Artificial aldolases from peptide dendrimer combinatorial libraries†

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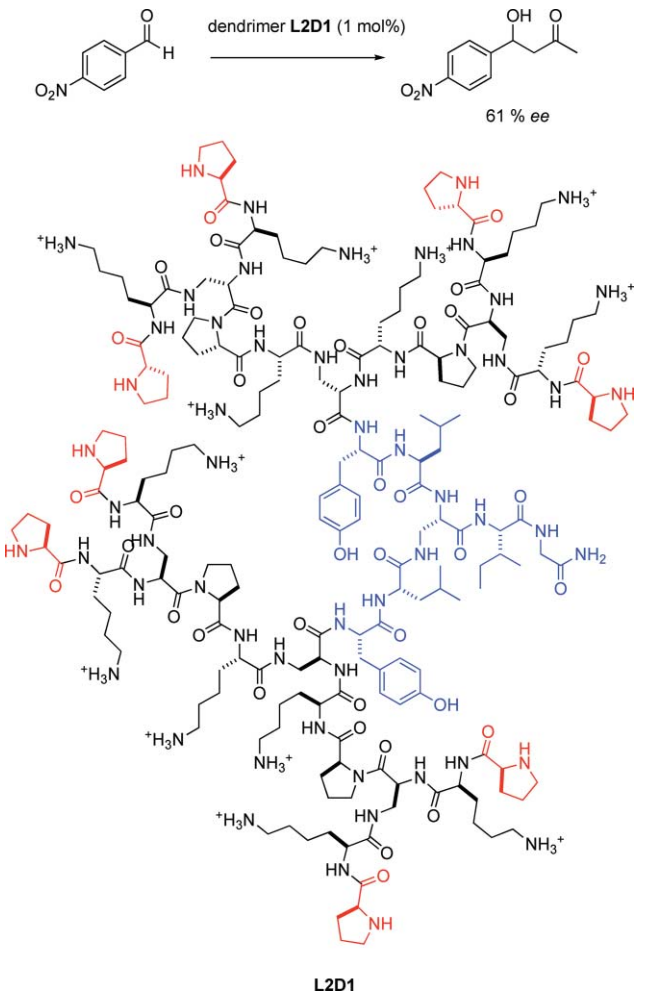
Peptide dendrimers were investigated as synthetic models for aldolase enzymes. Combinatorial libraries were prepared with aldolase active residues such as lysine and proline placed at the dendrimer core or near the surface. On-bead selection for aldolase activity was carried out using the dye-labelled 1,3-diketone **1a**, suitable for covalent trapping of enamine-reactive side-chains, and the fluorogenic enolization probe **6**. Aldolase dendrimers catalyzed the aldol reaction of acetone, dihydroxyacetone and cyclohexanone with nitrobenzaldehyde. Much like enzymes, the dendrimers exhibited strong aldolase activity in aqueous medium, but were also active in organic solvent. Dendrimer-catalyzed aldol reactions reached complete conversion in 3 h at 25 °C with 1 mol% catalyst and gave aldol products with up to 65% ee. A positive dendritic effect in catalysis was observed with both lysine and proline based aldolase dendrimer catalysts.

Introduction

The aldol reaction is one of the most important C–C bond forming reactions in organic synthesis. The reaction can be catalyzed in water by enzymes, which operate by an enamine (type I) or an enolate (type II) mechanism.[1] Type I aldolase mimics are known based on catalytic antibodies acting primarily in water,[2] and small molecule organocatalysts such as proline, which are usually more active in organic solvent.[3,4] Zn–proline is a type II aldolase mimic acting in water.[5]

We asked the question whether a synthetic macromolecular enzyme model could be obtained that would reproduce the type I aldolase activity in an aqueous environment, working on the basis of peptide dendrimers as a framework. Dendrimers are tree-like macromolecules under investigation for various uses in technology and medicine.[6,7] We recently reported artificial esterases on a peptide dendrimer basis,[8] showing that these macromolecules are suitable as catalysts in an aqueous environment.[9] A recent report showed that multivalent prolines at the surface of a poly(propylene) imine dendrimer exhibit comparable activities to proline itself for aldolization in organic solvent, however the study did not address the issue of aqueous catalysis as an enzyme model.[10] Herein, we report the discovery of aldolase peptide dendrimers by functional selection from dendrimer combinatorial libraries using probes specific for aldolase active residues. The aldolase dendrimers are shown to operate by a type I mechanism in water. The most active catalysts, such as **L2D1**, display multiple *N*-terminal prolines or primary amines as catalytic groups (Scheme 1). The activity of proline residues in the dendritic multivalent display is enhanced compared to monovalent catalysts.



Scheme 1 Peptide dendrimer catalyzed aldol reaction. Conditions: 1 mM dendrimer in DMSO–acetone (4 : 1, *v*/*v*), 36 h, rt.

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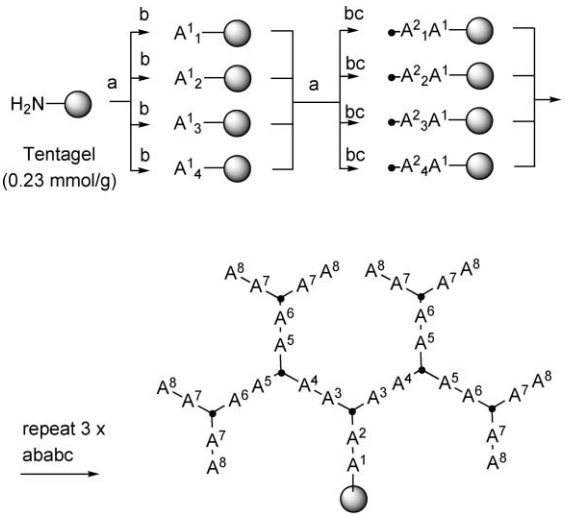
Results and discussion

Combinatorial discovery of aldolase dendrimers

We recently reported a combinatorial approach to peptide dendrimers based on functional screening of split-and-mix[11] libraries on a solid support.[12] The peptide dendrimers in these libraries contain eight variable amino acid positions along three successive branches. Using four different amino acids per variable position results in a combinatorial library of $4^8 = 65\,536$ members (Fig. 1).[12] We reasoned that focused dendrimer libraries incorporating the essential features of known aldolase enzymes and catalysts might contain catalytic aldolase dendrimers, and that these functional dendrimers could be discovered using appropriate probes for screening.

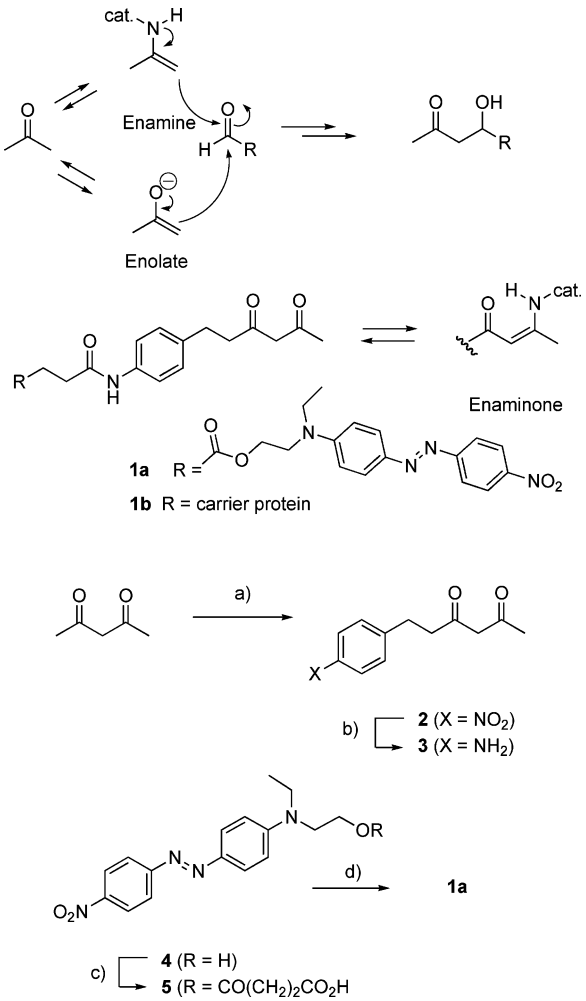


	L1				L2			
	1	2	3	4	1	2	3	4
A^1, A^3	Lys	Ala	Gly	Val	Ala	Gly	Leu	Val
A^2, A^4	Asp	Phe	Ser	Trp	Phe	Tyr	Ile	β-Ala
A^5, A^7	His	Thr	Tyr	Ile	Lys	His	Arg	Asp
A^6, A^8	Pro	Leu	Glu	Arg	Pro	Glu	Ser	Thr

Fig. 1 Combinatorial split-and-mix synthesis of peptide dendrimers. ● = L-2,3-diaminopropanoic acid (Dap). Library **L1** is acetylated at the *N*-terminus. Using 4 amino acids per variable position A^i (i = 1–8) gives $4^8 = 65\,536$ members. Conditions: a) suspend the whole resin batch in DMF–DCM (2 : 1, *v*/*v*), mix *via* nitrogen bubbling for 15 min, and split the batch in four equal portions 1–4; b) in each portion x = 1–4: 2.5 eq. Fmoc–A^i_x–OH, 2.5 eq. PyBOP, 6.0 eq. DIEA, DMF, $2^g \times 60$ min (where g = generation number), then DMF–piperidine (4 : 1, v/v), 2 × 10 min; c) same as b) with Fmoc–Dap(Fmoc)–OH.

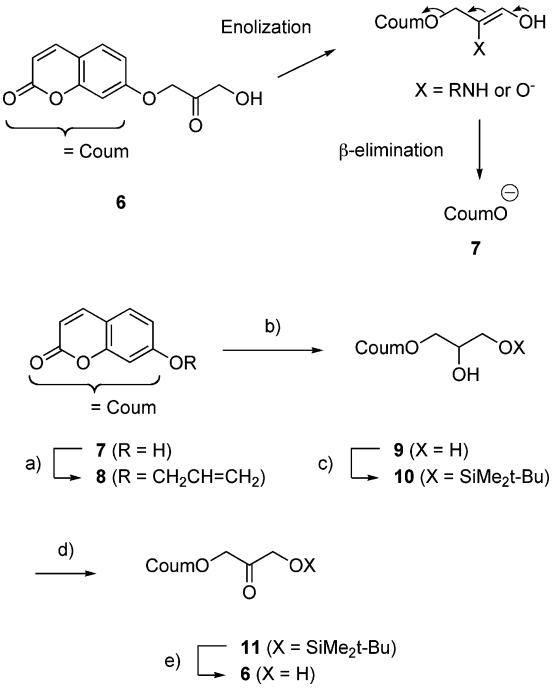
A first library (**L1**) was designed to mimic type I aldolase enzymes and catalytic antibodies by using lysine residues as one of the variable residues for the core positions A^1 and A^3 (Fig. 1).[13] The core also featured hydrophobic and aromatic residues to create a hydrophobic microenvironment, while most other charged and polar residues were placed near the surface. The library was acetylated at the *N*-terminus and the side chains deprotected to yield library **L1**. A second library (**L2**) featured multiple catalytic residues at the surface (free *N*-termini, *N*-terminal proline, or lysine), with the aim of exploiting a possible dendritic effect in aldolase catalysis similar to that observed with esterase peptide dendrimers with multiple histidines.[8d] Thus, lysine was used as one of the variable amino acids for positions A^5 and A^7, and proline as one of the variable amino acids for positions A^6 and A^8. The other amino acids were distributed evenly in the available variable positions. In this case, the library was left with a free *N*-terminus after removal of the last Fmoc protecting groups and the side chains deprotected, giving library **L2**.

Four different probes were prepared for functional screening of the dendrimer libraries. The first probe was the dye-labelled 1,3-diketone **1a**. Such 1,3-diketones react rapidly with lysine side-chains to form a stable enaminone, and the method has been used to select catalytic antibodies by reactive immunization with hapten **1b** (Scheme 2).[2] The probe was obtained by alkylation of acetylacetone with nitrobenzyl bromide at the terminal position *via* the dienolate to intermediate **2**, hydrogenation of the nitro group to the unstable aniline **3**, and coupling with monoester **5**, obtained by acylation of Disperse Red 1 (**4**) with succinic anhydride.



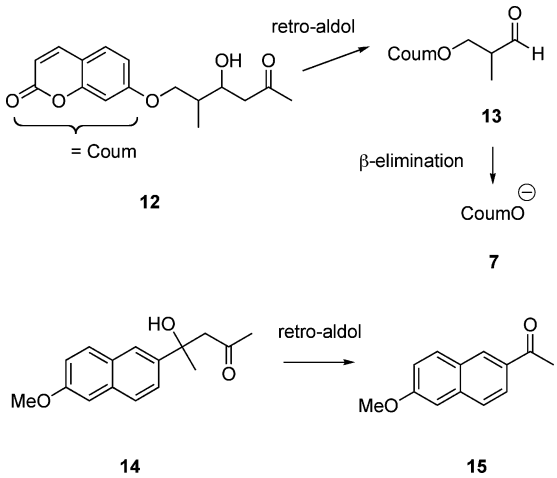
Scheme 2 Aldolization mechanisms and enaminone formation with diketone probe **1a**. Synthesis: (a) 2.1 eq. LDA, THF, HMPA, −78 °C, 2 h, 4-nitrobenzyl bromide (40%); (b) H_2, Pd/C (10 mol%), DCM, rt, 18 h, not isolated; (c) succinic anhydride, DMAP, Et_3N, DCM, rt, 5 h, (33%); (d) **3**, EDC, HOBt, DCM, 0 °C to rt, 18 h, (95%).

The dihydroxyacetone derivative **6** was used as a second screening probe (Scheme 3). Ketone **6** undergoes enolization in the presence of aldolase catalysts by rate-limiting deprotonation of the α-carbon atom, followed by β-elimination of the fluorescent product umbelliferone **7**. The probe detects both enamine (type I) aldolase catalysts such as catalytic antibody 38C2 and enolate (type II) aldolase catalyst such as $Zn(Pro)_2$.[14] Ketone **6** was prepared from umbelliferone **7** by alkylation with allylbromide to form allyl ether **8**, dihydroxylation to form glycerol ether **9**, protection of the primary alcohol to silyl ether **10**, oxidation of the secondary alcohol to ketone **11**, and acidic deprotection and purification by reverse-phase HPLC.



Scheme 3 Fluorogenic enolization probe **6**. Synthesis conditions: (a) allyl bromide, K_2CO_3, acetone, reflux, 18 h, (85%); (b) NMO, OsO_4, *t*-BuOH–H_2O (2 : 1, *v*/*v*), rt, 18 h, (95%); c) TBDMSCl, imidazole, DCM–DMF (3 : 1, *v*/*v*), rt, 18 h, (58%); d) oxalyl chloride, DMSO, Et_3N, 1 h, −78 °C, 78% yield; e) TFA–H_2O (9 : 1, *v*/*v*), rt, 1 h, (33%).

Two additional aldolase screening assays were carried out using the known retro-aldolization probes **12**[15] and **14**,[16] which were prepared using the published procedures. Theses substrates undergo a fluorogenic retro-aldolization reaction and are suitable for detecting aldolase antibodies by fluorescence (Scheme 4). Indeed, the aldol reaction between a ketone and an aldehyde is a near-equilibrium process, and the direction of the reaction is given by the concentration of the reactants,[17] allowing the use of retro-aldolization as a test for aldol catalysis.



Scheme 4 Fluorogenic probes for retro-aldolization.

Activity screening was carried out several times for each of the probes. Each screening used a 50 mg batch of the library corresponding to approximately 62 500 beads,[18] ensuring 60% coverage of the library.[19] Since the beads were acidic after removal of the protecting group with trifluoroacetic acid, they were equilibrated several times with aqueous phosphate buffer saline pH 7.4 (PBS) and DMF for neutralization before each assay.

The binding assay with diketone **1a** was carried out in a 1 : 1 mixture of PBS and dimethylsulfoxide (DMSO), followed by washing. The cosolvent was necessary to solubilize the probe, and the conditions were also favorable for aldol catalysis. The concentration of **1a** was adjusted to 50 μM and the incubation time to 30 min to produce only very few beads (*ca.* 50, 0.1%) per screening batch (Fig. 2). There was no detectable staining with up to 10 mM Disperse Red (**3**) alone under the screening conditions, showing the specificity of the staining reaction with the diketone probe **1a**. The beads stained by diketone **1a** in library **L1** contained dendrimers with one or three lysine residues, with a strong selection for lysine at position A^1, consistent with enaminone formation

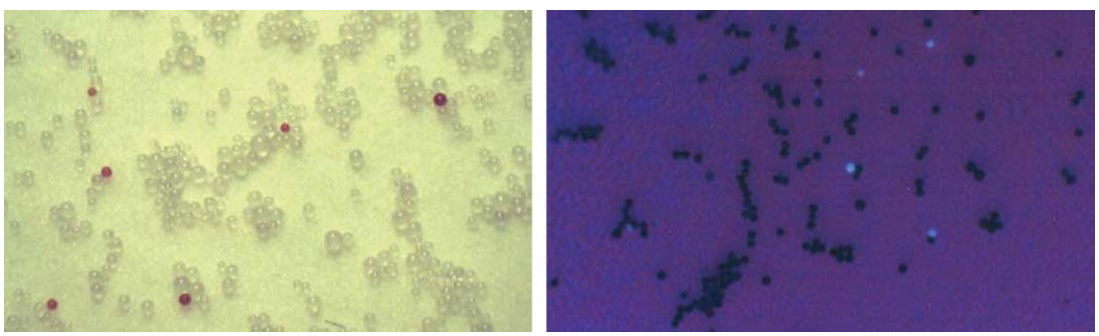


Fig. 2 High throughput screening of the libraries. Left: diketone probe **1b**, the beads containing binding sequences are deep red, beads with inactive sequences are colorless. Right: enolization probe **6**, the beads containing catalytic sequences appear in light blue, beads with inactive sequences are black.

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Table 1 Peptide dendrimer sequences[a] identified by amino acid analysis of active beads from the combinatorial libraries **L1** and **L2**. Sequences **L1D1**–**L1D7** and **L2D1**–**L2D9**; hits for the diketone **1a** with library **L1** and **L2**. Sequences **L2K1**–**L2K8**; hits for the ketone **6** with library **L2**

Assay	Dendrimer	A8	A7	A6	A5	A4	A4	A2	A1
Enaminone formation with **1a**[b]	**L1D1**	Leu	Ile	Glu	Thr	Ser	Val	Ser	Lys
	L1D2	Glu	Thr	Glu	Tyr	Phe	Gly	Ser	Lys
	L1D3	Glu	Thr	Arg	Ile	Phe	Ala	Asp	Gly
	L1D4	Leu	Ile	Arg	Thr	Trp	Gly	Phe	Ala
	L1D5	Glu	Tyr	Arg	Thr	Phe	Lys	Ser	Lys
	L1D6	Arg	Tyr	Glu	Tyr	Phe	Lys	Ser	Lys
	L1D7	Arg	Ile	Leu	Thr	Phe	Val	Asp	Lys
	L2D1	Pro	Lys	Pro	Lys	Tyr	Leu	Ile	Gly
	L2D2	Pro	Lys	Pro	Lys	Ile	Ala	Tyr	Ala
	L2D3	Glu	Lys	Thr	Asp	Tyr	Leu	Phe	Val
	L2D4	Thr	Lys	Glu	Lys	Ile	Ala	Tyr	Gly
	L2D5	Glu	Lys	Ser	Lys	Tyr	Ala	Phe	Val
	L2D6	Ser	Lys	Ser	Lys	Tyr	Gly	Phe	Gly
	L2D7	Pro	Lys	Glu	Arg	β-Ala	Gly	Phe	Val
	L2D8	Ser	Lys	Pro	Arg	Ile	Gly	Tyr	Gly
	L2D9	Glu	Lys	Ser	Asp	Ile	Gly	Phe	Gly
Catalysis of direct β-elimination of ketone **6**[c]	**L1**	No hits with this library were observed							
	L2K1	Pro	Lys	Ser	His	β-Ala	Ala	Ile	Val
	L2K2	Thr	Arg	Ser	Arg	β-Ala	Val	Tyr	Gly
	L2K3	Pro	Lys	Thr	Lys	β-Ala	Ala	Phe	Gly
	L2K4	Pro	Lys	Thr	His	Tyr	Gly	Phe	Val
	L2K5	Pro	Lys	Ser	Asp	Ile	Gly	Ile	Leu
	L2K6	Ser	Asp	Pro	His	β-Ala	Gly	Tyr	Gly
	L2K7	Pro	Lys	Ser	Arg	β-Ala	Val	Tyr	Leu
	L2K8	Glu	Lys	Glu	Asp	Ile	Gly	Tyr	Ala

[a] Dendrimer structure according to Fig. 1. Dendrimers marked bold were resynthesized by Fmoc–SPPS and purified. [b] In library **L1** *N*-termini were acetylated; in library **L2** *N*-termini were free amine. Screening conditions: beads shaken in DMSO–PBS buffer (pH = 7.4) (1 : 1, *v*/*v*) with 50 μM diketone **1a** for 30 min, 25 °C. Hits are deep red (see Fig. 2). [c] In library **L1** *N*-termini were acetylated; in library **L2** *N*-termini were free amine. Beads soaken in aqueous bicine buffer pH = 8.50 with 1% acetonitrile with 100 μM probe **6** for 40 min, 25 °C. Hits are light blue under radiation with a TLC UV-lamp set at 356 nm (see Fig. 2). Statistically significant selections for **L1**: Lys at A^1, Phe(Trp) at A^4, Arg and Glu at A^6/A^8. **L2** with **1a**: Lys at A^7. L2 with **6**: Pro at A^8.

(Table 1). The positive selection of lysine residues by probe **1a** at position A^1 was confirmed by sequencing of 7 beads from library **L1** picked at random. In this case residues at positions A^1 corresponded to statistical distribution (Lys: 21%, Ala: 21%, Gly: 43%, Val: 15%). The situation was similar for the hits from library **L2** featuring surface amine residues. The selection of lysine residues at position A^5 and A^7 was surprising in this case since *N*-terminal amino groups might also engage in enaminone formation. As for **L1**, random picking of beads from **L2** gave a statistically even representation of amino acids at positions A^5 and A^7 (6 beads, Lys: 25%, His 21%, Arg: 31%, Asp: 23%).

The enolization probe **6** was assayed in aqueous bicine buffer pH = 8.50 with 1% acetonitrile at 100 μM, under which conditions it was fully soluble. The beads were first equilibrated with bicine buffer, washed, and suspended in a freshly prepared solution of probe **6**. The beads were then plated out onto a silica gel plate, which absorbed most of the solution and left each bead well separated from the others as a free-standing microreactor. Under these conditions the fluorescent product umbelliferone (**7**) cannot diffuse away and remains inside the polymer bead, ensuring that it can be detected by fluorescence. Library **L1** gave no detectable fluorescent beads with this assay. This was surprising, since ketone **6** reacts well with catalytic antibody 38C2 in an active site lysine specific reaction.[14] Screening of library **L2** featuring surface catalytic residues produced fluorescent beads, which appeared slowly over the course of the first 40 min (Fig. 2). The beads were picked and sequenced as above (Table 1). The majority of active beads with probe **6** featured *N*-terminal proline residues, in agreement with the known aldolase reactivity of *N*-terminal prolyl peptides.[4d] Lysine was also often present.

The retro-aldolization probes **12** and **14** were tested at 100 μM concentration in aqueous borate buffer pH 9 with 5% acetonitrile, which are the conditions under which catalytic antibodies[15] show activity. The screening protocol for probe **6** above was used. None of the beads gave any detectable reaction with these probes in either of the libraries.

Aldol catalysis with peptide dendrimers

Hits from the combinatorial experiment were synthesized on Tentagel at 0.25 mmol g^{-1}, deprotected and cleaved, and purified by preparative HPLC (Table 2). The dendrimers were investigated for catalysis of the aldol reaction of acetone with nitrobenzaldehyde **16** to give aldol **17** (Scheme 5).[4] The reaction was first tested in organic solvent (DMSO–acetone 4 : 1, *v*/*v*), typical for organocatalysis of this reaction using 0.1 M aldehyde and 1 mM (1 mol%) peptide dendrimer. While dendrimers from library **L1** with core lysines were not active, all the peptide dendrimers from library **L2** were catalytically active (Table 3). The four dendrimers containing *N*-terminal proline residues (**L2D1**, **L2D7**, **L2K4**, **L2K7**) were clearly more active than the others, and showed moderate enantioselectivity (up to 61% ee for (*R*)-**17** with dendrimer **L2D1**).

The dendrimers were also tested with acetone and aldehyde **17** under aqueous conditions (acetone–water 1 : 1, pH 8.5) mimicking those of an enzyme-catalyzed reaction. While the dendrimers from library **L1** were again not active, the dendrimers from library **L2**

Table 2 Yield and molecular weight of the synthesized peptide dendrimers from **L1** and **L2**[a]

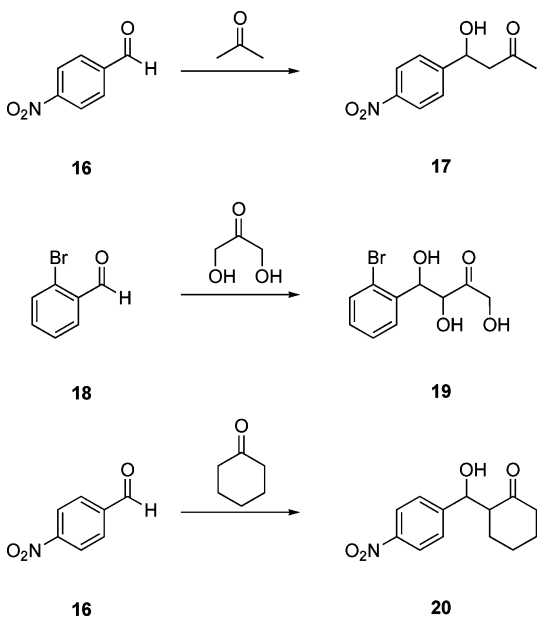
Origin	Dendrimer		Yield as TFA-salts/mg (%)	Expected mass $[M + H]^+$	Observed mass $[M + H]^+$
Enaminone formation with **1a**	AcEY·RT·FK·SK	**L1D5**	18 mg, 9%	5088.35	5088.38
	AcRY·EY·FK·SK	**L1D6**	21 mg, 10%	5444.65	5444.25
	PK·PK·YL·IG	**L2D1**	86 mg, 25%	4046.00	4045.88
	EK·SKYA·FV	**L2D5**	20 mg, 8%	3942.30	3942.12
	SK·SK·YG·FG	**L2D6**	33 mg, 13%	3846.15	3846.13
	PK·ER·βAG·FV	**L2D7**	19 mg, 7%	4064.36	4064.38
β-Eliminaton of ketone **2**	PK·TH·TG·FV	**L2K4**	11 mg, 4%	4060.28	4060.38
	PK·SR· βAV.YL	**L2K7**	28 mg, 10%	4010.42	4010.75
	EK·ED·IG·YA	**L2K8**	17 mg, 6%	4228.05	4228.50

[a] The peptide dendrimers were synthesized on NovasynTGR resin (200 mg, 0.25 mmol g^{-1}) using Fmoc SPPS and were purified by RP-HPLC.

Table 3 Activities of the peptide dendrimers for the aldol reaction of acetone with nitrobenzaldehyde **16**

Dendrimer		Conditions[a]	Time/h	Conversion (%)[b]	ee (%)[c]
PK·PK·YL·IG	**L2D1**	DMSO–acetone (4 : 1, *v*/*v*)	36	69	61
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
EK·SKYA·FV	**L2D5**	DMSO–acetone (4 : 1, *v*/*v*)	36	<5	N.a.
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	14	N.a.
SK·SK·YG·FG	**L2D6**	DMSO–acetone (4 : 1, *v*/*v*)	36	<5	N.a.
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	11	N.a.
PK·ER·βAG·FV	**L2D7**	DMSO–acetone (4 : 1, *v*/*v*)	72	37	41
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
PK·TH·TG·FV	**L2K4**	DMSO–acetone (4 : 1, *v*/*v*)	36	68	35
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	92	<5
PK·SR· βAV.YL	**L2K7**	DMSO–acetone (4 : 1, *v*/*v*)	36	95	46
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
EK·ED·IG·YA	**L2K8**	DMSO–acetone (4 : 1, *v*/*v*)	36	16	N.a.
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	34	<5

[a] Conditions: 100 mM aldehyde, 1 mM peptide dendrimer in the indicated solvent mixture. The aqueous buffer is bicine pH 8.5. The DMSO was buffered *x* eq. of NMM (where *x* = number of TFA–amine salts for the dendrimer tested). [b] Conversion was measured by RP-HPLC at 254 nm. [c] Enantiomeric excess of (*R*)-**17** was determined by HPLC at 254 nm on a Daicel Chiralpak AS column.



Scheme 5 Direct aldol reactions investigated with aldolase peptide dendrimers.

were active and showed much stronger activity than in organic solvent, particularly for dendrimers with *N*-terminal proline residues. With three dendrimers (**L2D1**, **L2D7** and **L2K7**), the aldol reaction with 1 mol% catalyst was complete in 60 min at room temperature (>99% conversion), compared to 40–95% conversion after 36 h in organic solvent (Table 3). Unfortunately, the reactions were not enantioselective under these aqueous conditions.

The aldol reaction of dihydroxyacetone with 2-bromobenzaldehyde **18** to give aldol **19** was investigated next. Hydroxyketones are typical substrates for enzyme-catalyzed transaldolase processes.[1] The reaction was carried out under aqueous conditions (MeOH–water 1 : 1, pH 8.5) using 0.1 M aldehyde, 5 M dihydroxyacetone and 1 mM (1 mol%) peptide dendrimer.[20] The **L1** dendrimers were once more inactive. However, the **L2** dendrimers showed good to moderate activity for this aldol reaction (Table 4). In fact, only the dendrimers derived from screening with enolization probe **2** showed significant activity, while those identified with the 1,3-diketone probe **1a** were only weakly active. There was no significant diastereoselectivity.

The dihydroxyacetone aldolase reactivity of the selected dendrimers **L2K4**, **L2K7** and **L2K8** might be a general-base type reactivity which is much weaker in the other dendrimers. Indeed, we showed recently that aldol reactions of dihydroxyacetone in water are catalyzed by general bases not capable of enamine formation such as *N*-methylmorpholine, while typical enamine-forming catalysts such as L-proline are poor catalysts for this substrate.[14]

The selection of dihydroxyacetone aldolase reactive dendrimers during library screening with probe **6** could not be traced back to a

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Table 4 Activities of the peptide dendrimers for the aldol reaction of dihydroxyacetone with bromobenzaldehyde **18**.[a] Dendrimers **L1D5** and **L1D6** were not active for this reaction

Dendrimer		Conversion (%)[b] (*anti* : *syn*)ratio[b]
PK·PK·YL·IG	**L2D1**	7 (1.0 : 1.0)
EK·SK·YA·FV	**L2D5**	8 (1.0 : 1.5)
SK·SK·YG·FG	**L2D6**	16 (1.0 : 1.3)
PK·ER·βAG·FV	**L2D7**	<5 (n.a. : n.a.)
PK·TH·TG·FV	**L2K4**	40 (1.0 : 1.4)
PK·SR· βAV.YL	**L2K7**	74 (1.0 : 1.5)
EK·ED·IG·YA	**L2K8**	36 (1.0 : 1.8)

[a] Conditions: 5 M dihydroxyacetone, 100 mM aldehyde, 1 mM peptide dendrimer in methanol–aq. bicine buffer pH 8.5 (1 : 1, *v*/*v*) 66 h, room temperature. [b] Conversion and diastereomeric excess of *syn*-**19** was measured by RP-HPLC at 254 nm.

specific reactivity with this probe. Indeed, all dendrimers catalyzed the fluorogenic reaction of probe **6** (see the ESI). Although the three dendrimers derived from the screening with **6** are the most reactive, the difference is less than 2-fold and is not sufficient to explain their reactivity with dihydroxyacetone. Probe **6** shows relatively low K_M values with the dendrimers, which probably indicates hydrophobic substrate–dendrimer interactions. These interactions should be much weaker or even absent with the more polar dihydroxyacetone, resulting in a more differentiated behavior of the dendrimers with this substrate.

Cyclohexanone, which was recently shown to be an excellent substrate for organocatalyzed aldolization,[4f] was investigated as a third substrate (Table 5). In this case dendrimer **L1D5** (AcEY·RT·FK·SK), equipped with catalytic lysine residues at the dendrimer core, was catalytically active, but showed no stereoselectivity. Very high conversions were also observed with dendrimer **L2K8** (EK·ED·IG·YA) featuring only primary amines as possible catalytic groups, namely the lysine side chains and the *N*-terminal amino groups. The reactions showed moderate diastereo- and enantioselectivities. These results were surprising to us because of the lack of activity with acetone with these dendrimers under the same conditions, suggesting a substrate specific interaction with the ketone, which might originate from hydrophobic binding of the cyclohexanone as compared to acetone.

Cyclohexanone is not miscible with water, allowing this aldol reaction to be run as an emulsion, conditions which seem to be favorable for stereoselective organocatalysis as suggested by recent reports.[4h] The peptide dendrimers might form micelles under these conditions in which the reaction takes place. The most promising dendrimer **L1D5**, **L2D1** and **L2K8** were studied in water–cyclohexanone emulsion. Indeed, we observed enantiomeric excess in the aldol product under these aqueous conditions with dendrimer **L2K8** displaying multiple primary amines. On the other hand, dendrimer **L1D5** with core lysines showed negligible stereoselectivity.

Mechanistic investigations

The mechanism of the aldol reaction of acetone and cyclohexanone with aldehyde **16** was investigated. Four of the most active aldolase dendrimers (**L2D1**, **L2D7**, **L2K4** and **L2K7**) featured an *N*-terminal Pro–Lys dyad. This catalytic dyad was therefore used to prepare a regular dendritic series **R2** of increasing generation number to investigate a possible dendritic effect in catalysis. A regular dendrimer series **R1** featuring the known[4l] catalytic dipeptide Pro–Thr in its branches was also prepared (Table 6). All dendritic peptides of the regular series **R1** and **R2** were

Table 5 Activities of the peptide dendrimers for the aldol reaction of cyclohexanone with nitrobenzaldehyde **16** in DMSO and aqueous buffer[a]

		DMSO		Aqueous buffer (pH 8.5)	
Catalyst/dendrimer		Conversion[a,b] (*anti* : *syn* ratio)	ee[b] (*anti*/*syn*, %)	Conversion[c,b] (*anti* : *syn* ratio)	ee[b] (*anti*/*syn*, %)
L-Proline[d]		95 (1.4 : 1.0)	70/80		
AcEY·RT·FK·SK	**L1D5**	32 (1.0 : 2.2)	14/18	32 (2.2 : 1.0)	5/8
AcRY·EY·FK·SK	**L1D6**	18 (1.0 : 1.3)	<5/<5		
PK·PK·YL·IG	**L2D1**	96 (1.0 : 2.0)	40/12	94 (1.6 : 1.0)[e]	12/12
SK·SK·YG·FG	**L2D6**	97 (1.5 : 1.0)	22/16		
PK·TH·TG·FV	**L2K4**	26 (1.0 : 1.9)	64/24		
PK·SR· βAV.YL	**L2K7**	88 (1.0 : 1.5)	14/16		
EK·ED·IG·YA	**L2K8**	98 (1.0 : 2.4)	50/28	54 (1.9 : 1.0)	65/45

[a] Conditions: 100 mM aldehyde, 1 mM peptide dendrimer in DMSO–cyclohexanone (1 : 1, *v*/*v*) buffered with *x* eq. of NMM (where *x* = number of TFA–amine salts for the dendrimer tested), 18 h, room temperature. [b] Conversion and diastereomeric excess of **20** was measured by RP-HPLC at 268 nm. Enantiomeric excess of *anti*-**20** and *syn*-**20** respectively were measured by chiral phase HPLC at 268 nm on a ChiralPak OD-H column. [c] Conditions: 100 mM aldehyde, 1 mM peptide dendrimer in cyclohexanone–aq. bicine buffer pH 8.5 (1 : 1, *v*/*v*) 4 d, room temperature. [d] 30 mol% catalyst was used. [e] Reaction time 18 h.

Table 6 Yield and molecular weight for synthesis of the regular series **R1** and **R2**[a]

Origin	Dendrimer		Yield (as TFA-salts) (mg, %)	Expected mass $[M + H]^+$	Observed mass $[M + H]^+$
Regular series Pro–Thr **R1**	PT·PT	**R1G1**	7 mg, 79%	696.78	697.38
	PT·PT·PT	**R1G2**	15 mg, 78%	1661.84	1661.88
	PT·PT·PT·PT	**R1G3**	14 mg, 26%	3591.97	3591.84
Regular series Pro–Lys **R2**	PK·PK	**R2G1**	9 mg, 85%	777.99	778.50
	PK·PK·PK	**R2G2**	17 mg, 58%	1851.32	1851.33
	PK·PK·PK·PK	**R2G3**	18 mg, 20%	3997.99	3998.00

[a] The peptide dendrimers were synthesized on NovasynTGR resin (200 mg, 0.25 mmol g^{-1}) using Fmoc SPPS and were purified by RP-HPLC.

Table 7 Activities of the regular series of peptide dendrimers for the aldol reaction of acetone and cyclohexanone with nitrobenzaldehyde **16**

Dendrimer		Conditions[a]	Time/h	Conversion (%)[b]	ee (%)[c]
PT	**R1G0**	DMSO–acetone (4 : 1, *v*/*v*)	36	20	N.a.
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	15	<5
PT·PT	**R1G1**	DMSO–acetone (4 : 1, *v*/*v*)	36	80	44
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	68	<5
PT·PT·PT	**R1G2**	DMSO–acetone (4 : 1, *v*/*v*)	36	>99	20
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	16 (1.3 : 1.0)	54/26
PT·PT·PT·PT	**R1G3**	DMSO–acetone (4 : 1, *v*/*v*)	36	>99	34
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	26 (1.4 : 1.0)	59/20
PK	**R2G0**	DMSO–acetone (4 : 1, *v*/*v*)	36	8	N.a.
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	14	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	<5	N.a.
		aq. buffer–cyclohexanone (1 : 1, *v*/*v*)	2	<5	N.a.
PK·PK	**R2G1**	DMSO–acetone (4 : 1, *v*/*v*)	36	27	52
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	41	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	<5	N.a.
		aq. buffer–cyclohexanone (1 : 1, *v*/*v*)	2	<5	N.a.
PK·PK·PK	**R2G2**	DMSO–acetone (4 : 1, *v*/*v*)	36	87	49
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	93	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	32 (1.0 : 2.2)	46/8
		aq. buffer–cyclohexanone (1 : 1, *v*/*v*)	2	43 (2.1 : 1.0)	2/16
PK·PK·PK·PK	**R2G3**	DMSO–acetone (4 : 1, *v*/*v*)	36	81	60
		aq. buffer–acetone (1 : 1, *v*/*v*)	3	>99	<5
		DMSO–cyclohexanone (1 : 1, *v*/*v*)	18	56 (1.0 : 2.5)	40/22
		aq. buffer–cyclohexanone (1 : 1, *v*/*v*)	2	96 (1.2 : 1.0)	30/22

[a] Conditions: 100 mM aldehyde, 1 mM peptide dendrimer in the indicated solvent mixture. The aqueous buffer is bicine pH 8.5, room temperature. The DMSO was buffered *x* eq. of NMM (where *x* = number of TFA–amine salts for the dendrimer tested). [b] For aldol **17**: conversion was measured by RP-HPLC at 254 nm; for aldol **20**: conversion and *anti* : *syn* ratio were measured by RP-HPLC at 268 nm. [c] For aldol **17**: enantiomeric excess of (*R*)-**17** was determined by HPLC at 254 nm on a Daicel Chiralpak AS column; for aldol **20**: enantiomeric excess of *anti*-**20** and *syn*-**20** were determined by HPLC at 268 nm on a Daicel Chiralpak OD–H column.

catalytically active in both organic solvent and aqueous conditions with diastereo- and enantioselectivities comparable to those of the library-derived dendrimers (Table 7). The **R1** (Pro–Thr) series was more active with acetone, while in the case of cyclohexanone the **R2** (Pro–Lys) series was more active, in agreement with the fact that dendrimers from library **L1** with core active site lysine residues showed activity with this ketone.

The catalytic mechanism of the peptide dendrimer-catalyzed aldol reactions with acetone and cyclohexanone should involve enamine formation. Reductive alkylation of amino groups was investigated with dendrimer **R2G3** featuring both *N*-terminal proline residues and core lysine residues. Incubation with acetone and $NaBH_4$ under the reaction conditions produced *N*-isopropyl derivatives of the dendrimer. ^{1}H NMR analysis showed that four *N*-terminal prolines were alkylated in **R2G3**, as indicated by the integral ratio between the two different α-H (11 : 4, proline–*N*-isopropyl proline) and the appearance of a signal at *ca.* 1.2 ppm corresponding to the isopropyl group. The formation of the iminium intermediate between acetone and the *N*-terminal proline under the reaction conditions supports an enamine mechanism for catalysis.[14] A small extent of alkylation at lysine residues was also observed. Nevertheless, an enamine at the ε-amino group of lysine is probably not significant with acetone because lysine itself is not an efficient aldolase catalyst for acetone.

The activity data shows that acetone aldol catalysis is most efficient with *N*-terminal proline residues, while cyclohexanone reacts preferentially with primary amino groups, as in the case of dendrimers **L1D5**, **L2D6** and **L2K8**. Incubation of *N*-α-acetyl-L-lysine with cyclohexanone in water and subsequent reduction with $NaBH_4$ gave approximately 50% alkylation of the ε-amino group. The same experiment with L-proline gave approximately 75% alkylation of the pyrrolidine nitrogen. A similar alkylation experiment was attempted with dendrimer **L1D5**, however due to the complexity of the ^{1}H NMR spectrum it was not possible to estimate the extent of alkylation. Nevertheless, *N*-alklyation of both lysine and proline with cyclohexanone is consistent with an enamine mechanism for both primary-amine and proline-based catalysts, including our peptide dendrimers.

The reactions under aqueous conditions were followed by HPLC to obtain a precise measure of catalytic efficiency (Fig. 3). In the case of acetone, the activity per *N*-terminal proline residue increased strongly with dendrimer size in the regular series **R1** and **R2** up to the second generation dendrimers with four *N*-terminal residues, with a stronger activity in the **R1** (Pro–Thr) series (Fig. 4A). Although the G3 dendrimers were slightly less active per *N*-terminal residue than the G2 dendrimers, there was a further increase in activity per *N*-terminal residue in the library derived G3 dendrimers **L2D1** (PK·PK·YL·IG) and **L2K7** (PK·SR·βAV·YL) relative to the related regular series **R2** (Pro–Lys). Dendrimer **L2K7** was even more active than **R1G2** of the Pro–Thr series. The higher activity of these library derived dendrimers suggests that the hydrophobic core residues present in both dendrimers play a role in enhancing catalysis.

The rate data for the dendritic series **R2** was also investigated with cyclohexanone since lysine containing dendrimers were particularly active with this substrate (Fig. 3B and 4B). In this

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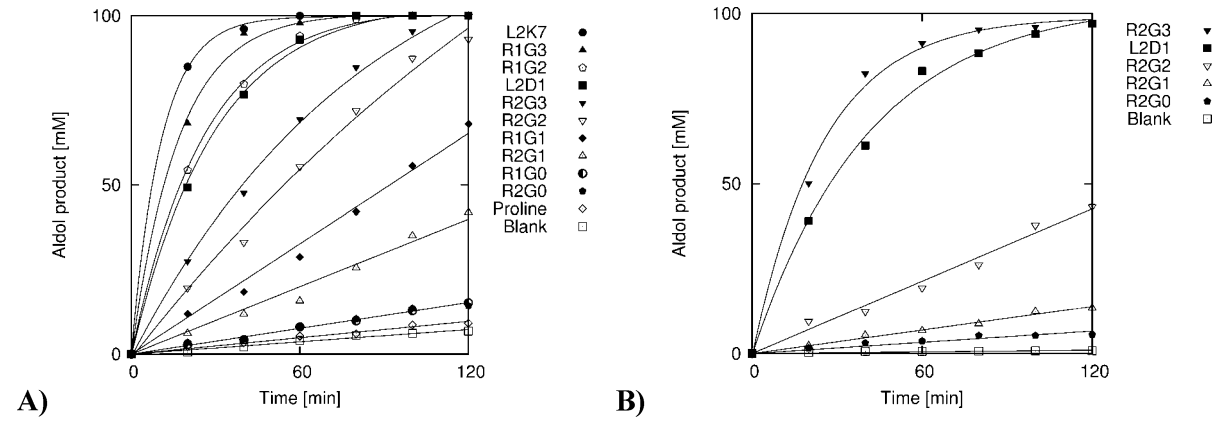

Fig. 3 (A) Plot of concentration of **17** *versus* time in the peptide dendrimer catalyzed direct aldol reaction of **16** with acetone. Conditions: 100 mM aldehyde **16**, 1 mM dendrimer in acetone–aq. bicine buffer pH = 8.5 (1 : 1, *v*/*v*). Reaction was followed by RP-HPLC at 254 nm. (B) Plot of concentration of **20** *versus* time in the peptide dendrimer catalyzed direct aldol reaction of **16** with cyclohexanone. Conditions: 100 mM aldehyde **16**, 1 mM dendrimer in aq. bicine buffer pH = 8.5–cyclohexanone (1 : 1. *v*/*v*). Reaction was followed by RP-HPLC at 268 nm.

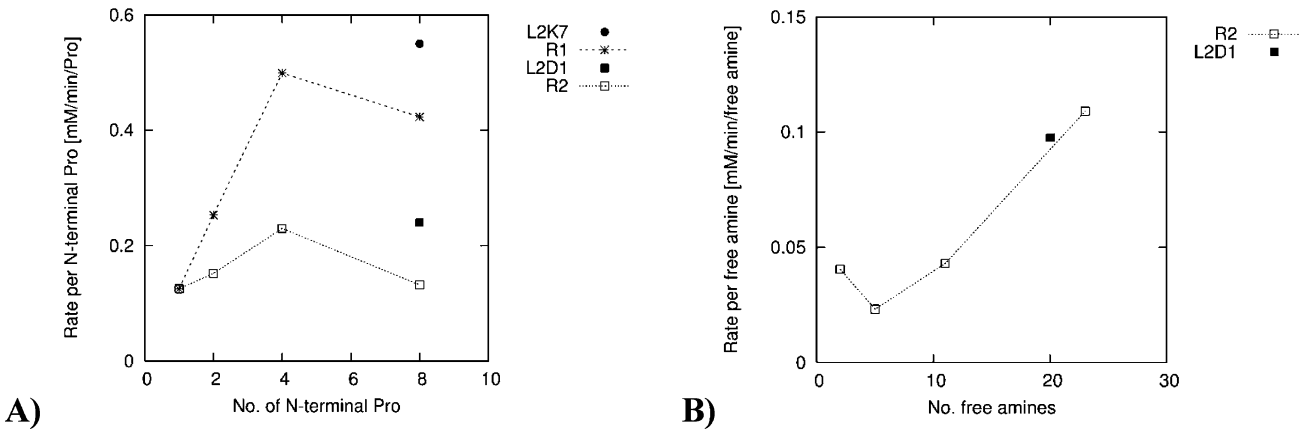

Fig. 4 (A) Rate of aldol product **17** formation per *N*-terminal proline *versus* number of *N*-terminal prolines in the regular series **R1** and **R2** and catalytic peptide dendrimers **L2K7** and **L2D1**. Conditions are as in Fig. 3A. (B) Rate of aldol product **20** formation per *N*-terminal proline *versus* number of *N*-terminal prolines in the regular series **R1** and **R2** and catalytic peptide dendrimers **L2K7** and **L2D1**. Conditions are as in Fig. 3B.

case a positive dendritic effect in catalysis occurred up to the third generation dendrimer **R2G3**, which had similar activity to **L2D1**, the most active dendrimer under water emulsion conditions with cyclohexanone. It should be noted that with cyclohexanone, dendrimer **R2G1** (featuring three core lysine residues and two *N*-terminal prolines) was less active (<5% conversion, Table 7) than the library derived dendrimer **L1D5** with three core lysine residues (32% conversion, Table 5), highlighting the particular activity of the library-derived dendrimer.

Modulation of the pK_a of the *N*-terminal proline in the dendrimers might influence catalytic activity and thus explain the positive dendritic effect observed in aldol catalysis. For example, hydroxyproline, with a pK_a of 9.73 for the pyrrolidine, is more efficient than proline ($pK_a = 10.60$) for aldol catalysis under aqueous conditions.[14] However, the apparent pK_a of the *N*-terminal proline residues was found to be largely independent of dendrimer size, and decreased from $pK_a = 8.43$ in the Pro–Thr–NH_2 dipeptide **R1G0** to $pK_a = 8.13$ in dendrimer **R1G3** (Fig. 5). The much lower pK_a value for the *N*-terminal prolinamide compared to free proline ($pK_a = 10.60$) can be explained by the change from carboxylate to carboxamide, which removes the electrostatic component

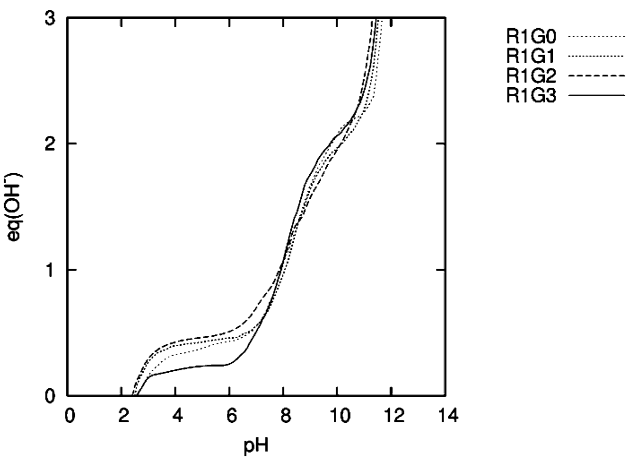

Fig. 5 Acid–base titration of peptide dendrimers **R1G0**–**R1G3** at equimolar concentration with respect to *N*-terminal prolines. pK_a values obtained: **R1G0**: 8.43, **R1G1**: 8.39, **R1G2**: 8.21 and **R1G3**: 8.13.

stabilizing the protonated pyrrolidine in free proline. However, considering that **R1G0** (Pro–Thr–NH_2) catalyzes the aldol only

slightly better than L-Proline, the small downward shift in pK_a between **R1G0** (Pro–Thr) and **R1G3** cannot explain the increased aldolase activity in higher generation peptide dendrimers.

We propose that the positive dendritic effect observed in aldolase catalysis is caused by a hydrophobic effect either increasing substrate binding or shifting the imine formation equilibrium towards the reactive enamine, which both would be favorable for catalysis. Such an effect might also explain the enhanced reactivity of the library derived dendrimers **L2D1** and **L2K7** which both feature a strongly hydrophobic core in comparison to the regular series dendrimers **R1** and **R2**. The results obtained could also indicate bifunctional catalysis, with a free amino group involved in enamine formation being assisted by another protonated amino group for activation of the aldehyde towards aldolization. The fact that only four alkylated prolines were observed in the iminium trapping experiment corroborates this assumption. Bifunctional catalysis is an accepted hypothesis for the proline catalyzed aldol reaction supported by computational studies indicating that H-bonding activation of the aldehyde electrophile may lower the activation barrier for aldol addition by as much as 17 kcal mol^{-1}.[21]

Conclusion

Peptide dendrimer libraries were screened for aldolase reactivity using four different probes, including the 1,3-diketone **1a** suitable for enaminone formation with enamine reactive side chains, the fluorogenic coumarin ether of dihydroxyacetone **6** selective for enolization, and the two known retro-aldolase fluorogenic substrates **12** and **14**. Library **L1**, with core active-site lysines gave positive hits with diketone **1a**. Library **L2** with catalytic residues at the surface gave positive hits with diketone **1a** and with probe **6**. Most hit sequences showed *N*-terminal proline residues.

Peptide dendrimers selected from library **L1** showed aldolase activity with nitrobenzaldehyde and cyclohexanone as substrates. The activity must be attributed to primary amines from lysine side-chains at the dendrimer core since these are the only aldolase catalytic groups available. Peptide dendrimers selected from library **L2** catalyzed the aldol reaction of nitrobenzaldehyde with both acetone and cyclohexanone, under organic or aqueous conditions. Aldol (*S*)-**17** was formed from acetone with 61% ee with dendrimer **L2D1** (PK·PK·YL·IG) in DMSO, and *anti*-**20** was formed from cyclohexanone with 65% ee with dendrimer **L2K8** (EK·ED·IG·YA) in water–cyclohexanone mixture. In the case of acetone, the reaction was much faster in water (complete conversion in 3 h at 25 °C with 1 mol% catalyst) but not enantioselective. Dendrimers selected with the enolization probe **6** also showed good activity with dihydroxyacetone as the substrate, which was not found in any of the other dendrimers in the study. The reaction with dihydroxyacetone presumably involves general-base reactivity *via* an enolate intermediate.

The reactions with acetone and cyclohexanone involve an enamine intermediate, as evidenced by reductive trapping of the imminium with $NaBH_4$. Dendritic effects in catalysis were investigated using regular series dendrimers featuring the catalytic dyad Pro–Lys (**R2**) and Pro–Thr (**R1**) with increasing generation number. A positive dendritic effect was observed with both acetone (maximum activity per *N*-terminal proline residue with 2nd generation dendrimer **R1G2**) and cyclohexanone (maximum activity per free amino-group with 3rd generation dendrimer **R2G3**). The effect might be caused by the macromolecular nature of the peptide dendrimer as enzyme model, such as a hydrophobic microenvironment in the larger dendrimers favoring substrate binding and/or enamine formation, or by bifunctional catalysis.

The present study clearly points to the pyrrolidine ring as the residue of choice for catalyzing acetone aldolizations, and primary amines (either *N*-termini or lysine side-chains) for catalyzing cyclohexanone aldolizations in the peptide dendrimer enzyme model. The multivalent dendritic display is advantageous for catalytic efficiency, as evidenced by the positive dendritic effect on reaction rates observed in regular peptide dendrimer series of increasing generation number. On the other hand, the presence of multiple, stereochemically non-equivalent catalytic sites might be deleterious for obtaining stereoselectivity. Future experiments towards aldolase peptide dendrimer enzyme models will use analogs of library **L1** with a single core catalytic residue containing either a primary amine for cyclohexanone aldolization, or a pyrroldine for reactions with acetone.

Experimental section

General

Reagents were purchased in the highest quality available from Fluka, Sigma, Bachem, Novabiochem, NeoMPS or Aldrich. All solvents used in reactions were bought in *p.a.* quality or distilled and dried prior to use. Solvents for extractions were distilled from technical quality. Sensitive reactions were carried out under nitrogen or argon, the glassware being heated under HV. Chromatographic purifications (flash) were performed with silica gel 60 from Merck or Fluka (0.04 ± 0.063 nm; 230 ± 400 mesh ASTM). Preparative RP-HPLC (flow rate 100 mL min^{-1}) was performed with a Waters Delta Prep 4000 system with a Waters Prepak Cartridge (500 g) as column and Waters 486 Tunable Absorbance Detector. Semi-preparative RP-HPLC (flow rate 4 mL min^{-1}) was performed with a Water 510 Pump operated with a Waters Automated Gradient Controller and Jasco VU-2075 Plus Detector on a Vydac 218 TP (1.0 × 25 cm) column. Analytical RP-HPLC was performed on Waters 600E systems with a Waters Atlantis (4.6 mm × 100 mm, dC18, 5 μm) column, UV detection with Waters 996 photodiode array detector). Eluents for all systems were: A: water and 0.1% TFA; D: acetonitrile, water and TFA (3/2/0.1%). TLC monitoring was performed with Alugram SIL G/UV254 silica gel sheets (Macherey-Nagel), followed by coloration with cerium solution (10.5 g Ce(IV)sulfate, 21 g phosphomolybdic acid, 60 mL conc. H_2SO_4 in 900 mL water), anisaldehyde stain and heating or observation under a UV lamp. MS and HRMS analyses were provided by the mass spectrometry service of the Department of Chemistry and Biochemistry, University of Berne. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 300 (300 MHz) and DRX 500 or Avance 500 (500 MHz) instruments. Chemical shifts δ are given in ppm, coupling constants (J) in Hertz (Hz).

6-(4-Nitro-phenyl)hexane-2,4-dione (2)[2a]

To a solution of acetyl acetone (750 μL, 7.30 mmol) (freshly distilled) in dried THF (5 mL) was added under N_2 at room temperature a commercial solution of LDA (2N, 7.5 mL) The

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solution was warmed to 40 °C for 1 h. After cooling to −78 °C 4-nitrobenzyl bromide (1.50 g, 7.30 mmol) in HMPA (5 mL) was added dropwise *via* a syringe. The reaction was stirred for at −78 °C and followed by TLC. The solution was quenched carefully with water and evaporated to dryness, taken up in CH_2Cl_2, extracted with 1 N HCl, brine and water, dried ($MgSO_4$) and flash chromatographed using hexane–ethyl acetate (2 : 1) to give the title compound (673 mg, 40%). $R_f = 0.26$ (*n*-hexane–ethyl acetate (2 : 1)). ^{1}H NMR (300 MHz, $CDCl_3$) enol $\delta = 15.25$ (s, 1H), 8.13 (d, 2H, $J = 8.67$ Hz), 7.33 (s, 2H, $J = 8.67$ Hz), 5.49 (s, 1H), 3.05 (t, 2H, $J = 7.63$ Hz), 2.68 (t, 2H, $J = 7.63$ Hz), 2.08 (s, 3H) ppm. FAB MS(+): m/z 236 ($M^+ + 1$).

Succinic acid mono-(2-{ethyl-[4-(4-nitrophenylazo)phenyl]amino}ethyl) ester (5)[22]

To a solution of Disperse Red 1 **4** (200 mg, 0.636 mmol), Et_3N (117 μL, 0.837 mmol) and DMAP (8 mg, 0.0646 mmol) in CH_2Cl_2 (8 mL) was added succinic acid anhydride (76 mg, 0.761 mg). The reaction was followed by TLC. After stirring overnight the solution was evaporated to dryness and the residue was purified by flash chromatography (CH_2Cl_2–MeOH (20 : 1), 0.1% AcOH) yielding **5** as a red solid (86 mg, 33%). $R_f = 0.18$ (CH_2Cl_2–MeOH (20 : 1), 0.1% AcOH). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 8.33$ (d, 2H, $J = 9.05$ Hz), 7.93 (dd, 4H, $J = 6.60$, 8.95 Hz), 6.81 (d, 2H, $J = 9.24$ Hz), 4.34 (t, 2H, $J = 6.40$ Hz), 3.71 (t, 2H, $J = 6.22$ Hz), 3.54 (q, 2H, 7.16 Hz), 2.71–2.55 (m, 4H), 1.32 (t, 3H, $J = 6.97$ Hz) ppm. ESI MS(+): calcd for $[M + H]^+$ $C_{20}H_{23}N_4O_6^+$ 415.42, found 415.20.

N-[4-(3,5-Dioxohexyl)phenyl]succinamic acid 2-{ethyl-[4-(4-nitrophenylazo)phenyl]amino}ethyl ester (1a)

The diketone **2** was dissolved in CH_2Cl_2 and Pd/C (10 mole%) was added and the solution was degassed with N_2 three times. H_2 was bubbled through the solution for 1 min and the solution was stirred under H_2 (atm) for 2 h. The reaction was followed by TLC. The solution was filtered through celite and evaporated to give the crude amine **3** which was used directly for the next coupling step. A solution of amine **3** was taken up in CH_2Cl_2 and EDC, HOBt and **5** was added at 0 °C. The solution was stirred for 2 h at 0 °C and then it was allowed to warm up to room temperature overnight. The reaction was followed by TLC. An aqueous workup (1 N HCl, 10% $NaHCO_3$ and brine successively), following by drying with $MgSO_4$, evaporation and flash chromatography (CH_2Cl_2–MeOH (40 : 1 *v*/*v*)) yielded the title compound as a red solid (39 mg, 95%). M.p. = 125–127 °C. $R_f = 0.21$ (CH_2Cl_2–MeOH (20 : 1). IR (neat) $\tilde{\nu} = 3320$, 2899, 2360, 1727, 1667, 1589, 1515, 1411, 1387, 1335, 1313, 1160, 1139, 1106, 998, 858, 821 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) enol $\delta = 15.35$ (s, 1H), 8.33 (d. 2H, $J = 9.04$ Hz), 7.81 (dd, 4H, $J = 7.15$, 9.04 Hz), 7.35 (d, 2H, $J = 8.10$ Hz), 7.04 (d, 2H, $J = 8.1$ Hz), 6.67 (d, 2H, $J = 9.24$ Hz), 5.39 (s, 1H), 4.22 (t, 2H, $J = 6.21$ Hz), 3.62 (t, 2H, $J = 6.21$ Hz), 3.47 (m, 2H), 2.85–2.78 (m, 2H), 2.71–2.62 (m, 2H), 2.59–2.52 (m, 2H), 2.51–2.44 (m, 2H), 1.99 (s, 3H), 1.23 (t, 3H, $J = 6.74$ Hz) ppm. ^{13}C NMR (300 MHz, $CDCl_3$) enol $\delta = 193.4$, 172.9, 169.8, 169.3, 156.7, 151.3, 147.5, 147.5, 143.8, 137.1, 128.8, 126.3, 124.7, 122.7, 119.9, 111.5, 100.1, 61.7, 55.6, 48.7, 45.7, 39.9, 32.5, 31.8, 29.3, 12.3 ppm. ESI MS(+): calcd for $[M + H]^+$ $C_{32}H_{36}N_5O_7^+$ 602.26, found 602.46.

7-(Allyloxy)-2*H*-chromen-2-one (8)

A solution of umbelliferone **7** (810 mg, 5.00 mmol), allyl bromide (360 μL, 4.2 mmol) and potassium carbonate (690 mg, 5.00 mmol) in acetone (50 mL) was stirred overnight at reflux. The reaction mixture was evaporated to dryness and the residue taken up in ethyl acetate (50 mL). The organic phase was extracted with 1 N NaOH (50 mL) and brine (50 mL), dried (Na_2SO_4) and evaporated to yield a white solid (yield 950 mg, 85%). M.p. = 78–82 °C. $R_f = 0.42$ (*n*-hexane–ethyl acetate (2 : 1)). IR (neat) $\tilde{\nu} = 3081$, 1712, 1710, 1603, 1600, 1557, 1511, 1424, 1398, 1367, 1354, 1283, 1225, 1208, 1124, 1012, 994, 940, 892, 856, 838 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 7.59$ (d, $J = 9.4$ Hz, 1H), 7.39 (d, $J = 8.5$ Hz, 1H), 6.74 (dd, $J = 2.45$, 8.48 Hz, 2H), 6.76 (d, $J = 2.5$ Hz, 1H), 6.17 (d, $J = 9.4$ Hz, 1H), 5.96 (m, 1H), 5.35 (ddd, $J = 1.3$, 2.6 Hz, 17.2, 1H), 5.30 (ddd, $J = 1.5$, 3.0, 10.6 Hz, 1H), 4.51 (dt, $J = 1.4$, 5.3 Hz, 2H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$) $\delta = 162.5$, 161.8, 156.6, 144.0, 132.8, 129.4, 119.2, 113.9, 113.8, 102.4, 70.0 ppm. ESI MS(+): calcd for $[M + H]^+$ $C_{12}H_{11}O_3^+$ 203.07, found 203.03.

7-(2,3-Dihydroxypropoxy)-2*H*-chromen-2-one (9)

To a solution of **8** (800 mg, 4.00 mmol) in *tert*-butanol–water mixture (40 mL, 2 : 1), was added *N*-methylmorpholine-*N*-oxide (703 mg, 6.00 mmol) and a solution of osmium tetroxide (2.5% in *tert*-butanol, 0.3 mL). After stirring overnight, sodium sulfite solution 10% (30 mL) was added and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. After purification on silica gel (*n*-hexane–ethyl acetate (2 : 3)), the desired diol **9** was obtained as a white solid (890 mg, 95%), m.p. = 118–122 °C. $R_f = 0.17$ (*n*-hexane–ethyl acetate (2 : 3)). IR (neat) $\tilde{\nu} = 3309$, 3065, 2939, 1698, 1618, 1606, 1550, 1508, 1398, 1294, 1236, 1138, 1118, 1102, 1054, 1036, 1002, 944, 885, 837 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) $\delta =$ 7.79 (d, $J = 9.4$ Hz, 1H), 7.58 (d, $J = 8.7$ Hz, 1H), 7.02 (dd, $J =$ 2.5, 8.7 Hz, 1H), 6.96 (d, $J = 2.7$ Hz, 1H), 6.26 (d, $J = 9.4$ Hz, 1H), 3.96 (dd, $J = 6.26$, 11.1 Hz, 1H), 3.40 (m, 1H), 2.95 (m, 1H), 2.80 (m, 1H) ppm. ^{13}C NMR (300 MHz, CD_3OD) $\delta = 162.0$, 161.5, 156.1, 143.9, 129.4, 113.7, 113.3, 113.2, 102.0, 69.7, 50.2, 44.9 ppm. ESI MS(+): calcd for $[M + Na]^+$ $C_{12}H_{10}O_5Na^+$ 259.06, found 259.00.

7-(2-Hydroxypropoxy-3-*tert*-butyldimethylsilyloxy)-2*H*-chromen-2-one (10)

The diol **9** (890 mg, 3.9 mmol) was dissolved in dichloromethane–dimethylformamide (3 : 1) (50 mL), *tert*-butyldimethyl silyl chloride (570 mg, 3.7 mmol) and imidazole (385 mg, 5.7 mmol) were added and the mixture was stirred overnight. After addition of dichloromethane (50 mL) and water (100 mL) the organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography *n*-hexane–ethyl acetate (2 : 1) to give **10** (yield 533 mg, 58%) as an oil. $R_f = 0.67$ (*n*-hexane–ethyl acetate (2 : 1)). IR (neat) $\tilde{\nu} = 3320$, 2928, 2856, 1725, 1712, 1612, 1556, 1507, 1471, 1404, 1349, 1292, 1279, 1249, 1238, 1197, 1105, 1027, 937, 824, 775 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.59$ (d, $J = 9.5$ Hz, 1H), 7.32 (d, $J = 8.5$ Hz, 1H), 6.79 (dd, $J = 2.40$, 8.50 Hz, 1H), 6.75 (d, $J = 2.4$ Hz, 1H), 6.12 (d, $J = 9.50$ Hz, 1H), 3.96 (m, 3H), 3.68 (m, 3H), 0.80 (s, 9H), −0.03 (s, 6H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) $\delta = 143.7$, 129.2, 113.8, 113.2, 102.1,

70.3, 69.4, 63.9 ppm. ESI MS(+): calcd for $[M + H]^+$ $C_{18}H_{27}O_5Si^+$ 351.16, found 351.11.

7-(2-Oxopropoxy-3-*tert*-butyldimethylsilyloxy)-2*H*-chromen-2-one (11)

To oxalyl chloride (240 μL, 3.2 mmol) in dry CH_2Cl_2 (5 mL) at −78 °C was added dry dimethyl sulfoxide (410 μL, 5.8 mmol). After 15 min **10** (450 mg, 1.29 mmol) was added and the mixture was stirred for 1 h at −78 °C. Triethylamine (1170 μL, 8.39 mmol) was added and the mixture was stirred for 1.5 h. The reaction mixture was warmed to rt and quenched with sat. NH_4Cl (20 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was dried (Na_2SO_4), concentrated *in vacuo* and flash chromatographed (*n*-hexane–ethyl acetate, 2 : 1) to yield **11** as an oil (350 mg, 78%). $R_f = 0.60$ (*n*-hexane–ethyl acetate (2 : 1)). IR (neat) $\tilde{\nu}$ = 2928, 2856, 1725, 1608, 1508, 1424, 1402, 1348, 1277, 1232, 1109, 1055, 995, 893, 833, 775 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) $\delta = 7.50$ (d, $J = 9.4$ Hz, 1H), 7.26 (d, $J = 8.7$ Hz, 1H), 6.74 (dd, $J = 2.50, 8.70$ Hz, 1H), 6.59 (d, $J = 2.50$ Hz, 1H), 6.14 (d, $J =$ 9.60 Hz, 1H), 4.85 (s, 2H), 4.26 (s, 2H), 0.82 (s, 9H), −0.01 (s,6H) ppm. ^{13}C NMR (500 MHz, $CDCl_3$) $\delta = 204.9, 161.3, 161.2, 156.1, 143.6, 129.4, 114.1, 113.3, 102.0, 26.1, 18.6, 14.6, -5.2$ ppm. ESI MS(+): calcd for $C_{18}H_{25}O_5Si^+$ $[M + H]^+$ 349.15 found 349.08.

7-(3-Hydroxy-2-oxopropoxy)-2*H*-chromen-2-one (6)

A solution of **11** (40 mg, 0.115 mmol) in TFA–H_2O (9 : 1) (10 mL) was stirred for 1 h. The TFA–H_2O mixture was evaporated under high vacuum to yield an oily film which was taken up in H_2O–CH_3CN (85 : 15) (50 mL) and purified directly using preparative RP-HPLC (RP-HPLC conditions: H_2O–CH_3CN (85 : 15) with 0.1% TFA to H_2O–CH_3CN (50 : 50) in 70 min, $\lambda = 254$ nm and flow rate = 100 mL min^{-1}) to yield **6** (9 mg, 33%). M.p. 138–142 °C. $R_f = 0.31$ (*n*-hexane–ethyl acetate (2 : 1)). IR (neat) $\tilde{\nu}$ = 3422, 2922, 1699, 1615, 1558, 1507, 1418, 1400, 1353, 1283, 1230, 1158, 1123, 1010, 985, 836, 815 cm^{-1}. ^1H NMR (500 MHz, d_6-acetone) $\delta =$ 7.78 (d, 1H, $J = 9.60$ Hz), 7.49 (d, 1H, $J = 8.67$ Hz), 6.84 (dd, 1H, $J = 2.45, 8.67$ Hz), 6.09 (d, 1H, $J = 9.42$ Hz), 4.99 (s, 2H), 4.31 (s, 2H) ppm. ^{13}C NMR (500 MHz, d_6-acetone) $\delta = 206.2, 161.4, 160.6, 155.6, 144.6, 129.8, 113.1, 113.0, 101.7, 80.9, 66.1$ ppm. ESI MS(+): calcd for $C_{12}H_{11}O_5^+$ $[M + H]^+$ 235.0606 found 235.0605.

4-(4-Nitrophenyl)-4-hydroxybutan-2-one (17)[4a]

Catalysis in water. 50 μL of a 200 mM solution of 4-nitrobenzaldehyde in acetone and 50 μL of a 2 mM dendrimer catalyst solution in 100 mM aqueous bicine buffer pH = 8.5 was shaken in an Eppendorf® PP-tube for 2–3 h. Final concentrations: 100 mM aldehyde and 1 mM catalyst. 10 μL aliquots of the reaction mixture were diluted with 100 μL A and injected on analytical RP-HPLC running isocratic 66% A, 34% D.

Catalysis in DMSO. 50 μL of a 200 mM solution of 4-nitrobenzaldehyde in DMSO–acetone (4 : 1, *v*/*v*) and 50 μL of a 2 mM dendrimer solution in DMSO–acetone (4 : 1, *v*/*v*) buffered with *x* eq. of NMM (where *x* = number of TFA–amine salts for the dendrimer tested) was shaken in an Eppendorf® PP-tube for 36–72 h. Final concentrations: aldehyde 100 mM, catalyst 1 mM. 10 μL aliquots of the reaction mixture were diluted with 100 μL A and injected on analytical RP-HPLC. The aldol product was purified by diluting the reaction mixture with A and injecting on semi-preparative RP-HPLC and the pure aldol product was injected on chiral phase HPLC separating the enantiomers.

^1H NMR (300 MHz, $CDCl_3$) $\delta = 8.20$ (d, $J = 8.5$ Hz, 1 H), 7.55 (d, $J = 8.5$ Hz, 1 H), 5.25 (m, 1 H), 3.56 (d, $J = 3.2$ Hz, 1 H), 2.85 (m, 2 H), 2.25 (s, 3 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$) $\delta = 208.51, 150.04, 147.22, 126.37, 123.71, 123.57, 68.94, 51.43, 30.67$ ppm. Anal. chiral HPLC [Daicel Chiralpak AS, *i*PrOH–hexane (20 : 80), UV 254 nm, flow rate 2.0 mL min^{-1}]: t_R (major) = 8.47 min; t_R (minor) = 10.02 min.

4-(2-Bromophenyl)-1,3,4-trihydroxybutan-2-one (19)[10d]

Catalysis in water. 50 μL of a 200 mM solution of 2-bromobenzaldehyde in methanol, dihydroxyacetone (0.5 mmol, 45 mg) and 50 μL a 2 mM dendrimer catalyst solution in 100 mM aqueous bicine buffer pH = 8.5 was shaken in an Eppendorf® PP-tube for 66 h. Final concentrations: 5 M dihydroxyacetone, 100 mM aldehyde and 1 mM catalyst. 10 μL aliquots of the reaction mixture were diluted with 100 μL A and injected on analytical RP-HPLC separating the diastereomers. Anal. HPLC (Waters Atlantis, UV 254 nm, gradient 90% A, 10% D to 50% A, 50% D in 10 min), t_R (major, *syn*) = 4.96 min and t_R = (minor, *anti*) 4.37 min.

***syn*-19.** ^1H NMR (300 MHz, CD_3OD) $\delta = 7.70$ (d, $J = 8.3$ Hz, 1H), 7.56 (d, $J = 8.3$ Hz, 1H), 7.39 (app. t, 1H), 7.16–7.21 (m, 1H), 5.45 (d, $J = 2.07$ Hz, 1H), 4.59 (s, 2 H), 4.41 (d, $J = 2.07$ Hz, 1H) ppm. ^{13}C NMR (CD_3OD) $\delta = 213.31, 142.09, 133.79, 131.35, 130.54, 128.72, 122.67, 79.00, 74.72, 68.44$ ppm. HR ESI MS(+): calcd for $C_{10}H_{11}BrNaO_4$ 296.9738; found 296.9749. Anal. HPLC (254 nm; gradient 90% A, 10% D to 50% A, 50% D in 10 min): $t_R = 5.0$ min.

***anti*-19.** ^1H NMR (300 MHz, CD_3OD) $\delta = 7.50–7.77$ (m, 2H), 7.35 (app. t, 1H), 7.1–7.2 (m, 1H), 5.22 (d, $J = 4.9$ Hz, 1H), 4.35–4.50 (m, 3H) ppm. ^{13}C NMR (300 MHz, CD_3OD) $\delta = 212.17, 141.69, 133.91, 130.66, 130.60, 128.86, 124.16, 79.47, 75.89, 68.79$ ppm. HR ESI MS(+): calcd. For $C_{10}H_{11}BrNaO_4$ 296.9738; found 296.9732. Anal. HPLC (254 nm; gradient 90% A, 10% D to 50% A, 50% D in 10 min): $t_R = 4.4$ min.

2-[Hydroxy-(4-nitrophenyl)methyl]cyclohexanone (20)

Catalysis in DMSO. 100 μL of a 200 mM solution of 4-nitrobenzaldehyde in cyclohexanone and 100 μL of a 2 mM dendrimer solution in DMSO buffered with *x* eq. of NMM (where *x* = number of TFA–amine salts for the dendrimer tested) was shaken in an Eppendorf® PP-tube for 18 h. Final concentrations: Aldehyde 100 mM, catalyst 1 mM. 10 μL aliquots of the reaction mixture were diluted with 100 μL A and injected on analytical RP-HPLC. The aldol product was purified by diluting the reaction mixture with A and injecting on semi-preparative RP-HPLC separating the diastereomers. The pure aldol products were injected on chiral phase HPLC separating the enantiomers.

Catalysis in water. 50 μL of a 200 mM solution of 4-nitrobenzaldehyde in cyclohexanone and 50 μL a 2 mM dendrimer catalyst solution in 100 mM aqueous bicine buffer pH = 8.5 was shaken in an Eppendorf® PP-tube for 2 h. Final concentrations: 100 mM aldehyde and 1 mM catalyst. 10 μL aliquots of the

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reaction mixture were diluted with 100 μL A and injected on analytical RP-HPLC. The aldol product was purified by diluting the reaction mixture with A and injecting on semi-preparative RP-HPLC separating the diastereomers. The pure aldol products were injected on chiral phase HPLC separating the enantiomers.

***anti*-20.** IR (neat) $\tilde{\nu}$ = 3504, 1688, 1604, 1531, 1508, 1342, 1131, 1044, 855, 842, 800, 702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 8.22 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.7 Hz, 2H), 4.95 (d, J = 8.3 Hz, 1H), 4.09 (br, 1H), 2.32–2.71 (m, 3H), 2.12–2.29 (m, 1H), 1.31–1.87 (m, 5H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$) δ = 214.7, 145.6, 145.2, 127.8, 123.5, 74.0, 57.2, 42.7, 30.8, 27.6, 24.7 ppm. ESI MS(+): calcd for $C_{13}H_{15}NNaO_4$ 272.3; found 272.5. Anal. HPLC (268 nm; gradient 90% A, 10% D to 10% A, 90% D in 15 min): t_R = 11.3 min. Anal. chiral HPLC [Daicel Chiralpak OD–H, *i*PrOH–hexane (10 : 90), UV 268 nm, flow rate 1.5 mL min^{-1}]: t_R (major); = 16.1 min. t_R (minor) = 22.5.

***syn*-20.** IR (neat) $\tilde{\nu}$ = 3491, 1693, 1602, 1508, 1447, 1343, 1186, 1131, 1091, 852, 796, 703 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 8.22 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.9 Hz, 2H), 5.45 (d, J = 2.1 Hz, 1H), 3.05 (br, 1H), 2.51–2.61 (m, 1H), 2.29–2.49 (m, 2H), 1.95–2.11 (m, 1H), 1.41–1.82 (m, 5H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$) δ = 214.0, 144.6, 143.8, 126.6, 123.5, 70.2, 56.8, 42.6, 27.8, 25.9, 24.8 ppm. ESI MS(+): calcd for $C_{13}H_{15}NNaO_4$ 272.3; found 272.4. Anal. HPLC (268 nm; gradient 90% A, 10% D to 10% A, 90% D in 15 min): t_R = 11.7 min. Anal. chiral HPLC [Daicel Chiralpak OD–H, *i*PrOH–hexane (10 : 90), UV 268 nm, flow rate 1.5 mL min^{-1}]: t_R (minor) = 13.5 min; t_R (major) = 15.1 min.

Library synthesis and screening

Coupling of the Fmoc-protected amino acids. The resin was washed and swollen inside the reactor with DCM (2 × 5 mL) and DMF (1 × 5 mL). The NovasynTGR (Tentagel with Rink linker) (0.25 mmol g^{-1}) was acylated with 2.5 equivalents of *N*-Fmoc amino acid in the presence of 2.5 equivalents of PyBOP ((benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) and 6 equivalents of DIEA (*N*,*N*′-diisopropylethylamine) in DMF. After 2^g × 60 min (where g = generation number) the resin was washed (3 × each) with DMF, DCM and MeOH and controlled with the TNBS (trinitrobenzenesulfonic acid) or chloranil test followed by acetylation.

Resin mixing and splitting. The resin was suspended in DMF–DCM (2 : 1, *v*/*v*), and mixed *via* nitrogen bubbling for 15 min, and then distributed in four equal portions.

On-bead assay with diketone 1a. 50 mg library resin was swollen overnight in 20 mM DMSO–PBS buffer pH = 7.4 (1 : 1, *v*/*v*). The swelling mixture was removed by filtration and 1 mL of 50 μM solution of **1a** in 20 mM DMSO–PBS buffer pH = 7.4 (1 : 1, *v*/*v*) was added. The resin was shaken for 30 min and washed extensively with PBS buffer, DMSO, DMF, MeOH, DCM, MeOH, DMF and finally with PBS buffer again (3 × each). A suspension of the resin in DMF was transferred to a silica gel plate and the beads were observed under a microscope. Single red colored beads were transferred *via* a syringe needle to amino acid analysis vials.

On-bead assay with enolization probe 6. 50 mg library resin was swollen overnight in 20 mM bicine buffer pH = 8.5. The swelling mixture was removed by filtration and 1 mL of 100 μM solution of **6** in 20 mM bicine buffer pH = 8.5 with 1% acetonitrile was added. The bead suspension was then plated out onto a silica gel plate and incubated for 40 min. The beads were observed under a microscope with a UV lamp irradiating at 365 nm. Single blue fluorescent beads were transferred *via* a syringe needle to amino acid analysis vials.

On bead assay with retro-aldolase substrates 12 and 14. 50 mg library resin was swollen overnight in 20 mM borate buffer pH = 8.8. The swelling mixture was removed by filtration and 1 mL of 100 μM solution of **12** or **14** in 20 mM borate buffer pH = 8.8 with 5% acetonitrile was added. The bead suspension was then plated out onto a silica gel plate and incubated for up to 2 h. The beads were observed under a microscope with a UV lamp irradiating at 365 nm. No hits were observed.

Bead analysis. Single dendrimer-containing resin beads were hydrolyzed with aqueous HCl (6 M) at 110 °C for 22 h, and their amino acid composition was determined quantitatively by HPLC after derivatization with phenyl isothiocyanate (PITC). Such amino acid analyses are routine for protein composition analysis. The analysis detects as little as 5 pmol per amino acid, which is sufficient for single resin bead analysis, as these contain 50–200 pmol of dendrimers. False positives are most likely due to manipulation errors during bead picking. Sometimes more than one bead is transferred to the pipette, and the fluorescence staining is diluted when the beads are washed down the pipette used for picking, which does not allow staining to be rechecked afterwards.

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

***N*-Acetylation.** The resin was acetylated with a solution of acetic acid anhydride–DCM (1 : 1, *v*/*v*) for 10 min. After filtration, the procedure was repeated and then washed (3 × each) with DMF, DCM and MeOH.

TFA cleavage. The cleavage was carried out using TFA–H_2O–TIS (triisopropylsilane) as a (95 : 2.5 : 2.5, *v*/*v*) solution for 6 h. The peptide was precipitated with methyl *tert*-butyl ether then dissolved in water–acetonitrile mixture. All the dendrimers were purified by preparative RP-HPLC.

Yields, purification method and characterization of all dendrimers

The dendrimers were synthesized using the same conditions as for the library synthesis.

Dendrimer **L1D5** $(Ac–Glu–Tyr)_8(Dap–Arg–Thr)_4(Dap–Phe–Lys)_2(Dap–Ser–Lys)$: from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L1D5** was obtained as colorless foamy solid after preparative HPLC purification (18 mg, 9%, as TFA-salt); anal. RP-HPLC: t_R = 4.75 min; ESI MS(+): calcd for $C_{228}H_{326}N_{60}O_{74}$: 5088.35, found: 5088.38.

Dendrimer **L1D6** $(Ac–Arg–Tyr)_8(Dap–Glu–Tyr)_4(Dap–Phe–Lys)_2(Dap–Ser–Lys)$: from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L1D6** was obtained as colorless foamy solid after preparative HPLC purification (21 mg, 10%, as TFA-salt); anal. RP-HPLC (70% A, 30% D to 30% A, 70% D in 10 min):

t_R = 4.44 min; ESI MS(+): calcd for $C_{252}H_{352}N_{72}O_{66}$: 5444.65, found: 5444.25.

Dendrimer **L2D1** (Pro–Lys)$_8$(Dap–Pro–Lys)$_4$(Dap–Tyr–Leu)$_2$(Dap–Ile–Gly): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2D1** was obtained as colorless foamy solid after preparative HPLC purification (86 mg, 25%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 10 min): t_R = 6.74 min; ESI MS(+): calcd for $C_{191}H_{327}N_{56}O_{40}$: 4046.00, found: 4045.88.

Dendrimer **L2D5** (Ser–Lys)$_8$(Dap–Pro–Arg)$_4$(Dap–Ile–Gly)$_2$-(Dap–Tyr–Gly): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2D5** was obtained as colorless foamy solid after preparative HPLC purification (20 mg, 8%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 10 min): t_R = 7.87 min; ESI MS(+): calcd for $C_{166}H_{302}N_{64}O_{47}$: 3942.00, found: 3942.12.

Dendrimer **L2D6** (Ser–Lys)$_8$(Dap–Ser–Lys)$_4$(Dap–Tyr–Gly)$_2$-(Dap–Phe–Gly): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2D6** was obtained as colorless foamy solid after preparative HPLC purification (33 mg, 13%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 10 min): t_R = 6.83 min; ESI MS(+): calcd for $C_{162}H_{284}N_{56}O_{52}$: 3846.15, found: 3846.13.

Dendrimer **L2D7** (Pro–Lys)$_8$(Dap–Glu–Arg)$_4$(Dap–β-Ala–Gly)$_2$-(Dap–Phe–Val): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2D7** was obtained as colorless foamy solid after preparative HPLC purification (19 mg, 7%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 10% A, 90% D in 15 min): t_R = 5.13 min; ESI MS(+): calcd for $C_{177}H_{306}N_{64}O_{46}$: 4064.36, found: 4064.38.

Dendrimer **L2K4** (Pro–Lys)$_8$(Dap–Thr–His)$_4$(Dap–Tyr–Gly)$_2$-(Dap–Phe–Val): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2K4** was obtained as colorless foamy solid after preparative HPLC purification (11 mg, 4%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 10% A, 90% D in 15 min): t_R = 3.43 min; ESI MS(+): calcd for $C_{185}H_{295}N_{61}O_{43}$: 4060.28, found: 4060.38.

Dendrimer **L2K7** (Pro–Lys)$_8$(Dap–Ser–Arg)$_4$(Dap–β-Ala–Val)$_2$-(Dap–Tyr–Leu): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2K7** was obtained as colorless foamy solid after preparative HPLC purification (28 mg, 10%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 10% A, 90% D in 15 min): t_R = 5.34 min; ESI MS(+): calcd for $C_{176}H_{313}N_{65}O_{42}$: 4010.42, found: 4010.75.

Dendrimer **L2K8** (Glu–Lys)$_8$(Dap–Glu–Asp)$_4$(Dap–Ile–Gly)$_2$-(Dap–Tyr–Ala): from NovasynTGR (200 mg, 0.25 mmol g^{-1}), **L2K8** was obtained as colorless foamy solid after preparative HPLC purification (17 mg, 6%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 10% A, 90% D in 15 min): t_R = 4.10 min; ESI MS(+): calcd for $C_{173}H_{388}N_{53}O_{70}$: 4228.05, found: 4228.50.

Dendrimer **R1G1** (Pro–Thr)$_2$(Dap–Pro–Thr): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R1G1** was obtained as colorless foamy solid after preparative HPLC purification (7 mg, 79%, as TFA-salt); anal. RP-HPLC (100% A, 0% D to 10% A, 90% D in 15 min): t_R = 4.92 min; ESI MS(+): calcd for $C_{30}H_{51}N_9O_{10}$: 696.78, found: 697.38.

Dendrimer **R1G2** (Pro–Thr)$_4$(Dap–Pro–Thr)$_2$(Dap–Pro–Thr): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R1G2** was obtained as colorless foamy solid after preparative HPLC purification (17 mg, 58%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 15 min): t_R = 4.75 min; ESI MS(+): calcd for $C_{71}H_{119}N_{21}O_{24}$: 1661.84, found: 1661.88.

Dendrimer **R1G3** (Pro–Thr)$_8$(Dap–Pro–Thr)$_4$(Dap–Pro–Thr)$_2$(Dap–Pro–Thr): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R1G3** was obtained as colorless foamy solid after preparative HPLC purification (14 mg, 26%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 15 min): t_R = 5.22 min; ESI MS(+): calcd for $C_{156}H_{256}N_{45}O_{52}$: 3591.97, found: 3591.84.

Dendrimer **R2G1** (Pro–Lys)$_2$(Dap–Pro–Lys): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R2G1** was obtained as colorless foamy solid after preparative HPLC purification (9 mg, 85%, as TFA-salt); anal. RP-HPLC (100% A, 0% D to 10% A, 90% D in 15 min): t_R = 4.10 min; ESI MS(+): calcd for $C_{36}H_{67}N_{12}O_7$: 777.99, found: 778.50.

Dendrimer **R2G2** (Pro–Lys)$_4$(Dap–Pro–Lys)$_2$(Dap–Pro–Lys): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R2G2** was obtained as colorless foamy solid after preparative HPLC purification (17 mg, 58%, as TFA-salt); anal. RP-HPLC (95% A, 5% D to 70% A, 30% D in 15 min): t_R = 6.38 min; ESI MS(+): calcd for $C_{86}H_{155}N_{28}O_{17}$: 1851.32, found: 1851.33.

Dendrimer **R2G3** (Pro–Lys)$_8$(Dap–Pro–Lys)$_4$(Dap–Pro–Lys)$_2$-(Dap–Pro–Lys): from NovasynTGR (100 mg, 0.25 mmol g^{-1}), **R2G3** was obtained as colorless foamy solid after preparative HPLC purification (18 mg, 20%, as TFA-salt); anal. RP-HPLC (90% A, 10% D to 50% A, 50% D in 15 min): t_R = 5.794 min; ESI MS(+): calcd for $C_{186}H_{331}N_{60}O_{37}$: 3997.97, found: 3998.00.

Reductive alkylation of R2G3

Dendrimer **R2G3** (6.0 mg, 1 μmol) was stirred in 100 μL 100 mM bicine buffer pH 8.5. Then, 100 μL of acetone was added and the resulting mixture was stirred for 3 h at room temperature. A solution of $NaBH_4$ (4.0 mg, 100 μmol) in H_2O (100 μL) was added and the mixture was stirred overnight at room temperature. Then, acetic acid was added (100 μL) and the mixture was lyophilized. The ^{1}H NMR spectrum was recorded.

Reductive alkylation of L1D5

Dendrimer **L1D5** (5.4 mg, 1 μmol) was stirred in 100 μL 100 mM bicine buffer pH 8.5. Then, 100 μL of cyclohexanone was added and the resulting mixture was stirred for 3 h at room temperature. A solution of $NaBH_4$ (4.0 mg, 100 μmol) in H_2O (100 μL) was added and the mixture was stirred overnight at room temperature. Then, acetic acid was added (100 μL) and the mixture was lyophilized. The ^{1}H NMR spectrum was recorded.

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