Chemoselective Multicomponent One-Pot Assembly of Purine Precursors in Water

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Abstract: The recent development of a sequential, high-yielding route to activated pyrimidine nucleotides, under conditions thought to be prebiotic, is an encouraging step toward the greater goal of a plausible prebiotic pathway to RNA and the potential for an RNA world. However, this synthesis has led to a disparity in the methodology available for stepwise construction of the canonical pyrimidine and purine nucleotides. To address this problem, and further explore prebiotically accessible chemical systems, we have developed a high-yielding, aqueous, one-pot, multicomponent reaction that tethers masked-sugar moieties to prebiotically plausible purine precursors. A pH-dependent three-component reaction system has been discovered that utilizes key nucleotide synthons 2-aminooxazole and 5-aminoimidazoles, which allows the first divergent purine/pyrimidine synthesis to be proposed. Due to regiospecific aminoimidazole tethering, the pathway allows N9 purination only, thus suggesting the first prebiotically plausible mechanism for regiospecific N9 purination.

Introduction

A plausible prebiotic synthesis of the canonical nucleotides has long been a major goal in origins of life research.1–3 The oligomerization of hydrogen cyanide—one of the major products of spark discharge in atmospheres containing methane and nitrogen—to furnish the HCN tetramer dianimoniomalonitrile (1), its near-quantitative intramolecular photochemical rearrangement to 5-aminoimidazole-4-carbonitrile (AICN, 2), and the subsequent hydrolysis of AICN to 5-aminoimidazole-4-carboxamide (AICA, 3) were demonstrated over 50 years ago.4 However, the further elaboration of aminoimidazoles 2 and 3 to give the purine nucleobases and, after ribosylation and phosphorylation, the purine nucleotides is both very low yielding and, most importantly, not selective for the canonical nucleotides over many other isomers.5 Thus, irrespective of the prebiotic availability of ribose,6 the formation of adenosine (and inosine) is in low yield, alongside a multitude of regiosomers and pyranosyl isomers. Furthermore, guanine, cytosine, and uracil do not give any of their respective nucleosides under similar conditions.6 Recently, a stepwise route from simple abiotic molecules considered to be prebiotically available to activated pyrimidine nucleotides was demonstrated (Scheme 1B).1 However, this route does not solve the problem of purine synthesis. Ideally, further development of this chemistry would also lead to a stepwise synthetic route for purine nucleotide assembly, thus providing a plausible pathway by which the four nucleotides required for an information-rich RNA coding system would be prebiotically available.

The key to effective pyrimidine synthesis was the stepwise increase in molecular complexity from simple aldehydes and cyanamide 4 through in situ formation of 2-aminooxazole (2AO, 5) as a hybrid sugar and nucleobase synthon. Therefore, we considered ways in which 5 could additionally participate in

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3 (1) Powner, M. W.; Gerland, B.; Sutherland, J. D. Nature 2009, 495, 239.
the synthesis of purines. The diversity of chemical species likely to have been present under prebiotic conditions and the proven utility of multicomponent reactions (MCRs) to yield biologically relevant structures suggested that the development of novel MCRs could lead to the rapid buildup of molecular complexity and, in particular, to the structures that are central to molecular biology. The formation of 1 is catalyzed by simple aldehydes via the formation of cyanohydrins during hydrogen cyanide oligomerization (Scheme 1A). It therefore seemed important to assess the chemistry of hydrogen cyanide tetramers with simple aldehydes in aqueous solution toward the prebiotic synthesis of purine ribonucleotides. Specifically, we proposed that the addition of 2 or 3 to the reaction of 5 with aldehydes might yield regiospecifically tethered aminoimidazole nucleotide intermediates. The close relationship of aminoimidazole tethering and pyrimidine synthesis is depicted in Scheme 1B, wherein we propose that divergent access to purines and pyrimidines may be achieved by the addition of 5 to an imine or an aldehyde, respectively.

Results and Discussion

As the five carbon atoms of ribose are contiguous, the formation of the canonical purine ribonucleotides from simple aldehydes should be considered both in the context of one C2 and one C3 fragment, and of one C1 and two C2 fragments. We first assessed the efficiency of forming an aminoimidazole-tethered masked C3-aldehyde upon reaction of 2AO 5 with formaldehyde and AICN 2 or AICA 3, which could ultimately translate into a C5'-tethered aminoimidazole. Upon mixing

\[ \text{5} + 2 \rightarrow \text{6} \]

\[ \text{5} + 3 \rightarrow \text{7} \]

The [5,6,5]-fused tricyclic structures were proven by X-ray diffraction analysis of a single crystal of 7 crystallized at pH 6.0 and a single crystal of the hemi-hydrochloride salt of 6 crystallized at pH 5.0.
formaldehyde, 5, and 2 or 3 in water at room temperature and near neutral pH, the rapid, efficient synthesis of rac-tetrahydroimidazo[1′,3′]-2′′-aminooxazolo[1′,2′]-pyrimidines 6 or 7 was observed (Scheme 2). Synthesis of 6 and 7 demonstrates the selective sequestration of 2 and 3 through sequential formation of iminium ion 8 at equilibrium, followed by intermolecular carbon–carbon bond formation to furnish 9 and 6-exo-trig intramolecular imidazole–iminium trapping (Scheme 3). These reactions were found to be scalable to multigram levels, and the products crystallized from concentrated aqueous solution, giving 6 and 7 in 89 and 80% isolated yield, respectively.

To further explore the generality of the MCR, two additional aldehydes were investigated. The reaction was shown to tolerate acetaldheyde to yield 11 and 12 as a 2:1 (erythro:three (e:t)) mixture of diastereomers. The minor rac-[2′S,3′R]-isomer 12 was then isolated by precipitation and crystallization, which allowed the structure to be proven unambiguously by single-crystal X-ray diffraction. To explore this reaction outside of the prebiotic context, an aromatic aldehyde, 13, was shown to undergo reaction in the presence of DMSO as a co-solvent, giving a 3:2 e:t ratio of products 14 and 15 (Scheme 4).

Three-Component Reaction with α-Hydroxyaldehydes. To continue our investigation into the potential of this MCR for prebiotic nucleotide assembly, we studied the α-hydroxyaldehydes glycolaldehyde and glyceraldehyde. The reaction of these α-hydroxyaldehydes with AICN 2 or AICA 3 and 2AO 5 was very clean and high yielding for the expected C₄ (C₂ + C₂) and C₃ (C₂

"Pyrimidine nucleotide precursors result from two-component chemistry (2CR), " while three-component chemistry (3CR) leads to potential purine nucleotide precursors.

**Scheme 3. Proposed Mechanism for Nucleoside Precursor Generation by Equilibrium Imine Formation Followed by Intermolecular Carbon–Carbon Bond Formation by Nucleophilic Attack and Subsequent Intramolecular Iminium Trapping**

**Scheme 4. One-Pot Multicomponent Assembly of rac-3′-Methyltetrahydroimidazo[1′,3′]-2′′-aminooxazolo[1′,2′]-pyrimidines 11 and 12 and rac-3′-(Pyridin-2-yl)tetrahydroimidazo[1′,3′]-2′′-aminooxazolo[1′,2′]-pyrimidines 14 and 15, Shown with Crystal Structures of 12 and 14."
+ C3) products.11 pH-dependent reactivity was observed, with three-component chemistry dominating at lower pH values and two-component chemistry dominating at higher pH values (vide infra). Notably, the products were uncontaminated with homo-aldolization or homo-Mannich byproducts, and the three-component products exhibited a high diastereoselectivity for rac-

\[ \text{Scheme 5. One-Pot Multicomponent Assembly of rac-3'-(Hydroxymethyl)tetrahydroimidazo[1',3']-2'\text{-}aminooxazolo[1',2']-pyrimidines from Glycolaldehyde, AICN 2 or AICA 3, and 2AO 5, with Crystal Structures of 17 and 22} \]

\[ \text{Scheme 6. One-Pot Multicomponent [3'R,4'R]-Selective Assembly of rac-3'-(Dihydroxyethyl)tetrahydroimidazo-2'\text{-}aminooxazolopyrimidines from Glyceraldehyde with Crystal Structures of 24 and 30} \]

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Glycolaldehyde. Proposed routes for RNA synthesis must be continually evaluated for their potential to yield alternative structures capable of Watson–Crick base-pairing. Glycolaldehyde, as a C3 sugar synthon, has the potential to furnish precursors of threose nucleic acid (TNA) upon reaction with 5.12 At pH 5.0, the reaction of glycolaldehyde, AICN 2, and 5 gave a 4:1 mixture of diastereoisomers 16 and 17, alongside 10% two-component tetrose aminooxazoline products 18 and 19. At pH 4.0, the reaction gave a near-quantitative conversion to three-component products; however, 21% of these products were 20 and 21, which result from 5-exo-trig ring closure of intermediate 9.13 An improvement in diastereoselectivity accrued upon reaction with AICA 3 in lieu of AICN 2. Atp D5 . 0,a7 : 1 mixture of diastereoisomers 22 and 23 (95% yield as determined by 1H NMR integration) was observed, with complete 6-exo-trig selectivity. Compound 22 was isolated in 74% yield and crystallized to allow the \( \text{C3'-stereochemical relationship to be proven by X-ray crystallography. Interestingly, 22 and 16 are related to TNA via C3'-stereochemical inversion.} \]

(11) Intramolecular trapping of iminium ion 9 can presumably occur reversibly, but it is thought that 6-exo-trig reaction dominates for thermodynamic reasons. (a) See ref 1. (b) Powner, M. W.; Sutherland, J. D. Angew. Chem., Int. Ed. 2010, 49, 4641.

Glyceraldehyde. Glyceraldehyde is a C_3 sugar synthet; therefore, its reaction with the C_2 sugar synthet 2AO 5 can yield precursors of pentose nucleic acids (e.g., RNA). However, a high-yielding synthesis of ribo-purininosides via C_3-tethered aminimidazoles would require high [3'R,4'R]-diastereoselectivity (lyxosylol), a reversal of the facial selectivity of glyceraldehyde upon bimolecular reaction with 5. At pH 5.0, the reaction of glyceraldehyde, 5, and AICA 3 furnished lyxotetrahydroimidazo[1,3′]−2′−aminooxazolo[1,2′−pyrimidinones 28−31 and rac−3′−aminimidazo[2−aminooxazolines 32−35. The three-component reaction of glyceraldehyde with 2 or 3 and 5 is highly lyxo-selective, yielding 66% of 28/32 or 60% of 24, respectively, whereas the bimolecular reaction of glyceraldehyde and 5 is highly ribulaborabino-selective (ribo 36 = 44% and arabino 37 = 30%). The high lyxo-selectivity of the three-component reaction establishes the required C1′, C3′, and C4′ stereochemistry for the proposed ribo-nucleotide synthesis by C3′ stereocentric inversion, while the ribulaborabino-selectivity of the two-component reaction establishes the required C3′ and C4′ stereochemistry for pyrimidine synthesis.1,11b The stereochemical preference for lyxosylol three-component products or ribulaborabino two-component products likely derives from the control of facial selectivity of the imine or aldehyde component, respectively, coupled with the obligatory Z-geometry of the nucleophilic C=C bond of 5 (see Supporting Information, Figure S36), though calculations to support this conjecture have not been carried out.

Interestingly, with regard to the furanosyl selectivity of ribo-nucleotide synthesis, isolated 32 is observed to equilibrate with 28, but not with detectable amounts of the pyranosyl isomer 40. Though lyxosylfuranosyl aminooxazoline 39−f equilibrate to give significant amounts of the pyranosyl isomer (39−p/39−f, 5:1) (Scheme 7).17 Equilibration of 28 and 32 is proposed to occur via intermediate 9 and to be controlled by imidazole protonation; therefore, at low pH, protonation of the imidazole moiety results in complete furanosyl selectivity (see Supporting Information, Figure S27, for 1H NMR spectra of 28/32 at pH 1−12).

Two-Component Reaction with α-Hydroxyaldehydes. Although efficient three-component reactivity is observed at pH values lower than 6.0, the reaction profile of α-hydroxyaldehydes with AICN 2 or AICA 3 and 2AO 5 was observed to shift to form only bimolecular aminooxazoline products at higher pH’s, even in the presence of a large excess of 2 or 3. The reaction of glycaldehyde and 5 with 2 or 3, above pH 6.5 and 7.0, respectively, was found to predominantly yield tetrose aminooxazolines 18 and 19 in a 1:2:1 [2′R,3′R]:[2′S,3′R] ratio.14 Similarly, the reaction of glycolaldehyde, 2 or 3, and 5 at pH 7.0 yields a mixture of pentose aminooxazolines 36−39.

(13) The structures of the 3′-aminimidazole-2-aminooxazolines were proposed and differentiated on the basis of NMR spectroscopy (see Supporting Information, Figures S13−S16, S19−S22, S28, and S29); 1H−13C HSQC assigned C1′ and H−(C1′) chemical shifts; for 16, 17, and 28−31, δC = 60−65 ppm, δH = 6.3−6.5 ppm; for 20, 21, and 32−35, δC = 87−90 ppm, δH = 6.0−6.2 ppm. The C1′ upfield shift and the H−(C1′) downfield shift of tetrydroimidazo[1′,3′]−C2′−aminooxazolo[1′,2′]−pyrimidinones are most likely due to the difference between the electron-withdrawing effect of nitrogen relative to oxygen and the deshielding effect of the aromatic imidazole ring, respectively. We observed 1H−13C HMBC correlations of H−(C1′)−C5 and H−(C1′)−C4′ but not H−(C1′)−C5. 1H−13C HMQC and 1H−13C HMQC coupled with 1H−13C TOCSY assigned H−(C2′) proton−proton coupling constants: 16 J(H−(C1′)=C5) = 5.5 Hz, J(H−(C1′)=C5) = 5.5 Hz, J(H−(C1′)=C4′) = 5.4 Hz, J(H−(C1′)=C4′) = 5.4 Hz. Cf. 6-exo-trig product 28, J(H−(C1′)=C5) = 7.4 Hz, and 5-exo-trig product 36, J(H−(C1′)=C5) = 5.5 Hz, J(H−(C1′)=C4′) = 5.5 Hz, J(H−(C1′)=C4′) = 5.5 Hz). 1H−13C HSQC and 1H−13C HMBC spectra were used to assign the connectivity of the imidazole nitrogens with respect to H−(C1′). Diastereoisomers were differentated by a combination of crystallography and 2D NOESY spectroscopy.

(14) M. A. Crowe, M. W.; Sutherland, J. D. Angew. Chem., Int. Ed. 2006, 45, 1676.

(15) Equilibration resulting in a 1:4 mixture of 28:32 is observed over 3 d at 60°C.

(16) No pyranosyl isomers were observed upon 1H−13C HSQC and 1H−13C HMBC analysis of the products of the reaction of glycaldehyde, 2 or 3 (see Supporting Information, Figure S28).

in a 1.5:1 [2′R,3′R]-[2′S,3′R] ratio.14 The more or less complete switch between two- and three-component reactivity is observed to occur over 2 pH units at an aminimidazole pH-dependent pH. However, surprisingly, at pD 7.0, formaldehyde and 2 or 3 were observed to react selectively with 5 as imine/iminium 8, resulting in the synthesis of 6 or 7 (Schemes 2 and 3). Even at elevated pH, and in the absence of 2 and 3, no hydroxymethylation of 5 to give 41 was observed—a standard sample of this latter compound being prepared by the reaction of glyceraldehyde with cyanamide 4 (Scheme 8).18 It is possible that, due to the stability of the hemiaminal 42 formed between the amino group of 5 and formaldehyde, there is insufficient free aldehyde form of formaldehyde to react with free 5 at an observable rate.19 The selective MCR of formaldehyde, and the absence of hydroxymethylation of 5, may have interesting implications for the selectivity of prebiotic nucleotide synthesis. In effect, glycolaldehyde may be sequestered as 5 by the action of cyanamide 4 even in the presence of other aldehydes, such as formaldehyde, that cannot undergo oxazole or aminooxazoline formation.

As an additional point, aldehydes are known to accelerate the rate of hydrolysis of syn-disposed β-aminonitriles at elevated pH values,10,20 but no concurrent nitrile hydrolysis was observed under the conditions of tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidine Cyanovinylation. Selective cyanovinylation of the aminooxazoline moiety of the tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidines is one of several routes that may allow for the subsequent removal of those atoms derived from cyanamide 4 (Scheme 3) through photoactivated hydrolytic (or otherwise) removal of the resultant cytosine moiety, thereby affording products constitutionally closer to purine nucleotides. To investigate this potential for pyrimidine nucleobase elaboration and potential subsequent loss, a series of cyanovinylation experiments was undertaken to show that the aminooxazoline moiety of the tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidine structure could be selectively cyanovinylated. Because there are several potentially nucleophilic heteroatoms in the generic tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidine structure that could react with an alkylating agent, we were initially unsure as to the likely regioselectivity of cyanovinylation upon treatment with aqueous cyanacetylene. Nonetheless, with regard to the nucleophilicity of N1″, we were encouraged by the observation that 6 crystallized as the hemihydrate salt at pH 5.0, wherein two molecules of 6 were hydrogen-bonded via a common proton between N1″ (see Supporting Information, Figure S3). Furthermore, we thought that the tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidines were likely to be intrinsically protected from C3″-NH cyanovinylation by delocalization of the nitrogen lone pair into the imidazole ring. Interestingly, and very pleasingly, highly selective cyanovinylation was observed upon treatment of 6, 17, 21, 24, and 30 with cyanacetylene. Even in the presence of 5 equiv of cyanacetylene, clean conversion of 6, 17, 21, 24, and 30 to 4-amino-1-(3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-a]-pyrimidin-4-yl)pyrimidin-2-(2H)-ones 43–47 was observed over the period of 1-3 days at 60 °C in unbuffered aqueous solution in 80–95% yield.21 This range of products demonstrates the efficacy this cyanovinyl in both tetrose and pentose series for both 2,3′-cis- and 2,3′-trans-tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidines. Additionally, compounds 43 and 44 were isolated and crystallized from D2O, providing samples for X-ray diffraction to unambiguously prove the novel structural motif (Scheme 9).
Experiments to investigate the propensity for cytosine loss from these compounds under a variety of conditions are now underway.

**Outlook and Summary**

We have described a concise and high-yielding route to the tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidines. On the basis of these key intermediates, one can envisage several stepwise regioselective purine/beta2-ribofuranosyl nucleotide syntheses. Cytidines are known to undergo both pyrimidine loss and C2′-epimerization upon UV-irradiation, possibly due to the photochemical generation of nucleobase-iminium ions.22 These intermediates can undergo dissociative hydrolytic processes that are similar to those observed during the acid-catalyzed hydrolysis of beta2-D-uridine,23 but at near-neutrality. It is of note that the MCR described provides a high yield of \([3′R,4′R]-products (\sim 70\% \text{ lyxo/xylo}), with the lyxo and xylo products being stereochemically related via C2′-epimerization.24 Upon cyanovinylation and dissociative cytosine cleavage, compounds 27, 31, and 34 should, via intramolecular ring closure, furnish 5,3′-anhydro-AICN-riboside 49 (where R′ = CH2OH), which has an obvious relationship to AICA-riboside 50, an intermediate in the de novo stepwise biosynthesis of canonical nucleotides.

(21) Although we did not observe N3′ cyanovinylation, within the limits of NMR spectroscopy, we did detect on the order of 5–8% cytidine modification when tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1′,2′]-pyrimidines were treated with an excess of cyanoacetylene. 48 (R = CN, R′ = CH2OH) was isolated from the reaction of 17 with cyanoacetylene by precipitation from concentrated aqueous solution. See Supporting Information, Figure S35, for comparative 1H–1H COSY analysis.


(24) C2′-stereochemical inversions have been observed in related molecules under both thermal and photochemical condition; see refs 1, 11b, and 22.
base-pairing interactions. It is of note that the proposed phosphorylation of the 2′-hydroxyl group of 49 could lead to purines via a C3′ stereochemical inversion, which would be directly analogous to the C2′ inversion previously described to give access to the pyrimidine nucleotides.

In summary, we have described additional predisposed complex structures that can be accessed through the participation of the key intermediate 2-aminooxazole 5 in a novel aqueous multicomponent reaction system. This MCR has proved to be scalable and high-yielding, and it generates a pH-dependent product distribution. Further, the intrinsically controlled cyanovinylolation of the low-pH MCR products generates potential precursors of the purine nucleotides. These reactions help to define the chemical structural space and chemical distribution likely to be accessible under plausible prebiotic chemical scenarios. In particular, we have demonstrated the concurrent synthesis of 37 and the tetrahydroimidazo[1′,3′]-2′-aminooxazolo[1,2]-pyrimidines in the pH range in which activated pyrimidine synthesis can occur (pH < 6.5, where hydrolysis of 53 is prevented), suggesting that both the pyrimidine and the purine ribonucleotides could be made together at the same time, in the same place, and under the same conditions. The reactions may therefore be of direct relevance to the problem of abiogenesis of a full set of canonical ribonucleotides and hence the chemical origins of molecular biology.

Experimental Section

General Methods. Reagents and solvents were purchased from Sigma-Aldrich, TCI America, Frontier Scientific, or Cambridge Isotope Laboratories. Flash column chromatography was carried out using Merck 9385 silica gel 60 (230–400 mesh). NMR spectroscopy was carried out on a Varian NMR spectrometer (Oxford AS-400) operating at 20 °C probe temperature (unless specified). Where possible, the chemical shift of the corresponding solvent was used as a reference. Chemical shift values are reported relative to TMS (Oxford AS-400) operating at 20 °C.

(25) Proposed six-member imidazole cyclization to furnish 49.


(29) Alternatively, the pH-dependent profiles of the multicomponent reaction described provide a route to 36–39 in the presence of 2 and 3. Therefore, subsequent incorporation of the pyrimine fragment with 36, 38, or 39 (or derivatives thereof) still remains a plausible route to purine molecules under prebiotic constraints for their concurrent synthesis with pyrimidine nucleotides accessed via 37.
rac-1,2',3',N-Tetrahydroimidazo[1',3'-][2',3']-pyrimidine-4-carboxamide (7). Method A: 5-Aminomidazole-4-carboxamide 3 (29.0 mg, 0.23 mmol) and 2-aminoazoxazole 5 (20 mg, 0.23 mmol) were dissolved in D$_2$O (2.0 mL) at pH 5.0, 6.0, or 7.0. Formaldehyde (37%, 18.6 mg, 0.23 mmol) was added in D$_2$O (0.5 mL), the pH was rechecked, and the reaction volume was adjusted to 2 mL with D$_2$O. The reactions were incubated for 15 h, and the progress of the reaction was assessed by NMR spectroscopy (see Supporting Information, Figure S4, for 1H NMR spectra at pH 5.0, 6.0, and 7.0). The formation of tetrathymidazol[1',3'-][2',3']-N-aminoazoxazolo[1',2']-pyrimidine 7 was observed at all pH values. 7 was then crystallized by cooling the reaction solution to 4 °C for 1 d. 7 was isolated by filtration and washed with ice-cold water.

Method B: 5-Aminimidazole-4-carboxamide 3 (1.50 g, 11.9 mmol) and 2-aminoazoxazole 5 (1.00 g, 11.9 mmol) were dissolved in H$_2$O (50 mL). Formaldehyde (77%, 0.96 g, 11.9 mmol) was added, the pH was rechecked, and the reaction volume was adjusted to 20 mL with H$_2$O. After 20 min a white precipitate had formed in the reaction. The reaction was mechanically stirred for 15 h. The solids present were isolated by filtration, and the filtrate was lyophilized. The combined solids were redissolved in water by heating, agitated, and then allowed to cool to give 1.95 g of 7 as a yellow solid. The solids were dissolved in hot water (4 mL), and the product was allowed to crystallize upon cooling. 7 was isolated by filtration, washed with methanol (10 mL), and air-dried to give 1.30 g (49%) of 7 as a white solid. A further portion of methanol was added to the filtrate, and the solution was then cooled to give a second batch (0.82 g, 31%) of 7 as an off-white powder. An analytical sample was then recrystallized from H$_2$O for X-ray analysis.

IR (KBr, cm$^{-1}$): 3365 (NH), 3096, 2979, 2765, 2660 (C–H), 1676 (N=C), 1640 (C=O). 1H NMR (400 MHz, D$_2$O): $\delta$ 7.21 (s, 1H, H-(C2)); 3.83 (d, $J = 7.8$ Hz, 1H, H-(C1)); 5.03 (dt, $J = 7.8$, 1.8 Hz, 1H, H-(C2')); 3.53 (abs, $J = 13.8$, 1.8 Hz, 1H, H2-(C3')); 3.18 (abs, $J = 13.8$, 1.8 Hz, 1H, H1-(C3')). 13C NMR (101 MHz, D$_2$O): $\delta$ 166.2 (C=O); 164.1 (C=O); 144.3 (C5); 130.0 (C2); 110.4 (C4); 78.4 (C2'); 66.1 (C1'); 43.4 (C3'). ES-MS (pos. $m/z$): 223 (100%, [M + H$^+$]). HRMS (m/z): [M$^+$]$^+$ calculated for C$_3$H$_3$N$_6$O$_2$, 223.0938; found, 223.0835. X-ray diffraction structure solved; deposited as CCDC 784245.

rac-(1S,2'S,3'S,3''S)-2',2'-N,N-dimethylimidazo[1',3'-][2',3'']-pyrimidine-4-carboxamide (16) and rac-(1S',2'S',3'R,3''S)-2',2'-N,N-dimethylimidazo[1',3'-][2',3'']-pyrimidine-4-carboxamide (17). Method A: Glycolaldehyde (14.2 mg, 0.23 mmol), 5-aminimidazole-4-carbonitrile 2 (24.8 mg, 0.23 mmol), and 2-aminoazoxazole 5 (20 mg, 0.23 mmol) were dissolved in D$_2$O (2 mL) at pH 4.0, 5.0, 5.5, or 6.0. The reactions were incubated for 5 d, and the progress of the reaction was assessed by $^1$H NMR spectroscopy (see Table 1 and Supporting Information, Figure S7). The progress of the reaction was followed by $^1$H NMR spectroscopy, and the crystals were dissolved in the supernatant by warming, and NMR spectroscopy showed a 2:1 ratio of 11:12. The crystals were observed to form a second 24 h at room temperature. The crystals were isolated by filtration (18 mg), and an analytical sample was dissolved in DMSO-d$_6$ (0.75 mL) for NMR spectroscopy. The remaining crystals were recrystallized from slowly cooling H$_2$O to provide a sample for X-ray diffraction.

11. $^1$H NMR (400 MHz, D$_2$O): $\delta$ 7.18 (s, 1H, H–Ar); 5.81 (d, $J = 7.6$ Hz, 1H, H-(C1)); 4.74 (dd, $J = 7.6$, 2.9 Hz, 1H, H-(C2')); 3.65 (dq, $J = 6.9$, 2.9 Hz, 1H, H-(C3')); 0.90 (d, $J = 6.9$ Hz, 3H, Me). ES-MS (pos. $m/z$): 237 (100%, [M + H$^+$]). HRMS (m/z): [M$^+$]$^+$ calculated for C$_9$H$_3$N$_6$O$_2$, 237.0935; found, 237.1099.

12. IR (KBr, cm$^{-1}$): 3413 (NH), 3187, 2908, 2960 (C=H), 1664, 1663 (N=C), 1616 (C=O). $^1$H NMR (400 MHz, D$_2$O): $\delta$ 7.15 (s, 1H, H-(C2)); 5.81 (d, $J = 7.7$ Hz, 1H, H-(C1')); 4.83 (dd, $J = 7.7$, 2.0 Hz, 1H, H-(C2')); 3.32 (dq, $J = 6.6$, 2.0 Hz, 1H, H-(C3')); 1.16 (d, $J = 6.6$ Hz, 3H, Me). $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 7.16 (s, 1H, H–Ar); 6.68 (br d, 2H, NH$_2$); 6.40 (br s, 2H, NH$_2$); 5.79 (d, $J = 7.4$ Hz, 1H, H-(C1')); 5.59 (br s, 1H, NH); 4.70 (dd, $J = 7.4$, 0.5 Hz, 1H, H-(C2')); 3.41 (dd, $J = 6.0$, 0.5 Hz, 1H, H-(C3')); 1.29 (d, $J = 6.0$ Hz, 3H, Me). $^1$C NMR (101 MHz, DMSO-d$_6$): $\delta$ 166.7 (C=O); 166.7 (C2'); 142.0 (C5); 129.5 (C2); 112.5 (C4); 79.7 (C2'); 73.6 (C1'); 47.9 (C3'); 17.9 (Me). ES-MS (pos. $m/z$): 237 (100%, [M + H$^+$]). HRMS (m/z): [M$^+$]$^+$ calculated for C$_9$H$_3$N$_6$O$_2$, 237.1095; found, 237.1099. X-ray diffraction structure solved; deposited as CCDC 784245.
Table 1. NMR (1H NMR 400 MHz; 13C NMR 101 MHz; 15N NMR 40 MHz) Spectral Relationships of rac-3′-(4′-Hydroxymethyl)-1′,2′,3′-N-tetrahydroimidazol[1′,3′]-2′-aminoxazolo[1′,2′]-pyrimidine-4-carbonitriles 16 and 17 and rac-3′-(1H-S-aminomidoazo)iminooxazolines 20 and 21

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<td>δ_C1</td>
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<td>H(C1′)–C(C4′)</td>
<td>H(C1′)–N1(C3′)</td>
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NOE intensity volumes are given as strong (s), medium (m), weak (w), or not observed (−) relative to the volume of H(C1′)–H(C2′) of the same compound which is defined as strong.

D2O: δ_H1 7.16 (s, 1H, H-Ar); 5.78 (d, J = 7.7 Hz, 1H, H-C(1′)); 4.8 (dd, J = 7.7, 1.9 Hz, 1H, H-C(2′)); 3.63 (abs, J = 11.4, 6.6 Hz, 1H, H-C(4′)); 3.56 (abs, J = 11.4, 6.8 Hz, 1H, H-C(4′)); 3.56 (app td, J = 5.7, 1.9 Hz, 1H, H-C(3′)). 1H NMR (400 MHz, DMSO-d6, 25 °C): δ_H1 7.35 (s, 1H, H-Ar); 6.83 (s, 1H, HN); 5.59 (s, 2H, NH2): 5.87 (d, J = 7.5 Hz, 1H, H-C(1′)); 5.08 (t, J = 5.85 Hz, 1H, OH); 4.92 (d, J = 7.5 Hz, 1H, H-C(2′)); 3.74 (abs, J = 10.5, 6.0 Hz, 1H, H-C(4′)); 3.60 (abs, J = 10.5, 6.7 Hz, 1H, H-C(4′)); 3.42 (m, 1H, H-C(3′)). 13C NMR (101 MHz, DMSO-d6, 25 °C): 13C 163.6 (C2); 145.4 (C5); 131.9 (C2); 116.9 (CN); 88.4 (C4); 75.5 (C2); 73.5 (C1); 60.3 (C3′); 53.6 (C3′). ES-MS (pos. m/z): 235 (100%, [M + H]+), 257 (10%, [M + Na]+). ES-MS (neg. m/z): 233 (70%, [M – H]-). HRMS (m/z): [M+] calcd for C10H15N6O4, 283.1094; found, 283.1094.

rac-′(1′S,2′S,3′S)-3′-(Hydroxymethyl)-1′,2′,3′-N-tetrahydroimidazol[1′,3′]-2′-aminoxazolo[1′,2′]-pyrimidine-4-carboxamide (22).

Method A: Glycolaldehyde (14.3 mg, 0.23 mmol), 5-aminoimidazole-4-carboxamide 3 (29.0 mg, 0.23 mmol), and 2-aminooxazole 2′ (29.0 mg, 0.23 mmol) were dissolved in D2O (2 mL) at pH 5.0, 6.0, 6.5, or 7.0. The reactions were incubated for 5 d, and the progress of the reaction was assessed by 1H NMR spectroscopy (see Supporting Information, Figure S17, for 1H NMR spectra at pH 5.0, 6.0, and 7.0). The formation of 22 (78%) and 23 (11%) was observed at pH 5.0. 22 was isolated by precipitation with methanol and studied by 2D homo- and heteronuclear NMR spectroscopy (see Supporting Information, Figures S19–S21). The formation of 18 (60%) and 19 (40%) was observed at pH 7.0.

Method B: Glycolaldehyde (357 mg, 5.9 mmol), 5-aminoimidazole-4-carboxamide 3 (743 mg, 5.9 mmol), and 2-aminooxazole 5 (200 mg, 5.9 mmol) were dissolved in H2O (20 mL) at pH 6.0. The reaction was incubated for 15 h, after which a white precipitate had formed. The precipitate was isolated by filtration and washed with ice-cold water to give 1.1 g (74%) of 22. An analytical sample of 22 was then reprecipitated five times from aqueous ethanol for X-ray diffraction analysis.

IR (KBr, cm−1): 3443 (NH), 3305 (O–H), 2866, 2807, 2662 (C–H), 1678 (C=O) 1652, 1577 (N=C), 1′H NMR (400 MHz, D2O): δ_H1 7.16 (s, 1H, H–Ar); 5.72 (d, J = 7.4 Hz, 1H, H–(C1′)); 4.93 (dd, J = 7.4, 2.4 Hz, 1H, H–(C2′)); 3.72 (dd, J = 4.2, 2.4 Hz, 1H, H–(C3′)); 3.63 (app q, J = 5.2 Hz, 1H, H–(C4′)); 3.50 (abs, J = 11.7, 5.0 Hz, 1H, H–(C5′)); 3.42 (abs, J = 11.7, 6.2 Hz, 1H, H–(C5′)). 13C NMR (101 MHz, D2O): δ_C1 168.1 (C=O); 161.0 (C3); 141.3 (C5); 131.0 (C2); 110.4 (C4); 78.4 (C2); 71.3 (C1); 62.5 (C4′); 53.3 (C5′); 48.9 (C3′). ES-MS (pos. m/z): 283 (100%, [M + H]+). HRMS (m/z): [M+] calcd for C14H14N6O5, 283.1149; found, 283.1154. X-ray diffraction structure solved; deposited as CCDC 784247.

rac-′(1′S,4′,5′-Dihydroxymethyl)-1′,2′,3′-N-tetrahydroimidazol[1′,3′]-2′-aminoxazolo[1′,2′]-pyrimidine-4-carbonitrile (30) and rac-′(1′H-5-Aminomidoazo)iminooxazine (32).

Method A: Glycolaldehyde (20.7 mg, 0.23 mmol), 5-aminoimidazole-4-carboxamide 2 (49.6 mg, 0.46 mmol), and 2-aminooxazole 5 (20 mg, 0.23 mmol) were dissolved in D2O (2 mL) at pH 4.0, 4.5, 5.0, 6.0, and 7.0. The reactions were incubated for 5 d, and the progress of the reaction was assessed by 1H NMR spectroscopy (see Table 2 and Supporting Information, Figure S26, for 1H NMR spectra at pH 4.0, 4.5, 5.0, 6.0, and 7.0). At pH 4.0, the formation of four rac-tetrahydroimidazol[1′,3′]-2′-aminoxazolo[1′,2′]-pyrimidines, 28–31 (46%, 13: 2.11:1 ratio), and four 3′-imidazolidinooxazolines, 32–35 (43%, 16:22:1:1 ratio), was observed by 1H NMR spectroscopy (see Supporting Information, page S17, for partial NMR characterization of compounds 28, 29, 31, 33, 34, and 35). The predominant formation of pentose aminoazolines 36 (44%), 37 (30%), 38 (8%), 35-f (2%), and 35-p (10%) was observed at pH 7.0.

Method B: Glycolaldehyde (207 mg, 2.3 mmol), 5-aminoimidazole-4-carboxamide 2 (496 mg, 2.3 mmol), and 2-aminooxazole 5 (200 mg, 2.3 mmol) were dissolved in H2O (10 mL) at pH 4.0. The reaction was incubated for 2 d, lyophilized, and dissolved in MeOH (10 mL). SiO2 (2 g) was added, the mixture was concentrated to a fine, free-flowing powder in vacuo, and the compounds were eluted with 1:4 to 1:3 methanol:CH2Cl2. Those fractions containing 32 were concentrated in vacuo to give white solids. The solids were dissolved in aqueous methanol (H2O:MeOH 0.5:10 mL), and EtOAc (6 mL) was added to partially precipitate the mixture. The solids were filtered and washed with methanol to give 98 mg of 32 (16%). Those fractions eluted from silica gel that predominately contained 28–31 were concentrated in vacuo to a white foam. The foam was dissolved in D2O and studied by 1H, 1H–H COSY, and 1H–15N NOESY NMR. The solution was lyophilized,
**Table 2.** NMR (1H NMR 400 MHz; 13C NMR 101 MHz; 15N NMR 40 MHz) Spectral Relationships of rac-3'-4-(5'-Dihydroxymethyl)-1',2',3',N-tetrahydroimidazo[1',5-][1,3],2'-aminooxazolo[1',2'-]pyrimidine-4-carbinolines 28–31 and rac-3'(1H-5-Aminomidoazolo)-aminooxazolines 32–35

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<th>HMBC (1H-15N)</th>
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<td>H(C1')-H(C4')</td>
</tr>
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</table>

* NOE intensity volumes are given as strong (s), medium (m), weak (w), or not observed (−) relative to the volume of H(C1')-H(C2') of the same compound which is defined as strong.

and the solid was dissolved in warm aqueous ethanol. Upon cooling, white crystalline solids formed; these were isolated by centrifugation and then dissolved in D2O. H NMR analysis showed that the solids were enriched in one three isomer. Repeated recrystallization of the crystalline solids from H2O afforded 20 mg of 30 as fine, clear needles (see Supporting Information, Figure S25).

30. IR (KBr, cm⁻¹): 3440 (NH), 3323 (O-H), 2928 (CH3), 1738 (C=O), 1666 (C=C), 1537 (C=C), 1452 (C=C), 1309 (C=C), 1195 (C=C), 1132 (C=C), 1036 (C=C), 967 (C=C), 684 (C=C), 648 (C=C), 624 (C=C), 43.5 (C=C).


32. 1H NMR (400 MHz, D2O, pD 1.0): δ 8.21 (s, 1H, H(C2')); 5.97 (dd, J = 5.4 Hz, 1H, H(C1')); 4.17 (dd, J = 10.0, 5.4 Hz, 1H, H(C3')); 3.87 (dd, J = 10.0, 3.9 Hz, 1H, H(C4')); 3.77 (dd, J = 12.5, 2.0 Hz, 1H, H(C5')); 3.55 (dd, J = 12.5, 3.9 Hz, 1H, H(C6)); 4.29 (d, J = 2.0 Hz, 1H, H(C5)); 7.13 (s, 1H, H(C2')); 6.76 (d, J = 2.0 Hz, 1H, H(C1')); 5.96 (d, J = 7.4 Hz, 1H, H(C5)); 4.27 (d, J = 2.7 Hz, 1H, H(C2')); 3.77–3.87 (m, 1H, H(C4')); 3.67–3.77 (m, 2H, H(C3'), H(C4')); 13C NMR (101 MHz, D2O): δ 167.6 (C2'); 157.4 (C4'); 147.2 (C5'); 131.1 (C2'); 116.5 (C5N); 96.3 (C5'); 88.6 (C4); 66.0 (C2'); 62.2 (C1'); 60.4 (C4'); 55.2 (C3'). UV-vis: λmax 252 nm (ε 10 100). ES-MS (pos. m/z): 304 (100%, [M + H]+). HRMS (m/z): [M]+ calcd for C11H12N7O4, 304.1152; found, 304.1149. X-ray diffraction structure solved; deposited as CCDC 784251.

33. 1H NMR (400 MHz, D2O): δ 7.28 (d, J = 7.4 Hz, 1H, H(C6)); 7.13 (s, 1H, H(C2')); 6.76 (d, J = 2.7 Hz, 1H, H(C1')); 5.96 (d, J = 7.4 Hz, 1H, H(C5)); 4.27 (d, J = 2.7 Hz, 1H, H(C2')); 3.77–3.87 (m, 1H, H(C4')); 3.67–3.77 (m, 2H, H(C3'), H(C4')); 13C NMR (101 MHz, D2O): δ 167.6 (C2'); 157.4 (C4'); 147.2 (C5'); 131.1 (C2'); 116.5 (C5N); 96.3 (C5'); 88.6 (C4); 66.0 (C2'); 62.2 (C1'); 60.4 (C4'); 55.2 (C3'). UV-vis: λmax 252 nm (ε 9760). ES-MS (pos. m/z): 355 (100%, [M + H]+). HRMS (m/z): [M]+ calcd for C11H12N7O4, 355.1261; found, 355.1261.
nm. ES-MS (pos. m/z): 304 (100%, [M + H+]\(^+\)). HRMS (m/z): [M]\(^+\) c,aled for C\(_{12}\)H\(_{16}\)N\(_7\)O\(_4\), 322.1258; found, 322.1284.

\((2R,3S,4S)-4-(4-Amino-2-oxopyrimidin-1(2H)-yl)-2-((R)-1,2-dihydroxyethyl)-3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-a]-pyrimidine-8-carboxonitride (46). Method C: 30 mg (0.019 mmol) and cyanoacetylene (0.98 M in H\(_2\)O, 50 \(\mu\)L) were incubated at 60 \(^\circ\)C for 24 h. The formation of 46 was observed in 82% yield by \(^1\)H NMR integration (see Supporting Information, Figure S34, for crude \(^1\)H NMR).

\(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta\)H 7.16 (d, \(J = 7.6 \text{ Hz}, 1\text{H}, \text{H-C}\(_6\))\(^\prime\prime\)); 7.03 (s, 1H, \text{H-(C2)}); 6.74 (d, \(J = 4.3 \text{ Hz}, 1\text{H}, \text{H-(C5)}\))\(^\prime\prime\)); 5.84 (d, \(J = 7.6 \text{ Hz}, 1\text{H}, \text{H-(C5)}\)); 4.30 (d, \(J = 3.1, 1.0 \text{ Hz}, 1\text{H}, \text{H-(C2)}\)); 3.66–3.77 (m, 2H, \text{H-(C4)}\(^\prime\)), \text{H a-(C5)}\(^\prime\)); 3.58 (abx, \(J = 12.1, 5.6 \text{ Hz}, 1\text{H}, \text{H-b-(C5)}\)); 3.51 (app d, \(J = 8.6 \text{ Hz}, 1\text{H}, \text{H-(C3)}\)). 13CN M R (101 MHz, D\(_2\)O): \(\delta\)C 165.6 (C\(_2\)\(^\prime\prime\)); 157.5 (C\(_4\)\(^\prime\prime\)); 147.2 (C\(_5\)); 142.0 (C\(_6\)\(^\prime\prime\)); 130.8 (C\(_2\)); 116 (C\(_4\)); 96.0 (C\(_5\)); 65.9 (C\(_1\)); 61.7 (C\(_2\)); 69.0 (C\(_4\)); 62.3 (C\(_5\)); 55.2 (C\(_3\)). UV-vis: \(\lambda_{\text{max}}\) 254 nm. ES-MS (pos. m/z): 352 (100%, [M + H\(^+\)]\(^+\)). HRMS (m/z): [M\(^+\)] c,aled for C\(_{13}\)H\(_{16}\)N\(_7\)O\(_4\), 352.1364; found, 352.1363.

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Supporting Information Available: NMR spectra of reaction mixtures, purified compounds, and pH stacked plots of reaction profiles; CIF files of crystal structures obtained. This material is available free of charge via the Internet at http://pubs.acs.org. JA108197S