A CARBOCYCLIC 7-DEAZAPURINE–PYRIMIDINE HYBRID NUCLEOSIDE

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contributions to the area of nucleic acid chemistry.

The design, synthesis and rationale for the first example of a carbocyclic 7-deazapurine–pyrimidine hybrid nucleoside is described. The marriage of key structural features from the purine and pyrimidine nucleobase scaffolds has given rise to novel hybrid nucleobases designed to be recognized by biologically relevant purine- and pyrimidine-metabolizing enzymes. Pairing these hybrid bases with chemotherapeutically beneficial carbocyclic "sugars", in addition to utilizing the highly effective 7-deaza ring system, should result in chemotherapeutically useful nucleosides. The approach to realize the first 7-deazapurine–pyrimidine hybrid nucleoside **3** is presented herein.

Keywords: Carbocyclic nucleosides; Carbanucleosides; Isoadenosine; 7-Deazapurines; Pyrrolopyrimidines; Pyrimidines; Nucleobases.

Recent efforts in our laboratories have focused on the strategic manipulation of key aspects of the nucleoside scaffold in an effort to probe structure, function, and chemotherapeutic activity. Increasing reports of viral and antibacterial resistance have rendered many of the current chemotherapeutics less effective, thus the design of an inhibitor that could be recognized by either a purine- or a pyrimidine-metabolizing enzyme might lead to a dual inhibitor capable of disrupting two different enzymatic steps in the same mechanistic pathway, or in different pathways. This could prove to be a highly effective approach for overcoming resistance mechanisms involving binding site mutations.

In that regard, exploitation of the nonstandard connectivity of the purine nucleoside isoadenosine (**1**; IsoA, Fig. 1) was envisioned as a starting point for constructing hybrid nucleosides that resemble both purines and

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pyrimidines. IsoA is a structural isomer of adenosine where the purine ring is connected to the sugar moiety at N3 (purine numbering), rather than the traditional N9 (refs[1,2]). Interestingly, N3-glycosolated purines can be viewed as 5,6-disubstituted pyrimidines, and conversely, 5,6-disubstituted pyrimidines are often considered as purine mimics, thus providing impetus for our goal[3,4].

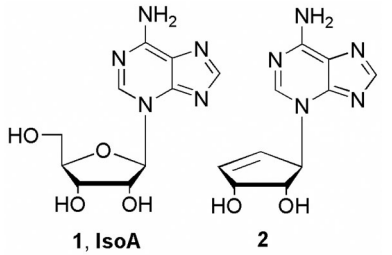


FIG. 1
Isoadenosine and (–)-3-(2′,3′-dihydroxycyclopent-4′-enyl)adenine

Next, incorporation of the carbocyclic nucleoside scaffold was also viewed as strategic, since as a class, carbocyclic purine nucleosides are known inhibitors of *S*-adenosylhomocysteine hydrolase (SAHase), and indirectly, DNA methyltransferase (DNA MeTase), both critical enzymes in the replication cycle of many viruses, parasites, and cancers[5,6]. In addition, some carbocyclic pyrimidines have proven to be potent inhibitors of CTP synthetase[7,8], while many 5- and 6-substituted pyrimidine nucleosides have exhibited potent broad-spectrum chemotherapeutic activity[9], as well as inhibition of thymidine phosphorylase and thymidine synthase[10,11]. Drawing upon these leads, it appeared that a hybrid of the pyrimidine and purine ring systems merged with the carbocyclic scaffold might offer forth a successful strategy for the design of dual inhibitor analogues.

While our initial synthetic efforts had begun with the construction of the parent carbocyclic isoA analogue (**2**; DHCe-IsoA, Fig. 1)[12] as an entry into the N3-glycosylated series, our ultimate goal was to construct a hybrid of the pyrimidine and purine ring systems, thus the next targets considered were the fused uridine-imidazole analogues depicted in Fig. 2. Preliminary ab initio calculations (data not shown) indicated that the imidazole proton that tautomerizes between N7 and N9 preferred to reside on N7. Unfortunately, this also suggested that an intramolecular hydrogen bond (Fig. 3) due to the formation of a five-membered pseudo cyclic structure was highly favorable. This was undesirable, since it would deactivate two key structural

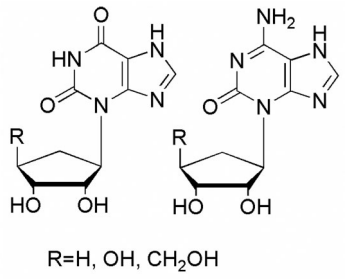


FIG. 2
Carbocyclic purine–pyrimidine hybrid nucleosides

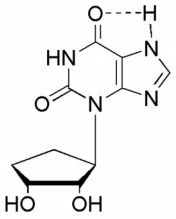


FIG. 3
Intramolecular H-bonding in a carbocyclic nucleoside

contacts critical for recognition in enzyme binding sites. To investigate the implications of this, the isosteric exchange of a methine group for the N7 nitrogen to afford a 7-deazapurine-like or pyrrolopyrimidine nucleoside was considered (Fig. 4). This structural modification would allow for retention of the aromaticity desired for the heterocyclic base while removing the proton involved. In addition, given the rich history of biological activity for many 7-deaza carbocyclic nucleosides[13–16], the pursuit of this target was deemed especially attractive.

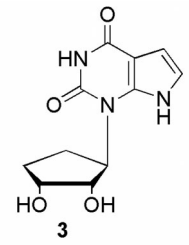
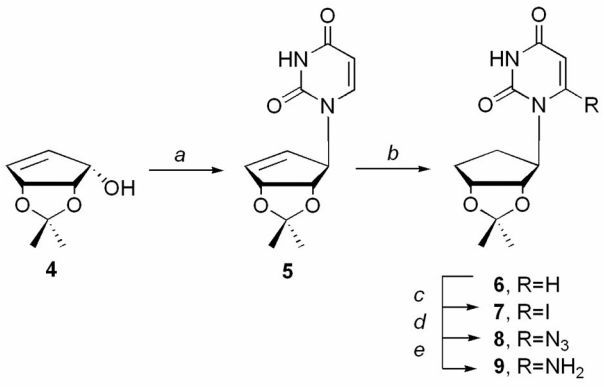


FIG. 4
Carbocyclic pyrrolopyrimidine hybrid nucleosides

The route to our previously reported[12] isoA analogue **2** (and its corresponding enantiomer) had relied upon nucleophilic displacement of an activated alcohol or halide by adenine in the absence of base. This approach had proven unsatisfactory due to disappointingly low yields. In the absence of base the N3-glycosylated product was the major isomer obtained, however, the N7, N9 and N1 isomers were also formed, thereby significantly lowering the yield of the desired N3 isomer. As a result, a new, more efficient coupling procedure, was sought. In that regard, Mitsunobu coupling[17] has been one of the traditionally used methods in the synthesis of carbocyclic nucleosides, although it is generally higher yielding for purine nucleosides, since the pyrimidines suffer from competition between O- and N-alkylation. Fortunately, a recent study in the literature[18] provided a valuable insight on how to tip the scales to favor almost exclusive formation of the desired N-alkylated product by using the benzoyl protecting group for the N3 of uracil.

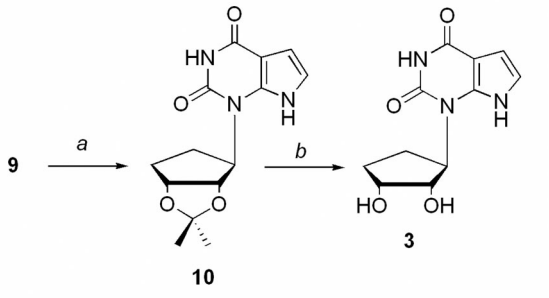
As shown in Scheme 1, N^3-benzoyl uracil[19] was coupled to the known **4** [14,20], which, after removal[19] of the benzoyl group gave **5** in a 65% yield for the two steps. Reduction of the double bond of the cyclopentene ring was then necessary, as we have found that direct iodination of the base results in a significant amount of cleavage of the base, probably due to the formation of an allylic carbocation. The reduction was accomplished in quantitative yield using mild palladium-catalyzed hydrogenation[15] to afford **6**, which, following iodination with LDA [10,21] gave **7** (60%). Next, dis-



Reagents and conditions: *a*, i) PPh_3, DIAD, N^3-benzoyluracil, CH_3CN, 0 °C to rt, 24 h; ii) NaOH, MeOH, rt, 12 h, 65 %; *b*, Pd/C, H_2, MeOH, rt, 20 min, quant.; *c*, LDA, THF, I_2, -78 °C, 5 h, 60 %; *d*, NaN_3, DMF, rt, 1h, 82 %; *e*, Pd/C, H_2, 0.17MPa, rt, 1h, 85 %.

SCHEME 1

placement of the iodo functionality with sodium azide provided **8** (82%), followed by reduction to the 6-aminopyrimidine intermediate **9** in quantitative yield[10]. Manipulation of the amine functionality of **9** to form the pyrrole ring (Scheme 2) of **10** was accomplished by refluxing **9** with chloroacetaldehyde and sodium acetate for 1 day[22], albeit in only a moderate yield (40%). Finally, standard deblocking of the isopropylidene group on the 2′- and 3′-hydroxyl groups of **10** (quantitative) gave the desired 7-deaza target **3** in 7 steps with an overall yield of 9.6%. Efforts are currently underway to optimize this route so that more extensive biological studies of **3** can be undertaken. In the meantime, the initial broad-screen biological testing on **3** has begun and the results will be described in due course.



Reagents and conditions: *a*, chloroacetaldehyde, NaOAc, EtOH, 1 d, reflux, 40 %; *b*, TFA : H_2O (2:1), rt, 2 h,70.5 %.

SCHEME 2

In summary, a short and reasonably efficient synthetic route to a new class of 7-deazapurine–pyrimidine hybrid nucleosides has been introduced. While it remains to be seen if they will ultimately act as dual inhibitors, the significant biological activity of a variety of 7-deaza nucleosides including 7-deazaaristeromycin, 7-deaza-5′-noraristeromycin[13,16], tubercidin, toyocamycin and sangivamycin[14,23–25] certainly offers forth strong impetus for the pursuit of the corresponding hybrid analogues of these potent nucleosides. Efforts are currently underway to realize these, and reports of their synthesis will be forthcoming as they become available.

EXPERIMENTAL

General

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were operated at 400 and 100 MHz, respectively, all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman

Diamond silica gel 60-F_{254} precoated plates. Column chromatography was performed on Whatman silica, 200–400 mesh, 60 Å and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

Preparation of 1-[(4,5-(Propane-2,2-diyldioxy)cyclopent-2-en-1-yl]uracil (**5**)

To (+)-4,5-(propane-2,2-diyldioxy)cyclopent-2-en-1-ol[14,20] (**4**) (6.50 g, 41.67 mmol), N^3-benzoyluracil[19] (18.02 g, 83.33 mmol) and PPh_3 (21.88 g, 83.33 mmol) in anhydrous CH_3CN (500 ml) at 0 °C was added dropwise diisopropylazodicarboxylate (16.85 g, 83.33 mmol, 16.52 ml). After stirring at room temperature for 15 h, the mixture was concentrated and purified by column chromatography (EtOAc/hexane, 3:1) to afford 9.74 g of the coupled product as a white solid, 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6): 1.23 (3 H, s); 1.32 (3 H, s); 4.71 (1 H, d); 5.27 (1 H, s); 5.29 (1 H, d); 5.81 (1 H, d); 5.84 (1 H, dd); 6.23 (1 H, dt); 4.71 (1 H, d); 5.29 (2 H, d); 5.84 (1 H, d); 5.85 (1 H, d); 6.22 (1 H, m); 7.51 (1 H, d); 7.57 (2 H, t); 7.78 (1 H, t); 7.97 (2 H, m). ^{13}C NMR (75 MHz, DMSO-d_6): 24.5, 26.2, 69.2, 83.2, 84.6, 101.1, 111.8, 128.9, 129.1, 130.2, 131.6, 135.0, 139.0, 143.0, 145.0, 163.0, 168.8.

NaOH (10 ml, 1% in MeOH) was added to the coupled product (0.45 g, 1.27 mmol) and the mixture allowed to stir at room temperature for 12 h, then neutralized with 1 M HCl[19]. The mixture was evaporated and the resulting residue dissolved in EtOAc (50 ml), washed with H_2O (25 ml), dried (anhydrous $MgSO_4$), and the solvent evaporated under vacuum. The residue was then purified by column chromatography (EtOAc/hexane, 4:1) to afford 0.25 g of **5** as a white solid, 79% yield. ^{1}H NMR (400 MHz, CD_3OD): 1.22 (3 H, s); 1.31 (3 H, s); 4.53 (1 H, d); 5.27 (2 H, m); 5.52 (1 H, dd); 5.77 (1 H, dd); 6.19 (1 H, d); 7.23 (1 H, d); 11.3 (1 H, s). ^{13}C NMR (75 MHz, CD_3OD): 24.5, 26.2, 68.4, 83.4, 84.6, 101.2, 111.7, 129.4, 138.6, 142.5, 151.3, 165.0.

Preparation of 1-[(2,3-(Propane-2,2-diyldioxy)cyclopentyl]uracil (**6**)

To **5** (0.25 g, 1.0 mmol) in MeOH (10 ml) was added Pd/C (10%, 0.025 g) and the mixture subjected to hydrogenation at a pressure of 0.17 MPa for 20 min[14]. The mixture was filtered and the filtrate concentrated to afford 0.26 g of **6** as a white solid (quantitative). ^{1}H NMR (400 MHz, DMSO-d_6): 1.20 (3 H, s); 1.35 (3 H, s); 1.65–1.82 (2 H, m); 1.95–2.15 (2 H, m); 4.63 (1 H, m); 4.64 (1 H, m); 4.71 (1 H, dd); 5.52 (1 H, d); 7.58 (1 H, d); 11.3 (1 H, s). ^{13}C NMR (75 MHz, DMSO-d_6): 24.5, 27.3, 29.1, 31.2, 63.9, 80.4, 84.4, 101.8, 110.0, 143.9, 152.6, 163.8

Preparation of 6-Iodo-1-[(2,3-(propane-2,2-diyldioxy)cyclopentyl]uracil (**7**)

To a flame-dried flask under an Ar atmosphere was added anhydrous THF (5 ml) and freshly distilled diisopropylamine (0.23 g, 2.27 mmol). The temperature was lowered to –78 °C and n-BuLi added dropwise (2.5 M in hexanes, 2.27 mmol, 0.21 ml). Maintaining the temperature at –78 °C, the mixture was stirred for 15 min, then a solution of **6** (0.26 g, 1.03 mmol) in anhydrous THF (10 ml) added and the mixture stirred for an additional 30 min. Still maintaining the temperature at –78 °C, a solution of I_2 (0.58 g, 2.27 mmol) in anhydrous THF (5 ml) was added and the mixture stirred for an additional 2 h[10,21]. The reaction was then quenched with acetic acid (10 drops) and $NaHCO_3$ (1 ml), the solvent removed by evaporation, and the resulting residue dissolved in EtOAc (50 ml), washed with sodium thiosulfate (25 ml) and brine (25 ml), dried (anhydrous $MgSO_4$), and the solvent removed

by evaporation. The residue was purified by column chromatography (EtOAc/hexane, 4:1) to afford 0.23 g of **5** as a white solid, 60% yield. ^{1}H NMR (400 MHz, CD_3OD): 1.26 (3 H, s); 1.46 (3 H, s); 1.81–1.88 (1 H, m); 1.92 (1 H, m); 2.31 (2 H, m); 4.99 (2 H, s); 5.01 (1 H, m); 6.38 (1 H, s). ^{13}C NMR (75 MHz, CD_3OD): 23.6, 26.4, 29.2, 31.7, 74.9, 82.2, 84.7, 110.8, 115.1, 118.1, 148.3, 162.9.

Preparation of 6-Azido-1-[(2,3-(propane-2,2-diyldioxy)cyclopentyl]uracil (**8**)

A solution of **7** (0.10 g, 0.29 mmol) and NaN_3 (0.094 g, 1.45 mmol) in anhydrous DMF (3 ml) was stirred at room temperature for 30 min [10]. H_2O (20 ml) was added and the mixture extracted with EtOAc (3 × 50 ml), washed with brine (50 ml), dried (anhydrous $MgSO_4$), and concentrated to yield 0.066 g of **8** as a white solid, 85% yield. ^{1}H NMR (400 MHz, CD_3OD): 1.25 (3 H, s); 1.43 (3 H, s); 1.79–1.83 (1 H, m); 1.95 (1 H, m); 2.29 (2 H, m); 4.83 (2 H, m); 4.97 (1 H, dd); 5.52 (1 H, s). ^{13}C NMR (75 MHz, CD_3OD): 23.8, 26.2, 28.5, 31.8, 62.2, 81.9, 84.4, 87.9, 111.5, 146.2, 151.7, 163.1.

Preparation of 6-Amino-1-[(2,3-(propane-2,2-diyldioxy)cyclopentyl]uracil (**9**)

To a solution of **8** (0.066 g, 0.23 mmol) in absolute EtOH (50 ml) was added Pd/C (10%, 10 mg). This mixture was subjected to hydrogenation at a pressure of 0.17 MPa for 20 min. The reaction mixture was filtered and the filtrate concentrated to afford 0.060 g of **9** as a white solid (quantitative yield). ^{1}H NMR (400 MHz, CD_3OD): 1.23 (3 H, s); 1.43 (3 H, s); 1.91 (2 H, m); 2.32 (2 H, m); 4.39 (1 H, m); 4.81 (1 H, s); 4.88 (1 H, m); 5.06 (1 H, dd). ^{13}C NMR (75 MHz, CD_3OD): 23.5, 26.1, 29.6, 32.6, 62.2, 76.3, 82.1, 84.9, 110.5, 151.5, 157.8, 164.9.

Preparation of 1-[(2,3-(Propane-2,2-diyldioxy)cyclopentyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (**10**)

To a stirred suspension of **9** (0.06 g, 0.22 mmol) in EtOH (10 ml) was added NaOAc (0.07 g, 0.51 mmol) and the mixture was heated to 70 °C. Chloroacetaldehyde (0.02 ml, 0.31 mmol) and NaOAc (0.07 g, 0.51 mmol) were then added and the mixture was stirred at 70 °C for an additional 24 h [22]. The EtOH was removed under vacuum and the product was dissolved in EtOAc (25 ml), washed with H_2O (2 × 10 ml) and brine (2 × 10 ml), dried (anhydrous $MgSO_4$), and concentrated. The residue was purified by column chromatography (EtOAc/hexanes, 4:1) to afford 0.028 g of **10** as a white solid, 51% yield. ^{1}H NMR (400 MHz, CD_3OD): 1.24 (3 H, s); 1.44 (3 H, s); 1.89 (2 H, m); 2.34 (2 H, m); 3.28 (1 H, m); 4.44 (1 H, s); 4.89 (1 H, s); 5.21 (1 H, s); 6.43–6.47 (1 H, m); 6.63 (1 H, m); 9.98 (1 H, s). ^{13}C NMR (75 MHz, CD_3OD): 23.2, 25.8, 29.5, 32.1, 64.0, 81.9, 84.2, 99.8, 103.8, 110.5, 117.2, 140.5, 150.8, 160.9.

Preparation of 1-(2,3-Dihydroxycyclopentyl)-7*H*-Pyrrolo[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (**3**)

Compound **10** (0.028 g, 0.09 mmol) was dissolved in a 1:1 mixture of TFA and H_2O (25 ml) and the reaction mixture was stirred at room temperature for 12 h. The resulting solution was concentrated and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 5:1) to afford 0.012 g of **3** as a white solid 70.5% yield. ^{1}H NMR (400 MHz, CD_3OD): 1.71

(1 H, m); 2.14 (3 H, m); 4.12 (1 H, m); 4.54 (1 H, m); 4.76 (1 H, m); 6.42 (1 H, d); 6.71 (1 H, d). ^{13}C NMR (75 MHz, CD_3OD): 22.9, 29.3, 62.8, 72.1, 74.1, 99.9, 103.5, 116.9, 141.4, 150.6, 161.1. For $C_{11}H_{13}N_3O_4 \cdot 0.3H_2O$ calculated: 51.48% C, 5.35% H, 16.38% N; found: 51.39% C, 5.20% H, 16.26% N.

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