Conformational analysis of 4-amido-2,4-dimethylbutyric acid derivatives

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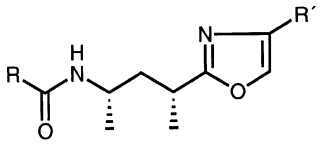
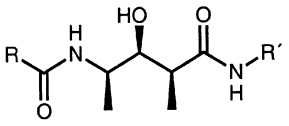
Received* (*in Montpellier, France*) *20th December 1999, Accepted 14th January 2000

Both *syn*- and *anti*-4-amido-2,4-dimethylbutyric acid derivatives **5** and **6** were found to populate a conformation in which the amido group is *gauche* to the main chain of the molecule. In the *anti* series (**6**) a single conformation predominates, in which the acid carbonyl group is also *gauche* to the main chain. In the *syn* series (**5**) two local conformers prevail about the C-2–C-3 bond.

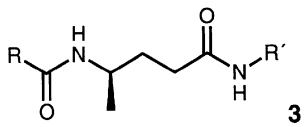
In certain natural products such as bleomycin A_2[1] or calyculin C,[2] nature connects two effector domains of a molecule by a short linker chain, *cf.* **1** and **2**. In both cases the linker is a derivative of a γ-aminobutyric acid, which carries methyl groups in the 2- and 4-positions. It can be envisaged that these methyl groups serve to give the linker unit a preferred conformation,[3] while maintaining full conformational flexibility.



Linker unit in Calyculin C **1**



Linker unit in Bleomycin A_2 **2**



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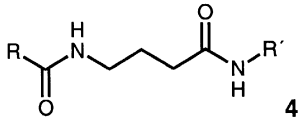

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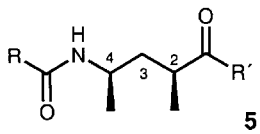
In fact, for both **1**[2] and **2**[4] NMR coupling constants indicate the predominance of a single conformation of the linker region in solution. The importance of this conformational preorganization for its biological activity has been probed for bleomycin A_2 by synthesizing analogs containing, for example, the linker units **3** and **4**. The observed decrease in biological activity has been attributed[4] to a lower conformational preorganization in the molecules containing the linkers **3** and **4**.

These phenomena were of interest to us in the context of learning more about flexible molecular backbones with a preferred conformation.[3] In particular, we were interested to learn whether the conformational preferences observed in **1** and **2** are an inherent property of the dimethylated γ-amidobutyric acid derivatives **5** and **6**, or whether they are mainly a consequence of the flanking groups present in calyculin C or bleomycin A_2. For this reason we prepared a series of compounds having the structural units **5** or **6** and studied their conformational behaviour.[5] We report here our results in detail.



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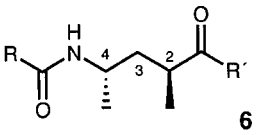

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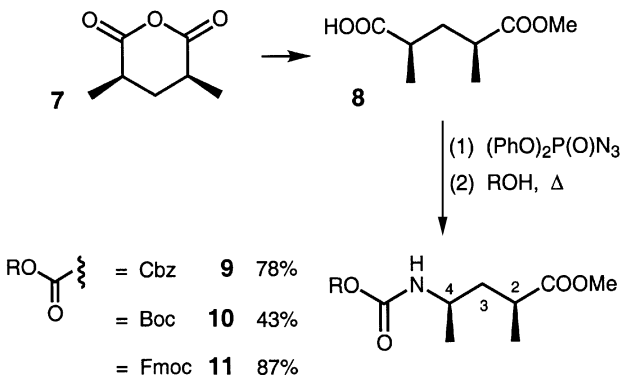
Results and discussion

Synthesis

Compounds of both the *syn* and *anti* series **5** and **6** have been synthesized in enantiomerically pure form from alanine by Koskinen and Pihko.[6,7] Since racemic material suffices for our study we explored a shorter route to the (2*R**,4*S**) series **5** starting from the *meso*-anhydride **7**[8] (Scheme 1).

Thus, opening of **7** with methanol led to the half ester **8**, which may be resolved if optically pure material is desired.[9] Curtius degradation of the acid **8**, in the presence of benzyl alcohol led us to the Cbz-protected ester **9**. The Boc- and Fmoc-protected derivatives **10** and **11** were obtained in a like manner.



Scheme 1

In order to prepare dipeptides containing the (2*R**,4*S**)-4-amino-2,4-dimethylbutyric acid (*cf.* **5**) the Boc group was cleaved with trifluoroacetic acid to form the ester **12**. However, any attempts to couple activated amino acids to the

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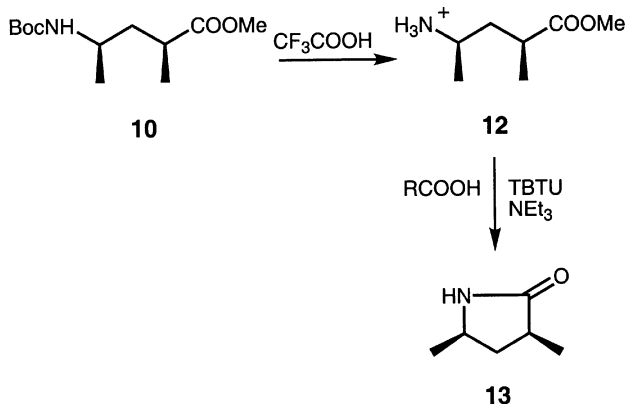


Scheme 2

resulting amino ester **12** led only to the formation of the γ-lactam **13** (Scheme 2).

This necessitated a more circuituous route, in which the ester **9** was first saponified with aqueous lithium hydroxide to give the acid **14** (Scheme 3). The protecting group in **14** was changed to Fmoc (*viz.* **15**). Next, the acid **15** was attached to chlorotrityl resin. It could then be successfully coupled with the *N*-protected amino acids **16** and **17** using *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate as coupling reagent. Apparently, the undesired closure to the lactam **13** is sufficiently slower in the chlorotrityl ester to allow dipeptide formation to proceed. The dipeptides **18** and **19** were eventually cleaved from the resin using standard techniques.

In order to gain information on the conformational behaviour of more extended systems, the heptenoic acid derivatives **25** and **27** (Scheme 4) were required. Thus, the enantiomerically pure half-ester *ent*-**8** was converted to the aldehyde **21**.[10] Wittig reaction with the phosphorane **22**[11] furnished the α,β-unsaturated ester **23**. Hydrolysis of the methyl ester with aqueous lithium hydroxide and Curtius rearrangement generated the desired heptenoate **25**.

The stereoisomeric heptenoate **27** was synthesized in an analogous manner from the (*R**,*R**) half-ester **26**.[12]

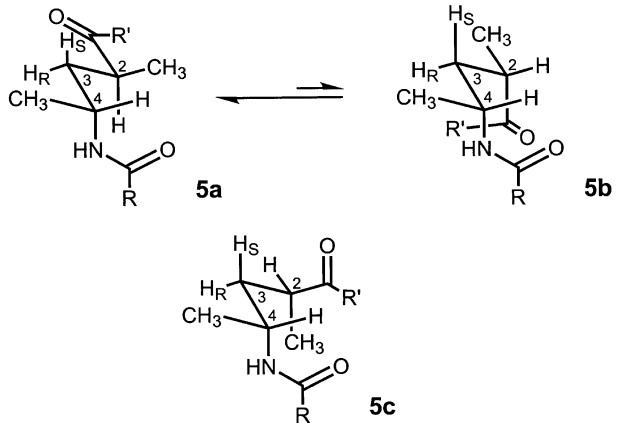
Two additional compounds of interest, **29** and **30** (Scheme 5), were prepared following the route described by Koskinen and Pihko.[6,7] The former compound was converted *via* the γ-amino acid to the Fmoc-protected derivative **30** in 89% overall yield.

In order to identify the preferred conformation of the 4-amido-2,4-dimethylbutyric acid derivatives **5** and **6** on the basis of $^3J_{H,H}$ coupling constants the signals of the individual diastereotopic protons at C-3 needed to be assigned. This was done for compounds **33** and **34** by stereospecific deuterium labelling. To this end, the pyrrolinone **31**[6,7] was reduced (Scheme 6) with a "copper deuteride" reagent.[13] This produced a 1 : 1.5 mixture of the C-2 epimeric pyrrolidinones **32**, the spectral data of which corresponded to those given by Pihko and Koskinen.[7]

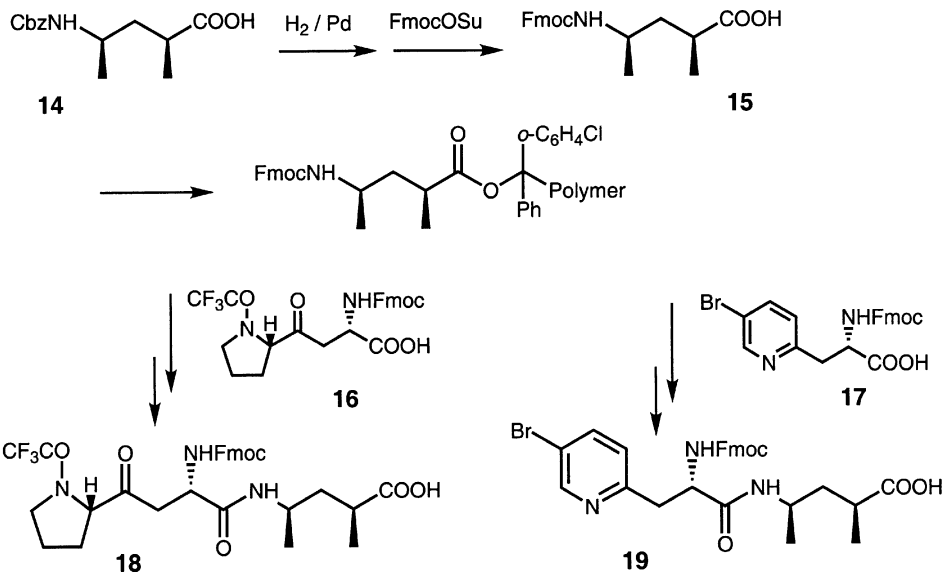
We presume that the deuterium was incorporated into compound **31** *trans* to the C-4 methyl group. The pyrrolidinones **32** were then converted into the esters **33** and **34** by hydrolysis with aqueous lithium hydroxide, followed by a diazomethane treatment. The esters **33** and **34** were separated by preparative HPLC. The ^{1}H-NMR spectrum of **33** allowed signal assignments to the individual diastereotopic C-3 protons in **10**. Likewise, the spectrum of **34** suggested an assignment of the diastereotopic protons in the corresponding acid **29**.

Conformational analysis

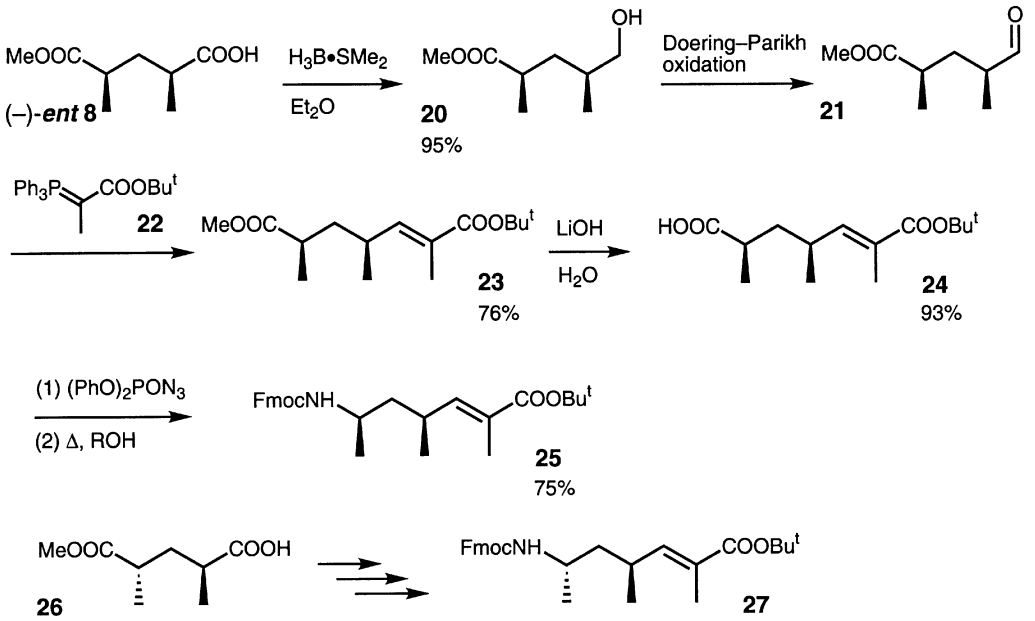
In the (2*R**,4*S**) series **5** MM3* calculations suggest a preference to populate the conformation **5a**, which corresponds to the one found in solution for calyculin C[2] and the one suggested for desoxybleomycin A_2.[4]



According to the calculations, conformation **5b** should also be easily accessible, whereas conformation **5c** should be substantially higher in energy (+7 kJ mol^{-1} relative to **5a**). It should be noted that the acylamido group is *gauche* to the main chain of the molecule in all of the conformers **5a** to **5c**. Any conformational preference, such as this feature, should be manifest from the vicinal ^{1}H-NMR coupling constants between C-2-H, the diastereotopic C-3-H protons and C-4-H. Determination of the coupling constants from the ^{1}H-NMR



Scheme 3



Scheme 4

spectra was limited in several cases by signal overlap and line broadening due to the presence of amide rotamers. Nevertheless, the coupling constants could be derived for a representative set of compounds, *cf.* Table 1.

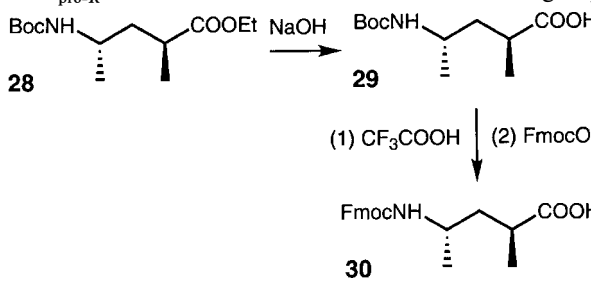
More insight into the nature of the preferred conformation can be gained when the $^3J_{H,H}$ coupling constants can be related to the individual diastereotopic protons at C-3. In all compounds of type **5** the two C-3-H protons give rise to a high-field signal around δ 1.4 and a low-field signal around δ 1.9 ppm. Stereospecific deuterium labelling of **33** as outlined above, allowed the assignment of the $\delta = 1.4$ signal to $H_{pro\text{-}S}$ and the $\delta = 1.7$ signal to $H_{pro\text{-}R}$, defined in the table.[14] This assignment of the signals of the diastereotopic protons at C-3 is consistent with that made for the linker unit in calyculin C,[2] the data of which are also included in the table. In view of this consistency we assume that the same assignment holds for all of the compounds corresponding to **5** listed in the table.

The coupling constants refer to a weighted average of the values of the individual conformers contributing to the conformer equilibrium. The high (*ca.* 10 Hz) coupling constant for C-3-$H_{pro\text{-}R}$ to C-4-H indicates that indeed the amido group at C-4 is arranged predominantly *gauche* to the main chain. The "average" coupling constant between C-3-$H_{pro\text{-}R}$ to C-2-H indicates that more than one conformation around the C-2–C-3 bond is populated. This is in line with the few values recorded for the coupling constants between C-3-$H_{pro\text{-}S}$ and C-2-H. We interpret this as indicating that both conformers **5a** and **5b** contribute significantly to the conformer equilibrium.

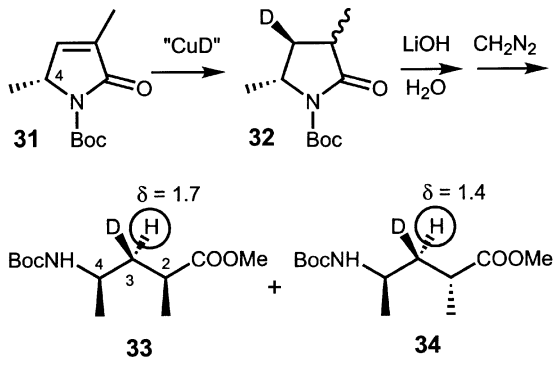
This contrasts with the situation found in calyculin C, where the coupling constants indicate that a single conformer **5a** dominates the conformer equilibrium. This finding cannot simply be ascribed to the presence of a flanking group on the N-terminus of **5**, as the dipeptides **18** and **19** show the same behaviour as the model compound **11**. An extension at the C-terminus of **5**, *viz.* the α,β-unsaturated ester **25**, did not alter the conformational preferences either. This suggests that there is an inherent preference in structures of the type **5** to have a single conformation about the C-3–C-4 bond, whereas the molecules remain biconformational with regard to the C-2–C-3 bond. This may hold as well for the linker unit in deoxybleomycin A_2. In calyculin C, however, there is an additional—as yet undetermined—effect, which renders the linker unit monoconformational also about the C-2–C-3 bond.

In the (2*R**,4*R**) series **6**, deuterium labelling of **34** allowed the assignment of the high-field and low-field C-3-H signals to the individual diastereotopic hydrogen atoms. It is assumed that this assignment holds also for compounds **27**, **29**, and **30**. The coupling constants listed in the table for **29** and **30** are large, both for the coupling between C-3-$H_{pro\text{-}R}$ to C-2-H and of C-3-$H_{pro\text{-}S}$ to C-4-H. This indicates that a single conformer **6a** predominates substantially in the conformer equilibrium, a conformation identical to the conformation found for **29** in the crystalline state.[7,15]

The conformation **6a** corresponds to the arrangement deduced for the helical structure of γ-peptides[16] derived from 4-substituted γ-aminobutyric acids of the type **35**. It may be



Scheme 5



Scheme 6

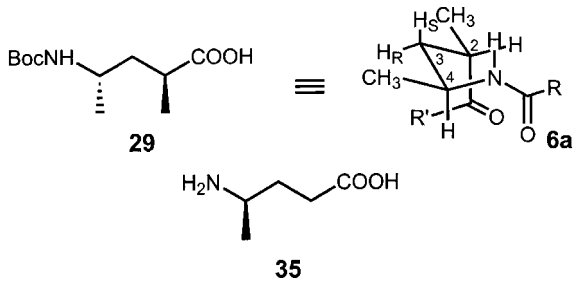
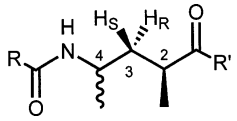
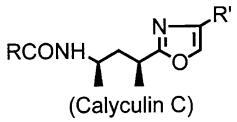
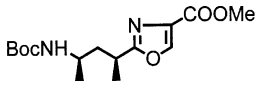
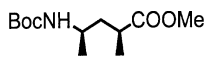
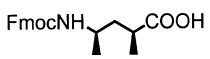
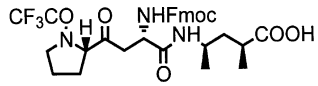
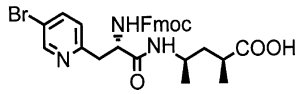
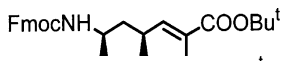
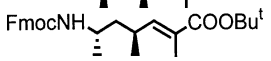
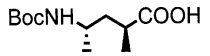
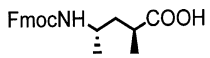


Table 1 Characteristic $^3J_{H,H}$ coupling constants for 4-amido-2,4-dimethylbutyric acid derivatives

		Solvent	T/K	C-3-H$_{(pro-R)}$ δ	^3J to H-2	^3J to H-4	C-3-H$_{(pro-S)}$ δ	^3J to H-2	^3J to H-4
1[a]	 (Calyculin C)	C_6D_6	298	2.18	1.5	13.0	1.84	12.5	1.5
[b]		$CDCl_3$	298	1.94	6.2	9.6	1.68	6.7	n.d.
10		$CDCl_3$	298	1.7	7	10	1.4	7[c]	n.d.
9		$CDCl_3$	333	1.85	7.2	9.6	1.48	6.7[c]	4.8
15		$CDCl_3$	333	1.87	7.2	9.8	1.48	n.d.	n.d.
18		$CDCl_3$	298	1.89	8.0	9.9	1.49	n.d.	n.d.
19		$CDCl_3$	328	1.86	7.6	10.3	1.49	6.5	3.9
25		C_6D_6	298	1.11	6.6	8.7	1.01	7.7	n.d.
27		$CDCl_3$	298	1.48	9.4[c]	n.d.	1.48	5.4[c]	n.d.
29		$CDCl_3$	253	1.75	10.7	3.3	1.4	n.d.	n.d.
30		$CDCl_3$	298	1.80	9.6	4.5	1.43	4.5	9.5

[a] Values from ref. 2. [b] Values from ref. 7. [c] Taken from the coupling pattern of the C-2-H signal. n.d. = not determined.

anticipated that the conformational preference, and hence, the helix-forming and helix-stabilizing abilities will be even more marked in γ-peptides derived from the conformationally preorganized amino acids **6**. This is indeed borne out by a recent study[17] in which an octapeptide derived from γ-amino acids of the type **6** had a helical secondary structure, whereas the analogous octapeptide from γ-amino acids corresponding to the biconformational amino acid **5** did not adopt a helical arrangement.[17]

We conclude that the (*R**,*R**)-4-amido-2,4-dialkylbutyric acid derivatives **6** have a strong tendency to populate a conformation shown as **6a**. In the (*R**,*S**) series, *cf.* **5**, the compounds have a biconformational behaviour. In both conformers, however, the amido substituent is *gauche* to the main chain. This information is valuable for the design of conformationally preorganized linker units for novel functional molecules.

Experimental

All temperatures quoted are not corrected. Reactions were carried out under dry nitrogen or argon. Boiling range of the petroleum ether used is 40–60 °C. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker ARX-200, AC-300 and AMX-500 spectrometers. Spectra were recorded for *ca.* 0.2 mM solutions in $CDCl_3$ (99% d), which was also used as an internal standard. Flash chromatography was run using silica gel Si 60 (40–63 μm, E. Merck AG, Darmstadt).

1. Methyl (2*S**,4*R**)-4-(benzyloxycarbonylamino)-2-methylpentanoate(9)

Triethylamine (0.7 ml, 5 mmol) was added to a solution of (2*R**,4*S**)-2,4-dimethylpentanedioic acid monomethyl ester (**8**, 870 mg, 5.0 mmol) in toluene (3.3 ml). After establishing a nitrogen atmosphere diphenylphosphoryl azide (1.4 g, 5 mmol) was added and the solution was heated to 80 °C. After nitrogen evolution had ceased benzyl alcohol (595 mg, 5.5 mmol) was added and stirring was continued for 6 h at 80 °C. The solvent was removed under reduced pressure, the residue was taken up in diethyl ether (25 ml) and the solution was washed with water (3 × 15 ml). The organic phase was dried ($MgSO_4$) and concentrated. 3,4-Dihydro-2*H*-pyran (220 mg, 2.5 mmol) and a solution of *p*-toluenesulfonic acid (1 mg, 0.005 mmol) in CH_2Cl_2 (5 ml) was added at 0 °C to the residue. After stirring for 10 min at 0 °C and 2.5 h at room temperature brine (15 ml), saturated aqueous $NaHCO_3$ solution (15 ml) and water (30 ml) were added and the mixture was extracted with diethyl ether (3 × 20 ml). The combined organic layers were dried ($MgSO_4$) and concentrated. Flash chromatography of the residue with pentane–diethyl ether (65 : 35) yielded 1.09 g (78%) of the product **9** as a colourless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.18 (d, J = 6.8 Hz, 3 H), 1.20 (d, J = 6.6 Hz, 3 H), 1.52 (m, 1 H), 1.86 (ddd, J = 14.0, 9.9 and 7.5 Hz, 1 H), 2.54 (sextet, J = 6.8 Hz, 1 H), 3.61 (s, 3 H), 3.81 (m, 1 H), 4.61 (br d, J = 7.4 Hz, 1 H), 5.08 (s, 2 H), 7.29–7.35 (m, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 17.8, 22.5, 37.3, 41.2, 46.1, 52.1, 66.9, 128.5, 128.9, 137.0,

156.2, 177.7. HR-MS: $C_{15}H_{21}NO_4^+$ requires 279.1471; found 279.1476.

2. Methyl (2*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-2-methylpentanoate (10)

This compound was prepared as described for **9** using *tert*-butanol as the alcohol component. The product was purified by flash chromatography with pentane–ethyl acetate (4 : 1) to give 43% of **10** as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.06$ (d, $J = 6.6$ Hz, 3 H), 1.11 (d, $J = 7.0$ Hz, 3 H), 1.35 (s, 9 H), 1.40 (m, 1 H), 1.73 (ddd, $J = 14.0$, 10.0, and 7.0 Hz, 1 H), 2.44 (sextet, $J = 7.0$ Hz, 1 H), 3.60 (s, 3 H), 3.67 (m, 1 H), 4.3 (m, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 17.3$, 22.1, 28.3, 36.9, 40.9, 44.9, 51.7, 79.0, 155.3, 177.4. $C_{12}H_{23}NO_4$ requires C 58.75, H 9.45, N 5.71; found C 57.99, H 9.08, N 6.36%.

3. Methyl (2*R**,4*S**)-4-(fluorenylmethoxycarbonylamino)-2-methylpentanoate (11)

This compound was prepared as described for **9** using fluorenylmethanol as the alcohol component. The product was purified by flash chromatography with pentane–ethyl acetate (4 : 1) to give 87% of the product **11**. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.14$–1.19 (m, 6 H), 1.50 (m, 1 H), 1.84 (m, 1 H), 2.49 (sextet, $J = 6.9$ Hz, 1 H), 3.62 (s, 3 H), 3.79 (m, 1 H), 4.18 (m, 1 H), 4.39 (m, 2 H), 4.64 (br d, $J = 8.1$ Hz, 1 H), 7.13–7.40 (m, 4 H), 7.58 (d, $J = 7.4$ Hz, 2 H), 7.74 (d, $J = 7.3$ Hz, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 17.3$, 22.0, 36.9, 40.7, 45.6, 47.3, 51.6, 66.3, 119.9, 124.9, 127.0, 127.6, 141.3, 144.0, 155.8, 177.2. $C_{14}H_{19}NO_4$ requires C 71.83, H 6.71, N 3.75; found C 71.91, H 6.86, N 3.81%.

4. (2*S*,4*R*)-4-(Benzyloxycarbonylamino)-2-methylpentanoic acid (14)

Aqueous LiOH solution (5 M, 5 mL) was added to a solution of **9** (*ca.* 4 mmol) in dimethoxyethane (16 mL) and water (10 mL). After stirring for 30 min water (200 mL) was added and the mixture was extracted with ether (3 × 25 mL). The aqueous layer was acidified with hydrochloric acid (1 M) and extracted with dichloromethane (3 × 25 mL). The combined organic phases were washed with water (3 × 20 mL), dried ($MgSO_4$) and concentrated to give 0.95 g of the acid **14**. $[\alpha]_D^{20} = +6.3$ ($c = 20.19$, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.14$–1.25 (m, 6 H), 1.49 (m, 1 H), 1.85 (m, 1 H), 2.51 (m, 1 H), 3.84 (m, 1 H), 4.83 (br d, $J = 6.6$ Hz, 1 H), 5.07 (m, 2 H), 7.33 (br s, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 17.5$, 22.4, 37.1, 41.0, 45.9, 67.0, 128.4, 128.5, 128.9, 136.9, 156.4, 182.4. HR-MS: $C_{14}H_{19}NO_4^+$ requires 265.1314; found 265.1310.

5. (2*S*,4*R*)-4-(Fluorenylmethoxcarbonylamino)-2-methylpentanoic acid (15)

10% Pd on charcoal (92 mg) was added to a solution of **14** (920 mg, 3.5 mmol) in methanol (25 mL). The suspension was stirred 15 h under an atmosphere of hydrogen. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was taken up in water (14 mL); Na_2CO_3 (1.84 g, 17.4 mmol) was added and the solution was cooled to 0 °C. A solution of *N*-fluorenylmethoxycarbonyloxysuccinimide (Fmoc-OSu,[18] 1.75 g, 5.2 mmol) in dioxane (28 mL) was added in increments over 5 min. The pH of the solution was adjusted to 9 by adding 10% aqueous Na_2CO_3 solution. After stirring for 1 h at 0 °C and 2 h at room temperature the mixture was diluted with water (100 mL) and was washed with diethyl ether–ethyl acetate (1 : 1, 3 × 50 mL). The combined organic layers were extracted with aqueous Na_2CO_3 (2%, 2 × 30 mL) and the combined aqueous layers were brought to pH 1 with aqueous 1 M HCl. The resulting precipitate was dissolved by extraction with ethyl acetate (3 × 30 mL). The organic solution was concentrated and the residue was crystallized from ethyl acetate–pentane (1 : 3) to give 3.24 g (97%) of the product **15** of mp 132–133 °C. $[\alpha]_D^{20} = -0.98$ ($c = 10.2$, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 333 K): $\delta = 1.16$ (m, 6 H), 1.48 (m, 1 H), 1.87 (ddd, $J = 14.1$, 9.8, and 7.8 Hz, 1 H), 2.49 (m, 1 H), 3.80 (m, 1 H), 4.22 (br t, $J = 6.6$ Hz, 1 H), 4.44 (br d, $J = 6.6$ Hz, 2 H), 4.59 (m, 1 H), 7.31 (m, 2 H), 7.39 (t, $J = 7.5$ Hz, 2 H), 7.58 (d, $J = 7.5$ Hz, 2 H), 7.75 (d, $J = 7.5$ Hz, 2 H), 9.96 (br s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 16.9$, 21.9, 36.6, 40.4, 45.3, 47.2, 66.4, 119.9, 125.0, 127.0, 127.6, 141.2, 143.9, 155.9, 182.3. $C_{21}H_{23}NO_4$ requires C 71.37, H 6.56, N 3.96; found C 71.11, H 6.50, N 3.91%.

6. *tert*-Butyl (2*S*)-2-(fluorenylmethoxycarbonylamino)-4-oxo-4-[(2*S*)-1-trifluoroacetylpyrrolidin-2-yl]butyrate

To a solution of (*S*)-*N*-trifluoroacetyl proline (0.249 g, 1.125 mmol) in dry toluene (1 mL) was added freshly distilled oxalyl chloride (0.202 mL, 2.25 mmol) at 0 °C under nitrogen. Adding one drop of dry dimethylformamide started the reaction. After 20 min stirring at 0 °C the gas evolution stopped. Solvents were removed using a rotary evaporator and the resulting solid was redissolved in dry toluene. This operation was repeated twice and the crude (*S*)-*N*-trifluoroacetylproline acid chloride[19] was used directly in the next step.

A solution of β-iodo-*N*-Fmoc-L-alanine *tert*-butyl ester[20] (0.370 g, 0.75 mmol) in dry toluene (3 mL) and dry DMF (0.20 mL) was added to a dry nitrogen purged flask charged with zinc–copper couple[21] (0.090 g). The resulting mixture was stirred at 50 °C under nitrogen for 25 min until no starting material remained (as judged by TLC visualized with Kagi–Mösher solution). Bis(triphenylphosphine)palladium dichloride (0.028 g, 0.04 mmol) was added, followed by the freshly prepared *N*-trifluoroacetylproline acid chloride solution (1.125 mmol) in toluene (1 mL) and the mixture was stirred at 50 °C under nitrogen for a further 90 min. Ethyl acetate (50 mL) was added to the cooled reaction mixture, which was filtered into a separating funnel. Sequential washing with brine (25 mL) and extraction with ethyl acetate (50 mL), followed by drying over anhydrous sodium sulfate, filtration and concentration under reduced pressure gave a crude product. Flash chromatography over silica gel (light petroleum–ethyl acetate 9 : 1) gave *N*-Fmoc-L-alanine *tert*-butyl ester (150 mg, 55%) and the title compound (196 mg, 46%). The resulting solid was recrystallized from dichloromethane by addition of warm *n*-hexane until turbidity was reached to afford 162 mg of fine needles of mp 155–157 °C; $[\alpha]_D^{25} = -26.3$, ($c = 1.09$, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.45$ (s, 9 H), 1.94–2.05 (m, 3 H), 2.16–2.23 (m, 1 H), 3.07 (dd, $J = 18.3$ and 3.7 Hz, 1 H), 3.29 (dd, $J = 18.3$ and 4.6 Hz, 1 H), 3.71–3.76 (m, 1 H), 3.79–3.84 (m, 1 H), 4.24 (t, $J = 7.3$ Hz, 1 H), 4.31–4.38 (m, 2 H), 4.53–4.57 (m, 1 H), 4.61–4.64 (m, 1 H), 5.87 (d, $J = 8.5$ Hz, 1 H), 7.29–7.33 (m, 2 H), 7.40 (t, $J = 7.3$ Hz, 2 H), 7.62 (d, $J = 7.7$ Hz, 2 H), 7.76 (d, $J = 7.3$ Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 24.9$, 27.1, 27.8, 30.0, 42.0, 47.5, 50.4, 65.6, 67.3, 82.5, 116.2 (q, $J = 285$ Hz), 120.0, 125.3, 127.0, 127.7, 141.3, 143.8, 143.9, 155.8 (q, $J = 38$ Hz), 156.2, 169.8, 205.0. IR (film, cm^{-1}): 3362, 1728, 1689, 1152. MS (electrospray): $m/z = 459$. $C_{29}H_{31}F_3N_2O_6$ requires C 62.14, H 5.57, N 5.00; found: C 61.40, H 5.58, N 4.83%.

7. 2-(Fluorenylmethoxycarbonylamino)-4-oxo-4-[(2*S*)-1-(2,2,2-trifluoroacetyl)pyrrolidin-2-yl]butanoic acid (16)

The *tert*-butyl ester described in section 6 (150 mg, 0.27 mmol) was dissolved in a 1 : 1 mixture of dry dichloromethane and trifluoroacetic acid (2 mL). The mixture was stirred at room temperature for 3 h, and then the volatiles were removed to

afford the crude acid (*ca.* 130 mg), which was used in the coupling step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.95$, 2.12 (2 m, 4 H), 3.06 (dd, $J = 15.5$ and 4.0 Hz, 1 H), 3.26 (dd, $J = 15.5$ and 3.8 Hz, 1 H), 3.68 (m, 2 H), 4.16 (t, $J = 7.2$ Hz, 1 H), 4.30 (d, $J = 7.2$ Hz, 2 H), 4.58–4.64 (m, 2 H), 5.88 (d, $J = 8.6$ Hz, 1 H), 6.21 (br s, 1 H), 7.19–7.34 (m, 4 H), 7.53 (d, $J = 7.2$ Hz, 2 H), 7.68 (d, $J = 7.5$ Hz, 2 H). ^{13}C NMR [50 MHz, $(CD_3)_2CO$]: $\delta = 25.0$, 27.0, 41.5, 47.4, 47.8, 49.7, 66.3, 66.8, 116.5 (q, $J = 150.0$ Hz), 120.2, 125.6, 127.4, 128.0, 141.5, 144.4, 154.5 (q, $J = 55.0$ Hz), 156.3, 172.2, 204.1. ^{19}F NMR (188 MHz, $CDCl_3$): $\delta = 73.0$.

8. (2*S*,4*R*)-4-[(1*S*)-1-(Fluorenylmethoxcarbonylamino)-3-oxo-3-[(2*S*)-1-(2,2,2-trifluoroacetyl)pyrrolidine-2-yl]propylcarboxamido]-2-methylpentanoic acid (18)

To a suspension of 2-chlorotrityl chloride resin (200–400 mesh, 1% divinylbenzene crosslinked, 219 mg, *ca.* 0.3 mmol) in dry dichloromethane (1.1 ml) was added diisopropylethylamine (131 µl, 0.75 mmol) and **15** (110 mg, 0.31 mmol). The mixture was stirred over 19 h at room temperature. Methanol (38 µl) and diisopropylethylamine (39 µl) were added and the resin was stirred for an additional 15 min. The resin was washed with dichloromethane (3 × 5 ml) and methanol (3 × 5 ml) and dried in vacuo over KOH. For Fmoc deprotection the resin was treated with a 10% piperidine solution in dichloromethane (3 × 3 ml, 10 min each) and subsequently washed with dimethylformamide (3 × 5 ml), dichloromethane (3 × 5 ml), methanol (3 × 5 ml), and dichloromethane (3 × 5 ml). After drying *in vacuo* 260 mg of the loaded resin was obtained.

A portion of the above resin (105 mg, *ca.* 0.1 mmol) was suspended in a solution of **16** (126 mg, 0.25 mmol) in dimethylformamide (1 ml). Diisopropylethylamine (88 µl, 0.5 mmol) was added and *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (161 mg, 0.5 mmol) in DMF (1 ml) was added. After stirring for 1 day the resin was washed with dimethylformamide (3 × 5 ml), dichloromethane (3 × 5 ml), methanol (3 × 5 ml), and dichloromethane (3 × 5 ml). The resin was dried *in vacuo* and suspended in a 1 : 1 : 3 mixture of trifluoroethanol–acetic acid–dichloromethane (2 ml) for 1 h at room temperature. The resin was washed with the same mixture (2 × 1 ml) and the filtrates were concentrated *in vacuo* to give crude **18**, which was purified by flash chromatography over silica gel with pentane–ethyl acetate–acetic acid (4 : 6 : 0.5) to give 25 mg (41%) of compound **18**, mp 119–120 °C. $[\alpha]_D^{20} = -14.8$ ($c = 0.27$, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.12$ (d, $J = 6.5$ Hz, 3 H), 1.18 (d, $J = 6.8$ Hz, 3 H), 1.49 (m, 1 H), 1.89 (ddd, $J = 14.2$, 9.9, and 8.0 Hz, 1 H), 2.00–2.23 (m, 4 H), 2.46 (m, 1 H), 2.87 (dd, $J = 18.1$ and 4.8 Hz, 1 H), 3.35 (m, 1 H), 3.72 (m, 2 H), 4.00 (m, 1 H), 4.23 (t, $J = 7.0$ Hz, 1 H), 4.40 (m, 2 H), 4.57 (m, 1 H), 4.62 (dd, $J = 8.4$ and 4.6 Hz, 1 H), 6.24 (d, $J = 9.0$ Hz, 1 H), 6.45 (d, $J = 8.2$ Hz, 1 H), 7.31 and 7.39 (2t, $J = 7.5$ Hz, 4 H), 7.61 (m, 2 H), 7.76 (d, $J = 7.5$ Hz, 2 H), 7.76 (d, $J = 7.5$ Hz, 2 H), 7.96 (br s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 17.4$, 20.8, 24.8, 27.0, 37.1, 40.1, 41.7, 44.5, 47.0, 47.5, 51.0, 65.8, 67.5, 115.2 (q, $J = 285.0$ Hz), 120.0, 125.2, 127.1, 127.8, 141.3, 143.7, 155.8 (q, $J = 37.5$), 156.5, 170.0, 181.5, 206.3. $C_{31}H_{34}N_3O_7F_3$ requires C 60.29, H 5.55, N 6.80; found C 60.10, H 5.33, N 6.67%.

9. Methyl (2*S*)-2-(fluorenylmethoxycarbonylamino)-3-(5′-bromo-2′-pyridyl)propionate

Zinc dust (0.27 g, 4.2 mmol) was heated to *ca.* 100 °C in a 50 mL flask with side arm, then evacuated and flushed with nitrogen 3 times. Dry DMF (0.5 mL) and 1,2-dibromoethane (18 µL, 0.21 mmol) were added and the mixture was heated in a hot water bath (90 °C) with vigorous stirring for 30 min. The reaction mixture was allowed to cool. Trimethylsilyl chloride (5 µL, 0.042 mmol) was added and the mixture was allowed to stir for a further 30 min. Methyl (2*S*)-2-(fluorenylmethoxycarbonylamino)-3-iodopropionate[20] (0.31 g, 0.7 mmol) was dissolved in dry DMF (0.9 mL), and transferred *via* syringe to the reaction mixture, which was then heated to 35 °C. Monitoring the reaction by TLC, using petroleum ether–ethyl acetate (2 : 1), showed complete consumption of starting material after 1 h. 2,5-Dibromopyridine (0.22 g, 0.93 mmol) was added, followed by bis(triphenylphosphine)-palladium(II) chloride (0.026 g, 0.037 mmol). The reaction was heated at 50 °C for 3 h and then allowed to cool to room temperature. The mixture was diluted with EtOAc (50 mL), washed with water (30 mL) and with brine (30 mL), dried with $MgSO_4$ and filtered through a Celite layer. Flash column chromatography over silica gel, with an appropriate petroleum ether–ethyl acetate gradient, furnished the title compound (0.20 g, 60%), mp 99–101 °C. $[\alpha]_D^{20} + 22.5$ ($c = 1$, CH_2Cl_2) IR (cap. film, cm^{-1}): 3333 (N–H), 3064 (Ar–H), 2951 (Me), 1721 (C=O), 1516 (NH), 1450 (=C–H), 759 (3H adj), 739 (4H adj). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.29$ (dd, $J = 4.5$ and 15.0 Hz, 1 H), 3.34 (dd, $J = 5.5$ and 15.0 Hz, 1 H), 3.72 (s, 3 H), 4.22 (t, $J = 7.0$ Hz, 1 H), 4.37 (d, $J = 7.0$ Hz, 2 H), 4.78–4.74 (m, 1 H), 6.04 (d, $J = 7.6$ Hz, 1 H), 7.03 (d, $J = 8.2$ Hz, 1 H), 7.30 (t, $J = 7.6$ Hz, 2 H), 7.40 (t, $J = 7.6$ Hz, 2 H), 7.57 (t, $J = 7.6$ Hz, 2 H), 7.76–7.73 (m, 3 H), 8.58 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 172.1$, 155.7, 155.0, 150.2, 143.9, 141.3, 139.4, 127.7, 127.0, 125.1, 125.1, 119.9, 118.6, 67.1, 53.2, 52.5, 47.2, 38.4. MS (EI): $m/z = 482$ (M^+, 20%), 480 (M^+, 19), 421 ($M^+ - CO_2Me$, 12), 423 ($M^+ - CO_2Me$, 12), 284–286 (M^+ – fluorenylmethyloxy, 80–82), 257–259 (M^+ – Fmoc, 32), 178 ($FmocCH_2$, 100), 165 (fluorene$^+$, 62). $C_{24}H_{21}N_2O_4Br$ requires C 59.9, H 4.4, N 5.8; found: C 60.1, H 4.1, N 5.8%.

10. (2*S*)-2-(Fluorenylmethoxycarbonylamino)-3-(5′-bromo-2′-pyridyl)propionic acid (17)

The methyl ester obtained in section 9 (0.57 g, 1.19 mmol) was dissolved in THF (12 mL) and aqueous LiOH (0.2 N, 12 mL, 2.4 mmol) was added. The reaction was stirred at room temperature and monitored by TLC. After complete consumption of the starting material, the solvent was removed under reduced pressure. The residue was dissolved in a minimum of water (10 mL) and then washed with EtOAc (5 mL). The aqueous phase was acidified to pH 3.8 with AcOH. The product was extracted with EtOAc (2 × 10 mL). The combined extracts were dried with $MgSO_4$ and concentrated by rotary evaporation, yielding the carboxylic acid **17** (0.49 g, 90%) as a white solid, mp 219–221 °C. IR (KBr disc, cm^{-1}): 3321 (N–H and O–H), 3037 (Ar–H), 1695 (C=O), 1421 (=C–H), 838 (2H adj), 757 (4H adj). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.40$–3.34 (m, 2 H), 4.23 (t, $J = 7.0$ Hz, 1 H), 4.41 (dd, $J = 10.5$ and 6.5 Hz, 1 H), 4.48 (dd, $J = 10.5$ and 7.0 Hz, 1 H), 4.62–4.56 (m, 1 H), 5.97–5.90 (br s, 1 H), 7.09 (d, $J = 8.0$ Hz, 1 H), 7.33 (t, $J = 7.5$ Hz, 2 H), 7.42 (d, $J = 7.5$ Hz, 2 H), 7.59 (d, $J = 7.5$ Hz, 2 H), 7.78 (d, $J = 7.5$ Hz, 2 H), 7.85 (d, $J = 8.0$ Hz, 1 H), 8.65 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 175.5$, 172.7, 155.6, 146.2, 143.7, 141.3, 139.3, 127.7, 127.1, 125.7, 125.1, 123.0, 119.9, 66.8, 52.3, 47.2, 38.1. MS (EI): $m/z = 468$–466 (M^+, 13%), 450–448 ($M^+ - H_2O$, 63), 244–242 (M^+ – Fmoc, 25), 229–227 (M^+ – NHFmoc, 80), 196 (fluorenylmethyloxy$^+$, 17), 178 ($FmocCH_2$, 100), 165 (fluorene$^+$, 80). HR-MS: $C_{23}H_{19}N_2O_4Br^+$ requires 466.0528; found 466.0497.

11. (2*S*,4*R*)-4-[(1*S*)-1-(Fluorenylmethoxycarbonylamino)-2-(5-bromo-2-pyridyl)ethylcarboxamidol-2-methylpentanoic acid (19)

This material was prepared from another portion of the resin obtained in section 6 by coupling with the acid **17** (117 mg, 0.25 mmol) to give 21 mg (37%) of compound **19**, mp 80–

81 °C. $[\alpha]_D^{20} = +6.3$ ($c = 0.48$, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 328 K): $\delta = 1.06$ (d, $J = 6.5$ Hz, 3 H), 1.15 (d, $J = 6.8$ Hz, 3 H), 1.49 (ddd, $J = 14.0$, 6.5, and 3.9 Hz, 1 H), 1.86 (ddd, $J = 14.0$, 10.3 and 7.6 Hz, 1 H), 2.38 (m, 1 H), 3.17 (dd, $J = 14.3$ and 6.9 Hz, 1 H), 3.23 (dd, $J = 14.3$ and 5.4 Hz, 1 H), 4.04 (m, 1 H), 4.10 (t, $J = 6.7$ Hz, 1 H), 4.32 (d, $J = 6.7$ Hz, 2 H), 4.59 (m, 1 H), 5.96 (br s, 1 H), 6.39 (d, $J = 7.1$ Hz, 1 H), 6.61 (d, $J = 8.2$ Hz, 1 H), 7.26–7.75 (m, 1 H), 8.53 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 17.7$, 20.6, 22.0, 25.4, 29.7, 44.4, 47.0, 66.5, 67.3, 140.3, 141.3, 149.4, 156.1, 156.4, 170.3, 180.6. $C_{29}H_{30}N_3O_5Br$ requires C 60.01, H 5.21, N 7.24; found C 59.89, H 5.15, N 7.17%.

12. *tert*-Butyl (2*E*,4*S*,6*R*)-6-methoxycarbonyl-2,4-dimethyl-2-heptenoate (23)

To a solution of the phosphorane **22**[11] (1.95 g, 5.0 mmol) in CH_2Cl_2 (9 mL) was added a solution of the aldehyde **21**[10] (668 mg, 4.2 mmol) in CH_2Cl_2 (4 mL). After heating at reflux for 19 h the solvents were removed and the residue was triturated with pentane (50 mL). Triphenylphosphine oxide was removed by filtration and the filtrate was concentrated *in vacuo*. Flash chromatography of the residue with pentane–ethyl acetate (9 : 1) furnished 870 mg (76%) of **23** as a colourless oil. $[\alpha]_D^{22} = +5.1$ ($c = 2.4$, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): $\delta = 0.96$ (d, $J = 6.6$ Hz, 3 H), 1.08 (d, $J = 6.9$ Hz, 3 H), 1.60 (m, 1 H), 1.44 (s, 9 H), 1.70 (m, 1 H), 1.74 (d, $J = 1.1$ Hz, 3 H), 2.40 (m, 1 H), 2.50 (m, 1 H), 3.60 (s, 3 H), 6.33 (d, $J = 10.1$ Hz, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 12.5$, 17.4, 20.1, 28.1, 31.2, 37.6, 40.5, 51.5, 80.0, 128.4, 145.5, 167.6, 177.0. HR-MS: $C_{15}H_{26}NO_4^+$ requires 270.1831; found 270.1819.

***tert*-Butyl (2*E*,4*S*,6*S*)-6-(methoxycarbonyl-2,4-dimethyl-2-heptenoate.** The half-ester **26** was converted to the corresponding aldehyde[12] and subjected to the Wittig reaction as above to give 58% of the title compound. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.99$ (d, $J = 6.7$ Hz, 3 H), 1.14 (d, $J = 7.0$ Hz, 3 H), 1.35 (m, 1 H), 1.49 (s, 9 H), 1.75 (d, $J = 1.3$ Hz, 3 H), 1.82 (m, 1 H), 2.39 (m, 1 H), 2.52 (m, 1 H), 3.67 (s, 3 H), 6.37 (d, $J = 10.2$ Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.4$, 17.9, 20.2, 28.1, 31.3, 37.5, 40.9, 51.4, 79.9, 128.5, 145.5, 167.5, 177.0.

13. *tert*-Butyl (2*E*,4*S*,6*R*)-6-(fluorenylmethoxycarbonyl-amino)-2,4-dimethyl-2-heptenoate (25)

Aqueous lithium hydroxide (5 M, 2.8 mL, 14 mmol) was added to a solution of the diester **23** (592 mg, 2.2 mmol) in dimethoxyethane (9 mL) and water (5.6 mL). After stirring for 17 h the mixture was diluted with water (10 mL) and extracted with diethyl ether (3 × 10 mL). The aqueous layer was acidified at 0 °C with aqueous hydrochloric acid and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine (3 × 5 mL), dried ($MgSO_4$) and concentrated to leave 518 mg (92%) of the carboxylic acid as a colourless oil. $[\alpha]_D^{22} = +7.8$ ($c = 2.56$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.99$ (d, $J = 6.6$ Hz, 3 H), 1.14 (d, $J = 7.0$ Hz, 3 H), 1.30–1.40 (m, 1 H), 1.45 (s, 9 H), 1.70–1.80 (m, 1 H), 1.77 (d, $J = 1.4$ Hz, 3 H), 2.40 (m, 1 H), 2.50 (m, 1 H), 6.36 (d, $J = 10.1$ Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.5$, 17.0, 20.0, 28.1, 30.9, 37.3, 40.0, 80.1, 128.6, 145.2, 167.6, 182.8.

The acid obtained was dissolved in toluene (1.3 mL). Triethylamine (0.3 mL, 2.0 mmol) and diphenylphosphorylazide (0.43 mL, 2.0 mmol) were added. The mixture was heated to 80 °C and after 2 h, when nitrogen evolution had ceased, fluorenylmethanol (784 mg, 4.0 mmol) was added. After 6 h at 80 °C the solvents were removed under reduced pressure and the residue was taken up in diethyl ether (20 mL). The solution was washed with water (3 × 5 mL), dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography with pentane–ethyl acetate (4 : 1) afforded 683 mg (75%) of the ester **25** as colourless crystals, mp 63–64 °C. $[\alpha]_D^{22} = +27.1$ ($c = 3.06$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.95$ (d, $J = 6.5$ Hz, 3 H), 1.06 (d, $J = 6.3$ Hz, 3 H), 1.30–1.40 (m, 2 H), 1.40 (s, 9 H), 1.71 (d, $J = 1.4$ Hz, 3 H), 2.40 (m, 1 H), 3.60 (m, 1 H), 4.14 (t, $J = 6.6$ Hz, 1 H), 4.33 (d, $J = 6.8$ Hz, 2 H), 4.40 (m, 1 H), 6.38 (d, $J = 9.9$ Hz, 3 H), 7.20 (dt, $J = 7.4$ and 1 Hz, 2 H), 7.30 (t, $J = 7.4$ Hz, 2 H), 7.50 (d, $J = 7.4$ Hz, 2 H), 7.70 (d, $J = 7.4$ Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.7$, 19.9, 21.9, 28.3, 30.4, 44.2, 47.5, 66.5, 80.2, 120.1, 125.1, 127.1, 127.8, 141.5, 144.2, 145.8, 155.2, 165.6. HR-MS: $C_{28}H_{35}NO_4^+$ requires 449.2566; found 449.2562.

***tert*-Butyl (2*E*,4*S*,6*S*)-6-(fluorenylmethoxycarbonylamino)-2,4-dimethyl-2-heptenoate (27).** This compound was prepared in an analogous manner from the corresponding *tert*-butyl (2*E*,4*S*,6*S*)-6-methoxycarbonyl-2,4-dimethylheptenoate. Mp 94–95 °C. $[\alpha]_D^{20} = +16.3$ ($c = 1.53$, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.99$ (d, $J = 6.3$ Hz, 3 H), 1.12 (d, $J = 6.3$ Hz, 3 H), 1.48 (m, 11 H), 1.75 (d, $J = 1.3$ Hz, 3 H), 2.57 (m, 1 H), 3.60 (m, 1 H), 4.19 (t, $J = 6.7$ Hz, 1 H), 4.39 (d, $J = 6.9$ Hz, 2 H), 4.67 (m, 1 H), 6.40 (d, $J = 10.0$ Hz, 1 H), 7.29 (dt, $J = 7.4$ and 1.1 Hz, 2 H), 7.38 (t, $J = 7.2$ Hz, 2 H), 7.58 (t, $J = 7.3$ Hz, 2 H), 7.74 (d, $J = 7.4$ Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.4$, 20.0, 21.5, 28.0 30.2, 43.8, 47.3, 66.2, 79.9, 119.8, 124.9, 126.9, 127.5, 128.4, 141.2, 143.9, 145.4, 155.5, 167.4. HR-MS: $C_{28}H_{35}NO_4$ requires 449.2566; found 449.2560.

14. (2*S*,4*S*)-4-(Fluorenylmethoxycarbonylamino)-2-methyl-pentanoic acid (30)

To a solution of (2*S*,4*S*)-4-*tert*-butoxycarbonylamino-2-methyl-2-pentanoic acid (**29**)[7] (1.16 g, 5.0 mmol) in CH_2Cl_2 (25 mL) was added dropwise at 0 °C trifluoroacetic acid (4.59 mL, 60 mmol). After stirring for 1 h at room temperature the solution was concentrated, diethyl ether (15 mL) was added and the solution was concentrated again. The residue was taken up in water (20 mL), Na_2CO_3 (2.65 g, 25 mmol) was added and the mixture was cooled to 0 °C. A solution of Fmoc-OSu[18] (2.53 g, 7.5 mmol) in dioxane (35 mL) was added in several increments over 5 min. The pH of the solution was adjusted to 9 by adding 10% aqueous Na_2CO_3 solution. The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. Water (130 mL) was added and the aqueous solution was extracted with diethyl ether–ethyl acetate (1 : 1, 250 mL). The combined organic extracts were washed with 2% aqueous Na_2CO_3 solution (125 mL) and the aqueous layers were acidified to pH 1 with 1 M aqueous hydrochloric acid. The precipitated white solid was extracted with ethyl acetate (2 × 250 mL), the extracts were concentrated and the residue was crystallized from ethyl acetate–hexane (1 : 3, 60 mL) to give 1.57 g (89%) of the desired acid, mp = 102 °C. $[\alpha]_D^{20} = +18.9$ ($c = 1.5$, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.15$ (d, $J = 6.6$ Hz, 3 H), 1.18 (d, $J = 7.6$ Hz, 3 H), 1.40–1.58 (m, 1 H), 1.80 (ddd, $J = 13.9$, 9.1 and 4.8 Hz, 1 H), 2.40–2.60 (m, 1 H), 3.50–3.88 (m, 1 H), 4.21 (br t, $J = 6.1$ Hz, 1 H), 4.43 (br d, $J = 6.0$ Hz, 2 H), 4.73 (br d, $J = 7.8$ Hz, 1 H), 7.28 (t, $J = 7.4$ Hz, 2 H), 7.39 (t, $J = 7.3$ Hz, 2 H), 7.58 (d, $J = 7.4$ Hz, 2 H), 7.75 (d, $J = 7.4$ Hz, 2 H), 10.2 (br s, 1 H). Irradiation at $\delta = 3.8$ collapses the 4.8 Hz coupling at $\delta = 1.80$. Irradiation at $\delta = 2.4$ collapses the 4.5 Hz coupling at $\delta = 1.43$, ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 17.4$, 21.3, 36.4, 41.0, 45.6, 47.3, 66.5, 119.8, 125.0, 127.1, 127.7, 141.3, 143.1, 156.3, 180.7. $C_{21}H_{23}NO_4$ (353.4) requires C 71.37, H 6.56, N 3.96; found C 70.73, H 6.76, N 3.89%.

15. (3*R*/*S*,4*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-4-deutero-3,5-dimethylpyrolidine-2-one (32)

Methanol-OD (0.24 mL, 6 mmol) was added to a solution of lithium aluminium deuteride (84 mg, 2 mmol) in THF (2 mL).

The mixture was stirred for 30 min and added to a suspension of CuBr (123 mg, 0.86 mmol) in THF (3.1 mL) at 0 °C. After stirring for 30 min the resulting dark brown suspension was cooled to −78 °C and a solution of (5*R*)-1-*tert*-butoxycarbonyl-3,5-dimethyl-3-pyrrolin-2-one (**31**)[6,7] (95 mg, 0.45 mmol) in THF (1 mL) was added rapidly. The mixture was allowed to warm slowly to −20 °C and was stirred at this temperature for 90 min. Methanol (2 mL) was added and the mixture was poured into brine (10 mL). The mixture was extracted with ether (3 × 5 mL) and the combined organic phases were washed with water (2 × 3 mL), dried ($MgSO_4$) and concentrated. Purification of the yellow residue (73 mg) by flash chromatography with pentane–ethyl acetate (4 : 1) afforded a 1 : 1.5 mixture of the epimeric pyrrolidinones **32** (22 mg, 60%) as a colourless oil. The NMR data were consistent with those given in ref. 7.

16. Methyl (2*R*/*S*,3*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-deutero-2-methylpentanoate (33 and 34)

Aqueous lithium hydroxide (1 M, 0.26 mL) was added to a solution of the pyrrolidinones **32** (18 mg, 0.084 mmol) in THF (0.42 mL) at 0 °C. After stirring for 6 h at 0 °C water (10 mL) was added, the phases were separated and the aqueous phase was extracted with diethyl ether (3 × 5 mL). The aqueous layer was acidified at 0 °C with aqueous hydrochloric acid and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine (3 × 3 mL), dried ($MgSO_4$), and concentrated to afford 15 mg (77%) of acids, which were taken up in diethyl ether (1 mL). A solution of diazomethane in ether was added dropwise until the yellow colour persisted. The solution was stirred for 16 h and concentrated to afford 16 mg (100%) of a mixture of the esters **33** and **34** as a colourless oil. The esters were separated by preparative HPLC using pentane–*tert*-butyl methyl ether (8 : 2) as eluent.

Methyl (2*S*,3*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-deutero-2-methylpentanoate (33). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (d, J = 6.5 Hz, 3 H), 1.11 (d, J = 7.0 Hz, 3 H), 1.49 (s, 9 H), 1.71 (dd, J = 10.0 and 7.4 Hz, 1 H), 2.44 (quint, J = 7.0 Hz, 1 H), 3.61 (s, 3 H), 3.68 (m, 1 H), 4.20 (m, 1 H).

Methyl (2*R*,3*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-deutero-2-methylpentanoate (34). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.02 (d, J = 6.6 Hz, 3 H), 1.10 (d, J = 7.1 Hz, 3 H), 1.37 (s, 9 H), 1.40 (m, 1 H), 2.50 (quint, J = 7 Hz, 1 H), 3.61 (s, 3 H), 3.60 (m, 1 H), 4.20 (m, 1 H).

Acknowledgement

This work has been supported by the European Commission through the TMR network ERB FMRX CT-960011.

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Paper a910179i