PERKIN 1

Highly efficient C-8 oxidation of substituted xanthines with substitution at the 1-, 3-, and 7- positions using biocatalysts

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A bacterial consortium consisting of strains belonging to the genus *Klebsiella* and *Rhodococcus* quantitatively converts 1-, 3- and 7-substituted xanthines to their respective 8-oxo compounds.

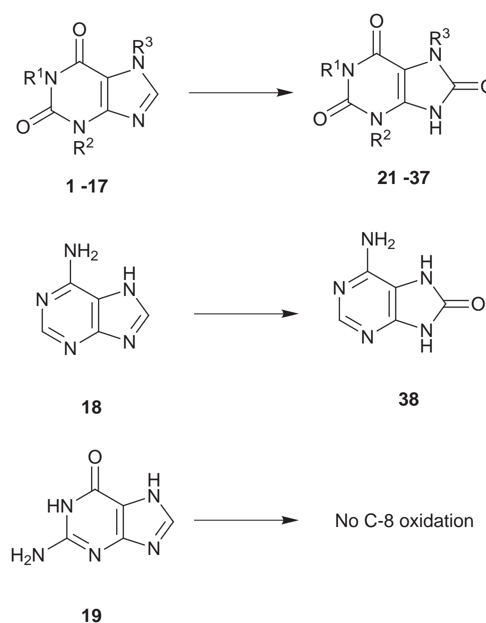
The C-8 oxidation of purine DNA bases is one of the major forms of oxidative base damage and this chemical modification has biological implications particularly in DNA replication, mutagenicity and aging.[1,2] In fact patients with leukemia excrete higher levels of 8-oxoguanine in urine than normal humans suggesting the significance of C-8 oxidation of purine bases.[3] It is also known that 8-oxo derivatives of alkyl and substituted xanthines are biologically active and have numerous applications in the formulations of drugs and cosmetics. 8-Oxocaffeine (1,3,7-trimethyluric acid) has been classified as a good radical scavenger and a potent antioxidant in model systems.[4] Studies carried out *in vitro* have demonstrated that 8-oxoxanthine (uric acid) protects erythrocytes against damage by singlet oxygen.[5] Uric acid has also been shown to inhibit lipid peroxidation,[6] protect against oxidant damage to DNA,[7] and act as a scavenger of hydroxyl radicals.[8] In fact it has been suggested that uric acid can serve as an important physiological antioxidant against oxidative injury and thus can play a role in the prevention of aging and cancer. It is interesting to note that 8-oxomethylxanthines and their derivatives are used as one of the components in obesity-treating pharmaceuticals, cosmetic skin and antidandruff preparations.[9–11] However, details of these studies are not available as they are covered by patents. To explore further the utility of various C-8 oxidized alkylxanthines and related compounds, studies have been carried out to develop synthetic routes to such compounds.[12,13]

8-Oxocaffeine is one of the metabolites of caffeine in the mammalian system.[14] Although several xanthines substituted at the 1-, 3-, and 7- positions (caffeine analogues) such as pentoxyfylline, lisofylline, enprofylline, are used as drugs,[15,16] the corresponding 8-oxo derivatives have never been prepared so far and hence these compounds are not available for biological evaluation. Since many of these substituted xanthines are water soluble, we were interested to explore the possibility of using biocatalysts as a reagent to selectively carry out C-8 oxidation in these compounds. A search for such a microbial system led to the isolation of a naturally occurring bacterial consortium consisting of strains belonging to the genus *Klebsiella* and *Rhodococcus* capable of utilizing caffeine (**1**, 1,3,7-trimethylxanthine) as the sole source of carbon and energy. Mixed cultures normally display a variety of degradative activities against substances of natural origin and in recent years they have been successfully used in the synthesis of valuable products.[17,18]

We report here that the mixed culture isolated by the enrichment culture technique using caffeine **1** as the carbon source, selectively and efficiently carries out C-8 oxidation of adenine and various 1-, 3- and 7-substituted xanthines. Many of the 8-oxo compounds (uric acids) prepared in the present investigation were hitherto unknown.

Results and discussion

A mixed culture grown on caffeine **1** transformed quantitatively theophylline (1,3-dimethylxanthine, **2**), theobromine (3,7-dimethylxanthine, **3**) and paraxanthine (1,7-dimethylxanthine, **4**) to their corresponding C-8 oxidized compounds **22**–**24** (Table 1 and Scheme 1). The HPLC analyses revealed that the conversions were quantitative and the isolated yields were 95–97%. The 8-oxo compounds **22**–**24** formed were fully characterized by comparison (NMR, MS, HPLC) with authentic samples and also the spectral characteristics agreed with the earlier reports for these compounds.[19,20] When caffeine **1** was used as the substrate, it was rapidly metabolized and although all the substrate **1** was transformed, the amount of the 8-oxo compound (1,3,7-trimethyluric acid, **21**) present in the incubation medium after 12 h of incubation was significantly low (35%, on the basis of HPLC analysis). However, incubation carried out in the presence of *N*-methylmaleimide (see Experimental section) resulted in the quantitative conversion of **1** into **21**. This suggests that enzymes involved in the further metabolism of **21** are inhibited by the thiol-group blocking reagent. The spectral characteristics (NMR, MS, HPLC) of compound **21** were in good agreement with the authentic sample of 1,3,7-trimethyluric acid **21**.



Scheme 1

Table 1 C-8 oxidation of substituted xanthines with substitution at the 1-, 3-, and 7-positions using biocatalysts

Substrate	R^1	R^2	R^3	Product
1[a]	CH_3	CH_3	CH_3	**21**
2	CH_3	CH_3	H	**22**
3	H	CH_3	CH_3	**23**
4	CH_3	H	CH_3	**24**
5	CH_3CH_2	CH_3	CH_3	**25**
6	$CH_3CH_2CH_2$	CH_3	CH_3	**26**
7	$CH_3CH_2CH_2CH_2$	CH_3	CH_3	**27**
8	$CH_3CH_2CH_2CH_2CH_2$	CH_3	CH_3	**28**
9	CH_2CH_2OH	CH_3	CH_3	**29**
10	$PhCH_2$	CH_3	CH_3	**30**
11	$CH_3COCH_2CH_2CH_2$-CH_2	CH_3	CH_3	**31**
12	CH_3COCH_2	CH_3	CH_3	**32**
13	CH_2=$CHCH_2$	CH_3	CH_3	**33**
14	$CH{\equiv}CCH_2$	CH_3	CH_3	**34**
15	CH_2=$CHCH_2CH_2$	CH_3	CH_3	**35**
16	H	$CH_3CH_2CH_2$	H	**36**
17	CH_3	$CH_3CH_2CH_2$	CH_3	**37**

[a] Incubation of **1** and **18** were carried out in the presence of *N*-methylmaleimide (1 mM). Details are given in the Experimental section.

In order to investigate the specificity of this bacterial consortium for inserting an oxygen atom at the C-8 position, we synthesized a range of 1-, 3-, and 7- substituted xanthines **5–17** (Table 1) and used them as substrates. It was observed that caffeine **1** grown cells convert quantitatively analogues of theobromine **3** with N-1-H replaced by various groups such as alkyl (**5–8**), hydroxyethyl (**9**), benzyl (**10**), 5-oxohexyl (**11**), 2-oxopropyl (**12**), allyl (**13**), propynyl (**14**) and but-3-enyl (**15**) to their corresponding C-8 oxidized compounds (**25–35**, Table 1). The compounds **25–35** (Table 1) were fully characterized by various spectral analyses (see Experimental section) and as far as we know these uric acids **25–35** were hitherto unknown. The optimal size of alkyl substituent at the N-1 position appears to be pentyl and any substitution higher than pentyl, for example 1-hexyl-3,7-dimethylxanthine was not accepted by the mixed culture as a substrate. However, pentoxyfylline **11** with a $-(CH_2)_4COCH_3$ substitution at the N-1 position was readily accepted by the bacterial consortium and quantitatively converted into its corresponding C-8 oxidized product **31** (Table 1). It is interesting to note that theophylline **2** analogues prepared by replacing N-7-H with alkyl substitution higher than methyl, for example, ethyl, propyl and butyl, were not accepted by the mixed culture as substrates, possibly due to the proximity of the bulky substituents to the C-8 position. However, the bacterial consortium readily accepted 3-propylxanthine **16** (enprofylline) and 3-propyl-1,7-dimethylxanthine **17** as substrates and both these compounds (**16**, **17**) were converted quantitatively into their corresponding 8-oxo derivatives **36**, **37**. The compounds **36** and **37** appear to be hitherto unknown and have been fully characterized by various spectral analyses (see the Experimental section). These results suggest that replacement of the N-3 methyl in caffeine **1** with a higher alkyl group such as propyl has no effect on the C-8 oxidation, whereas a substituent at the N-7 position with an alkyl group higher than methyl is not accepted by the mixed culture as a substrate.

Since purine bases (**18**, **19**) have considerable structural similarities with caffeine **1**, we were interested in finding out whether the mixed culture has the ability to carry out C-8 oxidation of these compounds. Although caffeine **1** grown cells readily metabolized both adenine **18** and guanine **19**, the HPLC analyses of the incubation mixture indicated the absence of any 8-oxo derivatives of **18** and **19**. However, incubation of **18** carried out in the presence of *N*-methylmaleimide (see the Experimental section) resulted in the accumulation (70%, Table 1) of a metabolite which was identified as 8-oxoadenine **38** based on spectral analyses.[21] Under these experimental conditions, we could not demonstrate the formation of 8-oxoguanine from gaunine. It is quite possible that guanine is metabolized following a different pathway. It is interesting to note that the mixed culture does not accept adenosine **20** as the substrate.

Enzymatic hydroxylation at the C-8 position of xanthine derivatives is effected either by a mixed function oxidase or xanthine oxidase.[22] We have noticed that cell-free extract prepared from the mixed culture readily carries out C-8 oxidation of various substituted xanthines in the presence of PES (phenazine ethosulfate)† (unpublished observation). So it is quite possible that the C-8 oxidation is mediated by xanthine oxidase. However, xanthine oxidases isolated from various microbial sources have never been shown to accept trimethyl- and dimethylxanthines as substrates.[23,24] The exceptional ability of the mixed culture to convert 1,3,7-trimethylxanthine and various 1-, 3-, and 7- substituted xanthines to their corresponding 8-oxo derivatives in quantitative yields clearly distinguishes the xanthine oxidase from this mixed culture from previously reported xanthine oxidases from various microbial sources.

The results of the present investigation document a microbial method for carrying out selectively and efficiently the C-8 oxidation of substituted xanthines (**1–17**). In all the cases, no *N*-oxide derivatives were formed suggesting that the organism is not capable of addition of O_2 onto the nitrogen atom(s) of the imidazole or purine ring. In all the cases, the products formed were free from the substrates and there were no side products and hence did not require any further purification. Considering the fact that derivatives of uric acids have numerous applications, the scope for the biocatalyst mediated process for the preparation of these compounds appears to be unlimited.

Experimental

IR spectra were recorded using a Perkin-Elmer spectrometer. NMR studies were carried out at 90 MHz using a JEOL FT-90 spectrometer or at 300 MHz using a JEOL JNM-LA 300 FT spectrometer. *J* values are quoted in Hz and chemical shifts are reported relative to TMS. Mass spectra were determined using a JEOL JMS-DX 303 spectrometer. HPLC analysis was carried out on a Shimadzu CR7A instrument, on a reversed phase ODS column using either NaOAc (0.1 M)–CH_3OH–CH_3CN (70:20:10, v/v) or H_2O–CH_3OH–CH_3CN (70:10:20, v/v) and the solvent system and eluents (1 ml min^{-1}) were monitored with a UV detector at 254 nm. TLC analyses were performed on silica gel GF_{254} plates (0.5 mm) developed with $CHCl_3$–CH_3OH (92:8, v/v).

Materials

Caffeine **1**, theophylline (1,3-dimethylxanthine, **2**), theobromine (3,7-dimethylxanthine, **3**), paraxanthine (1,7-dimethylxanthine, **4**), 1,3-dimethyluric acid, **22**, 3,7-dimethyluric acid **23**, 1,7-dimethyluric acid **24**, 1,3,7-trimethyluric acid **21**, adenine **18**, and guanine **19** were purchased from Sigma. Chloroacetone was purchased from Fluka. Enprofylline (3-propylxanthine, **16**) was a generous gift from Dr Hans Jurgen Federsel, Astra Production Chemicals AB, Sweden. Chlorohexane was prepared following the method reported earlier.[25] Other bromides used in the present study were prepared according to the standard procedure given in Vögel.[26]

Synthesis of *N*-1 substituted theobromines (5–15)

Substituted theobromines **5–15** were synthezised as reported earlier.[27] A mixture of theobromine (1.0 g, 5.55 mmol), sodium hydroxide (8 cm^3, 3.2 mmol), alkyl/allyl/propynyl/but-3-enyl/

† The IUPAC name is *N*-ethylphenazin-5-ium ethylsulfate.

hydroxyethyl/benzyl bromides (11 mmol) and propan-2-ol (12 cm^3) was taken in a sealed tube and heated at 120 °C for 24 h. The reaction mixture was allowed to cool to room temperature and extracted with chloroform (50 cm^3 × 4). The organic layer, evaporated and purified by column chromatography over silica gel using $CHCl_3$–CH_3OH (97 : 3, v/v), yielded pure **5**–**15** and yields varied from 45–83%.

Synthesis of 3-propyl-1,7-dimethylxanthine (17)

The compound **17** was prepared following the published procedure.[28] To a stirred suspension of 3-propylxanthine (0.97 g, 5 mmol) and anhydrous K_2CO_3 in 8 cm^3 of DMF was added dropwise 15 mmol of methyl iodide. The reaction mixture was heated at 35 °C for 4 h and the volatile material removed under vacuum. The product was isolated by adding water and extracted with ethyl acetate. The crude product was purified by column chromatography over silica gel using $CHCl_3$–CH_3OH (97 : 3, v/v) and yielded pure **17**. Following the same procedure,[28] *N*-7-substituted theophyllines were prepared using theophylline **2** and alkyl halide.

Microorganism and growth media

The mixed culture used in this study was isolated from the garden soil using caffeine **1** as the carbon source. It was shown to be a mixed population of two bacterial strains. Based on various morphological, cultural and biochemical characteristics, the two organisms were identified as belonging to the genus *Klebsiella* and *Rhodococcus*. The mixed culture was maintained at 3 °C on nutrient agar slants containing 0.04% of caffeine **1** and 0.1% glucose. It was also maintained regularly in a liquid mineral salts medium[29] containing 0.04% caffeine **1** and 0.1% glucose. The starter culture was prepared by transferring an aliquot (5 cm^3) from the maintenance culture to a 100 cm^3 sterilized liquid mineral salts medium (pH 7.2) containing 0.04% caffeine **1** and 0.1% glucose and incubating this on a rotary shaker (220 rpm) at 29–30 °C for 36 h. Although the mixed culture accepts caffeine **1** as the sole source of carbon, the growth rate is slow. Hence to enhance the growth rate, 0.1% glucose was added to the medium.

General procedure for biotransformation

A batch of 15 flasks containing 100 cm^3 of sterile mineral salts medium[29] (pH 7.2) containing 0.1% glucose and 0.04% of **1** were inoculated from a 36 h old culture (5 cm^3, $A_{660} = 1.1$) and incubated on a rotary shaker at 29–30 °C for 36 h. At the end of the incubation period, the cells were harvested by centrifugation (3000 *g* for 20 min), washed well with phosphate buffer (0.03M, pH 7.2) and suspended in the same buffer (~1.5 g wet weight in 100 cm^3). To this cell suspension (100 cm^3), substrate (**2**–**17**, 100 mg) was added and incubated on a rotary shaker for 12 h at 29–30 °C. In the case of caffeine **1**, adenine **18**, and guanine **19**, the incubation mixture also contained *N*-methylmaleimide (1 mM) and the rest of the conditions were the same as that used for substrates **2**–**17**. At the end of the incubation period, the assay mixture was acidified to pH 5–6 and centrifuged. An aliquot from the supernatant was subjected to HPLC analyses which indicated the absence of substrate and its complete conversion to the corresponding C-8 oxidized product (**21**–**37**). For isolating the biotransformed product, the supernatant was extracted with $CHCl_3$–CH_3OH (2 : 1, v/v), the organic phase dried over Na_2SO_4 and solvent removed under reduced pressure. The residue was passed through a small pad of silica gel, eluting with $CHCl_3$–CH_3OH (95 : 5, v/v) to obtain pure 8-oxo compounds (uric acids **21**–**37**, 8-oxoadenine **38**) which were completely characterized by various spectral analyses (IR, NMR, MS). Although the HPLC analyses indicated the complete conversion (100%) of the substrates (**1**–**17**) to the corresponding C-8 oxidized products, the isolated yields of the uric acids (**21**–**37**) varies from 95–97% (Table 1). The isolated yield of 8-oxoadenine **38** was only 70%. The spectral characteristics of the 8-oxo compounds **25**–**38** are given below.

1-Ethyl-3,7-dimethyluric acid (25). Mp 295 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1695 (–NC=O–); δ_H(300 MHz, $CDCl_3$) 1.2 (3H, t, *J* 7.2, CH_3CH_2–), 3.5 (3H, s, $–NCH_3$), 3.58 (3H, s, $–NCH_3$), 4.0 (2H, q, *J* 7.2, CH_2CH_3); δ_C(75 MHz, $CDCl_3$) 14.2, 30.0, 30.8, 41.2, 99.0, 136.7, 150.9, 153.7, 154.0; *m*/*z* 224 (M^+, 65%), 153 (40%), 82 (100%), 67 (55%); HRMS: found M^+, 224.0900. $C_9H_{12}N_4O_3$ requires 224.0909.

1-Propyl-3,7-dimethyluric acid (26). Mp 280 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1695 (–NC=O–); δ_H(300 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.2, $CH_3CH_2CH_2$–), 1.67 (2H, m, $CH_3CH_2CH_2$–), 3.5 (3H, s, $–NCH_3$), 3.56 (3H, s, $–NCH_3$), 3.9 (2H, t, *J* 7.2, $–NCH_2$–); δ_C(75 MHz, $CDCl_3$) 12.4, 22.3, 29.0, 31.2, 41.4, 98.9, 136.7, 150.0, 153.0, 154.5; *m*/*z* 238 (M^+, 100%), 219 ($M^+ - H_2O$, 50%), 196 ($M^+ - C_3H_7$, 70%), 153 (60%), 82 (75%); HRMS: found M^+, 238.1061. $C_{10}H_{14}N_4O_3$ requires 238.1065.

1-Butyl-3,7-dimethyluric acid (27). Mp 268–270 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1700 (–NC=O–); δ_H(300 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.2, $CH_3(CH_2)_3$–), 1.4–1.6 (4H, m, CH_2CH_2–CH_2–N–), 3.5 (3H, s, $–NCH_3$), 3.57 (3H, s, $–NCH_3$), 3.97 (2H, t, *J* 7.2, $–NCH_2$-); δ_C(22.5 MHz, $CDCl_3$) 13.8, 20.2, 28.8, 30.0, 30.8, 41.4, 99.0, 136.0, 150.0, 153.0, 153.7; *m*/*z* 252 (M^+, 100%), 225 ($M^+ - H_2O$, 55%), 196 ($M^+ - C_4H_9$, 95%), 153 (90%), 82 (80%); HRMS: found M^+, 252.1218. $C_{11}H_{16}N_4O_3$ requires 252.1222.

1-Pentyl-3,7-dimethyluric acid (28). Mp 265 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1695 (–NC=O–); δ_H(200 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.2, CH_3–$(CH_2)_4$–), 1.35 (4H, m, $CH_3CH_2CH_2$), 1.6 (2H, m, $–CH_2CH_2CH_2$–N–), 3.5 (3H, s, $–NCH_3$), 3.56 (3H, s, $–NCH_3$), 3.97 (2H, t, *J* 7.2, $–NCH_2$–); δ_C(75 MHz, $CDCl_3$) 13.9, 22.3, 27.6, 29.0, 29.5, 33.4, 41.0, 107.0, 141.0, 148, 151, 155; *m*/*z* 250 (M^+, 70%), 180 ($M^+ - C_5H_{11}$, 100%); HRMS: found M^+, 250.1435. $C_{12}H_{18}N_4O_2$ requires 250.1429.

1-(2-Hydroxyethyl)-3,7-dimethyluric acid (29). Mp 255 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 3300 ($–CH_2OH$), 1640, 1680, 1690 (–NC=O–); δ_H(90 MHz, DMSO-d_6) 3.58 (6H, s, 2 × NCH_3), 3.8 (2H, t, *J* 7.2, $–CH_2$–OH), 4.15 (2H, t, *J* 7.2, $–CH_2N$–); δ_C(22.5 MHz, DMSO-d_6) 29.0, 30.8, 45.6, 59.0, 97.0, 136.1, 150.2, 153.3, 153.9; *m*/*z* 240 (M^+, 70%), 196 ($M^+ - CH_2$-CH_2OH, 60%), 153 (65%), 82 (100%); HRMS: found M^+, 240.0852. $C_9H_{12}N_4O_4$ requires 240.0858 (Found: C, 44.89; H, 4.92; N, 23.12. $C_9H_{12}N_4O_4$ requires C, 45.0, H, 5.0, N, 23.37%).

1-Benzyl-3,7-dimethyluric acid (30). Mp 315 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1695 (–NC=O–); δ_H(200 MHz, $CDCl_3$) 3.4 (3H, s, $–NCH_3$), 3.5 (3H, s, $–NCH_3$), 5.0 (2H, s, $–NCH_2$–), 7.2–7.4 (5H, m, aromatic); δ_C(22.5 MHz, DMSO-d_6) 28.0, 30.8, 43.0, 97.0, 126.9, 127.3, 127.6, 136.9, 137.5, 149.8, 151.6, 152.5; *m*/*z* 286 (M^+, 100%), 195 (30%), 91 (C_6H_5, 75%); HRMS: found M^+, 286.2896. $C_{14}H_{14}N_4O_3$ requires 286.2899 (Found: C, 58.64; H, 4.81; N, 19.48. $C_{14}H_{14}N_4O_3$ requires C, 58.74; H, 4.89; N, 19.58%).

1-(5-Oxohexyl)-3,7-dimethyluric acid (31). Mp 223–225 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1700 (–C=O–), 1640, 1680 (–NC=O–); δ_H(300 MHz, $CDCl_3$) 1.65 (4H, m, $–CH_2CH_2$), 2.1 (3H, s, CH_3–CO–), 2.5 (2H, t, *J* 7.2, –CO–CH_2–), 3.5 (3H, s, $–NCH_3$), 3.57 (3H, s, $–NCH_3$), 3.9 (2H, t, *J* 7.2, $–NCH_2$–); δ_C(22.5 MHz, $CDCl_3$) 21.0, 27.4, 28.9, 29.7, 30.8, 41.6, 43.0, 99.2, 136.0, 150.2, 153.3, 153.5, 208.0; *m*/*z* 294 (M^+, 100%), 196

($M^+ - C_6H_{11}O$, 95%), 153 (70%), 82 (55%); HRMS: found M^+, 294.1295. $C_{13}H_{18}N_4O_4$ requires 294.1325 (Found: C, 52.88; H, 6.04; N, 18.95. $C_{13}H_{18}N_4O_4$ requires C, 53.06; H, 6.12; N, 19.04%).

1-(2-Oxopropyl)-3,7-dimethyluric acid (32). Mp 240 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1700 (–C=O–), 1640, 1680 (–NC=O–); δ_H(90 MHz, $CDCl_3$) 2.1 (3H, s, CH_3CO–), 3.5 (3H, s, –NCH_3), 3.57 (3H, s, –NCH_3), 4.7 (2H, s, –NCH_2–); δ_C(75 MHz, $CDCl_3$) 28.0, 30.6, 33.8, 47.8, 98.2, 136.1, 150.0, 153.3, 153.5, 204.0; *m/z* 252 (M^+, 100%), 209 ($M^+ - CH_3CO$, 75%), 196 ($M^+ - C_3H_5O$, 85%); HRMS: found M^+, 252.0852. $C_{10}H_{12}N_4O_4$ requires 252.0858.

1-Allyl-3,7-dimethyluric acid (33). Mp 305–306 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1690 (–NC=O–); δ_H(200 MHz, $CDCl_3$) 3.5 (3H, s, –NCH_3), 3.56 (3H, s, –NCH_3), 4.59 (2H, td, *J* 4.5, 13, –N–CH_2–CH=CH_2), 5.2 (1H, d, *J* 10, *cis* CH=CH_2), 5.3 (1H, d, *J* 17, *trans* CH=CH_2), 5.97 (1H, tdd, *J* 17, 10.3, 4.5, C*H*=CH_2); δ_C(22.5 MHz, DMSO-d_6) 28.2, 30.9, 42.5, 97.8, 116.5, 133.0, 136.8, 149.5, 151.8, 152.3; *m/z* 236 (M^+, 100%), 219 ($M^+ - H_2O$, 15%), 153 (40%), 82 (45%); HRMS: found M^+, 236.0900. $C_{10}H_{12}N_4O_3$ requires 236.0909.

1-Prop-2-ynyl-3,7-dimethyluric acid (34). Mp 276 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680 (–NC=O–); δ_H(300 MHz, $CDCl_3$) 2.1 (1H, t, *J* 2.4, HC=C–CH_2), 3.5 (3H, s, –NCH_3), 3.59 (3H, s, –NCH_3), 4.6 (2H, d, *J* 2.7, –NCH_2–); δ_C(22.5 MHz, $CDCl_3$) 29.7, 30.3, 34.0, 71.4, 79.1, 97.0, 136.0, 150.7, 153.0, 153.8; *m/z* 234 (M^+, 100%), 153 (25%), 84 (45%), 82 (85%), 67 (57%); HRMS: found M^+, 234.0749. $C_{10}H_{10}N_4O_3$ requires 234.0752.

1-But-3-enyl-3,7-dimethyluric acid (35). Mp 262 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1690 (–NC=O–); δ_H(90 MHz, $CDCl_3$) 2.4 (2H, q, CH_2=CH–CH_2–CH_2), 3.5 (3H, s, –NCH_3), 3.58 (3H, s, –NCH_3), 4.0 (2H, t, *J* 7.2, –N–CH_2–CH_2), 5.0 (1H, d, *J* 10, *cis* CH=CH_2), 5.2 (1H, d, *J* 17, *trans* CH=CH_2), 5.8 (1H, tdd, *J* 17, 10.3, 4.5, C*H*=CH_2); δ_C(22.5 MHz, $CDCl_3$) 28.0, 30.7, 31.8, 40.3, 97.8, 116.6, 135.1, 136.4, 149.5, 151.5, 152.5; *m/z* 250 (M^+, 40%), 196 ($M^+ - C_4H_7$, 100%), 153 (45%), 82 (50%); HRMS: found M^+, 250.1061. $C_{11}H_{14}N_4O_3$ requires 250.1065 (Found: C, 52.73; H, 5.58; N, 22.18. $C_{11}H_{14}N_4O_3$ requires C, 52.8; H, 5.6; N, 22.4%).

3-Propyluric acid (36). Mp 279 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680 (–NC=O–); δ_H(300 MHz, DMSO-d_6) 0.95 (3H, t, *J* 6.6, CH_3CH_2–), 1.64 (2H, m, $CH_3CH_2CH_2$–), 3.9 (2H, t, *J* 7.5, –NCH_2–), 11.0 (1H, s, –NH); δ_C(75 MHz, DMSO-d_6) 10.9, 21.5, 44.5, 98.9, 137.7, 151, 153.4, 153.9; *m/z* 210 (M^+, 100%), 168 ($M^+ - C_3H_7$, 95%), 137 ($M^+ - C_2H_2NO_2$, 75%); HRMS: found M^+, 210.0748. $C_8H_{10}N_4O_3$ requires 210.0752.

3-Propyl-1,7-dimethyluric acid (37). Mp 282 °C ($CHCl_3$–MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680 (–NC=O–); δ_H(90 MHz, $CDCl_3$) 1 (3H, t, *J* 7.2, CH_3CH_2–), 1.6 (2H, m, $CH_3CH_2CH_2$–), 3.4 (3H, s, –NCH_3), 3.5 (3H, s, –NCH_3), 3.9 (2H, t, *J* 7.2, –NCH_2–); δ_C(75 MHz, $CDCl_3$) 10.8, 21.6, 28, 28.9, 46.0, 99.2, 135.7, 150.0, 153.0, 153.9; *m/z* 238 (M^+, 100%), 196 ($M^+ - C_3H_7$, 70%), 152 ($M^+ - C_3H_4NO_2$, 50%), 139 (65%); HRMS: found M^+, 238.1059. $C_{10}H_{14}N_4O_3$ requires 238.1065.

8-Oxoadenine (38). Mp 301 °C (decomposes)(MeOH); ν_{max} (Nujol)/cm^{-1} 1640, 1680, 1695 (–NC=O–) 3310 (–NH_2); δ_H(300 MHz, DMSO-d_6) 8.02 (H-2), 6.22 (–NH_2); *m/z* 152 ($M^+ + 1$, 25%), 136 ($M^+ + 1 - NH_2$, 100%), 109 (30%), 54 (25%), 44 (50%). The spectral characteristics agreed with the earlier report for 8-oxoadenine.[21]

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