Arch. Pharm. Res. Vol. 17, No. 2, pp. 60-65, 1994

Synthesis and Quarternization of 6-(Substitutedamino)-Purines with Antitumor Activity Screening

Khairy A. M. El-Bayouki, Wahid M. Basyouni, S. M. El-Din and A. G. Habeeb

National Research Center, Cairo, Egypt.

(Received January 11, 1993)

Reaction of 6-chloro-9-benzyl-8-(methylthio)purine **3** with primary amines afforded, the corresponding 6-(substitutedamino)purines **4a-g**. The latter products when methylated with methyl iodide yielded smoothly N^3-methyl purinium iodide salts **5a-f** rather than the probable N^1- and N^7-derivatives. 9-Benzyl-3-methyl-6-(methylimino)-8-(methylthio)purine **8** was obtained upon treating the purinium iodide **5a** with alkali. Most of the synthesized compounds were screened for their antitumor activity.

Key words: Substifutedamino-purines, Quarternization, Antitumor activity

INTRODUCTION

In last years there has been a growing interest in the synthesis and evaluation of many purine derivatives as potential antitumor and antiviral agents (Kelly and Soroka, 1986; Kyoto *et al.*, 1987; Kozalka *et al.*, 1989; McGee *et al.*, 1985). Some 6-(substitutedamino) purine derivatives have been reported for their antitumor activity against Ehrlich Ascities Carcinoma (Bharat and Brumo, 1989; Moharram and Osman, 1989). Also, the clinical usefulness of N^6-benzyladenosines in neoplastic descease and growth inhibitory activity in tumor systems was reported (Dutta *et al.*, 1975; Fleysher *et al.*, 1969).

During the course of our work on substituted purines as potential antitumor and antiviral agents, it was aimed in the present work to synthesize newer substituted purines and purinium salts for the same purpose.

MATERIALS AND METHODS

Chemistry

Melting points are uncorrected. Microanalyses were carried out by the Microanalytical Laboratory, NRC, Cairo, Egypt. IR spectra were recorded with Schimadzu Spectrophotometer Model IR 435. UV spectra were recorded with Varian 634 and Schimadzu Spectrophotometer Model UV 265. Mass spectra were recorded at 70 e.v. on VG ZAB-3F mass spectrometer. ^{1}H-NMR spectra were measured at 60, 90 and 200 MHz in DMSO-d_6 or $CDCl_3$ (where indicated) with chemical shift in δ from internal TMS using Varian Gemini 200 Spectrometer. ^{13}C-NMR spectra were measured by Varian Gemini 200 and JNM-GX-400 Fa Jeol Tokyo Spectrometer.

5-Amino-2-(methylthio)-1-benzyl-1H-imidazole-4-carboxamide 1b: In an aqueous sodium hydroxide solution (150 ml, 2%), the imidazole **1a** (10 g) was dissolved and then dimethyl sulfate (10 ml) was added while stirring. The separated solid after 5-10 min. was filtered off and crystallized to give **1b** (Table I).

Synthesis of 9-benzyl-8-(methylthio)-9H-purine-6-one 2:

Method 1: A mixture of the imidazole **1b** (2 g) and formamide (10 ml) was heated at 180-190℃ (oil-bath temperature) for 1 hr and then left to cool. The solid product which separated out was filtered off and crystalized to give **2** (Table I).

Method 2: The imidazole **1b** (2 g) was added to a mixture of ethyl chloroformated (10 ml) and N,N-dimethylformamide (5 ml). The reaction mixture was left to react at room temperature with slight warming for 10-15 min. The solid product obtained was filtered off, washed with cold ethanol, dried and crystallized to give **2**. Undepressed when admixed with the product obtained from method 1.

9-Benzyl-6-chloro-8-(methylthio)-9H-purine 3: A mixture of **2** (2 g) and phosphoryl chloride (5 ml) was

Correspondence to: Khairy A. M. El-Rayouki, National Research Center, Dokki, Cairo, Egypt.

Table I. Characterization of the Synthesized Products

Compound	m.p. °C (solvent)[a]	Yield, % (Time, hr)	IR (KBr) cm $^{-1}$	Analysis Found (Calcd) C	H	N%	Mass M^{+} (%)
1b	215- 6 (E)	65	3410-3100, 3000-2900 1670	55.00 (54.96)	5.50 (5.34)	21.18 (21.37)	262 (30)
2	234- 5 (A)	72	3500-2800, 1750	57.39 (57.35)	4.51 (4.41)	20.62 (20.58)	
3	155- 7 (A)	80	3000-2900	54.20 (53.70)	4.00 (3.78)	18.98 (19.28)	
4a	136- 8 (C)	68 (3)	3150, 3050, 2900	58.70 (58.95)	5.30 (5.26)	24.23 (24.56)	
b	107- 8 (C)	65 (3)	3350, 3050, 2900	60.00 (60.20)	5.55 (5.68)	23.00 (23.41)	
c	90- 2 (B)	50 (5)	3380, 3050-2900	60.94 (61.34)	5.97 (6.07)	22.36 (22.36)	
d	87- 9 (B)	55 (3)	3250, 3050-2900	61.15 (61.34)	6.00 (6.07)	22.00 (22.36)	
e	>250 (E)	50 (15)	3250, 3100-2800	65.25 (65.70)	4.79 (4.89)	20.14 (20.17)	
f	149-50 (A)	62 (17)	3300, 3100-3000	66.79 (66.48)	5.23 (5.26)	19.32 (19.39)	
g	127- 9 (B)	60 (17)	3400, 3000-2800	63.50 (63.66)	4.99 (5.03)	18.24 (18.56)	
5a	>250 (A)	75	3150, 3100-3000	42.50 (42.15)	4.45 (4.22)	16.34 (16.39)	
b	196- 7 (F)	70	3300, 3050-2800	43.80 (43.53)	4.60 (4.53)	15.70 (15.87)	314 (100)
c	200- 2 (A)	60	3400, 3100-2800	45.00 (44.83)	4.80 (4.83)	15.55 (15.38)	
d	216- 7 (D)	50	3300, 3100-3000	44.80 (44.83)	4.90 (4.83)	15.50 (25.38)	
e	>250 (A)	65	3300, 3150-2900	49.25 (49.07)	4.00 (4.08)	14.00 (14.30)	
f	127- 9 (B)	55	3300, 3100-2900	48.64 (48.55)	4.43 (4.23)	13.48 (13.48)	
8	154- 6 (A)	52	3100-2900	59.73 (60.00)	5.95 (6.00)	23.00 (23.33)	299 (38.5)

[a](A) Ethanol, (B) Aqueous ethanol; (C) Cyclohexane; (D) Iso-propanol; (E) Benzene/methanol; (F) Ethyl acetate/iso-propanol.

heated under reflux for 1 h. The reaction mixture was poured on crushed ice and the solid product which separated out was filtered off, washed with water and crystallized to give **3** (Table I).

9-Benzyl-8-(methylthio)-6-(substitutedamino)-9H-purine Derivatives 4a-g:

General procedure: The chloropurine **3** (0.01 mol) was added to the desired amine (0.01 mol) in absolute ethanol (20 ml) containing few drops of triethylamine and the whole mixture was heated under reflux for the appropriate time (3-17 h) until the reaction was completed (TLC). The reaction mixture was then diluted with cold water, the precipitated solid was filtered off and crystallized to give **4a-g** (Table I).

9-Benzyl-3-methyl-6-(substitutedamino)-8-(methylthio)-9-H-purinium Iodide Salts 5a-f:

General procedure: To a solution of **4a-e** and **4g** (0.01 mol) in N,N-dimethylformamide (15 ml), methyl iodide (7 ml) was added and the reaction mixture was left over night at room temperature. Upon treating the reaction mixture with dry diethyl ether a precipitate was obtained. The solid product was then filtered off and crystallized to give **5a-f** (Table I).

9-Benzyl-3-methyl-6-(methylimino)-8-(methylthio)-9 H-purine 8: To a solution of the purinium salt **5a** (1 g) in dilute ethanol (50% 100 ml), sodium hydroxide solution (2 ml, 1 N) was added. The reaction mixture was heated on a steam-bath for 1 hr and then left to cool. The solid product obtained was filtered off

and crystallized to give **8** (Table I).

Cytotoxic Activity

Compounds **1b-5** were screened for cytotoxic activity at the National Institute of Cancer, Cairo University.

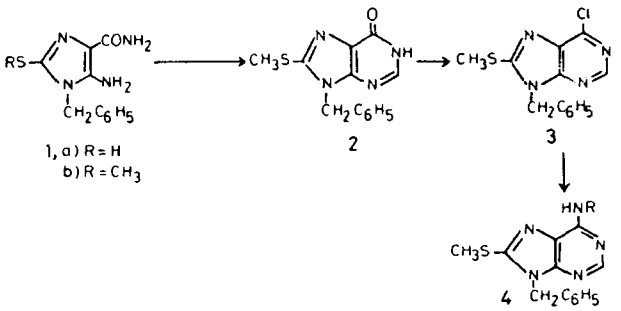
In a set of sterile test tubes 0.1 ml of culture of Ehrlich Ascites Carcinoma (2.5×10^5 cell/ml) suspension, 0.8 ml RPMI and 0.1 ml of each tested compound (corresponded to 100, 50 and 25 µg/ml) were mixed. The test tubes were incubated at 37°C for 2 hr. Trypan blue (Mclimans *et al.*, 1957) exclusion test was carried out to culture percentage of non-viable cells after 2 hr incubation.

RESULTS AND DISCUSSION

5-Amino-1-benzyl-2-(methylthio)-4-imidazolecarboxamide **1b** was synthesized by methylation of 2-mercaptoimidazole **1a** (Sen & Sibbos, 1976) with dimethyl sulfate. **1b** was then cyclized to the corresponding hypoxanthine **2** by using either formamide (Shaw, 1950) or ethyl chloroformate/N,N-dimethylformamide mixture (El-Bayouki *et al.*, 1989). Upon treating **2** with phosphorus oxychloride, the corresponding chloropurine **3** was readily afforded.

Structures of compounds **1b**, **2** and **3** were assigned on the basis of elemental analysis and by studying of their IR and ^{1}H-NMR spectroscopic data (Tables I and II).

The chloropurine **3**, when reacted with several primary amines (alkyl and aryl) inthe presence of an acid scavenger, gave the corresponding 6-substitutedaminopurines **4a-g** in good yields.

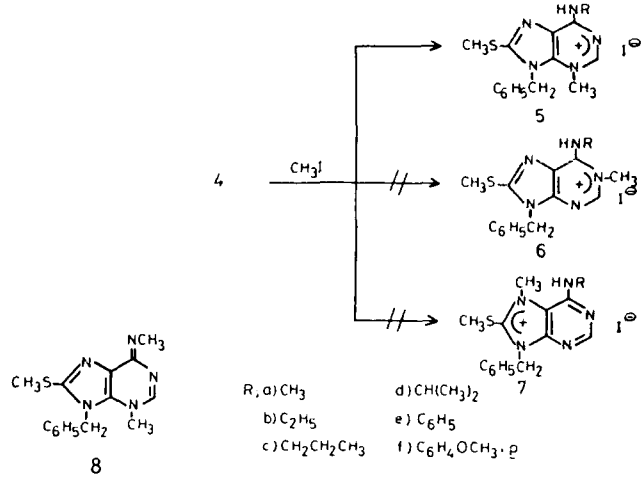


4	R	4	R
a	CH_3	e	C_6H_5
b	C_2H_5	f	$C_6H_4CH_3$-p
c	$CH_2CH_2CH_3$	g	$C_6H_4OCH_3$-p
d	$CH(CH_3)_2$		

IR spectra of the obtained 6-(alkylamino)purines **4a-d** displayed a sharp absorption band in 3380-3250 cm^{-1} region, characteristic for NH group. For instance, this absorption was noticed around 3400-3150 cm^{-1} region for compounds **4e-g**. ^{1}H-NMR spectra of compounds **4** revealed C_2-H (purine), CH_2 (benzyl) and SCH_3 beside the aromatic proton signals. The spectra showed also NH proton signals at δ: 5.70-5.90 ppm for compounds **4c** and **d** and at δ: 9.60 ppm for **4f** (D_2O-exchangeable) (El-Bayouki *et al.*, 1990) (Table II).

Previous reaction (Johannsen *et al.*, 1986) of some furo[2,3-d]pyrimidine-4-amines with alkyl halides has led to their alkylation at N^1-position; however, in case of some other 9-methyl-6-(diethylamino)-purine derivatives, the methylation has been reported (Neiman and Bergman, 1967) to occur at n^3-position.

In the present work it was of interest to study the methylation of the synthesized 6-(substitutedamino) purines **4** with methyl iodide.



Theoretically, the methyl radical could be incorporated either on N^3- or N^1- or N^7-position of the purine ring to afford the probable structure of type **5** or **6** or **7**. Practically, when compounds **4a-e** and **g** were treated with methyl iodide in N,N-dimethylformamide at room temperature, the corresponding N^3-methyl derivatives **5a-f** were isolated as quarternary iodide salts in good yields rather than the probable products of type **6** or **7**. This result could be explained on the following basis:

^{1}H-NMR spectra of products **5**, generally, revealed the characteristic signals noticed in the spectra of their parents **4**, beside the signals due to the incorporated CH_3 groups as a result of alkylation. The proton signals of CH_3 and NH were exhibited in all the investigated spectra of compounds **5** around δ: 3.80 and 8.00-9.00 ppm region respectively (Table II). ^{13}C-NMR spectrum of product **5a** revealed C-6 signal at 154.75 ppm, which is deshielded than that of the parent **4a** (at 153.42 ppm). The spectrum also, cleared both C-2 and C-4 signals at 151.31 and 147.80 ppm respectively, whereas their corresponding of **4a** were deshielded and appeared at 152.22 and 151.02 ppm respec-

Table II. UV, ^{1}H-NMR and ^{13}C-NMR Spectral Data of Compounds **1b-5** and **8**

Compound	UV	^{1}H-NMR	^{13}C-NMR
	λ_{maxnm} (ε)	δ ppm	δ ppm
1b	218 (3345) 260 (6132) 282 (7060)	2.30 (s, 3H, CH_3); 5.10 (s, 2H, CH_2); 5.90 (s, 2H, NH_2, D_2O-exchangeable); 6.70 (s, 2H, NH_2, D_2O-exchangeable); 7.15 (m, 5H, C_6H_5).	
2	220 (9645) 269 (12539)	2.50 (s, 3H, CH_3); 5.15 (s, 2H, CH_2); 7.15 (m, 5H, C_6H_5); 8.00 (s, 1H, C_2-H); 13.25 (s, 1H, NH, D_2O-exchangeable)$_b$.	
3	220 (13391) 290 (17100)		
4a		2.55 (s, 3H, SCH_3); 3.15 (s, 3H, NCH_3); 5.30 (s, 2H, CH_2); 5.80 (s, 1H, NH, D_2O-exchangeable); 7.30 (m, 5H, C_6H_5); 8.30 (s, 1H, C_2-H)$_b$.	C-6 (153.426); C-2 (152.015)' C-4 (151.028); C-8 (149.343); C_6H_5 (135.349, 128.411, 128.047, 127.698, 127.470); C-5 (119.759); N-CH_2 (45.807); N^6-CH, (27.456); SCH_3 (14.250)$_b$.
b		1.30 (t, 3H, CH_3-ethyl); 2.20 (s, 3H, SCH_3; 3.60 (q, 2H, CH_2-ethyl); 5.20 (s, 2H, CH_2); 5.70 (s, 1H, NH); 7.30 (m, 5H, C_6H_5); 8.25 (s, 1H, C_2-h)^a.	
c	207 (16800) 225 (13400) 285 (12500)	1.25 (t, 3H, CH_3-prop.); 1.90 (sex, 2H, CH_2-prop.); 2.90 (s, 3H, SCH_3); 3.80 (q, 2H, CH_2-prop.); 5.45 (s, 2H, CH_2); 5.90 (s, 1H, NH); 7.40 (m, 5H, C_6H_5); 8.50 (s, 1H, C_2-H)$_a$.	
b	207 (26400) 225 (21120) 285 (16200)		
f		2.30 (s, 3H, SCH_3); 2.80 (s, 3H, CH_3); 2.40 (s, 2H, CH_2); 7.10-7.30 (m, 9H, $2C_6H_5$); 8.40 (s, 1H, C_2-H); 9.60 (s, 1H, NH)^a.	
g	220 (8289) 250 (5068) 305 (13815)		
5a	222 (29100) 285 (12240)	2.70 (s, 3H, SCH_3); 3.25 (s, 3H, CH_3); 3.70 (s, 3H, CH_3); 5.30 (s, 2H, CH_2); 7.20 (m, 5H, C_6H_5); 8.60 (s, 1H, C_2-H); 8.80 (s, 1H, NH)^b.	C-6 (154.758); C-2 (151.317); C-4 (147.884); C-8 (146.313); C_6H_5 (134.268, 129.641, 129.339, 127.575); C-5 (119.582); N_6-CH_2 (47.26); N^3-CH_3 (43.104); N^6-CH_3 (32.546); SCH_3 (14.666)^b.
5b	225 (29100) 283 (9200)	1.40 (t, 3H, CH_3-ethyl); 2.80 (s, 3H, SCH_3); 3.90 (s, 3H, CH_3); 4.20 (q, 2H, CH_2-ethyl); 5.40 (s, 2H, CH_2); 7.30 (m, 5H, C_6H_5); 8.80 (s, 1H, C_2-H); 9.00 (s, 1H, NH)^a.	
c	205 (23698) 248 (9479) 305 (22750)		
8	210 (8254) 220 (8413) 282 (5397)	2.45 (s, 3H, SCH_3); 3.40 (s, 3H, CH_3); 3.50 (s, 3H, CH_3); 5.20 (s, 2H, CH_2); 7.30 (m, 5H, C_6H_5); 8.20 (s, 1H, NH)$_a$.	

a) DMSO-d_6 b) $CDCl_3$

tively. Presumably, such shielding for C-2 and C-4 in case of **5a** is attributed to the incorporation of the methyl radical at N^3- of the purine ring, which known as electron releasing group and cause an increase of the electron density at C-2 and C-4 (Table II).

UV absorption spectrum of **5c** exhibited λ_{max} at 308 nm; whereas that of **4c** exhibited λ_{max} 285 nm. Obviously, the alkylation on N^3-position was considered the main reason for such bathochromic shift this is because if alkylation of **4** occured at N^1-(as in the probable structure of type **6**) this bathochromic shift could not be realized. This has previously reported

Table III. *In Vitro* Cytotoxic Activity of the synthesized Compounds

Compound	Inhibition of Cell Viability		
	100	50	25 µg/ml
1b	100	100	85
2	100	90	25
3	100	100	95
4a	100	100	90
d	100	100	85
e	100	100	90
g	100	100	90
5a	100	85	50
b	100	100	95
d	100	100	75

(Marsico and Goldman, 1965) in the UV spectrum of 1-methyladeninesd and its N^3-alkylated analogue (Table II).

The mass spectrum of **5a** revealed m/e=314 (100%, i.e. M^+-I). This observation suggested the splitting of iodide fragment from **5b** in the spectrometer (El-Bayouki *et al.*, 1986) (Table II).

Furthermore, when product **5a** was warmed in ethanolic sodium hydroxide solution, its dequarternized derivative: 9-benzyl-3-methyl-6-(methylimino)-8-(methylthio)-9H-purine **8** was isolated in a satisfactory yield. This was considered as a complementary support for the proposed structure of type **5** and thus structure of type **7** could be excluded; since some 9-methyladeninium iodide derivatives bearing alkyl radicals at N^7-position have been found to be unstable under such mild alkaline treatment (Saito *et al.*, 1990).

The ^{1}H-NMR spectrum of product **8** was found to lack NH proton signal. Its mass spectrum cleared m /e=299 (M^+, 38%).

Antitumor Activity

The results of the *in vitro* cytotoxic screening for compounds **1b-5** were presented in Table III.

All the tested compounds gave a 100% activity at 100 µg/ml concentration. Also, most of the tested compounds gave a 100% activity at 50 µg/ml concentration. Moreover, at 25 µg/ml concentration the activity ranged from 95 to 25%. From the results it was generally concluded that, the quarternization led to decrease in the attained activity. For instance, the activity of compounds **4a** & **d** was dropped from 90 & 85% to 50 & 75% in case of compounds **5a** and **5d** respectively.

Compounds producing more than 70% non viable cells are considered active (El-Merzabani *et al.*, 1979).

REFERENCES CITED

Bharat, T. and Brumo, R., N^6-Disubstituted adenosines: Synthesis and structure-activity relationships. *J. Med. Chem.*, 32(8), 1667-1673 (1989).

Dutta, S. P., Tritsch, G. L., Cox, C. and Chheda, G. B., Synthesis and biological activities of some N^6-(nitro- and amino-benzyl)adenosines. *J. Med. Chem.*, 18 (8), 780-783 (1975).

El-Bayouki, Kh. A. M., Hammad, M., Osman, S. and Basyouni, W. M., Synthesis and quaternization of 5-amino-1-arylimidazole-4-carboxamides and their related imidazo-pyrazines. *Oriental J. Chem.*, 2, 45-50 (1986).

El-Bayouki, Kh. A. M., El-Sayed, A. S. and Basyouni, W. M., Ethyl chloroformate/DMF in organic synthesis (I): A novel reagent for ring closure of 5-amino-1-aryl-4-imidazole carboxamides to their hypoxanthine derivatives. *Gazzetta Chimica Italiana,* 119, 163-165 (1989).

El-Bayouki, Kh. A. M., Basyouni, W. M. and El-Sayed, M. M., Molluscicidal activity of newly synthesised triazolo- and tetrazolothiazolopyrimidines and related derivatives. *Egypt. J. Bilharziasis,* 12, 45-58 (1990).

El-Merzabani, M. M., El-Aaser, A. A., Attia, M. A., El-Duweini, A. K. and Ghazal, A. M., Screening system for Egyptien plants with potential antitumor activity. *J. Med. Plant. Res. (Planta Medica)*, 36, 150-155 (1979).

Fleysher, M. M., Bloch, A., Hakala, M. T. and Nicol, C. A., Synthesis and biological activity of some new N^6-substituted purine nucleosides. *J. Med. Chem.*, 12, 1056-1061 (1969).

Johannsen, F., Jargensen, A., El-Bayouki, Kh. A. M. and Pedersen, E., Phosphorus pentaoxide in organic synthesis XXVI: Preparation and alkylation of furo(2,3-d) pyrimidin-4-amines. *Chemica Scripta,* 26, 337-342 (1986).

Kelly, J. L. and Soroka, F. E., 9-(2-Fluorobenzyl)-6-(methylamino)-9H-purine hydrochloride: Synthesis and anticonvulsant activity. *J. Med. Chem.*, 29, 1133-1134 (1986).

Kiyoto, I., Hajime, K. and Kiyoshi, I., 7-Hydroxyguanine compounds and their use as antitumor agents. *European Patent Applied EP* 204,251 (1985); *C.A.*, 106, 156161 (1987).

Kozalka, G., Krentsky, T., Rideout, J. and Burns, C., Preparation of 9-β-2'-3'-dideoxyribofuranosylpurines for treatment and prophylaxix of human immunodeficiency virus infection. *European Patent,* EP 286,425 (1987); *C.A.*, 111, 23910 (1989).

Marsico, J. and Goldman, L., Site of alkylation of N^6,N^6-di-alkyl-9-substituted adenosines: Synthesis and alkaline degradation of 6-diethylamino-3,9-dimethylpurinium iodide. *J. Org. Chem.*, 30, 3597-3600 (1965).

McGee, D. P. C., Martin, J. C., Smee, D. F., Matthews, T. R. and Verheyden, J. P. H., Synthesis and antiherbes simplex virus activity of 9-[(1,3-dihydroxy-2-propylthio)methyl]guanine. *J. Med. Chem.*, 28(9), 1242-

1245 (1985).

McLimans, W. F., Davis, E. V., Golve, F. I. and Rake, G. W., The submerged culture of mammalien cells: The spinner culture. *J. Immunol.*, 79, 428-433 (1957).

Moharram, H. and Osman, A., Reactions of purine derivatives with phosphorus pentaoxide and triethylamine and their antitumor effects. *Arch. Pharm. Res.*, 12(1), 1-4 (1989).

Neiman, Z. and Bergman, F., Benzylation of 6-alkylthiopurines in aprotic solvents. *Isracel J. Chem.*, 5, 243-245 (1967).

Saito, T., Inous, I. and Fuji, T., Purines XLI: An alernative synthesis and the chemical behaviour of 7,9-dialkyladeninium salts. *Chem. Pharm. Bull.*, 38(6), 1536-1547 (1990).

Sen, A. and Sibbos, R., Synthesis of compounds related to 4(5)-aminoimidazole-5(4)-carboxamides: Part II. *Ind. J. Chem.*, 14B, 222-225 (1976).

Shaw, E., A new synthesis of the purines adenine, hypoxanthine, xanthine and isoguanine, *J. Biol. Chem.*, **185**, 439-447 (1950).