Amino Acids
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ORIGINAL ARTICLE

Preparation of enantiopure methionine, arginine, tryptophan, and proline benzyl esters in green ethers by Fischer–Speier reaction

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Abstract

The simplest way to prepare the tosylate salts of amino acid benzyl esters, whose enantiomers are very important synthetic intermediates, is treatment of amino acid with benzyl alcohol and *p*-toluenesulfonic acid in a refluxing water-azeotroping solvent (Fischer–Speier esterification). However, to this day, the literature proposes only hazardous solvents, such as benzene, carbon tetrachloride, and chloroform, which must be absolutely avoided, or solvents, such as toluene and benzyl alcohol, which cause racemization because of too high boiling water azeotropes. On the other hand, the alternative successful use of cyclohexane, which we have recently reported for several amino acid benzyl esters, is inapplicable or not very efficient for 'problematic' amino acid such as tryptophan, arginine, and methionine, for which, indeed, the simple Fischer–Speier esterification is not described or poorly exemplified in the literature. Therefore, more polar solvents, in particular the green ethers CPME, TAME, and Me-THF, were selected and first considered for the preparation of methionine benzyl ester, previously accomplished in cyclohexane with modest yield. After discarding CPME and TAME, because causing racemization and decomposing under acidic conditions, respectively, we focused on Me-THF. In this ether, the benzyl esters of Met, Arg, and Trp could be obtained in good yield and, as proved by chiral HPLC or H NMR analysis, enantiomerically pure. The procedure was successfully extended to proline benzyl ester, which could be prepared enantiomerically pure and in quantitative yield both in cyclohexane and in Me-THF, thus avoiding the recently reported use of carbon tetrachloride.

Keywords Amino acid benzyl ester · Water azeotrope · Racemization · Chiral HPLC · Mosher's acid · TAME · CPME · Me-THF

Introduction

Amino acid esters are very important synthetic intermediates (Bolchi et al. 2007; Liu et al. 2007; Verdié et al. 2008; Bolchi et al. 2009; Cerić et al. 2010; Liu et al. 2010; Pallavicini et al. 2010; Tekkam et al. 2013; Straniero et al. 2014; Bolchi et al. 2015b; Mao et al. 2016). However, despite widespread and long documented use, the attainment of the large majority of them as enantiomers of certified purity and through sustainable methods remains an open challenge, too often neglected by both synthetic and analytical chemists. Recently, we have reported the conversion of 11 L or D amino acids (Ala, Phe, Phg, Tyr, Val, Leu, Met, Ser, Asp, Glu, and Lys) into enantiopure benzyl esters *p*-toluenesulfonate salts by treatment with benzyl alcohol and slightly more than stoichiometric *p*-toluenesulfonic acid under reflux in a water-azeotroping solvent according to the Fischer–Speier procedure (Bolchi et al. 2015a, 2017a) (Chart 1).

For the first time, we have successfully used cyclohexane in such amino acid esterifications instead of banned benzene, carbon tetrachloride, and chloroform or of toluene, which unexpectedly causes complete or partial racemization of all the prepared benzyl esters, with the exception of that of valine, because of its higher boiling point. We have also demonstrated that even moderate racemizations, resulting in relatively high enantiomeric excesses (> 75%), can be deleterious for the many amino acid benzyl esters tosylates

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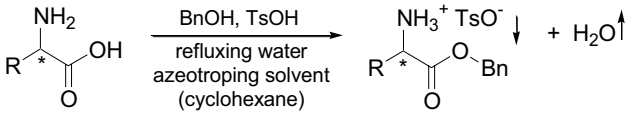

Chart 1 General method to prepare amino acids benzyl esters and to isolate them as *p*-toluenesulfonic acid salts

forming racemic compounds. In fact, the enantiomeric excesses corresponding to the eutectics in the respective binary melting point and ternary solubility phase diagrams are often higher than the enantiomeric excesses of the these partially racemized benzyl esters, and thus, their successive enantioenrichment by crystallization/trituration processes is precluded (Bolchi et al. 2017b).

In a continuation of our effort to prepare enantiopure amino acid benzyl esters under acceptable conditions, we have extended our investigation over further amino acids. Again, our intent was to develop efficient Fischer–Speier esterification procedures avoiding racemization and using safer solvents. We focused on L-proline, L-tryptophan, L-arginine, and L-methionine benzyl esters salts **1**–**4** (Chart 2). The direct conversion of these four amino acids into benzyl esters salts by simple treatment with benzyl alcohol was a problematic challenge in itself, as stated already 60 years ago in a systematic study on the synthesis of amino acid benzyl ester *p*-toluenesulfonates by Izumiya and Makisumi (1957). Unlike for the previously studied amino acids, the literature provides not only a very poor and inadequate analytical characterization for both chemical and enantiomeric purity of these benzyl esters, but also very few and often inadvisable or little reliable procedures. Therefore, we first verified whether Fischer–Speier esterification is applicable to these amino acids by screening acceptable water-azeotroping solvents and then whether enantiomeric purity is preserved under the selected esterification conditions. Here, we report the results of our double-focused research.

Results and discussion

For the four amino acids, results of literature survey are briefly detailed hereafter.

- Proline, a unique amino acid for its rigid architecture, and proline derivatives, such as the benzyl ester, have been extensively used as chiral synthons and auxiliaries and widely investigated for the attractive conformational properties of diproline, oligoprolines, polyprolines, and proline-rich peptides (Dai and Etzkorn 2009; Martin et al. 2013; Bräuer et al. 2016; Zanna et al. 2016; Liu and Wang 2017). However, although enantiomeric purity is an indispensable requisite for such uses and studies, we found no chiral HPLC determinations of the enantiomeric excess, for instance, of starting L- or D-proline benzyl ester. On the other hand, the only reported Fischer–Speier esterification with benzyl alcohol was recently accomplished in carbon tetrachloride (Dai and Etzkorn 2009).
- Tryptophan is the most degradable of the proteinogenic amino acids and literature on its direct conversion into benzyl ester is very elusive. Only one and two dated procedures are reported to obtain the benzyl ester *p*-toluenesulfonate and hydrochloride, respectively, under conditions unsuitable for scaling-up (Wilchek and Patchornik 1962; Otsuka and Inouye 1964; Arai and Muramatsu 1983). Analytical characterization is limited to melting point and elemental analysis and, only for the hydrochloride, also to the optical rotatory power. No information is available on the enantiomeric purity. Few more recent reports refer to old preparations or propose very small-scale procedures which are not ameliorative and do not provide new or updated analytical data (Magnus et al. 1989; Biondini et al. 2010). Suffice it to say that the first and unique NMR spectra of the unsalified benzyl ester date to 2015 (Taglang et al. 2015). Poor and costly commercial availability confirms that tryptophan benzyl ester, free, or salified is a tricky compound to work around.
- Arginine is another amino acid *sui generis*, because of the highly basic distal guanidine residue, and the mono- and di-*p*-toluenesulfonate of its benzyl ester are not routine derivatives. We found only one example of Fischer–Speier esterification, accomplished in 1/1 benzyl alcohol/benzene, without NMR characterization and analytical data on enantiomeric purity (Dorman and Cheng 1976).
- Methionine had been already investigated by us. As previously said, we have recently reported its conversion to

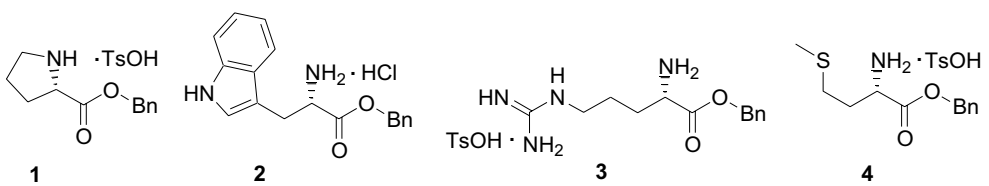

Chart 2 L-Amino acid benzyl esters salts prepared by Fischer–Speier esterification with benzyl alcohol

enantiopure benzyl ester by treatment with benzyl alcohol in refluxing cyclohexane. Our procedure avoided the use of benzene and the almost complete racemization observed in toluene. However, because of degradation of the substrate, its yield was only 40%, lower than that reported in benzene (56%) (Kawasaki et al. 1980) and far from that claimed in toluene (75%) (Fayad et al. 2015).

L-Methionine benzyl ester *p*-toluenesulfonate (4)

Within this context, we started our investigation just from methionine to improve the yield of its transformation into benzyl ester *p*-toluenesulfonate by the Fischer–Speier method while maintaining product enantiopurity high. We reasoned that, in the case of the easily degradable methionine, refluxing reaction in an apolar solvent such as cyclohexane, in which the amino acid is highly insoluble, could advantage substrate degradation over its conversion into ester, thus lessening yield of the desired ester product. Therefore, we searched for alternative solvents forming heterogeneous water azeotropes, similar to benzene and cyclohexane for relatively low boiling point, water-rich azeotropic composition, and modest reciprocal solubility with water, but more polar than hydrocarbons. Ethers were the natural candidates to satisfy such requirements. The most recent solvent selection guides and green chemistry literature, which classify solvents on the basis of EHS (environmental, health, and safety) properties, unanimously rank the classical ethers as 'hazardous' or 'highly hazardous', but provide discordant indications on the new ethers (Prat et al. 2013, 2016; Byrne et al. 2016). These, developed to circumvent the issues of the previous ones, are ranked as 'recommended' or 'problematic' depending on the weight given to each of the selected solvent properties (flash point, autoignition temperature, volatility, explosive limits, freezing point, solubility in water, persistence in the environment, peroxide formation, stability to acids and bases, synthetic accessibility, derivation from renewable resources, toxicity, etc.). Nevertheless, rankings based on the most stringent criteria converge to identify TAME (*tert*-amyl-methyl ether), CPME (cyclopentyl-methyl ether), and Me-THF (2-methyl-tetrahydrofuran) as acceptable green alternatives to hazardous traditional ethers, such as diisopropyl ether, methyl-*tert*-butyl ether, and THF, and also to banned or hazardous halogenated hydrocarbons. Therefore, we considered the replacement of cyclohexane with these ethers. First, we tested CPME, which is particularly recommended for its high flash point, high stability to acids, low peroxide formation, low miscibility with water, and low toxicity (Azzena et al. 2015; Byrne et al. 2016). However, its relatively high boiling point (106 °C) raised concerns of possible racemization of the benzyl ester, as we had previously observed esterifying other amino acids in boiling toluene (Bolchi et al. 2015a, 2017a). Therefore, we first prepared L-phenylalanine benzyl ester in refluxing CPME according to the reported procedures in cyclohexane and in toluene. Chiral HPLC analysis showed that the ester product, obtained in high yield, was extensively racemized. We thus abandoned CPME and considered 20 degrees lower boiling TAME, whose water azeotrope boils at 74 °C, a few degrees higher than the water azeotropes of cyclohexane and benzene. TAME is ranked as a 'recommended' solvent by CHEM21 selection guide (Prat et al. 2016). Unfortunately, attempts at preparing amino acid benzyl esters by treatment with benzyl alcohol and *p*-toluenesulfonic acid in this water-azeotroping solvent were unsuccessful. The most reliable cause was the instability of tertiary ethers to acids: boiling TAME in the presence of *p*-toluenesulfonic acid over-night resulted in at least 40% degradation of the ether to 2-methyl-2-butene, 2-methyl-1-butene, and methanol. On paper, the third option, Me-THF, a green alternative to THF for better health and environmental score (Byrne et al. 2016; Aul and Comanita 2007; Aycock 2007), looked very well: low boiling point (71 °C) and stability to acids so as to avoid product racemization and solvent degradation, respectively. Indeed, the treatment of L-methionine with a large excess of benzyl alcohol and a slight excess of *p*-toluenesulfonic acid in boiling Me-THF over-night and the subsequent work-up according to the previously reported procedure in cyclohexane afforded L-methionine benzyl ester *p*-toluenesulfonate with 95.7% e.e. and in 62% yield, one and a half relative to the yield obtained in cyclohexane. In Me-THF, maximum yield coincided with 10:1 benzyl alcohol/methionine molar ratio. Lower ratios led to some percentage points lower yields, while higher ratios were avoided, because the consequent boiling point enhancement of the reaction mixture could cause degradation and racemization.

L-Arginine benzyl ester mono-*p*-toluenesulfonate (3)

Prompted by this improvement, we decided to address arginine and tryptophan, two 'difficult' amino acids, which we had previously tried to transform into benzyl ester in cyclohexane or in toluene, but to no avail. To our knowledge, the only detailed and reliable example of preparation of L-arginine benzyl ester can be found in a patent (Dorman and Cheng 1976), where the ester is isolated in 85% yield as di-*p*-toluenesulfonate hydrate and characterized for melting point, optical activity, and elemental analysis. Unfortunately, the reaction is accomplished in benzene. We developed a Fischer–Speier procedure in boiling Me-THF and worked up the reaction to isolate the mono-*p*-toluenesulfonate, which was found to have a higher melting point than di-*p*-toluenesulfonate (99 vs 67 °C) and to be not hygroscopic. We maintained the 10:1 benzyl alcohol/arginine ratio, identical to that adopted for methionine and similar to that reported

in the above patent (11:1). At the end of the reaction, after removing the upper Me-THF phase, the downer phase was washed with ethyl acetate, dried under vacuum, and poured into DCM/aqueous Na_2CO_3 to remove *p*-toluenesulfonic acid. Concentration of the DCM phase afforded pure arginine benzyl ester salified only at the more basic guanidine residue with *p*-toluenesulfonic acid as a white solid in 58% yield. A similar yield was also obtained using 5:1 benzyl alcohol/arginine molar ratio. Attempts at removing both *p*-toluenesulfonic acid molecules to recover free arginine benzyl ester were unsuccessful: treatment with an organic solvent and water containing stronger bases than carbonate caused ester hydrolysis and no arginine benzyl ester could be recovered from the organic phase. This behavior seriously hampered our ability to determine the enantiomeric excess of the arginine benzyl ester by chiral HPLC according to the analytical procedures applied to the other amino acid benzyl esters previously synthesized. Difficulty in desalifying the guanidine moiety without ester hydrolysis suggested that we desist and rather salify also the liberated alpha-amino group, but with the enantiomer of a chiral acid so as to diastereomerize the two arginine benzyl ester enantiomers. Salification with α-methoxy-α-trifluoromethylphenylacetic acid (Mosher acid) offered the promising chance of determining the enantiomeric excess of arginine benzyl ester mono-*p*-toluenesulfonate by H NMR. Therefore, we salified racemic arginine benzyl ester mono-*p*-toluenesulfonate with the *R* enantiomer of Mosher's acid. The resulting 1:1 diastereomeric mixture of L-arginine benzyl ester tosylate (*R*)-Mosher carboxylate and D-arginine benzyl ester tosylate (*R*)-Mosher carboxylate was analyzed by H NMR in CD_3OD at 60 mM concentration to check whether salification with a single Mosher acid enantiomer induced anisochrony (Δδ) between the signals of the two arginine ester enantiomers. Spectral non-equivalence (0.07 ppm Δδ) was detected for the proton linked to the stereogenic arginine carbon: the chemical shift of its triplet ($J = 6.4$ Hz) was 4.00 ppm in the L*R* stereoisomer and 4.07 ppm in the D*R* stereoisomer (Fig. 1, spectrum E). The Δδ and *J* values were such as to avoid any overlapping between the two triplets. NMR spectra at 600 MHz of prepared non-equimolar L*R*/D*R* mixtures containing decreasing percentages of D*R* diastereomer showed that the presence of the CH downfield triplet of the minor diastereomer was clearly detectable when higher or equal to 1%. Based on these results and on the inspection of the H NMR spectrum of the (*R*)-Mosher carboxylate of the L-arginine benzyl ester mono-*p*-toluenesulfonate prepared in Me-THF, we concluded that conversion of L-arginine into benzyl ester in refluxing Me-THF occurs without racemization and providing the ester with > 98% e.e. (Fig. 1, spectra, B, C and D).

L-Tryptophan benzyl ester hydrochloride (2)

The successive challenge was the preparation of tryptophan benzyl ester, which has never been obtained by the

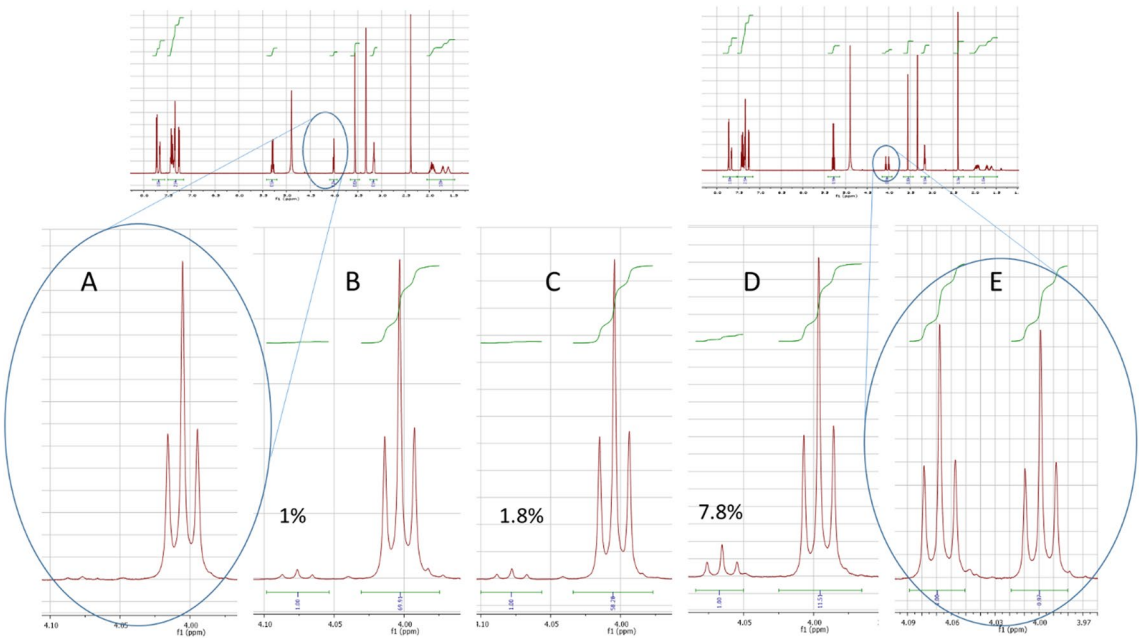

Fig. 1 H NMR signal of the proton linked to the stereogenic carbon of L-arginine benzyl ester *p*-toluenesulfonate (*R*)-Mosher carboxylate (**A**), of its mixtures with increasing amounts of D-arginine benzyl ester *p*-toluenesulfonate (*R*)-Mosher carboxylate (**B**–**D**) and of *rac*-arginine benzyl ester *p*-toluenesulfonate (*R*)-Mosher carboxylate (**E**)

Fischer–Speier procedure. Considering the instability of tryptophan at acidity and high temperature, some researchers have proposed a procedure based on the conversion into *N*-carboxy-α-amino acid anhydride by treatment with phosgene in dioxane and subsequent reaction with benzyl alcohol and diethyl ether saturated with hydrogen chloride at 0 °C (Wilchek and Patchornik 1962) or, alternatively, the transesterification with equimolar benzyl *p*-toluenesulfonate, formed from TsCl and benzyl alcohol in situ, in benzyl alcohol at 80 °C and in the presence of equimolar *p*-toluenesulfonic acid (Arai and Muramatsu 1983). In both cases, the benzyl ester was isolated as hydrochloride. More recently, a very small-scale preparation has been reported, which utilizes benzyl chloride in ionic liquids affording the product in a modest 34% yield (Biondini et al. 2010). Our attempts at esterifying L-tryptophan with benzyl alcohol in the presence of *p*-toluenesulfonic acid by azeotropical removal of water were completely unsuccessful in both boiling toluene and cyclohexane, whereas they succeeded when we used Me-THF and 10:1 or 5:1 benzyl alcohol/amino acid molar ratio, as in the case of arginine. After refluxing for 24 h and removing Me-THF under vacuum, the residue was poured into EtOAc/aqueous Na_2CO_3. Concentration of the organic layer afforded crude tryptophan benzyl ester, which was isolated as a hydrochloride in 62% by treatment with hydrochloric dioxane in TAME at 0 °C. Chiral HPLC analysis under conditions effective in enantioseparation did not show any trace of D-tryptophan benzyl ester.

L-Proline benzyl ester *p*-toluenesulfonate (1)

We have previously reported that the benzyl esters tosylates of neutral amino acids with unfunctionalized alkyl side chains, such as leucine and valine, undergo limited or no racemization when prepared in boiling toluene, whereas those of amino acids with electron-with-drawing side chains, aromatic or not, such as phenylglycine, tyrosine, and methionine, extensively racemize under the same conditions (Bolchi et al. 2017a). Beside the electron-withdrawing capacity of the side chain, the protonation of the α-amine function is the other important factor that facilitates racemization favoring methine dissociation and stabilizing the incipient carbanion (Smith and Sivakua 1983). Compared to the amino acids with unfunctionalized alkyl side chain, proline stands out for the higher basicity of its amine function (pKa 10.60), secondary because alkylated by the side chain (Dwyer 2005). Therefore, higher susceptibility to racemization was expected for proline than for leucine. Indeed, Fischer–Speier esterification of L-proline with benzyl alcohol in boiling toluene provided the benzyl ester *p*-toluenesulfonate in quantitative yield but with only 85% enantiomeric excess, a value determined by chiral HPLC that was significantly lower than that previously registered for leucine (90%) (Bolchi et al. 2017a). On the contrary, reaction in boiling cyclohexane or Me-THF quantitatively afforded the benzyl ester *p*-toluenesulfonate without any racemization. Comparison of our preparations with literature Fischer–Speier esterification in carbon tetrachloride cannot be made, because optical rotation is not provided by the authors and the oily consistency of the *p*-toluenesulfonate hampers evaluation of melting points (Dai and Etzkorn 2009). It is, nevertheless, presumable that also the esterification in carbon tetrachloride, a, however, banned solvent, yields enantiomerically pure proline benzyl ester because of the low refluxing temperature. On the other hand, L-proline benzyl ester hydrochloride, which is a high-melting solid, is somewhat better characterized than *p*-toluenesulfonate in the literature, although only for optical rotation and melting point (Neuman and Smith 1951; Adam and Fleš 1959; Hwu et al2002). However, in the absence of any enantiomeric composition data, its enantiomeric purity is also questionable depending on the preparation conditions. Our results indicate that temperatures little higher than cyclohexane and Me-THF boiling point can be deleterious for the L-proline benzyl ester enantiomeric purity, which cannot thus be taken for granted in the case of its hydrochloride either.

Conclusions

Preparing enantiopure benzyl ester salts of methionine, arginine, tryptophan, and proline by the simple Fischer–Speier procedure is, for several reasons, a non-trivial challenge, as suggested by the very few examples in the literature. Poor solubility and instability of the substrates add to pronounced tendency to racemization common to almost all the amino acids, here further documented by proline benzyl ester in boiling toluene and phenylalanine benzyl ester in CPME at reflux. Reference analytical characterization is largely faulty and, for enantiomeric purity, completely absent. Moreover, cyclohexane, which can successfully replace banned or unsuitable solvents to azeotropically remove water in the esterification of several amino acids, is totally ineffective or little efficient for the esterification of these amino acids, except for proline. Therefore, we searched for an alternative solvent and developed a straightforward procedure to obtain enantiopure methionine, arginine, tryptophan, and proline benzyl esters in good-to-excellent yield by treatment of the amino acid with benzyl alcohol and *p*-toluenesulfonic acid in refluxing Me-THF, a green ether whose low boiling water azeotrope allows any racemization to be avoided. Other two green ethers were considered, CPME and TAME. However, the former was discarded, because it causes racemization for its high boiling point and the latter was not used for its instability to acidity. The enantiomeric excesses were determined by chiral HPLC and, in the case of arginine benzyl ester, by

H NMR analysis of the *p*-toluenesulfonate (*R*)-mosherate. The present procedures widen the application field of the Fischer–Speier esterification from amino acids containing OH, NH_2, and COOH functions in the side chain to amino acids, such as tryptophan, methionine, and arginine, whose susceptibility to degradation, due to 3-indolyl, methylthio, and guanidyl residue, respectively, is well documented (Unger and Holzgrabe 2018).

Materials and methods

^{1}H NMR spectra were recorded on a Varian Gemini 300 operating at 300 MHz and ^{13}C NMR at 75 MHz. Chemical shifts are reported in ppm relative to residual solvent ($CHCl_3$ and methanol) as internal standard. HRMS: stock solution was prepared by dissolution in 200 µL of CH_3OH; prior analysis, an aliquot of stock solution was diluted in water containing 0.1% HCOOH. Sample analysis: sample was introduced by flow injection directly into the ESI interface (Finnigan IonMax). Nebulization was achieved by 1 unit of nitrogen and applying 5 kV spray voltage. Spectra were acquired using the orbitrap analyzer of a LTQ Orbitrap XL instrument. Resolution was set at 100,000 (FWHM at 400 *m/z*) and scan range was 150–700 *m/z*. The melting points were determined by DSC analysis and the melting peak maximum is reported for each compound. The DSC curves were recorded and integrated with the aid of a TA Instruments DSC 2010 apparatus. Optical rotations were determined in a 1 dm cell of 5 mL capacity using a Perkin-Elmer 241 polarimeter. The enantiomeric excess of the benzyl esters of L-proline, L-tryptophan, and L-methionine was determined by chiral HPLC, while that of L-arginine by H NMR analysis of the *p*-toluenesulfonate (*R*)-Mosher carboxylate salt. Reference racemic samples of the four amino acid benzyl esters were prepared from the corresponding racemic amino acids as described for the L enantiomers.

L-Proline benzyl ester *p*-toluenesulfonate (1)

(a) In cyclohexane. A mixture of L-Proline (0.05 mol), *p*-toluenesulfonic acid (0.06 mol), benzyl alcohol (0.25 mol), and cyclohexane (30 mL) was heated at reflux with a Dean–Stark trap under vigorous stirring for 4 h. The reaction mixture was cooled to rt and the upper layer was removed. The obtained residue was washed twice with cyclohexane (60 ml). After vacuum pump drying, **1** was obtained as a pure oil in quantitative yield. $[\alpha]_D^{25} = -22.5$ (*c* 1, MeOH); 100% e.e. (determined by HPLC analysis on a Phenomenex Lux 3 µ Amyloae-2 column, 150 × 4.6 mm i.d.; hexane/2-PrOH 8/2; 1 mL/min; 220 nm, $t_R = 8.91$ min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82–2.12 (m, 3H), 2.35 (s, 4H), 3.52 (m, 2H), 4.52 (m, 1H), 5.09 (d, *J* = 12.1 Hz, 1H), 5.17 (d, *J* = 12.1 Hz, 1H) 7.14 (d, *J* = 8.2 Hz, 2H), 7.22–7.32 (m, 5H), 7.72 (d, *J* = 8.2 Hz, 2H), 8.75 (bs, 1H), 9.58 (bs, 1H); ^{13}C NMR (75 MHz,CD_3OD) δ 168.54, 141.99, 140.44, 134.99, 128.51, 128.35, 128.32, 128.22, 125.55, 67.91, 59.41, 45.84, 27.88, 23.04, 19.97. HRMS (ESI) *m/z* calcd for $C_{12}H_{16}NO_2^+$ (MH^+) 206.11756, found 206.11741.

(b) In Me-THF. A mixture of L-proline (0.05 mol), *p*-toluenesulfonic acid (0.06 mol), benzyl alcohol (0.25 mol), and Me-THF (30 mL) was heated at reflux with a Dean–Stark trap under vigorous stirring for 4 h. The reaction mixture was cooled to rt and concentrated under vacuum. The obtained residue was washed twice with cyclohexane (60 ml). After vacuum pump drying, **1** was obtained as a pure oil in quantitative yield.

L-Tryptophan benzyl ester hydrochloride (2)

A mixture of L-tryptophan (0.05 mol), *p*-toluenesulfonic acid (0.06 mol), benzyl alcohol (0.50 mol), and Me-THF (120 mL) was heated at reflux with a Dean–Stark trap under vigorously stirring for 24 h. The reaction mixture was cooled to rt and the solvent was evaporated under vacuum. The obtained residue was poured into EtOAc/aqueous Na_2CO_3 and, after removing the water layer, the organic phase was evaporated under vacuum. The residue was diluted with TAME and cooled to 0 °C, and hydrochloric dioxane was added to the solution. The precipitated hydrochloride was collected by filtration and washed twice with ethyl acetate to give **2** in 62% yield. M.p. = 207.20; $[\alpha]_D^{25} = +4.56$ (*c* 2, MeOH); 100% e.e. (determined by HPLC analysis on a Phenomenex Lux 3 µ Cellulose-2 column, 150 mm × 4.6 mm i.d.; hexane/2-PrOH 9/1; 1 mL/min; 280 nm, $t_R = 13.57$ min); ^{1}H NMR (300 MHz, CD_3OD) δ 3.34 (dd, *J* = 7.0, 15.2, Hz, 1H), 3.43 (dd, *J* = 6.4, 15.2, Hz, 1H),4.35 (dd, *J* = 6.4, 7.0, Hz, 1H), 5.16 (d, *J* = 12.3 Hz, 1H), 5.21 (d, *J* = 12.3 Hz, 1H) 7.05-7.41 (m, 8H), 7.52 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.90, 136.86, 134.74, 128.29, 128.21, 126.86, 124.26, 121.50, 118.93, 117.49, 111.31, 106.02, 67.80, 53.37, 26.27. HRMS (ESI) *m/z* calcd for $C_{18}H_{19}N_2O_2^+$ (MH^+) 295.14410, found 295.14426.

L-Arginine benzyl ester mono-*p*-toluenesulfonate (3)

A mixture of L-arginine (0.05 mol), *p*-toluenesulfonic acid (0.11 mol), benzyl alcohol (0.50 mol), and 2-Me-THF (100 mL) was heated at reflux with a Dean–Stark trap under vigorously stirring for 24 h. The reaction mixture was cooled to rt and the upper layer was removed. The lower layer was washed with ethyl acetate twice and, after vacuum pump drying, poured into DCM/aqueous Na_2CO_3. Water was removed and DCM was evaporated to give **3** as a white solid in 58% yield. M.p. = 98.94 °C; $[\alpha]_D^{25} = -2.26$ (*c* 1, MeOH); > 98% e.e. (determined by H NMR analysis); ^{1}H NMR (300 MHz,

CD_3OD) δ 1.53-1.81 (m, 4H), 2.35 (s, 3H), 3.13 (t, J = 6.4 Hz, 2H), 3.49 (t, J = 5.8 Hz, 1H), 5.14 (d, J = 12.2 Hz, 1H), 5.19 (d, J = 12.2 Hz, 1H) 7.22 (d, J = 8.2 Hz, 2H), 7.31-7.42 (m, 5H), 7.70 (d, J = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.33, 157.25, 141.55, 140.48, 135.60, 128.97, 128.56, 128.30, 128.15, 125.68, 66.68, 53.79, 40.85, 30.57, 25.01, 21.25. HRMS (ESI) *m/z* calcd for $C_{13}H_{21}N_4O_2^+$ (MH^+) 265.16590, found 265.16592. The (*R*)-Mosher carboxylates were prepared by adding an equimolar amount of (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid to a methanolic solution of L-arginine benzyl ester mono-p-toluenesulfonate (**3**) or of *rac*-arginine benzyl ester mono-p-toluenesulfonate and evaporating the solvent under vacuum. L*R*-Salt ^{1}H NMR (600 MHz, CD_3OD) δ 1.51-1.80 (m, 2H), 1.83-2.07 (m, 2H), 2.39 (s, 3H), 3.16 (m, 2H), 3.56 (s, 3H), 4.00 (t, J = 6.4 Hz, 1H), 5.27 (d, J = 12.0 Hz, 1H), 5.31 (d, J = 12.0 Hz, 1H), 7.26 (d, J = 8.2 Hz, 2H), 7.29-7.46 (m, 8H), 7.66 (m, 2H), 7.72 (d, J = 8.2 Hz, 2H). L*R*/D*R*-Salt: ^{1}H NMR (600 MHz, CD_3OD) δ 1.52–1.82 (m, 2H), 1.83–2.07 (m, 2H), 2.39 (s, 3H), 3.16 (dt, J = 6.4, 2.0 Hz, 2H), 3.56 (s, 3H), 4.00 (t, J = 6.4 Hz, 0.5H), 4.07 (t, J = 6.4 Hz, 0.5H), 5.27 (d, J = 12.0 Hz, 1H), 5.31 (d, J = 12.0 Hz, 1H), 7.26 (d, J = 8.2 Hz, 2H), 7.29–7.46 (m, 8H), 7.66 (m, 2H), 7.72 (d, J = 8.2 Hz, 2H). L*R*-Salt ^{13}C NMR (75 MHz, CD_3OD) δ 170.61, 169.02, 157.29, 141.90, 140.56, 135.75, 135.01, 128.58, 128.40, 128.36, 128.26, 127.49, 125.55, 124.87 (q, J = 288.0 Hz), 85.30 (q, J = 24.8 Hz), 67.75, 54.07, 52.17, 40.13, 27.28, 23.92, 19.99; ^{19}F NMR (282 MHz, CD_3OD) δ -67.45.

L-Methionine benzyl ester *p*-toluenesulfonate (4)

A mixture of L-methionine (0.05 mol), *p*-toluenesulfonic acid (0.06 mol), benzyl alcohol (0.50 mol), and Me-THF (120 mL) was heated at reflux with a Dean–Stark trap under vigorous stirring for 24 h. The reaction mixture was cooled to rt and the solvent was evaporated under vacuum. The obtained residue was poured into DCM/aqueous Na_2CO_3 and, after removing the water layer, the organic phase was evaporated under vacuum. The residue was diluted with TAME, cooled to 0 °C, and treated with equimolar *p*-toluenesulfonic acid to give **4** as a white solid in 62% yield. M.p. = 129.51 °C, $[\alpha]_D^{25} = +0.95$ (*c* 1, MeOH); 95.7% e.e. (determined by HPLC analysis on a Phenomenex Lux 3 μ Amylose-2 column, 150 mm × 4.6 mm i.d.; hexane/iPrOH 80/20 1 mL/min; 210 nm, t_R = 11.08 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.84 (s,3H), 2.10 (m, 2H), 2.30 (s, 3H), 2.43 (m, 2H), 4.14 (m, 1H), 5.00 (d, J = 12.3 Hz, 1H), 5.12 (d, J = 12.3 Hz, 1H), 7.08 (d, J = 7.0 Hz, 2H), 7.24–7.30 (m, 5H), 7.73 (d, J = 7.0 Hz, 2H), 8.35 (bs, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.76, 142.07, 140.34, 134.96, 128.44, 128.42, 128.32, 125.55, 67.83, 51.49, 29.38, 28.51, 19.95, 13.58. HRMS (ESI) *m/z* calcd for $C_{12}H_{18}NO_2S^+$ (MH^+) 240.10527, found 240.10531.

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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