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Conformationally Constrained Analogues of Diacylglycerol. Interaction of γ-Lactones with the Phorbol Ester Receptor of Protein Kinase C

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Abstract: Four 2-deoxy-*erythro*-1,4-lactones in the D- and L-series and the four corresponding 2-deoxy-*threo*-1,4-lactones, bearing myristic acid acyl groups at either primary or secondary alcohol functions, were synthesized from L-ascorbic and D-isoascorbic acids. These eight pentonolactones which represent all possible isomers in this series were designed as rigid analogues of 1,2-diacylglycerol (DAG). The inhibition by these compounds of the binding of [^{3}H]phorbol-12,13-dibutyrate to protein kinase C (PK-C) demonstrated the importance in this context of stereochemistry and particularly of the orientation of the fatty acid side chain. The most effective inhibitor (**13d**, K_i = 2.3 μM) has a fixed conformation which is presumed to be similar to the conformation adopted by DAG when binding to PK-C. A three-point attachment model which has been previously used to rationalize the similar behavior of DAG and phorbol esters was extended to include additional points of equivalence between these two PK-C agonists. This extended model addresses the disposition of the lipophilic myristic acid side chain and the orientation of the lactone carbonyl group which functions as a hydrogen bond acceptor. In this new model, the most active isomer **13d** provides the best fit of the eight pentonolactones to phorbol myristate acetate.

Introduction

The response of cells to external stimuli is mediated by transducing mechanisms that involve the production of specific second messengers. One such family of compounds includes the diacyl glycerols (DAGs), which are released from membrane phospholipids as a result of a signaling event that activates the phospholipase C-mediated cleavage of a polyphosphoinositide.[1] This endogenously generated DAG binds the inactive cytoplasmic enzyme, protein kinase C (PK-C), causing its translocation to the membrane.[2,3] At the membrane and in the presence of other membrane phospholipids, such as phosphatidyl serine, PK-C becomes fully activated.[2,3] In addition to increasing the affinity of PK-C for the membrane, DAG also increases the affinity of the enzyme for calcium, reducing the requirement of this third cofactor down to physiological levels.[2,3] This calcium and phospholipid dependent enzyme (PK-C) catalyzes protein phosphorylation at serine and threonine residues, and it is believed to play a crucial role in many cellular processes including growth, cell differentiation, tumor promotion, and onocogenic expression.[2–6] Evidence for a constitutive activation of PK-C in transformed cells[7,8] as well as antitumor drug resistance resulting from induced elevation of PK-C activity[9] suggests that PK-C may be a potential target enzyme for anticancer therapy.[10,11]

Phorbol esters and other tumor promoters have also been shown to activate PK-C by acting as stable, highly potent DAG equivalents.[2,3] Contrary to DAG, whose presence in the membrane is transient, the phorbol esters are not metabolized and therefore are able to activate PK-C chronically, bypassing the phosphoinositide-derived DAG pathway.[2,3,12] PK-C has been identified as the DAG/phorbol ester receptor.[13] Furthermore, DAG inhibits the binding of phorbol diesters to PK-C in a competitive manner which suggests that both compounds interact at the same binding site on the enzyme-phospholipid complex.[14,15] In phorbol diesters[16,17] as well as in DAGs[18–20] the ability to bind and activate PK-C is highly stereospecific and involves the stereoisomers shown in Figure 1.

Several attempts have been made to derive a receptor model that would explain the stereospecific hydrophilic interactions of DAGs, phorbol esters, and other agonists with PK-C.[16,17,21–25] All proposed models suggest the existence of at least a three-point attachment between the active pharmacophores and the receptor protein. For DAG, the carbonyl ester oxygens attached to positions C-1 (*sn*-1) and C-2 (*sn*-2) are postulated to function as hydrogen bond acceptors, whereas the C-3 hydroxyl (*sn*-3) would donate

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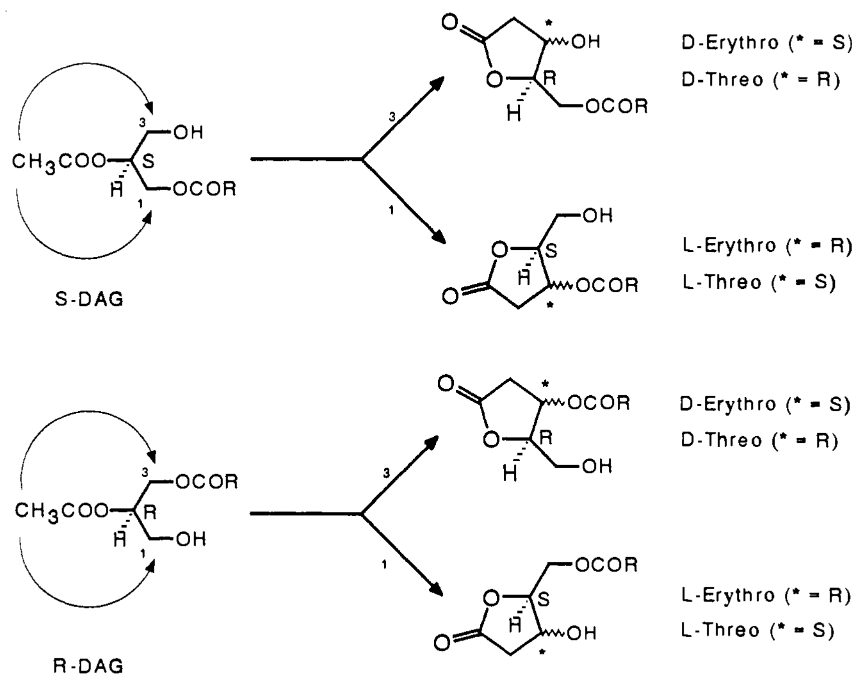
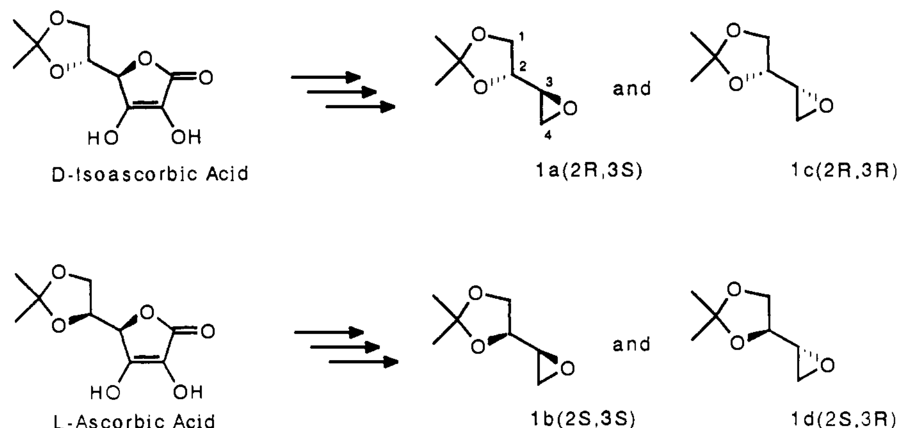
Scheme I



Scheme II



a hydrogen to a hydrogen bond acceptor in the receptor.[21,24] Phorbol esters appear to show a similar spatial bonding pattern, but the proposed models lack unanimity with regard to the identity of the functional groups in the phorbol molecule that are supposed to mimic DAG. For example, in one model, a conformer of 1,2-diacetyl-*sn*-glycerol that is energetically only 3.1 Kcal/mol higher than the global minimum could be superimposed nearly perfectly on the relevant area of 12,13-diacetylphorobol.[25] This analogy of DAG to the "northern" portion of phorbol esters was suggested previously[2,21] although it shows substantial inconsistencies with the known structure-activity relationships.[24] Other models that correlate the same atoms in DAG with a different set of oxygen atoms in the "southern" part of phorbol have been reported.[16,17,24] These models have also been extended to accommodate a number of structurally different PK-C activators.[16,17,22,24] Additional refinements on these models include the generation of a hypothetical receptor cavity in which the spatial disposition of physical and chemical domains (hydrophobic, electrostatic and hydrogen bonding) are defined.[23] Among these published models, the one that postulates the involvement of oxygens at C-3, C-9, and C-20 of phorbol seems among the most consistent with the structure-activity data for known PK-C activators.[24] This model will be used in this paper as a frame of reference in interpreting our results (vide infra).

There appears to be no stereospecificity, however, associated with the interaction between DAGs, or phorbol esters, with the hydrophobic domain of PK-C. Nevertheless, this interaction is essential for activity as it provides an anchoring site in the membrane bilayer, thus contributing both to the local concentration and proper orientation of the molecule at the active site.[16,17,20-25] Binding and PK-C activation are known to be affected by the length of the fatty acid side chain in DAGs and phorbol ester.[26-30]

Design of Conformationally Restricted DAGs. Despite the stereochemical selectivity observed for both DAGs and phorbols in their hydrophilic interactions with PK-C, the DAGs are substantially less potent and even the most potent DAGs bind to PK-C several orders of magnitude less tightly than the corresponding phorbol esters.[12,31] Several structure-activity studies with DAGs have shown that a range of modifications of the glycerol backbone

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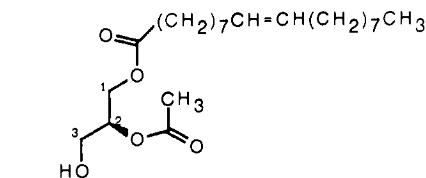

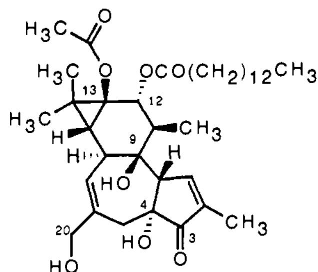

Figure 1. Structures of DAG and phorbol diester analogues.

result in a substantial decrease of activity. These changes included homologation of the backbone by one or two carbon atoms,[34] elimination or replacement of the C-3 hydroxyl group by other moieties,[30] and construction of rigid analogues of DAGs through the use of cyclic templates.[34,20] This cyclic template concept has also been extended, without success, to mimicking the cyclohexane C-ring structure of phorbol diesters comprising the hydroxyl groups at either C-9 or C-20 and the acyl groups at C-12 and C-13, respectively.[35,36] The construction of cyclic templates for DAG, inspired by the structural rigidity of the phorbol esters, is intended to reduce the number of possible rotameric forms of DAG in the hope that one of the rigid rotamers would approximate the actual conformation of the physiologically active DAG. Such a rigid rotamer of DAG was expected to bind more tightly to PK-C due to a favorable, smaller entropy decrease in relation to the more flexible DAG.[20] However, from the various published structure-activity studies for phorbol esters and DAGs,[16,17,21-25] it is possible to conclude that for a successful interaction with PK-C a rigid DAG analogue would necessitate more than just a good correlation with the centers of mass of key oxygen atoms in the phorbol ester pharmacophore. Good alignment of orbitals, necessary for strong hydrogen-bonding interactions, are very important, and these could be compromised in the conformationally constrained molecule, resulting in the cancellation of any entropy gain.

In order to minimize this problem, we propose the development of a rigid analogue constructed from DAG itself. As seen in Scheme I, hypothetical intramolecular cyclizations performed on DAG lead to a series of pentonolactone analogues that would contain the glycerol moiety embedded within them. Since PK-C has a great deal of tolerance with respect to the size of the *sn*-2 acyl group in DAG, even to the point that an acetyl group is acceptable [(*S*)-1-oleyl-2-acetyl-DAG is an important PK-C activator, Figure 1],[37,38] the structure of an (*S*)-2-acetyl-DAG was selected for this "cyclization" study. Joining the methyl group of the acetyl moiety to either the *sn*-1 or *sn*-3 carbons of the glycerol backbone generates the corresponding lactones. A similar operation for the *R*-isomer can also be performed (Scheme I). These operations resulted in the creation of an extra chiral center which could be useful in further discrimination of the affinities of these compounds for PK-C. Evidence exists that a second chiral center in DAG created by the introduction of a methyl group at C-3 resulted in the specific recognition of the 2-(*S*),3-(*R*)-isomer by PK-C.[39]

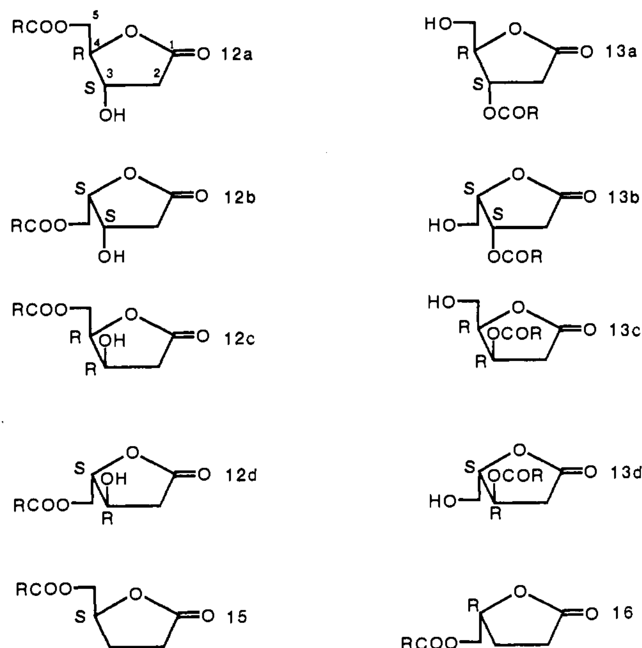
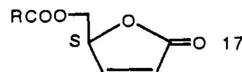

Figure 2. Target pentonolactones.

Given the structure of the pentonolactones generated by the hypothetical cyclization process, it is easy to compute the total number of isomeric DAG analogues. Because there are two chiral carbon atoms there will be four isomers: the 2-deoxy-*erythro*- and 2-deoxy-*threo*-1,4-lactones in both D and L series. The hydrophobic side chain, which we chose not to vary, was myristic acid in all of the analogues proposed. Since in each of the four generated isomers the myristoyl group could be incorporated at either the C-3 or the C-5 hydroxyl, the total number of possible isomers is doubled (**12a–d** and **13a–d**, Figure 2). The proposed hypothesis is that with these molecules one could probe the PK-C receptor and find out if any of these rigid forms binds to PK-C with an affinity that approximates that of DAG. In theory, assuming a gain in binding from entropy considerations diminished in part from any imperfections in the alignment of hydrogen bonds, it is proposed that the most potent analogue of this series should approximate the conformation with which DAG binds to PK-C.

Chemistry. Although one could conceivably synthesize all the required isomeric 2-deoxypentonolactones from the corresponding D- or L-ribonic and -xylonic acids, not all of the precursors are readily available. Consequently, a more general methodology, capable of generating all stereoisomers from plentiful precursors, was sought. Recently, the synthesis of all isomeric 2-deoxy-

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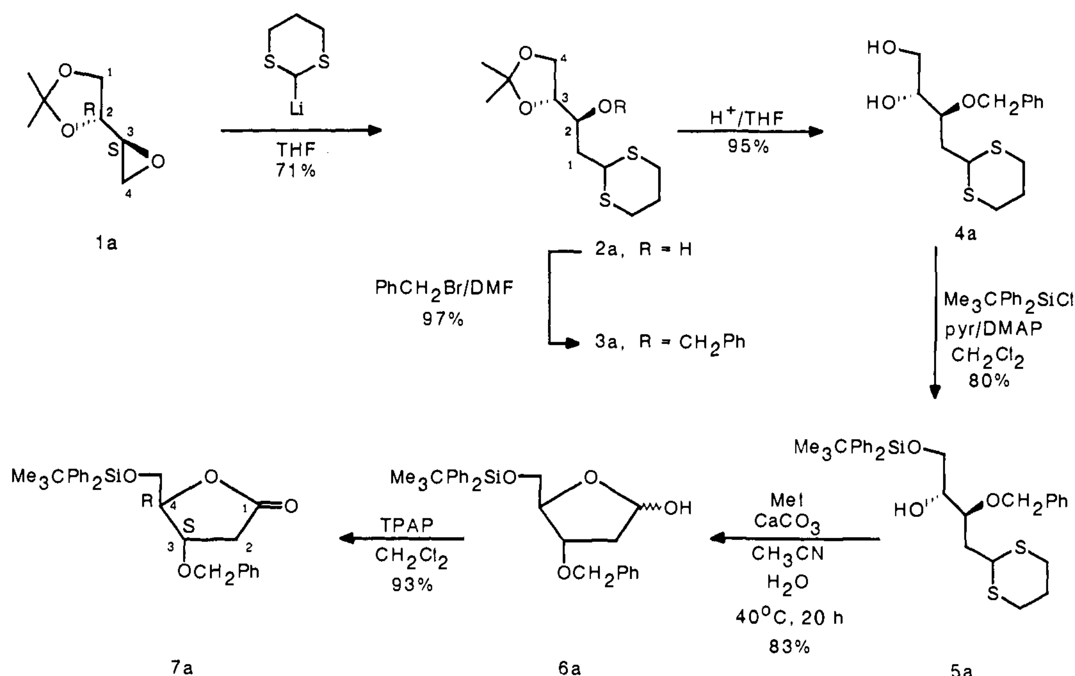
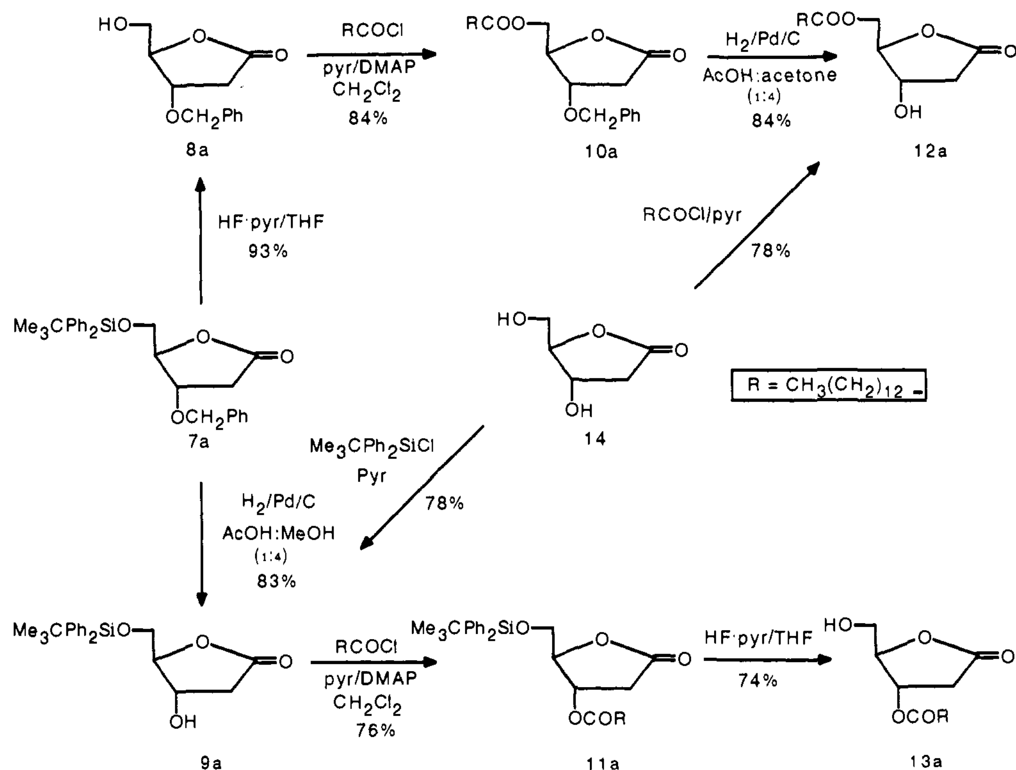
Scheme III



Scheme IV



pentofuranoses from L-ascorbic and D-isoascorbic acids was reported.[40] The success of this methodology hinged upon the efficient generation of the four stereoisomers of 3,4-epoxy-1,2-*O*-isopropylidenebutane-1,2-diol (**1a–1d**) (*RS*, *RR*, *SS*, and *SR*) which were individually converted to the desired isomeric sugars.[40] An identical set of epoxyacetonides has been reported by a different laboratory.[41] We were able to reproduce this chemistry and the desired epoxides (**1a–1d**) were obtained in good yield (Scheme II). From these epoxydiols, the required one-carbon homologation was achieved in each instance via the regiospecific ring opening of the epoxide ring with the lithium salt of 1,3-dithiane[42] [Scheme III illustrates the complete reaction sequence for the *RS* isomer (**1a**)]. The resulting secondary alcohol (**2a**) was benzylated, and the isopropylidene group was removed with dilute HCl in THF to afford **4a**. At this stage, selective protection of the primary alcohol as the *tert*-butyldiphenylsilyl ether was performed. Conversion of the dithiane ring to the aldehyde was achieved with methyl iodide and calcium carbonate in a mixture of acetonitrile and water, and the expected cyclization to the desired 2-deoxy-D-ribopentofuranoside (**6a**) ensued. Oxidation of this hemiacetal (mixture of C-1 anomers) to a single lactone product (**7a**) was

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then readily performed with tetrapropylammonium perruthenate (TPAP).[43] From this point, the selective removal of the individual hydroxyl-protecting groups facilitated the specific formation of myristic acid esters at positions 3 and 5 of the ribonolactone (Scheme IV). From **7a**, cleavage of the *tert*-butyldiphenylsilyl group with HF/pyridine freed the 5-hydroxyl group which was then acylated with myristoyl chloride to give intermediate **10a**. This compound was converted to the target compound (**12a**) by hydrogenolysis of the 3-*O*-benzyl group. Alternatively, when hydrogenolysis was performed first on **7a**, the 3-hydroxyl group was freed, and then acylation with myristoyl chloride afforded intermediate **11a**. This compound was converted to the alternate target (**13a**) in the D-ribo series after removal of the *tert*-butyldiphenylsilyl group with HF/pyridine. The sequence illustrated in Scheme IV worked very well for the preparation of the (*R*,*S*)-D-ribonolactone series (target compounds **12a** and **13a**) as well as for the (*S*,*R*)-L-ribonolactone series (target compounds **12d** and **13d**) in which the disposition of the substituents at C-3 and C-4 is trans. In the (*R*,*R*)-D-xylo (target compounds **12c** and **13c**) and (*S*,*S*)-L-xylo series (target compounds **12b** and **13b**), where the disposition of the groups is cis, care had to be exercised to avoid trans esterification of the myristoyl group in compounds **13b** and **13c**. Such trans esterification from the secondary to the primary alcohol occurred readily during silica gel chromatography, and compounds **13b** and **13c** rearranged to **12b** and **12c**, respectively. This trans esterification reaction was successfully suppressed by using pyridine in the eluant mixture during chromatography.

To corroborate the ability of our synthetic method to produce the desired absolute stereochemistry for the eight target compounds (**12a–12d** and **13a–13d**), the synthesis of the D-ribo analogues **12a** and **13a** was undertaken from chiral 2-deoxy-D-ribonolactone (**14**) which was easily prepared from commercial D-ribonolactone (Scheme IV). Following the method of Joullie and Chen[44] (corrected later by Baggett et al.[45] in terms of the proposed structure of the benzylidene acetal intermediate), 2-deoxy-D-ribonolactone (**14**) was synthesized.[46,47] Conversion of **14** to **12a** and **9a**, both identical to the materials prepared previously, corroborated the accuracy of the stereochemical control achieved with the process that began with the epoxyacetonide **1a**. This operation also supported the stereochemical assignments made for the rest of the target compounds.

Another set of simpler myristoylated compounds (**15–17**, Figure 2), readily prepared from commercially available precursors, was used to investigate lactones lacking a free hydroxyl group at the polar end of the molecule. These compounds were prepared by direct myristoylation of *S*-(−)-5-hydroxymethyl-2(5*H*)-furanone, 2,3-dideoxy-D-glyceropentono-1,4-lactone, and 2,3-dideoxy-L-glyceropentono-1,4-lactone, respectively. The last two compounds were available after the detritylation of commercially available γ-(trityloxymethyl)-γ-butyrolactone precursors.

Biological Results. The various target compounds designed as conformationally restricted analogues of diacylglycerols (DAG) were evaluated for their ability to inhibit [20-^{3}H]phorbol-12,13-dibutyrate (PDBU) binding to PK-C from mouse brain as described previously.[14,28] Through the use of this technique, which allows separate manipulation of the lipid and protein portions of the receptor, Blumberg et al.[14] have demonstrated the competitive nature of the process by which 1,2-*sn*-DAG derivatives inhibit phorbol ester binding. Since we expect our target ribonolactones to behave as DAG surrogates, the results from this technique should be helpful in confirming this presumption. In this particular assay the test compounds were directly incorporated into the phospholipid used to reconstitute the receptor.[28] The inhibition curves obtained for the active compounds were of the type expected for competitive inhibition, and the ID_{50} values were determined by a least-squares fit of the data points to a theoretical competition curve. The K_i for inhibition of binding was calculated from the ID_{50} value (see Experimental Section).

Table I. Apparent K_i Values for Various Pentonolactones Assayed as Inhibitors of [^{3}H]PDBU Binding to Protein Kinase C

compd	K_i (μM)	compd	K_i (μM)
12a	21.1 ± 7.3 (*n* = 3)	**12d**	58
13a	5.3 ± 1.5 (*n* = 3)	**13d**	2.5 ± 0.3 (*n* = 3)
12b	>300	**15**	110
13b	31.00	**16**	150
12c	>300	**17**	>300
13c	>300	GMA[a]	0.5 ± 0.0 (*n* = 2)

[a] GMA is *rac-glycerol*-1-myristate-2-acetate. A K_i value of 1.3 μM has also been reported.[31]

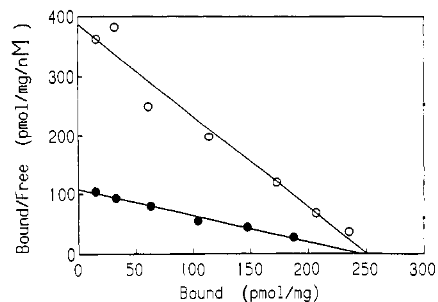

Figure 3. Scatchard analysis of [3H]PDBU binding to PK-C in the absence (○) or presence (●) of **13a**. A second experiment gave similar results.

The K_i values obtained are listed in Table I. To further confirm that the mechanism of the inhibition was competitive, we examined the binding of [^{3}H]PDBU in the presence or absence of 14.5 μM **13a**. As expected, **13a** reduced the binding affinity of [^{3}H]PDBU with no effect on the number of binding sites (Figure 3). Although the K_i value given represents the nominal concentration of the active inhibitor in the assay mixture, it does not reflect the critical concentration of the inhibitor at the level of the phospholipid layer where the actual competition for the [^{3}H]PDBU binding site occurs. To account for this variability, particularly when dealing with a series of widely different compounds, the K_i could be expressed as a percent relative to phosphatidylserine concentration.[14] However, in this situation, where myristic acid was selected as a common fatty acid for the series, the hydrophobic/hydrophilic balance was expected to remain constant. Therefore, the K_i values expressed in μM concentrations in the assay mixture should give an accurate estimate of the relative potencies among the compounds assayed. This is important because it has been demonstrated that differences in the lipid environment can produce different binding affinities within a similar set of compounds.[28]

The results in Table I suggest that there is a clear relationship between the stereochemical disposition of the polar groups in the pentonolactones and their ability to compete for the phorbol ester receptor. The differences in potencies observed for this uniformly amphiphilic series—ranging from inactive to very potent compounds—argue against nonspecific binding as the responsible factor. However, PK-C does not have the ability to appreciably discriminate between enantiomers in this pentonolactone series. The small difference in potency (ca. 2-fold) that exists between the two most active optical antipodes (compounds **13a** and **13d**) is unexpected given the competitive nature of the interaction and the previous stereospecificity observed for DAG. In DAG biological activity resides primarily with the *S*-isomer as revealed by the [^{3}H]PDBU binding assay as well as the DAG-mediated PK-C activation assay.[18,19,34,39] In terms of PK-C activation we found that in agreement with the binding assay data, the two optical antipodes **13a** and **13d** showed a nearly identical activation profile for this enzyme (Figure 4) and inhibited [^{125}I]EGF binding to the same extent when added to culture medium of confluent NIH 3T3 cells (data not shown). The enantiomers **12a** and **12d** behaved as significantly weaker PK-C activators which were better discriminated by the enzyme (Figure 4).

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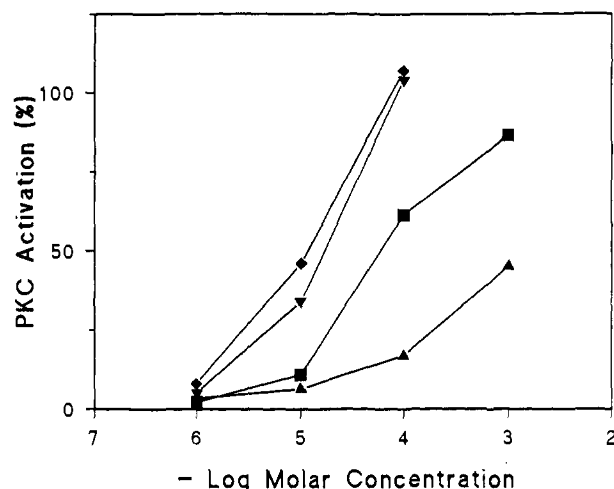

Figure 4. Activation of protein kinase C by pentonolactones **12a** (■), **12d** (▲), **13a** (▼), and **13d** (♦). The figure represents the average of 2–3 experiments.

Table II. RMS of Different Superimpositions of Pentonolactones on Phorbols

	fit A	fit B		
compd	3-Pt	3-Pt	5-Pt	6-Pt
12a	0.447	0.798	∞	∞
12b	1.462	1.178	2.237	2.137
12c	1.462	1.178	2.276	2.193
12d	0.447	0.798	2.578	2.483
13a	1.237	1.176	1.968	1.921
13b	0.950	1.088	2.659	2.581
13c	0.950	1.088	2.474	2.351
13d	1.237	1.176	1.175	1.130

The location of the myristoyl group appears to be very critical (compare the K_i values of **12d** vs **13d**) and agrees with the structure-activity requirements delineated for various nonrigid DAG analogues[26,30] which indicate that the presence of a primary alcohol function is critical for good activity. The synthesis of compounds **15–17** (Figure 2) was prompted by a recent publication which described the ability of diol lipids (i.e., dioleoylethylene glycol) to activate PK-C at relatively low concentrations.[48] Compounds **15–17** represent conformationally restricted forms of this diol lipid. However, these compounds showed very poor affinities, underscoring the importance of a free hydroxyl function for interaction with PK-C (compare compound **12a** with **15** and **12d** with **16**, respectively).

The last entry in Table I corresponds to the DAG analogue (glycerol-1-myristate acetate, GMA) that is equivalent to the target pentonolactones in terms of the nature of the substituents. The K_i value for GMA was five times lower than that of the most potent pentonolactone analogue (**13d**) which would indicate that, of all the pentonolactones examined, compound **13d** might provide the best approximation of what the conformation of GMA would be in binding to PK-C. This observation was further supported by the modeling experiments discussed below.

Molecular Modeling and Discussion. As discussed earlier, the three-point attachment model which implicates the oxygen atoms at C-3, C-9, and C-20 of phorbol (Figure 1) appeared to be most consistent with the available information and, accordingly, this was used in the fitting studies. The pentonolactones, however, can be superimposed upon these three oxygens of phorbol in two different ways as exemplified in Figure 5 for structures **13** (no stereochemistry implied). In all cases, the free hydroxyl oxygen

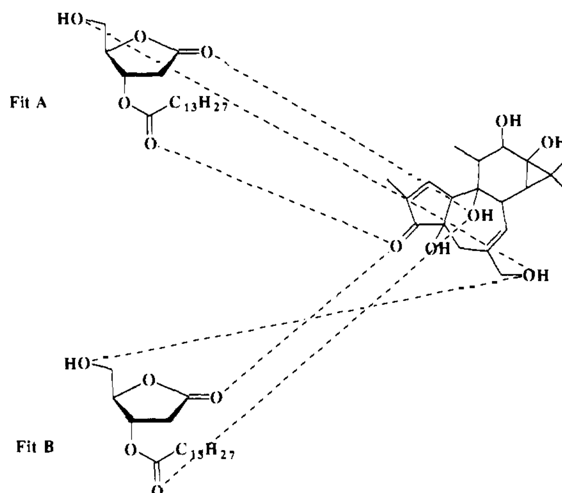

Figure 5. Three-point fits of pentonolactones to phorbol.

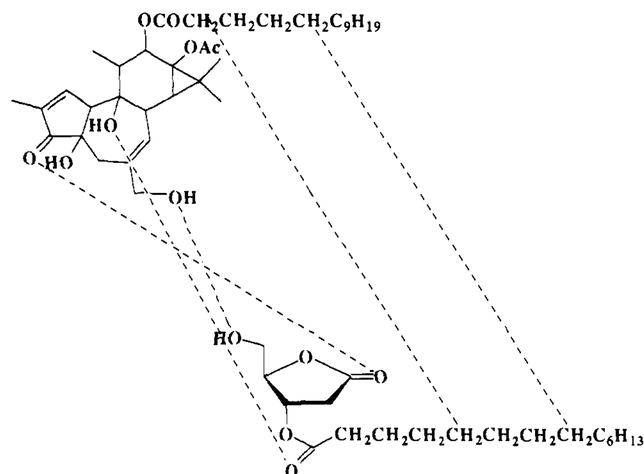

Figure 6. Five-point fits of pentonolactones to PMA.

of the pentonolactone is matched to the hydroxyl oxygen at C-20 of phorbol. In fit A, the ring carbonyl oxygen of the lactone is then fit to phorbol's C-9 hydroxyl oxygen, and the myristoyl carbonyl oxygen is superimposed upon the C-3 carbonyl oxygen of the phorbol. In the other fit (fit B), the ring carbonyl oxygen of the lactone is identified with the C-3 carbonyl oxygen of phorbol. The quality of either fit to phorbol, as determined by the square root of the sum of the squares of the $O_{phorbol} - O_{lactone}$ distances (the RMS value), did not reveal any discriminating trends compatible with the biological data (Table II). Moreover, inspection of the superimposed pairs of molecules revealed that the long hydrocarbon side chain of the pentonolactone was in some cases oriented in a direction opposite to the C-12 and C-13 oxygens of phorbol which normally bear the lipophilic substituents in phorbol esters such as PMA. It was concluded from this that the three-point fit was not adequate and that the orientation of tetradecanoyl side chains in the pentonolactones relative to the corresponding acyl side chain in PMA had to be taken into account. After careful inspection of the models, two methylene carbons on each of the side chains of the lactones and of phorbol myristate acetate (PMA) were included in a five-point fit to assess the directionality of the side chain. The selected carbon atoms for this exercise are shown in Figure 6 for an orientation according to fit B, since the results from fit A resulted in signifiant deterioration of the RMS value (data not shown). In this five point fit, the RMS value for **13d** was the lowest (1.175), followed by that of its optical antipode. Finally, an attempt was made to deal with the directionality of the groups presumed to be involved in hydrogen bonding at the active site. Since the two hydroxyl groups at C-9 and C-20 of PMA can rotate freely, only the position and not the orientation of the oxygen atom would appear to be im-

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(48) Severin, S. E., Jr.; Tovmasyan, E. K.; Shvets, V. I.; Molotokovsky, J. G.; Bergelson, L. D. *FEBS Lett.* **1988**, *232*, 286.

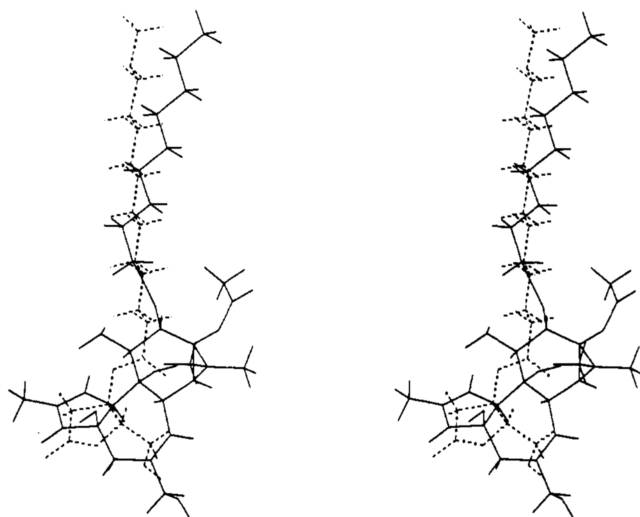

Figure 7. Superposition of pentonolactone **13d** on PMA.

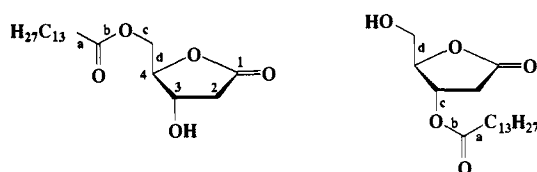

Figure 8. Rotatable bonds in pentonolactones **12** (left) and **13** (right).

portant. However, in the case of the carbonyl group at C-3, the orientation of the oxygen, which is presumed to be a hydrogen bond acceptor, is critical. In order to measure the degree of conformance of the orientation of this carbonyl with respect to the C-1 carbonyl of the pentonolactones, the carbons of the carbonyl groups were added to produce a six-point fit. This resulted in an even better RMS value (1.130) for **13d** which remained the best of the eight isomers (Table II). A stereoscopic view of the superimposition of **13d** on PMA is shown in Figure 7. The only anomaly observed in this correlation was compound **12a**, which according to the modeling data, should have had the poorest affinity towards PK-C. This indicates that perhaps there are other alternative modes of binding to PK-C that, although less efficient, could be operating in the case of the C-5 esters (compounds **12a–12d**) which lack a primary alcohol function.

The possibility that compound **13d** might approximate the conformation with which DAG binds to PK-C is interesting in view of a recent report that indicates that the preferred conformation for DAG in hydrated bilayers should be one in which the glycerol backbone is approximately parallel to the acyl chains.[49] This conformation, which is different from DAG in the crystalline state, is precisely the conformation that cyclization into a pentonolactone forces the molecule to have, specially in the case of the C-3 myristic acid ester lactones (**13a–13d**).

In summary, two of these conformationally restricted DAG surrogates (enantiomers **13d** and **13a**) appear to mimic DAG in its physiological reactions and may indeed represent good approximations of the active conformation of DAG. The competitive nature of their inhibition to phorbol binding indicates that they interact with the PK-C receptor. However, their diminished potency with respect to the equivalent DAG analogue (GMA) and the fact that both enantiomers possess nearly similar activities could indicate that perhaps there is an alternative mode of binding to PK-C not considered in the present model. The goal of elucidating the precise conformation of DAG when inserted into membranes should contribute to the design of drugs which may be of value in preventing the effects of tumor promoters as well as controlling other PK-C mediated effects.

Experimental Section

General Experimental. All chemical reagents were commercially available. Melting points were determined on a Mel-Temp II apparatus, Laboratory Devices, USA, and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvents indicated for the individual experiments. Proton and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise indicated at 200 and 50 MHz, respectively, in a Varian XL-200 instrument. Chemical shifts are expressed as δ values with reference to Me_4Si. In the carbon spectra the signs + and − refer to the peaks above or below the base line in the fully decoupled attached proton test (APT). Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR and specific rotations were measured in a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

Analysis of Inhibition of [^{3}H]PDBU Binding by Nonradioactive Ligands. Protein kinase C was partially purified through the DEAE–cellulose chromatography step as described by Jeng et al.[50] [20-^{3}H]PDBU (specific activity = 19.1 (Ci/mmol)) was from New England Nuclear, Boston, MA. Phosphatidylserine and bovine immunoglobulin G were from Sigma, St. Louis, MO.

The affinity of phorbol ester analogues for protein kinase C was determined by competition for binding of [20-^{3}H]PDBU. The assay procedure was measured as follows: In a total volume of 250 μL was incubated [^{3}H]PDBU (1–3 nM), protein kinase C, 0.1 mM $CaCl_2$, 50 mM Tris-Cl (pH 7.4), 100 μg/mL phosphatidylserine, variable concentrations of the competing ligand, and 4 mg/mL bovine immunoglobulin G for 5 min at 37 °C. The samples were then chilled for 5 min at 0 °C, 200 μL of 30% polyethylene glycol in 50 mM Tris-Cl, pH 7.4, was added, and the samples were further incubated at 0 °C for 15 min to induce precipitation of the protein kinase C. The samples were then centrifuged for 15 min at 12000 rpm in a Beckman microfuge 12 at 4 °C. A 100-μL aliquot of the supernatant was removed and subjected to liquid scintillation counting to determine the free concentration of the [^{3}H]PDBU. The remainder of the supernatant was removed by aspiration and by blotting the pellet with a Kimwipe. The tip of the centrifuge tube was then cut off, and the radioactivity in the pellet was measured to determine total bound [^{3}H]PDBU. Nonspecific binding was determined by inclusion of 30 μM nonradioactive PDBU in the incubation mixture.

In each competition experiment, typically 6–7 concentrations of the competing ligand were used. Because of potential low solubility of the competing ligand, the compounds were dissolved in methanol and added to the phosphatidylserine, which was dissolved in chloroform. After mixing, the solvent was removed under a stream of nitrogen, and the phosphatidylserine was dispersed in 0.05 M Tris-HCl, pH 7.4, by sonication. It was then added to the assay mixture as indicated above. At each ligand concentration, three determinations of binding in the absence of nonradioactive PDBU and one determination of binding in the presence of nonradioactive PDBU were made. The partition coefficient of [^{3}H]PDBU between supernatant and pellet was calculated from the measured values in the presence of the nonradioactive PDBU. Specific binding represents the difference between total and nonspecific binding, where nonspecific binding for each tube was calculated from the measured free [^{3}H]PDBU in that tube and the partition coefficient of [^{3}H]PDBU under these conditions. ID_{50} values were determined by fitting a theoretical sigmoidal competition curve to the binding data. The K_i was calculated from the ID_{50} according to the relationship

$$K_i = ID_{50}/(1 + L/K_d)$$

where L is the concentration of free [^{3}H]PDBU at the ID_{50} and K_d is the dissociation constant for [^{3}H]PDBU under these assay conditions.

Measurement of Protein Kinase C Activity. Protein kinase C activation was assayed by measuring ^{32}P transferred from [γ-^{32}P]ATP to histone H_1 in the presence of calcium and phospholipids. The reaction mixture contained, in a total volume of 50 μL, 50 mM Tris-Cl (pH = 7.4), 0.10 mM calcium chloride, 7.5 mM magnesium acetate, 250 μg/mL bovine serum albumin, 750 μg/mL histone IIls, 100 μg/mL phospholipid (phosphatidylcholine/phosphatidylserine, 4:1 w/w), 25 μM [γ-^{32}P]ATP, protein kinase C, and the corresponding compound. The incubation was carried out at 30 °C for 10 min and stopped by chilling the tubes on ice for 5 min. 32-Phosphorus incorporation was determined using phosphocellulose paper.[51] Activation produced by PDBU (0.3 μM) was considered as 100%.

Molecular Modeling. The eight pentonolactones (**12a–12d** and **13a–13d**) were computer-modeled, and the initial minimum-energy structures were identified using an adopted basis Newton–Raphson (ABNR) algorithm for 500 iterations or to convergence. The typical potential energy

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at this stage was on the order of −5 kcal/mol. Subsequently, optimum values of the torsion angles corresponding to the four rotatable exocyclic single bonds identified as a, b, c, and d in Figure 8 were determined in each case by means of a conformational search through 360° in 36 steps of 10° each. For every conformation, 50 steps of ABNR minimization were applied to find a minimum energy. This energy was then plotted against the angle, and the angle corresponding to the minimum energy was determined from the curve. This angle was then set in the model. The conformational analysis of the four bonds a, b, c, and d can be done cooperatively, in which case two bonds (b and c, for example) are rotated through 360° for every rotational position of the third bond d. Alternatively, each bond can be analyzed independently of the others. The cooperative approach takes much longer, because it is an n^4 calculation, requiring in this case, 36^4 = 1 679 616 conformations, and when it was tested, it was shown to give the same results as noncooperative analysis, which requires only 3 × 36 or 108 conformations to be examined. Accordingly, the four bonds were rotated separately, and the overall minimum energy structure of the lactone was determined. This process was completed for each of the C-3 esters (**13a–13d**), and the analogous procedure was then carried out for the C-5 esters (**12a–12d**), producing a total of eight minimum-energy structures whose fit to phorbol was then studied. The remaining C–C bonds in the fatty acid side chain can also rotate, but as the side chain was directed away from the lactone ring and all the C–C bonds were fully skewed, no lower energy conformations were found.

The structure of phorbol that was used was based upon the atomic coordinates that were determined by X-ray diffraction.[52] These coordinates were imported into the molecular modeling environment and used for the comparisons of phorbol with the pentonolactones. This model of phorbol was also used as the basis for the model of phorbol 12-*O*-tetradecanoyl-13-*O*-acetate (phorbol myristate acetate, PMA) which was developed by the addition of the ester side chains in the modeling program.

3,4-Epoxy-1,2-*O*-isopropylidenebutane-1,2-diols (1a–1d). These compounds were prepared according to the procedure of Abushanab et al.[40,53]

(2*R*,3*S*)-Isomer (1a): obtained from D-isoascorbic acid in five steps (53% yield); $[\alpha]^{22}_D$ +8.16° (*c* 1.47, $CHCl_3$); $[\alpha]^{22}_D$ + 11.47° (*c* 3.75, EtOH) [lit.[53] $[\alpha]_D$ +8.67° (*c* 2.215, EtOH)].

(2*S*,3*S*)-Isomer (1b): obtained from L-ascorbic acid in five steps (56% yield); $[\alpha]^{22}_D$ = −6.54° (*c* 1.88, $CHCl_3$); $[\alpha]^{22}_D$ −0.21° (*c* 1.92, EtOH) [lit.[53] $[\alpha]_D$ −3.23° (*c* 4.025, EtOH)].

(2*R*,3*R*)-Isomer (1c): obtained from D-isoascorbic acid in six steps (54% yield); $[\alpha]^{24}_D$ +4.24° (*c* 2.10, $CHCl_3$); $[\alpha]^{23}_D$ + 0.36° (*c* 2.66, EtOH) [lit.[40] $[\alpha]^{25}_D$ −0.69° (*c* 4.15, EtOH)].

(2*S*,3*R*)-Isomer (1d): obtained from L-ascorbic acid in six steps (50% yield); $[\alpha]^{20}_D$ −9.80° (*c* 1.46, $CHCl_3$); $[\alpha]^{23}_D$ −6.92° (*c* 3.75, EtOH) [lit.[40] $[\alpha]^{25}_D$ −10.96° (*c* 2.55, EtOH)].

2-[3,4-*O*-Isopropylidene-2,3,4-trihydroxybut-1-yl]-1,3-dithianes (2a–2d). These compounds were prepared according to the procedure of Abushanab et al.[40]

(2*S*,3*R*)-Isomer (2a): obtained in 71% yield from **1a**; $[\alpha]^{24}_D$ −9.22° (*c* 1.60, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.18; mp 72.1–72.7 °C; IR (KBr) 3450 cm^{-1}; ^1H NMR δ 1.34 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3), 1.79–2.15 (m, 4 H, H-$1_{a,b}$, $SCH_2CH_2CH_2S$), 2.28 (d, 1 H, *J* = 3.2 Hz, D_2O exchangeable, *OH*), 2.82–2.94 (m, 4 H, 2 × CH_2S), 3.89–4.06 (m, 4 H, H-2, H-3, H-$4_{a,b}$), 4.25 (dd, 1 H, *J* = 9.5, 5.0 Hz, SC*H*S); ^{13}C NMR δ 25.17 (q, −, CH_3), 25.82 (t, +, $SCH_2CH_2CH_2S$), 26.52 (q, −, CH_3), 29.79 and 30.13 (t, +, 2 × CH_2S), 38.35 (t, +, C-1), 43.81 (d, −, SCHS), 65.26 (t, +, C-4), 68.55 (d, −, C-2), 78.32 (d, −, C-3), 109.26 (s, +, OCO). Anal. Calcd for $C_{11}H_{20}O_3S_2$: C, 50.00; H, 7.58; S, 24.24. Found: C, 49.78; H, 7.48; S, 24.42.

(2*S*,3*S*)-Isomer (2b): obtained in 71% yield from **1b**; $[\alpha]^{22}_D$ −22.69° (*c* 1.67, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.18; mp 90.0–90.8 °C; IR (KBr) 3446 cm^{-1}; ^1H NMR δ 1.35 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 1.66–2.17 (m, 4 H, H-$1_{a,b}$, $SCH_2CH_2CH_2S$), 2.28 (d, 1 H, *J* = 5.9 Hz, D_2O exchangeable, *OH*), 2.76–2.99 (m, 4 H, 2 × CH_2S), 3.72–4.06 (m, 4 H, H-2, H-3, H-$4_{a,b}$), 4.31 (dd, 1 H, *J* = 10.2, 4.3 Hz, SC*H*S); ^{13}C NMR δ 25.16 (q, −, CH_3), 25.88 (t, +, $SCH_2CH_2CH_2S$), 26.51 (q, −, CH_3), 29.91 and 30.40 (t, +, 2 × CH_2S), 39.54 (t, +, C-1), 43.50 (d, −, SCHS), 65.94 (t, +, C-4), 68.48 (d, −, C-2), 78.56 (d, −, C-3), 109.58 (s, +, OCO). Anal. Found: C, 49.95; H, 7.64; S, 24.31.

(2*R*,3*R*)-Isomer (2c): obtained in 78% yield from **1c**; $[\alpha]^{22}_D$ + 23.08° (*c* 1.07, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.20; mp 90.6–92.0 °C. The ^1H NMR and ^{13}C NMR spectra were identical to the optical antipode **2b**. Anal. Found: C, 49.73; H, 7.68; S, 24.53.

(2*R*,3*S*)-Isomer (2d): obtained in 74% yield from **1d**; $[\alpha]^{22}_D$ +9.34° (*c* 3.49, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.22; mp 71.2–72.4 °C. The ^1H NMR and ^{13}C NMR spectra were identical to the optical antipode **2a**. Anal. Found: C, 50.25; H, 7.92; S, 24.47.

2-[2-*O*-Benzyl-3,4-*O*-isopropylidene-2,3,4-trihydroxybut-1-yl]-1,3-dithianes (3a–3d). These compounds were prepared according to the procedure of Abushanab et al.[40]

(2*S*,3*R*)-Isomer (3a): obtained in 96% yield from **2a**; $[\alpha]^{24}_D$ −18.07° (*c* 1.76, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.21; ^1H NMR (C_6D_6) δ 1.29 (s, 3 H, CH_3), 1.37–1.70 (m, 2 H, $SCH_2CH_2CH_2S$), 1.42 (s, 3 H, CH_3), 2.02–2.08 (m, 2 H, H-$1_{a,b}$), 2.23–2.43 (m, 4 H, 2 × CH_2S), 3.75 (m, 1 H, H-2), 3.89 (m, 1 H, H-4_a), 3.96 (m, 1 H, H-4_b), 4.01 (m, 1 H, H-3), 4.27 (dd, 1 H, *J* = 8.1, 6.4 Hz, SC*H*S), 4.70 (AB q, 2 H, *J* = 11.5 Hz, $PhCH_2O$), 7.10–7.23 (m, 3 H, Ph), 7.34–7.37 (m, 2 H, Ph); ^{13}C NMR δ 25.25 (q, −, CH_3), 25.93 (t, +, $SCH_2CH_2CH_2S$), 26.46 (q, −, CH_3), 29.79 and 30.25 (t, +, 2 × CH_2S), 37.66 (t, +, C-1), 43.63 (d, −, SCHS), 65.85 (t, +, C-4), 73.64 (t, +, $PhCH_2O$), 75.79 (d, −, C-2), 78.24 (d, −, C-3), 109.24 (s, +, OCO), 127.70, 127.97, and 128.37 (d, −, Ph), 138.49 (s, +, Ph). Anal. Calcd for $C_{18}H_{26}O_3S_2$: C, 61.02; H, 7.34; S, 18.08. Found: C, 61.18; H, 7.29; S, 18.06.

(2*S*,3*S*)-Isomer (3b): obtained in 97% yield from **2b**; $[\alpha]^{24}_D$ −35.00° (*c* 1.76, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.22; ^1H NMR δ 1.35 (s, 3 H, CH_3), 1.45 (s, 3 H, CH_3), 1.70–2.16 (m, 4 H, $SCH_2CH_2CH_2S$, H-$1_{a,b}$), 2.70–2.85 (m, 4 H, 2 × CH_2S), 3.72 (dd, 1 H, *J* = 8.4, 7.0 Hz, H-4_a), 3.81 (m, 1 H, H-2), 3.98 (dd, 1 H, *J* = 8.4, 6.6 Hz, H-4_b), 4.11 (dd, 1 H, *J* = 9.9, 4.6, Hz, SC*H*S), 4.22 (q, 1 H, *J* = 6.8 Hz, H-3), 4.77 (AB q, 2 H, *J* = 11.5 Hz, $PhCH_2O$), 7.25–7.35 (m, 5 H, Ph); ^{13}C NMR δ 25.21 (q, −, CH_3), 25.94 (t, +, $SCH_2CH_2CH_2S$), 26.47 (q, −, CH_3), 29.83 and 30.34 (t, +, 2 × CH_2S), 36.59 (t, +, C-1), 43.67 (d, −, SCHS), 65.69 (t, +, C-4), 73.47 (t, +, $PhCH_2O$), 76.40 (d, −, C-2), 77.03 (d, −, C-3), 109.50 (s, +, OCO), 127.64, 128.08 and 128.34 (d, −, Ph), 138.65 (s, +, Ph). Anal. Found: C, 61.34; H, 7.41; S, 18.19.

(2*R*,3*R*)-Isomer (3c): obtained in 93% yield from **2c**; $[\alpha]^{21}_D$ +32.63° (*c* 1.56, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.18. The ^1H NMR and ^{13}C NMR spectra were identical to those of the optical antipode **3b**. Anal. Found: C, 61.02; H, 7.34; S, 18.08.

(2*R*,3*S*)-Isomer (3d): obtained in 95% yield from **2d**; $[\alpha]^{20}_D$ +13.80° (*c* 2.14, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.22. The ^1H NMR and ^{13}C NMR spectra were identical to those of the optical antipode **3a**. Anal. Found: C, 60.86; H, 7.28; S, 17.85.

2-(2-*O*-Benzyl-2,3,4-trihydroxybut-1-yl)-1,3-dithianes (4a–4d). A stirred solution of **3a–3d** (10 mmol) in 75 mL of THF was treated with 25 mL of 10% aqueous HCl at room temperature for 4 h. The reaction mixture was then diluted with 150 mL of ether, and the organic layer was subsequently washed with 5% aqueous $NaHCO_3$ (10 mL) and water (10 mL). The ethereal solution was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash column chromatography with a mixture of $CHCl_3$/EtOH (10:1) as eluant to give **4a–4d** as colorless syrups.

(2*S*,3*R*)-Isomer (4a): obtained in 99% yield from **3a**; $[\alpha]^{22}_D$ −10.74° (*c* 1.89, $CHCl_3$); R_f ($CHCl_3$/EtOH, 10:1) 0.52; IR (neat) 3395 cm^{-1}; ^1H NMR (C_6D_6 + 1 drop of D_2O) δ 1.36–1.69 (m, 2 H, $SCH_2CH_2CH_2S$), 2.04–2.16 (m, 2 H, H-$1_{a,b}$), 2.30–2.50 (m, 4 H, 2 × CH_2S), 3.42–3.60 (m, 3 H, H-2, H-$4_{a,b}$), 3.89 (distorted ddd, 1 H, H-3), 4.23 (dd, 1 H, *J* = 9.1, 5.4 Hz, SC*H*S), 4.55 (AB q, 2 H, *J* = 11.7 Hz, $PhCH_2O$), 7.07–7.38 (m, 5 H, Ph); ^{13}C NMR δ 25.83 (t, +, $SCH_2CH_2CH_2S$), 29.96 and 30.34 (t, +, 2 × CH_2S), 36.66 (t, +, C-1), 43.56 (d, −, SCHS), 63.20 (t, +, C-4), 72.79 (d, −, C-3), 73.07 (t, +, $PhCH_2O$), 77.43 (d, −, C-2), 127.74, 128.07 and 128.52 (d, −, Ph), 138.04 (s, +, Ph). Anal. Calcd for $C_{15}H_{22}O_3S_2$: C, 57.32; H, 7.01. Found: C, 57.09; H, 7.29.

(2*S*,3*S*)-Isomer (4b): obtained from **3b** in 95% yield; $[\alpha]^{25}_D$ +13.30° (*c* 0.97, $CHCl_3$); R_f ($CHCl_3$/EtOH, 10:1) 0.48; IR (neat) 3417 cm^{-1}, ^1H NMR ($CDCl_3$ + 1 drop of D_2O) δ 1.80–2.00 (m, 2 H, $SCH_2CH_2CH_2S$), 2.01–2.12 (m, 2 H, H-$1_{a,b}$), 2.70–2.90 (m, 4 H, 2 × CH_2S), 3.65–3.80 (m, 3 H, H-3, H-$4_{a,b}$), 3.87 (m, 1 H, H-2), 4.10 (t, 1 H, *J* = 7.2 Hz, SC*H*S), 4.65 (AB q, 2 H, *J* = 11.3 Hz, $PhCH_2O$), 7.25–7.45 (m, 5 H, Ph); ^{13}C NMR δ 25.83 (t, +, $SCH_2CH_2CH_2S$), 30.07 and 30.29 (t, +, 2 × CH_2S), 36.52 (t, +, C-1), 43.71 (d, −, SCHS), 63.84 (t, +, C-4), 73.06 (t, +, $PhCH_2O$), 73.09 (d, −, C-3), 76.48 (d, −, C-2), 127.99, 128.16 and 128.52 (d, −, Ph), 137.95 (s, +, Ph). Anal. Found: 57.37; H, 7.07.

(2*R*,3*R*)-Isomer (4c): obtained from **3c** in 94% yield; $[\alpha]^{21}_D$ −10.40° (*c* 2.97, $CHCl_3$); R_f ($CHCl_3$/EtOH, 10:1) 0.54. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **4b**. Anal. Found: C, 57.08; H, 7.22.

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(2*R*,3*S*)-Isomer (4d): obtained from **3d** in 98% yield; $[\alpha]^{21}_D$ +9.68° (*c* 4.77, $CHCl_3$); R_f ($CHCl_3$/EtOH, 10:1) 0.55. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **4a**. Anal. Found: 56.90; H, 7.32.

2-[2-*O*-Benzyl-4-*O*-(*tert*-butyldiphenylsilyl)-2,3,4-trihydroxybut-1-yl]-1,3-dithianes (5a–5d). A stirred, ice-cold (0–5 °C) mixture of **4a–4d** (9 mmol), pyridine (36 mmol), and DMAP (4 × 10^{-2} mmol) in 80 mL of CH_2Cl_2 was treated with *tert*-butyldiphenylsilyl chloride (13–15 mmol) via syringe in one portion. The reaction mixture was then warmed up to room temperature and stirred for an additional 16–24 h. The resultant mixture was diluted with 150 mL of ether and washed with 5% aqueous HCl (2 × 30 mL) and water (100 mL). The organic layer was dried ($MgSO_4$) and concentrated at reduced pressure. The residue was purified by column chromatography with hexanes/EtOAc (4:1) as eluant to give **5a–5d** as colorless syrups.

2*S*,3*R*-Isomer (5a): obtained from **4a** in 93% yield; $[\alpha]^{25}_D$ −16.45° (*c* 1.24, $CHCl_3$); R_f (hexane/EtOAc, 4:1) 0.35; IR (neat) 3428 cm^{-1}; ^1H NMR (C_6D_6) δ 1.17 (s, 9 H, CH_3), 1.25–1.70 (m, 2 H, $SCH_2CH_2CH_2S$), 2.22 (d, 1 H, *J* = 3.9 Hz, D_2O exchangeable, O*H*), 2.26–2.40 (m, 6 H, H-$1_{a,b}$, 2 × CH_2S), 3.79–3.92 (m, 3 H, H-3, H-$4_{a,b}$), 4.07–4.16 (m, 1 H, H-2), 4.35 (dd, 1 H, *J* = 7.5, 6.3 Hz, SC*H*S), 4.70 (AB q, 2 H, *J* = 11.6 Hz, $PhCH_2O$), 7.07–7.37 (m, 11 H, Ph), 7.57–7.78 (m, 4 H, Ph); ^{13}C NMR δ 19.28 (s, +, Me_3C), 26.03 (t, +, $SCH_2CH_2CH_2S$), 26.97 (q, −, CH_3), 29.88 and 30.31 (t, +, 2 × CH_2S), 36.46 (t, +, C-1), 43.86 (d, −, SCHS), 64.43 (t, +, C-4), 72.88 (t, +, $PhCH_2O$), 73.20 (d, −, C-3), 76.31 (d, −, C-2), 127.71, 128.87, 128.05, and 129.91 (d, −, Ph), 133.07 (s, +, Ph), 135.63 (d, −, Ph), 138.37 (s, +, Ph). Anal. Calcd for $C_{31}H_{40}O_3S_2Si$: C, 67.39; H, 7.25. Found: C, 67.68; H, 7.21.

(2*S*,3*S*)-Isomer (5b); obtained in 97% yield from **4b**; $[\alpha]^{23}_D$ −4.15° (*c* 2.92, $CHCl_3$); R_f (hexanes/EtOAc, 4:1) 0.38; IR (neat) 3470 cm^{-1}; ^1H NMR δ 1.06 (s, 9 H, CH_3), 1.80–2.19 (m, 4 H, $SCH_2CH_2CH_2S$, H-$1_{a,b}$), 2.39 (br s, 1 H, D_2O exchangeable, O*H*), 2.76–2.89 (m, 4 H, 2 × CH_2S), 3.66–3.76 (m, 3 H, H-3, H-$4_{a,b}$), 3.97–4.16 (m, 1 H, H-2), 4.06 (m, 1 H, SC*H*S), 4.62 (AB q, 2 H, *J* = 11.4 Hz, $PhCH_2O$), 7.26–7.50 (m, 11 H, Ph), 7.66–7.62 (m, 4 H, Ph); ^{13}C NMR δ 19.22 (s, +, Me_3C), 25.94 (t, +, $SCH_2CH_2CH_2S$), 26.98 (q, −, CH_3), 29.99 and 30.29 (t, +, 2 × CH_2S), 36.85 (t, +, C-1), 43.92 (d, −, SCHS), 64.42 (t, +, C-4), 73.18 (d, −, C-3), 73.43 (t, +, $PhCH_2O$), 75.24 (d, −, C-2), 127.77, 128.81, 128.02, 128.37, and 129.81 (d, −, Ph), 133.18 (s, +, Ph), 135.58 (d, −, Ph), 138.31 (s, +, Ph). Anal. Found: C, 67.19; H, 7.34.

(2*R*,3*R*)-Isomer (5c): obtained in 93% yield from **4c**; $[\alpha]^{20}_D$ +1.49° (*c* 1.48, $CHCl_3$); R_f (hexanes/EtOAc, 4:1) 0.40. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **5b**. Anal. Found: C, 67.13; H, 7.34.

(2*S*,3*R*)-Isomer (5d): obtained in 86% yield from **4d**; $[\alpha]^{20}_D$ +15.76° (*c* 2.62, $CHCl_3$); R_f (hexanes/EtOAc, 4:1) 0.36. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **5a**. Anal. Found: C, 66.94; H, 7.25.

General Procedure for the Preparation of Hemiacetals 6a–6d. A stirred mixture of **5a–5d** (8 mmol) and $CaCO_3$ (30 mmol) in a 4:1 mixture of CH_3CN/H_2O (100 mL) was treated with iodomethane (200 mmol) in one portion at room temperature. The reaction mixture was warmed to 47 °C and stirred at that temperature for 16–20 h. After cooling to room temperature, it was diluted with 50 mL of H_2O and then extracted with CH_2Cl_2 (3 × 50 mL). The combined extract was washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated at reduced pressure. The residue was purified by flash column chromatography using CH_2Cl_2/EtOAc (10:1) as eluant to afford **6a–6d** as colorless viscous oils.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-D-*ribo*-furanose (6a): obtained in 90% yield from **5a** as a 67:33 mixture of anomers; R_f (CH_2Cl_2/EtOAc, 10:1) 0.55; ^1H NMR δ 5.44 (dd, 1 H, *J* = 10.8, 4.1 Hz, H-1 for major anomer), 5.50–6.00 (m, 1 H, H-1 for minor anomer). Anal. Calcd for $C_{28}H_{34}O_4Si$: C, 72.73; H, 7.46. Found: C, 72.44; H, 7.09.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-L-*xylo*-furanose (6b): obtained in 83% yield from **5b** as a 62:38 mixture of anomers; R_f (CH_2Cl_2/EtOAc, 10:1) 0.51; ^1H NMR δ 5.37 (dd, 1 H, *J* = 11.9, 4.8 Hz, H-1 for major anomer), 5.67 (m, 1 H, H-1 for minor anomer). Anal. Calcd for $C_{28}H_{34}O_4Si$: C, 72.73; H, 7.46. Found: C, 72.48; H, 7.46.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-D-*xylo*-furanose (6c): obtained in 86% yield from **5c** as a 62:38 mixture of anomers; R_f (CH_2Cl_2/EtOAc, 10:1) 0.48. The ^1H NMR spectra was identical to that of **6b**. Anal. Calcd for $C_{28}H_{34}O_4Si$: C, 72.73; H, 7.46. Found: C, 72.39; H, 7.40.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-L-*ribo*-furanose (6d): obtained in 79% yield from **5d** as a 67:33 mixture of anomers; R_f (CH_2Cl_2/EtOAc, 10:1) 0.52. The ^1H NMR spectra was identical to that of **6a**. Anal. Calcd for $C_{28}H_{34}O_4Si$: C, 72.73; H, 7.46. Found: C, 73.12; H, 7.08.

General Procedure for the Synthesis of 2-Deoxypentonolactones 7a–7d. A suspended mixture of **6a–6d** (6–7 mmol), 4-methylmorpholine *N*-oxide (9–11 mmol), and activated powdered 4A molecular sieves (10–12 g) in 40 mL of CH_2Cl_2 was treated with tetrapropylammonium perruthenate (TPAP, 0.5 mmol) in one portion. The reaction mixture was stirred at room temperature until no starting material was observed by TLC analysis (ca. 4 h). The suspension was filtered, and the solid obtained was rinsed with several small portions of acetone. The combined filtrate and washings were concentrated under vacuum, and the residue was purified by flash column chromatography with hexanes/EtOAc (3:1) as eluant to give **7a–7b** as colorless syrups.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-D-ribonolactone (7a): obtained in 93% yield from **6a**; $[\alpha]^{22}_D$ +27.14° (*c* 4.69, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.47. A small sample (ca. 0.1 g) was recrystallized from 95% EtOH to give colorless prisms: mp 70.6–71.8 °C; IR (KBr) 1783 cm^{-1}; ^1H NMR δ 1.02 (s, 9 H, CH_3), 2.62 (dd, 1 H, *J* = 18.1, 2.2 Hz, H-2_α), 2.90 (dd, 1 H, *J* = 18.1, 6.9 Hz, H-2_β), 3.70 (dd, 1 H, *J* = 11.5, 2.6 Hz, H-5_a), 3.84 (dd, 1 H, *J* = 11.5, 3.2 Hz, H-5_b), 4.32 (ddd, 1 H, *J* = 6.9, 6.7, 2.2 Hz, H-3), 4.53 (AB q, 2 H, *J* = 11.8 Hz, $PhCH_2O$), 4.55 (m, 1 H, H-4), 7.28–7.46 (m, 11 H, Ph), 7.57–7.64 (m, 4 H, Ph); ^{13}C NMR δ 19.21 (s, +, Me_3C), 26.85 (q, −, CH_3), 35.98 (t, +, C-2), 63.84 (t, +, C-5), 71.21 (t, +, $PhCH_2O$), 75.96 (d, −, C-3), 85.11 (d, −, C-4), 127.82, 128.02, 128.14, 128.66, and 130.15 (d, −, Ph), 132.16 and 132.68 (s, +, Ph), 135.54 (d, −, Ph), 135.68 (d, −, Ph), 137.14 (s, +, Ph), 175.53 (s, +, C-1). Anal. Calcd for $C_{28}H_{32}O_4Si$: C, 73.04; H, 6.96. Found: C, 72.84; H, 6.87.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-L-xylonolactone (7b): obtained in 94% yield form **6b**; $[\alpha]^{22}_D$ −2.79° (*c* 3.05, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.44. Attempts to crystallize this compound were unsuccessful: IR (neat) 1786 cm^{-1}; ^1H NMR δ 1.07 (s, 9 H, CH_3), 2.68 (dd, 1 H, *J* = 17.2, 6.5 Hz, H-2_β), 2.85 (dd, 1 H, *J* = 17.2, 4.8 Hz, H-2_α), 3.98 (dd, 1 H, *J* = 14.2, 4.8 Hz, H-5_a), 4.05 (dd, 1 H, *J* = 14.2, 4.2 Hz, H-5_b), 4.44 (m, 1 H, H-3), 4.56 (AB q, 2 H, *J* = 11.6 Hz, $PhCH_2O$), 4.58 (m, 1 H, H-4), 7.25–7.47 (m, 11 H, Ph), 7.69–7.72 (m, 4 H, Ph); ^{13}C NMR δ 19.5 (s, +, Me_3C), 26.76 (q, −, CH_3), 35.64 (t, +, C-2), 61.43 (t, +, C-5), 72.15 (t, +, $PhCH_2O$), 76.60 (d, −, C-3), 82.20 (d, −, C-4), 127.60, 127.86, 128.06, 128.60, and 129.91 (d, −, Ph), 132.86 and 132.91 (s, +, Ph), 135.66 (d, −, Ph), 137.26 (s, +, Ph), 174.78 (s, +, C-1). Anal. Calcd for $C_{28}H_{32}O_4Si$: C, 73.04; H, 6.96. Found: C, 72.90; H, 6.90.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-D-xylonolactone (7c): obtained in 99% yield from **6c**; $[\alpha]^{25}_D$ +4.81° (*c* 1.58, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.50. Attempts to crystallize this compound were unsuccessful. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **7b**. Anal. Calcd for $C_{28}H_{32}O_4Si$: C, 73.03; H, 6.96. Found: C, 72.89; H, 7.04.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-L-ribonolactone (7d): obtained in 91% yield from **6d**; $[\alpha]^{22}_d$ −26.43° (*c* 2.30, $CHCl_3$); R_f (hexanes/EtOAc, 3:1) 0.47. A small sample (ca. 0.1 g) was recrystallized from 95% EtOH to give colorless prisms: mp 72.0–72.4 °C. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **7a**. Anal. Calcd for $C_{28}H_{32}O_4Si$: C, 73.03; H, 6.96. Found: C, 72.87; H, 6.95.

General Desilylation Procedure for the Synthesis of 8a–8d. A stirred, ice-cold (0–5 °C) solution of **7a–7d** (2 mmol) in 10 mL of THF was treated with 2 mL of HF–pyridine (70% HF in pyridine) for 4 h. The reaction mixture was then poured into an ether–water mixture (5:1) which contained 10–15 g of crushed ice. The organic layer was washed with H_2O (10 mL), dried ($MgSO_4$), and concentrated at reduced pressure. The residue was purified by flash column chromatography with CH_2Cl_2/EtOAc (10:1) as eluant to give **8a–8d** as colorless viscous oils.

3-*O*-Benzyl-2-deoxy-D-ribonolactone (8a): obtained in 93% yield from **7a**; $[\alpha]^{22}_D$ +48.80° (*c* 0.83, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 10:1) 0.22. An analytical sample (ca. 0.05–0.08 g) was recrystallized from ether/hexanes to give needlelike crystals: mp 68.0–68.8 °C; IR (KBr) 3388 and 1776 cm^{-1}; ^1H NMR δ 2.18 (t, 1 H, *J* = 6.6 Hz, D_2O exchangeable, O*H*), 2.60 (dd, 1 H, *J* = 18.0, 3.4 Hz, H-2_α), 2.87 (dd, 1 H, *J* = 18.0, 7.2 Hz, H-2_β), 3.70 (ddd, 1 H, *J* = 12.5, 6.6, 3.2 Hz, H-5_a), 3.92 (ddd, 1 H, *J* = 12.5, 6.6, 3.0 Hz, H-5_b), 4.31 (ddd, 1 H, *J* = 7.2, 7.0, 3.4 Hz, H-3), 4.54 (AB q, 2 H, *J* = 11.8 Hz, $PhCH_2O$), 4.57 (m, 1 H, H-4), 7.26–7.40 (m, 5 H, Ph); ^{13}C NMR δ 35.99 (t, +, C-2), 62.14 (t, +, C-5), 71.37 (t, +, $PhCH_2O$), 75.63 (d, −, C-3), 85.89 (d, −, C-4), 127.79, 128.11, and 128.61 (d, −, Ph), 137.04 (s, +, Ph), 176.44 (s, +, C-1). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.76; H, 6.39.

3-*O*-Benzyl-2-deoxy-L-xylonolactone (8b): obtained in 89% yield from **7b**; $[\alpha]^{23}_D$ +32.98° (*c* 1.68, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 10:1) 0.14. Attempts to crystallize this compound were unsuccessful: IR (neat) 3434 and 1779 cm^{-1}; ^1H NMR (C_6D_6 + 1 drop of D_2O) δ 1.84 (dd, 1 H, *J* = 17.5, 6.5 Hz, H-2_β), 2.22 (dd, 1 H, *J* = 17.5, 3.4 Hz, H-2_α), 3.39 (distorted ddd, 1 H, H-3), 3.59 (dd, 1 H, *J* = 12.2, 4.9 Hz, H-5_a), 3.68 (dd,

1 H, J = 12.2, 5.2, H-5$_b$), 3.82 (AB q, 2 H, J = 12.0 Hz, PhCH_2O), 3.86 (q, 1 H, J = 5.1 Hz, H-4), 7.01–7.16 (m, 5 H, Ph); ^{13}C NMR δ 35.40 (t, +, C-2), 60.91 (t, +, C-5), 71.88 (t, +, PhCH$_2$O), 74.74 (d, –, C-3), 82.55 (d, –, C-4), 127.73, 128.31, and 128.70 (d, –, Ph), 136.70 (s, +, Ph), 174.71 (s, +, C-1). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.03; H, 6.77.

3-*O*-Benzyl-2-deoxy-D-xylonolactone (8c): obtained in 90% from **7c**; $[\alpha]^{22}_D$ −30.56° (c 1.43, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.15. Attempts to recrystallize this compound were unsuccessful. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **8b**. Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.19; H, 6.45.

3-*O*-Benzyl-2-deoxy-L-ribonolactone (8d): obtained in 89% yield from **7d**; $[\alpha]^{23}_D$ −42.91° (c 1.03, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.18. An analytical sample (ca. 0.03–0.05 g) was recrystallized from ether/hexanes to give needlelike crystals: mp 66.0–67.2 °C. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of the optical antipode **8a**. Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 65.08; H, 6.41.

General Hydrogenolytic Debenzylation Procedure for the Synthesis of 9a–9d. A solution of **7a–7d** (2 mmol) in 5 mL of acetone was added to a suspension of 10% Pd/C (3–5 g) in a 4:1 mixture of MeOH/HOAc (50 mL) contained in a 500-mL pressure bottle. The bottle was evacuated and flushed three times with hydrogen and finally filled with hydrogen at 50 psi. The reactions were conducted in a Parr hydrogenator at room temperature for 3 days. The suspended material was filtered off through a pad of Celite which was immediately rinsed several times with small portions of acetone. The combined filtrate and washings were concentrated at reduced pressure to leave a yellowish crude mixture (6–10 mL). This crude mixture was diluted with 100 mL of ether and washed successively with 5% aqueous NaHCO$_3$ (2 × 20 mL), H$_2$O (20 mL), and brine (20 mL). The organic layer was dried (MgSO$_4$) and concentrated at reduced pressure. The residues obtained were purified by flash column chromatography with CH$_2$Cl$_2$/EtOAc (10:1) as eluant to afford **9a–9d** as colorless syrups.

5-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-D-ribonolactone (9a). Method A (general procedure): obtained in 80% yield from **7a**; $[\alpha]^{22}_D$ +20.99° (c 1.51, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.28; IR (neat) 3438 and 1760 cm^{-1}; ^{1}H NMR δ 1.03 (s, 9 H, CH_3), 2.36 (d, 1 H, J = 4.3 Hz, D$_2$O exchangeable, OH), 2.50 (dd, 1 H, J = 18.1, 2.4 Hz, H-2$_\alpha$), 3.02 (dd, 1 H, J = 18.1, 6.9 Hz, H-2$_\beta$), 3.78 (dd, 1 H, J = 15.5, 2.7 Hz, H-5$_a$), 3.86 (dd, 1 H, J = 15.5, 3.2 Hz, H-5$_b$), 4.43 (m, 1 H, H-4), 4.63 (m, 1 H, H-3), 7.35–7.48 (m, 6 H, Ph), 7.60–7.65 (m, 4 H, Ph); ^{13}C NMR δ 19.10 (s, +, Me$_3$$C$), 26.71 (q, –, CH$_3$), 38.79 (t, , C-2), 63.60 (t, +, C-5), 69.67 (d, –, C-3), 87.56 (d, –, C-4), 127.93 and 130.06 (d, –, Ph), 131.98 and 132.57 (s, +, Ph), 135.45 and 135.59 (d, –, Ph), 176.09 (s, +, C-1). Anal. Calcd for $C_{21}H_{26}O_4Si$: C, 68.11; H, 7.03. Found: C, 67.63; H, 7.44.

Method B. 2-Deoxy-D-ribonolactone (**14**, 0.074 g, 0.56 mmol) was dissolved in dry pyridine (6 mL). The solution was cooled to 0 °C, and *tert*-butyldiphenylsilyl chloride (0.16 g, 0.56 mmol) was added via syringe. The flask was warmed to room temperature and stirred for 12 h. Water was added, and the mixture was extracted with ether (3×). The combined organic extracts were washed with brine (3×), dried (MgSO$_4$), and reduced to dryness. Flash chromatography on silica gel with hexanes/EtOAc (3:1) afforded 0.16 g (78%) of **9a** which was spectroscopically identical to the material prepared under method A.

5-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-L-xylonolactone (9b): obtained in 84% yield from **7b**; $[\alpha]^{22}_D$ −25.33 (c 5.61, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.48; IR (neat) 3452 and 1778 cm^{-1}; ^{1}H NMR δ 1.07 (s, 9 H, CH_3), 2.61 (dd, 1 H, J = 17.8, 3.0 Hz, H-2$_\alpha$), 2.78 (dd, 1 H, J = 17.8, 6.3 Hz, H-2$_\beta$), 3.31 (d, 1 H, J = 5.1 Hz, D$_2$O exchangeable, OH), 4.03 (dd, 1 H, J = 16.4, 5.3 Hz, H-5$_a$), 4.11 (dd, 1 H, J = 16.4, 4.2 Hz, H-5$_b$), 4.44 (m, 1 H, H-4), 4.69 (m, 1 H, H-3), 7.36–7.50 (m, 6 H, Ph), 7.65–7.71 (m, 4 H, Ph); ^{13}C NMR δ 19.10 (s, +, Me$_3$$C$), 26.74 (q, –, CH$_3$), 38.61 (t, +, C-2), 61.94 (t, +, C-5), 68.78 (d, –, C-3), 81.84 (d, –, C-4), 127.85, 128.02, and 130.20 (d, –, Ph), 131.93 and 132.31 (s, +, Ph), 135.46 and 135.57 (d, –, Ph), 175.34 (s, +, C-1). Anal. Calcd for $C_{21}H_{26}O_4Si$: C, 68.11; H, 7.03. Found: C, 68.03; H, 6.89.

5-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-D-xylonolactone (9c): obtained in 79% yield from **7c**; $[\alpha]^{21}_D$ +31.86° (c 1.18, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.47. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **9b**. Anal. Calcd for $C_{21}H_{26}O_4Si$: C, 68.11; H, 7.03. Found: C, 67.96; H, 6.99.

5-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-L-ribonolactone (9d): obtained in 87% yield from **7d**; $[\alpha]^{23}_D$ −24.37° (c 1.26, CHCl$_3$); R_f (CH$_2$Cl$_2$/EtOAc, 10:1) 0.30. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **9a**. Anal. Calcd for $C_{21}H_{26}O_4Si$: C, 68.11; H, 7.03. Found: C, 68.02; H, 6.90.

General Procedure for the Myristoylation of Alcohols 8a–8d and 9a–9d. A stirred solution of **8a–8d**/**9a–9d** (1 mmol), pyridine (4 mmol), and DMAP (0.02 mmol) in 20 mL of CH$_2$Cl$_2$ was treated with a single portion of myristoyl chloride (2 mmol) and kept at room temperature for 16–20 h. The reaction mixture was then diluted with ether, and the ethereal solution was washed with 10% aqueous HCl (2 × 20 mL) and H$_2$O (30 mL). The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure. The residue was purified by flash column chromatography using mixtures of hexanes/EtOAc as eluant (3:1 for **10a–10b** and 9:1 for **11a–11b**) to give the corresponding esters as colorless syrups. Compounds **10a** and **10d** were crystallized from 95% EtOH affording white needles. Attempts to recrystallize the remaining esters were unsuccessful.

5-*O*-Tetradecanoyl-3-*O*-benzyl-2-deoxy-D-ribonolactone (10a): obtained from **8a** in 84% yield; $[\alpha]^{22}_D$ +30.85° (c 1.41, CHCl$_3$); R_f (hexanes/EtOAc, 3:1) 0.40; mp 57.0–57.4 °C; IR (KBr) 1756 and 1742 cm^{-1}; ^{1}H NMR δ 0.87 (t, 3 H, J = 6.9 Hz, CH_3), 1.25 (br s, 20 H, CH$_3$(CH_2)$_{10}$CH$_2$CH$_2$CO), 1.52–1.62 (m, 2 H, CH$_3$(CH$_2$)$_{10}$CH_2CH$_2$CO), 2.29 (t, 2 H, J = 7.4 Hz, CH$_3$(CH$_2$)$_{10}$CH$_2$CH_2CO), 2.62 (dd, 1 H, J = 18.6, 3.5 Hz, H-2$_\alpha$), 2.81 (dd, 1 H, J = 18.6, 6.9 Hz, H-2$_\beta$), 4.15 (m, 1 H, H-3), 4.17 (dd, 1 H, J = 12.2, 4.0 Hz, H-5$_a$), 4.30 (dd, 1 H, J = 12.2, 3.4 Hz, H-5$_b$), 4.53 (AB q, 2 H, J = 11.8 Hz, PhCH_2O), 4.68 (m, 1 H, H-4), 7.24–7.40 (m, 5 H, Ph); ^{13}C NMR δ 14.09 (q, –, CH$_3$), 22.65, 24.74, 29.06, 29.20, 29.33, 29.41, 29.62, 31.89, and 33.93 (t, +, –CH$_2$–), 35.35 (t, +, C-2), 63.21 (t, +, C-5), 71.59 (t, +, PhCH$_2$O), 75.29 (d, –, C-3), 82.11 (d, –, C-4), 126.76, 128.28, and 128.66 (d, –, Ph), 136.55 (s, +, Ph), 173.06 (s, +, myristoyl C=O), 174.26 (s, +, C-1). Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.22; H, 9.26. Found: C, 72.31; H, 9.26.

5-*O*-Tetradecanoyl-3-*O*-benzyl-2-deoxy-L-xylonolactone (10b): obtained in 96% yield from **8b**; $[\alpha]^{22}_D$ −6.72° (c 1.31, CHCl$_3$); R_f (hexanes/EtOAc, 3:1) 0.24; IR (neat) 1789 and 1743 cm^{-1}; ^{1}H NMR δ 0.87 (t, 3 H, J = 6.8 Hz, CH_3), 1.24 (br s, 20 H, CH$_3$(CH_2)$_{10}$CH$_2$CH$_2$CO), 1.55–1.65 (m, 2 H, CH$_3$(CH$_2$)$_{10}$CH_2CH$_2$CO), 2.31 (t, 2 H, J = 7.7 Hz, CH$_3$(CH$_2$)$_{10}$CH$_2$CH_2CO), 2.62–2.73 (m, 2 H, H-2$_{\alpha,\beta}$), 4.34 (m, 1 H, H-3), 4.41 (dd, 1 H, J = 17.7, 5.0 Hz, H-5$_a$), 4.49 (dd, 1 H, J = 17.7, 3.8 Hz, H-5$_b$), 4.51 (m, 2 H, PhCH_2O), 4.68 (m, 1 H, H-4), 7.25–7.41 (m, 5 H, Ph); ^{13}C NMR δ 14.08 (q, –, CH$_3$), 22.65, 24.82, 29.09, 29.23, 29.33, 29.43, 29.62, 31.89, and 34.07 (t, +, –CH$_2$–), 35.16 (t, +, C-2), 62.09 (t, +, C-5), 71.61 (t, +, PhCH$_2$O), 74.07 (d, –, C-3), 80.39 (d, –, C-4), 127.68, 128.20, and 128.61 (d, –, Ph), 136.68 (s, +, Ph), 173.39 (myristoyl C=O), 174.11 (s, +, C-1). Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.22; H, 9.26. Found: C, 72.38; H, 9.18.

5-*O*-Tetradecanoyl-3-*O*-benzyl-2-deoxy-D-xylonolactone (10c): obtained in 94% yield from **8c**; $[\alpha]^{20}_D$ +6.83° (c 1.42, CHCl$_3$); R_f (hexanes/EtOAc, 3:1) 0.28. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **10b**. Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.22; H, 9.26. Found: C, 72.41; H, 9.22.

5-*O*-Tetradecanoyl-3-*O*-benzyl-2-deoxy-L-ribonolactone (10d): obtained in 92% yield from **8d**; $[\alpha]^{22}_D$ −26.46° (c 1.75, CHCl$_3$); R_f (hexanes/EtOAc, 3:1) 0.45; mp 55.0–55.6 °C. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **10a**. Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.22; H, 9.26. Found: C, 72.44; H, 9.38.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-tetradecanoyl-2-deoxy-D-ribonolactone (11a): obtained in 89% yield from **9a**; $[\alpha]^{22}_D$ +10.31° (c 2.61, CHCl$_3$); R_f (hexanes/EtOAc, 9:1) 0.31; IR (neat) 1790 and 1741 cm^{-1}; ^{1}H NMR δ 0.90 (t, 3 H, J = 6.1 Hz, CH_3), 1.03 (s, 9 H, C(CH_3)$_3$), 1.24 (br s, 20 H, CH$_3$(CH_2)$_{10}$CH$_2$CH$_2$CO), 1.54–1.64 (m, 2 H, CH$_3$(CH$_2$)$_{10}$CH_2CH$_2$CO), 2.32 (t, 2 H, J = 7.2 Hz, CH$_3$(CH$_2$)$_{10}$CH$_2$CH_2CO), 2.57 (dd, 1 H, J = 18.5, 1.2 Hz, H-2$_\alpha$), 3.13 (dd, 1 H, J = 18.5, 7.4 Hz, H-2$_\beta$), 3.86 (m, 2 H, H-5$_{a,b}$), 4.48 (br s, 1 H, H-4), 5.43 (br d, 1 H, H-3), 7.35–7.45 (m, 6 H, Ph), 7.60–7.66 (m, 4 H, Ph); ^{13}C NMR δ 14.12 (q, –, CH$_3$), 19.10 (s, +, Me$_3$$C$), 22.70 and 24.20 (t, +, –CH$_2$–), 26.73 (q, –, C(CH$_3$)$_3$), 29.06, 29.21, 29.34, 29.41, 29.63, 31.91, and 34.04 (t, +, –CH$_2$–), 35.76 (t, +, C-2), 63.81 (t, +, C-5), 71.97 (d, –, C-3), 85.17 (d, –, C-4), 127.95 and 130.06 (d, –, Ph), 131.84 and 132.40 (s, +, Ph), 135.45 and 135.60 (d, –, Ph), 173.30 (s, +, myristoyl C=O), 174.84 (s, +, C-1). Anal. Calcd for $C_{35}H_{52}O_5Si$: C, 72.41; H, 8.97. Found: C, 72.17; H, 9.01.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-tetradecanoyl-2-deoxy-L-xylonolactone (11b): obtained in 86% yield from **9b**; $[\alpha]^{23}_D$ −6.50° (c 2.23, CHCl$_3$); R_f (hexanes/EtOAc, 9:1) 0.38; IR (neat) 1794 and 1744 cm^{-1}; ^{1}H NMR δ 0.87 (t, 3 H, J = 6.8 Hz, CH_3), 1.04 (s, 9 H, C(CH_3)$_3$), 1.24 (br s, 20 H, CH$_3$(CH_2)$_{10}$CH$_2$CH$_2$CO), 1.46–1.64 (m, 2 H, CH$_3$(CH$_2$)$_{10}$CH_2CH$_2$CO), 2.24 (overlapping triplets, 2 H, CH$_3$(CH$_2$)$_{10}$CH$_2$CH_2CO), 2.72 (dd, 1 H, J = 17.9, 4.6 Hz, H-2$_\alpha$), 2.89 (dd, 1 H, J = 17.9, 7.0 Hz, H-2$_\beta$), 3.84 (dd, 1 H, J = 11.2, 4.5 Hz, H-5$_a$), 3.92 (dd, 1 H, J = 11.2, 4.9 Hz, H-5$_b$), 4.65 (q, 1 H, J = 4.9 Hz, H-4), 5.54 (m, 1 H, H-3), 7.30–7.49 (m, 6 H, Ph), 7.62–7.69 (m, 4 H, Ph); ^{13}C NMR δ 14.09 (q, –, CH$_3$), 19.06 (s, +, Me$_3$$C$), 22.68 and 24.66 (t, +, –CH$_2$–), 26.64 (q, –, C(CH$_3$)$_3$), 29.06, 29.18, 29.35, 29.39, 29.63, 31.91 and 33.92 (t, +, –CH$_2$–), 35.85 (t, +, C-2), 61.17 (t, +, C-5), 69.17 (d, –, C-3), 80.74 (d, –, C-4), 127.83 and 129.95 (d, –, Ph), 132.45 and

132.68 (s, +, Ph), 135.54 (d, –, Ph), 172.76 (s, +, myristoyl *C*=O), 173.59 (s, +, C-1). Anal. Calcd for $C_{35}H_{52}O_3Si$: C, 72.41; H, 8.97. Found: C, 72.30; H, 9.16.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-tetradecanoyl-2-deoxy-D-xylonolactone (11c): obtained in 88% yield from **9c**; $[\alpha]^{20}_D$ +8.24° (*c* 1.65, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.31. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **11b**. Anal. Calcd for $C_{35}H_{52}O_3Si$: C, 72.41; H, 8.97. Found: C, 72.63; H, 9.19.

5-*O*-(*tert*-Butyldiphenylsilyl)-3-*O*-tetradecanoyl-2-deoxy-L-ribonolactone (11d): obtained in 97% yield from **9d**; $[\alpha]^{22}_D$ –10.71° (*c* 3.09, $CHCl_3$); R_f (hexanes/EtOAc, 9:1) 0.37. The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **11a**. Anal. Calcd for $C_{35}H_{52}O_3Si$: C, 72.41; H, 8.97. Found: C, 72.58; H, 8.92.

Synthesis of 5-*O*-Myristoylated 2-Deoxypentonolactones (12a–12d). These products were obtained following the same debenzylation procedure described earlier for the syntheses of **9a–9d**. The crude compounds were purified by flash column chromatography with CH_2Cl_2/EtOAc (5:1) as eluant to give, after recrystallization from ether/hexanes, white crystalline solids.

5-*O*-Tetradecanoyl-2-deoxy-D-ribonolactone (12a). Method A (general procedure): obtained from **10a** in 84% yield; $[\alpha]^{21}_D$ +13.57° (*c* 1.12, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.35; mp 66.0–66.7 °C (needles); IR (KBr) 3436 and 1743 cm^{-1}; ^1H NMR δ 0.86 (t, 3 H, *J* = 6.7 Hz, CH_3), 1.24 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.56–1.60 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.17 (d, 1 H, *J* = 4.0 Hz, D_2O exchangeable, O*H*), 2.32 (t, 2 H, *J* = 7.6 Hz, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.55 (dd, 1 H, *J* = 18.1, 3.8 Hz, H-2_α), 2.89 (dd, 1 H, *J* = 18.1, 6.9 Hz, H-2_β), 4.23 (dd, 1 H, *J* = 12.4, 4.1 Hz, H-5_a), 4.33 (dd, 1 H, *J* = 12.4, 3.3 Hz, H-5_b), 4.47 (m, 1 H, H-3), 4.55 (m, 1 H, H-4); ^{13}C NMR δ 14.09 (q, –, CH_3), 22.65, 24.75, 29.06, 29.18, 29.33, 29.41, 29.55, 29.62, 31.89, and 33.98 (t, +, $-CH_2-$), 37.87 (t, +, C-2), 62.95 (t, +, C-5), 69.03 (d, –, C-3), 84.44 (d, –, C-4), 173.47 (s, +, myristoyl *C*=O), 174.43 (s, +, C-1). Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.74; H, 9.89. **Method B**: from **14**. 2-Deoxy-D-ribonolactone (**14**, 0.1 g, 0.75 mmol) was dissolved in dry pyridine (7 mL) and cooled to 0 °C. Myristoyl chloride (0.5 g, 2 mmol) was added dropwise via syringe after which the flask was warmed to room temperature and stirred for 12 h. Water was added, and the mixture was extracted with EtOAc (3×). The combined extracts were washed with brine (4×), dried ($MgSO_4$), and concentrated to dryness. Flash chromatography on silica gel with hexanes/EtOAc (5:1) afforded 0.20 g (78%) of **12a** which was identical to the material obtained under method A: $[\alpha]^{22}_D$ +13.20° (*c* 2.50, $CHCl_3$).

5-*O*-Tetradecanoyl-2-deoxy-L-xylonolactone (12b): obtained in 93% yield from **10b**; $[\alpha]^{21}_D$ –58.6° (*c* 1.05, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.32; mp 75.8–76.5 °C (needles); IR (KBr) 3436 and 1742 cm^{-1}; ^1H NMR (C_6D_6 + 1 drop of D_2O) δ 0.94 (t, 3 H, *J* = 6.7 Hz, CH_3), 1.26 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.52–1.59 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.89 (dd, 1 H, *J* = 17.6, 4.6 Hz, H-2_β), 2.11 (m, 3 H, H-2_α, $CH_3(CH_2)_{10}CH_2CH_2CO$), 3.59 (m, 1 H, H-3), 3.87 (m, 1 H, H-4), 4.19 (dd, 1 H, *J* = 12.2, 6.6 Hz, H-5_a), 4.40 (dd, 1 H, *J* = 12.2, 4.9 Hz, H-5_b); ^{13}C NMR δ 14.04 (q, –, CH_3), 22.62, 24.76, 29.05, 29.20, 29.30, 29.41, 29.59, 31.86, and 34.02 (t, +, $-CH_2-$), 38.53 (t, +, C-2), 61.67 (t, +, C-5), 67.74 (d, –, C-3), 81.65 (d, –, C-4), 174.32 (s, +, myristoyl *C*=O), 175.64 (s, +, C-1). Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.91; H, 9.94.

5-*O*-Tetradecanoyl-2-deoxy-D-xylonolactone (12c): obtained in 97% yield from **10c**; $[\alpha]^{18}_D$ +46.34° (*c* 1.23, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.35; mp 74.3–75.2 °C (prisms). The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **12b**. Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.43; H, 10.08.

5-*O*-Tetradecanoyl-2-deoxy-L-ribonolactone (12d): obtained in 85% yield from **10d**; $[\alpha]^{21}_D$ –15.00° (*c* 0.76, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.33; mp 65.0–65.7 °C (flakes). The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **12a**. Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.74; H, 9.89.

Synthesis of 3-*O*-Myristoylated 2-Deoxypentonolactones (13a–13d). These products were obtained following the same desilylation procedure described earlier for the syntheses of **8a–8d**. The crude compounds were purified by flash column chromatography using either CH_2Cl_2/EtOAc (5:1) for compounds **13a** and **13d** or CH_2Cl_2/EtOAc/pyridine (85:15:2) for compounds **13b** and **13c** as eluant. After recrystallization from ether/hexanes the compounds were obtained as white crystalline solids.

3-*O*-Tetradecanoyl-2-deoxy-D-ribonolactone (13a). Method A (general procedure): obtained in 87% yield from **11a**; $[\alpha]^{22}_D$ –23.08° (*c* 0.78, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.25; mp 60.1–60.8 °C (needles); IR (KBr) 3414, 1763, and 1729 cm^{-1}; ^1H NMR ($CDCl_3$ + 1 drop of D_2O) δ 0.86 (t, 3 H, *J* = 6.8 Hz, CH_3), 1.23 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.55–1.68 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.31 (t, 2 H, *J* = 7.3 Hz, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.53 (dd, 1 H, *J* = 18.6, 1.8 Hz, H-2_α), 3.06 (dd, 1 H, *J* = 18.6, 7.5 Hz, H-2_β), 3.90 (m, 2 H, H-$5_{a,b}$), 4.50 (br q, 1 H, H-4), 5.34 (br dt, 1 H, H-3); ^{13}C NMR δ 14.09 (q, –, CH_3), 22.65, 24.68, 29.03, 29.18, 29.32, 29.60, 31.86, and 34.02 (t, +, $-CH_2-$), 35.43 (t, +, C-2), 62.18 (t, +, C-5), 71.53 (d, –, C-3), 85.58 (d, –, C-4), 173.47 (s, +, myristoyl *C*=O), 175.36 (s, +, C-1). Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.53; H, 10.04. **Method B**: from **14**. Following the sequence **14** → **9a** → **11a** → **13a**, this compound was also obtained in pure optical form: $[\alpha]^{22}_D$ –23.70° (*c* 1.60, $CHCl_3$).

3-*O*-Tetradecanoyl-2-deoxy-L-xylonolactone (13b): obtained in 47% yield from **11b**; $[\alpha]^{22}_D$ –24.06° (*c* 0.76, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.22; mp 74.2–75.8 °C (flakes); IR (KBr), 3468, 1770, 1736 cm^{-1}; ^1H NMR ($CDCl_3$ + 1 drop of D_2O) δ 0.87 (t, 3 H, *J* = 6.9 Hz, CH_3), 1.24 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.56–1.65 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.36 (t, 2 H, *J* = 7.7 Hz, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.65 (dd, 1 H, *J* = 18.2, 2.4 Hz, H-2_α), 2.93 (dd, 1 H, *J* = 18.2, 6.4 Hz, H-2_β), 3.75 (dd, 1 H, *J* = 12.4, 5.2 Hz, H-5_a), 3.95 (dd, 1 H, *J* = 12.4, 5.8 Hz, H-5_b), 4.65 (br q, 1 H, H-4), 5.54 (m, 1 H, H-3); ^{13}C NMR δ 14.08 (q, –, CH_3), 22.65, 24.72, 29.03, 29.16, 29.32, 29.61, 31.89, and 33.98 (t, +, $-CH_2-$), 36.12 (t, +, C-2), 60.05 (t, +, C-5), 69.94 (d, –, C-3), 81.91 (d, –, C-4), 173.42 (s, +, myristoyl *C*=O), 173.56 (s, +, C-1). Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.43; H, 10.13.

3-*O*-Tetradecanoyl-2-deoxy-D-xylonolactone (13c): obtained in 65% yield from **11c**; $[\alpha]^{22}_D$ +29.05° (*c* 2.63, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.20; mp 77.2–77.9 °C (flakes). The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **13b**. Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.40; H, 9.91.

3-*O*-Tetradecanoyl-2-deoxy-L-ribonolactone (13d): obtained in 97% yield from **11d**; $[\alpha]^{22}_D$ +19.40° (*c* 1.16, $CHCl_3$); R_f (CH_2Cl_2/EtOAc, 5:1) 0.25; mp 61.4–62.2 °C (needles). The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **13a**. Anal. Calcd for $C_{19}H_{34}O_5$: C, 66.67; H, 9.94. Found: C, 66.99; H, 10.07.

2-Deoxy-D-ribonolactone (14).[46] This compound was prepared from D-ribonolactone in four steps via 3,4-*O*-benzylidene-D-ribono-1,5-lactone and 3,4-*O*-benzylidene-2-*O*-[(methylthio)thiocarbonyl]-D-ribono-1,5-lactone as reported in the literature.[44,45]

5-*O*-Tetradecanoyl-2,3-dideoxy-D-*glycero*-pentono-1,4-lactone (15). A solution of 1.83 g (5.08 mmol) of 5-*O*-trityl-2,3-dideoxy-D-*glycero*-pentono-1,4-lactone in THF (60 mL) was treated with 10 mL of 10% aqueous HCl at room temperature for 45 min. After the solvent was removed under vacuum, the residue was purified by silica gel flash column chromatography with a mixture of $CHCl_3$/EtOH (10:1) as eluant. The product-containing fractions were pooled and concentrated at reduced pressure to give 0.556 g (94%) of the crude deprotected lactone. The crude lactone (0.521 g, 4.5 mmol) was dissolved in a mixture consisting of pyridine (1.26 g, 16 mmol) and CH_2Cl_2 (20 mL). After adding a catalytic amount of DMAP (ca. 1 mg), myristoyl chloride (1.38 g, 5.6 mmol) was slowly added to the mixture, while stirring under an argon atmosphere. After 24 h, the reaction mixture was diluted with ether (100 mL), and the ethereal solution was washed successively with 5% aqueous HCl and water. The organic layer was dried (Mg SO_4) and concentrated at reduced pressure. The residue was purified by silica gel column chromatography with a mixture of hexanes/EtOAc (1:1) as eluant to give 1.40 g (92%) of **15** which was recrystallized from hexanes/Et_2O as white needles: mp 38.8–39.6 °C; IR (KBr) 1768 and 1735 cm^{-1}; $[\alpha]^{18}_D$ +21.7° (*c* 3.56, $CHCl_3$); ^1H NMR δ 0.88 (t, 3 H, *J* = 6.8 Hz, CH_3), 1.26 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.56–1.66 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.04 (m, 1 H, H-$3_a$), 2.35 (t, 2 H, *J* = 7.7 Hz, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.36 (m, 1 H, H-$3_b$), 2.60 (m, 2 H, H-$2_{a,b}$), 4.15 (dd, 1 H, *J* = 12.2, 5.4 Hz, H-5_a), 4.33 (dd, 1 H, *J* = 12.2, 3.2 Hz, H-5_b), 4.75 (m, 1 H, H-4); ^{13}C NMR δ 13.04 (q, –, CH_3), 21.62, 22.84, 23.76, 27.09, 28.03, 28.17, 28.29, 28.38, 28.52, 28.59, 30.85 (t, +, $-CH_2-$ and C-3), 32.93 (t, +, C-2), 64.04 (t, +, C-5), 77.30 (d, –, C-4), 172.26 (s, +, myristoyl *C*=O), 175.53 (s, +, C-1). Anal. Calcd for $C_{19}H_{34}O_4$: C, 69.94; H, 10.43. Found: C, 70.02; H, 10.73.

5-*O*-Tetradecanoyl-2,3-dideoxy-L-*glycero*-pentono-1,4-lactone (16). This compound was obtained in a similar fashion as **15** starting from the corresponding 5-*O*-trityl-2,3-dideoxy-L-*glycero*-pentono-1,4-lactone: mp 40.8–41.4 °C (hexanes/Et_2O); $[\alpha]^{25}_D$ –23.31° (*c* 2.65, $CHCl_3$). The IR, ^1H NMR, and ^{13}C NMR spectra were identical to those of its optical antipode **15**. Anal. Calcd for $C_{19}H_{34}O_4$: C, 69.94; H, 10.43. Found: C, 69.75; H, 10.52.

5-*O*-Tetradecanoyl-2,3-didehydro-2,3-dideoxy-D-*glycero*-pentono-1,4-lactone (17). This compound was obtained as a white solid in 97% yield from commercially available *S*-(–)-5-(hydroxymethyl)-2-(5*H*)-furanone under similar acylation conditions as performed for the synthesis of compounds **15** and **16**: mp 37.2–39.0 °C; $[\alpha]^{22}_D$ –59.14° (*c* 1.17, $CHCl_3$); IR (KBr) 1749 and 1731 cm^{-1}; ^1H NMR δ 0.87 (t, 3 H, *J* = 6.7 Hz, CH_3), 1.25 (br s, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.51–1.62 (m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.30 (t, 2 H, *J* = 7.7 Hz, CH_3-

$(CH_2)_{10}CH_2CH_2CO)$, 4.34 (d, 2 H, $J = 4.5$ Hz, H-$5_{a,b}$), 5.22 (m, 1 H, H-4), 6.20 (dd, 1 H, $J = 5.7, 2.0$ Hz, H-3), 7.40 (dd, $J = 5.7, 1.5$ Hz, H-2); ^{13}C NMR δ 14.08 (q, −, CH_3), 22.65, 24.25, 29.03, 29.16, 29.31, 29.38, 29.59, 31.88, 33.08, and 33.89 (t, +, $-CH_2-$), 62.24 (t, +, C-5), 80.83 (d, −, C-4), 123.25 (d, −, C-3), 152.32 (d, −, C-2), 172.16 (s, +, myristoyl C=O), 173.33 (s, +, C-1). Anal. Calcd for $C_{19}H_{32}O_4$: C, 70.37; H, 9.88. Found: C, 70.32; H, 10.14.

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Cooperation of Cyclodextrin and Alkali-Metal Halide for Regioselective Cleavage of Ribonucleoside 2′,3′-Cyclic Phosphates[1]

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Abstract: Regioselective catalysis of β- and γ-cyclodextrins (β- and γ-CyDs) for the P–O(3′) cleavage of adenosine 2′,3′-cyclic phosphate (A>p) to adenosine 2′-phosphate is significantly promoted, with respect to both selectivity and reaction rate, by cooperation with NaCl, KCl, RbCl, CsCl, KBr, and KF. A selectivity of 94% is achieved at pH 9.5 and 30 °C by the combination of β-CyD and KCl (3.0 M); the values with β-CyD alone and with KCl alone are 79% and 41%, respectively. By contrast, LiCl and KI reduce the regioselectivity. The logarithm of the 2′-phosphate/3′-phosphate ratio decreases linearly with an increase in the logarithm of the mean ionic activity coefficient of the medium. The α-CyD-induced regioselective P–O(2′) cleavage of A>p and U>p to the 3′-phosphates is also enhanced by KCl. The difference in chemical environment of the P–O(2′) and P–O(3′) bonds provided by CyDs on complex formation with the cyclic phosphates is amplified by the metal salts, resulting in the increase in regioselectivity.

Introduction

Preparation of artificial nucleases has been a most challenging topic. Site-selective fission of deoxyribonucleic acids by oxidative processes was successfully accomplished.[2] Furthermore, artificial systems for efficient hydrolysis of ribonucleic acids (RNAs) were reported.[3-7] However, none of them (except, it is assumed, for the conjugates[7] using natural enzymes as catalytic sites) could mimic the regioselective catalysis of ribonuclease. The enzyme selectively cleaves the P–O(2′) bond of 2′,3′-cyclic phosphate of ribonucleotide as an intermediate, providing an RNA fragment having the terminal phosphate at the 3′-position.[8]

Breslow reported modified cyclodextrins bearing two imidazolyl residues as elegant models of ribonuclease.[9] Cyclic phosphate of 1,2-dihydroxybenzene, a model compound of the intermediate in RNA hydrolysis, is regioselectively cleaved. Furthermore, the manner of cooperation of the two imidazole residues was investigated in detail by use of precisely modified cyclodextrins.[10]

Recently,[11] we succeeded in regioselective cleavage of 2′,3′-cyclic phosphates of ribonucleosides and ribonucleotides by use of cyclodextrins (CyDs) as catalysts.[12] The direction of regioselective catalysis depends largely on the kind of CyD: P–O(2′) bonds are selectively cleaved by α-CyD, whereas β- and γ-CyDs enhance P–O(3′) cleavage. The regioselective catalysis is ascribed to the formation of a complex between CyD and the cyclic phosphates, in which the P–O(2′) and the P–O(3′) bonds are differentiated.

The present work reveals that both the selectivity and the reaction rate for the CyD-induced regioselective cleavage of ribonucleoside 2′,3′-cyclic phosphates are further increased by cooperation with alkali-metal halides. The cooperative catalysis is kinetically and spectroscopically investigated, and a reaction mechanism is proposed.

Experimental Section

Kinetics. Cleavage of 2′,3′-cyclic phosphates of adenosine, guanosine, cytidine, and uridine (A>p, G>p, C>p, and U>p) was carried out at 30 °C and pH 9.5 unless otherwise noted. Reaction mixtures were prepared by addition of metal salts to aqueous carbonate buffer solutions (ionic strength 0.01 M), followed by adjustment of pH when necessary. The rate constant and regioselectivity in the absence of the metal salts were evaluated by extrapolation to zero buffer concentration.

All the reaction vessels and the water were sterilized immediately before use, and special caution was taken throughout the experiments to avoid contamination of ribonuclease and other nucleases.[13] Rate constants of the cleavage as well as ratios (2′/3′) of the 2′-phosphate of ribonucleoside to the 3′-phosphate in the products were determined by periodic analysis with HPLC, as described previously.[11] All the reactions

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(13) Absence of contamination of ribonuclease is further confirmed by the preferable formation of adenosine 2′-phosphate in the cleavage of A>p catalyzed by a combination of β- or γ-CyD and the metal halide. The enzyme if any should produce the 3′-phosphate in 100% selectivity.[8]

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