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First Total Synthesis of Astin G

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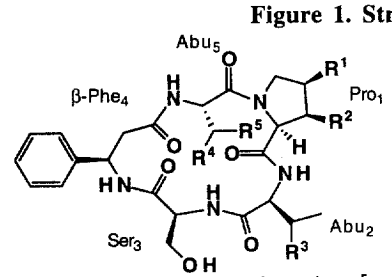
Abstract: The astin family of cyclopentapeptides contains several noncoded amino acids, including β-phenylalanine, α-aminobutyric acid and several substituted prolines. The difficult cyclization of the macrocycle and the subsequent first total synthesis of a member of this family, astin G, are discussed herein. This synthesis provides a model for the syntheses of the astins which contain the highly sensitive 3,4-dichloroproline.

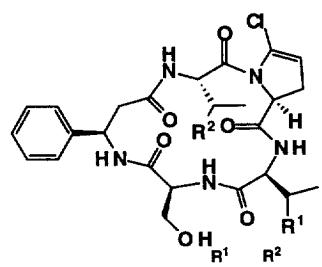
Keywords: peptides, amino acids and derivatives, macrocycles.

The first member of the astin family of cyclopentapeptides was isolated in 1993 from the biologically active extracts of the root of *Aster tataricus* (Compositae).[1] This flowering plant was used previously in Chinese medicinal teas.[2] To date, cyclic astins A-I have been isolated and subsequently characterized using degradation and NMR experiments.[1,3-6] In addition, a crystal structure of astin B has been reported.[7] Each of these cyclic pentapeptides exhibits one *cis* peptide bond between Abu_5 and Pro_1. This structural feature is one of the main differences between the solid state structures of the astins, cyclochlorotine[8] and islanditoxin.[9] The latter two compounds are toxic metabolites of yellow rice mold, *Penicillium islandicum* Sopp., whose occurrence on a variety of foodstuffs constitutes a human health hazard. The isolation of these toxins has been difficult because of their low availability, water solubility and instability.[10] Since its characterization in 1968, no synthesis of cyclochlorotine has been reported due, in part, to its instability, but mostly due to its toxicity.[11] In

Figure 1. Structures of astins A-H



	R1	R2	R3	R4	R5
1 astin A	Cl	Cl	H	OH	Me
2 astin B	Cl	Cl	OH	H	Me
3 astin C	Cl	Cl	H	H	Me
4 astin F	H	Cl	H	H	Me
5 astin G	**H**	**H**	**H**	**H**	**Me**
6 astin I	Cl	OH	H	H	Me
7 cyclochlorotine	Cl	Cl	OH	H	H



	R1	R2
8 astin D	H	H
9 astin E	OH	H
10 astin H	H	OH

PII: S0040-4039(98)02423-X

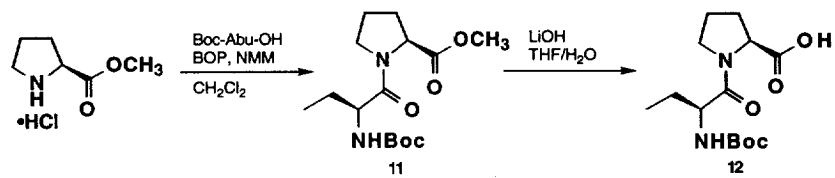
cyclochlorotine only the astin Abu_5 residue is replaced with serine, yet the molecule adopts a stable type I β-turn conformation with a *trans* proline amide bond and a transannular hydrogen bond. Cyclochlorotine causes major peripheral damage to the liver globule and the effects are very rapid (5 minutes after dosing, LD_{50} = 0.47 mg/kg).[12] The physical, chemical and biological characteristics of islanditoxin were very similar to cyclochlorotine. These two compounds were later shown to have the same structure.[13] Though there are few differences between the peptide sequences of the astins and those of cyclochlorotine, the astins exhibit antitumor activity and only a fraction of the hepatatoxicity shown by cyclochlorotine and islanditoxin.[3] The fact that minor structural changes cause such a noticeable change in biological activity makes both the astins and the toxins interesting synthetic targets.

Though several studies on the solution and solid state conformations have been reported for astins A-C,[14,15] no synthetic studies have appeared to date. As part of a broad program aimed at the synthesis of all astins, we have synthesized all of the noncoded amino acids present in the astins.[16,17] We now report the first total synthesis of a member of the astin family, astin G (**5**). Our main objective was to design a general synthetic route toward the astin macrocycle using the congener astin G (**5**), which contains an unsubstituted proline analog as a model. This approach is shown in the following schemes.

α-Aminobutyric acid is commercially available, yet at some expense. Several syntheses of this compound have been reported,[18-22] but most are inefficient and inconvenient. More efficient syntheses of this amino acid and β-phenylalanine[23] were reported by us.[16] Included in this previous communication was the synthesis of the tripeptide fragment (β-Phe-Ser-α-Abu) common to astins C, D, F, G, and I which was accomplished in 68% overall yield. Coupling of β-phenylalanine and serine and subsequent coupling of the resulting dipeptide to α-aminobutyric acid were achieved utilizing FDPP (1.5 eq, pentafluorophenyl diphenylphosphinate),[24] DIEA (3 eq), in DMF at room temperature and afforded the dipeptide and tripeptide in 85% and 80% yields respectively. In each case, the *tert*-butoxycarbonyl group was removed in nearly quantitative yields using 3 M HCl•dioxane at room temperature.

The synthesis of the α-Abu-Pro dipeptide proceeded in 62% overall yield from the commercially available proline methyl ester hydrochloride salt (**Scheme 1**). BOP [benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexafluorophosphate][25] coupling was used as the coupling method of choice since other astin analogs contain unprotected secondary hydroxyl substituents. The dipeptide was converted to the carboxylic acid using standard ester saponification conditions.

Scheme 1

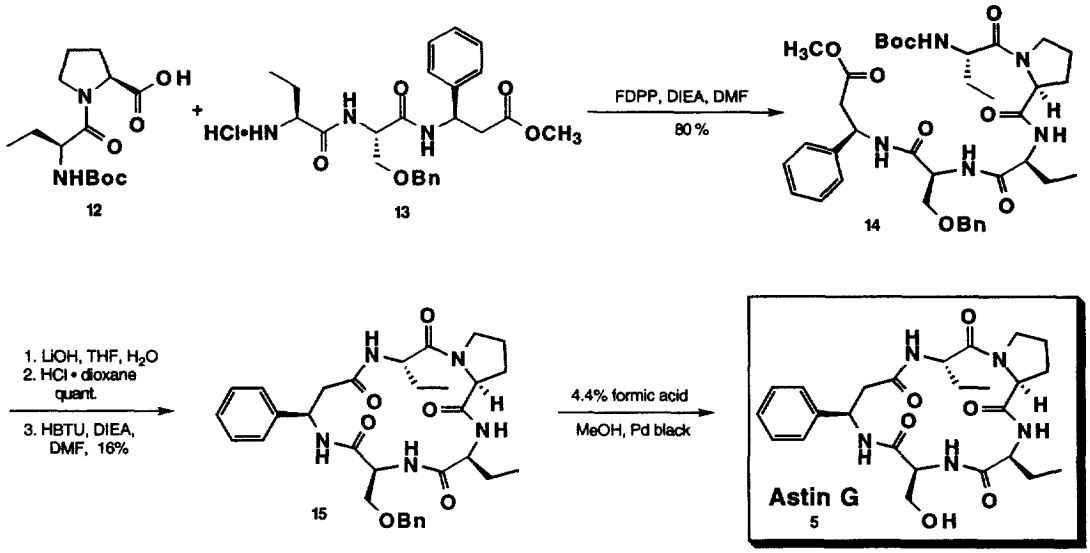


11 12

Both FDPP and BOP activated couplings were utilized to afford pentapeptide **14** with similar yields (**Scheme 2**). Hydrolysis of **14** yielded the corresponding carboxylic acid. The Boc group was removed with HCl•dioxane and the pentapeptide in DMF (0.0015M) was treated with equal amounts of TBTU [2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborate][26] and HOBt, followed by DIEA. The macrocyclization took 8-15h. Ethyl acetate extraction, workup and chromatography (1-10% MeOH/ CH_2Cl_2

gradient system) gave 16% of benzyl-protected macrocycle **15.** Previous cyclization attempts using either FDPP/DIEA/DMF or DPPA/$NaHCO_3$/DMF[27] yielded little or no product. Although the novel "metal ion-assisted peptide cyclization" technique reported by Tam[28] was very successful in the cyclization of linear precursors containing hydroxyl substituents on proline,[29] it failed to increase the macrocyclization yield in this case, mostly because of solubility problems.

Scheme 2



The deprotection of the macrocycle to afford astin G (**5**) was accomplished in 87% yield using transfer hydrogenolysis.[30] Though several deprotection protocols were attempted, 4.4% formic acid/ palladium black/ methanol were the only conditions which afforded astin G. The physical constants of the product were in agreement with those of the natural product.[5]

In conclusion, the first synthesis of a member of the astin family of cyclic pentapeptides has been completed. The application of this general route to the synthesis of all other astins isolated to date is in progress.

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Compound (10). R_f 0.55 (5% MeOH/ CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 5.23 (d, J=7.92 Hz, 1H), 4.50 (dd, J=8.40, 4.58 Hz, 1H), 4.36 (m, 1H), 3.72- 3.69 (m, 1H), 3.68 (s, 3H), 3.62- 3.58 (m, 1H), 2.20-2.17 (m, 1H), 2.03- 1.93 (m, 3H), 1.82- 1.78 (m, 1H), 1.63- 1.59 (m, 1H), 1.39 (s, 9H), 0.95 (t, J=7.74 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 171.2, 155.5, 79.5, 58.7, 52.9, 52.1, 46.9, 28.9, 28.3, 26.0, 24.9, 9.3; IR ($CHCl_3$) 3319, 2974, 2879, 2360, 1747, 1710, 1646 cm^{-1}; HRMS *m/z* calc'd for $C_{15}H_{26}N_2O_5$ (M + H): 315.1920, found 315.1931; $[\alpha]_D^{25}$ -71.52 (c=1.15, $CHCl_3$).

Compound (13). m.p. 85 °C; R_f 0.24 (5% MeOH/ CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, J=8.41 Hz, 1H), 7.38 (d, J=5.13 Hz, 1H), 7.30- 7.17 (m, 10H), 6.98 (d, J=7.38 Hz, 1H), 5.44 (m, 1H), 5.21 (d, J=7.92 Hz, 1H), 4.55 (m, 1H), 4.44 (s, 2H), 4.31 (m, 1H), 4.16 (m, 1H), 3.98 (dd, J=9.42, 3.34 Hz, 1H), 3.70- 3.63 (m, 1H), 3.51- 3.48 (m, 1H), 2.92 (dd, J=15.37, 7.34 Hz, 1H), 2.82 (dd, J=15.47, 6.62 Hz, 1H), 2.07- 1.85 (m, 4H), 1.80- 1.66 (m, 2H), 1.56- 1.54 (m, 1H), 1.43 (s, 9H), 0.92 (t, J=7.66 Hz, 3H), 0.90 (t, J=7.69 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 172.2, 171.1, 171.0, 168.9, 155.6, 140.8, 137.6, 128.4, 128.3, 127.7, 127.6, 127.3, 126.5, 79.8, 73.31, 73.25, 69.6, 60.1, 56.0, 53.5, 53.2, 51.8, 50.0, 47.4, 40.4, 28.3, 27.1, 25.9, 25.2, 24.7, 10.1, 9.8; IR ($CHCl_3$) 3297, 2972, 2360, 1738, 1644 cm^{-1}; HRMS *m/z* calc'd for $C_{31}H_{47}N_5O_9$ (M + H): 724.3921, found 724.3943; $[\alpha]_D^{25}$ -39.62 (c=1.32, $CHCl_3$).

Compound (14). R_f 0.52 (10% MeOH/CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J=9.21 Hz, 1H), 7.61 (d, J=5.99 Hz, 1H), 7.36-7.22 (m, 10H), 6.89 (d, J=5.90 Hz, 1H), 6.64 (d, J=4.22 Hz, 1H), 5.05 (m, 1H), 4.59 (m, 1H), 4.49 (s, 3H), 4.29 (m, 1H), 4.19 (dd, J=4.08, 9.85 Hz, 1H), 3.93 (dd, J=4.07, 9.80 Hz, 1H), 3.77 (dd, J=4.01, 9.78 Hz, 1H), 3.65- 3.59 (m, 3H), 2.81 (dd, J=13.70, 4.79 Hz, 1H), 2.58 (q, 6.38H), 2.40 (dd, J=13.40, 11.51 Hz, 1H), 2.10 (m, 1H), 1.98- 1.95 (m, 1H), 1.91- 1.87 (m, 1H), 1.26 (s, 9H), 1.02 (t, J=7.41 Hz, 3H), 1.00 (t, J=7.22 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.23, 171.20, 171.1, 169.0, 141.2, 137.3, 128.7, 128.5, 128.0, 127.7, 127.4, 125.9, 73.4, 70.5, 68.2, 61.3, 56.2, 54.4, 54.1, 51.5, 46.9, 42.5, 31.6, 29.7, 24.6, 24.2, 22.1, 10.6, 10.1; IR ($CHCl_3$) 3286, 2926, 2360, 1634 cm^{-1}; HRMS *m/z* calc'd for $C_{26}H_{37}N_5O_6$ (M + H): 592.3135, found 592.3158; $[\alpha]_D^{25}$ -77.3 (c=0.24, $CHCl_3$).

Compound (5). ^1H NMR (500 MHz, CD_3OD) δ 8.57 (s, 1H), 8.49 (d, J=9.31 Hz, 1H), 7.90 (d, J=5.58 Hz, 1H), 7.76 (d, J=5.96 Hz, 1H), 7.26- 7.20 (m, 4H), 7.15- 7.13 (m, 1H), 4.85 (dd, J=11.67, 4.55 Hz, 1H), 4.52 (d, J=7.83 Hz, 1H), 4.47- 4.44 (m, 1H), 4.16 (dd, J=7.55, 6.15 Hz, 1H), 3.97 (dd, J=5.66, 4.30 Hz, 1H), 3.80- 3.73 (m, 2H), 3.47- 3.44 (m, 2H), 2.80 (dd, J=13.09, 4.57 Hz, 1H), 2.43 (q, J=6.22 Hz, 1H), 2.22 (dd, J=13.03, 11.79 Hz, 1H), 2.08- 2.00 (m, 2H), 1.93- 1.81 (m, 2H), 1.71- 1.62 (m, 3H), 0.97 (t, J=7.46 Hz, 3H), 0.92 (t, J=7.30 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.7, 173.6, 173.48, 173.47, 171.8, 143.0, 129.6, 128.3, 126.9, 62.8, 61.5, 60.1, 56.0, 55.7, 53.6, 48.0, 43.2, 32.1, 24.9, 24.3, 23.0, 11.2, 10.4; HRMS *m/z* calc'd for $C_{25}H_{35}N_5O_6$ (M + H): 502.2666, found 502.2672, $[\alpha]_D^{25}$ -112.8 (c=0.25, MeOH); lit.[5] $[\alpha]_D^{25}$ -107.9 (c=1.14, MeOH).