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A chemo-enzymatic synthesis of the N-terminal aminoacid lactone of Nikkomycin B

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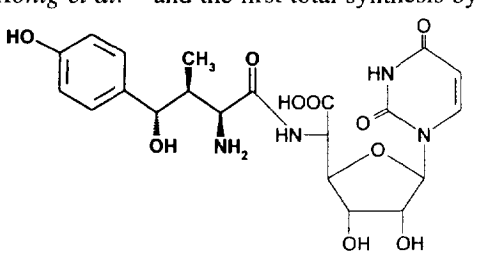
Abstract: (2*S*,3*S*,4*S*)-4-(4-Acetoxyphenyl)-2-amino-3-methylbutan-4-olide (***S,S,S*)-11**, a precursor for the aminoacid part of Nikkomycin B, was prepared in a five step synthesis in 12% yield, involving two enzymatic steps. First enantiopurity was achieved by resolution of racemic ester **3** using protease from *Aspergillus oryzae* and second in lactone (***RS,S,S*)-10** the N-acetyl group was removed distereo- and chemoselectively to give the final aminolactone (***S,S,S*)-11** by application of acylase from *Aspergillus sp.* © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

Nikkomycin B **1** which inhibits the growth of several fungi has been isolated from the fermentation broth of *Streptomyces tendae* Tü 901. This compound represents one of a class of similar nucleoside antibiotics[1] which are potent chitin synthetase inhibitors and exhibit fungicidal, insecticidal and acaricidal activities[1–3]. Nikkomycin B **1** was first isolated 1976 by *Dähn et al.*[3], the first partial synthesis was performed by *König et al.*[4a] and the first total synthesis by *Barrett et al.*[4d].



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Several synthetic pathways for the synthesis of the racemic or enantiomerically pure N-terminal side chain of Nikkomycin B **1**, 2-amino-4-hydroxy-4-(4-hydroxyphenyl)-3-methylbutanoic acid and derivatives, are known in the literature.

In the first synthesis by *König et al.*[5] the corresponding carboxamides with (*R*)-α- or (*S*)-α-phenylethylamine were used for the resolution of ***rac*-3**. The diastereomers were separated by fractional crystallisation and the amides cleaved afterwards by alkaline hydrolysis. Recently, the preparation of lactones **10** and **11**, although with other protective groups and in part racemic, has been also described using other synthetic strategies. *Weinreb et al.*[4b] started with (*E*)-p-methoxycinnamyl chloride, *Barrett et al.*[4c] used the reaction of 4-pivaloyloxybenzaldehyde with (−)-(*E*)-crotyldiisopinocamphenylborane as chiral reagent or 2-hydroximino butanamides[4d] and *Barluenga et al.*[4e] started with furyl substituted 1-aza-butadienes. *Hanaoka et al.*[4f] applied chromonium(0) complexed benzaldehydes, *Palomo et al.*[4g] started with 3,4-substituted α-lactames, *Akita et al.*[4h] used a similar pathway as *Barrett et al.*[4d] combined with enzymatic hydrolysis of an intermediate monoacetylated diol and finally *Bloch et al.*[4i] started the synthesis with enantiomerically pure tricycles.

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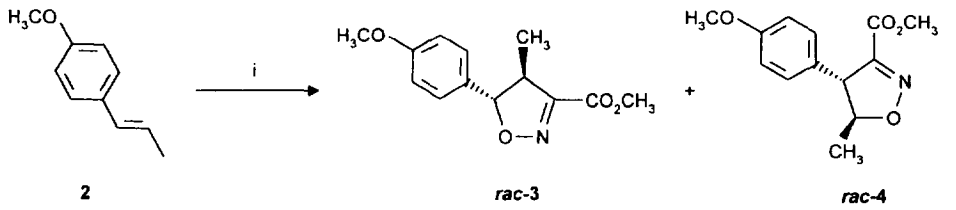
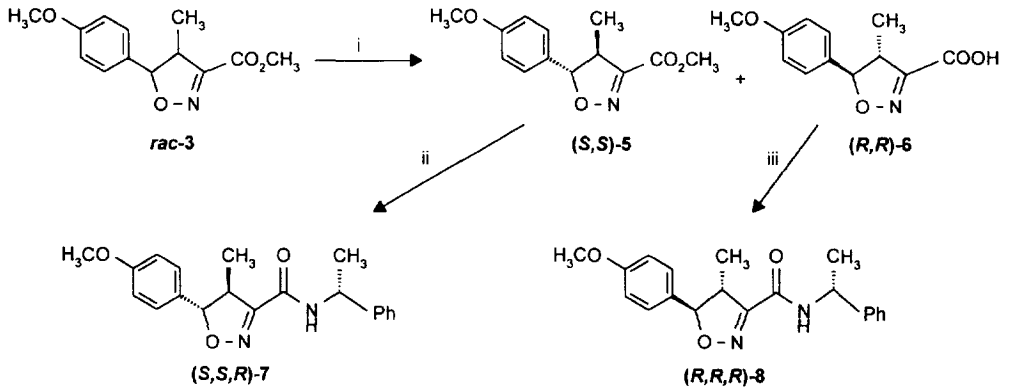


2 ***rac*-3** ***rac*-4**

Scheme 1. i (a) $CH_3O_2CC(Cl)=NOH$, (b) Et_3N/Et_2O; only one enantiomer is drawn.



***rac*-3** **(*S,S*)-5** **(*R,R*)-6**

(*S,S,R*)-7 **(*R,R,R*)-8**

Scheme 2. i Protease from *Aspergillus oryzae*, toluene/buffer pH 7.0; ii (a) NaOH, (b) $CHCl_2OCH_3$, (c) (*R*)-α-phenylethylamine; iii (a) $CHCl_2OCH_3$, (b) (*R*)-α-phenylethylamine.

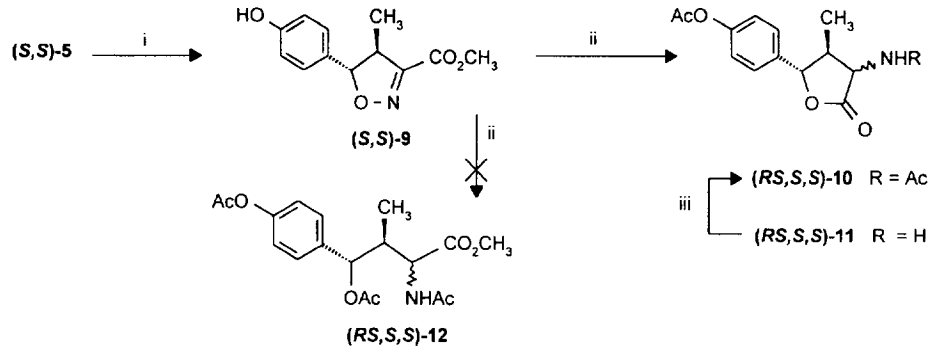
In continuation of our work on the synthesis of carbocyclic nucleoside analogues of biological interest[6] and on the preparation of various isoxazolines[7] we present a short and effective strategy involving two enzymatic steps for the resolution of enantiomeric and diastereomeric intermediates of the N-terminal side chain of Nikkomycin B **1**.

Results and discussion

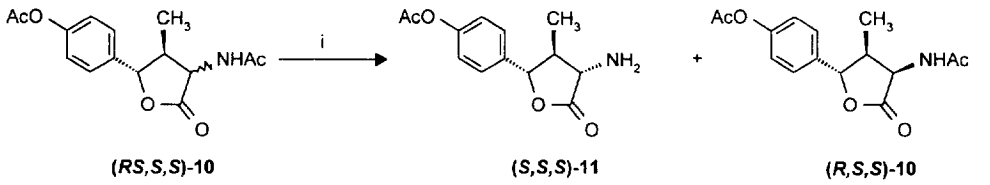
For the first step in the preparation of lactone **(*S,S,S*)-11** as the final intermediate for the synthesis of the aminoacid part of Nikkomycin B **1** a 1,3-dipolar cycloaddition was performed as described by *König et al.*[8]. Investigations by *Huisgen*[9] showed when starting with pure (*E*)-anethol **2** only two of the four possible products are formed. Instead of the ethyl chlorooximidoacetate described we used methyl chlorooximidoacetate (prepared as published by *Kozikowski*[10]), because we found, that the allergy causing by-product[7] bis(methoxycarbonyl)furoxan could be separated more easily than bis(ethoxycarbonyl)furoxan[5] (Scheme 1).

The methyl ester has also the advantage of a more rapid saponification with lipases (see Scheme 2) and for the determination of the enantiomeric excess of acid **(*R,R*)-6** by NMR methods using the $Eu(hfc)_3$ shift reagent the methyl ester (first formed with CH_2N_2) is more easily prepared than an ethyl ester *via* the corresponding acid chloride (for the preparation of the acid chloride see Scheme 2). The ratio of the regioisomers ***rac*-3** to ***rac*-4** was 93 to 7 in favour of isoxazoline ***rac*-3** and the relative configuration of the enantiomeric mixture was determined to be *trans* by correlation of the ^1H coupling constants with that of the corresponding ethyl ester of ***rac*-3** prepared by *König et al.*[5].

The resolution was performed with the protease from *Aspergillus oryzae* (Sigma Chemical Co., Type XXIII) in a biphasic system toluene/phosphate buffer (0.1 *M*, pH 7.0) and the pH value was maintained at pH 7.0 with 1 *N* sodium hydroxide. The reaction was run to 50% conversion.



Scheme 3. i (a) BBr_3, (b) MeOH; ii Zn/Cu HOAc/Ac_2O; iii Ac_2O/Pyr.



Scheme 4. i Acylase EC 3.5.1.14 from *Aspergillus sp.* immobilised on Eupergit C, buffer pH 7.0.

Determination of the absolute configuration of ester **(*S,S*)-5** and carboxylic acid **(*R,R*)-6** was performed by the synthesis of the corresponding carboxamides with (*R*)-α-phenylethylamine and comparison of their specific rotation values with the known[5] **(*S,S,S*)-8** enantiomer with that of **(*R,R,R*)-8** and of the known[5] **(*R,R,S*)-7** enantiomer with that of **(*S,S,R*)-7**.

The methyl ether of isoxazoline **(*S,S*)-5** was cleaved with $BBr_3/CH_2Cl_2/-80°C$[5]. Quenching of the reaction mixture with MeOH afforded **(*S,S*)-9** in 87% yield. Isoxazoline **(*S,S*)-9** was reduced in a similar manner as described by *König*[5,11] with Zn/Cu couple in HOAc/Ac_2O. We could only isolate the lactones **(*RS,S,S*)-10** and about 5%–10% of the N-unprotected lactones **(*RS,S,S*)-11**. The aminoacid **(*RS,S,S*)-12** was not detected[5]. Lactones **(*RS,S,S*)-11** were acetylated with Ac_2O/Py to give **(*RS,S,S*)-10**. Lactone **(*R,S,S*)-11** was isolated for characterisation (Scheme 3).

The diastereomeric separation was performed on **(*RS,S,S*)-10** with acylase EC 3.5.1.14 from *Aspergillus sp.*[13] immobilised on Eupergit C (supplier Fluka) to yield enantiomerically pure amine **(*S,S,S*)-11** in 48% yield. Unreacted acetylated amine **(*R,S,S*)-10** was also isolated but not fully characterised. The last enzymatic step also has the great advantage that the deprotection of the amine is done with the right enantiomer, because alkaline deprotection might cause epimerisation at carbon atom 2[4d] (Scheme 4).

The relative configuration of amine **(*S,S,S*)-11** was determined by correlation of known[4e,g] ^1H NMR shift values and corresponding coupling constants. Since the absolute configuration of carbon atoms 3 and 4 of the lactone ring has been assigned at an earlier stage of the reaction sequence (see Scheme 2) the stereochemistry was unequivocally determined. For the opening of lactone **(*S,S,S*)-11** and derivatives and for further reactions see *Barrett et al.*[4d], *König et al.*[5], *Saksena et al.*[12] and *Jäger et al.*[14].

Experimental

Melting points were obtained on a Büchi–Tottoli apparatus and are uncorrected. Column chromatography was performed on silica gel 60, 230–400 mesh (Merck, Darmstadt), and TLC on aluminium sheets coated with silica gel 60 F_{254} (Merck, Darmstadt). Optical rotations were determined on a Jasco DIP 370 polarimeter. GC analysis was performed on a DANI 8500 gas chromatograph, column DB 1701. ^1H and ^{13}C NMR spectra were recorded on a Bruker MSL 300 instrument (TMS as internal standard, δ-values in ppm, $CDCl_3$ as solvent unless otherwise indicated). IR spectra were determined as films on a Bomem Michelson 100 FT-spectrophotometer. MS spectra were recorded on a Kratos Profile spectrometer. The elemental analyses were performed at the Institute of Organic Chemistry, University of Graz. The enantiomeric excesses for esters were determined by ^1H NMR spectroscopy using the Eu(hfc)$_3$ shift reagent, acids were first esterified with CH_2N_2.

*(4*RS*,5*RS*)-Methyl 4,5-dihydro-5-(4-methoxyphenyl)-4-methylisoxazole-5-carboxylate,* **rac-3**

To a vigorously stirred solution of 10.0 g (72.7 mmol) of methyl chlorooximidoacetate and 10.0 g (67.5 mmol) of (*E*)-1-methoxy-4-propenylbenzene **2** in 170 ml of dry diethyl ether was added dropwise over a 5 h period triethylamine (11.0 ml, 78.9 mmol) in 100 ml of dry diethyl ether. Triethylamine hydrochloride was filtered off, the organic layer was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product, a mixture of 93% of ***rac*-3** and 7% of ***rac*-4** (determined *via* gas chromatographic analysis) was purified by recrystallisation from cyclohexane to yield 14.0 g (83%) of ***rac*-3** (purity>99%). ^1H NMR: δ 1.44 (d, 3H, J=6.9 Hz, CH–C*H*$_3$), 3.50 (m, 1H, C*H*–CH$_3$), 3.81 (s, 3H, –COOC*H*$_3$), 3.90 (s, 3H, COOCH$_3$), 5.22 (d, 1H, J=8.0 Hz, Ar–C*H*), 6.90 (d, 2H, J=8.7 Hz, H-3′ and H-5′), 7.23 (d, 2H, J=8.7 Hz, H-2′ and H-6′). ^{13}C NMR: δ 17.15 (C4-*C*H$_3$), 49.55 (C-4), 52.63 (COO*C*H$_3$), 55.43 (OCH$_3$), 92.52 (C-5), 114.44 (C-3′ and C-6′), 127.38 (C-3′ and C-5′), 131.07 (C-1′), 154.62 (C=N), 160.18 (C-4′), 161.12 (C=O). Anal. calcd. for $C_{13}H_{15}NO_4$ [249.27]: C, 62.64%; H, 6.07%; N, 5.62%. Found: C, 62.55%; H, 5.94%; N, 5.52%.

*(4*S*,5*S*)-(+)-Methyl 4,5-dihydro-5-(4-methoxyphenyl)-4-methylisoxazole-3-carboxylate* **(S,S)-5** *and (4*R*,5*R*)-(−)-4,5-dihydro-5-(4-methoxyphenyl)-4-methylisoxazole-3-carboxylic acid* **(R,R)-6**

Protease from *Aspergillus oryzae* (2.0 g, Sigma Chemical Co., Type XXIII) was suspended in a two-phase system prepared from 200 ml of phosphate buffer (0.1 *M*, pH 7.0) and 150 ml of toluene. The suspension was stirred vigorously and the pH value was kept at pH 7.0 with 1.0 *N* sodium hydroxide by means of an autoburette. Isoxazoline ***rac*-3** (3.0 g, 12 mmol) was added and consumption of alkali was followed. After 50% conversion (about 15 h) the phases were filtered over a pad of Celite® 545 (Merck) and the Celite® was washed with 50 ml of toluene. The layers were separated and the aqueous phase extracted twice with 100 ml of toluene each. The combined organic phases were dried over Na_2SO_4, concentrated *in vacuo*, and the crude ester **(*S,S*)-5** was purified by recrystallisation from cyclohexane to yield 1.06 g (35%) of enantiomerically pure **(*S,S*)-5**. $[\alpha]_D^{20}$=+522 (c 1.06, $CHCl_3$; e.e.>99%). mp 63–64°C. NMR — data were identical to those of compound ***rac*-3**.

The aqueous layer was acidified with HCl conc. to pH 2, extracted with ethyl acetate, filtered over a pad of Celite® 545 (Merck) and the Celite® was washed with 50 ml of ethyl acetate. The layers were separated and the aqueous phase extracted twice with 50 ml of ethyl acetate each. The combined organic phases were dried over Na_2SO_4, concentrated *in vacuo* to yield 2.70 g (49%) of **(*R,R*)-6**. $[\alpha]_D^{20}$=−274 (c 1.5, $CHCl_3$; e.e. 91.4%). mp 117°C. All other physical data were in accordance with the literature[8].

*(4*S*,5*S*)-(+)-4,5-Dihydro-5-(4-methoxyphenyl)-4-methyl-*N*-[(*R*)-1-phenylethyl]isoxazole-3-carboxamide* **(S,S,R)-7**

To a solution of 3.93 g (15.8 mmol) of **(*S,S*)-5** in 10 ml of methanol were added at 0°C 40 ml of 2.5 *N* NaOH and stirred for 2 h. The reaction mixture was acidified with 2 *N* HCl and extracted with ethyl acetate (3 × 100 ml). The combined organic layers were dried over Na_2SO_4 and evaporated *in*

vacuo. The crude carboxylic acid was treated with 2.0 ml (22 mmol) of dichloromethylmethyl ether and stirred for 2 h at 80°C. The solution was diluted with 40 ml of CH_2Cl_2, cooled to −78°C and 3.0 g (12 mmol) of (*R*)-α-phenylethylamine and 3 ml of Et_3N, diluted with 10 ml of CH_2Cl_2, were added. After 2 h the reaction mixture was extracted with 50 ml of water and the aqueous layer was reextracted twice with 50 ml of CH_2Cl_2. The combined organic layers were extracted twice with 2 *N* HCl (2 × 50 ml each), washed with $NaHCO_3$ solution (10 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and chromatographic purification of the residue (toluene/ethyl acetate 9/1 v/v) and an additional recrystallisation from hexane/ethyl acetate yielded 4.40 g (82.5%) of compound **(*S,S,R*)-7**. $[\alpha]_D^{20}$=+309 (c 1.0, $CHCl_3$). mp 121–122°C. All other physical data were in accordance with the enantiomer described by *König*[5].

*(4*R,*5*R*)-(−)-4,5-Dihydro-5-(4-methoxyphenyl)-4-methyl*-N-*[(*R*)-1-phenylethyl]isoxazole-3-carboxamide* **(R,R,R)-*8***

1.0 g of carboxylic acid **(*R,R*)-6** were treated with 0.50 g (4.4 mmol) of dichloromethylmethyl ether and stirred for 2 h at 80°C. The solution was diluted with 40 ml of CH_2Cl_2, cooled to −78°C and 0.60 g (5 mmol) of (*R*)-α-phenylethylamine and 0.5 ml of Et_3N, diluted with 10 ml of CH_2Cl_2, were added. After additional 2 h the reaction mixture was extracted with 50 ml of water and the aqueous layer was reextracted twice with 30 ml of CH_2Cl_2. The combined organic layers were extracted twice with 2 *N* HCl (2 × 20 ml each), washed with $NaHCO_3$ solution (5 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and chromatographic purification of the residue (toluene/ethyl acetate 9/1 v/v) and an additional recrystallisation from hexane/ethyl acetate yielded 0.82 g (57%) of compound **(*R,R,R*)-8**. $[\alpha]_D^{20}$=−186 (c 1.0, $CHCl_3$). mp 105–107°C. All other physical data were in accordance with the enantiomer described by *König*[5].

*(4*S,*5*S*)-(+)-Methyl 4,5-dihydro-5-(4-hydroxyphenyl)-4-methylisoxazole-3-carboxylate* **(S,S)-*9***

A solution of 1.0 g of **(*S,S*)-5** in 20 ml CH_2Cl_2 was cooled to −80°C and under vigorous stirring a 10-fold excess of BBr_3 (1 *N* in dichloromethane) was added through a syringe under argon atmosphere. The solution was slowly warmed to room temperature (1 h) and stirred at 20°C for two hours (until complete conversion). The resulting dark brown solution was again cooled to −80°C and carefully quenched by the addition of 50 ml of dry methanol. After warming up to room temperature, the solution was stirred overnight. Solvents were removed *in vacuo* and the resulting brown oil was treated 5 times with 20 ml of dry methanol each, followed by evaporation. The remaining brown residue was purified by flash chromatography (ethyl acetate/petrol ether 2/1 v/v) to yield 0.82 g (87%) of **(*S,S*)-9** as slightly yellow oil. $[\alpha]_D^{20}$=+71.5 (c 1.0, $CHCl_3$). ^{1}H NMR: δ 1.39 (d, 3H, J=6.9 Hz, CHC*H*$_3$), 3.46 (m, 1H, C*H*CH$_3$), 3.84 (s, 3H, CH$_3$), 5.17 (d, 1H, J=8.3 Hz, –CH–O), 6.84 (d, 2 × 1H, J=8.4 Hz, H-2′ and H-6′), 7.10 (d, 2 × 1H, J=8.4 Hz, H-3′ and H-5′), 7.27 (bs, 1H, OH). ^{13}C NMR: δ 16.95 (CH*C*H$_3$), 49.17 (C-4), 52.83 (COO*C*H$_3$), 92.73 (C-5), 115.92 (C-3′ and C-5′), 127.54 (C-2′ and C-6′), 130.21 (C-1′), 154.78 (C=N), 156.73 (C-4′), 161.15 (C=O). Anal. calcd. for $C_{12}H_{13}NO_4$ [235.24]: C, 61.27%; H, 5.57%; N, 5.95%. Found: C, 61.11%; H, 5.87%; N, 5.92%.

*(2*RS,*3*S,*4*S*)-4-(4-Acetoxyphenyl)-2-acetylamino-3-methylbutan-4-olide* **(RS,S,S)-*10***

Preparation of the Zn/Cu couple

20 g of Zn-powder were activated under stirring for 10 min with 1 *N* HCl, filtered through a glass filter funnel and washed with dry MeOH (50 ml each) for 3 times. This activated Zn powder was suspended in 100 ml of MeOH, 100 ml of saturated Cu(II)acetate in methanol were added and stirred until decolourisation occurred. The layer was separated and another 100 ml of saturated Cu(II)acetate were added. This procedure was repeated until no more decolourisation occurred (about 10 times). The red Zn/Cu couple was filtered under N_2 atmosphere through a glass filter funnel, washed with dry MeOH twice (50 ml each), dried *in vacuo* to yield 11.7 g of highly active Zn/Cu couple. To a solution of 0.50 g (2.13 mmol) of **(*S,S*)-9** in a mixture of 15 ml of acetic acid and 7.5 ml of acetic anhydride under N_2-atmosphere was added freshly prepared Zn/Cu couple in small portions (about

0.5 g) every 30 min (the reaction was monitored by TLC) until complete turnover (about 7 h). The solution was filtered, washed twice with 30 ml of CH_2Cl_2, concentrated *in vacuo* and coevaporated with toluene three times to remove traces of acetic acid and acetic anhydride. The remaining residue was a mixture of about 90%–95% of amides (***RS,S,S*)-10** and 5%–10% of amines (***RS,S,S*)-11.** The remaining residue was diluted with 20 ml of CH_2Cl_2 and 3.3 ml (42.5 mmol) of pyridine, cooled to 0°C and 2.0 ml (21.3 mmol) of acetic anhydride were added. The solution was stirred at room temperature overnight. The reaction mixture was diluted with 100 ml of CH_2Cl_2, extracted with 1 *N* HCl, washed with saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated to yield in quantity 0.62 g of a diastereomeric mixture of (***RS,S,S*)-10**. NMR data were not recorded. EIMS z/e (relative intensity %): 291 (M^+, 40), 249 (44), 232 (9), 221 (12), 204 (8), 190 (22), 162 (53), 146 (62), 134 (78), 121 (100), 100 (73), 57 (49), 43 (92). Anal. calcd. for $C_{15}H_{17}NO_5$ [291.30]: C, 61.85%; H, 5.88%; N, 4.81%. Found: C, 61.42%; H, 5.97%; N, 4.62%.

(2R,3S,4S)-4-(4-Acetoxyphenyl)-2-amino-3-methylbutan-4-olide **(R,S,S)-*11***

Amine (***R,S,S*)-11** was isolated from another reaction mixture (as described above) before acetylation. ^{1}H NMR (CD_3OD): δ 1.03 (d, 3H, J=7.0 Hz, $CHCH_3$), 2.07 (s, 3H, $OCOCH_3$), 2.70 (m, 1H, $CHCH_3$), 3.38 (m, 2H, $CD_3OH \cong NH_2$), 4.61 (d, 1H, J=8.9 Hz, H-2), 5.19 (d, 1H, J=6.7 Hz, H-4), 6.85 (d, 2 × 1H, J=8.5 Hz, H-2′ and H-6′), 7.23 (d, 2 x 1H, J=8.5 Hz, H-3′ and H-4′). ^{13}C NMR (CD_3OD): δ 9.50 ($CHCH_3$), 22.50 (CH_3CO), 41.38 (C-3), 56.18 (C-2), 83.04 (C-4), 116.57 (C-3′ and C-5′), 127.98 (C-1′), 128.09 (C-2′ and C-6′), 158.65 (C-4′), 173.90 and 177.11 (C=O). IR (neat, KBr) 3292, 2945, 1765, 1656, 1518, 1451, 1373, 1214, 1166, 978, 838 (cm^{-1}).

(2S,3S,4S)-(−)-4-(4-Acetoxyphenyl)-2-amino-3-methylbutan-4-olide **(S,S,S)-*11***

0.50 g (1.72 mmol) of (***RS,S,S*)-10** were diluted with 125 ml of K_2HPO_4/KH_2PO_4 buffer pH 7.00 (supplied from Riedel-de Haen) and treated with 0.27 mg of acylase (acylase EC 3.5.1.14 from *Aspergillus sp.* immobilised on Eupergit C, supplier Fluka). The reaction course was monitored *via* TLC ($CHCl_3$/MeOH 6/1 v/v). After complete turnover (about 48 h) the reaction mixture was quenched with 2 ml of Et_3N and extracted with CH_2Cl_2. The combined organic extracts were concentrated *in vacuo* to yield after flash chromatography ($CHCl_3$/MeOH 40/1 v/v) 204 mg (48%) of amine (***S,S,S*)-11** as colourless foam. $[\alpha]_D^{20} = -53.9$ (c 0.5, MeOH). ^{1}H NMR (CD_3OD): δ 1.10 (d, 3H, J=6.6 Hz, $CHCH_3$), 2.07 (s, 3H, $OCOCH_3$), 2.58 (ddq, 1H, $J_{3,2}$=11.7 Hz, $J_{3,4}$=10.2 Hz, J_q=6.6 Hz, $CHCH_3$), 3.34 (m, 2H, $CD_3OH \cong NH_2$), 4.46 (d, 1H, J=11.7 Hz, H-2), 4.96 (d, 1H, J=10.2 Hz, H-4), 6.85 (d, 2 × 1H, J=8.5 Hz, H-2′ and H-6′), 7.29 (d, 2 × 1H, J=8.5 Hz, H-3′ and H-4′). ^{13}C NMR (CD_3OD): δ 13.92 ($CHCH_3$), 22.79 (CH_3CO), 46.39 (C-3), 58.25 (C-2), 86.96 (C-4), 116.78 (C-3′ and C-5′), 129.34 (C-1′), 129.94 (C-2′ and C-6′), 159.84 (C-4′), 173.82 and 176.42 (C=O). IR (neat, KBr) 2923, 2853, 1760, 1651, 1517, 1456, 1374, 1239, 1165, 981, 834 (cm^{-1}). Anal. calcd. for $C_{13}H_{15}NO_4$ [249.27]: C, 62.64%; H, 6.07%; N, 5.62%. Found: C, 62.42%; H, 5.87%; N, 5.82%.

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