Total Synthesis of the Cyclic Peptide Argyrin B

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Keywords: Dehydroalanine / Immunology / Peptides / Total synthesis / Tryptophan

The details of the total synthesis of Argyrin B, a natural product with immunosuppressive properties, are reported below. The two most unusual amino acid residues of this cyclic peptide are 4-methoxytryptophan and dehydroalanine, which were obtained by modifying known synthetic methods. The linear peptide was assembled in a most convergent fashion then cyclisation followed by generation of the sensitive dehydroalanine afforded synthetic Argyrin B (**1**).
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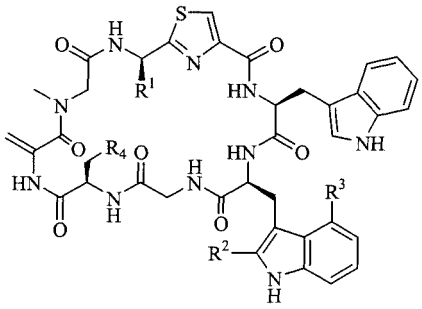
Introduction

During the screening of myxobacteria[1] as a potential source of new antibiotics, a group of cyclic peptides were isolated from the *Archangium gephyra* strain and named the Argyrins (Figure 1).[2] Further biological evaluation at Novartis identified Argyrin B (**1**) as an immunosuppressant and a possible drug development candidate in xenotransplantation.[2,3]



Argyrin	R^1	R^2	R^3	R^4
A	CH_3	H	OCH_3	H
B (**1**)	CH_3	H	OCH_3	CH_3
C	CH_3	CH_3	OCH_3	H
D	CH_3	CH_3	OCH_3	CH_3
E	CH_3	H	H	H
F	CH_2OH	H	OCH_3	H
G	CH_2OH	H	OCH_3	CH_3
H	H	H	OCH_3	H

Figure 1. Structure of the Argyrins A-H

By using known biological assays for both murine[4–6] and human B-cells,[7,8] Argyrin B was shown to be a potent inhibitor of T-cell independent antibody formation. Similarly the two way murine mixed-lymphocyte reaction (MLR), a cellular model for alloantigenic-mediated T-cell activation and proliferation,[9] was also inhibited by Argyrin B. Cytotoxicity was low, since it did not affect the proliferation of human Jurkat T-cells. Argyrin B was further shown to be a potent inhibitor of in vitro IgG production by CD40 L-stimulated murine and human B-cells.

The structure of Argyrin B (**1**) and its congeners was determined by a combination of NMR spectroscopy, chemical degradation and X-ray crystallographic analysis.[10] Argyrin B (**1**) is a non-ribosomal bicyclic octapeptide with the cyclic dipeptide 2-(1-aminoethyl)thiazole resulting from the heterocyclisation of cysteine to the preceding carboxy group of alanine.[11] The most unusual features in this molecule are the novel 4-methoxytryptophan and the dehydroalanine residues. Both tryptophans have the L-configuration while aminobutyric acid and the alanine-derived thiazole have the D-configuration. Another unusual component is the sarcosine unit, which is an *N*-methylated amino acid.

Results and Discussion

Owing to its interesting biological profile and these unusual structural features, we have embarked on a total synthesis of **1** and here wish to report full details of these efforts.[12] In order to undertake this task we chose to use a solution-phase synthesis as being the most reasonable approach to a molecule with this level of complexity. Although a large variety of alternative coupling strategies for the various amino acid fragments could be conceived, we devised a plan, outlined in Figure 2, that would protect the stereogenic centres at crucial stages of the synthesis. This route also facilitates deprotection reactions, coupling methods and the important late stage cyclisation process.

The route required, therefore, the preparation of suitably protected fragments such as the thiazole **2**, the tripeptide **3** and the tripeptide **4** containing the dehydroalanine com-

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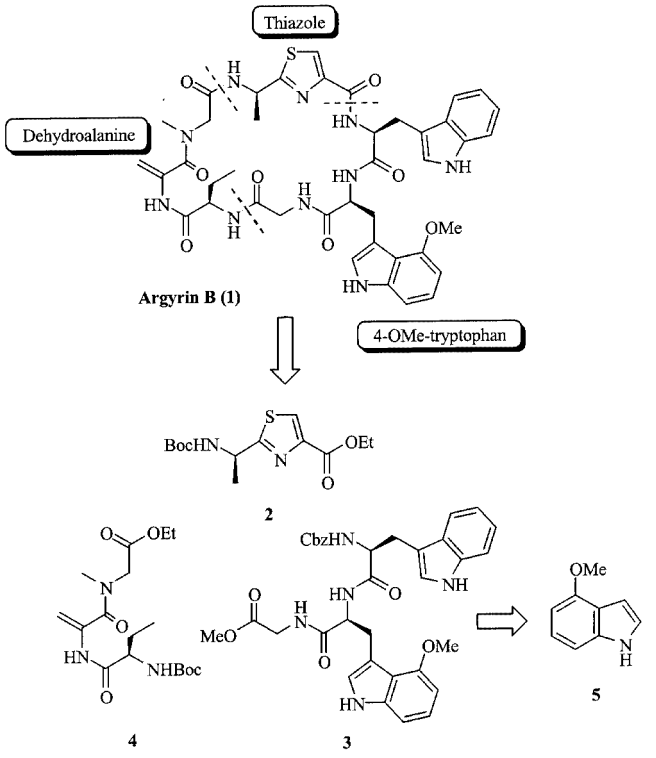
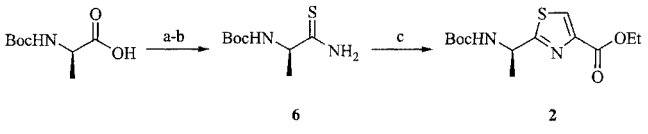


Figure 2. Retrosynthetic analysis of Argyrin B

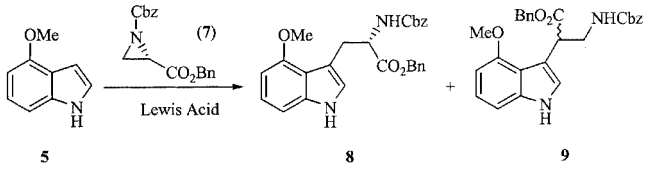
ponent or a synthetic equivalent. Tripeptide **3** incorporates the 4-methoxytryptophan unit, which itself derives from 4-methoxyindole (**5**). The α-centre to the *C*-terminus of all three fragments is achiral and therefore no epimerisation problems arise during fragment coupling.

For the preparation of **2**, we followed a straightforward route[13] from *N*-Boc-D-alanine that involves transformation to the thioamide **6** via the intermediate amide and thiolation with Belleau's reagent[14] (Scheme 1). Compound **6** was then reacted with ethyl bromopyruvate to afford an intermediate which, on treatment with trifluoroacetic anhydride and 2,6-lutidine, gave the thiazole **2**.[15]

Next, the tripeptide **3** was assembled but this first required us to develop an efficient synthesis of the amino acid 4-methoxy-L-tryptophan. Initially we chose to rely on the known regioselective opening of aziridine **7**[16] by 4-methoxyindole in the presence of a Lewis acid such as $Zn(OTf)_2$ [17] or $Sc(OTf)_3$ [18] (Scheme 2). Despite steric and electronic factors favouring the nucleophilic attack at the methylene centre of **7**, we obtained a mixture of regioisomers **8** and **9** which were extremely difficult to separate; the combined yield was below 50%.

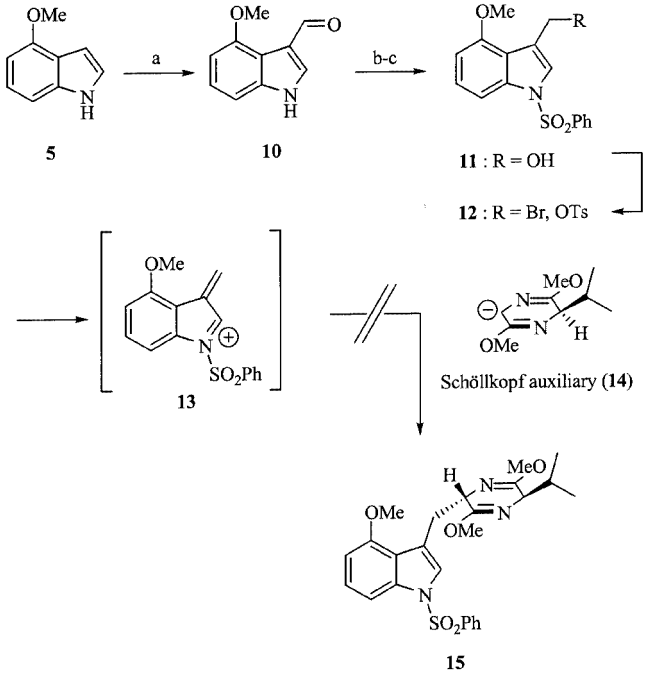


Scheme 1. Synthesis of fragment **2**; reagents and conditions: (a) EDC, HOBt, NH_3, CH_2Cl_2, 0 °C; (b) Belleau's reagent, THF, 0 °C (quantitative, 2 steps); (c) i. $BrCH_2COCO_2Et$, $KHCO_3$, DME; ii. TFAA, 2,6-lutidine, DME, −15 °C (41%)



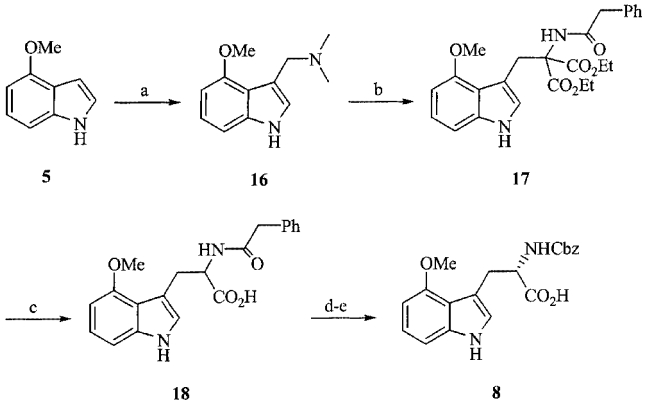
Scheme 2. Synthesis of 4-methoxytryptophan with aziridine

We next examined the use of the Schöllkopf auxiliary to build the stereogenic centre of the amino acid (Scheme 3).[19,20] Indole **5** was converted in three steps to **11**. We then anticipated that conversion of the alcohol to a suitable leaving group followed by substitution with the chiral auxiliary **14** should generate adduct **15**. However, all attempts to isolate the intermediate **12** or to react it in situ with **14** resulted in decomposition of the starting material. We attribute these results to the high instability of the carbocation intermediate **13** compared to the relatively low reactivity of **14** under the reaction conditions.



Scheme 3. Synthesis of 4-methoxytryptophan with Schöllkopf auxiliary; reagents and conditions: (a) $POCl_3$, DMF, room temp. (84%); (b) NaH, $PhSO_2Cl$, THF, room temp. (97%); (c) $NaBH_4$, $EtOH/CH_2Cl_2$, room temp. (68%)

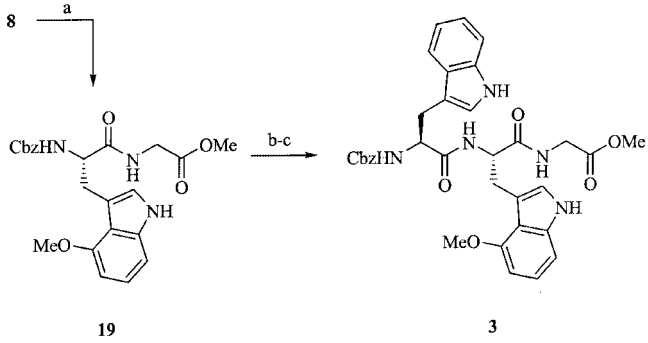
The third approach relied on an enzyme resolution route using an immobilised Penicillin G acylase.[21,22] This required the synthesis of the racemic 4-methoxytryptophan derivative **18** (Scheme 4). Indole **5** was converted quantitatively to the gramine derivative **16**. Methylation followed by quenching with the enolate of 2-phenylacetylaminomalonic acid diethyl ester gave **17**. Saponification of the diester and decarboxylation gave pure racemic phenylacetamide **18** after recrystallisation. The three-step sequence from indole **5** was readily performed on a ten gram scale, without the need for column chromatography, in an overall 56% yield.



Scheme 4. Synthesis of 4-methoxy-L-tryptophan by enzymatic resolution; reagents and conditions: (a) $CH_2NMe_2^+ \cdot I^-$, CH_3CN, room temp. (99%); (b) $(C_2H_5O_2C)_2CHNHCOCH_2Ph$, EtONa then **16** and $MeSO_4Me$, EtOH, room temp. (82%); (c) i. NaOH, MeOH/dioxane, 50 °C; ii. dioxane, 100 °C; iii. NaOH, MeOH/dioxane, 50 °C (69%); (d) Penicillin G acylase immobilised, MeOH/H_2O, room temp.; (e) CbzCl, $NaHCO_3$, THF/H_2O, room temp. (44%, 2 steps)

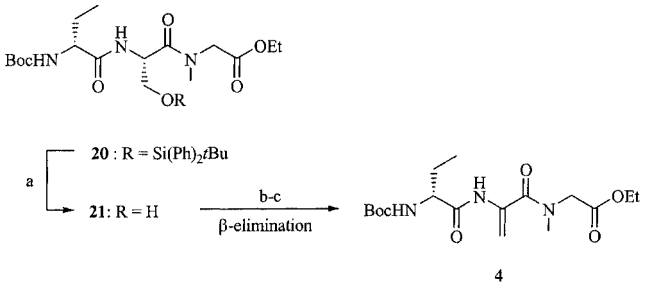
Phenylacetamide **18** was treated with Penicillin G acylase on the solid support, and, following filtration, washing of the aqueous solution to remove unchanged starting material and trapping of the free amino acid with CbzCl, provided carbamate **8** in a 44% yield (maximum yield 50%). We did not investigate other methods, most notably the recently published asymmetric hydrogenation which gives access to both configurations of tryptophan derivatives.[23,24]

This amino acid was then coupled with methyl glycine by standard peptide coupling techniques and, after removal of the Cbz group, reacted with N^{α}-Cbz-L-tryptophan to afford the tripeptide **3** in excellent yield (Scheme 5). The high enantioselectivity of the enzymatic hydrolysis was confirmed by ^1H NMR spectroscopy of tripeptide **3**, which showed only one diastereoisomer to be present.



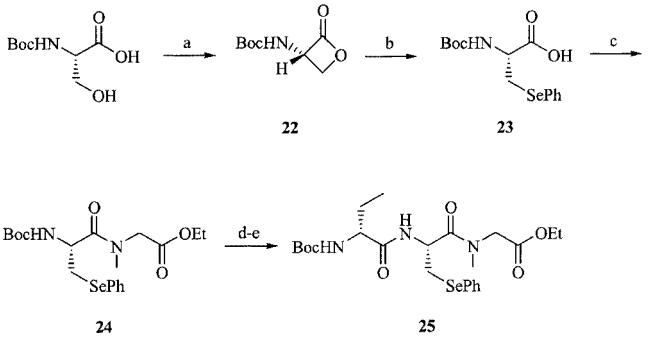
Scheme 5. Synthesis of fragment **3**; reagents and conditions: (a) HCl·Gly-OMe, EDC, HOBt, DIPEA, CH_2Cl_2, room temp. (94%); (b) H_2, Pd/C, MeOH/aqueous HCl, room temp.; (c) N^{α}-Cbz-L-Trp, EDC, HOBt, DIPEA, CH_2Cl_2, room temp. (81%, 2 steps)

Finally, the remaining coupling component **4** required synthesis with dehydroalanine introduced as a masked form, since this is known to be unstable and prone to nucleophilic attack. Most routes affording dehydroalanine in peptide synthesis rely on the β-elimination of serine or cysteine as the final step. Therefore, as a first approach to the third fragment, we prepared the tripeptide **20** in which the serine side chain was protected as a silyl ether (Scheme 6). Although a model deprotection-dehydration sequence was successful with **20** in generating dehydroalanine **4**, all our attempts to synthesise the real cyclic peptide failed to provide the target molecule.



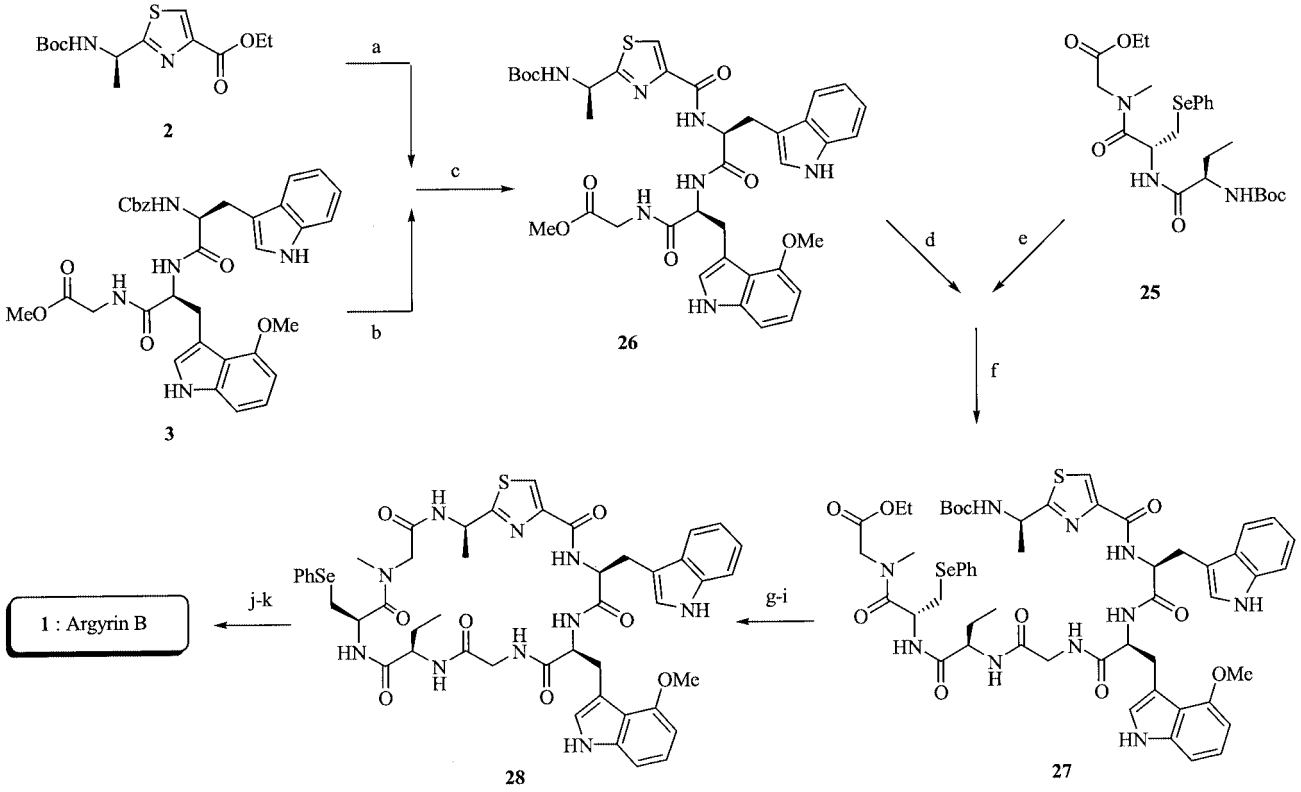
Scheme 6. Model study for dehydroalanine formation; reagents and conditions: (a) $nBu_4N^+ \cdot F^-$, THF; (b) MsCl, NEt_3, CH_2Cl_2; (c) NEt_3, CH_2Cl_2

Consequently, we decided to use phenylselenocysteine to generate dehydroalanine via oxidative β-elimination.[25,26] The tripeptide **25**, containing the phenylseleno group, was constructed again following established protocols from *N*-Boc-L-Serine (Scheme 7). This route proceeded via β-lactone **22**,[27] which, after ring opening, afforded selenide **23**. This was coupled with ethyl sarcosine using PyBroP® [28] to give **24** which, after deprotection to remove the Boc group, was coupled with *N*-Boc-D-aminobutyric acid to give **25**.



Scheme 7. Synthesis of fragment **4** equivalent; reagents and conditions: (a) DEAD, PPh_3, THF, −78 °C to room temp.; (b) PhSeSePh, $NaBH(OMe)_3$, EtOH then **22**, room temp. (41%, 2 steps); (c) HCl-Sar-OEt, PyBroP©, DIPEA, CH_2Cl_2, room temp. (80%); (d) TFA/CH_2Cl_2, room temp.; (e) Boc-D-Abu, EDC, HOBt, DIPEA, CH_2Cl_2, room temp. (84%, 2 steps)

With all the fragments in place, we could now apply the proposed end-game strategy (Scheme 8). Removal of the Cbz protection from **3** and coupling with the free acid from **2** gave pentapeptide **26** in 96% yield. Similarly, hydrolysis of **26** to the acid and coupling with the Boc-deprotected fragment from **25** gave the fully assembled peptide **27**. De-



Scheme 8. Synthesis of Argyrin B; reagents and conditions: (a) LiOH, THF/MeOH/H_2O, room temp.; (b) H_2, Pd/C, MeOH, room temp.; (c) EDC, HOBt, CH_2Cl_2, room temp. (97%, 2 steps); (d) LiOH, THF/MeOH/H_2O, room temp.; (e) TFA/CH_2Cl_2, room temp.; (f) EDC, HOBt, DIPEA, CH_2Cl_2 (80%, 2 steps); (g) LiOH, THF/MeOH/H_2O, room temp.; (h) Anisole/TFA, room temp.; (i) TBTU, HOBt, DIPEA, CH_2Cl_2, room temp. (50−60%, 3 steps); (j) $NaIO_4$, dioxane/H_2O, room temp.; (k) $NaHCO_3$, CH_3CN/H_2O, room temp. (66%, 2 steps)

protection of **27** at the two termini and cyclisation (TBTU, HOBt) afforded **28** in an overall yield of 50−60%. Lastly, *syn*-elimination of the selenide was achieved using periodate and bicarbonate to give Argyrin B (**1**), identical in all respects to an authentic sample of the natural product.[29] ^1H and ^{13}C NMR spectra of a mixture of equivalent amounts of synthetic and natural samples were identical to both separate samples, and $[\alpha]_D$ values clearly matched.

Conclusion

In summary, we have achieved the first total synthesis of a new cyclic peptide Argyrin B from 4-methoxyindole in a 5.8% overall yield with the longest linear sequence being 17 steps (23.4% yield from the enantiopure N^{α}-Cbz- 4-methoxy-L-tryptophan **8**, 12 steps). An enzymatic resolution proved to be the most efficient and stereoselective method to generate one L-tryptophan component of this synthesis, while oxidation and β-elimination of phenylselenocysteine was the most reliable way to generate the dehydroalanine residue in the fully assembled molecule.

Experimental Section

General Remarks: All reactions not involving aqueous reagents were carried out under an argon atmosphere in oven-dried glassware cooled under vacuum. Acetonitrile, dioxane, DME, DMF, methanol and ethanol were used as supplied. THF was distilled over sodium/benzophenone and dichloromethane was distilled over calcium hydride.

TLC was carried out on Merck 60 F254 silica gel plates and visualised by UV irradiation (254 nm) or by staining with iodine absorbed on silica gel, ninhydrin solution or with aqueous acidic ammonium molybdate solution as appropriate. LCMS were recorded on a Micromass Platform MS using positive and negative electrospray ionisation with a Hewlett Packard 1050 LC instrument with detection by UV diode array. Flash column chromatography was performed on silica gel with a Biotage Flash 40i system.

Melting points were measured on a Reichert hot-stage apparatus and are uncorrected. Mass spectra and accurate mass measurements were obtained on a Kratos Q-TOF spectrometer using electrospray (+ESI) or a Kratos MS890MS spectrometer using electron impact (EI) or fast atom bombardment (FAB) techniques at the Department of Chemistry, University of Cambridge. Optical rotations were measured on a Perkin−Elmer model 343 polarimeter. $[\alpha]_D$ values are reported in 10^{-1} deg·cm^{-2}·g^{-1}, concentration (*c*) in g per 100 mL. Infrared spectra were obtained on a Perkin−Elmer "Spectrum One" spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory. ^1H NMR spectra were recorded at room temperature unless specified on DPX-400 and DRX-600 Bruker spectrometers, at 400 or 600 MHz, with residual protic solvent $CHCl_3$ as the internal reference (δ_H = 7.26 ppm). ^{13}C NMR spectra were recorded at room temperature unless specified on the same spectrometers at 100 or 150 MHz with

the central peak of $CHCl_3$ as the internal reference (δ_C = 77 ppm). DEPT 135 and two dimensional NMR spectroscopy (COSY, HMQC and HMBC) were used for the assignment of signals in the ^1H and ^{13}C NMR spectra. For convenience in the peak assignment of NMR spectra, the residues were numbered as follows: AlaThz = 1, Trp= 2, 4-OMe-Trp= 3, Gly = 4, Abu= 5, Sec/Dha = 6, Sar= 7 (e.g. 2-CH_2 stands for the methylene in Trp). Microanalysis were performed in the microanalytical laboratories at the Department of Chemistry, University of Cambridge.

List of Abbreviations: Abu: α-aminobutyric acid; Ala: alanine; Bn: benzyl; Boc: *tert*-butoxycarbonyl; Cbz: carbobenzyloxy; DEAD: diethyl azodicarboxylate; Dha: dihydroalanine; DIPEA: *N*,*N*-diisopropylethylamine; DME: 1,2-dimethoxyethane; EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Gly: glycine; HOBt: 1-hydroxybenzotriazole; Ig: immunoglobulin; IPA: 2-propanol; PyBroP®: bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; Phac: phenylacetyl; Sar: sarcosine; Sec: selenocysteine; TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA: trifluoroacetic acid; TFAA: trifluoroacetic anhydride; Thz: thiazole; Trp: tryptophan.

Peptide Synthesis: Peptides were synthesised by solution-phase methods using a racemisation-free fragment condensation (EDC and HOBt). Boc or Cbz groups were used to protect the *N*-terminus and were cleaved respectively by TFA in CH_2Cl_2 and hydrogenolysis with Pd/C in MeOH. The *C*-terminus was protected as a methyl or ethyl ester and cleaved by saponification with a slight excess of LiOH in THF/MeOH/H_2O. Reactions were monitored by LCMS and TLC. Reaction mixtures were worked up with CH_2Cl_2 or in case of poor solubility with $CHCl_3$/IPA (3:1). Purification was performed by flash column chromatography with CH_2Cl_2 and MeOH. Free *N*- and *C*-terminus intermediates were isolated and used directly in the next step without purification.

***tert*-Butyl (*R*)-(1-Thiocarbamoylethyl)carbamate (6):** EDC (5.57 g, 29.1 mol) was added to a suspension of Boc-L-alanine (5 g, 26.4 mmol) and 1-hydroxybenzotriazole (3.92 g, 29 mmol) in CH_2Cl_2 (250 mL) at 0 °C. The solution was warmed to room temperature and stirred for 30 minutes. The solution was cooled to 0 °C and ammonia was condensed into the solution (20 drops). The reaction mixture was warmed to room temperature, stirred for 30 minutes and then filtered. The mother liquor was concentrated and purified by column chromatography (CH_2Cl_2 with 4% then 6% MeOH) to give the amide as a white solid (4.99 g, 100%); m.p. 132 °C (ref.[30] m.p. 120−121 °C); R_f = 0.22 (CH_2Cl_2 with 6% MeOH). MS: *m*/*z* (+ESI) found 211.1068 [MNa^+], $C_8H_{16}N_2O_3$ (M, 188.1) requires 211.1059. $[\alpha]_D^{25}$ = 36.2 (*c* = 1, $CHCl_3$). IR (solid): $\tilde{\nu}$ = 3390, 3353, 3196, 1682, 1644, 1520, 1325, 1250 and 1165 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.36 (d, *J* = 7 Hz, 3 H, $CHCH_3$), 1.43 [s, 9 H, $C(CH_3)_3$], 4.21 (br, 1 H, $CHCH_3$), 5.21 (br, 1 H, α-NH), 5.93 (br, 1 H, NH_AH_B), 6.42 (br, 1 H, NH_AH_B) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.4 ($CHCH_3$), 28.3 [$C(CH_3)_3$], 49.6 ($CHCH_3$), 80.1 [$C(CH_3)_3$], 155.6 and 175.5 ppm. $C_8H_{16}N_2O_3$ (188.2): calcd. C 51.05, H 8.57, N 14.88; found C 50.95, H 8.55, N 14.77.

Belleau's reagent (5.05 g, 9.55 mmol) was added to a solution of the amide (3 g, 15.9 mmol) in THF (100 mL) cooled at 0 °C. The solution was warmed to room temperature, stirred for 40 minutes and partitioned between ice (150 mL) and saturated aqueous $NaHCO_3$ (150 mL). This was extracted with Et_2O (3 × 150 mL). The organic layers were combined, washed with brine (150 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 8:2 then 7:3) to give thioamide **6** as a light yellow solid (3.25 g, 100% yield); m.p. 104−105 °C; R_f = 0.29 (toluene/EtOAc, 3:2). MS: *m*/*z* (+ESI) found 204.0929 [M], $C_8H_{16}N_2O_2S$ (M, 204.1) requires 204.0932. $[\alpha]_D^{25}$ = 69.1 (*c* = 1, $CHCl_3$). IR (solid): $\tilde{\nu}$ = 3373, 3289, 3191, 1674, 1512, 1243, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.43 [s, 9 H, $C(CH_3)_3$], 1.45 (d, *J* = 7 Hz, 3 H, $CHCH_3$), 4.59 (br, 1 H, $CHCH_3$), 5.53 (br, 1 H, α-NH), 8.07 (br, 1 H, NH_AH_B), 8.37 (br, 1 H, NH_AH_B) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 ($CHCH_3$), 28.4 [$C(CH_3)_3$], 55.1 ($CHCH_3$), 80.5 [$C(CH_3)_3$], 155.6 (CO_2), 210.6 ($CSNH_2$) ppm. $C_8H_{16}N_2O_2S$ (204.3): calcd. C 47.03, H 7.89, N 13.71; found C 46.99, H 7.81, N 13.75.

Ethyl (*R*)-2-(1-*tert*-Butoxycarbonylaminoethyl)thiazole-4-carboxylate (2): Ethyl bromopyruvate (1.33 mL, 10.6 mmol) was added to a suspension of finely powdered $KHCO_3$ (2.55 g, 25.5 mmol) and thioamide **6** (0.65 g, 3.18 mmol) which had been stirred in DME (5 mL) at −15 °C for 5 minutes. After 1 minute, the mixture was treated with a solution of 2,6-lutidine (3.15 mL, 27 mmol) and trifluoroacetic anhydride (2.67 g, 12.7 mmol) in DME (3 mL) at −15 °C. The reaction mixture was stirred for 3 hours at the same temperature, poured into water (25 mL) and extracted with $CHCl_3$ (3 × 25 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 6:1 then 5:1) and recrystallised from hexane/Et_2O to give thiazole **2** as a light yellow solid (0.39 g, 41% yield); m.p. 85−86 °C (ref.[31] m.p. 89.5 °); R_f = 0.21 (EtOAc/hexane, 1:1); R_t = 17.13 min [R_t (Boc-L-ThzAla-OEt) 13.87 min], Chiralcel OD from DAICEL, 0.46 × 25 cm, hexane/IPA, 95:5, 1 mL/min. MS: *m*/*z* (+ESI) found 300.1158 [M], $C_{13}H_{20}N_2O_4S$ (M, 300.1) requires 300.1144. $[\alpha]_D^{25}$ = 40.8 (*c* = 1, $CHCl_3$). IR (solid): $\tilde{\nu}$ = 3367, 3114, 1716, 1685, 1495, 1228 and 1156 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 1.39 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.43 [s, 9 H, $C(CH_3)_3$], 1.61 (d, *J* = 6.5 Hz, 3 H, $CHCH_3$), 4.40 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 5.10 (br. s, 1 H, α-CH), 5.22 (br. s, 1 H, α-NH) and 8.07 (s, 1 H, C^5-H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 14.3 (CH_2CH_3), 21.7 ($CHCH_3$), 28.3 [$C(CH_3)_3$], 48.9 (α-CH), 61.4 (CH_2CH_3), 80.2 [$C(CH_3)_3$], 127.1 (C^5-H), 147.2, 154.8, 161.3 and 174.8 (4 quat. C) ppm.

Diethyl 2-Phenylacetylaminomalonate: Phenylacetyl chloride (5.95 mL, 45 mmol) was added dropwise to a solution of diethyl aminomalonate hydrochloride (10 g, 47 mmol) and sodium hydrogen carbonate (9.92 g, 118 mmol) in Et_2O (100 mL) and water (100 mL) cooled at 0 °C. The solution was warmed to room temperature, stirred for 1 hour and acidified with 3 N aqueous HCl to pH 2. The organic layer was washed with water (50 mL) then brine (50 mL), dried (Na_2SO_4) and concentrated to give the amide as a white fluffy solid (12.9 g, 98% yield); m.p. 68 °C; R_f = 0.23 (Et_2O/hexane, 3:2). MS: *m*/*z* (+ESI) found 294.1341 [MH^+], $C_{15}H_{19}NO_5$ (M, 293.1) requires 294.1341. IR (solid): $\tilde{\nu}$ = 3315, 1740, 1646, 1533, 1235, 1175 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7 Hz, 6 H, OCH_2CH_3), 3.64 (s, 2 H, $COCH_2$), 4.23 (m, 4 H, OCH_2CH_3), 5.13 (d, *J* = 7 Hz, 1 H, NHC*H*), 6.45 (br, 1 H, N*H*CH), 7.35 (m, 5 H, arom. CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9 (OCH_2CH_3), 43.1 ($COCH_2$), 56.5 (CHNH), 62.6 (OCH_2CH_3), 127.5 (arom. CH), 129 (arom. CH), 129.4 (arom. CH), 134.1 (arom. C), 166.1 (CO), 170.6 (CO) ppm. $C_{15}H_{19}NO_5$ (293.3): calcd. C 61.42, H 6.53, N 4.78; found C 61.39, H 6.43, N 4.77.

(4-Methoxy-1*H*-indol-3-ylmethyl)dimethylamine (16): A solution of 4-methoxy indole (10 g, 68 mmol) in CH_3CN (50 mL) was added over 20 minutes to a suspension of *N*,*N*-dimethylmethyleneammonium iodide (15 g, 81 mmol) in CH_3CN (50 mL) and AcOH (25 mL) at room temperature. The solution was stirred for 2 hours

and partitioned between IPA and $CHCl_3$ (1:3, 250 mL) and 10% aqueous NaOH (250 mL). The aqueous layer was extracted with IPA/$CHCl_3$ (1:3, 250 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated to give gramine **16** as a white solid (13.80 g, 99% yield); m.p. 137−138 °C (ref.[32] 136−138 °C). MS: *m*/*z* (+ESI) found 205.1345 [MH^+], $C_{12}H_{16}N_2O$ (M, 204.1) requires 205.1341. IR (solid): $\tilde{\nu}$ = 3100, 1588, 1515, 1447, 1244, 1085, 995, 726 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.32 [s, 6 H, $N(CH_3)_2$], 3.82 (s, 2 H, CH_2), 3.92 (s, 3 H, OCH_3), 6.49 (d, 1 H, arom. CH), 6.95 (d, 1 H, arom CH), 6.99 (br, 1 H, C^2-H), 7.07 (m, 1 H, arom. CH), 8.18 (br, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 45 (NCH_3), 55 (OCH_3), 55 (CH_2), 99.5, 104.6, 112.8, 117.6, 122.4, 122.9, 137.9, 154.8 ppm. $C_{12}H_{16}N_2O$ (204.3): calcd. C 70.56, H 7.90, N 13.71; found C 70.36, H 7.81, N 13.42.

Diethyl 2-(4-Methoxy-1*H*-indol-3-ylmethyl)-2-phenylacetylaminomalonate (17): A solution of sodium ethoxide (21% weight in EtOH, 14.4 mL, 39 mmol) was added to a solution of diethyl 2-acetylamino-2-phenylmalonate (9.43 g, 32 mmol) stirred in absolute EtOH (100 mL) at 0 °C. After 5 minutes gramine **16** (6.70 g, 32 mmol) was added in one portion. After 5 minutes dimethyl sulfate (4.6 mL, 48 mmol) was added dropwise. The solution was warmed to room temperature, stirred for 2 hours and partitioned between Et_2O (150 mL) and water (150 mL). The reaction mixture was acidified with 3 N aqueous HCl to pH 3. The organic layer was washed with water (100 mL), dried (Na_2SO_4) and concentrated. The residue was granulated in Et_2O/hexane (3:2, 50 mL) to give malonate **17** as a white solid (11.9 g, 82% yield); m.p. 136−138 °C; R_f = 0.34 (Et_2O). MS: *m*/*z* (+ESI) found 475.1839 [MNa^+], $C_{25}H_{28}N_2O_6$ (M, 452.2) requires 475.1845. IR (solid): $\tilde{\nu}$ = 3380, 3271, 1732, 1654, 1510, 1197, 1083 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.18 (t, *J* = 7 Hz, 6 H, OCH_2CH_3), 3.47 (s, 2 H, CH_2Ph), 3.84 (s, 3 H, OCH_3), 3.95 (s, 2 H, CCH_2), 4.13 (dq, *J* = 11 and 7 Hz, 2 H, $OCH_AH_BCH_3$), 4.22 (dq, *J* = 11 and 7 Hz, 2 H, $OCH_AH_BCH_3$), 6.46 (d, 1 H, arom. CH), 6.59 (d, *J* = 8 Hz, 1 H, C^2-H), 6.68 (s, 1 H, NHCO), 6.94 (d, 1 H, arom. CH), 7.07 (m, 1 H, arom. CH), 7.11 (m, 2 H, arom. CH); 7.21 (m, 3 H, arom. CH), 8.20 (br, 1 H, indole NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3 (OCH_2CH_3), 29.9 (CCH_2), 43.8 (CH_2Ph), 55.4 (OCH_3), 62.5 (OCH_2CH_3), 68 (CCH_2), 100, 105.1, 109.3, 118.3, 122.9, 123, 127.4, 129.1, 129.7, 135, 137.9, 154.8, 168.5, 169.8 ppm. $C_{25}H_{28}N_2O_6$ (452.5): calcd. C 66.36, H 6.24, N 6.19; found C 66.34, H 6.23, N 6.22.

3-(4-Methoxy-1*H*-indol-3-yl)-2-phenylacetylaminopropionic Acid (18): A suspension of malonate **17** (11.9 g, 26 mmol) in MeOH/dioxane (1:1, 120 mL) and 1.5 N aqueous NaOH (60 mL) was stirred for 2 hours at 50 °C. The solution was cooled to 0 °C, acidified with 3 N aqueous HCl to pH 2 and extracted with CH_2Cl_2 (100 mL). The organic layer was partially concentrated, diluted with dioxane (30 mL) and stirred for 30 minutes at 100 °C. The solution was cooled to 0 °C and 1.5 N aqueous NaOH (60 mL) was added. The solution was stirred for 1 hour at room temperature, partitioned with CH_2Cl_2 (100 mL) and acidified with 3 N aqueous HCl to pH 2. The aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated. The residue was recrystallised from EtOAc/Et_2O to give two crops of racemic amino acid **18** as a white solid (6.4 g, 69% yield); m.p. 153−156 °C; R_f = 0.25 (EtOAc with 2% AcOH). MS: *m*/*z* (+ESI) found 375.1322 [MNa^+], $C_{20}H_{20}N_2O_4$ (M, 352.1) requires 375.1321. IR (solid): $\tilde{\nu}$ = 3291, 1717, 1622, 1511, 1433, 1259, 1240 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO): δ = 3.00 (dd, *J* = 14 and 10 Hz, 1 H, $NHCHCH_AH_B$), 3.35 (m, 1 H, $NHCHCH_AH_B$), 3.39 (s, 2 H, CH_2Ph), 3.81 (s, 3 H, OCH_3), 4.50 (m, 1 H, $NHCHCH_2$), 6.42 (m, 1 H, arom. CH), 6.93 (m, 3 H), 7.09 (m, 2 H), 7.13−7.22 (m, 3 H), 8.22 (d, *J* = 8.5 Hz, 1 H), 10.76 (br, 1 H, indole NH), 12.35 (br, 1 H, CO_2H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 29.2 ($NHCHCH_2$), 42.5 (CH_2Ph), 54.1 and 55.4 (OCH_3 and $NHCHCH_2$), 99.3, 105.3, 110.6, 117.3, 122.2, 122.8, 126.6, 128.5, 129.3, 136.7, 138.3, 154.5, 170.3, 174.3 ppm. $C_{20}H_{20}N_2O_4$ (352.4): calcd. C 68.17, H 5.72, N 7.95; found C 67.93, H 5.80, N 7.84.

(*S*)-2-Benzyloxycarbonylamino-3-(4-methoxy-1*H*-indol-3-yl)propionic Acid (8): Aqueous 0.5 N NaOH was added to a solution of tryptophan **18** (0.815 g, 2.31 mmol) in MeOH (15 mL) and water (120 mL) to set the pH to 7.6. The volume was adjusted with water to 200 mL and penicillin G acylase immobilised (Penicillin G amidase immobilised from E. coli, 150 U/g, [EC 3.5.1.11], from Fluka (cat. number: 76428); 0.08 g, 9.6 U) was added. The reaction mixture was shaken at room temperature in the dark. When the reaction did not proceed further, as measured by HPLC, or when nearly 50% conversion was measured by HPLC or NMR spectroscopy, the solution was filtered. It was then acidified with 3 N aqueous HCl to pH 1.5 and washed with EtOAc (3 × 70 mL). The pH was adjusted to 7 with 10% aqueous NaOH. $NaHCO_3$ (0.21 g, 2.5 mmol), THF (50 mL) and carbobenzyloxy chloride (0.19 mL, 1.3 mmol) were added successively. The reaction mixture was stirred overnight at room temperature. The solution was partially concentrated, acidified with 3 N aqueous HCl to pH 2 and extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 3:2 with 0.5% AcOH) to give carbamate **8** as a white solid (0.376 g, 44% yield, maximum 50%); m.p. 152 °C; R_f = 0.27 (EtOAc/hexane, 3:1 with 1% AcOH). MS: *m*/*z* (+ESI) found 391.1277 [MNa^+], $C_{20}H_{20}N_2O_5$ (M, 368.1) requires 391.1270. $[\alpha]_D^{25}$ = −52.8 (*c* = 0.5, MeOH). ^1H NMR (400 MHz, [D_6]DMSO, 60 °C): δ = 2.95 (dd, *J* = 14 and 10 Hz, 1 H, $NHCH_AH_B$), 3.35 (dd, *J* = 14 and 4 Hz, 1 H, $NHCH_AH_B$), 3.83 (s, 3 H, OCH_3), 4.30 (m, 1 H, $NHCHCH_2$), 4.95 (m, 2 H, CH_2Ph), 6.44 (d, *J* = 7 Hz, 1 H, arom. CH), 6.9−7 (m, 3 H, arom. CH), 7.24−7.34 (m, 5 H, arom. CH), 7.41 (d, *J* = 8 Hz, 1 H, $NHCHCH_2$), 10.75 (br. s, 1 H, indole NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 28.9 ($NHCHCH_2$), 55.4 (OCH_3), 55.9 ($NHCHCH_2$), 65.5 (CH_2Ph), 99.2, 105.1, 110.6, 117.1, 122.1, 123, 127.8, 128, 128.6, 137.4, 138.2, 154.3, 156.3, 174.4 ppm.

***N*$^{\alpha}$-Cbz-(4-OMe)Trp-Gly-OMe (19):** Diisopropylethylamine (0.19 mL, 1.08 mmol) and EDC (172 mg, 0.9 mmol) were added successively to a suspension of carboxylic acid **8** (265 mg, 0.72 mmol), glycine methyl ester hydrochloride (135 mg, 1.08 mmol) and 1-hydroxybenzotriazole (145 mg, 1.08 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between 0.1 N aqueous HCl (4 mL) and CH_2Cl_2 (4 mL). The organic layer was washed with saturated aqueous ammonium chloride (4 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2 with 2% MeOH) to give dipeptide **19** (298 mg, 94% yield) as an oil; R_f = 0.38 (CH_2Cl_2 with 5% MeOH). MS: *m*/*z* (+ESI) found 462.1653 [MNa^+], $C_{23}H_{25}N_3O_6$ (M, 439.2) requires 462.1641. $[\alpha]_D^{25}$ = −19.2 (*c* = 1, MeOH). IR (film): $\tilde{\nu}$ = 3316, 1703, 1665, 1507, 1244, 1211 cm^{-1}. ^1H NMR (400 MHz, [D_8]toluene, 60 °C): δ = 3.25 (s, 3 H, CO_2CH_3), 3.39 (m, 1 H, $NHCHCH_AH_B$), 3.53 (dd, *J* = 14 and 5.2, 1 H, $NHCHCH_AH_B$), 3.63 (s, 3 H, OCH_3), 3.69 (m, 2 H, $NHCH_2$), 4.69 (m, 1 H, $NHCHCH_2$), 4.92 (s, 2 H, CH_2Ph), 6.18 (d, *J* = 7 Hz, 1 H, $NHCHCH_2$), 6.32 (d, *J* = 8 Hz, 1 H, arom. CH), 6.43 (br, 1 H, $NHCH_2$), 6.66 (br, 1 H, ind. C^2 H), 6.79 (d, *J* = 8 Hz, 1 H, arom. CH), 6.9−7.1 (m, 6 H, arom. CH), 7.79 (br, 1 H, arom.

NH) ppm. ^{13}C NMR (100 MHz, [D$_8$]toluene, 60 °C): δ = 29.4 (NHCH*C*H$_2$), 40.8 (NH*C*H$_2$), 50.9 (CO$_2$*C*H$_3$), 54.5 (OCH$_3$), 57.5 (NH*C*HCH$_2$), 66.3 (*C*H$_2$Ph), 99.6, 105.1, 110.8, 117.7, 122.4, 122.6, 127.4, 127.6, 128.1, 137.1, 138.3, 154.2, 156.3, 169.6, 172 ppm. $C_{23}H_{25}N_3O_6$ (439.5): calcd. C 62.86, H 5.73, N 9.56; found C 62.72, H 5.78, N 9.46.

N^{α}-Cbz-Trp-(4-OMe)Trp-Gly-OMe (3): Palladium on charcoal (30 mg, 10% weight) was added to a solution of carbamate **19** (297 mg, 0.68 mmol) and 3 N aqueous HCl (0.24 mL) in methanol (10 mL). The reaction mixture was purged with hydrogen three times and stirred for 4 hours at room temperature. The suspension was filtered through a plug of Celite®, washed with methanol (2 × 5 mL) and concentrated by coevaporation with toluene (2 × 5 mL). The amine was used directly in the next step.

Diisopropylethylamine (0.13 mL, 0.74 mmol) and EDC (162 mg, 0.84 mmol) were added successively to a suspension of *N*-Carbobenzyloxy-L-tryptophan (3.43 g, 1.01 mmol), the amine and 1-hydroxybenzotriazole (139 mg, 1.01 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between 0.1 N aqueous HCl (10 mL) and CH_2Cl_2 (10 mL). The organic layer was washed with saturated aqueous ammonium chloride (10 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2 with 2.5% MeOH) to give tripeptide **3** as a glassy solid (342 mg, 81% yield); m.p. 119−124 °C; R_f = 0.22 (CH_2Cl_2 with 4% MeOH). MS: *m*/*z* (+ESI) found 648.2431 [MNa$^+$], $C_{34}H_{35}N_5O_7$ (M, 625.3) requires 648.2434. $[\alpha]_D^{25}$ = −34 (*c* = 1, MeOH). IR (solid): $\tilde{\nu}$ = 3421, 3305, 1742, 1697, 1640, 1531 and 1224 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 60 °C): δ = 2.87 (dd, 1 H, NHCHC*H*$_A$H$_B$), 3.07 (dd, 1 H, NHCHCH$_A$*H*$_B$), 3.12 (dd, 1 H, NHCHC*H*$_C$H$_D$), 3.38 (dd, 1 H, NHCHCH$_C$*H*$_D$), 3.63 (s, 3 H, CO$_2$CH$_3$), 3.84 (m, 2 H, 4-CH$_2$), 3.84 (s, 3 H, OCH$_3$), 4.30 (m, 1 H, NHC*H*CH$_2$), 4.65 (m, 1 H, NHC*H*CH$_2$), 4.92 (s, 2 H, C*H*$_2$Ph), 6.44 (d, 1 H, arom. CH), 6.90−7.07 (m, 7 H, arom. CH), 7.15−7.35 (m, 6 H, arom. CH and N*H*CHCH$_2$), 7.55 (d, 1 H, arom. CH), 7.84 (dd, 1 H, 4-α-NH), 7.92 (d, *J* = 12 Hz, 1 H, N*H*CHCH$_2$), 10.6 (br. s, 2 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, 60 °C): δ = 28.3 (NHCH*C*H$_2$), 29.6 (NHCH*C*H$_2$), 41.2 (4-CH$_2$), 52 (4-CH$_3$), 54.5 (NHC*H*CH$_2$), 55.6 (3-OCH$_3$), 56.2 (NH*C*HCH$_2$), 65.9 (*C*H$_2$Ph), 99.5, 105.4, 110.6, 110.8, 111.7, 117.7, 118.7, 118.8, 121.2, 122.1, 122.5, 124.1, 127.82, 127. 85, 127.88, 128, 128.7, 136.6, 137.4, 138.4, 154.7 (4-CO$_2$), 170.5 (CO), 171.9 (CO), 172.4 (CO) ppm. $C_{34}H_{35}N_5O_7$ (625.7): calcd. C 65.27, H 5.64, N 11.19; found C 65.06, H 5.66, N 11.06.

(*R*)-2-*tert*-Butoxycarbonylaminobutyric Acid: Dioxane (20 mL) and di-*tert*-butyl dicarbonate (4.1 g, 19.3 mmol) were added successively to a solution of (*R*)-2-aminobutyric acid (2 g, 19.4 mmol) and NaOH (0.77 g, 19.3 mmol) in water (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 hours. The solution was partially concentrated, acidified with 3 N aqueous HCl to pH 2 and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (EtOAc/petroleum ether, 1:3 with 1% AcOH) to give the amide (3.45 g, 88% yield) as an oil; R_f = 0.63 (EtOAc with 2.5% AcOH). MS: *m*/*z* (+ESI) found 226.1046 [MNa$^+$], $C_9H_{17}NO_4$ (M, 203.1) requires 226.1055. $[\alpha]_D^{25}$ = −9.0 (*c* = 1, CHCl$_3$). IR (solid): $\tilde{\nu}$ = 1710, 1510, 1394, 1368, 1159 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$, rotamer ratio 3:2, the asterisk denotes minor rotamer peak): δ = 0.94 and 0.96* (m, 3 H, CH$_2$C*H*$_3$), 1.41 [s, 9 H, C(C*H*$_3$)$_3$], 1.71 and 1.86 (m, 2 H, C*H*$_2$CH$_3$), 4.04* and 4.26 (br, 1 H, α-CH), 5.14 and 6.45* (br, 1 H, α-NH), 11.19 (s, 1 H, CO$_2$H) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 9.5 and 9.7* (CH$_2$*C*H$_3$), 25.6 (*C*H$_2$), 27.3* and 28.2 [C(*C*H$_3$)$_3$], 54.4 and 55.7* (α-CH), 80 and 81.6* [*C*(CH$_3$)$_3$], 155.6 and 157* (CO), 177.1 (CO) ppm. $C_9H_{17}NO_4$: calcd. C 53.19, H 8.43, N 6.89; found C 53.20, H 8.34, N 6.75.

(*S*)-2-*tert*-Butoxycarbonylamino-3-phenylselanylpropionic Acid (23): Diethyl azodicarboxylate (1.53 mL, 9.75 mmol) was added to a solution of triphenylphosphane (2.55 g, 9.75 mmol) in THF (90 mL) at −78 °C. This was allowed to warm to room temperature and then cooled to −78 °C. A solution of *N*-*tert*-butoxycarbonyl-L-serine (2 g, 9.75 mmol) in THF (15 mL) was added over 20 minutes. After 20 minutes, the reaction mixture was warmed to room temperature, stirred for 2 hours then concentrated. The residue was triturated in EtOAc/hexane (20 mL, 1:1) and filtered. The liquor was concentrated and purified by column chromatography (EtOAc/hexane, 3:7). The β-lactone was used directly in the next step (NB Storage of the β-lactone results in decomposition. It is recommended to use the residue directly or to store the chromatography fractions containing the product in the fridge overnight and concentrate the solution just prior to use.).

Sodium trimethoxyborohydride (0.84 g, 6.57 mmol) was added to a solution of diphenyl diselenide (1.02 g, 3.27 mmol) in dry ethanol (25 mL) at room temperature under argon. The resulting pale peach solution was stirred for 30 minutes then a solution of the β-lactone in dry ethanol (5 mL) was added. The reaction mixture was stirred for 2 hours and concentrated. The residue was partitioned between aqueous saturated $NaHCO_3$ (50 mL) and Et_2O (50 mL). The aqueous layer was washed with Et_2O (20 mL), acidified with 3 N aqueous HCl to pH 2 and extracted with EtOAc (3 × 50 mL). The organic layers were combined, washed with brine (50 mL), dried (Na_2SO_4) and concentrated to give amino acid **23** as a white solid (1.37 g, 41% yield); m.p. 94−95 °C (ref.[26] m.p. 91−92 °C); R_f = 0.44 (CH_2Cl_2/MeOH, 9:1 with 2% AcOH). MS: *m*/*z* (+ESI) found 368.0370 [MNa$^+$], $C_{14}H_{19}NO_4Se$ (M, 345) requires 368.0377. $[\alpha]_D^{25}$ = −36.8 (*c* = 1, MeOH) (ref.[26] $[\alpha]_D^{25}$ = −38.1 (*c* = 1, MeOH)]. IR (solid): $\tilde{\nu}$ = 3300, 2930, 2519, 1729, 1652, 1413, 1220 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 1.41 [s, 9 H, C(C*H*$_3$)$_3$], 3.35 (br, 2 H, CH$_2$), 4.64 (br, 1 H, α-CH), 5.28 (br, 1 H, α-NH), 7.26 (br, 3 H, arom. CH), 7.55 (br, 2 H, arom. CH) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 28.6 (CH$_3$), 30.4 (CH$_2$), 53.8 (α-CH), 81 [*C*(CH$_3$)$_3$], 128.1 (arom. CH), 129.2 (arom. C), 129.6 (arom. CH), 134.1 (arom. CH), 155.7 (CO), 175.5 (CO) ppm.

Boc-(Ph)Sec-Sar-OEt (24): Diisopropylethylamine (1.38 mL, 7.91 mmol) was added to a suspension of carboxylic acid **23** (0.91 g, 2.64 mmol), sarcosine ethyl ester hydrochloride (0.445 g, 2.90 mmol) and PyBroP© (1.35 g, 2.90 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 hours. The solution was partitioned between saturated aqueous ammonium chloride (20 mL) and CH_2Cl_2 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (20 mL). The organic layers were combined, washed with water (20 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 3:7) to give dipeptide **24** as an oil (0.94 g, 80% yield); R_f = 0.51 (EtOAc/hexane, 1:1). MS: *m*/*z* (+ESI) found 467.1041 [MNa$^+$], $C_{19}H_{28}N_2O_5Se$ (M, 444.1) requires 467.1061. $[\alpha]_D^{25}$ = −1.5 (*c* = 1, CHCl$_3$). IR (solid): $\tilde{\nu}$ = 3306, 2978, 2934, 1745, 1706, 1647, 1478, 1199, 1164 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$, 4:1 rotamer ratio, the asterisk denotes minor rotamer peak): δ = 1.25* and 1.26 (t, *J* = 7 Hz, 3 H, CH$_2$C*H*$_3$), 1.41* and 1.43 [s, 9 H, C(C*H*$_3$)$_3$], 2.90* and 2.99 (s, 3 H, NCH$_3$), 3.10 (dd, *J* = 13 and 7 Hz, 1 H, CHC*H*$_A$H$_B$), 3.21 (dd, *J* = 13 and 6 Hz, 1 H, CHCH$_A$*H*$_B$), 3.93 (d, *J* = 17 Hz, 1 H, C*H*$_A$H$_B$NCH$_3$), 4.03 (d, *J* =

17 Hz, 1 H, $CH_AH_BNCH_3$), 4.12 (q, J = 7 Hz, 1 H, OCH_A-H_BCH_3), 4.18 (q, J = 7 Hz, 1 H, $OCH_AH_BCH_3$), 4.67* and 4.89 m, 1 H, NHC*H*), 5.30* and 5.38 (d, J = 8 Hz, 1 H, N*H*CH), 7.26 (m, 3 H, arom. CH), 7.54*and 7.58 (m, 2 H, arom. CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.4* and 14.5 (CH_2CH_3), 28.6* and 28.7 [$C(CH_3)_3$], 31 ($CHCH_2$), 35.6* and 36.1 (NCH_3), 48.8 ($CHCH_2$), 50.1 (CH_2NCH_3), 61.7 (CH_2CH_3), 80.3 [$C(CH_3)_3$], 127.8 (arom. CH), 129.5 (arom. CH), 130.1 (arom. C), 133.4* and 133.7 (arom. CH), 155.4 (CO), 169 (CO), 171.8 (CO) ppm. $C_{19}H_{28}N_2O_5Se$ (443.4): calcd. C 51.47, H 6.37, N 6.32; found C 51.24, H 6.37, N 6.29.

***N*-Boc-D-Abu-(Ph)Sec-Sar-OEt (25):** Trifluoroacetic acid (5 mL) was added dropwise to a solution of carbamate **24** (0.319 g, 0.72 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 30 minutes. The solution was concentrated by coevaporation with toluene (3 × 5 mL) and the resulting ammonium salt was used directly in the next step.

EDC (0.172 g, 0.90 mmol) and diisopropylethylamine (0.14 mL, 0.79 mmol) were added successively (addition of the base prior to EDC results in the rapid formation of the diketopiperazine) to a solution of the ammonium salt, (*R*)-2-*tert*-butoxycarbonylaminobutyric acid (0.175 g, 0.86 mmol) and 1-hydroxybenzotriazole (0.153 g, 1.13 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between 0.1 N HCl (10 mL) and CH_2Cl_2 (10 mL). The organic layer was washed with aqueous saturated ammonium chloride (10 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2 with 1% then 2% MeOH) to give tripeptide **25** as an oil (0.319 g, 84% yield); R_f = 0.26 (CH_2Cl_2 with 5% MeOH). MS: *m*/*z* (+ESI) found 552.1584 [MNa^+], $C_{23}H_{35}N_3O_6Se$ (M, 529.2) requires 552.1589. $[\alpha]_D^{25}$= 3.6 (*c* = 1, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3309, 2972, 1744, 1716, 1642, 1493, 1366 and 1206 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 3:1 rotamer ratio, the asterisk denotes minor rotamer peak): δ = 0.93 and 0.92* (t, J = 7.5 Hz, 3 H, 5-CH_3), 1.25 and 1.22* (t, J = 7 Hz, 3 H, OCH_2CH_3), 1.43 [s, 9 H, $C(CH_3)_3$], 1.6 (m, 1 H, 5-CH_AH_B), 1.84 (m, 1 H, 5-CH_AH_B), 2.94 and 2.87* (s, 3 H, NCH_3), 3.1 (dd, J = 13 and 7 Hz, 1 H, 6-CH_AH_B), 3.22 (dd, J = 13 and 7 Hz, 1 H, 6-CH_AH_B), 3.91 (d, 1 H, 7-CH_AH_B), 4 (d, 1 H, 7-CH_AH_B), 4.09 (m, 1 H, 5-CH), 4.16 (q, J = 7 Hz, 2 H, OCH_2CH_3), 5.16 and 4.90* (m, 1 H, 6-CH), 5.04 (br. s, 1 H, 5-NH), 6.95 (d, J = 7 Hz, 1 H, 6-NH), 7.25 (m, 3 H, arom. CH), 7.56 and 7.52* (m, 2 H, arom CH) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 9.8(5-CH_3), 14.1 and 14* (OCH_2CH_3), 26.1 (5-CH_2), 28.3 [$C(CH_3)_3$], 29.7 and 29.9* (6-CH_2), 36.4 and 35.2* (NCH_3), 48.4 (6-CH), 49.7 (7-CH_2), 51.4 (5-CH), 61.3 and 61.7* (OCH_2CH_3), 79.9 [$C(CH_3)_3$], 127.5 and 127.3* (arom. CH), 129.2 (arom. CH), 129.4 (quat. C), 133.2 and 133* (arom. CH), 155.4 [$CO_2C(CH_3)_3$], 168.5 (CO), 170.9 and 171* (CO), 171.6 (CO) ppm.

***N*-Boc-D-AlaThz-Trp-(4-OMe)Trp-Gly-OMe (26):** A 0.5 N aqueous solution of LiOH (0.8 mL, 0.4 mmol) was added to a solution of ester **2** (0.1 g, 0.33 mmol) in THF/MeOH/water (4:1:2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between 0.1 N aqueous HCl (10 mL) and CH_2Cl_2 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (10 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated. The carboxylic acid was used directly in the next step.

Palladium on charcoal (15 mg, 10% weight) was added to a solution of the carbamate **3** (161 mg, 0.26 mmol) in methanol (10 mL). The reaction mixture was purged with hydrogen three times and stirred for 2 hours at room temperature. The suspension was filtered through a plug of Celite®, washed with methanol (2 × 5 mL) and concentrated by coevaporation with toluene (2 × 5 mL). The resulting amine was used directly in the next step.

EDC (62 mg, 0.32 mmol) was added to a suspension of the carboxylic acid, the amine and 1-hydroxybenzotriazole (52 mg, 0.39 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between water (5 mL) and CH_2Cl_2 (5 mL) and the aqueous layer was extracted with CH_2Cl_2 (5 mL). The organic layers were combined, washed with aqueous saturated ammonium chloride (10 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2 with 4.5% MeOH) to give pentapeptide **26** as a sticky solid (186 mg, 97% yield); R_f = 0.42 (CH_2Cl_2 with 10% MeOH). MS: *m*/*z* (+ESI) found 768.2800 [MNa^+], $C_{37}H_{43}N_7O_8S$ (M, 745.3) requires 768.2792. $[\alpha]_D^{25}$ = −45.0 (*c* = 1, $CHCl_3$). IR (solid): $\tilde{\nu}$ = 3320, 1654, 1507, 1246, 1164 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (m, 3 H, 1-$CHCH_3$), 1.47 [s, 9 H, $C(CH_3)_3$], 3−3.2 (m, 3 H, $NHCHCH_2$ and $NHCHCH_AH_B$), 3.44 (m, 1 H, $NHCHCH_AH_B$), 3.64 (s, 3 H, 3-CH_3), 3.68 (s, 3 H, 4-OCH_3), 3.81 (dd, J = 18 and 5 Hz, 1 H, 4-CH_AH_B), 4.01 (dd, J = 18 and 6 Hz, 1 H, 4-CH_AH_B), 4.64 (m, 2 H, 2-$NHCHCH_2$ and 3-$NHCHCH_2$), 4.91 (m, 1 H, 1-$CHCH_3$), 5.25 (d, J = 7 Hz, 1 H, 1-NH), 6.36 (m, 1 H, arom. CH), 6.59 (m, 2 H), 6.95 (m, 1 H, 4-NH), 6.95 (m, 1 H, arom. CH), 7.03 (m, 1 H, $NHCHCH_2$), 7.03 (m, 2 H, arom. CH), 7.13 (m, 1 H, arom. CH), 7.27 (m, 1 H, arom. CH), 7.52 (m, 1 H, arom. CH), 7.65 (m, 1 H, $NHCHCH_2$), 7.90 (s, 1 H, 1-C^5-H), 8.09 (br, 1 H, arom. NH), 8.94 (br, 1 H, arom. NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6 (1-$CHCH_3$), 26.9 ($NHCHCH_2$), 27.9 ($NHCHCH_2$), 28.3 [$C(CH_3)_3$], 41.1 (4-CH_2), 49.1 (1-$NHCHCH_3$), 52.1 (4-CH_3), 54.9 ($NHCHCH_2$), 55.2 (3-OCH_3), 55.6 ($NHCHCH_2$), 80.7 [$C(CH_3)_3$], 99.7, 105.3, 109.4, 110, 111.4, 117.4, 118.5, 119.5, 122, 122.5, 122.7, 123.2, 123.6, 127.2, 136.1, 138.1, 148.7, 153.8, 155.1, 161.6, 170.1, 171.6, 172.1, 174.8 ppm.

***N*-Boc-D-AlaThz-Trp-(4-OMe)Trp-Gly-D-Abu-(Ph)Sec-Sar-OEt (27):** A 0.5 N aqueous solution of LiOH (0.45 mL, 0.22 mmol) was added to a solution of ester **26** (151 mg, 0.20 mmol) in THF/MeOH/water (6:1.5:3 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 90 minutes. The solution was partitioned between 0.1 N aqueous HCl (5 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated. The resulting carboxylic acid was used directly in the next step.

Trifluoroacetic acid (2 mL) was added dropwise to a solution of carbamate **25** (117 mg, 0.22 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 hour. The solution was concentrated by coevaporation with toluene (3 × 5 mL) and the resulting ammonium salt was used directly in the next step.

Diisopropylethylamine (39 μL, 0.22 mmol) and EDC (48 mg, 0.25 mmol) were added successively to a suspension of the carboxylic acid, the ammonium salt and 1-hydroxybenzotriazole (43 mg, 0.32 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 90 minutes. The solution was partitioned between 0.1 N aqueous HCl (10 mL) and CH_2Cl_2 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The organic layers were combined, washed with aqueous saturated ammonium chloride (20 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column

chromatography (CH_2Cl_2 with 5% MeOH) to give octapeptide **27** as a sticky solid (185 mg, 80% yield); R_f = 0.15 (CH_2Cl_2 with 6% MeOH). MS: *m/z* (+ESI) found 1165.3669 [MNa^+], $C_{54}H_{66}N_{10}O_{11}SSe$ (M, 1142.4) requires 1165.3696. $[\alpha]_D^{25}$ = 16.8 (*c* = 1, $CHCl_3$). IR (solid): $\tilde{\nu}$ = 3309, 3055, 2977, 2933, 1645, 1531, 1506, 1253, 1164, 732 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO, 90 °C): δ = 0.86 (t, *J* = 7.4 Hz, 3 H, 5-CH_3), 1.18 (m, 3 H, 7-CH_2CH_3), 1.40 [s, 9 H, 1-C(CH_3)$_3$], 1.43 (d, *J* = 7 Hz, 3 H, 1-$CHCH_3$), 1.61 (m, 1 H), 1.73 (m, 1 H), 2.80−3.35 (m, 7 H), 2.98 (s, 3 H, 7-NCH_3), 3.37 (m, 1 H), 3.76 (m, 2 H), 3.81 (s, 3 H, 3-CH_3), 4.08 (m, 2 H, 7- CH_2CH_3), 4.08 (m, 1 H), 4.27 (m, 1 H), 4.58 (m, 1 H), 4.70 (m, 1 H), 4.84 (m, 1 H, 1-CHCH_3), 6.41 (dd, *J* = 7 and 1.5, 1 H), 6.85−6.95 (m, 4 H), 7.01 (t, *J* = 7.6 Hz, 1 H), 7.09 (d, *J* = 2 Hz, 1 H), 7.20−7.60 (m, 10 H), 7.79 (d, *J* = 7.8 Hz, 1 H), 7.96 (d, *J* = 7.4 Hz, 1 H), 8 (s, 1 H, 1-C^5 H), 8.14 (br, 1 H), 10.42 (s, 1 H), 10.48 (s, 1 H) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 90 °C): δ = 10.3, 14.3, 20.9, 25.9, 28.4, 28.7, 29.4, 36.2, 43, 49.2, 49.3, 54.2, 54.4, 55.3, 55.6, 60.9, 79.1, 99.8, 105.5, 110.3, 110.9, 111.7, 117.8, 118.7, 118.9, 121.3, 122.1, 122.6, 123.8, 124.2, 127.4, 128.1, 129.5, 129.7, 130.5, 132.8, 136.8, 138.5, 149.6, 154.7, 155.4, 160.7, 169, 170.8, 171.5, 171.6, 172.2, 176.5 ppm.

***Cyclo*[D-AlaThz-Trp-(4-OMe)Trp-Gly-D-Abu-(Ph)Sec-Sar] (28):** A 0.5 N aqueous solution of LiOH (0.15 mL, 75 μmol) was added to a solution of the ester **27** (74 mg, 65 μmol) in THF/MeOH/water (2:0.5:1 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 hours. The solution was partitioned between 0.1 N aqueous HCl (5 mL) and CH_2Cl_2 (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated. The resulting carboxylic acid was used directly in the next step.

The residue was dissolved in anisole (0.5 mL) and the solution was cooled to 0 °C before trifluoroacetic acid (5 mL) was added. The reaction mixture was warmed to room temperature and stirred for 20 minutes. The solution was concentrated by coevaporation with toluene (3 × 5 mL). The resulting amine was used directly in the next step.

Diisopropylethylamine (45 μL, 250 μmol) and TBTU (42 mg, 130 μmol) were added successively to a solution of the linear peptide and 1-hydroxybenzotriazole (17 mg, 130 μmol) in CH_2Cl_2 (100 mL) at room temperature. The reaction mixture was stirred for 2 hours, concentrated and purified by column chromatography (CH_2Cl_2 with a gradient of 0.5 to 4% MeOH) to give the cyclic peptide **28** as a sticky solid (32 mg, 50% yield); R_f = 0.2 (CH_2Cl_2 with 5% MeOH). MS: *m/z* (+ESI) found 1019.2786 [MNa^+], $C_{47}H_{52}N_{10}O_8SSe$ (M, 996.3) requires 1019.2753. $[\alpha]_D^{25}$ = 55.8 (*c* = 1, $CHCl_3$). IR (film): $\tilde{\nu}$ = 3304, 1653 and 1536 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 7 Hz, 3 H, 5-CH_3), 1.21 (m, 1 H, 4-CH_AH$_B$) 1.73 (d, *J* = 7 Hz, 3 H, 1-CH_3), 1.81 (m, 1 H, 5-CH_AH$_B$), 1.97 (m, 1 H, 5-CH_AH_B), 2.87 (dd, *J* = 15 and 3 Hz, 1 H, 2-CH_AH$_B$), 3.12 (s, 3 H, 7-CH_3), 3.16 (m, 1 H, 6-CH_AH$_B$), 3.18 (m, 1 H, 7-CH_AH$_B$) 3.28 (dd, *J* = 15 and 4 Hz, 1 H, 3-CH_AH$_B$), 3.33 (dd, *J* = 13 and 8 Hz, 1 H, 6-CH_AH_B), 3.44 (m, 1 H, 4-CH_AH_B), 3.44 (m, 1 H, 3-CH_AH_B), 3.53 (dd, *J* = 15 and 3 Hz, 1 H, 2-CH_AH_B), 3.92 (m, 1 H, 5-CH), 4.18 (d, 1 H, 3-α-CH), 4.31 (s, 3 H, 3-CH_3), 4.48 (m, 1 H, 4-NH), 4.48 (m, 1 H, 6-α-CH), 4.90 (d, *J* = 17 Hz, 1 H, 7-CH_AH_B), 5.04 (m, 1 H, 2-α-CH), 5.26 (m, 1 H, 1-α-CH), 5.54 (m, 1 H, arom. 2-CH), 6.42 (m, 1 H, arom. 2-CH), 6.57 (br. s, 1 H, 5-NH), 6.76 (br. s, 1 H, ind. C^2 H), 6.92 (m, 1 H, arom. 3-CH), 6.92 (m, 1 H, arom. 2-CH), 7.01 (d, *J* = 2 Hz, 1 H, ind. C^2 H), 7.13 (d, *J* = 8 Hz, 1 H, arom. 2-CH), 7.29 (m, 3 H, arom. 6-CH), 7.33 (m, 2 H, arom. 3-CH), 7.56 (m, 2 H, arom. 6-CH), 8 (s, 1 H, 1-C^5 H), 8.27 (s, 1 H, 6-NH), 8.32 (d, *J* = 7 Hz, 1 H, 1-α-NH), 8.45 (br. s, 1 H, ind. NH), 8.64 (d, *J* = 7 Hz, 1 H, 2-α-NH), 8.66 (m, 1 H, 3-α-NH), 10.96 (br. s, 1 H, ind. NH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 10.7 (5-CH_3), 20 (1-CH_3), 21.6 (5-CH_2), 26.8 (2-CH_2), 26.9 (3-CH_2), 27.94 (6-CH_2), 36.8 (7-CH_3), 40.9 (4-CH_2), 46.3 (1-α-CH), 50.2 (6-CH), 52 (2-α-CH), 52.1 (7-CH_2), 55 (5-CH), 56.1 (3-CH_3), 57.6 (3-α-CH), 101.2 (arom. 3-CH), 106.2 (arom. C), 106.6 (arom. 3-CH), 108.3 (arom. C), 111.2 (arom. 2-CH), 116.4 (arom. 2-CH), 117.4 (arom. C), 119.3 (arom. 2-CH), 121.4 (arom. 2-CH), 122.7 (1-C^5 H), 123.5 (arom. 3-CH), 123.6 (arom. 3-CH), 124.7 (arom. 2-CH), 126.5 (arom. C), 128.1 (arom. 6-CH), 128.1 (arom. 6-C), 129.5 (arom. 6-CH), 133.2 (arom. 6-CH), 134.7 (arom. C), 138.4 (arom. C), 150.1 (1-C^4), 152.4 (arom. C), 159.8, 167.5, 170.1, 171.6, 171.9, 172, 172.6 and 173.1 (7 CO and 1-C^2) ppm.

Argyrin B (1): Sodium periodate (17 mg, 80 μmol) was added to a solution of phenyl selenide (**28**; 20 mg, 20 μmol) in water (4 mL) and dioxane (4 mL) at room temperature. The solution was stirred for 2 hours then diluted with water (10 mL) and extracted with $CHCl_3$/IPA (3:1, 2 × 20 mL). The organic layers were combined, washed with water (10 mL) and concentrated. The residue was dissolved into acetonitrile (4 mL) then water (2 mL) and saturated aqueous $NaHCO_3$ (2 mL) were added successively. The reaction mixture was stirred for 2 days, diluted with water (10 mL) and extracted with $CHCl_3$/IPA (3:1, 2 × 20 mL). The organic layers were combined, washed with water (10 mL), dried (Na_2SO_4) and concentrated. This was purified by column chromatography (CH_2Cl_2 with a gradient of 1 to 4% of MeOH) to give Argyrin B (**1**; 11.2 mg, 66% yield) as a sticky solid; R_f = 0.5 (CH_2Cl_2 with 10% MeOH). MS: *m/z* (+ESI) found 861.3113 [MNa^+], $C_{41}H_{46}N_{10}O_8S$ (M, 838.3) requires 861.3119. $[\alpha]_D^{25}$ (synthetic product) = 92.2 (*c* = 0.27, acetone), $[\alpha]_D^{25}$ (natural product) = 104 (*c* = 2.9, acetone) (personal communication Heinrich Steinmetz, GBF Naturstoffchemie, Braunschweig, Germany. IR (film): $\tilde{\nu}$ = 3296, 1650, 1535 and 1254 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 7 Hz, 3 H, 5-CH_3), 1.05 (dd, *J* = 17 and 5 Hz, 1 H, 4-CH_AH$_B$), 1.72 (d, *J* = 7 Hz, 3 H, 1-CH_3), 1.88 (m, 1 H, 5-CH_AH$_B$), 2 (m, 1 H, 5-CH_AH_B), 2.85 (dd, *J* = 15 and 3 Hz, 1 H, 2-CH_AH$_B$), 3.09 (s, 3 H, 7-CH_3), 3.32 (dd, *J* = 15 and 4 Hz, 1 H, 3-CH_AH$_B$), 3.39 (d, *J* = 17.1 H, 7-CH_AH$_B$), 3.49 (m, 1 H, 3-CH_AH_B), 3.49 (m, 1 H, 4-CH_AH_B), 3.55 (dd, *J* = 15 and 3 Hz, 1 H, 2-CH_AH_B), 3.98 (m, 1 H, 5-CH), 4.21 (m, 1 H, 3-α-CH), 4.34 (s, 3 H, 3-CH_3), 4.53 (m, 1 H, 4-NH), 4.71 (d, 1 H, 6-CH_AH$_B$), 4.97 (d, *J* = 17 Hz, 1 H, 7-CH_AH_B), 5 (d, 1 H, 6-CH_AH_B), 5.07 (m, 1 H, 2-α-CH), 5.42 (d, *J* = 8 Hz, 1 H, 2-C^4 H), 5.48 (m, 1 H, 1-α-CH), 6.34 (m, 1 H, 2-C^5 H), 6.81 (d, *J* = 7 Hz, 1 H, 5-NH), 6.82 (d, *J* = 2 Hz, 1 H, 3-C^2 H), 6.89 (m, 1 H, 2-C^6 H), 6.91 (m, 1 H, arom. 3-CH), 6.96 (d, *J* = 2 Hz, 1 H, 2-C^2 H), 7.05 (d, *J* = 8 Hz, 1 H, 2-C^7 H), 7.33 (m, 2 H, arom. 3-CH), 8.04 (s, 1 H, 1-C^5 H), 8.35 (d, *J* = 2 Hz, 1 H, arom. 3-NH), 8.59 (d, *J* = 7 Hz, 1 H, 2-α-NH), 8.77 (d, 1 H, 3-α-NH), 8.81 (d, *J* = 9 Hz, 1 H, 1-α-NH), 9.36 (s, 1 H, 6-NH), 10.63 (br. s, 1 H, arom. 2-NH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 10.4 (5-CH_3), 20.4 (1-CH_3), 21.1 (5-CH_2), 26.6 (2-CH_2), 26.9 (3-CH_2), 37.2 (7-CH_3), 40.5 (4-CH_2), 45.2 (1-α-CH), 51 (7-CH_2), 52.1 (2-α-CH), 54.3 (5-CH), 56.1 (3-CH_3), 57.8 (3-α-CH), 99.3 (6-CH_2), 101.3 (arom. 3-CH), 105.7 (arom. C), 106.6 (arom. 3-CH), 108.4 (arom. C), 111.3 (arom. 2-CH), 115.9 (arom. 2-CH), 117.4 (arom. C), 119.2 (arom. 2-CH), 121.2 (arom. 2-CH), 122.7 (1-C^5 H), 123.6 (arom. 3-CH), 123.7 (arom. 3-CH), 125.5 (arom. 2-CH), 126.5 (arom. C), 134.8 (arom. C), 136.9 (6-α-C), 138.4 (arom. C), 150.4 (1-C^4), 152.3 (arom. C), 159.8 (CO), 166.8 (CO), 168.2 (CO), 169.3 (CO), 170 (CO), 170.8 (1-C^2), 171.4 (CO), 172.9 (CO) ppm.

X-ray Crystal Structure determination of 8: Crystal data: $C_{20}H_{20}N_2O_5$, M_w = 368.38, colourless prism 0.42 × 0.25 ×

0.10 mm, monoclinic $P2_1$ (No. 4), a = 11.5678(10), b = 5.0122(2), c = 15.9392(13) Å, β = 107.451(3)°, V = 881.62(11) Å^3, T = 230(2) K, D_X = 1.388 g.cm^{-3}, λ = 0.71073 Å, μ = 0.101 mm^{-1}, Nonius Kappa CCD diffractometer, 1.85° < θ < 27.79°, 5220 measured reflections, 3510 independent, 2961 with $I > 2\sigma(I)$. The structure was solved by direct methods (*SHELXS*-97) and refined by least-squares (*SHELXL*-97) using Chebyshev weights on F_0^2 to $R1$ = 0.046, $wR2$ = 0.113 [$I > 2\sigma(I)$], 249 parameters, all H atoms in calculated positions except H10 (the carboxylic acid proton) which was located and refined successfully, goodness-of-fit on F^2 1.16, residual electron density 0.33 e·Å^{-3}.

CCDC-187559 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgments

This work was financially supported by the Novartis Research Fellowship (SVL), the BP Endowment (SVL) and the Cambridge European Trust (AP). We thank Prof. Dr. G. Höfle from the GBF, Braunschweig, for an authentic sample of Argyrin B (**1**) and Dr J. E. Davies for determining the crystal structure of **8**.

[1] H. Reichenbach, G. Höfle, in *Drug Discovery from Nature* (Eds.: S. Grabley, R. Thiericke), Springer-Verlag, Berlin Heidelberg New York, **2000**, pp. 149−179.
[2] F. Sasse, H. Steinmetz, T. Schupp, F. Petersen, K. Memmert, H. Hofmann, C. Heusser, V. Brinkmann, P. von Matt, G. Höfle, H. Reichenbach, *J. Antibiot.* **2002**, *55*, 543−551.
[3] For a review on natural products as immunosuppressive agents see: J. Mann, *Nat. Prod. Rep.* **2001**, *18*, 417−430.
[4] R. R. Skelly, P. Munkenbeck, D. C. Morisson, *Infect. Immun.* **1979**, *23*, 287−293.
[5] B. L. Pike, G. J. V. Nossal, *J. Immunol.* **1984**, *132*, 1687−1695.
[6] E. L. Morgan, W. O. Weigle, *J. Immunol.* **1983**, *130*, 1066−1070.
[7] V. Brinkmann, C. Kristofic, *J. Immunol.* **1995**, *154*, 3078−3087.
[8] F. Levy, C. Kristofic, C. Heusser, V. Brinkmann, *Int. Arch. Allergy Appl. Immunol.* **1997**, *112*, 49−58.
[9] J. J. Sanglier, V. Quesniaux, T. Fehr, H. Hofmann, M. Mahnke, K. Memmert, W. Schuler, G. Zenke, L. Gschwind, C. Maurer, W. Schilling, *J. Antibiot.* **1999**, *52*, 466−473.
[10] L. Vollbrecht, H. Steinmetz, G. Höfle, L. Oberer, G. Rihs, G. Bovermann, P. von Matt, *J. Antibiot.* **2002**, *submitted*.
[11] R. S. Roy, A. M. Gehring, J. C. Milne, P. J. Belshaw, C. T. Walsh, *Nat. Prod. Rep.* **1999**, *16*, 249−263.
[12] For a preliminary communication of this work see: S. V. Ley, A. Priour, C. Heusser, *Org. Lett.* **2002**, *4*, 711−714.
[13] H. Kigoshi, S. Yamada, *Tetrahedron* **1999**, *55*, 12301−12308.
[14] G. Lajoie, F. Lepine, L. Maziak, B. Belleau, *Tetrahedron Lett.* **1983**, *24*, 3815−3818.
[15] E. Aguilar, A. I. Meyers, *Tetrahedron Lett.* **1994**, *35*, 2473−2476.
[16] K. Nakajima, F. Takai, T. Tanaka, K. Okawa, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1577−1578.
[17] K. Sato, A. P. Kozikowski, *Tetrahedron Lett.* **1989**, *30*, 4073−4076.
[18] Y. L. Bennani, G. D. Zhu, J. C. Freeman, *Synlett* **1998**, 754−756.
[19] U. Schöllkopf, U. Groth, C. Deng, *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 798−799.
[20] U. Schöllkopf, R. Lonsky, P. Lehr, *Liebigs Ann. Chem.* **1985**, 413−417.
[21] M. Nettekoven, M. Psiorz, H. Waldmann, *Tetrahedron Lett.* **1995**, *36*, 1425−1428.
[22] B. Meseguer, D. Alonso-Diaz, N. Griebenow, T. Herget, H. Waldmann, *Chem. Eur. J.* **2000**, *6*, 3943−3957.
[23] W. Wang, C. Y. Xiong, J. Q. Yang, V. J. Hruby, *Tetrahedron Lett.* **2001**, *42*, 7717−7719.
[24] W. Wang, C. Y. Xiong, J. Y. Zhang, V. J. Hruby, *Tetrahedron* **2002**, *58*, 3101−3110.
[25] N. M. Okeley, Y. T. Zhu, W. A. van der Donk, *Org. Lett.* **2000**, *2*, 3603−3606.
[26] M. Sakai, K. Hashimoto, H. Shirahama, *Heterocycles* **1997**, *44*, 319−324.
[27] S. V. Pansare, L. D. Arnold, J. C. Vederas, *Org. Syn.* **1991**, *70*, 10−17.
[28] J. Coste, E. Frerot, P. Jouin, B. Castro, *Tetrahedron Lett.* **1991**, *32*, 1967−1970.
[29] We note the data are remarkably similar to those of antibiotic A21459 A: E. Selva, L. Gastaldo, G. S. Saddler, G. Toppo, P. Ferrari, G. Carniti, B. P. Goldstein, *J. Antibiot.* **1996**, *49*, 145−149.
[30] Z. P. Xia, C. D. Smith, *J. Org. Chem.* **2001**, *66*, 3459−3466.
[31] U. Schmidt, P. Gleich, H. Griesser, R. Utz, *Synthesis* **1986**, 992−998.
[32] F. Yamada, K. Kobayashi, A. Shimizu, N. Aoki, M. Somei, *Heterocycles* **1993**, *36*, 2783−2804.

Received July 2, 2002
[O02363]