

Supporting Information for

Chemical cell-surface receptor engineering using affinity-guided, multivalent organocatalysts

Hangxiang Wang,¹ Yoichiro Koshi,¹ Daishiro Minato,¹ Hiroshi Nonaka,¹ Shigeki Kiyonaka,¹ Yasuo Mori,¹ Shinya Tsukiji³ & Itaru Hamachi^{*,1,2}

¹*Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering Kyoto University, Katsura, Kyoto 615-8510, Japan.* ²*Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, 5 Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan.* ³*Top Runner Incubation Center for Academia-Industry Fusion, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan.*

*Correspondence should be addressed to I.H. (ihamachi@sbchem.kyoto-u.ac.jp)

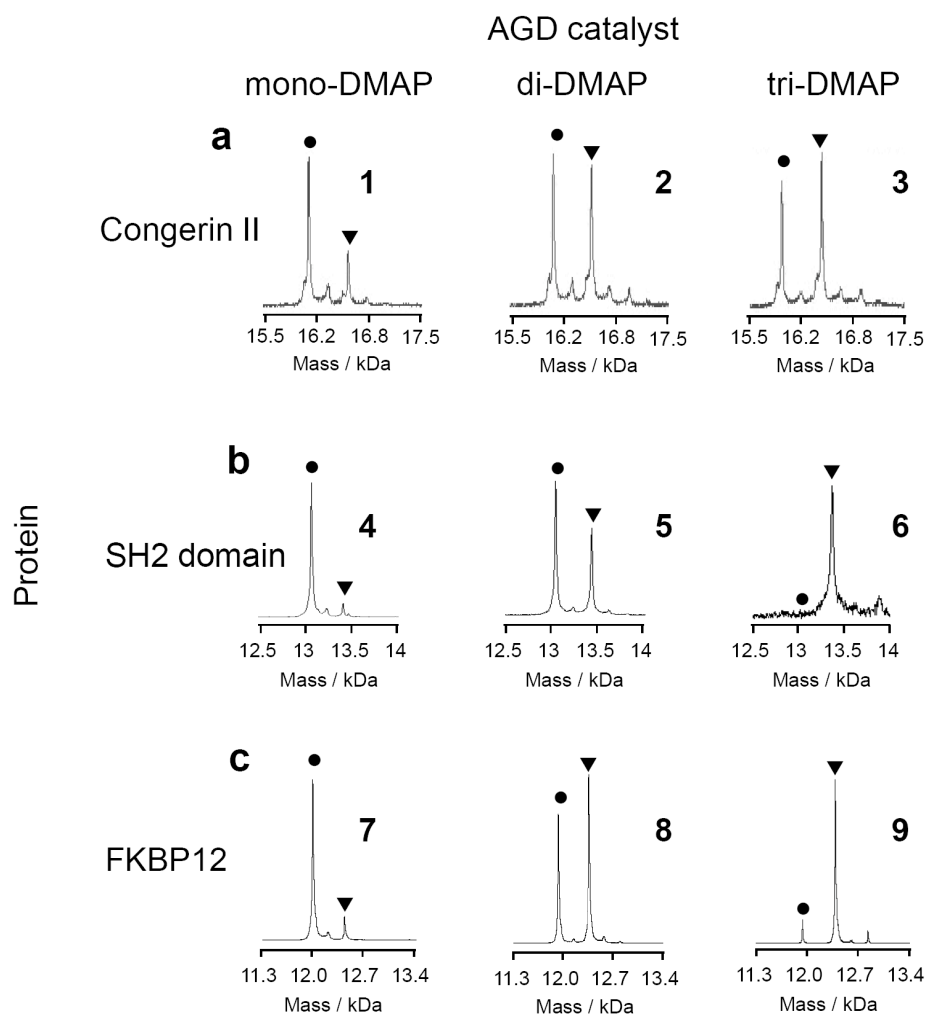


Figure S1. *In vitro* chemical protein acylation using mono-, di- and trivalent AGD catalysts: (a) congerin II, (b) SH2 domain and (c) FKBP12. Reaction conditions: 10 μ M (purified) protein, 10 μ M AGD catalyst, 50 μ M fluorescein-type acyl donor **12** in 50 mM HEPES buffer, pH 8.0. After incubation at 25 $^{\circ}$ C for 30 min for congerin II, at 25 $^{\circ}$ C for 1 h for SH2 domain and at 37 $^{\circ}$ C for 15 min for FKBP12, the reaction mixtures were analyzed by MALDI-TOF MS. ●, unmodified protein; ▼, single fluorescein-labeled protein.

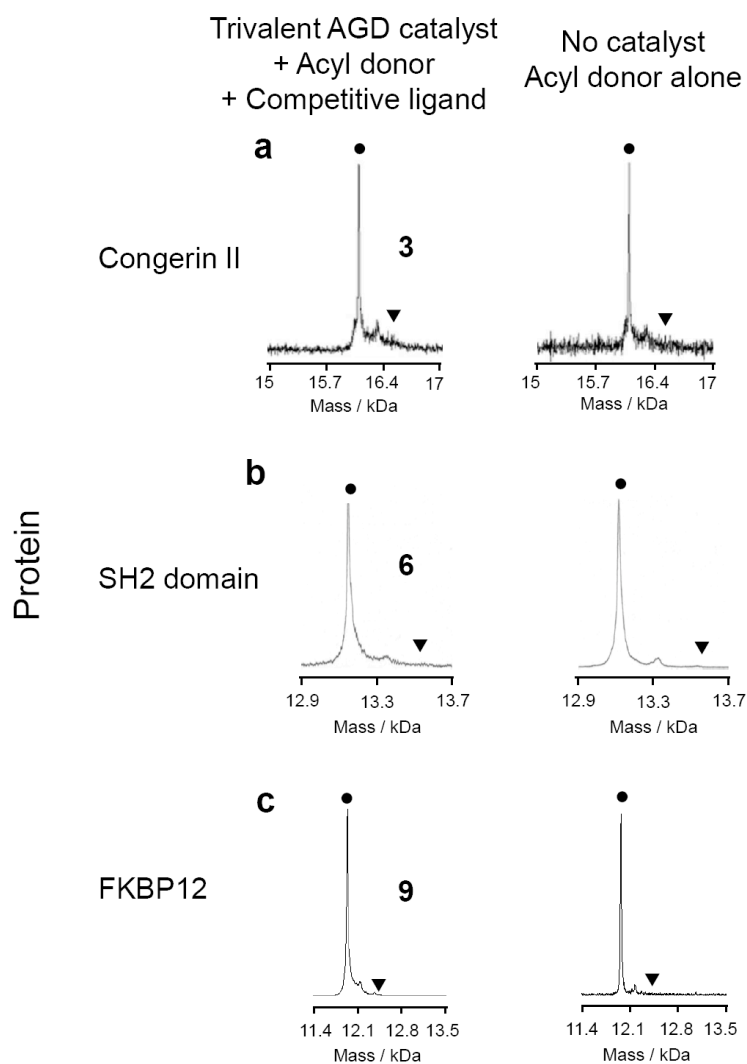


Figure S2. Negative control experiments for *in vitro* chemical protein acylation: (a) congerin II, (b) SH2 domain and (c) FKBP12. Labeling reactions were performed as described in **Figure S1** using trivalent AGD catalysts, (left) in the presence of a competitive ligand or (right) without catalyst. Competitive ligands: (a) lactose (1 mM), (b) AcHN-VDpYVPMLD-CONH₂ peptide (1 mM), (c) FK506 (100 μ M).

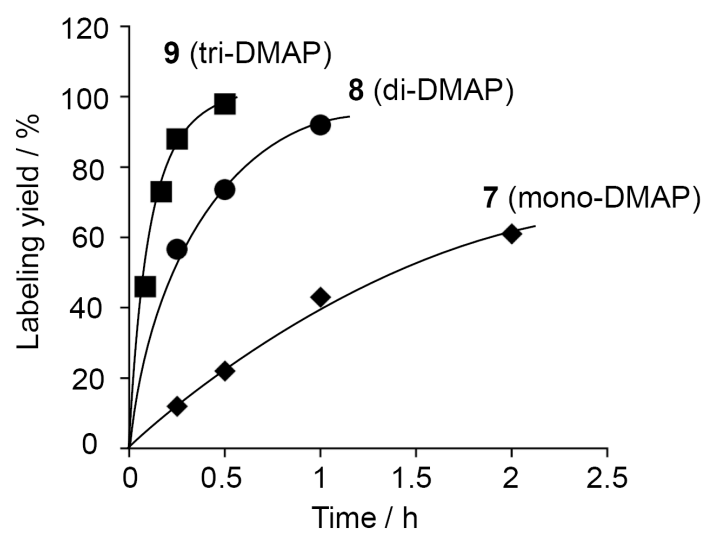


Figure S3. Reaction kinetics of FKBP12 labeling using mono-, di-, and trivalent AGD catalysts. Labeling reactions were performed as described in **Figure S1**.

a

³³³GSPGIQLQDAE ³³³WYWGDISREE ³⁴³VNEKLRDTAD
³⁵³GTFLVRDAST ³⁶³KMHGDTTLTL ³⁷³RKGGNNKLIK
³⁸³IFHRDGKYGF ³⁹³SDPLTFSSVV ⁴⁰³ELINHYRNES
⁴¹³LAQYNPKLDV ⁴²³KLLYPVSKYQ ⁴³³QDQ

L1 L2
 L3 L4 L5
 L6 L7 L8

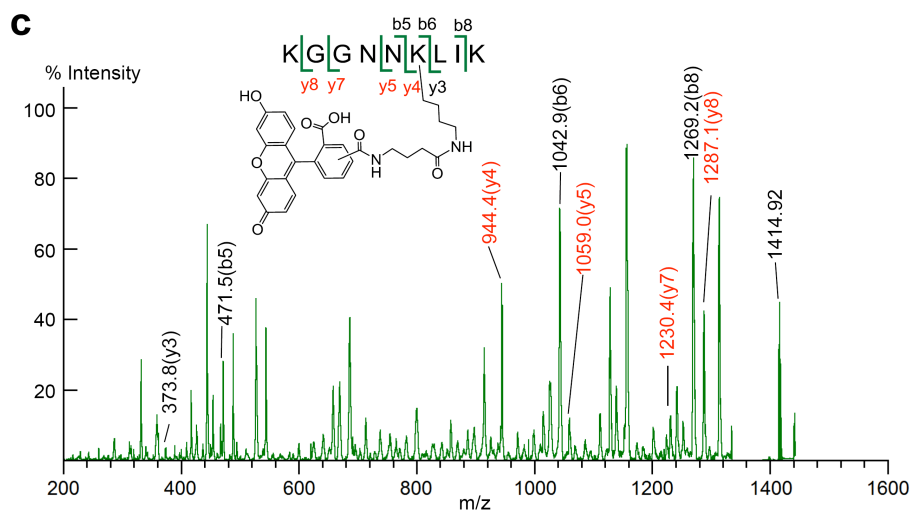
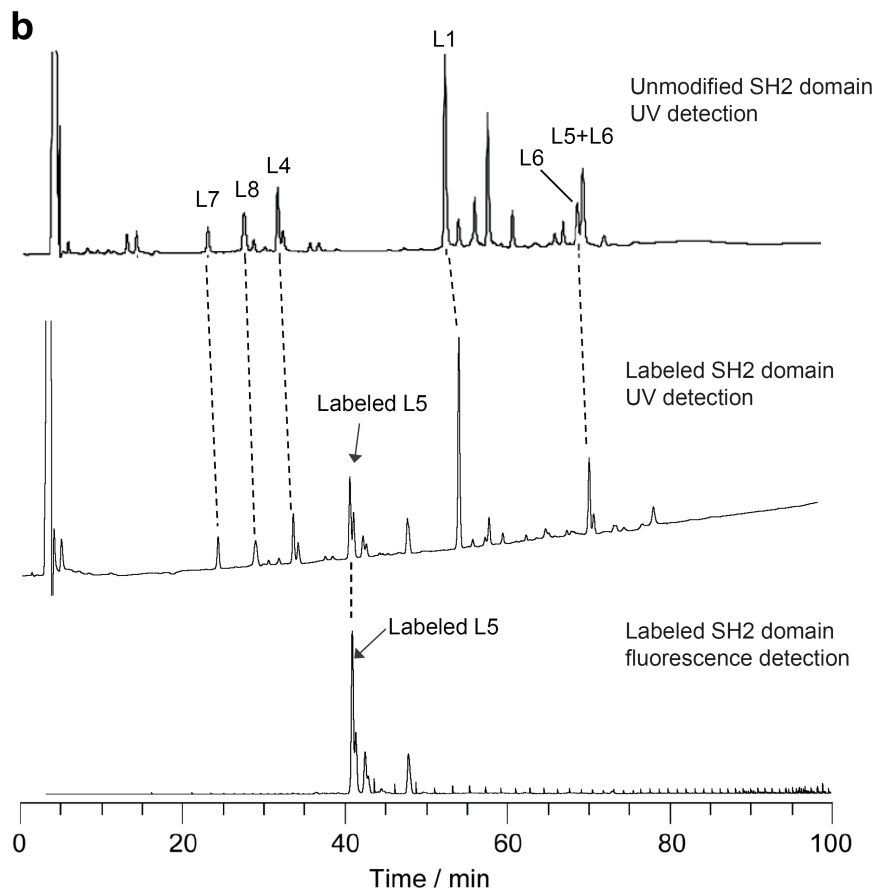


Figure S4. Identification of the labeling site of the SH2 domain treated with monovalent AGD catalyst **4** and acyl donor **12**. (a) The primary sequence of the SH2 domain, which is derived from the p85 α subunit of human PI3K, and the assignment of each fragment generated by trypsin digestion. The extra amino acids shown in gray (GSPGIQ) are derived from the expression vector. Sequence numbers correspond to human p85 α . (b) Reversed-phase HPLC analysis of trypsin-digested, unmodified SH2 domain (top) and fluorescein-labeled SH2 domain (middle and bottom). A gradient of 5–55% of solvent A over 100 min was used with UV detection at 220 nm (top and middle) and fluorescence detection at 515 nm (excitation at 480 nm, bottom). The labeled L5 fragment was characterized by MALDI-TOF MS. MALDI-TOF MS (CHCA): calcd for $[M+H]^+ = 1414.70$, obsd = 1414.92. (c) MALDI-TOF MS/MS analysis of the fluorescein-labeled L5 fragment.

*Note that the labeling site was identical when the SH2 domain was labeled using trivalent AGD catalyst **6** (data not shown).*

a GGG¹GVQVETISPG¹¹DGRTFPKRGQ²¹TCVVHYTG
 ML³¹EDG KKF^{L2}DSSR^{L3}DRN^{L3'}KPF^{L4}KFML^{L5}GKQEVIRG
 WE⁶¹EGVAQMSVGQ⁷¹RAKLTISPDY⁸¹AYGATGHPG
 I⁹¹IPPHATLVFD¹⁰¹VELLKLE
 L6

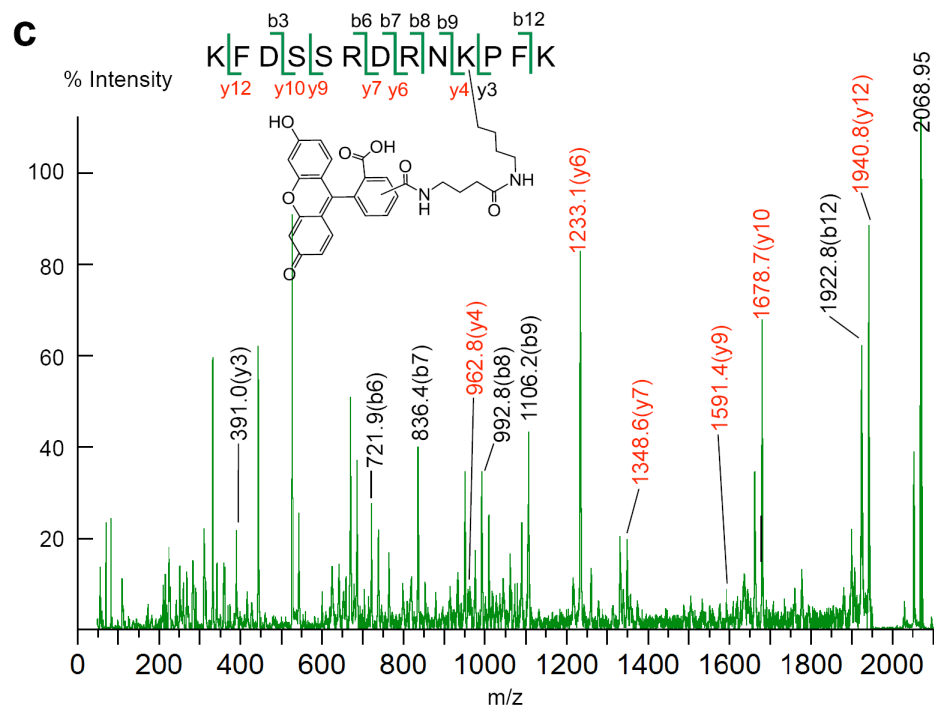
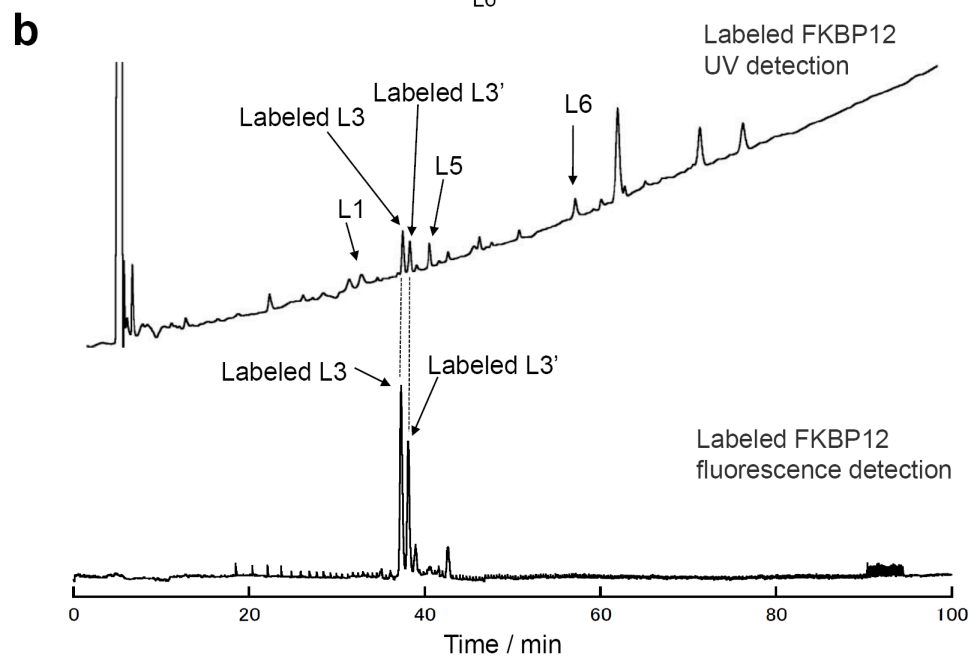
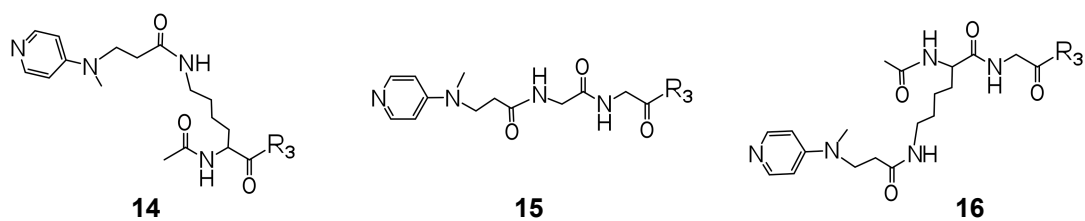


Figure S5. Identification of the labeling site of FKBP12 treated with trivalent AGD catalyst **9** and acyl donor **12**. **(a)** The primary sequence of FKBP12 and the assignment of each fragment generated by lysyl endopeptidase (LEP) digestion. The extra amino acids shown in gray (GGG) are derived from the expression vector. **(b)** Reversed-phase HPLC analysis of LEP-digested, fluorescein-labeled FKBP12. A gradient of 5—55% of solvent A over 100 min was used with UV detection at 220 nm (top) and fluorescence detection at 515 nm (excitation at 480 nm, bottom). The labeled L3 fragment was characterized by MALDI-TOF MS. MALDI-TOF MS (CHCA): calcd for $[M+H]^+ = 2068.92$, obsd = 2068.95. The second major peak could be assigned to labeled L3' fragment (see **(a)**). **(c)** MALDI-TOF MS/MS analysis of the fluorescein-labeled L3 fragment.



$R_3 = \text{Val-Asp-pTyr-Val-Pro-Met-Leu-Asp-CONH}_2$

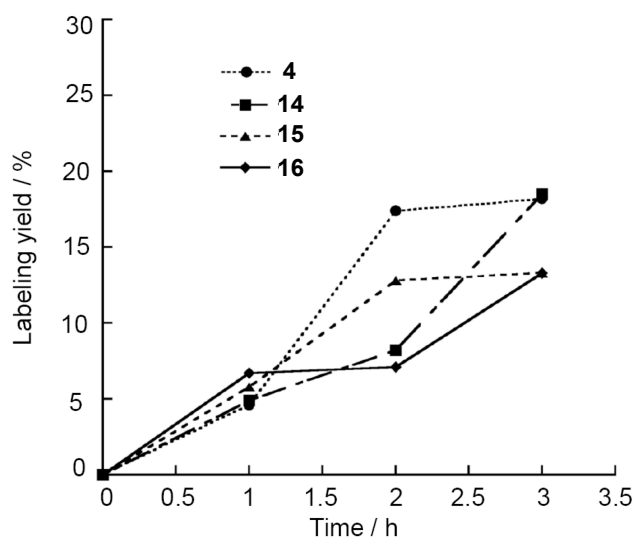


Figure S6. The SH2 domain labeling using monovalent AGD catalysts with different spacer lengths. Reaction conditions: 10 μM SH2 domain, 10 μM AGD catalyst, 50 μM acyl donor **12** in 50 mM HEPES buffer, pH 8.0, at 25 °C. ●, **4**; ■, **14**; ▲, **15**; ◆, **16**.

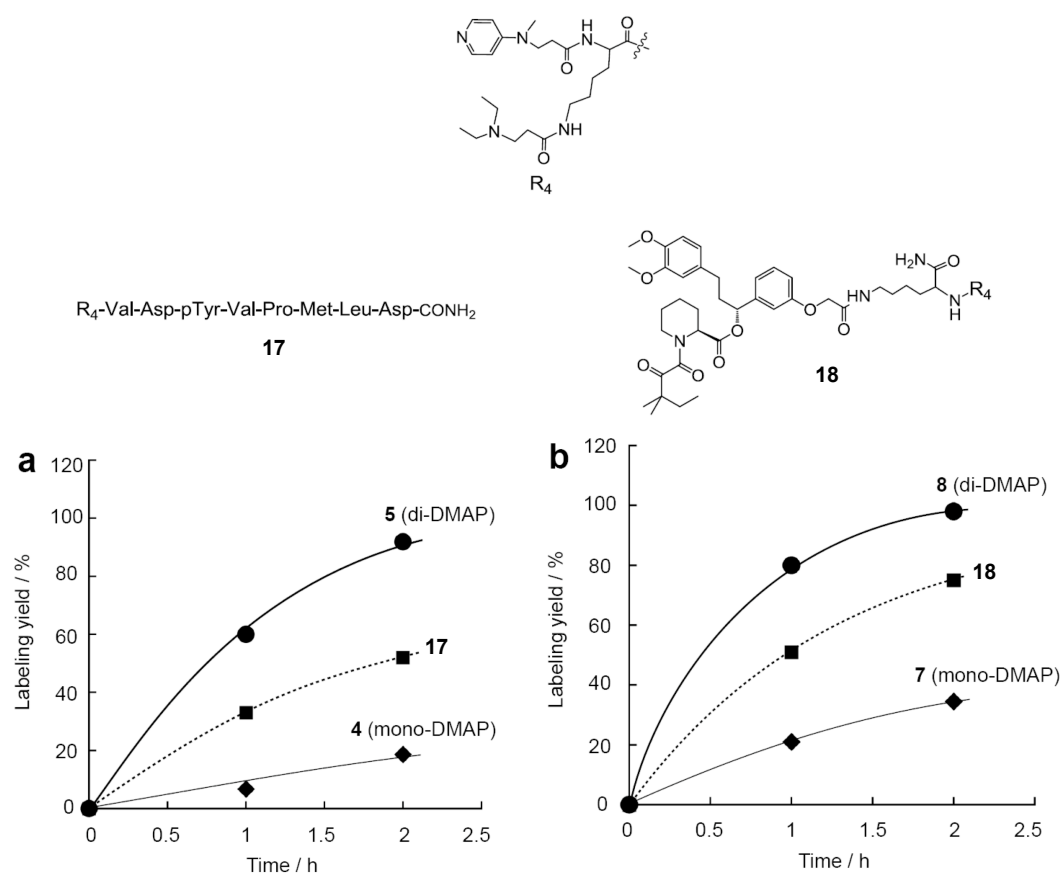


Figure S7. Chemical acylation of (a) the SH2 domain and (b) FKBP12 using AGD catalysts containing one DMAP and one TEA groups. Reaction conditions: 10 μ M protein, 10 μ M AGD catalyst, 50 μ M acyl donor **12** in 50 mM HEPES, pH 8.0, at 25 °C.

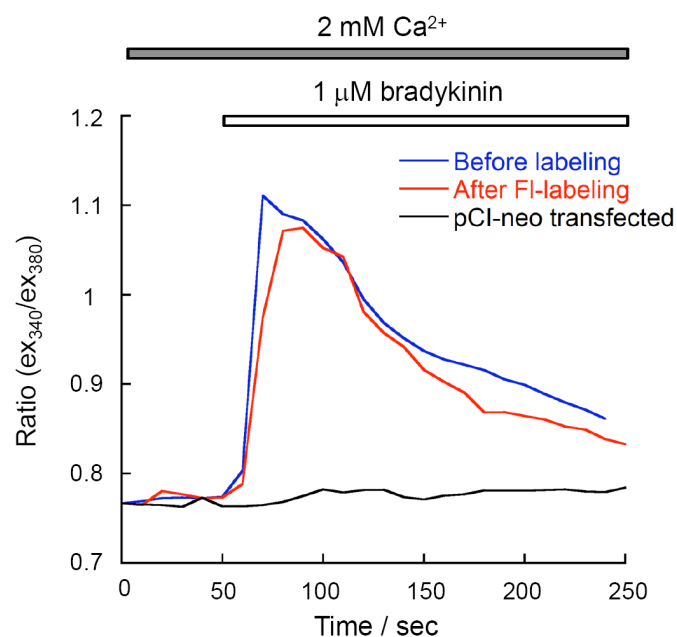


Figure S8. Evaluation of the B₂R activity. The B₂R-mediated Ca²⁺ influx was measured using fura-2 upon stimulation with 1 μM bradykinin in HEK 293 cells transfected with B₂R before (blue line) and after (red line) fluorescein labeling. The black line shows the response of the HEK 293 cells transfected with control vehicle vector under the same condition.

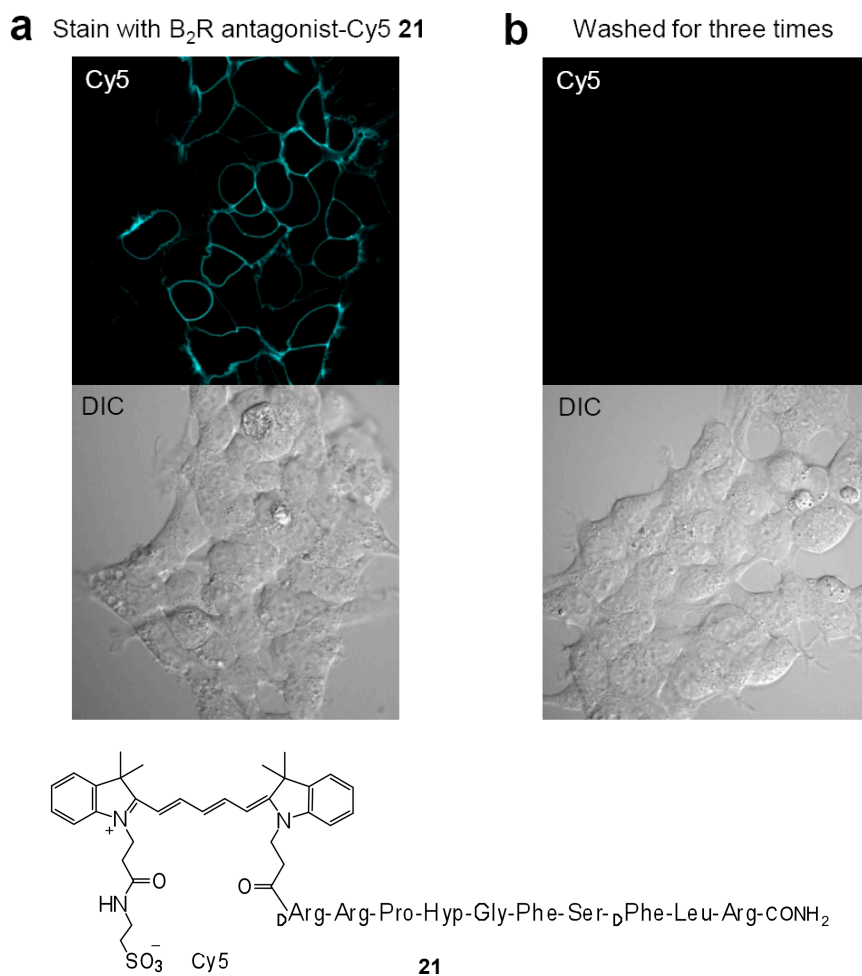


Figure S9. Removability of the antagonist peptide, ${}^{\text{D}}\text{Arg}^0[\text{Hyp}^3, {}^{\text{D}}\text{Phe}^7, \text{Leu}^8]\text{BK}$, by a washing procedure. (Left) CLSM images obtained after HEK 293 cells expressing B₂R were stained (incubated) with 1 μM Cy5-conjugated antagonist peptide **21**.^{S1} (Right) CLSM images obtained after washing the cells three times with PBS.

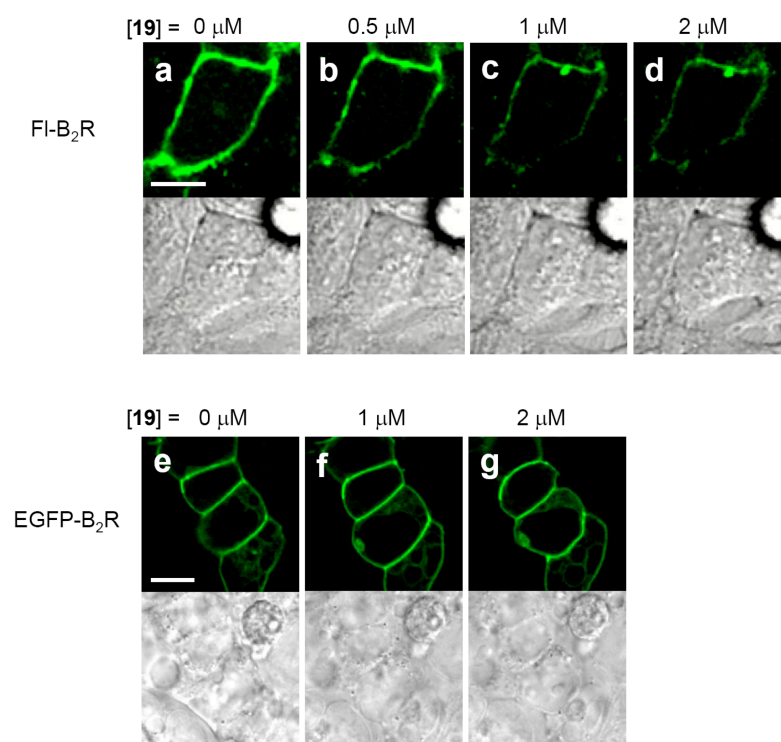


Figure S10. Fluorescence quenching of FI-B₂R and EGFP-B₂R upon the addition of DABCYL-conjugated B₂R ligand **19**. HEK 293 cells displaying FI-B₂R or EGFP-B₂R were incubated with various concentrations of **19** and observed by CLSM. Scale bars, 10 μ m.

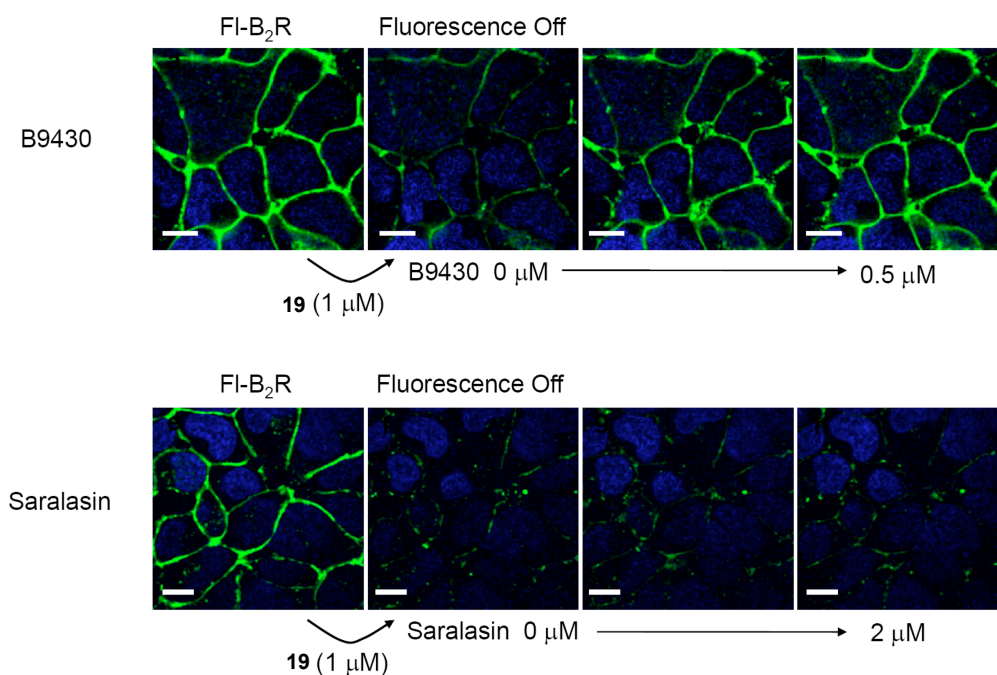


Figure S11. The BFQR-based assay of B₂R ligands using FI-B₂R on the cell surface. The post-labeling HEK293 cells, which display FI-B₂R, were treated with **19** (1 μ M) to quench fluorescein fluorescence. Subsequently, (top) B9430 or (bottom) saralasin was added to the culture medium. The cell nucleus was stained with Hoechst 33342. Scale bars, 10 μ m.

Table S1. Initial rates ($\mu\text{M}\cdot\text{h}^{-1}$) of fluorescein labeling of congerin II, SH2 domain and FKBP12 using mono-, di- and trivalent AGD catalysts. Labeling reactions were performed under the conditions described in **Figure S1**, and monitored at early time points to determine the initial rates.

	AGD catalyst		
	mono-DMAP	di-DMAP	tri-DMAP
Congerin II	1: 5.6 ± 0.4	2: 20.4 ± 0.4	3: 25.6 ± 2
SH2 domain	4: 0.9 ± 0.2	5: 6.0 ± 1.2	6: 15.5 ± 4.9
FKBP12	7: 4.8	8: 22.6	9: 55.2

Table S2. Peptide ligands used in the Fl-B₂R-based BFQR assay.

Peptide ligands	Sequence
HOE 140	^D Arg-Arg-Pro-Hyp-Gly-Thi-Ser- ^D -Tic-Oic-Arg
B9430	^D Arg-Arg-Pro-Hyp-Gly-Igl-Ser- ^D -Igl-Oic-Arg
^D Arg ⁰ [Hyp ³ , ^D Phe ⁷ , Leu ⁸]BK	^D Arg-Arg-Pro-Hyp-Gly-Phe-Ser- ^D -Phe-Leu-Arg
des-Arg ⁹ -BK	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe
Lys-des-Arg ⁹ -BK	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe
Saralasin	Sar-Arg-Val-Tyr-Val-His-Pro-Ala

Abbreviations: Thi, β -(2-thienyl)alanine; Tic, tetrahydroisoquinoline-3-carboxylic acid; Oic, octahydroindole-2-carboxylic acid; Igl, α -(2-indanyl)glycine; Sar, sarcosine.

Supplementary Methods

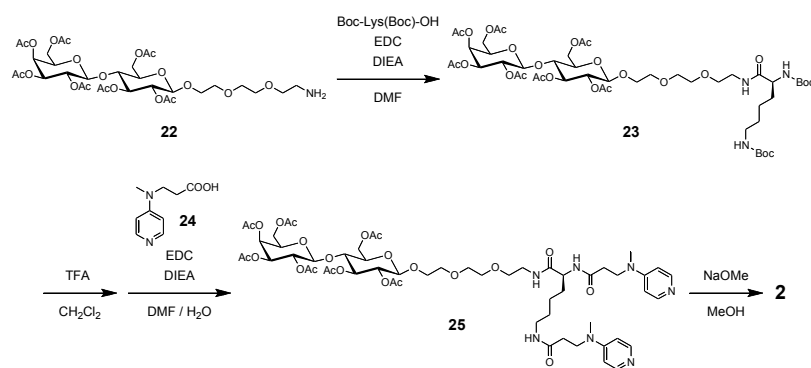
General materials and methods for organic synthesis

All chemical reagents and solvents were obtained from commercial suppliers (Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries, Acros Organics, Sasaki Chemical, or Watanabe Chemical Industries) and used without further purification.

All reactions were carried out under an atmosphere of argon or nitrogen unless otherwise noted. Thin layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ precoated aluminium sheets (Merck) and visualized by fluorescence quenching or ninhydrin staining. Chromatographic purification was accomplished using flash column chromatography on silica gel 60 N (neutral, 40–50 μ m, Kanto Chemical). ¹H NMR spectra were recorded in deuterated solvents on a Varian Mercury 400 (400 MHz) spectrometer and calibrated to the residual solvent peak or tetramethylsilane (= 0 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet. ¹³C NMR spectra were recorded in deuterated solvents on a Varian Mercury 400 (400 MHz) spectrometer, a JEOL JNM-ECS400 (400 MHz) spectrometer and a JEOL JNM-ECA600 (600 MHz) spectrometer. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra were recorded on an Autoflex II instrument (Bruker Daltonics) using α -cyano-4-hydroxycinnamic acid (CHCA) as the matrix. High-resolution fast atomic bombardment mass spectrometry (HR-FAB MS) spectra and high-resolution electrospray ionization quadrupole fourier transform mass spectrometry (HR-ESI Qq-LTMS) spectra were acquired on a JEOL JMS-HX110A mass spectrometer using 3-nitrobenzyl alcohol (NBA) as the matrix and on a Bruker apex-ultra (7T) mass spectrometer, respectively, by Dr. Keiko Kuwata (Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University). Reversed-phase HPLC (RP-HPLC) was carried out on a Hitachi LaChrom L-7100 system equipped with a LaChrom L-7400 UV detector, and a YMC-Pack ODS-A column (5 μ m, 250 \times 10 mm) at a flow rate of 3.0 mL/min. UV detection was at 220 nm. All runs used linear gradients of acetonitrile containing 0.1% TFA (solvent A) and 0.1% aqueous TFA (solvent B).

Synthesis of lactose-tethered AGD catalysts **2** and **3**

The synthesis of AGD catalyst **1** has been reported previously.^{S2}



Scheme S1. Synthetic scheme of **2**

Compound **23**

To a solution of **22**^{S2} (135 mg, 176 μ mol) in anhydrous DMF (10 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (33.7 mg, 176 μ mol), HOBt (25 mg, 176 μ mol), Boc-Lys(Boc)-OH (74.3 mg, 141 μ mol) and *N,N*-diisopropylethylamine (DIEA) (61 μ L, 352 μ M). The reaction mixture was stirred at room temperature for 2 h, and evaporated under vacuum. The residue was dissolved in ethyl acetate and washed with 5% aqueous citric acid, aqueous NaHCO₃ and brine. The organic layer was dried over anhydrous MgSO₄. The crude residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane 20:1) to give compound **23** as a colorless oil (93 mg, 48 %).

¹H NMR (400 MHz; CDCl₃): δ 6.65–6.55 (m, 1H), 5.35 (dd, 1H, *J* = 3.2, 0.8 Hz), 5.20 (t, 1H, *J* = 9.6 Hz), 5.25–5.15 (m, 1H), 5.11 (dd, 1H, *J* = 10.0, 8.0 Hz), 4.96 (dd, 1H, *J* = 10.0, 4.4 Hz), 4.90 (dd, 1H, *J* = 9.6, 8.0 Hz), 4.72–4.64 (m, 1H), 4.56 (d, 1H, *J* = 8.0 Hz), 4.52–4.46 (m, 2H), 4.23 (t, 1H, *J* = 4.8 Hz), 4.17–4.04 (m, 4H), 3.96–3.85 (m, 2H), 3.85–3.50 (m, 13H), 3.10 (d, 2H, *J* = 6.4 Hz), 2.15 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H), 1.90–1.20 (m, 6H), 1.44 (s, 18H).

MALDI-TOF-MS (CHCA): calcd for [M+Na]⁺ = 1118.4738, obsd 1118.4738.

Compound **25**

Compound **23** (93 mg, 84 μ mol) was dissolved in dichloromethane (DCM) (3 mL)

containing 50 % trifluoroacetic acid. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was co-evaporated twice with toluene (5 mL).

The oily residue was dissolved in DMF (3 mL) containing EDC (48.7 mg, 252 μ mol) and DIEA (44 μ L, 252 μ mol), and then a solution of **24**^{S2} (45 mg, 252 μ mol) in water (300 μ L) was added. The reaction mixture was stirred at room temperature for 1 h, and then evaporated under vacuum. The crude residue was purified by gel filtration chromatography (sephadex LH-20, MeOH) to give compound **25** as a white solid (57 mg, 55 %).

¹H NMR (400 MHz; CDCl₃): δ 8.15-8.05 (m, 4H), 6.72-6.62 (m, 4H), 5.90-5.65 (m, 1H), 5.55-5.40 (m, 1H), 5.35 (dd, 1H, J = 3.2, 0.8 Hz), 5.19 (t, 1H, J = 9.2 Hz), 5.10 (dd, 1H, J = 10.0, 8.0 Hz), 4.97 (dd, 1H, J = 10.0, 3.6 Hz), 4.88 (dd, 1H, J = 9.6, 8.0 Hz), 4.72-4.64 (m, 1H), 4.56-4.46 (m, 3H), 4.23 (t, 1H, J = 5.6 Hz), 4.16-4.05 (m, 5H), 3.95-3.87 (m, 2H), 3.85-3.10 (m, 17H), 3.07 (s, 3H), 3.04 (s, 3H), 2.64-2.50 (m, 4H), 2.15 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.90-1.20 (m, 6H).

MALDI-TOF-MS (CHCA): calcd for [M]⁺ = 1219.5384, obsd 1219.5405.

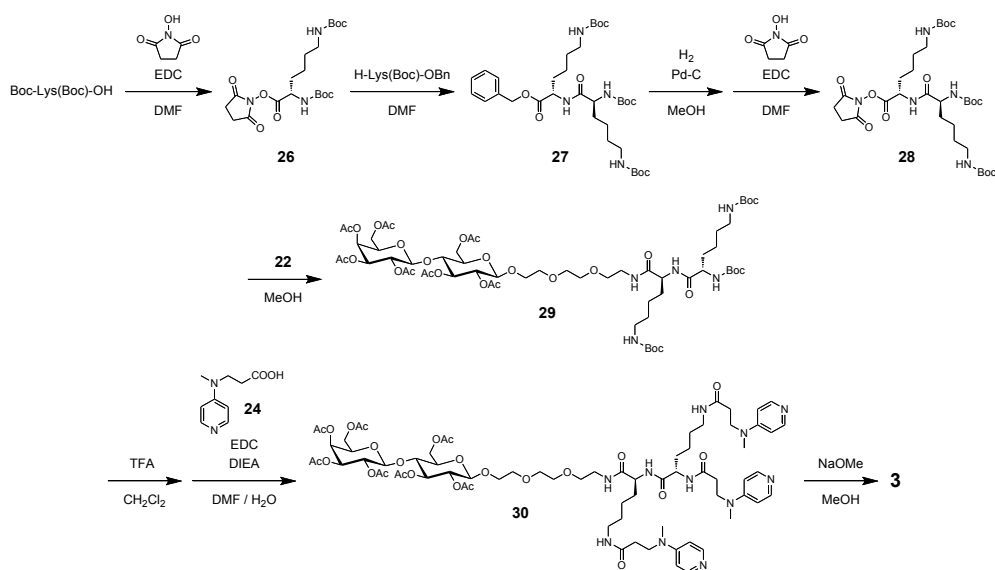
Compound **2**

To a solution of compound **25** (25 mg, 20.5 μ mol) in methanol (3 mL) was added a solution of sodium methoxide (51 μ mol) in methanol (9.6 mL). The reaction mixture was stirred at room temperature for 1 h and purified by RP-HPLC. The fraction containing the target compound was collected and lyophilized to give compound **2** (12 mg, 64 %).

¹H NMR (400 MHz; CD₃OD): δ 8.16 (d, 4H, J = 6.0 Hz), 7.08 (d, 4H, J = 69.6 Hz), 4.43-4.35 (m, 2H), 4.25-4.18 (m, 1H), 4.04-3.95 (m, 1H), 3.95-3.15 (m, 34H), 3.23 (s, 6H), 3.15-3.08 (m, 2H), 2.75-2.65 (m, 2H), 2.65-2.58 (m, 2H), 1.80-1.20 (m, 6H).

¹³C NMR (100 MHz; CD₃OD): δ 174.5, 172.9, 172.7, 158.7, 158.7, 140.4, 140.0, 108.7, 108.7, 105.2, 103.9, 80.8, 77.1, 76.5, 76.2, 74.8, 74.7, 72.5, 71.4, 71.2, 71.1, 70.7, 70.3, 69.6, 56.3, 56.3, 55.2, 49.9, 49.7, 40.3, 39.0, 39.0, 38.5, 34.3, 34.0, 32.6, 29.8, 24.3.

HR-FAB MS (NBA): calcd for C₄₂H₆₈N₇O₁₆ [M]⁺ = 926.4717; obsd 926.4673.



Scheme S2. Synthetic scheme of **3**

Compound **26**

To a solution of Boc-Lys(Boc)-OH (500 mg, 947 μmol) in dry DMF (5 mL) was added EDC (217 mg, 1.14 mmol), *N*-hydroxysuccinimide (NHS) (131 mg, 1.14 mmol) and DIEA (198 μL , 1.14 mmol). The reaction mixture was stirred overnight at room temperature. After evaporation under vacuum, the residue was dissolved in ethyl acetate and washed with 5% aqueous citric acid, aqueous NaHCO_3 and brine. The organic layer was dried over anhydrous MgSO_4 . The solution was filtered and evaporated under vacuum to afford **26** (941 mmol, 99 %) as a white solid.

^1H NMR (400 MHz; CDCl_3): δ 5.20-5.08 (m, 1H), 4.70-4.60 (m, 2H), 3.13 (d, 2H, J = 6.0 Hz), 2.83 (s, 4H), 2.02–1.20 (m, 6H), 1.44 (s, 18H).

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{Na}]^+ = 466.2160$, obsd 466.2240.

Compound **27**

Compound **26** (202 mg, 456 μmol) and H-Lys-OBn (200mg, 537 μmol) were dissolved in DCM (5 mL) containing DIEA (94 μL , 537 μmol). The reaction mixture was stirred at room temperature for 1 h. After evaporation, the residue was diluted with chloroform (100 mL) and washed with 5% aqueous citric acid, aqueous NaHCO_3 and brine. The organic layer was dried over anhydrous MgSO_4 . The solution was filtered and evaporated to afford **27** as a colorless oil (291 mg, 91 %).

^1H NMR (400 MHz; CDCl_3): δ 7.40-7.25 (m, 5H), 6.70-6.60 (m, 1H), 5.23-5.05 (m, 2H), 4.75-4.50 (m, 3H), 4.12-3.98 (m, 1H), 3.18-2.95 (m, 4H), 1.95-1.20 (m, 12H), 1.44 (s, 27H).

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{Na}]^+ = 687.3940$, obsd 687.3971.

Compound **28**

To a solution of **27** (291 mg, 438 μmol) in methanol (5 mL) was added 10% palladium-carbon (Pd-C) (15 mg). The mixture was stirred at room temperature under hydrogen atmosphere. After 1 h, the solution was filtered and evaporated under vacuum. The residue was dissolved in DMF (10 mL) containing EDC (95 mg, 497 μmol), NHS (57 mg, 497 μmol) and DIEA (87 μL , 497 μmol). The mixture was stirred at room temperature for 2 h. After evaporation, the residue was dissolved in ethyl acetate and washed with 5% aqueous citric acid, aqueous NaHCO_3 and brine. The organic layer was dried over anhydrous MgSO_4 . The solution was filtered and evaporated to afford **28** as a white solid (280 mg, 95 %).

^1H NMR (400 MHz; CDCl_3): δ 6.95-6.80 (m, 1H), 5.30-5.18 (m, 1H), 5.00-4.88 (m, 1H), 4.88-4.75 (m, 1H), 4.75-4.60 (m, 1H), 4.18-4.03 (m, 1H), 3.20-3.00 (m, 4H), 2.84 (s, 4H), 2.08-1.20 (m, 12H), 1.43 (s, 27H).

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{Na}]^+ = 694.3634$, obsd 694.3607.

Compound **29**

To a solution of **22**^{S2} (103 mg, 134 μmol) in DCM (4 mL) was added **28** (81 mg, 121 μmol). The reaction mixture was stirred at room temperature for 2 h. After evaporation, the residue was dissolved in DCM (150 mL) and washed with 5% aqueous citric acid, aqueous NaHCO_3 and brine. The organic layer was dried over anhydrous MgSO_4 . The crude residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane 2:1) to give compound **29** as a colorless oil (47 mg, 29 %).

^1H NMR (400 MHz; CDCl_3): δ 6.82-6.70 (m, 1H), 6.70-6.60 (m, 1H), 5.34 (d, 1H, $J = 4.0$ Hz), 5.19 (t, 1H, $J = 9.2$ Hz), 5.09 (dd, 1H, $J = 10.0, 8.0$ Hz), 4.95 (dd, 1H, $J = 9.6, 3.6$ Hz), 4.90-4.60 (m, 3H), 4.89 (dd, 1H, $J = 9.2, 8.4$ Hz), 4.54 (d, 1H, $J = 7.6$ Hz), 4.52-4.49 (m, 2H), 4.44-4.36 (m, 1H), 4.13-3.93 (m, 4H), 3.96-3.78 (m, 3H), 3.67-3.58 (m, 10H), 3.51-3.47 (m, 2H), 3.20-2.96 (m, 4H), 2.14 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03 (s, 9H), 1.95 (s, 3H), 1.90-1.20 (m, 12H), 1.43 (s, 27H).

MALDI-TOF-MS (CHCA): calcd for $[M+Na]^+ = 1346.6212$, obsd 1346.6226.

Compound **30**

Compound **29** (47 mg, 35.5 μ mol) was dissolved in DCM (4 mL) containing 50 % trifluoroacetic acid. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was co-evaporated twice with toluene (5 mL).

The oily residue was dissolved in dry DMF (3 mL) containing EDC (34 mg, 178 μ mol) and DIEA (31 μ L, 178 μ mol), and then a solution of **24** (32 mg, 178 μ mol) in water (300 μ L) was added. The reaction mixture was stirred at room temperature for 2 h and evaporated under vacuum. The crude residue was purified by gel filtration chromatography (sephadex LH-20, MeOH) to give compound **30** as a white solid (47 mg, 88 %).

^1H NMR (400 MHz; CD_3OD): δ 8.13 (d, 6H, $J = 6.4$ Hz), 7.03 (d, 6H, $J = 6.4$ Hz), 5.36 (d, 1H, $J = 3.6$ Hz), 5.18 (t, 1H, $J = 8.8$ Hz), 5.12 (dd, 1H, $J = 10.4, 3.2$ Hz), 5.05–4.96 (m, 1H), 4.90–4.76 (m, 2H), 4.75–4.65 (m, 2H), 4.51 (d, 1H, $J = 12.0$ Hz), 4.30–4.20 (m, 2H), 4.20–4.10 (m, 2H), 4.00–3.80 (m, 8H), 3.80–3.50 (m, 13H), 3.32 (s, 9H), 3.13 (t, 4H, $J = 6.8$ Hz), 2.68–2.50 (m, 6H), 2.13–1.93 (m, 21H), 1.75–1.29 (m, 12H).

MALDI-TOF-MS (CHCA): calcd for $[M+H]^+ = 1510.7199$, obsd 1510.7230.

Compound **3**

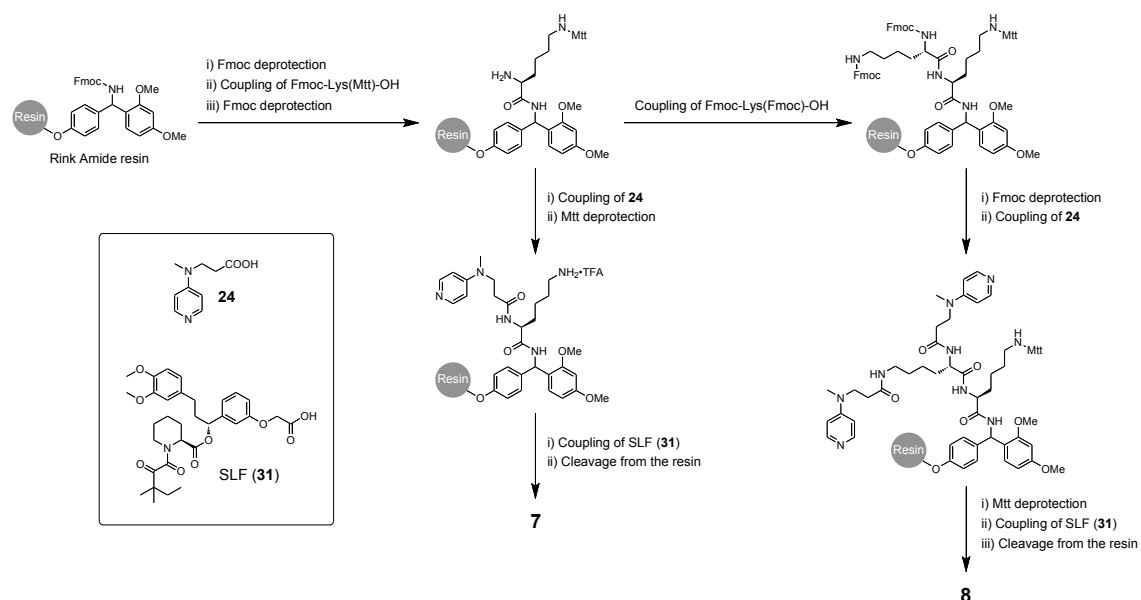
To a solution of compound **30** (12 mg, 7.96 μ mol) in methanol (3 mL) was added a solution of sodium methoxide (15.9 μ mol) in methanol (4 mL). The reaction mixture was stirred at room temperature for 1 h, and then purified by RP-HPLC. The fraction containing the target compound was collected and lyophilized to give **3** (1.6 mg, 17 %).

^1H NMR (400 MHz; CD_3OD): δ 8.15 (d, 6H, $J = 6.4$ Hz), 7.08 (d, 6H, $J = 6.8$ Hz), 4.43–4.35 (m, 2H), 4.25–4.18 (m, 2H), 4.04–3.95 (m, 1H), 3.95–3.30 (m, 35H), 3.28–3.08 (m, 5H), 3.23 (s, 9H), 2.75–2.65 (m, 2H), 2.62–2.55 (m, 4H), 1.85–1.35 (m, 12H).

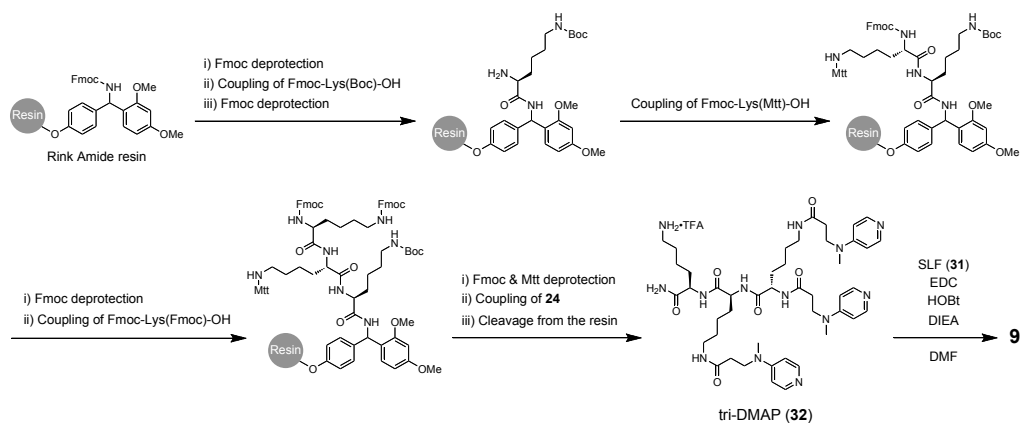
^{13}C NMR (100 MHz; CD_3OD): δ 174.4, 174.2, 173.1, 172.7, 172.7, 158.8, 158.8, 158.8, 140.5, 140.2, 140.0, 108.7, 108.7, 108.7, 105.2, 103.9, 80.9, 77.1, 76.5, 76.2, 74.8, 74.8, 74.8, 72.5, 71.4, 71.2, 71.0, 70.7, 70.3, 69.6, 62.6, 61.8, 55.3, 54.9, 49.9, 49.7, 49.2, 40.3, 40.2, 40.2, 39.1, 39.0, 34.3, 33.9, 32.7, 32.4, 32.1, 29.8, 29.8, 24.3, 24.2.

HR-FAB MS (NBA): calcd for $\text{C}_{57}\text{H}_{90}\text{N}_{11}\text{O}_{18} [M]^+ = 1216.6460$; obsd 1216.6466.

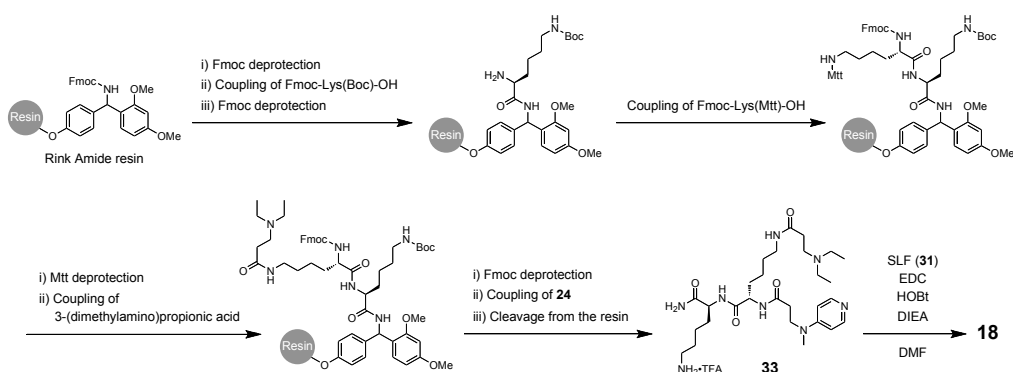
Synthesis of SLF-tethered AGD catalysts **7–9** and **18**



Scheme S3. Synthetic scheme of **7** and **8**



Scheme S4. Synthetic scheme of **9**



Scheme S5. Synthetic scheme of **18**

AGD catalysts **7** and **8** were totally synthesized by solid-phase synthesis. AGD catalyst **9** was synthesized by coupling SLF (**31**)^{S3} with tri-DMAP derivative **32**, which was prepared by solid-phase synthesis, in solution phase.

TEA-containing AGD catalyst **18** was synthesized by coupling SLF (**31**) with **33**, which was prepared by solid-phase synthesis, in solution phase.

General materials and methods for solid-phase synthesis

The solid-phase synthesis was carried out using Rink Amide resin (Novabiochem). Fmoc-Lys(Boc)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Lys(Fmoc)-OH were used as building blocks. Fmoc deprotection was performed with 20% piperidine in *N*-methylpyrrolidone (NMP), and 4-methyltrityl (Mtt) deprotection was performed in DCM containing 1% TFA and 5% triisopropylsilane (TIS). Amino acid coupling reactions were performed with a mixture of Fmoc-amino acid (4 eq.), HBTU (4 eq.), HOBt (4 eq.), and DIEA (8 eq.) in NMP. The coupling of **24**, SLF (**31**) or 3-(diethylamino)propanoic acid was performed with a mixture of the compound (4 eq.), HBTU (4 eq.), HOBt (4 eq.), and DIEA (8 eq.) in NMP. All coupling and Fmoc and Mtt deprotection steps were monitored by the Kaiser test.^{S4} Following assembly, global deprotection and cleavage from the resin was performed with TFA containing 1% TIS, 2.5% ethanedithiol, and 2.5% H₂O. The crude peptide products were purified by RP-HPLC.

Compound **7**

¹H NMR (400 MHz; CD₃OD): δ 8.04 (d, 2H, *J* = 6.8 Hz), 7.30 (t, 1H, *J* = 8.0 Hz), 7.03-6.89 (m, 3H), 6.84 (d, 1H, *J* = 8.0 Hz), 6.82-6.75 (m, 1H), 6.75-6.69 (m, 1H), 6.66

(d, 2H, $J = 6.8$ Hz), 5.77-5.69 (m, 1H), 5.24-5.18 (m, 1H), 4.49 (s, 2H), 4.30-4.20 (m, 1H), 3.80-3.58 (m, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 3.43-3.12 (m, 4H), 2.97 (s, 3H), 2.68-2.45 (m, 4H), 2.38-2.00 (m, 2H), 1.80–1.20 (m, 14H), 1.20 (s, 3H), 1.19 (s, 3H), 0.88 (t, 3H, $J = 8.0$ Hz).

^{13}C NMR (100 MHz; CD_3OD): δ 209.0, 176.8, 173.7, 171.0, 170.9, 169.1, 159.3, 155.3, 150.4, 149.4, 143.3, 135.2, 131.0, 121.7, 121.0, 115.5, 114.3, 113.6, 113.3, 108.1, 77.9, 68.3, 56.6, 56.5, 54.3, 52.8, 47.7, 45.7, 39.7, 39.2, 37.8, 34.2, 33.7, 33.6, 32.7, 32.2, 30.1, 27.3, 25.9, 24.1, 24.1, 23.8, 23.7, 22.1, 9.1.

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{H}]^+ = 873.4757$, obsd 873.4760.

Compound **8**

^1H NMR (400 MHz; CD_3OD): δ 8.17-8.02 (m, 4H), 7.32 (t, 1H, $J = 8.0$ Hz), 7.20-6.90 (m, 7H), 6.86 (d, 1H, $J = 8.0$ Hz), 6.78 (d, 1H, $J = 8.0$ Hz), 6.71 (d, 1H, $J = 8.0$ Hz), 5.80-5.70 (m, 1H), 5.23-5.19 (m, 1H), 4.54 (s, 2H), 4.32-4.20 (m, 2H), 3.93-3.82 (m, 4H), 3.81 (s, 3H), 3.80 (s, 3H), 3.45-3.05 (m, 10H), 3.20 (s, 3H), 3.17 (s, 3H), 2.70-2.50 (m, 4H), 2.30-2.00 (m, 2H), 1.80–1.20 (m, 18H), 1.21 (s, 6H), 0.89 (t, 3H, $J = 8.0$ Hz).

^{13}C NMR (100 MHz; CD_3OD): δ 209.1, 176.7, 174.2, 172.7, 172.6, 171.0, 171.0, 171.0, 159.3, 158.8, 158.7, 150.4, 148.9, 143.5, 140.5, 140.4, 135.2, 131.0, 121.7, 120.9, 115.5, 114.3, 113.6, 113.2, 108.6, 108.5, 78.0, 68.3, 56.5, 56.5, 55.0, 54.2, 52.8, 49.8, 49.0, 47.7, 45.7, 40.2, 39.6, 39.3, 38.9, 38.8, 34.2, 33.7, 33.6, 32.7, 32.6, 32.2, 30.0, 29.9, 27.4, 25.9, 24.2, 24.0, 23.8, 23.7, 22.2, 9.1.

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{H}]^+ = 1163.6499$, obsd 1163.6487.

Compound **32**

^1H NMR (400 MHz; CD_3OD): δ 8.12 (d, 6H, $J = 6.0$ Hz), 7.06 (d, 6H, $J = 63.2$ Hz), 4.35–4.17 (m, 3H), 3.95–3.85 (m, 6H), 3.21 (s, 9H), 3.18–3.05 (m, 4H), 3.00–2.90 (m, 2H), 2.68–2.50 (m, 6H), 1.90–1.25 (m, 18H).

MALDI-TOF-MS (CHCA): calcd for $[\text{M}+\text{H}]^+ = 888.5567$, obsd 888.5589.

Compound **9**

To a solution of SLF (**31**) (3 mg, 5 μmol) in anhydrous DMF (0.5 mL) was added tri-DMAP derivative **32** (3 mg, 3.4 μmol), HOBt (1.0 mg, 6.7 μmol), EDC (1.3 mg, 6.7 μmol), and DIEA (2.4 μL , 13.5 μmol) at room temperature. The crude residue was

purified by RP-HPLC and lyophilized to give a yellow solid (1.7 mg, 35%).

¹H NMR (400 MHz; CD₃OD): δ 8.10 (d, 6H, *J* = 6.4 Hz), 7.30 (t, 1H, *J* = 8.0 Hz), 7.00–6.93 (m, 9H), 6.84 (d, 1H, *J* = 8.0 Hz), 6.77 (d, 1H, *J* = 8.0 Hz), 6.70 (d, 1H, *J* = 8.0 Hz), 5.74–5.71 (m, 1H), 5.30 (m, 1H), 4.52 (s, 2H), 4.29–4.23 (m, 3H), 3.92–3.85 (m, 6H), 3.80 (s, 3H), 3.79 (s, 3H), 3.20 (s, 3H), 3.18 (s, 3H), 3.18 (s, 3H), 3.16–3.14 (m, 2H), 3.12–3.05 (m, 6H), 2.65–2.50 (m, 8H), 2.35 (d, 2H, *J* = 14.4 Hz), 2.03 (m, 2H), 1.85–1.30 (m, 24H), 1.19 (s, 6H), 0.86 (t, 3H, *J* = 8.0 Hz).

¹³C NMR (150 MHz; CD₃OD): δ 209.0, 176.6, 174.4, 174.0, 173.7, 173.5, 173.5, 171.0, 170.9, 159.3, 155.7, 155.6, 155.6, 150.4, 148.9, 148.6, 148.6, 148.5, 143.4, 135.2, 131.0, 121.7, 120.9, 115.6, 115.5, 114.4, 113.6, 113.3, 108.1, 108.1, 108.1, 78.0, 68.3, 56.6, 56.5, 55.0, 54.7, 54.3, 52.8, 49.8, 49.1, 49.1, 48.9, 47.7, 45.7, 40.2, 40.2, 39.7, 39.3, 37.9, 37.9, 37.9, 34.6, 34.6, 34.1, 33.6, 32.8, 32.6, 32.4, 32.2, 31.6, 29.9, 29.8, 27.4, 25.9, 24.2, 24.0, 23.8, 23.7, 22.2, 9.1.

MALDI-TOF-MS (CHCA): calcd for [M+H]⁺ = 1453.8242, obsd 1453.8224.

Compound 18

To a solution of SLF (**31**) (6 mg, 10 μmol) in anhydrous DMF (2 mL) was added TEA derivative **33** (4 mg, 7 μmol), HOBt (1.5 mg, 10 μmol), and EDC (2 mg, 10 μmol) at room temperature. The crude residue was purified by RP-HPLC and lyophilized to give a yellow solid (2 mg, 18%).

¹H NMR (400 MHz; CD₃OD): δ 8.13–8.00 (m, 2H), 7.31 (t, 1H, *J* = 8.0 Hz), 7.13–7.02 (m, 1H), 7.01 (d, 1H, *J* = 9.2 Hz), 6.98–6.95 (m, 3H), 6.85 (d, 1H, *J* = 8.4 Hz), 6.77 (d, 1H, *J* = 6.8 Hz), 6.68 (d, 1H, *J* = 8.4 Hz), 5.78–5.75 (m, 1H), 5.23–5.18 (m, 1H), 4.53 (s, 2H), 4.27–4.21 (m, 2H), 3.89–3.80 (m, 2H), 3.79 (s, 3H), 3.79 (s, 3H), 3.42–3.32 (m, 2H), 3.32–3.10 (m, 8H), 3.16 (s, 3H), 2.76–2.51 (m, 6H), 2.36 (d, 1H, *J* = 14.4 Hz), 2.30–2.15 (m, 2H), 2.15–2.00 (m, 1H), 1.85–1.30 (m, 26H), 1.20 (s, 6H), 0.86 (t, 3H, *J* = 8.0 Hz).

¹³C NMR (150 MHz; CD₃OD): δ 209.1, 176.7, 174.1, 172.7, 171.7, 171.0, 169.1, 161.2, 158.7, 154.1, 150.4, 148.9, 143.5, 140.2, 139.9, 135.2, 131.0, 121.7, 121.0, 115.5, 114.3, 113.6, 113.3, 108.6, 78.0, 68.3, 56.6, 56.5, 55.0, 54.2, 52.9, 49.6, 49.3, 48.6, 47.8, 45.7, 40.2, 39.6, 39.3, 38.9, 33.7, 33.6, 32.7, 32.6, 32.2, 30.0, 29.8, 27.5, 27.4, 26.0, 25.9, 24.1, 24.0, 23.8, 23.7, 22.2, 9.2.

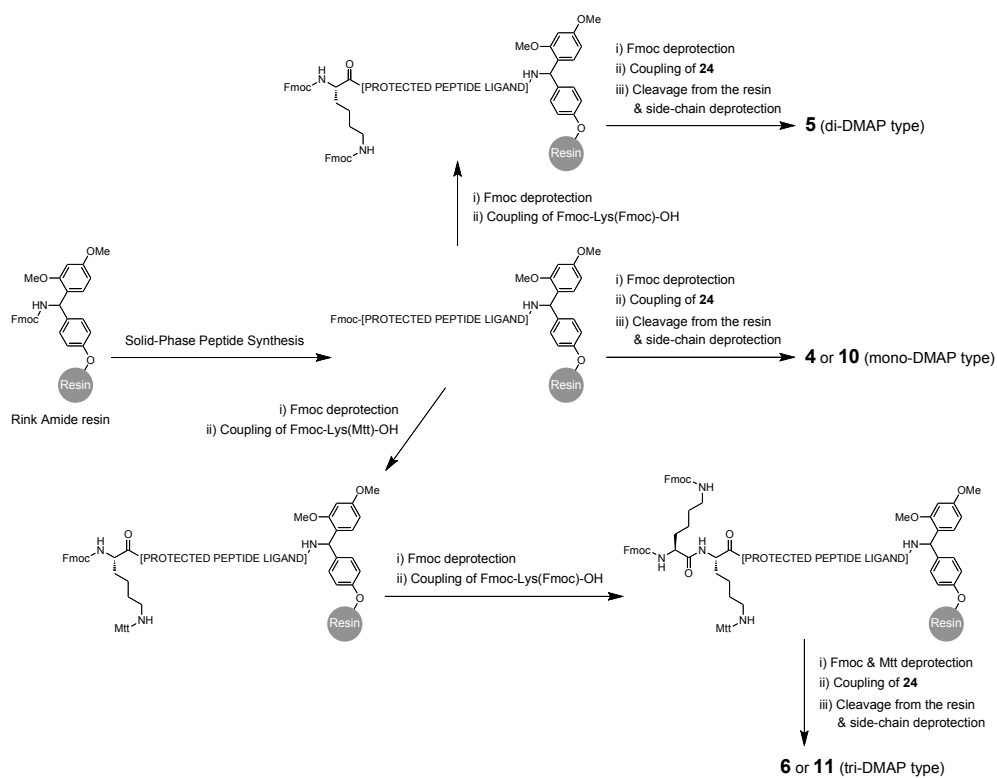
MALDI-TOF-MS (CHCA): calcd for [M+H]⁺ = 1128.6703, obsd 1128.6715.

Synthesis of peptide ligand-tethered AGD catalysts 4-6, 10, 11, 14-17, and other related peptides

General materials and methods for solid-phase peptide synthesis

The peptides were synthesized manually on Rink Amide resin (Novabiochem) by standard Fmoc-based solid-phase peptide synthesis protocol. Fmoc-Arg(Pbf)-OH, Fmoc-D-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Hyp(tBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-D-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, and Fmoc-Tyr(PO(OBzl)OH)-OH were used as building blocks. Fmoc deprotection was performed with 20% piperidine in *N*-methylpyrrolidone (NMP), and 4-methyltrityl (Mtt) deprotection was performed in DCM containing 1% TFA and 5% triisopropylsilane (TIS). Coupling reactions were performed with a mixture of Fmoc-amino acid (4 eq.), HBTU (4 eq.), HOBt (4 eq.), and DIEA (8 eq.) in NMP. The N-terminal modification was carried out using **24**, 5(6)-carboxyfluorescein, or 4-dimethylaminophenylazobenzene-4-carboxylic acid (DABCYL) (4 eq.) with HBTU (4 eq.), HOBt (4 eq.), and DIEA (8 eq.) in NMP. Acetylation was carried out with a mixture of acetic anhydride (10 eq.) and DIEA (10 eq.) in NMP. All coupling and Fmoc, Mtt deprotection steps were monitored by the Kaiser test.^{S3} Following chain assembly, global deprotection and cleavage from the resin was performed with TFA containing 1% TIS, 2.5% ethanedithiol, and 2.5% H₂O. The crude peptide products were precipitated by diisopropyl ether and purified by RP-HPLC.

Synthesis of peptide ligand-tethered AGD catalysts 4-6, 10, and 11



Scheme S6. Synthetic scheme of **4-6**, **10** and **11**

MALDI-TOF-MS (CHCA):

4: calcd for $[M+H]^+ = 1295.5$, obsd 1295.8

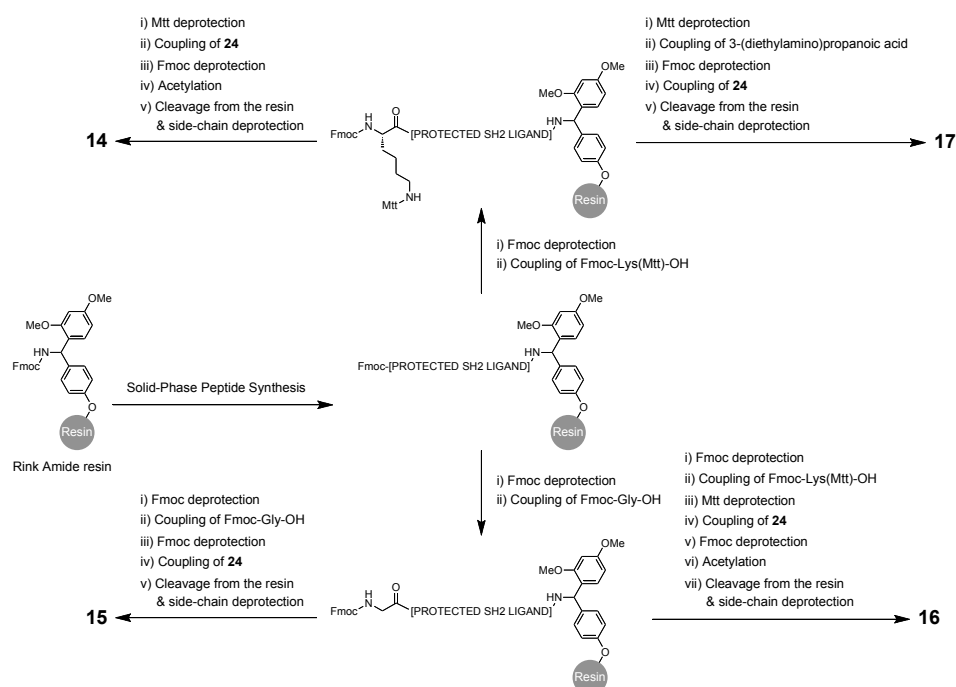
5: calcd for $[M+H]^+ = 1586.7$, obsd 1586.3

6: calcd for $[M+H]^+ = 1875.9$, obsd 1876.3

10: calcd for $[M+H]^+ = 1579.9$, obsd 1580.8

11: calcd for $[M+H]^+ = 1990.1$, obsd 1990.7

Synthesis of peptide ligand-tethered AGD catalysts 15-18



Scheme S7. Synthetic scheme of 14-17

MALDI-TOF-MS (CHCA):

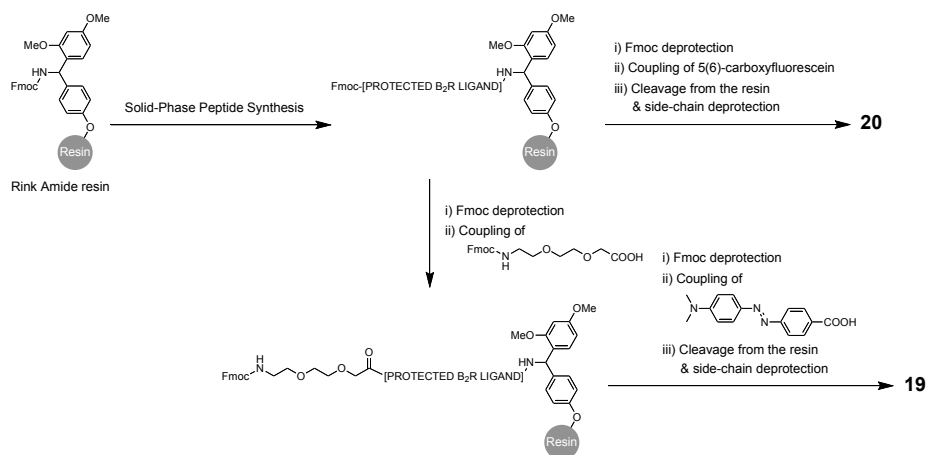
14: calcd for $[M+H]^+ = 1362.9$, obsd 1362.6

15: calcd for $[M+H]^+ = 1306.8$, obsd 1306.6

16: calcd for $[M+H]^+ = 1419.8$, obsd 1419.6

17: calcd for $[M+H]^+ = 1447.7$, obsd 1448.1

Synthesis of B₂R antagonist-DABCYL conjugate **19** and antagonist-fluorescein conjugate **20**



Scheme S9. Synthetic scheme of **19** and **20**

MALDI-TOF-MS (CHCA):

19: calcd for $[M+H]^+ = 1643.9$, obsd 1644.7

20: calcd for $[M+H]^+ = 1605.8$, obsd 1606.1

MALDI-TOF-MS (CHCA) of peptide ligands used for the BFQR assay:

$_D\text{Arg}^0[\text{Hyp}^3, _D\text{Phe}^7, \text{Leu}^8]\text{BK}$: calcd for $[M+H]^+ = 1247.7$, obsd 1247.7

des-Arg⁹-BK: calcd for $[M+H]^+ = 903.5$, obsd 903.4

Lys-des-Arg⁹-BK: calcd for $[M+H]^+ = 1031.6$, obsd 1031.4

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