Peptide dendrimer enzyme models for ester hydrolysis and aldolization prepared by convergent thioether ligation

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Supporting Information

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Materials and Reagents

All reagents were purchased in the highest quality available either from Sigma Aldrich, Bachem, Acors Organics, Carl Roth GmbH or GE Healthcare. PyBOP, amino acids and their derivatives were purchased from Advanced ChemTech (Giessen, Germany) or Novabiochem. For SPPS amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Amb-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Dap(Fmoc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-DGlu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Pro-OH, Fmoc-DPro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH. Tenta Gel S RAM® resin (loading: 0.24 or 0.26 mmol·g⁻¹) was purchased from Rapp Polymere (Tübingen, Germany). Peptide dendrimer syntheses were performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper. Ligation reactions were done in solution using standard glass ware. All solvents used in reactions on solid phase and in solution were bought in p.a. quality and distilled prior to use. Analytical RP-HPLC was performed with a Waters Chromatography System (996 Photo diode array detector) using an Atlantis® column (dC18, 5 µm, 4.6 × 100 mm, flow rate 1.4 mL·min⁻¹) or a chiral Daicel Chiralpak AS® column (amylose tris[(S)-α-methylbenzylcarbamate] coated on a silica support, 10 µm, 4.6 × 250 mm, flow rate 2.0 mL·min⁻¹) for ee determination. Analytical RP-UPLC was performed with an Ultimate 3000 Rapid Separation LC System (DAD-3000RS diode array detector) using an Acclaim® RSLC 120 C18 column (2.2 µm, 120 Å, 3.0 × 50 mm, flow 1.2 ml·min⁻¹) from Dionex. Data recording and processing was either done with Waters Empower2 software (analytical RP-HPLC) or with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). Preparative RP-HPLC was performed with a Waters Prep LC4000 Chromatography System using a Delta-Pak® column (C18, 15 µm, pore size 300 Å, flow rate 80 mL·min⁻¹) or an Atlantis OBD column (dC18, 5 µm, 19 × 100 mm, flow rate 20 mL·min⁻¹). Compounds
were detected by UV absorption at 214 nm using a Waters 486 Tunable Absorbance Detector. The following elution solutions were used for all RP-HPLC: A mQ-deionized H₂O with 0.1% TFA; D mQ-deionized H₂O/HPLC-grade CH₃CN (40:60) with 0.1% TFA. MS spectra, recorded on either a Thermo Scientific LTQ OrbitrapXL, an AB Sciex QTrap or an AB Sciex API 2000 (LC-MS) and amino acid analysis were provided by the MS and protein analytical services of the Department of Chemistry and Biochemistry at the University of Berne. ¹H-NMR were recorded on a Bruker DRX500. Kinetic measurements were carried out using a CytoFluor Series 4000 multi-well plate reader from PerSeptive Biosystems. SDS-PAGE was done with equipment purchased from Bio-Rad.
Dendrimer Spectra

Peptide Dendrimer G2C1

\((\text{ClAc-His-Ser})_4(D\text{ap-His-Ser})_2D\text{ap-His-Ser-NH}_2\)

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G2C1 was obtained as foamy colourless solid after preparative RP-HPLC (156.7 mg, 53.1 µmol, 44%). Analytical RP-HPLC: \(t_R = 9.071\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

\(\text{C}_{80}\text{H}_{109}\text{Cl}_4\text{N}_{35}\text{O}_{28}\) found/calc. 2150.0/2150.8 [M]\textsuperscript{+}; 2189.0/2189.9 [M+K]\textsuperscript{+}.

Mass spectrum, MS (ESI+):

![Analytical RP-HPLC chromatogram](image)

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2C2

(\text{ClAc-Pro-Ser})_4(\text{Dap-Pro-Ser})_2\text{Dap-Pro-Ser-NH}_2

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G2C2 was obtained as foamy colourless solid after preparative RP-HPLC (104.0 mg, 55.6 \mu\text{mol}, 46\%). Analytical RP-UPLC: \(t_R = 1.405\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI\textsuperscript{+}):

\(\text{C}_{73}\text{H}_{109}\text{Cl}_{4}\text{N}_{21}\text{O}_{28}\) found/calc. 1869.6/1870.6 \[\text{M}^+\].

Mass spectrum, MS (ESI\textsuperscript{+}):

![Mass spectrum](image)

Analytical RP-UPLC chromatogram:

![Analytical RP-UPLC chromatogram](image)
Peptide Dendrimer G3C1

\[(\text{ClAc-His-Ser})_8(\text{Dap-His-Ser})_4(\text{Dap-His-Ser})_2\text{Dap-His-Ser-NH}_2\]

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g\(^{-1}\)), G3C1 was obtained as foamy yellowish solid after preparative RP-HPLC (116.2 mg, 18.4 µmol, 15%). Analytical RP-HPLC: \(t_R = 9.766\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

\[\text{C}_{172}\text{H}_{233}\text{Cl}_8\text{N}_{75}\text{O}_{60}\text{ found/calc. 4594.0/4594.8 [M]+.}\]

Mass spectrum, MS (ESI+):

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G3C2

(\text{ClAc-Pro-Ser})_8(Dap-Pro-Ser)_4(Dap-Pro-Ser)_2Dap-Pro-Ser-NH_2

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g\(^{-1}\)), G3C2 was obtained as foamy yellowish solid after preparative RP-HPLC (119.9 mg, 30.0 µmol, 25%). Analytical RP-UPLC: \(t_R = 1.515\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI+):

\[\text{C}_{157}\text{H}_{233}\text{Cl}_{8}\text{N}_{45}\text{O}_{60}\] found/calc. 3993.9/3994.4 \([M]^+\); 4015.4/4017.4 \([M+Na]^+\); 4034.3/4033.5 \([M+K]^+\).

Mass spectrum, MS (ESI+):

Analytical RP-UPLC chromatogram:
Peptide Dendrimer G3C3

\((\text{ClAc-Pro-Ser})_8(\text{Lys-Pro-Ser})_4(\text{Lys-Pro-Ser})_2\text{Lys-Pro-Ser-NH}_2\)

From Tenta Gel S RAM\(^\circledR\) resin (800 mg, 0.24 mmol \cdot g\(^{-1}\)), \textbf{G3C3} was obtained as foamy colourless solid after preparative RP-HPLC (149.7 mg, 34.9 µmol, 18%). Analytical RP-UPLC: \(t_R = 1.510\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI+):

\[
\text{C}_{178}\text{H}_{275}\text{Cl}_8\text{N}_{45}\text{O}_{60} \text{ found/calc. 4287.9/4289.0 [M]}^+; 4310.0/4312.0 [M+Na]^+; 4326.7/4328.1 [M+K]^+; 4348.8/4351.1 [M+Na+K]^+; 4364.1/4367.2 [M+2K]^+.
\]

Mass spectrum, MS (ESI+):
Peptide Dendrimer G2M1

$\text{(Ac-His-Ser)}_4\text{(Dap-His-Ser)}_2\text{Dap-Cys-NH}_2$

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g$^{-1}$), G2M1 was obtained as foamy colourless solid after preparative RP-HPLC (123.0 mg, 47.8 µmol, 40%). Analytical RP-HPLC: $t_R = 7.682$ min (A/D 100/0 to 50/50 in 10 min, $\lambda = 214$ nm). MS (ESI\textsuperscript{+}): C$_{74}$H$_{106}$N$_{32}$O$_{26}$S found/calc. 1891.0/1891.9 [M]$^+$.

Mass spectrum, MS (ESI\textsuperscript{+}):

![Analytical RP-HPLC chromatogram](image)

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M2

\((\text{Ac-His-Ser})_4(\text{Dap-His-Ser})_2\text{Dap-His-Ser-Cys-NH}_2\)

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G2M2 was obtained as foamy colourless solid after preparative RP-HPLC (147.9 mg, 50.7 µmol, 42%). Analytical RP-HPLC: \(t_R = 7.569\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

C\textsubscript{83}H\textsubscript{118}N\textsubscript{36}O\textsubscript{29}S found/calc. 2116.0/2116.1 [M]\textsuperscript{+}.

Mass spectrum, MS (ESI+):

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M3

(Pro-Ser)$_4$(Dap-Pro-Ser)$_2$Dap-Pro-Ser-Cys-NH$_2$

From Tenta Gel S RAM$^\text{®}$ resin (500 mg, 0.24 mmol·g$^{-1}$), G2M3 was obtained as foamy colourless solid after preparative RP-HPLC (118.8 mg, 56.0 µmol, 47%). Analytical RP-HPLC: $t_R = 9.142$ min (A/D 100/0 to 50/50 in 10 min, $\lambda = 214$ nm). MS (ESI+):

C$_{68}$H$_{110}$N$_{22}$O$_{25}$S found/calc. 1668.0/1667.8 [M]$^+$; 1709.0/1708.8 [M+2H+K]$^+$. 

Mass spectrum, MS (ESI+):

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M4

\((\text{Pro-Asp})_4(\text{Dap-Pro-Asp})_2\text{Dap-Pro-Asp-Cys-NH}_2\)

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g\(^{-1}\)), G2M4 was obtained as foamy colourless solid after preparative RP-HPLC (110.5 mg, 59.3 µmol, 49%). Analytical RP-HPLC: \(t_R = 9.258\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

C\(_{75}\)H\(_{110}\)N\(_{22}\)O\(_{32}\)S found/calc. 1863.0/1863.9 \([M]^+\); 1902.0/1903.0 \([M+K]^+\).

Mass spectrum, MS (ESI+):

![Mass spectrum](image)

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M5

$\text{(Pro-Asp)}_4(\text{Dap-Pro-Asp})_2\text{Dap-Cys-NH}_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g$^{-1}$), G2M5 was obtained as foamy colourless solid after preparative RP-HPLC (109.0 mg, 66.0 µmol, 55%). Analytical RP-HPLC: $t_R = 8.330$ min (A/D 100/0 to 50/50 in 10 min, $\lambda = 214$ nm). MS (ESI+):

$\text{C}_{66}\text{H}_{98}\text{N}_{20}\text{O}_{28}\text{S}$ found/calc. 1651.0/1651.7 [M]$^+$; 1690.0/1690.8 [M+K]$^+$. 

Mass spectrum, MS (ESI+):

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M6

\((\text{Pro-Lys})_4(\text{Dap-Pro-Lys})_2\text{Dap-Pro-Lys-Cys-NH}_2\)

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G2M6 was obtained as foamy colourless solid after preparative RP-HPLC (195.9 mg, 71.2 µmol, 59%). Analytical RP-HPLC: \(t_R = 9.523\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

\[\text{C}_{89}\text{H}_{159}\text{N}_{29}\text{O}_{18}\text{S}\]

found/calc. 1955.0/1955.5 \([M]^+\); 1996.0/1994.6 \([M+K]^+\); 2069.0/2069.5 \([M+TFA]^+\); 2183.0/2183.5 \([M+2TFA]^+\); 2297.0/2297.5 \([M+3TFA]^+\); 2411.0/2411.5 \([M+4TFA]^+\); 2525.0/2525.5 \([M+5TFA]^+\).

Mass spectrum, MS (ESI+):

![Mass spectrum](image)

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G2M7

\((\text{DPro-Pro-Glu})_4(\text{Dap-Pro-Asp})_2\text{Dap-Pro-Asp-Cys-NH}_2\)

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G2M7 was obtained as foamy colourless solid after preparative RP-HPLC (149.9 mg, 54.2 µmol, 45%). Analytical RP-UPLC: \(t_R = 1.267\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI+):

\(\text{C}_{99}\text{H}_{146}\text{N}_{26}\text{O}_{36}\text{S}\) found/calc. 2307.5/2308.4 [M]\textsuperscript{+}.

Mass spectrum, MS (ESI+):

Analytical RP-UPLC chromatogram:
Peptide Dendrimer G3M1

\((\text{Pro-Ser})_8(\text{Dap-Pro-Ser})_4(\text{Dap-Pro-Ser})_2\text{Dap-Pro-Ser-Cys-NH}_2\)

From Tenta Gel S RAM\textsuperscript{®} resin (500 mg, 0.24 mmol·g\textsuperscript{-1}), G3M1 was obtained as foamy colourless solid after preparative RP-HPLC (149.0 mg, 42.8 µmol, 36%). Analytical RP-HPLC: \(t_R = 8.995\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm). MS (ESI+):

\[
\text{C}_{144}\text{H}_{230}\text{N}_{46}\text{O}_{53}\text{S} \text{found/calc. 3485.0/3485.7 [M]}^+; 3527.0/3526.8 [M+2H]^+; 3568.0/3567.9 [M+4H]^+; 3610.0/3610.0 \text{[M+7H]^+}; 3650.0/3650.2 \text{[M+8H]^+}.
\]

Mass spectrum, MS (ESI+):

Analytical RP-HPLC chromatogram:
Peptide Dendrimer G4E

$\text{(Ac-Glu-Ala)}_{16}(\text{Lys-Amb-Tyr})_8(\text{Dap-Cys(Asp)}-\text{x-His-Ser})_4(\text{Dap-His-Ser})_2\text{Dap-His-Ser-NH}_2$

From starting materials G2C1 and B1G2 using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), G4E was obtained as colourless solid after preparative RP-HPLC (8.23 mg). Amino Acid Analysis: protein content 52.5% (4.32 mg, 0.40 µmol, yield 28%); found/calc. (%) Glu 17.6/18.8, Ala 17.3/18.8, Dap 11.8/8.2, Tyr 10.0/9.4, Amb 9.5/9.4, Lys 9.2/9.4, His 7.8/8.2, Ser 7.1/8.2, Asp 4.8/4.7, CM-Cys 4.8/4.7 (AMBA $t_R = 7.03$ min, 1552.4 pmol, Dap coeluted with Phe, 1941.7 pmol). Analytical RP-UPLC: $t_R = 1.467$ min (A/D 100/0 to 0/100 in 5 min, $\lambda = 214$ nm). MS (ESI-): $C_{464}H_{629}N_{119}O_{160}S_4$ (4-fold ligation product) found/calc. 10562.0/10561.9 [M]; 10624.0/10624.0 [M+Na+K]. No incomplete ligation products observed (3-fold ligation product calc. 8459.1 [M], 2-fold ligation product calc. 6356.3 [M]). $D = 10.25 \cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$; $R_h = 1.98 \pm 0.08$ nm.

Mass spectrum, MS (ESI-):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:
$^1$H-NMR:

![NMR spectrum image]

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Peptide Dendrimer G5E

(Ac-Glu-Ala)$_{32}$(Lys-Amb-Tyr)$_{16}$(Dap-Cys(Asp)-x-His-Ser)$_{8}$(Dap-His-Ser)$_{4}$(Dap-His-Ser)$_{2}$Dap-His-Ser-NH$_{2}$

From starting materials G3C1 and B1G2 using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), G5E was obtained as foamy colourless solid after preparative RP-HPLC (13.3 mg). Amino Acid Analysis: protein content 32.3% (4.29 mg, 0.19 µmol, yield 30%); found/calc. (%) Ala 19.2/18.5, Glu 15.2/18.5, Dap 13.1/8.7, Tyr 9.8/9.2, His 9.7/8.7, Lys 9.2/9.2, Amb 9.0/9.2, Ser 8.7/8.7, CM-Cys 4.2/4.6, Asp 1.9/4.6 (Dap coeluted with Phe, 1324.4 pmol). Analytical RP-UPLC: t$_{R}$ = 1.480 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI-): C$_{940}$H$_{1273}$N$_{243}$O$_{324}$S$_{8}$ (8-fold ligation product, main peak) found/calc. 21417.0/21416.1 [M-H]$^{-}$, C$_{844}$H$_{1143}$ClN$_{222}$O$_{291}$S$_{7}$ (7-fold ligation product with one unreacted chloroacetyl group, peak height 33% of main peak) found/calc. 19311.0/19313.3 [M-H]$^{-}$, no 6-fold ligation product observed (calc. 17210.5 [M-H]$^{-}$). D = 8.63·10$^{-11}$ m$^{2}$·s$^{-1}$; R$_{h}$ = 2.35 ± 0.02 nm.

Mass spectrum, MS (ESI-):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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Total: 189,773 1924,580 11627,32
\(^1\text{H-NMR:}\)
Peptide Dendrimer G4H1

\((\text{Ac-His-Ser})_{16}(\text{Dap-His-Ser})_{8}(\text{Dap-Cys-}x\text{-His-Ser})_{4}(\text{Dap-His-Ser})_{2}\text{Dap-His-Ser-NH}_2\)

From starting materials G2C1 and G2M1 using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), G4H1 was obtained as foamy colourless solid after preparative RP-HPLC (4.02 mg). Amino Acid Analysis: protein content 46.6% (1.87 mg, 0.17 µmol, yield 12%); found/calc. (%) His 47.8/38.3, Ser 31.5/38.3, Dap 16.3/18.5, CM-Cys 4.4/4.9. Analytical RP-UPCL: \(t_R = 1.228\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI+): \(\text{C}_{376}\text{H}_{529}\text{N}_{163}\text{O}_{132}\text{S}_4\) (4-fold ligation product) found/calc. 9572.0/9572.5 \([M]^+\). No incomplete ligation products observed (3-fold ligation product calc. 7717.1 \([M]^+\), 2-fold ligation product calc. 5861.6 \([M]^+\)). \(D = 7.70 \times 10^{-11}\) m²·s⁻¹, \(R_h = 2.63 \pm 0.06\) nm.

Mass spectrum, MS (ESI+):

![Mass spectrum, MS (ESI+)](image)
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<td>480.82</td>
<td>1768.3</td>
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Total: 11532.76
$^1$H-NMR:

Representative Michaelis-Menten plots for determination of $k_{cat}$ and $K_M$:

**MM G4H1 with 8-acetoxypyrene-1,3,6-trisulfonate (citrate pH 5.5)**

**MM G4H1 with isobutyryl fluorescein (citrate pH 5.5)**
**Peptide Dendrimer G4H2**

$$(\text{Ac-His-Ser})_{16}(\text{Dap-His-Ser})_8(\text{Dap-His-Ser-Cys-x-His-Ser})_4(\text{Dap-His-Ser})_2\text{Dap-His-Ser-NH}_2$$

From starting materials G2C1 and G2M2 using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), G4H2 was obtained as foamy colourless solid after preparative RP-HPLC (2.24 mg). Amino Acid Analysis: protein content 59.7% (1.34 mg, 0.11 µmol, yield 8%); found/calc. (%) His 48.8/39.3, Ser 32.1/39.3, Dap 14.7/16.9, CM-Cys 4.3/4.5. Analytical RP-UPLC: $t_R = 1.230$ min (A/D 100/0 to 0/100 in 5 min, $\lambda = 214$ nm). MS (ESI+): $C_{412}H_{577}N_{179}O_{144}S_4$ (4-fold ligation product, main peak) found/calc. 10471.0/10470.4 [M+H]$^+$; 10511.0/10510.5 [M+2H+K]$^+$. $C_{329}H_{460}ClN_{143}O_{115}S_3$ (3-fold ligation product with one unreacted chloroacetyl group, peak height 9% of main peak) found/calc. 8392.0/8390.7 [M+H]$^+$, no 2-fold ligation product observed (calc. 6310.1 [M]$^+$). $D = 6.94 \times 10^{-11}$ m$^2$·s$^{-1}$; $R_h = 2.92 \pm 0.09$ nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:
$^1$H-NMR:

Representative Michaelis-Menten plots for determination of $k_{cat}$ and $K_M$:
Peptide Dendrimer G5H

\((\text{Ac-His-Ser})_{32}(\text{Dap-His-Ser})_{16}(\text{Dap-His-Ser-Cys-x-Pro-Ser})_{8}(\text{Dap-Pro-Ser})_{4}(\text{Dap-Pro-Ser})_{2}\text{Dap-Pro-Ser-NH}_2\)

From starting materials G3C2 and G2M2 using the general procedure described above (solvent: DMF/H\(_2\)O (1/1 v/v), 20 eq KI), G5H was obtained as foamy colourless solid after preparative RP-HPLC (13.7 mg). Amino Acid Analysis: protein content 64.1% (8.75 mg, 0.38 \(\mu\)mol, yield 50%); found/calc. (%) His 37.2/30.9, Ser 31.8/39.2, Dap 15.6/17.1, Pro 10.9/8.3, CM-Cys 4.5/4.4. Analytical RP-UPLC: \(t_R = 1.243\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (LC-MS+): \(C_{821}H_{1169}N_{333}O_{292}S_8\) (8-fold ligation product, main peak) found/calc. 20634.0/20633.6 \([M+2H]^+\). \(C_{738}H_{1053}N_{297}O_{264}S_7\) (7-fold ligation product with one hydroxyacetyl group, peak height 23% of main peak) found/calc. 18535.0/18534.5 \([M+H]^+\), no 6-fold ligation product observed (calc. 16655.3 \([M]^+\)). \(D = 8.07 \times 10^{-11}\) m\(^2\) s\(^{-1}\); \(R_h = 2.51 \pm 0.07\) nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>PVI (50%)</th>
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<th>Height (mAU)</th>
<th>n.a. (n.a.)</th>
<th>Amount (pmol)</th>
<th>Peak Name</th>
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S30
$^1$H-NMR:

Representative Michaelis-Menten plots for determination of $k_{cat}$ and $K_M$:
Peptide Dendrimer G4P

\[(Pro-Ser)_{16}(Dap-Pro-Ser)_8(Dap-Pro-Ser-Cys-x-Pro-Ser)_4(Dap-Pro-Ser)_2Dap-Pro-Ser-NH_2\]

From starting materials G2C2 and G2M3 using the general procedure described above (solvent: DMF/H\textsubscript{2}O (1/1 v/v), 20 eq KI), G4P was obtained as foamy colourless solid after preparative RP-HPLC (2.05 mg). Amino Acid Analysis: protein content 65.8\% (1.35 mg, 0.15 \textmu{}mol, yield 55\%); found/calc. (\%) Pro 44.0/39.3, Ser 33.8/39.3, Dap 16.9/16.9, CM-Cys 5.4/4.5. Analytical RP-HPLC: \(t_R = 9.156 \text{ min}\) (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214 \text{ nm}\)). MS (LC-MS+): C\textsubscript{345}H\textsubscript{545}N\textsubscript{109}O\textsubscript{128}S\textsubscript{4} (4-fold ligation product) found/calc. 8393.0/8395.9 [M]+. No incomplete ligation products observed (3-fold ligation product calc. 6856.0 [M]+, 2-fold ligation product calc. 5316.2 [M]+). \(D = 9.72 \cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}\); \(R_h = 2.09 \pm 0.07 \text{ nm}\).

Mass spectrum, MS (ESI+):
Analytical RP-HPLC chromatogram:

Amino Acid Analysis:

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<th>RT (min)</th>
<th>RT (STD)</th>
<th>PWR(%)</th>
<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
<th>n.a.</th>
<th>Amount (pmol)</th>
<th>Peak Name</th>
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</table>
$^1$H-NMR:
Peptide Dendrimer G5P1

(Pro-Asp)$_{32}$(Dap-Pro-Asp)$_{16}$(Dap-Cys-x-Pro-Ser)$_{10}$(Dap-Pro-Ser)$_{4}$(Dap-Pro-Ser)$_{2}$Dap-Pro-Ser-NH$_2$

From starting materials G3C2 and G2M5 using the general procedure described above (solvent: 0.1 M phosphate buffer pH 8.5, 20 eq KI), G5P1 was obtained as foamy colourless solid after preparative RP-HPLC (10.3 mg). Amino Acid Analysis: protein content 76.5% (7.86 mg, 0.43 μmol, yield 57%); found/calc. (%) Pro 39.4/38.2, Asp 30.7/29.1, Dap 19.0/18.8, Ser 6.3/9.1, CM-Cys 4.7/4.8. Analytical RP-UPLC: $t_R = 1.245$ min ($A/D$ 100/0 to 0/100 in 5 min, $\lambda = 214$ nm). MS (ESI+): $C_{685}H_{1009}N_{205}O_{284}S_8$ (8-fold ligation product) found/calc. 16916.0/16916.1 [M]$^+$. No incomplete ligation products observed (7-fold ligation product calc. 15392.3 [M]$^+$, 6-fold ligation product calc. 13868.6 [M]$^+$). $D = 7.36 \cdot 10^{-11}$ m$^2$·s$^{-1}$; $R_h = 2.76 \pm 0.05$ nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>P(k%)</th>
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Total: 511.837, 3837.522, 27668.43
$^1$H-NMR:

![NMR spectrum image]
**Peptide Dendrimer G5P2**

\[(\text{Pro-Ser})_{32}(\text{Dap-Pro-Ser})_{16}(\text{Dap-Pro-Ser-Cys-x-Pro-Ser})_{8}(\text{Dap-Pro-Ser})_{4}(\text{Dap-Pro-Ser})_{2}\text{Dap-Pro-Ser-NH}_2\]

From starting materials G3C2 and G2M3 using the general procedure described above (solvent: DMF/H\textsubscript{2}O (1/1 v/v), 20 eq KI), G5P2 was obtained as foamy colourless solid after preparative RP-HPLC (1.48 mg). Amino Acid Analysis: protein content 62.4% (0.92 mg, 0.05 µmol, yield 40%); found/calc. (%) Pro 44.1/39.2, Ser 33.3/39.2, Dap 17.2/17.1, CM-Cys 5.4/4.4. Analytical RP-HPLC: \(t_R = 9.132\) min (A/D 100/0 to 50/50 in 10 min, \(\lambda = 214\) nm).

MS (ESI\(^+\)): \(C_{701}H_{1105}N_{221}O_{260}S_8\) (8-fold ligation product) found/calc. 17043.0/17045.1 [M]\(^+\). No incomplete ligation products observed (7-fold ligation product calc. 15505.2 [M]\(^+\), 6-fold ligation product calc. 13965.4 [M]\(^+\)). \(D = 5.93 \times 10^{-11}\) m\(^2\)·s\(^{-1}\); \(R_h = 3.42 \pm 0.04\) nm.

Mass spectrum, MS (ESI\(^+\)):
Analytical RP-HPLC chromatogram:

Amino Acid Analysis:

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<th>RT (STD)</th>
<th>PWH%</th>
<th>Area</th>
<th>Height</th>
<th>n.a.</th>
<th>Amount (µmol)</th>
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Total: 501.211 | 4201.917 | 24707.35
$^1$H-NMR:
Peptide Dendrimer G5P3

(Pro-Asp)$_{32}$(Dap-Pro-Asp)$_{16}$(Dap-Pro-Asp-Cys-x-Pro-Ser)$_{8}$(Dap-Pro-Ser)$_{4}$(Dap-Pro-Ser)$_{2}$Dap-Pro-Ser-NH$_{2}$

From starting materials G3C2 and G2M4 using the general procedure described above (solvent: 0.5 M NaHCO$_{3}$ buffer pH 8.0, 20 eq KI), G5P3 was obtained as foamy colourless solid after preparative RP-HPLC (14.7 mg). Amino Acid Analysis: protein content 63.6% (9.33 mg, 0.46 µmol, yield 62%); found/calc. (%) Pro 40.4/39.2, Asp 31.9/30.9, Dap 17.1/17.1, Ser 6.6/8.3, CM-Cys 4.1/4.4. Analytical RP-UPLC: $t_{R} = 1.245$ min (A/D 100/0 to 0/100 in 5 min, $\lambda = 214$ nm). MS (LC-MS+): $C_{757}H_{1103}N_{221}O_{316}S_{8}$ (8-fold ligation product, main peak) found/calc. 18620.0/18619.7 [M+6H]$^{+}$. $C_{682}H_{996}IN_{199}O_{284}S_{7}$ (7-fold ligation product with one unreacted iodoactyl group, peak height 26% of main peak) found/calc. 16876.0/16877.7 [M]$^{+}$, no 6-fold ligation product observed (calc. 15141.9). $D = 6.50 \cdot 10^{-11}$ m$^{2}$·s$^{-1}$; $R_{h} = 3.12 \pm 0.04$ nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:
$^1$H-NMR:
Peptide Dendrimer G5P4

\((\text{Pro-Lys})_{32}(\text{Dap-Pro-Lys})_{16}(\text{Dap-Pro-Lys-Cys-x-Pro-Ser})_{8}(\text{Dap-Pro-Ser})_{4}(\text{Dap-Pro-Ser})_{2}\text{Dap-Pro-Ser-NH}_2\)

From starting materials G3C2 and G2M6 using the general procedure described above (solvent: 0.5 M NaHCO\(_3\) buffer pH 8.0, 20 eq KI), G5P4 was obtained as crystalline colourless solid after preparative RP-HPLC (16.4 mg). Amino Acid Analysis: protein content 82.3% (13.5 mg, 0.58 µmol, yield 77%); found/calc. (%) Pro 40.8/39.2, Lys 31.5/30.9, Dap 17.8/17.1, Ser 6.4/8.3, CM-Cys 3.5/4.4. Analytical RP-UPLC: \(t_R = 1.255\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (LC-MS+): \(C_{869}H_{1497}N_{277}O_{204}S_8\) (8-fold ligation product, main peak) found/calc. 19353.0/19352.4 \([M+6H]^+\). \(C_{780}H_{1340}N_{248}O_{187}S_7\) (7-fold ligation product with one hydroxyacetyl group, peak height 10% of main peak) found/calc. 17411.0/17411.0 \([M+2H]^+\), no 6-fold ligation product observed (calc. 15471.5 \([M]^+\)).

\[D = 4.27 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}; R_h = 4.74 \pm 0.15 \text{ nm}.\]

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>RT (STD) (min)</th>
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<th>Height (mAU)</th>
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<th>Amount (pmol)</th>
<th>Peak Name</th>
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Total: 607,403, 4028.835, 25558.22
$^1$H-NMR:
Peptide Dendrimer G5P5

\((\text{Pro-Ser})_{32}(\text{Dap-Pro-Ser})_{16}(\text{Dap-Pro-Ser-Cys-x-His-Ser})_{8}(\text{Dap-His-Ser})_{4}(\text{Dap-His-Ser})_{2}\text{Dap-His-Ser-NH}_2\)

From starting materials G3C1 and G2M3 using the general procedure described above (solvent: 0.5 M NaHCO\(_3\) buffer pH 8.0/CH\(_3\)CN (2/1 v/v), 20 eq KI), G5P5 was obtained as foamy colourless solid after preparative RP-HPLC (2.77 mg). Amino Acid Analysis: protein content 52.2\% (1.45 mg, 0.07 µmol, yield 11\%); found/calc. (%) Pro 37.5/30.9, Ser 31.2/39.2, His 10.7/8.3, Dap 16.0/17.1, CM-Cys 4.6/4.4. Analytical RP-UPLC: \(t_R = 1.144\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (ESI+): \(C_{716}H_{1105}N_{251}O_{260}S_8\) (8-fold ligation product, main peak) found/calc. 17645.0/17645.5 [M]+. \(C_{648}H_{997}N_{229}O_{236}S_7\) (7-fold ligation product with one hydroxyacetyl group, peak height 19\% of main peak) found/calc. 15995.0/15995.7 [M]+, no 6-fold ligation product observed (calc. 14565.8 [M]+). \(D = 5.57 \times 10^{-11}\) m\(^2\) s\(^{-1}\); \(R_h = 3.64 \pm 0.10\) nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>n.a.</th>
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$^1$H-NMR:

Representative Michaelis-Menten plots for determination of $k_{cat}$ and $K_M$:
Peptide Dendrimer G5P6

\[(\text{Pro-Asp})_{32}(\text{Dap-Pro-Asp})_{16}(\text{Dap-Pro-Asp-Cys-x-Pro-Ser})_{8}(\text{Lys-Pro-Ser})_{4}(\text{Lys-Pro-Ser})_{2}\text{Lys-Pro-Ser-NH}_2\]

From starting materials G3C3 and G2M4 using the general procedure described above (solvent: DMF/H\textsubscript{2}O (1/1 v/v), 20 eq KI), G5P6 was obtained as foamy colourless solid after preparative RP-HPLC (11.8 mg). Amino Acid Analysis: protein content 80.0\% (9.41 mg, 0.46 \mu\text{mol}, yield 66\%); found/calc. (\%) Pro 39.6/39.2, Asp 31.7/30.9, Dap 12.3/13.3, Ser 7.5/8.3, CM-Cys 4.6/4.4, Lys 4.3/3.9 (Dap coeluted with Phe, 3501.1 pmol). Analytical RP-UPLC: 

- \text{t}_{R} = 1.214 \text{ min (A/D 100/0 to 0/100 in 5 min, } \lambda = 214 \text{ nm). MS (LC-MS+):}
- \text{C}_{778}\text{H}_{1147}\text{N}_{221}\text{O}_{316}\text{S}_{8} (8\text{-fold ligation product, main peak) found/calc. 18907.0/18908.2 [M]^+}. 
- \text{C}_{703}\text{H}_{1038}\text{IN}_{199}\text{O}_{284}\text{S}_{7} (7\text{-fold ligation product with one unreacted iodoacetyl group, peak height 28\% of main peak) found/calc. 17165.0/17172.3 [M]^+}. 
- \text{C}_{628}\text{H}_{929}\text{I}_{2}\text{N}_{177}\text{O}_{252}\text{S}_{6} (6\text{-fold ligation product with two unreacted iodoacetyl groups, peak height 11\% of main peak) found/calc. 15467.0/15436.3 [M]^+}. 

- D = 6.61 \cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}; R_b = 3.07 \pm 0.05 \text{ nm.}

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>RT (min)</th>
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Total: 31162.91
$^1$H-NMR:
Peptide Dendrimer G5P7

\([\text{DPro-Pro-Glu}]_{32}(\text{Dap-Pro-Asp})_{16}(\text{Dap-Pro-Asp-Cys-x-Pro-Ser})_{8}(\text{Lys-Pro-Ser})_{4}(\text{Lys-Pro-Ser})_{2}\text{Lys-Pro-Ser-NH}_2\]

From starting materials G3C3 and G2M7 using the general procedure described above (solvent: DMF/H\(_2\)O (1/1 v/v), 20 eq KI), G5P7 was obtained as foamy colourless solid after preparative RP-HPLC (15.6 mg). Amino Acid Analysis: protein content 60.7% (9.46 mg, 0.40 \(\mu\)mol, yield 56%); found/calc. (%) Pro 51.7/48.4, Glu 15.3/15.0, Asp 11.6/11.3, Dap 10.6/11.3, Ser 6.6/7.0, CM-Cys 4.2/3.8, Lys 3.7/3.3 (Dap coeluted with Phe, 2208.5 pmol).

Analytical RP-UPLC: \(t_R = 1.304\) min (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214\) nm). MS (LC-MS+): \(C_{970}H_{1435}N_{253}O_{348}S_8\) (8-fold ligation product, main peak) found/calc. 22459.0/22464.8 [M]+. \(C_{871}H_{1290}IN_{227}O_{312}S_7\) (7-fold ligation product with one unreacted iodoacetyl group, peak height 12% of main peak) found/calc. 20270.0/20271.9 [M]+, no 6-fold ligation product observed (calc. 18117.8 [M]+). \(D = 6.31 \times 10^{-11}\) m\(^2\) s\(^{-1}\); \(R_h = 3.21 \pm 0.08\) nm.

Mass spectrum, MS (ESI+):
Analytical RP-UPLC chromatogram:

Amino Acid Analysis:

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<th>RT (min)</th>
<th>RT (STD) min</th>
<th>PW(50%) min</th>
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<th>Height mAU</th>
<th>n.a. n.a.</th>
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S54
$^1$H-NMR:
Peptide Dendrimer G6P1

\[(\text{Pro-Ser})_{64}(\text{Dap-Pro-Ser})_{32}(\text{Dap-Pro-Ser-Cys-x-Pro-Ser})_{8}(\text{Dap-Pro-Ser})_{4}(\text{Dap-Pro-Ser})_{2}\text{Dap-Pro-Ser-NH}_2\]

From starting materials G3C2 and G3M1 using the general procedure described above (solvent: DMF/H\textsubscript{2}O (1/1 v/v), 20 eq KI), G6P1 was obtained as foamy colourless solid after preparative RP-HPLC (17.7 mg). Amino Acid Analysis: protein content 65.8% (11.6 mg, 0.34 \mu\text{mol}, yield 45%); found/calc. (\%) Pro 44.2/39.6, Ser 30.3/39.6, Dap 22.9/18.5, CM-Cys 2.6/2.3. Analytical RP-UPLC: \(t_R = 1.189 \text{ min} \) (A/D 100/0 to 0/100 in 5 min, \(\lambda = 214 \text{ nm}\)). MS: \(C_{1309}H_{2065}N_{413}O_{484}S_8\) (8-fold ligation product) found/calc. N.d./31588.4 [M]+. \(D = 3.68 \cdot 10^{-11}\) m\(^2\) s\(^{-1}\); \(R_h = 5.51 \pm 0.07 \text{ nm}\).

Analytical RP-UPLC chromatogram:
Amino Acid Analysis:

\[
\begin{array}{cccccc}
\text{RT (STD)} & \text{PW(50%)} & \text{Area mAUC/min} & \text{Height mAUC/min} & \text{n.a. mAU/min} & \text{Amount pmol} \\
1.20 & 1.19 & 0.089 & 4.82 & 52.17 & 472.8 \\
2.55 & 2.55 & 0.077 & 10.19 & 125.26 & 675.6 \\
3.40 & 3.41 & 0.092 & 141.75 & 1357.27 & 7859.0 \\
5.29 & 5.32 & 0.101 & 247.89 & 2223.93 & 11480.3 \\
11.46 & 11.59 & 0.111 & 169.16 & 1275.08 & 6890.4 \\
\hline
\text{Total} & & & 26450.22 & \\
\end{array}
\]

\[\text{^1H-NMR:}\]
Peptide Dendrimer G6P2

\((\text{Pro-Ser})_{64}(\text{Dap-Pro-Ser})_{32}(\text{Dap-Pro-Ser})_{16}(\text{Dap-Pro-Ser-Cys-x-Pro-Ser})_{8}(\text{Lys-Pro-Ser})_{4}(\text{Lys-Pro-Ser})_{2}\text{Lys-Pro-Ser-NH}_2\)

From starting materials G3C3 and G3M1 using the general procedure described above (solvent: DMF/H$_2$O (1/1 v/v), 20 eq KI), G6P2 was obtained as foamy colourless solid after preparative RP-HPLC (18.2 mg). Amino Acid Analysis: protein content 68.6% (12.5 mg, 0.36 µmol, yield 51%); found/calc. (%) Pro 44.2/39.6, Ser 32.9/39.6, Dap 17.4/16.4, CM-Cys 3.0/2.3, Lys 2.6/2.1 (Dap coeluted with Phe, 4658.9 pmol). Analytical RP-UPLC: $t_R = 1.202$ min (A/D 100/0 to 0/100 in 5 min, $\lambda = 214$ nm). MS: C$_{1330}$H$_{2107}$N$_{413}$O$_{484}$S$_8$ (8-fold ligation product) found/calc. N.d./31883.0 [M]$^+$, D = 4.15·10$^{-11}$ m$^2$·s$^{-1}$; $R_h = 4.88 \pm 0.18$ nm.

Analytical RP-UPLC chromatogram:
Amino Acid Analysis:

\[ \text{H-NMR:} \]

\[ ^1H-NMR: \]
Amino Acid Analysis

Samples were hydrolyzed in the gas phase with 6M HCl containing 0.1% (v/v) phenol for 22 h at 115 °C under N₂ vacuum according to Chang and Knecht.¹ The liberated amino acids were coupled with phenylisothiocyanate (PITC), and the resulting phenylthiocarbamoyl (PTC) amino acids were analyzed by RP-HPLC on a Nova Pack C18 column (4 µm, 3.9 mm × 150 mm, Waters) with a Dionex Summit® HPLC system with an automatic injection system according to Bidlingmeyer et al.² The corresponding ammonium acetate buffer replaced the 0.14 M sodium acetate buffer, pH 6.3. Cysteine was detected as carboxymethyl cysteine (CM-Cys); AMBA = Amb = 4-aminomethylbezoic acid. Dap coeluted with Phe. For some compounds the amount of Dap (pmol) was calculated based on a separate standard and is indicated within the characterization data.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

To cast the gels (84 × 73 × 0.75 mm), Bio-Rad short plates/spacer plates were filled with resolving gel until 2 cm under the glass rim and covered with mQ-deionized H₂O. When polymerization was finished (~ 20 min) the water was removed and stacking gel was poured on top of the resolving gel. The pocket forming comb was inserted. Polymerization of the stacking gel was generally finished after 5 min. The gels were used in electrophoresis boxes purchased from Bio-Rad. After the addition of electrophoresis buffer, the gel pockets were washed with the latter and compounds (6-36 µg dissolved in a mixture of 12.5 µL mQ-deionized H₂O and 2.5 µL sample buffer) or molecular marker (2.5 µL of the prepared solution) were added using a 50 µL glass syringe. Power was supplied by a Consort E452

(200 V) during 0.7 to 1.3 h. To develop the gels, they were removed from the glass plates and stained in a staining/fixation bath for 3 min in a microwave oven at 600 W followed by a background destaining bath (4 × 15 min). The gels were washed in a mixture of mQ-deionized H$_2$O/glycerol and slowly dried at rt between two permeable jam foils. Pictures of the gels were obtained by the use of a flat-bed scanner.

**Preparation.** All stock solutions and buffers were filtered prior to use.

**Resolving gel 20% (for two gels).** 1.5 M Tris·Base pH 8.7 (2.5 mL), aq. solution of SDS (10%, 0.1 mL), aq. solution of acrylamide (Rotiphorese Gel A®, 30%, 6.65 mL), aq. solution of bisacrylamide (Rotiphorese Gel B®, 2%, 0.325 mL), aq. solution of APS (10%, 33 µL), TMED (5 µL).

**Stacking gel 4.3% (for two gels).** mQ-deionized H$_2$O (2 mL), 0.25 M Tris·Base pH 6.8 with 0.2% of SDS and Coomassie® Brilliant Blue R-250 (2.5 mL), aq. solution of acrylamide (30%) and bisacrylamide (0.8%) (0.75 mL), aq. solution of APS (10%, 50 µL), TMED (10 µL).

**Sample buffer.** SDS (5 g), 1M Tris·Base pH 6.8 (15 mL), glycerol (22.5 mL), β-mercaptoethanol (12.5 mL), bromphenol blue.

**Molecular Marker.** Calibration Kit 17-0446-01 purchased from GE Healthcare was used as molecular marker. The protein mixture containing phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) was dissolved in mQ-deionized H$_2$O (160 µL) and sample buffer (40 µL). The solution was heated at 100 °C for 5 min and cooled back down to rt prior to use on the gel.
**Electrophoresis buffer.** To a solution of glycine (72 g) and Tris-Base (15 g) in mQ-deionized H$_2$O (975 mL) a solution of SDS (10%, 25 mL) was added.

**Staining/fixation bath.** MeOH (0.5 L), acetic acid (99%, 0.125 L), mQ-deionized H$_2$O (0.625 L), Coomassie® Brilliant Blue R-250 (2.5 g).

**Background destaining bath.** MeOH (2.5 L), acetic acid (99%, 0.5 L), mQ-deionized H$_2$O (2.5 L).

**Catalysis**

![Graph showing pH dependent activity profile of G6 dendrimer G6P1](image)

**Figure S1.** pH dependent activity profile of G6 dendrimer G6P1 resulting from a bell-shaped fit assuming bifunctional catalysis (v = c [HA][A]), plotted for pKa(HA) = 7.4.

**Table S1.** Enantioselectivities (%) observed for aldol 6.

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<th>G5P2</th>
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Determined after dendrimer-catalyzed aldol reaction in $^a$0.1 M HEPES buffer pH 7.0/acetone (1/1, v/v) or $^b$DMSO/acetone (1/1, v/v), buffered with x eq. of NMM (where x = number of formic acid-amine salts for the dendrimer tested) on a chiral Daicel Chiralpak AS® column (amylose tris[(S)-α-methylbenzylcarbamate] coated on a silica support, 10 μm, 4.6 × 250 mm, flow rate 2.0 mL·min$^{-1}$); $^c$no reaction.