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Total Synthesis of the Cyclic Heptapeptide Argyrin B: A New Potent Inhibitor of T-Cell Independent Antibody Formation

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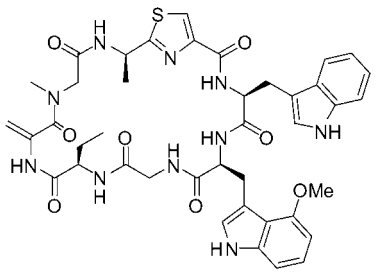
ABSTRACT



Argyrin B (**1**)

The total synthesis of Argyrin B (1) is presented using a synthetic plan that is convergent and flexible and conserves the stereogenic centers. The unusual amino acid 4-methoxy tryptophan (6) was obtained via an enzymatic resolution. Cyclization followed by oxidative elimination of the phenylseleno cysteine to the sensitive dehydroalanine afforded synthetic 1.

In a continuing search for new drugs that selectively inhibit antibody formation and subsequently may prove to be effective in xenotransplantation by modulating the progression of antibody-mediated rejection events, the cyclic heptapeptide Argyrin B (**1**)[1,2] has been recently identified at Novartis as a potential candidate. Argyrin B and its congeners were originally discovered at GBF Braunschweig during the screening of myxobacteria for the production of new antibiotics.[3]

Using established assays for both murine[4] and human B-cells,[5] Argyrin B was found to be a potent inhibitor of T-cell independent antibody formation. Moreover the two way murine mixed lymphocyte reaction (MLR), a cellular model for alloantigenic-mediated T-cell activation and proliferation,[6] was also inhibited by Argyrin B. Cytotoxicity was low, since **1** 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 CD40L-stimulated murine and human B-cells.

Owing to this interesting biological profile and the unusual features of this natural product we have undertaken a

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(1) Full details of the biological data, the isolation, and the X-ray crystal structure characterization will be reported separately.

(2) Höfle, G. *GBF Scientific Annual Report*; Walsdorff, H.-J., Ed.; GBF: Braunschweig, 1996; p 112.

(3) Revised structure and absolute configuration can be found in: Höfle, G.; Vollbrecht, L. *GBF Scientific Annual Report*; Walsdorff, H.-J., Ed.; GBF: Braunschweig, 2000/2001; p 99.

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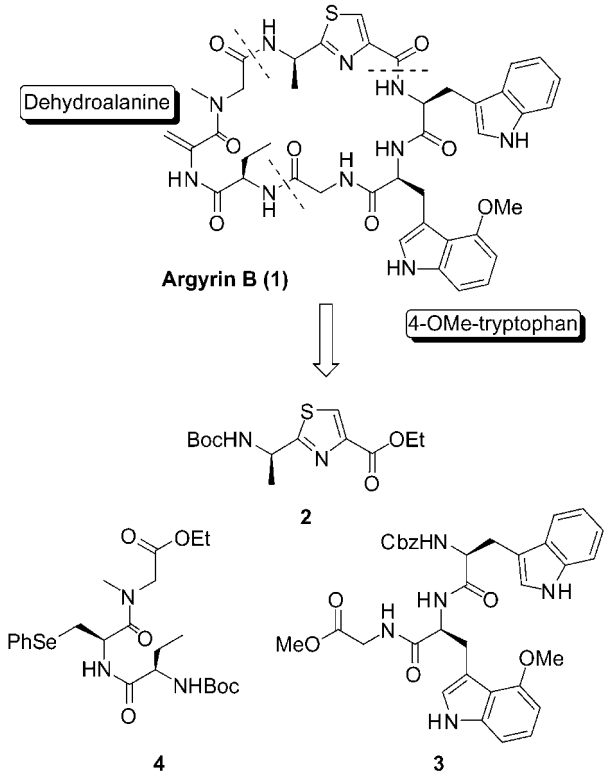
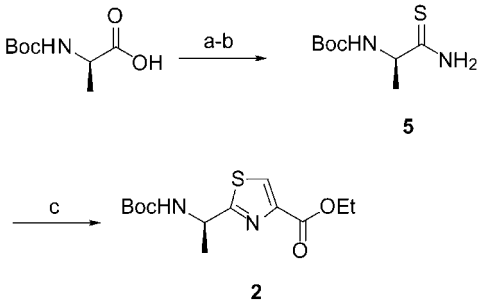


Figure 1. Structure and retrosynthetic analysis of Argyrin B.

synthesis of **1**, the details of which are reported below. In particular we were interested in the absolute stereochemistry, which was not known at the start of the work, and especially we wished to determine methods for the introduction of both the unusual 4-methoxy tryptophan and dehydroalanine units.

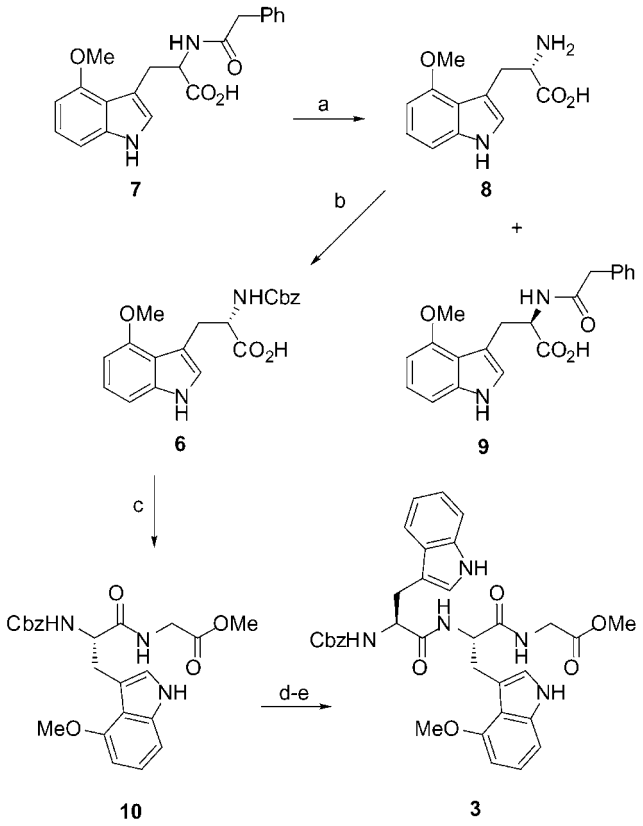
While it is possible to conceive of a large variety of alternative coupling arrangements for the various amino acid fragments, we have chosen a plan outlined in Figure 1

Scheme 1. Synthesis of Fragment **2**[a]



[a] Reagents and conditions: (a) DCC, HOBt, NH_3, CH_2Cl_2, 0 °C (97%); (b) Belleau's reagent, THF, 0 °C (79%); (c) (i) $BrCH_2COCO_2Et$, $KHCO_3$, DME, (ii) TFAA, 2,6-lutidine, DME, −15 °C (41%). DCC = *N*,*N*′-dicyclohexylcarbodiimide; HOBt = *N*-hydroxybenzotriazole; Belleau's reagent = 2,4-bis(4-phenoxyphenyl)-1,3,2,4-dithiaphosphetane-2,4-disulfide; DME = 1,2-dimethoxyethane; TFAA = trifluoroacetic anhydride.

Scheme 2. Synthesis of Fragment **3**[a]



[a] Reagents and conditions: (a) penicillin G acylase immobilized, $MeOH/H_2O$, rt; (b) CbzCl, $NaHCO_3$, THF/H_2O, rt (44%, 2 steps); (c) Gly-OMe, EDC, HOBt, CH_2Cl_2, rt (94%); (d) H_2, Pd/C, MeOH/aquous HCl, rt; (e) Cbz-L-Trp, EDC, HOBt, DIPEA, CH_2Cl_2, rt (81%, 2 steps). DIPEA = *N*,*N*-diisopropylethylamine; EDC = 1-ethyl-3-(3′-dimethyl aminopropyl)carbodiimide hydrochloride.

because this best serves to protect the stereogenic centers at crucial stages of the synthesis. The route also should facilitate later deprotection, coupling, and the difficult cyclizing event as required.

This approach therefore requires the synthesis of appropriately protected fragments, namely, the thiazole **2**, the tripeptide unit **3** containing the 4-methoxy tryptophan, and the selenophenyl substituted tripeptide **4**, which in turn derives from D-aminobutyric acid and sarcosine.

For the preparation of **2**, this follows a straightforward route[7] from *N*-Boc-D-alanine requiring transformation to the thioamide **5** via the intermediate amide and thiolation with Belleau's reagent[8] (Scheme 1). The compound **5** was reacted with bromo ethyl pyruvate to afford an intermediate that on treatment with trifluoroacetic anhydride and 2,6-lutidine gave **2**.[9]

Next the tripeptide **3** was assembled; however, this first required an efficient synthesis of the key 4-methoxy tryp-

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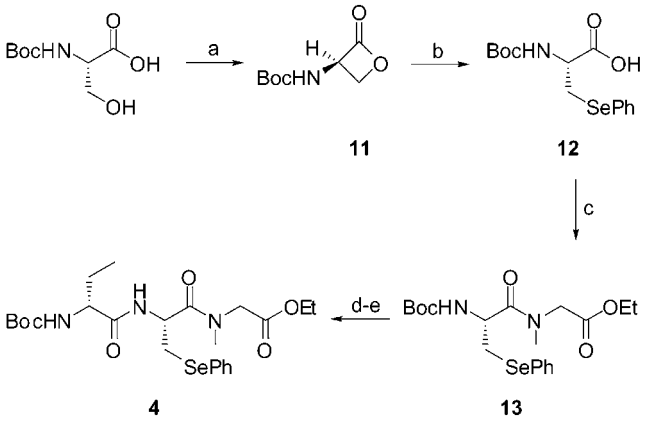
Scheme 3. Synthesis of Fragment **4**[a]



[a] Reagents and conditions: (a) DEAD, PPh_3, THF; −78 °C to rt; (b) PhSeSePh, $NaBH(OMe)_3$, EtOH then **11**, rt (34%, 2 steps); (c) HCl-Sar-OEt, PyBroP, DIPEA, CH_2Cl_2, rt (84%); (d) TFA/CH_2Cl_2, rt; (e) Boc-D-Abu, EDC, HOBt, DIPEA, CH_2Cl_2, rt (71%, 2 steps). DEAD = diethyl Azodicarboxylate; Sar = sarcosine; PyBroP = bromo tripyrrolidino phosphonium hexafluorophosphate; TFA = trifluoro acetic acid; Abu = aminobutyric acid.

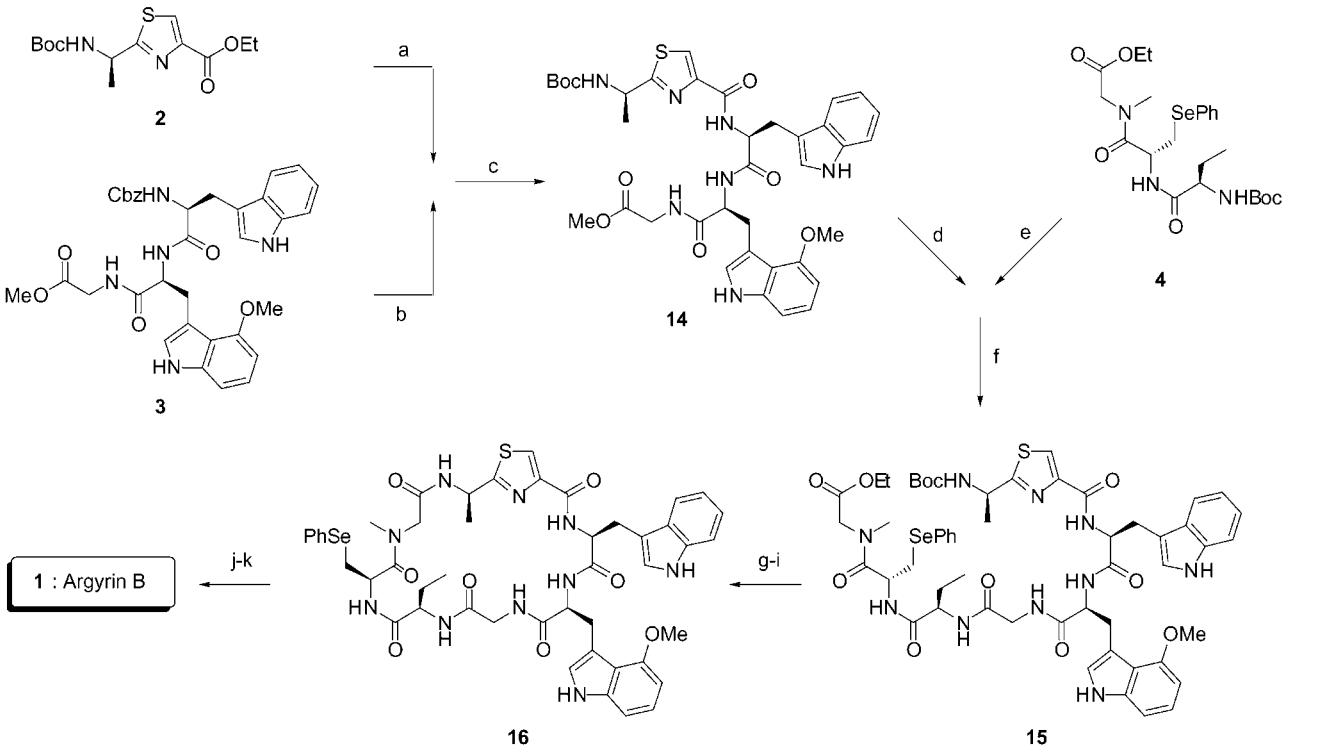
tophan amino acid derivative **6** (Scheme 2). Although we investigated a number of alternative methods to this fragment, none proved satisfactory on a gram scale until we studied the enzyme resolution route using immobilized Penicillin G acylase.[10] This was achieved by highly selective hydrolysis of the racemic amide **7**,[11] which afforded **8** and the non-hydrolyzed R-isomer **9**. Protection of **8** with the Cbz group gave **6** in an overall 44% yield.

This compound was then coupled using standard peptide coupling techniques with methyl glycine and after removal of the Cbz group reacted with *N*α-Cbz-L-tryptophan to afford the tripeptide in excellent yield (Scheme 2).

Last, the tripeptide containing the selenophenyl group **4** was constructed again following the established protocols from *N*-Boc-L-serine (Scheme 3). This route proceeded via the β-lactone **11**, which after ring opening afforded the selenide **12**.[12] This was coupled with ethyl sarcosine using PyBroP[13] to give **13**, which after deprotection to remove the butyloxycarbonyl group was coupled with *N*-Boc-D-amino-butyric acid to give **4**.

With all the fragments in hand we could now progress these to the natural product (Scheme 4). Removal of the Cbz protection from **3** and coupling with the free acid from **2** gave the product **14** in 96% yield. Similarly, hydrolysis of **14** to the acid and coupling with the Boc deprotected fragment from **4** gave the fully assembled heptapeptide **15**. This was then efficiently deprotected at the two termini and finally cyclized to **16** in an overall yield of 60%. Lastly, syn-elimination of the selenide was achieved using periodate

Scheme 4. Synthesis of Argyrin B[a]



[a] Reagents and conditions: (a) LiOH, THF/MeOH/H_2O, rt; (b) H_2, Pd/C, MeOH, rt; (c) EDC, HOBt, CH_2Cl_2, rt (60%, 3 steps); (d) LiOH, THF/MeOH/H_2O, rt; (e) TFA/CH_2Cl_2, rt; (f) EDC, HOBt, DIPEA, CH_2Cl_2 (90%, 2 steps); (g) LiOH, THF/MeOH/H_2O, rt; (h) TFA/Anisole/CH_2Cl_2, rt; (i) TBTU, HOBt, DIPEA, CH_2Cl_2, rt (60%, 2 steps); (j) $NaIO_4$, CH_3CN/H_2O, rt; (k) $NaHCO_3$, CH_3CN/H_2O, rt (52%, 2 steps). TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

and bicarbonate to give Argyrin B **1** identical in all respects to an authentic sample of the natural product.[14]

In summary, we have achieved the first synthesis of a new cyclic heptapeptide Argyrin B from 4-methoxy indole in 5.6% overall yield with the longest linear sequence being 18 steps (20.5% yield from the enantiopure *N*α-Cbz-L-4-methoxy-tryptophan **6**, 13 steps).

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Supporting Information Available: Characterization data for Argyrin B (**1**)[15] and synthetic intermediates **2**, **3**, **4**, **6**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The racemic *N*-phenylacetyl-4-methoxy tryptophan was obtained from 4-methoxy indole (4 steps, 63% yield).

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(14) We thank Prof. Dr. G. Höfle from the GBF, Braunschweig for an authentic sample of Argyrin B (**1**).

(15) We note the data are remarkably similar to those of antibiotic A21459 A (Selva, E.; Gastaldo, L., Saddler, G. S., Toppo, G, Ferrari, P.; Carniti, G.; Goldstein, B. P. *J. Antibiot.* **1996**, *49*, 150.