An Unusual Iodinated 5′-Deoxyxylofuranosyl Nucleoside from an Okinawan Ascidian, *Diplosoma* sp.

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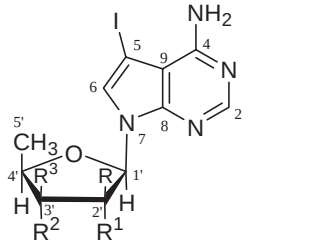
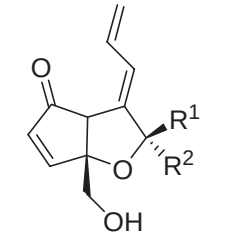
An unusual nucleoside, 4-amino-7-(5′-deoxy-β-D-xylofuranosyl)-5-iodopyrrolo[2,3-*d*]pyrimidine (**1**), was isolated from an ascidian, *Diplosoma* sp., and its structure was successfully determined by spectroscopic and chemical analysis. The known didemnenones **2**–**5** were also isolated. Compound **1** was found to inhibit the division of fertilized sea urchin eggs.

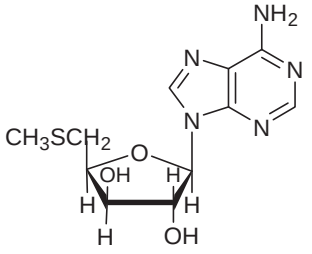
It has been amply demonstrated that ascidians are a prolific source of novel bioactive secondary metabolites.[1] As part of our continuing search for bioactive metabolites from Okinawan marine organisms, we examined *Diplosoma* sp.[2] ascidians collected off the coast of Hateruma island. Bioassay-guided fractionation led to the isolation of a novel iodinated 5′-deoxyxylofuranosyl nucleoside **1**, together with four known (+)-didemnenones **2**–**5** (Chart 1). Although many uncommon nucleosides have been isolated from terrestrial and marine organisms, nucleosides containing xylofuranose or its derivatives, iodinated bases and/or pyrrolo[2,3-*d*]pyrimidine are rare in nature.[3,4] Several uncommon nucleosides have been isolated from marine organisms, including iodinated 5′-deoxyribofuranosyl nucleoside **6** and 5′-(methylsulfanyl)-5′-deoxyxylofuranosyl nucleoside **7**.[5]



1 R, R^2 = H; R^1, R^3 = OH
6 R^1, R^2 = OH; R, R^3 = H
8 R, R^2 = H; R^1 = $OC(Me)_2OMe$; R^3 = OH
10 R^1, R^3 = *p*-bromobenzoyloxy



2 R^1 = H, R^2 = OH
3 R^1 = OH, R^2 = H
4 R^1 = H, R^2 = OMe
5 R^1 = OMe, R^2 = H



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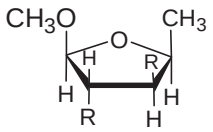

9 R = *p*-bromobenzoyloxy

Chart 1.

Samples of the green encrusting ascidian *Diplosoma* sp. (900 g, wet weight) overgrown on dead coral were collected by hand from the coast of Hateruma island, Okinawa, and stored at −15 °C before extraction with acetone. The acetone extract was suspended in aqueous MeOH (1:1) and then successively partitioned with hexanes, $CHCl_3$, and 1-BuOH. Repeated purification of the $CHCl_3$ extract by a series of chromatographic processes, including a silica gel column, an ODS column, HPLC on Si60, and reverse-phase HPLC on ODS, led to the isolation of **1** (0.018%), **2** and **3** (≈1:1 mixture, 0.0070%), **4** (0.0010%), and **5** (0.0003%). (+)-Didemnenones A (**2**) and B (**3**) and acetals **4** and **5** were unambiguously identified by comparison of their spectral data with those described in the literature.[6]

Analysis of **1** by ^{13}C NMR (Table 1) and HR-FABMS [m/z $(M)^+$ 376.0016, calcd for $C_{11}H_{13}IN_4O_3$, 376.0033] provided a molecular formula of $C_{11}H_{13}IN_4O_3$, which could be accounted for by seven degrees of unsaturation.[7] The MS spectrum [base peak at m/z 260 ($C_6H_5IN_4$)][7] and NMR data (Table 1) suggested that **1** was a nucleoside of an iodinated deazapurine base. The base was identified as 4-amino-5-iodo[2,3-*d*]pyrimidine by analysis of HMBC and HMQC data and by comparison of its ^1H and ^{13}C NMR resonances with those of the known nucleoside **6** (Table 1).[5a] ^1H NMR data for the sugar moiety in **1** showed some similarity to those of **6**, indicating that the sugar was a

Table 1. NMR data for **1** and **6**

	1[a]		**6**[b]	
C No.	^{13}C	^1H(Hz)	^{13}C	^1H(Hz)
2	151.9	8.10 (s)	151.9	8.11 (s)
4	156.9		156.9	
5	51.4		52.1	
6	128.0	7.59 (s)	126.7	7.60 (s)
8	149.5		150.1	
9	102.9		103.4	
1′	89.3	5.95 (d, 1.8)	86.9	6.00 (d, 5.2)
2′	81.9	4.12 (dd, 1.8, 3.7)	79.2	4.39 (q, 5.2)
3′	76.5	3.81 (dd, 3.7, 4.3)	73.3	3.84 (q, 5.2)
4′	77.9	4.20 (dq, 3.7, 6.7)	74.4	3.89 (dq, 5.2, 6.3)
5′	13.9	1.27 (d, 6.7)	18.9	1.27 (d, 6.3)
2′-OH		5.77 (d, 3.7)		5.31 (d, 5.2)
3′-OH		5.63 (d, 4.3)		5.08 (d, 5.2)
NH_2		6.65 (brs)		6.55 (brs)

[a]Recorded at 500 MHz ^1H NMR and 125 MHz ^{13}C NMR in $(CD_3)_2SO$. [b]Recorded at 400 MHz ^1H NMR and 100 MHz ^{13}C NMR in $(CD_3)_2SO$.[5a]

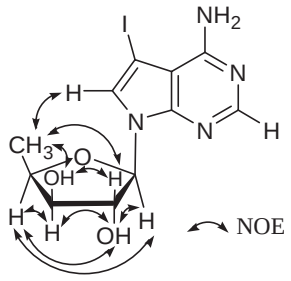

Figure 1. Selected NOEs for compound **1**.

5′-deoxypentose. Major differences in the ^{1}H–^{1}H coupling constants of **1** and **6** were observed for H1′ [5.95 (d, $J = 1.8$ Hz) in **1**, 6.00 (d, $J = 5.2$ Hz) in **6**], H2′ [4.12 (dd, $J = 1.8$, 3.7 Hz) in **1**, 4.39 (q, $J = 5.2$ Hz) in **6**], H3′ [3.81 (dd, $J = 3.7$, 4.3 Hz) in **1**, 3.84 (q, $J = 5.2$ Hz) in **6**] and H4′ [4.20 (dq, $J = 3.7$, 6.7 Hz) in **1**, 3.89 (dq, $J = 5.2$, 6.3 Hz) in **6**]. The sugar in **1** differed significantly from that of **6** in terms of ^{13}C NMR chemical shifts (Table 1).

The relative stereochemistry of the sugar moiety in **1** was determined by NOE experiment (Figure 1), chemical transformation (acetalization), and comparison of the appropriate ^{1}H NMR resonances with those of the known nucleoside **7** and synthetic β-xylofuranosyl nucleosides.[5b,8] The coupling constants for H1′/H2′, H2′/H3′, H3′/H4′, and H4′/H5′ in **1** ($J_{1',2'} = 1.8$ Hz, $J_{2',3'} = 0$ Hz, $J_{3',4'} = 3.7$ Hz, and $J_{4',5'} = 6.7$ Hz) were comparable to those of a β-xylofuranoside derivative **7** ($J_{1',2'} = 1.4$ Hz, $J_{2',3'} = 0$ Hz, $J_{3',4'} = 6.7$, 3.2 Hz, and $J_{4',5'} = 6.8$ Hz). A cis relationship between H1′ and H4′ was inferred from the NOE data, while the data obtained for H1′/OH2′, H2′/OH3′, H4′/OH2′, CH$_3$5′/OH3′, and CH$_3$5′/H2 implied that the sugar in **1** was β-5′-deoxyxylose (Figure 1).

Treatment of **1** with 2,2-dimethoxypropane and CSA (24 h at rt and then 4 h at 45 °C) afforded acetal **8**[9] rather than the acetonide. In the ^{1}H NMR spectrum of this compound, the proton signal [5.77 (d, $J = 3.7$ Hz)] corresponding to OH2′ in **1** disappeared, and two new methyl proton signals [1.17 (3H, s), 1.32 (3H, s)] and a methoxy proton signal [2.95 (3H, s)] appeared, indicating the presence of an acetal group in **8**. In addition, a proton signal [4.12 (dd, $J = 1.8$, 3.7 Hz)] assigned as H2′ in **1** was shifted to δ_H 4.22 (s) in **8**. These changes revealed that the sterically less hindered OH group at C2′ underwent addition to 2,2-dimethoxypropane. This result, with no formation of acetonide, provided evidence for a trans relationship between OH2′ and OH3′. Thus, the sugar moiety in **1** was concluded to be β-5′-deoxyxylose. All attempts to hydrolyze **1** were unsuccessful, resulting in decomposition of the reaction products. Naturally occurring xylose is known to be a D-series sugar. However, in view of the fact that a small but significant amount of both (+)- and (−)-isomers are present in marine natural products,[6,10] we attempted to determine the absolute stereochemistry of marine metabolite **1** by CD measurement. A pronounced negative Cotton effect which was seen in the CD spectrum of **1** [λ_{ext} 242 ($\Delta\varepsilon$ −1.9) and 210 ($\Delta\varepsilon$ −2.6) nm, EtOH] suggested purin-9-yl β-D-xylofuranosides.[11] In addition, to confirm the absolute stereostructure of the 5-deoxy-β-xylofuranose unit of **1**, we synthesized dibenzoates **9** and **10**[12] by treatment of methyl 5-deoxy-β-L-xylofuranoside[13,14] and **1** with 4-bromobenzoyl chloride, DMAP and pyridine (24 h at rt). The CD spectrum [λ_{ext} 253 ($\Delta\varepsilon$ −20.3) and 236 ($\Delta\varepsilon$ +1.9) nm, MeOH] of dibenzoate **9** was similar to that of **10** [λ_{ext} 253 ($\Delta\varepsilon$ +20.4) and 237 ($\Delta\varepsilon$ −8.1) nm, MeOH][15] except for the sign. Therefore, the absolute configuration of the 5-deoxy-β-xylofuranose unit of **1** was determined to be D.

Compounds **1** and **6** were isolated from two unrelated marine organisms, the ascidian *Diplosoma* sp. and the alga *Hypnea valendiae*, which supports the possibility of microbial origin of these compounds. Compound **1** was found to cause complete inhibition of cell division in fertilized sea urchin eggs at a concentration of 1 μg/mL and showed weak activity against HCT116 cells (human colorectal cancer cells) with an IC_{50} of >20 ppm.

References and Notes

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7 **1**: $[\alpha]_D^{26}$ −69° (*c* 0.1, MeOH); IR (film) 3461, 3317, 3132, 1633, 1584, 1474, 1084, 755 cm^{-1}; UV (MeOH) λ_{max} 283 nm (ε 3900); LR-EIMS *m/z* (rel. %) 376 (M^+, 7), 303 (3), 289 (13), 261 (33), 260 (100), 233 (18).

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9 **8**: HR-FABMS *m/z* 449.0677 $(M + H)^+$ (calcd for $C_{15}H_{22}IN_4O_4$, 449.0686); ^{1}H NMR [$(CD_3)_2SO$, 500 MHz] δ 1.17 (3H, s, Me), 1.23 (3H, d, $J = 4.6$ Hz, Me5′), 1.32 (3H, s, Me), 2.95 (3H, s, OMe), 3.88 (1H, dd, $J = 3.4$, 4.6 Hz, H3′), 4.12 (1H, qd, $J = 3.4$, 6.4 Hz, H4′), 4.22 (1H, brs, H2′), 5.83 (1H, d, $J = 4.6$ Hz, OH3′), 6.04 (1H, d, $J = 2.0$ Hz, H1′), 7.63 (1H, s, H8), 8.11 (1H, s, H2).

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12 **9**: HR-ESIMS *m/z* 534.9340 $(M + Na)^+$ (calcd for $C_{20}H_{18}Br_2NaO_6$: 534.9368); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (d, $J = 8.8$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.59 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 8.8$ Hz, 2H), 5.51 (d, $J = 5.4$ Hz, 1H), 5.44 (s, 1H), 5.01 (s, 1H), 4.69 (m, 1H), 3.46 (s, 3H), 1.32 (d, $J = 6.8$ Hz, 3H); **10**: HR-ESIMS *m/z* 740.8857 $(M + H)^+$ (calcd for $C_{25}H_{20}Br_2IN_4O_5$: 740.8845); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21 (s, 1H), 7.90 (d, $J = 8.3$ Hz, 2H), 7.89 (d, $J = 8.3$ Hz, 2H), 7.66 (d, $J = 8.3$ Hz, 2H), 7.60 (d, $J = 8.3$ Hz, 2H), 7.43 (s, 1H), 6.51 (s, 1H), 5.74 (s, 1H), 5.64 (d, $J = 3.4$ Hz, 1H), 4.69 (m, 1H), 1.42 (d, $J = 6.4$ Hz, 3H).

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15 Positive chirality between the *p*-bromobenzoyl chromophores of **10** also indicated that the sugar moiety in **1** was of the D-xylose series (2′R and 3′S configuration).[16]

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