Synthesis and antiischemic activity of dicarboxylic nitroxyalkylamides and nitroxyalkylimides

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A number of dicarboxylic *N*-(2-nitroxyalkyl)amides and *N*-(2-nitroxyalkyl)imides were synthesized and their antiischemic activity was studied. The ratio of the areas of necrotic and ischemic zones was used as a criterion for evaluation of antiischemic activity. The maximum values were close to antiischemic activity of Nicorandil, with acute toxicity of compounds synthesized being considerably lower.

Key words: reaction of diethyl dicarboxylates with amino alcohols, nitration of oxyalkylamides and imides with nitric acid and its mixtures with sulfuric acid and acetic anhydride, solutions of nitric acid in chloroalkanes, succinic and citric nitroxyalkylamides, succinic nitroxyalkylimides, acute toxicity, antiischemic activity.

Modern medicinal chemistry is an independent complex branch of organic chemistry, which is fundamentally based, besides fine organic synthesis, on achievements of bioorganic chemistry, quantum chemistry, and pharmacology. Fundamental works in this field include studies of detailed mechanisms of biological effects of pharmacologically active agents or pharmacophoric groups in their composition. Lately, a vast body of works were devoted to the synthesis of the so-called nitrogen monoxide donors[1] as one of the ways for development of new medicinal agents containing groups capable of generating nitrogen monoxide during biotransformation. In this connection, the works dealing with the directed synthesis of biologically active compounds which use metabolites are of great interest. In the present work, we synthesized nitroxyalkyl derivatives based on the intermediates of biosynthesis of ATP in the cycle of tricarboxylic acids — a key macroergic metabolite determining all the energy dependent processes of metabolism, including the transmembrane ion transport processes. The main attention is paid to dicarboxylic and tricarboxylic acids as the dehydrogenase substrates, which transfer electrons to the respiratory chain, where synthesis of ATP takes place.

Cardio-vascular pathologies belong to the category of socially significant diseases and occupy the first place in the incidence of mortality. In the alcohol nitrates (nitroglycerin, isosorbide dinitrate and mononitrate) used at the present time in medical practice, compounds from carbohydrate class serve as the nitrate group carriers.[2] However, their use does not limit a possibility of application for this purpose of other metabolites providing synthesis of ATP. Note that carbohydrates are the primary substrates of biosynthesis of ATP. It can be suggested that some other products of the aerobic glycolysis, for example, dicarboxylic acids, can be more promising carriers of nitrate groups as compared to carbohydrates, since they are involved in the Krebs Cycle as intermediate products and are in the intermediate steps closer to the final steps of the synthesis of ATP. It is known that the use of nicotinamide (vitamin PP) as a nitrate group metabolite-carrier compound made it possible to accomplish synthesis on its basis of *N*-(2-nitroxyethyl)nicotinamide (commercial names Nicorandil, Sigmart, Nicorel).[3—5] Pharmacological and clinical tests of this agent showed its efficient prolonged coronodilatation effect, as well as an ability to prevent the formation of blood clots and optimize the metabolic processes of the myocardium. Therefore, it can be suggested that, in fact, the use of other nitrate group metabolites-carriers allows one to obtain medicines with high efficiency of treatment.

This circumstance prompted us to begin the search for efficient and low toxic antiischemic agents from the class of dicarboxylic and tricarboxylic acids, which would have been stable during preparation, storage, and recycling and would not have caused side effects when used as antiischemic agents.

We believe that the high antiischemic activity of Nicorandil is determined by the structural specifics, namely, a combination in its molecule of two biologically active fragments: the nitrate groups (as a source of NO in the

* Based on the Materials of the First Russian Conference on Medicinal Chemistry (MedChem Russia-2013) with international participation (September 8—12, 2013, Moscow).

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 0843—0847, May, 2014.

1066-5285/14/6305-01119

organism) and nicotinamide which is involved in the biosynthesis of nicotinamide coenzymes, including dehydrogenases. Our approach to the synthesis of compounds with antiischemic activity consists in the choice of substituted dicarboxylic acid amides as nitrate group carriers, first of all, substituted amides and imides of succinic acid and citric acid amide.

Nitroxyalkyl groups, functioning in biotransformations as an NO generator, were added to the acid amides and in the case of succinic acid, also to imide. Succinic acid was chosen because of its physiological activity:[6] it stimulates the nervous system, strengthens the activity of kidneys and intestines, is used as an antiinflammatory and antistress agent.[6] Succinic acid is a central component of the Krebs Cycle and the cycle of tricarboxylic acids and facilitates synthesis of ATP. Citric acid, like succinic acid, is the intermediate product of the cycle of tricarboxylic acids.

The purpose of the present work is the synthesis of *N*-(2-nitroxyalkyl)amides and *N*-(2-nitroxyalkyl)imides of succinic acid and some other dicarboxylic acids, actively involved in the metabolytic processes taking place in living organisms.

Results and Discussion

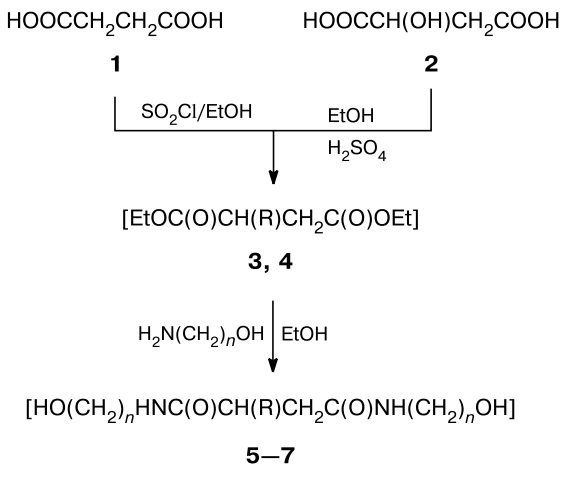
Synthesis of succinic acid nitroxyalkylamides. Succinic acid nitroxyalkylamides were synthesized by the reactions given in Schemes 1 and 2. First, succinic acid was converted to succinoyl chloride upon treatment with thionyl chloride.[7] After excess of thionyl chloride was evaporated at reduced pressure, the residue without isolation was treated with anhydrous ethanol to obtain diethyl succinate, which was involved in the reaction with 2-aminoethanol or 3-aminopropanol in ethanol. Esters **3**—**4** also without isolation were treated with aminoalcohols in anhydrous ethanol. Then, dicarboxylic acid hydroxyethylamides **5**—**7** were nitrated with a mixture of H_2SO_4—HNO_3 (see Scheme 2), the nitration products were isolated as crystalline compounds. The nitration process of alkylamides was carried out within the temperature range from 0 to 20 °C, then, the reaction mixture was poured into a mixrure ice—water, the crystalline reaction products were filtered off, washed with ice-cold water, and dried in air. Compounds **8** and **9** were characterized by elemental analysis and ^{1}H NMR spectroscopy. Malic acid diethyl ester was obtained upon treatment of malic acid with ethanol in the presence of concentrated H_2SO_4, which was then treated with 2-aminoethanol to obtain substituted amide of malic acid (**7**), which was also subjected to nitration (Scheme 3).

Nitration of compounds **5** and **6** with a mixture of concentrated HNO_3 and concentrated H_2SO_4 led to dinitrates of *N*,*N*′-(bishydroxyalkyl)succindiamides (compounds **8** and **9**) in 42—44% yield.

Note that aminoalcohols can be successfully nitrated either with concentrated HNO_3 or its mixture with concentrated H_2SO_4 with their v/v ratio 1 : (2—5). Better results were obtained with the 1 : (2—3) wt/v ratio of amide and a nitrating agent. Good yields of nitroxyalkylamides **8** and **9** were also obtained by nitration with a mixture of nitric acid and acetic anhydride at the molar ratio 1 : (1—3). In this case, the yields of nitrates were 60—70%. However this modification cannot be recommended because of a possibility of uncontrolled decomposition of the reaction mixture, which has an explosive character.

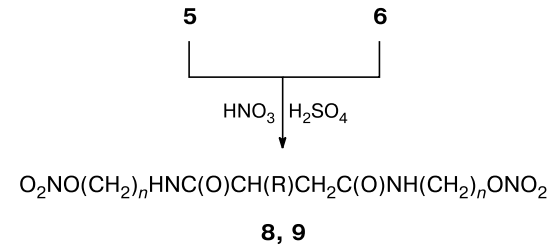
Nitration of hydroxysuccindiamide **7** containing a primary and a secondary alcohol group gave both dinitrate **10** and trinitrate **11** (see Scheme 3).

Scheme 1



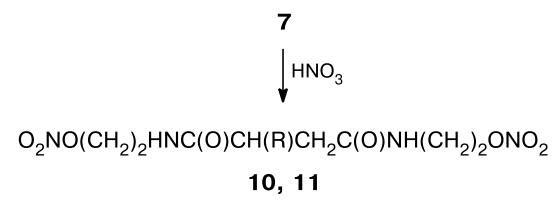
R = H (**3**, **5**, **6**), OH (**4**, **7**); *n* = 2 (**5**, **7**), 3 (**6**)

Scheme 2



R = H; *n* = 2 (**8**), 3 (**9**)

Scheme 3

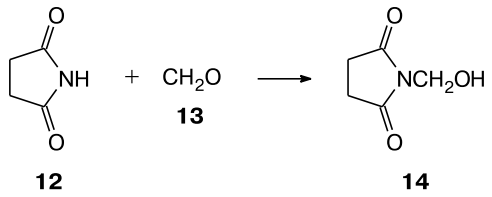


R = OH (**10**), ONO_2 (**11**)

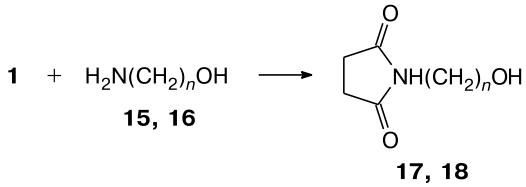
When nitration was carried out with less than 90% concentration of HNO_3, dinitrate **10** was obtained in 56% yield. Trinitrate **11** was obtained in 46% yield after treatment of triol **7** with concentrated HNO_3.

Synthesis of succinic acid nitroxyalkylimides. Succinimide (**12**) reacted with formaldehyde (**13**) according to Scheme 4 to give *N*-hydroxymethylsuccinimide (**14**). The reaction of compounds **1**, **15**, and **16** according to Scheme 5 led to the synthesis of *N*-(2-hydroxyethyl)- and *N*-(3-hydroxypropyl)succinimides (**17**, **18**).

Scheme 4



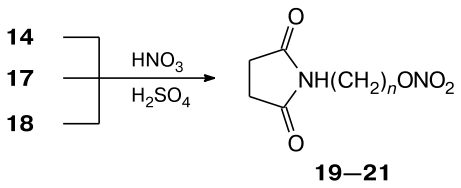
Scheme 5



n = 2 (**15**, **17**), 3 (**16**, **18**)

Nitration with a mixture of H_2SO_4—HNO_3 gave compounds **14**, **17**, and **18** (Scheme 6) in 65—86% yields and nitrates of *N*-(hydroxyalkyl)succinimides (**19—21**).

Scheme 6

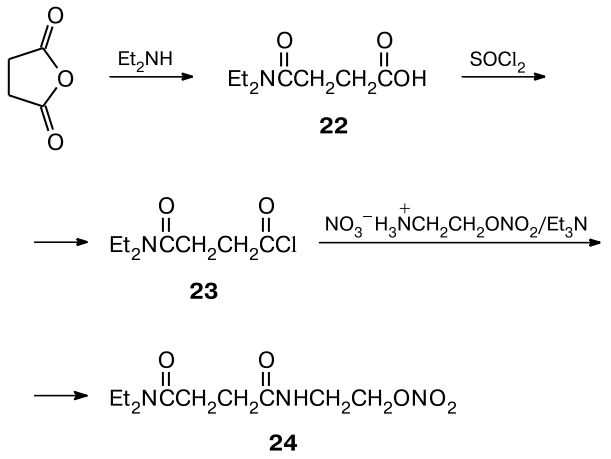


n = 1 (**19**), 2 (**20**), 3 (**21**)

We found that the nitration process of alkanolsuccinimides, like the process of nitration of succinalkyldiamides, can be successfully carried out in concentrated nitric acid, in sulfuric acid—nitric acid mixtures, as well as in 8—13% solutions of nitric acid in dichloromethane or 1,2-dichloroethane, that makes the process safe. However, nitration of hydroxyalkylsuccinimides with 8—13% solutions of nitric acid in chloroalkanes is the most preferable. The yields of nitrates in this case reach 76—81% depending on temperature of the process and reaction time after mixing reagents. The 8—13% solutions of nitric acid in chloroalkane (dichloroethane or dichloromethane) as a nitrating agent can be also successfully used for nitration of dicarboxylic acid oxysuccindiamides. In this case, after the nitration process is complete and the reaction mixture is diluted with water, nitroxyalkylamides or imides can be isolated either by filtration or extraction with organic solvent, for example, ethyl acetate. In conclusion, using *N*,*N*′-bis(hydroxyalkyl)succindiamides and imides as examples, we showed a possibility of selective *O*-nitration with nitrogen atom of the amide group remaining intact, as well as a possibility of selective nitration of primary and secondary hydroxy groups.

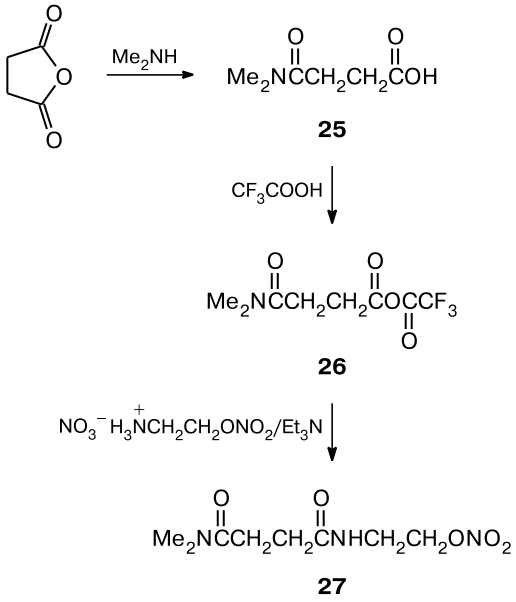
Synthesis of nitrates of unsymmetric *N*,*N*-dialkyl-*N*′-(hydroxyalkyl)succindiamides. The synthesis of nitrates of unsymmetric *N*,*N*-dialkyl-*N*′-(hydroxyalkyl)succindiamides was performed using two pathways (Schemes 7 and 8): either through *N*,*N*′-dialkylsuccinamide acyl chloride (**23**) in the presence of 2-nitroxyethylammonium nitrate[9] (see Scheme 7) or through the mixed anhydride of *N*,*N*-dimethylamide of succinic acid and trifluoroacetic acid (**26**) (see Scheme 8).

Scheme 7

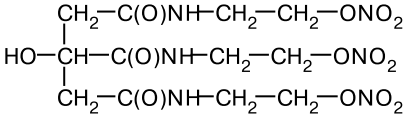


Using this scheme of the synthesis, we obtained high yields of dimethyl- and diethyl-substituted succinic acid amides with nitrate groups. Testing these compounds in the Russian Research Center for Security of Biological Active Substances (RRC BAS, Kupavna, Moscow Region) for antiischemic activity showed their lower therapeutic activity as compared to succinic acid nitroxyalkylamides (the ratio of the areas of necrotic and ischemic zones for compounds **24** and **27** was 56—57% with 68% in control). In another words, their activity is considerably lower than that of succinic acid nitroxyalkylamides and imides and its hydroxy analogs. Though, it is possible that these nitrates can be used as antiarythmic and antihypoxic agents.

Scheme 8



Besides, substituted citric acid amide **28** was converted to trinitrate **29**.



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This compound is not toxic in the dose 1000 mg kg^{-1} and possesses antiischemic activity (Table 1).

Antiischemic activity of compounds synthesized. Antiischemic activity was tested in the RRC BAS in the Prof. L. N. Sernov´s laboratory according to the known procedure.[10] Artificial myocardial infarction in white rats (weight 240 g) was caused by the coronary artery occlusion for 4 h, which was registered with instruments, after which a tested substance was administered in the amount of 10 mg kg^{-1}, further all subsequent operations were performed according to the procedure given in the literature.[10] Table 1 summarizes the data on the acute toxicity and antiischemic activity of compounds synthesized by us based on substituted succinic acid nitroxyalkylimides and amides, which were tested in the RRC BAS. Four of the nitrates synthesized (**10**, **11**, **19**, and **20**) have the following ratios of the areas of necrotic and ischemic zones: 39.3±5.4, 36.4±5.6, 42.2±6.6, and 44.3±6.7%. In another words, these nitrates possess antiischemic activity similar to that of Nicorandil (42.2±5.7), which we consider as the analog for its intended purpose. As to the acute toxicity, these all four nitrates are favorably distinguished from Nicorandil. Thus, LD_{50} for Nicorandil is 470 mg kg^{-1}, while for nitrates synthesized by us, it is 675—1150 mg kg^{-1}.

Table 1. Properties of substituted nitroxyalkylamides and nitroxyalkylimides of succinic and citric acids

Compound	M.p./°C	LD_{50}/mg kg^{-1}	γ* (%)
8	100—101.5	870	62.0±10.3
9	105—106	580	58.1±9.7
10	90—91	>1000	39.3±5.4
11	108—109	780	36.4±5.6
19	112—113	>1000	42.2±6.6
20	61.5—62.5	675	44.3±6.7
21	35—36	315	45.7±5.3
24	85—86	>1000	57.0±6.4
27	94—95	860	53.0±7.7
29	92—93	580	49.0±7.6

* The ratio of the areas of necrotic and ischemic zones (in control 68%).

In conclusion, we developed an approach to the preparation of *N*-(2-nitroxyalkyl)succinamides and *N*-(2-nitroxyalkyl)succinimides by selective *O*-nitration without affecting nitrogen atom of the amide group. We also elaborated a procedure for selective *O*-nitration of primary and secondary hydroxy groups, which allows us to obtain nitrates either at the primary hydroxy groups or at the primary and secondary hydroxy groups. We synthesized two *N*-(2-nitroxyethyl)amides and two *N*-(nitroxyalkyl)-imides of succinic acid, which have antiischemic activity on the level with Nicorandil, with their acute toxicity (LD_{50} = 680—1150 mg kg^{-1}) being lower than that of Nicorandil (LD_{50} = 475 mg kg^{-1}).

Experimental

Succinic and citric acid ethyl esters were obtained upon treatment of malic and citric acids with ethanol in the presence of sulfuric acid.[11] Diethyl succinate was obtained according to the known procedure[7] by the reaction of succinic acid with thionyl chloride with subsequent treatment of the reaction mixture with anhydrous ethanol and then with ethanolamine or propanolamine. Both dicarboxylic acyl chlorides and amides were not purified and used in subsequent reactions without isolation in the pure form after evaporation of excess of thionyl chloride at reduced pressure. Substituted succinic and citric acid amides were synthesized from the dicarboxylic acid ethyl esters by the reaction with aminoalcohols in anhydrous ethanol.[11]

IR spectra were recorded on a Specord M-80 IR spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Bruker AC-200 spectrometer.

***N*,*N*´-Bis(2-hydroxyethyl)succindiamide (5).** A mixture of diethyl succinate (17 g, 97.6 mmol), anhydrous ethanol (20 mL), and monoethanolamine (17.65 g, 288 mmol) was refluxed for 3.5 h, then cooled to room temperature. A white crystalline precipitate was filtered off, washed with ethanol, dried in a fume hood, and immediately was used in the nitration reaction.

***N*,*N*´-Bis(3-hydroxypropyl)succindiamide (6).** The synthesis was performed similarly to that of compound **5**, except that

3-aminopropanol was used in the reaction instead of monoethanolamine. The reaction product was filtered off, dried in air, and subjected to nitration reaction.

***N,N′*-Bis(2-hydroxyethyl)oxysuccindiamide (7).** Malic acid (6.7 g, 0.05 mol) was added to ethanol (75 mL) at 0—50 °C with stirring, followed by addition of concentrated H_2SO_4 (4.5 mL). After mixing the reagents, the reaction mixture was heated to 800 °C and allowed to stand at this temperature with stirring for another 2 h. The azeotropic mixture of ethanol—water (15 mL) was distilled off *in vacuo*, followed by addition of ethanol (15 mL) into the reaction flask and heating for another 1 h. Then, the reaction mixture was cooled to room temperature and poured into a mixrure ice—water (120 mL), neutralized with K_2CO_3, and extracted with dichloroethane (DCE) (2×70 mL). The combined extracts were dried with $MgSO_4$, DCE was evaporated *in vacuo* using a water-aspirator pump. Malic acid diethyl ester (5.94 g) was obtained in the residue as an oil, to which monoethanolamine (2.5 g) was added. After mixing the reagents, the reaction mixture was heated to 85—90 °C and allowed to stand at this temperature for 1 h. The reaction mixture crystallized during standing. Diethyl ether (20 mL) was added into the flask, the crystalline substance formed was triturated, colorless crystalline precipitate was filtered off. The yield of product **7** was 4.08 g (91% calculated on ethanolamine), m.p. 119.5—121.0 °C. IR, ν/cm^{-1}: 683.6 w, 691.5 w, 758.2 m, 811.1 w, 829.4 w, 854 w, 829.3 m, 854.1 m, 896 m, 962.2 w, 1051.3 s, 1087.5 s, 1094 s, 11.86 s, 12.18 m, 12.87 m, 1323 m,13.51 m, 1379 w, 14.42 m, 15.47 s, 17.35 m, 2033 w, 2886 w, 2941 w, 2972.6 w, 3003 w, 313913 s, 3652.4 w, 3759 w, 3781.4 w. ^{1}H NMR (DMSO-d_6, $SiMe_4$), δ: 2.30 (s, 4 H, $CH_2C(O)$); 3.10 (dt, 4 H, NH$\underline{CH_2}$, $^3J_{CH,CH} \approx {}^3J_{CH,NH} \approx 5.7$ Hz); 3.37 (dt, 4 H, $\underline{CH_2}OH$, $^3J_{CH,CH} \approx 5.7$ Hz, $^3J_{CH,OH} \approx 5.0$ Hz); 4.61 (t, 2 H, OH, $^3J_{OH,CH} \approx 5.0$ Hz); 7.79 (br.t, 2 H, NH, $^3J_{NH,CH} \approx 5.7$ Hz).

***N,N′*-Bis(2-nitroxyethyl)succindiamide (8).** *N,N′*-(2-Hydroxyethyl)succindiamide **5** (7 g, 34.27 mmol) was added to a mixture of conc. H_2SO_4 (28 mL) and conc. HNO_3 (14 mL) at 0—5 °C, then, the reaction mixture was allowed to stand for another 6 h at 0—5 °C and poured onto crushed ice (250 mL), neutralized with potassium hydroxide, and extracted with ethyl acetate (5×25 mL). The extracts were combined and dried with $MgSO_4$. Compound **8** (6.82 g, 68.1%) was obtained after evaporation of ethyl acetate at reduced pressure, m.p. 100—101.5 °C (chloroform). Found (%): C, 32.50; H, 4.82; N, 19.16. $C_8H_{16}N_4O_8$. Calculated (%): C, 32.65; H, 4.76; N, 19.04.

***N,N′*-Bis(3-nitroxypropyl)succindiamide (9).** Succinic acid (5.9 g, 0.05 mol) was added to a solution of conc. H_2SO_4 (4.5 mL) in ethanol (75 mL) at 0—50 °C with stirring. The reaction mixture was heated to 80 °C and allowed to stand at this temperature for 3 h. The azeotropic mixture (ethanol—water, 15 mL) was distilled off *in vacuo* using a water-aspirator pump, followed by addition of ethanol (15 mL) to the mixture and heating for another 1 h. Then, the content of the flask was poured into a mixrure ice—water (120 mL), neutralized with dry Na_2CO_3 to pH 8—9, and extracted with DCE (2×75 mL). The combined extracts were dried with $MgSO_4$, DCE was separated, and the solvent was evaporated *in vacuo* using a water-aspirator pump to obtain an oily precipitate (6 g), which without purification was used in the subsequent reaction. Namely, 3-aminopropanol (3 g) was added to this oily precipitate, the reaction mixture was heated to 90 °C and allowed to stand at this temperature for 2 h with stirring. The reaction mixture obtained was evacuated using an oil pump to obtain compound **6** (6.7 g) as a dense oil. ^{1}H NMR (DMSO-d_6, $SiMe_4$), δ: 1.53 (q, 4 H, CH_2, $^3J_{CH,CH} = 6.5$ Hz); 2.31 (s, 4 H, $CH_2C(O)$); 3.11 (dt, 4 H, NH$\underline{CH_2}$, $^3J_{CH,CH} \approx {}^3J_{CH,NH} \approx 5.7$ Hz); 3.40 (dt, 4 H, $\underline{CH_2}OH$, $^3J_{CH,CH} \approx 5.7$, $^3J_{CH,OH} \approx 5.0$ Hz); 4.43 (t, 2 H, OH, $^3J_{OH,CH} \approx 5.0$ Hz); 7.96 (br.t, 2 H, NH, $^3J_{NH,CH} \approx 5.7$ Hz). Then, this oil was treated with a mixture of conc. H_2SO_4 (24 mL) and conc. HNO_3 (8 mL) and allowed to stand at 50 °C with stirring for 3.5 h. The content of the flask was poured into a mixrure ice—water (200 mL), neutralized with KOH to pH 7—7.5, and extracted with ethyl acetate (3×50 mL). The combined extracts were dried with $MgSO_4$. Compound **9** (4.75 g) was obtained after evaporation of ethyl acetate at reduced pressure, m.p. and ^{1}H NMR spectrum are given in Table 2.

***N,N′*-Bis(2-nitroxyethyl)oxysuccindiamide (10).** *N,N′*-Bis(2-hydroxyethyl)oxysuccindiamide (**7**) (4.2 g, 19.07 mmol) was added to 75% aqueous HNO_3 (21 mL) at 18—20 °C. After mixing the reagents, the reaction mixture was stirred for 1.5—2 h at this temperature and poured into a mixrure ice—water, neutralized with $NaHCO_3$, and extracted with ethyl acetate (5×15 mL). The extracts were combined and dried with $MgSO_4$. Compound **10** (3.3 g, 55.9%) was obtained after usual treatment, m.p. 90—91 °C (from DCE). Found (%): C, 30.84; H, 5.14; N, 18.11. $C_8H_{14}N_4O_9$. Calculated (%): C, 30.97; H, 5.42; N, 18.06. ^{1}H NMR spectrum is given in Table 2.

***N,N′*-Bis(2-nitroxyethyl)nitroxysuccindiamide (11).** *N,N′*-Bis(2-hydroxyethyl)oxysuccindiamide **7** (5 g, 23.78 mmol) was added in portions to HNO_3 (20 mL, $d = 1.51$) at room temperature with stirring. After mixing the reagents, the reaction mixture was stirred for 3 h, then poured onto crushed ice. A colorless precipitate formed was filtered off, washed with ice-cold water, and dried in air to obtain nitrate **11** (5.24 g, 62%), m.p. 108—109 °C. Found (%): C, 30.48; H, 3.92; N, 18.11. $C_8H_{13}N_5O_{11}$. Calculated (%): C, 30.05; H, 3.69; N, 19.71. ^{1}H NMR spectrum is given in Table 2.

***N*-Nitroxymethylsuccinimide (19).** *N*-Hydroxymethylsuccinimide[11] (1 g, 7.74 mmol) was added to a mixture of Ac_2O (1.1 mL) and HNO_3 (0.5 mL, $d = 1.5$) at 10—15 °C with stirring. The reaction mixture was allowed to stand at 15 °C for 2 h with stirring, then poured onto crushed ice. A colorless precipitate formed was filtered off and dried in air to obtain colorless crystals (1.05 g) with m.p. 111.5—112.5 °C. Found (%): C, 34.06; H, 3.15; N, 15.84. $C_5H_6N_2O_5$. Calculated (%): C, 34.5; H, 3.47; N, 16.09. ^{1}H NMR spectrum is given in Table 2.

***N*-(2-Nitroxyethyl)succinimide (20).** *N*-(2-Hydroxyethyl)-succinimide **17** (6 g, 41.92 mmol) was added to a solution of HNO_3 (15 mL) in CH_2Cl_2 (55 mL) at 15—20 °C with stirring. After mixing the reagents, the reaction mixture was stirred for 2 h, then poured onto crushed ice, and neutralized with K_2CO_3 to pH = 7.2—7.0. The solution obtained was extracted with ethyl acetate (3×15 mL). The extracts were combined and dried with $MgSO_4$. Compound **20** (6.8 g, 86%) was obtained after evaporation of the solvent *in vacuo*, white crystals, m.p. 61.5—62.5 °C (DCE). ^{1}H NMR spectrum is given in Table 2.

***N*-(3-Nitroxypropyl)succinimide (21).** *N*-(3-Hydroxypropyl)-succinimide (**18**) (1 g, 6.21 mmol) was added to a mixture of HNO_3 (2 mL) and H_2SO_4 (6 mL) at −5—0 °C with stirring and the reaction mixture was stirred for 5 h at 0 °C, then neutralized with K_2CO_3, and extracted with ethyl acetate (3×15 mL). The combined extracts were dried with $MgSO_4$. The usual treatment gave *N*-(3-nitroxypropyl)succinimide **21** (0.91 g, 70.8%) with

Table 2. ^{1}H NMR spectra of substituted nitroxyalkylamides and imides of succinic and citric acids

Compound	Solvent	δ (*J*/Hz)
8	CD_3CN	2.41 (s, 4 H, $CH_2C(O)$); 3.50 (q, 4 H, CH_2N, $^3J \approx 6.0$); 4.52 (t, 4 H, CH_2ONO_2, $^3J \approx 6.0$); 6.80 (br.s, 2 H, NH)
9	CD_3CN	1.84 (quint, 4 H, CH_2, $^3J = 6.4$); 2.36 (s, 4 H, $CH_2C(O)$); 3.19 (q, 4 H, CH_2N, $^3J = 6.4$); 4.47 (t, 4 H, CH_2ONO_2, $^3J = 6.4$); 6.67 (br.s, 2 H, NH)
10	DMSO-d_6	2.36 (2 H, CH_2, AB-part of ABX-spectrum, $\Delta\nu = 70.3$, $\lvert^2J\rvert = 14.4$, $^3J_{AX} = 3$; $^3J_{BX} = 9.5$); 3.30—3.50 (m, 4 H, CH_2N, $^3J \approx 5.2$); 4.25 (1 H, CH, AB-part of ABX-spectrum); 4.50 (br.s, 1 H, OH); 4.52 (4 H, CH_2ONO_2,$^3J = 5.2$); 8.12 (br.t, 1 H, NH, ^3J =5.2); 8.18 (br.t, 1 H, NH, $^3J = 5.2$)
11	CD_3CN	2.78 (2 H, CH_2, AB-part of ABX-spectrum, $\Delta\nu = 27.3$, $\lvert^2J_{gem}\rvert = 16.2$, $^3J_{AX}$ =4.7; $^3J_{BX}$ =7.6); 3.45—3.62 (m, 4 H, CH_2, $^3J \approx 4.6$); 4.50 (t, 2 H, CH_2ONO_2, $^3J = 4.6$); 4.53 (t, 2 H, CH_2ONO_2, $^3J = 4.6$); 5.60 (H, CH, X-part of ABX-spectrum); 6.95 (br.s, H, NH); 7.34 (br.s, H, NH)
19	CD_3CN	2.70 (s, 4 H, CH_2); 5.78 (s, 2 H, CH_2ONO_2)
20	CD_3CN	2.64 (s, 4H, CH_2); 3.78 (t, 2 H, NCH_2, $^3J = 6.0$); 4.55 (t, 2 H, CH_2ONO_2, $^3J = 6.0$)
21	CD_3CN	1.90 (quint, 2 H, CH_2, $^3J = 5.8$, $^3J = 6.4$); 2.58 (s, 4 H, $CH_2C(O)$); 3.49 (t, 2 H, NCH_2, $^3J = 6.4$); 4.48 (t, 2 H, CH_2ONO_2, $^3J = 5.8$)
24	CD_3CN	1.05 (t, 3 H, CH_3, $^3J = 7.0$); 1.14 (t, 3 H, CH_3, $^3J = 7.0$); 2.40 (t, 2 H, CH_2CO, $^3J = 6.1$); 2.60 (t, 2 H, CH_2CO, $^3J = 6.1$); 3.30 (q, 2 H, CH_2N, $^3J = 7.0$); 3.35 (q, 4 H, CH_2N, $^3J = 7.0$); 3.46 (dt, 2 H, CH_2, $^3J = 5.2$); 4.50 (t, 2 H, CH_2ONO_2, $^3J = 5.2$); 7.03 (br.s, H, NH)
27	CD_3CN	2.37 (t, 2 H, CH_2CO, $^3J = 6.6$); 2.55 (t, 2 H, CH_2CO, $^3J = 6.6$); 2.82 (s, 3 H, CH_3); 2.96 (s, 3 H, CH_3); 3.44 (dt, 2 H, NCH_2, $^3J_{CH,CH} \approx {}^3J_{CH,NH} \approx 5.2$); 4.48 (t, 2 H, CH_2ONO_2, $^3J = 5.2$); 6.86 (br.s, H, NH)
29	DMSO-d_6	3.55 (dt, 4 H, NCH_2, $^3J_{CH,CH} \approx {}^3J_{CH,NH} \approx 5.0$); 4.58 (t, 4 H, CH_2ONO_2, $^3J = 5.0$); 6.12 (s, 2 H, $CHONO_2$); 9.00 (t, 2 H, NHC(O), $^3J = 5.0$)

m.p. 35—36 °C. Found (%): C, 41.38; H, 4.86; N, 13.91. $C_7H_{10}N_2O_5$. Calculated (%): C, 41.59; H, 4.98; N, 13.86. ^{1}H NMR spectrum is given in Table 2.

[*N*-(2-Nitroxyethyl-*N*′,*N*′-(diethyl)]succindiamide (24). A solution of diethylamine (1 g, 15.86 mmol) in CH_2Cl_2 (5 mL) was added to a solution of succinic anhydride (1 g, 9.99 mmol) in CH_2Cl_2 (10 mL) at 0—5 °C with stirring. The mixture was stirred for 30 min at room temperature and evacuated using a water-aspirator pump, thus removing residual diethylamine. Then, a solution of SO_2Cl (2 g, 6.8 mmol) in CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred for 3 h at room temperature. Then, the solution was washed with cold water and 1% aqueous Na_2CO_3 (5 mL). The organic layer was separated and dried with magnesium sulfate, the solvent was evaporated. Ethanol (10 mL) and ethanolamine (0.7 g, 11.46 mmol) were added to the residue and the mixture was refluxed with a reflux condenser for 2 h. A solid compound formed after cooling was filtered off, dried in air, and added to 75% aqueous HNO_3 (16 mL) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature and poured onto crushed ice. The product formed was filtered off and dried in air. The yield of compound **24** was 1.2 g, m.p. 85—86 °C. Found (%): C, 45.64; H, 7.13; N, 15.82. $C_{10}H_{18}N_3O_5$. Calculated (%): C, 45.97, H, 7.33; N, 16.08. ^{1}H NMR spectrum is given in Table 2.

***N*-(2-Nitroxyethyl)-*N*′,*N*′-(dimethyl)succindiamide (27).** A solution of dimethylamine (1.2 g, 26.7 mmol) in CH_2Cl_2 (10 mL) was added to a solution of succinic anhydride (1 g, 9.99 mmol) in CH_2Cl_2 (12 mL). The reaction mixture was stirred for 10 min at room temperature, excess of dimethylamine was evaporated at reduced pressure. Trifluoroacetic anhydride (2 g, 9.52 mmol) in CH_2Cl_2 (15 mL) was added to the residue of the reaction mixture, which was stirred for 5 h at room temperature, followed by addition of a solution of nitroxyethylammonium nitrate (1.69 g, 10 mmol) in water (5 mL) and stirring for 1 min at room temperature. Then, triethylamine (1.02 g, 10.1 mmol) was added, the mixture was stirred for 15 min, the upper layer was decanted, the aqueous layer was extracted with ethyl acetate (2×15 mL). The extracts were combined and dried with $MgSO_4$. Ethyl acetate was evaporated *in vacuo*. The yield of compound **27** was 1.4 g, white crystals, m.p. 94—95 °C. Found (%): C, 40.94; H, 6.1; N, 18.44. $C_8H_{15}N_3O_5$. Calculated (%): C, 41.20; H, 6.43; N, 18.02. ^{1}H NMR spectrum is given in Table 2.

***N*,*N*′,*N*′′-Tris(2-nitroxyethyl)citratetriamide (29).** Concentrated H_2SO_4 (6 mL) was added to ethanol (100 mL) at 0—5 °C with stirring, then citric acid (9 g, 53.3 mmol) was added to the solution obtained. The content of the flask was heated to 80 °C and allowed to stand at this temperature for 3 h. Then, the azeotropic mixture of ethanol—water (20 mL) was distilled off *in vacuo* using a water-aspirator pump, whereas a fresh portion of ethanol (20 mL) was added into the flask, which was heated for another 1 h. Ethanol (90 mL) was evaporated *in vacuo*. The residue was poured into a mixrure ice—water (120 mL) and neutralized with dry Na_2CO_3 to pH 8—9. The mixture obtained was placed in a separatory funnel and extracted with ethyl acetate (2×70 mL). The extracts were combined, ethyl acetate was evaporated *in vacuo* using a water-aspirator pump. The residual crystalline product was added to ethanolamine (3.4 g) without purification and identification. Temperature was elevated to 80 °C and the mixture was allowed to stand for 4.5 h, during which it completely solidified. After addition of diethyl ether to the crystal-

line substance, it was triturated until homogeneity. The precipitate was filtered off from the ether, washed with diethyl ether (15 mL). The *N*,*N*´,*N*´´-tris(2-hydroxyethyl)citratetriamide (**28**) obtained was not purified and identified, but immediately involved in the nitration reaction by treatment with 80% aq. HNO_3 (32 mL). After mixing the reagents, the reaction mixture was stirred for 4.5 h at room temperature, then poured onto crushed ice (400 mL). A precipitate formed was filtered off, washed with ice-cold water, and dried in air to obtain *N*,*N*´,*N*´´-tris(2-nitroxyethyl)citratetriamide (**29**) (5.6 g, 23.5 % calculated on citric acid), colorless crystals, m.p. 92—93 °C. Found (%): C, 35.2; H, 4.52; N, 19.94. $C_{12}H_{20}N_6O_{13}$. Calculated (%): C, 35.64; H, 4.95; N, 20.79. ^{1}H NMR spectrum is given in Table 2.

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Received August 27, 2013;
in revised form January 23, 2014