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ARTICLE *in* NATURE PROTOCOL · FEBRUARY 2008

Impact Factor: 9.67 · DOI: 10.1038/nprot.2007.524 · Source: PubMed

Simple protocols for NMR analysis of the enantiomeric purity of chiral primary amines

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Published online 17 January 2008; doi:10.1038/nprot.2007.524

A simple three-component chiral derivatization protocol for determining the enantiopurity of chiral primary amines by ^{1}H NMR spectroscopic analysis is described here. The method involves condensation of the amines with 2-formylphenylboronic acid and enantiopure 1,1′-bi-2-naphthol. This approach affords a mixture of diastereoisomeric iminoboronate esters whose ratio can be determined by the integration of well-resolved diastereotopic resonances in their ^{1}H NMR spectra, thus enabling the enantiopurity of the parent amine to be determined easily. The protocol, as described, takes less than 90 min to complete.

INTRODUCTION

Chiral primary amines have many important chemical and pharmaceutical applications, and, as a consequence, a wide range of methodologies have been developed for their asymmetric synthesis[1–10]. Therefore, the development of inexpensive and versatile chiral derivatization protocols that enable their enantiomeric excess (ee) to be simply determined by NMR spectroscopic analysis is of great interest to the scientific community. The most widely used approach for determining the enantiomeric purity of chiral amines using NMR spectroscopy[11–18] involves their derivatization with the relevant chiral derivatization agent (CDA) to afford either methoxy(trifluoromethyl)-phenyl acetyl (Mosher)[19] or methoxyphenyl acetyl (Trost)[20] amides. Despite its popularity, this methodology has its limitations, including the moisture sensitivity of the acid chloride reagents used for derivatization and the need to run both ^{1}H and ^{19}F NMR experiments for an accurate determination of enantiomeric purity[21]. Another drawback of this approach derives from the potential for different conversion rates in the reaction between the enantiomers of sterically demanding amines and the CDAs (kinetic resolution)[22,23]. Such an effect would in turn lead to enantiomer ratios of the solutions of the derivatized amines that are different from those of the parent compounds.

We have recently described the development of a series of simple and versatile ^{1}H NMR spectroscopic protocols[24,25] for determining the ee of a wide range of chiral primary amines[26] and chiral diols[27]. The enantiopurity of chiral primary amines is determined by carrying out a three-component coupling reaction between 1.0 equiv. of the amine, 1.0 equiv. of 2-formylphenylboronic acid and 1.1 equiv. of enantiopure (*S*)-1,1′-bi-2-naphthol (BINOL) in chloroform-d (see **Fig. 1**; ref. 24). This reaction results in quantitative complexation that affords a mixture of structurally rigid diastereoisomeric iminoboronate esters[28,29] whose *N*-fragments experience different anisotropic effects, resulting in significantly different ^{1}H NMR spectra. As this three-component self-assembly reaction proceeds with no kinetic resolution, comparison of the relative intensities of integrals of pairs of diastereotopic resonances enables the enantiopurity of the parent amine to be easily determined[24]. Furthermore, the possibility afforded by this approach to accurately measure the integrals of more than one pair of diastereotopic resonances ($\Delta\delta$ = 0.02–0.67 ppm) ensures a high degree of accuracy in quantifying the enantiomeric purity of chiral amines. We have demonstrated that the detection limit of these protocols for determining the ee of chiral amines lies well within the accepted limits for CDA analysis using NMR spectroscopy (±5%), which compares well with other techniques used for ee determination such as chiral GC or HPLC analysis[30]. From a practical perspective, both enantiopure BINOL and 2-formylphenylboronic acid used for amine derivatization are inexpensive, commercially available reagents that are stable to moisture and so can be used without the need to operate in an inert atmosphere. The simplicity and speed of this derivatization procedure enables the enantiopurity of chiral primary amines to be determined in less than 90 min in all cases.

The range of chiral amines that have been successfully analyzed are shown in **Figure 2** (see ref. 24) and include α-arylethylamines, α-methylalkylamines, β-amino ethers, α-amino esters and β-amino esters. In all cases, derivatization of chiral amines proceeded quantitatively to afford mixtures of diastereoisomeric iminoboronate esters whose ^{1}H NMR spectra exhibited more than one

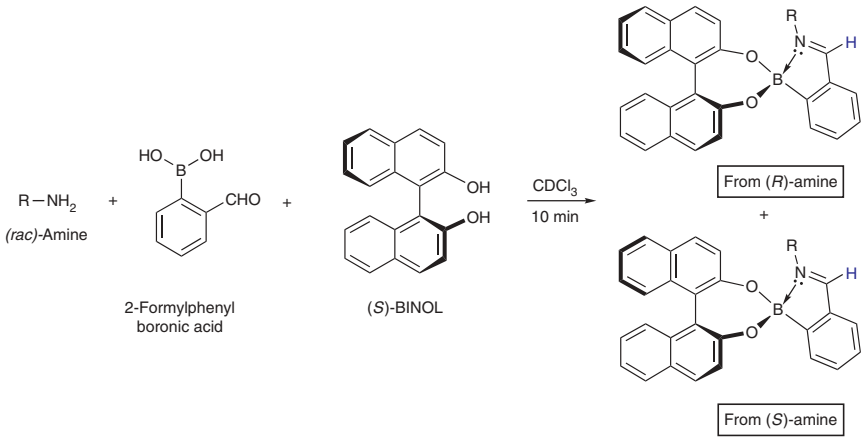

Figure 1 | Chiral derivatization protocol used for determining the ee of a chiral primary amine via derivatization with 2-formylphenyl boronic acid and (*S*)-BINOL. The imino proton (shown in blue) of the resultant diastereoisomeric iminoboronate ester complexes are always baseline resolved.

pair of baseline-resolved diastereotopic resonances. Furthermore, the diastereotopic imino resonances of each pair of iminoboronate esters were always baseline-resolved in a region of the NMR spectrum where no other resonances occur, thus providing a useful set of diagnostic resonances for integration that is independent of the structure of the amine being analyzed. This three-component derivatization approach may also be used to determine the enantiopurity of primary amines that contain remote stereogenic centers[31], with diastereotopic resonances being observed for substituents located up to five bonds away from the imino functionality[24]. The relative chemical shifts of the diastereoisomeric iminoboronate esters may also be used to predict the absolute configuration of certain classes of chiral amine, including α-arylethylamines and α-amino esters that are widely used as chiral building blocks in drug discovery and life-science applications[24].

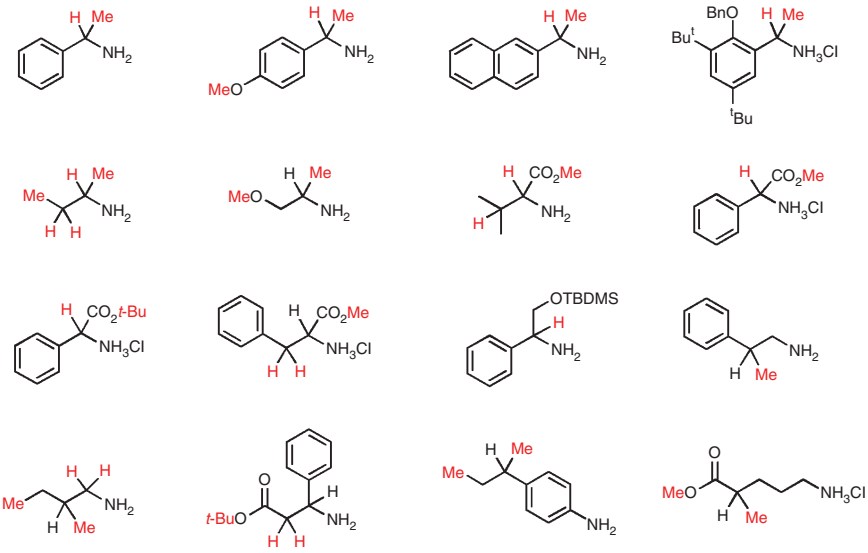

Figure 2 | Range of racemic primary amines successfully derivatized to afford diastereoisomeric iminoboronate esters that display one or more sets of baseline-resolved diastereotopic resonances in their ^{1}H NMR spectra. The protonic fragments highlighted in red are those to which the mentioned diastereotopic resonances are attributed.

As a consequence of the simplicity and speed of this derivatization technique, we now describe representative protocols for using this CDA approach for ^{1}H NMR spectroscopic analysis of the ee of chiral amines.

MATERIALS

REAGENTS

- 2-Formylphenylboronic acid (Frontier Scientific, cat. no. F6355)
- (*S*)-BINOL (Sigma-Aldrich, cat. no. 246948); (*R*)-BINOL (Sigma-Aldrich, cat. no. 246948) is also available
- Cesium carbonate (optional, see Step 7; Sigma-Aldrich, cat. no. 441902)
- Chloroform-d (Sigma-Aldrich, cat. no. 151858)
- Molecular sieves, 4 Å (optional, see **Table 1**; Sigma-Aldrich, cat. no. 208590)

EQUIPMENT

- Glass round-bottomed flask, 10 ml (Barloworld Scientific, cat. no. FR50/3M)
- Teflon-coated magnetic stirring bar (Sigma-Aldrich, cat. no. Z329207)
- Hamilton microsyringe: 800 series, cemented needle, volume, 50 μl; needle size, 22 G bevel tip (Sigma-Aldrich, cat. no. 24544)
- Fortuna Optima glass syringe (Sigma-Aldrich, cat. no. Z314358) and syringe needles (Neolus)
- Weighing paper, 4 inch × 4 inch (Whatman Schleicher and Schuell; Sigma-Aldrich, cat. no. Z134120)
- NMR tubes, 5 mm (Wilmad Ultra Imperial GR)
- NMR spectrometer (Bruker, AV400 or AV500)

EQUIPMENT SETUP

- Dry a 10-ml round-bottomed flask before use as the reaction vessel by placing it for at least 2 h in an oven set at 200 °C.

PROCEDURE

1| Take a 10-ml round-bottomed flask from hot oven at 200 °C and cool to room temperature (15 °C; at least 30 min).

2| Place a Teflon-coated magnetic stir bar into the round-bottomed flask. Turn the magnetic stirrer on.

3| Weigh out 60 mg (0.40 mmol) of 2-formylphenylboronic acid onto a sheet of weighing paper and transfer to the round-bottomed flask.

4| Transfer 5 ml of chloroform-d to the reaction vessel using a glass syringe fitted with a disposable needle.
▲ CRITICAL STEP 2-Formylphenylboronic acid is only sparingly soluble in chloroform-d. Therefore, take care to ensure that the entire amount of reagent is transferred into the solvent in the round-bottomed flask.

5| Weigh out 128 mg (0.44 mmol) of (*S*)-BINOL onto a sheet of weighing paper and transfer to the round-bottomed flask.

6| Add 0.40 mmol of the chiral amine under analysis to the round-bottomed flask, and stir until a homogeneous solution is obtained.

7| (OPTIONAL) If the parent amine is only available as its corresponding ammonium salt, add one equivalent of caesium carbonate (130 mg, 0.40 mmol) to generate its free amino functionality *in situ*.

8| Transfer a further 1 ml of chloroform-d to the round-bottomed flask.

▲ CRITICAL STEP (*S*)-BINOL is sparingly soluble in chloroform; therefore, take care to ensure that all the reagent is transferred into the solution in the round-bottomed flask.

9| Stir the reaction mixture for 10 min at room temperature.
■ PAUSE POINT The reaction mixture can be left to stir overnight.

10| Remove an aliquot (0.7 ml) of the reaction mixture using a glass syringe fitted with a disposable needle. Transfer to an NMR tube and fit with a lid. Please note that if the reaction mixture contains caesium carbonate, then any insoluble inorganic solids should be removed via filtration through a small plug of cotton wool, before transferring the amine solution to the NMR tube.

11| Run ^{1}H NMR spectrum referencing the resonance peaks in relation to either the chloroform signal (singlet at 7.27 ppm) or TMS (singlet at 0.00 ppm).

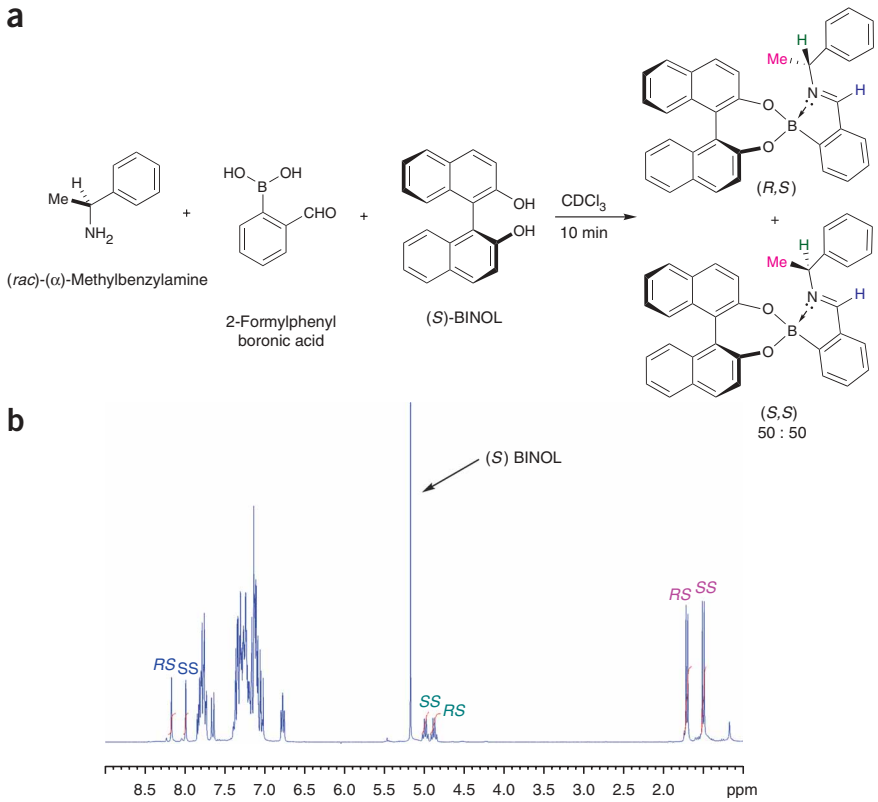

Figure 3 | Derivatization of (*rac*)-α-methylbenzylamine. Derivatization of (**a**) (*rac*)-α-methylbenzylamine with 2-formylphenylboronic acid and (*S*)-BINOL affords (**b**) a ^{1}H NMR spectrum containing a 50:50 mixture of diastereoisomeric (*R*,*S*)- and (*S*,*S*)-iminoboronate esters.

● TIMING
Steps 1–3, 30 min; Step 4, 5 min; Steps 5 and 6, 5 min; Step 7, 5 min; Step 8, 10 min; Step 9, 10 min; Steps 10 and 11, 20 min (20 min if filtration is required to remove caesium carbonate)

? TROUBLESHOOTING
Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Problem	Possible reason	Solution
The chiral amine substrate whose ee is being determined is not fully derivatized, as testified by the presence of its resonances in the ^{1}H NMR spectrum	Insufficient quantities of the derivatizing reagents were added to the reaction	Repeat experiment ensuring accurate weights of starting materials and derivatizing agents. To ensure that the correct amounts of reactants are introduced, dry BINOL should be employed for derivatization. A slight excess of BINOL 1.1 equivalents is used to ensure the complexation reaction proceeds to completion. Excess unreacted BINOL can afford a residual singlet at δ 5.2–5.3 ppm in $CDCl_3$
	2-Formylphenylboronic acid and other reactants with poor solubility in chloroform-d were not fully washed into the reaction mixture	Repeat the derivatization reaction, ensuring that all of the derivatizing agents are transferred to the round-bottomed flask
	The parent amine, boronic acid or chloroform-d was wet	Add 0.50 g activated 4 Å molecular sieves to the derivatization reaction to remove any adventitious water
The diasterotopic resonances of the iminoboronate esters are not sufficiently	Diastereotopic resonances of iminoboronate esters are not baseline-resolved	Run the sample using an NMR spectrometer with higher field strength of 500 MHz or above

TABLE 1 | Troubleshooting table (continued).

Problem	Possible reason	Solution
well resolved in the ^{1}H NMR spectrum for accurate integration to be carried out	under standard conditions using a 400 MHz NMR spectrometer	Run the ^{1}H NMR spectrum in a different solvent. To accomplish this, remove the chloroform-d using a rotary evaporator *in vacuo* at ~40 °C (5 min), immediately dissolve the residue in 2 ml of an alternative deuterated solvent and acquire a new ^{1}H NMR spectra (repeat Steps 10–11). Alternative deuterated solvents suggested for this analysis are acetone-d6, toluene-d8 or methanol-d4. Reference the ^{1}H NMR spectrum to residual solvent peaks or to TMS
		Run the ^{1}H NMR spectrum at lower temperature to strengthen the N→B coordinate bond of the imino-boronate esters, which may improve the resolution of the diastereotopic signals

ANTICIPATED RESULTS

The amine derivatization reaction affords a quantitative yield of iminoboronate ester diastereoisomers, whose ratio may be determined from comparison of the integral ratios of appropriate pairs of diastereotopic protons in their ^{1}H NMR spectra. As no kinetic resolution occurs in this reaction, the diastereoisomeric excess value obtained from integral analysis is equivalent to the ee of the parent amine.

Representative ^{1}H NMR spectra obtained in determining the ee of α-methylbenzylamine

The ^{1}H NMR spectrum of a racemic sample of α-methylbenzylamine derivatized with 2-formylphenylboronic acid and (*S*)-BINOL reveals the presence of two distinct diastereoisomers with three pairs of well-resolved diastereotopic protons available for integration (see **Fig. 3b**). As can be seen, resonances corresponding to the C*H*=N NC*H*(Me) and NC(H)*Me* protons of the two diastereoisomers are well resolved and their integrals display a ~50:50 ratio, as expected for derivatization of a racemic sample of α-methylbenzylamine (see also **Table 2**). A peak at δ 5.10 ppm corresponding to the hydroxyl protons of the 10% excess BINOL is observed in ^{1}H NMR spectra run in $CDCl_3$; however, it appears in a region of the spectra free of other resonances used for integral analysis. The ^{1}H NMR spectrum of a derivatized sample of scalemic (*R*)-α-methylbenzylamine of 80% ee (**Fig. 4**, see panel **b**) clearly reveals how integration of any one (or all) of these three pairs of resolved diastereotopic resonances may be used to accurately determine its 80% diastereoisomeric excess (see also **Table 3**).

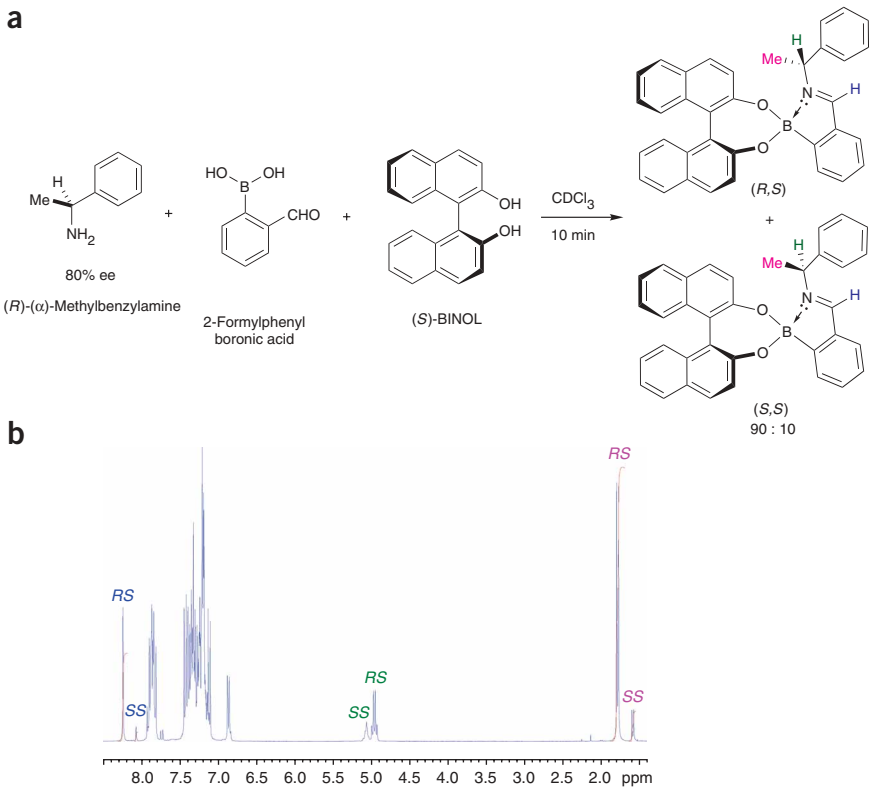

Figure 4 | Determination of the enantiomeric exces of scalemic α-methylbenzylamine. Derivatization of (**a**) scalemic (*R*)-α-methylbenzylamine of 80% ee with 2-formylphenylboronic acid and (*S*)-BINOL affords (**b**) a ^{1}H NMR spectrum containing a 90:10 mixture of diastereoisomeric (*R*,*S*)- and (*S*,*S*)-iminoboronate esters.

^{1}H NMR spectroscopic data for diastereoisomerically pure iminoboronate esters

^{1}H NMR data for (*R*,*S*)-diastereoisomer: δ_H (300 MHz; $CDCl_3$) 8.25 (1H, s, C*H*=N), 7.90–7.80 (4H, m, Ar*H*), 7.43-7.08 (16H, m, Ar*H*), 6.85 (1H, d, *J* 7.0, Ar*H*), 4.95 (1H, q, *J* 6.9, C*H*(CH_3)N), 1.77 (3H, d, *J* 7.0, CH_3C(H)N).

TABLE 2 | Important signals observed in the ^{1}H NMR spectrum of the 50:50 mixture of diastereoisomeric (*R*,*S*)- and (*S*,*S*)-iminoboronate esters derived from (*S*) BINOL.

Proton	δ_H (ppm) (*R*)	δ_H (ppm) (*S*)	Integration (*R*):(*S*) I_{maj}:I_{min}	ee% $\left(\frac{I_{maj}-I_{min}}{I_{maj}+I_{min}}\right)\times 100$
C*H*N	8.17	7.99	1.0:1	0.0
C*H*(Me)N	4.88	4.98	1.0:1	0.0
CH(*Me*)N	1.72	1.51	1.0:1	0.0

TABLE 3 | Important signals observed in the ^{1}H NMR spectrum of the 90:10 mixture of diastereoisomeric (*R*,*S*)- and (*S*,*S*)-iminoboronate esters derived from (*S*) BINOL.

Proton	δ_H (ppm) (*R*)	δ_H (ppm) (*S*)	Integration (*R*):(*S*) I_{maj}:I_{min}	ee% $\left(\frac{I_{maj}-I_{min}}{I_{maj}+I_{min}}\right)\times 100$
C*H*N	8.24	8.08	11.1:1	83.5
CH(*Me*)N	1.79	1.57	8.3:1	78.5

^{1}H NMR data for (*S*,*S*)-diastereoisomer: δ_H (300 MHz; $CDCl_3$) 8.05 (1H, s, C*H*=N), 7.89–7.67 (4H, m, Ar*H*), 7.47–7.07 (16H, m, Ar*H*), 6.84 (1H, d, *J* 6.9, Ar*H*), 5.03 (1H, q, *J* 6.8, C*H*(CH_3)N), 1.57 (3H, d, *J* 6.9, CH_3C(H)N).

ACKNOWLEDGMENTS We wish to thank the University of Bath, the EPSRC, the Leverhulme Trust and the Royal Society for financial support.

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