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Synthesis of Hydroxylamine Analogues of Polyamines

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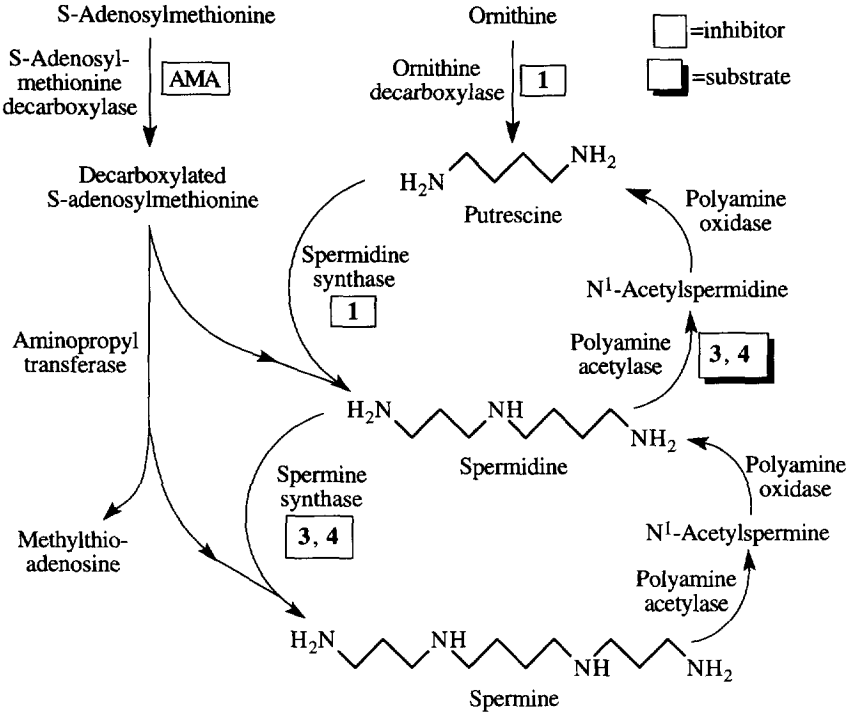
Abstract: Novel analogues of spermine and spermidine with terminal H_2N-CH_2-group substituted by H_2N-O-group, were prepared starting the synthesis from EtO(Me)C=NOH and subsequent extension of a polyamine backbone. To prepare their earlier unknown tritium labelled analoques, ω-[[(1'-ethoxy-ethylidene)amino]oxy]-poly-(iminomethylene) nitriles were reduced to amines by $NaBT_4/CoCl_2$ complex, which did not effect the N-O or C=N bonds of ethoxyethylidene group, whereas aminooxy group deprotection was performed at the final step of synthesis by mild acidic hydrolysis. Novel monoacetylated (AcHN- or AcNHO-) analoques of spermidine were also synthesised.

Polyamines - spermine (Spm), spermidine (Spd) and putrescine (Put), are essential factors of cell growth and differentiation. They are important components of the architecture of nucleic acids and proteins. Their role can not be explained only in terms of organic polycations that are needed in an amounts depending on the requirements of cellular metabolism. Polyamines participate in the regulation of the activities of several enzymes. Inhibitors of polyamine biosynthesis together with polyamine analogues are of considerable interest as anticancer and, recently, as antimalarial agents.[1,2]

Biosynthesis of polyamines *(Scheme 1)* is started from enzymatic decarboxylation of ornithine (Orn) and S-adenosylmethionine (AdoMet). The former enzyme is pyridoxal-5'-phosphate (PLP) dependent and the latter has a protein-bound pyruvate residue. This was the reason to design product like 3-[(amino)oxy]-1-amino-propane (**1**, APA) and [2-[(amino)oxy]ethyl](5'-deoxyadenos-5'-yl)(methyl)sulphonium (AMA) for specific inhibition of Orn decarboxylase (ODC) and AdoMet decarboxylase, respectively. At low concentrations these two O-substituted hydroxylamines irreversibly inhibited the cognate enzymes and appeared to be useful tools in investigation of the cellular functions of polyamines[3], as well as the peculiarities of their biosynthesis and transport[4].



Scheme 1. Metabolism of polyamines and its inbition by aminooxy compounds.

The transfer of aminopropyl group from decarboxylated AdoMet to Put and Spd, catalysed by the corresponding aminopropyl transferases, leads to Spd and Spm. Acetylation of Spm and Spd are the key steps of polyamine catabolism *(Scheme* 1). Aminopropyl transferases and polyamine acetylases, unlike ODC and AdoMetDC, do not contain catalytically important carbonyl group.

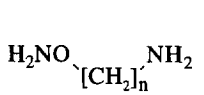
Among the large family of polyamine analogues, there are currently no derivatives with the polyamine backbone and having amino group(s) with predominantly modified properties. O-alkyl hydroxylamines may be considered as isosteric analogues of alkylamines and their pK_a values (about 5) are much lower than those of the amino groups of natural polyamines. Hence, isosteric hydroxylamine analogues of polyamines with H_2NO-group instead of terminal H_2NCH_2-group, seem to be a rational choice to study the role(s) of the cationic terminal amine group(s) of polyamines.

Thus, APA, a specific carbonyl reagent developed for ODC, is also an isosteric analogue of Put. APA was found to be a competitive inhibitor of Spd aminopropyl transferase, IC_{50} 2 μM[5]. The structural similarity of APA and Put was a likely reason also for the inhibition of melanin biosynthesis in *Pyricularia oryzae* by **1**[6].

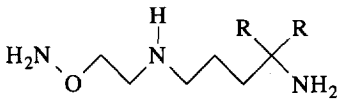
Isosteric aminooxy analogues of Spd, **3** and **4** (synthesis shortly reported in preliminary paper[7]), turned to be effective competitive inhibitors of Spd aminopropyl transferase. The affinity of **3** and **4** to the enzyme was determined by the position of the aminooxy group in the analogue. Hence, the affinity of **4** was 10 times better than that of Spd, while **3** had 10 times worse affinity as compared to Spd.[8]

In the present paper we describe the synthesis of hydroxylamine analogues of the substrates and the products of the enzymes of polyamine biosynthesis and catabolism. This group of compounds covers novel

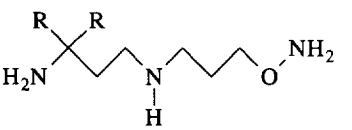
aminooxy analogues of Spm, **9**, **10** and **11**; aminooxy analogues of Spd, **3** and **4** (each of them is prepared by two independent methods); and the new ^{3}H-labelled derivatives **3*** and **4***. Syntheses of earlier unknown monoacetylated derivatives **5-8** are also reported.



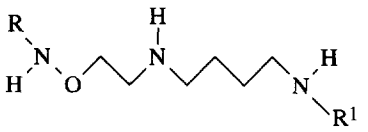
1 n=3, **2** n=2



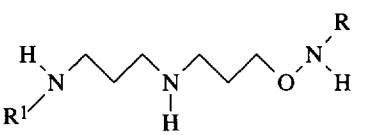
3 R=H, **3*** R=^{3}H



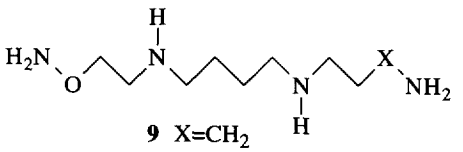
4 R=H, **4*** R=^{3}H



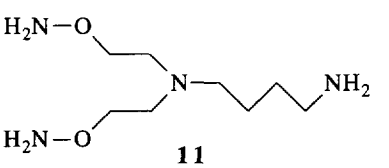
5 R=Ac, R^1=H, **6** R=H, R^1=Ac



7 R=Ac, R^1=H, **8** R=H, R^1=Ac



9 X=CH_2
10 X=O



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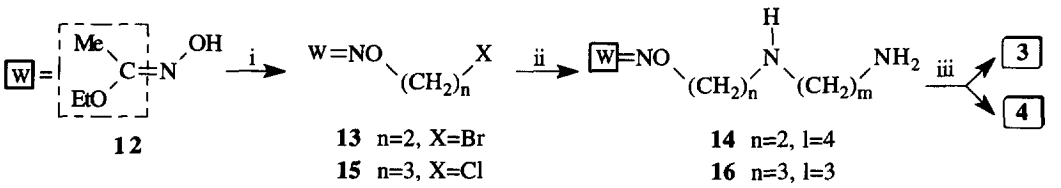
RESULTS AND DISCUSSION

The peculiarities of polyamine structure make their modification rather complicate. One of the strategies to synthesise polyamine analogues is based on the transformation of nitrile or amide group of the appropriate precursor into H_2N- or HN-group(s). Another approach is based on the alkylation of the corresponding amine. A set of Put and Spd derivatives with different N-protecting groups at each nitrogen atom were developed to simplify modification.[9]

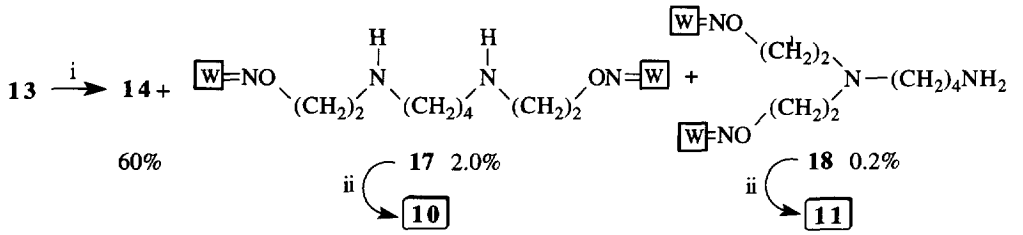
General strategy of the syntheses of target hydroxylamine analogues **3-11** consisted the introduction of the protected aminooxy group into a starting synthon and subsequent extension of the poly-iminomethylene chain (see *Schemes* 2 and 6). Among the known aminooxy precursors, ethyl N-hydroxyacetimidate (**12**) was selected because it is easily alkylated and the alkoxy fragment of the resulting esters may undergo different types of transformations, while C=N and N-O bonds remain unaffected.[10,11] Furthermore, the aminooxy group may be deblocked under mild conditions. The original protocol[11] requires treatment with equivalent amounts of strong acid and water within few minutes at room temperature and does not require heating in 2 M HCl[12]. As a result, the ethoxyethylidene derivatives are converted into target O-substituted hydroxylamines with an almost quantitative yield.[10,11]

The iminomethylene backbones of **3** and **4** were started to build up by alkylating **12** with 1,2-dibromoethane or 1-bromo-3-chloropropane to give the key halides **13** and **15** (*Scheme* 2). Alkylation of an excess of 1,4-diaminobutane with **13** in 2-propanol afforded **14**, the required precursor of **3**, in 60% isolated yield.



Scheme 2. i) $Br(CH_2)_nX$; ii) $H_2N(CH_2)_mNH_2$; iii) HCl/H_2O

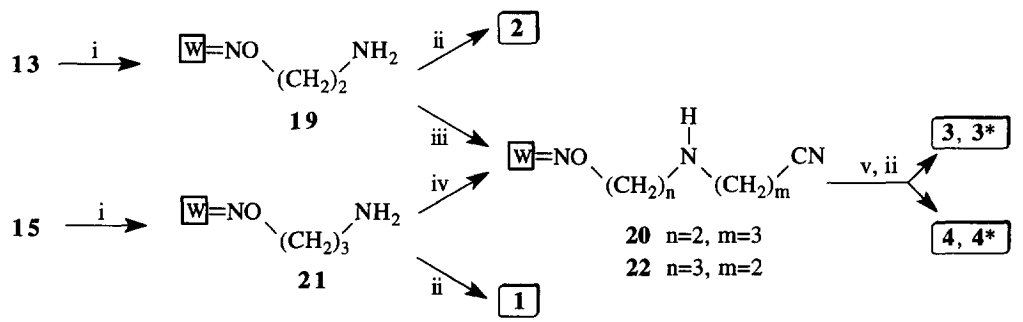
NMR analysis of a small higher-boiling fraction proved it to be a mixture of **17** and **18** (8:2), which were separated using flash-chromatography and affording pure **17** and **18** (*Scheme* 3). Mild acidic hydrolysis of **14**, **17** and **18** gave the expected products **3**, **10** and **11** with high yields. Another possibility to separate **17** and **18** is based on the ability of **17** to form alcohol-insoluble complexes with $CoCl_2$. Subsequent acidic hydrolysis and chromatography on Dowex 50X8 resin in H^+-form afforded pure **10** and **11** (data not shown).



Scheme 3. i) $H_2N(CH_2)_4NH_2$; ii) HCl/H_2O

The same approach was also used to synthesise **16**, starting from 1,3-diaminopropane and **15**, but in this case the alkylation was less successful and clearly slower than the above mentioned aminooxyethylation of putrescine and resulted in only 29% isolated yield. Obviously, alkylation of trimethylenediamine must give rise to several other products similar to those described above, but detailed analysis of the reaction mixture was not performed. Mild acidic hydrolysis of **16** produced the required **4** in 97% yield.

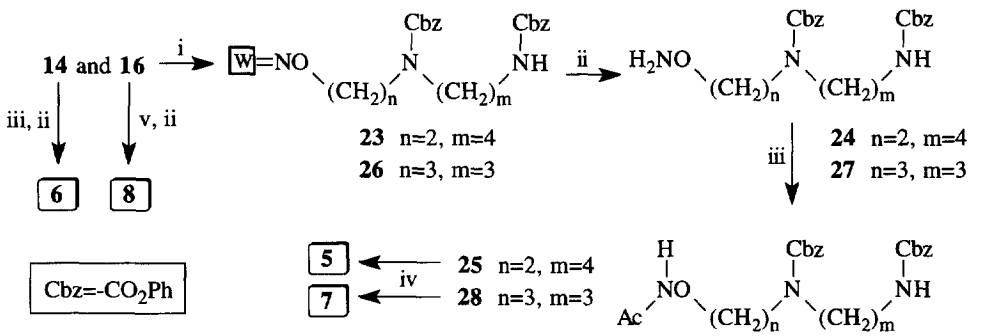
It was established that ω-{[(1'-ethoxyethylidene)amino]oxy}-poly-(iminomethylene) nitriles could be reduced to amines using $NaBH_4$-$CoCl_2$ complex as reducing reagent, while N-O and C=N bonds of ethoxyethylidene aminooxy group were not affected. The reduction of $EtO(Me)C{=}NO(CH_2)_2CN$ at +2...+5°C resulted in **1** with the yield of 55-65%. The increase of the reaction temperature to 25...27°C halved the yield.



Scheme 4. i) NH_3/liq.; ii) HCl/H_2O; iii) $Cl(CH_2)_3CN$; iv) $H_2C{=}CH{-}CN$; v) $NaBH_4(NaBT_4)/CoCl_2$

The precursors to ^{3}H-labelled aminooxy spermidine analogues **3*** and **4*** were the nitriles **20** and **22**, respectively (*Scheme* 4). To prepare nitrile **22**, synthesis was started from chloride **15** by amination with liquid ammonia at 85°C and under 60 atm (steel bomb) followed by cyanoethylation. The same procedure was used to prepare **20** starting from bromide **13** *via* amine **19**. The last compound was alkylated with 4-chlorobutyronitrile in boiling 2-propanol in the presence of K_2CO_3 to give the required nitrile **20**. Both **20** and **22** were selectively reduced to the corresponding amines by treating with $NaBH_4$ ($NaBT_4$) in the presence of $CoCl_2$ at +2...+5°C. ^{3}H-Labelled **3*** and **4***, as well as **3** and **4**, were isolated with good yields by ion-exchange chromatography after deprotection of aminooxy group.[13]

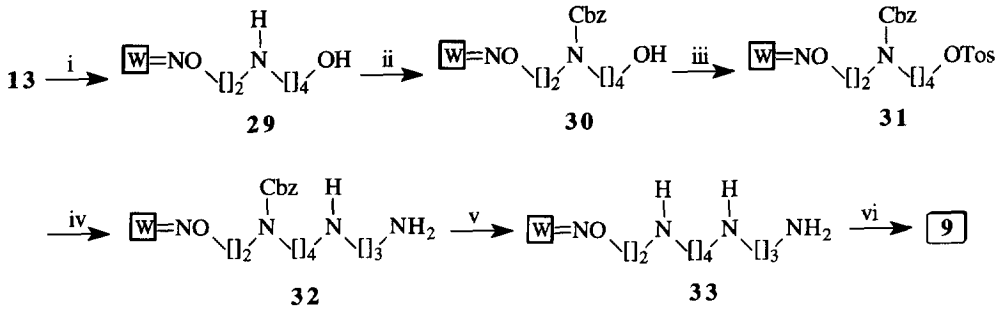
Monoacetylated derivatives **5**-**8** (either AcHNO- or -NHAc derivatives), which are the products of enzymatic acetylation of **3** and **4**[14], were synthesised (*Scheme* 5). Acetyl chloride treatment of an excess of **14** and subsequent aminooxy group deprotection resulted in **6**, which was purified by ion-exchange chromatography. An attempt to prepare **8** using the same procedure was unsuccessful and resulted mostly in bisacetylated derivative of **4** and only a small amount of the required **8**. However, **8** was obtained in a yield of about 60%, when small excess of **16** in THF was treated with acetic acid N-hydroxysuccinimide ester (Ac-NOSu) at +4°C followed by acidic hydrolysis and purification.



Scheme 5. i) CbzCl; ii) HCl/H_2O; iii) AcCl; iv) Pd/H_2; v) Ac-NOSu

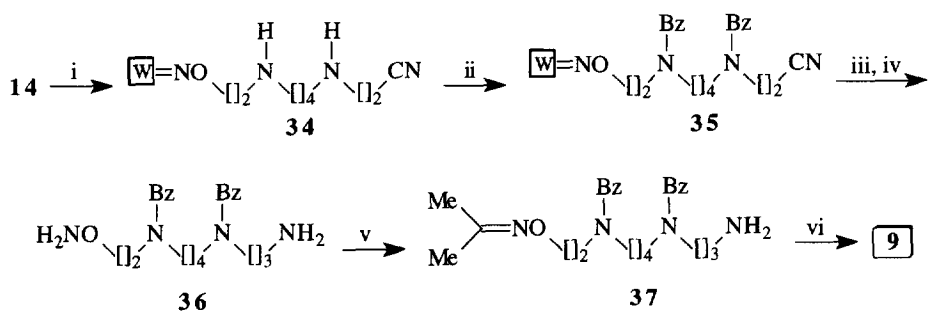
To prepare monoacetylated derivatives **5** and **7**, amino and imino groups of **14** and **16** were first protected with benzyloxycarbonyl group, giving corresponding bis-Cbz-derivatives **23** and **26**, respectively. The aminooxy groups were then selectively deprotected with equimolar amounts of HCl and water to give the corresponding O-substituted hydroxylamines **24** and **27**. The formed free H_2NO-groups were first acetylated and then the benzyloxycarbonyl groups were removed to give the required monoacetylated **5** and **6**, respectively.

To prepare **9** an excess of 4-amino-1-butanol was first alkylated with **13** in boiling 2-propanol in the presence of K_2CO_3, yielding **29** (*Scheme* 6). Subsequent carbobenzoxylation of imine nitrogen of **29** gave **30**, which was converted into p-toluenesulfonate **31** (intermediates **30** and **31** were purified by flash-chromatography). Treatment of **31** with an excess of 1,3-diaminopropane gave **32**. Stepwise deprotection (catalytic hydrogenation and mild acidic hydrolysis) afforded **9** with the overall yield of 14% calculated from **13**.



Scheme 6. i) $H_2N(CH_2)_4OH/K_2CO_3/(CH_3)_2CHOH$); ii) CbzCl; iii) TosCl; iv) $H_2N(CH_2)_3NH_2$; v) Pd/H_2; vi) HCl/H_2O.

An alternative route to prepare **9** is based on reduction of corresponding nitrile **34**, prepared from **14** by cyanoethylation, with $NaBH_4/CoCl_2$ complex (*Scheme* 7). However, the reduction of **34** according to the protocol used in the case of nitriles **20** and **22**, turned out to be unsuccessful, because **34** formed alcohol-insoluble complex with $CoCl_2$. Benzoylation of both imino groups gave compound **35**, which was easily reduced by $NaBH_4/CoCl_2$ complex. Subsequent deprotection of aminooxy group resulted in crude **36**, which was purified by flash-chromatography in the form of its acetone oxime **37**. Subsequent acidic hydrolysis of **37** and purification by ion-exchange chromatography afforded pure **9** with on overall yield of 4%, calculated from **34**.



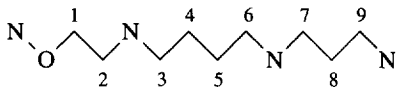
Scheme 7. i) $H_2C{=}CH{-}CN$; ii) BzCl; iii) $NaBH_4/CoCl_2$; iv) Dowex-50-H^+; v) Me_2CO; vi) HCl/H_2O.

The structure of the compounds were identified using ^1H and ^{13}C NMR spectroscopy and accurate mass measurements. The ^1H NMR spectra for the molecules containing a protected aminooxy group showed a triplet at ca. 4 ppm (=CNOC<u>H</u>$_2$-) and for the unprotected form at ca. 4.2 ppm (H_2NOC<u>H</u>$_2$-), which is about 0.5-0.7 ppm higher than the corresponding shift for the primary alcohols. The rest of the proton chemical shift were assigned using 2D ^1H-^1H COSY experiment for the products **3-11**. Because of the solvents, $CDCl_3$ and D_2O, exchangeable protons were not observed. However, to detect these protons the dihydrochloride of **2** was dissolved in dry DMSO-d_6 and as expected, both aminooxy and amine protons were detected at 11.12 and 8.18 ppm as broad singlets (other protons 4.13 t, 2.86 m 1.93 m).

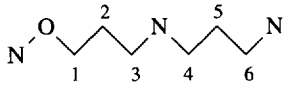
In the ^{13}C NMR spectra the downfield shift induced by the neighbouring aminooxy moiety was even more characteristic being ca. 10 ppm compared to primary alcohols (see Table 1 and 2). The compounds **3-11**

containing several similar carbons next to the nitrogen were assigned combining the information from 2D COSY and ^{1}H-^{13}C correlated NMR experiments. Compound **9**, in which signals for $H_2NO\underline{C}H_2$- (72.83 ppm) and $\underline{C}H_2NH_2$ (39.5 ppm) were easily assigned, contained also four signals at 50.1-47.5 ppm (carbons bound a secondary nitrogen) and three signals at 26 ppm were more difficult to assign. C-2 at 48.28 ppm and C-8 at 26.66 ppm were directly obtained from the ^{1}H-^{13}C correlated spetrum and the rest of the signals were assigned from a long-range ^{1}H-^{13}C correlated (COLOC) experiment based either on $^{3}J_{CH}$ coupling over nitrogen (C-3 and C-6) or $^{2}J_{CH}$ coupling (C-7, C-4 and C-5).

Table 1. ^{13}C NMR chemical shifts for compounds **1-11**.



[Amino(oxy)]ethyl derivatives



[Amino(oxy)]propyl derivatives

Comp.	1	2	3	4	5	6	7	8	9	C=O	CH_3
1	75.47	28.21	39.67	-	-	-	-	-	-	-	-
2	73.80	40.58	-	-	-	-	-	-	-	-	-
3	72.82	50.06	48.29	25.48	26.78	41.74	-	-	-	-	-
4	75.17	27.20	47.73	47.59	26.60	39.52	-	-	-	-	-
5	74.22	48.31	49.83	25.60	26.73	41.71	-	-	-	174.77	21.50
6	78.02	27.04	49.24	47.56	26.59	39.52	-	-	-	173.89	21.36
7	72.86	50.42	48.13	25.74	28.37	41.49	-	-	-	177.11	24.84
8	75.19	28.35	48.13	47.50	27.15	39.01	-	-	-	177.57	24.69
9	72.83	48.28	50.05	25.55	25.67	49.93	47.48	26.66	39.52	-	-
10	69.34	44.70	46.55	22.01	22.01	46.55	44.70	69.34	-	-	-
11	71.44	54.76	56.56	26.79	23.21	41.74	-	-	-	-	-

Table 2. ^{13}C NMR chemical shifts for selected precursors to compounds **1-11**.

Comp.	1	2	3	4	5	6	CH_3	$-CH_2O$	CH_3C	C=N
13	72.88	30.18	-	-	-	-	14.37	62.32	13.78	163.32
14	72.86	48.93	49.78	31.68'	27.60'	42.22	14.39	62.17	13.61	162.33
15	69.95	32.17	41.99	-	-	-	14.39	62.20	13.60	162.43
16	71.97	29.56	47.37	47.95	34.10	40.65	14.41	62.12	13.62	162.18
17	72.72	48.83	49.71	27.95	-	-	14.37	62.10	13.54	162.18
18	72.04	53.07	55.31	30.89	24.98	41.78	14.40	62.11	13.68	162.15
19	75.77	41.46	-	-	-	-	14.39	62.19	13.62	162.41
20	72.66	48.62	47.84	25.88	14.87	119.78	14.39	62.21	13.65	162.47
21	71.37	33.05	39.48	-	-	-	14.40	62.13	13.61	162.17
22	71.63	29.40	46.45	45.22	18.73	118.74	14.39	62.10	13.61	162.22
29	72.07	48.52	49.44	28.63	32.44	62.54	14.38	62.20	13.63	162.49

EXPERIMENTAL

General. - Melting points were determined in open capillary tubes and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker AM 400 WB or AMX 400 instrument operating at 100.6 MHz and 400.1 MHz, respectively. The sample solutions were prepared in 5 mm tubes using TMS ($CDCl_3$) or TSP (D_2O) as internal standard. Spectra were acquired using 32 kW data points with resolution enhancement and zero filling to point resolution better than 0.1 Hz. The accurate mass measurements were recorded on a VG 70-250SE mass spectrometer using a direct insertion probe, or on MSBX (Ukraine) using plasm-desorbtion. The progress of reactions and chromatographic separations were controlled by TLC on Silufol UV_{254} (Kavalier, Czechoslovakia) silica gel plates. Systems for TLC: 2-propanol-25% NH_4OH-H_2O, 7:1:2 (A); $CHCl_3$-MeOH, 9:1 (B); $CHCl_3$-MeOH, 9.5:0.5 (C); 1-butanol-AcOH-pyridine-H_2O, 4:2:1:2 (D); $CHCl_3$ (E); all ratio are in v/v. Flash chromatography was performed with Chemapol (Czechoslovakia) silica gel (40-100 μ), systems are indicated in the text. All solvents were purified in accordance with usual procedures. Acetic acid N-hydroxysuccinimide ester was from "Sigma" and ethyl N-hydroxyacetimidate was prepared as previously descriped.[11]

2-[[(1'-Ethoxyethylidene)amino]oxy]-1-bromoethane (**13**). - To a mixture of ethyl N-hydroxyacetimidate (103.1 g, 1.0 mol) and 1,2-dibromoethane (275 mL, 3.2 mol) in water (150 mL) was added 10 M NaOH (150 mL) at 95°C with stirring over 1.5 h. Stirring and heating were continued until the pH of the mixture was 7-8. On cooling to ambient temperature organic layer was separated and water was extracted with $CHCl_3$ (2x75 mL), combined organic layers were dried ($MgSO_4$) and after third distillation gave **13** (59 g, 28%): bp 76-76.5°C/10 mmHg; n_D^{20} 1.4631. NMR ($CDCl_3$): δ_H 4.16 (2H, t, J=6.3), 4.00 (2H, q, J=7.1), 3.54 (2H, t, J=6.3), 1.96 (3H, s), 1.27 (3H, t, J=7.1). Calcd for $C_6H_{12}BrNO_2$: C 34.30; H 5.76; N 6.67%, $M^{+\bullet}$ 209.00518. Found: C 34.28; H 5.73; N 6.44%, $M^{+\bullet}$ 209.00514.

3-[[(1'-Ethoxyethylidene)amino]oxy]-1-chloropropane (**15**). - To a solution of sodium ethylate [from sodium (4.6 g, 0.2 mol) and abs. ethanol (100 mL)], ethyl N-hydroxyacetimidate (20.6 g, 0.2 mol) followed by 1-bromo-3-chloropropane (157 g, 1.0 mol) were added with stirring and the mixture was kept overnight at ambient temperature. Solvent was evaporated, the residue was pooled in water, organic layer was separated and water was extracted with $CHCl_3$ (3x50 mL). Combined organic fractions were dried ($MgSO_4$) and distiled twice to give **15** (18 g, 50%): bp 84-86°C/13 mmHg; n_D^{20} 1.4448. NMR ($CDCl_3$): δ_H 4.03 (2H, t, J=5.9), 3.99 (2H, q, J=7.1), 3.63 (2H, t, J=6.6), 2.10 (2H, m), 1.92 (3H, s), 1.27 (3H, t). Calcd for $C_7H_{14}ClNO_2$: $M^{+\bullet}$ 179.07130. Found: $M^{+\bullet}$ 179.07131.

2-[[(1'-Ethoxyethylidene)amino]oxy]-1-aminoethane (**19**). - A solution of **13** (31.0 g, 0.148 mol) in dry ethanol (25 mL) and liquid NH_3 (350 mL) was heated in steel bomb at 95°C (pressure about 60 atm) for 16 h. On cooling and removal of NH_3, KOH (9.8 g, 0.15 mol) in MeOH (75 mL) was added to the residue, precipitated crystals were filtered off and solvents from the filtrate were evaporated. The residue was pooled into 5.0 M NaCl (25 mL) and extracted with ether (4x30 mL). Ether extracts were dried (KOH) and distiled to give **19** (11.45 g, 52%): bp 75.5-76°C/13 mmHg; n_D^{20} 1.4445; R_f 0.70 (A). NMR ($CDCl_3$): δ_H 4.00 (2H, q, J=7.1), 3.92 (2H, m), 2.93 (2H, m), 1.95 (3H, s), 1.27 (3H, t, J=7.1). Calcd for $C_6H_{15}N_2O_2$: $[M+H]^+$ 147.11334. Found: $[M+H]^+$ 147.11335.

2-[(Amino)oxy]-1-aminoethane hydrochloride (**2**). - To **19** (2.35 g, 0.016 mol) in 2-propanol (40 mL) 37% HCl (8 mL) was added and, after 30 min at room temperature, the precipitated crystals were filtered off giving **2** (2.24 g, 94%): mp 195-6°C (dec., lit.[13]: 181-3°C); R_f 0.16 (A); R_f 0.44 (D). NMR (D_2O): δ_H 4.37 (2H, m), 3.39 (2H, m). Calcd. for $C_2H_9N_2O$: $[M+H]^+$ 77. Found: $[M+H]^+$ 77.

3-[[(1'-Ethoxyethylidene)amino]oxy]-1-aminopropane (**21**). - Prepared as **19** at 80°C, but starting from **15** (16.0 g, 84 mmol) to give **21** (8.7 g, 63%) after second distillation: bp. 85°C/9 mmHg; n_D^{20} 1.4478; R_f 0.72 (A). NMR ($CDCl_3$): δ_H 4.00 (2H, q, J=7.1), 3.97 (2H, t, J=6.2), 2.80 (2H, J=6.9), 1.92 (3H, s), 1.78 (2H, m), 1.27 (3H, t). Calcd for $C_7H_{16}N_2O_2$: $M^{+\bullet}$ 160.12117. Found: $M^{+\bullet}$ 160.12118.

3-[(Amino)oxy]-1-aminopropane dihydrochloride (**1**). - To **21** (3.2 g, 20 mmol) in 2-propanol (50 mL) 37% HCl (10 mL) was added, after cooling crystals were collected by filtration, washed with 2-propanol and dried *in vacuo* over P_2O_5/KOH to give **1** (3.17 g, 90%): mp 198-9°C (dec.); R_f 0.13 (A); R_f 0.43 (D). NMR (D_2O): δ_H 4.19 (2H, t, J=5.9), 3.15 (2H, t, J=7.6), 2.09 (2H, m). Calcd for $C_3H_{10}N_2O$*2HCl: C 22.22; H 7.46; N 17.28%. Found: C 22.15; H 7.34; N 16.94%.

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-aza-1-aminoheptane (**14**). - A solution of **13** (21.0 g, 0.1 mol) and 1,4-diaminobutane (44.0 g, 0.5 mol) in 2-propanol (40 mL) was kept for 24 h at room temperature. The precipitate was filtered off, washed with 2-propanol and filtrate was evaporated. The residue was pooled into ether (250 mL), dried (KOH) and evaporated. The residue was distiled to give **14** (13.5 g, 62%): bp 73.5-74°C/0.08 mm Hg; n_D^{20} 1.4648; R_f 0.33 (A). NMR ($CDCl_3$): δ_H 4.00 (2H, q, J=7.1), 2.86 (2H, m), 2.70 (2H, t, J=6.8), 2.65 (2H, m), 1.92 (3H, s), 1.50 (5H, m), 1.26 (3H, t, J=7.1), 1.16 (2H, bs). Calcd for $C_{10}H_{24}N_3O_2$: $[M+H]^+$ 218.18684. Found: $[M+H]^+$ 218.18685.

7-[(Amino)oxy]-5-aza-1-aminoheptane trihydrochloride (**3**). - To **14** (7.8 g, 35 mmol) in MeOH (120 mL) 37% HCl (27 mL) and hot 2-propanol (40 mL)was added. After cooling to +4°C the crystals were collected by filtration, washed with 2-propanol and dried *in vacuo* over P_2O_2/KOH to give **3** (8.75 g, 94%): mp 187-188°C (dec.); R_f 0.30 (D). NMR (D_2O): δ_H 4.34 (2H, m), 3.44 (2H, m), 3.17 (2H, m), 3.05 (2H, t, J=7.5 Hz), 1.86-1.72 (4H, m). Calcd for $C_6H_{17}N_3O$*3HCl: C 28.23; H 7.90; N 16.47%. Found: C 28.08; H 7.86; N 16.14%.

7-[(Amino)oxy]-5-aza-1-(N-acetamido)-heptane dihydrochloride (**7**). - Acetyl chloride (0.4 g, 5 mmol) in THF (10 mL) was gradually added with stirring at +10°C to a solution of **14** (1.54 g, 7 mmol) and TEA (1.0 mL, 7 mmol) in THF (50 mL). Stirring was continued for 2 h at +10°C, precipitate was collected by filtration, washed with THF and combined filtrates were evaporated. The residue was dissolved into ethanol (20 mL) followed with 37% HCl (1.5 mL) and evaporated to dryness. The residue was dissolved in water (150 mL) and applied to Dowex 50Wx8 (H^+ form) column (V = 15 mL) followed by step-wise elution with water, 1.8% HCl and 3.7% HCl. Fractions containing the product were evaporated to dryness *in vacuo* and rechromatographed as before on Dowex (V = 15 mL) to give **7** (0.69 g, 52%) after crystallisation from water-ethanol: R_f 0.47 (D). NMR (D_2O): δ_H 4.36 (2H, m), 3.42 (2H, m), 3.20 (2H, t, J=6.9), 3.12 (2H, m), 1.98 (3H, s), 1.72 (2H, m), 1.58 (2H, m).

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]-aminoheptane (**23**). - To **14** (2.21 g, 10 mmol) and TEA (3.1 mL, 22 mmol) in THF (40 mL) benzyl chloroformate (3.6 g, 21 mmol) in THF (20 mL) was added with stirring in three portions at +4°C. After 2 h at 20°C, the precipitate was filtered off, washed with THF and the filtrates were evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (40 mL), washed with 1.0 M $NaHCO_3$ (20 mL) and water, dried ($MgSO_4$) and concentrated to 10 mL. This solution was chromatographed on silica gel using $CHCl_3$ as eluent to give **23** (3.24 g, 67%): R_f 0.67 (B); R_f 0.16 (E). NMR (CD_3OD/CD_3CN; 8/2): δ_H 7.4-7.2 (10H, m), 5.09 (2H, s), 5.05 (2H, s), 4.05-3.85 (4H, m), 3.50 (2H, m), 3.30 (2H, m), 3.10 (2H, m), 1.84 (3H, bs), 1.56 (2H, m), 1.44 (2H, m), 1.21 (3H, m). δ_C 162.41 s, 156.41 s, 156.18 s, 137.49 s, 136.82 s, 128.57 d, 128.49 d, 128.38 d, 128.08 d, 127.93 d, 127.78 d, 71.72 t, 67.01 t, 66.55 t, 62.22 t, 47.72 t, 46.35 t, 40.74 t, 27.12 t, 25.52 t, 14.36 q, 13.66 q. Calcd for $C_{26}H_{35}N_3O_6$: $M^{+\bullet}$ 485.25257. Found: $M^{+\bullet}$ 485.25259.

7-[(Amino)oxy]-5-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]-aminoheptane (**24**). - To **23** (1.94 g, 4 mmol) in 2-propanol (8 mL) water (1.8 mL) and 37% HCl (0.8 mL) were added and after 5 min at 20°C, the reaction mixture was evaporated to dryness. The residue was dissolved in water (17 mL), neutralized with aqueous NaOH, extracted with benzene (3x15 mL) and the extracts were dried (K_2CO_3) and evaporated to dryness. The residual oil was purified on silica gel (75 g) eluting first with $CHCl_3$ and then with $CHCl_3$-MeOH (9:1, v/v) to give **24** (1.4 g, 84%): R_f 0.48 (B). NMR ($CDCl_3$): δ_H 7.4-7.2 (10H, m), 5.12 (2H, s), 5.08 (2H, s), 3.77 (2H, m), 3.46 (2H, m), 3.35-3.08 (4H, m), 1.65-1.38 (4H, m). δ_C 156.63 s, 156.46 s, 136.68 s (2C), 128.92-127.89 d (Ar-C), 73.77 t, 73.04 t, 67.15 t, 66.87 t, 47.40 t, 45.57 t, 40.58 t, 27.11 t, 25.41 t.

7-[(Acetamido)oxy]-5-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]-aminoheptane (**25**). - A solution of acetyl chloride (0.25 g, 3.2 mmol) in THF (4 mL) was added dropwise at +4°C with stirring to a mixture of **24** (1.29 g, 3.2 mmol) and TEA (0.46 mL, 3.3 mmol) in THF (10 mL). Stirring at +4°C was continued for 30 min and the reaction mixture was allowed to warm to room temperature. The precipitate was filtered off, filtrate was evaporated to dryness and resulted oil was purified on silica gel (75 g) eluting first with CHCl3 and then with $CHCl_3$-MeOH (9:1, v/v) to give **25** (1.22 g, 83%): R_f 0.41 (B). NMR ($CDCl_3$): δ_H 7.4-7.2 (10H, m), 5.13 (2H, s), 5.08 (2H, s) 3.93 (2H, m), 3.49 (2H, t, J=4.7), 3.28 (2H, t, J=7.3), 3.14 (2H, m), 1.89 (3H, s), 1.56 (2H, m), 1.45 (2H, m). δ_C 167.61 s, 157.37 s, 156.54 s, 136.53 s, 136.34 s, 128.72-127.77 d (Ar-C), 72.35 t, 67.51 t, 66.66 t, 4679 t, 45.08 t, 40.45 t, 27.16 t, 25.51 t, 19.99 q. Calcd. for $C_{24}H_{32}N_3O_6$: $[M+H]^+$ 458.2. Found: $[M+H]^+$ 457.9.

7-[(Acetamido)oxy]-5-aza-1-aminoheptane dihydrochloride (**5**). - A solution of **25** (0.32 g, 0.7 mmol) in AcOH-MeOH (10 mL, 1:1, v/v) was stirred with Pd black under H_2 until the end of CO_2 evolution. After filtration and washing with MeOH 37% HCl (1.4 mL) was added to the combined filtrates and the resulting solution was evaporated to dryness; the residue solidified on drying in *vacuo* over P_2O_5/KOH, to give **5** (0.178 g, 97%): R_f 0.04 (A); R_f 0.34 (D). NMR (D_2O): δ_H 4.19 (2H, m), 3.35 (2H, m), 3.17 (2H, m), 3.07 (2H, m), 1.96 (3H, s), 1.85-1.75 (4H, m). Calcd. for $C_8H_{20}N_3O_2$: $[M+H]^+$ 190.2. Found: $[M+H]^+$ 190.3.

7-[[(1'-Ethoxyethylidene)amino]oxy]-4-aza-1-aminoheptane (**16**). - Prepared as **14** from **15** (17.9 g, 0.1 mol) and 1,3-diaminopropane (44.5 g, 0.6 mol) in 2-propanol (50 mL), but reaction time was 5 days. After third distillation **16** (6.22 g, 29%) was obtained: bp 123-125°C/0.8 mm Hg; n_D^{20} 1.4669, R_f 0.25 (A). NMR

($CDCl_3$): δ_H 4.01 (2H, q, J=7.1), 3.96 (2H, t, J=6.2), 2.76 (2H, t, J=6.9), 2.70 (2H, t, J=7.1), 2.68 (2H, t, J=7.0), 1.93 (3H, s), 1.82 (2H, m), 1.63 (2H, m), 1.27 (3H, t, J=7.1). Calcd for $C_{10}H_{24}N_3O_2$: $[M+H]^+$ 218.18684. Found: $[M+H]^+$ 218.18680.

7-[(Amino)oxy]-4-aza-1-aminoheptane trihydrochloride (**4**). - To **16** (4.0 g, 18 mmol) in MeOH (60 mL) was added 37% HCl (14 mL) and hot 2-propanol (20 mL). After cooling to +4°C crystals were collected by filtration, washed with cold 2-propanol and dried in *vacuo* over P_2O_5/KOH, giving **4** (4.52 g, 97%): mp 188-9°C (dec.); R_f 0.30 (D). NMR (D_2O): δ_H 4.16 (2H,t, J=5.8), 3.20 (4H, m), 3.11 (2H, m), 2.11 (4H, m). Calcd for $C_6H_{17}N_3O*3HCl*H_2O$: C 26.37; H 8.12; N 15.38%. Found: C 26.60; H 7.82; N 15.11%.

7-[(Amino)oxy]-4-aza-1-(N-acetamido)heptane dihydrochloride (**8**). - To solution of **16** (0.87 g, 4 mmol) in THF (25 mL) at +4°C was added dropwise with stirring the solution of acetic acid N-hydroxysuccinimide ester (0.47 g, 3 mmol) in THF (10 mL). Stirring was continued at +4°C for 3 hr, resulting precipitate was collected by centrifugation, washed with a small portion of cold THF, dissolved into H_2O and after addition of 37% HCl (1 mL) the reaction mixture was evaporated to dryness. The residue was dissolved into H_2O and applied to Dowex 50Wx8 (H^+ form, *V* = 15 mL) followed by step-wise elution with water, 1.8% HCl and 3.7% HCl. Fractions containing the product were evaporated to dryness, and rechromatographed as before on Dowex (*V* = 8 mL). Required fractions were evaporated to dryness, coevaporated with H_2O and dried in *vacuo* over P_2O_5/KOH to give crystalline **8** (0.55 g, 71%): R_f 0.42 (D). NMR (D_2O): δ_H 4.22 (2H, t, J=5.8), 3.29 (2H, t, J=6.6, 3.20 (2H, t, J=7.6), 3.09 (2H, t, J=7.6), 2.13 (2H, m), 2.01 (3H, s), 1.91 (2H, m).

7-[[(1'-Ethoxyethylidene)amino]oxy]-4-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]amino-heptane (**26**). - Prepared as **23**, but starting from **16** (2.21 g, 10 mmol) to give **26** (2.7 g, 57%). NMR ($CDCl_3$): δ_H 7.4-7.2 (10H, m), 5.11 (2H, s), 5.09 (2H, s), 3.97 (2H, q, J=7.2), 3.89 (2H, t, J=5.6), 3.40-3.25 (4H, m), 3.16 (2H, m), 1.91 (2H, m), 1.86 (3H, bs), 1.69 (2H, m), 1.25 (3H, t). δ_C 162.31 s, 156.87 s, 156.56 s, 136.82 s, 136.71 s, 128.59 d, 127.72 d (Ar-C), 70.69 t, 66.47 t, 62.18 t, 44.32 t, 44.19 t, 37.60 t, 28.19 t, 28.02 t, 14.40 q, 13.58 q. Calcd for $C_{26}H_{35}N_3O_6$: $M^{+\bullet}$ 485.25257. Found: $M^{+\bullet}$ 485.25258.

7-[(Amino)oxy]-4-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]-aminoheptane (**27**). - Prepared as **24**, but starting from **26** (2.6 g, 5.35 mmol) to give **27** (1.96 g, 88%). NMR ($CDCl_3$): δ_H 7.4-7.2 (10H, m), 5.12 (2H, s), 5.08 (2H, s), 3.77 (2H, m), 3.46 (2H, m), 3.35-3.08 (4H, m), 1.65-1.38 (4H, m). δ_C 156.63 s, 156.46 s, 136.68 s (2C), 128.92-127.89 d (Ar-C), 73.77 t, 73.04 t, 67.15 t, 66.87 t, 47.40 t, 45.57 t, 40.58 t, 27.11 t, 25.41 t.

7-[(Acetamido)oxy]-4-[[N-(benzyl)oxy]carbonyl]-aza-1-[[N-(benzyl)oxy]carbonyl]-aminoheptane (**28**). - Prepared as **25**, but starting from **27** (1.43 g, 3.45 mmol) to give **28** (1.15 g, 73%). Calcd. for $C_{24}H_{32}N_3O_6$: $[M+H]^+$ 458.2. Found: $[M+H]^+$ 457.9.

7-[(Acetamido)oxy]-4-aza-1-aminoheptane dihydrochloride (**6**). - Prepared as **5** starting from **28** (0.345 g, 0.75 mmol) that resulted after coevaporation with H_2O in a solid, which was dried *in vacuo* over P_2O_5/KOH and afforded **6** (0.183 g, 93%): R_f 0.35 (D). NMR (D_2O): δ_H 4.06 (2H, m), 3.27 (2H, m), 3.19 (2H, m),

3.13 (2H, m), 2.12 (2H, m), 2.05 (2H, m), 1.95 (3H, s). Calcd. for $C_8H_{19}N_3O_2$: $[M+H]^+$ 190.2. Found: $[M+H]^+$ 190.1.

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-aza-heptanoic acid nitrile (**20**). **19** (5.84 g, 0.04 mol), freshly distiled 4-chlorobutyronitrile (3.11 g, 30 mmol) and K_2CO_3 (10.4 g, 75 mmol) in dry 2-propanol (50 mL) were refluxed with stirring for 96 h. After cooling the precipitate was filtered off, washed with 2-propanol and from combined filtrates solvent was evaporated. The residue was treated with ether (50 mL), insoluble material was filtered off, ether was removed in *vacuo* and the residue was distiled to give **20** (4.07 g, 63%): bp 94-5°C/0.9 mmHg; n_D^{20} 1.4592; R_f 0.47 (B); NMR ($CDCl_3$): δ_H 4.00 (2H, q, J=7.1), 3.99 (2H, m), 2.85 (2H, m), 2.77 (2H, t, J=6.7), 2.46 (2H, t, J=7.1), 1.93 (3H, s), 1.81 (2H8, m), 1.27 (3H, t, J=7.1). Calcd. for $C_{10}H_{20}N_3O_2$: $[M+H]^+$ 214.15554. Found: $[M+H]^+$ 214.15556.

7-[(Amino)oxy]-5-aza-1-aminoheptane trihydrochloride (**3**) from **20**. - To stirred solution of **20** (0.16 g, 0.75 mmol) and $CoCl_2 \cdot 6H_2O$ (0.5 M in ethanol, 1.5 mL) in ethanol (13.5 mL) was added $NaBH_4$ (0.150 g, 0.42 mmol) in ethanol (15 mL) at +5°C. After stirring at +4°C for 20 h HCl (1.0 M, 20 mL) was added to the reaction mixture and resulted solution was evaporated to dryness. The residue was dissolved in water and applied to Dowex 50Wx8 (H^+ form) column (V = 8 mL) followed by step-wise elution with water, 1.8% HCl, 3.7% HCl and 7.4% HCl. Fractions containing the crude product were evaporated to dryness, and rechromatographed using column (V = 4 mL) and the same eluent system, that gave **3** (60 mg, 31%), being identical to the sample obtained from **14**.

7-[(Amino)oxy]-5-aza-1-amino(1,1'-$^3H_2$)heptane trihydrochloride (**3***) from **20**. - Tritium-labelled **3*** was prepared from **20** (40.5 mg, 0.19 mmol), $NaBH_4$ (44.1 mg, 1.26 mmol), $NaBT_4$ (100 mCi, 287 mCi/mg, Amersham) and $CoCl_2 \cdot 6H_2O$ (0.5 M in ethanol, 0.45 mL) in ethanol (4.6 mL) as described for the synthesis of **3** from **20**, giving **3*** (15 mg) with sp. activity of 55.6 mCi/mmol.

7-[[(1'-Ethoxyethylidene)amino]oxy]-4-azaheptanoic acid nitrile (**22**). - To freshly distiled acrylonitrile (2.0 mL, 0.03 mol) was added **21** (4.8 g, 0.03 mol) dropwise with stirring at +2°C and allowed to stirring 2 h at each 2°C, 20°C, 50°C and 100°C temperature. Distillation gave **22** (4.8 g, 75%): bp 102°C/0.35 mm Hg; n_D^{20} 1.4600; R_f 0.45 (B). NMR ($CDCl_3$): δ_H 4.00 (2H, t, J=7.1), 3.96 (2H, t, J=6.1), 2.93 (2H, t, J=6.7), 2.74 (2H, t, J=6.9), 2.51 (2H, t, J=6.7), 1.92 (3H, s), 1.82 (2H, t+t), 1.27 (3H, t, J=7.1). Calcd for $C_{10}H_{20}N_3O_2$: $[M+H]^+$ 214.15554. Found: $[M+H]^+$ 214.15555.

7-[(Amino)oxy]-4-aza-1-aminoheptane trihydrochloride (**4**) from **22**. - To stirred solution of **22** (0.213 g, 1 mmol) and $CoCl_2.6H_2O$ (0.5 M in ethanol, 2 mL) in ethanol (3.5 mL) was added $NaBH_4$ (0.18 g, 5 mmol) in ethanol (15 mL) at +4°C. After stirring at +4°C for 18 h HCl (2.0 M, 10 mL) was added to the mixture and resulted solution was evaporated to dryness. The residue was dissolved in water and applied to Dowex 50Wx8 (H^+ form) column (V = 8 mL) followed by stepwise elution with water, 1.8% HCl, 3.7% HCl and 7.4% HCl. Fractions containing the product were evaporated to dryness the residue was coevaporated with 2-propanol, suspended into hot 2-propanol, crystals were filtrated off, and dried in *vacuo* over P_2O_5/KOH, giving 4 (0.14 g, 53%), being identical to the sample obtained from **16**.

7-[(Amino)oxy]-4-aza-1-amino(1,1'-$^3H_2$)heptane trihydrochloride (**4***) from **22**. - Tritium-labelled **4*** was prepared from **22** (53 mg, 0.25 mmol), $NaBH_4$ (40 mg, 1.1 mmol), $NaBT_4$ (50 mCi, 287 mCi/mg, Amersham) and $CoCl_2.6H_2O$ (0.5 M in ethanol, 2.0 mL) in ethanol (4.5 mL) as described for the synthesis of **4** from **22**, giving **4*** (35 mg) with sp.activity of 21.9 mCi/mmol.

1,10-bis[[(1'-Ethoxyethylidene)amino]oxy]-3,8-diaza-decane (**17**) and *N^1,N^1-bis[[[(1'-Ethoxyethylidene)-amino]oxy]ethyl]-1,4-diaminobutane* (**18**). - On continuation of the distillation of **14**, a mixture of **17** and **18** (1.11 g) in a ratio of 7:3 (according to ^1H NMR) was obtained: bp 139-144°C/0.07-0.08 mm Hg. The mixture was separated on silica gel (150 g) with MeOH-TEA (8:2) and fractions containing **17** and **18** were rechromatographed on silica gel (75 g) with the same solvent to give **17** (0.4 g, 1.2%): R_f 0.76 (A) and **18** (0.08 g, 0.23%): R_f 0.63 (A). NMR ($CDCl_3$) **17**: δ_H 4.01 (4H, m), 3.99 (4H, t, J=7.1), 2.87 (4H, m), 2.66 (4H, m), 1.92 (6H, s), 1.54 (4H, m), 1.27 (6H, t, J=7.1). **18**: δ_H 4.00 (4H, q, J=7.1), 3.98 (4H, t, J=6.1), 2.80 (4H, t, J=6.0), 2.70 (2H, m), 2.56 (2H, m), 1.92 (6H, s), 1.48 (4H, m), 1.27 (6H, t, J=7.1). Calcd. for $C_{16}H_{35}N_4O_4$: $[M+H]^+$ 347.26581. Found for **17**: $[M+H]^+$ 347.26583; **18**: 347.26581.

1,10-Bis[(amino)oxy]-3,8-diaza-decane tetrahydrochlodide (**10**). - To **17** (0.38 g, 1.1 mmol) in MeOH (4.0 mL) was added 37% HCl (1.0 mL) following with hot ethanol (4.0 mL) and after cooling to +4°C crystals were collected by filtration, washed with cold ethanol and dried in *vacuo* over P_2O_5/KOH, giving **10** (0.27 g, 77%): mp. 210-1°C (dec.); R_f 0.32 (D). NMR (D_2O): δ_H 4.39 (4H, m), 3.44 (4H, m), 3.15 (4H, m), 1.80 (4H, m). Calcd. for $C_8H_{22}N_4O_2$: $M^{+\bullet}$ 206.2. Found: $M^{+\bullet}$ 206.6.

N^1,N^1-Bis[[(amino)oxy]ethyl]-1,4-diaminobutane tetrahydrochloride (**11**). - To **18** (0.05 g, 0.2 mmol) in MeOH (1.0 mL) was added 37% HCl (0.15 mL) following with hot ethanol (3.0 mL) and after cooling to -20°C crystals were collected by filtration, washed with cold ethanol and dried in *vacuo* over P_2O_5/KOH, giving **11** (0.035 g, 50%). NMR (D_2O): δ_H 4.35 (4H, m), 3.65 (4H, m), 3.37 (2H, m), 3.06 (2H, m), 1.85 (2H, m), 1.76 (2H, m). Calcd. for $C_8H_{22}N_4O_2$: $M^{+\bullet}$ 206.2. Found:$M^{+\bullet}$ 206.5.

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-aza-1-heptanol (**29**). - **13** (6.3 g, 30 mmol), 4-amino-1-butanol (8.1 g, 90 mmol) and K_2CO_3 (8.3 g, 60 mmol) in 2-propanol (50 mL) was refluxed with stirring for 5 h. After cooling to ambient temperature salts were filtered off, washed with 2-propanol and the combined filtrates were evaporated to dryness. The residue was distiled to give **29** (3.9 g, 60%): bp 96-98°C/0.7 mm Hg; n_D^{20} 1.4665; R_f 0.78 (A). NMR ($CDCl_3$): δ_H 4.00 (2H, m), 3.99 (2H, q, J=7.1), 3.58 (2H, m), 2.87 (2H, m), 2.68 (2H, m), 1.93 (3H, s), 1.70-1.58 (4H, m), 1.27 (3H, t). Calcd for $C_{10}H_{23}N_2O_3$: $[M+H]^+$ 219.17085. Found: $[M+H]^+$ 219.17087.

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-[[N-(benzyl)oxy]carbonyl]-aza-1-heptanol (**30**). - To **29** (3.3 g, 15 mmol) and $NaHCO_3$ (2.5 g, 30 mmol) in water (45 mL) containing 10 M NaOH (1.5 mL) was added benzyl chloroformate (3.15 mL, 153 mmol) in three portions with stirring at +4°C. Stirring was continued for 1 h at +4°C and 3 h at 20°C. The mixture was evaporated to half a volume and extracted with EtOAc (3x20 mL), combined extracts were washed with water (5 mL), dried ($MgSO_4$) and evaporated to dryness giving crude **30** (4.9 g). The product was chromatographed on silica gel (150 g), elution with $CHCl_3$ followed by $CHCl_3$/MeOH

(9:1, v/v), to give **30** (3.9 g, 74%) as a colourless oil: R_f 0.57 (B); R_f 0.41 (C). NMR ($CDCl_3$): δ_H 7.35-7.20 (5H, m), 5.12 (2H, s), 3.99 (4H, m), 3.70-3.45 (4H, m), 3.34 (2H, m), 1.88 (3H, s), 1.70-1.45 (4H, m), 1.25 (3H, t, J=7.0). δ_C 162.45 s, 156.30 s, 136.86 s, 128.47 d, 127.93 d, 127.81 d, 71.77 t, 67.05 t, 62.42 t, 62.22 t, 47.92 t, 46.36 t, 29.72 t, 24.70 t, 14.36 q, 13.65 q. Calcd for $C_{18}H_{29}N_2O_5$: $[M+H]^+$ 353.20763. Found:$[M+H]^+$ 353.20765.

7-[[(1'-Ethoxyethylidene)amino]oxy]-5-[[N-(benzyl)oxy]carbonyl)-aza-1-heptanol p-toluenesulfonate (**31**). - 4-Toluenesulfonyl chloride (1.36 g, 7.2 mmol) was added at +4°C with stirring to **30** (2.6 g, 7.4 mmol) in pyridine (10 mL) and stirring was continued at +4°C for two days. Crystals were filtered off, filtrate was evaporated to 1/3 of volume, pooled into ice water (50 mL) and extracted with cold CH_2Cl_2 (4x5 mL). Combined extracts were washed fast with cold 1.0 M citric acid (4x25 mL) and by cold water (4x10 mL). CH_2Cl_2 layer was dried ($MgSO_4$) and evaporated to dryness, giving crude **31** (2.8 g) as a slightly yellow oil. Chromatography on silica gel (150 g) with $CHCl_3$/MeOH (9:0.5, v/v), gave **31** (2.4 g, 64%) as a colourless oil: R_f 0.83 (C). NMR ($CDCl_3$): δ_H 7.76 (2H, m), 7.40-7.24 (7H, m), 5.10 (2H, bs), 4.10-3.90 (6H, m), 3.55-3.35 (4H, m), 2.43 (3H, s), 1.87 (3H, bs), 1.80-1.50 (4H, m), 1.25 (3H, t, J=6.9). δ_C 162.39 s, 156.17 s, 144.72 s, 136.78 s, 133.21 s, 129.85 d, 128.48 d, 127.90 d, 127.86 d, 127.78 d, 71.73 t, 70.11 t, 67.05 t, 62.22 t, 47.37 t, 46.34 t, 29.75 t, 24.30 t, 21.59 q, 14.36 q, 13.63 q.

11-[[(1'-Ethoxyethylidene)amino]oxy]-9-[[N-(benzyl)oxy]carbonyl)-aza-4-aza-1-aminoundecane (**32**). - The solution of **31** (0.8 g, 1.6 mmol) and 1,3-diaminopropane (1.0 mL, 12 mmol) in 2-propanol (2.0 mL) was kept for 3 days at room temperature. Reaction mixture was evaporated to dryness at 40°C (0.1 mm Hg), and the residual oil was chromatographed on silica gel (75 g) with MeOH-TEA (8:2, v/v), giving **32** (0.5 g, 75%) as a colourless oil: R_f 0.21 (A); R_f 0.56 (D). NMR ($CDCl_3$): δ_H 7.40-7.25 (5H, m), 5.12 (2H, s), 4.10-3.95 (4H, m), 3.51 (2H, m), 3.31 (2H, m), 2.76 (2H, m), 2.70-2.52 (4H, m), 1.88 (3H, s), 1.70-1.40 (6H, m), 1.25 (3H, J=7). δ_C 162.41 s, 156.20 s, 136.92 s 128.46 d, 127.90 d, 127.79 d, 71.75 t, 66.99 t, 62.22 t, 49.66 t, 48.05 t, 47.83 t, 46.36 t, 40.46 t, 33.39 t, 27.17 t, 26.10 t, 14.37 q, 13.66 q. Calcd for $C_{21}H_{37}N_4O_4$: $[M+H]^+$ 409.28146. Found: $[M+H]^+$ 409.28148.

11-[(Amino)oxy]-4,9-diaza-1-aminoundecane tetrahydrochloride (**9**). - A solution of **32** (0.5 g, 1.2 mmol) in AcOH-MeOH (10 mL, 1:1) was stirred with Pd black under H_2 atmosphere until the evolution of CO_2 has ceased, catalyst was filtered, washed with MeOH and combined filtrates were evaporated to dryness. The residue was co-evaporated with 2-propanol to give **33** as a colourless oil which then was converted to **9**, as descriped for the synthesis of **10** from **17**, to give **9** (0.27 g, 64%): mp. 221-223°C (dec.); R_f 0.22 (D). NMR (D_2O): δ_H 4.36 (2H, m), 3.44 (2H, m), 3.20-3.08 (8H, m), 2.10 (2H, m), 1.84-1.77 (4H, m). Calcd. for $C_9H_{25}N_4O$: $[M+H]^+$ 205.2. Found: $[M+H]^+$ 204.8.

11-[[(1'-Ethoxyethylidene)amino]oxy]-4,9-diaza-undecane acid nitrile (**34**). - Prepared from **14** (4.34 g, 20 mmol) and freshly distiled acrylonitrile (1.35 mL, 20 mmol) as **22**, giving **34** (3.47 g, 67%) after second distillation: bp 149-150°C/0.16 mmHg; n_D^{20} 1.4723; R_f 0.80 (A). NMR ($CDCl_3$): δ_H 4.00 (2H, t, J=7.1 Hz), 4.00 (2H, m), 2.92 (2H, t, J=6.7 Hz), 2.86 (2H, m), 2.65 (4H, m), 2.51 (2H, t, J=6.7), 1.93 (3H, s), 1.54 (4H, m), 1.27 (3H, t, J=7.1). δ_C 162.38 s, 118.70 s, 72.85 t, 62.19 t, 49.70 t, 49.13 t, 48.92 t, 45.13 t,

27.95 t, 27.88 t, 18.79 t, 14.40 q, 13.63 q. Calcd. for $C_{13}H_{27}N_4O_2$: $[M+H]^+$ 271.21339. Found: $[M+H]^+$ 271.21341

11-[[N-isopropylidene(amino)oxy]-4,9-[N,N'-bis(benzoyl)]-diaza-1-aminoundecane (**37**). - To a solution of **34** (1.35 g, 5 mmol) and TEA (1.9 mL, 13 mmol) in THF (30 mL) was added benzoyl chloride (1.54 g, 11 mmol) in THF (5 mL) with stirring in three portions at +4°C. After 2 h at 20°C the precipitate was filtered off, washed with THF and filtrates were evaporated to dryness. The residue was dissolved in $CHCl_3$ (20 mL), washed with 1.0 M $NaHCO_3$ (5 mL), water, dried ($MgSO_4$) and concentrated under reduced pressure to 8 mL. Chromatography of this solution on a silica gel using $CHCl_3$ as eluent afforded **35** (1.93 g, 79%): R_f 0.45 (B). A part of the product **35** (1.19 g, 2.5 mmol) was dissolved in ethanol (11 mL) followed by adding $CoCl_2 \cdot 6H_2O$ (0.5 M in ethanol, 5.2 mL) and $NaBH_4$ (0.825 g, 22.5 mmol) in ethanol (60 mL) at +5°C. After stirring at +4°C for 18 h HCl (2.0 M, 100 mL) was added and the mixture was evaporated to dryness in *vacuo*, the residue was dissolved in water and applied to Dowex 50Wx8 (H^+ form, V = 30 mL) column followed by step-wise elution with water, 1.8% HCl; 3.7% HCl and 7.4% HCl. The fractions containing the product were evaporated to dryness giving crude **36**, R_f 0.52 (A), which was then dissolved into water:aceton (40 mL, 3:1) mixture and pH was adjusted to 5-6. After 2 h at ambient temperature the mixture was evaporated to dryness followed by co-evaporated with water and then with 2-propanol. After drying the product was suspended into 2-propanol, the insoluble material was filtered off, filtrate was evaporated to dryness *in vacuo* and the residue was purified on silica gel (150 g) with MeOH-TEA (8:2, v/v) to give **63** (0.22 g of ca. 75% purity according to ^1H NMR): R_f 0.80 (A). NMR ($CDCl_3$): δ_H 7.5-7.3 (10H, m), 4.20 (2H, m), 3.65-3.40 (6H, m), 3.31 (2H, m), 3.05 (2H, m), 2.17 (2H, m), 1.9-1.7 (10H, m).

11-[(Amino)oxy]-4,9-diaza-1-aminoundecane tetrahydrochloride (**9**) from **37**. - **37** (0.22 g) was refluxed with 20% HCl (20 mL) for 4 h, the mixture was washed with ether (3x5 mL) and water phase was evaporated to dryness in *vacuo*. The residue was dissolved in water and applied to Dowex 50Wx8 (H^+ form, V = 5 mL) column followed by step-wise elution with water, 1.8% HCl; 3.7% HCl and 7.4% HCl. The required fractions were evaporated to dryness in vacuo and the residue was crystalised from water-ethanol giving **9** (0.07 g, 4% calculated from **35**), which was identical to the sample obtained from **33**.

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