One-Pot TiO_2-Catalyzed Synthesis of Nucleic Bases and Acyclonucleosides from Formamide: Implications for the Origin of Life

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A novel one-pot TiO_2-catalyzed synthesis of nucleobases and acyclonucleosides from formamide is reported. Since formamide can be formed under prebiotic conditions, these reactions have implications for the origin of life. While a number of purine derivatives have been found as products of non-TiO_2-catalyzed reactions, important compounds that would not otherwise occur (namely, thymine, 5-hydroxymethyluracil, and acyclonucleosides) are formed in acceptable yields by TiO_2-catalyzed reactions. Moreover, TiO_2 selectively affects the rates of degradation of nucleobases, as single units and when embedded in polynucleotides.

KEYWORDS:

catalysis · formamide · nucleic acids · nucleobases · prebiotic syntheses

Introduction

Hydrogen cyanide (HCN) chemistry provides a preferential route for the prebiotic syntheses of purines and pyrimidines.[1] A major problem for the accumulation of the nucleobases adenine (A), uracil (U), guanine (G), cytosine (C), and thymine (T) on early Earth is the rapid rates of their degradation.[2–3] At high concentrations of HCN and at relatively low temperatures, their syntheses might possibly be preferred over their degradations;[2] catalysts could play a major role in this balance.

In addition to condensation into nucleobases, HCN hydrolyzes to form formamide and then formic acid,[1, 4, 5] thereby making formamide a likely candidate for the synthesis of nucleobases.[1, 6] Purine is synthesized from HCN or neat formamide,[7–9] and the synthesis of both purine and pyrimidine derivatives from formamide under catalytic conditions[10] provides a plausible prebiotic route. In the presence of $CaCO_3$, silica, alumina, kaolin, and zeolite as prebiotic models of heterogeneous catalysts, purine, adenine, 4(3*H*)-pyrimidinone and, notably, cytosine were formed, which points to a possible role of inorganic materials in the prebiotic synthesis of nucleic acids from formamide.[10] The role of catalysts was not limited to the improvement of the yield but also provided high selectivity, which affected the product distribution.

Formamide is also able to degrade purine and pyrimidine bases.[11, 12] When the reaction is performed on polynucleotides, the degradation of nucleic bases is followed by scission of the glycosidic linkages through a β-elimination.[13] This reaction pattern is the basis for novel chemical DNA sequencing procedures.[14, 15]

Thus, to evaluate the prebiotic relevance of any formamide-based synthesis of nucleic acid precursors, the study of the degradative pathway under similar experimental conditions is necessary. Here we describe the unprecedented one-pot TiO_2-catalyzed synthesis of nucleobases, N^9-formylpurines, and acyclonucleosides from formamide. The selective TiO_2-induced degradation of nucleosides by formamide, and the chemical degradation of the same compounds when embedded in polynucleotides are also described.

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The use of TiO_2 allows the synthesis of important compounds that would not otherwise occur (namely, thymine, 5-hydroxymethyluracil, and acyclonucleosides) and selectively affects the rates of degradation of nucleobases.

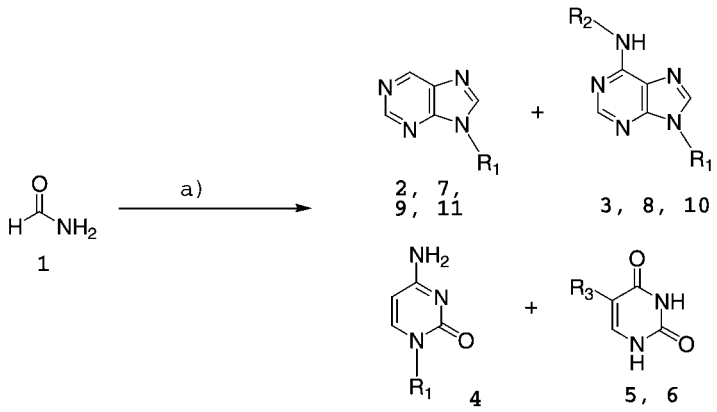
Results and Discussion

Syntheses

To provide further information on the effect of inorganic oxides on the synthesis of purines and pyrimidines, we analyzed the reaction of formamide in the presence of titanium(IV) oxide (TiO_2, microcrystalline anatase powder). TiO_2 powders are prebiotic materials and are widely employed as catalysts[16] in hydrocarbon-selective oxidations,[17] in solar energy conversion,[18] and in other processes.[19, 20] Among photocatalysts, TiO_2 is remarkable for its high photoreactivity[21] and stability.[22] TiO_2 particles selectively adsorb nucleic acids and their components[23] and upon exposure to UVA and UVB induce degradation of these molecules by generation of hydroxyl radicals.[24] However, the application of TiO_2 particles to cell surfaces reduced the extent of UVA-induced DNA pyrimidine dimer formation, with no effect on DNA repair.[25] Apart from research on the radiochemical synthesis of nucleoside analogues starting from purine bases and sugars,[26] no data are available on the role of TiO_2 in the prebiotic synthesis of nucleic acids.

The most favorable set of conditions for these syntheses is high formamide concentration, presence of catalysts, and a temperature between 100 and 180 °C.[10] All experiments were performed in a flask containing neat formamide (5.7 g, 5 mL, 0.12 mol) at 160 °C for 48 h in the presence of TiO_2 (2% w/w) under sunlight illumination. A complex mixture of reaction products was obtained. We focused our attention on the characterization of the most abundant purine and pyrimidine derivatives by gas chromatography–mass spectroscopy by comparison with authentic samples and, when necessary, by spectroscopic analysis of purified samples (Scheme 1, Table 1).

Without TiO_2, purine (**2**) was obtained as the only recovered product in low yield (34.1 mg **2** per gram formamide (**1**)[1]). In the presence of TiO_2, purine (**2**) was again recovered as the main reaction product (15 mg g^{-1}) accompanied by adenine (**3**; 0.3 mg per gram formamide), cytosine (**4**; 0.8 mg g^{-1}) and, remarkably, by thymine (**5**; 0.2 mg g^{-1}), and 5-hydroxymethyluracil (5-HMU, **6**; 0.3 mg g^{-1}) These results are shown in Figure 1.



2, 3, 4: $R_1 = R_2 = H$
5: $R_1 = H$; $R_3 = CH_3$
6: $R_1 = H$; $R_3 = CH_2OH$
7, 8: $R_1 = R_2 = CHO$
9: $R_1 = COCH_2OH$
10: $R_1 = COCH_2OH$; $R_2 = COH$
11: $R_1 = COCH(OH)CH_2OH$

Scheme 1. *Synthesis of purine and pyrimidine bases, N^9-formylpurines, and acyclonucleosides from formamide. a) TiO_2 (2% w/w), 160 °C, 48 h.*

The low yield obtained for purine (**2**) with respect to previously described syntheses may be due to the presence of several other competitive products (see above), even if low efficiency of TiO_2 as a catalyst cannot be completely ruled out.

Compounds **2**–**4** were previously synthesized from formamide under catalytic conditions. In contrast, the formation of thymine (**5**) and 5-HMU (**6**) from formamide alone was not observed. Dehydrogenation of dihydrouracil,[6] cyanide polymerization,[27] and the condensation of the C-3 fragments cyanoacetaldehyde[28] and cyanoacetylene[29] with cyanate[30] and urea, are among the most commonly encountered prebiotic pyrimidine syntheses.[31] Thymine (**5**) and 5-HMU (**6**) were obtained by treatment of uracil, a product of the hydrolysis of cytosine,[32] with formaldehyde and formic acid in aqueous solution at 140 °C.[33, 34] Thymine was also synthesized by methylation of

Table 1. *Selected mass spectrometric data.*

Products	*m/z* (abundance [%])
2	120 (*M*, 100), 93 (*M* − HCN, 37), 86 (*M* − 2HCN, 19)
3	135 (*M*, 100), 108 (*M* − HCN, 38), 81 (*M* − 2HCN, 18), 66 (*M* − 69, 21), 54 (*M* − 3HCN, 28)
4	111 (*M*, 100), 95 (*M* − NH_2, 20), 83 (*M* − CO, 28), 69 (*M* − NCO, 45), 41 (*M* − HNCO − HCN, 58)
5	126 (*M*, 100), 83 (*M* − HNCO, 20), 55 (*M* − HNCO − CO, 50)
6	142 (*M*, 100), 124 (*M* − H_2O, 38), 113 (*M* − HCO, 50), 96 (*M* − H − HNCO, 20), 70 (*M* − H − HCO − HNCO, 60)
7	148 (*M*, 100), 121 (*M* − HCN, 8), 92 (*M* − HCN − HCO, 18), 66 (*M* − 2HCN − HCO, 28)
8	191 (*M*, 100), 164 (*M* − HCN, 10), 147 (*M* − H − HNCO, 80), 118 (*M* − HNCO − H_2CO, 15)
9	178 (*M*, 100), 162 (*M* − OH, 28), 151 (*M* − HCN, 21), 124 (*M* − 2HCN, 18), 120 (*M* − $COCH_2OH$, 28)
10[a]	295 (*M*, 100), 222 (*M* − $Si(CH_3)_3$, 29), 205 (*M* − $Si(CH_3)_3$ − OH, 80), 135 (*M* − $COCH(OH)OSi(CH_3)_3$ − H_2NCO, 35)
11[a]	280 (*M*, 100), 249 (*M* − H − H_2CO, 19), 207 (*M* − $Si(CH_3)_3$, 21), 149 (*M* − $Si(CH_3)_3$ − H_2CO − CO, 89), 119 (*M* − $COCH(OH)OSi(CH_3)_3$, 21)

[a] Detected as the trimethylsilyl derivative after treatment of the reaction mixture with bistrifluoroacetamide (BSTFA)/pyridine.

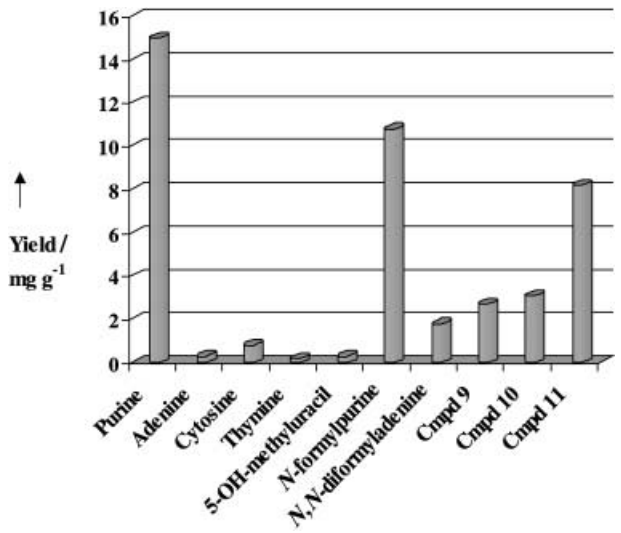


Figure 1. *Quantitative profile of purine and pyrimidine derivatives obtained from the formamide – TiO_2 system. Reactions were performed in the presence of 2.0% w/w TiO_2. Products were identified by comparison of their retention times and mass spectra with those of authentic samples. Quantitative evaluations were performed by capillary gas-chromatographic analysis with an HP5890II gas chromatograph with a FID detector equipped with an ALLTECH AT20 column; a temperature program of 100 – 280 °C (rate 10 °C min^{-1}) with helium as carrier gas was used. 6-Methoxypurine was used as the internal standard. As a result of the uncertainty of the number of formamide molecules involved in the synthesis of the recovered products, the yields were calculated as milligram of product formed per gram of formamide.*

uracil with formaldehyde and hydrazine.[35] 5-HMU was obtained from formaldehyde and uracil under basic conditions.[36] The one-pot synthesis of thymine and 5-HMU starting from a C-1 fragment has never been reported previously.

Formaldehyde, formic acid, and traces of uracil were recovered from our reaction mixture by gas chromatography – mass spectrometry. Aldehydes were isolated in appreciable amounts and are formed by the photooxidation of amides;[37] TiO_2-photocatalyzed degradation of amides has been reported[38, 39] and can be accompanied by the release of formaldehyde.[40] These data suggest that the observed compounds **5** and **6** may be formed by reaction of uracil with the formaldehyde and formic acid produced by TiO_2-catalyzed photooxidation of formamide.[41]

The presence of formaldehyde and formic acid in the reaction mixture may also be responsible for the recovery of two additional families of nucleic acid derivatives—formylpurines **7** and **8**, and acyclonucleosides **9** – **11** (Scheme 2)—not previously observed in prebiotic procedures.

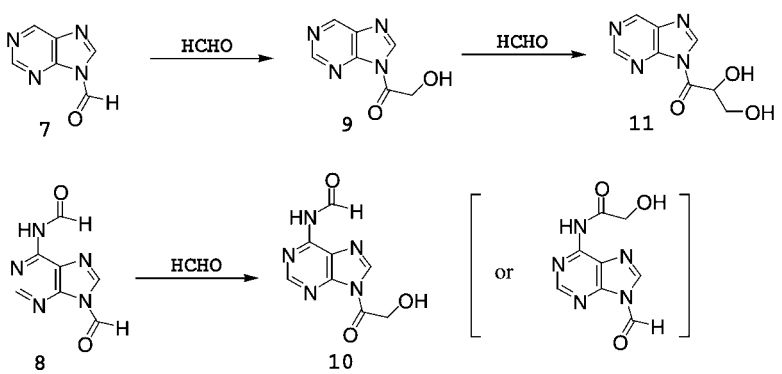
N^9-Formylpurine (**7**) and N^6,N^9-diformyladenine (**8**) were recovered in 10.8 mg g^{-1} and 1.8 mg g^{-1} yields, respectively, after chromatographic purification. *N*-Formylpurines and pyrimidines are usually prepared by treatment of the free bases with formic acid and its derivatives.[42, 43] This reaction is also operative in the formamide – TiO_2 procedure, even if the incorporation of a whole formamide molecule as an N^9-formyl fragment cannot be completely ruled out.

Acyclonucleosides 9-(hydroxyacetyl)purine (**9**), N^6-formyl-9-(hydroxyacetyl)adenine (**10**), and 9-[2,3-dihydroxy-1-(oxo)propyl]purine (**11**) appear to be products of "formose condensation" on **7** and **8**. On formose condensation, formaldehyde is converted into a mixture of monosaccharides by an aldol-like reaction.[44, 45] Alumina, and naturally occurring aluminosilicates, kaolinite, illite, and hydroxyapatite may catalyze this transformation and several sugars, including ribose and deoxyribose, may be formed by the action of ultraviolet light on a dilute solution of formaldehyde.[46, 47] TiO_2 may catalyze the formose condensation by stabilizing the anion resulting from the ionization of formaldehyde hydrate and thereby rendering the N^9-formyl moiety much more susceptible to nucleophilic attack.[48] Examples of carbon – carbon bond formation through the aldolization of aldehydes on TiO_2 have been described.[49]

We assumed that nucleotides are produced by the condensation of the sugar with preformed purine and pyrimidine bases. However, major problems arose in demonstrating this condensation under prebiotic experimental conditions.[50] Adenine and guanine gave low yields of the corresponding nucleosides when the reactions were performed in the presence of inorganic salts.[51] Apart from the synthesis of cytosine arabinonucleoside,[52] no prebiotic condensation of uracil or cytosine with ribose is known. The possibility of building the sugar moiety stepwise by starting from formylpurine derivatives in which a masked glycosidic bond is already preformed is a potential alternative route to nucleosides. High-molecular-weight nucleoside derivatives recovered in the reaction mixture are being characterized.

Degradations

The evolution of self-replicating information-bearing molecules requires chemically stable compounds and high but not absolute accuracy in the replication of information. Chemical stability is necessary for the accumulation of precursors under the physicochemical conditions in order to allow their synthesis and polymerization into oligomers, and for low-error template processes. To evaluate the prebiotic relevance of TiO_2-catalyzed synthesis of nucleic acid precursors, we analyzed the degrada-



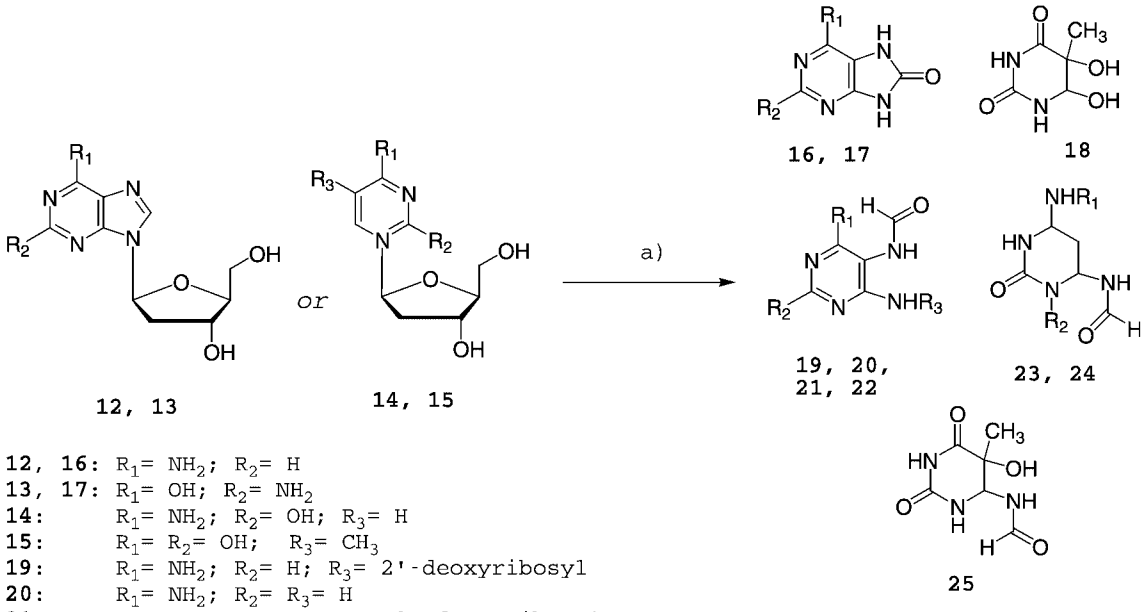
Scheme 2. *Proposed synthesis of acyclonucleoside analogues from N^9-formylpurines by the formose reaction.*

tion of nucleosides and polynucleotides under the experimental conditions described above.

The degradation pathway of nucleosides in the formamide – TiO_2 system was analyzed for 2′-deoxyadenosine (**12**), 2′-deoxyguanosine (**13**), 2′-deoxycytidine (**14**), and thymidine (**15**).[11] The nucleoside (1.0 mmol) was added to formamide (5 mL) in the presence of 2% w/w TiO_2 and the mixture was heated at 160 °C for 2 days. The stirred suspension was irradiated at 254 nm by a mercury arc in air. Complex mixtures of reaction products were obtained and analyzed by gas chromatography – mass spectroscopy and, when necessary, by spectroscopic analysis of purified samples (Scheme 3, Table 2).

As expected, we observed products that arise from the synthetic formamide – TiO_2 pathway independent of the nucleoside studied. Moreover, small amounts of products resulting from hydroxyl radical damage of nucleobases, such as 8-oxoadenine (**16**),[53] 8-oxoguanine (**17**),[54] and 5,6-dihydroxy-5,6-dihydrothymine (*cis*- and *trans*-**18**)[55] were recovered from the reaction mixture. Hydroxyl radical damage to nucleobases has been reviewed.[56] TiO_2 exerts photobiological activity in prokaryotic and eukaryotic cells,[57] and photoirradiation of uracil and thymine solutions in the presence of TiO_2 led to decomposition of substrates by hydroxylation of the C5,6 double bond.[58] The chemistry of nucleosides with formamide provides the main degradative pathway and accompanies that of hydroxyl radical damage.

6-Amino-5-formamido-4-[*N*-(2′-deoxy-β-D-ribofuranosyl)]pyrimidine (**19**), 4,6-diammino-5-formamidopyrimidine (**20**), 2-amino-4-oxo-5-formamido-6-[*N*-(2′-deoxy-β-D-ribofuranosyl)]pyrimidine (**21**), and 2,6-diamino-4-oxo-5-formamidopyrimidine (**22**), were recovered from the reaction mixture as products of selective C8 addition of formamide to **12** and **13**, respectively (Figure 2).[11] 6-Formylamino-5,6-dihydro-4-amino-1-[β-D-ribofuranosyl]-2-(1*H*)-pyrimidinone (**23**), and 4,6-di(formylamino)-5,6-



Scheme 3. *Formamide degradation of 2′-deoxynucleosides. a) TiO_2 (2% w/w), 160 °C, 48 h.*

Table 2. *Selected mass spectrometric data.*

Products	*m/z* (abundance [%])
18	160 (*M*, 100), 143 (*M* − OH, 63), 125 (*M* − OH − HO, 51), 117 (*M* − HCNO, 39)
19[a]	413 (*M* as dimethylsilyl derivative, 28), 358 (*M* − HCN − CO, 33), 339 (*M* − $HSi(Me)_3$, 100), 311 (*M* − CO − $HSi(Me)_3$, 53), 265 (*M* − 2 $HSi(Me)_3$, 38)
20	153 (*M*, 78), 124 (*M* − CHO, 69), 109 (*M* − NHCHO, 100)
21	285 (*M*, 100), 256 (*M* − CHO, 63), 241 (*M* − NHCHO, 53)
22	169 (*M*, 100), 140 (*M* − CHO, 71), 125 (*M* − NHCO, 43), 82 (*M* − NHCHO − HCNO, 39)
23[a]	344 (*M* as monotrimethylsilylderivative, 100), 314 (*M* − 2 Me, 78), 299 (*M* − 3 Me, 63), 270 (*M* − $HSi(Me)_3$, 61), 225 (*M* − $HSi(Me)_3$ − NH_2CHO, 58)
24[a]	516 (*M* as tristrimethylsilylderivative, 100), 413 (*M*-2 Me-$HSi(Me)_3$, 51), 367 (*M*-2 $HSi(Me)_3$, 62), 297 (*M*-3 $Si(Me)_3$, 48), 255 (*M* − 3′,5′-bistrimethylsilyl-1-α-D-ribofuranosyl, 47)
25	187 (*M*, 100), 170 (*M* − OH, 79), 143 (*M* − NHCHO, 68), 115 (*M*-NHCHO-CO, 43)

[a] Detected as the trimethylsilyl derivative after treatment of the reaction mixture with BSTFA/pyridine.

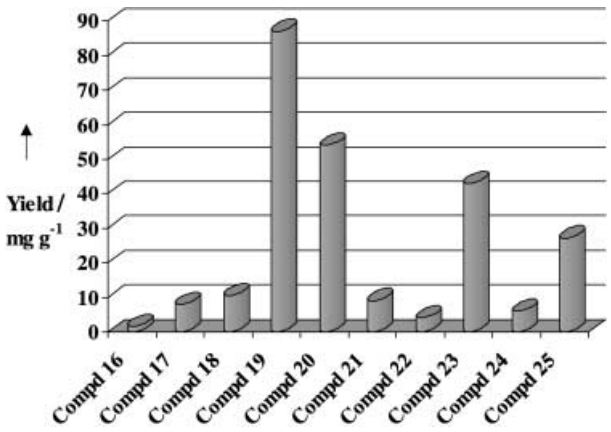


Figure 2. *Quantitative profile of 2′-deoxynucleoside degradation by the formamide – TiO_2 system. For experimental details, see the legend of Figure 1.*

dihydro-1-[β-D-ribofuranosyl]-2-(1*H*)-pyrimidinone (**24**), obtained by the addition of formamide to the electrophilic C6 and C4 positions of the pyrimidine ring, were recovered from the degradation of 2′-deoxycytidine (**14**).[12]

The degradation reaction of thymidine (**15**) deserves notice. In the absence of TiO_2, **15** did not react over a prolonged reaction time,[12] while efficient degradation was observed in the presence of TiO_2. In the latter case, 6-formylamino-5-methyl-5,6-dihydrouracil (**25**), derived from C6 addition of formamide to a reactive 5,6-oxiranyl-5,6-dihydrouracil intermediate, was recovered along with small amounts of hydroxyl damage products. 5,6-Oxiranyl-5,6-dihydrouracil derivatives are formed as initial photooxidation products of pyrimidine nucleic acid components.[12, 47, 59]

The differential effects of TiO_2 on the degradation of polynucleotides were studied for homogeneous or mixed-sequence polymers. The overall approach consisted of the analysis of the degradation products of the following synthetic 5′-labeled oligonucleotides: 1) homogeneous segments: two short mixed-sequence tails (10 and 6 bases, respectively) and a central 30-base-long homogeneous stretch of G, A, C, and T bases; 2) a mixed-sequence segment: a heterogeneous 40-base-long sequence.

The labeled oligonucleotides were treated (20 min, 110 °C) with formamide in the presence of varying amounts of TiO_2 (Figure 3). DNA was then recovered and analyzed (see the Experimental Section). Oligonucleotides used in the degradation of homogeneous sequences were: Oli1 (5′-ACCTAACCGG[G]$_{30}$CCGGTT-3′), Oli2 (5′-ACCTAACCGG[A]$_{30}$CCGGTT-3′), Oli3 (5′-CCCGAACCGG[C]$_{30}$CCGGTT-3′), and Oli4 (5′-CCCGAACCGG[T]$_{30}$CCGGTT-3′). These oligonucleotides were designed pairwise to be complementary (that is, Oli1 with Oli3, and Oli2 with Oli4). Upon annealing the oligonucleotides leave four-nucleotide-long 5′-protruding tails at both extremities of the DNA that can be used for selective labeling. Figure 3 shows the resulting degradation profiles. A comparison of the four

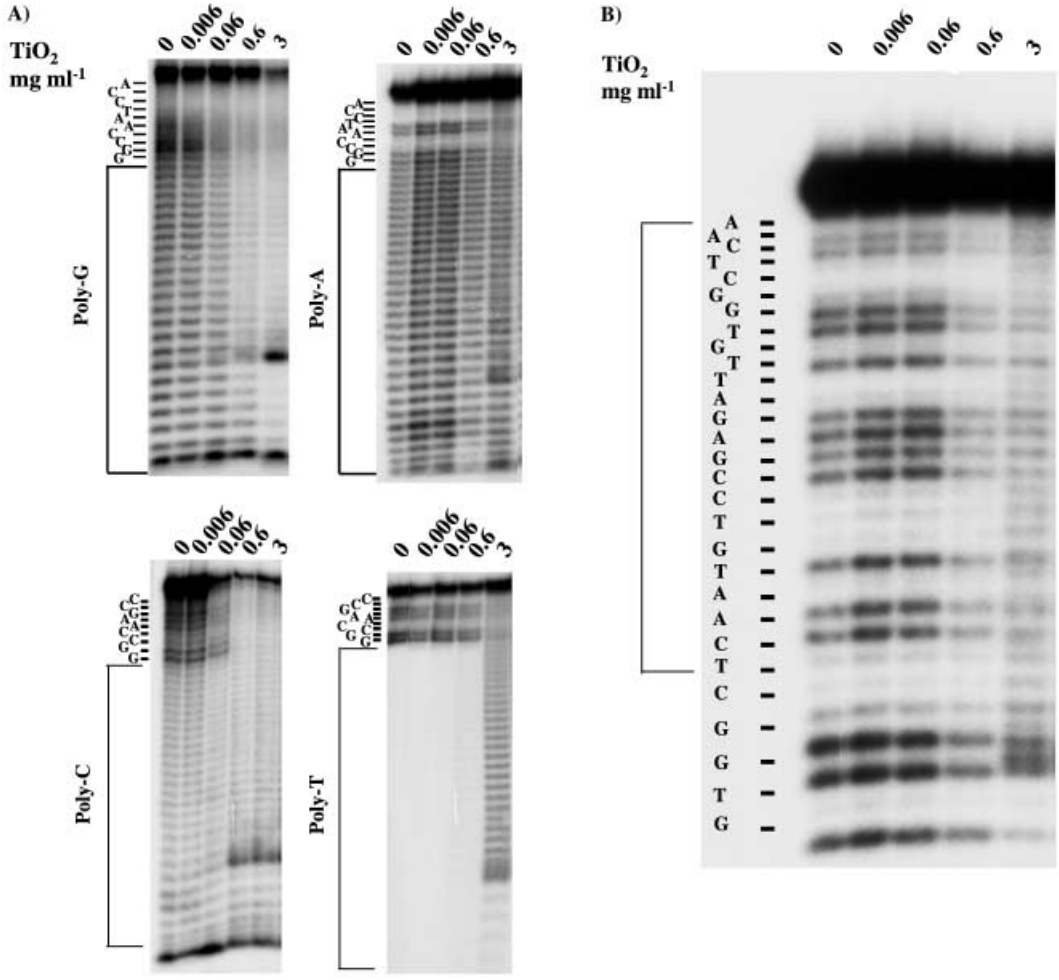


Figure 3. *Degradation of 46-base-long oligonucleotides containing homo- or heterogeneous base stretches by formamide – TiO_2. The treatment of 5′-labeled oligonucleotides (20 min, 110 °C) was performed in the presence of the indicated amounts of TiO_2 followed by purification and gel analysis. Panel A: (from left) Oli1 (poly-G), Oli2 (poly-A), Oli3 (poly-C), Oli4 (poly-T). The mixed sequence containing the 5′-labeled extremity is in the run-out section of the gel. Panel B: as above but with the mixed sequence oligonucleotide.*

degradation profiles obtained in the absence of TiO_2 (first lanes) leads to the observations expected from the described differential sensitivity of the nucleic bases to hot formamide ($G \geq A > C > T$).[14, 15] The poly-G and poly-A degradations are not enhanced by low concentrations of TiO_2; both poly-G and poly-A are actually digested to a lesser extent at higher concentrations. Degradation of poly-C is enhanced by TiO_2 only at high concentration, while a major enhancement effect on poly-T is observed between 0.6 and 3 mg mL^{-1} which causes an essentially complete and regular digestion of the homogenous sequence tract.

The mixed sequence 5′-GTAACTCGGTGTTAGAGCCTG-TAACTCGGTGTTAGAGCCT-3′ was analyzed as described above (Figure 3B), which revealed the same selectivity of TiO_2 on the degradation pathway; thymine was unexpectedly degraded.[14, 15] Figure 4 compares the sensitivity of the four bases to hot formamide as a function of the concentration of TiO_2. For both homo- and heterogeneous oligonucleotides, the degradation conditions used in this assay cause less than one hit per molecule, as shown by the regularity of the cleavage patterns and by the presence of a substantial amount of unreacted molecules. Thus, the decreased intensity of the cleavage at G and A bases above 0.6 mg mL^{-1} TiO_2 is not a kinetic artifact. In conclusion, the degradation profiles of polynucleotides by formamide with and without TiO_2 are profoundly different. At a TiO_2 concentration of 3 mg mL^{-1}, the sensitivity of all the bases becomes comparable.

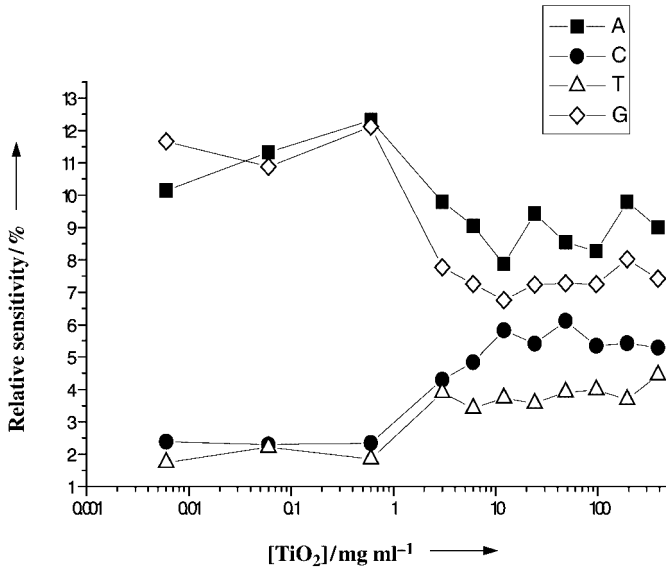


Figure 4. *The relative sensitivity (ordinate) of the four bases as a function of increasing concentration of TiO_2 (abscissa). Each experimental lane of the gels was scanned; the total intensity of the lane was taken as 100%; the values reported for each base represent the average intensity (%) of the bands of each of the four bases. Values are from the electrophoreses in Figure 3 and from data not shown.*

Conclusion

While a number of purine derivatives have been found as products of prebiotic synthesis from HCN, novel compounds that would not otherwise occur (namely, thymine, 5-hydroxymethyluracil, and acyclonucleosides) are formed in a single step and in acceptable yields from formamide in the presence of TiO_2. As formamide is a compound that can be formed under prebiotic conditions, these reactions have implications for the origin of life. Moreover, TiO_2 selectively affects the rates of degradation of nucleobases, both as single molecules and when embedded in polynucleotides.

One of the major unresolved problems for any plausible chemical origin-of-life scenario is that of the stability of the nucleic acid precursors. Given that formamide both synthesizes and degrades such precursors, our results are relevant to the regulation of the availability of the substrates for prebiotic nucleic acid syntheses.

Experimental Section

General: Formamide (Fluka, >99%), TiO_2 (Aldrich), and 6-methoxypurine (Aldrich) were used without further purification. Gas chromatography analysis and mass spectrometry were performed with an HP5890II gas chromatograph and a Shimadzu GC-MS QP5050A instrument equipped with an Alltech AT-20 column (0.25 mm, 30 m). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported as δ values. Microanalyses were performed with a C. Erba 1106 analyzer. Chromatographic purifications were performed on columns packed with Merck silica gel, 230–400 mesh for flash chromatography. TLC was carried out on silica-coated plates (Merck platten Kieselgel 60 F_{254}). Melting points were determined on a Reichert Kofler apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer Paragon 500 FT-IR spectrophotometer.

Formamide condensation: Formamide (5.7 g, 5 mL, 0.12 mmol) was heated at 160 °C for 48 h in the presence of TiO_2 (2% w/w). The reaction mixture was filtered to remove the catalyst and evaporated under high vacuum. Gas chromatography–mass spectrometry of a portion of the crude reaction was performed by using an isothermal temperature profile of 100 °C for the first 2 min, followed by a 10 °C min^{-1} temperature gradient to 280 °C, and finally an isothermal period at 280 °C for 40 min. The injector temperature was 280 °C. Chromatography grade helium was used as the carrier gas. The fragmentation patterns were compared with those of authentic samples. 6-Methoxypurine was used as internal standard. The crude reaction mixture was also purified by flash chromatography ($CHCl_3$/CH_3OH, 9:1) and the structures of isolated products were confirmed by spectroscopic techniques and by comparison with authentic commercial samples.

***N*^{9}-Formylpurine (7)**: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.87$ (s, 1H; H_8), 9.29 (s, 1H; H_6), 9.48 (s, 1H; H_2), 10.22 (s, 1H; CHO) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 140.87$ (C), 144.30 (CH), 148.22 (C), 149.30 (CH), 152.96 (CH), 160.26 (CHO) ppm; elemental analysis calcd (%) for $C_6H_4N_4O$: C 48.65, H 2.72, N 37.82; found: C 47.98, H 2.67, N 37.18.

***N*^{6},*N*^{9}-Diformyladenine (8)**: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.24$ (s, 1H; CHO), 8.79 (s, 1H; H_2), 9.28 (s, 1H; CH), 11.22 (s, 1H; CHO), 12.14 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 128.56$ (C), 144.16 (CH), 146.47 (C), 149.04 (C), 156.46 (CH), 159.59 (CHO), 162.43

(CHO) ppm; elemental analysis calcd (%) for $C_7H_5N_5O_2$: C 43.99, H 2.64, N 36.64; found: C 43.56, H 2.67, N 36.56.

9-(Hydroxyacetyl)purine (9): ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.65$ (s, 2H; CH_2), 7.23 (br s, 1H; OH), 8.98 (s, 1H; H_8), 9.29 (s, 1H; H_6), 9.41 (s, 1H; H_2) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 58.60$ (CH_2), 129.80 (C), 146.66 (CH), 147.16 (C), 149.77 (C), 157.10 (CH), 161.4 (CHO), 167.56 (CHO) ppm; elemental analysis calcd (%) for $C_7H_6N_4O_2$: C 47.19, H 3.33, N 31.45; found: C 47.33, H 3.37, N 31.39.

***N*^{6}-Formyl-9-(hydroxyacetyl)adenine (10)**: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 4.63$ (s, 2H; CH_2), 6.86 (br s, 1H; OH), 8.23 (s, 1H; CHO), 8.76 (s, 1H; H_2), 9.14 (s, 1H; H_6), 12.35 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 59.60$ (CH_2), 129.12 (C), 146.56 (CH), 147.87 (C), 150.78 (C), 157.12 (CH), 158.32 (CHO), 168.18 (CHO) ppm; elemental analysis calcd (%) for $C_8H_7N_5O_3$: C 43.44, H 3.19, N 31.66; found: C 43.48, H 3.06, N 31.88.

9-[2,3-Dihydroxy-1-(oxo)propyl]purine (11): ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 3.96$ (d, $J = 6.92$ Hz, 2H; CH_2), 5.27 (t, $J = 6.92$ Hz, 1H; CH), 5.79 (s, 1H; CHO), 8.76 (s, 1H; H_2), 9.14 (s, 1H; H_6), 12.35 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 63.25$ (CH_2), 72.50 (CH), 144.41 (C), 146.57 (CH), 148.81 (C), 150.11 (CH), 154.59 (CH), 168.39 (CHO) ppm; elemental analysis calcd (%) for $C_8H_8N_4O_3$: C 46.16, H 3.87, N 26.91; found: C 46.09, H 3.73, N 27.01.

Degradation of 2′-deoxynucleosides (12 – 15) with formamide:

General procedure: A 2′-deoxynucleoside **12 – 15** (1 mmol) was dissolved in formamide (5 mL) in the presence of TiO_2 (2% w/w). The reaction mixture was heated at 160 °C for 48 h, then filtered to remove the catalyst and evaporated under high vacuum. Gas chromatography – mass spectrometry of a portion of the crude reaction was performed by using an isothermal temperature profile of 100 °C for the first 2 min, followed by a 10 °C min^{-1} temperature gradient to 280 °C and finally an isothermal period at 280 °C for 40 min. The injector temperature was 280 °C. Chromatography grade helium was used as the carrier gas. The fragmentation patterns were compared with those of authentic samples. 6-Methoxypurine was used as internal standard. The crude reaction mixture was also purified by flash chromatography ($CHCl_3$/CH_3OH, 9.5:0.5), and the structures of isolated products were confirmed with spectroscopic techniques.

7,8-Dihydro-8-oxoadenine (16): ^{1}H NMR (200 MHz, $CDCl_3$/CD_3OD): $\delta = 3.85$ (br s, 2H; NH_2), 7.62 (s, 1H; H_2), 10.87 (s, 1H; NH), 11.80 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 114.74$ (C), 150.38 (C), 150.48 (C), 151.14 (C), 165.96 (CH) ppm; elemental analysis calcd (%) for $C_5H_5N_5O$: C 39.74, H 3.33, N 46.34; found: C 39.76, H 3.21, N 46.18.

7,8-Dihydro-8-oxoguanine (17): ^{1}H NMR (200 MHz, $CDCl_3$/CD_3OD): $\delta = 4.08$ (br s, 1H; OH), 4.12 (br s, 2H; NH_2), 10.65 (s, 1H; NH), 13.06 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 109.96$ (C), 147.66 (C), 153.21 (C), 155.33 (C), 158.82 (C) ppm; elemental analysis calcd (%) for $C_5H_5N_5O_2$: C 35.93, H 3.02 N 41.90; found: C 35.88, H 3.06, N 41.79.

5,6-Dihydroxy-5,6-dihydrothymine (18): ^{1}H NMR (200 MHz, $CDCl_3$/CD_3OD): $\delta = 1.42$ (s, 3H; CH_3), 5.58 (d, $J = 7.71$ Hz, 1H; H_6), 6.48 (br s, 1H; NH), 6.56 (d, $J = 7.71$ Hz, 1H; OH), 8.85 (br s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 17.75$ (CH_3), 76.52 (C), 77.87 (CH), 153.87 (C), 173.17 (C) ppm; elemental analysis calcd (%) for $C_5H_8N_2O_4$: C 37.50, H 5.04, N 17.49; found: C 37.61, H 5.12, N 17.37.

6-Amino-5-formamido-4-[*N*-(2′-deoxy-β-D-ribofuranosyl)]pyrimidine (19): M.p.: 158 – 160 °C; IR (KBr): $\tilde{\nu} = 3351$ (OH), 3202 (NH), 1680 (CO), 1637 (C=C), 1280, 970 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 2.20 – 2.85 (m, 2H; H-2′, H-2″), 3.30 – 3.95 (m, 4H; H-3′, H-4′, H-5′, H-5″), 6.09 – 6.15 (m, 1H; H-1′), 6.71 – 7.08 (br s, 4H; NH), 8.18 (br s, 1H; CHO), 8.29 (s, 1H; H-2) ppm; elemental analysis calcd (%) $C_{10}H_{15}N_5O_4$: C 44.61, H 5.61, N 26.03; found: C 44.69, H 5.62, N 26.30.

4,6-Diamino-5-formamidopyrimidine (20): M.p.: 224 – 226 °C; IR (KBr): $\tilde{\nu} = 3215$ (NH), 1677 (CO), 1637 (C=C), 1280, 970 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.80 - 7.12$ (br s, 5H; NH and NH_2), 8.05 – 8.13 (br s, 1H; CHO), 8.19 (s, 1H; H-2) ppm; elemental analysis calcd (%) for $C_5H_7N_5O$: C 39.21, H 4.60, N 45.73; found: C 39.26, H 4.57, N 45.79.

2-Amino-4-oxo-5-formamido-6-[*N*-(2′-deoxy-β-D-ribofuranosyl)]-pyrimidine (21): M.p.: 180 – 185 °C; IR (KBr): $\tilde{\nu} = 3360$ (OH), 3202 (NH), 1690 (CO), 1637 (C=C), 1280, 970 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.28 - 2.85$ (m, 2H; H-2′, H-2″), 4.05 – 5.20 (m, 4H; H-3′, H-4′, H-5′, H-5″), 5.30 – 5.48 (m, 1H; H-1′), 6.50 – 6.71 (br s, 2H; NH_2), 6.85 (br s, 1H; NH), 7.89 – 7.95 (br s, 1H; CHO) ppm; elemental analysis calcd (%) for $C_{10}H_{15}N_5O_5$: C 42.10, H 5.30, N 24.55; found: C 42.03, H 5.29, N 24.67.

2,6-Diamino-4-oxo-5-formamidopyrimidine (22): IR (KBr): $\tilde{\nu} = 3230$ (NH), 1780 (CO), 1679 (CO), 1640 (C=C), 1290, 970 cm^{-1}; elemental analysis calcd (%) for $C_5H_7N_5O_2$: C 35.50, H 4.17, N 41.40; found: C 35.55, H 4.16, N 41.38.

6-Formylamino-5,6-dihydro-4-amino-1-[β-D-ribofuranosyl]-2-(1*H*)-pyrimidinone (23): IR (KBr): $\tilde{\nu} = 3400 - 3350$ (OH and NH), 2825 (CHO), 1723 (CO), 1678 (CO), 1637 (C=C), 1280, 970 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.12 - 2.43$ (m, 2H; H-2′, H-2″), 4.10 – 4.87 (m, 4H; H-3′, H-4′, H-5′, H-5″), 4.09 – 4.25 (m, 1H; H-1′), 4.89 – 5.46 (m, 2H; H-5), 9.10 (s, 1H; CHO), 9.35 (br s, 2H; NH_2) ppm; elemental analysis calcd (%) for $C_{10}H_{16}N_4O_5$: C 44.12, H 5.92, N 20.50; found: C 44.07, H 5.95, N 20.53.

4,6-Di(formylamino)-5,6-dihydro-1-[β-D-ribofuranosyl]-2-(1*H*)-pyrimidinone (24): IR (KBr): $\tilde{\nu} = 3492 - 3350$ (OH and NH), 2850 (CHO), 1723 (CO), 1669 (CO), 1629 (C=C), 1280, 970 cm^{-1}; m.p.: 178 – 181 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.23 - 2.57$ (m, 2H; H-2′, H-2″), 4.10 – 4.23 (m, 4H; H-3′, H-4′, H-5′, H-5″), 4.16 – 4.25 (m, 1H; H-1′), 4.89 – 5.46 (m, 2H; H-5), 9.10 (s, 1H; CHO), 9.21 (s, 1H; CHO) ppm; elemental analysis calcd (%) for $C_{11}H_{16}N_4O_6$: C 44.00, H 5.37, N 18.66; found: C 44.10, H 5.39, N 18.79.

6-Formylamino-5-methyl-5,6-dihydrouracil (25): ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.45$ (s, 3H; CH_3), 6.72 (s, 1H; H_6), 8.47 (s, 1H; CHO), 11.21 (s, 1H; NH) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 19.89$ (CH_3), 79.80 (C), 81.22 (CH), 164.30 (C), 170.22 (CH), 178.90 (C) ppm; elemental analysis calcd (%) for $C_6H_9N_3O_4$: C 38.51, H 4.85, N 22.45; found: C 38.76, H 4.46, N 23.09.

Degradations of oligonucleotides by formamide – TiO_2: A 2-μg sample of each oligonucleotide was annealed with the same amount of the complementary molecule and labeled with [α^{32}P]dATP (Oli3 and Oli4) or with [α^{32}P]dCTP (Oli1 and Oli2). Labeling was performed by using the T7 Sequenase (USBC – Amersham Biosciences); the labeled oligonucleotide was purified on a 10% denaturing acrylamide (acrylamide/bisacrylamide, 19:1) gel. The polyacrylamide was removed by a NuncTrap Probe Purification Column (Stratagene); 2 pmol (typically 300 000 cpm) DNA was processed for each sample. The DNA was precipitated with ethanol, resuspended in formamide (5 μL, Fluka), and added to 97% formamide (10 μL) containing the indicated amounts of TiO_2. After 20 min at 110 °C, H_2O was added to give a final volume of 100 μL, then the samples were precipitated with ethanol and resuspended in H_2O (5 μL). Formamide (4 μL) was added to 1-μL aliquots of the treated samples, heated for 2 min at 95 °C, and loaded onto a 10% denaturing polyacrylamide gel (acrylamide/bisacrylamide, 19:1). The oligonucleotides (labeled with [α^{32}P]dCTP) used for the analysis of the effect of formamide – TiO_2 on a heterogeneous sequence were the 40-base-long Oli5 (5′-GTAACTCGGTGTTAGAGCCTGTAACTCGGTGTTAGAGCCT-3′) and the

44-base-long Oli6 (5′-CCGAAGGCTCTAACACCGAGTTACAGGCTCTAA-CACCGAGTTAC-3′).

Irregularities in the lowest part of the digested samples shown in Figure 3, typically encompassing approximately the shortest segment (17 – 18 bases long) of the digested oligonucleotide, were constantly observed for all samples treated with TiO_2 at a concentration higher than 0.6 mg mL^{-1}. These irregularities are present in both homo- and heterogeneous sequence digests, are indicated by a vertical bar on the left side of the gel in the figure, and were attributed to the presence of aggregates. Under different experimental conditions the presence of TiO_2 in the gel electrophoresis caused a "smiling" effect and, on defined samples, a marked blurring. Although methodologically unpleasant, these unavoidable effects do not interfere with the interpretation of the data nor with their reproducibility.

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