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ARTICLE

A Simple and Rapid Approach for Testing Enantiopurity of Hydroxy acids and their Derivatives using ^{1}H NMR Spectroscopy

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A rapid and the simple chiral derivatizing protocol involving the coupling of 2-formylphenylboronic acid and an optically pure [1,1-binaphthalene]-2,2-diamine is introduced for the accurate determination of the enantiopurity of hydroxy acids and their derivatives, possessing one or two optically active centers, using ^{1}H NMR spectroscopy.

Introduction

A single optical centre possessing hydroxy acids find enormous utility, such as, pharmacologicals,[1] synthetic precursors[2,3], cosmeceuticals[4,5], etc. The hydroxy acid containing two optical centers at α and β positions, e.g. (2*R*, 3*S*)-(-)-2,3-dihydroxy-3-phenylpropionate is a precursor of taxol. The taxol is known to be an important drug effective against ovarian, breast, and cell lung cancers[6]. The enantiodiscrimination[7-9] and the knowledge of enantiomeric excess (*ee*)[10,11] is highly important in numerous areas, such as, chiral synthesis[8,12], pharmacology[13], catalysis[14] and biochemistry[13]. The measurement of enantiomeric composition[10,11] by Nuclear Magnetic Resonance (NMR) spectroscopy is well known in the literature since several decades. The discrimination of enantiomers using NMR spectroscopy in isotropic solutions requires the conversion of the enantiomers into diastereomers, which is always accomplished by utilizing a chiral auxiliary, such as, chiral derivatizing agent (CDA)[15-18], chiral solvating agent (CSA)[15-18], or a chiral lanthanide shift reagent[15-19]. Number of chiral reagents containing diverse functional groups, and macrocyclic compounds, that belong to the above mentioned family of auxiliaries, has been reported for discrimination of chiral acids[19,23] and amino acids[24]. Nevertheless most of them have certain limitations, such as, very small chemical shift differences between diastereomeric peaks, involves tedious multistep procedure for synthesis, and lack spectral resolution. For example the MTPA (Mosher), MPA (Trost) amide,[25,26] crown ethers and their derivatives[27-29] are employed for the enantio-discrimination of amines. When MPA or MTPA are employed as chiral reagents the problems of kinetic resolution[30,31] persist, and the precise measurement of ee severely hindered. It is difficult to achieve differentiation of enantiomeric peaks at lower concentrations of crown ethers. At higher concentrations of crown ethers, excessive broadening of the ^{1}H NMR peaks severely hampers the precise measurement of enantiomeric excess. As a consequence continuous research is being carried out to discover reliable and efficient chiral reagents for the accurate determination of the enantiopurity of chiral molecules of diverse functionality. The simple three-component derivatization protocols for the accurate assessment of enantiopurity, using 2-formylphenylboronic acid and (*S*)-BINOL, have been reported for chiral primary amines[32,33], diamines[34], amino alcohols[35] and hydroxy amines[36]. For discrimination of different diols the mixture of enantiopure α-methylbenzylamine and 2-formylphenylboronic acid has been reported as a CDA[37-39]. Our group has also introduced a three-component derivatizing protocol for enantiodiscrimination of primary amines and alpha hydroxy acids[40], which is not only economical but also highly efficient over the existing protocols for the enantiodiscrimination of hydroxy acids[41]. The ^{1}H NMR spectrum is generally overcrowded due to the overlap of peaks arising from hydroxy acids and the primary amines employed as a chiral reagent. As a consequence the accurate measurement of *ee* is largely hindered from the protocols reported in the literature. Due to this there is a urgent requirement of a protocol that can is able to overcome such a problem.

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Electronic Supplementary Information (ESI) available: See
DOI: 10.1039/x0xx00000x

Journal Name

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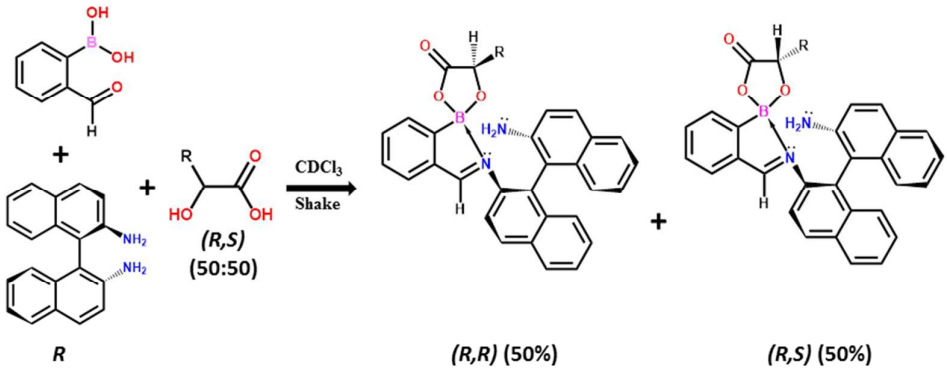
Results and discussion

To circumvent such a problem, in the present study, we are introducing an efficient and a simple three-component protocol for the precise and rapid measurement of enantiomeric purity of hydroxy acids. The proposed protocol is demonstrated to be more efficient and useful compared to any of the reported chiral derivatizing agents, as far as the discrimination of hydroxy acids is concerned. The new protocol involves the coupling of 2-formylphenylboronic acid and optically pure [1,1-binaphthalene]-2,2-diamine in 1:1 molar ratio, at very low concentration in the solvent $CDCl_3$ at 25°C. The derivatization reaction can be accomplished in the NMR tube itself rendering it a rapid technique. The schematic representation of the protocol is reported in scheme 1.



Scheme 1: The protocol for the coupling between 2-formylphenylboronic acid, (*R*)-[1,1-binaphthalene]-2,2-diamine, and (*rac*) hydroxy acid to derivatize two different diastereomers of iminoboronate esters (*R*,*R*) and (*R*,*S*).

Due to the absence of aliphatic protons in [1,1-binaphthalene]-2,2-diamine the clean and overlap free ^{1}H NMR spectra of the chiral analytes are obtained. In the aliphatic region the spectra are also devoid of any interfering peaks from the chiral reagent. A representative example of the ^{1}H NMR spectrum of (*R/S*)-4-trifluoromethyl mandelic acid acquired on a 400 MHz NMR spectrometer using the present protocol is reported in Fig. 1 and the spectra of the other investigated molecules are reported in ESI.

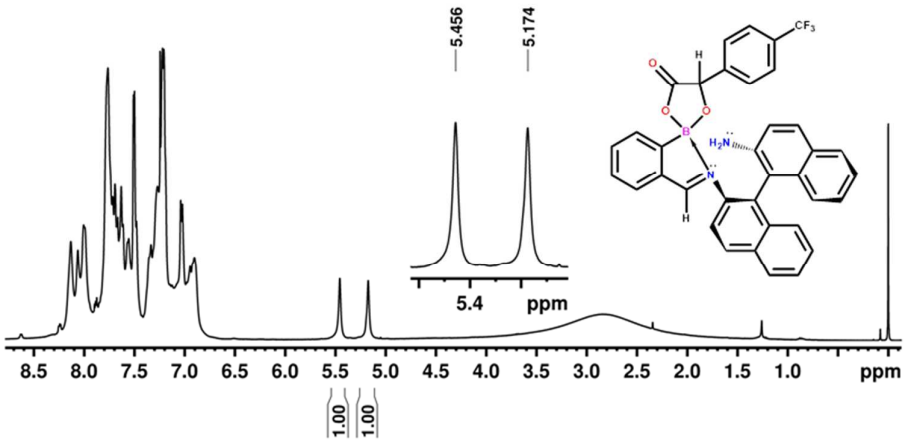


Figure 1: 400 MHz ^{1}H NMR spectrum of 4-trifluoromethyl mandelic acid performed using the proposed protocol.

The proper baseline resolution for the distinct set of resonances is obtained for all the investigated molecules. Interestingly in each molecule there is at least one chemically inequivalent proton site with significantly large Δδ value. The ^{1}H Δδ values for the investigated molecules ranged from 0.29-0.66 ppm for at least one of the proton resonances. The discrimination could also be achieved in the ^{13}C NMR spectra. The 2D ^{1}H-^{13}C HSQC NMR spectrum of the molecule 4-bromo-mandelic acid and 2-chloro-mandelic acid are reported in the Figs. 2 and 3.

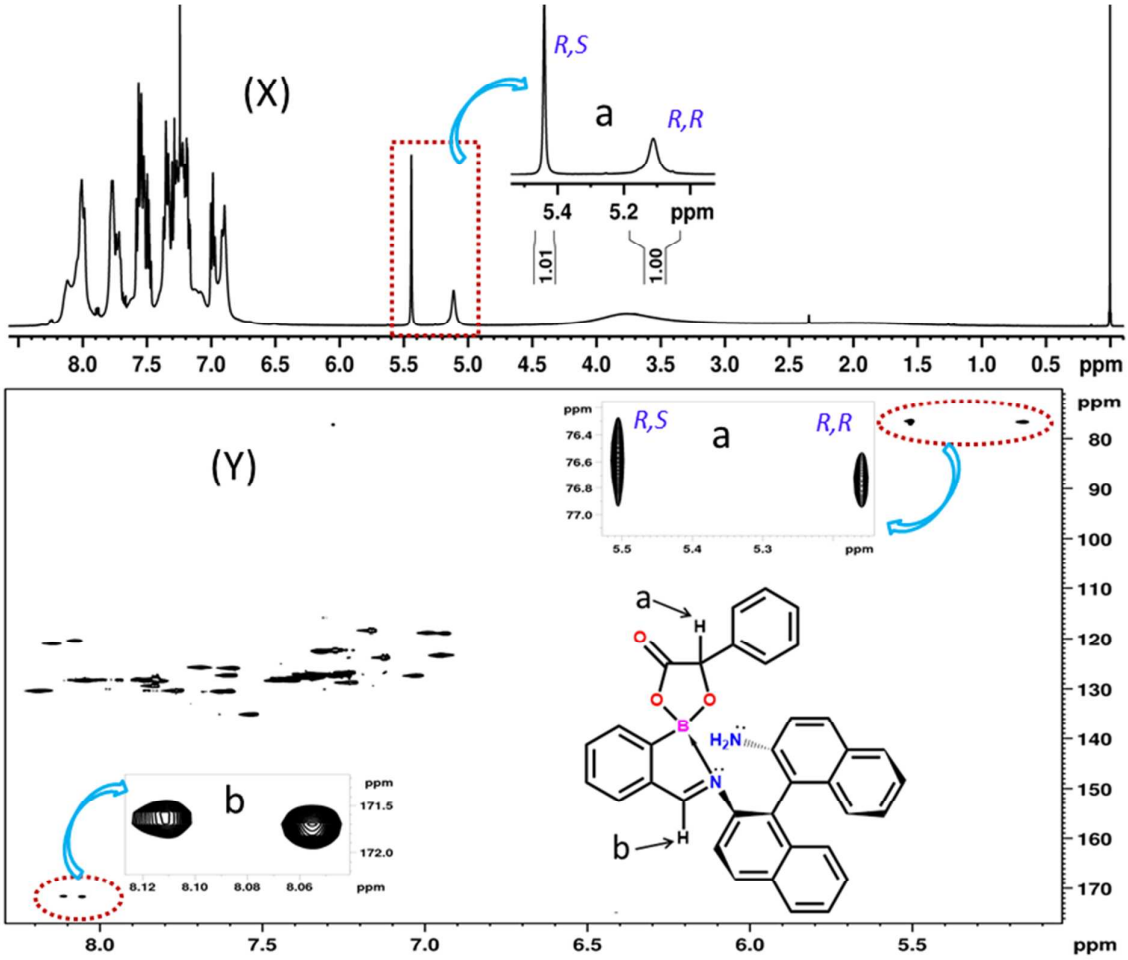


Fig.2. The 400 MHz (X) ^{1}H and (Y) ^{1}H-^{13}C-HSQC NMR spectrum of *R/S*-4-Bromo-mandelic acid in iminoboronate complex. The discriminated peaks are marked inside the dotted circle and expanded region is given as an inset.

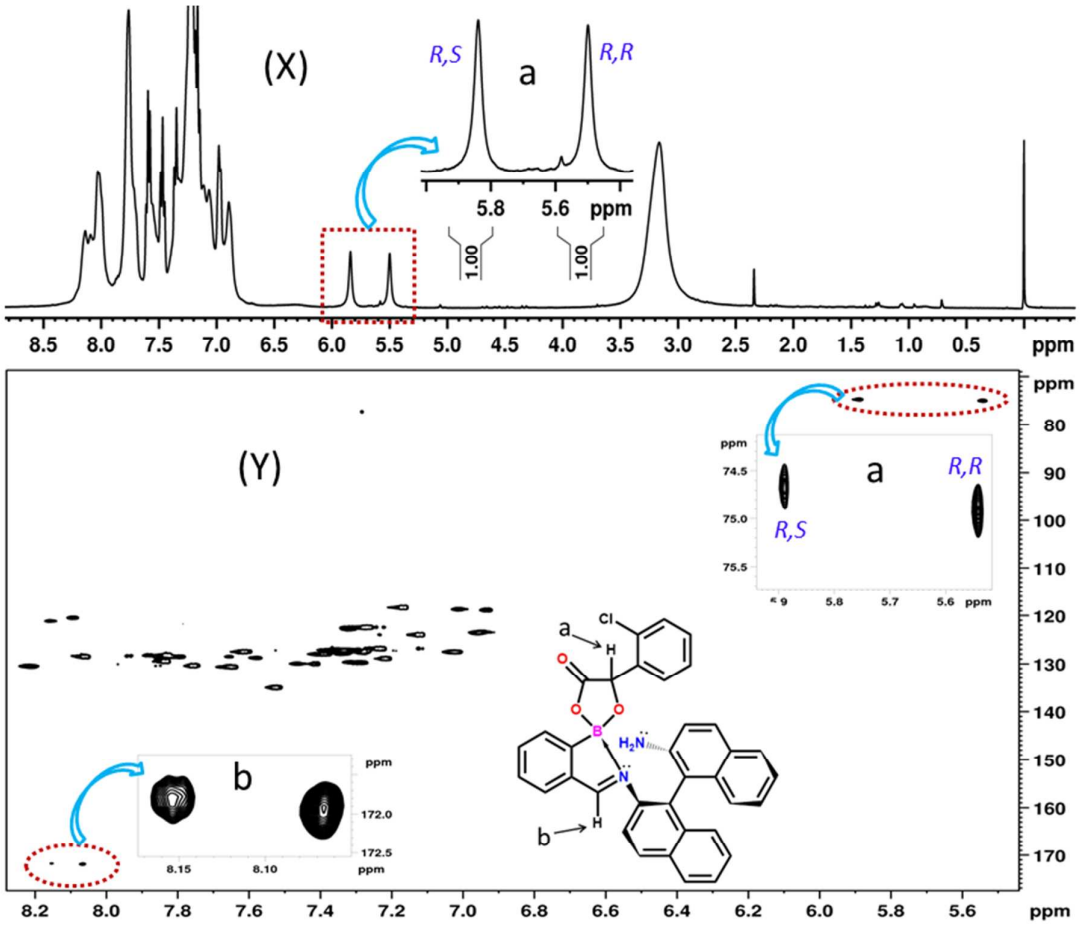
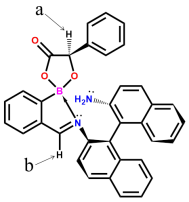
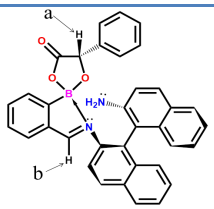
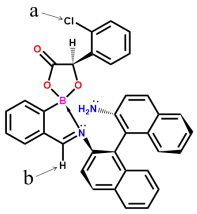
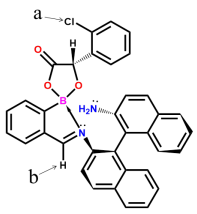
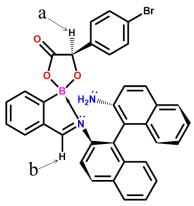
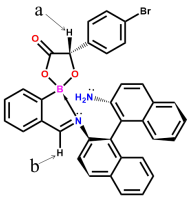
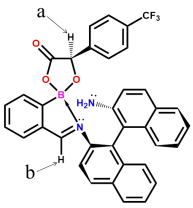
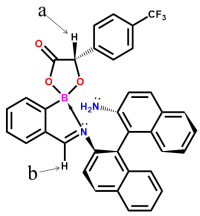
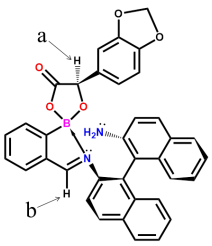
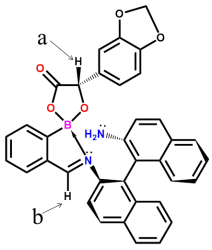
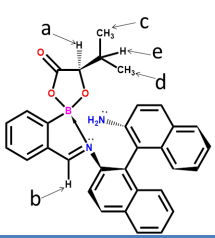
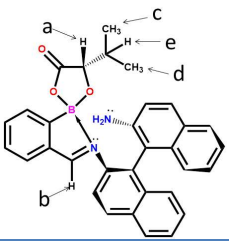


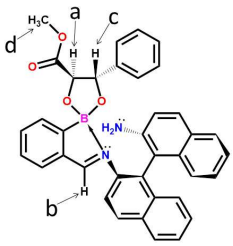
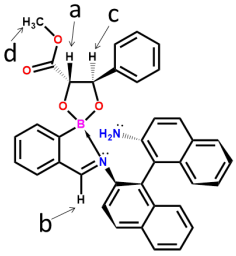
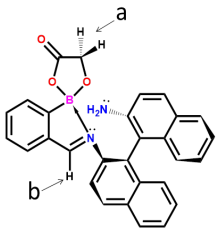
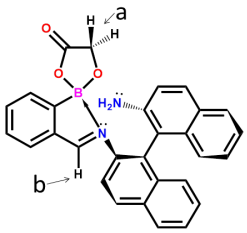
Fig.3. The 400 MHz (X) ^{1}H and (Y) ^{1}H-^{13}C-HSQC NMR spectrum of *R/S*-2-Chloro-mandelic acid in iminoboronate complex. The discriminated peaks are marked inside the dotted circle and expanded region is given as an inset.

From the Figs. 2 and 3 it is clearly evident that the discrimination is achieved in both the dimensions i.e. ^{1}H and ^{13}C, for alpha protons of hydroxy acid and imide proton. The ^{1}H-^{13}C HSQC spectra pertaining to other investigated molecules are reported in ESI. The proton resonance belongs to the imide site overlapped with the other aromatic resonances and is difficult to assign in majority of the in ^{1}H NMR spectra. This problem is circumvented in the ^{1}H-^{13}C HSQC spectra, where these peaks are well resolved. The experimentally measured Δδ values of discriminated sites in both ^{1}H and ^{13}C spectrum along with the derivatizing protocols for all the investigated molecules are assimilated in Table 1.

Table.1: Chemical shift differences (Δδ) for the diastereomers in the racemic mixtures of different hydroxy acids containing one or two stereogenic centers, recorded on a 400 MHz NMR spectrometer.

Entry	Investigated *rac*-hydroxy acids	Diastereomeric Boronate esters formed with (*R*)- [1,1-binaphthalene]-2,2-diamine	Δδ ^{1}H NMR (ppm)	Δδ ^{13}C NMR[2] (ppm)	δ ^{11}B NMR[4] (ppm)

1	Mandelic acid (1)			a)= 0.32 b)= 0.06	a)= 0.14 b)=0.11	13.63
		(R,R)[1]	*(R,S)*			
2	2-Cl-mandelic acid (2)			a)= 0.35 b)= 0.09	a)= 0.25 b)=0.15	13.62
		(R,R)	*(R,S)*			
3	4-Br-mandelic acid (3)			a)= 0.31 b)= 0.07	a)= 0.16 b)=0.04	13.53
		(R,R)	*(R,S)*			
4	4-trifluoromethyl mandelic acid (4)			a)= 0.29 b)= 0.08	a)= 0.16 b)=0.01	13.51
		(R,R)	*(R,S)*			
5	3,4-(Methylenedioxy) mandelic acid			a)= 0.34 b)=0.08	a)= 0.05 b)=0.02	16.68
		(R,R)	*(R,S)*			
6	2-Hydroxy-3-Methyl Butyric acid (6)			a)= 0.35 b)= 0.10 c)= 0.05 d)= 0.13 e)= 0.03	a)= 0.25 b)=0.13 c)=0.04 d)=0.19 e)=0.01	13.52

		(R,R)	*(R,S)*			
7	Methyl-2,3-dihydroxy-3-phenyl propionate (7)			a)= 0.07 b)=0.19 c)=0.45 d)=0.66	a)= 0.48 b)=0.45 c)=0.55 d)=0.48	16.78
		(R,(2R,3S))	*(R,(2S,3R))*			
8	Glyconic acid[3] (8)			a)=0.04		14.13
		(R,pro-R/S)				

[1]While naming the diastereomer the first word belongs to (R)-[1,1-binaphthalene]-2,2-diamine. [2]The Δδ for ^{13}C NMR is obtained from ^{1}H-^{13}C-HSQC spectra. [3]prochiral molecule,^{11}B NMR[4]. All the spectra are reported in ESI.

The ^{1}H NMR spectrum of the racemic mixture of investigated hydroxy acids gave two diastereomeric peaks of equal integral areas thereby discarding any possibility of kinetic resolution[30,31]. Therefore the application of the protocol permits the accurate measurement of enantiomeric excess, from the ratiometric analysis of the integral areas of the discriminated peaks[10,11]. The diastereomeric excess[42] measurement was thus carried out for the molecule 2-hydroxy-3-methylbutyric acid with different predetermined ratios of two different enantiomers as reported in the Fig. 4. It was possible to experimentally measure *ee* upto 98%. The experimentally determined values agreed with the gravimetrically prepared ratios within the experimental error[43,44] of 2-3%.

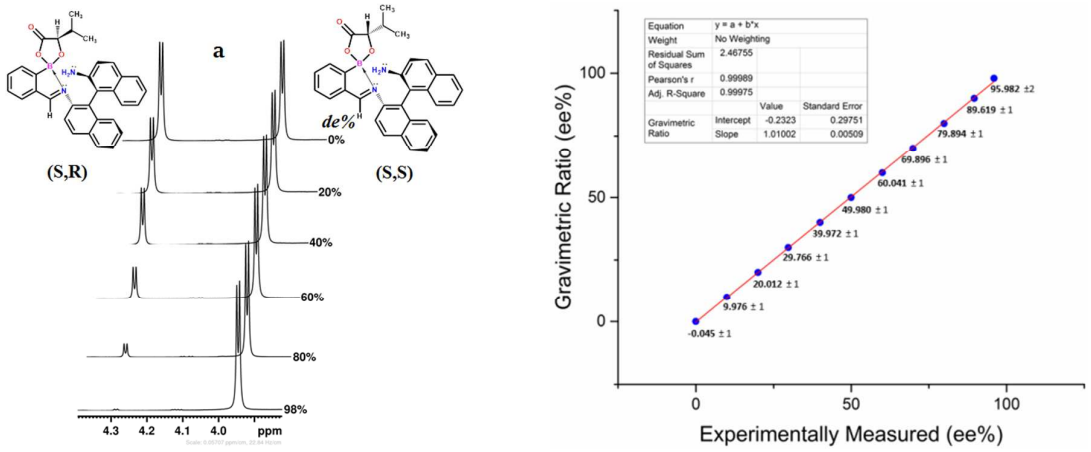


Figure 4. (a) The stacked plots of the expanded alpha proton regions of the ^{1}H NMR spectra of 2-hydroxy-3-methylbutyric acid with different enantiomeric ratios derived using 2-formylphenylboronic acid and (*R*)-[1,1-binaphthalene]-2,2-diamine(1:1). (b) The comparison

of gravimetric ratio with the experimentally measured values. The experiments were repeated to derive the maximum possible errors in the measurement of *ee* and largest measurement error is mentioned.

It is evident from Fig. 2b that the gravimetric ratios are in excellent agreement with the experimentally determined *ee* depicting the excellent reliability of the present protocol. Another interesting observation is that the resonances of homochiral diastereomers are showing deshielding effect and the resonance pertaining to heterochiral diastereomers gets more shielded in the present protocol. Using the directional displacement of proton peaks, the protocol can be employed as a tool for the assignment of the unknown configuration[45-50]. This is an interesting observation and however, in the present work, we do not want to make any strong claim for this.

Experimental section

The 2-formylphenylboronic acid (2 mg) and (*R*)-[1,1-binaphthaline]-2,2-diamine (3.8 mg) was added in to the NMR sample tube containing 400 µl of $CDCl_3$ and was thoroughly shaken. Subsequently the hydroxy acid to be investigated (1:1:1) was added to it. The two diastereomers (*R,S*) and (*R,R*) of iminoboronate ester were formed, which was confirmed by ^{1}H NMR spectrum acquired on a 400 MHz NMR spectrometer. The same procedure was adopted for all other investigated molecules. For measurement of *ee*, the samples with different enantiomeric ratios were prepared using the similar procedure and ^{1}H NMR spectra were obtained using a 400 MHz NMR spectrometer.

Conclusions

In conclusion, in the present study we are reporting a novel three-component chiral derivatizing protocol for testing the enantiopurity of hydroxy acids and their derivatives possessing one or two optically active sites, using a conventional one dimensional ^{1}H and/or ^{13}C NMR. The present approach is rapid, simple, efficient and can be applied for the range of hydroxy acids and their derivatives. We strongly believe that, it will have enormous utility in the field of pharmacology and asymmetric synthesis.

Acknowledgements

SKM likes to thank UGC for SRF and NS gratefully acknowledges the generous financial support by Board of Research in Nuclear Sciences, Mumbai (Grant No. 2013/37C/4/BRNS) and the Science and Engineering Research Board, Department of Science and Technology, New Delhi (grant No. SR/S1/PC-42/2011).

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Graphical Abstract