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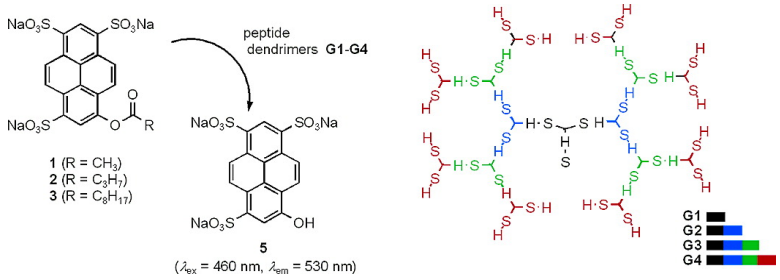

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A Strong Positive Dendritic Effect in a Peptide Dendrimer-Catalyzed Ester Hydrolysis Reaction

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Dendrimers are treelike molecules with various uses in chemistry and biology such as catalysis, drug delivery, artificial vaccines, and gene delivery into cells.[1] Most applications in catalysis consist of attaching a catalytic module to the dendrimer surface to afford an easily separable macromolecular catalyst.[2] This multiple display at the dendrimer surface, however, can induce a decrease in catalytic efficiency per catalyst unit due to steric crowding.[3] A strong positive dendritic effect was observed in the oxidation of bromide by H_2O_2 catalyzed by dendritic phenylselenides.[4] Herein we report the first strong positive dendritic effect for a peptide dendrimer-catalyzed ester hydrolysis reaction and show that the effect originates from cooperativity between substrate binding and catalysis.

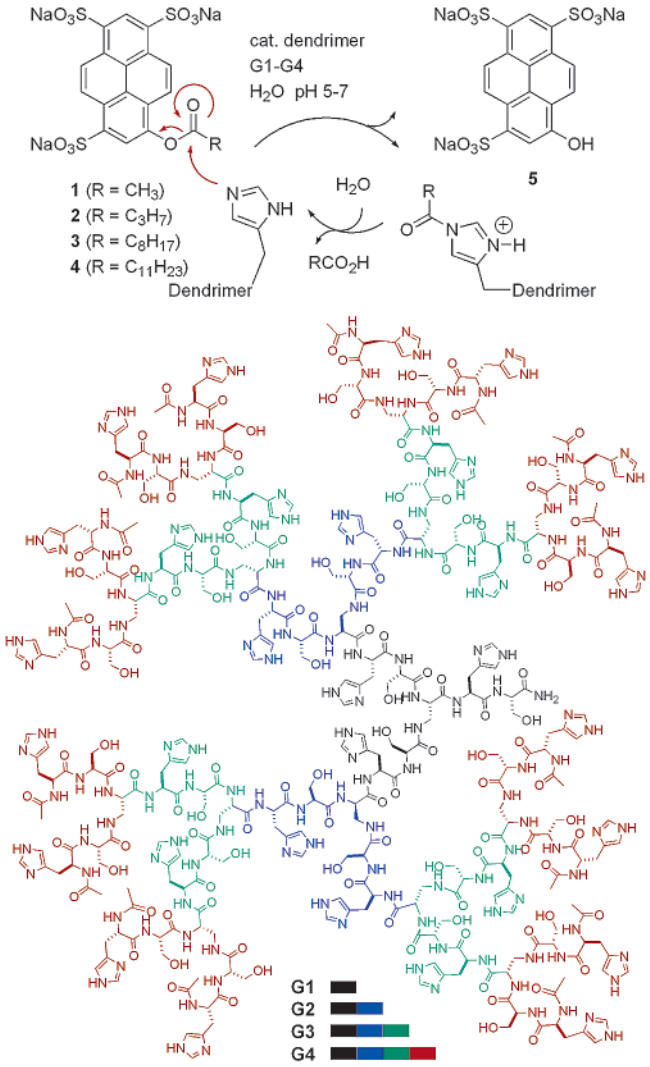
During our investigation of peptide dendrimers as enzyme models, we reported that peptide dendrimers displaying multiple histidine residues at their surface catalyze ester hydrolysis reactions.[5] In particular, dendrimers with surface His-Ser catalyzed the hydrolysis of pyrene trisulfonate esters **1**−**3**.[6] Hydrolysis seems to occur by nucleophilic catalysis by the histidine side-chain without participation of serine.[6a] To determine the contribution of the dendrimer structure in catalysis, we have investigated a systematic peptide dendrimer series of increasing generation number containing a catalytic consensus sequence His-Ser in all branches (Scheme 1).

Synthesis was carried out by Fmoc strategy on NovaSyn Tentagel resin (0.25 mmol/g), affording the peptide dendrimers in good yields (**G1** 15%, **G2** 44%, **G3** 32%, **G4** 4.6%). Hydrolysis of pyrene trisulfonate esters **1**−**4** (0.01−1.0 mM) catalyzed by dendrimers **G1**−**G4** (**G1** 10.6 μM, **G2** 6.4 μM, **G3** 3.5 μM, **G4** 1.9 μM) or 4-methyl-imidazole (0.04−1 mM) was followed by fluorescence (λ_{exc} = 460 nm, λ_{em} = 530 nm) in a 96 well microtiter-plate setup. All dendrimers were catalytically active with multiple turnover and displayed enzymelike Michaelis−Menten kinetics. The pH rate profile with substrate **2** indicated an optimal activity in the range pH 5−6 (Figure 1).

Kinetic parameters showed that catalysis increased with increasing generation number (Table 1). The catalytic rate constants k_{cat} and the substrate binding constants $1/K_M$ with substrates **1**−**3** were directly proportional to the total number of histidines per dendrimer, resulting in a quadratic increase in catalytic efficiency k_{cat}/K_M as a function of dendrimer size (Table 1, Figure 2). In the best case of dendrimer **G4** and nonanoyl ester **3**, the dendrimer was 140 000-fold more efficient than 4-methyl-imidazole as a reference catalyst, amounting to a 4500-fold acceleration per histidine side-chain. The positive dendritic effect in catalysis can be measured by the increase in histidine reactivity $(k_{cat}/K_M)/k_2$/His between **G1** and **G4**, which amounts to 24-fold for substrate **1**, 25-fold for **2**, and 100-fold for **3**.

Binding of the dendrimers to the slow reacting substrate **4**[7] or product **5** was investigated by isothermal titration calorimetry (ITC) (Table 2). Binding was strongly exothermic in both cases. Substrate **4** binding was 15−300 times stronger than product binding and increased in higher generation number with an overall 50-fold

Scheme 1. Peptide Dendrimer **G1**−**G4**-Catalyzed Ester Hydrolysis



increase from **G1** to **G4**, following the trend in K_M observed with the reactive substrates **1**−**3** (Table 1). By contrast, product **5** binding remained constant across the series **G1**−**G4**. Therefore, the positive dendritic effect on substrate binding $1/K_M$ is best interpreted in terms of hydrophobic interactions between the substrate's acyl chains and the dendrimers.

The experiments above show that the esterolytic activity of peptide dendrimers involves a strong positive dendritic effect resulting from cooperative binding and catalysis (Figure 2). Catalytic efficiency increases with generation despite steric crowding of catalytic groups at the periphery, which must occur in higher generation dendrimers. The effect of increasing dendrimer size

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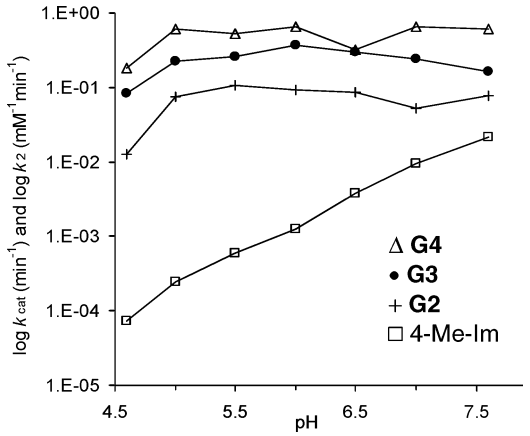


Figure 1. pH rate profile for substrate **2** (4-Me-Im = 4-methyl-imidazole).

Table 1. Michaelis−Menten Parameters for Dendrimers **G1**−**G4**[a]

		G1	G2	G3	G4
1	K_M (μM)	840	110	41	35
	k_{cat} (min^{-1})	0.080	0.16	0.30	0.86
	k_{cat}/k_{uncat}[b]	1800	3600	6800	20 000
	$(k_{cat}/K_M)/k_2$[c]	140	2000	10 500	35 000
	$(k_{cat}/K_M)/k_2$/His	46	280	700	1100
2	K_M (μM)	450	140	63	29
	k_{cat} (min^{-1})	0.031	0.11	0.24	0.55
	k_{cat}/k_{uncat}[b]	2200	8000	17 000	39 000
	$(k_{cat}/K_M)/k_2$[c]	140	1600	7900	38 000
	$(k_{cat}/K_M)/k_2$/His	48	230	530	1200
3	K_M (μM)	1600	67	13	5.8
	k_{cat} (min^{-1})	0.099	0.096	0.15	0.39
	k_{cat}/k_{uncat}[b]	4500	4400	6700	18 000
	$(k_{cat}/K_M)/k_2$[c]	130	3000	23 000	140 000
	$(k_{cat}/K_M)/k_2$/His	44	420	1600	4500

[a] Conditions: 5 mM aq citrate pH 5.5, 27 °C. [b] $k_{uncat}(min^{-1}) = 4.4 \times 10^{-5}$ (**1**), 1.4×10^{-5} (**2**), 2.2×10^{-5} (**3**). [c] k_2(4-Me-Im) (mM^{-1} min^{-1}) = 7.0×10^{-4} (**1**), 4.9×10^{-4} (**2**), 4.8×10^{-4} (**3**).

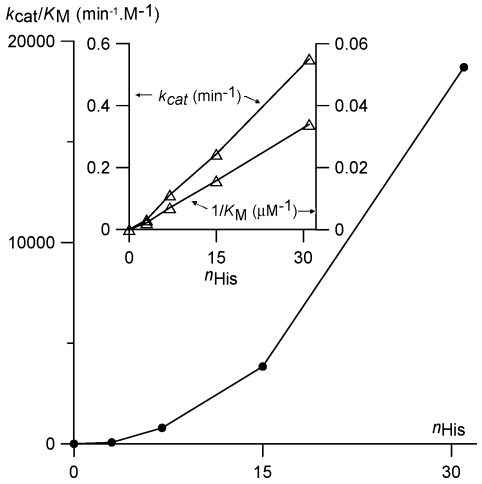
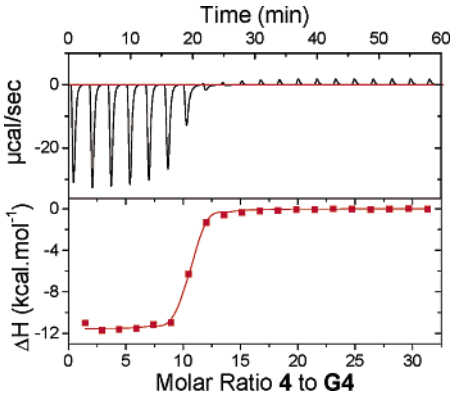


Figure 2. Dendritic effect on dendrimer-catalyzed hydrolysis of butyrate **2**.

rather induces catalytically productive interactions such as (1) modulation of the histidine side-chain's pK_a, as evidenced from the very different pH rate profile of k_{cat} for the dendrimers vs 4-methyl-imidazole (Figure 1) and (2) creation of a hydrophobic microenvironment allowing substrate binding by the acyl chains. Fine-tuning of this dendritic effect toward selective substrate recognition and turnover might enable the preparation of more efficient and selective dendritic catalysts.

Table 2. Calorimetric Titration of **4** and **5** Added to **G1**−**G4**[a]



		n	K_a ($\times 10^4$ M^{-1})
G1	**4**	2.36	1.66 (±0.51)
G2	**4**	1.86	10.5 (±3.2)
G3	**4**	5.83	56.2 (±12.0)
G4	**4**	9.88	80.8 (±15.0)
G1	**5**	0.84	0.11 (±0.02)
G2	**5**	1.69	0.27 (±0.03)
G3	**5**	4.78	0.29 (±0.05)
G4	**5**	9.43	0.28 (±0.07)

[a] Association constants (K_a) and number of binding sites (n); raw data for titration of **4** added to **G4** and enthalpogram corrected for the heat of dilution. Conditions: **G1** 0.5 mM, **G2** 0.2 mM, **G3** 0.1 mM, **G4** 0.05 mM, **4** 10 mM, **5** 10 mM in citrate buffer pH 5.5 (5 mM), at 27 °C.

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Supporting Information Available: Synthetic procedures and data for all dendrimers and details of kinetic and ITC measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Dodecanoyl ester **4** was a poor substrate (see Figure S1, Supporting Information) and competitively inhibited catalysis for the shorter chain substrates, making this substrate an ideal model for studying substrate binding to the dendrimers.

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