Synthesis of Cysteine and Homoglutathione Conjugates of Crop Protection Agents Containing Electrophilic Centers

Gary A. Roth[1]

DowElanco Discovery Process Research, Midland, Michigan 48640

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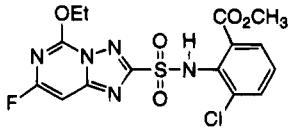
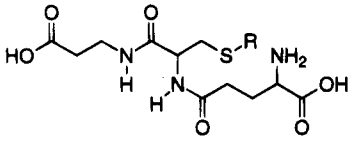
It has been well documented that pesticides containing electrophilic centers can be metabolized in legumes *via* conjugation with the tripeptides glutathione and homoglutathione (γ-(*R*)-glu-(*R*)-cys-β-ala).[2] In some instances, cysteine conjugates also appear as a result of peptide cleavage. Soybean metabolism studies on the sulfonamide herbicide DE-565[3] (**1**) have led to the isolation of several polar metabolites. In view of the many reports of homoglutathione conjugation in such species and as a part of our continued interest in the synthesis/identification of pesticide metabolites,[4] we initiated studies directed at a practical synthesis of such conjugates.[5] Reported herein is a general method for the preparation of homoglutathione and cysteine conjugates of electrophiles and specifically the synthesis of the homoglutathione and cysteine conjugates of DE-565 (**1**).



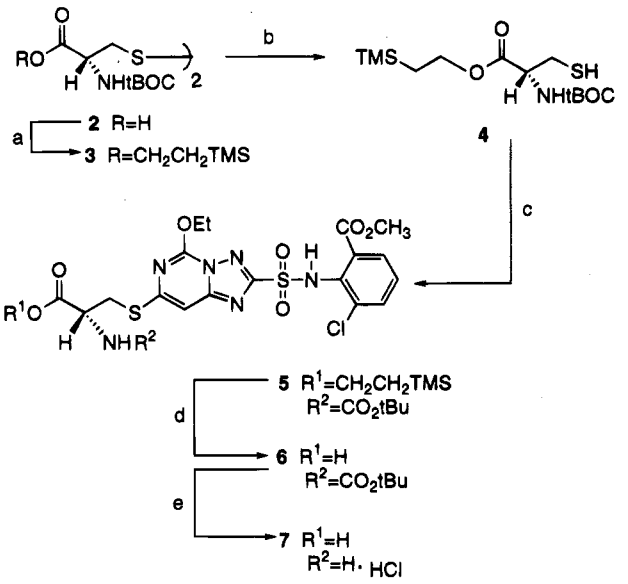
DE-565

1



Homoglutathione conjugate of R

Scheme 1[a]



[a] Key: (a) CH_3CN, DMF, pyr, $TMSCH_2CH_2OH$, DCC; (b) Zn, HOAc; (c) DMSO, K_2CO_3, 1; (d) TBAF, THF; (e) HCl, dioxane.

The synthetic studies began with the synthesis of an appropriately protected cystine as depicted in Scheme 1. Formation of the 2-(trimethylsilyl)ethyl ester[6] (**3**) of *N,N'*-bis-*t*-BOC-(*R*)-cystine (**2**) followed by zinc/acetic acid reduction of the disulfide provided thiol **4**. Reaction of **4** with **1** in the presence of potassium carbonate in DMSO afforded an excellent yield of the protected cysteine conjugate **5** following flash chromatography.[7] A convenient method of deprotection involved treatment of **5** with tetrabutylammonium fluoride in THF followed by dilution with 1 M hydrochloric acid and trituration, thus affording carboxylic acid **6**. Exposure of **6** to 4 M hydrogen chloride in dioxane provided the hydrochloride salt **7** as a white crystalline solid.[8] The implementation of the trimethylsilyl ethyl and *N-t*-BOC protecting groups served two purposes: (1) deprotection was convenient, and (2) these lipophilic groups made the physical properties (i.e., solubility) of **5** very desirable for purification and characterization purposes.

With the completion of an efficient synthesis of the cysteine conjugate of **1**, we focused our attention on the application of a similar strategy for the preparation of the homogutathione conjugate. Formation of the 2-(trimethylsilyl)ethyl ester of *N-t*-Boc-β-alanine (**8**, Scheme 2) yielded **9**, which upon removal of the *N-t*-Boc protecting group provided the amine hydrochloride **10**. Coupling of **10** with pentafluorophenyl (PFP) ester **11** afforded disulfide **12**.

The requisite glutamate **16** was prepared as shown in Scheme 3. Commericially available *N-t*-Boc-(*R*)-gutamic acid γ-benzyl ester (**13**) was converted to the corresponding 2-(trimethylsilyl)ethyl ester **14** under standard conditions. Removal of the benzyl protecting group gave **15**,

(1) Present address: Dow Chemical Co., Agricultural Products Process Research, 1710 Building, Midland, MI 48640.

(2) (a) Lamoureux, G. L.; Rusness, D. G. *Pestic. Biochem. Physiol.* **1989**, *34*, 187. (b) Brown, H. M.; Neighbors, S. M. *Pestic. Biochem. Physiol.* **1987**, *29*, 112. (c) Breaux, E. J. *Weed Sci.* **1987**, *35*, 463. (d) Breaux, E. J.; Patanella, J. E.; Sanders, E. F. *J. Agric. Food Chem.* **1987**, *35*, 474. (e) Breaux, E. J. *J. Agric. Food Chem.* **1986**, *34*, 884. (f) Frear, D. S.; Swanson, J. R.; Mansager, E. R. *Pestic. Biochem. Physiol.* **1985**, *23*, 56. (g) Frear, D. S.; Swanson, H. R.; Mansager, E. R. *Pestic. Biochem. Physiol.* **1983**, *20*, 299. (h) Lamoureux, G. L.; Stafford, L. E.; Tanaka, F. S. *J. Agric. Food Chem.* **1971**, *19*, 346 and references cited therein.

(3) For herbicides representative of this sulfonamide class see the following patents: (a) Van Heertum, J. C.; Gerwick, B. C., III; Kleschick, W. A. EP 343 752; *Chem. Abstr.* **1990**, *112*, 198409. (b) Van Heertum, J. C.; Gerwick, B. C., III; Kleschick, W. A.; Johnson, T. C. US 5,163,995; *Chem. Abstr.* **1993**, *118*, 213106. (c) Costales, M. J.; Van Heertum, J. C.; Kleschick, W. A.; Ehr, R. J.: Ray, P. G. US 5,201,938; *Chem. Abstr.* **1993**, *119*, 180806.

(4) Roth, G. A.; McClymont, E. L. *J. Agric. Food Chem.* **1991**, *38*, 612. (b) Roth, G. A.; McClymont, E. L. *Synth. Commun.* **1992**, *22*, 411.

(5) Previous syntheses of the naturally occurring tripeptides glutathione and homoglutathione have been reported; for example, see: (a) Camble, R.; Purkayastha, R.; Young, G. T. *J Chem. Soc. C* **1968**, 1219 and references cited therein. (b) Kasai, T.; Yoshinari, S.; Sakamura, S. *Phytochemistry* **1986**, *25*, 679. The *in vitro* preparation of the tripeptide conjugates have been reported, ref 1; however, we are unaware of a convenient totally synthetic route, with accompanying physical and spectral characterization, to homoglutathione conjugates.

(6) Sieber, P. *Helv. Chim. Acta* **1977**, *60*, 2711.

(7) Still, W; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978**, *43*, 2923.

(8) Although the stereochemical integrity of the asymmetric center was not experimentally proven, other couplings under the described reaction conditions did not induce racemization. See ref 10.

(9) For a general discussion see: Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984.

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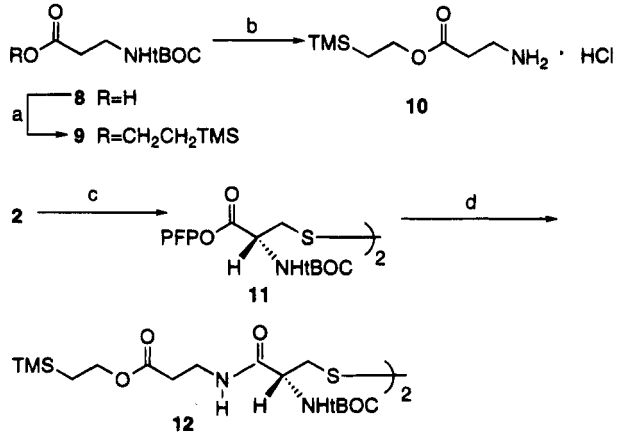
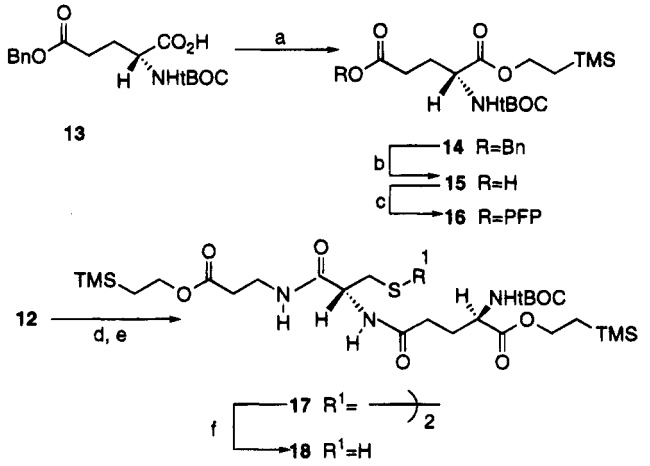
Scheme 2[a]



[a] Key: (a) CH_3CN, $TMSCH_2CH_2OH$, pyr, DCC; (b) HCl, dioxane; (c) pentafluorophenol, DCC, THF; (d) CH_2Cl_2, NEt_3, **10**.

Scheme 3[a]



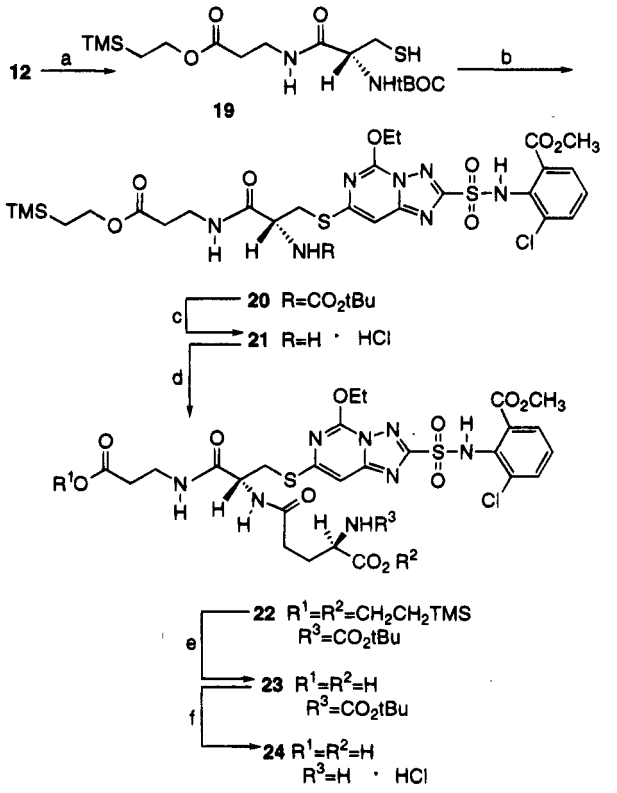
[a] Key: (a) CH_3CN, $TMSCH_2CH_2OH$, pyr, DCC; (b) $Pd(OH)_2/C$, EtOH, cyclohexene; (c) pentafluorophenol, DCC, THF; (d) HCl, dioxane; (e) CH_3CN, NEt_3, **16**; (f) Zn, HOAc.

which was activated as the PFP ester **16**. Removal of the *N*-*t*-Boc group of **12** and subsequent coupling with activated ester **16** yielded the desired disulfide **17**. Reductive cleavage provided the fully protected homoglutathione thiol **18**.

All of our attempts at coupling thiol **18** with DE-565 (**1**) met with failure, most likely due to the lack of electrophilic reactivity of **1** combined with competing intramolecular acyl transfer reactions of **18**. However, this problem was circumvented by connecting an appropriate dipeptide with **1**, followed by installation of the final glutamyl peptide residue late in the synthesis. Toward this end, reductive cleavage of **12** (Scheme 4) furnished the desired thiol **19** which upon treatment with **1**, potassium carbonate, and DMSO yielded the dipeptide conjugate **20**. Deblocking of the *N*-*t*-Boc nitrogen and reaction with activated ester **16** afforded the fully protected homoglutathione conjugate of DE-565 (**22**).[10] Concomitant removal of the silylethyl protecting groups provided diacid **23**, which when exposed to anhydrous hydrogen chloride in dioxane furnished the desired homoglutathione conjugate **24**.

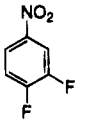
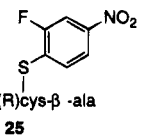
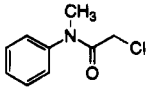
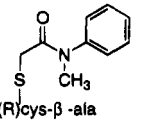
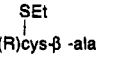
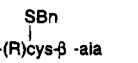
(10) Other diasteromers were not detected (^1H NMR, HPLC, TLC); thus, we concluded that racemization during dipeptide (**19**) coupling with **1** had not occurred.

Scheme 4[a]



[a] Key: (a) Zn, HOAc; (b) K_2CO_3, DMSO, **1**; (c) HCl, dioxane; (d) CH_3CN, NEt_3, **16**; (e) TBAF, THF/HCl; (f) HCl, dioxane.

Table 1. Synthesis of Protected Homoglutathione Conjugates of Various Electrophiles

electrophile	product [a]	yield (%)
	 γ-(R)glu-(R)cys-β -ala **25**	85
	 γ-(R)glu-(R)cys-β -ala **26**	78
EtI	 γ-(R)glu-(R)cys-β -ala **27**	86
BnBr	 γ-(R)glu-(R)cys-β -ala **28**	91

[a] The amino acids glu and ala contain protecting groups as in **18**.

Although we were unable to directly couple tripeptide thiol **18** with **1** presumably due to the unreactive nature of the electrophile, the scope of S-alkylation of **18** was worth exploring as a general solution to the synthesis of other homoglutathione conjugates. Table 1 presents the results of alkylation of **18** with four representative electrophiles. In each case, mild reaction conditions and short reaction times produced high yields of S-alkylated products.

In summary, a new method for the synthesis of homoglutathione and cysteine conjugates of electrophiles

has been developed. The synthetic sequence reported here should allow for the preparation of a variety of biologically significant conjugates. This procedure has the distinct advantage of allowing for isolation, purification, and characterization of conjugates of polar electrophiles since all of the peptide functional groups are initially protected with lipophilic moieties.

Experimental Section

Reagents and solvents were reagent grade and used as received. Thin layer chromatography (TLC) was routinely used to monitor reactions. TLC was conducted on precoated Analtech silica GF/UV_{254} plates (250 μm layer). Column chromatography was conducted using silica gel 60 (230–400 mesh, E. Merck Inc.). Analytical reversed-phase HPLC analyses were conducted on a Waters Novapak C18 colunm using gradient elution from 100% water (0.5% HOAc) to 100% CH_3CN (0.5% HOAc). Detection was accomplished using a photodiode array detector. Reported melting points are uncorrected. Solvents were evaporated *in vacuo* (25–45 mmHg). Low field NMR spectra were determined at 90 MHz. High field ^1H NMR and ^{13}C NMR spectra were determined at 400 MHz in the solvent noted. Infrared spectra were recorded either as a neat film or KBr pellet. Chemical ionization (methane) mass spectra were obtained in the positive ion mode. FAB mass spectra were recorded in a thioglycerol: dithiothreitol:dithioerythritol (300/5/1, magic bullet) matrix. The FAB HRMS was obtained at 10 000 resolving power using a glycerol matrix with PEG-600 internal mass reference. Elemental analyses were carried out by Oneida Research Services, Whitesboro, NY.

N,N'-Bis-*t*-Boc-(*R*)-cystine, Bis-2-(trimethylsilyl)ethyl Ester (3). To a stirred, cooled (0 °C) solution of **2** (8.5 g, 19.3 mmol) in CH_3CN (100 mL), DMF (20 mL) and pyridine (6.24 mL, 77.2 mmol) and 2-(trimethylsilyl)ethanol (6.64 mL, 46.3 mmol) was added DCC (8.76 g, 42.5 mmol) and the mixture was stirred at 0 °C for 1 h, warmed to rt and stirred for 14h. Solid citric acid (0.6 g) was added and stirring continued for 0.5h. The solids were filtered and washed well with CH_3CN. Removal of the solvents afforded an oil which was purified by flash chromatography on silica gel (hexane/EtOAc, 7/1) providing **3** as a thick colorless oil which slowly crystallized on standing (10.47 g, 85%): mp 45–47 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ 0 (s, 9H), 1.0 (m, 4H), 1.4 (s, 18H), 3.15 (d, J = 5 Hz, 4H), 4.2 (m, 4H), 4.5 (m, 2H), 5.3 (bd, 2H); MS (CH_4 CI) 641 (16), 485 (62), 457 (75), 429 (100); IR (film) 3400, 3000, 2980, 1720 (b). Anal. Calcd for $C_{26}H_{52}N_2O_8S_2Si_2$: C, 48.72; H, 8.18; N, 4.37. Found: C, 48.45; H, 8.01; N, 4.27.

***N*-*t*-Boc-(*R*)-cysteine, 2-(Trimethylsilyl)ethyl Ester (4).** Disulfide **3** (3.45 g, 5.39 mmol) was dissolved in HOAc (35 mL) and the solution warmed to 55-60 °C. Zinc dust (2.0 g, 31 g atoms) was added in portions with good stirring over a 4 h period. After the addition was complete the mixture was stirred at 60 °C for an additional 1.5h. The warm mixture was filtered through Celite and the filter pad washed well with EtOAc. The solvents were removed and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 10/1) providing **4** as a clear colorless oil (3.15 g, 91%): ^1H NMR ($CDCl_3$, 90 MHz) δ 0.0 (s,9H), 1.0 (m, 2H), 1.3 (s, 9H), 1.35 (d, 1H), 2.9 (dd, J = 5,8 Hz, 2H), 4.2 (m, 2H), 4.5 (m, 1H), 5.35 (bd, 1H); IR (film) 3430, 3380 (b), 2580, 1720; MS (CH_4 CI) 322 (30), 238 (100). Anal. Calcd for $C_{13}H_{27}NO_4SSi$: C, 48.57; H, 8.46; N, 4.36. Found: C, 48.29; H, 8.32; N, 4.12.

***N*-*t*-Boc-(*R*)-cys-OTMSE Conjugate (5).** To a stirred solution of thiol **4** (3.0 g, 9.3 mmol) and **1** (2.68 g, 6.23 mmol) in DMSO (40 mL) was added K_2CO_3 (2.67 g, 19.3 mmol) and the mixture stirred at rt for 4 h. The reaction was poured into ice–water (200 mL) and the aqueous mixture taken to pH 2 by the addition of concd HCl. The resulting precipitate was collected and washed well with water. The solid was dissolved in $CHCl_3$ (ca. 80 mL) and the residual water separated. The organics were dried (Na_2SO_4) and then filtered through Celite. Silica gel (ca. 25 g) was added and the solvents were removed, leaving the crude product preadsorbed on silica gel. Flash chromatography (hexane/acetone, 2/1) provided **5** as a white amorphous solid (4.1 g, 90%): ^1H NMR ($CDCl_3$, 400 MHz) δ 0.0 (s, 9H), 0.98 (m, 2H), 1.36 (s, 9H), 1.54 (t, J = 7.1 Hz, 3H), 3.43 (dd, J = 6, 13 Hz, 1H), 3.78 (s, 3H), 3.86 (dd, J = 5, 13 Hz, 1H), 4.22 (m, 2H), 4.64 (m, 1H), 4.80 (two overlapping quartets, J = 7 Hz, 2H), 5.29 (bd, J = 7.6 Hz, 1H), 7.15 (s, 1H), 7.24 (m, 1H), 7.56 (dd, J = 1.5, 7 Hz, 1H), 7.79 (dd, J = 1.5, 9 Hz, 1H); ^{13}C NMR −1.6, 14.1, 17.5, 28.2, 33.4, 52.8, 53.5, 64.5, 67.4, 80.3, 100.6, 127.6, 127.9, 129.4, 133.1, 133.7, 134.8, 148.2, 155.0, 155.3, 157.1, 165.2, 166.7, 170.5; IR (KBr) 3400 (b), 3250 (b), 2970, 1715; MS (CH_4 CI) 731 (8), 444 (64), 160 (100). Anal. Calcd for $C_{28}H_{39}ClN_6O_9S_2Si$: C, 45.99; H, 5.38; N, 11.49. Found: C, 45.78; H, 5.22; N, 11.36.

***N*-*t*-Boc-(*R*)-Cys Conjugate 6.** Protected cystine conjugate **5** (650 mg, 0.89 mL) was treated with 1 M tetrabutylammonium fluoride/THF (10 mL) and the solution allowed to stand at rt for 1 h. Dilution with 1 M HCl (70 mL) caused a gummy solid to form, which upon trituration solidified. The solid was collected and washed well with water. Drying to a constant weight afforded **6** as an off-white solid (510 mg, 91%): mp 135–137 °C dec; ^1H NMR (acetone-d_6, 90 MHz) 1.4 (s, 9H), 1.6 (t, J = 7 Hz, 3H), 3.75 (s, 3H), 3.9 (m, 2H), 4.65 (m, 1H), 4.85 (q, J = 7 Hz, 2H), 6.5 (bd, J = 9 Hz, 1H), 7.35 (s, 1H), 7.5–8.0 (m, 3H); MS (FAB) 631 (75), 242 (85), 184 (100); IR (KBr) 3390, 3270, 2990, 1720 (b).

(*R*)-Cys Conjugate 7. Carboxylic acid **6** (430 mg, 0.68 mmol) was treated with 4 M HCl in dioxane and the solution stirred under a drying tube for 20 min. The sovent was removed *in vacuo* and the residue triturated with ether. The solid was collected and washed well with ether (398 mg crude). The crude material was recrystallized from water (15 mL, hot filtration) to provide **7** (152 mg, 39%) as a fluffy white solid. Reversed-phase HPLC (UV at 254 nm) showed the product to be 97+% pure: mp 138–148 °C dec; ^1H NMR (methanol-d_4, 400 MHz) 1.54 (t, J = 7.1 Hz, 3H), 3.33 (dd, J = 9.7, 14.8 Hz, 1H), 3.75 (s, 3H), 3.94 (dd, J = 3.6, 9.7 Hz, 1H), 4.19 (dd, J = 3.6, 14.8 Hz, 1H), 4.86 (overlapping q's of OCH_2CH_3 obscured by the methanol solvent), 7.30 (s, 1H), 7.39 (m, 1H), 7.61 (dd, J = 1.5, 8.8 Hz, 1H) 7.73 (dd, J = 1.5, 7.8 Hz, 1H); MS (FAB) 533, 531; IR (KBr) 3400 (b), 3050 (b), 1710; Anal. Calcd for $C_{18}H_{19}Cl_2N_6O_7S_2$: C, 38.17; H, 3.38; N, 14.84. Found: C, 37.94; H, 3.63; N, 14.77.

***N*-*t*-Boc-β-ala-OTMSE (9).** To a stirred cooled (0 °C) solution of **8** (10 g, 52.9 mmol) in CH_3CN (100 mL), pyridine (8.6 mL, 106 mmol), and 2-(trimethylsilyl)ethanol (8.0 mL, 56 mmol) was added DCC (11.56 g, 56 mmol) and the mixture stirred at 0 °C for 1 h, warmed to rt, and stirred overnight. The urea was filtered and the filter cake washed with CH_3CN. The solvent was removed *in vacuo* and the residue filtered through a plug of silica gel (ca. 250 g) using CH_2Cl_2. After removal of the solvent, the product was obtained as a clear colorless liquid (12.8 g, 84%): ^1H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.0 (m, 2H), 1.4 (s, 9H), 2.5 (t, J = 7 Hz, 2H), 3.35 (m,2H), 4.2 (m, 2H), 5.1 (bs, 1H); IR (film) 3400 (b), 2990, 1720; MS (FAB) 290 (100), 206 (75), 162 (90).

Bis-*N*-*t*-Boc-(*R*)-cystine-bis-OPFP (11). To a stirred, cooled (0 °C) solution of **2** (10 g, 22.7 mmol) and pentafluorophenol (9.2 g, 50 mmol) in THF (60 mL) and EtOAc (100 mL) was added DCC (10.4 g, 50 mmol) and the mixture stirred at 0 °C for 0.5h, warmed to rt, and stirred for 1 h. The urea was filtered and the filter cake washed well with EtOAc. The solvent was removed *in vacuo* and the crude product recrystallized from CH_2Cl_2/hexane. The mother liquor was concentrated and a second crop obtained, affording a total of 16.4 g (93%) of **11** as a white crystalline solid: mp 165–166 °C; ^1H NMR (DMSO-d_6, 90 MHz) δ 1.45 (s, 9H), 3.3 (m, 2H), 4.8 (m, 1H), 7.3 (bd, J = 8 Hz, 1H); IR (KBr) 3400, 1810, 1790, 1695; MS (FAB) 773 (100). Anal. Calcd for $C_{28}H_{26}F_{10}N_2O_8S_2$: C, 43.53; H, 3.39; N, 3.63. Found: C, 43.43; H, 3.35; H, 3.59.

Bis-TMSEO-β-ala-bis-*N*-*t*-Boc-(*R*)-cystine (12). Ester **9** (7.0 g, 24.2 mmol) was treated with 4 M HCl in dioxane (20 mL) and the solution stirred at or below 30 °C under a drying tube for 1.25 h. The solvent was removed *in vacuo*, leaving **10** as a thick yellow oil which was not characterized. The oil was dissolved in CH_2Cl_2 (300 mL) and **11** was added, followed by addition of NEt_3 (3.4 mL, 24.2 mmol). The mixture was stirred at rt for 1.5 h and then washed with 1/2 saturated Na_2CO_3 (2 × 200 mL) and water (2 × 200 mL). The organics were dried (Na_2SO_4), and the solvent was removed *in vacuo*, affording a white solid. The solid was dissolved in CH_2Cl_2 and flash chromatographed on silica gel (5/4, hexane/EtOAc), providing **12** as a white solid (7.2 g). Recrystallization from hexane (two crops)

gave **12** as a fluffy white solid (5.8 g, 61%): mp 105–107 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.0 (s, 9H), 1.0 (m, 2H), 1.4 (s, 9H), 2.5 (t, J = 7 Hz, 2H), 3.0 (d, J = 7 Hz, 2H), 3.5 (m, 2H), 4.2 (m, 2H), 4.7 (m, 1H), 5.6 (d, J = 9 Hz, 1H), 7.7 (bm, 1H); IR (KBr) 3360 (b), 1740, 1690; MS (FAB) 783 (60), 499 (30), 73 (100). Anal. Calcd for $C_{32}H_{62}N_4O_{10}S_2Si_2$: C, 49.08; H, 7.98; N, 7.15. Found: C, 49.06; H, 7.75; N, 7.07.

γ-OBn-α-OTMSE-*N*-*t*-Boc-(*R*)-glu (14). The procedure described for the preparation of **9** was utilized. After flash chromatography, **14** was obtained as a clear colorless oil (6.3 g, 97%): ^{1}H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.0 (m, 2H), 1.46 (s, 9H), 1.6–2.2 (m, 2H), 2.4 (m, 2H), 4.2 (m, 2H), 5.1 (s and overlapping bd, 3H), 7.3 (s, 5H); MS (FAB) 438 (100), 310 (80), 192 (95).

γ-OPFP-α-OTMSE-*N*-Boc-(*R*)-glu (16). A solution of benzyl ester **14** (4.1 g, 9.38 mmol) in EtOH (20 mL) and cyclohexene (4 mL) was treated with $Pd(OH)_2$/C (200 mg, 20% Pd) and the mixture heated to reflux and held there for 1.25 h. The warm reaction mixture was filtered through a Celite pad and the pad washed with EtOAc. The solvents were removed *in vacuo*, providing **15** as a thick colorless oil. The crude carboxylic acid was only characterized by ^{1}H NMR: ^{1}H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.0 (m, 2H), 1.4 (s, 9H), 1.7–2.2 (m, 2H), 2.4 (m, 2H), 4.2 (m, 3H), 5.2 (bs, 1H), 10.2 (bs, 1H).

Carboxylic acid **15** was converted to **16** using the procedure described for the preparation of **11**. Purification *via* flash chromatography provided **16** as a colorless oil which crystallized on standing (3.2 g, 66%): mp 52–54 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.0 (m, 2H), 1.4 (s, 9H), 1.8–2.5 (m, 2H), 2.75 (m, 2H), 4.25 (m, 3H), 5.2 (bd, J = 8 Hz, 1H); IR (KBr) 3400, 2990, 1780, 1730; MS (CH_4 CI) 514 (10), 430 (100), 386 (80). Anal. Calcd for $C_{21}H_{28}F_5NO_6Si$: C, 49.12; H, 5.50; N, 2.73. Found: C, 49.22; H, 5.49; N, 2.80.

Bis-TMSEO-β-ala-bis-*N*-(α-TMSEO-*N*-*t*-Boc-(*R*)-glu)-(*R*)-cystine (17). Disulfide **12** (1.84 g, 2.35 mmol) was treated with 4 M HCl in dioxane (6 mL) and the solution stirred at rt for 40 min. The solvent was removed *in vacuo*, providing the bis hydrochloride salt as a white solid. The salt was suspended in CH_3CN (20 mL) and treated with NEt_3 (657 uL, 4.7 mmol). After the mixture was stirred for ca. 20 min, pentafluorophenyl ester **16** (2.4 g, 4.7 mmol) was added and the mixture stirred for 16 h. The solvent was removed and the residue dissolved in EtOAc (100 mL). The organics were successively washed with 1/2 saturated brine (100 mL), 1/2 saturated Na_2CO_3 (2 × 100 mL), and 1/2 saturated brine (100 mL). After drying (Na_2SO_4), the solvent was removed affording a pale yellow foam. Purification by flash chromatography on silica gel (2/1 hexane/EtOAc and then 1/1 hexane/EtOAc) provided **17** as a white crystalline solid (2.0 g, 67%): mp 119–120 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.36 (s, 9H), 0.48 (s, 9H), 1.00 (m, 4H), 1.44 (s, 9H), 1.91 (m, 1H), 2.21 (m, 1H), 2.38 (m, 1H), 2.55 (m, 2H), 2.95 (m, 2H), 3.56 (m, 2H), 4.22 (m, 4H), 4.34 (m, 1H), 5.24 (m, 1H), 5.33 (d, J = 8.2 Hz, 1H), 6.80 (bd, J = 8.2 Hz, 1H), 8.16 (m, 1H); ^{13}C NMR −1.5, −1.3, 17.4, 17.5, 28.4, 28.9, 32.5, 34.2, 35.4, 45.8, 53.0, 53.5, 62.9, 64.0, 80.1, 155.7, 170.1, 171.7, 172.3, 172.4; IR (KBr) 3300, 1735, 1695; MS (FAB) 1242 (5), 73 (100). Anal. Calcd for $C_{52}H_{100}N_6O_{16}S_2Si_4$: C, 50.29; H, 8.12; N, 6.77. Found: C, 50.14; H, 8.01; N, 6.71.

TMSEO-β-ala-*N*-(α-TMSEO-*N*-*t*-Boc-(*R*)-glu)-(*R*)-cys-SH (18). The protected homoglutathione **18** was prepared by the procedure described for the synthesis of **4**. Purification by flash chromatography (hexane/EtOAc, 2/1) provided **18** as a white wax (1.27 g, 84%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.01 (s, 9H), 0.03 (s, 9H), 1.00 (m, 4H), 1.38 (d, 1H), 1.85 (m, 1H), 2.22 (m, 1H), 2.34 (m, 2H), 2.50 (t, J = 6.8 Hz), 3.15–3.58 (m, 4H), 4.23 (m, 4H), 4.38 (m, 1H), 4.52 (q, J = 7.3 Hz, 1H), 5.32 (bd, 1H), 6.75 (m, 1H), 7.01 (m, 1H); MS (FAB) 622 (10), 522 (40), 73 (100). Anal. Calcd for $C_{26}H_{51}N_3O_8SSi_2$: C, 50.21; H, 8.27; N, 6.76. Found: C, 50.29; H, 8.15; N, 6.48.

TMSEO-β-ala-*N*-*t*-Boc-(*R*)-cys-SH (19). Thiol **19** was prepared by the procedure described for the synthesis of **4**. Purification by flash chromatography (2/1, hexane/EtOAc) provided **19** as a colorless oil (2.3 g, 92%): ^{1}H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.4 (s, 9H), 2.5 (t, J = 7 Hz, 2H), 2.9 (m, 2H), 3.5 (q, J = 7 Hz, 2H), 4.2 (m, 3H), 5.55 (bd, J = 8 Hz, 1H), 6.95 (m, 1H); IR (film) 3325 (b), 1720; MS (CH_4 CI) 393 (40), 309 (100).

TMSEO-β-ala-*N*-*t*-Boc-(*R*)-cys Conjugate 20. Thiol **19** (2.2 g, 5.6 mmol) and DE-565 (1,1.9 g, 4.4 mmol) were dissolved in DMSO (20 mL) and treated with K_2CO_3 (2.8 g, 20 mmol). The mixture was stirred at rt for 4.5 h and then poured into ice cold 0.5 M HCl (500 mL). The resulting precipitate was collected, washed with water, and allowed to air dry overnight. The solid was dissolved in acetone (40 mL) and treated with reversed-phase column packing material (C18, 40 um, 20 g). The solvent was removed *in vacuo*, leaving the crude product preadsorbed on the packing material. Reversed-phase flash chromatography provided **20** as a white amorphous solid (2.15 g, 48%): ^{1}H NMR ($CDCl_3$, 90 MHz) 0.0 (s, 9H), 1.0 (m, 2H), 1.4 (s, 9H), 1.5 (t, J = 7 Hz, 3H), 2.5 (bt, J = 6 Hz, 2H), 3.55 (5, 4H), 3.8 (s, 3H), 4.2 (m, 2H), 4.5 (bq, J = 8 Hz, 1H), 4.8 (q, J = 7 Hz, 2H), 5.6 (bd, J = 8 Hz, 1H), 7.1 (bt, J = 6 Hz, 1H), 7.1–7.8 (m, 4H); MS (FAB) 802 (20), 718 (25), 73 (100). Anal. Calcd for $C_{31}H_{44}ClN_7O_{10}S_2Si$: C, 46.40; H, 5.53; N, 12.22. Found: C, 45.96; H, 5.52; N, 11.83.

TMSEO-β-ala-*N*-(α-TMSEO-*N*-*t*-Boc-(*R*)-glu)-(*R*)-cys Conjugate 22. Dipeptide conjugate **20** (961 mg, 1.2 mmol) was treated with 4 M HCl in dioxane (3 mL) and the mixture stirred at rt for 40 min. The solvent was removed *in vacuo*, affording **21** as a white powder. The powder was dissolved in CH_3CN (10 mL) and treated with NEt_3 (168 μL, 1.2 mmol). Pentafluorophenyl ester **16** (0.8 g, 1.56 mmol) was added and the mixture stirred at rt for 19 h. The CH_3CN was removed and the residue dissolved in EtOAc (30 mL). Successive washing with saturated $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL), drying (Na_2SO_4/$MgSO_4$), and solvent removal yielded a pale yellow foam. Flash chromatography on silica gel (2/1, hexane/acetone) provided **22** as a white amorphous solid (795 mg, 65%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.0 (s, 18 H), 0.99 (m, 2H), 1.56 (t, J = 7.1 Hz, 3H), 1.85 (m, 1H), 2.19 (m, 1H), 2.34 (t, J = 7.0 Hz, 2H), 2.50 (t, J = 6.2 Hz, 2H), 3.52 (m, 3H), 3.73 (dd, J = 7, 14 Hz, 1H), 3.82 (s, 3H), 4.15 (m, 2H), 4.24 (m, 2H), 4.32 (m, 1H), 4.72 (q, J = 7 Hz, 1H), 4.82 (q, J = 7.1 Hz, 2H), 5.31 (bd, J = 7.8 Hz, 1H), 6.98 (t, J = 6 Hz, 1H), 7.04 (bd, J = 6 Hz, 1H), 7.19 (s, 1H), 7.26 (m, 1H), 7.57 (dd, J = 1.5, 8.3 Hz, 1H), 7.81 (dd, J = 1.5, 8 Hz, 1H), 9.00 (bs, 1H); ^{13}C NMR −1.5, 14.2, 17.3, 17.5, 28.3, 29.3, 32.1, 32.3, 33.9, 35.2, 52.8, 53.0, 63.1, 64.2, 67.5, 80.3, 100.5, 127.7, 128.3, 129.4, 133.2, 133.6, 134.6, 148.2, 155.3, 156.0, 157.1, 165.1, 166.6, 169.5, 172.2, 172.2; IR (KBr) 3350(b), 1730; MS (FAB) 931 (20), 73 (100). Anal. Calcd for $C_{41}H_{63}ClN_8O_{13}S_2Si_2$: C, 47.73; H, 6.15; N, 10.86. Found: C, 47.59; H, 6.06; N, 10.74.

HO-β-ala-*N*-(α-HO-*N*-*t*-Boc-(*R*)-glu)-(*R*)-cys Conjugate 23. The procedure described for the preparation of **6** was utilized. The crude material was preadsorbed on silica gel and then purified by flash chromatography (13/1/1, $CHCl_3$/CH_3OH/HOAc), affording **23** as an off-white amorphous solid (258 mg, 72%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 1.40 (s, 9H), 1.51 (t, J = 7.1 Hz, 3H), 1.95 (m, 1H), 2.15 (m, 1H), 2.40 (m, 2H), 2.52 (t, J = 6.6 Hz, 2H), 3.49 (m, 3H), 3.67 (dd, J = 6.6, 14 Hz, 1H), 3.71 (s, 3H), 4.19 (m, 1H), 4.78 (m, 1H), 4.84 (q, J = 7.1 Hz, 2H), 7.31 (s, 1H), 7.45 (m, 1H), 7.58 (bt, J = 13 Hz, 1H), 7.65 (bm, 1H), 7.68 (dd, J = 3, 8 Hz, 1H), 7.77 (dd, J = 1.5, 7.7 Hz, 1H); IR (KBr) 3400–2900 (b), 1715, 1610; MS (FAB) 831 (20), 217 (80), 91 (100).

Homoglutathione Conjugate 24. The procedure described for the preparation of **7** was utilized starting with **23** (178 mg, 0.21 mmol). After drying, **24** was obtained as an off-white amorphous solid (163 mg, 0.21 mmol): reversed-phase HPLC indicated the material was 94+% pure and contained a single more polar impurity; ^{1}H NMR (methanol-d_4, 400 MHz) δ 1.53 (t, J = 7.0 Hz, 3H), 2.19 (m, 2H), 2.52 (m, 4H), 3.42 (m, 4H), 3.74 (s, 3H), 4.02 (bm, 1H), 4.70 (t, J = 7 Hz, 1H), 4.81 (q, J = 7.0 Hz, 2H), 7.25 (s, 1H), 7.40 (apparent t, J = 7.9 Hz, 1H), 7.61 (dd, J = 1.3, 8.0 Hz, 1H), 7.73 (dd, J = 1.3, 7.7 Hz, 1H); ^{13}C NMR 15.4, 28.1, 33.3, 34.2, 35.4, 37.5, 54.1, 54.6, 55.2, 67.9, 101.9, 131.5, 131.5, 133.8, 135.3, 135.6, 137.3, 150.6, 157.8, 160.4, 167.1, 168.6, 172.5, 172.9, 175.3, 176.1; a DEPT[11] experiment allowed assignment of the carbon NMR; IR (KBr) 3700–2400 (b), 1715 (b); HRMS (FAB/ glycerol) calcd for $C_{26}H_{32}N_8O_{11}S_2Cl$ 731.1321, found 731.1328.

General Procedure for the Reaction of 18 with Electrophiles. Thiol **18** (0.17 mmol) was dissolved in CH_3CN (2 mL) and treated with the electrophile (1.1 equiv, Table 1) and

(11) For a discussion of the DEPT method see: Breitmaier, E.; Voilter, W. *Carbon-13 NMR Spectroscopy*; VHC Publishers: Weinheim, Federal Republic of Germany, 1987; pp 78–83.

K_2CO_3 (2.0–2.5 equiv). The mixture was stirred at rt until the reaction was determined to be complete as judged by TLC (2.0–5.5 h). The solvent was removed and the residue slurried in CH_2Cl_2 and flashed chromatographed on silica gel (hexane/EtOAc).

25: pale yellow amorphous solid; mp 70–74 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.02 (s, 9H), 0.03 (s, 9H), 0.98 (m, 4H), 1.41 (s, 9H), 1.85 (m, 1H), 2.20 (m, 1H), 2.32 (m, 2H), 2.47 (t, J = 6 Hz, 2H), 3.32–3.58 (m, 4H), 4.20 (m, 4H), 4.33 (m, 1H), 4.49 (q, J = 7.0 Hz, 1H), 5.28 (bd, 1H), 6.77 (m, 1H), 7.01 (m, 1H), 7.71 (m, 1H), 7.87 (dd, J = 2.3, 9.5 Hz, 1H), 8.03 (dd, J = 2.2, 10.7 Hz, 1H); IR (KBr) 3305 (b), 1730 (b); MS (FAB) 761 (20), 661 (65), 73 (100). Anal. Calcd for $C_{32}H_{53}FN_4O_{10}SSi_2$: C, 50.50; H, 7.02; N, 7.36. Found: C, 50.61; H, 7.06; N, 7.26.

26: colorless glass; ^{1}H NMR ($CDCl_3$, 400 MHz) 0.041 (s, 9H), 0.043 (s, 9H), 1.00 (m, 4H), 1.43 (s, 9H), 1.97 (m, 1H), 2.20 (m, 1H), 2.36 (t, J = 7.3 Hz, 2H), 2.57 (t, J = 6.5 Hz, 2H), 2.69 (dd, J = 7.2, 14.3 Hz, 1H), 3.02 (dd, J = 5.2, 14.3 Hz, 1H), 3.15, 3.24 (ABq, J = 15 Hz, 2H), 3.32 (s, 3H), 3.57 (m, 2H), 4.21 (m, 2H), 4.28 (m, 1H), 4.57 (m, 1H), 5.31 (bd, 1H), 7.27 (m, 1H), 7.37 (m, 2H), 7.44 (m, 2H), 7.77 (m, 1H); MS (FAB) 769 (45), 669 (50), 73 (100); IR (film) 3310 (b), 1730. Anal. Calcd for $C_{35}H_{60}N_4O_9SSi_2$: C, 54.66; H, 7.86; N, 7.28. Found: C, 54.40; H, 7.79; N, 7.12.

27: colorless glass; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.05 (s, 18H), 1.00 (m, 4H), 1.27 (t, J = 7.3 Hz, 3H), 1.44 (s, 9H), 1.93 (m, 1H), 2.20 (m, 1H), 2.34 (m, 2H), 2.52 (m, 2H), 2.60 (dq, J = 2, 7 Hz, 2H). 2.80 (dd, J = 7.5, 13.9 Hz, 1H), 2.95 (dd, J = 5.5, 13.9 Hz, 1H), 3.54 (m, 2H), 4.21 (m, 4H), 4.34 (m, 1H), 4.44 (m, 1H), 5.26 (bd, J = 7 Hz, 1H), 6.74 (m, 1H), 6.89 (m, 1H); IR (film) 3310 (b), 1730 (b); MS (FAB) 650 (50), 550 (100), 73 (85). Anal. Calcd for $C_{28}H_{55}N_3O_8SSi_2$: C, 51.74; H, 8.53; N, 6.46. Found: C, 51.69; H, 8.61; N, 6.37.

28: colorless glass; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.044 (s, 9H), 0.045 (s, 9H), 1.00 (m, 4H), 1.44 (s, 9H), 1.91 (m, 1H), 2.17 (m, 1H), 2.28 (m, 2H), 2.50 (m, 2H), 2.73 (dd, J = 7, 14 Hz, 1H), 2.88 (m, 2H), 3.51 (m, 2H), 3.74, 3.77 (ABq, J = 10 Hz, 2H), 4.19 (m, 4H), 4.33 (m, 1H), 4.42 (q, J = 7 Hz, 1H), 6.60 (bs, 1H), 6.70 (bt, 1H), 7.33 (m, 5H); IR (film) 3310 (b), 1730 (b); MS (FAB) 712 (55), 612 (95), 91 (95), 73 (100). Anal. Calcd for $C_{33}H_{57}N_3O_8SSi_2$: C, 55.66; H, 8.07; N, 5.90. Found: C, 55.40; H, 7.79; N, 5.65.

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