## "Molecular Chameleons". Design and Synthesis of C-4-Substituted Imidazole Fleximers

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## ABSTRACT



The synthesis of two flexible nucleosides is presented. The "fleximers" feature the purine ring system split into its imidazole and pyrimidine components. This modification serves to introduce flexibility to the nucleoside while still retaining the elements essential for molecular recognition. As a result, these structurally innovative nucleosides can more readily adapt to capricious binding sites and, as such, should find use for investigating enzyme–coenzyme as well as nucleic acid–protein interactions.

One focus for our research has involved the design and synthesis of structurally unique nucleosides to explore fundamental aspects of nucleic acid structure, function, and stability, as well as to investigate enzyme binding site parameters. With the ever-increasing number of crystal structures for biologically important enzyme—substrate complexes, it has become apparent that many binding sites are more flexible than previously thought and can therefore adjust to fit a wide range of substrates. Significant to this observation, it has recently been shown that (i) flexible inhibitors can overcome drug resistance mutations in viral HIV<sup>1–4</sup> and (ii) the binding site of *S*-adenosyl-L-homocysteine hydrolase (SAHase), an enzyme critical in the replication mechanism of viruses, parasites, and cancer, is quite flexible and exhibits a large difference between the

"open" and "closed" conformations.<sup>5</sup> As a possible means to explore this phenomenon, we have strategically designed a series of structurally innovative nucleosides that possess a heteroaromatic bicyclic purine ring split into its two components (for example, an imidazole and pyrimidine ring), thereby conferring additional degrees of conformational freedom and torsional flexibility to the ligand. As a result, these molecular "chameleons" can adapt to the environment of the flexible binding site in order to maximize and complement structural interactions, without losing the integrity of the crucial contacts involved in the enzyme's mechanism of action. Simply stated, the flexibility of the potential drug complements the structural changes in the drug target, and the result should be a more potent inhibitor, with broad implications for overcoming viral mutations.

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The lead<sup>6</sup> provided by the unusual inhibitory activity exhibited by our guanosine *dist*-fleximer against SAHase, an adenosine-metabolizing enzyme, prompted us to expand our initial efforts with these structurally unique analogues. In contrast to our previously reported<sup>7,8</sup> fleximers, the C-4 analogues are connected at the C-4 of the imidazole and the C-5 of the pyrimidine (as in **1**, Figure 1), rather than the C-5 of the imidazole and the C-6 of the pyrimidine (as in **3**, Figure 1).



Figure 1. C-4- and C-5-substituted imidazole fleximers.

To our knowledge, the only other example in the literature of split nucleosides was recently introduced by Weisz et al. to investigate triplex helix formation.<sup>9,10</sup> The connectivity of these analogues differs from the fleximers, however, as the C-4 of the imidazole is substituted with either a pyridine or a benzene ring attached at the C-3 of the benzene ring (or the C-2 of the pyridine ring), rather than C-5 or C-6 of the pyrimidine ring. As such, these analogues diverge from the standard purine motif we have chosen to endow the fleximers with, since we felt it was critical for our investigations to retain the classic purine design in order to provide a unique perspective on enzyme/ligand interactions.

The synthetic efforts to realize the C-4 imidazole fleximers focused on the synthesis of the adenosine (1), as well as the inosine (2) analogues, since 2 could be obtained from 1 in one step (Scheme 1). The initial approach to the C-4 imidazole fleximer scaffold was envisioned from traditional



Coupling methods: Stille, Negishi, Grignard, Suzuki

organometallic coupling methods to attach the imidazole and pyrimidine rings (as depicted in Scheme 1). The plethora of available coupling methods in the literature made this effort more attractive and straightforward than the nontrivial linear synthesis that had been required to achieve the previously reported C-5 fleximers. While there were examples of both the imidazole and the pyrimidine serving as either the electrophilic or nucleophilic component, we chose to approach the coupling with 4-iodoimidazole analogue **4**, since this intermediate was in hand from other synthetic efforts.

As depicted in Figure 2, a variety of cross coupling approaches and conditions were attempted, including the



Coupling Method III (Suzuki): (i) *n*-BuLi or EtMgBr; (ii) B(O<sup>i</sup>Pr)<sub>3</sub>; (iii) HCl; or (i) bis(pinacolato)diboron, PdCl<sub>2</sub>(dppf); (ii) 5-bromoor 5-iodo-2-hydroxypyrimidine, PdCl<sub>2</sub>(dppf), K<sub>2</sub>CO<sub>3</sub>

Figure 2. Attempted cross-couplings.

Stille,<sup>11–13</sup> Kumada,<sup>14,15</sup> and Negishi<sup>16,17</sup> methods. Unfortunately, all efforts to obtain the C-4 imidazole scaffold in this manner proved fruitless; altering the nature of the halogen on the pyrimidine, the choice of solvent, the reaction temperature, or the catalyst all failed to yield the desired C-4/C-5 coupled product, from which the two desired fleximers **1** and **2** could be realized. Negishi coupling had been shown<sup>16</sup> to work well with C-4 iodo-substituted imidazole systems, and since we were successful in forming

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**1982**, *18*, 117–122. (17) Erdik, E. *Tetrahedron* **1992**, *48*, 9577–9648. the nucleophilic imidazole moiety, both in situ and as an isolated intermediate, we reasoned that the electronically deficient pyrimidine system was simply insufficiently activated to participate in the cross coupling.

At this point we turned to construction of the heterocyclic moieties by means of a linear approach. Starting with commercially available histidine hydrochloride **6** (Scheme 2), treatment with commercially available sodium hypochlorite (household bleach)<sup>18</sup> afforded **7** in a 70% yield.<sup>19,20</sup>



Once **7** was in hand, standard nucleoside coupling<sup>21</sup> to commercially available tetraacetate-protected ribose with bis(trimethylsilyl)acetamide (BSA) and trimethylsilyltriflate (TMSOTf) was accomplished (Scheme 2), albeit in less than desirable yields to give **8** (36%) and **9** (32%).<sup>22</sup> The coupling products were readily separated by column chromatography.

(21) Bennua-Skalmowski, B.; Krolikiewicz, K.; Vorbruggen, H. Tetrahedron Lett. 1995, 36, 7845-7848. Literature has shown that by NMR analysis it is possible to distinguish between the two products. The characteristic downfield shift of the H-5 proton as compared to the shift for H-2 is indicative of the 1,4-disubsituted imidazole (8), while for the 1,5-disubstituted imidazole (9), one would see a shift upfield for H-4 as compared to H-2.<sup>23</sup>

Subsequent in situ formation of **1** from **8** was accomplished by a [4 + 2] Diels–Alder cycloaddition using sodium methoxide and 1,3,5-triazine,<sup>24</sup> which was followed by a retro-Diels–Alder fragmentation of the intermediate that formed (Scheme 3). Concurrent deblocking of the acetate



protecting groups also occurred. The yield for the mechanistically complex reaction to give **1** was only 15%, resulting in a 3.8% overall yield in three steps from histidine.<sup>25</sup>

Inosine fleximer **2** was then realized from **1** using standard diazotization conditions,<sup>26</sup> followed by hydrolysis to give **2** in 68% yield.<sup>27</sup> While admittedly the yields for these steps are not optimal, it is notable that the overall yield is comparable to the typical yields obtained for other modified

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<sup>(20)</sup> **4(5)-Cyanomethylimidazole (7).** Commercial bleach Clorox (active ingredient: sodium hypochlorite, 30 mL, 95 mmol) was added dropwise to a stirred suspension of histidine monohydrochloride monohydrate (10.4 g, 50 mmol) in H<sub>2</sub>O (30 mL) at 0 °C. The resulting yellow solution was kept at 10–20 °C for 3 h and the stirring allowed to continue for 18 h at rt. Solid sodium carbonate was added until the pH reached 8.0. The solution was evaporated to dryness, and the residue triturated with refluxing EtOAc (4 × 150 mL). The organic extracts were combined, washed with brine (300 mL), dried over MgSO<sub>4</sub> and the solvent removed under vacuum. Recrystallization of the residue in ethanol gave **7** as a yellow crystalline solid (4.5 g, 70%): mp 135–137 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.84 (s, 2 H), 7.05 (s, 1 H), 7.64 (s, 1 H), 12.09 (s, 1 H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  16.5, 114.4, 118.8, 129.5, 135.6.

<sup>(22)</sup> **2,3-Diactoxy-5-acetoxymethyl-1-(4-cyanomethylimidazol-3-yl)***β*-**D-ribofuranose (8).** A mixture of **7** (2.18, 2.03 mmol), 1,2,3,5-tetra-*O*-acetate-*β*-D-ribofuranose (7.1 g, 2.25 mmol), and BSA (20 mL, 8.1 mmol) in acetonitrile (100 mL) was stirred at rt for 3 h under Ar. The solution was cooled to 0 °C, and TMSOTf (4.3 mL, 2.25 mmol) was added dropwise. The mixture was stirred at 60 °C for 18 h under Ar, and then cooled to 0 °C and quenched with aqueous NaHCO<sub>3</sub> (50 mL). The organic solvent was removed and the residue extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The organic extracts were combined, washed with brine (100 mL), and dried over MgSO<sub>4</sub>, and the solvent removed under vacuum to give a brown syrup. Column chromatography eluting with 2% ethanol in CH<sub>2</sub>Cl<sub>2</sub> gave **8** as a pale yellow syrup (1.6 g, 35%): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.05 (s, 3 H), 2.08 (s, 3 H), 2.11 (s, 3 H), 3.81 (d, 4.0 Hz, 1 H), 4.36–4.42 (m, 3 H), 5.79 (d, 6.0 Hz, 1 H), 7.16 (s, 1 H), 7.77 (s, 1 H); MS(ESI) calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>7</sub> 365, found (M+1) 366.

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<sup>(25)</sup> **1-[4-(4-Aminopyrimidin-5-yl)imidazol-1-yl]-1-β-D-ribofuranose-2,3,5-triol (1).** A mixture of **8** (1.6 g, 4.38 mmol), sodium methoxide (2 equiv, made fresh from dissolving 202 mg of sodium metal in 10 mL of anhydrous methanol), and 1,3,5-triazine (356 mg, 4.38 mmol) was heated under Ar at 40 °C for 18 h. Silica gel was added to the reaction mixture, and the solvent was removed under reduced pressure. The silica gel containing the product was loaded on silica gel column and eluted with EtOAc/acetone/EtOH/H<sub>2</sub>O (8:1:1:0.5) to give **1** as a hygroscopic foam (272 mg, 15%): <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 3.65 (dd, 3.6 Hz, 12.8 Hz, 1 H), 3.72 (dd, 3.2 Hz, 12.8 Hz, 1 H), 3.97 (q, 3.5 Hz, 1 H), 4.15 (dd, 4.0 Hz, 5.2 Hz, 1 H), 8.26 (t, 5.2 Hz, 1 H), 5.36 (d, 6.0 Hz, 1 H), 7.08 (s, 1 H), 8.05 (s, 1 H), 8.22 (s, 1 H), 8.39 (s, 1 H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 61.5, 70.7, 75.9, 85.7, 89.3, 126.6, 128.7, 137.3, 156.2, 158.1,162.8, 172.9; MS(ESI) calcd for C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>O4 293, found (M + 1) 294.

nucleosides, since 1 (and subsequently, 2) are possible in only three (or four) steps using cheap, commercially available materials. As a result, while we will continue to optimize these yields, we feel this is not a hindrance to using this route.

With the first two C-4 imidazole fleximers now in hand, we can proceed with our investigations into exploring the confines of biologically significant enzymes. By comparing the results already obtained with the C-5-substituted fleximers, it will be possible to see if altering the position of the fleximer bond has a significant effect on recognition with SAHase and the other biologically relevant enzymes we are investigating.

In summary, a series of organometallic cross-coupling methods using a variety of catalysts and conditions were explored in an effort to realize the synthesis of two new C-4 substituted imidazole fleximers. It is clear that the imidazole and pyrimidine ring systems are temperamental at best and that much work remains before we will fully understand the ideal conditions to manipulate these heterocycles more efficiently. We are, however, encouraged by the results finally obtained with a linear approach, and we will continue to optimize this promising route as we pursue additional analogues. Synthesis of the guanosine and additional C-4 analogues is presently in progress by another route, and the results of these efforts, as well as the broad screen biological testing underway will be reported as they become available.

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<sup>(27)</sup> **1-[4-(Pyrimidin-5-yl-2-one)imidazol-1-yl]-1-**β-**D-ribofuranose-2,3,5-triol (2).** To a solution of **1** (400 mg, 1.36 mmol) in a 1:1 mixture of THF and H<sub>2</sub>O (60 mL) was added sodium nitrite (430 mg, 6.23 mmol), followed by glacial acetic acid (0.54 mL, 9.69 mmol). The mixture was heated to 80 °C and stirred for 5 h, at which point the TLC showed no traces of starting material. The solution was cooled to room temperature, neutralized with concentrated NH<sub>4</sub>OH (1 mL), and evaporated under reduced pressure. The crude product was purified by column chromatography eluting with EtOAc/acetone/EtOH/H<sub>2</sub>O (8:1:1:0.5) to give **2** as a white powder (272 mg, 68%): <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 3.68 (dd, 3.5 Hz, 12.8 Hz, 1 H), 3.75 (dd, 3.2 Hz, 12.8 Hz, 1 H), 4.01 (q, 3.1 Hz, 1 H), 4.11, (dd, 3.8 Hz, 5.1 Hz, 1 H), 4.29 (dd, 2 H), 5.43 (d, 3.6, 1 H), 7.69 (s, 1 H), 7.92 (s, 1 H), 8.01 (s, 1 H), 8.14 (s, 1 H), 8.48 (bs, 1 H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 6.1, 70.1, 75.6, 85.6, 89.8, 119.1, 123.2, 129.0, 135.7, 158.9, 164.1, 169.7.