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CONTROL OF THE ENANTIOMERIC PURITY OF N-BOC N-METHYLAMINO ACID BUILDING BLOCKS BY A CONVENIENT NMR METHOD

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Abstract : The enantiomeric purity of N-Boc N-methylamino acid methyl esters could be easily and accurately established by measuring at 250 MHz in presence of Eu(hfc)3 the enantiomeric shift separation of N-Boc and/or N-methyl signals of one of the conformers.

N-Methylamino acids have attracted special interest as constituents of biologically active natural peptides[1]. Furthermore, replacing amino acids by N-methyl derivatives is commonly used to obtain information about peptide conformations[2].

Peptide synthesis requires enantiomerically pure building blocks. However, as many methods of N-methylamino acid synthesis are not totally enantioselective[3,4], determination of the optical purity is of particular importance. Some methods have been specifically applied to N-methylamino acids:

-The technique of MANNING and MOORE which has been discussed by BENOITON[4];

-separation by gas chromatography of the diastereoisomers formed by esterification with a chiral alcohol[6];

-gas chromatography analysis of N-methyloxazolidine-2,5-diones obtained by reaction with phosgene[7];

-capillary GLC analysis of ethyl ester derivatives[8];

-recording of NMR spectra of methyl esters[9], in some cases in the presence of a shift reagent[10,11] (we will see later on that this last

method cannot be used with total confidence).

N-Boc N-methylamino acids are particularly interesting substrates that can be directly used in peptide synthesis and are conveniently obtained by N-methylation of the parent N-Boc amino acid at room temperature[12]. It was therefore of interest to look for a method allowing determination of the enantiomeric purity with minimal derivatization of this substrate.

Recently we described a general and accurate method allowing determination of the enantiomeric purity of α-amino acids or α-amino acid derivatives

In this method, NMR spectra were run in the presence of a chiral lanthanide salt after derivatization of the substrate by N-acetylation. Under these conditions, the major coordination site with the chiral shift reagent (N-acetyl) corresponded to the NMR observation site; this afforded a very good signal separation and allowed detection of less than 1% of the minor enantiomer. We thought that in the case of N-Boc N-methylamino acids, the urethane function would be a strong coordination site. However, one difficulty could be the possible broadening of methyl signals following addition of the lanthanide salt, thus preventing any accurate measurement. This was indeed observed with non N-methylated N-Boc amino acid which could not be analyzed using this NMR method[13].

To apply the method to N-Boc N-methylamino acids, we had first to esterify the substrate with diazomethane in order to increase the solubility.

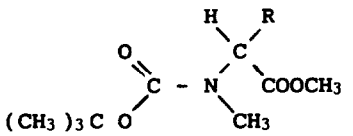
In order to select the most suitable experimental conditions and demonstrate the efficiency of the method, we recorded the NMR spectra of a reference product, (D,L) methyl N-Boc N-methyl leucinate, with successive modification of the solvent, the chiral reagent, the substrate concentration and the amount of lanthanide salt. Best results were obtained with a C_6D_6 solution of 50 moles of substrate in the presence of 0.5 equiv. $Eu(hfc)_3$.

The method was applied to four racemic N-Boc N-methylamino acid methyl esters, these being the Val, Ala, Leu and Phe derivatives.

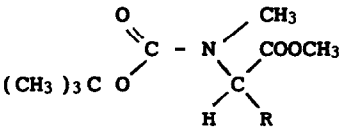
NMR spectra of N-methylated amino acid derivatives are more complex

than those of the parent non-methylated compounds, the latter showing signals of only the trans conformer[14].

In the absence of $Eu(hfc)_3$, we observed a splitting of the N-methyl signals consistent with the presence of conformers I and II (60% and 40% respectively) (there is no splitting of the N-Boc and OMe signals).



transoid form I



cisoid form II

In agreement with the literature[14], N-methyl signals of transoid conformers I are always upfield from those of cisoid conformers II. This is the result of a higher effect of the aromatic solvent on the N-methyl protons trans to the carboxymethyl group.

As soon as a small amount of the chiral shift reagent is added, a splitting of each conformer signal occurs[15,16]. With additional amounts of europium salt, signals corresponding to the formation of diastereoisomeric complexes of each conformer can be seen.

In Tables 1 and 2, the chemical shift variation ($\Delta\delta$) and the enantiomeric shift separation ($\Delta\Delta\delta$) are given for each conformer when 0.5 equiv. $Eu(hfc)_3$ is added to the solution.

		Val (D,L)	Ala (D,L)	Leu (D,L)	Phe (D,L)
O C-O-Bu^t	I	1,17	1,61	0,41	1,15
	II	4,51	3,09	2,81	4,04
$N-CH_3$	I	1,65	1,78	0,56	1,51
	II	5,06	3,80	3,03	4,96
OCH_3	I	0,65	0,63	0,22	0,34
	II	1,11	1,21	0,81	0,86

$\Delta\delta$ values in ppm of N-Boc, N-methyl and methoxy signals of racemic 0.1 molar N-Boc N-Me aminoacid methyl esters with 0.5 equiv. $Eu(hfc)_3$ (solvent C_6D_6).

- Table 1 -

We observed in all cases a much larger chemical shift variation ($\Delta\delta$) for each N-Boc or N-methyl signal than for the methoxy signal. That probably means that whatever the steric hindrance of the amino acid lateral chain, preferential complexation with the europium salt takes place with the urethane function.

		Val (D,L)	Ala (D,L)	Leu (D,L)	Phe (D,L)
O C-O-Bu^t	I	0,14	0,18	0,01	0,02
	II	0	0,14	0,17	0,23
N-CH_3	I	0,10	0	0,03	0,06
	II	0,05	0,28	0,28	0,12
O-CH_3	I	0,06	0,07	0,06	0
	II	0,06	0,03	0,05	0

$\Delta\Delta\delta$ values in ppm of N-Boc, N-methyl and methoxy signals of racemic 0.1 molar N-Boc N-Me aminoacid methyl esters with 0.5 equiv. Eu(hfc)$_3$ (solvent C_6D_6).

- Table 2 -

Furthermore, chemical shift variations ($\Delta\delta$) are larger for the cisoid than for the transoid form. This is likely to be the result of stronger complexation of the cisoid form which allows a sterically easier approach of the lanthanide salt to the urethane function.

Measurement of the enantiomeric shift separations ($\Delta\Delta\delta$) corresponding to N-Boc as well as to N-methyl signals allowed accurate determination of the optical purity. $\Delta\Delta\delta$ of the methoxy signals appeared to be less useful and indeed no separation occured with the phenylalanine derivative.

Moreover, we have checked that whatever the amounts of Eu(hfc)$_3$ added to the C_6D_6 solution, the conformer ratio and the ratio of diastereoisomeric complexes formed with each of the conformers were not modified. Therefore, it is sufficient to measure the relative signal intensities of only one conformer of each enantiomer. Conformers of each amino acid have distinct $\Delta\Delta\delta$ values. As can be seen in Table 2, $\Delta\Delta\delta$ values corresponding to the N-Boc signal are best measured with conformer I of Ala

and Val and with conformer II of Leu and Phe. Studies with a range of enantiomeric mixtures showed that it is possible with our methodology to detect as little as 1 % of the minor enantiomer.

In order to establish a comparison with our previous results , it was appealing to acetylate N-methylamino esters and to analyze them under the same standard conditions.

Chemical shift variations ($\Delta\delta$) and enantiomeric shift separations ($\Delta\Delta\delta$) obtained with four methylated or non-methylated N-acetylamino esters are given in Table 3.

		alanine (D,L)		valine (D,L)		leucine (D,L)		phenylalanine (D,L)	
		NH	NCH_3	NH	NCH_3	NH	NCH_3	NH	NCH_3
CH_3-CO-NH	$\Delta\delta$	3,51	1,76	3,24	1,72	3,36	1,63	3,05	1,04
	$\Delta\Delta\delta$	0,06	0,25	0,16	0,31	0,40	0,47	0,06	0,19
CO-OCH_3	$\Delta\delta$	0,54	0,30	0,50	0,21	0,54	0,25	0,37	0,12
	$\Delta\Delta\delta$	0,02	0,01	0,02	0,04	0,09	0,05	0,04	0
N-CH_3	$\Delta\delta$		0,90		1,05		1,05		0,66
	$\Delta\Delta\delta$		0,06		0,07		0,15		0,11

$\Delta\delta$ and $\Delta\Delta\delta$ values in ppm of N-acetyl, N-methyl and methoxy signals of racemic 0.1 molar N-Ac N-Me aminoacid methyl esters with 0.3 equiv. $Eu(hfc)_3$ (solvent C_6D_6).

- Table 3 -

In all cases, enantiomeric shift separations ($\Delta\Delta\delta$) corresponding to both N-methyl and N-acetyl signals allowed accurate measurement of the optical purity. Moreover, we observed with the N-methyl derivatives better separation for the N-acetyl signals whereas that for the methoxy signals was smaller.

In conclusion, the enantiomeric purity of N-Boc N-methylamino acids could be easily and accurately measured after derivatization to the corresponding methyl esters. It appeared quite sufficient to measure the enantiomeric shift separations of the N-Boc and/or N-methyl signals of one

single conformer of each amino acid.

EXPERIMENTAL:

NMR spectra were recorded with a "BRUKER" AC-250 spectrometer.

N-Boc (D,L) amino acids were prepared by standard procedures as described by MORODER et al (17).

N-Boc N-methyl (D,L) amino acids were synthesised by the method of CHEUNG and BENOITON (12). Absence of unreacted starting products was controlled by reverse phase HPLC (18)

N-Boc N-methylamino acid methyl esters were obtained in quantitative yields by addition of an ether solution of diazomethane to a methanolic solution of N-Boc N-methylamino acids.

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