A Facile Synthesis of 2-(Aminomethyl)purines

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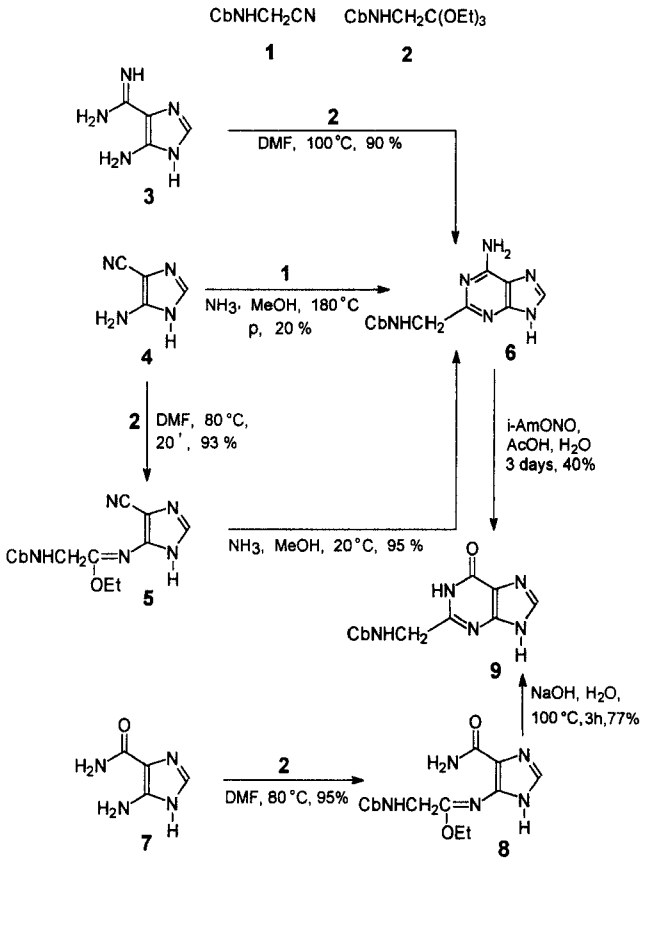
A simple synthesis of 2-(aminomethyl)purines is reported. Imidate intermediates are formed by condensation of the Cb-glycine orthoester with 4-aminoimidazole-5-carboxylic acid derivatives, and are further cyclized to 2-(Cb-aminomethyl)purines.

(Aminomethyl)purines are interesting homologues of biogenic purine bases; their biological activity, however, has not yet been reported. While the 6-(aminomethyl)purines are prepared by the catalytic reduction of 6-cyanopurines,[1,2] the only known synthesis of the 2-(aminomethyl)purine derivatives is a multistep procedure[3] starting from guanosine via its 2-phenylsulfonyl and 2-cyano derivatives. Both 2- and 6-(aminomethyl)purines are relatively unstable compounds due to their strongly basic benzylamine-type amino group and they have been isolated and characterized as their stable *N*-acetyl[2] or *N*-trifluoroacetyl[3] derivatives.

In our efforts to evaluate the biological activity of their nucleotide and nucleoside analogues, it was essential to develop a method for preparation of the 2-(aminomethyl)purine bases in larger quantities. We report here on a simple, straightforward synthesis of 2-benzyloxycarbonyl-(Cb) protected (aminomethyl)purines via purine synthesis[4] from imidazoles.

The glycine derivatives Cb-aminoacetonitrile **1** and triethyl Cb-aminoorthoacetate **2**, which were prepared[5] from commercially available aminoacetonitrile, were successfully condensed with the 4-aminoimidazole-5-carboxylic acid derivatives. Thus 4-aminoimidazole-5-carboxamidine **(3)** reacted with the orthoester **2** to give the adenine derivative **6** in 90% yield after column chromatography purification (method A). On the other hand, in analogy to a synthesis of 2-alkylpurines,[6] the reaction of 4-aminoimidazole-5-carbonitrile **4** with the acetonitrile **1** gave the adenine derivative **6** in a low yield of 20% (method B). The imidazolecarbonitrile **4** reacted readily with the orthoester **2** to give the imidate **5** which was then converted into **6** by standing overnight in methanolic ammonia. Pure crystalline compound **6** was obtained in a good yield of 95% (method C). 5-Aminoimidazole-4-carboxamide **(7)** reacted quantitatively with the orthoester **2** to afford the imidate **8** which was cyclized to the hypoxanthine derivative **9** under basic conditions. Compound **9** was also prepared by deamination of the adenine derivative **6** with isoamyl nitrite in acetic acid solution.

Both purines **6** and **9** were fully characterized. The imidate intermediates **5** and **8** were also isolated and characterized. Structural assignment of the carbon signals was based on J-modulated ^{13}C NMR ("attached proton test pulse sequence"). While C-4 and C-5 signals of the compounds **5** and **6** were broad due to the 1H–3H (7H–9H) tautomerism, all carbon signals of the imidate **8** were sharp. In case of the hypoxanthine derivative **9** a mixture of 7H and 9H isomers (1:1) was observed. Low-field shifts (8.0 and 4.8 ppm) of C-4 and C-8 and upfield shifts (−8.5 and −0.9 ppm) of C-5 and C-6 are in agreement with the reported[7] shift differences of the 9- and 7-substituted hypoxanthines.



In conclusion, 2-(Cb-aminomethyl)adenine **(6)** (method C) and 2-(Cb-aminomethyl)hypoxanthine **(9)** (method A) can be easily prepared by the above mentioned methods in multigram amounts. The only limitation is the requirement of at least fourfold excess of the orthoester[5] **2**, which must be used in order to reach a quantitative conversion.

Unless otherwise mentioned, solvents were evaporated at 40 °C/2 kPa and substances were dried at 60 °C/2 kPa over P_2O_5. Melting points were determined on a Kofler block melting point apparatus and are uncorrected. TLC was performed on Silufol UV254 plates in $CHCl_3$/MeOH (80:20) mixture. Column chromatography was performed with silica gel (30 μm, Kavalier Votice, Czech Rep.). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using the FAB (ionization by Xe, accelerating voltage 8 kV) technique, with glycerol and disulfide matrices. NMR spectra were measured on Varian Unity 500 (500 MHz for ^1H and 125.7 MHz for ^{13}C NMR) in DMSO-d_6, referenced to the solvent signals 2.5 ppm for ^1H and 39.7 ppm for ^{13}C NMR. Aminoacetonitrile was

purchased from Janssen (Belgium); 4-aminoimidazole-5-carboxamide hydrochloride (**7**) from Sigma (USA). 4-Aminoimidazole-5-carboxamidine[9] (**3**) was prepared and used as dihydrochloride salt; 4-aminoimidazole-5-carbonitrile[8] (**4**) as hydrochloride salt. DMF was distilled from P_2O_5 and stored over molecular sieves. Satisfactory microanalyses were obtained for all compounds: C ± 0.18, H ± 0.15, N ± 0.26.

5-[(*N*-Benzyloxycarbonylaminomethyl)(ethoxy)methylimino]imidazole-4-carbonitrile (5):

A mixture of **2** (850 mg, 2.73 mmol), **4**[8] (100 mg, 0.69 mmol) and DMF (4 mL) was stirred at 80 °C for 20 min, the solvent was then evaporated and, after addition of Et_2O (20 mL), **5** precipitated as a yellow powder; yield: 210 mg (93%); mp 168–171 °C (dec.); R_F = 0.60 ($CHCl_3$/MeOH, 80:20).

MS (FAB): *m/z* (%) = 328 [(M + H)$^+$, 100].

^{1}H NMR (DMSO-d_6): 1.25 (t, 3 H, *J* = 7.1 Hz, CH_3), 3.91 (m, 2 H, CH_2N), 4.23 (q, 2 H, *J* = 7.1 Hz, CH_2O), 5.04 (s, 2 H, CH_2Ph), 7.25–7.35 (m, 5 H, H-Ph), 7.63 (br, 1 H, H-2), 7.69 (br, 1 H, NHCO), 12.63 (br, 1 H, NH).

^{13}C NMR (DMSO-d_6): 13.91 (CH_3), 40.69 (CH_2N), 63.22 (CH_2O), 65.77 (CH_2Ph), 116.50 (C-5), 127.83 (C–Ph), 128.01 (C–*p*-Ph), 128.35 (CN), 128.52 (C–Ph), 137.35 (C–*i*-Ph), 137.53 (C-2), 144.50 (C-4), 156.38 (C–OEt), 163.34 (CO).

IR (KBr): *ν* = 2219 m (CN), 1689 vs, 1666 vs (C=O) cm^{-1}.

2-(*N*-Benzyloxycarbonylaminomethyl)adenine (6):

Method A: A mixture of **3**[9] (100 mg, 0.50 mmol), **2** (800 mg, 2.73 mmol) and DMF (5 mL) was stirred at 100 °C for 1 h, neutralized with methanolic ammonia (1.7 M, 1 mL), and evaporated to dryness. The crude product was purified by column chromatography (5 g silica gel, $CHCl_3$/MeOH, 90:10) to yield **6** as a brown powder; yield: 135 mg (90%).

Method B: A suspension of **4** (400 mg, 2.76 mmol) in MeOH (10 mL) was neutralized with 1 M methanolic NaOMe (2.76 mL) and after addition of **1** (2.12 g, 11 mmol) and sat. methanolic ammonia (20 mL) the mixture was heated at 180 °C in an autoclave for 6 h. The solvent was evaporated and the residue was chromatographed on a column (30 g silica gel, $CHCl_3$/MeOH, 90:10) to give **6** as a brown powder; yield: 160 mg (20%).

Method C: **5** (2.1 g, 6.42 mmol) was dissolved in sat. methanolic ammonia (40 mL). The solution was filtered and allowed to stand at 20 °C for 48 h. **6** crystallized as colourless needles; yield: 1.8 g (95%); mp 248–250 °C (dec.); R_F = 0.40 ($CHCl_3$/MeOH, 80:20).

MS (FAB): *m/z* (%) = 299 [(M + H)$^+$, 45], 91 [(C_7H_7)$^+$, 100].

^{1}H NMR (DMSO-d_6): 4.19 (d, 2 H, *J* = 5.9 Hz, CH_2N), 5.04 (s, 2 H, CH_2O), 7.11 (bs, 2 H, NH_2), 7.29–7.38 (m, 5 H, Ph), 7.49 (t, 1 H, *J* = 5.9 Hz, NHCO), 8.06 (s, 1 H, H-8), 12.80 (b, 1 H, NH).

^{13}C NMR (DMSO-d_6): 46.41 (CH_2N), 65.45 (CH_2Ph), 117.49 (C-5), 127.87, 127.88, 128.53, 137.43 (all C–Ph), 140.00 (C-8), 151.00 (C-4), 155.87 (C-6), 156.45 (C-2), 160.80 (CO).

UV (H_2O): *λ* (*ε*) = pH 7, 262 (11900); pH 2, 266 (11900); pH 12, 269 (10700).

IR (KBr): *ν* = 3475 m (NH_2), 1691 vs (CO), 1645 vs (NH_2), 1612 s, 1588 s (adenine ring).

5-[(*N*-Benzyloxycarbonylaminomethyl)(ethoxy)methylimino]imidazole-4-carboxamide (8):

A mixture of **2** (850 mg, 2.73 mmol), **7** (100 mg, 0.61 mmol) and DMF (4 mL) was stirred at 80 °C for 1 h. The solvents were evaporated and, after addition of Et_2O (20 mL), **8** precipitated as a white powder; yield: 200 mg (95%). An analytical sample was recrystallized from MeOH; mp 216–220 °C; R_F = 0.57 ($CHCl_3$/MeOH, 80:20).

MS (FAB): *m/z* = 346 [(M + H)$^+$, 40].

^{1}H NMR (DMSO-d_6): 1.24 (t, 3 H, *J* = 7.1 Hz, CH_3), 4.16 (q, 2 H, *J* = 7.1 Hz, CH_2O), 4.32 (d, 2 H, *J* = 6.1 Hz, CH_2N), 5.04 (s, 2 H, CH_2Ph), 7.34 (m, 6 H, H-2 and Ph), 7.40 and 7.60 (bs, 2 × 1 H, NH_2), 7.54 (t, 1 H, *J* = 6.1 Hz, NHCO), 12.10 (b, 1 H, NH).

^{13}C NMR (DMSO-d_6): 13.99 (CH_3), 41.84 (CH_2N), 62.28 (CH_2O), 65.54 (CH_2Ph), 115.69 (C-5), 127.77 (2C, *o*-Ph), 127.95 (*p*-Ph), 128.51 (2C, *m*-Ph), 134.71 (C-2), 137.34 (*i*-Ph), 144.37 (C-4), 156.43 (C–OEt), 161.27 (COO), 163.17 ($CONH_2$).

IR (KBr): *ν* = 1704 s, 1669 s (C=O).

2-(Benzyloxycarbonylaminomethyl)hypoxanthine (9):

Method A: A mixture of **7** (100 mg, 0.61 mmol), **2** (850 mg, 2.73 mmol) and DMF (5 mL) was stirred at 80 °C for 1 h and 1% aq NaOH (10 mL) was added. Heating was continued at 100 °C for 3 h, the solvents were then evaporated and the residue was chromatographed on a column (10 g silica gel, MeOH/$CHCl_3$, 10:90) to afford **9** as a white powder; yield: 140 mg (77%).

Method B: A mixture of **6** (100 mg, 0.34 mmol), isoamyl nitrite (0.5 mL), acetic acid (3 mL) and water (0.5 mL) was allowed to stand at 20 °C for 3 d, then the solvents were evaporated and the product was chromatographed on a column (5 g silica gel, $CHCl_3$/MeOH, 95:5) to give **9** as a yellow powder; yield: 40 mg (40%). An analytical sample was recrystallized from MeOH/H_2O; mp 143–146 °C (dec.); R_F = 0.38 ($CHCl_3$/MeOH, 80:20).

MS (FAB): *m/z* = 318 [(M + 19)$^+$, 15], 300 [(M + H)$^+$, 25].

NMR – a 1:1 mixture of 9 H (a) and 7 H (b) isomers was obtained:

^{1}H NMR (DMSO-d_6): 4.20 (d, 4 H, *J* = 6.1 Hz, CH_2N), 5.06 (s, 4 H, CH_2Ph), 7.10–7.40 (m, 10 H, Ph), 7.74 (t, 2 H, *J* = 6.1 Hz, NHCO), 7.99 (bs 1 H, H-8b), 8.19 (bs, 1 H, H-8a), 12.11 (bs, 1 H, NHb), 12.12 (bs, 1 H, NHa), 13.15 (bs, 1 H, NHb), 13.41 (bs, 1 H, NHa).

^{13}C NMR (DMSO-d_6): 42.72 (CH_2N), 65.84 (CH_2Ph), 113.84 (C-5b), 122.30 (C-5a), 127.92, 128.02, 128.59, 137.17 (all C–Ph), 137.40 (C-8a), 142.20 (C-8b), 149.36 (C-4a), 153.88 (C-6b), 154.73 (C-6a), 155.36 (C-2b), 155.50 (C-2a), 156.67 (C=O), 157.31 (C-4b).

UV (H_2O): *λ* (*ε*) = pH 7, 251 (5800); pH 2, 251 (6200); pH 12, 261 (5300).

IR (KBr): *ν* = 1700 vs, 1675 vs (C=O), 1624 m, 1605 s (hypoxanthine ring).

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