# $N^3$,5′-Cycloxanthosine, the First Natural Occurrence of a Cyclonucleoside

Robert J. Capon* and Nicholas S. Trotter

*Centre for Molecular Biodiversity, Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, 4072, Australia*

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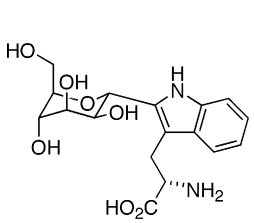
An *Eryus* sp. of marine sponge from the Great Australian Bight has yielded the first reported natural occurrence of a cyclonucleoside, $N^3$,5′-cycloxanthosine. The structure of $N^3$,5′-cycloxanthosine was confirmed by detailed spectroscopic analysis and total synthesis.

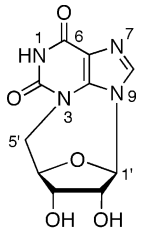
During investigations into bioactive metabolites from Australian marine sponges we had cause to examine an *Eryus* sp. (class, Demospongia; order, Astrophorida; family, Geodiidae) obtained in July 1995 during scientific trawling operations in the Great Australian Bight. Although the aqueous EtOH extract of this specimen did not demonstrate significant biological activity in our target bioassays (antimicrobial and antiparasitic), we were intrigued by the presence of unidentified "aromatic" compounds in the $H_2O$-soluble partition. In past years we have observed that sponge extracts can yield novel $H_2O$-soluble metabolites, such as the unprecedented sulfur sugar 5-thio-D-mannose,[1] the nematocidal amino acid analogues echinobetaines A[2] and B[3], and the acetylcholine mimic esmodil.[4] In each of these cases, the structural proofs were supported by detailed spectroscopic analysis and total synthesis. Encouraged by these experiences and the prospect that the *Eryus* sp. may also contain novel metabolites, we embarked on a detailed chemical analysis.

The freeze-dried $H_2O$-soluble partition of an aqueous EtOH extract of the *Eryus* sp. was fractionated by elution through Sephadex G-10 ($H_2O$) followed by $C_{18}$ HPLC, to yield the two pure "aromatic" metabolites. The first of these metabolites was identified as $C^2$-α-D-mannosylpyranosyltryptophan (**1**). This unusual *C*-glycosylated tryptophan was first reported in 1994 by Hofsteenge et al.[5] during an investigation into novel post-translational modification in human RNases and was subsequently detected by the same authors in a range of other human proteins including IL-12[6] and the erythropoietin receptor.[7] Although first recognized as a rare post-translational *C*-glycosylation event in human proteins, the free glycosylated amino acid **1** was subsequently reported in 2000 by Riguera et al.[8] from a marine ascidian. Following this study, in 2001 $C^2$-α-D-mannosylpyranosyltryptophan (**1**) was acknowledged by Yonemura et al.[9] as a novel marker of renal function, being an accurate measure of insulin clearance in patients with chronic renal failure. Our isolation of $C^2$-α-D-mannosylpyranosyltryptophan (**1**) from an Australian marine sponge, *Eryus* sp., suggests that tryptophan *C*-glycosylation as a biosynthetic event may be more widespread than previously documented.

While the reisolation of $C^2$-α-D-mannosylpyranosyltryptophan was noteworthy, of greater interest to us was the discovery of the co-metabolite, **2**. On the basis of high-resolution ESI(+)MS measurements (M + Na Δmmu −0.2), **2** was attributed the molecular formula $C_{10}H_{10}N_4O_5$, requiring eight double-bond equivalents. The $^1$H NMR data



**1**



**2**

for **2** (see Table 1) were deceptively simple, indicative of a nuclear base bearing a single aromatic proton attached to a sugar moiety, suggestive of a modified nucleoside. Lack of a measurable $^1$H NMR coupling or 2D NMR COSY correlation to the proposed anomeric proton H-1′ ($\delta$ 6.15) was interpreted as evidence of a restricted conformation featuring dihedral angles that disfavored $J_{1',2'}$ coupling. This analysis was further tempered by the observation of 2D NMR gHMBC correlations from H-1′ to C-2′, C-3′, and C-4′. Connection of the sugar moiety through $N^9$ was supported by 2D NMR gHMBC correlations from H-1′ to C-4 and C-8. The UV and 2D NMR data for **2**, while supportive of a modified nucleoside, did not provide conclusive evidence from which to assign unambiguously a molecular structure. To more fully explore this possibility and provide an unambiguous assignment of molecular structure, we embarked on a structure proof by total synthesis.

An early and plausible candidate structure for **2** was that of 8,5′-*O*-cycloinosine; however, although known as a synthetic compound since 1976,[10] the published spectroscopic data for 8,5′-*O*-cycloinosine proved inadequate for modern comparison purposes. Employing literature procedures,[10−13] we completed a total synthesis of 8,5′-*O*-cycloinosine in five steps from adenosine, as outlined in Scheme 1 (for experimental details see the Supporting Information). To our disappointment, 8,5′-*O*-cycloinosine did not coelute on HPLC with, and the $^1$H NMR ($d_6$-DMSO or $d_4$-MeOH) data clearly differed from that of, the natural product **2**. Despite these differences, the data were sufficiently similar for us to conclude that **2** is indeed a cyclonucleoside, just not 8,5′-*O*-cycloinosine.

A reappraisal of the data for **2** suggested an alternative cyclonucleoside structure, namely, $N^3$,5′-cycloxanthosine. First synthesized in 1963,[14] and further characterized by ORD studies,[15] $N^3$,5′-cycloxanthosine remained dormant in the scientific literature until 2004, at which time it came to our attention through the publication[16] of a convenient one-step synthesis from xanthosine. Although the $^1$H and $^{13}$C NMR ($d_6$-DMSO) data reported for $N^3$,5′-cycloxan-

* Corresponding author. Tel: +61-7-33462979. Fax: +61-7-33462101. E-mail: r.capon@imb.uq.edu.au.

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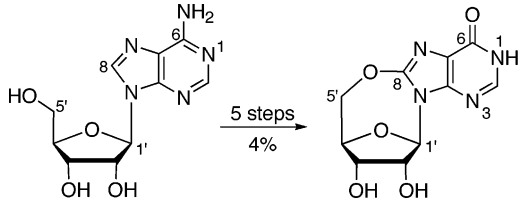
**Table 1.** NMR ($d_6$-DMSO, 600 MHz) Data for $N^3$,5′-Cycloxanthosine (**2**) and 8,5′-*O*-Cycloinosine

| | **2** | | | | 8,5′-*O*-cycloinosine | |
|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ (m, *J* (Hz)) (m,J) | COSY | gHMBC[a] | $\delta_C$ | $\delta_H$ [m, *J* (Hz)] |
| 1 | | | | | | 7.00 (br s) |
| 2 | 151.0 | | | H-5′a | 145.8 | 8.04 (s) |
| 3 | | | | | | |
| 4 | 140.7 | | | H-8, H-1′, H-5′a | 145.8 | |
| 5 | 117.8 | | | H-8 | 119.3 | |
| 6 | 157.4 | | | H-8 | 155.9 | |
| 7 | | | | | | |
| 8 | 134.5 | 7.81 (s) | | H-1′ | 152.5 | |
| 9 | | | | | | |
| 1′ | 92.5 | 6.15 (s) | | OH-2′, H-4′, H-5′a | 89.1 | 5.93 (s) |
| 2′ | 75.8 | 3.87 (dd, 5.2, 5.2) | OH-2′, H-3′ | H-1′, OH-2′, OH-3′ | 77.1 | 4.27 (d, 6.0) |
| 3′ | 70.1 | 4.20 (br m) | H-2′, OH-3′, H-4′ | H-1′, OH-3′, H-4′, H-5′a,b | 71.0 | 4.45 (d, 6.1) |
| 4′ | 83.7 | 4.56 (m) | H-3′, H-5′a,b | H-1′, H-2′, OH-3′, H-5′b | 88.2 | 4.56 (s) |
| 5′a | 51.3 | 4.56 (m) | H-4′, H-5′b | H-3′, H-4′ | 74.5 | 4.56 (d, 13.1) |
| 5′b | 51.3 | 3.71 (dd, 15.2, 3.2) | H-4′, H-5′a | H-3′, H-4′ | 74.5 | 4.05 (d, 13.1) |
| OH-2′ | | 5.50 (d, 5.0) | H-2′ | | | |
| OH-3′ | | 5.44 (d, 6.6) | H-3′ | | | |

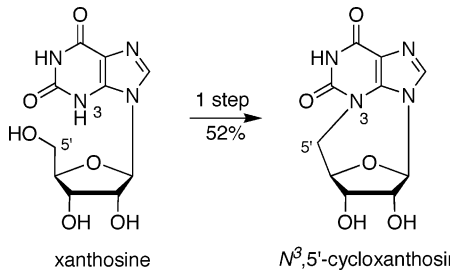
[a] Protons correlated to carbon resonances in the $\delta_C$ column.

**Scheme 1.** Synthesis of 8,5′-*O*-Cycloinosine[a]




[a] Reagents: (i) $Br_2$, NaOAc buffer; (ii) *p*-TsOH, acetone; (iii) NaH, 1,4-dioxane; (iv) 1 M $H_2SO_4$; (v) $NaNO_2$, acetic acid.

**Scheme 2.** Synthesis of $N^3$,5′-Cycloxanthosine (**2**)[a]




[a] Reagents: (i) $PPh_3$, diisopropylazodicarboxylate, DMF.

thosine in 2004 was a close match to the natural product **2**, small discrepancies and the absence of an optical rotation required that we repeat the synthesis and carry out an independent comparison. On repeating the synthesis of $N^3$,5′-cycloxanthosine (Scheme 2) (for experimental details see the Supporting Information), we observed that the natural product **2** was in fact identical in all respects ($^1$H and $^{13}$C NMR, UV/vis, $[\alpha]_D$, and coelution on HPLC) to $N^3$,5′-cycloxanthosine.

The discovery of novel nucleosides from marine sponges is not without precedence. Indeed, the discovery of spongothymidine and spongouridine from a Caribbean sponge in the 1950s by Bergmann et al.[17−19] has been credited[20] with inspiring development of the commercial antileukemic agent Ara-C and the antiviral agent Ara-A. It has even been suggested[20] that knowledge of these sponge nucleosides inspired development of the anti-AIDS drug AZT.

With respect to cyclonucleosides, although a target for synthetic chemists as early as the 1960s, the scientific literature is sparse on their application against biological targets, with no primary literature on the biological screening or biological properties of the purine cyclonucleosides 8,5′-*O*-cycloinosine or $N^3$,5′-cycloxanthosine. By contrast, 8,3′-*O*-cycloadenosine has been described as an inhibitor of rhodopsin kinase,[21] and the pyrimidine analogues, exemplified by 2,2′-*O*-cyclocytidine and 2,5′-*O*-cyclocytidine, have been acknowledged as antitumor[22] and antiviral agents,[23] respectively. Carbocyclic cyclonucleoside analogues such as 8,5′-cycloadenosine are known as products of oxidative damage to DNA.[24] Most recently, in 2004, the value of cyclonucleosides has been revisited, with $N^3$,5′-cycloxanthosine (**2**) being patented[25] as a member of a family of "synthetic" nucleosides with antiviral properties.

## Experimental Section

**General Experimental Procedures.** See Supporting Information.

**Biological Material.** The marine sponge *Eryus* sp. (Museum of Victoria Registry Number MVF83533) was collected by beam trawl in July 1995 from the Great Australian Bight at a depth of 50 m at position 33°35′ S, 114°46′ E. The sponge was diced, steeped in EtOH, and kept at −20 °C prior to extraction.

**Extraction and Isolation.** The EtOH extract was decanted and concentrated in vacuo to yield a crude extract (4.68 g). Trituration with DCM returned insoluble material (4.62 g, 99%), which was partitioned between *n*-BuOH (2.32 g, 50%) and $H_2O$ (2.25 g, 48%). The freeze-dried $H_2O$-soluble material was fractionated by Sephadex G-10 ($H_2O$) and $C_{18}$ HPLC (2.5 mL min$^{-1}$ 5% isocratic $CH_3CN/H_2O$ through a 10 $\mu$m Phenomenex Spherex 5 $C_{18}$ 250 × 10 mm column) chromatography to return the previously reported $C^2$-α-D-mannosylpyranosyltryptophan[8,26,27] (**1**) (10.0 mg, 0.21%) and (−)-$N^3$,5′-cycloxanthosine[14−16] (**2**) (9.5 mg, 0.20%). In subsequent isolations of natural products **1** and **2**, the $C_{18}$ HPLC column of choice proved to be a 5 $\mu$m Agilent Zorbax SB-Aq 150 × 9.4 mm column (4.2 mL min$^{-1}$ $H_2O$ elution). All yields for purified metabolites were calculated against the crude ethanolic extract (4.68 g).

***C*$^2$-α-D-Mannosylpyranosyltryptophan**[8,26,27] **(1):** white solid. Natural product **1** was identified by spectroscopic analysis [$^1$H and $^{13}$C NMR, ESI(±)MS], optical rotation ($[\alpha]_D$), and comparison with literature data.

**(−)-*N*$^3$,5′-Cycloxanthosine**[14−16] **(2):** white solid; $[\alpha]_D$ −18° (*c* 0.021, DMSO); UV ($H_2O$) $\lambda_{max}$ (log $\epsilon$) 236 (3.91), 266 (3.99) nm; $^1$H NMR ($d_6$-DMSO, 600 MHz), see Table 1; ($d_4$-MeOH, 600 MHz) $\delta$ 7.80 (s, H-8), 6.15 (s, H-1′), 4.76 (ddd, $J$ = 14.4, 2.3, 0.7 Hz, H-5′a), 4.68 (dt, $J$ = 3.6, 2.6, 2.6 Hz, H-4′), 4.34 (dd, $J$ = 5.5, 3.8 Hz, H-3′), 4.04 (d, $J$ = 5.6 Hz, H-2′), 3.87 (dd, $J$ = 14.4, 2.7 Hz, H-5′b); $^{13}$C NMR ($d_6$-DMSO, 150 MHz), see Table 1; $^{13}$C NMR ($d_4$-MeOH, 100 MHz) $\delta$ 159.7 (C, C-6), 152.9 (C, C-2), 142.5 br (C, C-4), 136.3 br (CH, C-8), 119.4 br (C,

C-5), 94.9 (CH, C-1′), 85.8 (CH, C-4′), 77.7 (CH, C-2′), 71.9 (CH, C-3′) and 53.1 ($CH_2$, C-5′); ESI(+)MS (100V) *m/z* 555 $[2M + Na]^+$, 533 $[2M + H]^+$, 267 $[M + H]^+$; ESI(−)MS (100V) *m/z* 531 $[2M - H]^-$, 265 $[M - H]^-$; HRESI(+)MS *m/z* 289.0547 ($[M + Na]^+$, $C_{10}H_{10}N_4O_5Na$ requires 289.0549).

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**Supporting Information Available:** General experimental procedures, synthetic schemes, experimental procedures, and references and notes for the synthesis of compounds **2** and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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