THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS.

IX. SYNTHESIS OF CYCLOHEXAPEPTIDES CONTAINING RESIDUES OF N-METHYL(AMINO ACID)S AND α-HYDROXYISOVALERIC ACID

V. T. Ivanov, I. A. Lavrinovich,
V. V. Lapshin, P. V. Kostetskii,
and Yu. A. Ovchinnikov

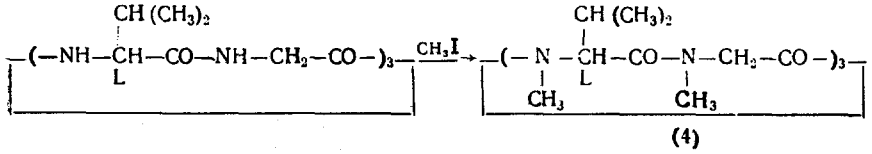
UDC 547.964.4

In the development of our study of the conformational states of cyclohexapeptides with limited conformational mobility [1], we have synthesized a series of compounds in which the positions and numbers of the hydrogen bonds are limited because of the presence of N-methylamide (compounds 1-4) or ester (compounds 6-10)* bonds. The study of compounds (1)-(4) and (6)-(10) is also of interest for explaining the properties of natural depsipeptides of the group of enniatine B [3] and the sporidesmolides [4, 5, 6, 7], which have structural features in common with them (18-membered ring and the presence of residues of N-methyl(amino acid)s and of α-hydroxyisovaleric acid).†

Cyclo (-L-Val-Gly-L-Val-Sar-L-Val-Gly-) (1)
Cyclo (-L-Val-Sar-L-Val-Sar-L-Val-Gly-) (2)
Cyclo (-L-Val-Sar-L-Val-Sar-L-Val-Sar-) (3)
Cyclo (-L-MeVal-Sar-L-MeVal-Sar-L-MeVal-Sar-) (4)
Cyclo (-D-Val-D-Leu-L-Hylv-D-Val-D-Leu-L-Hylv-) (6)
Cyclo (-L-Val-L-MeLeu-L-Hylv-L-Val-L-MeLeu-L-Hylv-) (7)
Cyclo (-D-Val-L-MeLeu-L-Hylv-D-Val-L-MeLeu-L-Hylv-) (8)
Cyclo (-L-Val-L-MeIle-D-Hylv-L-Val-L-MeIle-D-Hylv-) (9)
Cyclo (-D-Val-L-MeIle-D-Hylv-D-Val-L-MeIle-D-Hylv-). (10)

The sarcosine-containing cyclopeptides (1)-(3) were obtained by Scheme 1. The majority of the intermediates consisted of oils, which were purified by chromatography on alumina. The cyclization of the hexapeptides (28), (29), and (30) took place with extremely low yields (5-8%) and was accompanied by the formation of cyclo(L-valylsarcosine) (5) (5-10%). A similar phenomenon has been observed in the cyclization of the 2,4,5-trichlorophenyl esters of sarcosine-containing tetrapeptides [9-11].

The cyclopeptide (4) was obtained by the methylation of cyclo[-(L-valyl-glycyl)$_3$-] [1] by Lederer's method [12-14]:

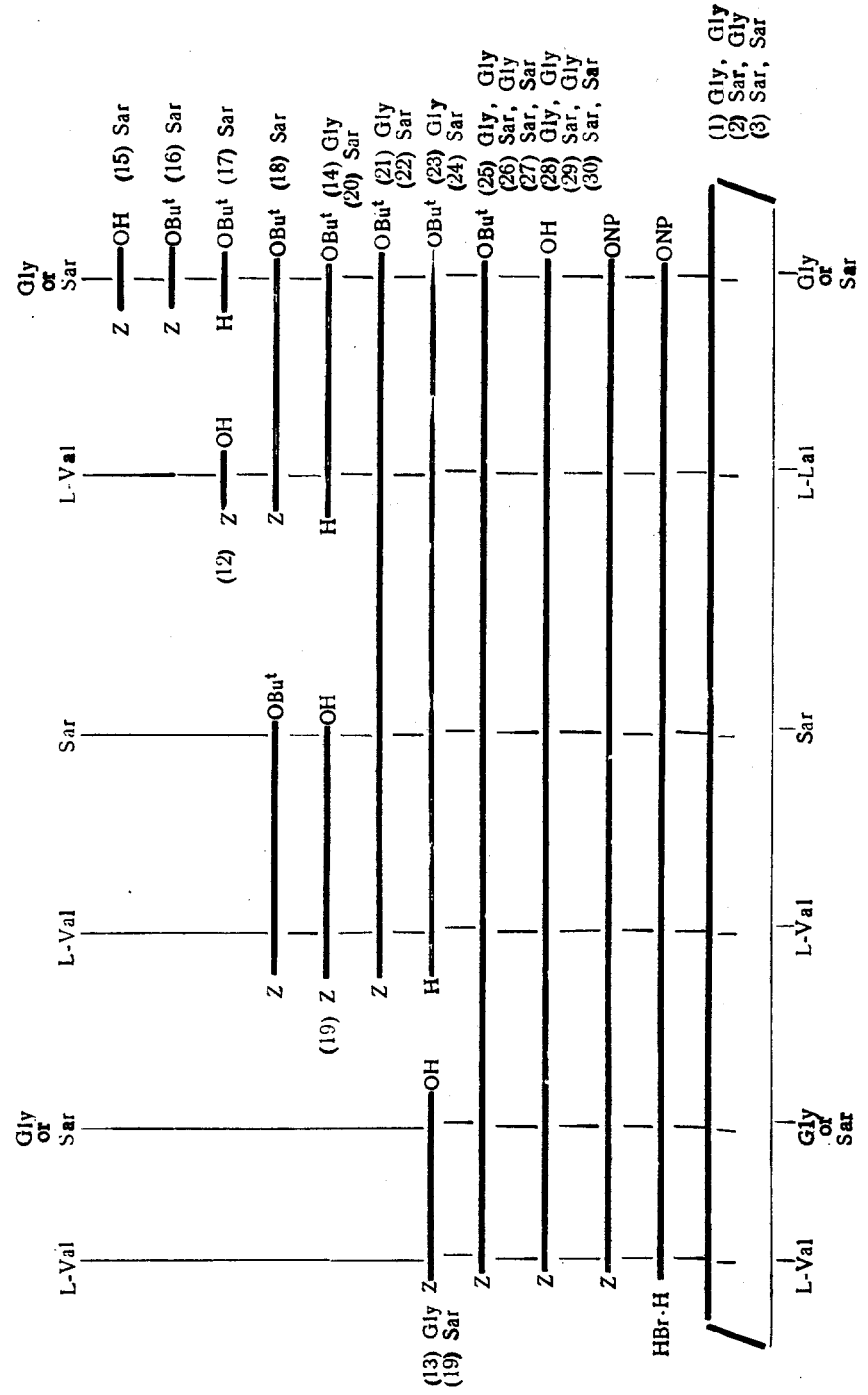


(4)

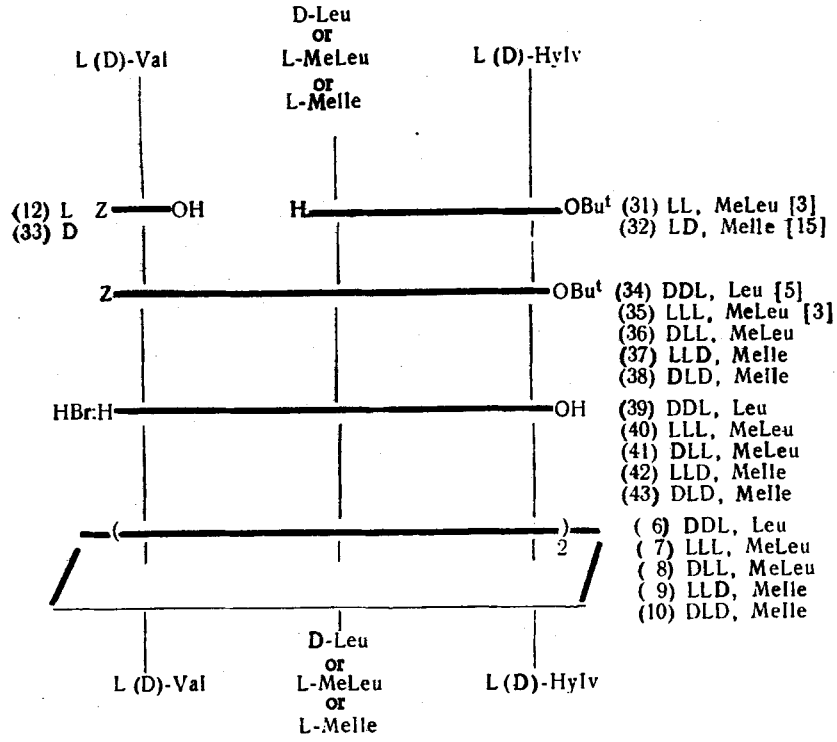
*For a preliminary communication on the synthesis of compounds (6-10), see [2].
†To denote the amino acid residues we use the nomenclature recommended by the International Union of Pure and Applied Chemistry [8].

M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 63-69, January-February, 1975. Original article submitted July 26, 1973.

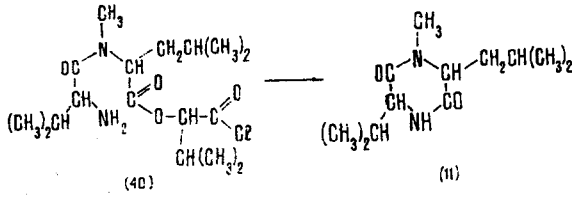
SCHEME 1


L-Val
Gly or Sar
L-Val
Sar
L-Val
Gly or Sar
L-Val
Sar
L-Val
Gly or Sar
Z
OH
(15) Sar
Z
OBut
(16) Sar
(12) Z
OH
H
OBut
(17) Sar
Z
OBut
(18) Sar
(19) Z
OH
H
OBut
(14) Gly
(20) Sar
Z
OBut
(21) Gly
(22) Sar
(13) Gly
(19) Sar
Z
OH
H
OBut
(23) Gly
(24) Sar
Z
OBut
(25) Gly, Gly
(26) Sar, Gly
(27) Sar, Sar
Z
OH
(28) Gly, Gly
(29) Sar, Gly
(30) Sar, Sar
Z
ONP
HBr·H
ONP
(1) Gly, Gly
(2) Sar, Gly
(3) Sar, Sar

SCHEME 2



Compounds (6)-(10) were obtained by doubling the corresponding tridepsipeptides (Scheme 2). To activate the C-terminal hydroxy-acid residue we used the acid chloride method, which gives excellent results in the cyclization of depsipeptides [15-18]. In the cyclization of the tridepsipeptide (40), in addition to the cyclohexadepsipeptide (7) we isolated the corresponding diketopiperazine, cyclo(-N-methyl-L-leucyl-D-valyl-) (11) (14%), apparently formed as the result of intramolecular aminolysis of the ester bond activated by the neighboring acid chloride group.



(40) (11)

As well as by the results of thin-layer chromatography, elementary analysis, and mass spectroscopy, the structure and purity of all the cyclopeptides were confirmed by their IR and NMR spectra, which will be considered in subsequent communications.

EXPERIMENTAL

All the temperatures are uncorrected. The individuality of each of the compounds obtained was checked by thin-layer chromatography in alumina (activity grade II) and on Eastman silica gel plates. For all the compounds the results of elementary analysis agreed satisfactorily with the calculated values for the C, H, and N contents. The contents of the intermediate compounds are given in Table 1, and the constants and yields of the cyclopeptides in Table 2.

1. tert-Butyl Ester of Benzyloxycarbonyl-L-valylsarcosine (18). At 0°C, a solution of 2.47 g (12 mmole) of N,N'-dicyclohexylcarbodiimide in 30 ml of methylene chloride was added to a solution of 1.75 g (12 mmole) of the tert-butyl ester of sarcosine (17) [19] and 3 g (12 mmole) of benzyloxycarbonyl-L-valine (12) in 60 ml of methylene chloride. After the mixture had been stirred at 0°C for 1 h and at 20°C for 12 h, the precipitate of N,N'dicyclohexylurea was filtered off, the filtrate was evaporated, the residue was dissolved in 60 ml

TABLE 1. Physicochemical Constants of the Linear Intermediate Compounds

Cpd. No.	mp, °C	$[\alpha]_D^{20}$	c	Solvent
15	Oil	—	—	—
18	Oil	−26,0	1	EtOH
19	64	−24,1	1	EtOH
21	Amorphous	−17,7	0,65	EtOH
22	Amorphous	−40,0	1	EtOH
25	157—158	+18,8	0,37	EtOH
26	Amorphous	−31,6	0,71	EtOH
27	Amorphous	−53,4	1	EtOH
28	151—152	−28,4	0,63	EtOH
29	120—121	−46,8	0,59	EtOH
30	Amorphous	−53,0	1	EtOH
36	Oil	−16,0	2,0	C_6H_6
37	Oil	−54,0	1,9	C_6H_6
38	Oil	−50,0	2,0	C_6H_6
39	Amorphous	+2,8	2,0	EtOH
40	Amorphous	−6,8	5,0	EtOH
41	Amorphous	−32,0	1,1	EtOH
42	Amorphous	−48,0	1,9	EtOH
43	Amorphous	−43,0	1,7	EtOH

of ethyl acetate, and the solution was washed with 5% HCl, water, and saturated $NaHCO_3$ solution, dried with $MgSO_4$, and evaporated. The product obtained was chromatographed on a column of alumina (activity grade II) in the benzene–ethyl acetate system (gradient elution). Yield 60%.

2. Benzyloxycarbonyl-L-valylsarcosine (19). A solution of 10 mmole of the tert-butyl ester of benzyloxycarbonyl-L-valylsarcosine (18) in 30 ml of anhydrous CF_3COOH was kept at room temperature for 30 min and evaporated. The residual oil was dissolved in absolute benzene and the solution was evaporated again. The new residue was dissolved in ethyl acetate and the solution was extracted with a saturated solution of $NaHCO_3$, the bicarbonate extracts were acidified with concentrated HCl and extracted with ethyl acetate, and the ethyl acetate extract was dried with $MgSO_4$ and evaporated.

3. tert-Butyl Esters of Benzyloxycarbonyltetra- and -hexapeptides (21), (22), and (25)-(27). By the dicyclohexylcarbodiimide method (as in experiment 1), 20 mmole of a benzyloxycarbonyldipeptide (13) or (19) and 20 mmole of an amino ester (14), (20), (23), or (24) (obtained by the catalytic hydrogenolysis [1] of the corresponding benzyloxy derivatives) gave the esters (21), (22), and (25)-(27) with a yield of 40-60%.

4. The Benzyloxycarbonylhexapeptides (28)-(30). A solution of 10 mmole of a tert-butyl ester of a benzyloxycarbonylhexapeptide (25)-(27) in 30 ml of trifluoroacetic acid was kept at room temperature for 40 min. Then the solution was evaporated in vacuum and the residue was treated with dry ether, filtered off, and dried over KOH in vacuum (yield 93-98%).

5. The Cyclohexapeptides (1)-(3). Compounds (1)-(3) were obtained by the cyclization of the benzyloxycarbonylhexapeptides (28)-(30) using the p-nitrophenyl ester method [1]; yield 5-8%.

When the products of the cyclization of the hexapeptides (28), (29), and (30) were chromatographed, a substance was isolated which was eluted before the corresponding cyclohexapeptides. According to its mass spectrum (mol. wt. 170) and NMR spectrum (C—CH_3, δ =

TABLE 2. Physicochemical Properties of the Cyclohexapeptides (1)-(4) and (6)-(10)

Cpd. No.	Compound	mp, °C	$[\alpha]_D^{20}$, c; solvent	mol. wt. (mass spectrum or thermoelectrically — t/e)	Yield on cyclization, %
1	Cyclo(-L-Val-Gly-L-Val-Sar--L-Val-Gly-)	283 (decomp.)	−45,5 0,31; 87% EtOH	482 (mass spectrum)	5
2	Cyclo (-L-Val-Sar-L-Val-Sar--L-Val-Gly-)	182—3	−25,8 0,31; EtOH	496 (mass spectrum)	8
3	Cyclo [-(L-Val-Sar)$_3$-]	Amorphous	−99,0 0,5; EtOH	510 (mass spectrum)	7
4	Cyclo [-(L-MeVal-Sar)$_3$-]	230—3	−90,2 0,5; EtOH	553 (mass spectrum)	37
6	Cyclo [-(D-Val-D-Leu-L--Hyiv)$_2$-]	295—7	+112 0,9; $CHCl_3$	647; 629 (t/e in CF_3COOH)	32
7	Cyclo [-(L-Val-L-MeLeu-L--Hyiv)$_2$-]	237—8	−268 1,4; $CHCl_3$	641; 652 (t/e in CF_3COOH)	43
8	Cyclo [-(D-Val-L-MeLeu-L--Hyiv)$_2$-]	245—6	−5,8 1,6; $CHCl_3$	640; 644 (t/e in CF_3COOH)	33
9	Cyclo [-(L-Val-L-MeIle-D--Hyiv)$_2$-]	222—3	−179 3,1; $CHCl_3$	634; 657 (t/e in CF_3COOH)	28
10	Cyclo [-(D-Val-L-MeIle-D--Hyiv)$_2$-]	215—6	+7,1 1,7; $CHCl_3$	654; 680 (t/e in CF_3COOH)	26

0.91, 1.05; $C^{\beta}H$, δ = 2.42; $N-CH_3$, δ = 2.99; $C^{\alpha}H$, δ = 3.87; NH, δ = 7.5; solvent $CDCl_3$; c = 0.27 M), it is represented by the structure cyclo(-L-Val-Sar-) [5]. mp 139-140°C, $[\alpha]_D^{20}$ + 6.6° (c 0.3; C_2H_5OH).

6. The Cyclohexapeptide (4). A solution of the methylsulfinyl carbanion (obtained from 50 ml of NaH and 20 ml of dimethyl sulfoxide) was added to a suspension of 500 mg (1.07 mmole) of cyclo(-L-valylglycyl-L-valylglycyl-L-valylglycyl-) [1] in 5 ml of dimethyl sulfoxide freshly distilled over calcium hydride. The reaction mixture was stirred in a current of nitrogen at 100°C for 15 min. Then it was cooled to room temperature, and an excess of freshly distilled methyl iodide was added and stirring was continued for 1 h. The solution was diluted with water and extracted with chloroform. The combined extracts were dried with $MgSO_4$ and evaporated. The resulting product was chromatographed on a column of neutral alumina (activity grade II) in the benzene–ethyl acetate system (gradient elution). The product was crystallized from a mixture of chloroform and petroleum ether. Yield 220 mg (37%).

7. tert-Butyl Esters of Benzyloxycarbonyltridepsipeptides (36)-(38). To a solution of 2.63 g (10.5 mmole) of a benzyloxycarbonylvaline (13) or (33) and 10 mmole of an amino ester (31) or (32) in 20 ml of methylene chloride was added 2.27 g (11 mmole) of DCHDI in 5 ml of methylene chloride. The mixture was left overnight at room temperature, and then the precipitate was filtered off and the filtrate was washed with 1 N HCl and with 5% $NaHCO_3$ solution, dried with $MgSO_4$, and evaporated. The residue was dissolved in a small amount of ethyl acetate and left overnight. The dicyclohexylurea that deposited was filtered off, the filtrate was evaporated, and the residue was chromatographed on neutral alumina in the benzene–ethyl acetate system (gradient elution). Yield 90%.

8. Hydrobromides of Tridepsipeptides (39)-(43). To a solution of 10 mmole of a tert-butyl ester of a benzyloxycarbonyltridepsipeptide (34)-(38) in 5 ml of acetic acid was added 40 ml of 35% HBr/CH_3COOH, and the mixture was kept at room temperature for 30 min. Then the acetic acid was distilled off, the residue was extracted with water and with ether, the ethereal extract was washed twice with water, and the combined aqueous extracts were evaporated. The residue was dissolved in a small amount of methanol, and the solution was diluted with toluene and evaporated; yield 70-90%.

9. The Cyclohexadepsipeptides (6)-(10). A solution of 5 mmole of a hydrobromide (39)-(43) in 15 ml of thionyl chloride was left at 25°C for 30 min, the excess of thionyl chloride was distilled off, the residue was treated with dry toluene, and the mixture was evaporated in vacuum. The acid chloride formed was dissolved in 400 ml of dry benzene, and the resulting solution was added dropwise (20°C, 8 h), simultaneously with a solution of 1.5 g (15 mmole) of triethylamine in 400 ml of benzene to two liters of dry benzene, with stirring. The mixture was left overnight, and then 3 ml of triethylamine was added, and after 2 h the reaction mixture was evaporated to dryness. The residue was dissolved in methylene chloride, and the solution obtained was washed with 1 N HCl, and with 5% $NaHCO_3$ solution, dried with $MgSO_4$, and evaporated. Chromatography of the residue on 800 g of neutral alumina (activity grade II) in the benzene–ethyl acetate system (gradient elution) yielded the corresponding cyclohexadepsipeptide (6)-(10) with a yield of 25-45%.

After the chromatography of the products of the cyclization of the tridepsipeptide (41), two fractions were isolated. Fraction I consisted of cyclo(-D-valyl-N-methyl-L-leucyl-L-α-hydroxyvaleryl-D-valyl-N-methyl-L-leucyl-L-α-hydroxyvaleryl-) (8); yield 540 mg (33%). Fraction II consisted of cyclo(-D-valyl-N-methyl-L-leucyl-) (11); yield 160 mg (14%), mp 97-98°C (from hexane), $[\alpha]_D^{20}$ +130° (c 0.9; $CHCl_3$). Molecular weight 211; 225 (thermoelectrically in trifluoroacetic acid). $C_{12}H_{22}N_2O_2$; mol. wt. 226.3.

SUMMARY

The synthesis of ten cyclic hexapeptides containing residues of N-methyl (amino acid)s and of α-hydroxyisovaleric acid has been performed.

LITERATURE CITED

1. V. T. Ivanov, I. A. Lavrinovich, Ya. Bernat, P. V. Kostetskii, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 57 (1975) [in this issue].

2. Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and M. M. Shemyakin, Dokl. Akad. Nauk SSSR, 153, 122 (1963).
3. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Malenkov, A. V. Evstratov, I. A. Laine, and I. D. Ryabova, J. Membr. Biol., 1, 402 (1969).
4. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, Tetrahedron, 19, 995 (1963).
5. Yu. A. Ovchinnikov, A. A. Kiryushkin, V. T. Ivanov, and M. M. Shemyakin, Tetrahedron Lett., 1927 (1963).
6. Yu. A. Ovchinnikov, A. A. Kiryushkin, and M. M. Shemyakin, Tetrahedron Lett., 1111 (1965).
7. A. A. Kiryushkin, Yu. A. Ovchinnikov, and M. M. Shemyakin, Tetrahedron Lett., 143 (1965).
8. J. Biol. Chem., 247, 977 (1972).
9. J. Dale and K. Titlestad, Chem. Commun., 656 (1969).
10. K. Titlestad, Chem. Commun., 1527 (1971).
11. J. Shaug, Acta Chem. Scand., 25, 2771 (1971).
12. E. Vilkas and E. Lederer, Tetrahedron Lett., 3089 (1968).
13. S. I. Hakomori, J. Biochem., 55, 205 (1964).
14. E. J. Corey and M. Chaykovsky, J. Amer. Chem. Soc., 84, 866 (1962).
15. M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, Izv. Akad. Nauk SSSR, Ser. Khim., 579 (1963).
16. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, Tetrahedron, 19, 581 (1963).
17. M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, Izv. Akad. Nauk SSSR, Ser. Khim., 1623 (1965).
18. I. I. Mikhaleva, I. D. Ryabova, T. A. Romanova, T. I. Tarasova, T. V. Ivanov, Yu. A. Ovchinnikov, and M. M. Shemyakin, Zh. Obshch. Khim., 38, 1228 (1968).
19. J. P. Marsh and L. Goodman, Can. J. Chem., 44, 799 (1966).