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^{1}H NMR spectroscopy in the presence of Mosher acid to rapidly determine the enantiomeric composition of amino acid benzyl esters, chirons susceptible to easy racemization

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Abstract

Amino acid benzyl esters are very useful chiral synthons, whose enantiomeric purity needs to be carefully verified because of their susceptibility to easy racemization. Alternative to chiral HPLC, ^{1}H NMR in the presence of a chiral solvating agent (CSA) can allow a more rapid and acceptably accurate determination of the enantiomeric composition, if explicit spectral non-equivalence of one or more protons of the analyte enantiomers is found. Here, we have studied the enantiodiscrimination of thirteen amino acid benzyl esters by ^{1}H NMR in the presence of (*R*)-Mosher acid and in different solvents proving that, for five of them (Ala, Pro, Glu, Met, Ser), efficient enantiodifferentiation can be achieved and ≤ 98% enatiomeric excesses accurately determined. Generally, as expectable, the best enantiodifferentiated proton was that on the amino acid stereogenic α-carbon, but also the spectral non-equivalence of methyl protons and of protons on the β-carbon and on the benzylic carbon could be exploited to distinguish the two enantiomers and to quantify the minor one. Structural feature favoring the amino acid ester enantiodiscrimination by the CSA seems to be low sterical hindrance at the amino acid β-carbon.

Keywords

amino acid benzyl ester · ^{1}H NMR spectroscopy · Mosher’s acid · chiral solvating agent (CSA) · enantiodiscrimination

Introduction

The enantiomers of amino acid benzyl esters are very important and versatile chiral synthons as documented by widespread literature. An example for all is dibenzyl aspartate, a very useful C4 chiral building block (Jobbins and Miller 2014; Soicke et al. 2014; Kozikowski et al. 1996; Lynch et al. 1998; Meiresonne et al. 2012; Rudolph et al. 2001; Bergmeier et al. 1993; Bolchi et al. 2015). From the fifties, reported synthetic efforts are mainly based on the Fischer-Speier procedure (Zervas et al. 1957; Izumiya and Makisumi 1957). According to this simple and generally efficient method, the L or D amino acids are converted into benzyl esters *p*-toluenesulfonate salts by treatment with benzyl alcohol and slightly more than stoichiometric *p*-toluenesulfonic acid in a water-azeotroping solvent under reflux. Surprisingly, the search for water-azeotroping solvents alternative to banned benzene, carbon tetrachloride and chloroform is very poorly exemplified and the assessment of the enantiomeric excess of the resulting benzyl ester almost completely neglected despite the well-known high susceptibility of amino acids, even more if esterified, to racemization (Matsuo et al. 1967; Sato et al. 1970; Bada 1972; Smith and Evans 1980; Dhaon et al. 1982; Smith and Sivakua 1983). Proof is incautious replacement with high boiling toluene or benzyl alcohol leading, as we have demonstrated, to complete or partial racemization (Bolchi et al. 2017a). Recently, we have reported the Fischer-Speier efficient preparation of several amino acid benzyl esters with very high enantiomeric excess in cyclohexane or in the green ether Me-THF at reflux (Bolchi et al. 2017a; Bolchi et al. 2018). Differential scanning calorimetry investigations and development of chiral HPLC analytical methods specific for any amino acid benzyl ester allowed us to validate these new procedures and to discard unsuitable solvents such as toluene, but also as the high boiling ether CPME (Bolchi et al. 2017a; Bolchi et al. 2018; Bolchi et al. 2017b).

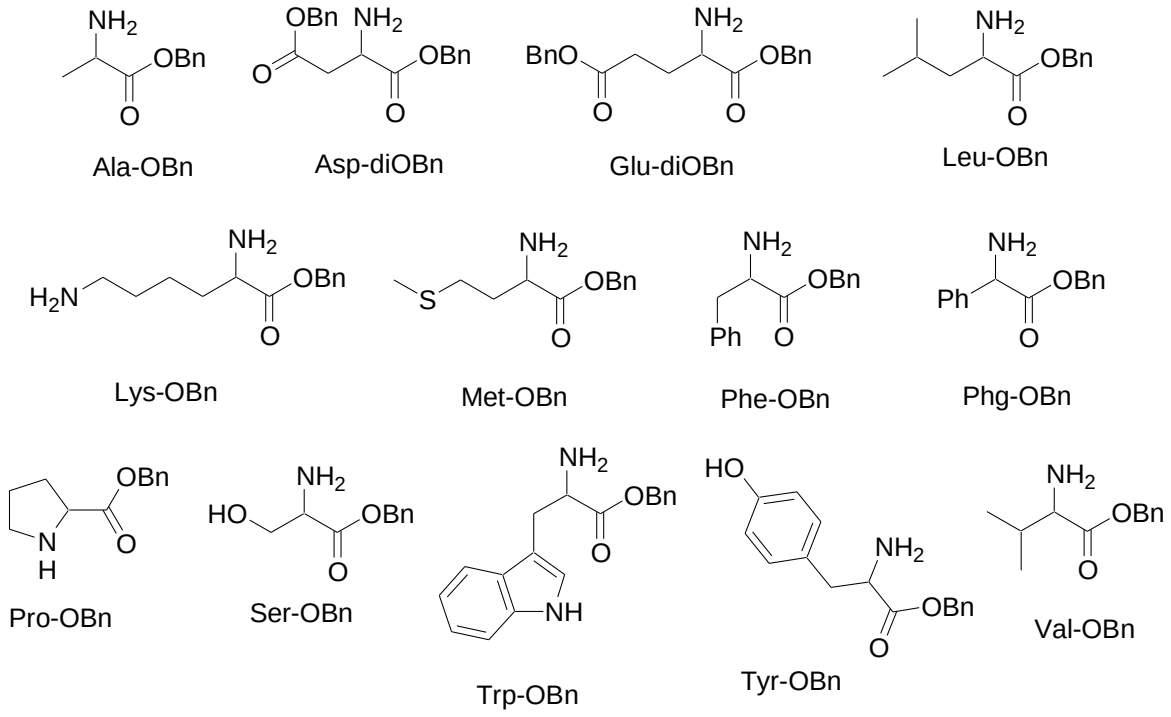


Chart 1. The thirteen amino acid benzyl esters, whose enantiodiscrimination by Mosher acid in ^{1}H NMR spectroscopy was investigated.

NMR spectroscopy associated to the use of chiral auxiliaries can be alternative to chiral HPLC and its attractiveness is much higher if enantiodifferentiation is achieved by simply adding chiral solvating agents (CSA), which form rapidly

reversible diastereomeric complexes with the two dissolved enantiomers of the analyte via noncovalent interactions and thus without need of any substrate derivatization (Wenzel and Chisholm 2011; Parker 1991; Seco et al. 2004; Seco et al. 2012; Perez-Trujillo et al. 2013). To our knowledge, such an approach has not been applied to amino acid benzyl esters before. The only example is the determination of the enantiomeric excess of L-arginine benzyl ester mono-*p*-toluenesulfonate we have recently reported together with its preparation by treatment with benzyl alcohol and *p*-toluenesulfonic acid in refluxing Me-THF (Bolchi et al. 2018). Difficulty in desalifying the guanidine moiety without benzyl ester hydrolysis suggested us salification of the alpha-amino group with (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (Mosher acid, MTPA) (Dale et al. 1969) and ^{1}H NMR analysis of the resulting benzyl ester tosylate (*R*)-Mosher carboxylate. Sufficiently great spectral non-equivalence of the proton linked to the stereogenic arginine carbon between the L*R* and the D*R* diastereomer allowed us to clearly detect the minor D*R* diastereomer with a lower limit of detection of 1%.

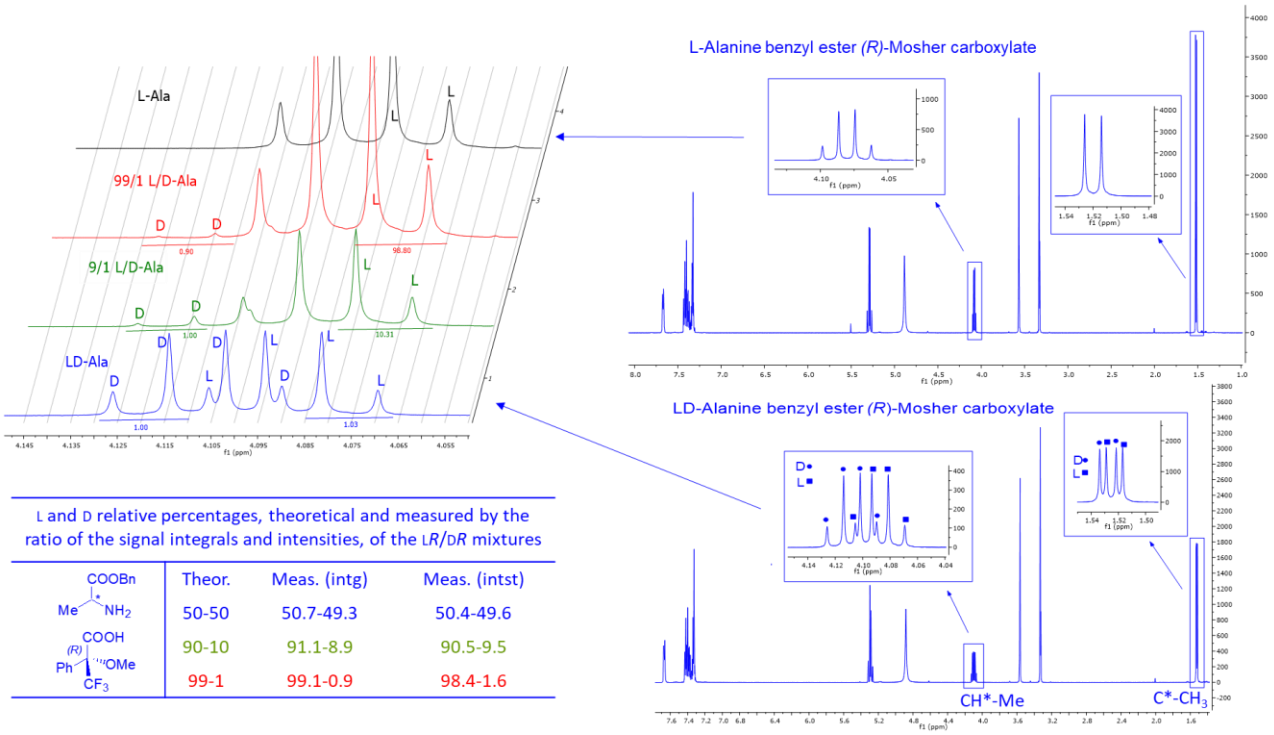
Based on this positive result and prompted by simplicity and easiness of the procedure, we extended it to the other thirteen amino acid benzyl esters we had previously synthesized and checked for enantiopurity by chiral HPLC (Chart 1). Here, we describe the ^{1}H NMR enantiodifferentiation of their salts with (*R*)-MTPA showing and briefly discussing the good potential of the method to rapidly determine the enantiomeric composition of some of these esters and thus to establish whether the esterification conditions cause racemization.

Results and discussion

First, we prepared the (*R*)-Mosher carboxylates of the thirteen racemic amino acid benzyl esters by mixing (*R*)-Mosher acid with equivalent racemic benzyl ester or, when unavailable the racemate, half equivalent amounts of both the benzyl ester enantiomers in dichloromethane and then concentrating to dryness. All the resultant (*R*)-Mosher carboxylates were solid except for that of DL-proline benzyl ester, which was an oil. DL-Lysine benzyl ester was salified with both one and two equivalents of (*R*)-Mosher acid. The ^{1}H NMR spectra of all the prepared 1/1 diastereomeric salts mixtures were preliminarily recorded at 300 MHz in concentrations ranging between 30 and 35 mM. Various solvents, such as chloroform, methanol, benzene and DMSO, were used which can profoundly influence, due to their different polarity, the strength of the interactions between ion pairs and consequently the relative value of diastereomeric complex formation constants K_{DR} and K_{LR}. As well known, difference in these association constants and in transient conformations are the main causes of spectral anisochrony Δδ, expressed as the difference in chemical shift of particular ^{1}H nuclei of the two diastereoisomers (Nemes et al. 2015). Such a preliminary reconnaissance was made to observe the best enantiorecognition phenomena, characterized by sufficiently large Δδ values and peaks baseline resolution so as to allow individual peak integration. When effective, enantiodiscrimination was expected to mainly involve the stereogenic methine (C*H) and, less explicitly, protons on β-carbon and on benzylic carbon. Furthermore, irrespective on their position, methyl groups signals were obviously on watch-list. Their high integration and low multiplicity could result in fairly enantiodifferentiated peaks despite modest Δδ values between the diastereoisomeric salts. The 300 MHz spectra, where we observed exploitable differentiation of the above signals, are given in Supplementary materials.

The stereogenic methine. We observed significant chemical shift differences between the C*H quartets of D and L-alanine benzyl ester (*R*)-Mosher carboxylates and between the C*H triplets of other eight diastereomeric (*R*)-mosherates, namely those of proline, serine, leucine, methionine, phenylalanine and tyrosine benzyl ester and of

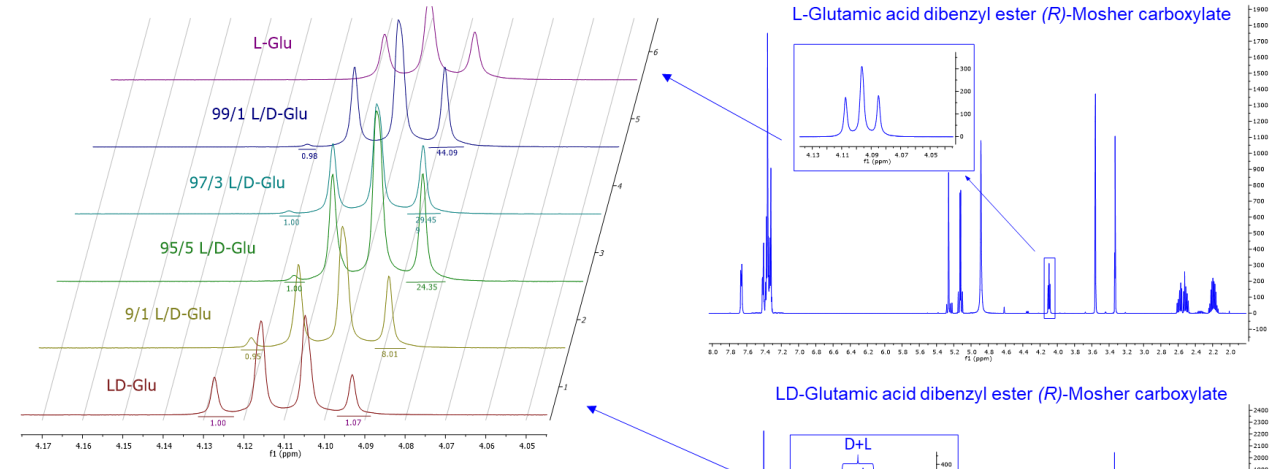
aspartic and glutamic acid dibenzyl ester (see the 300 MHz spectra in Supplementary materials). The best differentiation of these signals was achieved in CD_3OD and, in the only cases of proline and serine, in $CDCl_3$. Alanine, proline and glutamic acid benzyl esters showed the greatest enantiodiscrimination and the best chance of individual peak integration. For these three amino acid benzyl esters, we could not only reveal, at 300 MHz, the presence of both the enantiomers in racemic or near-racemic mixtures but also to detect and quantify, at 600 MHz, the minor enantiomer with good accuracy down to a 1% percentage relative to the major one (98% e.e.) in non-racemic mixtures, obtained by mixing *rac*- and L-amino acid benzyl ester (*R*)-mosherates. Figure 1 shows the expanded views of the C*H quartets and the relative integrations of three differently proportioned mixtures of D and L-alanine benzyl ester (*R*)-Mosher carboxylates. The signals of the C*H of the minor D*R* diastereoisomer are still detectable and integrated with very good accuracy when its relative percentage is lowered to 1%. In Figure 2, analogous phenomena and detectability are depicted for the C*H triplets of glutamic acid benzyl ester. They show $\Delta\delta = J$ and thus they result in four clearly distinct signals, of which the two outermost ones can be relatively integrated to quantify the enantiomers to each other with sufficient accuracy. Figure 3 shows the situation of proline benzyl ester, which has the best differentiation of the C*H pseudo-triplets/double doublets (0.17 ppm $\Delta\delta$). Despite such a good spectral non-equivalence, quantification of the minor enantiomer was accurately reliable above 5%. Below this percentage, the minor enantiomer was still easy detectable, but impurities deriving from the rapid degradation of proline benzyl ester interfered with its integration. The tables enclosed into the Figures 1-3 report also the relative L/D percentages measured on the basis of the relative heights of selected peaks of the two anisochronic signals: the values are consistent with those resulting from the relative integrations.



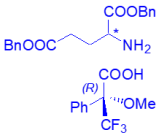
L and D relative percentages, theoretical and measured by the ratio of the signal integrals and intensities, of the L*R*/D*R* mixtures

COOBn, Me, NH2	Theor.	Meas. (intg)	Meas. (intst)
	50-50	50.7-49.3	50.4-49.6
COOH, (R), Ph, OMe, CF3	90-10	91.1-8.9	90.5-9.5
	99-1	99.1-0.9	98.4-1.6

Figure 1. On the right: full ^{1}H NMR spectra (600 MHz) of the (*R*)-Mosher carboxylates of *rac*- and L-alanine benzyl ester in CD_3OD. On the left: expanded view of the two quartets of the stereogenic methine for differently proportioned mixtures of the two diastereomeric salts (upper part) and, tabulated (lower part), the respective relative percentages ^{1}H NMR measured on the basis of the subscripted relative integrations and of the relative height of the second peak from the right compared to the seventh one.

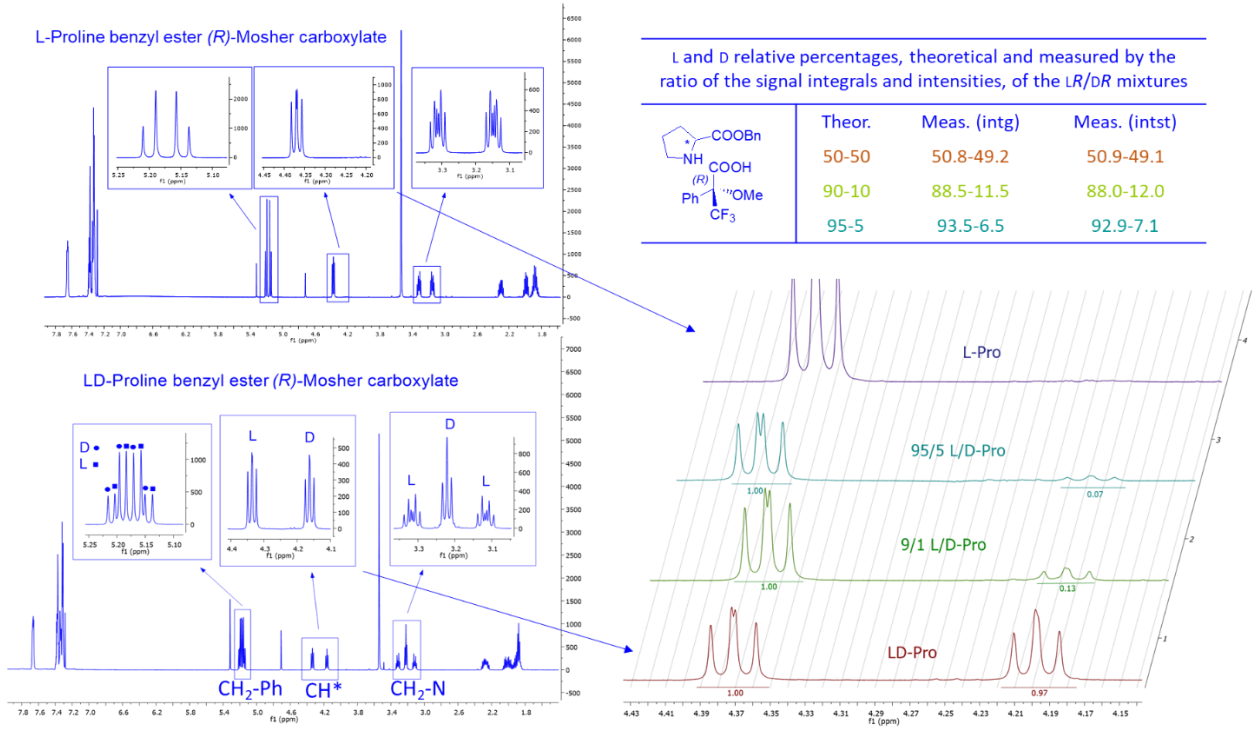


L and D relative percentages, theoretical and measured by the ratio of the signal integrals and intensities, of the LR/DR mixtures



Theor.	Meas. (intg)	Meas. (intst)
50 -50	51.7-48.3	51.7-48.3
90-10	89.4-10.6	87.8-11.2
95-5	96.0-4.0	95.1-4.9
97-3	96.7-3.3	95.9-4.1
99-1	97.8-2.2	97.1-2.9

Figure 2. On the right: full ^{1}H NMR spectra (600 MHz) of the (*R*)-Mosher carboxylates of *rac*- and L-glutamic acid dibenzyl ester in CD_3OD. On the left: expanded view of the two triplets of the stereogenic methine for differently proportioned mixtures of the two diastereomeric salts (upper part) and, tabulated (lower part), the respective relative percentages ^{1}H NMR measured on the basis of the subscripted relative integrations and of the relative height of the first peak from the right compared to the fourth one.

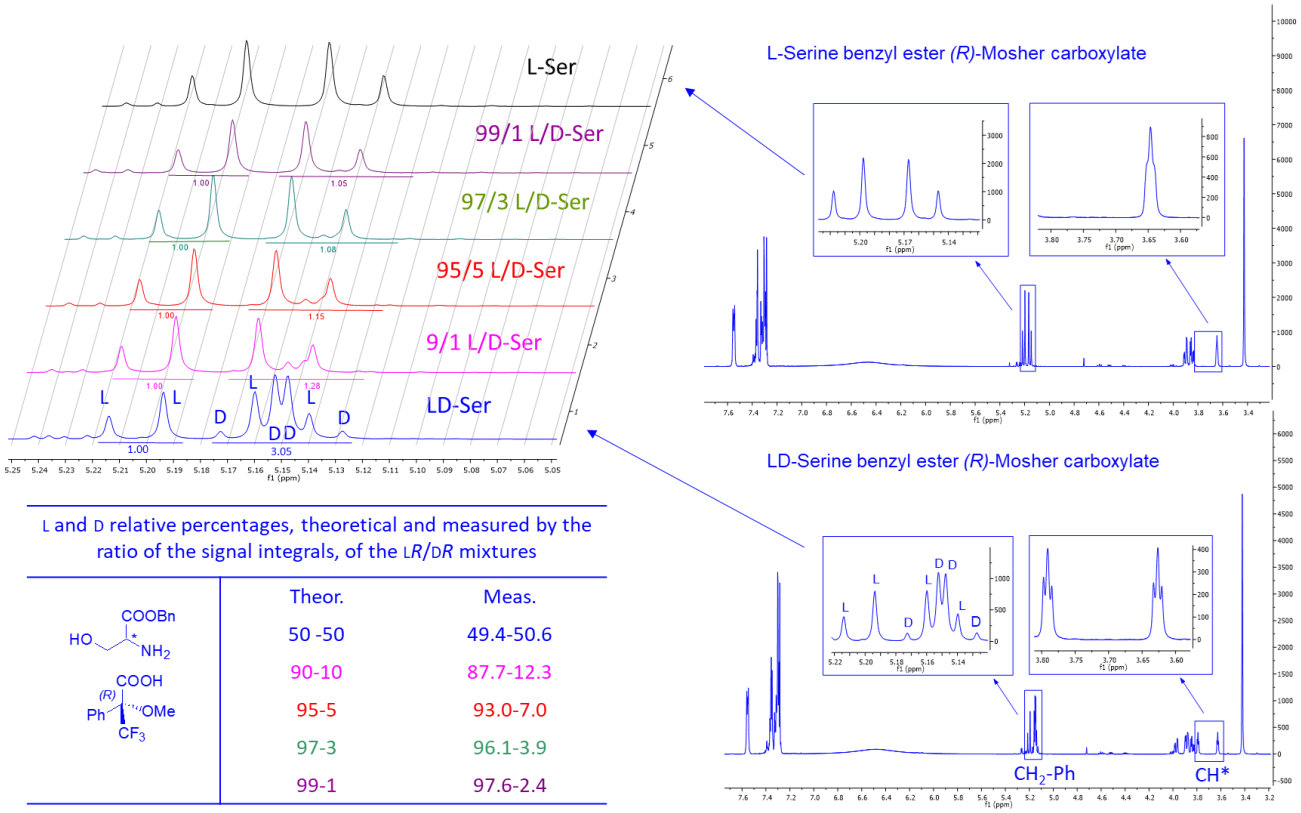


L and D relative percentages, theoretical and measured by the ratio of the signal integrals and intensities, of the LR/DR mixtures

Theor.	Meas. (intg)	Meas. (intst)
50-50	50.8-49.2	50.9-49.1
90-10	88.5-11.5	88.0-12.0
95-5	93.5-6.5	92.9-7.1

Figure 3. On the left: full ^{1}H NMR spectra (600 MHz) of the (*R*)-Mosher carboxylates of *rac*- and L-proline benzyl ester in $CDCl_3$. On the right: expanded view of the double doublets/pseudo-triplets of the stereogenic methine for differently proportioned mixtures of the two diastereomeric salts (lower part) and, tabulated (upper part), the respective relative percentages ^{1}H NMR measured on the basis of their relative heights and subscripted relative integrations.

The benzylic methylene. The benzylic CH_2 also offered some chances of enantiodiscrimination. As shown in Figures 1, 3 and 6, four doublets are recognizable in the equimolar mixtures of alanine, proline and methionine benzyl esters (*R*)-Mosher carboxylates. Analogous differentiation was found for leucine benzyl ester in $CDCl_3$ (see Supplementary materials). However, multiplicity and insufficient Δδ hamper relative quantification of the enantiomers on the basis of the signals of protons on benzylic carbon. Serine benzyl ester in $CDCl_3$ was a special case (Figure 4). Although larger differences in chemical shift were found between the two C*H signals and also between the two pairs of β-CH_2 double doublets, it was the anisochrony of the benzylic methylene protons that could be exploited to quantify the minority presence of the D enantiomer. In the reverse case, i.e. predominance of the D enantiomer, not contemplated in our experiments based on the availability of the analytes in L and racemic form, the completely isolated signal of C*H at 3.63 δ ppm should allow the accurate quantitative determination of the L enantiomer.



L and D relative percentages, theoretical and measured by the ratio of the signal integrals, of the L*R*/D*R* mixtures

Theor.	Meas.
50 -50	49.4-50.6
90-10	87.7-12.3
95-5	93.0-7.0
97-3	96.1-3.9
99-1	97.6-2.4

Figure 4. On the right: full ^{1}H NMR spectra (600 MHz) of the (*R*)-Mosher carboxylates of *rac*- and L-serine benzyl ester in $CDCl_3$. On the left: expanded view of the pairs of doublets of the benzylic CH_2 for differently proportioned mixtures of the two diastereomeric salts and, tabulated, the respective relative percentages ^{1}H NMR measured on the basis of the relative integrations of the benzylic CH_2 signals.

The β-methylene. The best enantiodifferentiation of the two protons on the amino acid β-carbon was observed in serine benzyl ester in $CDCl_3$, as above mentioned, and in aspartic acid dibenzyl ester in benzene (see Supplementary materials). In both cases, almost all the sixteen signals deriving from two anisochronic pairs of double doublets were

detectable. For serine, in particular, we observed an isolated double doublet (3.97 δ ppm) for one of the two methylenic protons of the D enantiomer (Figure 5). We could have used its integration relative to the isolated C*H (3.63 δ ppm) signal of the L enantiomer to determine the enantiomeric compositions as a support to the integration of the benzylic CH_2 signals outlined in Figure 4. Indeed, the lower part of Figure 5 shows the almost identical integrations of the D enantiomer double doublet at 3.97 ppm and of the L enantiomer C*H signal at 3.63 ppm in the spectrum of *rac*-serine benzyl ester (*R*)-mosherate, while the upper part the near 10% relative integration of the same double doublet in the spectrum of the 9/1 L/D mixture.

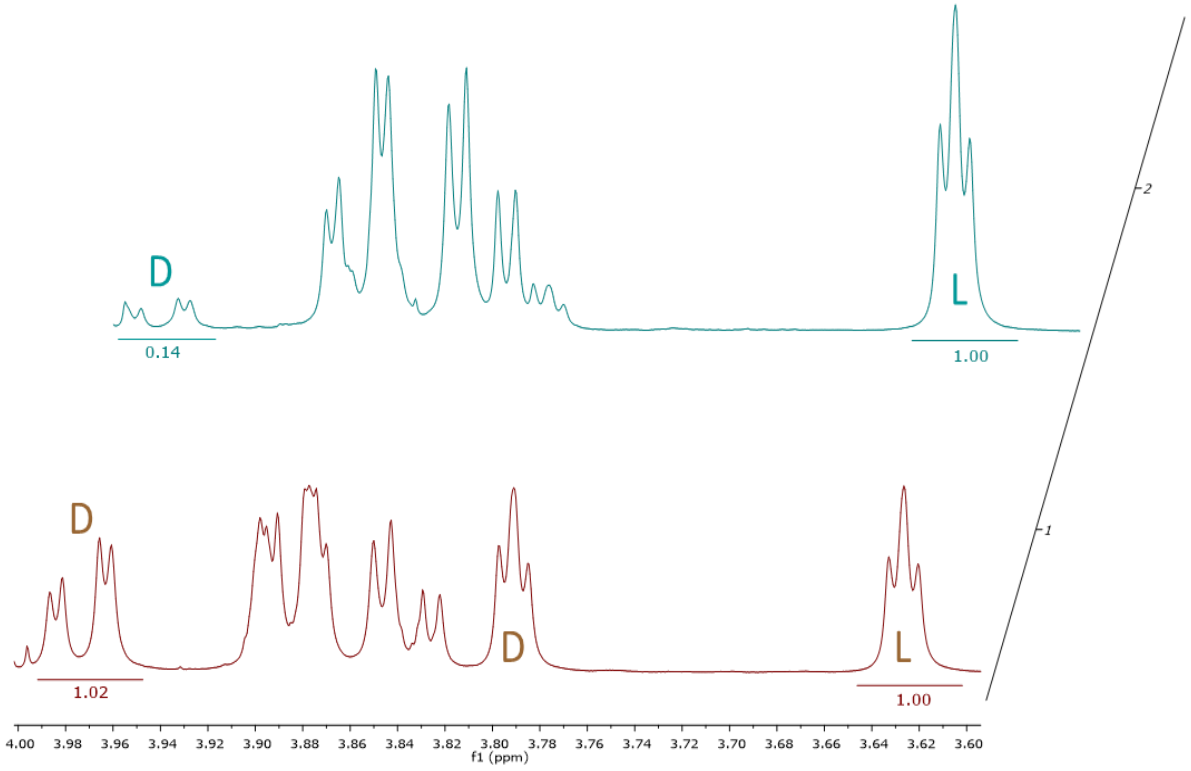


Figure 5. C*H and β-methylene signals in the ^{1}H NMR spectra (600 MHz, $CDCl_3$) of the (*R*)-Mosher carboxylates of *rac*-serine benzyl ester (lower trace) and of a 9/1 L/D serine benzyl ester mixture (upper trace). The relative integrations of the L enantiomer C*H and of one of the two methylenic protons of the D enantiomer are evidenced.

The methyl groups. Several differently positioned methyl groups were present in our analytes: attached to stereogenic methine (alanine), geminal on the β- or γ-carbon (valine and leucine) and bonded to terminal heteroatom (methionine). Because of its position, the most promising one was that of alanine. Indeed, two almost baseline resolved pairs of peaks were observable but unfortunately, as shown in Figure 1, the alternate belonging of the four peaks to the two enantiomers precluded individual integration and thus enantiomer quantification. In the case of valine benzyl ester (*R*)-Mosher carboxylate spectrum in DMSO, one methyl showed anisochrony (two distinct doublets), whereas the other methyl was not differentiated (one doublet) (see Supplementary materials). However, the peaks were far from being base-line resolved. Unexpectedly, it was the terminal methyl of methionine benzyl ester that gave, although remote from the stereogenic carbon, signals differentiated enough to allow detection and accurate quantification of the two enantiomers. As shown in Figure 6, despite the modest Δδ, the relative integration and the relative intensity measurement of the two methyl singlets in four different mixtures of the respective (*R*)-Mosher carboxylates were feasible and consistent with the theoretical values.

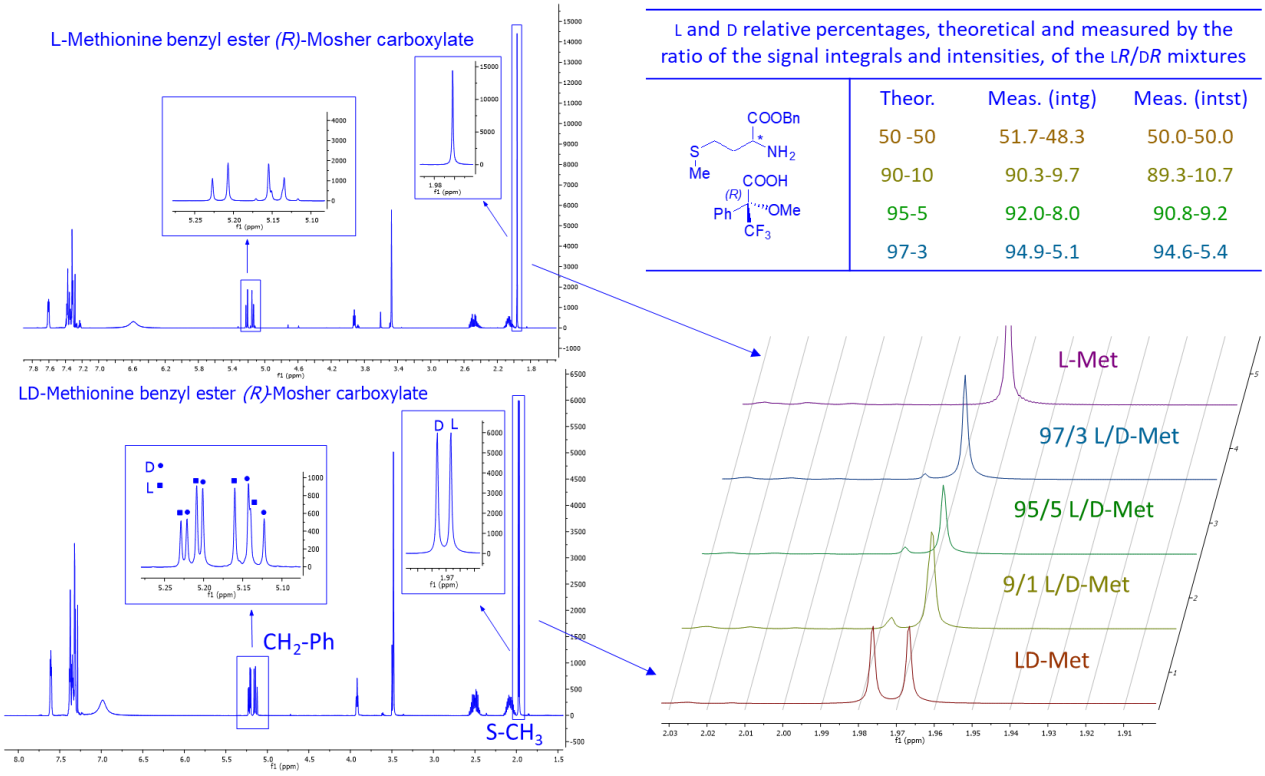


Figure 6. On the left: full ^{1}H NMR spectra (600 MHz) of the (*R*)-Mosher carboxylates of *rac*- and L-methionine benzyl ester in $CDCl_3$. On the right: expanded view of the singlets of the methylthio group for differently proportioned mixtures of the two diastereomeric salts (lower part) and, tabulated (upper part), the respective relative percentages ^{1}H NMR measured on the basis of the relative integrations and heights of the methylthio signals.

Conclusions

In summary, we studied the enantiodiscrimination of thirteen amino acid benzyl esters (Chart 1) by ^{1}H NMR spectroscopy in the presence of (*R*)-Mosher acid as a chiral salifying agent. At 300 MHz, except for tryptophan, phenylglycine, valine and lysine benzyl esters, the other nine amino acid benzyl esters showed, in the presence of Mosher acid, spectral non-equivalence of some protons large enough to clearly detect the presence of both the enantiomers in racemates and also of the minor enantiomer in non-racemic mixtures. Such protons were that of the stereogenic CH (alanine, leucine, proline, serine, phenylalanine, tyrosine, glutamic acid, aspartic acid and methionine), those linked to the benzylic carbon (alanine, proline, methionine and, above all, serine), those on the β-carbon (aspartic acid and serine) and those of methyl (methionine and alanine). Furthermore, the relative determination of the minor enantiomer in non-racemic samples was feasible at 600 MHz for five of the above nine benzyl esters, namely those of alanine, proline and glutamic acid exploiting the enantiodiscrimination of methine proton and those of methionine and serine on the basis of the differentiation of methyl and benzylic methylene protons respectively. As shown in the figures, one per cent relative amount of the minor enantiomer, that is 98% enantiomeric excess, could be easily determined and, for these five substrates, ^{1}H NMR spectroscopy with CSA can be considered alternative to chiral HPLC.

The results of this investigation can be tentatively rationalized considering what the structures of the here best resolved amino acid benzyl esters have in common. We think that the structural feature mainly favoring discrimination between the two enantiomers of these analytes by the Mosher acid is that their stereogenic CH, which is in any case benzyloxycarbonyl and amino substituted, is not overcrowded by a fourth bulky substituent depressing the enantioselectivity of the CSA approach. It is not by chance that substituents such as CH_3 (alanine) and relatively not hindered CH_2 (arginine, methionine, proline, serine, glutamic acid and aspartic acid) coincide with good spectroscopic resolvability of the enantiomers, whereas substituents such as tertiary carbon (valine), phenyl (phenylglycine) or hindered CH_2 (tryptophan, phenylalanine, tyrosine) with lower or null enantiodiscrimination.

Materials and methods

^{1}H NMR spectra were recorded on a Bruker Avance spectrometer at 600 MHz. Chemical shifts are reported in ppm relative to residual solvent as internal standard. 300 MHz ^{1}H NMR spectra, recorded on a on a Varian Gemini 300, can be found in Supplementary materials.

***rac*-Alanine benzyl ester (*R*)-Mosher carboxylate:** ^{1}H NMR (600 MHz, MeOD) δ 7.74 – 7.60 (m, 4H), 7.49 – 7.26 (m, 16 H), 5.31(d, *J* = 12.3, 2H), 5.27(d, *J* = 12.3, 2H), 4.11 (q, J = 7.2 Hz, 1H), 4.08 (q, J = 7.2 Hz, 1H), 3.57 (m, 6H), 1.53 (d, J = 7.2, 3H), 1.52 (d, J = 7.2, 3H).

L-Alanine benzyl ester (*R*)-Mosher carboxylate: ^{1}H NMR (600 MHz, MeOD) δ 7.74 – 7.61 (m, 2H), 7.50 – 7.26 (m, 8H), 5.31(d, *J* = 12.3, 1H), 5.27(d, *J* = 12.3, 1H), 4.08 (q, *J* = 7.2 Hz, 1H), 3.56 (m, 3H), 1.52 (d, *J* = 7.2 Hz, 3H).

***rac*-Glutamic acid dibenzyl ester (*R*)-Mosher carboxylate:** ^{1}H NMR (600 MHz, MeOD) δ 7.75 – 7.57 (m, 4H), 7.51 – 7.22 (m, 26H), 5.27 (m, 4H), 5.14 (d, *J* = 12.3 Hz, 2H), 5.10 (d, *J* = 12.3 Hz, 2H), 4.12 (t, *J* = 7.0 Hz, 1H), 4.10 (t, *J* = 7.0 Hz, 1H), 3.56 (m, 6H), 2.67 – 2.43 (m, 4H), 2.27 – 2.11 (m, 4H).

L-Glutamic acid dibenzyl ester (*R*)-Mosher carboxylate: ^{1}H NMR (600 MHz, MeOD) δ 7.7 – 7.60 (m, 2H), 7.52 – 7.22 (m, 13H), 5.27 (d, *J* = 12.1 Hz, 1H), 5.25 (d, *J* = 12.1 Hz, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 4.10 (t, *J* = 7.0 Hz, 1H), 3.56 (m, 3H), 2.67 – 2.42 (m, 2H), 2.29 – 2.12 (m, 2H).

***rac*-Proline benzyl ester (*R*)-Mosher carboxylate:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (m, 4H), 7.47 – 7.23 (m, 16H), 5.21 (d, *J* = 12.1 Hz, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.16 (d, *J* = 12.1 Hz, 1H), 5.15 (d, *J* = 12.1 Hz, 1H), 4.34 (dd, *J* = 8.4, 7.2 Hz, 1H), 4.16 (pt, *J* = 8.2, 7.4, 1H), 3.54 (s, 6H), 3.39 – 3.30 (m, 1H), 3.27 – 3.19 (m, 2H), 3.21 – 3.08 (m, 1H), 2.40 – 2.22 (m, 2H), 2.09 – 1.79 (m, 6H).

L-Proline benzyl ester (*R*)-Mosher carboxylate: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (m, 2H), 7.47 – 7.31 (m, 8H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.15 (d, *J* = 12.1 Hz, 1H), 4.37 (dd, *J* = 8.4, 7.2 Hz, 1H), 3.53 (s, 3H), 3.39 – 3.30 (m, 1H), 3.21 – 3.08 (m, 1H), 2.38 – 2.19 (m, 1H), 2.08 – 1.94 (m, 1H), 1.94 – 1.79 (m, 2H).

***rac*-Serine benzyl ester (*R*)-Mosher carboxylate:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (m, 4H), 7.44 – 7.23 (m, 16H), 6.48 (bs, 8H), 5.20 (d, *J* = 12.1 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 5.15 (d, *J* = 12.1 Hz, 1H), 5.14 (d, *J* = 12.2 Hz, 1H), 3.97 (dd, *J* = 12.5, 3.1 Hz, 1H), 3.88 (m, 2H), 3.84 (dd, *J* = 12.5, 4.3 Hz, 1H), 3.79 (m, 1H), 3.63 (m, 1H), 3.42 (s, 6H).

L-Serine benzyl ester (*R*)-Mosher carboxylate: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (m, 2H), 7.43 – 7.21 (m, 8H), 6.46 (s, 4H), 5.20 (d, *J* = 12.1 Hz, 1H), 5.15 (d, *J* = 12.1, 1H), 3.90 (dd, *J* = 12.5, 3.2 Hz, 1H), 3.84 (dd, *J* = 12.5, 4.3, 1H), 3.63 (m, 1H), 3.42 (s, 3H).

***rac*-Methionine benzyl ester (*R*)-Mosher carboxylate:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (m, 4H), 7.43 – 7.29 (m, 16H), 7.05 (bs, 6H), 5.22 (d, *J* = 12.1, 1H), 5.21 (d, *J* = 12.1, 1H), 5.15 (d, *J* = 12.1, 1H), 5.13 (d, *J* = 12.1, 1H), 3.92 (m, 2H), 3.48 (s, 6H), 2.64 – 2.38 (m, 4H), 2.20 – 2.01 (m, 4H), 1.98 (s, 3H), 1.97 (s, 3H).

L-Methionine benzyl ester (*R*)-Mosher carboxylate: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (m, 2H), 7.43 – 7.28 (m, 8H), 6.58 (s, 3H), 5.22 (d, *J* = 12.1 Hz, 1H), 5.15 (d, *J* = 12.1 Hz, 1H), 3.92 (pt, *J* = 5.7, 6.7 Hz , 1H), 3.47 (s, 3H), 2.59 – 2.35 (m, 2H), 2.17 – 2.01 (m, 2H), 1.97 (s, 3H).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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