Dynamic polythioesters via ring-opening polymerization of 1,4-thiazine-2,5-diones

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Dynamic polythioesters *via* ring-opening polymerization of 1,4-thiazine-2,5-diones†

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We describe the preparation and characterization of polythioesters composed of alternating α-amino acid and α-thioglycolic acid residues that undergo dynamic constitutional exchange under mild conditions. The polymers are assembled *via* reversible ring-opening polymerizations of 1,4-thiazine-2,5-diones and related monomers in solution-phase conditions that do not require the use of transition metal catalysts. Because 1,4-thiazine-2,5-diones can be derived in part from α-amino acids, a variety of side chain functionalized monomers in optically pure forms could readily be accessed. In addition, the resulting polythioesters have the potential for intra- and inter-chain hydrogen bonding, which is known to impart materials properties to other previously studied polyamides. The studies reported here could be useful in advancing a new class of biodegradable polymers and furthermore suggest that dynamic constitutional exchange could be exploited to modify many known synthetic and natural polythioesters.

Introduction

Most conventional polymers can be classified as constitutionally exchange-inert by virtue of their practically irreversible covalent bond connections. In contrast, although constitutionally dynamic polymers hold considerable promise in offering unique functional and materials attributes, polymers assembled *via* covalent bond connections that are reversible under ambient conditions have surprisingly received little attention until recently.[1,2]

Thioesters are known to undergo rapid, reversible transthioesterification reactions under mild conditions in the presence of thiols, and the utility of transthioesterification as a reversible covalent linkage has previously been established by the construction of dynamic combinatorial thioester libraries[3] and native chemical ligation.[4] Even so, to the best of our knowledge the potential for constitutional exchange in polythioesters[5] has only been previously explored in the context of short cyclic oligomers (n = 3–4).[6] Polythioesters have recently been designated as biomaterials based on the discovery of poly(3-mercaptoalkanoate) biosynthesis in wild-type and engineered bacteria,[7] providing a new avenue for polythioester preparation and making a better understanding of the properties of such polymers more desirable. In contrast to polythioesters, polyesters and polyamides have already been shown to undergo dynamic interchange, although relatively extreme conditions (250–300 °C, usually with transition metal catalysts) are necessary to observe exchange reactions at useful rates for these polymers.[8]

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† Electronic supplementary information (ESI) available: Schemes S1–S3, Figures S1, S2. See DOI: 10.1039/b903612a

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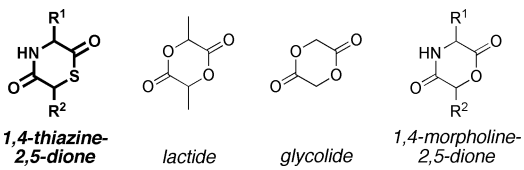
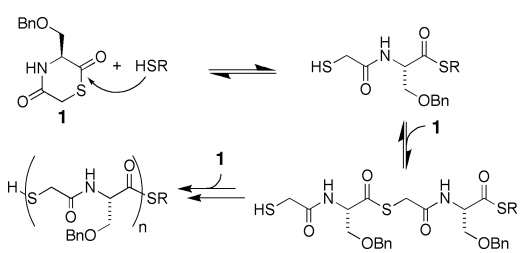
Results and discussion

To study the potentially dynamic properties of thioester-based polymers, we first required an efficient method to prepare such materials. We were somewhat surprised to find that the 1,4-thiazine-2,5-dione had not been used in polythioester synthesis despite its structural similarity to widely used monomers such as lactide,[9,10] glycolide,[10] and 1,4-morpholine-2,5-diones (Scheme 1),[11–13] and despite previous examples of polythioester preparation through catalyzed ring-opening polymerizations of five-, six-, or seven-membered thiolactones.[5] We reasoned that the nucleophilic attack by a thiol initiator onto a 1,4-thiazine-2,5-dione monomer would yield the corresponding ring-opened α-thiol-terminated dipeptide thioester that could participate in subsequent ring-opening reactions to propagate polymer growth (Scheme 2).

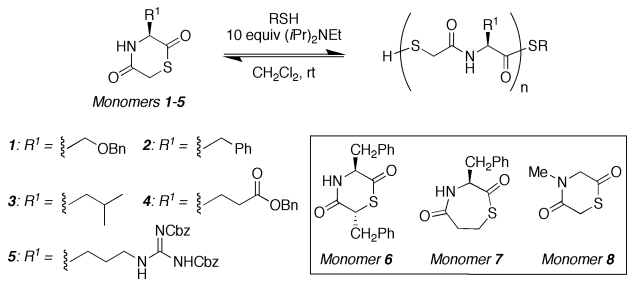


Scheme 1 Structure of the 1,4-thiazine-2,5-dione and other commonly used monomers for ring-opening polymerization.



Scheme 2 Proposed dynamic ring-opening polymerization.

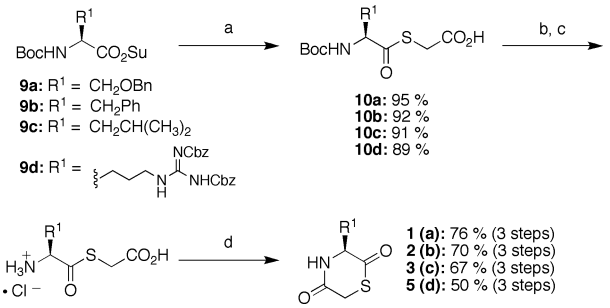
Table 1 Monomer conversions, polymer yields, and observed M_n's for polymerizations under various conditions[a]



Entry	Monomer	Thiol equiv.[b]	Time (h)	Conv. (%)	Yield (%)	M_n (Da)[c]	M_w (Da)[c]	PDI (M_w/M_n)[c]
1	**1**	0.1	20	80	77	9800	16100	1.6
2	**2**	0.1	15	81	67	8800	16600	1.9
3	**3**	0.1	20	92	84	Insol.	Insol.	Insol.
4	**4**	0.1	20	80	77	9000	16000	1.8
5	**5**	0.1	20	88	81	9700	13300	1.4
6	**1**	0.01	20	53	35	7300	13300	1.8
7	**1**	1.0	20	81	79	6800	11200	1.6
8	**1**	0.1 (BnSH)	20	81	67	9400	15300	1.6
9	**1**	0.1 (pentylSH)	20	80	70	10100	16800	1.7
10	**1**	0.1	0.5	55	28	7500	12100	1.6
11	**1**	0.1	4	66	49	10200	17800	1.7
12	**6**	0.1	18	39	13	3800	5700	1.5
13	**1** (1 eq), **3** (1 eq)	0.1	15	75, 80	61	12700	30600	2.4
14	**1** (3 eq), **3** (1 eq)	0.1	15	83, 82	80	15500	30300	2.0
15	**1** (1 eq), **2** (1 eq)	0.1	21	77, 77	72	8200	15300	1.9

[a] See ESI for full experimental details.† Conversion refers to monomer conversion as determined by HPLC. Yield refers to isolated yield of polymer precipitates. N.D. = not detected. [b] Unless otherwise noted, $HSCH_2CO_2Me$ was used as the thiol initiator. [c] Determined using size exclusion chromatography calibrated with polystyrene standards.

We therefore prepared monomers **1–8** derived from standard amino acids to evaluate their suitability for polymerization. Substrates **1–3** and **5** were prepared by coupling thioglycolic acid to an *N*-protected, activated amino acid, removing the amine protecting group, and then cyclizing under dilute conditions to form the 1,4-thiazine-2,5-dione (Scheme 3). Monomers **7** and **8** were likewise prepared by replacing thioglycolic acid with thiopropionic acid and a standard amino acid with *N*-methylglycine, respectively (Scheme S2†). Monomers **4** and **6** were prepared by a slightly different procedure involving conversion of an amino acid into the corresponding amino thioacid, followed by reaction of the thioacid with an unsubstituted or substituted bromoacetic acid derivative, respectively, to generate the substrate for cyclization (Scheme S1†).



Scheme 3 Synthesis of monomers **1–3**, **5**. *Reagents and conditions:* (a) $HSCH_2CO_2H$ (1.1 eq), $(i\text{Pr})_2\text{NEt}$ (2.1 eq), CH_2Cl_2, 0 °C to rt, 18 h; (b) TFA (50 eq), Et_3SiH (5.0 eq), CH_2Cl_2, rt, 4 h; (c) 1 N HCl; (d) EDC·HCl (1.2 eq), HOBt·H_2O (1.2 eq), $(i\text{Pr})_2\text{NEt}$ (2.2 eq), CH_2Cl_2, 40 mM, 0 °C to rt, 3 h.

Incubation of monomers **1–5** (~200 mM, room temperature, no catalyst, 10 eq $(i\text{Pr})_2\text{NEt}$) with methyl thioglycolate (0.1 eq) as an initiator yielded precipitates that started to appear generally within a few hours. Analysis of these precipitates by gel permeation chromatography (GPC) indicated polymers having uncorrected polystyrene-calibrated number average molecular masses (M_n) around 9000 Da (Table 1).[14] In the case of monomer **3**, the resulting polymer was insoluble in the GPC solvent (DMF), preventing M_n characterization. In contrast, copolymerizations of monomer **3** with **1** (Table 1, entries 13–14) provided good yields of soluble polymers having M_n values up to 15 500 Da. Polythioester polydispersity was somewhat higher for the copolymerizations (Table 1, entries 13–15) than for reactions initiated with a single monomer.

Polymer yields and M_n values were similar for three different thiol initiators (Table 1, entries 1, 8–9). The optimal concentration of thiol initiator was about 0.1 equivalents. Reduced yields were obtained for reaction periods shorter than ~15 h, although M_n values remained similar to those of the longer reactions (Table 1, entries 10–11). A survey of reaction solvents other than CH_2Cl_2 using monomer **2** indicated that no polymerization occurred in more polar solvents such as DMF, NMP, or 1,4-dioxane, while polymerization proceeded moderately in acetonitrile. Buffered water was examined as a solvent using side chain-deprotected derivatives of monomers **4** or **5** (see Schemes S1 and S3 for synthesis†); no polymerization was observed.

Compared to substrates **1–5**, monomer **6** polymerized poorly (Table 1) and appeared by HPLC to epimerize during the reaction (presumably at the methine carbon adjacent to sulfur). These findings are consistent with reports for analogous 1,4-morpholine-2,5-diones.[11] Monomers **7** and **8** both failed to polymerize under our experimental conditions. Considering that alleviation of ring strain provides the driving force for ROP, this result for the seven-membered monomer **7** was surprising. Although the simple seven-membered ring thiocaprolactone was reported to polymerize more efficiently than the analogous six-membered compound,[15] ring-opening polymerization of seven-membered analogs of the morpholinedione or thiazinedione monomers have never been reported. Furthermore, a seven-membered diester compound more similar to monomer **7** than thiocaprolactone was reported not to homopolymerize.[16] It is therefore possible that the conformational or hydrogen-bonding properties of monomer **7** and similar species drive the monomer/polymer equilibrium to disfavor polymerization. *N*-Methylated derivatives[13,17] of 1,4-morpholine-2,5-diones have been reported not to homopolymerize efficiently, consistent with the failure of monomer **8**.

To confirm the reversibility of the polythioesters, we treated a sample of poly-**2** (prepared as in Table 1, entry 2) with a large excess of base and thiol (>10 eq each) in DMF. After five minutes, HPLC analysis indicated the polymer was cleanly depolymerized to yield cyclic monomer **2** and the ring-opened monomeric derivative in a ~2:1 ratio, respectively (Figure S1†). To establish the feasibility of dynamic constitutional exchange, homopolymers of **1** and **3** (prepared as in Table 1, entries 11 and 3) were dissolved in a ~1:1 ratio and analyzed using MALDI-TOF mass spectrometry. As expected, we observed the presence of only homopolymeric species (Fig. 1). After incubating the mixture for

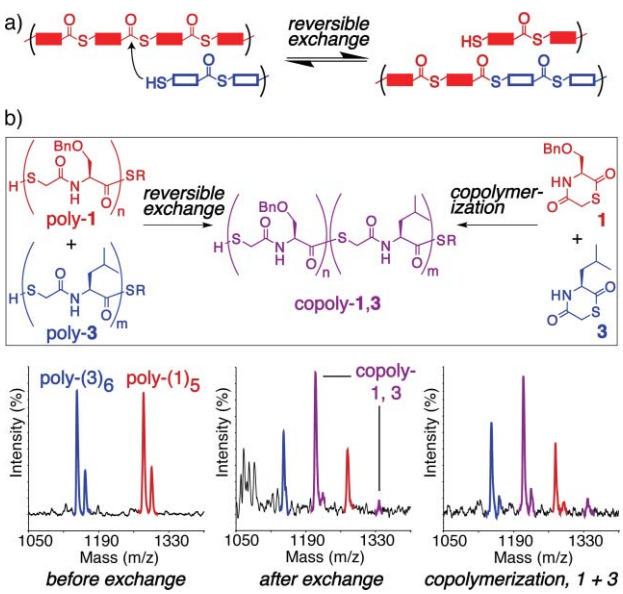


Fig. 1 (a) Schematic illustration of dynamic constitutional exchange in polythioesters. (b) Homopolymers of substrates **1** and **3** were dissolved in a stoichiometric ratio in hexafluoroisopropanol. MALDI-TOF analysis of the mixture before (left) and after (center) a 17 h incubation with $(i\text{Pr})_2\text{NEt}$ and $\text{HSCH}_2\text{CO}_2\text{Me}$ indicated the development of product peaks corresponding to heteropolymers. Comparative analysis of an independent copolymerization of **1** and **3** (right) gave a similar peak distribution as the product polymer generated *via* dynamic exchange.

17 h with base and thiol (~0.3 eq each), however, MS analysis indicated the presence of additional heteropolymeric products that matched those formed in an independent copolymerization of monomers **1** and **3** (prepared as in Table 1, entry 13). These findings are consistent with a dynamic process in which subunits were reversibly exchanged between the original homopolymers to generate hetero-copolymers.

Conclusions

1,4-Thiazine-2,5-diones should be suitable for copolymerizations with lactide, glycolide, and other substrates for ring-opening polymerization, making possible the synthesis of other novel materials. Furthermore, the dynamic aspect of the polymers demonstrated here is likely a common feature of many polythioesters, and therefore could be exploited to alter the properties of other synthetic or bio-engineered polythioesters.

Experimental

Polymerization reactions

Typical reaction procedure: monomer **1** (5.7 mg, 22.6 μmol) was dissolved in CH_2Cl_2 (63 μL) containing 10 mM acetanilide (an internal concentration standard) in an eppendorf vial. A 1.0 μL aliquot of this solution was removed and quenched in 1% TFA/ACN (200 μL); this aliquot was analyzed by HPLC as a time zero point for determining the initial concentration of monomer compared to the internal standard. $(i\text{Pr})_2\text{NEt}$ (37.4 μL, 226 μmol) and $\text{HSCH}_2\text{CO}_2\text{Me}$ (11.3 μL of a 200 mM stock solution in CH_2Cl_2, 2.3 μmol) were added to the solution to initiate the polymerization reaction. The vial was sealed and the solution stirred at room temperature for ~20 h. After this time, 1.0 μL of the suspension was removed and dissolved in 1% TFA/ACN (200 μL) for HPLC determination of the amount of monomer remaining relative to the internal standard. Et_2O (400 μL) was added to the reaction suspension and the mixture was vortexed. The mixture was centrifuged and supernatant decanted. The white polymer precipitates were washed several times using Et_2O (400 μL) and dried. After weighing the dried precipitate (4.4 mg, 77%), 0.6 mg of the precipitate was dissolved in 300 μL of the GPC solvent (DMF containing 100 mM LiBr) to give a 0.2% solution that was used for GPC analysis. Polymerization of monomer **8** was carried out at 100 mM due to poor monomer solubility. Copolymerizations were conducted as described above by dissolving the two monomers in the CH_2Cl_2/acetanilide solution. Polymer precipitates were stored at −20 °C.

Attempted aqueous polymerizations

Reactions using side chain-deprotected derivatives of monomers **4** and **5** (compounds **13** and **14**, respectively, see Schemes S1 and S3†) were attempted using buffered water as solvent. Two conditions were examined: buffer 1 (50 mM PIPES buffer, 10 mM tris-carboxyethyl phosphine [TCEP] as reducing agent, pH 6.5) or buffer 2 (50 mM HEPES buffer, 10 mM TCEP, pH 8.0). Reactant concentrations and reaction times were similar to those described above. Polymers did not form, although slow monomer consumption as a result of hydrolysis was observed (15% of compound **14** hydrolyzed in 24 h at pH 6.5).

Depolymerization reaction

A portion (~0.1 mg) of the precipitate obtained from the polymerization of **2** (obtained as in Table 1, entry 2) was dissolved in DMF (15 μL). A 1.0 μL aliquot of this solution was removed and quenched in 1% TFA/ACN (200 μL) for HPLC analysis. An excess of (iPr)$_2$NEt (8.0 μL, neat) and methyl thioglycolate (0.4 μL, neat) were added and the solution was left at rt for 5 min, after which time another aliquot was removed for HPLC analysis. The difference in HPLC spectra before and after the reaction clearly show that a depolymerization of the oligomers occurred to give the ~2:1 mixture of monomer **2** and the ring-opened monomeric derivative (Figure S1†).

Dynamic polymer exchange

Portions of the precipitate obtained from the polymerization of **1** (0.8 mg) (obtained as in Table 1, entry 11) and of **3** (0.7 mg) (obtained as in Table 1, entry 3) were mixed and dissolved in hexafluoroisopropanol (60 μL). To 20 μL of this solution, (iPr)$_2$NEt (3.0 μL) and HSCH$_2$CO$_2$Me (3.0 μL of a 200 mM stock solution in hexafluoroisopropanol) were added. The reaction was stirred at rt for 17 h, after which time Et$_2$O (400 μL) was added to precipitate the polymers. The mixture was centrifuged, the supernatant decanted, and the solids were washed several times with Et$_2$O. The obtained solid was analyzed using MALDI-TOF spectrometry.

MALDI-TOF MS conditions

For routine analysis of the polymers, a portion of the obtained precipitates was suspended in a solution saturated with α-cyano-3-hydroxycinnamic acid (in 1:1 H$_2$O/ACN containing 0.1% TFA) and sonicated for a period of 3 minutes. For analysis, 1.0 μL of the suspension was spotted on a MALDI plate and was analyzed in the positive mode. In some cases, the obtained precipitates and 3-aminoquinoline (usually 1:100 ratio by weight) were ground together using a mortar and pestle, and the obtained powder was pressed to make a thin disc. A piece of the disc was mounted on MALDI plate with double-sided tape and was analyzed in the positive mode.[18]

GPC conditions and calibration

Polymer M_n and M_w values were determined by means of gel permeation chromatography using a Hitachi D-7000 HPLC system monitoring UV absorbance at 265 nm. DMF (HPLC grade) containing 0.1 M LiBr was used as the eluent at a flow rate of 0.6 mL min^{-1}. A Waters Styragel HR 4E column (7.8 × 300 mm) heated to 40 °C was used. Molecular weights were calculated on the basis of polystyrene standards without further correction.

HPLC conditions

Analytical reverse-phase HPLC was performed at 257 nm using Phenomenex Jupiter Proteo or Zorbax 300-SB C-18 columns connected to a Hitachi D-7000 HPLC system. Solvent system (1.5 mL/min): binary gradients of solvent A (99% H$_2$O, 0.9% acetonitrile, 0.1% TFA) and solvent B (90% acetonitrile, 9.9% H$_2$O, 0.07% TFA). Preparative HPLC was carried out using the same solvents (10 mL/min) with a Phenomenex Jupiter Proteo column (250 × 21.2 mm).

Preparation and characterization of new compounds

Preparation of compounds 10a–d. (Scheme 1). Representative synthetic procedure for Boc-Phe-SCH$_2$CO$_2$H (**10b**): to a dry CH$_2$Cl$_2$ solution (20 mL) of Boc-Phe-OSu (**9b**) (1.55 g, 4.28 mmol, from Novabiochem or Bachem), thioglycolic acid (0.34 mL, 4.88 mmol) and (iPr)$_2$NEt (1.60 mL, 9.16 mmol) were added at 0 °C. The mixture was stirred at rt overnight. The solvent was removed under vacuum. The residue was taken up in EtOAc (30 mL) and washed with 0.5 M citric acid (10 mL × 2) and brine. The organic layer was dried over Na$_2$SO$_4$, and subsequent removal of the solvent gave a white solid. To the solid, dicyclohexylamine (0.85 mL, 4.28 mmol) and hexane were added and triturated. The obtained white precipitate was collected and washed with hexane. The precipitate was suspended in EtOAc and the dicyclohexylamine was extracted with 0.5 M citric acid (10 mL × 3). The organic layer was washed with brine and dried over Na$_2$SO$_4$. Filtration and removal of solvent under vacuum gave **10b** as a white solid (1.34 g, 3.96 mmol, 92%). Compounds **10a**, **10c**, and **10d** were prepared in a similar manner.

Compound 10a. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.37–7.28 (m, 5H), 5.54 (d, J = 8.7 Hz, 1H), 4.53–4.50 (m, 1H), 4.50 (s, 2H), 3.99 (dd, J = 2.9, 9.7 Hz, 1H), 3.79 (d, J = 16.2 Hz, 1H), 3.66 (d, J = 16.9 Hz, 1H), 3.66 (dd, J = 3.6, 9.6 Hz, 1H), 1.47 (s, 9H). ESI-MS (m/z) 370.1327 [M + H]$^+$ (MW$_{calcd}$ = 370.1319).

Compound 10b. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.77 (br s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.29–7.18 (m, 5H), 4.28 (ddd, J = 4.0, 8.4, 11.0 Hz, 1H), 3.65 (s, 2H), 3.07 (dd, J = 4.1, 13.9 Hz, 1H), 2.77 (dd, J = 11.1, 13.9 Hz, 1H), 1.32 (s, 9H). ESI-MS (m/z) 340.1209 [M + H]$^+$ (MW$_{calcd}$ = 340.1213).

Compound 10c. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.71 (br s, 1H), 7.67 (d, J = 7.9 Hz, 1H), 4.10–4.04 (m, 1H), 3.60 (s, 2H), 1.68–1.43 (m, 3H), 1.41 (s, 9H), 0.87 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H). ESI-MS (m/z) 306.1362 [M + H]$^+$ (MW$_{calcd}$ = 306.1370).

Compound 10d. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.74 (br s, 1H), 9.15 (br s, 2H), 7.68 (d, J = 8.0 Hz, 1H), 7.43–7.29 (m, 10H), 5.25 (d, J = 12.5 Hz, 1H), 5.21 (d, J = 12.5 Hz, 1H), 5.05 (s, 2H), 4.06–4.02 (m, 1H), 3.94-3.64 (m, 2H), 3.59 (s, 2H), 1.69-1.41 (m, 4H), 1.39 (s, 9H). ESI-MS (m/z) 617.2266 [M + H]$^+$ (MW$_{calcd}$ = 617.2276).

Preparation of monomers 1–3, 5. (Scheme 1). Representative synthetic procedure for **2**: to a dry CH$_2$Cl$_2$ solution (50 mL) of **10b** (1.20 g, 3.54 mmol), Et$_3$SiH (2.83 mL, 17.7 mmol) and TFA (13.1 mL) were added at 0 °C. The reaction mixture was stirred at rt for 3–4 h. Volatile materials were removed under vacuum, and the residue was azeotroped with toluene (× 3) to give a white solid. For compounds **10a**, **10c**, and **10d**, the obtained TFA salts were converted to hydrochloride salts by treatment with 1 N HCl followed by removal of the solvent under vacuum. To a CH$_2$Cl$_2$ suspension (80 mL) of the deprotected compound (1.10 g, 3.25 mmol), (iPr)$_2$NEt (1.18 mL, 7.15 mmol) was added at 0 °C. HOBt·H$_2$O (0.60 g, 3.91 mmol) and EDC·HCl (0.75 g, 3.91 mmol) were then added to the reaction mixture at 0 °C,

and the mixture was warmed up to rt. After stirring for 3–4 h, the reaction mixture was washed with aqueous $KHSO_4$ (5%). CH_2Cl_2 was used to extract the aqueous layer (×2). The combined organic layers were washed with water and brine, and dried over Na_2SO_4. Filtration and removal of solvent under vacuum gave a colorless oil, which solidified upon cooling in a refrigerator. Silica gel column chromatography (hexane:EtOAc = 1:1 to 1:3) gave **2** (550 mg, 2.49 mmol, 70%) as a white solid. Compounds **1**, **3**, and **5** were prepared in a similar manner.

Compound 1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.30 (m, 5H), 6.22 (br s, 1H), 4.58 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.7 Hz, 1H), 4.25 (ddd, J = 2.6, 4.1, 6.7 Hz, 1H), 3.87 (dd, J = 4.1, 10.1 Hz, 1H), 3.81 (dd, J = 6.8, 10.2 Hz, 1H), 3.81 (d, J = 15.1 Hz, 1H), 3.64 (dd, J = 1.6, 15.1 Hz, 1H). ESI-MS (m/z) 252.0682 $[M + H]^+$ (MW_{calcd} = 252.0689).

Compound 2. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.20 (m, 5H), 6.20 (brs, 1H), 4.27 (ddd, J = 3.0, 4.5, 9.0 Hz, 1H), 3.75 (d, J = 15.2 Hz, 1H), 3.38 (dd, J = 4.6, 14.4 Hz, 1H), 3.35 (dd, J = 1.0, 15.6 Hz, 1H), 2.90 (dd, J = 9.1, 14.6 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.60, 167.42, 134.90, 129.21 (2C), 129.19 (2C), 127.73, 63.62, 36.05, 31.44. ESI-MS (m/z) 222.0584 $[M + H]^+$ (MW_{calcd} = 222.0583).

Compound 3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.32 (br s, 1H), 4.03 (ddd, J = 3.7, 4.7, 9.3 Hz, 1H), 3.84 (d, J = 15.1 Hz, 1H), 3.60 (dd, J = 1.5, 15.1 Hz, 1H), 1.89 (ddd, J = 4.8, 9.1, 14.0 Hz, 1H), 1.82–1.72 (m, 1H), 1.55 (ddd, J = 5.1, 9.2, 14.2 Hz, 1H), 1.00 (d, J = 6.5 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H). ESI-MS (m/z) 188.0739 $[M + H]^+$ (MW_{calcd} = 188.0740).

Compound 5. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.15 (br s, 2H), 8.29 (s, 1H), 7.44–7.30 (m, 10H), 5.25 (s, 2H), 5.05 (s, 2H), 4.22 (ddd, J = 3.2, 4.8, 7.8 Hz, 1H), 4.01 (d, J = 14.7 Hz, 1H), 3.95–3.83 (m, 2H), 3.48 (dd, J = 1.5, 14.7 Hz, 1H), 1.77–1.51 (m, 4H). ESI-MS (m/z) 499.1656 $[M + H]^+$ (MW_{calcd} = 499.1646).

Preparation of 11. (Scheme S1†). Isobutyl chloroformate (1.35 mL, 10.4 mmol) was added to a solution of Boc-Glu(OBz)-OH (3.37 g, 10 mmol) and (iPr)$_2$NEt (1.65 mL, 10 mmol) in anhydrous THF (30 mL) cooled at –20 °C under argon. The mixture was allowed to stir for 20 min at –20 °C, after which time a white suspension was formed. A solution of NaSH (0.67 g, 12 mmol) in DMF (5 mL) was then added and the yellow solution was stirred at 0 °C for 4 h, after which time the TLC (silica, EtOAc/hexane/HOAc 3:7:0.5) showed completion of the reaction. The solution was then acidified to pH = 3 with 1 N HCl and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (1 × 20 mL), dried (Na_2SO_4), and evaporated under vacuum to afford the oily product **11**. The product was used without further purification.

Preparation of 12. (Scheme S1†). t-Butyl bromoacetate (2.0 g, 10 mmol) was added to a stirred solution of crude **11** (2.5 g, 7.0 mmol) in MTBE (50 mL) at 0 °C followed by the addition of Cs_2CO_3 (2.0 g). The cooling bath was then removed and the mixture was stirred at rt. The reaction was followed by TLC (silica, EtOAc/hexane/HOAc 1:1:0.1) and was complete within 2 h. EtOAc (30 mL) and water (30 mL) were added to the reaction mixture and then the organic layer was separated, washed with water (2 × 15 mL) and brine (1 × 15 mL), dried (Na_2SO_4), and evaporated under vacuum to leave an oily residue. The product was purified by column chromatography on silica using a gradient eluent of EtOAc/hexane starting with a 1:9 and reaching a 1:1 ratio to give **12** (1.5 g, 3.2 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.35 (m, 5H), 5.23 (d, 1 H), 5.13 (s, 2H), 4.52 (m, 1H), 3.60 (dd, 2H), 2.49 (m, 2H), 2.24 (m, 1H), 2.01 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H). SSI-LCMS: (m/z) 489.7 $[M + Na]^+$; 506.5 $[M + K]^+$ (MW_{calcd} = 490.2 $[M + Na]^+$; 506.2 $[M + K]^+$).

Preparation of monomer 4. (Scheme S1†). Triethylsilane (1.2 mL, 7.5 mmol) was added to a solution of **12** (1.4 g, 3 mmol) in CH_2Cl_2 (6.2 mL) under argon. TFA (2.89 mL, 39 mmol) was then added. The reaction was monitored by HPLC and LCMS and required 24 hrs to reach 95% completion. The solvent was removed under vacuum and the residue was dissolved in water and lyophilized to give a solid (1.2 g, 2.6 mmol, 87%). Without any further purification, a solution of the deprotected product (1.0 g, 2.2 mmol) and (iPr)$_2$NEt (0.8 mL, 4.7 mmol) in CH_2Cl_2 (120 mL) was added dropwise to a CH_2Cl_2 (130 mL) solution of PyBop (1.5 g, 2.8 mmol) over a period of 2 h. The reaction was then followed by LCMS and TLC (silica, EtOAc/hexane 1:1) and was complete after 1 additional hr. The organic solution was washed with water (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), and evaporated under vacuum to leave an oily residue. The product was purified by column chromatography on silica using EtOAc/pentane (1:1) to afford an oily residue that solidified upon sitting (400 mg, 1.4 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.36 (m, 5H), 7.17 (bs, 1H), 5.14 (s, 2H), 4.10 (m, 1H), 3.84 (d, J = 15.2 Hz, 1H), 3.53 (dd, J = 1.6, 15.2 Hz 1H), 2.61 (t, J = 6.8 Hz, 2H), 2.35 (m 1H), 2.10 (m, 1H). ESI-MS (m/z) 294.0796 $[M + H]^+$ (MW_{calcd} = 294.0795).

Preparation of 13. (Scheme S1†). HF (10 mL) was introduced to a mixture of monomer **4** (118 mg, 0.4 mmol) and anisole (88 µL, 0.8 mmol) at –78 °C. The solution was then warmed to 0 °C and the reaction stirred for 1 h. The excess HF was removed by flowing N_2. The solid was dissolved in Et_2O (5 mL) and extracted with water (5 × 10 mL). The combined aqueous extracts were concentrated under reduced pressure and lyophilized to yield a white solid identified as compound **13** (70 mg, 0.34 mmol, 90%). The solid was purified by preparative HPLC. In column chromatography (SiO_2, CH_2Cl_2:MeOH 95/5) the material decomposed. ^{1}H NMR (CD_3OD): δ 1.94 (td, 1H, J = 7.3, 14.7 Hz,), 2.20 (dtd, 1H, J = 7.3, 13.4, 14.7 Hz,), 2.48 (t, 2H, J = 7.3 Hz), 3.56 (d, 1H, J = 15.0 Hz), 4.05 (d, 1H, J = 15.0 Hz), 4.23 (dd, 1H, J = 5.45, 7.87 Hz). ESI-MS (m/z) 204.0323 $[M + H]^+$ (MW_{calcd} = 204.0330).

Preparation of 14. (Scheme S3†). HF (10 mL) was introduced to a mixture of monomer **5** (200 mg, 0.4 mmol) and anisole (88 µL, 0.8 mmol) at –78 °C. The solution was then warmed to 0 °C and the reaction stirred for 1 h. The excess HF was removed by flowing N_2. The solid was dissolved in Et_2O (10 mL) and extracted with 0.1 M HCl (3 × 10 mL). The aqueous phase was washed with Et_2O (2 × 15 mL), concentrated under vacuum, and lyophilized to afford a white solid identified as **14** (91 mg, 0.39 mmol, 98%). ^{1}H NMR (CD_3OD) δ 1.68–2.14 (m, 6H), 3.67 (d, 1H, J = 15.0 Hz,), 4.19 (d, 1H, J = 15.0 Hz), 4.3–4.38 (m, 1H). ESI-MS (m/z) 231.0909 $[M + H]^+$ (MW_{calcd} = 231.0910).

Preparation of 17. (Scheme S1†). Compounds **15**[19] and **16**[20] were prepared as reported previously. To a MTBE solution (5 mL)

of **15** (286 mg, 1.0 mmol) and **16** (233 mg, 1.0 mmol), K_2CO_3 (141 mg, 1.0 mmol) was added in one portion at 0 °C. After stirring at rt for 7 h (the reaction was followed by HPLC), the reaction mixture was quenched with 3 mL of 1 N HCl, and was extracted with EtOAc (× 2). The combined organic layers were dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by preparative HPLC, to yield **17** as a white solid (207 mg, 0.48 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30–7.08 (m, 11H), 4.65 (q, J = 7.1 Hz, 1H), 4.41 (t, J = 7.3 Hz, 1H), 3.36–3.24 (m, 2H), 3.10–2.97 (m, 2H), 1.39 (s, 9H).

Preparation of monomer 6. (Scheme S1†). To a dry CH_2Cl_2 solution (6 mL) of **17** (192 mg, 0.45 mmol), Et_3SiH (0.36 mL, 2.26 mmol) and TFA (1.68 mL, 22.6 mmol) were added at 0 °C. The reaction mixture was stirred at rt for 3 h. Volatile materials were removed under vacuum, and the residue was azeotroped with $CHCl_3$ (× 3) to give a white solid. To a CH_2Cl_2 suspension (5 mL) of this solid (85 mg, 0.20 mmol), (iPr)$_2$NEt (74 μL, 0.45 mmol) was added at 0 °C, and the mixture stirred at 0 °C for 20 min. HOBt·H_2O (35 mg, 0.23 mmol) and EDC·HCl (44 mg, 0.23 mmol) were then added, and the mixture stirred at 0 °C for 6 h. Aqueous $KHSO_4$ (5%) and CH_2Cl_2 were added and separated. The aqueous layer was further extracted with CH_2Cl_2 (× 2). The combined organic layers were washed with water and brine and dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (hexane: EtOAc = 10:1 to 2:1) to give monomer **6** (30 mg, 0.1 mmol, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.08 (m, 10H), 5.63 (br s, 1H), 3.89 (dd, J = 4.6, 8.3 Hz, 1H), 3.58 (ddd, J = 3.0, 4.1, 9.6 Hz, 1H), 3.38 (dd, J = 4.6, 14.2 Hz, 1H), 3.23 (dd, J = 4.4, 14.0 Hz, 1H), 3.15 (dd, J = 8.3, 14.3 Hz, 1H), 2.80 (dd, J = 9.6, 14.3 Hz, 1H). ESI-MS 312.1051 (m/z) $[M + H]^+$ (MW_{calcd} = 312.1053).

Preparation of 18. (Scheme S2†). To a dry CH_2Cl_2 solution (35 mL) of Boc-Phe-OSu **9b** (3.00 g, 8.28 mmol), 3-mercaptopropionic acid (0.82 mL, 9.44 mmol) and (iPr)$_2$NEt (3.09 mL, 18.7 mmol) were added at 0 °C. The mixture was stirred at rt for 14 h. After removal of solvent under vacuum, EtOAc (30 mL) was added and the solution was washed with 0.5 M citric acid (10 mL × 2) and brine (10 mL × 1). The organic layer was dried over Na_2SO_4. After filtration and removal of solvent, Et_2O and DCHA (1.48 mL, 7.45 mmol) were added to the residue, and the solvent was removed. The obtained white solid was triturated with pentane, and was collected by suction filtration. The solid was recrystallized from hexane/benzene. Insoluble solid was removed by filtration while the mixture was hot. The filtrate was cooled to rt, then to 0 °C. The white solid that appeared was collected by suction filtration, and was washed with pentane. The solid was suspended in EtOAc, and DCHA was extracted with citric acid (0.5 M, 10 mL × 3). The organic layer was washed with brine, and was dried over Na_2SO_4. Filtration and drying under vacuum gave **18** as a white solid (2.55 g, 7.22 mmol, 87%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.38 (s, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.29–7.18 (m, 5H), 4.26-4.20 (m, 1H), 3.04 (dd, J = 4.3, 13.9 Hz, 1H), 2.95 (t, J = 6.9 Hz, 2H), 2.77 (dd, J = 10.9, 13.8 Hz, 1H), 2.49–2.47 (m, 2H), 1.32 (s, 9H). ESI-MS (m/z) 354.1358 $[M + H]^+$ (MW_{calcd} = 354.1370).

Preparation of monomer 7. (Scheme S2†). To a dry CH_2Cl_2 solution (11 mL) of **18** (500 mg, 1.41 mmol), Et_3SiH (1.13 mL, 7.05 mmol) and TFA (5.24 mL, 70.5 mmol) were added at 0 °C. The reaction mixture was stirred at rt for 3 h. Volatile materials were removed under vacuum, and the residue was azeotroped with $CHCl_3$ (× 3) and rotovapped to dryness. To a CH_2Cl_2 solution (12 mL) of the obtained compound (105 mg, 0.29 mmol), DCC (77 mg, 0.37 mmol) was added at 0 °C, and the mixture was stirred at rt for 2 h. The white precipitates that appeared were filtered off, and the filtrate was washed with H_2O and brine and then dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (hexane: EtOAc = 20:1 to 1:2). The obtained white solid contained a small amount of by-products which were removed by preparative HPLC to give monomer **7** (25 mg, 0.11 mmol, 38%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.28 (m, 3H), 7.13–7.11 (m, 2H), 6.71 (d, J = 8.1 Hz, 1H), 4.98 (dt, J = 8.5, 6.1 Hz, 1H), 3.27–3.13 (m, 4H), 2.69 (t, J = 7.0 Hz, 2H). ESI-MS (m/z) 236.0732 $[M + H]^+$ (MW_{calcd} = 236.0740).

Preparation of 19. (Scheme S2†). To a CH_2Cl_2 solution (20 mL) of Boc-Sar-OH (3.80 g, 20.0 mmol), Et_3N (3.15 mL, 22.6 mmol) and $ClCO_2Et$ (2.10 mL, 22.0 mmol) were added dropwise at 0 °C. During addition of $ClCO_2Et$, white precipitates appeared. The reaction mixture was allowed to warm to rt and was stirred for 30 min. A mixture of $HSCH_2CO_2H$ (1.40 mL, 20.2 mmol) and Et_3N (3.15 mL, 22.6 mmol) were added dropwise to the above mixture at 0 °C, then the reaction mixture was warmed to rt and stirred overnight. The mixture was filtered, and the filtrate was dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (hexane: CH_2Cl_2:AcOH = 15:3:1 to 6:3:1) to give **19** (2.99 g, 11.4 mmol, 57%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.06 (s, 2H), 3.73 (s, 2H), 2.99 (s, 3H), 1.43 (s, 9H).

Preparation of monomer 8. (Scheme S2†). To a dry CH_2Cl_2 solution (100 mL) of **19** (2.43 g, 9.23 mmol), Et_3SiH (7.38 mL, 46.2 mmol) and TFA (34 mL, 461.5 mmol) were added at 0 °C. The reaction mixture was stirred at rt for 4 h. Volatile materials were removed under vacuum, and the residue was azeotroped with toluene (× 2). 1 N HCl (50 mL) was added, and the solvent was removed to give a sticky oil, which gradually solidified upon cooling at −15 °C to give white solid. To a CH_2Cl_2 suspension (170 mL) of this solid (1.34 g, 6.71 mmol) and PyBroP (4.71 g, 10.1 mmol), (iPr)$_2$NEt (3.50 mL, 21.2 mmol) was added at 0 °C, and the mixture was stirred at rt for 11 h. At the initial stage of the reaction, sonication was applied to dissolve the undissolved white solid. AcOH (1.15 mL, 20.1 mmol) was added, and the reaction mixture was then treated with 5% $KHSO_4$ and was extracted with CH_2Cl_2. The aqueous layer was further extracted with CH_2Cl_2 (× 2). The combined organic layers were washed with water and brine, and dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (hexane: EtOAc = 1:1 to 1:4) to give monomer **8** (613 mg, 4.22 mmol, 63%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.13 (s, 2H), 3.71 (s, 2H), 3.08 (s, 3H). ESI-MS (m/z) 168.0094 $[M + Na]^+$ (MW_{calcd} = 168.0090).

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