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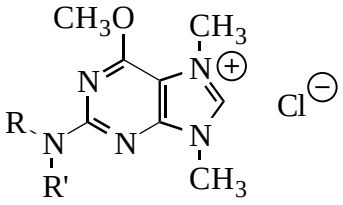
SYNTHESIS OF HETEROMINE C FROM GUANINE

Erik Jakobsen and Lise-Lotte Gundersen*†

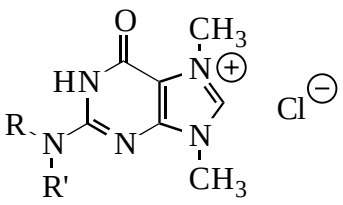
Department of Chemistry, University of Oslo, P. O. Box 1033, Blindern, *N*-0315 Oslo, Norway

Abstract - Heteromine C previously isolated from a Taipei folk medicine plant, has been synthesised for the first time by selective methylation reactions starting from guanine. Thermal rearrangement to 1-methylherbipoline takes place when heteromine C is heated. It is shown that treatment of O^6,9-dimethylguanine with methyl iodide gives the O^6,7,9-trimethylguaninium iodide with complete selectivity, while similar reaction on O^6,7-dimethylguanine results in methylation both in the 3- and 9-position.

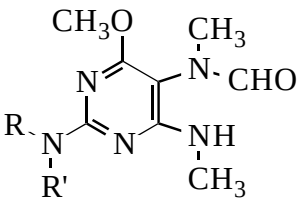
Heteromines A-H (Figure 1) have been isolated from *Heterostemma brownii* Hay (Asclepiadaceae).[1,2] This plant has been used for the treatment of tumors in Taipei folk medicine and heteromines are shown to be cytotoxic to several cancer cell lines.[2] So far only heteromine A have been synthesised and the published synthesis involved construction of the purine ring system.[3] Heteromines A, B and C are all O^6,7,9-trimethylguaninium salts and we thought that relatively inexpensive guanine would be an attractive starting material, if the methyl substituents could be introduced by chemo- and regioselective reactions in reasonably high yields. We herein reports that heteromine C (**1c**) is available from guanine in five steps.



Heteromine A (**1a**): R=R'=CH_3
Heteromine B (**1b**): R=CH_3, R'=H
Heteromine C (**1c**): R=R'= H



Heteromine D (**1d**): R=R'=CH_3
Heteromine E (**1e**): R=CH_3, R'=H



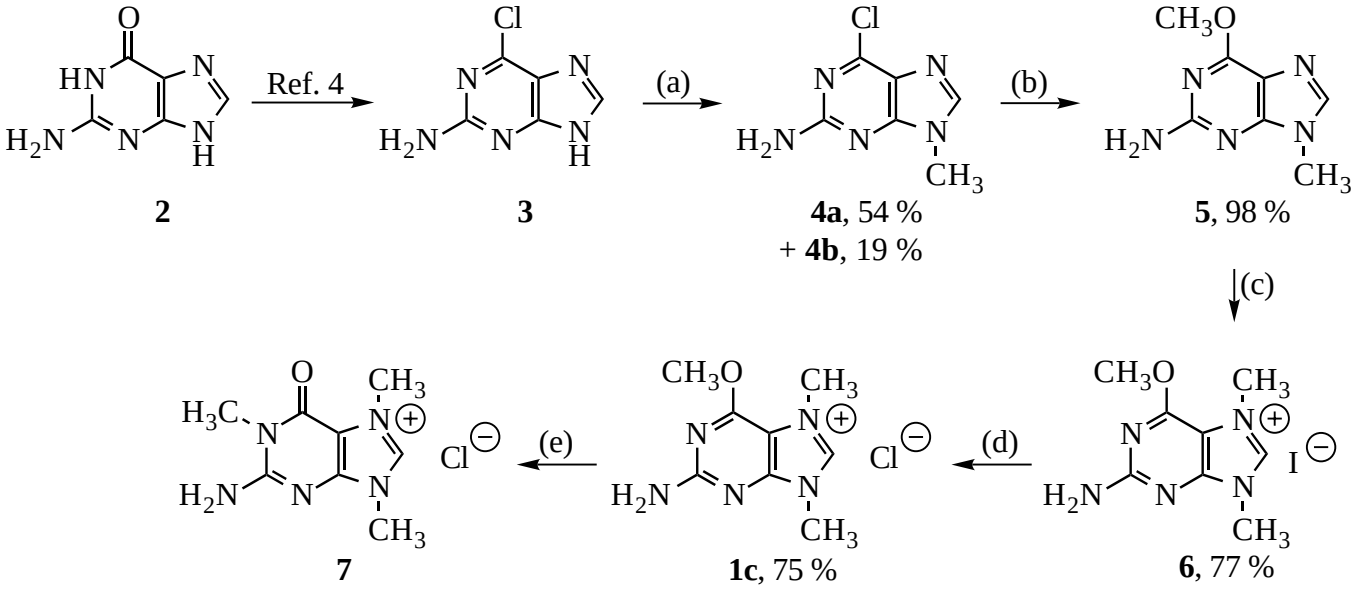
Heteromine F (**1f**): R=R'=CH_3
Heteromine G (**1g**): R=CH_3, R'=H
Heteromine H (**1h**): R=R'=H

Figure 1

The first step in our synthesis of heteromine C (Scheme 1) is conversion of guanine (**2**) to 2-amino-6-chloropurine (**3**) by standard methods.[4] *N*-Methylation of compound (**3**) with methyl iodide and K_2CO_3

† E-mail: l.l.gundersen@kjemi.uio.no

in DMF, essentially as we have reported for *N*-alkylation of halopurines before,[5] was rather slow. On the other hand, treatment of the purine (**3**) with methyl iodide in the presence of tetrabutylammonium fluoride (TBAF) as described for 9-methylation of adenine,[6] resulted in full conversion of the starting material in only 2 h and the 9-methylpurine (**4a**) and the 7-methyl isomer (**4b**) were formed in a *ca*. 4 : 1 ratio. The isomers (**4a**) and (**4b**) were isolated in 56 and 19 % yields, respectively. In contrast to the literature procedure[6] where a large excess of TBAF was used, only 1.5 equivs. TBAF were required for swift *N*-methylation of compound (**3**). Essentially the same results were obtained if TBAF was replaced with tetramethylammonium fluoride.



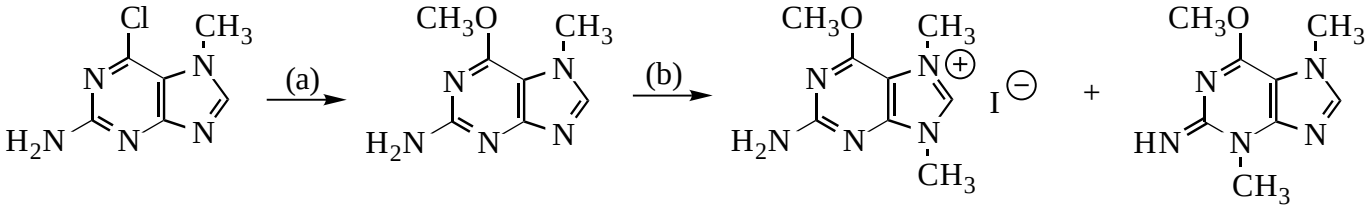
(a) CH_3I, TBAF, THF; (b) CH_3ONa, CH_3OH; (c) CH_3I, acetone; (d) 1. Ag_2SO_4 (aq), 2. $BaCl_2$ (aq); (e) 60-100 ^oC

Scheme 1

Reaction of the 9-methylpurine (**4a**) with sodium methoxide in methanol gave the methoxypurine (**5a**) in nearly quantitative yield. An alternative strategy where the 6-chloropurine first was reacted with sodium methoxide and then *N*-methylated, was abandoned because ring-opening during *N*-methylation of 2-amino-6-methoxypurine in the presence of base has been observed[7] and low *N*-9 selectivity in *N*-alkylation has been reported for 2-amino-6-methoxypurine compared with 2-amino-6-chloropurine (**3**).[8] Treatment of purine (**5**) with an excess methyl iodide in acetone gave only the purinium iodide (**6**) in high yield. The iodide was replaced with chloride when compound (**6**) was reacted with silver sulfate followed by barium chloride, to give the target heteromine C (**1c**) in 75 % yield. When heat was employed in the drying of compound (**1c**), migration of the methyl group situated on O^6 was observed[9] and 1-methylherbipoline (**7**) was formed. Bioactive 1-methylherbipoline salts have previously been isolated from marine sponges.[10]

The chloropurine (**4b**) was easily converted to the corresponding methoxypurine (**8**) (Scheme 2), but the reactivity of compound (**8)** towards methyl iodide in acetone was quite different from that of the 9-methyl isomer (**5**) (*vide supra*). The reaction was much faster but also considerably less selective. The desired

purinium iodide (**6**) was formed, but also two more lipophilic products were observed and the isolated yield of compound (**6**) was only 38 %. The major by-product was shown by HMQC[11] and HMBC[12] NMR spectroscopy to be the 3-methylated imine (**9**).[13] The second by-product was only formed in a small amount, *ca*. 10 % relative to compound (**9**), and we were not able to confirm the structure. However, decomposition of purine (**9**) to 3,7-dimethylguanine has been reported before,[7] and the NMR spectra strongly indicated that the compound was a dimethylguanine isomer where the two methyl substituents were situated on two of the ring nitrogens.



4b **8**, 92 % **6**, 38 % **9**

(a) CH_3ONa, CH_3OH; (b) CH_3I, acetone

Scheme 2

The total yield of the purinium iodide (**6**) from 6-chloroguanine (**3**) as described above is 48 %; 41 % *via* (**4a**) and 7 % *via* **4b**. When we omitted the separation of isomers (**4a**) and (**4b**), and isomers (**5**) and (**8**), we were able to convert compound (**3**) into the purinium salt (**6**) in 37 % total yield. Methylation of the chloropurine (**3**) as described in Scheme 1 gave a mixture of **4a** and **4b** in 66 % yield. Further treatment with sodium methoxide in methanol gave 98 % of the methoxypurines (**5**) and (**8**) and this mixture gave the desired purinium iodide (**6**) in 57 % yield when reacted with methyl iodide. The imine (**9**) was also formed in the last step according to the ^{1}H NMR spectrum of the crude product. Since the formation of compound (**9**) in methylation of the *N*-7 alkylated compound (**8**) reduced the amount of compound (**6**) formed and furthermore complicated the isolation of the iodide (**6**), a better yield of heteromine C is obtained when only the 9-methylpurine (**4a**) is used in the reaction sequence, compared to when the target molecule is prepared from a mixture of isomers (**4a**) and (**4b**).

EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument, at 300 MHz with a Bruker Avance DPX 300, or at 200 MHz with a Bruker Avance DPX 200 instrument. The ^{13}C NMR spectra were recorded at 125, 75 or 50 MHz using instruments mentioned above. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. MS spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionising voltage, and are presented as *m*/*z* (% rel. int.). Electrospray MS spectra were recorded with a Bruker Apex 47e FT-ICR mass spectrometer. Melting points are uncorrected. Silica gel for flash chromatography was purchased from Merck, Darmstadt, Germany (Merck No. 9385). THF was distilled from Na/benzophenone, methanol from iodine and magnesium, and acetone from boric anhydride. Tetrabutylammonium fluoride trihydrate was dried by azeotropic distillation with benzene. 2-Amino-6-chloro-9*H*-purine can be prepared

according to literature procedures,[4] and the compound is also commercially available. All other reagents were commercially available and used as received.

*2-Amino-6-chloro-9-methyl-9*H*-purine (**4a**) and 2-amino-6-chloro-7-methyl-7*H*-purine (**4b**).* A mixture of dry tetrabutylammonium fluoride (2.844 g, 9.0 mmol), 2-amino-6-chloro-9*H*-purine (**2**) (1.020 g, 6.0 mmol) and iodomethane (1.08 mL, 18 mmol) in dry THF (100 mL) was stirred at ambient temperatures for 2 h and evaporated *in vacuo*. The residue was stirred in methanol, the solid was filtered off and the filtrate evaporated *in vacuo*. The residue was treated with chloroform and the mixture was filtered. The solid isolated was combined with the solid formed after treatment with methanol and subjected to flash chromatography eluting with EtOAc-hexane (4:1) to give the 9-methyl isomer (**4a**) followed by EtOH-EtOAc (1:9) to give compound (**4b**).

*2-Amino-6-chloro-9-methyl-9*H*-purine (**4a**).* Yield 594 mg (54 %) colorless powdery crystals. mp 268-270 °C (EtOAc-hexane) (lit.,[14] 262-263 °C, decomp). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.62 (s, 3 H, CH_3), 6.88 (br s, 2 H, NH_2), 8.06 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 29.8 (CH_3), 123.6 (C-5), 144.1 (C-8), 149.5 (C-2), 154.8 (C-4), 160.1 (C-6). MS (EI): 185/183 (33/100, M^+), 184 (8), 148 (70), 121 (15).

*2-Amino-6-chloro-7-methyl-7*H*-purine (**4b**).* Yield 210 mg (19 %) colorless powdery crystals. mp > 350 °C (EtOAc-hexane) (lit.,[15] > 350 °C). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.89 (s, 3 H, CH_3), 6.59 (br s, 2 H, NH_2), 8.02 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 32.6 (CH_3), 115.7 (C-5), 142.5 (C-6), 149.8 (C-8), 159.9 (C-2), 164.0 (C-4). MS (EI): 185/183 (33/100, M^+), 184 (9), 149 (7), 148 (86), 141 (5), 121 (9), 94 (5), 80 (5), 67 (15).

*2-Amino-6-methoxy-9-methyl-9*H*-purine (**5**).* 2-Amino-6-chloro-9-methyl-9*H*-purine (**4a**) (184 mg, 1.0 mmol) was dissolved in a solution of sodium methoxide in methanol (0.4 M, 5.0 mL, 2.0 mmol CH_3ONa) and the mixture was stirred under N_2-atmosphere for 16 h, quenched with saturated aqueous ammonium chloride and evaporated *in vacuo*. The product was isolated by flash chromatography eluting with EtOH-$CHCl_3$ (1:1) to yield 175 mg (98 %) colorless powdery crystals. mp 193-194 °C (EtOH-$CHCl_3$) (lit.,[7] 194-195 °C, decomp). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.85 (s, 3 H, NCH_3), 3.94 (s, 3 H, OCH_3), 6.42 (br s, 2 H, NH_2), 7.80 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 29.2 (NCH_3), 53.1 (OCH_3), 113.7 (C-5), 140.3 (C-8), 154.5 (C-4), 159.8 (C-2), 160.6 (C-6). MS (EI): 179 (100, M^+), 150 (20), 149 (21), 148 (14), 147 (11), 133 (9), 122 (6), 107 (13), 82 (5).

*2-Amino-6-methoxy-7,9-dimethyl-7*H*-purinium iodide (**6**).*

Method A: A mixture of 2-amino-6-methoxy-9-methyl-9*H*-purine (**5**) (144 mg, 0.8 mmol) and iodomethane (1.2 mL, 20 mmol) in dry acetone (16 mL) was stirred under N_2-atmosphere for 13 days. The reaction mixture was evaporated *in vacuo*. The residue was washed with acetone (10×3 mL) and ether (5×3 mL) and dried *in vacuo* (*ca*. 1 mmHg) for 80 h to yield 199 mg (77 %) colorless powdery crystals. mp 284-286 °C (lit.,[7] 285-286 °C, decomp). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.74 [s, 3 H, N(9)CH_3], 3.98 [s, 3 H, N(7)CH_3], 4.05 (s, 3 H, OCH_3), 7.33 (br s, 2 H, NH_2), 9.28 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 31.2 [N(7)CH_3], 36.0 [N(9)CH_3], 54.6 (OCH_3), 104.7 (C-5), 140.2 (C-8), 152.0 (C-4), 158.3 (C-6), 161.6 (C-2). MS (electrospray): 194 (M^+).

Method B: A mixture of 2-amino-6-methoxy-7-methyl-7*H*-purine (**8**) (86 mg, 0.48 mmol) and iodomethane (0.29 mL, 4.75 mmol) in dry acetone (15 mL) was stirred under N_2-atmosphere for 48 h. The solid thus formed was filtered off and dried *in vacuo* to give compound (**6**) (59 mg, 38 %). The filtrate was evaporated to give a waxy material shown by NMR spectroscopy to consist mainly of the imine (**9**).[13]

*3,7-Dihydro-6-methoxy-3,7-dimethyl-2*H*-purin-2-imine (**9**)*: ^{1}H NMR (500 MHz, DMSO-d_6): 3.71 [s, 3 H, N(3)CH_3], 3.92 [s, 3 H, N(7)CH_3], 4.13 (s, 3 H, OCH_3), 8.41 (s, 1 H, H-8), 8.0-9 0 (br, =NH). ^{13}C NMR (50 MHz, DMSO-d_6): 32.3 [N(3)CH_3], 34.0 [N(7)CH_3], 55.6 (OCH_3), 107.4 (C-5), 146.4 (C-8), 152.2 (C-4), 154.0 (C-2), 158.8 (C-6).

*2-Amino-6-methoxy-7,9-dimethyl-7*H*-purinium chloride (heteromine C) (**1c**).* 2-Amino-6-methoxy-7,9-dimethylpurinium iodide (**6**) (160 mg, 0.5 mmol) was dissolved in water (9 mL) and silver sulfate (78 mg, 0.25 mmol) in water (6 mL) was added. The resulting mixture was stirred at ambient temperature for 30 min and filtered through celite before barium chloride dihydrate (61 mg, 0.25 mmol) was added. The mixture was stirred for an additional 5 min, filtered through celite and evaporated *in vacuo*. The residue was washed with acetone (10×3 mL) and ether (5×3 mL) and dried *in vacuo* (*ca*. 1 mmHg) for 80 h to yield 87 mg (75 %) colorless powdery crystals. mp 266-268 ^{o}C (lit.,[1] 269-270 ^{o}C). Spectroscopic data were identical to those reported for the corresponding iodide (**6**) above. Heating **1c** at 60 ^{o}C *in vacuo* (*ca*. 1 mmHg) for 120 h and 100 ^{o}C for 47 h gave *ca*. 90 % pure 1-methylherbipoline (**7**) as a colorless solid.

*2-Amino-6,9-dihydro-1,7,9-trimethyl-6-oxo-1*H*-purinium chloride (1-methylherbipoline) (**7**)*. ^{1}H NMR (500 MHz, DMSO-d_6): 3.34 (s, 3 H, CH_3), 3.69 (s, 3 H, CH_3), 4.00 (s, 3 H, CH_3), 7.82 (br s, 2 H, NH_2), 9.21 (s, 1 H, H-8). ^{1}H NMR (300 MHz, CD_3OD): 3.45 (s, 3 H, CH_3), 3.79 (s, 3 H, CH_3), 4.11 (s, 3 H, CH_3), protons of NH_2 and H-8 were completely exchanged by deuterium. ^{13}C NMR (75 MHz, CD_3OD): 28.8 (CH_3), 31.4 (CH_3), 36.0 (CH_3), 108.1 (C-5), 140.1 (C-8), 150.1 (C-4), 155.0, (C-6) 158.1 (C-2). NMR data are in accordance with those reported in the literature.[10a]

*2-Amino-6-methoxy-7-methyl-7*H*-purine (**8**).* The title compound was prepared from 2-amino-6-chloro-7-methyl-7*H*-purine (**4b**) (184 mg, 1.0 mmol) and sodium methoxide in methanol (0.4 M, 6.0 mL, 2.4 mmol CH_3ONa) as described for **5** above except that the reaction time was 24 h, to yield 166 mg (92 %) colorless powdery crystals. mp 249-250 ^{o}C (EtOAc-hexane) (lit.,[16] 252-253 ^{o}C). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.81 (s, 3 H, NCH_3), 3.95 (s, 3 H, OCH_3), 6.09 (br s, 2 H, NH_2), 7.98 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 33.4 (NCH_3), 53.2 (OCH_3), 106.5 (C-5), 145.6 (C-8), 157.3 (C-6), 159.5 (C-2), 163.6 (C-4). MS (EI): 179 (100, M^+), 178 (12), 150 (10), 148 (9), 122 (12), 96 (7).

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REFERENCES

1. Y.-L. Lin, H.-P. Lee, J.-C. Ou, and Y.-H. Kuo, *Heterocycles*, 1996, **43**, 781.

2. Y.-L. Lin, R.-L. Huang, C.-M. Chang, and Y.-H. Kuo, *J. Nat. Prod*., 1997, **60**, 982.
3. C. Peinador, J. M. Quintela, and M. J. Moreira, *Tetrahedron*, 1997, **53**, 8269.
4. See for instance: R. Ramert, and R. Sobotta, *Ger Offen. DE 4,342,131*, 1995 [*Chem. Abstr*., 1995, **123**, 55598]; M. Igi and T. Hayashi, *Eur. Pat. Appl., EP 543,095*, 1993 [*Chem. Abstr*., 1993, **119**, 180812]; J. C. Hanson, *PCT Int. Appl., WO 92 13,859*, 1992 [*Chem. Abstr*., 1992, **117**, 233720].
5. L.-L. Gundersen, A. K. Bakkestuen, A. J. Aasen, H. Øverås, and F. Rise, *Tetrahedron,* 1994, **50**, 9743; L.-L. Gundersen, *Acta Chem. Scand*., 1996, **50**, 58; G. Langli, L.-L. Gundersen, and F. Rise, *Tetrahedron*, 1996, **52**, 5625.
6. K. K. Ogilvie, S. L. Beaucage, and M. F. Gillen, *Tetrahedron Lett*., 1978, 1663.
7. K. Kohda, K. Baba, and Y. Kawazoe, *Tetrahedron Lett.,* 1987, **28**, 6285.
8. G. R. Geen, T. J. Grinter, P. M. Kincey, and R. L. Jarvest, *Tetrahedron,* 1990, **46**, 6903.
9. For other examples of thermal lactim-lactam rearrangement of alkoxypurines, see for instance: E. Bergmann and H. Heimhold, *J. Chem. Soc*., 1935, 955; A. Kowalska, A. Maslankiewicz, B. Syrek, and P. Cieplinski, *Monatsh. Chem.*, 1985, **116**, 341.
10. H. Yagi, S. Matsunaga, and N. Fusetani, *J. Nat. Prod*., 1994, **57**, 837; J. Kimura, E. Ishizuka, Y. Nakao, W. Y. Yoshida, P. J. Scheuer, and M. Kelly-Borges, *J. Nat. Prod*., 1998, **61**, 248.
11. R. E. Hurd, and B. K. John, *J. Magn. Reson.,* 1991, **91**, 648; G. W. Vuister, R. Boelens, R. Kaptein, R. E. Hurd, B. K. John, and P. C. M. Van Zijl, *J. Am. Chem. Soc.,* 1991, **113**, 9688.
12. P. L. Rinaldi and P. Keifer, *J. Magn. Reson., Ser A*, 1994, **108**, 259.
13. K. Yamauchi, T. Tanabe, and M. Kinoshita, *J. Org. Chem*., 1976, **41**, 3691.
14. I. M. Ovcharova and E. S. Golovchinskaya, *Zh. Obshch. Khim*., 1964, **34**, 3247 [*Chem. Abstr*., 1965, **62**, 14675].
15. V. S. Korsunskii and E. S. Golovchinskaya, *Khim. Pharm. Zh.,* 1972, **6**, 28 [*Chem. Abstr*., 1972, **77**, 88447].
16. K. Kohda, N. Sawada, and Y. Kawazoe, *Chem. Pharm. Bull.,* 1991, **39**, 801.