Synthesis and Enzymatic Resolution of C^{α}-Dialkylated α-Azido Carboxamides: New Enantiopure α-Azido Acids as Building Blocks in Peptide Synthesis

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Abstract: α-Azido carboxylic acids have recently emerged as versatile *N*-protected equivalents for α-amino acids, especially valuable when the sterically hindered C^{α}-dialkylated α-amino acids have to be incorporated. Unsymmetrically substituted C^{α}-dialkylated α-azido carboxylic acids can be obtained in enantiomerically pure form by enzymatic resolution of α-azido carboxamides. A L-amidase from *Ochrobactrum anthropi* NCIMB 40321 accepts 2-azido-2,4-dimethylpentanamide as the substrate and provides both the corresponding *S*-configured α-azido carboxylic acid and the *R*-configured α-azido carboxamide in excellent enantiomeric purity. The former is a valuable synthetic precursor of α-methylleucine [(α-Me)Leu] in peptide synthesis, as demonstrated by the successful synthesis of a (α-Me)Leu containing efrapeptin C analogue.

Key words: azides, bioorganic chemistry, enantiomeric resolution, enzymes, peptides

α-Azido carboxylic acids may be used as synthetic precursors for α-amino acids and are thus equivalent to *N*-protected α-amino acids, which are the standard building blocks in peptide synthesis for the stepwise elongation of the peptide chain from the *C*-terminus to the *N*-terminus. There are several examples for the application of α-azido carboxylic acids in peptide synthesis. The solid phase synthesis of a large number of small peptides has been reported by Pelletier et al.[2] using α-azido-substituted derivatives as substitutes for amino acids. The azido acids are coupled following the DCC–HOBt protocol (*N*,*N*′-dicyclohexylcarbodiimide/*N*-hydroxybenzotriazole) and the azido group is reduced on the solid support by treatment with trimethylphosphine in aqueous dioxane. DalPozzo et al.[3] reported the solution phase synthesis of a homooctamer of α-methylvaline using the corresponding azido acid bromide. The use of α-azidoisobutyryl chloride (Azib-Cl) as building block for the incorporation of Aib-residues in solid phase peptide synthesis has been reported by Meldal et al.[4] We used a similar protocol for the total synthesis of the naturally occurring peptide antibiotic efrapeptin C.[5] The efrapeptins are Aib-rich peptides produced by the fungus *Tolypocladium niveum*. They are inhibitors of F_1-ATPase,[6] show insecticidal[7] and antiproliferative[8] properties and are active against the malaria pathogen *Plasmodium falciparum*.[9] Recently, we reported on the synthesis of ^{2}H-labelled α-azidoisobutyryl chloride (D_6-Azib-Cl) as D_6-Aib equivalent building block in peptide synthesis.[10] Thus it was shown that α-azido carboxylic acids are versatile building blocks especially useful for the synthesis of sterically hindered peptide sequences, i.e. for the incorporation of C^{α}-dialkylated amino acids.

For the synthesis of peptides containing chiral C^{α}-dialkylated amino acids, however, the necessary building blocks have to be available in enantiopure form. C^{α}-Dialkylated amino acids can be obtained by enzymatic resolution of the racemic amino acid carboxamides.[11] They can be converted into the α-azido acids by diazo transfer with triflyl azide.[2]

We wondered whether the enantiopure α-azido acids could be obtained alternatively by direct enzymatic resolution of α-azido carboxamides. The enzymatic resolution of α-H-α-azido carboxylic acids with an amidase from *Pseudomonas putida* has already been reported for two substrates,[12] but the resolution of C^{α}-dialkylated α-azido carboxamides has not yet been described.

In this paper we report on the synthesis and enzymatic resolution of two C^{α}-dialkylated α-azido carboxamides, 2-azido-2,4-dimethylpentanamide (**6a**) and 2-azido-2-methyl-3-phenylpropanamide (**6b**). The enantiopure (*S*)-2-azido-2,4-dimethylpentanoic acid [(*S*)-**5a**] thus obtained was used as a building block for the introduction of an α-methylleucine residue into an analogue of efrapeptin.

The synthesis of the racemic substrates **6a** and **6b** (Scheme 1) for the enzymatic resolutions started from simple ethyl esters **1** bearing already the side chains at the α-carbon atom. The α-methyl groups were introduced by alkylation of the ester enolates of **1** with iodomethane. The esters **2** obtained were again converted to the enolates and treated with CBr_4,[13] thus yielding α-bromo esters **3**. Substitution of the bromide in **3** by treatment with sodium azide in dimethyl sulfoxide at room temperature overnight proceeded smoothly and gave rise to the α-azido esters **4**. The ethyl esters **4** were saponified by reaction with KOH in EtOH. The carboxylic acids **5** were activated by conversion to the mixed anhydride with ethyl chloroformate and reacted with NH_3 to yield the desired C^{α}-dialkylated α-azido carboxamides **6**, which were obtained in high purity after recrystallization.

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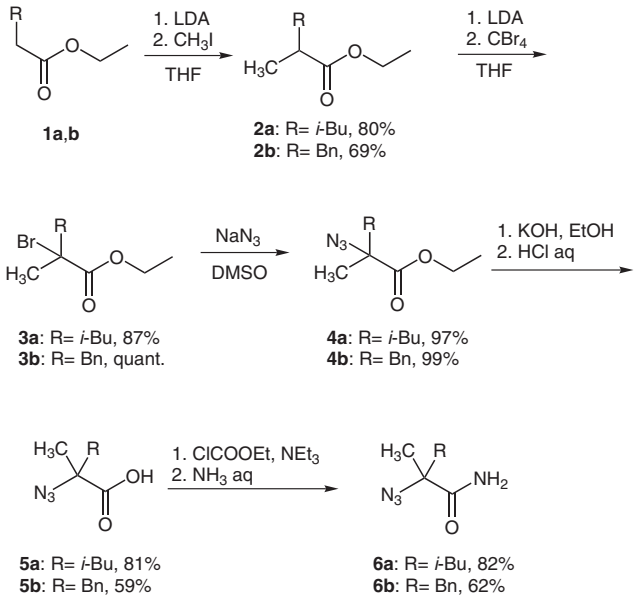
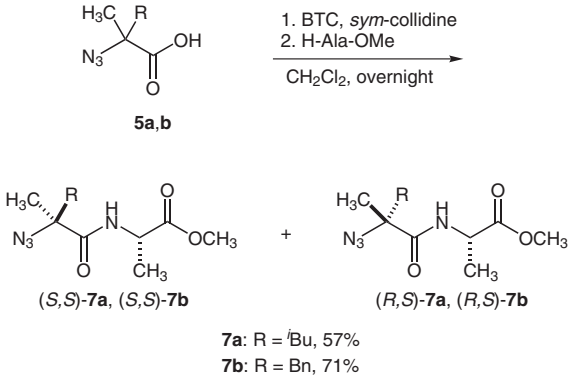


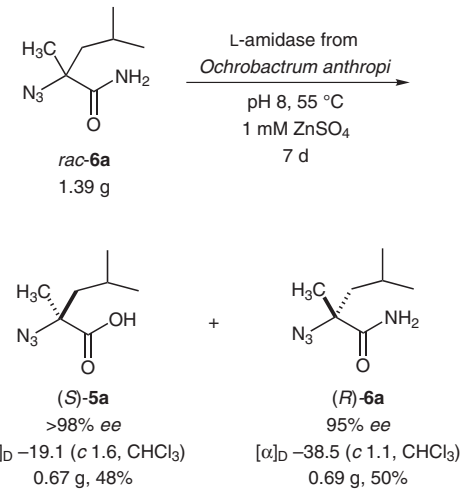
Scheme 1 Synthesis of α-azido carboxamides **6**

As it was planned to use the enantiomerically pure carboxylic acids **5** as building blocks in peptide syntheses, we investigated the coupling of the racemic mixtures to L-alanine methyl ester. Best results were obtained by activation of the carboxylic acids with BTC (bis(trichloromethyl) carbonate, triphosgene) in the presence of *sym*-collidine (Scheme 2).[14] After overnight reaction and chromatographic workup, the diastereomeric mixtures **7a** and **7b** were isolated in good yields.



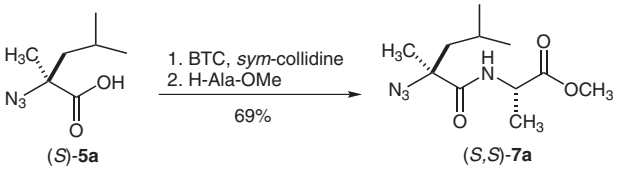
Scheme 2 Coupling of the racemic carboxylic acids **5** to L-alanine methyl ester

Preliminary studies on the enzymatic resolution of 2-azido-2,4-dimethylpentanamide (**6a**) and 2-azido-2-methyl-3-phenylpropanamide (**6b**) on an analytical scale revealed that the enzymatic activities are very low. This was expected as compounds **6** profoundly differ from the natural substrates of amidases.[11] In the case of **6b** only a moderate stereoselectivity was observed, while the resolution of **6a** proceeded with very high stereoselectivity. Therefore, the resolution of this compound was also performed on a preparative scale (Scheme 3). The carboxamide *rac*-**6a** was incubated at 55 °C and pH 8 for several days with the L-amidase from *Ochrobactrum anthropi* NCIMB 40321, which was obtained by expression of the *lam*A gene in *E. coli*. The carboxylic acid (*S*)-**5a** and the carboxamide (*R*)-**6a** were isolated in yields of 48% and 50%, respectively. The enantiomeric excess values of (*S*)-**5a** and (*R*)-**6a** were determined by HPLC analysis on a chiral stationary phase as being 96% and 95%, respectively, indicating enzymatic conversion slightly higher than 50% (E-ratio 150). The proof of the absolute configuration of (*S*)-**5a** as well as a confirmation of the enantiomeric excess of >98% was obtained by conversion to the corresponding α-methylleucine by catalytic hydrogenation and comparison with authentic samples of (*S*)-α-methylleucine[15] by chiral HPLC.



Scheme 3 Enzymatic resolution of α-azido carboxamide **6a**

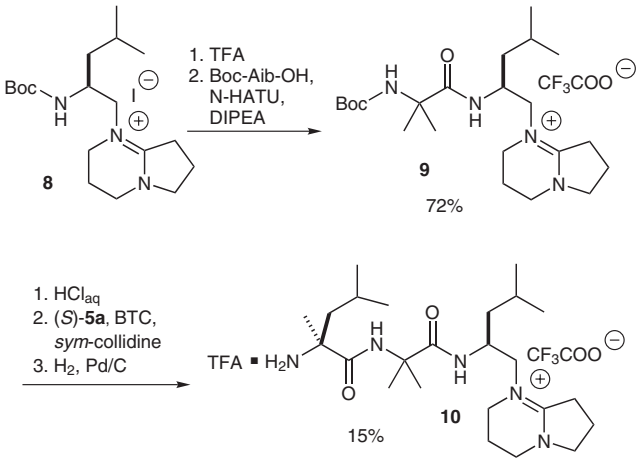
Coupling of the enantiomerically pure 2-azido-2,4-dimethylpentanoic acid [(*S*)-**5a**] with L-alanine methyl ester under the same conditions as described above for the racemic mixtures yielded the diastereomerically pure dipeptide (*S*,*S*)-**7a** (Scheme 4). There are no traces of (*R*,*S*)-**7a** or (*S*,*R*)-**7a** detectable in the NMR spectra of crude (*S*,*S*)-**7a**.



Scheme 4 Synthesis of diastereomerically pure dipeptide (*S*,*S*)-**7a**

Recently, we reported on the first total synthesis of the peptide antibiotic efrapeptin C **14a** (Scheme 6).[5] The full Aib-rich sequence was assembled by fragment condensations of an *N*-terminal fragment (Pip^1-Gly^8), a central fragment (Aib^9-Gly^{13}) and a *C*-terminal fragment containing the residues Leu^{14}-Aib^{15} as well as the unusual cationic head group derived from DBN and leucinol. In the course of our structure-activity relationship studies, we intended to incorporate (*S*)-**5a** in an analogue of efrapeptin

C containing α-methylleucine instead of leucine in position 14. The synthesis of the modified *C*-terminal fragment **10** is shown in Scheme 5. The Boc protected amino amidinium salt **8** was deprotected with TFA and one Aib residue was added by *N*-HATU mediated condensation with Boc-Aib-OH.



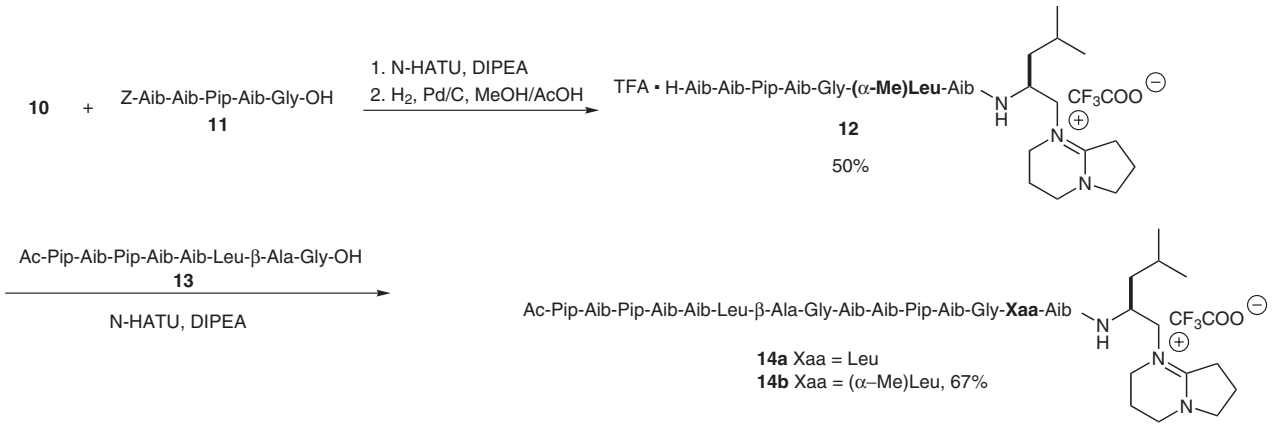
Scheme 5 Synthesis of the modified *C*-terminal fragment **10** of efrapeptin C

It was found that the use of TFA for the removal of the Boc group in **9** leads to the formation of trifluoroacetylated products in the following coupling reaction. Therefore, it was necessary to remove the Boc group in **9** by treatment with hydrochloric acid. In the next step the α-methylleucine residue was introduced by coupling of (*S*)-**5a** with deprotected **9** followed by catalytic hydrogenation giving rise to **10**.

With the modified fragment **10** in hand, the synthesis of the complete efrapeptin analogue **14b** was completed in the same way as described for the natural product **14a** (Scheme 6).[5] The *N*-terminally Z-protected central fragment **11** and the *N*-terminal fragment **13** were synthesized by solid phase methods employing α-azidoisobutyryl chloride as carboxy activated Aib equivalent. Condensation of **11** with **10** followed by hydrogenolytic cleavage of the Z group gave rise to compound **12**. The final segment condensation of **12** and **13** completed the synthesis of the efrapeptin analogue **14b**.

In conclusion we developed an efficient synthetic procedure for the synthesis of C^{α}-dialkylated α-azido carboxylic acids and the corresponding carboxamides starting from simple ethyl esters with overall yields up to 55% (4 steps) for the α-azido carboxylic acids and up to 45% (5 steps) for the corresponding carboxamides. We demonstrated that compounds **6a** and **6b** are substrates of an L-amidase from *Ochrobactrum anthropi* NCIMB 40321 and we could obtain the α-azido arboxylic acid (*S*)-**5a** in enantiopure form by enzymatic resolution of the carboxamide **6a**. Furthermore, we demonstrated the usefulness of C^{α}-dialkylated α-azido carboxylic acids as building blocks in peptide synthesis by the synthesis of small peptides and the incorporation of (*S*)-**5a** into an analogue of the peptide antibiotic efrapeptin.

All moisture and air-sensitive reactions were performed in flame-dried glassware under Ar or N_2. THF was distilled from sodium benzophenone ketyl immediately prior to use. CH_2Cl_2 was distilled over CaH_2. All other reagents were used as received. CAUTION: Low molecular weight azido compounds should be handled with great care. Differential scanning calorimetry analyses (DSC) of compounds **5** and **6** revealed that they start to decompose at temperatures around 100 °C with evolution of large amounts of heat. Flash column chromatography was carried out with silica gel 60 (40–63 μm) from Merck. IR spectra were recorded on a FT/IR-410 (Jasco) spectrometer from the neat oil film (NaCl plates) or from a solid in a KBr pellet. ^{1}H NMR spectra were recorded on AC-250-P or DRX-500 instruments (Bruker) in $CDCl_3$ solution, unless otherwise noted, at 250 MHz or 500 MHz; ^{13}C NMR spectra were recorded at 63 MHz or 126 MHz. TMS was used as an internal standard, shift values are reported in ppm. CI mass spectra were recorded on an Autospec X instrument (Vacuum Generators) using NH_3 as the reactant gas. ESI mass spectra were recorded on an Esquire 3000 ion trap instrument (Bruker). MALDI-ToF mass spectra were recorded on a Voyager-DE BioSpectrometry instrument (PerSeptive Biosystems) using 2,5-dihydroxybenzoic acid (DHB) as a matrix. Microanalyses were performed on a CHNS-932 (Leco) apparatus. Preparative RP-HPLC was performed with a Vydac 218TP102 efficiency protein and peptide C18-column (10 μm particle size, 250 ×



Scheme 6 Synthesis of the analogue **14b** of efrapeptin C (**14a**) containing α-methylleucine instead of leucine in position 14

22 mm) using mixtures of water and MeCN containing 0.1% TFA as the mobile phase.

Abbreviations: BTC: bis(trichloromethyl)carbonate; DBN: 1,5-diazabicyclo[4.3.0]non-5-ene; DCC: *N*,*N*′-dicyclohexylcarbodiimide; DIPEA: diisopropylethylamine; *N*-HATU: 1-[bis-(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-b]pyridine-3-oxide hexafluorophosphate; HOBt: *N*-hydroxybenzotriazole; TFA: trifluoroacetic acid.

Ethyl 2,4-Dimethylpentanoate (2a)

A solution of *n*-BuLi in hexane (1.6 M, 82.5 mL, 132 mmol, 1.1 equiv) was added dropwise at 0 °C to a stirred solution of diisopropyl amine (20.2 mL, 144 mmol, 1.2 equiv) in THF (200 mL). The mixture was stirred for 30 min at this temperature, cooled to –78 °C and ethyl 4-methylpentanoate (**1a**; Fluka, 19.9 mL, 120 mmol, 1.0 equiv) was added dropwise. The mixture was stirred for 30 min and then iodomethane (12.0 mL, 192 mmol, 1.6 equiv) was added slowly. The reaction mixture was allowed to warm up slowly to r.t. and was stirred overnight. The mixture was quenched with a sat. solution of NH_4Cl (200 mL) and petroleum ether (150 mL) was added. The phases were separated, the organic phase was dried (Na_2SO_4) and filtered. After evaporation of the solvent the residue was purified by vacuum distillation to afford a colorless liquid (15.1 g, 96 mmol, 80%); bp 99 °C/150 mbar [Lit.[16] bp 105 °C/175 mbar].

IR (neat): 2959, 2873, 1736, 1467, 1376, 1336, 1279, 1256, 1222, 1181, 1152, 1090, 1047, 1027 cm^{-1}.

^1H NMR (250 MHz): δ = 0.87 (d, *J* = 6.3 Hz, 3 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 1.13 (d, *J* = 6.9 Hz, 3 H), 1.20 (m, 1 H), 1.25 (t, *J* = 7.1 Hz, 3 H), 1.50–1.68 (m, 2 H), 2.49 (m, 1 H), 4.12 (q, *J* = 7.1 Hz, 2 H).

^{13}C NMR (63 MHz): δ = 14.3, 17.5, 22.5, 26.0, 37.7, 43.1, 60.1, 177.2.

MS (CI): *m*/*z* (%) = 176 (100), 159 (45).

Ethyl 2-Methyl-3-phenylpropanoate (2b)

Following the procedure described for **2a**, starting from 3-phenyl propionic acid ethyl ester **1b** (Merck, 17.6 mL, 100 mmol, 1.0 equiv) **2b** was obtained as a colorless liquid (13.3 g, 69 mmol, 69%) after vacuum distillation; bp 57–60 °C/1 × 10^{-1} mbar [Lit.[17] bp 68 °C/8 ×10^{-1} mbar].

IR (neat): 3086, 3063, 3029, 2978, 2936, 2876, 1733, 1605, 1496, 1454, 1376, 1350, 1284, 1252, 1174, 1117, 1028, 745, 700 cm^{-1}.

^1H NMR (250 MHz): δ = 1.15 (d, *J* = 6.6 Hz, 3 H), 1.18 (t, *J* = 7.1 Hz, 3 H), 2.60–2.80 (m, 2 H), 3.02 (m, 1 H), 4.08 (q, *J* = 7.1 Hz, 2 H), 7.10–7.32 (m, 5 H).

^{13}C NMR (63 MHz): δ = 14.1, 16.8, 39.8, 41.5, 60.3, 126.3, 128.3, 129.0, 139.5, 176.1.

MS (CI): *m*/*z* (%) = 210 (100), 193 (50).

Ethyl 2-Bromo-2,4-dimethylpentanoate (3a)

A solution of *n*-BuLi in hexane (1.6 M, 68.8 mL, 110 mmol, 1.1 equiv) was added dropwise at 0 °C to a stirred solution of diisopropyl amine (16.9 mL, 120 mmol, 1.2 equiv) in THF (200 mL). The mixture was stirred for 30 min at this temperature, cooled to –78 °C and ethyl ester **2a** (15.8 g, 100 mmol, 1.0 equiv) was added dropwise. The mixture was stirred for 30 min and then a solution of tetrabromomethane (33.2 g, 100 mmol, 1.0 equiv) in THF (20 mL) was added slowly. The mixture was warmed to r.t., quenched with a sat. solution of NH_4Cl (200 mL) and Et_2O (200 mL) was added. The phases were separated, the organic phase was washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and filtered. After evaporation of the solvent a black liquid was obtained, which was purified by vacuum distillation to afford a colorless liquid (20.6 g, 87 mmol, 87%); bp 57–60 °C (1.5 × 10^{-1} mbar).

IR (neat): 2961, 2935, 2906, 2872, 1735, 1467, 1381, 1368, 1305, 1228, 1154, 1125, 1067, 1040, 1021, 866, 669 cm^{-1}.

^1H NMR (250 MHz): δ = 0.88 (d, *J* = 6.7 Hz, 3 H), 0.97 (d, *J* = 6.7 Hz, 3 H), 1.31 (t, *J* = 7.1 Hz, 3 H), 1.76 (m, 1 H), 1.93 (s, 3 H), 2.09 (dd, *J* = 14.1, 5.3 Hz, 1 H), 2.25 (dd, ^2J = 14.1, 7.1 Hz, 1 H), 4.23 (q, *J* = 7.1 Hz, 2 H).

^{13}C NMR (63 MHz): δ = 13.9, 22.7, 24.0, 26.7, 28.1, 50.9, 61.1, 62.0, 171.7.

MS (CI): *m*/*z* (%) = 254, 256 (100), 237, 239 (4).

Ethyl 2-Bromo-2-methyl-3-phenylpropanoate (3b)

Following the procedure described for **3a**, starting from **2b** (10.0 g, 52.0 mmol) **3b** was obtained as a colorless liquid (14.3 g, 52.8 mmol, quantitative yield); bp 115 °C/1.6 × 10^{-1} mbar [Lit:[18] 85 °C/2 × 10^{-2} Torr].

IR (neat): 3987, 3063, 3031, 2980, 2935, 2872, 1735, 1496, 1453, 1380, 1299, 1279, 1254, 1223, 1195, 1166, 1101, 1055, 1022, 744, 702 cm^{-1}.

^1H NMR (250 MHz): δ = 1.31 (t, *J* = 7.1 Hz, 3 H), 1.82 (s, 3 H), 3.36 (d, *J* = 13.8 Hz, 1 H), 3.62 (d, *J* = 13.8 Hz, 1 H), 4.24 (q, *J* = 7.1 Hz, 2 H), 7.18–7.32 (m, 5 H).

^{13}C NMR (63 MHz): δ = 13.9, 27.4, 48.0, 60.2, 62.1, 127.3, 128.3, 130.5, 135.9, 171.0.

MS (CI): *m*/*z* (%) = 288, 290 (100).

Ethyl 2-Azido-2,4-dimethylpentanoate (4a)

A mixture of NaN_3 (6.3 g, 98 mmol, 1.5 equiv), **3a** (15.4 g, 65 mmol, 1.0 equiv) and DMSO (100 mL) was stirred at r.t. overnight. The mixture was poured carefully into water (400 mL) and Et_2O (200 mL) was added. The phases were separated and the organic phase was washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and filtered. After evaporation of the solvent a light yellowish liquid (12.5 g, 63 mmol, 97%) was obtained, which was used in the following step without further purification.

IR (neat): 2960, 2872, 2116 (vs, N_3), 1737, 1465, 1388, 1378, 1367, 1258, 1227, 1152, 1091, 1075, 1020 cm^{-1}.

^1H NMR (250 MHz): δ = 0.90 (d, *J* = 6.3 Hz, 3 H), 0.94 (d, *J* = 6.3 Hz, 3 H), 1.32 (t, *J* = 7.1 Hz, 3 H), 1.48 (s, 3 H), 1.57–1.85 (m, 3 H), 4.23 (q, *J* = 7.1 Hz, 2 H).

^{13}C NMR (63 MHz): δ = 14.1, 23.0, 23.5, 23.8, 24.6, 46.3, 61.8, 66.2, 172.9.

MS (CI): *m*/*z* (%) = 217 (100), 200 (5), 172 (68).

Ethyl 2-Azido-2-methyl-3-phenylpropanoate (4b)

Following the procedure described for **4a**, azide **4b** was obtained starting from **3b** (14.3 g, 52.8 mmol) as a light yellowish liquid (12.2 g, 52.4 mmol, 99%), which was used in the subsequent step without further purification.

IR (neat): 3088, 3064, 3032, 2983, 2937, 2874, 2114 (vs, N_3), 1739, 1497, 1454, 1378, 1254, 1195, 1109, 1019, 860, 767, 743, 702 cm^{-1}.

^1H NMR (250 MHz): δ = 1.27 (t, *J* = 7.1 Hz, 3 H), 1.45 (s, 3 H), 2.98 (d, *J* = 13.7 Hz, 1 H), 3.09 (d, *J* = 13.7 Hz, 1 H), 4.21 (q, *J* = 7.1 Hz, 2 H), 7.17–7.32 (m, 5 H).

^{13}C NMR (63 MHz): δ = 14.1, 22.3, 43.8, 62.0, 66.9, 127.2, 128.2, 130.4, 135.1, 172.1.

MS (CI): *m*/*z* (%) = 91 (100).

2-Azido-2,4-dimethylpentanoic Acid (5a)

A mixture of KOH (9.3 g, 125 mmol, 2.0 equiv) and **4a** (12.5 g, 63 mmol, 1.0 equiv) in EtOH (150 mL) and water (15 mL) was refluxed for 40 min. The mixture was cooled in an ice bath and acidified to pH 2 with concd HCl. The precipitated inorganic salts were

dissolved by addition of water (300 mL) and the mixture was extracted with Et_2O (3 × 60 mL). The combined organic layers were dried (Na_2SO_4) and filtered. After evaporation of the solvent a viscous, colorless oil (8.7 g, 51 mmol, 81%) was obtained, which was used in the following step without further purification.

IR (neat): 2960, 2873, 2119 (vs, N_3), 1713, 1463, 1256, 1156, 1050, 920, 781 cm^{-1}.

^1H NMR (250 MHz): δ = 0.94 (d, *J* = 6.7 Hz, 3 H), 0.97 (d, *J* = 6.6 Hz, 3 H), 1.54 (s, 3 H), 1.61–1.90 (m, 3 H), 10.1 (s, 1 H).

^{13}C NMR (63 MHz): δ = 23.0, 23.4, 23.8, 24.7, 46.2, 66.0, 179.2.

MS (CI): *m*/*z* (%) = 189 (47), 100 (100).

2-Azido-2-methyl-3-phenylpropionic Acid (5b)

Following the procedure described for **5a**, azide **5b** was obtained starting from **4b** (10.2 g, 43.8 mmol) as a colorless and highly viscous oil (5.3 g, 25.7 mmol, 59%), which was used in the following step without further purification.

IR (neat): 3032, 2110 (vs, N_3), 1711, 1497, 1454, 1413, 1257, 1120, 700 cm^{-1}.

^1H NMR (250 MHz): δ = 1.51 (s, 3 H), 3.01 (d, *J* = 13.7 Hz, 1 H), 3.15 (d, *J* = 13.7 Hz, 1 H), 7.20–7.35 (m, 5 H), 9.55 (s, 1 H).

^{13}C NMR (63 MHz): δ = 22.1, 43.5, 66.7, 127.4, 128.4, 130.4, 134.6, 178.0.

MS (CI): *m*/*z* (%) = 223 (1), 134 (100).

2-Azido-2,4-dimethylpentanamide (6a)

Et_3N (5.9 mL, 42.6 mmol, 1.0 equiv) and ethyl chloroformate (5.1 mL, 42.6 mmol, 1.0 equiv) were added at –10 °C to a solution of **5a** (7.3 g, 42.6 mmol, 1.0 equiv) in THF (100 mL). The resulting suspension was stirred for 10 min and was subsequently poured into an ice cooled aqueous solution of NH_3 (25%, 70 mL). The reaction mixture was warmed up to r.t. within 1 h and was extracted with Et_2O (200 mL). The organic phase was washed with brine (80 mL), dried (Na_2SO_4) and filtered. After evaporation of the solvent a solid was obtained, which was purified by crystallization from hexane to give a colorless, crystalline solid (5.9 g, 34.8 mmol, 82%); mp 69 °C.

IR (KBr): 3518, 3474, 3402, 2964, 2936, 2873, 2119 (vs, N_3), 1688, 1570, 1523, 1388, 1159, 1046, 929 cm^{-1}.

^1H NMR (250 MHz): δ = 0.92 (d, *J* = 6.6 Hz, 3 H), 1.00 (d, *J* = 6.6 Hz, 3 H), 1.54 (s, 3 H), 1.59 (dd, *J* = 14.1, 6.2 Hz, 1 H), 1.78 (m, 1 H), 1.97 (dd, *J* = 14.1, 6.3 Hz, 1 H), 6.18 (s, 1 H), 6.50 (s, 1 H).

^{13}C NMR (63 MHz): δ = 23.3, 23.6, 24.5, 24.9, 46.1, 67.2, 174.9.

MS (CI): *m*/*z* (%) = 188 (49), 171 (100).

Anal. Calcd for $C_7H_{14}N_4O$ (170.21): C, 49.39; H, 8.29; N, 32.92. Found: C, 49.60; H, 8.01; N, 32.64.

2-Azido-2-methyl-3-phenylpropanamide (6b)

Following the procedure described for **6a**, **6b** was obtained starting from **5b** (4.5 g, 22.0 mmol) as a colorless solid (2.8 g, 13.6 mmol, 62%) after crystallization from a mixture of $CHCl_3$ and hexane; mp 100 °C.

IR (KBr): 3473, 3416, 3294, 3220, 2120 (vs, N_3), 1687, 1627, 1495, 1455, 1217, 1099, 909, 757, 737, 704 cm^{-1}.

^1H NMR (250 MHz): δ = 1.62 (s, 3 H), 2.95 (d, *J* = 13.7 Hz, 1 H), 3.24 (d, *J* = 13.7 Hz, 1 H), 5.77 (s, 1 H), 6.24 (s, 1 H), 7.20–7.35 (m, 5 H).

^{13}C NMR (63 MHz): δ = 22.4, 44.2, 68.0, 127.2, 128.3, 130.2, 135.2, 174.1.

MS (CI): *m*/*z* (%) = 222 (96), 205 (89).

Anal. Calcd for $C_{10}H_{12}N_4O$ (204.23): C, 58.81; H, 5.92; N, 27.43. Found: C, 58.83; H, 5.89; N, 27.35.

Enzymatic Resolution of C^{α}-Dialkylated-α-azidocarboxamides; General Description of the Screening Reactions

The amidase activity and stereoselectivity for 2-azido-2,4-dimethylpentanamide (**6a**) and 2-azido-2-methyl-3-phenylpropanamide (**6b**) were tested according to the following general protocol:

A concentrated enzyme solution (500 μL) containing the L-amidase (1 mg) from *Ochrobactrum anthropi* NCIMB 40321 (produced via expression of the *lam*A gene in *E. coli* XL-1 Blue MRF′/*p*-TrcLAM[19]) was added at 55 °C to a suspension (15 mL) of the substrate (concentration 30 mM in 100 mM HEPES-NaOH buffer, pH 8.0, and 1 mM $ZnSO_4$). The reaction mixture was stirred at 55 °C in an orbital shaker. The time course of the reaction was monitored by taking 1 mL samples where the enzyme activity was stopped by addition of 500 μL of 1 M H_3PO_4 solution. The samples were analyzed by HPLC [column Chirobiotic R (250 × 4.6 mm); eluent: 15 mM NH_4OAc (pH 4.1)–MeOH (80:20); flow rate: 1.0 mL/min; T 20 °C; UV detection at 215 nm] with respect to conversion and *ee* of the azido acid **5a**/**5b**. With substrate **6a** conversion was 11% with an *ee* of 97% after 30 h, and 30% with an *ee* of 96% after 198 h, corrected for 0.5% racemic α-azido acid **5a** in the substrate **6a**. Identically, with substrate **6b** a conversion of 45% with an *ee* of 33% was obtained after 30 h. No work-up of these screening reactions was performed.

In a blank reaction (identical conditions; no enzyme added) no hydrolysis occurred for either substrate.

Preparative Enzymatic Resolution of 2-Azido-2,4-dimethylpentanamide (6a)

The same concentrated L-amidase solution (27 mL) as used in the screening reaction was added to a solution of racemic **6a** (1.4 g, 8.2 mmol) in water [518 mL, containing 100 mM HEPES-NaOH buffer (pH 8.0) and 1 mM $ZnSO_4$]. After stirring for 2 d at 55 °C in an orbital shaker a conversion of 25% was reached. Additional L-amidase solution (27 mL) was added and stirring was continued for 5 d (total reaction time 166 h). At that time a constant conversion of 45% was reached which did not change after addition of fresh L-amidase solution. Precipitated proteins were removed by centrifugation (30 min, 28,000 × g) and the supernatant (pH 8.0) was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. Thus, NMR-pure (*R*)-**6a** (690 mg, 4.1 mmol, 50%) was obtained as cream-colored crystals with an *ee* of 95% (*R*). The *ee* was determined by chiral HPLC: column Chiralpak AD (250 × 4.6 mm); eluent: *n*-hexane–*i*-PrOH–TFA, 98.8:1.2:0.05; flow rate 2.0 mL/min; T 20 °C; UV detection at 210 nm.

The aqueous layer after extraction was acidified to pH 1 with concd HCl solution and again extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with water, dried over Na_2SO_4. After removal of the solvent under reduced pressure (*S*)-**5a** (670 mg, 3.9 mmol, 48%) was obtained as a NMR-pure oil. The *ee* of 96% (*S*) was determined by chiral HPLC (column Chirobiotic R (250 × 4.6 mm) + Chirobiotic T (50 × 4.6 mm) in line; eluent: 15 mM NH_4OAc (pH 4.1)–MeOH, 80:20; flow rate: 1.0 mL/min; T 20 °C; UV detection at 215 nm).

The absolute stereochemistry of the resolved azido acid **5a** was determined by transferring the α-azido acid into the corresponding α-amino acid: Ammonium formate (310 mg, 5 mmol) and Pd/C (250 mg, 5 wt%) were added to a solution of the azido acid (30 mg, 0.18 mmol) in MeOH (5 mL). The mixture was stirred for 30 min at ambient temperature followed by heating to reflux for 5 min. The Pd/C was filtered and washed with MeOH (10 mL). The combined MeOH layers were concentrated under reduced pressure yielding (*S*)-α-methylleucine (52 mg) as a white solid. The *ee* and absolute

configuration of > 98% of the (*S*)-enantiomer were determined with chiral HPLC by comparison with authentic samples of (*S*)- and (*RS*)-α-methylleucine.[15] HPLC conditions: column Sumichira OA 5000 (150 × 4.6 mm); eluent: 2 mM aq $CuSO_4$–*i*-PrOH, 90:10; flow rate: 1.0 mL/min; T 35 °C; post-column reaction *o*-phthalic aldehyde (OPA)/mercaptoethanol (MCE); fluorescence detection with excitation at 338 nm and emission at > 420 nm).

N-[2-Azido-2,4-dimethylpentanoyl]-L-alanine Methyl Ester (7a)

sym-Collidine (616 μL, 4.7 mmol, 4.0 equiv) was added at 0 °C to a solution of *rac*-**5a** (200 mg, 1.17 mmol, 1.0 equiv) and BTC (139 mg, 0.47 mmol, 0.4 equiv) in CH_2Cl_2 (4 mL). The resulting suspension was stirred for 10 min and then L-alanine methyl ester hydrchloride (181 mg, 1.8 mmol, 1.5 equiv), dissolved in DMF, (1 mL) was added. After stirring overnight Et_2O (40 mL) was added and the mixture was washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4) and filtered. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether–EtOAc, 10:1) to afford the product as a colorless oil.

IR (neat): 3348, 2957, 2873, 2116 (vs, N_3), 1746, 1670, 1517, 1454, 1275, 1215, 1151, 1058 cm^{-1}.

^1H NMR (250 MHz): δ = 0.87 (d, *J* = 6.6 Hz, 3 H, *S*,*S*), 0.88 (d, *J* = 5.7 Hz, 3 H, *R*,*S*), 0.99 (d, *J* = 6.6 Hz, 3 H, *S*,*S*), 1.00 (d, *J* = 6.6 Hz, 3 H, *R*,*S*), 1.42 (d, *J* = 7.2 Hz, 3 H, *S*,*S*), 1.43 (d, *J* = 7.2 Hz, 3 H, *R*,*S*), 1.53 (s, 6 H, both diastereomers), 1.58 (dd, *J* = 14.3, 7.1 Hz, 1 H, *R*,*S*), 1.59 (dd, *J* = 14.4, 6.3 Hz, 1 H, *S*,*S*), 1.73 (m, 1 H, *S*,*S*), 1.75 (m, 1 H, *R*,*S*), 1.98 (dd, *J* = 14.3, 6.0 Hz, 1 H, *R*,*S*), 2.00 (dd, *J* = 14.4, 6.3 Hz, *S*,*S*), 3.75 (s, 3 H, *R*,*S*), 3.76 (s, 3 H, *S*,*S*), 4.53 (dq, *J* = 7.3, 7.3 Hz, 1 H, *R*,*S*), 4.54 (dq, *J* = 7.2, 7.2 Hz, 1 H, *S*,*S*), 7.06 (d, *J* = 6.9 Hz, 1 H, *R*,*S*), 7.10 (d, *J* = 6.9 Hz, 1 H, *S*,*S*).

^{13}C NMR (63 MHz): δ = 18.16 (*R*,*S*), 18.25 (*S*,*S*), 23.26 (*S*,*S*), 23.40 (*R*,*S*), 23.49 (*R*,*S*), 23.57 (*S*,*S*), 24.51 (*S*,*S*), 24.60 (*R*,*S*), 24.78 (*R*,*S*), 24.88 (*S*,*S*), 46.14 (both diastereomers), 48.10 (*R*,*S*), 48.19 (*S*,*S*), 52.42 (*R*,*S*), 52.49 (*S*,*S*), 67.29 (*S*,*S*), 67.37 (*R*,*S*), 171.24 (*S*,*S*), 171.28 (*R*,*S*), 172.92 (*R*,*S*), 173.07 (*S*,*S*).

MS (CI): *m*/*z* (%) = 274 (5), 257 (100).

Anal. Calcd for $C_{11}H_{20}N_4O_3$ (256.30): C, 51.55; H, 7.87; N, 21.86. Found: C, 51.60; H, 7.60; N, 21.47.

N-[(*S*)-2-Azido-2,4-dimethylpentanoyl]-L-alanine Methyl Ester [(*S*,*S*)-7a]

Following the procedure described for the mixture of diastereomers **7a**, the pure (*S*,*S*)-diastereomer was obtained starting from (*S*)-**5a** (50 mg, 0.29 mmol) as a colorless oil (50 mg, 0.20 mmol, 69%); $[\alpha]_D^{25}$ +23.2 (*c* = 0.3, $CHCl_3$)

N-[2-Azido-2-methyl-3-phenylpropanoyl]-L-alanine Methyl Ester (7b)

Following the procedure described for **7a** the diastereomeric mixture **7b** was obtained starting from *rac*-**5b** (205 mg, 1.00 mmol) as a colorless oil (207 mg, 0.71 mmol, 71%) after flash column chromatography (petroleum ether–EtOAc, 4:1).

IR (neat): 3406, 3354, 3062, 3030, 2989, 2953, 2879, 2848, 2116 (vs, N_3), 1745, 1674, 1518, 1454, 1377, 1271, 1213, 1167, 1090, 1061, 743, 702 cm^{-1}.

^1H NMR (250 MHz): δ = 1.15 (d, *J* = 7.2 Hz, 3 H), 1.37 (d, *J* = 7.2 Hz, 3 H), 1.58 (s, 3 H), 1.63 (s, 3 H), 2.94 (d, *J* = 13.8 Hz, 1 H), 2.97 (d, *J* = 13.8 Hz, 1 H), 3.20 (d, *J* = 13.8 Hz, 1 H), 3.23 (d, *J* = 13.8 Hz, 1 H), 3.67 (s, 3 H), 3.70 (s, 3 H), 4.44 (dq, *J* = 7.2, 7.2 Hz, 1 H), 4.45 (dq, *J* = 7.3, 7.3 Hz, 1 H), 6.77 (d, *J* = 7.8 Hz, 1 H), 6.86 (d, *J* = 7.1 Hz, 1 H), 7.16–7.32 (m, 10 H).

^{13}C NMR (63 MHz): δ = 17.99, 18.27, 22.12, 22.58, 44.21, 44.26, 48.00, 48.19, 52.39, 52.43, 67.85, 68.12, 127.14, 127.16, 128.24 (both diastereomers), 130.19, 130.25, 135.18, 135.33, 170.36, 170.89, 172.55, 172.89.

MS (CI): *m*/*z* (%) = 308 (22), 291 (100).

Anal. Calcd for $C_{14}H_{18}N_4O_3$ (290.32): C, 57.92; H, 6.25; N, 19.30. Found: C, 57.90; H, 5.95; N, 19.06.

2-[*N*-*tert*-Butyloxycarbonyl)amino]isobutyryl-*N*′-{(2*S*)-1-(1,5-diazabicyclo[4.3.0]non-5-ene-5-yliumyl)-4-methylpent-2-yl}amide Trifluoroacetate (9)

TFA (5 mL) was added at 0 °C to a solution of amidinium salt **8**[5] (600 mg, 1.33 mmol, 1.0 equiv) in CH_2Cl_2 (3 mL). The mixture was warmed to r.t. and after 30 min the volatiles were removed in vacuo and the residue was lyophilized from water. DIPEA (1.36 mL, 7.98 mmol, 6.0 equiv) was added at 0 °C to a stirred solution of Boc-Aib-OH (Novabiochem, 352 mg, 1.73 mmol, 1.3 equiv) and *N*-HATU (657 mg, 1.73 mmol, 1.3 equiv) in DMF. After 10 min a solution of the amino component obtained above in CH_2Cl_2 (6 mL) was added. After 6 h the volatiles were removed in vacuo and the product was isolated by RP-HPLC as a colorless solid (504 mg, 0.96 mmol, 72%).

^1H NMR (500 MHz): δ = 0.86 (d, *J* = 6.3 Hz, 3 H), 0.89 (d, *J* = 6.3 Hz, 3 H), 1.13 (m, 1 H), 1.39 (s, 9 H), 1.40 (s, 3 H), 1.52 (s, 3 H), 1.61–1.72 (m, 2 H), 2.03–2.29 (m, 4 H), 3.02 (m, 1 H), 3.26 (m, 1 H), 3.31–3.46 (m, 4 H), 3.66–3.84 (m, 4 H), 4.31 (m, 1 H), 5.41 (s, 1 H), 7.67 (d, *J* = 8.5 Hz, 1 H).

^{13}C NMR (126 MHz): δ = 18.2, 18.8, 21.3, 23.2, 24.6, 24.9, 26.3, 28.3, 30.9, 40.2, 42.4, 44.8, 45.2, 54.5, 56.5, 57.3, 79.1, 154.8, 165.3, 176.1.

MS (MALDI–ToF): *m*/*z* = 409.29 $[M]^+$, monoisotopic mass calculated for the cation $[C_{22}H_{41}N_4O_3]^+$: 409.32.

(*S*)-2-Methylleucyl-2-aminoisobutyryl-*N*-[(2*S*)-1-(1,5-diazabicyclo[4.3.0]non-5-ene-5-yliumyl)-4-methylpent-2-yl]amide Trifluoroacetate (10)

Concd HCl (3 mL) was added at 0 °C to a suspension of **9** (120 mg, 230 μmol) in water (0.5 mL). Water (20 mL) was added after 1 h and the mixture was lyophilized. *sym*-Collidine (121 μL, 915 μmol) was added slowly at 0 °C to a stirred solution of (*S*)-**5a** (30 mg, 172 μmol) and BTC (21 mg, 72 μmol) in CH_2Cl_2 (1 mL). After 5 min a solution of the amino component obtained above, dissolved in DMF (1 mL) and CH_2Cl_2 (1 mL) was added. The mixture was warmed to r.t. and after 2 h another portion of BTC (11 mg, 36 μmol) was added. The mixture was stirred 72 h at r.t., the volatiles were removed in vacuo and the coupling product was isolated from the residue by RP-HPLC (39 mg, 68 μmol, 30%). Palladium on activated charcoal (10%, 10 mg) was added to a solution of a part of this coupling product (10 mg, 17 μmol) in MeOH (4 mL). The mixture was stirred vigorously under an atmosphere of H_2 for 2 h, the catalyst was filtered off and washed with MeOH. The volatiles were removed in vacuo and the product was isolated by RP-HPLC as a colorless solid (6.0 mg, 8.3 μmol, 49%).

^1H NMR (500 MHz, CD_2Cl_2): δ = 0.84 (d, *J* = 6.3 Hz, 3 H), 0.88 (d, *J* = 6.3 Hz, 3 H), 0.91 (d, *J* = 6.3 Hz, 3 H), 0.94 (d, *J* = 6.6 Hz, 3 H), 1.13 (m, 1 H), 1.44 (s, 3 H), 1.46 (s, 3 H), 1.53 (s, 3 H), 1.53–1.64 (m, 2 H), 1.71–1.81 (m, 2 H) 1.85 (dd, *J* = 14.0, 5.8 Hz, 1 H), 2.02 (m, 1 H), 2.08–2.20 (m, 2 H), 2.26 (m, 1 H), 2.83 (ddd, *J* = 17.0, 10.4, 6.0 Hz, 1 H), 3.19–3.30 (m, 3 H), 3.31–3.45 (m, 2 H), 3.61–3.70 (m, 2 H), 3.79–3.89 (m, 2 H), 4.32 (m, 1 H), 7.07 (d, *J* = 9.1 Hz, 1 H), 7.74 (s, 1 H), 8.96 (br s, 3 H).

^{13}C NMR (126 MHz): δ = 18.6, 19.3, 21.5, 21.7, 23.2, 24.0, 24.1, 24.3, 25.1, 26.8, 31.2, 40.4, 42.8, 44.8, 45.4, 46.9, 55.0, 57.0, 58.2, 60.9, 165.7, 170.5, 175.1.

MS (ESI): *m*/*z* = 436.3, monoisotopic mass calculated for the cation $[C_{24}H_{45}N_5O_2]^+$: 435.36.

2-Aminoisobutyryl-2-aminoisobutyryl-(*S*)-pipecolinyl-2-aminoisobutyryl-glycyl-(*S*)-2-methylleucyl-2-aminoisobutyryl-*N*-[(2*S*)-1-(1,5-diazabicyclo[4.3.0]non-5-ene-5-yliumyl)-4-methylpent-2-yl]amide Bis(trifluoroacetate) (12)

[(*S*)-α-Methylleucine[14]]efrapeptin C-(9-15)-peptide bis(trifluoroacetate) (**12**)

DIPEA (16.1 μL, 94.5 μmol, 4.2 equiv) was added at 0 °C to a stirred solution of Z-Aib-Aib-Pip-Aib-Gly-OH **11**[5] (14.2 mg, 24.7 μmol, 1.1 equiv) and *N*-HATU (10.3 mg, 27.0 μmol, 1.2 equiv) in DMF (1.5 mL). After 10 min a solution of **10** (14.9 mg, 22.5 μmol, 1.0 equiv) in CH_2Cl_2 (1.5 mL) was added. The mixture was left overnight at 0 °C. The volatiles were removed in vacuo and the residue was redissolved in MeOH (3 mL), then HOAc (0.3 mL) and palladium on activated charcoal (10%, 10 mg) were added and the mixture was stirred vigorously under an atmosphere of H_2 for 1 h. The catalyst was removed by filtration and washed carefully with MeOH (15 mL). After removal of the solvent in vacuo, the product was isolated by RP-HPLC as a colorless solid (12.3 mg, 11.3 μmol, 50%).

MS (ESI): *m*/*z* = 859.7, monoisotopic mass calculated for the cation $[C_{44}H_{79}N_{10}O_7]^+$: 859.61.

[(*S*)-α-Methylleucine[14]]efrapeptin C (14b)

To a stirred solution of **13**[5] (3.1 mg, 4.0 μmol, 1.3 equiv) and *N*-HATU (1.5 mg, 4.0 μmol, 1.3 equiv) in DMF (0.5 mL) was added at 0 °C DIPEA (1.7 L, 10.2 μmol, 3.3 equiv). After 10 min a solution of **12** (3.4 mg, 3.1 μmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL) was added. The mixture was left at 0 °C overnight, the volatiles were removed in vacuo and the product was isolated by RP-HPLC as a colorless solid (3.6 mg, 2.1 mmol, 67%).

MS (ESI): *m*/*z* = 1620.4, monoisotopic mass calculated for the cation $[C_{81}H_{139}N_{18}O_{16}]^+$: 1620.06.

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References

(1) Present address: Università di Padova, Dipartimento di Scienze Chimiche, 35131 Padova, Italy.
(2) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781.
(3) DalPozzo, A.; Ni, M.; Muzi, L.; Caporale, A.; de Castiglione, R.; Kaptein, B.; Broxterman, Q. B.; Formaggio, F. *J. Org. Chem.* **2002**, *67*, 6372.
(4) (a) Meldal, M.; Juliano, M. A.; Jansson, A. M. *Tetrahedron Lett.* **1997**, *38*, 2531. (b) Tornøe, C. W.; Davis, P.; Porreca, F.; Meldal, M. *J. Pept. Sci.* **2000**, *6*, 549.
(5) Jost, M.; Greie, J.-C.; Stemmer, N.; Wilking, S. D.; Altendorf, K.; Sewald, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 4267; *Angew. Chem.* **2002**, *114*, 4438.
(6) Abrahams, J. P.; Buchanan, S. K.; van Raau, M. J.; Fearnley, I. M.; Leslie, A. G. W.; Walker, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9420.
(7) (a) Gupta, S.; Krasnoff, S. B.; Roberts, D. W.; Renwick, J. A. A.; Brinen, L. S.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 707. (b) Gupta, S.; Krasnoff, S. B.; Roberts, D. W.; Renwick, J. A. A.; Brinen, L. S.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 2306. (c) Matha, V.; Weiser, J.; Olejnicek, J. *Folia Parasitol.* **1988**, *35*, 379. (d) Bandani, A. R.; Khambay, B. P. S.; Faull, J. L.; Newton, R. M.; Deadman, M.; Butt, T. M. *Mycol. Res.* **2000**, *104*, 537.
(8) Papathanassiu, A. E. (Ergon Pharmaceuticals LLC) U. S. Patent, 6528489, **2003**.
(9) Nagaraj, G.; Uma, M. V.; Shivayogi, M. S.; Balaram, H. *Antimicrob. Agents Chemother.* **2001**, *45*, 145.
(10) Weigelt, S.; Sewald, N. *Synlett* **2004**, 726.
(11) Sonke, T.; Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Schoemaker, H. E.; Hans, E.; Kamphuis, J.; Formaggio, F.; Toniolo, C.; Rutjes, F. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, **2000**, 23.
(12) Tornøe, C. W.; Sonke, T.; Maes, I.; Schoemaker, H. E.; Meldal, M. *Tetrahedron: Asymmetry* **2000**, *11*, 1239.
(13) Arnold, R. T.; Kulenovic, S. T. *J. Org. Chem.* **1978**, *43*, 3687.
(14) (a) Falb, E.; Yechezkel, T.; Salitra, Y.; Gilon, C. *J. Pept. Res.* **1999**, *53*, 507. (b) Thern, B.; Rudolph, J.; Jung, G. *Tetrahedron Lett.* **2002**, *43*, 5013. (c) Thern, B.; Rudolph, J.; Jung, G. *Angew. Chem. Int. Ed.* **2002**, *41*, 2307; *Angew. Chem.* **2002**, *114*, 2401. (d) Sewald, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 4661; *Angew. Chem.* **2002**, *114*, 4855.
(15) Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Peters, P. H. J.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1113.
(16) Göttlich, R.; Schopfer, U.; Stahl, M.; Hoffmann, R. W. *Liebigs Ann./Recl.* **1997**, 1757.
(17) Meyers, A. I.; Tempel, D. L.; Nolen, R. L.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2778.
(18) Roberts, A.; Jaguelin, S.; Guinamant, J. L. *Tetrahedron* **1986**, *42*, 2275.
(19) Sonke, T.; Ernste, S.; Tandler, R. F.; Kaptein, B.; Peeters, W. P. H.; van Assema, F. B. J.; Wubbolts, M. G.; Schoemaker, H. E. An L-Selective Amidase with Extremely Broad Substrate Specificity from *Ochrobactrum anthropi* NCIMB 40321, submitted.