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SYNTHESIS OF TUNICHROMES Mm-1 AND Mm-2, BLOOD PIGMENTS OF THE IRON-ASSIMILATING TUNICATE, *MOLGULA MANHATTENSIS*

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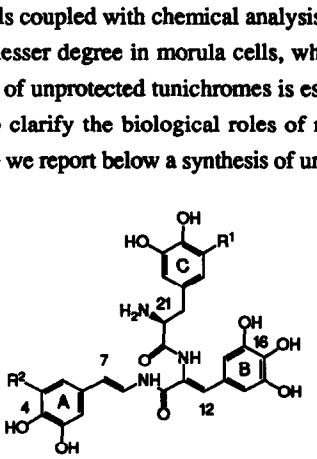
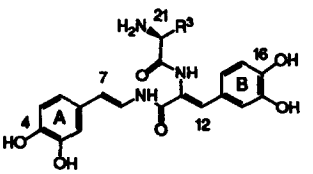
Abstract : Unstable poly-phenolic pigments, tunichromes Mm-1 and Mm-2, blood pigments of the iron-assimilating tunicate, *Molgula manhattensis*, have been synthesized by a modified versatile route.

The mechanism for the assimilation of certain tunicates (sea squirts) to assimilate transition metals, vanadium (V), Fe, etc., to extraordinary levels has remained an enigma since 1911.[1] Certain V- assimilating tunicates such as *Ascidia nigra* and *A. ceratodes* store V in their blood cell vacuoles (vanadophores) at concentrations up to 0.15 M and 1 M, respectively, *ca.* 10^7-fold greater than background sea water levels;[2] other species specifically sequester Fe. In these tunicates, V(V) from the sea water is reduced and stored primarily in the oxygen-sensitive V(III) state,[3] which is not expected to survive without assistance of certain stabilizing factors because of the instability of V(III) at physiological pH.[4] The focal point of tunicate research[5] has been to understand the mechanism of these phenomena, e.g., the assimilation of V, the reduction of V(V) to V(III), the stabilization of V(III) *in vivo*, and the interaction between V and tunichromes. Tunichromes, An-1~3 and Mm-1, 2 are pigments abundantly present in the blood of *Ascidia nigra* (V collector) and *Molgula manhattensis* (Fe collector), respectively.[6,7,8] Fluorescence activated cell sorting (FACS) of *A. nigra* and *A. ceratodes* blood cells coupled with chemical analysis of separated cells indicated that V was present in signet ring cells and to a lesser degree in morula cells, whereas free tunichromes were detected mainly in morula cells.[7] Availability of unprotected tunichromes is essential to investigate the interaction of V and/or Fe with tunichromes and to clarify the biological roles of metals and tunichromes. We reported the synthesis of unprotected An-1;[9a] we report below a synthesis of unprotected Mm-1 and Mm-2[9b] by a more versatile route.



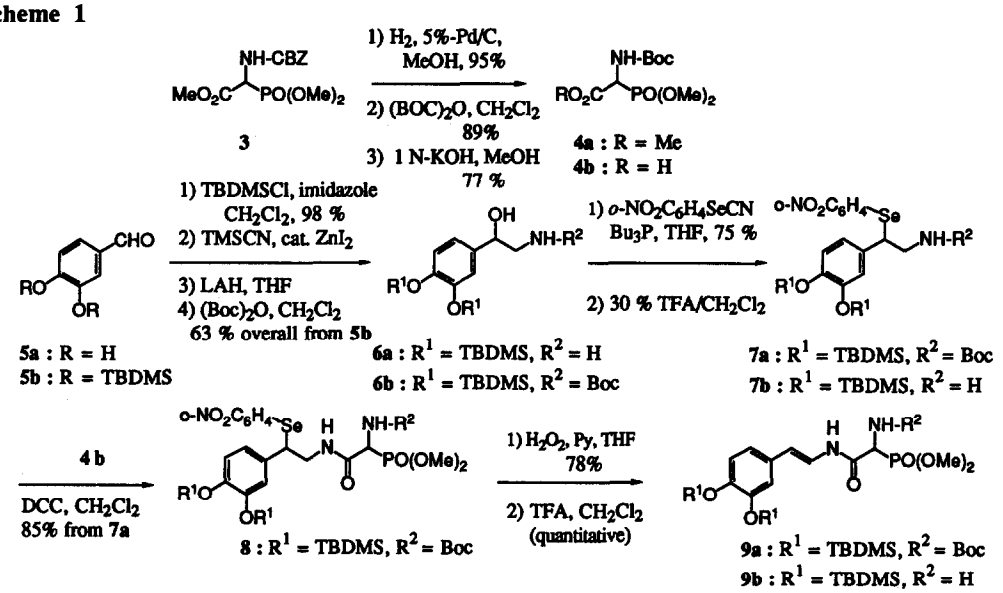
1a : An-1, $R^1 = R^2 = OH$
1b : An-2, $R^1 = H$, $R^2 = OH$
1c : An-3, $R^1 = R^2 = H$



2a : Mm-1, $R^3 = H$
2b : Mm-2, R^3 = i-Bu

The simplest tunichrome Mm-1 (**2a**), containing a glycine unit instead of the hydroxy-Dopa in the C-ring moiety of An, could represent the minimal structural requirement for tunichromes to exert their biological activity; furthermore, the reactivity of An and Mm toward V and Fe is similar (preliminary results). It would thus be advantageous to carry out investigations with the simplest tunichrome, Mm-1 **2a**. Herein we report the synthesis of two analogs, Mm-1 (**2a**) and Mm-2 (**2b**), using the common intermediate **9b** (Scheme 1);[10] the route follows the previous method leading to An-1[9] up to selenide **7**.

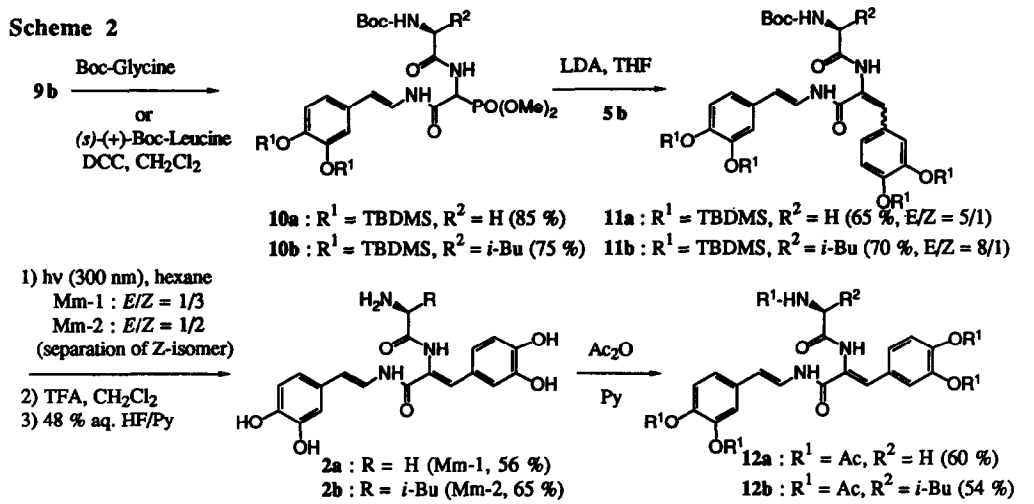
Scheme 1



As in An-1 synthesis,[9] the two olefinic functions were introduced by selenoxide elimination[11] and Horner-Emmons Wittig condensation.[12] The key starting material, phosphonoglycinate **4b**, was prepared from the known methyl carbobenzoxyphosphonoglycinate **3**[12] in three steps, hydrogenolysis (95 %), N-Boc protection (89 %) and hydrolysis (77 %). Catechualdehyde **5a** was used as a starting material for construction of the enamide moiety of **9**. The addition of TMS cyanide to TBDMS aldehyde **5b** in the presence of ZnI_2 afforded TMS-cyanohydrin quantitatively.[13] Subsequent LAH reduction followed by protection of the amino group with di-*tert*-butyl dicarbonate provided protected (±)-norephinephrine **6b** (63 % overall from **5b**). Treatment of alcohol **6b** with *o*-nitrophenylselenocyanate and tri-*n*-butylphosphine gave the selenide **7a** (75 %),[14] which was converted to the β-selenodopamine **7b** by trifluoroacetic acid (TFA) quantitatively.

Phosphonoglycine **4b** was then coupled with β-selenodopamine **7b** to provide selenyl dipeptide **8** as a diastereomeric mixture in 85 % yield. While the oxidation/elimination of the selenide with $NaIO_4$ formed the desired enamide **9a** in low yield (25%),[9b] the reaction with H_2O_2 (10 eq.)/Py (10 eq.) (THF, rt, 2 h) gave exclusively *E*-enamide **9a** (J_{vic} = 14.6 Hz) in satisfactory yield (78 %).[15] Deprotection of Boc-group with TFA gave the desired amine **9b**, a useful common intermediate for the synthesis of both Mm-1 and Mm-2 (Scheme 2) and other analogs.

Scheme 2



The amine **9b** was coupled with Boc-glycine to provide phosphonate **10a** in 85 % yield. The Horner-Emmons Wittig reaction[12] of **10a** with aldehyde **5b** afforded protected Mm-1 (**11a**) as a 5/1 mixture of E- and Z-isomers (65%)[16] as determined by the ^{1}H NMR integration of characteristic vinyl protons[17] When a solution of pure E-isomer was irradiated (300 nm Rayonet lamps, degassed hexane in quartz tube, 3 h),[18] the E/Z ratio reversed to enrich the desired Z-isomer **11aZ** (E/Z = 1/3),[19] which was separated by prep TLC (yield 60 %; 20 % of **11aE** was recovered). Both protecting groups, Boc- and TBDMS-groups of **Z-11a**, were removed by TFA (CH_2Cl_2, rt, 1 h) and 48 % HF/Pyridine (rt, 4-6 h), respectively. The separation of deprotected Mm-1 (**2a**) from the crude mixture was achieved by employing the fractional precipitation technique[9] in which precipitation of the highly polar tunichrome was induced by addition of CH_2Cl_2 and hexane. Three repetitions of MeOH dissolution and precipitation with CH_2Cl_2 afforded bright yellow tunichrome Mm-1 (**2a**).[20] Peracetylated Mm-1 (**12a**) was identical with naturally derived Mm-1 peracetate as evidenced by ^{1}H NMR and mass spectra.[21] For the synthesis of Mm-2 (**2b**) Boc-glycine was replaced by *(s)*-(+)-Boc-leucine to give the phosphonate **10b**, which was converted to natural Mm-2 (**2b**),[20] following the same sequence as in the Mm-1 synthesis. A comparison of the CD spectrum of peracetylated Mm-2 (**12b**) with that of naturally derived Mm-2 peracetate confirmed the absolute stereochemistry at C-21 to be *S*.[21]

An Mm-1 analog from [$^{13}C_1$]glycine has been prepared by this scheme; such analogs containing ^{13}C should be useful for structural studies of the poorly soluble precipitates formed from tunichromes and V or Fe by solid state NMR. Intermediate **9b** can also be employed for the synthesis of tunichrome analogs containing various moieties in place of moiety C in An-1. These analogs should play an important role in clarifying the biological role as well as the chemical reactivity, e.g., with metals, of the tunichromes.

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16. **11aE**: R_f 0.24 (H/E = 3/1), UV (hexane) λ_{max} = 313 nm (ε = 15200); **11aZ**: R_f 0.44, UV λ_{max} = 328 nm (ε = 34000).; LRMS (identical for both isomers) 941 (M + 1), 886, 740, 507, 446, 379.
17. ^1H NMR (250 MHz, $CDCl_3$): 7-CH (**11aE**): δ 5.43 (d, J = 14.5 Hz), (**11aZ**): δ 6.32 (d, J = 14.6 Hz). The B unit geometries of both olefinic double bonds were established by NOE results and UV data (see: reference 9 and Rich, D. H.; Mathiaparanam, P. *Tetrahedron Lett.* **1974**, 4037).
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19. Prolonged irradiation (10 h) at this wavelength resulted in complete decomposition of the products.
20. (a) Mm-1 (**2a**): ^1H NMR (250 MHz, CD_3OD): δ 7.50 (d, 1 H, J = 14.7 Hz), 7.06 (s, 1 H), 7.00-6.59 (m, 6 H), 6.24 (d, 1 H, J = 14.7 Hz), 3.88 (broad S, 2 H). HRMS calcd for $C_{19}H_{20}N_3O_6$ (M + 1) 386.1352, found 386.1346.; LRMS 386 (M + 1), 368, 351, 326, 307, 279, 257. (b) Mm-2 (**2b**): ^1H NMR (250 MHz, CD_3OD): δ 7.24 (d, 1 H, J = 14.6 Hz), 7.02 (s, 1 H), 7.00-6.68 (m, 6 H), 6.18 d, 1 H, J = 14.6 Hz), 3.75 (m, 1 H), 1.76-1.40 (m, 3 H), 0.96 (d, 3 H, J = 5.8 Hz), 0.92 (d, 1 H, J = 5.7 Hz).; HRMS calcd for $C_{23}H_{28}N_3O_6$ (M + 1) 442.1978, found 442.2021.; LRMS: 442 (M + 1), 428, 413, 399, 391, 383.
21. (a) Mm-1 Ac (**12a**): ^1H NMR (250 MHz, $CDCl_3$): δ 9.05 (d, 1 H, J = 10.5 Hz), 7.62 (s, 1 H), 7.52 (dd, 1H, J = 14.6, 10.5 Hz), 7.43 (s, 1 H), 7.27-7.06 (m, 6 H, Ar's), 6.56 (t, 1 H, J = 4.7 Hz), 6.36 (d, 1 H, J = 14.6 Hz), 3.88 (d, 2 H, J = 4.7 Hz), 2.30-2.25 (4 s, 12 H, OAc's), 2.03 (s, 3 H, NAc).; HRMS calcd for $C_{29}H_{30}N_3O_{11}$ (M + 1) 596.1880, found 596.1898.; LRMS : 596 (M + 1), 553, 497, 454, 361. (b) Mm-2 Ac (**12b**): ^1H NMR (250 MHz, $CDCl_3$): δ 9.07 (d, 1 H, J = 10.7 Hz), 7.51 (s, 1 H), 7.51 (dd, 1H, J = 14.5, 10.7 Hz), 7.44 (s, 1 H), 7.27-7.05 (m, 6 H, Ar's), 6.37 (d, 1 H, J = 14.5 Hz), 6.11 (d, 1 H, J = 4.8 Hz), 4.22 (m. 1 H), 2.28-2.25 (4 s, 12 H, OAc's), 2.03 (s, 3 H, NAc), 1.78-1.48 (m, 3 H), 0.98 (d, 3 H, J = 5.7 Hz), 0.93 (d, 3 H, J = 5.8 Hz).; HRMS calcd for $C_{33}H_{37}N_3O_{11}Na$ (M + Na) 674.2325, found 674.2286.; LRMS 652 (M + 1), 596, 568, 491, 417, 333, 277.; UV (CH_3CN): λ_{max} = 225 (sh, 12700), 274 (ε = 13300), 313 nm (ε = 15500).; CD (CH_3CN) 293 ($\Delta\varepsilon$ = -0.5), 229 nm ($\Delta\varepsilon$ = +1.2).

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