Synthesis and Enzymatic Phosphorylation of a Photoactivatable Dolichol Analogue

D. Grassi, V. Lippuner, M. Aebi,[†] J. Brunner,[‡] and A. Vasella*[,§]

Contribution from the Institutes of Microbiology, Biochemistry, and Organic Chemistry, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland

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Abstract: The synthesis of the photochemical probes **6** and **7** is described. These photoprobes are analogues of dolichol and dolichol phosphate, obligatory intermediates in the *N*-linked glycosylation pathway in the endoplasmic reticulum. The synthesis of **6** and **7** follows a new strategy. It involves the sequential alkylation of a monoterpenoid hydroxysulfonyl dianion with allyl chlorides. The photoreactive group, a 3-(trifluoromethyl)-3-aryldiazirine, was connected to the hydroxylated methyl group of the β-prenyl unit of the fully assembled polyprenyl chain. The photoactivatable dolichol analogue **6** is a substrate for dolichol kinase from yeast membranes, an essential enzyme involved in the *N*-linked glycosylation pathway.

Dolichols[1] (**1**), a family of polyisoprenoid alcohols, are found in all eukaryotic cells and have also been identified in *Archaebacteria*.[2] Dolichols (**1**) occur as mixtures differing only in the number ($n = 9-20$)[3] of (*E*)-isoprene units. The number of isoprene units shows a Gaussian distribution around a mean value, which appears to increase with the complexity of the organism.[4]

Dolichol (Dol, **1**), dolichol phosphate (Dol-P, **2**), and dolichol pyrophosphate (Dol-P-P, **3**) are involved in the biosynthesis of glycoproteins in eukaryotes and prokaryotes.

In eukaryotes, *N*-linked glycosylation of secretory and membrane proteins occurs at the endoplasmic reticulum (ER) and follows a highly conserved pathway.[5] In this pathway dolichol kinase phosphorylates the free alcohol **1**[6−13] to produce Dol-P (**2**), an obligatory intermediate,[14−16] which in turn is a substrate for several enzymes involved in oligosaccharide assembly. The concentration of Dol-P (**2**) in the ER is believed to be rate limiting in these glycosylations.[17,18]

Dol-P-P (**3**) is the membrane anchor and carrier of the oligosaccharide $GlcNAc_2Man_9Glc_3$, which is assembled in a stepwise manner,[16] UDP-GlcNAc,[19] GDP-Man, Dol-P-Man, and Dol-P-Glc serving as glycosyl donors.[5,16] The Dol-P-P-bound oligosaccharide $GlcNAc_2Man_9Glc_3$ is finally transferred to the amido nitrogen of an Asn residue of the triplet Asn-Xaa-Ser-(Thr) of nascent acceptor proteins.[20]

Dol-P (**2**) is also involved in the process of GPI-anchoring in eukaryotes and in *O*-linked glycosylations in fungal cells.[21−27]

In *Archaebacteria*, Dol-P (**2**) is used in the biosynthesis of cell surface glycoproteins.[28]

To investigate the distribution of Dol (**1**) and Dol-P (**2**) in the ER and their possible topological relation to specific proteins recognizing these lipids, we decided to pursue photoaffinity labeling[29,30] using the radiolabeled Dol and Dol-P analogues **4** and **5**, respectively (Figure 1). As a first step toward this goal we report a new strategy for the preparation of functionalized, long-chain dolichols, leading to the synthesis and characterization of the Dol analogue **6** and the corresponding phosphate **7**. We also demonstrate that the photoactivatable dolichol derivative **6** is a substrate for dolichol kinase from yeast.

Results and Discussion

Design of the Photolabeling Reagents 4−7. The proper design of a coenzyme-mimicking photolabeling reagent should solve the problem of introducing a photoactivatable group in a

[†] Institute of Microbiology.
[‡] Laboratory of Biochemistry.
[§] Laboratory of Organic Chemistry.
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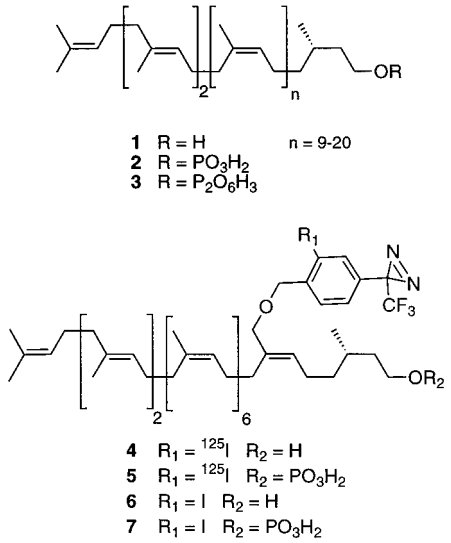

Figure 1.

position that leads to specific labeling of the protein without interfering with the recognition. The inability of mutant Chinese hamster ovary cells to reduce the α-isoprene unit of polyprenols to form Dol results in a severe alteration of *N*-linked glycosylation,[31] suggesting an essential role of the saturated unit in the recognition of Dol. Therefore, this recognition site should be present in the photoactivatable analogue, and the photolabile functionality should be close to this segment to increase the labeling specificity. To trace the photoadducts, the photoprobe must bear a reporter group, preferentially a radioisotope with a high specific radioactivity, such as ^{125}I.

Dolichol analogues **4**/**5** are expected to satisfy these requirements. The chain length of 11 isoprene units was chosen, since authentic Dol of this chain length (Dol-11) is a good substrate for dolichol kinase in mammalian cells[13] and Dol-P-11 is readily accepted by mammalian Dol-P-Man synthase[13] and by UDP-GlcNAc:Dol-P *N*-acetylglucosamine-1-P transferase from yeast.[32]

A 3-(trifluoromethyl)-3-aryldiazirine was chosen as photolabile substituent. In the absence of UV light, this diazirine is stable under a wide range of physical and chemical conditions. It is stable to temperatures up to 80 °C, strong bases, strong acids, oxidizing conditions, and mild reducing agents.[33] Upon irradiation with light of a wavelength of around 350 nm, the diazirine is rapidly photolyzed to generate N_2 and a carbene capable of reacting with the full range of functional groups occurring in biomolecules, including nonactivated C−H bonds.[33]

Synthesis of the Photoactivatable, Non-radiolabeled Dolichol Analogue 6 and the Corresponding Phosphate 7. The syntheses of Dol (**1**) have been reviewed.[34−36] The longest dolichol, Dol-20, the main component of human dolichols, has been prepared by Sato et al. in a convergent way[37,38] (Figure 2). Their synthesis is based on the preparation of **10** from the sulfone **8** and the allyl chloride **9**. The product **10** was

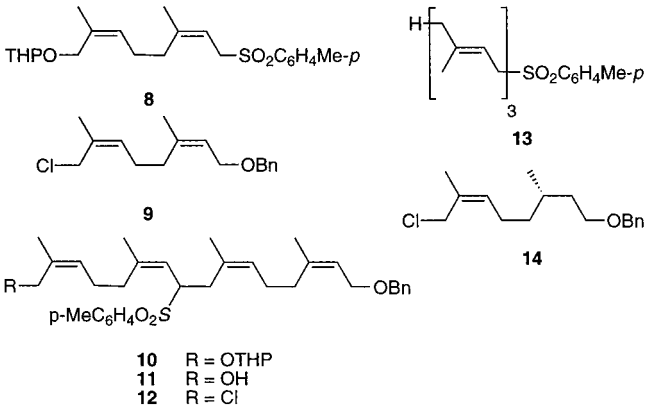

Figure 2.

transformed into **11** and **12**, and chain extended toward the unsaturated ("left") end, using building blocks derived from **12** and the farnesyl sulfone **13** to give a pentadecaprenyl derivative. This was debenzylated and desulfonylated under conditions of the Birch reaction and chain extended toward the saturated ("right") end, using a building block derived from the sesquiterpenoid analogue of **8** and the allyl chloride **14**.

This strategy has been applied to the synthesis of other long-chain prenol derivatives, allowing one to study the behavior of prenols of defined chain length and geometry in biological systems.[39−41] Despite its advantages, the strategy suffers from a significant drawback. The reductive cleavage of the C−S bonds is accompanied by double bond migration and leads to isomeric byproducts (≤15%) from which the desired isomer is separated only with considerable difficulty.[37,42]

We envisaged a shorter synthesis free of this drawback. For this, we conceived a linear synthesis, starting at the saturated hydroxyl terminus while still using the alkylation of sulfonyl anions as one of the key steps. However, we decided to use phenyl sulfones instead of *p*-tolyl sulfones, to avoid the deprotonation of the tolylsulfonyl group with the strong bases required in the coupling steps.[43,44] The synthesis from the reduced end obviates the use of benzyl protecting groups. Both the removal of all sulfonyl groups in one step at the end of the chain assembly and the use of the hydroxysulfone **21** (Scheme 1) rather than the corresponding THP derivative should further reduce the number of steps. In addition, desulfonylation with $LiBEt_3H$ in the presence of a Pd catalyst[45] should proceed without formation of isomeric byproductes.[46]

The synthesis started with the preparation of the acrylate **20** and the hydroxy sulfone **21** (Scheme 1). The acrylate **20** was prepared from (*S*)-citronellol (**15**). Ozonolysis of the triisopropylsilyl ether **16** afforded aldehyde **17** in 65−83% yield. Baylis−Hillman reaction of **17** with methyl acrylate (**18**) yielded 62−79% of acrylate **19** but proceeded very slowly.[47] Acetylation led to the desired building block **20** as an approximately

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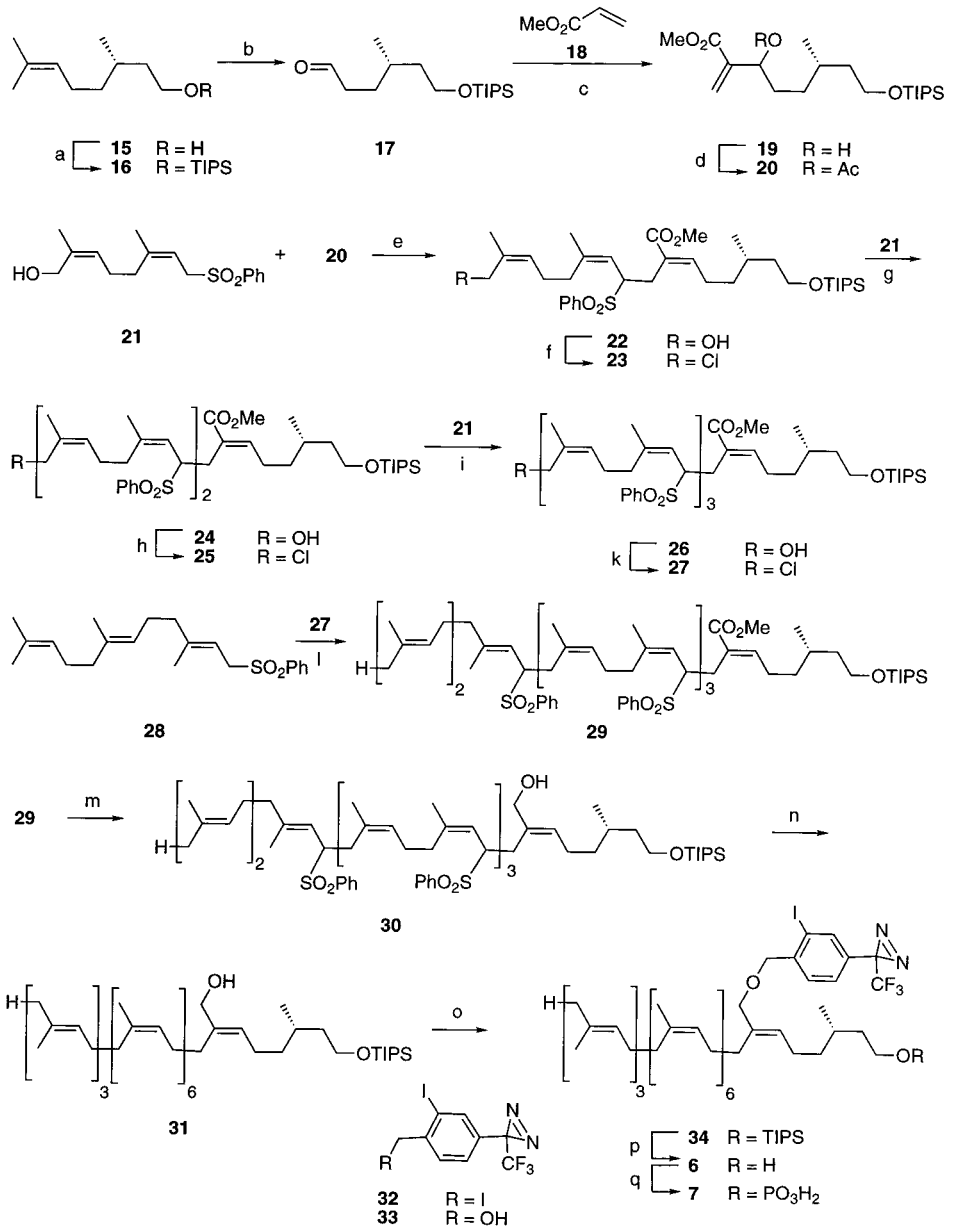
Scheme 1[a]



[a] (a) TIPSCl, imidazole, CH_2Cl_2; (b) 1. O_3, CH_2Cl_2, −78° 2. Me_2S (65−83% from **15**); (c) DABCO (62−79%); (d) Ac_2O, pyridine, DMAP, CH_2Cl_2 (90−95%); (e) BuLi, THF, DMTP, −78° (69%); (f) MsCl, LiCl, collidine, DMF, 0°; (g) BuLi, THF, DMTP, −78° (52% from **22**); (h) MsCl, LiCl, collidine, DMF, 0° (92%); (i) BuLi, THF, DMTP, −78° (76%); (k) MsCl, LiCl, collidine, 0° (89%); (l) BuLi, THF, DMTP, −78° (66%); (m) DIBAH, CH_2Cl_2, −78° (78%); (n) $LiEt_3BH$, [$PdCl_2$(dppp)], THF, 0° (43−51%); (o) **32**, benzene, NaOHaq, TBAI; (p) THF, TBAF (56% from **31**); (q) $POCl_3$, NEt_3, hexane (68%).

1:1 mixture of two diastereoisomers. The hydroxysulfone **21** was prepared according to Sato et al.[41,48]

Alkylation with **20** of the dianion derived from **21** afforded 69% of the ester **22**. There was no advantage in protecting the hydroxyl group of **21**, since coupling of either the alcohol or its THP derivative with **20** proceeded with similar yields. The (*E*)-configuration of the acrylic double bond was evidenced by the typical chemical shift (6.83 ppm) of the acrylic H.[49] The alcohol **22** was transformed into the allyl chloride **23**. Alkylation of the dianion derived from **21** with **23** led to the triterpene **24** in an overall yield of 52% from **22**. Deoxyhalogenation of **24** gave allyl chloride **25**, which was treated with the same dianion derived from **21** to afford the tetraterpene **26** (70% from **24**). The chloride **27**, obtained in 89% yield, was used to alkylate the anion derived from the farnesyl sulfone **28**, yielding 66% of the tetrasulfone **29**.

Desulfonylation of **29** with $LiBEt_3H$ in the presence of [$PdCl_2$(dppp)] (dppp = 1,3-bis(diphenylphosphino)propane) was accompanied by partial (50%, NMR) 1,4-reduction of the acrylic ester function. Therefore, **29** was first reduced with diisobutylaluminium hydride (DIBAH) to give the alcohol **30** (78%) and then desulfonylated with $LiBEt_3H$ in the presence of 30 mol % [$PdCl_2$(dppp)].[45,46] Although the desulfonylation was incomplete, we isolated 43−51% of **31**, uncontaminated by isomeric byproducts.

Benzyl iodide **32** is available by treating the known alcohol **33**[33] with methyltriphenoxyphosphonium iodide.[50] Alkylation of allylic alcohol **31** with **32** under phase transfer conditions led to **34**, which was desilylated with tetrabutylammonium

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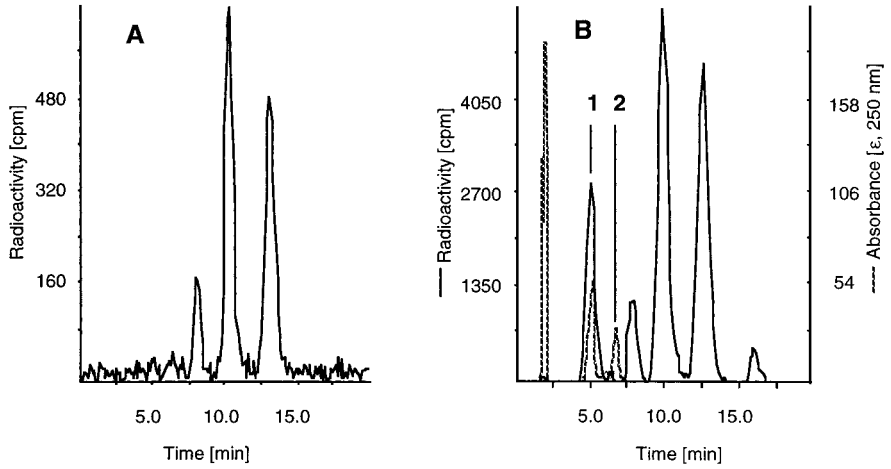

Figure 3. Enzymatic phosphorylation of dolichol analogue **6**. Membranes derived from dolichol kinase overexpressing yeast cells and [γ-^{32}P]-CTP were incubated under assay conditions; lipids were extracted and subjected to reversed phase HPLC. ^{32}P-radioactivity and/or absorption at 250 nm were monitored. (A) Analysis of extract from an assay containing only yeast membrane lipids; Dol-14-^{32}P, Dol-15-^{32}P, and Dol-16-^{32}P give rise to distinct peaks. (B) Analysis of extract from an assay containing yeast membranes (dolichol kinase and endogenous dolichols) plus Dol analogue **6**. After incubation, extracted lipids were mixed with synthetic, nonradioactive reference compound **7**. Enzymatically phosphorylated **6** (1, detected by radioactivity) coelutes with synthetic reference compound **7** (detected by UV absorbance, dashed line). The second UV signal (2) derives from **6** added to the kinase assay. The radio signals with higher retention time derive from Dol-14-^{32}P, Dol-15-^{32}P, Dol-16-^{32}P, and Dol-17-^{32}P.

fluoride (TBAF) in THF, yielding the desired photoactivatable dolichol derivative **6** (56% from **31**). It was phosphorylated[51] with phosphorous oxychloride, followed by aqueous workup. The product **7** was purified by ion exchange and size exclusion chromatography to give the phosphate **7** in 68% yield.

Enzymatic Phosphorylation of Dolichol Analogue 6. To test if photoprobe **6** is a substrate for dolichol kinase, we incubated **6** with membranes[9] derived from dolichol kinase overproducing yeast cells, in the presence of [γ-^{32}P]-CTP as phosphoryl donor. Since the membrane extract contains endogenous yeast dolichols, the phosphorylation of **6** competes with the phosphorylation of the endogenous substrate.

In the absence of **6**, HPLC analysis (Figure 3A, radio detection) of the lipid extract from the kinase assay showed a characteristic pattern of ^{32}P-labeled yeast dolichol phosphates (Dol-14, Dol-15, Dol-16).[52] When **6** was added, the chromatogram of the lipid extract showed an additional radio signal possessing the same retention time as the chemically synthesised phosphate **7** (Figure 3B, 1). The second UV-detected signal (2) is derived from excess photoprobe **6**, not phosphorylated in the assay. These results provide strong evidence that the dolichol analogue **6** is phosphorylated by dolichol kinase in the membrane preparation.

To evaluate the substrate properties of **6**, the apparent affinity of this lipid for dolichol kinase was compared to that of Dol-11, as described in the Experimental Section. Dol-11 was chosen as reference, since it contains the same number of isoprene units as **6** and is a good substrate for dolichol kinase.[13] Although the photoprobe **6** is a substrate for the kinase, it proved approximately 170 times less stimulatory to the the kinase activity than Dol-11, indicating that the additional photoactivatable functionality interferes with the phosphorylation of **6**. We therefore assume that the photolabile group is located close to the active site of the kinase in the enzyme−substrate complex and that the radiolabeled analogue of **6** (**4**) is likely to be useful for studying the active site of this enzyme. In addition, these results open the possibility to specifically label other Dol or Dol-P recognizing enzymes with the photoprobes **4** and **5**.

Experimental Section

General Methods. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 200−500 MHz on Varian instruments, chemical shifts are reported in ppm, and coupling constants (*J*) are given in hertz. IR spectra were recorded on a Perkin Elmer 1600 Series FT-IR spectrometer, FAB mass spectra were recorded on a VG-SABSEQ, and ESI mass spectra on a Finnigan MAT TSQ 7000 instrument. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany), column chromatography was performed with silica gel 60 (230−400 mesh) from Fluka (Buchs, Switzerland), gel filtration was performed with Sephadex LH-20 from Pharmacia Biotech (Uppsala, Sweden), and ion exchange chromatography was performed with DEAE Sepharose CL-6B from Pharmacia Biotech. Dolichol-11 was purchased from Sigma (St. Louis, MO). (*E*,*E*)-Farnesol (>98%) and (*S*)-citronellol (ee >98%) were gifts from R. K. Müller, F. Hoffmann-La Roche AG (Basel, Switzerland). [γ-^{32}P]-CTP (25 Ci/mmol, 10 mCi/mL) was purchased from ICN (Irvine, CA). For quantification of ^{32}P, β-counting system LS 180S from Beckman was used, with IRGA-SAFE PLUS (Packard) as scintillation fluid. Plasmid pSec5920 was a gift from R. Schekman, University of California, Berkeley.

Dolichol Kinase Assay. Microsomal membranes were prepared according to Knauer and Lehle[53] from 4 L cultures with the following modifications. Unless otherwise specified, all steps were performed at 4 °C. Cells from *Saccharomyces cerevisiae* strain DBY746 transformed with the plasmid pSec5920, which contains the *S. cerevisiae* dolichol kinase gene, were grown at 30 °C in synthetic minimal medium supplemented with adenine, histidine, uracil, and tryptophan according to Sherman[54] to $OD_{546nm} = 1.0$. The cells were pelleted, washed once with water, resuspended in 300 mL buffer containing 50 mM Tris HCl (pH 7.69), 10 mM 2-mercaptoethanol and protease inhibitors (1 mM PMSF, 2 μg/mL each of pepstatin A, chymostatin, antipain, aprotonin), and broken in a bead beater (Biospec Products, U.S.A.) at 0 °C using six 1 min pulses with 1 min intervals. Cell wall and unlysed cells were removed by centrifugation in a Sorvall GS3 rotor at 3000 rpm, and the supernatant was recentrifuged at 22000 rpm in a Beckman Ti45 rotor for 30 min. The membrane pellet was washed once in the same buffer. The membranes were resuspended in 3 mL buffer containing 50 mM Tris HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 30% glycerol and were homogenized using a Potter homogenizer yielding a concentration of 14 μg/μL protein as determined by the BioRad protein assay using bovine serum albumin

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(BSA) as a standard. Aliquots of extract were frozen in liquid nitrogen and stored at −80 °C.

Dolichol kinase activity was assayed using a modified procedure of Heller et al.[9] A known amount of **6** (8.4−170 μg) or Dol-11 (0.038−0.77 μg) dissolved in $CHCl_3$ or CCl_4 was placed in a pointed test tube, and the solvent was evaporated under reduced pressure. Nikkol (10 μL, 4%, BL−8SY, Nikko Chemicals Co LTD, Tokyo) was added and mixed with the dolichol derivative using a Hamilton syringe followed by incubation at 37 °C for 30 min and sonification for 5 min. Buffer was added, and the enzymatic reaction was started by the addition of 300 μg of membrane protein. The final volume was 100 μL, containing 0.05 M TrisHCl (pH 7.5), 0.01 M UTP, 0.03 M $CaCl_2$, 15 μCi [γ-^{32}P]-CTP (25 Ci/mmol, 0.6 nmol), 0.9 nmol unlabeled CTP (total 15 μm CTP), and 0.4% nikkol. The mixture was incubated at 24 °C for 20 min, and the reaction was stopped by the addition of 750 μL of KOH in MeOH (1 M) followed by a 25 min incubation at 37 °C to hydrolyze alkali-labile lipids (especially phosphatidic acid[9]). Water (1 mL) was added, and the lipids were extracted with $CHCl_3$/MeOH (2:1, 3 × 2.3 mL). The combined organic phases were washed with 1 mL of the upper phase of a mixture containing $CHCl_3$/MeOH/H_2O 2.6:1.3:1 and 0.02 M KCl. An aliquot of the organic phase (1 mL) was transferred into a scintillation vial and evaporated, and the activity (cpm) was determined. The remainder (4 mL) was evaporated under a stream of N_2, dissolved in 2-propanol/MeOH/H_2O 40:60:5 (20 mM H_3PO_4, 250 μL) filtered through a Teflon filter (Chromafil, Typ O-20/3, Macherey-Nagel, Germany) and subjected to HPLC analysis.[55]

High-Pressure Liquid Chromatography (HPLC). HPLC[55] was performed on a Merck system with a radiochromatography detector (series A-500) from Packard, using a Hypersil ODS 3 column (100 × 4.6 mm) from Supelco (Buchs, Switzerland). Solvents were degassed by sonification for 10 min. Dol-P derivatives were eluted with 2-propanol/MeOH/H_2O 40:60:5 containing 20 mM H_3PO_4 for 25 min. After each run, the column was regenerated with acetonitrile for 10 min. The flow rate was 1 mL/min, and the injection volume was 40−80 μL. The apparent affinity of **6** for dolichol kinase was compared to that of Dol-11. Reversed phase HPLC allowed the separation of the different Dol-P species, and their relative amount was determined by integration of the peak area. The ratio of Dol-P formed by phosphorylation of the exogenous/endogenous substrate was determined by dividing the peak area of the endogenous Dol-P (phosphate of **6** or Dol-11-P) by the sum of the peak areas of the yeast dolichol phosphates. In order to obtain a 1:5 ratio of exogenous/endogenous Dol-P, the concentration of **6** in the kinase assay had to be about 170 times higher than the one of Dol-11 (505 μM, 3 μM respectively).

(3*S*)-3,7-Dimethyloct-6-en-1-yl Triisopropylsilyl (TIPS) Ether ((*S*)-Citronellyl Triisopropylsilyl Ether, 16). Triisopropylchlorosilane (7.06 mL, 33.3 mmol) was added slowly at 0 °C to a solution of (*S*)-citronellol (**15**, 4.956 g, 31.71 mmol) and imidazole (4.32 g, 63.45 mmol) in CH_2Cl_2 (75 mL). The mixture was allowed to warm to room temperature (rt), stirred for 1 h, and treated with a saturated aqueous (aq) NH_4Cl solution (20 mL). The organic phase was separated, washed with H_2O (3 × 20 mL) and brine (20 mL), and dried over Na_2SO_4. Evaporation gave **16** (10.134 g, quantitative), which was used for the next reaction without further purification. TLC: R_f (hexane/Et_2O 1:1) 0.84. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.13−5.07 (m, 1H), 3.74−3.67 (m, 2H), 2.03−1.96 (m, 2H), 1.70−0.98 (m, 32H), 0.88 (d, J = 6.9, Me).

(4*S*)-4-Methyl-6-(triisopropylsilyloxy)hexanal (17). Ozone was bubbled through a solution of **16** (8.983 g, 28.74 mmol) in CH_2Cl_2 (74 mL) at −78 °C for 4.5 h. After Me_2S (4.8 mL) was added, the solution was allowed to warm to 25 °C and stirred overnight. Evaporation and flash column chromatography (hexane/EtOAc 15:1) gave **17** (6.812 g, 83% yield) as a colorless oil. TLC: R_f (hexane/CH_2Cl_2 2:1) 0.18. IR ($CHCl_3$): 2944s, 2867s, 2727m, 1722s, 1603w, 1463s, 1410w, 1382m, 1367m, 1248m, 1100s, 1070s, 1013s, 996s, 919m, 883s, 838w, 657s, 560w, 543w, 530w, 514w, 502w cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.77 (t, J = 1.8, 1H), 3.72−3.65 (m, 2H), 2.48−2.40 (m, 2H), 1.80−1.20 (m, 5H), 1.20−0.96 (m, 21H), 0.91 (d, J = 6.4, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.93 (d), 61.33 (t), 41.73 (t), 39.69 (t), 29.23 (d), 29.02 (t), 19.43 (q), 18.05 (6q), 12.00 (3d). EI-MS: *m/z* 285 (0.5, M^+), 243 (100), 213 (13), 201 (63), 187 (24), 171 (24), 157 (15), 145 (19), 131 (67), 119 (15), 115 (13), 103 (40), 101(13), 95 (23), 87 (12), 81 (13), 75 (33), 69 (14), 61 (19), 59 (12). Anal. Calcd for $C_{16}H_{34}O_2Si$ (286.53): C, 67.07; H, 11.96. Found: C, 66.90; H, 11.90.

Methyl (1′*RS*,4′*S*)-2-[1-Hydroxy-4-methyl-6-(triisopropylsilyloxy)-hexyl]acrylate (19). A mixture of **17** (6.708 g, 23.4 mmol), methyl acrylate (**18**, 4.059 mL, 47.1 mmol), and 1,4-diazabicyclooctane (533 mg, 4.75 mmol) was stirred at 25 °C for 37 d. Evaporation of excess methyl acrylate and flash column chromatography (hexane/EtOAc 10:1) of the residue gave **19** (5.431 g, 62% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 10:1) 0.11. IR ($CHCl_3$): 3608w, 3007w, 2945s, 2867s, 1713s, 1653w, 1628w, 1463m, 1441m, 1382w, 1337w, 1158m, 1098s, 1069m, 1014w, 996w, 960w, 883m cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.23 (d, J = 1, 1H), 5.80 (m, 1H), 4.38−4.34 (m, 1H), 3.79 (s, OMe), 3.74−3.67 (m, 2H), 2.58−2.52 (m, 1H), 1.71−1.43 (m, 5H), 1.43−1.23 (m, 2H), 1.14−1.02 (m, 21H), 0.90 (d, J = 6.5, Me). ^{13}C NMR (75 MHz, $CDCl_3$, 1:1.1 mixture): δ 142.5/142.00 (2s), 125.1/125.0 (2t), 72.3/72.1 (2d), 61.6 (t), 51.9 (q), 40.1/39.9 (2t), 33.7 (2t), 33.2/33.1 (2t), 29.4/29.3 (2d), 19.7/19.6 (2q), 18.1 (6q), 12.0 (3d). EI-MS: *m/z* 341 (5), 329 (6), 181 (10), 149 (84), 131 (43), 125 (15), 121 (100), 119 (42), 115 (34), 107 (12), 103 (23), 95 (19), 93 (40), 87 (17), 83 (19), 81 (13), 79 (17), 77 (12), 75 (38), 73 (12), 69 (15), 61 (25), 59 (27, 55 (17). Anal. Calcd for $C_{20}H_{40}O_4Si$ (372.63): C, 64.47; H, 10.82. Found: C, 64.44; H, 10.83.

Methyl (1′*RS*,4′*S*)-2-[1-Acetoxy-4-methyl-6-(triisopropylsilyloxy)-hexyl]acrylate (20). Pyridine (3.35 mL, 41.5 mmol), acetic anhydride (2.4 mL, 25.4 mmol), and 4-(dimethylamino)pyridine (33.5 mg, 0.27 mmol) were added at 24 °C to a solution of **19** (3.056 g, 8.2 mmol) in CH_2Cl_2 (86 mL). The mixture was stirred for 2 h and then poured into a saturated aq solution of NH_4Cl (20 mL). The organic phase was washed with $CuSO_4$ (20% in H_2O, 2 × 15 mL) and brine (3 × 20 mL). Drying over Na_2SO_4 and evaporation, followed by flash column chromatography (hexane/EtOAc 10:1), gave **20** (3.121 g, 92% yield) as a colorless oil. TLC: R_f (hexane/Et_2O 10:1) 0.20. IR ($CHCl_3$): 2945s, 2867s, 1729s, 1632w, 1463m, 1440m, 1371m, 1248s, 1159m, 1100s, 1068m, 1028m, 996m, 959m, 908w, 883m, 657w cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.28 (br s, 1H), 5.76−5.75 (m, 1H), 5.62−5.55 (m, 1H), 3.78 (s, OMe), 3.75−3.64 (m, 2H), 2.09 (s, Ac), 1.88−1.01 (m, 28H), 0.88 (d, J = 6.2, Me). ^{13}C NMR (75 MHz, $CDCl_3$, 1:1.2 mixture): δ 170.3 (s), 166.1 (s), 140.5/140.4 (2s), 125.5/125.3 (2t), 72.3/72.2 (2d), 61.6/61.6 (2t), 52.1 (q), 40.1/39.9 (2t), 32.6/32.5 (2t), 31.9/31.8 (2t), 29.4/29.3 (2d), 21.1 (q), 19.7/19.5 (2q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 415 (10, M^+), 371 (31), 355 (13), 313 (15), 312 (34), 311 (100), 181 (10), 174 (10), 173 (50). Anal. Calcd for $C_{22}H_{42}O_5Si$ (414.67): C, 63.73; H, 10.21. Found: C, 63.85; H, 10.45.

Methyl (2*E*,4*RS*,5*Z*,9*Z*,4′*S*)-4-(Benzenesulfonyl)-11-hydroxy-6,10-dimethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]undeca-5,9-dienoate (22). Under Ar atmosphere at −78 °C, *n*-butyllithium (1.3 m in hexane, 15.2 mL, 19.8 mmol) was added dropwise to a solution of **21** (2.325 g, 7.90 mmol) in THF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (49 mL/12 mL), leading to an orange precipitate. The mixture was warmed to −10 °C, kept at this temperature for 10 min until phase separation, cooled to −78 °C, and treated dropwise within 70 min with **20** (3.274 g, 7.90 mmol) in THF (21 mL). The mixture was stirred and warmed to −30 °C (2 h), leading to a homogeneous pale yellow solution. The mixture was poured into saturated aq NH_4Cl (60 mL). The aqueous phase was extracted at 0 °C with hexane/Et_2O 1:1 (4 × 20 mL). The combined organic layers were washed with brine (30 mL) and dried over Na_2SO_4. Flash column chromatography (hexane/EtOAc 3:1) of the residue (5.85 g) gave **22** (3.544 g, 69% yield) as a colorless oil. TLC: R_f (hexane/Et_2O 1:1) 0.08. IR ($CHCl_3$): 3523w, 3007m, 2945s, 2867s, 1772w, 1706s, 1646m, 1586w, 1462s, 1448s, 1438s, 1380m, 1305s, 1146s, 1085s, 998s, 943w, 883s, 836w, 657m, 601m, 549m, 512m cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.89−7.84 (m, 2H), 7.63−7.49 (m, 3H), 6.83 (t, J = 7.3, 1H), 5.07−4.98 (m, 2H), 4.15 (dt, J = 3.7, 10.5, 1H), 4.01 (s, 2H), 3.74−3.64 (m, 2H), 3.65 (s, OMe), 2.92 (dd, J = 3.7, 13.2, 1H), 2.67 (dd, J = 10.5, 13.2, 1H), 2.25−2.04 (m, 2H), 1.88−0.98 (m, 37H), 0.90 (d, J = 6.3, Me). ^{13}C NMR (75 MHz, $CDCl_3$, 1:1 mixture): δ 167.8 (s), 146.8 (d), 145.6 (s), 138.1 (s), 135.4 (s), 133.5

(55) Elmberger, P. G.; Eggens, I.; Dallner, G. *Biomed. Chromatogr.* **1989**, *3*, 20−28.

(d), 129.1 (2d), 128.8 (2d), 126.8 (d), 126.7 (s), 116.6 (d), 63.2 (d), 61.4 (t), 61.3 (t), 51.9 (q), 40.0/39.9 (2t), 36.0 (t), 32.4 (t), 29.3 (d), 26.6/26.5 (2t), 26.1 (t), 25.8 (t), 23.5 (q), 21.3 (q), 19.4 (q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 650 (18), 649 (38, M^+), 507 (16), 491 (14), 490 (41), 489 (97), 475 (15), 463 (17), 457 (16), 454 (12), 431 (13), 413 (16), 379 (12), 283 (20), 273 (12), 267 (10), 257 (10), 256 (17), 255 (69), 241 (13), 229 (12), 227 (16), 215 (17), 213 (14), 201 (17), 199 (17), 191 (15), 189 (17), 187 (31), 185 (20), 183 (11), 179 (11), 177 (11), 175 (15). Anal. Calcd for $C_{36}H_{60}O_6SSi$ (649.02): C, 66.62; H, 9.32; S, 4.94. Found: C, 66.46; H, 9.38; S, 4.71.

Methyl (2*E*,4*RS*,5*Z*,9*Z*,4′*S*)-4-(Benzenesulfonyl)-11-chloro-6,10-dimethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]undeca-5,9-dienoate (23). Mesyl chloride (1.6 mL, 20 mmol) was added dropwise to a solution of **22** (4.440 g, 6.65 mmol), LiCl (0.808 g, 19.1 mmol), and *sym*-collidine (4.46 g, 36.8 mmol) in *N*,*N*-dimethylformamide (DMF, 46 mL) at −3 °C. The mixture was allowed to warm to 3 °C within 1 h leading to the formation of a colorless precipitate after 15 min. The mixture was poured into saturated aq $NaHCO_3$ (30 mL). The solution was extracted with hexane/Et_2O 1:1 (3 × 30 mL), and the combined organic phases were dried over Na_2SO_4 and evaporated. Crude **23** (5.917 g) was used for the next step without further purification. TLC: R_f (hexane/EtOAc 5:1) 0.16. IR ($CHCl_3$): 2945s, 2867s, 1707s, 1646w, 1462m, 1447s, 1438m, 1380m, 1305s, 1281s, 1146s, 1085s, 1037w, 1014w, 998m, 918w, 883m, 837w, 657w, 604w, 548w, 516w, 502w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.91−7.88 (m, 2H), 7.65−7.52 (m, 3H), 6.83 (t, J = 7.3, 1H), 5.16 (t, J = 6.8, 1H), 5.03 (d, J = 10.6, 1H), 4.14 (dt, J = 3.5, 10.8, 1H), 4.02−3.93 (m, CH_2Cl), 3.75−3.67 (m, 2H), 3.66 (s, OMe), 2.94 (dd, J = 3.4, 13.4, 1H), 2.72 (dd, J = 10.8, 13.4, 1H), 2.22−2.05 (m, 2H), 1.90−1.50 (m, 10H), 1.50−0.95 (m, 26H), 0.90 (d, J = 6.4, Me). ^{13}C NMR (75 MHz, $CDCl_3$, 1:1 mixture): δ 167.8 (s), 146.8 (d), 145.4 (s), 138.3 (s), 133.8 (d), 132.3 (s), 130.1 (d), 129.3 (2d), 129.1 (2d), 127.0/126.9 (2s), 117.2 (d), 63.4 (d), 61.5 (t), 51.9 (q), 43.5 (t), 40.0/39.9 (2t), 36.1 (t), 31.8 (t), 29.4/29.3 (2d), 26.6/26.5 (2t), 26.0 (t), 25.9 (t), 23.5 (q), 21.5 (q), 19.4 (q), 18.1 (6q), 12,0 (3d). FAB-MS (NOBA): *m/z* 670 (12), 669 (30), 668 (28), 667 (59, M^+), 625 (11), 623 (22), 528 (17), 527 (49), 526 (43), 525 (100), 493 (14), 489 (15), 483 (16), 482 (11), 481 (31), 449 (14), 379 (13), 291 (15), 256 (11), 255 (45), 187 (12), 185 (11), 173 (10), 171 (12), 169 (12), 165 (14), 161 (12), 159 (18), 157 (44), 155 (13), 154 (21), 151 (11).

Methyl (2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,4′*S*)-4,12-Di(benzenesulfonyl)-19-hydroxy-6,10,14,18-tetramethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonadeca-5,9,13,17-tetraenoate (24). Under an Ar atmosphere at −78 °C, *n*-butyllithium (9.33 mL, 1.6 m in hexane, 14.9 mmol) was added dropwise to a solution of **21** (1.759 g, 5.98 mmol) in THF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (69 mL/17 mL), leading to an orange solution. It was warmed to −15 °C, kept at this temperature for 25 min, cooled to −78 °C, and treated dropwise with crude **23** (5.917 g) in THF (21 mL) within 30 min. The solution was allowed to warm to −40 °C within 80 min, leading to a yellow solution. The mixture was poured into saturated aq NH_4Cl (80 mL). The aqueous phase was extracted at 0 °C with hexane/Et_2O 1:1 (3 × 30 mL). The organic phases were combined, washed with brine (30 mL), and dried over Na_2SO_4. Evaporation followed by flash column chromatography (hexane/EtOAc 3:1) gave a mixture of **24** and *sym*-collidine from the preceding deoxyhalogenation step (3.604 g, 1:1 mixture), containing approximately 3.190 g of **24** (52% yield from **22**). A sample of **24** was purified by dissolving it in CH_2Cl_2 and washing twice with 0.1 M HCl at 0 °C. TLC: R_f (hexane/EtOAc 1:1) 0.32. IR ($CHCl_3$): 3528w, 3008w, 2944s, 2867s, 1706s, 1646w, 1458m, 1447s, 1438m, 1379w, 1304s, 1145s, 1085s, 998m, 909m, 883m, 657w, 598m, 549w, 536w, 528w, 502w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.88−7.81 (m, 4H), 7.65−7.48 (m, 6H), 6.85−6.78 (t, J = 7.7, 1H), 5.10−4.88 (m, 4H), 4.20−4.09 (m, 1H), 4.09−3.95 (m, 2H), 3.88−3.65 (m, 3H), 3.64 (s, OMe), 2.91 (dd, J = 3.5, 13.3, 1H), 2.74−2.58 (m, 2H), 2.43−2.28 (m, 1H), 2.22−2.04 (m, 2H), 1.90−1.00 (m, 47H), 0.89 (d, J = 6.5, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.8 (s), 146.9 (d), 145.7 (s), 145.2 (s), 138.2 (s), 138.0 (s), 135.5 (s), 133.9 (d), 133.8 (d), 130.9/130.7 (2s), 129.4 (2d), 129.3 (2d), 129.1 (2d), 129.0 (2d), 128.0 (d), 127.1 (d), 126.9 (s), 118.0 (d), 116.8 (d), 63.4 (d), 63.3 (d), 61.5 (t), 61.4 (t), 51.9 (q), 40.0/39.9 (2t), 36.1 (t), 32.4 (t), 32.0 (t), 31.8 (t), 30.3 (t), 29.4/29.3 (2d), 26.6/26.5 (2t), 26.0/25.9 (2t), 25.7 (t), 23.7 (q), 23.5 (q), 23.4 (q), 21.4 (q), 19.4 (q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 1057 (11), 927 (6), 926 (10, M^+), 768 (17), 767 (39), 766 (70), 642 (17), 626 (19), 625 (50), 624 (100), 621 (11), 609 (17), 591 (15), 501 (16), 475 (11), 255 (23), 201 (21), 199 (11), 187 (11), 175 (24), 173 (16), 171 (10), 165 (12), 161 (15), 159 (27), 157 (34), 154 (26), 151 (14). Anal. Calcd for $C_{52}H_{80}O_8S_2Si$ (925.40): C, 67.49; H, 8.71; S, 6.93. Found: C, 67.32; H, 8.83; S, 7.13.

Methyl (2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,4′*S*)-4,12-Di(benzenesulfonyl)-19-chloro-6,10,14,18-tetramethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonadeca-5,9,13,17-tetraenoate (25). Mesyl chloride (0.8 mL, 10.2 mmol) was added dropwise to a solution of **24** (3.476 g, contaminated with *sym*-collidine, approximately 3.44 mmol), LiCl (0.432 g, 10.2 mmol) and *sym*-collidine (2.25 mL, 17 mmol) in DMF (36 mL) at −3 °C. The mixture was allowed to warm to 3 °C within 75 min, leading to the formation of a colorless precipitate after 15 min. The mixture was poured into saturated aq $NaHCO_3$ solution (30 mL) and extracted with hexane/Et_2O 1:1 (3 × 20 mL). The combined organic phases were washed with aq NH_4Cl (20 mL) and brine (20 mL) and dried over Na_2SO_4. Evaporation followed by flash column chromatography (hexane/EtOAc 3:1) gave **25** (2.963 g, 92% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 1:1) 0.82. IR ($CHCl_3$): 2944s, 2867s, 1708s, 1646w, 1462m, 1447s, 1379m, 1304s, 1248m, 1145s, 1085s, 1014w, 998w, 883m, 658w, 601m, 548w, 536w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.93−7.82 (m, 4H), 7.73−7.49 (m, 6H), 6.90−6.78 (m, 1H), 5.23−5.13 (m, 1H), 5.07−4.92 (m, 3H), 4.24−4.10 (m, 1H), 3.97 (s, CH_2Cl), 3.92−3.69 (m, 3H), 3.66 (s, OMe), 3.02−2.9 (m, 1H), 2.80−2.58 (m, 2H), 2.50−2.30 (m, 1H), 2.28−2.10 (m, 2H), 1.94−0.98 (m, 46H), 0.92 (d, J = 6.6, Me). ^{13}C NMR (75 MHz, $CDCl_3$, mixture): δ 167.7 (s), 146.7 (d), 145.6/145.5 (2s), 144.7 (s), 138.3 (s), 138.1 (s), 133.8 (d), 133.8 (d), 132.4 (s), 130.8/130.7 (2s), 130.1 (d), 129.4 (2d), 129.3 (2d), 129.1 (2d), 129.0 (2d), 128.1 (d), 126.9 (s), 118.3 (d), 116.9 (d), 63.3 (2d), 61.5 (t), 51.8 (q), 43.4 (t), 40.0/39.9 (2t), 36.1 (t), 31.8 (several t), 30.2 (t), 29.4/29.3 (2d), 26.6/26.5 (2t), 25.9−25.8 (several t), 23.5 (q), 23.4 (q), 23.3 (q), 21.6 (q), 19.4 (q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 946 (3), 945 (7), 944 (7), 943 (11, M^+), 803 (16), 802 (16), 801 (29), 662 (19), 661 (46), 660 (48), 659 (100), 657 (12), 627 (14), 501 (17), 255 (10), 211 (11), 157 (12).

Methyl (2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,20*RS*,21*Z*,25*Z*,4′*S*)-4,12,20-Tri(benzenesulfonyl)-6,10,14,18,22,26-hexamethyl-27-hydroxy-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]heptacosa-5,9,13,17,21,25-hexaenoate (26). Under an Ar atmosphere at −78 °C, *n*-butyllithium (4.6 mL, 1.6 m in hexane, 7.37 mmol) was added dropwise to a solution of **21** (943.7 mg, 3.21 mmol) in THF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (37 mL/9 mL), leading to an orange solution, which was warmed to −20 °C, kept at this temperature for 20 min, cooled to −78 °C, and treated dropwise with **25** (2.924 g, 3.1 mmol) in THF (21 mL) within 20 min. This mixture was allowed to warm to −40 °C within 120 min, leading to a yellow solution that was poured into saturated aq NH_4Cl (50 mL). The aqueous phase was extracted at 0 °C with hexane/Et_2O 1:1 (3 × 20 mL). The combined organic phases were washed with brine (20 mL) and dried over Na_2SO_4. Evaporation followed by flash column chromatography (hexane/EtOAc 3:1) gave **26** (2.824 g, 76% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 1:1) 0.33. IR ($CHCl_3$): 3536 w, 2944s, 2867m, 1708m , 1660w, 1447m, 1378m, 1304s, 1248m, 1145s, 1085s, 999w, 883w, 658w, 596m, 550w, 506w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.91−7.78 (m, 6H), 7.70−7.44 (m, 9H), 6.81 (t, J = 7.4, 1H), 5.10−4.82 (m, 6H), 4.23−4.05 (m, 1H), 4.05−3.95 (m, 2H), 3.90−3.64 (m, 4H), 3.63 (s, OMe), 2.98−2.85 (m, 1H), 2.78−2.52 (m, 3H), 2.50−1.98 (m, 4H), 1.98−1.00 (m, 57H), 0.89 (d, J = 6.5, Me). ^{13}C NMR (75 MHz, $CDCl_3$, mixture): δ 167.8 (s), 146.9 (d), 145.7 (s), 145.2 (s), 145.0 (s), 138.3 (s), 138.0 (s), 135.5 (s), 133.8 (several d), 130.7 (several s), 129.4−129.1 (several d), 128.0 (d), 127.1 (d), 126.9 (s), 118.0 (2d), 116.8 (d), 63.3 (several d), 61.5 (2t), 51.80 (q), 39.9 (t), 36.1 (t), 32.3−32.0 (several t), 30.2 (t), 29.3 (d), 26.1−25.6 (several t), 23.5 (several q), 21.4 (q), 19.4 (q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 1223.6 (8, $[M + Na]^+$), 1043 (16), 1042 (22), 919 (14), 918 (22), 916 (11), 902 (26), 901 (53), 900 (80), 868 (12), 778 (11), 777 (21), 776 (35), 774 (10), 760 (24), 759 (57), 758 (93), 756 (11), 751 (15), 745 (10), 744 (17), 727 (10), 726 (16), 610 (11), 609 (20), 502 (13), 501

(30), 476 (13), 475 (31), 379 (10), 267 (17), 255 (31), 229 (10), 227 (11), 213 (11), 201 (26), 199 (16), 187 (18), 185 (13), 175 (19), 173 (20), 171 (16), 169 (11), 167 (10), 165 (16), 163 (12), 161 (23), 159 (42), 158 (11), 157 (38), 155 (20), 154 (40), 152 (13), 151 (13). Anal. Calcd for $C_{68}H_{100}O_{10}S_3Si$ (1201.80): C, 67.96; H, 8.39; S, 8.00. Found: C, 67.79; H, 8.63; S, 7.95.

Methyl (2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,20*RS*,21*Z*,25*Z*,4′*S*)-4,12,20-Tri(benzenesulfonyl)-27-chloro-6,10,14,18,22,26-hexamethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]heptacosa-5,9,13,17,21,25-hexaenoate (27). Mesyl chloride (0.42 mL, 5.4 mmol) was added dropwise to a solution of **26** (2.159 g, 1.80 mmol), LiCl (0.229 g, 5.4 mmol) and *sym*-collidine (1.2 mL, 9.0 mmol) in DMF (36 mL) at −3 °C. The mixture was stirred at −3 °C for 135 min, leading to the formation of a colorless precipitate after 15 min. The mixture was poured into saturated aq. $NaHCO_3$ (20 mL) and extracted with hexane/Et_2O 1:1 (3 × 15 mL). The combined organic phases were dried over Na_2SO_4 and evaporated. Purification by flash column chromatography (hexane/EtOAc 3:1) gave **27** (1.953 g, 89% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 3:1) 0.16. IR ($CHCl_3$): 2944s, 2867s, 2361w, 2341w, 1772w, 1706m, 1684w, 1670w, 1662w, 1654w, 1647w, 1636w, 1612w, 1586w, 1570w, 1558w, 1540w, 1522w, 1508w, 1498w, 1489w, 1458m, 1447s, 1379m, 1304s, 1145s, 1085s, 1014w, 998w, 883w, 843w, 658w, 596m, 550w, 537w, 526w, 517w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.93−7.78 (m, 6H), 7.70−7.43 (m, 9H), 6.82 (t, J = 7.5, 1H), 5.20−5.08 (m,1H), 5.03−4.83 (m, 5H), 4.15 (dt, J = 3.7, 10.8, 1H), 3.93 (s, CH_2Cl), 3.90−3.65 (m, 4H), 3.64 (s, OMe), 2.93 (dd, J = 3.7, 10.8, 1H), 2.79−2.55 (m, 3H), 2.53−2.03 (m, 4H), 1.98−1.00 (m, 56H), 0.89 (d, J = 6.2, Me). ^{13}C NMR (75 MHz, $CDCl_3$, mixture): δ 167.5 (s), 146.5 (d), 145.4 (s), 144.6 (s), 144.4 (s), 138.1 (s), 137.9 (s), 133.6 (several d), 132.2 (s), 130.7 (s), 130.5 (s), 130.3 (s), 129.9−127.8 (several d), 126.7 (s), 118.1 (d), 117.9 (d), 116.7 (d), 63.2−63.1 (3d), 61.4 (t), 51.7 (q), 43.4 (t), 40.0 (t), 39.9 (t), 36.0 (t), 31.9−30.1 (several t), 29.4 (d), 26.5−25.6 (several t), 23.5−23.3 (several q), 21.6 (q), 19.4 (q), 18.1 (6q), 12.0 (3d). FAB-MS (NOBA): *m/z* 1241.5 (9, $[M + Na]^+$, 1078 (12), 939 (19), 938 (40), 937 (44), 936 (68), 904 (10), 797 (25), 796 (54), 795 (56), 794 (100), 792 (10), 761 (16), 752 (10), 751 (18), 610 (13), 609 (23), 502 (12), 501 (30), 475 (22), 303 (13), 255 (16), 237 (20), 213 (10), 211 (15), 201 (11).

Methyl (2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,20*RS*,21*Z*,25*Z*,28*RS*,29*E*,33*E*,4′*S*)-4,12,20,28-Tetra(benzenesulfonyl)-6,10,14,18,22,26,30,34,38-nonamethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonatriaconta-5,9,13,17,21,25,29,33,37-nonaenoate (29). Under an Ar atmosphere at −78 °C, *n*-butyl lithium (1.75 mL, 1.5 m in hexane, 2.63 mmol) was added dropwise to a solution of **28** (720.8 mg, 2.08 mmol) in THF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (25 mL/6 mL). The resulting orange solution was warmed to −20 °C, kept at this temperature for 25 min, cooled to −78 °C, and treated dropwise with **27** (1.953 g, 1.6 mmol) in THF (14 mL). The mixture was allowed to warm to −30 °C within 130 min, leading to a yellow solution that was poured into saturated aq. NH_4Cl (30 mL). The aqueous phase was extracted at 0 °C with hexane/Et_2O 1:1 (3 × 10 mL). The combined organic phases were washed with brine (20 mL) and dried over Na_2SO_4. Evaporation followed by flash column chromatography (hexane/EtOAc 3:1) gave **29** (1.612 g, 66% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 2:1) 0.19. IR ($CHCl_3$): 3038w, 2943m, 2866m, 1706m, 1661w, 1602w, 1447m, 1379w, 1304s, 1178w, 1145s, 1085s, 999w, 884w, 595m, 552w, 539w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.91−7.78 (m, 8H), 7.70−7.47 (m, 12H), 6.87−6.79 (m, 1H), 5.12−4.85 (m, 9H), 4.16 (dt, J = 3.7, 10.7, 1H), 3.91−3.77 (m, 3H), 3.77−3.67 (m, 2H), 3.65 (s, OMe), 2.99−2.89 (m, 1H), 2.80−2.55 (m, 4H), 2.52−2.32 (m, 3H), 2.29−1.00 (m, 78H), 0.91 (d, J = 6.3, Me). ^{13}C NMR (75 MHz, $CDCl_3$, mixture): δ 167.7 (s), 146.7 (d), 145.4−144.8 (several s), 138.1 (several s), 133.8 (several d), 131.6−130.6 (several s), 129.4−127.6 (several d), 126.9 (s), 124.3 (d), 123.4 (d), 118.0 (d), 117.3 (d), 116.8 (d), 63.5−63.2 (4d), 61.8 (t), 51.8 (q), 39.7 (several t), 36.1 (t), 31.9 (several t), 30.0 (several t), 29.3 (d), 26.7−25.7 (several t), 23.3 (several q), 19.4 (q), 18.1 (6q), 17.7 (q), 16.2 (q), 16.0 (q), 12.0 (3d). FAB-MS (NOBA): *m/z* 1552.4 (14, $[M + Na]^+$), 1248 (11), 1247 (28), 1246 (37), 1107 (12), 1106 (32), 1105 (1), 1104 (100), 1102 (17), 1072 (12), 964 (27), 963 (63), 961 (91), 960 (14), 929 (14), 886 (13), 752 (13), 752 (24), 744 (11), 656 (10), 614 (16), 611 (12), 610 (23), 514 (10), 501 (24), 475 (23), 471 (13), 405 (11), 379 (11), 337 (21), 271 (19), 269 (10), 255 (29), 229 (11), 227 (14), 215 (14), 213 (19), 211 (12), 203 (20), 201 (26), 199 (23), 197 (12), 191 (10), 189 (17), 187 (38), 185 (22), 183 (12), 177 (11), 175 (25), 173 (29), 171 (20), 169 (12), 165 (15), 163 (14), 161 (43), 160 (12). Anal. Calcd for $C_{89}H_{128}O_{11}S_4Si$ (1530.42): C, 69.85; H, 8.43; S, 8.38. Found: C, 70.03; H, 8.70; S, 8.26.

(2*E*,4*RS*,5*Z*,9*Z*,12*RS*,13*Z*,17*Z*,20*RS*,21*Z*,25*Z*,28*RS*,29*E*,33*E*,4′*S*)-4,12,20,28-Tetra(benzenesulfonyl)-6,10,14,18,22,26,30,34,38-nonamethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonatriaconta-5,9,13,17,21,25,29,33,37-nonaen-1-ol (30). Under an Ar atmosphere at −75 °C, a solution of **29** (1.338 g, 0.907 mmol) in CH_2Cl_2 (43 mL) was treated with diisobutylaluminium hydride (3.63 mL, 1 m in hexane) over 20 min. The solution was allowed to warm to −30 °C within 3 h, diluted with CH_2Cl_2 (20 mL), and poured into saturated aq NH_4Cl (20 mL). The aqueous phase was extracted with hexane/Et_2O 1:1 (3 × 10 mL), and the extracts were washed with brine (30 mL) and evaporated to give 1.542 g of crude **30**. Flash column chromatography (hexane/EtOAc 4:1) afforded **30** (1.061 g, 78% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 2:1) 0.11. IR ($CHCl_3$): 2942m, 2866m, 1447m, 1379w, 1303s, 1178w, 1145s, 1084s, 999w, 883w, 595m, 551w, 538m, 514w, 507w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.92−7.80 (m, 8H), 7.72−7.43 (m, 12H), 5.58−5.44 (m, 1H), 5.15−4.83 (m, 9H), 4.22−4.04 (m, 1H), 4.04−3.97 (m, 2H), 3.97−3.62 (m, 5H), 2.93−2.30 (m, 8H), 2.18−0.97 (m, 79H), 0.89 (d, J = 6.2, Me). ^{13}C NMR (75 MHz, $CDCl_3$, mixture): δ 145.5−144.9 (several s), 138.1 (s), 138.0 (s), 136.0 (s), 133.9−131.2 (several d), 130.9−130.7 (several s), 129.4−127.7 (several d), 124.4 (d), 123.5 (d), 118.0 (several d), 117.3 (d), 67.6 (t), 63.5−63.2 (4d), 61.7 (t), 40.2 (t), 40.1 (t), 39.8 (t), 37.1 (t), 32.0−31.9 (several t), 29.9 (several t), 29.4 (d), 27.1−26.2 (several t), 25.8 (q), 25.4 (t), 23.5−23.3 (several q), 19.6 (q), 18.1 (6q), 17.8 (q), 16.2 (q), 16.0 (q), 12.0 (3d). FAB-MS (NOBA): *m/z* 1343 (12, $[M - TIPS]^+$), 1342 (15), 1202 (24), 1201 (42), 1200 (519), 1061 (19), 1060 (23), 1059 (76), 1057 (100), 1056 (11), 917 (30), 915 (35), 884 (42), 742 (14), 705 (14), 255 (17), 201 (10), 187 (16), 173 (12), 161 (18), 159 (24).

(2*E*,5*Z*,9*Z*,13*Z*,17*Z*,21*Z*,25*Z*,29*E*,33*E*,4′*S*)-6,10,14,18,22,26,30,34,38-nonamethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonatriaconta-5,9,13,17,21,25,29,33,37-nonaen-1-ol (31). Under an Ar atmosphere, **30** (509.7 mg, 0.339 mmol) in THF (8.8 mL) was added to a mixture of $[PdCl_2(dppp)]$ (61 mg, 0.1 mmol) in THF (2.5 mL). The mixture was cooled to 0 °C, and a solution of $LiEt_3BH$ (3.4 mL, 1 M in THF, 3.4 mmol) in THF was added dropwise over 3.5 h. The mixture was stirred for additional 2.5 h at 0 °C and diluted with Et_2O (35 mL). The color of the mixture changed from orange (during the addition of $LiEt_3BH$) to black. The mixture was washed with NaCN (10 mL, 1 M in H_2O) and brine (10 mL); the organic phase was dried over Na_2SO_4 and evaporated. Flash chromatography (hexane/EtOAc 40:1) afforded **31** (163.9 mg, 51% yield) as a colorless oil. TLC: R_f (hexane/EtOAc 10:1) 0.13. IR ($CHCl_3$): 3599w, 2930s, 2865s, 2729w, 1662w, 1602w, 1456s, 1378m, 1317w, 1099m, 1070m, 996m, 918w, 884m, 841w, 657w, 570w, 529w, 520w, 514w, 5005m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 5.44 (t, J = 7.3, 1H), 5.22−5.08 (m, 9H), 4.05 (d, J = 5.8, 2H), 3.78−3.68 (m, 2H), 2.19−1.92 (m, 38H), 1.71 (s, 7Me), 1.62 (s, 3Me), 1.52−1.00 (m, 27H), 0.92 (d, J = 6.6, Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.8 (s), 135.8−135.2 (several s), 131.5 (s), 127.9 (d), 125.3−124.4 (several d), 67.5 (t), 61.7 (t), 40.2−39.8 (several t), 37.3 (t), 32.3−32.1 (several t), 29.4 (d), 28.5 (t), 26.9−26.5 (several t), 25.8 (q), 25.1 (t), 23.5 (several q), 19.7 (q), 18.1 (6q), 17.7 (q), 16.0 (several q), 12.1 (3d). Anal. Calcd for $C_{64}H_{112}O_2Si$ (941.68): C, 81.63; H, 11.99. Found: C, 81.46; H, 11.91.

(2*E*,5*Z*,9*Z*,13*Z*,17*Z*,21*Z*,25*Z*,29*E*,33*E*,4′*S*)-4-(1-Azi-2,2,2-trifluoroethyl)-1-{[6,10,14,18,22,26,30,34,38-nonamethyl-2-[4′-methyl-6′-(triisopropylsilyloxy)hexylidene]nonatriaconta-5,9,13,17,21,25,29,33,37-nonaen-1-oxy]methyl}-2-iodobenzene (34). Aqueous NaOH (0.25 mL 50%) was added to a solution of **31** (40 mg, 42.5 μmol), **32** (50.9 mg, 112.6 μmol), and tetrabutylammonium iodide (12 mg, 32 μmol) in benzene (0.25 mL). The emulsion was stirred in the dark at 25 °C for 23 h, treated with benzene (0.2 mL), and stirred for 49 h at 25 °C. Ice water (3mL) and hexane (2 mL) were added, and the aqueous phase was extracted with hexane/Et_2O 10:1 (2 × 2 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to give 76 mg

of crude extract. Flash column chromatography gave **32** (elution with hexane), **34** (49.5 mg, elution with hexane/Et_2O 10:1) containing an unknown contamination, and **31** (6.2 mg, 6.6 μmol, elution with hexane/Et_2O 1:1). TLC: R_f (hexane/Et_2O 10:1) 0.74. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63−7.47 (m, 2H), 7.32−7.20 (m, 1H), 5.49 (t, J = 7.2, 1H), 5.20−5.05 (m, 9H), 4.63 (s, 2H, contamination), 4.41 (s, CH_2-Ar), 4.02 (s, CH_2OBn), 3.79−3.66 (m, 2H), 2.19−1.90 (m, 38H), 1.70 (s, 7Me), 1.62 (s, 3Me), 1.50−0.99 (m, 26H), 0.92 (d, J = 6.7, Me).

(3*S*,6*E*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*Z*,34*E*,38*E*,42*E*)-7-{[4-(1-Azi-2,2,2-trifluoroethyl)-2-iodophenyl]methoxymethyl}-3,11,15,19,23,27,31,-35,39,43-decamethyltetratetraconta-6,10,14,18,22,26,30,34,38,42-decaen-1-ol (6). Tetrabutylammonium fluoride trihydrate (24 mg, 68 μmol) was added in the dark to a solution of slightly impure **34** (49.5 mg) in THF (0.8 mL) at rt. The mixture changed from colorless to black. After 4.5 h, ice water (2 mL) and hexane (3 mL) were added. The aqueous phase was extracted with hexane/Et_2O 10:1 (3 × 3 mL), and the combined organic phases were washed with brine (3 mL), dried over Na_2SO_4, and evaporated. Flash column chromatography (hexane/EtOAc 40:1) afforded pure **6** (26.6 mg) in 56% yield from **31**. TLC: R_f (hexane/Et_2O 1:1) 0.47. IR ($CHCl_3$): 3005m, 2963s, 2930s, 2856m, 2090w, 1720w, 1451m, 1378m, 1348w, 1322w, 1260m, 1162s, 1112s, 1034w, 999w cm^{-1}. UV (EtOH): λ_{max} 355 nm (ϵ 314). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, J = 1.85, 1H), 7.48 (d, J = 8.1, 1H), 7.22 (d, J = 8.1, 1H), 5.46 (t, J = 7.2, 1H), 5.14−5.08 (m, 9H), 4.39 (s, CH_2Ar), 4.00 (s, CH_2OBn), 3.71−3.65 (m, 2H), 2.16−1.96 (m, 38H), 1.68 (s, 7Me), 1.62−1.60 (m, 10H), 1.47−1.36 (m, 2H), 1.27−1.20 (m, 2H), 1.15−1.13 (m, 1H), 0.92 (d, J = 6.6, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.1 (s), 136.8 (d), 135.6−135.0 (several s), 131.3 (s), 130.0 (d), 129.7 (s), 128.5 (d), 126.5 (d), 125.0−124.2 (several d), 121.9 (q, $^1J_{CF}$ = 275), 97.2 (s), 75.3 (t), 74.9 (t), 61.1 (t), 39.9−39.7 (several t), 37.1 (t), 32.3−32.0 (several t), 29.3 (d), 28.6 (t), 27.6 (q, $^2J_{CF}$ = 41), 26.8−26.4 (several t), 25.7 (q), 25.1 (t), 23.5−23.4 (several q), 19.5 (q), 17.7 (q), 16.0 (q). FAB-MS (NOBA): *m/z* 1131.5 (11, [M + Na]$^+$), 768 (11), 189 (12). Anal. Calcd for $C_{64}H_{96}F_3IN_2O_2$ (1109.54): C, 69.29; H, 8.72; I, 11.44; N, 2.53. Found: C, 69.20; H, 8.93; I, 11.25; N, 2.23.

Diammonium (3*S*,6*E*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*Z*,34*E*,38*E*,42*E*)-7-{[4-(1-Azi-2,2,2-trifluoroethyl)-2-iodophenyl]methoxymethyl}-3,11,-15,19,23,27,31,35,39,43-decamethyltetratetraconta-6,10,14,18,22,-26,30,34,38,42-decaen-1-yl Phosphate (7). Under an Ar atmosphere, triethylamine (184 μL, absolute) was added dropwise to a solution of $POCl_3$ (123 μL) in hexane (6.15 mL). The solution was stirred for 15 min at 25 °C, while a colorless precipitate formed. An aliquot (0.65 mL) of this mixture was transferred into another flask, where **6** (8.9 mg, 8.02 μmol) in hexane (0.41 mL, 15 min) was added under an Ar atmosphere. The mixture was stirred for 20 min at 25 °C and poured into a mixture of acetone, H_2O, and NEt_3 (88:10:2, 6 mL). After 20 h, the solution was concentrated, forming a milky liquid. The evaporation was repeated after addition of 2-propanol (2 mL). The residue was treated with toluene (3 mL) and evaporated. Evaporation with toluene was repeated (2 × 3 mL). The residue was dissolved in dry chloroform (1 mL) and treated with toluene (3 mL), leading to the precipitation of a colorless solid. The suspension was concentrated to a volume of 3 mL and was left at −20 °C for 1 h. The crystalline precipitate (NH_4-Cl) was removed by filtration and washed with absolute toluene. The filtrate was evaporated to give crude phosphate **7** (16 mg) which was dissolved in chloroform/methanol (2:1, 30 mL) and applied to a DEAE sepharose column (11.5 × 1.2 cm). Increasing concentrations of ammonium acetate in $CHCl_3$/MeOH 2:1 were used for elution (30 mL 0.01 M, 30 mL 0.02 M, 30 mL 0.04 M). The fractions containing **7** (monitored by TLC, $CHCl_3$/MeOH/H_2O 65:25:4) were combined and evaporated to give 8.8 mg of a mixture of **7** and ammonium acetate. The mixture was dissolved in $CHCl_3$/MeOH 2:1 (0.5 mL) and ammonium acetate was removed by gel filtration (21.5 × 1 cm, $CHCl_3$/MeOH 2:1). The progress of the filtration was followed by TLC ($CHCl_3$/MeOH/H_2O 65:25:4); fractions containing **7** were combined and evaporated to give **7** (6.7 mg, 68% yield) as a colorless solid. TLC: R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.58. IR ($CHCl_3$): 2961s, 2929s, 2855s, 1617w, 1585w, 1452m, 1377m, 1340w, 1262m, 1161s, 1097s, 1050s, 1030s, 1012s, 952m. cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.47−7.46 (m, 1H), 7.30−7.19 (m, 1H), 5.45−5.40 (m, 1H), 5.13−5.08 (m, 9H), 4.37 (s, 2H), 4.00−3.90 (m, 4H), 2.61 (s br, 8H, NH_4), 2.24−1.94 (m, 38H), 1.67−1.65 (m, 7 Me), 1.60 (s, Me), 1.59 (s, 2 Me), 1.50−1.12 (m, 5H), 0.89 (d, J = 6.5, Me), two additional signals were observed.3.88 (s br, 1H), 3.22 (s br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.1 (s), 136.8 (d), 135.5−134.9 (several s), 131.2 (s), 130.0 (d), 129.7 (s), 128.4 (d), 126.4 (d), 125.0−124.2 (several d), 97.2 (s), 75.4 (t), 75.0 (t), 39.8 (several t), 37.2 (t), 32.2−32.0 (several t), 29.7 (t), 29.7 (d), 28.6 (t), 26.8−26.4 (several t), 25.7 (s), 25.0 (t), 23.4 (several q), 19.1 (q), 17.7 (q), 16.0 (q), (multiplets of CF_3, CN_2, CH_2OP, missing). ^{31}P NMR (121MHz, $CDCl_3$): 1.25. ESI-MS: *m/z* (MeOH) 1188 (M^-).

4-(1-Azi-2,2,2-trifluoroethyl)-2-iodo-1-(iodomethyl)benzene (32). Methyltriphenoxyphosphonium iodide (132 mg, 302 μmol) was added to a solution of 2-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol (**33**, 35.6 mg, 109 μmol) in acetonitrile (0.2 mL). After 23 h in the dark, the solution was diluted with Et_2O (5mL) and extracted with aqueous NaOH (1 m, 2 mL). The organic phase was washed with water and dried over $MgSO_4$. Flash column chromatography (hexane/Et_2O 3:1) gave **32** (39.9 mg, 84% yield) as a yellowish solid. TLC: R_f (hexane/Et_2O 1:1) 0.77. IR ($CHCl_3$): 1616w, 1600w, 1547w, 1491w, 1339m, 1160s, 1035w, 953w, 830w cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.55−7.46 (m, 2H), 7.20−7.16 (m, 1H), 4.52 (s, 2H). ^{13}C NMR (50 MHz): δ 143.6 (s), 138.0 (d), 130.3 (s), 129.7 (d), 127.1 (d), 99.7 (s), 10.2 (t), (multiplets of CF_3 and CN_2 missing). FAB-MS (NOBA): *m/z* 557 (12), 543 (11), 499 (11), 478 (11), 463 (20), 462 (19), 448 (11), 447 (31), 425 (11), 424 (15), 413 (12), 393 (11), 392 (28), 391 (100), 389 (12), 372 (11), 371 (32), 369 (12), 355 (10), 342 (15), 341 (13), 339 (12), 329 (11), 327 (18), 325 (92 [M]$^+$), 315 (10), 313 (16), 311 (12), 309 (12), 308 (13), 307 (37).

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