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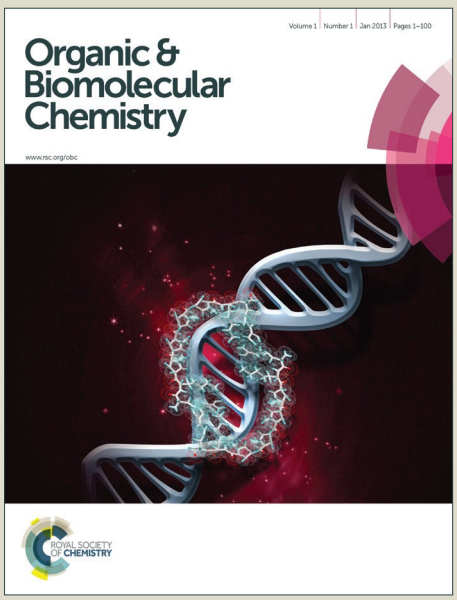

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RNA Nucleosides as Chiral Sensing Agents in NMR Spectroscopy

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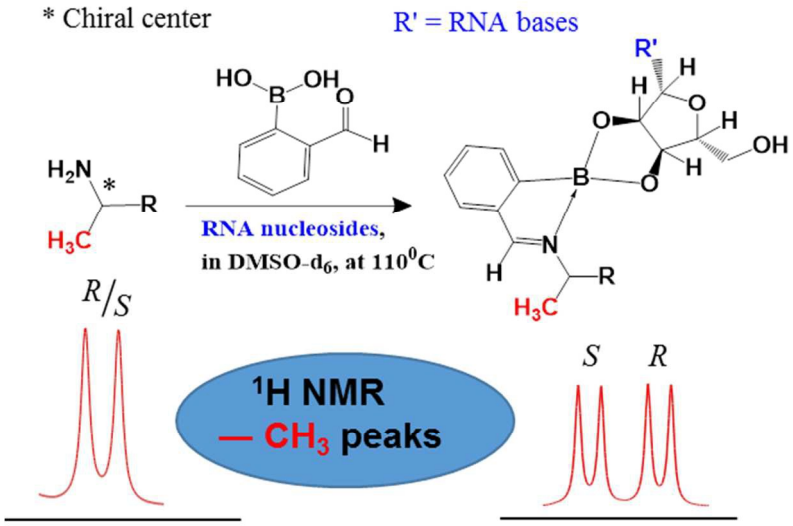


Abstract: The study reports chiral sensing property of RNA nucleosides. Adenosine, guanosine, uridine and cytidine are used as chiral derivatizing agents to differentiate chiral 1^0-amines. A three component protocol has been adopted for complexation of nucleoside and amine. The chiral differentiating ability of nucleosides are examined for different amines based on the ^{1}H NMR chemical shift differences of diastereomers ($\Delta\delta^{R,S}$). Enantiomeric differentiation has been observed at multiple chemically distinct proton sites. Adenosine and guanosine exhibit large chiral differentiation ($\Delta\delta^{R,S}$) due to the presence of purine ring. The measured diastereomeric excess (d.e.) by using adenosine is in good agreement with the gravimetric values.

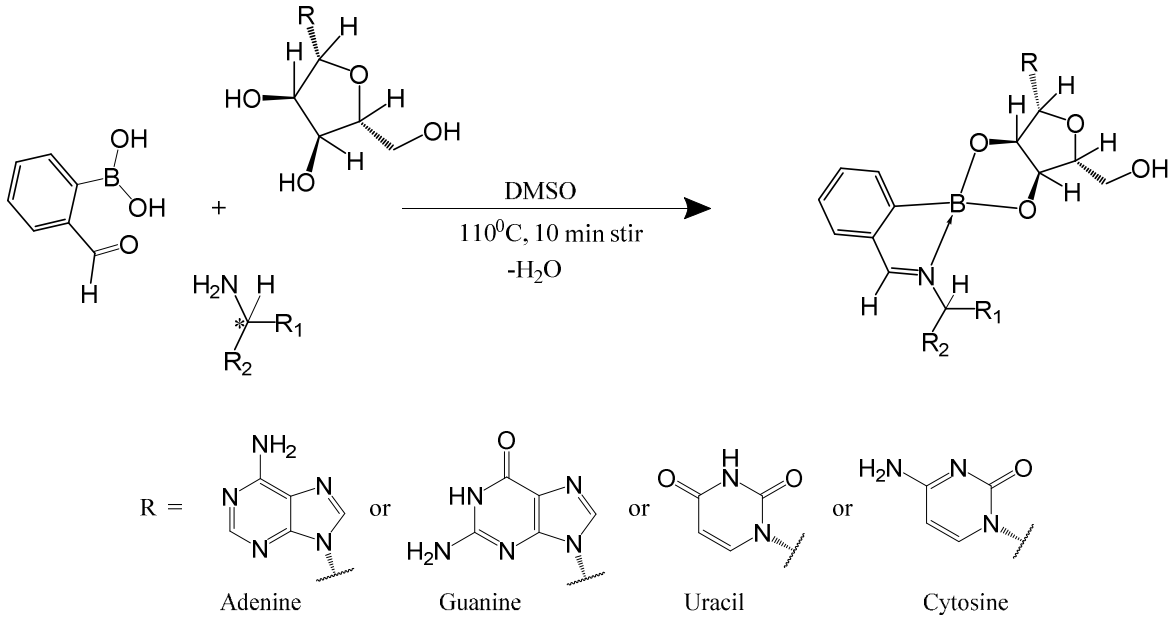
Introduction:

Most of the drug interactions with biological system are enantio specific due to inherent chiral nature of biological molecules, such as, RNA, DNA and proteins.[1, 2] Therefore, the chiral sensing or enantiomeric differentiation has gained enormous importance in the asymmetric synthesis and in pharmaceutical research.[3-6] Most commonly used techniques to analyze enantiomers are NMR,[7-10] X-ray,[11, 12] ORD, CD[13] and chromatographic methods.[14, 15] The easy accessibility and available experimental techniques renders NMR as an important analytical tool for chiral analysis. NMR differentiates enantiomers through chiral auxiliaries[7-10, 16-18] and through chiral alignment media,[19-21] which induces diastereomeric interactions and differential ordering effect (DOE) respectively. Numbers of chiral auxiliaries have been reported for efficient differentiation of enantiomers, assignment of absolute configuration and also for the measurement of enantiomeric excess (*ee)*.[7-10, 16-18] Most of these auxiliaries are synthetically designed, that requires more time, synthetic skills and is also uneconomical. On the other hand chiral biomolecules are cheaply available, more abundant and can be utilized for many applications.[22-32] Recently, several studies have shown for bio-molecular chiral sensing, which are DNA, xanthan, folic acid and guanosine based weak chiral aligning media to differentiate enantiomers using NMR.[19, 20, 33, 34] There are also reports of DNA and polysaccharides based chiral stationary phase in chromatography to differentiate enantiomeric peptides and chiral molecules,[27, 35, 36] epigallocatechin gallate (EGCG) as chiral solvating agent to differentiate chiral amino acids[24] and nucleotides for recognition of L-cysteine and D-cysteine by colorimetric technique. [32]

In general chiral auxiliaries contain chiral unit (viz. chiral center, chiral axis) to induce diastereomeric interactions and delocalize electrons (eg. aromatic ring) to induce shielding and

deshielding effects, causing enantiomeric differentiation. Nucleosides, on the other hand, possess both chiral unit (ribose sugar) and delocalized electrons (nuclear bases), and promise to be efficient chiral sensing agents.

The present study aimed at exploring the chiral sensing ability of RNA nucleosides, such as, adenosine, guanosine, uridine and cytidine, to differentiate chiral 1^0 amines using ^{1}H NMR. Reported three component protocol was employed to complex nucleoside and chiral amine in DMSO-d_6 by 2-formylphenylboronic acid.[37-39] The general scheme of the preparation of 3-component complex is given in scheme 1. Each nucleoside was tested for its differentiating ability of chiral amines, and the ^{1}H chemical shift separation ($\Delta\delta^{R,S}$) of diastereomers formed from *R*- and *S*- amines was used as an indicator. The unambiguous measurement of diastereomeric excess (d.e.) was demonstrated on a nucleoside and amine pair. The formed diastereomeric complexes of *R* and *S* amines exhibit discrimination at multiple proton sites, the peak assignment and the structure of complex are reported using 2D NMR (COSY, NOESY) techniques, spiking experiment and utilizing knowledge of the reported analogous structures. [37-39]



Scheme 1. A three-component protocol for derivatization of 1^0 chiral amines and RNA nucleosides using 2-formylphenylboronic acid. * indicates chiral center, R_1 and R_2 are different alkyl groups .

Experimental Section

All the chemicals were purchased and used without any further purification. The nucleosides used are adenosine, guanosine, cytidine and uridine. The chiral amines used for testing enantiodiscrimination are α-methylbenzylamine, 4-methoxy-α-methylbenzylamine, 1-(2-naphthyl)ethanamine, 1-cyclohexylethylamine and sec-butylamine. Other chemicals used are 2-formylphenylboronic acid (purchased from commercial sources and used as received) and DMSO-d_6. NMR spectra were recorded at 298 K on 500 MHz and 800 MHz spectrometers. DMSO-d_5 was used as ^{1}H reference in all the spectra. For diastereomeric excess (d.e.) measurement a longer inter-scan delay (10 sec) was used.

For derivatization, 1.3 equivalence of each nucleoside, 1 equivalence of racemic mixture of each amine and 1.3 equivalence of 2-formylphenylboronic acid were taken in 600 µl DMSO-d_6 and stirred at 110^0C for about 10 minutes. The excess quantities of nucleoside and 2-formylphenylboronic acid were used to ensure maximum conversion of enantiomeric amines to diastereomeric complexes. The high boiling point of DMSO helped to carry out the reaction above 100^0C, which removed any existed or formed water in the reaction. This completed the reaction. The prepared solutions were used for NMR experiments without any further purification.

Results and discussion

Initially guanosine was used as a chiral derivatizing agent to differentiate α-methylbenzylamine. 10 mg of guanosine, 5.5 mg of 2-formylphenylboronic acid and 3.3 mg of racemic mixture of α-methylbenzylamine (α-MBA) were taken in 600 µl of DMSO-d_6 and were stirred for 10 minutes at about 110^0 C. The possible complex formation is reported in scheme 1, which was further substantiated by mass and NMR spectroscopic analysis (spectrum is given in ESI). The recorded ^{1}H NMR spectrum is given in Fig. 1**,** the peaks were assigned based on 2D NMR (COSY, NOESY, spectra were given in ESI) experiments and spiking technique. The spectrum shows two doublets for methyl protons (peak 'c' in Fig. 1), suggesting formation of two diastereomeric complexes from two enantiomeric (*R* & *S*) forms of α-methylbenzylamine, which unambiguously confirms the enantiomeric differentiation. The chiral differentiation is observed

at many proton sites (shown as expanded regions in Fig. 1). The peak identified as 'a' pertains to imine proton and those identified with number '8' corresponds to nuclear base proton. The peaks marked 1', 2', 3', 4' 5' and 6' are from the protons of sugar unit. Better discrimination ($\Delta\delta^{R,S}$ in ppm) was observed for sugar ring protons and nuclear base proton. For quick observation of chiral discrimination and also for diastereomeric excess (d.e.) measurement, one can use the methyl proton peaks (marked as 'c' around 1.5 ppm) or the imine proton peaks (marked as 'a' around 9 ppm).

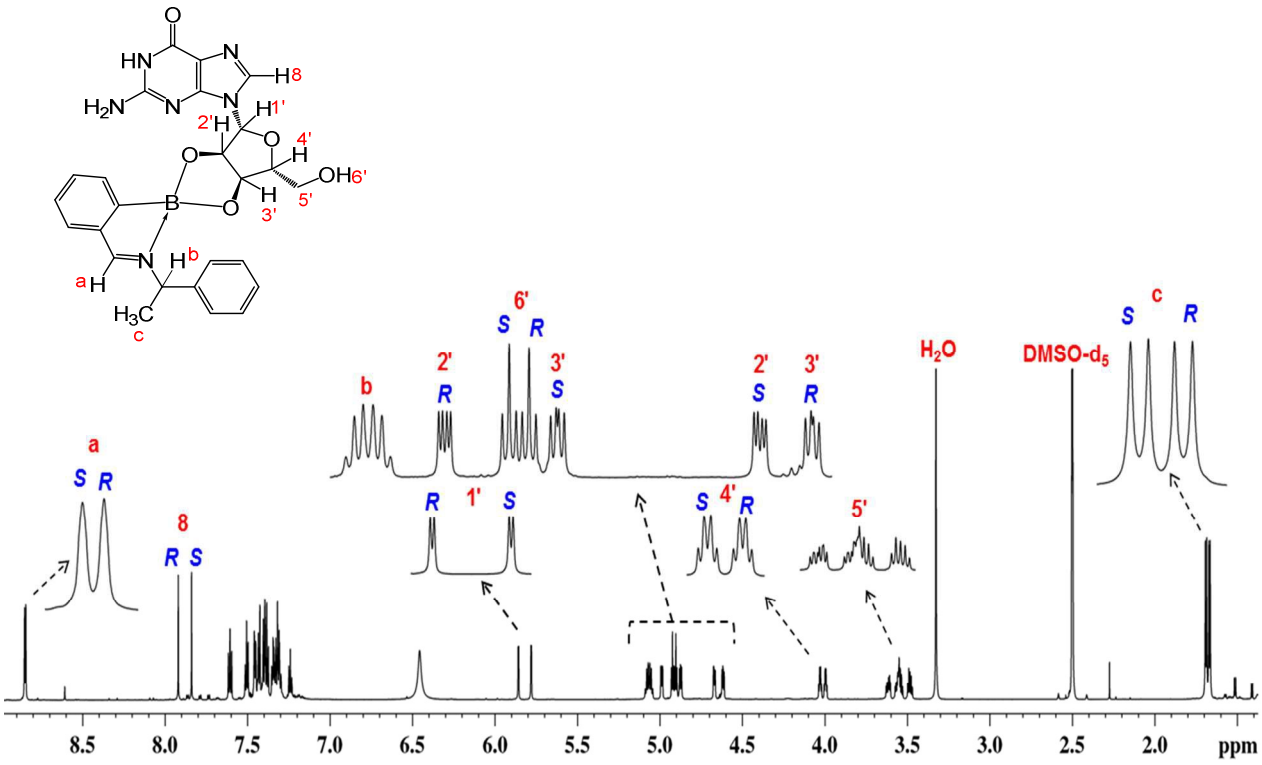


Fig 1. 800 MHz ^{1}H NMR spectrum and chemical structure of diastereomeric complex, formed from guanosine, α-methylbenzylamine and 2-formylphenylboronic acid. The protons and peaks showing enantiomeric differentiation are labeled with alphabets and numbers.

In the subsequent step the study was focused on verifying the chiral discrimination ability of other three nucleosides, adenosine, uridine and cytidine. Individual samples of three-component diastereomeric complexes of α-methylbenzylamine were prepared with each of the nucleoside (using identical procedure discussed earlier). The recorded ^{1}H NMR spectra for all the samples are given in Fig. 2. The spectrum given in the bottom trace pertains to pure α-methylbenzylamine in DMSO-d_6. The other labeled spectra G, A, U and C were recorded from the solutions of

diastereomeric complexes of α-methylbenzylamine and respective nucleosides (G-guanosine, A-adenosine, U-uridine and C-cytidine). The methyl and imine proton peaks were monitored for quick and easy identification of discrimination, which are reported in the expanded scale. Appearance of pair of doublets for methyl protons and doublet for imine protons (expanded region) in each spectrum confirms enantiomeric discrimination ability of all the four nucleosides. The extent of chemical shift separation ($\Delta\delta^{R,S}$ in ppm) between two doublets suggests chiral discrimination strength. As expected, more separation was observed in the case of adenosine, then guanosine followed by uracil and cytosine. This is attributed due to the presence of purine ring (more delocalized electrons) in adenosine and guanosine. However a opposite pattern was observed for imine proton peaks.

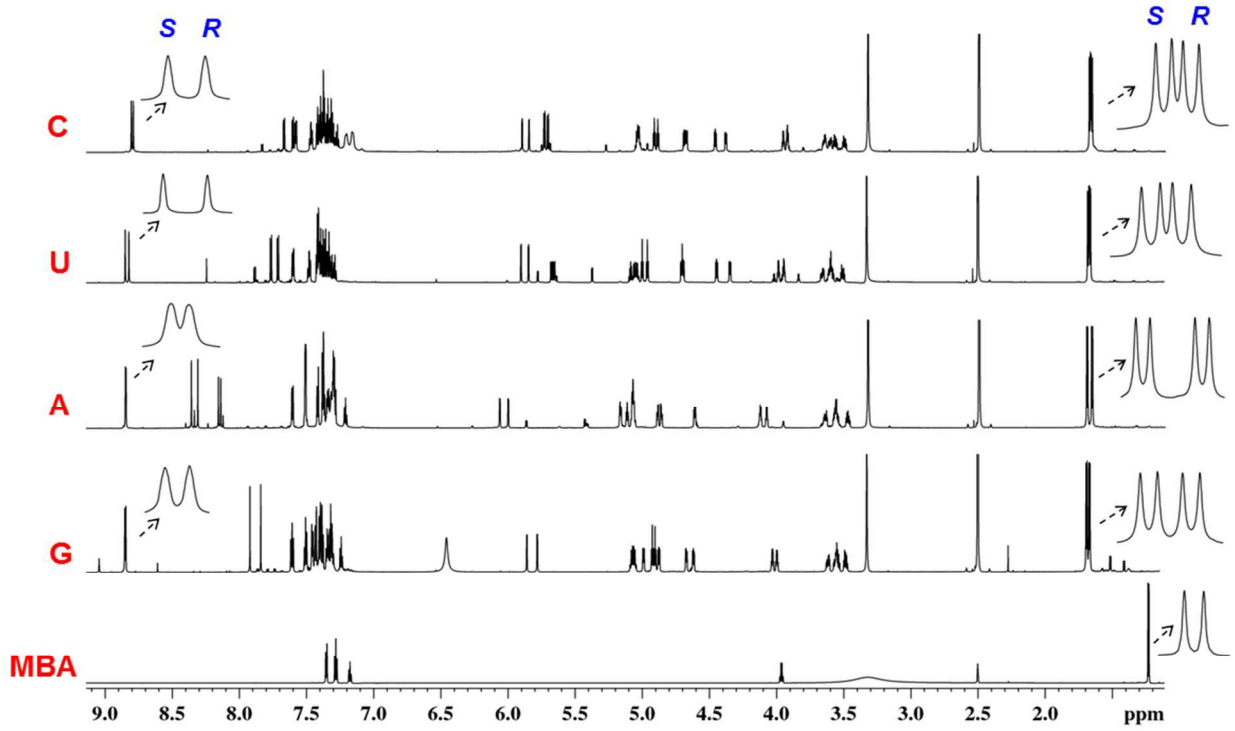


Fig 2. From bottom to top: 800 MHz ^{1}H NMR spectra of α-methylbenzylamine (MBA), and diastereomeric complex spectra of guanosine: α-methylbenzylamine:2-formylphenylboronic acid (G), adenosine: α-methylbenzylamine:2-formylphenylboronic acid (A), uridine:α-methylbenzylamine:2-formylphenylboronic acid (U) and cytidine:α-methylbenzylamine: 2-formylphenylboronic acid (C).

For establishing the wide generality of the enantiomeric discrimination property of nucleosides, many diastereomeric complexes were prepared from different types of 1^{0}-amines (aliphatic-, cyclic- and aromatic- amines) with each of the selected nucleosides using the identical procedure discussed earlier. All the recorded ^{1}H NMR spectra are given in ESI. The enantiomeric

discrimination and extent of discrimination of all the nucleosides with different amines are summarized in Fig. 3. The plot in Fig. 3 represents ^{1}H chemical shift separation ($\Delta\delta^{R,S}$) of *R* and *S* diastereomeric complexes of chosen peaks of amines with the corresponding nucleosides. The derivatized nucleosides are indicated as A, G, U and C for adenosine, guanosine, uridine and cytidine respectively. The amines used are 4-methoxy-α-methylbenzylamine, 1-(2-naphthyl)ethanamine, α-methylbenzylamine, sec-butylamine and 1-cyclohexylethylamine, which are numbered as 1, 2, 3, 4 and 5 respectively (chemical structures are given in Fig. 3). The chemical shift separation was measured for CH_3 protons marked in red colour. Adenosine exhibited more enantiomeric discrimination for all the investigated five amines, which is followed by guanosine, uracil and cytosine. In case of guanosine and adenosine, the methoxy-protons of 4-methoxy- α-methylbenzyl amine showed better discrimination. In all nucleosides the methyl protons of 1-(2-naphthyl)ethanamine are better discriminated, which is due to aromaticity of naphthyl ring, which induces more shielding and deshielding effects. The observed *R* and *S* chemical shift separation ($\Delta\delta^{R,S}$) in other three amines are almost same.

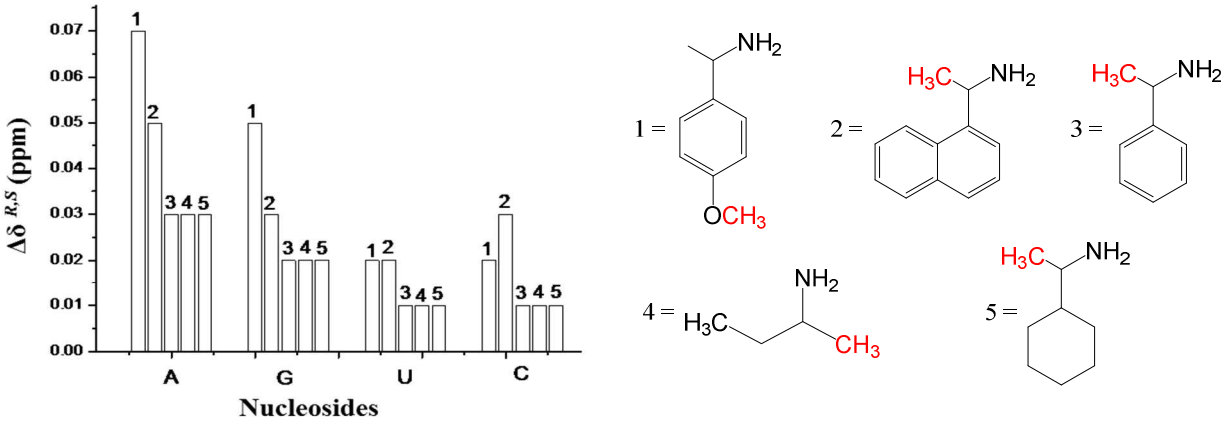


Fig 3. The plot of chemical shift separation of selected protons (identified in red colour in the structures above) of diastereomeric complexes formed from *R* and *S* amines versus different diastereomeric complexes (left). Nucleosides were labeled as A, G, U and C for adenosine, guanosine, uridine and cytidine respectively. The chemical structures of amines, 4-methoxy-α-methylbenzylamine (1), 1-(2-naphthyl)ethanamine (2), α-methylbenzylamine (3), sec-butylamine (4) and 1-cyclohexylethylamine (5) are also given.

Finally the nucleoside was tested for the precise measurement of diastereomeric excess (d.e.). A series of adenosine and 4-methoxy-α-methylbenzylamine derivative complexes were prepared with varying ratios of *R* and *S* mixture of amines (details are given in *ESI*). For each sample the 1D ^{1}H NMR spectra were recorded and the methyl peaks were used to calculate the

diastereomeric excess. The intensity variation of *R* and *S* methyl peaks with varying *R* and S amine concentration are reported in Fig. 4. The figure also contains the plot of diastereomeric excess calculated by gravimetric measurement and those derived from ^{1}H NMR spectrum. The obtained R^2 = 0.9993 suggests excellent agreement between the gravimetric measurement and those obtained by NMR experiment. This clearly establishes the applicability of nucleosides for *ee* measurement.

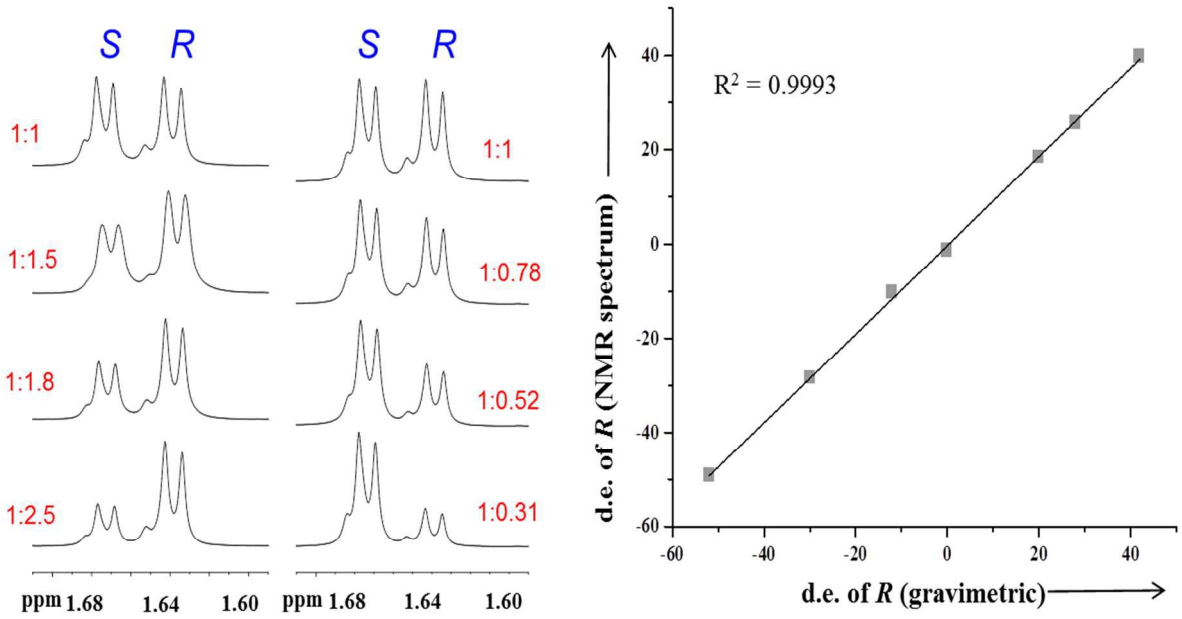


Fig 4: 800 MHz ^{1}H NMR spectrum pertaining to methyl peaks of adenosine:methoxy-α-methylbenzylamine:2-formylphenylboronic acid diastereomeric complex with varied *R* and *S* concentration of 4-methoxy α-methylbenzylamine, the used gravimetric amine ratio *S: R* are shown (left). Plot of diastereomeric excess of *R*-4-methoxy-α-methylbenzylamine measured by gravimetric method versus NMR experimental measurement (right).

Conclusions:

In summary, RNA nucleosides have been demonstrated as chiral derivatizing agents for efficient NMR spectroscopic discrimination of chiral 1^0 amines. A detailed analysis of differentiation ability of nucleosides have been carried out using ^{1}H NMR. The enantiomeric differentiation ability were observed for all RNA nucleosides. The better differentiation (Δδ in ppm for *R* & *S*) was observed for adenosine and guanosine than that of uracil and cytosine at methyl proton region due to the presence of purine ring but a opposite pattern was observed for imine protons. Adenosine has been used to demonstrate the precise measurement of diastereomeric excess

measurement, which is in good agreement with the gravimetrically prepared ratios. The present study demonstrated another important application of biomolecules and helps in exploring possibility of using different chiral biomolecules for chiral sensing applications.

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