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Determination of the chiral purity of benzylic amines using Marfey's reagent

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Abstract : *1-Fluoro-2,4-dinitrophenyl-5-(S)-alanine amide (Marfey's reagent) was used as a chiral derivatizing agent for benzylic amines in order to determine optical purities.*

During the course of our work on the stereoselective protonation of benzylic amines supported on a chiral polymer, we needed to determine the chiral purity of a number of samples (1). NMR (2-7) and liquid chromatography methods using chiral derivatizing reagents (7, 8) or HPLC chiral stationnary phases (7, 9-11) have been previously used to determine the optical purity of chiral amines.

Marfey's reagent, 1-Fluoro-2,4-dinitrophenyl-5-(S)-alanine amide, is a chiral derivatizing reagent frequently used for the enantiomeric purity determination of aminoacids and peptides by HPLC analysis (12, 13) or more recently by capillary electrophoresis (14). This reagent possess several advantages : (i) it is easily prepared from 1,5-difluoro-2,4-dinitrobenzene by substitution of one of the two fluorine atoms by (S)-alanine amide; (ii) it reacts rapidly and quantitatively under alkaline conditions with amino-substrates to supply diastereomeric derivatives; (iii) these diastereomers can be generally resolved by reverse phase HPLC and have a high absorption coefficient allowing them to be detected by UV at 340 nm with a picomole sensitivity.

Therefore, we decided to use this reagent for the chiral purity determination of six representative benzylic amines.

As in the case of aminoacids (12), the fluorine atom of Marfey's reagent was rapidly and quantitatively substituted under alkaline conditions by racemic amines.

We have gathered in Table 1 the appropriate gradients involved as well as the elution times obtained for Marfey's reagent and for each derivatized enantiomer. The large ΔTR values obtained in most cases allowed the integration of the two diastereomeric signals with good precision (0.1%). But the failure of the method for racemic 1-amino indan under all analytical conditions used showed that this HPLC method lacks generality.

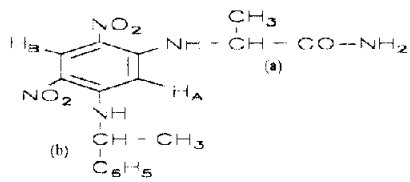
These results led us to explore the possibility of using Marfey's reagent as an NMR derivatizing reagent, which to our knowledge has not previously been attempted.

Table 1

(RS) Amines	Gradient	TR_0	TR_1, TR_2	$\Delta TR_{1,2}$
α-methyl benzylamine	I	8.30	16.38 - 17.67	1.29
α-naphthyl ethylamine	II	8.35	17.46 - 19.09	1.63
1,2,3,4-tetrahydro-α-naphthylethylamine	I	8.35	11.93 - 13.73	1.80
1-methyl 3-phenylpropylamine	III	11.05	50.74 - 52.06	1.32
1-aminoindan	I	8.30	16.86	0
	II	8.35	19.60	0
	III	11.05	45.52	0
α-methyl p-nitrobenzylamine	III	11.05	21.20 - 22.80	1.60

Gradient elution : A : water; B : acetonitrile; **I** : 0-20min : 40 to 70%B, 20-25min : 70%B, 25-27min : 70 to 40%B, **II** : 0-30min : 40 to 80%B, 30-34min : 80%B, 34-36min :80 to 40%B; **III** : 0-45min : 35 to 50%B, 45-50min : 50%B, 50-54min : 50 to 35%B. TR_0, TR_1, TR_2 : elution times (min) of Marfey's reagent and derivatized amines; $\Delta TR_{1,2}$: Difference between the elution times.

In order to check this possibility, we first recorded the ^1H NMR spectrum of a reference mixture of enantiomers : a racemic mixture of (R) and (S) α-methyl benzylamines previously derivatized with Marfey's reagent.



No separation of any diastereomeric signal was observed in $CDCl_3$. On the other hand, DMSO gave rise to a more complex spectrum where some diastereomeric protons have non equivalent chemical shifts We have reported in Table 2 the chemical shift (δ ppm) and the diastereomeric shift separation (Δδ ppm) of each signal. None of these signals is suitable for accurate integration and therefore for the determination of an enantiomeric composition. In addition, broad signals are observed for CHα (b) and NH that prevent accurate integration in spite of their large Δδ. The methyl signals (CH_3 (a)) showing a strong chemical shift separation (0.55 ppm) should allow a good determination of the diastereomeric composition but unfortunately this measurement is made inaccurate by the presence of the methyl signal of Marfey's reagent at 1.52 ppm which is always employed in excess to achieve complete derivatization.

On the other hand, two single signals at 5.58 and 5.76 ppm are observed for the aromatic proton H_A in a zone free from other signals, allowing detection of the two diastereomers with precise integration. Using the H_A signals, we have verified that one per cent of one of the two diastereomers in 99/1 mixture (prepared by weighing) can be readily detected. It can be noted that the diastereomer chemical shifts are identical for the second aromatic proton H_B.

Table 2

(RS) α-methyl benzylamine	δ (ppm)	Δδ (ppm)
CH_3 (a)	0.89(d) -1.44(d)	0.55
CH_3 (b)	1.62(d)	0
CHα (a)	4.06(m) - 4.76(m)	0.70
CHα (b)	4.78(m) -4.92(m)	(0.14)
H_A	5.58(s) - 5.76(s)	0.18
$H_{aro} + NH_2$	7.3-8	
NH	8.6-8.8(2dd)	(0.2)
H_B	8.95	

δ : chemical shifts; Δδ : diastereomeric shift separations

To illustrate the general application of Marfey's reagent as an NMR derivatizing auxiliary, spectra of the racemic mixtures of five other derivatized benzylic amines have been recorded. We have gathered in Table 3 the chemical shifts of H_A and H_B signals as well as the diastereomeric shift separations of H_A signals ($\Delta\delta\ H_A$). In all cases, large values of $\Delta\delta\ H_A$ were obtained allowing rapid and accurate determination of the diastereomeric composition. As with α-methyl benzylamine, other resonance signals cannot be generally used for these measurements and in no case is separation of the H_B signals observed.

Table 3

(R,S) Amines	$\delta\ H_A$ (ppm)	$\Delta\delta\ H_A$(ppm)	$\delta\ H_B$(ppm)
α-methyl benzylamine	5.58 - 5.76	0.18	8.95
α-naphthyl ethylamine	5.52 - 5.77	0.25	8.97
1,2,3,4-tetrahydro-			
α-naphthyl ethylamine	5.94 - 6.08	0.14	9.00
1-methyl 3-phenylpropylamine	5.76 - 6.06	0.30	9.12
1-aminoindan	5.97 - 6.06	0.09	9.00
α-methyl p-nitrobenzylamine	5.48 - 5.59	0.11	8.92
1-methyl propylamine	5.80	0	9.00
1-cyclohexyl ethylamine	5.78	0	8.99
Marfey's reagent	6.96		8.96

$\delta\ H_A$ and $\delta\ H_B$: chemical shifts of H_A and H_B signals; $\Delta\delta\ H_A$: diastereomeric shift separations of H_A signals

It would also be interesting to check if Marfey's reagent could be successfully used to analyse aliphatic amines. Unfortunately, for the two compounds studied, no separation of diastereomeric signals was observed (Table 3). It seems that an aromatic substituent is required to obtain chemical shift non equivalence,

perhaps because of a π-π interaction. We therefore considered using an aromatic solvent such as benzene, but this was not possible owing to the low solubility of our derivatives.

In conclusion, the above data indicate that Marfey's reagent is an effective NMR chiral auxiliary that induces an adequate chemical shift non equivalence for the two diastereomers formed with benzylic amines thus allowing good integration in NMR spectra.

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