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Synthesis and resolution of 2-methyl analogues of GABA

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Abstract—*E*-4-Amino-2-methylbut-2-enoic acid, (\pm)-4-amino-2-methylbutanoic acid, (+)-(*S*)- and (-)-(*R*)-4-amino-2-methylbutanoic acid, which are analogues of the inhibitory neurotransmitter GABA (γ -aminobutyric acid, 4-aminobutanoic acid), were synthesised from ethyl 2-methyl-4-phthalimidobut-2-enoate, ethyl 2-methyl-4-phthalimidobutanoate, (+)-[(2*R*-(3,3-dimethylbutyro-1,4-lactonyl)]-(2*S*)-methyl-4-phthalimidobutanoate and (-)-[(2*R*-(3,3-dimethylbutyro-1,4-lactonyl)]-(2*R*)-methyl-4-phthalimidobutanoate and (-)-[(2*R*-(3,3-dimethylbutyro-1,4-lactonyl)]-(2*R*)-methyl-4-phthalimidobutanoate and (-)-[(2*R*-(3,3-dimethylbutyro-1,4-lactonyl)]-(2*R*)-methyl-4-phthalimidobutanoate acid was based on the X-ray crystallographic structure of the (+)-(*R*,*S*)-diastereoisomer, and direct comparison of specific rotations with the published data for (-)-(*R*)-4-amino-2-methylbutanoic acid.

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1. Introduction

4-Aminobutanoic acid (γ -aminobutyric acid, GABA) **1** (Fig. 1) is the major inhibitory neurotransmitter in the brain. GABA mediates its action via GABA receptors, ionotropic GABA_A and GABA_C receptors and G-protein coupled GABA_B receptors.¹ GABA_A and GABA_C receptors are neurotransmitter gated chloride channel receptors. GABA receptor channels open in response to the binding of GABA. The opening of chloride channels allows chloride ions to follow their electrochemical gradient and enter the neuronal cell. Influx of chloride ions is considered inhibitory as it lowers the membrane potential and restores stability to excited neuronal membranes.

The GABA recognition site at GABA_C receptors is proposed to be narrow² and a substituent on the GABA backbone especially on C2 can have a strong effect on the receptor activity. *E*-4-Amino-2-methylbut-2-enoic acid (*trans*-2-methylcrotonic acid, 2-MeTACA) **3** (Fig. 1) is a moderately potent antagonist at GABA_C receptors (K_B 31 µM) while the parent compound *E*-4-amino-



Figure 1. Structures of GABA_C receptor agonists and antagonists.

but-2-enoic acid [*trans*-aminocrotonic acid (TACA)] **2** (Fig. 1) is a potent agonist (K_D 2.4 μ M) exhibiting a maximal response of 93% of GABA-activated current, which is not significantly different from a full

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agonist response.² (\pm)-4-Amino-2-methylbutanoic acid (2-MeGABA) **4** (Fig. 1) is a weak partial agonist at GABA_C receptors (K_D 189 µM) with low intrinsic activity reaching only to 12% maximal response of GABA. Compound **4** is more potent at blocking the effect of GABA (IC₅₀ 21 µM) than activating the receptor.²

The configuration of C2 on GABA backbone may also have a strong effect on GABA_C receptor activity as suggested by our previous findings.³ We found that enantiomers of the cyclopropane analogue of GABA, 2-(aminomethyl)cyclopropanecarboxylic acid, (\pm) CAMP 5 (Fig. 1), exhibited opposite pharmacology at GABA_C receptors.³ The (+)-(1*S*,2*R*)-enantiomer, (+)-CAMP **5a** (Fig. 1), is a moderately potent full agonist at GABA_C receptors (K_D 40 µM) whereas the (-)-(1*R*,2*S*)-enantiomer, (-)-CAMP **5b** (Fig. 1), is a weak antagonist (IC₅₀ 890 µM) and the observed activity of the racemic CAMP **5** (K_D 65 µM) reflects combined activity of the enantiomers.³

Enantiomers of 2-MeGABA 4, (+)-S-4-amino-2-methylbutanoic acid [(+)-(2S)-MeGABA] 4a (Fig. 1) and (-)-(R)-4-amino-2-methylbutanoic acid [(-)-(2R)-MeGABA] 4b (Fig. 1) were required to study the effect of the configuration of C2 substituted GABA analogues on their activity at GABA_C receptors. (+)-(2S)- and (-)-(2R)-MeGABA, 4a and 4b, respectively, were resolved by chromatographic separation of diastereoisomeric esters formed between 4-phthalimido-2-methylbutanoic acid and (-)-(R)-pantolactone. The assignment of the absolute configuration of 4a and 4b was made based on the X-ray crystallographic structure of the (+)-(R,S)- diastereoisomer and direct comparison of our specific rotation data with the published data for (-)-(R)-4-amino-2-methylbutanoic acid.

2. Result and discussion

2-MeTACA **3** has been synthesised previously by ammonolysis of 4-bromo-2-methylbut-2-enoic acid⁴ and (\pm) -2-MeGABA **4** by hydrolysis of *N*-phenyl-2methyl-4-phthalimidobutanamide.⁵ (-)-(2*R*)-MeGABA has been prepared by hydrazinolysis of (-)-2-methyl-4phthalimidobutanoic acid⁵ and enantioselective syntheses via cyanomethylation of a chiral enolate⁶ and palladium catalysed allylic substitution.⁷ These published procedures are not readily amenable to the preparation of a range of 2-methyl analogues of GABA including (+)-(2*S*)- and (-)-(2*R*)-MeGABA **4a** and **4b** required for the structure relationship study at GABA_C receptors, and a new procedure was developed.

The synthetic route incorporating a resolution step is shown in Scheme 1. The procedure enabled the preparation of 2-MeTACA 3, (\pm) -2-MeGABA 4 and its enantiomers 4a and 4b. The carbon skeleton of the required analogues is provided by the readily available 2-methylbut-2-enoic acid (tiglic acid). Tiglic acid was esterified and allylically brominated then coupled with potassium phthalimide to give ethyl 4-phthalimido-2methylbut-2-enoate 6. The nitrogen atom introduced as a phthalimido group remained masked until the amino acid was deprotected. Compound 6 was hydrolysed to give 2-MeTACA 3 and catalytically hydrogenated to



Scheme 1. Reagents and conditions: (i) NBS, CCl₄, reflux; (ii) potassium phthalimide, DMF, 140 °C; (iii) 10% Pd on C, H₂, EtOH, 4 atm; (iv) 6 M HCl, AcOH, 90 °C; (v) SOCl₂, benzene and (-)-(*R*)-pantolactone, CH₂Cl₂; (vi) short column vacuum chromatography; (vii) 6 M HCl, AcOH, 140 °C.

give 7. Complete hydrolysis of 7 gave (\pm) -2-MeGABA 4 while partial hydrolysis (ester removal) gave (\pm) -4-phthalimido-2-methylbutanoic acid 8.

The resolution was based on the chromatographic separation of diastereoisomers obtained by esterification of 8 with (-)-(2R)-(3,3-dimethylbutyro-1,4-lactone [(-)-(R)-pantolactone]. (-)-(R)-Pantolactone has been introduced to resolve the acid moiety of various synthetic pyrethroid insecticides⁸ and also successfully employed to resolve the amino acids (+)- and (-)-CAMP, **5a** and **5b**, respectively.⁹ In the resolution of (\pm) -2-MeGABA 4, (-)-(R)-pantolactone was condensed with the (\pm) -4-phthalimido-2-methylbutanoic acid 8 to form a mixture of diastereoisomeric pantolactone esters 9, which was separated using short column vacuum chromatography^{10,11} to give the (R,S)-diastereoisomer 9a and the (R,R)-diastereoisomer **9b**. (-)-(R)-Pantolactone was also condensed with 4-phthalimido-2-methylbut-2enoic acid 10 obtained from partial hydrolysis of 6 to give the corresponding pantolactone ester 11. The pantolactone ester 11 was used to assist with the ¹H and ¹³C NMR assignment of the diastereoisomers **9a** and **9b**.

The ¹H NMR singlet resonances of the pantolactone moiety enabled the diastereoisomer **9a** and **9b** to be clearly distinguished. The geminal dimethyl singlets (**9a**: δ 1.17, 1.27 and **9b**: δ 1.15, 1.23) and the methine singlet which corresponds to the resonance of the proton attached to the stereogenic carbon (C2'H) (**9a**: δ 5.40 and **9b**: δ 5.38) of the pantolactone moiety were clearly distinguishable. In addition, the methyl doublet which corresponds to the resonances of the methyl group attached to the stereogenic carbon of the GABA backbone (2-CH₃) [**9a**: δ 1.33 (J = 7.2 Hz) and **9b**: δ 1.35 (J = 7.0 Hz)] was also readily distinguishable. The high intensity of these resonances was an excellent measure of



Figure 2. A general view of the X-ray crystallographic structure of (+)-[(2R)-(3,3-dimethylbutyro-1,4-lactonyl)]-(2S)-methyl-4-phthalimidobutanoate**9a**. The ellipsoids are drawn at the 50% probability level.¹²

diastereoisomeric purity and contamination above 0.3% by the other diastereoisomer was detectable. ¹³C NMR resonances of the stereogenic carbons (C2 and C2') also gave good indication of diastereoisomeric purity [9a: δ 17.3 and 37.0, 9b: δ 17.1 and 37.3].

X-ray crystallography showed that the diastereoisomer **9a** had the molecular composition of $C_{19}H_{21}O_6N$ with the absolute configuration at C2 established as S relative to the R configuration of the pantolactone moiety (Fig. 2), thus establishing the (2'R,2S) configuration for **9a** and (2'R,2R) for **9b**. The pure diastereoisomer **9a** was cleaved to give (+)-(2S)-MeGABA **4a** $[\alpha]_D^{25} = +2.5$ and diastereoisomer **9b** (-)-(2R)-MeGABA **4b** $[\alpha]_D^{25} = -2.7$.

3. Conclusion

The method developed has enabled a number of 2-methyl analogues of GABA to be synthesised. Compound 6, the immediate precursor of 2-MeTACA 3, served as a common synthetic intermediate for all compounds synthesised in this study. Compound 7, the saturated analogue of 6, was the immediate precursor of (\pm) -2-MeGABA 4 and the racemic 4-phthalimido-2-methylbutanoic acid 8, which formed the diastereoisomers 9 on esterification with (-)-(R)-pantolactone. Conversion of the pure diastereoisomer 9a and 9b afforded (+)-(2S)-MeGABA 4a and (-)-(2R)-MeGABA 4b. The absolute configuration of 4a was inferred from the X-ray crystallographic structure of its precursor 9a, while the absolute configuration of 4b was established by comparison of the sign of the specific rotation with published data.

4. Experimental

4.1. General procedures

Melting points were determined on a Gallenkamp melting point apparatus and are reported uncorrected. Elemental analyses were carried out by the Microanalysis Unit, Department of Chemical Engineering, the University of Sydney and the Microanalytical Unit, the Australian National University. High resolution mass measurements were carried out on a Bruker BioApex-II 7T FTICR mass spectrometer equipped with both an on- and off-axis Analytical ESI source at the University of New South Wales. Low resolution ESI mass spectra were carried out on a Thermo Finigan TSQ 7000 LCMS San Jose CA instrument (APCI, flow injection, positive ions, vaporised temperature 475 °C, capillary temperature 190 °C and a source voltage 5 kV). Low resolution chemical ionisation mass spectral determinations using CH₄ as the reagent gas were carried out on a Thermo Finnigan Polaris Q GCMS Austin TX instrument. Optical rotations were recorded on a JASCO DIP-1000 digital polarimeter.

1D ¹H (300 Mz), ¹³C (75 MHz) and 2D NMR experiments were performed at 20 °C using a 300 Mz Varian-Gemini 300 spectrometer. For ¹H NMR, chemical shift

values are given in ppm relative to internal TMS for spectra measured in CDCl₃ and HOD (4.75 ppm) for spectra measured in D₂O, respectively. For ¹³C NMR, chemical shift values are given in ppm relative to CDCl₃ (77.0 ppm) for compounds measured in CDCl₃ and relative to internal dioxan (67.4 ppm) for compounds measured in D₂O. Standard pulse sequences were used for COSY, DEPT and HETCOR experiments.

Solvents were dried and distilled using a standard procedure. Short column vacuum chromatography was carried out following the method described^{10,11} using silica gel H60 packed under reduced pressure and monitored by thin-layer chromatography (TLC) using aluminium plates precoated with silica gel 60F254 (Merck).

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 229633. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44-01223-336033 or email: deposit@ccdc. cam.ac.uk].

4.2. Ethyl 2-methyl-4-phthalimidobut-2-enoate 6

Compound **6** was prepared by the coupling reaction between potassium phthalimide and ethyl E-4-bromo-2-methylbut-2-enoate, the brominated product of ethyl E-2-methylbut-2-enoate.

4.2.1. Ethyl *E***-2-methylbut-2-enoate.** To a solution of tiglic acid (50 g, 0.5 mol) in dry benzene (200 mL) and anhydrous EtOH (200 mL) was added *p*-toluenesulfonic acid (190 mg, 1 mmol) and the mixture was refluxed with stirring under Dean Stark condition until the entrainment of H₂O ceased. Removal of solvents by distillation gave crude title compound (60 g, 94%). ¹H NMR (CDCl₃) δ 1.19 (3H, t, J = 7.2 Hz, OCH₂CH₃), 1.69 (3H, d, J = 6.9 Hz, CH₃CH=C), 1.73 (3H, unresolved q, 2-CH₃), 4.08 (2H, q, J = 7.2 Hz, OCH₂CH₃), 6.75 (1H, qq, J = 7.2, 1.5 Hz, CH₃CH=C). ¹³C NMR (CDCl₃) δ 11.9 (2-CH₃), 14.16 (OCH₂CH₃), 14.22 (C4), 60.3 (OCH₂CH₃), 128.3 (C2), 136.7 (C3), 168.1 (CO).

4.2.2. Ethyl *E*-4-bromo-2-methylbut-2-enoate. To a solution of ethyl *E*-2-methylbut-2-enoate (60 g, 0.47 mol) in CCl₄ (250 mL) was added *N*-bromosuccinimde (90 g, 0.5 mol) and dibenzoylperoxide (50 mg). The mixture was refluxed under stirring for 18 h then filtered. Removal of CCl₄ by distillation gave a residue (80.6 g, 82.6%) containing *E*-4-bromo-2-methylbut-2-enoate and ethyl *E*-2-(bromomethyl)but-2-enoate as the major products (~4:3). Ethyl *E*-4-bromo-2-methylbut-2-enoate: ¹H NMR (CDCl₃) δ 1.30 (3H, t, J = 7.1 Hz, OCH₂CH₃), 1.917 (3H, d, J = 2.5 Hz, 2-CH₃), 4.03 (2H, br d, J = 8.5 Hz, BrCH₂CH=C), 4.21 (2H, q, J = 7.1 Hz, OCH₂CH₃), 6.92 (1H, tq, J = 8.5, 1.5 Hz, BrCH₂CH=C). Ethyl *E*-2-(bromomethyl)but-2-

enoate: δ 1.31 (3H, t, J = 7.1 Hz, OCH₂CH₃), 1.918 (3H, d, J = 7.5 Hz, H_3 CCH=C), 4.21 (2H, q, J = 7.1 Hz, OCH₂CH₃), 4.24 (2H, d, J = 1.2 Hz, HC=CCH₂Br), 7.07 (1H, q, J = 7.1 Hz, CH₃CH=CCH₂Br).

The mixture of ethyl E-2-bromomethylbut-2-enoate and ethyl E-4-bromo-2-methylbut-2-enoate (\sim 4:3) from above (40 g, 0.25 mol) was added dropwise to a stirring mixture of potassium phthalimide (46g, 0.25 mol) in DMF (200 mL). The resulting mixture was heated under stirring at 140 °C for 18 h, cooled and filtered. The filtrate was evaporated under reduced pressure to give a brown viscous oil which was taken up in ethyl acetate (1 L), washed with water $(5 \times 400 \text{ mL})$, dried (Na₂SO₄) and evaporated under reduced pressure to give a dark semi solid residue (60.3 g). The residue was purified by short vacuum column chromatography using ethyl acetate/light petroleum (1:2) to give the required product 6, which crystallised from ethyl acetate and light petroleum as needles. Recrystallisation from cyclohexane afforded **6** as white needles mp 86–87 °C (subl.), (14 g, 35%). ¹H NMR (CDCl₃) δ 1.26 (3H, t, J = 7.1 Hz, OCH₂CH₃), 2.02 (3H, m, 2-CH₃), 4.17 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.44 (2H, dq, J = 6.8, 1.0 Hz, PhthNCH₂ CH=), 6.46 (1H, tq, J = 6.8, 1.5 Hz, PhthNCH₂CH=), 7.71-7.77 (2H, m, PhthN: H4, H5), 7.84-7.90 (2H, m, PhthN: H3, H6). ¹³C NMR (CDCl₃) δ 12.5 (2-CH₃), 14.1 (OCH₂CH₃), 35.6 (C4), 60.7 (OCH₂CH₃), 123.2 (PhthN: C4, C5), 131.2 (C2), 131.9 (PhthN: C2A, C7A) 134.0 (C3), 134.04 (PhthN: C3, C6), 167.2 and 167.6 (CO, PhthN: 2× CO). MS (CI): m/z (%) 228 (100), 200 (41). MS (APCI): m/z (%) 306 (5), 274 (M+H, 9), 260 (11), 228 (100), 200 (23), 163 (24). HRMS calcd for $C_{15}H_{15}NO_4Na$ (M+Na): 296.0892. Found (M+Na): 296.0894. Anal. Calcd for C₁₅H₁₅NO₄: C, 65.93; H, 5.53; N, 5.13. Found: C, 65.54; H, 5.58; N, 5.10%.

4.3. 2-Methyl-4-phthalimidobut-2-enoic acid 10

To a solution of ester 6 (1.6 g, 5.9 mmol) in acetic acid (10 mL) was added 6 M HCl (2 mL) and the mixture was heated (90 °C) under stirring for 2 days. The phthalimido unsaturated acid 10 (300 mg) crystallised on cooling. The filtrate was evaporated under reduced pressure to give an off white residue which crystallised from acetic acid. Recrystallisation from acetonitrile afforded 10 as large white needles, mp 203-204 °C (subl.), (1.15 g, 80%). ¹H NMR (CDCl₃) δ 2.02 (3H, d, $J = 1.2 \text{ Hz}, 2\text{-CH}_3$, 3.65–3.85 (1H, br s, CO₂H), 4.46 (2H, dd, J = 6.8, 0.9 Hz, PhthNCH₂CH=), 6.70 (1H, tq, $J = 6.8, 0.9 \text{ Hz}, \text{PhthNCH}_2\text{C}H = 0, 7.73 - 7.79 (2H, m)$ PhthN: H4, H5), 7.85–7.91 (2H, m, PhthN: H3, H6). ¹³C NMR (CDCl₃) δ 12.4 (2-CH₃), 35.8 (C4), 123.4 (PhthN: C4, C5), 130.3 (C2), 132.2 (PhthN: C2A, C7A), 134.1 (PhthN: C3, C6), 136.6 (C3), 167.7 (PhthN: 2× CO), 170.5 (CO). MS (CI): m/z (%) 228 (100), 200 (38). MS (APCI): *m*/*z* (%) 228 (74), 200 (100), 163 (62). HRMS calcd for $C_{13}H_{11}NO_4Na$ (M+Na): 268.0579. Found (M+Na): 268.0581. Anal. Calcd for C₁₃H₁₁NO₄: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.59; H, 4.50; N, 5.67%.

4.4. Ethyl 2-methyl-4-phthalimidobutanoate 7

To a solution of 6 (3.9 g, 0.014 mol) in EtOH (150 mL) was added 10% Pd on C (45 mg) and the mixture was hydrogenated at 4 atm for 20 h. The mixture was filtered and the filtrate evaporated under reduced pressure to give 7 as a colourless oil (3.84 g, 98%). ¹H NMR (CDCl₃) δ 1.24 (3H, d, J = 7.0 Hz, 2-CH₃), 1.26 (3H, t, $J = 7.1 \text{ Hz}, \text{ OCH}_2\text{C}H_3$, 1.78 (1H, ddd, J = 13.6, 7.0,6.6 Hz, PhthNCH₂CH₂CH), 2.10 (1H, ddd, J = 13.7, 7.7, 7.2 Hz, PhthNCH₂CH₂CH), 2.47 (1H, tq, J = 7.1, 7.0 Hz, PhthNCH₂CH₂CH), 3.75 (2H, t, J = 7.1 Hz, PhthNC H_2 CH $_2$ CH), 4.11 (2H, q, J = 7.1 Hz, OCH2CH3), 7.69-7.75 (2H, m, PhthN: H4, H5), 7.82-7.89 (2H, m, PhthN: H3, H6). ¹³C NMR (CDCl₃) δ 14.1 (OCH₂CH₃), 17.1 (2-CH₃), 31.9 (C3), 35.9 (C4), 37.2 (C2), 60.4 (OCH₂CH₃), 123.2 (PhthN: C4, C5), 132.1 (PhthN: C2A, C7A), 133.9 (PhthN: C3, C6), 168.2 (PhthN: 2× CO), 175.7 (CO). MS (CI): m/z (%) 230 (100), 202 (38), 160 (36). MS (APCI): m/z (%) 276 (M+H, 100), 262 (14), 230 (5). HRMS calcd for C₁₅H₁₇NO₄Na (M+Na): 298.1052. Found (M+Na): 298.1050. Anal. Calcd for C₁₅H₁₇NO₄: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.16; H, 6.13; N, 4.94%.

4.5. 2-Methyl-4-phthalimidobutanoic acid 8

HCl (6 M, 60 mL) was added to a well-stirred solution of 7 (5.24 g, 19 mmol) in glacial acetic acid (100 mL). The mixture was heated (90 °C) under stirring for 3 days and evaporated under reduced pressure to give a colourless oil. The oil was crystallised from light petroleum/ CH₂Cl₂ and recrystallised from light petroleum/ethyl acetate to give the saturated acid 8 as fine white needles (4.6 g, 98%), mp 111-112 °C (subl.), lit.^{5,13} mp 114-115 °C, 112–113 °C. ¹H NMR (CDCl₃) δ 1.27 (3H, d, $J = 7.0 \text{ Hz}, 2\text{-CH}_3$, 1.78 (1H, ddd, J = 13.9, 7.0, 6.8 Hz, $1 \times PhthNCH_2CH_2CH)$, 2.13 (1H, ddd, J = 13.9, 7.0, 6.8 Hz, $1 \times PhthNCH_2CH_2CH$), 2.50 (1H, tq, J = 7.1, 7.0 Hz, PhthNCH₂CH₂CH), 3.78 (2H, dt, J = 7.0, 1.4 Hz, PhthNCH₂CH₂CH), 7.69–7.75 (2H, m, PhthN: H4, H5), 7.82–7.88 (2H, m, PhthN: H3, H6). ¹³C NMR $(CDCl_3) \delta 16.9 (2-CH_3), 31.9 (C3), 35.9 (C4), 37.0 (C2),$ 123.3 (PhthN: C4 and C5), 132.2 (PhthN: C2A, C7A), 133.9 (PhthN: C3, C6), 168.3 (PhthN: $2 \times CO$), 181.1 (CO). MS (CI): m/z (%) 230 (100), 202 (60), 160 (68). MS (APCI): m/z (%) 262 (100), 248 (M+H, 36), 230 (12). Anal. Calcd for C₁₃H₁₃NO₄: C, 63.15; H, 5.30; N, 5.66. Found: C, 63.29; H, 5.30; N, 5.62%.

4.6. [(2*R*)-(3,3-Dimethylbutyro-1,4-lactonyl)]-2-methyl-4-phthalimidobut-2-enoate 11

To a suspension of the unsaturated acid **10** (735 mg, 3 mmol) in anhydrous benzene (20 mL) was added SOCl₂ (1 mL, 14 mmol). The mixture was refluxed under stirring until the evolution of HCl ceased (\sim 3 h). The volatile materials were removed by distillation leaving a pale brown residue, which crystallised as light brown needles. The residue was dissolved in anhydrous CH₂Cl₂ (10 mL) then added dropwise to a solution of (-)-(*R*)-

pantolactone (390 mg, 3 mmol) in anhydrous CH_2Cl_2 (10 mL) and anhydrous pyridine (2 mL) under stirring. After stirring at room temperature for 18 h, the mixture was evaporated under reduced pressure to give a pale brown oil. The oil was purified by short column vacuum chromatography and eluted with CH₂Cl₂ to give a pale yellow oil, which crystallised from CH₂Cl₂/ether to give large colourless cubic crystals (1 g, 93%), mp 107–108 °C (subl.). ¹H NMR (CDCl₃) δ 1.12, 1.21 (2× 3H, 2× s, $C(CH_3)_2$, 2.10 (3H, q, J = 1.0 Hz, 2-CH₃), 4.05, 4.07 $(2H, J_{A,B} = 9.0 \text{ Hz}, C4'H_2O), 4.41-4.55 (2H, m,$ PhthNCH₂CH=), 5.43 (1H, s, C2'H), 6.79 (1H, tq, J = 6.7, 1.5 Hz, PhthNCH₂CH=), 7.72–7.78 (2H, m, PhthN: H4, H5), 7.85–7.91 (2H, m, PhthN: H3, H6). ¹³C NMR (CDCl₃) & 12.7 (2-CH₃), 20.0, 23.1 (C(CH₃)₂), 35.7 (C4), 40.3 (C3'(CH₃)₂), 75.4 (C4'), 76.2 (C2'), 123.4 (PhthN: C4, C5), 130.1 (C2), 132.1 (PhthN: C2A, C7A) 134.1 (PhthN: C3, C6), 136.4 (C3), 165.9 (CO), 167.6 (PhthN: 2× CO), 172.1 (C'O). MS (CI): m/z (%) 386 (M+29, 6), 358 (M+H, 2), 228 (100), 202 (30). MS (APCI): *m*/*z* (%) 375 (53), 358 (M+H, 53), 228 (100), 200 (35), 163 (23). HRMS calcd for C₁₉H₁₉NO₆Na (M+Na): 380.1106. Found (M+Na): 380.1105. Anal. Calcd for C₁₃H₁₁NO₄: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.93; H, 5.35; N, 3.90%.

4.7. [(2*R*)-(3,3-Dimethylbutyro-1,4-lactonyl)]-2-methyl-4-phthalimidobutanoate 9

To a solution of the saturated acid 8 (1.6 g, 6.5 mmol) in anhydrous benzene (40 mL) was added SOCl₂ (2 mL, 26 mmol). After standing at room temperature for 18 h, the solvent was removed from the mixture by distillation leaving a brown liquid residue. The residue was added to a solution of (-)-(R)-pantolactone (1.2 g, 9.4 mmol) in CH_2Cl_2 (50 mL) and anhydrous pyridine (10 mL). The mixture was left standing at room temperature for 18 h then evaporated under reduced pressure to give a light brown semi-solid. Purification by short column vacuum chromatography (CH_2Cl_2) afforded a mixture of the required pantolactonyl diastereoisomeric esters, the (R,R)-diastereoisomer and the (R,S)-diastereoisomer (2.26 g, 85%). ¹H NMR (CDCl₃) δ 1.15, 1.17, 1.23, 1.27 $(4 \times 3H, 4 \times s, 2 \times C(CH_3)_2), 1.33 (3H, d, J = 7.2 Hz, 2-$ CH₃), 1.69 (3H, d, J = 7.0 Hz, 2-CH₃), 1.78–1.90 (2H, m, PhthNCH₂CH₂CH), 2.10–2.22 (2H, m, PhthNCH₂ CH₂CH), 2.58–2.71 (2H, m, PhthNCH₂CH₂CH), 3.76– 3.81 (4H, m, 2× PhthNCH₂CH₂CH), 4.02–4.10 (4H, m, 2× C4'H₂O), 5.388 (1H, s, C2'H), 5.404 (1H, s, C2'H), 7.69–7.75 (4H, m, 2× PhthN: H4, H5), 7.82–7.89 (4H, m, $2 \times$ PhthN: H3, H6). The diastereoisomers were separated by short column vacuum chromatography using silica gel H60 (column i.d. 4.5×8.5 cm) and eluted with light petroleum/ethyl acetate (7:3) in 50 mL fractions. Fractions were analysed by TLC using two solvent systems, light petroleum/ethyl acetate (2:1) and CHCl₃. 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 . Fractions enriched with the less polar diastereoisomer were combined and evaporated under reduced pressure to give a white solid residue. Crystallisation from cyclohexane/ethyl acetate

afforded (+)-(R,S)-diastereoisomer. The more polar (-)-(R,R)-diastereoisomer, isolated initially as a viscous oil, crystallised from the pure fraction after several months standing. Complete separation of the whole mixture of diastereoisomers required repeat column separations and crystallisations.

4.8. (+)-[(2*R*)-(3,3-Dimethylbutyro-1,4-lactonyl)]-(2*S*)methyl-4-phthalimidobutanoate 9a

Recrystallisation of the less polar (+)-(R,S)-diastereoisomer from ethyl acetate/cyclohexane gave white crystals mp 109–110 °C (subl.), $[\alpha]_D^{25} = +13.6$ (c 1, CHCl₃). ¹H NMR (CDCl₃) δ 1.17, 1.27 (2× 3H, 2× s, C(CH₃)₂), 1.33 (3H, d, J = 7.2 Hz, 2-CH₃), 1.83 (1H, ddd, $J = 13.3, 7.3, 6.0 \text{ Hz}, \text{PhthNCH}_2\text{CH}_2\text{CH}), 2.10-2.22$ (1H, m, PhthNCH₂CH₂CH), 2.58–2.70 (1H, m, PhthNCH₂CH₂CH), 3.78 (2H, td, J = 7.0, 1.1 Hz, PhthNC H_2 CH $_2$ CH), 4.04, 4.07 (2H, $J_{A,B} = 9.0$ Hz, C4'H₂O), 5.40 (1H, s, C2'H), 7.70–7.76 (2H, m, PhthN: H4, H5), 7.82–7.88 (2H, m, PhthN: H3, H6).¹³C NMR (CDCl₃) δ 17.3 (2-CH₃), 19.9, 23.0 (C3'(CH₃)₂), 32.0 (C3), 35.7 (C4), 37.0 (C2), 40.2 $(C3'(CH_3)_2)$, 75.0 (C4'H₂O), 76.3 (C2'H), 123.3 (PhthN: C4, C5), 132.2 (PhthN: C2A, C7A) 133.9 (PhthN: C3, C6), 168.2 (PhthN: 2× CO), 172.1 (C'O), 174.6 (CO). MS (CI): *m*/*z* (%) 388 (M+29, 8), 230 (100), 202 (39), 160 (43). MS (APCI): *m*/*z* (%) 377 (10), 360 (M+H, 100), 262 (40). Anal. Calcd for C₁₉H₂₁NO₆: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.54; H, 5.93; N, 3.87%.

4.9. (-)-[(2*R*)-(3,3-Dimethylbutyro-1,4-lactonyl)]-4-phthalimido-(2*R*)-methylbutanoate 9b

Recrystallisation of the less polar (-)-(R,R)-diastereoisomer from cyclohexane gave white crystals mp 60-62 °C (subl.), $[\alpha]_D^{25} = -3.3$ (c 1, CHCl₃). ¹H NMR $(CDCl_3) \delta 1.15, 1.23 (2 \times 3H, 2 \times s, C(CH_3)_2), 1.35 (3H, d, d)$ $J = 7.0 \text{ Hz}, 2\text{-CH}_3$, 1.83 (1H, ddd, J = 13.3, 7.3, 6.0 Hz, PhthNCH₂CH₂CH), 2.10–2.22 (1H, m, PhthNCH₂CH₂ CH), 2.58–2.70 (1H, m, PhthNCH₂CH₂CH), 3.78 (2H, td, J = 7.0, 1.1 Hz, CH₂N), 4.04, 4.07 (2H, $J_{A,B} = 9.0$ Hz, C4'H₂O), 5.38 (1H, s, C2'H), 7.70-7.76 (2H, m, PhthN: H4, H5), 7.82-7.88 (2H, m, PhthN: H3, H6). ¹³C NMR $(CDCl_3) \delta 17.1 (2-CH_3), 19.9, 23.1 (C3'(CH_3)_2), 32.0$ (C3), 35.8 (C4), 37.3 (C2), 40.2 (C3'(CH₃)₂), 75.1 (C4'H₂O), 75.8 (C2'H), 123.3 (PhthN: C4, C5), 132.2 (PhthN: C2A, C7A) 133.9 (PhthN: C3, C6), 168.2 (PhthN: $2 \times$ CO), 172.0 (C'O), 174.4 (CO). MS (CI): m/z(%) 388 (M+29, 7), 360 (M+H, 3), 230 (100), 202 (36), 160 (35). MS (APCI): m/z (%) 377 (7), 360 (M+H, 100), 262 (22). Anal. Calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.62; H, 6.00; N, 3.80%.

4.10. 4-Amino-2-methylbut-2-enoic acid 3

To a solution of ethyl 4-phthalimido-2-methylbut-2enoate (1.6g, 6.5 mmol) in glacial acetic acid (50 mL) was added 6 M HCl (100 mL). The resulting mixture was heated at 140 °C under stirring for 18 h. Evaporation under reduced pressure gave a brown solid. Purification (ion exchange chromatography, Dowex 50, 1 M pyridine) gave the free amino acid 2-MeTACA **3** as a white powder (650 mg, 86%), mp 195–196 °C, Lit.⁴ mp 194–196 °C. Its TLC (*n*-butanol/acetic acid/water (3:1:1), $R_{\rm f}$ 0.4, ninhydrin) was identical to that of an authentic sample. ¹H NMR (D₂O) δ 1.74 (3H, dt, J = 1.5, 1.0 Hz, CH₃), 3.65 (2H, dq, J = 7.0, 1.0 Hz, CH₂NH₂), 6.11 (1H, tq, J = 7.0, 1.5 Hz, CH₂CH=CCH₃) consistent with the published data.⁴ ¹³C NMR (D₂O) δ 14.2 (2-CH₃), 38.2 (C4), 125.3 (C3), 141.1 (C2), 177.2 (CO). MS (APCI): m/z (%) 116 (M+H, 100), 99 (100).

4.11. (±)-4-Amino-2-methylbutanoic acid 4

The saturated ester 7 (2.75 g, 10 mmol) was converted to (±)-2MeGABA 4 (753 mg, 65%) using the method described for the preparation of **3**. The racemic amino acid was obtained as a white powder, which was recrystallised from EtOH, mp 183–184 °C, lit.³ mp 183– 185 °C. TLC (*n*-butanol/acetic acid/water (3:1:1), $R_{\rm f}$ 0.4, ninhydrin). ¹H NMR (D₂O, HOD δ 4.75 ppm) δ 1.04 $(3H, dt, J = 7.1 \text{ Hz}, CHCH_3), 1.58-1.85$ (2H, m, m)CH₂CH₂NH₂), 2.23–2.35 (1H, m appearing as a sextet, CHCH₃), 2.83–2.97 (2H, m, CH₂CH₂NH₂). ¹H NMR (D₂O, dioxan δ 3.75 ppm) δ 1.11 (3H, dt, J = 7.1 Hz, CHCH₃), 1.65–1.92 (2H, m, CH₂CH₂NH₂), 2.30–2.42 (1H, m appearing as a sextet, CHCH₃), 2.90-3.05 (2H, m, $CH_2CH_2NH_2$) consistent with the published data.⁶ ¹³C NMR (D_2O) δ 18.2 (2-CH₃), 32.2 (C3), 38.9 (C2), 41.1 (C4), 185.2 (CO₂H). MS (CI): *m*/*z* (%) 100 (100). MS (APCI): m/z (%) 118 (M+H, 5), 100 (100).

4.12. (+)-4-Amino-2S-methylbutanoic acid 4a

The less polar (+)-(*R*,*S*)-diastereoisomer (400 mg, 1.1 mmol) was converted to (2*S*)-MeGABA **4a** (103 mg, 77%) using the procedure described for the preparation of **4**. Recrystallisation of the crude product from EtOH gave white crystals, mp 189–190 °C, $[\alpha]_D^{25} = +2.5$ (*c* 1, H₂O). TLC, ¹H NMR and mass spectra of **4a** were identical to those of **4**. Anal. Calcd for C₅H₁₁NO₂: C, 51.26; H, 9.46; N, 11.96. Found: C, 49.96; H, 9.36; N, 11.49%.

4.13. (-)-4-Amino-2*R*-methylbutanoic acid 4b

The more polar (-)-(*R*,*R*)-diastereoisomer (240 mg, 0.65 mmol) was converted to (2*R*)-MeGABA **4b** (73 mg, 78%) using the same procedure for the preparation of **4**. Recrystallisation of the crude product from EtOH gave white crystals, mp 191–192 °C, lit.^{5–7} mp 196–197, 187–190, 192–193 °C. $[\alpha]_D^{25} = -2.7$ (*c* 1, H₂O). Lit.^{5,7} $[\alpha]_D^{24} = -6.7$ (*c* 2.8, H₂O), $[\alpha]_D^{20} = +3.5$ (*c* 0.4, H₂O). TLC, ¹H NMR and mass spectra of **4b** were identical to those of **4**. Anal. Calcd for C₅H₁₁NO₂: C, 51.26; H, 9.46; N, 11.96. Found: C, 51.42; H, 9.70; N, 11.89%.

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References and notes

- 1. Chebib, M.; Johnston, G. A. R. J. Med. Chem. 2000, 43, 1427–1447.
- 2. Chebib, M.; Vandenberg, R. J.; Johnston, G. A. R. Br. J. Pharmacol. 1997, 122, 1551–1560.
- Duke, R. K.; Chebib, M.; Balcar, V. J.; Allan, R. D.; Mewett, K. N.; Johnston, G. A. R. J. Neurochem. 2000, 75, 2602–2609.

- Allan, R. D.; Twitchin, B. Aust. J. Chem. 1978, 31, 2283– 2289.
- Adams, R.; Files, D. J. Am. Chem. Soc. 1959, 81, 4946– 4951.
- Azam, S.; D'Souza, A. A.; Wyatt, P. B. J. Chem. Soc., Perkin Trans. 1 1996, 621–627.
- 7. Martin, C. J.; Rawson, D. J.; Williams, M. J. Tetrahedron: Asymmetry 1998, 3723–3730.
- Duke, C. C.; Wells, R. J. Aust. J. Chem. 1987, 40, 1641– 1654.
- Duke, R. K.; Allan, R. D.; Chebib, M.; Greenwood, J. R.; Johnston, G. A. R. *Tetrahedron: Asymmetry* 1998, 2533– 2548.
- 10. Ravi, B. N.; Wells, R. J. Aust. J. Chem. 1982, 35, 129-144.
- Grech, J. N.; Qian, L.; Roufogalis, B. D. R.; Duke, C. C. J. Nat. Prod. 1994, 57, 1682–1687.
- 12. Farrugia, L. J. J. Appl. Cryst. 1997, 30, 565-566.
- 13. Robinson, R.; Suginome, H. J. Chem. Soc. 1932, 304-317.