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Selective prebiotic formation of RNA pyrimidine and DNA purine nucleosides

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28 **deoxyadenosine and deoxyinosine. Our synthesis utilizes key intermediates in the**
29 **prebiotic synthesis of the canonical pyrimidine ribonucleosides, and we show**
30 **that, once generated, the pyrimidines persist throughout the synthesis of the**
31 **purine deoxyribonucleosides, ultimately leading to a mixture of deoxyadenosine,**
32 **deoxyinosine, cytidine, and uridine. These results support the notion that purine**
33 **deoxyribonucleosides and pyrimidine ribonucleosides may have coexisted before**
34 **the emergence of life³.**

35 **Introduction**

36 The advent of life requires informational inheritance mediated by a suitable
37 polymer that can undergo replication in the absence of enzymes. The RNA world
38 hypothesis invokes RNA as this polymer^{4, 5}. Considerable progress in the prebiotic
39 synthesis of the pyrimidine ribonucleosides of RNA, cytidine (C) **1** and uridine (U) **2**,
40 and their 2-thio derivatives, **3** and **4**^{6, 7}, together with recent advances in non-
41 enzymatic RNA replication⁸⁻¹⁰ have given credence to the RNA world theory.
42 Progress towards the abiotic synthesis of purine nucleosides has been made, but only
43 using routes that employ as starting materials chemically and enantiomerically pure
44 sugars¹¹⁻¹⁵, which are not likely to have been found on the primordial earth.
45 Additionally, no prebiotically plausible route has been shown to provide a mixture
46 containing a competent set of nucleosides for information storage at the polymeric
47 level.

48 Extant biology, in contrast to the proposed RNA world, utilizes DNA as the central
49 information-carrying molecule. This discrepancy between the RNA world and
50 modern biology requires a ‘genetic takeover’ that invokes the power of primitive
51 biosynthetic machinery and natural selection operating over millions of years,
52 ultimately resulting in an ancestral biosynthetic route to DNA¹⁶. The superior

53 hydrolytic stability and replication fidelity¹⁷ of DNA could have resulted in selection
54 of primitive organisms capable of synthesizing DNA, and thus its rise to prominence
55 in the central dogma, but the feasibility of this evolutionary process in a pre-DNA
56 world is debated¹. To circumvent this potentially problematic transition, an R/DNA
57 world has been proposed, in which nascent biology had access to both RNA and DNA
58 building blocks from the outset, without requiring elaborate biosynthesis¹⁸⁻²⁰. In such
59 a world, heterogeneous polymers would have initially been most common, but
60 polymers with increased homogeneity, and hence properties closer to either that of
61 RNA or DNA, would have been selected for over their mixed counterparts². For the
62 R/DNA world to be plausible, an efficient prebiotic synthesis of DNA building blocks
63 is required, and one that provides building blocks for both RNA and DNA in the same
64 localized geochemical scenario is preferable. We recently demonstrated proof of this
65 principle by showing that 2'-deoxy-2-thiouridine **5** – a non-canonical deoxynucleoside
66 – can be synthesized from thioanhydrouridine **6** – an RNA derivative – by way of a
67 prebiotically plausible, hydrogen sulfide-mediated photoreduction³. Although this
68 finding provides an important prebiotic link between RNA and DNA building blocks,
69 the lability of **5** to hydrolysis may limit its phosphorylation and subsequent
70 oligomerization^{21, 22}. Additionally, the synthesis of canonical deoxyadenosine (dA) **7**
71 from **5** and adenine **8** was low yielding (4%), and generated a more abundant
72 undesired side product, the α -anomer of **7** (6%). Using guidance from a geochemical
73 scenario²³, we now demonstrate a synthesis of purine deoxynucleosides that is based
74 on prebiotically plausible reactions and substrates. We then evaluate our route at a
75 systems level by enacting the synthesis on mixtures of materials likely to arise in a
76 primordial environment, culminating in the demonstration of multiple reaction

77 sequences able to selectively furnish a mixture of U (1), C (2), dA (7) and
 78 deoxyinosine (dI, 9).

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82 **Results and Discussion**

83 **Prebiotically Guided Route to Purine Deoxyribonucleosides**

84 A route to purine nucleosides that diverges from a prebiotic RNA synthesis is
 85 attractive because it implies that the constituents of a set of nucleosides capable of
 86 storing information – pyrimidines and purines – may have formed in the same
 87 location on a primordial Earth, rather than having been necessarily brought together
 88 by environmental processes after their separate formation. To develop such a route,
 89 we evaluated intermediates in the prebiotic RNA pyrimidine nucleoside synthesis^{6, 7}

90 as ribosyl donors (Fig. 1). The RNA synthesis proceeds from RAO **10** which reacts
91 with cyanoacetylene **11** to provide α -anhydrocytidine **12**. Thiolysis of **12** in
92 formamide produces α -2-thiocytidine **13** which undergoes efficient UV-mediated
93 photoanomerisation to 2-thiocytidine **3**, which hydrolyses to the canonical
94 pyrimidines cytidine **1** and uridine **2**, and biologically important non-canonical
95 pyrimidine **4**. Alternatively, in the dark, **13** is hydrolysed to α -2-thiouridine **14**⁷.
96 Whilst **14** appeared initially only a by-product that would be produced in the dark on
97 the early Earth, it is readily cyclised to anhydrouridine **15** at 80 °C (63% yield in
98 water or 89% yield in formamide, Extended Data Fig. 2). We recognised α -
99 anhydropyrimidines **12** and **15** as ideal glycosyl donors for 1',2'-cis tethered
100 glycosylation²⁴. Since the sugar of **12** and **15** is fixed in its furanosyl form, the
101 formation of pyranosyl nucleosides – one of the critical downfalls of previous
102 strategies – should be excluded. Additionally, the α -stereochemistries of C1' and C2'
103 of **12** and **15** led us to expect transglycosylation to provide only β -anomers, the
104 correct stereochemistry at C1' for all natural (deoxy)ribonucleosides. Finally, since **12**
105 and **15** are ultimately derived from ribo-aminooxazoline (RAO) **10**, which crystallizes
106 enantiopure from solutions of minimally enantioenriched carbohydrates or amino
107 acids^{25, 26}, this route offered the so-far unmet potential to deliver enantio- and
108 diastereomerically pure furanosyl-nucleosides by glycosylation.

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110 Accordingly, we evaluated 8-mercaptadenine **16** and 8-mercaptoguanine **17**
111 as potential nucleophiles to participate in transglycosylation with **12** and **15** (Fig 2).
112 Although **17** proved unreactive, **16** reacts with **12** and **15** at 150 °C in the dry state
113 (Fig. 2), to provide two new β -configured nucleoside products in moderate yields
114 (14% and 16% respectively from **15**, trace amounts from **12**). The minor product was

provision of 8-mercaptoadenine **16**, due to its widely accepted prebiotic plausibility as a relatively stable pentamer of hydrogen cyanide^{29, 30}. Remarkably, despite the reactivity of related purines³¹, adenine did not react with elemental sulfur at temperatures up to 300 °C. However, adenine does undergo (slow) hydrolysis in aqueous media. Miller et. al. reported a half-time for hydrolysis of adenine of about 1 year at 100 °C, and identified (but did not quantify) 4,5,6-triaminopyrimidine **21** (TAP) among the products of hydrolysis³². We reinvestigated this hydrolysis of adenine **8**, under conditions more suited to a laboratory time-scale (138 °C, phosphate buffer pH 8), and at partial conversion after 10–12 days confirmed the presence of TAP in yields of 2–3% (8–9% based on recovered adenine) (Fig. 2). Due to the differential solubilities of adenine and TAP, the supernatants of adenine hydrolysis reactions are enriched in TAP after cooling. A typical supernatant contains 5-aminoimidazole-4-carboxamide **22**, TAP **21**, and adenine **8** in a 4:2:1 ratio, and formate **23** as the only other major component (See Fig. S1–S5 for full details). We found that TAP (either commercially supplied or that in the crude adenine hydrolysate) is converted to 8-mercaptoadenine **16** by heating in the dry state with either ammonium thiocyanate **24** or thiourea **25**. **24** is an inevitable by-product of the photochemistry of hydrogen cyanide and hydrogen sulfide³³, two precursors likely to have been abundant on the primordial earth, and heavily implicated in the origin of life by our cyanosulfidic chemical network²³. Thiourea **25** has also been widely invoked as a prebiotically plausible reagent³⁴. Thus, we envision that a primordial environment supplied with adenine and water would continuously generate TAP, which can be enriched in aqueous solution by moving down a thermal gradient. Ammonium thiocyanate **24** can be mixed with the TAP at any stage, and eventual evaporation and dry state reaction leads to 8-mercaptoadenine **16**. This method of

155 accumulation of TAP also improves the plausibility of some aspects of other prebiotic
156 syntheses¹².

157 With thioanhydropurine nucleosides **18** and **19** in hand, we moved on to
158 evaluate their photoreduction chemistry to see if we might directly generate
159 deoxyadenosine. Our previous synthesis of a deoxypyrimidine *via* a
160 thioanhydropyrimidine **6** (Fig. 1) proceeded by the reduction of a C–S to a C–H bond
161 mediated by a hydrated electron, generated by UV irradiation of hydrosulfide^{3, 33}. **18**
162 and **19** were separately subjected to UV irradiation at 254 nm in water with hydrogen
163 sulfide (H₂S) as the reductant (Fig. 2). In the photoreduction of **18**, the natural regio-
164 isomer *N*⁹-deoxyadenosine **7** (dA) was detected in 39% yield, along with 15% of 8-
165 mercapto-deoxyadenosine **26**. **26** was demonstrated to be a competent intermediate in
166 the reaction by desulfurization to give **7** (dA) either by UV irradiation³⁵, or treatment
167 with nitrous acid, which is produced from common atmospheric gases, nitrogen and
168 carbon dioxide³⁶. Nucleobase loss was also apparent (8-mercaptopadenine **16** in 10%
169 yield and adenine **8** in 17% yield). The same reaction starting with **19** gave *N*⁷-
170 deoxyadenosine **20** in 23% yield with no other nucleoside products. Our proposed
171 intermediate in this process, 8-mercapto-*N*⁷-deoxyadenosine **27**, is either fully
172 converted to **20** or photochemically destroyed. Photoreduction was also carried out on
173 a mixture of **18** and **19** compatible with our synthesis by tethered transglycosylation.
174 The ratio of *N*⁹:*N*⁷ regioisomers was increased from 38:62 of **18:19** in the starting
175 mixture to 56:44 of **7:20** after photoreduction (31% yield for **7**, 17% yield for **20**),
176 indicating an enhanced stability of intermediates or products bearing the natural *N*⁹
177 glycosidic linkage, compared to *N*⁷ isomers. Replacing hydrosulfide as the electron
178 donor with bisulfite (HSO₃⁻, pH 7)³⁷, which is readily formed by the dissolution of
179 atmospheric SO₂ in water³⁸, improved both the yield and selectivity of

180 photoreduction. Photoreduction with bisulfite of **18** alone provided deoxyadenosine **7**
181 (dA) in 51% yield and 8-mercapto-deoxyadenosine **26** in 23% yield, while a similar
182 reaction with the N^7 -regio-isomer **19** led only to its photochemical destruction.
183 Photoreduction of a mixture of **18** and **19** with bisulfite led only to N^9 -linked
184 products, **7** and **26** in 44% and 18% yield respectively (Extended Data Fig. 3).
185 Separate experiments probing the stability of starting materials and products under the
186 reaction conditions indicated that the relative stabilities of intermediates are the cause
187 of this selectivity. This strikingly selective destruction is highly suggestive of a
188 potential mechanism by which primordial nucleosides were restricted to a near-
189 canonical set^{39, 40}. We found further evidence for such restriction in the hydrolysis
190 rates of the N^9 and N^7 isomers of deoxyadenosine. In acetate buffer (pH 4, room
191 temperature), the natural isomer **7** (dA) is more than 70 times more stable than **20**
192 (half-lives of 1565 and 22 hours respectively), which is consistent with the reported
193 difference in stabilities towards acid hydrolysis between the corresponding isomers of
194 adenosine^{41, 42}.

195 Photoreduction Mechanism

196 To provide mechanistic rationale for the observed photochemical selectivity,
197 we performed quantum chemical calculations using density functional theory and
198 algebraic diagrammatic construction to the second order [ADC(2)] methods^{43, 44}.
199 These calculations revealed, in the case of bisulfite, two possible competing
200 mechanisms that explain the difference in reactivity of the two regioisomers. **18** and
201 **19** can both undergo photoexcitation, but generate dissimilar biradical species (Fig.
202 3a). Photoexcitation of **18** leads to rupture of the C2'-S bond on the surface of the
203 lowest excited singlet (S_1) state, generating biradical **28** (Fig 3a, N^9 ; Extended Data
204 Fig. 4a). Reduction of this species by intermolecular hydrogen atom transfer (HAT)

205 or proton-coupled electron transfer (PCET) is likely to lead to C2'-reduced species **29**,
 206 and ultimately, *via* a second HAT or PCET and subsequent photolysis of the C8–S
 207 bond of **26**³⁵, deoxyadenosine **7** (dA) (Fig. 3a, *N*⁹). In contrast, photoexcitation of **19**
 208 leads to N7–C8 bond rupture through the S₁/S₀ state crossing (Fig. 3a, *N*⁷; Extended
 209 Data Fig. 4b), generating **30**, which is likely to undergo decomposition without C2'–S
 210 reduction. Since bisulfite is well-known to provide a hydrated electron upon
 211 irradiation⁴⁵, a second possibility is the reduction by hydrated electrons of **18** and **19**
 212 in the ground state. Again, calculations suggest different fates of **18** and **19** upon
 213 reduction. Reduction of **18** is predicted to proceed with concomitant barrierless C2'–S
 214 bond rupture to give radical anion intermediate **31** (Fig. 3b, *N*⁹; Extended Data Fig. 5)
 215 whereas reduction of **19** is predicted to lead to formation of a C8, N9 radical anion **32**
 216 which also is likely to undergo decomposition rather than C2' reduction (Fig. 3b *N*⁷,
 217 Extended Data Fig. 5). In the absence of any reducing agent, both **18** and **19** undergo
 218 (equally) slow photochemical decomposition, presumably via the calculated biradical
 219 structures **28** and **30**, but in the presence of bisulfite, reduction of the ground state or
 220 photochemically generated intermediates results in remarkably different fates.

221 The successful reduction of **19** alongside **18** when using hydrosulfide as the
 222 reducing agent is explained by a distinct mechanism. Calculations located stable
 223 encounter complexes, **33** and **34**, between HS[−] and thioanhydronucleosides **18** and **19**,
 224 respectively (Fig. 3c, Extended Data Fig. 4c and 4d). This interaction is
 225 predominantly stabilized by electrostatic and dispersion interactions and our
 226 interaction energy decomposition demonstrates its stability in aqueous solution (see
 227 the SI for detail). Similar S⋯S interactions were recently identified in intramolecular
 228 complexes and were classified as chalcogen bonds⁴⁶. Such an encounter complex
 229 facilitates charge transfer (CT) from the hydrosulfide anion to the thioanhydropurine

230 fragment almost immediately after UV absorption by the complex to the S_1 state.
231 Subsequent relaxation on the S_1 surface enables practically barrierless C2'-S bond
232 breaking completed by a peaked S_1/S_0 state crossing for both intermediates **31** and **35**,
233 thus facilitating C2'-S reduction of both **18** and **19** (Extended Data Fig. 4c and 4d).
234 The products of this photochemical transformation, **26** and **27**, may further undergo
235 photochemical sulfur cleavage through the mechanism described by Roberts *et al.*³⁵
236 (Fig. 3c). Thus, a HS⁻ thioanhydropurine encounter complex facilitates C-S bond
237 cleavage and partially protects N^7 isomer **19** from the photodestruction observed in
238 the presence of bisulfite. This finding not only explains the distinctive outcomes of
239 photoreduction between the two reducing agents, but also points towards a potentially
240 important stabilising role for hydrosulfide in prebiotic chemistry and photochemistry
241 in general.

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245 Prebiotic Route to A Purine/Pyrimidine Genetic System

246 Whilst our attempts to glycosylate 8-mercaptoguanine **17** to provide
 247 thioanhydroguanosine (and ultimately deoxyguanosine) failed, the triple selectivity
 248 and high yield of our route to deoxyadenosine combined with recent results from the
 249 Szostak group⁴⁷ suggest a possible alternative genetic alphabet that does not include
 250 (deoxy)guanosine. Guanosine is yet to succumb to a plausible prebiotic synthesis, but
 251 Szostak *et al.* have recently shown that inosine (I), which is capable of base-pairing
 252 with cytosine, can replace guanosine in non-enzymatic RNA replication systems with
 253 no loss of rate or fidelity. (Deoxy)adenosine **7** (dA) is readily converted to

(deoxy)inosine **9** (dI) (Fig. 2) by deaminative hydrolysis, which spontaneously occurs very slowly in nucleic acid polymers⁴⁸, and is greatly accelerated by the presence of nitrous acid⁴⁹. To demonstrate that this conversion can occur under mild conditions consistent with our primordial geochemical scenario⁵⁰, we treated deoxyadenosine **7** (dA) with nitrous acid at pH 4 (the same conditions by which we could effect desulfurization of **26**). After four days at room temperature, approximately 40% of **7** (dA) had been converted to **9** (dI), providing a 60:40 mixture of **7** (dA) and **9** (dI) (Fig 2). A control experiment monitoring the decomposition of deoxyadenosine **7** (dA) at pH 4, without nitrous acid, showed only a trace of depurination ($t_{1/2} = 1600$ h). When a 67:33 mixture of **7** and **26**, representative of the outcome of photoreduction, was submitted to the reaction conditions, **26** underwent relatively rapid desulfurization first, with deoxyadenosine **7** (dA) undergoing slower deaminative hydrolysis to ultimately provide a 65:35 mixture of **7** (dA) and **9** (dI). Thus, mixtures of deoxyadenosine **7** (dA) and deoxyinosine **7** (dA) are readily obtainable from partial deaminative hydrolysis of deoxyadenosine **7** (dA) or its precursor **26**, thereby supplying half of a potential primordial alphabet. Despite the potential for a mismatch in reactivity between deoxypurines and pyrimidines, a 47:53 mixture of deoxyadenosine **7** (dA) and cytidine **1** (C) underwent nitrous acid-promoted deamination to provide all four (deoxy)nucleosides deoxyadenosine **7** (dA), deoxyinosine **9** (dI), cytidine **1** (C), and uridine **2** (U) (30:17:42:11 ratio) (Extended Data Fig. 6). A similar primordial mixture may have been a starting point for genetic information storage. Furthermore, in the absence of significant geochemically plausible sources of pyrimidine deoxynucleotides and purine ribonucleotides, heteropolymers made from a mixture of purine deoxyribonucleotides and pyrimidine ribonucleotides should possess heritable backbone heterogeneity and thus a 1:1

phenotype to genotype correspondence, which is potentially advantageous in the evolution of catalytic activity¹⁸.

Systems Level Prebiotic Plausibility

Having demonstrated the potential of a divergent route to yield a local mixture of **7** (dA), **9** (dI), **1** (C) and **2** (U), we sought to evaluate the key question of whether all four nucleosides could persist after divergence in the sequence. We chose a 1:1 mixture of α - and β -2-thiouridines **13** and **3** as our starting point, which could be obtained from the partial photoanomerisation of **13**, and evaluated two particular combinations of reactions as representative permutations of a primordial geochemical process (Fig. 4, Route A and B). In route A, exposure of the mixture to nitrous acid (pH 4) generates a mixture of **12** and **1** (100% yield for **12** from **13**, 54% yield for **1** from **3**). **12** is formed from **13** by intramolecular addition of the C2' hydroxyl to C2 of an S-nitrosyl intermediate, and subsequent elimination of SNO⁻. Dry state glycosylation of **16** and a 1:1 mixture of **12** and **1** (C), in the presence of MgCl₂, leads to a mixture of **18** and **19** as described in our route development above, however, critically, 95% of **1** persists in this mixture. Subsequent photoreduction in the presence of ferrocyanide and bisulfite generates the expected mixture of purine nucleosides **7** (dA), **26**, **20** and **27** alongside **1** (C). Finally, a second exposure to nitrous acid converts this mixture into the components of a competent genetic system, **7** (dA), **9** (dI) (10% and 9% yield respectively from **12** for 3 steps), **1** (C) and **2** (U) (84% combined persistence after 3 steps) with no significant nucleoside impurities. Products derived from **19** – with the wrong *N*⁷ regiochemistry – are hydrolysed in the last step. It is noteworthy that this route is only viable from a systems level approach – for instance, the pyrimidines are fairly rapidly destroyed in the photoreduction step

304 in the absence of the thioanhydropurines (Extended Data Fig. 7). Route B presents an
305 alternative in which initial hydrolysis of the mixture of **13** and **3** generates glycosyl
306 donor **15** (26% yield) alongside pyrimidine nucleosides (4% of **1** (C), 2% of **2** (U),
307 92% **3** remaining). **3** has previously been shown to hydrolyse to **1** (C) and **2** (U) in
308 greater yields (44%) over longer periods⁷. A representative mixture of **15**, **1** (C) and **2**
309 (U) (2:1:1) was then subjected to tethered glycosylation, resulting in **18** and **19** as
310 above (30% and 50% yield respectively) with 80% and 95% persistence of **1** (C) and
311 **2** (U). Photoreduction of the mixture, this time with hydrogen sulfide, provides purine
312 products **7**, **26**, **20** and **27** alongside the pyrimidines **1** (C) and **2** (U). Finally,
313 nitrosation furnished the key mixture of **7** (dA) and **9** (dI) (6% for each from **15** for
314 three steps) alongside pyrimidine nucleosides (43% persistence over 3 steps, final
315 ratio of dA:dI:C:U in the mixture is 14:13:45:28, Extended Data Fig. 8). Thus,
316 sequences comprised of various orders of operations and various photoreduction
317 conditions, which might plausibly emulate a terrestrial geochemical scenario, generate
318 the components of a mixed genetic system alongside one another. The exact ratio of **1**
319 (C) and **2** (U) (ribosylpyrimidines) to **7** (dA) and **9** (dI) (deoxyribosylpurines) in the
320 final mixture will depend on the ratio of α -(anhydro)pyrimidines (**13**, **12**, and **15**) to
321 β -(thio)pyrimidines (**1**, **2** and **3**) earlier in the sequence, which will vary based on
322 environmental conditions.

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In conclusion, a highly efficient synthesis of both deoxyadenosine 7 (dA) and deoxyinosine 9 (dI), requiring only prebiotically plausible reagents and conditions, is reported. In contrast to all previous attempts to synthesize purine nucleosides, our synthesis is both prebiotically plausible and strictly stereo-, regio-, and furanosyl-selective for the only isomer of the deoxypurine nucleosides used in modern biology. The pathway proceeds mostly via simple hydrolysis or dry state processes, with a key reduction step promoted by UV irradiation supported by distinct mechanisms. The (photo)chemical selection exhibited by this route hints at an explanation for Nature's

333 choice of one isomer of nucleic acid from the many that are conceivable. Critically,
334 we have demonstrated that sequences leading selectively to both RNA pyrimidine and
335 DNA purine nucleosides can occur together simultaneously, providing mixtures
336 which could conceivably complete a genetic alphabet. The fact that DNA building
337 blocks can be co-produced with the RNA pyrimidine nucleosides is consistent with
338 and perhaps evidence for the coexistence of RNA and DNA building blocks at the
339 dawn of life.

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343 **Data and materials availability:** Supplementary Information is available containing
344 all procedures, characterization data, NMR spectra, HPLC traces, X-Ray data and
345 CCDC numbers, and theoretical methods and data. Any additional data are available
346 from the corresponding author upon reasonable request.

347

348 **Code availability:** All custom code used to generate the data in this study is available
349 upon reasonable request.

350

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480

481 **Fig. 1. Previous synthesis of RNA pyrimidine nucleosides 1 (C), 2 (U) and a**
482 **deoxypyrimidine nucleoside 5, and the present work.** RAO **10** is a starting point in
483 the network since it crystallises in enantiopure form from minimally enantio-enriched
484 solutions. It can be elaborated via **12** and **3** to the pyrimidine nucleosides. Although
485 we had developed a low-yielding route to deoxyadenosine **7** (dA) from **6** via **5**, we
486 recognized that **12** and **15** are ideal candidates for tethered glycosylation with **16**. The
487 products, thioanhydropurines **18** and **19**, are reduced photochemically in a similar
488 way to **6**, providing an efficient route to deoxynucleosides. Critically, once produced,
489 pyrimidines **1** (C) and **2** (U) survive the sequence that produces purines **7** (dA) and **9**
490 (dI), and we show that the four nucleosides **1** (C), **2** (U), **7** (dA) and **9** (dI) can be
491 produced alongside one another.

492

493 **Fig. 2. Prebiotic route to purine deoxyribonucleosides, 7 (dA) and 9 (dI).** The
494 route starts with α -anhydropyrimidines **12** and **15**, which are intermediates in the

RNA pyrimidine synthesis, and 8-mercaptoadenine **16**, which is available from adenine **8** via hydrolysis and reaction with ammonium thiocyanate or thiourea. Dry state tethered glycosylation of **16** and **12** or **15** provides thioanhydropurines **18** and **19**, which can be photochemically reduced by two routes. If bisulfite is the reductant, only N^9 -configured products **7** (dA) and **26** are formed. **26** can be converted to **7** by further irradiation, or by nitrosation. If hydrosulfide is used as the reductant, both N^9 -configured **7** (dA) and **26** as well as N^7 -configured **20** is formed. **20** has a half-time of hydrolysis nearly two orders of magnitude lower than **7** (dA) and so is selectively degraded. To generate deoxyinosine **9** (dI) alongside deoxyadenosine **7** (dA), the products of either photoreduction are treated with nitrous acid at pH 4.

Fig. 3 Proposed mechanism of photoreduction of N^7 -8,2'-anhydro-thioadenosine **18 and N^9 -8,2'-anhydro-thioadenosine **19** nucleosides.** a) Potential mechanism involving bisulfite proceeding with initial photoexcitation of the thioanhydronucleosides to **28**, followed by reduction of C2', sulfur, and C8. Photoexcitation of the N^7 isomer **19** to **30** leads to decomposition. b) Potential mechanism involving bisulfite proceeding via initial reduction of ground state thioanhydronucleosides, followed by desulfurisation of **26**. Reduction of **19** gives **32** which leads to decomposition. c) Distinct mechanism involving reduction of thioanhydronucleoside-hydrosulfide encounter complexes, **33** and **34**, which both undergo charge transfer and concomitant C–S bond cleavage to produce **31** and **35**. **31** and **35** undergo reduction at C2' and desulfurisation to furnish **7** (dA) and **20**.

Fig. 4. A systems-level approach to a potential primordial genetic alphabet composed of **1 (C), **2** (U), **7** (dA) and **9** (dI).** A mixture of the α - and β -epimers of

2-thiocytidine **13** and **3**, which interconvert in UV light, can generate a mixture containing **1** (C), **2** (U), **7** (dA) and **9** (dI). A general route is shown at left. The thiopyrimidines are initially converted into the canonical pyrimidines (cytidine **1** and uridine **2**) and the α -anhydropyrimidines **12** and **15**. The latter undergo tethered glycosylation and then photoreduction to selectively provide purine deoxyribonucleosides **7** (dA) and **9** (dI) as depicted in Fig. 2. The pyrimidines **1** (C) and **2** (U) persist through each step of this sequence, ultimately generating a mixture of all four nucleosides. Specific conditions and yields for two possible particular routes (Routes A and B) are shown at right.

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544 **Correspondence and requests for materials** should be addressed to J. D. S.

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548

549 **Extended Data Fig. 1. A summary of the main findings of the work.** Previously, a
550 prebiotically plausible synthesis of beta-riboypyrimidines C and U has been identified
551 using α -thiocytidine. Herein, we demonstrate that the same intermediate can undergo
552 a distinct prebiotically plausible process that could have happened in a similar, or the
553 same, environment. The new process furnishes β -*D*-*N*⁹-deoxyribopurine nucleosides,
554 dA and dI, alongside the pyrimidines. Remarkable selectivity enforced by UV
555 irradiation and hydrolysis operates throughout the reported ribosylpyrimidine
556 synthesis and the newly discovered deoxyribosylpurine synthesis, resulting in a set of
557 nucleosides with only the canonical regio- and stereochemistry. The coexistence in
558 one location of a set of nucleosides similar to this is thought by many to be a
559 precondition for the spontaneous emergence of life on Earth.

560

561
562 **Extended Data Fig. 2. ¹H NMR spectra of conversion of α -anhydrouridine **15****
563 **from α -thiouridine **14**.** a) ¹H NMR spectrum of α -anhydrouridine **15**; b) ¹H NMR
564 spectrum of the reaction mixture after heating α -thiouridine **14** in H₂O; c) ¹H NMR
565 spectrum of the reaction mixture after heating α -thiouridine **14** in formamide.

566

567 **Extended Data Fig. 3. ¹H NMR spectra of photoreduction of *N*⁷-8,2'-anhydro-**
568 **thioadenosine **18** and *N*⁹-8,2'-anhydro-thioadenosine **19** mixture with bisulfite. a)**

¹H NMR spectrum of the crude mixture before irradiation (the ratio of N^7 : N^9 isomer was 4 : 5); b) ¹H NMR spectrum of the mixture after irradiation for 7 hrs (the N^9 isomers dA **7** and **26** are the only detectable products).

Extended Data Fig. 4. Potential energy surfaces and S_1/S_0 state crossings of the key photochemical steps in deoxyadenosine synthesis calculated at the ADC(2)/ma-def2-TZVP level (see the SI for more details). a) C-S bond opening may spontaneously occur in **18** leading to a peaked S_1/S_0 state crossing, however, a reducing agent is necessary to maintain that geometry after reaching the S_0 state; b) N7-C8 bond rupture is the lowest energy photochemical process in **19** and results in destruction of the purine ring; c) and d) encounter complexes of **18** and **19** with HS^- , which readily undergo photochemical C-S bond rupture induced by charge transfer from HS^- to chromophore.

Extended Data Fig. 5. Equilibrium geometries of C2, S8 radical anion **31 and C8, N9 radical anion **32** radical anions which may be formed after accepting a hydrated electron from the environment and the adiabatic electron affinities calculated at the ω B97X-D/IEFPCM/ma-def2-TZVP.**

Extended Data Fig. 6. ¹H NMR spectra for the reactions of deoxyadenosine **7 and cytidine **1** with nitrous acid.** a) ¹H NMR spectrum of the mixture of deoxyadenosine **7** and cytidine **1**; b) ¹H NMR spectrum of the reaction mixture after 4 days, showing the ratio of all four (deoxy)nucleosides deoxyadenosine **7**, deoxyinosine **9**, cytidine **1**, and uridine **2** is 30:17:42:11.

595 **Extended Data Fig. 7. ^1H NMR spectra for stability study of cytidine 1 and**
596 **uridine 2 at 254 nm irradiation with bisulfite.** a) ^1H NMR spectrum of the mixture
597 of cytidine 1, bisulfite and $\text{K}_4\text{Fe}(\text{CN})_6$ in the dark; b) as a), ^1H NMR spectrum after 10
598 hours of irradiation; c) ^1H NMR spectrum of the mixture of uridine 2, bisulfite and
599 $\text{K}_4\text{Fe}(\text{CN})_6$ in the dark; d) as c), ^1H NMR spectrum after 10 hours of irradiation; e) ^1H
600 NMR spectrum of the mixture of cytidine 1, uridine 2, N^9 -thioanhydroadenosine 18,
601 bisulfite and $\text{K}_4\text{Fe}(\text{CN})_6$ in the dark; f) as e), ^1H NMR spectrum after 10 hours of
602 irradiation.

603
604 **Extended Data Fig. 8. ^1H NMR spectra for sequential reactions with the mixture**
605 **of α -anhydrouridine 15, cytidine 1 and uridine 2.** a) ^1H NMR spectrum of the
606 mixture after heating with 8-mercaptopadenine 16 and magnesium chloride at 150 °C
607 for 1.5 days; b) ^1H NMR spectrum of the same mixture after irradiation with
608 hydrogen sulfide at 254 nm; c) ^1H NMR spectrum of the same mixture after reacting
609 with nitrous acid for 2 days (dA 7:dl 9:C 1:U 2= 14:14:44:28).

610

Previously: pyrimidine ribonucleosides

This work: purine deoxynucleosides

a) Photocleavage first

b) Reduction first

c)

General Route

Pyrimidine anhydronucleosides
(glycosyl donors) and nucleosides

Thioanhydropurine and pyrimidine nucleosides

Purine deoxyribonucleosides and
pyrimidine ribonucleosides

Conditions and Yields

13 + 3 (1:1 mixture)
(see Fig. 1)

Route A

NaNO₂
(pH 4)

From 13: 12 (100%)
From 3: C 1 (54%)

Representative mixture
12 and 1 (1:1)

MgCl₂, 16,
150 °C

From 12: 18 (26%)
and 19 (34%)
95% C 1 remaining

HSO₃⁻
Fe(CN)₆²⁻
h

From 18: dA 7 and 26
From 19: 20 and 27
C 1 persists
(yields below)

NaNO₂, pH 4

Purine deoxyribonucleosides (19%)
dA 7 (10%) and dI 9 (9%)
(yields for 3 steps from 12)

Pyrimidine ribonucleosides
84% persistence after 3 steps

dA:dI:C:U = 10:9:67:14

Route B

H₂O
(pH 5)

From 13: 15 (26%)
From 3: C 1 (4%)
and U 2 (2%)
(87% 3 remains after 24 d)
(Ref 7: 1 + 2 44% in 24 d)

Representative mixture
15, 1 and 2 (2:1:1)

MgCl₂, 16,
150 °C

From 15: 18 (30%) and
19 (50%)
80% C 1 remaining
95% U 2 remaining

HS⁻
h

From 18: dA 7 and 26
From 19: 20 and 27
C 1 and U 2 persist
(yields below)

Purine deoxyribonucleosides (12%)
dA 7 (6%) and dI 9 (6%)
(yields for 3 steps from 15)

Pyrimidine ribonucleosides
43% persistence after 3 steps

dA:dI:C:U = 14:14:44:28