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ORIGINAL ARTICLE

Synthesis of a conformationally constrained δ-amino acid building block

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Abstract Conformationally restricted amino acids are important components in peptidomimetics and drug design. Herein, we describe the synthesis of a novel, non-proteinogenic constrained delta amino acid containing a cyclobutane ring, *cis*-3(aminomethyl)cyclobutane carboxylic acid (ACCA). The synthesis of the target amino acid was achieved in seven steps, with the key reaction being a base induced intramolecular nucleophilic substitution. A small library of dipeptides was prepared through the coupling of ACCA with proteinogenic amino acids.

Keywords Non-proteinogenic amino acid · Dipeptides · Conformational restriction · Cyclobutane ring

Introduction

Many neurotransmitters, neuromodulators and hormones are peptides which interact with receptors and effect biological processes. Peptide agonists or antagonists have the potential to be used as therapeutic agents by mimicking the messenger molecule and disrupting the biological process. However, studies have shown that bioactive peptides often display poor bioavailability and can easily be degraded by peptidases (Giannis and Kolter 1993). Another limitation associated with the use of peptides as drugs is their high hydrophilicity and high flexibility. Peptidomimetics have been designed to mimic bioactive peptides and display enhanced pharmacological properties, such as increased bioavailability and metabolic stability (Gante 1994; Smith et al. 2002; Giannis and Kolter 1993). Further to this, it is proposed that appropriate conformational restriction of lead compounds can increase the binding affinity of the drug for its receptor (Boger et al. 2000). Conformationally constrained amino acids are a useful way of tailoring the rigidity of peptides. There have been a number of reports on the synthesis of conformationally constrained amino acids (Miller and Grubbs 1995) and in particular, great interest has been shown for amino acids containing a cyclobutane moiety (Burgess et al. 1997; Aguilera et al. 2008a; Andre' et al. 2011). Some of these residues have been incorporated into peptides (Aguilera et al. 2008b), peptide dendrimers (Gutierrez-Abad et al. 2010) and even into nucleotides (Fernandez et al. 1995). The presence of these amino acids can lead to unusual conformations and hydrogen-bonding interactions in the modified dipeptides (Izquierdo et al. 2005; Gorrea et al. 2011) and peptides (Fernandez-Tejada et al. 2009; Torres et al. 2009; Gutiérrez-Abad et al. 2011; Celis et al. 2012).

The incorporation of ACCA into peptides can result in well-defined conformations and molecular rigidity. Cyclobutane rings retain considerable flexibility due to flipping between the two conformations through low energy barriers and this may favour interactions between peptidomimetics and receptors or active sites (Allan et al. 1990).

Further to their application in the field of peptidomimetics, cyclobutane derivatives have been widely exploited in organic synthesis (Lee-Ruff and Mladenova 2003;

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Namyslo and Kaufmann 2003) and the strained motif features in several natural products and synthetic compounds which often display interesting biological properties (Dembitsky 2008).

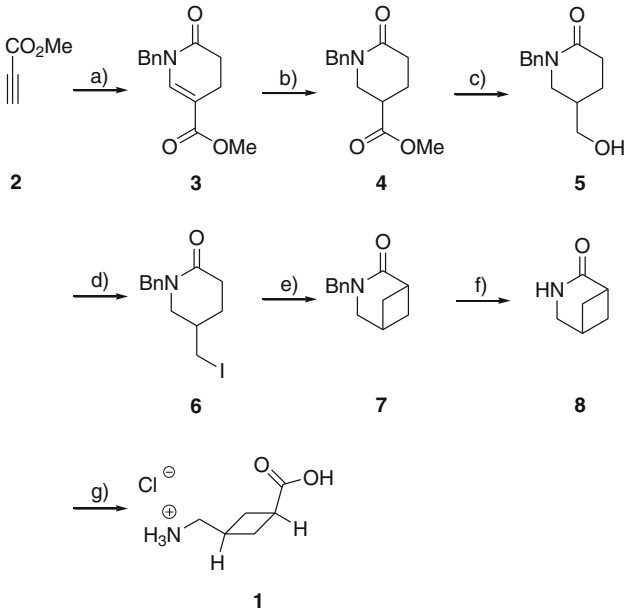
In this context, we describe a seven steps synthesis for the preparation of a novel achiral, conformationally constrained amino acid (Fig. 1). An enantioselective synthesis of the 2,2-dimethyl derivative of **1** has been previously reported starting from α-pinene (Fernandez et al. 1995). We have also synthesised a small library of dipeptides by coupling amino acid **1** with the proteinogenic amino acids glycine, valine and phenylalanine as well as self-coupling of **1**.

Results and discussion

Our approach to the synthesis of amino acid **1** involved the construction of nitrogen heterocycle **3** (Scheme 1), whose synthesis in two steps has previously been reported (Cook et al. 1994). The reaction involved the formation of an enamine via the conjugate addition of benzylamine to methyl propiolate **2**, followed by the aza-annulation reaction with acryloyl chloride, giving ester **3** in 53 % yield over 2 steps (Cook et al. 1994). Hydrogenation of **3** gave racemic ester **4** in 97 % yield. Treatment of **4** with lithium borohydride afforded racemic alcohol **5**, which was converted to the iodo-derivative **6** passing through a mesylated intermediate with 93 % yield over the 2 steps. Direct iodination of **5** was also achieved, albeit with lower yield (Garegg and Samuelsson 1980). It is noteworthy that a kinetic resolution of racemic ester **4** has previously been reported, providing access to the enantio-enriched compounds in high yields (Gray and Gallagher 2006). In the same study, the direct bromination of **5** has also been reported. Treatment of **6** with LHMDS resulted in a rapid intramolecular nucleophilic substitution, yielding **7** in 93 % yield. This type of intramolecular cyclisation has been previously reported. Galeazzi et al. employed this strategy for the synthesis of both enantiomers of *cis*-2-aminomethylcyclobutane (Galeazzi et al. 1999). Compound **7** was deprotected under Birch conditions affording bicyclic derivative **8** in 94 % yield. The formation of **8** was confirmed by X-ray crystallography (Fig. 2).

Acidic hydrolysis of **8** afforded amino acid salt **1**·HCl in quantitative yield. The nature of the cyclic precursor means that only the *cis* isomer was obtained upon hydrolysis due to **8** being "conformationally locked". ^{1}H NMR data suggested that no epimerization had occurred during hydrolysis and the X-ray crystal structure of the Boc-protected amino acid **13** supported this result (Fig. 3). In order to investigate the possibility of epimerisation, amino acid **1** was also treated with a base [NaOH (2 M) and stirred overnight at 100 °C] with no changes noticed in this ^{1}H-NMR spectrum denoting a great stability of this compound.

A small library of dipeptides containing ACCA **1** was then prepared (Schemes 2, 3). The carboxylic acid moiety was first protected through esterification. Lewis acids such as $TiCl_4$ and $ZrCl_4$ have previously been employed for highly successful esterifications (Singh et al. 2008; Shang et al. 2007). A catalytic quantity of zirconium chloride was found to be an effective catalyst for the esterification of amino acid **1**. The reaction proceeded with high efficiency in *n*-propanol yielding amino ester **9** in 92 % yield.



Scheme 1 Synthesis of conformationally constrained amino acid salt **1**·HCl. Reagents and conditions: *a)* (i) $BnNH_2$, Et_2O, −10 °C rt, 1 h; (ii) acryloyl chloride, THF, rt, 12 h 53 %; *b)* H_2, Pd/C, Na_2CO_3, EtOH, rt, 12 h, 97 %; *c)* $LiBH_4$, THF, 0 °C rt, 12 h, 77 %; *d)* (i) CH_3SO_2Cl, TEA, CH_2Cl_2, −10 °C rt, 16 h; (ii) NaI, acetone, reflux, 24 h, 93 %; or PPh_3, imidazole, I_2, toluene, 3 h, 68 %; *e)* LHMDS, THF, −20 °C, 1 h, 93 %; *f)* NH_3(l), Li(s), THF, *t*BuOH, −78 °C, 94 %; *g)* HCl 2 M, reflux, 12 h, 99 %

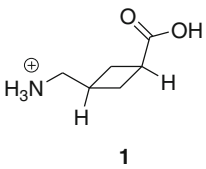


Fig. 1 δ-Amino acid 1

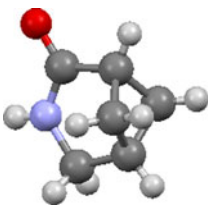


Fig. 2 X-ray crystal structure of bicyclic amide 8

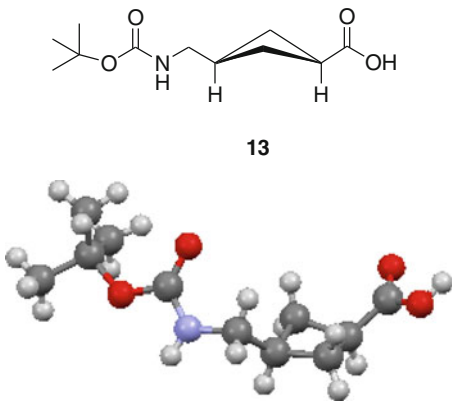


Fig. 3 X-ray crystal structure of Boc-protected ACCA **13**

Amino acids **10 a–c** were protected at the N-terminus with *tert*-Butyloxycarbonyl (*t*-Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc) and then coupled with ester **9** to achieve the protected dipeptides **11 a–c**. Best results were achieved using *O*-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) as the coupling agent. Base-catalysed hydrolysis was employed for deprotection of the C-terminus ester. The deprotection of the N-terminus Fmoc took place under the same conditions while the deprotection of the N-terminus *t*-Boc in **11a** was achieved under standard acidic conditions using HCl/Et_2O. In this case, the non-aqueous conditions aid isolation of the deprotected amino acid, which precipitates as a solid.

The same strategy was followed for the synthesis of **15**. NMR spectra of the dipeptides indicated the formation of a single product, again with no epimerization observed.

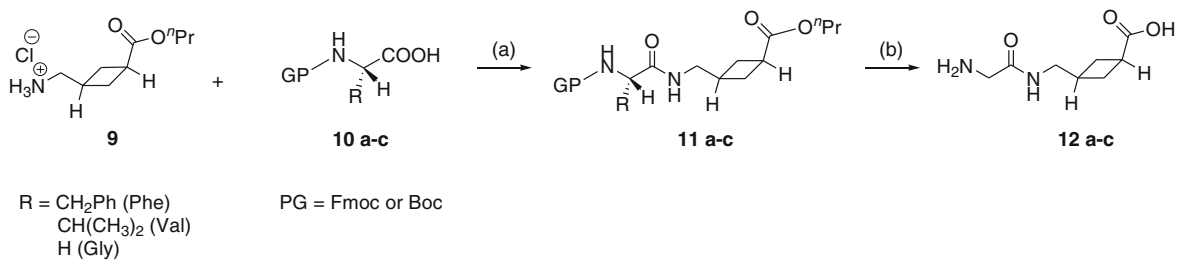
In the case of the Gly-ACCA dipeptide (**12c**), unusual behaviour was observed. Initially, ^{1}H-NMR analysis indicated a splitting of the signals, which was attributed to epimerization of the compound. However, 2D NMR studies and variable temperature NMR experiments indicated that neither diasteromers nor rotamers were involved in the phenomenon. To investigate this further, an alternative stepwise strategy was adopted for the deprotection of compound **11c**. Piperidine was initially employed for the selective cleavage of the Fmoc group, however, the product could not be isolated with a sufficient degree of purity. Triethylamine yielded the best results, and compound **12c** was obtained after an overnight reflux and subsequent purification with ion exchange chromatography in excellent yields. The ion exchange resin purification step was sufficient to catalyse also the hydrolysis of the ester protecting group, affording the free carboxylic acid. The efficiency of this hydrolysis technique was already demonstrated in the literature (Basu et al. 1989; Qureshi et al. 1968).

Experimental

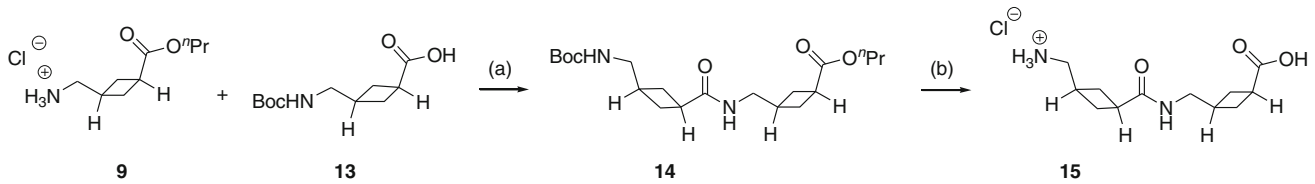
General methods

All chemicals were purchased from Sigma Aldrich. Dry solvents were obtained from a Puresol Grubbs Speciality Chemicals and Stream. When necessary, all reactions were performed under an atmosphere of nitrogen using oven dried glassware. Oxygen-free nitrogen was obtained from BOC gases and used without further drying.

Proton and carbon nuclear magnetic resonance spectra (^{1}H and ^{13}C-NMR, respectively) were recorded on 400 MHz (operating frequencies: ^{1}H, 399.75 MHz; ^{13}C, 101.00), 500 MHz (operating frequencies: ^{1}H, 499.72 MHz; ^{13}C, 125.65) and 600 MHz (operating frequencies: ^{1}H, 599.78 MHz; ^{13}C, 150.82) FT spectrometers. Tetramethylsilane was used as an internal reference in the deuterated chloroform ($CDCl_3$) ($\delta = 0.00$ ppm) for ^{1}H NMR spectra. The middle $CDCl_3$ solvent peak was referenced to 77.16 ppm for ^{13}C NMR spectra. The residual solvent peak in deuterated methanol (CD_3OD) was referenced to 3.31 and 49.00 ppm for ^{1}H NMR and ^{13}C NMR spectra, respectively. The residual solvent peak in deuterated water (D_2O) was referenced to 4.79 ppm for ^{1}H NMR. The coupling constants (J) are in Hz and the chemical shifts (δ) are given in parts per million. High resolution mass spectra were obtained on a Waters/Micromass instrument. Melting points are uncorrected. Evaporation in vacuo refers to the removal of solvent on a Büchi rotary



Scheme 2 Synthesis of dipeptides **12 a–c**. Reagents and conditions: *a)* HATU, ^{i}Pr$_2$EtN, CH_2Cl_2, 18 h, 38–92 %; *b)* for **12a**: (i) NaOH 2 M, CH_3CN, 45 °C, 3 h; (ii) HCl/Et_2O, CH_2Cl_2, 54 %; for **12b**: NaOH 2 M, dioxane/water 7:3, 45 °C, 3 h, 90 %; for **12c**: CH_2Cl_2/TEA 1:1, 45 °C, 24 h, 90 %



Scheme 3 Synthesis of self-coupling dipeptide **15**. Reagents and conditions*: a)* HATU, $^{i}Pr_2EtN$, CH_2Cl_2, 18 h, 58 %; *b)* (i) NaOH 2 M, CH_3CN, 45 °C, 3 h; (ii) HCl/Et_2O, CH_2Cl_2 57 %

evaporator with an integrated vacuum pump. Thin-layer chromatography (TLC) was carried out on aluminium backed 60 F254 silica gel.

Compounds **3** and **4** were prepared as described in the work of Cook and colleagues (1994). Methods and characterization are reported in the supporting information.

Preparation of (±)1-benzyl-5-(hydroxymethyl)piperidin-2-one (5)

(±)**4** (5.23 g, 23.85 mmol) was dissolved in anhydrous THF (35 mL) and cooled to 0 °C. $LiBH_4$ (1.04 g, 47.70 mmol) was added and the solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched at 0 °C with water (20 mL) and then with 10 % HCl. The organic layer was separated and the aqueous layer was extracted with EtOAc (4 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was purified by silica gel column chromatography (cyclohexane/EtOAc, 60:40) to give (±)7 (4.03 g, 77 % yield) as a clear oil. (Rf = 0.11, cyclohexane/EtOAc, 50:50). δH (600 MHz; $CDCl_3$) 7.33–7.18 (5H, m, PhH), 4.59 (1H, d, J = 14.6, $PhCH_2$), 4.53 (1H, d, J = 14.6, $PhCH_2$), 3.53 (1H, dd, J = 10.7 and 5.6, CH_2OH), 3.44 (1H, dd, J = 10.7 and 7.2, CH_2OH), 3.30 (1H, ddd, J = 12.2, 5.2 and 1.5, NCH_2), 2.99 (1H, dd, J = 12.2 and 10.1, NCH_2), 2.92 (1H, br s, OH), 2.51 (1H, ddd, J = 17.8, 5.8 and 3.4, C = OCH_2), 2.39 (ddd, J = 17.8, 11.3 and 6.5, C = OCH_2), 2.04–1.95 (1H, m, $OHCH_2CH$), 1.89–1.82 (1H, m, C = OCH_2CH_2), 1.54–1.46 (1H, m, C = OCH_2CH_2); δC (151 MHz; $CDCl_3$) 170.0, 137.0, 128.5, 127.9, 127.3, 64.3, 50.3, 49.8, 36.4, 31.1, 23.8; m/z (ES) 220.1329 ($M + H^+$ $C_{13}H_{18}NO_2$ requires 220.1338).

Preparation of (±) 1-benzyl-5-(iodomethyl)piperidin-2-one (6)

(±)**5** (3.92 g, 13.18 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL). The solution was cooled to −10 °C and TEA (2.0 g, 19.77 mmol) was added, followed by the slow addition of CH_3SO_2Cl (1.81 g, 15.19 mmol). The solution was allowed to warm slowly to room temperature and stirred overnight. The organic layer was concentrated *in vacuo* and dissolved in acetone (50 mL) and NaI (3.98 g, 26.58 mmol) was added to the stirring solution. The mixture was stirred under reflux for 24 h. The organic solvent was removed in vacuo and H_2O (40 mL) was added along with EtOAc (50 mL), the organic layer was separated and the aqueous layer was extracted with EtOAc (5 × 20 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was purified by silica gel column chromatography (cyclohexane/EtOAc, 70:30) to give **6** (3.36 g, 96 % yield) as a yellow/orange oil. (Rf = 0.12, cyclohexane/EtOAc, 50:50). δH (500 MHz; $CDCl_3$) 7.3–7.22 (5H, m, PhH), 4.63 (1H, d, J = 14.7, $PhCH_2$), 4.54 (1H, d, J = 14.7, $PhCH_2$), 3.34 (1H, m, NCH_2), 3.08 (2H, m, CH_2I), 2.97 (1H, dd, J = 12.0 and 9.5, NCH_2), 2.56 (1H, ddd, J = 17.7, 6.2 and 3.6, C = OCH_2), 2.45 (1H, ddd, J = 17.7, 11.2 and 6.2, C = OCH_2), 2.06–1.95 (2H, m, C = OCH_2CH_2), 1.66–1.54 (1H, m, $CHCH_2$); δC (126 MHz; $CDCl_3$) 169.0, 136.8, 128.5, 128.0, 127.4, 52.3, 50.1, 35.9, 30.7, 27.7, 7.9; m/z (EI) 329.0273 ($M + H^+$. $C_{13}H_{16}INO$ requires 329.0277).

Preparation of 3-benzyl-3-aza-bicyclo[3.1.1]heptan-2-one (7)

(±)**6** (3.2 g, 9.72 mmol) was dissolved in anhydrous THF (5 mL) and LHMDS (1 M soln./THF, 9.72 mL) was added at −20 °C. The solution was stirred for 1 h before being quenched with H_2O (15 mL). EtOAc (20 mL) was added and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was filtered through a short silica column (cyclohexane/EtOAc, 2:1) to give **7** (1.82 g, 93 % yield) as a yellow/orange oil. (Rf = 0.24, cyclohexane/EtOAc, 50:50). δH (500 MHz; $CDCl_3$) 7.36–7.22 (5H, m, PhH), 4.61 (2H, s, $PhCH_2$), 3.26 (2H, d, J = 1.5, NCH_2), 2.84 (1H, q, J = 5.7 Hz, C = OCH), 2.72–2.65 (1H, m, NCH_2CH), 2.38–2.28 (2H, m, C = $OCH(CHH)_2$, 1.73–1.63 (2H, m, C = $OCH(CHH)_2$; δC (126 MHz; $CDCl_3$) 175.7, 137.4, 128.5, 128.0, 127.2, 50.0, 48.0, 41.1, 33.2, 30.9; m/z (ES) 202.1222 ($M + H^+$ $C_{13}H_{16}NO$ requires 202.1232).

Preparation of 3-aza-bicyclo[3.1.1]heptan-2-one (8)

7 (1.5 g, 7.45 mmol) was dissolved in anhydrous THF/*t*BuOH, 10:1 (10 mL) and the mixture was added to a stirring liquid ammonia at −78 °C. Metallic Li pellets were added slowly to the stirring solution until a constant blue/black colour was observed. The reaction was then quenched by the addition of solid ammonium chloride. After removal of the NH_3, EtOAc (30 mL) and H_2O (8 ml) were added. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was filtered through a short silica column (cyclohexane/EtOAc, 1:1) to give **8** (800 mg, 94 % yield) as a yellow oil. (Rf = 0.1, cyclohexane/EtOAc, 50:50). δH (500 MHz; CD_3OD) 7.46–7.13 (1H, m, NH) 3.43 (2H, d, $J = 2.0$, NCH_2), 2.81–2.71 (1H, m, NCH_2CH), 2.60 (1H, q, $J = 5.6$ Hz, C = OCH), 2.48–2.37 (2H, m, C = OCH(CHH)$_2$, 1.70–1.62 (2H, m, C = OCH(CHH)$_2$). δC (126 MHz; CD_3OD) 181.5, 46.3, 42.0, 34.3, 31.4; m/z (EI+) 111.0684 ($M + H^+$ C_6H_9NO requires 111.0684).

Preparation of cis-3-(aminomethyl)cyclobutanecarboxylic acid (1 HCl)

8 (220 mg, 1.98 mmol) was refluxed in 2 M HCl (10 mL) overnight. The H_2O was removed in vacuo to give **1** (328 mg, quantitative yield) as a beige solid with no further purification required. Mp: 169–170 °C. δH (500 MHz; CD_3OD) 3.18–3.08 (1H, m, C = OCH), 2.96 (2H, d, $J = 7.3$, NCH_2), 2.63–2.51 (1H, m, NCH_2CH), 2.46–2.37 (2H, m, C = OCH(CHH)$_2$, 2.02 (2H, m, C = OCH(CHH)$_2$; δC (126 MHz; CD_3OD) 176.8, 45.5, 34.9, 30.2, 29.9; m/z (ES^+) 130.0863 ($M + H^+$ $C_6H_{12}NO_2$ requires 130.0868).

Preparation of (3-(Propoxycarbonyl)cyclobutyl) methanaminium chloride (9)

1·HCl (370 mg, 2.86 mmol) was dissolved in *n*-propanol (10 mL) and $ZrCl_4$ (133 mg, 0.572 mmol, 20 mol %) was added. The solution was refluxed for 24 h before being concentrated in vacuo. The crude oil was dissolved in $CHCl_3$ (2 mL) and left overnight. The precipitate was filtered off and the solution concentrated in vacuo to give **11** (570 mg, 92 % yield) as an off-white solid. Mp: 159–160.5 °C. δH (400 MHz; CD_3OD) 3.46 (2H, t, $J = 6.7$, $CH_2CH_2CH_3$), 3.08 (1H, m, C = OCH), 2.93 (2H, d, $J = 7.4$, NCH_2), 2.60–2.49 (1H, m, NCH_2CH), 2.43–2.33 (2H, m, C = OCH(CHH)$_2$, 1.98 (2H, ddd, $J = 18.7$, 9.3 and 2.4, C = OCH(CHH)$_2$, 1.56–1.43 (2H, m, CH_2CH_3), 0.88 (3H, t, $J = 7.4$, CH_2CH_3); δC (101 MHz; CD_3OD) 176.7, 64.7, 45.4, 34.8, 30.1, 29.9, 26.6, 10.5; m/z (ES+) 172.1342 ($M + H^+$ $C_9H_{18}NO_2$ requires 172.1338).

Preparation of 3-(((tert-butoxycarbonyl)amino) methyl)cyclobutanecarboxylic acid (13)

1 HCl (150 mg, 0.9 mmol) was dissolved in dioxane and water 2:1 (12 mL) and cooled to 0 °C. 1 M NaOH (5 mL) was added and the solution was stirred for 10 min before adding 2 eq of bis-*tert*-butyl dicarbonate (390 mg, 1.8 mmol). The reaction was left under stirring for 3 h before being concentrated in vacuo. The aqueous layer was extracted with Et_2O (15 mL × 2) and adjusted to pH 3 by the addition of 1 M HCl. The acidified aqueous layer was extracted with CH_2Cl_2 (15 mL × 5) and the combined CH_2Cl_2 layers were dried over $MgSO_4$ and concentrated in vacuo to give **13** (227 mg, 95 % yield) as a brown solid. No further purification was required. (Rf = 0.28, EtOAc/CH_3OH 90:10). Mp: 63–65 °C. δH (400 MHz; $CDCl_3$) 5.99 (1H, br s, COOH), 4.61 (1H, br s, NH), 3.13 (2H, s, NCH_2), 3.07–2.98 (1H, quin, J 8.9 Hz, C = OCH), 2.51–2.36 (1H, m, NCH_2CH), 2.37–2.25 (2H, m, C = OCH(CHH)$_2$, 2.00–2.09 (2H, m, C = OCH(CHH)$_2$, 1.44 (9H, s, $C(CH_3)_3$); δC (101 MHz; $CDCl_3$) 180.8, 156.5, 79.5, 45.7, 34.5, 31.4, 28.8, 28.4; m/z (ES+) 252.1212 ($M + Na^+$ $C_{11}H_{19}NO_4Na$ requires 252.1212).

General method: coupling of protected dipeptides

9, the N-protected amino acid (**10a–c** and **13**), and *O*-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU, 1 eq) were dissolved in anhydrous THF (20 mL). The solution was cooled to 0 °C and *N,N*-diisopropylethyl amine (3 eq) was added. The reaction was stirred at room temperature overnight. The solvent was evaporated in vacuo and the crude oil dissolved in CH_2Cl_2 (15 mL) and washed with $NaHCO_3$ (15 mL), acetic acid 10 % (15 mL × 2) and brine (15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo.

Preparation of propyl (S)-3-((2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)methyl)cyclobutanecarboxylate (11a)

11a was prepared following the General Procedure, by reacting **9** (300 mg, 1.45 mmol) with *N*-Boc-L-Phenylalanine **10a** (500 mg, 1.89 mmol). The crude oil was purified by silica gel column chromatography (cyclohexane/EtOAc, 100:0 then 70:30) to give **11a** as a white solid (557 mg, 92 % yield). (Rf = 0.65, cyclohexane/EtOAc, 50:50). $[\alpha]_D^{20}$: +1.6° (c $13{\cdot}10^{-3}$ $CHCl_3$). Mp: 106–108 °C. δH

(500 MHz; $CDCl_3$) 7.33–7.17 (5H, m, PhH), 5.85 (1H, s, N*H*C = OCH), 5.08 (1H, s, NHBoc), 4.27 (1H, dd, $J = 14.6$, 7.3 Hz, C*H*CH_2Ph), 4.02 (2H, t, $J = 6.7$ Hz, C$H_2$$CH_2CH_3$), 3.26–3.12 (2H, dd, $J = 12.8$ and 6.5 Hz, NCH_2), 3.11–3.04 (1H, dd, $J = 6.5$ and 13.6 Hz, CH_2Ph), 3.04–2.98 (1H, dd, $J = 7.7$ and 13.6 Hz, CH_2Ph), 2.97–2.88 (1H, m, C = OCH), 2.34–2.27 (1H, m, NCH_2 C*H*), 2.25–2.12 (2H, m, C = OCH(C*H*H)$_2$, 1.93–1.79 (2H, m, C = OCH(CH*H*)$_2$), 1.68–1.61 (2H, m, C$H_2$$CH_3$), 1.41 (9H, s, C$(CH_3)_3$), 0.93 (3H, t, $J = 7.4$ Hz, CH_2CH_3). δC (125.65 MHz; $CDCl_3$) 175.2, 171.6, 155.7, 137.3, 129.7, 128.9, 127.1, 80.2, 66.2, 56.4, 45.0, 39.4, 34.4, 30.8, 29.1, 28.8, 22.3, 10.6; m/z (ES+) 417.2370 (M-H$^+$. $C_{23}H_{33}N_2O_5$ requires 417.2389).

Preparation of propyl (S)-3-((2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)methyl) cyclobutanecarboxylate (11b)

11b was prepared according to General Procedure, by reacting **9** (180 mg, 0.86 mmol) with *N*-Fmoc-L-Valine **10b** (580 mg, 1.7 mmol). The crude oil was purified through a silica gel column chromatography (cyclohexane/EtOAc from 100:0 to 40:60) giving **11b** as a yellow solid (155 mg, 38 % yield). (Rf = 0.86, EtOAc). $[\alpha]_D^{20}$: −13.0° (c 10.2×10^{-3} $CHCl_3$). Mp: 152–154 °C. δH (500 MHz; CD_3OD) 7.75 (2H, d, $J = 7.5$ Hz, Ar), 7.58 (2H, d, $J = 7.4$ Hz, Ar), 7.39 (2H, t, $J = 7.4$ Hz, Ar) 7.29 (2H, m, Ar), 6.29 (1H, s, N*H*C = OCH), 5.57 (1H, d, $J = 8.2$ Hz, NHFmoc), 4.43–4.39 (1H, m, CH_2Fmoc), 4.35–4.32 (1H, m, CH_2Fmoc), 4.19 (1H, t, $J = 7.0$ Hz, FmocCH), 4.01 (2H, t, $J = 6.7$ Hz, C$H_2$$CH_2CH_3$), 3.96–3.94 (1H, m, C*H*CH$(CH_3)_2$), 3.36–3.27 (1H, m, NCH_2), 3.27–3.18 (1H, m, NCH_2), 3.05–2.93 (1H, m, C = OCH), 2.53–2.38 (1H, m, NCH_2C*H*), 2.29–2.24 (2H, m, C = OCH(C*H*H)$_2$), 2.16–2.07 (1H, m, C*H*$(CH_3)_2$), 2.01–1.90 (2H, m, C = OCH(CH*H*)$_2$), 1.68–1.56 (2H, m, C$H_2$$CH_3$), 0.92 (6H, m, CH(C$H_3$)$_2$ and 3H, t, $J = 7.4$ Hz, CH_2CH_3); δC (125.65 MHz; $CDCl_3$) 175.5, 171.4, 156.6, 144.0, 141.6, 128.2, 127.4, 125.3, 120.1, 67.3, 66.3, 60.8, 47.3, 44.2, 34.0, 31.1, 30.7, 28.8, 28.7, 22.2, 19.4, 18.1, 10.6 m/z (ES+) 515.2521 (M + Na$^+$. $C_{29}H_{36}N_2O_5Na$ requires 515.2522).

Preparation of propyl 3-((2-((((9H-fluoren-9-yl)methoxy) carbonyl)amino)acetamido)methyl) cyclobutanecarboxylate (11c)

11c was prepared according to General Procedure, by reacting **9** (240 mg, 1.15 mmol) with *N*-Fmoc-Glycine **10c** (350 mg, 1.18 mmol). The crude oil was purified through a silica gel column chromatography (cyclohexane/EtOAc from 100:0 to 20:80) giving **11c** as a white solid (437 mg, 0.97 mmol, 84 % yield). (Rf = 0.75, EtOAc/CH_3OH, 90:10). Mp: 111–113 °C. δH (500 MHz; $CDCl_3$) 7.79–7.75 (2H, d, $J = 7.6$ Hz, Ar), 7.61–7.6 (2H, d, $J = 7.4$ Hz, Ar), 7.41–7.38 (2H, t, $J = 7.5$ Hz, Ar), 7.34–7.29 (2H, m, Ar), 6.15 (1H, s, N*H*C = OCH_2), 5.49 (1H, s, NHFmoc), 4.44 (1H, d, $J = 6.9$ Hz, CH_2Fmoc), 4.23 (1H, t, $J = 6.9$ Hz, CHFmoc), 4.02 (1H, t, $J = 6.7$ Hz, C$H_2$$CH_2CH_3$), 3.86 (2H, d, $J = 4.6$ Hz, NHCH_2C = O), 3.30 (2H, t, $J = 6.1$ Hz, NCH_2), 3.03–2.96 (1H, quin, $J = 8.8$ Hz, C = OCH), 2.53–2.44 (1H, m, NCH_2C*H*), 2.35–2.26 (2H, m, C = OCH(C*H*H)$_2$), 2.02–1.92 (2H, m, C = OCH (CH*H*)$_2$), 1.67–1.60 (1H, m, CH_2C$H_2$$CH_3$), 0.92 (3H, t, $J = 7.4$ Hz, CH_2CH_3); δC (125.65 MHz; $CDCl_3$) 175.5, 169.0, 143.6, 141.6, 127.7, 127.2, 125.5, 120.0, 67.3, 66.2, 47.4, 44.3, 44.0, 34.2, 30.7, 28.4, 21.8, 10.3; m/z (ES+) 473.2033 (M + Na$^+$. $C_{26}H_{30}N_2O_5Na$ requires 473.2052).

Preparation of propyl 3-((3-(((tert-butoxycarbonyl) amino)methyl)cyclobutanecarboxamido)methyl) cyclobutanecarboxylate (14)

14 was prepared according to General Procedure, by reacting **9** (181 mg, 0.87 mmol) with **13** (195 mg, 0.85 mmol). The crude oil was purified through a silica gel column chromatography (cyclohexane/EtOAc from 100:0 to 0:100) giving **14** as a clear yellow solid (194 mg, 58 % yield). (Rf = 0.13, cyclohenane/EtOAc 50:50). Mp: 62–64 °C. δH (400 MHz; $CDCl_3$) 5.60 (1H, s, N*H*C = OCH), 4.72 (1H, s, N*H*Boc), 4.02 (2H, t, $J = 6.5$ Hz, C$H_2$$CH_2CH_3$), 3.25 (2H, t, $J = 5.9$, NCH_2), 3.13–3.11 (2H, m, BocNCH_2), 3.04–2.95 (1H, m, C*H*C = OO^n*Pr*), 2.92–2.79 (1H, m, CHC = ON), 2.55–2.37 (2H, m, NCH_2C*H* and BocNCH_2C*H*), 2.33–2.21 (4H, m, OC = OCH(C*H*H)$_2$ and NC = OCH(C*H*H)$_2$), 2.01–1.92 (4H, m, OC = OCH(CH*H*)$_2$ and NC = OCH (CH*H*)$_2$), 1.69–1.60 (2H, m, CH_2CH_3), 1.44 (9H, s, C$(CH_3)_3$), 0.92 (3H, t, $J = 7.4$ Hz, CH_2CH_3). δC (101 MHz; $CDCl_3$) 175.7, 175.4, 156.4, 79.6, 66.5, 45.9, 44.6, 36.6, 34.4, 31.2, 28.9, 28.8, 22.4, 10.7; m/z (ES−) 381.2408 (M − H$^-$ $C_{20}H_{33}N_2O_5$ requires 381.2389).

Preparation of (S)-1-(((3-carboxycyclobutyl)methyl) amino)-1-oxo-3-phenylpropan-2-aminium chloride (12a)

11a (550 mg, 1.31 mmol) was dissolved in 20 mL of CH_3CN and NaOH 2 M (8 mL) was added. The reaction was left under stirring for 2 h at 45 °C before bringing the solution to pH 3 with HCl 1 M and extracting it with CH_2CH_2 (15 mL × 5). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The white solid obtained was dissolved in CH_2CH_2 (20 mL) and HCl in Et_2O 3 M (0.4 mL) was added at 0 °C. The reaction was stirred for 4 h. The white precipitate was filtered off and

washed with Et_2O (190 mg, 0.69 mmol) with an overall yield (over the 2 steps) of 53 %. (Rf = 0.08, EtOAc/CH_3OH, 90:10). $[\alpha]_D^{20}$: +26.0° (c 7.6 × 10^{-3} MeOH). Mp: 170–172 °C. δH (400 MHz; CD_3OD) 7.46–7.18 (5H, m, PhH), 4.08 (1H, t, J = 7.4 Hz, C*H*CH_2Ph), 3.25–3.05 (4H, m, NCH_2 and CH_2Ph), 3.05–2.93 (1H, m, C = OCH), 2.45–2.26 (1H, m, NCH_2C*H*), 2.28–2.15 (2H, m, C = OCH(C*H*H)$_2$), 1.90–1.81 (2H, m, C = OCH(CH*H*)$_2$); δC (101 MHz; CD_3OD) 177.1, 169.6, 135.7, 130.6, 129.9, 128.7, 55.8, 45.4, 38.6, 34.7, 31.8, 29.9; m/z (ES+) 277.1552 (M + H^+ $C_{15}H_{21}N_2O_3$ requires 277.1552).

Preparation of (S)-3-((2-amino-3-methylbutanamido) methyl)cyclobutanecarboxylic acid (12b)

11b (48 mg, 0.09 mmol) was dissolved in dioxane/methanol 7:3 (7 mL) and NaOH 2 M (2 mL) was added. The reaction was left under stirring for 4 h at 45 °C. Dioxane was evaporated in vacuo and the water left was extracted with Et_2O (10 mL) and CH_2CH_2 (10 mL × 2). The aqueous layer was neutralised with HCl 1 M and purified with DOWEX 50WX8-200 ion exchange column affording **12b** as a yellow solid (18 mg, 0.078 mmol, 90 % yield). (Rf = 0.55, CH_3OH). Mp could not be measured because the compound is hydroscopic. $[\alpha]_D^{20}$: +118.0° (c 0.5 × 10^{-3} MeOH). δH (500 MHz, CD_3OD) 3.50 (1H, brs, C*H*CH$(CH_3)_2$), 3.23 (2H, d, J = 6.2 Hz, NCH_2), 2.90 (1H, quint, J = 8.9 Hz, C = OC*H*$(CH_2)_2$), 2.50–2.39 (1H, m, NCH_2C*H*), 2.35–2.20 (2H, m, C = OCH(C*H*H)$_2$), 2.08–2.16 (1H, m, C*H*$(CH_3)_2$), 1.99–1.92 (2H, m, C = OCH(CH*H*)$_2$), 1.06–0.98 (3H, d, J = 6.8, Hz, CH$(CH_3)_2$ and 3H, d, J = 6.8, Hz, CH$(CH_3)_2$). δC (125.65 MHz; CD_3OD) 180.1, 169.4, 60.4, 45.3, 37.1, 31.8, 30.4, 30.2, 19.2, 18.2; m/z (ES+) 229.1546 (M + H^+ $C_{11}H_{21}N_2O_3$ requires 229.1552).

Preparation of cis-3-((2-aminoacetamido)methyl) cyclobutanecarboxylic acid (12c)

11c (185 mg, 0.41 mmol) was dissolved in CH_2Cl_2/TEA 1:1 (20 mL). The reaction was left under stirring for 24 h at 45 °C. The white precipitate was filtered off and washed with acetonitrile. The filtrate solution was concentrated in vacuo and the crude yellow solid was purified with DOWEX 50WX8-200 ion exchange column, affording **12c** as white solid (69 mg, 0.37 mmol, 90 % yield). (Rf = 0.3, CH_3OH). Decomposition temperature: 200 °C. δH (500 MHz; D_2O) 3.87 (2H, br s, NHCH_2C = O), 3.30 (2H, d, J = 6.4 Hz, NCH_2), 3.00–2.92 (2H, m, C = OCH), 2.52–2.42 (1H, m, NCH_2C*H*), 2.33–2.27 (2H, m, C = OCH(C*H*H)$_2$), 1.88–1.81 (2H, m, C = OCH(CH*H*)$_2$). δC (125.65 MHz; D_2O) 184.9, 166.8, 44.2, 40.5, 36,5, 29.5, 29.3; m/z (ES+) 187.1082 (M + H^+ $C_8H_{15}N_2O_3$ requires 187.1083).

Preparation of (3-(((3-carboxycyclobutyl)methyl) carbamoyl)cyclobutyl)methanaminium chloride (15)

14 (180 mg, 0.47 mmol) was dissolved in CH_3CN (15 mL) and NaOH 2 M (5 mL) was added. The reaction was left under stirring for 2 h at 45 °C before bringing the solution to pH 3 with HCl 1 M and extracting it with CH_2CH_2 (15 mL × 5). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo giving **15** as a dark yellow oil, 84 mg (0.25 mmol) of which was dissolved in CH_2CH_2 (10 mL). HCl in Et_2O 5 M (0.1 mL) was added and the reaction was stirred for 4 h. The clear yellow precipitate was filtered off and washed with Et_2O (38 mg, 0.14 mmol) affording **15** in an overall yield (over the 2 steps) of 56 %. (Rf = 0.025, EtOAc/CH_3OH 90:10). Mp: 156–158 °C. δH (500 MHz; CD_3OD) 3.17 (2H, d, J = 6.5 Hz, C = ONHCH_2), 3.06–2.94 (4H, m, C = OCH, NC = OCH, CH_2N), 2.60–2.50 (1H, m, C = ONCH_2C*H*), 2.48–2.39 (1H, m, NCH_2C*H*), 2.37–2.32 (2H, m, OC = OCH(C*H*H)$_2$), 2.29–2.23 (2H, m, NC = OCH(C*H*H)$_2$), 2.03–1.92 (4H, m, OC = OCH(CH*H*)$_2$ and NC = OCH(CH*H*)$_2$). δC (101 MHz; CD_3OD) 177.2, 176.9, 45.3, 45.0, 36.3, 34.9, 32.2, 29.9, 29.7, 29.6; m/z (ES+) 241.1564 (M + H^+ $C_{12}H_{21}N_2O_3$ requires 241.1552).

Conclusions

We have developed an efficient route for the preparation of an achiral δ-amino acid bearing a cyclobutane core. The synthetic strategy employed means that only the *cis* isomer is formed upon hydrolysis, providing *cis*-3(aminomethyl)cyclobutane carboxylic acid in an overall yield of 34 %. NMR and X-ray crystallography data confirm that no epimerization of the amino acid occurs under the reaction conditions employed. This conformationally constrained δ-amino acid has applications in the field of peptidomimetics as well as being a useful synthetic building block.

We have prepared a small library of dipeptides by coupling amino acid **1** to a small panel of proteinogenic amino acids. The dipeptide of amino acid **1** was also synthesised.

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