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Synthesis of enantiopure azetidine 2-carboxylic acids and their incorporation into peptides

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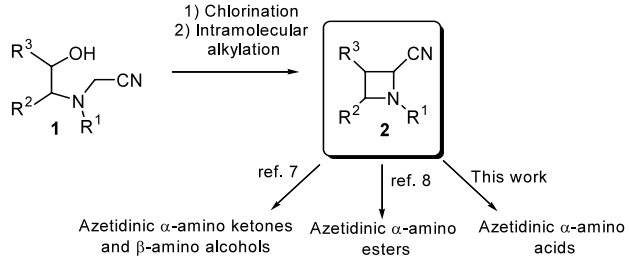
Abstract—Enantiopure azetidine 2-carboxylic acids were prepared by hydrolysis of the corresponding 2-cyano azetidines, without ring cleavage of the azetidine or epimerization. The produced amino acids, which are conformationally constrained analogues of phenylalanine, can be cleanly debenzylated and used for the synthesis of tripeptides. In the course of the synthesis of new enantiopure 2-cyano azetidines through intramolecular alkylation of a metallated amino nitrile, it was found that the involved anionic cyclisation can be thermodynamically controlled, thus enhancing its diastereoselectivity.

1. Introduction

Much synthetic effort has been recently devoted to the preparation of conformationally constrained α-amino acids.[1] In this field, the modification of the conformation of bioactive peptides by the inclusion of such synthetic analogues of proteinogenic α-amino acids into the peptide sequence is an intensive area of research in biomedical chemistry.[2] In this vast topic, the asymmetric synthesis of cyclic constrained α-amino acids in which the nitrogen atom of the amino moiety is part of a ring (e.g. constrained imino acids) is of particular interest since these amino acids, when incorporated in peptides, are unable to engage a hydrogen bond and can therefore deeply influence the spatial conformation of the peptide.[1d] Moreover, peptides including such *N*-alkyl α-amino acids are usually less prone to enzymatic proteolytic cleavage. For those reasons, the asymmetric synthesis of five-membered ring (proline derivatives)[3] and six-membered ring[4] (pipecolic acid derivatives) α-amino acids have been particularly studied. In contrast, the asymmetric synthesis of 4-membered ring (azetidinic α-amino acids) has been much less reported,[5] reflecting the difficult access to those strained heterocycles, especially in enantiomerically pure form. We have recently reported a straightforward preparation of 2-cyano azetidines, starting from commercially available enantiopure β-amino alcohols (Scheme 1).[6] In these compounds, the α-amino nitrile moiety can be conveniently transformed into either an α-amino ketone, or a β-amino alcohol[7] or a α-amino ester.[8] Since α-amino nitriles are also direct precursors to α-amino acids through the Strecker synthesis,[9] we herein report the preparation of azetidinic 2-carboxylic acids by hydrolysis of the amino nitrile and the incorporation of such amino acids into a simple peptidic sequence.



Scheme 1. Synthesis of enantiopure functionalized azetidines from *N*-cyanomethyl β-amino alcohols.

2. Results

2.1. Synthesis of 2-cyano azetidines

Cyano azetidines **3-5** were prepared as previously described from (1*R*,2*S*)-ephedrine **3** and **4** and (*R*)-phenylglycinol **5**.[6] *N*-Benzyl analogues **9** and **10** were prepared following a similar procedure depicted in Scheme 2 from (1*R*,2*S*)-*N*-benzyl norephedrine **6**.

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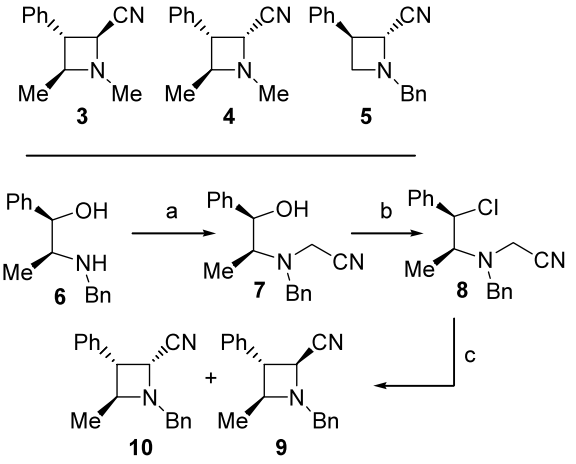
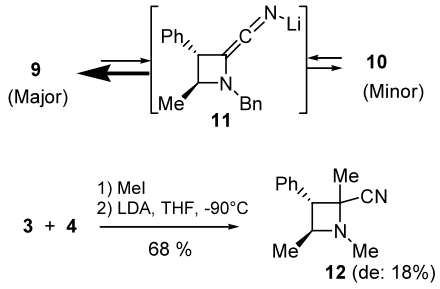
Cyanomethylation of **6** followed by chlorination gave **8** as a single isomer. Treatment of **8** by LiHMDS resulted in intramolecular alkylation, to give an inseparable mixture of *N*-benzyl-2-cyano azetidines **9** and **10** in good yield.



Scheme 2. Azetidinic substrates choosen for their transformation into α-amino acids. *Reagents and conditions*: (a) $BrCH_2CN$, K_2CO_3, CH_3CN, 97%; (b) $SOCl_2$, CH_2Cl_2, 77%; (c) 1.2 equiv. LiHMDS, −78°C to −50°C, 1 h: **10**/**9**: 60/40, 78%; 2 equiv. LiHMDS, −78°C to −30°C, 2 h, **10**/**9**: 30/70; 2 equiv. LiHMDS, −78°C to −30°C, 5 h, **10**/**9**: 8/92, 84%.

In the course of the synthesis of these new 2-cyano azetidines, we have brought to light a very important feature concerning the anionic cyclisation. The diastereoselectivity of this ring closure was found to be highly dependent on the amount of base used and also on the duration of the experiment (Scheme 2). Protracted experiments at low temperatures with an excess of base give *trans*-**9** with 84% d.e. while the *cis*-isomer **10** is predominantly produced when the reaction time is shortened. These results suggest that thermodynamic control is operative in this reaction, leading to the more stable *trans*-isomer.[10] This equilibration is slow and proceeds through the metallated amino nitrile **11**: it should be noted that this anion is only produced in low concentrations at −30°C, since a quench with CD_3OD at this temperature afforded a mixture of **9** and **10** with less than 10% of deuterium incorporation at C-2. However this is sufficient for the equilibration to proceed. The occurrence of such a metallated azetidine was best seen using azetidines **3** and **4** (mixture of isomers at C-2) as the substrates. In these compounds, bearing a *N*-methyl instead of a *N*-benzyl group, the acidic proton is sterically less hindered and complete metallation occurs readily with LDA at −90°C. This anion can then be trapped with methyl iodide, to give **12** with no selectivity. It should be mentioned that this anion proved to be particularly reactive, leading to degradation products if the alkylating agent was not added in the reaction mixture before the base (Scheme 3).

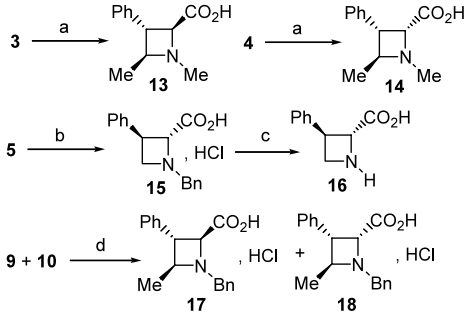
Having in hand a set of enantiopure 2-cyano azetidines, we next focused on the conditions for their hydrolysis into amino acids.



Scheme 3. Metallated aminonitrile **11** accounts for the equilibration of **10** into **9**.

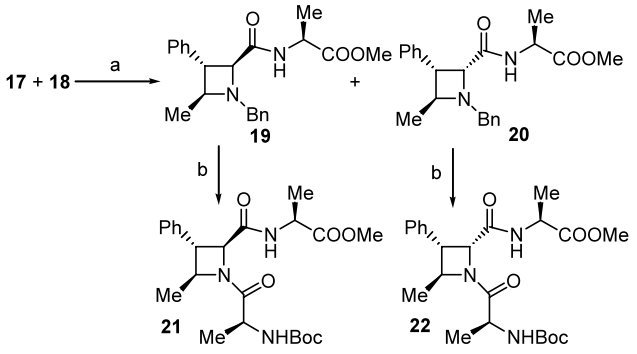
2.2. Synthesis of azetidinic α-amino acids

Hydrolysis of 2-cyano azetidines into amino acids required very harsh conditions: prolonged heating in concentrated HCl (35% wt.) was necessary to completely hydrolise the intermediate amide. Moreover, in some cases, a co-solvent such as CCl_4 (for **5**), or AcOH (for **9**+**10**) had to be used because of the low solubility of the intermediates in the reaction mixture. Nevertheless, under these drastic conditions, neither the ring opening of the azetidine ring[11] nor epimerization was observed. The amino acids **13** and **14** were isolated with excellent yield after purification by ion exchange chromatography, and hydrochloride **15** was cleanly debenzylated prior to purification by ion exchange chromatography, yielding amino acid **16** that can be viewed as a conformationally constrained analogue of phenylalanine.[12] Finally, *cis* or *trans*-enriched epimeric mixtures of **9**+**10** obtained following the above conditions gave the same epimeric mixtures of amino acids **17**+**18** (Scheme 4).



Scheme 4. Hydrolysis of azetidinic amino nitriles. *Reagents and conditions*: (a) 35% HCl, 50°C, 72 h, then DOWEX (50X8-200), 97% (**13**), 97% (**14**); (b) 35% HCl, CCl_4, 80°C, 7 days (quant.); (c) H_2, Pd/C, then DOWEX (50X8-200), 78%; (d) 35% HCl, AcOH, 100°C, 72 h.

This crude epimeric mixture of amino acids was then coupled with (*L*)-Ala-OMe to give the diastereoisomeric dipeptides **19** and **20,** which were easily separated by flash chromatography. Debenzylation of these compounds, followed by coupling to (*L*)-*N*-Boc-Ala, gave tripeptides **21** and **22** in modest to good yield (Scheme 5). These tripeptides were shown by NMR to exist as a roughly 1/1 mixture of rotamers in $CDCl_3$. Recording



Scheme 5. Peptide chemistry with azetidinic α-amino acids. *Reagents and conditions*: (a) HOBt, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, HCl, Et_3N, (*L*)-Ala-OMe, HCl, CH_2Cl_2, 58% overall from a crude 92/8 mixture of **9**/**10**, **19**, 72% from stereoisomerically pure *cis*-**18** **20**; (b) (i) TMSCl/MeOH, (ii) H_2, Pd/C then HOBt, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, HCl, Et_3N, (*L*)-*N*-Boc-Ala, CH_2Cl_2, 32% overall **21**, 78% overall **22**.

the spectra of **22** in DMSO-d_6 modified the ratio of rotamers (3/7) and at higher temperature (355 K) some of the signals began to fuse indicating that the temperature of coalescence was near.

In summary, we have described a straightforward synthesis of azetidinic α-amino acids from 2-cyano azetidines. These amino acids are constrained analogues of phenylalanine and can be conveniently incorporated into a peptidic sequence. Furthermore, we have also demonstrated that the anionic cyclisation leading to 2-cyano azetidines can be thermodynamically controlled, thus increasing the diastereoselectivity of the process.

3. Experimental section

General comments. ^1H and ^{13}C spectra ($CDCl_3$ solution unless otherwise stated) were recorded on a Bruker AC 200 or 300 spectrometer at 200, 300 (^1H), 50.3 and 75.5 (^{13}C) MHz; chemical shifts are reported in ppm from TMS. Optical rotations were determined with a Perkin Elmer 241 instrument. All reactions were carried out under argon. Column chromatography was performed on silica gel 230–400 mesh by using various mixtures of diethyl ether (E), ethyl acetate (AcOEt), cyclohexane (CyH) and petroleum ether (PE). TLCs were run on Merck Kieselgel $60F_{254}$ plates. Melting points are uncorrected. THF was distilled from sodium/benzophenone ketyl. The mention of 'usual workup' means: (i) decantation of the organic layer; (ii) extraction of the aqueous layer with ether; (iii) washing the combined organic layers with brine and drying of the combined organic phases over $MgSO_4$; (iv) solvent evaporation under reduced pressure. Compositions of stereoisomeric mixtures were determined by NMR analysis on crude products before any purification.

3.1. (4*S*,5*R*)-[Benzyl-(2-hydroxy-1-methyl-2-phenyl-ethyl)-amino]-acetonitrile 7

To a solution of *N*-benzyl norephedrine **6** (3.0 g, 12.6 mmol) in acetonitrile (120 mL) was added potassium carbonate (2.6 g, 18.9 mmol) and bromoacetonitrile (2.6 mL, 37.8 mmol). After 12 h of reflux, the mixture was concentrated under reduced pressure and the residue was taken up in water and ether. Usual workup followed by flash chromatography (E/CyH 1/1) gave title compound as an oil (3.42 g, 97%). Rf: 0.52 (E/CyH: 1/1); $[\alpha]_D^{20} = -8.8$ (*c* 1, $CHCl_3$); ^1H NMR: 1.19 (d, $J = 6.8$ Hz, 3H, Me), 2.80 (bs, 1H, OH), 3.09 (quadd, $J = 4.0$ and 6.8 Hz, 1H, C*H*Me), 3.51 (s, 2H, CH_2CN), 3.91 (AB syst, $J = 13.4$ Hz, 2H, CH_2Ph), 5.01 (d, $J = 2.6$ Hz, 1H, PhC*H*), 7.25–7.43 (m, 10H, Ar); ^{13}C NMR: 10.6 (CH_3), 55.6 (CH_2), 38.1 (CH_2), 62.7 (CH), 74.0 (CH), 116.8 (CN) 125.9, 127.4, 127.8, 128.3, 128.8, 128.9 (CHAr), 137.0, 142.0 (CqAr); Anal. Calc. for $C_{18}H_{20}N_2O$: C: 77.11; H: 7.19; N: 9.99. Found: C: 76.84; H: 7.32; N: 9.56.

3.2. (4*S*,5*R*)-[Benzyl-(2-chloro-1-methyl-2-phenyl-ethyl)-amino]-acetonitrile 8

To a solution of **7** (1.02 g, 3.65 mmol) in CH_2Cl_2 (22 mL) was added thionyl chloride (530 μl, 7.3 mmol). After 1 h of reflux, the mixture was neutralized by the addition of a saturated aqueous solution of $NaHCO_3$. Usual workup, followed by flash chromatography (E/CyH: 1/9) gave **8** as a pale yellow solid (842 mg, 77%). Rf: 0.70 (E/CyH: 1/1); Mp: 100°C; $[\alpha]_D^{20} = -32.4$ (*c* 0.4, $CHCl_3$); ^1H NMR: 1.48 (d, $J = 6.6$ Hz, 3H, Me), 3.35 (ABsyst, $J = 17.7$ Hz, 2H, CH_2CN), 3.46 (quint, $J = 6.6$ Hz, 1H, C*H*Me), 3.73 (d, 1H, $J = 13.4$ Hz, C*H*HPh), 3.87 (d, 1H, $J = 13.4$ Hz, CH*H*Ph), 4.95 (d, $J = 7.6$ Hz, 1H, PhC*H*), 7.00–7.05 (m, 2H, Ar), 7.22–7.28 (m, 3H, Ar), 7.33–7.39 (m, 5H, Ar); ^{13}C NMR: 12.1 (CH_3), 39.0 (CH_2), 54.4 (CH_2), 63.7 (CH), 66.3 (CH), 116.8 (CN) 127.7, 127.9, 128.4, 128.7, 128.8 (CHAr), 137.0, 141.1 (CqAr); Anal. Calc. for $C_{18}H_{19}N_2Cl$: C: 72.35; H: 6.41; N: 9.37. Found: C: 72.51; H: 6.45; N: 9.12.

3.3. (2*R*,3*S*,4*S*)-1-Benzyl-4-methyl-3-phenyl-azetidine-2-carbonitrile 9 and (2*S*,3*S*,4*S*)-1-Benzyl-4-methyl-3-phenyl-azetidine-2-carbonitrile 10

To a solution of chloride **8** (1 mmol) in THF (15 mL) was added dropwise at −78°C a solution of LiHMDS (1 M solution in THF, 1.2 to 3 equiv.). The mixture was stirred for the time and temperature specified in Scheme 2 and then quenched by the addition of an aqueous, saturated solution of NH_4Cl. Usual workup, followed by flash chromatography (E/CyH: 1/9) gave mixtures (see text) of cyanoazetidines **9** and **10** as oils. Compound *trans*-**9**: Rf: 0.57 (E/CyH: 3/7); ^1H NMR: 1.21 (d, 3H, $J = 5.7$ Hz, Me), 3.32–3.45 (m, 1H, H_4), 3.51 (t, 1H, $J = 8.3$ Hz, H_3), 3.68 (d, 1H, $J = 8.3$ Hz, H_2) 3.80 (AB syst., 2H, $J = 12.7$ Hz, CH_2Ph), 7.14–7.45 (m, 10H, Ar); ^{13}C NMR: 20.9 (CH_3), 49.6 (CH), 55.3 (CH_2), 60.8, 66.8 (CH), 119.2 (CN), 120.7, 127.5, 127.8, 128.5, 128.8, 129.1, 129.4 (CHAr), 135.9, 137.6 (CqAr). Compound *cis*-**10**: Rf: 0.62 (E/CyH: 3/7); ^1H NMR: 1.26 (d,

3H, $J=5.0$ Hz, Me), 3.64 (t, 1H, $J=8.3$ Hz, H_3), 3.80–3.94 (m, 1H, H_4), 3.87 (AB syst., 2H, $J=13.2$ Hz, CH_2Ph), 4.56 (dd, $J=0.9$ and 7.5 Hz, H_2), 7.17–7.48 (m, 10H, Ar); ^{13}C NMR: 20.1 (CH_3), 47.0 (CH), 56.4 (CH_2), 57.8, 65.4 (CH), 116.2 (CN), 127.0, 127.5, 127.8, 127.9, 128.5, 128.8, 129.1, 129.4 (CHAr), 135.7, 137.0 (CqAr); Anal. Calc. for $C_{18}H_{18}N_2$: C: 82.41; H: 6.92; N: 10.68. Found: C: 81.93; H: 7.03; N: 10.72.

3.4. (2*RS*,3*S*,4*S*)-1,2,4-trimethyl-3-phenyl-azetidine-2-carbonitrile 12

To a solution of 7/3 epimeric mixture of **3** and **4** (194 mg, 1.04 mmol) and methyl iodide (100 μL, 1.6 mmol) in THF (10 mL) cooled to −90°C, was added a freshly prepared solution of LDA (0.6 M in THF, 3.5 mL, 2.6 mmol). The mixture was stirred at −90°C for 10 min, gradually warmed to −60°C, and quenched at this temperature by the addition of aqueous NH_4Cl. Usual workup, followed by flash chromatography (AcOEt/EP: 8/92) gave **12** as a 41/58 mixture of isomers. *Minor isomer*: Rf: 0.74 (E/PE: 7/3); $[\alpha]_D^{20}=-76$ (*c* 1.9, $CHCl_3$) ^1H NMR: 1.01 (s, 3H, Me), 1.28 (d, 3H, $J=6.2$ Hz, Me), 2.26 (s, 3H, Me), 3.52 (dq, $J=8.0$ and 6.2 Hz, 1H, H_4), 3.42 (d, 1H, $J=8.0$, H_3), 7.11–7.31 (m, 5H, Ar); ^{13}C NMR: 14.9, 19.2, 33.9 (CH_3), 53 (CH), 59.7 (Cq), 61.7 (CH), 122.7 (CN), 127.7, 128.1, 128.8 (CHAr), 135.0 (CqAr). *Major isomer*: Rf: 0.60 (E/PE: 7/3); $[\alpha]_D^{20}=-48$ (*c* 1.3, $CHCl_3$) ^1H NMR: 1.19 (d, 3H, $J=6.0$ Hz, Me), 1.54 (s, 3H, Me), 2.35 (s, 3H, Me), 3.07 (d, 1H, $J=9.0$, H_3), 3.52 (dq, $J=9.0$ and 6.0 Hz, 1H, H_4), 7.14–7.31 (m, 5H, Ar); ^{13}C NMR: 19.7, 25.2, 36.8 (CH_3), 55.9, 63.3 (CH), 67.8 (Cq), 118.0 (CN), 127.8, 128.0, 128.7 (CHAr), 135.4 (CqAr).

3.5. (2*R*,3*S*,4*S*)-(1,4-Dimethyl-3-phenyl-azetidin-2-yl)-carboxylic acid 13

Compound **3** (381 mg, 2.04 mmol) was melted and then dissolved into an aqueous solution of HCl (35% wt., 35 mL) with vigorous stirring. The solution was then heated at 50°C for 72 h, and evaporated to dryness under reduced pressure. The residual white solid was then dissolved into a small amount of distilled water, and purified on an ion-exchange resin (Dowex 50X8-200; 15 g). Elution with water until neutrality, followed by 1% aqueous NH_4OH, gave fractions of the title compound that were concentrated under reduced pressure. Drying under high vacuum (0.5 mmHg/70°C for 48 h) gave amino acid **13** as a colorless glass (407 mg, 97%). Mp: 94–96°C; $[\alpha]_D^{20}=-61$ (*c* 1.4, H_2O); ^1H NMR (D_2O): 1.51 (d, $J=7.0$ Hz, 3H, Me), 2.81 (s, 3H, NMe), 4.01 (dd, $J=10.5$ and 7.7 Hz, 1H, H_3), 4.67–4.79 (m, 2H, $H_{2,4}$), 7.28–7.41 (m, 5H, Ar); ^{13}C NMR (D_2O): 13.7 (CH_3), 33.4 (CH), 44.9 (CH), 67.4 (CH), 70.3 (CH), 127.7, 128.1, 128.6 (CHAr), 134.9 (CqAr), 170.0 (C=O); Anal. Calc. for $C_{12}H_{15}NO_2$: C: 70.22; H: 7.37; N: 6.82. Found: C: 70.13; H: 7.31; N: 6.85.

3.6. (2*S*,3*S*,4*S*)-(1,4-Dimethyl-3-phenyl-azetidin-2-yl)-carboxylic acid 14

Following the above procedure and starting from cyanoazetidine **4** (289 mg, 1.55 mmol), amino acid **14** was obtained as a colorless glass (309 mg, 97%). Mp: 100–103°C; $[\alpha]_D^{20}=+64$ (*c* 1.4, H_2O); ^1H NMR (D_2O): 1.57 (d, $J=6.5$ Hz, 3H, Me), 2.991 (s, 3H, NMe), 3.70 (t, $J=9.7$ Hz, 1H, H_3), 4.24 (dq, $J=9.7$ and 6.5 Hz, 1H, H_4), 4.60 (d, $J=9.7$ Hz, 1H, H_2), 7.28–7.41 (m, 5H, Ar); ^{13}C NMR (D_2O): 16.4 (CH_3), 39.5 (CH), 48.4 (CH), 69.4 (CH), 71.2 (CH), 127.5, 128.7, 129.5 (CHAr), 136.4 (CqAr), 172.4 (C=O); Anal. Calc. for $C_{12}H_{15}NO_2$: C: 70.22; H: 7.37; N: 6.82. Found: C: 70.32; H: 7.49; N: 6.75

3.7. (2*R*,3*R*)-(1-Benzyl-3-phenyl-azetidine-2-yl)-carboxylic acid 15,HCl

Cyano azetidine **5** (289 mg, 1.15 mmol) was dissolved into a biphasic mixture of aqueous HCl (35% wt., 20 mL) and carbon tetrachloride (20 mL). The solution was then heated with vigorous stirring at 80°C for 7 days and concentrated under reduced pressure. The residue was triturated in diethyl ether, and the obtained white solid (370 mg) engaged without further purification in the debenzylation step. Mp: 185–190°C (dec); $[\alpha]_D^{20}=+57$ (*c* 0.6, DMSO); ^1H NMR (DMSO-d_6): 4.04–4.12 (m, 3H, $H_{3,4}$), 4.52 (s, 2H, CH_2Ph), 5.30 (d, $J=9.3$ Hz, 1H, H_2), 7.35–7.65 (m, 10H, Ar); ^{13}C NMR (DMSO-d_6): 38.7 (CH), 55.1 (CH_2), 56.5 (CH_2), 69.2 (CH), 70.3 (CH), 127.7, 127.9, 128.6, 128.8, 129.5 (CHAr), 130.8, 136.7 (CqAr), 167.7 (C=O).

3.8. (2*R*,3*R*)-(3-Phenyl-azetidine-2-yl)-carboxylic acid 16

A suspension of hydrochloride **15** (237 mg, 0.78 mmol) and Pd/C (10% wt., 200 mg) in MeOH (20 mL) and water (20 mL) was vigorously stirred under an atmosphere of hydrogen for 6 h. The suspension was then filtrated over Celite, and the filtrate concentrated under reduced pressure. The residual solid was purified on an ion-exchange resin (Dowex 50X8-200; 6 g). Elution with water until neutrality, followed by 1% aqueous NH_4OH, gave fractions of the title compound that were then concentrated under reduced pressure. Drying under high vacuum (0.5 mmHg/70°C for 48 h) gave amino acid **16** as a white solid (106 mg, 78%). Mp: 175–185°C (dec); $[\alpha]_D^{20}=-118$ (*c* 2, H_2O); ^1H NMR (D_2O): 4.07–4.21 (m, 3H, $H_{3,4}$), 4.88 (d, $J=7.4$ Hz, 1H, H_2), 7.33–7.44 (m, 5H, Ar); ^{13}C NMR (D_2O): 38.7 (CH), 48.8 (CH_2), 65.8 (CH), 42.0 (CH), 127.4, 128.6, 129.5 (CHAr), 138.3 (CqAr), 173.3 (C=0). HRMS–FAB (*m*/*z*): [M+H$^+$] calcd for $C_{10}H_{12}NO_2$, 178.0868; found, 178.0863.

3.9. (2*R*,3*S*,4*S*)-1-Benzyl-4-methyl-3-phenyl-azetidine-2-carboxylic acid 17, HCl and (2*S*,3*S*,4*S*)-1-benzyl-4-methyl-3-phenyl-azetidine-2-carboxylic acid 18, HCl

To a solution of epimeric mixtures of cyanoazetidines **9** and **10** (0.58 mmol) in glacial acetic acid (5 mL) was added concentrated HCl (35% wt., 10 mL). The mixture was heated under vigorous stirring for 72 h at 100°C and then concentrated under reduced pressure. Starting

with epimeric mixtures in which the *cis*-compound **10** was the major isomer (60/40 mixture), a solid residue was obtained. Washing this solid with a 1/1 mixture of ether/acetone gave pure *cis*-**10**. Starting with *trans*-cyano azetidine **9** (92/8 mixture) an oil was obtained and engaged without further purification in the next step. *Trans*-**17** (dechlorhydrated by treatment with a phosphate buffer, followed by extraction in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): 1.39 (d, 3H, $J=5.7$ Hz, Me), 3.65–3.90 (m, 1H, H_4), 3.83 (t, 1H, $J=8.8$ Hz, H_3), 4.04 (d, 1H, $J=13.2$ Hz, C*H*HPh), 4.16 (d, 1H, $J=8.8$ Hz, H_2), 4.47 (d, 1H, $J=13.2$ Hz, CH*H*Ph), 7.12–7.61 (m, 10H, Ar), 8.39 (bs, 1H, COOH); ^{13}C NMR: 18.0 (CH_3), 47.2 (CH_2), 57.9, 64.8, 68.9 (CH), 126.9, 128.7, 129.0, 130.9 (CHAr), 138.4 (CqAr), 171.2 (CO); MS (IC, CH_4): *m*/*z* 282 (MH^+, 100), 236 (13), 134 (10), 91 (8); HRMS (IC, CH_4): [M+H$^+$] calcd for $C_{18}H_{20}NO_2$ 282.1494; found 282.1491. *Cis*-**18**, HCl: Mp 270–290°C; $[\alpha]_D^{20}=+5.5$ (*c* 0.3, H_2O); ^{1}H NMR (200 MHz, D_2O+ NaOD): 0.84 (d, 3H, $J=5.9$ Hz, Me), 3.25 (t, 3H, $J=8.2$ Hz, H_3), 3.58 (d, 1H, $J=12.5$ Hz, C*H*HPh), 3.71 (d, 1H, $J=12.4$ Hz, CH*H*Ph), 3.92 (d, 1H, $J=9.2$ Hz, H_2), 4.02 (quint, 1H, $J=6.6$ Hz, H_4), 6.92–7.13 (m, 10H, Ar); ^{13}C NMR: 21.3 (CH_3), 48.9 (CH_2), 56.3, 65.8, 72.1 (CH), 129.3, 129.9, 130.1, 131.1, 131.2, 132.1 (CHAr), 140.9, 141.7 (CqAr), 180.2 (CO); MS (IC, CH_4): *m*/*z* 282 (MH^+, 40), 281 (100), 236 (18), 154 (22), 135(25), 124 (15), 106 (20), 91 (6); HRMS (IC, CH_4): [M+H$^+$] calcd for $C_{18}H_{20}NO_2$ 282.1494; found 282.1492.

3.10. Coupling with (*L*)-Ala-OMe: general procedure

To a solution of crude *N*-Bn azetidinic amino acid, HCl **17** or **18** (0.094 mmol) in dichloromethane (2 mL) was added 2-hydroxybenzotriazole (0.19 mmol), (*L*)-Ala-OMe, HCl (0.14 mmol), 1-(3-dimethyl-aminopropyl)-3-ethyl carbodiimide (0.11 mmol) and triethylamine (0.47 mmol). After 12 h of stirring at rt, the reaction was quenched by the addition of water. Usual workup and purification by flash chromatography (AcOEt/CyH: 4/6) gave dipeptides **19** or **20**. Dipeptide **19**: 58% (starting from a 92/8 crude epimeric mixture of **17**/**18**); Rf = 0.58 (AcOEt/CyH = 1/1); $[\alpha]_D^{25}=-2.4$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): 1.14 (d, 3H, $J=5.9$ Hz, Me), 1.45 (d, 3H, $J=7.3$ Hz, Me), 3.09 (t, 1H, $J=7.9$ Hz, CHPh), 3.39 (quadd, 1H, $J=5.9$ and 7.9 Hz, CHMe), 3.65 (d, 1H, $J=12.5$ Hz, C*H*HPh), 3.69 (d, 1H, $J=7.9$ Hz, H_2), 3.77 (s, 3H, OMe), 3.82 (d, 1H, $J=12.3$, CH*H*Ph), 4.52 (quint, 1H, $J=7.5$, NHC*H*Me), 7.18–7.43 (m, 10H, Ar), 7.88 (d, 1H, $J=7.5$ Hz, NH); ^{13}C NMR: 18.8, 22.1 (Me) 47.6, 49.9 (CH), 52.5 (Me), 61.5 (CH_2), 65.9, 69.8 (CH),126.9, 127.1, 127.6, 128.6, 128.7, 129.7 (CHAr), 137.4, 140.2 (CqAr), 172.1, 173.3 (CO). Dipeptide **20**: 72% (starting from isomerically pure *cis*-**18**); Rf = 0.65 (AcOEt/CyH = 8/2); $[\alpha]_D^{25}=-70.2$ (*c* 0.5, $CHCl_3$); Mp 138–140°C; ^{1}H NMR (200 MHz, $CDCl_3$): 0.95 (d, 3H, $J=7.0$ Hz, Me), 1.42 (d, 3H, $J=6.6$ Hz, Me), 3.45 (dd, 1H, $J=3.1$ and 9.2 Hz, CHPh), 3.68 (s, 3H, OMe), 3.69 (d, 1H, $J=13.2$ Hz, C*H*HPh), 3.92 (quint, 1H, $J=7.0$ Hz, CHMe), 3.94 (d, 1H, $J=13.6$ Hz, CH*H*Ph), 4.02–4.11 (m, 1H, NHC*H*Me), 4.30 (d, 1H, $J=9.2$ Hz, H_2) 7.18–7.43 (m, 11H, Ar, NH); ^{13}C NMR: 15.7, 18.7 (Me) 46.9, 47.8 (CH), 52.3 (Me), 54.2 (CH_2), 60.4, 68.5 (CH), 126.9, 127.4, 128.1, 128.6, 128.7, 128.9 (CHAr), 138.3, 138.4 (CqAr), 169.5, 173.0 (CO); Anal. Calc. for $C_{22}H_{26}N_2O_3$: C: 72.11; H: 7.15; N: 7.64. Found: C: 71.06; H: 7.24; N: 7.56.

3.11. Coupling with (*L*)-Boc-Ala-OH: general procedure

To a solution of dipeptide **19** or **20** (0.3 mmol) in MeOH (8 mL) was added TMSCl (0.6 mmol), followed by Pd/C (10% wt., 40 mg) after 5 min. The suspension was vigorously stirred under an atmosphere of hydrogen for 24 h, filtrated over Celite, and concentrated under reduced pressure. Crude debenzylated dipeptides were used without purification for the next step. To a solution of dipeptide (0.36 mmol) in dichloromethane were added 2-hydroxybenzotriazole (0.72 mmol), (*L*)-Boc-Ala-OH (0.43 mmol), 1-(3-dimethyl-aminopropyl)-3-ethyl carbodiimide (0.54 mmol) and triethylamine (1.08 mmol). After 12 h of stirring at rt, the addition of water was followed by usual workup (CH_2Cl_2) and purification by flash chromatography (AcOEt/CyH: 6/4 for **21** and AcOEt for **22**) and gave tripeptides **21** or **22**. Tripeptide **21**: (yield: 32%); Rf = 0.68 (AcOEt); $[\alpha]_D^{25}=-47$ (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, 263 K, $CDCl_3$): Mixture of two rotamers: 1.29 (d, 3H, $J=7.2$ Hz, Me), 1.30 (d, 3H, $J=7.2$ Hz, Me), 1.38 (d, 3H, $J=7.2$ Hz, Me), 1.39 (s, 9H, Boc), 1.42 (d, 3H, $J=7.2$ Hz, Me), 1.45 (s, 9H, Boc), 1.59 (d, 3H, $J=6.2$ Hz, Me), 1.60 (d, 3H, $J=6.2$ Hz, Me), 3.35 (t, 1H, $J=5.3$ Hz, CHPh), 3.68 (t, 1H, $J=6.0$, CHPh), 3.69 (s, 3H, OMe), 3.73 (s, 3H, OMe), 4.17 (quint, 1H, $J=6.9$ Hz, CHMe), 4.27 (quint, 1H, $J=6.9$ Hz, CHMe), 4.51 (quint, 1H, $J=7.2$ Hz, CHMe), 4.54 (quint, 1H, $J=6.0$ Hz, CHMe), 4.60–4.84 (m, 2H, CHMe), 4.63 (d, 1H, $J=5.6$ Hz, H2), 4.75 (d, 1H, $J=6.6$ Hz, H2), 5.02 (d, 1H, $J=5.6$ Hz, NH), 5.20 (d, 1H, $J=8.5$ Hz, NH), 7.24–7.40 (m, 10H, Ar), 7.98 (d, 1H, $J=6.9$ Hz, NH), 8.78 (d, 1H, $J=8.5$ Hz, NH); ^{13}C NMR: 18.2, 19.1, 22.5 (broad, CH_3), 28.4 (CH_3), 45.1, 46.2 (broad, CH), 48.2, 52.5 (CH_3), 64.5, 66.2 (broad, CH), 80.3 (Cq), 127.2, 127.6, 129.0 (CHAr), 139.5 (CqAr), 155.5 (broad, NCOO), 169.8, 173.0, 178.7 (broad, CO). Tripeptide **22**: (yield: 78%); Rf = 0.48 (AcOEt); $[\alpha]_D^{25}=-4.2$ (*c* 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, 298 K, $CDCl_3$): Mixture of two rotamers: 0.68 (d, 3H, $J=7.0$ Hz, Me), 1.04 (d, 3H, $J=7.3$ Hz, Me), 1.28 (d, 3H, $J=6.8$ Hz, Me), 1.38 (d, 3H, $J=6.8$ Hz, Me), 1.45 (s, 9H, Boc), 1.47 (s, 9H, Boc), 1.60 (d, 3H, $J=5.9$ Hz, Me), 1.62 (d, 3H, $J=5.6$ Hz, Me), 3.61–3.71 (m, 2H, CHPh), 3.68 (s, 3H, OMe), 3.69 (s, 3H, OMe), 3.89 (quint, 1H, $J=7.3$ Hz, CHMe), 4.24 (quint, 2H, $J=7.0$ Hz, CHMe), 4.37 (quint, 1H, $J=7.2$ Hz, CHMe), 4.78 (quint, 1H, $J=5.9$ Hz, CHMe), 5.00 (d, 1H, $J=9.8$, H2), 5.02 (d, 1H, $J=8.2$ Hz, NH), 5.09 (quint, 1H, $J=6.2$, CHMe), 5.27 (d, 1H, $J=6.5$ Hz, NH), 5.54 (d, 1H, $J=9.4$ Hz, NH), 5.94 (d, 1H, $J=6.8$ Hz, NH), 7.01 (d, 1H, $J=6.3$ Hz, NH), 7.20–7.37 (m, 10H, Ar); ^{13}C NMR: 16.8, 17.5, 17.9, 18.0 (CH_3), 20.0, 22.1, 28.3, 46.0, 46.6, 46.8, 47.1, 47.5, 47.7 (CH), 52.1, 52.3 (CH_3), 61.7, 62.9, 64.2, 65.9 (CH), 79.5, 79.8 (Cq), 127.6, 127.7, 128.2, 128.4, 128.6, 128.7

(CHAr), 135.1, 135.9 (CqAr), 1554, 155.6 (NCOO), 166.1, 166.9, 172.7, 173.0, 173.4, 174.0 (CO); Anal. Calc. for $C_{23}H_{33}N_3O_6$: C: 61.73; H: 7.43; N: 9.39. Found: C: 61.68; H: 7.55; N: 9.24.

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