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Synthesis of Modular Headgroup Conjugates Corresponding to All Seven Phosphatidylinositol Polyphosphate Isomers for Convenient Probe Generation

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Phosphatidylinositol polyphosphate lipids (PIP_ns) play key roles in important biological pathways, and defects in the signaling activities of these molecules have been implicated in a number of disease states. As such, it is necessary to understand the complex roles of these lipids in biological pathways, which often involve their actions as site-specific ligands that recruit receptors to the surfaces of cellular membranes. However, the complex structures of PIP_n family members, of which seven biologically active isomers exist, complicate studies. Derivatized analogs of the PIP_n structures are beneficial as chemical tools for elucidating signaling and binding activities. Herein, we present an efficient approach to probe the generation in which amino conjugates of PIP_n headgroups can be conveniently functionalized in the final step of the synthesis to obtain a number of derivatized analogs of use for different studies. In addition to the application of this strategy to generate PI-(4,5)-P_2 headgroup probes, we also report the synthesis of PIP_n–amine conjugates corresponding to all seven naturally existing isomers. This approach will be invaluable for generating the range of probe structures that is required to elucidate the intricacies of PIP_n signaling.

Introduction

The phosphatidylinositol polyphosphates (PIP_ns) are vital signaling lipids that regulate a litany of key cellular processes.[1–7] As is common with signaling lipids, the PIP_ns act as ligands for the recruitment of peripheral protein receptors to membrane surfaces, events that control both protein function and subcellular localization.[8–10] Signaling lipids are particularly prominent in the regulation of crucial cellular processes as their activities transmit information from membranes into cellular pathways. As such, defects in lipid-signaling events have been implicated in the onset of diseases including cancer, diabetes and leukemia.[11] A particularly well-understood example pertains to the conversion of phosphatidylinositol-4,5-bisphosphate [PI-(4,5)-P_2] to phosphatidylinositol-3,4,5-trisphosphate [PI-(3,4,5)-P_3], the forward and reverse reactions of which are catalyzed by phosphoinositide 3-kinase (PI3K)[12] and the phosphatase PTEN,[13] respectively. The product, PI-(3,4,5)-P_3, binds and activates protein kinase B (Akt), which activates numerous pathways that control cell proliferation. Both PI3K[14] and the tumor suppressor PTEN[15] are among the most frequently mutated enzymes in tumorigenesis.

Whereas recent advances in the study of protein–PIP_n association have yielded the elucidation of important details, many aspects of these events remain poorly understood. For example, numerous protein-binding modules that target PIP_ns have been identified, including the PH, PX, FYVE, ENTH, ANTH, FERM, Tubby, and PROPPIN domains. However, the diversity present within these families provides an impediment to detailed understanding, particularly with the PH domains, for which sequence homology searches predict over 250 members.[7] Here, variations in binding details complicate full elucidation of the affinities and specificities that each particular receptor exhibits for the seven PIP_n isomers. Furthermore, diversity in the structural features required for binding also exist, as certain proteins require the presence of the membrane, whereas others show high affinity for isolated PIP_n headgroups.[9,16,17] In particular, certain PH domains contain minimal domains for membrane penetration, and thus interactions are believed to be primarily driven by headgroup association.[7,18] In such cases, simplified headgroup analogs can be effective for probing and perturbing binding.[19]

The structural complexity of the PIP_ns complicates probe synthesis, as these phospholipids contain phosphodiester-linked *myo*-inositol headgroups that are phosphorylated at every combination of the 3-, 4-, and 5-positions. Nevertheless, a number of effective approaches for PIP_n synthesis have been reported,[20–33] including the recent development of peptide catalysts effective for stereoselective phosphorylation of different *myo*-inositol-based substrates.[34–38] Whereas these strategies have resulted in the generation of probes that have proven effective for characterizing binding, the complex synthesis that is required for each reporter-

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derivatized probe generally limits the scope of probes that can be produced. Herein, we report the design and synthesis of modular PIP_n headgroup scaffolds that can be conveniently derivatized for efficient access to a range of functionalized headgroup analogs for use as probes.

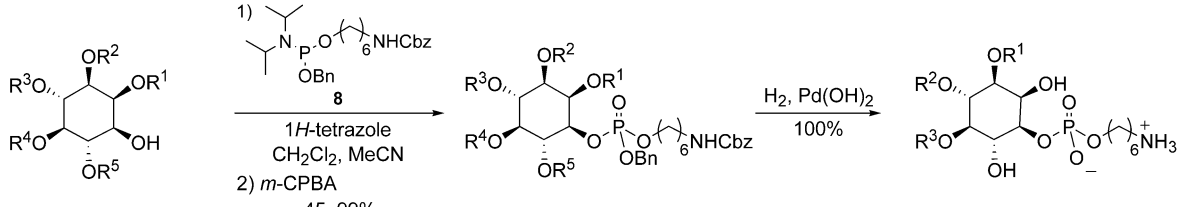
Results and Discussion

A particularly advantageous approach to the production of a range of derivatized probes corresponding to a biomolecule target is to install a reactive functional group that can be modified in the final step of the synthesis. To implement this strategy for the production of PIP_n headgroup probes, we designed modular analogs of type **1a**–**g** (Scheme 1), which consist of each PIP_n headgroup tethered to an amino group that can be conveniently functionalized by coupling chemistry. The amino group is linked through a phosphodiester moiety at the 1-position, similar to the connection of the headgroup to the glycerolipid backbone in the natural lipid. A similar derivatization approach has previously proven effective in the development of tetherable analogs of inositol 1,4,5-trisphosphate (IP_3).[39–42] Finally, a hexyl linker was installed between the headgroup and amino moieties of **1a**–**g** to introduce hydrophobicity and in part mimic the non-polar properties of the lipid backbone. These enantiomerically pure modular headgroup analogs were synthesized as described below.

In the synthesis of **1a**–**g**, it was desirable to obtain these final compounds by a global deprotection in the final step through a clean hydrogenolysis process, an approach that has previously been advantageous.[24–26] Thus, we sought to prepare these from precursors **2a**–**g**, in which each functional group is protected by a group that can be removed by hydrogenolysis, including amino protection using a benzyloxycarbonyl (Cbz) group, hydroxy moieties protected as benzyl (Bn) or benzyloxymethyl (BOM) ethers, and the phosphates masked as benzyl phosphotriesters.[24,26] The initial synthetic routes to headgroup analogs corresponding to the PI-4P (**1a**), PI-5P (**1b**), PI-(4,5)-P_2 (**1c**), and PI-(3,4,5)-P_3 (**1d**) isomers are detailed in Scheme 2. First, intermediates **3a**–**c**, containing benzoyl groups at the location of the eventual phosphate moieties, were synthesized in four steps from *myo*-inositol as described previously.[22] PI-(3,4,5)-P_3 precursor **3d** was generated by appending a third benzoyl group onto compound **3c**. The remaining hydroxy groups in compounds **3a**–**d** were then protected with BOM groups to generate **4a**–**d**, followed by benzoyl deprotection to access **5a**–**d**. Next, phosphoramidite chemistry was used to install the phosphotriester moieties of **6a**–**d**, followed by silyl deprotection to produce **7a**–**d**.

For the synthesis of headgroup analogs of PI-3P (**1e**), PI-3,4-P_2 (**1f**), and PI-3,5-P_2 (**1g**), the corresponding intermediates **7e**–**g** (Scheme 1), containing Bn rather than BOM protecting groups, were synthesized in 9–12 steps as described previously.[21] The synthesis of compounds **7a**–**g**, in which all functional groups are protected except for the P-1 hydroxy moiety, set the stage for phosphodiester coupling to produce **1a**–**g** (Scheme 1). Here, compounds **7a**–**g** were each coupled to phosphoramidite reagent **8**, obtained from Cbz-protected aminohexanol and benzyloxybis(diisopropylamino)phosphane, to install the phosphodiester linkage of **2a**–**g**. Finally, global deprotection by hydrogenolysis yielded quantitative access to modular PIP_n–amine conjugates **1a**–**g**.

Upon obtaining our initial PIP_n–amine conjugate, that corresponding to PI-(4,5)-P_2 (**1c**), we set out to showcase the efficacy of this modular scaffold for probe development (Scheme 3). Derivatized PI-(4,5)-P_2 headgroup analogs **9**–**11** were conveniently synthesized in one step by coupling the amine of **1c** to the corresponding succinimidyl esters (**12a**–**c**). We first introduced a tetraethyleneglycol (TEG)–biotin group to produce probe **9**, of use for immobilizing the headgroup onto surfaces or for pull-down studies. Recently, we implemented this compound in the development of a microplate-based assay for the detection of protein–PIP_n headgroup binding interactions.[43] These studies provided further evidence that proteins could bind to PIP_n headgroups with high affinity outside of the membrane environment, and showcased the efficacy of the described de-

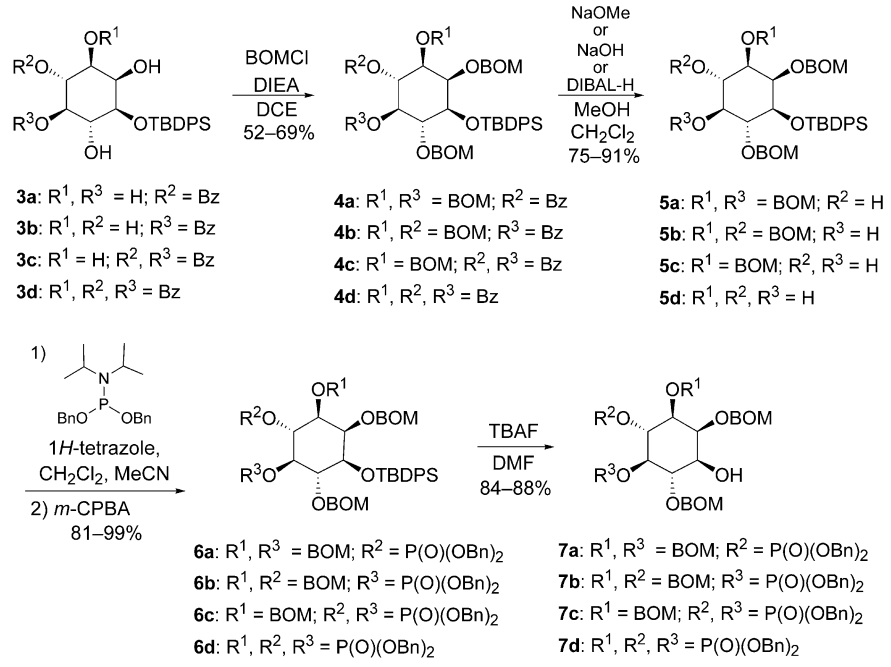


7a: R^1, R^2, R^4, R^5 = BOM; R^3 = P(O)(OBn)$_2$
7b: R^1, R^2, R^3, R^5 = BOM; R^4 = P(O)(OBn)$_2$
7c: R^1, R^2, R^5 = BOM; R^3, R^4 = P(O)(OBn)$_2$
7d: R^1, R^5 = BOM; R^2, R^3, R^4 = P(O)(OBn)$_2$
7e: R^1, R^3, R^4, R^5 = Bn; R^2 = P(O)(OBn)$_2$
7f: R^1, R^4, R^5 = Bn; R^2, R^3 = P(O)(OBn)$_2$
7g: R^1, R^3, R^5 = Bn; R^2, R^4 = P(O)(OBn)$_2$

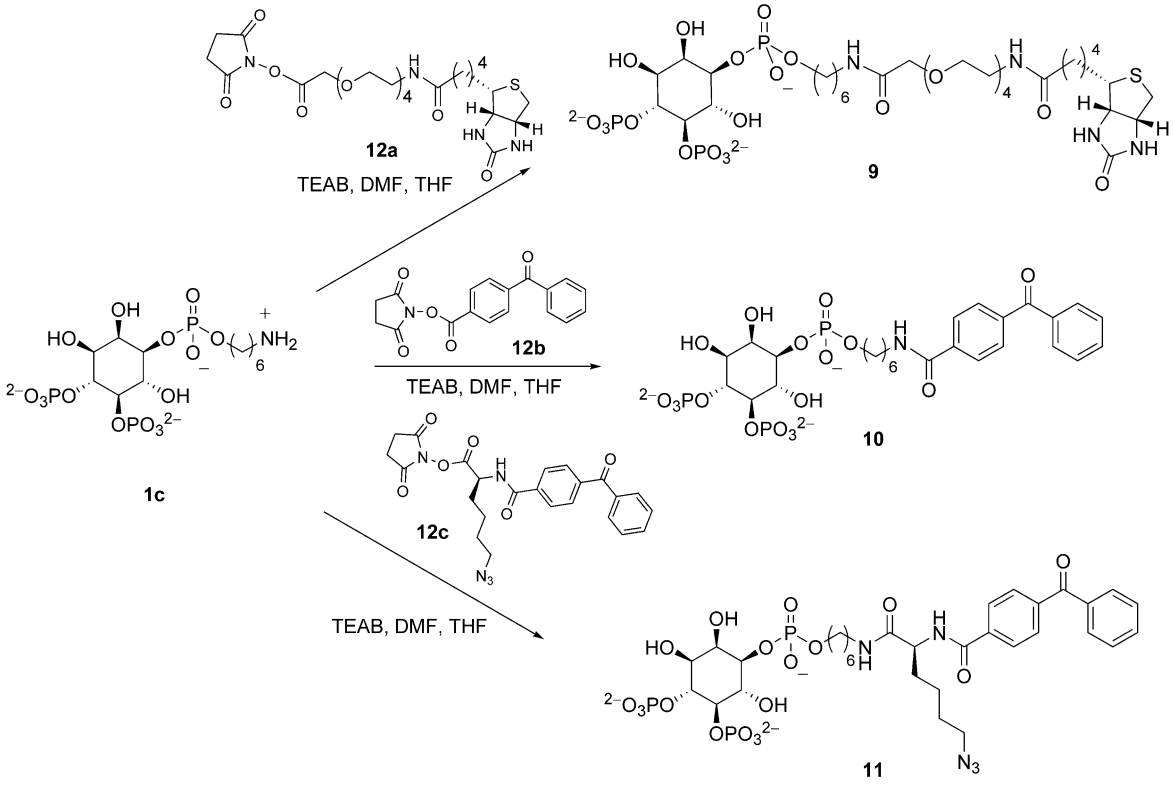
2a: R^1, R^2, R^4, R^5 = BOM; R_3 = P(O)(OBn)$_2$
2b: R^1, R^2, R^3, R^5 = BOM; R^4 = P(O)(OBn)$_2$
2c: R^1, R^2, R^5 = BOM; R^3, R^4 = P(O)(OBn)$_2$
2d: R^1, R^5 = BOM; R^2, R^3, R^4 = P(O)(OBn)$_2$
2e: R^1, R^3, R^4, R^5 = Bn; R^2 = P(O)(OBn)$_2$
2f: R^1, R^4, R^5 = Bn; R^2, R^3 = P(O)(OBn)$_2$
2g: R^1, R^3, R^5 = Bn; R^2, R^4 = P(O)(OBn)$_2$

1a: R^1, R^3 = H; R^2 = PO_3^{2-}
1b: R^1, R^2 = H; R^3 = PO_3^{2-}
1c: R^1 = H; R^2, R^3 = PO_3^{2-}
1d: R^1, R^2, R^3 = PO_3^{2-}
1e: R^2, R^3 = H; R^1 = PO_3^{2-}
1f: R^3 = H; R^1, R^2 = PO_3^{2-}
1g: R^2 = H; R^1, R^3 = PO_3^{2-}

Scheme 1. Synthetic route to modular PIP_n headgroup–amine conjugates of all seven naturally occurring isomers as modular scaffolds.



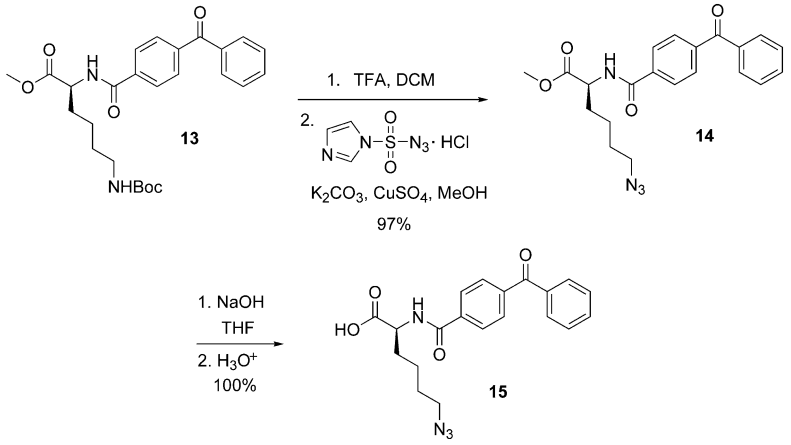
Scheme 2. Synthesis of fully protected *myo*-inositol headgroup intermediates.



Scheme 3. Convenient coupling of PIP_n headgroup–amine conjugates for modular access to derivatized probe structures.

rivatized headgroup analogs for probing and perturbing these binding events. In addition, this approach shows great prospects for high-throughput characterization of protein–PIP_n binding interactions.

We have since further derivatized **1c** to introduce other reporter groups of use for investigations, such as the installation of a benzophenone tag (**10**) for photo-crosslinking studies.[44] In addition, bifunctional probes exemplified by **11** are targeted for use in purifying, identifying and characterizing PIP_n-binding receptors from a complex sample such as a cell extract. This strategy for probe development is inspired by the concept of activity-based protein profiling (ABPP), which employs bifunctional analogs as probes for the mechanism-based collective labeling of enzyme targets



Scheme 4. Synthesis of bifunctional reporter tag **15** for coupling onto PIP_n headgroups.

based on their activity.[45–49] In the current approach, probe **11** contains both a photo-crosslinking group (benzophenone) for attachment to cognate receptors, as well as a secondary tag (azide) for purification of successfully crosslinked proteins from a complex sample. Similar bifunctional designs have previously proven effective for this purpose,[50,51] including a recent study that utilized a phosphatidylcholine (PC) probe to identify receptors that target this lipid.[52] The bifunctional tag of **11** was synthesized beginning with benzophenone–lysine conjugate **13**,[53] as shown in Scheme 4. This was performed by Boc-deprotection and direct azido transfer to azide **14**, followed by ester hydrolysis to **15**. We are currently implementing probe **11** for identifying PIP_n-binding proteins, studies that exemplify the powerful advantages of chemical approaches employing probes as tools for efficiently elucidating the intricacies of complex biological binding and signaling events. Although these probes may be subject to enzymatic modification during biological studies, such as by phosphodiesterases, an effective remedy for this problem is to introduce non-hydrolyzable phosphorothioate groups.[54]

Conclusion

Whereas the signaling lipids that compose the phosphatidylinositol family play crucial roles in numerous cellular processes, these activities are difficult to study due to the complex synthesis required to access derivatized probes of these structures. As such, versatile strategies for probe production are imperative for generating the diverse probes that are necessary to elucidate the complex details of lipid signaling processes. The modular approach to probe generation presented herein has proven successful for the convenient production of biologically active analogs of use for direct characterization of protein–PIP_n binding interactions. We are currently extending these studies to scrutinize all seven naturally occurring headgroup isomers.

Experimental Section

General Experimental: Generally, reagents were purchased from Acros or Aldrich and used as received. Dry solvents were obtained from a Pure Solv solvent delivery system purchased from Innovative Technology, Inc. Column chromatography was performed by using 230–400 mesh silica gel purchased from Sorbent Technologies. NMR spectra were obtained by using a Bruker AC250 spectrometer updated with a TecMag data collection system, a Varian Mercury 300 spectrometer, and a Bruker Avance 400 spectrometer. Mass spectra were obtained with JEOL DART-AccuTOF and ABI DE Pro MALDI spectrometers with high-resolution capabilities. Optical rotation values were obtained by using a Perkin–Elmer 241 polarimeter. The synthesis of initial modular PI-(4,5)-P_2 scaffold **1c**, including the procedures for intermediates **2c**–**6c** and **8c** as well as biotin conjugate **9**, were previously described.[43]

4-*O*-Benzyl-2,3,5,6-*O*-tetra(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (4a): Diisopropylethylamine (1.0 mL, 5.76 mmol) and benzyl chloromethyl ether (0.8 mL, 5.76 mmol) were added to a solution of **3a** (0.251 g, 0.48 mmol) in 1,2-dichloroethane (10 mL). The reaction mixture was stirred at reflux for 24 h. Diisopropylethylamine (1.0 mL, 5.76 mmol) and benzyl chloromethyl ether (0.8 mL, 5.76 mmol) were added to the solution, and the mixture was stirred at reflux for another 24 h. After the reaction was finished, water was added and the mixture extracted with dichloromethane (2 × 50 mL). The combined organic phases were dried with magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (hexane/acetone, 5:1) to give **4a** (0.275 g, 57%) as a syrup. $[\alpha]_D^{296\,K}$ = +8.3 (c = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.00 (d, J = 7.2 Hz, 2 H), 7.71–7.80 (m, 4 H), 7.13–7.44 (m, 25 H), 6.95–6.98 (m, 2 H), 6.86–6.89 (m, 2 H), 5.80 (t, J = 9.6 Hz, 1 H), 5.21 (d, J = 6.3 Hz, 1 H), 5.03–5.05 (m, 2 H), 4.75–4.86 (m, 5 H), 4.64 (d, J = 11.7 Hz, 1 H), 4.48 (d, J = 12.3 Hz, 1 H), 4.28–4.42 (m, 3 H), 4.14–4.22 (m, 2 H), 4.07 (d, J = 6.9 Hz, 1 H), 3.95–4.01 (m, 2 H), 3.78 (t, J = 9.3 Hz, 1 H), 3.51 (d, J = 9.9 Hz, 1 H), 3.44 (s, 1 H), 1.12 (s, 9 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 165.7, 138.2, 137.9, 137.7, 136.2, 136.0, 134.2, 133.0, 133.0, 130.3, 130.1, 129.8, 128.5, 128.5, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.4, 127.3, 97.3, 96.5, 95.4, 91.8, 79.2, 78.8, 74.7, 74.5, 73.5, 73.3, 70.7, 70.5, 69.4, 68.9, 27.4, 19.4 ppm. MALDI-HRMS: calcd. for $C_{61}H_{66}O_{11}SiNa$ $[M + Na]^+$ 1025.4267; found 1025.4282.

5-*O*-Benzyl-2,3,4,6-*O*-tetra-(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (4b): Diisopropylethylamine (1.6 mL, 9.25 mmol) and benzyl chloromethyl ether (1.3 mL, 9.38 mmol) were added to a solution of **3b** (0.40 g, 0.765 mmol) in 1,2-dichloroethane (16 mL). The reaction mixture was stirred at reflux for 24 h.

Diisopropylethylamine (1.6 mL, 9.25 mmol) and benzyl chloromethyl ether (1.3 mL, 9.38 mmol) were added to the solution, and the mixture was stirred at reflux for another 24 h. After the reaction was finished, water was added and the mixture extracted with dichloromethane (2 × 50 mL). The combined organic phases were dried with magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (hexane/acetone, 5:1) to provide **4b** (0.526 g, 69%) as a syrup. $[\alpha]_D^{296\ K}$ = +15.7 (*c* = 1.9, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.08 (d, *J* = 10.2 Hz, 2 H), 7.70–7.74 (m, 4 H), 7.11–7.44 (m, 25 H), 6.93–6.96 (m, 4 H), 5.36 (t, *J* = 9.6 Hz, 1 H), 5.05 (d, *J* = 6.6 Hz, 1 H), 4.90 (d, *J* = 6.6 Hz, 1 H), 4.69–4.84 (m, 5 H), 4.19–4.49 (m, 11 H), 4.04 (d, *J* = 9.6 Hz, 1 H), 3.50–3.54 (m, 2 H), 1.09 (s, 9 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 166.0, 138.1, 137.7, 137.7, 137.6, 135.9, 135.8, 133.8, 132.8, 132.8, 130.1, 129.9, 129.8, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 127.5, 127.3, 127.3, 127.2, 127.2, 127.1, 96.7, 95.7, 95.5, 92.4, 79.2, 78.4, 76.6, 75.3, 75.2, 74.5, 73.9, 69.9, 69.7, 69.3, 69.1, 27.2, 19.3 ppm. MALDI-HRMS: calcd. for $C_{61}H_{66}O_{11}SiNa$ $[M + Na]^+$ 1025.4267; found 1025.4296.

3,4,5-*O*-Tribenzyl-2,6-*O*-bis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (4d): Diisopropylethylamine (0.43 mL, 2.6 mmol) and benzyl chloromethyl ether (0.36 mL, 2.6 mmol) were added to a solution of **3d** (0.236 g, 0.323 mmol) in 1,2-dichloroethane (3 mL). The reaction mixture was stirred at reflux for 24 h. After the reaction was finished, water was added and the mixture extracted with dichloromethane (2 × 50 mL). The combined organic phases were dried with magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (hexane/acetone, 10:1) to yield **4d** (0.158 g, 52%) as a syrup. $[\alpha]_D^{296\ K}$ = +68.9 (*c* = 4.8, $CHCl_3$). Other characterizations match previous reports.[26]

2,3,5,6-*O*-Tetrakis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (5a): DIBAL-H (2.0 mL, 2.0 mmol) was added to a solution of **4a** (0.27 g, 0.27 mmol) in dichloromethane (15 mL) at –78 °C. After 90 min, ethyl acetate (2 mL) was added to quench the reaction. The solution was partitioned between ethyl acetate (60 mL) and water (80 mL), and the aqueous phase was extracted with ethyl acetate (60 mL). The combined organic phases were washed with brine (50 mL), dried with magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (hexane/acetone, 10:1) to give **5a** (0.225 g, 91%) as a syrup. $[\alpha]_D^{296\ K}$ = +16.5 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.77–7.83 (m, 4 H), 7.25–7.47 (m, 26 H), 5.19 (d, *J* = 6.6 Hz, 1 H), 5.01 (d, *J* = 6.9 Hz, 1 H), 4.83–4.93 (m, 7 H), 4.53–4.66 (m, 5 H), 4.24–4.45 (m, 4 H), 4.06 (t, *J* = 9.6 Hz, 1 H), 3.97 (d, *J* = 9.9 Hz, 1 H), 3.69 (s, 1 H), 3.32–3.40 (m, 2 H), 1.17 (s, 9 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 138.2, 138.1, 137.8, 136.8, 135.9, 135.8, 133.8, 132.9, 130.0, 129.8, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 127.4, 127.2, 96.7, 96.5, 95.1, 92.7, 78.0, 75.0, 74.7, 74.4, 71.9, 70.1, 69.8, 69.1, 69.0, 27.2, 19.2 ppm. MALDI-HRMS: calcd. for $C_{54}H_{62}O_{10}SiNa$ $[M + Na]^+$ 921.4004; found 921.4011.

2,3,4,6-*O*-Tetrakis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (5b): DIBAL-H (4.4 mL, 4.4 mmol) was added to a solution of **4b** (0.52 g, 0.518 mmol) in dichloromethane (30 mL) at –78 °C. After 90 min, ethyl acetate (5 mL) was added to quench the reaction. The solution was partitioned between ethyl acetate (100 mL) and water (100 mL), and the aqueous phase was extracted with ethyl acetate (80 mL). The combined organic phases were washed with brine (80 mL), dried with magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (hexane/acetone, 10:1) to provide **5b** (0.405 g, 87%) as a syrup. $[\alpha]_D^{296\ K}$ = +36.2 (*c* = 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.69–7.72 (m, 4 H), 7.14–7.39 (m, 26 H), 4.29–5.03 (m, 17 H), 4.01 (t, *J* = 9.3 Hz, 1 H), 3.91 (t, *J* = 9.6 Hz, 1 H), 3.81–3.84 (m, 2 H), 3.47 (dd, *J* = 9.9, 1.8 Hz, 1 H), 3.35 (t, *J* = 8.4 Hz, 1 H), 1.10 (s, 9 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 138.1, 137.9, 137.7, 137.1, 135.8, 135.7, 133.7, 133.5, 129.9, 129.6, 128.4, 128.3, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.3, 96.5, 95.8, 95.3, 92.9, 84.1, 79.1, 76.0, 74.7, 74.0, 73.3, 70.0, 69.6, 69.1, 69.1, 27.0, 19.3 ppm. MALDI-HRMS: calcd. for $C_{54}H_{62}O_{10}SiNa$ $[M + Na]^+$ 921.4004; found 921.4033.

2,6-*O*-Bis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol (5d): A 1% sodium hydroxide solution (2 mL) and dichloromethane (2 mL) were added to a solution of **4d** (0.13 g, 0.134 mmol) in methanol (5 mL). The mixture was stirred at room temp. under nitrogen for 18 h. The solvent was removed and the residue purified by chromatography on silica gel (hexane/acetone, 4:1) to give **5d** (0.66 g, 75%) as a syrup. $[\alpha]_D^{296\ K}$ = +60.5 (*c* = 2.6. $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.68 (t, *J* = 7.2 Hz, 4 H), 7.45–7.25 (m, 16 H), 4.94 (d, *J* = 6.8 Hz, 1 H), 4.75–4.65 (m, 3 H), 4.54–4.51 (m, 3 H), 4.42 (d, *J* = 11.6 Hz, 1 H), 4.30 (br. s, 1 H), 3.81–3.72 (m, 2 H), 3.66–3.54 (m, 3 H), 3.20–3.13 (m, 2 H), 2.75 (br. s, 1 H), 1.04 (s, 9 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 136.3, 136.2, 135.4, 135.3, 133.1, 129.6, 129.3, 128.1, 127.6, 127.5, 127.3, 127.1, 96.5, 96.2, 84.7, 82.6, 73.3, 72.2, 70.4, 70.0, 69.9, 26.5, 18.9 ppm. DART-HRMS: calcd. for $C_{38}H_{45}O_8Si$ $[M - H]^-$ 657.2884; found 657.2888.

2,3,5,6-*O*-Tetrakis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol 4-(Dibenzyl phosphate) (6a): Dibenzyl *N*,*N*-diisopropylphosphoramidite (0.16 mL, 0.489 mmol) was added to a solution of alcohol **5a** (0.220 g, 0.245 mmol) and 1*H*-tetrazole (1.63 mL, 0.735 mmol, 0.45 M in acetonitrile) in dichloromethane (10 mL) under nitrogen, and the mixture was stirred at room temp. for 18 h. The reaction solution was cooled to –60 °C, *meta*-chloroperbenzoic acid (*m*CPBA, 0.25 g, 57–86%) was added, and the mixture was stirred at this temperature for 60 min before slowly warming to room temp. by removal of the cooling bath. The solution was diluted to 60 mL with dichloromethane, and washed with saturated sodium bicarbonate (2 × 30 mL). The aqueous phase was extracted with dichloromethane (60 mL). The combined organic phases were dried with magnesium sulfate, filtered, concentrated, and the residue was purified by chromatography on silica gel (hexanes/acetone, 7:1) to yield product **6a** (0.258 g, 91%) as a syrup. $[\alpha]_D^{296\ K}$ = +10.1 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 6.0 Hz, 2 H), 7.67–7.69 (m, 2 H), 7.17–7.38 (m, 34 H), 7.05–7.08 (m, 2 H), 5.19 (d, *J* = 6.3 Hz, 1 H), 5.12 (d, *J* = 6.6 Hz, 1 H), 4.98–5.06 (m, 6 H), 4.62–4.86 (m, 8 H), 4.18–4.43 (m, 5 H), 4.10 (d, *J* = 12.3 Hz, 1 H), 3.91 (d, *J* = 9.6 Hz, 1 H), 3.65 (t, *J* = 9.1 Hz, 1 H), 3.45 (s, 1 H), 3.35 (d, *J* = 10.2 Hz, 1 H), 1.09 (s, 9 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 138.3, 138.1, 138.1, 137.9, 136.0, 136.0, 135.8, 133.9, 132.7, 130.1, 129.9, 128.4, 128.3, 128.2, 128.2, 128.2, 128.0, 127.8, 127.8, 127.7, 127.7, 127.7, 127.5, 127.5, 127.4, 127.3, 127.2, 126.8, 97.0, 96.4, 95.3, 92.7, 77.7, 77.3, 77.2, 75.1, 74.3, 73.7, 70.6, 69.2, 69.1, 68.3, 27.2, 19.2 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.05 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{68}H_{75}O_{13}PSiNa$ $[M + Na]^+$ 1181.4607; found 1181.4605.

2,3,4,6-*O*-Tetrakis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol 5-(Dibenzyl phosphate) (6b): Dibenzyl *N*,*N*-diisopropylphosphoramidite (0.30 mL, 0.89 mmol) was added to a solution of alcohol **5b** (0.40 g, 0.445 mmol) and 1*H*-tetrazole (3.0 mL, 1.34 mmol, 0.45 M in acetonitrile) in dichloromethane

(20 mL) under nitrogen, and the mixture was stirred at room temp. for 18 h. The reaction solution was cooled to –60 °C, and *m*CPBA (0.45 g, 57–86%) was added. The mixture was then stirred at this temperature for 60 min and then slowly warmed to room temp. following removal of the cooling bath. The solution was diluted to 80 mL with dichloromethane and washed with saturated sodium hydrogen carbonate (2 × 50 mL). The aqueous phase was extracted with dichloromethane (60 mL). The combined organic phases were dried with magnesium sulfate, filtered, and concentrated, and the residue was purified by chromatography on silica gel (hexanes/acetone, 7:1) to yield product **6b** (0.481 g, 93%) as a syrup. $[\alpha]_D^{296\ K}$ = +16.3 (*c* = 1.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, *J* = 6.0 Hz, 2 H), 7.67–7.70 (m, 2 H), 7.12–7.37 (m, 36 H), 5.23 (d, *J* = 6.3 Hz, 1 H), 4.86–5.09 (m, 8 H), 4.56–4.76 (m, 6 H), 4.14–4.49 (m, 8 H), 3.90 (d, *J* = 8.1 Hz, 1 H), 3.41 (s, 1 H), 3.31 (d, *J* = 9.9 Hz, 1 H), 1.10 (s, 9 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 138.3, 138.2, 138.1, 137.8, 136.2, 136.0, 135.8, 133.9, 132.7, 130.1, 130.0, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.0, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 96.6, 96.5, 95.4, 92.8, 76.4, 75.8, 75.3, 74.6, 74.2, 70.6, 70.1, 69.4, 69.3, 69.3, 69.0, 27.3, 19.3 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.25 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{68}H_{75}O_{13}PSiNa$ $[M + Na]^+$ 1181.4607; found 1181.4595.

2,6-*O*-Bis(benzyloxymethyl)-1-*O*-(*tert*-butyldiphenylsilyl)-D-*myo*-inositol 3,4,5-Tris(dibenzyl phosphate) (6d): Dibenzyl *N*,*N*-diisopropylphosphoramidite (0.40 mL, 1.22 mmol) was added to a solution of alcohol **5d** (0.134 g, 0.203 mmol) and 1*H*-tetrazole (4.0 mL, 1.8 mmol, 0.45 M in acetonitrile) in dichloromethane (4 mL) under nitrogen, and the mixture was stirred at room temp. for 18 h. The reaction solution was cooled to –20 °C, and *m*CPBA (0.315 g, 57–86%) was added. The mixture was warmed to room temp. and stirred for 3 h. The solvent was removed and the residue purified by flash chromatography on silica gel (hexanes/acetone, 8:1) to access **6d** (0.289 g, 99%) as a syrup. $[\alpha]_D^{296\ K}$ = +15.5 (*c* = 2.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.76–7.67 (m, 4 H), 7.38–7.05 (m, 46 H), 5.15–4.88 (m, 12 H), 4.83–4.64 (m, 7 H), 4.54–4.48 (m, 2 H), 4.37–4.26 (m, 2 H), 4.08 (t, *J* = 12 Hz, 1 H), 3.98–3.91 (m, 2 H), 1.07 ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 138.2, 137.8, 136.2, 136.2, 136.1, 136.1, 136.0, 135.9, 135.9, 135.8, 135.7, 133.3, 132.5, 130.1, 130.0, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 96.6, 96.0, 75.1, 75.1, 75.1, 73.4, 70.7, 69.9, 69.6, 69.5, 69.5, 69.4, 69.3, 69.2, 69.1, 69.0, 27.2, 19.4 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.07, –0.04, –0.28 ppm. MALDI-HRMS: calcd. for $C_{80}H_{85}O_{17}P_3SiNa$ $[M + Na]^+$ 1461.4666; found 1461.4603.

2,3,5,6-*O*-Tetrakis(benzyloxymethyl)-D-*myo*-inositol 4-(Dibenzyl phosphate) (7a): A solution of fully protected inositol **6a** (0.255 g, 0.22 mmol) in dimethylformamide (10 mL) was treated with tetrabutylammonium fluoride trihydrate (0.222 g, 0.70 mmol), and stirred at room temp. for 12 h. After the reaction was finished, the solution was partitioned between ethyl acetate (80 mL) and water (80 mL). The aqueous layer was further extracted with ethyl acetate (60 mL), and the combined organic layers were washed with brine (60 mL), dried with magnesium sulfate and filtered. Following concentration, the residue was purified by chromatography on silica gel (hexanes/acetone, 3:1) to give alcohol **7a** (0.175 g, 86%) as a syrup. $[\alpha]_D^{296\ K}$ = –18.7 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.17–7.33 (m, 30 H), 4.82–5.07 (m, 11 H), 4.65–4.77 (m, 6 H), 4.45–4.57 (m, 4 H), 4.26 (s, 1 H), 4.09 (d, *J* = 3.9 Hz, 1 H), 3.84 (t, *J* = 9.3 Hz, 1 H), 3.64–3.71 (m, 2 H), 3.52–3.56 (m, 1 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 138.0, 137.8, 137.5, 136.9, 136.0, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 96.8, 96.2, 95.7, 94.4, 83.3, 79.5, 79.5, 78.6, 78.5, 76.2, 75.3, 71.1, 70.4, 70.1, 69.7, 69.6, 69.3, 69.3, 69.2 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.23 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{52}H_{57}O_{13}PNa$ $[M + Na]^+$ 943.3429; found 943.3421.

2,3,4,6-*O*-Tetrakis(benzyloxymethyl)-D-*myo*-inositol 5-(Dibenzyl phosphate) (7b): A solution of fully protected inositol **6b** (0.475 g, 0.41 mmol) in dimethylformamide (20 mL) was treated with tetrabutylammonium fluoride trihydrate (0.414 g, 1.31 mmol) at room temp. for 12 h. After the reaction was finished, the solution was partitioned between ethyl acetate (100 mL) and water (100 mL). The aqueous layer was further extracted with ethyl acetate (2 × 60 mL), and the combined organic layers were washed with brine (80 mL), dried with magnesium sulfate and filtered. Following concentration, the residue was purified by chromatography on silica gel (hexanes/acetone, 3:1) to give alcohol **7b** (0.324 g, 86%) as a syrup. $[\alpha]_D^{296\ K}$ = –21.1 (*c* = 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.17–7.31 (m, 30 H), 4.36–4.85 (m, 21 H), 4.24 (t, *J* = 9.6 Hz, 2 H), 4.14 (d, *J* = 3.3 Hz, 1 H), 3.83 (t, *J* = 9.3 Hz, 1 H), 3.67 (dd, *J* = 9.9, 2.1 Hz, 1 H), 3.52 (dd, *J* = 7.5, 3.0 Hz, 1 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 138.1, 137.8, 137.4, 136.9, 135.9, 135.9, 128.5, 128.4, 128.3, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 96.8, 96.1, 95.7, 94.2, 83.1, 80.8, 80.7, 76.1, 76.1, 76.0, 75.9, 70.7, 70.3, 70.1, 69.7, 69.5, 69.4, 69.3, 69.2, 69.2 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.06 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{52}H_{57}O_{13}PNa$ $[M + Na]^+$ 943.3429; found 943.3408.

2,6-*O*-Bis(benzyloxymethyl)-D-*myo*-inositol 3,4,5-Tris(dibenzyl phosphate) (7d): A solution of fully protected inositol **6d** (0.027 g, 0.019 mmol) in tetrahydrofuran (1 mL) was treated with tetrabutylammonium fluoride trihydrate (0.018 g, 0.056 mmol) and stirred at room temp. for 18 h. Following concentration, the residue was purified by chromatography on silica gel (hexanes/acetone, 3:1) to give alcohol **7d** (0.019 g, 84%) as a syrup. $[\alpha]_D^{296\ K}$ = –15.4 (*c* = 0.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.15 (m, 40 H), 5.14–4.84 (m, 16 H), 4.70–4.60 (m, 5 H), 4.48–4.40 (m, 3 H), 4.28–4.19 (m, 2 H), 3.85 (t, *J* = 9.4 Hz, 1 H), 3.48–3.44 (m, 1 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 137.6, 136.9, 136.1, 136.0, 135.9, 135.9, 135.8, 135.7, 135.6, 135.6, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.6, 97.1, 96.2, 82.4, 78.7, 76.8, 76.3, 75.4, 70.4, 70.1, 69.7, 69.6, 69.6, 69.5, 69.4, 69.3, 69.3 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = –0.04, –0.48, –0.58 ppm. MALDI-HRMS: calcd. for $C_{64}H_{67}O_{17}P_3Na$ $[M + Na]^+$ 1223.3489; found 1223.3498.

Cbz-Aminohexanol Phosphoramidite (8): 1*H*-Tetrazole (4.6 mL of a 0.45 M solution in acetonitrile, 2.07 mmol) and benzyloxybis(diisopropylamino)phosphane (1.392 g, 4.11 mmol) were added to a solution of cbz-aminohexanol (0.694 g, 2.76 mmol) in dichloromethane (30 mL) under nitrogen. This mixture was stirred at room temp. for 2 h, at which point the solution was extracted with dichloromethane (3 × 80 mL) from saturated sodium hydrogen carbonate (80 mL). The combined organic phases were dried with magnesium sulfate, filtered, and concentrated, and the residue was purified by chromatography on silica gel (hexane/ethyl acetate/triethylamine, 100:10:1). The resulting colorless liquid (0.983 g, 2.0 mmol) was passed on to the next step without further purification.

General Procedure for Phosphoramidite Chemistry to Convert 2a–g to 7a–g: Cbz-aminohexanol phosphoramidite **8** was added to a solution of an alcohol of type **7a**–**g** and 1*H*-tetrazole in dichloromethane (15 mL) under nitrogen, and the mixture was stirred at room temp. for 18 h. The reaction solution was cooled to –60 °C, and *m*CPBA was added. The mixture was stirred at this tempera-

ture for 60 min and slowly warmed to room temp., after removal of the cooling bath. The solution was diluted to 60 mL with dichloromethane, washed with saturated aqueous sodium hydrogen carbonate (2 × 30 mL), and the aqueous phase was extracted with dichloromethane (60 mL). The combined organic phases were dried with magnesium sulfate, filtered, and concentrated, and the residue was purified by chromatography on silica gel to yield the corresponding product of type **2a**–**g**.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,3,5,6-*O*-Tetrakis-(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 4-(Dibenzyl phosphate) (2a): Alcohol **7a** (0.173 g, 0.188 mmol), 1*H*-tetrazole (1.25 mL, 0.564 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.23 g, 0.47 mmol), and *m*CPBA (0.191 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 3:1) was used to afford product **2a** (0.181 g, 73%) as a syrup. $[\alpha]_D^{296\ K}$ = –6.9 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.16–7.33 (m, 40 H), 4.60–5.07 (m, 24 H), 4.48 (s, 3 H), 4.19–4.24 (m, 2 H), 3.90 (t, *J* = 6.3 Hz, 2 H), 3.67 (d, *J* = 9.0 Hz, 2 H), 3.06–3.08 (m, 2 H), 1.17–1.46 (m, 8 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 156.3, 138.0, 137.9, 137.5, 136.7, 136.0, 135.9, 128.6, 128.5, 128.5, 128.3, 128.3, 128.1, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 96.7, 96.4, 95.5, 94.1, 79.0, 79.0, 78.2, 76.1, 76.0, 74.6, 70.7, 70.4, 70.3, 69.6, 69.3, 68.0, 68.0, 67.8, 67.8, 66.5, 40.8, 30.0, 29.9, 29.7, 26.0, 24.9 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.06 (d, *J* = 10.2 Hz, 1 P), 0.15 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{73}H_{83}NO_{18}P_2Na$ $[M + Na]^+$ 1346.4978; found 1346.4988.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,3,4,6-*O*-Tetrakis-(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 5-(Dibenzyl phosphate) (2b): Alcohol **7b** (0.320 g, 0.348 mmol), 1*H*-tetrazole (2.3 mL, 1.05 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.424 g, 0.869 mmol), and *m*CPBA (0.27 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 3:1) was used to access product **2b** (0.256 g, 56%) as a syrup. $[\alpha]_D^{296\ K}$ = –2.3 (*c* = 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.20–7.31 (m, 40 H), 4.86–5.11 (m, 15 H), 4.72–4.81 (m, 5 H), 4.49–4.67 (m, 6 H), 4.18–4.35 (m, 4 H), 3.92 (t, *J* = 6.0 Hz, 2 H), 3.63–3.67 (m, 1 H), 3.08 (dd, *J* = 12.9, 6.3 Hz, 2 H), 1.34–1.47 (m, 4 H), 1.18 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 156.4, 138.1, 138.1, 137.9, 137.4, 136.7, 135.9, 135.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 127.9, 127.8, 127.7, 127.7, 127.5, 127.4, 127.4, 96.4, 96.3, 95.6, 93.9, 80.6, 80.5, 75.9, 75.2, 75.1, 74.7, 70.3, 70.3, 70.2, 69.6, 69.5, 69.5, 69.3, 69.3, 68.0, 67.9, 67.8, 66.4, 40.8, 29.9, 29.9, 29.6, 26.0, 24.9 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = –0.05 (d, *J* = 3.4 Hz, 1 P), 0.19 (d, *J* = 4.5 Hz, 1 P) ppm. MALDI-HRMS: calcd. for $C_{73}H_{83}NO_{18}P_2Na$ $[M + Na]^+$ 1346.4978; found 1346.4985.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,6-*O*-Bis(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 3,4,5-Tris(dibenzyl phosphate) (2d): Alcohol **7d** (0.180 g, 0.15 mmol), 1*H*-tetrazole (1.0 mL, 0.45 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.183 g, 0.375 mmol), and *m*CPBA (0.78 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 3:1) was used to afford product **2d** (0.238 g, 99%) as a syrup. $[\alpha]_D^{296\ K}$ = –14.7 (*c* = 5.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.17 (m, 50 H), 5.13–4.81 (m, 21 H), 4.76–4.52 (m, 5 H), 4.45–4.17 (m, 4 H), 3.94–3.82 (m, 2 H), 3.09–3.03 (m, 2 H), 1.46–1.18 (m, 8 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 155.9, 137.6, 137.3, 136.3, 135.6, 135.6, 135.5, 135.3, 135.2, 135.2, 135.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.6, 127.2, 127.0, 126.9, 96.0, 95.7, 95.6, 81.9, 77.5, 75.1, 75.0, 74.8, 74.7, 74.3, 74.1, 74.0, 70.0, 69.9, 69.6, 69.3, 69.1, 69.0, 68.9, 68.9, 68.8, 67.7, 67.6, 67.5, 67.4, 66.0, 52.9, 40.4, 29.5, 29.4, 29.1, 25.5, 24.4 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 0.13, –0.03, –0.21, –0.56 ppm. MALDI-HRMS: calcd. for $C_{85}H_{93}NO_{22}P_4Na$ $[M + Na]^+$ 1626.5032; found 1626.5042.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,4,5,6-*O*-tetra-(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 3-(Dibenzyl phosphate) (2e): Alcohol **7e** (0.354 g, 0.442 mmol), 1*H*-tetrazole (2.4 mL, 1.06 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.432 g, 0.884 mmol), and *m*CPBA (0.36 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 2:1) was used to yield product **2e** (0.47 g, 88%) as a syrup. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.18–7.33 (m, 40 H), 4.77–5.08 (m, 17 H), 4.50 (d, *J* = 8.8 Hz, 1 H), 4.30 (d, *J* = 8.8 Hz, 2 H), 4.03 (d, *J* = 9.2 Hz, 2 H), 3.86–3.92 (m, 2 H), 3.47–3.48 (m, 1 H), 3.10 (m, 2 H), 1.38–1.51 (m, 4 H), 1.18 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 156.4, 138.6, 138.3, 138.2, 136.7, 135.9, 135.7, 128.4, 128.4, 128.4, 128.3 128.0, 127.9, 127.8, 127.7, 127.7, 127.5, 127.2, 82.7, 79.9, 78.3 77.9, 76.1, 75.7, 75.6, 69.4, 69.3, 68.0, 66.5, 40.9, 30.0, 29.7, 26.1, 25.0, 24.9 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = –1.57–1.37 (m, 2 P) ppm. MALDI-HRMS: calcd. for $C_{69}H_{75}NO_{14}P_2Na$ $[M + Na]^+$ 1226.4555; found 1226.4502.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,5,6-*O*-Tris(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 3,4-Bis(dibenzyl phosphate) (2f): Alcohol **7f** (0.37 g, 0.381 mmol), 1*H*-tetrazole (2.2 mL, 0.953 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.372 g, 0.762 mmol), and *m*CPBA (0.36 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 3:1) was used to give product **2f** (0.42 g, 80%) as a syrup. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.04–7.33 (m, 45 H), 4.73–5.08 (m, 21 H), 4.62 (s, 1 H), 4.29–4.34 (m, 2 H), 4.07 (t, *J* = 9.6 Hz, 1 H), 3.84–3.88 (m, 2 H), 3.44–3.51 (m, 1 H), 3.09–3.11 (m, 2 H), 1.17–1.51 (m, 8 H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 156.4, 138.3, 138.0, 136.7, 136.0, 135.8, 135.8, 135.6, 128.5, 128.5, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 127.2, 80.7, 79.6, 78.0, 77.8, 76.0, 75.8, 75.5, 69.8, 69.8, 69.6, 69.3, 68.1, 67.9, 66.4, 40.1, 30.8, 29.9, 29.6, 26.0, 24.9 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = –0.211 (s, 1 P), –0.31 (d, *J* = 22.8 Hz, 1 P), –0.87 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{76}H_{82}NO_{17}P_3Na$ $[M + Na]^+$ 1396.4688; found 1396.4740.

Benzyl (6-Benzyloxycarbonylaminohexyl) 2,4,6-*O*-Tris(benzyloxymethyl)-D-*myo*-inosit-1-yl Phosphate 3,5-Bis(dibenzyl phosphate) (2g): Alcohol **7g** (0.22 g, 0.227 mmol), 1*H*-tetrazole (1.3 mL, 0.568 mmol, 0.45 M in acetonitrile), Cbz-aminohexanol phosphoramidite **8** (0.221 g, 0.453 mmol), and *m*CPBA (0.22 g, 57–86%) were used. Purification by chromatography on silica gel (hexanes/acetone, 3:1) was used to provide product **2g** (0.271 g, 92%) as a syrup. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.97–7.41 (m, 45 H), 4.48–5.17 (m, 21 H), 4.32–4.35 (m, 2 H), 4.09 (t, *J* = 9.0 Hz, 2 H), 3.85–3.86 (m, 2 H), 3.08–3.10 (m, 2 H), 1.15–1.38 (m, 8 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 156.4, 138.4, 138.3, 138.0, 138.0, 137.2, 137.1, 137.1, 136.9, 136.9, 135.8, 135.8, 135.7, 135.7, 135.6, 135.5, 135.4, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.3, 78.1, 78.0, 78.0, 77.9, 77.9, 77.9, 77.9, 77.3, 75.7, 74.5, 69.6, 69.5, 69.3, 69.3, 69.2, 69.2, 66.4, 40.8, 29.6, 26.0, 24.8 ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = –0.32 (d, *J* = 5.6 Hz, 1 P), –0.59 (d, *J* = 16.2 Hz, 1 P), –0.65 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{76}H_{82}NO_{17}P_3Na$ $[M + Na]^+$ 1396.4688; found 1396.4722.

General Procedure for the Global Deprotection of 2a–g to 1a–g by Hydrogenolysis: Palladium hydroxide (20%) on charcoal was added to a solution of a compound of type **2a**–**g** in methanol (15–40 mL).

The mixture was then stirred at room temp. with 1 atm of hydrogen (balloon) for 3 d. The catalyst was removed by filtration and the residue washed with methanol. The solvent was removed in vacuo, and the crude product was dissolved in water and stirred with Chelex 100 resin (Sigma, Na^+ form) for 3 h. The resin was removed by filtration and the filtrate lyophilized to give the corresponding amino conjugate of type **1a**–**g**.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 4-(Disodium phosphate) (1a): Compound **2a** (0.18 g, 0.137 mmol) and 20% palladium hydroxide on charcoal (0.20 g) provided amino conjugate **1a** (70 mg, 100%) as a white solid. $[\alpha]_D^{296\ K} = -7.0$ ($c = 0.64$, H_2O). ^1H NMR (300 MHz, D_2O): $\delta = 4.23$ (s, 1 H), 4.12 (dd, $J = 16.8$, 9.0 Hz, 1 H), 3.88–3.98 (m, 3 H), 3.81 (t, $J = 9.6$ Hz, 1 H), 3.63 (dd, $J = 9.9$, 2.7 Hz, 1 H), 3.45 (t, $J = 9.3$ Hz, 1 H), 2.99 (t, $J = 7.2$ Hz, 2 H), 1.62–1.66 (m, 4 H), 1.40 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, D_2O): $\delta = 78.8$, 78.7, 78.7, 76.5, 74.1, 73.6, 69.0, 42.1, 32.2, 29.4, 27.8, 27.1 ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 5.67$ (s, 1 P), 1.13 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{12}H_{28}NO_{12}P_2$ (free acid, $[M + H]^+$) 440.1081; found 440.1088.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 5-(Disodium phosphate) (1b): Compound **2b** (0.25 g, 0.189 mmol) and 20% palladium hydroxide on charcoal (0.27 g) afforded amino conjugate **1b** (96 mg, 100%) as a white solid. $[\alpha]_D^{296\ K} = -17.4$ ($c = 0.69$, H_2O). ^1H NMR (300 MHz, D_2O): $\delta = 4.23$ (s, 1 H), 3.75–3.97 (m, 6 H), 3.57–3.61 (m, 1 H), 2.98 (t, $J = 7.5$ Hz, 2 H), 1.62–1.68 (m, 4 H), 1.40 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, D_2O): $\delta = 80.8$, 78.6, 74.6, 73.8, 73.6, 73.3, 68.9, 42.1, 32.0, 29.2, 27.7, 27.0 ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 4.62$ (s, 1 P), 0.92 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{12}H_{28}NO_{12}P_2$ (free acid, $[M + H]^+$) 440.1081; found 440.1063.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 3,4,5-Tris(disodium phosphate) (1d): Compound **2d** (0.23 g, 0.143 mmol) and 20% palladium hydroxide on charcoal (0.30 g) yielded amino conjugate **1d** (88 mg, 100%) as a white solid. $[\alpha]_D^{296\ K} = -7.2$ ($c = 0.68$, H_2O). ^1H NMR (400 MHz, D_2O): $\delta = 4.04$ (br. s, 2 H), 4.22–3.95 (m, 6 H), 3.02 (br. s, 2 H), 1.68 (br. s, 4 H), 1.44 (br. s, 4 H) ppm. ^{13}C NMR (100.6 MHz, D_2O): $\delta = 78.2$, 76.1, 75.3, 74.5, 70.9, 70.7, 66.3, 39.5, 29.4, 26.6, 25.1, 24.4 ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 2.75$, 2.54, 1.78, 0.89 ppm. MALDI-HRMS: calcd. for $C_{12}H_{31}NO_{18}P_4Na$ $[M + Na]^+$ 624.0389; found 624.0301.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 3-(Disodium phosphate) (1e): Compound **2e** (0.457 g, 0.34 mmol) and 20% palladium hydroxide on charcoal (0.45 g) afforded amino conjugate **1e** (171 mg, 100%) as a white solid. $[\alpha]_D^{296\ K} = -2.3$ ($c = 0.72$, H_2O). ^1H NMR (400 MHz, D_2O): $\delta = 4.39$ (s, 1 H), 3.93–4.03 (m, 4 H), 3.82 (dd, $J = 21.2$, 9.6 Hz, 2 H), 3.42 (t, $J = 9.6$ Hz, 1 H), 3.03 (t, $J = 7.2$ Hz, 2 H), 1.67–1.73 (m, 4 H), 1.45–1.47 (m, 4 H) ppm. ^{13}C NMR (100.5 MHz, D_2O): $\delta = 78.6$, 76.7, 76.7, 74.8, 74.1, 73.7, 69.0, 42.1, 32.1, 29.3, 27.8, 27.1 ppm. ^{31}P NMR (161.8 MHz, D_2O): $\delta = 6.86$ (d, $J = 7.9$ Hz, 1 P), 2.69 (d, $J = 3.2$ Hz, 1 P) ppm. MALDI-HRMS: calcd. for $C_{12}H_{28}NO_{12}P_2$ (free acid, $[M + H]^+$) 440.1081; found 440.1078.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 3,4-Bis(disodium phosphate) (1f): Compound **2f** (0.42 g, 0.306 mmol) and 20% palladium hydroxide on charcoal (0.50 g) provided amino conjugate **1f** (191 mg, 100%) as white solid. $[\alpha]_D^{296\ K} = +3.4$ ($c = 0.60$, H_2O). ^1H NMR (400 MHz, D_2O): $\delta = 4.47$ (s, 1 H), 4.27 (dd, $J = 18.0$, 4.8 Hz, 1 H), 3.95–4.06 (m, 4 H), 3.87 (t, $J = 9.6$ Hz, 1 H), 3.57 (t, $J = 9.2$ Hz, 1 H), 3.04 (t, $J = 7.6$ Hz, 2 H), 1.70–1.72 (m, 4 H), 1.46 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, D_2O): $\delta = 78.7$, 78.3, 76.7, 76.5, 73.9, 73.4, 69.0, 42.1, 32.1, 29.3, 27.8, 27.0 ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 5.89$ (s, 1 P), 4.48 (s, 1 P), 1.05 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{12}H_{29}NO_{15}P_3$ (free acid, $[M + H]^+$) 520.0745; found 520.0730.

1-(6-Aminohexyl sodium phosphate)-D-*myo*-inositol 3,5-Bis(disodium phosphate) (1g): Compound **2g** (0.270 g, 0.197 mmol) and 20% palladium hydroxide on charcoal (0.30 g) gave amino conjugate **1g** (122 mg, 100%) as a white solid. $[\alpha]_D^{296\ K} = -4.8$ ($c = 0.65$, H_2O). ^1H NMR (300 MHz, D_2O): $\delta = 4.43$ (s, 1 H), 3.89–4.03 (m, 7 H), 2.99 (t, $J = 7.5$ Hz, 2 H), 1.63–1.69 (m, 4 H), 1.41 (m, 4 H) ppm. ^{13}C NMR (100.6 MHz, D_2O): $\delta = 80.9$, 78.1, 77.3, 73.9, 73.6, 72.9, 69.0, 42.1, 32.0, 29.2, 27.7, 27.0 ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 4.05$ (s, 1 P), 2.48 (s, 1 P), 0.91 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{12}H_{29}NO_{15}P_3$ (free acid, $[M + H]^+$) 520.0745; found 520.0728.

1-[6-(14-Biotinamido-3,6,9,12-tetraoxatetradecanamido)hexyl sodium phosphate]-D-*myo*-inositol 4,5-Bis(disodium phosphate) (9): Succinimidyl ester **12a** was dissolved in dimethylformamide (1 mL), and compound **1c** (7 mg, 0.011 mmol), dissolved in triethylammonium hydrogen carbonate (TEAB, 0.5 M, 1 mL), was added. The mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the residue was washed with acetone (4 × 2 mL) and dried in vacuo. The resulting white solid was dissolved in water, and the solution was stirred with Chelex 100 resin (Sigma, Na^+ form) for 3 h. The resin was removed by filtration, and the filtrate was lyophilized to give the product (13 mg, 100%) as white solid. ^1H NMR (300 MHz, D_2O): $\delta = 4.56$–4.58 (m, 1 H), 4.38–4.41 (m, 1 H), 4.04–4.25 (m, 3 H), 3.82–3.95 (m, 4 H), 3.58–3.74 (m, 14 H), 2.93–3.40 (m, 14 H), 2.72–2.85 (m, 2 H), 2.24 (d, $J = 6.9$ Hz, 2 H), 1.33–1.79 (m, 16 H) ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 5.79$ (s, 1 P), 5.70 (s, 1 P), 0.87 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{32}H_{61}N_4O_{22}P_3SH$ (free acid, $[M + H]^+$) 979.2784; found 979.2775.

1-[6-(4-Benzoylbenzamido)hexyl sodium phosphate]-D-*myo*-inositol 4,5-Bis(disodium phosphate) (10): Compound **1c** (8 mg, 0.0127 mmol) in triethylammonium hydrogen carbonate (TEAB, 0.5 M, 0.85 mL) was added to a solution of succinimidyl ester **12b** (8 mg, 0.0254 mmol) in dimethylformamide (0.85 mL). Tetrahydrofuran (0.45 mL) was then added, and the mixture was stirred at room temp. for 24 h. The solvent was removed under reduced pressure, and the residue was washed with acetone (4 × 2 mL) and dried in vacuo. The white solid was dissolved in water and filtered, and the filtrate was lyophilized to afford product **10** (12 mg, 100%) as a white solid, containing a small amount of triethylammonium buffer salt. ^1H NMR (300 MHz, D_2O): $\delta = 7.70$–7.85 (m, 7 H), 7.57 (t, $J = 7.8$ Hz, 2 H), 4.25–4.28 (m, 2 H), 3.87–4.00 (m, 5 H), 3.69 (d, $J = 9.0$ Hz, 1 H), 3.40 (t, $J = 6.6$ Hz, 2 H), 1.64 (m, 4 H), 1.41 (m, 4 H) ppm. ^{31}P NMR (121.5 MHz, D_2O): $\delta = 3.47$ (s, 1 P), 2.46 (s, 1 P), 0.91 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{26}H_{37}NO_{17}P_3$ $[M + H]^+$ 728.1269; found 728.1245.

Bifunctional PI-(4,5)-P_2–Benzophenone/Azide Conjugate 11: *N*-Hydroxysuccinimide (3 mg, 0.0254 mmol) and dicyclohexylcarbodiimide (5.5 mg, 0.0254 mmol) were added to a solution of carboxylic acid **15** (9.6 mg, 0.0254 mmol) in tetrahydrofuran (1 mL), and the mixture was stirred at room temp. for 24 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was washed with hexane/diethyl ether (1:1) and dried in vacuo. The resulting white solid was dissolved in dimethylformamide (1 mL), and compound **1c** (8 mg, 0.0127 mmol) in triethylammonium hydrogen carbonate (TEAB, 0.5 M, 1.3 mL) was added. Tetrahydrofuran (0.5 mL) was added, and the mixture was stirred at room temp. for 24 h. The solvent was removed under reduced pressure, and the residue was washed with acetone (5 × 2 mL) and dried in vacuo. The resulting white solid was dis-

solved in water, and the solution was stirred with Chelex 100 resin (Sigma, Na^+ form) for 3 h. The resin was removed by filtration, and the filtrate was lyophilized to give the product **11** (12 mg, 95%) as a white solid. ^{1}H NMR (300 MHz; D_2O/CD_3OD, 1:1): δ = 7.74–8.00 (m, 7 H), 7.62 (t, J = 7.8 Hz, 2 H), 4.52 (t, J = 7.5 Hz, 1 H), 4.22–4.25 (m, 2 H), 3.93–4.03 (m, 6 H), 3.70–3.73 (m, 1 H), 3.18–3.28 (m, 2 H), 1.89–1.92 (m, 2 H), 1.32–1.71 (m, 14 H) ppm. ^{31}P NMR (121.5 MHz, D_2O/CD_3OD = 1:1): δ = 5.80 (s, 1 P), 5.72 (s, 1 P), 0.87 (s, 1 P) ppm. MALDI-HRMS: calcd. for $C_{32}H_{47}N_5O_{18}P_3$ $[M + H]^+$ 882.2124; found 882.2179.

Benzophenone-Lys(N_3)-OMe (14): Benzophenone-Lys(Boc)-OMe (**13**)[53] (112 mg, 0.238 mmol) was dissolved in dichloromethane (2 mL). With stirring, trifluoroacetic acid (2 mL) was added, and stirring was continued for 2 h. The solvent was removed under reduced pressure, and the residue was dried under high vacuum for 2 h to remove excess trifluoroacetic acid. The residue was dissolved in methanol (2.5 mL), and potassium carbonate [1.27 mL, 0.804 M (aqueous), 1.03 mmol] and copper sulfate·$5H_2O$ [238 μL, 0.01 M (aqueous), 10 mol-%] were added. Imidazole-1-sulfonyl azide hydrochloride[55] (60 mg, 0.286 mmol) was added, and stirring was continued at room temp. for 3.5 h. The reaction mixture was extracted with ethyl acetate (2 × 100 mL), which was subsequently washed with 2 N hydrochloric acid (50 mL). The organic phase was dried with magnesium sulfate, filtered, and concentrated under reduced pressure. Column chromatography (silica gel; ethyl acetate/hexanes, 1:1) afforded **14** as a clear glass (91 mg, 97%). $[\alpha]_D^{296\,K}$ = +22.95(c = 2.296, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94–7.79 (m, 6 H), 7.63 (t, J = 7.4 Hz, 1 H), 7.53–7.48 (m, 2 H), 6.85 (d, J = 7.5 Hz, 1 H), 4.91–4.84 (m, 1 H), 3.82 (s, 3 H), 3.03 (t, J = 6.6 Hz, 2 H), 2.08–1.46 (m, 6 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 195.9, 172.8, 166.3, 140.3, 137.0, 136.9, 133.0, 130.14, 130.09, 128.5, 127.1, 52.7, 52.5, 51.0, 32.1, 28.4, 22.5 ppm. DART-HRMS: calcd. for $C_{21}H_{23}N_4O_4$ $[M + H]^+$ 395.17193; found 395.16983.

Benzophenone-Lys(N_3)-OH (15): Benzophenone-Lys(N_3)-OMe (**14**) was dissolved in tetrahydrofuran (2 mL). With stirring, 2 N sodium hydroxide (2 mL) was added, and stirring was continued for 70 min. At this point, the solution was neutralized to pH ≈ 4 with Dowex® 50WX8-200H^+ ion resin. The solution was then filtered, and the filtrate was concentrated under reduced pressure to afford **15** as a clear solid (43 mg, quant.). $[\alpha]_D^{296\,K}$ = +17.97(c = 2.02, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90–7.87 (m, 2 H), 7.78–7.73 (m, 4 H), 7.60 (t, J = 7.5 Hz, 1 H), 7.49–7.44 (m, 2 H), 4.79 (br. s, 1 H), 3.28 (t, J = 6.3 Hz, 2 H), 2.03–1.87 (m, 2 H), 1.63–1.53 (m, 4 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 196.1, 167.2, 140.4, 136.73, 136.69, 133.1, 130.13, 130.10, 128.5, 127.2, 51.0, 51.0, 31.5, 28.4, 22.7 ppm. DART-HRMS: calcd. for $C_{20}H_{21}N_4O_4$ $[M + H]^+$ 381.15628; found 381.15494.

Supporting Information (see footnote on the first page of this article): Spectra for novel compounds.

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