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Chemoselective Protection of Glutathione in the Preparation of Bioconjugates: The Case of Trypanothione Disulfide

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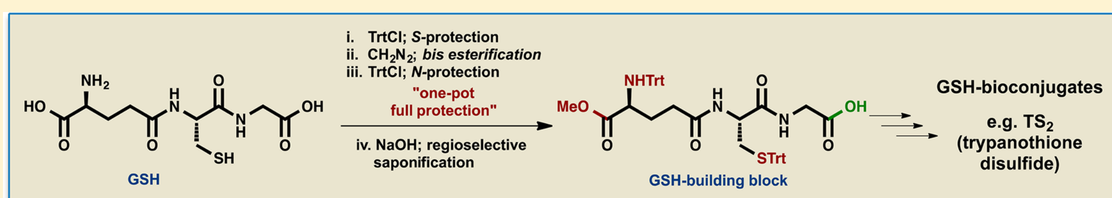
Supporting Information



ABSTRACT: A novel synthetic route to the chemoselectively protected *N,S*-ditritylglutathione monomethyl ester is described involving the chemical modification of the commercially available glutathione (GSH). The synthetic value of this building block in the facile preparation of GSH bioconjugates in a satisfying overall yield was exemplified by the case of trypanothione disulfide (TS_2), a GSH-spermidine bioconjugate, involved in the antioxidative stress protection system of parasitic protozoa, such as trypanosoma and leishmania parasites.

Glutathione (GSH; **1**, Figure 1) is a ubiquitous thiol present in many biological systems. It is involved in important reactions and in numerous metabolic pathways within mammalian cells.[1]

GSH participates in detoxification systems[2] and can complex metals for transport and storage through non-enzymatic reactions.[3] While GSH is also a therapeutic agent, its use is hampered by unfavorable biochemical properties and water solubility. To bypass this limitation, GSH levels in target cells can be increased by the use of reversible bioconjugates,[4] which are able to cross the cellular membrane. In recent years, many efforts have been devoted to the preparation of GSH bioconjugates. These compounds have been successfully introduced as synthetic prodrugs and codrugs with demonstrated inhibitory activities against cancers dependent on GSH enzymes.[5] GSH bioconjugates have also been proposed as specific molecular probes in medicinal chemistry and for the development of glutathione reductase inhibitors.[6] Self-assembling micro- and hydrogels, gold nanoparticles, biosensors, and bioadhesives based on the GSH structure are of great current interest in nanotechnologies and in biomedical research.[7]

GSH is the tripeptide γ-L-glutamyl-L-cysteinyl glycine. It displays four "free" functional groups, of which the sulfhydryl group of cysteine is a key functional element, capable of generating glutathione disulfide (GSSG) and glutathione conjugates (GSR).[8] Methods of bioconjugation are highly site-specific, and amide linkages are highly attractive for this purpose.[9] In general, the preparation of reversible GSH bioconjugates involves the COOH group of the terminal glycine moiety. It is evident that orthogonal protection is required to selectively mask the cysteine thiol, the glutamic acid carboxyl and amino, and glycine carboxyl groups in preparing the desired conjugates. Most of the reported[10] non-enzymatic liquid and solid-phase synthetic routes to GSH-based versatile building blocks and bioconjugates proceed through a significant number of masking/unmasking steps used for the assembly of the GSH core. Isolation and recovery of the required intermediates are often difficult, demanding extensive and time-consuming chromatographic purifications. These drawbacks diminish the reliability of synthetic step-by-step GSH conjugation strategies, and consequently, the design of methods allowing easy access to orthogonally protected GSH-based building blocks is of high importance. The possibilities of the chemical modification of commercially available low-cost, natural GSH, in combination with a reduced number of reaction steps, easy product isolation, and high yields are the most important advantages of the proposed synthetic route to GSH building blocks, which could then be utilized in preparing bioconjugates with potential pharmaceutical applications.

In connection with our studies of the preparation of polyamine conjugates with biologically active compounds, we decided to examine the possibility of developing a protocol for the chemical modification of GSH, leading to a suitable building block for conjugation approaches through the −COOH function of the glycine residue. Thus, we now present a novel and rapid preparation of the orthogonally

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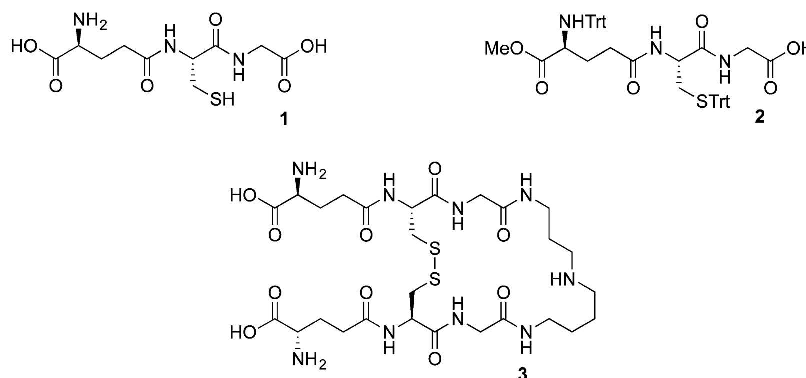
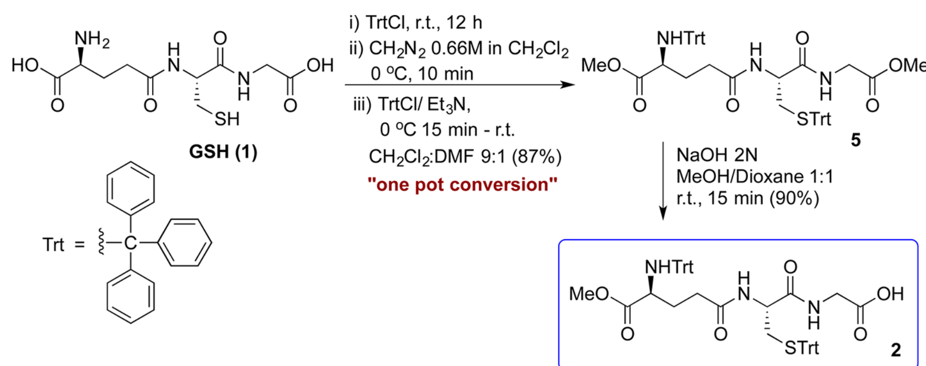


Figure 1. GSH (**1**), the GSH-based building block **2**, and trypanothione disulfide (TS_2, **3**).

Scheme 1. Preparation of GSH-Based Building Block 2



protected *N*,*S*-ditritylglutathione monomethyl ester (**2**, Figure 1) from commercially available GSH. A yield of 87% was achieved through a three-step one-pot procedure, followed by a regioselective saponification. Due to the usefulness of synthon **2** as a suitable building block for such bioconjugation protocols, we demonstrated in the case of the synthesis of trypanothione disulfide, namely, N^1,N^8-bis(glutathionyl) spermidine disulfide (TS_2, **3**; Figure 1), a substrate of trypanothione reductase, the essential enzyme of the antioxidant defenses of trypanosoma and leishmania parasites.[11]

This new synthetic strategy exploited the advantages of the triphenylmethyl (trityl, Trt) protecting group in preserving both amino and thiol[12] groups. This provides orthogonal protection of the amino and thiol functions, which permits a selective amino group deprotection. In addition, the high lipophilicity offered by the Trt group to the protected molecules facilitates any required chromatographic procedures,[13] and its bulkiness prevents the carboxy deprotection of the glutamic acid residue methyl ester and thus allows the selective regiospecific saponification of the glycine methyl ester.

Preparation of building block **2** involves a three-step one-pot procedure leading to the diester **5**. Treatment of GSH with TrtCl takes advantage of the different reactivity of the S- and N-nucleophilic sites of GSH and results in regioselective S-tritylation. Subsequent addition of a diazomethane solution[14] masks both carboxyl groups as methyl esters, and the sequence is completed by TrtCl-mediated amino protection of the glutamic residue (Scheme 1). The fully protected GSH derivative **5** was isolated in an 87% overall yield by a simple aqueous workup of the reaction mixture. The structure of **5** was unequivocally confirmed by ^{1}H and ^{13}C NMR spectroscopy as well as HRMS MALDI and MALDI MS/MS experiments.

Compound **5** was obtained pure enough to be used in the next step without further purification. The target building block **2** was finally obtained by subjecting **5** to saponification, according to our previously established methodology.[15] This procedure was regiospecific, due to the steric hindrance effect exerted by the trityl group placed on the α-amino function of the glutamic acid residue, affording compound **2** as the sole product in excellent yield and without the need for further chromatographic purification. Thus, our novel protocol offers significant advantages over other established synthetic procedures involving multistep peptide synthesis of GSH building blocks for bioconjugates. Compound **2** can be easily obtained by a short and highly efficient chemical modification of commercial GSH and can be considered as a high value synthon for synthetic routes requiring GSH conjugation to other biomolecules.

Having in our hands the key compound **2**, and wishing to demonstrate its usefulness toward the synthesis of glutathione bioconjugates, we proceeded with its application in the synthesis of trypanothione disulfide (TS_2, **3**). Compound **3** and its analogues are important compounds for the development of new attractive chemotherapeutics for clinical treatment of several lethal tropical diseases caused by trypanosomatids, such as African sleeping sickness, Chagas' disease, and the three forms of leishmaniasis.[16] However, TS_2 **3** is not widely available as a result of some drawbacks of the existing synthetic methods. Until now, the preparation of **3** and its analogues has been confined to a restricted number of stepwise and multistage liquid or solid-phase peptide synthesis protocols.[17] All of these methods suffer from the need for the step-by-step assembly of the GSH peptide core, together with a variable number of chromatographic purifications, and afford trypanothione

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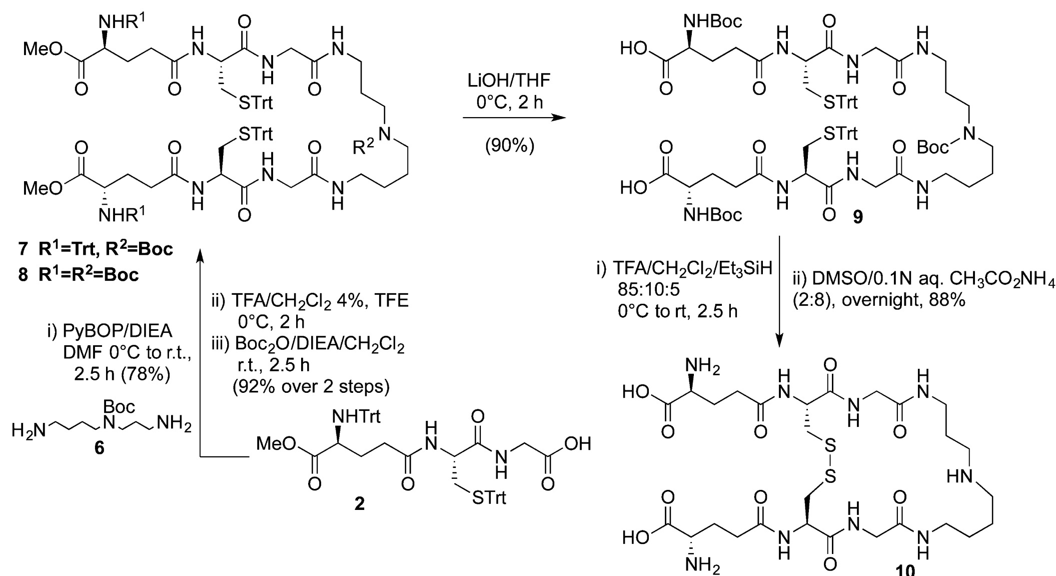
Scheme 2. Synthesis of Trypanothione Disulfide Trifluoroacetate Salt 10 from 2



(TSH_2) and its disulfide (TS_2) in low to moderate (10−38%) overall yields. Accordingly, the use of the GSH-based building block **2** was exploited in the synthesis of TS_2. The proposed synthetic route to **3** required only a few steps, as illustrated in Scheme 2. We started with the double conjugation of the sterically hindered building block **2** to the primary amino functions of the N^4-Boc-spermidine (**6**, Scheme 2).

Although the bulky Trt groups might be expected to limit the effectiveness of our approach, the coupling between **2** and **6** was easily carried out with the PyBOP/DIEA system in DMF, affording the corresponding conjugate **7** in a 78% yield after flash column chromatography (FCC). At this point, the Trt amino protection was switched to Boc, in order to reduce the steric hindrance and facilitate the saponification of the glutamyl α-carboxy ester moiety (Scheme 2). Thus, the chemospecific N-detritylation of **7** was carried out under mild conditions using a dilute solution (4% in DCM) of trifluoroacetic acid (TFA) in the presence of trifluoroethanol (TFE), and the crude product was directly treated with the Boc_2O/DIEA system in DCM to give **8**. Compound **8** was isolated in excellent yield (92%) over two steps and successfully converted to **9** by saponification with LiOH in THF. The dicarboxylic acid **9** was isolated in very high yield (90%) without any further chromatographic purification and used directly in the next step.

Our synthetic route to TS_2 required the installation of a disulfide bridge as the final step. The transformation of **9** into **10** was undertaken in two steps involving full deprotection and oxidation.[18] The complete cleavage of **9** through acidolysis with TFA in DCM using Et_3SiH as the scavenger provided the corresponding tris-trifluoroacetate salt, which was used in the final oxidation step without further purification, and provided the TS_2 tris trifluoroacetate salt (**10**) after overnight stirring in DMSO/0.1 N aq $CH_3CO_2NH_4$ (2:8) at room temperature followed by semipreparative RP-HPLC separation and freeze-drying in order to obtain a pure, white solid (58.9 mg, 56% overall yield, based on the amount of orthogonally protected GSH).

In conclusion, we have established a short and advantageous synthetic method to convert natural GSH into the corresponding *N*,*S*-ditritylmonomethyl ester **2**. The procedure does not require the use of expensive auxiliaries and reagents, minimizes chromatography, and improves the overall yield. The power of our strategy was tested in a divergent approach to trypanothione disulfide, a compound not readily attainable by most of the previously reported solution-phase synthetic schemes. The successful preparation of this GSH bioconjugate demonstrates that the sterically congested and orthogonally protected monomethyl ester derivative **2** may provide a new tool in the synthesis of a wide variety of structurally complex GSH bioconjugates.

■ EXPERIMENTAL SECTION

General Experimental Information. All solvents were dried and/or purified according to standard procedures prior to use. Anhydrous Na_2SO_4 was used for drying solutions, and the solvents were then routinely removed at ca. 40 °C under reduced pressure using a rotary vacuum evaporator. All reagents employed in the present work were commercially available and used without further purification. ^1H NMR spectra were obtained at 400.13 MHz and ^{13}C NMR spectra at 100.62 MHz. Chemical shifts (δ) are indicated in parts per million (ppm) and refer to the residual solvents, as reported elsewhere (for D_2O and $CDCl_3$) and to 2.05 ppm for CD_3COCD_3 (central line of the quintet in the proton spectra). Coupling constants (*J*) are reported in hertz. MALDI-MS and MS/MS spectra were obtained under the conditions previously published.[19] Melting points were determined with a hot stage apparatus and are uncorrected. When required, reactions were carried out under an inert atmosphere (dry N_2) in preflamed glassware. Flash column chromatography on silica gel 60 (230−400 mesh) and analytical thin layer chromatography (TLC) were performed on silica gel F_{254} precoated aluminum foils (0.2 mm film). Spots on the TLC plates were visualized with UV light at 254 nm and charring agents. The eluent systems used for chromatography were as follows: (A) toluene/AcOEt 1:1, (B) $CHCl_3$/MeOH 9.5:0.5, (C) $CHCl_3$/MeOH 9:1, (D) $CHCl_3$/MeOH 8.5:1.5, (E) $CHCl_3$/MeOH/NH_4OH 9:1:0.2, (F) $CHCl_3$/MeOH/AcOH 9:1:0.1, and (G) MeCN/H_2O 5:1. RP-HPLC: Trypanothione disulfide trifluoroacetate salt (**10**) was analyzed on a C-8 column (5 μm particle size; 4 × 125 mm) using a linear gradient from 0 (5 min to 0) to 40% B over 35 min, where A = 0.08% TFA in water and B = 0.08% TFA in MeCN, at a flow rate of 1 mL/min, monitored at 214 nm and purified a semipreparative column RP-18 (5 μm particle size; 10 × 250 mm); using a linear gradient from 0 (5 min to 0) to 40% B over 35 min, where A = 0.08% TFA in water and B = 0.08% TFA in MeCN, at a flow rate of 4 mL/min, monitored at 214 nm.

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One-Pot Preparation of Diester 5. Triphenylmethyl chloride (TrtCl, 2.33 g, 8.4 mmol) was added portionwise over a period of 30 min to a suspension of glutathione (GSH, **1**; 2.46 g, 8.0 mmol) in DCM/DMF (9:1 v/v, 10.7 mL), and the reaction mixture was stirred for 12 h at rt. The resulting colorless solution was then cooled to 0 °C and treated with a solution of diazomethane[15] in DCM (0.66 M; 27.5 mL, 24 mmol), which was added dropwise until a persistent yellow color was observed (CAUTION!). The completion of the reaction was verified by TLC (eluent system A), and the reaction mixture was then left under vigorous stirring in an open vessel in the hood until the yellow color disappeared. At this point, the mixture was cooled again to 0 °C, and a solution of TrtCl (2.33 g, 8.4 mmol) and Et_3N (2.5 mL, 16.8 mmol) in DCM (25 mL) was added dropwise. After completion of the addition, the mixture was left to reach rt, where it was stirred for an additional 60 min. The reaction mixture was then placed in a separatory funnel, diluted with DCM (50 mL), and the organic phase sequentially washed with 5% aqueous citric acid, H_2O, and brine to give the diester **5** as a foam after evaporation under vacuum in satisfactory purity for the next step. An analytical sample of **5** was obtained after FCC (eluent system, toluene/AcOEt 7:3) of the crude product and subjected to complete structural characterization by instrumental analysis.

5: Pale yellow foam (6.96 g, 87% yield over three steps, based on the initial amount of **1**); TLC (eluent system A) R_f 0.31; ^{1}H NMR ($CDCl_3$) δ 7.49−7.42, 7.33−7.22, and 7.21−7.15 (3 m, 30H), 6.63 (br s, 1H), 5.71 (br s, 1H), 4.17 (q, 1H, *J* = 12.8 Hz), 3.91 and 3.83 (2 dd, 2H, *J* = 5.6, 18, 18.4 Hz), 3.70 (s, 3H), 3.40 (br s, 1H), 3.15 (s, 3H), 2.82 (dd, 1H, *J* = 6.8, 12.8 Hz), 2.56 (dd, 1H, *J* = 5.2, 13.2 Hz), 2.29−2.19 (m, 1H), 2.17−2.07 (m, 2H), 2.05−1.95 (m, 1H), 1.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 175.1, 172.2, 170.4, 169.8, 145.8, 144.5, 129.6, 128.8, 128.1, 127.9, 127.0, 126.5, 71.2, 67.3, 55.2, 52.3, 51.9, 51.7, 41.2, 33.2, 31.7, 30.6; MALDI-MS calcd for $C_{50}H_{49}N_3O_6NaS^+$ 842.3234, found 842.3272.

Preparation of Acid 2. To a solution of diester **5** (3.5 g, 4.27 mmol) in MeOH/dioxane/DMSO (10:10:0.45, 36 mL) was added 2 N aqueous NaOH (5.1 mL), and the mixture was stirred at rt. The reaction went to completion in 15 min as indicated by TLC (eluent system D). It was then diluted with Et_2O (100 mL) and first washed once with 1 N aqueous NaOH (10 mL) and then twice with distilled water (2 × 20 mL). The aqueous layers were combined, cooled to 0 °C, acidified to pH 5 with 5% aqueous citric acid, and then extracted with AcOEt (2 × 25 mL). The organic layers were combined and washed once with brine, dried (Na_2SO_4), filtered, and evaporated to dryness under vacuum to obtain the corresponding monocarboxylic acid **2**: pale yellow foam (2.93 g, 90% yield); TLC (eluent system D) R_f 0.22; ^{1}H NMR ($CDCl_3$) δ 7.49−7.39, 7.32−7.20, and 7.19−7.13 (3 m, 30H), 6.93 (unresolved t, 1H), 6.08 (d, 1H, *J* = 7.6 Hz), 4.35 (q, 1H, *J* = 13.2 Hz), 3.94 and 3.81 (2 dd, 2H, *J* = 4.4, 6.8, 18.4 Hz), 3.37 (t, 1H, *J* = 5.6 Hz), 3.12 (s, 3H), 2.70 (dd, 1H, *J* = 7.2, 12.8 Hz), 2.56 (dd, 1H, *J* = 5.6, 13.2 Hz), 2.31−2.20 (m, 1H), 2.17−2.04 (m, 2H), 2.03−1.91 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 175.1, 172.8, 171.9, 170.5, 145.6, 144.3, 129.5, 128.7, 128.0, 127.8, 126.8, 126.4, 71.1, 67.1, 55.2, 51.8, 51.7, 41.5, 33.6, 31.7, 30.7; MALDI-MS calcd for $C_{49}H_{47}N_3O_6NaS^+$ 828.3116, found 828.3078.

Synthesis of Compound 7. (Benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP, 167 mg, 0.32 mmol) and N^4-Boc-spermidine (36.8 mg, 0.15 mmol) were added to a solution of **2** (243 mg, 0.3 mmol) in DMF (0.7 mL). The resulting suspension was cooled to 0 °C, and DIEA (0.11 mL, 0.6 mmol) was added dropwise. The mixture was then left under magnetic stirring at rt for the reaction to be completed (2.5 h), and it was then diluted with AcOEt (25 mL) and placed in a separatory funnel. The organic layer was washed with 5% aqueous $NaHCO_3$ (3 × 10 mL), distilled water (3 × 10 mL), 5% aqueous citric acid (3 × 10 mL), and once with brine (10 mL). After being dried (Na_2SO_4), the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC (eluent system B) to give product **7**: white foam (218 mg, 78% yield); TLC (eluent system B) R_f 0.3; ^{1}H NMR (CD_3COCD_3) δ 7.68 (t, 2H, *J* = 5.6 Hz), 7.52−7.44, 7.43−7.35, 7.34−7.29, 7.28−7.21, and 7.20−7.41 (5 m, 60H), 3.76 and 3.68 (2 dd, 4H, *J* = 4.4, 6.0, 16.8 Hz), 3.41−3.31 (m, 2H), 3.16−3.09 (m, 10H), 3.08−2.99 (m, 2H), 2.91−2.74 (m, 10H), 2.71−2.56 (m, 4H), 2.49−2.35 (m, 2H), 2.32−2.19 (m, 2H), 2.15−2.07 (m, 2H), 2.03−1.97 (m, 1H), 1.96 (s, 1H), 1.68−1.55 (m, 2H), 1.54−1.44 (m, 2H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.6, 172.5, 170.3, 168.5, 155.3, 146.1, 144.7, 129.5, 128.8, 128.0, 127.8, 126.8, 126.4, 78.4, 71.1, 66.7, 55.5, 55.4, 54.1, 53.2, 51.0, 46.7, 42.8, 38.2, 33.3, 31.1, 30.6, 27.8, 26.5, 19.6, 13.6; MALDI-MS calcd for $C_{110}H_{117}N_9O_{12}NaS_2^+$ 1842.8155, found 1842.8247.

Preparation of Compound 8. A solution of **7** (1.2 g, 0.66 mmol) and TFE (0.4 mL) in DCM (4 mL) was cooled to 0 °C, and TFA (0.25 mL) in DCM (2 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, and completion of the reaction was monitored by TLC (eluent system E). At the end, Et_2O was added dropwise until full precipitation of the corresponding double trifluoroacetate salt as a white solid, which was collected upon filtration under vacuum and used in the next step without further purification. The intermediate double trifluoroacetate salt was characterized by mass spectrometry: MALDI-MS calcd for $C_{49}H_{47}N_3O_6NaS^+$ 828.3116, found 828.3078.

An ice-cold solution of the recovered white solid dissolved in DCM (2.5 mL) was prepared, and Boc_2O (202 mg, 0.92 mmol) was added prior to the dropwise addition of DIEA (0.27 mL, 1.54 mmol). The amounts of reagents were calculated by assuming that the previously described acidolysis was quantitative. After 10 min, the ice bath was removed and the reaction mixture was left to stir for a further 3.5 h at rt. Subsequently, the reaction mixture was cooled to 0 °C, diluted with AcOEt (10 mL), and washed with 5% aqueous citric acid (3 × 5 mL), distilled water (3 × 5 mL), and once with brine (5 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness under vacuum to give a residue, which on trituration from a cold 3:1 mixture of AcOEt/Et_2O and filtration under vacuum afforded the product with no need for further purification.

8: White solid (615 mg, 92% yield over two steps; 92% yield based on the initial amount of **7**); TLC (eluent system C) R_f 0.36; mp 113.7−114.9 °C; ^{1}H NMR (CD_3COCD_3) δ 7.74 (br s, 1H), 7.57 (br s, 1H), 7.43−7.37, 7.36−7.28. and 7.27−7.21 (3 m, 30H), 6.40 (d, 2H, *J* = 7.6 Hz), 4.26−4.14 (m, 2H), 4.08−3.97 (m, 1H), 3.90−3.77 (m, 2H), 3.66 (s, 7H), 3.25−3.06 (m, 7H), 2.82 (s, 6H overlapped with H_2O) 2.73−2.61 (m, 4H,), 2.43−2.31 (m, 4H), 2.21−2.10 (m, 2H), 1.93−1.82 (m, 2H), 1.67 (br s, 2H), 1.43 (s, 9H), 1.39 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 172.6, 172.5, 170.2, 168.5, 155.8, 144.7, 129.5, 128.0, 126.8, 78.6, 78.4, 66.7, 65.2, 53.1, 51.4, 46.7, 42.8, 38.3, 33.1, 31.4, 27.8, 27.7, 27.3, 26.5, 22.4, 14.7, 13.4; MALDI-MS calcd for $C_{82}H_{105}N_9O_{16}NaS_2^+$ 1558.7013, found 1558.7091.

Preparation of 9. A precooled aqueous solution (0.175 mL) of LiOH monohydrate (16.4 mg, 0.36 mmol) was added dropwise to an ice-cold solution of **8** (133 mg, 0.086 mmol) in THF (0.35 mL). The resulting mixture was stirred at 0 °C for 2 h, acidified to pH 4 with 5% aqueous citric acid, and extracted with AcOEt (3 × 5 mL). The combined organic layers were washed once with distilled water (10 mL) and once with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated under vacuum to give the dicarboxylic acid **9**: white solid (116.7 mg, 90% yield); TLC (eluent system F) R_f 0.26; mp 222.3 °C decomp.; ^{1}H NMR (CD_3COCD_3) δ 7.85 (br s, 2H), 7.67 (d, 2H, *J* = 5.2 Hz), 7.44−7.36, 7.35−7.28. and 7.26−7.20 (3 m, 31H), 6.24 (d, 1H, *J* = 8.0 Hz), 4.29−4.12 (m, 2H), 4.01−3.93 (m, 1H), 3.92−3.77 (m, 2H), 3.69−3.57 (m, 2H), 3.23−3.06 (m, 10H), 2.74−2.64 (m, 4H), 2.44−2.35 (m, 4H), 2.25−2.11 (m, 2H), 2.00−1.90 (m, 2H), 1.67 (br s, 2H), 1.43 (s, 9H), 1.40 (s, 18H); ^{13}C NMR (CD_3OD) δ 174.4, 173.8, 171.2, 170.1, 156.7, 145.6, 130.4, 128.9, 127.7, 79.5, 79.4, 67.6, 54.5, 53.7, 47.6, 43.7, 39.4, 33.8, 33.2, 28.7, 28.6, 28.3, 27.3, 20.5; MALDI-MS calcd for $C_{80}H_{101}N_9O_{16}S_2K^+$ 1546.6439, found 1546.6509.

Preparation of the Trypanothione Disulfide Tris-trifluoroacetate Salt (10). A solution of **9** (95 mg, 0.063 mmol) in TFA/DCM/Et_3SiH (85:10:5) (0.2 mL) was stirred for 30 min at 0 °C and then for 2 h at room temperature. Then, Et_2O was added dropwise until full precipitation of the corresponding tris-trifluoroacetate salt as a white solid, which was collected upon filtration under vacuum and

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used in the next step without further purification. Thus, 40 mg (0.037 mmol) of the tris-trifluoroacetate salt was dissolved in DMSO/0.1 N aq $CH_3CO_2NH_4$ (2:8, 40 mL) and stirred overnight at room temperature. The resultant mixture was purified by semipreparative RP-HPLC using gradient elution [0% MeCN (5 min delay) to 40% MeCN in 35 min] to afford **10**, which had identical data to those reported in the literature:[20] white solid (58.9 mg, 88% over two steps; 56% overall yield based on the initial amount of **2**); ^{1}H NMR (D_2O) δ 4.71 (quintet, 2H, J = 4.4 Hz), 3.96 (t, 2H, J = 6.4 Hz), 3.91 (d, 2H, J = 16.4 Hz), 3.83 (d, 1H, J = 6.8 Hz), 3.78 (d, 1H, J = 6.6 Hz), 3.32 (t, 2H, J = 6.6 Hz), 3.29−3.16 (dd buried under t, 4H), 3.10−2.92 (m, 6H), 2.65−2.46 (m, 4H), 2.25−211 (m, 4H), 1.88 (quintet, 2H, J = 12.8 Hz), 1.70−1.52 (m, 4H); ^{13}C NMR (D_2O) δ 174.8, 172.8, 172.3, 171.8, 171.2, 162.9 (q, J_{CO} = 34.8 Hz), 113.4 (q, J_{CF} = 293.0 Hz), 52.9, 52.5, 52.3, 51.3, 47.2, 44.5, 38.6, 38.1, 37.6, 35.6, 30.8, 25.5; MALDI-MS calcd for $C_{27}H_{48}N_9O_{10}S_2^+$ 722.2989, found 722.2960; 96.53% purity in HPLC, t_R = 10.79 min (λ = 214 nm).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00300.

Copies of ^{1}H and ^{13}C NMR spectra for compounds **2**, **5**, **7**, **8**, **9**, and **10** and MALDI-MS and MS/MS spectra for compounds **2**, **5**, **7**, **8**, **9**, and **10** (PDF)

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The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to the memory of Professor Paul Cordopatis.

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