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The synthesis of 4-arylsulfanyl-substituted kainoid analogues from *trans*-4-hydroxy-L-proline

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Abstract—The potent neuroexcitatory activity of kainoid amino acids in the mammalian CNS places new analogues in high demand as tools for neuropharmacological research. A range of 4-arylsulfanyl-substituted kainoids has been synthesised in a parallel fashion via mesylate displacement by a number of aromatic thiolates upon (2*S*,3*S*,4*R*)-1-benzoyl-3-tert-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9**, which is obtainable in eight steps from *trans*-4-hydroxy-L-proline **5**.

1. Introduction

The kainoids are a class of non-proteinogenic amino acids of general structure **1**, which have been isolated from a number of marine and fungal sources (Fig. 1).[1] In addition to their insecticidal[2] and anthelmintic properties,[3] they have been shown to display powerful neuroexcitatory activity in the mammalian central nervous system, where they act as conformationally restricted analogues of L-glutamic acid upon the kainate sub-class of ionotropic glutamate receptors.[4] New kainoid compounds which exhibit selective agonistic or antagonistic behaviour on such receptors are highly sought after as tools for pharmacological research into the mechanisms of neurogenerative disorders such as epilepsy,[5] Huntington's chorea[6] and senile dementia.[7] Structure–activity studies[8] performed on the parent compound kainic acid **2**, first isolated in 1953 from the Japanese marine red alga *Digenea simplex*,[9] and also acromelic acid A **3**, another naturally occurring kainoid isolated from the Japanese mushroom *Clitocybe acromelalga*,[10] have established the main criteria for bioactivity. The most potent analogue disclosed thus far is the *o*-anisyl compound **4**,[11] and since this observation was made, several other syntheses of phenylkainoids have been published.[12] Recent efforts within this laboratory have focussed on developing methodology for the synthesis of further analogues of arylkainoids, in a manner that may be applicable to parallel synthesis.[13] We recently reported[14] the synthesis of a novel class of 4-arylsulfanyl substituted kainoids, based on the cheap and readily available *trans*-4-hydroxy-L-proline **5**, a by-product of collagen hydrolysis.[15] We now discuss our findings in detail.

2. Results and discussions

Previous work has shown that the ketone **6** is obtainable in multigram quantities, 36% overall yield, from *trans*-4-hydroxy-L-proline **5** in a six step sequence (Scheme 1).[16] Treatment of the ketone **6** with sodium borohydride in methanol afforded the alcohol **7** as a single diastereomer. It was envisaged that hydride attack occurs from the side of the carbonyl unhindered by the 2-C ester substituent, giving a product with the stereostructure **7**. Unfortunately, NOED

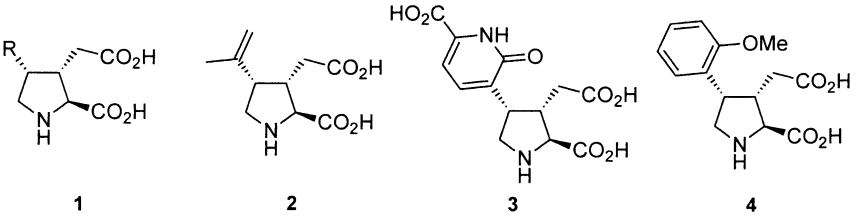

Figure 1.

Keywords: kainoids; pyrrolidines; substitution; thiols.
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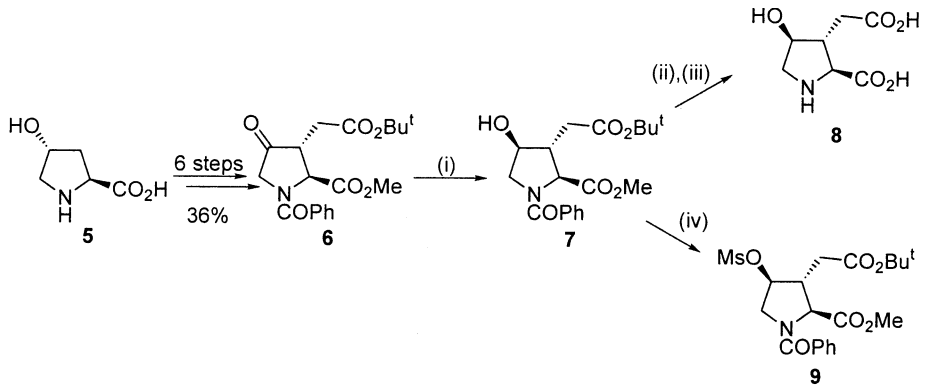


Scheme 1. (i) $NaBH_4$, MeOH; (ii) 6 M $HCl_{(aq)}$; Δ; (iii) Dowex® 50WX8-100 ion exchange resin; (iv) MsCl, Et_3N, CH_2Cl_2, 91% (over 2 steps).

spectroscopic experiments on this product were unsuccessful, but treatment of the alcohol **7** with 6 M aqueous hydrochloric acid afforded a single product **8** in quantitative yield after ion-exchange chromatography (Dowex® 50WX8-100).

NOED spectroscopic experiments on the hydrochloride salt of compound **8** established that the stereochemistry was as indicated. A particularly diagnostic finding in the data was that irradiation of the signal pertaining to 3-H enhanced the signals of 4- and 2-H by 3.2 and 2.7%, respectively (Fig. 2). From these observations, it was deduced that 3- and 4-H and 3- and 2-H have a similar distance between them, and therefore bear a *trans*-disposition to one another.

The alcohol **7** was reacted with methanesulfonyl chloride and triethylamine in dichloromethane, which afforded the mesylate **9** in 91% yield over two steps. It was envisaged that the mesylate **9** could be made to undergo S_N2 displacement by a range of appropriate nucleophiles, thus setting up the desired kainoid stereochemistry around the pyrrolidine ring and providing access to a range of compounds, from a common intermediate.

Young and his co-workers[17] had unsuccessfully attempted displacement of a mesylate by Grignard reagents and organocuprates on a similar pyrrolidine system; the present authors observed similar unreactivity when the mesylate **9** was reacted with a range of carbon nucleophiles. This was presumably due to a combination of steric hindrance from the 2-C ester substituent, and the fact that the mesylate is secondary.

The use of more powerful nucleophiles was explored. It was proposed that a sulfur anion would be more likely to displace the hindered mesylate. Initial attempts at the displacement of the mesylate of compound **9** with 2-mercapto-1-methylimidazole in the presence of either triethylamine or sodium hydride, at ambient or elevated temperatures, were unsuccessful. It was found, however, that the mesylate group of compound **9** could be displaced by sulfur anions, affording a range of 4-arylsulfanyl-substituted kainoid analogues in protected form. For example, treatment of the mesylate **9** with the sodium salt of 2-mercapto-1-methylimidazole in dimethyl sulfoxide at 90°C afforded compound **10a** in 55% yield (Scheme 2). ^{1}H NMR studies on the reaction showed that only one new compound was being formed, however yields are lower than expected due to difficulties encountered during the work-up of the reaction. The process was shown to be applicable to a wider range of heterocyclic thiols, as shown in Table 1.

The stereochemistry of compound **10e** was confirmed by NOED spectroscopic experiments, the key data from which is presented in Fig. 3. Irradiation of the signal pertaining to

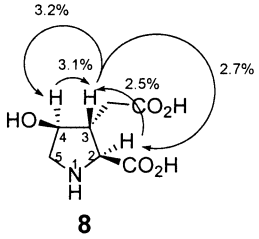
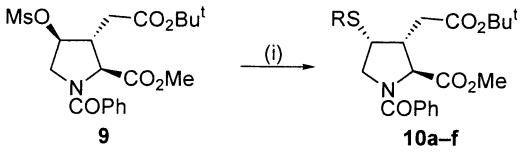
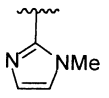
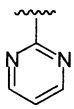
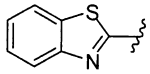
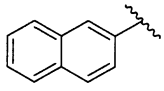


Figure 2.



Scheme 2. (i) RS^-Na^+ (5 mol equiv.), Me_2SO, 90°C.

Table 1. Reaction of the mesylate **9** with thiolates

Entry	R	Product	Yield (%)
1		**10a**	55
2	Ph	**10b**	53
3	*o*-$MeOC_6H_4$	**10c**	53
4		**10d**	47
5		**10e**	53
6		**10f**	25

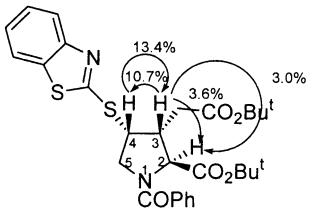
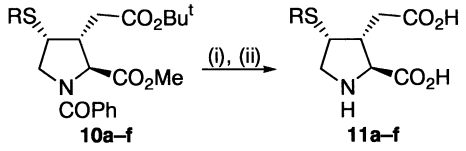


Figure 3.



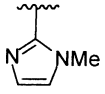
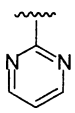
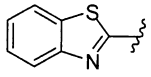
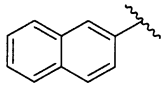
Scheme 3. (i) 6 M $HCl_{(aq)}$, Δ; (ii) Dowex® 50WX8-100 ion exchange resin.

4-H enhanced the 3-H signal by a large 13.4%; an equally high enhancement of the 4-H signal (10.7%) was observed when the 3-H signal was irradiated, thus suggesting that the 3- and 4-H bore a *cis*-relationship. Irradiation of the signal pertaining to 3-H only enhanced the 2-H signal by 3.6%, implying a greater distance between the two nuclei, and thus a *trans*-relationship between 2- and 3-H.

In addition, the signals attributed to the methylene group adjacent to the *tert*-butyl ester in the ^{1}H NMR spectrum of **10a–f** had a characteristic splitting pattern, which was observed on similar compounds with the same stereochemistry.[18] Other analogues prepared were assumed to have the same configuration.

Compounds **10a–f** were treated with 6 M aqueous hydrochloric acid at reflux, and then subjected to ion-exchange chromatography (Dowex® 50WX8-100) (Scheme 3). As indicated in Table 2, deprotected amino acids **11a–c** were obtained in good to excellent yields. In cases **11d** and **e**, where the pyrimidine and benzothiazole functionality were susceptible to hydrolysis, isolation of the desired material was not possible. Compound **11f** proved to be highly insoluble, making handling and characterisation impractical.

Table 2. Deprotection of compounds **10a–f**

Entry	R	Product	Yield (%)
1		**11a**	77
2	Ph	**11b**	71
3	*o*-$MeOC_6H_4$	**11c**	97
4[a]		**11d**	–
5[a]		**11e**	–
6[b]		**11f**	–

[a] Partial decomposition of the product occurred.
[b] Product insoluble.

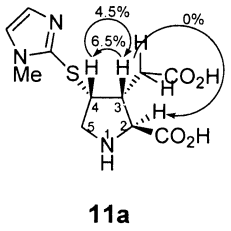


Figure 4.

The stereochemistry of compound **11a** was confirmed by NOED spectroscopic experiments, the key data from which is presented in Fig. 4. Irradiation of the signal pertaining to 4-H enhanced the 3-H signal by 4.5% and, conversely, irradiation of the 3-H signal enhanced the 4-H signal by 6.5%. There was no enhancement of the 3-H proton upon irradiation of the 2-H signal, neither was there a through-ring interaction between 2- and 4-H. Additionally, the signals in the ^{1}H NMR spectrum of the methylene group adjacent to the carboxyl group of compounds **11a–c** had a characteristic splitting pattern, which has been observed in analogous compounds.[18]

3. Conclusions

In summary, a novel class of 4-arylsulfanyl-substituted kainoid amino acids have been synthesised from commercially available *trans*-4-hydroxy-L-proline **5**. Despite some limitations, this route should allow the preparation of more 4-C heteroaromatic kainoid analogues by parallel nucleophilic substitutions on the mesylate **9**. The prepared compounds are currently undergoing biological evaluation for their use as neuropharmacological tools for the study of kainate receptors in the CNS.

4. Experimental

4.1. General

Melting points were obtained using a Büchi 510 capillary apparatus and are uncorrected.

Specific optical rotations, given in 10^{-1} deg cm^2 g^{-1}, were determined with a Perkin–Elmer 241 automatic polarimeter with a cell of path length 1 dm. Concentrations are given in g 100^{-1} cm^{-3}.

Infrared spectra were recorded on a Perkin–Elmer Paragon 1000 Fourier transform spectrometer, with major absorbances only being quoted. The following abbreviations are used: w, weak; m, medium; s, strong; br, broad.

^{1}H NMR spectra were recorded at 200, 400 or 500 MHz using a Varian Gemini 200, or Bruker AC200, DPX400, AM500 or AMX500 instruments. For ^{1}H NMR spectra recorded in $CDCl_3$ or D_2O, chemical shifts are quoted in parts per million, and referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are quoted to the nearest 0.5 Hz.

^{13}C NMR spectra were recorded at either 50.3 MHz using Varian Gemini 200 or Bruker AC200 instruments, 100.63 MHz on a Bruker DPX400 or at 125.8 MHz on a Bruker AM500 or AMX500 instrument, using DEPT editing to assist assignment where necessary. Chemical shifts are quoted in parts per million and are referenced to $CDCl_3$.

Low-resolution mass spectra were recorded on a Fisons VG Platform APCI instrument or a Micromass Autospec OA-TOF instrument. Only molecular ions, fragments from molecular ions and other major peaks are reported. High-resolution mass spectra were recorded on a Micromass Autospec OA-TOF instrument.

Thin layer chromatography was performed on aluminium or glass backed plates pre-coated with Merck silica-gel (60 F_{254}), which were visualised by quenching of UV fluorescence or by staining with iodine vapour or 10% ammonium molybdate in 2 M sulfuric acid (followed by heat), as appropriate. Flash chromatography was effected with Sorbsil C60 (40–63 mm, 230–240 mesh) silica-gel as stationary phase.

Evaporation refers to the removal of solvent at ≤40°C under reduced pressure using a Büchi rotary evaporator fitted with a water or dry ice condenser as necessary.

All solvents and reagents were purified by standard techniques reported in Perrin and Armarego,[19] or used as supplied from commercial sources, as appropriate.

The various thiolate salts required were prepared from their corresponding thiols, as previously described for *p*-chlorophenylthiolate by Bowman and Rakshit.[20] General procedure: to a stirred mixture of sodium methoxide (1 mol equiv.) in methanol was added the thiol (1 mol equiv.). After 3 h, the mixture was evaporated, the residue washed with diethyl ether to remove excess thiol traces, and then dried in vacuo to give the thiolate in essentially quantitative yield.

4.1.1. (2*S*,3*S*,4*R*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-hydroxypyrrolidine-2-carboxylic acid methyl ester 7. To a stirred solution of (2*S*,3*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-oxo-pyrrolidine-2-carboxylic acid methyl ester **6**[16] (0.500 g, 1.38 mmol) in methanol (20 cm^3) was added sodium borohydride (0.057 g, 1.51 mmol). After 1.5 h, the mixture was poured onto saturated ammonium chloride solution and extracted with ethyl acetate. The combined organic extracts were washed with 0.1 M hydrochloric acid, followed by saturated brine and then dried ($MgSO_4$) and evaporated to a white solid (0.49 g, ca. 98%), identified as the title compound **7**, which was used in subsequent syntheses without further purification; δ_H (400 MHz; $CDCl_3$) (major rotamer) 1.46 (9H, s, CO_2Bu^t), 2.47–2.60 (2H, m, 3-H and C*H*HCO_2Bu^t), 2.81 (1H, dd, *J*=15.5, 3.5 Hz, CH*H*CO_2Bu^t), 3.68–3.89 (2H, m, 5-H), 3.84 (3H, s, CO_2Me), 4.02–4.07 (1H, m, 4-H), 4.33 (1H, d, *J*=7 Hz, 2-H), 7.38–7.53 (3H, m, Ph*H*) and 7.58–7.60 (2H, m, Ph*H*); *m*/*z* (CI) 386 (*M*Na$^+$, 10%), 364 (*M*H$^+$, 17) and 308 ($C_{15}H_{18}NO_6^+$, 100).

4.1.2. (2*S*,3*S*,4*R*)-3-Carboxymethyl-4-hydroxypyrrolidine-2-carboxylic acid 8. (2*S*,3*S*,4*R*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-hydroxypyrrolidine-2-carboxylic acid methyl ester **7** (0.101 g, 0.28 mmol) was heated in 6 M hydrochloric acid (4 cm^3) at reflux for 3.5 h. The resulting solution was cooled and evaporated to a white solid (0.086 g), identified as largely the hydrochloride salt of the title compound **8**; δ_H (200 MHz; D_2O) 2.67 (2H, d, *J*=7 Hz, C*H*$_2$CO$_2$H), 2.85–2.93 (1H, m, 3-H), 3.40 and 3.58 [each 1H, dd (*J*=12.5, 2.5 Hz) and dd (*J*=12.5, 5 Hz), 5-H$_2$], 4.29 (1H, d, *J*=5.5 Hz, 2-H) and 4.35–4.40 (1H, m, 4-H).

The material was dissolved in water (10 cm^3) and loaded onto a pre-activated column of acidic ion-exchange resin (Dowex® 50WX8-100). After flushing the column with water (100 cm^3), the compound was eluted with 2 M aqueous ammonia solution (50 cm^3). The resulting solution was evaporated to give the title compound **8** (0.050 g, ca. 100%) as an off-white gum; ν_{max}/cm^{-1} (KBr) 3190 (br), 1623 (s) and 1399 (s); δ_H (500 MHz; D_2O) 2.32 and 2.52 [each 1H, dd (*J*=15.5, 9.5 Hz) and dd (*J*=15.5, 5.5 Hz), C*H*$_2$CO$_2$H], 2.78–2.79 (1H, m, 3-H), 3.40 and 3.53 [each 1H, d (*J*=12.5 Hz) and dd (*J*=16, 4.5 Hz), 5-H$_2$], 3.88 (1H, d, *J*=4.5 Hz, 2-H) and 4.28–4.29 (1H, m, 4-H); δ_C (125 MHz; D_2O) 38.98 (*C*H$_2$CO$_2$H), 47.89 (3-C), 50.90 (5-C), 64.35 (2-C), 73.53 (4-C), 173.45 and 178.69 (2×CO_2H); *m*/*z* (CI) 190 (*M*H$^+$, 49%), 172 (100), 146 (43) and 126 (38). Found: *M*H$^+$ 190.0718. $C_7H_{11}NO_5$ requires *M*H$^+$ 190.0716.

4.1.3. (2*S*,3*S*,4*R*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester 9. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-hydroxypyrrolidine-2-carboxylic acid methyl ester **7** (0.490 g, 1.38 mmol) in dry dichloromethane (20 cm^3) under argon was added triethylamine (0.38 cm^3, 2.76 mmol), followed by methanesulfonyl chloride (0.12 cm^3, 1.52 mmol). After 16 h, the material was washed with water and the aqueous phase re-extracted with dichloromethane. The combined organic extracts were washed with saturated brine, dried ($MgSO_4$) and evaporated to a white solid (0.590 g). The material was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (9:1) as eluent], which afforded one major fraction. The eluted material, obtained as a white solid (0.556 g, 91%), was identified as the title compound **9**; mp 132–133°C; $[\alpha]_D$=−82 (*c* 0.25, CH_2Cl_2); ν_{max}/cm^{-1} (KBr) 1753 and 1724 (2×ester C=O) and 1637 (amide C=O); δ_H (400 MHz; $CDCl_3$) (2 rotamers) 1.44–1.48 (9H, m, CO_2Bu^t), 2.36–2.66 (2H, m, C*H*$_2$CO$_2$Bu^t), 2.99–3.24 (4H, m, $MeSO_3$ and 3-H), 3.69–4.02 (5H, m, CO_2Me and 5-H$_2$), 4.30–4.40 and 4.61–4.63 (1H, each m, 2-H), 4.95–5.08 (1H, m, 4-H) and 7.46–7.57 (5H, m, COPh); δ_C (50 MHz; $CDCl_3$) (major rotamer) 27.83 [$CO_2C(CH_3)_3$], 35.17

($CH_2CO_2Bu^t$), 38.03 (CH_3SO_3), 43.56 (3-C), 52.64 (4-C), 53.12 (5-C), 61.37 (2-C), 81.86 [$CO_2C(CH_3)_3$], 127.45, 128.70, 130.97, 135.00 (*Ph*), 169.68 and 170.96 (*C*OPh, *C*O_2Me and *C*O_2Bu^t); *m/z* (CI) 442 (MH^+, 100%) and 386 ($C_{16}H_{20}NO_8S^+$, 50). Found: MH^+ 442.1537. $C_{20}H_{27}NO_8S$ requires MH^+ 442.1536.

4.1.4. (2*S*,3*S*,4*S*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-(1-methyl-1*H*-imidazol-2-sulfanyl)pyrrolidine-2-carboxylic acid methyl ester 10a. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.106 g, 0.24 mmol) in dimethyl sulfoxide (5 cm^3) was added 2-mercapto-1-methylimidazole sodium salt (0.165 g, 1.20 mmol). The solution was heated at 90°C; a blue colour was observed after 2 min. After 1.5 h, the mixture was cooled, poured onto saturated brine and extracted with dichloromethane. The combined organic extracts were washed with water, dried ($MgSO_4$) and evaporated to a brown oil (0.088 g). The material was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (9:1) as eluent], which afforded one major fraction. The eluted material, obtained as a pale-yellow oil (0.060 g, 55%), was identified as the title compound **10a**; $[\alpha]_D$=+44.5 (*c* 0.38, CH_2Cl_2); ν_{max}/cm^{-1}(KBr) 1725 (ester C=O) and 1636 (amide C=O); δ_H (400 MHz; $CDCl_3$) (major rotamer) 1.44 (9H, s CO_2Bu^t), 2.71 and 2.86 [each 1H, dd (*J*=17.5, 6 Hz) and dd (*J*=17.5, 8.5 Hz), C*H*$_2CO_2Bu^t$), 3.03–3.09 (1H, m, 3-H), 3.49 (3H, s, NMe), 3.78 (3H, s, CO_2Me), 3.68–3.75 and 4.29 [each 1H, m and dd (*J*=14.5, 6.5 Hz), 5-H_2], 4.42–4.44 (1H, m, 4-H), 4.43 (1H, d, *J*=9.5 Hz, 2-H), 6.85 (1H, s, ArH), 6.96 (1H, s, ArH), 7.35–7.44 (3H, m, PhH) and 7.54–7.56 (2H, m, PhH); δ_C (100 MHz; $CDCl_3$) (major rotamer) 28.03 [$CO_2C(CH_3)_3$], 33.29 (NMe), 34.65 (*C*$H_2CO_2Bu^t$), 42.56 (3-C), 51.01 and 52.58 (2-C and CO_2CH_3), 56.36 (5-C), 62.75 (4-C), 81.31 [$CO_2C(CH_3)_3$], 122.76, 127.59, 128.25, 129.85, 130.64, 134.83, 138.55 (CO*Ph* and Ar-C), 169.59, 170.18 and 171.58 (*C*OPh, *C*O_2Me and *C*O_2Bu^t); *m/z* (CI) 482 (MNa^+, 10%) and 460 (MH^+, 100). Found: MH^+ 460.1907. $C_{23}H_{29}N_3O_5S$ requires MH^+ 460.1906.

4.1.5. (2*S*,3*S*,4*S*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-phenylsulfanylpyrrolidine-2-carboxylic acid methyl ester 10b. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.070 g, 0.16 mol) in dimethyl sulfoxide (2 cm^3) was added benzenethiol sodium salt (0.106 g, 0.80 mmol). The solution was heated at 90°C for 15 h, and then cooled to ambient temperature. The reaction mixture was then poured onto saturated brine, and extracted with dichloromethane. The combined organic extracts were concentrated and redissolved in diethyl ether. The ethereal phase was washed with water, dried ($MgSO_4$) and evaporated to a pale-brown oil (0.073 g). The material was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (9:1) as eluent], which afforded one major fraction. The eluted material, obtained as a white foam (0.039 g, 53%), was identified as the title compound **10b**; $[\alpha]_D$=+47.0 (*c* 0.20, CH_2Cl_2); ν_{max}/cm^{-1} (KBr) 1724 (ester C=O) and 1640 (amide C=O); δ_H (400 MHz; $CDCl_3$) (5:1 mixture of rotamers) 1.40 and 1.45 (1.5 and 7.5H, each s, CO_2Bu^t), 2.30–2.50 and 2.64–3.09 (0.16 and 2.84H, each m, C*H*$_2CO_2Bu^t$ and 3-H), 3.48 and 3.80 (0.48 and 2.52H, each s, CO_2Me), 3.59 and 3.91–4.05 [0.83 and 2.17 H, dd (*J*=11, 2 Hz) and m, 4-H and 5-H_2], 4.27 and 4.48 [0.16 and 0.84H, d (*J*=5.5, 8.5 Hz), 2-H] and 7.17–7.59 (5H, m, PhH); δ_C (50 MHz; $CDCl_3$) (major rotamer) 28.01 [$CO_2C(CH_3)_3$], 34.38 (*C*$H_2CO_2Bu^t$), 42.81 (3-C), 51.19 and 52.52 (4-C and CO_2*Me*), 55.56 (5-C), 62.50 (2-C), 81.23 [$CO_2C(CH_3)_3$], 127.38, 127.71, 128.22, 128.40, 129.15, 130.42, 132.54 (PhS and CO*Ph*), 170.0, 170.56 and 171.72 (*C*O_2Me, *C*O_2Bu^t and *C*OPh); *m/z* (CI) 456 (MH^+, 50%) and 400 ($C_{21}H_{22}NO_5S^+$, 100). Found: MH^+ 456.1850. $C_{25}H_{29}NO_5S$ requires MH^+ 456.1845.

4.1.6. (2*S*,3*S*,4*S*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-[(2-methoxyphenyl)sulfanyl]pyrrolidine-2-carboxylic acid methyl ester 10c. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.104 g, 0.24 mmol) in dimethyl sulfoxide (5 cm^3) was added 2-methoxybenzenethiol sodium salt (0.195 g, 1.20 mmol). The solution was heated at 90°C for 17 h, and then cooled to ambient temperature and evaporated (Kugelrohr). The residue was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (19:1) as eluent], which afforded one major fraction. The eluted material, obtained as a pale-yellow oil (0.062 g, 53%), was identified as the title compound **10c**; $[\alpha]_D$=+95.3 (*c* 0.34, CH_2Cl_2), ν_{max}/cm^{-1} (KBr) 1727 (ester C=O) and 1639 (amide C=O); δ_H (500 MHz; $CDCl_3$) (major rotamer) 1.49 (9H, s, CO_2Bu^t), 2.71 (1H, dd, *J*=17, 5.5 Hz, C*H*HCO_2Bu^t), 2.96–3.07 (2H, m, 3-H and CH*H*CO_2Bu^t), 3.64 and 3.98 [each 1H, dd (*J*=11.5, 2.5 Hz) and dd (*J*=11.5, 5 Hz), 5-H_2], 3.78 and 3.84 (each 3H, s, 2×OMe), 4.11–4.13 (1H, m, 4-H), 4.55 (1H, d, *J*=8.5 Hz, 2-H), 6.82–6.85 (2H, m, ArH), 7.25–7.32 (2H, m, ArH), 7.39–7.47 (3H, m, PhH) and 7.57–7.59 (2H, m, PhH); δ_C (125 MHz; $CDCl_3$) (major rotamer) 28.02 [$CO_2C(CH_3)_3$], 34.32 (*C*$H_2CO_2Bu^t$), 42.84 (3-C), 49.14, 51.90 and 55.56 (CO_2*Me*, OMe and 4-C), 55.51 (5-C), 62.61 (2-C), 81.09 [$CO_2C(CH_3)_3$], 111.00 (3′-C), 121.06 (6′-C), 127.47, 128.14, 129.73, 130.35, 134.72, 135.31 (CO*Ph* and 3×Ar-C), 159.08 (3′-C), 169.80, 170.64 and 171.93 (*C*O_2Bu^t, *C*O_2Me and *C*OPh); *m/z* (CI) 508 (MNa^+, 10%), 486 (MH^+, 8) and 430 ($C_{22}H_{24}NO_6S^+$, 100). Found: MH^+ 486.1937. $C_{26}H_{31}NO_6S$ requires MH^+ 486.1950.

4.1.7. (2*S*,3*S*,4*S*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-(pyrimidin-2-ylsulfanyl)pyrrolidine-2-carboxylic acid methyl ester 10d. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.149 g, 0.34 mmol) in dimethyl sulfoxide (5 cm^3) was added 2-mercaptopyrimidine sodium salt (0.226 g, 1.69 mmol). The solution was heated at 90°C for 1.5 h, and then cooled to ambient temperature. The reaction mixture was then poured onto saturated brine, and extracted with dichloromethane. The combined organic extracts were concentrated and redissolved in diethyl ether. The ethereal phase was washed with water, dried ($MgSO_4$) and evaporated to a yellow foam (0.138 g). The material was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (9:1) as eluent], which afforded one major fraction. The eluted material, obtained as a clear, colourless oil (0.074 g, 47%), was identified as the title compound **10d**; $[\alpha]_D$=+38.5 (*c*

0.33, CH_2Cl_2); ν_{max}/cm^{-1} (KBr) 1752 and 1734 (2×ester C=O) and 1642 (amide C=O); δ_H (200 MHz; $CDCl_3$) (major rotamer) 1.39 (9H, s, CO_2Bu^t), 2.63 and 2.77 (each 1H, dd, J=17.5, 7.5 Hz, $CH_2CO_2Bu^t$), 3.15–3.21 (1H, m, 3-H), 3.73 and 4.23 [each 1H, dd (J=11.5, 3.5 Hz) and dd (J=11.5, 5.5 Hz), 5-H_2], 3.80 (3H, s, CO_2Me), 4.45 (1H, d, J=8 Hz, 2-H), 4.69–4.76 (1H, m, 4-H), 6.95 (1H, t, J=5 Hz, 5′-H), 7.32–7.41 (3H, m, PhH), 7.53–7.58 (3H, m, PhH) and 8.45 (2H, d, J=5 Hz, 4′- and 6′-H); δ_C (50 MHz; $CDCl_3$) 27.95 [$CO_2C(CH_3)_3$], 34.94 ($CH_2CO_2Bu^t$), 41.84 (3-C), 46.85 (CO_2Me), 52.54 (2-C), 56.15 (5-C), 63.26 (4-C), 81.17 [$CO_2C(CH_3)_3$], 117.02 (5′-C), 126.62, 127.46, 128.25, 128.44, 130.43 (CO*Ph* and 2′-C), 157.39 (4′- and 6′-C), 169.68, 170.25 and 171.60 (CO_2Bu^t, CO_2Me and COPh); m/z (CI) 458 (MH^+, 100%) and 402 ($C_{19}H_{20}N_3O_5S^+$, 46). Found: MH^+ 458.1755. $C_{23}H_{27}N_3O_5S$ requires MH^+ 458.1750.

4.1.8. (2*S*,3*S*,4*S*)-4-(Benzothiazol-2-ylsulfanyl)-1-benzoyl-3-*tert*-butoxycarbonylmethylpyrrolidine-2-carboxylic acid methyl ester 10e. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.190 g, 0.43 mmol) in dimethyl sulfoxide (5 cm^3) was added 2-mercaptobenzothiazole sodium salt (0.320 g, 1.69 mmol). The solution was heated at 90°C for 1 h, and then cooled to ambient temperature. The reaction mixture was then poured onto saturated brine, and extracted with dichloromethane. The combined organic extracts were concentrated and redissolved in diethyl ether. The ethereal phase was washed with water, dried ($MgSO_4$) and evaporated to a yellow solid (0.263 g). The material was subjected to silica-gel column chromatography (CH_2Cl_2 as eluent), which afforded one major fraction. The eluted material, obtained as a clear, colourless oil (0.116 g, 53%), was identified as the title compound **10e**; $[\alpha]_D$=+42.3 (c 0.80, CH_2Cl_2); ν_{max}/cm^{-1} (KBr) 1729 (ester C=O) and 1640 (amide C=O); δ_H (200 MHz; $CDCl_3$) (major rotamer) 1.38 (9H, s, CO_2Bu^t), 2.63–2.87 (2H, m, $CH_2CO_2Bu^t$), 3.16–3.31 (1H, m, 3-H), 3.81 (3H, s, CO_2Me), 3.90 and 4.29 [each 1H, dd (J=11.5, 2 Hz) and dd (J=11.5, 5 Hz), 5-H_2], 4.46 (1H, d, J=9 Hz, 2-H), 4.84–4.93 (1H, m, 4-H), and 7.18–7.90 (9H, m, PhH and 4×Ar-H); δ_C (50 MHz; $CDCl_3$) (major rotamer) 27.92 [$CO_2C(CH_3)_3$], 34.96 ($CH_2CO_2Bu^t$), 42.45 (3-C), 50.34 and 52.63 (2-C and CO_2Me), 56.45 (5-C), 62.86 (4-C), 81.43 [$CO_2C(CH_3)_3$], 120.97, 121.86, 124.64, 126.11, 127.43, 128.31, 128.39, 130.59, 134.88 (CO*Ph* and 4×Ar-C), 170.00 and 171.43 (CO_2Bu^t, CO_2Me and COPh); m/z (CI) 513 (MH^+, 30%) and 457 ($C_{22}H_{21}N_2O_5S_2^+$, 100). Found: MH^+ 513.1517. $C_{26}H_{28}N_2O_5S_2$ requires MH^+ 513.1518.

4.1.9. (2*S*,3*S*,4*S*)-1-Benzoyl-3-*tert*-butoxycarbonylmethyl-4-(napthalen-2-ylsulfanyl)pyrrolidine-2-carboxylic acid methyl ester 10f. To a stirred solution of (2*S*,3*S*,4*R*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-methanesulfonyloxypyrrolidine-2-carboxylic acid methyl ester **9** (0.100 g, 0.23 mmol) in dimethyl sulfoxide (5 cm^3) was added 2-thionapthol sodium salt (0.206 g, 1.13 mmol). The solution was heated at 90°C for 6 h, and then cooled to ambient temperature. The reaction mixture was then poured onto saturated brine, and extracted with dichloromethane. The combined organic extracts were concentrated and redissolved in diethyl ether. The ethereal phase was washed with water, dried ($MgSO_4$) and evaporated to a brown oil. The material was subjected to silica-gel column chromatography [CH_2Cl_2–EtOAc (19:1) as eluent], which afforded one major fraction. The eluted material, obtained as a pale-yellow oil (0.029 g, 25%), was identified as the title compound **10f**; $[\alpha]_D$=+32.0 (c 0.50, CH_2Cl_2); ν_{max}/cm^{-1} (KBr) 1723 (ester C=O) and 1640 (amide C=O); δ_H (400 MHz; $CDCl_3$) (major rotamer) 1.42 (9H, s, CO_2Bu^t), 2.75 and 2.91 [each 1H, dd (J=17.5, 5.5 Hz) and dd (J=17.5, 9.5 Hz), $CH_2CO_2Bu^t$], 3.03–3.08 (1H, m, 3-H), 3.66 and 3.96 [each 1H, dd (J=11.5, 1.5 Hz) and dd (J=11.5, 4.5 Hz), 5-H_2], 3.81 (3H, s, CO_2Me), 4.12–4.14 (1H, m, 4-H), 4.53 (1H, d, J=9 Hz, 2-H) and 7.32–7.81 (12H, m, PhH and Ar-H); δ_C (100 MHz; $CDCl_3$) (major rotamer) 28.00 [$CO_2C(CH_3)_3$], 34.44 ($CH_2CO_2Bu^t$), 42.94 (3-C), 51.29 and 52.57 (4-C and CO_2Me), 55.58 (5-C), 62.48 (2-C), 81.25 [$CO_2C(CH_3)_3$], 126.56, 126.66, 127.35, 127.39, 127.61, 128.24, 128.44, 128.93, 129.65, 130.33, 130.44, 131.96, 132.46, 133.49 and 135.23 (CO*Ph* and napthyl-C), and 170.00, 170.62 and 171.76 (CO_2Bu^t, CO_2Me and COPh); m/z (CI) 506 (MH^+, 60%) and 450 ($C_{25}H_{24}NO_5S^+$, 100). Found: MH^+ 506.1984. $C_{29}H_{31}NO_5S$ requires MH^+ 506.2001.

4.1.10. (2*S*,3*S*,4*S*)-3-Carboxymethyl-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl)pyrrolidine-2-carboxylic acid 11a. To (2*S*,3*S*,4*S*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-(1-methyl-1*H*-imidazol-2-ylsulfanyl) pyrrolidine-2-carboxylic acid methyl ester **10a** (0.050 g, 0.109 mmol) was added 6 M hydrochloric acid (2 cm^3). The mixture was heated at reflux for 16 h. The resulting solution was then cooled to ambient temperature, washed with dichloromethane, and evaporated to an off-white solid (0.033 g). The residue was dissolved in water (10 cm^3) and loaded onto a pre-activated column of acidic ion exchange resin (Dowex® 50WX8-100). After flushing the column with water (100 cm^3), the compound was eluted with 2 M aqueous ammonia solution (100 cm^3). The resulting solution was evaporated to give the title compound **11a** (0.024 g, 77%); $[\alpha]_D$=+64.1 (c 0.46, H_2O); ν_{max}/cm^{-1} (KBr) 3500–2000 (brs), 1622 (brs) and 1394 (s); δ_H (400 MHz; D_2O) 2.45 and 2.72 [each 1H, dd (J=16.5, 11 Hz) and dd (J=16.5, 4.5 Hz), CH_2CO_2H], 2.77–2.85 (1H, m, 3-H), 3.37 and 3.50 [each 1H, d (J=12.5 Hz) and dd (J=12.5, 5 Hz), 5-H_2], 3.62 (3H, s, NMe), 3.77 (1H, d, J=10.5 Hz, 2-H), 4.05 (1H, t, J=5 Hz, 4-H), 6.97 and 7.16 (1H, s, imidazole 4- and 5-H); δ_C (50 MHz; D_2O) 33.47 and 36.61 (NMe and CH_2CO_2H), 44.20 (3-C), 50.50, 51.14 (2- and 5-C), 63.50 (4-C), 125.17, 128.64 (Ar 4- and 5-C), 137.08 (Ar 2-C), 173.55 and 178.62 (2×CO_2H); m/z (CI) 286 (MH^+, 100%). Found: MH^+ 286.0858. $C_{11}H_{15}N_3O_4S$ requires MH^+ 286.0862.

4.1.11. (2*S*,3*S*,4*S*)-3-Carboxymethyl-4-phenylsulfanylpyrrolidine-2-carboxylic acid 11b. To (2*S*,3*S*,4*S*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-phenylsulfanylpyrrolidine-2-carboxylic acid methyl ester **10b** (0.027 g, 0.059 mmol) was added 6 M hydrochloric acid (2 cm^3). The reaction mixture was heated at reflux for 5 h. The resulting solution was then cooled to ambient temperature, washed with dichloromethane, and evaporated to an off-white solid. The residue was dissolved in water (10 cm^3) and loaded onto a pre-activated column of acidic ion

exchange resin (Dowex® 50WX8-100). After flushing the column with water (100 cm^3), the compound was eluted with 2 M aqueous ammonia solution (50 cm^3). The resulting solution was evaporated to give the title compound **11b** (0.012 g, 71%); $[\alpha]_D$=+74.0 (*c* 0.14, H_2O); ν_{max}/cm^{-1} (KBr) 3500–2000 (brs), 1620 (brs) and 1390 (s); δ_H (500 MHz; D_2O) 2.49 and 2.70 [each 1H, dd (*J*=16.5, 11 Hz) and dd (*J*=16.5, 4.5 Hz), CH_2CO_2H], 2.83–2.89 (1H, m, 3-H), 3.30 and 3.54 [each 1H, dd (*J*=12.5, 2 Hz) and dd (*J*=12.5, 5 Hz), 5-H_2], 3.85 (1H, d, *J*=10 Hz, 2-H), 4.16–4.19 (1H, m, 4-H), 7.29–7.33 (3H, m, PhH) and 7.36–7.43 (3H, m, PhH); δ_C (125 MHz; D_2O) 36.72 (*C*H_2CO_2H), 44.22 (3-C), 50.41 and 50.80 (2- and 5-C), 63.57 (4-C), 128.13, 129.57, 132.38 (PhS), 173.15 and 178.92 (2×CO_2H); *m/z* (CI) 282 (*M*H^+, 100%). Found: *M*H^+ 282.0797. $C_{13}H_{15}NO_4S$ requires *M*H^+ 282.0800.

4.1.12. (2*S*,3*S*,4*S*)-3-Carboxymethyl-4-[(2-methoxyphenyl)sulfanyl]pyrrolidine-2-carboxylic acid 11c. To (2*S*,3*S*,4*S*)-1-benzoyl-3-*tert*-butoxycarbonylmethyl-4-(2-methoxyphenylsulfanyl)-pyrrolidine-2-carboxylic acid methyl ester **10c** (0.049 g, 0.10 mmol) was added 6 M hydrochloric acid (4 cm^3). The reaction mixture was heated at reflux for 15 h. The resulting solution was then cooled to ambient temperature, and evaporated to an off-white solid. The residue was dissolved in water (10 cm^3) and loaded onto a pre-activated column of acidic ion exchange resin (Dowex® 50WX8-100). After flushing the column with water (100 cm^3), the compound was eluted with 2 M aqueous ammonia solution (50 cm^3). The resulting solution was evaporated to a clear, colourless oil (0.030 g, 97%), identified as the title compound **11c**; $[\alpha]_D$=+78.3 (*c* 0.35, MeOH); ν_{max}/cm^{-1} (KBr) 3500–2000 (brs), 1620 (brs) and 1495 (s); δ_H (500 MHz; D_2O) 2.86 and 3.08 [each 1H, dd (*J*=16.5, 11 Hz) and dd (*J*=16.5, 4.5 Hz), CH_2CO_2H], 2.96–2.99 (1H, m, 3-H), 3.30 and 3.63 [each 1H, dd (*J*=12.5, 2 Hz) and dd (*J*=12.5, 5 Hz), 5-H_2], 3.85 (1H, d, *J*=10 Hz, 2-H), 3.90(3H, s, OMe) 4.31–4.33 (1H, m, 4-H), 6.96 (1H, t, *J*=7 Hz, PhH), 7.04 (1H, d, *J*=7 Hz, PhH) and 7.36–7.47 (2H, m, PhH); δ_C (125 MHz; D_2O) 36.65 (*C*H_2CO_2H), 42.11 (3-C), 47.64 and 51.23 (2- and 5-C), 55.73 (OMe) 65.32 (4-C), 111.37, 121.02, 127.51, 130.02, 157.21 (6×ArS), 172.43 and 173.26 (2×CO_2H); *m/z* (CI) 329 (*M*NH_4^+, 82%) and 312 (*M*H^+, 100). Found: *M*H^+ 312.0900. $C_{14}H_{17}NO_5S$ requires *M*H^+ 312.0906.

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