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Synthesis of 1D-3-Deoxy- and -2,3-Dideoxyphosphatidylinositol

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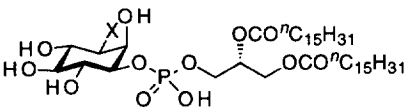
Abstract: Both 1D-3-deoxy- and -2,3-dideoxyphosphatidylinositol (**3** and **18**) were synthesized using the regioisomeric mixture of viburnitol 1,2:4,5- and 1,2:5,6- diacetonides as starting material. Selective acidic hydrolysis and subsequent benzylation or deoxygenation afforded **11a,b** as important intermediates. Compound **3** and **18** were of interest as putative antimetabolites of phosphatidylinositol-3-phosphate and as inhibitors of cancer cell colony formation. © 1997 Elsevier Science Ltd.

INTRODUCTION

Phosphatidylinositol phospholipase C (PI-PLC) plays a key role in the metabolism of membrane phospholipids by hydrolyzing phosphatidylinositol 4,5-bisphosphate to *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol.[1] Ins(1,4,5)P_3 is a ubiquitous second messenger which interacts stereospecifically with a membrane receptor to promote the release of Ca^{2+} from intracellular stores whereas diacylglycerol is an activator of protein kinase C (PKC). The increase in the cytoplasmic Ca^{2+} concentration and the activation of PKC lead to a sequence of events that culminate in DNA synthesis and cell proliferation.[1, 2] Because of the minute quantities which are available from biological sources, as well as the desire to elucidate structure-activity relationships, this class of compounds remains a popular target for synthesis.[3]

Recently, a second PI signaling pathway has been identified and linked to the action of some growth factors and oncogenes.[4] PI-3-kinase is associated with several protein tyrosine kinases activated by a number of peptide hormones.[5] The PI 3-K isozymes have been characterized, and each is a heterodimer comprised of a regulatory 85 kDa domain and a catalytic 110 kDa domain.[6, 4b] PI 3-K phosphorylates the D-3 position of the inositol ring of phosphatidylinositols, for example PI(4,5)P_2 to form PI(3,4,5)P_3, a second messenger recognized as an effector in the phosphorylation of pleckstrin and in the activation of Akt/PKB kinase, as well as a ligand for centaurin, a brain protein linking extracellular events to cytoskeletal changes.[7]



1 X = F
2 X = Cl
3 X = H

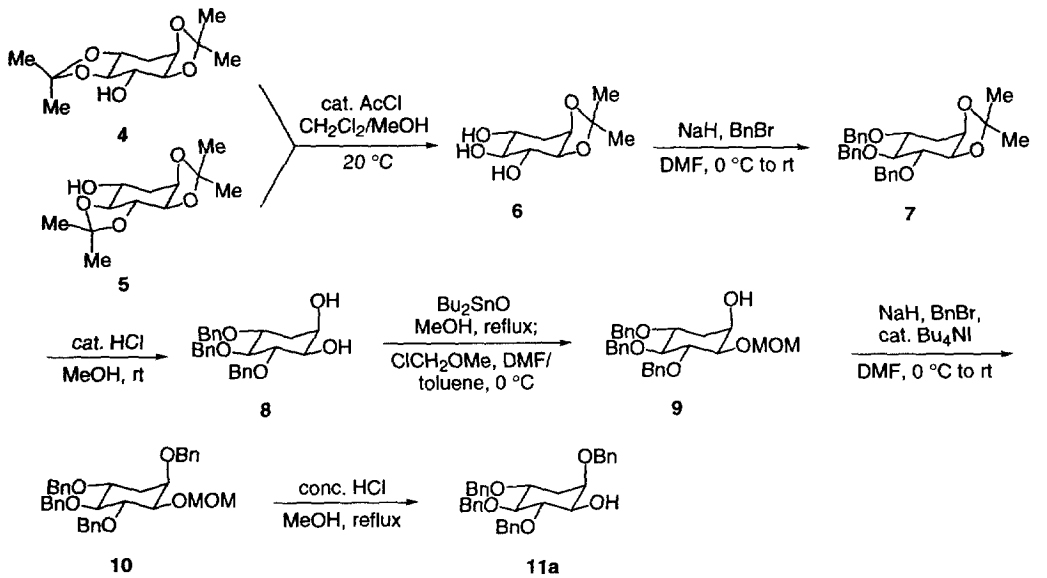
Figure 1

The PI-3-phosphates are not substrates for PI-PLC[8] and appear to exert their signaling action independently of the inositol phosphate pathway. In the course of our program to investigate analogues of PI as potential antimetabolites of PI 3-K,[9] we have synthesized 1D-3-deoxyphosphatidyl-*myo*-inositol (**3**)[10] as well as its 3-fluoro (**1**) and 3-chloro derivatives (**2**). It was found that compounds **1** and **3** inhibit colony formation by HT-29 human colon carcinoma cells with IC_{50}'s of 37 and 35 μM, respectively, whereas **2** displayed only low activity. Compound **1** is an inhibitor of PI 3-K (IC_{50} 30 μM) while **3** has no effect at concentrations up to 250 μM. This result suggests that **3** can be used in whole cell lysates to measure PI 3-K activity as the difference between [γ^{32}P]ATP-dependent phosphorylation of PI and **3**. In the present paper, we would like to give a full account of the synthesis of this compound, as well as the corresponding 2,3-dideoxy derivative **18**, from L-quebrachitol. Compound **18** was chosen as a target because the deleted 2-hydroxyl group is intimately involved in the mechanism of hydrolysis of phosphatidylinositols by phospholipase C.[11] It was therefore hoped that **18**, if it remained biologically active, could be used as a more stable substitute for **3**.

SYNTHESIS

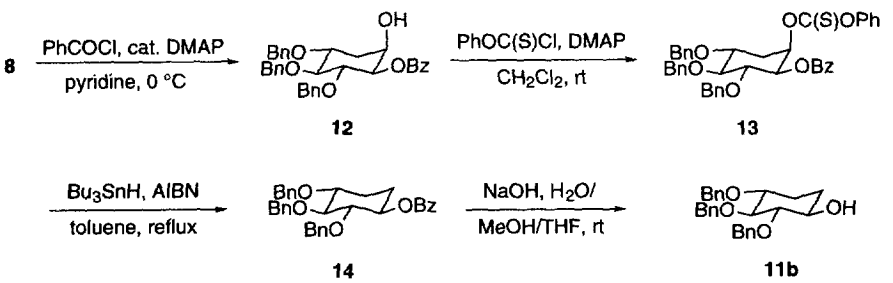
Starting material for both PI analogues is the regioisomeric mixture of viburnitol (*i. e.*, 3-deoxy-*myo*-inositol) 1,2:4,5- and 1,2:5,6-diacetonides (**4**/**5**) (Scheme 1), obtained from L-quebrachitol as described previously.[12] Whereas in our original synthesis of DPI (**3**) we had proceeded through the 5,6-di-*O*-benzoyl-4-*O*-benzyl derivative (not shown) of the 1,2-monoacetonide **6** as a consequence of its availability as a sideproduct in the preparation of deoxyinositol trisphosphates,[12] we now chose to use the monoacetonide itself as an intermediate and prepared it by controlled acidic hydrolysis of the more labile trans-acetonide moieties in the diacetonide mixture in 79% yield. Additionally, 6% of unreacted diacetonides and 14% of viburnitol were recovered and could be recycled. All of the three required O-benzyl groups were then introduced simultaneously with benzyl bromide and NaH in DMF (74% yield), and the remaining cis-acetonide was removed by acidic hydrolysis (96% yield). The resulting diol **8** was protected selectively at the equatorial 1-hydroxyl by reacting its cyclic dibutylstannylene derivative[13] with chloromethyl methyl ether. Taking recovered starting material into account, the yield in this step was improved to 79%. An attempt at introducing the protecting group without organotin derivatization (1 M solution of diol **8**, 1.2 eq. $ClCH_2OMe$, 1.5 eq. $(i\text{-Pr})_2NEt$,

CH_2Cl_2, 0 °C, then 26 h at rt) proved unsatisfactory: besides 29% of starting material and 17% of its bis(methoxymethyl) ether, an approx. 1:1 mixture of the desired 1-(methoxymethyl) ether **9** and its 2-isomer was obtained. This result stands in stark contrast to the facile discrimination of the two hydroxyls by benzoylation (see below). Subsequent benzylation of the 2-hydroxyl (73% yield) and acidic hydrolysis of the MOM ether (77% yield) afforded the key intermediate, 2,4,5,6-tetra-*O*-benzylviburnitol (**11a**) in crystalline form.



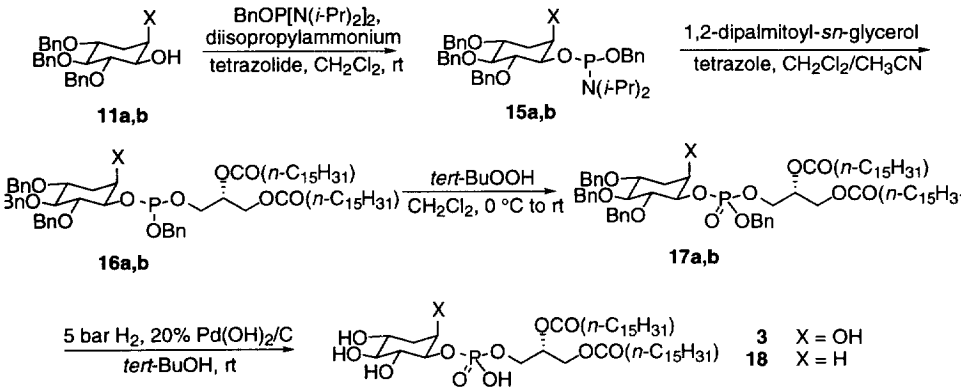
Scheme 1

Controlled benzoylation of diol **8** (Scheme 2) afforded the pure 1-*O*-benzoyl derivative **12** in 88% yield besides small amounts of starting material and dibenzoate; no 2-*O*-benzoyl derivative was



Scheme 2

detected. Intermediate **12** was transformed into the thionocarbonate **13** (86% yield besides 6% of **12**), and this compound was deoxygenated with $(n\text{-Bu})_3SnH/AIBN$[14] (85% yield) as reported previously for similar compounds.[12] Basic saponification then delivered 4,5,6-tri-*O*-benzyl-2,3-dideoxy-*myo*-inositol (**11b**) in 87% yield.



(**a** series: X = OBn; **b** series: X = H)

Scheme 3

The intermediates **11a,b** were transformed into the corresponding phosphatidylinositols in accordance with established protocols (Scheme 3).[9] Thus, phosphitylation[15] with *O*-benzyl *N,N,N',N'*-tetraisopropylphosphorodiamidite catalyzed by diisopropylammonium tetrazolide gave rise to the phosphoramidites **15a,b** in quantitative yield which were coupled with 1,2-dipalmitoyl-*sn*-glycerol in the presence of tetrazole (60/71% yield). The resulting phosphites **16a,b** were oxidized to the phosphates **17a,b** with *tert*-butyl hydroperoxide (90/89% yield). Final hydrogenolysis then delivered 3-deoxy- and 2,3-dideoxyphosphatidylinositol (**3**) and (**18**) in good purity, and in 94% yield each. While **18** remained unchanged on storage at -20 °C during one year, **3** was found to have undergone extensive decomposition after extended storage.

EXPERIMENTAL SECTION

General Methods: NMR spectra were acquired at proton frequencies of 270 and 300 MHz, using $CDCl_3$ as solvent unless noted otherwise. ^1H chemical shifts were reported with Me_4Si (δ = 0.00 ppm) or $CHCl_3$ (δ = 7.26 ppm) as internal standards, ^{31}P chemical shifts relative to external aqueous 85% H_3PO_4 (δ = 0.00 ppm), and ^{13}C chemical shifts with $CHCl_3$ (δ = 77.00 ppm) or TMS (δ

= 0.00 ppm) as internal standards. Mass spectra were obtained in electron impact ionization mode at 70 eV. Optical rotations were measured at rt. For solvent purification, chromatography, and melting points, see ref. 9c.

1D-3-Deoxy-1,2-*O*-isopropylidene-*myo*-inositol (6). To a solution of 6.83 g (28.0 mmol) of 1D-3-deoxy-1,2:4,5- and -1,2:5,6-di-*O*-isopropylidene-*myo*-inositol (**4/5**) in 280 mL of CH_2Cl_2 was added 70 mL of methanol, followed by 40 µL (560 µmol) of acetyl chloride. The mixture was stirred at 20 °C (water bath) under close TLC control for 17 min, then the reaction was quenched by addition of 240 µL (1.7 mmol) of Et_3N. After addition of 40 g of silica gel, the mixture was evaporated, and the residue was chromatographed on silica gel. Unreacted starting material (0.43 g, 6%) was eluted with CH_2Cl_2/MeOH 11:1, the desired product (4.52 g, 79%) with CH_2Cl_2/MeOH 4:1, and 1D-3-deoxy-*myo*-inositol (0.66 g, 14%) with isopropanol/water 9:1. The product is a glass which on storage forms an amorphous solid: mp 113-120 °C; ^{1}H NMR (D_2O) δ 4.46 (m, 1 H), 4.04 (dd, 1 H, *J* = 5.5, 8 Hz), 3.73 (ddd, 1 H, *J* = 5, 9, 14 Hz), 3.51 (dd, 1 H, *J* = 8, 10 Hz), 3.24 (t, 1 H, *J* = 10 Hz), 2.38 (ddd, 1 H, *J* = 2, 5, 14.5 Hz), 1.82 (ddd, 1 H, *J* = 4.5, 11.5, 15.5 Hz), 1.51 (s, 3 H), 1.39 (s, 3 H); IR (nujol) 3310 (br), 1098, 1034, 995, 877 cm^{-1}; MS *m/z* 189 (M^+ - CH_3), 111, 83, 59, 57, 43 (100%).

1D-4,5,6-Tri-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-*myo*-inositol (7). To a suspension of 4.24 g (106 mmol) of NaH (60% in oil) in 21 mL of DMF was added under N_2 with ice cooling 12.6 mL (106 mmol) of benzyl bromide. A solution of 4.32 g (21.2 mmol) of triol **6** in 21 mL of DMF was added dropwise within 10 min, and the mixture was stirred in the thawing cold bath for 2 h, then at rt for 5.5 h. The reaction was quenched by dropwise addition of 2 mL of water with ice cooling, the mixture stirred at rt for 10 min, and the volatiles pumped into a -78 °C trap (bath ≤ 50 °C). The residue was taken up in 50 mL of water, the product extracted into 2 x 25 mL of EtOAc/hexane 1:6, and the combined organic phases washed with 25 mL of water. After evaporation, the residue was chromatographed on silica gel with EtOAc/hexane (1:6, then 1:3) to obtain 7.43 g (74%) of the product as a light-yellow oil: $[\alpha]_D$ -44.2° (*c* 18.6 gL^{-1}, $CHCl_3$); ^{1}H NMR δ 7.40-7.29 (m, 15H), 4.88, 4.76 (ABq, 2H, *J* = 11.5 Hz), 4.79 (s, 2H), 4.69, 4.64 (ABq, 2H, *J* = 11.5 Hz), 4.35 (m, 1H), 4.17 (t, 1H, *J* = 6.5 Hz), 3.81 (ddd, 1H, *J* = 4.5, 8, 12.5 Hz), 3.65 (dd, 1H, *J* = 7.5, 8.5 Hz), 3.46 (t, 1H, *J* = 8.5 Hz), 2.37 (dt, 1H, *J* = 4 Hz (t), 14.5 Hz (d)), 1.84 (ddd, 1H, *J*= 4.5, 10.5, 14 Hz), 1.45 (s, 3H), 1.36 (s, 3H); ^{13}C NMR δ 138.59, 138.47, 128.36, 128.29, 128.21, 128.04, 127.97, 127.59, 127.55, 127.47, 108.95, 83.80, 83.19, 79.86, 76.69, 74.87, 74.02, 72.88, 72.48, 30.07, 27.90, 25.84; IR (film) 2929, 1497, 1454, 1367, 1218, 1087, 1071, 1048, 737, 697 cm^{-1}; MS *m/z* 459 (M^+ - Me, 0.2%), 383 (M^+ - Bn, 9%), 277, 219, 91 (100%). Anal. Calcd for $C_{30}H_{34}O_5$ (474.60) C, 75.92; H, 7.22. Found: C, 76.00; H, 7.21.

1D-4,5,6-Tri-*O*-benzyl-3-deoxy-*myo*-inositol (8). A solution of 15.4 g (32.5 mmol) of **7** in 300 mL of methanol was stirred with 150 μL of conc. HCl at rt for 48 h. Evaporation of the solvent gave 13.7 g (97%) of the diol as white crystals: mp 115-116 °C; $[\alpha]_D$ -49.0° (*c* 20 gL^{-1}, $CHCl_3$); IR (film) 3440 (br), 3030, 2904, 1362, 1067, 732, 697 cm^{-1}; ^1H NMR δ 7.4-7.3 (m, 15H), 5.02, 4.64 (ABq, 2H, *J* = 11.5 Hz), 5.00, 4.83 (ABq, 2H, *J* = 11 Hz), 4.68 (s, 2H), 4.08 (br s, 1H), 3.94 (ddd, 1H, *J* = 5, 9.5, 14 Hz), 3.68 (t, 1H, *J* = 9.5 Hz), 3.56-3.48 (m, 2H), 2.38 (br s, 1H), approx. 2.35 (overlapping, 1H), 2.34 (br s, 1H), 1.45 (br dd, 1H, *J* = 11.5, 14 Hz); ^{13}C NMR δ 138.65, 138.50, 128.68, 128.36, 127.95, 127.89, 127.71, 127.56, 85.61, 81.08, 77.71, 75.46, 75.40, 73.88, 72.73, 67.40, 32.64; MS *m/z* 343 (M^+ - Bn, 13%), 111, 107, 91 (100%). Anal. Calcd for $C_{27}H_{30}O_5$ (434.53): C, 74.62; H, 6.96. Found: C, 74.75 ; H, 6.47.

1D-4,5,6-Tri-*O*-benzyl-3-deoxy-1-*O*-(methoxymethyl)-*myo*-inositol (9). A solution of 13.3 g (30.6 mmol) of the diol **8** in 300 mL of dry methanol was refluxed under N_2 with 8.4 g (33.6 mmol) of di-*n*-butyltin oxide until a clear solution was obtained (approx. 2 h). The cooled solution was evaporated, and the residue was dried in vacuo and taken up in 200 mL of dry DMF. A solution of 2.63 mL (33.6 mmol) of $ClCH_2OMe$ in 20 mL of dry toluene was added dropwise with ice cooling under N_2. Stirring was continued for 1 h at 0 °C. The solvent was then evaporated under reduced pressure, the residue was dissolved in 400 mL of CH_2Cl_2, and the solution was cautiously washed with water (2 x 100 mL, ready emulsification) and dried over $MgSO_4$. Chromatography on silica gel with EtOAc/hexane 1:2, then 1:1 gave 7.6 g (52%) of the product as colorless crystals followed by 4.5 g (34%) of the starting material. Compound **9**: mp 101-102 °C; $[\alpha]_D$ +25.7° (*c* 4.0 gL^{-1}, $CHCl_3$); ^1H NMR δ 7.31 (m, 15H), 4.96-4.64 (4 overlapping ABq, 8H), 4.11 (narrow m, 1H), 3.92 (ddd, 1H, *J* = 4.5, 8, 11.5 Hz), 3.81 (t, 1H, *J* = 9.5 Hz), 3.59 (dd, 1H, *J* = 3, 9.5 Hz), 3.48 (t, 1H, *J* = 9.5 Hz), 3.38 (s, 3H), 2.39 (br s, 1H), 2.35 (dt, 1H, *J* = 4 Hz (t), 14 Hz (d)), 1.45 (br dd, 1H, *J* = 11.5, 14 Hz); ^{13}C NMR δ 138.83, 138.71, 138.62, 128.35, 128.31, 127.89, 127.82, 127.74, 127.56, 127.53, 127.47, 96.89, 85.84, 81.22, 80.54, 77.04, 75.94, 75.70, 72.91, 67.60, 55.75, 32.61; IR 3496, 2903, 1102, 1084, 1039, 901, 733, 695 cm^{-1}; MS *m/z* 433 (M^+ - CH_2OMe, 0.7%), 387 (M^+ - Bn, 12%), 249, 111, 91 (100%), 45 (78%). Anal. Calcd for $C_{29}H_{34}O_6$ (458.59): C, 72.78; H, 7.16. Found: C, 72.66; H, 6.99.

1D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-*myo*-inositol (11a). To a stirred solution of 664 mg of NaH (60% in mineral oil, 16.6 mmol) in 20 mL of anhydrous DMF was added with ice cooling under N_2 a solution of 5.25 g (11.1 mmol) of compound **9** in 40 mL of DMF. After 0.5 h, 1.59 mL (13.3 mmol) of benzyl bromide and 0.5 g of Bu_4NI were added, and the mixture was stirred at rt overnight. The reaction was quenched by adding 150 mL of ice water, and the mixture was extracted with 2 x 150 mL of ether. The combined organic layers were washed with 2 x 100 mL of water, then with brine, and dried over Na_2SO_4. After concentration, the residue was purified by

chromatography on silica gel with EtOAc/hexane 1:5, and the evaporated eluate was dried in vacuo to give 4.6 g (73%) of intermediate **10** as a colorless oil. This compound was dissolved in 300 mL of methanol and 6 mL of conc. HCl. The mixture was refluxed under TLC control until the starting material disappeared (approx. 2 h). After concentration, the residue was crystallized from EtOAc/hexane to give 3.3 g (77%) of **11a** as white needles: mp 66-67 °C; $[\alpha]_D$ -7.34° (*c* 6.2 gL^{-1}, $CHCl_3$); ^1H NMR δ 7.40-7.20 (m, 20 H), 4.94, 4.80 and 4.90, 4.84 (2 overlapping ABq, 4H), 4.65, 4.55 (ABq, 2H, *J* = 11.5 Hz), 4.49 (narrow ABq, 2H) 3.86-3.76 (m, 2H), 3.73 (t, 1H, *J* = 9.5 Hz), 3.61-3.54 (m, 1H), 3.49 (t, 1H, *J* = 9 Hz), 2.41 (br d, 1H, *J* = 6.5 Hz), 2.25 (dt, 1H, *J* = 4 Hz (t), 14 Hz (d)), 1.31 (br t, *J* = 13 Hz); ^{13}C NMR δ 138.70, 138.48, 138.18, 128.36, 128.34, 128.30, 127.95, 127.93, 127.87, 127.66, 127.61, 127.50, 85.59, 82.63, 77.00, 75.65, 75.59, 75.51, 74.73, 72.80, 71.69, 30.77; IR (film) 3466, 3030, 2871, 1454, 1360, 1087, 1070, 736, 697 cm^{-1}; MS *m/z* 433 (M^+ -Bn, 10%), 327, 219, 181, 111, 91 (100%). Anal. Calcd for $C_{34}H_{36}O_5$ (524.66): C, 77.83; H, 6.92. Found: C, 77.55; H, 6.94.

1D-1-*O*-Benzoyl-4,5,6-tri-*O*-benzyl-3-deoxy-*myo*-inositol (12). To a solution of 2.55 g (5.62 mmol) of the diol **8** and 69 mg (0.56 mmol) of 4-(dimethylamino)pyridine (DMAP) in 25 mL of anhydrous pyridine was added dropwise within 15 min with ice cooling and exclusion of moisture 0.72 mL (6.2 mmol) of benzoyl chloride. Stirring at 0 °C was continued for 4.5 h, then the mixture was kept at 4 °C for 44 h. After evaporation, 50 mL of 5% aq. HCl was added, and the product was extracted into 3 x 30 mL of EtOAc/hexane 1:1. The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was taken up in a small volume of $CHCl_3$ and applied on a silica gel column. EtOAc/hexane 1:4 eluted 0.13 g (3.5%) of the dibenzoate; the 1-benzoate (2.67 g, 88%) was eluted with EtOAc/hexane 1:2; finally, 0.13 g (5%) of **8** were recovered by further elution with EtOAc. Recrystallization of the 1-benzoate from EtOAc/hexane 10:3 (reflux to -20 °C) gave colorless, cotton-like crystals: mp 142-142.5 °C; $[\alpha]_D$ -73.8°, $[\alpha]_{546}$ -88.4° (*c* 46 gL^{-1}, EtOAc); ^1H NMR δ 8.01 (dd, 2H, *J* = 1.5, 8.5 Hz), 7.58 (m, 1H), 7.44 (t, 2H, *J* = 7.5 Hz), 7.37-7.27 (m, 10H), 7.12 (narrow m, 5H), 5.18 (dd, 1H, *J* = 3, 10 Hz), 4.96, 4.85 (ABq, 2H, *J* = 10.5 Hz), 4.84, 4.70 (ABq, 2H, *J* = 10.5 Hz), 4.74, 4.69 (ABq, 2H, *J* = 10.5 Hz), 4.28 (narrow m, 1H), 4.08 (t, 1H, *J* = 9.5 Hz), 4.02 (ddd, 1H, *J* = 4.5, 9, 11.5 Hz), 3.64 (t, 1H, *J* = 9 Hz), 2.37 (dt, 1H, *J* = 4.5 Hz (t), 14 Hz (d)), 2.01 (t, 1H, *J* = 2 Hz), 1.61 (ddt, 1H, *J* = 2 Hz (t), 12 Hz (d), 14 Hz (d)); IR (nujol) 3500, 1698, 1286, 1116, 1085, 1074, 732, 713, 695 cm^{-1}; MS *m/z* 447 (M^+ - Bn, 3%), 341, 181, 105, 91 (100%). Anal. Calcd for $C_{34}H_{34}O_6$ (538.64): C, 75.82; H, 6.36. Found: C, 75.72; H, 6.16.

1D-1-*O*-Benzoyl-4,5,6-tri-*O*-benzyl-3-deoxy-2-*O*-[phenoxy(thiocarbonyl)]-*myo*-inositol (13). To a solution of 2.40 g (4.46 mmol) of **12** and 0.76 g (6.2 mmol) of DMAP in 20 mL of anhydrous CH_2Cl_2 was added dropwise with ice cooling 0.83 mL (6.0 mmol) of phenyl chlorothionoformate. The mixture was stirred at rt for 139 h, then washed with 2 x 10 mL of 5% aq. HCl and dried over

$MgSO_4$. The evaporation residue was separated by chromatography on silica gel using EtOAc/hexane mixtures. With a solvent ratio of 1:9, a byproduct (presumably *O,O*-diphenyl thionocarbonate) was eluted; with a ratio of 1:4, the title compound (2.58 g, 86%), immediately followed by 48 mg of the corresponding inositol phenyl carbonate; with a ratio of 1:3, 38 mg of the bis(inositol) carbonate, followed by 155 mg (6%) of the starting material. The approximate R_f values of these compounds in EtOAc/hexane 1:4 are 0.70, 0.40, 0.30, 0.16, and 0.12, respectively. The product **13** was obtained as a yellowish foam or glass: ^{1}H NMR δ 8.01 (m, 2H), 7.56 (m, 1H), 7.44 (m, 2H), 7.39-7.23 (m, 13H), 7.18-7.10 (m, 5H), 6.99 (m, 2H), 5.96 (narrow m, 1H), 5.32 (dd, 1H, *J* = 3, 10 Hz), 4.96, 4.88 (ABq, 2H, *J* = 10.5 Hz), 4.83, 4.74 (ABq, 2H, *J* = 10.5 Hz), 4.75, 4.70 (ABq, 2H, *J* = 11.5 Hz), 4.06 (t, 1H, *J* = 9.5 Hz), 3.89 (ddd, 1H, *J* = 5, 9, 11.5 Hz), 3.71 (t, 1H, *J* = 9 Hz), 2.64 (dt, 1H, *J* = 4.5 Hz (t), 14.5 Hz (d)), 1.78 (ddd, 1H, *J* = 2, 11.5, 14.5 Hz); IR (film) 1725, 1292, 1273, 1214, 1192, 1094, 735, 711, 698 cm^{-1}; MS *m/z* 583 (M^+ - Bn, 0.7%), 477, 429, 191, 181, 105, 91 (100%).

1D-1-*O*-Benzoyl-4,5,6-tri-*O*-benzyl-2,3-dideoxy-*myo*-inositol (14). Under N_2, a solution of 2.58 g (3.82 mmol) of **13**, 1.02 mL (3.8 mmol) of tributyltin hydride, and 94 mg (0.57 mmol) of azobis(isobutyronitrile) (AIBN) in 60 mL of toluene was inserted into a preheated 125 °C oil bath and heated under reflux for 10 min. A solution of 2.04 mL (7.6 mmol) of Bu_3SnH and 188 mg (1.15 mmol) of AIBN in 30 mL of toluene was added at reflux temperature within 2.5 h. After an additional 40 min, TLC (SiO_2, EtOAc/toluene 1:4; R_f approx. 0.65 and 0.53 for **14** and **13**, resp.) indicated the persistence of some starting material. Additional 2.04 mL of Bu_3SnH and 188 mg of AIBN in 5 mL of toluene were added all at once, and the mixture was refluxed for an additional 5.5 h. After evaporation, the residue was chromatographed on silica gel with EtOAc/toluene 1:9 to remove traces of starting material and most of the organotin byproduct, and again with EtOAc/hexane 1:5, then 1:3. The evaporation residue was taken up in 25 mL of EtOAc/hexane 1:9 and set aside at -20 °C for crystallization, to obtain two fractions of the product totalling 1.70 g (85%). The analytical sample was recrystallized from EtOAc/hexane and dried in vacuo at 60 °C: colorless crystals; mp 86-87.5 °C; $[\alpha]_D$ -62.2°, $[\alpha]_{546}$ -74.4° (*c* 30 gL^{-1}, EtOAc); ^{1}H NMR δ 7.99 (m, 2H), 7.55 (m, 1H), 7.41 (m, 2H), 7.37-7.25 (m, 10H), 7.15 (m, 5H), 5.12 (m, 1H), 4.95, 4.84 (ABq, 2 H, *J* = 11 Hz), 4.83, 4.74 (ABq, 2H, *J* = 10.5 Hz), 4.70 (narrow ABq, 2H), 3.69-3.47 (m, 3H), 2.13 (m, 2H), 1.60-1.25 (m, 2H); IR (nujol) 1712, 1276, 1114, 1097, 1072, 735, 710, 695 cm^{-1}; MS *m/z* 431 (M^+ - Bn, 2%), 325, 105, 91 (100%). Anal. Calcd for $C_{34}H_{34}O_5$ (522.64): C, 78.14; H, 6.56. Found: C, 77.93; H, 6.51.

1D-4,5,6-Tri-*O*-benzyl-2,3-dideoxy-*myo*-inositol (11b). A solution of 1.61 g (3.08 mmol) of **14** in 15 mL each of THF and methanol was stirred at rt with 1.2 mL of 5 M aq. NaOH for 170 min. After partial evaporation, the mixture was extracted with 20 + 10 mL of $CHCl_3$, and the

combined organic phases were dried over Na_2SO_4 and evaporated. Chromatography on silica gel with EtOAc/hexane 1:4, then 1:1 gave a crude product which crystallized on addition of EtOAc/hexane 1:9. Crystallization was completed at -20 °C to yield two fractions totalling 1.11 g (87%) of a colorless solid: mp 87-88.5 °C; $[\alpha]_D$ +2.35°, $[\alpha]_{546}$ +3.15° (*c* 35 gL^{-1}, EtOAc); ^1H NMR δ 7.43-7.25 (m, 15H), 5.02, 4.65 (ABq, 2H, *J* = 11.5 Hz), 4.99, 4.82 (ABq, 2H, *J* = 11 Hz), 4.68 (narrow ABq, 2H), 3.60-3.43 (m, 3H), 3.25 (m, 1H), 2.34 (d, 1H, *J* = 2 Hz), 2.15-1.90 (m, 2H), 1.42-1.18 (m, 2H); IR (nujol) 3300 (br), 1090, 1048, 733, 695 cm^{-1}; MS *m/z* 327 (M^+ - Bn, 6%), 223, 203, 91 (100%).

D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-*myo*-inositol 1-(*O*-Benzyl-*N*,*N*-diisopropylphosphor amidite) (15a). To a suspension of 540 mg (3.15 mmol) of diisopropylammonium tetrazolide[16] in 10 ml of dry CH_2Cl_2 was added dropwise under N_2 at rt (water bath) 2.73 mL (7.65 mmol) of *O*-benzyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite,[17] followed by a solution of 3.30 g (6.29 mmol) of alcohol **11a** in 2 mL of dry CH_2Cl_2. The mixture was stirred in the water bath for 24 h, the solvent was evaporated, and the residue was chromatographed on silica gel previously deactivated with Et_3N using EtOAc/hexane 1:10. Evaporation and drying in vacuo afforded 4.82 g (100%) of **15a** as a colorless syrup: ^1H NMR (signals of minor diastereoisomer omitted if separated from those of the major isomer) δ 7.40-7.20 (m, 25H), 4.98-4.44 (m, 10H), 3.98-3.82 (m, 3H), 3.79-3.61 (m, 3H), 3.47 (t, 1H, *J* = 9.5 Hz), 2.13 (dt, 1H, *J* = 4.5 Hz (t), 14 Hz (d)), 1.23 (m, 1H), 1.14 (t, 12H, *J* = 6 Hz); ^{31}P NMR δ 150.26, 147.42 (major/minor diastereoisomer); IR (film) 3030, 2965, 1496, 1362, 1089, 1071, 1027, 975, 732, 696 cm^{-1}.

D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-*myo*-inositol 1-[Benzyl (*R*)-2,3-bis(hexadecanoyloxy)-propyl phosphite] (16a). To 3.94 g (6.92 mmol) of 1,2-dipalmitoyl-*sn*-glycerol and 970 mg (13.84 mmol) of tetrazole in 22 mL of dry CH_2Cl_2 was added under N_2 at rt a solution of 4.80 g (6.29 mmol) of phosphoramidite **15a** in 22 mL of anhydrous CH_3CN. The resulting mixture was stirred for 20 h at rt, then 10 mL of $NaHCO_3$ solution was added, and the organic solvents were evaporated under reduced pressure. The residue was extracted with 200 mL of ether, and the organic phase was washed with aq. $NaHCO_3$ and brine and dried over Na_2SO_4. After evaporation, the residue was purified by chromatography on silica gel with EtOAc/hexane 1:7 to yield 4.65 g (60%) of **16a** as a colorless oil: ^1H NMR δ 7.37-7.20 (m, 25H), 5.08 (m, 1H), 4.93-4.42 (m, 9H), 4.22-3.75 (m, 8H), 3.50 (m, 1H), 2.38-2.18 (m, 5H), 1.58 (br s, 4H), 1.24 (br s, 49H), 0.88 (t, 6H, *J* = 7 Hz); ^{31}P NMR δ 140.58, 140.42 (minor/major diastereoisomer); IR (film) 2925, 2853, 1742, 1455, 1091, 1070, 733, 697 cm^{-1}. Anal. Calcd for $C_{76}H_{109}O_{11}P$ (1229.67): C, 74.23; H, 8.93. Found: C, 73.93; H, 8.55.

D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-*myo*-inositol 1-[Benzyl (*R*)-2,3-bis(hexadecanoyloxy)-propyl phosphate] (17a). To an ice-cooled solution of 4.64 g (3.78 mmol) of phosphite **16a** in 100

mL of anhydrous CH_2Cl_2 was added under N_2 1.58 mL of anhydrous *tert*-butyl hydroperoxide (3.35 M in CH_2Cl_2, 5.29 mmol) diluted with 20 mL of CH_2Cl_2. Stirring was continued in the ice bath for 90 min, then at rt for 1 h. The mixture was evaporated, and the residue was chromatographed on silica gel with EtOAc/hexane 1:3 to give 4.23 g (90%) of **17a** as a colorless waxy solid: ^{1}H NMR δ 7.38-7.18 (m, 25H), 5.12-4.40 (m, 11H), 4.32-3.78 (m, 8H), 3.50 (m, 1H), 2.22 (m, 5H), 1.57 (m, 4H), 1.25 (br s, 49H), 0.88 (t, 6H, J = 6.5 Hz); ^{31}P NMR δ -1.07, -1.17; IR (film) 2923, 2853, 1743, 1455, 1147, 1091, 1023, 735, 697 cm^{-1}. Anal. Calcd for $C_{76}H_{109}O_{12}P$ (1245.67): C, 73.28; H, 8.82. Found: C, 73.40; H, 8.90.

1D-4,5,6-Tri-*O*-benzyl-2,3-dideoxy-*myo*-inositol 1-[Benzyl (*R*)-2,3-bis(hexadecanoyloxy)-propyl phosphate] (17b). To a solution of 82 mg (0.48 mmol) of diisopropylammonium tetrazolide[16] in 3.5 mL of anhydrous CH_2Cl_2 was added dropwise at rt (water bath) under N_2 0.43 mL (1.2 mmol) of neat *O*-benzyl *N,N,N',N'*-tetraisopropylphosphorodiamidite.[17] Subsequently, a solution of 403 mg (963 μmol) **11b** in 5.5 mL of anhydrous CH_2Cl_2 was added dropwise within 20 min. The mixture was stirred in the water bath for 21 h, then 10 mL of saturated aq. $NaHCO_3$ was added, the phases were separated, and the aqueous phase was extracted with 2 x 10 mL of CH_2Cl_2. After drying over Na_2SO_4, the evaporation residue was filtered rapidly with EtOAc/hexane 1:4 over 60 g of silica gel which had previously been deactivated with 1 mL of Et_3N to obtain 647 mg (nominally 102%) of the phosphoramidite **15b**.

To a solution of 603 mg (1.06 mmol) of 1,2-dipalmitoyl-*sn*-glycerol and 135 mg (1.93 mmol) of tetrazole in 3.5 mL of anhydrous CH_2Cl_2 was added dropwise under N_2 the solution of 647 mg of **15b** in 3.5 mL of anhydrous CH_3CN. The mixture was stirred at rt for 16 h and at 36 °C for 69 h before quenching with 10 mL of saturated aq. $NaHCO_3$. Extraction with 3 x 10 mL of CH_2Cl_2 was followed by drying over Na_2SO_4 and rapid column chromatography on silica gel with EtOAc/hexane 1:9, then 1:6 to obtain 748 mg (69% over both steps) of the phosphite **16b** as a yellowish oil.

To a solution of 748 mg (666 μmol) of **16b** in 6.7 mL of anhydrous CH_2Cl_2 was added dropwise within 1 h with ice cooling under N_2 0.28 mL (0.93 mmol) of a 3.35 M solution of *tert*-butyl hydroperoxide in CH_2Cl_2. The mixture was stirred at 0 °C for 1 h and allowed to revert to rt over a period of 2 h; it was then evaporated and again evaporated with toluene. Column chromatography of the residue on silica gel with EtOAc/hexane 1:2 gave 678 mg (89%) of **17b** as a yellowish glass. The analytical sample was dried overnight at 80 °C/0.2 torr: ^{1}H NMR δ 7.38-7.20 (m, 20H), 5.17-4.74 (m, 7H), 4.66 (narrow ABq, 2H), 4.36-3.87 (m, 5H), 3.46 (m, 3H), 2.30-2.12 (m, 5H), 2.05 (m, 1H), 1.65-1.3 (m, 6H), 1.25 (s, 48H), 0.88 (t, 6H, J = 6.5 Hz); IR (film) 2919, 2850, 1738,

1093, 1025, 735, 697 cm^{-1}. Anal. Calcd for $C_{69}H_{103}O_{11}P$ (1139.54): C, 72.83; H, 8.84. Found: C, 72.73; H, 9.11.

1D-3-Deoxy-*myo*-inositol 1-[(*R*)-2,3-bis(hexadecanoyloxy)propyl hydrogen phosphate] (3). A solution of 1.036 g (0.832 mmol) of **17a** in 30 mL of *tert*-butanol was hydrogenated for 24 h in a Parr shaker under 70 psi of H_2 at rt, using 580 mg of 20% $Pd(OH)_2/C$ (Aldrich, containing 50% of water) as the catalyst. The solution was filtered from the catalyst, and the solvent was evaporated. Drying in vacuo left 621 mg (94%) of **3** as a white powder: mp 110-111 °C (after sintering); $[\alpha]_D$ -7.0° (*c* 2.0 gL^{-1}, $CHCl_3$/MeOH 2:1); ^1H NMR ($CDCl_3/CD_3OD$, TMS) δ 5.28 (br s, 1H), 4.42 (dd, 1H, *J* = 3, 12 Hz), 4.21 (narrow m, 4H), 4.04 (br t, 1H, *J* = 7 Hz), 3.88-3.72 (m, 2H), 3.21 (t, 1 H, *J* = 9.5 Hz), 2.36 (t, 2H, *J* = 7.5 Hz), 2.33 (t, 2H, *J* = 7 Hz), 2.13 (dt, 1H, *J* = 4, 13.5 Hz), 1.62 (narrow m, 4H), 1.49 (t, 1H, *J* = 13.5 Hz), 1.27 (br s, 48 H), 0.89 (t, 6H, *J* = 7 Hz); ^{13}C NMR ($CDCl_3/CD_3OD$, TMS) δ 174.42, 174.03, 81.85 (d, *J* = 7 Hz), 78.42, 71.96 (d, *J* = 5.5 Hz), 70.39 (d, *J* = 8 Hz), 68.38, 67.67, 65.54 (d, *J* = 5.5 Hz), 62.66, 35.67, 34.62, 34.49, 32.41, 30.17, 30.14, 29.99, 29.84, 29.60, 29.58, 25.35, 23.13, 14.28; ^{31}P NMR ($CDCl_3/CD_3OD$) δ -0.33. Anal. Calcd for $C_{41}H_{79}O_{12}P$: C, 61.94; H, 10.02. Found: C, 62.08; H, 10.07.

1D-2,3-Dideoxy-*myo*-inositol 1-[(*R*)-2,3-Bis(hexadecanoyloxy)propyl hydrogen phosphate] (18). The precursor **17b** (101 mg, 88.6 μmol) was dissolved by sonication in 9 mL of *tert*-butanol, 40 mg of 20% $Pd(OH)_2/C$ (Aldrich, ≤ 50% H_2O) was added and dispersed by further sonication, and the mixture was hydrogenated under 5 bar of H_2 for 38 h. The catalyst was removed by centrifugation to leave 66 mg (96%) of the product as an off-white amorphous solid: no defined mp; $[\alpha]_D$ +0.4°, $[\alpha]_{546}$ +0.5° (*c* 14 gL^{-1}, $CHCl_3$/MeOH 2:1); ^1H NMR ($CDCl_3/CD_3OD$ 2.7:1 v/v, TMS) δ 5.25 (m, 1H), approx. 4.4 (1H, partially concealed by OH), 4.24-4.12 (m, 3H), 4.07 (br, 1H), 3.47-3.32 (m, 2H), 3.20 (t, 1H, *J* = 9 Hz), 2.40-2.30 (m, 4H), 2.11 (m, 1H), 1.94 (m, 1H), 1.62 (br, 4H), 1.49 (m, 1H), 1.27 (s, 49H), 0.89 (t, 6H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3/CD_3OD$ 2.7:1 v/v, TMS) δ 174.13, 173.69, 79.61 (d, *J* = 5.5 Hz), 77.69, 76.15 (d, *J* = 4.5 Hz), 72.00, 69.95 (d, *J* = 7.5 Hz), 65.11 (d, *J* = 3.5 Hz), 62.33, 34.35 (d, *J* = 9.5 Hz), 32.16, 29.94, 29.89, 29.75, 29.60, 29.56, 29.54, 29.36, 29.33, 28.06, 27.73, 25.09, 22.91, 22.88, 14.20; ^{31}P NMR ($CDCl_3/CD_3OD$ 2.7:1 v/v) δ -0.44; IR (nujol) 3360 (br), 1739, 1038 cm^{-1}. Anal. Calcd for $C_{41}H_{79}O_{11}P$: C, 63.21; H, 10.22. Found: C, 62.52; H, 9.53.

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REFERENCES

1. (a) Berridge, M. J.; Irvine, R. F. *Nature (London)* **1989**, *341*, 197. (b) Berridge, M. J. *ibid.* **1993**, *361*, 315.

2. De Camilli, P.; Emr, S. D.; McPherson, P. S.; Novick, P. *Science* **1996**, *271*, 1533.

3. (a) Aneja, S. G.; Parra, A.; Stoenescu, C.; Xia, W.; Aneja, R. *Tetrahedron Lett.* **1997**, *38*, 803 and references cited therein. (b) Mills, S. J.; Potter, B. V. L. *J. Org. Chem.* **1996**, *61*, 8980. (c) Thum, O.; Chen, J.; Prestwich, G. D. *Tetrahedron Lett.* **1996**, *37*, 9017 (d) Prestwich, G. D. *Acc. Chem. Res.* **1996**, *29*, 503. (e) Ryan, M.; Smith, M. P.; Vinod, T. K.; Lau, W. L.; Keana, J. F. W.; Griffith, O. H. *J. Med. Chem.* **1996**, *39*, 4366.

4. (a) Carpenter, C. L.; Cantley, L. C. *Biochemistry* **1990**, *29*, 11147. (b) Carpenter, C. L.; Cantley, L. C. *Curr. Opin. Cell. Biol.* **1996**, *8*, 153. (c) Shepherd, P. R.; Reaves, B. J.; Davidson, H. W. *Trends Cell Biol.* **1996**, *6*, 92.

5. (a) Hawkins, P. T.; Jackson, T. R.; Stephen, L. R. *Nature* **1992**, *358*, 157. (b) Wennstorm, S.; Hawkins, P.; Cooke, F.; Hara, K.; Yonezawa, K.; Kasuga, M.; Jackson, T.; Claesson-Welch, L.; Stephens, L. *Curr. Biol.* **1994**, *4*, 385.

6. Shibasaki, F.; Homma, Y.; Takenawa, T. *J. Biol. Chem.* **1991**, *266*, 8108.

7. (a) Zhang, J.; Falck, J. R.; Reddy, K. K.; Abrams, C. S.; Zhao, W.; Rittenhouse, S. E. *J. Biol. Chem.* **1995**, *270*, 22807. (b) Burgering, B. M. T.; Coffer, P. J. *Nature* **1995**, *376*, 599. (c) Franke, T. F.; Yang, S.; Chan, T. O.; Datta, K.; Kazlauskas, A.; Morrison, D. K.; Kaplan, D. R.; Tsichlis, P. N. *Cell* **1995**, *81*, 727. (d) Hammonds-Odie, L. P.; Jackson, T. R.; Profit, A. A.; Blader, I. J.; Turck, C. W.; Prestwich, G. D.; Theibert, A. B. *J. Biol. Chem.* **1996**, *261*, 18859. (e) Franke, T. F.; Kaplan, D. R.; Cantley, L. C.; Toker, A. *Science* **1997**, *275*, 665.

8. (a) Lips, D. L.; Majerus, P. W. *J. Biol. Chem.* **1989**, *264*, 19911. (b) Serunian, L. A.; Haber, M. T.; Fukui, T.; Kim, J. W.; Rhee, S. G.; Lowenstein, J. M.; Cantley, L. C. *J. Biol. Chem.* **1989**, *264*, 17809.

9. (a) Kozikowski, A. P.; Fauq, A. H.; Malaska, M. J.; Tückmantel, W.; Ognyanov, V. I.; Powis, G. *Curr. Med. Chem.* **1994**, *1*, 1. (b) Kozikowski, A. P.; Tückmantel, W.; Powis, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1379. (c) Kozikowski, A. P.; Powis, G.; Fauq, A. H.; Tückmantel, W.; Gallegos, A. *J. Org. Chem.* **1994**, *59*, 963.

10. Kozikowski, A. P.; Kiddle, J. J.; Frew, T.; Berggren, M.; Powis, G. *J. Med. Chem.* **1995**, *60*, 1053.

11. review: Bruzik, K. S.; Tsai, M.-D. *Bioorg. Med. Chem.* **1994**, *2*, 49.

12. Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 4429.

13. review: David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

14. Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.

15. (a) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acid Res.* **1984**, *12*, 4051. (b) Beaucage, S. L. *Tetrahedron Lett.* **1984**, *25*, 375.

16. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J.-Y. *Methods Enzymol.* **1987**, *154*, 287.

17. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175.

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