Synthesis and Protein Kinase C Inhibitory Activities of Acyclic Balanol Analogs That Are Highly Selective for Protein Kinase C over Protein Kinase A

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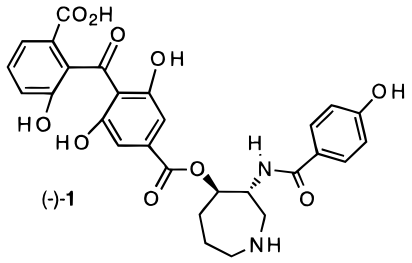
A series of balanol analogs in which the perhydroazepine ring and the *p*-hydroxybenzamide moiety were combined into an acyclic linked unit have been prepared and evaluated for their inhibitory properties against the serine/threonine kinase PKC. Several low-micromolar to low-nanomolar inhibitors of the α, β_I, β_{II}, γ, δ, ϵ, and η PKC isozymes were prepared. In general, these acyclic balanol analogs were found to be highly selective for PKC over the serine/threonine kinase PKA. The type and number of atoms linking the benzophenone ester to the *p*-hydroxyphenyl group necessary for optimal PKC inhibition were investigated. The most potent compounds contained a three-carbon linker in which the carboxamide moiety of balanol had been replaced by a methylene group. The effect of placing substituents on the three-carbon chain was also investigated. The preferred compounds contained either a 2-benzenesulfonamido (**6b**) or a 1-methyl (**21b**) substituent. The preferred compounds **6b** and **21b** were tested against a panel of serine/threonine kinases and found to be highly selective for PKC. The more active enantiomer of **6b**, (*S*)-**12b**, was 3−10-fold more active than the *R*-enantiomer against the PKC isozymes. The effect of making the analogs more rigid by making the three-carbon chain part of a five-membered ring, but with retention of the methylene replacement for the carboxamide moiety, led to potent PKC inhibitors including *anti*-substituted pyrrolidine analog **35b** and the most potent PKC inhibitor in the series, *anti*-substituted cyclopentane analog **29b**. The *anti* cyclopentane analog **29b** was a low-micromolar inhibitor of the PMA-induced superoxide burst in neutrophils, and its carboxylic ester was a high-nanomolar inhibitor of neutrophils. Finally esterification of **21b**, (*S*)-**12b**, and **35b** turned these potent PKC inhibitors into low-micromolar inhibitors of neutrophils.

Introduction

Protein kinase C (PKC) is a family of phospholipid dependent, serine/threonine specific kinases that plays a key role in signal transduction pathways which lead to cellular proliferation and differentiation.[1] Activated PKC phosphorylates numerous proteins which are involved in many biological systems, and enhanced activation of PKC has been implicated in many disease states. Inhibitors of PKC may be useful in therapeutic treatment of a number of diseases such as cancer, asthma, rheumatoid arthritis, diabetic complications, psoriasis, and central nervous system disorders.[2]

As part of our efforts directed toward finding therapeutic agents for the treatment of PKC-mediated disorders, we discovered balanol, (−)-**1**, one of the most potent PKC inhibitors. Balanol was isolated here as a metabolite of the fungus *Verticillium balanoides*[3] and later from a species of *Fusarium* at Nippon Roche.[4] Its low-nanomolar potency and novel structure make balanol an exciting target for total synthesis and structure−activity relationship (SAR) studies. These reasons coupled with its low availability from natural sources have led to three total syntheses of balanol: from our laboratory,[5] from Nicolaou's,[6] and by a team at Rhone-Poulenc Rorer.[7]

Balanol consists of three different parts: the tetra-substituted benzophenone, the *p*-hydroxybenzamide moiety, and the perhydroazepine ring. Due to the complexity of their synthesis, more readily accessible



(-)-**1**

replacements were desired for both the benzophenone ester and the perhydroazepine portions.

Balanol has been shown to be an ATP competitive inhibitor of PKC.[4,8] Balanol is not selective for PKC over PKA, the c-AMP dependent kinase,[8,9] which is not surprising considering the high degree of homology found among the catalytic sites of protein kinases. In order for an inhibitor to be a useful therapeutic agent, it will need to be selective for one kinase. Herein, we would like to report the syntheses and activity of potent PKC inhibitors which are selective for PKC over PKA and in which the perhydroazepine ring and the *p*-hydroxybenzamide moiety have been combined into an acyclic linked unit.

Chemistry

In general, the acyclic analogs were made by first preparing the perhydroazepine ring−hydroxybenzamide replacement which was then coupled to a protected, fully functionalized benzophenone. The syntheses of the

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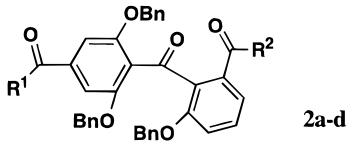
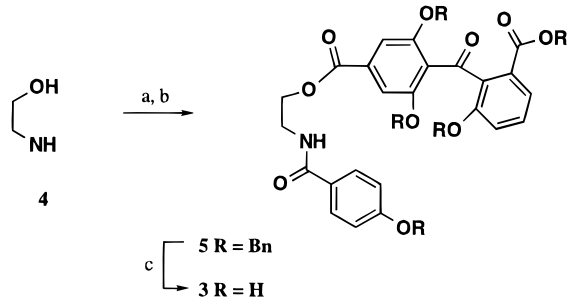
Table 1. Protected Benzophenones



2a-d

	R^1	R^2
2a	Cl	OBn
2b	OH	OBn
2c	Cl	OMe
2d	1-imidazoyl	OBn

Scheme 1[a]



[a] Reagents: (a) *p*-$BnOC_6H_4COCl$, TEA, DMAP, DCM; (b) **2a**, Et_3N, DMAP, DCM; (c) H_2, $Pd(OH)_2$, EtOAc, EtOH.

appropriately protected benzophenones **2** (Table 1) have been previously reported.[10]

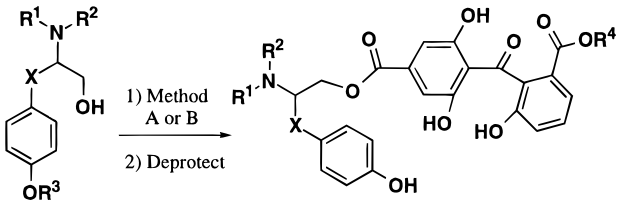
The acylic analog **3**, which most closely resembles balanol, was prepared as outlined in Scheme 1. Analog **3** was synthesized from ethanolamine (**4**) by sequential *N*-acylation with *p*-(benzyloxy)benzoyl chloride, *O*-acylation with benzophenone acid chloride **2a**, and hydrogenation using Pearlman's catalyst.

For the remainder of this section, the syntheses of the perhydroazepine−hydroxybenzamide replacements (referred to as alcohols) are described first. The couplings of the alcohols to the benzophenone portion are then summarized in Tables 2−4.

Alcohols **6**−**8** (Table 2) were prepared as outlined in eq 1 of Scheme 2. Starting with tyrosine methyl ester hydrochloride (**9**), they were synthesized by first functionalizing the nitrogen, followed by protecting the phenol, and lastly reducing the carboxylic ester to an alcohol as illustrated for alcohol **6**. Treatment of racemic tyrosine methyl ester hydrochloride (**9**) in a biphasic mixture of aqueous 1 N NaOH (2.3 equiv) and DCM with benzenesulfonyl chloride (1.1 equiv) over 2 h gave a mixture of *N*,*O*-bis(phenylsulfonyl)-DL-tyrosine methyl ester, *N*-(phenylsulfonyl)-DL-tyrosine methyl ester, and their corresponding acids. Stirring the mixture of products in a solution of aqueous KOH in ethanol cleaved the methyl ester and the *O*-sulfonyl group to give **10** (40% over two steps). It was later found that the yield could be improved to about 60% by using 2−3 equiv of benzenesulfonyl chloride. Treatment of **10** with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine in CH_3CN gave trialkylated ester **11**. Reduction with $LiBH_4$ then gave the corresponding alcohol **6**.

The synthetic strategy used to prepare alcohols **12** and **13** (Table 2) became the preferred method (eq 2, Scheme 2). These alcohols were prepared by first functionalizing the nitrogen, then reducing the carboxylic acid, and finally selectively protecting the phenol as its Cbz derivative. In the example shown, compound L-**10** was derived from L-**9** in the same manner as

Table 2[a]



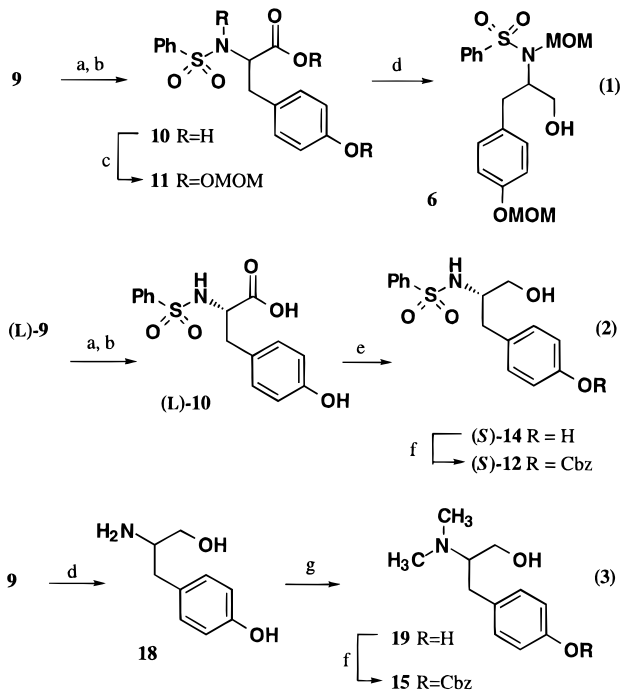
6, 7, 8, (*S*)-**12,** (*R*)-**12, 13, 15, 16, 17, and 26**

6b, 7b, 8b, (*S*)-**12b,** (*S*)-**12c,** (*R*)-**12b, 13b, 15b, 16b, 17b, and 26b**

	R^1	R^2	R^3	X
6	$PhSO_2$	MOM	MOM	CH_2
6b	$PhSO_2$	H		CH_2
7	$MeSO_2$	H	TBDMS	CH_2
7b	$MeSO_2$	H		CH_2
8	PhCO	H	MOM	CH_2
8b	PhCO	H		CH_2
(*S*)-**12**	$PhSO_2$	H	Cbz	CH_2
(*S*)-**12b**	$PhSO_2$	H		CH_2
(*S*)-**12c**	$PhSO_2$	H		CH_2
(*R*)-**12**	$PhSO_2$	H	Cbz	CH_2
(*R*)-**12b**	$PhSO_2$	H		CH_2
13	$PhSO_2$	H	Cbz	
13b	$PhSO_2$	H		
15	Me	Me	Cbz	CH_2
15b	Me	Me		CH_2
16	2-$C_{10}H_7SO_2$	H	H	CH_2
16b	2-$C_{10}H_7SO_2$	H		CH_2
17	$BnOC_6H_4CO$	H	Cbz	CH_2
17b	HOC_6H_4CO	H		CH_2
26	$PhSO_2$	H	Bn	OCH_2
26b	$PhSO_2$	H		OCH_2

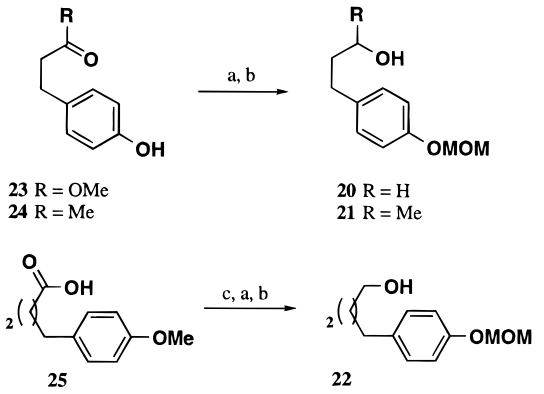
[a] R^4 = H except for (*S*)-**12c**, where $R^4 = CH_3$. Coupling method A was used except in the preparation of **6b** and **8b**, for which coupling method B was utilized. See text for descriptions of the coupling methods.

Scheme 2[a]



[a] Reagents: (a) $PhSO_2Cl$, 1 N NaOH, DCM; (b) 1 N KOH/3:1 EtOH:H_2O; (c) MOMCl, iPr_2NEt, CH_3CN; (d) $LiBH_4$, THF; (e) BH_3, THF, reflux; (f) CBzCl, Et_3N, DCM; (g) HCHO, $NaBH_4$.

Scheme 3[a]



[a] Reagents: (a) MOMCl, iPr_2NEt, CH_3CN; (b) [H], THF; (c) NaSEt, DMF, reflux.

described above for the preparation of DL-**10** from DL-**9**. The reason the conditions used to hydrolyze the intermediate *N*,*O*-bissulfonylated tyrosine did not lead to racemization is most likely because the sulfonamide was deprotonated during the hydrolysis. The carboxylic acid L-**10** was then reduced with borane which provided (*S*)-**14**. The phenol of (*S*)-**14** was then selectively protected in the presence of the primary alcohol using CbzCl (1.5 equiv) and Et_3N in DCM to afford (*S*)-**12**. This selective protection of the phenol in the presence of the primary alcohol proved useful for the synthesis of several acyclic analogs. The Cbz group was the preferred protecting group since all of the protecting groups could be removed from the penultimate intermediate in a single step by hydrogenation. Note that (*R*)-**12** was prepared from D-**9** in the exact same way as described for the preparation of (*S*)-**12** from L-**9**. The alcohols (*S*)-**12** and (*R*)-**12** were prepared with at least 95% ee.[11]

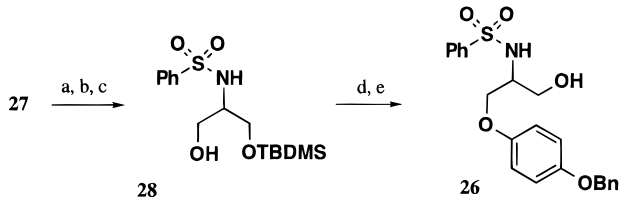
Alcohols **15**−**17** (Table 2) were prepared using the following synthetic strategy: The ester was first reduced to provide the primary alcohol, the nitrogen was then funtionalized, and finally the phenol was protected (eq 3, Scheme 2). The synthesis of the illustrated example, **15**, began with $LiBH_4$ reduction of **9**. Reductive alkylation of **18** then provided dimethylated amine **19**. The phenol of **19** was then selectively protected using CbzCl which afforded **15**.

The preparation of alcohols **20**−**22** is described in Scheme 3. Protection of the phenol **23** as its methoxymethyl ether followed by $LiBH_4$ reduction of the ester functionality gave alcohol **20**. Similarly, phenol **24** was protected as a methoxymethyl ether, and then the carbonyl was reduced with $LiAlH_4$ to yield alcohol **21**. Demethylation of the methyl ether **25** followed by simultaneous esterfication and etherification using chloromethyl methyl ether and subsequent $LiAlH_4$ reduction of the ester functionality led to alcohol **22**.

The synthesis of alcohol **26** began with *N*-sulfonylation of serine methyl ester hydrochloride (**27**) (Scheme 4). The primary alcohol was then protected as its *tert*-butyldimethylsilyl ether. Reduction of the carboxylic ester gave **28**. The (benzyloxy)phenyl ether was introduced into **28** using Mitsonobu chemistry, and then the silyl protecting group was removed to give **26**.

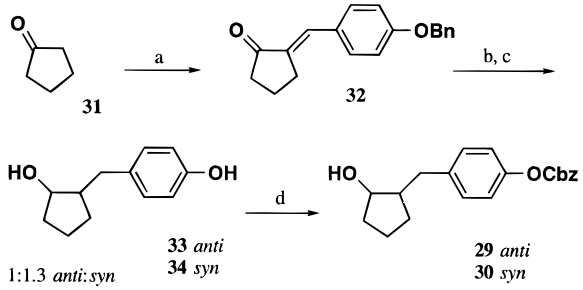
Alcohols **29** and **30** were prepared as illustrated in Scheme 5. Aldol condensation of cyclopentanone **31** with *p*-(benzyloxy)benzaldehyde provided the desired aldol product **32** in 61% yield along with some cyclo-

Scheme 4[a]



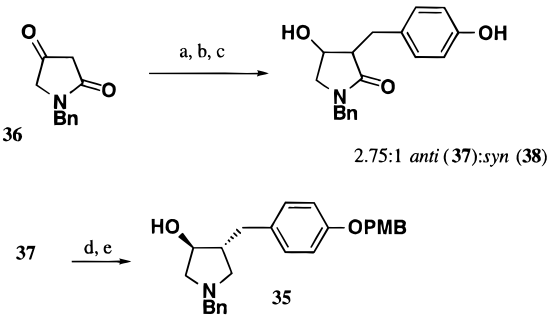
[a] Reagents: (a) $PhSO_2Cl$, iPrNEt, THF; (b) TBDMSCl, ImH, DMF; (c) $LiBH_4$; (d) *p*-$BnOC_6H_4OH$, Ph_3P, DEAD, THF; (e) TBAF, THF.

Scheme 5[a]



[a] Reagents: (a) 4-$BnOC_6H_4CHO$, KOH, H_2O, reflux; (b) $NaBH_4$, THF, MeOH; (c) H_2, Raney Ni; (d) CbzCl, Et_3N, DCM.

Scheme 6[a]

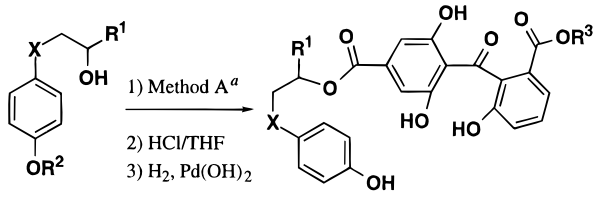


[a] Reagents: (a) 4-$BnOC_6H_4CHO$, $LiN(SiMe_3)_2$, THF, −50 to 35 °C; (b) $NaBH_4$, THF, MeOH; (c) H_2, Raney Ni; (d) 4-$MeOC_6H_4CH_2Cl$, K_2CO_3, DMF, 65 °C; (e) $LiAlH_4$, THF, reflux.

pentanone self-condensation product and double-aldol product. Reduction of the carbonyl group of **32** using $NaBH_4$ followed by Raney nickel hydrogenation of the olefin provided a 1:1.3 mixture of the *anti*- and *syn*-substituted cyclopentanones **33** and **34**, which were easily separated by column chromatography. The phenol functionalities of both **33** and **34**, which had lost their protecting groups during the olefin reduction step, were then reprotected as their Cbz derivatives **29** and **30**.

The synthesis of alcohol **35** is shown in Scheme 6. Dione **36** was obtained by *N*-benzylation and subsequent enol ether hydrolysis of commercially available 4-methoxy-3-pyrrolin-2-one. As in the preceding case, dione **36** was condensed with *p*-(benzyloxy)benzaldehyde, and then the ketone and the olefin were sequentially reduced to provide a 2.75:1 mixture of the *anti*- and *syn*-substituted pyrrolidinones **37** and **38** which were easily separated by column chromatography. The phenol of the desired *anti* isomer **37** was reprotected as its *p*-methoxybenzyl ether and the amide functionality was reduced to an amine using $LiAlH_4$, providing **35**. The last coupling precursor **39** (Table 4) was prepared in a single step by the addition of *p*-(benzyloxy)phenol to cyclopentene oxide (**40**).

Table 3



20 21, and 22 **20b, 21b, 21c, and 22b**

	R^1	R^2	R^3	X
20	H	MOM		CH_2
20b	H		H	CH_2
21	Me	MOM		CH_2
21b	Me		H	CH_2
21c	Me		Me	CH_2
22	H	MOM		$(CH_2)_2$
22b	H		H	$(CH_2)_2$

[a] See text for a description of the coupling method.

Tables 2−4 summarize the general procedures used to couple the alcohols to the benzophenones. The alcohols listed in Table 2 were coupled to the benzophenone portion of the molecule using one of two methods.[12] Method A consisted of acylating the alcohol with benzophenone acid chloride **2a** or **2c** in the presence of catalytic DMAP and either Et_3N or *N*,*N*-diisopropylethylamine, with DCM as the solvent. Method B consisted of 1,1′-carbonyldiimidazole coupling of the alcohol with benzophenone acid **2b** with DBU as the base and THF as the solvent. After coupling, any methoxymethyl protecting groups were removed with HCl, and any silyl protecting groups were removed using tetrabutylammonium fluoride. The benzyl and any benzyloxycarbonyl protecting groups were then removed by catalytic hydrogenation to provide the acyclic balanol analogs.

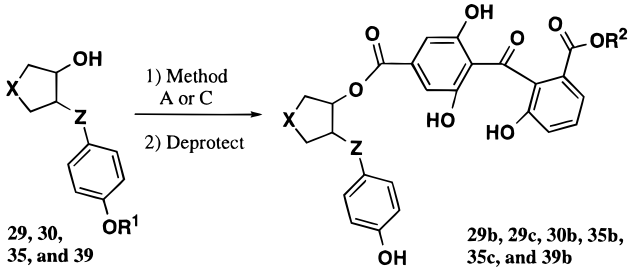
The alcohols shown in Table 3 were coupled to the benzophenone portion of the molecule using method A as described for Table 2 above. The methoxymethyl groups were then removed with aqueous HCl/THF, and subsequently the benzyl and benzyloxycarbonyl protecting groups were removed by hydrogenation using Pearlman's catalyst to provide the target compounds.

The target compounds in Table 4 were prepared by first coupling the alcohols with the benzophenone portion using method A as described above followed by removal of the benzyl and Cbz protecting groups using catalytic hydrogenation. Target compounds **35b**,**c** were exceptions. Compound **35b** was prepared by coupling the lithium salt of **35** with the preformed imidazolide **2d** (method C). The benzyl protecting groups were then removed by catalytic hydrogenation followed by removal of the *p*-methoxybenzyl group with TFA which provided analog **35b**. Treatment of analog **35b** with thionyl chloride in absolute MeOH provided its methyl ester **35c** in 52% yield.

Discussion

Although efficient syntheses[5−7,13] of the appropriately substituted perhydroazepine ring necessary to make balanol had been devised, they were longer than desired for large scale preparation of research materials. For this reason, and more importantly the possibility that modification could lead to selectivity for PKC over other kinases, the effect of replacing the perhydroazepine ring with simple readily accessible acyclic units was investigated.

Table 4[a]



29, 30, 35, and 39 **29b, 29c, 30b, 35b, 35c, and 39b**

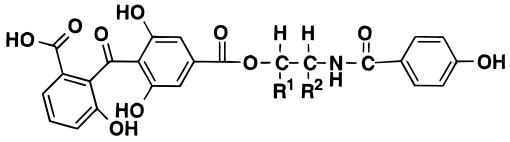
	X	R^1	R^2
29 (*anti*)	CH_2	Cbz	
29b (*anti*)	CH_2		H
29c (*anti*)	CH_2		CH_3
30 (*syn*)	CH_2	Cbz	
30b (*syn*)	CH_2		H
35 (*anti*)	NBn	PMB	
35b (*anti*)	NH		H
35c (*anti*)	NH		CH_3
39 (*anti*)	CH_2	Bn	
39b (*anti*)	CH_2		H

[a] Z = CH_2 except for **39** and **39b**, for which Z = O. Coupling method A was used except in the preparation of **35b** and **35c**, for which coupling method B was utilized. See text for descriptions of the coupling methods.

Substitution of the perhydroazepine linker of balanol with an ethyl linker as in **3** (Table 5) resulted in a 10−300-fold loss in activity when compared to racemic balanol (±)-**1**. This is especially significant since cyclopentane analog **41**[14] is just as potent as racemic balanol (more potent for the δ and η isozymes). Taken together these results suggest that inhibition of PKC is strongly dependent upon the spatial orientation of the benzophenone ester and the *p*-hydroxybenzamide substituents. This is supported by a study which demonstrated a marked effect on PKC inhibitory activity as the perhydroazepine was contracted to six- and five-membered rings.[14] Thus it initially appeared that spatial orientation of the benzophenone ester and the *p*-hydroxybenzamide substituents needed for potent PKC inhibition was not energetically favorable in an open chain form.

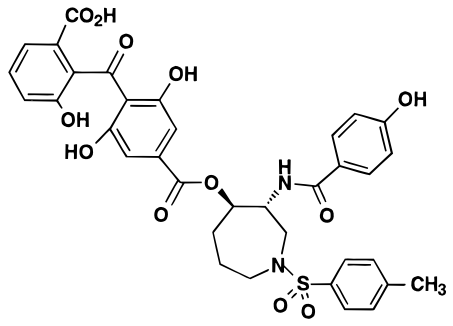
It occurred to us that a tyrosine balanol analog might provide a readily accessible perhydroazepine replacement. A tyrosine analog was appealing for several reasons. Both enantiomers of tyrosine are commercially available, and this amino acid has a *p*-hydroxybenzyl group which might provide a suitable replacement for the *p*-hydroxybenzamide side chain of balanol. It contains a carboxylic acid which, after reduction to an alcohol, could be used for introduction of the benzophenone. Finally, it possesses a nitrogen which provided a handle for the introduction of several different groups. *N*-(Phenylsulfonyl)tyrosine was chosen because it had been observed that, although sulfonamide derivatives of balanol such as **42** (Table 6) were less potent PKC inhibitors, they were selective for PKC over PKA.[15] In addition, many of the sulfonamide derivatives of balanol were selective for β_I and β_{II} among the PKC isozymes as exemplified by **42**.[15] The tyrosine analog **6b** (Table 7) was as potent as racemic balanol against the β_{II} and η isozymes while only 2−9 times less potent against the remaining isozymes. In addition, **6b** was inactive

Table 5. PKC and PKA Inhibitory Activities (IC_{50}s) of Two-Carbon Amide-Linked Acyclic Analogs (μM)



	R[1]	R[2]	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ϵ	PKC η	PKA
3	H	H	11	4.5	7.5	6.6	0.41	13	0.23	8.4
(±)-**1**	$CH_2CH_2CH_2NHCH_2$		0.067	0.03	0.03	0.03	0.023	0.038	0.02	0.06
41	$CH_2CH_2CH_2$		0.04	0.04	0.05	0.01	0.0009	0.05	0.0006	0.03

Table 6. PKC Inhibitory Activities (IC_{50}s) of the *N*-Tosyl Derivative (**42**) of Balanol (μM)



	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ϵ	PKC η	PKA
42	6.1	0.07	0.02	3.5	4.2	15	0.10−1.5	>150

against PKA at the highest concentration tested (50 μM) but, unlike its congener **42**, did not show selectivity for the β isozymes.

The two enantiomers of **6b** were then prepared in hopes of finding increased activity since the natural isomer of balanol was 25−100-fold more potent against PKC than the unnatural isomer.[5d] Indeed the *S*-enantiomer of the tyrosine analog (*S*)-**12b** was found to be 4−13-fold more potent than racemic **6b** against the β_I, β_{II}, δ, and η isozymes (the activities against α, γ, and ϵ were about the same). The more active *S*-enantiomer (*S*)-**12b** was 3−10-fold more potent than the *R*-enantiomer (*R*)-**12b**, not quite as large of a difference as seen between balanol and its enantiomer.

Given the improved kinase selectivity of **6b**, further modifications were investigated. The size of the sulfonamide group had varying effects on the inhibitory activities of the individual PKC isozymes. Replacement of phenylsulfonamide with methylsulfonamide (**7b**) did not affect activity toward β_I; however, activity against the rest of the isozymes decreased 10−210-fold. Alternatively, increasing the size to naphthylsulfonamide (**16b**) resulted in a 10−1750-fold loss in activity. Simply replacing the benzenesulfonamide with benzenecarboxamide (**8b**) resulted in a 90−900-fold loss in activity for the isozymes tested, whereas replacement with dimethylamino (**15b**) resulted in a smaller loss in activity (4−100-fold).

Interestingly, the simple three-carbon-linked analog **20b** which lacks the benzenesulfonamide unit was fairly potent. Compound **20b** was as potent as **6b** against the β_I and η isozymes while only 4−17-fold less potent against the remaining isozymes. The simple methyl-branched analog **21b**, which would be expected to be more resistant to enzymatic hydrolysis of the ester linkage, was essentially as active against PKC as the racemic tyrosine analog **6b**. Importantly, it shared the tyrosine analog's selectivity for PKC over PKA.

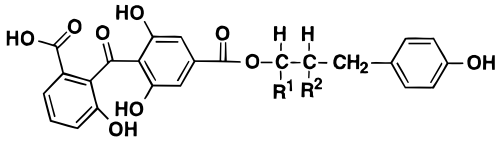
Analog **17b** was designed to investigate the possibility that the benzenesulfonamide moiety present in **6b** was actually serving as a replacement for the *p*-hydroxybenzamide side chain in balanol while the *p*-hydroxyphenyl of **6b** was actually serving as a replacement for the perhydroazepine ring of balanol. The low activity of **17b** suggests that this is unlikely.

The effect that the number and type of atoms in the linker between the benzophenone ester and the *p*-hydroxyphenyl had on activity was also investigated (Table 8). Shortening the length of the three-carbon linker in **6b** by one carbon as in **13b** greatly reduced activity (22− to >455-fold), while increasing the length by adding an oxygen as in **26b** resulted in a large loss in activity (6−164-fold). Increasing the length of the chain between the benzophenone ester and the *p*-hydroxyphenyl by one carbon reduced potency in the straight chain series (**22b** vs **20b**).

Since the acyclic analogs were selective for PKC over PKA, the two best racemic analogs, **6b** and **21b**, were tested against a panel of other kinases. Table 9 compares their inhibitory activities along with those of synthetic (−)-balanol against four kinases. Protein kinase A, casein kinase, and Ca^{2+}/calmodulin (CaM) dependent kinase are, like PKC, serine/threonine specific kinases, while src tyrosine kinase is a tyrosine specific kinase. Neither balanol nor the acyclic analogs **6b** and **21b** inhibited casein kinase at the highest concentrations tested. While balanol was a potent inhibitor of CaM kinase (30 nM), the tyrosine analog **6b** was a weak inhibitor (32 000 nM, which is 150−1600-fold less than its activity toward the PKC isozymes), and **21b** did not inhibit at all at the highest concentration tested (50 μM). Neither **6b** nor balanol inhibited src tyrosine kinase. Balanol has also been reported to be ineffective against the epidermal growth factor receptor (EGFR) protein tyrosine kinase.[8] Thus, like balanol, **6b** appears to be ineffective against tyrosine protein kinases. Unlike balanol, **6b** and **21b** appear to be selective for PKC over other serine/threonine kinases in general, at least among the kinases tested.

Due to the surprisingly good activity of the simple methyl-branched analog **21b**, several less flexible analogs were prepared in which part of the three-carbon linkage was tied back in a five-membered ring (Table 10).[16] It was proposed that the rigidity might provide greater activity and that with secondary alcohol ester linkages they might be more hydrolytically stable than

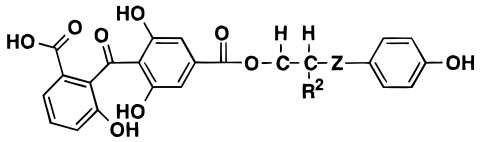
Table 7. Effects of R Groups on the PKC and PKA Inhibitory Activities (IC_{50}s) of Three-Carbon-Linked Acyclic Balanol Analogs (μM)[a]



	R^1	R^2	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ϵ	PKC η	PKA
6b	H	$NHSO_2C_6H_5$	0.11	0.22	0.03	0.22	0.04	0.32	0.02	>50
(*S*)-**12b**	H	$NHSO_2C_6H_5$	0.21	0.03	0.007	0.47	0.003	0.46	0.003	>50
(*R*)-**12b**	H	$NHSO_2C_6H_5$	1.9	0.23	0.02	2.6	0.03	2.3	0.02	>50
7b	H	$NHSO_2CH_3$	23	0.22	0.43	5.0	0.41	>50	0.39	>50
16b	H	$NHSO_2$-2-naphthyl	3.2	2.8	1.8	2.7	2.6	39	35	>50
8b	H	$NHCOC_6H_5$	45	NT	2.8	NT	22	>50	18	>50
15b	H	$N(CH_3)_2$	4.0	0.88	0.50	4.2	0.27	30	1.4	>50
20b	H	H	1.9	0.21	0.27	0.83	0.13	4.1	<0.05	>50
21b	CH_3	H	0.41	0.04	0.03	0.25	0.03	0.28	0.02	>50
17b	H	$NHCOC_6H_4$-4-OH	18	3.6	3.0	2.9	0.82	>50	2.3	>50
(−)-**1**			0.03	0.01	0.01	0.01	0.02	0.02	0.003	0.04

[a] NT = not tested.

Table 8. Effects of the Length and Type of Linker on PKC and PKA Inhibitory Activities (IC_{50}s, μM)[a]



	R^2	Z	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ϵ	PKC η	PKA
13b	$NHSO_2C_6H_5$		>50	11	2.8	4.8	1.9	>50	3.3	>50
26b	$NHSO_2C_6H_5$	CH_2O	18	1.6	0.83	1.6	0.22	47	0.50	>50
22b	H	CH_2CH_2	3.3	0.96	0.37	1.4	0.29	30	0.25	>50
6b	$NHSO_2C_6H_5$	CH_2	0.11	0.22	0.03	0.22	0.04	0.32	0.02	>50
20b	H	CH_2	1.9	0.21	0.27	0.83	0.13	4.1	<0.05	>50

[a] NT = not tested.

Table 9. Comparison of the Kinase Inhibitory Properties (IC_{50}s) of **6b** and **21b** versus Balanol (μM)[a]

	PKA	casein kinase	CaM kinase	srcTyrK
(−)-**1**	0.30−0.80	>50	0.03−0.05	>10
6b	>50	>50	32	>12.5
21b	>50	>50	>50	NT

[a] NT = not tested.

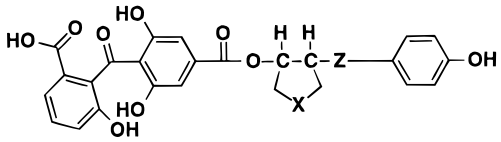
6b. The *anti* cyclopentane **29b** analog was 1−10-fold more active than racemic balanol against the PKC isozymes with the exception of ϵ (it was 3-fold less active than racemic balanol) and 3−55-fold more active than **6b**. The *syn* cyclopentane **30b** was 3−7-fold less active than the *anti* derivative, not as large of a difference as was anticipated since a marked preference for an *anti* relationship between the benzophenone and the *p*-hydroxyphenyl moieties has been observed in other balanol analogs.[17] The *anti* pyrrolidine analog **35b** had activity similar to racemic balanol (within ±5-fold) with the exception of ϵ (it was 50-fold less active than racemic balanol). The *anti* cyclopentane ether **39b** also had similar activity to balanol (within ±3-fold) with the exceptions of δ and ϵ (against which it was 8- and 6-fold less active, respectively, than racemic balanol). The *anti* cyclopentane analog **29b** and the *anti* cyclopentane ether **39b** were not active against PKA, while the pyrrolidine analog **35b** displayed modest activity (IC_{50} = 4.3 μM).

Several acyclic balanol analogs which were potent inhibitors of the isolated enzyme and selective for PKC over PKA were investigated in cellular assays. The neutrophil assay, an assay which measures the inhibition of the phorbol-12-myristate-13-acetate (PMA)-induced release of superoxide in human neutrophils, a process thought to be mediated by PKC,[18] was chosen. Like balanol, most of the acyclic analogs initially tested were not active in this assay at the highest concentration tested (10 μM). However, both the *anti* and *syn* cyclopentane analogs **29b** and **30b** were low-micromolar inhibitors of the superoxide burst in neutrophils (3.1 and 10 μM, respectively, Table 10), and the carboxylic ester of the *anti* cyclopentane analog, **29c**, was a high-nanomolar inhibitor of neutrophils (260 nM). In addition, esterification of the tyrosine analog (*S*)-**12b**, methyl-substituted acyclic analog **21b**, and pyrrolidine analog **35b** provided low-micromolar inhibitors of the neutrophil response, (*S*)-**12c**, **21c**, and **35c**, respectively (Table 11). Previous studies in our laboratories had shown that while masking the carboxylic acid group of balanol analogs as an ester slightly decreased PKC inhibitory activities,[19] it greatly enhanced the cellular activity.[10b]

Conclusions

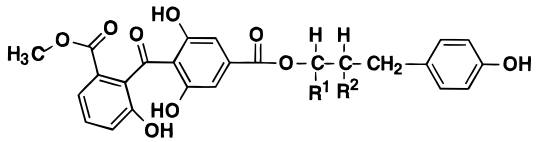
In conclusion we have prepared a series of balanol analogs in which the perhydroazepine ring and the *p*-hydroxybenzamide moiety have been combined into a simple, readily accessible acyclic linked unit. A three-

Table 10. Effects of Making the Three-Carbon Chain Part of a Five-Membered Ring on PKC, PKA, and Neutrophil Inhibitory Activities (IC_{50}s, μM)



	X	Z	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ε	PKC η	PKA	neutrophils
29b (*anti*)	CH_2	CH_2	0.030	0.004	0.003	0.040	0.005	0.120	0.003	>50	3.1−4.2
30b (*syn*)	CH_2	CH_2	0.160	0.020	0.020	0.130	0.030	0.500	0.010	>50	10
35b (*anti*)	NH	CH_2	0.260	0.010	0.030	0.040	0.005	1.8	0.020	4.3	>10
39b (*anti*)	CH_2	O	0.220	0.020	0.020	0.250	0.020	0.240	0.010	>50	>10

Table 11. Effects of Esterification on PKC and Neutrophil Inhibitory Activities (IC_{50}s, μM)



	R^1	R^2	PKC α	PKC β_I	PKC β_{II}	PKC γ	PKC δ	PKC ε	PKC η	PKA	neutrophils
(*S*)-**12c**	H	$NHSO_2C_6H_5$	18	2.2	0.34	4.5	0.36	38	<0.05	>50	0.93−1.1[a]
21c	H	H	7.8	0.46	0.75	4.1	0.30	13	0.17	NT	1.3−2.7[a]
29c	$CH_2CH_2CH_2$ (*anti*)		1.4	0.10	0.09	1.7	0.05	4.5	0.04	>50	0.26[a]
35c	CH_2NHCH_2 (*anti*)		2.2	0.11	0.25	0.38	0.10	4.0	0.50	4.1	4.3
6b			0.11	0.22	0.03	0.22	0.04	0.32	0.02	>50	>10

[a] 1 h preincubation at 4 °C. NT = not tested.

carbon linkage between the benzophenone ester and the *p*-hydroxyphenyl substituent, in which the carboxamide moiety has been replaced by a methylene, was found to be optimal. Substitution on the three-carbon chain was investigated, and the most active analogs found were the 1-methyl-substituted analog **21b**, the 2-benzensulfonamido- substituted analog **6b**, and its enantiomers (*S*)-**12b** and (*R*)-**12b**. The more potent enantiomer, (*S*)-**12b,** had low-nanomolar activity against three of the PKC isozymes and was 3−10-fold more active than the *R*-enantiomer (*R*)-**12b** against the PKC isozymes overall. In general, the acyclic analogs have been shown to be highly selective for PKC over PKA. Moreover, compounds **21b** and **6b** appeared to be selective for PKC over other serine/threonine kinases in general. Reintroduction of a ring for the perhydroazepine replacement in the form of an *anti*-substituted pyrrolidine and an *anti*-substituted cyclopentane ring, but with retention of the methylene replacement for the carboxamide moiety, led to the potent analog **35b** and the most potent analog **29b**, respectively. Besides being equipotent to balanol against PKC and selective for PKC over PKA, the *anti* cyclopentane analog **29b** was a low-micromolar inhibitor of neutrophils, and its carboxylic ester **29c** was a high-nanomolar inhibitor of neutrophils. In addition to having a better pharmacological profile, **29b** was also much easier to synthesize than balanol itself. Finally esterification of **21b**, (*S*)-**12b**, and **35b** turned these potent PKC inhibitors into low-micromolar inhibitors of the superoxide burst in neutrophils.

Experimental Section

All final products were purified on a Rainin HPLC using Dynamax-60 C18 columns (unless otherwise noted). Appropriate linear gradients of the solvent systems A and B (A, 5% CH_3CN in H_2O + 0.1% TFA; B, 100% CH_3CN) were employed. The solvent was then removed by lyophilization. For intermediates, column chromatography was performed using silica gel 60 (230−400 mesh, E. Merck) at ca. 5 psig. Proton (^1H) NMRs were obtained on a 300 MHz Varian Gemini instrument. Proton chemical shifts are reported in δ values (parts per million) relative to tetramethylsilane. Mass spectra were performed on a Finnigan MAT 211 mass spectrometer in the FAB (positive) mode by Analytical Instrument Group, Inc., Raleigh, NC. Optical rotations were recorded using a Perkin-Elmer polarimeter 241. Data are reported as follows: $[\alpha]_D$ (concentration in g/100 mL, solvent). Elemental analyses were obtained on a Carlo Erba CHNS elemental analyzer 1108, and the results are within 0.4% of the theoretical values, except where noted. Melting points were determined in open glass capillaries utilizing a Mel-temp apparatus and are uncorrected. Solvents and reagents were obtained from commercial suppliers and used as received. Dichloromethane is abbreviated as DCM.

Benzyl 2-[[4-[[2-[[[4-(Benzyloxy)phenyl]carbonyl]amino]ethoxy]carbonyl]-2,6-bis(benzyloxy)phenyl]carbonyl]-3-(benzyloxy)benzoate (5). To an ice cold solution of ethanolamine (**4**) (122 mg, 2 mmol) in THF (6 mL) was added 2.0 N KOH (1 mL). Slowly, 4-(benzyloxy)benzoyl chloride (493 mg, 2 mmol) was added. After the reaction mixture stirred for 4 h at room temperature, it was diluted with DCM and washed with H_2O and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was triturated with Et_2O/hexanes to give *N*-(2-hydroxyethyl)-4-(benzyloxy)-benzamide (**43**) as a white solid (410 mg, 76%): mp 141−143 °C; ^1H NMR (CD_3OD) δ 7.79 (d, 2H, J = 9 Hz), 7.36 (m, 5H), 7.04 (d, 2H, J = 9 Hz), 5.13 (s, 2H), 3.69 (t, 2H, J = 5.9 Hz), 3.47 (t, 2H, J = 5.8 Hz). Anal. ($C_{16}H_{17}NO_3$) C, H, N.

To a stirred solution of 4-[6-(benzyloxy)-2-(benzyloxycarbonyl)benzoyl]-3,5-bis(benzyloxy)benzoic acid (**2b**) (339 mg, 0.50 mmol) in DCM (5 mL) was added DMF (3 drops) followed by oxalyl chloride (0.75 mL, 2.0 M in DCM, 1.5 mmol) dropwise at room temperature under N_2. The reaction mixture was then stirred at room temperature for 2 h and the solvent evaporated *in vacuo*. The residue of benzyl 2-[[4-(chloroformyl)-3,5-bis-

(benzyloxy)phenyl]carbonyl]-3-(benzyloxy)benzoate (**2a**) was used "as is" in the following reaction without any further purification.

To a stirred mixture of **43** (136 mg, 0.50 mmol) in DCM (10 mL) were added DMAP (8 mg, 65 μmol) and Et_3N (0.20 mL, 1.5 mmol) followed by the slow addition of a solution of freshly prepared **2a** from above in DCM (5 mL). The reaction mixture was stirred at room temperature for 2 h under N_2, and then the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography (hexane:EtOAc, 7:3 then 1:1) to afford **5** (400 mg, 86%).

2-[[2,6-Dihydroxy-4-[[2-[[(4-hydroxyphenyl)carbonyl]-amino]ethoxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic Acid (3). To a solution of **5** (380 mg, 407 μmol) in EtOAc:EtOH (3:1, 8 mL) was added $Pd(OH)_2$ (30 mg). The reaction mixture was then stirred at room temperature under H_2 (1 atm) for 18 h. The mixture was filtered through Celite and the solvent evaporated *in vacuo*. The residue was purified by column chromatography [EtOAc (100%), MeOH:EtOAc (5:95, 10:90), and then MeOH (100%)] to give **3** (130 mg, 66%) as a light yellow powder: mp 198−205 °C dec; ^{1}H NMR (CD_3OD) δ 7.68 (d, 2H, J = 8.6 Hz), 7.37 (d, 1H, J = 7.6 Hz), 7.19 (t, 1H, J = 7.8 Hz), 6.93 (s, 2H), 7.07 (d, 1H, J = 8.1 Hz), 6.80 (d, 2H, J = 8.7 Hz), 4.42 (t, 2H, J = 6 Hz), 3.70 (t, 2H, J = 6 Hz); MS m/z 482 (M + 1).[20]

***N*-(Phenylsulfonyl)-DL-tyrosine (10).** To a suspension of **9** (5.0 g, 21.6 mmol) in DCM (25 mL) was added benzenesulfonyl chloride (3.0 mL, 23.7 mmol) followed by 1 N NaOH (45 mL). After 2 h, the mixture was poured into EtOAc (200 mL) and washed with water (2 × 100 mL) followed by brine (100 mL). The organic layer was dried ($MgSO_4$) and concentrated. Column chromatography (EtOAc/hexanes, 30−50%) provided the *N*,*O*-bis(phenylsulfonyl)-DL-tyrosine (**44**) as a white foam (2.93 g, 40%).

Intermediate **44** was dissolved in a solution of 1 N KOH/EtOH/water (1.4 g/20 mL/2 mL) and heated at 60 °C. After 24 h, another 1.4 g of KOH was added to the system. After another 24 h, the solvent was evaporated and the byproduct was recrystallized from ethanol. The side product (benzenesulfonic acid) was filtered off, and the filtrate was evaporated to dryness. The residue was triturated with toluene (5×) and then dried in a vacuum oven for 16 h. A tan solid of **10** (4.72 g, quantitative) was obtained: mp 140 °C; ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 1H, J = 9 Hz), 7.63 (s, 1H), 7.56−7.51 (m, 1H), 7.43 (d, 1H, J = 8 Hz), 7.39 (s, 1H), 6.92 (d, 2H, J = 8.5 Hz), 6.60 (d, 2H, J = 8.5 Hz), 3.96 (dd, 1H, J = 8, 5 Hz), 2.94 (dd, 1H, J = 13.5, 5 Hz), 2.74 (dd, 1H, J = 13.5, 8 Hz). Anal. ($C_{15}H_{15}NO_5S$) C, H, N, S.

***N*-(Phenylsulfonyl)-*N*,*O*-bis(methoxymethylene)-DL-tyrosine Methoxymethylene Ester (11).** To a suspension of **10** (4.6 g, 14.3 mmol) and *N*,*N*-diisopropylethylamine (6.3 mL, 35.8 mmol) in CH_3CN (70 mL) at 0 °C was slowly added chloromethyl methyl ether (2.7 mL, 35.8 mmol). After 1.5 h the solid material was filtered off and discarded. The filtrate was concentrated, poured into a 1:1 mixture of saturated aqueous NH_4Cl, and then extracted with EtOAc (300 mL). The organic layer was washed with brine (75 mL) and then dried ($MgSO_4$). After concentration, the crude oil was chromatographed (EtOAc/hexanes, 25%) to give **11** as a yellow oil (1.17 g, 46%).

General Procedure for the $LiBH_4$ Reduction of Carboxylic Esters to Alcohols: (±)-3-[4-[(Methoxymethylene)oxy]phenyl]-2-[*N*-[(methoxymethylene)oxy]-*N*-(phenylsulfonyl)amino]-1-propanol (6). To a solution of **11** (1.16 g, 2.56 mmol) in THF (9 mL) was added $LiBH_4$ (1.5 mL, 2 M in THF, 3.00 mmol). After 18 h, the reaction was quenched with saturated aqueous NH_4Cl (9 mL) and the mixture poured into EtOAc (60 mL). After washing with brine (30 mL), the organic layer was dried (Na_2SO_4), concentrated to a clear oil, and then dried under high vacuum to give **6** (901 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 1H, J = 8 Hz), 7.81 (d, 1H, J = 8.5 Hz), 7.61−7.56 (m, 1H), 7.50 (d, 1H, J = 8 Hz), 7.48 (d, 1H, J = 13 Hz), 6.89 (s, 2H), 6.88 (s, 2H), 5.14 (s, 2H), 5.09 (d, 1H, J = 11.5 Hz), 4.65 (d, 1H, J = 11.5 Hz), 4.02−3.90 (m, 1H), 3.64−3.50 (m, 1H), 3.48 (s, 3H), 3.46 (s, 3H), 3.13 (t, 1H, J = 6.5 Hz), 2.87 (dd, 1H, J = 9.5, 13.5 Hz), 2.54, (dd, 1H, J = 5, 13.5 Hz).

(*S*)-3-(4-Hydroxyphenyl)-2-[(phenylsulfonyl)amino]-1-propanol [(*S*)-14]. To a solution of L-**10** (12.2 g, 42.2 mmol) in anhydrous THF (500 mL) under an atmosphere of N_2 was added BH_3·THF complex (253 mL, 1.0 M in THF, 253 mmol), and the reaction mixture was heated at reflux overnight. The reaction was quenched by the dropwise addition of saturated aqueous NH_4Cl. The reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine (75 mL). The EtOAc layer was dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 1:1) to provide a white solid of (*S*)-**14** (10.4 g, 80%): ^{1}H NMR (DMSO-d_6) δ 9.13 (br s, 1H), 7.68−7.42 (m, 5H), 6.82 (d, 2H, J = 8.5 Hz), 6.55 (d, 2H, J = 8.5 Hz), 3.30−3.10 (m, 3H), 2.67 (dd, 1H, J = 14, 6 Hz), 2.31 (dd, 1H, J = 14, 6 Hz). Anal. ($C_{15}H_{17}NO_4S$) C, H, N.

General Procedure for the Cbz Protection of Phenols: (*S*)-3-[4-[(Benzyloxycarbonyl)oxy]phenyl]-2-[(phenylsulfonyl)amino]-1-propanol [(*S*)-12] and (*R*)- 3-[4-[(Benzyloxycarbonyl)oxy]phenyl]-2-[(phenylsulfonyl)amino]-1-propanol [(*R*)-12]. To a suspension of (*S*)-**14** (9.15 g, 29.8 mmol) and Et_3N (6.22 mL, 44.6 mmol) in anhydrous THF (200 mL) was added benzyl chloroformate (4.46 mL, 31.3 mmol) at 0 °C. After stirring for 30 min, the reaction mixture was diluted with EtOAc and washed with water and brine. The EtOAc layer was dried ($MgSO_4$) and concentrated to afford (*S*)-**12** as a white solid (11.5 g, 87%): $[\alpha]_D = -20°$ (0.51, EtOAc);[11] ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 2H, J = 8 Hz), 7.58−7.38 (m, 8H), 6.98 (s, 4H), 5.28 (s, 2H), 4.78 (d, 1H, J = 7 Hz), 3.69−3.39 (m, 3H), 2.81 (dd, 1H, J = 7, 14 Hz), 2.69 (dd, 1H, J = 7, 14 Hz), 2.01 (dd, 1H, J = 6, 5.5 Hz). Anal. ($C_{23}H_{23}NO_6S$) C, H, N, S.

Treatment of (*R*)-**14** using the same conditions provided (*R*)-**12**: $[\alpha]_D = 20°$ (0.66, EtOAc).[11]

(±)-2-Amino-3-(4-hydroxyphenyl)-1-propanol (18). Compound **9** was reduced using the same procedure described for the preparation of **6** from **11** which provided alcohol **18** (2.0 g, quantitative yield): ^{1}H NMR (DMSO-d_6) δ 9.20 (br s, 1H) 6.96 (d, 2H, J = 8.3 Hz), 6.67 (d, 2H, J = 8.3 Hz), 5.05−4.70 (m, 2H), 3.52−3.15 (m, 3H), 2.9 (d, 1H, J = 12 Hz), 2.65−2.46 (m, 2H). Anal. ($C_9H_{13}NO_2 \cdot 1.6H_2O$) C, H, N.

(±)-2-(*N*,*N*-Dimethylamino)-3-(4-hydroxyphenyl)-1-propanol (19). A solution of **18** (0.38 g, 2.26 mmol) in MeOH (2 mL) was treated with formaldehyde (0.40 mL, 37% aqueous, 15.0 mmol) and 1 drop of HOAc. Sodium borohydride (427 mg, 11.3 mmol) was added portionwise, and the reaction mixture was allowed to stir at room temperature for 20 min. A slurry was obtained. The reaction mixture was diluted with DCM (2 mL) and stirred for an additional 1 h. Solvents were removed *in vacuo*; the resulting residue was quenched with water (3 mL) and then extracted with DCM (8 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford **19** as a white solid (353 mg, 80%). Anal. ($C_{11}H_{17}NO_2 \cdot 0.35H_2O$) C, H, N.

(±)-2-(*N*,*N*-Dimethylamino)-3-[4-[(benzyloxycarbonyl)-oxy]phenyl]-1-propanol (15). Compound **15** was prepared from **19** using the general procedure described for the preparation of (*S*)-**12** from (*S*)-**14**. After stirring at room temperature for 2 h, the reaction mixture was loaded onto a silica gel column and eluted with 5−10% MeOH/DCM to afford **15** (340 mg, 60%).

3-[4-[(Methoxymethylene)oxy]phenyl]-1-propanol (20). To a solution of methyl 3-(4-hydroxyphenyl)propanoate (**23**) (2.00 g, 11.0 mmol) in CH_3CN (15 mL) under an atmosphere of N_2 at 0 °C was added *N*,*N*-diisopropylethylamine (2.89 mL, 11.6 mmol) followed by the dropwise addition of chloromethyl methyl ether (1.26 mL, 11.6 mmol) over 10 min. The reaction mixture was allowed to stir at room temperature for 96 h during which time additional *N*,*N*-diisopropylethylamine (11.6 mL, 64.4 mmol) and chloromethyl methyl ether (5.04 mL, 64.4 mmol) were added over four portions. The reaction mixture was diluted with EtOAc (350 mL) and washed with water (3 × 50 mL), 1 N NaOH (4 × 50 mL), water (20 mL), and brine (50 mL). The EtOAc layer was dried ($MgSO_4$) and concentrated to provide an oil of methyl 3-[4-[(methoxymethylene)-

oxy]phenyl]-1-propanoate (**45**) (625 mg, 90%) which was used as is in the next step: ^{1}H NMR ($CDCl_3$) δ 7.13 (d, 2H, J = 9 Hz), 6.97 (d, 2H, J = 9 Hz), 5.16 (s, 2H), 3.68 (s, 3H), 3.48 (s, 3H), 2.91 (t, 2H, J = 8 Hz), 2.61 (t, 2H, J = 8 Hz).

Compound **20** was obtained from **45** using a method similar to that described for the preparation of **6** from **11**. The crude residue was purified by column chromatography (MeOH/$CHCl_3$, 2%) to provide **20** as an oil (1.55 g, 82%): ^{1}H NMR ($CDCl_3$) δ 7.12 (d, 2H, J = 9 Hz), 6.97 (d, 2H, J = 9 Hz), 5.16 (s, 2H), 3.68 (dt, 2H, J = 6, 5 Hz), 3.48 (s, 3H), 2.67 (t, 2H, J = 7 Hz), 1.88 (m, 2H), 1.28 (t, 1H, J = 6 Hz). Anal. ($C_{11}H_{16}O_3$) C, H.

(±)-4-[4-[(Methoxymethylene)oxy]phenyl]-2-butanol (21). Using conditions similar to those described for the preparation **45** from **23**, **24** was converted into 4-[4-[(methoxymethylene)oxy]phenyl]-2-butanone (**46**) (2.67 g, 70%), a golden yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.11 (d, 2H, J = 9 Hz), 6.96 (d, 2H, J = 9 Hz), 5.15 (s, 2H), 3.48 (s, 3H), 2.89−2.82 (m, 2H), 2.78−2.71 (m, 2H), 2.14 (s, 3H). Anal. ($C_{12}H_{16}O_3$) C, H.

To a solution of **46** (1.25 g, 6.00 mmol) in anhydrous THF (75 mL) under an atmosphere of N_2 at 0 °C was added $LiAlH_4$ (6.60 mL, 1 M in THF, 6.60 mmol) dropwise over 10 min. The reaction mixture was allowed to stir while warming to room temperature over 2 h. The reaction was quenched by the sequential dropwise addition of water (250 μL), 15% NaOH (250 μL), and water (875 μL). The reaction mixture was diluted with EtOAc (200 mL), dried ($MgSO_4$), and concentrated to provide a colorless oil of **21** (1.26 g, 100%): ^{1}H NMR ($CDCl_3$) δ 7.13 (d, 2H, J = 9 Hz), 6.97 (d, 2H, J = 9 Hz), 5.16 (s, 2H), 3.88−3.78 (m, 1H), 3.48 (s, 3H), 2.78−2.57 (m, 2H), 1.79−1.70 (m, 2H), 1.23 (d, 3H, J = 6 Hz). Anal. ($C_{12}H_{18}O_3$) C, H.

4-[4-[(Methoxymethylene)oxy]phenyl]butanol (22). To a suspension of NaH (1.65 g, 60% in mineral oil, 41.2 mmol) in anhydrous DMF (30 mL) under an atmosphere of N_2, at 0 °C, was added ethanethiol (3.05 mL, 41.2 mmol) dropwise over 20 min. The reaction mixture was allowed to warm to room temperature over 1 h. After recooling to 0 °C a solution of 4-(4-methoxyphenyl)butyric acid (**25**) (2.00 g, 10.3 mmol) in anhydrous DMF (15 mL) was added dropwise over 15 min. The reaction mixture was heated at reflux for 7 h. After cooling to room temperature the reaction was quenched with 2 N HCl (100 mL) and the mixture was diluted with EtOAc (350 mL). The layers were separated, and the EtOAc layer was washed with 2 N HCl (100 mL), water (5 × 75 mL), and 1 N NaOH (3 × 75 mL). The combined 1 N NaOH layers were acidified with 1 N HCl and then extracted with EtOAc (2 × 125 mL). The latter two EtOAc layers were combined and washed with water (2 × 50 mL) and brine (50 mL). The EtOAc layer was dried ($MgSO_4$) and concentrated to provide a white solid of 4-(4-hydroxyphenyl)butanoic acid (**47**) (1.69 g, 91%): mp 106−108 °C; ^{1}H NMR (DMSO-d_6) δ 12.02 (br s, 1H), 9.18 (br s, 1H), 6.96 (d, 2H, J = 8.5 Hz), 6.66 (d, 2H, J = 8.5 Hz), 2.45 (t, 2H, J = 7.5 Hz), 2.17 (t, 2H, J = 7.5 Hz), 1.72 (tt, 2H, J = 7.5, 7.5 Hz). Anal. ($C_{10}H_{12}O_3$) C, H.

Using conditions similar to those described for the preparation of **45** from **23, 47** was converted into a golden yellow oil of methoxymethyleneoxy 4-[4-[(methoxymethylene)oxy]phenyl]-butanoate (**48**) (1.67 g, 69%) which was used as is in the next step: ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 2H, J = 8.5 Hz), 6.97 (d, 2H, J = 8.5 Hz), 5.23 (s, 2H), 5.16 (s, 2H), 3.48 (s, 3H), 3.47 (s, 3H), 2.62 (t, 2H, J = 7.5 Hz), 2.37 (t, 2H, J = 7.5 Hz), 1.95 (tt, 2H, J = 7.5, 7.5 Hz).

Lithium aluminum hydride reduction of **48** using a procedure similar to the one described for the preparation of **21** from **46** afforded a colorless oil of **22** (540 mg, 96%): ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 2H, J = 8.5 Hz), 6.96 (d, 2H, J = 8.5 Hz), 5.16 (s, 2H), 3.65 (t, 2H, J = 6 Hz), 3.48 (s, 3H), 2.60 (t, 2H, J = 7 Hz), 1.56−1.74 (m, 4H).

(±)-*N*-[2-[(*tert*-Butylsilyl)oxy]-1-(hydroxymethyl)ethyl]-benzenesulfonamide (28). To a suspension of **27** (3.89 g, 25.0 mmol) in THF (100 mL) at 0 °C was added benzenesulfonyl chloride (3.5 mL, 27 mmol). To this mixture was added dropwise over 10 min a solution of *N*,*N*-diisopropylethylamine (9.6 mL, 55 mmol) in THF (10 mL), and the mixture was stirred for 1.5 h at 0 °C and then at room temperature. After 20 h the mixture was diluted with ether (200 mL), washed with 2 N HCl and brine, dried ($MgSO_4$), and evaporated to a crude residue. Recrystallization from EtOAc/hexanes afforded 4.05 g (63%) of *N*-(phenylsulfonyl)-DL-serine methyl ester (**49**) as a white solid: mp 93−96 °C. Anal. ($C_{10}H_{13}NO_5S$) C, H, N, S.

To a solution of **49** (1.04 g, 4.01 mmol) in DMF (4 mL) were added imidazole (0.408 g, 5.99 mmol) and *tert*-butyldimethylsilyl chloride (0.603 g, 4.00 mmol), and the mixture was stirred at room temperature under a N_2 atmosphere. After 16 h the mixture was diluted with EtOAc (50 mL), washed with water and brine, dried ($MgSO_4$), and evaporated to give 1.47 g (99%) of *O*-(*tert*-butyldimethylsilyl)-*N*-(phenylsulfonyl)-DL-serine methyl ester (**50**) as a white solid: mp 57−59 °C. Anal. ($C_{16}H_{27}NO_5SSi$) C, H, N, S.

Treatment of **50** with $LiBH_4$, using conditions similar to the general procedure described for the reduction of **11** to **6**, provided 0.70 g (100%) of **28** as a colorless oil, which was carried on without further purification.

(±)-*N*-[2-[4-(Benzyloxy)phenoxy]-1-(hydroxymethyl)-ethyl]benzenesulfonamide (26). To a solution of **28** (0.173 g, 0.501 mmol) in THF (2 mL) at 0 °C were added 4-(benzyloxy)phenol (0.100 g, 0.499 mmol), Ph_3P (0.170 g, 0.648 mmol), and diethyl azodicarboxylate (102 μL, 0.648 mmol), and the mixture was stirred at room temperature under a N_2 atmosphere for 4 h, after which it was heated to reflux. After 16 h the mixture was cooled to room temperature and treated with KOtBu (0.5 mL, 1.0 M in THF, 0.5 mmol). The deep red mixture was stirred for an additional 24 h at room temperature, after which it was diluted with EtOAc (20 mL), washed with 2 N HCl and brine, dried ($MgSO_4$), and evaporated to give a residue. The crude product was chromatographed (hexane:EtOAc, 4:1) to give 78.6 mg of partially purified (±)-*N*-[2-[4-(benzyloxy)phenoxy]-1-[[(*tert*-butyldimethylsilyl)oxy]-methyl]ethyl]benzenesulfonamide (**51**), which was carried on as is.

The above 78.6 mg of partially purified **51** was dissolved in THF (1 mL), and the solution was treated with tetrabutylammonium fluoride (0.224 mL, 1.0 M in THF, 0.224 mmol). The mixture was stirred for 3 h at room temperature, after which it was diluted with 20 mL of EtOAc, washed with water and brine, dried ($MgSO_4$), and evaporated to a residue. Column chromatography (DCM:EtOAc, 4:1) afforded 17.8 mg (8.6% overall) of **26** as a glass, which was used without further characterization.

(±)-*anti*-2-(4-Hydroxybenzyl)cyclopentanol (33) and (±)-*syn*-2-(4-Hydroxybenzyl)cyclopentanol (34). To 4-(benzyloxy)benzaldehyde (12.4 g, 58.2 mmol) was added a solution of KOH (1.45 g, 25.9 mmol) in H_2O (25.5 mL), and the suspension was heated to 65 °C. Cyclopentanone (**31**) (5.72 mL, 64.7 mmol) was added dropwise over 0.25 h, and the reaction mixture was brought to reflux. After refluxing for 5 h the reaction mixture was allowed to stir overnight at room temperature. After acidifying with 1 N HCl (150 mL) and diluting with EtOAc (700 mL), a suspension was obtained. The suspension was filtered, and the biphasic filtrate was discarded. The solid was triturated with $CHCl_3$ (500 mL), filtered, and discarded. The filtrate was washed with brine (100 mL), dried ($MgSO_4$), and concentrated to provide aldol adduct **32** (9.38 g, 61%), a yellow solid, which was used as is in the next step: ^{1}H NMR ($CDCl_3$) δ 7.60−7.32 (m, 8H), 7.02 (d, 2H, J = 9 Hz), 5.12 (s, 2H), 2.99−2.92 (m, 2H), 2.41 (t, 2H, J = 6 Hz), 2.08−1.98 (m, 2H).

To a suspension of the aldol adduct **32** (9.37 g, 33.6 mmol) in anhydrous 1:1 THF:MeOH (240 mL) under an atmosphere of N_2 at 0 °C was added $NaBH_4$ (1.76 g, 46.5 mmol), and the reaction mixture was allowed to stir while warming to room temperature over 4 h. The reaction mixture was filtered, and the solid was washed with MeOH. The volatiles were removed from the filtrate, and the resulting residue was purified by column chromatography (hexane:EtOAc, 5:1−2:1) to provide an off-white solid of the allylic alcohol (8.21 g, 87%): mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ 7.49−7.30 (m, 7H), 6.97 (d, 2H, J = 9 Hz), 6.52 (s, 1H), 5.68 (s, 2H), 4.60 (br s, 1H), 2.80−2.48 (m, 2H), 2.07−1.60 (m, 4H). Anal. ($C_{19}H_{20}O_2$) C, H.

To a solution of the allylic alcohol (4.00 g, 4.30 mmol) in absolute EtOH (75 mL) under an atmosphere of N_2 was added Raney nickel (1/3 tsp). The reaction mixture was placed under H_2 (55 psi) overnight. The reaction mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography (hexane:EtOAc, 4:1−2.5:1) which provided a white solid of the *anti* alcohol **33** (1.10 g, 40%): mp 104−106 °C; ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 2H, J = 9 Hz), 6.76 (d, 2H, J = 9 Hz), 4.10 (ddd, 1H, J = 6, 4, 1 Hz), 2.78 (dd, 1H, J = 8, 15 Hz), 2.63 (dd, 1H, J = 7.5, 15 Hz), 2.05−1.42 (m, 7H). Anal. ($C_{12}H_{16}O_2$) C, H.

Further elution provided a white solid of the *syn* alcohol **34** (1.46 g, 53%): mp 79−81 °C; ^{1}H NMR ($CDCl_3$) δ 7.05 (d, 2H, J = 8.5 Hz), 6.72 (d, 2H, J = 8.5 Hz), 3.93 (ddd, 1H, J = 9, 6, 4 Hz), 2.67−2.49 (m, 2H), 2.08−1.51 (m, 7H). Anal. ($C_{12}H_{16}O_2$) C, H.

(±)-*anti*-2-[4-[(Benzyloxycarbonyl)oxy]benzyl]cyclopentanol (29). Treatment of **33** under the same conditions described for the preparation of (*S*)-**12** from (*S*)-**14** provided a colorless oil of **29** (1.15 g, 99%): ^{1}H NMR ($CDCl_3$) δ 7.49−7.35 (m, 5H), 7.19 (d, 2H, J = 9 Hz), 7.09 (d, 2H, J = 9 Hz), 5.28 (s, 2H), 4.14−4.05 (m, 1H), 2.86 (dd, 1H, J = 8, 15 Hz), 2.68 (dd, 1H, J = 8, 15 Hz), 2.05−1.43 (m, 7H). Anal. ($C_{20}H_{22}O_4$) C, H.

(±)-*syn*-2-[4-[(Benzyloxycarbonyl)oxy]benzyl]cyclopentanol (30). Treatment of **34** under the same conditions described for the preparation of (*S*)-**12** from (*S*)-**14** provided a colorless oil of **30** (1.28 g, 100%): ^{1}H NMR ($CDCl_3$) δ 7.49−7.35 (m, 5H), 7.20 (d, 2H, J = 9 Hz), 7.10 (d, 2H, J = 9 Hz), 5.27 (s, 2H), 3.90 (ddd, 1H, J = 6, 6, 6 Hz), 2.80 (dd, 1H, J = 6.5, 15 Hz), 2.50 (dd, 1H, J = 9, 15 Hz), 2.10−1.52 (m, 7H). Anal. ($C_{20}H_{22}O_4$) C, H.

1-(Phenylmethyl)pyrrolidine-2,4-dione (36). A solution of solid KOtBu (10.0 g, 89 mmol) in anhydrous THF (165 mL) under N_2 was cooled on an ice bath and treated in portions with 4-methoxy-3-pyrrolin-2-one (9.04 g, 80 mmol) so that the pot temperature remained below 10 °C. After the addition, the mixture was stirred at 5 °C for 15 min and then treated dropwise with a solution of benzyl bromide (10.7 mL, 15.4 g, 90 mmol) in anhydrous THF (15 mL). The mixture was heated to 50 °C, stirred for 2 h, then cooled (10 °C), treated with saturated aqueous $NaHCO_3$ (35 mL), and filtered through Celite. The filtrate was separated, and the lower aqueous layer was discarded. The organic solution was dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (DCM:acetone, 2:1) provided 1-benzyl-4-methoxy-3-pyrrolin-2-one (**52**) (11.1 g, 68%) as a tan solid.

A solution of **52** (4.06 g, 20 mmol) in DCM (40 mL) was treated with 50% aqueous H_2SO_4 (16 g), and the biphasic mixture was stirred for 15 h. Additional DCM (60 mL) was added, and the organic layer was separated. The acidic aqueous layer was extracted with DCM (30 mL), and the combined organic solution was washed with water (2 × 30 mL, each back-washed with DCM), dried (Na_2SO_4), and concentrated *in vacuo* to afford **36** (3.67 g, 97%) as a viscous oil containing 8% unhydrolyzed enol ether.

(±)-*anti*-4-Hydroxy-3-(4-hydroxybenzyl)-1-benzylpyrrolidin-2-one (37). A cooled (−60 °C) solution of **36** (0.378 g, 2.0 mmol) and 4-(benzyloxy)benzaldehyde (0.467 g, 2.2 mmol) in anhydrous THF (4 mL) under N_2 was treated dropwise with lithium bis(trimethylsilyl)amide (2.0 mL, 1 M in THF, 2.0 mmol) so as to keep the pot temperature below −50 °C; then the mixture was allowed to warm to 35 °C over 30 min and was stirred at room temperature for 3 h. The solution was plunged into a stirred mixture of saturated aqueous $NaHCO_3$ (10 mL) and DCM (15 mL) and then stirred for 5 min, during which time the organic layer became bright yellow and was separated. The aqueous layer was extracted with DCM (15 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed (acetone/DCM, 1−2%) to afford 3-[4-(benzyloxy)benzyl]-1-benzylpyrrolidine-2,4-dione (**53**) (0.35 g, 46%) as a bright yellow solid: mp 135−137 °C.

A cooled (5 °C) suspension of **53** (1.07 g, 2.8 mmol) in 2:1 DCM:methanol (15 mL) was treated with $NaBH_4$ (granular, 0.14 g, 3.7 mmol), and the mixture was warmed to room temperature, stirred for 15 min, and then concentrated *in vacuo*. The residue was taken up in 1:1 MeOH:EtOH (40 mL), placed in a Parr bottle, and treated with Raney nickel ($^1/_2$ tsp). The bottle was charged with H_2 on a Parr apparatus, shaken at 45 psi for 1.5 h, and then carefully evacuated of H_2. The solution was filtered through Celite, and the filter cake was rinsed with methanol (but not allowed to dry). The filtrate was concentrated *in vacuo*, and the residue was chromatographed on silica gel (2-propanol/DCM, 5%, 10%, and then 15%) to afford the *anti* isomer **37** (0.578 g, 69%) as a colorless glass followed by a minor amount (0.21 g, 25%) of the *syn* isomer **38**.

(±)-*anti*-3-Hydroxy-4-[4-[(4-methoxybenzyl)oxy]benzyl]-1-benzylpyrrolidine (35). A solution of **37** (0.297 mg, 1.0 mmol) in anhydrous DMF (2.5 mL) under N_2 was treated with 4-methoxybenzyl chloride (0.19 g, 1.2 mmol) and K_2CO_3 (0.166 g, 1.2 mmol), and the mixture was heated to 65 ± 5 °C for 2.5 h. Additional 4-methoxybenzyl chloride (30 mg, 0.2 mmol) was added, and heating was continued for 4.5 h longer. The mixture was cooled and added to water (20 mL), whereupon a precipitate formed. This was collected by filtration, washed with water, air-dried, dissolved in DCM, dried (Na_2SO_4), and concentrated *in vacuo*. The residual solid was chromatographed on silica gel (2-propanol/DCM, 3%) to afford (±)-*anti*-4-hydroxy-3-[4-[(4-methoxybenzyl)oxy]benzyl]-1-benzylpyrrolidin-2-one (**54**) (0.34 g, 81%) as a white solid.

A cooled (−5 °C) solution of **54** (0.313 g, 0.75 mmol) in anhydrous THF (4 mL) under N_2 was treated dropwise with 1.0 N $LiAlH_4$/THF (1.9 mL, 1.9 mmol) at a rate to keep the pot temperature below 10 °C, then allowed to warm to room temperature, and stirred overnight. The mixture was refluxed for 3 h, cooled (5 °C), and treated dropwise sequentially with water (0.08 mL), 15% NaOH (0.08 mL), and water (0.24 mL). The suspension was stirred for 30 min and filtered, and the filter cake was washed with THF. The filtrate was concentrated *in vacuo*, and the residue was chromatographed on silica gel (MeOH/DCM, 10%) to afford **35** (0.246 g, 81%) as an off-white solid.

(±)-*anti*-1-[4-(Benzyloxy)phenoxy]-2-hydroxycyclopentane (39). A mixture of 4-(benzyloxy)phenol (0.402 g, 2.01 mmol), cyclopentene oxide (**40**) (43.6 mL, 5.00 mmol), and DBU (74.8 mL, 5.00 mmol) was heated to nearly 100 °C under an atmosphere of N_2. After 3 h additional cyclopentene oxide (43.6 mL, 5.00 mmol) was added, and heating was continued for 40 h. The crude reaction mixture was chromatographed twice on silica gel (DCM/hexane/EtOAc, 90/8/2, and then DCM/EtOAc, 97/3) to give **39** (119 mg, 42%) as a white solid: mp 76−79 °C. Anal. ($C_{18}H_{20}O_3$) C, H.

Method A. (*S*)-Benzyl 2-[[4-[[[3-[4-[(Benzyloxycarbonyl)oxy]phenyl]-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]-2,6-bis(benzyloxy)phenyl]carbonyl]-3-(benzyloxy)benzoate (55) and (*R*)-Benzyl 2-[[4-[[[3-[4-[(Benzyloxycarbonyl)oxy]phenyl]-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]-2,6-bis(benzyloxy)phenyl]carbonyl]-3-(benzyloxy)benzoate (56). To a solution of (*S*)-**12** (197 mg, 0.334 mmol), Et_3N (140 μL, 1.00 mmol), and DMAP (5.3 mg, 43.5 μmol) in DCM (5 mL) under an atmosphere of N_2 was added a solution of freshly prepared **2a** (201 mg, 0.290 mmol) in DCM (7 mL) at 0 °C. The ice bath was removed, and the reaction mixture was allowed to stir overnight. The reaction mixture was diluted with EtOAc (75 mL) and washed with 0.5 N HCl (2 × 20 mL) and brine (25 mL). The EtOAc layer was dried ($MgSO_4$) and concentrated. The crude residue was purified by column chromatography (DCM−2% acetone/DCM) to provide **55** (259 mg, 81%): mp 61−64 °C; $[\alpha]_D$ = −1.40° (0.43, $CHCl_3$);[11] ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 2H, J = 8 Hz), 7.50−7.02 (m, 34H), 6.96 (dd, 1H, J = 8, 2 Hz), 6.90 (d, 2H, J = 7 Hz), 5.28 (s, 2H), 5.16 (s, 2H), 4.81 (s, 4H), 4.75 (s, 2H), 4.32−4.10 (m, 2H), 3.89−3.78 (m, 1H), 2.81 (d, 2H, J = 7 Hz). Anal. ($C_{66}H_{55}NO_{13}S$) C, H, N, S.

Compound **56** was obtained from (*R*)-**12** using the same procedure: $[\alpha]_D$ = 1.10° (0.42, $CHCl_3$).[11]

General Procedure for the Palladium(II) Hydroxide on Carbon-Catalyzed Debenzylation: (*S*)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxy-

benzoic Acid [(*S*)-12b] and (*R*)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]-propyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic Acid [(*R*)-12b]. To a solution of **55** (252 mg, 0.229 mmol) and TFA (53 μL) in 1:1 EtOAc:EtOH (20 mL) under an atmosphere of N_2 was added Pearlman's catalyst (63 mg, 20% by wt on C, 25% by wt), and the reaction mixture was placed under 1 atmosphere of H_2. The reaction mixture was allowed to stir overnight. Purification by HPLC provided (*S*)-**12b** (120 mg, 83%): mp 148−150 °C; $[\alpha]_D$ = 1.02° (0.59, EtOH);[11] ^{1}H NMR (CD_3OD) δ 7.66 (d, 2H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz), 7.44 (dd, 1H, J = 8, 8 Hz), 7.37−7.25 (m, 3H), 7.04 (d, 1H, J = 8 Hz), 6.93 (d, 2H, J = 9 Hz), 6.78 (s, 2H), 6.62 (d, 2H, J = 8 Hz), 4.20 (dd, 1H, J = 4, 11 Hz), 4.07 (d, 1H, J = 7, 11 Hz), 3.77−3.68 (m, 1H), 2.83−2.66 (m, 2H); HRMS m/z 608.1247 [calcd for $C_{30}H_{25}NO_{11}S$ (M + 1) 608.1227]. Anal. ($C_{30}H_{25}NO_{11}S\cdot1.5H_2O$) C, H, N, S.

(*R*)-**12b** was obtained from **56** using the same procedure (94 mg, 74%): mp 148−150 °C; $[\alpha]_D$ = −2.86° (0.70, EtOH);[11] HRMS m/z 608.1253 [calcd for $C_{30}H_{25}NO_{11}S$ (M + 1) 608.1227]. Anal. ($C_{30}H_{25}NO_{11}S\cdot2H_2O$) C, H, N, S.

(*S*)-Methyl 2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoate [(*S*)-12c]: mp 123−125 °C; $[\alpha]_D$ = −3.95° (0.38, EtOH);[11] ^{1}H NMR (CD_3OD) δ 7.68 (d, 2H, J = 8 Hz), 7.51 (d, 1H, J = 7.5 Hz), 7.45 (d, 1H, J = 7.5 Hz), 7.38−7.28 (m, 3H), 7.07 (d, 1H, J = 8 Hz), 6.94 (d, 2H, J = 8.5 Hz), 6.82 (s, 2H), 6.63 (d, 2H, J = 8.5 Hz), 4.22 (dd, 1H, J = 11, 4.5 Hz), 4.10 (dd, 1H, J = 11, 6.5 Hz), 3.81−3.69 (m, 4H), 2.84−2.66 (m, 2H); MS m/z 622 (M + 1). Anal. ($C_{31}H_{27}NO_{11}S\cdot0.2TFA\cdot0.8H_2O$) C, H, N, S.

Method B. (±)-Benzyl 2-[[2,6-Bis(benzyloxy)-4-[[[2-[*N*-(methoxymethylene)-*N*-(phenylsulfonyl)amino]-3-[4-[(methoxymethylene)oxy]phenyl]propyl]oxy]carbonyl]-phenyl]carbonyl]-3-(benzyloxy)benzoate (57). A solution of **2b** (370 mg, 0.545 mmol) in THF (6 mL) was treated with 1,1′-carbonyldiimidazole (97 mg, 0.600 mmol) and stirred for 6 h at room temperature. A mixture of **6** (313 mg, 0.818 mmol) and DBU (90 μL, 0.600 mmol) in THF (6 mL) was then added to the reaction mixture. After 72 h, the mixture was poured into EtOAc (50 mL) and washed with water (50 mL) followed by brine (25 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (EtOAc/hexanes, 25−40%) and then purified further on a chromatotron (acetone/DCM, 1%) to afford 295 mg of **57** as a clear glass (51%): ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 2H, J = 7 Hz), 7.43 (d, 1H, J = 7.5 Hz), 7.36 (d, 2H, J = 8 Hz), 7.28−7.18 (m, 16H), 7.15 (d, 1H, J = 6 Hz), 7.12−7.08 (m, 6H), 6.96 (d, 1H, J = 2 Hz), 6.92 (d, 4H, J = 3.5 Hz), 6.88 (d, 1H, J = 1.5 Hz), 5.15 (s, 2H), 5.14 (s, 2H), 4.90 (s, 2H), 4.83 (s, 4H), 4.75 (s, 2H), 4.43−4.24 (m, 3H), 3.48 (s, 3H), 3.35 (s, 3H), 2.88−2.70 (m, 2H). Anal. ($C_{62}H_{57}NO_{13}S$) C, H, N, S.

(±)-Benzyl 2-[[2,6-Bis(benzyloxy)-4-[[[3-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]-phenyl]carbonyl]-3-(benzyloxy)benzoate (58). Compound **57** (280 mg, 0.265 mmol) was dissolved in ∼0.5 N HCl/dioxane (20 mL), and 2 drops of concentrated HCl was added. After 24 h, the mixture was concentrated to an oil and dried under high vacuum to give 255 mg (100%) of **58**.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]-carbonyl]-3-hydroxybenzoic Acid (6b). To a suspension of **58** (250 mg, 0.258 mmol) in 15:1 MeOH:EtOAc (32 mL) was added Pearlman's catalyst (50 mg, 20% by wt). The suspension was stirred under a H_2 atmosphere at ambient conditions for 20 h. The catalyst was filtered through Celite, and the filtrate was concentrated. The crude material was purified by reverse phase HPLC (0−100% B over 1 h; A = 5% CH_3CN in water with 0.1% TFA, B = CH_3CN) to give 60 mg of **6b** as a light yellow powder (38%): mp 143−145 °C; ^{1}H NMR see (*S*)-**12b**; MS m/z 608 (M + 1). Anal. ($C_{30}H_{25}NO_{11}S\cdot0.2TFA\cdot0.8H_2O$) C, H, N, S.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(methylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]-carbonyl]-3-hydroxybenzoic acid (7b): mp 92−100 °C dec; ^{1}H NMR (CD_3OD) δ 7.29 (d, 1H, J = 8.7 Hz), 7.07 (dd, 1H J = 7.9, 8.0 Hz), 6.91 (d, 2H, J = 8.4 Hz), 6.82 (d, 1H, J = 7.2 Hz), 6.77 (s, 2H), 6.54 (d, 2H, J = 8.4 Hz), 4.11 (dd, 1H, J = 11.2, 4.8 Hz), 3.99 (dd, 1H, J = 11.3, 6.1 Hz), 3.70−3.60 (m, 1H), 2.70 (dd, 1H, J = 13.9, 5.6 Hz), 2.48 (dd, 1H, J = 13.8, 9.0 Hz), 2.26 (s, 3H); HRMS m/z 546.1060 [calcd for $C_{25}H_{24}NO_{11}S$ (M + 1) 546.1070]. Anal. ($C_{25}H_{23}NO_{11}S\cdot0.4TFA\cdot0.5H_2O$) C, H, N, S.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(phenylcarbonyl)amino]propyl]oxy]carbonyl]phenyl]-carbonyl]-3-hydroxybenzoic acid (8b): mp 140−148 °C dec; ^{1}H NMR (CD_3OD) δ 8.44 (d, NH), 7.68 (d, 2H), 7.45 (m, 4H), 7.26 (t, 1H), 7.12 (d, 2H), 7.06 (d, 1H), 6.92 (s, 2H), 6.71 (d, 2H), 4.61 (m, 1H), 4.44 (dd, 1H), 4.30 (dd, 1H), 2.91 (m, 2H); HRMS m/z 572.1551 [calcd for $C_{31}H_{26}NO_{10}$ (M + 1) 572.1557]. Anal. ($C_{31}H_{25}NO_{10}\cdot0.3TFA\cdot H_2O\cdot0.1CH_3CN$) C, H, N.

(±)-2-[[2,6-Dihydroxy-4-[[2-(4-hydroxyphenyl)-2-[(phenylsulfonyl)amino]ethoxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (13b): mp 144−146 °C dec; ^{1}H NMR (CD_3OD) δ 7.66 (d, 2H, J = 7 Hz), 7.52 (d, 1H, J = 8 Hz), 7.42−7.24 (m, 4H), 7.12−7.03 (m, 3H), 6.72 (s, 2H), 6.67 (d, 2H, J = 9 Hz), 4.68−4.61 (m, 1H), 4.37−4.21 (m, 2H); MS m/z 594 (M + H). Anal. ($C_{29}H_{23}NO_{11}S\cdot0.75H_2O$) C, H, N, S.

(±)-2-[[2,6-Dihydroxy-4-[[[2-(*N*,*N*-dimethylamino)-3-(4-hydroxyphenyl)propyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid, trifluoroacetic acid salt (15b): mp 124−127 °C dec; ^{1}H NMR (CD_3OD) δ 7.49 (d, 1H, J = 8.7 Hz), 7.28 (t, 1H, J = 8.7 Hz), 7.12 (d, 2H, J = 8.6 Hz), 7.03 (d, 1H, J = 8.7 Hz), 6.97 (s, 2H), 6.76 (d, 2H, J = 8.6,), 4.52 (dd, 1H, J = 13.7, 2.4 Hz), 4.39 (dd, 1H, J = 13.7, 5.8 Hz), 3.94 (m, 1H), 3.21 (m, 1H), 2.98 (m, 1H), 3.03 (s, 6H); MS m/z 496 (M + 1). Anal. ($C_{26}H_{25}NO_9\cdot1.6TFA\cdot1.0H_2O$) C, H, N.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[(naphthylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]-carbonyl]-3-hydroxybenzoic acid (16b): MS m/z 658 (M + 1).

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-2-[[(4-hydroxyphenyl)carbonyl]amino]propyl]oxy]carbonyl]-phenyl]carbonyl]-3-hydroxybenzoic acid (17b): mp 133−137 °C dec; ^{1}H NMR (CD_3OD) δ 7.60 (d, 2H, J = 8.7 Hz), 7.48 (d, 1H, J = 7.9 Hz), 7.26 (t, 1H, J = 7.7 Hz), 7.09 (d, 2H, J = 8.4 Hz), 7.00 (d, 1H, J = 7.9 Hz), 6.91 (s, 2H), 6.78 (d, 2H, J = 8.7 Hz), 6.69 (d, 2H, J = 8.4 Hz), 4.58 (m, 1H), 4.39 (dd, 1H, J = 13.9, 4.4 Hz), 4.28 (dd, 1H, J = 13.9, 7.3 Hz), 3.00−2.80 (m, 2H); MS m/z 588 (M + 1). Anal. ($C_{31}H_{25}NO_{11}\cdot0.8TFA\cdot1.0CH_3CN$) C, H, N.

2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)propyl]oxy]-carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (20b): mp 124−127 °C; ^{1}H NMR (CD_3OD) δ 7.51 (d, 1H, J = 8 Hz), 7.28 (dd, 1H, J = 8, 8 Hz), 7.07−7.01 (m, 3H), 6.93 (s, 2H), 6.71 (d, 2H, J = 8 Hz), 4.26 (t, 2H, J = 7 Hz), 2.68 (t, 2H, J = 7 Hz), 2.02 (tt, 2H, J = 7, 7 Hz); MS m/z 453 (M + H). Anal. ($C_{24}H_{20}O_9\cdot0.5H_2O$) C, H.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenyl)-1-methylpropyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (21b): mp 130−133 °C; ^{1}H NMR (CD_3OD) δ 7.51 (d, 1H, J = 8 Hz), 7.28 (dd, 1H, J = 8, 8 Hz), 7.03 (d, 1H, J = 8 Hz), 6.99 (d, 2H, J = 8.5 Hz), 6.91 (s, 2H), 6.68 (d, 2H, J = 8.5 Hz), 5.10−5.00 (m, 1H), 2.71−2.51 (m, 2H), 2.08−1.81 (m, 2H), 1.33 (d, 3H, J = 6 Hz); MS m/z 467 (M + H). Anal. ($C_{25}H_{22}O_9\cdot0.5H_2O$) C, H.

(±)-Methyl 2-[[2,6-dihydroxy-4-[[[3-(4-hydroxyphenyl)-1-methylpropyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoate (21c): mp 92−95 °C dec; ^{1}H NMR (CD_3OD) δ 7.48 (dd, 1H, J = 8, 1 Hz), 7.29 (dd, 1H, J = 8, 8 Hz), 7.05 (dd, 1H, J = 8, 1 Hz), 6.99 (d, 2H, J = 8.5 Hz), 6.92 (s, 2H), 6.68 (d, 2H, J = 8.5 Hz), 5.10−4.98 (m, 1H), 3.72 (s, 3H), 2.71−2.53 (m, 2H), 2.08−1.81 (m, 2H), 1.34 (d, 3H, J = 6 Hz); MS m/z 481 (M + H). Anal. ($C_{26}H_{24}O_9\cdot0.5H_2O$) C, H.

2-[[2,6-Dihydroxy-4-[[[4-(4-hydroxyphenyl)butyl]oxy]-carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (22b): mp 124−127 °C; ^{1}H NMR (CD_3OD) δ 7.50 (d, 1H, J = 8 Hz), 7.27 (dd, 1H, J = 8 Hz), 7.03 (d, 1H, J = 8 Hz), 7.01 (d, 2H, J = 8.5 Hz), 6.89 (s, 2H), 6.69 (d, 2H, J = 8.5 Hz), 4.29 (t, 2H, J = 6 Hz), 2.59 (t, 2H, J = 7 Hz), 1.81−1.66 (m, 4H); MS m/z 467 (M + H). Anal. ($C_{25}H_{22}O_9\cdot0.5H_2O$) C, H.

(±)-2-[[2,6-Dihydroxy-4-[[[3-(4-hydroxyphenoxy)-2-[(phenylsulfonyl)amino]propyl]oxy]carbonyl]phenyl]-carbonyl]-3-hydroxybenzoic acid (26b): mp 114−135 °C; ^{1}H NMR (CD_3OD) δ 7.80 (d, 2H, J = 7 Hz), 7.52 (dd, 1H, J = 1, 9 Hz), 7.48−7.34 (m, 3H), 7.29 (t, 1H, J = 10 Hz), 7.04 (dd, 1H, J = 1, 8 Hz), 6.71 (s, 2H), 6.70−6.65 (m, 4H), 4.43 (dd, 1H, J = 4, 12 Hz), 4.32 (dd, 1H, J = 7, 12 Hz), 4.04−3.98 (m, 1H), 3.95−3.89 (m, 2H); MS m/z 623 (M + H). Anal. ($C_{30}H_{25}NO_{12}S\cdot0.1TFA\cdot1.2H_2O$) C, H, N, S.

(±)-*anti*-2-[[2,6-Dihydroxy-4-[[[2-(4-hydroxybenzyl)cyclopentyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (29b): mp 141−144 °C; ^{1}H NMR (CD_3OD) δ 7.51 (d, 1H, J = 8 Hz), 7.28 (dd, 1H, J = 8, 8 Hz), 7.04 (d, 1H, J = 8 Hz), 6.95 (s, 2H), 6.94 (d, 2H, J = 8.5 Hz), 6.64 (d, 2H, J = 8.5 Hz), 5.15 (dd, 1H, J = 4, 4 Hz), 2.76 (dd, 1H, J = 8, 15 Hz), 2.62 (dd, 1H, J = 8, 15 Hz), 2.30−2.22 (m, 1H), 2.06−1.56 (m, 6H); MS m/z 493 (M + H). Anal. ($C_{27}H_{24}O_9\cdot1.25H_2O$) C, H.

(±)-*anti*-Methyl 2-[[2,6-dihydroxy-4-[[[2-(4-hydroxybenzyl)cyclopentyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoate (29c): mp 111−114 °C; ^{1}H NMR (CD_3OD) δ 7.48 (dd, 1H, J = 8, 1 Hz), 7.29 (dd, 1H, J = 8, 8 Hz), 7.05 (dd, 1H, J = 8, 1 Hz), 6.96 (s, 2H), 6.95 (d, 2H, J = 8.5 Hz), 6.65 (d, 2H, J = 8.5 Hz), 5.15 (dd, 1H, J = 4, 4 Hz), 3.72 (s, 3H), 2.77 (dd, 1H, J = 8, 14 Hz), 2.63 (dd, 1H, J = 8, 14 Hz), 2.31−2.28 (m, 1H), 2.06−1.57 (m, 6H); MS m/z 507 (M + H). Anal. ($C_{28}H_{26}O_9\cdot1.25H_2O$) C, H.

(±)-*syn*-2-[[2,6-Dihydroxy-4-[[[2-(4-hydroxybenzyl)cyclopentyl]oxy]carbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (30b): mp 129−132 °C; ^{1}H NMR (CD_3OD) δ 7.50 (d, 1H, J = 8 Hz), 7.27 (dd, 1H, J = 8, 8 Hz), 7.02 (d, 1H, J = 8 Hz), 7.00 (d, 2H, J = 8.5 Hz), 6.83 (s, 2H), 6.67 (d, 2H, J = 8.5 Hz), 5.05−4.95 (m, 1H), 2.75 (dd, 1H, J = 6, 14 Hz), 2.49 (dd, 1H, J = 9, 14 Hz), 2.41−2.38 (m, 1H), 2.18−2.02 (m, 1H), 1.93−1.68 (m, 4H), 1.45−1.34 (m, 1H); MS m/z 493 $(M + H)^+$; analytical HPLC eluted in 24.3 min (area = 100%) on C18 using 0−100% B over 45 min with A = 5% CH_3CN + 0.1% TFA in water and B = CH_3CN. Anal. ($C_{27}H_{24}O_9\cdot1H_2O\cdot0.1DMF$) C, H, N.

Method C. (±)-*anti*-3-[[3,5-Bis(benzyloxy)-4-[6-(benzyloxycarbonyl)-2-(benzyloxy)benzoyl]benzoyl]oxy]-4-[4-[(4-methoxybenzyl)oxy]benzyl]-1-benzylpyrrolidine (59). A cooled (−20 °C) solution of **35** (0.34 g, 0.84 mmol) in anhydrous THF (5 mL) under N_2 was treated with nBuLi/hexane (0.35 mL, 2.45 N, 0.86 mmol); then the mixture was stirred at room temperature for 10 min, concentrated *in vacuo*, and placed under high vacuum. Meanwhile, a solution of **2b** (0.62 g, 0.90 mmol) in anhydrous THF (3 mL) under N_2 was treated with 1,1′-carbonyldiimidazole (0.17 g, 1.05 mmol) and then stirred for 2 h at room temperature after gentle warming with a heat gun. This acylimidazole solution was transferred into the flask containing the above alcoholate under N_2, and the mixture was stirred at room temperature for 16 h and at 55 °C for 1 h. The solution was concentrated *in vacuo*, and the residue was chromatographed (EtOAc:hexane, 1:1) to afford **59** (0.66 g, 74%) as a white foam.

(±)-*anti*-3-[[4-(6-Carboxy-2-hydroxybenzoyl)-3,5-hydroxybenzoyl]oxy]-4-(4-hydroxybenzyl)pyrrolidine, Trifluoroacetic Acid Salt (35b). A solution of **59** (0.70 g, 0.658 mmol) in 3:1 EtOH:EtOAc (40 mL) in a 500 mL Parr bottle was purged with N_2. Trifluoroacetic acid (0.15 mL) was added followed by 20% $Pd(OH)_2$/C (0.2 g), and the vessel was promptly charged without delay with H_2 (45 psi) on a Parr apparatus and shaken for 18 h. The bottle was carefully evacuated of H_2, the solution was filtered through Celite, and then the filter cake was washed with EtOH but **not** allowed to dry. The filtrate was concentrated *in vacuo* to a yellow foam, dissolved in TFA (12 mL), and then heated to 55 °C for 2 h under N_2 in order to remove the 4-methoxybenzyl protecting group. The solution was concentrated *in vacuo* and the residue purified by C18 HPLC (0−100% B over 1 h; A = 5% CH_3CN/H_2O + 0.5% TFA, B = CH_3CN) to afford **35b** (0.235 g, 54%) as a voluminous yellow solid: mp 186−191 °C; R_f (6:1:1 nBuOH:AcOH:H_2O) 0.50; ^{1}H NMR (DMSO-d_6) δ 12.95 (br s, 1H), 11.79 (s, 2H), 9.96 (s, 1H), 9.28 (s, 1H), 9.15 (br s, 1H), 9.00 (br s, 1H), 7.40 (d, 1H, J = 8 Hz), 7.30 (t, 1H, J = 8 Hz), 7.09 (d, 1H, J = 8 Hz), 6.98 (d, 2H, J = 8 Hz), 6.97 (s, 2H), 6.66 (d, 2H, J = 8 Hz), 5.27 (br s, 1H), 3.30−3.60 (m, 3H), 3.10 (m, 1H), 2.70 (m, 3H); MS m/z 494 (M + H). Anal. ($C_{26}H_{23}NO_9\cdot1.3TFA\cdot H_2O$) C, H, N.

(±)-*anti*-3-[[3,5-Dihydroxy-4-[2-hydroxy-6-(methoxycarbonyl)benzoyl]-benzoyl]oxy]-4-(4-hydroxybenzyl)pyrrolidine, Trifluoroacetic Acid Salt (35c). A cooled (−20 °C) solution of **35b** (63 mg, 0.095 mmol) in absolute MeOH (1.5 mL) was treated dropwise with thionyl chloride (0.15 mL, 2.0 mmol), placed under a drying tube, then warmed to 50 °C over 30 min, and stirred at 50 °C for 2 h. The solution was concentrated *in vacuo*, and the residue was dissolved in DMF (0.5 mL) and purified by C18 HPLC (0−100% B over 1 h; A = 5% CH_3CN/H_2O + 0.5% TFA, B = CH_3CN) to afford **35c** (0.034 g, 52%) as a voluminous orange solid: mp 144−147 °C; R_f (75:24:1 DCM:MeOH:NH_3) 0.50; ^{1}H NMR (DMSO-d_6) δ 11.82 (s, 2H), 10.14 (s, 1H), 9.34 (s, 1H), 9.18 (br s, 1H), 9.00 (br s, 1H), 7.44 (d, 1H, J = 8 Hz), 7.35 (t, 1H, J = 8 Hz), 7.17 (d, 1H, J = 8 Hz), 7.05 (s, 2H), 6.99 (d, 2H, J = 8 Hz), 6.71 (d, 2H, J = 8 Hz), 5.30 (br s, 1H), 3.69 (s, 3H), 3.30−3.60 (m, 3H), 3.05−3.20 (m, 1H), 2.70 (m, 3H); MS m/z 508 (M + H). Anal. ($C_{27}H_{25}NO_9\cdot1.4TFA\cdot H_2O$) C, H, N.

(±)-*anti*-2-[[2,6-Dihydroxy-4-[[[2-[(4-hydroxyphenyl)-oxy]cyclopentyl]oxyc]arbonyl]phenyl]carbonyl]-3-hydroxybenzoic acid (39b): mp 114−138 °C; MS m/z 494 (M + H). Anal. ($C_{26}H_{22}O_{10}\cdot0.75\ H_2O\cdot CH_3CN$) C, H, N.

Protein Kinase C Expression and Purification. The α, β_I, β_{II}, γ, δ, ϵ, and η recombinant human PKC enzymes were produced using a baculovirus expression system in SF9 cells.[20] The Ca^{2+} independent isozymes (δ, ϵ, and η) were purified as described in the literature by Bronson et al.[21] The Ca^{2+} dependent isozymes (α, β_I, β_{II}, and γ) were purified using a modification of a method described by Kochs et al.[22] After the Ca^{2+} dependent isozyme was released by EGTA treatment, it was purified on a Poros Q (Perspective Biosystems) anion exchange column using 0−500 mM NaCl. Each fraction was assayed for PKC activity, and the peak activity for each recombinant PKC was pooled and used in these studies. Purities range from 50% to 90% depending on isozyme subtype.

Protein Kinase C Assay. PKC was assayed by quantitating the incorporation of ^{32}P from [γ-^{32}P]ATP into histone type IIIS. The reaction mixture (250 μL) contained 30 mg of phosphatidylserine (Avanti), 20 mM Hepes buffer (pH 7.5; Sigma), 10 mM $MgCl_2$, 47.5 μM EGTA, 100 mM $CaCl_2$, 200 μg/mL histone (Sigma), 10 μL of DMSO or 10 μL of a solution of inhibitor in DMSO, 30 μM [γ-^{32}P]ATP (DuPont), the enzyme, and diacylgylcerol. The amount of diacylglycerol necessary for 50% maximal activation of the enzyme was used. This amount was isozyme dependent. The assay was incubated for 10 min at 30 °C and terminated with 500 μL of 25% trichloroacetic acid and 100 μL of bovine serum albumin (1 mg/mL; Sigma). The reaction mixtures were filtered onto glass fiber filters and quantified by counting in a β scintillation counter. The reported IC_{50}s are single determinations. Most IC_{50}s were determined by testing the compounds at 0.05, 0.5, 5, and 50 μM and then at 0.5, 5, 50, and 500 nM, respectively. The more pertinent of the two values was reported. Assay controls included a maximal lipid-activated PKC assay and a no-lipid PKC assay. The no-lipid activity was subtracted from the maximal lipid dependent activity to account for background nonspecific kinase activities. The PKC inhibitor sphingosine, which inhibits all the PKC isozymes, was included as a control inhibitor for all of the PKC assays.[23]

cAMP Dependent Protein Kinase Assay. The assay components were in a total volume of 250 μL: 20 mM Hepes buffer (pH 7.5; Sigma), 200 μg/mL histone type HL (Worthington), 10 mM $MgCl_2$ (Sigma), 10 μL of DMSO or 10 μL of a solution of inhibitor in DMSO, and 30 μM [γ-^{32}P]ATP (DuPont). The reaction was initiated by the addition of bovine heart cAMP dependent kinase catalytic subunit (Sigma), incubated at 30 °C for 10 min, and stopped by adding 0.5 mL of ice cold trichloroacetic acid (Amresco) followed by 100 μL of 1 mg/mL bovine serum albumin (Sigma). The precipitate was collected by vacuum filtration on glass fiber filters employing a TomTec cell harvester and quantified by counting in a β scintillation counter.

Casein Protein Kinase II Assay. The assay components were in a total volume of 250 μL: 20 mM Tris-HCl (pH 7.5), 5 mM sodium fluoride, 50 mg/mL casein (Sigma), 10 mM $MgCl_2$ (Sigma), 10 μL of DMSO or 10 μL of a solution of inhibitor in DMSO, and 30 μM [γ-^{32}P]ATP (DuPont). The reaction was initiated by addition of casein protein kinase II (isolated from rat brain homogenate), incubated at room temperature for 10 min, and stopped by the addition of 0.5 mL of ice cold trichloroacetic acid (Amresco) followed by 100 μL of 1 mg/mL bovine serum albumin (Sigma). The precipitate was collected by vacuum filtration on glass fiber filters and quantified by counting in a β scintillation counter.

Ca^{2+} Calmodulin Dependent Protein Kinase Assay. Assay conditions were as follows: 50 mM Tris-HCl (pH 7.5), 5 mM β-mercaptoethanol, 400 μM EGTA, 700 μM calcium chloride (Sigma), 10 mM $MgCl_2$ (Sigma), 200 μg/mL histone type HA (Worthington), 10 μL of DMSO or 10 μL of a solution of inhibitor in DMSO, and 30 μM [γ-^{32}P]ATP (DuPont). The reaction was initiated by addition of Ca^{2+} calmodulin dependent protein kinase (isolated from rat brain homogenate), incubated at room temperature for 10 min, and stopped by adding 0.5 mL of ice cold trichloroacetic acid (Amresco) followed by 100 μL of 1 mg/mL bovine serum albumin (Sigma). The precipitate was collected by vacuum filtration on GFC filters and quantified by counting in a β scintillation counter.

src Protein Tyrosine Kinase Assay. The assay was carried out according to a protocol obtained from Oncogene Science, Inc. except that Triton X-100 was used in place of Brij 35. Protein tyrosine kinase ($p60^{c-src}$, #PK03) and Raytide kinase substrate (#PK02) were obtained from Oncogene Science.

Neutrophil Assay. Phorbol-12-myristate-13-acetate (PMA; 15 ng/mL) was added to lucigenin in reaction HBSS containing a neutrophil suspension (2×10^6 cells/mL). Cuvettes were loaded into a luminometer, and chemiluminescence at 550 nm was measured for 15 cycles at 37 °C. Determinations made in the presence of test compounds were compared to maximum response values. IC_{50}s were determined using a four-point curve of 10-fold dilutions.

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