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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 diaminomaleonitrile (**1**), its near-quantitative intramolecular photochemical rearrangement to 5-aminoimidazole-4-carbonitrile (AICN, **2**), and the subsequent hydrolysis of AICN **2** 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 isomers thereof. For example, adenine and hypoxanthine have been condensed under dry conditions at high temperatures with an excess of ribose in the presence of a high concentration of magnesium salts to yield 3 and 15% of the respective canonical N9-linked β-D-ribofuranonucleosides in complex mixtures with many other isomers.[5] Thus, irrespective of the prebiotic availability of ribose,[6,7] the formation of adenosine (and inosine) is in low yield, alongside a multitude of regioisomers 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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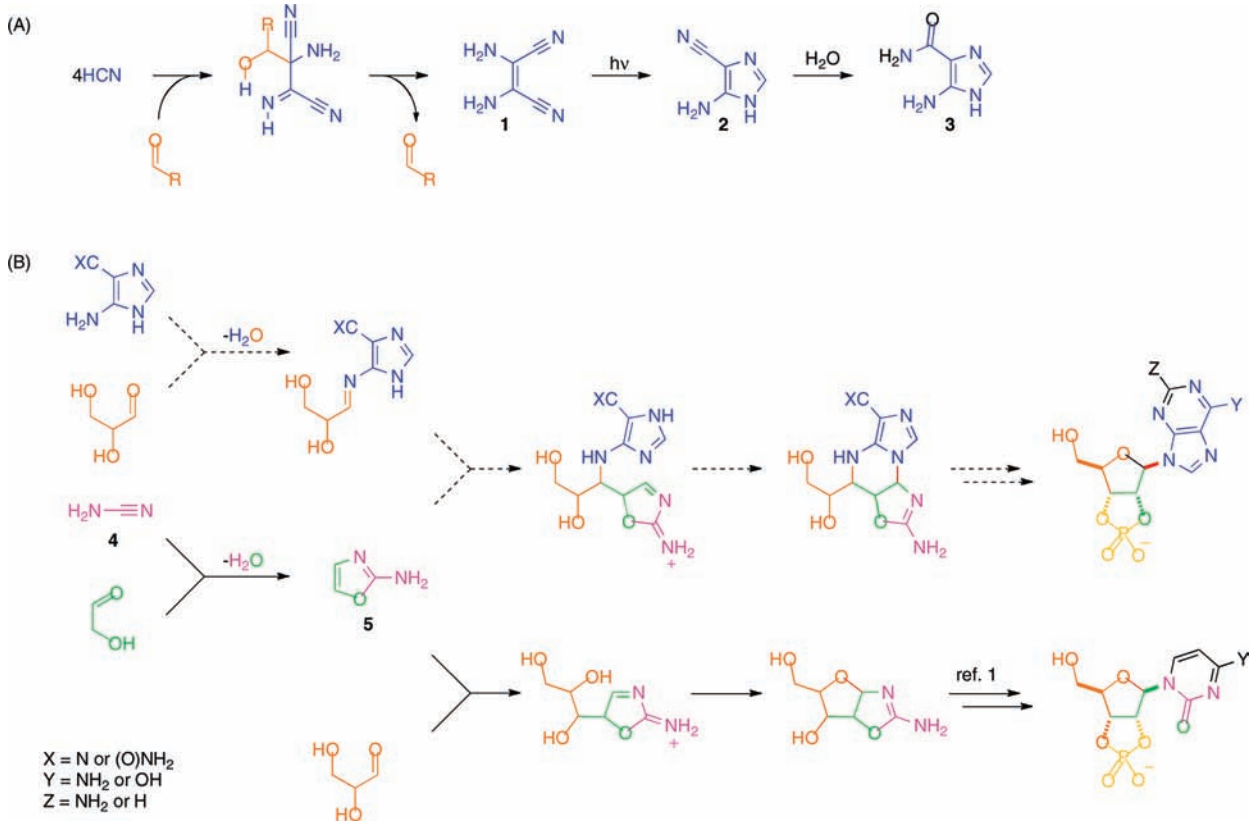
Scheme 1. (A) Multicomponent Assembly of Hydrogen Cyanide Tetramers and (B) Proposed Development of Activated Pyrimidine Synthesis To Concurrently Yield Purine Precursors[a]



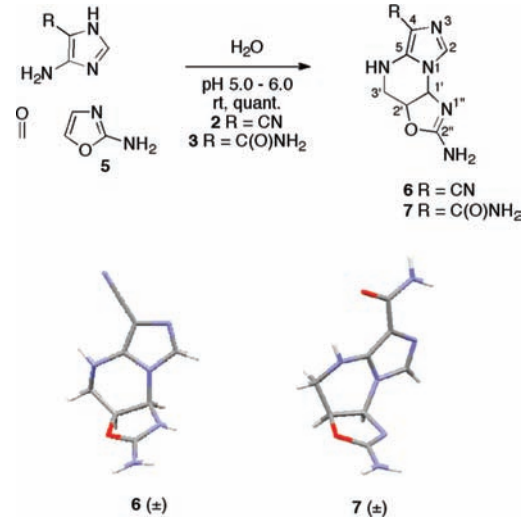
[a] Solid arrows, reported chemistry; dashed arrows, proposed chemistry.

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[4,5,8] 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).[9] It therefore seemed important to assess the chemistry of hydrogen cyanide tetramers with simple aldehydes in aqueous solution toward the prebiotic synthesis of purine *ribo*-nucleotides.[10] 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 C_2 and one C_3 fragment, and of one C_1 and two C_2 fragments. We first assessed the efficiency of forming an aminoimidazole-tethered masked C_3-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

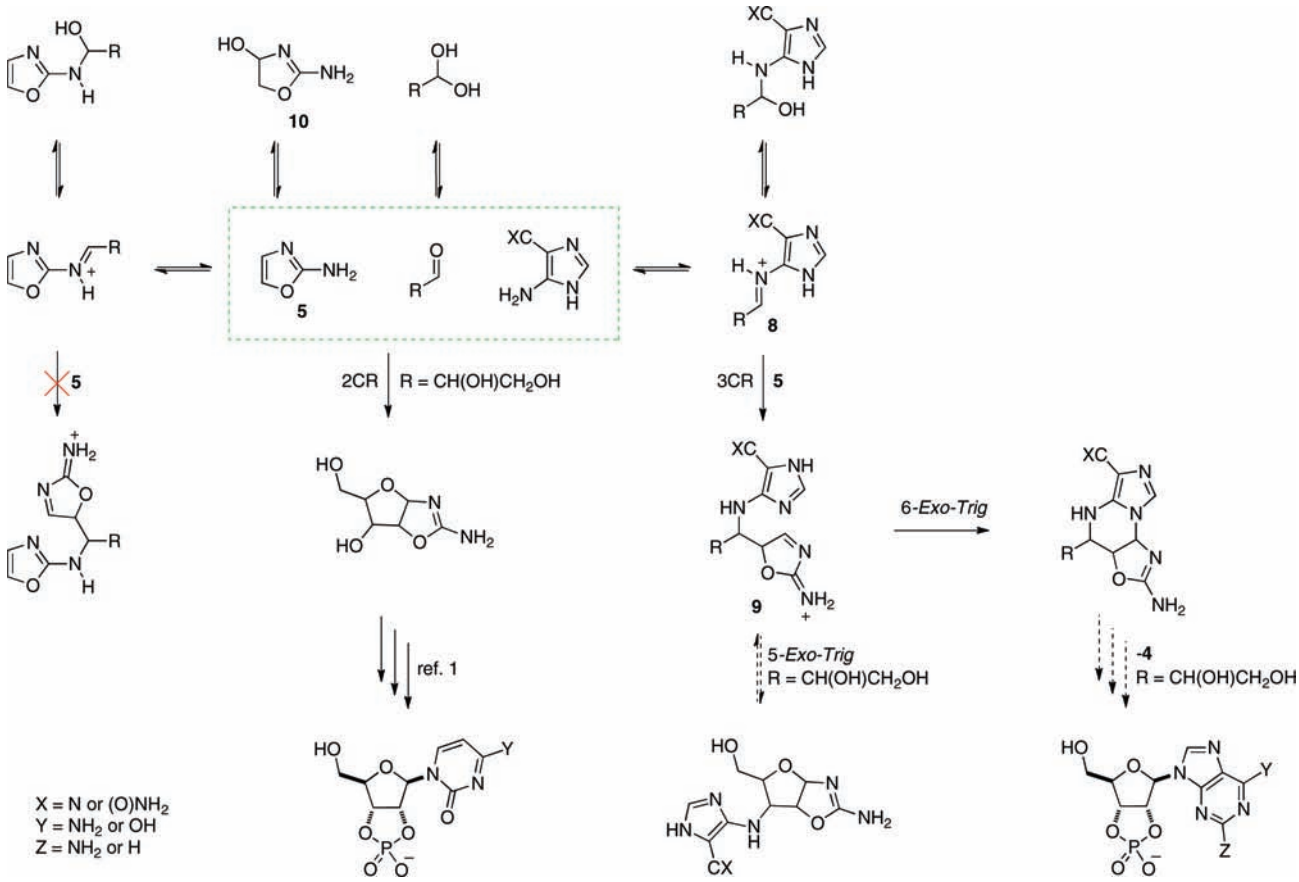
Scheme 2. One-Pot Multicomponent Assembly of *rac*-Tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines **6** and **7**[a]



[a] 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.

(8) (a) Strecker, A. *Liebigs Ann. Chem.* **1850**, *75*, 27. (b) Strecker, A. *Liebigs Ann. Chem.* **1854**, *91*, 349. (c) Miller, S. L.; Urey, H. C. *Science* **1959**, *130*, 245. (d) Mullen, L. B.; Sutherland, J. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 8063.
(9) (a) Schwartz, A. W.; Goverde, M. *J. Mol. Evol.* **1982**, *18*, 351. (b) Eschenmoser, A. *Chem. Biodiv.* **2007**, *4*, 554.
(10) Koch, K.; Schweizer, W. B.; Eschenmoser, A. *Chem. Biodiv.* **2007**, *4*, 541.

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[a]



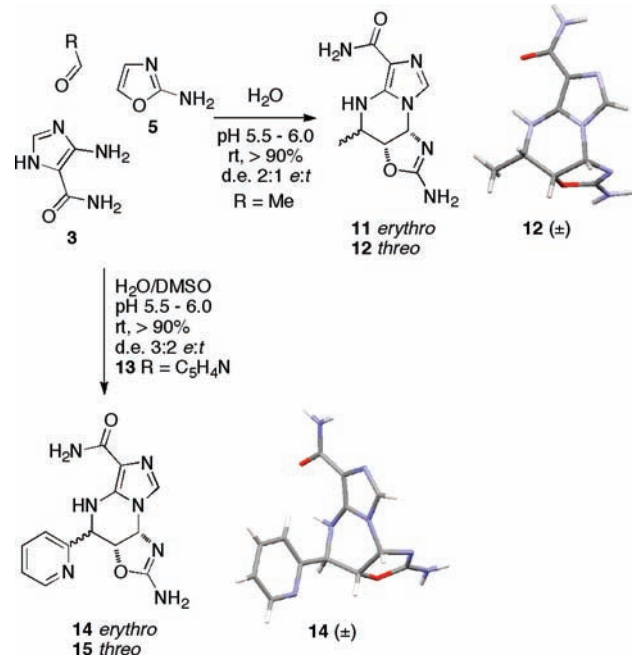
[a] Pyrimidine nucleotide precursors result from two-component chemistry (2CR),[1] while three-component chemistry (3CR) leads to potential purine nucleotide precursors.

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.

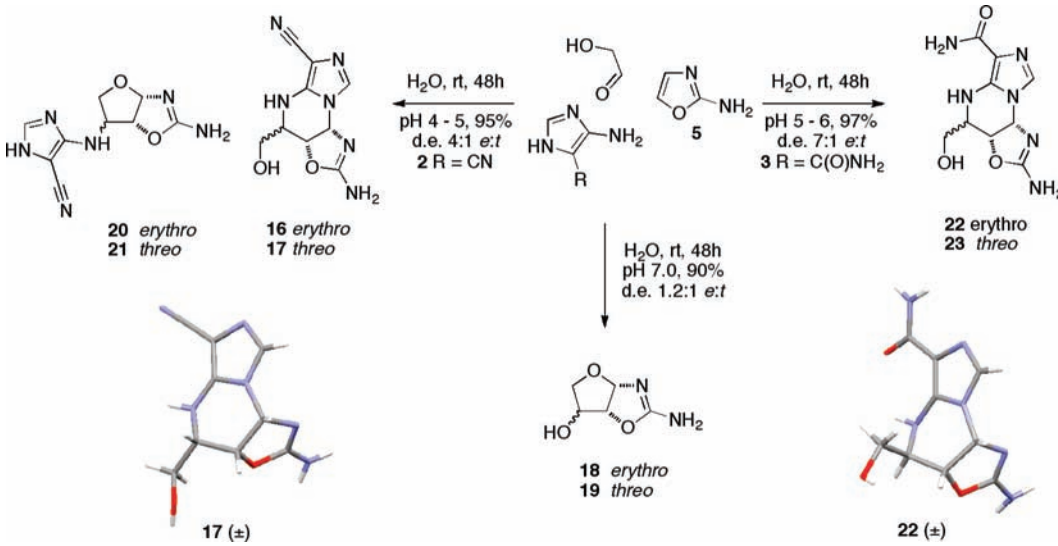
To further explore the generality of the MCR, two additional aldehydes were investigated. The reaction was shown to tolerate acetaldehyde to yield **11** and **12** as a 2:1 (*erythro*:*threo (e*:*t*)) mixture of diastereomers. The minor *rac*-[2′*S*,3′*R*]-isomer **12** was then isolated by precipitation 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 C_4 ($C_2 + C_2$) and C_5 (C_2

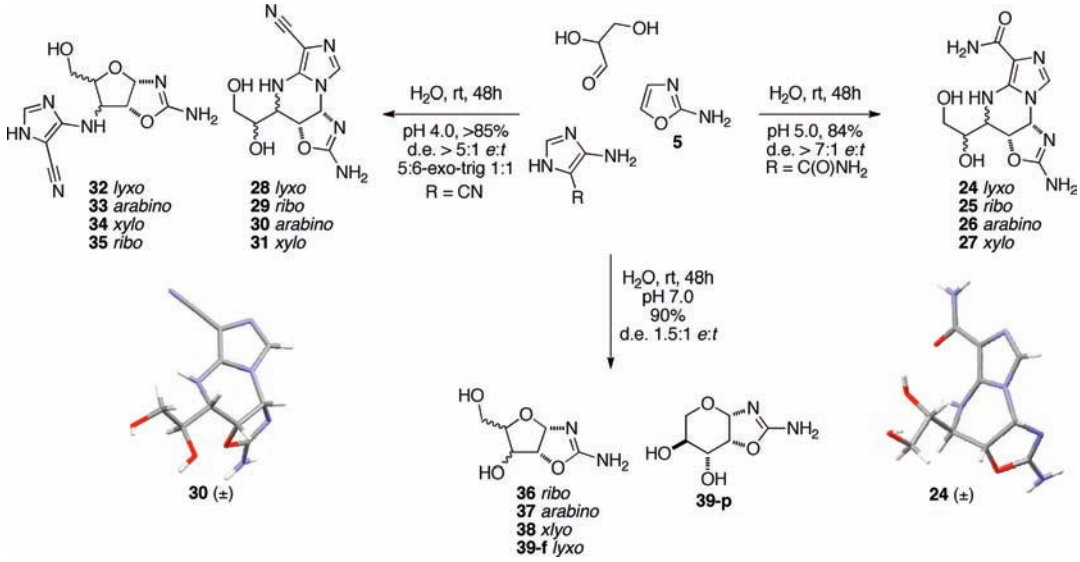
Scheme 4. One-Pot Multicomponent Assembly of *rac*-3′-Methyltetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines **11** and **12** and *rac*-3′-(Pyridin-2-yl)tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines **14** and **15**, Shown with Crystal Structures of **12** and **14**



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



Scheme 6. One-Pot Multicomponent [3′*R*,4′*R*]-Selective Assembly of *rac*-3′-(Dihydroxyethyl)tetrahydroimidazo-2″-aminooxazolopyrimidines from Glyceraldehyde with Crystal Structures of **24** and **30**



+ C_3) products.[11] pH-dependent reactivity was observed, with three-component chemistry dominating at lower pH values and two-component chemistry dominating a 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*-[3′*R*,4′*R*]-products—important for realization of a *β-ribo*-nucleotide synthesis by C3′-stereochemical inversion (Schemes 5 and 6).

Glycolaldehyde. Proposed routes for RNA synthesis must be continually evaluated for their potential to yield alternative structures capable of Watson−Crick base-paring. Glycolaldehyde, as a C_2 sugar synthon, has the potential to furnish precursors of threose nucleic acid (TNA) upon reaction with **5**.[12] At pD 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 pD 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**. At pD 5.0, a 7:1 mixture of diastereoisomers **22** and **23** (95% yield as determined by ^{1}H NMR integration) was observed, with complete 6-*exo-trig* selectivity. Compound **22** was isolated in 74% yield and crystallized to allow the [2′*R*,3′*R*]-stereochemical relationship to be proven by X-ray crystallography. Interestingly, **22** and **16** are related to TNA via C3′-stereochemical inversion.[12]

(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.

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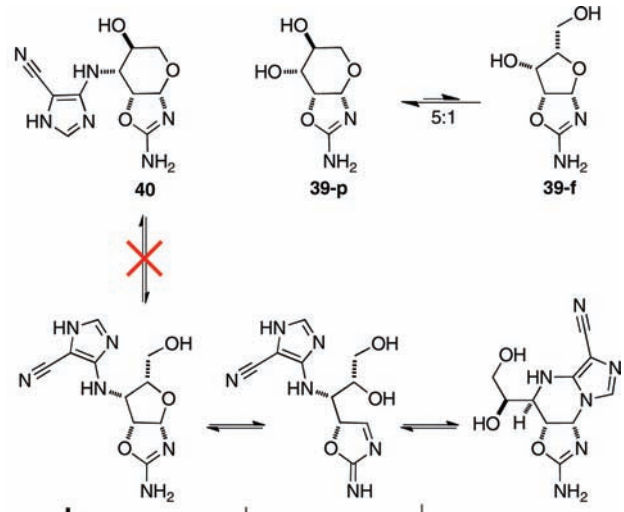
Glyceraldehyde. Glyceraldehyde is a C_3 sugar synthon; therefore, its reaction with the C_2 sugar synthon 2AO **5** can yield precursors of pentose nucleic acids (e.g., RNA).[1] However, a high-yielding synthesis of *ribo*-purinosides via C3′-tethered aminoimidazoles would require high [3′*R*,4′*R*]-diastereoselectivity (*lyxo*/*xylo*), a reversal of the facial selectivity of glyceraldehyde upon bimolecular reaction with **5**.[14] At pD 5.0, the reaction of glyceraldehyde, **5**, and AICA **3** furnished *lyxo*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine (**24**) in 60% yield (by ^{1}H NMR analysis; 46% isolated yield), alongside three other diastereoisomers, **25**−**27**, in 11, 5, and 8% yields, respectively (by ^{1}H NMR analysis). At pD 4.0, the reaction of AICN **2**, **5**, and glyceraldehyde was highly selective for three-component products (89% by ^{1}H NMR analysis). However, likely due to an increased Thorpe−Ingold effect in **9** (where R = $CH(OH)CH_2OH$ relative to R = CH_2OH) and the lower pH required for selective three-component reaction of **2** relative to **3**, competitive 6-*exo*-*trig* imidazole cyclization and 5-*exo*-*trig* hydroxyl cyclization resulted in a 1:1 mixture of *rac*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines **28**−**31** and *rac*-3′-aminoimidazole-2-aminooxazolines **32**−**35**.[13,15] 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 *ribo*/*arabino*-selective (*ribo* **36** = 44% and *arabino* **37** = 30%).[14] 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′ stereochemical inversion, while the *ribo*/*arabino*-selectivity of the two-component reaction establishes the required C3′ and C4′ stereochemistry for pyrimidine synthesis.[1,11b] The stereochemical preference for *lyxo*/*xylo* three-component products or *ribo*/*arabino* two-component products likely derives from the control of facial selectivity of the imine or aldehyde component, respectively,[14] 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**,[16] though *lyxo*-furanosyl aminooxazoline **39-f** equilibrates 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 ^{1}H NMR spectra of **28**/**32** at pD 1−12).

Scheme 7. pH-Controlled Isomerization of **32** to **28** in Water after 3 Days at Room Temperature



pD	32	9	28
1	>50		1
2	20		1
4	4		1
6	4		1
7	1.25		1
8	1		1
9	3		1
12	5		1

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 furnish only bimolecular aminooxazoline products at higher pH's, even in the presence of a large excess of **2** or **3**. The reaction of glycolaldehyde and **5** with **2** or **3**, above pD 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 glyceraldehyde, **2** or **3**, and **5** at pD 7.0 yields a mixture of pentose aminooxazolines **36**−**39**

(13) The structures of the 3′-aminoimidazole-2-aminooxazolines were proposed and differentiated on the basis of NMR spectroscopy (see Supporting Information, Figures S13−S16, S19−S22, S28, and S29); ^{1}H−^{13}C HSQC assigned C1′ and H−(C1′) chemical shifts: for **16**, **17**, and **28**−**31**, δ_C = 60−65 ppm, δ_H = 6.3−6.5 ppm; for **20**, **21**, and **32**−**35**, δ_C = 87−90 ppm, δ_H = 6.0−6.2 ppm). The C1′ upfield shift and the H−(C1′) downfield shift of tetrahydroimidazo[1′,3′]-C2″-aminooxazolo[1′,2′]-pyrimidines 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 ^{1}H−^{13}C HMBC correlations of H−(C3′)−C5 and H−(C1′)−C4′ but not H−(C1′)−C5. ^{1}H, ^{1}H−^{1}H COSY, and ^{1}H−^{1}H TOCSY assigned H−(C2′) proton−proton coupling constants: **20**, $J_{H\text{-}(C1')\text{-}H\text{-}(C2')}$ = 5.5, $J_{H\text{-}(C2')\text{-}H\text{-}(C3')}$ = 5.5 Hz; **32**, $J_{H\text{-}(C1')\text{-}H\text{-}(C2')}$ = 5.4 Hz, $J_{H\text{-}(C2')\text{-}H\text{-}(C3')}$ = 5.4 Hz. Cf. 6-*exo*-*trig* product **28**, $J_{H\text{-}(C1')\text{-}H\text{-}(C2')}$ = 7.4 Hz, $J_{H\text{-}(C2')\text{-}H\text{-}(C3')}$ = 1.6 Hz; and 5-*exo*-*trig* product **36**, $J_{H\text{-}(C1')\text{-}H\text{-}(C2')}$ = 5.5 Hz, $J_{H\text{-}(C2')\text{-}H\text{-}(C3')}$ = 5.5 Hz). ^{1}H−^{15}N HSQC and ^{1}H−^{15}N HMBC spectra were used to assign the connectivity of the imidazole nitrogens with respect to H−(C1′). Diastereoisomers were differentiated by a combination of crystallography and 2D NOESY spectroscopy.

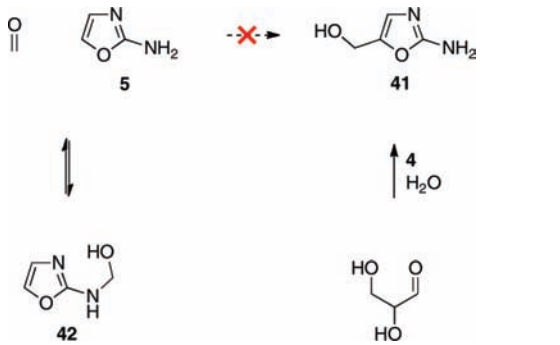
(14) Anastasi, C.; Crowe, M. A.; Powner, M. W.; Sutherland, J. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 6176.

(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 ^{1}H−^{13}C HSQC and ^{1}H−^{13}C HMBC analysis of the products of the reaction of glyceraldehyde, **2**, and **5** (see Supporting Information, Figure S28).

(17) Saewan, N.; Crowe, M. A.; Helliwell, M.; Raftery, J.; Chantranpromma, K.; Sutherland, J. D. *Chem. Biodiv.* **2005**, *2*, 66.

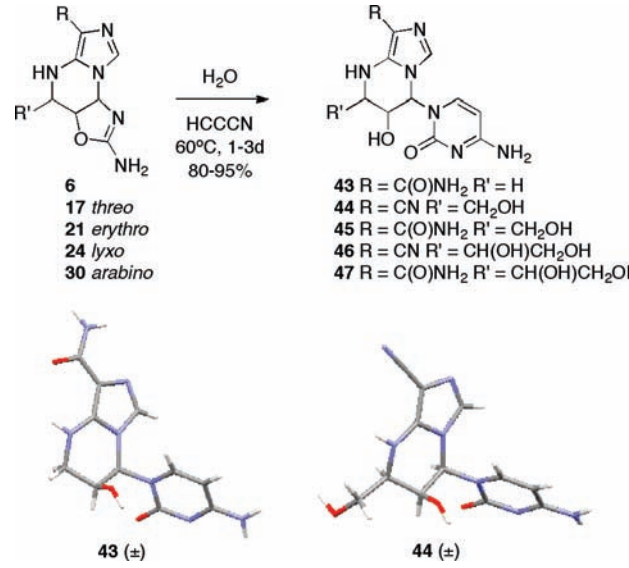
Scheme 8. Potential Hydroxymethylation of 2-Aminooxazole **5**



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 aminoimidazole pK_a-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 formation. As the nitrile of derivatives of **2** retains the potential to give divergent access to adenine and guanine nucleotides, it is of note that no significant nitrile hydrolysis was observed in our experiments.

Scheme 9. Cyanovinylation of Tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines with Unbuffered Aqueous Cyanoacetylene



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 cyanoacetylene. Nonetheless, with regard to the nucleophilicty of N1″, we were encouraged by the observation that **6** crystallized as the hemihydrochloride 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 cyanoacetylene. Even in the presence of 5 equiv of cyanoacetylene, 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-(1H)-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 D_2O, providing samples for X-ray diffraction to unambiguously prove the novel structural motif (Scheme 9).

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(19) Though it is possible that the reaction of formaldehyde and **5** is reversible, **41** was not observed to undergo retro-aldol decomposition during synthesis from glyceraldehyde and **4** or upon heating in aqueous solution. Furthermore, it should be noted that neither intramolecular cyclization nor aromatization is required for product trapping to prevent retro-aldol reaction. The reversible interchange of pentose aminooxazolines and their respective oxazoles with open-chain hydrates has been observed in the absence of retro-aldol reaction (see ref 11b). The reaction of 4-methoxybenzaldehyde and 2-amino-4-methyloxazole has been reported (albeit in hot toluene) wherein, due to the lack of a suitably positioned nucleophilic moiety, cyclization to an aminooxazoline cannot occur (ref 19a). Finally, in aqueous solution, the reaction of glyceraldehyde-2-phosphate and **5** furnishes a diastereomeric mixture of open-chain hydrates. Despite the potential for 6-*exo-trig* hydroxyl cyclization to afford pyranosyl−aminooxazoline products, heating these hydrate species leads to aromatization not cyclization or retro-aldol decomposition. (a) Khan, H. R.; Crank, G. *Tetrahedron Lett.* **1987**, *28*, 3381. (b) Anastasi, C.; Buchet, F. F.; Crowe, M. A.; Helliwell, M.; Raftery, J.; Sutherland, J. D. *Chem. Eur. J.* **2008**, *14*, 2375.

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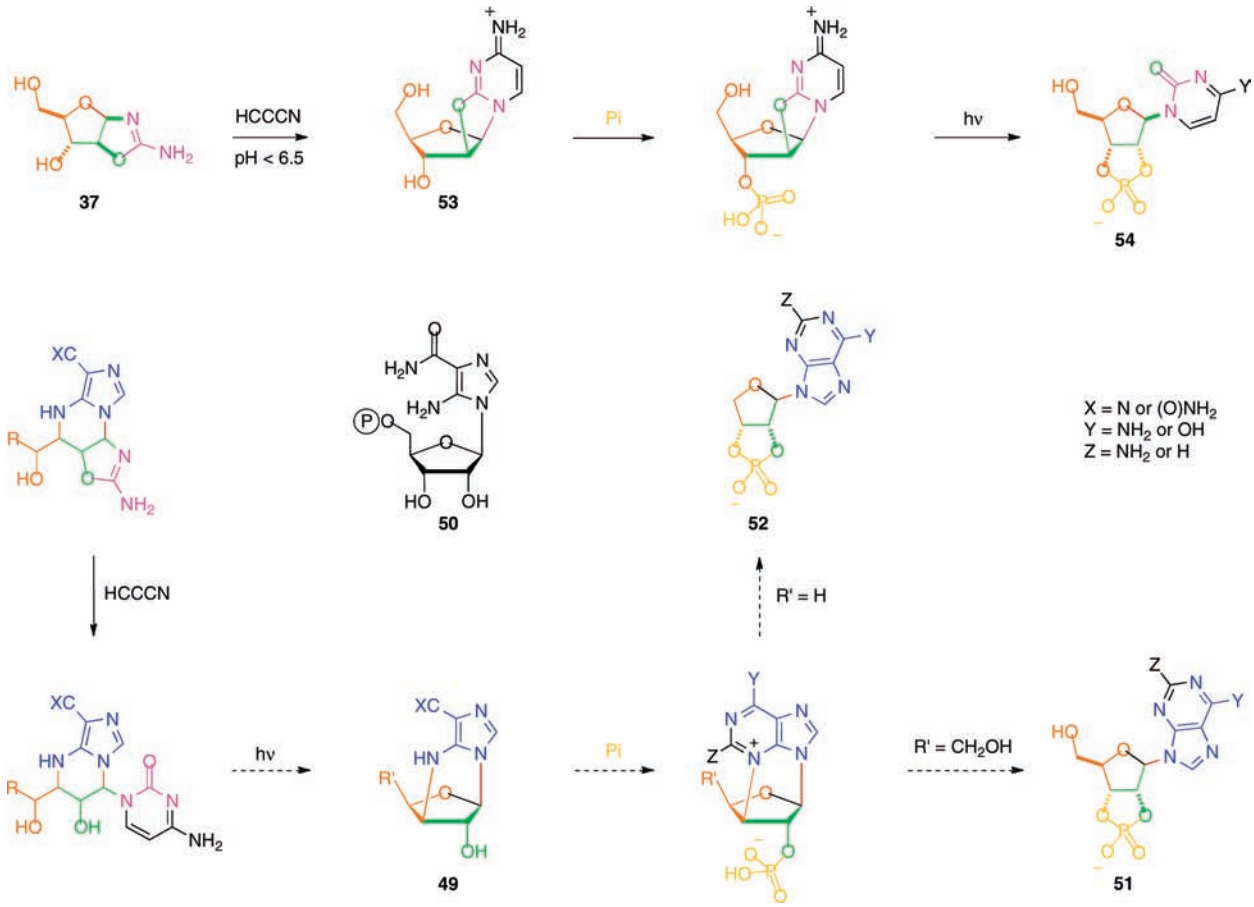
Scheme 10. β-Ribofuranosyl-pyrimidine Nucleotide Assembly and Potential Stepwise Regioselective β-Ribofuranosyl-purine Assembly Pathway via the Intermediacy of Tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines[a]



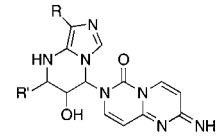
[a] Center: comparison to AICA-riboside **50**, an intermediate in the *de novo* stepwise biosynthesis of canonical nucleotides.

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 β-ribofuranosylnucleotide 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 β-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 (∼70% *lyxo*/*xylo*), reversing the facial selectivity of glyceraldehyde observed in the biomolecular reaction with 2AO **5** (∼70% *ribo*/*arabino*), 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′ = CH_2OH), which has an obvious relationship to AICA-riboside **50**, an intermediate in the *de novo* biosynthesis of purines (Scheme 10).[25,26] Furthermore, those compounds with *trans*-2′-hydroxyl-3′-anhydronucleoside relationships, such as **49**, have the clear potential to allow 2′,3′-cyclic phosphate formation under urea-mediated phosphoryl-transfer conditions,[1] thus generating **51**. Access to **49** should allow further study of the selectivity issues involved in synthesizing the canonical nucleotides as opposed to closely related chemical structures capable of Watson−Crick

(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′]-C2″-aminooxazolo[1′,2′]-pyrimidines were treated with an excess of cyanoacetylene. **48** (R = CN, R′ = CH_2OH) 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.



48

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(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.[1,3,4,9b,12,27] 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.[1,28]

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 cyanovinylation 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),[1] suggesting that both the pyrimidine and the purine ribonucelotides could be made together at the same time, in the same place, and under the same conditions.[29] 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 in parts per million (ppm). Electrospray mass spectrometry was recorded on a Bruker Daltonics Esquire 6000 ESI-MS. High-resolution mass spectrometry was carried out on a Waters Q-ToF micro LC/MS/MS system. Infrared spectra were recorded as manually pressed potassium bromide (KBr) discs on a PerkinElmer spectrum 100 series FT-IR spectrometer. Single-crystal X-ray crystallography was carried out with a Bruker APEX II CCD diffractometer (Mo Kα radiation, $\lambda = 0.71073$ Å), equipped with an Oxford Cryosystems nitrogen flow apparatus, at 100 K. Data integration down to 0.76 Å resolution was carried out using SAINT V7.46 A (Bruker diffractometer, 2009) with reflection spot size optimization. Absorption corrections were made with the program SADABS (Bruker diffractometer, 2009). The structure was solved by the direct methods procedure and refined by least-squares methods against F^2 using SHELXS-97 and SHELXL-97 (Sheldrick, 2008). Non-hydrogen atoms were refined anisotropically.

General Synthetic Methods. *Method A:* A solution of aminoimidazole **2** or **3** (≥1 equiv) and 2-aminooxazole **5** (1 equiv) in D_2O was adjusted to the desired pD. A solution of aldehyde (1 equiv) in D_2O and, if required, DMSO-d_6 was added. The pD was adjusted as necessary by addition of DCl or NaOD, and the reaction was volumetrically adjusted to the required concentration and then incubated at the specified temperature. The progress of reactions in deuterated solvents was directly monitored by NMR spectroscopy.

Method B: A solution of aminoimidazole **2** or **3** (≥1 equiv) and 2-aminooxazole **5** (1 equiv) in H_2O was adjusted to the desired pH. A solution of aldehyde (1 equiv) in H_2O was added. The pH was adjusted as necessary by addition of HCl or NaOH, and the reaction was volumetrically adjusted to the required concentration and then incubated at the specified temperature. Reaction mixtures were lyophilized and dissolved in deuterated solvents for NMR spectroscopic studies. One or more diastereomeric products were isolated from each reaction for charaterization.

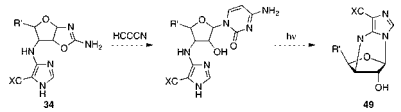
Method C: An aqueous solution of cyanoacetylene (0.98 M, 3−5 equiv) was added to tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines (20 mM), and the solution was then heated at 60 °C for 1−3 d with stirring. Analytical samples were removed and lyophilized to assess reaction progress by ^{1}H NMR spectroscopy.

***rac*-1′,2′,3′,*N*-Tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitrile (6).** *Method A:* 5-Aminoimidazole-4-carbonitrile **2** (24.8 mg, 0.23 mmol) and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (1.0 mL) at pD 5.0, 6.0, or 7.0. Formaldehyde (37%, 18.6 mg, 0.23 mmol) was added in D_2O (0.5 mL), the pD was rechecked, and the reaction volume was adjusted to 2 mL by the addition of D_2O. The reactions were incubated at room temperature, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S4, for ^{1}H NMR spectra at pD 5.0, 6.0, and 7.0). The formation of *rac*-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitrile **6** was observed at all pD values. **6** was then crystallized by cooling the reaction at pD 5.0 to 4 °C for 2 d. **6** was isolated by filtration and washed with ice-cold water, and a single crystal was removed for X-ray diffraction.

Method B: 5-Aminoimidazole-4-carbonitrile **2** (1.29 g, 11.9 mmol) and 2-aminooxazole **5** (1.00 g, 11.9 mmol) were dissolved in H_2O (10 mL) at pH 5.0. Formaldehyde (37%, 0.96 g, 11.9 mmol) was added, the pH was rechecked, and the reaction volume was adjusted to 20 mL with H_2O. After 20 min a white precipitate had formed in the reaction. The reaction was mechanically stirred for a further 15 h. The solids present were isolated by filtration, and the filtrate was lyophilized. The combined solids were redissolved in aqueous methanol by heating and agitation. Slowly cooling to room temperature gave 1.68 g (69%) of **6** as a white solid. Recrystallization of the lyophilized filtrate afforded a further 0.5 g (20%) of **6**.

IR (KBr, cm^{-1}): 3467 (N−H), 3380 (O−H), 3115, 2986, 2938 (C−H), 2212 (C≡N) 1698, 1610 (N═C). ^{1}H NMR (400 MHz, D_2O): δ_H 7.39 (s, 1H, H-(C2)); 6.08 (d, $J = 7.8$ Hz, 1H, H-(C1′)); 5.30 (ddd, $J = 7.8$, 2.0, 1.8 Hz, 1H, H-(C2′)); 3.54 (abx, $J = 14.2$, 1.8 Hz, 1H, H_a-(C3′)); 3.37 (abx, $J = 14.2$, 2.0 Hz, 1H, H_b-(C3′)) [peak partially obscured by HOD peak in DMSO; see Supporting Information, Figure S2]. ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 166.6

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



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(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 purine 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**.

(C═O); 164.0 (C2″); 142.1 (C5); 129.6 (C2); 112.7 (C4); 76.4 (C2′); 72.8 (C1′); 42.3 (C3′). ES-MS (pos. *m*/*z*): 205 (100%, $[M + H^+]^+$), 227 (10%, $[M + Na^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_8H_9N_6O$, 205.0832; found, 205.0831. X-ray diffraction structure solved; deposited as CCDC 784250.

***rac*-1′,2′,3′,*N*-Tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carboxamide (7).** *Method A:* 5-Aminoimidazole-4-carboxamide **3** (29.0 mg, 0.23 mmol) and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2.0 mL) at pD 5.0, 6.0, or 7.0. Formaldehyde (37%, 18.6 mg, 0.23 mmol) was added in D_2O (0.5 mL), the pD was rechecked, and the reaction volume was adjusted to 2 mL with D_2O. The reactions were incubated for 15 h, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S4, for ^{1}H NMR spectra at pD 5.0, 6.0, and 7.0). The formation of tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine **7** was observed at all pD 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-Aminoimidazole-4-carboxamide **3** (1.50 g, 11.9 mmol) and 2-aminooxazole **5** (1.00 g, 11.9 mmol) were dissolved in H_2O (10 mL) at pH 5.0. Formaldehyde (37%, 0.96 g, 11.9 mmol) was added, the pH was rechecked, and the reaction volume was adjusted to 20 mL with H_2O. 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 thrice recrystallized from H_2O for X-ray analysis.

IR (KBr, cm^{-1}): 3365 (NH), 3096, 2979, 2765, 2660 (C−H), 1676 (N═C), 1640 (C═O). ^{1}H NMR (400 MHz, D_2O): δ_H 7.21 (s, 1H, H-(C2)); 5.83 (d, J = 7.8 Hz, 1H, H-(C1′)); 5.03 (dt, J = 7.8, 1.8 Hz, 1H, H-(C2′)); 3.53 (abx, J = 13.8, 1.8 Hz, 1H, H_a-(C3′)); 3.18 (abx, J = 13.8, 1.8 Hz, 1H, H_b-(C3′)). ^{13}C NMR (101 MHz, D_2O): δ_C 166.2 (C═O); 164.1 (C2″); 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*): $[M]^+$ calcd for $C_8H_{11}N_6O_2$, 223.0938; found, 223.0935. X-ray diffraction structure solved; deposited as CCDC 784244.

***rac*-(1′*S*,2′*S*,3′*S*)-3′-Methyl-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carboxamide (11) and *rac*-(1′*S*,2′*S*,3′*R*)-3′-Methyl-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carboxamide (12).** *Method A:* Acetaldehyde (10 mg, 0.23 mmol), 5-aminoimidazole-4-carboxamide **3** (86.9 mg, 0.69 mmol), and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2 mL) at pD 6.0. The reaction was incubated for 15 h, and crystals (colorless needles) were observed to form. The supernatant was removed, and two products were observed by NMR spectroscopy (see Supporting Information, Figure S7, for ^{1}H NMR spectrum). 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 re-form over 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_2O to provide a sample for X-ray diffraction.

11. ^{1}H NMR (400 MHz, D_2O): δ_H 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*): $[M]^+$ calcd for $C_9H_{13}N_6O_2$, 237.1095; found, 237.1099.

12. IR (KBr, cm^{-1}): 3413 (NH), 3178, 3087, 2960 (C−H), 1684, 1663 (N═C), 1616 (C═O). ^{1}H NMR (400 MHz, D_2O): δ_H 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): δ_H 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). ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 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*): $[M]^+$ calcd for $C_9H_{13}N_6O_2$, 237.1095; found, 237.1099. X-ray diffraction structure solved; deposited as CCDC 784245.

***rac*-(1′*R*,2′*R*,3′*R*)-3′-(Pyridin-2-yl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carboxamide (14).** *Method A:* 2-Pyridine carboxaldehyde **13** (24.6 mg, 0.23 mmol), 5-aminoimidazole-4-carboxamide **3** (58 mg, 0.42 mmol), and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O:DMSO-d_6 (10:1, 2 mL) at pD 6.0. The reaction was incubated for 15 h, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S9, for ^{1}H NMR spectrum). The formation of two products, **14**/**15**, was observed in a 3:2 ratio by ^{1}H NMR spectroscopy. Compound **14** was observed to crystallize from the solution over 3 d and was isolated by centrifugation, washed with methanol, and dried to give 15 mg (20%) of **14**. An analytical sample of crystalline **14** was then recrystallized from ethanol three times for single-crystal X-ray diffraction.

IR (KBr, cm^{-1}): 3412 (N−H), 2937, 2528, 2458 (C−H), 1693 (N═C), 1669, 1637 (C═O) 1591. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 9.1 (s, NH); 8.43 (dd, J = 4.8, 0.8 Hz, 1H, Ar); 7.75 (td, J = 7.8, 1.8 Hz, 1H, Ar); 7.36 (d, J = 7.8 Hz, 1H, Ar); 7.24 (ddd, J = 7.8, 4.8, 1.8 Hz, 1H, Ar); 5.78 (d, J = 7.8 Hz, 1H, H-(C1′)); 5.40 (dd, J = 7.6, 2.5 Hz, 1H, H-(C2′)); 4.88 (d, J = 2.5 Hz, 1H, H-(C3′)). ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 166.6 (C═O); 163.7 (C2″); 159.0 (Ar); 149.6 (C5); 139.9 (Ar−H); 137.9 (Ar−H); 129.8 (C2); 123.6 (Ar−H); 122.2 (Ar−H); 112.7 (C4); 78.6 (C2′); 72.3 (C1′); 56.2 (C3′). ES-MS (pos. *m*/*z*): 300 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{13}H_{14}N_7O_2$, 300.1203; found, 300.1205. X-ray diffraction structure solved; deposited as CCDC 784252.

***rac*-(1′*S*,2′*S*,3′*S*)-3′-(Hydroxymethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitrile (16) and *rac*-(1′*S*,2′*S*,3′*R*)-3′-(Hydroxymethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitrile (17).** *Method A:* Glycolaldehyde (14.2 mg, 0.23 mmol), 5-aminoimidazole-4-carbonitrile **2** (24.8 mg, 0.23 mmol), and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2 mL) at pD 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 S11, for ^{1}H NMR spectra at pD 5.0, 6.0, and 7.0). The formation of **16** (60%), **17** (16%), **20** (14%), and **21** (7%) was observed at pD 4.0 (see Supporting Information, Figure S16, for ^{1}H, ^{1}H−^{1}H COSY, and 2D NOESY spectra). **17** was isolated by precipitation from warm methanol and recrystallized four times from ethanol and once by vapor diffusion (chloroform into methanol) to provide a sample for X-ray crystallography. **17** was studied by 2D homo- and heteronuclear NMR spectroscopy (see Supporting Information, Figures S13−S15). The formation of **18** (60%) and **19** (40%) was observed at pD 7.0.

16. ^{1}H NMR (400 MHz, D_2O): δ_H 7.22 (s, 1H, H-(C2)); 5.81 (d, J = 7.4 Hz, 1H, H-(C1′)); 4.99 (dd, J = 7.4, 2.7 Hz, 1H, H-(C2′)); 3.74 (dt, J = 5.7, 2.7 Hz, 1H, H-(C3′)); 3.50 (t, J = 5.7 Hz, 2H, H-(C4′)). ES-MS (pos. *m*/*z*): 235 (100%, $[M + H^+]^+$), 257 (10%, $[M + Na^+]^+$), 273 (3%, $[M + K^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_9H_{11}N_6O_2$, 235.0938; found, 235.0944.

17. IR (KBr, cm^{-1}): 3467 (N−H), 3380 (O−H), 3115, 2986, 2938 (C−H), 2212 (C≡N) 1698, 1610 (N═C). ^{1}H NMR (400 MHz,

Table 1. NMR (^{1}H NMR 400 MHz:^{13}C NMR 101 MHz:^{15}N NMR 40 MHz) Spectral Relationships of *rac*-3′-(4′-Hydroxymethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitriles **16** and **17** and *rac*-3′-(1*H*-5-aminoimidazolo)aminooxazolines **20** and **21**

	chemical shift (ppm)		NOE[a]			J coupling (Hz)		HMBC (^{1}H:^{13}C)			HMBC (^{1}H:^{15}N)		
	$\delta_{H1'}$	$\delta_{C1'}$	H(C1′)−H(C2)	H(C1′)−H(C3′)	H(C1′)−H$_2$(C4′)	$J_{H(C1')-H(C2')}$	$J_{H(C2')-H(C3')}$	H(C1′)−C2	H(C1′)−C4′	H(3′)−C5	H(C1′)−N1	H(C1′)−N1″	H(C3′)−N(C3′)
17	6.53	63.58	m	m	−	7.7	1.9	√	−	√	√	√	√
16	6.39	62.84	m	w	m	7.4	2.4	√	−	√	√	√	√
21	6.17	89.16	−	−	m	5.1	−	−	√	√	−	√	√
20	6.14	89.37	−	m	w	5.5	5.5	−	√	√	−	√	√

[a] 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.

D_2O): δ_H 7.16 (s, 1H, H-Ar)); 5.78 (d, J = 7.7 Hz, 1H, H-(C1′)); 4.8 (dd, J = 7.7, 1.9 Hz, 1H, H-(C2′)); 3.63 (abx, J = 11.4, 6.6 Hz, 1H, H_a-(C4′)); 3.56 (abx, J = 11.4, 6.8 Hz, 1H, H_b-(C4′)); 3.56 (app td, J = 6.7, 1.9 Hz, 1H, H-(C3′)). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ_H 7.35 (s, 1H, H-Ar); 6.83 (s, 1H, HN); 6.55 (s, 2H, NH_2); 5.87 (d, J = 7.5 Hz, 1H, H-(C1′)); 5.08 (t, J = 5.85 Hz, 1H, OH); 4.92 (d, J = 7.5 Hz, 1H, H-(C2′)); 3.74 (abx, J = 10.5, 6.0 Hz, 1H, H_a-(C4′)); 3.60 (abx, J = 10.5, 6.7 Hz, 1H, H_b-(C4′)); 3.42 (m, 1H, H-(C3′)). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C): δ_C 163.6 (C2); 145.4 (C5); 131.9 (C2); 116.9 (CN); 88.8 (C4); 75.5 (C2′); 73.5 (C1′); 60.3 (C4′); 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 $C_9H_{11}N_6O_2$, 235.0938; found, 235.0944. X-ray diffraction structure solved; deposited as CCDC 784246.

***rac*-(1′*S*,2′*S*,3′*S*)-3′-(Hydroxymethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[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 **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2 mL) at pD 5.0, 6.0, or 7.0. The reactions were incubated for 5 d, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S17, for ^{1}H NMR spectra at pD 5.0, 6.0, and 7.0). The formation of **22** (78%) and **23** (11%) was observed at pD 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 pD 7.0.

Method B: Glycolaldehyde (357 mg, 5.9 mmol), 5-aminoimidazole-4-carboxamide **3** (743 mg, 5.9 mmol), and 2-aminooxazole **5** (500 mg, 5.9 mmol) were dissolved in H_2O (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 recrystallized five times from aqueous ethanol for X-ray diffraction analysis.

22. IR (KBr, cm^{-1}): 3315 (O−H), 3188 (N−H), 2922, 2850 (C−H), 1711 (N═C), 1659 (C═O), 1614, 1575 (N═C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.14 (s, 1H, H-(C2)); 6.65 (bd, 2H, NH_2)); 6.45 (s, 2H, NH_2)); 6.18 (s, 1H, NH_2)); 5.74 (d, J = 7.4 Hz, 1H, H-(C1′)); 5.04 (t, J = 5.12 Hz, 1H, HO)); 4.78 (dd, J = 7.4, 2.9 Hz, 1H, H-(C2′)); 3.49 (m, 1H, H-(C3′)); 3.44 (abx, J = 11.0, 5.0 Hz, 1H, H_a-(C4′)); 3.38 (abx, J = 11.0, 5.3 Hz, 1H, H_b-(C4′)). ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 166.7 (C═O); 163.4 (C═N); 140.4 (C5); 129.2 (C2); 112.2 (C4); 76.3 (C1′); 72.5 (C2′); 62.4 (C3′); 54.2 (C4′). ES-MS (pos. m/z): 253 (100%, $[M + H^+]^+$). HRMS (m/z): $[M]^+$ calcd for $C_9H_{13}N_6O_3$, 253.1044; found, 253.1049. X-ray diffraction structure solved; deposited as CCDC 784243.

***rac*-3′-(4′,5′-Dihydroxyethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carboxamide (24).** Glyceraldehyde (20.7 mg, 0.23 mmol), 5-aminoimidazole-4-carboxamide **3** (29.0 mg, 0.23 mmol), and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2 mL) at pD 5.0, 6.0, 6.5, or 7.0. The reaction was incubated for 5 d, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Supporting Information, Figure S23, for ^{1}H NMR spectra at pD 5.0, 6.0, 6.5, and 7.0). The predominant formation of four tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines (84%) **24** (60%), **25** (11%), **26** (5%) and **27** (8%) was observed at pD 5.0. The predominant formation of pentose aminoxaolines **36** (44%), **37** (30%), **38** (8%), **35-f** (2%), and **35-p** (10%) was observed at pD 7.0. The reaction initiated at pD 5.0 was lyophilized and dissolved in methanol (5 mL). **24** was isolated by precipitation with anhydrous diethyl ether (10 mL) and crystallized from methanol to give 29 mg (46%) of **24** as colorless crystals. An analytical sample of **24** was crystallized three times from aqueous ethanol and then recrystallized from methanol:CH_2Cl_2 (1.5:1) by evaporation.

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, D_2O): δ_H 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 (abx, J = 11.7, 5.0 Hz, 1H, H_a-(C5′)); 3.42 (abx, J = 11.7, 6.2 Hz, 1H, H_b-(C5′)). ^{13}C NMR (101 MHz, D_2O): δ_C 168.1 (C═O); 165.1 (C2″); 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 $C_{10}H_{15}N_6O_4$, 283.1149; found, 283.1154. X-ray diffraction structure solved; deposited as CCDC 784247.

***rac*-3′-(4′,5′-Dihydroxyethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitrile (30) and *rac*-3′-(1*H*-5-Aminoimidazolo)aminooxazoline (32).** *Method A:* Glyceraldehyde (20.7 mg, 0.23 mmol), 5-aminoimidazole-4-carbonitrile **2** (49.6 mg, 0.46 mmol), and 2-aminooxazole **5** (20 mg, 0.23 mmol) were dissolved in D_2O (2 mL) at pD 4.0, 4.5, 5.0, 6.0, or 7.0. The reactions were incubated for 5 d, and the progress of the reaction was assessed by ^{1}H NMR spectroscopy (see Table 2 and Supporting Information, Figure S26, for ^{1}H NMR spectra at pD 4.0, 4.5, 5.0, 6.0, and 7.0). At pD 4.0, the formation of four *rac*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidines, **28**−**31** (46%, 13:2.2:1 ratio), and four 3′-imidazoloaminooxazolines, **32**−**35** (43%, 16:2.2:1 ratio), was observed by ^{1}H 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 aminoxaolines **36** (44%), **37** (30%), **38** (8%), **35-f** (2%), and **35-p** (10%) was observed at pD 7.0.

Method B: Glyceraldehyde (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 H_2O (10 mL) at pH 4.0. The reaction was incubated for 2 d, lyophilized, and dissolved in MeOH (10 mL). SiO_2 (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:CH_2Cl_2. Those fractions containing **32** were concentrated *in vacuo* to give white solids. The solids were dissolved in aqueous methanol (H_2O: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 D_2O and studied by ^{1}H, ^{1}H−^{1}H COSY, and ^{1}H−^{1}H NOESY NMR. The solution was lyophilized,

Table 2. NMR (^{1}H NMR 400 MHz:^{13}C NMR 101 MHz:^{15}N NMR 40 MHz) Spectral Relationships of *rac*-3′-(4′,5′-Dihydroxyethyl)-1′,2′,3′,*N*-tetrahydroimidazo[1′,3′]-2″-aminooxazolo[1′,2′]-pyrimidine-4-carbonitriles **28**−**31** and *rac*-3′-(1*H*-5-Aminoimidazolo)-aminooxazolines **32**−**35**

	chemical shift (ppm)		NOE[a]			*J* coupling (Hz)		HMBC (^{1}H−^{13}C)			HMBC (^{1}H−^{15}N)		
	$\delta_{H1'}$	$\delta_{C1'}$	H(C1′)−H(C2)	H(C1′)−H(C3′)	H(C1′)−H(C4′)	$J_{H1'-H2'}$	$J_{H2'-H3'}$	H(C1′)−C2	H(C1′)−C4′	H(C3′)−C5	H(C1′)−N1	H(C1′)−N1″	H(C3′)−N(C3′)
31	6.49	63.94	m	m	−	7.8	1.7	√	−	√	√	√	√
30	6.48	63.69	m	m	−	7.4	1.9	√	−	√	√	√	√
29	6.40	62.58	m	−	m	7.6	1.8	√	−	√	√	√	√
28	6.34	62.92	m	−	m	7.4	1.6	√	−	√	√	√	√
33	6.17	88.01	−	−	m	5.1	−	−	√	√	−	√	√
32	6.12	88.18	−	m	w	5.3	5.3	−	√	√	−	√	√
34	6.03	87.84	−	−	m	5.5	−	−	√	√	−	√	√
35	6.01	87.50	−	m	−	5.2	5.2	−	√	√	−	√	√

[a] 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 D_2O. ^{1}H NMR analysis showed that the solids were enriched in one *threo* isomer. Repeated recrystallization of the crystalline solids from H_2O afforded 20 mg of **30** as fine, clear needles (see Supporting Information, Figure S25).

30. IR (KBr, cm^{-1}): 3440 (NH), 3323 (O−H), 2873, 2800, 2658 (C−H), 2216 (C≡N), 1579 (N═C). ^{1}H NMR (400 MHz, D_2O): δ_H 7.40 (s, 1H, H-(C2)); 6.00 (d, J = 7.4 Hz, 1H, H-(C1′)); 5.22 (dd, J = 7.4, 1.9 Hz, 1H, H-(C2′)); 3.93 (ddd, J = 8.1, 5.6, 3.9 Hz, 1H, H-(C4′)); 3.83 (abx, J = 12.1, 3.9 Hz, 1H, H_a-(C5′)); 3.73 (abx, J = 12.1, 5.6 Hz, 1H, H_b-(C5′)); 3.55 (dd, J = 8.1, 1.9 Hz, 1H, H-(C3′)). ES-MS (pos. *m*/*z*): 265 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{10}H_{12}N_6O_3$, 265.144; found, 265.1050. X-ray diffraction structure solved; deposited as CCDC 784248.

32. ^{1}H NMR (400 MHz, D_2O, pD 1.0): δ 8.21 (s, 1H, H-(C2)); 5.97 (d, J = 5.4 Hz, 1H, H-(C1′)); 5.45 (t, J = 5.4 Hz, 1H, H-(C2′)); 4.17 (dd, J = 10.0, 5.4 Hz, 1H, H-(C3′)); 3.87 (ddd, J = 10.0, 3.9, 2.0 Hz, 1H, H-(C4′)); 3.77 (abx, J = 12.5, 2.0 Hz, 1H, H_a-(C5′)); 3.55 (abx, J = 12.5, 3.9 Hz, 1H, H_b-(C5′)). ^{13}C NMR (400 MHz, D_2O): 162.7 (C2″); 151.4 (C5); 135.5 (C2); 114.9 (CN); 88.2 (C1′); 84.9 (C2′); 84.0 (C4); 78.0 (C4′); 59.1 (C5′); 55.5 (C3′). ES-MS (pos. *m*/*z*): 265 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{10}H_{12}N_6O_3$, 265.144; found, 265.1051.

5-(Hydroxymethyl)-2-aminooxazole (41). Glyceraldehyde (1.8 g, 20 mmol) and cyanamide **4** (840 mg, 20 mmol) were dissolved in H_2O (10 mL) at pH 10 and heated at 60 °C for 2 h. The solution was then cooled, diluted with H_2O (10 mL), and extracted in EtOAc (5 × 20 mL). The organics were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in EtOAc and recrystallized by the addition of heptane to give 1.5 g (66%) of 5-(hydroxymethyl)-2-aminooxazole **41** as a white solid.

^{1}H NMR (400 MHz, MeOD): δ_H 6.58 (s, 1H, H-(C4)); 4.40 (s, 2H, CH_2). ^{13}C NMR (400 MHz, MeOD): δ_C 163.9 (C2); 146.2 (C5); 124.5 (C4); 55.2 (CH_2). MS ES (pos., *m*/*z*): 115 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_4H_7N_2O_2$, 115.0502; found, 115.0507.

4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carboxamide (43). *Method C:* **7** (100 mg, 0.49 mmol) and cyanoacetylene (0.98 M in H_2O, 1.5 mL) were incubated at 60 °C for 72 h. The product was then purified by silica gel chromatography (eluting with 1:5 to 1:1 CH_2Cl_2:MeOH), and the fractions containing the product (R_f 0.3; 1:5 CH_2Cl_2:MeOH) were concentrated *in vacuo*. The product was crystallized from aqueous ethanol to give 120 mg of **43** (85%) as clear, colorless crystals.

^{1}H NMR (400 MHz, D_2O): δ_H 7.25 (d, J = 7.6 Hz, 1H, H-(C6)); 7.08 (s, 1H, H-Ar); 6.75 (d, J = 3.8 Hz, 1H, H-(C1′)); 5.93 (d, J = 3.8 Hz, 1H, H-(C5)); 4.37 (ddd, J = 5.3, 3.8, 2.2 Hz, 1H, H-(C2′)); 3.53 (abx, J = 13.0, 2.2 Hz, 1H, H-(C3′)); 3.41 (abx, J = 11.7, 5.0 Hz, 1H, H-(C3″)). ^{13}C NMR (101 MHz, D_2O): δ_C 168.2 (C═O); 166.3 (C2″); 157.8 (C4″); 144.0 (C6″); 142.7 (C5); 130.6 (C2); 109.5 (C5″); 96.6 (C4); 64.8 (C2′); 62.4 (C1′); 43.5 (C3′). UV−vis: λ_{max} 277 nm (ε 9800). ES-MS (pos. *m*/*z*): 292 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{11}H_{12}N_7O_4$, 292.1153; found, 292.1146. X-ray diffraction structure solved; deposited as CCDC 784249.

(2*R*,3*S*,4*S*)-4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-3-hydroxy-2-(hydroxymethyl)-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carbonitrile (44) and (2*R*,3*S*,4*S*)-3-Hydroxy-2-(hydroxymethyl)-4-(2-imino-6-oxo-2*H*-pyrimido[1,6-*a*]-pyrimidin-7(6*H*)-yl)-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carbonitrile (48). *Method C:* **17** (117 mg, 0.50 mmol) and cyanoacetylene (0.98 M in H_2O, 2.0 mL) were incubated at 60 °C for 48 h. (2*R*,3*S*,4*S*)-3-Hydroxy-2-(hydroxymethyl)-4-(2-imino-6-oxo-2*H*-pyrimido[1,6-*a*]-pyrimidin-7(6*H*)-yl)-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carbonitrile (**48**) was observed to precipitate from solution over 4 h and was removed by filtration to give 2 mg of **48** as a yellow solid. (2*R*,3*S*,4*S*)-4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-3-hydroxy-2-(hydroxymethyl)-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carbonitrile (**44**) was observed to crystallize from aqueous solution over 48 h. The large colorless crystals were used directly for X-ray diffraction.

44. ^{1}H NMR (400 MHz, D_2O): δ_H 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_a-(C4′)); 3.67−3.77 (m, 2H, H-(C3′), H_b-(C4′)). ^{13}C NMR (101 MHz, D_2O): δ_C 166.2 (C2″); 157.4 (C4″); 147.2 (C5); 142.7 (C6″); 131.1 (C2); 116.5 (CN); 96.3 (C5″); 88.6 (C4); 66.0 (C2′); 62.2 (C1′); 60.4 (C4′); 55.2 (C3′). UV−vis: λ_{max} 252 nm (ε 10 100). ES-MS (pos. *m*/*z*): 304 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{12}H_{14}N_7O_3$, 304.1152; found, 304.1119. X-ray diffraction structure solved; deposited as CCDC 784251.

48. ^{1}H NMR (400 MHz, D_2O): δ_H 8.34 (s, 1H, H-(C2)); 7.62 (d, J = 7.5 Hz, 1H, H-(C6″)); 7.33 (d, J = 9.4 Hz, 1H, H-(C7″)); 6.10 (d, J = 3.5 Hz, 1H, H-(C1′)); 5.96 (d, J = 7.5 Hz, 1H, H-(C5″)); 5.55 (d, J = 9.4 Hz, 1H, H-(C8″)); 4.48 (dd, J = 3.5, 2.0 Hz, 1H, H-(C2′)); 4.43 (dd, J = 9.8, 4.0 Hz, 1H, H_a-(C4′)); 4.26 (app dt, J = 3.0, 1.9 Hz, 1H, H-(C3′)); 4.0 (dd, J = 9.8, 1.9 Hz, 1H, H_b-(C4′)). UV−vis: λ_{max} 252 nm (ε 9760), 320 (ε 6400). ES-MS (pos. *m*/*z*): 355 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{15}H_{15}N_8O_3$, 355.1261; found, 355.1261.

(2*S*,3*S*,4*S*)-4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-3-hydroxy-2-(hydroxymethyl)-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carboxamide (45). *Method C:* **21** (50 mg, 0.20 mmol) and cyanoacetylene (0.98 M in H_2O, 0.5 mL) were incubated at 60 °C for 24 h. The formation of **45** was observed in 80% yield by ^{1}H NMR integration.

^{1}H NMR (400 MHz, D_2O): δ_H 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′)). ^{13}C NMR (101 MHz, D_2O): δ_C 167.6 (C═O); 165.6 (C2″); 157.3 (C4″); 142.4 (C5); 142.0 (C6″); 129.5 (C2); 108.4 (C4); 96.2 (C5″); 63.3 (C1′); 62.9 (C2′); 60.9 (C4′); 54.1 (C3′). UV−vis: λ_{max} 272

nm. ES-MS (pos. *m*/*z*): 304 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{12}H_{16}N_7O_4$, 322.1258; found, 322.1284.

(2*R*,3*S*,4*S*)-4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-2-((*R*)-1,2-dihydroxyethyl)-3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carbonitrile (46). *Method C:* **30** (5 mg, 0.019 mmol) and cyanoacetylene (0.98 M in H_2O, 50 μL) were incubated at 60 °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_2O): δ_H 7.16 (d, $J = 7.6$ Hz, 1H, H-(C6″)); 7.03 (s, 1H, H-(C2)); 6.64 (d, $J = 3.1$ Hz, 1H, H-(C1′)); 5.84 (d, $J = 7.6$ Hz, 1H, H-(C5″)); 4.30 (d, $J = 3.1$, 1.0 Hz, 1H, H-(C2′)); 3.66−3.77 (m, 2H, H-(C4′), H_a-(C5′)); 3.58 (abx, $J = 12.1$, 5.8 Hz, 1H, H_b-(C5′)); 3.51 (app d, $J = 8.6$ Hz, 1H, H-(C3′)). ^{13}C NMR (101 MHz, D_2O): δ_C 165.6 (C2″); 157.5 (C4″); 147.2 (C5); 142.0 (C6″); 130.8 (C2); 116 (CN); 96.0 (C4); 96.0 (C5″); 65.9 (C1′); 61.7 (C2′); 69.0 (C4′); 62.3 (C5′); 55.2 (C3′). UV−vis: λ_{max} 254 nm. ES-MS (pos. *m*/*z*): 334 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{13}H_{16}N_7O_4$, 334.1258; found, 334.1251.

(2*R*,3*R*,4*R*)-4-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-2-((*R*)-1,2-dihydroxyethyl)-3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-*a*]-pyrimidine-8-carboxamide (47). *Method C:* **24** (20 mg, 0.07 mmol) and cyanoacetylene (0.98 M in H_2O, 0.35 mL) were incubated at 60 °C for 24 h. The formation of **47** was observed in 87% yield by ^{1}H NMR integration (see Supporting Information, Figure S34, for crude ^{1}H NMR).

^{1}H NMR (400 MHz, D_2O): δ_H 7.16 (d, $J = 7.5$ Hz, 1H, H-(C6″)); 7.00 (s, 1H, H-(C2)); 6.74 (d, $J = 4.3$ Hz, 1H, H-(C1′)); 5.81 (d, $J = 7.5$ Hz, 1H, H-(C5″)); 4.22 (dd, $J = 5.8$, 4.3 Hz, 1H, H-(C2′)); 3.68 (ddd, $J = 5.6$, 4.8, 2.7 Hz, 1H, H-(C4′)); 3.60 (abx, $J = 11.8$, 4.8 Hz, 1H, H-(C5′)); 3.53 (abx, $J = 11.8$, 5.6 Hz, 1H, H-(C5′)); 3.50 (dd, $J = 5.8$, 2.7 Hz, 1H, H-(C3′)). ^{13}C NMR (101 MHz, D_2O): δ_C 168.3 (C═O); 166.1 (C4″); 157.8 (C2″); 143.2 (C5); 142.6 (C6″); 129.8 (C2); 109.0 (C4); 96.7 (C5″); 69.7 (C4′); 64.4 (C2′); 63.7 (C1′); 63.6 (C5′); 55.5 (C3′). UV−vis: λ_{max} 272 nm. ES-MS (pos. *m*/*z*): 352 (100%, $[M + H^+]^+$). HRMS (*m*/*z*): $[M]^+$ calcd for $C_{13}H_{16}N_7O_4$, 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.

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