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Stereoisomers of 4-amino-3-hydroxy-1-cyclohexanecarboxylic acid and 4-amino-3-oxo-1-cyclohexanecarboxylic acid as mimetics of a twisted *cis*-amide bond

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Abstract—Stereoselective synthesis of the title compounds was performed. The relative configuration of methyl t-4-(*tert*-butoxycarbonylamino)-c-3-hydroxy-r-1-cyclohexanecarboxylate and methyl *trans*-4-(*tert*-butoxycarbonylamino)-3-oxo-r-1-cyclohexanecarboxylate was confirmed by X-ray diffraction methods. Analogues of cyclolinopeptide A (CLA) containing these twisted *cis*-amide bond mimetics were then synthesised. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Recently we have presented the synthesis of new mimetics of a twisted *cis*-peptide bond moiety—*cis*-4-amino-3-oxo-1-cyclohexanecarboxylic acid (cAOC) and its precursor c-4-amino-t-3-hydroxy-r-1-cyclohexanecarboxylic acid (ctAHC).[1] We have since expanded our investigations to study *trans*-4-amino-3-oxo-1-cyclohexanecarboxylic (tAOC) and t-4-amino-c-3-hydroxy-r-1-cyclohexanecarboxylic (tcAHC) acids—the diastereoisomers with *trans*-arrangement of the amino and carboxyl groups. Both cAOC and tAOC are mimetics of the *cis*/*trans* isomerisation transition state structures (torsion angles Ω are the same—about 50°) but correspond to different peptide chain conformations about the twisted *cis*-peptide bond (Fig. 1).

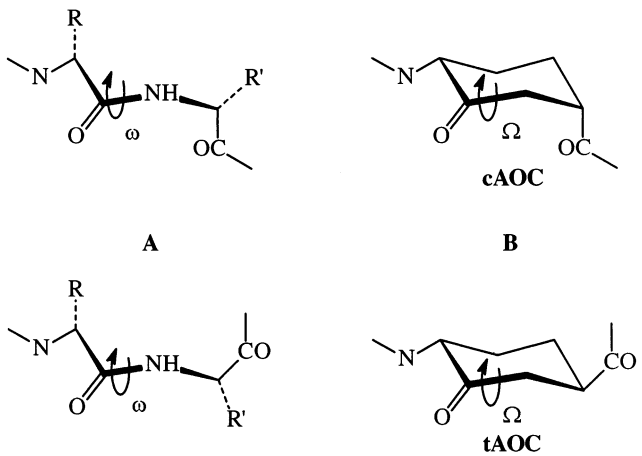

Figure 1. A schematic representation of the twisted *cis*-peptide bond (**A**) and its mimetic (**B**).

Moreover, the (1*S*,4*S*)-enantiomer of the tAOC system [t(*S*)AOC] mimics the conformation of the cyclosporin A (CsA) backbone fragment (residues 11 and 1) in the complex with cyclophilin A (CyPA, PPI-ase, Fig. 2).[2] The position of this fragment in relation to the CyPA catalytic domain corresponds to the position of Ala-Pro residues of the peptide substrates in their complexes with PPI-ases, but the directions of the CsA backbone and the substrate backbone are opposite.[3,4]

cAOC (in particular the (1*S*,4*R*)-enantiomer) mimics the Pro-Pro fragment of cyclolinopeptide A [CLA, c-(Leu-Ile-Ile-Leu-Val-**Pro-Pro**-Phe-Phe)], a nonapeptide isolated from linseed,[5] which exhibits a distinct immunosuppressive activity,[6] and has a *cis*-amide bond between proline residues. The tAOC residue mimics this fragment less well if a conformation of CLA, as indicated by the X-ray structure, is considered.[7] Both CsA and CLA affect the same cyclophilin binding site.

Analysis of the dependence of immunosuppressive activity of CLA analogues on conformational restrictions introduced by cAOC and tAOC may well prove useful in predicting the biologically active conformation of CLA.

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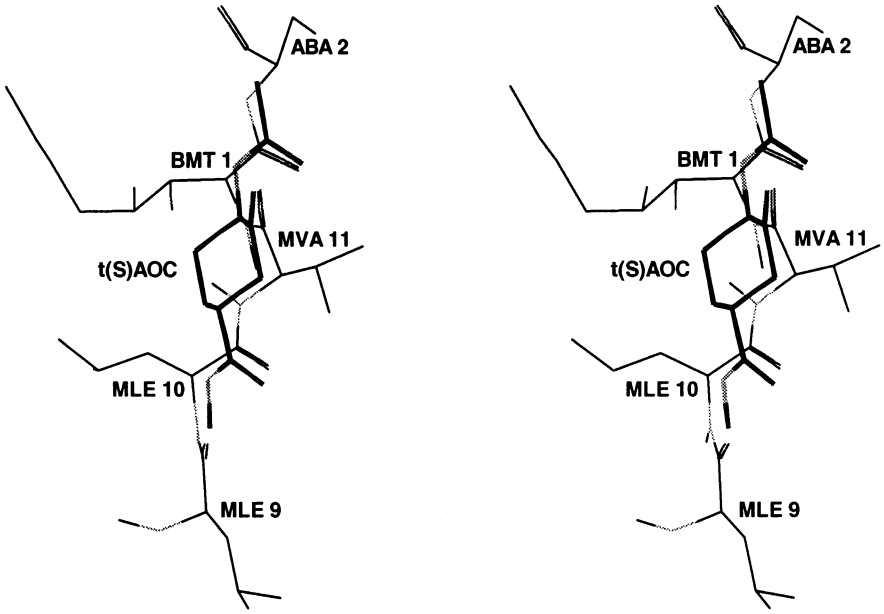
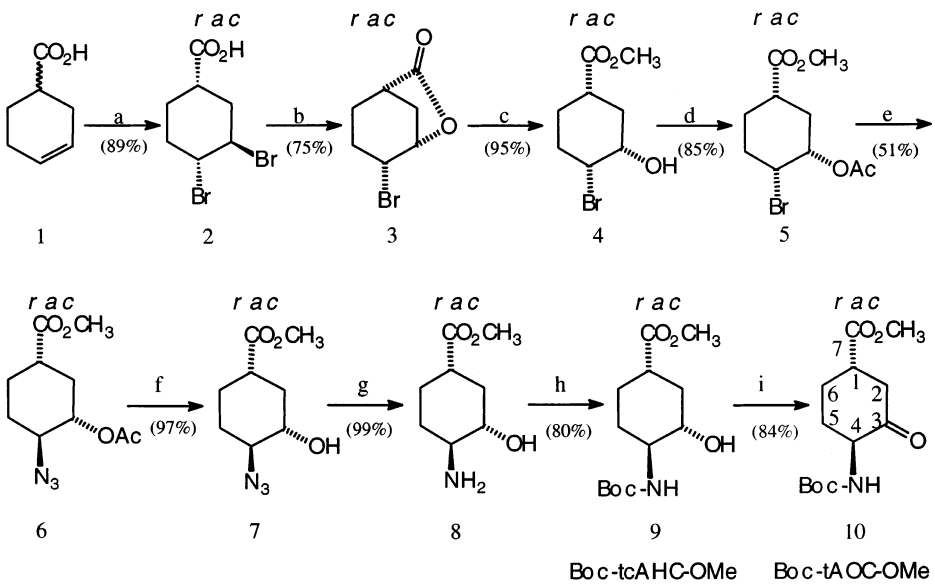


Figure 2. The stereoview of superimposition of tc(*S*)AHC moiety (**thick line**) and the 9-2 fragment of CsA (bonded to CyPA).

2. Results and discussion

The synthetic methods used for synthesis of tcAHC and ctAHC are different, but the method of oxidation is the same for both diastereoisomers. We obtained methyl t-4-amino-c-3-hydroxy-r-1-cyclohexanecarboxylate **8** (tcAHC-OMe) as a racemic mixture and also as the single enantiomer **8i** ((1*S*,3*S*,4*S*)-, tc(*S*)AHC-OMe). The Boc-derivatives **9** and **9i** were prepared from tcAHC-OMe and tc(*S*)AHC-OMe, and then oxidised to give the racemic methyl *trans*-4-(*tert*-butoxycarbonylamino)-3-oxo-1-cyclohexanecarboxylate **10** (Boc-tAOC-OMe), and the (1*S*,4*S*)-enantiomer **10i** (Boc-t-(*S*)AOC-OMe), respectively. X-Ray structures were obtained for both model compounds **9** and **10**.

As shown in Scheme 1, compound **8** was prepared from 3-cyclohexene-1-carboxylic acid **1**. Bromination of the double bond of **1** with Br_2 in $CHCl_3$ afforded the dibromide **2** which was subjected to base induced lactonisation to give the bromolactone **3**. The bromination gave a diastereoisomeric mixture of 90% of **2** and 10% of c-3,t-4-dibromo-r-1-cyclohexanecarboxylic acid, but only **2** underwent lactonisation (step **b**) under the conditions employed. The *cis*-bromolactone **3** ring was then opened to afford **4** in 95% yield by treatment with $MeOH/NaHCO_3$. Protection of the hydroxyl group of **4**



Scheme 1. (a) Br_2, $CHCl_3$; (b) NaOH, H_2O, Δ; (c) $NaHCO_3$, MeOH; (d) Ac_2O, Pyr, DCM; (e) NaN_3, DMF, Δ; (f) MeONa, MeOH then amberlite; (g) H_2, 10% Pd–C, MeOH; (h) Boc_2O, DMAP, acetone; (i) PDC, DMF.

was necessary to avoid any formation of methyl 3-azido-4-hydroxy-1-cyclohexanecarboxylate,[8] and acetate protection of the hydroxyl group followed by reaction of **5** with NaN_3 in DMF cleanly afforded the azide **6**. The acetate protecting group was then removed from **6** to give methyl t-4-azido-c-3-hydroxy-r-1-cyclohexanecarboxylate **7**, which was hydrogenated in the presence of catalytic palladium to give the amino alcohol **8**.

Methyl t-4-(*tert*-butoxycarbonylamino)-c-3-hydroxy-r-1-cyclohexanecarboxylate **9** was prepared from **8** by reaction with Boc_2O in the presence of *N*,*N*-dimethylaminopyridine; the X-ray structure of **9** confirmed its relative configuration (Fig. 6). Oxidation of **9** with pyridinium dichromate (PDC)[9] afforded Boc-tAOC-OMe **10**. Again, the relative configuration and conformation of **10** were confirmed on the basis of its X-ray structure (Fig. 7).

The synthesis of homochiral compounds **2i**–**10i** was performed analogously, starting from (*S*)-3-cyclohexene-1-carboxylic acid **1i**, with an e.e. of 93% (prepared via diastereoselective Diels–Alder reaction and D-pantolactone ester hydrolysis).[10] The 96% e.e. of **8i** was determined by analysis of its dansyl derivative by chiral HPLC (Fig. 3). Enantiomeric enrichment (from 93 to 96%) was seen only when *cis*-bromolactone was formed and then recrystallised from hot water (step **b**).

Racemic methyl t-4-amino-c-3-hydroxy-r-1-cyclohexanecarboxylate was used for the synthesis of diastereoisomeric dipeptides (Val-tcAHC) by acylation with Boc-Val and deprotection. These dipeptide diastereoisomers were then separated (as previously for Val-ctAHC diastereoisomers)[1] by preparative HPLC. To determine the absolute configuration of the tcAHC residue in both of the HPLC fractions of Val-tcAHC, we performed a synthesis of Val-tc(*S*)AHC from **8i**. After comparison of retention times (Fig. 4) we found that the diastereoisomer with the shorter retention time contained the (1*S*,3*S*,4*S*)-4-amino-3-hydroxy-1-cyclohexanecarboxylic acid residue (tc(*S*)AHC) and the diastereoisomer with the longer retention time contained (tc(*R*)AHC), the (1*R*,3*R*,4*R*)-4-amino-3-hydroxy-1-cyclohexanecarboxylic acid residue.

The homochiral dipeptides Val-tc(*R*)AHC-OH and Val-tc(*S*)AHC-OH, as well as the Val-ct(*R*)AHC-OH and Val-ct(*S*)AHC-OH synthesised previously,[1] were used in solid-phase syntheses of CLA analogues.

The linear peptides (Val-**AHC**-Phe-Phe-Leu-Ile-Ile-Leu-OH) were formed by coupling Boc-Val-**AHC**-OH with the Merrifield resin bonded hexapeptide Phe-Phe-Leu-Ile-Ile-Leu-. The resulting octapeptides were cleaved from the resin using the 'low TFMSA' method[11] and linear CLA analogues were cyclised after purification by preparative HPLC. The cyclisations were successful in the case of peptides containing ct(*R*)AHC and ct(*S*)AHC residues, but failed in the case of peptides containing tc(*R*)AHC and tc(*S*)AHC residues. These results are consistent with the structural properties of both types of residue: in tcAHC residues the substituents at the C-(1) and C-(4) atoms are equatorial and their relative configuration is *trans*-, so tcAHC residues induce extended conformations of peptides containing them, which disfavours the peptide cyclisation process. In contrast, the ctAHC residues, with *cis*-relative configuration of the substituents at C-(1) and C-(4) are backbone-turn inducers and facilitate cyclisation.

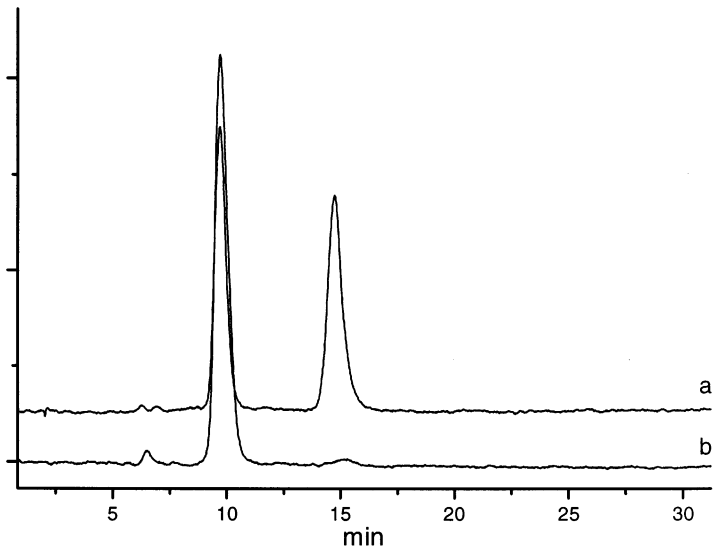


Figure 3. The HPLC chromatograms of (a) DNS-tcAHC-OMe and (b) DNS-tc(*S*)AHC-OMe.

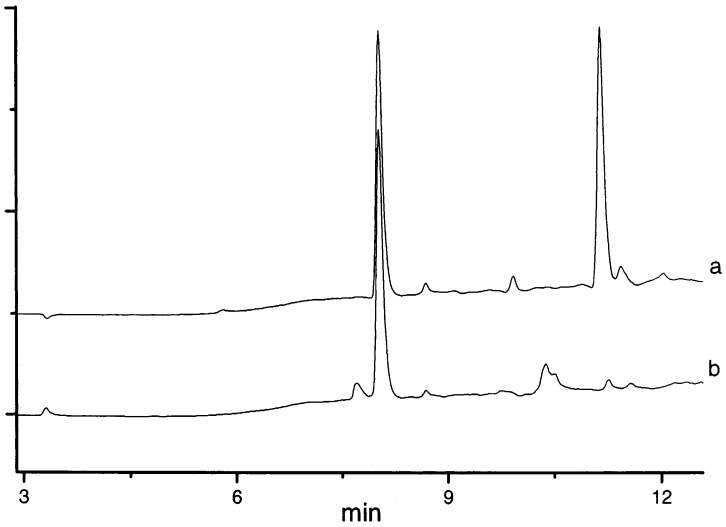


Figure 4. The HPLC chromatograms of (a) Val-tcAHC-OH and (b) Val-tc(*S*)AHC-OH.

The ct(*R*)AHC and ct(*S*)AHC residues in the cyclic peptides were oxidised with PDC in DMF to c(*R*)AOC and c(*S*)AOC residues. All obtained peptides were characterised by HPLC analysis (purity $\geq 93\%$) and ESIMS spectra.

Comparison of the CD spectra of the cyclic analogues and CLA (the amide bond region, Fig. 5) showed that the analogue with the c(*S*)AOC residue is the most conformationally similar to CLA.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer in the indicated solvents; chemical shifts are given in ppm. The NMR assignment was based on COSY and HETCOR experiments. CD spectra were measured on a Jasco-600 spectropolarimeter in methanol. ESIMS spectra were recorded on a Finnigan MAT TSQ700 instrument. HPLC analyses were performed on a reverse phase ODS column (Vydac 218TP) eluted with increasing linear gradient of CH_3CN (1.6%/min) in the presence of 0.1% TFA, or on a chiral β-cyclodextrin column (Merck ChiraDex) eluted with a MeOH:water (40:60) mixture. Melting and boiling points are uncorrected. The ^{1}H, ^{13}C NMR and ESIMS data for the racemic **1**–**10** and the enantiomeric **1i**–**10i** were identical.

3.2. Preparation of t-3,c-4-dibromo-r-1-cyclohexanecarboxylic acid 2

To a solution of 3-cyclohexene-1-carboxylic acid **1** (7.56 g, 60 mmol) in $CHCl_3$ (200 mL) was added a 2 M solution of Br_2 in $CHCl_3$ in small portions until the

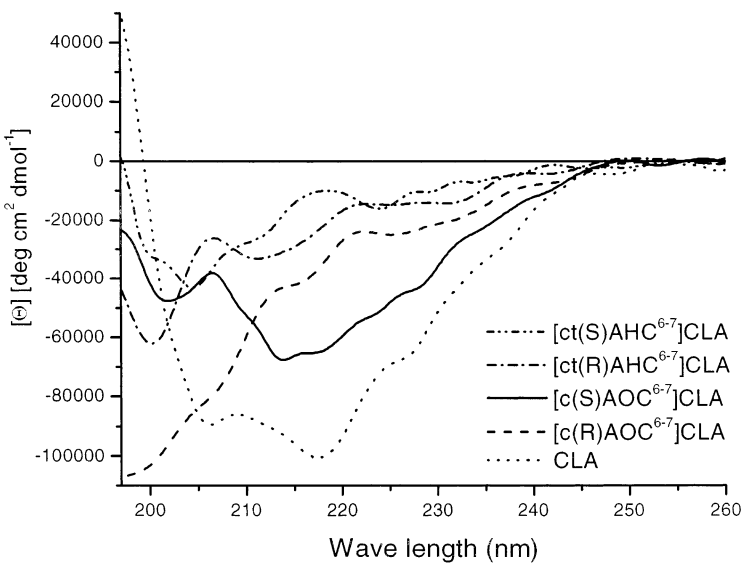


Figure 5. Comparison of the CD spectra (an amide region) of the cyclic CLA analogues containing ct(*R*)AHC, ct(*S*)AHC, c(*R*)AOC, c(*S*)AOC residues and CLA.

yellow colour persisted (65 mmol Br_2). The solution was stirred at room temperature for 15 min and extracted with satd $NaHCO_3$ aq. (2×100 mL). To the aqueous layers was added H_2SO_4 aq. (0.5 M) to give pH 1 (oil precipitates). The mixture was extracted with Et_2O (3×100 mL). The organic layers were separated, dried ($MgSO_4$) and concentrated under reduced pressure. The residual colourless oil crystallised slowly. This product contained 10% (GC–MS) t-3,c-4-dibromo-r-1-cyclohexanecarboxylic acid. Yield: 15.25 g (89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.96 (bs, 1-COO**H**), 4.70 (dd, J=6.3, 3.2, 1-H3), 4.60 (dd, J=5.6, 3.2, 1-H4), 2.96 (m, 1-H1), 2.62 (m, J=14.9, 11.7, 3.3, 1-H2′), 2.53 (m, 1-H5′), 2.23 (dddd, J=14.9, 5.0, 3.5, 1.5, 1-H2), 2.08–1.95 (m, 3-H5, H6′, H6). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.97 (**C**O), 51.83 (C4), 51.79 (C3), 37.36 (C1), 30.55 (C2), 28.09 (C5), 22.93 (C6).

3.3. Preparation of (1*S*,3*R*,4*R*)-3,4-dibromo-1-cyclohexanecarboxylic acid 2i

Conditions were the same as for synthesis of **2** (except the reaction scale). Yield: 1.256 g (85%).

3.4. Preparation of *endo*-4-bromo-6-oxabicyclo[3.2.1]octan-7-one 3

A mixture of **2** (14.30 g, 50.0 mmol) and water (80 mL) was neutralised (phenolphthalein) with 1 M NaOH aq. (50.8 mL). The solution was then heated for 30 min at 60°C, wherein white crystals of **3** formed. The mixture was cooled to room temperature and filtered. The crystals were washed with water. The filtrate was again heated for 30 min at the same temperature, and a second portion of crystals was collected to give a total of 7.68 g (75%). Mp: 100–101°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.91 (d, J=6.5, 1-H3), 4.17 (ddd, J=11.0, 6.1, 0.8, 1-H4), 2.72 (m, 1-H1), 2.55 (ddddd, J=12.2, 6.5, 5.4, 2.8, 0.4, 1-H2′), 2.42 (ddd, J=13.4, 6.9, 6.1, 1-H5′), 2.17 (dddd, J=14.2, 13.1, 11.2, 6.5, 1-H5), 1.99 (ddddd, J=11.0, 9.3, 4.1, 2.7, 1.4, 1-H6′), 1.87 (d, J=12.2, 1-H2), 1.68 (dddd, J=13.3, 13.3, 5.7, 2.2, 1-H6). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.31 (**C**O), 81.69 (C3), 47.51 (C4), 37.60 (C2), 37.20 (C1), 30.62 (C5), 27.62 (C6). Anal. calcd for $C_7H_9O_2Br$: C, 41.00; H 4.42. Found: C, 40.98; H, 4.56%.

3.5. Preparation of (1*S*,4*R*,5*S*)-4-bromo-6-oxabicyclo[3.2.1]octan-7-one 3i

Conditions were the same as for synthesis of **3** (except the reaction scale). Yield: 529 mg (59%). Mp: 79–80°C, $[\alpha]_D^{20}$ +68.3 (c 1.2, $CHCl_3$).

3.6. Preparation of methyl c-4-bromo-c-3-hydroxy-r-1-cyclohexanecarboxylate 4

To a solution of **3** (6.15 g, 30 mmol) in MeOH (150 mL) was added $NaHCO_3$ (2.64 g, 31.5 mmol) and the mixture was stirred for 1 h. The solvent was evaporated under reduced pressure and $CHCl_3$ (75 mL) was added. After filtration and evaporation of $CHCl_3$, **4** was obtained as white crystals. Yield: 6.75 g (95%). Mp: 63–64°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (m, 1-H4), 3.69 (s, 3-C**H$_3$**), 3.50 (ddd, J=10.4, 4.2, 3.2, 1-H3), 2.41 (dddd, J=11.0, 11.0, 4.1, 4.1, 1-H1), 2.33–2.23 (m, 1-H5′), 2.20 (bs, 1-O**H**), 2.05–1.72 (m, 5-H2′, H2, H5, H6′, H6). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.41 (**C**O), 70.03 (C3), 61.12 (C4), 51.84 (**C**H$_3$), 40.59 (C1), 32.42 (C2), 31.49 (C5), 22.86 (C6). Anal. calcd for $C_8H_{13}O_3Br$: C, 40.53; H, 5.53. Found: C, 40.20; H, 5.51%.

3.7. Preparation of methyl (1*S*,3*S*,4*R*)-4-bromo-3-hydroxy-1-cyclohexanecarboxylate 4i

Conditions were the same as for synthesis of **4** (except the reaction scale). Yield: 448 mg (94%). Mp: 67–68°C, $[\alpha]_D^{20}$ −33.1 (c 1.0, $CHCl_3$).

3.8. Preparation of methyl c-3-acetoxy-c-4-bromo-r-1-cyclohexanecarboxylate 5

To a solution of **4** (5.93 g, 25 mmol) in DCM (70 mL) under nitrogen were added sequentially pyridine (3.0 g, 38 mmol), DMAP (75 mg, 0.61 mmol) and Ac_2O (3.8 g, 38 mmol). The solution was stirred for 4 h. After DCM evaporation, the residue was shaken with water (100 mL) and hexane (100 mL), and the aqueous layer was extracted with hexane (100 mL). The combined organic layers were washed with aq. 5% $CuSO_4$ and dried ($MgSO_4$). Hexane was removed under reduced pressure affording yellowish oil **6**. Yield: 5.95 g (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (ddd, J=10.8, 4.3, 3.2, 1-H3), 4.51 (m, 1-H4), 3.60 (s, 3-C**H$_3$**), 2.45–2.34 (m, 1-H1), 1.99 (s, 3-C**H$_3$**CO), 2.20–1.70 (m, 6-H5′, H2′, H2, H5, H6′, H6). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.60 (**C**O), 169.76 (CH$_3$**C**O), 71.43 (C3), 53.16 (C4), 51.59 (**C**H$_3$), 40.23 (C1), 31.44 (C2), 28.52 (C5), 22.52 (C6), 20.75 (**C**H$_3$CO). Anal. calcd for $C_{10}H_{15}O_4Br$: C, 43.03; H, 5.42. Found: C, 43.08; H, 5.53%.

3.9. Preparation of methyl (1*S*,3*S*,4*R*)-3-acetoxy-4-bromo-1-cyclohexanecarboxylate 5i

Conditions were the same as for synthesis of **5** (except the reaction scale). Yield: 430 mg (91%). $[\alpha]_D^{20}$ −39.4 (c 1.2, $CHCl_3$).

3.10. Preparation of methyl c-3-acetoxy-t-4-azido-r-1-cyclohexanecarboxylate 6

To a stirred solution of **5** (5.58 g, 20 mmol) in DMF (40 mL) was added NaN_3 (5.84 g, 90 mmol) and 15-crown-5 (five drops) under nitrogen. The mixture was stirred under nitrogen at 75°C for 120 h. Half of the solvent was evaporated under reduced pressure. The residue was diluted with water (120 mL) and extracted with Et_2O (3×40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated to give a yellow liquid which was purified by flash chromatography (silica gel, EtOAc:petroleum 1:6), after which pure **6** was obtained as a colourless liquid (2.52 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.68 (ddd, J=11.3, 9.8, 4.6, 1-H3), 3.63 (s, 3-C**H$_3$**), 3.38 (ddd, J=11.3, 9.8, 4.5, 1-H4), 2.42

(dddd, J=12.0, 12.3, 3.7, 3.7, 1-H1), 2.34–2.24 (m, 1-H2′), 2.11–1.98 (m, 2-H5′, H6′), 2.06 (s, 3-C**H**$_3$CO), 1.56–1.28 (m, 3-H2, H5, H6). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.84 (**C**O), 169.97 (CH$_3$**C**O), 74.33 (C3), 62.34 (C4), 51.78 (**C**H$_3$), 40.42 (C1), 32.61 (C2), 29.12 (C5), 26.54 (C6), 20.90 (**C**H$_3$CO).

3.11. Preparation of methyl (1*S*,3*S*,4*S*)-3-acetoxy-4-azido-1-cyclohexanecarboxylate 6i

Conditions were the same as for the synthesis of **6** (except the reaction scale). Yield: 177 mg (49%). $[\alpha]_D^{20}$ +25.0 (c 1.2, $CHCl_3$).

3.12. Preparation of methyl t-4-amino-c-3-hydroxy-r-1-cyclohexanecarboxylate 8

To a solution of MeONa (prepared by addition of Na metal (0.12 g, 5.2 mmol) to MeOH (10 mL)) was added a solution of **6** (2.41 g, 10 mmol) in MeOH (30 mL). The solution was stirred for 4 h under nitrogen and then neutralised by the addition of Amberlite IRC-50 (3.6 g, pH 6). After filtration, 10% palladium on carbon (100 mg) was added and the mixture was stirred under hydrogen for 1 h. After filtration and evaporation, **8** was obtained as a colourless oil that crystallised very slowly (1.66 g, 9.6 mmol, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.66 (s, 3-C**H**$_3$), 3.28 (ddd, J=11.1, 9.4, 4.4, 1-H3), 2.57 (ddd, J=11.4, 9.4, 3.8, 1-H4), 2.40 (dddd, J=12.4, 12.4, 3.4, 3.4, 1-H1), 2.26–2.12 (m, 1-H2′), 2.00–1.90 (m, 2-H5′, H6′), 1.46 (ddd, J=11.1, 13.0, 12.4, 1-H2), 1.52–1.37 (m, 1-H6), 1.25 (dddd, J=11.4, 13.6, 11.8, 3.3, 1-H5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.94 (**C**O), 73.65 (C3), 56.07 (C4), 51.73 (**C**H$_3$), 41.56 (C1), 35.81 (C2), 32.20 (C5), 27.40 (C6).

3.13. Preparation of methyl (1*S*,3*S*,4*S*)-4-amino-3-hydroxy-1-cyclohexanecarboxylate 8i

Conditions were the same as for synthesis of **8** (except the reaction scale). Yield: 112 mg (88%). $[\alpha]_D^{20}$ +29.2 (c 0.5, MeOH), 96% e.e. Samples (1.5 mg) of **8** and **8i** were converted to their dansyl (DNS-) derivatives by reaction (4 h at 40°C) with dansyl chloride (5 mg) in an acetone/saturated $NHCO_3$ aq. (1:1, 2 mL) solution and extraction with Et_2O. After solvent evaporation the derivatives were dissolved in MeOH and analysed by chiral HPLC (β-cyclodextrin stationary phase), eluting with a MeOH:water (40:60) mixture (Fig. 3, **8i**—96% e.e.).

3.14. Preparation of methyl t-4-(*tert*-butoxycarbonylamino)-c-3-hydroxy-r-1-cyclohexanecarboxylate 9

To a solution of **8** (52 mg, 0.30 mmol) in acetone was added (10 mL) Boc_2O (100 mg, 0.46 mmol) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.29 mmol). The solution was stirred at 26°C for 20 h. The solvent was removed under reduced pressure affording a yellowish oil which was purified by flash chromatography (silica gel, CH_3OH:$CHCl_3$, 5:95) and crystallisation from Et_2O/hexane, after which pure **6** was obtained as white crystals. Yield: 66 mg (80%). Mp: 162–163°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (s, 3-C**H**$_3$), 3.33 (ddd, J=10.5, 9.7, 4.0, 1-H3), 3.27 (ddd, J=9.7, 9.5, 3.7, 1-H4), 2.32 (dddd, J=12.5, 12.5, 3.5, 3.5, 1-H1), 2.26 (dddd, J=13.1, 4.0, 3.5, 2.0, 1-H2′), 2.03 (dddd, J=13.0, 3.5, 3.7, 3.3, 1-H5′), 1.93 (dddd, J=13.3, 3.5, 3.7, 3.3, 1-H6′), 1.66–1.54 (m, 2-H6, H2), 1.44 (s, 9-3C**H**$_3$, Boc; m, 1-H5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.71 (**C**O), 157.03 (**C**=O, Boc), 79.98 (C, Boc), 73.71 (C3), 55.91 (C4), 51.69 (**C**H$_3$), 41.12 (C1), 36.19 (C2), 30.27 (C5), 28.22 (3**C**H$_3$, Boc), 27.20 (C6). ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5} M NaCl) 296.4 (100%, M+Na$^+$), 569.8 (10%, 2M+Na$^+$). Anal. calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.31; H, 8.40; N, 4.89%.

3.15. Preparation of methyl (1*S*,3*S*,4*S*)-4-(*tert*-butoxycarbonylamino)-3-hydroxy-1-cyclohexanecarboxylate 9i

Conditions were the same as for the synthesis of **9** (except the reaction scale). Yield: 21 mg (46%). Mp: 100–105°C, $[\alpha]_D^{20}$ +11.4 (c 1.9, MeOH).

3.16. Preparation of methyl *trans*-4-(*tert*-butoxycarbonylamino)-3-oxo-1-cyclohexanecarboxylate 10

To a solution of **9** (41 mg, 0.15 mmol) in DMF (2 mL) was added PDC[9] (339 mg, 0.9 mmol). The solution was stirred at 23°C for 24 h, diluted with water (30 mL) and extracted with Et_2O (3×10 mL). The combined organic layers were washed with 5% $CuSO_4$ aq. (10 mL) and dried ($MgSO_4$). After Et_2O evaporation, **10** was obtained as a white solid. Yield: 30 mg (84%). Mp: 128–130°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (ddd, J=11.8, 5.6, 5.6, 1-H4), 3.69 (s, 3-C**H**$_3$), 2.76–2.59 (m, 4-H1, H2′, H2, H5′), 2.17 (dddd, J=14.0, 6.9, 3.5, 2.3, 1-H6′), 1.90 (dddd, J=14.1, 13.6, 11.1, 3.4, 1-H6), 1.44 (s, 9-3C**H**$_3$, Boc; m 1-H5). ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.19 (C3), 173.19 (**C**O), 155.12 (**C**=O, Boc), 79.71 (C, Boc), 58.43 (C4), 52.05 (**C**H$_3$), 44.13 (C1), 42.50 (C2), 33.64 (C5), 28.22 (3**C**H$_3$, Boc), 27.12 (C6). ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5} M NaCl) 294.2 (M+Na$^+$). Anal. calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.29; H, 7.58; N, 5.06%.

3.17. Preparation of methyl (1*S*,4*S*)-4-(*tert*-butoxycarbonylamino)-3-oxo-1-cyclohexanecarboxylate 10i

Conditions were the same as for synthesis of **10** (except the reaction scale). Yield: 17 mg (85%). Mp: 109–113°C, $[\alpha]_D^{20}$ +1.8 (c 1.5, MeOH). CD (MeOH): 300 nm ($[\Theta]$=−581, $n\rightarrow\pi^*$).

3.18. General procedure of preparation of dipeptides Boc-Val-AHC-OMe

To a stirred solution of AHC-OMe (0.951 g, 5.50 mmol) and Boc-Val-OH (1.196 g, 5.50 mmol) in DCM (10 mL) cooled to −3°C was added a solution of DCC (1.134 g, 5.50 mmol) in DCM (15 mL). The solution was stirred at 0°C for 30 min and then for a further 2.5 h at room temperature. The mixture was then cooled to 0°C and filtered. The filtrate was diluted with EtOAc (60 mL) and washed with solutions of $NaHCO_3$, $KHSO_4$, $NaHCO_3$ and water, dried ($MgSO_4$) and the solvent was then evaporated to give a white foam. Yields: Boc-Val-ctAHC-OMe (1.972 g, 97%) and Boc-Val-tcAHC-OMe (1.911 g, 94%). ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5}

M NaCl) 395.2 ($M+Na^+$), 767.5 ($2M+Na^+$) (identical for both dipeptides).

3.19. General procedure for the preparation of dipeptides Boc-Val-AHC-OH

To a stirred solution of Boc-Val-AHC-OMe (1.860 g, 5.00 mmol) in THF (10 mL) was added LiOH (0.420 g, 10.00 mmol) in water (12 mL). The solution was stirred at 23°C for 2 h. The reaction mixture was acidified with $KHSO_4$ aq. to pH 2 and extracted with Et_2O (2×50 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated to give a white foam. Yields: 1.701 g (95%, Boc-Val-ctAHC-OH) and 1.736 g (97%, Boc-Val-tcAHC-OH). ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5} M NaCl), 381.3 ($M+Na^+$), 739.6 ($2M+Na^+$) (identical for both dipeptides).

3.20. General procedure for the preparation of homochiral dipeptides Val-AHC-OH

To a stirred solution of Boc-Val-AHC-OH (1.074 g, 3.00 mmol) in DCM (10 mL) was added TFA (10 mL). The solution was stirred for 45 min and the solvent removed under reduced pressure affording a yellowish oil. The product was purified by gel chromatography (Sephadex G-10, eluent: 0.1% AcOH aq.). Yields: 0.763 g (80%, Val-ctAHC-OH AcOH) and 0.887 g (93%, Val-tcAHC-OH AcOH).

The mixtures of diastereoisomeric dipeptides (Val-ct(*R*)AHC-OH, Val-ct(*S*)AHC-OH and Val-tc(*R*)AHC-OH, Val-tc(*S*)AHC-OH) were separated via HPLC on a preparative reverse phase column (C-18, gradient from water to 20% acetonitrile in water, with constant concentration of TFA—0.1%).

Yields: 307 mg (55%, Val-ct(*R*)AHC-OH·TFA), 245 mg (44%, Val-ct(*S*)AHC-OH·TFA), 296 mg (53%, Val-tc(*R*)AHC-OH·TFA) and 295 mg (53%, Val-tc(*S*)-AHC-OH·TFA). ESIMS (m/z, MeOH:water:AcOH, 1:1:0.05) 259.3 ($M+H^+$) (identical for all dipeptides).

3.21. Synthesis of linear CLA analogues containing ct(*R*)AHC, ct(*S*)AHC, tc(*R*)AHC and tc(*S*)AHC residues

The hexapeptide Phe-Phe-Leu-Ile-Ile-Leu- was synthesised on Merrifield resin using standard Boc strategy with DIC as a coupling reagent. Its purity was checked by HPLC and ESIMS. The homochiral dipeptides were coupled with the hexapeptide after Boc protection of the amine group. The coupling was performed in DMF over 3 h using Boc-Val-AHC-OH (1.5 equiv.), PyBOP (1.5 equiv.), HOBt (1.5 equiv.) and DIEA (3.0 equiv.).

Cleavage of the peptide from Merrifield resin was effected by use of a mixture of TFA (5 mL): TFMSA (1 mL): DMS (3 mL): *m*-cresol (1 mL) per 1 g of resin at 0°C over 4 h. The resulting mixture was filtered into Et_2O (100 mL). Hexane was added to the solution until the peptide precipitation, the mixture was kept at −24°C for 1 h and filtered. The crude peptide was purified by gel chromatography (Sephadex LH-20, eluent: MeOH) and preparative HPLC. ESIMS (m/z, CH_3Cl:MeOH , 1:1, 1×10^{-5} M NaCl) Val-**ct(*R*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-OH 1027.8 ($M+Na^+$), Val-**ct(*S*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-OH 1027.7 ($M+Na^+$), Val-**tc(*R*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-OH 1027.9 ($M+Na^+$), Val-**tc(*S*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-OH 1027.8 ($M+Na^+$).

3.22. General procedure of cyclisation of CLA analogues

To the solution of the linear octapeptide (100 mg, 0.1 mmol) in a DCM:DMF mixture (4:1, 2.0 L) BOP (0.4 mmol), HOBt (0.4 mmol) and DMAP (0.5 mmol) were added sequentially and the solution was stirred for 7 days at room temperature. The solvent was evaporated under reduced pressure and the residue was washed with water (2×10 mL) and purified by preparative HPLC.

ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5} M NaCl) c-(-Val-**ct(*R*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-) 1009.6 ($M+Na^+$), c-(-Val-**ct(*S*)AHC**-Phe-Phe-Leu-Ile-Ile-Leu-) 1009.8 ($M+Na^+$).

The linear peptides containing tc(*R*)AHC and tc(*S*)AHC did not give the cyclic peptides under the above indicated conditions.

3.23. Preparation of CLA analogues containing c(*R*)AOC and c(*S*)AOC residues

To a solution of PDC (46 mg, 0.12 mmol) in DMF (0.2 mL) the cyclic octapeptide (10 mg, 0.01 mmol) in DMF (0.2 mL) was added. The solution was shaken at room temperature for 46 h, then water (5 mL) was added and the mixture was extracted with EtOAc (2×10 mL). The combined organic layers were washed twice with water and dried ($MgSO_4$). After filtration the solvent was removed in a stream of N_2. Yields: 4.5 mg (45%, c-(-Val-c(*R*)AOC-Phe-Phe-Leu-Ile-Ile-Leu-)) and 5.0 mg (50%, c-(-Val-c(*S*)AOC-Phe-Phe-Leu-Ile-Ile-Leu-)). ESIMS (m/z, CH_3Cl:MeOH, 1:1, 1×10^{-5} M NaCl) c-(-Val-**c(*R*)-AOC**-Phe-Phe-Leu-Ile-Ile-Leu-) 1007.7 ($M+Na^+$), c-(-Val-**c(*S*)AOC**-Phe-Phe-Leu-Ile-Ile-Leu-) 1007.8 ($M+Na^+$).

3.24. X-Ray structural analysis of 9 and 10

$C_{13}H_{23}NO_5$ **9**, $M_r=273.32$ g mol^{-1}, white, crystal size 0.10×0.10×0.20 mm, $a=5.9890(5)$, $b=26.377(2)$, $c=9.3591(7)$ Å, $\beta=100.33(3)°$, $V=1454.5(2)$ Å^3, $T=110$ K, monoclinic, $P2_1/c$, $Z=4$, $D_{calcd}=1.248$ g cm^{-3}, $\mu=0.095$ mm^{-1}, extinction coefficient 0.008(2) final $R_1=0.0447$, $wR_2(F^2)=0.1257$, $S=1.072$ for 265 parameters and 3520 unique observed reflections ($3.5<\theta<28.8°$) with $I\geq2\sigma(I)$. $C_{13}H_{21}NO_5$ (**10**), $M_r=271.31$ g mol^{-1}, white, crystal size 0.10×0.15×0.20 mm, $a=14.655(1)$, $b=5.7924(4)$, $c=17.398(7)$ Å, $\beta=109.60(1)°$, $V=1391.3(2)$ Å^3, $T=110$ K, monoclinic, $P2_1/c$, $Z=4$, $D_{calcd}=1.295$ g cm^{-3}, $\mu=0.099$ mm^{-1}, extinction coefficient 0.004(1) final $R_1=0.0393$, $wR_2(F^2)=0.0969$, $S=1.114$ for 257 parameters and 3343 unique observed reflections ($3.7<\theta<28.8°$) with $I\geq2\sigma(I)$.

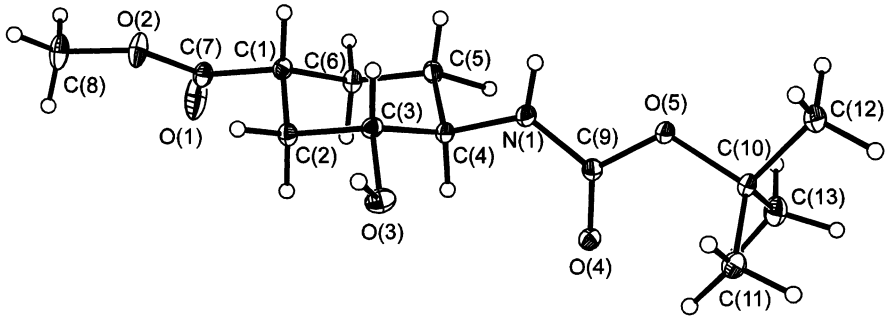


Figure 6. ORTEP[14] drawing of the molecular structure of **9** (one enantiomer).

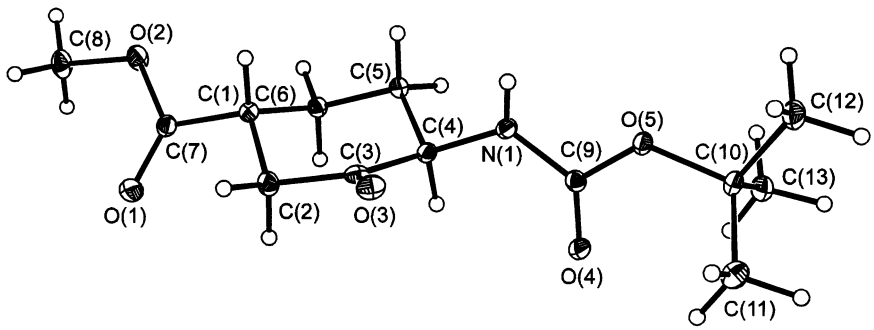


Figure 7. ORTEP[14] drawing of the molecular structure of **10** (one enantiomer).

The crystals of both compounds **9** and **10** were grown from EtOAc/hexane solutions. All measurements of crystals were performed at temperature 110 K on a Kuma KM4CCD κ-axis diffractometer with graphite-monochromated Mo Kα radiation and an Oxford cryosystem device. Each crystal was positioned 65 mm from the KM4CCD camera and 612 frames were measured at 0.75° intervals with a counting time of 30 s. The data were corrected for Lorentz and polarisation effects. No absorption correction was applied. Data reduction and analysis were carried out with the Kuma Diffraction (Wrocław) programs. Both structures were solved by direct methods (program SHELXS-97[12]) and refined by the full-matrix least-squares method on all F^2 data using the SHELXL-97[13] program. Non-hydrogen atoms were refined with anisotropic thermal parameters; hydrogen atoms were included from the $\Delta\rho$ maps and refined with isotropic thermal parameters.

The structure of **9** (Fig. 6) indicates that it possesses a chair conformation with equatorial substituents. Comparison of the structures of **9** (Fig. 6) and **10** (Fig. 7) indicates only small conformational changes. The rings of **9** and **10** are different only at C-(3). The conformation of Boc-NH- is the same in both structures, but the carboxyl group has a slightly different orientation with regard to the ring. Both structures have a similar packing arrangement.

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