Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

# Supporting Information



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# Cleavage Agents for Soluble Oligomers of Amyloid β Peptides

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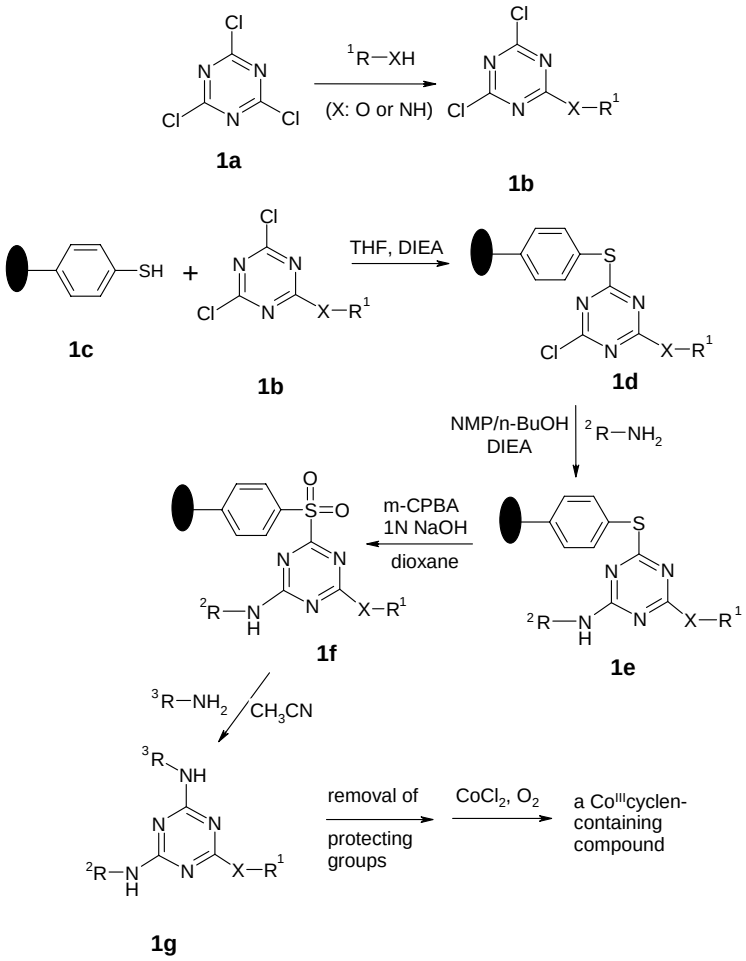
## Contents

**(1) Procedures for Preparation of Combinatorial Library of Candidates for Cleavage Agents**

Combinatorial library of $Co^{III}$cyclen-containing candidates for cleavage agents of soluble oligomers of amyloid β peptides was constructed according to the route of Scheme S1. Here, the procedures for the solid phase synthesis were taken from the literature (S. M. Khersonsky, Y. T. Chang, *J. Comb. Chem.* **2004**, *6*, 474-477).




***Scheme S1.*** Synthetic route for construction of the chemical library of $Co^{III}$cyclen-containing candidates for cleavage agents of soluble oligomers of amyloid β peptides

Cyanuric chloride (**1a**) (0.20 g 1.1 mmol), $^{1}$R-XH (0.20 g 0.90 mmol), and diisopropylethylamine (DIEA) (0.38 mL, 2.7 mmol) were mixed together in tetrahydrofuran (THF) (50 mL) and the mixture was

stirred for 4-8 hours in an ice bath. The residue obtained by evaporation of the mixture was purified by column chromatography to obtain **1b**. Here, $^{1}$R-XH was an amine or an alcohol.

To a THF solution (1.5 mL) of **1b** (0.15 mmol) were added PS-Thiophenol resin (**1c**) (purchased from Argonaut Technologies) (50 mg, 0.074 mmol) and DIEA (0.10 mL, 0.74 mmol). The mixture was placed in a heating block set at 65$^{o}$C overnight. After filtration, the resulting resin (**1d**) was washed with *N*,*N*-dimethylformamide (DMF), methylene chloride (MC), methanol (MeOH), and MC (each 3 mL × 3) and dried under nitrogen gas.

To the suspension of **1d** in *N*-methyl-2-pyrrolidinone (NMP) (1 mL) were added *n*-butanol (1 mL) and amine $^{2}$R-$NH_2$ (0.58 mmol), followed by DIEA (120 μL, 0.87 mmol). The mixture was placed in a heating block set at 120 $^{o}$C for 8 hours. After filtration, the resulting resin (**1e**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.

To **1e** was added the mixture of a solution of *m*-chloroperoxybenzoic acid (m-CPBA) (13 mg, 0.074 mmol) in 1,4-dioxane (1.8 mL) and aqueous 1 N NaOH (16 μL). The mixture was gently shaken for 4 hours at room temperature. After filtration, the resulting resin (**1f**) was washed with 1,4-dioxane and MC (each 3 mL × 3) and was dried under nitrogen gas.

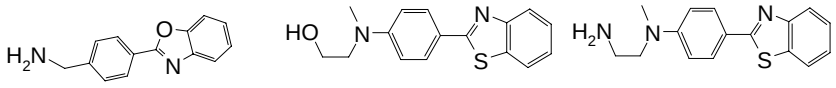
To the suspension of **1f** in acetonitrile (1.5 mL) were added PS-DIEA resin (purchased from Argonaut Technologies) (3 mg, 0.012 mmol) and amine $^{3}$R-$NH_2$ (7.4 μmol). The reaction tube was placed in a heating block at 80 $^{o}$C for 8 hours. The mixture was filtered and the resin was washed with MC (1 mL × 3). The combined filtrate and washing were evaporated in vacuo and the residue was dried with a centrifugal vacuum concentrator (Hanil Model 3180C) to obtain crude **1g**.

Crude **1g** (5 mg) was treated with 50 % trifluoroacetic acid (TFA) in MC (50 μL) for 5 hours and diethyl ether (1 mL) was added to the TFA solution to remove the boc protecting groups attached to cyclen moieties. The precipitate was separated by centrifugation, washed with diethyl ether several times, and dried under nitrogen gas to obtain the TFA salt of the ligand of the candidate for cleavage agents, which was used without further purification. To the MeOH (500 μL) solution of the TFA salt was added LiOH (~4.5 equiv.) followed by $CoCl_2$•$6H_2O$ (~2 equil.), and the resulting solution was shaken overnight

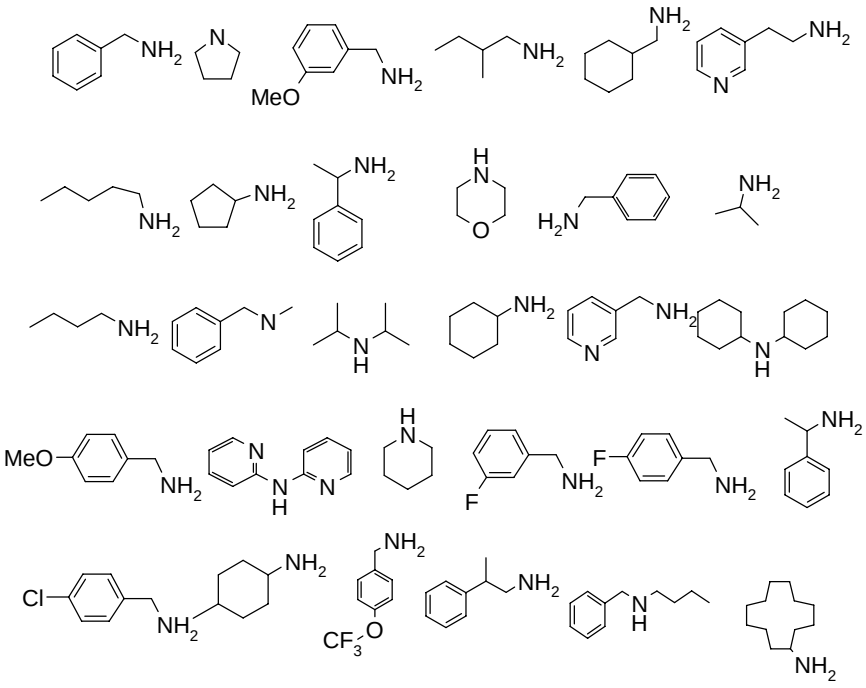
under the air (S. E. Castillo-Blum, M. E. Sosa-Torres, *Polyhedron* **1995**, *14*, 223–229). Oxidation of $Co^{II}$ to $Co^{III}$ was accompanied by appearance of deep violet color. The solvent was evaporated completely with a centrifugal vacuum concentrator (Hanil Model 3180C) and water (500 μL) was added. The aqueous solution of the resulting $Co^{III}$ complex was kept at room temperature for several days or weeks before testing its ability to cleave the substrate.

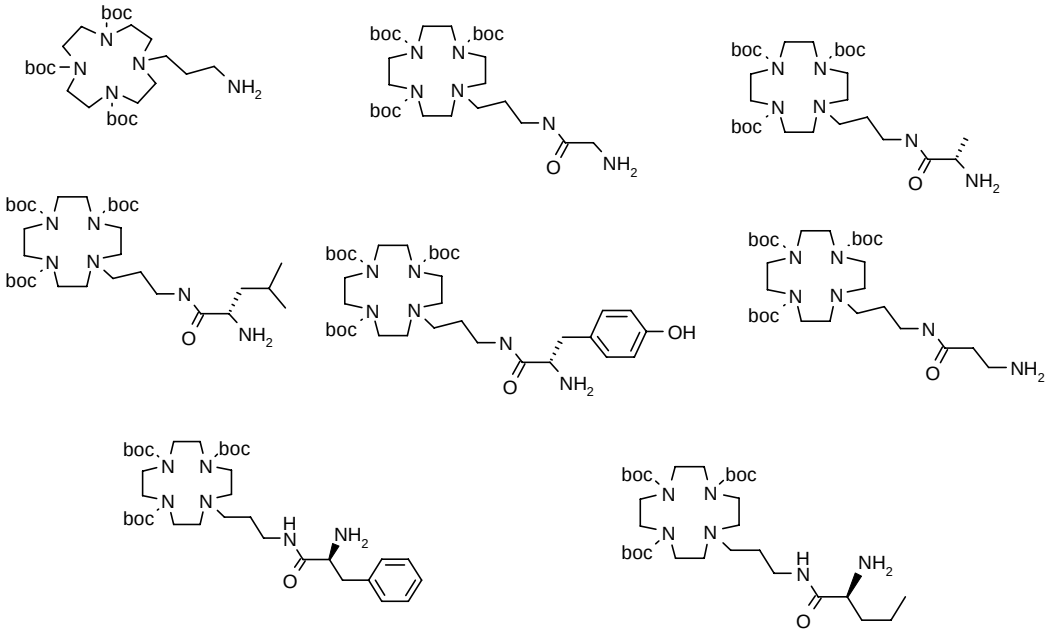
## (2) Components Used in Construction of the Library

The following compounds were used to introduce component $^{1}$R-XH:

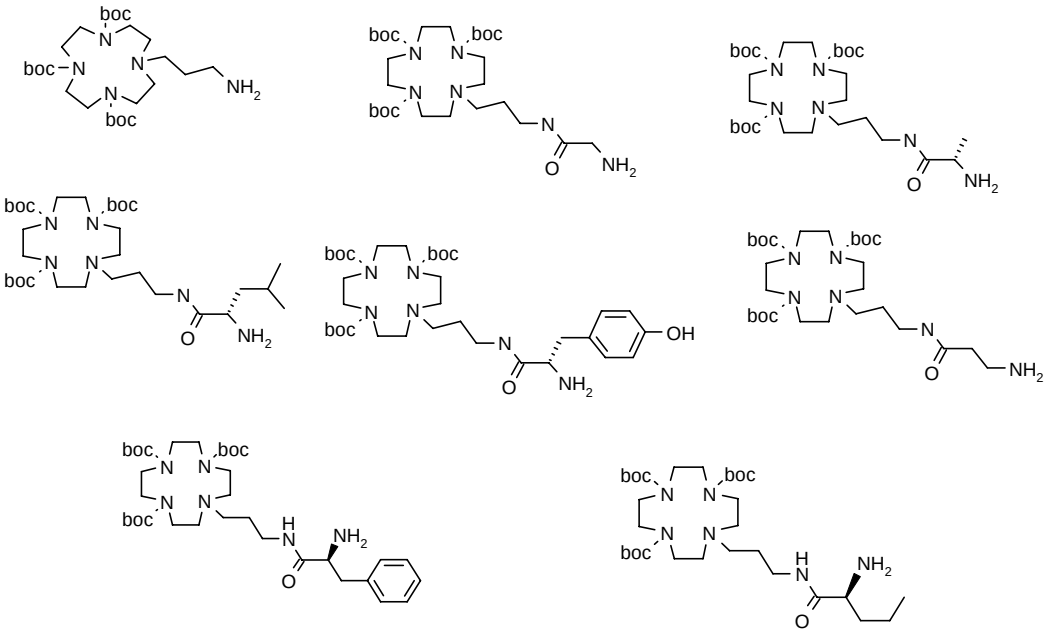


The following compounds were used to introduce component $^{2}$R-$NH_2$:





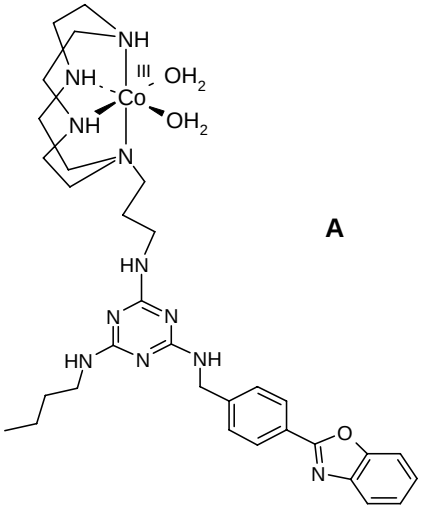
The following compounds were used to introduce component $^{3}R\text{-}NH_2$:



**(3) Procedures for Synthesis of A-D**

a) Synthesis of **A**

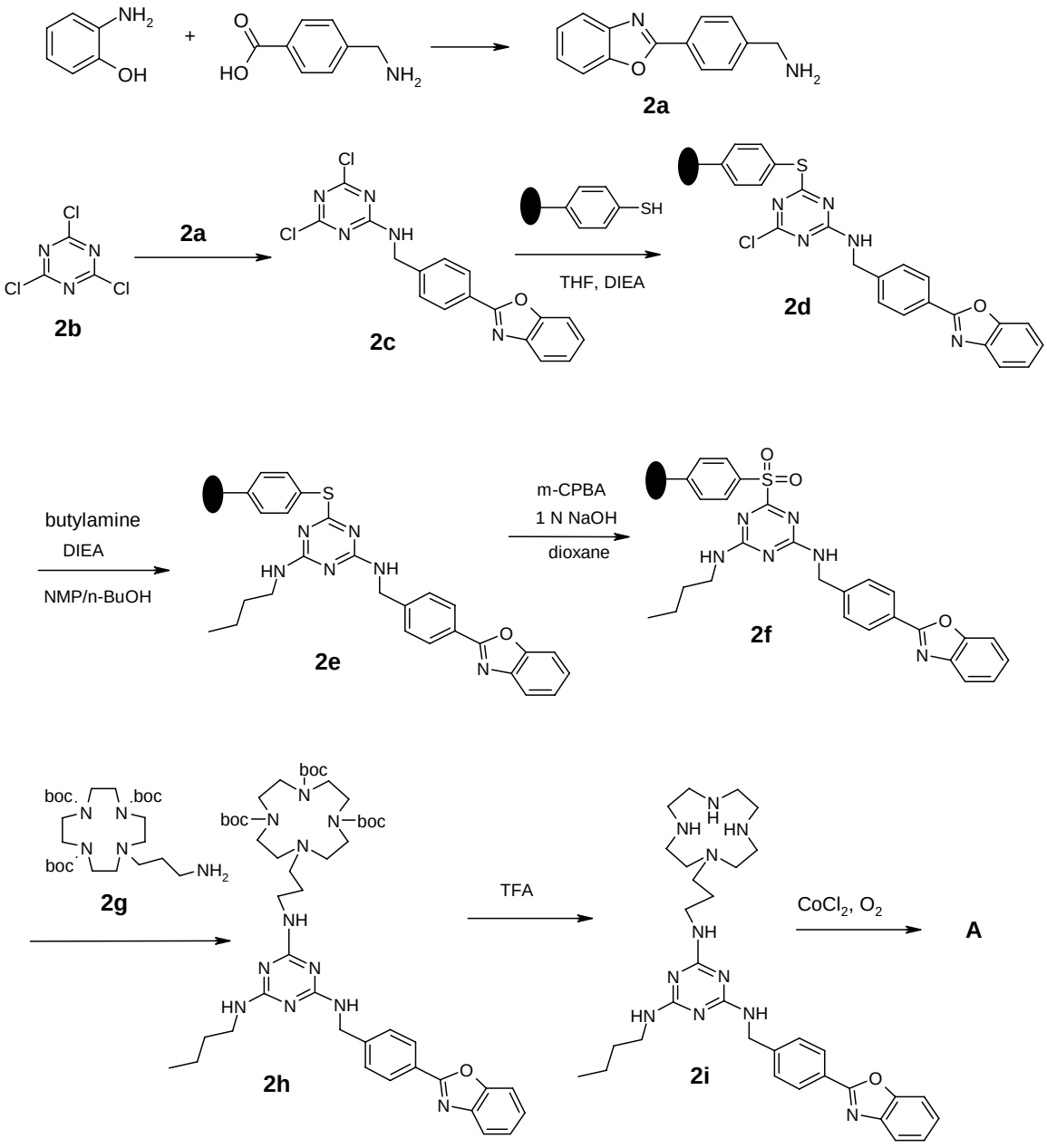
Cleavage agent **A** was synthesized according to the route described in Scheme S2.




**A**

4-Aminomethyl-benzoic acid (1.0 g, 6.8 mmol) and 2-aminophenol (0.69 g, 7.4 mmol) were mixed together with polyphosphoric acid (10 g) and heated to 170°C under $N_2$ atmosphere for 1.5 hours according to a general procedure reported in the literature (C. A. Mathis, Y. Wang, D. P. Holt, G.-F. Huang, M. L. Debnath, W. E. Klunk, *J. Med. Chem.* **2003**, *46*, 2740-2754). The reaction mixture was cooled to room temperature and poured into 10 % $K_2CO_3$ solution. The precipitate was filtered under reduced pressure. The precipitate was recrystallized from acetone-water followed by treatment with activated charcoal in THF-water to obtain 4-benzooxazol-2-yl-benzylamine (**2a**). $R_f$ 0.65 (EA/hexane 1:2); $^1$H NMR ($CDCl_3$): δ 8.20 (d, 2 H), 7.76 (dd, 1 H), 7.66 (dd, 1 H), 7.55 (d, 2 H), 7.41 (m, 2 H), 3.90 (s, 2 H); MS (MALDI-TOF) *m/z* 225.33 $(M+H)^+$ calcd for $C_{14}H_{13}N_2O_1$ 225.09. Cyanuric chloride (**2b**) (0.20 g 1.1 mmol), **2a** (0.20 g 0.90 mmol), and DIEA (0.38 mL, 2.7 mmol) were mixed together in THF (50 mL) and the mixture was stirred for 4 hours in an ice bath. The residue obtained by evaporation of the mixture was purified by column chromatography to obtain (4-benzooxazol-2-yl-benzyl)-(4,6-dichloro-[1,3,5]triazin-2-yl)-amine (**2c**). $R_f$ 0.7 (EA/hexane 1:4); $^1$H NMR ($CDCl_3$): δ 8.13 (d, 2H), 7.70 (d, 1H), 7.51 (d, 1H), 7.40 (d, 2H), 7.27 (d, 2H), 4.58 (d, 2H); $^{13}$C NMR ($CDCl_3$): δ 171.26, 170.17, 166.04,

162.43, 150.72, 141.96, 139.84, 128.16, 128.09, 126.96, 125.34, 124.73, 120.06, 110.65, 76.60, 45.05; MS (MALDI-TOF) *m/z* 372.28 $(M+H)^+$, calcd for $C_{17}H_{12}Cl_2N_5O$ 372.03.




***Scheme S2.*** Synthetic route for **A**.

To a THF solution (1.5 mL) of **2c** (55 mg, 0.15 mmol) were added PS-Thiophenol resin (purchased from Argonaut Technologies) (50 mg, 0.074 mmol) and DIEA (0.10 mL, 0.74 mmol). The mixture was placed in a heating block set at 65°C overnight. After filtration, the resulting resin (**2d**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.

To the suspension of **2d** in NMP (1 mL) were added *n*-butanol (1 mL) and butylamine (49 μL, 0.58 mmol), followed by DIEA (120 μL, 0.87 mmol). The mixture was placed in a heating block set at 120 °C for 8 hours. After filtration, the resulting resin (**2e**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.

To **2e** was added the mixture of a solution of m-CPBA (130 mg, 0.74 mmol) in 1,4-dioxane (1.8 mL) and aqueous 1 N NaOH (160 μL). The mixture was gently shaken for 4 hours at room temperature. After filtration, the resulting resin (**2f**) was washed with 1,4-dioxane and MC (each 3 mL × 3) and was dried under nitrogen gas. To the suspension of **2f** in acetonitrile (1.5 mL) were added PS-DIEA resin (purchased from Argonaut Technologies) (30 mg, 0.12 mmol) and **2g** (P. S. Chae, M. Kim, C. Jeung, S. D. Lee, H. Park, S. Lee, J. Suh, *J. Am. Chem. Soc.* **2005**, *127*, 2396-2397) (39 mg, 0.074 mmol). The reaction tube was placed in a heating block at 80 °C for 8 hours. The mixture was filtered and the resin was washed with MC (1 mL × 3). The combined filtrate and washing were evaporated in vacuo and the residue was purified by column chromatography to obtain 10-{3-[4-(4-benzooxazol-2-yl-benzylamino)-6-butylamino-[1,3,5]triazin-2-ylamino]-propyl}-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester (**2h**). $R_f$ 0.5 (EA only); $^1$H NMR ($CDCl_3$): δ 8.13 (d, 2H), 7.70 (d, 1H), 7.51 (d, 1H), 7.40 (d, 2H), 7.27 (d, 2H), 4.58 (d, 2H), 3.8-3.2 (br, 14H), 2.5 (br, 6H), 2.1 (br, 2H), 1.63 (br, 2H), 1.5-1.3 (m, 31H), 0.9-0.8 (dd 3H); $^{13}$C NMR ($CDCl_3$): δ 164.89, 161.91, 154.33, 149.67, 142.56, 141.06, 126.72, 124.82, 124.01, 123.53, 118.89, 109.53, 78.51, 78.31, 75.58, 53.92, 52.91, 48.92, 48.08, 43.31, 39.35, 37.97, 30.86, 28.67, 27.65, 19.03, 13.10; MS (MALDI-TOF) *m/z* 902.99 $(M+H)^+$, calcd for $C_{47}H_{72}N_{11}O_7$ 902.55.

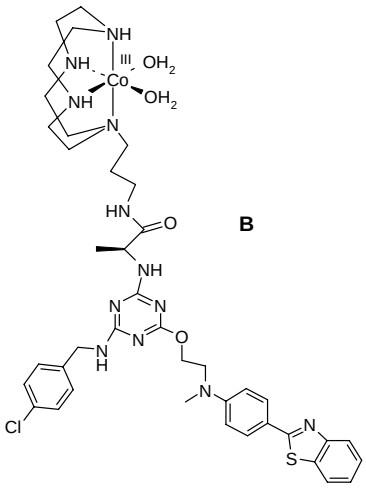
Compound **2h** (5 mg) was treated with 50 % TFA in MC (50 μL) for 5 hours and diethyl ether (1 mL) was added to the TFA solution. The precipitate was separated by centrifugation, washed with diethyl ether several times, and dried under nitrogen gas to obtain the TFA salt of *N*-(4-benzooxazol-2-yl-benzyl)-*N*'-butyl-*N*''-[3-(1,4,7,10tetraaza-cyclododec-1-yl)-propyl]-[1,3,5]triazine-2,4,6-triamine (**2i**). The TFA salt of **2i** was used for NMR and MS characterization; $^1$H NMR ($CDCl_3$): δ 8.17 (d, 2 H), 7.73 (d, 1 H), 7.55 (d, 1H), 7.43 (d, 2H), 7.33 (d, 2H), 4.42 (br, 2H), 3.4-3.0 (br, 14H), 2.85 (br, 6H), 2.62 (br, 2H),

1.68 (br, 2H), 1.36-1.23 m, 4H), 0.9-0.8 (dd 3H); $^{13}$C NMR ($CDCl_3$): δ 164.89, 162.67, 150.62, 141.76, 127.80, 126.12, 125.30, 124.73, 119.83, 110.65, 76.60, 51, 48.90, 44.87, 42.80, 42.32, 38.07, 30.89, 29.70, 23.71, 19.83, 14.07; MS (MALDI-TOF) *m/z* 602.73. $(M+H)^+$, calcd for $C_{32}H_{48}N_{11}O$ 602.40; HRMS *m/z* 602.4043. $(M+H)^+$, calcd for $C_{32}H_{48}N_{11}O$ 602.4038.

To the MeOH (500 μL) solution of the TFA salt of **2i** (3.0 mg, 0.0050 mmol) were added LiOH (1.0 mg, 0.025 mmol) followed by $CoCl_2{\cdot}6H_2O$ (1.2 mg, 0.0050 mmol), and the solution was shaken overnight under the air (S. E. Castillo-Blum, M. E. Sosa-Torres, *Polyhedron* **1995**, *14*, 223-229). Oxidation of $Co^{II}$ to $Co^{III}$ was accompanied by appearance of deep violet color. The resulting $Co^{III}$ complex was purified with HPLC (Hewlett-Packard 1050 series) by using a gradient of two solvent systems consisting of 0.1 % TFA in water and 0.1 % TFA in HPLC-grade acetonitrile, a 10 μm C-18 column (300 × 3.9 mm, purchased from Phenomenex), and a UV (545 nm) detector. The portion containing $Co^{III}$ complexes was evaporated, dissolved in 0.1 M NaOH, incubated for 1 hour at 37$^o$C, neutralized to pH 6-8, and kept at room temperature for several days to obtain the stock solution of **A**. Concentration of the stock solution was determined through quantification of cobalt by ICP-AES (Shimadzu ICPS-1000IV model) or ICP-MS (Perkin Elmer ELAN 6100 model).

b) Synthesis of **B**.

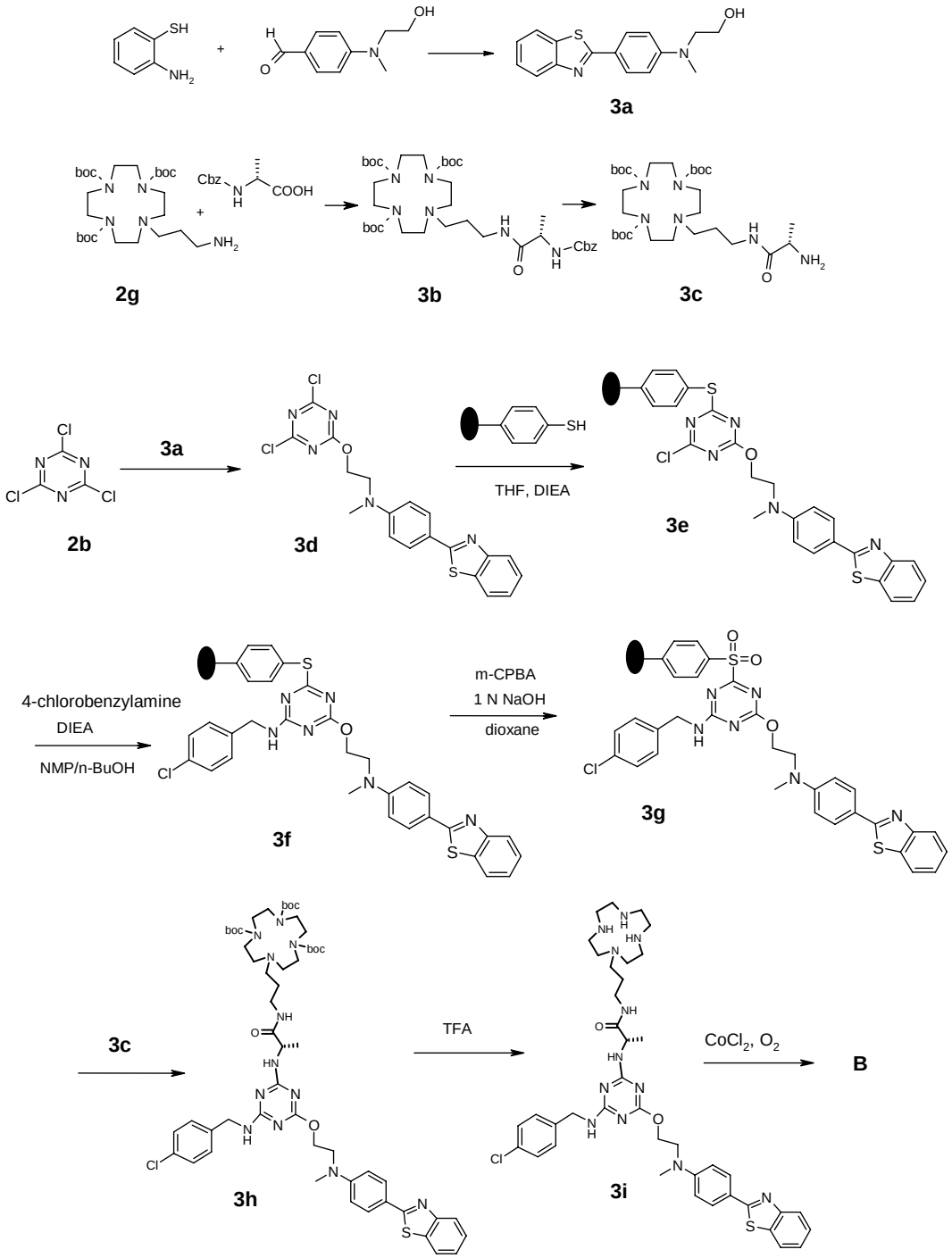
Cleavage agent **B** was synthesized according to the route described in Scheme S3.




A mixture of 2-aminothiophenol (1.3 g, 10 mmol) and *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (1.8 g, 10 mmol) in dimethyl sulfoxide (10 mL) was heated to 170 °C for 1.5 hours. After cooling to room temperature, the reaction mixture was poured into water. The resulting mixture was extracted with ethyl acetate (EA) (50 mL × 2). The combined organic layers were dried over $Na_2SO_4$. The residue obtained by evaporation of the solvent was recrystallized from acetonitrile to afford 2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethanol (**3a**) as a yellow solid. $R_f$ 0.20 (EA/hexane 1:1); $^1$H NMR ($CDCl_3$): δ 7.97 (d, 1H), 7.95 (d, 2H), 7.85 (d, 1H), 7.45 (t, 1H), 7.32 (t, 1H), 6.81 (d, 2H), 3.88 (t, 2H), 3.60 (t, 2H), 1.80 (br s, 3H); $^{13}$C NMR ($CDCl_3$): δ 154.09, 151.61, 134.38, 129.03, 126.10, 124.34, 122.23, 121.67, 111.96, 77.02, 60.19, 54.66, 39.03; MS (MALDI-TOF) 285.35 *m/z* $(M+H)^+$ calcd for $C_{16}H_{17}NOS$ 285.10.

To the stirred solution of **2g** (2.9 g, 5.5 mmol) in acetonitrile (100 mL) were added *N*-α-Cbz-L-alanine (1.2 g, 5.5 mmol) and DIEA (2.9 mL, 17 mmol). To the reaction mixture was added *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluroniumhexafluoro phosphate (2.1 g, 5.5 mmol) and the mixture was stirred for 1 hour. The residue obtained by evaporation of the solution was dissolved in EA (100 mL). The EA solution was washed with 5% aq. citric acid (50 mL), 5% aq. $Na_2CO_3$ (50 mL), and brine (50 mL), and dried over $Na_2SO_4$. The solvent was evaporated off and column chromatography afforded 10-[(*S*)-3-(2-benzyloxycarbonylamino-propionylamino)-propyl]-1,4,7,10-tetraaza-cyclododecane-1,4,7-

tricarboxylic acid tri-*tert*-butyl ester (**3b**) as a colorless oil. $R_f$ 0.2 (EA/hexane 1:1). $^1$H NMR ($CDCl_3$): δ 7.30 (s, 5H), 5.02 (s, 2), 3.50-3.10 (br, 15H) 2.60-2.30 (br, 6H), 1.57-1.49 (br, 2H), 1.39-1.36 (m, 27H), 1.18 (s, 3H); $^{13}$C NMR ($CDCl_3$): δ 171.58, 154.82, 154.79, 154.22, 135.42, 127.55, 78.49, 65.71, 53.42, 49.56, 48.92, 47.57, 47.18, 46.53, 45.25, 37.68, 29.92, 27.64, 18.32; MS (MALDI-TOF) *m/z* 735.88 $(M+H)^+$ calcd for $C_{37}H_{63}N_6O_9$ 735.46.




***Scheme S3.*** Synthetic route for **B**.

A suspension of **3b** (2.0g, 2.7 mmol) and 1.0 g of 10 % Pd/C in 80 mL of EtOH was stirred under 1 atm of $H_2$ for 24 hours. The catalyst was filtered off on Celite, and the solvent was evaporated off to afford 10-[(*S*)-3-(2-benzyloxycarbonylamino-propionylamino)-propyl]-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (**3c)** as an amorphous solid; $^1$H NMR ($CDCl_3$): δ 7.49 (s, 1H), 3.63-3.18 (br, 15H), 2.72-2.52 (br, 6H), 1.88-1.75 (br, 2H), 1.73-1.60 (m, 2H), 1.50-1.40 (m, 27H), 1.40-1.30 (d, 3H); $^{13}$C NMR ($CDCl_3$): δ 171.98, 155.03, 154.69, 154.25, 78.57, 78.42, 78.28, 53.60, 52.95, 49.72, 49.01, 47.80, 47.07, 46.54, 46.18, 37.56, 29.92, 27.64, 22.99; MS (MALDI-TOF) 601.58 *m/z* $(M+H)^+$ calcd for $C_{29}H_{57}N_6O_7$ 601.42.

Cyanuric chloride (**2b**) (0.20 g 1.1 mmol), **3a** (0.20 g 0.70 mmol), and DIEA (0.38 mL, 2.7 mmol) were mixed together in THF (50 mL) and the mixture was stirred 8 hours at room temperature. The residue obtained by evaporation of the solvent was purified by column chromatography to obtain (4-benzothiazol-2-yl-phenyl)-[2-(4,6-dichloro-[1,3,5]triazin-2-yloxy)-ethyl]-methyl-amine (**3d**). $R_f$ 0.7 (EA/hexane 1:4); $^1$H NMR ($CDCl_3$): δ 7.97 (t, 3H), 7.85 (d, 1H), 7.45 (t, 1H), 7.36 (t, 1H), 6.78 (d, 2H), 4.67 (t, 2H), 3.84 (t, 2H), 3.12 (br, 3H); $^{13}$C NMR ($CDCl_3$): δ 172.53, 171.84, 168.15, 155.13, 151.39, 135.02, 129.21, 126.27, 124.59, 122.66, 122.46, 121.74, 112.14, 67.78, 50.58, 38.89; MS (MALDI-TOF) *m/z* 432.29 $(M+H)^+$ calcd for $C_{19}H_{16}Cl_2N_5OS$ 432.04.

To a THF solution (1.5 mL) of **3d** (62 mg, 0.15 mmol) were added PS-Thiophenol resin (purchased from Argonaut Technologies)(50 mg, 0.074 mmol) and DIEA (0.10 mL 0.74 mmol). The mixture was placed in a heating block set at 65$^o$C overnight. After filtration, the resulting resin (**3e**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.

To a suspension of **3e** in NMP (1 mL) were added *n*-butanol (1 mL) and 4-chlorobenzylamine (71 μL, 0.58 mmol), followed by DIEA (120 μL, 0.87mmol). The mixture was placed in a heating block set at 120 $^o$C for 8 hours. After filtration, the resulting resin (**3f**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.

To **3f** was added the mixture of a solution of m-CPBA (130 mg, 0.74 mmol) in 1,4-dioxane (1.8 mL) and aqueous 1 N NaOH (160 μL). The mixture was gently shaken for 4 hours at room temperature. After

filtration, the resulting resin (**3g**) was washed with 1,4-dioxane and MC (each 3 mL × 3) and was dried under nitrogen gas.

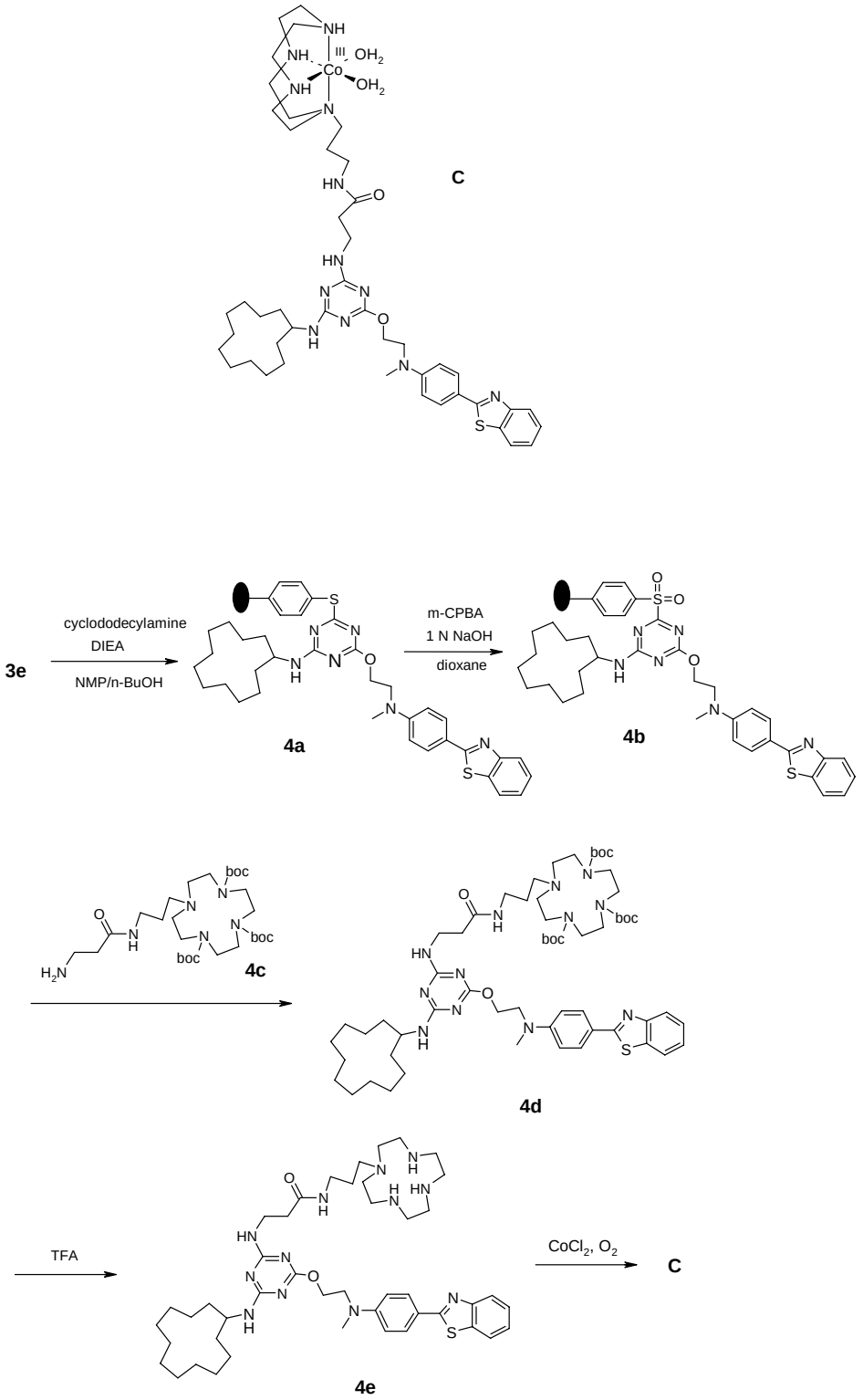
To the suspension of **3g** in acetonitrile (1.5 mL) was added PS-DIEA resin (purchased from Argonaut Technologies) (30 mg, 0.12 mmol) and **3c** (39 mg, 0.074 mmol). The reaction tube was placed in a heating block at 80 $^{o}$C for 8 hours. The mixture was filtered and the resin was washed with MC (1 mL × 3). The combined filtrate and washing were evaporated in vacuo and the resulting residue was purified by column chromatography to obtain 10-(3-{2-[4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-(4-chloro-benzylamino)-[1,3,5]triazin-2-ylamino]-(S)-propionylamino}-propyl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (**3h**). $R_f$ 0.2 (EA only); $^{1}$H NMR ($CDCl_3$): δ 8.13 (d, 2H), 7.70 (d, 1H), 7.51 (d, 1H), 7.40 (d, 2H), 7.27 (d, 2H), 4.58 (d, 2H), 3.8-3.2 (br, 12H), 2.5 (br, 6H), 2.1 (br, 2H), 1.63 (br, 4H), 1.5-1.3 (m, 31H), 0.9-0.8 (dd, 3H); $^{13}$C NMR ($CDCl_3$): δ 164.89, 161.91, 154.33, 149.67, 142.56, 141.06, 126.72, 124.82, 124.01, 123.53, 118.89, 109.53, 78.51, 78.31, 75.58, 53.92, 52.91, 48.92, 48.08, 43.31, 39.35, 37.97, 30.86, 28.67, 27.65, 19.03, 13.10; MS (MALDI-TOF) *m/z* 1102.59 $(M+H)^+$, calcd for $C_{55}H_{78}ClN_{12}O_8S$ 1102.54.

Compound **3h** was treated with TFA as described above for **2h** to obtain the TFA salt of 2-[4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-(4-chloro-benzylamino)-[1,3,5]triazin-2-ylamino]-*N*-[3-(1,4,7,10tetraaza-cyclododec-1-yl)-propyl]-(S)-propionamide (**3i**). The TFA salt of **3i** was used for NMR and MS characterization; $^{1}$H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.92-7.7.85 (q, 3H), 7.45 (t, 1H), 7.37-7.28 (m, 5H), 6.79 (t, 2H), 4.70-4.40 (br, 2H), 3.78 (br, 2H), 3.13-2.70 (br, 15H), 2.74 (s, 3H), 2.58-2.44 (br, 6H), 1.93 (m, 1H), 1.66-1.55(br, 2H), 1.44-1.19 (m, 5H); $^{13}$C NMR ($CDCl_3$) δ 173.57, 169.69, 168.84, 163.26, 162.01, 155.42, 152.65, 138.23, 135.63, 132.63, 130.76, 130.52, 130.07, 127.63, 125.90, 123.26, 123.08, 122.51, 113.29, 69.08, 67.48, 52.18, 51.49, 50.44, 45.22, 43.73, 43.18, 39.98, 31.51, 24.91; MS (MALDI-TOF) *m/z* 801.57 $(M+H)^+$, calcd for $C_{40}H_{54}ClN_{12}O_2S$ 801.39; HRMS *m/z* 801.3907. $(M+H)^+$, calcd for $C_{40}H_{54}ClN_{12}O_2S$ 801.3896.

The stock solution of **B** was obtained from **3i** as described for **A**.

c) Synthesis of **C**.

Cleavage agent **C** was synthesized according to the route described in Scheme S4.




***Scheme S4.*** Synthetic route for **C**.

To a suspension of **3e** (50 mg, 0.046 mmol) in NMP (1 mL) were added *n*-butanol (1 mL) and cyclododecylamine (66 μL, 0.51 mmol), followed by DIEA (63 μL, 0.36 mmol). The mixture was placed in a heating block set at 120 °C for 8 hours. After filtration, the resulting resin (**4a**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas. To **4a** was added the mixture of a solution of m-CPBA (80 mg, 0.46 mmol) in 1,4-dioxane (1.8 mL) and aqueous 1 N NaOH (93 μL). The mixture was gently shaken for 4 hours at room temperature. After filtration, the resulting resin (**4b**) was washed with 1,4-dioxane and MC (each 3 mL × 3) and was dried under nitrogen gas.

To the suspension of **4b** in acetonitrile (1.5 mL) was added PS-DIEA resin (purchased from Argonaut Technologies) (19 mg, 0.075 mmol) and **4c** (P. S. Chae, M. Kim, C. Jeung, S. D. Lee, H. Park, S. Lee, J. Suh, *J. Am. Chem. Soc.* **2005**, *127*, 2396-2397) (28 mg, 0.046 mmol). The reaction tube was placed in a heating block at 80 °C for 8 hours. The mixture was filtered and the resin was washed with MC (1 mL × 3). The combined filtrate and washing were evaporated in vacuo and the residue was purified by column chromatography to obtain 10-{3-[3-(4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-cyclododecylamino-[1,3,5]triazin-2-ylamino)-propionylamino]-propyl}-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (**4d**). $R_f$ 0.3 (EA only); $^1$H NMR ($CDCl_3$): δ 7.95 (t, 3H), 7.83 (d, 1H), 7.41 (t, 1H), 7.33 (br, 1H), 6.78 (m, 2H), 4.67 (br, 2H), 3.75 (br, 2H), 3.67 (br, 2H), 3.55-3.18 (br, 14H), 3.10 (s, 3H), 2.60 (br, 5H), 2.50 (br, 4H), 1.61-1.58 (br, 6H), 1.45-1.41 (m, 27H), 1.33 (br, 18H); $^{13}$C NMR ($CDCl_3$): δ 184.41, 173.21, 171.70, 169.97, 168.67, 156.28, 155.60, 155.24, 154.34, 150.84, 134.47, 129.75, 128.97, 125.99, 125.01, 124.21, 122.21, 121.35, 111.57, 111.46, 79.46, 62.94, 56.52, 55.11, 51.07, 49.97, 48.36, 47.41, 46.54, 40.92, 39.27, 36.76, 35.63, 29.69, 28.62, 23.50, 21.32; MS (MALDI-TOF) *m/z* 1144.02 $(M+H)^+$, calcd for $C_{60}H_{95}N_{12}O_8S$ 1143.70,

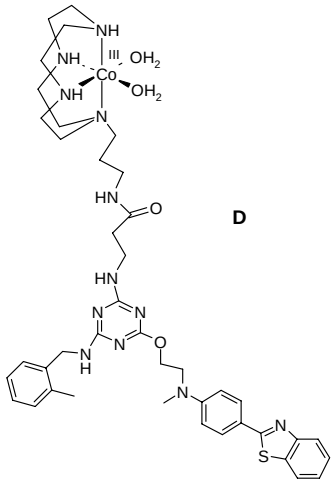
Compound **4d** was treated with TFA as described above for **2h** to obtain the TFA salt of 3-(4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-cyclododecylamino-[1,3,5]triazin-2-ylamino)-*N*-[3-(1,4,7,10-tetraaza-cyclododec-1-yl)-propyl]-propionamide (**4e**). The TFA salt of **4e** was used for NMR and MS characterization; $^1$H NMR ($CDCl_3$): δ 7.94 (br, 3H), 7.83 (d, 1H), 7.45 (br, 1H), 7.35 (br, 1H), 6.75 (d, 2H), 4.57 (br, 2H), 3.81 (br, 2H), 3.63 (br, 2H), 3.3-2.9 (br, 17H), 2.76 (br, 5H), 2.44 (br, 4H),

1.65-1.61 (br, 6H), 1.31 (br, 18H); $^{13}$C NMR ($CDCl_3$): δ 188.78, 188.78, 172.02, 169.91 169.79, 169.44, 157.24, 151.78, 133.60, 129.56, 126.89, 125.41, 125.16, 121.81, 120.57, 112.28, 112.02, 66.26, 65.57, 60.62, 50.69, 49.67, 48.68, 44.63, 42.51, 39.33, 39.16, 37.01, 30.20, 29.94, 23.82, 23.51, 21.48; MS (MALDI-TOF) *m/z* 843.79 $(M+H)^+$, calcd for $C_{45}H_{71}N_{12}O_2S$ 843.55; HRMS *m/z* 843.5547. $(M+H)^+$, calcd for $C_{45}H_{71}N_{12}O_2S$ 843.5538.

The stock solution of **C** was obtained from **4e** as described for **A**.

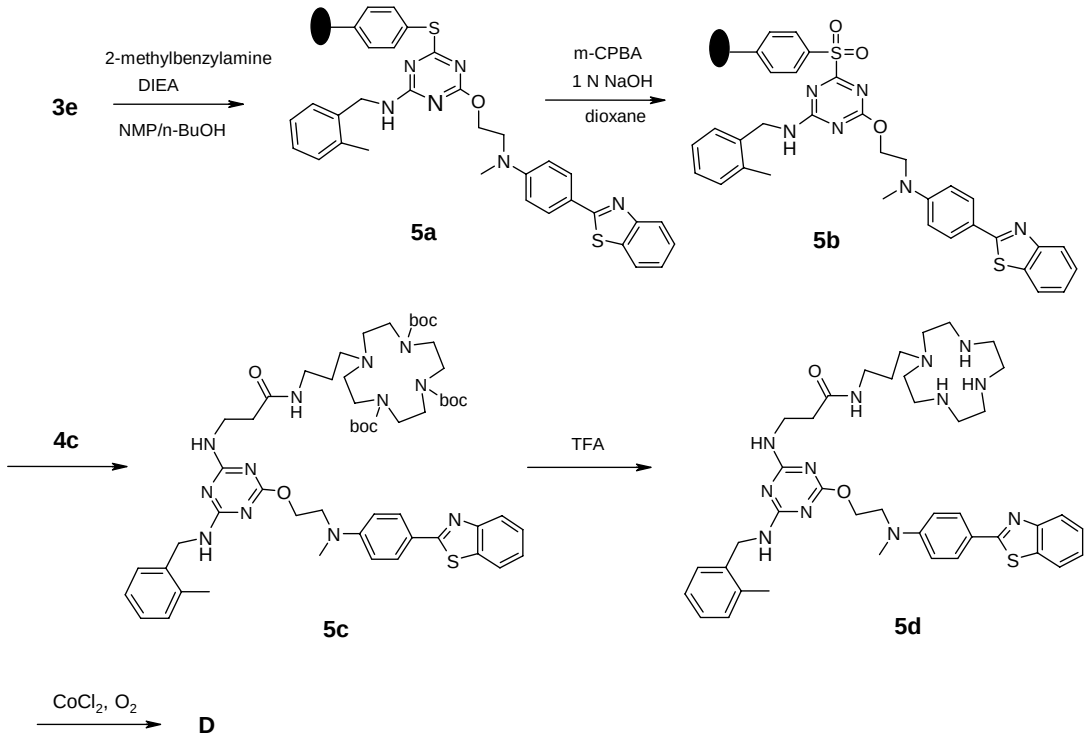
d) Synthesis of **D**.

Cleavage agent **D** was synthesized according to the route described in Scheme S5.




**D**

To a suspension of **3e** (50 mg, 0.046 mmol) in NMP (1 mL) were added *n*-butanol (1 mL) and 2-methylbenzylamine (63 μL, 0.51 mmol), followed by DIEA (63 μL, 0.36 mmol). The mixture was placed in a heating block set at 120 °C for 8 hours. After filtration, the resulting resin (**5a**) was washed with DMF, MC, MeOH, and MC (each 3 mL × 3) and dried under nitrogen gas.




***Scheme S5.*** Synthetic route for **D**.

To **5a** was added the mixture of a solution of m-CPBA (80 mg, 0.46 mmol) in 1,4-dioxane (1.8 mL) and aqueous 1 N NaOH (93 μL). The mixture was gently shaken for 4 hours at room temperature. After filtration, the resulting resin (**5b**) was washed with 1,4-dioxane and MC (each 3 mL × 3) and was dried under nitrogen gas. To the suspension of **5b** in acetonitrile (1.5 mL) was added PS-DIEA resin (purchased from Argonaut Technologies) (19 mg, 0.075 mmol) and **4c** (28 mg, 0.046 mmol). The reaction tube was placed in a heating block at 80 °C for 8 hours. The mixture was filtered and the resin was washed with MC (1 mL × 3). The combined filtrate and washing were evaporated in vacuo and the residue was purified by column chromatography to obtain 10-(3-{3-[4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-(2-methyl-benzylamino)-[1,3,5]triazin-2-ylamino]-propionylamino}-propyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (**5c**). $R_f$ 0.4 (EA only); $^1$H NMR ($CDCl_3$): δ 7.96 (t, 3H), 7.83 (d, 1H), 7.43 (t, 1H), 7.32 (br, 1H), 7.15 (br, 4H), 6.77 (d, 2H), 4.53 (br, 4H), 3.75 (br, 2H), 3.66 (br, 2H), 3.5-3.1 (br, 14H), 3.09 (s, 3H), 2.58 (br, 4H), 2.49 (br, 4H), 2.32 (s, 3H),

1.60 (br, 2H), 1.46-1.41 (m, 27H); $^{13}$C NMR ($CDCl_3$): δ 186.87, 179.99, 173.28, 171.18, 168.68, 155.26, 154.35, 150.86, 136.88, 136.10, 134.50, 130.27, 128.99, 127.27, 126.09, 126.01, 125.02, 124.24, 122.24, 121.45, 121.38, 111.55, 80.31, 79.51, 63.54, 56.56, 55.10, 54.95, 50.98, 49.95, 48.35, 47.65, 40.01, 39.03, 36.93, 29.71, 28.65, 19.10; MS (MALDI-TOF) *m/z* 1081.89 $(M+H)^+$, calcd for $C_{56}H_{81}N_{12}O_8S$ 1081.59.

Compound **5c** was treated with TFA as described above for **2h** to obtain the TFA salt of 3-[4-{2-[(4-benzothiazol-2-yl-phenyl)-methyl-amino]-ethoxy}-6-(2-methyl-benzylamino)-[1,3,5]triazin-2-ylamino]-*N*-[3-(1,4,7,10-tetraaza-cyclododec-1-yl)-propyl]-propionamide (**5d**). The TFA salt of **5d** was used for NMR and MS characterization; $^1$H NMR ($CDCl_3$): δ 7.96 (t, 3H), 7.85 (d, 1H), 7.49 (br, 1H), 7.37 (br, 1H), 7.15 (br, 4H), 6.80 (d, 2H), 4.56 ( m, 4H), 3.58 (br, 2H), 3.3-2.8 (br, 17H), 2.33 (br, 8H), 2.31 (s, 3H), 1.48 (br, 2H); $^{13}$C NMR ($CDCl_3$): δ 186.11, 180.06, 171.65, 169.85, 169.01, 151.51, 151.17, 136.08, 134.83, 132.77, 130.66, 129.65, 129.42, 127.99, 127.93, 127.82, 126.31, 126.13, 125.33, 121.76, 121.05, 112.09, 111.90, 66.12, 62.87, 53.89, 50.44, 49.72, 44.38, 44.22, 42.34, 42.09, 39.36, 36.63, 29.71, 19.07; MS (MALDI-TOF) *m/z* 781.73 $(M+H)^+$, calcd for $C_{41}H_{57}N_{12}O_2S$ 781.44; HRMS *m/z* 781.4457. $(M+H)^+$, calcd for $C_{41}H_{57}N_{12}O_2S$ 781.4443.

The stock solution of **D** was obtained from **5d** as described for **A**.

**(4) Stock Solutions of Aβ Peptides**

Recombinant $A\beta_{40}$ treated with NaOH, purchased form r-Peptide, was dissolved in water and voltexed for 5 seconds to obtain the stock solution right before use in measurements. Recombinant $A\beta_{42}$ treated with hexafluoroisopropanol (0.50 mg), purchased form r-Peptide, was dissolved in 0.50 mL of 50 mM NaOH and the solution was sonicated for 1 minute at room temperature and lyophilized. The lyophilized sample was redissolved in 1.0 mL water, voltexed for 5 seconds and sonicated for 30 seconds right before use in measurements.

**(5) Procedures for Filtration Experiments**

A solution of $A\beta_{40}$ or $A\beta_{42}$ (4.0 μM) was obtained by adding the stock solution to a 50 mM sodium phosphate solution (pH 7.50) and then voltexing for 5 sec. After the solution was incubated at 37 °C for various periods without shaking, 100 μL of the solution was loaded to a 10 kDa MWCO filter (Millipore Microcon centrifugal filter device YM-10) and then was partially filtered through using a high speed centrifuge (Hanil Model Supra 21K) at 14000 × g for 10 min at 20 °C. Then, 20 μL of the filtrate was transferred to a 500 μL microtube to perform amino acid analysis. After addition of 10 μL of 13.5 M NaOH, the mixture was voltexed briefly, centrifuged at 7000 × g for 5 sec with a micro centrifuge (Hanil Model Micro 12), and kept at 120 $^{o}$C in an autoclave for 2 hours for alkaline hydrolysis of the peptide. After the mixture was cooled to room temperature, the mixture was neutralized with methanesulfonic acid (15.4 M, 7.5 μL), and then pH of the reaction mixture was adjusted to pH 9.0 ± 0.2 with a boric acid solution (700 mM, 48 μL). After brief voltexing and centrifugation, a solution of fluorescamine (S. Udenfriend, S. Stein, P. Bőhlen, W. Dairman, W. Leimgruber, M. Weigele, *Science* **1972**, *178*, 871-872) in acetonitrile (5.0 mg/mL, 10 μL) was added to the mixture. After additional brief voltexing and centrifugation, the mixture was loaded to a 96 well plate (F96 Cert. Maxisorp, Nunc-Immuno Plate). The relative value of fluorescence of the resulting solution was measured with an imaging instrument (Alpha Imager Model 1220 INT) and compared with a standard curve constructed under the same conditions with standard solutions of $A\beta_{40}$ or $A\beta_{42}$. Buffer solutions were filtered with 0.45 μm Millipore microfilter and autoclaved before use in measurements in the present study.

**(6) Results of Filtration Experiments**

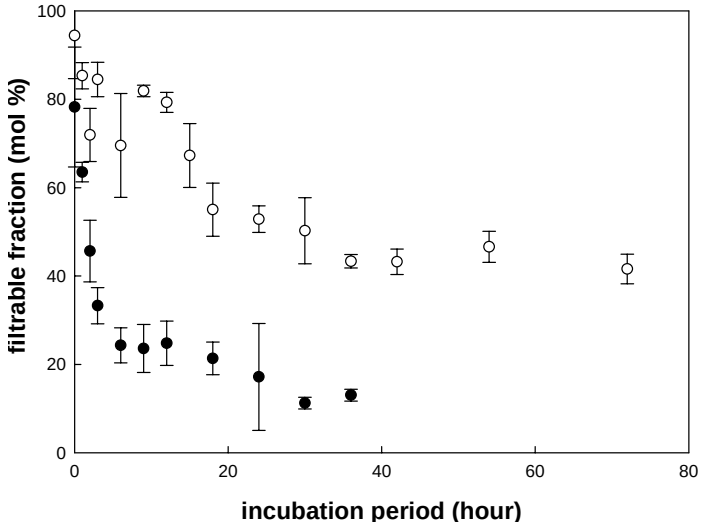



***Figure S1.*** The fraction of $A\beta_{40}$ (○) or $A\beta_{42}$ (●) (initial concentration: 4.0 μM) passing the membrane with cut-off MW of 10000 after incubation at pH 7.50 and 37°C. Each data point represents the average of at least 5 measurements.

**(7) MALDI-TOF Mass Spectra of Products for Cleavage by A-D**

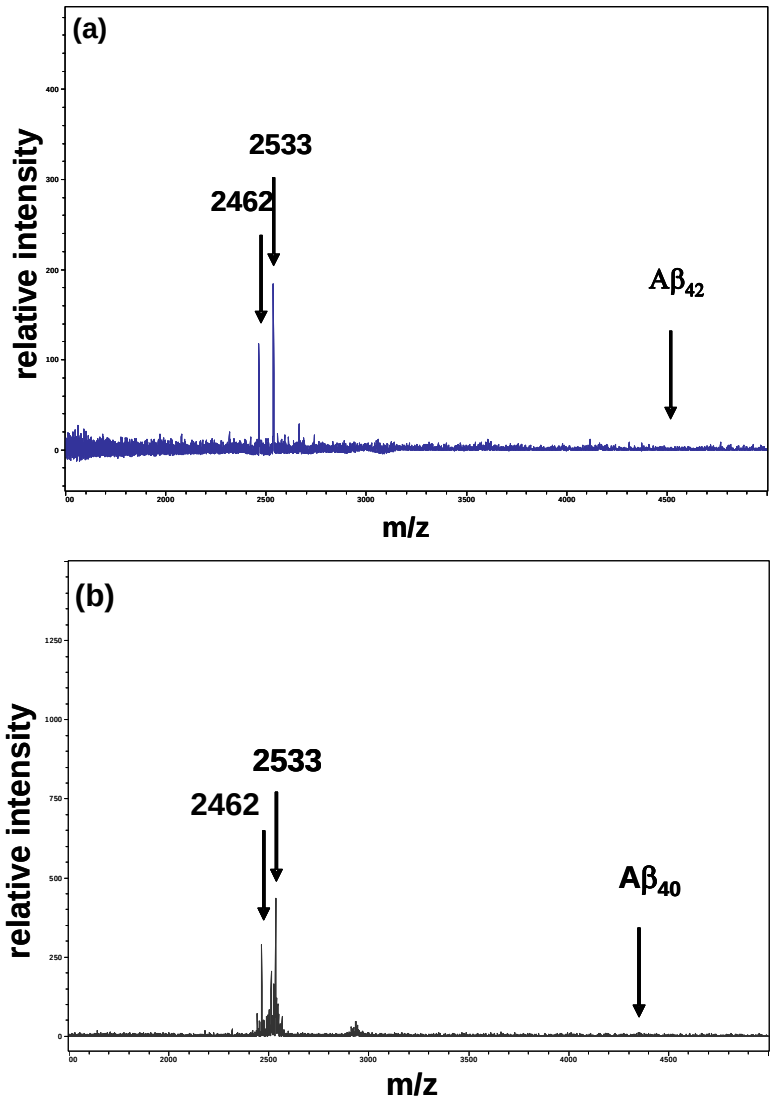



***Figure S2.*** MALDI-TOF mass spectrum of the product solution obtained by incubation of $A\beta_{42}$ (4.0 μM) with **A** (1.0 μM) (a) or $A\beta_{40}$ (4.0 μM) with **A** (3.0 μM) (b) at pH 7.50 (0.050 M phosphate) and 37°C. The incubation period was 36 hours for $A\beta_{40}$ and 24 hours for $A\beta_{42}$. The peaks with *m/z* of 2462 and 2533 correspond to $A\beta_{1\text{-}20}$ and $A\beta_{1\text{-}21}$, respectively. MALDI-TOF MS measurements were carried out with a Bruker Daltonics Autoflex II MALDI-TOF/TOF Mass Spectrometer.

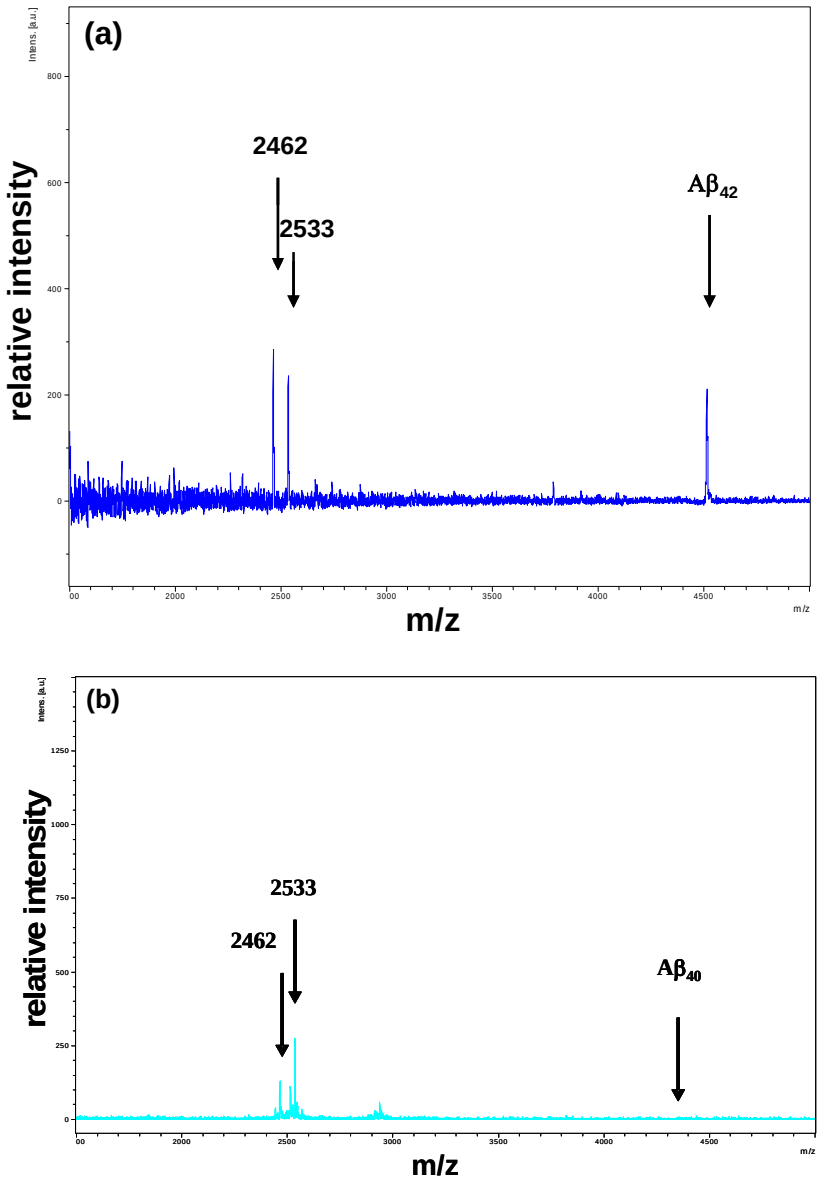



***Figure S3.*** MALDI-TOF mass spectrum of the product solution obtained by incubation of $A\beta_{42}$ (4.0 μM) with **B** (0.50 μM) (a) or $A\beta_{40}$ (4.0 μM) with **B** (3.0 μM) (b) at pH 7.5 (0.050 M phosphate) and 37°C. The incubation period was 36 hours for $A\beta_{40}$ and 24 hours for $A\beta_{42}$. The peaks with *m/z* of 2462 and 2533 correspond to $A\beta_{1\text{-}20}$ and $A\beta_{1\text{-}21}$, respectively.

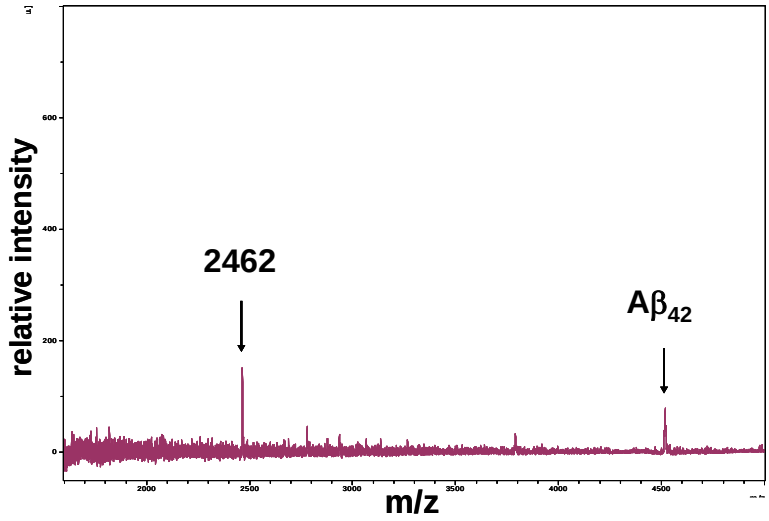



***Figure S4.*** MALDI-TOF mass spectrum of the product solution obtained by incubation of $A\beta_{42}$ (4.0 μM) with **C** (1.0 μM) at pH 7.50 (0.050 M phosphate) and 37°C for 24 hours. The peak with *m/z* of 2462 corresponds to $A\beta_{1\text{-}20}$.

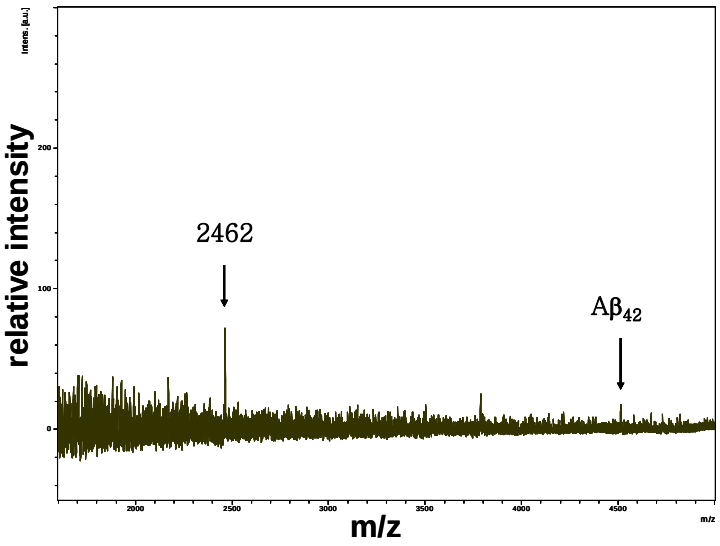



***Figure S5.*** MALDI-TOF mass spectrum of the product solution obtained by incubation of $A\beta_{42}$ (4.0 μM) with **D** (1.0 μM) at pH 7.50 (0.050 M phosphate) and 37°C for 24 hours. The peak with *m/z* of 2462 corresponds to $A\beta_{1\text{-}20}$.

**(8) Procedures for Estimation of Cleavage Yields**

Unless noted otherwise, the stock solution (20 μL) of $A\beta_{40}$ or $A\beta_{42}$ was added to a phosphate buffer solution (50 mM, pH 7.50, 480 μL) of a cleavage agent to initiate the cleavage reaction. After incubation at 37°C, the solution was fully filtered through a 10 kDa MWCO filter (Millipore Microcon centrifugal filter device YM-10) at approximately 10000 × g for 30 min at room temperature with a micro centrifuge (Hanil Model Micro 12). The filtrate was subjected to HPLC separation to isolate the oligopeptide fragments formed from the cleavage of $A\beta_{40}$ or $A\beta_{42}$. HPLC analysis was carried out with a Agilent Technology 1100 series HPLC. The mixture was separated by gradient elution at room temperature on a reverse phase C18 column (Grace VYDAC 218TP54) with a flow rate of 1.0 mL/min. The eluents were 0.1 % TFA in water (A) and 0.1 % TFA in acetonitrile (B) (gradient: 0 %-20 % B in 2 min, 20 %-50 % B in 10 min, 50 %-80 % B in 3 min). Fractions were examined by MALDI-TOF MS to ensure exclusion of uncleaved $A\beta_{40}$ or $A\beta_{42}$. The solution of the oligopeptide fragments thus collected was evaporated to dryness with a centrifugal vacuum concentrator (Hanil Model 3180C) and then 50 μL water was added to the residue. After the residue was dissolved by sonication, the oligopeptide fragments were quantified with fluorescamine according to the procedure described above, by using 13.5 M NaOH (20 μL), 15.4 M methanesulfonic acid (15 μL), 700 mM boric acid (96 μL), and an acetonitrile solution (5.0 mg/mL, 20 μL) of fluorescamine. Control samples lacking the cleavage agents were analyzed by the same procedure and fluorescence values obtained with the control samples were subtracted from those obtained with the reaction products.

**(9) Prediction of Cleavage Yields under the Conditions of Patients of Alzheimer's Disease**

For **A**-**D**, the plateau values of cleavage yield for $A\beta_{42}$ obtained at high concentrations of the cleavage agents are 10-30 %. Since isolation of the oligopeptide fragments during the measurement of cleavage yields inevitably involves some losses, the cleavage yields are somewhat underestimated. As $A\beta_{42}$ oligomers exist as transient intermediates, the cleavage of an $A\beta_{42}$ oligomer by **A**-**D** competes with conversion of the target $A\beta_{42}$ oligomer into protofibrils and fibrils. While cleavage of $A\beta_{42}$ with **A**-**D** is first order in the peptide concentration, oligomerization of $A\beta_{42}$ is expected to be at least second order in the peptide concentration. When the total concentration of $A\beta_{42}$ is decreased, the half-life of the target oligomer due to the conversion into protofibrils and fibrils is to become longer. On the other hand, the half-life of the target oligomer due to cleavage by **A**-**D** is not affected by the reduction of the total concentration of $A\beta_{42}$ as far as the concentration of the cleavage agent is fixed. The yield for cleavage of $A\beta_{42}$ is, therefore, expected to be higher at lower total concentration of $A\beta_{42}$. In the brains of patients of AD, the total concentration of $A\beta_{42}$ is lower than 1 nM [ L. F. Lue, Y. M. Kuo, A. E. Roher, L. Brachova, Y. Shen, L. Sue, T. Beach, J. H. Kurth, R. E. Rydel, J. Rogers, *Am. J. Pathol.* **1999**, *155*, 853-862]. At such low concentrations of $A\beta_{42}$, the efficiency for cleavage of $A\beta_{42}$ by **A**-**D** would be much higher than that summarized in Figure 1.

**(10) Results of Preincubation Experiments**

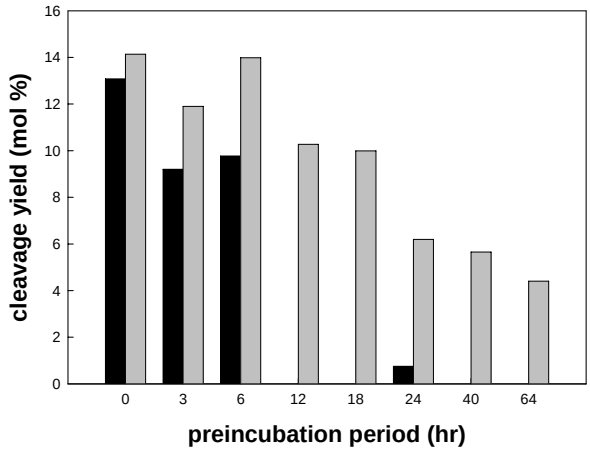



***Figure S6.*** Effects of period of preincubation of $A\beta_{40}$ (gray bars) or $A\beta_{42}$ (dark bars) on yield of cleavage by **A** (3.0 μM) measured after reaction for 36 hours at 37°C and pH 7.50.

**(11) Results of Kinetic Experiments**

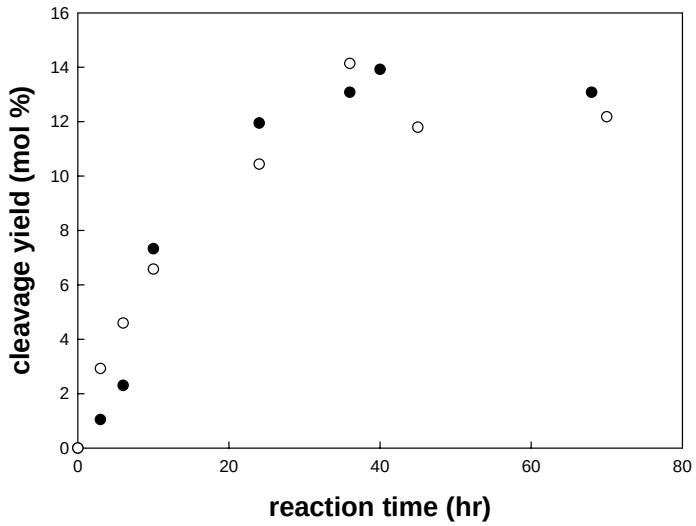



***Figure S7.*** Plot of the cleavage yield against reaction time for cleavage of $A\beta_{40}$ (○) or $A\beta_{42}$ (●) (4.0 μM) by **A** (3.0 μM) at 37°C and pH 7.50.

**(12) Control Experiments**

Two kinds of scrambled $A\beta_{42}$, having the same 42 amino acids as $A\beta_{42}$ in a scrambled sequence [type 1: KVKGLIDGAHIGDLVYEFMDSNSAIFREGVGAGHVHVAQVEF (purchasede from r-Peptide), and type 2: AIAEGDSHVLKEGAYMEIFDVQGHVFGGKIFRVVDLGSHNVA (purchased from AnaSpec)] were used as the controls. No peptide cleavage was detected when the scrambled $A\beta_{42}$ peptides (4.0 μM) were incubated with **A**-**D** (3.0 μM) for 36 hours at 37°C and pH 7.50. Also used as controls are proteins such as horse heart myoglobin, bovine serum γ-globulin, bovine serum albumin, human serum albumin, chicken egg white lysozyme, chicken egg ovalbumin, and bovine pancreas insulin (each 2-7 μM). When **A**-**D** (5.0 μM) was incubated with those proteins or $Co^{III}$cyclen (20 μM) was incubated with $A\beta_{40}$ or $A\beta_{42}$ (4.0 μM) at pH 7.50 and 37°C for 36 hours, no peptide cleavage was detected.

**(13) Complete References**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