Synthesis of 2-Amino-2-deoxo-5-deazaflavins and Related Compounds

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Received April 2, 1991

10-Alkyl-2-amino-2-deoxo-5-deazaflavins were prepared by the condensation of 2-amino-6-chloro-5-formylpyrimidin-4(3*H*)-one with the corresponding *N*-alkylanilines. 2-Amino-10-*p*-tolyl-2-deoxo-5-deazaflavin was prepared by the condensation of 2-amino-6-*p*-toluidinopyrimidin-4(3*H*)-one with *o*-chlorobenzaldehyde. Some reactivities of 2-aminopyrimidin-4(3*H*)-ones are described.

J. Heterocyclic Chem., **28**, 1537 (1991).

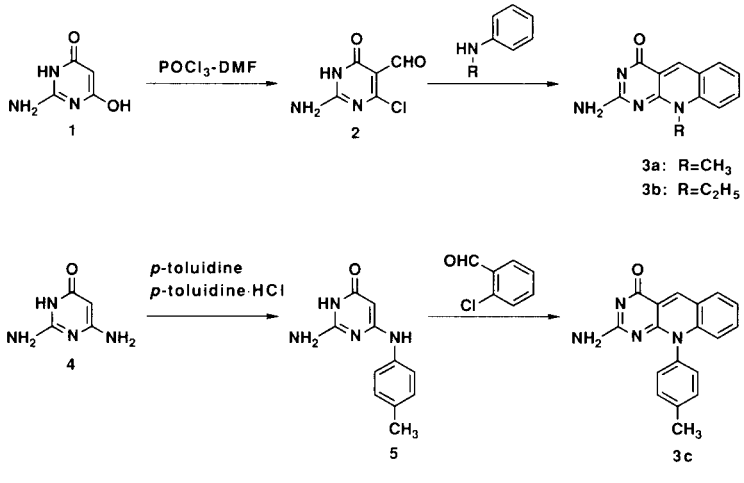
Despite the large number of publication on the chemistry of 5-deazaflavin and related compounds including 5-deazaflavin coenzymes [1], information of the 2-amino analogs of 5-deazaflavin is nil. As part of a program to develop the synthetic methodology of a wide variety of 5-deazaflavins and analogs and to explore their biological activities, we have now synthesized some 2-amino-2-deoxo-5-deazaflavins and related 2-aminopyrimidine derivatives.

The requisite starting material, 2-amino-6-chloro-5-formylpyrimidin-4(3*H*)-one (**2**) [2] was prepared by the treatment of 2-amino-4,6-dihydroxypyrimidine (**1**) with Vilsmeier reagent (DMF: phosphoryl chloride = 1:7). Compound **2** was fused with *N*-methylaniline without solvent to give 2-amino-10-methyl-2-deoxo-5-deazaflavin (**3a**) in 20% yield. 2-Amino-10-ethyl-2-deoxo-5-deazaflavin (**3b**) was likewise prepared from **2** and *N*-ethylaniline in 20% yield.

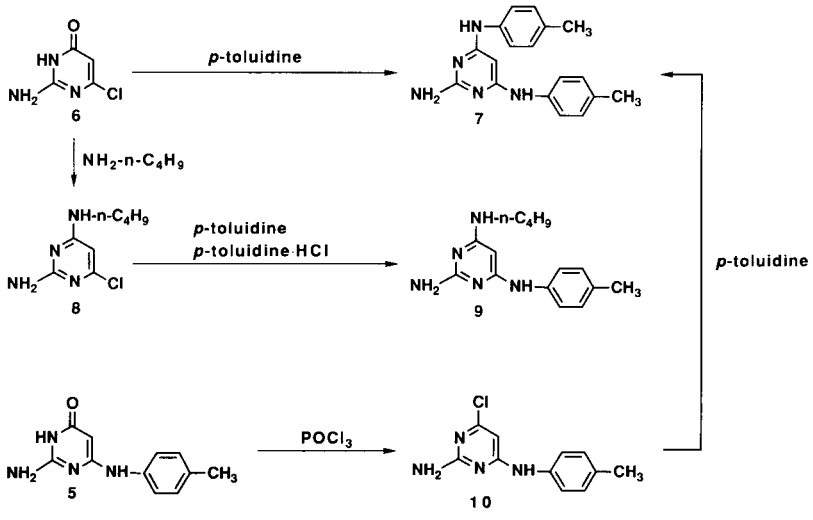
Next, we have planned to synthesize 10-aryl-2-deoxo-5-deazaflavin derivative. Thus, compound **1** was treated with phosphoryl chloride to give 2-amino-6-chloropyrimidine-4(3*H*)-one (**6**) [3]. The reaction of compound **6** with *p*-toluidine (both with or without *p*-toluidine hydrochloride) gave only 2-amino-4,6-di(*p*-toluidino)pyrimidine (**7**) in fairly good yield. However, the required 2-amino-6-*p*-toluidinopyrimidine-4(3*H*)-one (**5**) was not obtained under any reaction condition. On the other hand, the reaction of 2,6-diaminopyrimidin-4(3*H*)-one (**4**) with a mixture of *p*-toluidine and *p*-toluidine hydrochloride gave 2-amino-6-*p*-toluidinopyrimidin-4(3*H*)-one (**5**). Compound **5** thus obtained was condensed with *o*-chlorobenzaldehyde in DMF to give the expected 2-amino-10-*p*-tolyl-2-deoxo-5-deazaflavin (**3c**) in 20% yield.

In the meantime, reaction of compound **6** with excess *n*-bytylamine under reflux gave 2-amino-4-*n*-butylamino-6-chloropyrimidine (**8**) in quantitative yield. The treatment of compound **8** with a mixture of *p*-toluidine and *p*-toluidine hydrochloride gave 2-amino-4-*n*-butylamino-6-*p*-toluidinopyrimidine (**9**) in quantitative yield. Next, 2-amino-6-*p*-toluidinopyrimidin-4(3*H*)-one (**5**) was treated with phosphoryl chloride to give 2-amino-4-chloro-6-*p*-toluidinopyrimidine (**10**) in moderate yield. Then the fusion of compound **10** with excess *p*-toluidine gave 2-amino-4,6-di(*p*-

Scheme 1



Scheme 2



toluidino)pyrimidine (**7**) in high yield, which was identical in all respects with the compound **7** obtained above from compound **6** and excess *p*-toluidine.

2-Amino-10-methyl-2-deoxo-5-deazaflavin (**3a**) exhibited fairly strong inhibitory effects against calf liver dihydrofolate reductase. It roughly decreases enzyme activity to *ca* 50% at concentrations less than 20 μmoles, whereas 2-amino-10-ethyl-2-deoxo-5-deazaflavin (**3b**) had hardly any effect on the enzyme. Further enzymological study will be published elsewhere.

EXPERIMENTAL

All melting points were determined on a Yanagimoto hot-stage apparatus, and are uncorrected. The ir spectra were obtained on a Shimazu IR-400 spectrometer and ^{1}H nmr spectra on a JEOL FX 200 spectrometer. Mass spectra were taken on a JEOL JMS OISG-2 instrument by direct insertion at 70 eV. Column chromatography was carried out with Silica gel 60 (E. M. Merck, 230 mesh).

2-Amino-6-chloro-5-formylpyrimidin-4(3*H*)-one (**2**) [2].

2-Amino-4,6-dihydroxypyrimidine (**1**) (3.81 g, 30 mmoles) was dissolved in DMS (5 ml) and to the mixture phosphoryl chloride (35 ml) was added drop by drop under ice cooling and stirring. Then the mixture was stirred at 100° for 2.5 hours. After the reaction, the mixture was evaporated under reduced pressure. The residue was treated with ice water to precipitate crude product which was washed with water and methanol and dried in desiccator to yield pale yellow powder (1.46 g, 28%). As this compound was unstable, it was used for the next procedure without purification; ir (Nujol): 3400, 3320, 3200, 1688, 1662 cm^{-1}, ^{1}H nmr (200 MHz, DMSO-d_6): δ 8.51 (3H, bs, N*H* and NH_2), 10.07 (1H, s, C*H*O); ms: m/z 173 (M$^+$).

2-Amino-10-methyl-2-deoxo-5-deazaflavin (**3a**).

A mixture of compound **2** (863 mg, 5 mmoles) and *N*-methylaniline (803 mg, 7.5 mmoles) was fused at 60° for 20 minutes under argon atmosphere. The reaction mixture was diluted with methanol to precipitate crystals, which were filtered off. The crude product was purified with column chromatography (chloroform-methanol) and recrystallized from methanol to give yellow needles (166 mg, 15%), mp 288° dec; ir (Nujol): 3270, 3100, 1713, 1620 cm^{-1}; ^{1}H nmr (200 MHz, DMSO-d_6): δ 4.12 (3H, s, N-CH_3), 7.56 (3H, m, NH_2 and 7-*H*), 7.95 (2H, m, 8-*H* and 9-*H*), 8.20 (1H, d, J = 8.1 Hz, 6-*H*), 8.96 (1H, s, 5-*H*); ms: m/z 226 (M$^+$).

Anal. Calcd. for $C_{12}H_{10}N_4O$: C, 63.70; H, 4.46; N, 24.77. Found: C, 63.62; H, 4.47; N, 24.39.

2-Amino-10-ethyl-2-deoxo-5-deazaflavin (**3b**).

Compound **2** (485 mg, 2.8 mmoles) was suspended in 1-butanol (6 ml) and *N*-ethylaniline (467 mg, 3.9 mmoles) in 3 ml 1-butanol was added drop by drop under reflux. After the mixture was refluxed for 1 hour, 1-butanol was evaporated. The residue was purified with column chromatography (chloroform-methanol) and recrystallized from methanol to give yellow needles (99 mg, 15%), mp 266° (dec); ir (Nujol): 3320, 3200, 1712, 1626 cm^{-1}; ^{1}H nmr (200 MHz, DMSO-d_6): δ 1.37 (3H, t, J = 6.6 Hz, NCH$_2$CH_3), 4.83 (2H, m, N-CH_2CH$_3$), 7.57 (2H, m, *H* of NH_2 and 7-*H*), 7.80 (1H, bs, *H* of NH_2), 8.01 (2H, m, 8-*H* and 9-*H*), 8.23 (1H, d, J = 8.1 Hz, 6-*H*), 9.00 (1H, s, 5-*H*).

Anal. Calcd. for $C_{13}H_{12}N_4O$: C, 64.98; H, 5.03; N, 23.32. Found: C, 65.14; H, 5.10; N, 23.17.

2-Amino-6-chloropyrimidin-4(3*H*)-one (**6**).

Compound **1** (5.08 g, 40 mmoles) was added to phosphoryl chloride (50 ml) and the mixture was heated at 100-110° for 6 hours. The reaction mixture was evaporated under reduced pressure and the residue was crushed in ice water to give precipitate which was collected by filtration. The precipitate was dissolved in chloroform-methanol under warming and then filtered. The filtrate was concentrated to a small volume to give crystals, which were filtered off and dried in desiccator to yield crude product (2.40 g, 41%). This compound was used for the next procedure without further purification; ir (Nujol): 3390, 3320, 3230, 1661 cm^{-1}; ^{1}H nmr (200 MHz, DMSO-d_6): δ 6.87 (1H, s, 5-*H*), 7.60 (3H, bs, N*H* and NH_2); ms: m/z 145 (M$^+$).

2-Amino-6-*p*-toluidinopyrimidin-4(3*H*)-one (**5**).

A mixture of 2,6-diaminopyrimidin-4(3*H*)-one (**4**) (882 mg, 7 mmoles), *p*-toluidine hydrochloride (1.00 g, 7 mmoles) and *p*-toluidine (1.50 g, 14 mmoles) was heated at 130-140° for 50 minutes under argon atmosphere. After the reaction, the mixture was purified with column chromatography (chloroform-methanol) and recrystallized from methanol to give colorless needles (923 mg, 61%), mp 277°; ir (Nujol): 3390, 3340, 1684, 1645 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 2.24 (3H, s, C_6H_4-CH_3), 4.79 (1H, s, 5-*H*), 6.34 (2H, bs, NH_2), 7.05 and 7.29 (each 2H, d, J = 8.4 Hz, aryl *H*), 8.52 (1H, s, N*H*-C_6H_4-CH_3), 9.99 (1H, bs, N_3-*H*); ms: m/z 216 (M^+).

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.01; H, 5.59; N, 25.91. Found: C, 60.87; H, 5.50; N, 25.65.

2-Amino-10-*p*-tolyl-2-deoxo-5-deazaflavin (**3c**).

Compound **5** (648 mg, 3 mmoles) and *o*-chlorobenzaldehyde (843 mg, 6 mmoles) were dissolved in DMF (6 ml) and the solution was refluxed for 90 minutes. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was purified with column chromatography (chloroform-methanol) and recrystallized from methanol to give yellow needles (139 mg, 15%), mp >300°; ir (Nujol): 3430, 1637 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 2.46 (3H, s, $C_6H_4C H_3$), 6.73 (1H, d, J = 8.8 Hz, 9-*H*), 7.18 (1H, bs, *H* of NH_2), 7.35 (3H, m, toluidino 2*H* and *H* of NH_2), 7.49 (3H, m, toluidino 2*H* and 8-*H*), 7.70 (1H, t like dd, J = 7.5 Hz, 7-*H*), 8.23 (1H, d, J = 8.0 Hz, 6-*H*), 9.03 (1H, s, 5-*H*); ms: m/z 302 (M^+).

Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53. Found: C, 71.44; H, 4.80; N, 18.21.

2-Amino-4,6-di(*p*-toluidino)pyrimidine (**7**).

A mixture of compound **6** (145.5 mg, 1 mmole) and *p*-toluidine (321 mg, 3 mmoles) was fused at 130-140° under argon atmosphere for 1 hour. After the reaction, the reaction mixture was purified with column chlomatography (ethyl acetate-hexane) and recrystallized from methanol to give colorless prisms (186 mg, 61%), mp 218°; ir (Nujol): 3460, 3420, 3150, 1637, 1585 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 2.23 (6H, s, 2 x C_6H_4-CH_3), 5.47 (1H, s, 5-*H*), 5.86 (2H, s, NH_2), 7.04 and 7.44 (each 4H, d, J = 8.4 Hz, tolyl-*H*), 8.51 (2H, s, 2 x N*H*-C_6H_4-CH_3).

Anal. Calcd. for $C_{18}H_{19}N_5$: C, 70.79; H, 6.27; N, 22.94. Found: C, 70.83; H, 6.15; N, 22.69.

2-Amino-4-*n*-butylamino-6-chloropyrimidine (**8**).

Compound **6** (141 mg, 0.97 mmole) was dissolved in 1-butylamine (1 ml) and the solution was refluxed for 30 minutes. After the reaction, the reaction mixture was evaporated under reduced pressure and the residue was purified with column chromatography (ethyl acetate-hexane) to give colorless oil (194 mg, 97%); ir (Nujol): 3420, 1586 cm^{-1}; ^1H nmr (200 MHz, deuteriochloroform): δ 0.88 (3H, t, J = 7.4 Hz, $NHCH_2CH_2CH_2CH_3$), 1.21-1.53 (4H, m, $NHCH_2CH_2CH_2CH_3$), 3.19 (2H, m, $NHCH_2CH_2CH_2CH_3$), 5.72 (1H, s, 5-*H*), 6.35 (2H, s, NH_2), 7.07 (1H, m, N*H*-*n*-Bu); ms: m/z 200 (M^+).

Anal. Calcd. for $C_8H_{13}N_4Cl$: C, 47.88; H, 6.53; N, 27.92. Found: C, 47.65; H, 6.48; N, 27.68.

2-Amino-4-*n*-butylamino-6-*p*-toluidinopyrimidine (**9**).

Compound **8** (81 mg, 0.4 mmoles) was mixed with a mixture of *p*-toluidine (95 mg, 0.88 mmole) and *p*-toluidine hydrochloride (64 mg, 0.44 mmole), and the mixture was fused at 120-130° for 30 minutes. The reaction mixture was purified with column chromatography (ethyl acetate-hexane) and recrystallized from methanol to give colorless powder (106 mg, 96%), mp 91°; ir (Nujol): 3420, 1590 cm^{-1}; ^1H nmr (200 MHz, deuteriochloroform): δ 0.89 (3H, t, J = 7.1 Hz, $NHCH_2CH_2CH_2CH_3$), 1.25-1.49 (4H, m, $NHCH_2CH_2CH_2CH_3$), 2.22 (3H, s, $C_6H_4CH_3$), 3.11 (2H, dt, J = 5.3, 6.2 Hz, $NHCH_2CH_2CH_2CH_3$), 5.14 (1H, s, 5-*H*), 5.63 (2H, s, NH_2), 6.27 (1H, t, N*H*-*n*-Bu, J = 5.3 Hz), 7.02 and 7.40 (each 2H, d, J = 8.4 Hz, tolyl-*H*), 8.34 (1H, s, N*H*$C_6H_4CH_3$).

Anal. Calcd. for $C_{15}H_{21}N_5$: C, 66.39; H, 7.80; N, 25.81. Found: C, 66.43; H, 7.85; N, 25.62.

2-Amino-4-chloro-6-*p*-toluidinopyrimidine (**10**).

Compound **5** (108 mg, 0.5 mmole) was dissolved in phosphoryl chloride (3 ml) and the solution was stirred for 4 hours at 90°. The reaction mixture was evaporated under reduced pressure and the residue was treated with ice water and extracted with chloroform. The extracts were washed with water, dried with sodium sulfate, and evaporated. The residue was purified with column chromatography (ethyl acetate-hexane) to give colorless prisms (29 mg, 25%), mp 246°; ir (Nujol): 3480, 3150, 1652, 1608 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 2.26 (3H, s, $C_6H_4C H_3$), 5.95 (1H, s, 5-*H*), 6.67 (2H, bs, NH_2), 7.09 and 7.52 (each 2H, d, J = 8.1 Hz, tolyl-*H*), 9.20 (1H, s, N*H*$C_6H_4CH_3$); ms: m/z 234 (M^+).

Anal. Calcd. for $C_{11}H_{11}N_4Cl$: C, 56.29; H, 4.72; N, 23.58. Found: C, 56.41; H, 4.68; N, 23.59.

Alternative Synthesis of 2-Amino-4,6-di(*p*-toluidino)pyrimidine (**7**).

Compound **10** (23 mg, 0.098 mmole) was added to *p*-toluidine (200 mg, 1.87 mmoles) and the mixture was heated at 130-140° for 1 hour. The reaction mixture was purified with column chromatography (ethyl acetate-hexane) to give compound **7** (25 mg, 84%), which was identical in all respects with the authentic sample of **7** described above.

REFERENCES AND NOTES

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