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The first direct ammonolysis of 2-thiouracil nucleosides to 2-thiocytosine nucleosides

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Abstract

Sugar-peracetylated 1-(β -D-glucopyranosyl)-, 1-(β -D-galactopyranosyl)-, and 1-(β -D-xylopyranosyl)-6-aryl-5-cyano-2-thiouracils **2–4** and the corresponding 1-pyranosyl-2-(pyranosylthio)pyrimidines **5–7** undergo efficient ammonolysis (NH_3/MeOH , $0 \rightarrow 23^\circ\text{C}$, 16 h) to give the corresponding 1-(β -D-pyranosyl)-6-aryl-5-cyano-2-thiocytosines. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: 6-aryl-5-cyano-2-thiouracils; 1-(β -D-pyranosyl)-2-thiocytosines.

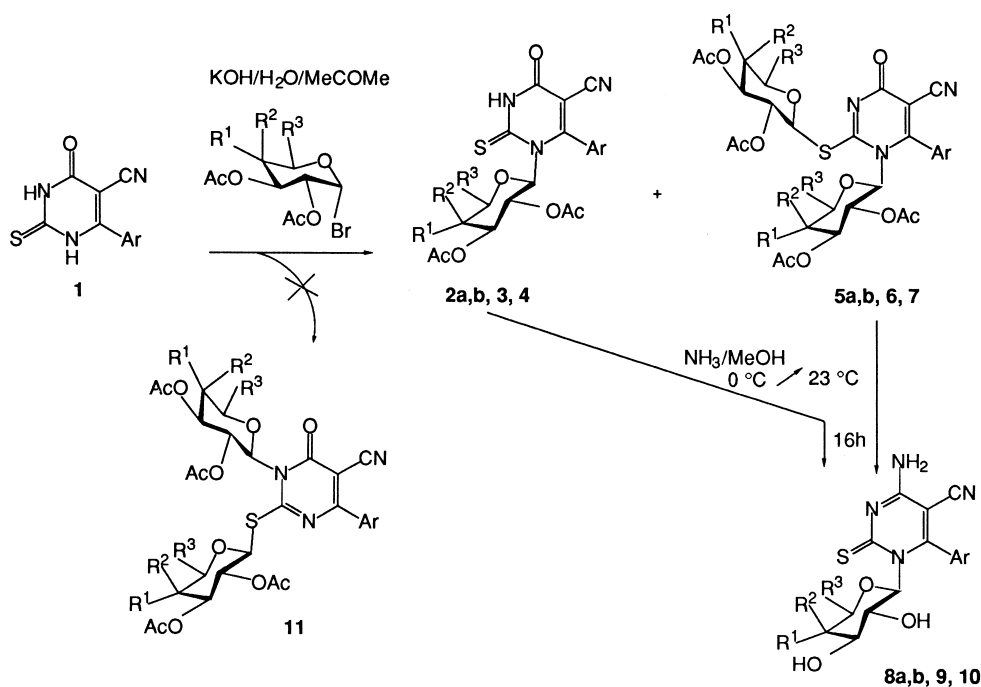
Unnatural nucleosides continue to draw attention as potential biologically active agents, and many diverse structures are being designed and synthesized. In particular, it has been reported recently¹ that the reaction of a dianion derived from a 2-thiouracil **1** with 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide or its α -D-galactopyranosyl isomer gives the corresponding *N*3,*S*²-bis(pyranosyl)pyrimidin-4-one **11** (Scheme 1). It has also been claimed that the treatment of these products with ammonia in methanol furnishes the corresponding *N*3-pyranosyluracils.

In this paper, we report a reinvestigation of the research discussed above and show that the bis-nucleosides (the major products) derived from **1** are *N*1,*S*²-bis(pyranosyl) derivatives **5** and **6** rather than the claimed *N*3,*S*²-isomers **11** and they are accompanied by *N*1-pyranosylpyrimidines **2** and **3**. Xylopyranosylpyrimidines of analogous structures **4** and **7** were also obtained as part of this work (Scheme 1). Most important, however, in our hands the deacetylation of **2–7** by treatment with ammonia in methanol, in addition to removal of the pyranosyl function from the *S*² atom at the pyrimidine of **5–7**, was also accompanied by an unprecedented ammonolysis to furnish *N*1-(β -D-pyranosyl)-2-thiocytosines **8–10** in high yields. These results are fundamentally different from those reported, and consistent results were obtained in all cases studied.

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2-10	Ar	R ¹	R ²	R ³
glucosides				
2a	phenyl	OAc	H	CH ₂ OAc
5a	phenyl	OAc	H	CH ₂ OAc
8a	phenyl	OH	H	CH ₂ OH
2b	<i>p</i> -tolyl	OAc	H	CH ₂ OAc
5b	<i>p</i> -tolyl	OAc	H	CH ₂ OAc
8b	<i>p</i> -tolyl	OH	H	CH ₂ OH
galactosides				
3	phenyl	H	OAc	CH ₂ OAc
6	phenyl	H	OAc	CH ₂ OAc
9	phenyl	H	OH	CH ₂ OH
xylosides				
4	2-naphthyl	OAc	H	H
7	2-naphthyl	OAc	H	H
10	2-naphthyl	OH	H	H

Scheme 1.

Our findings have important ramifications for the synthesis of novel cytosine nucleosides and analogs. First, in addition to the starting materials **1**, a large number of other 5-cyano-2-thiouracils are readily available.^{1–3} Second, the products **8–10** are potential substrates for the preparation of nucleosides that contain a fused heterocyclic system because the 4-amino-5-cyano functionality is a well known synthon for annulation of the pyrimidine to pyrazolopyrimidines, pyridopyrimidines, and pyrimidopyrimidines.⁴

The treatment of a solution of **1** (Ar = Ph, 10 mmol) and KOH (22 mmol) in water (6 mL) with 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (22 mmol) in acetone (30 mL) and stirring the mixture¹ (25 °C, 4 h) resulted in the disappearance of **1** and the formation of two products that

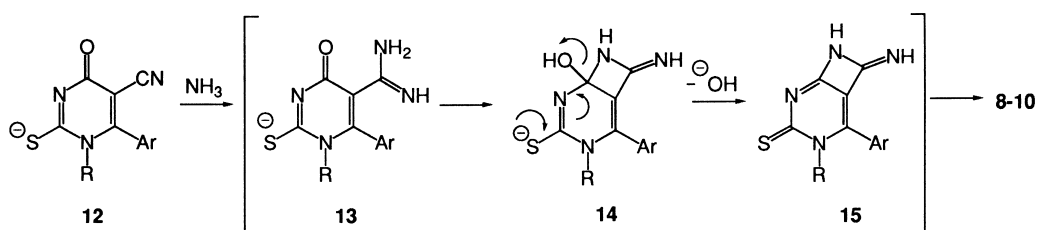
were separated by silica gel chromatography (hexanes:ether:chloroform, 1:2:2). The structures of the major and minor products were established as **5a** and **2a**, respectively, by elemental analyses, IR, and ^1H NMR including COSY and NOESY methods. In particular, the IR spectrum of **2a** shows absorptions for acetoxy carbonyl groups ($1720\text{--}1750\text{ cm}^{-1}$), and a strong signal at 1698 cm^{-1} that is also present in the IR spectrum of **1** and characteristic for the pyrimidin-4-one.⁵ In the ^1H NMR spectrum of **2a** the anomeric proton $\text{H1}'$ of the glucopyranosyl moiety gives a doublet at δ 5.99 with a coupling constant $J(\text{H1}'\text{--H2}')$ of 10.9 Hz for the diaxial interaction. First, this large coupling constant is characteristic for a β -glucopyranosyl anomer.⁶ Second, the chemical shift is typical for *N1*-substitution of 2-thiouracils, and the structurally related *N3*-substituted 2-thiouracils give a resonance for $\text{H1}'$ at a much lower magnetic field.⁷ The *N1*-substitution in **2a** was confirmed by an NOE experiment in which the irradiation at δ 5.99 for $\text{H1}'$ gave a strong signal at δ 8.08 corresponding to *ortho* protons of the adjacent phenyl group. Additional enhancements were observed at δ 5.35 and 5.15 for $\text{H3}'$ and $\text{H5}'$, respectively, and all these interactions were confirmed by analysis of the NOESY spectrum of **2a**. These conclusions derived from the NOE studies are fully supported by the results of molecular modeling by using a PC Model program. The conformational analysis of **2a** gave two low-energy conformations, *syn* (shown) and *anti*, in both of which the plane of the pyrimidine bisects the sugar moiety while the phenyl ring is twisted relative to the pyrimidine. In the more stable (by 0.15 kcal/mol) energy-minimized *syn*-structure the distances between $\text{H1}'$ of the sugar and the *ortho* protons of the phenyl are 2.59 and 3.84 Å, which is fully consistent with the observed NOE enhancements. By contrast, such distances are beyond the limit of NOE interactions for the alternative *N3*-isomer of **2a**. In the ^1H NMR spectrum of **5a** a doublet for $\text{H1}'$ at δ 5.98, $J(\text{H1}'\text{--H2}') = 10.4\text{ Hz}$, is indicative of the presence of the β -glucopyranosyl moiety at position *N1* of the pyrimidine, as in **2a**. Analysis of the NOESY spectrum of **5a** confirmed this result. The chemical shift for the anomeric proton $\text{H1}''$ of the second β -glucopyranosyl group in **5a** at δ 6.50 is too small for an *N3*-nucleoside, and the *O4*-nucleoside can be ruled out on the basis of a strong infrared absorption at 1685 cm^{-1} .

Interestingly, the reaction of **1** ($\text{Ar}=\text{Ph}$, 1 equiv.) in the presence of KOH (2.2 equiv.) with increasing amounts of 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide, from 0.5 equiv. to 2.2 equiv. required for a consumption of **1**, always furnished a mixture of **2a** and **5a**. It can be suggested that **2a** and an *S*²-linked mononucleoside are formed initially and both compounds are precursors to **5a**. Consistent with this suggestion are calculated HOMO electron densities for anions derived from **1** ($\text{Ar}=\text{Ph}$) and its *S*-methyl derivative (Fig. 1). The latter compound is a model for the presumed *S*²-linked mononucleoside. As can be seen, the sulfur atom in the dianion **1-N1-N3**[−] and monoanions **1-N1**[−], **1-N3**[−] is the most nucleophilic site. A competitive formation of the *N1*-nucleoside **2a** from each of the monoanions should be possible, as observed. On the basis of the calculated HOMO electron densities and the favorable heat of formation for **MeS1**[−] the presumed *S*²-nucleoside should be easily transformed into the *N1,S*²-bis-nucleoside **5a**, also as observed.

Ammonolysis of mononucleosides **2-4**, dinucleosides **5-7** or the mixtures **2/5**, **3/6** and **4/7** furnished the corresponding 1-(β -D-pyranosyl)-2-thiocytosines **8-10** in yields of 84–87%. In a typical experiment a solution of **5a** (1 mmol) in MeOH (10 mL) was added at 0°C to a saturated solution of NH_3 in MeOH (25 mL) and the mixture was stirred at 0°C for 4 h and then at 23°C for an additional 12 h. Concentration followed by silica gel chromatography (MeOH: CHCl_3 , 1:19) gave **8a**. Analysis of the NOESY spectra showed that products **8-10** contain a pyranosyl moiety linked to *N1* of the pyrimidine. The experimental and computer-simulated ^{13}C NMR spectra of **8-10** closely matched each other. In comparison to the infrared spectra of **2-7**, the 2-thiocytosine nucleosides **8-10** lack absorption at $1680\text{--}1690\text{ cm}^{-1}$ that is characteristic for

the pyrimidin-4-one. Their IR spectra are dominated by strong CN, OH, and NH₂ absorptions. Two sets of resonances for OH and NH₂, both exchangeable with D₂O, are observed in the ¹H NMR spectra of **8–10** as well.[§]

The suggested mechanism for **8–10** is given in Scheme 2. Removal of a sugar moiety from *S*-nucleosides under certain basic/nucleophilic conditions to give an anion such as **12** is well known.^{7–9} In addition to the standard deacetylation at the pyranosyl moiety of **12**, the cyano group of **12** may also undergo an addition reaction with ammonia to generate an amidine **13**. The subsequent intramolecular cyclization of **13** to **14** is consistent with the computational work of Fig. 1 which shows that the C4 atom in the analogous anion **1-N3⁻** is electrophilic in nature. Elimination of hydroxide ion from **14** (*sp*³-C4→*sp*²-C4) would generate a less strained system **15** which is the suggested precursor to **8–10**.



Scheme 2.

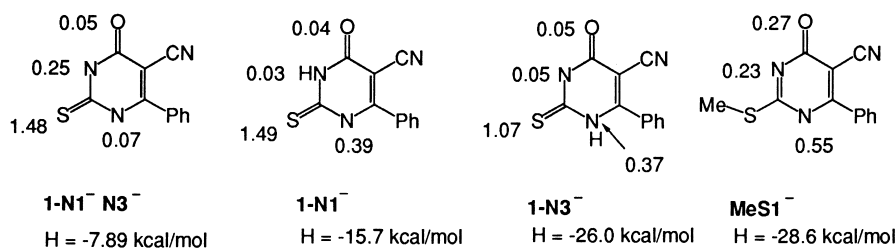


Figure 1. Calculated (PM3-AQ) HOMO electron densities for anions derived from **1** (Ar=Ph) and **MeS1** by deprotonation at the indicated sites. H denotes the calculated heat of formation

[§] Products **2–7** and **8–10** were crystallized from 95% EtOH and 95% MeOH, respectively. All compounds gave satisfactory microanalysis results (C, ± 0.3 ; H, ± 0.2 ; N, ± 0.2 ; S, ± 0.3) as exemplified for **8a**·1.5 H₂O. Calcd/found: C, 48.92/48.84; H, 5.03/5.10; N, 13.42/13.33; S, 7.67/7.73. The elemental analysis of the remaining compounds **2–10** also showed the presence of crystallization water that was demonstrated independently by the use of ¹H NMR. All yields given below are for analytically pure products. The yields of **8–10** are for ammonolysis of the respective mixtures **2a/5a**, **2b/5b**, **3/6** and **4/7**. Compound, yield, mp, α_D^{25} (*c*=2 mg/mL, CHCl₃ for **2–7** and MeOH for **8–10**): **2a**·2H₂O, 20%, 214–216°C, 38.3; **2b**·2H₂O, 30%, 189–192°C, 23.0; **3**·2.5 H₂O, 23%, 232–234°C, 36.4; **4**·H₂O, 20%, 165–168°C, 26.7; **5a**·0.5 H₂O, 40%, 114–116°C, 45.0; **5b**·0.5H₂O, 40%, 118–120°C, 38.0; **6**·H₂O, 35%, 128–130°C, 23.0; **7**·H₂O, 35%, 123–125°C, 21.6; **8a**·1.5H₂O, 86%, 190–193°C, 21.0; **8b**·2H₂O, 86%, 190–193°C, 25.5; **9**·1.5H₂O, 87%, 192–195°C, 22.6; **10**·2H₂O, 84%, 196–198°C, 16.8. Selected ¹H NMR spectra (400 MHz, 25°C, CDCl₃/TMS): **2a**, δ 1.90–2.14 (4s, 12H), 4.03 (m, 2H), 4.43 (s, 1H), 5.15 (m, 1H), 5.35 (m, 1H), 5.54 (m, 1H), 5.99 (d, *J*=10.9 Hz, 1H), 7.64 (m, 3H), 8.08 (m, 2H); **5a**, δ 1.83–2.01 (8s, 24H), 4.25 (m, 6H), 5.11 (m, 4H), 5.55 (m, 2H), 5.98 (d, *J*=10.4 Hz, 1H), 6.50 (d, *J*=8.0 Hz, 1H), 7.62 (m, 3H), 8.08 (m, 2H); **8a**, δ 3.21 (m, 4H), 3.48 (m, 1H), 3.68 (m, 1H), 4.80 (m, exchangeable with D₂O, 4H), 5.37 (d, *J*=10.0 Hz, 1H), 7.18 (bs, exchangeable with D₂O, 2H), 7.47 (m, 3H), 7.80 (m, 2H).

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