Structure−Immunosuppressive Activity Relationships of New Analogues of 15-Deoxyspergualin. 2. Structural Modifications of the Spermidine Moiety

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Received March 22, 1999

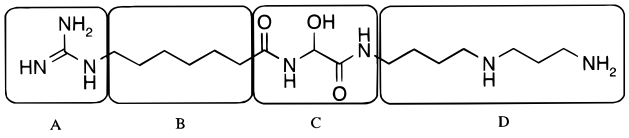
A series of new analogues of 15-deoxyspergualin (DSG), an immunosuppressive agent commercialized in Japan, was synthesized and tested in a graft-versus-host disease (GVHD) model in mice. Various substitutions of the spermidine "D" region were made in order to determine its optimum structure in terms of in vivo immunosuppressive activity. Various positions of methylation were first investigated leading to the discovery of the monomethylated malonic derivative **56h** in which the *pro-R* hydrogen of the methylene α to the primary amine of the spermidine moiety has been replaced by a methyl group. Synthesis of the similarly methylated analogue of the previously reported glycolic derivative LF 08-0299 afforded **60e** which demonstrated a powerful activity at a dose as low as 0.3 mg/kg in the GVHD model and was much more potent than DSG in the demanding heart allotransplantation model in rats. The improvement of in vivo activity was supposed to be related to an increase of the metabolic stability of the methylated analogues compared to the parent molecules. Due to its very low active dose, compatible with a subcutaneous administration in humans, and its favorable pharmacological and toxicological profile, **60e** was selected as a candidate for clinical evaluation.

Introduction

(−)-15-Deoxyspergualin ((−)DSG) represents the prototype of a new class of immunosuppressive agents[1a] derived from the natural product (−)-spergualin discovered by Umezawa in 1981.[2] We decided to investigate structure−immunosuppressive activity relationships in this series in order to better define its structural requirements and to design, if possible, improved analogues. The linear structure of this polyamine derivative can be dissected into four regions according to Chart 1. In a previous paper[3] we have described our results dealing with the "C" region modulation. Here, we present our study of analogues in which the spermidine "D" region has been modified. Umezawa's group has already published biological results[4] obtained with analogues of DSG where the spermidine moiety has been replaced by other polyamines such as spermine, norspermidine, octanediamine, and putrescine. Unfortunately, all these analogues were inactive;[1a] in particular the putrescine derivative which is the main metabolite of DSG in humans[5a] is not immunosuppressive.[5b] The very precise structural requirements observed are reminiscent of that found with the "C" region.[3,6] However, it was important to extend the study of this "D" region especially because it represents the main site of in vivo metabolism of DSG.

Influence of substitution, such as alkylation or fluorination of the skeleton of naturally occurring polyamines, on their biological activity has been reported.[7] These changes may modulate the biological activity, either by a direct effect on the affinity of the molecule for its biological target or by modifying its metabolism. Such effects may induce changes either in the potency or in the toxicity of the resulting compounds. For example, the catabolism of polyamines involves enzymatic oxidations which lead to potentially toxic species.[8] Similarly, human DSG catabolism seems to be related to that of natural polyamines involving a desaminopropylation sequence leading to the above-mentioned inactive putrescine derivative and to other minor oxidized metabolites.[1a] We could thus expect that substitution (e.g. alkylation) of the spermidine "D" region may induce activity and/or toxicity improvement. To make the target compounds more readily accessible, we decided to first synthesize derivatives of our previously reported[1b,3] DSG analogues where the "C" region consists of a malonyl unit instead of directly making compounds encompassing the labile hydroxyglycine central amino acid of DSG. This approach avoids the formation of mixtures of diastereomers which would result from the coupling of racemic monosubstituted spermidines with the hydroxyglycine-containing precursor.

Chart 1. (±)-15-Deoxyspergualin (DSG)



(±)-15-Deoxyspergualin

Chemistry

a. Preparation of the N-Protected Alkylated Spermidines. Compounds synthesized according to Schemes 1, 2, 4, and 5 are racemic. The N-protected

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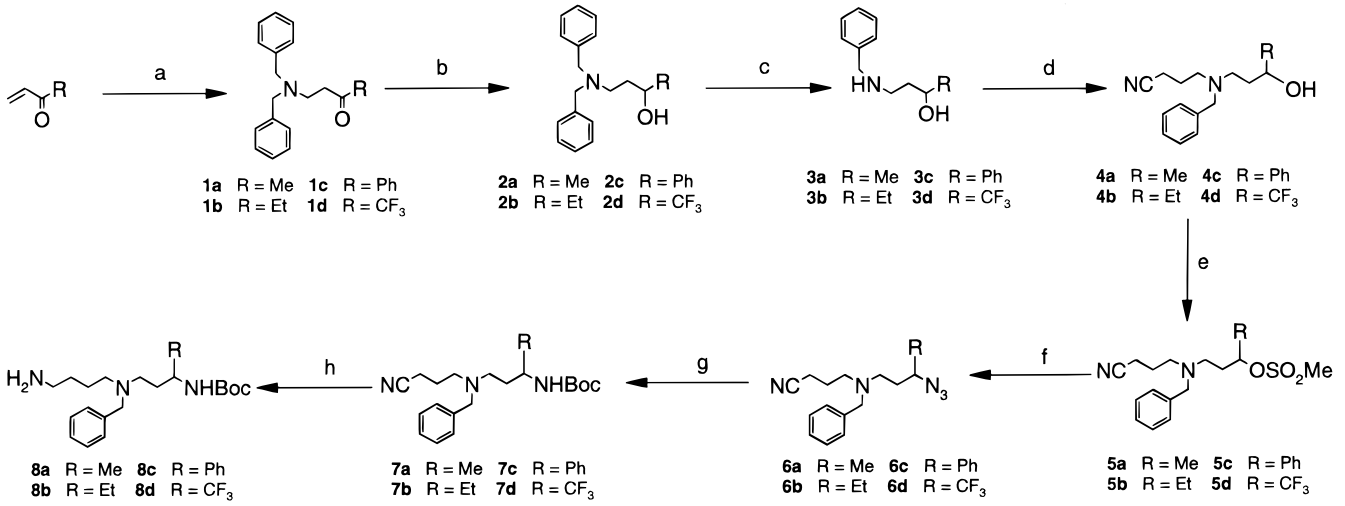
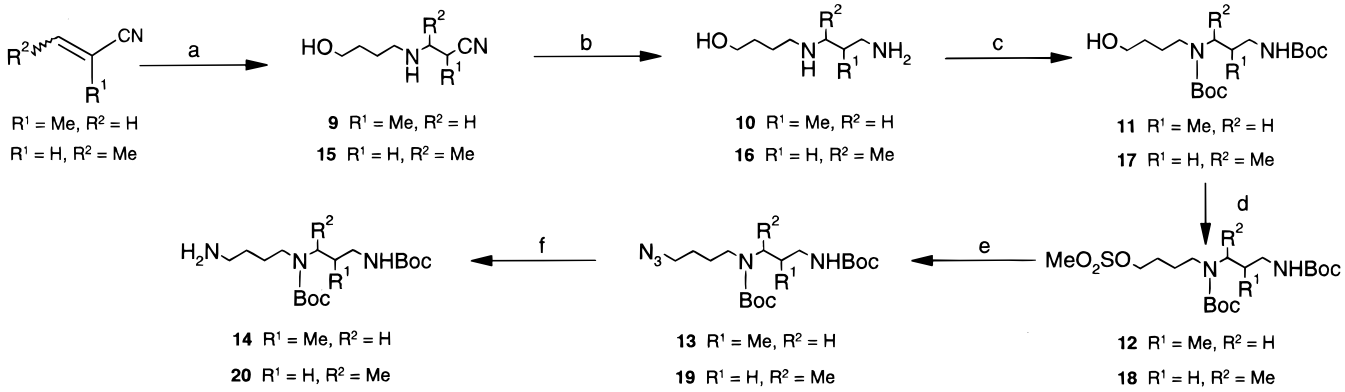
10.1021/jm991043x CCC: $18.00
Published on Web 10/28/1999

Scheme 1[a]



[a] Reagents: (a) dibenzylamine, MeOH, 40 °C; (b) $NaBH_4$, EtOH, 10 °C; (c) H_2, Pd/C (10%), MeOH; (d) $Cl(CH_2)_3CN$, KI, Na_2CO_3, *n*-BuOH, reflux; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C; (f) NaN_3, DMSO, 40 °C; (g) H_2, 4 bar, Pd/C (10%), $(Boc)_2O$, EtOAc; (h) H_2, 6 bar, Raney Ni, EtOH saturated with NH_3.

Scheme 2[a]



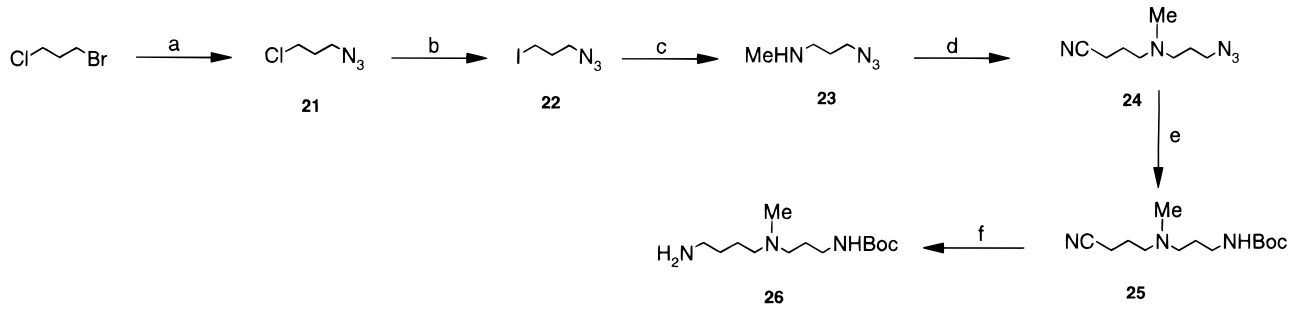
[a] Reagents: (a) $HO(CH_2)_4NH_2$, EtOH; (b) H_2, PtO_2, EtOH, HCl; (c) $(Boc)_2O$, NaOH (1 M), CH_2Cl_2, H_2O; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C; (e) NaN_3, DMSO, 40 °C; (f) H_2, 4 bar, Pd/C (10%), MeOH.

1-alkyl- and 1-arylspermidine synthons **8a**−**d** were obtained by a specific and versatile route illustrated in Scheme 1. It gives access to a wide variety of groups depending on the Michael acceptors. Alkyl and aryl vinyl ketones reacted with dibenzylamine in methanol with good yield to give *β*-amino ketones **1a**−**d** which were reduced by $NaBH_4$ in ethanol to afford amino alcohols **2a**−**d**. The removal of one benzyl group yielded **3a**−**d** with good purities after purification by distillation. Alkylation of the secondary amine by 4-chlorobutyronitrile in refluxing *n*-butanol in the presence of KI and Na_2CO_3 led to **4a**−**d**. Conversion of the alcohols to the azido derivatives was performed by nucleophilic displacement of the corresponding methanesulfonates with sodium azide in DMSO. Chemoselective palladium-catalyzed hydrogenation of the previous azido derivatives **6a**−**d** in the presence of $(Boc)_2O$ in EtOAc afforded **7a**−**d** in very good yields. Finally, the nitrile derivatives were reduced by hydrogenation catalyzed by Raney nickel in EtOH saturated with NH_3 to produce the protected 1-substituted spermidines **8a**−**d** in good yields.

The protected 2-methyl- and 3-methylspermidine synthons **14** and **20** were obtained according to Scheme 2. The Michael addition of 4-aminobutanol to methacrylonitrile was performed in refluxing ethanol for 48 h leading to **9** in quantitative yield. The reaction of crotonitrile required only 3 h in ethanol at 40 °C to obtain **15** quantitatively. The following steps were identical for the two series: the nitrile function of **9** and **15** was then hydrogenated in an ethanolic hydrochloric acid solution at atmospheric pressure in the presence of PtO_2. Protection of the two amines of **10** and **16** was performed with $(Boc)_2O$ in a CH_2Cl_2/H_2O mixture containing 2 equiv of NaOH. Finally, conversion of the alcohol function to the primary amine was achieved as mentioned above to afford the desired synthons **14** and **20**.

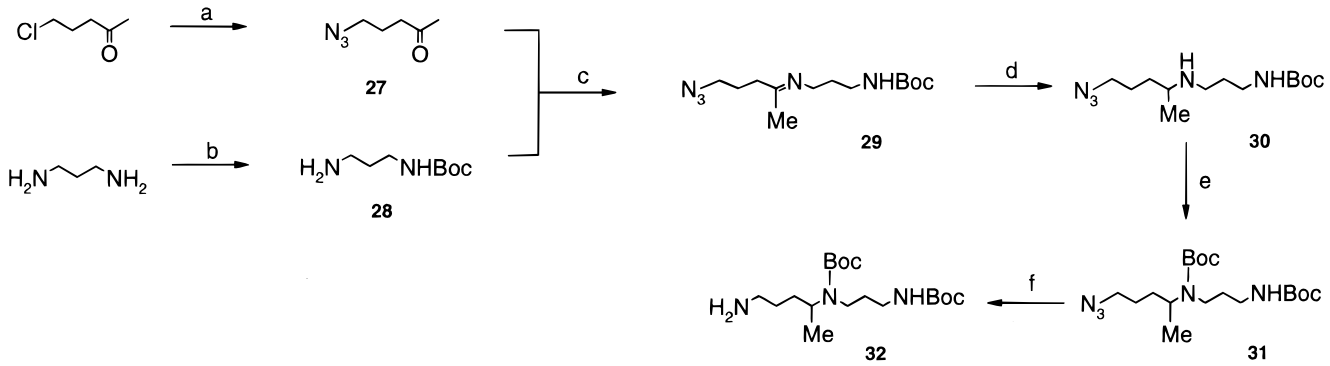
The protected 4-methylspermidine synthon **26** was synthesized by the route illustrated in Scheme 3 starting from 1-bromo-3-chloropropane. This reagent reacted with sodium azide at room temperature in DMSO to give **21** with a good chemoselectivity (90% yield). Exchange of chlorine by iodine with sodium iodide was achieved in refluxing acetone. The nucleophilic substitution of the iodine by $MeNH_2$ in large excess led to **23** with 65% yield. Successive alkylation of the secondary amine with 4-chlorobutyronitrile, reduction of the azido group in the presence of $(Boc)_2O$, and hydrogenation of the nitrile group were performed as mentioned above to produce the desired synthon **26**.

Scheme 3[a]



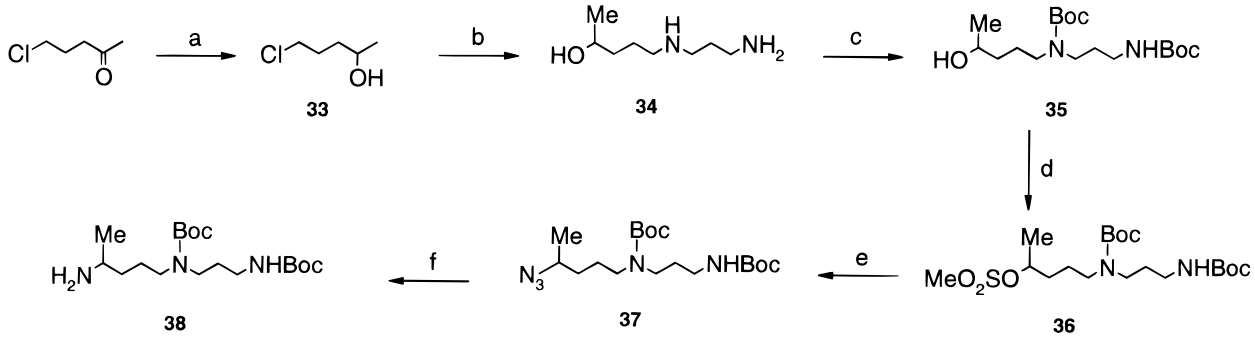
[a] Reagents: (a) NaN_3, DMSO; (b) NaI, acetone, reflux; (c) $MeNH_2$, EtOH; (d) $Cl(CH_2)_3CN$, KI, Na_2CO_3, *n*-BuOH, reflux; (e) H_2, 4 bar, Pd/C (10%), $(Boc)_2O$, EtOAc; (f) H_2, 6 bar, Raney Ni, EtOH saturated with NH_3.

Scheme 4[a]



[a] Reagents: (a) NaN_3, NaI, DMSO, 50 °C; (b) $(Boc)_2O$, THF; (c) Et_2O, 3 Å molecular sieves; (d) $NaBH_4$, EtOH, 10 °C; (e) $(Boc)_2O$, THF, 10 °C; (f) H_2, 4 bar, Pd/C (10%), MeOH.

Scheme 5[a]



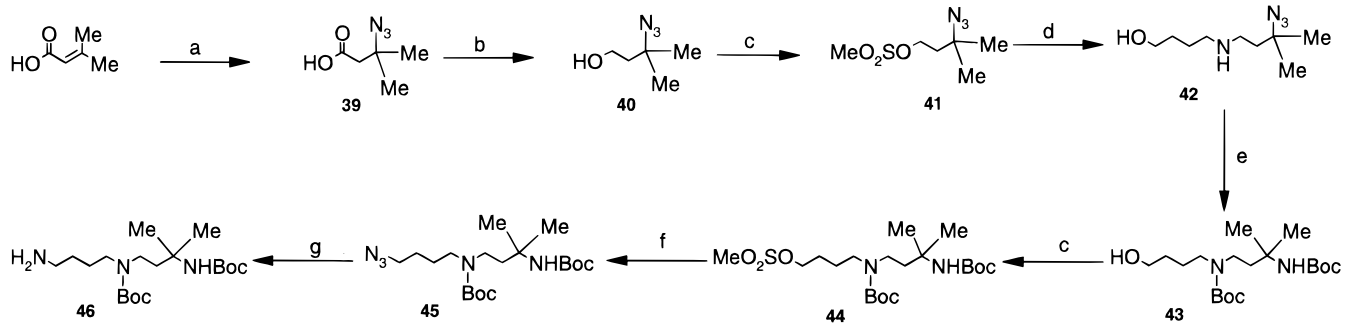
[a] Reagents: (a) $NaBH_4$, EtOH, 10 °C; (b) $H_2N(CH_2)_3NH_2$, KI, Na_2CO_3, *n*-BuOH, reflux; (c) $(Boc)_2O$, THF, 10 °C; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C; (e) NaN_3, DMSO, 40 °C; (f) H_2, 4 bar, Pd/C (10%), MeOH.

The protected 5-methylspermidine **32** was obtained according to a convergent synthetic route illustrated in Scheme 4 from 5-chloropentan-2-one and 1,3-diaminopropane. The mono-Boc-protected 1,3-diaminopropane **28** was obtained by reaction of $(Boc)_2O$ with a large excess of 1,3-diaminopropane.[9] 5-Azidopentan-2-one **27** was synthesized according to the literature from 5-chloropentan-2-one.[16] Reductive amination of **27** to **30** was achieved in two steps: imine **29** was first obtained starting from **27** and **28** and then was further reduced to **30** with $NaBH_4$. Finally, protection of the secondary amine with a Boc group using $(Boc)_2O$ followed by reduction of the azido group gave the desired synthon **32**.

The protected 8-methylspermidine synthon was prepared by the route illustrated in Scheme 5. 5-Chloropentan-2-one was reduced with $NaBH_4$ in absolute ethanol into the corresponding alcohol derivative **33** which further reacted with 1,3-diaminopropane in refluxing *n*-butanol in the presence of KI and Na_2CO_3 to give **34**. Protection of the amines with Boc groups and conversion of the alcohol to the primary amine resulted in the desired synthon **38**.

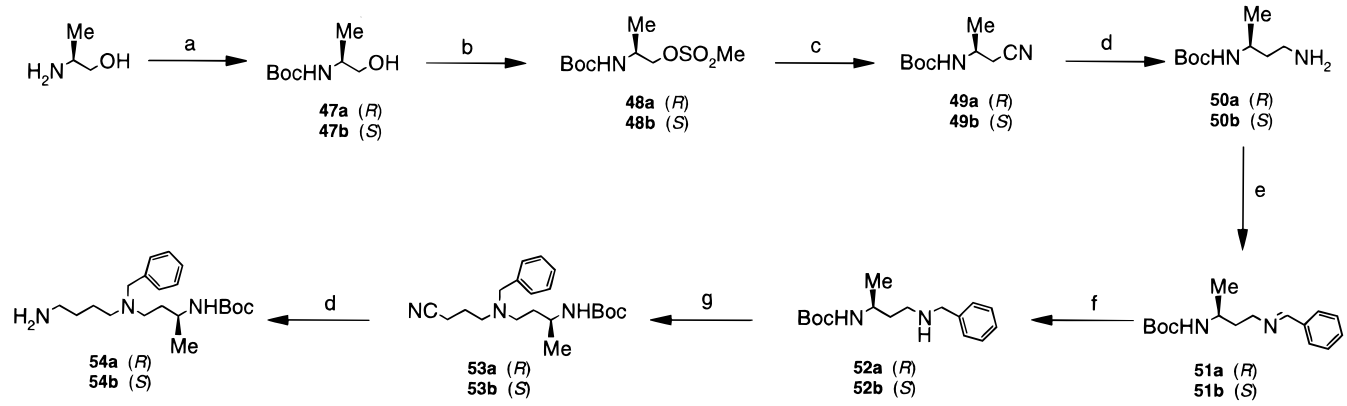
The synthesis of the protected 1,1-dimethylspermidine synthon **46** is illustrated in Scheme 6. A Michael reaction using β,β-dimethylacrylic acid and sodium azide in acidic conditions[7a] afforded **39** which was further chemoselectively reduced into the corresponding alcohol derivative **40** by BH_3-Me_2S in THF. Conversion of the alcohol function of **40** into the methanesulfonate derivative **41** allowed its nucleophilic displacement with 4-aminobutanol in the presence of DBU in refluxing *n*-butanol to yield **42**. The one-step conversion of **42** to the Boc-protected diamine **43** was accomplished by hydrogenation in the presence of $(Boc)_2O$. Then the conversion of the hydroxyl of **43** into an amino group

Scheme 6[a]



[a] Reagents: (a) NaN_3, AcOH, H_2O, reflux; (b) $BH_3 \cdot Me_2S$, THF; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C; (d) $HO(CH_2)_4NH_2$, DBU, *n*-BuOH, reflux; (e) H_2, 4 bar, Pd/C (10%), $(Boc)_2O$, EtOAc; (f) NaN_3, DMSO, 40 °C; (g) H_2, 4 bar, Pd/C (10%), MeOH.

Scheme 7[a]



[a] Reagents: (a) $(Boc)_2O$, THF; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C; (c) NaCN, DMSO, 40 °C; (d) H_2, 6 bar, Raney Ni, EtOH saturated with NH_3; (e) PhCHO, 3 Å molecular sieves, Et_2O; (f) $NaBH_4$, EtOH, 10 °C; (g) $Cl(CH_2)_3CN$, KI, Na_2CO_3, *n*-BuOH, reflux.

using the methanesulfonate−azide sequence produced the desired synthon **46**.

To synthesize the optically active *R* and *S* N-protected 1-methylspermidines **54a**,**b**, we chose to use commercially available alaninol (*R* or *S*; Scheme 7). Protection of the primary amine and conversion of the hydroxyl group into a nitrile group via the methanesulfonate derivative were performed under mild experimental conditions as mentioned in Scheme 7 in order to avoid, as much as possible, degradation and racemization reactions. Reduction of the nitrile group carried out with Raney nickel in ethanol saturated with ammonia afforded **50a** or **50b** in good yield. Introduction of a benzyl group on the primary amine made possible the clean monoalkylation of the resulting secondary amine with 4-chlorobutyronitrile. This reaction was conducted in the presence of KI and Na_2CO_3 in refluxing *n*-butanol to afford **53a** or **53b**. Final reduction of the nitrile group led to the desired synthon **54a** or **54b** in good yield and high optical purity measured by the Mosher's method.[10]

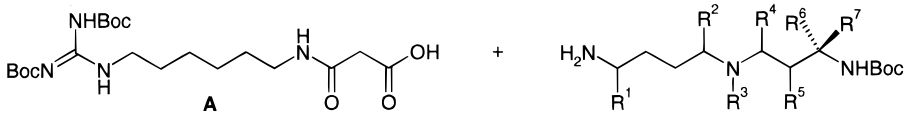
b. Preparation of the Target Derivatives. Target compounds of the malonic series **56a**−**i** were obtained according to Scheme 8 by the coupling of N-protected methylspermidine synthons (**8a**, **14**, **20**, **26**, **32**, **38**, **46**, **54a**,**b**) with synthon A, the synthesis of which has been described in the previous paper.[3] Standard coupling techniques were used followed by removal of protecting groups by hydrogenolysis and/or acidic treatment.

Final glycolic derivatives **60a**−**g** were synthesized according to Scheme 9. N-Protected 1-alkylspermidine synthons (**8a**−**d** and **54a**,**b**) and N-protected 1,4-diaminobutane reacted with methyl 2-[(phenoxycarbonyl)oxy]-acetate in toluene at 80 °C. The resulting esters **57a**−**g** were treated with NaOH in DME to give the acids **58a**−**g**. The coupling of the acids **58a**−**g** with synthon B using the DCC, HOBT procedure led to the intermediates **59a**−**g** which were finally deprotected to afford the target compounds **60a**−**g**.

Results and Discussion

Pharmacological and analytical data are shown in Tables 1−3. Immunosuppressive activity was first assayed in vivo in a mouse graft-versus-host disease (GVHD) model as described in our previous paper.[3] All compounds were administered daily by ip route during 10 days (day 6 omitted), and the survival of the mice was followed during 2 months. Most of the compounds were studied at 3 and 0.3 mg/kg. The mean survival time of the control group was 15 ± 2 days. In such conditions, LF 07-0109, which was used as the reference (unsubstituted) compound for the malonic series, increased survival time to 52 ± 20 days at 3 mg/kg and was inactive at 0.3 mg/kg. Table 1 shows the results obtained with analogues of LF 07-0109 in which one or two methyl groups have been introduced on different positions of the spermidine moiety. We investigated first the positions α to nitrogens. Compounds **56a**,**b**,**d** were clearly inactive. In contrast, the racemic compound **56f** showed a powerful activity at 3 mg/kg. However at 0.3

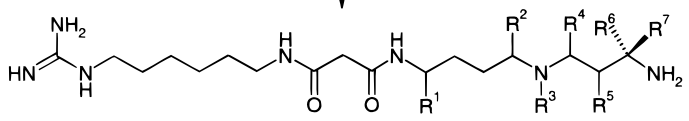
Scheme 8[a,b]



38 R^1 = Me, R^3 = Boc
32 R^2 = Me, R^3 = Boc
26 R^3 = Me
20 R^3 = Boc, R^4 = Me
14 R^3 = Boc, R^5 = Me

8a R^3 = Bn, R^6 = Me or H, R^7 = Me or H
46 R^3 = Boc, R^6 = Me, R^7 = Me
54a R^3 = Bn, R^7 = Me
54b R^3 = Bn, R^6 = Me

a, b

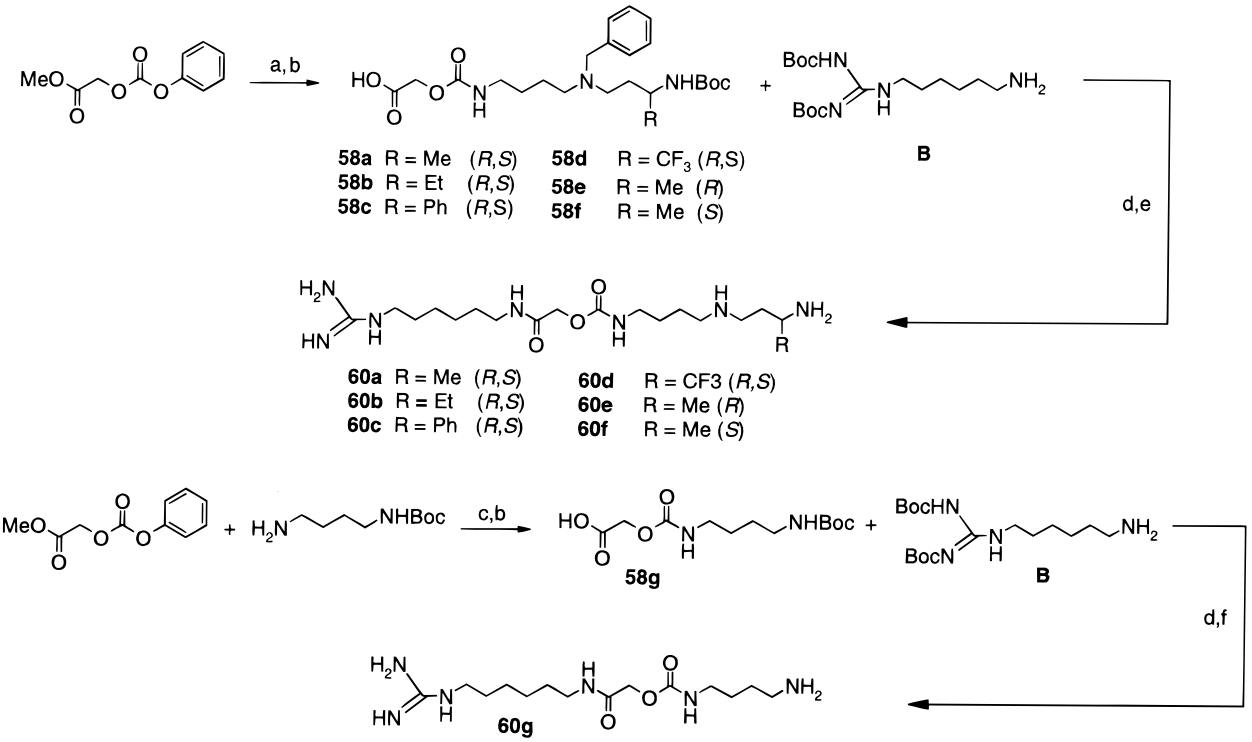


56a R^1 = Me
56b R^2 = Me
56c R^3 = Me
56d R^4 = Me
56e R^5 = Me

56f R^6 = Me or H, R^7 = Me or H
56g R^6 = Me, R^7 = Me
56h R^7 = Me
56i R^6 = Me

[a] Reagents: (a) DCC, HOBT, $CHCl_3$; (b) with R^3 = Me or Boc: TFA, CH_2Cl_2, with R^3 = Bn: H_2, Pd/C (10%), EtOAc, then TFA, CH_2Cl_2. [b] R = H unless otherwise indicated.

Scheme 9[a]



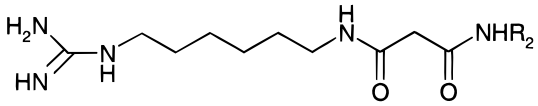
58a R = Me (*R*,*S*)
58b R = Et (*R*,*S*)
58c R = Ph (*R*,S)
58d R = CF_3 (*R*,S)
58e R = Me (*R*)
58f R = Me (*S*)

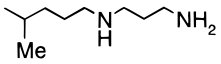
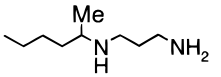
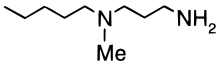
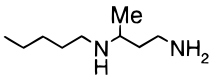
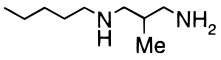
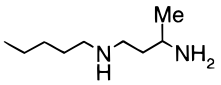
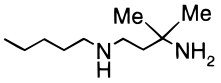
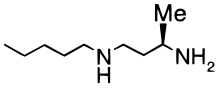
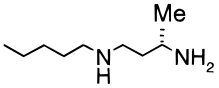
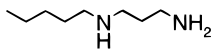
[a] Reagents: (a) **8a**−**d** or **54a**,**b**, toluene, 80 °C; (b) NaOH (1 M), DME; (c) THF; (d) DCC, HOBT, $CHCl_3$; (e) H_2, Pd/C (10%), EtOAc, then TFA, CH_2Cl_2; (f) TFA, CH_2Cl_2.

mg/kg, no activity was observed. The corresponding two enantiomers were thus synthesized: the *S* isomer **56i** was totally inactive at 3 mg/kg, but in contrast the *R* isomer **56h** was active at 3 mg/kg and slightly active at 0.3 mg/kg. These results seemed to indicate that the replacement of the *pro-R* hydrogen α to the primary amine may improve the activity of LF 07-0109, at least in terms of the minimum active dose. Unfortunately, gem-dimethylation, which could alleviate problems of stereochemistry,[7a] yielded a completely inactive derivative **56g**. Two other positions of methylation were also studied with compounds **56c**,**e** which were totally inactive.

To better define the elements of structure−immunosuppressive activity relationships, we then investigated the influence of the nature of the substituent α to the primary amine. This study was performed starting from the glycolic derivative LF 08-0299 (see Table 2). This

Table 1. Physicochemical and Biological Data of Malonic Analogues of DSG with Structural Modifications of the Spermidine Moiety



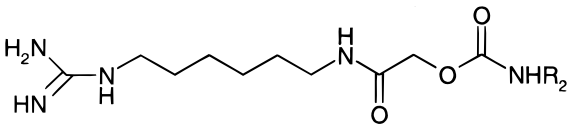
compd	R_2	chirality	HRMS calcd	HRMS found	GVHD mean survival (days) (M ± SD)[a] 3mg/kg[b]	0.3mg/kg[b]
56a		*R,S*	386.3243	386.3246	21 ± 4	
56b		*R,S*	386.3243	386.3248	14 ± 3	11 ± 1
56c			386.3243	386.3235	14 ± 5	14 ± 2
56d		*R,S*	386.3243	386.3262	12 ± 2	11 ± 2
56e		*R,S*	386.3243	386.3238	18 ± 2	18 ± 6
56f		*R,S*	386.3243	386.3247	60 ± 1	17 ± 1
56g			400.3400	400.3383	15 ± 2	15 ± 2
56h		*R*	386.3243	386.3255	49 ± 14	26 ± 6
56i		*S*	386.3243	386.3226	17 ± 3	
LF 07-0109[c]					52 ± 20	17 ± 1

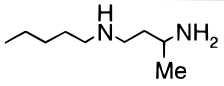
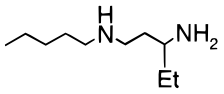
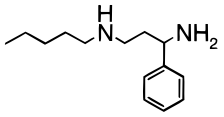
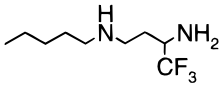
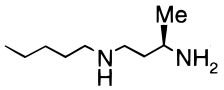
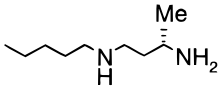
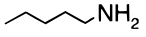
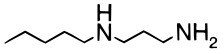
[a] The mean survival time of control was 15 ± 2 days. [b] All compounds were administered daily by ip route using groups of 8 mice/dose. [c] See ref 3.

product which is currently under clinical development showed the following biological profile:[3] in our GVHD model, it increased survival to 58 ± 6 days at 3 mg/kg and was inactive at 0.3 mg/kg. We first synthesized the racemate **60a**. As in the malonic series, methylation of the position α to the primary amine led to an active compound. Moreover, in this series, the activity observed at 0.3 mg/kg was quite impressive. Surprisingly, the methyl group seemed to be the only suitable one considering the loss of activity observed with ethyl, phenyl, and trifluoromethyl analogues (**60b**−**d**). As in the malonic series, we synthesized the enantiomers of **60a**. The *S* isomer **60f** was found inactive, whereas the *R* isomer **60e** was the most potent of all compounds synthesized so far. Indeed, the maximum activity was observed starting from the lowest dose of 0.3 mg/kg. A complete dose−effect study of this compound on the GVHD model showed that the minimal active dose was about 0.1 mg/kg. These results seemed to indicate that the structure−immunosuppressive activity relationships observed in these two series were similar. However, the influence of the structural modification of the spermidine part of the molecule seemed to be more pronounced in the glycolic series.

Following our screening strategy, the best compounds were then studied on a heart allotransplantation model in the rat (Table 3). At 6 mg/kg, LF 07-0109, the nonmethylated malonic derivative, was inactive. Racemic **56f** was slightly active, whereas its *R* enantiomer **56h** was quite active. In the glycolic series, at 2.5 mg/kg the unsubstituted compound LF 08-0299 was inactive. At this dosage, the racemate **60a** was very active. In comparison, the *S* isomer **60f** was inactive even at 6 mg/kg, whereas the *R* isomer **60e** was the most active compound, capable of inducing graft tolerance as shown by the long-term (>100 days) graft survival observed

Table 2. Physicochemical and Biological Data of Glycolic Analogues of DSG with Structural Modifications of the Spermidine Moiety



compd	R_2	chirality	HRMS calcd	HRMS found	GVHD mean survival (days) (M ± SD)[a] 3mg/kg[b]	GVHD mean survival (days) (M ± SD)[a] 0.3mg/kg[b]
60a		*R,S*	402.3193	402.3190	57 ± 9	53 ± 14
60b		*R,S*	416.3349	416.3370	21 ± 5	16 ± 1
60c		*R,S*	464.3349	464.3331	15 ± 1	15 ± 1
60d		*R,S*	456.2910	456.2913	17 ± 5	16 ± 4
60e		*R*	402.3193	402.3198	60 ± 0	60 ± 1
60f		*S*	402.3193	402.3198	25 ± 14	16 ± 1
60i			331.2458	331.2453	14 ± 1	14 ± 1
LF 08-0299[c]					58 ± 6	15 ± 2

[a–c] See corresponding footnotes in Table 1.

Table 3. Prevention of Graft Rejection after Heart Allotransplantation in the Dark Agouti to Lewis Rat Combination

compd	dose[a] (mg/kg)	survival (day)	mean survival (days) (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[c]	38 ± 30	S
LF 07-0109	6	5, 7, 8, 10, 10, 13	9 ± 3	NS
56f	6	13, 19, 20, 22, 30, 32, 34	24 ± 8	S
56h	6	37, 45, 45, 62, 87, 99,[c] 99[c]	68 ± 27	S
LF 08-0299	2.5	9, 13, 13, 14, 16, 17, 27	16 ± 6	S
60f	6	8, 8, 8, 9, 9, 10, 10	9 ± 1	S
60a	2.5	37, 44, 50, 50, 54, 59, 97	56 ± 19	S
60e	2.5	33, 41, 88, 99,[c] 99,[c] >100,[c] >100[c]	80 ± 30	S

[a] All compounds were administered daily by ip route for 10 days starting the day after surgery. [b] Manntel & Haenszel test according to SAS (Cary, NC). [c] Allotransplanted recipients were autopsied at the end of experiment; transplanted hearts with sign of rejection were noted 99 days, while hearts free of rejection signs were noted >100.

with some animals treated at 2.5 mg/kg. These results confirmed those obtained in our primary screening in the mouse: the replacement of the *pro-R* hydrogen α to the primary amine by a methyl group induces a strong improvement of the immunosuppressive activity in both the mouse and the rat.

To better understand these results, we have synthesized **60i** which corresponds to the desaminopropyl-LF08-0299 (see Table 2). Similarly to the desaminopropyl-DSG, **60i** was not immunosuppressive. During the preclinical and clinical development of LF 08-0299, it has been shown that its major metabolic pathway leads to **60i** in rat, monkey, or human (unpublished results). In contrast, preliminary metabolic studies of **60e** seemed to indicate that **60i** was not formed in vivo, in the rat. With these data, it was tempting to attribute the improvement of activity of **60e** compared to LF 08-0299 to an increase of its plasma level due to its better resistance to degradation into inactive metabolites. Figure 1 shows the pharmacokinetic profiles of LF 08-

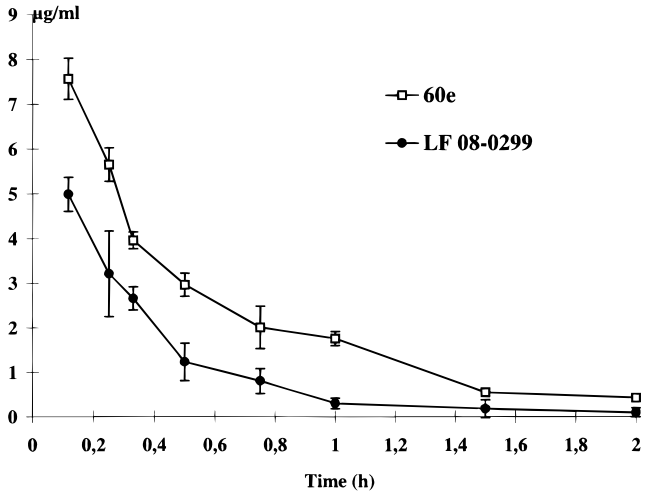


Figure 1. Concentration (μg/mL) of **60e** and LF 08-0299 in plasma after intravenous administration of [^{14}C]**60e** and [^{14}C]-LF 08-0299 (4 mg/kg) to male Sprague−Dawley rats.

0299 and **60e** in the rat after iv administration of a same dose of the corresponding ^{14}C-labeled compounds. The AUC_{0-2h} of **60e** is clearly higher than that of LF 08-0299 at the same dosage confirming our hypothesis, at least for the rat. Pharmacokinetic and metabolism data seem to indicate that this improvement is due to the blockade of the metabolism of the aminopropyl part of the molecule. This blockade obtained by the replacement of the *pro-R* hydrogen α to the primary amine by a methyl group deserves further investigation. Referring to polyamine catabolism,[8e] we can explain the desaminopropylation observed with DSG and LF 08-0299 by two possible mechanisms (Scheme 10). A direct oxidation α to the primary amine could lead to the formation of an intermediate aldehyde. This β-amino aldehyde is supposed to be rather unstable leading to the desaminopropylamine and acrolein by a retro-Michael reaction. An alternative route could first involve an acetylation step of the primary amine followed by an oxidation α to the secondary amine leading to the desaminopropylamine and 3-acetamidopropanal. Although the methylation could prevent a priori either the oxidation of the primary amine (pathway 1) or the acetylation step (pathway 2), our preliminary metabolic studies seemed to indicate that LF 08-0299 (and probably DSG) is metabolized via the initial pathway 1 (unpublished data).

Obviously, more experiments are required in order to better understand the nature of the enzymes involved in this oxidative process. The search for the putative initially formed aldehyde derived from LF 08-0299 would help to definitively confirmed our hypothesis. These experiments are in progress and will be reported later. The change in the metabolic pathway induced by the methyl group is clearly documented and is very likely related to the potency improvement observed. However, part of the improvement could also be due to a better affinity of the methylated analogues for their molecular target. Unfortunately, the mechanism of action of these molecules is not yet fully elucidated. Thus, it is difficult to get an accurate measure of their intrinsic activity. However, heat shock cognitive protein 70 (Hsc 70) has been postulated as one of the possible targets of DSG.[11] We have recently shown[12] that LF 08-0299 and **60e** bind to Hsc 70. In contrast, we were unable to detect any binding of the inactive *S* enantiomer **60f**. These results seem to indicate that the binding of DSG analogues to Hsc 70 could be at least one step of their mechanism of action.We may thus tentatively conclude that if α-methylation of the primary amine is necessary in order to block the oxidative catabolism, replacement of only the *pro-R* hydrogen is compatible with the molecular recognition of HSP 70 which seems to be required for the immunosuppressive activity.

In summary, this study shows that the possibility to modulate the spermidine part of DSG analogues is extremely restricted. In particular, the aminopropyl moiety can only accommodate one methyl group, replacing the *pro-R* hydrogen α to the primary amine. Other stereochemistry, other substituents, or other sites of methylation are incompatible with a good immunosuppressive activity. This strict structural requirement seems to indicate a very precise molecular recognition. Fortunately, the only possible monomethylation required by this recognition is fully compatible with the blockade of the main catabolism pathway and allowed us to discover new improved analogues of DSG. Among these analogues, **60e**, which presents very low active doses compatible with a subcutaneous route of administration in humans, has been selected as a candidate for clinical evaluation.

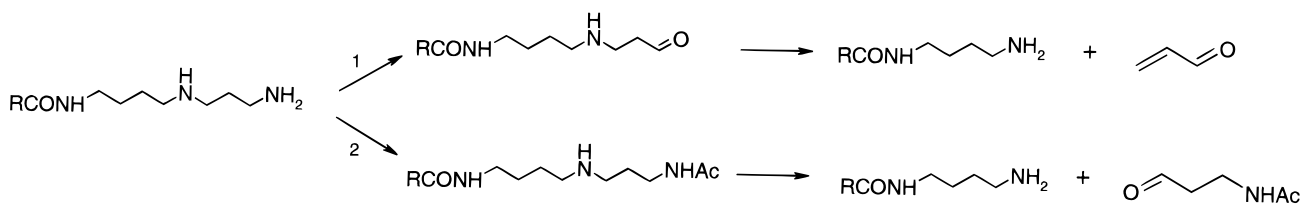
Experimental Section

Chemistry. ^{1}H NMR 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 confirmed 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 60 F_{254} plates or RP-18$F_{254\ s}$ (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. The purity of all tested compounds was analyzed by HPLC using a Zorbax C18 column with an

Scheme 10



acetonitrile/water/Pic B7 gradient as eluent with UV detection at 205 nm and was found to be >95%.

General Procedure for the Preparation of Compounds 8a−d. Compounds of the general structure **8a−d** (Scheme 1) were synthesized from vinyl ketone derivatives by the representative procedure illustrated for analogue **8a** in eight steps as follows.

4-[Bis(phenylmethyl)amino]butan-2-one (1a). To a stirred solution of dibenzylamine (20.0 g, 100.0 mmol) in methanol (90 mL) was added a solution of methyl vinyl ketone (7.7 g, 110.0 mmol) in methanol (10 mL). The mixture was stirred at 40 °C for 2 h. The solvent was evaporated off under reduced pressure and the obtained residue was recrystallized (*i*-Pr_2O) to give **1a** (24.71 g, 93%) as a white solid: mp 59 °C; ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3H), 2.56 (t, 2H), 2.75 (t, 2H), 3.53 (s, 4 H), 7.15−7.40 (m, 10H).

4-[Bis(phenylmethyl)amino]-2-butanol (2a). $NaBH_4$ (6.0 g, 137.0 mmol) was added in small portions to a solution of **1a** (24.5 g, 92.0 mmol) in dry EtOH (200 mL) at 10 °C. After stirring for 3 h, the mixture was concentrated under vacuum. The residue was taken up in water (150 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The obtained oily residue was purified by distillation to yield **2a** (23.3 g, 94%) as a colorless oil: $bp_{0.05}$ 130−140 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (d, 3H), 1.40−1.55 (m, 1H), 1.60−1.80 (m, 1H), 2.45−2.60 (m, 1H), 2.65−2.80 (m, 1H), 3.23 (d, 2H), 3.72 (m, 1H), 3.85 (d, 2H), 5.65 (br s, 1H), 7.20−7.36 (m, 10H).

4-[(Phenylmethyl)amino]-2-butanol (3a). A mixture of **2a** (21.4 g, 80.0 mmol) and 10% palladium on carbon (1.0 g) in methanol (200 mL) was stirred at room temperature and under a hydrogen atmosphere for 6 h at atmospheric pressure. The catalyst was then filtered off and the organic phase was evaporated to give a residue which was purified by distillation to yield **3a** (8.1 g, 56%): $bp_{0.05}$ 80−85 °C (lit.[13] bp_{12} 152−154 °C); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H), 1.75−1.20 (m, 2H), 2.90−3.15 (m, 2H), 3.90−4.0 (m, 1H), 4.1 (d, 1H), 4.15 (d, 1H), 7.20−7.70 (m, 5H), 9.1 (br s, 2H).

4-[(3-Hydroxybutyl)(phenylmethyl)amino]butanenitrile (4a). 4-Chlorobutyronitrile (7.4 g, 70.0 mmol) was added into a solution of **3a** (5.0 g, 28.0 mmol), potassium iodide (1.06 g, 7.0 mmol), and sodium carbonate (3.56 g, 34.0 mmol) in *n*-butanol (60 mL). The mixture was stirred at reflux for 20 h and then concentrated under vacuum. The residue was taken up in CH_2Cl_2 (100 mL) and the insoluble salts were removed by filtration and washed with CH_2Cl_2 (2 × 50 mL). The organic layers were combined and concentrated under vacuum. The obtained oily residue was purified by distillation to afford **4a** (6.2 g, 87%) as a colorless oil: $bp_{0.05}$ 160−170 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 3H), 1.47−1.83 (m, 4H), 2.15−2.80 (m, 6H), 3.33 (d, 1H), 3.78 (d, 1H), 3.80−3.90 (m, 1H), 4.80 (br s, 1H) 7.20−7.40 (m, 5H).

Methanesulfonic Acid, 1-Methyl-3-[(3-cyanopropyl)-(phenylmethyl)amino]propyl Ester (5a). To a stirred solution of **4a** (9.11 g, 37.0 mmol) and triethylamine (11.23 g, 111.0 mmol) in CH_2Cl_2 (100 mL) cooled to 0 °C was added dropwise a solution of methanesulfonyl chloride (4.66 g, 40.0 mmol) in CH_2Cl_2 (50 mL). The stirring was continued for 1 h; the solution was then washed with saturated $NaHCO_3$ solution. The layers were separated; the organic phase was dried over K_2CO_3 and concentrated under vacuum to give **5a** as a viscous yellow oil which was used in the next step without further purification (11.73 g, 98%): ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3H), 1.65−2.00 (m, 4H), 2.37 (t, 2H), 2.45−2.60 (m, 4H), 2.89 (s, 3H), 3.48 (d, 1H), 3.57 (d, 1H), 4.80−4.90 (m, 1H), 7.15−7.37 (m, 5H).

4-[(3-Azidobutyl)(phenylmethyl)amino]butanenitrile (6a). To a stirred solution of **5a** (10.59 g, 33.0 mmol) in DMSO (50 mL) was added NaN_3 (2.58 g, 39.6 mmol). The stirring was continued for 15 h at 40−45 °C. After cooling at room temperature, water (100 mL) was added to the solution and the mixture was extracted with Et_2O. The organic layers were combined, washed with a brine solution (3 × 20 mL), dried over $MgSO_4$, and concentrated under vacuum. The oily residue was then purified by flash chromatography on silica gel (Et_2O/heptane, 3/7) to give **6a** as a viscous colorless oil (7.8 g, 87%): ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3H), 1.50−1.90 (m, 4H), 2.34 (t, 2H), 2.40−2.60 (m, 4H) 3.40−3.65 (m, 3H), 7.15−7.40 (m, 5H).

[3-[(3-Cyanopropyl)(phenylmethyl)amino]-1-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (7a). A mixture of **6a** (3.00 g, 11.0 mmol), $(Boc)_2O$ (2.85 g, 13.0 mmol), and 10% palladium on carbon (0.3 g) in EtOAc (30 mL) was stirred at room temperature under a hydrogen atmosphere (4 bar) for 15 h. The catalyst was then filtered off and the organic phase was evaporated under reduced pressure. The oily residue was purified by flash chromatography on silica gel (Et_2O/heptane, 55/45) to yield **7a** as a viscous colorless oil (3.47 g, 91%): ^{1}H NMR ($CDCl_3$) δ 1.06 (d, 3H), 1.35−1.85 (m, 13 H), 2.33 (t, 2H), 2.38−2.62 (m, 4H), 3.47 (d, 1H), 3.57 (d, 1H), 3.60−3.85 (m, 1H), 5.08 (br s, 1H), 7.20−7.40 (m, 5H).

[3-[(4-Aminobutyl)(phenylmethyl)amino]-1-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (8a). A mixture of **7a** (2.95 g, 8.0 mmol) and Raney nickel (1 g) in dry EtOH saturated with ammonia was stirred at room temperature under a hydrogen atmosphere and under a pressure of 6 bar for 15 h. Then, the catalyst was filtered off and the filtrate was evaporated under reduced pressure to give an oily residue which was purified by flash chromatography on silica gel (Et_2O/MeOH/NH_4OH, 90/10/1) to give **8a** as a viscous colorless oil (2.70 g, 91%): ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H), 1.31 (s, 2H), 1.35−1.80 (m, 15H), 2.25−2.70 (m, 6H), 3.43 (d, 1H), 3.60 (d, 1H), 3.65−3.75 (m, 1H), 5.74 (br s, 1H) 7.15−7.35 (m, 5H).

Procedure for the Preparation of Compounds 14 and 20. Compounds **14** and **20** (Scheme 2) were synthesized, respectively, from methacrylonitrile and crotonitrile by the representative procedure illustrated for analogue **14** in six steps as follows.

3-[(4-Hydroxybutyl)amino]-2-methylpropanenitrile (9). Methacrylonitrile (3.36 g, 50.0 mmol) was added into a solution of 4-aminobutan-1-ol (1.80 g, 20.0 mmol) in dry ethanol (40 mL). The mixture was stirred at reflux for 48 h. Then, the mixture was warmed to room temperature, filtered, and concentrated under vacuum to give **9** as a yellow oil which was used without further purification (3.10 g, quantitative yield): ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3H), 1.50−1.75 (m, 4H), 2.60−2.93 (m, 5H), 3.05 (br s, 2H), 3.59 (t, 2H).

4-[(3-Amino-2-methylpropyl)amino]-1-butanol, Bis(hydrochloride) (10). To a solution of **9** (2.80 g, 17.9 mmol) in ethanol (30 mL) was added 9 mL of concentrated hydrochloric acid. The mixture was then hydrogenated in the presence of PtO_2 (0.4 g) at room temperature and atmospheric pressure. The catalyst was then filtered off and the filtrate was evaporated under reduced pressure to give a solid residue which was recrystallized (EtOH/Et_2O) to give **10** (3.5 g, 83%) as a white solid: ^{1}H NMR (D_2O) δ 1.14 (d, 3H), 1.50−1.85 (m, 4H), 2.30−2.40 (m, 1H), 2.85−3.20 (m, 6H), 3.61 (t, 2H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methylpropyl](4-hydroxybutyl)carbamic Acid, 1,1-Dimethylethyl Ester (11). To a stirred solution of **10** (3.2 g, 13.7 mmol) in water (20 mL) cooled to 10 °C were added 28 mL of a molar sodium hydroxide solution and then dropwise a solution of $(Boc)_2O$ (6.58 g, 30.1 mmol) in CH_2Cl_2 (50 mL). The stirring was continued at room temperature for 20 h. The layers were separated and the organic phase was washed with water (25 mL) then with a 5% $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude product was purified by flash chromatography on silica gel (Et_2O) to afford **11** as a viscous colorless oil (4.07, 82%): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3H), 1.30−1.75 (m, 22H), 1.90−1.95 (m, 1H), 2.34 (br s, 0.5H), 2.48 (br s, 0.5H), 2.80−3.35 (m, 6H), 3.63 (t, 2H), 4.85 (br s, 0.5H), 5.44 (br s, 0.5H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methylpropyl][4-(methylsulfonyloxy)butyl]carbamic Acid, 1,1-Dimethylethyl Ester (12). 12 (4.10 g, 94%) was obtained as a viscous yellow oil according to the method described for **5a** starting from **11** (3.60 g, 10.0 mmol) and methanesulfonyl

chloride (1.26 g, 11.0 mmol): ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3H), 1.35−2.00 (m, 23H), 2.80−3.40 (m, 9H), 4.25 (t, 2H), 4.88 (br s, 0.5H), 5.40 (br s, 0.5H).

(4-Azidobutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (13). 13 (2.32 g, 87%) was obtained in the form of a viscous yellow oil according to the procedure described for **6a** starting from **12** (3.0 g, 6.8 mmol) and NaN_3 (1.35 g, 20.8 mmol) after purification on silica gel (Et_2O/heptane, 6/4): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3H), 1.30−1.75 (m, 22H), 1.85−1.95 (m, 1H), 2.80−3.40 (m, 8H), 4.83 (br s, 0.4H), 5.38 (br s, 0.6H).

(4-Aminobutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (14). A mixture of **13** (1.77 g, 4.06 mmol) and 10% palladium on carbon (0.3 g) in methanol (30 mL) was stirred at room temperature under a hydrogen atmosphere (4 bar) for 20 h. The catalyst was then filtered off and the filtrate was evaporated under vacuum. The oily residue was purified by flash chromatography on silica gel (Et_2O/MeOH/NH_4OH (32%), 90/10/1) to yield **14** as a yellow viscous oil (1.40 g, 85%): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3H), 1.30−1.75 (m, 22H), 1.85−1.95 (m, 1H), 2.80−3.44 (m, 8H), 4.83 (br, 0.4H), 5.38 (br s, 0.6 H).

Preparation of Compound 26 (Scheme 3). 1-Azido-3-chloropropane (21). To a stirred solution of 1-bromo-3-chloropropane (23.85 g, 150.0 mmol) in DMSO (100 mL) was added a solution of NaN_3 (9.85 g, 150 mmol) in DMSO (50 mL). The stirring was continued for 20 h at room temperature. Then, water (100 mL) was added to the solution and the mixture was extracted with Et_2O (3 × 100 mL). The organic layers were combined, washed with a brine solution (3 × 50 mL), dried over $MgSO_4$, and concentrated under vacuum. The oily residue was then purified by distillation to afford **21** as a colorless oil (16.27 g, 90%): bp_{18} 45−50 °C (lit.[14] bp_{15} 45−50 °C); ^{1}H NMR ($CDCl_3$) δ 2.03 (q, 2H), 3.50 (t, 2H), 3.65 (t, 2H).

1-Azido-3-iodopropane (22). To a stirred solution of **21** (15.64 g, 131.0 mmol) in acetone (130 mL) was added NaI (40.0 g, 262.0 mmol). The stirring was continued for 20 h at reflux. After cooling at room temperature, the solvent was evaporated under reduced pressure; the obtained residue was taken up in water (100 mL) and then extracted with Et_2O (3 × 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was then purified by distillation to afford **22** as a colorless oil (21.7 g, 81%): $bp_{0.1}$ 25−30 °C (lit.[15] $bp_{0.1}$ 38−40 °C); ^{1}H NMR ($CDCl_3$) δ 2.04 (q, 2H), 3.25 (t, 2H), 3.43 (t, 2H).

3-Azido-*N*-methyl-1-propanamine (23). To a commercial solution of methylamine in water (40%, 20 equiv) was added a solution of **22** (11.33 g, 53.6 mmol) in methanol. The mixture was stirred at room temperature for 20 h and then extracted with Et_2O (3 × 30 mL). The organic layers were combined, dried over K_2CO_3, filtered, and concentrated under vacuum. The oily residue was purified by distillation to afford **23** (4.0 g, 65%) as a colorless oil: bp_{18} 48−50 °C (lit.[14] bp_{15} 46 °C); ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 1H), 1.76 (q, 2H), 2.43 (s, 3H), 2.66 (t, 2H), 3.36 (t, 2H).

4-[(3-Azidopropyl)methylamino]butanenitrile (24). 24 (7.43 g, 72%) was obtained in the form of a yellow oil according to the procedure described for **4a** starting from **23** (6.50 g, 57.0 mmol), Na_2CO_3 (7.25 g, 70.0 mmol), KI (2.36 g, 14.0 mmol), and chlorobutyronitrile (12.04 g, 114.0 mmol) after purification on silica gel (Et_2O/heptane, 4/6, then Et_2O): ^{1}H NMR ($CDCl_3$) δ 1.60−1.90 (m, 4H), 2.18 (s, 3H), 2.35−2.50 (m, 6H) 3.37 (t, 2H).

[3-[(3-Cyanopropyl)methylamino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (25). A mixture of **24** (3.63 g, 20.0 mmol), $(Boc)_2O$ (4.63 g, 21.0 mmol), and 10% palladium on carbon (0.3 g) in EtOAc (30 mL) was stirred at room temperature under a hydrogen atmosphere (4 bar) for 15 h. The catalyst was then filtered off and the organic phase was evaporated under reduced pressure. The oily residue was purified by flash chromatography on silica gel (Et_2O/heptane, 3/7) to yield **25** (4.78 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.64 (q, 2H), 1.80 (q, 2H) 2.18 (s, 3H), 2.35−2.50 (m, 6H), 3.17 (q, 2H), 5.28 (br s, 1H).

[3-[(4-Aminobutyl)methylamino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (26). 26 (2.43 g, 97%) was obtained in the form of a viscous yellow oil according to the procedure described for **8a** starting from **25** (2.55 g, 10.0 mmol) and after purification by flash chromatography on silica gel (MeOH/NH_4-OH (32%), 100/1): ^{1}H NMR ($CDCl_3$) δ 1.30−1.55 (m, 13H), 1.64 (q, 2H), 1.80 (s, 2H) 2.19 (s, 3H), 2.32 (t, 2H), 2.38 (t, 2H), 2.70 (t, 2H), 3.16 (q, 2H), 5.63 (br s, 1H).

Preparation of Compound 32 (Scheme 4). [3-[(4-Azido-1-methylbutylidene)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (29). To a stirred solution of **27** (2.0 g, 16.0 mmol) and 3 Å molecular sieves (5 g) in anhydrous Et_2O (60 mL) was added a solution of **28** (2.74 g, 16.0 mmol) in anhydrous Et_2O (40 mL). The mixture was stirred at room temperature for 20 h and then filtered. The solvent was evaporated under vacuum to give **29** (4.14 g, 93%) as a colorless viscous oil which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.30−2.00 (m, 16H), 2.33 (t, 2H), 3.00−3.45 (m, 6H), 5.21 (br s, 1H).

[3-[(4-Azido-1-methylbutyl)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (30). $NaBH_4$ (0.7 g, 18.0 mmol) was added in small portions to a solution of **29** (4.0 g, 14.1 mmol) in dry ethanol (100 mL) at 10 °C. After stirring for 3 h, the mixture was concentrated under vacuum. The residue was taken up in water (100 mL) and extracted with 3 × 50 mL. The organic layers were combined, dried over K_2CO_3, filtered, and concentrated under vacuum to give **30** (3.63 g, 90%) as a viscous yellow oil which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.05 (d, 3H), 1.30−1.80 (m, 16H), 2.50−2.80 (m, 3H), 3.20 (q, 2H), 3.28 (t, 2H), 5.21 (br s, 0.5H), 5.46 (br s, 0.5H).

(4-Azido-1-methylbutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (31). To a stirred solution of **30** (3.53 g, 12.4 mmol) in THF (50 mL) cooled to 0 °C was added dropwise a solution of $(Boc)_2O$ (2.73 g, 12.4 mmol) in THF (20 mL). The stirring was continued for 1 h at room temperature and the solvent was evaporated under vacuum. The residue was taken up in Et_2O (100 mL) and the solution washed respectively with a 0.01 N HCl solution (25 mL), then with water (25 mL), and finally with a 5% $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum. The oily residue was purified by flash chromatography on silica gel (Et_2O/heptane, 4/6) to afford **31** as a viscous colorless oil (3.44 g, 73%): ^{1}H NMR ($CDCl_3$) δ 1.19 (d, 3H), 1.35−1.85 (m, 24H), 2.90−3.20 (m, 4H), 3.29 (t, 2H), 3.70−3.80 (m, 0.5H), 4.10−4.20 (m, 0.5H), 4.79 (br s, 0.5H), 5.32 (br s, 0.5H).

(4-Amino-1-methylbutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (32). 32 (1.35 g, 98%) was obtained in the form of a viscous yellow oil according to the procedure described for **14** starting from **31** (1.47, 3.8 mmol) and after purification by flash chromatography on silica gel (MeOH/NH_4OH (32%), 100/1): ^{1}H NMR ($CDCl_3$) δ 1.14 (d, 3H), 1.70−1.80 (m, 24H), 2.00 (s, 2H) 2.69 (t, 2H), 2.95−3.25 (m, 4H), 3.70−3.80 (m, 0.5H), 4.00−4.10 (m, 0.5H), 4.85 (br s, 0.5H), 5.40 (br s, 0.5H).

Preparation of Compound 38 (Scheme 5). 5-Chloro-2-pentanol (33). 33 (7.20 g, 98%) was obtained in the form of a colorless oil according to the procedure described for **2a** starting from 5-chloro-2-pentanone (7.23 g, 60.0 mmol) and $NaBH_4$ (2.75 g, 72 mmol) and was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.20 (d, 3H), 1.40−1.55 (m, 2H), 1.75−1.95 (m, 2H), 3.39 (s, 1H), 3.58 (t, 2H), 3.80−3.90 (m, 1H).

5-[(3-Aminopropyl)amino]-2-pentanol (34). To a stirred solution of **33** (5.46 g, 44.5 mmol) in *n*-butanol (200 mL) were added Na_2CO_3 (5.66 g, 53.3 mmol), KI (1.85 g, 11.1 mmol), and 1,3-diaminopropane (32.98 g, 445.0 mmol). The stirring was continued at reflux for 20 h; then the solution was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (100 mL) and the solution was filtered. The filtrate was evaporated under vacuum and the oily residue was purified by distillation to give **34** as a colorless oil (2.64 g, 37%): $bp_{0.05}$

110−120 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 3H), 1.10−1.60 (m, 6H), 2.25−2.60 (m, 10H), 3.45−3.55 (m, 1H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]propyl](4-hydroxypentyl)carbamic Acid, 1,1-Dimethylethyl Ester (35). 35 (4.21 g, 77%) was obtained in the form of a viscous oil according to the procedure described for **31** starting from **34** (2.44 g, 15.2 mmol) and $(Boc)_2O$ (7.94 g, 36.5 mmol) and after purification by flash chromatography on silica gel (Et_2O): ^{1}H NMR ($CDCl_3$) δ 1.17 (d, 3H), 1.35−1.75 (m, 24H), 2.26 (br s, 0.4H), 2.64 (br s, 0.6H), 3.0−3.35 (m, 6H), 3.75−3.85 (m, 1H), 4.85 (br s, 0.4H), 5.4 (br s, 0.6H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]propyl][4-(methylsulfonyloxy)butyl]carbamic Acid, 1,1-Dimethylethyl Ester (36). 36 (4.63 g, 97%) was obtained in the form of a viscous yellow oil according to the method described for **5a** starting from **35** (3.94 g, 10.9 mmol), triethylamine (1.66 g, 16.4 mmol), and methanesulfonyl chloride (1.38 g, 12.0 mmol): ^{1}H NMR ($CDCl_3$) δ 1.40−1.50 (m, 21 H), 1.55−1.75 (m, 6H), 3.01 (s, 3H), 3.05−3.35 (m, 9H), 4.75−4.90 (m, 1.4H), 5.3 (br s, 0.6H).

(4-Azidopentyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (37). 37 (3.66 g, 97%) was obtained in the form of a yellow oil according to the procedure described for **6a** starting from **36 (**4.27 g, 9.7 mmol) and NaN_3 (1.90 g, 29.1 mmol) and was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H), 1.35−1.75 (m, 24H), 3.00−3.85 (m, 6H), 3.45−3.55 (m, 1H), 4.81 (br s, 0.4H), 5.32 (br s, 0.6H).

(4-Aminopentyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (38). 38 (2.66 g, 90%) was obtained in the form of a viscous yellow oil according to the procedure described for **14** starting from **37** (3.20 g, 8.3 mmol) and after purification by flash chromatography on silica gel (Et_2O/MeOH/NH_4OH (32%), 90/10/1): ^{1}H NMR ($CDCl_3$) δ 1.05 (d, 3H), 1.20−1.75 (m, 26H), 2.80−2.90 (m, 1H), 3.00−3.85 (m, 6H), 4.97 (br s, 0.4H), 5.41 (br s, 0.6H).

Preparation of Compound 46 (Scheme 6). 3-Azido-3-methylbutanoic Acid (39). This compound was obtained from dimethylacrylic acid (10.4 g, 100 mmol) according to the procedure described by Nagarajan and Ganem[7a] as a colorless oil (11.8 g, 83%): $bp_{0.05}$ 85−90 °C (lit.[7a] $bp_{0.05}$ 92−95 °C); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H), 2.50 (s, 2H), 11.40 (br s, 1H).

3-Azido-3-methyl-1-butanol (40). To a stirred solution of **39** (10.0 g, 70 mmol) in anhydrous THF (100 mL) under a nitrogen atmosphere was added dropwise a solution of BH_3·Me_2S (7 mL, 70 mmol) in THF (100 mL). The stirring was maintained under N_2 for 3 h at room temperature and then cooled with an ice−water bath. MeOH (10 mL) was added dropwise to the solution. Then the mixture was evaporated to dryness and dissolved in Et_2O (100 mL). The solution was washed, respectively, with a 10% sodium hydroxide solution (20 mL) and a brine solution (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by distillation to afford **40** as a colorless oil (7.8 g, 86%): $bp_{0.05}$ 110−120 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 6H), 1.76 (t, 2H), 2.50 (br s, 1H), 3.76 (t, 2H).

Methanesulfonic Acid, 3-Azido-3-methylbutyl Ester (41). 41 (7.89 g, 95%) was obtained in the form of yellow oil according to the method described for **5a** starting from **40** (5.17 g, 40.0 mmol), triethylamine (6.07 g, 60.0 mmol), and methanesulfonyl chloride (5.04 g, 44.0 mmol) and was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6H), 1.95 (t, 2H), 3.03 (s, 3H), 4.33 (t, 2H).

4-[(3-Azido-3-methylbutyl)amino]-1-butanol (42). To a stirred solution of **41** (4.32 g, 20.8 mmol) in *n*-butanol (100 mL) were added a solution of 4-amino-1-butanol (3.78 g, 40.0 mmol) in *n*-butanol (20 mL) and 10 drops of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). The stirring was continued at reflux for 20 h. After cooling, the mixture was concentrated under vacuum. The residue was purified by flash chromatography on silica gel (MeOH/NH_4OH (32%), 100 /1) to afford **42** as a viscous colorless oil (2.94 g, 79%): ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 6H), 1.65−1.75 (m, 6H), 2.60−2.75 (m, 4H), 3.56 (t, 2H), 3.70 (br s, 2H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-methylbutyl](4-hydroxybutyl)carbamic Acid, 1,1-Dimethylethyl Ester (43). 43 (5.53 g, 97%) was obtained in the form of a colorless viscous oil according to the method described for **7a** starting from **42** (3.04 g, 15.2 mmol) and $(Boc)_2O$ (7.02 g, 31.8 mmol) and after purification by flash chromatography on silica gel (Et_2O): ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 6H), 1.35−1.80 (m, 22H), 1.80−2.00 (m, 2H), 2.24 (br s, 0.6H), 2.62 (br s, 0.44), 3.05−3.30 (m, 4H), 3.65 (t, 2H), 4.46 (br s, 0.6 H), 4.62 (br s, 0.4H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-methylbutyl][4-(methylsulfonyloxy)butyl]carbamic Acid, 1,1-Dimethylethyl Ester (44). 44 (5.45 g, 96%) was obtained in the form of a viscous yellow oil according to the method described for **5a** starting from **43** (5.05 g, 13.5 mmol), triethylamine (2.05 g, 20.2 mmol), and methanesulfonyl chloride (1.70 g, 14.8 mmol) and was used without purification: ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 6H), 1.44 (s, 9H), 1.46 (s, 9H), 1.65−1.80 (m, 4H), 1.82−1.95 (m, 2H), 3.02 (s, 3H), 3.10−3.30 (m, 4H), 4.25 (t, 2H), 4.58 (br s, 0.5H), 4.62 (br s, 0.5H).

(4-Azidobutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutyl]carbamic Acid, 1,1-Dimethylethyl Ester (45). 45 (3.83 g, 79%) was obtained in the form of a viscous colorless oil according to the procedure described for **6a** starting from **44** (4.90 g, 11.6 mmol) and NaN_3 (2.28 g, 35.0 mmol), after purification by flash chromatography on silica gel (Et_2O/heptane, 2/8): ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 6H), 1.35−1.75 (m, 22H), 1.80−2.00 (m, 2H), 3.10−3.40 (m, 6H), 4.46 (br s, 0.5H), 4.57 (br s, 0.5H).

(4-Aminobutyl)[3-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutyl]carbamic Acid, 1,1-Dimethylethyl Ester (46). 46 (2.05 g, 90%) was obtained in the form of a viscous yellow oil according to the procedure described for **14** starting from **45** (2.05 g, 5.14 mmol) and after purification by flash chromatography on silica gel (MeOH/NH_4OH (32%), 100/1): ^{1}H NMR ($CDCl_3$) δ 1.05−1.75 (m, 32H), 1.80−2.00 (m, 2H), 2.70 (t, 2H), 3.05−3.30 (m, 4H), 4.53 (br s, 0.5H), 4.66 (br s, 0.5H).

Preparation of Compound 54a (Scheme 7). (2-Hydroxy-1(*R*)-methylethyl)carbamic Acid, 1,1-Dimethylethyl Ester (47a). To a stirred solution of D-alaninol (5.18 g, 69.0 mmol) in THF (50 mL) cooled to 0 °C was added a solution of $(Boc)_2O$ (15.05 g, 68.3 mmol) in THF (20 mL). After stirring for 1 h at room temperature, the solution was concentrated under vacuum. The residue was taken up in Et_2O (100 mL) and the solution washed, respectively, with a 0.01 N HCl solution (25 mL), then with water (25 mL), and finally with a 5% $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum. The resulting residue was recrystallized from heptane to afford **47a** as white crystals (9.90 g, 82%): mp 60 °C (lit.[17] mp 52−53 °C); $[\alpha]_D^{23} = +12.0$ (c = 1.0, $CHCl_3$) (lit.[17] $[\alpha]_D^{23} = +10.0$ (c = 1.0, MeOH)); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H), 1.44 (s, 9H), 3.35−3.65 (m, 4H), 4.99 (br s, 1H).

(2-Hydroxy-1(*S*)-methylethyl)carbamic Acid, 1,1-Dimethylethyl Ester (47b). Starting from L-alaninol and following the same procedure was obtained **47b** as white crystals (80%): mp 60 °C; $[\alpha]_D^{23} = -12.0$ (c = 1.0, $CHCl_3$).

[2-(Methylsulfonyloxy)-1(*R*)-methylethyl]carbamic Acid, 1,1-Dimethylethyl Ester (48a). To a stirred solution of **47a** (9.76 g, 55.7 mmol) and triethylamine (8.45 g, 83.6 mmol) in CH_2Cl_2 (60 mL) cooled to 0 °C and under N_2 atmosphere was added dropwise a solution of methanesulfonyl chloride (7.65 g, 61.3 mmol) in CH_2Cl_2 (60 mL). The stirring was continued for 2 h at room temperature and then the solution was poured into water (100 mL). The layers were separated and the organic phase was washed with a 0.1 N hydrochloric acid solution and then with a 5% $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered, and evaporated under vacuum to afford **48a** (11.64 g, 86%) as a white solid which was used without further purification: mp 76 °C (lit.[18] mp 75−76 °C); $[\alpha]_D^{22} = +30.0$ (c = 1.0, $CHCl_3$) (lit.[18] $[\alpha]_D^{23} = +29.9$ (c

= 1.0, $CHCl_3$)); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H), 1.45 (s, 9H), 3.05 (s, 3H), 3.95−4.0 (m, 1H) 4.10−4.30 (m, 2H), 4.80 (br s, 1H).

[2-(Methylsulfonyloxy)-1(*S*)-methylethyl]carbamic Acid, 1,1-Dimethylethyl Ester (48b). Starting from **47b** and following the same procedure **48b** was obtained as a white solid (92%) which was used without further purification: mp 76 °C (lit.[18] mp 75−76 °C); $[\alpha]_D^{22} = -30.0$ (c = 1.0, $CHCl_3$) (lit.[18] $[\alpha]_D^{23} = -30.2$ (c = 1.0, $CHCl_3$)).

(2-Cyano-1(*R*)-methylethyl)carbamic Acid, 1,1-Dimethylethyl Ester (49a). To a stirred solution of **48a** (11.00 g, 45.2 mmol) in DMSO (40 mL) was added NaCN (6.65 g, 135.7 mmol). The stirring was continued for 15 h at 40−45 °C. After cooling at room temperature, water (40 mL) was added to the solution and the mixture was extracted with Et_2O (3 × 50 mL). The organic layers were combined, washed with a brine solution (3 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. The obtained residue was recrystallized from heptane/*i*-Pr_2O to give **49a** as white crystals (5.80 g, 70%): mp 70 °C; $[\alpha]_D^{23} = +93.0$ (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3H), 1.45 (s, 9H), 2.54 (dd, 1H), 2.76 (dd, 1H) 3.90−4.00 (m, 1H), 4.67 (br s, 1H). Anal. ($C_9H_{16}N_2O_2$) C, H, N.

(2-Cyano-1(*S*)-methylethyl)carbamic Acid, 1,1-Dimethylethyl Ester (49b). Starting from **48b** and following the same procedure was obtained **49b** as white crystals (72%): mp 70 °C; $[\alpha]_D^{23} = -93.0$ (c = 1.0, $CHCl_3$).

(3-Amino-1(*R*)-methylpropyl)carbamic Acid, 1,1-Dimethylethyl Ester (50a). A mixture of **49a** (5.60 g, 30.4 mmol) and Raney nickel (1 g) in dry EtOH saturated with ammonia was stirred at room temperature under a hydrogen atmosphere (6 bar) for 15 h. Then the catalyst was filtered off and the filtrate was evaporated under reduced pressure to give a residue which was purified by flash chromatography on silica gel (Et_2O/MeOH/NH_4OH (32%), 90/10/1) to afford **50a** as a viscous colorless oil which crystallized (5.50 g, 96%): $bp_{0.05}$ 80−85 °C; $[\alpha]_D^{23} = -12.0$ (c = 2.0, $CHCl_3$); ee > 99%; ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H), 1.30−1.70 (m, 13H), 3.36 (t, 2H), 3.70−3.80 (m, 1H) 4.94 (m, 1H).

(3-Amino-1(*S*)-methylpropyl)carbamic Acid, 1,1-Dimethylethyl Ester (50b). Starting from **49b** and following the same procedure was obtained **50b** as a colorless oil (94%): $bp_{0.05}$ 80−85 °C; $[\alpha]_D^{23} = +13.0$ (c = 2.0, $CHCl_3$); ee > 99%.

[1(*R*)-Methyl-3-[(phenylmethylidene)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (51a). To a stirred solution of **50a** (4.60 g, 24.4 mmol) and 3 Å molecular sieves (5 g) in anhydrous Et_2O (80 mL) was added a solution of benzaldehyde (2.59 g, 24.4 mmol) in anhydrous Et_2O (40 mL). The mixture was stirred at room temperature for 20 h and then filtered. The solvent was evaporated under vacuum to give **51** (6.75 g, 98%) as a white solid which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.20 (d, 3H), 1.42 (s, 9H), 1.70−1.95 (m, 2H), 3.55−3.90 (m, 3H), 5.12 (br s, 1H), 7.33−7.77 (m, 5H), 8.26 (s, 1H).

[1(*S*)-Methyl-3-[(phenylmethylidene)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (51b). Starting from **50b** and following the same procedure was obtained **51b** as a white solid which was used without further purification (98%).

[1(*R*)-Methyl-3-[(phenylmethyl)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (52a). $NaBH_4$ (1.40 g, 36.6 mmol) was added in small portions to a solution of **51a** (6.75 g, 24.4 mmol) in dry ethanol (100 mL) at 10 °C. After stirring for 3 h, the mixture was concentrated under vacuum. The residue was taken up in water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The obtained residue was purified by recrystallization from heptane as white crystals (4.89 g, 86%): mp 79 °C; $[\alpha]_D^{22} = -5.2$ (c = 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 3H), 1.35−1.80 (m, 12H), 2.60−2.80 (m, 2H), 3.65−3.85 (m, 3H), 5.22 (br s, 1H), 7.19−7.35 (m, 5H).

[1(*S*)-Methyl-3-[(phenylmethyl)amino]propyl]carbamic Acid, 1,1-Dimethylethyl Ester (52b). Starting from **51b** and following the same procedure was obtained **52b** as a white solid (84%): mp 79 °C; $[\alpha]_D^{22} = +5.1$ (c = 2.0, $CHCl_3$). Anal. ($C_{16}H_{26}N_2O_2$) C, H, N.

[3-[(3-Cyanopropyl)(phenylmethyl)amino]-1(*R*)-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (53a). 4-Chlorobutyronitrile (3.03 g, 28.7 mmol) was added into a mixture of **52a** (5.33 g, 19.1 mmol), potassium iodide (0.80 g, 4.8 mmol), and sodium carbonate (2.43 g, 22.9 mmol) in butanol (50 mL). The mixture was stirred at reflux for 20 h and then concentrated under vacuum. The residue was taken up in Et_2O (100 mL) and the insoluble salts were filtered and washed with Et_2O (2 × 50 mL). The organic layers were combined and concentrated under vacuum. The obtained residue was purified by flash chromatography on silica gel (Et_2O) to afford **53a** (6.58 g, 98%) as a viscous colorless oil: $[\alpha]_D^{22} = -6.90$ (c = 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 3H), 1.47−1.83 (m, 4H), 2.15−2.80 (m, 6H), 3.33 (d, 1H) 3.78 (d, 1H), 3.80−3.90 (m, 1H), 4.80 (br s, 1H), 7.20−7.40 (m, 5H).

[3-[(3-Cyanopropyl)(phenylmethyl)amino]-1(*S*)-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (53b). Starting from **52b** and following the same procedure was obtained **53b** as a viscous colorless oil (93%): $[\alpha]_D^{22} = +7.1$ (c = 2.0, $CHCl_3$).

[3-[(4-Aminobutyl)(phenylmethyl)amino]-1(*R*)-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (54a). A mixture of **53a** (5.90 g, 14.5 mmol) and Raney nickel (1 g) in dry EtOH saturated with ammonia was stirred at room temperature under a 6 bar hydrogen atmosphere for 15 h. Then the catalyst was filtered off and the filtrate was evaporated under reduced pressure to give after purification **54a** (5.44 g, 92%) as a viscous colorless oil: $[\alpha]_D^{22} = -1.4$ (c = 1.1, $CHCl_3$); ee > 95%;[10] ^{1}H NMR δ 1.04 (d, 3H), 1.31 (s, 2H), 1.35−1.80 (m, 15H), 2.25−2.70 (m, 6H), 3.43 (d, 1H), 3.60 (d, 1H), 3.65−3.75 (m, 1H), 5.74 (br s, 1H), 7.15−7.35 (m, 5H).

[3-[(4-Aminobutyl)(phenylmethyl)amino]-1(*S*)-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (54b). Starting from **53b** and following the same procedure was obtained **54b** as a viscous colorless oil (90%): $[\alpha]_D^{22} = +1.6$ (c = 1.2, $CHCl_3$); ee > 95%.[10]

General Procedure for the Preparation of Compounds 56a−i. Compounds of the general structure **56a−i** (Scheme 8) were synthesized from N-protected methylated spermidine derivatives (**8a**, **14**, **20**, **26**, **32**, **38**, **46**, **54a**,**b**) and 3-[[(1,1-dimethylethoxy)carbonyl]amino]-12-oxo-2,4,11-triazatetradec-2-enedioic acid, 1-(1,1-dimethylethyl ester) (**A**) using a standard coupling method to give **55a−i** followed by removal of protecting groups by hydrogenolysis and/or acidic treatment.

Preparation of Derivatives 55a−i. Standard Procedure. To a stirred solution of 3-[[(1,1-dimethylethoxy)carbonyl]amino]-12-oxo-2,4,11-triazatetradec-2-enedioic acid, 1-(1,1-dimethylethyl ester) (1 equiv) in CH_2Cl_2 were added at 0 °C DCC (1.5 equiv) and HOBT (0.2 equiv). The mixture was allowed to stand 0.5 h at 0 °C. Then the appropriate N-protected methylated spermidine (1 equiv) in CH_2Cl_2 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 overnight. The solvent was evaporated off under reduced pressure and the obtained residue was purified by flash chromatography as indicated.

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]propyl][17-[[(1,1-dimethylethoxy)carbonyl]amino]-4,21,21-trimethyl-6,8,19-trioxo-20-oxa-5,9,16,18-tetraaza-17-docosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (55a). From **38**; colorless oil, yield 91% after purification by flash chromatography on silica gel (methylcyclohexane/EtOAc, 6/4, then EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.05−1.75 (m, 53H), 3.0−3.3 (m, 11H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 and 7.2 (br s, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[3-[[(1,1-Dimethylethoxy)carbonyl]amino]propyl][17-[[(1,1-dimethylethoxy)carbonyl]amino]-1,21,21-trimethyl-6,8,19-trioxo-20-oxa-5,9,16,18-tetraaza-17-docosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (55b). From **32**;

translucent white amorphous solid, yield 90% after purification by flash chromatography on silica gel (methylcyclohexane/EtOAC, 1/1, then EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.05−1.75 (m, 53H), 3.0−3.35 (m, 11H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 and 7.2 (br s, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[21-[[(1,1-Dimethylethoxy)carbonyl]amino]-4,25,25-trimethyl-10,12,23-trioxo-24-oxa-4,9,13,20,22-pentaaza-21-hexacosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (55c). From **26**; yellow oil, yield 89% after purification by flash chromatography on silica gel (EtOAc then EtOAc/EtOH/NH_3): ^{1}H NMR ($CDCl_3$) δ 1.05−1.85 (m, 44H), 2.3−2.55 (m, 2H), 2.6−2.7 (m, 3H), 3.15−3.55 (m, 9H), 5.3−5.5 (br s, 1H), 7.1−7.3 (br s, 1H), 7.5−7.7 (br s, 1H), 8.3 (t, 1H) 11.5 (s, 1H).

[17-[[(1,1-Dimethylethoxy)carbonyl]amino]-21,21-dimethyl-6,8,19-trioxo-20-oxa-5,6,16,18-tetraaza-17-docosen-1-yl][3-[[(1,1-dimethylethoxy)carbonyl]amino]-1-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (55d). From **20**; colorless oil, yield 82% after purification by flash chromatography on silica gel (methylcyclohexane/EtOAc, 2/8, then EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.05−1.75 (m, 53H), 3.0−3.3 (m, 11H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 and 7.2 (br s, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[17-[[(1,1-Dimethylethoxy)carbonyl]amino]-21,21-dimethyl-6,8,19-trioxo-20-oxa-5,9,16,18-tetraaza-17-docosen-1-yl][3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (55e). From **14**; yellow amorphous solid, yield 89% after purification by flash chromatography on silica gel (methylcyclohexane/EtOAc, 1/1, then EtOAc): ^{1}H NMR ($CDCl_3$) δ 0.9 (d, 3H), 1.05−1.75 (m, 49H), 2.85−3.0 (m, 2H), 3.1−3.35 (m, 10H), 3.4 (q, 2H), 4.8 and 5.3 (br s, 1H), 6.8 and 7.2 (br s, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[21-[[(1,1-Dimethylethoxy)carbonyl]amino]-1,25,25-trimethyl-10,12,23-trioxo-4-(phenylmethyl)-24-oxa-4,9,13,-20,22-pentaaza-21-hexacosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (55f). From **8a**; yellow amorphous solid, yield 80% after purification by flash chromatography on silica gel (EtOAc then EtOAc/EtOH, 9/1): ^{1}H NMR (DMSO-d_6) δ 1.0−1.9 (m, 44H), 2.35−2.60 (m, 42H), 3.00−3.25 (m, 7H), 3.35−3.70 (m, 4H), 6.0 (br s, 1H), 7.15−7.35 (m, 5H), 7.75−7.95 (m, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[17-[[(1,1-Dimethylethoxy)carbonyl]amino]-21,21-dimethyl-6,8,19-trioxo-20-oxa-5,9,16,18-tetraaza-17-docosen-1-yl][3-[[(1,1-dimethylethoxy)carbonyl]-3-methylbutyl]-carbamic Acid, 1,1-Dimethylethyl Ester (55g). From **46**; yellow oil, yield 84% after purification by flash chromatography on silica gel (EtOAc/methylcyclohexane, 1/1, then EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.05−1.70 (m, 54H), 3.1−3.35 (m, 12H), 3.4 (q, 2H), 4.6 (br s, 1H), 7.0 (br s, 1H), 7.2 (br s, 1H), 8.3 (t, 1H), 11.5 (s, 1H).

[21-[[(1,1-Dimethylethoxy)carbonyl]amino]-1,25,25-trimethyl-10,12,23-trioxo-4-(phenylmethyl)-24-oxa-4,9,13,-20,22-pentaaza-21-hexacosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (*R*) (55h). From **54a**; colorless oil, yield 80% after purification by flash chromatography on silica gel (EtOAc/EtOH, 3/1, then EtOAc/EtOH/NH_3, 9/1/0.1): $[\alpha]_D^{23.5} = -4.1$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.0 (d, 3H), 1.2−1.8 (m, 39H), 2.3−2.6 (m; 3H), 3.1−3.8 (m, 8H), 5.3 (br s, 1H), 7.1 (br s, 1H), 7.2−7.4 (m, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

[21-[[(1,1-Dimethylethoxy)carbonyl]amino]-1,25,25-trimethyl-10,12,23-trioxo-4-(phenylmethyl)-24-oxa-4,9,13,-20,22-pentaaza-21-hexacosen-1-yl]carbamic Acid, 1,1-Dimethylethyl Ester (*S*) (55i). From **54b**; colorless oil, yield 88% after purification by flash chromatography on silica gel (EtOAc/EtOH, 9/1, then EtOAc/EtOH/NH_3, 9/1/0.1): $[\alpha]_D^{23.5} = +4.3$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.0 (d, 3H), 1.2−1.8 (m, 39H), 2.3−2.6 (m; 3H), 3.1−3.8 (m, 8H), 5.3 (br s, 1H), 7.1 (br s, 1H), 7.2−7.4 (m, 2H), 8.3 (t, 1H), 11.5 (s, 1H).

Preparation of Derivatives 56a−e,g. Standard Procedure. The appropriate derivative **55** was dissolved in a solution of trifluoroacetic acid and anhydrous CH_2Cl_2 (v/v). The reaction mixture was stirred for 12 h at room temperature and the solvents were then removed under reduced pressure. The oily residue was then taken up in water and washed with EtOAc (×3). The aqueous phase was lyophilized and the obtained residue was purified by MPLC on RP18 silica gel (type grafted, particle size 5−20 μm) as indicated.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-aminopropyl)amino]-1-methylbutyl]propanediamide, Tris(trifluoroacetate) (56a).** From **55a**; colorless oil, yield 68% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1/8/1): ^{1}H NMR (DMSO-d_6) δ 1.0 (d, 3H), 1.25−1.65 (m, 12H), 1.9 (m, 2H), 2.85−3.15 (m, 12H), 3.75 (m, 1H), 7.0−8.0 (m, 12H); ^{13}C NMR (D_2O) δ 20.54, 22.26, 23.80, 25.75, 25.97, 28.38, 28.92, 32.87, 36.18, 38.55, 41.27, 43.41, 43.72, 43.82, 46.60, 156.80, 166.30, 166.78.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-aminopropyl)amino]pentyl]propanediamide, Tris(trifluoroacetate) (56b).** From **55b**; colorless oil, yield 86% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 2/7/1): ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H), 1.25−1.55 (m, 10H), 1.85 (m, 2H), 2.85−3.15 (m, 13H), 6.8−7.5 (br s, 3H), 7.6 (t, 1H), 7.8−8.15 (m, 6H), 8.35−8.65 (m, 2H); ^{13}C NMR (D_2O) δ 15.67, 24.00, 24.86, 25.72, 25.94, 28.36, 28.87, 29.49, 36.25, 38.12, 38.55, 40.65, 41.09, 43.36, 53.14, 156.78, 166.72, 166.91.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-aminopropyl)methylamino]butyl]propanediamide, Tris(trifluoroacetate) (56c).** From **55c**; translucent white amorphous solid, yield 55% after purification by MPLC on a RP18 silica gel (EtOH/H_2O/TFA, 2/7.5/0.5): ^{1}H NMR (DMSO-d_6) δ 1.2−1.7(m, 12H), 1.9 (m, 2H), 2.7(s, 3H), 2.85 (t, 2H), 2.95−3.2 (m,12H); 6.7−7.4 (br s, 4H), 7.70 (t, 1H), 7.75−8.1 (m, 5H), 9.7 (br s, 2H); ^{13}C NMR (D_2O) δ 21.74, 22.72, 26.21, 26.36, 28.56, 28.74, 28.84, 37.27, 39.42, 40.32, 40.39, 41.87, 44.31, 53.54, 56.62, 157.53, 170.01, 170.36.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-amino-1-methylpropyl)amino]butyl]propanediamide, Tris(trifluoroacetate) (56d).** From **55d**; colorless oil, yield 61% after purification by MPLC on a RP 18 silica gel (CH_3CN/H_2O/TFA, 2/7/1): ^{1}H NMR (DMSO-d_6) δ 1.2−1.7 (m, 15H), 1.8 (m, 1H), 2.0 (m, 1H), 2.8−3.2 (m, 12H), 3.3 (m, 1H), 6.7−7.4 (m, 4H), 7.6 (t, 1H), 7.8−8.1 (m, 5H), 8.5 (br s, 2H); ^{13}C NMR (D_2O) δ 15−91, 23.68, 26.19, 26.25, 26.34, 28.55, 28.82, 30.91, 36.66, 39.47, 40.30, 41.88, 45.27, 52.66, 157.61, 170.21, 170.41.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-amino-2-methylpropyl)amino]butyl]propanediamide, Tris(trifluoroacetate) (56e).** From **55e**; translucent white amorphous solid, yield 65% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1/8/1): ^{1}H NMR (DMSO-d_6) δ 1.0 (d, 3H), 1.25−1.65 (m, 12H), 2.15 (q, 1H), 2.65−3.15 (m, 14H), 7.1 (br s, 3H), 7.65 (t, 1H), 7.8−8.1 (m, 6H), 8.4−8.6 (br s, 2H); ^{13}C NMR (D_2O) δ 14.97, 23.56, 26.20, 26.26, 26.34, 28.55, 28.83, 30.21, 39.50, 40.31, 41.88, 43.15, 44.29, 48.80, 51.30, 157.55, 170.01, 170.33.

***N*^{1}-[6-[(Aminoiminomethyl)amino]hexyl]-*N*^{3}-[4-[(3-amino-3-methylbutyl)amino]butyl]propanediamide, Tris(trifluoroacetate) (56g).** From **55g**; white translucent amorphous solid, yield 75% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1.3/8/0.7): ^{1}H NMR (DMSO-d_6) δ 1.2−1.65 (m, 18H), 1.85−1.95 (m, 2H), 2.85−3.15 (m, 12H), 6.8−7.5 (br s, 4H), 7.65 (t, 1H), 7.9−8.15 (m, 5H), 8.5−8.7 (br s, 2H).

Preparation of Derivatives 56f,h,i. Standard Procedure. A solution of the appropriate derivative **55** in methanol/1 M hydrochloric acid solution (200/1 v/v) was hydrogenated in the presence of palladium chloride (0.1 equiv) at room temperature and atmospheric pressure for 6 h. The catalyst was then filtered off and the filtrate was evaporated under reduced pressure to give a residue which was taken up in a solution of trifluoroacetic acid and anhydrous CH_2Cl_2 (v/v). The reaction mixture was stirred for 24 h at room temperature and the solvents were then removed under reduced pressure. The oily residue was then taken up in water and washed with EtOAc (×3). The aqueous phase was lyophilized and the obtained residue was purified by MPLC on RP18 silica gel (type grafted, particle size 5−20 μm) as indicated.

***N*¹-[4-[(3-Aminobutyl)amino]butyl]-*N*³-[6-[(aminoiminomethyl)amino]hexyl]propanediamide, Tris(trifluoroacetate) (56f).** From **55f**; translucent white amorphous solid, yield 47% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 8/1/1): ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H), 1.25−1.7 (m, 15H), 1.7−1.9 (m, 1H), 2.85−3.15 (m, 15H), 4 (s, 2H), 6.87 (m, 5H), 7.7 (t, 1H), 7.85−795 (t, 1H), 7.95−8.2 (m, 3H), 8.7−8.9 (br s, 2H); ^{13}C NMR (D_2O) δ 18.02, 23.65, 26.19, 26.28, 26.76, 28.56, 29.93, 31.22, 39.77, 40.59, 41.88, 44.61, 46,11, 48.22, 63.76, 157,54, 158.21, 171.45.

***N*¹-[4-[(3-Aminobutyl)amino]butyl]-*N*³-[6-[(aminoiminomethyl)amino]hexyl]propanediamide, Tris(trifluoroacetate) (*R*) (56h).** From **55h**; translucent white amorphous solid, yield 46% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1/8/1): $[\alpha]_D^{23} = +1.1$ (c = 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H), 1.35−1.7 (m, 12H), 1.75 (m, 1H), 1.9 (m, 1H), 2.85−3.15 (m, 12H), 3.3 (m, 1H), 6.6−7.5 (br s, 4H), 7.65 (t, 1H), 7.8−8.15 (m, 5H), 8.35−8.7 (2H); ^{13}C NMR (D_2O) δ 18.02, 23.71, 26.18, 26.23, 26.35, 28.56, 28.84, 31.23, 39.50, 40.30, 41.88, 44.30, 44.61, 46,10, 48.15, 158.91, 170.01, 170.33.

***N*¹-[4-[(3-Aminobutyl)amino]butyl]-*N*³-[6-[(aminoiminomethyl)amino]hexyl]propanediamide, Tris(trifluoroacetate) (*S*) (56i).** From **55i**; colorless oil, yield 61% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 30/65/5): $[\alpha]_D^{23} = -0.9$ (c = 2, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.2−1.65 (m, 12H), 1.8 (m, 1H), 1.95 (m, 1H), 2.8−3.15 (m, 12H), 3.3 (m, 1H), 6.8−7.6 (br s, 4H), 7.75 (t, 1H), 7.9−8.15 (m, 5H), 8.5−8.7 (m, 2H).

General Procedure for the Preparation of Compounds 60a−g. Compounds of the general structure **60a−g** (Scheme 9) were synthesized from the N-Protected α-alkylated spermidine intermediates (**8a−d** and **54a**,**b**) or mono-N-Boc-protected 1,4-diaminobutane with methyl 2-[(phenoxycarbonyl)-oxy]acetate and [(6-aminohexyl)carbonimidoyl]bis(carbamic acid), bis(1,1-dimethylethyl ester) (**B**), the syntheses of which were described in a previous paper.[3] The syntheses of compounds **60a−g** were performed according to the representative procedure illustrated for **60e**.

[3-[[4-(2,4-Dioxo-3-oxazolidinyl)butyl](phenylmethyl)-amino]-1-methylpropyl]carbamic Acid, 1,1-Dimethylethyl Ester (*R*) (57e). To a solution of methyl 2-[(phenoxycarbonyl)oxy]acetate (1.57 g, 7.5 mmol) and triethylamine (0.8 g, 8 mmol) in toluene (100 mL) was added a solution of **54a** (1.8 g, 5.15 mmol) in toluene (50 mL). The reaction mixture was stirred at 80 °C 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, then EtOAc) to afford **57e** (4.5 g, 90%) as an oil which crystallized as white needles: $[\alpha]_D^{22} = -1.0$, (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H), 1.4−1.7 (m, 15H), 2.3−2.6 (m, 4H), 3.4−3.75 (m, 5H), 4.66 (s, 1H), 5.3 (br s, 1H), 5.3 (br s, 1H), 7.2−7.4 (m, 5H).

2,2,6-Trimethyl-4,15-dioxo-9-(phenylmethyl)-3,16-dioxa-5,9,14-triazaoctadecan-18-oic Acid (*R*) (58e). 57e (2 g, 4.62 mmol) was dissolved in a mixture of 1 N NaOH (20 mL) and DME (20 mL). The reaction mixture was stirred for 15 h at room temperature, concentrated to one-third of its volume, and then acidified to pH 2 with 1 N HCl. The aqueous solution was then lyophilized to give **58e** (2.5 g) as a white pasty solid which contained sodium chloride but which can be used without further purification in the next step: $[\alpha]_D^{22} = -3.9$ (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.17 (d, 3H), 1.25−2.1 (m, 15H), 2.35−3.3 (m, 8H), 3.45−3.7 (m, 1H), 4.0−4.35 (m, 2H), 4.8−5.2 (m, 1H), 7.15−7.8 (m, 6H), 12.1−12.7 (br s, 1H).

[4-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]-(phenylmethyl)amino]butyl]carbamic Acid, 11-[[(1,1-Dimethylethoxy)carbonyl]amino]-15,15-dimethyl-2,13-dioxo-14-oxa-3,4,12-triaza-11-hexadecen-1-yl Ester (*R*) (59e). A solution of isobutyl chloroformate (0.68 g, 5.0 mmol) in THF (5 mL) was added dropwise to a solution, cooled to −30 °C, of **58e** (2.5 g, 5.6 mmol) and *N*-methylmorpholine (1.51 g, 15.0 mmol) in THF (50 mL). The reaction mixture was stirred for 0.5 h and a solution of [(6-aminohexyl)carbonimidoyl]bis(carbamic acid), bis(1,1-dimethylethyl ester) (1.97 g, 50 mmol) in THF (20 mL) was added. Stirring was maintained for 0.5 h at −30 °C and then 2 h at room temperature. After evaporation of the solvent under vacuum, the obtained residue was purified by flash chromatography on silica gel (EtOAc then EtOAc/EtOH, 9/1) to afford **59e** in the form of a colorless oil (3.6 g, 81%): $[\alpha]_D^{22} = -2.3$ (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.01 (d, 3H), 1.3−1.8 (m, 41H), 2.3−2.5 (m, 3H), 2.5−2.65 (m, 1H), 3.05−3.2 (m, 2H), 3.2−3.3 (m, 2H), 3.35−3.5 (m, 3H), 3.6 (d, 1H), 3.65−3.8 (m, 1H), 4.5 (s, 2H), 5.35−5.6 (m, 2H), 6.25−6.4 (br s, 1H), 7.2−7.35 (m, 5H), 8.3 (t, 1H), 11.5 (s, 1H).

[4-[(3-Aminobutyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (*R*) (60e). This compound was obtained in the form of a translucent white amorphous solid (2.48 g, 73%) according to procedure described for **56f** and starting from **59e** (3.6 g, 4.5 mmol): $[\alpha]_D^{22} = +1.1$ (c = 2.0, CH_3OH); ee > 99%; ^{1}H NMR (DMSO-d_6) δ 1.18 (d, 3H), 1.25−1.65 (m, 12H), 1.65−1.85 (m, 1H), 1.85−2.0 (m, 1H), 2.85−3.15 (m, 10H), 3.2−3.35 (m, 1H), 4.33 (s, 2H), 6.8−7.3 (br s, 3H), 7.32 (t, 1H), 7.62 (t, 1H), 7.86 (t, 1H), 7.9−8.05 (br s, 4H), 8.5−8.7 (br s, 2H); ^{13}C NMR (D_2O) δ 18.01, 23.64, 26.19, 26.28, 26.76, 28.56, 28.93, 31.21, 39.77, 40.58, 41.87, 44.60, 46.10, 48.21, 63.75, 157.55, 157.89, 171.44.

[4-[(3-Aminobutyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (60a). From **59a**; colorless oil, yield 89% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 2/7.5/0.5): ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H), 1.25−1.7 (m, 12H), 1.8 (m, 1H), 1.95 (m, 1H), 2.80−3.15 (m, 10H), 3.3 (m, 1H), 4.3 (s, 2H), 6.9−7.5 (m, 5H), 7.7 (t, 1H), 7.75−8.3 (m, 4H), 8.8 (br s, 2H); ^{13}C NMR (D_2O) δ 18.02, 23.65, 26.19, 26.28, 26.76, 28.56, 28.93, 31.22, 39.77, 40.59, 41.88, 44.61, 46.11, 48.22, 63.76, 157.54, 158.21, 171.45.

[4-[(3-Aminopentyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (60b). From **59b**; translucent white amorphous solid, yield 76% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1/8/1): ^{1}H NMR (DMSO-d_6) δ 0.9 (t, 3H), 1.2−1.65 (m, 14H), 1.85 (m, 2H), 2.85−3.25 (m, 11H), 4.3 (s, 2H), 6.7−7.35 (m, 5H), 7.6 (t, 1H), 7.8−8.1 (m, 4H), 8.55 (br s, 2H); ^{13}C NMR (D_2O) δ 9.06, 23.65, 25.39, 26.20, 26.30, 26.77, 28.57, 28.94, 29.01, 39.78, 40.58, 41.88, 44.53, 48.19, 51.24, 52.08, 63.75, 158.44, 158.51, 198.17.

[4-[(3-Amino-3-phenylpropyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (60c). From **59c**; colorless oil, yield 96% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 2/7.5/0.5): ^{1}H NMR (DMSO-d_6) δ 1.2−1.6 (m, 12H), 2.1−2.35 (m, 2H), 2.8−3.15 (m, 10H), 4.25−4.45 (m, 3H), 6.6−7.7 (m, 11H), 7.9 (m, 1H), 8.4−8.8 (m, 6H); ^{13}C NMR (D_2O) δ 23.58, 26.27, 26.36, 26.78, 28.64, 29.01, 30.56, 39.85, 40.62, 41.96, 44.60, 48.16, 51.74, 51.76, 53.71, 63.84, 128.14, 130.60, 131.01, 135.04, 158.26, 158.60, 171.52.

[4-[(3-Amino-4,4,4-trifluorobutyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]-amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (60d). From **59d**; colorless oil, yield 94% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 2/7.5/0.5): ^{1}H NMR (DMSO-d_6) δ 1.25−1.65 (m, 12H), 1.9 (m, 1H), 2.05 (m, 1H), 2.9−3.2 (m, 10H), 4.05 (m, 1H), 4.35 (s, 2H), 6.6−7.4 (m, 5H), 7.6 (t, 1H), 7.9 (t, 1H), 8.0−8.8 (m, 5H); ^{13}C NMR (D_2O) δ 23.63, 24.48, 26.20, 26.29, 26.75, 28.57, 28.94, 39.77, 40.55, 41.86, 43.83,48.27, 50.86, 63.74, 115.24, 119.10, 122.44, 126.15, 157.91, 158.24, 171.47.

[4-[(3-Aminobutyl)amino]butyl]carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (*S*) (60f). From **59f**; translucent white amorphous solid, yield 82% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1.5/8/0.5): $[\alpha]_D^{23} = -0.95$ (c = 2, CH_3OH); ee > 99%; ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H), 1.25−1.7 (m, 12H), 1.75 (m, 1H), 1.9 (m, 1H), 2.85−3.10 (m, 10H), 3.30 (m, 1H), 4.35 (s, 2H), 6.8−7.4 (m, 5H), 7.6 (t, 1H), 7.75−8.1 (m, 4H), 8.4−8.6 (m, 2H); ^{13}C NMR (D_2O) δ

15.01, 20.64, 23.19, 23.28, 23.76, 25.56, 25.93, 28.22, 36.77, 37.59, 38.88, 41.60, 43.10, 45.21, 60.75, 154.87, 155.20, 168.43.

(3-Aminobutyl)carbamic Acid, 2-[[6-[(Aminoiminomethyl)amino]hexyl]amino]-2-oxoethyl Ester, Tris(trifluoroacetate) (60g). From **59g**; colorless oil, yield 81% after purification by MPLC on a RP18 silica gel (CH_3CN/H_2O/TFA, 1.5/8/0.5): ^{1}H NMR (DMSO-d_6) δ 1.2−1.6 (m, 12H), 2.75 (m, 2H), 2.95−3.2 (m, 6H), 4.35 (s, 2H), 6.9−7.6 (m, 5H), 7.75−8.1 (m, 5H); ^{13}C NMR (D_2O) δ 23.75, 24.81(2C), 26.21, 26.24, 26.35, 28.56, 28.84, 36.32, 39.50, 40.31, 41.87, 43.54, 44.30, 48.17, 53.69, 158.2, 170.02, 170.34.

Biology. Experimental graft-versus-host disease (GVHD) was induced in cyclophosphamide-immunosuppressed B6D2F1 mice[19] by intravenous injection of 2×10^8 viable spleen cells from B6 origin. Untreated control animals died generally between days 14 and 21 post-GVHD induction. Compounds were dissolved in 0.9% NaCl solution and administered from days 1 to 10 (day 6 omitted) by intraperitoneal route. In such experimental conditions, control vehicle was unable to modify survival. Survival was followed until day 60 post-cell injection.

Heterotopic abdominal heart transplant was performed according to the method of Ono and Lindsey.[20] 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 and confirmed after autopsy. Treatment was performed 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).

Acknowledgment. The authors thank Valérie Lepais, Philippe Peralba, Anne-Marie Dhennequin, Gilles Martin-Gousset, Florence Chirade, Agnès Martinien, Marina Tripier, Hervé Duclos, and Martine Page for their skillful experimental work.

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JM991043X