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# Regiospecific Synthesis of 3,5-Bis(bromomethyl)benzoic Acid, A Cysteine Crosslinking Agent

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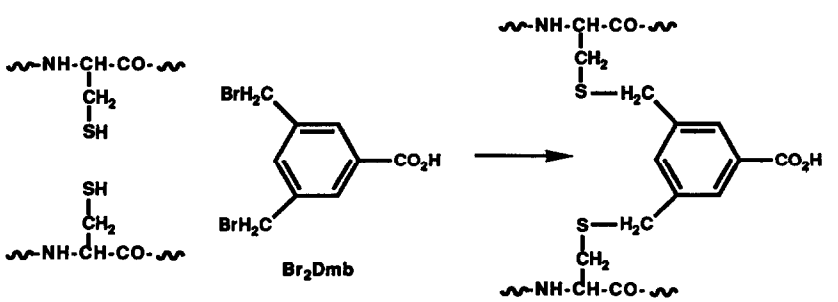
***Abstract*** : Bridging two molecules of an organic thiol with the 3,5-dimethylenebenzoic acid (Dmb) group produces a rigid five-carbon Dmb crosslink that is stable to disulfide reducing agents. Radical bromination of 3,5-dimethylbenzoic acid or its succinimido ester gave four α-brominated products. So NMR-pure 3,5-bis(bromomethyl)benzoic acid ($Br_2$Dmb) was regiospecifically synthesized in 38% overall yield by a four-step route from 1,3,5-benzenetricarboxylic acid. S-Alkylation of cysteine methyl ester, a model thiol, with $Br_2$Dmb provided the 2:1 adduct (Cys-$OCH_3$)$_2$Dmb in 79% yield. Subsequent N-protection with di(*tert*-butyl) dicarbonate gave (Boc-Cys-$OCH_3$)$_2$Dmb in 60% overall yield from cysteine methyl ester. This protected diamino acid is suitable for assembly of two Dmb-crosslinked peptide chains.

Two molecules of a peptide thiol are readily crosslinked through air oxidation by formation of a disulfide bond. The resulting crosslink is readily broken by a mild reducing agent (2-mercaptoethanol, dithiothreitol, tributylphosphine). Engineering of novel protein structures through organic synthesis would benefit from a method of joining two molecules of a peptide thiol by a covalent S-to-S crosslink that is stable to reagents that reduce disulfide bonds.[1] The 3,5-dimethylenebenzoic acid (Dmb) group is such a crosslink. It can join two sulfur atoms by a rigid chain of five carbon atoms (S-$C^{\alpha'}$-$C^{3'}$-$C^{4'}$-$C^{5'}$-$C^{\alpha'}$-S). The distance between the two $C^{\alpha'}$ atoms of the Dmb group is 5.0 Å. The Dmb crosslink is stable to disulfide reducing agents.



A convenient reagent for introducing a Dmb crosslink is 3,5-bis(bromomethyl)benzoic acid ($Br_2$Dmb). This symmetric carboxylic acid bears two reactive benzylic bromide groups. $Br_2$Dmb could be used to crosslink two molecules of a peptide thiol through S-alkylation. The two Cys $C^{\alpha}$ atoms of the resulting $Cys_2$Dmb moiety are separated by 13 Å when the chain of nine intervening atoms ($C^{\alpha}$-$C^{\beta}$-S-$C^{\alpha'}$-$C^{3'}$-$C^{4'}$-$C^{5'}$-$C^{\alpha'}$-S-$C^{\beta}$-$C^{\alpha}$) is in the fully extended conformation. Previously, $Br_2$Dmb and its acid chloride have been used as intermediates in the synthesis of a 3,5-bis(methoxymethyl)benzamido porphyrin.[2] In addition, we have used $Br_2$Dmb methyl ester in the synthesis of two new symmetric aromatic diamino acids, 3,5-bis(aminomethyl)benzoic acid and 3,5-bis(aminoethyl)benzoic acid.[3]

## RESULTS AND DISCUSSION

*Radical Bromination.* Coupling of 3,5-dimethylbenzoic acid (**1a**) and *N*-hydroxysuccinimide (HOSu) with *N,N'*-dicyclohexylcarbodiimide (DCC) in tetrahydrofuran (THF) afforded the ester succinimido 3,5-dimethylbenzoate[4] (**1b**) in 55% yield. α-Bromination of active ester **1b** with two equivalents of *N*-bromosuccinimide (NBS) and a catalytic amount of dibenzoyl peroxide in hot $CH_2Cl_2$ produced a mixture of four brominated esters: the α-monobromide **2b**, the desired α,α'-dibromide **3b**, the α,α-dibromide **4b**, and the α,α,α'-tribromide **5b** (Fig. 1).

Radical bromination often generates multiple brominated products.[5,6] Electron-withdrawing groups on the benzene ring decrease yields and increase reaction times for α-bromination.[5] The ratio of the symmetric dibromide **3b** to the unsymmetric dibromide **4b** is higher for solvents of higher refractive index.[6] Based on

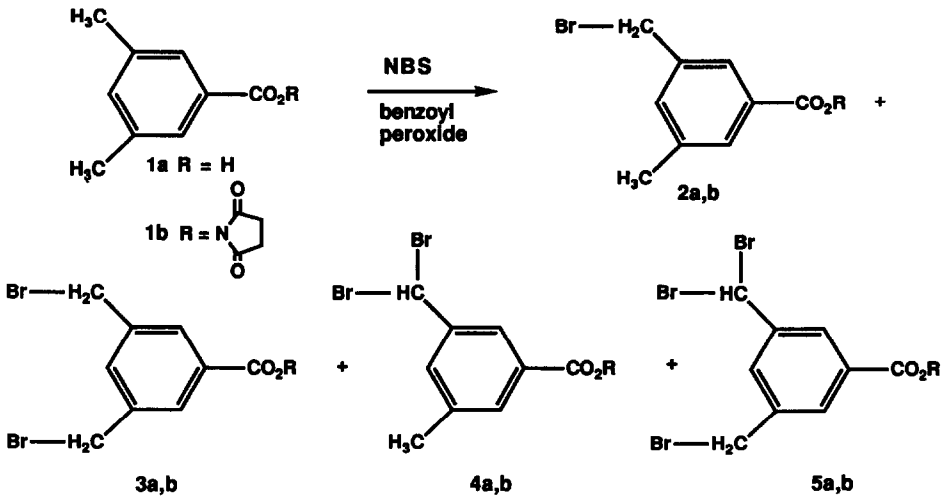



Fig. 1. Formation of four α-brominated products on radical bromination of 3,5-dimethylbenzoic acid (**1a**) or its succinimido ester (**1b**).

Table 1. Radical Bromination of Succinimido 3,5-Dimethylbenzoate: Effect of Reaction Conditions on Product Yields

| | | | | Product (mol %)[a] | | | | |
|---|---|---|---|---|---|---|---|---|
| Reagent | Equiv | Solvent | Conditions | **1b** | **2b** | **3b** | **4b** | **5b** |
| NBS | 0.5 | $CCl_4$ | reflux, <1 h | 19 | 60 | 12 | 7 | 2 |
| NBS | 2.0 | $CH_2Cl_2$ | hν, room temp, 4 d | 10 | 50 | 25 | 10 | 5 |
| $Br_2$ | 2.0 | $CH_2Cl_2$ | hν, reflux | 4 | 50 | 29 | 9 | 7 |
| NBS | 2.0 | $CCl_4$ | reflux, <1 h | 0 | 15 | 32 | 21 | 32 |
| NBS | 2.1 | $CH_2Cl_2$ | hν, reflux, <1 h | 0 | 19 | 49 | 13 | 19 |
| NBS | 2.0 | $CH_2Cl_2$ | hν; dark, 2 d; hν, 1.5 h | 0 | 28 | 55 | 10 | 7 |
| NBS | 2.0 | $CH_2Cl_2$ | hν, reflux, <1 h | 0 | 20 | 59 | 10 | 11 |

[a] Molar percentages for the recovered ester **1b** and the four α-brominated esters **2b-5b** (Fig. 1) were measured by determining the relative intensities of the CH and $CH_2$ singlets in the NMR spectrum of the reaction mixture.

these studies, we examined several solvents and reaction conditions for radical bromination of ester **1b** (Table 1). The composition of each product mixture was measured by proton NMR spectrometry (Table 2). The highest analytical yield (59 mol%) observed for the desired symmetric dibromo ester (succinimido 3,5-bis(bromomethyl)benzoate, $Br_2$Dmb-OSu, **3b**) was obtained by photoinitiated bromination of a solution of ester **1b** in $CH_2Cl_2$ at reflux.

Table 2. Proton NMR Data for Succinimido 3,5-Dimethylbenzoate and Four of Its α-Brominated Derivatives

| | | Chemical shift (δ, ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | Code | $C^2H$ | $C^4H$ | $C^6H$ | $C^{\alpha}H$ | $C^{\alpha}H_2$ | $C^{\alpha}H_3$ | $(CH_2)_2$ |
| parent ester | **1b** | 7.75 | 7.30 | 7.75 | - | - | 2.38 | 2.91 |
| α-monobromide | **2b** | 7.95 | 7.52 | 7.89 | - | 4.48 | 2.43 | 2.92 |
| α,α'-dibromide | **3b** | 8.08 | 7.75 | 8.08 | - | 4.50 | - | 2.93 |
| α,α-dibromide | **4b** | a | a | a | 6.63 | - | 2.47 | 2.93 |
| α,α,α'-tribromide | **5b** | a | a | a | 6.65 | 4.52 | - | 2.93 |

[a] Not determined.

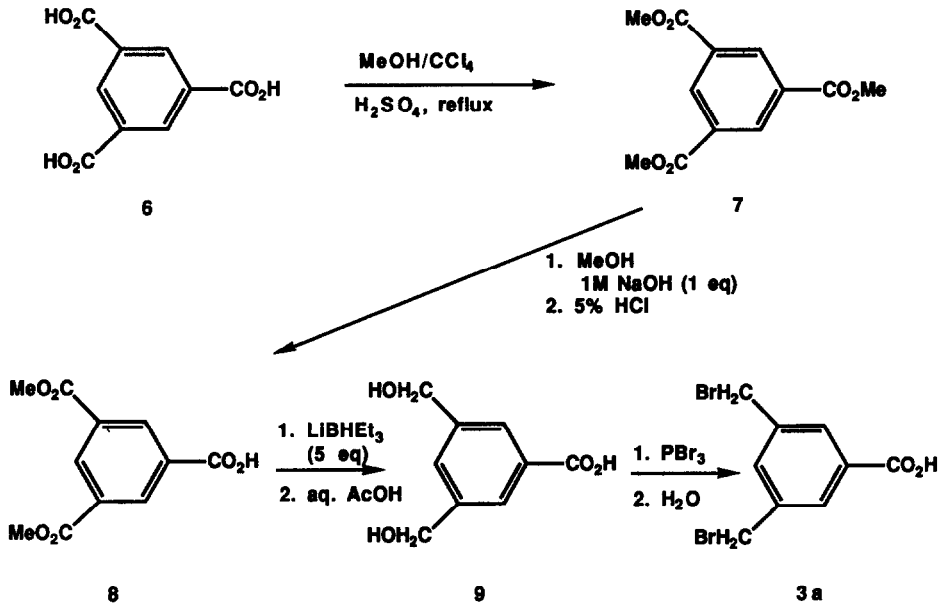



Fig. 2. A regiospecific four-step synthesis of 3,5-bis(bromomethyl)benzoic acid (**3a**) from 1,3,5-benzenetricarboxylic acid (6)

In our hands, NBS bromination of the corresponding acid, 3,5-dimethylbenzoic acid (**1a**), gave similar mixtures of the four brominated acids **2a-5a** (data not shown). Young and Chang[2] reported that they obtained the acid **3a** in 38% yield by NBS bromination of acid **1a** followed by recrystallization from methanol/$CH_2Cl_2$. But they did not mention the amounts of the by-products **2a**, **4a**, and **5a** present in their samples of **3a** before or after crystallization or the melting point of their crystalline sample of **3a**.

We attempted to separate the succcinimido ester **1b** and its brominated products **2b-5b** by several different methods. The esters were eluted in the order **1b**, **2b**, **3b**, **4b**, and **5b** from a silica column washed with 12:5:3 (v/v) hexane/ethyl acetate/$CH_2Cl_2$ . Even when as little as 100 mg of this product mixture was loaded onto a long silica column (1 m x 2 cm), the desired $\alpha,\alpha'$-dibromo ester **3b** was not obtained in pure form. Finally, three successive crystallizations of the product mixture from ethyl acetate/hexane provided the symmetric dibromo ester **3b** as a solid that was 97% pure by NMR spectrometry but was isolated in only 5% yield.

*Regiospecific Synthesis.* We avoided the formation of this mixture of bromomethyl compounds by devising and executing a regiospecific four-step synthesis of $Br_2$Dmb from 1,3,5-benzenetricarboxylic acid (**6**). As shown in Fig. 2, the key step was formation of dimethyl ester **8** by monodemethylation of trimethyl ester **7**.

Esterification of triacid **6** proceeded better when using $CCl_4$ instead of $CH_2Cl_2$ as the co-solvent because $CCl_4$ selectively dissolved the final product, trimethyl 1,3,5-benzenetricarboxylate (**7**). Triacid **6** was only

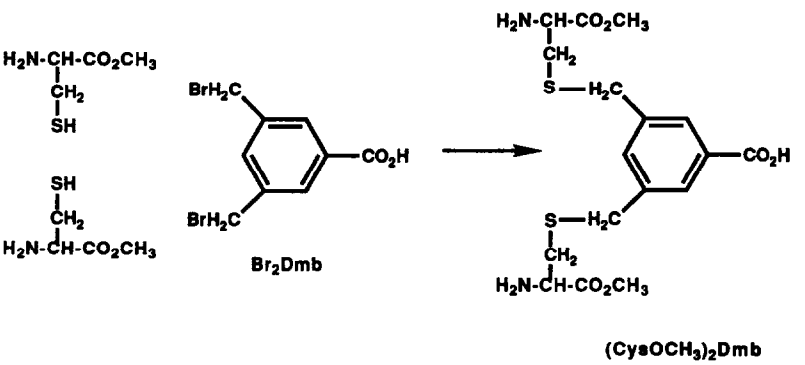
partially soluble in the $CCl_4$/methanol solvent mixture but dissolved as the reaction proceeded. When the reaction was performed on a 50-mmol scale, esterification was driven to completion by removing water through rotary evaporation. NMR-pure trimethyl ester **7** was isolated in 96% yield.

Attempted monodemethylation of triester **7** with *N,N*-dimethylhydrazine[7] gave an impure product in low yield. Therefore, saponification at room temperature with one equivalent of sodium hydroxide was examined in several different solvents. The use of methanol gave the desired 3,5-bis(methoxycarbonyl)benzoic acid (**8**) as the major product as determined by TLC. Since $CCl_4$ readily dissolved triester **7** but not the desired diester **8**, most of the unreacted triester **7** was removed by trituration with $CCl_4$. The recovered triester **7** was recycled. Finally, flash chromatography of the triturated solid on silica gel furnished NMR-pure diester **8** in 90% net isolated yield.

Diester **8** was reduced to 3,5-bis(hydroxymethyl)benzoic acid (**9**) with 1 M lithium triethylborohydride in THF (Super Hydride). The borate intermediate was not cleaved when the reaction was quenched with 3 N HCl, probably because the triethylborate forms a complex with dihydroxy acid **9**. When the reaction was quenched with 10% acetic acid, however, the volatile triethylborate/THF complex could be removed by rotary evaporation. The remaining mixture of lithium 3,5-bis(hydroxymethyl)benzoate and lithium acetate was dissolved in water and separated by anion-exchange chromatography. Elution with formic acid gave NMR-pure dihydroxy acid **9** in 71% isolated yield.

Dihydroxy acid **9** was transformed into 3,5-bis(bromomethyl)benzoic acid ($Br_2$Dmb, **3a**) by reaction with phosphorus tribromide in 5:1 (v/v) diethyl ether/THF. THF was needed to dissolve the dihydroxy acid. When only THF was used, a major by-product was 4-bromo-1-butanol, which was formed by attack of $PBr_3$ on THF. Recrystallization from ethyl acetate/hexane furnished NMR-pure dibromo acid **3a**, which was obtained in 62% isolated yield. Specifically, the resulting $Br_2$Dmb contained none of the α-bromo acids **2a**, **4a**, and **5a** as measured by proton NMR spectrometry. This regiospecific synthesis of the symmetric α,α'-dibromo acid **3a** from triacid **1a** proceeded in 38% overall yield over four steps.

*Thiol Crosslinking.* S-Alkylation of a model organic thiol, cysteine methyl ester (Cys-$OCH_3$), with $Br_2$Dmb was carried out in an organic solvent in the presence of a tertiary amine. A solution of Cys-$OCH_3$, $Br_2$Dmb, and *N,N*-diisopropylethylamine (DIEA) in $(CD_3)_2SO$ was monitored by proton NMR spectrometry



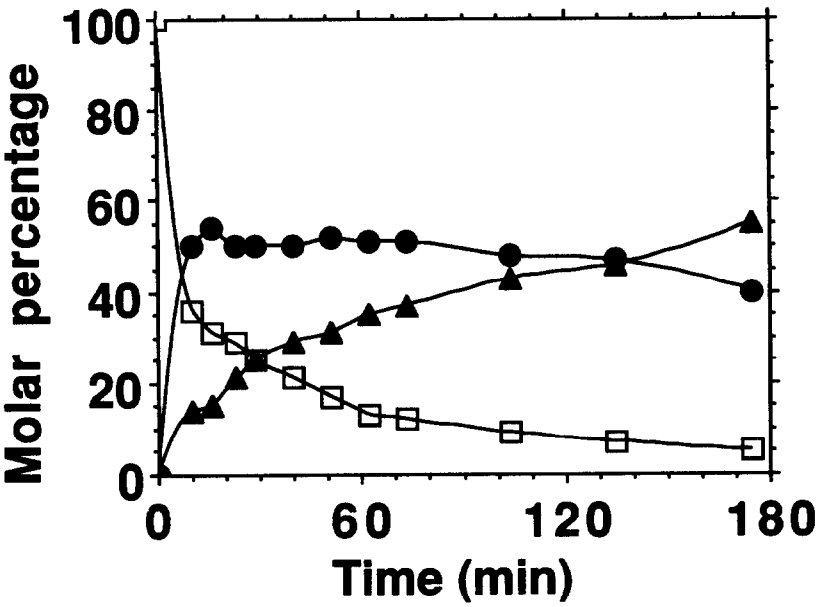
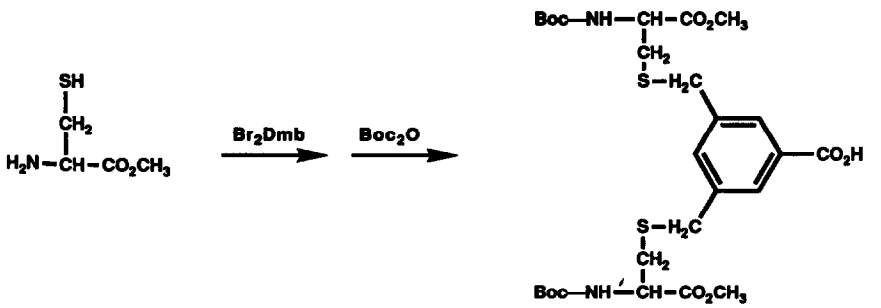



Fig. 3. Course of the S-alkylation of cysteine methyl ester (2.8 equiv) by $Br_2Dmb$ (2.0 equiv) in DIEA (2.8 equiv) and $(CD_3)_2SO$. The reaction mixture was monitored by proton NMR spectrometry to determine the molar percentages of $Br_2Dmb$ (squares), an intermediate (circles), and (Cys-$OCH_3)_2Dmb$ (triangles) versus time.

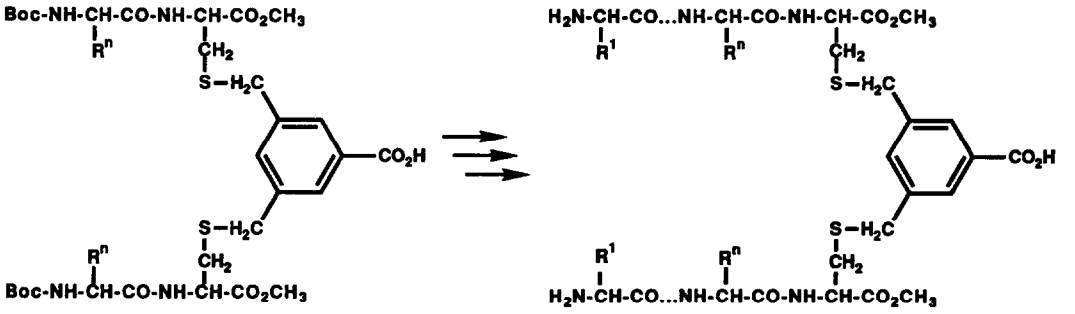
(Fig. 3). Between 10 min and 130 min, about half of the aromatic components was a single intermediate between $Br_2Dmb$ and (Cys-$OCH_3)_2Dmb$, presumably the 1:1 adduct (Br,Cys-$OCH_3$)Dmb. After 24 h the desired 2:1 adduct, (Cys-$OCH_3)_2Dmb$, was formed in 89% analytical yield and was isolated in 79% preparative yield. Thus the presence of the unprotected amino group of Cys-$OCH_3$ is compatible with these conditions for crosslinking with $Br_2Dmb$. S-Alkylation proceeded even faster when more DIEA was present to neutralize the HBr generated during the reaction. For example, increasing the amount of DIEA from 2.8 equiv to 4.8 equiv provided (Cys-$OCH_3)_2Dmb$ in 75% analytical yield after S-alkylation for only 50 min.

Once two molecules of Cys-$OCH_3$ were crosslinked with $Br_2Dmb$, subsequent addition of di(*tert*-butyl) dicarbonate ($Boc_2O$, where Boc is *tert*-butyloxycarbonyl) and more DIEA to the reaction vessel successfully blocked both amino groups of (Cys-$OCH_3)_2Dmb$ with the Boc protecting group. The net one-pot conversion of Cys-$OCH_3$ into (Boc-Cys-$OCH_3)_2Dmb$ by successive treatment with $Br_2Dmb$ and $Boc_2O$ proceeded in 60% isolated yield.




(Boc-Cys-$OCH_3$)$_2$Dmb

The protected diamino acid (Boc-Cys-$OCH_3$)$_2$Dmb is suitable for use in the solid-phase assembly of two peptide chains joined by the five-atom Dmb crosslink. For example, through standard procedures[8] of solid-phase peptide synthesis, after the free carboxyl group of (Boc-Cys-$OCH_3$)$_2$Dmb is covalently attached to a solid support, the two Boc groups could be removed, the two protonated amino groups could be neutralized, and the two free amino groups could be coupled with a suitable Boc-amino acid to give (Boc-aminoacyl-Cys-$OCH_3$)$_2$Dmb sites. By repetition of this three-step synthetic cycle, a series of amino acid residues could be sequentially added to each of the two peptide chains growing from the crosslinked Cys residues of (Cys-$OCH_3$)$_2$Dmb to furnish (peptidyl-Cys-$OCH_3$)$_2$Dmb after cleavage from the solid support. We have used this assembly strategy with a related diamino acid during solid-phase assembly of a potential β-barrel protein.[9] Briefly, the protected diamino acid (Boc-Bal)$_2$Bab-Bal, where Bal is 3-aminopropionic acid and Bab is 3,5-bis(aminoethyl)benzoic acid,[3] was covalently attached to a solid support. Then the two identical 32-residue peptide chains of betabellin 7 were simultaneously assembled by stepwise addition of 31 Boc-amino acids to the two amino groups of the peptide chains growing from the crosslinked Bal residues.[9] Finally, we have proposed that a receptor-adhesive modular protein containing two identical 39-residue peptide chains that form an α-helical coiled coil might be covalently joined through a Dmb crosslink.[1]




(Boc-aminoacyl-Cys-$OCH_3$)$_2$Dmb

(Peptidyl-Cys-$OCH_3$)$_2$Dmb

## EXPERIMENTAL PROCEDURES

$^1$H NMR spectra were recorded on a Bruker AC200 or a Varian XL400 spectrometer. Monoisotopic mass ratios ($M_r$) were determined on a VG Model 70/SEQ hybrid tandem mass spectrometer. Melting points were measured in glass capillaries and are uncorrected.

**Succinimido 3,5-Dimethylbenzoate (1b).** A solution of 3,5-dimethylbenzoic acid (**1a**, 11.14 g, 74 mmol) and *N*-hydroxysuccinimide (8.53 g, 74 mmol) in freshly distilled THF (100 mL) was stirred in an ice bath as DCC (15.28 g, 74 mmol) was added. After the reaction mixture was stirred overnight at 7 °C, the dicyclohexylurea was removed by filtration and THF was removed from the filtrate by rotary evaporation. The pale yellow solid was recrystallized twice from 2-propanol to give NMR-pure ester **1b** (10.16 g, 56% yield) as a white solid: mp 149-151°C; wt% calcd (found) for $C_{13}H_{13}NO_4$, C 63.15 (63.05), H 5.30 (5.33), N 5.66 (5.63); $^1$H NMR ($CDCl_3$) δ 2.38 (s, 6 H, $CH_3$), 2.91 (s, 4 H, $CH_2$), 7.30 (s, 1 H, $C^4H$), and 7.75 ppm (s, 2 H, $C^2H$ and $C^6H$).

**Succinimido 3,5-Bis(bromomethyl)benzoate (3b).** A mixture of ester **1b** (2.48 g, 10 mmol), NBS (3.74 g, 21 mmol), and dibenzoyl peroxide (42 mg) in $CH_2Cl_2$ (55 mL) was heated at reflux and irradiated with a UV lamp placed 5 cm from the reaction flask. After 5 min the turbid solution turned orange and clear. After the solution was irradiated for 1 h at reflux, the solution turned yellow. The organic layer was washed three times with water and was dried over anhydrous sodium sulfate, and $CH_2Cl_2$ was removed by rotary evaporation. The pale yellow solid was recrystallized three times from ethyl acetate/hexane to yield the α,α'-dibromo ester **3b** as a white crystalline solid (0.2 g, 5% yield) that was 97% pure by NMR: mp 171-172 °C; wt% calcd (found) for $C_{13}H_{11}Br_2NO_4$, C 38.80 (38.62), H 2.76 (2.75), Br 39.05 (39.34), N 3.48 (3.42); $^1$H NMR ($CDCl_3$) δ 4.50 (s, 4 H, $CH_2Br$), 7.75 (br s, 1 H, $C^4H$), and 8.08 ppm (d, 2 H, $C^2H$ and $C^6H$).

**Trimethyl 1,3,5-Benzenetricarboxylate (7).** A solution of 1,3,5-benzenetricarboxylic acid (**6**, 10.5 g, 50 mmol) in $CCl_4$ (320 mL), methanol (80 mL), and 18 N sulfuric acid (0.5 mL) was heated at reflux until esterification was complete as measured by TLC in 95:5:0.1 (v/v) $CH_2Cl_2$/methanol/acetic acid. After the solvents were removed by rotary evaporation, the solid was dissolved in ethyl acetate (250 mL). The organic layer was washed with saturated sodium bicarbonate solution (100 mL) and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was evaporated to give NMR-pure triester **7** (12.1 g, 96% yield) as a white solid: mp 141-143 °C; wt% calcd (found) for $C_{12}H_{12}O_6$, C 57.14 (57.20), H 4.80 (4.82); $M_r$ calcd (found) for $(C_{12}H_{12}O_6)^+$, 252.0625 (252.0633); $^1$H NMR ($CDCl_3$) δ 3.98 (s, 9 H, $OCH_3$), and 8.86 ppm (s, 3 H, $C_6H_3$).

**3,5-Bis(methoxycarbonyl)benzoic Acid (8).** Trimethyl 1,3,5-benzenetricarboxylate (**7**, 2.52 g, 10 mmol) was dissolved in methanol (200 mL) and 0.95 N sodium hydroxide (10.5 mL, 10 mmol) by stirring at room temperature for 1 h. Stirring was continued for another 16 h at pH 7-8. After removal of solvents by rotary evaporation, the residual solid was dissolved in ethyl acetate (100 mL) and the solution was washed with 0.6 N HCl and was dried over anhydrous sodium sulfate. Solvent was removed by rotary

evaporation and the solid was triturated with $CCl_4$ (75 mL) and filtered. Flash chromatography of the remaining solid (2.26 g) on silica gel with 19:1 (v/v) $CHCl_3$/methanol furnished the diester **8** (2.14 g, 90% yield) as a white solid: mp 149.5-150.5 °C; wt% calcd (found) for $C_{11}H_{10}O_6$, C 55.46 (55.20), H 4.23 (4.31); $M_r$ calcd (found) for $(C_{11}H_{10}O_6)^+$, 238.0476 (238.0473); $^1H$ NMR ($CD_3OD$) δ 3.97 (s, 6 H, $OCH_3$), 8.74 (t, 1 H, $C^4H$), and 8.77 ppm (d, 2 H, $C^2H$ and $C^6H$).

**3,5-Bis(hydroxymethyl)benzoic Acid (9).** A solution of 3,5-bis(methoxycarbonyl)benzoic acid (**8**, 1.34 g, 5.6 mmol) in freshly distilled THF (20 mL) was added dropwise to 1.0 M lithium triethylborohydride in THF (29 mL, 29 mmol, Super Hydride, Aldrich Chemical Co.). The colorless solution turned yellow and then deep red and gas was evolved. After being stirred at room temperature overnight, the solution contained no starting acid **8** as measured by TLC in 800:200:1 (v/v) $CHCl_3$/methanol/acetic acid. Aqueous 10% acetic acid was added until the solution turned colorless. The triethylborate-THF complex was removed by rotary evaporation. The resulting rubbery solid was redissolved in water and passed through an anion-exchange column (AG 50x5, formate form, BioRad Laboratories). Lithium acetate was eluted with water and then the desired acid **9** was eluted with 1.0 M formic acid. Fractions containing acid **9** as measured by TLC were combined and freed of solvent by rotary evaporation to furnish NMR-pure dihydroxy acid **9** (0.73 g, 71% yield) as a white solid: mp 171-173 °C; wt% calcd (found) for $C_9H_{10}O_4$, C 59.33 (59.26), H 5.53 (5.54); $M_r$ calcd (found) for $(C_9H_{10}O_4)^+$, 182.0578 (182.0576); $^1H$ NMR ($CD_3OD$) δ 4.65 (s, 4 H, $CH_2$), 7.56 (br s, 1 H, $C^4H$), and 8.92 ppm (d, 2 H, $C^2H$ and $C^6H$) in agreement with Young and Chang.[2]

**3,5-Bis(bromomethyl)benzoic Acid (3a, $Br_2$Dmb).** A solution of 3,5-bis(hydroxymethyl)benzoic acid (**9**, 0.50 g, 2.7 mmol) in diethyl ether (25 mL), THF (5 mL), and $PBr_3$ (0.027 mL, 2.84 mmol) was stirred at room temperature for 3 h until no starting acid was present as measured by TLC. Water (25 mL) was added, the organic phase was washed with water (2 x 25 mL), and the combined aqueous phase was extracted with ether (2 x 25 mL). The combined organic phase was dried over anhydrous sodium sulfate and freed of solvent by rotary evaporation. The residual white solid was crystallized from ethyl acetate/hexane to furnish NMR-pure dibromo acid **3a** (0.518 g, 62% yield) as white needles: mp 161-163 °C; wt% calcd (found) for $C_9H_8Br_2O_2$, C 35.10 (35.21), H 2.62 (2.65), Br 51.89 (51.84); $M_r$ calcd (found) for $(C_9H_8Br_2O_2)^+$, 305.8891 (305.8888); $^1H$ NMR ($CDCl_3$) δ 4.50 (s, 4 H, $CH_2$), 7.74 (t, 1 H, $C^4H$), and 8.08 ppm (d, 2 H, $C^2H$ and $C^6H$).

**3,5-Bis((2-amino-2-(methoxycarbonyl)ethyl)thiomethyl)benzoic Acid** ($(Cys\text{-}OCH_3)_2$Dmb). The $^1H$ NMR spectrum of a solution of Cys-$OCH_3$ (17.1 mg, 100 μmol, 2.8 equiv), $Br_2$Dmb (**3a**, 11.1 mg, 36 μmol, 2.0 equiv), and DIEA (17.4 μL, 100 μmol, 2.8 equiv) in $(CD_3)_2SO$ (1.0 mL) was monitored at 12 time points over 3 h (see Fig. 3). In addition, the amounts of $(Cys\text{-}OCH_3)_2$Dmb and $Br_2$Dmb present were 80 mol% and 20 mol% at 8 h and 89 mol% and 11 mol% at 25 h, respectively. Formation of the disulfide $(Cys\text{-}OCH_3)_2$ was not observed. Reversed-phase HPLC separation of the reaction mixture on octyl-silica (μBondapak C8, Millipore Corp.) afforded the NMR-pure title diamino acid (12 mg, 79% yield) as a white solid: $M_r$ calcd (found) for $(C_{17}H_{24}N_2O_6S_2 + H)^+$, 417.1156 (417.1147); $^1H$ NMR ($(CD_3)_2SO$) δ 2.89 (m, 4 H, $C^\beta H_2$), 3.75 (s, 6 H, $OCH_3$), 3.87 (s, 4 H, $SC^{\alpha'}H_2$), 4.38 (m, 2 H, $C^\alpha H$), 7.54 (s, 1 H, $C^{4'}H$), 7.84 (s, 2 H, $C^{2'}H$ and $C^{6'}H$), and 8.50 ppm (br s, 4 H, $NH_2$).

**3,5-Bis((2-(*tert*-butyloxycarbonylamino)-2-(methoxycarbonyl)ethyl)thiomethyl)benzoic Acid ((Boc-Cys-$OCH_3$)$_2$Dmb).** A solution of Cys-$OCH_3$ (0.392 g, 2.3 mmol, 2.6 equiv), $Br_2$Dmb (**3a**, 0.308 g, 1.0 mmol, 2.0 equiv), and DIEA (1.04 mL, 6.0 mmol, 12 equiv) in $(CH_3)_2SO$ (2.5 mL) was kept at room temperature for 30 min until $Br_2$Dmb was no longer detected by TLC in 950:50:1 (v/v) $CHCl_3$/methanol/acetic acid. Di(*tert*-butyl) dicarbonate (0.77 mL) and DIEA (0.52 mL) were added and the solution was allowed to stand for 1 h. The solvent was removed under vacuum and the residue was dissolved in ethyl acetate and washed with water (3 x 25 mL). The aqueous layer was acidified with 5% HCl and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent was removed by rotary evaporation. The residual yellow solid was recrystallized from ethyl acetate/hexane to furnish NMR-pure (Boc-Cys-$OCH_3$)$_2$Dmb (0.374 g, 60% yield) as a white solid: mp 105-107 °C; wt% calcd (found) for $C_{27}H_{40}N_2O_{10}S_2$, C 52.58 (52.65), H 6.54 (6.56), N 4.54 (4.46), S 10.38 (10.30); $^1$H NMR ($CDCl_3$) δ 1.44 (s, 18 H, $CCH_3$), 2.83 (m, 4 H, $C^{\beta}H_2$), 3.75 (s, 10 H, $SC^{\alpha'}H_2$, $OCH_3$), 4.53 (m, 2 H, $C^{\alpha}H$), 5.33 (d J = 7.3 Hz, 2 H, NH), 7.52 (s, 1 H, $C^{4'}H$), and 7.91 ppm (s, 2 H, $C^{2'}H$ and $C^{6'}H$).

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