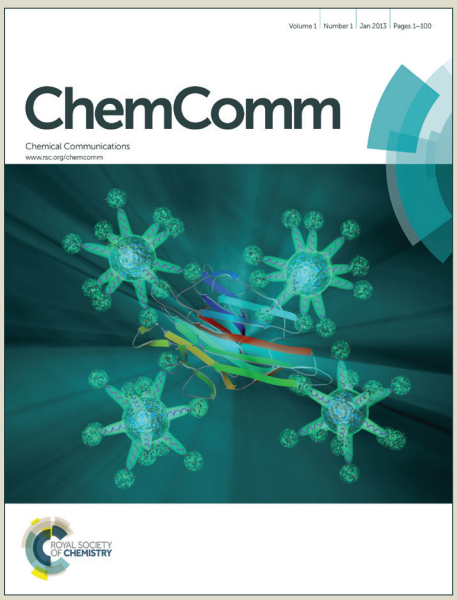
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

Enantio-differentiation of Molecules with diverse functionalities by a Single probe

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

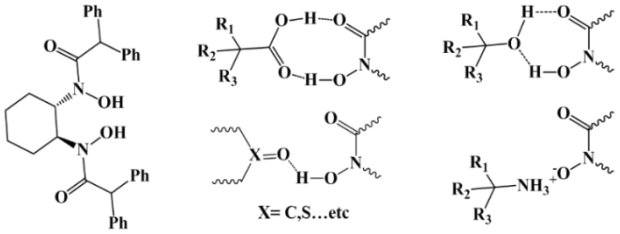
DOI: 10.1039/x0xx00000x

www.rsc.org/

A. Lakshmipriya,[a,c] S. R. Chaudari[b] and N. Suryaprakash[a,c,*]

The study reports (1*S*,2*S*)-N,N'-Dihydroxy-N,N'-bis(diphenylacetyl)-1,2-cyclohexanediamine, a C_2 symmetric chiral hydroxamic acid ((*S*)-CBHA-DPA), as a single and unique probe for discrimination of molecules with diverse functionalities. The proposed CSA is also utilized for the accurate measurement of enantiomeric excess.

Chiral recognition is extremely important in various fields. Its knowledge is essential in understanding the interactions in biological systems, chromatographic chiral analysis and asymmetric synthesis [1, 2]. Among the available techniques [3, 4], NMR spectroscopy has proven to be an extremely useful technique for chiral analysis and enantiomeric excess (*ee*) measurement since the NMR analysis is simple and straightforward [5]. NMR spectroscopic discrimination of enantiomers is achieved by using one of the chiral auxiliaries, viz., chiral derivatizing agent (CDA), chiral solvating agent (CSA) or chiral lanthanide shift reagent (CLSR)[5]. The use of the CDA requires cumbersome and time consuming synthetic procedures [6]. The major problem encountered in the use of CLSR is enormous line broadening in the spectrum [7]. The use of CSA, on the other hand, is an attractive option [5] as it involves the formation of diastereomers using non-covalent interactions and is generally free from the problems encountered in CDA and CLSR [5]. In addition, the process of chiral discrimination by the CSA involves mixing and shaking with the chiral analyte rendering it a convenient technique for rapid analysis and is also less prone to errors [6]. A pool of chiral auxiliaries are available in the literature for chiral analysis [5], each being specific to molecules containing a specific functional group(s) [7] and some of them demand multistep synthesis [8]. A single chiral auxiliary for discrimination of molecules possessing wide varieties of functional groups [7, 8] though advantageous, is rarely encountered. As a result there is a dire necessity to discover a chiral auxiliary which serves as a single probe for enantio discrimination of varieties of molecules containing diverse functional groups. Consequent to limited range of recognition ability, the selection of an appropriate auxiliary for enantiomeric analysis of wide range of molecules continues to be a challenging task. Recent addition to the CSA library using BI[illegible] derivative is applicable to wide variety of the molecules and found to be ubiquitous [6b]. Interestingly, the authors have not demonstrated its applicability for molecules containing amine grou[illegible] and also the CSA need to be synthesized [6b]. Thus the search for novel auxiliary either for broader utility or dedicated to chir[illegible] molecules with particular functional group is always an open field, thereby has attracted the constant efforts of many researchers [6].



Scheme 1. Chemical structure of (1*S*,2*S*)-N,N'-Dihydroxy-N,N'-bis(diphenylacetyl)-1,2 cyclohexanediamine and its possible ways of interactions with different guest molecules,where R_1, R_2 and R_3 are substitutents.

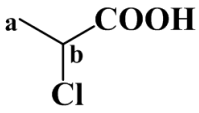
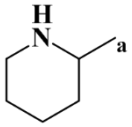
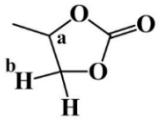
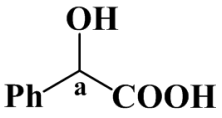
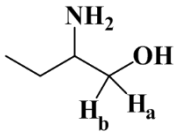
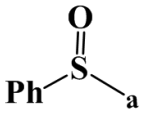
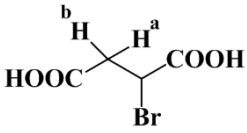
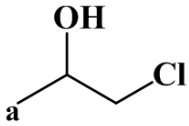
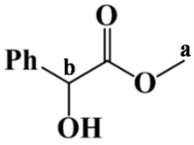
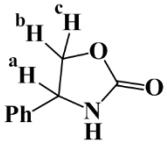
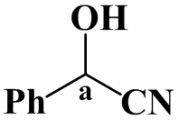
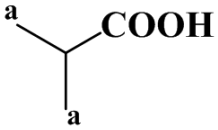
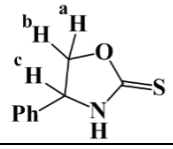
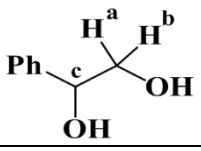
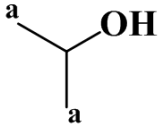
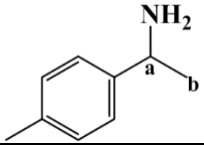
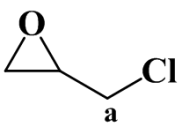
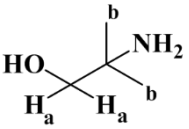
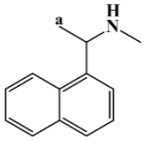
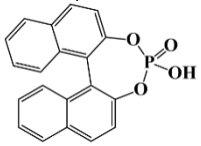
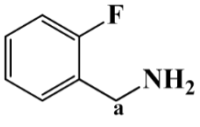
The (S)-CBHA-DPA (chemical structure is given in scheme 1[illegible]) possess both hydrogen-bond donor and acceptor sites and thus has enormous potential to get involved in the formation of hydroge[illegible] bond with variety of functional groups. The possible ways [illegible] interactions with different guest molecules is reported in scheme [illegible]. Such type of interactions are widely known in supramolecular chemistry [11], and are thus very attractive for their potenti[illegible] application as a CSA. In case of amino alcohols (2-amino-1-butanol), and primary and secondary amines (1(4-methylphe[illegible]yl) ethylamine, N-methyl-1-(naphthalene-1-yl) ethanamine, 2-flu[illegible]ro benzyl amine) the OH peak of N-OH in CSA disappeared. This indicates that in amines and amino alcohols the diastereom[illegible] formation occurs through ion pair mechanism.

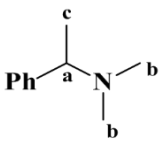
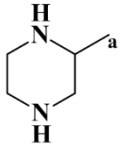
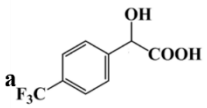
[a]*NMR Research Centre,* [C]*Solid State and Structural Chemistry Unit, Bangalore-560012, India,*
[b]*Present Address: Centre de RMN a TresHauts Champs, University of Lyon, UMR5280 CNRS / ENS Lyon / UCB Lyon 1, 69100 Villeurbanne, France*

Journal Name

COMMUNICATION

Table 1. The measured chemical shift difference ($\Delta\delta^{R/S}$) values of discriminated protons for the molecules of different functionality. 1:1 ratio of analyte and CSA was used. {*, § Indicates the values measured at 233 K temperature and in the presence of DMAP(4-dimethylaminopyridine) respectively.}

Entry	Guest	$\Delta\delta^{R/S}$ (ppm)	Entry	Guest	$\Delta\delta^{R/S}$ (ppm)	Entry	Guest	$\Delta\delta^{R/S}$ (ppm)
1	Acid 	a 0.05 b 0.01	9	piperidine 	a 0.06	17	Carbonates 	a 0.01 b 0.01
2	Alpha Hydroxy acid 	a 0.04	10	Amino alcohols 	a 0.04 b 0.09	18	Sulfoxide 	a 0.002
3	Diacid 	a 0.11 b 0.04	11	Alcohol 	a 0.03*	19	Hydroxy ester 	a 0.01 b 0.02
4	Oxazolidinone 	a 0.13 b 0.07 c 0.03	12	Cyanohydrin 	a 0.3	20	Prochiral acid 	a 0.02 0.03*
5	Thiones 	a 0.04 b 0.02 c 0.01	13	Diols§ 	a 0.04 b 0.02 c 0.02	21	Prochiral alcohol 	a 0.02
6	Primary Amine 	a 0.07 b 0.02	14	Epoxide 	a 0.15*	22	Prochiral amino alcohol 	a 0.07 b 0.06
7	Secondary amine 	a 0.03	15	Phosphoric acid 	^{31}P 0.06 0.15§	23	Prochiral amine 	a 0.08 0.1*

8	Tertiary amine 	a 0.01 b 0.02* c 0.01*	16	piperazine 	a 0.02	24	Hydroxy acid 	^{19}F 0.03

The ^{1}H-NMR spectrum of 2-methyl piperidine, given in Fig. 1A, exhibits a doublet for the methyl group. The addition of one equivalent of (*S*)-CBHA-DPA to it resulted in the separate peaks for methyl group of each enantiomer with a chemical shift difference ($\Delta\delta^{R/S}$) of 0.04 ppm, convincingly establishing that (*S*)-CBHA-DPA has a enantio-discrimination ability. It is well known that, $\Delta\delta^{R/S}$ depends on the concentration of CSA [5]. Hence the ^{1}H-NMR spectra were obtained at varied concentrations of CSA. The enhanced concentration of CSA resulted in increased $\Delta\delta^{R/S}$. This is clearly evident from Fig. 1A. The $\Delta\delta^{R/S}$ for the discriminated peaks also has a strong temperature dependence [5], especially when the chiral auxiliary is a solvating agent. Thus it is possible to iterate these two physical parameters to maximize the separation between the discriminated peaks for unambiguous differentiation and the precise measurement of *ee.*

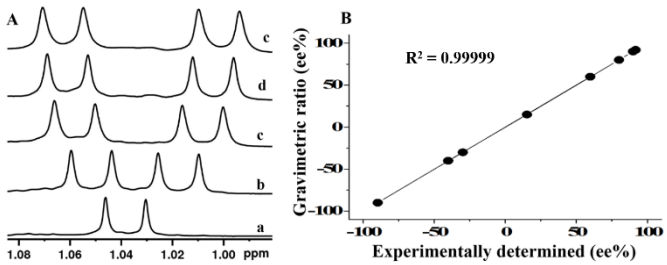


Fig. 1 A] (a) 400 MHz ^{1}H-NMR spectrum pertaining to methyl region of 2-methyl piperidine; (b-e) with 1:1, 1:2, 1:3 and 1:4 equivalents of 2-methyl piperidine and (S)-CBHA-DPA; B] Plot of experimentally measured ee values verses the gravimetrically prepared ratios for eight different non-racemic mixtures of mandelic acid in $CDCl_3$.

In order to explore the generality and wide applicability of the proposed CSA, the molecules with different functional groups were tested. Interestingly the proposed CSA allows the discrimination of large number of molecules with functional groups, such as, acids, hydroxy acids, diacids, primary, secondary and tertiary amines, alcohols, amino alcohols, oxazolidones, oxathiones, carbonates, diols, epoxides, phosphoric acids, cyanohydrins, piperidine, piperazine, hydroxy esters, sulphoxides, prochiral amines, prochiral acids, prochiral alcohols and prochiral amino alcohols. This versatility of proposed CSA can be attributed to multiple hydrogen bonding sites present in the molecule (scheme 1). In some cases (diols, phosphoric acids) where there is no proper base line separation, we employed our recent strategy of ternary ion pair formation [13], where addition of DMAP enhanced the chiral resolution. The measured $\Delta\delta^{R/S}$ values for chosen molecules are compiled in Table 1. In all the investigated molecules we are able to achieve the discrimination at multiple chemical sites with the proper baseline correction. This unequivocally establishes that the proposed CSA serves as a single probe for testing enantio purity of wide variety of molecules. A point that can be highlighted is that the present CSA permitted better discrimination [12] and performed well for the molecules which are usually hard to discriminate by other reported methods [6,7]. Furthermore we also explored the utility of heteronuclei, such as, ^{13}C, ^{19}F and ^{31}P for chiral analysis.[6b,7d,7e] The corresponding spectra are reported in the supporting information. The adaptability of this CSA became evident, as we are able to observe distinct peaks for each enantiomer, even in ^{13}C, ^{19}F and ^{31}P spectra. All the spectra were recorded in the solvent $CDCl_3$. To ascertain the enantio-discrimination ability of this CSA in different solvents, the spectra of the molecule 2-methylpiperidine were also acquired in solvents such as, CD_2Cl_2, benzene-d_6, toluene-d_8, acetonitrile-d_3. If the chiral analyte is not soluble in the above mentioned solvents, one can also use the solvent mixture of 10-20% DMSO in chloroform for achieving discrimination. These spectra are given in supporting information. However the solvent DMSO has a tendency to form the hydrogen bond with host-guest complex, results in the destabilizes or weakens the host-guest interactions, thereby not favorable for enantio discrimination. This is the well known major limitation for the use of CSA for chiral discrimination.

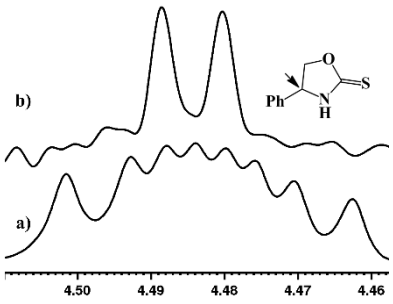


Fig. 2 a) 500 MHz ^{1}H-NMR spectrum; b) Pure shift spectrum obtained using Zanger – Sterk technique[15c], pertaining to alpha-proton region of 4-phenyl oxazolidine-2-thione.

After successful demonstration of (S)-CBHA-DPA as a promising CSA for discrimination of large variety of the molecules, its utility for the accurate determination of *ee* was explored. Therefore eight different non-racemic solutions of mandelic acid in $CDCl_3$ were prepared. The experimentally measured *ee* from the well discriminated *R* and *S* peaks, is in close agreement with the gravimetrically prepared samples, within the experimental error. This is graphically illustrated in Fig. 1B.

Furthermore for the complex system where spectrum is severely overcrowded due to multiplicity pattern and overlapping of peaks from both *R* and *S* enantiomers, even when the chemical shift separation is large enough, one can use our previously reported methods such as, ω_1-decoulpled COSY, MQT, *J*-Resolved and RE-TOCSY, for unraveling the spectra of enantiomers.[14] In the present study the utility of pure shift NMR approach for achieving the resolution of the overlapped peaks is demonstrated. This approach is well known in unraveling peaks from the complex multiplet pattern [15, 16]. The spectrum of the molecule 4-phenyl oxazolidine-2-

thione exhibits severe overlapping of peaks of *R* and *S* enantiomers at alpha position. The pure shift spectrum decoupled all the coupled protons and gave single peak at the chemical shift positions of both the enantiomers (Fig. 2), enabling the visualization of discrimination. The drawback of the utility of the pure shift NMR in the accurate determination of *ee* comes into picture when the chemical shift difference is very small and is of the order of 4-6 Hz[16]. However in such situations two dimensional RES-TOCSY experiment can be utilized for the determination of *ee* of enantiorich samples[14].

In conclusion, we have introduced a versatile chiral solvating agent (*S*)-CBHA-DPA, which exhibits better discrimination on large number of molecules, each containing a different functional group. The proposed CSA has a unique property that it possesses both hydrogen bond donor and acceptor sites and establishes hydrogen bond interactions with the molecules containing different functional groups enabling chiral recognition. The synthetic accessibility[10,17] and commercially availability of this CSA from sigma Aldrich, in addition to its enantio-discrimination ability for molecules of diverse functionalities, makes it a practically convenient and versatile reagent. Besides the enantiomeric discrimination, its utility for accurate measurement of *ee* has also been demonstrated. The use of pure shift NMR opens up a new door in the chiral analysis for unraveling the NMR spectra of enantiomers, whenever there is severe overlap.

ALP would like to thank CSIR, New Delhi, for JRF. NS gratefully acknowledges the generous financial support by the 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).

Notes and references

1 a) M.Hiramatsu and A. K.Cho, *Neuropharmacology,*1990, **29**, 269; b)E. Francotte, W. Lindner, R. Mannhold, H. Kubinyi and G.Folkers, *Chirality in Drug Research,* Wiley-VCH Verlag GmbH @ Co. KGaA, 2006, vol. 33.

2 a) R. E.Gawley andJ.Aube, *Principles of Asymmetric Synthesis*, Pergamon, Oxford, UK, 1996; b) P.M. Dewick, *Medicinal Natural Products*, John Wiley and Sons, Chichester, UK, 2nd edn, 2001.

3 N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy – Exciton Coupling in Organic Stereochemistry*, University Science Book, Mill Valley, CA, 1983.

4 J. D. Dunitz, *X-Ray Analysis and the Structure of Organic Molecules,* 2nd Corrected Reprint; Verlag Helvetica ChimicaActa, Basel and VCH, New York, 1995.

5 a) T. J. Wenzel, *Discrimination Of Chiral Compounds Using NMR Spectroscopy*, Wiley, Hoboken, 2007; b) T. J. Wenzel and C. D.Chisholm, *Prog. Nucl. Magn. Reson. Spectrosc.,* 2011, **59**, 1; c) J. M. Seco, E. Quiñoá and R. Riguera, *Chem. Rev.,* 2004, **104**, 17.

6 a) J. Chin, D. C. Kim, H.-J. Kim, F. B. Panosyan and K. M.Kim, *Org. Lett.,* 2004, **6**, 2591; b) T. Ema, D. Tanida and T. Sakai, *J. Am. Chem. Soc.,* 2007, **129**, 10591; c) Q. Ma, M. Ma,H. Tian, X. Ye,H. Xiao, L. Chen and X. Lei, *Org. Lett.*, 2012, **14**, 5813; d) C. Wolf, S. Liu and B. C.Reinhardt, *Chem. Commun.,*2006, **40**, 4242; e) R. Carrillo, M. López-Rodríguez,V. S. Martín and T. Martín, *Angew. Chem. Int. Ed. Engl.,* 2009, **48**, 7803; f) H. Huang,R. Nandhakumar, M. Choi, Z. Su andK. M. Kim, *J. Am. Chem. Soc.,* 2013, **135**, 2653.

7 a) T. J. Wenzel,*NMR Shift Reagents*, CRC Press, Inc, Boca Raton, FL, 1978; b) F. Cuevas, P. Ballester andM. A.Pericàs, *Org. Lett.,* 2005, **7**, 5485; c) D. Yang, X. Li, Y. Fan and D. Zhang, *J. Am. Chem. Soc.,* 2005, **127**, 7996; d) S. R. Chaudhari and N. Suryaprakash, *Org. Biomol. Chem*., 2012, **10**, 6410; e)Z. Pakulski, O. M. Demchuk, R. Kwiatosz, P. W. Osiński, W. Świerczyńska and K. M. Pietrusiewicz, *Tetrahedron: Asymmetry*, 2003, **14**, 1459.

8 a) G. Bian,H. Fan, H. Huang, S. Yang, H. Zong, L. Song and C. Yang, *Org. Lett.,* 2015, 17, 1369; b) Q. Ma, M. Ma, H. Tian, X. Ye, H. Xiao, L. Chen and X.Lei, *Org. Lett.,* 2012, **14**, 5813; c) A. E. Lovely and T. J.Wenzel, *J. Org. Chem.,* 2006, **71**, 9178.

9 S. R. Chaudhari and N. Suryaprakash, *J. Org. Chem.,* 2012, **77**, 648; b) D. Kumari, P. Bandyopadhyay and N. Suryaprakash, *J. Org. Chem.,* 2013, **78**, 2373.

10 Z. Li and H. Yamamoto, *Acc. Chem. Res.,* 2013,**46**, 506.

11 a) S. P. Gupta, Ed., *Hydroxamic Acids*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013; b) P. Politzer and J. S.Murrey, *The Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids,*John Wiley and Sons, Chichester, UK, 2nd edn, 2009; c) D. kaur and R. Kohli, *Struct. Chem.,* 2011, 161.

12 A. Couffin, O.Thillaye du Boullay, M.Vedrenne, C. Navarro, E. Martin-Vaca and D. Bourissou, *Chem. Commun.,* 2014, **50**, 5997.

13 a)I. Pal, S. R . Chaudhari and N. Suryaprakash, *New J. Chem.* 2014, **38**, 4908; b) S. R. Chaudhari and N. Suryaprakash, *New J. Chem.,* 2013, **37**, 4025.

14 a) N. Nath, D. Kumari and N. Suryaprakash, *Chem. Phys. Lett.,* 2011, **508**, 149; b) D. Kumari, S. Hebbar and N. Suryaprakash, *RSC Adv.,* 2013, **3**, 3071; c) S. R. Chaudhari and N. Suryaprakash, *Chem. Phys. Lett.,* 2014, **602**, 40; d) Lokesh, S. R. Chaudhariand N. Suryaprakash, *Org. Biomol. Chem.,* 2014, **12**, 993.

15 a) J. A. Aguilar, S. Faulkner, M. Nilsson and G. A. Morris, *Angew. Chemie Int. Ed.,* 2010, **49**, 3901; b) N. H. Meyer and K. Zangger, *Angew. Chem. Int. Ed. Engl.,* 2013, **52**, 7143.; c) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486

16 a) N. Lokesh, S. R. Chaudhari and N. Suryaprakash, *Chem. Commun.,* 2014, 50, 15597; b)M. Pérez-Trujillo, L. Castañar, E. Monteagudo, L. T. Kuhn, P. Nolis, A. Virgili, R. T. Williamson and T. Parella, *Chem. Commun. (Camb).*, 2014, **50**, 10214; c) M. Pérez-Trujillo, T. Parella and L. T. Kuhn, *Anal. Chim. Acta*, 2015, **876**, 63; d) L. Castañar, M. Pérez-Trujillo, P. Nolis, E. Monteagudo, A. Virgili and T. Parella, *Chemphyschem*, 2014, **15**, 854.

17 a) A. Basak, A. U. Barlan and H. Yamamoto, *Tetrahedron: Asymmetry*, 2006, **17**, 508; b) W. Zhang, A. Basak, Y. Kosugi, Y. Hoshino and H. Yamamoto, *Angew. Chem. Int. Ed. Engl.,* 2005, **44**, 4389; c) A. U. Barlan, A. Basak and H. Yamamoto, *Angew. Chem. Int. Ed. Engl.,*2006, **45**, 5849.