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Graphical Abstract

Synthesis of 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 Asciidiacea, 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-7432 and the didemnins3) 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 (dcDOPA) or 3,4,5-trihydroxyphenylalanine (dcTOPA) 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 formation12 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 1H, COSY and TOCSY NMR data. Edman degradation studies defined the peptide as L-DOPA-L-DOPA-Gly-L-Pro-dcDOPA.

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 and HATU as the coupling reagent. Cleavage from the resin was effected by 2,2,2-trifluoroethanol:CH₂Cl₂ 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 transformation¹⁸ of 5 to the corresponding (E)-vinyl iodide (6, 31% yield) was carried out by reaction with N-iodosuccinimide in CH₂Cl₂.

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-NH₂ under standard Buchwald conditions
using catalytic CuI, N,N'-dimethylethylenediamine as the bidentate ligand and Cs₂CO₃ as the base failed to yield any product. Suspecting that the secondary amine present in L-Pro-NH₂ could compete with N,N'-dimethylethylenediamine as a copper ligand, the reaction was repeated using Fmoc-L-Pro-NH₂ 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 [H₂O: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 ¹H, COSY and TOCSY spectroscopic data (DMSO-d₆) for tunichrome Sp-1 (1) was in good agreement with data for the natural product published by Tincu and Taylor (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 ¹H spectroscopic data and our own. While the relatively broad appearance and overlapped nature of the ¹H resonances in DMSO-d₆ precluded determination of the nature of this minor component, re-acquisition and complete assignment of NMR data in CD₃OD provided ample evidence to identify it as the cis-prolyl conformer of Sp-1 (Figure 2). While detection of a NOESY correlation between Gly-CH₂ and Pro-δCH₂ 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). 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).

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://.

References and notes

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 v max (ATR) 3284, 2973, 1666, 1638, 1187, 1103, 954, 721 cm⁻¹; [α]D²⁰ -71 (c 1.0, CH₂OH); ¹H NMR (500 MHz, DMSO-d₆) δ 6.70* (1H, d, J = 10.0 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), 7.84–7.68 (1H, m, DOPA(2)-NH), 6.81 (1H, t, J = 4.9 Hz, Gly(3)-NH), 7.91 (2H, br s, DOPA(1)-NH₂), 7.10* (1H, dd, J = 14.6, 10.0 Hz, dcΔDOPA(5)-cH), 7.05 (1H, dd, J = 14.6, 10.0 Hz, dcΔDOPA(5)-cH), 6.78–6.47 (9H, m, DOPA(1)-cH, DOPA(2)-cH, dcΔDOPA(5)-ArH), 6.13* (1H, d, J = 14.6 Hz, dcΔDOPA(5)-bCH), 0.60 (1H, d, J = 14.6 Hz, dcΔDOPA(5)-bCH), 6.05 (1H, m, 1H, m, DOPA(1)-bCH), 3.42 (1H, dd, J = 8.3, 3.5 Hz, Pro(4)-cH), 4.04 (1H, dd, J = 17.1, 5.5 Hz, Gly(3)-cH), 3.90 (1H, dd, J = 17.1, 4.9 Hz, Gly(3)-cH), 3.85–3.81 (1H, br, m, Pro(4)-cH), 3.61–3.35 (1H, br, m, Pro(4)-cH), 3.04–2.99 (1H, m, DOPA(1)-bCH), 2.96–2.85 (1H, m, DOPA(2)-bCH), 2.68–2.59 (2H, m, DOPA(1)-bCH), DOPA(2)-bCH), DOPA(2)-bCH, 2.15–2.05 (1H, m, Pro(4)-bCH), 1.99–1.84 (2H, m, Pro(4)-cH), 1.91–1.79 (1H, m, Pro(4)-cH); ¹³C NMR (125 MHz, DMSO-d₆) δ 171.0 (DOPA(2)-cO), 169.5 (Pro(4)-cO), 168.2 (DOPA(1)-cO), 166.9 (Gly(3)-cO), 145.5 (DOPA(1)-Ar-O), 145.2 (DOPA(2)-Ar-O), 144.4 (DOPA(2)-Ar-O), 144.4 (dcdΔDOPA(5)-Ar-O), 143.8 (dcdΔDOPA(5)-Ar-O), 128.3 (DOPA(2)-cC), 127.8 (dcdΔDOPA(5)-cC), 125.5 (DOPA(1)-cC), 120.6 (dcdΔDOPA(5)-cCH), 120.4 (DOPA(1)-Ar-H), 120.0 (DOPA(2)-Ar-H), 117.0 (dcdΔDOPA(5)-ArH), 116.8 (DOPA(1)-Ar-H), 116.7 (DOPA(2)-Ar-H), 115.9 (dcdΔDOPA(5)-ArH), 115.6 (DOPA(1)-Ar-H), 115.3 (DOPA(2)-Ar-H), 112.7 (dcdΔDOPA(5)-bCH), 111.9 (dcdΔDOPA(5)-bCH), 59.8 (Pro(4)-cH), 54.6 (DOPA(2)-cH), 53.6 (DOPA(1)-cH), 46.0 (Pro(4)-cH), 41.5 (Gly(3)-cH), 37.1 (DOPA(2)-cCH), 36.6 (DOPA(1)-cCH), 29.4 (Pro(4)-cH), 24.4 (Pro(4)-cH); ¹H NMR (500 MHz, CD₃OD) δ 7.23* (1H, d, J = 14.6 Hz, dcΔDOPA(5)-cH), 7.16 (1H, d, J = 14.6 Hz, dcΔDOPA(5)-cH), 6.80–6.50 (9H, m, DOPA(1)-ArH, DOPA(2)-ArH, dcdΔDOPA(5)-ArH), 6.23* (1H, d, J = 14.6 Hz, dcΔDOPA(5)-cH), 6.16 (1H, d, J = 14.6 Hz, dcΔDOPA(5)-cH), 4.66 (1H, d, J = 7.1 Hz, DOPA(2)-cCH), 4.60–4.51* (1H, m, Pro(4)-cCH), 4.44 (1H, m, Pro(4)-cCH), 3.56–3.47 (1H, m, Pro(4)-cCH), 3.06–2.96 (2H, m, DOPA(1)-bCH), DOPA(2)-bCH), 2.90 (1H, dd, J = 14.0, 7.5 Hz, DOPA(1)-bCH), DOPA(2)-bCH), 2.79 (1H, dd, J = 14.0, 8.5 Hz, DOPA(2)-bCH), 2.39–2.33* (1H, m, Pro(4)-cCH), 2.26–2.18 (1H, m, Pro(4)-cCH), 2.18–2.09* (1H, m, Pro(4)-cCH), 2.10–1.97 (2H, m, Pro(4)-cCH), 2.06–1.94 (1H, m, Pro(4)-cCH); ¹³C NMR (125 MHz, CD₃OD) δ 173.1 (DOPA(2)-cO), 171.9 (Pro(4)-cO), 171.2* (Pro(4)-cO), 169.5 (DOPA(2)-cO), 169.4 (Gly(3)-cO), 164.6 (Pro(4)-cO), 146.1 (DOPA(2)-Ar-O), 146.0 (DOPA(2)-Ar-O), 145.7 (dcdΔDOPA(5)-ArO), 145.2 (dcdΔDOPA(5)-ArO), 129.7 (DOPA(2)-cC), 129.6 (dcdΔDOPA(5)-cC), 126.5 (DOPA(1)-cC), 122.0 (DOPA(1)-ArH), 121.7 (DOPA(2)-ArH), 121.1 (dcdΔDOPA(5)-ArH), 119.1 (dcdΔDOPA(5)-ArH), 117.6 (DOPA(1)-ArH), 117.4 (DOPA(2)-ArH), 116.8 (dcdΔDOPA(5)-ArH), 116.5 (DOPA(1)-ArH), 116.3 (DOPA(2)-ArH), 116.1 (dcdΔDOPA(5)-ArH), 113.2 (dcdΔDOPA(5)-ArH), 61.9 (Pro(4)-cCH), 61.2* (Pro(4)-cCH), 56.3 (DOPA(2)-cH), 55.5 (DOPA(1)-cH), 47.9 (Pro(4)-cH), 47.7* (Pro(4)-cH), 43.1 (Gly(3)-cH), 43.0* (Gly(3)-cH), 38.2 (DOPA(1)-cH), 37.9 (DOPA(2)-

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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.