ALKYLATION AND COVALENT ADDUCT FORMATION OF 2-OXO-PURINE

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Abstract - 2-Oxopurine reacted with benzyl bromide and ethanol to give the covalent adduct 1,3,7-tribenzyl-6-ethoxy-2-oxopurine, as well as dibenzylated products. Carbon - carbon bond formation was observed in the reaction between 2-oxopurine, dry silica gel, and benzyl bromide, giving rise to 6-hydroxy-1,3,8-tribenzyl-2-oxopurine.

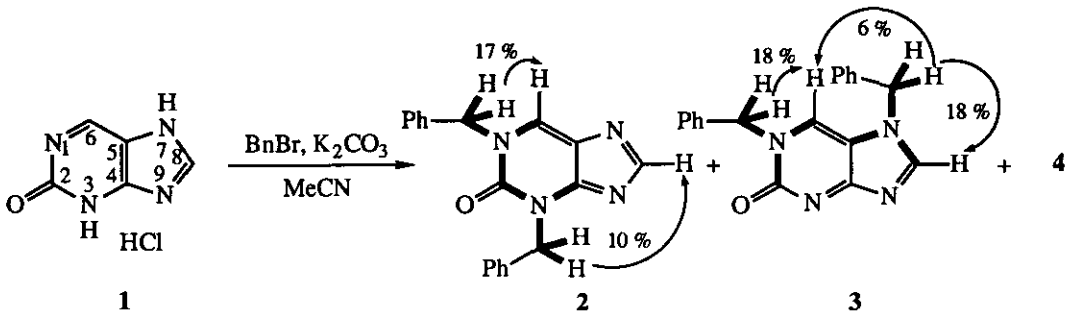
One subgroup of the biologically important purines, which to the best of our knowledge is not found in the nature and has not attracted much synthetic interest yet, is the group of 2-oxopurines. These compounds are exceptionally polarized molecules as shown by calculation of the dipole moment of the parent molecule.[1] Aromatic stabilization is absent, resulting in highly polarized double bonds with much of the electron density localized on the nitrogen atoms, thus leaving the carbon atoms electron deficient.[2] In particular, some of the 6-unsubstituted 2-oxopurines seem to be so π-electron deficient at the 6-position that almost any nucleophile adds to this electrophilic position. In many of their properties the polarized, π-electron deficient azine ring systems resemble the carbonyl group, e.g., in the 1:1 adduct formation with nucleophiles.[3,4] In related studies to the one reported here, we and others have recently exploited the corresponding propensity of pyrimidin-2-ones to form covalent adducts.[3]

6-Substituted 2-oxopurines are of considerable interest in several respects. For example, it has been reported that 6-substituted purines can inhibit endogenous protein degradation. 6-Substituted 2-oxopurines are therefore potentially useful as selective degradation inhibitors in treatment of cancer.[5] Hence, we investigated the possibility of employing the electrophilic property of the 2-oxopurines for selective introduction of substituents to the 6-position.

2-Oxopurine hydrochloride (**1**) was prepared by some modifications of the procedure previously reported by Holy.[6] Cytosine was nitrated at the 5-position, followed by reduction to the diamino compound. Final ring closure with triethyl orthoformate to form the imidazole moiety afforded the purine.

In the present communication we wish to report on some very intriguing results from our preliminary studies. It is known that the parent 2-oxopurine undergoes a slow covalent adduct formation with barbituric acid in protic solvents with concomitant decomposition.[7] To avoid the slow reaction and resulting decomposition problems we chose to work with alkylated derivatives. When 2-oxopurine hydrochloride (**1**) was alkylated

with an excess of benzyl bromide in acetonitrile, using potassium carbonate as the base, only the 1,3- and the 1,7- dialkylated isomers (**2** and **3**) were obtained.[8] The benzyl groups were chosen due to the nmr diagnostic value of the benzylic AB resonance quartets in the predicted covalent adducts,[3a-f] as well as to ensure high lipophilicity for easy chromatographic handling of the products. The regiochemical outcome of the alkylation of 2-oxopurine was revealed by long-range (3 bond) selective INEPT[9] experiments after prior assignment of the ^{1}H and ^{13}C resonances as well as by NOE difference spectroscopy[10] (Scheme 1). In the assignment of the ^{13}C resonances the general assumption that the signal at the highest field in the purine skeleton is due to C-5, is taken as the starting point.[11,12]

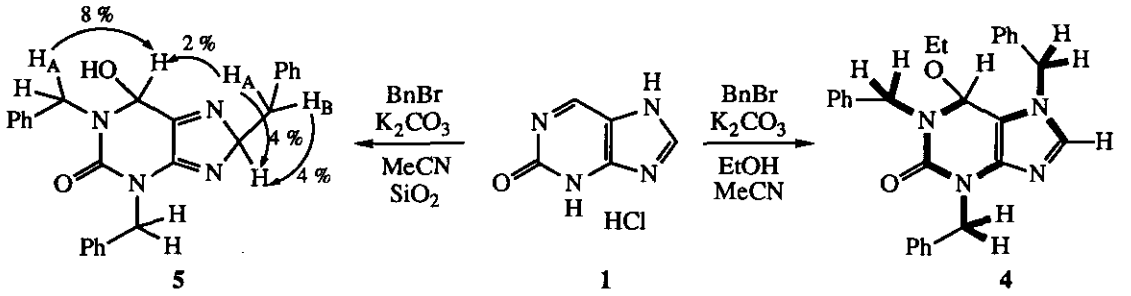


Selective INEPT correlations between benzylic protons and carbon atoms over three bonds, (solid lines in the formulas of the molecules **2** and **3**). Selected NOE effects are indicated by curved arrows.

Scheme 1

However, the major product obtained after flash chromatography with chloroform as the eluent was 1,3,7-tribenzyl-6-ethoxy-2-oxopurine (**4**) (Scheme 2).[13] Evidently a small amount of ethanol present as stabilizer in the solvent added to the 6-position, with concomitant further alkylation. Selective INEPT and NOE difference spectroscopies were again employed to prove the structure of the ethanol adduct (vide supra). When the alkylation of 2-oxopurine was carried out in the presence of a nucleophile such as ethanol, water,[14] or ethanethiol, the trialkylated ethoxy,[13] hydroxy,[14] or ethylthio adduct was the main heterocyclic product. In the reaction with ethanethiol, however, the yield of the adduct was reduced (30 %) due to the reactivity of ethanethiol towards benzyl bromide; a substantial amount of benzyl ethyl sulfide was formed. Treatment of either **2** or **3** with benzyl bromide, ethanol and potassium carbonate however, did not yield **4**. It is highly possible that 3,7-dibenzyl-2-oxopurine is the elusive intermediate giving rise to the ethoxy adduct (**4**). The ease of adduct formation was further illustrated by the fact that when 2-oxopurine was alkylated under dry conditions where every effort[15] was undertaken to exclude nucleophiles like ethanol and water, a trialkylated water adduct which is different from the other adducts was formed during the purification of the products on highly activated[15] silica gel. Nmr studies showed that the adduct formed most likely was 6-hydroxy-1,3,8-tribenzyl-2-oxopurine (**5**)[16] (Scheme 2). The ^{1}H nmr signals of **5** were assigned by COSY spectra and the position of the N-1 and C-8 substituents by NOE difference spectroscopy and decoupling experiments. The NOE's reported for **5** are small compared to the values reported for **2** and **3**, but this stems from the fact that the irradiated frequencies are very close to other nmr resonances in **5**[16] and irradiation of the selected resonances

were performed at very low power levels to ensure that the nearby resonances were not saturated.[17] Furthermore, a series of proton decoupling experiments were undertaken to verify the NOE results. Selective decoupling of H-8 (5.58 ppm) resulted in simplification of the resonances belonging to the nearby benzylic protons from an ABX pattern to a clean AB system. Similarly, decoupling of the resonances belonging to either H_A (3.23 ppm) or H_B (3.42 ppm) of the benzylic group resulted in simplification of the resonance belonging to H-8 thereby confirming the C-8 alkylation. Decoupling of the resonances belonging to the two other benzylic AB quartets resulted only in the expected simplification of the AB quartets themselves.



5 1 4

Scheme 2

The reason for the different substitution pattern of the water (**5**) and the ethanol adducts (**4**) and the unexpected and highly interesting finding of C-alkylation in the water adduct, are at the moment not fully understood. However, it is only formed in the presence of activated silica gel so that this must clearly have a role in the reaction. It has been known for a long time that C-8 alkylations can occur in purines, for instance sodium theophyllinate gives rise to C-8 substituted products when reacted with allylic and benzylic halides.[18] It has been suggested in the literature that C-8 alkylations of this type might occur more widely than is presently appreciated.[19] One possible explanation for the C-8 alkylation might be found in the fact that unsubstituted purines are at the same time both nitrogen and carbon acids (at C-8), as well as they have ylide character.[20] Alkylation using acetone as solvent gave a product due to addition of the enolate anion of the solvent.

This initial study of the reactivity of the 2-oxopurine shows that oxygen and sulfur substituents can easily be introduced to the 6-position by nucleophilic addition to the π-electron deficient ring system. The results reported here shed new light on the reasons for the low yields reported in the literature, and in previous studies of 2-oxopurine alkylations.[6] 2-Oxopurines are here shown to posses an inherent lability, which when manipulated carefully can be exploited in the construction of potentially valuable purines. We envisage that the chemistry reported here might readily be extended to the addition of carbon nucleophiles, such as organometallic reagents, to facilitate carbon-carbon bond formation at the 6-position.

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8. Benzyl bromide (375 µl, 3.15 mmol) was added to a mixture of 2-oxopurine hydrochloride (**1**) (173 mg, 1.00 mmol) and anhydrous potassium carbonate (276 mg, 2 mmol) in dry acetonitrile (10 ml). After stirring under N_2 at ambient temperature for 12 h the reaction mixture was filtered, the filtrate was evaporated, and the products were isolated by gradient flash chromatography on silica gel using chloroform-acetonitrile (10:1 to 1:1) for elution. 1,3-dibenzyl-2-oxopurine (**2**): mp 208-211 °C. Yield 77 mg, (24 %). ^{1}H Nmr (300 MHz, $CDCl_3$); δ 5.12 (s, 2H, N(1)-CH_2), 5.18 (s, 2H, N(3)-CH_2), 7.3 - 7.4 (m, 10H, Ph), 7.61 (s, 1H, H-8), 8.00 (s, 1H, H-6). ^{13}C Nmr (75 MHz, DMSO-d_6); δ 54.0 (CH_2), 54.6 (CH_2), 123.1 (C-5), 127.8, 127.8, 128.0, 128.1, 128.6, 128.7 and 128.9 (CH in Ph), 136.6 and 137.1 (C in Ph), 141.7 (C-6), 148.4 (C-8), 154.8 (C-2), 159.0 (C-4). Ms (E.I.); 317 (18, M+1), 316 (78, M$^+$), 225 (27), 211 (21), 91 (100), 65 (27). 1,7-dibenzyl-2-oxopurine (**3**): mp 190 °C (decomp.). Yield 51 mg (16 %). ^{1}H Nmr (300 MHz, acetone-d_6); δ 5.09 (s, 2H, N(1)-CH_2), 5.22 (s, 2H, N(7)-CH_2), 7.3 - 7.4 (m, 10H, Ph), 8.05 (s. 1H, H-6), 8.28 (s, 1H, H-8). ^{13}C Nmr (75 MHz, DMSO-d_6); δ 48.8 (CH_2), 53.8 (CH_2), 115.47 (C-5), 127.9, 128.2, 128.3, 128.4, 128.6 and 128.9 (CH in Ph), 135.0 (C-6), 135.9 and 137.0 (C in Ph), 155.37 (C-2), 155.41 (C-8), 168.7 (C-4).
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13. Benzyl bromide (375 µl, 3.15 mmol) and ethanol (1.0 ml, 17 mmol) was added to a mixture of 2-oxopurine hydrochloride (**1**) (174 mg, 1.00 mmol) and anhydrous potassium carbonate (280 mg, 2 mmol) in dry acetonitrile (10 ml). After stirring under N_2 at ambient temperature for 12 h the reaction mixture was filtered, the filtrate was evaporated, and the products were isolated by flash chromatography on silica gel using chloroform-acetonitrile (10:1 to 1:1) for elution. 6-ethoxy-1,3,7-tribenzyl-2-oxopurine (**4**) : Yield 243 mg (54 %); colourless oil, ^{1}H Nmr (300 MHz, $CDCl_3$); δ 0.93 (t, J 6.9 Hz, 3H, Me), 2.81 (m,

1H, CH_2O), 3.05 (m, 1H, CH_2O), 4.27 (AB, J 15.0 Hz, H_A, 1H, N(1)-CH_2), 4.95 (AB, J 14.9 Hz, H_A, 1H, N(7)-CH_2), 5.02 (AB, J 14.9 Hz, H_B, 1H, N(7)-CH_2), 5.14 (AB 1H, J 9.5 Hz, H_A, N(3)-CH_2), 5.17 (AB, 1H, J 9.5 Hz, H_B, N(3)-CH_2), 5.26 (AB, J 15.0 Hz, H_B, 1H, N(1)-CH_2), 5.69 (s, 1H, H-6), 7.0 - 7.3 (m, 16H, Ph and H-8). ^{13}C Nmr (75 MHz, $CDCl_3$); δ 14.6 (Me), 45.4 (N-CH_2), 47.5 (N-CH_2), 49.3 (N-CH_2), 55.6 (O-CH_2), 78.7 (C-6), 103.1 (C-5), 127 - 129 (CH in Ph), 134.8 (C in Ph), 135.5 (C-8), 137.6 (C in Ph), 138.2 (C in Ph), 141.6 (C-4), 153.0 (C-2). Ms (E.I.); 452 (23, M^+), 422 (9), 407 (37), 91 (100), 65 (17).

14. This reaction gave low yield due to formation of several other unidentified products.
15. The silica gel (E. Merck) was dried at 300 °C under high vacuum for 24 h and the reaction mixture was eluted with dry acetonitrile and chloroform. The chloroform was purified by extracting it three times with water and then dried ($MgSO_4$ and CaH_2).
16. 6-Hydroxy-1,3,8-tribenzyl-2-oxopurine (**5**): Colourless oil. ^1H Nmr (300 MHz, $CDCl_3$); δ 3.23 (dd, J 12.2 and 5.0 Hz, H_A, 1H, C(8)-CH_2), 3.42 (dd, J 12.2 and 5.0 Hz, H_B, 1H, C(8)-CH_2), 4.17 (AB, J 15.0 Hz, H_A, 1H, N(1)-CH_2), 5.02 (AB, J 16.1 Hz, H_A, 1H, N(3)-CH_2), 5.07 (AB, J 16.1 Hz, H_B, 1H, N(3)-CH_2), 5.17 (AB, J 15.0 Hz, H_B, 1H, N(1)-CH_2), 5.58 (m, 1H, H-8), 5.73 (s, 1H, H-6), 7.0 - 7.4 (m, 15H, Ph). Ms (E.I.); 424 (3, M^+), 423 (5), 422 (14), 408 (16), 407 (25), 317 (9), 316 (9), 289 (6), 225 (8), 91 (100), 65 (14).
17. Irradiation of other resonances than those of interest would of course give rise to other NOE effects and the results would be ambiguous. Furthermore, a short buildup time (6 sec) was used in the NOE study of this compound, resulting in small NOEs. The NOE effects reported for **5** are for this reason not the maximum equilibrium values.
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