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A lactate-derived chiral aldehyde for determining the enantiopurity of enantioenriched primary amines†

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In this paper we describe the use of a chiral aldehyde derived from lactate esters for determining the enantiopurity of primary amines, *via* the formation of diastereomeric imines. The method was shown to be suitable for reproducibly determining the enantiopurity of a diverse set of chiral amines. Both enantiomers of the aldehyde can be prepared in two steps from commercially available materials.

Introduction

During the course of many synthetic organic chemistry research projects, it is essential to be able to determine the enantiopurity of chiral intermediates or products in order to ascertain whether any racemisation has taken place. This is typically achieved either *via* chiral HPLC analysis (which generally requires a sample of the opposite enantiomer or of the racemic material), or *via* the formation of a derivative with a chiral reagent which enables the resulting diastereomeric species to be observed *via* standard spectroscopic techniques.[1–6] Ideally, the chiral derivatising reagent should be readily accessible in either enantiomeric form, and should undergo rapid and efficient reaction with the analyte in order to minimise errors which can occur due to the inherent diastereoselectivity of the reactions. For chiral alcohols, the formation of an ester using the chiral acid **1** (Fig. 1) originally developed by Mosher has become the standard method, enabling the determination of not only the enantiopurity, but also the absolute stereochemistry of the major isomer.[1] Acid **1** is available in both enantiomeric forms, and undergoes efficient reaction with secondary alcohols either *via* the corresponding acid chloride or *via* direct coupling reactions.

For chiral amines, however, derivatisation methods are somewhat more difficult. If amides are prepared using a chiral acid such as **1**, then the resulting compounds are frequently difficult to analyse *via* NMR due to the presence of rotational isomers caused by slow rotation around the C–N bond.[1,2] An

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†Electronic supplementary information (ESI) available: Experimental procedures, spectroscopic data and ¹H and ¹³C NMR spectra. See DOI: 10.1039/c5ob01398d
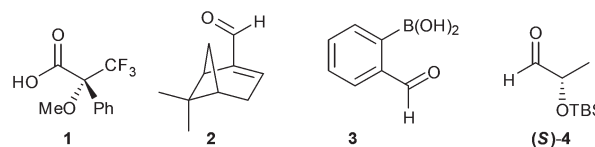
Fig. 1 Chiral derivatising agents.

alternative strategy is to use a chiral aldehyde to generate an imine.[3–5] This strategy is potentially useful as imine formation is often rapid. However, the aldehyde used must be fairly hindered in order to give the *E*-imine selectively as a single geometrical isomer, and it must also be stable towards racemisation under the conditions used for imine formation. There have been a few reports in this area, including the use of myrtenal **2**[2,3] as well as *o*-formylbenzene boronic acid **3** in combination with enantiomerically enriched BINOL.[4] Myrtenal **2**, has not been widely employed, with the original reports simply demonstrating that the diastereomeric imines derived from racemic amines could easily be distinguished by NMR. This is, however, very different from the measurement of the enantiopurity of an amine derived from a chemical reaction in which small quantities of the minor diastereoisomer must typically be quantified as accurately as possible. The enantiomer of **2** is also not readily available. Aldehyde **3**, in combination with chiral *bis*-phenols, has been used to measure the enantiopurity of a range of amines synthesised by various methods.[5] In our hands it has proved suitable for the measurement of the enantiopurity of simple branched amines (*e.g.* α-alkylbenzylamines), but we were not able to apply it to determine the enantiopurity of a series of α-aminoamides prepared in our amidation chemistry.[7]

In this paper, we describe the use of chiral aldehyde **4** as a new method for the determination of the enantiopurity of

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chiral amines. We demonstrate its application to a diverse set of commercially available chiral amines including samples covering a wide range of enantiopurities as well as samples of α-aminoamides obtained directly from amidation reactions.

Results and discussion

During the course of our work on borate-mediated amidation reactions,[7] we frequently needed to determine the enantiopurity of α-aminoamides in order to determine whether racemisation had taken place during the coupling reactions. In many cases, the enantiopurity could be determined *via* chiral HPLC analysis of the Boc derivative. However, in several cases, it was not possible to separate the enantiomers using column systems available in our laboratory. The requirement to prepare an authentic sample of the opposite enantiomer (or the racemate) also makes this approach extremely tedious when many samples need to be analysed. We therefore decided to explore the use of chiral aldehydes to see whether it would be possible to determine the enantiomeric purity of the corresponding free amine through imine formation and subsequent NMR analysis. Unfortunately, none of the reported amine derivatisation methods proved suitable for our needs,[3,4] with very slow imine formation reactions that did not readily go to completion, sometimes giving complex mixtures by NMR that were difficult to analyse accurately. We therefore elected to investigate an alternative chiral aldehyde for this purpose. Both enantiomers of aldehyde **4** can be prepared in two steps from commercially available lactate esters (Scheme 1) and have been widely used as chiral pool building blocks for a range of synthetic applications.[8] Importantly, no detectable racemisation occurs during the synthesis of **4**,† and it does not racemise readily on storage for up to one month,† despite the presence of an alpha proton.

To determine the suitability of **4** for the derivatisation of chiral amines we examined the imine formation reaction between racemic amino amide **7** and (*S*)-**4** in a range of deuterated NMR solvents (Scheme 2 and Table 1). Pleasingly, the corresponding diastereomeric mixture of imines **8a**/**8b** was formed rapidly at room temperature in a variety of different solvents.
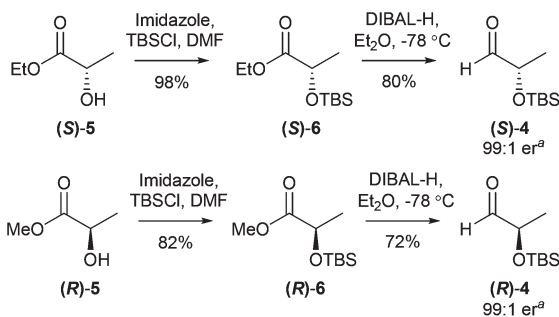
Scheme 1 Synthesis of both enantiomers of aldehyde **4**. [a]The enantiopurity was determined by imine formation with L-phenylalanine methyl ester.†
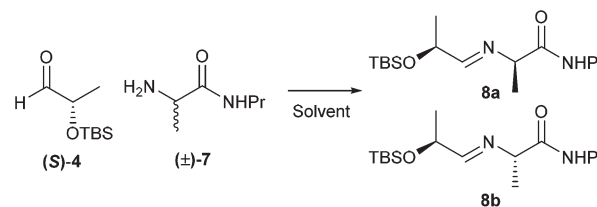
Scheme 2 Imine formation with aldehyde **4** in a range of NMR solvents.

Table 1 Separation of imine ^{13}C signals in diastereoisomers **8a**/**8b**

Solvent	Peak separation (ppm)
$CDCl_3$	0.11
DMSO-d_6	0.09
MeOH-d_4	0.21
C_6D_6	0.13

Unfortunately, the ^{1}H NMR did not offer sufficient resolution between the two diastereoisomers to enable accurate quantification. However, the ^{13}C NMR clearly showed two separate peaks for the imine carbons around 170 ppm which were readily resolved in all of the solvents used (Table 1). Fortunately, using modern NMR machines it is relatively straightforward to obtain accurate quantification from ^{13}C NMR, provided the carbon signals under scrutiny have similar relaxation times. Typically, a 15-minute ^{13}C NMR experiment was sufficient to obtain spectra with a strong enough signal to noise ratio, enabling efficient analysis of multiple samples in a short period of time.

With samples of both enantiomers of aldehyde **4** in hand, we explored the derivatisation of a selection of commercially available chiral amines **9–20** (Table 2). In the majority of cases, excellent resolution of the diastereomers was observed, enabling the enantiopurity[9] of the chiral amines to be determined. There was good agreement between the measurements obtained with the two enantiomeric aldehydes, and the two values were averaged to give a more accurate measurement. The method could be used to derivatise a selection of benzylamines (**10–12**, **16**, **19**) as well as amines containing only aliphatic substituents at the adjacent carbon atom (**9**, **13–15**). Bicyclic amines such as **17**, **18** and **20** could also be analysed, although in the latter case the two imine carbon signals were very close together so the ratio of the two diastereoisomers could only be quantified by peak height and not by integration.

A key test of any enantiomeric determination method is the ability to accurately and reproducibly measure the enantiomeric purity of a set of unknown samples. To this end, we challenged the method with a set of 'blind' tests (Table 3). Three 'unknown' scalemic samples of chiral amine were prepared by each of three researchers by weighing out appropriate quantities of commercially available amines **9–11**. The three samples were then analysed 'blind' by the other two researchers using both enantiomers of aldehyde **4**. The measured er in each case was calculated by taking the average of the values

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Table 2 Determination of the enantiomeric purity of commercial chiral amines 9–20 using aldehyde 4
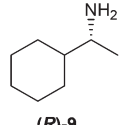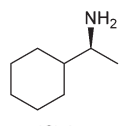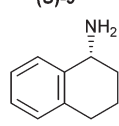
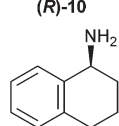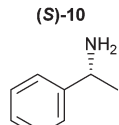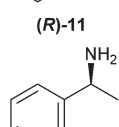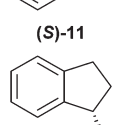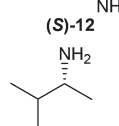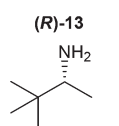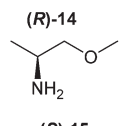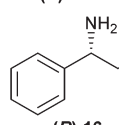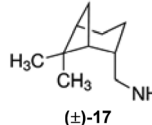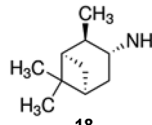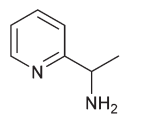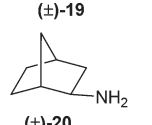| Amine	$\Delta(\delta_C)$/ppm (solvent)	Enantiomeric ratio[9]		
		(S)-4	(R)-4	Average
(R)-9		98:2	99:1	98.5:1.5
(S)-9	0.070 (d_4-MeOH)	98:2	99:1	98.5:1.5
(R)-10		98:2	99:1	98.5:1.5
(S)-10	0.093 (d_4-MeOH)	98:2	99:1	98.5:1.5
(R)-11		97:3	98:2	97.5:2.5
(S)-11	0.139 (d_4-MeOH)	98:2	99:1	98.5:1.5
(S)-12	0.163 (d_4-MeOH)	99:1	98:2	98.5:1.5
(R)-13	0.264 (CDCl$_3$)	99:1	98:2	98.5:1.5
(R)-14	0.792 (CDCl$_3$)	99:1	99:1	99:1
(S)-15	0.164 (d_4-MeOH)	99:1	99:1	99:1
(R)-16	0.186 (d_4-MeOH)	98:2	98:2	98:2

Table 2 (Contd.)

Amine	$\Delta(\delta_C)$/ppm (solvent)	Enantiomeric ratio[9]		
		(S)-4	(R)-4	Average
(±)-17	0.116 (d_4-MeOH)	97:3	96:4	96.5:3.5
18	0.173 (CDCl$_3$)	95:5	96:4	95.5:4.5
(±)-19	0.200 (d_4-MeOH)	51:49	50:50	50.5:49.5
(±)-20	0.036 (C$_6$D$_6$)	50:50	50:50	50:50[a]

[a] The ratio of imines was determined using peak height rather than integration.
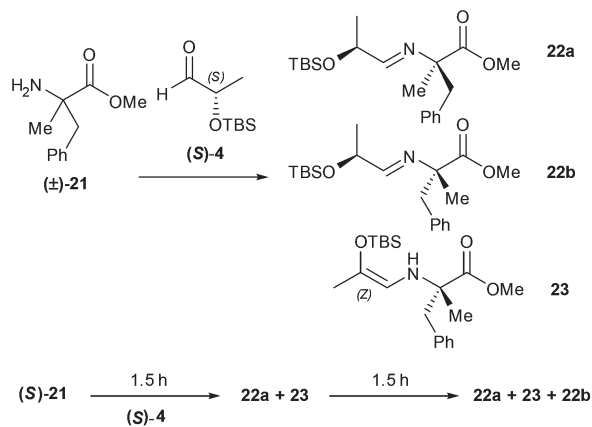
Table 3 Preparation and analysis of amine samples of 'unknown' enantiopurity using amines 9–11

Entry	Amine	Prepared by	Actual er[a] (R:S)	Measured er[b] (R:S)
1	11	RML	69:31	70:30 (LB) 69:31 (SMG)
2	10	RML	34:66	34:66 (LB) 35:65 (SMG)
3	9	RML	77:23	77:23 (LB) 78:22 (SMG)
4	11	LB	6:94	8:92 (RML) 9:91 (SMG)
5	10	LB	86:14	87:13 (RML) 85:15 (SMG)
6	9	LB	24:76	25:75 (RML) 25:75 (SMG)
7	11	SMG	89:11	88:12 (RML) 87:13 (LB)
8	10	SMG	53:47	53:47 (RML) 53:47 (LB)
9	9	SMG	16:84	17:83 (RML) 18:82 (LB)

[a] Calculated using weights of amines used and the measured er values for amines 9–11 from Table 2. [b] Average of values obtained with (S)-4 and (R)-4, see supporting information for original data and the corresponding NMR spectra.

obtained with (S)-4 and (R)-4. As can be seen from the results, extremely consistent measurements were obtained by the three researchers, and in all cases these were in very close agreement with the actual enantiopurity of the sample based on the original weighings. There is a small divergence in the measured

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Scheme 3   Reaction of quaternary amino acid ester **21** with aldehyde **4**.

Fig. 2   Imine/enamine formation from amine **21** over time: (a) reaction of (S)-**21** and (S)-**4**; (b) reaction of (S)-**21** and (R)-**4**.

Scheme 4   Determination of the enantiopurity of amidation products using aldehydes **4** and chiral HPLC.

enantiopurities at high er (entries 4 and 7), but this is reasonably expected as the errors in measuring the small integral for the minor isomer in these cases is likely to be relatively large.

Pleasingly, an amine (±)-**21** bearing an adjacent quaternary centre reacted with aldehyde **4** to give two easily resolved diastereomeric imines (Scheme 3). However, the formation of enamine **23** (as a single geometrical isomer) also took place, and significant amounts of **23** were observed before all of the starting amine **21** had been consumed. The formation of imine **22** was relatively slow between the enantioenriched amine (S)-**21** and (S)-**4**. As can be seen in Fig. 2, the proportion of enamine present in the sample is already ∼35% by the time the starting amine is completely consumed (∼1.5 hours). Eventually, after a total reaction time of three hours the diastereomeric imine **22b** is observed and its concentration gradually
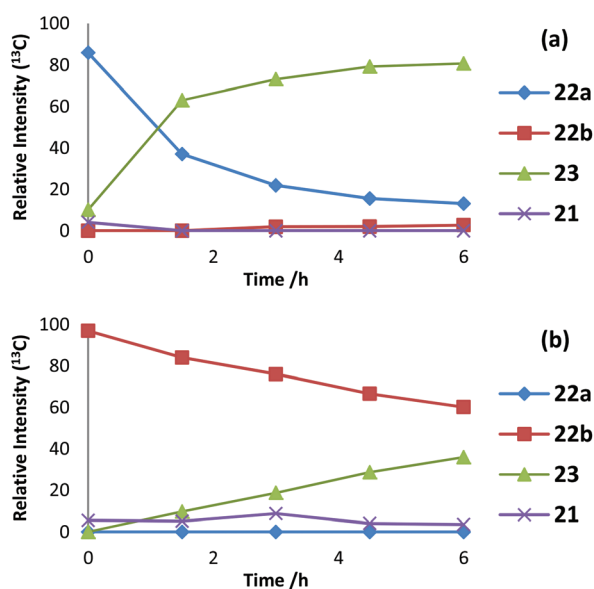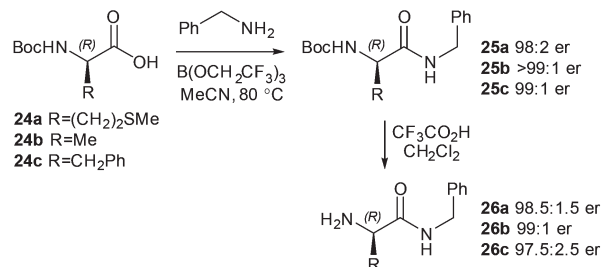
increases over time demonstrating that in this particular case racemisation of imine **22a** *via* the enamine **23** takes place. In the corresponding reaction between (S)-**21** and (R)-**4**, a similar reaction profile is observed, although the initial imine formation did not quite go to completion in this case. This nevertheless enables us to conclude that the original amine (S)-**21** is of high enantiopurity (∼99 : 1 er) as no minor imine **22b** is observed in the reaction of (S)-**21** and (S)-**4** until consumption of the amine **21** is complete. This demonstrates that either: (i) no detectable imine **22b** is formed (there is no significant quantity of (R)-**21** present); or (ii) imine **22b** is formed but is rapidly converted into the enamine **23**. The latter is unlikely to be the case as no significant quantity of the minor imine (in this case *ent*-**22a**) is observed when (S)-**21** is reacted with (R)-**4**.

In the case of all other amines analysed (Table 2), imine formation was observed to be much more rapid than enamine formation, enabling accurate determination of the enantiopurity in each case. However, enamine formation was seen to take place in some cases if samples of the imine were stored for prolonged periods of time. It is therefore recommended that NMR analysis should be carried out immediately after sample preparation in order to obtain accurate results.

We next went on to examine some compound samples obtained from our amidation chemistry. Amides **25a**–**25c** were prepared *via* direct borate-mediated amidation of the corresponding Boc-amino acids **24a**–**24c** (Scheme 4).[7] Acidic deprotection of the Boc group gave the corresponding amines as their trifluoroacetate salts which were converted to the free amine by filtration through a basic ion exchange resin. The enantiopurity of the amines **26** was then measured using both enantiomers of aldehyde **4**, and the enantiopurity of the Boc-protected compounds **25** was measured by chiral HPLC. In all three cases, good agreement was seen between the two methods, demonstrating that the amidation reactions and subsequent deprotection occur with very little loss in enantiopurity under these conditions.

Conclusions

In summary, we have demonstrated that aldehyde **4** is a suitable derivatising agent for the determination of the enantio-

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purity of chiral primary amines by NMR spectroscopy. Both enantiomers can be prepared on multigram scale in two steps from commercially available lactate esters, and they react rapidly with primary amines in a range of NMR solvents to enable straightforward analysis of the diastereomeric imines generated.

Acknowledgements

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