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Development of a Novel Hapten for Radioimmunoassay of the Lignan, Enterolactone in Plasma (Serum). Total Synthesis of (±)-*trans*-5-Carboxymethoxyenterolactone and Several Analogues

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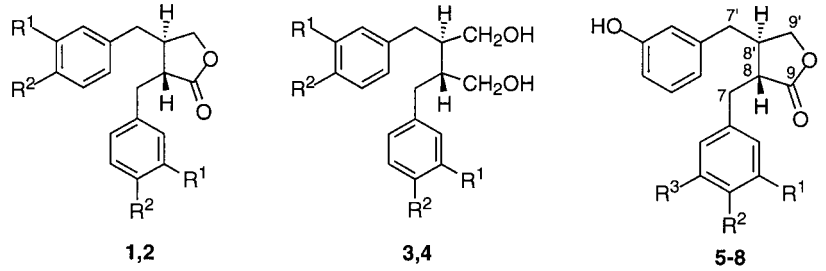
Abstract—A recently developed method for the analysis of the mammalian lignan, enterolactone **1**, is based on time-resolved fluoroimmunoassay (TR-FIA) using an europium chelate as a label. This RIA utilizes enterolactone derivatives carrying a carboxylic acid appendage for the production of antiserum and tracer. The synthesis of 5-carboxymethoxyenterolactone **6** and analogues **5**, **7** and **8** is described, and their suitability for the method are discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Lignans have attracted much interest over the years on account of their widespread occurrence in various plant species,[1] and their broad range of biological activity.[2] Since the discovery of the mammalian lignans enterolactone **1** and enterodiol **3** from human urine,[3–6] there has been much discussion about their biological function. Especially interesting is their suggested role as antiestrogens and anticarcinogens among other possible biological activities.[7] Enterolactone **1**, alone or together with enterodiol **3**, has more recently been detected in human plasma and other biological fluids as well.[8–10]

Human diet has been shown to contain plant lignans, which act as precursors for the mammalian lignans.[8,11,12] The enterolignans are produced by the action of intestinal microflora on the precursors (i.e. matairesinol **2** and secoisolariciresinol **4**) in dietary fiber. The precursor lignans have also been detected in human urine and plasma (Scheme 1).[13,14]

Available analytical methods for the detection and quantification of phytoestrogens in human biological fluids and in food samples are based on gas–liquid chromatography or high-performance liquid chromatography alone or in combination with mass spectrometry.[14–19] These expensive and time-consuming methods are not suitable for screening purposes in large populations. In addition, these procedures are not sensitive enough for the assay of conjugated phytoestrogens in plasma. These disadvantages led to the application of a new analytical method based on radioimmunoassay (RIA).[20]



1,2 **3,4** **5-8**

Scheme 1. **1**, **3** : R^1=OH, R^2=H; **2**, **4** : R^1=OMe, R^2=OH; **5** : R^1=OCH_2CO_2H; R^2=R^3=H; **6** : R^1=OH; R^2=H; R^3=OCH_2CO_2H; **7** : R^1=OH; R^2=$O(CH_2)_3CO_2H$; R^3=H; **8** : R^1=OH; R^2=$(CH_2)_2CO_2H$; R^3=H.

Keywords: lignans; lactones; carboxylic acids and derivatives; Michael reactions.

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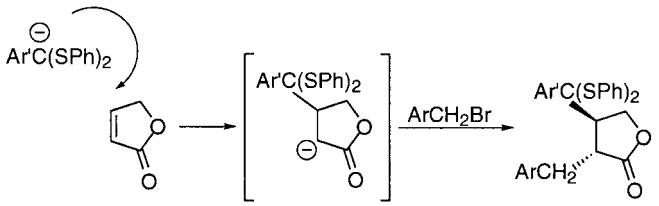


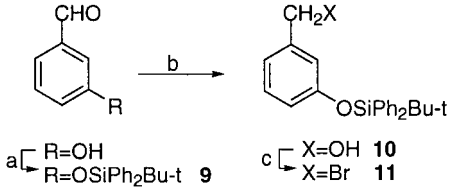
Scheme 2.

The first direct radioimmunoassay for unconjugated and total daidzein and genistein in human biological fluids was developed by Adlercreutz et al.[21,22] and as the next step, the use of time-resolved fluoroimmunoassay (TR-FIA) of plasma enterolactone was recently introduced.[23] This rapid technique has the advantages of other nonradioisotopic assays, such as stability of the reagent and lack of radiation. The increase in sensitivity and assay range compares well with the conventional enzyme immunoassay (EIA) and fluoroimmunoassay (FIA) methods. The method has very recently been applied to the quantitative determination of phytoestrogens in human urine.[24]

An essential part of the work was to find a properly substituted enterolactone derivative to be coupled to bovine serum albumin and then used in the immunization of rabbits in order to create an antiserum.[20] The same derivative would be used in preparing the tracer with europium label. A number of synthetic enterolactone derivatives carrying various carboxylic acid side chains were developed for this purpose. We present here the synthesis of 5-carboxymethoxyenterolactone **6** and analogous derivatives **5**, **7** and **8**, and discuss their suitability for radioimmunoassay.

Results and Discussion

The *trans*-α,β-dibenzyl-γ-butyrolactone framework is generally obtained by the Michael addition of an anion derived from dithioacetal to butenolide followed by benzylation in situ (Scheme 2).[25,26]



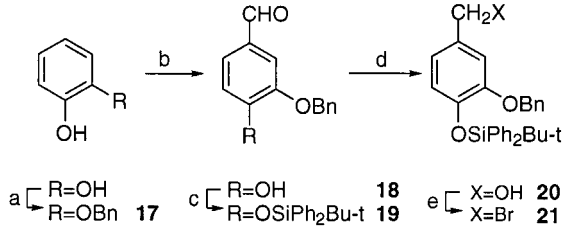
Scheme 3. Reagents: (a) *t*-$BuPh_2SiCl$, imidazole, DMF (99%); (b) $NaBH_4$, ethanol (99%); (c) PBr_3, ether, +4°C (86%).

Two approaches for attaching the carboxylate side chain were studied, either adding it to the finished molecular framework or incorporating it in one of the starting materials. The former approach was more successful, particularly for the attachment of side chain to the aromatic ring by a carbon–oxygen bond. As a matter of fact, the method was chosen for general use due to the flexibility in regard to the aromatic ring substituents. To allow a selective and controlled introduction of the side chain late in the synthesis, suitable protection of the phenolic hydroxy groups was required. For this purpose, benzyl and silyl ethers were chosen.

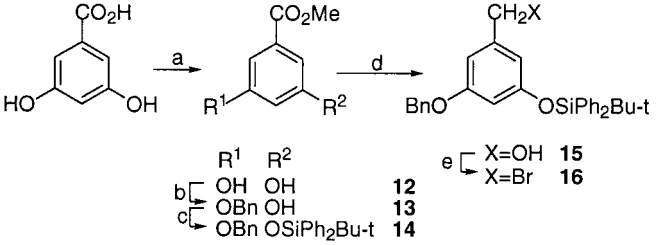
The latter approach was applied only in one case, where the side chain was attached directly to the aromatic ring by a carbon–carbon bond.

Since the carboxylic acid side chain was intended to be incorporated in the benzyl bromide moiety of the three-component synthesis (Scheme 2), the synthetic procedure of properly substituted bromides varied considerably with the availability of starting materials.

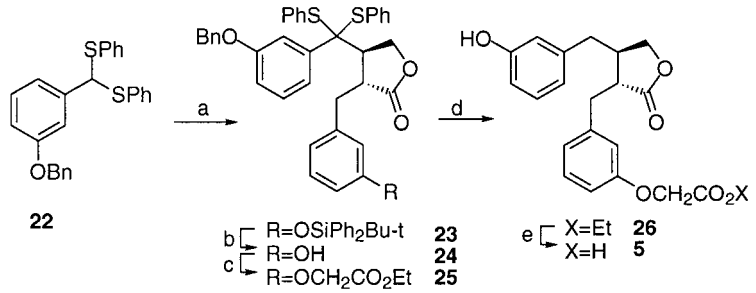
The benzyl bromide **11** for 3-carboxymethoxyenterolactone **5** was readily prepared via silyl ether[27] formation and the reduction of aldehyde **9** to alcohol **10** with $NaBH_4$ (Scheme 3). For the preparation of benzyl bromide **16** for 5-carboxymethoxyenterolactone **6**, methyl 3,5-dihydroxybenzoate



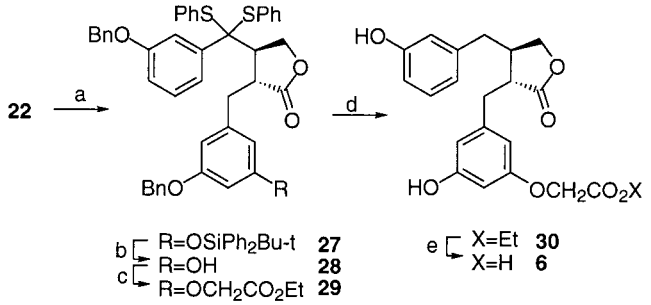
Scheme 5. Reagents: (a) benzyl chloride, KOH, DMF, 80–90°C (56%); (b) NaOH, ethanol, H_2O, $CHCl_3$, reflux (17%); (c) *t*-$BuPh_2SiCl$, imidazole, DMF (71%); (d) $NaBH_4$, ethanol (61%); (e) PBr_3, ether, +4°C (87%).



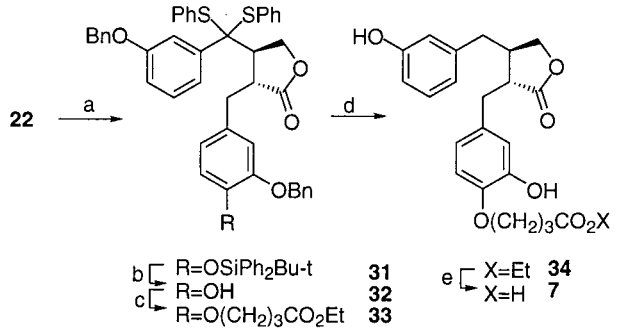
Scheme 4. Reagents: (a) MeOH, conc. H_2SO_4, reflux (89%); (b) benzyl bromide, K_2CO_3, acetone, reflux (33%); (c) *t*-$BuPh_2SiCl$, imidazole, DMF (99%); (d) $LiAlH_4$, ether, reflux (27%); (e) PBr_3, ether, +4°C (89%).



Scheme 6. Reagents: (a) *n*-butyllithium, THF, −78°C, then 2-butenolide, THF, −78°C, then benzyl bromide, **11**, HMPA, THF, −78°C (10%); (b) *n*-$Bu_4N^+F^-$, THF (99%); (c) $BrCH_2CO_2Et$, K_2CO_3, KI, acetone, reflux (96%); (d) Raney nickel, ethanol, reflux (43%); (e) KOH (10%), MeOH, H_2O (71%).



Scheme 7. Reagents: (a) *n*-butyllithium, THF, −78°C, then 2-butenolide, THF, −78°C, then benzyl bromide, **16**, HMPA, THF, −78°C (18%); (b) *n*-$Bu_4N^+F^-$, THF (99%); (c) $BrCH_2CO_2Et$, K_2CO_3, KI, acetone, reflux (99%); (d) Raney nickel, ethanol, reflux (38%); (e) KOH (10%), MeOH, H_2O (50%).



Scheme 8. Reagents: (a) *n*-butyllithium, THF, −78°C, then 2-butenolide, THF, −78°C, then benzyl bromide, **21**, HMPA, THF, −78°C (27%); (b) *n*-$Bu_4N^+F^-$, THF (98%); (c) $Br(CH_2)_3CO_2Et$, K_2CO_3, KI, acetone, reflux (98%); (d) Raney nickel, ethanol, reflux (97%); (e) KOH (10%), MeOH, H_2O (54%).

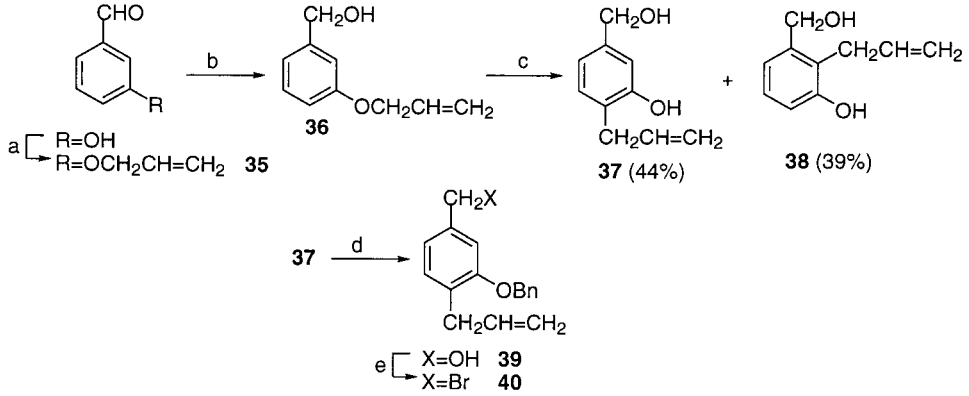
12[28] was monobenzylated[29] (Scheme 4). After subsequent silylation, methyl ester **14** was reduced to alcohol **15** with $LiAlH_4$. For the preparation of the benzyl bromide **21** for 4-carboxypropoxyenterolactone **7**, *O*-benzylcatechol **17**[30] was Reimer–Tiemann formylated with $CHCl_3$ in aqueous NaOH to afford the aldehyde **18**[31] (Scheme 5). Silyl ether formation and aldehyde reduction to alcohol **20** was accomplished in the usual manner.

Treatment of the corresponding benzyl alcohols **10**, **15** and **20** with phosphorous tribromide in ether afforded the desired benzyl bromides **11**, **16** and **21** (Schemes 3–5).[32]

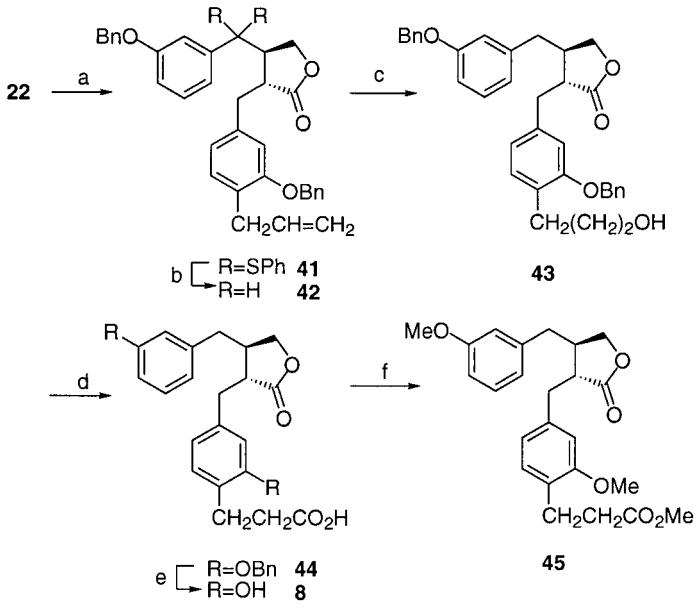
Thioacetal derivatives of dibenzylbutyrolactone **23**, **27** and **31** were prepared from the dithioacetal **22**[33] by reaction with *n*-butyllithium and 2-butenolide in THF at −78°C followed by in situ alkylation with the corresponding benzyl bromides **11**, **16** or **21** in the presence of HMPA (Schemes 6–8).[26]

Cleavage of the silyl ether protection with tetrabutylammonium fluoride,[27] and subsequent addition of ethyl bromoacetate or ethyl 4-bromobutyrate afforded the carboxylate side chain as ethyl ester in the lignan framework. Simultaneous desulfurization and debenzylation were achieved by treatment with Raney nickel in refluxing ethanol.[26] Ester hydrolysis under basic conditions completed the reaction sequence to afford the free acids, *trans*-2,3-dibenzylbutyrolactones **5**, **6** and **7** with the desired side chains.

As already mentioned, for the synthesis of a carbon–carbon



Scheme 9. (a) allyl bromide, K_2CO_3, ethanol, reflux, (75%); (b) $NaBH_4$, ethanol (79%); (c) *N*,*N*-dimethyl aniline, reflux; (d) benzyl chloride, *t*-BuOK, DMF, +70°C (74%); (e) PBr_3, ether, 0°C (86%).



Scheme 10. Reagents: (a) *n*-butyllithium, THF, −78°C, then 2-butenolide, THF, −78°C, then benzyl bromide, **40**, HMPA, THF, −78°C (41%); (b) n-$Bu_4N^+F^-$, AIBN, toluene, 90°C (69%); (c) $NaBH_4$, THF, DMSO, 35°C, then H_2O, 5°C, and then H_2O_2 (30%), NaOH (51%); (d) PDC, DMF (77%); (e) Raney nickel, ethanol, reflux (26%); (f) CH_2N_2, ether.

linked carboxylate side chain analogue a completely different approach was chosen. A functionalized side chain was generated by the preparation of benzyl bromide **40**, available by the Claisen rearrangement of allyl *m*-formylphenyl ether as a key step (Scheme 9).[34]

3-Allyloxybenzaldehyde **35** was reduced to the alcohol **36**, which in refluxing *N*,*N*-dimethylaniline underwent rearrangement to afford the two isomers of *o*-allyl phenol **37** and **38** in yields of 44% and 39%, respectively. The *O*-benzylated isomer **39** underwent bromination in the usual manner to afford the benzyl bromide **40**.

The tandem conjugate addition reaction with benzyl bromide **40** afforded the allylic dibenzylbutyrolactone **41** (Scheme 10).[26]

The phenylthio groups were eliminated with tributyltin hydride[35] in the presence of AIBN initiator affording the reduced product **42** without affecting the allyl group.[36] The desired carboxy group was generated by a reaction sequence involving the hydroboration–oxidation of alkene **42** to the primary alcohol **43**,[37] and subsequent oxidation with pyridinium dichromate in DMF,[38] yielding the carboxylic acid **44**. Benzyl ethers were cleaved with Raney nickel in refluxing ethanol to afford the desired 4-carboxyethylenterolactone **8**. As a confirmation of the successful synthesis of **8**, the product was converted to the dimethyl ether methyl ester **45** by prolonged exposure to ethereal diazomethane. The mass spectrum of the resulting product (412, M^+) confirmed the methylation of both the carboxyl group and the free phenolic hydroxy groups.

The developed analytical method utilized an enterolactone derivative for the production of antiserum and tracer.[23] The synthesis of a carboxyalkyl ether derivative introduces a carboxyl group to the molecular framework, that could be coupled to amino residues on protein and used after europium label as a tracer.[20]

The enterolactone derivative with carboxylic acid side chain was synthesized by attachment to the benzyl bromide moiety of the molecular framework either through a carbon–carbon or carbon–oxygen bond. The most favorable positions in the aromatic ring were assumed to be in *ortho*- and *meta*-position with respect to the phenolic hydroxyl group in order to avoid any steric hindrance and allow easy access to protein during the coupling procedure. The length of the chain was not restricted.

When the derivatives were used in analytical procedures, some problems concerning structural features appeared.[39] In compounds **7** and **8**, where the side chain is located next to the phenolic hydroxyl group, interaction between hydroxy and carboxy groups was observed, causing lactonization competing with the protein coupling. This problem is due to the favorable position of these groups, and the suitable length of the chain, which allows the intermolecular esterification reaction. The reason for difficulties concerning compound **5** is probably due to substitution of one of the phenolic hydroxyl groups, which either causes recognition problems or problems during europium labeling.

Taking into consideration the disadvantages discussed above, compound **6** should have most suitable structure for the experiments. Hydroxy and carboxy groups are far enough, and also the chain is short enough to avoid cyclization problems. As a matter of fact, the reported experimental results have demonstrated that the new TR-FIA method for detection and quantification of plasma enterolactone utilized successfully the synthesized derivative **6** in preparation of antiserum and tracer.[23]

Experimental

All experiments were monitored by thin layer chromatography using aluminum based, precoated silica gel sheets (Merck 60 F_{254}, layer thickness 0.2 mm). Silica gel 60 (230–400 mesh, Merck) was used for flash column chromatography. Melting points were determined on an Electrothermal melting point apparatus in an open capillary tube and are uncorrected. IR spectra were obtained with a Perkin–Elmer 125 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on 60 MHz JEOL JNM-PMX 60 or 200 MHz Varian GEMINI spectrometer in $CDCl_3$ or acetone-d_6 and chemical shifts are relative to tetramethylsilane (TMS) as an internal standard. EIMS and HRMS were obtained using JEOL JMS-SX102 spectrometer. THF was freshly distilled from sodium benzophenone ketyl, diethyl ether from sodium, and DMF and DMSO from CaH. Other solvents were of analytical grade. All commercially available chemicals were used as supplied by the manufacturers.

3-*tert*-Butyldiphenylsilyloxybenzaldehyde (9). 3-Hydroxybenzaldehyde (3.0 g, 0.025 mol) in dry DMF (7 mL) was stirred at room temperature and *tert*-butyldiphenylsilylchloride (10.1 g, 0.037 mol) and imidazole (3.3 g, 0.048 mol) was added to the solution. The reaction mixture was stirred for 5 h, poured into water and extracted with ether. The organic phase was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave **9** as a gum (8.8 g, 99%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.12 (9 H, s, 3 C*H*$_3$), 6.95 (m, ArH), 7.15–7.48 (m, 10 ArH), 7.68–7.77 (m, 3 ArH), 9.80 (1 H, s, C*H*O); ^{13}C NMR ($CDCl_3$) δ 19.34, 26.36, 120.35, 122.79, 125.97, 127.94, 129.57, 130.17, 132.25, 135.49, 137.73, 156.29, 192.19; HRMS *m*/*z* calcd for $C_{23}H_{24}O_2Si$ (M^+) 360.1546, found 360.1537.

3-*tert*-Butyldiphenylsilyloxybenzyl alcohol (10). To a stirred solution of **9** (8.8 g, 0.024 mol) in 94% ethanol (120 mL) was added in small portions $NaBH_4$ (1.85 g, 0.049 mol). The mixture was stirred at room temperature for 4 h, ethanol evaporated, and the residue was acidified with 2N H_2SO_4 and extracted with ether. The organic phase was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave **10** as a gum (8.7 g, 99%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.10 (9 H, s, 3 C*H*$_3$), 2.31 (1 H, br s, O*H*), 4.50 (2 H, s, C*H*$_2$), 6.62 (m, ArH), 6.83 (m, 2 ArH), 7.03 (m, ArH), 7.28–7.45 (m, 6 ArH), 7.68–7.78 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.36, 26.43, 65.03, 118.33, 118.89, 119.55, 127.79, 129.32, 129.93, 132.88, 135.56, 142.34, 155.84; HRMS *m*/*z* calcd for $C_{23}H_{26}O_2Si$ (M^+) 362.1702, found 362.1699.

3-*tert*-Butyldiphenylsilyloxybenzyl bromide (11). To a stirred solution of **10** (8.8 g, 0.024 mol) in dry ether (85 mL) in an ice bath was added dropwise PBr_3 (4.0 g, 0.015 mol). The solution was kept at +4°C for two days. The reaction mixture was washed with water (5×) and dried over Na_2SO_4. Evaporation of the solvent gave **11** as a gum (8.9 g, 86%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.10 (9 H, s, 3 C*H*$_3$), 4.30 (2 H, s, C*H*$_2$), 6.64 (m, ArH), 6.87 (m, 2 ArH), 7.02 (m, ArH), 7.29–7.48 (m, 6 ArH), 7.68–7.78 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.38, 26.44, 33.27, 119.82, 120.53, 121.73, 127.85, 129.51, 130.01, 132.70, 135.56, 138.95, 155.78; HRMS *m*/*z* calcd for $C_{23}H_{25}OSiBr$ (M^+) 424.0858, found 424.0870.

Methyl 3-benzyloxy-5-hydroxybenzoate (13). To a solution of **12** (20.0 g, 0.12 mol) in acetone (300 mL) was added benzyl bromide (20.3 g, 0.12 mol) and powdered K_2CO_3 (16.4 g, 0.12 mol). The reaction was refluxed for 5 h. The solid material was filtered off and washed with acetone. The filtrate, which consisted of starting material and both dibenzyl and monobenzyl ether, was evaporated. Most of the dibenzyl ether was removed by crystallization from ethanol, and the resulting residue was purified by flash column chromatography (EtOAc–hexane 1:1) to give **13** as a white solid (10.1 g, 33%): mp 98.5°C (lit.[40] mp 98–99°C); ^{1}H NMR (acetone-d_6) δ 3.85 (3 H, s, C*H*$_3$), 5.14 (2 H, s, C*H*$_2$), 6.74 (t, *J*=2.3 Hz, ArH), 7.11 (dd, *J*=1.1, 2.4 Hz, ArH), 7.15 (dd, *J*=1.4, 2.5 Hz, ArH), 7.35–7.51 (m, 5 ArH), 8.78 (1 H, s, O*H*).

Methyl 3-benzyloxy-5-*tert*-butyldiphenylsilyloxybenzoate (14). Following the same procedure as for **9**, **13** (10.0 g, 0.039 mol) was converted to **14** as a gum (19.0 g, 99%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.10 (9 H, s, 3 C*H*$_3$), 3.82 (3 H, s, C*H*$_3$), 4.79 (2 H, s, C*H*$_2$), 6.48 (t, *J*=2.4 Hz, ArH), 7.14 (dd, *J*=1.4, 2.2 Hz, ArH), 7.19 (dd, *J*=1.4, 2.4 Hz, ArH), 7.24–7.44 (m, 11 ArH), 7.71 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.41, 26.46, 52.07, 70.03, 108.89, 111.47, 114.06, 127.47, 127.91, 128.01, 128.56, 130.08, 131.83, 132.55, 135.58, 136.49, 156.63, 159.38, 166.81; HRMS *m*/*z* calcd for $C_{31}H_{32}O_4Si$ (M^+) 496.2070, found 496.2083.

3-Benzyloxy-5-*tert*-butyldiphenylsilyloxybenzyl alcohol (15). A solution of **14** (19.0 g, 0.038 mol) in dry ether (330 mL) was carefully added to $LiAlH_4$ (3.7 g, 0.097 mol) in dry ether (70 mL), and the reaction mixture was refluxed for 1 h. The mixture was treated carefully with ice-water and 1N NaOH (20 mL). The solids were filtered off and washed with ether, which was dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (pentane–ether 1:1) to give **15** as a white solid (4.9 g, 27%, mp 57°C): ^{1}H NMR ($CDCl_3$) δ 1.09 (9 H, s, 3 C*H*$_3$), 4.46 (1 H, d, *J*=6.2 Hz, C*H*$_2$), 4.77 (1 H, s, C*H*$_2$), 6.28 (t, *J*=2.2 Hz, ArH), 6.39 (m, ArH), 6.54 (m, ArH), 7.25–7.43 (m, 11 ArH), 7.68 (d, *J*=2.0 Hz, 2 ArH), 7.72 (d, *J*=1.6 Hz, 2 ArH); ^{13}C NMR ($CDCl_3$) δ 19.39, 26.46, 65.10, 69.81, 105.72, 106.49, 110.98, 127.44, 127.82, 127.89, 128.52, 129.97, 132.88, 135.59, 136.86, 143.05, 156.86, 159.78; HRMS *m*/*z* calcd for $C_{30}H_{32}O_3Si$ (M^+) 468.2121, found 468.2128.

3-Benzyloxy-5-*tert*-butyldiphenylsilyloxybenzyl bromide (16). Following the same procedure as for **11**, **15** (4.89 g, 0.010 mol) was converted to **16** as an oil (4.96 g, 89%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.09 (9 H, s, 3 C*H*$_3$), 4.25 (2 H, s, C*H*$_2$), 4.75 (2 H, s, C*H*$_2$), 6.28 (m, ArH), 6.44 (m, ArH), 6.55 (m, ArH), 7.25–7.44 (m, 11 ArH), 7.67 (m, 2 ArH), 7.71 (m, 2 ArH); ^{13}C NMR ($CDCl_3$) δ 19.39, 26.46, 33.34, 69.88, 106.57, 108.91, 113.37, 127.49, 127.88, 127.97,

128.54, 130.03, 132.70, 135.58, 136.65, 139.39, 156.77, 159.64.

3-Benzyloxy-4-*tert*-butyldiphenylsilyloxybenzaldehyde (19). Following the same procedure as for **9**, **18** (11.7 g, 0.051 mol) was converted to **19** as an oil (16.9 g, 71%), after purification by flash column chromatography (CH_2Cl_2): ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.12 (9 H, s, 3 C*H*$_3$), 5.16 (2 H, s, C*H*$_2$), 6.80–8.10 (m, 18 ArH), 10.00 (1 H, s, C*H*O).

3-Benzyloxy-4-*tert*-butyldiphenylsilyloxybenzyl alcohol (20). Following the same procedure as for **10**, **19** (8.7 g, 0.019 mol) was converted to **20** as a gum (5.3 g, 61%) after purification by flash column chromatography (acetone–cyclohexane 2:5): ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.20 (9 H, s, 3 C*H*$_3$), 2.52 (1 H, br s, O*H*), 4.45 (2 H, s, C*H*$_2$), 5.06 (2 H, s, C*H*$_2$), 6.79 (m, 2 ArH), 7.08 (m, ArH), 7.32–7.63 (m, 11 ArH), 7.84–8.10 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.60, 26.52, 65.21, 70.55, 113.07, 119.68, 120.09, 127.53, 127.61, 127.69, 128.33, 129.69, 133.41, 134.11, 135.44, 137.12, 144.94, 149.80.

3-Benzyloxy-4-*tert*-butyldiphenylsilyloxybenzyl bromide (21). Following the same procedure as for **11**, **20** (5.3 g, 0.011 mol) was converted to **21** as a white solid (5.1 g, 87%, mp 71°C) after crystallization from light petroleum (bp 40–60°C): ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.10 (9 H, s, 3 C*H*$_3$), 4.61 (2 H, s, C*H*$_2$), 5.18 (2 H, s, C*H*$_2$), 6.91 (m, 2 ArH), 7.20 (m, ArH), 7.40–7.80 (m, 11 ArH), 7.83–8.18 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.61, 26.48, 34.38, 70.61, 114.79, 120.13, 121.83, 127.57, 127.66, 127.78, 128.37, 129.78, 130.74, 133.22, 135.43, 136.90, 145.71, 149.81.

(±)-2-(3-*tert*-Butyldiphenylsilyloxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (23). To a stirred solution of **22** (5.0 g, 12 mmol) in dry THF (30 mL) maintained under argon at −78°C was added a solution of *n*-butyllithium (8.5 mL, 12 mmol) in *n*-hexane. The resulting solution was stirred for 2.5 h, and a solution of 2-butenolide (1.0 g, 12 mmol) dissolved in dry THF (4 mL) was added. The reaction mixture was stirred for a further 2.5 h at −78°C and then treated dropwise with a solution of **11** (5.1 g, 12 mmol) and HMPA (2.1 mL, 12 mmol) in dry THF (10 mL). The reaction mixture was allowed to reach room temperature overnight, and then the reaction was quenched with water. The mixture was extracted with EtOAc, washed with water and dried over Na_2SO_4. Evaporation of the solvent left a gum, which was purified by flash column chromatography (CH_2Cl_2–pentane 2:1) to give **23** as an amorphous solid (1.0 g, 10%): IR (film) 1770 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.09 (9 H, s, 3 C*H*$_3$), 2.68 (1 H, dd, *J*=5.8, 13.8 Hz, H-7), 2.90 (2 H, m and dd, overlapping, H-8′, H-7), 3.25 (1 H, m, H-8), 3.43 (1 H, dd, *J*=8.5, 10.4 Hz, H-9′), 4.32 (1 H, dd, *J*=3.0, 10.0 Hz, H-9′), 4.96 (2 H, s, C*H*$_2$), 6.40 (d, *J*=7.5 Hz, ArH), 6.51 (dd, *J*=2.6, 8.1 Hz, ArH), 6.61 (m, 2 ArH), 6.83 (m, 2 ArH), 7.16–7.42 (m, 23 ArH), 7.65 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.39, 26.47, 36.74, 44.56, 47.64, 67.97, 70.01, 72.90, 114.97, 116.33, 118.43, 120.90, 121.07, 122.36, 127.54, 127.80, 128.07, 128.64, 128.72, 129.26, 129.51, 129.95, 130.83, 132.28, 132.86, 133.34, 135.55, 135.95, 136.74, 138.25, 139.35, 155.82, 158.76, 178.43.

(±)-2-(3-Hydroxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (24). To a stirred solution of **23** (1.0 g, 1.2 mmol) in dry THF (5 mL) maintained under argon in an ice bath was added dropwise a 1 M solution of tetrabutylammoniumfluoride in THF (5.9 mL, 5.9 mmol). The reaction mixture was stirred in an ice bath for 10 min, the bath was removed and stirring continued at room temperature for 3 h. The reaction was quenched with water and the mixture was extracted with ether. The organic phase was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave **24** as an amorphous solid (0.71 g, 99%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 2.77 (1 H, dd, *J*=6.8, 13.2 Hz, H-7), 2.97 (1 H, m, H-8′), 3.09 (1 H, dd, *J*=4.6, 14.1 Hz, H-7), 3.31 (1 H, m, H-8), 3.55 (1 H, dd, *J*=8.5, 10.2 Hz, H-9′), 4.35 (1 H, dd, *J*=3.4, 10.2 Hz, H-9′), 4.98 (2 H, s, C*H*$_2$), 6.46 (m, ArH), 6.70 (m, ArH), 6.90 (m, 2 ArH), 7.06 (m, 2 ArH), 7.22–7.44 (m, 15 ArH), 7.72 (m, 2 ArH).

(±)-2-(3-Ethoxycarbonylmethoxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (25). To a solution of **24** (0.68 g, 1.12 mmol) in acetone (20 mL) was added ethyl bromoacetate (0.21 g, 1.24 mmol), K_2CO_3 (0.31 g, 2.25 mmol), and KI (0.09 g, 0.56 mmol). The reaction was refluxed for 4 h. The mixture was cooled, the solids were filtered off and rinsed with acetone. The solvent was evaporated and the residue extracted with ether. The organic phase was washed with 2 N NaOH solution and water, and dried over Na_2SO_4. Evaporation of the solvent afforded **25** as a gum (0.75 g, 96%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.29 (3 H, t, *J*=7.1 Hz, C*H*$_3$), 2.78 (1 H, dd, *J*=5.7, 13.5 Hz, H-7), 2.92 (1 H, m, H-8′), 3.09 (1 H, dd, *J*=5.1, 13.6 Hz, H-7), 3.29 (1 H, m, H-8), 3.51 (1 H, dd, *J*=8.6, 10.2 Hz, H-9′), 4.20–4.35 (3 H, q and dd, overlapping, C*H*$_2$, H-9′), 4.51 (2 H, s, C*H*$_2$), 4.96 (2 H, s, C*H*$_2$), 6.54 (m, 2 ArH), 6.75–6.91 (m, 2 ArH), 7.06–7.42 (m, 15 ArH), 7.70 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 14.08, 36.90, 44.39, 47.43, 61.35, 65.18, 68.02, 70.03, 72.96, 113.77, 115.07, 115.28, 116.32, 121.19, 122.92, 127.53, 128.02, 128.10, 128.67, 128.70, 128.80, 129.47, 129.59, 129.78, 133.01, 136.29, 138.50, 157.99, 158.79, 169.68, 178.50.

(±)-2-(3-Ethoxycarbonylmethoxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (26). A suspension of **25** (0.75 g, 1.1 mmol) in ethanol (75 mL) and W-2 Raney nickel (12 g) was refluxed for 3 h. The catalyst was removed by filtration and rinsed with acetone. Evaporation of the solvents gave a gum, which was purified by flash column chromatography (acetone–cyclohexane 1:2) to give **26** as a gum (0.18 g, 43%): IR (film) 1750, 1735 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 1.24 (3 H, t, *J*=7.0 Hz, C*H*$_3$), 2.51–3.02 (6 H, m, H-7′, H-8′, H-8, H-7), 3.89 (1 H, m, H-9′), 4.07 (1 H, m, H-9′), 4.20 (2 H, q, *J*=7.1 Hz, C*H*$_2$), 4.72 (2 H, s, C*H*$_2$), 6.59–6.72 (m, 3 ArH), 6.79–6.90 (m, 3 ArH), 7.10 (t, *J*=8.1 Hz, ArH), 7.24 (t, *J*=8.0 Hz, ArH), 8.23 (1 H, s, O*H*); HRMS *m*/*z* calcd for $C_{22}H_{24}O_6$ (M^+) 384.1573, found 384.1582; EIMS *m*/*z* 384 (M^+, 92), 277 (31), 193 (37), 191 (20), 108 (100), 107 (62); ^{13}C NMR (acetone-d_6) δ 14.43, 35.40, 38.72, 42.19, 46.83, 61.52, 65.73, 71.56, 113.72, 114.38, 116.60, 120.73, 123.44, 130.43, 130.51, 141.19, 141.41, 158.63, 159.40, 169.60, 178.90.

(±)-*trans*-2-(3-Carboxymethoxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (5). To a stirred solution of **26** (0.18 g, 0.47 mmol) in aqueous methanol (50%, 28 mL) at room temperature was added aqueous KOH (10%, 5.5 mL). The reaction was stirred for 3 h, methanol was evaporated, and water was added to the residue. The inorganic phase was washed with ether (5×), acidified with 2N H_2SO_4 and extracted with ether. The organic phase was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave **5** as an amorphous solid (0.12 g, 71%): IR (film) 2990 (br), 1760, 1710 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.48–2.79 (4 H, m, H-7′, H-8′, H-8), 2.85–3.08 (2 H, m, H-7), 3.89 (1 H, m, H-9′), 4.07 (1 H, m, H-9′), 4.72 (2 H, s, CH_2), 6.59–6.71 (m, 3 ArH), 6.78–6.92 (m, 3 ArH), 7.09 (t, J=8.0 Hz, ArH), 7.24 (t, J=8.0 Hz, ArH), 8.03 (1 H, s, OH); ^{13}C NMR (acetone-d_6) δ 35.42, 38.70, 42.20, 46.75, 65.26, 71.49, 113.50, 114.21, 116.43, 116.56, 120.64, 123.20, 130.30, 130.39, 141.02, 141.28, 158.35, 159.21, 178.68, 180.34; HRMS m/z calcd for $C_{20}H_{20}O_6$ (M^+) 356.1260, found 356.1261; EIMS m/z 356 (M^+, 63), 338 ($M-H_2O$, 3), 249 (27), 191 (18), 165 (28), 108 (100), 107 (42).

(±)-2-(3-Benzyloxy-5-*tert*-butyldiphenylsilyloxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (27). In a similar manner as for **23** using benzyl bromide 16 (4.96 g, 9.3 mmol) in place of benzyl bromide **11** to give **27** as an amorphous solid (1.63 g, 18%): IR (film) 1770 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.08 (9 H, s, 3 CH_3), 2.62 (1 H, dd, J=5.5, 13.6 Hz, H-7), 2.90 (2 H, m, H-8′, H-7), 3.24 (1 H, m, H-8), 3.49 (1 H, dd, J=8.4, 10.0 Hz, H-9′), 4.32 (1 H, dd, J=2.9, 10.2 Hz, H-9′), 4.64 (2 H, s, CH_2), 4.95 (2 H, s, CH_2), 6.18 (m, 2 ArH), 6.88 (m, ArH), 7.14–7.39 (m, 30 ArH), 7.69 (m, 4 ArH); ^{13}C NMR ($CDCl_3$) δ 19.42, 26.47, 36.94, 44.56, 47.86, 68.10, 69.63, 70.01, 72.86, 105.55, 109.28, 113.80, 114.98, 116.35, 121.09, 127.39, 127.55, 127.85, 128.06, 128.19, 128.50, 128.65, 128.70, 129.20, 129.48, 130.00, 130.91, 132.21, 132.88, 133.54, 135.22, 135.59, 135.68, 136.76, 136.82, 138.87, 139.47, 156.80, 158.72, 159.52, 178.50.

(±)-2-(3-Benzyloxy-5-hydroxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (28). Following the same procedure as for **24**, **27** (1.63 g, 1.7 mmol) was converted to **28** as a gum (1.21 g, 99%), which was used directly in the next step: ^1H NMR ($CDCl_3$) δ 2.72 (1 H, dd, J=6.0, 13.8 Hz, H-7), 2.96 (1 H, m, overlapping, H-8′), 3.05 (1 H, dd, J=5.0, 13.0 Hz, H-7), 3.28 (1 H, m, H-8), 3.62 (1 H, dd, J=8.4, 10.2 Hz, H-9′), 4.37 (1 H, dd, J=3.5, 10.1 Hz, H-9′), 4.93 (2 H, s, CH_2), 4.97 (2 H, s, CH_2), 6.02 (m, ArH), 6.20 (m, ArH), 6.36 (m, ArH), 6.90 (m, ArH), 7.16–7.45 (m, 20 ArH), 7.71 (m, 3 ArH).

(±)-2-(3-Benzyloxy-5-ethoxycarbonylmethoxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (29). Following the same procedure as for **25**, **28** (1.2 g, 1.7 mmol) was converted to **29** as a gum (1.34 g, 99%), which was used directly in the next step: ^1H NMR ($CDCl_3$) δ 1.28 (3 H, t, J=7.1 Hz, CH_3), 2.75 (1 H, dd, J=5.7, 13.4 Hz, H-7), 2.99 (1 H, m, overlapping, H-8′), 3.08 (1 H, dd, J=5.1, 13.5 Hz, H-7), 3.29 (1 H, m, H-8), 3.60 (1 H, dd, J=8.4, 10.1 Hz, H-9′), 4.26 (2 H, q, J=7.2 Hz, CH_2), 4.36 (1 H, dd, J=3.3, 10.2 Hz, H-9′), 4.49 (2 H, s, CH_2), 4.93 (2 H, s, CH_2), 4.96 (2 H, s, CH_2), 6.15 (m, ArH), 6.28 (m, ArH), 6.45 (m, ArH), 6.90 (m, ArH), 7.13–7.45 (m, 20 ArH), 7.71 (m, 3 ArH); ^{13}C NMR ($CDCl_3$) δ 14.07, 37.12, 44.40, 47.59, 61.37, 65.17, 68.16, 69.97, 70.01, 72.90, 101.09, 107.78, 109.34, 115.06, 116.32, 121.20, 127.53, 127.56, 128.08, 128.63, 128.66, 128.70, 128.76, 128.87, 129.42, 129.56, 133.13, 135.22, 136.09, 139.29, 158.76, 159.02, 160.06, 168.69, 178.65.

(±)-2-(3-Hydroxy-5-ethoxycarbonylmethoxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (30). Following the same procedure as for **26**, **29** (1.37 g, 1.7 mmol) was converted to **30** as an amorphous solid (0.26 g, 38%) after purification by flash column chromatography (EtOAc–hexane 4:1): IR (film) 1750, 1735 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.24 (3 H, t, J=7.1 Hz, CH_3), 2.56 and 2.68 (4 H, m and m, overlapping, H-7′, H-8′, H-8), 2.87 (2 H, m, H-7), 3.88 (1 H, m, H-9′), 4.05 (1 H, m, H-9′), 4.20 (2 H, q, J=7.1 Hz, CH_2), 4.65 (2 H, s, CH_2), 6.30 (t, J=2.3 Hz, ArH), 6.36 (dd, J=1.4, 2.2 Hz, ArH), 6.42 (dd, J=1.6, 1.8 Hz, ArH), 6.65 (m, 3 ArH), 7.10 (t, J=8.0 Hz, ArH), 8.22 (1 H, br s, OH) 8.41 (1 H, br s, OH); HRMS m/z calcd for $C_{22}H_{24}O_7$ (M^+) 400.1522, found 400.1516; EIMS m/z 400 (M^+, 55), 293 (11), 210 (100), 209 (6), 191 (3), 107 (15); ^{13}C NMR (acetone-d_6) δ 14.20, 35.27, 38.49, 41.91, 46.47, 61.26, 65.53, 71.30, 101.13, 107.55, 110.39, 114.14, 116.36, 120.54, 130.28, 141.23, 141.63, 158.40, 159.43, 160.29, 169.38, 178.68.

(±)-*trans*-2-(3-Hydroxy-5-carboxymethoxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (6). Following the same procedure as for **5**, **30** (0.22 g, 0.55 mmol) was converted to **6** as a white solid (0.10 g, 50%, mp 130–133°C) after crystallization from chloroform: IR (film) 2990 (br), 1750, 1710 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.56 and 2.68 (4 H, m and m, overlapping, H-7′, H-8′, H-8), 2.89 (2 H, m, H-7), 3.89 (1 H, m, H-9′), 4.06 (1 H, dd, J=7.0, 8.6 Hz, H-9′), 4.67 (2 H, s, CH_2), 6.32 (t, J=2.3 Hz, ArH), 6.38 (dd, J=1.4, 2.2 Hz, ArH), 6.43 (dd, J=1.4, 2.0 Hz, ArH), 6.64 (m, 3 ArH), 7.09 (t, J=8.1 Hz, ArH), 8.43 (2 H, s, OH); ^{13}C NMR (acetone-d_6) δ 35.50, 38.71, 42.16, 46.65, 65.31, 71.48, 101.16, 107.82, 110.37, 114.23, 116.46, 120.68, 130.40, 141.33, 141.72, 158.37, 159.48, 160.35, 178.72, 180.37; HRMS m/z calcd for $C_{20}H_{20}O_7$ (M^+) 372.1210, found 372.1212; EIMS m/z 372 (M^+, 43), 354 ($M-H_2O$, 2), 265 (11), 191 (7), 182 (100), 181 (12), 107 (22).

(±)-2-(3-Benzyloxy-4-*tert*-butyldiphenylsilyloxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (31). In a similar manner as for **23** using benzyl bromide **21** (6.7 g, 0.013 mol) in place of benzyl bromide **11** to give **31** as an amorphous solid (3.26 g, 27%): IR (film) 1770 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.07 (9 H, s, 3 CH_3), 2.62 (1 H, dd, J=5.6, 13.6 Hz, H-7), 2.78 (1 H, m, H-8′), 3.02 (1 H, dd, J=4.4, 13.6 Hz, H-7), 3.10–3.28 (2 H, m, H-8, H-9′), 4.28 (1 H, dd, J=2.9, 9.9 Hz, H-9′), 4.82 (2 H, s, CH_2), 4.97 (2 H, s, CH_2), 6.03 (1 H, dd, J=1.8, 8.0 Hz, ArH), 6.48 (1 H, d, J=8.2 Hz, ArH), 6.57 (1 H, d, J=2.0 Hz, ArH), 6.90 (m, ArH), 7.05–7.43 (m, 30 ArH), 7.71 (t, J=7.5 Hz, 3 ArH); ^{13}C NMR ($CDCl_3$) δ 19.57, 26.51, 36.65, 44.57, 47.12, 68.09, 70.05, 70.46, 73.02, 114.59, 114.93, 116.35, 120.25, 121.10, 121.80, 127.56, 127.62, 127.66, 127.71, 127.93, 128.09, 128.33, 128.66, 128.72,

129.42, 129.54, 129.82, 130.55, 132.38, 132.98, 133.29, 133.44, 135.55, 136.41, 136.73, 136.93, 139.23, 144.43, 149.90, 158.79, 178.87.

(±)-2-(3-Benzyloxy-4-hydroxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (32). Following the same procedure as for **24**, **31** (0.8 g, 0.84 mmol) was converted to **32** as a gum (0.59 g, 98%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 2.76 (1 H, dd, J=5.4, 13.6 Hz, H-7), 2.94 (1 H, m, H-8′), 3.07 (1 H, dd, J=4.7, 13.6 Hz, H-7), 3.28 (1 H, m, H-8), 3.52 (1 H, dd, J=8.4, 10.1 Hz, H-9′), 4.33 (1 H, dd, J=3.7, 10.1 Hz, H-9′), 4.96 (2 H, s, CH_2), 4.97 (2 H, s, CH_2), 6.36 (1 H, dd, J=1.8, 8.0 Hz, ArH), 6.66 (1 H, d, J=1.8 Hz, ArH), 6.74 (1 H, d, J=8.0 Hz, ArH), 6.90 (m, ArH), 7.17–7.41 (m, 21 ArH), 7.72 (m, 2 ArH).

(±)-2-(3-Benzyloxy-4-ethoxycarbonylpropyloxybenzyl)-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (33). In a similar manner as for **25** using ethyl 4-bromobutyrate (0.18 g, 0.91 mmol) in place of ethyl bromoacetate, **32** (0.59 g, 0.83 mol) was converted to **33** as a gum (0.67 g, 98%) after purification by flash column chromatography (acetone–cyclohexane 1:2): ^{1}H NMR ($CDCl_3$) δ 1.26 (3 H, t, J=7.0 Hz, CH_3), 2.14 (2 H, m, CH_2), 2.55 (2 H, t, J=7.0 Hz, CH_2), 2.76 (1 H, m, H-7), 2.93 (1 H, m, H-8′), 3.07 (1 H, dd, J=4.6, 12.8 Hz, H-7), 3.27 (1 H, m, H-8), 3.50 (1 H, dd, J=8.4, 10.1 Hz, H-9′), 4.04 (2 H, t, J=6.3 Hz, CH_2), 4.14 (2 H, q, J=7.1 Hz, CH_2), 4.32 (1 H, m, 9′-H), 4.97 (2 H, s, CH_2), 4.98 (2 H, s, CH_2), 6.40 (m, ArH), 6.68 (m, 2 ArH), 6.90 (m, ArH), 7.22–7.44 (m, 23 ArH).

(±)-2-(3-Hydroxy-4-ethoxycarbonylpropyloxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (34). Following the same procedure as for **26**, **33** (0.67 g, 0.81 mmol) was converted to **34** as a gum (0.34 g, 97%), which was used directly in the next step: IR (film) 1750, 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (3 H, t, J=7.1 Hz, CH_3), 2.16 (2 H, m, CH_2), 2.26–2.61 (4 H, m, H-7′, H-8, H-8′), 2.52 (2 H, t, J=6.8 Hz, CH_2), 2.79 (1 H, dd, J=7.2, 14.0 Hz, H-7), 3.02 (1 H, dd, J=4.4, 14.6 Hz, H-7), 3.89 (1 H, m, H-9′), 4.05–4.22 (5 H, m, t, q, overlapping, H-9′, 2 CH_2), 6.40 (m, ArH), 6.56–6.77 (m, 5 ArH), 7.12 (t, J=7.7 Hz, ArH); HRMS m/z calcd for $C_{24}H_{28}O_7$ (M^+) 428.1835, found 428.1823; EIMS m/z 428 (M^+, 15), 191 (3), 115 (100), 107 (6).

(±)-*trans*-2-(3-Hydroxy-4-carboxypropoxybenzyl)-3-(3′-hydroxybenzyl)butyrolactone (7). Following the same procedure as for **5**, **34** (0.34 g, 0.79 mmol) was converted to **7** as greyish solid (0.17 g, 54%, mp 135°C) after crystallization from chloroform: IR (film) 2990 (br), 1750, 1710 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 2.07 (2 H, m, overlapping, CH_2), 2.50–2.70 (6 H, m, overlapping, H-7′, H-8′, H-8), 2.53 (2 H, t, J=7.1 Hz, CH_2), 2.77–2.99 (2 H, m, H-7), 3.87 (1 H, m, H-9′), 4.04 (2 H, m, overlapping, H-9′), 4.07 (2 H, t, J=6.2 Hz, CH_2), 6.59–6.89 (m, 6 ArH), 7.09 (t, J=7.9 Hz, ArH), 8.30 (2 H, br s, OH); ^{13}C NMR (acetone-d_6) δ 25.44, 30.77, 34.72, 38.72, 41.99, 46.96, 68.71, 71.45, 113.34, 114.18, 116.41, 117.05, 120.65, 121.31, 130.40, 132.19, 141.40, 146.33, 147.52, 158.38, 174.78, 178.80; HRMS m/z calcd for $C_{22}H_{24}O_7$ (M^+) 400.1522, found 400.1526; EIMS m/z 400 (M^+, 27), 382 ($M-H_2O$, 5), 314 (35), 191 (7), 180 (28), 123 (100), 107 (20).

3-(2-Propenyloxy)benzaldehyde (35). To a solution of 3-hydroxybenzaldehyde (61.1 g, 0.50 mol) in ethanol (500 mL) was added allyl bromide (60.5 g, 0.50 mol) and powdered anhydrous K_2CO_3 (138.2 g, 1.0 mol). The reaction mixture was refluxed overnight, poured into water and extracted with ether. The organic phase was washed with 2% KOH and water, and dried over $MgSO_4$. Evaporation left a crude product, which was distilled at reduced pressure to afford **35** as a colorless liquid (61.1 g, 75%): bp 97°C (0.85 mm) [lit.[41] bp 71–74°C (0.3–0.4 mm)]: ^{1}H NMR ($CDCl_3$) δ 4.60 (2 H, d, J=7.0 Hz, CH_2), 5.39 (2 H, m, CH_2), 6.02 (1 H, m, CH), 7.18 (m, ArH), 7.45 (m, 3 ArH), 9.97 (1 H, s, CHO); HRMS m/z calcd for $C_{10}H_{10}O_2$ (M^+) 162.0681, found 162.0666.

3-(2-Propenyloxy)benzyl alcohol (36). To a stirred solution of **35** (61.0 g, 0.38 mol) in 94% ethanol (150 mL) at room temperature was added in small portions $NaBH_4$ (6.5 g, 0.17 mol). The reaction mixture was stirred for 5 h, glacial acetic acid was added, and the mixture was neutralized with 10% $NaHCO_3$ and extracted with toluene. The organic phase was dried over $MgSO_4$. Evaporation left a crude product, which was distilled under reduced pressure to give **36** as a colorless liquid (48.7 g, 79%): bp 120°C (1.3 mm): ^{1}H NMR ($CDCl_3$) δ 2.21 (1 H, br s, OH), 4.55 (2 H, d, J=7.0 Hz, CH_2), 4.64 (2 H, s, CH_2), 5.38 (2 H, m, CH_2), 6.08 (1 H, m, CH), 6.86 (dd, J=2.1, 8.0 Hz, ArH), 6.94 (m, 2 ArH), 7.28 (t, J=8.0 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 64.66, 68.55, 112.96, 113.76, 117.52, 119.18, 129.40, 133.16, 142.53, 158.67; HRMS m/z calcd for $C_{10}H_{12}O_2$ (M^+) 164.0837, found 164.0840.

2-(2-Propenyl)-3-hydroxybenzyl alcohol and 3-hydroxy-4-(2-propenyl)benzyl alcohol (37 and 38). A mixture of compound **36** (48.3 g, 0.29 mol) and *N*,*N*-dimethylaniline (95.6 g, 0.79 mol) was refluxed overnight. The reaction mixture was acidified with 1N H_2SO_4 and extracted with ether. The organic phase was dried over $MgSO_4$. Evaporation left a crude product, which was purified by flash column chromatography (CH_2Cl_2–EtOAc 4:1) to give compound **37** as an oil (21.3 g, 44%) and compound **38** as glassy crystals (18.7 g, 39%, mp 64.5–65.5°C).

Compound 37. ^{1}H NMR (acetone-d_6) δ 3.46 (2 H, d, J=6.0 Hz, CH_2), 4.03 (1 H, t, J=5.9 Hz, O$H_{\text{alcoholic}}$), 4.60 (2 H, d, J=6.0 Hz, CH_2), 4.82–5.02 (2 H, m, CH_2), 5.82–6.08 (1 H, m, CH), 6.78 (d, J=7.7 Hz, ArH), 6.99 (s, ArH), 7.34 (d, J=7.7 Hz, ArH), 8.16 (1 H, s, OH_{phenolic}); ^{13}C NMR (acetone-d_6) δ 34.72, 64.60, 114.30, 115.36, 118.77, 125.84, 130.62, 138.42, 142.84, 155.82; HRMS m/z calcd for $C_{10}H_{12}O_2$ (M^+) 164.0837, found 164.0846.

Compound 38. ^{1}H NMR (acetone-d_6) δ 3.37 (2 H, d, J=6.2 Hz, CH_2), 4.09 (1 H, t, J=6.0 Hz, O$H_{\text{alcoholic}}$), 4.54 (2 H, d, J=6.0 Hz, CH_2), 5.10 (2 H, m, CH_2), 5.89–6.09 (1 H, m, CH), 6.78 (d, J=8.0 Hz, ArH), 6.80 (m, ArH), 7.06 (dd, J=2.0, 7.7 Hz, ArH), 8.19 (1 H, s, OH_{phenolic}).

3-Benzyloxy-4-(2-propenyl)benzyl alcohol (39). To a stirred solution of **37** (9.4 g, 0.057 mol) in DMF (95 mL)

maintained under argon was added KOBu-*t* (8.5 g, 0.076 mol) and benzyl chloride (7.2 g, 0.057 mol). The reaction mixture was stirred at +70°C for 2 h, quenched with water, and extracted with ether. The organic phase was dried over $MgSO_4$. Evaporation of the solvent left a crude product, which was purified by flash column chromatography (CH_2Cl_2–EtOAc 9:1) to afford **39** as a solid (10.6 g, 74%, mp 42–48°C): ^{1}H NMR ($CDCl_3$) δ 3.43 (2 H, d, J=6.7 Hz, CH_2), 4.61 (2 H, s, CH_2), 5.00–5.11 (2 H, m, overlapping, CH_2), 5.07 (2 H, s, CH_2), 5.90–6.10 (1 H, m, C*H*), 6.88 (d, J=7.4 Hz, ArH), 6.95 (s, ArH), 7.14 (d, J=7.6 Hz, ArH), 7.30–7.46 (m, 5 ArH); ^{13}C NMR ($CDCl_3$) δ 34.18, 65.31, 69.87, 110.38, 115.55, 119.26, 127.17, 127.82, 128.53, 129.98, 136.90, 137.27, 140.34, 156.62; HRMS *m*/*z* calcd for $C_{17}H_{18}O_2$ (M^+) 254.1307, found 254.1316.

3-Benzyloxy-4-(2-propenyl)benzyl bromide (40). To a stirred solution of **39** (8.7 g, 0.034 mol) in dry ether (60 mL) in an ice bath was added dropwise PBr_3 (5.6 g, 0.021 mol). The reaction mixture was stirred for 30 min and quenched with water. The organic phase was washed with water (5×) and dried over $MgSO_4$. Evaporation of the solvent left a crude product, which was crystallized from light petroleum (bp 40–60°C) to give **40** as a white powder (9.3 g, 86%, mp 61–62°C): ^{1}H NMR ($CDCl_3$) δ 3.41 (2 H, d, J=6.0 Hz, CH_2), 4.48 (2 H, s, CH_2), 4.98–5.15 (2 H, m, overlapping, CH_2), 5.09 (2 H, s, CH_2), 5.88–6.07 (1 H, m, C*H*), 6.90–6.98 (m, 2 ArH), 7.12 (d, J=7.8 Hz, ArH), 7.32–7.49 (m, 5 ArH); ^{13}C NMR ($CDCl_3$) δ 33.87, 34.18, 69.91, 112.23, 115.83, 121.38, 127.21, 127.88, 128.54, 129.63, 130.08, 136.52, 136.93, 137.00, 156.53; HRMS *m*/*z* calcd for $C_{17}H_{17}OBr$ (M^+) 316.0463, found 316.0472.

(±)-2-[3-Benzyloxy-4-(2-propenyl)benzyl]-3-[3′-benzyloxy-α,α-bis(phenylthio)benzyl]butyrolactone (41). In a similar manner as for **23** using benzyl bromide **40** (3.92 g, 0.012 mol) in place of benzyl bromide **11** to give **41** as a white amorphous solid (3.7 g, 41%): IR (film) 1770, 1610, 1579, 1253, 1021, 911 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.82 (1 H, dd, J=5.8, 13.8 Hz, H-7), 2.93 (1 H, m, H-8′), 3.10 (1 H, dd, J=4.7, 13.6 Hz, H-7), 3.32 (1 H, m, H-8), 3.41 (2 H, d, J=6.3 Hz, CH_2), 3.54 (1 H, m, H-9′), 4.35 (1 H, dd, J=3.1, 10.1 Hz, H-9′), 4.93 (2 H, s, CH_2), 4.97 (2 H, s, CH_2), 5.02 (2 H, dd, overlapping, CH_2), 5.90–6.07 (1 H, m, C*H*), 6.45 (d, J=7.7 Hz, ArH), 6.62 (s, ArH), 6.86–6.98 (m, 2 ArH), 7.14–7.40 (m, 23 ArH); ^{13}C NMR ($CDCl_3$) δ 33.98, 36.77, 44.47, 47.30, 67.98, 69.70, 69.95, 72.86, 112.58, 114.90, 115.45, 116.29, 121.17, 121.82, 127.09, 127.44, 127.63, 127.72, 127.91, 128.01, 128.44, 128.58, 128.60, 128.66, 129.32, 129.51, 129.91, 130.61, 132.34, 132.94, 135.98, 136.17, 136.63, 136.88, 137.13, 139.17, 156.42, 158.71, 178.66.

(±)-2-[3-Benzyloxy-4-(2-propenyl)benzyl]-3-(3′-benzyloxybenzyl)butyrolactone (42). To a stirred solution of **41** (3.69 g, 5.0 mmol) in dry toluene (60 mL) maintained under argon at 90°C was added in small portions during 30 min, a mixture of tributyltin hydride[35] (6.0 g, 20.1 mmol) and AIBN (0.22 g, 1.3 mmol). The reaction mixture was stirred for 2 h, cooled and concentrated under reduced pressure. The crude product was purified by flash column chromatography (cyclohexane–toluene 1:1) and (EtOAc–cyclohexane 1:4) to give **42** as an oil (1.8 g, 69%): IR (film) 1770, 1610, 1580, 1257, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.41–2.62 (4 H, m, H-7′, H-8′, H-8), 2.95 (2 H, m, H-7), 3.40 (2 H, d, J=6.8 Hz, CH_2), 3.80 (1 H, dd, J=7.2, 9.0 Hz, H-9′), 4.05 (1 H, dd, J=6.4, 9.2 Hz, H-9′), 5.00 (2 H, s, CH_2), 5.03 (2 H, s, CH_2), 5.07 (2 H, m, overlapping, CH_2), 5.88–6.09 (1 H, m, C*H*), 6.57 (d, J=7.4 Hz, 2 ArH), 6.70 (d, J=9.2 Hz, 2 ArH), 6.82 (dd, J=2.1, 7.9 Hz, ArH), 7.06–7.51 (m, 12 ArH); ^{13}C NMR ($CDCl_3$) δ 34.07, 34.80, 38.40, 40.90, 46.34, 69.79, 69.90, 71.11, 112.62, 112.85, 115.57, 121.25, 121.71, 127.19, 127.49, 127.70, 127.78, 128.09, 128.54, 128.66, 129.80, 129.94, 136.83, 136.91, 137.25, 139.61, 156.53, 159.04, 178.67; HRMS *m*/*z* calcd for $C_{35}H_{34}O_4$ (M^+) 518.2457, found 518.2470; EIMS *m*/*z* 518 (M^+, 22), 427 (23), 106 (78), 91 (100).

(±)-2-[3-Benzyloxy-4-(3-hydroxypropyl)benzyl]-3-(3′-benzyloxybenzyl)butyrolactone (43). A solution of **42** (1.8 g, 3.5 mmol) in dry THF (40 mL) was added to $NaBH_4$ (0.09 g, 2.4 mmol). The reaction mixture was heated to 35°C, and dimethyl sulfate (0.26 g, 2.1 mmol) was added dropwise with stirring during 5 min. The reaction was stirred for 4 h, cooled to +5°C, and quenched carefully with water. The mixture was allowed to reach room temperature, and 2N NaOH (1.7 mL) was added in one portion. The mixture was cooled again, 30% H_2O_2 (0.6 mL) was added dropwise, and stirring was continued for 20 min at room temperature. The mixture was extracted with ether, washed with ice water and saturated NaCl solution, and dried over $MgSO_4$. Evaporation of the solvent left a crude product, which was purified by flash column chromatography (EtOAc–cyclohexane 1:1) to afford **43** as a gum (0.94 g, 51%): IR (film) 3430 (br), 1760, 1250, 1015 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.83 (2 H, t, J=7.0 Hz, CH_2), 2.42–2.63 (4 H, m, H-7′, H-8′, H-8), 2.72 (2 H, t, J=7.0 Hz, CH_2), 2.95 (2 H, m, H-7), 3.56 (2 H, t, J=6.0 Hz, CH_2), 3.81 (1 H, dd, J=7.4, 9.5 Hz, H-9′), 4.04 (1 H, dd, J=6.2, 9.2 Hz, H-9′), 5.01 (2 H, s, CH_2), 5.03 (2 H, s, CH_2), 6.58 (d, J=7.0 Hz, 2 ArH), 6.71 (d, J=7.1 Hz, 2 ArH), 6.83 (m, ArH), 7.06–7.43 (m, 12 ArH); ^{13}C NMR ($CDCl_3$) δ 25.73, 32.86, 34.76, 38.38, 40.93, 46.33, 61.90, 69.92, 70.03, 71.12, 112.67, 112.80, 115.66, 121.25, 121.91, 127.36, 127.50, 128.02, 128.12, 128.69, 129.07, 129.82, 130.32, 136.82, 137.01, 139.63, 156.79, 159.07, 178.58; HRMS *m*/*z* calcd for $C_{35}H_{36}O_5$ (M^+) 536.2563, found 536.2569; EIMS *m*/*z* 536 (M^+, 14), 445 (4), 106 (80), 91 (100).

(±)-2-[3-Benzyloxy-4-(2-carboxyethyl)benzyl]-3-(3′-benzyloxybenzyl)butyrolactone (44). A stirred mixture of **43** (0.94 g, 1.8 mmol) and pyridinium dichromate (2.3 g, 6.1 mmol) in dry DMF (20 mL) was maintained under argon at room temperature for two days. The reaction mixture was poured into water (215 mL), extracted with ether and dried over $MgSO_4$. Evaporation of the solvent gave **44** as an oil (0.74 g, 77%), which was used directly in the next step: IR (film) 2950 (br), 1770, 1715, 1270 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.32–2.72 (4 H, m, overlapping, H-7′, H-8′, H-8), 2.66 (2 H, t, J=7.7 Hz, CH_2), 2.91–3.06 (4 H, m and t, overlapping, H-7, CH_2), 3.82 (1 H, dd, J=7.1, 9.1 Hz, H-9′), 4.05 (1 H, dd, J=6.6, 9.2 Hz, H-9′), 5.00 (2 H, s, CH_2), 5.07 (2 H, s, CH_2), 6.60–6.84 (m, 5 ArH), 7.06–7.50 (m, 12 ArH); ^{13}C NMR ($CDCl_3$) δ 25.60, 33.38,

34.75, 38.36, 40.90, 46.29, 69.70, 69.98, 71.13, 112.53, 112.74, 115.74, 121.29, 121.69, 127.17, 127.53, 127.88, 128.14, 128.63, 128.69, 129.82, 130.20, 136.80, 137.39, 139.63, 156.40, 159.01, 178.60; HRMS *m/z* calcd for $C_{35}H_{34}O_6$ (M^+) 550.2355, found 550.2349; EIMS *m/z* 550 (M^+, 6), 459 (8), 106 (75), 91 (100).

(±)-*trans*-2-[3-Hydroxy-4-(2-carboxyethyl)benzyl]-3-(3′-hydroxybenzyl)butyrolactone (8). A suspension of **44** (0.74 g, 1.3 mmol) in ethanol (60 mL) and W-2 Raney nickel (7.5 g, 0.13 mol) was refluxed for 3 h. The catalyst was filtered by rinsing with acetone, and the solvents were evaporated. The residue was purified by extraction of the impurities from basic aqueous solution into ether. Acidification with 2N H_2SO_4, and extraction with ether afforded **8** as an amorphous solid (0.13 g, 26%): IR (film) 2990 (br), 1750, 1710 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.50–2.72 (6 H, m, H-7′, H-8, H-8′, CH_2), 2.80–3.12 (4 H, m, H-7, CH_2), 3.89 (1 H, dd, J=7.1, 9.1 Hz, H-9′), 4.04 (1 H, dd, J=6.6, 9.2 Hz, H-9′), 6.58–6.82 (m, 4 ArH), 7.00–7.20 (m, 2 ArH), 7.4 (m, ArH); ^{13}C NMR (acetone-d_6) δ 26.33, 34.63, 34.98, 38.77, 42.11, 46.90, 71.61, 114.29, 116.50, 117.07, 120.66, 121.72, 130.20, 130.76, 130.83, 138.60, 141.39, 145.58, 158.49, 175.50, 178.28; HRMS *m/z* calcd for $C_{21}H_{20}O_5$ ($M-H_2O$) 352.1311, found 352.1299; EIMS *m/z* 352 ($M-H_2O$, 57), 324 (4), 245 (23), 191 (11), 161 (32), 107 (26), 91 (100). Compound **8** was methylated for mass spectrum by diazomethane in ether solution at room temperature to give the trimethylated product **45**: EIMS *m/z* 412 (M^+).

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