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Efficient Coupling of Amino Acid Derivatives to Rigid Organic Scaffolds: Model Syntheses for *De Novo* Proteins.

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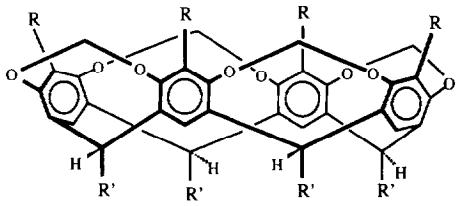
Abstract. We describe the coupling of amino acid derivatives to four different rigid organic macrocycles. The couplings were achieved in high yields, which augurs well for the coupling of polypeptides to the rigid macrocycles to create a new family of *de novo* proteins. We discuss the structure of the model compounds based on the hydrogen bonding patterns that are evident from their NMR and IR spectra.

INTRODUCTION

We are embarking on the design and synthesis of a new family of *de novo* proteins, using rigid organic macrocycles as scaffolds to organize helical bundles and beta sheets. We propose the name *caviteins* for these simple *de novo* proteins because they are hybrids of cavitands (rigid organic molecules that contain enforced cavities)[1] and proteins. Our goal is to use these caviteins to probe some of the noncovalent interactions that are essential to the formation of a protein's tertiary structure. The protein moiety will be covalently linked to the scaffold, which will preorganize the protein's secondary structural units and help them fold into the desired motif. Thus, the simplicity and stability of the caviteins should facilitate the detailed examination of noncovalent interactions such as packing. Others have linked peptides to porphyrins,[2] and to linear[3] and cyclic peptides,[4] using a variety of linkages.[5] We chose the reaction of halo-acetylated peptides (and their homologues) with phenols and thiophenols as our coupling method because of their synthetic viability and the short, but variable, linker groups that could be incorporated. We report model syntheses for four caviteins by the efficient coupling of amino acid esters and a dipeptide to four different macrocycles.

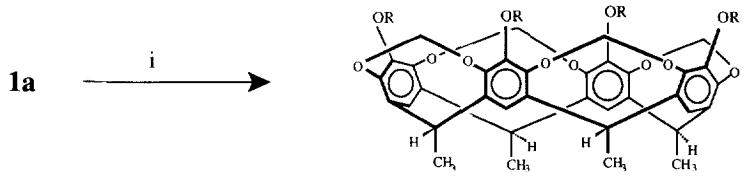


1a: R = OH, R' = CH_3
1b: R = OH, R' = CH_2CH_2Ph
2: R = Br, R' = CH_3
3: R = SH, R' = CH_3

RESULTS

Syntheses.

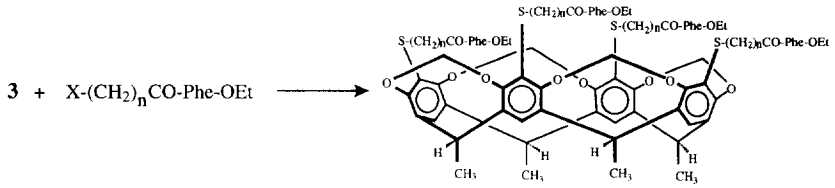
Tetrol **1b** is a rigid bowl-shaped molecule that contains an enforced cavity.[6] Recently, we have found that tetrol **1a** can be used to synthesize carceplexes and that the tetra sodium salt of **1a** gives a well-resolved ^1H NMR spectrum in D_2O while the corresponding spectrum of **1b** is broad.[7] Thus, tetrol **1a** holds promise as a non-aggregate-inducing scaffold for a cavitein. Tetrol **1a** was particularly attractive as a scaffold for a four-helix bundle because of its synthetic availability, its rigidity and because the four phenolic groups are about 7 Å apart, which is nearly ideal for the inter-helical distance found in four-helix bundles of natural proteins.[8] In addition, the enforced cavity may be used as a binding site for potential substrates in a "catalytic cavitein" or to bind drugs in a cavitein drug delivery system. Tetrol **1a** was alkylated with *N*-bromoacetyl-Phe-OEt (**4a**)[9] in dimethylacetamide (DMA) as solvent in the presence of Cs_2CO_3 as base (Scheme 1) to yield model cavitein **6a** in 66% yield. Similarly, alkylation using *N*-chloroacetyl-GlyGly-OEt (**5**) gave model cavitein **6b** in 89% yield. We also synthesized $PhOCH_2$-Phe-OEt (**7**) as a control, so we could compare its spectral properties with the model caviteins.



6a: R = CH_2CO–Phe–OEt
6b: R = CH_2CO–GlyGly–OEt

Scheme 1. Synthesis of model caviteins **6a** and **6b**. For **6a**, (i) = four equivalents of $BrCH_2CO$–Phe–OEt (**4a**), DMA, Cs_2CO_3. For **6b**, (i) = four equivalents of $ClCH_2CO$–GlyGly–OEt (**5**), DMA, K_2CO_3.

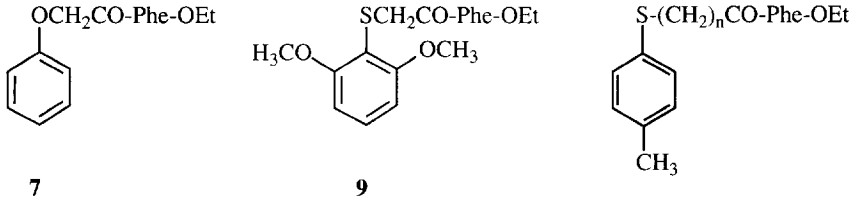
Tetrathiol **3** was synthesized in the hope that the more nucleophilic thiophenol would give higher yields of the caviteins than the less nucleophilic phenol, particularly when unprotected amino acid side chains are present, as will be the case when coupling long peptides to the scaffolds. Furthermore, the sulfide linkage may impart different conformational stabilizing/destabilizing effects to the helical bundles, as sulfides have different bond lengths and angles from ethers. Tetrathiol **3** was synthesized in 84% yield by metal-halogen exchange of bromo-bowl **2** followed by addition of S_8.[10] Tetrathiol **3** was alkylated using bromoacetyl **4a** to give model cavitein **8a** in 76% yield (Scheme 2). We are also interested in modeling the effect of the linker group length on the stability of the helical bundles and thus, synthesized model caviteins **8b**, **8c** and **8d**, where the linkages include 2, 3 and 4 methylenes, respectively (Scheme 2). These compounds were synthesized by alkylation of tetrathiol **3** with the corresponding alkyl halide: X–$(CH_2)_n$CO-Phe-OEt where n = 2, 3 or 4 (**4b**-**4d**, respectively). A series of control compounds were synthesized for comparative purposes: 2,6-$(CH_3O)_2$-C_6H_4S-CH_2CO-Phe-OEt (**9**) from 2,6-$(CH_3O)_2$-C_6H_4SH and *N*-chloroacetyl-Phe-OEt (**4e**); 4-CH_3-C_6H_4S–$(CH_2)_n$–CO-Phe-OEt where n = 1, 2, 3 or 4 (**10a**-**10d**, respectively) from thiocresol and halides **4e**, **4b**, **4c** and **4d**, respectively.



4a: n = 1, X = Br
4b: n = 2, X = Cl
4c: n = 3, X = Br
4d: n = 4, X = Br

8a: n = 1
8b: n = 2
8c: n = 3
8d: n = 4

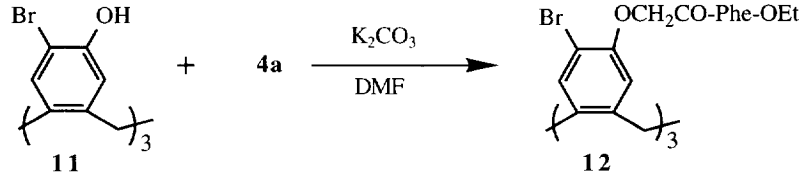
Scheme 2. Synthesis of model caviteins **8a-8d**.



7 **9**

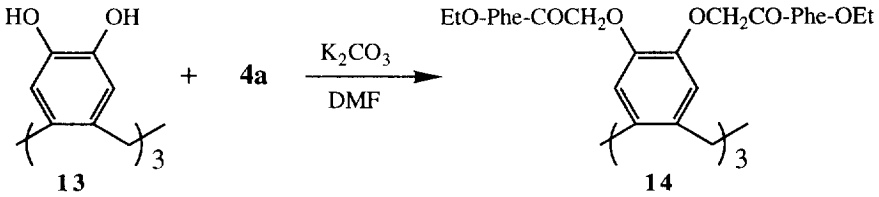
10a: n = 1
10b: n = 2
10c: n = 3
10d: n = 4

Next, we explored the utility of cyclotriveratrylene (CTV) macrocycles in both a tris- and hexa-funtionalized form. We synthesized a cavitein model, **12**, for a three helix bundle by alkylating CTV derivative **11**[11] with bromoacetyl **4a** in DMA as solvent in the presence of K_2CO_3 as base (Scheme 3) to yield model cavitein **12** in 20% yield. Finally, we synthesized a cavitein model (**14**) for a closed surface beta sheet by alkylating cyclotriveratrylene derivative **13**[12] with bromoacetyl **4a** in DMF as solvent in the presence of K_2CO_3 as base (Scheme 4) to yield model cavitein **14** in 90% yield. The yields for the syntheses of compounds **6-10**, **12**, and **14** are given in Table 1.



11 **12**

Scheme 3. Synthesis of model cavitein **12**.



13 **14**

Scheme 4. Synthesis of model cavitein **14**.

Table 1. Yields, Amide N–H Chemical Shifts and Stretching Frequencies for Cavitein Models and Control Compounds.

Compound	Abbreviated Formula	Yield (%)	δ_{NH} (ppm) in $CDCl_3$[b]	δ_{NH} (ppm) in DMSO-d_6	$\Delta\delta$ (ppm)[a]	Stretching Frequency (cm^{-1}) in $CDCl_3$[c]
6a	tetrol[CH_2CO-Phe-OEt]$_4$	66	8.05	8.00	-0.05	3403 (w), 3345 (s)
6b	tetrol[CH_2CO-GlyGly-OEt]$_4$	89	6.45[b], 8.11	8.03, 8.39	1.58, 0.28[d]	3414 (m), 3356 (m)[c]
7	$PhOCH_2CO$-Phe-OEt	83	6.99	8.43	1.44	3413
8a	tetrathiol[CH_2CO-Phe-OEt]$_4$	76	7.87	8.37	0.50	3418 (w), 3346 (s)
8b	tetrathiol[$(CH_2)_2CO$-Phe-OEt]$_4$	47	6.37	8.35	1.97	3422 (s), 3359 (w)
8c	tetrathiol[$(CH_2)_3CO$-Phe-OEt]$_4$	43	6.10	8.25	2.15	3426 (s), 3372 (w)
8d	tetrathiol[$(CH_2)_4CO$-Phe-OEt]$_4$	34	5.86	8.23	2.37	3428 (s), 3350 (vw)
9	2,6-$(CH_3O)_2$-$C_6H_4SCH_2CO$-Phe-OEt	90	8.10	8.26	0.16	3336
10a	4-CH_3-$C_6H_4SCH_2CO$-Phe-OEt	42	7[e]	8.53	~1.5	3373
10b	4-CH_3-$C_6H_4S(CH_2)_2CO$-Phe-OEt	59	5.97	8.38	2.41	3425
10c	4-CH_3-$C_6H_4S(CH_2)_3CO$-Phe-OEt	59	5.85	8.32	2.47	3427
10d	4-CH_3-$C_6H_4S(CH_2)_4CO$-Phe-OEt	45	5.80	8.26	2.46	3428
12	CTV[CH_2CO-Phe-OEt]$_3$	20	7.20	7.93	0.73	3406
14	CTV[CH_2CO-Phe-OEt]$_6$	90	7.18[e], 7.58	8.23, 8.33	~1.05, 0.75[f]	3410 (s), 3370 (w)

[a] $\Delta\delta = \delta_{DMSO} - \delta_{CDCl_3}$.

[b] The chemical shifts of the NH protons in $CDCl_3$ were largely independent of concentration: the $\Delta\delta$ ($\delta_{50\ mM} - \delta_{1\ mM}$) was +0.09 ppm for **10d**, +0.06 ppm for **10c** and <0.04 ppm for **6a**, **7**, **9**, **10a**, **10b**, **12**, and **14**. The $\Delta\delta$ ($\delta_{20\ mM} - \delta_{1\ mM}$) for **8a-8d** were <0.04 ppm. For **6b** at 1 mM, there was one signal at 6.45 ppm; at 20 mM, there were three peaks (6.67, 6.81 and 7.37 ppm); the signal at 8.11 ppm for **6b** was independent of concentration.

[c] The stretching frequencies were largely independent of concentration with the notable exception of **6b**, which showed equal intensity bands at 1 mM, but the 3356 band shifted to 3347 and grew in intensity with respect to the 3414 band at 20 mM. s = strong; w = weak; vw = very weak; m = medium.

[d] Or $\Delta\delta$ = -0.08, 1.94.

[e] Coincident with aromatic peaks; estimated from COSY.

[f] Or $\Delta\delta$ = ~1.15, 0.65.

^1H NMR Spectra.

The chemical shifts for the amide N–H protons for all of the model caviteins in $CDCl_3$ and DMSO-d_6 are listed in Table 1. Amide protons are typically hydrogen bonded (indicated by a downfield shift) to solvent molecules in DMSO-d_6, but not in $CDCl_3$. Thus, the $\Delta\delta$ ($\delta_{DMSO} - \delta_{CDCl_3}$) is indicative of the extent of intramolecular or intermolecular hydrogen bonding of the N–H group in $CDCl_3$. The following observations can be made about hydrogen bonding in $CDCl_3$ from the data in Table 1: The N–H's of model cavitein **6a** are more strongly hydrogen bonded than the N–H's in control compound **7**. Model cavitein **6b** has one set of N–H's that are more strongly hydrogen bonded than the other. The N–H's in cavitein model **8a** are more strongly hydrogen bonded than in cavitein models **8b**, **8c** or **8d**. The N–H's of control compound **9** are more strongly hydrogen bonded than in control compounds **10a-10d**.

The N–H 's of cavitein model **12** appear to be more hydrogen-bonded than control compound **7**, but this may be an artifact of the ortho bromo group as explained below. The spectrum of **12** in $CDCl_3$ was symmetric and clean; apparently, the two diastereomers (a 1:1 mixture of diastereomers is expected from the reaction of racemic **11** and optically pure **4a**) give coincident ^1H NMR signals. The ^1H NMR spectra of cavitein model **14** indicate that there are two sets of N–H protons, each of which is hydrogen bonded, one more strongly than the other. The ^1H NMR spectra of the cavitein models and control compounds were largely concentration independent as indicated in Table 1 (footnote b).

IR Spectra.

A non-hydrogen-bonded amide N–H in $CDCl_3$ typically appears at about 3437 cm^{-1}, whereas hydrogen bonding is indicated by a shift to lower frequency or smaller wave numbers.[13] If an N–H hydrogen exists in both a hydrogen-bonded and a non-hydrogen-bonded state, two bands will be observed in the IR spectrum, whereas the ^1H NMR spectrum usually yields a time-average. Table 1 lists the band position of the amide N–H stretch in the infrared spectra of all model caviteins and control compounds in $CDCl_3$. The N–H's of compounds **7**, **8b-8d**, and **10b-10d** are only very weakly hydrogen-bonded, with **10a** showing more significant hydrogen bonding. The N–H's of cavitein models **6a** and **8a** are mostly hydrogen-bonded, with an observable component that is only very weakly hydrogen-bonded. The N–H's of cavitein model **6b** exhibit hydrogen-bonded and non-hydrogen-bonded bands of equal intensity at 1 mM, whereas the hydrogen-bonded state predominates at 20 mM. The N–H's of compound **9** are completely hydrogen-bonded. The N–H's of cavitein model **12** are very weakly hydrogen-bonded. The N–H's of cavitein model **14** show a mixture of hydrogen-bonded and non-hydrogen-bonded species with the latter being in slight majority. The effect of concentration on the N–H stretches are indicated in Table 1 (footnote c).

DISCUSSION

Hydrogen Bonding.

It is important to understand the hydrogen bonding in the model caviteins so that a more complete picture will be available when studying the larger, more complex real caviteins, which require a definite set of hydrogen-bonding networks. Starting with the bowl (i.e., non-CTV) compounds and their controls, it is

evident that the compounds with the single methylene linker (**6a**, **6b**, **8a**, and **9**) each demonstrate significant hydrogen bonding, while the compounds with the longer linkers (**8b-8d** and **10b-10d**) show only modest hydrogen bonding. Likewise, single methylene-linked control compound **7** exhibits little or no hydrogen bonding, while control compound **10a** shows some hydrogen-bonding. The weak hydrogen bonding that is evident in compound **7** as well as compound **10a** is likely due to the five-membered ring that can form between the N–H's and the aryl ether oxygens[14] and aryl sulfide sulfurs,[15] respectively. What is available to control compound **9** as well as cavitein models **6a**, **6b**, and **8a**, but missing in **7** and **10a** are oxygens that are eight atoms away from the N–H's (in the methoxyls of **9** and in the bridges of **6a**, **6b**, and **8a**). It appears that the eight-membered ring that can form is stable[16] whereas the 9, 10, and 11-membered rings that are available in compounds **8b-8d** are not as stable (see Figure 1). For cavitein model **6b**, the N–H's that are closest to the bowl (on bowl-*Gly*Gly) presumably forms the eight-membered ring hydrogen bond while the other N–H's (on bowl-Gly*Gly*) are largely free from hydrogen bonding at 1 mM, but enter into a hydrogen bond at 20 mM. This concentration dependence suggests that cavitein model **6b** can form some type of aggregate, perhaps a dimer. All other cavitein models and control compounds exhibited concentration independent IR and NMR spectra as noted in the footnotes of Table 1.

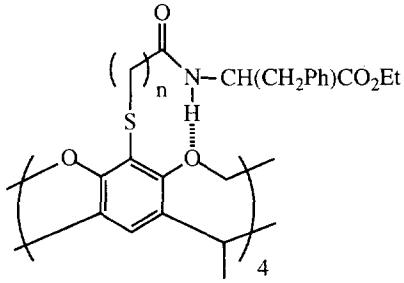


Figure 1. Hydrogen bonding in model caviteins **8a-8d**. The eight-membered ring in **8a** (n = 1) yields a significant hydrogen bond, whereas the 9-, 10- and 11-membered rings in **8b-8d** (n = 2, 3 and 4, respectively) do not yield significant hydrogen bonds. Similar eight-membered rings to that found in **8a** can be envisioned for model caviteins **6a** and **6b** and for control compound **9**.

CTV model cavitein **12** demonstrates only very weak hydrogen bonding by IR, but some more significant hydrogen bonding is suggested by the NMR data. This discrepancy is likely an artifact of the anisotropic effect of the ortho bromine. For the CTV model **14**, it appears that one set of three N–H's are very weakly hydrogen bonded, as in control compound **7**, while the other three N–H's are more strongly hydrogen bonded, but only part of the time.[17] The overall symmetry in the ^{1}H NMR spectrum of **14** in $CDCl_3$ is consistent with two sets of strands. Thus, there are two possible configurations for the hydrogen bonding network as depicted in Figure 2: There are either 16-membered interstrand, inter-catechol hydrogen bonds or 11-membered interstrand, intra-catechol hydrogen bonds in addition to weak 5-membered intrastrand hydrogen bonds. As well, each network could be either clockwise or counter-clockwise.

Potential for Caviteins.

The syntheses described bode well for coupling peptides to rigid scaffolds, particularly using tetrathiol **3** since there will be minimal competition with peptide side chains with the thiophenol nucleophile. Indeed, preliminary evidence suggests that four 14-residue peptides can be linked to tetrathiol **3** in high yield.[18]

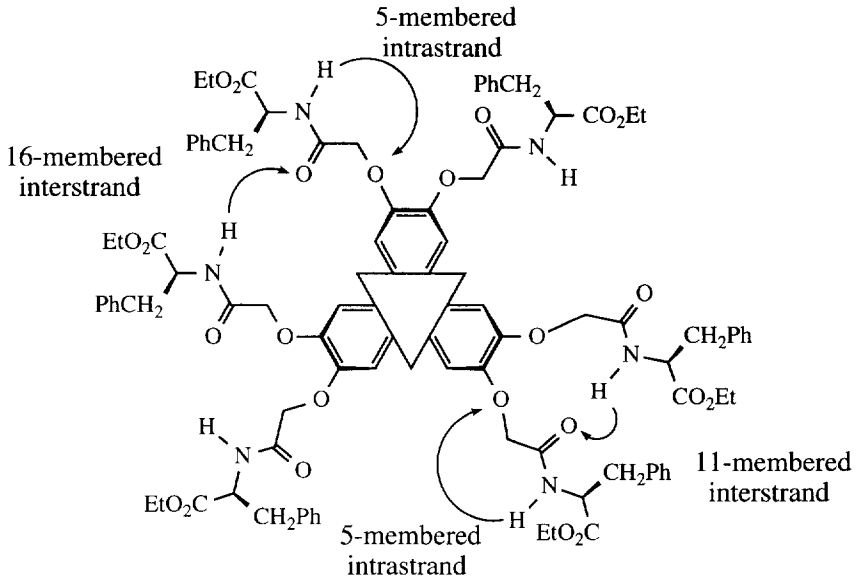


Figure 2. Possible hydrogen-bonding patterns for cavitein model **14**. Inter-catechol (16-membered interstrand) versus intra-catechol (11-membered interstrand) hydrogen bonding is possible. Both networks could, in principle, be either clockwise or counter-clockwise. Intrastrand (5-membered) hydrogen bonding is also indicated.

The tris-functionalized CTV **12** should be useful to study three-helix bundles.[19] The hexa-functionalized CTV **14** has demonstrated potential to form interstrand hydrogen bonds as are needed for a closed surface beta-sheet (or cylinder).[20] Cavitein model **6b** exhibits a tendency to aggregate, which may be useful as an anti-parallel beta-sheet if the aggregate is a dimer. Cavitein models **6a**, **6b** and **8a-8d** have good potential as four-helix bundles.[21] The hydrogen bonding to the bridge oxygens in cavitein models **6a**, **6b** and **8a** will have to be overcome to form hydrogen bonds to the amino acids further up the backbone to form an α-helix; fortunately, preliminary evidence suggests that a four-helix bundle based on tetrathiol **3** is helical.[18] It will be interesting to explore the effect on helix stability of longer linkers, which will impart more degrees of freedom, but will also disrupt the hydrogen bonding to the bridge oxygens.

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EXPERIMENTAL

General. Chemicals were reagent grade (Aldrich or BDH). DMF and CH_2Cl_2 were dried over 4 Å molecular sieves and degassed by bubbling with dry N_2 for 20-30 mins. DMA was stirred over BaO, then

distilled under N_2 onto 4 Å molecular sieves. THF was distilled under N_2 from sodium benzophenone ketyl. The ^1H NMR spectra were run on a Bruker AC-200E or WH-400 spectrometer. Residual ^1H signals from deuterated solvents were used as the reference. $CDCl_3$ and DMSO-d_6 were dried over 4 Å molecular sieves. Mass spectra were run on a Kratos Concept IIH32 using LSIMS with thioglycerol as the matrix unless otherwise noted. IR spectra were run on an ATI Mattson Genesis Series FTIR spectrometer using NaCl cells of path length 0.516 mm. Samples for IR were solutions in $CDCl_3$. Peaks were referenced to the 1600 cm^{-1} peak of polystyrene. Melting points were recorded on a Thomas Hoover Unimelt capillary melting point apparatus and are uncorrected. Silica gel (230-400 mesh, BDH) was used for column chromatography and silica gel glass-backed analytical plates (0.2 mm, Aldrich) were used for t.l.c. with UV detection and I_2 staining where necessary. All products were dried overnight at RT and 0.1 torr unless otherwise noted.

Tetrathiol 3.[10] A solution of bowl **2** (600 mg, 0.66 mmol) in THF (60 mL) was cooled to -78 °C and *n*-BuLi (4.9 mL of a 1.5 M solution in hexanes, 7.40 mmol) was added. The reaction was stirred for 2 min and sulphur (240 mg, 7.5 mmol) was added from a side arm. The reaction was allowed to warm to room temperature, water (15 mL) was added, and the reaction mixture was concentrated *in vacuo*. Water (150 mL) and EtOAc (300 mL) were added to the residue and the two layers were separated. The aqueous layer was adjusted to pH 6 with aqueous 5% HCl and was extracted with EtOAc (4 x 150 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried ($MgSO_4$), concentrated *in vacuo* and the residue was dissolved in $CHCl_3$ (3 mL) and triturated with hexanes. The precipitate was collected by filtration and recrystallized twice from $CHCl_3$/hexanes. Drying at 110 °C (0.1 mm Hg) for 24 h afforded 423 mg of tetrathiol **3** (81%). Removal of residual tristhiol was achieved via acetylation (pyridine and acetic anhydride), column chromatography (3:2, hexanes:acetone), deacetylation (0.2 M NaOH in DMF) and acidification (0.2 M HCl): mp > 220 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (s, 4 H, ArH), 5.95 (d, 4 H, outer OCH_2O, J = 7.0 Hz), 4.94 (q, 4 H, $CHCH_3$, J = 7.4 Hz), 4.36 (d, 4 H, inner OCH_2O, J = 7.0 Hz), 3.76 (s, 4 H, SH), 1.71 (d, 12 H, $CHCH_3$, J = 7.4 Hz); MS (DCI, NH_3) *m/z* 738 ($M^+ + NH_3 + 1$, 100%), 721 ($M^+ + 1$, 50%); Anal. Calcd for $C_{36}H_{32}S_4O_8$: C, 59.98; H, 4.47. Found: C, 60.20; H, 4.54.

$BrCH_2CO$-Phe-OEt 4a. To Phe-OEt·HCl[22] (0.50 g, 2.6 mmol) in a mixture of acetonitrile (20 mL) and 50% saturated sodium bicarbonate solution (30 mL), at 0 °C was added bromoacetyl bromide (1.05 g, 5.2 mmol) dropwise over 5 min, maintaining the pH between 8-10 by further addition of saturated sodium bicarbonate solution. The reaction mixture was stirred at 0-5 °C for 1h before being carefully poured into a mixture of ice (60 g) and concentrated HCl (15 mL) in an oversize container. The acidic mixture was extracted with diethyl ether (2 x 75 mL), and the combined organic extracts were washed with saturated sodium bicarbonate solution (75 mL) and brine (75 mL) and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* resulted in a pale yellow oil which was purified by column chromatography (CH_2Cl_2) to yield 0.61 g of **4a** (75%) as a white solid: mp 71-72 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.33-7.03 (m, 5H, Ph), 6.81 (d, 1H, NH, J = 7.1 Hz), 4.77 (dt, 1H, NCH, J = 7.1, 5.6 Hz), 4.13 (q, 2H, CH_2CH_3, J = 6.4 Hz), 3.79 (s, 2H, $BrCH_2$), 3.10 (d, 2H, CH_2Ph, J = 5.6 Hz), 1.18 (t, 3H, CH_2CH_3, J = 6.4 Hz); MS *m/z* 314/316 $(M+H)^+$; Anal. Calcd. for $C_{13}H_{17}BrNO_3$: C, 49.70; H, 5.13; N, 4.46. Found: C, 49.78; H, 5.12; N, 4.55.

$Cl(CH_2)_2CO$-Phe-OEt 4b. Procedure "A": A mixture of Phe-OEt[21] (500 mg, 2.59 mmol) and 3-bromopropionyl chloride (247 μL, 2.590 mmol) in DMF was stirred for 1 hour at RT. The DMF was removed *in vacuo* and the residue was purified by column chromatography (9:1, EtOAc:hexanes) to yield 586 mg of **4b**

(80%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.07 - 7.29 (m, 5H, Ph), 6.10 (d, 1H, NH, *J* = 7.3 Hz), 4.82 - 4.92 (m, 1H, NCH), 4.16 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.69 - 3.80 (m, 2H, $ClCH_2CH_2$), 3.12 (d, 2H, CH_2Ph, *J* = 5.6 Hz), 2.58 - 2.66 (m, 2H, $ClCH_2CH_2$), 1.24 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{14}H_{19}ClNO_3$ $(M + H)^+$, calcd 284.1053, found 284.1050.

$Br(CH_2)_3CO$-Phe-OEt 4c. Procedure "A" was employed using Phe-OEt (520 mg, 2.70 mmol) and 4-chlorobutyryl chloride (312 µl, 2.70 mmol) to yield 767 mg of **4c** (83%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.06 - 7.31 (m, 5H, Ph), 5.95 (d, 1H, NH, *J* = 7.3 Hz), 4.80 - 4.90 (m, 1H, NCH), 4.11 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.34 - 3.60 (m, 2H, $BrCH_2$), 3.06 - 3.12 (m, 2H, CH_2Ph), 2.30 - 2.38 (m, 2H, $BrCH_2CH_2CH_2$), 2.01 - 2.15 (m, 2H, $BrCH_2CH_2$), 1.23 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{15}H_{21}BrNO_3$ $(M + H)^+$, calcd 342.0705, found 342.0701.

$Br(CH_2)_4CO$-Phe-OEt 4d. Procedure "A" was employed using Phe-OEt (496 mg, 2.57 mmol) and 5-bromovaleryl chloride (344 µl, 2.57 mmol) to yield 900 mg of **4d** (98%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.05 – 7.29 (m, 5H, ArH), 5.93 (d, 1H, NH, *J* = 7.2 Hz), 4.80 - 4.90 (m, 1H, NCH), 4.15 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.36 (t, 2H, $BrCH_2$, *J* = , 3.06 - 3.12 (m, 2H, CH_2Ph), 2.15 - 2.22 (m, 2H, CH_2CON), 1.67 - 1.85 (m, 4H, $BrCH_2CH_2CH_2$); HRMS *m/z* for $C_{16}H_{23}BrNO_3$ $(M + H)^+$, calcd 356.0861, found 356.0856.

$ClCH_2CO$-Phe-OEt 4e. Procedure "A" was employed using Phe-OEt (50 mg, 0.26 mmol) and chloroacetyl chloride (22 µL, 0.29 mmoL) to yield 70 mg of **4e** (100%) as a white solid: mp 61-64 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.08 - 7.29 (m, 5H, Ph), 6.98 (d, 1H, NH, *J* = 7.1 Hz), 4.82 (m, 1H, NCH), 4.16 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 4.00 (s, 2H, $ClCH_2$), 3.13 (d, 2H, CH_2Ph, *J* = 5.9 Hz), 1.22 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{13}H_{17}ClNO_3$ $(M + H)^+$, calcd 270.0897, found 270.0897.

$ClCH_2CO$-GlyGly-OEt 5. A solution of GlyGly-OEt•HCl[21] (500 mg, 2.6 mmol) in DMF (15 mL) at 0 °C was treated with chloroacetyl chloride (0.6 mL, 7.8 mmol). The reaction was stirred for 1.5 h, concentrated *in vacuo*, and MeOH (30 mL) was added. The precipitate was collected by filtration and washed with MeOH, affording 308 mg of compund **5** (51%): mp 148-150 °C; ^{1}H NMR (200 MHz, $(CD_3)_2SO$) 8.47 (t, 1H, NH, *J* = 5.7 Hz), 8.37 (t, 1H, NH, *J* = 5.9 Hz), 4.12 (s, 2H, $ClCH_2CO$), 4.08 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.83 (d, 2H, NCH_2, *J* = 5.9 Hz), 3.78 (d, 2H, NCH_2, *J* = 5.7 Hz), 1.18 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_8H_{14}ClN_2O_4$ $(M + H)^+$, calcd 237.0642, found 237.0636.

Tetrol[CH_2CO-Phe-OEt]$_4$ 6a. A solution of tetrol **1a** (12 mg, 0.018 mmol), bromoacetyl **4a** (25 mg, 0.080 mmol) in DMA (2 mL) was stirred with excess Cs_2CO_3 at RT overnight and concentrated *in vacuo*. EtOAc (2 mL) was added to the residue and the slurry was filtered through silica gel. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography (7:3, EtOAc:hexanes) to yield 19 mg of **6a** (66%): mp 78 °C (decomp); IR ($CDCl_3$) 3370 (NH), 3344 (NH), 1742 (ester CO), 1674 (amide CO) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 4H, NH, *J* = 8.2 Hz), 7.07-7.21 (m, 20H, Ph), 6.94 (s, 4H, ArH), 5.67 (d, 4 H, outer OCH_2O, *J* = 7.1 Hz), 4.88-4.93 (m, 4H, NCH), 4.85 (q, 4H, $CHCH_3$, *J* = 7.4 Hz), 4.48 (AB quartet, 8H, OCH_2CO, *J* = 16.0, Δν = 23.4 Hz), 4.27 (d, 4H, inner OCH_2O, *J* = 7.1 Hz), 4.13 (q, 8H, CH_2CH_3, *J* = 7.1 Hz), 3.14 (two overlapping ABX dd, 8H, CH_2Ph, *J* = 6.2, 6.1, 13.9 Hz, Δν = 20.1 Hz), 1.74 (d, 12H, $CHCH_3$, *J* = 7.4 Hz), 1.19 (t, 12H, CH_2CH_3, *J* = 7.1 Hz); HRMS (NOBA) *m/z* for $C_{88}H_{93}O_{24}N_4$ $(M + H)^+$, calcd 1589.6151, found 1589.6179.

Tetrol[CH_2CO-GlyGly-OEt]$_4$ 6b. A solution of tetrol **1a** (12 mg, 0.018 mmol) and chloroacetyl **5** (20 mg, 0.080 mmol) in DMA (2 mL) was stirred with excess K_2CO_3 at RT overnight and concentrated *in vacuo*. EtOAc (1 mL) and MeOH (1 mL) were added to the residue and the slurry was filtered through silica gel, eluted (1:1 EtOAc:MeOH) and concentrated *in vacuo* to afford 26 mg of **6b** (90%): mp 73 °C (decomp); IR ($CDCl_3$) 3414 (NH), 3356 (NH), 1745 (ester CO), 1673 (amide CO) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07-8.10 (t, 4H, NH, *J* = 5.1 Hz), 6.98 (s, 4H, ArH), 6.46-6.48 (t, 4H, NH, *J* = 5.1 Hz), 6.09 (d, 4H, outer OCH_2O, *J* = 7.1 Hz), 4.92 (q, 4H, $CHCH_3$, *J* = 7.4 Hz), 4.55 (s, 8H, OCH_2CO), 4.46 (d, 4H, inner OCH_2O, *J* = 7.1 Hz), 4.20 (q, 8H, CH_2CH_3, *J* = 7.1 Hz), 4.06 (d, 16H, NCH_2, *J* = 5.1 Hz), 1.72 (d, 12H, $CHCH_3$, *J* = 7.4 Hz), 1.27 (t, 12H, CH_2CH_3, *J* = 7.1 Hz); HRMS (NOBA) *m/z* for $C_{68}H_{81}O_{28}N_8$ $(M + H)^+$, calcd 1457.5148, found 1457.5150.

$PhOCH_2CO$-Phe-OEt 7. To a mixture of phenoxyacetic acid (152 mg, 1 mmol) in dry CH_2Cl_2 (5 mL), was added oxalyl chloride (127 mg, 1 mmol), and 2 drops of DMF. The reaction mixture was stirred under dry conditions at room temperature for 2 h. The solvent was removed *in vacuo* and the oily green residue added to Phe-OEt (193 mg, 1 mmol) in DMF (5 mL). This mixture was stirred for 2 h at RT, the solvent was removed *in vacuo* and the reaction mixture was partitioned between diethyl ether (25 mL) and saturated sodium bicarbonate solution (20 mL). The organic layer was washed with 2N HCl (20 mL), brine (20 mL) and dried over $MgSO_4$. Evaporation of solvent resulted in a glass, which was recrystallized from diethyl ether / petroleum ether to produce 271 mg of **7** (83%) as fine needles: mp 75-76 °C; IR ($CDCl_3$) 3413 (NH), 1730 (ester CO), 1684 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.34-6.84 (m, 11H, Ph, ArO, NH), 4.93 (dt, 1H, NCH, *J* = 6.5, 8.2 Hz), 4.48 (s, 2H, $ArOCH_2$), 4.16 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.13 (d, 2H, CH_2Ph, *J* = 6.5 Hz), 1.22 (t, 3H, $CHCH_3$, *J* = 7.1 Hz); MS *m/z* 327 $(M+H)^+$; Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 69.70; H, 6.47; N, 4.28. Found: C, 69.53; H, 6.35; N, 4.20.

Tetrathiol[CH_2CO-Phe-OEt]$_4$ 8a. Procedure "B": A mixture of tetrathiol **3** (36 mg, 0.050 mmol), bromoacetyl **4a** (69 mg, 0.22 mmol) and DBU (33 µl, 0.22 mmol) in DMF (1 ml) was stirred at RT overnight under N_2. The DMF was removed *in vacuo* and the residue was purified by column chromatography (3:2, hexanes:acetone) to yield 63 mg of **8a** (76%) as a white solid: mp 94 °C (decomp); IR ($CDCl_3$) 3418 (NH), 3346 (NH), 1735 (ester CO), 1664 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.87 (d, 4H, NH, *J* = 8.2 Hz), 7.00 - 7.21 (m, 24H, CH_2Ph, ArH), 5.96 (d, 4H, outer OCH_2O, *J* = 7.4 Hz), 5.00 (q, 4H, $CHCH_3$, *J* = 7.4 Hz), 4.71 - 4.75 (m, 4H, NCH), 4.32 (d, 4H, inner OCH_2O, *J* = 7.4 Hz), 4.06 (q, 8H, CH_2CH_3, *J* = 7.1 Hz), 3.46 (AB quartet, 8H, SCH_2, *J* = 17.2 Hz, Δν = 21.4 Hz), 2.99 (two overlapping ABX dd, 8H, CH_2Ph, *J* = 6.3, 7.2, 13.9 Hz, Δν = 28.6 Hz), 1.72 (d, 12H, $CHCH_3$, *J* = 7.4 Hz), 1.11 (t, 12H, CH_2CH_3, *J* = 7.1 Hz); MS *m/z* 1654 $(M + H)^+$; Anal. Calcd for $C_{88}H_{92}N_4O_{20}S_4$: C, 63.91; H, 5.61; N, 3.39. Found: C, 63.55; H, 5.61; N, 3.41.

Tetrathiol[$(CH_2)_2CO$-Phe-OEt]$_4$ 8b. Procedure "B" was employed using tetrathiol **3** (78 mg, 0.11 mmol), chloropropionyl **4b** (135 mg, 0.477 mmol) and DBU (71 µl, 0.48 mmol) to yield 87 mg of **8b** (47%) as a white solid: mp 133 °C (decomp); IR ($CDCl_3$) 3422 (NH), 3359 (NH), 1734 (ester CO), 1668 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.10 - 7.30 (m, 24H, CH_2Ph, ArH), 6.37 (d, 4H, NH, *J* = 7.6 Hz), 5.68 (d, 4H, outer OCH_2O, *J* = 7.5 Hz), 4.98 (q, 4H, $CHCH_3$, *J* = 7.3 Hz), 4.80 - 4.90 (m, 4H, NCH), 4.11 - 4.22 (m, 12H, inner OCH_2O, CH_2CH_3), 2.94 - 3.16 (m, 16H, CH_2Ph, CH_2CH_2CO), 2.24 - 2.31 (m, 8H, SCH_2), 1.74 (d, 12H, $CHCH_3$, *J* = 7.4 Hz), 1.22 (t, 12H, CH_2CH_3, *J* = 7.1 Hz); MS *m/z* 1710 (M +

H)$^+$; Anal. Calcd for $C_{92}H_{100}N_4O_{20}S_4 \cdot H_2O$: C, 63.94; H, 5.95; N, 3.24. Found: C, 63.80; H, 5.96; N, 3.10.

Tetrathiol[$(CH_2)_3CO$-Phe-OEt]$_4$ 8c. Procedure "B" was employed using tetrathiol **3** (100 mg, 0.139 mmol), bromobutyryl **4c** (209 mg, 0.612 mmol) and DBU (92 µl, 0.612 mmol) to yield 106 mg of **8c** (43%) as a white solid: mp 52 °C (decomp); IR ($CDCl_3$) 3426 (NH), 3372 (NH), 1732 (ester CO), 1667 (amide CO) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.04 - 7.24 (m, 24H, CH_2Ph, ArH), 6.11 (d, 4H, NH, J = 8.0 Hz), 5.90 (d, 4H, outer OCH_2O, J = 7.5 Hz), 5.00 (q, 4H, $CHCH_3$, J = 7.4 Hz), 4.78 - 4.88 (m, 4H, NCH), 4.30 (d, 4H, inner OCH_2O, J = 7.5 Hz), 4.13 (q, 8H, CH_2CH_3, J = 7.0 Hz), 3.08 (two overlapping ABX dd, 8H, CH_2Ph, J = 6.2, 6.8, 14.2 Hz, Δν = 21.1 Hz), 2.72 - 2.89 (m, 8H, CH_2CH_2CO), 2.30 - 2.37 (m, 8H, SCH_2), 1.64 - 1.80 (m, 20H, $SCH_2CH_2CH_2$, $CHCH_3$), 1.20 (t, 12H, CH_2CH_3, J = 7.0 Hz); MS *m/z* 1766 (M + H)$^+$; Anal. Calcd for $C_{96}H_{108}N_4O_{20}S_4 \cdot H_2O$: C, 64.63; H, 6.21; N, 3.14. Found: C, 64.58; H, 6.13; N, 2.98.

Tetrathiol[$(CH_2)_4CO$-Phe-OEt]$_4$ 8d. Procedure "B" was employed using tetrathiol **3** (50 mg, 0.069 mmol), bromovaleryl **4d** (110 mg, 0.31 mmol) and DBU (46 µl, 0.31 mmol) to yield 54 mg of **8d** (34%) as a white solid: mp 99 °C (decomp); IR ($CDCl_3$) 3428 (NH), 1732 (ester CO), 1670 (amide CO) cm^{-1}; ^{1}H NMR (200MHz, $CDCl_3$) δ 7.05 - 7.26 (m, 24H, CH_2Ph, ArH), 5.88 (d, 8H, NH, outer OCH_2O, J = 7.5 Hz), 4.98 (q, 4H, $CHCH_3$, J = 7.2 Hz), 4.79 - 4.89 (m, 4H, NCH), 4.27 (d, 4H, inner OCH_2O, J = 7.3 Hz), 4.14 (q, 8H, CH_2CH_3, J = 7.1 Hz), 3.09 (two overlapping ABX dd, 8H, CH_2Ph, J = 5.7, 5.8, 14.2 Hz, Δν = 23.3 Hz), 2.75 - 2.82 (m, 8H, CH_2CH_2CO), 2.10 - 2.18 (m, 8H, SCH_2), 1.45 - 1.74 (m, 28H, $SCH_2CH_2CH_2$, $CHCH_3$), 1.21 (t, 12H, CH_2CH_3, J = 7.1 Hz); MS *m/z* 1822 (M + H)$^+$; Anal. Calcd for $C_{100}H_{116}N_4O_{20}S_4 \cdot 2H_2O$: C, 64.63; H, 6.51; N, 2.74. Found: C, 64.43; H, 6.29; N, 2.80.

2,6-$(CH_3O)_2$-$C_6H_4SCH_2CO$-Phe-OEt 9. A mixture of 2,6-dimethoxythiophenol[23] (71 mg, 0.26 mmol), chloroacetyl **4e** (45 mg, 0.26 mmol) and DBU (43 µl, 0.29 mmol) in dry DMF (1 ml) was stirred overnight at RT under N_2. The DMF was removed *in vacuo* and the residue was purified by column chromatography (7:3, hexanes:ethyl actetate) to yield 96 mg of **9** (90%) as a colorless oil: IR ($CDCl_3$) 3336 (NH), 1735 (ester CO), 1661 (amide CO) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10 (d, 1H, NH, J = 7.8 Hz), 7.00 - 7.30 (m, 6H, *para* ArH, CH_2Ph), 6.53 (d, 2H, *meta* ArH, 7 = 8.4 Hz), 4.76 (ddd, 1H, NCH, J = 6.5, 6.5, 8.0 Hz), 4.06 (q, 2H, CH_2CH_3, J = 7.1 Hz), 3.77 (s, 6H, OCH_3), 3.53 (AB quartet, 2H, SCH_2, J = 17.0 Hz, Δν = 21.1 Hz), 2.98 (two overlapping ABX dd, 2H, CH_2Ph, J = 6.5, 6.5, 13.9 Hz, Δν = 20.8 Hz), 1.13 (t, 3H, CH_2CH_3, J = 7.1 Hz); MS *m/z* 404 (M + H)$^+$; Anal. Calcd for $C_{21}H_{25}NO_5S$: C, 62.51; H, 6.25; N, 3.47. Found: C, 62.13; H, 5.93; N, 3.50.

4-CH_3-$C_6H_4SCH_2CO$-Phe-OEt 10a. Procedure "C": A mixture of thiocresol (50 mg, 0.186 mmol), chloroacetyl **4e** (23 mg, 0.186 mmol) and DBU (31 µl, 0.204 mmol) in dry DMF (1 ml) was stirred overnight at RT under N_2. The DMF was removed *in vacuo* and the residue was purified by column chromatography (9:1 hexanes:EtOAc, then 4:1 hexanes:ethyl actetate) to yield 28 mg of **10a** (42%) as a white solid: mp 66-67 °C; IR ($CDCl_3$) 3373 (NH), 1737 (ester CO), 1668 (amide CO) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.95 - 7.26 (m, 10H, ArH, CH_2Ph, NH), 4.79 (dd, 1H, NCH, J = 6.0, 7.9 Hz), 4.11 (q, 2H, CH_2CH_3, J = 7.1 Hz), 3.55 (s, 2H, SCH_2), 3.03 (d, 2H, CH_2Ph, J = 6.0 Hz), 2.29 (s, 3H, $ArCH_3$), 1.18 (t, 3H, CH_2CH_3, J = 7.1 Hz); HRMS *m/z* for $C_{20}H_{24}NO_3S$ (M + H)$^+$, calcd 358.1477, found 358.1478.

4-CH_3-$C_6H_4S(CH_2)_2CO$-Phe-OEt 10b. Procedure "C" was employed using thiocresol (65 mg, 0.52 mmol), chloropropionyl **4b** (74 mg, 0.26 mmol) and DBU (43 µl, 0.281 mmol) to yield 57 mg of **10b** (59%) as a yellow solid: mp 44 °C; IR ($CDCl_3$) 3425 (NH), 1735 (ester CO), 1673 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.06 - 7.22 (m, 9H, ArH, CH_2Ph), 6.01 (d, 1H, NH, *J* = 7.2 Hz), 4.79 - 4.87 (m, 1H, NCH), 4.15 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.06 - 3.13 (m, 4H, CH_2CH_2CO, CH_2Ph), 2.41 (t, 2H, SCH_2, *J* = 7.4 Hz), 2.30 (s, 3H, $ArCH_3$), 1.23 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{21}H_{26}NO_3S$ $(M + H)^+$, calcd 372.1633, found 372.1634.

4-CH_3-$C_6H_4S(CH_2)_3CO$-Phe-OEt 10c. Procedure "C" was employed using thiocresol (37 mg, 0.30 mmol) bromobutyryl **4c** and DBU (25 µl, 0.167 mmol) to yield 35 mg of **10c** (59%) as a yellow oil: IR ($CDCl_3$) 3427 (NH), 1735 (ester CO), 1673 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.05 - 7.27 (m, 9H, ArH, CH_2Ph), 5.89 (d, 1H, NH, *J* = 7.4 Hz), 4.79 - 4.89 (m, 1H, NCH), 4.15 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.08 (two overlapping ABX dd, 2H, CH_2Ph, *J* = 5.2, 5.8, 13.3, Δν = 18.0 Hz), 2.77-2.91 (m, 2H, CH_2CH_2CO), 2.26 - 2.33 (m, 2H, SCH_2CH_2), 2.29 (s, 3H, $ArCH_3$), 1.80 - 1.95 (m, 2H, SCH_2), 1.22 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{22}H_{28}NO_3S$ $(M + H)^+$, calcd 386.1790, found 386.1783.

4-CH_3-$C_6H_4S(CH_2)_4CO$-Phe-OEt 10d. Procedure "C" was employed using thiocresol (17 mg, 0.14 mmol), bromovaleryl **4d** (50 mg, 0.14 mmol) and DBU (23 µl, 0.15 mmol) to yield 25 mg of **10d** (45%) as a yellow oil: IR ($CDCl_3$) 3428 (NH), 1734 (ester CO), 1671 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.04 - 7.31 (m, 9H, ArH, CH_2Ph), 5.89 (d, 1H, NH, *J* = 7.5 Hz), 4.79 - 4.89 (m, 1H, NCH), 4.15 (q, 2H, CH_2CH_3, *J* = 7.1 Hz), 3.07 (two overlapping ABX dd, 2H, CH_2Ph, *J* = 5.9, 5.9, 13.7, Δν = 14.8 Hz), 2.84 (t, 2H, CH_2CH_2CO, *J* = 7.0 Hz), 2.29 (s, 3H, $ArCH_3$), 2.16 (t, 2H, SCH_2, *J* = 7.0 Hz), 1.51 - 1.77 (m, 4H, $SCH_2CH_2CH_2$), 1.23 (t, 3H, CH_2CH_3, *J* = 7.1 Hz); HRMS *m/z* for $C_{23}H_{30}NO_3S$ $(M + H)^+$, calcd 400.1946, found 400.1951.

CTV[CH_2CO-Phe-OEt]$_3$ 12. A mixture of 15 mL of DMF, compound **11** (50 mg, 0.09 mmol), bromoacetyl **4a** (104 mg, 0.32 mmol) and dry K_2CO_3 (42 mg, 30 mmol) were stirred under N_2 atmosphere at RT. After three days, the DMF was removed *in vacuo*. The residue was suspended in acetone and filtered. The acetone was removed *in vacuo* to give a white solid that was recrystallized with $CHCl_3$/hexanes and purified by column chromatography (3:1:1 $CHCl_3$:hexanes:EtOAc) to give 20 mg of **12** (20%): mp 105-107 °C; IR ($CHCl_3$) 3406 (NH), 1732 (ester CO), 1682 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.44 (s, 3H, ArH), 7.25 (d, 3H, NH, *J*= 7.4 Hz), 6.95 - 7.10 (m, 15H, Ph), 6.71 (s, 3H, ArH), 4.95 (m, 3H, NCH, *J*= 5.6, 7.4 Hz), 4.64 (d, 3H, $ArCH_2Ar$, *J*= 13.3 Hz), 4.48 (AB quartet, 6H, OCH_2O, *J*= 14.2 Hz, Δν= 10.2 Hz), 4.17 (q, 6H, CH_3CH_2O, *J*= 7.1 Hz), 3.56 (d, 3H, $ArCH_2Ar$, *J*= 13.3 Hz), 3.11 (d, 6H, *J*= 5.6 Hz, CH_2Ph), 1.23 (t, 9H, *J*= 7.1 Hz, CH_3CH_2O); HRMS (NOBA) *m/z* for $C_{60}H_{61}N_3O_{12}Br_3$ $(M + H)^+$, calcd 1256.1767, found 1256.1743.

CTV[CH_2CO-Phe-OEt]$_6$ 14. To a mixture of CTV **11** (13 mg, 0.036 mmol) and potassium carbonate (44 mg, 0.32 mmol) in DMF, was added bromoacetyl **4a** (100 mg, 0.32 mmol). The reaction mixture was stirred for 20 h at RT and the DMF was removed *in vacuo*. The residue was dissolved in ethyl acetate and filtered through a pad of silica gel with ethyl acetate as eluent producing 56 mg (90%) of **14** as a pale yellow oil. Further purification was achieved by column chromatography (3:1, diethyl ether:acetone) to produce a pale yellow glass: IR ($CDCl_3$) 3410 (NH), 3370 (NH), 1734 (ester CO), 1675 (amide CO) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.58 (d, 3H, NH , *J* = 7.2 Hz), 7.25-6.68 (m, 39H, NH, Ar, Ph), 4.88 (ddd, 3H, NCH, *J* =

7.2, 6.8, 6.8 Hz), 4.75 (ddd, 3H, NCH, J = 7.6, 6.4, 6.4 Hz), 4.63 (d, 3H, Ar_2CH_2 inner , J = 15.0 Hz), 4.42 (d, 3H, $ArOCH_2$, J = 15.2 Hz), 4.35 (s, 6H, $ArOCH_2$), 4.25 (d, 3H, $ArOCH_2$, J = 15.2 Hz), 4.10 (q, 12H, CH_2CH_3, J = 6.3 Hz), 3.50 (d, 3H, Ar_2CH_2 outer, J = 15.0 Hz), 3.12 (two overlapping ABX dd, 6H, CH_2Ph, J = 6.0, 7.0, 13.9 Hz, $\Delta\nu$ = 28.8 Hz), 2.92 (two overlapping ABX dd, 6H, CH_2Ph, J = 6.6, 7.2, 14.0 Hz, $\Delta\nu$ = 20.8 Hz), 1.16 (t, 18H, CH_2CH_3, J = 6.3 Hz); MS (NOBA) *m/z* 1766 $(M+H)^+$; Anal. Calcd. for $C_{99}H_{108}N_6O_{24}\bullet H_2O$: C, 66.65; H, 6.21; N, 4.71. Found: C, 66.66; H, 6.30; N, 4.51.

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