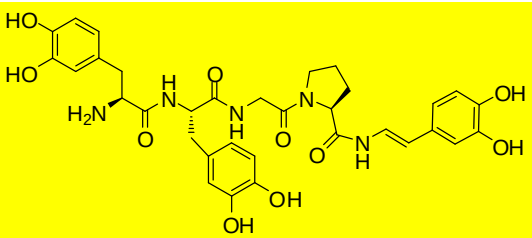
Graphical Abstract

Synthesis of tunichrome Sp-1

Michael A. Pullar, David Barker, Brent R. Copp*



tunichrome Sp-1

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Abstract – The first total synthesis of the ascidian blood pigment tunichrome Sp-1 is reported, with the modified pentapeptide prepared in a convergent manner using a combination of solid-phase peptide synthesis, Hunsdiecker decarboxylative iodination and Buchwald amidation reaction chemistry. The natural product was shown to exist as a mixture of *trans*- and *cis*-prolyl conformers, with the former dominating in a 5:1 ratio.

Keywords – tunichrome; DOPA; peptide; marine natural product; enamide

Ascidians, marine organisms of the Class Ascidiacea, are well known to produce a variety of bioactive marine natural products.[1] The 1980's and early 1990's was a particularly fruitful period, with the reporting of a number of unique structural classes of natural products that exhibited therapeutically useful biological activities (e.g. ecteinascidin-743[2] and the didemnins[3]) or were windows into the intriguing world of marine sessile invertebrate physiology (e.g. the tunichromes).[4,5] Publication of the isolation and structure elucidation of tunichrome B-1,[6-8] a modified 3,4,5-trihydroxyphenylalanine (TOPA)-containing tripeptide, was the culmination of six years of research into defining the chemical constituents of the blood pigments of *Ascidia nigra* that were thought to be responsible for the organisms ability to accumulate, amongst other metals, iron and vanadium. This research was hampered by the trace amounts of the pigment, co-occurrence with related pigments and by their sensitivity to water and oxygen. Since these initial reports, a number of related peptides have been reported from the blood cells of ascidians including *Ascidia nigra* (An-1, An-2, An-3),[9] *A. ceratodes*,[4] *Phallusia mammillata* (Pm-1, Pm-2, Pm-3),[10] *P. julinea*,[4,5] *Mogula manhattensis* (Mm-1, Mm-2)[9] and most recently *Styela plicata* (Sp-1).[11] Structurally, tunichromes are characterised as linear, low molecular weight peptides containing an oxidatively decarboxylated 3,4-dihydroxyphenylalanine (dcΔDOPA) or 3,4,5-trihydroxyphenylalanine (dcΔTOPA) residue at the C-terminus as well as one or more 3,4-dihydroxyphenylalanine (DOPA) or 3,4,5-trihydroxyphenylalanine (TOPA) residues or their α,β-unsaturated derivatives.[4,5] In addition to the initial associative link between tunichromes and vanadium or iron in blood cells suggesting that the natural products may act as chelators or 'vanadium-trappers',[5] alternative roles of wound repair, cross-linking/tunic formation[12] and action as a primitive clotting mechanism have also been proposed.[4,5] In an effort directed towards facilitating further studies of the role(s) played by these intriguing natural products, we now report the synthesis and structural confirmation of the most recently reported tunichrome, Sp-1 (**1**). Sp-1 was isolated in trace amounts (~ 800 μg) and characterized by ^{1}H, COSY and TOCSY NMR data. Edman degradation studies defined the peptide as L-DOPA-L-DOPA-Gly-L-Pro-dcΔDOPA.

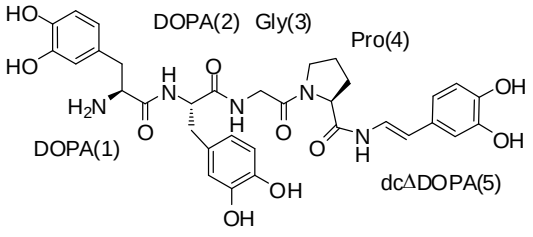

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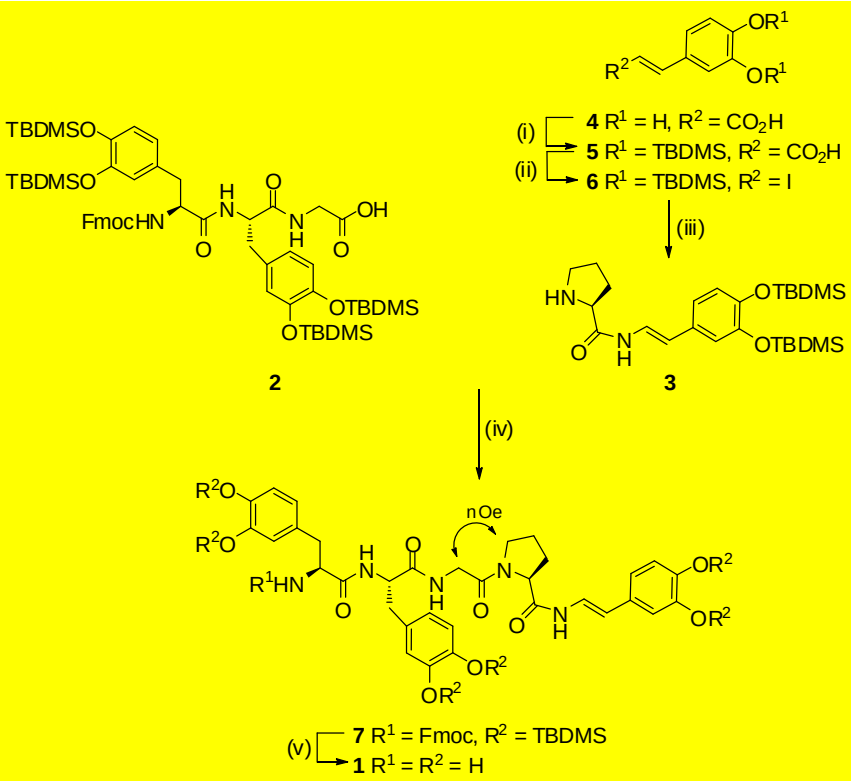
Figure 1. Structure of tunichrome Sp-1 (**1**).

We chose as a starting point to disconnect **1** at the Gly-Pro amide bond, requiring the synthesis of protected tripeptide L-DOPA-L-DOPA-Gly (**2**) and L-Pro-dihydroxystyrylenamide fragment (**3**) (Scheme 1). Tripeptide **2** was prepared by standard Fmoc solid-phase peptide synthesis procedures using 2-chlorotrityl resin, protected amino acids Fmoc-Gly-OH and Fmoc-DOPA(TBDMS)$_2$-OH[13] and HATU as the coupling reagent. Cleavage from the resin was effected by 2,2,2-trifluoroethanol:CH_2Cl_2 to afford **2** in 88% yield over 6 steps. Previous syntheses of tunichromes have relied upon the use of oxidation/elimination of phenylselenide derivatives to prepare the required enamide moiety.[5,14] We elected to explore an alternative route, making use of copper-catalysed Buchwald amidation methodology.[15] Attractions of this route include the mild reaction conditions required to effect the reaction between an amino acid carboxamide and a vinyl halide, proceeding with no epimerization of the amino acid stereocenter or isomerization of the enamide double bond.[16] The protected L-Pro-dihydroxystyrylenamide fragment **3** was prepared as shown in Scheme 1, starting with protection of 3,4-dihydroxycinnamic acid (**4**) as the TBDMS ether (**5**, 87% yield).[17] LiOAc-catalysed Hunsdiecker transformation[18] of **5** to the corresponding (*E*)-vinyl iodide (**6**, 31% yield) was carried out by reaction with *N*-iodosuccinimide in CH_2Cl_2.



Scheme 1. Reagents and conditions: Tripeptide **2** was synthesized upon chlorotrityl resin; (Resin loading) Fmoc-Gly-OH, DIPEA, CH_2Cl_2, rt, 1 h; (Fmoc-deprotection) piperidine, DMF, rt, 30 min; (Peptide coupling) Fmoc-DOPA(TBDMS)$_2$-OH, HATU, DIPEA, DMF, rt, 1 h; (Fmoc-deprotection) piperidine, DMF, rt, 30 min; (Peptide coupling) Fmoc-DOPA(TBDMS)$_2$-OH, HATU, DIPEA, DMF, rt, 1 h; (cleavage) TFE:CH_2Cl_2 (1:5, 20 mL), 2 h, 88% yield (6 steps); (i) TBDMSCl, imidazole, DMF, rt, 18 h, 87%; (ii) NIS, LiOAc, CH_2Cl_2, 0 °C, 1 h, 31%; (iii) Fmoc-Pro-NH_2, $(CH_3NHCH_2)_2$, CuI, Cs_2CO_3, dioxane, 85 °C, 18 h, 32%; (iv) EDC, HOBt, DMF, rt, 2 h, 53%; (v) piperidine, DMF, rt, 1 h, dried, then $Et_3N.3HF$, THF, rt, 1 h, 44%.

Previous studies regarding the Buchwald amidation of amino acid carboxamides with vinyl halides have concluded that protection of the α-nitrogen was not required, but that some amino acid sidechains competed for reaction.[16a] Preliminary efforts to undertake amidation of vinyl iodide **6** with L-Pro-NH_2 under standard Buchwald conditions

using catalytic CuI, *N*,*N*'-dimethylethylenediamine as the bidentate ligand and Cs_2CO_3 as the base[15] failed to yield any product. Suspecting that the secondary amine present in L-Pro-NH_2 could compete with *N*,*N*'-dimethylethylenediamine as a copper ligand, the reaction was repeated using Fmoc-L-Pro-NH_2 in the presence of stoichiometric CuI, affording (*E*)-enamide **3** in 32% yield. Of note was the concomitant cleavage of the Fmoc protecting group during the reaction, likely caused by the presence of the secondary amine base *N*,*N*'-dimethylethylenediamine. Peptide coupling (EDC, HOBt, DMF, 2 h) of enamide **3** and tripeptide acid **2** gave protected Sp-1 **7** in 53% yield. A two step, one-pot deprotection of the *N*-terminus (piperidine:DMF, 1 h), followed by subsequent deprotection of the catechol groups (triethylamine trihydrofluoride, THF, 1 h) gave the crude peptide that was purified by reversed-phase C18 column chromatography [H_2O:MeOH:TFA (79.99:20:0.01)], to afford tunichrome Sp-1 (**1**) in a 44% yield, present as a 5:1 mixture of *trans*- and *cis*-prolyl conformers.

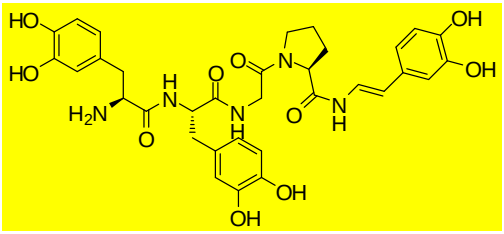


Figure 2. Structure of *cis*-prolyl tunichrome Sp-1

The ^{1}H, COSY and TOCSY spectroscopic data (DMSO-d_6) for tunichrome Sp-1 (**1**) was in good agreement with data for the natural product published by Tincu and Taylor[11] (see ESI). Although not mentioned in the original publication, the presence of a second set of (*E*)-enamide NH-CH=CH resonances were easily discernible in both the original ^{1}H spectroscopic data and our own. While the relatively broad appearance and overlapped nature of the ^{1}H resonances in DMSO-d_6 precluded determination of the nature of this minor component, re-acquisition and complete assignment of NMR data in CD_3OD[19] provided ample evidence to identify it as the *cis*-prolyl conformer of Sp-1 (Figure 2). While detection of a NOESY correlation between Gly-CH_2 and Pro-δCH_2 for the major component of the mixture identified it to be the *trans*-prolyl conformer, more telling were the observation of differences in the chemical shifts of the β and γ carbons (Δβγ) of the proline residue. It has been previously noted that a proline residue that adopts a *trans*-conformation about its amide bond characteristically has a smaller Δβγ value (ca. < 8) than a proline residue in the *cis*-conformation (ca. 9-15).[20] In the present case, Δβγ for the major component of the product mixture was 5.1 ppm (*trans*), while that of the minor component was 10.0 ppm (*cis*).[21]

In summary, we have described the first total synthesis of the natural product tunichrome Sp-1 (**1**), verified the structure that was proposed by Tincu and Taylor and characterised the originally present, but not reported, *cis*-prolyl conformer. The route used is amenable to the synthesis of un-natural analogues of **1** that will prove useful for investigation of the metal chelating and oxidation/reduction properties of the tunichromes.

Acknowledgments

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Supplementary data

Supplementary data (experimental details and compound characterisation) associated with this article can be found, in the online version, at http://.

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8. Note that between the publication of ref. 6 and 7, the name of tunichrome B-1 was changed to An-1.
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19. A 1:5 mixture of *cis*-prolyl tunichrome *Sp*-1* and *trans*-prolyl tunichrome *Sp*-1 was characterized; * are used to denote shifts for the *cis* conformer that differ from the corresponding signal in the *trans*-conformer: IR ν_{max} (ATR) 3284, 2973, 1666, 1638, 1513, 1187, 1130, 954, 721 cm^{-1}; $[\alpha]^{20}_{D}$ -71 (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6) δ 10.27* (1H, d, *J* = 9.9 Hz, dcΔDOPA(5)-NH), 9.96 (1H, br d, *J* = 10.0 Hz, dcΔDOPA(5)-NH), 8.94–8.62 (6H, m, 2 x DOPA(1)-OH, 2 x DOPA(2)-OH, 2 x dcΔDOPA(5)-OH), 8.74–8.68 (1H, m, DOPA(2)-NH) 8.18 (1H, t, *J* = 4.9 Hz, Gly(3)-NH), 7.91 (2H, br s, DOPA(1)-NH_2), 7.10* (1H, dd, *J* = 14.6, 10.0 Hz, dcΔDOPA(5)-αCH), 7.05 (1H, dd, *J* = 14.6, 10.0 Hz, dcΔDOPA(5)-αCH), 6.78–6.47 (9H, m, DOPA(1)-ArH, DOPA(2)-ArH, dcΔDOPA(5)-ArH), 6.13* (1H, d, *J* = 14.6 Hz, dcΔDOPA(5)-βCH), 6.05 (1H, d, *J* = 14.6 Hz, dcΔDOPA-βCH), 4.62–4.51 (1H, m, DOPA(2)-αCH), 4.32 (1H, dd, *J* = 8.3, 3.8 Hz, Pro(4)-αCH), 4.04 (1H, dd, *J* = 17.1, 5.5 Hz, Gly(3)-αCH_2a), 3.90 (1H, dd, *J* = 17.1, 4.9 Hz, Gly(3)-αCH_2b), 3.85–3.81 (1H, br m, DOPA(1)-αCH), 3.61–3.35 (1H, br m, Pro(4)-δCH_2), 3.04–2.99 (1H, m, DOPA(1)-βCH_2a), 2.96–2.85 (1H, m, DOPA(2)-βCH_2a), 2.68–2.59 (2H, m, DOPA(1)-βCH_2b, DOPA(2)-βCH_2b), 2.15–2.05 (1H, m, Pro(4)-βCH_2a), 1.99–1.84 (2H, m, Pro(4)-γCH_2), 1.91–1.79 (1H, m, Pro(4)-βCH_2b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.0 (DOPA(2)-C=O), 169.5 (Pro(4)-C=O), 168.2 (DOPA(1)-C=O), 166.9 (Gly(3)-C=O), 145.5 (DOPA(1)-Ar-O^b), 145.2 (DOPA(1)-Ar-O^b), 144.9 (DOPA(2)-Ar-O^b), 144.6 (DOPA(2)-Ar-O^b), 144.4 (dcΔDOPA(5)-Ar-O^b), 143.8 (dcΔDOPA(5)-Ar-O^b), 128.3 (DOPA(2)-γC^c), 127.8 (dcΔDOPA(5)-γC^c), 125.5 (DOPA(1)-γC^c), 120.6 (dcΔDOPA(5)-αCH^d), 120.4 (DOPA(1)-ArH^d), 120.0 (DOPA(2)-Ar-H^d), 117.0 (dcΔDOPA(5)-ArH), 116.8 (DOPA(1)-ArH^d), 116.7 (DOPA(2)-ArH^d), 115.9 (dcΔDOPA(5)-ArH^d), 115.6 (DOPA(1)-ArH^d), 115.3 (DOPA(2)-ArH^d), 112.7 (dcΔDOPA(5)-βCH), 111.9 (dcΔDOPA(5)-ArH), 59.8 (Pro(4)-αCH), 54.6 (DOPA(2)-αCH), 53.6 (DOPA(1)-αCH), 46.0 (Pro(4)-δCH_2), 41.5 (Gly(3)-αCH_2), 37.1 (DOPA(2)-βCH_2), 36.6 (DOPA(1)-βCH_2), 29.4 (Pro(4)-βCH_2), 24.4 (Pro(4)-γCH_2); ^1H NMR (500 MHz, CD_3OD) δ 7.23* (1H, d, *J* = 14.6 Hz, dcΔDOPA(5)-αCH), 7.16 (1H, d, *J* = 14.6 Hz, dcΔDOPA(5)-αCH), 6.80–6.50 (9H, m, DOPA(1)-ArH, DOPA(2)-ArH, dcΔDOPA(5)-ArH), 6.23* (1H, d, *J* = 14.6 Hz, dcΔDOPA-βCH), 6.16 (1H, d, *J* = 14.6 Hz, dcΔDOPA-βCH), 4.66 (1H, t, *J* = 7.1 Hz, DOPA(2)-αCH), 4.60–4.51* (1H, m, Pro(4)-αCH), 4.44 (1H, dd, *J* = 8.7, 4.2 Hz, Pro(4)-αCH), 4.08–4.00 (1H, m, DOPA(1)-αCH), 4.05–3.98 (2H, m, Gly(3)-αCH_2), 3.65–3.58 (1H, m, Pro(4)-δCH_2a), 3.56–3.47 (1H, m, Pro(4)-δCH_2b), 3.06–2.96 (2H, m, DOPA(1)-βCH_2a, DOPA(2)-βCH_2a), 2.90 (1H, dd, *J* = 14.0, 7.5 Hz, DOPA(1)-βCH_2b), 2.79 (1H, dd, *J* = 14.0, 8.5 Hz, DOPA(2)-βCH_2b), 2.39–2.33* (1H, m, Pro(4)-βCH_2a), 2.26–2.18 (1H, m, Pro(4)-βCH_2a), 2.18–2.09* (1H, m, Pro(4)-βCH_2b), 2.10–1.97 (2H, m, Pro(4)-γCH_2), 2.06–1.94 (1H, m, Pro(4)-βCH_2b) 1.96–1.88* (1H, m, Pro(4)-γCH_2); ^{13}C NMR (125 MHz, CD_3OD) δ 173.1 (DOPA(2)-C=O), 171.9 (Pro(4)-C=O), 171.2* (Pro(4)-C=O), 169.5 (DOPA(1)-C=O^a), 169.4 (Gly(3)-C=O^a), 146.6 (DOPA(1)-Ar-O^b), 146.4 (DOPA(1)-Ar-O^b), 146.1 (DOPA(2)-Ar-O^b), 146.0 (DOPA(2)-Ar-O^b), 145.7 (dcΔDOPA(5)-Ar-O^b), 145.2 (dcΔDOPA(5)-Ar-O^b), 129.7 (DOPA(2)-γC^c), 129.6 (dcΔDOPA(5)-γC^c), 126.5 (DOPA(1)-γC), 122.0 (DOPA(1)-ArH), 121.7 (DOPA(2)-Ar-H), 121.1 (dcΔDOPA(5)-αCH), 119.1 (dcΔDOPA(5)-ArH), 117.6 (DOPA(1)-ArH^d), 117.4 (DOPA(2)-ArH^d), 116.8 (dcΔDOPA(5)-ArH^d), 116.5 (DOPA(1)-ArH^d), 116.3 (DOPA(2)-ArH^d), 116.1 (dcΔDOPA(5)-βCH^d), 113.2 (dcΔDOPA(5)-ArH), 61.9 (Pro(4)-αCH), 61.2* (Pro(4)-αCH), 56.3 (DOPA(2)-αCH), 55.5 (DOPA(1)-αCH), 47.9 (Pro(4)-δCH_2), 47.7* (Pro(4)-δCH_2), 43.1 (Gly(3)-αCH_2), 43.0* (Gly(3)-αCH_2), 38.2 (DOPA(1)-βCH_2^e), 37.9 (DOPA(2)-βCH_2^e), 33.4* (Pro(4)-βCH_2), 30.8 (Pro(4)-βCH_2), 25.7 (Pro(4)-γCH_2) 23.4* (Pro(4)-γCH_2); (+)-HRESIMS m/z 664.2615 $[M+H]^+$ (Calcd for $C_{33}H_{38}N_5O_{10}$, 664.2613).
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21. All NMR resonances could be assigned to either the *cis*- or *trans*-prolyl conformers, showing that no diastereomers had formed during the Buchwald coupling step.