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SYNTHESIS OF OPTICALLY ACTIVE 9-PURINYL-α-AMINO ACIDS

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A general method for the synthesis of optically active 9-purinyl-α-amino acids by condensation of 5-amino-4,6-dichloropyrimidine with α,ω-diamino carboxylic acids and subsequent cyclization of the N_ω-(5-amino-4-chloro-6-pyrimidinyl)-amino acids with triethyl orthoformate was developed. A number of 6-substituted α-amino-ω-(9-purinyl) carboxylic acids were obtained by nucleophilic substitution of the chlorine atom in the α-amino-ω-(6-chloro-9-purinyl) carboxylic acids.

We first synthesized 9-purinyl-α-amino acids by the cyanohydrin method [1], after which they were obtained by other researchers [2-4]; however, the syntheses of only racemic 9-purinyl-α-amino acids have thus far been described.

Interesting data regarding the biological activity of 9-purinylamino acids were recently obtained. Thus some of the derivatives isolated from the growing seeds of lupine are of interest as new cytokinins [5]. It has also been established that 9-adeninyl-α-alanine is a strong activator of the enzyme adenylate cyclase, which is responsible for the synthesis of cyclic AMP in the cell [6].

The goal of the present research was to develop a method for the direct synthesis of the optically active 9-purinyl-α-amino acids that are necessary for further biological studies.

We have already reported [7] a new method for the synthesis of 6-substituted 9-purinyl-α-amino acids that is based on the cyclization of N_ω-(5-amino-4-chloro-6-pyrimidinyl)diamino carboxylic acids with ethyl orthoformate; this method made it possible to obtain DL-α-amino-ε-(6-chloro-9-purinyl)caproic acid [7].

Further studies showed that the method is general in character and that by using the corresponding α,ω-diamino carboxylic acids one can synthesize 9-purinyl-α-aminovaleric acids (regarding the synthesis of which no data had been previously reported), as well as 9-purinyl-α-aminopropionic and -α-aminobutyric acids, which had previously been obtained by other methods [2-4].

Optically active 9-purinyl-α-amino acids can also be obtained by this new method by using L- or D-α,ω-diamino carboxylic acids as the asymmetric agent, and optically active N_α-substituted ω-(9-purinyl)amino carboxylic acids that are suitable for the subsequent synthesis of peptides can also be obtained by using N_α-substituted diamino carboxylic acids.

The starting compounds were N_ω-(5-amino-4-chloro-6-pyrimidinyl)diamino acids II, which were obtained by condensation of 5-amino-4,6-dichloropyrimidine (I) with the corresponding α,ω-diamino carboxylic acids.

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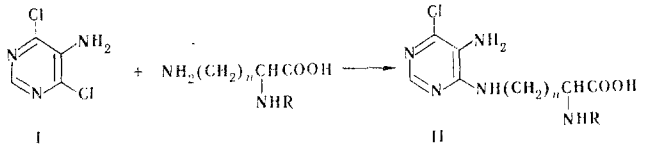


II a n=1, R=H; b n=2, R=H; c n=3, R=H; d n=4, R=H; e n=1, R=Tos

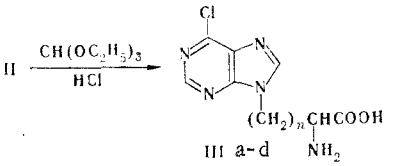
In connection with the fact that the amino acids are not soluble in organic solvents, the condensation was carried out in 1 N sodium hydroxide solution at high temperatures.

The side product of the reaction (5-amino-6-hydroxy-4-chloropyrimidine) can be separated efficiently by extraction with ethyl acetate. The yields of pyrimidinylamino acids II averaged 43% after crystallization.

The condensation of pyrimidine I with N_α-substituted diamino carboxylic acid is realized by refluxing for 3 h in Methylcellosolve in the presence of triethylamine. The yield of N_β-(5-amino-4-chloro-6-pyrimidinyl)-α-tosyldiaminopropionic acid averaged 60%.

The UV spectra of all diamino carboxylic acids IIa-e recorded at pH 1, 7, and 13 contain intense absorption maxima at 302, 265, 290, 263, and 291 nm, respectively.

α-Amino-ω-(6-chloro-9-purinyl)carboxylic acids III are obtained as a result of the formation of an imidazole ring in pyrimidinylamino acids II; the ring-closing reaction is carried out in triethyl orthoformate in the presence of hydrochloric acid at room temperature.

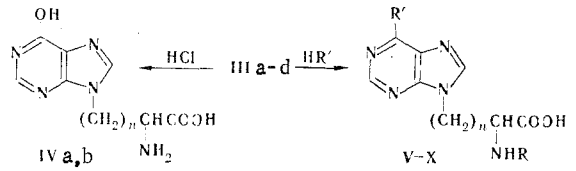


III a n=1; b n=2; c n=3; d n=4

We found that a mixture of N_α-tosyl-β-(6-chloro-9-purinyl)alanine and its ethyl ester is formed in the reaction of N_β-(5-amino-4-chloro-6-pyrimidinyl)-α-tosyldiaminopropionic acid with triethyl orthoformate in the presence of hydrochloric or ethanesulfonic acid. The structure of the ester was proved by the PMR spectrum, in which a multiplet at 3.2-3.7 ppm due to the methylene fragment of the ester grouping is observed. Only N_α-tosyl-β-(6-chloro-9-purinyl)alanine (IIIe) is formed when a mixture of triethyl orthoformate with acetic anhydride is used as the cyclizing agent.

An absorption maximum at 263-265 nm is observed in the UV spectra of all of the 6-chloro-9-purinyl-α-amino acids obtained, regardless of the pH of the medium; it is therefore convenient to use the data from UV spectroscopy in acidic media to monitor the cyclization. A hypsochromic shift on the order of 40 nm is observed on passing from 5-amino-4-chloro-6-amino-substituted pyrimidines to 9-substituted 6-chloropurines; a change in the amino acid radical in the 9 position of the purine ring does not have a substantial effect on the shift of the maximum of the UR spectrum.

The presence of a labile chlorine atom in ω-(6-chloro-9-purinyl)-α-amino acids III makes it possible to obtain a number of 6-substituted α-amino-ω-(9-purinyl)carboxylic acids.



IV a n=1, b n=3; V a-d R′=NH_2, R=H; a n=1, b n=2, c n=3, d n=4, e R=Tos, n=1; VI a, b R′=$C_6H_5CH_2NH$, R=H, a n=1, b n=3, c R=Tos, n=1; VII R′=$N(CH_3)_2$, n=3, R=H; VIII R′=morpholino, n=3, R=H; IX R′=$NHCH_3$, n=3, R=H; X R′=$NHCH_2CH{=}C(CH_3)_2$, R=H, a n=1, b n=3

The corresponding 9-hypoxanthinyl-α-amino acids IV were obtained in the hydrolysis of acids III, while the corresponding ω-adenyl- (V) and ω-(6-amino-9-purinyl)-α-amino acids (VI-X) were obtained in the case of amination with ammonia, amines, and amino acids.

TABLE 1. N_ω-(5-Amino-4-chloro-6-pyrimidinyl)diamino Acids

Compound	R_f			mp, °C	$[\alpha]_D^{20}$, deg	Found, %			Empirical formula	Calc., %			Yield, %
	1	2	3			C	H	N		C	H	N	
IIa[a]	0,72	0,20	—	190—192	+7,3 (c=1,0; 1 N NH_4OH)	32,3	4,8	27,5	$C_7H_{10}ClN_5O_2 \cdot 1{,}5H_2O$	32,5	5,1	27,1	34
IIa[b]	0,72	0,20	—	210	−7,4 (c=1,0; 1N NH_4OH)	32,4	5,2	27,3	$C_7H_{10}ClN_5O_2 \cdot 1{,}5H_2O$	32,5	5,1	27,1	36
IIb	0,58	0,28	0,52	181—182	—	32,3	5,9	22,9	$C_8H_{12}ClN_5O_2 \cdot 3H_2O$	32,1	6,0	23,4	41
IIc	0,69	0,28	0,47	246—247	—	38,6	5,8	25,1	$C_9H_{14}ClN_5O_2 \cdot H_2O$	38,9	5,8	25,2	46
IIc[a]	0,69	0,28	0,48	261	+2,3 (c=1,0; 1N NH_4OH)	38,9	6,0	25,4	$C_9H_{14}ClN_5O_2 \cdot H_2O$	38,9	5,8	25,2	45
IId[a]	0,72	0,23	0,42	252—253	+3,9 (c=1,0; H_2O)	41,3	6,4	24,2	$C_{10}H_{16}ClN_5O_2 \cdot H_2O$	41,2	6,2	24,0	30
IIe[a]	0,84	0,78	—	194—196	−19,6 (c=1; 1N NH_4OH)	41,6	4,4	17,3	$C_{14}H_{16}ClN_5O_4S \cdot H_2O$	41,6	4,5	17,3	58

[a]The L isomer. [b]The D isomer.

A report regarding the separation of DL-N_α-acetyl-β-(9-adeninyl)-α-alanine into its optical antipodes by stereoselective enzymatic hydrolysis [8] was published at the same time as we were conducting our studies of the synthesis of optically active 9-purinyl-α-amino acids. It should be noted that the signs and magnitudes of the specific rotations of the enantiomers of β-(9-adeninyl)-α-alanine that we synthesized and those obtained by enzymatic hydrolysis coincided.

All of the 9-purinyl-α-amino acids that we obtained are high-melting crystalline substances with amphoteric character that give a positive ninhydrin test and have intense absorption in the near-UV region. The specific rotations of the amino acid derivatives obtained did not change after many crystallizations.

EXPERIMENTAL

The UV spectra of solutions of the compounds in 0.1 N HCl, water, and 0.1 N NaOH were recorded with a Spectromom-204 spectrophotometer. The specific rotations were measured with a Perkin-Elmer-141 polarimeter. The course of the reaction and monitoring of the individuality of the compounds obtained were realized by means of thin-layer chromatography (TLC) on Silufol UV-254 plates in the following systems: 1) 2-propanol—25% ammonium hydroxide—water (7:1:2); 2) butanol—acetic acid—water (6:2:2); 3) water. The substances were detected on the chromatograms from their absorption in UV light and were developed with ninhydrin.

N_ω-(5-Amino-4-chloro-6-pyrimidinyl)diamino Acids (IIa-d). A solution of 10 mmole of 5-amino-4,6-dichloropyrimidine (I) and 10 mmole of the corresponding α,ω-diamino carboxylic acid in 30 ml of 1 N sodium hydroxide was heated on a water bath for 3 h, after which the cooled solution was extracted with ethyl acetate. The aqueous solution was evaporated *in vacuo* to approximately one third its original volume, and the concentrate was acidified to pH 5-6 by the addition of acetic acid. The solution was allowed to stand overnight, and the resulting precipitate was removed by filtration and crystallized from water (Table 1).

N_β-(5-Amino-4-chloro-6-pyrimidinyl)-α-tosyldiaminopropionic Acid (IIe). A solution of 1.64 g (10 mmole) of aminopyrimidine I and 2.58 g (10 mmole) of N_α-tosyl-α,β-diaminopropionic acid in 70 ml of Methylcellosolve and 20 ml of triethylamine was heated at 120°C for 7 h, after which the solution was evaporated *in vacuo*. Ether was added to the syrupy residue, and the mixture was allowed to stand overnight. The ether was then decanted, the residue was dissolved in water, and the aqueous solution was extracted with ether. The aqueous phase was acidified to pH 5 by the addition of dilute hydrochloric acid and cooled to precipitate 2.34 g (58%) of acid IIe (Table 1).

α-Amino-ω-(6-chloro-9-purinyl) Carboxylic Acids (IIIa-d). A solution of 1 mmole of diamino acid II in a mixture of 6 ml of triethyl orthoformate with 0.43 ml of hydrochloric acid was stirred at room temperature for 4-5 h, after which it was maintained at 0°C overnight. The precipitate was removed by filtration and crystallized from ethanol—ether (Table 2).

N_α-Tosyl-β-(6-chloro-9-purinyl)alanine (IIIe). A mixture of 0.8 g (2 mmole) of acid IIe with 2 ml of acetic anhydride and 2 ml of triethyl orthoformate was heated at 120°C for

TABLE 2. α-Amino-ω-(6-chloro-9-purinyl) Carboxylic Acids

Compound	R_f			mp, °C	$[\alpha]_D^{20}$, deg	Found, %			Empirical formula	Calc., %			Yield, %
	1	2	3			C	H	N		C	H	N	
IIIa[a]	0,78	0,14	0,61	198	−32 (c=1,0; H_2O)	34,7	3,4	25,0	$C_8H_8ClN_5O_2 \cdot HCl$	34,6	3,3	25,2	96
IIIa[b]	0,76	0,14	0,60	205	+32 (c=1,0; H_2O)	34,8	3,4	25,0	$C_8H_8ClN_5O_2 \cdot HCl$	34,6	3,3	25,2	85
IIIb	—	0,18	0,53	232	—	37,3	3,9	24,3	$C_9H_{10}ClN_5O_2 \cdot HCl$	37,0	3,8	24,0	87
IIIc	0,66	0,16	0,50	210	—	39,4	4,3	23,2	$C_{10}H_{12}ClN_5O_2 \cdot HCl$	39,2	4,3	22,9	88
IIIc[a]	0,65	0,16	0,51	217	+8 (c=1,0; H_2O)	39,2	4,1	22,6	$C_{10}H_{12}ClN_5O_2 \cdot HCl$	39,2	4,3	22,9	87
IIIe[a]	0,97	0,82	—	199	−106 (c=1,0; 1 N NH_4OH)	45,3	3,6	17,4	$C_{15}H_{14}ClN_5O_4S$	45,5	3,6	17,7	53

[a]The L isomer. [b]The D isomer.

3 h, after which the solution was evaporated to dryness *in vacuo*. The residue was washed with alcohol and crystallized from water to give 0.41 g (53%) of IIIe (Table 2).

α-Amino-ω-(6-hydroxy-9-purinyl) Carboxylic Acids (IVa, b). A solution of 1 mmole of α-amino-ω-purinyl carboxylic acid II in 7.6 ml of 1 N HCl was refluxed for 2 h, after which the hydrochloric acid was removed by distillation, and the residue was dissolved in the minimum amount of water. The solution was applied to a column filled with Dowex 50/H^+ ion-exchange resin, and the column was washed with water until the washings gave a negative test for chloride ions. The reaction product was eluted with 2 N ammonium hydroxide, the eluate was evaporated to dryness, and the residue was crystallized from aqueous ethanol (Table 3).

α-Amino-ω-(6-amino-9-purinyl) Carboxylic Acids (Va-d). A solution of 3 mmole of acid IIIa-d in 40 ml of 25% ammonium hydroxide was heated at 100°C for 15 h in a glass ampul, after which the solution was evaporated to dryness *in vacuo*, and ethanol was added to the residue. The precipitate was removed by filtration and dissolved in the minimum amount of water, and the solution was passed through a column filled with Sephadex G-25 (Table 3).

N_α-Tosyl-β-(6-amino-9-purynyl)-α-alanine (Ve). A solution of 0.39 g (1 mmole) of amino acid IIIe in 15 ml of 25% ammonium hydroxide was heated in a glass ampul at 100°C for 10 h, after which the solution was evaporated to dryness *in vacuo*, and water was added to the residue. The precipitate was removed by filtration and crystallized from water (to which a few drops of ammonium hydroxide had been added) to give 0.23 g of amino acid Ve (Table 3).

α-Amino-ω-(6-benzylamino-9-purinyl) Carboxylic Acids (VIa, b). A mixture of 5 mmole of acid III with 15 ml of benzylamine and 80 ml of butanol was refluxed for 4 h, after which the solution was evaporated to dryness *in vacuo*. The residue was washed with ether and crystallized from water (Table 3).

N_α-Tosyl-β-(6-benzylamino-9-purinyl)-α-alanine (IVc). A mixture of 1 g (2.5 mmole) of amino acid IIIe with 7 ml of benzylamine and 20 ml of butanol was refluxed for 5 h, after which the solution was evaporated to dryness *in vacuo*, and the residue was washed with alcohol. The mixture was cooled, and the solid material was removed by filtration and recrystallized from water (Table 3).

α-Amino-δ-(6-dimethylamino-9-purinyl)valeric Acid (VII). A solution of 5 mmole of acid IIIc in 20 ml of a freshly prepared 30% solution of dimethylamine was heated in a glass ampul at 100°C for 13 h, after which the solution was evaporated to dryness, and the residue was washed with alcohol, removed by filtration, and crystallized from water–ethanol (Table 3).

α-Amino-δ-(6-morpholino-9-purinyl)valeric Acid (VIII). A suspension of 1 mmole of amino acid IIIc in 2 ml of morpholine and 7 ml of butanol was refluxed for 5-6 h, after which it was cooled, and the precipitate was removed by filtration, washed with ether and ethyl acetate, and crystallized from 99% ethanol (Table 3).

α-Amino-δ-(6-methylamino-9-purinyl)valeric Acid (IX). A solution of 1 mmole of acid IIIc in 10 ml of 25% aqueous methylamine was heated in a steel autoclave at 135°C for 20 h, after which the solvent was removed by distillation, and the residue was recrystallized from absolute ethanol (Table 3).

TABLE 3. 6-Substituted α-Amino-ω-(9-purinyl) Carboxylic Acids

Compound	R_f			mp, °C	$[\alpha]_D^{20}$, deg	λ_{max}, nm			Found, %			Empirical formula	Calc., %			Yield, %
	1	2	3			pH 1	pH 7	pH 13	C	H	N		C	H	N	
IVa[a]	0,43	0,04	—	220—221	+19,8 (c=0,5; 1 N HCl)	249	248	255	38,2	5,0	28,2	$C_8H_9N_5O_3 \cdot 1{,}5H_2O$	38,4	4,8	28,0	46
IVb[a]	0,37	0,04	0,57	227	+23,8 (c=0,5; 1N HCl)	249	248	256	43,4	5,6	24,9	$C_{10}H_{13}N_5O_3 \cdot 1{,}5H_2O$	43,2	5,8	25,2	41
Va	0,65	0,06	0,49	248—250	—	258	259	262	39,8	5,1	34,6	$C_8H_{10}N_6O_2 \cdot H_2O$	40,0	5,0	34,9	72
V a[a]	0,65	0,06	0,49	249—251	+4,5 (c=0,5; H_2O), −11,8 (c=1,0; 1 N HCl)	258	259	262	40,3	4,8	34,7	$C_8H_{10}N_6O_2 \cdot H_2O$	40,0	5,0	34,9	68
IVa[b]	0,65	0,06	0,49	249—251	−4,5 (c=0,5; H_2O) +11,8 (c=1,0; 1 N HCl)	258	259	262	40,2	5,0	34,8	$C_8H_{10}N_6O_2 \cdot H_2O$	40,0	5,0	34,9	75
Vb	—	0,06	0,38	256—259	—	258	259	262	44,4	5,1	34,5	$C_9H_{12}N_6O_2 \cdot 0{,}5H_2O$	44,1	5,3	34,3	60
Vc	0,49	0,05	0,29	245—247	—	258	259	262	44,8	6,2	31,0	$C_{10}H_{14}N_6O_2 \cdot H_2O$	44,8	6,0	31,3	65
Vc[a]	0,49	0,05	0,29	254—257	+6,7 (c=0,5; H_2O)	258	259	262	44,9	5,8	31,0	$C_{10}H_{14}N_6O_2 \cdot H_2O$	44,8	6,0	31,3	45
Vd	0,53	0,17	—	232—235	—	258	259	262	48,7	6,1	30,8	$C_{11}H_{16}N_6O_2 \cdot 0{,}5H_2O$	48,3	6,3	30,8	39
Ve[a]	0,90	0,75	0,73	186—188	+11 (c=0,5; 4 N NH_4OH)	259	259	263	47,5	4,1	22,5	$C_{15}H_{16}N_6O_4S$	47,9	4,3	22,3	61
VIa[a]	0,69	0,06	0,53	226—228	+16 (c=1,0; 1N HCl)	267	271	269	57,5	5,3	26,6	$C_{15}H_{16}N_6O_2$	57,7	5,2	26,9	62
VIb[a]	0,70	0,12	0,48	222	+21,3 (c=1,0; 1 N HCl)	267	271	269	59,7	5,9	24,4	$C_{17}H_{20}N_6O_2$	60,0	5,9	24,7	38
VIc[a]	0,90	0,75	0,73	186—188	−28,5 (c=1,0; 1 N HCl)	268	270	269	56,8	4,9	18,3	$C_{22}H_{22}N_6O_4S$	56,6	4,8	18,0	63
VII[a]	0,62	0,04	0,16	213—216	+18 (c=1,0; 1 N HCl)	268	275	276	51,5	6,4	29,8	$C_{12}H_{18}N_6O_2$	51,8	6,5	30,2	35
VIII[a]	0,61	0,09	0,23	321	+3,2 (c=0,5; H_2O)	281	279	280	48,4	6,4	23,9	$C_{14}H_{20}N_6O_3 \cdot 1{,}5H_3O$	48,4	6,7	24,2	34
IX[a]	0,51	0,05	0,21	220	+4,8 (c=0,5; H_2O)	265	267	268	46,5	6,4	29,0	$C_{11}H_{16}N_6O_2 \cdot H_2O$	46,8	6,4	29,8	35
Xa[a]	0,75	0,40	0,17	186—187	−10,4 (c=1,0; H_2O)	268	271	271	53,8	6,4	28,7	$C_{13}H_{18}N_6O_2$	53,8	6,2	29,0	47
Xb[a]	0,68	0,37	0,09	193—194	−15 (c=1,0; H_2O)	268	270	271	56,8	7,1	26,2	$C_{15}H_{22}N_6O_2$	56,6	7,0	26,4	50

[a]The L isomer. [b]The D isomer.

α-Amino-ω-[6-(3-methyl-2-butenylamino)-9-purinyl] Carboxylic Acids (Xa, b). A mixture of 3 mmole of acid III with 6 mmole of 3-methyl-2-butenylamine hydrochloride, 6 ml of triethylamine, and 20 ml of butanol was refluxed for 3 h, after which it was evaporated to dryness *in vacuo*, and the residue was dissolved in water and passed through a column filled with Sephadex G-10 (Table 3).

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