Synthesis and Esterolytic Activity of Catalytic Peptide Dendrimers

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Abstract: Peptide dendrimers were prepared by solid-phase peptide synthesis. Monomeric dendrimers were first obtained by assembly of a hexapeptide sequence containing alternate standard α-amino acids with diamino acids as branching units. The monomeric dendrimers were then dimerized by disulfide-bridge formation at the core cysteine. The synthetic strategy is compatible with functional amino acids and different diamino acid branching units. Peptide dendrimers composed of the catalytic triad amino acids histidine, aspartate, and serine catalyzed the hydrolysis of *N*-methylquinolinium salts when the histidine residues were placed at the outermost position. The dendrimer-catalyzed hydrolysis of 7-isobutyryl-*N*-methylquinolinium followed saturation kinetics with a rate constant of catalysis/rate constant without catalysis (k_{cat}/k_{uncat}) value of 3350 and a rate constant of catalysis/Michaelis constant (k_{cat}/K_M) value 350-fold larger than the second-order rate constant of the 4-methylimidazole-catalyzed reaction; this corresponds to a 40-fold rate enhancement per histidine side chain. Catalysis can be attributed to the presence of histidine residues at the surface of the dendrimers.

Keywords: dendrimers · enzyme models · ester hydrolysis · peptides · solid-phase synthesis

Introduction

Enzyme catalysis is one of the most fascinating properties encountered in proteins. Enzyme catalysis is characterized by the emergence of selective molecular recognition, transition-state stabilization, and regulation processes in the folded protein which are not encountered at the level of the amino acid building blocks.[1] These processes appear as a consequence of the interplay between amino acids within the ordered three-dimensional structure produced by the folding process. Rational de novo enzyme design, which is one of the main goals of supramolecular chemistry and biochemistry, would require a complete understanding of both folding and catalysis. The key difficulty resides in choosing the right amino acid sequence and length among the combinatorial infinity of possibilities to fold into a stable functional structure.

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The analysis of protein structures over the years has given sufficient insights to permit the rational design and synthesis of single synthetic peptides that fold into predetermined topologies, such as four-helix bundles.[2] Nevertheless the approach is limited. The vast majority of artificial proteins are produced by semiempirical approaches which, in one way or another, circumvent the need for complete rational design by applying experimental or theoretical search algorithms inspired by natural selection and evolution. These methods are based on defining boundaries for active structures and randomly generating candidates within these boundaries; the candidates are then selected on the basis of their properties. The strategy concerns computational predictions of the three-dimensional structure of proteins,[3] catalytic antibodies,[4] and the directed evolution of enzymes.[5]

Our approach to de novo enzyme design is based on peptide dendrimers (Scheme 1). Dendrimers are ramified structures that adopt globular or disk-shaped structures as a consequence of topology rather than folding.[6] Catalytic dendrimers are known that are based on ether linkages with incorporation of catalytically active subunits such as metal complexes and cofactors, either at the surface or at the core of the dendrimer, which results in systems reproducing the catalytic properties of these cofactors.[7] The dendrimer is used either to provide a particular microenvironment or to increase molecular size and facilitate catalyst separation and recovery. We reasoned that dendrimeric architectures applied to a peptide sequence would provide an efficient strat-

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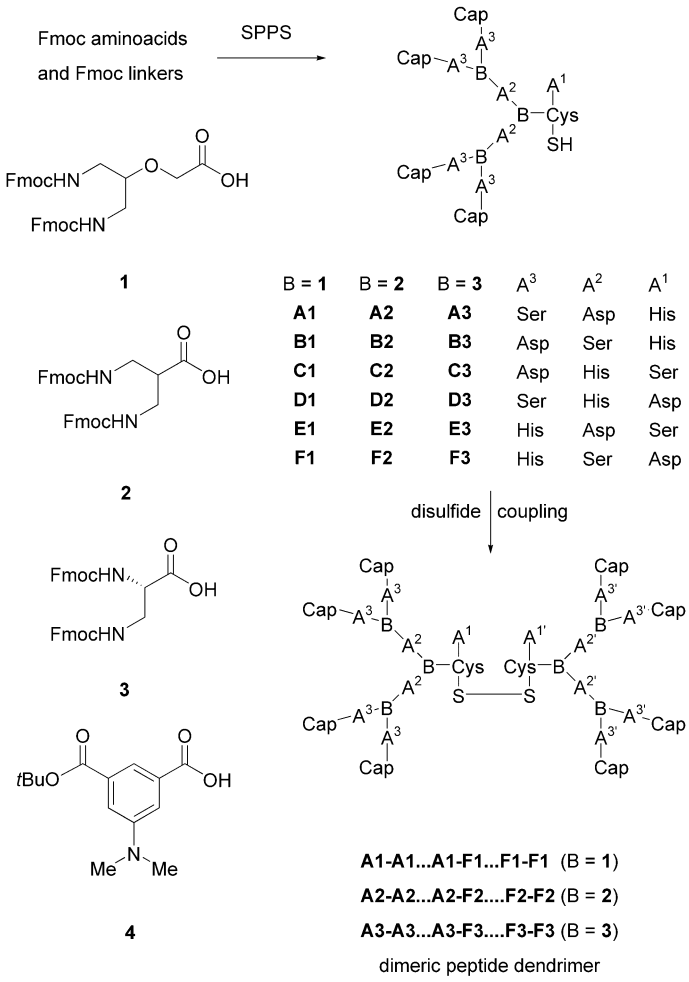
egy to circumvent the protein-folding problem. Such an approach would lead to protein-like structures where catalysis or molecular-recognition phenomena might appear by constructive interactions between amino acids as in natural proteins. Herein we report a versatile synthetic strategy for functionalized peptide dendrimers. More than one hundred different peptide dendrimers were prepared based on solid-phase peptide synthesis. The catalytic properties of esterolytic dendrimers obtained by using catalytic triad amino acids are discussed.[8]

Results

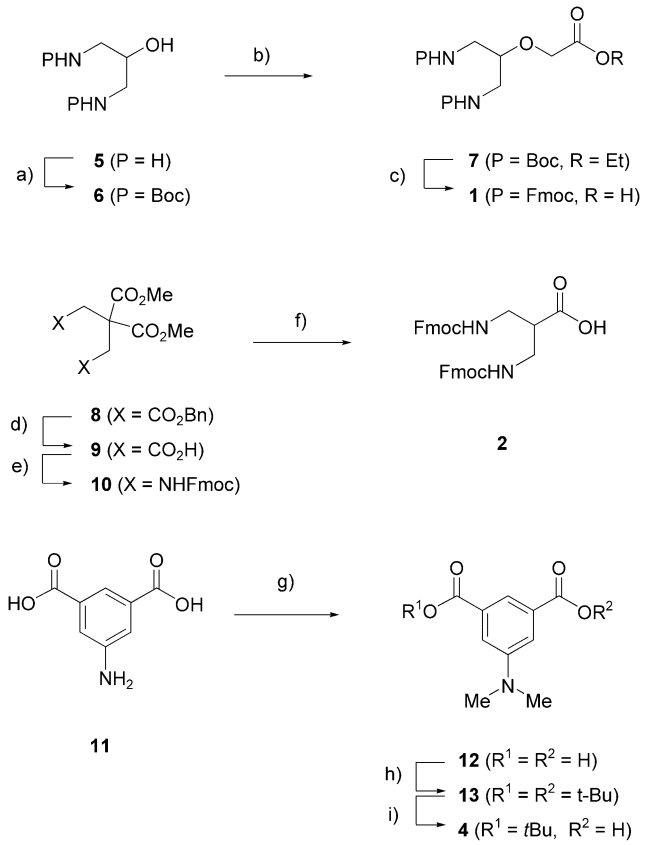
Strategy: Dendrimers are usually synthesized either by a divergent or a convergent strategy. In the divergent strategy sequential assembly starts from the dendrimer core and extends to the outside by addition of branching units and spacers. This approach is applied in polymerization or oligomerization protocols to produce statistical dendrimer populations with average molecular sizes. The convergent strategy proceeds in the opposite direction by condensing preassembled dendrons to a common core to form a new, larger dendron and by repeating this cycle iteratively. The approach is equivalent to the convergent strategy in natural product synthesis and is usually applied for the synthesis of dendrimers consisting of a single molecular species with defined molecular weight.

Peptides are most efficiently prepared by solid-phase peptide synthesis (SPPS), whereby *N*-protected amino acid building blocks are condensed sequentially to the free amino terminus of a growing peptide chain attached to the solid support.[9] The key advantage is the possibility of using a large excess of *N*-protected activated amino acid in solution to maximize the coupling efficiency to the amino terminus anchored on the solid support. SPPS can furthermore be automated and all common building blocks and reagents are commercially available. We envisioned a divergent synthesis of peptide dendrimers based on SPPS whereby standard α-amino acid building blocks would alternate with tailored diamino acids as branching units (Scheme 1). The exploration of synthetic peptide dendrimers would only be interesting if a certain level of structural diversity could be achieved. Diversity would be realized by using different α-amino acids at the variable positions between the branching diamino acids. We also took the opportunity to multiply structural diversity by chain dimerization through disulfide bridges. Disulfide coupling is an efficient synthetic protocol to assemble fragments of peptides or proteins and enables the formation of both homo- and heterodimers.[10] This design applied to second-generation dimeric dendrimers could provide as many as 1.7×10^9 different sequences from 19 proteinogenic α-amino acids and 3 different branching diamino acids. Our first attempt at preparing functional peptide dendrimers was aimed at finding peptide dendrimers that could catalyze an ester hydrolysis reaction. Following the well-known model of esterolytic enzymes, peptide dendrimers containing combinations of the catalytic triad amino acids aspartate, histidine, and serine were prepared.



Scheme 1. General structure of peptide dendrimers. A^n = α-amino acid, B = branching diamino acid, Fmoc = 9-fluorenylmethoxycarbonyl.

Design and synthesis of building blocks: Ordered polylysine dendrimers have been prepared on solid support as antigen-display units.[11] However, we wanted to use shorter, more symmetrical branching units than lysine to obtain symmetrically growing dendrimers. We therefore chose diamino acids **1**–**3** as branching units. The branching diamino acid **1** was prepared in three steps from 1,3-diamino-2-propanol (Scheme 2). *O*-alkylation of the Boc-protected derivative **6** with ethyl bromoacetate gave **7**. Saponification of the ethyl ester with LiOH in THF, followed by treatment with 3 N HCl, gave the free diamino acid, which was reprotected with Fmoc-Cl in 1,4-dioxane in the presence of sodium hydrogen carbonate to give the bis-Fmoc derivative **1**. The synthesis of branching diamino acid **2** started with double alkylation of dimethyl malonate with benzyl bromoacetate (NaH, THF, 98%). Reductive debenzylation gave diacid **9**, which was converted into the bis(acyl) azide via the acyl chloride. A thermal Curtius rearrangement in refluxing anhydrous toluene in the presence of 9-fluorenemethanol gave carbamate **10**. Finally, saponification and decarboxylation of the remaining diester function with AcOH and 5 N HCl under reflux conditions gave the desired branching unit **2**. The commercially available Fmoc-protected (*S*)-2,3-diaminopro-

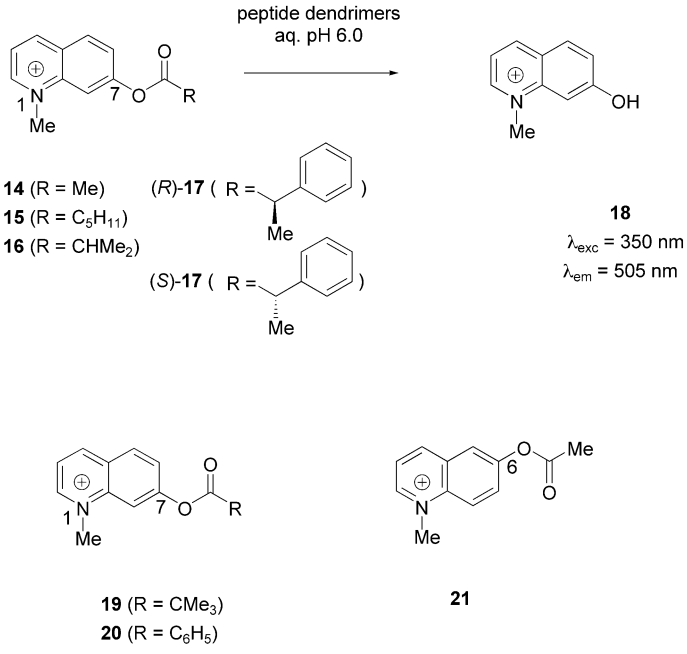


Scheme 2. Synthesis of dendrimer building blocks. Conditions: a) $(Boc)_2O$, Et_3N, CH_2Cl_2/MeOH, 2 h, 25 °C, 98 %; b) $BrCH_2CO_2Et$, NaH, THF, 25 °C, 5 h, 54 %; c) LiOH, THF/H_2O, 25 °C, 15 h; then 3 N aq. HCl, 60 °C, 3 h; then, Fmoc-Cl, $NaHCO_3$, H_2O/dioxane, 25 °C, 15 h, 66 %; d) H_2 (4 bars), Pd/C, EtOH, 14 h, 91 %; e) $(COCl)_2$, CH_2Cl_2, cat. DMF, 25 °C, 7 h; then $TMSN_3$, CH_2Cl_2, 25 °C, 20 h; then 9-fluorenemethanol, toluene, reflux, 18 h, 43 %; f) AcOH, 2 N HCl, reflux, 15 h, 65 %; g) 40 % aq. CH_2O, $NaBH_3CN$, DMF, 25 °C, 5 h, 83 %; g) $(COCl)_2$, CH_2Cl_2, cat. DMF, 25 °C, 2 h; then KO*t*Bu (3 equiv), CH_2Cl_2, 0 °C, 3 h, 67 %; h) KOH (1 equiv), *t*BuOH/CH_2Cl_2, 40 °C, 12 h; then HCl, 37 %. Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide, THF = tetrahydrofuran, TMS = trimethylsilyl.

panoic acid **3** was used as a third branching unit. In addition to the linkers, we also prepared the monoprotected 5-dimethylamino-isophthalic acid **4** from 5-amino-isophthalic acid **11**, to be used as capping group. Addition of this acid as the last building block at the dendrimer surface would provide homogeneous properties such as aqueous solubility at neutral pH values, by means of the negatively charged carboxylate, and easy traceability by HPLC due to the aminobenzamide chromophore.

SPPS of peptide dendrimers: The SPPS protocol for dendrimer synthesis was developed with diamino acid **1**, which was expected to be the least problematic in terms of steric crowding. We adopted an Fmoc strategy on polystyrene resin functionalized with Rink amide (0.47 mmol g^{-1}) and used 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU) or benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent. This protocol was used to prepare the six monomeric dendrimers, **A1**–**F1**, resulting from the six possible combinations of aspartate, histidine, and serine at positions A^1, A^2, and A^3. Each monomeric dendrimer was purified by preparative reversed-phase (RP) HPLC. The purified monomeric dendrimers were then dimerized with aldrithiol,[12] a reaction that allowed the preparation of all 21 possible combinations of disulfide-bridged dimeric dendrimers. The same protocol was used to prepare equivalent dendrimer families based on branching diamino acids **2** and **3** (Scheme 1). Somewhat to our surprise, the 2,3-diaminopropanoic acid branching unit **3** yielded to the same protocol and gave similar yields of isolated products in spite of it being the smallest branching unit.

Screening for esterolytic activity: The 60 dimeric dendrimers obtained were assayed for hydrolysis of fluorogenic and chromogenic ester substrates. We used a series of 7-hydroxycoumarin esters and acyloxymethyl ethers from our laboratory,[13] as well the commercially available esterase substrates 8-acetoxypyrene-1,3,6-trisulfonate, 7-acetoxycoumarin-3-carboxylic acid, and 7-acetoxy-1-methylquinolinium iodide (**14**). The dendrimers were assayed for activity in aqueous buffer (pH 7.0 or 6.0) in the presence of the different substrates in a microtiter-plate setup. The reactions were followed over several hours and the apparent rates of hydrolysis were compared to the rates in the absence of dendrimer. The rates of hydrolysis of 7-acetoxy-1-methylquinolinium iodide (**14**) were accelerated by some of the dendrimers at pH 6.0 (Scheme 3, Table 1). Calibration with the product **18** in the presence of the dendrimers showed that the effect observed was not due to a dendrimer-induced fluorescence enhancement. Product formation was confirmed by HPLC analysis of the reaction mixture. The catalytic den-



Scheme 3. Acyloxy-*N*-methylquinolinium derivatives assayed for hydrolysis by catalytic peptide dendrimers. Esters **14**–**17** are accepted as substrates to give the fluorescent product **18**. Ester **19**–**21** are not accepted as substrates.

Table 1. Initial screening results for the hydrolysis of quinolinium ester **14** by catalytic peptide dendrimers. The apparent rate enhancement (V_{net}/V_{uncat})[a] is reported for monomeric dendrimers and all combinations of dimers.

	Monomer	**A1**	**B1**	**C1**	**D1**	**E1**	**F1**
A1	0.5	0.2					
B1	0.0	0.7	0.5				
C1	0.6	0.3	0.6	0.4			
D1	0.4	0.2	0.5	0.7	0.4		
E1	2.1	3.1	1.9	1.6	1.4	9.2	
F1	1.7	2.1	0.7	1.8	1.0	12.0	6.4
	Monomer	**A2**	**B2**	**C2**	**D2**	**E2**	**F2**
A2	0.2	0.0					
B2	0.1	0.5	0.2				
C2	0.4	0.4	1.1	0.4			
D2	0.4	0.5	0.4	0.4	0.4		
E2	2.0	1.8	1.9	1.4	1.0	2.8	
F2	1.5	1.5	1.5	–	1.1	1.9	2.8
	Monomer	**A3**	**B3**	**C3**	**D3**	**E3**	**F3**
A3	2.2	0.3					
B3	0.8	0.2	0.4				
C3	2.3	–	2.1	0.2			
D3	1.8	0.9	1.4	0.4	1.9		
E3	12.2	0.4	–	4	7.6	8.4	
F3	9.7	3.2	0.9	5.6	3.7	5.8	4.6

[a] With 5 μM dendrimer and 200 μM substrate. For other conditions and methods, see the Experimental Section. V_{uncat} is the hydrolysis rate of **14** in buffer without catalysis. $V_{net} = V_{app} - V_{uncat}$, where V_{app} is the apparent hydrolysis rate of **14** in the presence of dendrimer.

drimers retained activity upon resynthesis from the amino acid constituents, a result showing that the observed effects were not due to impurities in the samples. The dendrimers appeared unchanged upon HPLC and MS analysis after the reactions; this shows that they were not permanently acylated during the reaction. Catalysis was further confirmed by the observation of multiple turnovers.

Kinetic studies: A pH-profile study under screening conditions (5 μM dendrimer, 200 μM substrate) showed a maximum of activity at pH 6.0, in agreement with a possible role of the histidine side chain as a catalytic side chain. All further measurements were therefore carried out at that pH value. Catalysis was proportional to the dendrimer concentration up to 10 μM dendrimer. However catalysis did not increase above that concentration, a fact suggesting the formation of catalytically inactive aggregates.

Catalysis with a range of additional quinoline-derived substrates was also examined. The substrates were prepared by esterification of hydroxyquinoline, followed by quaternization with dimethylsulfate.[14] The hexanoyl **15**, isobutyryl **16**, and (*R*)- and (*S*)-α-methylphenacetyl derivatives **17** were also accepted as substrates. By contrast, the more sterically hindered pivalate **19** and benzoate **20**, as well as the isomeric 6-acetoxy-1-methylquinolinium iodide (**21**), were not hydrolyzed by the dendrimers. The kinetics of hydrolysis with the reactive substrates **14**–**17** and the most active dendrimers were characterized in detail and followed the Michaelis–Menten model, with Michaelis constant (K_M; dissociation constant of the substrate–dendrimer complex) values in the range of 0.1–1 mM and rate constant of catalysis/rate constant without catalysis (k_{cat}/k_{uncat}) values in the range of 10^2–10^3. The best substrate across all dendrimers was the isobutyryl ester **16**. The dendrimers did not show any significant degree of enantioselectivity with the enantiomeric substrate pair **17** (Table 2, Figure 1, and Figure 2).

Dendrimer variations: Since the esterolytic activity of our catalytic peptide dendrimers was strongly correlated with the presence of histidine residues at the outermost position in the sequence, we set out to prepare a new dendrimer family incorporating histidine as the catalytic amino acid together with a broader selection of other amino acids. A new family of 22 dendrimers incorporating histidine together with the amino acids leucine, arginine, tryptophan, or phenylalanine, as well as aspartate and serine, was designed (Table 3). All the dendrimers were prepared with diamino acid **1** as the branching unit and were obtained in satisfactory yields. Aldrithiol-mediated dimerization was then carried out for all combinations. Disulfide-bond formation was much slower with hydrophobic dendrimers, with reaction times extending up to 24 h for the more hydrophobic dendrimers. Homo- and heterodimers were nevertheless obtained in good yields, with the exception of the **HWS–HWS** and **DHF–DHL** dendrimers, which were not formed even after repeated attempts under various conditions. In fact, the aldrithiol-activated **HWS** dendrimer did not undergo any reaction and the corresponding heterodimers were obtained by using the **HWS** dendrimer as a free thiol nucleophile in combination with another thiol-activated dendrimer.

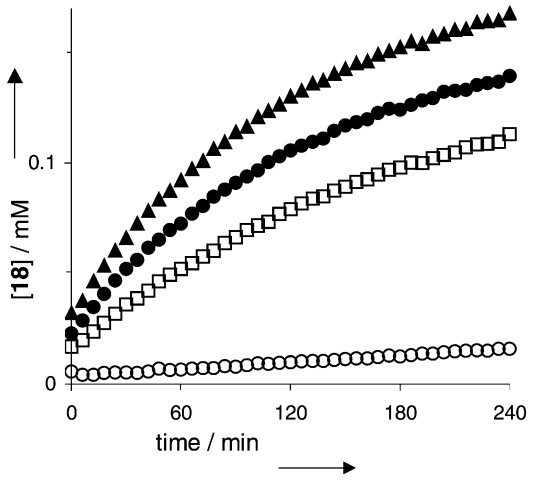


Figure 1. Time-course graph for the hydrolysis of **16** to form **18** in the absence (○) or presence of dendrimers **E1–F1** (●), **E2–E2** (□), or **E3–E3** (▲), with 200 μM substrate and 5 μM catalyst.

Table 2. Michaelis–Menten parameters for the six most active dendrimers on substrates **14**–**17**.[a]

		14	**15**	**16**	(*S*)-**17**	(*R*)-**17**	E[b]
–	k_{uncat} [min^{-1}]	3.6×10^{-4}	2.4×10^{-4}	1.2×10^{-4}	2.2×10^{-4}	2.2×10^{-4}	
4-methylimidazole	k_2[c] [$mM^{-1}min^{-1}$]	6.7×10^{-3}	5.6×10^{-3}	4.3×10^{-3}	5.0×10^{-3}	4.8×10^{-3}	
E1–E1	K_M [mM]	0.20	0.21	0.11	0.17	0.22	
	k_{cat} [min^{-1}]	0.31	0.26	0.28	0.21	0.20	
	k_{cat}/k_{uncat}	860	1050	2333	944	891	
	$k_{cat}/K_M{:}k_2$[c]	230	220	592	244	192	1.27
	V_{net}/V_{uncat}[d]	9.2	12.5	23.3	17.8	15.5	
E1–F1	K_M [mM]	0.14	0.12	0.23	0.12	0.15	
	k_{cat} [min^{-1}]	0.12	0.33	0.30	0.14	0.12	
	k_{cat}/k_{uncat}	340	1380	2455	637	487	
	$k_{cat}/K_M{:}k_2$[c]	130	360	280	229	148	1.55
	V_{net}/V_{uncat}[d]	12.0	18.8	26.5	13.0	10.4	
E2–E2	K_M [mM]	0.25	0.07	0.69			
	k_{cat} [min^{-1}]	0.10	0.09	0.25			
	k_{cat}/k_{uncat}	268	380	2010			
	$k_{cat}/K_M{:}k_2$[c]	57	226	83			
	V_{net}/V_{uncat}[d]	2.8	3.7	11.1			
E2–F2	K_M [mM]	0.27	0.24	0.48			
	k_{cat} [min^{-1}]	0.06	0.08	0.15			
	k_{cat}/k_{uncat}	173	322	1214			
	$k_{cat}/K_M{:}k_2$[c]	30	57	72			
	V_{net}/V_{uncat}[d]	1.9	2.3	6.3			
E3–E3	K_M [mM]	0.29	0.32	0.264	0.23	0.16	
	k_{cat} [min^{-1}]	0.14	0.17	0.41	0.18	0.18	
	k_{cat}/k_{uncat}	380	690	3350	816	828	
	$k_{cat}/K_M{:}k_2$[c]	70	90	360	154	243	
	V_{net}/V_{uncat}[d]	4.1	6.2	31.8	7.8	11.8	
E3–F3	K_M [mM]	0.23	0.14	0.634	0.16	0.08	
	k_{cat} [min^{-1}]	0.08	0.08	0.25	0.11	0.10	
	k_{cat}/k_{uncat}	220	330	2011	519	463	
	$k_{cat}/K_M{:}k_2$[c]	50	100	90	142	278	
	V_{net}/V_{uncat}[d]	2.7	4.9	16.2	6.9	6.4	

[a] With 5 μM dendrimer and 40–700 μM substrate. For other conditions and methods, see the Experimental Section. The kinetic constants given are derived from the linear double-reciprocal plots of $1/V_{net}$ against 1/[S] (Figure 1), with $r^2>0.95$. [b] $E=(k_{cat}/K_M((S)\text{-}\mathbf{17}))/(k_{cat}/K_M((R)\text{-}\mathbf{17}))$. [c] k_2 is the second-order rate constant for the reaction of the substrate with 4-methylimidazole. The ratio of k_{cat}/K_M to k_2 gives a quantitative comparison of dendrimer catalysis under the reaction conditions. [d] V_{net}/V_{uncat} was observed with 5 μM dendrimer and 200 μM substrate (see legend of Table 1).

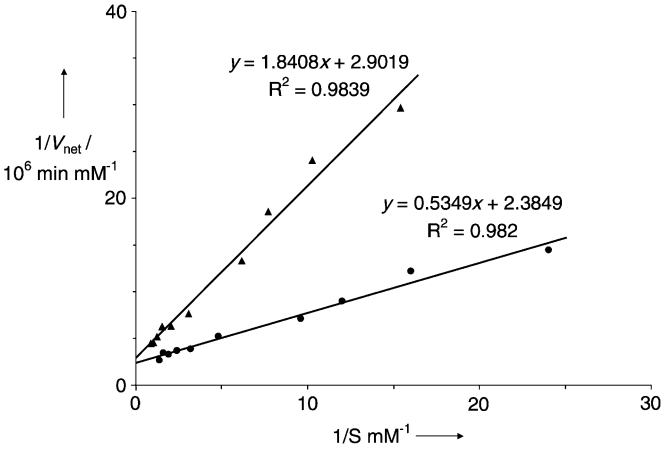


Figure 2. Double reciprocal plot for hydrolysis of ester **16** catalyzed by peptide dendrimers **E1–F1** (●) and **E3–F3** (▲).

We also prepared the all-histidine monomeric dendrimer (**HHH**) derived with branching unit **1**. The hydrophobic dendrimer **LRH** was coupled with this all-histidine monomeric dendrimer, as well as with a selection of dendrimers from the aspartate/histidine/serine family (Table 3).

The new series was screened for catalysis with the substrates used for the catalytic triad dendrimer series described above. There was no significant catalytic effect in any of the dendrimers tested, including **LRH–HHH**, which contained the all-histidine monomeric dendrimer. An all-histidine monomeric dendrimer was also prepared with diamino acid **3**, **3(HHH)**, and converted into the corresponding all-histidine homodimer **3(HHH–HHH)**. The all-histidine homodimer showed esterolytic activity with substrates **14, 15, 16**, (*S*)-**17**, and (*R*)-**17**, with apparent rates of hydrolysis (V_{net}/V_{uncat}) of 6.5, 6.7, 6.2, 5.3, and 6.1, respectively.

Discussion

The chemical synthesis of large molecules is intrinsically complex and synthetic availability is a key issue in supramolecular chemistry. In the context of enzyme models, synthesis planning should also incorporate diversity to allow for variations in binding and catalysis. The preparation of catalytic peptide dendrimers demonstrated here is advantageous in both respects. By using a carefully optimized peptide-coupling protocol we obtained yields of up to 30% after HPLC purification for the monomeric dendrimers, which is in the range expected for linear peptide sequences of similar length. Branching and resin loading did not affect synthesis significantly. The fact that synthesis succeeded with the standard Rink-amide resin at the usual loading of 0.47 mmol g^{-1} is noteworthy, as are the high yields obtained with the commercially available branching unit 2,3-diaminopropanoic acid **3**. Dendrimer synthesis was exemplified with a broad range of amino acids, including acidic, basic, polar, hydrophobic, and aromatic amino acids. Diversity was efficiently increased by the dimerization strategy through disulfide bridges, which enabled us to access a sizeable structural

Table 3. Hydrophobic dendrimers with branching unit **1.** Amino acids A^1, A^2, and A^3 correspond to one monomeric dendrimer and $A^{1'}$, $A^{2'}$, and $A^{3'}$ correspond to the second monomeric dendrimer.

Entry	A^3	A^2	A^1	$A^{1'}$	$A^{2'}$	$A^{3'}$	Yield[a] [%]
1	D	H	L				11
2	D	F	H				20
3	H	W	S				12
4	L	R	H				14
5	L	R	H	S	W	H	26
6	L	R	H	L	H	D	27
7	L	R	H	H	F	D	40
8	L	R	H	D	S	H	74
9	L	R	H	S	H	D	54
10	L	R	H	H	S	D	54
11	L	R	H	H	H	H	45
12	L	R	H	H	R	L	30
13	H	W	S	L	H	D	47
14	H	W	S	D	S	H	39
15	H	W	S	S	H	D	55
16	H	W	S	H	S	D	63
17	D	H	L	D	S	H	60
18	D	H	L	S	H	D	47
19	D	H	L	H	S	D	54
20	D	H	L	L	H	D	30
21	D	F	H	D	S	H	39
22	D	F	H	H	F	D	35

[a] Entries 1–4 give yields for the monomer after HPLC purification. Entries 5–22 give yields of dimerization after HPLC purification.

family from only a small number of monomeric dendrimer building blocks. Semipreparative HPLC on milligram quantities of dendrimers provided a convenient method to obtain each dendrimer in pure form. All the dendrimers prepared were very well behaved in terms of chemical stability. In particular, the heterodimers did not show any disproportionation, a fact indicating that disulfide-exchange reactions were slow. The low reactivity of the disulfide group was also evident from the long dimerization times of up to 24 h observed in some of the dendrimers. These results suggest that larger scale preparation of any dendrimer is possible and that the procedure can be automated.

While only proteinogenic L-amino acids were used here, structural diversity in the dendrimers could be further extended to the enantiomeric D-amino acids and to nonnatural amino acid side chains. Amino acids bearing catalytic side chains[15] and fluorescent groups[16] would be relevant for developing other catalytic or biosensor peptide dendrimers. Dendrimers based on β- or γ-amino acids[17] could also be considered. Combinatorial split-and-mix protocols were not addressed in this study since the dependence of coupling efficiency on sequence and on the nature of the diamino acid branching unit was not known.

The choice of an ester hydrolysis reaction was guided by a number of precedents in the history of enzyme models. Targeting ester hydrolysis naturally led to the choice of the catalytic triad amino acids aspartate, histidine, and serine as the variable amino acids. All substrates tested were catalytically hydrolyzed by 4-methylimidazole, which is the simplest model for the histidine side chain. The fact that catalytic activity only appeared when histidine was positioned at the outermost position in the dendrimer suggests that nucleophilic catalysis by the imidazole side chain is involved. A quantitative comparison of dendrimer catalysis along these lines is given by the ratio of second-order rate constants for the reaction of the substrates with the dendrimers (k_{cat}/K_M) and with 4-methyl-imidazole (k_2) under the reaction conditions (see Table 2). The best dendrimers accelerate hydrolysis by approximately 350-fold over the rate with 4-methylimidazole. Since these dendrimers display a total of eight histidines at their surface, this corresponds to a 40-fold rate enhancement per histidine side chain. Similar acceleration factors have been reported by Baltzer and co-workers for the hydrolysis of nitrophenyl esters by using designed helical peptides containing six histidines.[18]

Catalytic efficiency in dendrimers having the same amino acid sequence was strongly influenced by the linking diamino acid used. The best results were observed for the homodimer **E3–E3** with the diamino acid linker **3** on the isobutyryl ester **16**. Dendrimers prepared with the bis(β-alanine) linker **2** showed almost no activity with the acetate ester **14** and the hexanoate **15** and only showed sizable activity with the isobutyryl substrate **16**. By contrast, dendrimers made from linkers **1** and **3** were quite active across all the different quinolinium esters **14**–**17**.

Catalytic activity was also observed with the monomeric dendrimers derived from the shorter diamino acids **2** and **3**. The catalytic efficiency in these monomeric dendrimers was slightly higher than that of the dimeric dendrimers when referred to the number of catalytic histidine residues. By contrast, with the longer branching unit **1** there was almost no catalysis in the monomeric dendrimers. These observations suggest that a productive interplay between several histidine side chains only occurs under certain conditions. In the case of the longer linker **1**, dimerization is probably required to enforce steric crowding of the side chains and the monomeric dendrimers are too flexible to acquire strong activity. In the dendrimers made with the shorter linkers **2** and **3**, the amino acids are close enough for productive interaction in the monomeric dendrimers and dimerization does not enhance catalysis.

The observation of histidine-only catalysis was challenged with the preparation of a dendrimer family containing histidine and noncatalytic amino acids. To our surprise, there was no significant catalytic effect within this series with any of the substrates tested, in particular with the dendrimers displaying surface histidine side chains as in the catalytic series above. This was all the more puzzling since these dendrimers were similar to those of the histidine/aspartate/

serine series. Most strikingly, heterodimers containing one of the "catalytic" halves combined with a more hydrophobic half were also not active (**LRH–HHH**). These results indicate that other factors beyond histidine proximity at the surface control catalytic activity. The more hydrophobic dendrimers might adopt a compact, noncatalytic structure due to hydrophobic packing of the side chains.

Conclusion

Peptide dendrimers were prepared by solid-phase synthesis, alternating standard α-amino acids with diamino acid branching units, followed by disulfide dimerization of core cysteine residues. 103 different dendrimers were obtained from diamino acids **1**–**3** as branching units and a variety of acidic, basic, hydrophobic, aromatic, and hydrophilic amino acids. Dendrimers containing catalytic triad amino acids with the histidine at the outermost position catalyzed the hydrolysis of *N*-methylquinolinium esters **14**–**17** with enzyme-like kinetic behavior, thereby demonstrating that the dendrimers can display functionality. Catalysis was strongly influenced by the diamino acid branching unit and the actual amino acid sequence. Further experimentation will be required to discover catalytic dendrimers with practical catalytic potencies or selective molecular recognition properties. The general strategy for dendrimer assembly delineated here enables the assembly of billions of different structures. A combinatorial synthetic protocol[19] combined with activity screening can be considered to explore much larger numbers of dendrimers.

Experimental Section

General: All reagents were either purchased from Aldrich or Fluka or synthesized following literature procedures. Amino acids and their derivatives were purchased from Senn Chemicals or Novabiochem (Switzerland). Rink amide resin and TGR resin were purchased from Novabiochem. All solvents used were analytical grade. Chromatography (flash) was performed with Merck silica gel 60 (0.040 ± 0.063 mm). Analytical HPLC was performed in a Waters (996 photodiode array detector) chromatography system with a Vydac 218TP54 column (RP-C18, 300 Å pore size, 0.4×22 cm, flow rate of 1.5 mL min^{-1}) or a chromolith column (0.4×5 cm, flow rate of 5 mL min^{-1}). Preparative HPLC was performed with HPLC-grade acetonitrile and MilliQ deionized water in a Waters prepak cartridge (500 g; RP-C18, 300 Å pore size, 20 mm) installed on a Waters Prep LC4000 system from Millipore (flow rate of 100 mL min^{-1}, gradient of 0.5% CH_3CN per min). Fluorescence measurements were carried out with a spectraMAX fluorescence detector. NMR spectra were recorded on a Bruker AC-300 instrument (^{1}H, 300 MHz; ^{13}C, 75 MHz). Infrared spectroscopy was performed with a Perkin–Elmer 1600 series FTIR apparatus. Frequencies (ν) are given in cm^{-1}. Optical rotations were measured with a Perkin–Elmer 241 digital polarimeter with a 1-dm cell. Melting points were determined on a Büchi 510 apparatus and are not corrected. TLC was performed with fluorescent F254 glass plates. MS was provided by Dr. Thomas Schneeberger (University of Bern, Switzerland).

***N*-(3-(9-Fluorenylmethyloxycarbonylamino)-2-(carboxymethyloxy)prop-1-yl)carbamic acid 9-fluorenylmethyl ester (1)**: A solution of LiOH (164 mg, 6.85 mmol) in water (10 mL) was added to a stirred solution of **7** (858 mg, 2.28 mmol) in 10 mL THF. The mixture was stirred overnight. The solvent was evaporated and the residue was lyophilized to give a white solid. 3 N HCl (10 mL) was added to the solid and the mixture was stirred at 60 °C for 3 h. The pH value of the solution was adjusted to 5 with NaOH pellets and then to pH 8 with aq. sat. $NaHCO_3$ (10 mL). A solution of Fmoc-Cl (1.30 g, 5.02 mmol) in dioxane (15 mL) was added at 0 °C and the mixture was stirred overnight at room temperature. After addition of water (until the mixture became cloudy), the solution was washed twice with diethyl ether. The aqueous phase was acidified to pH 2 with aq. conc. HCl and extracted with EtOAc. The organic phase was evaporated and the residue purified by flash column chromatography (hexane/EtOAc/AcOH (3.8:6:0.2)) to give **1** (895 mg; 66%) as a colorless solid; m.p. 205 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.66$ (m, 4H), 7.40 (m, 4H), 7.31 (m, 8H), 4.23 (m, 6H), 4.10 (s, 2H), 3.36 (m, 1H), 3.12 (m, 4H) ppm; ^{13}C NMR (75 MHz, DMSO-d$_6$): $\delta = 162.07$, 145.87, 133.48, 130.32, 117.24, 116.70, 114.80, 109.72, 67.02, 55.87, 55.14, 36.33, 31.38 ppm; FABMS: 593 [M^+]; HRMS: calcd. for $C_{35}H_{32}N_2O_7$: 593.2288; found: 593.2287.

***N*-(3-(9-Fluorenylmethyloxycarbonylamino)-2-carboxyprop-1-yl)carbamic acid 9-fluorenylmethyl ester (2)**: A solution of **10** (2.89 g, 4.55 mmol) in AcOH (30 mL) and aq. 2 N HCl (5 mL) was stirred under reflux conditions for 15 h. The solution was cooled and toluene was added. The precipitate was filtered to gave **2** (1.67 g, 2.98 mmol, 65%) as a white solid; m.p. 201 °C; IR (KBr): $\tilde{\nu} = 3326$ (m), 2946 (s), 1699 (s), 1538 (m), 1450 (m), 1258 (w), 1450 (m), 1258 (w) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.35 (d, $J = 7.35$ Hz, 4H), 7.57 (d, $J = 7.71$ Hz, 4H), 7.27 (m, $J = 7.35$, 11.0 Hz, 8H), 5.74 (t, $J = 6.27$ Hz, 2H), 4.35 (d, $J = 7.0$ Hz, 4H), 4.18 (t, $J = 7.0$ Hz, 2H), 3.61 (m, 2H), 3.29 (m, 2H), 2.74 (t, $J = 5.5$ Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.22$, 157.49, 144.45, 14.86, 128.27, 127.65, 125.171, 120.53, 67.41, 47.87, 45.87, 39.51, 21.49 ppm; HRMS: calcd for $C_{34}H_{30}N_2O_6$: 563.2182; found: 563.2181.

5-Dimethylamino-isophthalic acid mono-*tert*-butyl ester (4): A solution of **13** (5.5 g, 17.11 mmol) in *tert*-butanol (55 mL) and dry CH_2Cl_2 (11 mL) at room temperature was treated with 20 N KOH (855 µL, 1 equiv) and heated at 40 °C for 12 h. After removal the solvent, the crude product was dissolved in CH_2Cl_2 and extracted with water (3×). The water layer was filtered over celite and then acidified with 1 N HCl to pH 4–5 to give **4** (1.66 g, 37%) as a white precipitate; m.p. 175–183 °C; ^{1}H NMR (300 MHz, DMSO): $\delta = 7.72$ (s, 1H), 7.43 (s, 1H), 7.39 (s, 1H), 2.98 (s, 6H), 1.55 (s, 9H) ppm; ^{13}C NMR (75 MHz, DMSO): $\delta = 167.2$, 164.9, 150.2, 132.3, 131.8, 117.0, 116.2, 115.8, 80.9, 39.9, 27.4 ppm; MS (EI): 265 [M^+]; HRMS (ESI): calcd for $C_{14}H_{20}NO_4$: 266.1392; found: 266.1398.

***N*-(3-(*tert*-Butoxycarbonylamino)-2-hydroxyprop-1-yl)carbamic acid *tert*-butyl ester (6)**: A solution of di-*tert*-butyl dicarbonate (4.80 g, 22 mmol) in CH_2Cl_2 (4 mL) was added slowly to a stirred solution of 1,3-diaminopropanol (**5**; 901 mg, 10.0 mmol) and triethylamine (164 µL, 1.18 mmol) in CH_2Cl_2/MeOH (1:5, 10 mL). The reaction was complete in 2 h (as monitored by TLC). The solution was concentrated and the oily residue was purified by flash column chromatography (hexane/EtOAc (1:4)) to give the Boc-protected intermediate **6**[20] as a colorless solid (2.83 g, 98%); ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 3.70$ (m, 1H), 3.20 (m, 4H), 1.27 (s, 18H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 157.27$, 80.07, 71.20, 43.61, 28.37 ppm.

***N*-(3-(*tert*-Butoxycarbonylamino)-2-(ethoxycarbonylmethyloxy)prop-1-yl)carbamic acid *tert*-butyl ester (7)**: Ethyl bromoacetate (1.18 mL, 10.7 mmol) was added to a stirred solution of **6** (1.23 g, 4.23 mmol) in dry THF (2 mL) at room temperature. Sodium hydride (0.47 g, 4.5 equiv) was added slowly over 1 h. After an additional 5 h the reaction mixture was filtered over celite and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc (8:2)) to give **7** (858 mg, 54%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.21$ (q, $J = 7$ Hz, 2H), 4.15 (s, 2H), 3.45 (m, 1H), 3.48–3.06 (m, 4H), 1.42 (s, 18H), 1.27 (t, $J =$ 7 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.09$, 156.37, 79.38, 78.91, 67.16, 61.13, 40.56, 28.35, 14.10 ppm; MS (FAB): 377 [$M+$H]$^+$; HRMS: calcd for $C_{17}H_{32}N_2O_7+$Na: 399.2113; found: 399.2107.

Dibenzyl-3,3-bis(methoxycarbonylmethyl)glutarate (8): A solution of dimethyl malonate (13.0 mL, 0.112 mol) in dry THF (170 mL) was added dropwise to a stirred suspension of NaH (10.66 g, 0.235 mol, 2.1 equiv; 55–65% oil dispersion) in dry THF (170 mL) under N_2. The solution was stirred at room temperature for 2 h and then a solution of benzyl 2-bromoacetate (35.2 mL, 0.224 mol, 2 equiv) in dry THF (160 mL) was added dropwise. The reaction mixture was stirred at room temperature for 4 h and then poured into aq. sat. NH_4Cl. After extraction with AcOEt (2×), the combined organic phases were dried with Na_2SO_4. Removal of the

solvents in vacuum gave **8** (47.9 g, 98%) as a yellow oil; IR ($CHCl_3$): $\tilde{\nu}$ = 3034 (s), 2955 (s), 1741 (s), 1170 (w), 1227 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 7.33 (s, 10H), 5.10 (s, 4H), 3.66 (s, 6H), 3.22 (s, 4H) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 170.88, 170.19, 136.14, 129.0, 128.96, 67.37, 53.81, 38.44, 30.38 ppm; MS (EI): 428 [M^+]; HRMS: calcd. for $C_{23}H_{24}O_8$: 429.147118; found: 428.147130.

3,3-Bis(methoxycarbonylmethyl)glutaric acid (9): A solution of **8** (48.6 g, 0.113 mol) in absolute EtOH (60 mL) was hydrogenated with H_2 (4 bars) over 10% Pd/C (4 g) in a Parr shaker for 14 h. The catalyst was removed by filtration through celite and the filtrate was concentrated. The solid residue was dissolved in a small amount of AcOEt and precipitated by addition of hexane. After filtration, **9** (25.6 g, 91%) was obtained as a white solid; m.p. 126°C; IR (KBr): $\tilde{\nu}$ = 3418 (br), 2924 (s), 2854 (m), 1731 (s), 1395 (s), 1220 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 3.71 (s, 6H), 3.13 (s, 4H) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 173.77, 171.38, 54.59, 53.46, 38.51 ppm; MS (FAB): 249 [M+H]; HRMS: calcd for $C_9H_{12}O_8$: 249.0610; found: 249.0599.

***N*-(3-(9-Fluorenylmethyloxycarbonylamino)-2,2-bis(methoxycarbonyl)-prop-1-yl)carbamic acid 9-fluorenylmethyl ester (10)**: A solution of **9** (8.15 g, 0.033 mol) in dry CH_2Cl_2 was treated at 25°C with oxalyl chloride (8.50 mL, 0.098 mol, 3 equiv) and DMF (1 drop) and the mixture was stirred for 7 h. Evaporation to dryness gave the bis-acyl chloride as an orange oil (8.66 g, 0.030 mol), which was dissolved in dry CH_2Cl_2 and treated with azidotrimethylsilane (14.90 mL, 0.113 mol, 3.7 equiv). After stirring for 20 h at 25°C, evaporation to dryness gave the corresponding bis-acyl azide as an orange oil. A solution of acyl azide (5.53 g, 18.50 mmol) and 9-fluorenemethanol (15.80 g, 80.51 mmol, 4.3 equiv) in dry toluene (120 mL) was stirred under reflux conditions for 18 h. After removal of the solvent, the crude product was purified by flash column chromatography (hexane/AcOEt (8:2)) to give **10** (5.51 g, 8.68 mmol, 43%) as a colorless solid; m.p. 75°C; IR ($CHCl_3$): $\tilde{\nu}$ = 3447 (m), 3014 (s), 2955(s), 1732 (s), 1516 (m), 1451(s), 1229 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, J = 7.4 Hz, 4H), 7.60 (d, J = 7.4 Hz, 4H), 7.32 (m, J = 6.6, 10.7 Hz, 8H), 5.58 (t, 2H), 4.44 (d, J = 6.99 Hz, 4H), 4.25 (q, J = 6.99 Hz, 2H), 3.76 (d, 4H), 3.74 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.91, 157.60, 144.49, 142.00, 128.42, 127.78, 125.77, 120.67, 67.79, 59.67, 53.73, 47.88, 42.11 ppm; MS (FAB): 635 [M^+]; HRMS: calcd for $C_{37}H_{34}N_2O_8$: 635.2393; found: 635.2362.

5-Dimethylamino-isophthalic acid (12): Formaldehyde (≈37 wt% in water, 24 mL, 10 equiv) was added to a solution of 5-amino-isophthalic acid (**11**; 5.50 g, 30.4 mmol) in DMF at 25°C for 20 minutes. The solution was cooled to 0°C and $NaBH_3CN$ (5.70 g, 90.7 mmol, 3 equiv) was slowly added. The solution was stirred for 5 h at 25°C. After evaporation of the solvent, the residue was dissolved in water and precipitated by addition of 1 N HCl. The white precipitate was filtrated and washed with water until the pH value of the filtrate was neutral, to yield **12** (5.28 g, 83%) as a colorless solid; IR (DMSO): $\tilde{\nu}$ = 3424 (s), 2151 (w), 1653 (m), 1437 (w), 1430 (w), 1420 (w), 1405 (w), 1347 (w), 1317 (w), 1229 (w) cm^{-1}; ^1H NMR (300 MHz, DMSO): δ = 7.78 (s, 1H), 7.43 (s, 2H), 2.97 (s, 6H) ppm; ^{13}C NMR (75 MHz, DMSO): δ = 167.3, 150.2, 131.8, 117.5, 116.2, 40.0 ppm; MS (EI): 209 [M^+]; HRMS (ESI): calcd for $C_{10}H_{12}NO_4$: 210.0766; found: 210.0767.

5-Dimethylamino-isophthalic acid di-*tert*-butyl ester (13): A solution of **12** (4.0 g, 19.1 mmol) in dry CH_2Cl_2 (80 mL) was treated with oxalyl chloride (4.9 mL, 57.1 mmol, 3 equiv) and a few drops of DMF and the mixture was stirred at 25°C for 2 h. Evaporation to dryness gave a crude yellow acyl chloride, which was dissolved in dry CH_2Cl_2 (80 mL) and cooled to 0°C. KO*t*Bu (6.4 g, 57.3 mmol, 3 equiv) was slowly added at 0°C. The solution was stirred for 3 h and then evaporated to dryness. Aqueous work-up (AcOEt/water) and purification by flash column chromatography (CH_2Cl_2) gave **13** (4.14 g, 67%) as a yellow solid; m.p. 149–151°C; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.67 (s, 1H), 7.27 (s, 2H), 2.79 (s, 6H), 1.37 (s, 18H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.9, 150.3, 132.7, 118.2, 116.7, 81.2, 40.5, 28.1 ppm; MS (EI): 321 [M^+]; HRMS (ESI): calcd for $C_{18}H_{28}NO_4$: 322.2018; found: 322.2029.

7-Hexanoyloxy-1-methylquinolinium trifluoroacetate (15): Hexanoyl chloride (145 μL, 1.04 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (DIEA; 250 μL, 1.44 mmol, 2 equiv) were added to a solution of 7-hydroxyquinoline (**18**; 103 mg, 0.71 mmol) in THF (4 mL). After 20 h at 25°C, the solvent was evaporated and the residue was dissolved in THF (3 mL). Dimethylsulfate (0.47 mL, 5.00 mmol, 7 equiv) was added and the solution was stirred for 12 h at 25°C. After solvent evaporation, the residue was purified by preparative RP HPLC (C-18, acetonitrile/water gradient containing 0.1% trifluoroacetic acid (TFA)). Lyophilization of the product-containing fractions gave **15** (71.6 mg, 0.245 mmol, 64%) as an orange oil; ^1H NMR (300 MHz, D_2O): δ = 9.14 (d, J = 5.84 Hz, 1H), 9.04 (d, J = 8.29 Hz, 1H), 8.34 (d, J = 9.04 Hz, 1H), 8.10 (d, J = 1.88 Hz, 1H), 7.93 (q, J = 8.47, 6.02 Hz, 1H), 7.72 (dd, J = 8.86, 1.89 Hz, 1H), 4.51 (s, 3H), 2.70 (t, J = 7.35 Hz, 2H), 1.71 (q, J = 7.35 Hz, 2H), 1.32 (m, 4H), 0.83 (t, J = 7.35 Hz, 3H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 117.24, 157.93, 152.33, 149.85, 142.21, 134.93, 130.37, 128.12, 123.88, 113.22, 47.81, 36.24, 32.94, 26.19, 24.13, 15.63 ppm; MS (EI): 258 [M^+]; HRMS: calcd for $C_{16}H_{20}NO_2$: 258.149290; found: 258.149404.

7-Isobutyryloxy-1-methylquinolinium trifluoracetate (16): The same procedure as for **15** was applied with 7-hydroxyquinoline (**18**; 50.0 mg, 0.34 mmol) in THF (2 mL), isobutyryl chloride (55 μL, 0.52 mmol, 1.5 equiv), and DIEA (118 μL, 0.69 mmol, 2 equiv), followed by dimethylsulfate (0.225 mL, 2.4 mmol, 7 equiv) to give, after lyophilization, **16** (71.6 mg, 0.245 mmol, 72%) as an orange oil; ^1H NMR (300 MHz, D_2O): δ = 9.14 (d, J = 5.84 Hz, 1H), 9.04 (d, J = 8.39 Hz, 1H), 8.34 (d, J = 9.04 Hz, 1H), 8.10 (d, J = 1.88 Hz, 1H), 7.93 (q, J = 8.39, 5.84 Hz, 1H), 7.73 (dd, J = 9.04, 1.88 Hz, 1H), 4.51 (s, 3H), 2.95 (m, 1H), 1.28 (d, J = 6.97 Hz, 6H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 180.42, 158.10, 152.32, 149.85, 134.92, 130.37, 128.11, 126.59, 123.88, 113.18, 47.83, 36.53, 20.41 ppm; MS (EI): 230 [M^+]; HRMS: calcd for $C_{14}H_{16}NO_2$: 230.117570; found: 230.118104.

(*S*)-7-(α-methylphenylacetoxy)-1-methylquinolinium trifluoracetate ((*S*)-17): A solution of 7-hydroxyquinoline (**18**; 66.6 mg, 0.46 mmol) in THF (4 mL) with (*S*)-(+)-2-phenylpropionic acid (75.8 mg, 0.51 mmol, 1.1 equiv), DCC (114.0 mg, 0.55 mmol, 1.2 equiv), and 4-dimethylaminopyridine (6.1 mg, 0.05 mmol, 0.1 equiv) was stirred for 15 h at 25°C. The solvent was evaporated and the residue was dissolved in THF (3 mL) and treated with dimethylsulfate (0.3 mL, 3.20 mmol, 7 equiv). After 12 h at 25°C, the solvent was evaporated and the residue was purified by preparative RP HPLC (C18, water/acetonitrile gradient containing 0.1% TFA). Lyophilization of the product-containing fractions gave (*S*)-**17** (71.6 mg, 0.245 mmol, 53%) as orange oil; ^1H NMR (400 MHz, D_2O): δ = 9.07 (d, J = 5.77 Hz, 1H), 8.96 (d, J = 8.43 Hz, 1H), 8.21 (d, J = 8.93 Hz, 1H), 7.91 (d, J = 1.83 Hz, 1H), 7.88 (q, J = 8.43, 5.77 Hz, 1H), 7.54 (dd, J = 8.93, 1.83 Hz, 1H), 7.40 (m, 4H), 7.31 (m, 1H), 4.40 (s, 3H), 4.15 (t, J = 6.91 Hz, 1H), 1.55 (d, J = 6.91 Hz, 3H) ppm; ^{13}C NMR (100 MHz, DMSO): δ = 172.23, 155.14, 150.72, 146.81, 132.14, 128.88, 127.68, 127.45, 125.54, 121.78, 111.29, 45.47, 44.67, 18.65 ppm; $[\alpha]_D^{20} = -70$ (c = 1 M, MeOH); MS (EI): 292 [M^+]; HRMS: calcd for $C_{19}H_{18}NO_2$: 292.133880; found: 292.133754.

(*R*)-(7-α-methylphenylacetoxy)-1-methylquinolinium trifluoracetate ((*R*)-17): The same procedure as above was applied with **18** (67 mg, 0.46 mmol) and (*R*)-(+)-2-phenylpropionic acid (76.0 mg, 0.51 mmol) to give (*R*)-**17** (84.6 mg, 63%) as an orange oil; $[\alpha]_D^{20} = +70$ (c = 1 M, MeOH).

7-(2,2-Dimethyl-propionyloxy)-1-methylquinolinium trifluoracetate (19): The same procedure as for **15** was applied with 7-hydroxyquinoline (**18**; 50.0 mg, 0.34 mmol) in THF (2 mL), pivaloylchloride (64 μL, 0.52 mmol, 1.5 equiv), and DIEA (118 μL, 0.69 mmol, 2 equiv), followed by dimethylsulfate (0.23 mL, 2.4 mmol, 7 equiv) to give, after lyophilization, **19** (41.5 mg, 0.167 mmol, 50%) as an orange oil; ^1H NMR (300 MHz, D_2O): δ = 9.14 (d, J = 5.86 Hz, 1H), 9.05 (d, J = 8.47 Hz, 1H), 8.35 (d, J = 9.03 Hz, 1H), 8.09 (d, J = 1.98 Hz, 1H), 7.94 (q, J = 8.47, 5.86 Hz, 1H), 7.74 (dd, J = 9.03, 1.98 Hz, 1H), 4.52 (s, 3H), 1.35 (s, 9H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 181.93, 158.40, 152.33, 149.87, 142.24, 134.92, 130.38, 128.12, 123.89, 113.14, 47.85, 41.61, 28.62 ppm; MS (EI): 244 [M^+]; HRMS: calcd for $C_{15}H_{18}NO_2$: 244.133710; found: 244.133754.

7-Benzoyloxy-1-methylquinolinium trifluoracetate (20): The same procedure as for **15** was applied with 7-hydroxyquinoline (**18**; 51 mg, 0.35 mmol) in THF (2 mL), benzoyl chloride (165 μL, 0.52 mmol, 1.5 equiv), and DIEA (118 μL, 0.69 mmol, 2 equiv), followed by dimethylsulfate (0.23 mL, 2.4 mmol, 7 equiv) to give, after lyophilization, **20** (64.0 mg, 0.242 mmol, 69%) as an orange oil; ^1H NMR (300 MHz, D_2O): δ = 9.18 (d, J = 5.84 Hz, 1H), 9.08 (d, J = 8.40 Hz, 1H), 8.40 (d, J = 9.04 Hz, 1H), 8.27 (d, J = 1.79 Hz, 1H), 8.17 (d, J = 7.16 Hz, 2H), 7.97

(dd, $J=8.40$, 5.84 Hz, 1H), 7.90 (dd, $J=9.04$, 1.79 Hz, 1H), 7.71 (t, $J=7.54$ Hz, 1H), 7.55 (t, $J=7.53$ Hz, 2H), 4.54 (s, 3H) ppm; ^{13}C NMR (75 MHz, D_2O): $\delta=168.83$, 158.21, 152.41, 149.93, 142.22, 137.44, 135.05, 132.74, 131.49, 130.44, 130.25, 128.15, 124.00, 113.34, 47.86 ppm; MS (EI): 264 [M^+]; HRMS: calcd for $C_{17}H_{14}NO_2$: 264.102450; found: 264.102454.

6-Acetoxy-1-methylquinolinium trifluoracetate (21): Acetyl chloride (53.0 μL, 0.75 mmol, 2.0 equiv) and DIEA (0.13 μL, 0.76 mmol, 2 equiv) were added to a solution of 7-hydroxyquinoline (**18**; 54.2 mg, 0.37 mmol) in THF (0.5 mL). The solution was stirred overnight at room temperature. The solvent was removed under vacuum and the residue was taken up into THF (0.5 mL). Dimethyl sulfate (0.25 mL, 2.6 mmol, 7 equiv) was added and the solution was stirred for 20 h at room temperature. The solvent was removed under vacuum and the residue was purified by preparative HPLC. After lyophilization, **21** (32.1 mg, 0.16 mmol, 43%) was obtained as a white solid; ^{1}H NMR (300 MHz, D_2O): $\delta=9.15$ (d, $J=5.74$ Hz, 1H), 9.00 (d, $J=8.43$ Hz, 1H), 8.41 (d, $J=9.42$ Hz, 1H), 8.04 (d, $J=2.45$ Hz, 1H), 7.97 (q, $J=8.43$, 5.77 Hz, 2H), 3.08 (s, 3H), 2.38 (d, $J=6.91$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO): $\delta=174.70$, 152.77, 151.85, 149.71, 133.28, 133.03, 124.82, 123.84, 122.93, 48.09, 22.93 ppm; HRMS: calcd for $C_{12}H_{12}NO_2$: 202.0868; found: 202.0862.

Dendrimer synthesis: The dendrimer synthesis was carried out by using standard Fmoc SPPS on Rink amide resin (0.47 mmol g^{-1}). The resin was acylated with each amino acid or diamino acid (3 equiv) in the presence of TBTU or BOP (3 equiv) and DIEA (5 equiv). After 30 min, the resin was successively washed with DMF, MeOH, and CH_2Cl_2 (2× with each solvent), then checked for free amino groups with the 2,4,6-trinitrobenzenesulfonic acid test. The Fmoc protecting group was removed with a solution of 20% piperidine in DMF for 2×10 min and the solution was then removed by filtration. The resin was washed with DMF, MeOH, and CH_2Cl_2 (2× with each solvent). At the end of the synthesis, the resin was acylated with three equivalents of the capping group in the presence of BOP (3 equiv) and DIEA (5 equiv) for 2 h. The resin was dried and the cleavage was carried out with TFA/1,2-ethanediothiol/H_2O/triisopropylsilane (94.5:2.5:2.5:1) for 4 h. The peptide was precipitated with methyl *tert*-butyl ether then dissolved in a water/acetonitrile mixture. All dendrimers were purified by preparative HPLC (flow rate of 100 mL min^{-1}; eluent A = water and 0.1% TFA; eluent B = acetonitrile, water, and TFA (3:2:0.1%); column: Waters prepak cartridge 500 g RP-C18, 20 μm, pore size: 300 Å, detection: $\lambda=214$ nm).

Dendrimer A1: From Rink amide resin (200 mg, 0.061 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{1}Asp)$_2$B^{1}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (27 mg, 16%); RP HPLC: $t_R=18.56$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{84}H_{111}N_{21}O_{34}S$: 1989.73; found: 1990.00.

Dendrimer B1: From Rink amide resin (200 mg, 0.061 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{1}Ser)$_2$B^{1}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (25 mg, 15%); RP HPLC: $t_R=16.96$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{86}H_{111}N_{21}O_{36}S$: 2045.72; found: 2046.00.

Dendrimer C1: From Rink amide resin (200 mg, 0.061 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{1}His)$_2$B^{1}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (23 mg, 12%); RP HPLC: $t_R=19.55$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{89}H_{113}N_{23}O_{35}S$: 2095.76; found: 2096.13.

Dendrimer D1: From Rink amide resin (400 mg, 0.061 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{1}His)$_2$B^{1}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (34 mg, 16%); RP HPLC: $t_R=17.48$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{86}H_{113}N_{23}O_{32}S$: 2011.78; found: 2012.13.

Dendrimer E1: From Tentagel resin (300 mg, 0.2 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{1}Asp)$_2$B^{1}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (18 mg, 12%); RP HPLC: $t_R=17.96$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{93}H_{117}N_{27}O_{31}S$: 2139.83; found: 2140.38.

Dendrimer F1: From Tentagel resin (400 mg, 0.2 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{1}Ser)$_2$B^{1}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (44 mg, 18%); RP HPLC: $t_R=17.25$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{92}H_{117}N_{27}O_{30}S$: 2111.83; found: 2112.38.

Dendrimer A2: From Rink amide resin (200 mg, 0.61 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{2}Asp)$_2$B^{2}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (29.8 mg, 12.9%); RP HPLC: $t_R=14.0$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{81}H_{105}N_{21}O_{31}S$: 1899.70; found: 1900.25.

Dendrimer B2: From Rink amide resin (200 mg, 0.47 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{2}Ser)$_2$B^{2}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (8.2 mg, 4.5%); RP HPLC: $t_R=15.6$ min (A/B = 70/30→40/60 in 30 min); MS (ES+): calcd for $C_{83}H_{105}N_{21}O_{33}S$: 1955.69; found: 1956.25.

Dendrimer C2: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{2}His)$_2$B^{2}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (14.7 mg, 8.0%); RP HPLC: $t_R=15.6$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{86}H_{107}N_{23}O_{32}S$: 2005.72; found: 2006.39.

Dendrimer D2: From TGR resin (400 mg, 0.20 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{2}His)$_2$B^{2}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (22.9 mg, 14.9%); RP HPLC: $t_R=24.8$ min (A/B = 90/10→50/50 in 40 min); MS (ES+): calcd for $C_{83}H_{107}N_{23}O_{23}S$: 1921.73; found: 1922.13.

Dendrimer E2: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{2}Asp)$_2$B^{2}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (9.7 mg, 5.2%); RP HPLC: $t_R=22.4$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{90}H_{111}N_{27}O_{26}S$: 2049.78; found: 2050.86.

Dendrimer F2: From TGR resin (400 mg, 0.20 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{2}Ser)$_2$B^{2}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (21.8 mg, 13.5%); RP HPLC: $t_R=17.2$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{89}H_{111}N_{27}O_{27}S$: 2021.79; found: 2022.25.

Dendrimer A3: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{3}Asp)$_2$B^{3}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (9.7 mg, 5.5%); RP HPLC: $t_R=34$ min ($\lambda=214$ nm, A/B = 90/10→60/40 in 60 min); MS (ES+): calcd for $C_{78}H_{99}N_{21}O_{31}S$: 1857.65; found: 1858.50.

Dendrimer B3: From Rink amide resin (100 mg, 0.47 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{3}Ser)$_2$B^{3}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (21.4 mg, 24%); RP HPLC: $t_R=34$ min ($\lambda=214$ nm, A/B = 97/3→50/50 in 47 min); MS (ES+): calcd for $C_{80}H_{100}N_{21}O_{33}S$: 1914.64; found: 1914.38.

Dendrimer C3: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{3}His)$_2$B^{3}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (7.0 mg, 4%); RP HPLC: $t_R=23$ min ($\lambda=214$ nm, A/B = 90/10→70/30 in 20 min, then →60/40 in 40 min); MS (ES+): calcd for $C_{83}H_{102}N_{23}O_{32}S$: 1964.87; found: 1964.13.

Dendrimer D3: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-Ser)$_2$B^{3}His)$_2$B^{3}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (50.4 mg, 29.3%); RP HPLC: $t_R=27$ min ($\lambda=214$ nm, A/B = 90/10→50/50 in 40 min); MS (ES+): calcd for $C_{80}H_{102}N_{23}O_{29}S$: 1880.69; found: 1880.13.

Dendrimer E3: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{3}Asp)$_2$B^{3}CysSerNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (17.1 mg, 9.3%); RP HPLC: $t_R=17$ min ($\lambda=214$ nm, A/B = 90/10→70/30 in 20 min, then →60/40 in 40 min); MS (ES+): calcd for $C_{87}H_{105}N_{27}O_{28}S$: 2008.73; found: 2008.73.

Dendrimer F3: From Rink amide resin (150 mg, 0.61 mmol g^{-1}), the sequence ((Cap-His)$_2$B^{3}Ser)$_2$B^{3}CysAspNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (47.2 mg, 26%); RP HPLC: $t_R=34$ min ($\lambda=214$ nm, A/B = 90/10→60/40 in 60 min); MS (ES+): calcd for $C_{86}H_{105}N_{27}O_{27}S$: 1981.99; found: 1980.38.

Dendrimer DFH: From Novasyn TGR resin (400 mg, 0.02 mmol g^{-1}), the sequence ((Cap-Asp)$_2$B^{2}Phe)$_2$B^{2}CysHisNH$_2$ was obtained as a colorless foamy solid after preparative HPLC purification (24.8 mg, 20%); RP

HPLC: $t_R = 28.4$ min (A/B = 80/20 → 40/60 in 40 min); MS (ES+): calcd for $C_{98}H_{119}N_{21}O_{34}S$: 2165.79; found: 2166.63.

Dendrimer HWS: From Rink amide resin (200 mg, 0.47 mmol g^{-1}), the sequence $((Cap\text{-}His)_2B^2Trp)_2B^2CysSerNH_2$ was obtained as a colorless foamy solid after preparative HPLC purification (26 mg, 12%); RP HPLC: $t_R = 24.6$ min (A/B = 80/20 → 40/60 in 40 min); MS (ES+): calcd for $C_{99}H_{123}N_{21}O_{34}S$: 2281.92; found: 2282.88.

Dendrimer LRH: From Novasyn TGR resin (400 mg, 0.02 mmol g^{-1}), the sequence $((Cap\text{-}Leu)_2B^2Arg)_2B^2CysHisNH_2$ was obtained as a colorless foamy solid after preparative HPLC purification (23.3 mg, 14%); RP HPLC: $t_R = 24.6$ min (A/B = 60/40 → 20/80 in 40 min); MS (ES+): calcd for $C_{99}H_{123}N_{21}O_{34}S$: 2176.09; found: 2177.13.

Dendrimer DHL: From Rink amide resin (200 mg, 0.47 mmol g^{-1}), the sequence $((Cap\text{-}Asp)_2B^2His)_2B^2CysLeuNH_2$ was obtained as a colorless foamy solid after preparative HPLC purification (21 mg, 11%); RP HPLC: $t_R = 22.1$ min (A/B = 80/20 → 40/60 in 40 min); MS (ES+): calcd for $C_{93}H_{121}N_{23}O_{33}S$: 2121.16; found: 2122.25

Dendrimer HHH: From Novasyn TGR resin (100 mg, 0.02 mmol g^{-1}), the sequence $((Cap\text{-}His)_2B^2His)_2B^2CysHisNH_2$ was obtained as a colorless foamy solid after preparative HPLC purification; MS (ES+): calcd for $C_{100}H_{123}N_{33}O_{26}S$: 2233.90; found 2235.26.

Dendrimer dimerization: Homodimers: The monomeric dendrimer *X* (1 mg) was dissolved in water (25 μL) and a solution of aldrithiol (0.0454 M in MeOH, 0.45 equiv) was added. The pH value was adjusted to 8 with 20 mM aq. NH_4HCO_3 and the solution was stirred for 30 min at 25 °C then acidified with TFA (1 drop). The homodimer *X*–*X* was purified by semipreparative RP HPLC (column: Vydac 218TP510, C18, 1.0 × 25 cm, flow rate of 4 mL min^{-1}; eluents A and B as above, gradient of eluents as indicated in the characterization; detection by UV, $\lambda = 220$ nm). Heterodimers: Dendrimer *X* (5 mg) was dissolved in water (125 μL) and aldrithiol (0454 M in MeOH, 25 equiv) was added. The methanol was evaporated and the excess of aldrithiol was removed by extraction with dichloromethane. The activated dendrimer solution was divided in five parts. A solution of dendrimer *Y* (1 mg in 25 μL of H_2O, 1 equiv) was added to each part and the final solution was adjusted to pH 8 by addition of 20 mM aq. NH_4HCO_3. The solution was stirred for 30 minutes and then acidified with TFA (1 drop). The heterodimer was purified by semipreparative RP HPLC as above.

Dendrimer A1–A1: Yield: 0.8 mg (0.19 μmol), 80%; RP HPLC: $t_R = 13.95$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{168}H_{222}N_{42}O_{68}S_2$: 3977.46; found: 3978.75.

Dendrimer B1–B1: Yield: 0.4 mg (0.09 μmol), 40%; RP HPLC: $t_R = 16.70$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{172}H_{222}N_{42}O_{72}S_2$: 4089.44; found: 4090.75.

Dendrimer C1–C1: Yield: 0.8 mg (0.17 μmol), 80%; RP HPLC: $t_R = 15.85$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{178}H_{226}N_{46}O_{70}S_2$: 4189.52; found: 4191.00.

Dendrimer D1–D1: Yield: 0.5 mg (0.11 μmol), 50%; RP HPLC: $t_R = 14.88$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{172}H_{226}N_{46}O_{64}S_2$: 4021.56; found: 4022.63.

Dendrimer E1–E1: Yield: 0.9 mg (0.17 μmol), 90%; RP HPLC: $t_R = 10.30$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{186}H_{234}N_{54}O_{62}S_2$: 4277.66; found: 4279.13.

Dendrimer F1–F1: Yield: 0.5 mg (1.0 μmol), 50%; RP HPLC: $t_R = 13.90$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{184}H_{234}N_{54}O_{60}S_2$: 4221.66; found: 4223.63.

Dendrimer A1–B1: Yield: 1.5 mg (0.35 μmol), 75%; RP HPLC: $t_R = 14.65$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{170}H_{222}N_{42}O_{70}S_2$: 4033.45; found: 4034.88.

Dendrimer A1–C1: Yield: 0.9 mg (0.20 μmol), 45%; RP HPLC: $t_R = 14.90$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{173}H_{224}N_{44}O_{69}S_2$: 4083.49; found: 4085.25.

Dendrimer A1–D1: Yield: 0.5 mg (0.11 μmol), 25%; RP HPLC: $t_R = 14.05$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{170}H_{224}N_{44}O_{66}S_2$: 3999.51; found: 4001.13.

Dendrimer A1–E1: Yield: 0.9 mg (0.19 μmol), 40%; RP HPLC: $t_R = 11.75$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{177}H_{228}N_{48}O_{65}S_2$: 4127.56; found: 4129.00.

Dendrimer A1–F1: Yield: 1.1 mg (0.23 μmol), 49%; RP HPLC: $t_R = 14.50$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{176}H_{228}N_{48}O_{64}S_2$: 4099.56; found: 4101.25.

Dendrimer B1–C1: Yield: 0.6 mg (0.13 μmol), 29%; RP HPLC: $t_R = 15.80$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{175}H_{224}N_{44}O_{71}S_2$: 4139.49; found: 4140.63.

Dendrimer B1–D1: Yield: 0.9 mg (0.20 μmol), 43%; RP HPLC: $t_R = 15.43$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{172}H_{224}N_{44}O_{68}S_2$: 4055.5; found: 4056.63.

Dendrimer B1–E1: Yield: 0.8 mg (0.17 μmol), 36%; RP HPLC: $t_R = 13.68$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{179}H_{228}N_{48}O_{67}S_2$: 4183.55; found: 4185.00.

Dendrimer B1–F1: Yield: 0.4 mg (0.08 μmol), 18%; RP HPLC: $t_R = 14.25$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{178}H_{228}N_{48}O_{66}S_2$: 4155.55; found: 4157.25.

Dendrimer C1–D1: Yield: 0.5 mg (0.11 μmol), 25%; RP HPLC: $t_R = 15.32$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{175}H_{226}N_{46}O_{67}S_2$: 4105.54; found: 4106.63.

Dendrimer C1–E1: Yield: 0.7 mg (0.08 μmol), 19%; RP HPLC: $t_R = 13.82$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{182}H_{228}N_{50}O_{66}S_2$: 4233.59; found: 4234.50.

Dendrimer C1–F1: Yield: 1.4 mg (0.29 μmol), 29%; RP HPLC: $t_R = 14.26$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{181}H_{230}N_{50}O_{62}S_2$: 4205.59; found: 4207.25.

Dendrimer D1–E1: Yield: 0.4 mg (0.08 μmol), 18%; RP HPLC: $t_R = 12.50$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{179}H_{230}N_{50}O_{63}S_2$: 4150.38; found: 4149.61.

Dendrimer D1–F1: Yield: 1.1 mg (0.23 μmol), 51%; RP HPLC: $t_R = 15.28$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{178}H_{228}N_{50}O_{62}S_2$: 4121.61; found: 4122.63.

Dendrimer E1–F1: Yield: 1.0 mg (0.19 μmol), 50%; RP HPLC: $t_R = 13.00$ min (A/B = 70/30 → 50/50 in 20 min); MS (ES+): calcd for $C_{185}H_{234}N_{54}O_{61}S_2$: 4249.66; found: 4251.88.

Dendrimer A2–A2: Yield: 0.6 mg (0.149 μmol), 60%; semipreparative RP HPLC: $t_R = 21.6$ min (A/B = 80/20 → A/B = 50/50 in 30 min); MS (ES+): calcd for $C_{162}H_{208}N_{42}O_{62}S_2$: 3797.39; found: 3798.75.

Dendrimer B2–B2: Yield: 0.5 mg (0.121 μmol), 50%; semipreparative RP HPLC: $t_R = 26.7$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{166}H_{208}N_{42}O_{66}S_2$: 3909.37; found: 3910.75.

Dendrimer C2–C2: Yield: 0.45 mg (0.10 μmol), 45%; semipreparative RP HPLC: $t_R = 27.1$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{172}H_{212}N_{46}O_{64}S_2$: 4009.42; found: 3910.75.

Dendrimer D2–D2: Yield: 0.45 mg (0.09 μmol), 45%; semipreparative RP HPLC: $t_R = 25.3$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{166}H_{212}N_{46}O_{58}S_2$: 3841.45; found: 3842.88.

Dendrimer E2–E2: Yield: 0.7 mg (0.14 μmol), 70%; semipreparative RP HPLC: $t_R = 22.5$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{180}H_{220}N_{54}O_{56}S_2$: 4097.55; found: 4099.13.

Dendrimer F2–F2: Yield: 0.9 mg (0.18 μmol), 45%; semipreparative RP HPLC: $t_R = 22.6$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{178}H_{220}N_{54}O_{54}S_2$: 4041.56; found: 4043.13.

Dendrimer A2–B2: Yield: 0.5 mg (0.12 μmol), 25%; semipreparative RP HPLC: $t_R = 22.6$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{164}H_{208}N_{42}O_{64}S_2$: 3853.38; found: 3854.88.

Dendrimer A2–C2: Yield: 0.5 mg (0.12 μmol), 25%; semipreparative RP HPLC: $t_R = 23.9$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{167}H_{210}N_{44}O_{63}S_2$: 3903.40; found: 3904.63.

Dendrimer A2–D2: Yield: 1.3 mg (0.31 μmol), 65%; semipreparative RP HPLC: $t_R = 24.7$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{164}H_{210}N_{44}O_{60}S_2$: 3819.42; found: 3820.63.

Dendrimer A2–E2: Yield: 0.8 mg (0.160 μmol), 40%; semipreparative RP HPLC: $t_R = 21.6$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{180}H_{220}N_{54}O_{56}S_2$: 4097.55; found: 3948.63.

Dendrimer A2–F2: Yield: 1.1 mg (0.245 μmol), 55%; semipreparative RP HPLC: $t_R = 21.8$ min (A/B = 80/20 → 50/50 in 30 min); MS (ES+): calcd for $C_{170}H_{214}N_{48}O_{58}S_2$: 3919.47; found: 3920.75.

Dendrimer B2–C2: Yield: 1.1 mg (0.256 μmol), 55%; semipreparative RP HPLC: $t_R = 24.7$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{169}H_{210}N_{44}O_{65}S_2$: 3959.39; found: 3961.74.

Dendrimer B2–D2: Yield: 0.5 mg (0.120 μmol), 25%; semipreparative RP HPLC: $t_R = 25.0$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{166}H_{210}N_{44}O_{62}S_2$: 3875.21; found: 3876.75.

Dendrimer B2–E2: Yield: 0.8 mg (0.175 μmol), 40%; semipreparative RP HPLC: $t_R = 22.6$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{173}H_{214}N_{48}O_{61}S_2$: 4003.46; found: 4005.13.

Dendrimer B2–F2: Yield: 0.6 mg (0.132 μmol), 30%; semipreparative RP HPLC: $t_R = 24.0$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{172}H_{214}N_{48}O_{60}S_2$: 3975.46; found: 3976.75.

Dendrimer C2–D2: Yield: 0.7 mg (0.160 μmol), 35%; semipreparative RP HPLC: $t_R = 24.0$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{169}H_{212}N_{46}O_{61}S_2$: 3925.43; found: 3927.54.

Dendrimer C2–E2: Yield: 1.0 mg (0.199 μmol), 50%; semipreparative RP HPLC: $t_R = 22.7$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{176}H_{216}N_{50}O_{60}S_2$: 4053.48; found: 4055.00.

Dendrimer D2–E2: Yield: 1.8 mg (0.387 μmol), 90%; semipreparative RP HPLC: $t_R = 21.6$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{173}H_{216}N_{50}O_{57}S_2$: 3969.50; found: 3970.63.

Dendrimer D2–F2: Yield: 1.3 mg (0.281 μmol), 65%; semipreparative RP HPLC: $t_R = 21.6$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{172}H_{216}N_{50}O_{56}S_2$: 3941.50; found: 3943.38.

Dendrimer E2–F2: Yield: 1.4 mg (0.281 μmol), 70%; semipreparative RP HPLC: $t_R = 20.4$ min (A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{179}H_{220}N_{54}O_{55}S_2$: 4069.55; found: 4071.00.

Dendrimer A3–A3: Yield: 0.4 mg, 40%; semipreparative RP HPLC: $t_R =$ 19.80 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{156}H_{197}N_{42}O_{62}S_2$: 3714.29; found: 3714.63.

Dendrimer B3–B3: Yield: 0.4 mg, 50%; semipreparative RP HPLC: $t_R =$ 21.75 min ($\lambda = 225$ nm, A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{166}H_{197}N_{42}O_{66}S_2$: 3826.27; found: 3726.63.

Dendrimer C3–C3: Yield: 0.4 mg, 40%; semipreparative RP HPLC: $t_R =$ 21.25 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{166}H_{201}N_{46}O_{64}S_2$: 3926.33; found: 3926.38.

Dendrimer D3–D3: Yield: 1.3 mg, 100%; semipreparative RP HPLC: $t_R = 21.00$ min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{160}H_{201}N_{46}O_{58}S_2$: 3758.36; found: 3758.75.

Dendrimer E3–E3: Yield: 0.5 mg, 50%; semipreparative RP HPLC: $t_R =$ 17.00 min ($\lambda = 225$ nm, A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{174}H_{209}N_{42}O_{60}S_2$: 4014.45; found: 4014.75.

Dendrimer F3–F3: Yield: 0.6 mg, 60%; semipreparative RP HPLC: $t_R =$ 16.20 min ($\lambda = 225$ nm, A/B = 80/20→50/50 in 30 min); MS (ESI): calcd for $C_{172}H_{209}N_{54}O_{54}S_2$: 3958.46; found: 3959.00.

Dendrimer A3–B3: Yield: 0.1 mg, 10%; semipreparative RP HPLC: $t_R =$ 42.80 min ($\lambda = 225$ nm, A/B = 80/20→70/30 in 20 min, then →40/60 in 40 min); MS (ES+): calcd for $C_{158}H_{197}N_{42}O_{64}S_2$: 3770.28; found: 3771.13.

Dendrimer A3–D3: Yield: 0.1 mg, 9%; semipreparative RP HPLC: $t_R =$ 30.25 min ($\lambda = 225$ nm, A/B = 90/10→50/50 in 40 min); MS: calcd for $C_{158}H_{199}N_{44}O_{60}S_2$: 3736.32; found: 3736.63.

Dendrimer A3–E3: Yield: 0.9 mg, 51%; semipreparative RP HPLC: $t_R =$ 18.00 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{165}H_{203}N_{48}O_{59}S_2$: 3864.37; found: 3865.00).

Dendrimer A3–F3: Yield: 1 mg, 58%; semipreparative RP HPLC: $t_R =$ 18.25 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{164}H_{203}N_{48}O_{58}S_2$: 3836.38; found: 3836.75.

Dendrimer B3–C3: Yield: 0.2 mg, 20%; semipreparative RP HPLC: $t_R =$ 39.00 min ($\lambda = 225$ nm, A/B = 90/10→70/30 in 20 min, then →A/B = 40/60 in 60 min); MS (ES+): calcd for $C_{163}H_{199}N_{44}O_{66}S_2$: 3876.30; found: 3777.13.

Dendrimer B3–D3: Yield: 0.9 mg, 74%; semipreparative RP HPLC: $t_R =$ 21.25 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{160}H_{199}N_{44}O_{62}S_2$: 3792.31; found: 3793.00.

Dendrimer B3–F3: Yield: 1.3 mg, 74%; semipreparative RP HPLC: $t_R =$ 18.50 min ($\lambda = 225$ nm, A/B = 80/20→50/50 in 30 min); MS (ES+): calcd for $C_{166}H_{203}N_{48}O_{60}S_2$: 3892.37; found: 3893.25.

Dendrimer C3–D3: Yield: 0.2 mg, 10%; semipreparative RP HPLC: $t_R =$ 22.00 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{163}H_{201}N_{46}O_{61}S_2$: 3842.34; found: 3842.88.

Dendrimer C3–E3: Yield: 0.7 mg, 39%; semipreparative RP HPLC: $t_R =$ 8.00 min ($\lambda = 225$ nm, A/B = 70/30→40/60 in 30 min); MS (ES+): calcd for $C_{170}H_{205}N_{50}O_{60}S_2$: 3970.39; found: 3970.50.

Dendrimer C3–F3: Yield: 1.4 mg, 70%; semipreparative RP HPLC: $t_R =$ 17.80 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{169}H_{205}N_{50}O_{59}S_2$: 3942.39; found: 3942.88.

Dendrimer D3–E3: Yield: 0.8 mg, 39%; semipreparative RP HPLC: $t_R =$ 17.80 min ($\lambda = 225$ nm, A/B = 80/20→40/60 in 40 min); MS (ES+): calcd for $C_{167}H_{205}N_{50}O_{57}S_2$: 3886.40; found: 3886.88.

Dendrimer D3–F3: Yield: 1.0 mg, 48%; semipreparative RP HPLC: $t_R =$ 18.80 min ($\lambda = 225$ nm, A/B = 90/10→40/60 in 40 min); MS (ES+): calcd for $C_{166}H_{205}N_{50}O_{56}S_2$: 3858.41; found: 3858.88.

Dendrimer E3–F3: Yield: 2.3 mg, 100%; semipreparative RP HPLC: $t_R = 6.50$ min ($\lambda = 225$ nm, A/B = 70/30→40/60 in 30 min); MS (ES+): calcd for $C_{173}H_{209}N_{54}O_{55}S_2$: 3986.46; found: 3986.88.

Dendrimer DHL–DHL: Yield: 0.6 mg (0.107 μmol), 60%; semipreparative RP HPLC: $t_R = 17.9$ min (A/B = 70/30→30/70 in 40 min); MS (+ ESI): calcd for $C_{184}H_{236}N_{46}O_{68}S_2$: 4241.59; found: 4243.38.

Dendrimer LRH–LRH: Yield: 0.6 mg (0.105 μmol), 60%; semipreparative RP HPLC: $t_R = 27.7$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{200}H_{296}N_{54}O_{52}S_2$: 4350.16; found: 4352.00.

Dendrimer DFH–DFH: Yield: 0.7 mg (0.127 μmol), 70%; semipreparative RP HPLC: $t_R = 27.7$ min (A/B = 70/30→30/70 in 40 min); MS (+ ESI): calcd for $C_{197}H_{240}N_{42}O_{68}S_2$: 4345.61; found: 4352.00.

Dendrimer LRH–HWS: Yield: 0.4 mg (0.088 μmol), 26%; semipreparative RP HPLC: $t_R = 21.1$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{207}H_{274}N_{56}O_{53}S_2$: 4455.99; found: 4458.00.

Dendrimer LRH–DHL: Yield: 0.4 mg (0.093 μmol), 27%; semipreparative RP HPLC: $t_R = 25.1$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{192}H_{266}N_{50}O_{60}S_2$: 4295.87; found: 4297.50.

Dendrimer LRH–DFH: Yield: 0.6 mg (0.138 μmol), 31%; semipreparative RP HPLC: $t_R = 16.3$ min (A/B = 50/50→10/90 in 40 min); MS (+ ESI): calcd for $C_{198}H_{266}N_{48}O_{60}S_2$: 4339.87; found: 4341.50.

Dendrimer LRH–HSD: Yield: 1.1 mg (0.257 μmol), 53%; semipreparative RP HPLC: $t_R = 35.6$ min (A/B = 70/30→30/70 in 40 min); MS (+ ESI): calcd for $C_{192}H_{264}N_{54}O_{56}S_2$: 4285.89; found: 4287.50.

Dendrimer LRH–DHS: Yield: 0.8 mg (0.188 μmol), 41%; semipreparative RP HPLC: $t_R = 16.5$ min (A/B = 50/50→10/90 in 40 min); MS (+ ESI): calcd for $C_{189}H_{260}N_{50}O_{61}S_2$: 4269.82; found: 4271.38.

Dendrimer LRH–DSH: Yield: 0.8 mg (0.190 μmol), 42%; semipreparative RP HPLC: $t_R = 19.2$ min (A/B = 50/50→10/90 in 40 min); MS (+ ESI): calcd for $C_{186}H_{258}N_{48}O_{62}S_2$: 4219.80; found: 4221.00.

Dendrimer LRH–HHH: Yield: 0.4 mg (0.091 μmol), 31%; semipreparative RP HPLC: $t_R = 22.7$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{200}H_{270}N_{60}O_{52}S_2$: 4407.98; found: 4409.99.

Dendrimer HWS–DHL: Yield: 0.6 mg (0.136 μmol), 33%; semipreparative RP HPLC: $t_R = 21.6$ min (A/B = 70/30→30/70 in 40 min); MS (+ ESI): calcd for $C_{199}H_{244}N_{52}O_{61}S_2$: 4401.72; found: 4403.13.

Dendrimer HWS–DHS: Yield: 0.7 mg (0.160 μmol), 39%; semipreparative RP HPLC: $t_R = 10.7$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{196}H_{238}N_{52}O_{62}S_2$: 4375.67; found: 4378.61.

Dendrimer HWS–DSH: Yield: 0.8 mg (0.185 μmol), 45%; semipreparative RP HPLC: $t_R = 11.4$ (A/B = 60/40→20/80 in 40 min); MS (+ESI): calcd for $C_{193}H_{236}N_{50}O_{63}S_2$: 4325.64; found: 4328.59.

Dendrimer HWS–HSD: Yield: 0.5 mg (0.114 μmol), 26%; semipreparative RP HPLC: $t_R = 11.8$ min (A/B = 60/40→20/80 in 40 min); MS (+ ESI): calcd for $C_{199}H_{242}N_{56}O_{57}S_2$: 4391.74, found: 4393.00.

Dendrimer DHL–HSD: Yield: 0.9 mg (0.213 μmol), 43%; semipreparative RP HPLC: $t_R = 6.7$ min (A/B = 60/40→20/80 in 40 min); MS (+ESI): calcd for $C_{184}H_{234}N_{50}O_{64}S_2$: 4231.62; found: 4233.55.

Dendrimer DHL–DHS: Yield: 0.7 mg (0.166 μmol), 35%; semipreparative RP HPLC: $t_R = 8.5$ min (A/B = 60/40→A/B = 20/80 in 40 min); MS (+ESI): calcd for $C_{181}H_{230}N_{46}O_{69}S_2$: 4215.55; found: 4217.31.

Dendrimer DHL–DSH: Yield: 0.8 mg (0.192 μmol), 42%; semipreparative RP HPLC: $t_R = 18.4$ min (A/B = 70/30→30/70 in 40 min); MS (+ ESI): calcd for $C_{178}H_{228}N_{44}O_{70}S_2$: 4165.52; found: 4166.38.

Dendrimer DFH–HSD: Yield: 0.6 mg (0.140 μmol), 28%; semipreparative RP HPLC: $t_R = 10.5$ min (A/B = 50/50→10/90 in 40 min); MS (+ ESI): calcd for $C_{191}H_{238}N_{48}O_{64}S_2$: 4275.61; found: 4277.25.

Assays and kinetic measurements: Dendrimers were kept as 1 mM stock solutions in acetonitrile/water (1:1) and preserved at 4°C in Eppendorf vials. Dendrimers were freshly diluted to 0.05 mM in 20 mM aq. bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BisTris, pH 6.0) before each measurement. The BisTris buffer (pH 6.0) was prepared with MilliQ deionized water and the pH value was adjusted with aq. NaOH and aq. HCl solutions. Assays were followed in individual wells of round-bottomed polystyrene 96-well plates (Costar) by using a SPECTRAMax fluorescence detector with preset values of the excitation and emission wavelengths corresponding to the measured substrate**.** All pipetting manipulations were done by hand with single pipettes (Pipetman form, Gilson). The measurement temperature inside the instrument was 25°C. Kinetic experiments were followed for 2 h. Fluorescence data were converted into product concentrations by means of a calibration curve. Initial reaction rates were calculated from the steepest part observed during the first 2000 s of each curve. In a typical experiment, 20 mM aq. BisTris (20 μL; pH 6.0) was added to a well first, followed by dendrimer solution (2.5 μL, 0.05 mM in aq. BisTris (pH 6.0), final concentration in the well = 5 μM) and then substrate solution (2.5 μL, 2 mM in acetonitrile/water (1:1), final concentration in the well = 200 μM). The rate observed under these conditions is the apparent rate V_{app}. V_{uncat} is the rate observed with 20 mM aq. BisTris (22.5 mL; pH 6.0) and substrate solution (2.5 μL, 2 mM in acetonitrile/water (1:1), final concentration in the well = 200 μM). The observed rate enhancement V_{net}/V_{uncat} is defined as $(V_{app}/V_{uncat})-1$. Michaelis–Menten parameters were obtained from the linear double reciprocal plot of $1/V_{net}$ against 1/[S], measured similarly with (final concentrations) of 5 μM dendrimer (V_{app}) or no dendrimer (V_{uncat}), 40, 60, 80, 100, 200, 300, 400, 500, 600, or 700 μM substrate, and 20 mM BisTris at 25°C. The reaction rate with 4-methylimidazole was obtained under the same conditions with 40, 60, 80, 100, 200, 300, 400, and 500 μM 4-methylimidazole and 200 μM substrate.

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