SYNTHESIS OF PYRIMIDYL-N_1-α-AMINO ACIDS

M. Yu. Lidak, R. A. Paégle,
M. G. Plata, and Yu. P. Shvachkin

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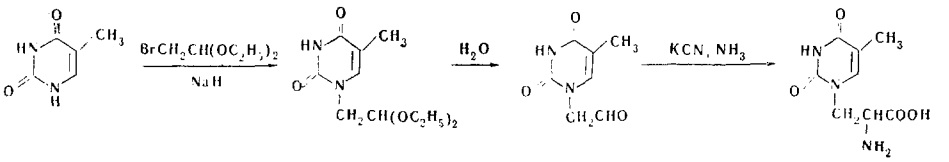
β-(5-Fluoro-N_1-uracilyl)-α-alanine, β-(5-bromo-N_1-uracilyl)-α-alanine, β-(5-chloro-N_1-uracilyl)-α-alanine, β-(N_1-thyminyl)-α-alanine, β-(N_1-cytosinyl)-α-alanine, β-(2-oxo-4-carboxymethylamino-1,2-dihydro-N_1-pyrimidyl)-α-alanine, γ-(N_1-uracilyl)-α-aminobutyric acid, γ-(N_1-thyminyl)-α-aminobutyric acid, and γ-(N_1-cytosinyl)-α-aminobutyric acid were synthesized by the reactions involving the cyanohydrin synthesis and other transformations. In addition, a number of substituted N_1-pyrimidylacetaldehydes and N_1-pyrimidylpropionaldehydes and their diethylacetals were synthesized.

In connection with a study of the purinyl peptides and nucleopeptides [1-5], we have synthesized a number of previously unknown N_1-pyrimidyl-α-amino acids. These compounds and the corresponding compounds of the purine series can be used to obtain peptide analogs of nucleic acids in which the pentosophosphate skeleton of the polynucleotide chain is replaced by a polypeptide chain, and the environment of the base chain of the macromolecule is retained by the corresponding pyrimidine and purine bases. In this connection, a study of the biological activity of purinylpeptides and nucleopeptides is of interest, and a study of the effect of such structures on the repression and depression of gene activity is particularly worthy of attention.

We examined the general principles of the synthesis of N_1-pyrimidylamino acids previously in [1,6,7]. The conditions for the preparation of a number of β-(N_1-pyrimidyl)-α-alanines are set forth in this paper, and methods for the synthesis of γ-(N_1-pyrimidyl)-α-aminobutyric acids are also described.

We have worked out methods for the preparation of a number of analogs of the natural nonproteinogenic amino acid willardiine [8-11]; specifically, we have synthesized the following compounds: β-(N_1-thyminyl)-α-alanine (I), β-(5-fluoro-N_1-uracilyl)-α-alanine (II), β-(5-bromo-N_1-uracilyl)-α-alanine (III), β-(5-chloro-N_1-uracilyl)-α-alanine (IV), β-(N_1-cytosinyl)-α-alanine (V), and β-(2-oxo-4-carboxymethylamino-1,2-dihydro-N_1-pyrimidyl)-α-alanine (VI).

The majority of the enumerated syntheses are based on conversion of the appropriate pyrimidine derivatives to N_1-diethylacetals, hydrolysis of these acetals to the corresponding N_1-pyrimidylacetaldehydes, and introduction of the latter into the cyanohydrin synthesis.



Thus the sodium salt of thymine, which can be introduced into reaction with 1-bromo-2,2-diethoxyethane without isolation from the reaction mixture, is formed by the reaction of thymine with sodium hydride in anhydrous dimethylformamide. Paper chromatography established that the product of this reaction is a mixture of two compounds, one of which is 1-(N_1-thyminyl)-2,2-diethoxyethane, while the other is apparently

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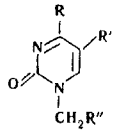
TABLE 1. N_1-Pyrimidyl-α-amino Acids



Compound	R	R′	R″	Mp, °C	UV spectra				Empirical formula	Found, %			Calc., %			Yield, %
					H_2O		0.1 *N* NaOH									
					λ_{max}, nm	$\varepsilon \cdot 10^3$	λ_{max}, nm	$\varepsilon \cdot 10^3$		C	H	N	C	H	N	
VII	OH	CH_3	$CH(OC_2H_5)_2$	78	268	8,84	268	6,18	$C_{11}H_{18}N_2O_4$	54,60	7,35	12,02	54,50	7,48	11,61	35
VIII	OH	F	$CH(OC_2H_5)_2$	114—115	275	8,24	272	6,24	$C_{10}H_{15}FN_2O_4$	49,28	6,07	11,21	48,78	6,09	11,38	7
IX	SCH_3	H	$CH(OC_2H_5)_2$	98—99	303	14,9	303	15,4	$C_{11}H_{18}N_2O_3S$	51,28	6,99	11,02	51,16	6,97	10,85	52
X	NH_2	H	$CH(OC_2H_5)_2$	242—243	272	9,5	272	10,9	$C_{10}H_{17}N_3O_3$	53,25	7,87	18,75	52,80	7,53	18,55	88
XI	$NHCH_2COOH$	H	$CH(OC_2H_5)_2$	130—131	—	—	—	—	$C_{12}H_{19}N_3O_5$	50,20	6,66	—	50,69	6,68	—	47
XVI	OH	H	$CH_2CH(OC_2H_5)_2$	Oil	267	6,5	267	5,3	$C_{11}H_{18}N_2O_4$	54,18	7,44	11,80	54,53	7,48	11,50	38
XVII	OH	CH_3	$CH_2CH(OC_2H_5)_2$	88—89	272	10,2	269	8,1	$C_{12}H_{20}N_2O_4$	56,68	7,57	10,78	56,27	7,99	10,93	39
XVIII	SCH_3	H	$CH_2CH(OC_2H_5)_2$	Oil	266	8,7	266	7,2	$C_{12}H_{20}N_2O_3S$	53,21	7,70	10,65	52,91	7,40	10,23	30
XIX	NH_2	H	$CH_2CH(OC_2H_5)_2$	187—188	272	8,34	270	6,24	$C_{11}H_{19}N_3O_3$	54,81	7,77	17,63	54,74	7,87	17,41	85
XII	OH	CH_3	CHO	300	268	30,4	270	9,1	$C_7H_8N_2O_3 \cdot H_2O$	45,35	5,52	15,31	45,19	5,85	15,05	78
XIII	OH	F	CHO	292	272	11,8	273	10,7	$C_6H_5FN_2O_3 \cdot H_2O$	37,80	4,01	15,12	37,87	3,71	14,78	85
XIV	NH_2	H	CHO	—	275	8,3	277	7,5	$C_6H_7N_3O_2 \cdot H_2O \cdot HCl$	34,99	5,22	20,36	34,71	4,85	20,24	70
XV	$NHCH_2COOH$	H	CHO	—	—	—	—	—	$C_8H_9N_3O_4 \cdot H_2O$	42,22	4,79	17,93	41,92	4,83	18,33	84
I	OH	CH_3	$CH(NH_2)COOH$	218	269	8,26	269	8,86	$C_8H_{11}N_3O_4 \cdot H_2O$	42,07	5,35	18,42	41,55	5,69	18,17	31
II	OH	F	$CH(NH_2)COOH$	213—215	270	7,84	270	12,9	$C_7H_8FN_3O_4 \cdot H_2O$	35,97	4,07	18,45	35,75	4,21	17,87	35
III	OH	Br	$CH(NH_2)COOH$	211—212	278	9,8	275	7,66	$C_7H_8BrN_3O_4 \cdot H_2O$	28,29	3,61	13,99	28,39	3,41	14,18	74
IV	OH	Cl	$CH(NH_2)COOH$	220—221	278	11,7	280	7,06	$C_7H_8ClN_3O_4$	36,10	3,69	17,59	35,98	3,45	15,21	41
V	NH_2	H	$CH(NH_2)COOH$	216—217	271	9,04	274	8,05	$C_7H_{10}N_4O_3 \cdot H_2O$	38,89	5,84	25,68	38,88	5,59	25,91	66
VI	$NHCH_2COOH$	H	$CH(NH_2)COOH$	192—194	—	—	—	—	$C_9H_{12}N_4O_5 \cdot H_2O$	36,25	5,42	20,04	35,75	5,14	20,43	50
XX	OH	H	$CH_2CH(NH_2)COOH$	256	265	10,8	265	8,2	$C_8H_{11}N_3O_4 \cdot H_2O$	41,86	5,24	18,47	41,56	5,62	18,17	14
XXI	OH	CH_3	$CH_2CH(NH_2)COOH$	254	271	9,2	270	8,0	$C_9H_{13}N_3O_4 \cdot H_2O$	45,41	6,01	17,52	45,76	5,93	17,72	61
XXII	NH_2	H	$CH_2CH(NH_2)COOH$	237	272	12,3	274	6,9	$C_8H_{12}N_4O_3 \cdot H_2O$	44,72	5,89	26,10	45,28	5,70	26,39	30

1,3-dialkylated thymine. 1-(N_1-Thyminyl)-2,2-diethoxyethane (VII) can be isolated from this mixture and used for subsequent syntheses.

The alkylation of 5-fluorouracil with 1-bromo-2,2-diethoxyethane proceeds via a similar scheme but in lower yields. In this case we established the formation of 1-(5-fluoro-N_1-uracilyl)-2,2-diethoxyethane (VIII); this compound was also isolated in pure form, the formation of an N_3-substituted 5-fluorouracil derivative was not observed. This is in agreement with the data in [12] regarding the alkylation of 5-fluorouracil with benzyl bromide and allyl bromide. According to Baker and Jackson [12], the direction of alkylation of the 5-fluorouracil ring with halo derivatives in these reactions depends on the conditions used to carry out the process and is also determined by the reactivity of the halogen atom in the alkylating agent.

Both VII and VIII are converted in good yields to the corresponding N_1-pyrimidylacetaldehydes (XII and XIII) by acid hydrolysis; XII and XIII are then introduced into the cyanohydrin synthesis.

An amino acid derivative of cytosine could be obtained from 4-methylthiouracil, which forms only 1-(4-methylthio-N_1-uracilyl)-2,2-diethoxyethane (IX) in good yield by alkylation with 1-bromo-2,2-diethoxyethane. Treatment of this derivative with ammonium hydroxide readily gives 1-(N_1-cytosinyl)-2,2-diethoxyethane (X), which is converted to N_1-cytosinylacetaldehyde (XIV) by acid hydrolysis.

The methylmercapto group in IX can be replaced by a glycine residue by a similar route to form acetal XI, and 2-oxo-4-carboxymethylamino-1,2-dihydro-N_1-pyrimidylacetaldehyde (XV) is obtained after acid hydrolysis in this case.

Compounds XIV and XV are converted to V and VI by the cyanohydrin synthesis.

In the course of our investigations of the synthesis of N_1-pyrimidyl-α-amino acids we also found that III is obtained by the treatment of willardiine with bromine in glacial acetic acid, while treatment of willardiine with N-chlorosuccinimide yields IV.

As expected, the UV absorption spectra of the compounds obtained in aqueous solutions and in 0.1 N sodium hydroxide do not display a bathochromic shift when the basicity of the medium is increased, which is characteristic for the UV spectra of N_1-substituted uracils [13]. A comparison of the UV spectra of the synthesized acetals, the substituted pyrimidylacetaldehydes, the aldehydes themselves, and the corresponding pyrimidylamino acids with the UV spectra of genuine samples of N_1-methyluracil and N_3-methyluracil indicated that the UV spectra of the compounds obtained differ from the absorption spectrum of N_3-methyluracil and are extremely similar to the absorption spectrum of N_1-methyluracil. Consequently, the side chain in the compounds that we synthesized is in the uracil ring at N_1.

There are currently no data in the literature regarding the synthesis and properties of N_1-pyrimidyl-α-aminobutyric acids. We have found that these compounds can be synthesized via a scheme similar to that which we used to obtain N_1-pyrimidyl-α-alanines.* Thus a mixture of the corresponding N_1-mono- and $N_{1,3}$-disubstituted derivatives is formed on treatment of the sodium salt of uracil or thymine with 1-chloro-3,3-diethoxypropene; these compounds can be separated because of their different solubilities. Thus, in particular, XVI and XVII were isolated in pure form.

Only the N_1-substituted derivative (XVIII) is readily formed in the reaction of 4-methylthiouracil with 1-chloro-3,3-diethoxypropane; XVIII can be converted to 1-(N_1-cytosinyl)-3,3-diethoxypropane (XIX) by treatment with ammonium hydroxide.

Since no bathchromic shift is observed in the UV spectra of monosubstituted derivatives when the pH of the medium is increased (see Table 1), one can conclude that the side chain in the acetals obtained is attached to the nitrogen atom in the 1-position of the pyrimidine ring.

In a study of the acid hydrolysis of the indicated acetals we found that better results are obtained when aqueous ammonium chloride rather than dilute hydrochloric acid is used as the hydrolyzing agent. We obtained N_1-uracilyl-, N_1-thyminyl-, and N_1-cytosinylpropionaldehydes, which were then subjected to the cyanohydrin synthesis to obtain γ-(N_1-uracilyl)-α-aminobutyric acid (XX), γ-(N_1-thyminyl)-α-aminobutyric acid (XXI), and γ-(N_1-cytosinyl)-α-aminobutyric acid (XXII).

*After the completion of this investigation, a communication regarding the synthesis of some N_1-pyrimidyl-α-aminobutyric acids by another method was published [14].

It should be noted that the synthesis of the aldehydes of the pyrmidine series noted above can also be accomplished by reduction of β-(N_1-pyrimidyl)propionitriles (previously obtained by us) with Raney nickel in anhydrous formic acid [15]. However, this method is less efficient since complications arise in the purification of the desired compounds when it is used.

Compounds XX-XXII dissolve readily in dilute acids and alkalis, are soluble in water, and give a blue-violet color when treated with ninhydrin. The electrophoretic mobilities of these compounds indicate their amphoteric character.

We are continuing our investigations of the synthesis of N_1-pyrimidyl-α-amino acids and the corresponding peptides.

EXPERIMENTAL

1-(N_1-Thyminyl)-2,2-diethoxyethane (VII). Sodium hydride [3.8 g (0.158 mole)] was added all at once to a suspension of 15.7 g (0.125 mole) of thymine in 150 ml of dimethylformamide, and the mixture was stirred for 2 h. A solution of 50.0 g (0.25 mole) of 1-bromo-2,2-diethoxyethane in 25 ml of dimethylformamide was added, the mixture was held at 80° for 12 h, and 25.0 g (0.125 mole) of 1-bromo-2,2-diethoxyethane was added. The temperature was then raised to 149-150° and held there for 30 min. After removal of the solvent in vacuo (1 mm), the residue was extracted with ethyl acetate. The organic layer was separated, washed with water, and dried with magnesium sulfate. Removal of the solvent in vacuo gave an oily residue which crystallized on standing in a refrigerator. The product was treated with 100 ml of petroleum ether–diethyl ether (1:1) to remove the disubstituted derivative, and the crystalline residue was filtered.

Compounds VIII and IX were similarly obtained, while XVI-XVIII were obtained from 3-chloro-1,1-diethoxypropane.

1-(N_1-Cytosinyl)-2,2-diethoxyethane (X). A mixture of 2.0 g of IX and 100 ml of ammonium hydroxide was heated for 14 h in a sealed ampule. The mixture was cooled, filtered, and washed with ammonium hydroxide. Compound XIX was similarly synthesized.

1-(N_1-Thyminyl)acetaldehyde (XII). Compound VII [2.42 g (0.01 mole)] was heated on a water bath with 20 ml of 0.5 N HCl for 45 min. The mixture was cooled, and the monohydrate of XII was filtered.

Compounds XIII-XV were similarly obtained. In the synthesis of XIV and XV the solution was evaporated to dryness after the hydrolysis, and the residue was recrystallized from methanol.

2-(N_1-Thyminyl)propionaldehyde. A mixture of 2.56 g (0.01 mole) of XVI with 5.0 g of NH_4Cl in 25 ml of water was heated on a water bath for 3 h. The other pyrimidylpropionaldehydes were similarly obtained. The products were used for the subsequent syntheses without additional purification.

β-(N_1-Thyminyl)-α-alanine (I). A mixture of 1.63 g (0.01 mole) of XII, 6 ml of water, 1.5 ml of ammonium hydroxide, 0.8 g of KCN, and 0.7 g of NH_4Cl was heated at 55-60° for 5 h, 20 ml of 10 N HCl was added, and the mixture was refluxed for 1 h and allowed to stand overnight. After removal of the solvent in vacuo, 12 ml of 10 N HCl was added, and the mixture was refluxed for 3 h. The residual HCl was removed by distillation, two 15-ml portions of water were removed by distillation, and the residue was dissolved in a small amount of water and neutralized to pH 4 with concentrated ammonium hydroxide. The precipitate was separated and washed with water.

Compounds II, V, and VI were similarly obtained.

β-(5-Bromo-N_1-uracilyl)-α-alanine (III). Bromine (2 ml) was added dropwise with stirring to a suspension of 2.17 g (0.01 mole) of β-(N_1-uracilyl)-α-alanine* in glacial acetic acid. The willardiine dissolved as the bromine was being added, and the solution was allowed to stand at room temperature overnight. The mixture was then evaporated to dryness, and the resulting crystals were washed with water to give 2.1 g (74%) of a product with mp 211-212° (water).

β-(5-Chloro-N_1-uracilyl)-α-alanine (IV). A mixture of 2.17 g (0.01 mole) of willardiine and 2.0 g of N-chlorosuccinimide in 50 ml of glacial acetic acid was heated on a water bath for 4 h. The mixture was cooled, and the resulting crystals were filtered and washed with water and ethanol to give 0.95 g (41%) of a product with mp 220-221° (from water).

*Obtained by the method in [14].

γ-(N_1-Thyminyl)-α-amonobutyric Acid (XXI). A mixture of 1.82 g (0.01 mole) of N_1-thyminylpropionaldehyde, 40 ml of water, 0.65 g of potassium cyanide, and 0.2 ml of ammonium hydroxide was stirred for 2 h at room temperature. The bath temperature was raised to 55-60° and held there for 10 h. The mixture was then cooled to 5-7°, 40 ml of 10 N hydrochloric acid was added dropwise, and the mixture was allowed to stand overnight. After this, the mixture was heated on a water bath for 2 h and evaporated to dryness. The residue was dissolved in 10-12 ml of water, the solution was neutralized with ammonium hydroxide (pH 5-6), and the mixture was introduced into a column containing Dowex-50 (H+) ion-exchange resin. The column was washed with water until the washings gave a negative test for chloride ions, and the amino acid was then eluted with ammonium hydroxide (20 ml of concentrated ammonium hydroxide was diluted to 500 ml with water). The presence of an amino acid in the eluate was monitored by means of its reaction with ninhydrin. The eluate was evaporated to dryness to give a colorless, amorphous substance which was chromatographically pure and had mp 254-256° (50% ethanol).

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