and Pituitary Program, University of Maryland School of Medicine. Data are plotted as the mean values for a given dose of peptide obtained by pooling the means from individual experiments done in quadruplicate. The number of experiments for each analogue is given in Table III. Potencies and 95% confidence intervals were calculated by four-point assay.[30]

(30) Pugsley, L. I. *Endocrinology* **1946**, *39*, 161.

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Registry No. I, 126821-35-0; II, 126789-51-3; III, 126789-52-4; IV, 126789-53-5; V, 126789-54-6; VI, 126789-55-7; VII, 126821-36-1; VIII, 126821-37-2; IX, 126821-38-3.

Cyclohexane Diester Analogues of Phorbol Ester as Potential Activators of Protein Kinase C†

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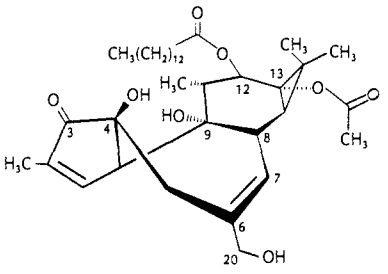
Phospholipid-dependent, Ca^{2+}-sensitive protein kinase (protein kinase C) is activated by the plant product phorbol ester at nanomolar concentrations and also in vivo at micromolar concentrations by diacylglycerols. We designed and synthesized cyclohexane diester analogues of the phorbol ester C ring as potential high-affinity activators of protein kinase C. We proposed that the necessary pharmacophore of phorbol ester could be mimicked by diesters of appropriately substituted cyclohexanediols. A series of 1,2-cyclohexanediol diesters with different substituents at position 4 was synthesized. These substituents were designed to mimic the 6,7-double bond and C-20 hydroxy of phorbol ester. Competitive binding vs [^{3}H]phorbol dibutyrate determined that these compounds have an affinity for protein kinase C of 1 mM or more, and thus they do not bind to nor are they activators of this enzyme.

Phorbol esters are tumor promoters which bind to and activate protein kinase C (PKC).[1] The activators of this enzyme that regulate it in vivo are diacylglycerols which are released upon receptor-mediated cleavage of inositol phospholipids.[2] Diacylglycerol binds to and activates PKC in concert with Ca^{2+} and membrane-associated phosphatidyl serine.[3] This activation is short-lived and well-controlled by the quick inactivation of diacylglycerol by diacylglycerol kinase[4] or diacylglycerol lipase.[5] The active phorbol esters, like TPA (1), all bind to and induce PKC



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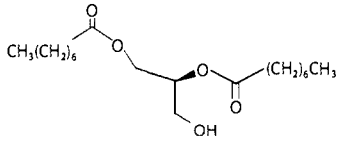

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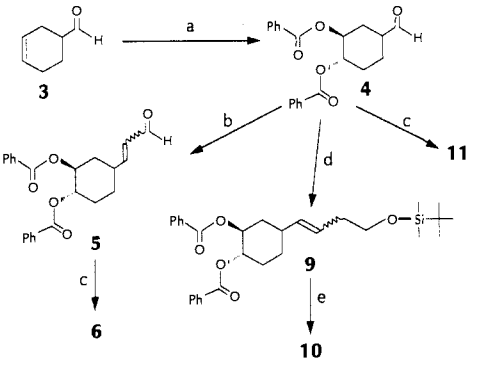
activity at nanomolar concentrations,[1f] whereas the diacylglycerols known to bind to PKC, like diC_8 (2), have affinities of 1–100 μM.[6] Phorbol esters thus can activate PKC for a prolonged period, leading to abnormally high levels of phosphorylated proteins.[7] This could be the reason for the cocarcinogenesis seen with tumor promoters. This enzyme is extremely important in signal transduction,

Scheme I. Synthesis of 1-Substituted-3,4-bis(benzoyloxy)cyclohexanes[a]



[a] (a) Silver benzoate, I_2; (b) (triphenylphosphoranylidene)acetaldehyde; (c) DBATO, PMHS; (d) butyllithium, compound 8; (e) tetrabutylammonium fluoride.

† Abbreviations used are as follows: PKC, protein kinase C; TPA, tetradecanoylphorbol acetate; diC_8, 1,2-dioctanoylglycerol; DBATO, dibutylacetyltin oxide; PMHS, poly(methoxyhydrosilane); PDBu, phorbol dibutyrate.

(1) (a) Ashendel, C. L. *Biochim. Biophys. Acta* **1985**, *822*, 219. (b) Nishizuka, Y. *Science* **1986**, *233*, 305. (c) Takai, Y.; Kaibuchi, K.; Tsuda, T.; Hoshiyima, M. *J. Cell. Biochem.* **1985**, *29*, 143. (d) Hecker, E. *Arzneim.-Forsch.* **1985**, *12*, 1890. (e) Wolf, M.; Cuatrecasas, P.; Sahyoun, N. *J. Bio. Chem.* **1985**, *260*, 15718. (f) Shoyab, M.; Todaro, G. J. *Nature* **1980**, *288*, 451. (g) Niedel, J. E.; Kahn, L. J.; Vandenbark, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 36. (h) Shoyab, M.; Todaro, G. *J. Biol. Chem.* **1982**, *257*, 434. (i) Shoyab, M.; Boaze, R. *Arch. Biochem. Biophys.* **1984**, *234*, 197. (j) Nishizuka, Y. *Cancer* **1989**, *63*, 1892.

(2) (a) Hannun, Y. A.; Loomis, C. R.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 7184. (b) DeChaffoy de Courcelles, P.; Roevens, P.; Van Belle, H. *J. Biol. Chem.* **1985**, *260*, 15762.

(3) Kishimoto, A.; Takai, Y.; Mori, T.; Kikkawa, N.; Nishizuka, Y. *J. Biol. Chem.* **1980**, *255*, 2273.

(4) Bishop, W. R.; Ganong, B. R.; Bell, R. M. *J. Biol. Chem.* **1985**, *261*, 6993.

(5) Chabbot, H.; Cabot, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3126.

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especially affecting the growth and differentiation of cells.[8]

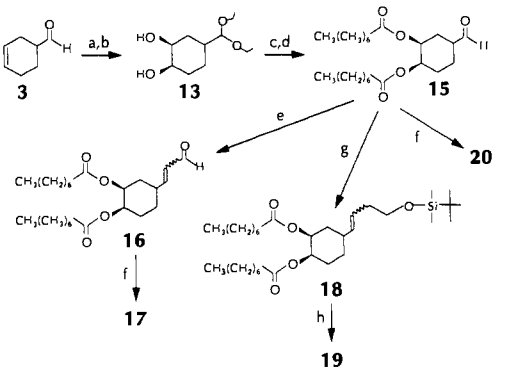
Given the effects that prolonged activation of PKC has on cell growth, the development of inhibitors of this enzyme might lead to useful chemotherapeutic agents. Many compounds inhibiting the activation of PKC have been discovered or synthesized, including acridines,[9] polymyxin B,[10] triphenylethylenes,[11] peptide analogues,[12] lipoidal amines,[13] proteins,[14] naphthalenesulfonamides,[15] and staurosporine.[16] These compounds act at either the ATP-binding site, the protein-binding site, or the diacylglycerol-regulatory site. None of them have proven to be selective, high-affinity inhibitors.

The structure–activity relationships of a variety of the phorbol esters have been determined[1d,17] and from this it is possible to determine the portions of the molecule that are important for biological activity. Studies have indicated that the C-20 hydroxy group is crucial for activity, with esterification, alkylation, or reduction of this group greatly reducing its ability to activate PKC.[1d,17] Also, at least one of the vicinal hydroxy groups at C_{12} and C_{13}, and preferably both, need to esterified.[18] The hydroxy group at position 4 is also critical, as is its β-configuration.[19] The 6,7-double bond is also important, as reduction of this to the saturated compound significantly reduces activity.[19] We focused on the C ring cyclohexane which contains the diesters and synthesized analogues with substituents which would mimic the 6,7-double bond and the C-20 hydroxy group.

Results

Chemistry. Our synthetic goal was a series of compounds which would be structurally similar to the C ring of phorbol ester (1). Since the cyclopropyl group fused to the cyclohexyl ring alters the relative configuration of the two ester groups, it was not known whether a cis or trans configuration would more closely mimic phorbol ester. To address this, we synthesized both the *cis*- and *trans*-diester isomers. Schemes I and II outline the syntheses used. The use of the 1,2,5,6-tetrahydrobenzaldehyde (**3**) as a starting material gave us access to both isomers. Scheme I outlines the route to *trans*-dibenzoyl ester **4**, which was made from **3** and silver benzoate by using the Prevost reaction.[20]

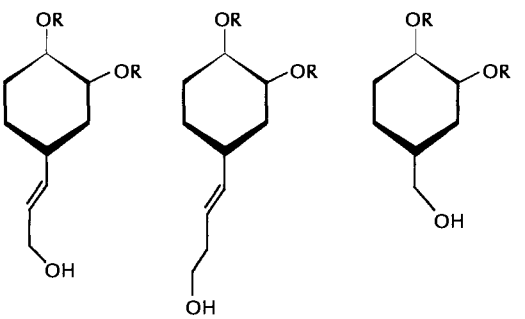
Scheme II. Synthesis of 1-Substituted-3,4-bis(octanoyloxy)cyclohexanes[a]



[a] (a) Triethyl orthoformate, Amberlyst; (b) OsO_4, *N*-methylmorpholine *N*-oxide; (c) octanoyl chloride, pyridine; (d) trifluoroacetic acid; (e) (triphenylphosphoranylidene)acetaldehyde; (f) DBATO, PMHS; (g) butyllithium, compound 8; (h) tetrabutylammonium fluoride.

Scheme II depicts the path to *cis*-dioctanoyl ester **14**, which was synthesized by treating protected **3** with osmium tetroxide[21] and esterifying the *cis*-diol with octanoyl chloride. Synthesis of the *cis*-diesters also yielded the separate axial and equatorial isomers at C-1. It has already been shown that the dioctanoyl derivatives of diacylglycerol and the dibenzoyl derivatives of phorbol ester are very active.[6,19]

The optimal distance of the important hydroxy group from the cyclohexane ring can be determined from the phorbol ester structure. In **2**, with micromolar binding affinity, this hydroxy is two carbons from the nearest acyl group, or 2.9 Å; whereas in **1**, with nanomolar affinity, there are six intervening carbons, or 8.1 Å. We synthesized **11**,



6: R= COPh; **17**: R= $CO(CH_2)_4CH_3$; **10**: R= COPh; **19**: R= $CO(CH_2)_4CH_3$; **11**: R= COPh; **20**: R= $CO(CH_2)_4CH_3$

20a, and **20b**, resulting in a four-carbon interval between the acyl and free hydroxy groups. Wittig chemistry[22] and subsequent reduction of the propenaldehyde with DBATO and PMHS[23] yielded **6a**, **6b**, **17a**, and **17b**, which directly mimic the distance between the hydroxy and acyl groups in phorbol ester. The one-carbon homologues **10**, **19a**, and **19b** were synthesized[22-24] to explore potential steric interference in binding to PKC.

(6) Kerr, D. E.; Kissinger, L. F.; Gentry, L. E.; Purchio, A. F.; Shoyab, M. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 776.
(7) May, W. S.; Tapetina, E. G.; Cuatrecasas, P. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1281.
(8) Nishizuka, Y. *Nature* **1984**, *308*, 693.
(9) Hannun, Y. A.; Bell, R. M. *J. Biol. Chem.* **1988**, *263*, 5124.
(10) Nel, A. E.; Wooten, M. W.; Goldschmidt-Clermont, P. J.; Miller, P. J.; Stevenson, H. C.; Galbraith, R. M. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 1364.
(11) O'Brian, C. A.; Fiskamp, R. M.; Solomon, D. H.; Weinstein, I. B. *Cancer Res.* **1985**, *45*, 2462.
(12) (a) Chan, K.-F. J.; Stoner, G. L.; Haskins, G. A.; Huang, K. P. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 1358. (b) Su, H.-D.; Kemp, B. E.; Turner, R. S.; Kuo, D. F. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 78.
(13) (a) Hannun, F. A.; Loomis, G. R.; Merril, A. H., Jr.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604. (b) Shoji, M.; Vogler, W. R.; Kew, J. F. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 590.
(14) McDonald, J. R.; Walsh, M. P. *Biochem. J.* **1985**, *232*, 559.
(15) Hidaka, H.; Inagaki, M.; Kowamoh, S.; Sasaki, Y. *Biochemistry* **1984**, *23*, 5036.
(16) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Makoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.
(17) Hecker, E. *Carcinogenesis*; Raven Press: New York, 1978; Vol. 2, pp 11–48.
(18) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. *J. Biol. Chem.* **1982**, *257*, 7847.
(19) Jeffrey, A. M.; Liskamp, R. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 241.
(20) McCasland, G. E.; Horswill, E. C. *J. Am. Chem. Soc.* **1954**, *76*, 1654.
(21) Van Rheenan, Z.; Cha, D. Y.; Hartley, W. M. *Org. Synth.* **1983**, *58*, 44.
(22) Bestman, H. J.; Hellwinkel, D.; Krebs, A.; Pommer, H.; Schoellkopf, U.; Thieme, P. C.; Vosrowsky, O.; Wilke, J. *Topics in Current Chemistry, Volume 109: Wittig Chemistry*; Springer-Verlag: Berlin, 1983.
(23) Lipkowitz, J.; Bowman, S. A. *J. Org. Chem.* **1973**, *38*, 162.
(24) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

Table I. [^{3}H]PDBu Binding Inhibition

compd	IC_{50}, μM	compd	IC_{50}, μM
1	0.010	**17a**	>1000
2	5	**17b**	>1000
6a	>1000	**19a**	>1000
6b	>1000	**19b**	>1000
10	>1000	**20a**	>1000
11	>1000	**20b**	1000

Molecular Modeling. The molecular modeling experiments were aimed at assessing the goodness of fit between O-3, O-4, C-1, C-6, and C-1–C of the cyclohexane derivatives with O-13, O-14, C-8, C-9, and C-7 of phorbol and then to determine the proximity of the C-20 hydroxy with the terminal hydroxy of the cyclohexane derivatives. Compounds **9**, **6b**, **10**, **11**, **17a**, **17b**, **19a**, **19b**, **20a**, and **20b** resulted in 40 different possible configurations. All of these compounds were minimized with the Alchemy minimization program. The compounds substituted at C-3 and C-4 with benzoate yielded 3,4-diequatorial *trans*-cyclohexane derivatives. The compounds substituted at C-3 and C-4 with octanoate gave us equatorial, axial compounds. Analysis of the phorbol structure revealed that whereas the configuration of 12,13-diesters was ambiguous, it more closely resembled the equatorial, equatorial configuration of the dibenzoate compounds. The fusion of the cycloheptane and cyclohexane rings of phorbol yields an equatorial configuration for C-7. The double bond at C-6,7 is trans and the distance from C-8 (equivalent to C-1 of the cyclohexane analogues) and the C-20 OH is three carbons. We surmised that compound **6a** would fit the best. Alchemy revealed that **6a** consisted of four different possible isomers. It exists in an axial, equatorial, equatorial (1,3,4) or equatorial, equatorial, equatorial configuration. We determined that the e,e,e configuration more closely resembled the phorbol structure than the a,e,e structure. The e,e,e configuration exists as an enantiomeric pair. The *R,R,R* configuration fitted best to phorbol with a mean separation between the five atoms tested of 0.47 Å. In this model O-3 and O-13 were separated by 0.40 Å, O-4 from O-12 by 0.44 Å and the terminal OH of the propenol from the C-20 OH by 0.77 Å. Of the various compounds synthesized, this was the best fit. Many of the other compounds also fit very well. The octanoyl derivatives, having a 3,4-axial,equatorial configuration did not fit well, and all of the compounds with C-1–C in an axial configuration did not fit well either.

Biology. The ability of TPA, diC_8, and the cyclohexane derivatives to compete with [^{3}H]PDBu for binding to PKC was measured. We have shown that a compound's binding affinity to PKC correlates well with the degree to which it can activate this enzyme.[6] The results of the competitive binding study (Table I) indicate that whereas TPA and diC_8 did bind well to PKC, the other compounds tested did not bind at all or with very low affinity, e.g. **20b**.

Discussion

The compounds made in this study were designed to be analogues of the phorbol esters. When the structures of diacylglycerols and phorbol esters are combined, the conclusion could be that the portions of the phorbol esters that are controlling much of the binding affinity and activation of PKC are contained within the fragment defined by the C ring cyclohexane, the C-12,13-vicinal diesters, and the C-20 hydroxy. Molecular modeling revealed that compound **6a** fit well with the integral parts of the putative (phorbol ester) pharmacophore. It was expected that this compound would bind well to PKC. This was not the case. This led us to conclude that more of the phorbol ester structure is responsible for its binding affinity and activity. The higher affinity displayed by **20b** is probably due to its approximation of diC_8.

The pharmacophores contained within the compounds known to bind and activate PKC (phorbol esters, teleocidin,[25] bryostatin,[26] and aplysiatoxin[27]) are very selective, i.e. more of each molecule is necessary to display its very high affinity for PKC. This is supported by the activity seen with simple compounds made to mimic the conserved structural characteristics of all these molecules.[28] These compounds exhibited only micromolar affinity for PKC. An explanation for this is that the formation of the complex of PKC, membrane phospholipids, Ca^{2+}, and an activator is a very complicated phenomenon that requires precise and multiple-site binding[29] and requires a larger portion of each of the active molecules mentioned above. Some researchers have contended that the vicinal diesters in the phorbol esters are equivalent to the vicinal diesters in diacylglycerol[8,29] and that they play an important role in recognition and anchoring the receptor complex to the membrane.[29] Other groups have proposed or synthesized compounds similar to the ones made in this study to determine the role of the diesters and C-20 or C-9 hydroxy in phorbol ester activity.[30] None of these compounds have proved to be highly active. New evidence derived from comparisons of the crystal structures and/or mathematical models of brysostatin, phorbol ester, and diacylglycerol has shown that the C-4 and C-9 hydroxys of phorbol ester are equivalent to the acyl oxygens of diacylglycerol.[32] Others have shown that the C-3 carbonyl is also important.[30] The data presented here would support these proposals.

Indolactam analogues of teleocidin have been synthesized[25] which are capable of eliciting teleocidin-like effects at nanomolar concentrations and which bind to PKC with K_D's of approximately 0.5 μM. These compounds incorporate most of the structural features of teleocidin, which is the reason for their success as analogues. This is the approach which would have to be taken with future analogues of phorbol ester. The pursuit of simple cyclohexane analogues seems to be unjustified, given the lack of activity of any of the compounds made. Any new compounds would need to include many more of the critical binding sites in the pharmacophore.

Experimental Section

All reagents were purchased from Aldrich. Solvents were from J. T. Baker and were used without further purification. Melting points were determined on a Fischer-Johns apparatus and are uncorrected. ^{1}H NMR's were recorded on either a Varian 300- or 360-MHz spectrometer relative to tetramethylsilane. Flash

(25) Fujiki, H. *Gann* **1984**, *75*, 866.
(26) Berkow, R.-L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109.
(27) (a) Horowitz, A.; Fujiki, H.; Weistein, I. B.; Jeffrey, A.; Okin, E.; Moore, R. E.; Sugimura, T. *Cancer Res.* **1983**, *43*, 1529. (b) Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9672.
(28) Wender, P. A.; Koehler, K. F.; Sharkey, N. A.; Dell'Aguila, M. L.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4214.
(29) Ganong, B. R.; Loomis, C. R.; Hannun, Y. A.; Bell, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1184.
(30) (a) Laughton, C. A.; Dale, I. L.; Gescher, A. *J. Med. Chem.* **1989**, *32*, 428. (b) Molleyres, L. P.; Rando, R. P. *J. Biol. Chem.* **1988**, *263*, 14832.
(31) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197.
(32) Pettersen, R. C.; Birnbaum, G. I.; Ferguson, G.; Islam, K. M. S.; Sime, J. G. *J. Chem. Soc. B* **1968**, 980.

chromatography was done with Kieselgel 60 from E. Merck. Elemental analyses were performed by the Bristol-Myers analytical department, Syracuse, New York, and are within ±0.4% unless otherwise noted.

3,4-Bis(benzoyloxy)cyclohexanecarboxaldehyde (4). Silver benzoate (4.37 g, 19.1 mmol) was suspended in 13 mL of dry benzene. To this was added, dropwise, a solution of 2.30 g (9.1 mmol) of iodine in 6 mL of benzene. After stirring for 30 min, 1.1 g of 1,2,3,4-tetrahydrobenzaldehyde (10 mmol) in 2 mL of benzene was added over 30 min. The reaction mixture was stirred at reflux for 3 h. Silver iodide was removed by filtration, the filtrate was washed with benzene, the benzene was evaporated, and the residue was chromatographed with 2:1 hexane/ether. Pure fractions were collected, evaporated, and dried under vacuum to yield 2.1 g of 4 as a white powder (11 mmol, 58% yield), mp 110 °C. This was most likely a mixture of 1,3,4-eq,eq,eq and -ax,eq,eq isomers: ^{1}H NMR ($CDCl_3$) δ 1.9–2.2 (m, 6 H, ring CH_2), 2.7 (m, 1 H, CHCO), 5.2 (d, 1 H, J = 4 Hz, HCOCO), 5.3 (d, 1 H, J = 4 Hz, HCOCO), 7.2–7.6 (m, 6 H, 3,4-ArH), 7.8–8.0 (m, 4 H, 2-ArH), 9.7 (s, 1 H, COH). Anal. ($C_{21}H_{20}O_5 \cdot 2H_2O$) C, H.

3-[3,4-Bis(benzoyloxy)cyclohexyl]-2-propen-1-al (5). (Triphenylphosphoranylidene)acetaldehyde (475 mg, 1.6 mmol) and 500 mg (1.4 mmol) of 4 were mixed in 50 mL of anhydrous toluene, refluxed for 4 h, and poured into 50 mL of water. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated. White crystals formed from ether/hexane to yield 415 mg of 5 (1.05 mmol, 75% yield): mp 95 °C; ^{1}H NMR ($CDCl_3$) δ 1.7–2.9 (m, 7 H, ring CH_2 and CHC=C), 5.4 (m, 2 H, CHOCO), 6.25 (m, 1 H, C=CH), 6.85 (m, 1 H, CH=C), 7.4–7.7 (m, 6 H, 3,4-ArH), 8.05 (m, 4 H, 2-ArH), 9.4 (m, 1 H, COH). Anal. ($C_{23}H_{22}O_5 \cdot 2.25H_2O$) C, H: calcd, 6.38; found, 5.66.

4-[3,4-Bis(benzoyloxy)cyclohexyl]-2-propen-1-ol (6). General procedure for the preparation of **6a**, **6b**, 11, **17a**, **17b**, **20a**, and **20b**: DBATO (40 mg, 0.066 mmol) and 250 mg (1.3 mmol) of 5 were mixed in 10 mL of 95% ethanol and brought to reflux. PMHS (91 mg, 1.45 mmol) was added, and after 1 h, 10 mL of H_2O was added and reflux was continued for 30 min. The reaction mixture was separated between H_2O and $CHCl_3$; the organic layer was dried ($MgSO_4$), evaporated, and chromatographed with 99:1 CH_2Cl_2/CH_3OH. This chromatography yielded separate *trans*- and *cis*-alkene isomers, 72 mg of trans (**6a**), 53 mg of cis (**6b**), and 120 mg of the mixture, all as clear oils. The overall yield was 0.245 g (0.65 mmol, 95% yield). **6a**: ^{1}H NMR ($CDCl_3$) δ 1.3–2.3 (m, 7 H, ring CH_2 and CHC=C), 3.6 (d, 1 H, J = 6.5 Hz, CHOH), 4.15 (m, 1 H, CHOH), 5.3 (m, 4 H, CHOCO), 5.6–5.7 (m, 2 H, CH=CH), 7.4 (m, 6 H, 3,4-ArH), 8.0 (m, 2-ArH). Anal. ($C_{23}H_{24}O_5 \cdot 0.5H_2O$) C, H. **6b**: ^{1}H NMR ($CDCl_3$) δ 1.6–2.1 (m, 7 H, ring CH_2 and CHC=C), 4.2 (m, 2 H, CH_2OH), 5.3 and 5.4 (q, 1 H, J = 5.5 Hz CHOCO), 5.8 (d, 2 H, CH=CH), 7.5 (m, 6 H, 3,4-ArH), 8.05 (m, 4 H, 2-ArH). Anal. ($C_{23}H_{24}O_5 \cdot 0.75H_2O$) C, H.

1-*O*-(Dimethyl-*tert*-butylsilyl)-3-bromopropanol (7). In 80 mL of dry CCl_4 were combined 10 g (0.07 mol) of 3-bromopropanol and 8.7 g (0.08 mol) of 2,6-lutidine. This was cooled to 0 °C and 21.2 g (0.08 mol) of chlorodimethyl-*tert*-butylsilane in 50 mL of CCl_4 was added dropwise. Stirring was continued for 4 h; the reaction was filtered, evaporated to a residue, and vacuum distilled. The desired compound distilled at 82 °C (0.01 mmHg) to yield 15.2 g (0.055 mol, 80% yield) of 7 as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 6 H, $SiCH_3$), 0.85 (s, 9 H, $C(CH_3)_3$), 1.95 (q, 2 H, J = 6.0 Hz, CCH_2C), 3.4 (t, 2 H, J = 6.5 Hz, CH_2O), 3.7 (t, 2 H, J = 5.5 Hz, CH_2Br). Anal. ($C_9H_{21}BrOSi$) C, H.

3-(Triphenylphosphonio)-1-*O*-(dimethyl-*tert*-butylsilyl)-1-propanol Bromide (8). Triphenylphosphine (3.5 g, 13 mmol) and 3.3 g (11.9 mmol) of 7 were combined in 50 mL of acetonitrile and refluxed for 18 h, at which time white crystals were collected by filtration and dried under vacuum to yield 5.1 g (10 mmol, 77% yield) of 8: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 0.0 (s, 6 H, $SiCH_3$), 0.85 (s, 9 H, $C(CH_3)_3$), 2.15 (q, 2 H, CCH_2C), 3.4 (t, 2 H, J = 6.5 Hz, CH_2O), 4.1 (t, 2 H, J = 7.0 Hz, CH_2P). Anal. ($C_{27}H_{36}BrOPSi \cdot 0.25H_2O$) C, H.

4-[3,4-Bis(benzoyloxy)cyclohexyl]-1-*O*-(dimethyl-*tert*-butylsilyl)-3-buten-1-ol (9). In 5 mL of anhydrous THF under nitrogen was suspended 0.73 g of 8 (1.4 mmol). To this was added 0.67 mL of a 2.5 M solution of butyllithium in hexane (1.7 mmol). After stirring for 1 h the red solution was added dropwise to 0.5 g of 4 (1.4 mmol) in 10 mL of THF. This was stirred overnight, and separated between ether and water. The combined ether extracts were washed, dried ($MgSO_4$), evaporated, and chromatographed with 1:1 hexane/ether to yield 0.56 g of 9 as a clear oil (1.1 mmol, 77% yield). The ^{1}H NMR indicated that this was a mixture of *cis*- and *trans*-olefins which could not be separated by flash chromatography: ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 6 H, $SiCH_3$), 0.8 (s, 9 H, $C(CH_3)_3$), 1.3–2.0 (m, 7 H, ring CH_2 and CHC=C), 2.25 (m, 2 H, CH_2C=C), 3.6 (t, 2 H, J = 6.5 Hz, CH_2OSi), 5.2–5.5 (m, 4 H, CH=CH and CHOCO), 7.3–7.5 (m, 6 H, 3,4-ArH), 7.8–8.1 (m, 4 H, 2-ArH). Anal. ($C_{30}H_{40}O_6Si$) C, H.

4-[3,4-Bis(benzoyloxy)cyclohexyl]-3-buten-1-ol (10). To 0.1 g of 9 (0.2 mmol) in 1 mL of THF was added 0.13 g of tetrabutylammonium fluoride (0.5 mmol). This was stirred for 4 h, evaporated to a residue, and chromatographed with 99:1 CH_2Cl_2/CH_3OH to yield 22 mg of 10 as a clear oil (57 μmol, 28% yield): ^{1}H NMR ($CDCl_3$) δ 1.4–1.9 (m, 7 H, ring CH_2 and CHC=C), 2.3 (m, 2 H, C=CCH_2), 3.6 (t, 2 H, J = 6.5 Hz, CH_2OH), 5.1–5.6 (m, 4 H, CHOCO and CH=CH), 7.6 (m, 6 H, 3,4-ArH), 7.9 (m, 2-ArH). Anal. ($C_{24}H_{26}O_5$) C, H.

3,4-Bis(benzoyloxy)cyclohexanemethanol (11). This was treated as for 6 and chromatographed with 95:5 dichloromethane/methanol to yield 50 mg of 11 (100% yield): ^{1}H NMR ($CDCl_3$) δ 1.5–1.9 (m, 7 H, ring CH_2's), 3.6 (dd, 2 H, J = 7.5 Hz, J = 2 Hz, CH_2OH), 5.3 and 5.4 (d, 1 H, J = 4 Hz, CHOCO), 7.45 (t, 4 H, J = 8 Hz, 3-ArH), 7.6 (t, 2 H, J = 7 Hz, 4-ArH), 8.1 (d, 4 H, J = 7.5 Hz, 2-ArH). Anal. ($C_{21}H_{22}O_5 \cdot 0.5H_2O$) C, H: calcd, 6.14; found, 7.05.

1,2,5,6-Tetrahydrobenzaldehyde Diethyl Acetal (12). Triethyl orthoformate (75.5 mL), 10.0 g (0.09 mol) of 1,2,3,4-tetrahydrobenzaldehyde, and 2.5 g of Amberlyst 15 were combined and stirred at 4 °C for 1 h. This mixture was then distilled at atmospheric pressure and the fraction distilling at 65 °C was collected to yield 15.6 g (0.085 mol, 93% yield) of 12 as a clear oil: ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 6 H, J = 7 Hz, CH_3), 1.5–2.0 (m, 7 H, ring CH_2 and CHC=C), 3.7 (q, 4 H, J = 6 Hz, CH_2O), 4.3 (m, 1 H, CHO_2), 5.7 (s, 2 H, CH=CH). Anal. ($C_{11}H_{20}O_2 \cdot 0.25H_2O$) C, H.

3,4-Dihydroxycyclohexanecarboxaldehyde Diethyl Acetal (13). In 60 mL of H_2O and 30 mL of THF were mixed 14.2 g of 12 (0.077 mol), 9.6 g (0.082 mol) of *N*-methylmorpholine *N*-oxide, and 20 mg of OsO_4. After 2 h, 0.7 g of Florisil and 70 mg of Na_2SO_4 were added, and the mixture was stirred for an additional hour. This was filtered through Celite and separated between ether and H_2O. The ether was dried ($MgSO_4$) and evaporated, and the residue was chromatographed with 95:5 ethyl acetate/CH_3OH to yield 12.2 g of 12 as a clear oil (0.056 mol, 73% yield): ^{1}H NMR ($CDCl_3$) δ 1.1 (t, 6 H, J = 7 Hz, CH_3), 1.4–1.9 (m, 6 H, ring CH_2), 2.1 (q, 1 H, J = 6 Hz, $CHCO_2$), 3.45 (m, 4 H, CH_2O), 3.7 (m, 1 H, CHO_2), 4.6 and 4.8 (m, 1 H, CHOCO). Anal. ($C_{11}H_{22}O_4$) C, H: calcd, 10.16; found, 11.04.

1,2-Dioctanoylcyclohexane-4-carboxaldehyde Diethyl Acetal (14). In 100 mL of dry pyridine were mixed 8.6 g (0.04 mol) of 13 and 9.4 g (0.12 mol) of octanoyl chloride. This was stirred at room temperature for 4 h, at which time the reaction was filtered, evaporated to a residue, and separated between water and ether. The water layer was extracted with ether, then the ether layers were combined, washed, dried ($MgSO_4$), and evaporated, and the residue was chromatographed with 3:1 hexane/ether. Pure fractions were pooled to yield 16.0 g of 14 as a clear oil (0.034 mmol, 85% yield): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 8 Hz, octyl-CH_3), 1.1 (overlapping t, 6 H, ethyl-CH_3), 1.3 (s, 20 H, octyl-CH_2), 1.4–2.0 (m, 6 H, ring-CH_2), 2.1 (q, 1 H, J = 6 Hz, $CHCO_2$), 2.2 and 2.3 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.45 (m, 4 H, CH_2O), 3.6 (m, 1 H, CHO_2), 4.8 and 5.2 (m, 1 H, CHOCO). Anal. ($C_{27}H_{50}O_6$) C, H.

3,4-Dioctanoylcyclohexanecarboxaldehyde (15a and 15b). An 11.0-g portion of 14 (0.023 mol), 80 mL of trifluoroacetic acid, 80 mL of H_2O, and 300 mL of $CHCl_3$ were combined and stirred at 0 °C for 1 h. The $CHCl_3$ layer was separated, washed with 5% sodium bicarbonate, dried ($MgSO_4$), and evaporated to a clear oil, yielding 9.3 g of pure 15 (100% yield). The diastereomers were separated by chromatography with 95:5 CH_2Cl_2/CH_3OH to yield 3.5 g of **15a**, which was the first compound eluted from the column, 3.0 g of the mixture, and 2.8 g of **15b**. The configuration of the carboxaldehyde relative to the *cis*-diesters was determined by homonuclear decoupling. In the ^{1}H NMR of **15b** the multiplet

at δ 1.7 was assigned to C_2H and the multiplet at δ 2.35 was assigned to C_1HCO. Irradiation at δ 1.7 yielded a doublet of doublets at δ 2.35 with J = 4.9 and 9.3 Hz. This was due to axial–equatorial and axial–axial coupling with C_6H. Therefore, the proton geminal to the carboxaldehyde is in the axial position. This results in an equatorial configuration for the carboxalaldehyde C-1. In **15a** decoupling was not possible, so this was assigned the axial configuration due to the assignment of **15b**. **15a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 8 Hz, CH_3), 1.25 (s, 20 H, CH_2), 1.4–2.0 (m, 6 H, ring CH_2), 2.2 and 2.3 (t, 2 H, J = 8.5 Hz, CH_2CO), 2.6 (m, 1 H, $CHCO$), 4.8 and 5.3 (m, 1 H, $CHOCO$), 9.6 (s, 1 H, COH). Anal. ($C_{23}H_{40}O_5 \cdot 1.25H_2O$) C, H. Compound **15b** was assigned the S configuration. The differences in the ^{1}H NMR for **15b** are δ 2.35 (m, 1 H, $CHCO$), 5.0 and 5.2 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{40}O_5 \cdot 1.75H_2O$) C, H: calcd, 10.75; found, 9.65. All subsequent **a** compounds are in the axial configuration at C-1 and **b** compounds are in the equatorial configuration at C-1.

3-[3,4-Bis(octanoyloxy)cyclohexyl]-2-propen-1-al (16a and 16b). In 10 mL of toluene were combined 0.15 g (0.4 mmol) of **15a** and 0.13 g (0.44 mmol) of (triphenylphosphoranylidene)-acetaldehyde. This was refluxed for 12 h and then poured into 10 mL of water. The organic layer was separated and the water was extracted with 20 mL of ether. The combined organic layers were dried ($MgSO_4$), evaporated to a residue, and chromatographed with 98:2 CH_2Cl_2/CH_3OH to yield 0.13 g (0.3 mmol, 75% yield) of **16a** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 6 Hz, CH_3), 1.3 (s, 20 H, CH_2), 1.5–2.1 (m, 7 H, ring CH_2 and CHC=C), 2.25 and 2.35 (t, J = 8 Hz, 2 H, CH_2CO), 4.85–5.35 (m, 2 H, $CHOCO$), 6.1 (m, 1 H, C=CH), 6.75 (dt, 1 H, J = 7 Hz, J = 14 Hz, CH=C), 9.5–9.7 (m, 1 H, COH). Anal. ($C_{25}H_{42}O_5 \cdot H_2O$) C, H. Compound **15b** was treated in the same manner to yield 0.11 g of **16b** as a clear oil (0.25 mmol, 64% yield). There were no differences in the ^{1}H NMR compared to that of **16a**. Anal. ($C_{25}H_{42}O_5 \cdot 0.5H_2O$) C, H.

3-[3,4-Bis(octanoyloxy)cyclohexyl]-2-propen-1-ol (17a and 17b). Compounds **16a** and **16b** were treated as for **5** to yield 75 mg of **17a** and **17b** (0.4 mmol, 100% yield). **17a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 6 Hz, CH_3), 1.3 (s, 20 H, CH_2), 1.5–2.0 (m, 7 H, ring CH_2), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.5 (dd, J = 6 Hz, J = 16 Hz, 2 H, CH_2OH), 4.8 and 5.3 (m, 1 H, $CHOCO$), 5.6 (m, 2 H, CH=CH). Anal. ($C_{25}H_{44}O_5$) C, H: calcd, 10.44; found, 11.86. ^{1}H NMR for **17b** is the same as that for **17a**. Anal. ($C_{25}H_{44}O_5$) C, H.

4-[3,4-Bis(octanoyloxy)cyclohexyl]-1-*O*-(dimethyl-*tert*-butylsilyl)-3-buten-1-ol (18a and 18b). These compounds were synthesized in the same manner as **8**, starting with 500 mg of **15a** (1.3 mmol) to yield 460 mg of **18a** (0.8 mmol, 63% yield): ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 6 H, $SiCH_3$), 0.8 (s, 15 H, $C(CH_3)_3$ and CH_3), 1.25 (m, 20 H, CH_2), 1.5–1.8 (m, 9 H, ring CH_2 and CHC=C, CH_2C=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.6 (t, 2 H, J = 7 Hz, CH_2OSi), 4.75 (m, 1 H, $CHOCO$), 5.15–5.3 (m, 3 H, CH=CH and $CHOCO$). Anal. ($C_{32}H_{60}O_5Si$) H, C: calcd, 69.51; found, 70.31. Starting with 750 mg of **15b** (1.9 mmol) yielded 650 mg of **18b** (1.2 mmol, 62% yield). Differences in ^{1}H NMR from that of **18a**: 4.8 and 5.2 (m, 1 H, $CHOCO$), 5.3 (m, 2 H, CH=CH). Anal. ($C_{32}H_{60}O_5Si \cdot H_2O$) C, H.

4-[3,4-Bis(octanoyloxy)cyclohexyl]-3-buten-1-ol (19a and 19b). These compounds were synthesized in the same manner as **10**, starting with 200 mg of **18a** (0.36 mmol) to yield 150 mg of **19a** (0.34 mmol, 95% yield): ^{1}H NMR ($CDCl_3$) δ 0.9 (m, 6 H, CH_3), 1.3 (m, 20 H, CH_2), 1.6–2.0 (m, 9 H, ring CH_2 and CHC=C, CH_2C=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.7 (dd, 2 H, J = 10.5 H, J = 5.5 Hz, CH_2OH), 4.85 and 5.3 (m, 1 H, $CHOCO$), 5.35 (m, 2 H, CH=CH). Anal. ($C_{26}H_{46}O_5 \cdot 0.5H_2O$) C, H. Starting with 200 mg of **18b** (0.36 mmol) yielded 130 mg of **19b** (0.29 mmol, 82% yield). Differences in ^{1}H NMR from that of **19a**: ($CDCl_3$) δ 5.35–5.5 (m, 2 H, CH=CH). Anal. ($C_{26}H_{46}O_5 \cdot 0.75H_2O$) C, H.

3,4-Bis(octanoyloxy)cyclohexanemethanol (20a and 20b). Compounds **15a** and **15b** were treated as for **6** to yield 100 mg of **20a** and **20b** (0.3 mmol, 100% yield). **20a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3 (m, 20 H, CH_2), 1.6–2.0 (m, 7 H, ring CH_2 and CHC=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.5 (d, 2 H, J = 6.5 Hz, CH_2OH), 4.8 and 5.35 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{41}O_5 \cdot 1.5H_2O$) C, H. Differences in ^{1}H NMR of **20b**: ($CDCl_3$) δ 4.85 and 5.3 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{41}O_5 \cdot 1.5H_2O$) C, H.

Molecular Modeling. Molecular modeling was performed with the program Alchemy II developed and distributed by Tripos, Inc., St. Louis, MO. The coordinates for phorbol were from the published crystal structure.[32] Coordinates for cyclohexane, benzene, and other portions of the compounds synthesized were from the internal data base. Conformational analysis and energy minimization were performed with internal programs. The lowest energy conformation of each isomer was determined independently.

Binding Assays. The binding assays were done as described before.[15] Essentially, murine brain fractions (50 μg of protein), [^{3}H]PDBu (5 nM), and the compounds at selected concentrations, or TPA at 5 μM, were placed into 200 μL of binding buffer. This was incubated for 1 h at 23 °C. This mixture was then filtered through GF/B glass-fiber filters with a Brandel filtration apparatus and washed with 5 mL of cold 10% polyethylene glycol in 1 mM Tris, pH 7.4. The filters were counted in a scintillation counter, and the percent bound was determined.

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Water-Soluble Renin Inhibitors: Design of a Subnanomolar Inhibitor with a Prolonged Duration of Action[1]

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Incorporation of nonreactive polar functionalities at the C- and N-termini of renin inhibitors led to the development of a subnanomolar compound (**21**) with millimolar solubility. This inhibitor demonstrated excellent efficacy and a long duration of action upon intravenous administration to monkeys. While activity was also observed intraduodenally, a comparison of the blood pressure responses indicated low bioavailability. Subsequent experiments in rats showed that, although the compound was absorbed from the gastrointestinal tract, extensive liver extraction severely limited bioavailability.

Renin is the first and rate-limiting enzyme in the well-known renin–angiotensin cascade that produces the pressor hormone angiotensin II, thus inhibition of this enzyme could lead to the introduction of a new class of

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