Structure−Immunosuppressive Activity Relationships of New Analogues of 15-Deoxyspergualin. 1. Structural Modifications of the Hydroxyglycine Moiety

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A series of new analogues of 15-deoxyspergualin (DSG), an immunosuppressive agent currently commercialized in Japan, was synthesized and tested in a graft-versus-host disease (GVHD) model in mice. Using the general concept of bioisosteric replacement, variations of the hydroxyglycine central "C" region were made in order to determine its optimum structure in terms of in vivo immunosuppressive activity. By this way, the malonic derivative **13a** was discovered as the first example of a new series of potent immunosuppressive agents encompassing a retro-amide bond linked to the hexyl-guanidino moiety. Structure−activity relationships of this series were studied by synthesizing compounds **13g**−**i** and **13k**−**s**. Variation of the "right-amide" of **13a** led to the urea **19a** and the carbamates **23** and **27a** which proved to be equally active as DSG in our GVHD model. Finally **27a** was found to be the most potent derivative, being slightly more active than DSG in a heart allotransplantation model in rats. Due to the absence of chiral center in its structure and to its improved chemical stability compared to DSG, **27a** was selected as a candidate for clinical evaluation.

Introduction

In the last three decades, major advances have been achieved in the development of immunosuppressive drugs which have made possible organ transplantation and treatment of autoimmune diseases. Azathioprine[1] was first introduced to control graft rejection and is still used in combination with other immunosuppressive agents. However, its myelotoxicity and other side effects limit its use. The discovery of cyclosporin A[2] allowed major improvements in transplantation. It induced dramatic increase of organ allograft survival which made possible liver and cardiac transplants. More recently, FK-506[3] has been introduced as a more potent immunosuppressant although mechanistically related to cyclosporin A. These two immunosuppressive compounds block T-cell activation via the inhibition of interleukin-2 expression.[4] Recently, mycophenolate mofetil[5] which inhibits the purine biosynthesis has been approved for the prevention of kidney graft rejection. However, although more specific than azathioprine, these drugs do show nephro-, neuro-, and myelotoxicities as well as other side effects.[6] Therefore, there is a need for new less toxic compounds and with new mechanisms of action, which could lead to a more specific immunosuppression.

Among the different compounds recently developed in this area (−)15-deoxyspergualin ((−)DSG)[7] appeared to be attractive considering its biological profile and its new mechanism of action.[8] (−)DSG is a derivative of (−)-spergualin,[9] a fermentation product isolated from *Bacillus laterosporus* by Umezawa's group. It was first obtained by dehydroxylation of (−)spergualin in 1982.[10] Its first total synthesis was published in 1987,[11] and despite its low overall yield,[12] this process afforded (−)-DSG and (+)DSG which could be separately evaluated. In vivo experiments clearly showed that (−)DSG is the only immunosuppressive enantiomer.[7] However, most of the biological, pharmacological, and clinical data have been obtained with the more readily available (+,−)-DSG whose first total synthesis was patented in 1982.[13] Since its discovery, DSG has been widely studied and demonstrated efficacy first as an anticancer agent,[14] then in animal transplantation either in allografts[15] or xenografts,[16] and more recently in graft-versus-host disease (GVHD) animal models.[17] DSG has also been widely investigated[18] in autoimmune diseases. The high potential of DSG to reverse renal graft rejection was first reported in 1990.[19] This drug was therefore launched in Japan (Spanidin) in this indication in 1994.[19]

However DSG suffers from three main drawbacks which limit its use. The first one is related to its low chemical stability in aqueous solution where hydrolysis of the hydroxyglycine part leads to 7-guanidinoheptanamide and glyoxyloylspermidine. This cleavage is a problem for large scale synthesis, purification, and standard galenic development. The second problem is due to the presence of the inactive (+)DSG isomer present in the racemic mixture. This compound is not reported to be immunosuppressive but can contribute to the acute toxicity.[7] Until now, industrial synthesis of the active (−)DSG form or another equivalent achiral compound was not available. The last limitation is related to a very low oral bioavailability (less than 5%[20]), preventing oral administration.

We present here the first part of our program aimed at the discovery of new DSG analogues (Chart 1), devoid of the above-mentioned drawbacks. For this the DSG structure has been divided into four regions. The purpose of the present study was to find the optimal "C" region which could alleviate problems associated with chirality and hydrolytic sensitivity brought by the hydroxyglycine moiety. Umezawa and co-workers have

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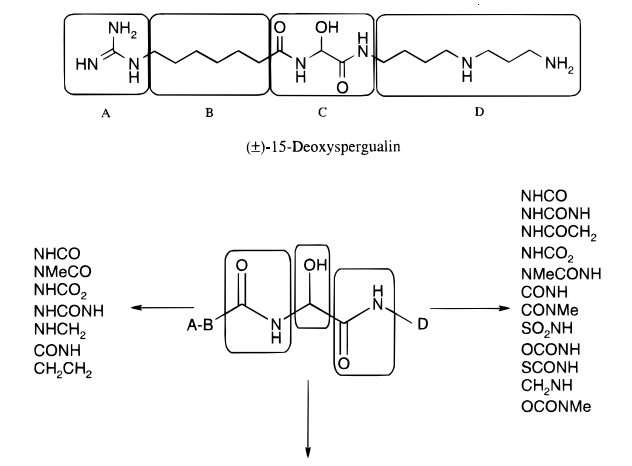
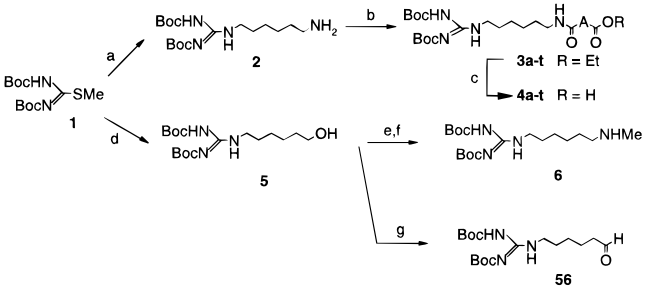
Chart 1



Scheme 1[a]



[a] Reagents: (a) hexanediamine, THF; (b) HOOCACOOEt, CH_2Cl_2, DCC, HOBT; (c) 1 M NaOH, DME (v/v); (d) amino-6-hexanol, THF; (e) Et_3N, CH_2Cl_2, −20 °C, methanesulfonyl chloride; (f) DME, $MeNH_2$ (40%); (g) PDC, CH_2Cl_2.

already explored the structure−activity relationship (SAR) of this part of the molecule, synthesizing numerous analogues where the hydroxyglycine has been replaced by various α-, *β*-, and *γ*-amino acids.[21] *O*-methylhydroxyglycine, L-serine, and glycine were the only tolerated α-amino acids. *γ*-Aminobutyric and *γ*-amino α- or *β*-hydroxybutyric acids were also tolerated, but none of these derivatives were as active as DSG. *β*-Amino acids and all other α-amino acids led to inactive compounds. This stringent structural requirement for this "C" region is remarkable. However, all these analogues retained the two amide bonds with the same orientation as in the parent DSG. We thus decided to extend the investigation of this crucial part of the molecule using concepts encountered in peptide medicinal chemistry such as retro-inverso approach and other bioisosteric replacements for the amide bonds. We also replaced the hydroxymethylene moiety by various linkers (Chart 1).

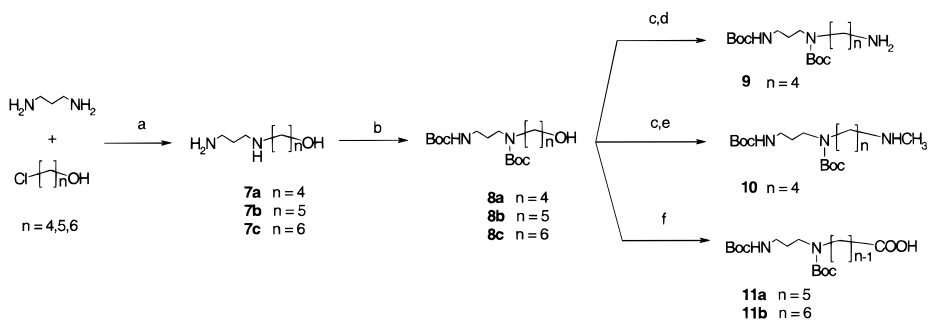
Chemistry

The wide range of structural modifications planned forced us to use rather diverse synthetic schemes. Moreover, the choice of these schemes was dictated by availability of certain starting materials.

The malonamide-related compounds **13a**−**t** and **15** were made by the route illustrated in Schemes 1, 2, and 3 from key intermediates **4a**−**t**, **9**, and **10**. Compounds **4a**−**t** were prepared in three steps from *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **1** which was obtained according to the procedure described by Bergeron et al.[22] (Scheme 1). Condensation of **1** with hexanediamine led to the intermediate **2** which was coupled to commercially available monoester malonic acid derivatives using standard coupling techniques. Saponification of the ester groups led to compounds **4a**−**t**. We used a shorter procedure (Scheme 2) than the one described by Bergeron et al.[23] to obtain **9**. Alkylation of propane diamine by *n*-chloroalkanol in refluxing butanol led to compounds **7a**−**c** with good yields. The amines were then protected by Boc groups to give **8a**−**c**. Conversion of alkanol **8a** to its methanesulfonate derivative followed by nucleophilic displacement with ammonia or methylamine yielded the desired spermidine derivatives **9** and **10**. Target compounds **13a**−**t** and **15** were obtained by the coupling of key intermediates **4a**−**t** with **9** or **4a** with **10** using standard coupling techniques followed by removal of Boc protecting groups of the intermediates **12a**−**t** or **14** with trifluoroacetic acid (TFA) in CH_2Cl_2 (Scheme 3).

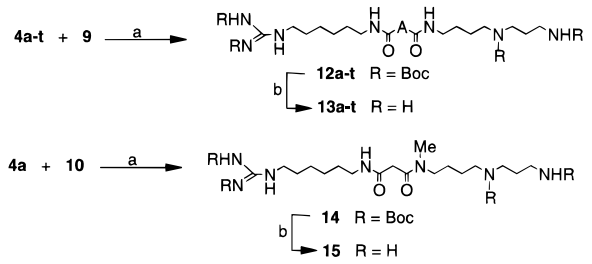
The synthetic sequences utilized for the preparation of derivatives **19a**−**c**, **21a**−**b**, **23**, **35**, and **42** are outlined in the Scheme 4. The key intermediates **17a**−**c** were obtained by coupling **2** with *N*-benzyloxycarbonylglycine, *N*-benzyloxycarbonyl-*N*-methyl-glycine, or *N*-benzyloxycarbonyl-*β*-alanine using standard coupling techniques followed by cleavage of the Cbz protecting group with Pd/C under a hydrogen atmosphere. The synthesis of urea derivatives (**19a**−**c**) was best achieved using a strategy involving condensation of **17a**−**c** with bis(4-nitrophenyl)carbonate followed by the action of **9** to give the N-Boc protected compounds **18a**−**c**. Final

Scheme 2[a]



[a] Reagents: (a) KI, K_2CO_3, n-BuOH reflux; (b) $(Boc)_2O$, dioxane, H_2O; (c) Et_3N, CH_2Cl_2, −20 °C, methanesulfonyl chloride; (d) EtOH, NH_4OH (v/v); (e) EtOH, $MeNH_2$; (f) PDC, DMF.

Scheme 3[a]



[a] Reagents: (a) DCC, HOBT, CH_2Cl_2; (b) TFA, CH_2Cl_2 (v/v).

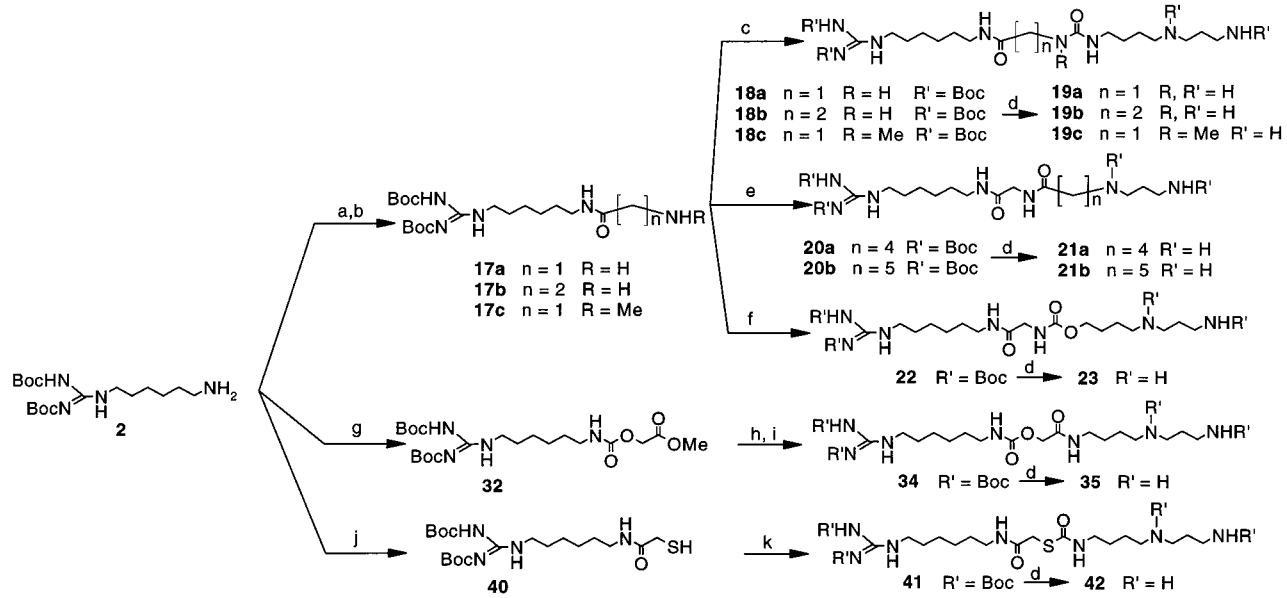
deprotection was then performed to provide the desired DSG analogues **19a**−**c**. In the same way, we adopted a similar strategy for the synthesis of the carbamate **23**. To the nonisolated intermediate nitrophenyl carbonate, generated by reaction of 4-nitrophenyl chloroformate with **8a**, was added **17a** to afford **22,** which was further deprotected to yield the desired carbamate derivative **23**. Oxidation of alkanol **8b**−**c** by pyridinium dichromate in DMF provided the carboxylic derivatives **11a**−**b** (Scheme 2). Compounds **21a**−**b** were obtained via the coupling of **17a** with intermediates **11a**−**b** to yield **20a**−**b**. Cleavage of the Boc protecting groups was then carried out by the standard method, i.e., TFA in CH_2Cl_2 to give the target compounds **21a**−**b**.

Addition of **2** to the methyl [(phenoxycarbonyl)oxy] acetate generated from methyl glycolate and phenyl chloroformate led to the intermediate **32**. Saponification of **32** with NaOH in DME followed by coupling with **9**, according to the standard techniques, and deprotection afforded **35**. Compound **42** was prepared via the key intermediate **40** which was obtained by the coupling of **2** with thioglycolic acid. Compound **40** was further converted to the thiocarbamate **42** by reaction of the nonisolated nitrophenyl thiocarbonate with **9** and deprotection with the standard acidic procedure.

The synthetic route leading to the DSG analogues **27a**−**d**, where the carbamate function is reversed compared to **23**, is outlined in Scheme 5. Acylation of spermidine derivatives **9** or **10** (Scheme 2) with phenyl carbonates derived from methyl hydroxyacetate or hydroxypropionate afforded, after saponification, **25a**−**c**. These acids were converted to the final products **27a**−**d** by coupling with **2** or **6** (Scheme 1) followed by removal of protecting groups.

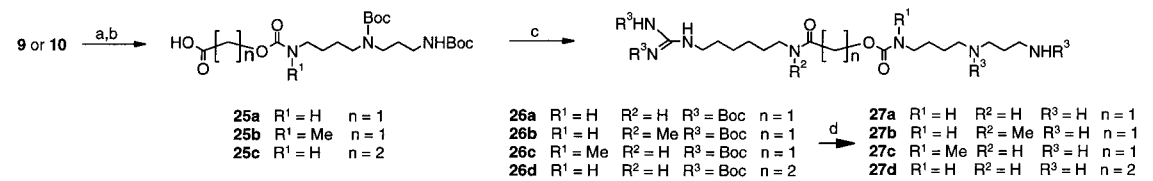
The compounds **31**, **39**, **46**, **50**, **55**, and **60** were made starting from **9** using specific routes illustrated in Scheme 6 and which are explained below. The intermediate 10-[(phenylmethoxy)carbonylamino]decanoic acid was coupled with **9** using standard coupling technique to give **28**. Removal of the Cbz group by hydrogenolysis, condensation with **1**, and final deprotection yielded the desired compound **31**. Carbobenzyloxyglycine was coupled to **9**, and the Cbz group of the coupling product **36** was then cleaved. The obtained intermediate was then

Scheme 4[a]



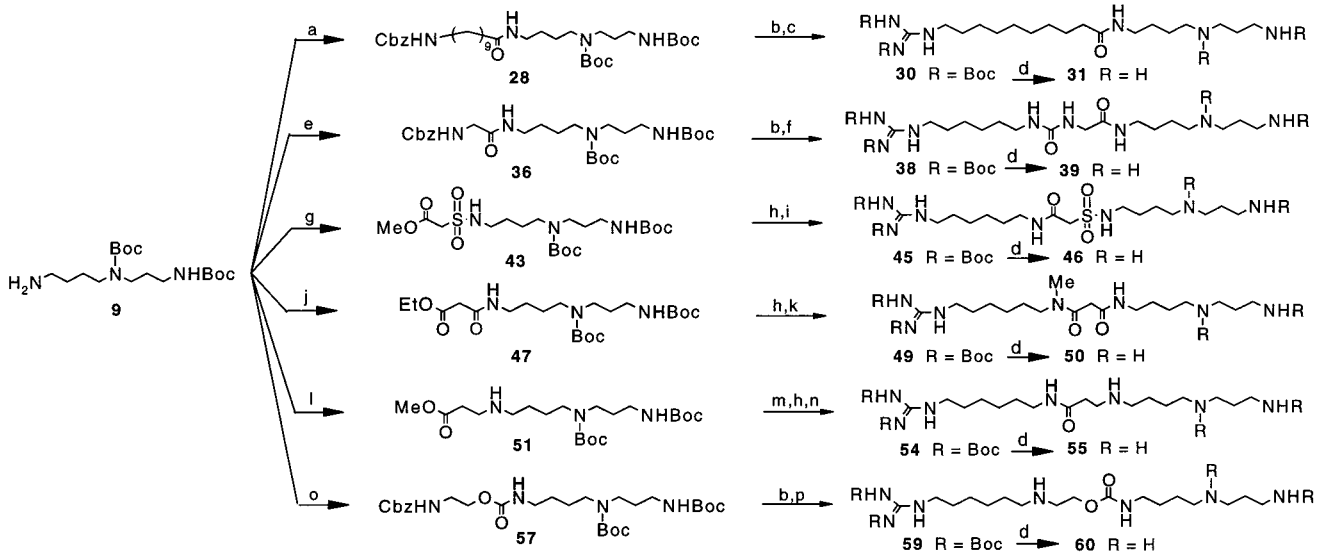
[a] Reagents: (a) $PhCH_2OCONR(CH_2)_nCO_2H$ with n = 1, 2 and R = H, CH_3, isobutylchloroformate, THF, −20 °C, NMM; (b) Pd/C 10%, EtOH, H_2; (c) bis-(4-nitrophenyl)carbonate, THF, **9**; (d) CH_2Cl_2, TFA (v/v); (e) **11a** or **11b**, isobutylchloroformate, THF, −30 °C, NMM; (f) **8a**, Et_3N, THF, 4-nitrophenyl chloroformate; (g) methyl[(phenoxycarbonyl)oxy]acetate, toluene, 40 °C; (h) 1 M NaOH, DME (v/v); (i) DCC, HOBT, CH_2Cl_2, **9**; (j) thioglycolic acid, carbonyldiimidazole, CH_2Cl_2; (k) 4-nitrophenyl chloroformate, Et_3N, THF then **9**.

Scheme 5[a]



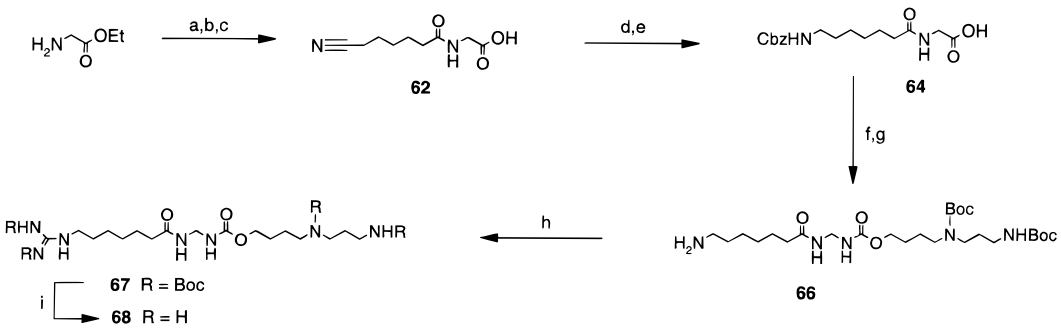
[a] Reagents: (a) $PhOCO_2(CH_2)_nCO_2Me$ with n = 1, 2, toluene, reflux; (b) 1 M NaOH, DME (v/v); (c) DCC, HOBT, CH_2Cl_2, **2** or **6**; (d) CH_2Cl_2, TFA (v/v).

Scheme 6[a]



[a] Reagents: (a) $CbzHN(CH_2)_9CO_2H$, $CHCl_3$, DCC, HOBT; (b) Pd/C 10%, EtOH, H_2; (c) **1**, THF; (d) CH_2Cl_2, TFA (v/v); (e) carbobenzyloxyglycine, $CHCl_3$, DCC, HOBT; (f) 4-nitrophenyl chloroformate, THF, Et_3N, **2**; (g) methyl 2-(chlorosulfonyl)acetate, CH_2Cl_2, −20 °C, Et_3N; (h) 1 M NaOH, DME (v/v); (i) carbonyldiimidazole, CH_2Cl_2, Et_3N, **2**; (j) ethyl hydrogen malonate, $CHCl_3$, DCC, HOBT; (k) CH_2Cl_2, DCC, HOBT, **6**; (l) methylacrylate, MeOH reflux; (m) $(Boc)_2O$, Et_3N, THF; (n) isobutyl chloroformate, THF, −30 °C, NMM, **2**; (o) $PhOCO_2(CH_2)_2NHCbz$, Et_3N, toluene; (p) EtOH, **56**, AcOH then $NaBH_3CN$.

Scheme 7[a]



[a] Reagents: (a) 6-bromohexanoic acid, $CHCl_3$, DCC, HOBT; (b) EtOH, reflux, KCN; (c) 1 M NaOH, DME (v/v); (d) H_2, Ni Raney, EtOH, 1 M NaOH; (e) EtOH, $NaHCO_3$, benzylchloroformate; (f) Et_3N, THF, DPPA then **8a**, toluene, reflux; (g) H_2, Pd/C 5%, EtOH; (h) THF, **1**; (i) CH_2Cl_2, TFA (v/v).

reacted with 4-nitrophenylchloroformate to give a reactive carbamate derivative which in the presence of **2** at room temperature provided the desired protected compound. Final Boc protecting groups cleavage was then performed to give **39**.

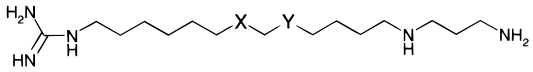
Methyl methyl 2-(chlorosulfonyl)acetate reacted with **9** in dichloromethane to afford the key intermediate **43**. Then usual sequence involving saponification, amide bond coupling of **2** using carbonyldiimidazole, and final deprotection was performed to obtain **46**.

The reactions carried out to obtain **50** are similar to those used for **46**; however, they include coupling **6** instead of **2** and using the DCC, HOBT procedure.

Michael addition of **9** to methylacrylate in refluxing methanol afforded the key intermediate **51** in which the amine was further protected by a Boc group. Saponification, coupling with **2** using the mixed anhydride method, and final deprotection allowed us to obtain the desired compound **55**. *N*-carbobenzyloxy-2-aminoethanol reacted with phenyl chloroformate in toluene to afford the reactive carbonate which in the presence of **9** led to the intermediate **57**. Removal of Cbz group of **57** by hydrogenolysis afforded the corresponding amine which was converted to the final compound **60** by a reductive amination reaction with **56** (see Scheme 1) in the presence of $NaBH_3CN$ followed by cleavage of Boc protecting groups.

The synthetic sequence leading to **68** with a reversed amide bond compared to **23** is outlined in Scheme 7. *N*-(6-bromohexanoyl)-glycine ethyl ester was obtained by coupling bromohexanoic acid with glycine ethyl ester. Nucleophilic substitution of the bromine by KCN followed by saponification of the ester allowed the production of the acid **62**. Hydrogenation of the nitrile group carried out with Raney nickel in the presence of sodium hydroxide afforded the amine which was protected by a Cbz group to give **64**. The crucial conversion of **64** to **66** was carried out as follows: reaction of **64** with diphenylphosphorazide gave the corresponding acyl azide derivative which upon Curtius rearrangement in toluene at reflux led to the corresponding isocyanate which was further reacted with **8a** (Scheme 2) to give **66** after cleavage of the Cbz protecting group. Reaction of the primary amine of **66** with **1** (Scheme 1) in THF and further acidic cleavage of the Boc protecting groups afforded the desired diacylaminal compound **68**.

Table 1. Physicochemical and Biological Data of 15-DSG Analogues with X and Y Variations



compd	X	Y	scheme	HRMS calcd	HRMS found	GVHD mean survival (days) (M ± SD)[a] 3 mg/kg[b]	GVHD mean survival (days) (M ± SD)[a] 1 mg/kg[b]
21a	NHCO	NHCO	4	372.3087	372.3089	22 ± 6	
13a	NHCO	CONH	3	372.3087	372.3086	52 ± 20	53 ± 13
31	CH_2CH_2	CONH	6	357.3342	357.3339	16 ± 1	
35	NHCOO	CONH	4	388.3036	388.3038	13 ± 1	
39	NHCONH	CONH	6	387.3196	387.3200	24 ± 7	
55	NHCO	CH_2NH	6	358.3294	358.3294	13 ± 4	
46	NHCO	SO_2NH	6	408.2757	408.2755	15 ± 1	
19a	NHCO	NHCONH	4	387.3196	387.3173	48 ± 12	42 ± 21
21b	NHCO	$NHCOCH_2$	4	386.3243	386.3243	18 ± 4	
27a	NHCO	OCONH	5	388.3036	388.3037	58 ± 6	46 ± 10
23	NHCO	NHCOO	4	388.3036	388.3035	48 ± 16	55 ± 4
68	CONH	NHCOO	7	388.3036	388.3034	58 ± 5	32 ± 3
42	NHCO	SCONH	4	404.2808	404.2805	17 ± 2	
27b	NMeCO	OCONH	5	402.3193	402.3193	14 ± 1	
50	NMeCO	CONH	6	386.3243	386.3247	14 ± 1	
15	NHCO	CONMe	3	386.3243	386.3246	12 ± 2	
19c	NHCO	NMeCONH	4	401.3352	401.3352	17 ± 4	
27c	NHCO	OCONMe	5	402.3193	402.3192	15 ± 2	
60	$NHCH_2$	OCONH	6	374.3243	374.3245	34 ± 9	

[a] The mean survival of control is 15 ± 2 days and, respectively, 38 ± 12 and 57 ± 7 days for animals treated with DSG at 1 mg/kg and 3 mg/kg. [b] All compounds were administered daily by i.p. route.

Results and Discussion

Biological and analytical data are shown in Tables 1, 2, and 3. Immunosuppressive activity was assayed in vivo in a mouse GVHD model which was adapted from the literature.[24] Briefly, GVHD is induced at day 0, treatments are then given from day 1 to day 10 (day 6 omitted), and survival is followed until sacrifice at day 60. The mean survival of the controls is 15 ± 2 days. In such conditions, DSG administered by i.p. route increases survival to 38 ± 12 days at 1 mg/kg/day and to 57 ± 7 days at 3 mg/kg/day. All compounds were then studied by the same route first at 3 mg/kg/day and then at 1 mg/kg/day if they demonstrated a significant activity. The first step of our investigation dealt with the modification of the two amide bonds. Our goal was to get more stable compounds that were easier to synthesize and devoid of chirality. Thus, we decided to make glycine instead of hydroxyglycine analogues of DSG. We first synthesized **21a** (Table 1) where the two amide bonds are reversed compared to DSG. This modification which destroys the spermidine integrity completely abolishes the immunosuppressive activity.

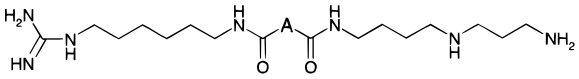
However **13a**, where only the "left amide" was reversed compared to DSG, was active at 3 and 1 mg/kg i.p. Other replacements of the "left amide" such as a two-methylene unit (**31**), a carbamate (**35**), or an urea (**39**) gave inactive compounds. Similarly, bioisosteric replacement of the "right amide" with a reduced amide (**55**) or a sulfonamide (**46**) was detrimental to activity. By contrast, **19a**, which can be seen as an analogue of inactive compound **21a** where the nitrogen of spermidine has been reintroduced giving a urea, was active at 3 mg/kg i.p.. Further modification of the urea of **19a** was successful and afforded potent and less toxic analogues.[25] Either NH can be favorably replaced by an O atom leading to **27a** and **23** which were very active at 3 and 1 mg/kg i.p. and which were in the same range of activity as that of DSG. However, NH cannot be replaced by a methylene (**21b**). It is worth noting that compound **23** was the first example of a very active analogue of DSG not encompassing the full spermidine moiety. The carbamate is clearly a very suitable function in this position whatever the orientation of the "left amide" (see activity of **68**). Interestingly the corresponding thiocarbamate (**42**) was inactive, possibly due to its poor stability at physiological pH. N-Methylation of either the amides **27b**, **50**, and **15**, the urea **19c**, or the carbamate **27c** was absolutely detrimental. Finally, reduction of the "left amide" of **27a** gave rise to the slightly active compound **60**.

Table 2 shows results obtained with analogues of **13a**, **19a**, and **27a** where the nature and length of the central linker have been modified. Obviously a single bond (**13e**) or a methylene (**13a**) were the only tolerated linkers. It is interesting to notice that a CH_2CH_2 linker leads to a totally inactive compound (**13c**) when its replacement with a CH_2O (**27a**) affords one of the most active derivatives of this series (Table 1).

In Umezawa's derivatives, the presence of OH or, to a lesser extent, of OCH_3 in the "C" region increases the potency compared to hydrogen. We investigated (Table 3) the effect of such groups or related ones such as F, NH_2, or NHAc on compound **13a**. We also introduced lipophilic aliphatic and aromatic substituents. Finally we made some disubstituted derivatives. In sharp contrast to the DSG series, introduction of OH (**13s**) or OCH_3 (**13i**) does not really increase activity. However, only OH, OCH_3, or F (**13t**) groups are tolerated in contrast to NH_2 (**13p**), NHAc (**13o**), or OCH_2Ph (**13r**) which, though susceptible to sharing a hydrogen bond, are detrimental for activity.

Disubstitution with OCH_3 (**13q**) and CH_2OH (**13l**) is also totally ineffective. Finally, as it is the case for DSG series, alkyl or aryl substituents led to inactive compounds **13g**, **13h**, **13k**, **13m**, and **13n**.

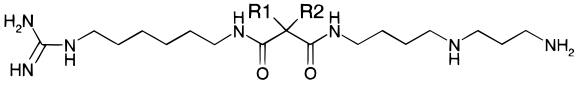
Table 2. Physicochemical and Biological Data of 15-DSG Analogues with A Modifications



compd	A	scheme	HRMS calcd	HRMS found	GVHD mean survival (days) (M ± SD)[a] 3 mg/kg[b]	GVHD mean survival (days) (M ± SD)[a] 1 mg/kg[b]
13b	CH=CH (Z)	3	384.3087	384.3084	17 ± 3	
13c	CH_2CH_2	3	386.3243	386.3246	16 ± 4	
13d	$CH_2CH_2CH_2$	3	400.3400	400.3403	15 ± 1	
13f	CH=CH (E)	3	384.3087	384.3086	17 ± 8	
13j	CH_2CH (OH)	3	402.3193	402.3184	15 ± 1	
13e		3	358.2930	358.2932	34 ± 8	53 ± 13
19b	CH_2CH_2NH	4	401.3352	401.3350	15 ± 3	
27d	CH_2CH_2O	5	402.3193	402.3196	13 ± 1	

[a,b] See footnotes in Table 1.

Table 3. Physicochemical and Biological Data of Malonic Derivatives with R1 and R2 Substituents



compd	R_1	R_2	HRMS calcd	HRMS found	GVHD mean survival (days) (M ± SD)[a] 3 mg/kg[b]	GVHD mean survival (days) (M ± SD)[a] 1 mg/kg[b]
13s	OH	H	388.3036	388.3030	53 ± 13	12 ± 1
13i	OCH_3	H	402.3193	402.3193	55 ± 12	27 ± 3
13t	F	H	390.2993	390.2996	60 ± 0	18 ± 11
13p	NH_2	H	387.3196	387.3196	16 ± 1	
13o	NHAc	H	429.3302	429.3299	16 ± 1	
13r	$OCH_2C_6H_5$	H	478.3506	478.3501	14 ± 2	
13q	OCH_3	OCH_3	432.3298	432.3298	12 ± 3	
13l	CH_2OH	CH_2OH	432.3298	432.3296	17 ± 2	
13h	CH_3	H	386.3243	386.3243	16 ± 2	
13m	CH_2CH_3	H	400.3400	400.3399	19 ± 3	
13k	C_6H_5	H	448.3400	448.3403	19 ± 4	
13g	$CH_2C_6H_5$	H	462.3556	462.3553	16 ± 2	
13n	CH_3	CH_3	400.3400	400.3407	15 ± 1	

[a,b] See footnotes in Table 1.

Table 4. Prevention of Graft Rejection after Heart Allotransplantation in the Dark Agouti to Lewis Rat Combination

compd	dose[a] (mg/kg)	survival (day)	mean survival (day) (M ± SD)	statistical analysis[b]
control	0	6, 6, 6, 7, 7, 7, 7	7 ± 1	
DSG	6	13, 20, 20, 26, 42, 46, >100	38 ± 30	S
27a	6	17, 27, 27, 45, 63, 99, >100	54 ± 34	S
13a	6	5, 7, 8, 10, 10, 13	9 ± 3	NS
13e	6	11, 11, 11, 11, 11, 11, 15	12 ± 2	S
19a	6	6, 6, 12, 12, 13, 15, 17	12 ± 4	S
13s	6	15, 20, 24, 25, 28, 37, 79	33 ± 22	S

[a] All compounds were administered daily by i.p. route for 10 days starting the day after surgery. [b] Manntel and Haenszel test according to SAS (Cary, NC).

Selection of the best immunosuppressor compounds obtained in this study was then made using a more demanding model. Some of the more active compounds were then tested in a model for acute organ rejection after heart allotransplantation in the rat. Genetic combination was selected to be completely histoincompatible, and the recipient was considered to be a high responder in which control of rejection would be especially difficult.[26,27] DSG induces a 38 ± 30 days survival of the graft in these experimental conditions (10 days, 6 mg/kg, i.p.) whereas rejection is observed at 7 ± 1 days in controls (Table 4). Despite its good activity in the mouse GVHD test, **13a** was unable to control graft rejection in rats at 6 mg/kg. Compounds **13e** and **19a** were only slightly active in this model, showing that it is a good discrimination step. However **13s**, which can be seen as a retro-analogue of DSG, induced a 4- to 5-fold graft survival increase compared to the control. Finally, **27a** proved to be the best tested compound of the series, being slightly more active than DSG. This compound was thus selected as a candidate for further preclinical investigation. In particular, it proved to be far more stable than DSG as assessed by its chemical stability studies. Figure 1 shows the kinetics of degradation of DSG and **27a** in aqueous solution at different pH at 37 °C. DSG chemical stability is clearly pH dependent. Its half-life is around 15 days at pH 1, 2 days at pH 7, and a few hours at pH 10. As anticipated, the stability of **27a** is much better: at acidic or neutral pH, **27a** degradation is less than 10% after 50 days. However at pH 10, its half-life is around 1 day. This high improvement in chemical stability makes the handling

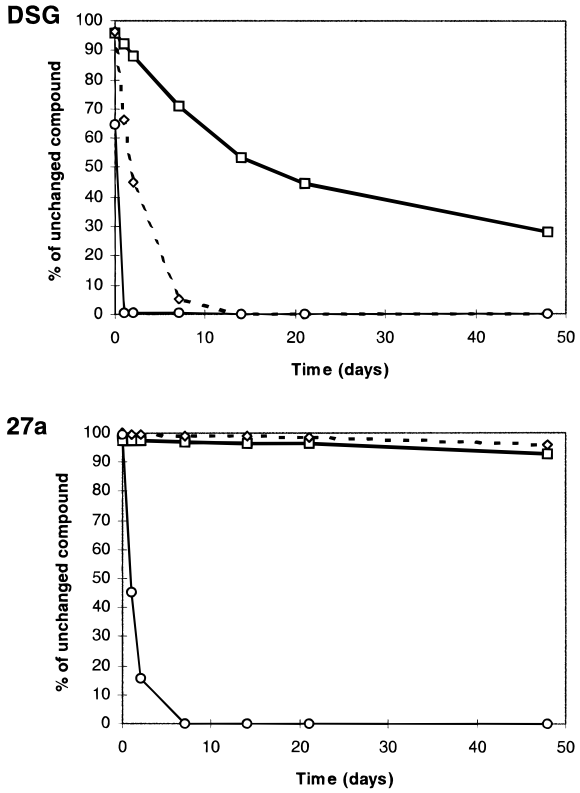


Figure 1. The chemical stability of DSG and **27a** in aqueous buffers at pH 1 (□), pH 7 (◇), and pH 10 (○) at 37 °C.

of **27a** much easier in aqueous medium. In particular, purification, formulation, and dosing can be carried out without any problem of degradation. This practical aspect of this freely water soluble compound intended to be administered by injection is crucial for its development.

Conclusion

In the present study we have synthesized a series of new DSG analogues in order to investigate the structural requirement for the so-called "C" region of the molecule in term of immunosuppressive activity. It proved to be rather stringent. However, we have shown that the left amide bond can be present in both directions but other functions such as urea, carbamate, and a two methylene unit are not compatible. N-Methylation of this amide is also precluded. Nevertheless the right amide bond can be favorably replaced by a carbamate in both directions and less successfully with an urea. N-Methylation of these functionalities led to inactive compounds. The central linker should be either a single bond or a methylene which can also be monosubstituted by a methoxy or hydroxyl group or by a fluorine atom.

This study allowed us to select some DSG analogues which have the same range of potency as DSG without containing chirality. Moreover, their chemical stability was strongly improved in comparison to DSG, which makes their industrial synthesis much more accessible. Due to its optimum biological profile in terms of immunosuppressive properties in the heart allograft model[28,29] and in a GVHD model after bone marrow transplantation[30] and also in terms of safety pharmacology and toxicology, **27a** was selected as a drug candidate and is currently undergoing clinical evaluation.

Experimental Section

Chemistry. ^{1}H Nuclear magnetic resonance spectra were obtained on a Bruker 300 at 300 MHz. The chemical shifts are expressed in δ values (part per million) relative to tetramethylsilane as internal standard. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons.

The identity of final compounds was verified by exact mass spectral determinations performed by the University of Rennes I (Centre Régional de Mesures Physiques de l'Ouest). High-resolution mass spectra (HRMS) and low resolution mass spectra (LRMS) were performed using a ZABSpec TOF from V. G. Analytical (L-SIMS ionization mode, Cs^+, mNBA).

Thin-layer chromatography was performed on silica gel $60F_{254}$ plates or RP-$18F_{254S}$ (Merck). Merck silica gel 60 (230−400 mesh) was used for flash column chromatography and lichroprep RP-18 Merck (5−20 μm) for medium-pressure liquid chromatography.

DSG was prepared following Umezawa's procedure.[14]

General Procedure for the Preparation of Compounds 13a−t and 15. Compounds of the general structure **13a−t** (Scheme 3) were synthesized from carboxylic acid derivatives **4a−t**, and 4-aminobutyl[3-[(1,1-dimethylethoxy)carbonylamino]propyl]carbamic acid, 1,1-dimethyl-ethyl ester **9** by the representative procedure illustrated for analogue **13a**.

Carboxylic acid derivatives **4a−t** were obtained in three steps (Scheme 1) from *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methoxy isothiourea **1** which was prepared according to the procedure described by R. J. Bergeron and J. S. MacManis.[23]

The compounds **9** and **10** were prepared in three steps as follows (Scheme 2).

4-[(3-Aminopropyl)amino]butanol (7a). 4-Chloro-1-butanol (2.2 g, 20.0 mmol) was added into a solution of 1,3-diaminopropane (3.0 g, 40.0 mmol), potassium iodide (0.33 g, 2.0 mmol), and potassium carbonate (1.4 g, 10.0 mmol) in butanol (50 mL). The mixture was stirred at reflux for 24 h. Then the mixture was slowly cooled to room temperature, filtered, and concentrated under vacuum to afford **7a** as a colorless oil which was used without further purification (2.8 g, 86% yield). ^{1}H NMR (DMSO-d_6) δ: 1.4−1.5 (m, 6H), 2.4−2.6 (m, 6H), 3.4−3.5 (m, 2H).

(4-Hydroxybutyl)[3-(1,1-dimethylethoxy)carbonylamino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (8a). This compound was obtained from 4-[(3-aminopropyl)amino]-butan-1-ol (2 g, 13.67 mmol) according to the procedure described by F. Veznik et al.[31] as a colorless oil (4.45 g, 94% yield).

(4-Aminobutyl)[3-[(1,1-dimethylethoxy)carbonylamino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9). To a stirred solution of **8a** (1 g, 2.9 mmol) and triethylamine (0.7 g, 3.76 mmol) in CH_2Cl_2 (10 mL) cooled to −20 °C was added dropwise a solution of methanesulfonyl chloride (0.43 g, 3.76 mmol) in CH_2Cl_2 (5 mL). The stirring was continued at −20 °C for 10 min, and then the reaction mixture was allowed to warm to room temperature. An additional portion of CH_2Cl_2 was added, and the solution was washed with saturated K_2CO_3 solution. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum.

The obtained crude product was taken up in ethanol (20 mL) and ammonium hydroxide (20 mL). The reaction mixture was then stirred for 48 h at room temperature. The solvents were removed under vacuum, and the oily residue was purified by flash chromatography on silica gel (EtOAc then EtOAc/EtOH/NH_4OH 6/3/0.1) to afford **9** as a clear oil (0.46 g, 46%). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.6 (m, 24H), 2.7 (t, 2H), 3.1−3.25 (m, 6H), 4.8 (s, 1H), 5.3 (s, 1H).

[4-(Methylamino)butyl] [3-[(1,1-dimethylethoxy)carbonyl amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (10). Starting with **8a**, the same procedure described for the synthesis of **9** but using methylamine as nucleophilic agent was applied to **10** obtained in 72% yield. ^{1}H NMR

($CDCl_3$) δ: 1.4−1.8 (m, 24H), 2.7 (s, 3H), 2.9−3.3 (m, 8H), 4.8−5.4 (br s, 1H), 8.1−9.5 (br s, 1H).

5-[*N*-[3[(1,1-dimethylethoxy)carbonylamino]-propyl]-*N*-[(dimethylethoxy)carbonyl]-amino]-pentanoic Acid (11a). Pyridinium dichromate (77.5 g, 206.0 mmol) was added in small portions to a solution of **8b** (21.2 g, 58.88 mmol) in DMF (100 mL). The reaction medium was stirred for 6 h at room temperature, then diluted with H_2O (700 mL) and extracted with Et_2O (3 × 150 mL). The organic phase was washed with a $CuSO_4$ solution, dried, concentrated, and purified by flash chromatography on silica gel (EtOAc/hexane 5/5 then EtOAc) to afford **11a** (20.0 g, 91% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.4−1.7 (m, 24H), 2.3 (t, 2H), 3.0−3.3 (m, 6H), 4.7 (br s, 1H), 5.3 (br s, 1H).

[(6-Aminohexyl)carbonimidoyl]bis-carbamic Acid, Bis-(1,1-dimethylethyl) Ester (2). Hexane-1,6-diamine (17.23 g, 0.148 mol) was added at room temperature to a stirred solution of *N*,*N*′-bis (*tert*-butoxycarbonyl)-*S*-methylisothiourea (43.0 g, 0.148 mol) in THF (300 mL). The reaction medium was stirred for 16 h. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel ($CHCl_3$/EtOH 3/1 then EtOAc/MeOH/32% aqueous ammonia mixture 6/3/0.1) to afford **2** (19.7 g, 37%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.25−1.60 (m, 28H), 2.7 (t, 2H), 3.5 (q, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-12-oxo-2,4,-11-triaza-tetradec-2-enedioic Acid, 1-[1,1-Dimethylethyl Ester]14-ethyl Ester (3a). To a stirred solution of ethyl hydrogen malonate (3.7 g, 28.0 mmol) in CH_2Cl_2 (50 mL) were added at 0 °C 1,3-dicyclohexyl carbodiimide (DCC) (5.8 g, 28.0 mmol) and 1-hydroxybenzotriazole hydrate (HOBT) (0.4 g, 3 mmol). The mixture was allowed to stand for half an hour at 0 °C. Then **2** (5 g, 14 mmol) in solution in CH_2Cl_2 (30 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature overnight. The solvent was evaporated off under reduced pressure, and the oily residue was purified by flash chromatography on silica gel (EtOAc/methylcyclohexane 1/1) to afford **3a** (6.27 g, 95% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 29H), 3.2−3.3 (m, 4H), 3.4 (q, 2H), 4.2 (q, 2H), 7.2 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-12-oxo-2,4,-11-triaza-tetradec-2-enedioic Acid, 1-[1,1-Dimethylethyl Ester] (4a). 3a (1.97 g, 4.19 mmol) was dissolved in a mixture of 1 N NaOH (20 mL) and DME (20 mL). The reaction mixture was stirred for 1 h at room temperature, concentrated to a third of its volume, and then acidified to pH 2 with 1 N HCl. Extraction of the water layer with 2 × 50 mL of $CHCl_3$, followed by evaporation of the organic phases under reduced pressure, afforded a residue which was purified by flash chromatography on silica gel (EtOAc/EtOH 1/9) to give **4a** (1.29 g, 70%). ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 24H), 3.1−3.4 (m, 6H), 7.7 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-20-[(1,1-dimethylethoxy)carbonyl]-12,14-dioxo-2,4,11,15,20,24-hexaazapentacosenedioic Acid, Bis(1,1-dimethylethyl) Ester (12a). To a stirred solution of **4a** (1.25 g, 2.8 mmol) in CH_2Cl_2 (20 mL) were added at 0 °C DCC (0.57 g, 2.8 mmol) and HOBT (0.07 g, 0.5 mmol). The mixture was allowed to stand for 0.5 h at 0 °C. Then **9** (1 g, 2.9 mmol) in solution in CH_2Cl_2 (10 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature and left to stand one night. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc/methylcyclohexane 1/1 then EtOAc/MeOH 9/1) to afford **12a** (1.6 g, 73%) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 50H), 3.1−3.3 (m, 12H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 and 7.15 (br s, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

***N*-[4-[[3-(Amino)propyl]amino]butyl]-*N*′-[6-[(aminoiminomethyl)amino]hexyl]propanediamide, Tris(trifluoroacetate) (13a). 12a** (0.7 g, 0.9 mmol) was dissolved in 10 mL of TFA and 10 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 24 h at room temperature, and the solvents were then removed under reduced pressure. The obtained residue was taken up in 150 mL of distilled water and then lyophilized. The residue was purified by MPLC (medium-pressure liquid chromatography) on a RP18 silica (type grafted, particle size 5 to 20 μm) using a water/CH_3CN/TFA mixture (7/2/1, v/v/v) as eluent to afford **13a** (0.43 g, 66% yield) in the form of a translucent white amorphous solid. ^{1}H NMR (DMSO-d_6) δ: 1.2−1.6 (m, 12H), 1.9 (m, 2H), 2.9−3.1 (m, 14H), 7.2 (br s, 4H), (t, 1H), 8 (m, 5H), 8.7 (br s, 2H). ^{13}C NMR (DMSO-d_6) δ: 22.5, 23.4, 25.4, 25.6, 25.7, 28.0, 28.5, 35.8, 37.6, 38.2, 40.3, 43.0, 43.5, 46.1, 156.6, 166.9 (2C).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-20-[(1,1-dimethylethoxy)carbonyl]-15-methyl-12,14-dioxo-2,4,11,-15,20,24-hexaazapentacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (14). 14 (1,15 g, 65%) was obtained in the form of an oil according to the method described for **12a** starting from **4a** (1 g, 2.25 mmol) and **10** (0.86 g, 2.4 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 50H), 3.1−3.3 (m, 15H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

***N*-[4-[(3-Aminopropyl)amino]butyl]-*N*-[6[(aminoiminomethyl)amino]hexyl]-*N*-methyl-propanediamide, Tris-(trifluoroacetate) (15). 15** (0.72 g, 72%) was obtained as an oil according to the method described for **13a** starting from **14** (1 g, 1.27 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.6 (m, 12H), 1.8−2.0 (m, 2H), 2.8−3.1 (m, 13H), 3.2−3.4 (m, 4H),7.8−8.0 (m, 3H), 8.0−8.1 (m, 1H), 8.5−8.8 (m, 4H). ^{13}C NMR (DMSO-d_6) δ: 23.5, 24.1, 24.5, 26.2, 26.3, 26.4, 28.1, 28.9, 35.2, 36.5, 37.3, 40.3, 41.9, 45.2, 48.2, 158.5, 168.6, 168.8.

General Procedure for the Preparation of Compounds 27a−d. Compounds of the general structure **27a−d** (Scheme 5) were synthesized from carboxylic acid derivatives **25a−d** and **2** or **6** by the representative procedure illustrated for **27a.** Carboxylic acid derivatives **25a−d** were obtained in two steps from **9** or **10**. **6** was prepared in three steps as follows (Scheme 1).

Preparation of Compound 6. [(6-Hydroxyhexyl)carbonimidoyl]biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (5). Amino-6 hexanol (4.24 g, 35.0 mmol) was added at room temperature, with stirring, to a solution of N,N′-bis (*tert*-butoxycarbonyl)-S-methylisothiourea (9,54 g, 32.8 mmol) in THF (100 mL). The reaction medium was stirred for 4 days. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 7.5/2.5) to afford **5** (9.05 g, 77% yield). ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 26H), 3.35−3.5 (m, 2H), 3.6−3.7 (m, 2H), 8.2−8.4 (br s, 1H), 11.5 (br s, 1H).

[(6-Aminomethylhexyl)carbonimidoyl]biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (6). To a stirred solution of **5** (2 g, 5.57 mmol) and triethylamine (1.4 g, 13.8 mmol) in CH_2Cl_2 (20 mL) cooled to − 20 °C was added dropwise a solution of methanesulfonyl chloride (0.8 g, 7 mmol) in CH_2Cl_2 (5 mL). The stirring was continued at − 20 °C for 15 min, and the mixture was then allowed to warm to room temperature. An additional portion of CH_2Cl_2 was added, and the solution was washed with a saturated K_2CO_3 solution. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The obtained crude product was taken up in DME (5 mL) and methylamine in aqueous solution (40%) (6.2 mL, 80 mmol). The reaction mixture was then stirred for 18 h. The solvents were removed under vacuum, and the oily residue was taken up in EtOAc (100 mL) and washed with a brine solution. The organic layer was dried over $MgSO_4$, filtered, and concentrated under vacuum. The obtained oily residue was purified by flash chromatography on silica gel (EtOAc/EtOH/NH_4OH 6/3/0.1) to afford **6** as a yellow oil (1.7 g, 83% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 26H), 2.50 (s, 3H); 2.68 (t, 2H), 3.40 (t, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

Preparation of Compound 27a. 6-[(1,1 Dimethylethoxy)-carbonyl]-13-oxa-12-oxo-2,6,11-triazapentadecanedioic Acid, 1-(1,1-Dimethylethyl) Ester 15-Methyl Ester (24a). A solution of **9** (7.4 g, 21.4 mmol) in toluene (20 mL) was added

to a stirred solution of methyl [(phenoxycarbonyl) oxy] acetate (4.5 g, 21.4 mmol) in toluene (100 mL). The reaction medium was refluxed for 15 h. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 7/3 and then EtOAc) to afford **24a** (8.4 g, 85%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.45−1.65 (m, 24H), 3.05−3.25 (m, 8H), 3.8 (s, 3H), 4.7 (s, 2H), 4.9 and 5.3 (br s, 1H).

6[(1,1-Dimethylethoxy)carbonyl]-13-oxa-12-oxo-2,6,11-triazapentadecanedioic Acid, 1-(1,1-Dimethylethyl Ester) (25a). This compound was obtained (6.7 g, 81% yield) as an oil by following a procedure analogous to preparation of **4a** and starting from **24a** (8.45 g, 18.3 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.8 (m, 24H), 3.10−3.25 (m, 8H), 4.7 (s, 2H), 5.0 (t, 1H), 6.8 (s, 1H).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-21-[(1,1-dimethylethoxy)carbonyl]-14-oxa-12,15-dioxo 2,4,11,16,21,-25-hexaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (26a). This compound was obtained (3.85 g, 73% yield) in the form of an oil by following a procedure analogous to preparation of **12a** and starting from **25a** (3.0 g, 6.7 mmol) and **2** (2.4 g, 6.7 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.35−1.65 (m, 50H), 3.10−3.30 (m, 12H), 4.55 (s, 2H), 5.2−5.5 (br s, 1H), 6.4 (br s, 1H).

4-[3-(Aminopropyl)amino]butylcarbamic Acid, 2-[[6-[(aminoiminomethyl)amino]-hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (27a). This compound was obtained as a translucent white amorphous solid (2.61 g, 73% yield) according to the procedure described for **13a** and starting from **26a** (3.85 g, 4.9 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.25−1.55 (m, 12H), 1.9 (m, 2H), 2.80−3.10 (m, 12H), 4.35 (s, 2H), 5.5−8.6 (m, 12H). ^{13}C NMR (D_2O) δ: 23.6, 24.5, 26.2, 26.3, 26.7, 28.6, 29.0, 37.3, 39.8, 40.6, 41.9, 45.2, 48.2, 63.7, 157.5, 158.2, 171.4.

General Procedure for the Preparation of Compounds 19a−c, 21a−b, and 23. Compounds of the general structure **19a−c**, **21a−b**, and **23** were synthesized according to the Scheme 4 by the representative procedures illustrated for analogues **19a**, **21a**, and **23**.

Preparation of Compound 19a. 3-[[(1,1-Dimethylethoxy)carbonyl]amino]-12-oxo-2,4,11,14-tetraazapentadec-2-enedioic Acid, 1-(1,1-Dimethylethyl) Ester 15-Phenylmethyl Ester (16a). A solution of isobutyl chloroformate (1.6 g, 14.0 mmol) in THF (5 mL) was added dropwise to a solution cooled to −30 °C of carbobenzyloxyglycine (3.0 g, 14.0 mmol) and *N*-methylmorpholine (2.8 g, 28 mmol) in THF (50 mL). The reaction medium was stirred for 0.5 h and a solution of **2** (5.4 g, 14 mmol) in THF (20 mL) was added. Stirring was maintained for 2 h at −30 °C and then for 24 h at room temperature. After filtration of the reaction medium and evaporation of the filtrate under vacuum, the residue obtained was purified by flash chromatography on silica gel (EtOAc/methylcyclohexane 1/1) to afford **16a** as an oil (7.16 g, 91% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 26H), 3.2 (q, 2H), 3.4 (q, 2H), 3.8 (d, 2H), 5.15 (s, 2H), 5.5 (br s, 1H), 6.0 (br s, 1H), 7.3 (s, 5H).

13-Amino-3-[[(1,1-dimethylethoxy)carbonyl]amino]-12-oxo-2,4,11-triazatridec-2-enoic Acid, 1,1-Dimethylethyl Ester (17a). A mixture of **16a** (7.1 g, 13 mmol) and 10% palladium on carbon (0.7 g) in ethanol (120 mL) was stirred at room temperature and under a hydrogen atmosphere for 2 h at atmospheric pressure. The catalyst was then filtered off, and the organic phase was evaporated to give **17a** (5.3 g, 98% yield) in the form of an oily residue which was used without further purification for the preparation of **18a**. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.6 (m, 28H), 3.25−3.45 (m, 6H), 7.3 (s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-21-[(1,1-dimethylethoxy)carbonyl]-12,15-dioxo-2,4,11,14,16,21,25-heptaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (18a). Bis(4-nitrophenyl)carbonate (4.3 g, 13 mmol) was added in small portions to a solution of **17a** (5.3 g, 12 mmol) in anhydrous THF (50 mL). The reaction medium was stirred for 1 h at room temperature, and a solution of **9** (4.5 g, 13 mmol) in anhydrous THF (50 mL) was added dropwise. Stirring was continued for 24 h at room temperature, and the solvent was evaporated off under reduced pressure. The obtained residue was purified by flash chromatography on silica gel (EtOAc) to afford **18a** (6.01 g, 64% yield) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 50H), 3.1−3.35 (m, 12H), 3.8 (d, 2H), 4.8 and 5.8 (br s, 3H), 6.9 (t, 1H), 8.3 (t, 1H); 11.5 (s, 1H).

***N*-[4-[[3-(Amino)propyl]amino]butyl]-*N′*-[[[6-[(aminoiminomethyl)amino]hexyl]amino]carbonyl]methyl]-urea, Tris(trifluoroacetate) (19a).** This compound was obtained as an oil (4.75 g, 86% yield) according to the procedure described for **13a** and starting from **18a** (6 g, 7.3 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.65 (m, 12H), 1.9 (m, 2H), 2.9−3.15 (m, 12H), 3.6 (d, 2H), 6.1 (t, 1H), 6.3 (t, 1H), 6.8−9 (m, 11H). ^{13}C NMR (DMSO-d_6) δ: 22.8, 23.7, 25.6, 25.8, 27.0, 28.3, 28.9, 36.1, 38.3, 38.5, 40.5, 42.7, 43.7, 46.5, 156.7, 157.9, 169.6.

Preparation of Compound 21a. 3-[(1,1-Dimethylethoxy)-carbonylamino]-20-[(1,1-dimethylethoxy)carbonyl]-12,-15-dioxo-2,4,11,14,20,24-hexaaza pentacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (20a). A solution of isobutyl chloroformate (0.97 g, 7.1 mmol) in THF (5 mL) was added dropwise to a solution cooled to −30 °C of **11a** (2.7 g, 7.1 mmol) and *N*-methylmorpholine (0.72 g, 7.1 mmol) in THF (40 mL). The reaction medium was stirred for 0.5 h and a solution of **17a** (2.9 g, 7.1 mmol) in THF (30 mL) was added. Stirring was maintained for 2 h at − 30 °C and then for 24 h at room temperature. After filtration of the reaction mixture and evaporation of the filtrate under vacuum, the residue obtained was purified by flash chromatography on silica gel (EtOAc) to afford **20a** as an oil (1.9 g, 34% yield). ^{1}H NMR ($CDCl_3$) δ: 1.1−1.6 (m, 50H), 2.1−2.2 (t, 2H), 2.8−2.9 (q, 2H), 3.0−3.1 (m, 6H), 3.2−3.3 (q, 2H), 3.6 (d, 2H), 6.7−6.9 (bs, 1 H), 7.7 (t, 1H), 8.0 (t, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

5-[(3-Aminopropyl)amino]-*N*-[2-[6-[(aminoiminomethyl)amino]hexylamino]-2-oxoethyl]pentanamide (21a). This compound was obtained as an oil (0.7 g, 90%) according to the procedure described for **13a** and starting from **20a** (0.84 g, 1.1 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.7 (m, 12H), 1.8−1.9 (m, 2H), 2.2 (t, 2H), 2.8−3.2 (m, 10H), 3.6 (d, 2H), 7.7 (t, 1H), 7.8−8.0 (m, 4H), 8.1 (t, 1H), 8.5−8.7 (m, 3H). ^{13}C NMR (D_2O) δ: 22.8, 24.6, 25.8, 26.2, 26.3, 28.6, 29.0, 35.4, 37.4, 40.0, 41.9, 43.4, 45.2, 48.2, 157.5, 172.0, 177.4.

Preparation of Compound 23. 3-[[(1,1-Dimethylethoxy)-carbonyl]amino]-21-[(1,1-dimethylethoxy)carbonyl]-1,2,-15-dioxo-16-oxa-2,4,11,14,21,25-hexaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (22). To a stirred solution of **8a** (1.0 g, 2.89 mmol) and triethylamine (0.45 g, 4.5 mmol) in THF (20 mL) was added a solution of 4-nitrophenyl chloroformate (0.58 g, 2.89 mmol) in THF (5 mL). The reaction mixture was stirred for 15 h at room temperature, and a solution of **17a** (1.2 g, 2.89 mmol) in THF (10 mL) was then added. The reaction mixture was heated to 40 °C and stirred for 5 h. After concentration of the reaction mixture under reduced pressure, the residue was purified by MPLC on silica gel (methylcyclohexane/EtOAc 7/3 and then EtOAc) to afford **22** (1.0 g, 44% yield) as a transparent oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.9 (m, 50H), 3.05−3.55 (m, 10H), 3.85 (d, 2H), 4.10 (t, 2H), 4.7−5.3 (br s, 1H), 5.4−5.6 (br s, 1H), 6.0−6.2 (br s, 1H), 8.3−8.5 (br s, 1H), 11.5 (s, 1H).

[4-[(3-Aminopropyl)amino]butoxycarbonylamino]-*N*-[6-[(aminoiminomethyl)amino]hexyl]acetamide, Tris-(trifluoroacetate) (23). This compound was obtained in the form of a translucent white amorphous solid (0.78 g, 84% yield) according to the procedure described for **13a** and starting from **22** (1.0 g, 1.25 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.55 (m, 8H), 1.6−1.75 (m, 4H), 1.8−1.95 (m, 2H), 2.8−3.1 (m, 10H), 3.55 (d, 2H), 3.95 (t, 2H), 6.7−7.4 (m, 4H), 7.5 (t, 1H), 7.75−8.0 (m, 4H), 8.5−8.65 (m, 3H). ^{13}C NMR (D_2O) δ: 23.0, 24.5, 26.1, 26.2, 26.3, 28.5, 28.9, 31.0, 37.3, 39.9, 41.8, 45.2, 48.1, 65.7, 158.0, 159.0, 178.5.

Procedure for the Preparation of Compound 31. 31 was synthesized from 10-[(phenylmethoxy)carbonyl amino]-decanoic acid and **9** in four steps as follows:

18-[(1,1-Dimethylethoxy)carbonyl]-12-oxo-2,13,18,22-tetraazatricosanedioic Acid, 23-(1,1-Dimethylethyl) Ester 1-Phenylmethyl Ester (28). To a stirred solution of 10-[(phenylmethoxy)carbonylamino]decanoic acid (0.92 g, 2.92 mmol) in $CHCl_3$ (30 mL) were added at 0 °C DCC (1.05 g, 5.1 mmol) and HOBT (69 mg, 0.51 mmol). The mixture was allowed to stand for half an hour at 0 °C. Then **9** (1.2 g, 3.47 mmol) in solution in $CHCl_3$ (20 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature overnight. The solvent was evaporated off under reduced pressure and the oily residue was purified by flash chromatography on silica gel (hexane/EtOAc 1/1 then EtOAc) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 38H), 2.1 (t, 2H), 3.0−3.3 (m, 10H), 4.7−4.9 (br s, 1H), 5.1 (s, 2H), 5.2 (br s, 1H), 5.7 (br s, 1H), 7.3−7.4 (m, 5H).

10-Amino-*N*-[4-[(1,1-dimethylethoxycarbonyl)-[3-[(1,1-dimethylethoxycarbonyl)amino]propyl]amino]butyl]decanamide (29). A mixture of **28** (1.1 g, 1.69 mmol) and 10% palladium on carbon (0.1 g) in ethanol (20 mL) was stirred at room temperature and under a hydrogen atmosphere for 4 h at atmospheric pressure. The catalyst was then filtered off, and the organic phase was evaporated to give an oily residue which was purified by flash chromatography on silica gel (EtOAc then EtOAc/MeOH/NH_4OH/6/4/0.1) to afford **29** (0.78 g, 90% yield). ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 38H), 2.1 (t, 2H), 2.3−2.5 (br s, 2H), 2.6−2.8 (br s, 2H), 3.0−3.3 (m, 8H), 5.1 (br s, 1H), 5.8 (br s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-20-[(1,1-dimethylethoxy)carbonyl]-14-oxo-2,4,15,20,24-pentaazapentacos-2-endioic Acid, Bis(1,1-dimethylethyl) Ester (30). 29 (0.78 g, 1.52 mmol) was added at room temperature to a stirred solution of *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **1** (1.76 g, 6.0 mmol) in THF (20 mL). The reaction mixture was stirred at room temperature for 3 days. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel (hexane/EtOAc 1/1 then EtOAc) to afford **30** (0.67 g, 59% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 56H), 2.1 (t, 2H), 2.3−3.3 (m, 8H), 3.4 (q, 2H), 5.1 (br s, 1H), 5.7 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

10-(Aminoiminomethylamino)-*N*-[4-[(3-aminopropyl)-amino]butyl]decanamide, Tris(trifluoroacetate) (31). This compound was obtained as a white amorphous solid (0.46 g, 75.4% yield) according to the procedure described for **13a** and starting from **30** (0.67 g, 0.88 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.35 (m, 10H), 1.4−1.6 (m, 8H), 1.8−1.9 (m, 2H), 2.0 (t, 2H), 2.8−3.1 (m, 10H),7.6 (t, 1H), 7.8−8.0 (m, 4H), 8.5−8.7 (br s, 3H). ^{13}C NMR (D_2O) δ: 23.8, 24.5, 26.2, 26.4, 26.5, 28.6, 28.9, 29.0, 29.1, 29.2, 36.6, 37.3, 39.2, 42.0, 45.2, 48.2.

Procedure for the Preparation of Compound 35. 35 was synthesized from **9** and 3-[(1,1-dimethylethoxy)carbonylamino]-13-oxa-12-oxo-2,4,11-triazapentadec-2-enedioic acid, 1-(1,1-dimethylethyl) ester, **33** which was obtained in two steps as follows.

3-[(1,1-Dimethylethoxy)carbonylamino]-13-oxa-12-oxo-2,4,11-triazapentadec-2-endioic Acid, 1-(1,1-Dimethylethyl) Ester 15-Methyl Ester (32). A solution of **2** (18.78 g, 47.6 mmol) in toluene (700 mL) was added to a stirred solution of methyl [(phenoxycarbonyl)oxy] acetate (10.0 g, 47.6 mmol) in toluene (100 mL). The stirred reaction mixture was heated at 40 °C for 5 h. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (hexane/EtOAc 8/2) to afford **32** (11.8 g, 52% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.6 (m, 26H), 3.2 (q, 2H), 3.4 (q, 2H), 3.8 (s, 3H), 4.6 (s, 2H), 4.8−5.0 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-13-oxa-12-oxo-2,4,11-triazapentadec-2-enedioic Acid, 1-(1,1-Dimethylethyl) Ester (33). This compound was obtained (8.9 g, 78.5% yield) as an oil by following a procedure analogous to preparation of **4a** and starting from **32** (11.7 g, 24.7 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 26H), 3.2−3.4 (m, 4H), 4.5 (s, 2H), 4.9 (t, 1H), 8.4 (s, 1H), 11.0−11.5 (br s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-21-[(1,1-dimethylethoxy)carbonyl]-13-oxa-12,15-dioxo-2,4,11,16,21,-25-hexaazacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (34). To a stirred solution of **33** (3.0 g, 6.52 mmol) in CH_2Cl_2 (60 mL) were added at 0 °C DCC (2.7 g, 13.1 mmol) and HOBT (0.176 g, 1.31 mmol). This mixture was allowed to stand for 0.5 h at 0 °C. Then **9** (2.25 g, 6.52 mmol) in solution in CH_2Cl_2 (30 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature and left to stand 2 days. The solvent was evaporated off under reduced pressure and the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 3/7) to afford **34** (4.5 g, 87.8% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.8 (m, 50H), 3.1−3.5 (m, 12H), 4.5 (s, 2H), 4.8−5.0 (br s, 1H), 5.2−5.4 (br s, 1H), 6.2−6.6 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

[6-(Aminoiminomethylamino)hexyl]carbamic Acid, 2-[[4-[(3-Aminopropyl)amino]-butyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (35). This compound was obtained in the form of a translucent white amorphous solid (4.0 g, 84.7% yield) according to the procedure described for **13a** and starting from **34** (4.5 g, 5.72 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.6 (m, 12H), 1.8−2.0 (m, 2H), 2.3−3.2 (m, 12H), 4.3 (s, 2H), 7.2 (t, 1H),7.6 (t, 1H), 7.8−8.0 (m, 4H), 8.5−8.7 (br s, 3H). ^{13}C NMR (D_2O) δ: 23.6, 24.5, 26.2, 26.3, 26.4, 28.5, 29.4, 37.3, 38.9, 41.8, 45.2, 48.1, 63.7, 157.9, 158.2, 172.0.

Procedure for the Preparation of Compound 39. 39 was synthesized from **9** and carbobenzyloxyglycine in four steps as follows.

10-[(1,1-Dimethylethoxy)carbonyl]-4-oxo-2,5,10,14-tetraazapentadecanedioic Acid, 1-Phenylmethyl 15-(1,1-Dimethylethyl) Ester (36). To a stirred solution of carbobenzyloxyglycine (7.06 g, 34.0 mmol) in $CHCl_3$ (100 mL) were added DCC (7.0 g, 34 mmol) and HOBT (0.95 g, 7 mmol). The mixture was allowed to stand for half an hour at 0 °C. Then **9** (9.32 g, 27 mmol) in solution in $CHCl_3$ (100 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature overnight. The solvent was evaporated off under reduced pressure, and the oily residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 1/1 then EtOAc) to afford **36** (12.5 g, 86% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 24H), 3.0−3.4 (m, 8H), 3.8 (d, 2H), 4.8−5.0 (br s, 1H), 5.1 (s, 2H),5.3−5.7 (br s, 1H), 6.1−6.6 (br s, 1H), 7.3−7.4 (m, 5H).

[3-[[(1,1-Dimethylethoxy)carbonyl][4-[2-amino-1-oxoethyl)amino]butyl]amino]propyl]carbamic Acid, (1,1-Dimethylethyl) Ester (37). A mixture of **36** (12.5 g, 23.32 mmol) and 10% palladium on carbon (0.88 g) in ethanol (200 mL) was stirred at room temperature under a hydrogen atmosphere for 5 h at atmospheric pressure. The catalyst was then filtered off, and the organic phase was evaporated to give **37** (9.3 g, quantitative yield) in the form of an oily residue which was used without further purification for the preparation of **38**. ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 24H), 1.9−2.0 (br s, 2H), 3.0−3.4 (m, 10H), 4.5 (br s, 1H), 5.3−5.4 (br s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-21-[(1,1-dimethylethoxy)carbonyl]-12,15-dioxo-2,4,11,13,16,21,25-heptaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (38). 4-Nitrophenyl chloroformate (0.5 g, 2.5 mmol) was added in small portions to a solution of **37** (1.0 g, 2.5 mmol) and triethylamine (2.5 mmol) in anhydrous THF (50 mL). The reaction medium was stirred for 1 h at room temperature, and then a solution of **2** (0.9 g, 2.5 mmol) in anhydrous THF (20 mL) was added dropwise. Stirring was continued overnight at room temperature, and the solvent was evaporated off under reduced pressure. The obtained residue was purified by flash chromatography on silica gel (EtOAc, MeOH 9.5/0.5) to afford **38** (1.1 g, 56% yield). ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 50H), 2.8−3.1 (m, 12H), 3.2 (q, 2H), 5.9 (t, 1H), 6.1 (t, 1H), 6.7−6.8 (br s, 1H), 7.8 (t, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

***N*-[6-(Aminoiminomethylamino)hexyl]-*N*′-[2-[[4-[(3-aminopropyl)amino]butyl]amino]-2-oxoethyl]urea, Tris(trifluoroacetate) (39).** This compound was obtained in the form

of an oil (0.58 g, 53% yield) according to the procedure described for **13a** and starting from **38** (1.2 g, 1.5 mmol). ^{1}H NMR (D_2O) δ: 1.2−1.7 (m, 12H), 1.9−2.1 (m, 2H), 3.0−3.2 (m, 14H). ^{13}C NMR (D_2O) δ: 23.8, 24.6, 26.3, 26.4, 26.5, 28.7, 29.7, 37.6, 38.3, 40.3, 41.9, 44.1, 45.1, 48.1, 157.8, 161.5, 174.3.

Procedure for the Preparation of Compound 42. 42 was synthesized from **2** and thioglycolic acid in three steps as follows.

[6[(2-Mercapto-1-oxoethyl)amino]hexyl]carbonimidoyl]-biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (40). Carbonyldiimidazole (1.4 g, 8.4 mmol) was added in small portions to a solution of thioglycolic acid (0.55 mL, 7.7 mmol) in CH_2Cl_2 (30 mL). The reaction medium was stirred overnight at room temperature, and then a solution of **2** (2.5 g, 7 mmol) in CH_2Cl_2 (20 mL) was added dropwise. Stirring was continued for 5 h at room temperature, and the solvent was evaporated off under reduced pressure. The obtained residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 1/1) to afford **40** as an oil (1.5 g, 55% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 26H), 1.9 (t, 1H), 3.2−3.4 (m, 4H), 3.5−3.6 (q, 2H), 6.6−6.8 (br s, 1H),8.3 (t, 1H), 11.5 (s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-21-[(1,1-dimethylethoxy)carbonyl]-12,15-dioxo-14-thia-2,4,11,16,-21,25-hexaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (41). To a stirred solution of **40** (1.37 g, 3.5 mmol) and triethylamine (0.7 g, 7.0 mmol) in THF (25 mL) was added a solution of 4-nitrophenyl chloroformate (0.7 g, 3.5 mmol) in THF (5 mL). The reaction mixture was stirred for 1 h at room temperature, and a solution of **9** (1.2 g, 3.5 mmol) in THF (10 mL) was then added. Stirring was continued for one night at room temperature. After concentration of the reaction medium under reduced pressure, the residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 6/4) to afford **41** (2.0 g, 71% yield) as a transparent oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.8 (m, 50H), 3.1−3.4 (m, 12H), 3.5 (s, 2H), 4.7−5.3 (br s, 1H), 6.7−6.8 (br s, 1H), 7.0−7.2 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

[4-[(3-Aminopropyl)amino]butyl]carbamothioic Acid, *S*-[2-[[6-(Aminoiminomethylamino)hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (42). This compound was obtained in the form of a translucent white amorphous solid (1.45 g, 77% yield) according to the procedure described for **13a** and starting from **41** (2 g, 2.5 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.7 (m, 12H), 1.8−2.0 (m, 2H), 2.9−3.2 (m, 12H), 3.5 (s, 2H), 7.5 (t, 1H), 7.8−8.0 (m, 5H), 8.3 (t, 1H), 8.5−8.6 (br s, 2H). ^{13}C NMR (D_2O) δ: 23.7, 24.5, 26.1, 26.3, 26.5, 28.5, 28.8, 33.8, 37.3, 40.1, 41.1, 41.8, 45.2, 48.1, 157.5, 169.1, 171.8.

Procedure for the Preparation of Compound 46. 46 was synthesized from **9** and methyl 2-(chlorosulfonyl)acetate in four steps as follows.

9-[(1,1-Dimethylethoxy)carbonyl]-3-thia-3,3-dioxo-4,9,-13-triazatetradecanedioic Acid, 14-(1,1-Dimethylethyl) 1-Methyl Ester (43). To a stirred solution of **9** (6.2 g, 18 mmol) and triethylamine (2.8 mL, 20 mmol) in CH_2Cl_2 (40 mL) cooled to −20 °C was added dropwise a solution of methyl 2-(chlorosulfonyl)acetate in CH_2Cl_2 (20 mL). The stirring was continued at −20 °C for 10 min, and the mixture was then allowed to warm to room temperature for 4 h. The solvent was evaporated off under reduced pressure, and the oily residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 1/1) to afford **43** (3.54 g, 41% yield). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.7 (m, 24H), 3.0−3.3 (m, 8H), 3.8 (s, 3H), 4.0 (s, 2H), 4.6−5.3 (br s, 2H).

9-[(1,1-Dimethylethoxy)carbonyl]-3-thia-3,3-dioxo-4,9,-13-triazatetradecanedioic Acid, 14-(1,1-Dimethylethyl) Ester (44). This compound was obtained (2.9 g, 85% yield) as a yellow oil by following a procedure analogous to preparation of **4a** and starting from **43** (3.5 g, 7.3 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 24H), 3.0−3.3 (m, 8H), 4.0 (s, 2H), 4.7−5.4 (br s, 2H).

3-[(1,1-Dimethylethoxy)carbonylamino]-20-[(1,1-dimethylethoxy)carbonyl]-14-thia-14,14-dioxo-2,4,11,15,-20,24-hexaazapentacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (45). To a stirred solution of **44** (2.7 g, 5.8 mmol) in CH_2Cl_2 (30 mL) was added carbonyldiimidazole (1.2 g, 6.5 mmol) in small portions and at room temperature. The reaction mixture was stirred for 3 h. Then **2** (2.28 g, 5.8 mmol) and triethylamine (0.9 mL, 6.0 mmol) in solution in CH_2Cl_2 (20 mL) were added dropwise at room temperature. After the addition was complete, stirring was continued overnight. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 1/1 then 4/1) to give **45** (2.17 g, 46% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 50H), 3.0−3.5 (m, 12H), 3.9 (s, 2H), 4.7−5.0 (br s, 1H), 5.1−5.3 (br s, 1H), 6.4−6.6 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

2-[[4-[(3-Aminopropyl)amino]butyl]aminosulfonyl]-*N*-[6-aminoiminomethylamino)hexyl]-acetamide, Tris(trifluoroacetate) (46). This compound was obtained as an oil (1.47 g, 73% yield) according to the procedure described for **13a** and starting from **45** (2.17 g, 2.7 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.7 (m, 12H), 1.9−2.0 (m, 2H), 2.9−3.2 (m, 12H), 3.9 (s, 2H), 7.2 (t, 1H), 7.7 (t, 1H), 7.8−8.0 (m, 4H), 8.2 (t, 1H), 8.6−8.7 (br s, 2H). ^{13}C NMR (D_2O) δ: 23.2, 24.3, 26.0, 26.1, 27.0, 28.3, 28.5, 37.2, 40.8, 41.7, 42.9, 45.0, 47.8, 58.1, 157.4, 173.3.

Procedure for the Preparation of Compound 50. 50 was synthesized from **6** and 1-(1,1-dimethylethyl)-6-[(1,1-dimethylethoxy)carbonyl]-12-oxo-2,6,11,triazatetradecane dioate **48** obtained in two steps as follows.

6-[(1,1-Dimethylethoxy)carbonyl]-12-oxo-2,6,11-triazatetradecanedioic Acid, 1-(1,1-Dimethylethyl)-14-ethyl Ester (47). To a stirred solution of ethyl hydrogen malonate (1.60 g, 12.0 mmol) in $CHCl_3$ (50 mL) were added at 0 °C DCC (2.50 g, 12.0 mmol) and HOBT (0.13 g, 1 mmol). The mixture was allowed to stand for half an hour at 0 °C. Then **5** (3.12 g, 9.0 mmol) in solution in $CHCl_3$ (20 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature overnight. The solvent was evaporated off under reduced pressure, and the oily residue was purified by flash chromatography on silica gel (EtOAc/hexane 1/1 then EtOAc) to afford **47** (2.67 g, 69%) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H), 1.4−1.7 (m, 24H), 3.10−3.35 (m, 10H), 4.2 (q, 2H).

6-[(1,1-Dimethylethoxy)carbonyl]-12-oxo-2,6,11-triazadecanedioic Acid, 1-(1,1-Dimethylethyl) Ester (48). This compound was obtained (0.75 g, 84% yield) as an oil by following a procedure analogous to preparation of **4a** and starting from **47** (0.95 g, 2.0 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.40 (s, 18H), 1.55 (m, 6H), 2.9 (m, 4H), 3.15 (m, 6H).

3-[(1,1-Dimethylethoxycarbonyl)amino]-11-methyl-20-[1,1-dimethylethoxycarbonyl)-12,14-dioxo-2,4,11,15,20,-24-hexaazapentacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (49). To a stirred solution of **48** (1.2 g, 2.7 mmol) in CH_2Cl_2 (50 mL) were added at 0 °C DCC (1.2 g, 5.4 mmol) and HOBT (0.2 g, 1.5 mmol). The mixture was allowed to stand 0.5 h at 0 °C. Then **6** (1.0 g, 2.7 mmol) in solution in CH_2Cl_2 (30 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature and left to stand one night. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc/EtOH 95/5) to afford **49** (1.8 g, 83% yield) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.7 (m, 50H), 2.9−3.3 (m, 15H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

***N*-Methyl-*N*-[6-(aminoiminomethylamino)hexyl]-*N′*-[4-[(3 aminopropyl)amino]butyl]-malonodiamide (50).** This compound was obtained in the form of a translucent white amorphous solid (1.44 g, 85% yield) according to the procedure described for **13a** and starting from **49** (1.8 g, 2.3 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.35 (m, 4H), 1.4−1.7 (m, 8H), 1.5−2.0 (m, 2H), 2.8−3.0 (m, 9H), 3.05−3.15 (m, 4H), 3.2−3.3 (m, 4H), 7.5−7.65 (br s, 1H), 7.75−7.9 (br s, 3H), 8.0−8.1 (br s, 1H), 8.4−8.6 (br s, 3H). ^{13}C NMR (D_2O) δ: 23.7, 24.5, 26.3, 26.4, 26.9, 28.0, 28.5, 36.6, 37.3, 39.5, 41.8, 45.2, 48.1, 48.7, 51.5, 157.5, 170.0, 170.5.

Procedure for the Preparation of Compound 55. 55 was synthesized from **2** and 3-[[4-[[(1,1-dimethylethoxy)car-

bonyl][3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]amino]-butyl] [(1,1-dimethylethoxy)carbonyl]amino]propanoic acid **53** which was obtained in three steps as follows.

3-[[4-[[(1,1-Dimethylethoxy)carbonyl][3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]amino]butyl]amino] propanoic Acid, Methyl Ester (51). Methylacrylate (0.5 g, 5.8 mmol) in solution in methanol (30 mL) was added with stirring to a solution of **9** (2.0 g, 5.8 mmol) in methanol (20 mL). The reaction was refluxed for 3 h. The solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc then EtOAc/EtOH 6/4) to afford **51** (0.98 g, 39%). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.9 (m, 25H), 2.9−3.3 (m, 12H), 3.72 (s, 3H), 4.7−5.4 (br s, 1H).

3-[[4-[[(1,1-Dimethylethoxy)carbonyl][3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]amino]butyl][(1,1-dimethylethoxy)carbonyl]amino]propanoic Acid, Methyl Ester (52). To a stirred solution of **51** (0.86 g, 2 mmol) and triethylamine (0.14 g, 1.4 mmol) in THF (30 mL) was added a solution of diterbutyl dicarbonate (0.48 g, 2 mmol) in THF (20 mL). The reaction mixture was stirred for 2 h at room temperature. Then the solvent was evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 5/5) to afford **52** (0.9 g, 85%). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 33H), 2.58 (t, 2H), 3.3−3.3 (m, 8H), 3.45 (t, 2H), 3.68 (s, 3H), 4.7−5.4 (br s, 1H).

3-[[4-[[(1,1-Dimethylethoxy)carbonyl][3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]amino]butyl][(1,1-dimethylethoxy)carbonyl]amino]propanoic Acid (53). This compound was obtained (0.74 g, 90% yield) as a yellow oil by following a procedure analogous to preparation of **4a** and starting from **52** (0.34 g, 1.6 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 33H), 2.61 (t, 2H), 3.0−3.3 (m, 8H), 3.5 (t, 2H), 4.7−5.4 (br s, 1H).

3-[(1,1-Dimethylethoxy)carbonylamino]-15,20-bis[(1,1-dimethylethoxy)carbonyl]-12-oxo-2,4,11,15,20,24-hexaazapentacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (54). A solution of isobutylchloroformate (0.165 mL, 1.3 mmol) in THF (5 mL) was added dropwise to a solution, cooled to −30 °C, of **53** (0.66 g, 1.3 mmol) and *N*-methylmorpholine (0.28 mL, 2.6 mmol) in THF (30 mL). The reaction medium was stirred for 0.5 h, and a solution of **2** (0.46 g, 1.3 mmol) in THF (20 mL) was added dropwise. Stirring was maintained for 2 h at −30 °C and then overnight at room temperature. After filtration of the reaction medium and evaporation of the filtrate in vacuo, the residue obtained was purified by flash chromatography on silica gel (EtOAc/cyclohexane 8/2) to afford **54** (0.9 g, 66% yield). ^{1}H NMR ($CDCl_3$) δ: 1.3−1.8 (m, 59H), 2.43 (t, 2H), 3.0−3.3 (m, 10H), 3.4 (t, 2H), 3.46 (t, 2H), 4.7−5.4 (br s, 1H), 6.2−6.4 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

3-[[4-[(3-Aminopropyl)amino]butyl]amino]-*N*-[6-(aminoiminomethylamino)hexyl]-propanamide, Tetrakis(trifluoroacetate) (55). This compound was obtained in the form of an oil (0.6 g, 74% yield) according to the procedure described for **7a** and starting from **54** (0.85 g, 1 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.5 (m, 8H), 1.55−1.7 (m, 4H), 1.8−2.0 (m, 2H), 2.5 (t, 2H), 2.8−3.2 (m, 14H), 7.67 (t, 1H), 7.8−8.0 (br s, 3H), 8.12 (t, 1H), 8.5−8.65 (br s, 2H), 8.7−8.85 (br s, 2H). ^{13}C NMR (D_2O) δ: 23.4, 23.5, 24.5, 26.2, 26.4, 28.5, 28.8, 31.8, 37.3, 40.2, 41.9, 44.3, 45.3, 47.6, 47.8, 157.4, 170.3.

Procedure for the Preparation of Compound 60. This compound was synthesized starting from [(6-oxohexyl)carboimidoyl]biscarbamic acid, bis(1,1-dimethylethyl) ester, **56** and *N*-[4[(2 aminoethoxy)carbonylamino]butyl]-1,3-propanebiscarbamic acid, bis(1,1-dimethyl ethyl ester, **58** obtained, respectively, in one step from [(6-aminomethylhexyl)carboimidoyl]biscarbamic acid, bis(1,1-dimethylethyl) ester, **6** and in two steps from carbamic acid, (2-hydroxyethyl)-phenylmethyl ester as follows.

[(6-Oxohexyl)carboimidoyl]biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (56). Pyridinium dichromate (2.6 g, 7.0 mmol) was added in small portions to a solution of **5** (1.4 g, 3.9 mmol) in CH_2Cl_2 (50 mL). The reaction medium was stirred for 1 day at room temperature, then filtered, and washed with CH_2Cl_2 (3 × 25 mL). The filtrates were evaporated off under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (EtOAc/methylcyclohexane 2.5/7.5) to afford **56** (0.42 g, 30%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.3−1.8 (m, 24H), 2.43 (t, 2H), 3.42 (t, 2H), 8.3 (t, 1H), 9.77 (t, 1H), 11.5 (s, 1H).

12-[1,1-Dimethylethoxycarbonyl]-5-oxa-6-oxo-2,7,12,-16-tetraazaheptadecanedioic Acid, 1-(Phenylmethyl)-17-(1,1-Dimethylethyl) Ester (57). To a stirred solution of carbamic acid (2-hydroxyethyl)-phenylmethyl ester (2.0 g, 10.25 mmol) and triethylamine (2.0 g, 20.5 mmol) in anhydrous toluene (100 mL) was added dropwise at room temperature a solution of phenylchloroformate (2.8 g, 10.25 mmol) in toluene (20 mL). The reaction mixture was stirred for 2 h at room temperature, and a solution of **9** (3.53 g, 10.25 mmol) in toluene (100 mL) was then added. The reaction mixture was heated to 60 °C and stirred for 16 h. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 8/2) to afford **57** (3.3 g, 57%). ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 24H), 3.0−3.3 (m, 8H), 3.4−3.5 (m, 2H), 4.1−4.2 (m, 2H), 4.7−5.0 (br s, 2H), 5.10 (s, 2H), 5.15−5.4 (br s, 1H), 7.3−7.4 (m, 5H).

***N*-[4[(2-Aminoethoxy)carbonylamino]butyl]-1,3-propane-biscarbamic Acid, Bis(1,1-dimethylethyl) Ester (58).** A mixture of **57** (3.3 g, 5.8 mmol) and 10% palladium on carbon (0.4 g) in ethanol (70 mL) was stirred at room temperature under a hydrogen atmosphere for 5 h at atmospheric pressure. The catalyst was then filtered off and the organic phase was evaporated to give **58** (2.45 g, 97% yield) in the form of an oily residue which was used without further purification for the preparation of **59**. ^{1}H NMR ($CDCl_3$) δ: 1.4−1.8 (m, 26H), 2.9 (t, 2H), 3.0−3.4 (m, 8H), 4.08 (t, 2H), 4.6−5.4 (br s, 2H).

3-[(1,1-Dimethylethoxycarbonylamino]-21-[1,1-dimethylethoxycarbonyl)-14-oxa-15-oxo-2,4,11,16,21,25-hexaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (59). To a stirred solution of **56** (0.43 g, 1.2 mmol) and **59** (0.52 g, 1.2 mmol) in EtOH (40 mL) acidified to pH 6 with AcOH was added in small portions $NaBH_3CN$ (0.15 g, 2.5 mmol). The reaction medium was stirred for 1 day. After concentration of the reaction medium under reduced pressure, the residue was taken up in CH_2Cl_2−H_2O (100 mL), and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, concentrated, and purified by flash chromatography on silica gel (EtOAc then EtOAc/EtOH/NH_4OH 8/2/0.02) to afford **59** (0.45 g, 48% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.2−2.2 (m, 50H), 2.7 (t, 2H), 2.8−3.0 (m, 2H), 3.05−3.35 (m, 8H), 3.5 (q, 2H), 4.2 (t, 2H), 4.8 (br s, 1H), 5.0 (br s, 1H), 5.3 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

[4-[(3-Aminopropyl)amino]butyl]carbamic Acid, 2[[6-(Aminoiminomethylamino)hexyl]amino]ethyl Ester (60). This compound was obtained in the form of a translucent white amorphous solid (0.4 g, 44% yield) according to the procedure described for **13a** and starting from **59** (0.85 g, 1.1 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.4 (m, 4H), 1.45−1.7 (m, 8H), 1.8−2.0 (m, 2H), 2.8−3.3 (m, 14H), 4.17 (t, 2H), 7.29 (t, 1H), 7.71 (t, 1H), 7.8−8.0 (br s, 3H), 8.5−8.8 (br s, 4H). ^{13}C NMR (D_2O) δ: 23.7, 24.5, 26.0, 26.1, 26.7, 28.4, 37.3, 40.6, 41.8, 45.2, 47.3, 48.0, 48.2, 48.5, 61.1, 158.4, 170.5.

Procedure for the Preparation of Compound 68. This compound was synthesized with *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **1** and 22-amino-6[(1,1-dimethylethoxy)-carbonyl]-12,16-dioxo-11-oxa-2,6,13,15-tetraaza-docosanoic acid, (1,1-dimethylethyl) ester **66** obtained in six steps from *N*-(6-bromohexanoyl)glycine, ethyl ester and **8a**.

***N*-(6-Cyanohexanoyl)glycine, Ethyl Ester (61).** Powdered potassium cyanide (6.5 g, 100 mmol) was added in small portions to a solution of *N*-(6-bromohexanoyl)glycine, ethyl ester (23.73 g, 84.7 mmol) in EtOH (200 mL). The reaction medium was refluxed for 15 h. After concentration of the mixture under reduced pressure, the residue was taken up with CH_2Cl_2, and the organic phase was washed with a brine solution. The organic phase was dried and concentrated under

reduced pressure to give **61** (19.0 g, 99%) which was used without purification. ^{1}H NMR ($CDCl_3$) δ: 1.29 (t, 3H), 1.45−1.55 (m, 2H), 1.6−1.8 (m, 4H), 1.27 (t, 2H), 2.36 (t, 2H), 4.03 (d, 2H), 4.22 (q, 2H), 5.35−6.05 (br s, 1H).

***N*-(6-Cyanohexanoyl)glycine (62).** This compound was obtained (10.2 g, 61% yield) as a yellow oil by following a procedure analogous to preparation of **4a** and starting from **61** (19.0 g, 84.0 mmol). ^{1}H NMR ($CDCl_3$) δ: 1.45−1.55 (m, 2H), 1.6−1.8 (m, 4H), 2.3 (t, 2H), 2.36 (t, 2H), 4.08 (d, 2H), 6.15−6.25 (br s, 1H).

***N*-(7-Aminoheptanoyl)glycine, Sodium Salt (63).** A mixture of *N*-(6-cyanohexanoyl)glycine (8.2 g, 41.4 mmol) and Raney nickel (800 mg) in ethanol (100 mL) and 1 M NaOH solution (60 mL) was stirred at room temperature under a hydrogen atmosphere and under a pressure of 3.5. 10^5 Pa for 8 h. Then 1 M HCl (20 mL) was added, and the mixture was concentrated under reduced pressure to give **63** (10.2 g) as a white pasty solid which contains the expected salt and sodium chloride and which can be used without further purification in the next step. ^{1}H NMR (DMSO-d_6) δ: 1.15−1.6 (m, 8H), 2.08 (t, 2H), 2.56 (t, 2H), 3.32 (d, 2H), 7.1−7.25 (br s, 1H).

***N*-[7-[(Phenylmethoxycarbonyl)amino]heptanoyl]glycine (64).** To a stirred solution of **63** (2 g, 9.9 mmol) in EtOH (50 mL) and water (50 mL) were added sodium carbonate (1.0 g, 10 mmol) and then dropwise benzylchloroformate (2.3 g, 19.8 mmol). After stirring overnight at room temperature, the reaction medium was brought to pH 1 with 1 M hydrochloric acid and then extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, dried, and concentrated under reduced pressure, and the obtained crude product was purified by flash chromatography on silica gel (EtOAc then EtOAc/EtOH/NH_4-OH 6/3/0.5) to afford **64** (1.3 g, 40% yield) as a white pasty solid. ^{1}H NMR (DMSO-d_6) δ: 1.2−1.6 (m, 8H), 2.09 (t, 2H), 2.98 (td, 2H), 3.64 (d, 2H), 5.01 (s, 2H), 7.25 (t, 1H), 7.3−7.4 (m, 5H), 7.94 (t, 1H).

19-[(1,1-Dimethylethoxy)carbonyl]-9,13-dioxo-14-oxa-2,10,12,19,23-pentaza-tetracosanedioic Acid, 1-(Phenylmethyl) 24-(1,1-Dimethylethyl) Ester (65). To a stirred solution of **64** (0.5 g, 1.49 mmol) and triethylamine (0.18 g, 1.8 mmol) in THF (25 mL) was added dropwise at 0 °C a solution of diphenylphosphoryl azide (0.46 g, 1.67 mmol). The reaction mixture was stirred at room temperature for 45 min. After concentration of the mixture under reduced pressure, the residue was taken up with toluene (20 mL). To this solution were added **8a** (1.04 g, 3.0 mmol) and triethylamine (0.18 g, 1.8 mmol). The solution medium was stirred at reflux for 24 h. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 1/1 to 1/9) to afford **65** (0.4 g, 40% yield). ^{1}H NMR ($CDCl_3$) δ: 1.2−1.7 (m, 32H), 2.15 (t, 2H), 3.05−3.3 (m, 8H), 4.05 (t, 2H), 4.5 (t, 2H), 4.7−4.9 (br s, 1H), 5.08 (s, 2H), 5.7−5.9 (br s, 1H), 6.4−6.6 (br s, 1H), 7.3−7.4 (m, 6H).

22-Amino-6-[(1,1-dimethylethoxy)carbonyl]-12,16-dioxo-11-oxa-2,6,13,15-tetraaza-docosanoic Acid, (1,1-Dimethylethyl) Ester (66). A mixture of **65** (0.33 g, 0.48 mmol) and 5% palladium on carbon (0.1 g) in ethanol (15 mL) was stirred at room temperature and under a hydrogen atmosphere for 5 h at atmospheric pressure. The catalyst was then filtered off, and the organic phase was evaporated to give **66** (0.26 g, quantitative yield) in the form of an oily residue which was used without further purification for the preparation of **67**. ^{1}H NMR ($CDCl_3$) δ: 1.2−1.75 (m, 32H), 2.2 (t, 2H), 2.8 (t, 2H), 2.85−3.3 (m, 8H), 4.07 (t, 2H), 4.53 (t, 2H), 4.7−5.4 (br s, 1H), 5.7−6.1 (br s, 1H), 6.4−7.1 (br s, 1H).

3-[[(1,1-Dimethylethoxyl)carbonyl]amino]-21-[(1,1-dimethylethoxy)carbonyl]-11,15-dioxo-16-oxa-2,4,12,14,21,-25-hexaazahexacos-2-enedioic Acid, Bis(1,1-dimethylethyl) Ester (67). 66 (0.26 g, 0.48 mmol) was added at room temperature to a stirred solution of *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **1** (0.28 g, 0.95 mmol) in THF (20 mL). The reaction medium was stirred at room temperature for 4 days. After evaporation of the solvent, the obtained residue was purified by flash chromatography on silica gel (methylcyclohexane/EtOAc 4/6) to afford **67** (0.10 g, 27% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.0−1.8 (m, 50H), 2.16 (t, 2H), 3.05−3.3 (m, 6H), 3.4 (q, 2H), 4.07 (t, 2H), 4.55 (t, 2H), 5.2−5.3 (br s, 1H), 5.7−5.8 (br s, 1H), 6.45−6.55 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

7-[(Aminoiminomethyl)amino]-*N*-[[4-[(3-aminopropyl)-amino]butoxy]carbonylaminomethyl]heptanamide, Tris-(trifluoroacetate) (68). This compound was obtained (0.050 g, 54% yield) in the form of a translucent white amorphous solid according to the procedure described for **13a** and starting from **67** (0.1 g, 0.127 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.2−1.35 (m, 4H), 1.4−1.7 (m, 8H), 1.8−1.95 (m, 2H), 2.06 (t, 2H), 2.8−3.1 (m, 8H), 3.96 (t, 2H), 4.32 (t, 2H), 6.85−7.4 (br s, 3H), 7.55−7.7 (m, 2H), 7.8−8.0 (m, 4H), 8.33 (t, 1H), 8.55−8.7 (m, 2H).

Biology. Experimental graft-versus-host disease was induced in cyclophosphamide immunosuppressed B6D2F1 mice[24] by intravenous injection of 2 × 10^8 viable spleen cells from B6 origin. Generally, untreated control animals died between days 14 and 21 post-GVHD induction. Compounds were dissolved in aqueous solution and administered from days 1 to 10 (day 6 omitted) by intraperitoneal route. In such experimental conditions, control vehicles were unable to modify survival. Survival was followed until day 60 post-cell injection.

Heterotopic abdominal heart transplant was done according to the method of Ono and Lindsey.[32] End-to-side anastomoses of the aorta and pulmonary artery of the donor heart (Dark Agouti) to the recipient (Lewis rat) abdominal aorta and vena cava were performed. Graft function was assessed by daily palpation until day 100, and rejection was defined by the cessation of palpable contractions. Treatment was done as previously described including day 6. All results were analyzed by the Manntel & Haenszel procedure for the nonparametric Log Rank test, validated by the Statistical Analysis System (SAS, Cary, NC).

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