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Kinetic resolution of 1-oxyl-3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine derivatives by lipase-catalyzed enantiomer selective acylation

József Bálint,[a] Violetta Kiss,[a] Gabriella Egri,[a] Tamás Kálai,[b] Ádám Demeter,[c] Mária Balog,[b] Elemér Fogassy[a] and Kálmán Hideg[b,*]

[a]*Department for Organic Chemical Technology, Technical University Budapest, P.O. Box 91, H-1521 Budapest, Hungary*
[b]*Institute of Organic and Medicinal Chemistry, University of Pécs, P.O. Box 99, H-7602, Hungary*
[c]*Spectroscopic Research Division, Gedeon Richter Ltd., P.O. Box 27, H-1475 Budapest, Hungary*

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Abstract—Three chiral stable free radicals containing a hydroxymethyl group have been resolved by biocatalysis. By recrystallization and/or re-esterification each molecule was produced in very high enantiomeric purity. The resolved 1-oxyl-3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine alcohols were converted to methanethiosulfonate spin labels, 1-oxyl-3-methanesulfonylthiomethyl-2,2,5,5-tetramethylpyrrolidines. Enantiomeric purities have been determined by the ^{19}F NMR method of the respective Mosher esters. Absolute configurations were assigned by comparing the chemical shifts of the Mosher esters and also by comparing the specific rotations obtained with the same enzyme preparations.

1. Introduction

Stable nitroxide free radicals find wide application as co-oxidants,[1] SOD mimics,[2] spin traps for carbon centred radicals[3] and mediating radicals in polymerization[4] making these paramagnetic *N*-oxyl heterocycles indispensable tools in analytical, organic and polymer chemistry. Another important role for nitroxide free radicals is spin labelling for exploration of protein structure, dynamics and function.[5] A new technique of site-directed spin labelling,[6] is based on the synthesis of cysteine point mutant peptides followed by labelling with the 1-oxyl-3-methanesulfonylthiomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole radical (MTS-SL).[7] However it was found that interaction of the pyrroline nitroxide ring with protein at some helix surface gives rise to EPR spectra degenerate with those at tertiary contact sites. It was found that this degeneracy can be resolved by using a saturated ring, for example, racemic 1-oxyl-3-methanesulfonylthiomethyl-2,2,5,5-tetramethylpyrrolidine radical (sat-MTS-SL).[8] The introduction of this new spin label containing a stereogenic centre raises the question of its interaction with the labelled peptide as a chiral molecule. This prompted us to prepare the two enantiomers of methanethiosulfonate spin label for further studies. However, methanethiosulfonates are sensitive and reactive esters, their direct resolution would be rather difficult, therefore the resolution must be attempted for their precursors. A large number of chiral nitroxides have been reported[9] but only a few were resolved and both enantiomers or at least one of the enantiomers isolated.[10]

Herein we report the resolution of three target molecules (1-oxyl-3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine **I**,[11] 1-oxyl-3-hydroxymethyl-4-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine, **II** and 1-oxyl-3-hydroxymethyl-4-nitromethyl-2,2,5,5-tetramethylpyrrolidine, **III**) as starting compounds for spin labels bound to proteins, which are chiral structures, thus obtaining their enantiomers is of interest. Racemates of compounds **II** and **III** are available by reduction of 1-oxyl-3-formyl-4-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine[12] and 1-oxyl-3-methoxycarbonyl-4-nitromethyl-2,2,5,5-tetramethylpyrrolidine,[13] respectively. As far as we know, they have not yet been reported to be resolved by biocatalysis, which can be a highly selective, easily manageable, economical and environmentally friendly method.

* Corresponding author. Tel.: +36-72-536220; fax: +36-72-536219; e-mail: kalman.hideg@aok.pte.hu

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Furthermore, the hydroxymethyl group indicates potential susceptibility for biocatalytic treatment. Herein we report on their lipase-catalyzed acylation.[14,15]

2. Results and discussion

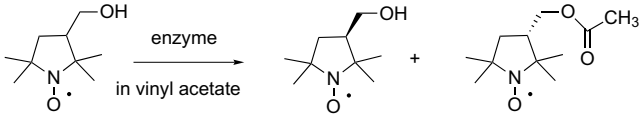
First, for each compound, several enzymes were tested in vinyl acetate. The results are shown in Tables 1–3. As it can be seen in Tables 1–3, at least one enzyme has been found for each compound (PPL and Lipozym IM® and Novozym 435® for **I**, Lipozym IM for **II** and Novozym for **III**) which afforded an enantiomeric excess high enough for further enantiomeric enrichment. Enantiomeric enrichment can be accomplished by recrystallization.

2.1. Enantiopure substances

The enantiomers of compounds **I**–**III** could be obtained by re-esterification or recrystallization or the combinations thereof, as described below.

(*R*)-**I** was obtained by recrystallization of the residual alcohol of the enzymatic reaction (Scheme 1). (*S*)-**I** needs a second esterification step and recrystallization from cyclohexane, therefore its overall yield is not so high but enantiomeric purity is around 98%.

Table 1. Enzymatic acetylation of **I**: screening with 7 commercially available enzyme preparations

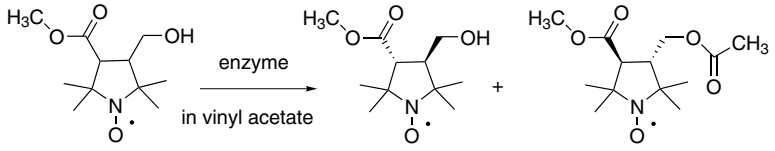


(*rac*)-**I** (*R*)-(+)-**I** (*S*)-(-)-**I-acetate**

Entry	Enzyme[a]		Time [h]	Remaining alcohol		Acetate formed	
	Name	[mg]		Y [%]	Ee [%]	Y [%]	Ee [%]
1	Amano AK[a]	50	13	38	34	65	19
2	CRL[a]	100	14	49	0	46	0
3	PPL[a]	100	13	42	80	34	65
4	Amano PS[a,b]	50	144	45	2	36	5
5	Amano PS-C[a,b]	50	24	44	4	54	6
6	Lipozym IM[a]	100	7	44	53	52	39
7	Novozym 435[a]	5	4	35	32	67	18

[a] Amano AK: lipase from *Pseudomonas fluorescens*, Amano PS: lipase from *Burkholderia cepacia*, Amano PS-C: lipase from *Burkholderia cepacia* immobilized on ceramic particles (>1000 U/g), PPL: lipase from porcine pancreas, CRL: lipase from *Candida rugosa* (*cylindracea*), Lipozym IM: lipase from *Mucor miehei*, Novozym 435: lipase B from *Candida antarctica* immobilized on arcylic resin.
[b] Reverse selectivity.

Table 2. Enzymatic acetylation of **II**: screening with 7 commercially available enzyme preparations

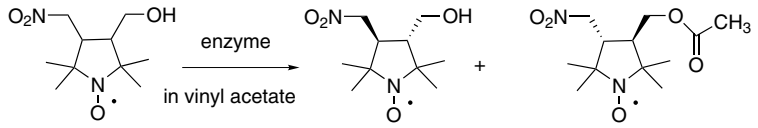


(*rac*)-trans **-II** (3*R*, 4*R*)-**II** (3*S*, 4*S*)-**II**-acetate

Entry	Enzyme		Time [h]	Remaining alcohol		Acetate formed	
	Name	[mg]		Y [%]	Ee [%]	Y [%]	Ee [%]
1	Amano AK[a]	100	7.5	33	37	74	15
2	CRL	100	13	70	11	31	32
3	PPL	100	48	100	n.m.[b]	0	n.m.[b]
4	Novozym 435[a]	100	22	45	55	52	51
5	Lipozym IM	100	10.5	46	72	53	65
6	Amano PS-C	100	4	0	n.m.[b]	100	n.m.[b]
7	Amano PS-C[a]	100	1.25	46	3	79	3

[a] Reverse selectivity.
[b] Not measured.

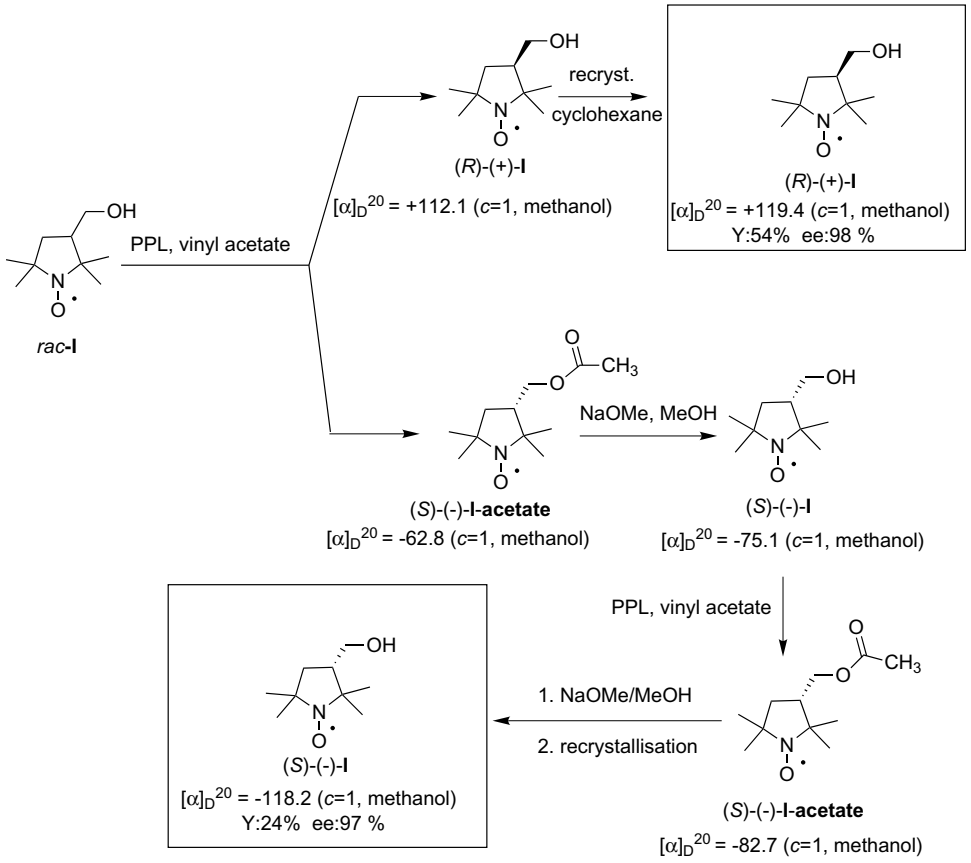
Table 3. Enzymatic acetylation of **III**: screening with commercially available enzyme preparations



(*rac*)-trans **-III** (3*S*, 4*R*)-**III** (3*R*, 4*S*)-**III**-acetate

Entry	Enzyme		Time [h]	Remaining alcohol		Acetate formed	
	Name	[mg]		Y [%]	Ee [%]	Y [%]	Ee [%]
1	Amano AK[a]	100	44	29	7	70	6
2	CRL	100	24	28	14	63	3
3	PPL	100	48	100	n.m.[b]	0	n.m.[b]
4	Novozym 435	100	24	51	69	54	65
5	Amano PS-C	100	24	44	14.1	56	14.3

[a] Reverse selectivity.
[b] Not measured.



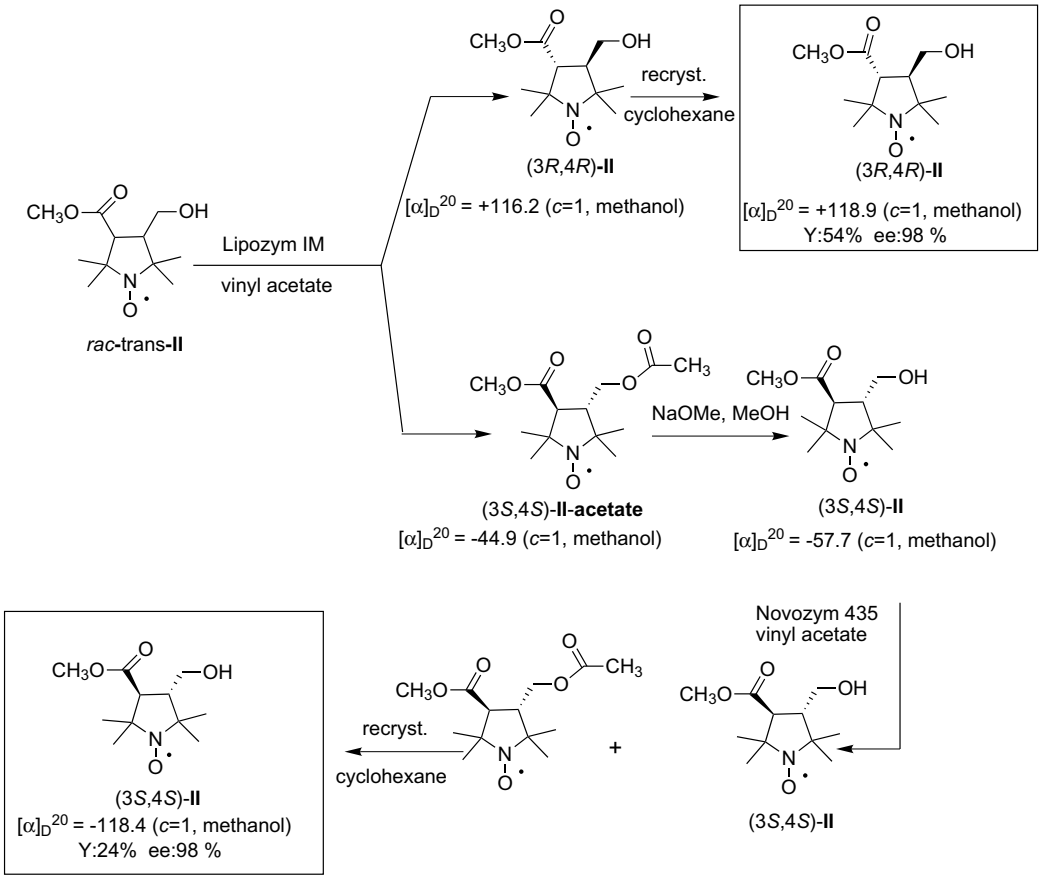
Scheme 1. Resolution of (*S*)- and (*R*)-**I**.

The (3*R*,4*R*)-**II** was obtained by Lipozym IM catalyzed acetylation as the remaining alcohol fraction. The acetate fraction ((3*S*,4*S*)-**II**-acetate) was methanolized and subjected to acetylation by the Novozym 435, showing opposite selectivity than Lipozym IM, yielding pure (3*S*,4*S*)-**II** (Scheme 2).

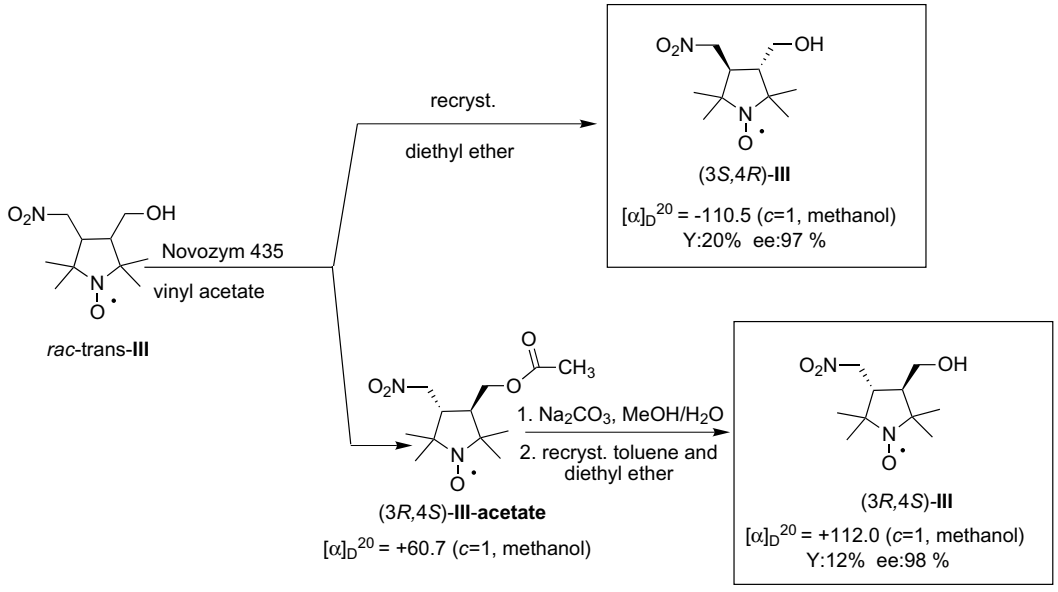
The racemic **III** was acetylated by Novozym 435, affording (3*S*,4*R*)-**III** and (3*R*,4*S*)-**III**-acetate, both in medium enantiomeric purity. The (3*R*,4*S*)-**III**-acetate was then hydrolyzed, yielding (3*R*,4*S*)-**III**. Both alcohols could be purified by repeated recrystallizations (Scheme 3).

2.2. Synthesis of thiolspecific spin label enantiomers

To get spin label reagents capable of modification peptides (*R*)-**I** and (*S*)-**I** alcohols were converted to mesylates in CH_2Cl_2 in the presence of triethylamine (TEA) and the mesylates were substituted with iodide by heating them in THF with excess NaI to give (*R*)-**IV** and (*S*)-**IV**.[8] The methanethiosulfonates (*R*)-**V** and (*S*)-**V**—capable of reacting with cysteine side chains—were achieved by treating iodides with $NaSSO_2CH_3$ in DMF without involvement of the stereogenic centre (Scheme 4).



Scheme 2. Resolution of (3*S*,4*S*)- and (3*R*,4*R*)-**II**.

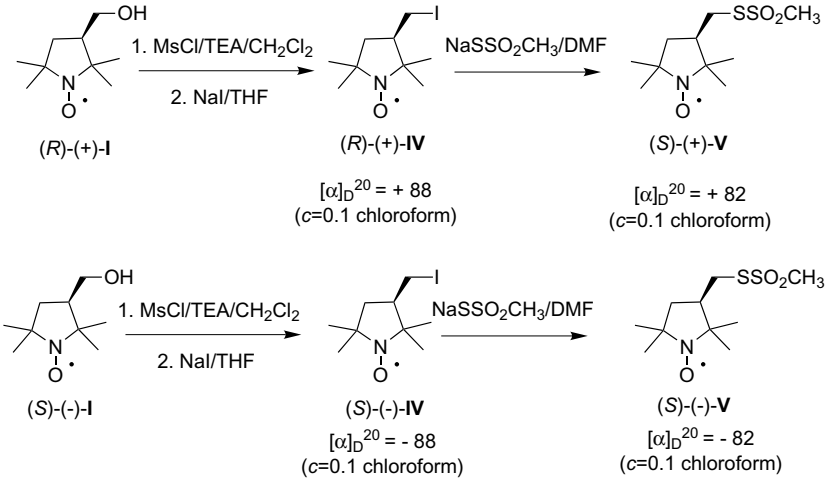


Scheme 3. Resolution of (3*R*,4*S*)- and (3*S*,4*R*)-**III**.

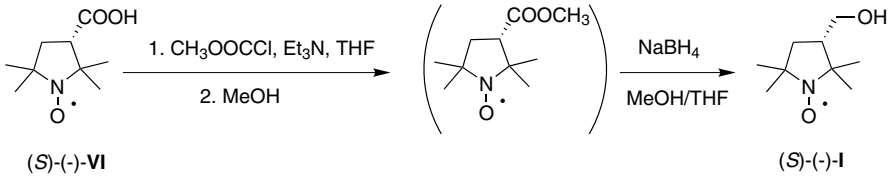
2.3. Enantiomeric purities and absolute configurations

Enantiomeric purities were determined by the fluorine NMR of the Mosher esters of the respective compounds **I**, **II** and **III**, whereas (*S*)-MTPA-Cl was used to give (*R*)-MTPA-esters. Enantiomeric purities of the acetate esters were deduced from their corresponding alcohol-MTPA-esters.

The absolute configuration of **I** was determined by a reaction sequence starting from a compound with an already assigned absolute configuration:[16] (*S*)-(−)-**VI**



Scheme 4. Synthesis of MTS spin label enantiomers.



Scheme 5. Absolute configuration of **I**.

was esterified by methanol, then it was reduced by sodium borohydride to give (*S*)-(−)-**I**, which, as the reactions did not influence the stereocentre, has the same configuration and is also (*S*) (Scheme 5). This result reinforced another literature finding ($[\alpha]_{578}^{25} = -136$ (*c* 0.55, EtOH))[17] in which the authors, although by a different way, came to the same conclusion.

The absolute configurations of **II** and **III** were assumed by NMR spectroscopy as detailed later and also by enzyme selectivity as follows: Lipozym IM, an enzyme which worked for all the three compounds, produced (+)-alcohol and (−)-acetate fractions. Similarly, Amano PS-C resulted in (−)-alcohols and (+)-acetates. Other enzymes may not be comparable as at least one of the formed alcohol and acetate fractions had an optical rotatory power close to zero, which means low selectivity and possibility of reversal of enantiomeric selectivity. However, the two comparable experiment series suggest that the configuration of (+)-**II** is (3*R*,4*R*) and (+)-**III** is (3*R*,4*S*). The measured optical rotation data of the respective enantiomers of **II** and **III** seems to correlate with that of **I**, which provides further confirmation regarding the configuration assignment.

2.4. Determination of the absolute configurations of II and III by NMR

In order to assess the absolute configurations in **II** and **III** the respective alcohols were allowed to react with (−)-α-methoxy-α-trifluoromethylphenylacetic acid to provide diastereomeric Mosher ester derivatives. The NMR assignment of the absolute configurations in **II** and **III** is based on the observed empirical correlation of the ^{19}F NMR chemical shifts of the respective Mosher esters of **II** and **III** with the Mosher ester of **I** of known configuration. The measured ^{19}F NMR spectra of the Mosher esters of **I**, **II** and **III** are shown in Figure 1. In (3*R*)-**I**-Mosher ester the CF_3 group gives a ^{19}F NMR signal that is narrower and resonates upfield relative to that of the (3*S*)-**I**-Mosher ester (Fig. 1). These two spectral features were used to assign the configurations in **II** and **III**. Similarly to **I**, the epimeric Mosher ester pairs of **II** and **III** give broader ^{19}F NMR signal that appears downfield from the signal exhibiting a smaller half-height width (Fig. 1). Based on these facts we assign (3*R*) and (3*S*) configuration to the stereoisomers of **II** and **III** with the upfield and downfield ^{19}F NMR signals, respectively, as shown in Figure 1. It should be noted that the above assignments are based on a purely empirical correlation that seems to be diagnostic of the C(3) configuration. In order to validate the generality of this correlation and to rationalize these observations, further investigations are needed. Nevertheless, the deduced configuration assignments are in line with those obtained from optical rotation as well as enzyme selectivity data.

3. Experimental

The NMR spectra were recorded on a Varian INOVA™ spectrometer operating at 500 MHz (^{1}H) by using a

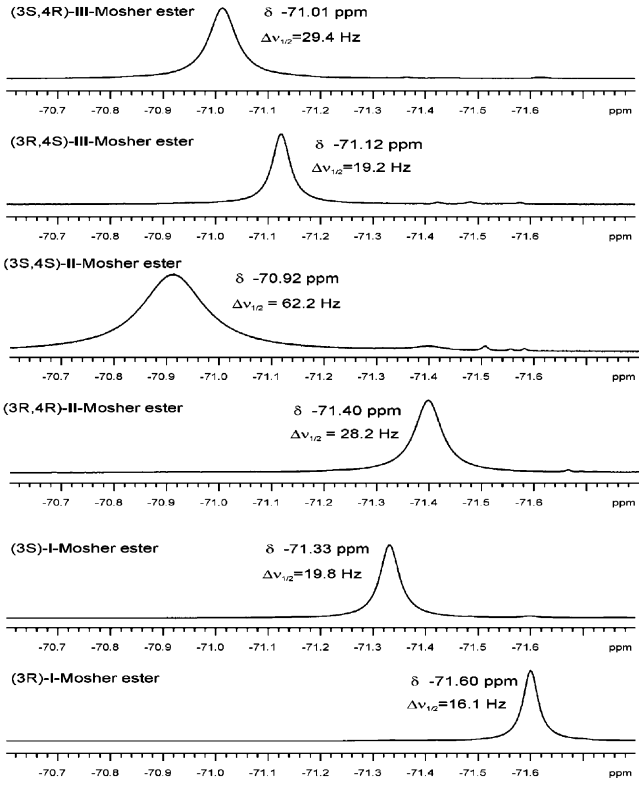


Figure 1. ^{19}F NMR spectra of the Mosher esters of **I**, **II** and **III** as measured in the absence of 1,2-diphenylhydrazine in $CDCl_3$ at 30 °C at ^{19}F 470 MHz. For each 3*S* and 3*R* pair the downfield signal appears characteristically broader as compared to the upfield signal.

Varian 5-mm $^1H\{^{13}C,^{15}N\}$ PFG Indirect NMR™ probe. ^1H NMR chemical shifts are given relative to TMS ($\delta_{TMS} = 0.00$ ppm), as measured in $CDCl_3$ at 30 °C in the presence of 1.5 mol equiv of 1,2-diphenylhydrazine. ^{19}F chemical shifts are given relative to CF_2Cl_2 internal standard ($\delta_{CF_2Cl_2} = 0.00$ ppm) and measured in the absence of 1,2-diphenylhydrazine. ^1H NMR assignments were straightforward by a concerted use of standard high-field one- and two-dimensional (2D) NMR methods: 1D DPFGSE-NOE (selective excitation by I-Burp2 shaped pulses) and 2D ^1H–^1H as well as ^{13}C–^1H shift correlations (PFG-HSQC, PFG-HMBC). The obtained scalar and NOE connectivities provided abundant information to ensure unambiguous spectral assignments. The numbering of structures used for the NMR assignments is depicted in Figure 2. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet).

Enantiomeric purity was determined as described in Section 2.3. IR spectra were recorded on a Specord 2000 spectrometer. Optical rotations were determined on a Perkin Elmer 241 and Perkin Elmer 343 polarimeters. Thin-layer chromatography (TLC) was made using Merck Kieselgel 60 F_{254} alumina sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative vacuum chromatography was performed using Merck Kieselgel 60 F_{254}. Porcine pancreatic lipase (PPL, type II) and CRL was obtained from Sigma.

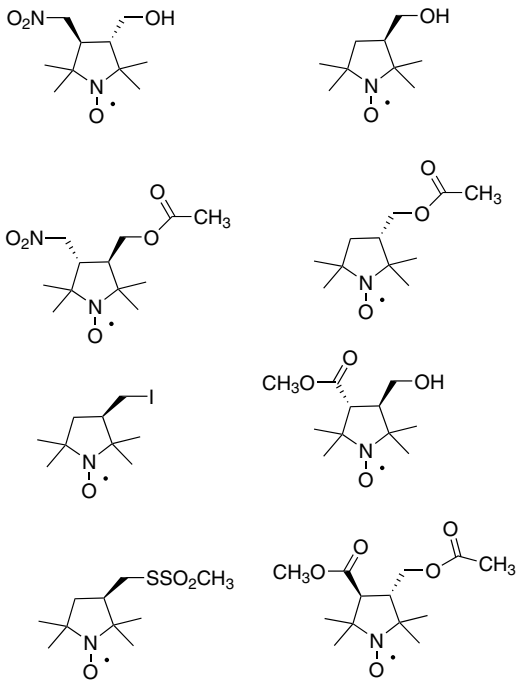


Figure 2.

Amano enzymes were courtesy of Amano. Novozym and Lipozym were courtesy of Novo Nordisk. Vinyl acetate was product of FLUKA. All solvents used were freshly distilled.

3.1. Absolute configuration

Absolute configuration of **I** is known from literature[17] and corresponds to our results.

(*S*)-**VI** (100 mg, 0,54 mmol, $[\alpha]_D^{20} = -76.5$ (*c* 0.37, ethanol), $[\alpha]_{578}^{20} = -85.4$ (*c* 0.37, ethanol)) was dissolved in THF (4 mL), and triethylamine (0.1 mL, 72.7 mg, 0.72 mmol) was added. Without cooling, methyl chloroformate (0.05 mL, 61.2 mg, 0.65 mmol) was also added. The solution (which slightly warmed up) was stirred for 15 min, then methanol was added (5 mL) and stirring continued for 30 min. After adding $NaBH_4$ (3 g, 79 mmol) the mixture was stirred for 20 min then solvent was evaporated. The residue was diluted with 15% Na_2CO_3 solution and was extracted by ethyl acetate (4×10 mL). The combined organic phase was dried over Na_2SO_4 and solvent was evaporated. The residue was purified by preparative vacuum column chromatography with eluent of hexane–EtOAc 10:2. Obtained: yellow crystalline material, 38.8 mg (0.23 mmol, 43%, $[\alpha]_D^{20} = -95.9$ (*c* 1, methanol), $[\alpha]_{578}^{20} = -142.9$ (*c* 0.55, ethanol)).

3.2. (*R*)-I

Racemic **I** (600 mg, 3.48 mmol) and PPL (lipase from porcine pancreas) (600 mg) were dissolved in vinyl acetate (12 mL). After 48 h vinyl acetate was evaporated in vacuum and the residue was separated by preparative

vacuum column chromatography (hexane–EtOAc 10:2) to obtain:

(a) (+)-**I** (206.6 mg, 1.20 mmol, 68%, $[\alpha]_D^{20} = +112.1$ (*c* 1, methanol)), recrystallized from cyclohexane: 162.4 mg (0.94 mmol, 54%, $[\alpha]_D^{20} = +119.4$ (*c* 1, methanol)), mp 114–117 °C off-white-brownish crystalline (R)-**I**, ee = 98% and
(b) (−)-**I**-acetate (453.9 mg, 2.12 mmol, 121%, $[\alpha]_D^{20} = -62.8$ (*c* 1, methanol)).

3.3. (*S*)-I

(−)-**I**-Acetate (453.9 mg, 2.12 mmol, 121%, $[\alpha]_D^{20} = -62.8$ (*c* 1, methanol)) and NaOMe (0.4 g) were dissolved in methanol (4 mL). The mixture was boiled up and left at room temperature for 1 h, when methanol was evaporated in vacuum. Water (5 mL) was added and it was extracted by ethyl acetate (4×10 mL). The combined organic phase was dried over Na_2SO_4 and solvent was evaporated to yield foxy crystalline (−)-**I** (306.8 mg, 84%, $[\alpha]_D^{20} = -75.1$ (*c* 1, methanol)).

The above obtained (−)-**I** and PPL (lipase from porcine pancreas) (300 mg) were stirred in vinyl acetate (3 mL). After 4 h vinyl acetate was evaporated in vacuum and the residue was separated by preparative vacuum column chromatography (hexane–EtOAc 10:2) to obtain:

(a) (−)-**I** (157.8 mg, 0.92 mmol, 43%, $[\alpha]_D^{20} = -55.9$ (*c* 1, methanol)) and
(b) (−)-**I**-acetate (182.5 mg, 0.85 mmol, 40%, $[\alpha]_D^{20} = -82.7$ (*c* 1, methanol)).

The acetate fraction was hydrolyzed in methanol (5 mL) by NaOMe (0.1 g) as described previously. The obtained yellow crystalline (−)-**I** (133.2 mg, 0.77 mmol, 91–36% overall, $[\alpha]_D^{20} = -101.8$ (*c* 1, methanol)), which was recrystallized twice from cyclohexane (15 mL) to yield yellowish crystalline (*S*)-**I** (88.4 mg, 0.51 mmol, 60–24% overall, $[\alpha]_D^{20} = -118.2$ (*c* 1, methanol), mp 115–117 °C).

3.4. Synthesis of racemic *trans*-II

To a solution of 1-oxyl-3-formyl-4-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (1.14 g, 5.0 mmol) in EtOH (10 mL), $NaBH_4$ (227 mg, 6.0 mmol) was added at 0 °C and kept at this temperature for 10 min, then $CHCl_3$ (20 mL) was added, the organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (eluent: hexane–EtOAc 10:2) to give the racemic compound **II** 713 mg (62%), mp 74–76 °C, MS (EI) *m*/*z*: 230 (M^+, 30), 216 (10), 197 (8), 115 (100).

3.5. Synthesis of racemic *trans*-III

To a refluxing solution of 1-oxyl-3-nitromethyl-4-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (2.59 g, 10.0 mmol) in dry *t*-BuOH (20 mL) and $NaBH_4$ (2.83 g, 75.0 mmol) mixture of *t*-BuOH (10 mL) and MeOH (4 mL) was added very slowly dropwise over 2–3 h under N_2. The mixture was concentrated in vacuo, quenched with water (40 mL), extracted with $CHCl_3$ (3×10 mL), dried ($MgSO_4$), filtered and evaporated. The crude product was purified by flash column chromatography (eluent: hexane–EtOAc 10:2) to give the racemic compound **III** 1.50 g (65%), mp 99–102 °C, MS (EI) *m*/*z*: 231 (M^+, 67), 217 (9), 198 (15), 110 (36), 71 (100).

3.6. (3*R*,4*R*)-II

Racemic *trans*-**II** (600 mg, 2.60 mmol) and Lipozym IM (lipase from *Mucor miehei*) (600 mg) were stirred in vinyl acetate (6 mL) for 48 h, then it was filtered and solvent was evaporated. The residue was subjected to preparative vacuum column chromatography (eluent: hexane–EtOAc 10:2) to obtain:

(a) (3*R*,4*R*)-**II** (198.2 mg, 60%, $[\alpha]_D^{20} = +116.2$ (*c* 1, methanol)) of oil, which transformed into yellowish oily crystalline material upon standing; recrystallized from 10 mL of cyclohexane: 152.4 mg (54%, $[\alpha]_D^{20} = +118.9$ (*c* 1, methanol), ee = 98%, mp 74–77 °C) of yellowish crystals and
(b) (3*S*,4*S*)-**II**-acetate (462.3 mg, 1.70 mmol, 130%, $[\alpha]_D^{20} = -44.9$ (*c* 1, methanol)). It was dissolved in methanol (2.5 mL), NaOMe (0.25 g) was added and the mixture was heated to boiling and then was let to cool down to rt. Brine (10 mL) was added and it was extracted by ethyl acetate (5×10 mL). The combined organic phase was dried over Na_2SO_4 and solvent was evaporated to yield (3*S*,4*S*)-**II** (362.2 mg, 120%, $[\alpha]_D^{20} = -57.7$ (*c* 1, methanol)).

3.7. (3*S*,4*S*)-II

(3*S*,4*S*)-**II** (362.2 mg, 1.57 mmol, $[\alpha]_D^{20} = -57.7$ (*c* 1, methanol)) and Novozym 435 (lipase from *Candida antarctica* immobilized on acrylic resin) (360 mg) were stirred in vinyl acetate (3.6 mL) for 48 h, then it was filtered and solvent was evaporated. The residue was subjected to preparative vacuum column chromatography (eluent: hexane–EtOAc 10:2) to obtain:

(a) (3*S*,4*S*)-**II** (133.6 mg, 0.58 mmol, 36%, $[\alpha]_D^{20} = -118.4$ (*c* 1, methanol)), after recrystallization from cyclohexane (8 mL): 111.2 mg (30%, mp 74–76 °C) and
(b) (3*S*,4*S*)-**II**-acetate (264.2 mg, 0.97 mmol, 61%, $[\alpha]_D^{20} = -15.8$ (*c* 1, methanol)).

The acetate fraction was hydrolyzed in methanol (1 mL) by NaOMe (0.1 g) as described previously to yield (3*R*,4*S*)-**II** (200.7 mg, 55%, $[\alpha]_D^{20} = -21.2$ (*c* 1, methanol)).

3.8. (3*S*,4*R*)-III

Racemic *trans*-**III** (600 mg, 2.59 mmol) and Novozym 435 (lipase from *Candida antarctica* immobilized on acrylic resin) (600 mg) were stirred in vinyl acetate (6 mL) for 24 h in room temperature. After filtration and

evaporation of the solvent, the residue was separated by preparative vacuum column chromatography to yield

(a) (3*S*,4*R*)-**III** (298.3 mg, 1.29 mmol, 99%, $[\alpha]_D^{20} = -79.7$ (*c* 1, methanol)), after recrystallization from toluene (1.5 mL): 159.7 mg, recrystallized from diethyl ether (2 mL, temperature −78 °C): yellow crystalline (3*S*,4*R*)-**III** (61.0 mg, 0.26 mmol, 20%, $[\alpha]_D^{20} = -110.5$ (*c* 1, methanol), mp 99–102 °C) and
(b) (3*R*,4*S*)-**III**-acetate (349.8 mg, 1.28 mmol, 100%, $[\alpha]_D^{20} = +60.7$ (*c* 1, methanol)).

3.9. (3*R*,4*S*)-III

The acetate fraction obtained above was hydrolyzed in methanol–water 1:1 (4 mL) by Na_2CO_3 (0.2 g) as described previously and the obtained material was purified by preparative vacuum column chromatography: 228.0 mg of (3*R*,4*S*)-**III** (0.99 mmol, 76%, $[\alpha]_D^{20} = +77.2$ (*c* 1, methanol)), which was recrystallized from toluene (1 mL) and then from diethyl ether (1.2 mL) to yield yellow crystalline (3*R*,4*S*)-**III** (38.5 mg, 0.17 mmol, 12%, $[\alpha]_D^{20} = +112.0$ (*c* 1, methanol), mp 99–102 °C).

3.10. (*R*)-IV, (*S*)-IV

The chiral alkyl iodide (*R*)-**IV**, (*S*)-**IV** compounds were synthesized from (*R*)-**I**, (*S*)-**I** alcohols (516 mg, 3.0 mmol) as published previously,[8] mp 86–88 °C (320 mg, 1.13 mmol, 37%, (*R*)-**IV** $[\alpha]_D^{20} = +88$ (*c* 0.1, chloroform)), (275 mg, 0.97 mmol, 32%, (*S*)-**IV** $[\alpha]_D^{20} = -88$ (*c* 0.1, chloroform)).

3.11. (*R*)-V, (*S*)-V

The chiral methanethiosulfonate (*R*)-**V**, (*S*)-**V** compounds were synthesized from (*R*)-**IV**, (*S*)-**IV** iodides (200 mg, 0.70 mmol) as published previously,[8] mp 85–87 °C (56 mg, 0.21 mmol, 30%, (*R*)-**V** $[\alpha]_D^{20} = +82$ (*c* 0.1, chloroform)), (52 mg, 0.19 mmol, 27%, (*S*)-**V** $[\alpha]_D^{30} = -82$ (*c* 0.1, chloroform)).

3.12. Mosher ester formation (general procedure)

The target compound (**I**, **II** or **III**, 0.1 mmol), pyridine (0.015 mL) and dimethylamino-pyridine (catalytic amount) were added into 5% (*S*)-MTPA-Cl solution in CCl_4 (0.7 mL). The mixture was kept at 50 °C for 3 h, then it was washed by 2% HCl (2×0.5 mL), 2% Na_2CO_3 (2×0.5 mL) and brine (0.5 mL), dried over $MgSO_4$, and solvent was evaporated. The residue was purified by vacuum column chromatography (eluent: hexane–EtOAc 10:2).

3.13. Analytical data

Hereby the ^{1}H NMR and FT-IR characterization as well as the elemental analysis data of the racemic **I**, **II** and **III** and their acetates are given. The reported ^{1}H chemical shifts are measured in the presence of 1.5 mol equiv of 1,2-diphenylhydrazine. The data of enantiomeric forms are identical or nearly identical to those of the racemate within experimental errors.

I: ^{1}H NMR ($CDCl_3$, TMS): δ 1.27 (s, 3H, H_3-8), 1.37 (s, 3H, H_3-7), 1.37 (s, 3H, H_3-9), 1.39 (s, 3H, H_3-6), 1.72 (dd, 1H, $^3J_{3,4\beta} = 8.2$ Hz and $^2J_{4\alpha,4\beta} = 13.1$ Hz, H_β-4), 2.07 (dd, 1H, $^3J_{3,4\alpha} = 8.1$ Hz and $^2J_{4\alpha,4\beta} = 13.1$ Hz, H_α-4), 2.13–2.22 (m, 1H, H-3), 3.65–3.74 (m, 2H, H-10), 4.20 (br s, OH). FT-IR (KBr, cm^{-1}): 3394 (br), 2972, 2882, 1637 (br), 1461, 1365, 1317, 1246, 1193, 1164, 1090, 1052, 956. Calcd for $C_9H_{18}NO_2$: C, 62.76; H, 10.53; N, 8.13. Found: C, 62.62; H, 10.55; N, 8.15.

I-OAc: ^{1}H NMR ($CDCl_3$, TMS): δ 1.06 (s, 3H, H_3-8), 1.21 (s, 3H, H_3-7), 1.22 (s, 3H, H_3-6), 1.25 (s, 3H, H_3-9), 1.49 (t, 1H, $^3J_{3,4\beta} = \sim 12.1$ Hz and $^2J_{4\alpha,4\beta} = 12.1$ Hz, H_β-4), 1.82 (dd, 1H, $^3J_{3,4\alpha} = 7.8$ Hz and $^2J_{4\alpha,4\beta} = 12.7$ Hz, H_α-4), 2.05 (s, 3H, OAc), 2.14–2.24 (m, 1H, H-3), 4.00–4.10 (m, 2H, $^3J_{3,10x} = 7.4$ Hz, $^3J_{3,10y} = 7.1$ and $^2J_{10x,10y} = 11.1$ Hz, H-10). FT-IR (KBr, cm^{-1}): 2976, 2952, 1744, 1464, 1368, 1236, 1164, 1036. Calcd for $C_{11}H_{20}NO_3$: C, 61.66; H, 9.41; N, 6.54. Found: C, 61.65; H, 9.43; N, 6.52.

(3*S*)-**I-Mosher**: ^{1}H NMR ($CDCl_3$, TMS): δ 1.06 (s, 3H, H_3-8), 1.19 (s, 3H, H_3-9), 1.21 (s, 3H, H_3-7), 1.24 (s, 3H, H_3-6), 1.51 (t, 1H, $^3J_{3,4\alpha} = \sim 12$ Hz and $^2J_{4\alpha,4\beta} = \sim 12$ Hz, H_α-4), 1.78 (dd, 1H, $^3J_{3,4\beta} = 7.7$ Hz and $^2J_{4\alpha,4\beta} = 12.9$ Hz, H_β-4), 2.20–2.30 (m, 1H, H-3), 3.52 (s, 3H, OMe), 4.18–4.40 (m, 2H, H-10), 7.38–7.54 (m, 5H, ArH).

(3*R*)-**I-Mosher**: ^{1}H NMR ($CDCl_3$, TMS): δ 1.02 (s, 3H, H_3-8), 1.18 (s, 3H, H_3-9), 1.21 (s, 3H, H_3-7), 1.22 (s, 3H, H_3-6), 1.51 (t,1H, $^3J_{3,4\beta} = \sim 12$ Hz and $^2J_{4\alpha,4\beta} = \sim 12$ Hz, H_β-4), 1.77 (dd, 1H, $^3J_{3,4\alpha} = 7.7$ Hz and $^2J_{4\alpha,4\beta} = 13.1$ Hz, H_α-4), 2.20–2.32 (m, 1H, H-3), 3.55 (s, 3H, OMe), 4.21 (dd, 1H, $^3J_{3,10x} = 7.3$ Hz and $^2J_{10x,10y} = 11.0$ Hz, H_x-10), 4.34 (dd, 1H, $^3J_{3,10y} = 7.1$ Hz and $^2J_{10x,10y} = 11.0$ Hz, H_y-10), 7.38–7.54 (m, 5H, ArH).

II: ^{1}H NMR ($CDCl_3$, TMS): δ 1.03 (s, 3H, H_3-8), 1.04 (s, 3H, H_3-7), 1.23 (s, 3H, H_3-9), 1.32 (s, 3H, H_3-6), 2.42 (ddd, 1H, $^3J_{3,4} = 11.2$ Hz, $^3J_{3,10x} = 7.5$ Hz and $^3J_{3,10y} = 5.8$ Hz, H-3), 2.60 (d, 1H, $^3J_{3,4\alpha} = 11.2$ Hz, H-4), 3.54 (dd, 1H, $^3J_{3,10x} = 7.5$ Hz and $^2J_{10x,10y} = 10.8$ Hz, H_x-10), 3.72 (s, 3H, COOMe), 3.75 (dd, 1H, $^3J_{3,10y} = 5.8$ Hz and $^2J_{10x,10y} = 10.8$ Hz, H_y-10), 3.77 (br s, 1H, OH). FT-IR (KBr, cm^{-1}): 3410 (br), 2976, 2934, 1721, 1463, 1435, 1363, 1277, 1263, 1181, 1050, 1019. Calcd for $C_{11}H_{20}NO_4$: C, 57.37; H, 8.75; N, 6.08. Found: C, 57.23; H, 8.77; N, 4.47.

II-OAc: ^{1}H NMR ($CDCl_3$, TMS): δ 1.09 (s, 3H, H_3-7); 1.10 (s, 3H, H_3-8); 1.29 (s, 3H, H_3-6); 1.36 (s, 3H, H_3-9); 2.02 (s, 3H, OAc); 2.58–2.67 (m, 2H, H-3, H-4); 3.73 (s, 3H, COOMe); 4.02 (dd, 1H, $^3J_{3,10x} = 5.7$ Hz and $^2J_{10x,10y} = 10.7$ Hz, H_x-10); 4.15 (dd, 1H, $^3J_{3,10y} = 6.2$ Hz and $^2J_{10x,10y} = 10.7$ Hz, H_y-10). FT-IR (KBr, cm^{-1}): 2976, 1744, 1440, 1412, 1340, 1236, 1168, 1152, 1036.

Calcd for $C_{13}H_{22}NO_5$: C, 57.34; H, 8.14; N, 5.14. Found: C, 57.23; H, 8.16; N, 5.16.

(3*R*,4*R*)-II-Mosher: ^{1}H NMR ($CDCl_3$, TMS): δ 0.97 (s, 3H, H_3-8); 1.05 (s, 3H, H_3-7); 1.15 (s, 3H, H_3-9); 1.31 (s, 3H, H_3-6); 2.53 (d, 1H, $^3J_{3,4} = 11.7$ Hz, H-4); 2.65 (ddd, 1H, $^3J_{3,4} = 11.7$ Hz, $^3J_{3,10x} = 4.8$ Hz and $^3J_{3,10y} = 7.9$ Hz, H-3); 3.53 (s, 3H, OMe); 3.68 (s, 3H, COOMe); 4.23 (dd, 1H, $^3J_{3,10x} = 4.8$ Hz and $^2J_{10x,10y} = 11.5$ Hz, H_x-10); 4.32 (dd, 1H, $^3J_{3,10y} = 7.9$ Hz and $^2J_{10x,10y} = 11.5$ Hz, H_y-10); 7.36–7.52 (m, 5H, ArH).

(3*S*,4*S*)-II-Mosher: ^{1}H NMR ($CDCl_3$, TMS): δ 0.99 (s, 3H, H_3-8); 1.02 (s, 3H, H_3-7); 1.16 (s, 3H, H_3-9); 1.29 (s, 3H, H_3-6); 2.53 (d, 1H, $^3J_{3,4} = 11.5$ Hz, H-4); 2.61 (ddd, 1H, $^3J_{3,4} = 11.5$ Hz, $^3J_{3,10x} = 5.1$ Hz and $^3J_{3,10y} = 7.2$ Hz, H-3); 3.51 (s, 3H, OMe); 3.64 (s, 3H, COOMe); 4.22–4.36 (m, 2H, $^3J_{3,10x} = 5.1$ Hz, $^3J_{3,10y} = 7.2$ Hz, and $^2J_{10x,10y} = 11.4$ Hz, H-10); 7.36–7.52 (m, 5H, ArH).

III: ^{1}H NMR ($CDCl_3$, TMS): δ 1.20 (s, 3H, H_3-8); 1.27 (s, 3H, H_3-7); 1.35 (s, 3H, H_3-6); 1.37 (s, 3H, H_3-9); 1.97 (ddd, 1H, $^3J_{3,4} = 11.0$ Hz, $^3J_{3,10x} = 7.3$ Hz and $^3J_{3,10y} = 4.8$ Hz, H-3); 2.82 (ddd, 1H, $^3J_{3,4} = 11.0$ Hz, $^3J_{4,11x} = 8.5$ Hz and $^3J_{4,11y} = 5.1$ Hz, H-4); 3.71 (dd, 1H, $^3J_{3,10x} = 7.3$ Hz and $^2J_{10x,10y} = 10.7$ Hz, H_x-10); 3.83 (dd, 1H, $^3J_{3,10y} = 4.8$ Hz and $^2J_{10x,10y} = 10.7$ Hz, H_y-10); 4.53 (dd, 1H, $^3J_{4,11x} = 8.5$ Hz and $^2J_{11x,11y} = 14.2$ Hz, H_x-11); 4.78 (dd, 1H, $^3J_{4,11y} = 5.1$ Hz and $^2J_{11x,11y} = 14.2$ Hz, H_y-11). FT-IR (KBr, cm^{-1}): 3382 (br), 2978, 2935, 1560, 1463, 1427, 1384, 1244, 1186, 1088, 1040. Calcd for $C_{10}H_{19}N_2O_4$: C, 51.93; H, 8.28; N, 12.11. Found: C, 52.01; H, 8.30; N, 12.09.

III-OAc: ^{1}H NMR ($CDCl_3$, TMS): δ 1.29 (s, 3H, H_3-8); 1.38 (s, 3H, H_3-7); 1.42 (s, 3H, H_3-6); 1.47 (s, 3H, H_3-9); 2.06 (s, 3H, H_3-OAc); 2.18–2.30 (br m, 1H, H-3); 2.80–2.90 (br m, 1H, H-4); 4.04 (dd, 1H, $^3J_{3,10x} = 6.7$ Hz and $^2J_{10x,10y} = 11.7$ Hz, H_x-10); 4.25 (dd, 1H, $^3J_{3,10y} = 5.5$ Hz and $^2J_{10x,10y} = 11.7$ Hz, H_y-10); 4.50–4.62 (m, 2H, H-11). FT-IR (KBr, cm^{-1}): 2984, 1736, 1560, 1466, 1432, 1384, 1236, 1040. Calcd for $C_{12}H_{21}N_2O_5$: C, 52.74; H, 7.74; N, 10.25. Found: C, 52.70; H, 7.74; N, 10.23.

(3*R*,4*S*)-III-Mosher: ^{1}H NMR ($CDCl_3$, TMS): δ 1.08 (s, 3H, H_3-8); 1.18 (s, 3H, H_3-7); 1.23 (s, 3H, H_3-6); 1.28 (s, 3H, H_3-9); 1.96 (m, 1H, H-3); 2.62 (ddd, 1H, $^3J_{3,4} = 11.6$ Hz, $^3J_{4,11x} = 4.6$ Hz and $^3J_{4,11y} = 9.2$ Hz, H-4); 3.51 (s, 3H, OMe); 4.16 (dd, 1H, $^3J_{3,10x} = 6.6$ Hz and $^2J_{10x,10y} = 11.8$ Hz, H_x-10); 4.20–4.32 (m, 2H, $^3J_{4,11x} = 4.6$ Hz, $^3J_{4,11y} = 9.2$ Hz and $^2J_{11x,11y} = 14.1$ Hz, H-11); 4.53 (dd, 1H, $^3J_{3,10y} = 5.8$ Hz and $^2J_{10x,10y} = 11.8$ Hz, H_y-10); 7.36–7.52 (m, 5H, ArH).

(3*S*,4*R*)-III-Mosher: ^{1}H NMR ($CDCl_3$, TMS): δ 1.00 (s, 3H, H_3-8); 1.02 (s, 3H, H_3-7); 1.10 (s, 3H, H_3-9); 1.12 (s, 3H, H_3-6); 1.76 (ddd, 1H, $^3J_{3,4} = 11.3$ Hz, $^3J_{3,10x} = 6.7$ Hz and $^3J_{3,10y} = 6.0$ Hz, H-3); 2.46 (ddd, 1H, $^3J_{3,4} = 11.3$ Hz, $^3J_{4,11x} = 4.5$ Hz and $^3J_{4,11y} = 9.5$ Hz, H-4); 3.52 (s, 3H, OMe); 4.20 (dd, 1H, $^3J_{4,11x} = 4.5$ Hz and $^2J_{11x,11y} = 13.7$ Hz, H_x-11); 4.27 (dd, 1H, $^3J_{3,10x} = 6.7$ Hz and $^2J_{10x,10y} = 11.6$ Hz, H_x-10); 4.30 (dd, 1H, $^3J_{4,11y} = 9.5$ Hz and $^2J_{11x,11y} = 13.7$ Hz, H_y-11); 4.37 (dd, 1H, $^3J_{3,10y} = 6.0$ Hz and $^2J_{10x,10y} = 11.6$ Hz, H_y-10); 7.36–7.52 (m, 5H, ArH).

VI: FT-IR (KBr, cm^{-1}): 2976 (br), 1732, 1464, 1412, 1348, 1304, 1252, 1200, 1152. Calcd for $C_9H_{16}NO_3$: C, 58.05; H, 8.66; N, 7.52. Found: C, 58.13; H, 8.67; N, 7.54.

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