membered $B_2C_2N_2O$ heterocycle in the

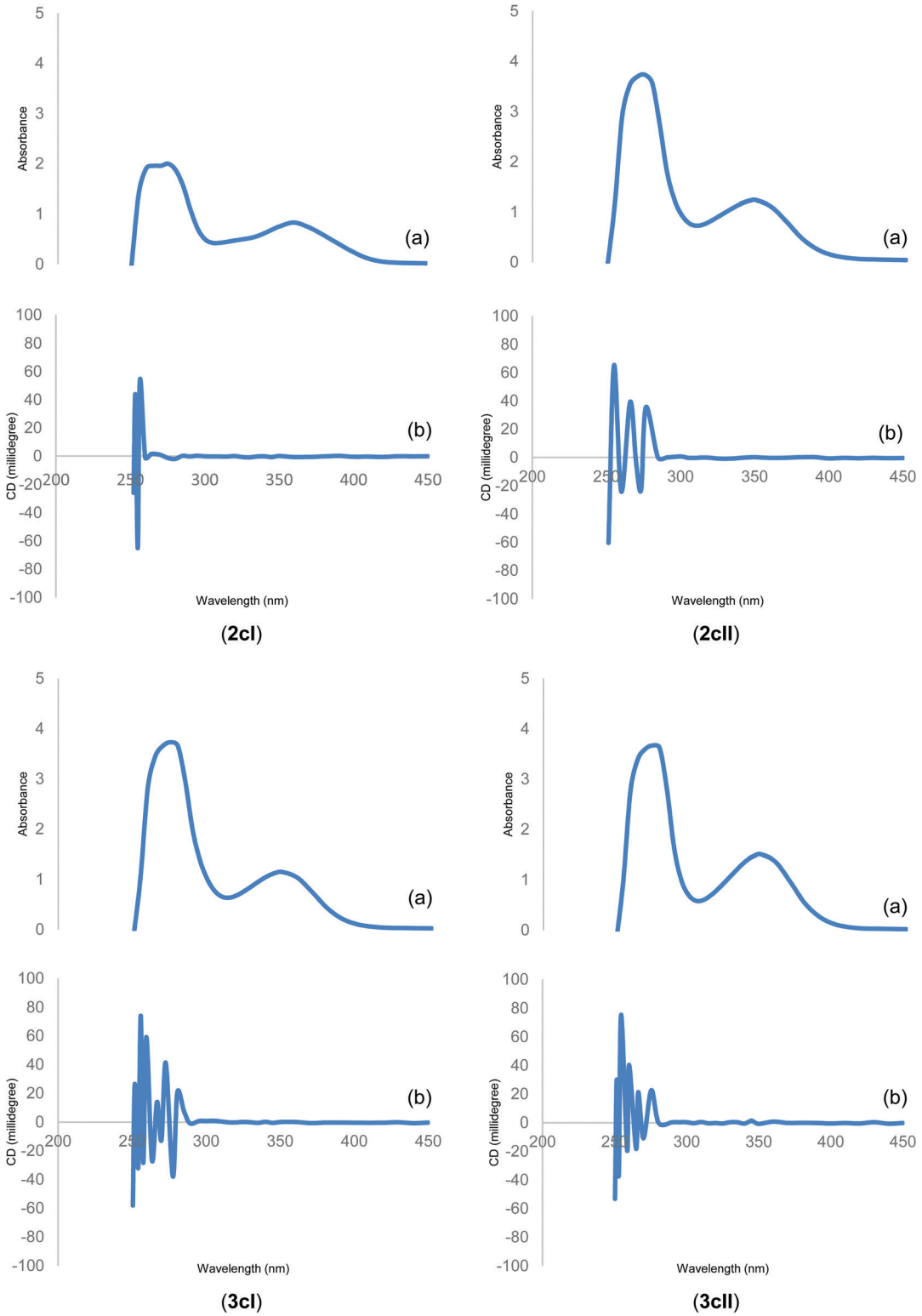
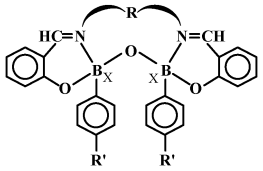
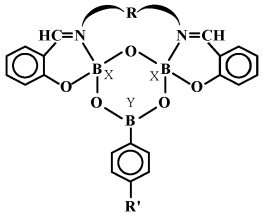


Fig. 3 (a) UV and (b) CD spectra of nine-membered dinuclear (**2bI** and **2aII**) and trinuclear (**3cI** and **3cII**) boron complexes. The spectra in the 400 to 250 nm region are from 1 cm path length cells, and for those in the 400 to 250 nm region the path lengths are 0.2 cm.

experimentally determined and theoretically calculated structures of **3aI** possesses twisted-chair and chair conformations, respectively, while the eight-membered $B_2C_3N_2O$ heterocycle in the theoretically calculated structure of **3bI** prefers a chair–chair conformation. However, in this literature, the issue of stereoisomerism was not discussed.[24] As a result, the trinuclear derivatives can have different configurations depending on their (B–O–B)–(N–R–N) heterocyclic ring conformations.

Table 2 ^{11}B NMR data (d_6-DMSO) of the dinuclear (**2aI–2cI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes (δ in ppm)





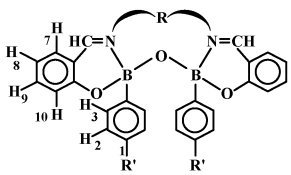
Compound	δB_X	Compound	δB_X	δB_Y
(**2aI**)	3.94	(**3aI**)	1.96	19.71
(**2bI**)	3.99	(**3bI**)	1.55	19.93
(**2cI**)	4.13	(**3cI**)	1.57	19.86
(**2aII**)	3.48	(**3aII**)	1.93	19.73
(**2bII**)	3.63	(**3bII**)	1.44	19.79
(**2cII**)	1.77	(**3cII**)	1.61	19.88

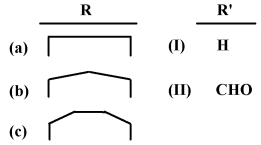
Moreover, the CHO group leads to the formation of one of the enantiomers of dinuclear complex **2bII**, and a *meso* isomer in trinuclear complex **3bII** for the $(CH_2)_3$ chain precursor. As a result, these compounds are likely to be conformationally-locked, and hence they are essentially chiral as they do not convert to their mirror images in the solid state. In the literature, a nice paper[28] asserts that one enantiomer of some conformationally-locked chiral compounds can crystallize spontanaously from the racemic or diastereomeric mixtures. In recent years, this situation has also been observed in some cyclotri[29] and cyclotetra[30] phosphazene derivatives.

All of the boron complexes have relatively high melting points and are stable to air. They (1×10^{-2} g) are insoluble, but decompose in about 2 days in 5 mL of water at room temperature. In boiling water the complexes easily and completely decompose in about 5 min. Moreover, they are practically insoluble in most polar and nonpolar organic solvents, but are sparingly soluble in DMSO. Especially **2cI** dissolves very poorly in DMSO. Hence, its ^{1}H and ^{13}C NMR spectra were not recorded. The boron complexes seem to be stable in DMSO at room temperature for more than three weeks but small quantities of decomposition products are observed in the NMR spectra.

The elemental analyses of the compounds are consistent with the structures, and fragments were observed under electrospray ionization mass spectrometry (ESI-MS) conditions. All the dinuclear boron complexes except **2cI** and **2cII** give protonated molecules $[MH]^+$, which are the parent peaks in relative intensity. In the mass spectra of the trinuclear boron complexes, the molecular ion peak does not appear, but the fragments corresponding to Schiff bases $[[HOArCH{=}N{-}(CH_2)_n{-}N{=}CHArOH\mathit{H}]^+]$ appear at m/z 269 for **3aI** and **3aII**, 283 for **3bI** and **3bII** and 297 for **3cI** and **3cII**.

Table 3 ^{1}H NMR data (d_6-DMSO) of dinuclear boron complexes (**2aI**, **2bI** and **2aII–2cII**) (δ in ppm, J in Hz, s: singlet, d: doublet, dd: doublet of doublets, t: triplet, dt: doublet of triplets, b: broad, and m: multiplet)





		2aI		**2bI**	**2aII**		**2bII**	**2cII**‡	
H		*A*	*B*	*meso*	*A*	*B*	Enantiomeric	*A*	*B*
NCH_2CH_2		—	—	1.96 (m,1H) (a) 2.48 (m,1H) (b)	—	—	2.02 (m,1H) (a) 2.35 (m,1H) (b)	1.48 (m,4H)	1.61 (m,4H)
NCH_2		3.45 (d,2H) (a) 3.75 (d,2H) (b) $^2J_{HaHb}$ = 10	3.72 (d,2H) (a) 3.87 (d,2H) (b) $^2J_{HaHb}$ = 8.2	3.49 (t,4H) $^3J_{HH}$ = 6.0	3.81 (m,2H) (a) 3.85 (m,2H) (b)	*	3.44 (m,2H) (a) 3.53 (m,2H) (b)	3.34 (m,2H) (a) 3.49 (m,2H) (b)	3.00 (m,2H) (a) 3.19 (m,2H) (b)
CHO		—	—	—	9.92 (s,2H)	9.82 (s,2H)	9.93 (s,2H)	9.88 (s,2H)	10.0 (s,2H)
HC=N		8.56 (s,2H)	8.49 (s,2H)	8.53 (s,2H)	8.58 (s,2H)	8.61 (s,2H)	8.60 (s,2H)	8.40 (s,2H)	8.64 (s,2H)
ArH	H_1	6.91 (d,2H)	6.95 (d,2H)	7.13 (m,2H)	—	—	—	—	—
	H_2	7.14 (t,4H)	7.11 (t,4H)	7.21 (t,4H)	7.72 (d,4H)	7.85 (d,4H)	7.76 (m,4H)	7.69	7.83
	H_3	7.17 (dd,4H)	7.45 (dd,4H)	7.57 (d,4H)	7.66 (d,4H)	7.97 (d,4H)	7.76 (m,4H)	7.6	7.71
	H_7	7.40 (dd,2H)	7.25 (dd,2H)	7.05 (d,2H)	7.29 (d,2H)	7.47 (d,2H)	7.07 (dd,2H)	7.17 (d)	7.24 (d)
	H_8	6.85 (t,2H)	6.69 (dt,2H)	6.48 (t,2H)	6.73 (t,2H)	7.22 (t,2H)	6.50 (dt,2H)	6.91	6.53 (b,2H)
	H_9	7.55 (dt,2H)	7.36 (dt,2H)	7.15 (m,2H)	7.39 (ti,2H)	7.56 (t,2H)	7.17 (dt,2H)	7.52	6.9
	H_{10}	6.84 (d,2H)	6.59 (d,2H)	6.36 (d,2H)	6.63 (d,2H)	7.15 (d,2H)	6.38 (d,2H)	6.85 (d)	6.28 (b,2H)
$^3J_{1-2}$		7.6	7.2	7.2	—	—	—	—	—
$^4J_{1-3}$		1.6	1.6	*	—	—	—	**	**
$^3J_{2-3}$		8.0	8.0	7.0	8.0	8.0	*	7.6	**
$^3J_{7-8}$		8.2	7.8	8.0	7.2	8.0	7.6	7.2	7.2
$^4J_{7-9}$		1.4	1.8	1.4	1.6	*	1.6	**	**
$^3J_{8-9}$		7.8	7.9	7.6	7.6	7.9	7.4	**	**
$^4J_{8-10}$		*	1.2	*	*	*	0.8	**	**
$^3J_{9-10}$		7.6	8.4	8.8	8.4	8.0	8.4	**	**

A designates a major diastereoisomer. *A*: *meso* and *B*: enantiomeric or *A*: enantiomeric and *B*: *meso* forms, ‡poor solubility, * not determined, ** not calculated. The notations a and b are used to show nonequivalent nuclei in NMR spectra.

These fragments show that the major fragmentation pathway involves the initial cleavage of the O–B and N–B bonds for the trinuclear boron complexes. The ion peak at *m*/*z* 297 corresponding to [[HOArCH═N–$(CH_2)_4$–N═CHArOHH]$^+$] is also attributed to the cleavage of O–B and N–B bonds in two dinuclear complexes **2cI** and **2cII** from the $(CH_2)_4$ chain precursor.

3.2. IR spectroscopy

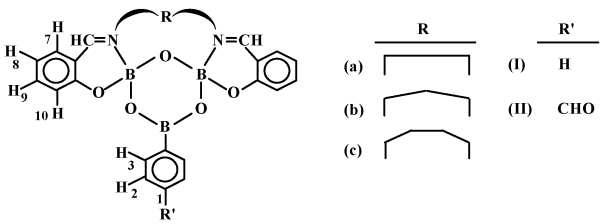
The IR spectra of the boron complexes (Fig. S2–S13, ESI†) show that the νC═N stretching frequencies (1638–1649 cm^{-1}) are shifted to higher wavenumbers ($\Delta\nu$ = 12–30 cm^{-1}) compared to the free SalenH_2 ligands [**1a** (1618 cm^{-1}), **1b** (1625 cm^{-1}) and **1c** (1624 cm^{-1})]. This is due to the N → B coordination, which serves to increase the electron density in the C═N bonds. In addition, compared with the IR spectra of the SalenH_2 ligands, the IR spectra of the complexes display new absorption bands appearing at 1557–1562 cm^{-1} and 1300–1319 cm^{-1} indicating the existence of N–B and O–B bonds, respectively.

3.3. NMR spectroscopy

The existence of N–B and O–B bonds was established by ^{11}B NMR (Fig. S14–S25, ESI†). It is not understandable from the ^{11}B NMR spectra whether the complexes contain a diastereomer mixture or not. For the dinuclear boron complexes (**2aI–2cI** and **2aII–2cII**), the signals at 4.13–1.77 ppm are characteristic of the tetrasubstituted boron atoms (Table 2). For the trinuclear complexes (**3aI–3cI** and **3aII–3cII**), this signal is slightly high-field-shifted $\Delta\delta$ = 2.56–0.16 ppm and additionally a less intense signal at 19.93–19.71 ppm is typical for a trisubstituted boron atom. Therefore, a trisubstituted boron atom is more acidic than the other two. The chemical shifts are indicative of tetra- and trisubstituted boron atoms and these shifts are in agrement with findings in the literature for tetra- and trisubstituted boron atoms.[31]

The ^{1}H and ^{13}C NMR spectral data establish the symmetrical nature of the boron complexes. The ^{1}H (Fig. S26–S36, ESI†) and ^{13}C NMR (Fig. S37–S47, ESI†) resonances of the boron complexes are summarized in Tables 3–5, respectively. The assignments were made unambiguously by HSQC (Fig. S48–S58, ESI†). The ^{1}H and ^{13}C NMR spectra reflect the differences between the two sets of isolated boron complexes. The spectra of **2bI**, **2bII**, **3bI** and **3bII** [R = $(CH_2)_3$] show one set of signals corresponding to only one diastereoisomer. However, the spectra of **2aI**, **2aII**, **3aI** and **3aII** [R = $(CH_2)_2$] and **2cII** [R = $(CH_2)_4$] show greater complexity due to doubling of the signals for the diastereoisomers. In the ^{1}H NMR spectra of **2aI**, **2aII**, **2cII**, **3aI** and **3aII**, the two sets of signals corresponding to the *meso* and enantiomeric isomers are readily assigned to HC═N (**2aI**, **2aII**, **2cII**, **3aI**), CHO (**2aII**, **2cII**, **3aII**), H_3 (**2aII**, **3aI**), H_7 (**2aI**, **2aII**, **3aII**) and H_9 (**2aI**, **2aII**) and integration of the signals is consistent with the populations of the two isomers in ratios of 65 : 35 (**2aI**), 96 : 4 (**2aII**), 86 : 14 (**2cII**), 53 : 47 (**3aI**), and 52 : 48 (**3aII**) (Tables 3 and 4). The disappearance of the singlet signals at 13.4 (**1a**), 13.5 (**1b**) and 13.6 (**1c**) ppm assigned to the Ar–OH protons of the SalenH_2 ligands (**1a–1c**) in the ^{1}H NMR spectra of the boron complexes indicates that the phenolic oxygen atoms are covalently bonded

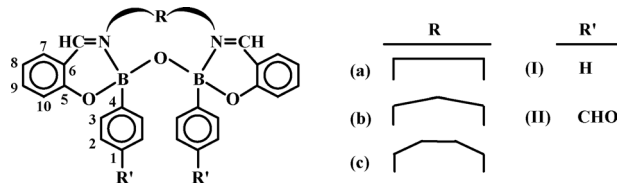
Table 4 ^{1}H NMR data (d_6-DMSO) of trinuclear boron complexes (**3aI–3cI** and **3aII–3cII**) (δ in ppm, *J* in Hz, s: singlet, d: doublet, t: triplet, and m: multiplet)

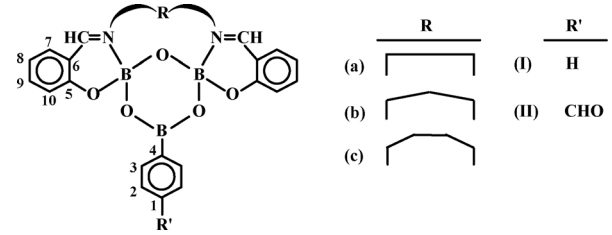


		3aI		3bI‡	3cI‡	3aII		3bII‡	3cII‡
H		*A*	*B*	Enantiomeric	Enantiomeric	*A*	*B*	meso	Enantiomeric
NCH_2CH_2		—	—	2.53 (m,2H)	1.77 (m,4H)	—	—	2.23 (m,2H)	1.93 (m,4H)
NCH_2		3.98 (m,4H)	4.07 (m,4H)	3.85 (t,4H)	3.76 (m,4H)	4.06 (m,4H)	3.99 (m,4H)	3.81 (m,8H)	3.80 (m,8H)
CHO		—	—	—	—	9.82 (s,2H)	9.91 (s,2H)	9.91 (s,2H)	9.92 (s,2H)
HC═N		8.63 (s,4H)		8.61 (s,2H)	8.65 (s,2H)	8.63 (s,4H)		8.62 (s,2H)	8.67 (s,2H)
ArH	H_1	7.18 (d,2H)		7.13 (d,1H)	7.11 (m,1H)	—	—	—	—
	H_2	7.17 (t,4H)		7.25 (m,2H)	7.21 (m,2H)	7.42 (d,4H)		7.59 (d,2H)	7.60 (d,2H)
	H_3	7.54 (d,2H)	7.57 (d,2H)	7.47 (d,2H)	7.39 (d,2H)	7.68 (d,2H)	7.47 (d,2H)	7.69 (d,2H)	7.87 (d,2H)
	H_7	7.53 (d,4H)		7.73 (m,2H)	7.68 (m,2H)	7.53 (d,2H)	7.65 (d,2H)	7.47 (d,2H)	7.55–7.41
	H_8	6.96 (t,2H)	6.91 (t,2H)	6.82 (m,2H)	6.82 (t,2H)	6.91 (t,4H)		6.90–6.80	6.97–6.80
	H_9	7.52 (t,4H)		7.41 (m,2H)	7.40 (m,2H)	7.50 (t,4H)		7.48 (t,2H)	7.55–7.41
	H_{10}	6.84 (d,2H)	6.90 (d,2H)	6.88 (d,2H)	6.85 (m,2H)	6.90 (d,4H)		6.90–6.80	6.97–6.80
$^3J_{1-2}$		6.0		6.4	**	—	—	—	—
$^3J_{2-3}$		8.4	8.4	8.0	7.2	8.0	7.6	7.6	8.0
$^3J_{7-8}$		8.8	7.8	**	7.6	7.6	8.0	7.6	**
$^3J_{8-9}$		7.6	7.6	**	7.2	7.6		8.0	**
$^3J_{9-10}$		7.8	7.6	7.6	**	7.2		8.0	**

A designates a major diastereoisomer. *A*: *meso* and *B*: enantiomeric or *A*: enantiomeric and *B*: *meso* forms, ‡poor solubility, ** not calculated.

Table 5 ^{13}C NMR data (d_6-DMSO) of dinuclear (**2aI**, **2bI** and **2aII–2cII**) and trinuclear (**3aI–3cI** and **3aII–3cII**) boron complexes (δ in ppm)





		2aI		**2bI**	**2aII**		**2bII**	**2cII**‡		**3aI**		**3bI**‡	**3cI**‡	**3aII**		**3bII**‡	**3cII**‡
C		*A*	*B*	*meso*	*A*	*B*	Enantiomeric	*A*	*B*	*A*	*B*	Enantiomeric	Enantiomeric	*A*	*B*	*meso*	Enantiomeric
$NCH_2\boldsymbol{C}H_2$		—	—	31.0	—	—	31.7	27.3	28.2	—	—	30.0	27.2	—	—	31.7	27.1
$N\boldsymbol{C}H_2$		56.6	53.0	52.5	53.0	*	52.9	53.2	52.9	54.7		50.8	52.1	54.8		51.5	52.3
$\boldsymbol{C}HO$		—	—	—	193.9	*	193.7	193.9	194.2	—	—	—	—	193.2	193.3	*	193.3
HC═N		165.5	164.9	163.6	166.5	*	164.9	163.9	163.1	162.9		163.3	162.7	162.9		163.8	163.4
Ar***C***	$\boldsymbol{C}_1$	126.6	126.3	126.0	135.3	135.2	135.3	135.5	135.3	127.0		126.7	126.6	134.5	134.8	*	134.8
	$\boldsymbol{C}_2$	127.0	127.3	126.7	128.7	128.6	128.7	128.8	128.9	131.8		127.2	127.2	132.3		132.6	132.1
	$\boldsymbol{C}_3$	132.2	131.6	131.3	132.2	*	132.3	132.8	132.7	132.0	132.1	131.5	131.4	128.3	128.0	128.8	128.1
	$\boldsymbol{C}_4$	*	*	*	*	*	*	*	*	163.3	164.4	*	*	165.2	165.8	*	*
	$\boldsymbol{C}_5$	160.7	161.6	160.6	161.4	*	160.8	160.3	160.0	159.6	159.5	159.4	159.4	159.8	160.7	160.2	159.7
	$\boldsymbol{C}_6$	116.3	117.0	115.5	116.9	*	115.9	116.7	116.1	116.3	116.2	116.3	116.0	116.4		116.9	116.1
	$\boldsymbol{C}_7$	132.6	132.3	131.1	132.5	*	131.8	132.6	132.5	134.2		134.0	134.0	131.8	131.7	132.1	131.5
	$\boldsymbol{C}_8$	118.0	117.9	116.7	118.3	*	117.6	119.1	118.1	118.6	118.5	118.4	118.3	118.6		118.9	118.4
	$\boldsymbol{C}_9$	137.9	137.5	136.5	137.8	*	137.3	138.0	137.6	136.5		136.3	136.5	136.5		136.8	136.9
	$\boldsymbol{C}_{10}$	118.9		117.9	118.9	*	118.4	118.8	118.0	118.4	117.6	116.4	116.2	118.5		118.6	118.0

A designates a major diastereoisomer. *A*: *meso* and *B*: enantiomeric or *A*: enantiomeric and *B*: *meso* forms, ‡poor solubility, *not determined.

to the boron atoms. The same result was confirmed by the IR spectra.

The singlet signals due to azomethine protons (*HC*═N) [8.59 (**1a**), 8.58 (**1b**) and 8.56 (**1c**) ppm in the free $SalenH_2$ ligands] are shifted towards higher fields for **2aI** and **2bI**, and lower fields for **2aII**, **2bII**, **2cII**, **3aI**, **3bI**, **3cI**, **3aII**, **3bII** and **3cII**, confirming the involvement of azomethine nitrogen in the formation of the boron complexes. Upfield shifts are expected for the δHC═N values of the boron complexes, compared to those of the $SalenH_2$ ligands (**1a–1c**). This situation depends on the electron density around the HC═N bonds increasing by electron delocalization throughout the six-membered BOPhCN rings after the N → B coordination, and this is in agreement with observations on similar structures.[32] However, this is observed for dinuclear boron complexes **2aI** and **2bI**. The downfield shifts for the δHC═N values of the dinuclear **2aII** and **2bII** and trinuclear **3aII** and **3cII** boron complexes can be attributed to the electronic changes induced by the *p*-CHO group in the B-phenyl moiety, which has an electron withdrawing effect. The existence of stereogenic centers at the two boron atoms gives rise to diastereotopic signals for the methylene hydrogens ($\Delta\delta$ = 0.04–0.30 ppm for NCH_2 and $\Delta\delta$ = 0.33–0.52 ppm for NCH_2CH_2).

The ^{13}C NMR spectra of the seven- (**2aI**, **2aII**, **3aI** and **3aII**) and nine- (**2cII**) membered heterocycles exhibit two sets of signals, which correspond to the *meso* and enantiomeric isomers (Fig. S48, S50, S52, S53, S56, ESI† and Table 4). The NMR signals of the aromatic *ipso* carbon atoms (C_4) for the boron complexes except **3aI** and **3aII** were not detectable, which may be due to the fast relaxation caused by the magnetic interaction with the boron nuclei.[33]

Consequently as explained above, the ^1H and ^{13}C NMR spectra of some of the complexes indicate that there are only a *meso* (*cis*) (*RS*/*SR*) isomer and two enantiomers (*RR*/*SS*) in the racemic (*trans*) mixtures. It is understood that both isomers do not form equally. One might expect to separate the diastereoisomers or enantiomers of these boron complexes into components by HPLC on a chiral column. However, the solubility problem of the boron complexes in common organic solvents is the biggest obstacle to this study. In addition, to our knowledge, no report has appeared in the literature on the performance to separate diastereoisomers or enantiomers of boron complexes derived from arylboronic acids and imine ligands and stereogenic only at B-atom/atoms.

3.5. Interactions of DNA with the compounds

3.5.1. Agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration is observed in the intact supercoiled form (Form I). If a scission occurs on one strand (nicked circular), then the supercoil form relaxes to generate a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between forms I and II is generated.[34] The cleaving activities of the complexes against pBR322 plasmid DNA were tested by agarose gel electrophoresis study. Two concentrations of the complexes were tried. In Fig. 4, C is the control line where no compounds have been added to the pBR322 plasmid DNA. There is mainly one large band of supercoiled DNA (Form I) and a low amount of the nicked DNA (Form II) above it. Form II becomes larger with a scission. When multiple scissions occurred in both strands, fast-moving small DNA pieces (Form III) were formed for **2bI**, **2aII**, **2bII** and **3bII**. While the strongest activity was found in the complexes from the $(CH_2)_3$ precursor, no activity was observed in the complexes from the $(CH_2)_4$ precursor. The scission activities can be put into order as **3bII** > **2bII** > **2bI** > **2aII** > **3cII**. The Schiff bases and starting materials showed no activity against pBR322 plasmid DNA at a very high [DNA]/[complex] ratio (Fig. 5).

3.5.2. UV titrations. The complexes with the $(CH_2)_3$ precursor (**2bI**, **2bII**, **3bI** and **3bII**) were titrated with DNA solutions for the evaluation of changes in the UV spectra (Fig. 6) because of their high cleavage activities against pBR322. When the DNA concentration increases, the degree of hypochromism also increases according to the UV spectra of the complexes, indicating

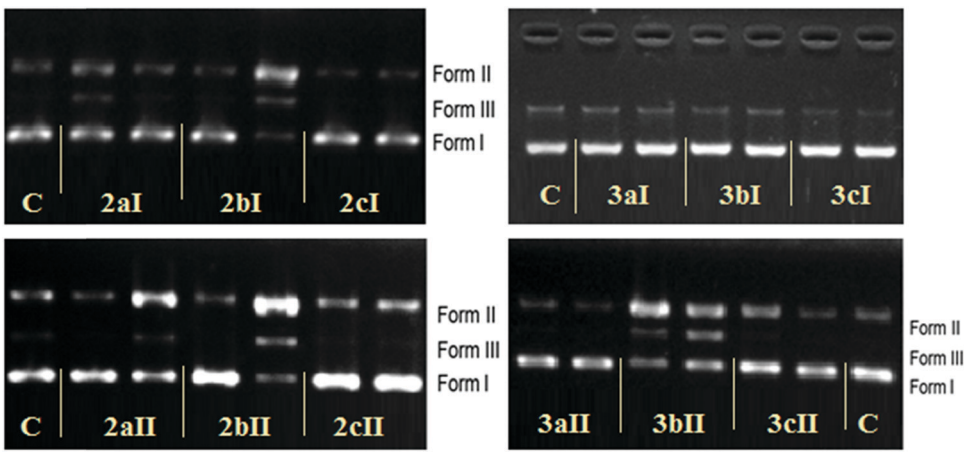


Fig. 4 The electrophoretograms of pBR322 plasmid DNA (15 μM) in the presence of the complexes at two concentrations where the [DNA]/[complex] ratios are 0.8 and 8, respectively, in 50 mM ammonium acetate buffer, pH 7.4. "C" is for control; only DNA, no compounds added.

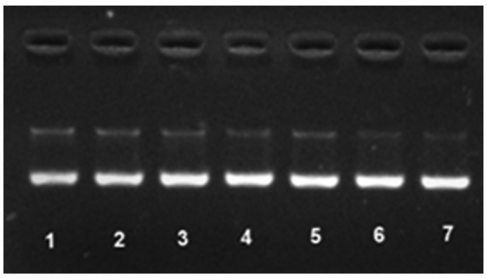


Fig. 5 The electrophoretograms of pBR322 plasmid DNA (15 μM) in 50 mM ammonium acetate buffer, pH 7.4, in the presence of the Schiff bases and the starting materials. Lane 1 is for the control; only DNA, no compounds added. Lanes 2–7 are for **1a**, **1b**, **1c**, boric acid, phenylboronic acid and 4-formylphenylboronic acid, respectively. The [DNA]/[compound] ratios are 12.

a mode of groove binding. In addition to the hypochromism, a bathochromic shift (2–3 nm) indicated a stronger insertion of complex **3bII** into the DNA grooves. For better evaluation and comparison between the complexes, the binding constants K_b were calculated:

$$[DNA]/(\varepsilon_A - \varepsilon_f) = [DNA]/(\varepsilon_B - \varepsilon_f) + 1/K_b(\varepsilon_B - \varepsilon_f)$$

where [DNA] is in base pairs, ε_A is the absorption coefficient of the complex for each measurement, and ε_f and ε_B are the absorptivity values of the free and the fully bound form of the complex. The binding constant K_b is given by the ratio of the slope to the intercept in the $[DNA]/(\varepsilon_A - \varepsilon_f)$ *vs.* [DNA] plot. The calculated binding constants were found to be around 10^4 M^{-1}, comparable to recent *spiro*-cyclotriphosphazenes containing 4-hydroxyphenylethyl pendant arms.[35] According to Table 6, the highest binding constant among the boron complexes with seven-membered heterocycles was observed for **3bII**, similar to its cleavage activity. On the other hand, the lowest binding value belongs to complex **3bI**, which has no activity against pBR322 plasmid DNA in the electrophoresis study.

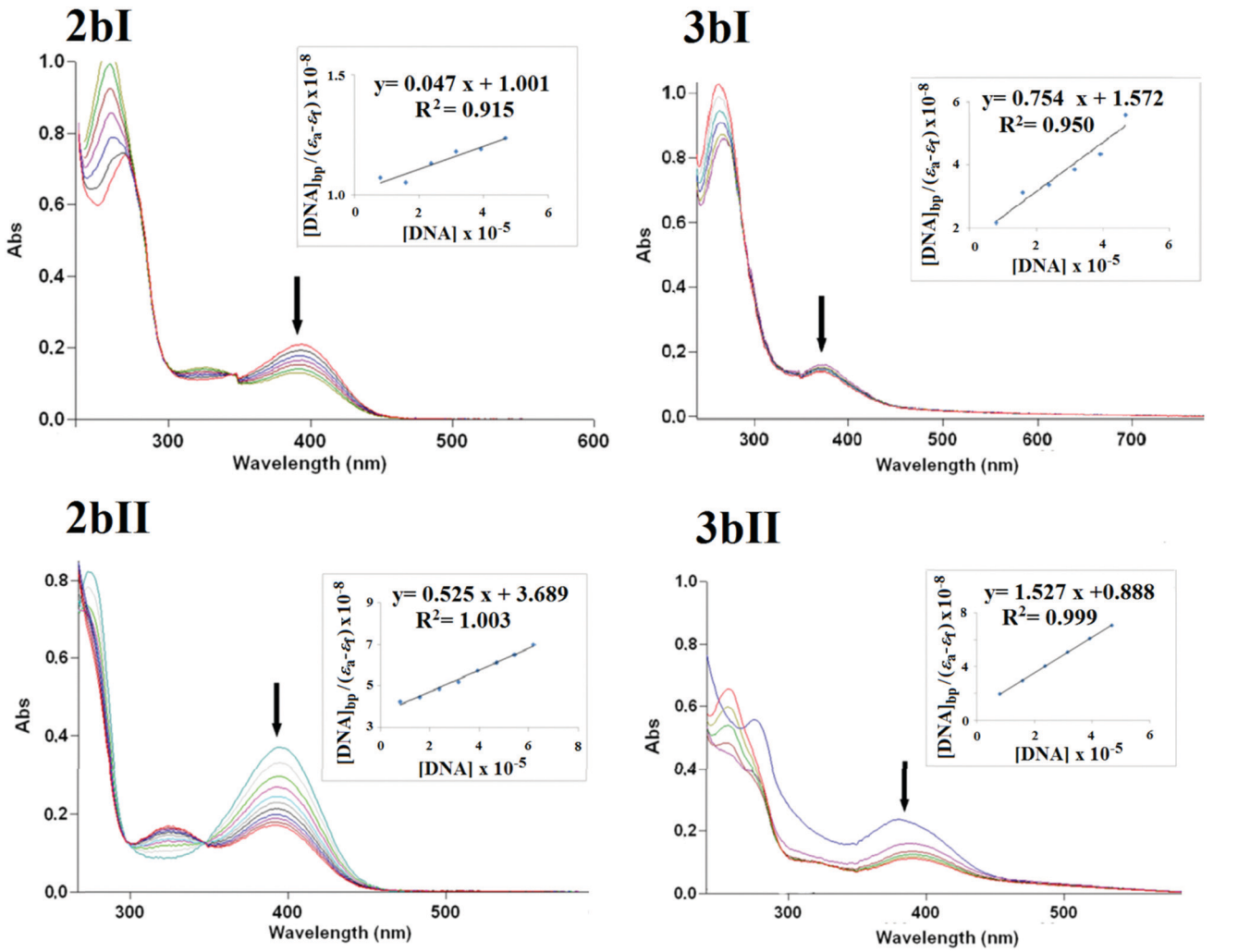


Fig. 6 The change in the absorption spectra of complexes from the $(CH_2)_3$ precursor (**2bI**, **2bII**, **3bI** and **3bII**) (20 μM) in 50 mM ammonium acetate buffer on the addition of CT-DNA: 0–32 μM. The arrows show the absorbance change with an increase in DNA concentration. The insets show plots of DNA concentration divided by the difference between the apparent absorption coefficient (ε_A) and the absorption coefficient of the free complexes (ε_f) *versus* DNA concentration.

Table 6 Binding constants of the complexes for double-stranded DNA (calf-thymus DNA)

Complexes	2bI	2bII	3bII	3bI
K_b ($\times 10^4$ M^{-1})	0.50	0.62	1.50	0.14

The stability of $SalenH_2$ ligands in aqueous solutions was always debated.[36] DNA titration of $SalenH_2$ ligand **1b** was also performed and a similar interaction pattern was observed (Fig. 7a). When the compound was studied without DNA addition in the titration conditions at room temperature, a new formation takes place within a short time after the compound is introduced into the buffer solution (Fig. 7b). Once the new species occurred in the solution it was again titrated with DNA for new possible interactions. However, there was no change in the UV spectrum of the "decomposed" $SalenH_2$ ligand (Fig. 7c), indicating that the decomposition products did not interact with DNA. The electrophoresis study of the other $SalenH_2$ ligands (**1a** and **1c**) gave similar results.

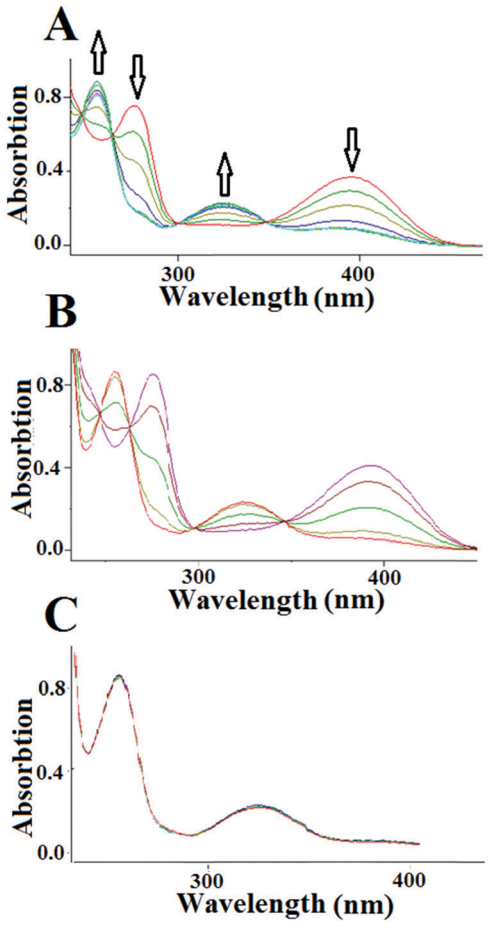


Fig. 7 The change in the absorption spectra of **1b** (20 μM) in 50 mM ammonium acetate buffer at pH 7.4 (a) on the addition of CT-DNA: 0–32 μM. The arrows show the absorbance change with an increase in DNA concentration. (b) (The stability titration) After **1b** is introduced into the solution the absorption peak at 400 nm started to decrease and completely disappeared at the 20th minute. However, the peak at 320 nm increased. (c) (After decomposition) No change can be seen upon addition of DNA.

Eventually, the complexes have moderate binding constants (Table 6) for the double-stranded DNA with the groove binding mode. There is a paper on DNA cleavage activity studies of boric acid complexes obtained from the reaction of citric, glucuronic and salicylic acids with boric acid in the literature.[19] The paper concluded that there was an intercalative interaction between the boric acid complexes and DNA. This result is consistent with the findings of the present study.

4. Conclusions

The synthesis, spectroscopic characterization and stereoisomerism of two different architectural types of tetrahedrally coordinated multi-heterocyclic boron compounds, namely di- and trinuclear boron complexes, were studied and compared with each other. These complexes have two equivalent chiral B-centers, giving rise to diastereoisomers. According to the ^{13}C and ^{1}H NMR spectra of the complexes, it was understood that two dinuclear (**2bI** and **2bII**) and two trinuclear (**3bI** and **3bII**) boron complexes from the $(CH_2)_3$ precursor and two trinuclear (**3cI** and **3cII**) from the $(CH_2)_4$ precursor were present as only one diastereomer, whereas two dinuclear (**2aI** and **2aII**) and two trinuclear (**3aI** and **3aII**) boron complexes from the $(CH_2)_2$ precursor and one dinuclear (**2cII**) complex from the $(CH_2)_4$ precursor were found to be diastereomeric mixtures. More reliable results were obtained by the combination of the NMR and CD spectra of the complexes. On the other hand, CD spectroscopy is a potential tool for determining which of the diastereoisomers in the diastereomeric boron complexes is present and whether the boron complex contains only a single diastereoisomer or a mixture of diastereoisomers.

Furthermore, when all DNA interaction studies were considered together, the seven-membered boron complexes from the $(CH_2)_3$ precursor were found to be the most effective ones in each architectural type of the boron complexes. Whether it is a dinuclear or trinuclear boron complex or whether there is a CHO group in the phenyl ring or not, the number of methylene groups in the $(CH_2)_n$ chain precursors or the number of members in the [(B–O–B)–(N–R–N)] heterocyclic ring was found to have a significant impact on the DNA cleavage activity. When compared to the DNA cleavage ability of dinuclear (**2bI** and **2bII**) and trinuclear (**3bI** and **3bII**) boron complexes with an eight-membered heterocycle, cleavage was found to be the most efficient in the presence of trinuclear boron complex **3bII**. This result can be explained by the fact that trinuclear boron complex **3bII** contains a planar phenyl ring and a CHO group compared to the dinuclear complexes, and this part may increase the groove binding mode of DNA by van der Waals interactions.

In addition, in the trinuclear boron complexes, two boron atoms have tetrahedral and one boron atom has trigonal planar geometry. Considering that the trigonal planar boron atom may still act as a Lewis acid, the trinuclear boron complexes might be useful as a catalyst for asymmetric synthesis.

As a result, this study answers the following questions: (1) The extent to which the reaction is stereospecific at the

two equivalent chiral boron centers, and what factors determine the major diastereoisomer produced by reaction of a SalenH_2 ligand. (2) The influence of the formyl group CHO attached to the benzene ring on the formation of diastereoisomers and DNA cleavage.

Conflicts of interest

There are no conflicts to declare.

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