Preparation of Guanine and Diaminopurine from Biuret

Part III[1])

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Because of their potential prebiotic origin and relative chemical stability, urea, biuret, formic acid, and glycine amide might have played a role in the assembly process of purine bases. In this paper, we describe a short reaction path to purine nucleobases from these acyclic precursors. The formation of different purines was verified by UV and NMR spectroscopy, as well as by mass spectrometry.

Introduction. – *Darwin*'s theory of evolution put forward a mechanism in which organisms might evolve over millennia from simple forms, but it did not address the original spark from which even simple organisms might have arisen [3]. However, he made the suggestion that life may have begun in a '*warm little pond, with all sorts of ammonia and phosphoric salts, lights, heat, electricity, etc. present,* [so] *that a protein compound was chemically formed ready to undergo still more complex changes*'. He went on to explain that '*at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed*' [4]. In 1936, *Oparin* argued in his book '*The Origin of Life on Earth*' [5] that a '*primeval soup*' of organic molecules could be created in an oxygen-free atmosphere through the action of sunlight. These would combine in ever-more complex fashion, until they dissolved into a coacervate droplet. The question on the origin of the nucleobases, as one of the important constituents of the primeval soup of organic matter leading to life, is still open today.

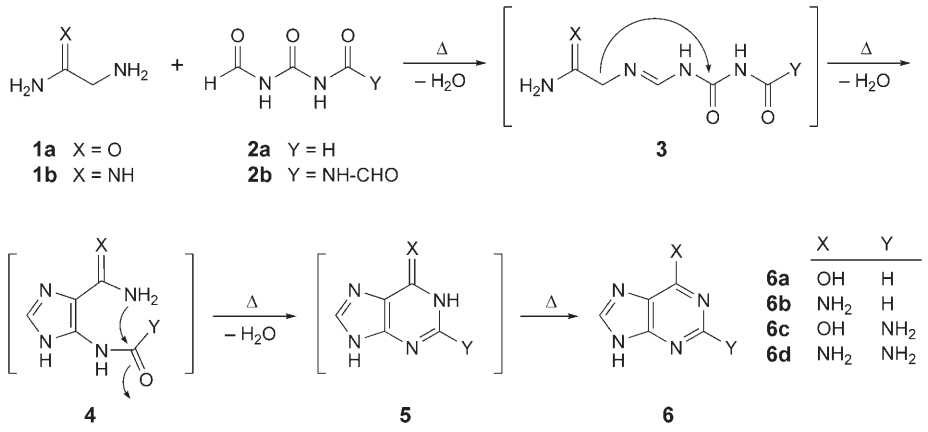
Herein, we have evaluated the potential to generate the nucleobase guanine, based on the same chemistry that was previously used to synthesize hypoxanthine [1][2].

Results and Discussion. – The prebiotic synthesis of purine bases, as proposed by *Oro* [6], and by *Ferris* and *Orgel* [7] starts from hydrogen cyanide (HCN). It has been demonstrated that adenine can be obtained by heating ammonium formate and HCN (present as the tetramer), or by heating formamide ($HCONH_2$) in a sealed tube [8–10]. Recently, we have shown that the simple organic compounds urea ($(NH_2)_2CO$), formic acid (HCOOH), and glycine might have been used for the formation of hypoxanthine; and we have proposed a possible reaction pathway [1][2]. As shown in the *Scheme*, glycinamide (**1a**) may be formed during the *Miller* experiment [11],

[1]) For Part I, see [1]; for Part II, see [2].

whereas the formation of the analogous 'amino-acetamidine' (=2-aminoethanimidamide; **1b**) under prebiotic conditions is not very likely. Biuret ($(NH_2CO)_2NH$[2]) is obtained as main product by pyrrolysis of urea, beside some triuret and melamine [12], and also has been found in astronomic-ice samples [13]. In analogy to urea, treatment of biuret with formic acid affords the diformyl derivative **2b**. However, a more sufficient approach, which has been used to prepare **2b** on a larger scale, has been reported by *Holý* [14]. Formally, three molecules of H_2O have to be abstracted from the amino acid precursors **1** and the urea derivatives **2** to afford, *via* the (so far elusive) intermediates **3**–**5**, the purine derivatives **6**.

Scheme. *Reaction Pathway for the Formation of the Purine Bases* **6** *by Condensation of an Amino Acid Derivative* **1** *with an Urea Derivative* **2**. To simplify the scheme, compounds **6a** and **6c** are given in form of the hydroxy tautomer.



In case of compounds **5b** or **5d** (X=NH), tautomerization will lead to the 6-amino purine derivatives **6b** (adenine) and **6d** (2,6-diamino-9*H*-purine), respectively. In contrast, for Y=NHCHO, spontaneous deformylation will yield the corresponding 2-amino purine derivates **6c** (guanine) and **6d**.

For the synthesis of compounds **6a** (hypoxanthine) and **6d**, best results were obtained in the condensation reaction by heating an equimolar mixture of the respective amino acid derivatives **1** and the urea derivative **2** with 3 equiv. of phosphorous pentoxide (P_2O_5). Due to the lability of the amidine **1b** and the diformylated biuret **2b**, which suffers decomposition to the triazines **7** and **8**, the yield of the amino-containing purine derivatives **6b**–**6d** was significantly lower than that of hypoxanthine proper (**6a**), which can be prepared from the more-stable intermediates **1a** and **2a**. Therefore, to increase the yield, the more stable starting material (*i.e.*, **2a** in case of **6b**, and **1a** in case of **6c**) was used in fourfold excess. The reaction was repeated six times each, giving average yields of 18, 7, 5, and <2% of **6a**, **6b**, **6c**, and **6d**, respectively (see *Table* in the *Exper. Part*).

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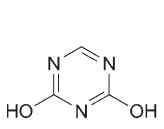
[2]) Systematic name: dicarbonimidic diamide.



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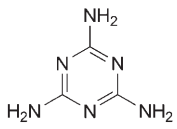

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1,3,5-Triazine-2,4-diol (**7**), which was isolated as a by-product of **6c** and **6d**, had been first described by *Bredereck et al.* [15] upon pyrrolysis of formylurea and formylamide. In our experiment, melamine (=1,3,5-triazine-2,4,6-triamine; **8**) was isolated as a major compound in the synthesis of **6b**–**6d**. Note that this compound is also formed as a pyrrolysis product of **2b** as well as of **1b** [16].

Conclusions. – As a follow up of the previous research on the synthesis of hypoxanthine from simple organic precursors, we have demonstrated that guanine can be obtained from the condensation reaction of *N*,*N*′-diformylbiuret with glycinamide in the presence of P_2O_5. In addition, when using amino-acetamidine as precursor, adenine and diaminopurine could also be obtained. These experiments should be considered as a generic approach to obtain the naturally occurring purine bases **6** from a single amino acid derivative **1** and an urea derivative **2**. Therefore, retrosynthetic analysis indicates a horizontal dissection of the purine skeleton in contrast to vertical dissection, as reported in the *Sarassin–Wegman* or *Traube* approaches towards purines. The question whether this chemistry was involved in the prebiotic synthesis of purine bases is not easy to answer. What speaks against such a theory is that this type of chemistry is certainly not adapted to early-Earth conditions and that the triazines, obtained as major compounds, are of no biochemical use. The latter observation may be rationalized by the hypothesis that the base-pairing properties (selectivity and strength) of potential prebiotic triazine heterocycles are inferior to those of the canonical nucleobases (in function of storage and transfer of information). Experimental evidence for this hypothesis has been reported recently based on 2,4-dioxotriazines as model compounds [17].

Experimental Part

1. *General*. TLC: *Merck* silica gel *60-F*$_{254}$ coated aluminum sheets. Prep. TLC: *Merck* silica gel *60-F*$_{254}$ (0.25 mm), eluting with CH_2Cl_2/EtOH/NH_4OH 2:1:0.5. HPLC: *C18* column, eluting with 25 mM aq. $NH_4^+HCO_2^-$. UV Spectra: *Varian Cary-Bio-300* spectrometer, in UV-grade MeOH. ^{1}H-NMR Spectra were recorded on a *Bruker AMX-300* spectrometer at 300 MHz. ESI-MS: *Q-Tof-2* apparatus (*Micromass*, Manchester, UK); samples were infused in a i-PrOH/H_2O 1:1 (3 μl/min).

2. *Preparation of Starting Materials.* 2.1. *Glycine Amide* (**1a**) *and 2-Aminoethanimidamide* (**1b**). Commercially available glycine amide hydrochloride (**1a**·HCl; 1 g) or amino-acetamidine bis(hydrobromide) (**1b**·2 HBr; 1.0 g), resp., was suspended in CH_2Cl_2 (30 ml). Et_3N (3 ml) was added, and the mixture was stirred for 48 h at r.t. The resulting precipitate was filtered off and dried at 25° over KOH under reduced pressure for 2 h. Yield 665 mg (94%) of **1a** and 318 mg (91%) for **1b**. Since salt-free **1b** is air-sensitive, it was used immediately in the next step.

2.2. *1,3-Diformylurea* (**2a**) *and* N,N′*-Diformyltricarbonodiimidic Diamide* (**2b**). Compound **2a** was prepared as described in [4]. The same protocol was applied also for the preparation of **2b** (18% yield), or an alternative one (60%) [14]. M.p. of **2b**: 180–182° (H_2O).

3. *Condensation to Purines.* In a glass vial, 1 equiv. (17.5 mmol) of the appropriate amino acid precursor **1**, 1 equiv. (17.5 mmol) of the corresponding urea derivative **2**, and 3 equiv. of P_2O_5 (50 mg, 35.2 mmol) were carefully mixed and flushed with nitrogen. The vial was sealed, and then heated at 170° (oil-bath temp.) for 2 h. The resulting black foam was treated with conc. aq. NH_3 (0.1 ml) and MeOH (1.9 ml) (or DMF in case of **6c**). The mixture was solubilized by sonication for 20 min. In case of the synthesis of **6b** or **6c**, the precursors **2a** and **1a**, resp., were used in four-fold excess (70 mmol) to increase the yield of the desired purine base.

4. *Preparation of UV Standards.* A total of 6.3 mmol of commercially available hypoxanthine (**6a**), adenine (**6b**), or 9*H*-purine-2,6-diamine (**6d**), resp., and P_2O_5 (20 mg) were dissolved in conc. aq. NH_3 (0.1 ml) and MeOH (1.9 ml). For baseline correction, P_2O_5 (20 mg) was dissolved in conc. aq. NH_3 (0.1 ml) and MeOH (1.9 ml), and 2 µl of this soln. was diluted with 998 µl of MeOH. Due to solubility problems in the case of guanine (**6c**), the reaction was quenched with conc. aq. NH_3 (0.1 ml), and DMF (1.9 ml) was added instead of MeOH, the baseline being recorded for the corresponding system containing DMF.

5. *Compound Purification.* Each condensation was carried out six times. The reaction was first analyzed by UV and MS. The crude yellow mixture was then purified by prep. TLC, eluting with EtOH/CH_2Cl_2/NH_4OH 1:2:0.25 or, in the case of **6c**, with EtOH/CH_2Cl_2/NH_4OH 1:1:0.25. The compounds were identified by MS analyses of all UV/VIS spots. The results are summarized in the *Table*.

Table. *Characterization of Condensation Products*

Product	Yield [%][a])	TLC R_f[b])	UV (MeOH) λ_{max} [nm]	^{13}C-NMR[c]) δ(C) [ppm]	$[M+H]^+$[d]) [*m*/*z*]
6a	18	0.68	254		137.0
6b	7	0.70	259		136.0
6c	5	0.45	248, 274		152.0
6d	<2	0.30	246, 280		151.0
7	12 (+**6b**)	0.76	255	152.2, 155.9	114.0
	17 (+**6c**)				
	15 (+**6d**)				
8	0 (+**6b**)	0.50	252	162.3	127.0
	12 (+**6c**)				
	10 (+**6d**)				

[a]) Average values ($n=6$). [b]) Solvent system: EtOH/CH_2Cl_2/NH_4OH 1:2:0.25 or, for **6c**, 1:1:0.25. [c]) Recorded at 75 MHz in (D_6)DMSO. [d]) FAB-MS.

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