



# THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

### Selective prebiotic formation of RNA pyrimidine and DNA purine nucleosides

**Citation for published version:**

Xu, J, Chmela, V, Green, NJ, Russell, DA, Janicki, MJ, Góra, RW, Szabla, R, Bond, AD & Sutherland, JD 2020, 'Selective prebiotic formation of RNA pyrimidine and DNA purine nucleosides', *Nature*, vol. 582, pp. 60-66. <https://doi.org/10.1038/s41586-020-2330-9>

**Digital Object Identifier (DOI):**

[10.1038/s41586-020-2330-9](https://doi.org/10.1038/s41586-020-2330-9)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Nature

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



1       **Selective prebiotic formation of RNA pyrimidine and DNA purine**  
2   **nucleosides**

3       Jianfeng Xu<sup>1</sup>†, Václav Chmela<sup>1</sup>†, Nicholas J. Green<sup>1</sup>, David A. Russell<sup>1</sup>, Mikołaj J.  
4           Janicki<sup>2</sup>, Robert W. Góra<sup>2</sup>, Rafał Szabla<sup>3,4</sup>, Andrew D. Bond<sup>5</sup> and John D.  
5   Sutherland<sup>1\*</sup>

6       <sup>1</sup>MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge  
7       Biomedical Campus, Cambridge, CB2 0QH, UK.

8       <sup>2</sup>Department of Physical and Quantum Chemistry, Wrocław University of Science  
9       and Technology, Faculty of Chemistry, Wybrzeże Wyspiańskiego 27, 50-370,  
10      Wrocław, Poland.

11      <sup>3</sup>EaStCHEM, School of Chemistry, University of Edinburgh, Joseph Black Building,  
12      David Brewster Road, Edinburgh, EH9 3FJ, UK.

13      <sup>4</sup>Institute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, PL-02668  
14      Warsaw, Poland.

15      <sup>5</sup>Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW, UK.

16      \*Correspondence to: johns@mrc-lmb.cam.ac.uk

17      †These authors contributed equally to this work.

18      **The nature of the first genetic polymer is the subject of major debate in the**  
19      **origin of life field<sup>1</sup>. Although the common ‘RNA world’ theory suggests RNA as**  
20      **the first replicable information carrier at the dawn of life, other evidence implies**  
21      **that life may have started with a heterogeneous nucleic acid genetic system**  
22      **including both RNA and DNA<sup>2</sup>. Such a theory streamlines the eventual ‘genetic**  
23      **takeover’ of homogeneous DNA from RNA as the principal information storage**  
24      **molecule in the central dogma, but requires a selective abiotic synthesis of both**  
25      **RNA and DNA building blocks in the same local primordial geochemical**  
26      **scenario. Herein, we demonstrate a high-yielding, completely stereo-, regio-, and**  
27      **furanosyl-selective prebiotic synthesis of the purine deoxyribonucleosides,**

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 life<sup>3</sup>.**

### 35 **Introduction**

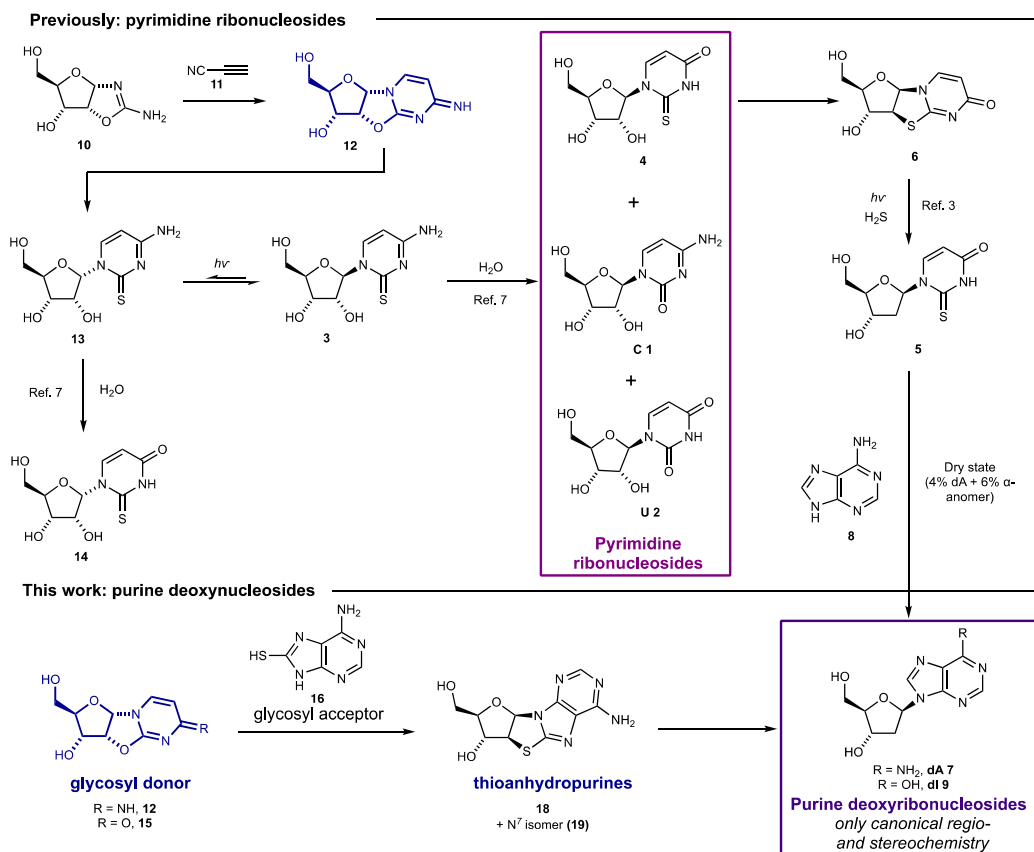
36         The advent of life requires informational inheritance mediated by a suitable  
37 polymer that can undergo replication in the absence of enzymes. The RNA world  
38 hypothesis invokes RNA as this polymer<sup>4, 5</sup>. Considerable progress in the prebiotic  
39 synthesis of the pyrimidine ribonucleosides of RNA, cytidine (C) **1** and uridine (U) **2**,  
40 and their 2-thio derivatives, **3** and **4**<sup>6, 7</sup>, together with recent advances in non-  
41 enzymatic RNA replication<sup>8-10</sup> 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 sugars<sup>11-15</sup>, which are not likely to be 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 DNA<sup>16</sup>. The superior

53 hydrolytic stability and replication fidelity<sup>17</sup> 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 debated<sup>1</sup>. 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 biosynthesis<sup>18-20</sup>. 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 counterparts<sup>2</sup>. 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 photoreduction<sup>3</sup>. 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<sup>21,22</sup>. 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  $\alpha$ -anomer of **7** (6%). Using guidance from a geochemical  
73 scenario<sup>23</sup>, 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**).

79



80

81

## 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<sup>6, 7</sup>

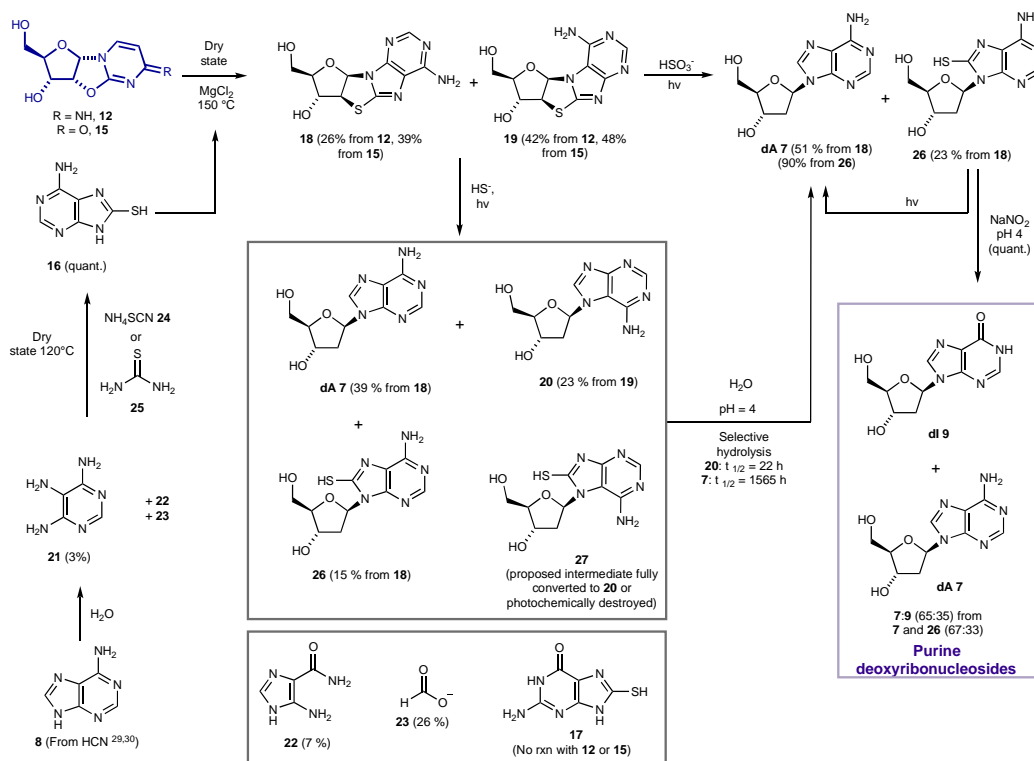
90 as ribosyl donors (Fig. 1). The RNA synthesis proceeds from RAO **10** which reacts  
91 with cyanoacetylene **11** to provide  $\alpha$ -anhydrocytidine **12**. Thiolysis of **12** in  
92 formamide produces  $\alpha$ -2-thiocytidine **13** which undergoes efficient UV-mediated  
93 photoanomerisation 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  $\alpha$ -2-thiouridine **14**<sup>7</sup>.  
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  $\alpha$ -  
99 anhydropyrimidines **12** and **15** as ideal glycosyl donors for 1',2'-cis tethered  
100 glycosylation<sup>24</sup>. 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  $\alpha$ -stereochemistries of C1' and C2'  
103 of **12** and **15** led us to expect transglycosylation to provide only  $\beta$ -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<sup>25, 26</sup>, this route offered the so-far unmet potential to deliver enantio- and  
108 diastereomerically pure furanosyl-nucleosides by glycosylation.

109

110         Accordingly, we evaluated 8-mercaptoadenine **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  $\beta$ -configured nucleoside products in moderate yields  
114 (14% and 16% respectively from **15**, trace amounts from **12**). The minor product was

115 determined to be *N*<sup>9</sup>-8, 2'-anhydro-thioadenosine **18** by X-ray crystallography and <sup>1</sup>H-  
 116 NMR spiking experiments with a synthetic standard. The major product was inferred  
 117 to be *N*<sup>7</sup>-8,2'-anhydro-thioadenosine **19**, the regioisomer of **18**, by its subsequent  
 118 conversion to 2'-deoxy-*N*<sup>7</sup>-adenosine **20**. The presence of magnesium chloride in the  
 119 reaction, presumably acting as a Lewis acid<sup>27</sup>, dramatically improved the yield of **18**  
 120 and **19** to 39% and 48% respectively from **15** (combined yield 87%) and 26% and  
 121 42% respectively from **12** (combined yield 68%). Thus, in a prebiotic environment  
 122 where **12** or **15** and **16** are brought together, perhaps by converging streams that then  
 123 undergo evaporation, **18** and **19** could be readily generated, especially in the presence  
 124 of magnesium ions<sup>28</sup>.

125



128 Any prebiotic synthesis requires a viable route to all reagents from plausible early-  
 129 Earth feedstocks. We were drawn towards adenine **8** as a starting point for the

130 provision of 8-mercaptoadenine **16**, due to its widely accepted prebiotic plausibility as  
131 a relatively stable pentamer of hydrogen cyanide<sup>29, 30</sup>. Remarkably, despite the  
132 reactivity of related purines<sup>31</sup>, adenine did not react with elemental sulfur at  
133 temperatures up to 300 °C. However, adenine does undergo (slow) hydrolysis in  
134 aqueous media. Miller et. al. reported a half-time for hydrolysis of adenine of about 1  
135 year at 100 °C, and identified (but did not quantify) 4,5,6-triaminopyrimidine **21**  
136 (TAP) among the products of hydrolysis<sup>32</sup>. We reinvestigated this hydrolysis of  
137 adenine **8**, under conditions more suited to a laboratory time-scale (138 °C, phosphate  
138 buffer pH 8), and at partial conversion after 10–12 days confirmed the presence of  
139 TAP in yields of 2–3% (8–9% based on recovered adenine) (Fig. 2). Due to the  
140 differential solubilities of adenine and TAP, the supernatants of adenine hydrolysis  
141 reactions are enriched in TAP after cooling. A typical supernatant contains 5-  
142 aminoimidazole-4-carboxamide **22**, TAP **21**, and adenine **8** in a 4:2:1 ratio, and  
143 formate **23** as the only other major component (See Fig. S1–S5 for full details). We  
144 found that TAP (either commercially supplied or that in the crude adenine  
145 hydrolysate) is converted to 8-mercaptoadenine **16** by heating in the dry state with  
146 either ammonium thiocyanate **24** or thiourea **25**. **24** is an inevitable by-product of the  
147 photochemistry of hydrogen cyanide and hydrogen sulfide<sup>33</sup>, two precursors likely to  
148 have been abundant on the primordial earth, and heavily implicated in the origin of  
149 life by our cyanosulfidic chemical network<sup>23</sup>. Thiourea **25** has also been widely  
150 invoked as a prebiotically plausible reagent<sup>34</sup>. Thus, we envision that a primordial  
151 environment supplied with adenine and water would continuously generate TAP,  
152 which can be enriched in aqueous solution by moving down a thermal gradient.  
153 Ammonium thiocyanate **24** can be mixed with the TAP at any stage, and eventual  
154 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 syntheses<sup>12</sup>.

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<sup>3,33</sup>. **18**  
162 and **19** were separately subjected to UV irradiation at 254 nm in water with hydrogen  
163 sulfide (H<sub>2</sub>S) as the reductant (Fig. 2). In the photoreduction of **18**, the natural regio-  
164 isomer *N*<sup>9</sup>-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 irradiation<sup>35</sup>, or treatment  
167 with nitrous acid, which is produced from common atmospheric gases, nitrogen and  
168 carbon dioxide<sup>36</sup>. Nucleobase loss was also apparent (8-mercaptoadenine **16** in 10%  
169 yield and adenine **8** in 17% yield). The same reaction starting with **19** gave *N*<sup>7</sup>-  
170 deoxyadenosine **20** in 23% yield with no other nucleoside products. Our proposed  
171 intermediate in this process, 8-mercapto-*N*<sup>7</sup>-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*<sup>9</sup>:*N*<sup>7</sup> 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*<sup>9</sup>  
177 glycosidic linkage, compared to *N*<sup>7</sup> isomers. Replacing hydrosulfide as the electron  
178 donor with bisulfite (HSO<sub>3</sub><sup>-</sup>, pH 7)<sup>37</sup>, which is readily formed by the dissolution of  
179 atmospheric SO<sub>2</sub> in water<sup>38</sup>, 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<sup>39, 40</sup>. 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<sup>41, 42</sup>.

#### 195 Photoreduction Mechanism

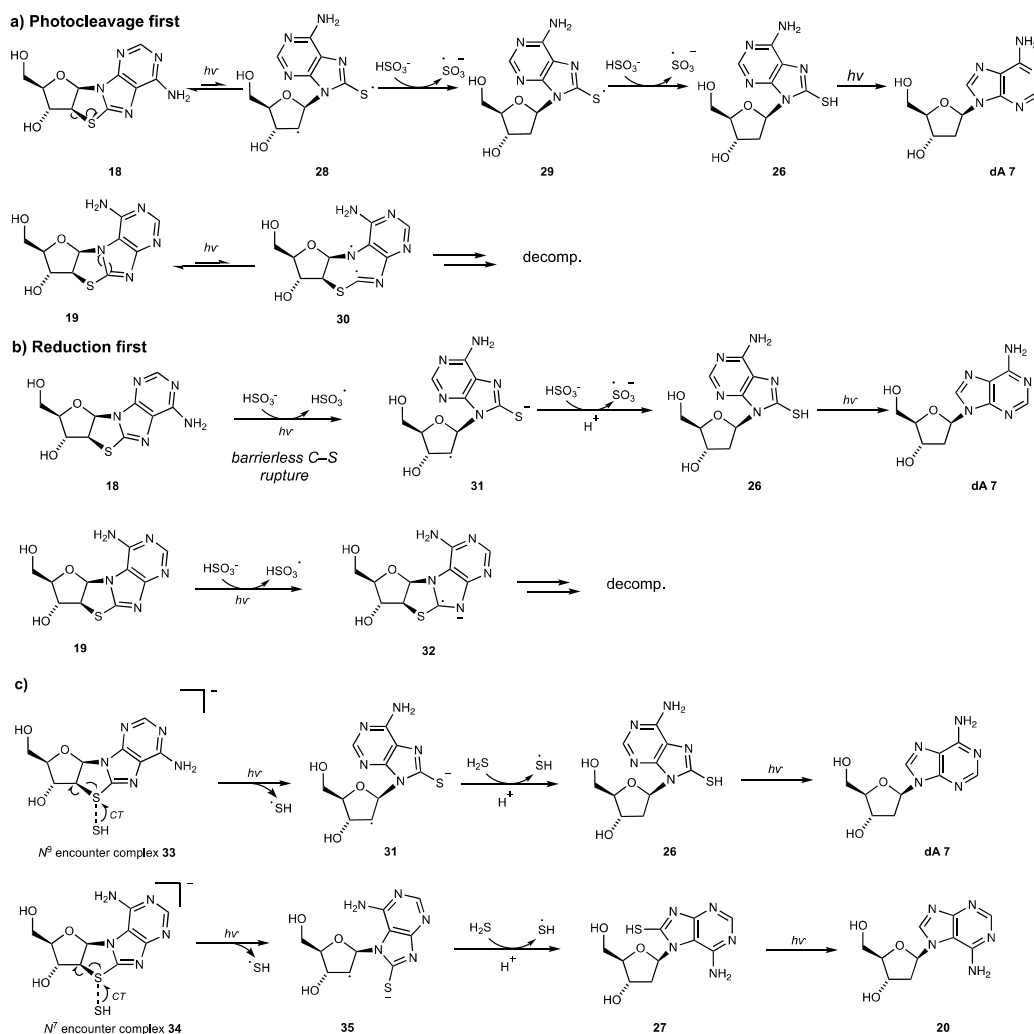
196 To provide mechanistic rationale for the observed photochemical selectivity,  
197 we performed quantum chemical calculations using density functional theory and  
198 algebraic diagrammatic construction to the second order [ADC(2)] methods<sup>43, 44</sup>.  
199 These calculations revealed, in 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**<sup>35</sup>, deoxyadenosine **7** (dA) (Fig. 3a, *N*<sup>9</sup>). In contrast, photoexcitation of **19**  
208 leads to N7–C8 bond rupture through the S<sub>1</sub>/S<sub>0</sub> state crossing (Fig. 3a, *N*<sup>7</sup>; 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 irradiation<sup>45</sup>, 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*<sup>9</sup>; 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*<sup>7</sup>,  
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<sup>-</sup> 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 bonds<sup>46</sup>. 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.*<sup>35</sup>  
236 (Fig. 3c). Thus, a HS<sup>-</sup> 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.

242



243

244

## 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 group<sup>47</sup> 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

254 (deoxy)inosine **9** (dI) (Fig. 2) by deaminative hydrolysis, which spontaneously occurs  
255 very slowly in nucleic acid polymers<sup>48</sup>, and is greatly accelerated by the presence of  
256 nitrous acid<sup>49</sup>. To demonstrate that this conversion can occur under mild conditions  
257 consistent with our primordial geochemical scenario<sup>50</sup>, we treated deoxyadenosine **7**  
258 (dA) with nitrous acid at pH 4 (the same conditions by which we could effect  
259 desulfurization of **26**). After four days at room temperature, approximately 40% of **7**  
260 (dA) had been converted to **9** (dI), providing a 60:40 mixture of **7** (dA) and **9** (dI) (Fig  
261 2). A control experiment monitoring the decomposition of deoxyadenosine **7** (dA) at  
262 pH 4, without nitrous acid, showed only a trace of depurination ( $t_{1/2} = 1600$  h). When  
263 a 67:33 mixture of **7** and **26**, representative of the outcome of photoreduction, was  
264 submitted to the reaction conditions, **26** underwent relatively rapid desulfurization  
265 first, with deoxyadenosine **7** (dA) undergoing slower deaminative hydrolysis to  
266 ultimately provide a 65:35 mixture of **7** (dA) and **9** (dI). Thus, mixtures of  
267 deoxyadenosine **7** (dA) and deoxyinosine **7** (dA) are readily obtainable from partial  
268 deaminative hydrolysis of deoxyadenosine **7** (dA) or its precursor **26**, thereby  
269 supplying half of a potential primordial alphabet. Despite the potential for a mismatch  
270 in reactivity between deoxypurines and pyrimidines, a 47:53 mixture of  
271 deoxyadenosine **7** (dA) and cytidine **1** (C) underwent nitrous acid-promoted  
272 deamination to provide all four (deoxy)nucleosides deoxyadenosine **7** (dA),  
273 deoxyinosine **9** (dI), cytidine **1** (C), and uridine **2** (U) (30:17:42:11 ratio) (Extended  
274 Data Fig. 6). A similar primordial mixture may have been a starting point for genetic  
275 information storage. Furthermore, in the absence of significant geochemically  
276 plausible sources of pyrimidine deoxynucleotides and purine ribonucleotides,  
277 heteropolymers made from a mixture of purine deoxyribonucleotides and pyrimidine  
278 ribonucleotides should possess heritable backbone heterogeneity and thus a 1:1

279 phenotype to genotype correspondence, which is potentially advantageous in the  
280 evolution of catalytic activity<sup>18</sup>.

281

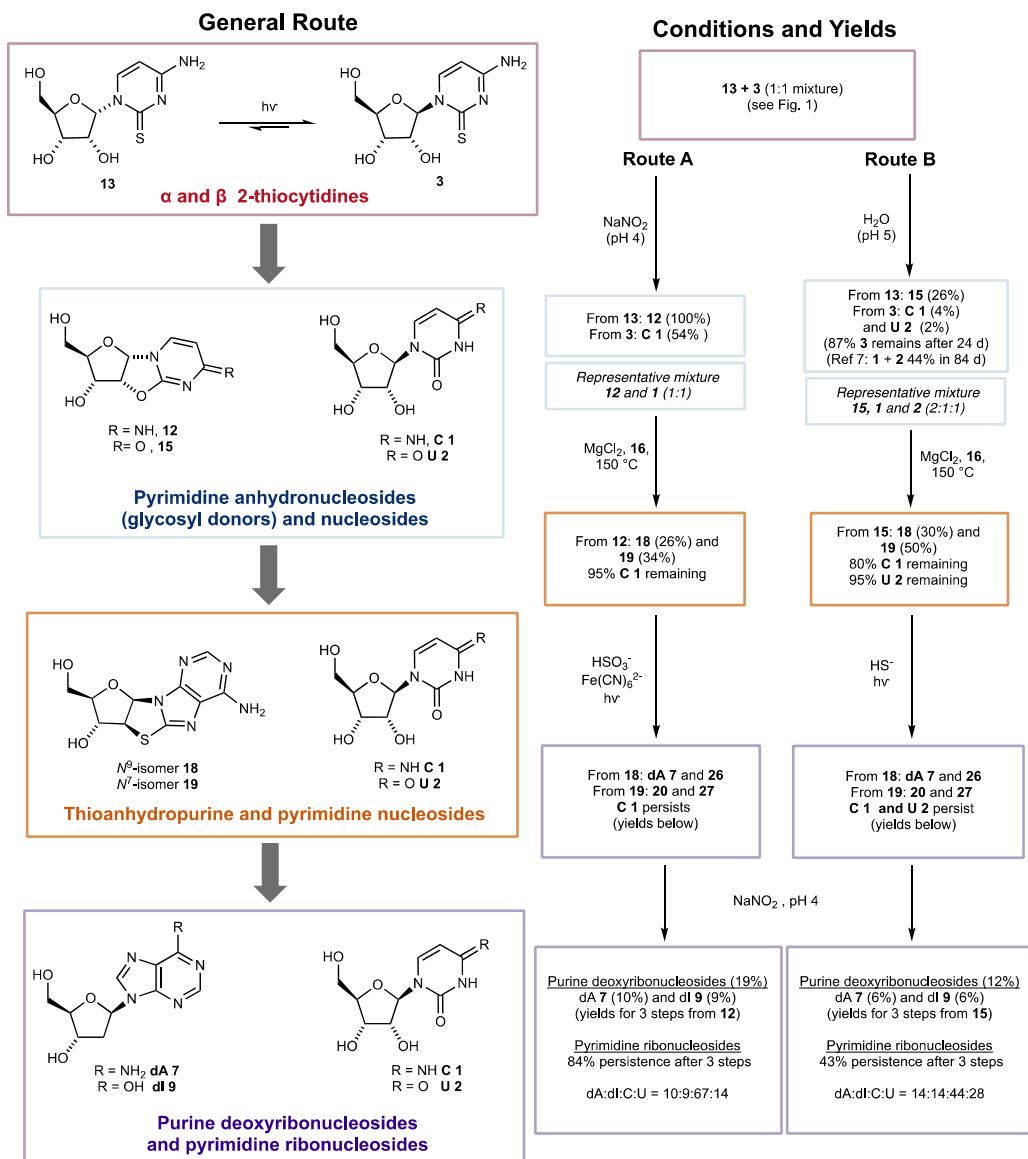
## 282 Systems Level Prebiotic Plausibility

283 Having demonstrated the potential of a divergent route to yield a local mixture of **7**  
284 (**dA**), **9** (**dI**), **1** (**C**) and **2** (**U**), we sought to evaluate the key question of whether all  
285 four nucleosides could persist after divergence in the sequence. We chose a 1:1  
286 mixture of  $\alpha$ - and  $\beta$ -2-thiouridines **13** and **3** as our starting point, which could be  
287 obtained from the partial photoanomerisation of **13**, and evaluated two particular  
288 combinations of reactions as representative permutations of a primordial geochemical  
289 process (Fig. 4, Route A and B). In route A, exposure of the mixture to nitrous acid  
290 (pH 4) generates a mixture of **12** and **1** (100% yield for **12** from **13**, 54% yield for **1**  
291 from **3**). **12** is formed from **13** by intramolecular addition of the C2' hydroxyl to C2 of  
292 an S-nitrosyl intermediate, and subsequent elimination of SNO<sup>-</sup>. Dry state  
293 glycosylation of **16** and a 1:1 mixture of **12** and **1** (**C**), in the presence of MgCl<sub>2</sub>, leads  
294 to a mixture of **18** and **19** as described in our route development above, however,  
295 critically, 95% of **1** persists in this mixture. Subsequent photoreduction in the  
296 presence of ferrocyanide and bisulfite generates the expected mixture of purine  
297 nucleosides **7** (**dA**), **26**, **20** and **27** alongside **1** (**C**). Finally, a second exposure to  
298 nitrous acid converts this mixture into the components of a competent genetic system,  
299 **7** (**dA**), **9** (**dI**) (10% and 9% yield respectively from **12** for 3 steps), **1** (**C**) and **2** (**U**)  
300 (84% combined persistence after 3 steps) with no significant nucleoside impurities.  
301 Products derived from **19** – with the wrong *N*<sup>7</sup> regiochemistry – are hydrolysed in the  
302 last step. It is noteworthy that this route is only viable from a systems level approach  
303 – 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 periods<sup>7</sup>. 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  $\alpha$ -(anhydro)pyrimidines (**13**, **12**, and **15**) to  
321  $\beta$ -(thio)pyrimidines (**1**, **2** and **3**) earlier in the sequence, which will vary based on  
322 environmental conditions.

323





324

325

326

327

328

329

330

331

332

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.

340

341

342

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

351 **References:**

352 1. Samanta, B & Joyce, G. F. A reverse transcriptase ribozyme. *Elife* **6**, e31153  
353 (2017).

- 354 2. Bhowmik, S. & Krishnamurthy, R. The role of sugar-backbone heterogeneity and  
355 chimeras in the simultaneous emergence of RNA and DNA. *Nat. Chem.* **11**, 1009-  
356 1018 (2019).
- 357 3. Xu, J., Green, N. J., Gibard, C., Krishnamurthy, R. & Sutherland, J. D. Prebiotic  
358 phosphorylation of 2-thiouridine provides either nucleotides or DNA building  
359 blocks via photoreduction. *Nat. Chem.* **11**, 457-462 (2019).
- 360 4. Gilbert, W. Origin of life: The RNA world. *Nature* **319**, 618 (1986).
- 361 5. Joyce, G. F. The antiquity of RNA-based evolution. *Nature* **418**, 214-221 (2002).
- 362 6. Powner, M. W., Gerland, B. & Sutherland, J. D. Synthesis of activated pyrimidine  
363 ribonucleotides in prebiotically plausible conditions. *Nature* **459**, 239-242 (2009).
- 364 7. Xu, J. *et al.* A prebiotically plausible synthesis of pyrimidine beta-ribonucleosides  
365 and their phosphate derivatives involving photoanomerization. *Nat. Chem.* **9**, 303-  
366 309 (2017).
- 367 8. Heuberger, B. D., Pal, A., Del Frate, F., Topkar, V. V. & Szostak, J. W. Replacing  
368 uridine with 2-thiouridine enhances the rate and fidelity of nonenzymatic RNA  
369 primer extension. *J. Am. Chem. Soc.* **137**, 2769-2775 (2015).
- 370 9. Walton, T. & Szostak, J. W. A highly reactive imidazolium-bridged dinucleotide  
371 intermediate in nonenzymatic RNA primer extension. *J. Am. Chem. Soc.* **138**,  
372 11996-12002 (2015).
- 373 10. Li, L. *et al.* Enhanced nonenzymatic RNA copying with 2-aminoimidazole  
374 activated nucleotides. *J. Am. Chem. Soc.* **139**, 1810-1813 (2017).
- 375 11. Fuller, W. D., Orgel, L. E. & Sanchez, R. A. Studies in Prebiotic Synthesis: VI.  
376 Solid-State Synthesis of Purine Nucleosides. *J. Mol. Evol.* **1**, 249-257 (1972).

- 377 12. Becker S. et al. A high-yielding, strictly regioselective prebiotic purine nucleoside  
378 formation pathway. *Science* **352**, 833-836 (2016).
- 379 13. Kim, H. & Benner, S. A. Prebiotic stereoselective synthesis of purine and  
380 noncanonical pyrimidine nucleotides from nucleobases and phosphorylated  
381 carbohydrates. *Proc. Nat. Acad. Sci. USA* **114**, 11315-11320 (2017).
- 382 14. Becker S. et al. Unified prebiotically plausible synthesis of pyrimidine and purine  
383 RNA ribonucleotides. *Science* **366**, 76-82 (2019).
- 384 15. Teichert, J. S., Kruse, F. M. & Trapp, O. Direct prebiotic pathway to DNA  
385 nucleosides. *Angew. Chem. Int. Ed.* **55**, 9944-9947 (2019).
- 386 16. Reichard, P. From RNA to DNA, why so many ribonucleotide reductases?  
387 *Science* **260**, 1773-1777 (1993).
- 388 17. Leu, K., Obermayer, B., Rajamani, S., Gerland, U. & Chen, I. A. The prebiotic  
389 evolutionary advantage of transferring genetic information from RNA to DNA.  
390 *Nucleic Acids Res.* **39**, 8135-8147 (2011).
- 391 18. Sutherland, J. D. & Whitfield, J. N. Prebiotic chemistry: a bioorganic perspective.  
392 *Tetrahedron* **53**, 11493-11527 (1997).
- 393 19. Trevino, S. G., Zhang, N., Elenko, M. P., Lupták, A. & Szostak, J. W. Evolution  
394 of functional nucleic acids in the presence of nonheritable backbone  
395 heterogeneity. *Proc. Nat. Acad. Sci. USA* **108**, 13492-13497 (2011).
- 396 20. Gavette, J. V., Stoop, M., Hud, N. V. & Krishnamurthy, R. RNA–DNA chimeras  
397 in the context of an RNA world transition to an RNA/DNA world. *Angew. Chem.*  
398 *Int. Ed.* **55**, 13204-13209 (2016).
- 399 21. Schoffstall, A. M. Prebiotic phosphorylation of nucleosides in formamide. *Orig.*  
400 *Life* **7**, 399-412 (1976).

- 401 22. Lohrmann, R. & Orgel, L. E. Urea-Inorganic Phosphate Mixtures as Prebiotic  
402 Phosphorylating Agents. *Science* **171**, 490-494 (1971).
- 403 23. Patel, B. H., Percivalle, C., Ritson, D. J., Duffy, C. D. & Sutherland, J. D.  
404 Common origins of RNA, protein and lipid precursors in a cyanosulfidic  
405 protometabolism. *Nat. Chem.* **7**, 301-307 (2015).
- 406 24. Ishiwata, A., Lee, Y. J. & Ito, Y. Recent advances in stereoselective glycosylation  
407 through intramolecular aglycon delivery. *Org. Biomol. Chem.* **8**, 3596-3608  
408 (2010).
- 409 25. Springsteen, G. & Joyce, G. F. Selective derivatization and sequestration of ribose  
410 from a prebiotic mix. *J. Am. Chem. Soc.* **126**, 9578-9583 (2004).
- 411 26. Anastasi, C., Crowe, M. A., Powner, M. W. & Sutherland, J. D. Direct Assembly  
412 of Nucleoside Precursors from Two- and Three-Carbon Units. *Angew. Chem. Int.*  
413 *Ed.* **45**, 6176-6179 (2006).
- 414 27. Vorbrüggen, H. & Ruh-Pohlenz, C. *Handbook of nucleoside synthesis*. (Wiley,  
415 2001).
- 416 28. Holm, N. G., Oze, C., Mousis, O., Waite, J. H. & Guilbert-Lepoutre, A.  
417 Serpentinization and the formation of H<sub>2</sub> and CH<sub>4</sub> on celestial bodies (planets,  
418 moons, comets). *Astrobiology* **15**, 587-600 (2015).
- 419 29. Sanchez, R. A., Ferris, J. P. & Orgel, L. E. Studies in prebiotic synthesis II:  
420 Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide.  
421 *J. Mol. Biol.* **80**, 223-253 (1967).
- 422 30. Hudson, J. S. *et al.* A unified mechanism for abiotic adenine and purine synthesis  
423 in formamide. *Angew. Chem. Int. Ed.* **51**, 5134-5137 (2012).

- 424 31. Giner-Sorolla, A., Thom, E. & Bendich, A. Studies on the Thiation of Purines. *J.*  
425 *Org. Chem.* **29**, 3209-3212 (1964).
- 426 32. Levy, M. & Miller, S. L. The stability of the RNA bases: implications for the  
427 origin of life. *Proc. Natl. Acad. Sci. USA* **95**, 7933-7938 (1998).
- 428 33. Ritson, D. J. & Sutherland, J. D. Synthesis of aldehydic ribonucleotide and amino  
429 acid precursors by photoredox chemistry. *Angew. Chem. Int. Ed.* **52**, 5845-5847  
430 (2013).
- 431 34. Robertson, M. P., Levy, M. & Miller, S. L. Prebiotic synthesis of  
432 diaminopyrimidine and thiocytosine. *J. Mol. Evol.* **43**, 543-550 (1996).
- 433 35. Roberts, S. J. *et al.* Selective prebiotic conversion of pyrimidine and purine  
434 anhydronucleosides into Watson-Crick base-pairing arabino-furanosyl nucleosides  
435 in water. *Nat. Commun.* **9**, 4073-4082 (2018).
- 436 36. Ranjan, S., Todd, Z. R., Rimmer, P. B., Sasselov, D. D. & Babbitt, A. R. Nitrogen  
437 oxide concentrations in natural waters on early Earth. *Geochem. Geophys. Geosy.*  
438 **20**, 2021-2039 (2019).
- 439 37. Xu, J. *et al.* Photochemical reductive homologation of hydrogen cyanide using  
440 sulfite and ferrocyanide. *Chem. Commun.* **54**, 5566-5569 (2018).
- 441 38. Marion, G. M., Kargel, J. S., Crowley, J. K. & Catling, D. C. Sulfite–sulfide–  
442 sulfate–carbonate equilibria with applications to Mars. *Icarus*, **225**, 342–351  
443 (2013).
- 444 39. Rios, A. C. & Tor, Y. On the origin of the canonical nucleobases: an assessment  
445 of selection pressures across chemical and early biological evolution. *Isr. J. Chem.*  
446 **53**, 469 – 483 (2013).

- 447 40. Rios, A. C., Yu, H. T. & Tor, Y. Hydrolytic fitness of *N*-glycosyl bonds:  
448 comparing the deglycosylation kinetics of modified, alternative, and native  
449 nucleosides. *J. Phys. Org. Chem.* **28**, 173-180 (2014).
- 450 41. Panzica, R. P., Rousseau, R. J., Robins, R. K., & Townsend, L. B. Relative  
451 stability and a quantitative approach to the reaction mechanism of the acid-  
452 catalyzed hydrolysis of certain 7- and 9- $\beta$ -D-ribofuranosylpurines. *J. Am. Chem.*  
453 *Soc.* **94**, 4708-4714 (1972).
- 454 42. Lindahl, T. & Nyberg, B. Rate of depurination of native deoxyribonucleic acid.  
455 *Biochemistry* **11**, 3610-3618 (1972).
- 456 43. Hättig, C. Structure Optimizations for Excited States with Correlated Second-  
457 Order Methods: CC2 and ADC(2). *Adv. Quantum Chem.* **50**, 37–60 (2005).
- 458 44. Dreuw, A. & Wormit, M. The algebraic diagrammatic construction scheme for the  
459 polarization propagator for the calculation of excited states. *Wiley Interdiscip. Rev.*  
460 *Comput. Mol. Sci.* **5**, 82–95 (2015).
- 461 45. Sauer, M. C., Crowell, R. A. & Shkrob, I. A. Electron Photodetachment from Aqueous  
462 Anions. 1. Quantum Yields for Generation of Hydrated Electron by 193 and 248 nm  
463 Laser Photoexcitation of Miscellaneous Inorganic Anions. *The Journal of Physical*  
464 *Chemistry A* **108**, 5490-5502 (2004).
- 465 46. Pascoe, D. J., Ling, K. B. & Cockroft, S. L. The origin of chalcogen-bonding interactions.  
466 *J. Am. Chem. Soc.* **139**, 15160-15167 (2017).
- 467 47. Kim, S. C., O'Flaherty, D. K., Zhou, L., Lelyveld, V. S. & Szostak, J. W. Inosine,  
468 but none of the 8-oxo-purines, is a plausible component of a primordial version of  
469 RNA. *Proc. Natl. Acad. Sci. USA* **115**, 13318-13323 (2018).

- 470 48. Karran, P. & Lindahl, T. Hypoxanthine in deoxyribonucleic acid: generation by  
471 heat-induced hydrolysis of adenine residues and release in free form by a  
472 deoxyribonucleic acid glycosylase from calf thymus. *Biochemistry* **19**, 6005-6011  
473 (1980).
- 474 49. Shapiro, R., & Pohl, S. H. Reaction of ribonucleosides with nitrous acid. Side  
475 products and kinetics. *Biochemistry* **7**, 448-455 (1968).
- 476 50. Mariani, A. D., Russell, A., Javelle, T. & Sutherland, J. D. A light-releasable  
477 potentially prebiotic nucleotide activating agent. *J. Am. Chem. Soc.* **140**,  
478 8657–8661 (2018).

479

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  $\alpha$ -anhydropyrimidines **12** and **15**, which are intermediates in the



495 RNA pyrimidine synthesis, and 8-mercaptoadenine **16**, which is available from  
496 adenine **8** via hydrolysis and reaction with ammonium thiocyanate or thiourea. Dry  
497 state tethered glycosylation of **16** and **12** or **15** provides thioanhydropurines **18** and  
498 **19**, which can be photochemically reduced by two routes. If bisulfite is the reductant,  
499 only  $N^9$ -configured products **7** (dA) and **26** are formed. **26** can be converted to **7** by  
500 further irradiation, or by nitrosation. If hydrosulfide is used as the reductant, both  $N^9$ -  
501 configured **7** (dA) and **26** as well as  $N^7$ -configured **20** is formed. **20** has a half-time of  
502 hydrolysis nearly two orders of magnitude lower than **7** (dA) and so is selectively  
503 degraded. To generate deoxyinosine **9** (dI) alongside deoxyadenosine **7** (dA), the  
504 products of either photoreduction are treated with nitrous acid at pH 4.

505

506 **Fig. 3 Proposed mechanism of photoreduction of  $N^7$ -8,2'-anhydro-thioadenosine**  
507  **$18$  and  $N^9$ -8,2'-anhydro-thioadenosine  $19$  nucleosides.** a) Potential mechanism  
508 involving bisulfite proceeding with initial photoexcitation of the  
509 thioanhydronucleosides to **28**, followed by reduction of C2', sulfur, and C8.  
510 Photoexcitation of the  $N^7$  isomer **19** to **30** leads to decomposition. b) Potential  
511 mechanism involving bisulfite proceeding via initial reduction of ground state  
512 thioanhydronucleosides, followed by desulfurisation of **26**. Reduction of **19** gives **32**  
513 which leads to decomposition. c) Distinct mechanism involving reduction of  
514 thioanhydronucleoside-hydrosulfide encounter complexes, **33** and **34**, which both  
515 undergo charge transfer and concomitant C-S bond cleavage to produce **31** and **35**. **31**  
516 and **35** undergo reduction at C2' and desulfurisation to furnish **7** (dA) and **20**.

517

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

520 2-thiocytidine **13** and **3**, which interconvert in UV light, can generate a mixture  
521 containing **1** (C), **2** (U), **7** (dA) and **9** (dI). A general route is shown at left. The  
522 thiopyrimidines are initially converted into the canonical pyrimidines (cytidine **1** and  
523 uridine **2**) and the  $\alpha$ -anhydropyrimidines **12** and **15**. The latter undergo tethered  
524 glycosylation and then photoreduction to selectively provide purine  
525 deoxyribonucleosides **7** (dA) and **9** (dI) as depicted in Fig. 2. The pyrimidines **1** (C)  
526 and **2** (U) persist through each step of this sequence, ultimately generating a mixture  
527 of all four nucleosides. Specific conditions and yields for two possible particular  
528 routes (Routes A and B) are shown at right.

529

530

531 **Acknowledgments:** The authors thank all JDS group members for fruitful  
532 discussions. This research was supported by the Medical Research Council  
533 (MC\_UP\_A024\_1009), the Simons Foundation (290362 to JDS, 494188 to RS), and a  
534 grant from the National Science Centre Poland (2016/23/B/ST4/01048 to RWG). MJJ  
535 acknowledges support within the “Diamond Grant” (0144/DIA/2017/46) from the  
536 Polish Ministry of Science and Higher Education and a computational grant from  
537 Wrocław Centre of Networking and Supercomputing (WCSS).

538

539 **Author contributions:** Experimental: J. X., V. C., N. J. G., D. A. R., A. D. B.;  
540 Theoretical: M. J. J., R. W. G., R. S.; Crystallography: A. D. B.; Supervision: J. D. S.;  
541 all authors co-wrote the manuscript.

542 **Competing interests:** The authors declare no competing financial interests.

543 **Supplementary Materials** is available for this paper.

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

545 **Reprints and permission information** is available at

546 <http://www.nature.com/reprints>.

547

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  $\alpha$ -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  $\beta$ -*D*-*N*<sup>9</sup>-deoxyribose 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. <sup>1</sup>H NMR spectra of conversion of  $\alpha$ -anhydrouridine 15**  
563 **from  $\alpha$ -thiouridine 14.** a) <sup>1</sup>H NMR spectrum of  $\alpha$ -anhydrouridine 15; b) <sup>1</sup>H NMR  
564 spectrum of the reaction mixture after heating  $\alpha$ -thiouridine 14 in H<sub>2</sub>O; c) <sup>1</sup>H NMR  
565 spectrum of the reaction mixture after heating  $\alpha$ -thiouridine 14 in formamide.

566

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

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

572

573 **Extended Data Fig. 4. Potential energy surfaces and S<sub>1</sub>/S<sub>0</sub> state crossings of the**  
574 **key photochemical steps in deoxyadenosine synthesis calculated at the**  
575 **ADC(2)/ma-def2-TZVP level (see the SI for more details).** a) C-S bond opening  
576 may spontaneously occur in **18** leading to a peaked S<sub>1</sub>/S<sub>0</sub> state crossing, however, a  
577 reducing agent is necessary to maintain that geometry after reaching the S<sub>0</sub> state; b)  
578 N7-C8 bond rupture is the lowest energy photochemical process in **19** and results in  
579 destruction of the purine ring; c) and d) encounter complexes of **18** and **19** with HS<sup>-</sup>,  
580 which readily undergo photochemical C-S bond rupture induced by charge transfer  
581 from HS<sup>-</sup> to chromophore.

582

583 **Extended Data Fig. 5. Equilibrium geometries of C2, S8 radical anion **31** and C8,**  
584 **N9 radical anion **32** radical anions which may be formed after accepting a**  
585 **hydrated electron from the environment and the adiabatic electron affinities**  
586 **calculated at the ωB97X-D/IEFPCM/ma-def2-TZVP.**

587

588

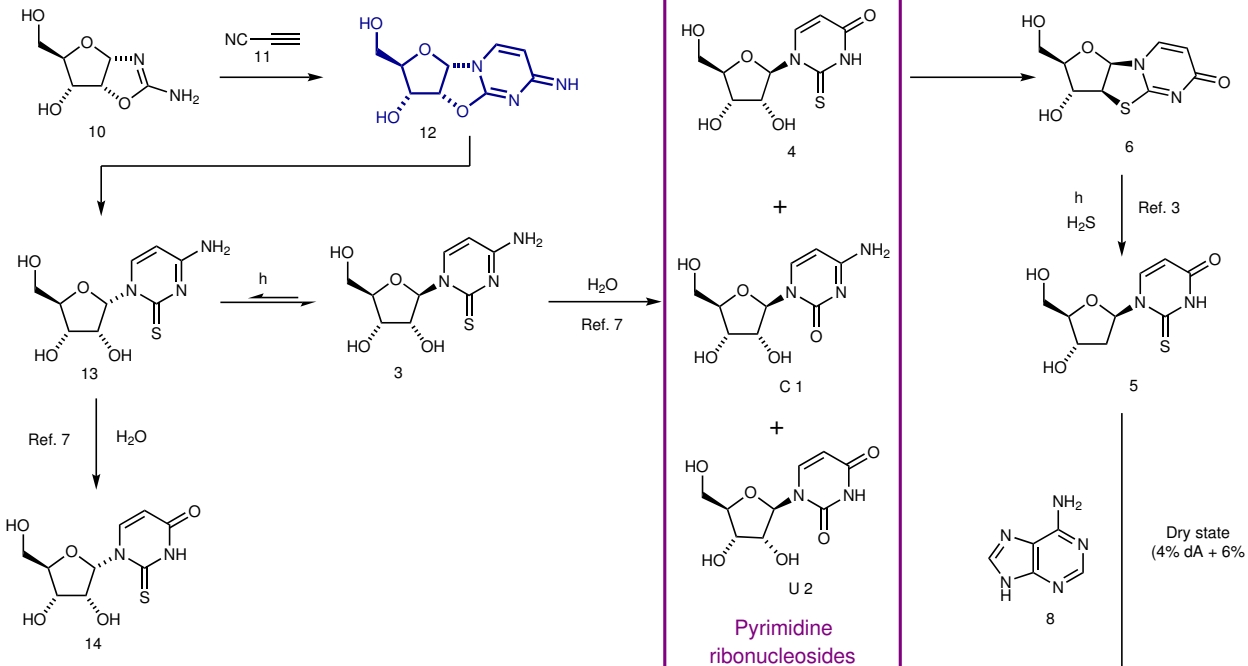
589 **Extended Data Fig. 6. <sup>1</sup>H NMR spectra for the reactions of deoxyadenosine **7** and**  
590 **cytidine **1** with nitrous acid.** a) <sup>1</sup>H NMR spectrum of the mixture of deoxyadenosine  
591 **7** and cytidine **1**; b) <sup>1</sup>H NMR spectrum of the reaction mixture after 4 days, showing  
592 the ratio of all four (deoxy)nucleosides deoxyadenosine **7**, deoxyinosine **9**, cytidine **1**,  
593 and uridine **2** is 30:17:42:11.

594

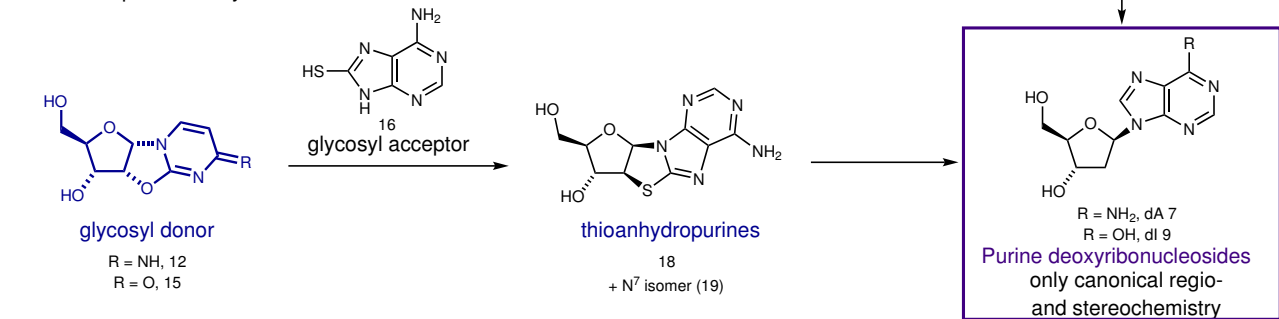
595 **Extended Data Fig. 7. <sup>1</sup>H NMR spectra for stability study of cytidine 1 and**  
596 **uridine 2 at 254 nm irradiation with bisulfite.** a) <sup>1</sup>H NMR spectrum of the mixture  
597 of cytidine **1**, bisulfite and K<sub>4</sub>Fe(CN)<sub>6</sub> in the dark; b) as a), <sup>1</sup>H NMR spectrum after 10  
598 hours of irradiation; c) <sup>1</sup>H NMR spectrum of the mixture of uridine **2**, bisulfite and  
599 K<sub>4</sub>Fe(CN)<sub>6</sub> in the dark; d) as c), <sup>1</sup>H NMR spectrum after 10 hours of irradiation; e) <sup>1</sup>H  
600 NMR spectrum of the mixture of cytidine **1**, uridine **2**, N<sup>9</sup>-thioanhydroadenosine **18**,  
601 bisulfite and K<sub>4</sub>Fe(CN)<sub>6</sub> in the dark; f) as e), <sup>1</sup>H NMR spectrum after 10 hours of  
602 irradiation.

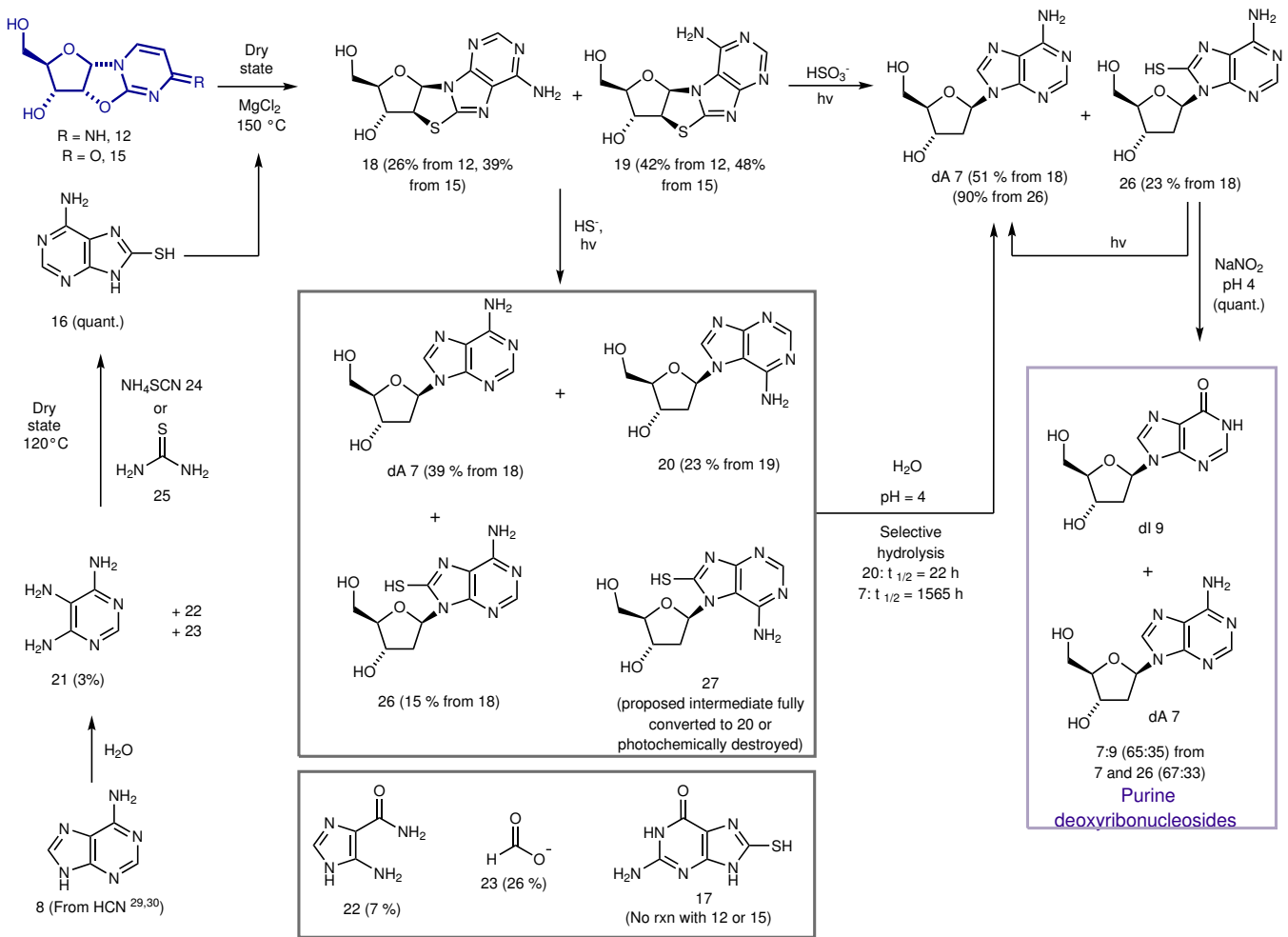
603  
604 **Extended Data Fig. 8. <sup>1</sup>H NMR spectra for sequential reactions with the mixture**  
605 **of α-anhydrouridine 15, cytidine 1 and uridine 2.** a) <sup>1</sup>H NMR spectrum of the  
606 mixture after heating with 8-mercaptopadenine **16** and magnesium chloride at 150 °C  
607 for 1.5 days; b) <sup>1</sup>H NMR spectrum of the same mixture after irradiation with  
608 hydrogen sulfide at 254 nm; c) <sup>1</sup>H NMR spectrum of the same mixture after reacting  
609 with nitrous acid for 2 days (dA 7:dI 9:C 1:U 2= 14:14:44:28).  
610

Previously: pyrimidine ribonucleosides

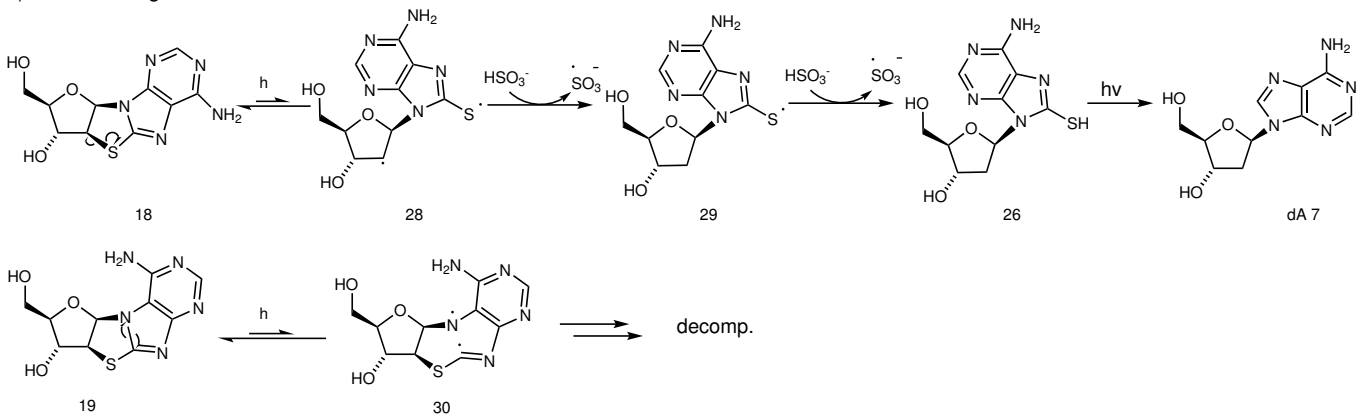


This work: purine deoxynucleosides

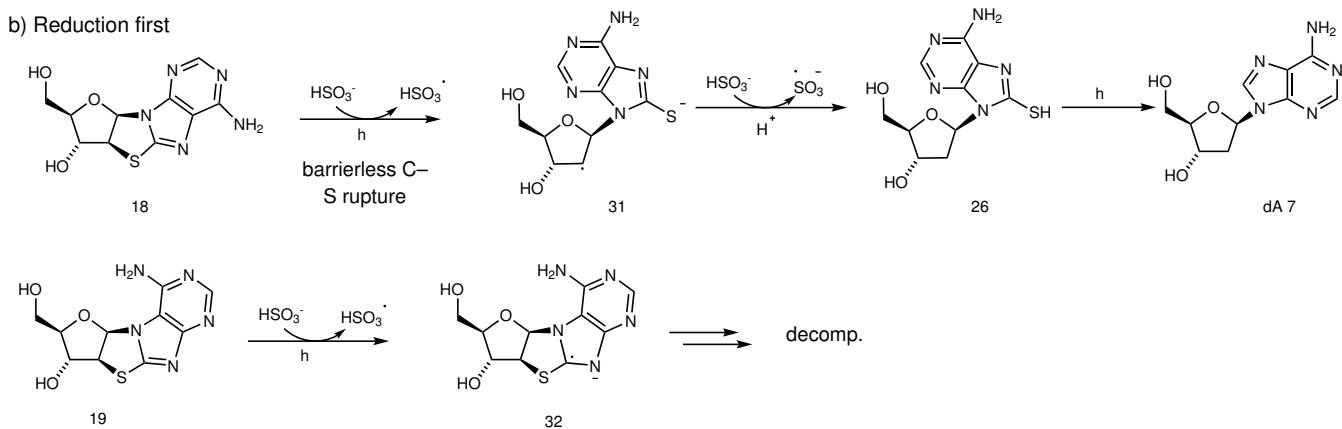




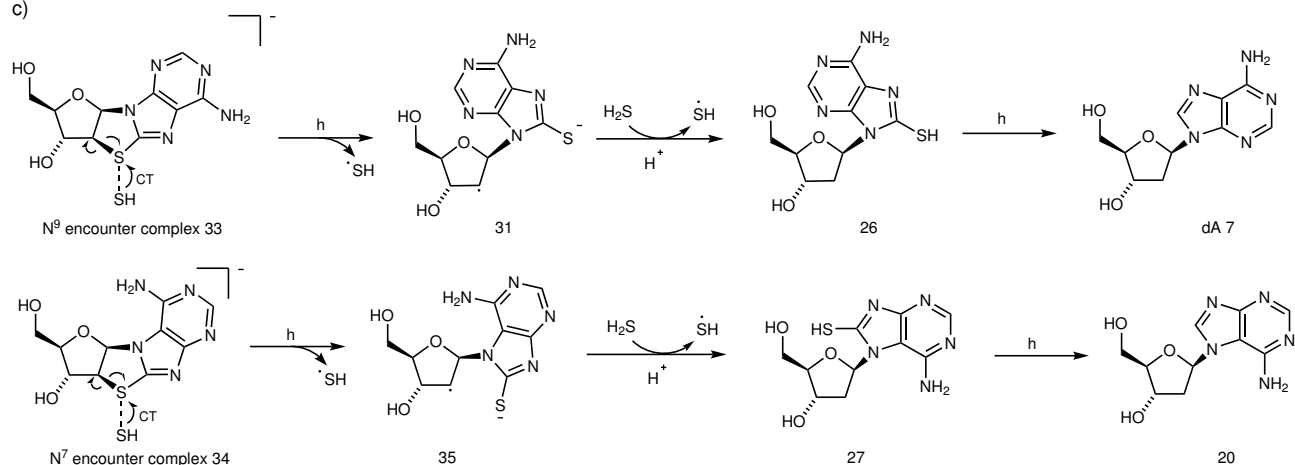
a) Photocleavage first



b) Reduction first

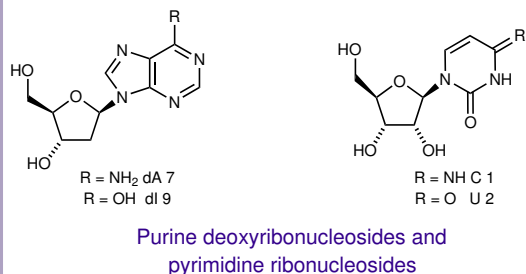
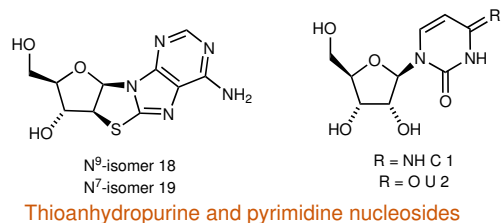
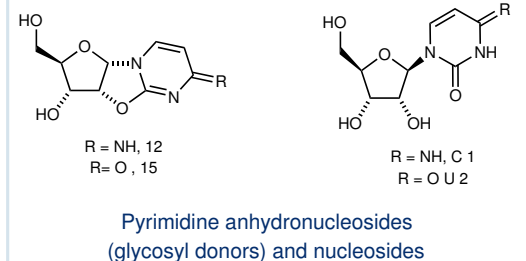
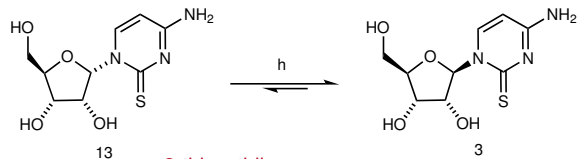


c)





## General Route



## Conditions and Yields

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

### Route A

NaNO<sub>2</sub>  
(pH 4)

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

Representative mixture  
12 and 1 (1:1)

MgCl<sub>2</sub>, 16,  
150 °C

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

HSO<sub>3</sub><sup>-</sup>  
Fe(CN)<sub>6</sub><sup>2-</sup>  
h

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

NaNO<sub>2</sub>, 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

H<sub>2</sub>O  
(pH 5)

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

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

MgCl<sub>2</sub>, 16,  
150 °C

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

HS<sup>-</sup>  
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