SYNTHESIS OF THE BIS(2'-CHLOROETHYL) AMIDE OF β-(8-GUANYL)PROPIONIC ACID

E. P. Studentsov and V. G. Nemets

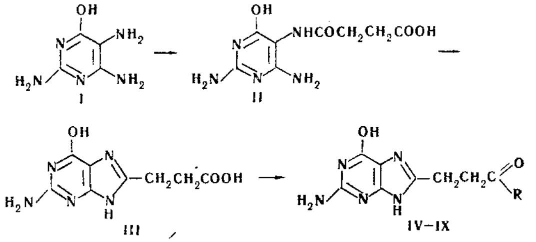
Khimiya Geterotsiklicheskikh Soedinenii, Vol. 4, No. 5, pp. 930–932, 1968

UDC 547.857.7.07

The synthesis of β-(8-guanyl)propionic acid and its chloride, methyl ester, bis(2'-hydroxyethyl)amide, and bis(2'-chloroethyl)amide has been effected. The latter readily rearranges under certain conditions into 2'-chloroethylaminoethyl β-(8-guanyl)propionate.

The 8-carboxylic acids of the natural purines and their derivatives have scarcely been studied. The present paper describes the synthesis of the bis(2'-chloroethyl) amide of β-(8-guanyl) propionic acid—a potential antitumoral compound with latent activity [1].

The preparation of the initial β-(8-guanyl) propionic acid has been described briefly in a German patent [2] without indication of the yield or the method of purification of the intermediate and final substances. We have refined the conditions for obtaining this acid by a series of experiments. The acylation of 2, 4, 5-triamino-6-hydroxypyrimidine (I) with succinic acid at 175° C for 20 min leads to the 5-succinamido derivative II. Heating the disodium salt of II in vacuum at 220° C for 3 hr gives β-(8-guanyl) propionic acid (III).



IV $R = OCH_3$; V $R = OC_2H_5$; VI $R = Cl$; VII $R = N(CH_2CH_2OH)_2$;
VIII $R = N(CH_2CH_2Cl)_2$; IX $R = OCH_2CH_2NHCH_2CH_2Cl$

The acids II and III are relatively stable: no decarboxylation was observed when they were recrystallized from water or subjected to prolonged drying at 120° C.

Compound III is readily esterified with anhydrous alcohols when the mixture is saturated with hydrogen chloride. The hydrochlorides of the methyl and ethyl esters (IV and V, respectively) are obtained, and the action of aqueous ammonia or triethylamine yields the free IV and V.

When III was boiled in a large excess of thionyl chloride, simultaneously with the formation of the acid chloride VI chlorination of the aliphatic side chain in the α-position with respect to the carboxyl took place. 8-Caffeinepropionic acid undergoes an analogous reaction under similar conditions [3]. It was possible to obtain the acid chloride VI with a high yield by carefully heating III with a small excess of thionyl chloride in absolute benzene. When VI was treated with absolute methanol, the hydrochloride of the methyl ester IV, identical with the sample obtained by the esterification of the acid, was obtained.

The bis(2'-hydroxyethyl)amide VII was synthesized in good yield by heating IV or V with an aqueous solution of diethanolamine or by the reaction of the acid chloride VI with diethanolamine in the absence of a solvent. In the replacement of the hydroxy groups in the side chain of VII by chlorine atoms in order to obtain the bis(2'-chloroethyl)amide VIII, we came up against some difficulties due to the ease of rearrangement of the VIII formed into the corresponding 2'-chloroethylaminoethyl ester IX and also to the cleavage of the amide bond. It is known that the amide-ether rearrangement takes place readily when amides are heated in polar solvents in the presence of catalytic amounts of mineral acids [4]. In actual fact, when VIII was heated with a small excess of thionyl chloride in chloroform the only reaction product was the hydrochloride of the ester IX. It was found that the conversion of VII into the hydrochloride of VIII without appreciable rearrangement can be carried out by treating VII with thionyl chloride at 20° C in an atmosphere of dry nitrogen. On dissolution in methanol, the hydrochloride of VIII is converted into the hydrochloride of IX in 48 hr. In the identification of the chlorine compounds mentioned, the ratio of ionic and covalently-bound chlorine atoms was established from the results of the elementary analysis of the hydrochlorides of VIII and IX and of the free bases obtained by treatment with an excess of triethylamine at room temperature.

The acid chloride VI readily reacts at room temperature with bis(2-chloroethyl) amine in chloroform in the presence of triethylamine, giving crude VIII in high yield, but this is difficult to isolate in the pure form by recrystallization from organic solvents because of its rearrangement into IX. Compound VIII is relatively stable on prolonged storage in the dry state.

EXPERIMENTAL

2, 4-Diamino-5-succinamido-6-hydroxyprimidine (II). In a Würtz flask, 10 g (0.071 mole) of 2, 4, 5-triamino-6-hydroxypyrimidine (I) [5], 33.5 g (0.28 mole) of succinic acid, and 30 ml of water were carefully mixed. The water was evaporated off under atmospheric pressure at 120° C, and the residue was kept at 175° C in a vacuum of 15 mm for 20 min. The yellow melt was dissolved in 250 ml of hot 10% aqueous NaOH, and the solution was treated with decolorizing carbon and acidified with 50% acetic acid. On cooling, a light yellow precipitate deposited which was filtered off, washed with ice water and acetone, and dried in vacuum over H_2SO_4. Recrystallization from water yielded 12.5 g (72.5%) of **II** in the form of light yellow crystals with mp 247° C (decomp.). Found, %: C 39.72; H 4.38; N 28.87. Calculated for $C_8H_{11}N_5O_4$, %: C 39.83; H 4.56; N 29.04. UV spectrum: pH 1—λ_{max} 262 nm (lg ε 4.23), pH 7—λ_{max} 264 nm (lg ε 3.94), pH 11—λ_{max} 262 nm (lg ε 4.01); pK_a 4.38.

β-(8-Guanyl)propionic acid (III). In a Würtz flask, 10 g (0.042 mole) of **II** was dissolved in 25 ml of 15% aqueous NaOH, the water was evaporated off at atmospheric pressure, and the residue was kept at 230° C in a vacuum of 15 mm for 3 hr. The melt was dissolved in 50 ml of boiling water, the solution was treated with decolorizing carbon, and the hot filtrate was acidified with 50% acetic acid. After cooling, the yellow precipitate was filtered off, washed with water and acetone, dried in vacuum at 60° C, and purified by reprecipitation from 15% aqueous NaOH with 50% acetic acid. The yield of **III** was 8.3 g (90%); yellow crystals with mp 300° C (decomp.). Found, %: C 43.21; H 3.88; N 31.19. Calculated for $C_8H_9N_5O_3$, %: C 43.05; H 4.06; N 31.38. UV spectrum: pH 1—λ_{max} 264–270 nm (plateau) (lg ε 3.97), λ_{max} 248 nm (lg ε 4.12), pH 11—λ_{max} 272 nm (lg ε 3.87); pK_a 4.55.

Methyl β-(8-guanyl)propionate (IV). A suspension of 4 g (0.018 mole) of **III** in 100 ml of absolute methanol was saturated with dry hydrogen chloride at 20° C with stirring and the mixture was left overnight. Then the precipitate was filtered off, washed with ether, dried, in the air, and recrystallized from a mixture of methanol and ether. The yield of the hydrochloride of **IV** was 2.6 g (50.1%); colorless crystals with mp 230° C (decomp.). Found, %: C 35.74; H 4.69; Cl 11.54; N 22.97. Calculated for $C_9H_{11}N_5O_4 \cdot HCl \cdot H_2O$, %: C 35.71; H 4.57; Cl 11.44; N 22.77. UV spectrum: pH 1—λ_{max} 262 nm (lg ε 3.96), λ_{max} 245 nm (lg ε 4.12), pH 7—λ_{max} 245 nm (lg ε 4.09), pH 11—λ_{max} 246 nm (lg ε 4.09), λ_{max} 273 nm (lg ε 3.95).

The action of aqueous ammonia or an excess of triethylamine on the hydrochloride of **IV** liberated the free base in quantitative yield, and this was recrystallized from water.

Ethyl β-(8-guanyl)propionate (V). This was obtained in a similar manner to **IV**. The yield of the hydrochloride of **V** was 57%; colorless crystals with mp 259° C (decomp., from ethanol). Found, %: C 37.23; H 4.97; Cl 10.95; N 21.84. Calculated for $C_{10}H_{13}N_5O_4 \cdot HCl \cdot H_2O$, %: C 37.37; H 5.02; Cl 11.02; N 21.77.

Hydrochloride of β-(8-Guanyl)propionyl chloride (VI). A mixture of 3 g (0.013 mole) of the acid **III** and 3.25 g (0.027 mole) of thionyl chloride in 25 ml of absolute benzene was boiled with stirring for 8 hr. The excess of thionyl chloride was distilled off in vacuum and the residue was suspended in absolute benzene, filtered off again, washed with dry ether, and immediately dried in vacuum over H_2SO_4. The yield of **VI** was 3.05 g (93%). The substance hydrolyzes rapidly in the air. Found, %: Cl 21.07; N 25.49. Calculated for $C_8H_8ClN_5O_2 \cdot HCl$, %: Cl 25.49; N 25.19.

When **VI** was treated with hot absolute methanol, the hydrochloride of **IV** was obtained with a yield of 67%.

Bis(2'-hydroxyethyl)amide of β-(8-guanyl)propionic acid (VII). a) Six grams (0.02 mole) of the hydrochloride of **V** was dissolved in 50 ml of hot water and, with stirring, 6 g (0.057 mole) of diethanolamine in 20 ml of water was gradually added to the solution. The free base **V** first precipitated, and this gradually redissolved when the mixture was boiled for 1 hr. The homogeneous solution was boiled for another 3 hr and the water was distilled off in vacuum; then the residue was treated with 50 ml of methanol and the precipitate was filtered off, washed with methanol and ether, and recrystallized from aqueous acetone. The yield of **VII** was 4.5 g (69%) in the form of colorless crystals with mp 170° C (decomp.). Found, %: C 43.98; H 5.89; N 25.87. Calculated for $C_{12}H_{18}N_6O_4 \cdot H_2O$, %: C 43.91; H 6.09; N 25.61. UV spectrum: pH 7—λ_{max} 250 nm (lg ε 3.84), λ_{max} 275 nm (lg ε 3.74). The water of crystallization was readily eliminated by drying in vacuum at 120° C. The yield of pure **VII** from the free bases **IV** or **V** under similar conditions was about 90%.

b) A mixture of 2 g (0.007 mole) of the freshly-prepared hydrochloride of **VI** and 10 g (0.095 mole) of diethanolamine was heated at 80° C with vigorous stirring for 3 hr. Then 25 ml of ethanol was added to the reaction mixture and it was left overnight in the refrigerator. The precipitate was filtered off, washed with ethanol and ether, and purified by crystallization from aqueous acetone. The yield of pure **VII** was 1.2 g (51.5%) in the form of colorless crystals with mp 170° C (decomp.). Found, %: C 44.01; H 6.13; N 25.79. Calculated for $C_{12}H_{18}N_6O_4 \cdot H_2O$, %: C 43.91; H 6.09; N 25.61.

Bis(2'-chloroethyl)amide of β-(8-guanyl)propionic acid (VIII). a) A mixture of 1 g (0.003 mole) of **VII** and 20 ml of freshly-distilled thionyl chloride was kept at room temperature for 96 hr in an atmosphere of dry nitrogen. The precipitate was filtered off, washed with absolute benzene, and dried in vacuum over KOH. The yield of the hydrochloride of **VIII** was 1.01 g (84.5%); light yellow crystals with mp 120° C (decomp.). Found, %: C 37.82; H 4.27; Cl 27.65; N 21.69. Calculated for $C_{12}H_{16}Cl_2N_6O_2 \cdot HCl$, %: C 37.71; H 4.33; Cl 27.72; N 21.91. UV spectrum (methanol): λ_{max} 250 nm (lg ε 3.89), λ_{max} 275 nm (lg ε 3.76). To isolate the free base **VIII**, 1 g of the hydrochloride was stirred with 15 ml of triethylamine at room temperature for 15 min. The precipitate was filtered off, washed with chloroform, and dried in vacuum over KOH. The yield was 0.75 g (81%). Found, %: Cl 20.81; N 23.99. Calculated for $C_{12}H_{16}Cl_2N_6O_2$, %: Cl 20.46; N 24.19.

b) With stirring, 1 g (0.1 mole) of triethylamine was slowly added to a mixture of 1 g (0.004 mole) of freshly-prepared **VI** and 1.5 g (0.01 mole) of bis(2-chloroethyl)amine in 10 ml of chloroform. The precipitate was filtered off, washed with chloroform, and dried in vacuum over KOH. The yield of **VIII** was 1.1 g (88%); yellow crystals with mp 110° C (decomp.). Found, %: Cl 18.91; N 24.51. Calculated for $C_{12}H_{16}Cl_2N_6O_2$, %: Cl 20.46; N 24.20.

2'-Chloroethylaminoethyl β-(8-guanyl)propionate (IX). a) A solution of 0.5 g (0.001 mole) of the hydrochloride of **VIII** in 30 ml of methanol was left for 24 hr at room temperature. Then 250 ml of ether was added and the mixture was left for another 48 hr in the refrigerator. The precipitate was filtered off and washed with ether to give 0.45 g of the hydrochloride of **IX**, which was treated at room temperature with 10 ml of triethylamine, after which the precipitate was filtered off, washed with chloroform, and dried in vacuum over KOH. The yield of **IX** was 0.3 g (72%); colorless crystals with mp 205° C (decomp.). Found, %: C 41.48; H 5.39; Cl 9.95; N 24.38. Calculated for $C_{12}H_{17}ClN_6O_3 \cdot H_2O$, %: C 41.38; H 5.45; Cl 10.24; N 24.24. UV spectrum (methanol): λ_{max} 247 nm (log ε 3.84).

b) A mixture of 0.6 g (0.002 mole) of **VII** and 0.6 g (0.005 mole) of thionyl chloride in 25 ml of dry chloroform was stirred at 70° C for 7 hr. On the following day the precipitate was filtered off and recrystallized from a mixture of ethanol and ether. The yield of the hydrochloride of **IX** was 0.6 g (91%). Found, %: Cl 19.72; N 23.01. Calculated for $C_{12}H_{17}ClN_6O_3 \cdot HCl$, %: Cl 19.47; N 23.08.

REFERENCES

1. W. Ross, Biological Alkylating Agents [Russian translation], Meditsina, Moscow, p. 187, 1964.
2. German patent 213711, Frdl., IX, 1009, 1909.
3. E. S. Golovchinskaya and E. S. Chaman, ZhOKh, 21, 528, 1951.
4. W. Ross and J. Wilson, J. Chem. Soc., 3616, 1959.
5. V. M. Berezovskii and L. I. Strel'chunas, Tr. VNII vitaminov, 5, 128, 1954.

13 July 1966

Lensovet Leningrad Technological Institute