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New Charge-Deficient Agmatine Analogues

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Abstract—*N*,*N*'-Di-Boc-*N*''-triflylguanidine was demonstrated to be an efficient guanidinylation reagent for *O*-substituted hydroxylamines. *N*-(3-Aminooxypropyl)- and *N*-(3-aminopropoxy)guanidines, previously unknown isosteric and charge-deficient agmatine analogues, have been synthesized. The possibilities of using these compounds in studying polyamine metabolism are discussed.

Key words: agmatine, polyamines, O-substituted hydroxylamines

INTRODUCTION

Agmatine is one of key arginine metabolites (Scheme 1).[2] It is widely distributed in bacteria, plants, and Invertebrata [1]. In mid-1990s, Agm has also been found in eukaryotes; however, its content is low (from a few to tens of mg per kg of tissue [2]). Agm is an effector of NO synthase; it is bound by imidazoline receptors and fulfills functions of neurotransmitter in brain (see review [1] and references therein). Agm is actively transported into cells by the system of Put transport and affects the polyamine homeostasis. In this case, the exhaustion of the intracellular pool of spermine and spermidine sharply increases the efficiency of Agm transport into cells [3].

The Agm conversion into Put is an initial stage of polyamine biosynthesis in bacteria and plants [1]. In animal cells, Put originates from ornithine. At the same time, there are data on the possibility of Put biosynthesis from Agm [3]; however, the existence of this metabolic pathway in eukaryotes is not generally admitted [4].

A productive approach to the clarification of role and functions of Agm in cell is the study of biochemical effects of its analogues. We describe here the synthesis of previously unknown isosteric charge-deficient Agm analogues *N*-(3-aminooxypropyl)- and *N*-(3-aminopropoxy)guanidines on the basis of 3-aminooxy-1-aminopropane. The possibility of using these compounds for the chemical regulation of the activity of polyamine metabolism enzymes is discussed.

RESULTS AND DISCUSSION

AO-Agm is an isosteric charge-deficient (pK_a of H_2NO group is ~5.0) analogue of Agm. Like other *O*-susbstituted hydroxylamines, it forms stable oximes with aldehydes and ketones, including pyridoxal 5'-phosphate (Fig. 1); the latter oxime is a coenzyme of ArgDC. The aminooxy analogues of the pyruvate- and pyridoxal 5'-phosphate-dependent enzymes of the amino acid metabolism are the efficient inhibitors of these enzyme (see review [5] and references therein), and, therefore, one can expect a high activity of AO-Agm (**I**) toward the ArgDC.

Some guanidine-containing diamine derivatives, including Agm and 1-guanidino-7-aminoheptane (an

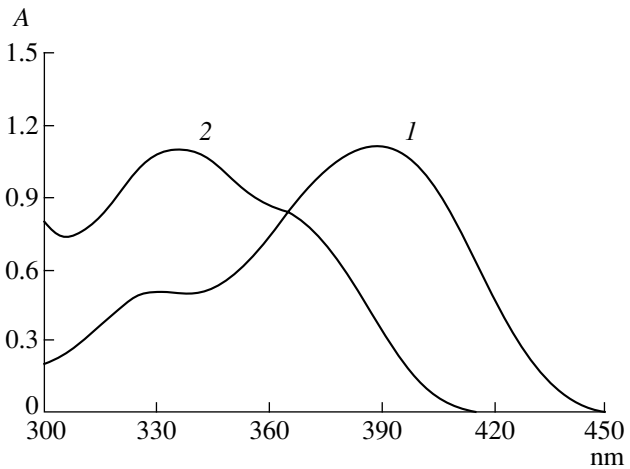

Fig. 1. UV spectra of (curve *1*) pyridoxal 5'-phosphate and (curve *2*) its oxime with AO-Agm. *1*, pyridoxal 5'-phosphate (2.28×10^{-4} M) in 0.1 M sodium acetate buffer, pH 5.0; *2*, the same but in the presence of 5×10^{-4} M AO-Agm (1 min after mixing).

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[2] Abbreviations: Agm, agmatine (1-guanidino-4-aminobutane); AO-Agm, *N*-(3-aminooxypropyl)guanidine; ArgDC, arginine decarboxylase; GAPA, *N*-(3-aminopropoxy)guanidine; ODC, ornithine decarboxylase; Put, putrescine (1,4-diaminobutane); and Tf, triflate (trifluoromethanesulfonate).

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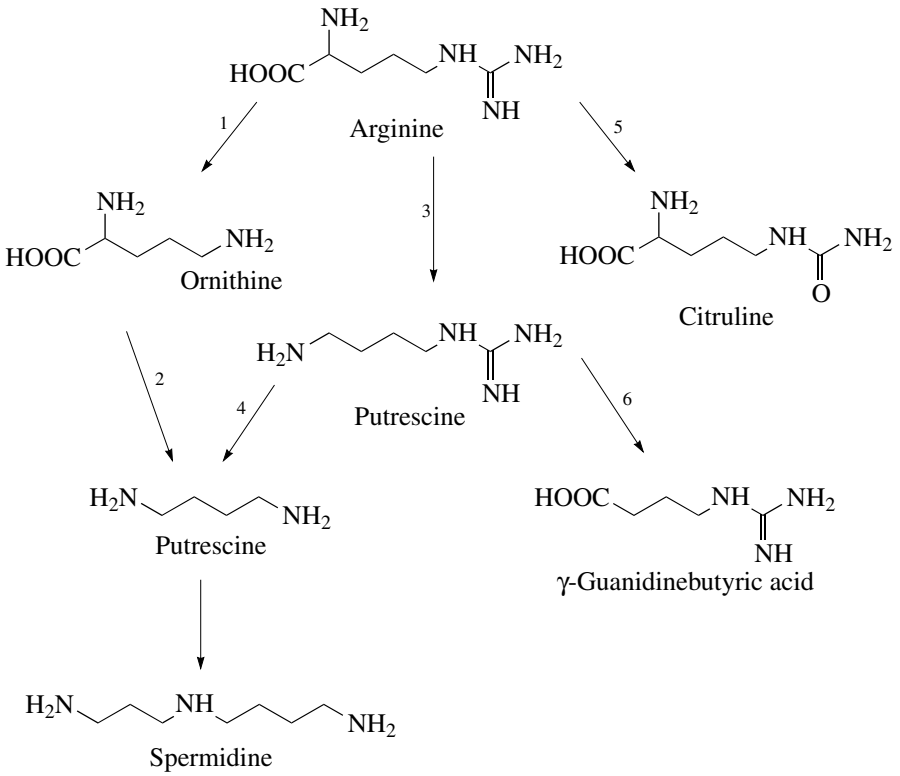
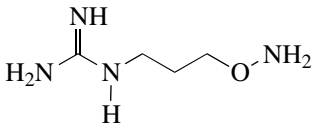
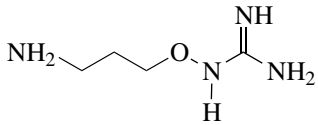


Scheme 1. Metabolism of agmatin [1]. 1, Arginase (EC 3.5.3.1); 2, ODC (ornithine decarboxylase, EC 4.1.1.17); 3, ArgDC (arginine decarboxylase, EC 4.1.1.19); 4, agmatinase (EC 3.5.3.11); 5, NO synthase (EC 1.14.13.39); and 6, diaminooxydase (EC 1.4.3.6).

efficient inhibitor of deoxyhypuzine synthase [6]), are actively transferred into cells [3]. Consequently, one can expect that GAPA (**II**), which, at physiological pH values, is a doubly protonated structural analogue of Agm (pK_a of the $H_2N(NH)CNHO$ group of GAPA is ~7.0–7.5, whereas the pK_a of NH_2 group is ~10.0), would be actively transported into cells exploiting the Put transport system. There are known the examples of catabolism of alkoxyguanidines. In particular, amino acid canavanine (α-amino-γ-guanidinooxybutyric acid) is widely distributed in the plants of family Leguminosae. This amino acid contains up to 13% of the whole bound nitrogen in the seeds of *Dioclea megacarpa* [7]. The larvae of the corn beetle *Caryedes brasiliensis* that are feeding with the seeds of this plant can cleave canavanine to canavaline (γ-aminooxy-α-aminopropionic acid) [7]. Accordingly, the possibility of intracellular degradation of GAPA to 3-aminooxy-1-aminopropane, one of the most efficient ODC inhibitors [8–10], cannot be excluded. Therefore, (**II**) is interesting for the study of special features of the Put active transport into cells; it can be regarded as an actively transportable proinhibitor of ODC.

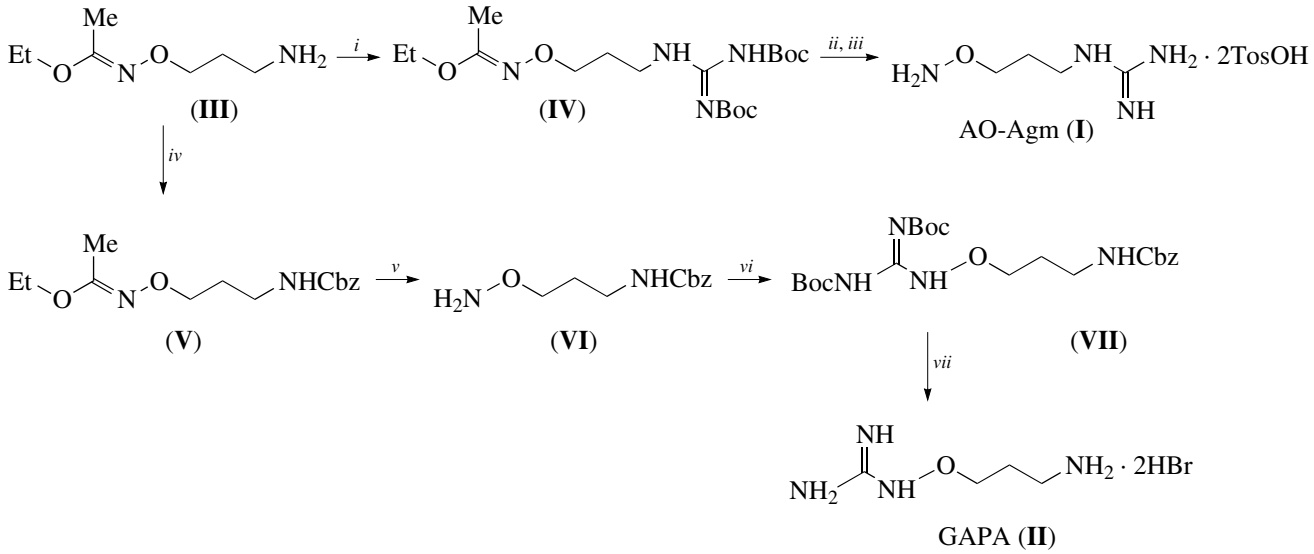


AO-Agm (**I**)



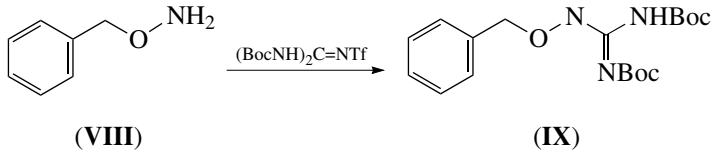
GAPA (**II**)

The guanidination of amino- and aminooxy groups was the key stage in the synthesis of AO-Agm and GAPA. A great number of guanidinating reagents are known from the chemistry of amino acids. Methyl ethers of urea and thiourea, their *N*-protected derivatives, and the corresponding pyrazoles are the most frequently used reagents among them [11–13]. However, the reaction rates are low in a number of cases, and the yields of target products are far from quantitative [6, 14]. Triflates of di-Boc, di-Cbz [15], and di-Fmoc [16] derivatives of guanidine have recently been suggested for the guanidinaion of amines. These reagents convert amines into the corresponding guanidines for several hours at room temperature in yields close to quantitative. Therefore, in this work, we guanidinated amino groups using the readily available di-Boc-guanidine triflate.



Reagents: *i*—$(BocNH)_2C{=}NTf/Et_3N/CH_2Cl_2$; *ii*—HCl/Pr^{i}OH/$H_2O$; *iii*—AG 1-X8 $TosO^-$-form; *iv*—Cbz-Cl/Et_3N/THF; *v*—HCl/EtOH/H_2O; *vi*—$(BocNH)_2C{=}NTf/Et_3N/CH_2Cl_2$/37°C; *vii*—HBr/AcOH,

Scheme 2. Synthesis of hydroxylamine-containing agmatine analogues.



Scheme 3. Synthesis of N^1-benzyloxy-N^2,N^3-di-*tert*-butyloxycarbonylguanidine (**IX**).

AO-Agm (**I**) was obtained starting from 3-(1'-ethoxyethylidene)aminooxy-1-aminopropane (**III**) (Scheme 2). The guanidination of free amino group in (**III**) with di-Boc-guanidine triflate was achieved by a standard procedure [15]. It led to di-Boc derivative (**IV**) in yield close to quantitative. The removal of protective groups by HCl/EtOH resulted in the AO-Agm dihydrochloride, which was slowly deliquesced in air and converted to a well crystallized tosylate (**I**).

The synthesis of GAPA (**II**) was a more complicated task, since, practically, the methods of guanidination of aminooxy group were not developed. The conversion of protected canaline into canavanine, described in [17], proceeds as a result of treatment with *S*-methylisothiourea sulfate (yield 10%) or a boiling with cyanamide in alcohol (yield 70%). We have used here di-Boc-guanidine triflate to obtain guanidinooxyalkanes for the first time. Its interaction with *O*-benzylhydroxylamine (**VIII**) (Scheme 3) showed that the corresponding guanidine derivative (**IX**) is formed in a high yield. However, the reaction proceeds slower (Fig. 2) than in the case of more nucleophilic aliphatic amines. The value of rate constant of the reaction of the second order (molar ratios of *O*-benzylhydroxylamine and di-Boc-guanidine triflate) determined by the NMR method according to the integral intensity of protons of CH_2 group of (**VIII**) (δ 4.63 ppm) and protons of CH_2 group of the di-Boc protected benzyloxyguanidine (**IX**) (δ

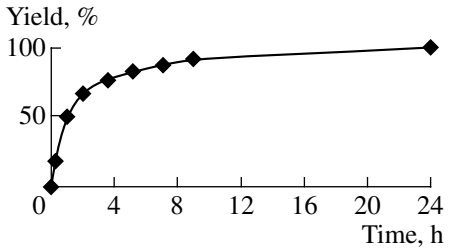


Fig. 2. The kinetics of guanidination of *O*-benzylhydroxylamine (0.3 M) with di-Boc-guanidine triflate (0.3 M) in the presence of triethylamine (0.3 M) in $CDCl_3$ (0.5 ml) at 37°C.

5.01 ppm) was shown to be 2.66×10^{-2} l mol^{-1} min^{-1} ($\tau_{1/2}$ 75 min at 37°C).

Analogue (**II**) was synthesized from 3-(1'-ethoxyethylidene)aminooxy-1-aminopropane (**III**) (Scheme 2). The free amino group of (**III**) was initially benzyloxycarbonylated, and the ethoxyethylidene protection was then removed by HCl in aqueous alcohol. This resulted in the corresponding hydrochloride, which was then converted into base (**VI**). The base was guanidinated by di-Boc-guanidine triflate (24 h at 37°C) to (**VII**) obtained in a high yield. The simultaneous removal of Boc and Cbz protective groups with HBr/AcOH at room temperature led to the crystalline dihydrobromide of (**II**).

Thus, we found that di-Boc-guanidine triflate effectively guanidinates not only amines, but also the substantially less nucleophilic *O*-alkylhydroxylamines, which allows the preparation of new isosteric charge-deficient analogues of Agm: AO-Agm and GAPA.

EXPERIMENTAL

The following reagents were used in this work: Cbz-Cl and absolute ethanol from Fluka and ion-exchange resin AG 1-X8 200–400 mesh from BioRad. 3-(1'-Ethoxyethylidene)aminooxy-1-aminopropane was synthesized as described in [18]; *N*,*N*'-di-Boc-guanidine triflate, as described in [15]; and *O*-benzylhydroxylamine base, as described in [19]. TLC was carried out on precoated Kieselgel 60 F_{254} plates (Merck) in the systems: (A) 4 : 1 chloroform–methanol; (B) 120 : 49 : 30 : 1 methanol–water–25% ammonia–trifluoroacetamide; (C) 95 : 5 CCl_4–MeOH; and (D) 7 : 3 dioxane–25% ammonia. Substances were detected on the chromatograms by the UV-light absorption and the color reaction with ninhydrin; aminooxy compounds, in the form of fluorescing oximes of pyridoxal 5'-phosphate. Silica gel of the trade mark Kieselgel (40–63 μm, Merck) was used for column chromatography; elution systems are given in the text.

NMR spectra were registered on a Bruker Avance 500 DRX instrument (Germany) at the working frequency of 500.1 MHz for ^{1}H and 125.8 MHz for ^{13}C nuclei. The substances were coevaporated with CD_3OD and then dissolved in $CDCl_3$. Tetramethylsilane (for $CDCl_3$ solutions) and sodium 3-trimethylsilyl-1-propanesulfonate (for D_2O solutions) were used as internal standards. Chemical shifts are given in ppm and spin–spin coupling constants in Hz.

***N*^{1}-[3-(1'-Ethoxyethylidene)aminooxypropyl]-*N*^{2}, *N*^{3}-di-*tert*-butyloxycarbonylguanidine (IV).** A solution of di-Boc-guanidine triflate (0.53 g (1.35 mmol) in dichloromethane (3 ml) added to a solution of (**III**) (0.24 g, 1.5 mmol) and triethylamine (0.21 ml, 1.5 mmol) in dichloromethane (3 ml). The reaction mixture was kept for 1.5 h at room temperature; diluted with an equal volume of dichloromethane; successively washed with 10% citric acid, water, 1 M $NaHCO_3$, water, and saturated NaCl; and evaporated in a vacuum to dryness. The residue was dried in a vacuum over phosphorus pentoxide, and get (**IV**); a viscous oil; yield 0.50 g (91%); R_f 0.76 (A); ^{1}H NMR ($CDCl_3$): 4.00–3.93 (4 H, m, $CH_3C\underline{H}_2$ + $NOCH_2$), 3.52 (2 H, m, $C\underline{H}_2NH$), 1.91 (3 H, s, $CH_3C{=}NO$), 1.89 (2 H, m, $CH_2C\underline{H}_2CH_2$), 1.48 [9 H, s, $C(CH_3)_3$], 1.46 [9 H, s, $C(CH_3)_3$], 1.24 (3 H, t, *J* 7.16, $C\underline{H}_3CH_2$).

***N*-(3-aminopropoxy)guanidine ditosylate (I).** A 37% solution of HCl (0.5 ml) was added to a solution of (**IV**) (0.34 g, 0.85 mmol) in isopropanol (5 ml). The reaction mixture was kept for 5 h at room temperature, evaporated in a vacuum to dryness; the oily residue was several times coevaporated with absolute isopropanol and dried in a vacuum over P_2O_5/KOH, to give crystalline slowly deliquescent *N*-(3-aminopropoxy)guanidine dihydrochloride; yield 0.22 g; R_f 0.28 (B); ^{1}H NMR (D_2O): 4.16 (2 H, t, *J* 6.0, $H_2NOC\underline{H}_2$), 3.33 (2 H, t, *J* 6.8, $C\underline{H}_2NH$), 1.99 (2 H, m, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (D_2O): 159.74 (s, NH_2CNH), 75.42 (t, H_2NOCH_2), 40.63 (t, CH_2NH), 29.43 (t, $CH_2C\underline{H}_2CH_2$). The dihydrochloride was converted into ditosylate on a column packed with ion-exchange resin AG 1-X8 in Tos^- form, which was recrystallized from absolute ethanol and dried in a vacuum over P_2O_5 to give (**I**); yield 0.33 g (83%); mp 195–196°C. Found, %: C 45.62, H 6.03, N 11.87. $C_{18}H_{28}N_4O_7S_2$. Calc., %: C 45.36, H 5.92, N 11.76.

3-Aminooxy-1-(*N*-benzyloxycarbonyl)aminopropane (VI). A solution of (**III**) (1.33 g, 8.3 mmol) and triethylamine (1.6 ml, 11 mmol) in THF (15 ml) was cooled to 0°C and treated at a vigorous stirring with Cbz-Cl (1.2 ml, 8.0 mmol) in three portions at 15-min intervals. The mixture was then stirred for 1 h at 0°C and for 3 h at room temperature. The triethylamine hydrochloride was filtered off; the filtrate was evaporated in a vacuum to dryness; and the residue was dissolved in chloroform (20 ml), cooled to 4°C, and successively washed with cool water, 10% citric acid, water, 1 M $NaHCO_3$, and water. The extract was dried with $MgSO_4$ and evaporated in a vacuum. The residue was dried in a vacuum over P_2O_5 to give (**V**) as a viscous oil; yield 2.39 g (92%); R_f 0.58 (C).

A solution of (**V**) (2.39 g, 7.7 mmol) in absolute ethanol (13 ml) was mixed with 37% HCl (1.7 ml), kept for 10 min at room temperature, and evaporated in a vacuum to dryness. The residue was recrystallized from EtOH–EtOAc and get (**VI**) hydrochloride; yield 1.47 g (73%); R_f 0.62 (E). A solution of (**VI**) hydrochloride (0.52 g, 2 mmol) in water (7 ml) was treated with 10 M NaOH (0.2 ml), extracted with chloroform, and the extract was dried with K_2CO_3. The solution was evaporated in a vacuum to dryness, and the solid residue was dried in a vacuum over P_2O_5 to give (**VI**); yield 0.38 g (86%); R_f 0.51 (A); ^{1}H NMR ($CDCl_3$): 7.34–7.30 (5 H, m, C_6H_5), 5.37 (2 H, s, $PhCH_2$), 3.70 (2 H, t, *J* 5.92,

$H_2NOC\underline{H}_2$), 3.29–3.24 (2 H, m, $C\underline{H}_2NHZ$), 1.79–1.73 (2 H, m, $CH_2C\underline{H}_2CH_2$).

***N*¹-(3-Benzyloxycarbonylaminopropoxy)-*N*²,*N*³-di-*tert*-butyloxycarbonylguanidine (VII).** A solution of di-Boc-guanidine triflate (0.49 g, 1.26 mmol) in chloroform (3 ml) was added to a solution of (**VI**) (0.32 g, 1.4 mmol) and 0.2 ml (1.4 mmol) of triethylamine in dichloromethane (3 ml).The reaction mixture was kept for 24 h at 37°C; twofold diluted with chloroform; successively washed with 10% citric acid, water, 1 M $CHCl_3$, water, saturated solution of NaCl; and evaporated in a vacuum. The residue was dried in a vacuum over P_2O_5 to give (**VII**) as a viscous oil; yield 0.49 g (88%); R_f 0.52 (A); ^{1}H NMR ($CDCl_3$): 7.31 (5 H, m, C_6H_5), 5.07 (2 H, s, $PhCH_2$), 4.07 (2 H, t, *J* 5.6, $NOCH_2$), 3.28 (2 H, m, $C\underline{H}_2NHZ$), 1.83 (2 H, m, $CH_2C\underline{H}_2CH_2$), 1.46 [9 H, s, $C(CH_3)_3$], 1.44 [9 H, s, $C(CH_3)_3$].

***N*-(3-Aminooxypropyl)guanidine dihydrobromide (II).** A 32% HBr/AcOH solution (3 ml) was added to a solution of (**VII**) (0.49 g, 1.1 mmol) in AcOH (5 ml). The reaction mixture was kept for 2 h at room temperature, and a 1 : 1 AcOH–Et_2O mixture (8 ml) was added to the resulting suspension. The precipitated solid was filtered, washed with ethyl ether, dried in a vacuum over KOH, and recrystallized from absolute ethanol. The precipitate of (**II**) was dried in a vacuum over P_2O_5/KOH; yield 0.26 g (70%); R_f 0.25 (D); mp 172–173°C (decomp.); ^{1}H NMR (D_2O): 4.06 (2 H, t, *J* 6.20, $HNOC\underline{H}_2$), 3.14 (2 H, t, *J* 7.50, $C\underline{H}_2NH_2$), 2.08 (2 H, m, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (D_2O): 161.54 (s, $NH_2(NH)C$), 77.07 (t, $HNOCH_2$), 39.67 (t, CH_2NH_2), 28.08 (t, $CH_2\underline{C}H_2CH_2$). Found, %: C 16.48, H 4.77, N 19.20. $C_4H_{14}Br_2N_4O$. Calc., %: C 16.34, H 4.80, N 19.06.

***N*¹-Benzyloxy-*N*²,*N*³-di-*tert*-butyloxycarbonylguanidine (IX).** Di-Boc-guanidine triflate (54.3 mg, 0.15 mmol) was added to a solution of *O*-benzylhydroxylamine (**VIII**) (18.8 mg, 0.15 mmol) and triethylamine (22 μl, 0.15 mmol) in $CDCl_3$ (0.5 ml), the mixture was kept for 24 h at 37°C, with registering ^{1}H NMR spectra after each hour. The reaction mixture was treated by the standard procedure [see the synthesis of (**VII**)], and (**IX**) was obtained as a viscous oil; yield 35 mg (71%); R_f 0.62 (G); ^{1}H NMR ($CDCl_3$): 7.42–7.31 (5 H, m, C_6H_5), 5.01 (2 H, s, $C_6H_5C\underline{H}_2$), 1.48 [9 H, s, $C(CH_3)_3$], 1.45 [9 H, s, $C(CH_3)_3$].

ACKNOWLEDGMENTS

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