Esterolytic Peptide Dendrimers with a Hydrophobic Core and Catalytic Residues at the Surface

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Abstract: (3*S*)-4-(9-Fluorenylmethoxycarbonylamino)-3-methyl(allyloxycarbonyl)aminoethyloxyacetic acid (**1**) was prepared from (*R*)-3-aminopropane-1,2-diol and used as branching unit for the synthesis of second generation peptide dendrimers with six individually addressable variable amino acid positions. Three pairs of diastereomeric dendrimers were prepared bearing a common hydrophobic core and permutations of the catalytic triad amino acids aspartate, histidine and serine at the surface. Dendrimers with two surface histidine residues catalyzed the hydrolysis of fluorogenic 8-acyloxypyrene-1,3,6-trisulfonates in aqueous buffer pH 6.0 with rate enhancement k_{cat}/k_{uncat} in the 10^3 range and Michaelis-Menten constants K_M in the 10^{-4} M range. Substrate recognition involves electrostatic interactions, as shown by competitive inhibition of catalysis observed with pyrene-1,3,6,8-tetrasulfonate. The 4-fold to 7-fold lowering in K_M between the butyryl and nonanoyl esters in the most active dendrimers provides evidence for a hydrophobic component in substrate binding, which is absent in a closely related, less active diastereomeric peptide dendrimer.

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

Introduction

Enzyme catalysis results from cooperative effects between amino acid side chains and cofactors that are made possible through the ordered folding of linear peptides.[1] In *de novo* enzyme design one attempts to use the knowledge of enzyme structure and function to build artificial enzymes from first principles.[2] Catalytic antibodies[3] and catalytic peptides[4–7] have provided convincing examples that catalysis on a protein basis is not restricted to natural enzymes. The field of organic catalysis has shown that efficient and selective catalytic cycles can be designed with small molecule catalysts of a much lower complexity level than enzymes, such as simple amino acids, suggesting that most of the protein structure can, in fact, be disposed of for the pure purpose of chemistry.[8]

Recently, we have started to explore *de novo* enzyme design on the basis of peptide dendrimers.[9] Dendrimers are tree-like structures that adopt a globular or disk-shape structure as a consequence of topology rather than folding.[10] The dendrimer strategy circumvents the protein folding problem. While catalytic dendrimers have been assembled from pre-existing catalytic subunits such as metal complexes and cofactors,[11] we are investigating catalysis arising from the interplay between amino acids within the peptide dendrimer structure as a protein model. Peptide dendrimers are formed by alternating functional amino acids with a branching diamino acid and can be prepared by standard solid phase peptide synthesis. Esterolytic dendrimers were obtained by combining the catalytic triad amino acids aspartate, histidine and serine within the dendrimer structure.[9]

Herein we report the preparation of catalytic peptide dendrimers based on an orthogonally protected diamino acid branching unit **1**. This strategy allows one to prepare dendrimers with differently functionalized branches, and was used to assemble different permutations of catalytic amino acids at the dendrimer surface around a common hydrophobic core. This arrangement follows the overall structural design of globular proteins where hydrophobic amino acids are usually found at the core, while catalytic sites are formed by pockets close to the protein surface. Our synthetic approach has not been applied before to dendrimer synthesis and the dendrimers reported here are among the very few examples of dendrimers with differentially functionalized layers.[12] The peptide dendrimers obtained catalyze the hydrolysis of pyrene-trisulfonate esters with enzyme-like properties of substrate binding and turnover in aqueous environment.

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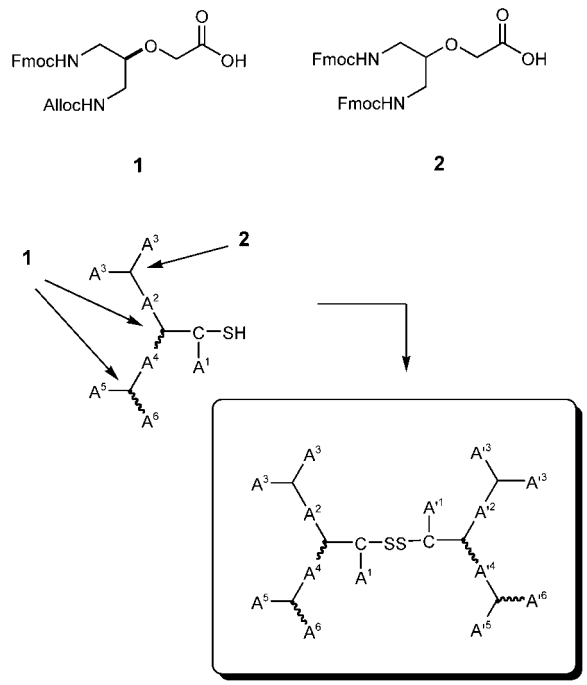
Results and Discussion

Synthesis

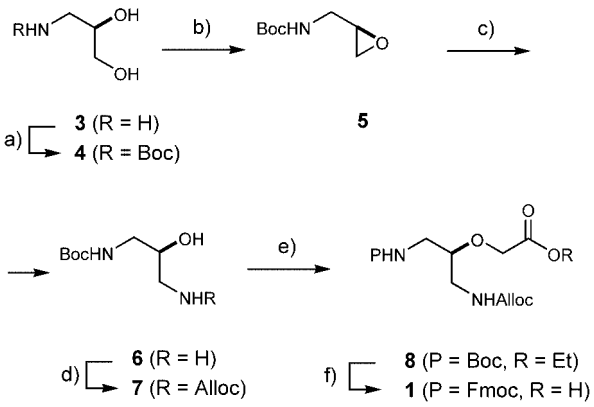
Our aim was to prepare catalytic peptide dendrimers with differentially functionalized layers on the basis of a selective protection scheme. Our peptide dendrimers are assembled as disulfide-bridged dimers of two second-generation peptide dendrimers containing three variable amino acids present in one, two, and four copies, separated by a common diamino acid branching unit (Scheme 1). We envisioned to synthesize the monomeric peptide dendrimers following the established solid-phase synthesis route, but employing a differentially protected diamino acid building block **1** at the first layer and at one of the second layer branching units, which would enable us to differentiate six individual positions, as opposed to only three when using only the symmetrically protected diamino acid building block **2**.

The realization of this strategy required the synthesis of a diamino acid with two orthogonal protecting groups. One amino group would be protected by the standard Fmoc (=9-fluorenylmethoxycarbonyl) protecting group for immediate functionalization, while the second would be blocked by the Alloc (=allyloxycarbonyl) protecting group, which is resistant to piperidine and can be removed selectively under neutral conditions with $Pd(PPh_3)_4$ and phenyltrihydrosilane ($PhSiH_3$).[13] The chiral branching diamino acid **1** was prepared in eight steps from commercially available (*R*)-*N*-*tert*-butyloxycarbonyl-3-aminopropane-1,2-diol **3**, in an overall yield of 23% (Scheme 2). Thus, protection of the amino group in **3** with Boc_2O gave **4**. The diol **4** was converted to epoxide **5** by treatment with methanesulfonyl chloride in pyridine followed by NaOH in aqueous DMSO (74%).[14] Chiral GC analysis gave an enantiomeric excess of 80% ee for epoxide **5**. The partial racemization was probably caused by the mesylation step due to an incomplete selectivity for the primary alcohol. Nucleophilic epoxide opening with ammonia gave amino alcohol **6**. Protection of the free amino group with allyl chloroformate then gave the Boc-Alloc intermediate **7**, which was alkylated at the secondary alcohol with ethyl bromoacetate to yield **8**. Ester hydrolysis with LiOH in aqueous THF was followed by treatment with aqueous HCl to remove the Boc protecting group, and finally reaction of the free amine with FmocCl in aqueous dioxane gave the orthogonally protected branching amino acid **1**. The preparation of the corresponding symmetrical bis-Fmoc protected (2-amino-1-aminomethyl-ethoxy)-acetic acid branching unit **2** has been described previously.[9]

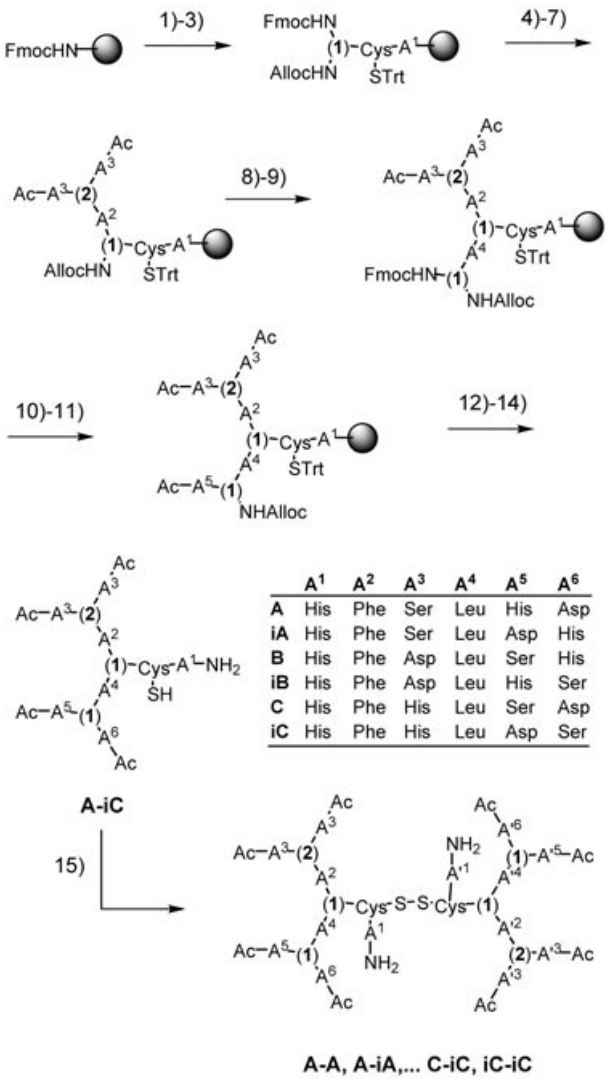
The peptide dendrimers were synthesized on a Rink amide resin (Scheme 3).[15] The synthetic sequence started with sequential attachment of the first amino acid A^1, cysteine, and the first branching unit **1** following the standard coupling methodology with BOP as *in situ* coupling reagent.[16] Fmoc deprotection then allowed us to attach amino acid A^2, followed by the branching unit **2**, and two copies of amino acid A^3. The first branch was completed by capping with acetic anhydride. The Alloc protecting group at the level of the first branching



Scheme 1. Structure of disulfide-bridged peptide dendrimers with differential functionalization of layers. (A^1–A^6, A'^1–A'^6 = variable amino acids, C = cysteine).



Scheme 2. Synthesis of chiral branching unit **1**. Conditions: a) Boc_2O, Et_3N, CH_2Cl_2/MeOH, 2 h, 25 °C, 94%; b) MsCl, Pyr, 0 °C, then NaOH, DMSO, 0 °C, 74%; c) NH_4OH, EtOH, 1.5 h, quant.; d) allyl chloroformate, EtOAc, $NaHCO_3$, 4 h, 87%; e) $BrCH_2CO_2Et$, NaH, dry THF, 5 h, 63%; f) i) LiOH, THF/H_2O, 25 °C, overnight, ii) aqueous 3 N HCl, 60 °C, 3 h, iii) Fmoc-Cl, $NaHCO_3$, H_2O/dioxane, 25 °C, overnight, 63%. Boc = *tert*-butoxycarbonyl, THF = tetrahydrofuran, Fmoc-Cl = 9-fluorenylmethoxycarbonyl chloride, MsCl = methanesulfonyl chloride.



	A^1	A^2	A^3	A^4	A^5	A^6
A	His	Phe	Ser	Leu	His	Asp
iA	His	Phe	Ser	Leu	Asp	His
B	His	Phe	Asp	Leu	Ser	His
iB	His	Phe	Asp	Leu	His	Ser
C	His	Phe	His	Leu	Ser	Asp
iC	His	Phe	His	Leu	Asp	Ser

Scheme 3. Solid phase synthesis of peptide dendrimers. Conditions: Deprotecting steps: i = DMF, piperidine (4:1); ii = $PhSiH_3$ (25 equivs.), CH_2Cl_2, 3 min, then $Pd(PPh_3)_4$ (0.1 equiv.). Coupling steps: iii = BOP/DIEA. Capping steps: iv = Ac_2O/CH_2Cl_2 (1:1). 1) i, then iii with FmocA^1OH; 2) i, then iii with FmocCys(Trt)OH; 3) i, then iii with **1**; 4) i, then iii with FmocA^2OH; 5) i, then iii with **2**; 6) i, then iii with FmocA^3OH; 7) i, then iv; 8) ii, then iii with FmocA^4OH; 9) i, then iii with **1**; 10) i, then iii with FmocA^5OH; 11) i, then iv; 12) ii, then iii with FmocA^6OH; 13) i, then iv; 14) TFA cleavage and HPLC purification; 15) disulfide coupling with 2,2′-dithiopyridine.

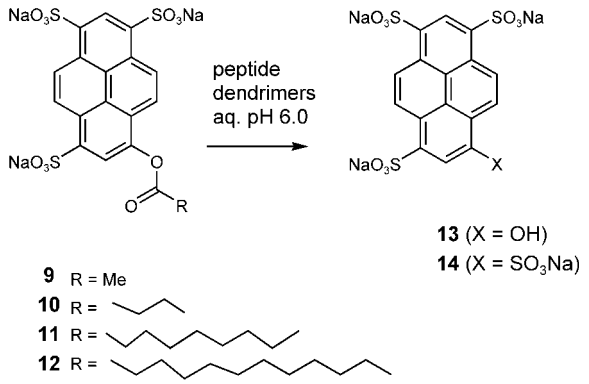
unit was removed by treatment with tetrakis(triphenylphosphine)palladium(0) and phenylsilane.[13] The fourth amino acid A^4 was introduced, followed again by the asymmetric branching unit **1**. Fmoc deprotection then allowed us to couple a single copy of an amino acid at position A^5, which was acetylated. Finally, removal of the second Alloc protection as above and peptide coupling allowed us to install the last amino acid A^6, which was acetylated.

Preparation of Esterase Dendrimers with Hydrophobic Core

The dendrimer synthesis was used to prepare a family of peptide dendrimers bearing a hydrophobic core at positions A^1, A^2 and A^4 and catalytic amino acids at the surface positions A^3, A^5 and A^6. All dendrimers were prepared with a conserved core consisting of phenylalanine and leucine at position A^2 and A^4. A histidine residue was placed at position A^1 to provide a positive charge in acidic pH and facilitate purification of the monomer by RP-HPLC, as well as to facilitate disulfide bridge formation. Indeed, we have previously experienced that disulfide bridge formation is very slow and sometimes impossible with dendrimers bearing hydrophobic residues at position A^1.[9b] Histidine has both polar and aromatic character. As will be seen below, histidine at position A^1 is not sufficient to induce catalysis of the hydrolytic reactions studied. The surface positions A^3, A^5 and A^6 were used to install permutations of the catalytic triad amino acids aspartate, histidine and serine, with the aim of mimicking a hydrolase-type enzyme. Since exchanging amino acids at position A^5 and A^6 gives diastereoisomeric products at the asymmetric branching unit **1** positioned in the second layer, the planned synthesis resulted in three pairs of diastereoisomeric peptide dendrimers **A/iA** (two serines at A^3), **B/iB** (two aspartates at A^3), and **C/iC** (two histidines at A^3). The dendrimers were obtained in yields of 20–30% after purification by semi-preparative HPLC, which is very satisfactory for a total of nine sequential peptide coupling steps, showing that the use of orthogonal protection with Alloc was very efficient. The monomeric dendrimers were then dimerized using aldrithiol[17] to give 21 combinations of disulfide-bridged dimeric dendrimers (**A-A** to **iC-iC**), which were isolated with 30–75% yields after purification by semi-preparative RP-HPLC. All dendrimers were purified by semi-preparative RP-HPLC and characterized by ESI MS.

Esterolytic Activity of Peptide Dendrimers

We expected that an esterolytic activity might arise from a cooperative effect between the catalytic triad amino acids aspartate, histidine and serine at positions A^3, A^5 and A^6 at the surface of the dendrimers. The hydrophobic core amino acids at positions A^2 and A^4 might assist catalysis by providing enhanced substrate binding through hydrophobic interactions. The dendrimers (6 monomers and 21 dimers) were assayed for esterolytic activity with various fluorogenic esters. The substrates (200 μM) were tested for hydrolysis in aqueous buffer at pH 6.0 in the presence of 2.5 mol % (5 μM) dendrimer catalyst in a microtiter-plate setup. There was no activity with esters of 7-hydroxy-*N*-methylquinolinium salts, even though these were active with our previous



Scheme 4. The hydrolysis of pyrene 1,3,5-trisulfonate esters is catalyzed by hydrophobic core peptide dendrimers with surface catalytic residues.

Table 1. Hydrolysis of pyrenesulfonate ester **10** in the presence of peptide dendrimers. The sequence of the amino acids on the surface are given in parenthesis.[a]

V_{net}/V_{uncat}	–	A	iA	B	iB	C	iC
A(SSHD)	4.0	4.5	/	/	/	/	/
iA (SSDH)	3.5	2.5	3.5	/	/	/	/
B (DDSH)	1.5	1.0	0.5	1.5	/	/	/
iB (DDHS)	0.5	1.5	0.5	0	1.0	/	/
C (HHSD)	**11.0**	3.0	**8.0**	3.0	5.5	**16.0**	/
iC (HHDS)	6.5	3.0	**9.0**	3.5	5.0	6.5	**10.0**

[a] Conditions: 200 μM **10**, 5 μM dendrimer, 25 °C, 20 mM aqueous bis-Tris pH 6.0. The ratio V_{net}/V_{uncat} is reported for monomeric dendrimers(1st column) and all combinations of dimers. V_{uncat} is the apparent spontaneous rate of hydrolysis of the butyrate ester **10** in buffer alone, and $V_{net} = V_{app} - V_{uncat}$, where V_{app} is the apparent rate of hydrolysis of **10** in the presence of dendrimer. The reactions were run in 96-well polystyrene half area microtiter plates and followed using a SpectraMAX fluorescence detector with $\lambda_{exc} = 460$ nm, $\lambda_{em} = 530$ nm. Fluorescence was converted to product concentration using a calibration curve, which was linear in the concentration range used.

series of peptide dendrimers,[9] or with aliphatic esters and acyloxymethyl ethers of umbelliferone, which are substrates suitable for esterolytic catalytic antibodies and enzymes.[18] We next turned out attention to the acyloxypyrene-1,3,6-trisulfonates **9**–**12** (Scheme 4). In this case, several dendrimers showed significant rate enhancements over the uncatalyzed background reaction. The best apparent catalytic effects with peptide dendrimers were observed with butyrate **10** and the two diastereoisomeric homodimers bearing two surface histidine residues **CC** and **iCiC**, which showed apparent catalytic effects of $V_{net}/V_{uncat} = 16$ and $V_{net}/V_{uncat} = 10$, respectively (Table 1). Catalysis was confirmed by the observation of multiple turnovers (Figure 2). Catalysis of butyryloxypyrene-1,3,6-trisulfonate **10** hydrolysis was

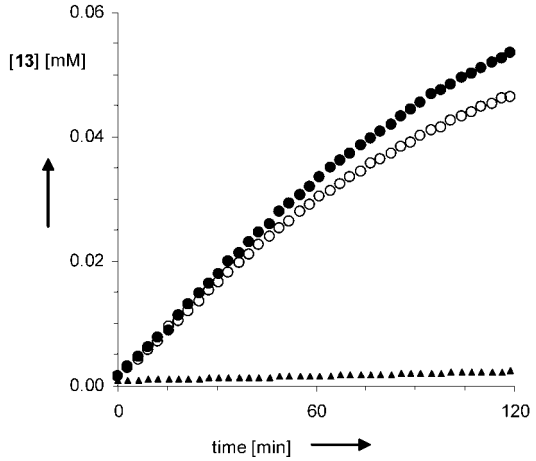


Figure 1. Time-course graph for the hydrolysis of **10** to form **13** in the absence (▲) or presence of dendrimers **iA-iC** (●), or **iA-C** (○).Conditions: 200 μM substrate **10**, 5 μM dendrimer, 26 °C, in 20 mM aqueous bis-Tris buffer pH 6.0. The reaction was followed using a SpectraMAX fluorescence plate reader ($\lambda_{exc} = 460$ nm, $\lambda_{em} = 530$ nm). Fluorescence was converted to product concentration using a calibration curve, which was linear in the concentration range used.

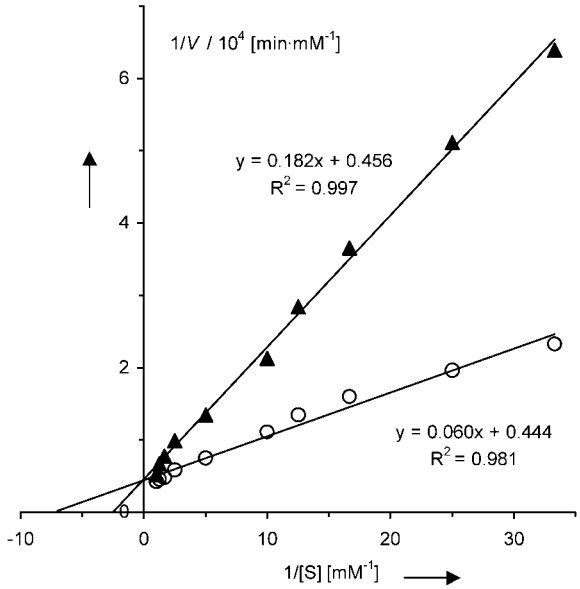


Figure 2. Double reciprocal plot for hydrolysis of ester **10** catalyzed by peptide dendrimers **iC-iC** (HHSD-HHSD) (○) and **iA-iC** (DDHS-HHDS) (▲).

also observed with the diastereoisomeric pair of heterodimers **iAC** ($V_{net}/V_{uncat} = 8$) and **iAiC** ($V_{net}/V_{uncat} = 9$), as well as with the monomeric dendrimers **C** ($V_{net}/V_{uncat} = 11$). Similar results were obtained with esters **9** and **11** as substrates. By contrast, there was no observable catalysis with the dodecanoyloxypyrene derivative **12**.

The kinetics of catalysis were characterized in detail for the five most reactive dendrimers (Table 2, Fig-

Table 2. Michaelis–Menten parameters for the most active dendrimers on pyrene substrates **9, 10** and **11**.

		9	**10**	**11**
4-MeIm	k_2 $(mM^{-1}\ min^{-1})$	1.1×10^{-3}	8.1×10^{-4}	8.2×10^{-4}
	k_{uncat} (min^{-1})	8.5×10^{-5}	4.4×10^{-5}	4.9×10^{-5}
C	K_M (mM)	0.18	0.19	0.046
(HHSD)	k_{cat} (min^{-1})	0.054	0.046	0.019
	k_{cat}/k_{uncat}	640	1100	400
	$k_{cat}/K_M/k_2$	280	300	500
	v_{net}/v_{uncat}	5.5	11	5.6
C-C	K_M (mM)	0.11	0.12	0.017
(HHSD-HHSD)	k_{cat} (min^{-1})	0.044	0.045	0.022
	k_{cat}/k_{uncat}	520	1000	450
	$k_{cat}/K_M/k_2$	380	470	1600
	v_{net}/v_{uncat}	7.6	16	11
iC-iC	K_M (mM)	0.11	0.20	0.10
(HHDS-HHSD)	k_{cat} (min^{-1})	0.031	0.037	0.033
	k_{cat}/k_{uncat}	370	840	680
	$k_{cat}/K_M/k_2$	260	230	390
	v_{net}/v_{uncat}	4.2	10.0	4.4
iA-C	K_M (mM)		0.37	
(SSDH-HHSD)	k_{cat} (min^{-1})		0.050	
	k_{cat}/k_{uncat}		1200	
	$k_{cat}/K_M/k_2$		180	
	v_{net}/v_{uncat}		8.0	
iA-iC	K_M (mM)		0.40	
(SSDH-HHSD)	k_{cat} (min^{-1})		0.050	
	k_{cat}/k_{uncat}		1100	
	$k_{cat}/K_M/k_2$		160	
	v_{net}/v_{uncat}		9.0	

Conditions and measurement method: 20 mM aqueous bis-Tris pH 6.0, 5 μM dendrimer and 50–800 μM substrate at 25 °C. The kinetic constants given are derived from the linear double-reciprocal plots of $1/v_{net}$ *vs.* 1/S, with $r^2 > 0.96$. The apparent rate enhancement V_{net}/V_{uncat} observed with S = 200 μM and 5 μM dendrimer. V_{uncat} is the hydrolysis rate of **9–11** in buffer alone, and $V_{net} = V_{app} - V_{uncat}$, where V_{app} is the apparent hydrolysis rate of **9–11** in the presence of dendrimer.

ure 2). Catalysis followed the Michaelis–Menten model, with substrate binding in the range of $K_M = 10^{-4}$ M and rate acceleration in the range of $k_{cat}/k_{uncat} = 10^3$, which is comparable to our previously reported catalytic peptide dendrimers for similar ester hydrolysis reactions. Catalysis was proportional to dendrimer concentration up to 50 μM dendrimer, suggesting that dendrimers do not aggregate in the buffer at pH 6. Butyryl ester **10** was the best substrate in terms of rate acceleration k_{cat}/k_{uncat}, with the best value being $k_{cat}/k_{uncat} = 1200$ in the case of dendrimer **iAC**. The catalytic rate constants k_{cat} and K_M were similar for acetate **9** and butyrate **10** in all cases, and the higher rate acceleration with the butyrate was due to the fact that the uncatalyzed reaction of butyrate **10** was only half that of acetate **9**.

In the case of dendrimers **C** and **CC**, the catalytic rate constant k_{cat} decreased two-fold between the butyrate ester **10** and the nonanoyl ester **11**, resulting in a correspondingly lower rate acceleration k_{cat}/k_{uncat} since the uncatalyzed reaction of the nonanoate **11** was similar to that of the butyrate **10**. Interestingly, the K_M values with these dendrimers also dropped significantly between butyrate **10** and nonanoate **11**. The longer aliphatic acid chain in this substrate induced a 4-fold lowering of K_M with **C** and a 7-fold lowering of K_M with **CC**. By contrast, the chain lengthening had no effect on either k_{cat} of K_M with the diastereoisomeric dendrimer **iCiC**, which showed comparable kinetic parameter with all three substrates tested. The dependence of K_M on the aliphatic chain length in the case of **C** and **CC** suggests that these dendrimers achieve substrate binding partly through hydrophobic interactions with the aliphatic chain, most likely *via* the hydrophobic core residues Phe and Leu. The effect, however, must depend on precise molecular interactions since it is completely absent in the diastereoisomeric dendrimer **iCiC**, whose monomeric form **iC** is also less active than its stereoisomer **C**.

Catalytic activity depends on the presence of two surface histidine residues. The fact that several dendrimers are not active at all is evidence that the core histidine res-

idue at position A^1 is not capable of inducing catalysis alone. The pair of surface histidine residues at position A^3 probably catalyzes the reaction by nucleophilic catalysis. Pyrene-1,3,6-trisulfonate esters **9–11** have been reported to be hydrolyzed in aqueous conditions by self-assembled peptide-based pores functionalized with multiple histidine residues.[19] The hydrolysis of these esters is also catalyzed by 4-methylimidazole in aqueous buffer at pH 6.0. The catalytic peptide dendrimers are between 260-fold and 1600-fold more reactive that 4-methylimidazole as measured by the ratio of the specificity constant k_{cat}/K_M to the second order rate constant for 4-methylimidazole catalysis k_2. This rate acceleration corresponds to between 60-fold and 400-fold rate acceleration per histidine residue if one assumes that each surface histidine residue acts independently.

Part of the reactivity enhancement observed for histidine side-chains within the dendrimers over the reaction of 4-methylimidazole in solution is explained by substrate binding, which is also taken into account for the calculation of the specificity constant k_{cat}/K_M. Since the imidazole side chains are partly protonated under the conditions of the assays, the histidine residues should participate in recognition of the anionic pyrene-1,3,6-trisulfonate group. A requirement for electrostatic binding can also explain the lack of catalysis with neutral and cationic fluorogenic substrates despite the fact that these are more reactive than the pyrene-trisulfonate esters. Further evidence for an electrostatic component in substrate binding was gained by investigating inhibition of catalysis by 1,3,6,8-pyrene-tetrasulfonate **14** (Scheme 4, Figure 3). This tetra-anionic ligand competitively inhibited dendrimer catalysis with $K_i = 60$ μM for **C**, $K_i = 20$ μM for **CC**. This competitive inhibition is best interpreted in terms of electrostatic interactions between the protonated histidine side chains and the sulfonate groups. Inhibition is, however, too weak to reflect transition state analogue type inhibition since the transition state dissociation constants for dendrimer catalysis lie in the range of $K_{TS} = K_M/(k_{cat}/k_{uncat}) = 0.4 - 0.04$ μM, which corresponds to a 100-fold to a 1000-fold stronger binding of the transition state compared to tetrasulfonate **14**.

On the basis of the experiments above one can propose a catalytic mechanism for the most active dendrimer **CC** (Figure 4). Substrate binding is mediated by electrostatic interactions between the sulfonate groups and the protonated histidine side chains of the dendrimer, and further increased by a hydrophobic interaction between the aliphatic chain of the acyl group and the hydrophobic core residues Phe and Leu in the case of nonanoate **11**. Hydrolysis proceeds by acyl group transfer to one of the histidine side chains and hydrolysis to regenerate the catalyst. The 8-hydroxypyrene-1,3,6-trisulfonate leaving group **13** diffuses away, and could also show a weak product inhibition analogous to that observed with the tetrasulfonate **14**.

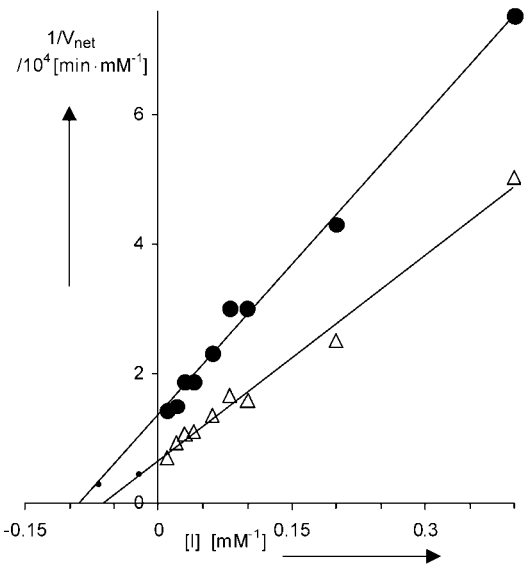


Figure 3. Dixon plot for competitive inhibition of dendrimer-catalyzed hydrolysis of acetate ester **9** by 1,3,6,8-pyrene-tetrasulfonate **14**. Measured in 20 mM bis-Tris pH 6.0, 0.2 mM **9**, and dendrimer **C** (●), and dendrimer **CC** (Δ). The competitive inhibition constant is the x-coordinate of the intercept between the fitted lines and the horizontal lines drawn at $y = 1/V_{max}$.

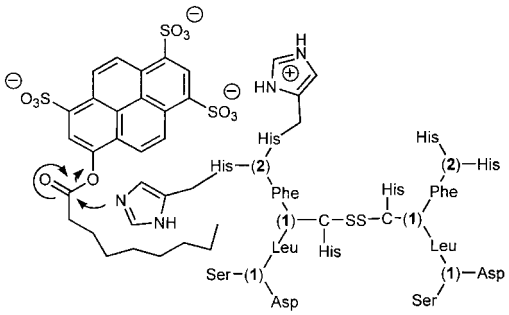


Figure 4. Hydrolysis of 8-nonanoyloxypyrene-1,3,6-trisulfonate **10** by catalytic peptide dendrimer **CC** involves electrostatic and hydrophobic substrate binding and nucleophilic catalysis by histidine side-chains.

Conclusions

The orthogonally protected diamino acid branching unit **1** was used to assemble peptide dendrimers with a hydrophobic core and differentiated surface catalytic residues. The preparation of a small 27-member library allowed us to observe esterolytic activity for the hydrolysis of 8-acyloxypyrene-1,3,6-trisulfonate esters. Substrate recognition can be understood in terms of electrostatic interactions between protonated histidines and the negatively charged sulfonate groups. In addition, the most active dendrimer **CC** showed enhanced substrate binding with long chain aliphatic acids, which may be explained

by hydrophobic interactions with the dendritic core. The catalysis itself was triggered by the presence of at least two surface histidine residues. Using a hydrophobic core for binding together with surface catalytic residues might provide a general strategy for the design of catalytic peptide dendrimers.

The efficiency of the dendrimer synthesis is noteworthy. The synthetic yields of the purified dendrimers are similar to those for linear peptides of similar length. One of the key advantages of peptide dendrimers as enzyme models is their synthetic availability including a potential for structural variations through variations of the amino acid building blocks. The synthetic strategy exposed here allows six individually variable positions and defines 64 million monomers and $2 \cdot 10^{15}$ dimers using the twenty proteinogenic amino acids. A combinatorial protocol should allow us to explore a much broader structural diversity of peptide dendrimers and to test their potential for selective catalysis.[9d]

Experimental Section

All reagents were either purchased from Aldrich or Fluka Chemica (Switzerland). Amino acids and their derivatives were purchased from Senn Chemicals or Novabiochem (Switzerland). Rink amide resin was purchased from Novabiochem (Switzerland). All solvents used were analytical grade. Peptide syntheses were performed manually in a glass reactor. Analytical RP-HPLC was performed in a Waters (996 photo diode array detector) chromatography system using a chromolith performance RP-18e, 4.6×100 mm, flow rate 3 mL min^{-1} column. Compounds were detected by UV absorption at 214 and 234 nm. Preparative RP-HPLC was performed with HPLC-grade acetonitrile and MilliQ deionized water in a Waters prepak cartridge 500 g (RP-C18 20 mm, 300 Å pore size) installed on a Waters Prep LC4000 system from Millipore (flow rate 100 mL min^{-1}, gradient 1% min^{-1} CH_3CN). Semipreparative RP-HPLC (flow rate, 4 mL/min; eluent A, water and 0.1% TFA; eluent B, acetonitrile, water and TFA, 3/2/0.1%); column, Vydac 218 TP ($1.0\,cm \times 25\,cm$, 300 Å pore size). Compounds were detected by UV absorption at 220 nm. Chromatography (flash) performed with Merck silica gel 60 (0.040 ± 0.063 mm), TLC with fluorescent F254 glass plates. Fluorescence measurements were carried out with a spectraMAX fluorescence detector. NMR spectra recorded on Bruker AC-300 (^1H, 300 MHz; ^{13}C, 75 MHz, δ in ppm), infrared spectroscopy with a Perkin-Elmer 1600 series FTIR [frequencies (ν) given in cm^{-1}]. Optical rotations 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. MS was provided by Dr. Thomas Schneeberger (University of Bern, Switzerland).

(2*R*)-*N*-*tert*-Butoxycarbonylaminopropane-1,2-diol (4)

Di-*tert*-butyl dicarbonate (5.75 g, 26.34 mmol) in CH_2Cl_2 (7 ml), was added slowly to a stirred solution of (*R*)-3-aminopropane-1,2-diol (**3**; 2 g, 21.95 mmol) in CH_2Cl_2-MeOH (1:5, 20 mL) and triethylamine (0.36 mL, 2.6 mmol). The reaction, which was followed by TLC, was complete in 2 h. After solvent evaporation, the oily residue was chromatographed (hexane/ethyl acetate 2/8) to give **4** as a white solid; yield: 3.97 g (95%); R_f (ethyl acetate): 0.5; mp 49 °C; IR (neat): $\nu = 3019$ *w*, 1653 *w*, 1393 *w*, 1369 *w*, 1215 *m*, 1045 cm^{-1} *w*; ^1H NMR ($CDCl_3$): $\delta = 4.92$ (s, 1H), 3.75 (m,1H), 3.59 (d, $J = 5.15$ Hz, 2H), 3.28 (broad s, 2H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta =$ 157.27, 80.07, 71.29, 63.68, 42.93, 28.37; HR-ESI-MS: calcd. for $C_8H_{17}NO_4$: 191.1158; found: 191.1055; $[\alpha]_D^{25}$: −10.0 (*c* 6.15 g mL^{-1} in CH_2Cl_2).

(2*S*)-*N*-*tert*-Butoxycarbonyl-1,2-epoxyethylaminopropane (5)

Methanesulfonyl chloride (46 μL, 0.577 mmol) was added to a stirred solution of **4** (100 mg; 0.525 mmol) in pyridine (0.645 ml) at 0 °C. After 10 min, the resulting solution was added over 5 min to a stirred solution of sodium hydroxide (64 mg; 1.6 mmol) in water (0.65 ml) plus dimethyl sulfoxide (0.43 mL) at 0 °C. After an additional 10 min of stirring, the solution was poured into ice-water and the mixture extracted with ether. The ether phase was washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was chromatographed (hexane/ethyl acetate 8/2) and the product **5** was obtained as a white solid; yield: 67.2 mg (74%); R_f (ethyl acetate/hexane 4/6): 0.65; mp 76–77 °C; IR (neat): $\nu = 3019$ *m*, 2981 *w*, 2931 *w*, 1713 *m*, 1698 *m*, 1511 *m*, 1505 *m*, 1393 *w*, 1369 *w*, 1215 cm^{-1} *s*; ^1H NMR ($CDCl_3$): $\delta = 4.81$ (s, 1H, NH), 3.50 (m, 1H), 3.15–3.25 (2dd, $J = 6.25$, 5.15, 6.2, 5.1 Hz, 1H), 3.08 (m, 1H), 2.77 (dd, $J = 4.2$, 4.2 Hz, 1H), 2.58 (dd, $J = 4.8$, 2.6 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 155.92$, 79.58, 50.83, 45.00, 41.62, 28.30; HR-ESI-MS: calcd. for $C_8H_{15}NO_3$: 173.1052; found: 173.0949; $[\alpha]_D^{25}$: −2.0 (*c* 7.05 g ml^{-1} in CH_2Cl_2). Chiral GC analysis of epoxide **5**[20] using a 25 m CST Restek, showed an enantiomeric ratio of 90 (*S*) to 10 (*R*) (80% ee).

(2*S*)-*N*-*tert*-Butoxycarbonyl-2-hydroxy-1,3-diaminopropane (6)

The epoxide **5** was mixed with a solution of 25% aqueous ammonia (5.2 mL) and ethanol (2.1 mL) at room temperature. The mixture was stirred for 90 min and the solvent evaporated. The product **6** was obtained quantitatively as a white solid; mp 45 °C; IR (neat): $\nu = 3022\,s, 2981\,w, 2931\,w, 1712\,m, 1698\,m, 1511\,m$, 1505 *m*, 1393 *w*, 1369 *w*, 1227 *m*, 1205 cm^{-1} *m*; ^1H NMR ($CDCl_3$): $\delta = 5.28$ (s, 1H, NH), 3.61 (m, 1H), 3.22 (m, 1H), 3.07 (m, 1H), 2.79 (NH_2), 2.75 (m, 1H), 2.61 (m, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 156.52$, 79.29, 70.61, 44.27, 43.91, 28.19; HR-ESI-MS: calcd. for $C_8H_{18}N_2O_3Na$: 213.1215; found: 213.1231; $[\alpha]_D^{25}$: +10.0 (c 7.75 g mL^{-1} in CH_2Cl_2).

(2*S*)-3-*N*-Allyloxycarbonyl-*N*-tert-butoxycarbonyl-2-hydroxy-1,3-diaminopropane (7)

To a stirred solution of **6** (1.9 g; 10 mmol) in ethyl acetate (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) was added allyl chloroformate (1.70 mL, 16 mmol). The mixture was stirred during 4 h, then washed with water, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and the solvents evaporat-

ed. The residue was chromatographed (hexane/ethyl acetate 6/4) to give product **7** as a colorless oil; yield: 2.37 g (87%); R_f (ethyl acetate): 0.73; IR (neat): $\nu = 3018$ *s*, 2980 *w*, 2935 *w*, 1720 *m*, 1716 *m*, 1698 *m*, 1524 *m*, 1515 *m*, 1393 *w*, 1368 *w*, 1215 *m*, 1117 cm^{-1} *w*; ^1H NMR ($CDCl_3$): $\delta = 5.93$ (m, 1H), 5.57 (broad s, 1H, NH), 5.30 (dd, $J = 1.5$, 17.1 Hz, 1H), 5.20 (overlap. dd and s, 2H), 4.56 (d, $J = 5.5$ Hz, 2H), 3.77 (m, 1H), 3.23 (m, 4H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 157.07$, 132.50, 117.59, 79.71, 70.43, 65.62, 43.60, 43.29, 28.14; HR-ESI-MS: calcd. for $C_{12}H_{22}N_2O_5$: 274.1529; found: 274.1606; $[\alpha]_D^{25}$: -3.0 (χ 5.7 g mL^{-1} in CH_2Cl_2).

(2*S*)-3-*N*-Allyloxycarbonyl-*N*-tert-butoxycarbonyl-2-(ethoxycarbonylmethoxy)-1,3-diaminopropane (8)

Ethyl bromoacetate (2.85 mL, 25.8 mmol) was added to a stirred solution of **7** (2.35 g, 8.6 mmol) in dry THF (2 mL) at room temperature. Then sodium hydride (1.14 g, 4.5 equivs.) was added during 1 h. After an additional 5 h the mixture was filtered on Celite and washed with THF and the solvent evaporated. The residue was chromatographed (hexane/ethyl acetate, 8/2) and the product **8** was obtained as a colorless oil; yield: 2.04 g (66%); R_f (ethyl acetate/hexane 1/1): 0.74; IR (neat): $\nu = 2984$ *w*, 2935 *w*, 1738 *m*, 1705 *m*, 1698 *m*, 1510 *m*, 1515 *m*, 1393 *w*, 1368 *w*, 1215 *m*, 1134 cm^{-1} *m*; ^1H NMR ($CDCl_3$): $\delta = 5.86$–5.82 (m, 1H plus 1H, NH), 5.35 (1H, NH), 5.25 (d, $J = 1.5$, 17.1 Hz, 1H), 5.15 (dd, $J = 1.1$, 10.3 Hz, 1H), 4.52 (d, $J = 4.5$ Hz, 2H), 4.14 (m, 4H), 3.49–3.44 (m, 1H), 3.35–3.31 (m, 2H), 3.25–3.08 (m, 2H), 1.39 (s, 9H), 1.24 (t, 3H); ^{13}C NMR ($CDCl_3$): $\delta = 171.22$, 156.72, 156.41, 132.90, 117.48, 79.14, 78.78, 67.02, 65.54, 61.17, 41.05, 40.55, 28.31, 14.09; HR-LSI-MS: calcd. for $C_{16}H_{28}N_2O_7 + H^+$: 361.197477; found: 361.197450; $[\alpha]_D^{25}$: -5.0 (*c* 8.4 g mL^{-1} in CH_2Cl_2).

(3*S*)-4-(9-Fluorenylmethoxycarbonylamino)-3-methyl(allyloxycarbonyl)aminoethoxyacetic Acid (1)

To a stirred solution of **8** (262 mg, 0.72 mmol) in THF (2 mL) was added a solution of LiOH (53 mg, 2.19 mmol) in water (2 mL). The mixture was stirred overnight. The THF was evaporated and residue lyophilized. Then 2.5 mL of 3 N HCl were added and the mixture was stirred at 60 °C during 3 h. The solution pH was adjusted to 5 with NaOH pellets and then to pH 8 with saturated aqueous $NaHCO_3$ (2.5 mL). A solution of 9-fluorenylmethyl chloroformate (Fmoc-Cl, 205 mg, 0.79 mmol) in dioxane (4.5 mL) was added at 0 °C and the mixture was stirred overnight at room temperature, diluted with water, and the aqueous phase was acidified to pH 2. After an extraction with ethyl acetate, the combined organic phases were concentrated and the residue chromatographed (hexane/ethyl acetate/acetic acid 3.8/6/0.2) to give **1** as white solid; yield: 198 mg (60%); R_f ($CHCl_3/CH_3OH$/acetic acid 180/10/5): 0.56; mp 98 °C; IR (neat): $\nu = 3019$ *w*, 1734 *m*, 1718 *m*, 1700 *m*, 1517 *m*, 1477 *m*, 1215 *s*, 1135 cm^{-1} *w*; ^1H NMR ($CDCl_3$): $\delta = 7.77$ (d, $J = 7.3$ Hz, 2H), 7.59 (d, $J = 7.32$ Hz, 2H), 7.37 (dd, $J = 8.4$, 14.3 Hz, 2H), 7.27 (dd, $J = 8.4$, 14.25 Hz, 2H), 5.91–5.82 (m, 1H), 5.74 (1H, NH), 5.30–5.21 (2 overlapping dd, 2H), 4.57 (d, $J = 5.8$ Hz, 2H), 4.39 (d, $J = 7.0$ Hz, 2H), 4.22 (m, 3H), 3.54 (m, 1H), 3.36–3.26 (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 177.01$, 157.26, 157.15, 143.78, 141.28, 132.62, 127.72, 127.07, 125.09, 119,99, 117.86, 78.65, 67.01, 66.83, 65.91, 47.16, 41.10, 40.97; HR-LSI-MS: calcd. for $C_{24}H_{26}N_2O_7 + H^+$: 455.181827; found: 455.181090; $[\alpha]_D^{25}$: 0.0 (c 9.1 g mL^{-1} in CH_2Cl_2).

Synthesis of the Dendrimers by SPPS

Coupling of the Fmoc-protected amino acids: The resin was washed and swelled inside the reactor with DCM (dichloromethane) (2×5 mL) and DMF (1×5 mL). The Rink amide resin (0.61 mmol/g) was acylated with 2.5 equivalents of *N*-Fmoc amino acid in the presence of 2.5 equivalents of BOP [benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate] and 6 equivalents of DIEA (*N*,*N*′-diisopropylethylamine) in DMF. After 30 min the resin was washed ($3 \times$ each) with DMF, DCM and MeOH and controlled with the TNBS (trinitrobenzenesulfonic acid) test.

Cleavage of the Fmoc protecting group: The Fmoc protecting group was removed with 5 mL of a solution of piperidine in DMF (1:4) for 10 min. After filtration, the procedure was repeated and then washed ($3 \times$ each) with DMF, DCM and MeOH.

Cleavage of the Alloc protecting group: The resin was treated with $PhSiH_3$ (25 equivs.) in 5 mL of anhydrous DCM for 3 minutes under argon. After addition of $Pd(PPh_3)_4$ (0.2 equivs.) the mixture was stirred for 20 min. The resin was washed with DCM (10 mL), dioxane : H_2O (9:1, 10 mL), DMF (10 mL) and DCM (2×10 mL). The procedure was repeated.

Capping of the N-terminus: At the end of the synthesis, the resin was acetylated with a solution of acetic anhydride in DCM (1:1) for 30 min. The resin was dried under vacuum and stored at -20 °C.

TFA cleavage: The cleavage was carried out using TFA (trifluoroacetic acid)/EDT (1,2-ethanedithiol)/H_2O/TIS (triisopropylsilane) as a 94/2/2/2 solution for 3 h. The peptide was precipitated with methyl *tert*-butyl ether then dissolved in a water/acetonitrile mixture. All the dendrimers were purified by preparative HPLC.

General procedure for homodimerization: The monomeric dendrimer X (1 mg) was dissolved in water (25 μL) and a methanolic solution of Aldrithiol was added (45 mM, 0.45 equivs.). The pH of the solution was adjusted to 8 with an $(NH_4)HCO_3$ buffer solution (20 mM). The solution was stirred for 30 minutes then acidified with one drop of TFA. The homodimer XX was purified by semi-preparative RP-HPLC.

General procedure for heterodimerization: To a solution of dendrimer X (1 mg/25 μL) in water was added a methanolic solution of Aldrithiol (45.4 mM, 5 equivs.). The thiol-activation was followed by HPLC and, after evaporation of methanol, the Aldrithiol excess was removed by extraction ($3 \times$) with DCM. To this activated dendrimer solution was added a solution of dendrimer Y (1 mg/25 μL, 1 equiv.) in water and the pH of the solution adjusted to 8 with an $(NH_4)HCO_3$ buffer solution (20 mM). The solution was stirred during 30 minutes then acidified with one drop of TFA. The heterodimer XY was purified by semi-preparative RP-HPLC.

Yields, purification method and characterization of all dendrimers are described in the Supporting Information.

Kinetic Measurements

The kinetic measurements were carried out by using a SPECTRAMax fluorescence detector with preset values of the excitation and emission wavelengths corresponding to the measured pyrene substrate ($\lambda_{ex} = 460$ nm, $\lambda_{em} = 530$ nm) at 25.2 °C. Assays were followed in individual wells of round-bottom polystyrene 96-well plates (Costar). Kinetic experiments were followed for 2 h. The dendrimers were stored at −20 °C in 1 mM stock solution in B (acetonitrile/water: 1/1). Dendrimer stock solutions were freshly diluted to 0.05 mM solution in 20 mM aqueous bis-Tris pH 6.0. The bis-Tris buffer, pH 6.0 was prepared using MilliQ deionized water. Fluorescence data were converted to product concentration by means of a calibration curve. Initial reaction rates were calculated from the steepest part observed during the first 2000 sec of each curve. In a typical experiment, 20 μL of aqueous bis-Tris pH 6.0 (20 mM) were first added in a well, then 2.5 μL of a dendrimer solution (0.05 mM in aqueous bis-Tris pH 6.0, final concentration in the well: 5 μM), and last 2.5 μL of substrate solution (2 mM in B, 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 22.5 μL aqueous bis-Tris pH 6.0 (20 mM) and 2.5 μL of substrate solution (2 mM in B, 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 $1/V_{net}$ *vs.* 1/[S] measured similarly with (final concentrations) 5 μM dendrimer (V_{app}) or no dendrimer (V_{uncat}) and 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800 μM substrate, 20 mM bis-Tris, 25 °C. Kinetic parameters such as maximum velocity (V_{max}) and Michaelis constant (K_M) were determined by the least squares method from a Lineweaver–Burk plot between $1/v_0$ (reciprocal value of initial velocity) and 1/[S]. The reaction activity reflected in k_{cat} for the hydrolysis (deacylation of substrates) was evaluated by $k_{cat} = V_{max}/[D]_0$, where $[D]_0$ indicates the initial concentration of dendrimers. The reaction rate with 4-methylimidazole (4-MeIm) was obtained under the same conditions with 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800 μM 4-MeIm and 200 μM substrate. The second order rate constants k_2 were calculated from linear regression of the experimentally measured pseudo-first order rate constants k′ as a function of *N*-methylimidazole concentrations [M] where [S] indicates the initial substrate concentration: $V_{net} = k'[S]$, $k' = k_2[M]$. The inhibition constant K_I was obtained from the linear reciprocal plot of $1/V_{net}$ *vs.* [I] measured similarly with (final concentrations) 5 μM dendrimer and 200 μM of substrate (V_{app}) and 0, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000 μM inhibitor, 20 mM bis-Tris, 25 °C. K_i was calculated from a Dixon plot of inhibition data and gave reproducible values with ±10% error.

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References and Notes

[1] a) L. Stryer, *Biochemistry*, New York, WH Freeman and Company, **1999**; b) A. Fersht, *Structure and Mechanism in Protein Science*, New York, WH Freeman and Company, **1999**.

[2] A. J. Kirby, *Angew. Chem.* **1996**, *108,* 770–790; *Angew. Chem. Int. Ed.* **1996**, *35*, 707–724.

[3] a) A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **1986**, *234*, 1566–1570; b) S. J. Pollack, J. W. Jacobs, P. G. Schultz, *Science* **1986**, *234*, 1570–1573; c) R. A. Lerner, S. J. Benkovic, P. G. Schultz, *Science* **1991**, *252*, 659–667; d) P. G. Schultz, R. A. Lerner, *Acc. Chem. Res.* **1993**, *26*, 391–395; e) P. G. Schultz, R. A. Lerner, *Science* **1995**, *269*, 1835–1842; f) G. MacBeath, D. Hilvert, *Chem. Biol.* **1996**, *3*, 433–445; g) N. R. Thomas, *Nat. Prod. Rep.* **1996**, *13*, 479–511; h) J. Hasserodt, *Synlett* **1999**, *12*, 2007–2022; i) J.-L. Reymond, *Top. Curr. Chem.* **1999**, *200*, 59–93; j) J. D. Stevenson, N. R. Thomas, *Nat. Prod. Rep.* **2000**, *17*, 535–577; k) P. G. Schultz, J. Yin, R. A. Lerner, *Angew. Chem.* **2002**, *114*, 4607–4618; *Angew. Chem. Int. Ed.* **2002**, *41*, 4427–4437.

[4] a) S. Juliá, J. Masana, J. C. Vega, *Angew. Chem.* **1980**, *19*, 968–969; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 929–930; b) S. Juliá, J. Guixer, J. Masana, J. Rocas, S. Colonna, R. Annuziata, H. Molinari, *J. Chem. Soc. Perkin Trans. 1* **1982**, 1317–1324; c) P. A. Bentley, R. W. Flood, S. M. Roberts, J. Skidmore, C. B. Smith, J. A. Smith, *Chem. Commun.* **2001**, 1616- 1617.

[5] a) L. K. Baltzer, S. Broo, H. Nilsson, J. Nilsson, *Bioorg. Med. Chem.* **1999**, *7,* 83–91; b) J. Nilsson, L. Baltzer, *Chem. Eur. J.* **2000**, *6,* 2214–2220; c) S. Kerstin, H. Nilsson, J. Nilsson, L. Baltzer, *J. Am. Chem. Soc.* **1998**, *120*, 10287–10295.

[6] K. W. Hahn, W. A. Klis, J. M. Stewart, *Science* **1990**, *248*, 1544–1547; b) K. Johnsson, R. K. Allemann, H. Widmer, S. A. Benner, *Nature* **1993**, *365*, 530–532.

[7] a) P. Krattiger, C. McCarthy, A. Pfaltz, H. Wennemers, *Angew. Chem.* **2003**, *115*, 1763–1766; *Angew. Chem. Int. Ed.* **2003**, *42*, 1722–1724; b) H. Wennemers, M. C. Nold, M. M. Conza, K. J. Kulicke, M. Neuburger, *Chem. Eur. J.*, **2003**, *9*, 442–448.

[8] For example, a) E. R. Jarvo, S. J. Miller, *Tetrahedron* **2002**, *58*, 2481–2495; b) B. List, *Tetrahedron* **2002**, *58*, 5573–5590; c) G. C. Fu, *Acc. Chem. Res.* **2000**, *33,* 412–420; d) M. H. Fonseca, B. List, *Curr. Opin. Chem. Biol.* **2004**, *8*, 319–326.

[9] a) A. Esposito, E. Delort, D. Lagnoux, F. Djojo, J. L. Reymond, *Angew. Chem.* **2003**, *115*, 1419–1421; *Angew. Chem. Int. Ed.* **2003**, *42*, 1381–1383; b) D. Lagnoux, E. Delort, C. Douat-Casassus, A. Esposito, J. L. Reymond, *Chem. Eur. J.* **2004**, *10*, 1215–1226; c) C. Douat-Casassus, T. Darbre, J. L. Reymond, *J. Am. Chem. Soc.* **2004**, *126*, 7817–7826; d) A. Clouet, T. Darbre, J.-L. Reymond, *Angew. Chem. Int. Ed.* **2004**, in press.

[10] a) A. W. Bosman, H. M. Janssen, E. W. Meijer, *Chem. Rev.* **1999**, *99*, 1665–1688; b) D. K. Smith, *Tetrahedron* **2003**, *59,* 3797–3798; c) S. Hecht, J. M. J. Fréchet, *Angew. Chem.* **2001**, *113,* 76–94; *Angew. Chem. Int. Ed.* **2001**, *40,*

74–91; d) G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH, Weinheim, **1996**.

[11] a) D. A. Tomalia, P. R. Dvornic, *Nature* **1994**, *372*, 617–618; b) J. W. J. Knapen, A. W. Van der Made, J. C. De Wilde, P. W. N. M. van Leeuwen, P. Wijkens, D. M. Grove, G. van Koten, *Nature* **1994**, *372*, 659–662; c) L. Balogh, D. A. Tomalia, *J. Am. Chem. Soc.* **1998**, *120*, 7355–7356; d) M. Zhao, R. M. Crooks, *Angew. Chem.* **1999**, *111*, 375–377; *Angew. Chem. Int. Ed.* **1999**, *38*, 364–366; e) B. Bhyrappa, G. Vaijayanthimala, *J. Am. Chem. Soc.* **1999**, *121*, 262–263; f) S. Nlate, J. Ruiz, V. Sartor, R. Navarro, J. C. Blais, D. Astruc, *Chem. Eur. J.* **2000**, *6*, 2544–2553; g) P. Weyermann, F. Diederich, *Helv. Chim. Acta* **2002**, *85*, 599–617; h) H. Sellner, P. B. Rheiner, D. Seebach, *Helv. Chim. Acta* **2002**, *85*, 352–387; i) L. J. Twyman, A. S. H. King, I. K. Martin, *Chem. Soc. Rev.* **2002**, *31*, 69–82.

[12] a) K. Sivanandan, D. Vutukuri, S. Thayumanavan, *Org. Lett*. **2002**, *4*, 3751–3753; b) W. Zhang, D. T. Nowlan, III, L. M. Thomson, W. M. Lackowski, E. E. Simaneck, *J. Am. Chem. Soc.* **2001**, *123*, 8914–8922 and references therein; c) S. M. Grayson, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2000**, *122*, 10335–10344.

[13] a) N. Thieriet, J. Alsina, E. Giralt, F. Guibé, F. Albericio, *Tetrahedron Lett*. **1997**, *38*, 7275–7278; b) E. J. Corey, J. W. Suggs, *J. Org. Chem*. **1973**, *38*, 3223–3224.

[14] J. C. Martin, D. F. Smee, J. P. H. Verheyden, *J. Org. Chem*. **1985**, *50*, 755–759.

[15] a) P. Lloyd-Williams, F. Albericio, E. Giralt, *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton, New York, **1997**; b) C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis: a practical approach*, Oxford Press, New York, **2000**.

[16] L. A. Carpino, A. El-Faham, *J. Am. Chem. Soc*. **1995**, *117*, 5401–5402.

[17] a) K. Akaji, K. Fujino, T. Tatsumi, Y. Kiso, *Tetrahedron Lett.* **1992**, *33*, 1073–1076; b) M. Ruiz-Gayo, M. Royo, I. Fernandez, F. Albericio, E. Giralt, M. Pons, *J. Org. Chem.* **1993**, *58*, 6319–6328.

[18] a) N. Bensel, M. T. Reymond, J.-L. Reymond, *Chem. Eur. J.*, **2001**, *7*, 4604; b) E. Leroy, N. Bensel, J.-L. Reymond, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2105–2108.

[19] a) N. Sakai, N. Sordé, S. Matile, *J. Am. Chem. Soc.* **2003**, *125*, 7776–7777; b) B. Baumeister, N. Sakai, S. Matile, *Org. Lett*. **2001**, *3*, 4229–4232.

[20] Obtained previously by kinetic resolution catalyzed by chiral (salen)Co^{III} complexes: S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunaga, K. B. Hansen, A. E. Gould, M. E. Furrow, E. N. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.

