

# Iodoaryl Analogues of Dioctanoylglycerol and 1-Oleoyl-2-acetylglycerol as Probes for Protein Kinase C

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Analogues of dioctanoylglycerol (diC<sub>8</sub>) and 1-oleoyl-2-acetylglycerol (OAG) containing an iodoaryl group have been synthesized and shown to compete with [<sup>3</sup>H]phorbol dibutyrate ([<sup>3</sup>H]PDBu) for binding to protein kinase C in a crude rat brain preparation. Phorbol diesters have been shown to bind specifically to protein kinase C and the PDBu receptor has been copurified with protein kinase C activity. All three diacylglycerol analogues were comparable to OAG in binding affinity. In an assay of protein kinase C activation, the diC<sub>8</sub> analogue was more active than the OAG analogues, thus demonstrating greater structural specificity under the conditions of this assay.

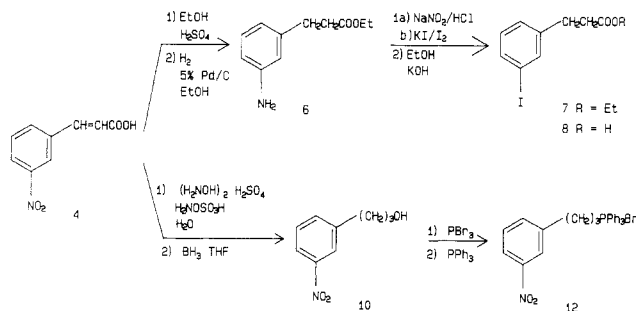
Diacylglycerols have been attracting attention since 1979, when it was discovered that they are the endogenous modulators of protein kinase C (PK-C).<sup>1,2</sup> PK-C is involved in cellular signal transduction and proliferation, as well as numerous other biological processes (for reviews, see ref 3-8). Diacylglycerol is produced transiently within cells when cellular receptor activation leads to the cleavage of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate and diacylglycerol. A complex forms among diacylglycerol, Ca<sup>2+</sup>, phosphatidylserine (PS), and PK-C, activating PK-C. Many proteins are phosphorylated by the activated kinase, but how this leads to initiation of biological responses is not yet understood.

In an effort to develop chemical probes that may be useful for elucidating events associated with the action of PK-C, we undertook the synthesis of a series of iodinated diacylglycerol analogues and analyzed them for their abilities to bind to and activate PK-C. The iodine atoms can be exchanged with radiiodine to facilitate identification in studies of subcellular localization and tissue distribution. In our preliminary studies,<sup>9</sup> iodine was incorporated into diacylglycerols by the use of iopanoic acid or its desethyl analogue as one of the acyl groups. Results of the binding studies indicated that there is little specificity for the size or stereochemistry of the acyl group in either the 1- or 2-position. Since the iopanoate-type acyl group in these molecules is very different from the fatty acyl groups that typically are found in endogenous diacylglycerols,<sup>10</sup> we decided to synthesize iodinated diacylglycerols that more closely resemble known diacylglycerols. Presently, the most commonly used diacylglycerols for such studies are dioctanoylglycerol (diC<sub>8</sub>) and 1-oleoyl-2-acetylglycerol (OAG) because they are able to activate PK-C in both intact and broken cells.<sup>11</sup> Analogues of these molecules, compounds 1 and 3, were chosen as target compounds (Figure 1). The positional isomer of 3, compound 2, was also synthesized to determine if there is a preference for the long-chain moiety in the 1- or 2-position. A terminal aromatic group was included to stabilize the iodine atom. All three diacylglycerol analogues were evaluated for their abilities to bind to and activate PK-C.

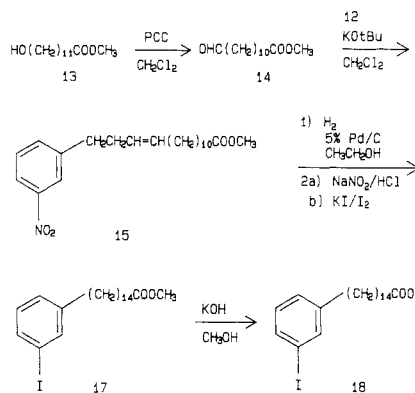
## Chemistry

The first step in the preparation of compounds 1-3 was the synthesis of the iodoacyl moieties. 3-Nitrocinnamic acid, 4, served as an appropriate starting material for both acids, as shown in Scheme I. For the synthesis of 3-(3-iodophenyl)propanoic acid, 8, the acid group of 4 was initially protected as the ethyl ester because difficulties were

Scheme I



Scheme II



encountered in recrystallization of the product from the Sandmeyer reaction when the acid was not protected. The nitro group and double bond were then reduced by hydrogen with 5% palladium on carbon as catalyst to produce ethyl 3-(3-aminophenyl)propanoate, 6. To convert the amino moiety to an iodide, a modification of the

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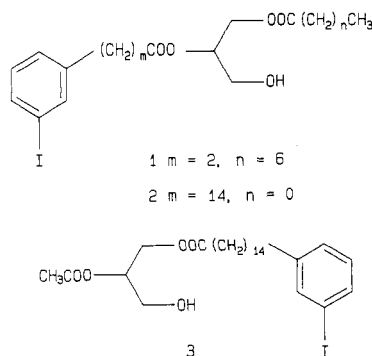
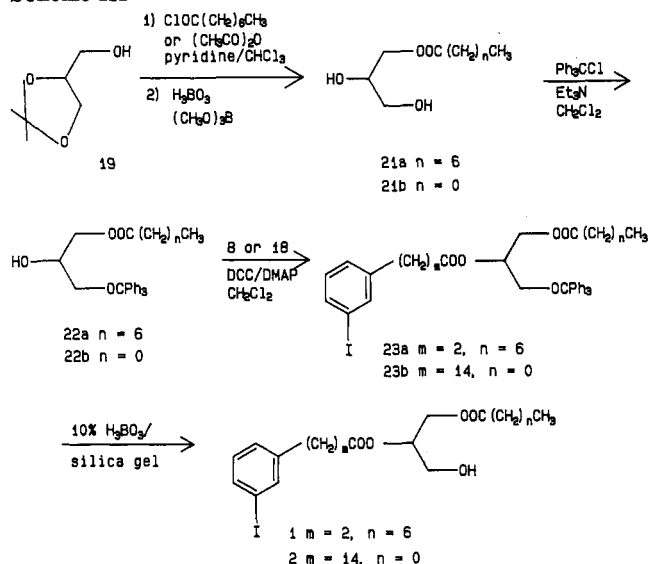


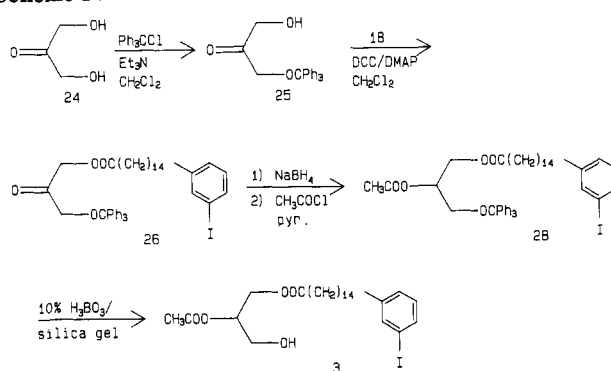
Figure 1. (Iodoaryl)diacylglycerol analogues.

## Scheme III



Sandmeyer reaction was used.<sup>12</sup> The product was easily purified by chromatography. Hydrolysis of the ethyl ester with potassium hydroxide in ethanol and recrystallization afforded the iodophenyl acid, 8. 15-(3-Iodophenyl)pentadecanoic acid, 18, was synthesized by utilizing a Wittig reaction to obtain the desired chain length (Schemes I and II). The olefinic bond of 4 was reduced to prevent subsequent reaction with diborane. Diimine was utilized for this purpose because it does not affect either nitro or acid groups.<sup>13</sup> Reduction of the acid with diborane afforded alcohol 10, which was converted to 3-(3-nitrophenyl)-1-bromopropane (11) by treatment with phosphorus tribromide. Treatment of 11 with triphenylphosphine in acetonitrile furnished the Wittig salt, 12. The aldehyde 14 used for the Wittig reaction was synthesized by pyridinium chlorochromate oxidation of methyl 12-hydroxydodecanoate, 13. Alcohol 13 was formed by Baeyer-Villiger oxidation of cyclododecanone followed by ring opening in the presence of methanol. The Wittig reaction was carried out between aldehyde 14 and salt 12 in methylene chloride by utilizing potassium *tert*-butoxide as base. The double bond and nitro group of product 15 were catalytically reduced with 5% palladium on charcoal in absolute ethanol. The amine was then converted to the iodide by the modified Sandmeyer reaction. The methyl ester 17 was easily hydrolyzed with potassium hydroxide in methanol to afford 15-(3-iodophenyl)pentadecanoic acid (18).

## Scheme IV



Diacylglycerols 1 and 2 were synthesized by the procedure outlined in Scheme III. Isopropylidene glycerol (19) was acylated with octanoyl chloride or acetic anhydride. The 1-acyl-2,3-*O*-isopropylideneglycerols could not be deprotected under normal acidic conditions because acid catalyzes migration of the acyl group to the secondary hydroxyl.<sup>14</sup> Deprotection was accomplished with the use of boric acid following the procedure of Jensen and Pitas.<sup>15</sup> The borate esters that were formed temporarily protected the products from acyl migration. The borate esters were readily hydrolyzed simply by washing with water, furnishing diols 21a and 21b. The primary hydroxyls of 21a,b were selectively protected by tritylation and the secondary hydroxyls were acylated with the acid of interest by a dicyclohexylcarbodiimide (DCC) coupling reaction to afford 23a and 23b. Detritylation of 23a,b was also carried out with boric acid to prevent acyl migration. In this case, Buchnea's procedure,<sup>16</sup> in which the acid was in a 10% mixture with silica gel, was utilized to simultaneously deprotect and purify the product.

It is desirable to add the iodinated moiety to the glycerol backbone as late in the synthesis as possible in order to minimize consumption of the acids and deiodination of intermediates. Since the removal of the isopropylidene group furnished low yields of diol, an alternate approach was sought for the synthesis of 3. The synthetic pathway shown in Scheme IV was developed to increase the yields of the diacylglycerol analogues with the iodinated moiety in the 1-position. One equivalent of trityl chloride was slowly added to 1,3-dihydroxy-2-propanone, 24, producing a mixture of products. The hydroxy ketone 25 could be isolated in sufficient quantities to esterify with the iodoaryl acid 18 to form 26. Reduction of 26 with sodium borohydride followed by acetylation and detritylation afforded the diacylglycerol 3. Although the acetyl group was particularly prone to migration to the 3-position, the 1,2- and 1,3-isomers could be separated by HPLC.

## Biological Results and Discussion

Diacylglycerol analogues were assayed for their ability to compete with [<sup>3</sup>H]phorbol dibutyrate ([<sup>3</sup>H]PDBu) for binding to PK-C in a crude rat brain preparation by a modification of the procedure of Sharkey and Blumberg.<sup>17</sup> All three compounds were similar to OAG and each other in their abilities to compete with [<sup>3</sup>H]PDBu at concentrations of 1–100  $\mu$ M (Figure 2). This indicates that the length of the acyl chains and the position of the longer

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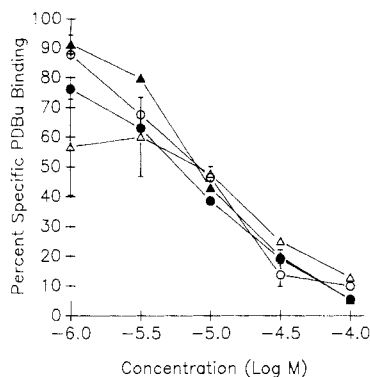
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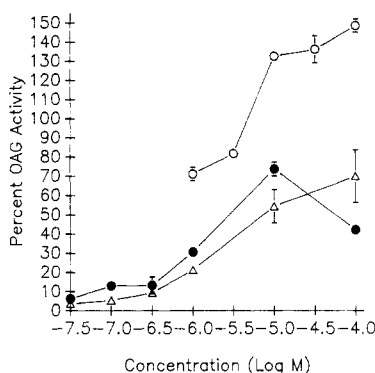
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**Figure 2.** Competitive binding analysis using [ $^3\text{H}$ ]PDBu (20 nM) and a crude rat brain membrane preparation: compound 1 (○), 2 (●), 3 (Δ), OAG (▲).



**Figure 3.** Activation of protein kinase C by diacylglycerol analogues. The amount of  $^{32}\text{P}$  transferred from [ $^{32}\text{P}$ ]ATP to histone III $\alpha$  was determined in the presence 1  $\mu\text{g}/\text{mL}$  PS and increasing concentrations of diacylglycerols: compound 1 (○), 2 (●), 3 (Δ).

chain does not influence the binding of these diacylglycerol analogues to PK-C in the presence of 100  $\mu\text{g}/\text{mL}$  PS. Similarly, a variety of triiodoaryl-substituted diacylglycerol analogues, some with a chiral center in the acyl group, demonstrated little structural specificity for the acyl group.<sup>9</sup> This is in agreement with the models for the diacylglycerol/PK-C/ $\text{Ca}^{2+}$ /PS complex that have been proposed by others.<sup>18,19</sup> In these models, the side chains are projected into the lipid bilayer so that their binding is nonspecific. The activation of PK-C by the (iodoaryl)diacylglycerol analogues was determined by measuring the transfer of [ $^{32}\text{P}$ ]ATP to histone according to the procedure of Leach and Blumberg.<sup>20</sup> In order to make comparisons between experiments carried out with different enzyme preparations, the data are presented as the percent of activation of the standard OAG (10  $\mu\text{M}$ ). Compound 1 was most comparable to OAG in its ability to activate PK-C at concentrations of 1–100  $\mu\text{M}$  (Figure 3). For compounds 2 and 3, concentrations below 0.3  $\mu\text{M}$  were ineffective, but from 1–10  $\mu\text{M}$  they showed dose-dependent activation of PK-C. The decrease in activation by 2 at 100  $\mu\text{M}$  may be due to the inability of 1  $\mu\text{g}/\text{mL}$  PS to fully solubilize the diacylglycerol. Difficulties in solubilization were previously encountered with other (iodoaryl)diacylglycerol analogues.<sup>9</sup>

The fact that compound 1 with two medium chains is more active than compounds 2 and 3 agrees with known structure–activity relationships in that diC<sub>8</sub> is more active

than OAG.<sup>11</sup> Differences in specificities for the acyl groups may have been found in the PK-C activation assay but not the competitive binding assay because of differences in experimental conditions. The binding assay contained 100-fold more PS than the activation assay. Previously, results of PDBu competition studies demonstrated that the  $K_i$  for diolein decreases with decreasing concentrations of PS.<sup>21</sup> Apparently, the concentration of diacylglycerol in the lipid environment, not the total concentration, determines the  $K_i$ . Bell and co-workers further investigated this with an assay system that utilized mixed micelles to regulate the mole fraction of PS and diacylglycerol.<sup>22</sup> They showed that PK-C activation was dependent on mole fraction of PS and diolein but not total concentration.

For compounds 1–3, 100  $\mu\text{g}/\text{mL}$  PS was apparently sufficient for maximal competition with PDBu. In the kinase assay, however, the PS concentration was 1  $\mu\text{g}/\text{mL}$ , and compounds 2 and 3 were less effective than compound 1 at activating PK-C. Compound 1 may be more soluble in the PS liposomes and thus more active than compounds 2 and 3. This variability in activity profiles between the analogues occurred only at low concentrations of PS. The dependency of the analogues on the lipid environment could be further investigated by the mixed micelle assay, where the relative concentration of the compounds in the phospholipid could be more strictly controlled.

Another possible explanation for the divergence in activation activities between the compounds is that they activate different isoforms of PK-C. Cloning studies have demonstrated that PK-C is a family of closely related genes which encode at least six different isoforms (for review, see ref 23). The isoforms are highly homologous, but some differences in the biochemical and immunological properties have been demonstrated.<sup>24–26</sup> For example, the sensitivity to calcium, as well as to activation by arachidonic acid, differs among the isoforms.<sup>27,28</sup> Ido et al.<sup>29</sup> showed that the EGF receptor is phosphorylated most rapidly by type III PK-C in vitro. Taken together, these results suggest that the different PK-C species may have different cofactor and substrate specificities. Both the binding and PK-C activation assays with compounds 1–3 were conducted with rat brain preparations that contained a mixture of PK-C isoforms. If they do activate individual PK-C isoforms, the analogues will be useful reagents to probe the biological function of the various PK-C isoforms.

In conclusion, iodoaryl analogues of diC<sub>8</sub> and OAG have been synthesized and shown to bind to and activate PK-C. Radioiodination of these compounds may provide very useful tools for further studies of PK-C, particularly if they, like diC<sub>8</sub> and OAG, can enter intact cells and if they interact differentially with PK-C isoforms.

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## Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected.  $^1\text{H}$  NMR spectra were obtained with a Varian EM 360A (60 MHz) or Bruker WP270SY (270 MHz) NMR in  $\text{CDCl}_3$  with TMS as internal standard. IR spectra were taken with either a Perkin-Elmer 281 or Nicolet 5DXB FT spectrometer. Elemental analyses were performed by Midwest Microlabs, Indianapolis, IN, and are within  $\pm 0.4\%$  of the calculated values. Thin-layer chromatography was carried out on either Merck silica gel-60 F<sub>254</sub> polyethylene-backed plates or Analtech silica gel GHLF glass-backed plates. Visualization was by UV and charring with 50% aqueous sulfuric acid or 5% methanolic sulfuric acid. Column chromatography was performed on Davisil 62 silica gel from Grace, Davison Chemical, Baltimore, MD. High-performance liquid chromatography was carried out either with an Altex 110A pump and Gilson 111 UV monitor or with an Isco dual-pump system with Model 2350 pumps and V<sup>4</sup> monitor (254 nm). Both systems were used with either a Whatman Partisil 10 Magnum 20 or a IBM silica gel (5  $\mu\text{m}$ , 10  $\times$  250 mm) column. All organic chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI. Unless specifically stated that water was used as a cosolvent, all solvents were dried before use by using standard methods. [ $^3\text{H}$ ]Phorbol dibutyrate and [ $^{32}\text{P}$ ]ATP were purchased from Du Pont-New England Nuclear, Boston, MA. Phosphatidylserine, bovine  $\gamma$ -globulin, phorbol dibutyrate, OAG, and histone were purchased from Sigma Chemical Co., St. Louis, MO.

**Ethyl 3-Nitrocinnamate (5).** 3-Nitrocinnamic acid (4, 10.00 g, 51.77 mmol), ethanol (50 mL), and concentrated  $\text{H}_2\text{SO}_4$  (10 drops) were combined in a 100-mL flask and heated to reflux overnight. The solvent was removed and the solid was redissolved in ether which was washed with saturated  $\text{NaHCO}_3$  (2 $\times$ ) and brine. The organic phase was dried ( $\text{MgSO}_4$ ) and the solvent was evaporated. The resulting solid was recrystallized from ethanol, producing 8.44 g of the ester 5 (74%): mp 71–72  $^\circ\text{C}$  (lit.<sup>30</sup> mp 78–79  $^\circ\text{C}$ ).

**Ethyl 3-(3-Aminophenyl)propanoate (6).** Ethyl 3-nitrocinnamate (5, 7.93 g, 35.85 mmol) and ethanol (150 mL) were placed in a flask for the Parr hydrogenator. The catalyst, 5% Pd/C, was added, and hydrogenation was carried out for 4 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness to yield 5.62 g of 6 (81%). NMR data (60 MHz) were as expected.

**Ethyl 3-(3-Iodophenyl)propanoate (7).** Ethyl 3-(3-aminophenyl)propanoate (6, 2.04 g, 10.56 mmol) was dissolved in acetic acid (5.2 mL), and HCl (2.1 mL) was added. Solutions of  $\text{NaNO}_2$  (0.77 g, 11.18 mmol) in water (3.7 mL) and KI (2.10 g, 12.65 mmol) and  $\text{I}_2$  (1.57 g, 6.18 mmol) in water (2.1 mL) were prepared and all solutions were cooled in the refrigerator. The flask containing the ester was placed in an ice bath and the  $\text{NaNO}_2$  solution was added slowly, and the temperature was maintained at 10  $^\circ\text{C}$ . The mixture was returned to the refrigerator for 45 mins and then put back in an ice bath for the addition of the iodide solution. The flask was equipped with a stir bar, septum, and gas outlet. The mixture was stirred below 10  $^\circ\text{C}$  for 2.5 h and then at room temperature overnight. It was diluted with water and washed with ether (2 $\times$ ). The ether layer was washed with 10%  $\text{NaHSO}_3$  (2 $\times$ ) and with brine, dried ( $\text{MgSO}_4$ ), and evaporated. Purification by chromatography (125 g of silica gel, hexanes/ethyl acetate 8:1) yielded 1.97 g of 7 (61%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{11}\text{H}_{13}\text{IO}_2$ ) C, H.

**3-(3-Iodophenyl)propanoic Acid (8).** Ethyl 3-(3-iodophenyl)propanoate (7, 1.85 g, 6.08 mmol), KOH (0.51 g, 9.12 mmol), and 95% ethanol (30 mL) were combined and heated to reflux overnight. The solvent was evaporated and the remaining solid was redissolved in ether and 0.5 N HCl. After separation of the layers, the aqueous phase was washed with ether, and the combined ether layers were washed with 0.5 N HCl and brine. The ether solution was dried ( $\text{MgSO}_4$ ) and the solvent was removed. Recrystallization of the residue from benzene/hexane afforded 1.36 g of the acid 8 (81%): mp 64–65  $^\circ\text{C}$ ; IR ( $\text{CHCl}_3$ ) 2790–3440 (OH, acid), 3025 (CH, arom), 2920 (CH, aliph), 1720

(C=O, acid dimer), 1592, 1563 (C=C, arom), 1285 (CO)  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.54–7.57 (d, 2 H, arom  $H_2$  and  $H_4$ ), 7.17–7.19 (d, 1 H, arom  $H_6$ ), 7.00–7.06 (t, 1 H, arom  $H_5$ ), 2.87–2.93 (t, 2 H,  $\text{PhCH}_2$ ), 2.64–2.70 (t, 2 H,  $\text{CH}_2\text{COO}$ ). Anal. ( $\text{C}_9\text{H}_9\text{IO}_2$ ) C, H, I.

**3-(3-Nitrophenyl)propanoic Acid (9).** 3-Nitrocinnamic acid (4, 5.00 g, 25.89 mmol) was added to water (200 mL) and cooled to 10  $^\circ\text{C}$ . Hydroxylamine sulfate (6.66 g, 40.58 mmol) and hydroxylamine-O-sulfonic acid (16.06 g, 142.01 mmol) were added, and the pH was adjusted to 6–7 with concentrated NaOH (20 mL). The pH and temperature were kept constant as the solution was stirred for 5.5 h. Additional base (6.9 mL) was required to maintain the pH at 6–7. The reaction mixture was filtered and acidified to pH 2 with 2 N  $\text{H}_2\text{SO}_4$ . The filtrate was refrigerated overnight and the product was collected by filtration. Recrystallization from ethanol and water produced off-white crystals (9, 3.57 g, 71%): mp 115–116  $^\circ\text{C}$  (lit.<sup>31</sup> mp 116–117  $^\circ\text{C}$ ). IR and 270-MHz NMR data were as expected.

**3-(3-Nitrophenyl)-1-propanol (10).** 3-(3-Nitrophenyl)propanoic acid (9, 1.59 g, 8.15 mmol) was dissolved in THF (4 mL) in a flame-dried, two-neck flask equipped with a condenser. The reaction mixture, under an atmosphere of nitrogen, was cooled with an ice bath. A 1 N solution of  $\text{BH}_3$  in THF (11 mL, 11 mmol) was added slowly and the mixture was stirred at room temperature for 2 h. Ice water was added slowly, then the mixture was washed with water (2 $\times$ ), saturated  $\text{NaHCO}_3$ , and brine. The ether layer was dried ( $\text{MgSO}_4$ ) and evaporated. The oily residue (1.35 g) was purified by chromatography (125 g of silica gel, hexanes/ethyl acetate/chloroform 6:2:1) to afford 1.06 g of pure alcohol 10 (72%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_9\text{H}_{11}\text{NO}_3$ ) C, H, N.

**3-(3-Nitrophenyl)-1-bromopropane (11).** The alcohol (10, 1.09 g, 6.02 mmol) and benzene (3.4 mL) were placed in a flame-dried, two-neck flask and cooled with an ice bath under a nitrogen atmosphere. Pyridine (5 drops) and phosphorus tribromide (0.33 mL, 3.56 mmol) were added slowly. The mixture was stirred at 0  $^\circ\text{C}$  for 1 h and then was allowed to warm to room temperature. It was heated to 65  $^\circ\text{C}$  for 5.5 h. The reaction mixture was then cooled by an ice bath and diluted with ether, and ice water was added slowly. The phases were separated, and the ether layer was washed (2 $\times$ ) with water, dried ( $\text{MgSO}_4$ ), and evaporated. The resulting orange oil (1.07 g) was purified by chromatography (100 g of silica gel, hexanes/ethyl acetate 7:1), affording 0.86 g of the desired product 11 (58%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_9\text{H}_9\text{BrNO}_2$ ) C, H.

**[3-(3-Nitrophenyl)propyl]triphenylphosphonium Bromide (12).** 3-(3-Nitrophenyl)-1-bromopropane (11, 5.87 g, 24.05 mmol) was combined in a flame-dried flask with triphenylphosphine (6.54 g, 24.93 mmol) and acetonitrile (70 mL). The mixture was refluxed for 44 h and then cooled to room temperature. The solvent was evaporated. A small amount of anhydrous ether was added to the residue and the mixture was cooled with an ice bath until the product solidified. The Wittig salt 12 (8.08 g, 66%) was isolated by filtration and utilized without further purification: mp 244–246  $^\circ\text{C}$ . IR and 270-MHz NMR data were as expected.

**Methyl 12-Hydroxydodecanoate (13).** Concentrated  $\text{H}_2\text{SO}_4$  (43.6 mL) was added slowly to water (14.8 mL) in a round-bottom flask immersed in ice. When it had cooled down below 15  $^\circ\text{C}$ ,  $\text{K}_2\text{S}_2\text{O}_8$  (30.76 g, 113.79 mmol) was added slowly, followed by methanol (62 mL). When the temperature had increased to 15  $^\circ\text{C}$ , cyclododecanone (10.00 g, 54.85 mmol) in methanol (40 mL) was added over 1 h and the temperature was maintained at 15  $^\circ\text{C}$ . It was stirred at room temperature for 45 h. The reaction mixture was saturated with  $\text{Na}_2\text{SO}_4$  and diluted with ethyl acetate. The phases were separated, and the organic phase was dried ( $\text{MgSO}_4$ ) and evaporated. The residue still weighed much more than theoretical yield, so it was redissolved in ether/hexanes 11:3, the phases were separated, and the organic phase was dried ( $\text{MgSO}_4$ ) and evaporated. Since the TLC (hexanes/ethyl acetate 3:1) showed the presence of lactone, the residue was heated to reflux in  $\text{CH}_3\text{OH}$  with  $\text{H}_2\text{SO}_4$  (3 drops) for 6 h. The reaction mixture was neutralized with  $\text{NH}_4\text{OH}$  and filtered and the solvent

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(31) Walter, M.; Besendorf, H.; Schnider, O. *Helv. Chim. Acta* 1961, 44, 1546.

was removed. Ether was added, and the mixture was dried ( $\text{MgSO}_4$ ) and evaporated. The crude product was divided into two portions for chromatography (150 g of silica gel each, hexanes/ethyl acetate 5:1), and pure fractions were combined to give 7.79 g of 13 (62%): mp 32–34 °C (lit.<sup>32</sup> mp 33 °C). NMR data (270 MHz) were as expected.

**Methyl 12-Oxododecanoate (14).** Pyridinium chlorochromate (4.31 g, 20.00 mmol) was stirred under nitrogen with  $\text{CH}_2\text{Cl}_2$  (27 mL) in a flame-dried flask. Methyl 12-hydroxydodecanoate (13, 3.07 g, 13.33 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was added. The resulting black solution was stirred at room temperature for 2 h, diluted with ether, and decanted. The residual black tar was washed with ether (4×), and the combined organic washings were filtered through Florisil covered with a pad of silica gel. The solvent was removed from the filtrate to give crude product (2.40 g). Purification by chromatography (140 g of silica gel, hexanes/ethyl acetate 12:1) afforded pure aldehyde<sup>33</sup> (14, 2.07 g, 68%). IR and 270-MHz NMR data were as expected.

**Methyl 15-(3-Nitrophenyl)pentadec-12-enoate (15).** Potassium *tert*-butoxide (1.14 g, 10.12 mmol) was placed in a flame-dried flask and  $\text{CH}_2\text{Cl}_2$  (16.5 mL) was added. The contents were cooled to –60 °C with an acetone/dry ice bath. The Wittig salt (12, 5.12 g, 10.12 mmol) was added with a dry addition funnel. After all the salt had been added, the mixture was stirred for 5 min. The aldehyde (14, 2.31 g, 10.12 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (5.5 mL) and added slowly. The purple solution was allowed to warm to room temperature and then stirred for 3 h. The solvent was evaporated and the residue was dissolved in ethyl acetate. The resulting solution was washed with water and saturated aqueous  $\text{NH}_4\text{Cl}$  (10 mL each) and dried ( $\text{MgSO}_4$ ) and the solvent was removed in vacuo to give a crude product (6.05 g). Chromatography (160 g of silica gel, hexanes/ethyl acetate 15:1) afforded 1.82 g of pure 15 (48%): IR ( $\text{CHCl}_3$ ) 2920, 2845 (C–H, aliph), 1728 (C=O, ester), 1525, 1352 ( $\text{NO}_2$ ), 1205, 1039 (CO)  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  8.04–8.06 (m, 2 H, arom  $H_2$  and  $H_4$ ), 7.41–7.53 (m, 2 H, arom  $H_5$  and  $H_6$ ), 5.30–5.49 (m, 2 H,  $\text{HC}=\text{CH}$ ), 3.67 (s, 3 H,  $\text{COOCH}_3$ ), 2.75–2.80 (t, 2 H,  $\text{PhCH}_2$ ), 2.36–2.43 (q, 2 H,  $\text{PhCH}_2\text{CH}_2\text{CH}=\text{C}$ ), 2.27–2.32 (t, 2 H,  $\text{CH}_2\text{COO}$ ), 1.92–1.94 (q, 2 H,  $\text{C}=\text{CHCH}_2$ ), 1.57–1.64 (quint, 2 H,  $\text{CH}_2\text{CH}_2\text{COO}$ ), 1.22–1.27 (s, 14 H,  $(\text{CH}_2)_7$ ). Anal. ( $\text{C}_{22}\text{H}_{33}\text{NO}_4$ ) C, H, N.

**Methyl 15-(3-Aminophenyl)pentadecanoate (16).** Methyl 15-(3-nitrophenyl)pentadec-12-enoate (15, 1.03 g, 2.74 mmol) was hydrogenated as described for compound 6 but with ethyl acetate (50 mL) as solvent. Recrystallization of the residue from petroleum ether afforded 0.86 g of 16 (91%): mp 50–53 °C. IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{22}\text{H}_{37}\text{NO}_2$ ) C, H, N.

**Methyl 15-(3-Iodophenyl)pentadecanoate (17).** The iodination was carried out as described for compound 7. Amine 16 (0.77 g, 2.22 mmol) was dissolved in glacial acetic acid (1.10 mL) and concentrated HCl (0.44 mL). Solutions of  $\text{NaNO}_2$  (0.16 g, 2.35 mmol) in water (0.77 mL), and KI (0.44 g, 2.66 mmol) and iodine (0.33 g, 1.30 mmol) in water (0.45 mL) were used. The crude product (0.81 g) was purified by chromatography (80 g of silica gel, hexanes/ethyl acetate 13:1) producing 0.35 g of pure 17 (34%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{22}\text{H}_{35}\text{IO}_2$ ) C, H, I.

**15-(3-Iodophenyl)pentadecanoic Acid (18).** The methyl ester (17, 0.30 g, 0.66 mmol) was hydrolyzed with KOH (0.04 g, 0.72 mmol) in methanol (2.5 mL). Recrystallization of the residue from petroleum ether afforded 0.18 g of pure acid 18 (61%): mp 62–64 °C; IR ( $\text{CHCl}_3$ ) 3500 (OH), 2921, 2852 (CH aliph), 1710 (C=O, acid dimer), 1590, 1563 (C=C, arom), 1465 ( $\text{CH}_2$ )  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.49–7.54 (m, 2 H, arom  $H_2$  and  $H_4$ ), 7.12–7.14 (d, 1 H, arom  $H_6$ ), 6.97–7.03 (t, 1 H, arom  $H_5$ ), 2.50–2.56 (t, 2 H,  $\text{PhCH}_2$ ), 2.32–2.38 (t, 2 H,  $\text{CH}_2\text{COOH}$ ), 1.55–1.66 (m, 4 H,  $\text{PhCH}_2\text{CH}_2$  and  $\text{CH}_2\text{CH}_2\text{COOH}$ ), 1.25–1.30 (s, 20 H,  $(\text{CH}_2)_{10}$ ). Anal. ( $\text{C}_{21}\text{H}_{33}\text{IO}_2$ ) C, H, I.

**1-Octanoyl-2,3-O-isopropylidene-*rac*-glycerol (20a).** 2,3-O-Isopropylidene-*rac*-glycerol (19, 10.95 g, 82.84 mmol) was placed

in a flame-dried flask with  $\text{CH}_2\text{Cl}_2$  (225 mL). Pyridine (10.54 mL, 131.57 mmol) was added, followed by octanoyl chloride (18.42 g, 113.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (75 mL). The reaction mixture was stirred overnight and poured into ice water, and the phases were separated. The organic phase was extracted with 1 N HCl (2×) and brine and dried ( $\text{MgSO}_4$ ). Removal of the solvent gave a crude product (29.14 g) that was sufficiently pure for use without further purification. An analytical sample (0.63 g) was obtained by chromatography (155 g of silica gel, hexanes/ethyl acetate 9:1). IR and 270-MHz NMR were as expected. Anal. ( $\text{C}_{14}\text{H}_{26}\text{O}_4$ ) C, H.

**1-Octanoyl-*rac*-glycerol (21a).** 1-Octanoyl-2,3-O-isopropylidene-*rac*-glycerol (20a, 26.04 g, 100.8 mmol), boric acid (31.19 g, 504.45 mmol), and trimethyl borate (185 mL) were combined. The reaction mixture was heated to 90 °C for 10 min whereupon the reflux condenser was exchanged for a distillation condenser. The system was put under vacuum to remove the solvent and was then heated at 90 °C for 15 min. The residue was dissolved in ether and water. The organic phase was washed with water (3×) and dried ( $\text{MgSO}_4$ ). The solid obtained upon removal of the solvent was recrystallized from petroleum ether/ether 3:1, producing 11.33 g of white crystalline 21a (63% from isopropylidene glycerol): mp 35.5–37.5 °C (lit.<sup>34</sup> mp 37 °C). IR and 270-MHz NMR were as expected.

**1-Octanoyl-3-O-trityl-*rac*-glycerol (22a).** 1-Octanoyl-*rac*-glycerol (21a, 10.00 g, 45.81 mmol) and a catalytic amount of DMAP were placed in a flame-dried flask, followed by triethylamine (13.0 mL, 93.27 mmol) and  $\text{CH}_2\text{Cl}_2$  (200 mL). When the solid had dissolved, trityl chloride (14.07 g, 50.47 mmol) was added and the mixture was stirred at room temperature for 2 days. The reaction mixture was poured into ice water, and the phases were separated. The organic layer was washed with 1 N HCl (3×) and water (2×) and dried ( $\text{MgSO}_4$ ). Removal of the solvent and purification of the residue by chromatography (done in 2 portions, 150 g of silica gel each, hexanes/ethyl acetate 8:1) furnished 9.96 g of 22a (47%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{30}\text{H}_{36}\text{O}_4$ ) C, H.

**1-Octanoyl-2-[3-(3-iodophenyl)propanoyl]-3-O-trityl-*rac*-glycerol (23a).** 1-Octanoyl-3-O-trityl-*rac*-glycerol (22a, 414 mg, 0.90 mmol), 3-(3-iodophenyl)propanoic acid (8, 250 mg, 0.91 mmol), and a catalytic amount of DMAP were dissolved in  $\text{CH}_2\text{Cl}_2$  (8.3 mL). DCC (206 mg, 1.00 mmol) was added and the mixture was stirred overnight. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , washed with 1 N HCl (2×), saturated  $\text{NaHCO}_3$ , water, and brine, and dried ( $\text{MgSO}_4$ ). Removal of the solvent and purification of the residue by chromatography (12 g of silica gel, hexanes/ethyl acetate 8:1) yielded 473.5 mg of pure 23a (73%): IR data were as expected; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.51–7.56 (m, 2 H, arom  $H_2$  and  $H_4$  on iodophenyl), 7.39–7.43 (m, 6 H, arom  $H_2$  and  $H_6$  on trityl), 7.21–7.33 (m, 9 H, arom  $H_3$ – $H_5$  on trityl), 7.14–7.16 (d, 1 H, arom  $H_6$  on iodophenyl), 6.94–7.00 (t, 1 H, arom  $H_5$  on iodophenyl), 5.22–5.26 (quint, 1 H,  $\text{COOCH}$ ), 4.31–4.37 (dd, 1 H,  $J_{\text{gem}} = 11.9$  Hz,  $J_{\text{vic}} = 3.7$  Hz) and 4.18–4.25 (dd, 1 H,  $J_{\text{gem}} = 11.9$  Hz,  $J_{\text{vic}} = 6.5$  Hz) ( $\text{CH}_2\text{OOC}$ ), 3.21–3.24 (m, 2 H,  $\text{CH}_2\text{OC}$ ), 2.86–2.92 (t, 2 H,  $\text{PhCH}_2$ ), 2.59–2.66 (t, 2 H,  $\text{PhCH}_2\text{CH}_2\text{COO}$ ), 2.18–2.23 (t, 2 H,  $\text{OOCCH}_2$ ), 1.51–1.56 (m, 2 H,  $\text{OOCCH}_2\text{CH}_2$ ), 1.26–1.29 (s, 8 H,  $(\text{CH}_2)_4$ ), 0.85–0.90 (t, 3 H,  $\text{CH}_3$ ). Anal. ( $\text{C}_{39}\text{H}_{43}\text{IO}_5$ ) C, H, I.

**1-Octanoyl-2-[3-(3-iodophenyl)propanoyl]-*rac*-glycerol (1).** 1-Octanoyl-2-[3-(3-iodophenyl)propanoyl]-3-O-trityl-*rac*-glycerol (23a, 373.6 mg, 0.52 mmol) was adsorbed to 10% boric acid/silica gel (400 mg) and placed on a column of 10% boric acid/silica gel (4 g). The column was eluted with petroleum ether (600 mL), petroleum ether/ether 95:5 (500 mL), and petroleum ether/ether 3:1 (200 mL). The fractions containing borate ester were combined, and the solvent was evaporated. The borate ester was dissolved in  $\text{CHCl}_3$ , extracted with water (4×), and dried ( $\text{MgSO}_4$ ), and the solvent was removed to give 218.1 mg of pure diacylglycerol 1 (88%): IR ( $\text{CHCl}_3$ ) 3010 (CH, arom), 2959, 2926, 2860 (CH, aliph), 1738 (C=O, ester), 1596, 1560 (C=C, arom), 1460 ( $\text{CH}_2$ ), 1375 ( $\text{CH}_3$ ), 1210, 1160 (CO)  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.54–7.57 (d, 2 H, arom  $H_2$  and  $H_4$ ), 7.16–7.19 (d, 1 H, arom  $H_6$ ),

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7.00–7.05 (t, 1 H, arom  $H_5$ ), 5.05–5.09 (quint, 1 H, COOCH), 4.27–4.33 (dd, 1 H,  $J_{\text{gem}} = 12.0$  Hz,  $J_{\text{vic}} = 4.4$  Hz) and 4.17–4.24 (dd, 1 H,  $J_{\text{gem}} = 12.0$  Hz,  $J_{\text{vic}} = 5.6$  Hz) ( $\text{CH}_2\text{OOC}$ ), 3.67–3.71 (t, 2 H,  $\text{CH}_2\text{OH}$ ,  $J = 7.5$  Hz), 2.88–2.94 (t, 2 H,  $\text{PhCH}_2$ ,  $J = 7.6$  Hz), 2.64–2.69 (t, 2 H,  $\text{PhCH}_2\text{CH}_2\text{COO}$ ), 2.28–2.34 (t, 2 H,  $\text{OOCCH}_2$ ), 1.91–1.96 (t, 1 H, OH,  $J = 6.5$  Hz), 1.59–1.63 (m, 2 H,  $\text{OOCCH}_2\text{CH}_2$ ), 1.28 (s, 8 H,  $(\text{CH}_2)_4$ ), 0.85–0.90 (t, 3 H,  $\text{CH}_3$ ). Anal. ( $\text{C}_{20}\text{H}_{29}\text{IO}_5$ ) C, H, I.

**1-Acetyl-2,3-O-isopropylidene-*rac*-glycerol (20b).** A 500-mL round bottom flask was flame dried and 2,3-O-isopropylidene-*rac*-glycerol (**19**, 1.4 mL, 11.35 mmol), acetic anhydride (27 mL, 286.87 mmol), triethylamine (15.8 mL, 113.25 mmol), and  $\text{CHCl}_3$  (300 mL) were added. The mixture was heated at reflux for 4 h. The solvent was removed under reduced pressure with heating; the residue was dissolved in  $\text{CHCl}_3$ , washed with water (2 $\times$ ), and dried ( $\text{MgSO}_4$ ). Since there was still some acetic anhydride present after removal of the solvent, it was dissolved in hexanes, washed with 2%  $\text{K}_2\text{CO}_3$  (2 $\times$ ) and water, and dried ( $\text{MgSO}_4$ ). Removal of the solvent afforded **20b**<sup>35</sup> (0.61 g, 31%), which was deprotected without further purification.

**1-Acetyl-*rac*-glycerol (21b).** The deprotection was carried out as for compound **21a** with glycerol **20b** (7.26 g, 41.68 mmol), boric acid (12.89 g, 208.52 mmol), and trimethyl borate (80 mL). The residue was taken up in water, stirred, and evaporated. This was repeated and then the solid was dissolved in ether, dried ( $\text{MgSO}_4$ ), and filtered to remove the boric acid and  $\text{MgSO}_4$ . Evaporation of the ether left pure **21b**<sup>30</sup> (2.93 g, 52%). IR and 270-MHz NMR data were as expected.

**1-Acetyl-3-O-trityl-*rac*-glycerol (22b).** The diol (**21b**, 2.69 g, 20.06 mmol), trityl chloride (6.16 g, 22.10 mmol), triethylamine (5.7 mL, 40.73 mmol), and a catalytic amount of DMAP in  $\text{CH}_2\text{Cl}_2$  (115 mL) were stirred under anhydrous conditions overnight. Workup was carried out as described for **22a**. Purification by chromatography (150 g silica gel, hexanes/ethyl acetate 5:1) produced 5.52 g of **22b** (73%), which was not recrystallized in order to avoid migration of the acetate group to the 2-position: mp 80–83 °C. An analytical sample was obtained by careful recrystallization from petroleum ether: mp 89–91 °C. IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{24}\text{H}_{24}\text{O}_4$ ) C, H.

**1-Acetyl-2-[15-(3-iodophenyl)pentadecanoyl]-3-O-trityl-*rac*-glycerol (23b).** 15-(3-Iodophenyl)pentadecanoic acid (18, 400.0 mg, 0.90 mmol), 1-acetyl-3-O-trityl-*rac*-glycerol (**22b**, 388.8 mg, 0.90 mmol), a catalytic amount of DMAP, and  $\text{CH}_2\text{Cl}_2$  (12 mL) were combined in a flame-dried flask. DCC (195.4 mg, 0.95 mmol) was added and the mixture was stirred overnight. Workup was carried out as described for **23a**. Chromatography of the residue (65 g of silica gel, hexanes/ethyl acetate 5:1) yielded 478.3 mg of pure **23b** (66%). IR data were as expected. 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.51–7.54 (m, 2 H, arom  $H_2$  and  $H_4$  on iodophenyl), 7.39–7.49 (m, 6 H, arom  $H_2$  and  $H_6$  on trityl), 7.20–7.33 (m, 9 H, arom  $H_3$ – $H_5$  on trityl), 7.11–7.14 (d, 1 H, arom  $H_6$  on iodophenyl), 6.96–7.02 (t, 1 H, arom  $H_5$  on iodophenyl), 5.23–5.26 (quint, 1 H, CHOO), 4.30–4.34 (dd, 1 H,  $J_{\text{vic}} = 3.8$  Hz) and 4.24–4.26 (dd, 1 H,  $J_{\text{vic}} = 6.6$  Hz) ( $\text{CH}_2\text{OOC}$ ), 3.22–3.25 (m, 2 H,  $\text{CH}_2\text{OC}$ ), 2.50–2.56 (t, 2 H,  $\text{PhCH}_2$ ), 2.31–2.37 (t, 2 H,  $\text{CH}_2\text{COO}$ ), 1.98 (s, 3 H,  $\text{OOCCH}_3$ ), 1.58–1.66 (m, 4 H,  $\text{PhCH}_2\text{CH}_2$  and  $\text{CH}_2\text{CH}_2\text{COO}$ ), 1.24 (s, 20 H,  $(\text{CH}_2)_{10}$ ). Anal. ( $\text{C}_{45}\text{H}_{55}\text{IO}_5$ ) C, H, I.

**1-Acetyl-2-[15-(3-iodophenyl)pentadecanoyl]-*rac*-glycerol (2).** The protected compound (**23b**, 428.6 mg, 0.52 mmol) was adsorbed to silica gel (0.5 g), placed on a 10% boric acid/silica gel (4.5 g) column and eluted with petroleum ether (600 mL), petroleum ether/ether 95:5 (125 mL), and petroleum ether/ether 3:1 (250 mL) as described for compound **23a**. Removal of the solvent and hydrolysis of the borate ester produced 243.6 mg of pure diacylglycerol **2** (84%): IR ( $\text{CHCl}_3$ ) 3000 (CH, arom), 2912, 2847 (CH, aliph), 1738 (CO, ester), 1590, 1563 (C=C, arom), 1468 ( $\text{CH}_2$ ), 1370 ( $\text{CH}_3$ ), 1235 (CO)  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.49–7.54 (m, 2 H, arom  $H_2$  and  $H_4$ ), 7.12 (d, 1 H, arom  $H_6$ ), 7.00–7.03 (t, 1 H, arom  $H_5$ ), 5.09 (quint, 1 H, CHOO), 4.29–4.30 (dd, 1 H,  $J_{\text{vic}} = 4.5$  Hz) and 4.24–4.26 (dd, 1 H,  $J_{\text{vic}} = 5.8$  Hz) ( $\text{CH}_2\text{COO}$ ), 3.72–3.76 (t, 2 H,  $\text{CH}_2\text{OH}$ ), 2.51–2.56 (t, 2 H,  $\text{PhCH}_2$ ), 2.32–2.38 (t, 2 H,  $\text{CH}_2\text{COO}$ ), 2.08 (s, 3 H,  $\text{OOCCH}_3$ ), 2.0 (t, 1 H, OH), 1.60–1.63 (m, 4 H,  $\text{CH}_2\text{CH}_2\text{Ph}$  and  $\text{CH}_2\text{CH}_2\text{COO}$ ), 1.26 (s,

20 H,  $(\text{CH}_2)_{10}$ ). Anal. ( $\text{C}_{26}\text{H}_{41}\text{IO}_5$ ) C, H, I.

**3-O-Trityl-1,3-dihydroxy-2-propanone (25).** 1,3-Dihydroxy-2-propanone dimer (**24**, 3.00 g, 16.65 mmol) and a catalytic amount of DMAP were placed in a flask.  $\text{CH}_2\text{Cl}_2$  (83 mL) and triethylamine (4.9 mL, 35.0 mmol) were added. Trityl chloride (9.28 g, 33.30 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (35 mL) and added slowly. The mixture was stirred overnight at room temperature and poured into ice water, and the phases were separated. The  $\text{CH}_2\text{Cl}_2$  layer was washed with 1 N HCl (2 $\times$ ) and brine and dried ( $\text{MgSO}_4$ ) and the solvent was removed. The residue was chromatographed in two batches (130 g of silica gel each, hexane/ethyl acetate 10:1) to afford 1.89 g of ketone **25** (17%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{22}\text{H}_{20}\text{O}_3$ ) C, H.

**1-O-[15-(3-Iodophenyl)pentadecanoyl]-3-O-trityl-1,3-dihydroxy-2-propanone (26).** 3-O-Trityl-1,3-dihydroxy-2-propanone (**25**, 0.38 g, 1.14 mmol), 15-(3-iodophenyl)pentadecanoic acid (**18**, 0.51 g, 1.14 mmol) and a catalytic amount of DMAP were dissolved in  $\text{CH}_2\text{Cl}_2$  (10.7 mL). DCC (0.26 g, 1.25 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was worked up as described for compound **23a**. Purification of the residue by chromatography (100 g of silica gel, hexanes/ethyl acetate 12:1) afforded 0.61 g of **26** (70%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{43}\text{H}_{51}\text{IO}_4$ ) C, H, I.

**1-[15-(3-Iodophenyl)pentadecanoyl]-3-O-trityl-*rac*-glycerol (27).** 1-[15-(3-Iodophenyl)pentadecanoyl]-3-O-trityl-1,3-dihydroxy-2-propanone (**26**, 0.52 g, 0.69 mmol) was placed in a round-bottom flask, followed by THF (6 mL), benzene (2 mL), and water (0.4 mL). The mixture was cooled to 5 °C and  $\text{NaBH}_4$  (42.5 mg, 1.12 mmol) was added. It was stirred for 30 min and acetic acid (23  $\mu\text{L}$ ) was added to destroy excess  $\text{NaBH}_4$ . The mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , extracted with saturated aqueous  $\text{NH}_4\text{Cl}$  (2 $\times$ ), water, and brine and dried ( $\text{MgSO}_4$ ). Removal of the solvent afforded an oil, which was chromatographed (50 g of silica gel, hexanes/ethyl acetate 8:1) to give 0.40 g of pure alcohol **27** (77%). IR and 270-MHz NMR data were as expected. Anal. ( $\text{C}_{43}\text{H}_{53}\text{IO}_4$ ) C, H.

**1-[15-(3-Iodophenyl)pentadecanoyl]-2-acetyl-3-O-trityl-*rac*-glycerol (28).** 1-[15-(3-Iodophenyl)pentadecanoyl]-3-O-trityl-*rac*-glycerol (**27**, 0.34 g, 0.45 mmol) was dissolved in ethanol-free  $\text{CHCl}_3$  (2.2 mL) in a two-neck flask. Pyridine (0.06 mL, 0.79 mmol) was added, followed by acetyl chloride (0.09 mL, 1.26 mmol). The reaction mixture was stirred for 7 h and water was added carefully to destroy the excess acid chloride. It was diluted with  $\text{CHCl}_3$ , washed with 1 N HCl (2 $\times$ ) and brine and dried ( $\text{MgSO}_4$ ). Removal of the solvent and chromatography (40 g of silica gel, hexanes/ethyl acetate 8:1) of the residue afforded 0.31 g of pure **28** (86%): IR data were as expected; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.50–7.55 (d, 2 H, arom  $H_2$  and  $H_4$  on iodophenyl), 7.4–7.5 (m, 6 H, arom  $H_2$  and  $H_6$  on trityl), 7.2–7.35 (m, 9 H, arom  $H_3$ – $H_5$  on trityl), 7.11–7.14 (d, 1 H, arom  $H_6$  on iodophenyl), 6.97–7.02 (t, 1 H, arom  $H_5$  on iodophenyl), 5.20–5.28 (quint, 1 H COOCH), 4.33–4.40 (dd, 1 H) and 4.20–4.28 (dd, 1 H) ( $\text{CH}_2\text{OOC}$ ), 3.22–3.25 (dd, 2 H,  $\text{CH}_2\text{OC}$ ), 2.50–2.56 (t, 2 H,  $\text{CH}_2\text{Ph}$ ), 2.21–2.26 (t, 2 H,  $\text{OOCCH}_2$ ), 2.08 (s, 3 H,  $\text{CH}_3\text{COO}$ ), 1.52–1.57 (m, 2 H,  $\text{OOCCH}_2\text{CH}_2$  and  $\text{CH}_2\text{CH}_2\text{Ph}$ ), 1.25 (s, 20 H,  $(\text{CH}_2)_{10}$ ). Anal. ( $\text{C}_{45}\text{H}_{55}\text{IO}_5$ ) C, H, I.

**1-[15-(3-Iodophenyl)pentadecanoyl]-2-acetyl-*rac*-glycerol (3).** The detritylation was carried out as described for compound **1** with **28** (379.7 mg, 0.47 mmol) adsorbed to silica gel (0.4 g) on a column of 10% boric acid/silica gel (3.7 g). The column was eluted with petroleum ether (600 mL), petroleum ether/ether 95:5 (200 mL), and petroleum ether/ether 3:1 (800 mL), affording **3** (195.6 mg, 74%). The 1,3-isomer was detected and removed by HPLC ( $\text{CHCl}_3$ /hexane 9:1; flow rate 2 mL/min; retention time (1,3-isomer) 26 min, (**3**) 33 min: IR ( $\text{CHCl}_3$ ) 3038 (CH, arom), 2932, 2858 (CH, aliph), 1736 (C=O, ester), 1462 ( $\text{CH}_2$ ), 1377 ( $\text{CH}_3$ ), 1231, 1052 (CO)  $\text{cm}^{-1}$ ; 270-MHz NMR ( $\text{CDCl}_3$ )  $\delta$  7.49–7.54 (m, 2 H, arom  $H_2$  and  $H_4$ ), 7.12–7.14 (d, 1 H, arom  $H_6$ ), 6.97–7.03 (t, 1 H, arom  $H_5$ ), 5.03–5.11 (quint, 1 H, COOCH), 4.30–4.36 (dd, 1 H,  $J_{\text{gem}} = 11.9$  Hz,  $J_{\text{vic}} = 4.6$  Hz) and 4.21–4.27 (dd, 1 H,  $J_{\text{gem}} = 12.0$  Hz,  $J_{\text{vic}} = 5.5$  Hz) ( $\text{CH}_2\text{OOC}$ ), 3.72–3.76 (t, 2 H,  $\text{CH}_2\text{OH}$ ), 2.51–2.56 (t, 2 H,  $\text{CH}_2\text{Ph}$ ), 2.31–2.36 (t, 2 H,  $\text{OOCCH}_2$ ), 2.11 (s, 3 H,  $\text{CH}_3\text{COO}$ ), 2.01–2.06 (t, 1 H, OH), 1.55–1.69 (m, 4 H,  $\text{OOCCH}_2\text{CH}_2$  and  $\text{CH}_2\text{CH}_2\text{Ph}$ ), 1.25 (s, 20 H,  $(\text{CH}_2)_{10}$ ). Anal. ( $\text{C}_{26}\text{H}_{41}\text{IO}_5$ ) C, H.

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**Competitive Binding Assay.** A modification<sup>36</sup> of the previously described method of Sharkey and Blumberg<sup>17</sup> was used to assess analogue affinity for PDBu-receptor binding. Briefly, rat brain cytosol was prepared from whole Sprague-Dawley rat brains by homogenization (Brinkman polytron at a setting of 60, two times for 10 s each) in an equal volume of 50 mM Tris-HCl buffer (pH 7.4) with 1 mM magnesium acetate, 1 mM phenylmethanesulfonyl fluoride, 10 mM ethylene glycol bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 2 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol. The homogenate was centrifuged at 100000g for 60 min at 4 °C. The supernatant was divided into aliquots and stored at -70 °C. Protein concentration was determined by the method of Bradford.<sup>37</sup>

Diacylglycerols were stored in chloroform at a concentration of  $10^{-3}$  M. Aliquots were added to test tubes along the 15  $\mu$ L of 10 mg/mL PS in chloroform, and the solvent was removed with a stream of nitrogen. To each tube, an aqueous solution of Tris-HCl, calcium chloride, and bovine  $\gamma$ -globulin (with concentrations such that the final concentrations in the reactions were as listed below) was added. Each tube was sonicated twice for 15 s at a setting of 3-4. A solution of [<sup>3</sup>H]PDBu was added to each tube and vortexed.

Reaction mixtures with a total volume of 250  $\mu$ L contained 0.02  $\mu$ M [<sup>3</sup>H]PDBu, rat brain cytosol (200  $\mu$ g/mL protein), 0.05 M Tris-HCl (pH 7.4), 0.1-4 mM calcium chloride, 100  $\mu$ g/mL PS, and 2 mg/mL bovine  $\gamma$ -globulin. Nonspecific binding was determined by measuring binding in parallel reactions containing 12  $\mu$ M PDBu. The reactions were carried out in polyethylene microfuge tubes, which were incubated at 37 °C for 30 min and then put on ice for 5 min. Polyethylene glycol (PEG, 187  $\mu$ L of a 35% solution in water) was added, and the tubes were vortexed and left on ice for 15 min. They were spun in a microfuge for 5-10 min. The supernate was removed, and the pellets were washed with 400  $\mu$ L of 15% PEG, cut from the centrifuge tubes, dried, and dissolved in scintillation fluid. Radioactivity was determined by liquid-scintillation spectroscopy with a Beckman LS8100 counter. All experiments were conducted in duplicate or triplicate.

**Protein Kinase C Activation Assay.** The enzyme was prepared as described by Leach and Blumberg.<sup>20</sup> Activity was

determined by measuring the transfer of <sup>32</sup>P from [<sup>32</sup>P]ATP to histone IIIs. The reaction mixture had a total volume of 50  $\mu$ L and contained 40 mM Tris-HCl (pH 7.4), 15 mM magnesium acetate, 1  $\mu$ g/mL PS, 50  $\mu$ M [<sup>32</sup>P]ATP (225-325 dpm/pmol), 75  $\mu$ g of histone IIIs, and 11  $\mu$ g of enzyme. Diacylglycerols were stored in stock CHCl<sub>3</sub> solutions. PS and an appropriate amount of diacylglycerol for the highest concentration to be assayed were measured into a tube and the solvent was removed by a stream of nitrogen. Tris-HCl buffer (0.5 mL) was added to the tube and the mixture was sonicated three times for 20 s each. Lower concentrations of diacylglycerol were obtained by diluting the sonicated mixture with a solution of Tris-HCl and PS and vortexing. The reactions were carried out in 1.5 mL tubes for 7 min at 30 °C. The tubes were placed on ice immediately after incubation, and 25  $\mu$ L from each was spotted on a 2  $\times$  2 cm square of phosphocellulose paper. The paper was washed five times with water and once with acetone, dried, and counted by liquid-scintillation spectroscopy. All experiments were carried out in triplicate.

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**Supplementary Material Available:** The IR and NMR data not included in the text (5 pages). Ordering information is given on any current masthead page.

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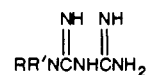
## Carbohydrate Biguanides as Potential Hypoglycemic Agents

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A series of monosaccharides containing a biguanide functionality was prepared and evaluated for hypoglycemic activity. Among the analogues prepared were those involving D-glucose substituted on the 6- or 1-position (19 and 24), D-galactose substituted on the 6-position (7), and D-arabinose (31). The target compounds were evaluated in a modified rat glucose-tolerance test (oral glucose load/oral drug, 100 mg/kg). Compounds 8 [6-biguanidino-1,2:3,5-bis-*O*-(1-methylethylidene)-6-deoxy- $\alpha$ -D-glucopyranoside] and 23 [methyl 6-biguanidino-6-deoxy-2,3,4-*O*-tribenzyl- $\alpha$ -D-glucopyranoside] were the most active, exhibiting nearly equivalent hypoglycemic activity to that of phenformin (1) and metformin (2), as measured by the inhibition of the rise of blood glucose. Compound 31 was somewhat less active with 26% inhibition, as compared to 64% inhibition with 1 and 41% inhibition with 2.

There are several approaches to the treatment of non-insulin-dependent diabetes mellitus (NIDDM). One fruitful avenue of investigation has centered on the use of biguanide-containing drugs, such as phenformin (1) and



1: R = PhCH<sub>2</sub>CH<sub>2</sub>, R' = H  
2: R = R' = Me

metformin (2), as oral hypoglycemic agents.<sup>1</sup> Unfortunately, since biguanide drugs can also cause fatal lactic

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