SYNTHESIS OF β-(9-PURINYL)ALANINES

M. Yu. Lidak, Ya. Ya. Shluke, and S. E. Poritere

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The corresponding 1-(6-substituted-9-purinyl)-2,2-diethoxyethanes were obtained by alkylation of 6-substituted purines with 1-bromo-2,2-diethoxyethane. Subsequent transformations of a large portion of the acetals gave 2-(6-substituted-9-purinyl) acetaldehydes, from which β-(6-substituted-9-purinyl)alanines were obtained by the cyanohydrin synthesis. The classification of the compounds as $N_{(9)}$-substituted purine derivatives was proved by means of UV spectroscopy.

In previous papers [1-3] we reported the synthesis of β-(2-amino-6-substituted-9-purinyl)alanines, α-amino-γ-(6-substituted-9-purinyl)butyric acids, and α-amino-ε-(9-purinyl)caproic acids.

We first described the general principle of the synthesis of β-(9-purinyl)alanines (XXII-XXX), i.e., those structures in which the α-amino acid residue is added to the nitrogen atom in the 9 position of the purine ring, several years ago [4,5]. In the present paper we examine the more detailed conditions for the synthesis of XXII-XXX and give a detailed experimental procedure. The starting compounds for the preparation of XXII-XXX were diethylacetals V-VIII, which were obtained by alkylation of 6-substituted purines (I-IV) with 1-bromo-2,2-diethoxyethane (XXXI) via the modified method of Montgomery and Temple [6]. The alkylation of 6-chloropurine (I), 6-methylthiopurine (II), 6-ethylthiopurine (III), and 6-benzylthiopurine (IV) was carried out in the presence of sodium hydride in dimethylformamide at elevated temperatures. The small amount of N_7 derivative impurity, which is formed in the alkylation, was separated by crystallization and was not isolated in pure form. Compounds IX-XV (Table 1) were synthesized from V and VI by reaction with nucleophilic reagents by known methods [6,7]. It should be noted that X-XIV are more conveniently obtained from VI.

TABLE 1. Properties of 1-(6-Substituted-9-purinyl)-2,2-diethoxyethanes

Comp.	mp, °C	Crystalliz. solvent	R_f		Empirical formula	Found, %			Calc., %			Yield, %
			1	2		C	H	N	C	H	N	
V	82—83	Hexane	0,92	0,93	$C_{11}H_{15}ClN_4O_2$	49,0	5,6	21,3	48,8	5,6	20,7	25
VI	76	Acetone–water (1:3)	0,91	0,88	$C_{12}H_{18}N_4O_2S$	51,4	6,3	19,7	51,0	6,4	19,8	33,5
VII	54—55	The same	0,93	0,95	$C_{13}H_{20}N_4O_2S$	52,7	6,4	18,9	52,9	7,0	19,5	42,5
VIII	90—91	Water	0,93	0,96	$C_{18}H_{22}N_4O_2S$	60,5	6,8	15,9	60,4	6,1	15,7	21
IX	300	Propanol	0,85	0,87	$C_{11}H_{16}N_4O_2S$	49,0	6,3	21,1	49,2	6,0	20,9	78
X	217—218	Ethanol	0,82	0,91	$C_{11}H_{17}N_5O_2$	52,6	6,8	27,6	52,6	6,8	27,9	63,5* 67,0 †
XI	94	Hexane	0,85	0,93	$C_{12}H_{19}N_5O_2$	54,2	7,3	26,0	54,3	7,2	26,4	70,5
XII	60	The same	0,93	0,95	$C_{13}H_{21}N_5O_2$	55 7	7,5	25,4	55,9	7,6	25,1	70,5
XIII	116	„ „	0,80	—	$C_{15}H_{25}N_5O_2$	58,6	8,3	22,8	58,3	8,1	22,7	64
XIV	128—129	„ „	0,95	0,91	$C_{16}H_{21}N_5O_2$	57,7	6,5	21,4	58,0	6,4	21,1	31,4

*Obtained by method A from V.

†Obtained by method B from VI.

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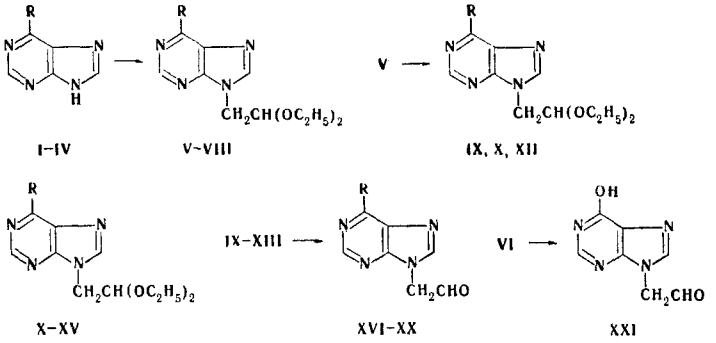
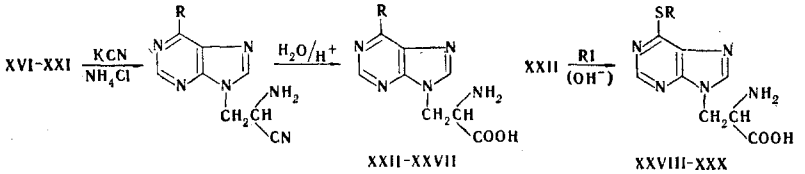
The corresponding 2-(9-purinyl)acetaldehydes (XVI-XX) were obtained by hydrolysis of IX-XIII by heating with 1 N-5 N hydrochloric acid. 2-(9-Hypoxanthinyl)acetaldehyde (XXI) was obtained by the action of chlorine on VI and hydrolysis of the reaction mixture.



I, V R=Cl; II, VI R=SCH_3; III, VII R=SC_2H_5; IV, VIII R=$SCH_2C_6H_5$; IX, XVI R=SH; X, XVII R=NH_2; XI, XVIII R=$NHCH_3$; XII, XIX R=$N(CH_3)_2$; XIII, XX R=$NH(CH_2)_3CH_3$; XIV R=$NHCH_2C_4H_3O$*; XV R=OH †

It is interesting to note that an attempt to obtain XXI by hydrolysis of V was unsuccessful. Because of their instability, XVIII-XX were not subjected to further purification but were introduced immediately into the Strecker–Zelinsky–Stadnikoff cyanohydrin synthesis. Exceptions to this were XVI and XVII, which were obtained in pure form.

The first phase in the cyanohydrin synthesis – preparation of the corresponding aminonitrile – was accomplished by heating a mixture of 2-(6-substituted-9-purinyl)acetaldehyde with an aqueous solution of potassium cyanide, ammonium chloride, and ammonia. The aminonitriles, without isolation from the reaction mixture, were then subjected to hydrolysis by heating with highly concentrated hydrochloric acid. The β-(6-alkylthio-9-purinyl)alanines (XXVIII-XXX) were obtained via the method in [8] by alkylation of XXII with the appropriate alkyl iodide in alkaline media.



XXII R=SH; XXIII R=NH_2; XXIV R=$NHCH_3$; XXV R=$N(CH_3)_2$; XXVI R=$NH(CH_2)_3CH_3$; XXVII R=OH; XXVIII R=CH_3; XXIX R=C_2H_5; XXX R=$CH(CH_3)_2$

The β-(6-substituted-9-purinyl)alanines (XXII-XXX) (Table 2) are high-melting crystalline substances of amphoteric character that have intense absorption in the near UV region. Compounds XXII-XXX give a positive ninhydrin reaction (blue-violet color).

The characteristic differences in the UV absorption between the 7-substituted and 9-substituted 6-dimethylaminopurine derivatives [9] served as proof that the substituent of the purinylalanines obtained (XXII-XXX) was attached to $N_{(9)}$. The fact that XXV, with a UV absorption maximum at 275 nm (pH 7), which is characteristic for 9-substituted purines, was obtained from XII, and that XII in turn was isolated from V and VI which are starting compounds for the synthesis for all of the other β-(9-purinyl)alanines, enables us to confirm the structure of XXII-XXX as 9-substituted purine derivatives.

EXPERIMENTAL

The UV spectra were recorded with an SF-4 spectrophotometer. Ascending chromatography on FN-11 paper in the following systems was used to identify the compounds obtained: $C_4H_9OH-H_2O$ (85:14) (system 1), $C_3H_7OH-NH_4OH-H_2O$ (6:3:1) (system 2), iso-$C_3H_7OH-NH_4OH-H_2O$ (7:1:2) (system 3), and

*C_4H_3O is furyl.

† Because of partial resinification of the product, 1-(9-hypoxanthinyl)-2,2-diethoxyethane was not isolated in analytically pure form.

TABLE 2. β-(6-Substituted-9-purinyl)alanines

Comp.	mp, °C*	R_f			Empirical formula	Found, %			Calc., %			UV spectra						Yield, %
												pH 1		pH 7		pH 13		
		2	3	4		C	H	N	C	H	N	λ_{max}, nm	lg ε	λ_{max}, nm	lg ε	λ_{max}, nm	lg ε	
XXII	225	0,66	—	0,26	$C_8H_9N_5O_2S \cdot H_2O$	37,6	4,3	27,2	37,3	4,3	27,2	322	4,36	320	4,38	310	4,34	33,3
XXIII	248—250	0,61	0,14	0,35	$C_8H_{10}N_6O_2 \cdot H_2O$	39,6	5,3	34,5	40,0	5,0	35,0	258	4,18	259	4,17	262	4,30	38,1
XXIV	247	—	0,27	0,55	$C_9H_{12}N_6O_2 \cdot H_2O$	42,16	5,2	33,4	42,6	5,6	33,1	263	4,19	265	4,16	266	4,19	30,6 †
XXV	243	0,77	0,32	0,61	$C_{10}H_{14}N_6O_2 \cdot H_2O$	45,1	5,8	31,5	44,1	6,1	31,3	268	4,29	275	4,30	273	4,29	43,5 †
XXVI	240	0,72	0,80	—	$C_{12}H_{18}N_6O_2 \cdot H_2O$	48,8	6,7	28,9	48,8	6,8	28,4	263	4,55	265	4,52	266	4,46	25,9 †
XXVII	219—220	0,66	—	0,25	$C_8H_9N_5O_3 \cdot 1\frac{1}{2}H_2O$	38,4	5,1	27,5	38,4	4,8	28,0	249	4,06	248	4,08	255	4,11	35,4 †
XXVIII	223—225	0,75	0,35	0,54	$C_9H_{11}N_5O_2S$	42,5	4,3	27,8	42,5	4,4	27,7	292	4,25	290	4,28	287	4,30	66,0
XXIX	219—220	—	—	0,78	$C_{10}H_{13}N_5O_2S \cdot \frac{2}{3}H_2O$	42,7	5,3	25,4	42,7	5,1	25,1	290	4,24			289	4,21	46,0
XXX	230	—	—	0,85	$C_{11}H_{15}N_5O_2S \cdot H_2O$	44,2	6,0	33,6	44,2	5,7	23,3	294	3,97	292	4,02	289	4,0	43,0

*Compounds XXII-XXX were recrystallized from water.

† Based on the acetal.

TABLE 3. Synthesis Conditions

Comp. obtained as a result of the reaction	DMF, ml	Alkylation conditions			Crystallization solvent
		time, h	temp., °C	temp. at end of reac., °C	
V	60	11	70	120	Hexane
VI	120	7	80	140	Acetone—water (1 : 3)
VII	70	7	80	140	The same
VIII	200	7	80	140	The same

TABLE 4. Reaction Conditions

Compounds obtained as result of reaction	Amine	Amt. of amine, ml	Reaction cond.	
			temp., °C	time, h
XI	25% aqueous methylamine	20	135	23
XII	30% aqueous dimethylamine	15	135	23
XIII	33% aqueous butylamine	20	142—144	25
XIV	Furfurylamine	10	150	24

$C_4H_9OH-CH_3COOH-H_2O$ (4:1:5) (system 4). The chromatograms were developed by UV absorption and with ninhydrin.

Alkylation of 6-Substituted Purines. A mixture of 0.04 mole of I-IV and 0.96 g (0.04 mole) of sodium hydride was stirred for 2 h in dry dimethylformamide, after which 15.76 g (0.08 mole) of XXXI was added, and the mixture was held at an elevated temperature for several hours. At the end of the reaction, the temperature was raised in the course of 1 h. The solvent and excess XXXI were removed by distillation, and the residue was extracted with ether. The ether extract was washed with water and evaporated, and the residue was crystallized. The conditions for the synthesis of V-VIII are presented in Table 3.

1-(6-Mercapto-9-purinyl)-2,2-diethoxyethane (IX). A mixture of 2.2 g (8.2 mmole) of V and 0.7 g (9.2 mmole) of thiourea in 50 ml of propyl alcohol was refluxed for 1.5 h in a flask equipped with a reflux condenser, after which the mixture was cooled, and the precipitate was removed by filtration and crystallized from propanol to give 1.7 g of IX.

1-(9-Adeninyl)-2,2-diethoxyethane (X). A. A solution of 1.36 g (5 mmole) of V in 20 ml of methanol, saturated with ammonia at 0°, was held at 115° in a sealed tube for 15 h. The solvent was removed by distillation, and the residue was crystallized from ethanol to give 0.8 g of X.

B. A solution of 3.0 g (0.011 mole) of VI in 40 ml of methanol, saturated with ammonia at 0°, was held at 145-150° in a sealed tube for 22 h. The solvent was removed by distillation, and the residue was crystallized from ethanol to give 1.8 g of X.

Synthesis of 1-(6-Substituted-9-purinyl)-2,2-diethoxyethanes (XI-XIV). A mixture consisting of 3.0 g (11 mmole) of VI and the amine or an aqueous solution of the amine was heated in an autoclave. The solvent was removed by distillation, and the residue was crystallized from hexane. The reaction conditions are given in Table 4.

2-(6-Mercapto-9-purinyl)acetaldehyde (XVI). A solution of 2.12 g (0.01 mole) of IX in 10 ml of 5 N HCl was heated to the boiling point, after which it was allowed to stand at room temperature for 1 h. The acid was removed by vacuum distillation, and the residue was crystallized from water to give 1.91 g (90%) of XVI as yellowish needles with mp > 200° (dec.). Found,%: C 39.2; H 4.2; N 26.4; S 14.8. $C_7H_6N_4OS \cdot H_2O$. Calculated,%: C 39.6; H 3.8; N 26.4; S 15.1.

β-(6-Mercapto-9-purinyl)alanine (XXII). A 1-g (4.7 mmole) sample of XVI, 0.32 g (6 mmole) of ammonium chloride, and 0.39 g (6 mmole) of potassium cyanide were dissolved in a mixture of 0.8 ml of ammonium hydroxide and 2.9 ml of water, and the resulting mixture was heated at 55-60° for 5 h. Concentrated HCl (10 ml) was added, and the mixture was heated on a water bath for 1 h and allowed to stand overnight at room temperature. It was then evaporated to dryness, and 5 ml of 10 N hydrochloric acid was added to the residue. The resulting solution was refluxed for 3 h, after which the hydrochloric acid was removed by vacuum distillation, and traces of the acid were removed by azeotropic distillation with water. The residue was dissolved in the minimum amount of water, and the pH of the solution was brought up to 5 by the addition of ammonium hydroxide. Crystallization of the resulting precipitate gave 0.4 g of XXII.

2-(9-Adeninyl)acetaldehyde Hydrochloride (XVII). A solution of 4.2 g (1.7 mmole) of XI in 35 ml of 1.5 N hydrochloric acid was heated on a water bath for 30 min and allowed to stand overnight. The precipitate was collected and dried over P_2O_5. The weight of the crude product, which was suitable for further treatment, was 3.6 g (87%).

β-(9-Adeninyl)alanine (XXXIII). A mixture consisting of 10 ml of water, 10 ml of ammonium hydroxide, 2.2 g (9 mmole) of XVII, 0.86 g (13 mmole) of potassium cyanide, and 0.7 g (13 mmole) of ammonium chloride was heated at 55-60° for 5 h with vigorous stirring. Concentrated HCl (50 ml) was added, and the mixture was heated on a water bath for 1 h and allowed to stand at room temperature overnight. The mixture was then evaporated to dryness, and 50 ml of 10 N hydrochloric acid was added to the residue. The mixture was then worked up as in the case of XXII to give 0.8 g of XXIII.

2-(6-Methylamino-9-purinyl)acetaldehyde Hydrochloride (XVIII). A solution of 2.65 g (0.01 mole) of XI in 20 ml of 1 N hydrochloric acid was heated on a water bath for 45 min. The acid was removed by vacuum distillation, the residue was dissolved in water, and the pH of the solution was brought up to 5 by the addition of ammonium hydroxide. The resulting precipitate was removed by filtration and crystallized from water to give 1.83 g (70%) of XVIII with mp 180°. Found,%: C 36.7; H 4.9; Cl 13.8; N 26.7. $C_8H_9N_5O \cdot HCl \cdot 2H_2O$. Calculated,%: C 36.4; H 5.4; Cl 13.4; N 26.6.

β-(6-Methylamino-9-purinyl)alanine (XXIV). A 1.7-g (6.5 mmole) sample of XI in 12 ml of 1 N hydrochloric acid was heated on a water bath for 45 min. The mixture was then evaporated to dryness, and the residue was subjected to azeotropic distillation with water in order to remove traces of hydrochloric acid. It was then crystallized from water to give 0.9 g of XVIII, which was sufficiently pure for further treatment. A mixture consisting of the XVIII, 2.9 g of ammonium chloride, 0.35 g of potassium cyanide, 5 ml of ammonium hydroxide, and 5 ml of water was processed as in the preparation of XXII to give, after recrystallization, 0.5 g of XXIV.

β-(6-Dimethylamino-9-purinyl)alanine (XXV). A 1.3-g (4.7 mmole) sample of XII in 9 ml of 1 N hydrochloric acid was heated on a water bath for 30 min and then evaporated to dryness. The residue was dissolved in 10 ml of water, and the pH was brought to 5-6 by the addition of ammonium hydroxide. The resulting precipitate of XIX was added to a solution of 0.29 g of ammonium chloride, 0.35 g of potassium cyanide, and 4 ml of ammonium hydroxide in 5 ml of water, and the mixture was processed as in the preparation of XXIV [the amount of hydrochloric acid used for saponification was as follows: 1) 12 ml of 10 N HCl and, 2) 10 ml of 10 N HCl] to give, after crystallization, 0.55 g of XXV.

β-(6-Butylamino-9-purinyl)alanine (XXVI). A solution of 2.0 g (6.6 mmole) of XIII in 12 ml of 1 N hydrochloric acid was heated on a water bath for 45 min and evaporated to dryness. The residue was dissolved in the minimum amount of water, and the pH was brought up to 5-6 by the addition of ammonium hydroxide. The resulting precipitate of crude XX was added, without additional purification, to a solution of 0.56 g of potassium cyanide, 0.46 g of ammonium chloride, and 6 ml of ammonium hydroxide in 8 ml of water. The mixture was processed as in the preparation of XXII [the amount of hydrochloric acid used for the saponification was as follows: 1) 12 ml of concentrated HCl and, 2) 10 ml of 10 N HCl] to give, after crystallization, 0.5 g of XXVI.

β-(9-Hypoxanthinyl)alanine (XXVII). Fluorine was bubbled with stirring into a solution of 2.0 g (7 mmole) of VI in 15 ml of absolute methanol with cooling in such a way that the temperature did not exceed 5° in the course of 20 min. The methanol was then removed by vacuum distillation, 10 ml of 5 N hydrochloric acid was added to the residue, and the mixture was refluxed. After 30 min, the hydrochloric acid was removed by azeotropic distillation with small portions of water, and the residue was dissolved in the minimum amount of water at 90°. The solution was brought up to pH 5 by the addition of ammonium hydroxide, and the resulting precipitate of XXI (0.85 g) was dissolved in 2 ml of water. The solution was treated with 0.4 g of ammonium chloride, 0.45 g of potassium cyanide, and 1.5 ml of 25% ammonium hydroxide, and the mixture was worked up as in the preparation of XXII to give, after crystallization, 0.63 g (35.4%, based on the acetal) of product.

β-(6-Methylthio-9-purinyl)alanine (XXVIII). A 0.1-g (4 mmole) sample of XXII was dissolved in a mixture of 1.3 ml of 1 N NaOH and 2 ml of water, after which 0.5 g of methyl iodide was added by drops with vigorous stirring at room temperature in the course of 30 min. The mixture was stirred for another hour, after which the reaction solution was brought down to pH 5 by the addition of acetic acid. The precipitate was crystallized to give 0.07 g of XXVIII.

β-(6-Ethylthio-9-purinyl)alanine (XXIX). A 0.4-g (16 mmole) sample of XXII was dissolved in a mixture of 2 ml of 2 N NaOH and 3 ml of water, after which 1.0 g of ethyl iodide was added by drops with vigorous stirring at 55-60°. Stirring was continued for another 30 h at the same temperature, and the mixture was then worked up as in the preparation of XXVIII to give 0.2 g of XXIX.

β-(6-Isopropylthio-9-purinyl)alanine (XXX). A 0.4-g (16 mmole) sample of XXII was dissolved in a mixture of 1.5 ml of 2 N NaOH and 3 ml of water, after which 1 ml of isopropyl iodide was added by drops with vigorous stirring at 60-70°. Stirring was continued for another 10 h at the same temperature, and the mixture was then worked up as in the preparation of XXVIII to give 0.2 g of XXX.

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