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Simultaneous enantioresolution and assignment of absolute configuration of secondary alcohols by directly coupled HPLC–NMR of 9-AMA esters

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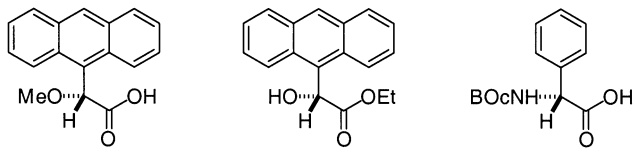
Abstract—Derivatization of mixtures of enantiomers (including racemates) of chiral secondary alcohols with a pure enantiomer of the auxiliary reagent, 9-anthrylmethoxyacetic acid [(*R*)- or (*S*)-9-AMA)] followed by directly coupled HPLC–NMR of the mixture of the esters allows separation of the two enantiomers, the determination of the e.e. and the assignment of their absolute configuration in a single operation and with just a few micrograms of substance. The procedure can also be applied to the assignment of the absolute configuration of a single enantiomer of the alcohol.

1. Introduction

The development of rapid and economical methods for the separation of enantiomers from mixtures, the evaluation of the e.e. and the assignment of their absolute configuration is of general interest in chemistry,[1,2] but is particularly important in pharmaceutical and medical applications and in cases where the amount of sample is limited and the need for automation high. It is in this context that techniques like HPLC–NMR–MS are most useful. Directly coupled HPLC–NMR has been successfully used for the rapid identification of compounds contained in complex mixtures and low amounts; important applications have been reported in several fields such as natural products,[3,4] and more especially, in the study of the metabolism and fate of metabolites of several drugs in humans and other animals.[5,6] In the last few years, we focused our interest on the study and design of methods and reagents for the assignment of absolute configuration by NMR and have amply demonstrated that derivatization of a chiral primary or secondary alcohol, a primary amine or a carboxylic acid with the two enantiomers of an appropriate auxiliary reagent, allows the assignment of the absolute configuration of the substrate simply by comparison of the proton NMR spectra of the resulting diastereoisomeric derivatives.[7,8]

The procedure is reliable, fast and economical, and the auxiliary reagents 9-AMA, 9-AHA and BPG (Fig. 1) have been demonstrated to be particularly useful for the analysis of chiral secondary[9–11] and primary alcohols,[12,13] carboxylic acids[14,15] and amines,[16] respectively.

Over these years, we have frequently observed that when the (*R*) and (*S*)-9-AMA ester derivatives of a chiral secondary alcohol were submitted to standard HPLC for analytical or purification purposes, well separated peaks for both derivatives could usually be obtained from both normal and reverse phase columns. This suggested to us that the derivatizing reagent 9-AMA might perhaps be used for both the HPLC separation of the enantiomeric alcohols and in their configurational assignment in a single operation using directly coupled HPLC–NMR. This concept has no precedent and if successful, would have a great impact on processes involving chiral compounds.



(*R*)-9-AMA (*R*)-9-AHA (*R*)-BPG

Figure 1.

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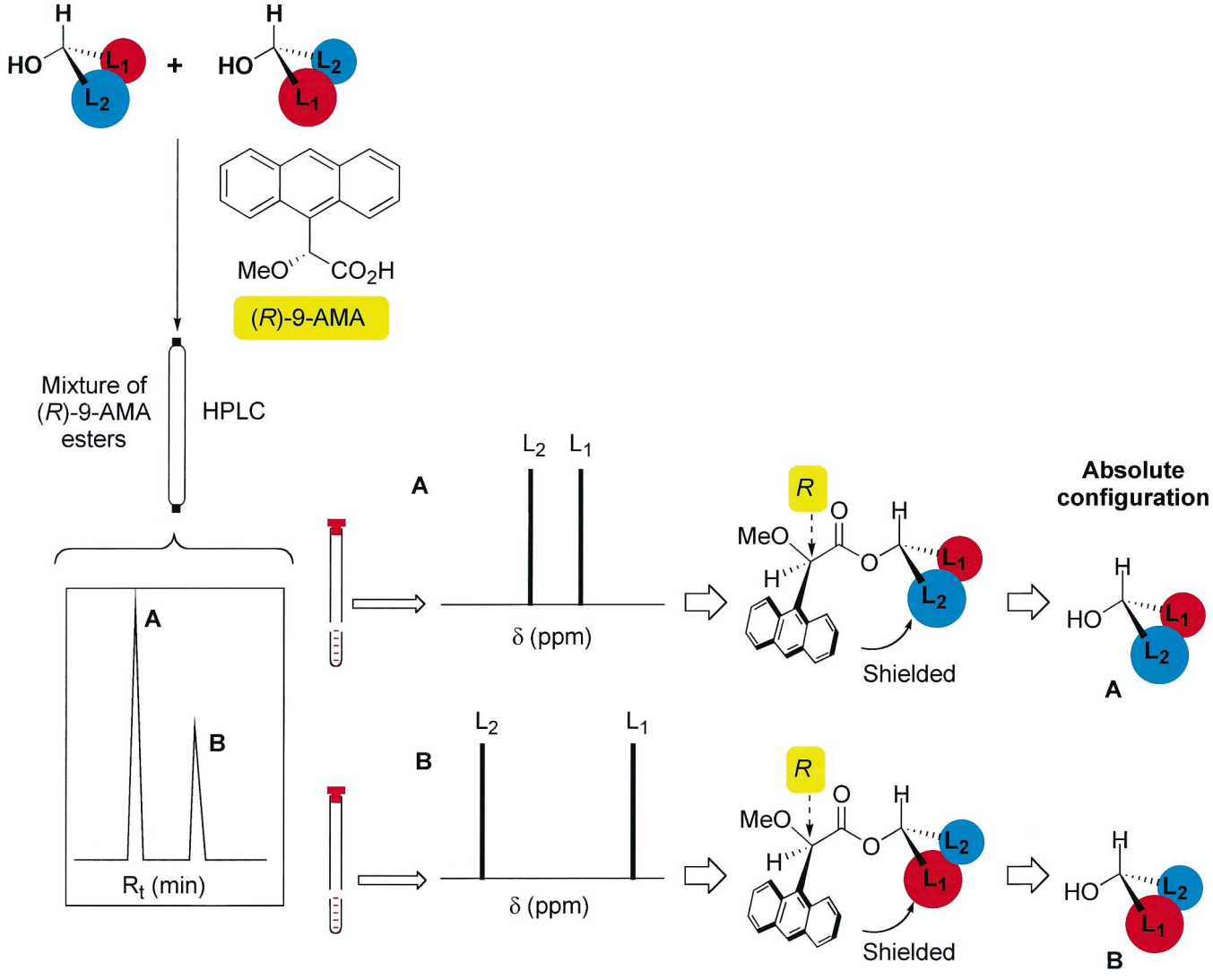
In this technique, the HPLC eluent is used as the solvent for the subsequent NMR analysis, and therefore, the search for adequate experimental conditions should respond to the following points:

(a) Good separations of a wide variety of alcohols should be achieved using HPLC eluents compatible with NMR.
(b) The configuration assigned using those eluents as NMR solvents should be undisputed.

Herein, we wish to communicate the successful application of tandem HPLC–NMR for on line separation and configurational assignment of secondary alcohols as 9-AMA derivatives. Conditions for semipreparative HPLC separation will be also shown.

HPLC–NMR experiments were carried out with less than 1 mg of the alcohol (mixture of enantiomers or a single enantiomer). If the absolute configuration of each enantiomer in a mixture (including racemic ones) has to be determined, the mixture of enantiomers is derivatized with a pure enantiomer of 9-AMA, as previously reported.[9–11] If the assignment of configuration of one enantiomer of the alcohol is the problem, the sample is derivatized with a 3:1 mixture of (*R*)- and (*S*)-9-AMA. In both cases, an aliquot containing 10–15 μg of the resulting mixture of 9-AMA esters is dissolved in acetonitrile-d_3, injected onto a reverse phase column and their ^{1}H NMR spectra recorded after separation.[†]

The two general situations where this methodology applies are described briefly next as a short guide to future users.



Scheme 1. Enantioresolution and assignment of the absolute configuration of the two enantiomers of a chiral secondary alcohol by derivatization of the mixture with (*R*)-9-AMA and tandem HPLC–NMR of the resulting esters. If (*S*)-9-AMA is used instead, the assignment is the opposite.

[†] 250×4 mm, C18 Purosphere-5 μm column.

2. Results and discussion

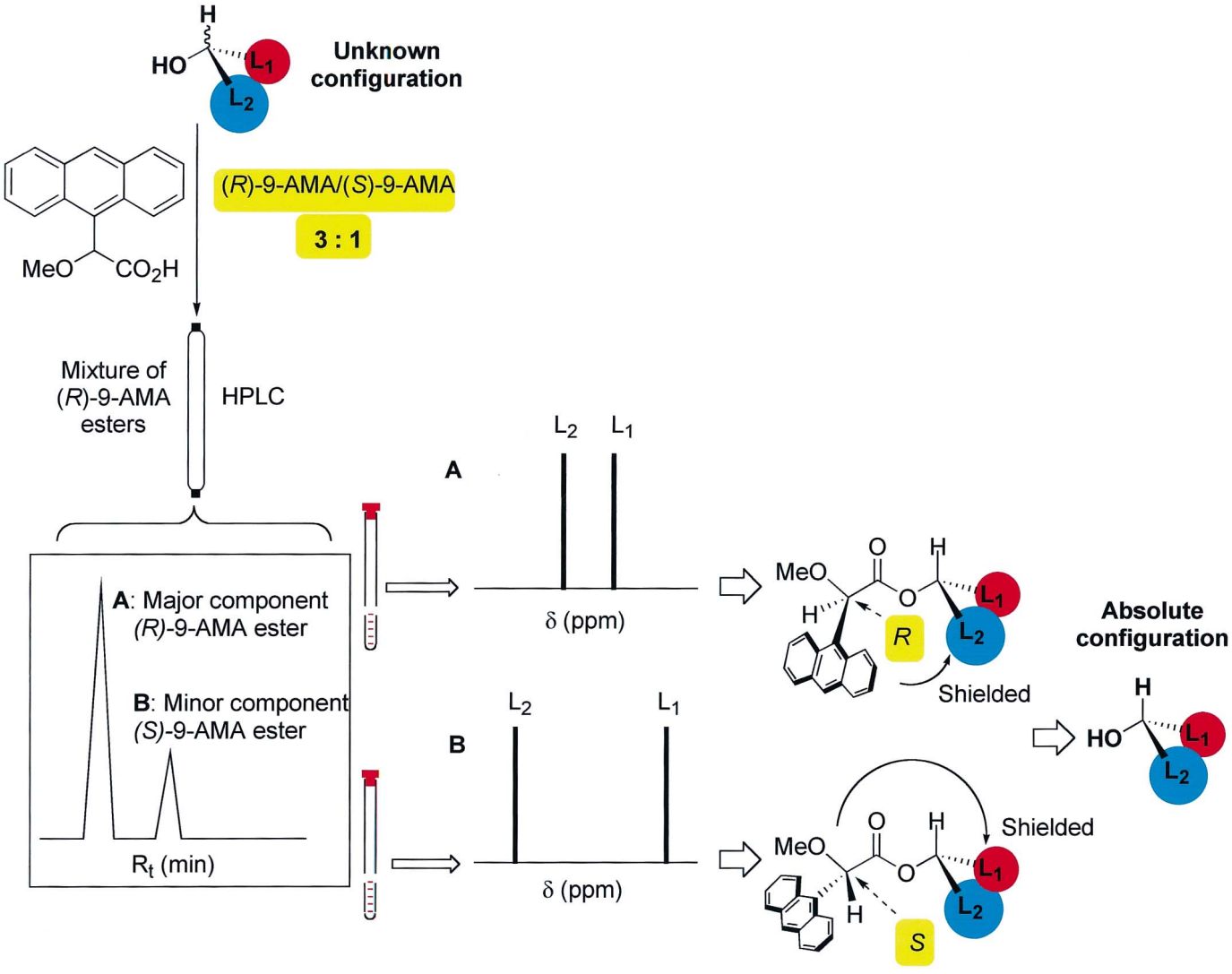
2.1. Enantioresolution and assignment of the absolute configuration of a mixture of two enantiomers

The mixture (that can be racemic) is derivatized with either (*R*) or (*S*)-9-AMA according to standard procedures.[9] The mixture of diastereomeric esters is submitted to HPLC–NMR and comparison of the ^{1}H NMR spectra of the two HPLC peaks allows the assignment of the configuration based on the shielding/deshielding effects caused in L_1/L_2 by the auxiliary reagent of known configuration (Scheme 1).[9–11]

2.2. Assignment of the absolute configuration of a single enantiomer

The alcohol is derivatized with a 3:1 mixture of (*R*)- and (*S*)-9-AMA.‡ The unequal mixture of diastereomeric esters is submitted to HPLC–NMR and the spectra of both peaks compared according to the standard procedure,[9–11] taking into account that the major component of the mixture corresponds to the (*R*)-ester and the minor one correspond to the (*S*)-isomer (Scheme 2).

The generality of this approach is demonstrated by its successful application to a variety of secondary alcohols



Scheme 2. Assignment of the absolute configuration of a chiral secondary alcohol of unknown configuration by derivatization with a (*R*)-9-AMA/(*S*)-9-AMA 3:1 mixture and tandem HPLC–NMR of the resulting esters.

‡ This is a recommended ratio that allows an easy assignment of the HPLC peaks: the major peak corresponds to the (*R*)-ester, while the minor one corresponds to the (*S*)-ester.

1–**18** of known absolute configuration shown in Fig. 2, including saturated, unsaturated, aromatic, acyclic and cyclic structures. As an example, Table 1 shows the retention times obtained for compounds **1**–**8** in the tandem experiments.

The reliability of the configuration predicted from those NMR spectra taken in deuterated acetonitrile-d_3/deuterium oxide/formic acid mixtures, but using a model developed for data obtained in deuterochloroform might be a cause for some concern. In order to clear up that point, we compared the NMR spectra of the (*R*)- and (*S*)-9-AMA esters of **1**–**8** taken in deuterochloroform with those obtained in the acetonitrile–water–formic acid mixtures used for HPLC and found no differences in the $\Delta\delta^{RS}$ signs relevant to the assignment of configuration.

We also examined the NMR spectrum of the (*R*)- and (*S*)-9-AMA esters of **1**–**8** in acetonitrile-d_3/deuterium oxide/formic acid mixtures of varying composition (from acetonitrile-d_3/deuterium oxide 75:25 plus 0.6% formic acid to acetonitrile-d_3/deuterium oxide 60:40 plus 0.6% formic acid) and found that the chemical shifts and $\Delta\delta^{RS}$ signs of the (*R*)- and (*S*)-9-AMA esters present no discrepancies with the trend observed in chloroform. From these experiments, we conclude that the use of acetonitrile-d_3/deuterium oxide/formic acid mixtures for HPLC–NMR produce reliable configurational assignments and can be applied to other alcohols with complete confidence.

Naturally, the enantioresolution and configurational assignment of secondary alcohols can be carried out in separate steps if actual isolation of the 9-AMA derivatives of the corresponding enantiomeric alcohols is needed for preparative purposes. In such a case, higher loading can be obtained when the HPLC separation is carried out under normal instead of reverse-phase conditions. In fact, we found that the (*R*)- and (*S*)-9-AMA

Table 1. Reverse-phase separation of 9-AMA esters of secondary alcohols

Alcohol	9-AMA	Rt (min)[a]
1	(*R*)/(*S*)	16.40/18.00
2	(*R*)/(*S*)	21.60/22.58
3	(*R*)/(*S*)	17.70/16.90
4	(*R*)/(*S*)	9.30/10.20
5	(*R*)/(*S*)	24.70/24.50
6	(*R*)/(*S*)	15.88/15.39
7	(*R*)/(*S*)	17.03/15.36
8	(*R*)/(*S*)	12.08/12.06

[a] Flow rate: 1 mL/min, for chromatographic conditions see Section 3.

Table 2. Semipreparative normal phase separation of 9-AMA esters of alcohols **9**–**18**

Alcohol	9-AMA	Rt (min)	Flux (mL/min)	Eluent (Hex:AcOEt)
9	(*R*)/(*S*)	31.10/31.40	2.0	80:20[a]
10	(*R*)/(*S*)	20.50/23.00	2.0	80:20[a]
11	(*R*)/(*S*)	34.73/31.94	2.0	96:4[a]
12	(*R*)/(*S*)	7.69/9.86	5.0	80:20[a]
13	(*R*)/(*S*)	13.19/10.76	4.0	90:10[a]
14	(*R*)/(*S*)	20.17/24.36	2.0	98:2[a]
15	(*R*)/(*S*)	52.94/54.02	2.0	98:2[a]
16	(*R*)/(*S*)	64.31/68.57	2.0	98:2[a]
17	(*R*)/(*S*)	31.00/29.30	1.2	96:4[b]
18	(*R*)/(*S*)	42.30/42.50	2.0	96:4[a]

[a] Spherisorb S5W 5 mm 10×250 mm.
[b] μ-Porasil 3 mm×250 mm.

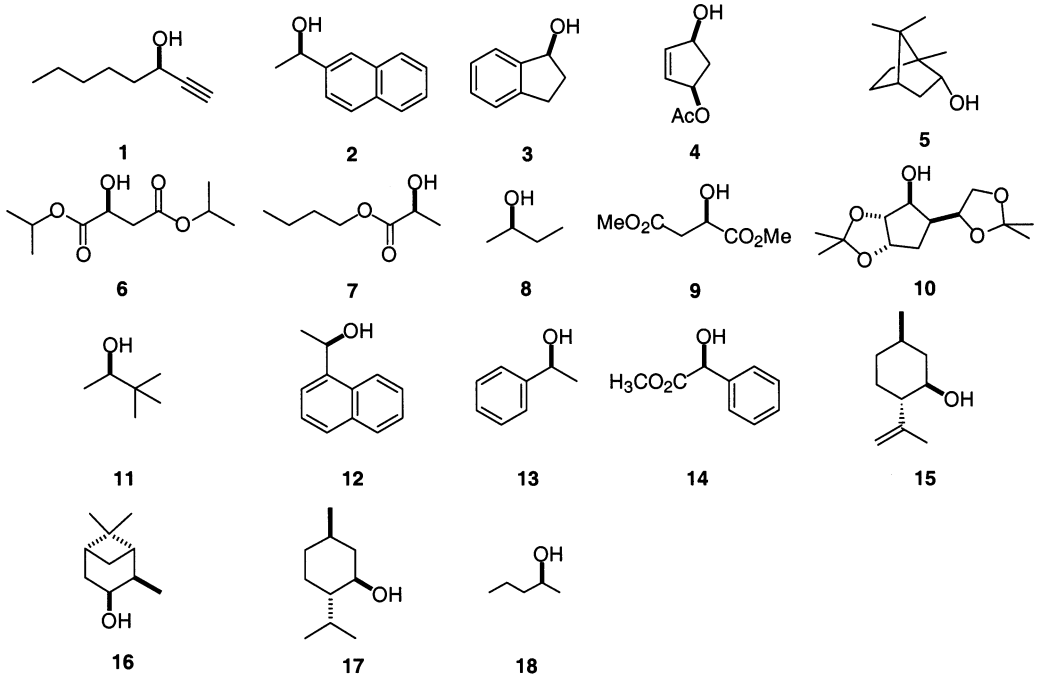


Figure 2. Chiral secondary alcohols studied.

esters of **1**–**18** can be easily separated (50–100 mg per injection) on a semi-preparative scale, using standard normal phase columns and eluting with hexane–ethyl acetate mixtures. The enantiomers are then isolated after simple hydrolysis.

Table 2 shows the experimental data for the isocratic normal phase, semi-preparative separation of a selection of substrates **9**–**18**.

In summary, we have shown that the enantioresolution and configurational assignment of secondary alcohols can be carried out by tandem HPLC–NMR of the corresponding 9-AMA esters. The number and structural variety of the alcohols of known absolute configuration **1**–**18** tested, indicate beyond doubt that this methodology is general for secondary alcohols and that it can be used in the analytical and semipreparative scales. The simplification involved in the use of the same auxiliary reagent for both the HPLC separation and the NMR configurational assignment, and its implementation in directly coupled HPLC–NMR has no precedent in the literature and should be extremely useful in the automation of many processes involving optically active compounds.

3. Experimental

The chromatographic system consisted of a quaternary gradient pump, an autosampler, and a column oven. The separations were achieved at 1 mL/min using a linear gradient of solvent A (deuterium oxide with 0.06% formic acid) and solvent B (acetonitrile-d_3) starting at a composition of 60% B and changing to 80% B in 20 min. Detection was carried out on a diode array detector and a 500 MHz NMR spectrometer. As interface, a peak sampling unit was used to store the separated peaks that were transferred to the NMR unit for measurement. NMR spectra were recorded using a selective inverse $^{1}H/^{13}C$ 500 MHz probe head equipped with a Z-gradient coil. Multiple solvent suppression was carried out using a modulated shaped pulse on channel f1. Channel f2 was used to decouple the carbon 13 satellites selectively. 64 scans were collected into 16k complex data points using a sweep width of 10000 Hz. The spectra were transformed to 16k points after multiplying the fid with an exponential function leading to a line broadening of 1 Hz.

Acknowledgements

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