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Resolution and conformational analysis of diastereoisomeric esters of *cis*- and *trans*-2-(aminomethyl)-1-carboxycyclopropanes

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Abstract

(1*R*,2*S*)-, (1*S*,2*R*)-, (1*R*,2*R*)- and (1*S*,2*S*)-2-(Aminomethyl)-1-carboxycyclopropanes, conformationally restricted analogues of the neurotransmitter γ-aminobutyric acid (GABA), have been resolved by chromatographic separation of the corresponding diastereoisomeric esters which were formed between the *cis*- and *trans*-2-(acetamidomethyl)-1-carboxycyclopropanes with (*R*)-(−)-pantolactone. ^{1}H NMR, semi-empirical conformational analysis, *ab initio* (DFT) structure and NMR shielding tensor calculations of the *cis*-diastereoisomers allowed the absolute configuration assignments of the *cis*-amino acids.

1. Introduction

γ-Aminobutyric acid, GABA **1**, the major inhibitory neurotransmitter in the mammalian central nervous system, activates three major classes of GABA receptors, the $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ receptors are neurotransmitter gated chloride ion channels which are blocked by the alkaloid bicuculline, and modulated by barbiturates, steroids and benzodiazepines.[1] In contrast, $GABA_B$ receptors operate *via* second messenger systems and are coupled to calcium and potassium channels through G-proteins. $GABA_B$ receptors are insensitive to bicuculline and $GABA_A$ modulators, are activated by (*R*)-baclofen and antagonised by the phosphonic and sulfonic analogues of baclofen, phaclofen and saclofen respectively.[2] $GABA_A$ and $GABA_C$ receptors share some common features; both are ionotropic, conducting chloride ions, and show marked differences from $GABA_B$ receptors such as being insensitive to (*R*)-baclofen. However, unlike $GABA_A$ receptors, $GABA_C$ receptors are not antagonised by bicuculline, nor are they modulated by barbiturates and benzodiazepines.[3,4] *Z*-4-Aminobut-2-enoic acid (*cis*-aminocrotonic acid, CACA) **2** (Fig. 1) is a selective partial agonist,[3,4] and

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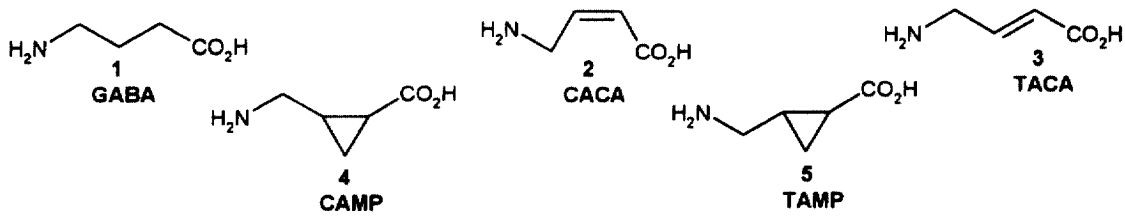


Fig. 1. $GABA_C$ receptor agonists and partial agonists

(1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) is a selective antagonist[5,6] at $GABA_C$ receptors.

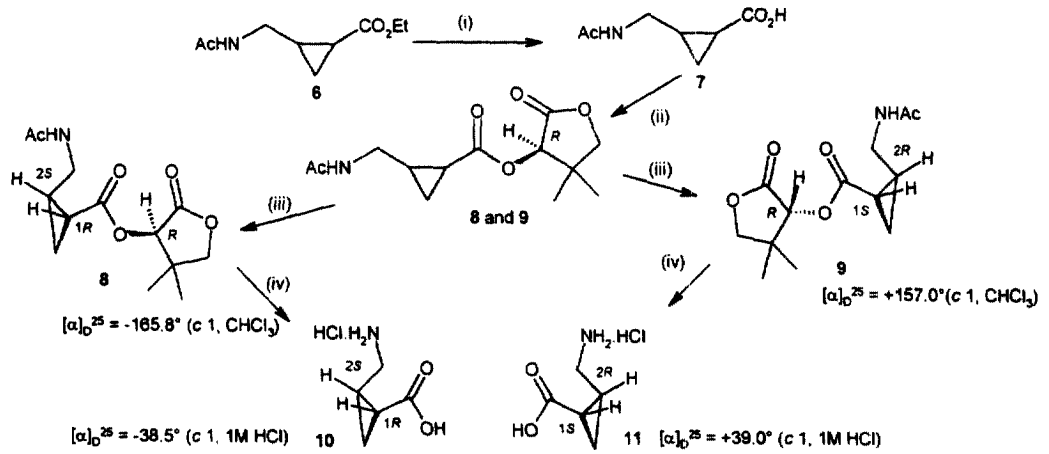
CACA **2** and *E*-4-aminobut-2-enoic acid (*trans*-aminocrotonic acid, TACA) **3** are unsaturated analogues of GABA with the four carbon atoms of the GABA chain constrained to lie in a plane, **2** in a folded conformation and **3** in an extended conformation. While **2** is a selective partial agonist at $GABA_C$ receptors, **3** is a potent partial agonist at both $GABA_A$ and $GABA_C$ receptors. The saturated carbocyclic analogues of GABA, *cis*-2-(aminomethyl)-1-carboxycyclopropane (CAMP) **4** and *trans*-2-(aminomethyl)-1-carboxycyclopropane (TAMP) **5** were synthesised[7] to evaluate the effects of constraining the carbon atoms of the GABA chain in a non-planar configuration by the cyclopropyl ring. While the folded conformation is retained in **4** and extended in **5**, the introduction of the cyclopropyl moiety on the GABA skeleton induces chirality, resulting in racemic mixtures which complicate pharmacological evaluation. (±)-CAMP **4** was found to show selectivity similar to that for CACA **2** and (±)-TAMP **5** was similar to TACA **3** in depressing neuronal firing at the bicuculline sensitive site of cat spinal neurones and in receptor binding assay.[7] The conclusion which may be drawn from these observations is that the folded conformation of the GABA chain was favoured for selectivity at $GABA_C$ receptors. When compounds **2–5** were tested on cloned $GABA_A$ and $GABA_C$ receptors, the *trans*-isomers, TACA **3** and (±)-TAMP **5**, were shown to be more potent at both receptors but less selective for $GABA_C$ receptors than the corresponding *cis*-isomers.[8,9] Since **4** and **5** were tested as racemates, the activities observed were the combined effect of the enantiomeric pair. The resolved enantiomers were required in order to associate activity directly with the stereochemical arrangement of binding groups required for GABA receptor activation.

Our initial attempts to obtain enantiomers of **4** and **5** using a chiral HPLC column were not successful. No detectable separation between the enantiomeric pairs of **4** and of **5** was observed by HPLC using a chiral Crown column which is commonly effective for the resolution of α-amino acids. However, separation *via* diastereoisomeric esters proved successful. This paper describes the resolution of **4** and **5** *via* (*R*)-(−)-pantolactonyl esters, as well as the prediction of the absolute configuration of the diastereoisomers of **4** by ^{1}H NMR, conformational analysis, *ab initio* density functional theory (DFT) and NMR shielding tensor calculations.

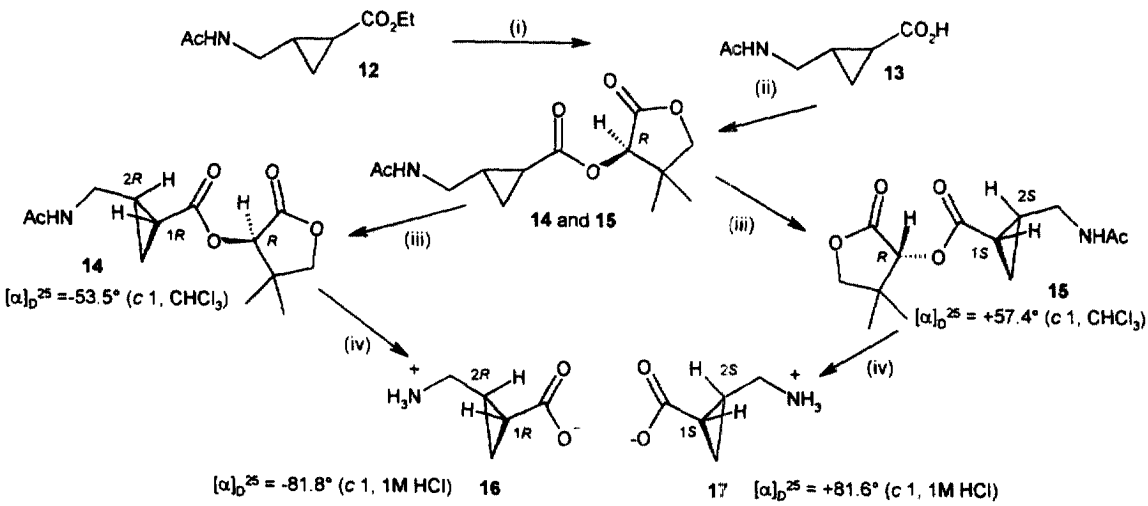
2. Discussion

Enantiomers **10**, **11** and **16** (Schemes 1 and 2) have been prepared previously by asymmetric syntheses using the Simmons–Smith reactions of *Z*- and *E*-allyl alcohol derivatives obtained from (*R*)-2,3-*O*-isopropylideneglyceraldehyde.[10] Enantiomer **10** has also been synthesised from the diastereoisomerically pure derivative of 3-aza-2-oxobicyclo[3.1.0]hexane prepared by an intramolecular cyclisation.[11] Our resolution has the practical advantage of providing all of the four enantiomers **10** and **11**, **16** and **17** which were required for pharmacological evaluation although in low yield. (*R*)-(−)-Pantolactone, which

was chosen for the formation of the diastereoisomeric esters, has the advantage of having three singlet resonances in the ^{1}H NMR spectrum which can be used in determining the purity of diastereoisomers.[12]



Scheme 1. (i) 6 M HCl/AcOH/RT, (ii) (*R*)-(−)-pantolactone, DCC, acetonitrile, (iii) chromatographic separation, (iv) 6 M HCl/AcOH/reflux



Scheme 2. (i) 6 M HCl/AcOH/RT, (ii) (*R*)-(−)-pantolactone, DCC, acetonitrile, (iii) chromatographic separation, (iv) 6 M HCl/AcOH/reflux

The synthetic route for the preparation of **10** and **11**, the enantiomers of **4**, is shown in Scheme 1. The *cis*-amido ester **6** was prepared by catalytic hydrogenation of *cis*-2-cyano-2-ethoxycarbonylcyclopropane in the presence of acetic anhydride.[7] Partial hydrolysis removed the ester and gave the amido acid **7** in good yield. Carbodiimide coupling of amido acid with *R*-(−)-pantolactone gave low to moderate yields of diastereoisomeric esters **8** and **9** which were separated using vacuum chromatography.[13] The pure diastereoisomers were cleaved with acid to give the corresponding amino acids **10** and **11**.

Compounds **16** and **17**, the enantiomers of **5**, were prepared by a similar procedure, as shown in Scheme 2, from the *trans*-amido ester **12**. **12** was prepared by catalytic hydrogenation of *trans*-2-cyano-2-ethoxycarbonylcyclopropane in the presence of acetic anhydride.[7]

The diastereoisomeric esters have different ^{13}C NMR and ^{1}H NMR resonances and therefore are completely distinguishable. The differences for particular peaks can be very large, up to 1 ppm, and all

resonances are separable when chemical shifts are measured to greater than two decimal places. ^{1}H and to a lesser extent ^{13}C NMR resonances also provide additional means for determining the diastereoisomeric composition and purity. As anticipated, the patterns and the peak heights of the geminal dimethyl singlets, as well as the singlet from the proton attached to the chiral carbon ($H_{2'}$) of the pantolactonyl moiety, proved to be excellent measures of the diastereoisomeric purity. Contamination above 0.3% by the other diastereoisomer could be detected. The intensity of the readily distinguishable geminal methyl singlet pair, acetyl singlet and $H_{2'}$ singlet of the diastereoisomers [(**8**, δ 1.261 and 1.228, 1.986, 5.44) (**9**, δ 1.234 and 1.134, 1.982, 5.47); (**14**, δ 1.207 and 1.134, 2.014, 5.36) (**15**, δ, 1.212 and 1.117, 2.003, 5.34)] allowed facile assessment of diastereoisomeric ratios. In addition, the signals of the methylene protons adjacent to the amide nitrogen were widely separated in three of the four diastereoisomers [(**8**, δ 4.32–4.23, m, 2.68–2.59, m), (**9**, δ 3.95, ddd, 2.95, ddd); (**14**, δ 3.52–3.44, m, 3.07–2.98, m), (**15**, δ 3.35–3.17, m)]. When the amide hydrogen was saturated or exchanged, these signals resolved to the simpler AM part of the AMX spin system in diastereoisomers **8** and **9** and the AB part of the ABX spin system in **14** and **15**. The strong couplings between the NH and these methylene protons were also clearly seen in the COSY spectra of all the diastereoisomers. The AM spin system indicates that one of the methylene protons is strongly shielded and the other strongly deshielded. To better understand the causes of the large difference in their magnetic environments, diastereoisomers **8** and **9** were investigated more rigorously.

The infrared spectra of the diastereoisomers recorded neat are very similar, with the exception of that of **8** which differs in the region of the NH and carbonyl stretching frequencies. Apart from very strong NH absorption at 3379 cm^{-1}, the amide and the lactone carbonyl stretching frequencies of **8** are approximately 10 cm^{-1} higher and lower, respectively, from the corresponding carbonyls of the other diastereoisomers. All four diastereoisomers show NH stretching vibrations varying in strength from 3610–3557 cm^{-1}, as well as multiple bands in the 3310–3082 cm^{-1} region in the infrared spectra, recorded neat, indicating that the amide groups are involved in various degrees and forms of hydrogen bonding. These multiple bands were absent in the spectra recorded with less concentrated samples (0.1 M, $CHCl_3$). The band at 3400 cm^{-1} in **8** was unaffected by dilution, but in **9** it was moderately affected, being weaker at 0.01 M, and weaker again on further dilution to 0.001 M. The bands at 3460 cm^{-1} in **14** and **15** were severely affected by dilution, becoming very weak at 0.01 M and almost absent on further dilution to 0.001 M. The results suggest that intramolecular hydrogen bonding is strong in **8**, weaker in **9** and not significant in **14** and **15**. The lowering in the lactone carbonyl stretching frequencies, 1780 cm^{-1} (0.1 M, $CHCl_3$) in **8** and 1785 cm^{-1} in **9** as compared with 1795 cm^{-1} in **14** and **15** are strongly indicative of intramolecular hydrogen bonding between the lactone carbonyls and the amide hydrogens. The amide carbonyl stretching frequencies in **8** and **9** are also lower, appearing at 1665 cm^{-1}, whereas those of **14** and **15** are at 1670 cm^{-1}. The NH exchange rates in the ^{1}H NMR of the diastereoisomers also correspond qualitatively to the relative strength of their intramolecular hydrogen bonding. On addition of D_2O, the NH resonances of the *trans*-diastereoisomers, **14** and **15** completely exchanged within 6 hours whereas complete exchange for the *cis*-diastereoisomers **9** and **8** requires up to 24 and 72 hours respectively. The slow exchange rate of **8** and **9** is possibly a result of the amide hydrogen being encased within the folded conformation of the *cis*-structure and therefore not readily accessible for interaction with D_2O. The comparative exchange rates of the two diastereoisomers may partly reflect the relative strength of intramolecular hydrogen bonding.

Each of the diastereoisomers exhibits a strong absorption in the region of 1544–1549 cm^{-1} which is characteristic of *trans*-geometry of the amide bond. Assuming the amide linkage to adopt a near-planar geometry, diastereoisomers **8** and **9** each possess five rotatable bonds which need to be considered, as

depicted in Fig. 2. Using the package Spartan 5.0,[14] a systematic search of the conformational space of the *trans*-amide forms was carried out.

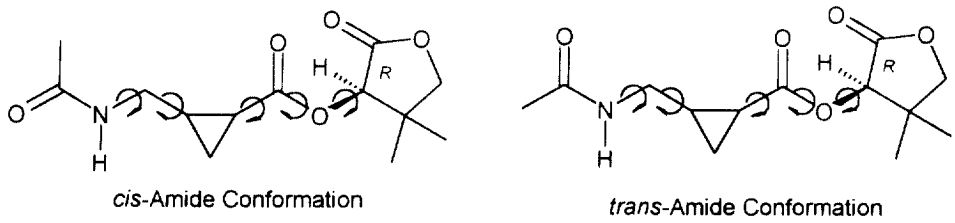
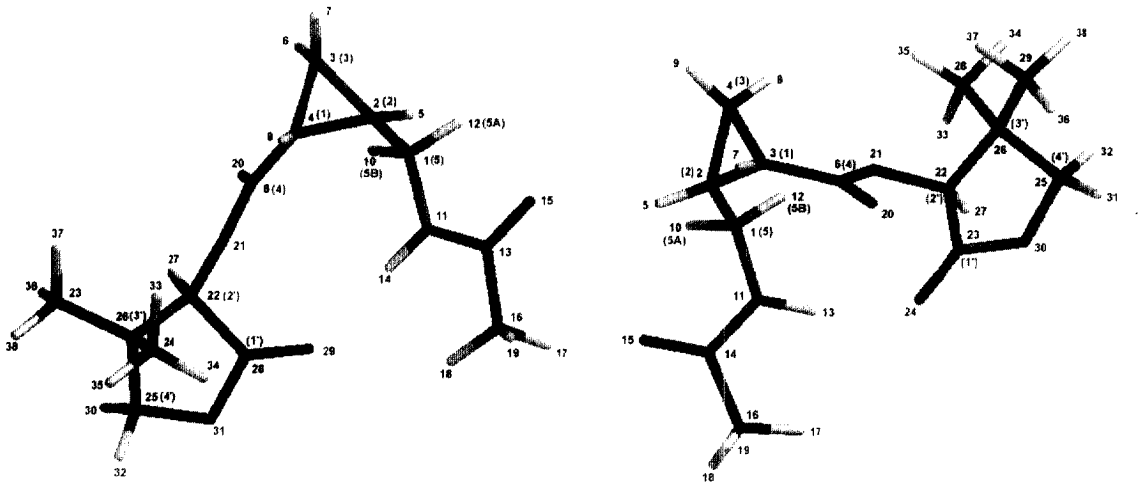


Fig. 2. Five rotatable bonds of diastereoisomers **8** and **9** to be considered when mapping conformational space

Three initial dihedral angles about each rotatable bond were selected, for a total number of 243 conformations per isomer. Each conformer was then geometry optimised using the semi-empirical AM1 method. One conformer of **8** was clearly favoured energetically, whereas three conformations of **9** lay close in energy. The lowest energy conformers of **8** and **9** shown in Fig. 3 possess an intramolecular lactone carbonyl–amide hydrogen bond, consistent with the lowering of the pantolactone carbonyl stretching frequencies observed in the infrared spectra. These conformers also show that H_{5B} in **8** is closer to the carbonyl oxygen of the acyclic ester while H_{5B} in **9** is closer to the saturated oxygen.



8 **9**

Fig. 3. Conformational global minima of diastereoisomers **8** and **9** as determined by exhaustive conformational analysis using AM1 theory and geometry optimisation using B3LYP/6-31G(d) theory. Atom labels in red and inside the brackets refer to chemical nomenclature used throughout the text

Conformers having *cis*-geometry about the amide bond were also subjected to the same calculation. According to AM1 semi-empirical method, the global minima of the *cis*-geometry structures were found to be higher than that of the *trans*-geometry, by 3.1 and 1.6 kcal mol^{-1} for **8** and **9**, respectively. Therefore, the amide bonds in **8** and **9** are likely to exist mainly in the *trans*-geometry consistent with the results obtained from the infrared spectra. Consequently, conformers with the *cis*-geometry were not subjected to further analysis.

The lowest energy conformer of **8**, as well as the five best conformers of **9**, as determined by the AM1 method, were submitted to geometry optimisation by high-level gas-phase *ab initio* density functional theory calculations (DFT) using the Gaussian 94 package.[15] Geometry optimisation was performed

using B3LYP theory and the basis set 6-31G(d), a theoretical method known to accurately reproduce experimental intramolecular hydrogen bonding lengths.[16] The results agreed qualitatively with the AM1 predictions, and, for **9**, the predicted global energy minimum conformer was 0.92 kcal mol^{-1} favoured over the next lowest energy local minimum, indicating the contribution of minor conformers in the approximate ratios of major:minors=82:17:1. The presence of the predominant minor conformer is consistent with an extra set of methylene resonances at δ 3.62 and δ 3.37 each integrating for slightly greater than 0.1H in the ^{1}H NMR spectrum and the corresponding ^{13}C–^{1}H resonance at δ 38.6 in the HETCOR spectrum of **9**.

The gas-phase structure of **8** and the lowest energy structure of **9** are shown in Fig. 3, and their geometries are given in Tables 1 and 2. Single-point energy calculations were then calculated with this geometry using Hartree–Fock theory, and the 6-311G(d,p) basis set. NMR shielding tensors were calculated using the gauge-independent atomic orbital (GIAO) method.[17,18] This model incorporating a triple ξ basis set is recommended by Cheeseman et al.[19] as providing accurate gas-phase geometries and reliable estimates of NMR chemical shifts. The calculated chemical shift values for the ^{13}C resonances shown in Table 3 and for ^{1}H resonances shown in Table 4, agreed qualitatively with observed values with a few minor exceptions.

While the calculated and observed chemical shifts of H_{5A} and H_{5B} in **8** are in excellent agreement, a large discrepancy is evident in the chemical shift value of H_{5B} in **9**. According to the calculated chemical shift, H_{5B} is strongly shielded (δ 2.22) which is inconsistent with the observed value of δ 2.95 ppm. In addition, H_{5B} of **9**, shown in Fig. 3, does not appear to be deep in the carbonyl shielding cone (dihedral angle OC_4OH_{5B}=147.5°, angles C_4OH_{5B}=19.6° and $H_{5B}C_4O$=152.9°), and is a long way away from the C_4 carbonyl (distances $H_{5B}O$=4.22 Å and $H_{5B}C_4$=3.11 Å), and therefore it is not expected to be greatly affected by the carbonyl shielding. In contrast, the carbonyl shielding of H_{5B} in **8** was predicted from the gas-phase structure and was observed experimentally. In the lowest energy conformer of **8** also shown in Fig. 3, H_{5B} lies well within the carbonyl shielding cone of the acyclic ester (dihedral angle, OC_4OH_{5B}=83.4°, angles C_4OH_{5B}=92.2° and $H_{5B}C_4O$=62.1°). This, together with its close proximity to the C_4 carbonyl group (distances $H_{5B}O$=2.47 Å and $H_{5B}C_4$=2.80 Å), results in H_{5B} being strongly shielded by the C_4 carbonyl consistent with the observed resonance for H_{5B} in **8** at δ 2.64 ppm. The large discrepancy between the observed and calculated chemical shifts of H_{5B} in **9** can be explained in terms of the higher conformational flexibility of **9** as indicated by the earlier calculations. Although the dielectric constant of chloroform is low, some conformational changes from the gas-phase structure may occur when the other conformations are close in energy. Present limitations in computing capability preclude the performance of higher level calculations on NMR shielding tensors.

The influence of carbonyl diamagnetic anisotropy could also be used to explain the difference between the chemical shifts of H_{5A} in **8** (δ 4.28) and **9** (δ 3.95). H_{5A} atoms in **8** and in **9** both lie in the deshielding cone of the C=O bond of the amide (**8**, dihedral angle, $OCNH_{5A}$=15.4°, angles COH_{5A}=78.3°, $H_{5A}CO$=72.1°; **9**, dihedral angle $OCNH_{5A}$=−22.9°, angles COH_{5A}=78.3°, $H_{5A}CO$=74.8°). Since the deshielding effect induced by the carbonyl is greatest along the transverse axis of the C=O bond, it is expected that H_{5A} in **8** would be more affected by the deshielding process than H_{5A} in **9**. Moreover, the distances between H_{5A} and the amide carbonyl, $H_{5A}O$=2.52 Å, $H_{5A}C$=2.62 Å in **8** and $H_{5A}O$=2.63 Å and $H_{5A}C$=2.66 Å in **9** also indicate that the deshielding is stronger for H_{5A} in **8**. These combined effects are likely to be the cause of the large difference observed in their chemical shifts.

There were no significant changes in the ^{1}H NMR spectra of **8** and **9** when the temperature was raised ($CDCl_3$, 20–40°C, in 10°C steps) indicating that **8** and **9** either exist in $CDCl_3$ solution mainly in the conformations predicted by the calculations or interconversion between conformations is not detectable using the standard pulse delay over the temperature range investigated. In **8**, the observed coupling

Table 1
Cartesian co-ordinates of conformational global minimum of diastereoisomer **8** as determined by geometry optimisation at B3LYP/6-31G(d) DFT theory

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.043095	0.517638	0.149474
2	6	-2.770437	-0.615733	1.125397
3	6	-2.991333	-2.047617	0.765663
4	6	-1.567242	-1.559587	0.949034
5	1	-2.942839	-0.333375	2.160796
6	1	-3.280799	-2.273055	-0.257320
7	1	-3.383255	-2.729253	1.515039
8	6	-0.716909	-1.381556	-0.246089
9	1	-1.029674	-1.821641	1.853977
10	1	-2.955765	0.169881	-0.882163
11	7	-2.143964	1.648223	0.328659
12	1	-4.064931	0.872232	0.312226
13	6	-2.337039	2.546453	1.338677
14	1	-1.258215	1.641668	-0.166124
15	8	-3.305330	2.493910	2.093424
16	6	-1.286723	3.643245	1.444458
17	1	-1.748534	4.600779	1.180011
18	1	-0.418744	3.478122	0.799864
19	1	-0.959965	3.717585	2.485864
20	8	-1.084975	-1.363495	-1.402398
21	8	0.592865	-1.188464	0.119087
22	6	1.483763	-0.808045	-0.918009
23	6	3.226746	-2.670248	-1.182715
24	6	3.332320	-1.105827	0.801833
25	6	3.646371	-0.172847	-1.531062
26	6	2.939001	-1.252489	-0.679542
27	1	1.104196	-1.181606	-1.877330
28	6	1.593513	0.716957	-1.021506
29	8	0.738777	1.543652	-0.816788
30	1	3.681174	-0.441966	-2.593321
31	8	2.851982	1.034579	-1.396915
32	1	4.655562	0.063954	-1.187403
33	1	2.737300	-1.774719	1.429049
34	1	3.180614	-0.083153	1.164420
35	1	4.390126	-1.358829	0.934508
36	1	2.954446	-2.787359	-2.238084
37	1	2.656961	-3.404471	-0.603128
38	1	4.289921	-2.915865	-1.076457

constants between the protons of the more flexible bonds are in complete agreement with the dihedral angles of the calculated structures. The coupling constants of 8.7 and 4.1 Hz which were clearly removed from the multiplets of H_{5A} and H_{5B} on addition of D_2O or irradiation of the NH peak are consistent with the calculated dihedral angles between NH and C_5H_{5A} of $-152.8°$ and NH and C_5H_{5B} of $-35.4°$ respectively. The dihedral angles, $H_2C_2C_5H_{5A}=-55.4°$ and $H_2C_2C_5H_{5B}=-174.4°$ are also consistent with the couplings of 3.3 and 9.8 Hz observed between H_2 and H_{5A}, H_2 and H_{5B} respectively. In **9**, the observed coupling constants between NH and H_{5A}, NH and H_{5B} ($J_{NH,H5A}$=6.1 Hz and $J_{NH,H5B}$=6.3 Hz) and between H_2 and H_{5B} ($J_{H2,HB}$=9.6 Hz) are in accordance with the respective calculated dihedral angles

Table 2
Cartesian co-ordinates of conformational global minimum of diastereoisomer **9** as determined by geometry optimisation at B3LYP/6-31G(d) DFT theory

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	2.074990	0.497103	-1.186777
2	6	2.772439	0.426452	0.163610
3	6	2.027174	0.223394	1.488358
4	6	2.914573	-0.865363	0.897853
5	1	3.586335	1.140361	0.252485
6	6	0.557395	0.104451	1.603031
7	1	2.423840	0.778665	2.331561
8	1	2.408260	-1.734709	0.487359
9	1	3.836175	-1.087962	1.428406
10	1	2.853922	0.492105	-1.957732
11	7	1.247115	1.678118	-1.365560
12	1	1.437344	-0.374699	-1.338699
13	1	0.240539	1.553025	-1.348207
14	6	1.802471	2.906436	-1.557195
15	8	3.019043	3.083808	-1.567952
16	6	0.817697	4.049056	-1.758987
17	1	-0.228975	3.730443	-1.753127
18	1	1.037837	4.538550	-2.712794
19	1	0.970011	4.792169	-0.969641
20	8	-0.106627	0.653377	2.454451
21	8	-0.001994	-0.708777	0.639112
22	6	-1.418338	-0.809408	0.635308
23	6	-2.005028	-0.249042	-0.660645
24	8	-1.596245	0.662109	-1.339285
25	6	-3.341690	-1.985469	0.024029
26	6	-1.968237	-2.249973	0.682360
27	1	-1.812432	-0.218520	1.471961
28	6	-1.130245	-3.198360	-0.193635
29	6	-2.104401	-2.799936	2.105507
30	8	-3.124368	-0.942191	-0.961785
31	1	-4.082166	-1.622592	0.746391
32	1	-3.749378	-2.849791	-0.504646
33	1	-1.070718	-2.851058	-1.231334
34	1	-1.577901	-4.198443	-0.198028
35	1	-0.110600	-3.282690	0.192154
36	1	-2.692234	-2.129757	2.742871
37	1	-1.118482	-2.922815	2.566600
38	1	-2.592454	-3.781760	2.100106

(HNC_5H_{5A}=132.6°, HNC_5H_{5B} of 15.5°, $H_2C_2C_5H_B$=166.3°). However, the coupling constant between H_{5A} and H_2 of 5.4 Hz is larger than the value of about 4 Hz predicted by the dihedral angle of 48.2°. This discrepancy may be accounted for in terms of conformational flexibility of **9**. The calculated dihedral angle is located in the steep part of the vicinal Karplus correlation curve and therefore a small deviation from this angle would result in a large change in the predicted coupling constant.

The *cis*-configuration of diastereoisomers **8** and **9** allows formation of intramolecular lactone carbonyl–amide hydrogen bond, thereby adding stability to the conformation. In the case of **8** and **9**, intramolecular hydrogen bonding also induces large differences in the chemical shift of the neighbouring

Table 3
Relative ^{13}C NMR chemical shifts of diastereoisomers **8** and **9** from magnetic shielding tensors calculated at HF/6-311G(d,p)/6-31G(d) in ppm relative to the observed chemical shift values of $C_{3'}$, **8** (δ 39.7), **9** (δ 40.0)

	8 (Calculated)	**8** (Observed)	**9** (Calculated)	**9** (Observed)
C_4O	187.9	173.6	186.1	173.3
$C_{1'}O$	187.2	171.9	181.6	171.7
CON	185.6	170.8	184.8	170.5
$C_{4'}$	75.2	76.7	74.9	76.6
$C_{2'}$	73.4	75.4	71.6	75.1
$C_{3'}$	39.7	39.7	40.0	40.0
CH_2N	39.0	37.3	41.8	38.6
CH_3CO	27.0	23.1	27.1	23.1
C_2	26.5	22.9	25.3	22.4
$C(CH_3)_2$	26.5, 24.5	22.7, 20.1	26.1, 24.2	22.9, 20.0
C_1	18.1	17.3	19.3	17.8
C_3	14.8	12.6	16.3	14.2

Table 4
Relative ^1H NMR chemical shifts of diastereoisomers **8** and **9** from magnetic shielding tensors calculated at HF/6-311G(d,p)/6-31G(d) in ppm relative to observed chemical shift values of $H_{2'}$, **8** (δ 5.44), **9** (δ 5.47)

	8 (Calculated)	**8** (Observed)	**9** (Calculated)	**9** (Observed)
NH	7.11	6.95-6.85	7.32	6.7-6.6
$H_{2'}$	5.44	5.44	5.47	5.47
OCH_2	4.16, 3.98	4.11, 4.11	3.79, 3.62	4.12, 4.09
H_{5A}	4.29	4.32-4.23	3.83	3.95
H_{5B}	2.65	2.68-2.59	2.22	2.95
H_2	2.22	2.20-1.75	2.23	2.10-1.89
$COCH_3$	2.22	1.99	1.80	1.98
H_1	1.62	2.20-1.75	1.26	1.88-1.79
$C(CH_3)_2$	1.58, 1.47	1.26, 1.23	1.05, 1.02	1.23, 1.13
H_3	1.47, 1.19	1.20-1.15	0.91, 0.86	1.32-1.23, 1.22-1.02

methylene protons. Conformational stability and large differences in the magnetic environment of the methylene protons, together with known chirality of pantolactone, enabled the absolute configuration to be predicted. The prediction was proven to be correct by comparison of the optical rotations of the enantiomers **10** and **11** with the literature values.[10,11] Using the same procedure as for **8** and **9**, diastereoisomers **14** and **15** were subjected to lowest energy conformer searches using the package

Spartan 5.0.[14] As anticipated for the *trans*-configuration, the low energy optimised conformers of **14** and **15** assume partially extended or extended conformations, and, consistent with the results obtained from infrared spectrometry, intramolecular carbonyl–amide hydrogen bonding was not observed.

3. Conclusion

cis- and *trans*-2-(Aminomethyl)-1-carboxycyclopropanes **4** and **5** were successfully resolved by the separation of diastereoisomeric esters prepared from the corresponding *cis*- and *trans*-2-(acetamidomethyl)-1-carboxycyclopropanes and (*R*)-(−)-pantolactone. The chirality of the *cis*-diastereoisomers, assigned by ^{1}H NMR, conformational analysis, and *ab initio* (DFT) calculations, was consistent with the absolute configurations of the corresponding amino acid enantiomers determined by comparison of the optical rotation and reference to literature values.

4. Experimental

4.1. General procedures

Melting points were determined on a Reichert hot-stage apparatus and are reported uncorrected. Elemental analyses were carried out by the Microanalysis Unit, Department of Chemical Engineering, the University of Sydney. High and low resolution chemical ionisation mass spectral determinations using CH_4 as the reagent gas were carried out on a VG Scientific ZAB-E high resolution magnetic sector mass spectrometer and a Finigan-MAT TSQ46 MS/MS instrument respectively. Solution infrared spectra were acquired on a Perkin–Elmer 177 grating spectrophotometer. Reflection infrared spectra of the neat material were acquired on a Bruker IFV66V FT-IR fitted with a microscope. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter.

1D ^{1}H (300 MHz), ^{13}C (75 MHz) and 2D NMR experiments were performed at 20°C using a 300 MHz Varian–Gemini 300 spectrometer. For ^{1}H NMR, chemical shift values are given in ppm relative to internal TMS for spectra measured in $CDCl_3$, and HOD (4.75 ppm) for spectra measured in D_2O respectively. For ^{13}C NMR, chemical shift values are given in ppm relative to $CDCl_3$ (77.0 ppm) for compounds measured in $CDCl_3$ and relative to dioxan (67.4 ppm) for compounds measured in D_2O. Standard pulse sequences were used for COSY, DEPT and HETCOR experiments.

AM1 semi-empirical conformational analyses were performed by Spartan 5.0[14] on a SGI O2 R5000 computer running under IRIX 6.3. *Ab initio* Hartree–Fock and density functional theory (DFT) calculations were performed by Gaussian 94[15] on an SGI Power Challenge R10000 network under IRIX 6.2.

4.2. (±)-cis-2-(Aminomethyl)-1-carboxycyclopropane 4

Crude (±)-CAMP **4** prepared by the method of Allan et al.[7] was adsorbed on a cationic exchange resin (Dowex 50) and eluted with 1 M pyridine. Removal of the eluant under reduced pressure gave a light brown solid which was further purified by gel filtration on Sephadex G10 (H_2O). Fractions were analysed by TLC (silica gel, *n*-butanol:acetic acid:water=3:1:1, ninhydrin). Fractions containing **4** which were homogeneous by TLC were combined and concentrated under reduced pressure to give a white crystalline solid. Recrystallisation from H_2O:EtOH gave colourless rectangular plates, m.p. 225–226°C (lit.[1] m.p. 225–226°C). *m/z* (%) $[M+1]^+$ 116 (47), 99 (76), 98 (100). ^{1}H NMR (D_2O) δ 3.16 (1H, dd,

J_{AB}=13.1 Hz, J_{AX}=7.1 Hz, CH_2N), 3.06 (1H, dd, J_{AB}=13.1 Hz, J_{BX}=7.7 Hz, CH_2N), 1.72–1.64 (1H, m, H_1), 1.40–1.27 (1H, m, H_2), 1.03–0.96 (1H, m, H_3), 0.82–0.76 (1H, m, H_3). ^{13}C NMR (D_2O) δ 181.0 (CO), 39.9 (CH_2N), 21.7 (C_2), 16.1 (C_1), 11.6 (C_3).

4.3. (±)-trans-2-(Aminomethyl)-1-carboxycyclopropane 5

Crude (±)-TAMP **5** prepared by the method of Allan et al.[7] was purified by the procedure described for the purification of **4**. The white crystalline solid obtained from ion exchange and gel filtration chromatographic purification was recrystallised from H_2O:EtOH to give colourless needles, m.p. 245°C (subl. from 240°C), [lit.[1] m.p. 270–275°C]. *m/z* (%) $[M+1]^+$ 116 (24), 99 (42), 98 (100). ^1H NMR (D_2O) δ 2.97–2.88 (1H, m, CH_2N), 2.83–2.74 (1H, m, CH_2N), 1.48–1.36 (2H, m, H_1 and H_2), 1.05–0.99 (1H, m, H_3), 0.75 (1H, ddd, *J*=7.9, 6.6, 4.7 Hz, H_3). ^{13}C NMR (D_2O) δ 182.3 (CO), 43.7 (CH_2N), 22.8 (C_2), 18.2 (C_1), 13.0 (C_3).

4.4. cis-2-(Acetamidomethyl)-1-ethoxycarbonylcyclopropane 6

Compound **6** was prepared by hydrogenation of *cis*-2-cyano-1-ethoxycarbonylcyclopropane catalysed by platinum oxide in the presence of acetic anhydride following the method described by Allan et al.[7] The mixture was concentrated to dryness under reduced pressure to give **6** as a light brown oil. ^1H NMR ($CDCl_3$) δ 5.9–5.7 (1H, br s, NH), 4.15 (2H, q, *J*=7.2 Hz, OCH_2), 3.68–3.58 (1H, m, CH_2N), 3.42–3.32 (1H, m, CH_2N), 1.98 (3H, s, $COCH_3$), 1.75–1.49 (2H, m, H_1 and H_2), 1.29 (3H, t, *J*=7.2 Hz, CH_3), 1.16–1.08 (1H, m, H_3), 1.06–1.00 (1H, m, H_3).

4.5. cis-2-(Acetamidomethyl)-1-carboxycyclopropane 7

A solution of compound **6** (15.6 g, 0.084 mol) in acetic acid (80 ml) was stirred with 6 M HCl (160 ml) at room temperature overnight. The mixture was concentrated under reduced pressure then dried under high vacuum over P_2O_5 to give *cis*-acid **7** as a very viscous pale brown oil (12.5 g, 95%). *m/z* (%) 198 (5), $[M+29]^+$ 186 (15), $[M+1]^+$ 158 (41), 140 (26), 98 (100). ^1H NMR (D_2O) δ 3.36 (1H, dd, J_{AB}=14.2 Hz, J_{AX}=6.3 Hz, CH_2N), 3.15 (1H, dd, J_{AB}=14.2 Hz, J_{BX}=8.8 Hz, CH_2N), 2.09 (3H, s, CH_3CO), 1.76–1.69 (1H, m, H_1), 1.60–1.40 (1H, m, H_2), 1.15–1.07 (1H, m, H_3), 0.97–0.91 (1H, m, H_3). ^{13}C NMR (D_2O) δ 179.4, 177.8 (2×CO), 39.8 (CH_2N), 22.4 (CH_3), 21.2 (C_2), 18.2 (C_1), 13.3 (C_3).

4.6. cis-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 8 and 9

To a solution of **7** (10.2 g, 65 mmol) in acetonitrile (250 ml) was added *R*-(−)-pantolactone (10.2 g, 78 mmol) and dicyclohexylcarbodiimide (21.8 g, 112 mmol). The mixture became homogeneous briefly when it was brought to reflux under stirring. A white precipitate of dicyclohexyl urea was observed, and refluxing and stirring was continued overnight. The mixture was cooled to room temperature and filtered. The filtrate was concentrated to dryness under reduced pressure and dissolved in CH_2Cl_2 (100 ml). The solution was refiltered if more precipitate appeared before being subjected to purification using vacuum chromatography[13] on a column (40 mm×40 mm i.d.) packed with silica gel H60 under reduced pressure. The mixture was eluted with 10% acetonitrile in benzene to remove most of the unreacted pantolactone and dicyclohexylcarbodiimide, and some side products. The partly purified diastereoisomers were separated on a longer (70 mm×40 mm i.d.) column, eluting in turn with $CHCl_3$

(2×100 ml), 1% MeOH in $CHCl_3$ (2×100 ml), 2% MeOH in $CHCl_3$ (4×100 ml), then collecting 20 ml fractions with 2% MeOH in $CHCl_3$ until the diastereoisomers were completely eluted. Fractions were analysed by TLC using two solvent systems, $CHCl_3$:MeOH=95:5 and benzene:acetonitrile=4:1. Generally, TLC plates were developed twice in the same solvent system before visualisation with 0.5% $KMnO_4$ in 2.5% aqueous K_2CO_3. Repeated column separations resulted in complete separation of the whole mixture of diastereoisomers.

4.7. (−)-(1R,2S)-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 8

The less polar diastereoisomer of CAMP **8** was isolated as a colourless viscous oil (2.0 g, 11.4%), $[\alpha]_D^{25}$ −165.8 (*c* 1, $CHCl_3$). IR (neat) 3610 sh, 3310 br, 3205 sh, 3379, 3289, 3083, 2969, 2935, 2878, 1778, 1737, 1669, 1549, 1467, 1445, 1404, 1389, 1375, 1338, 128, 1233 cm^{-1}, IR (0.1 M, CCl_4) 3405, 2990, 1780, 1750, 1685, 1145 cm^{-1}, IR (0.1 M, $CHCl_3$) 3400, 2995, 1785, 1740, 1665, 1150 cm^{-1}. HRMS (CI) calcd for $C_{13}H_{20}O_5N$: 270.1341. Found: 270.1341. *m/z* (%) $[M+29]^+$ 298 (24), $[M+1]^+$ 270 (100), 140 (19), 130 (7), 115 (5). ^1H NMR ($CDCl_3$) δ 6.95–6.85 (1H, br s, NH), 5.44 (1H, s, $H_{2'}$), 4.11 (2H, br s, OCH_2), 4.32–4.23 (1H, m, CH_2N), 2.68–2.59 (1H, m, CH_2N), 1.988 (3H, s, CH_3CO), 2.20–1.75 (2H, m, H_1 and H_2), 1.261 (3H, s, CH_3), 1.227 (3H, s, CH_3), 1.20–1.15 (2H, m, H_3). ^1H NMR ($CDCl_3$+D_2O) δ 5.44 (1H, s, $H_{2'}$), 4.12 (2H, br s, OCH_2), 4.29 (1H, dd, J_{AM}=14.5 Hz, J_{AX}=3.3 Hz, CH_2N), 2.64 (1H, dd, J_{AM}=14.7 Hz, J_{MX}=9.8 Hz, CH_2N), 1.986 (3H, s, CH_3CO), 2.20–1.80 (2H, m, H_1 and H_2), 1.261 (3H, s, CH_3), 1.228 (3H, s, CH_3), 1.20–1.15 (2H, m, H_3). ^{13}C NMR ($CDCl_3$) δ 173.6, 171.9, 170.8 (3×CO), 76.7 ($C_{4'}$), 75.4 ($C_{2'}$), 39.7 (CH_2N), 37.3 ($C_{3'}$), 23.1 (CH_3CO), 22.9 (C_2), 22.7 (CH_3), 20.1 (CH_3), 17.3 (C_1), 12.6 (C_3).

4.8. (+)-(1S,2R)-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 9

The more polar diastereoisomer of CAMP **9** was isolated as a colourless viscous oil (2.1 g, 12%), $[\alpha]_D^{25}$ +157.0 (*c* 1, $CHCl_3$). IR (neat) 3568, 3391, 3297, 3210 sh, 3088, 2968, 2932, 2878, 1788, 1738, 1656, 1546, 1467, 1445, 1403, 1390, 1373, 1336, 1285, 1232 cm^{-1}, IR (0.1 M, $CHCl_3$) 3400, 3000, 1790, 1735, 1665, 1160 cm^{-1}. HRMS (CI) calcd for $C_{13}H_{20}O_5N$: 270.1341. Found: 270.1341. *m/z* (%) $[M+29]^+$ 298 (25), $[M+1]^+$ 270 (100), 140 (27). ^1H NMR ($CDCl_3$) δ 6.7–6.6 (1H, br s, NH), 5.47 (1H, s, $H_{2'}$), 4.18 (0.1H, d, J_{AB}=7.2 Hz, OCH_2), 4.14 (0.1H, d, J_{AB}=7.2 Hz, OCH_2), 4.12 (0.9H, d, J_{AB}=9.1 Hz, OCH_2), 4.09 (0.9H, d, J_{AB}=9.1 Hz, OCH_2), 3.95 (0.87H, ddd, *J*=14.4, 6.1, 5.7 Hz, CH_2N), 3.62 (0.13H, ddd, *J*=14.3, 6.0, 6.0 Hz, CH_2N), 3.37 (0.13H, ddd, *J*=14.3, 8.3, 6.0 Hz, CH_2N), 2.95 (0.87H, ddd, *J*=14.4, 9.7, 6.3 Hz, CH_2N), 1.982 (3H, s, CH_3CO), 2.10–1.89 (1H, br m, H_2), 1.88–1.79 (1H, m, H_1), 1.31–1.23 (1H, m, H_3), 1.234 (3H, s, CH_3), 1.22–1.02 (1H, m, H_3), 1.134 (3H, s, CH_3). ^1H NMR ($CDCl_3$+D_2O) δ 5.47 (1H, s, $H_{2'}$), 4.18 (0.1H, d, J_{AB}=7.1 Hz, OCH_2), 4.14 (0.1H, d, J_{AB}=7.1 Hz, OCH_2), 4.12 (0.9H, d, J_{AB}=9.1 Hz, OCH_2), 4.09 (0.9H, d, J_{AB}=9.1 Hz, OCH_2), 3.95 (0.85H, dd, J_{AM}=14.4 Hz, J_{AX}=5.5 Hz, CH_2N), 3.62 (0.15H, dd, J_{AB}=14.3 Hz, J_{AX}=5.7 Hz, CH_2N), 3.37 (0.15H, dd, J_{AB}=14.3 Hz, J_{BX}=8.3 Hz, CH_2N), 2.95 (0.85H, dd, J_{AM}=14.4 Hz, J_{MX}=9.6 Hz, CH_2N), 2.04–1.88 (0.9H, m, H_2), 1.982 (3H, s, CH_3CO), 1.88–1.78 (0.9H, m, H_1), 1.75–1.69 (0.1H, m, H_1), 1.66–1.58 (0.1H, m, H_2), 1.31–1.23 (0.9H, m, H_3), 1.231 (3H, s, CH_3), 1.22–1.02 (0.9H, m, H_3), 1.134 (3H, s, CH_3), 1.08–0.82 (0.2H, m, H_3). ^{13}C NMR ($CDCl_3$) δ 173.3, 171.7, 170.5 (3×CO), 76.6 ($C_{4'}$), 75.1 ($C_{2'}$), 40.0 ($C_{3'}$), 38.6 (CH_2N), 23.1 (CH_3CO), 22.9 (CH_3), 22.4 (C_2), 20.0 (CH_3), 17.8 (C_1), 14.2 (C_3).

4.9. (−)-(1R,2S)-2-(Aminomethyl)-1-carboxycyclopropane hydrochloride 10

To a solution of the less polar (−)-diastereoisomer **8** (240 mg, 8.9 mmol) in acetic acid (15 ml) was added 6 M HCl (45 ml) and the mixture was heated under reflux with stirring overnight. Evaporation under reduced pressure gave a white solid. Purification (ion exchange chromatography, Dowex 50, 1 M pyridine) gave the free amino acid **10** as white powder. Its TLC (*n*-butanol:acetic acid:water=3:1:1, ninhydrin), R_f 0.4 and ^{1}H NMR (D_2O) spectrum were identical to (±)-CAMP **4**. The solid was redissolved in 6 M HCl and concentrated to dryness under reduced pressure to give the hydrochloride salt as a white crystalline solid. Recrystallisation (H_2O:EtOH) gave (1*R*,2*S*)-CAMP·HCl **10** (67 mg, 48%) as colourless rectangular plates, m.p. 244–246°C, [lit.[10,11] m.p. 239–241°C, 240–241°C], $[\alpha]_D^{25}$ −38.5 (*c* 1, 1 M HCl), [lit.[10,11] $[\alpha]_D^{30}$ −38.5 (*c* 0.99, 1 M HCl), $[\alpha]_D$ −38.1 (*c* 1, 1 M HCl)]. *m/z* (%) $[M+1]^+$ 116 (6), 99 (52), 98 (100). Anal. calcd for $C_5H_9O_2N$·HCl: C, 39.62; H, 6.65; N, 9.24. Found: C, 39.66; H, 6.45; N, 9.14.

4.10. (+)-(1S,2R)-2-(Aminomethyl)-1-carboxycyclopropane hydrochloride 11

The more polar (+)-diastereoisomer of CAMP **9** (270 mg, 1 mmol) was converted to (1*S*,2*R*)-CAMP·HCl **11** (120 mg, 80%), using the same procedure for the preparation of **10**. TLC of the free amino acid (*n*-butanol:acetic acid:water=3:1:1, ninhydrin) R_f 0.4 and ^{1}H NMR (D_2O) spectrum were identical to (±)-CAMP **4**. The hydrochloride salt **11** was recrystallised from H_2O:EtOH as colourless rectangular plates, m.p. 241–242°C, [lit.[10] m.p. 242–243°C], $[\alpha]_D^{25}$ +39.0 (*c* 1, 1 M HCl), [lit.[10] $[\alpha]_D^{26}$ +37.3 (*c* 0.99, 1 M HCl)]. *m/z* (%) $[M+1]^+$ 116 (9), 99 (29), 98 (100). Anal. calcd for $C_5H_9O_2N$·HCl: C, 39.62; H, 6.65; N, 9.24. Found: C, 39.88; H, 6.25; N, 9.04.

4.11. trans-2-(Acetamidomethyl)-1-ethoxycarbonylcyclopropane 12

Compound **12** was prepared by hydrogenation of ethyl *trans*-2-cyano-1-ethoxycarbonylcyclopropane over platinum oxide in the presence of acetic anhydride using the method described by Allan et al.[7] The mixture was concentrated to dryness under reduced pressure to give **12** as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 4.13 (2H, q, *J*=7.1Hz, OCH_2), 3.34–3.23 (1H, m, CH_2N), 3.18–3.09 (1H, m, CH_2N), 2.00 (3H, s, CH_3CO), 1.70–1.56 (1H, m, H_2), 1.57–1.51 (1H, m, H_1), 1.26 (3H, t, *J*=7.1Hz, CH_3), 1.24–1.17 (1H, m, H_3), 0.86 (1H, ddd, *J*=8.5, 6.2, 4.4 Hz, H_3). ^{13}C NMR ($CDCl_3$) δ 173.5, 170.2 (2×CO), 60.6 (OCH_2), 42.1 (CH_2N), 23.1 (C_2), 21.6 ($COCH_3$), 19.0 (C_1), 14.2 (CH_3), 13.7 (C_3).

4.12. trans-2-(Acetamidomethyl)-1-carboxycyclopropane 13

The *trans*-ester **12** was converted to the *trans*-acid **13** using the procedure described for the preparation of the *cis*-acid **7**. The *trans*-acid **13** obtained initially as a very viscous colourless oil (90%) which could be crystallised from either acetone or acetonitrile. Crystallisation from acetone gave **13** as colourless needles, m.p. 157–159°C (subl. from 150°C). *m/z* (%) $[M+29]^+$ 186 (14), $[M+1]^+$ 158 (21), 140 (100). ^{1}H NMR (D_2O) δ 3.18 (1H, dd, J_{AB}=14.2 Hz, J_{AX}=6.0 Hz, CH_2N), 2.98 (1H, dd, J_{AB}=14.2 Hz, J_{BX}=7.1 Hz, CH_2N), 1.89 (3H, s, CH_3CO), 1.59–1.42 (1H, m, H_2), 1.52–1.46 (1H, m, H_1), 1.14–1.08 (1H, m, H_3), 0.89 (1H, ddd, *J*=8.5, 6.3, 4.8 Hz, H_3). ^{13}C NMR (D_2O) δ 179.3, 174.8 (2×CO), 42.4 (CH_2N), 22.6 (CH_3), 22.5 (C_2), 19.3 (C_1), 14.5 (C_3). Anal. calcd for $C_7H_{11}O_3N$: C, 53.49; H, 7.05; N 8.91. Found: C, 53.09; H, 7.09; N, 9.18.

4.13. trans-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 14 and 15

trans-Acetamido acid **13** (4.7 g, 30 mmol) was converted to diastereoisomers **14** and **15** using the procedure described for the preparation of diastereoisomers **8** and **9**. Compounds **14** and **15** were separated using the same method as described for **8** and **9** with a slight increase in MeOH concentration to 3%. The separation between the TAMP-diastereoisomers was less than that of the CAMP-diastereoisomers and repeated column separations were required to achieve complete separation of the whole mixture of the diastereoisomers.

4.14. (−)-(1R,2R)-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 14

The less polar diastereoisomer **14** was isolated as a colourless viscous oil (2 g, 23%), $[\alpha]_D^{25}$ −53.5 (*c* 1, $CHCl_3$). IR (neat) 3558, 3375, 3292, 3205 sh, 3085, 2969, 2933, 2878, 1792, 1743, 1655, 1549, 1466, 1452, 1413, 1372, 1316, 1296, 1232, 1204 cm^{-1}, IR (0.1 M, $CHCl_3$) 3460, 2990, 1795, 1745, 1670, 1160 cm^{-1}. HRMS (CI) calcd for $C_{13}H_{20}O_5N$: 270.1341. Found: 270.1341. *m/z* (%) $[M+29]^+$ 298 (33), $[M+1]^+$ 270 (100), 140 (81). ^1H NMR ($CDCl_3$) δ 5.9–5.8 (1H, br s, NH), 5.36 (1H, s, $H_{2'}$), 4.07 (1H, d, J_{AB}=10.9 Hz, OCH_2) 4.04 (1H, d, J_{AB}=10.9 Hz, OCH_2), 3.52–3.44 (1H, m, CH_2N), 3.07–2.98 (1H, m, CH_2N), 2.014 (3H, s, CH_3CO), 1.76–1.66 (2H, m, H_1 and H_2), 1.36–1.30 (1H, m, H_3), 1.207 (3H, s, CH_3), 1.134 (3H, s, CH_3), 1.04–0.98 (1H, m, H_3). ^1H NMR ($CDCl_3$+D_2O) δ 5.37 (1H, s, $H_{2'}$), 4.08 (1H, d, J_{AB}=9.1 Hz, OCH_2), 4.04 (1H, d, J_{AB}=9.1 Hz, OCH_2), 3.49 (1H, dd, J_{AB}=14.1 Hz, J_{AX}=5.2 Hz, CH_2N), 3.00 (1H, dd, J_{AB}=14.1 Hz, J_{BX}=7.8 Hz, CH_2N), 2.01 (3H, s, CH_3CO), 1.76–1.66 (2H, m, H_1 and H_2), 1.36–1.30 (1H, m, H_3), 1.208 (3H, s, CH_3), 1.137 (3H, s, CH_3), 1.04–0.98 (1H, m, H_3). ^{13}C NMR ($CDCl_3$) δ 172.6, 170.2 (2×CO), 76.3 ($C_{4'}$), 75.2 ($C_{2'}$), 42.0 (CH_2N), 40.2 ($C_{3'}$), 23.2 (CH_3CO), 23.0 (CH_3), 22.9 (C_2), 19.9 (CH_3), 18.7 (C_1), 14.7 (C_3).

4.15. (+)-(1S,2S)-2-(Acetamidomethyl)-1-((R)-2-(3,3-dimethylbutyro-1,4-lactonyl)oxycarbonyl)cyclopropane 15

The more polar diastereoisomer **15** was isolated as a colourless viscous oil (1.5 g, 17%), $[\alpha]_D^{25}$ +57.4 (*c* 1, $CHCl_3$). IR (neat) 3568, 3391, 3297, 3205 sh, 3088, 2968, 2932, 2878, 1788, 1738, 1657, 1548, 1466, 1453, 1413, 1370, 1314, 1295, 1264, 1203 cm^{-1}, IR (0.1 M, $CHCl_3$) 3460, 2990, 1795, 1740, 1670, 1160 cm^{-1}. HRMS (CI) calcd for $C_{13}H_{20}O_5N$: 270.1341. Found: 270.1341. *m/z* (%) $[M+29]^+$ 298 (40), $[M+1]^+$ 270 (97), 140 (100). ^1H NMR ($CDCl_3$) δ 5.82–5.63 (1H, br s, NH), 5.34 (1H, s, $H_{2'}$), 4.06 (1H, d, J_{AB}=9.1 Hz, OCH_2), 4.03 (1H, d, J_{AB}=9.1 Hz, OCH_2), 3.35–3.17 (2H, m, CH_2N), 2.007 (3H, s, CH_3CO), 1.76–1.66 (2H, m, H_1 and H_2), 1.32 (1H, ddd, *J*=8.7, 8.7, 4.7 Hz, H_3), 1.212 (3H, s, CH_3), 1.117 (3H, s, CH_3), 1.00 (1H, ddd, *J*=8.5, 6.4, 4.6 Hz, H_3). ^1H NMR ($CDCl_3$+D_2O) δ 5.34 (1H, s, $H_{2'}$), 4.06 (1H, d, J_{AB}=9.0 Hz, OCH_2), 4.03 (1H, d, J_{AB}=9.0 Hz, OCH_2), 3.29 (1H, dd, J_{AB}=14.3 Hz, J_{AX}=6.6 Hz, CH_2N), 3.27 (1H, dd, J_{AB}=14.3 Hz, J_{BX}=6.8 Hz, CH_2N), 2.003 (3H, s, CH_3CO), 1.80–1.66 (2H, m, H_1 and H_2), 1.31 (1H, ddd, *J*=8.8, 8.7, 4.7 Hz, H_3), 1.212 (3H, s, CH_3), 1.117 (3H, s, CH_3), 1.00 (1H, ddd, *J*=8.4, 6.4, 4.7 Hz, H_3). ^{13}C NMR ($CDCl_3$) δ 172.8, 172.7, 170.6 (3×CO), 76.2 ($C_{4'}$), 75.1 ($C_{2'}$), 41.9 (CH_2N), 40.2 ($C_{3'}$), 23.2 (CH_3CO), 23.1 (CH_3), 22.7 (C_2), 19.9 (CH_3), 18.7 (C_1), 14.6 (C_3).

4.16. (−)-(1R,2R)-2-(Aminomethyl)-1-carboxycyclopropane 16

The less polar (−)-diastereoisomer **14** (99 mg, 0.37 mmol) was converted to (1*R*,2*R*)-TAMP **16** (41 mg, 96%) using the same procedure for the preparation of **10**. Its TLC (*n*-butanol:acetic acid:water=3:1:1, ninhydrin), R_f 0.4 and ^{1}H NMR (D_2O) spectrum were identical to (±)-TAMP **5**. (1*R*,2*R*)-TAMP **16** was recrystallised from H_2O:EtOH as square colourless plates, m.p. 270–273°C (subl. from 250°C), $[\alpha]_D^{25}$ −81.8 (*c* 1, 1 M HCl), [lit.[10] (1*R*,2*R*)-TAMP·HCl, $[\alpha]_D^{26}$ −65.5 (*c* 0.95, 1 M HCl)]. *m/z* (%) $[M+1]^+$ 116 (75), 100 (26), 99 (64), 98 (100). Anal calcd for $C_5H_9O_2N$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.90; H, 8.07; N, 12.16.

4.17. (+)-(1S,2S)-2-(Aminomethyl)-1-carboxycyclopropane 17

The more polar (+)-diastereoisomer **15** (120 mg, 0.45 mmol) was converted to (1*S*,2*S*)-TAMP **17** (38 mg, 74%) using the same procedure for the preparation of **10**. Its TLC (*n*-butanol:acetic acid:water=3:1:1, ninhydrin), R_f 0.4 and ^{1}H NMR (D_2O) spectrum were identical to (±)-TAMP **5**. Recrystallisation from H_2O:EtOH gave (1*S*,2*S*)-TAMP **17** as square colourless plates, m.p. 264–265°C (subl. from 230°C); $[\alpha]_D^{25}$ +81.6 (*c* 1, 1 M HCl). *m/z* (%) $[M+1]^+$ 116 (19), 100 (40), 99 (52), 98 (100). Anal. calcd for $C_5H_9O_2N$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.82; H, 7.69; N, 12.20.

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