DOI: 10.1002/ejoc.201001057

On the Compatibility of Azides in Phosphoramidite-Based Couplings: Synthesis of a Novel, Convertible Azido-Functionalized CyPLOS Analogue

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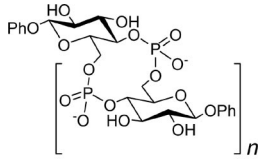
Keywords: Azides / Carbohydrates / Macrocycles / Phosphorylation

With the aim of preparing a library of ion transporters based on a CyPLOS (cyclic phosphate-linked oligosaccharide) backbone, we describe the synthesis of a novel azido-derivatized CyPLOS analogue that is tailored for practical post-synthetic functionalization with a variety of labels. As a prerequisite for the effective preparation of this phosphate-linked macrocycle, the compatibility of azido alcohols as coupling agents in phosphoramidite synthetic protocols was preliminarily investigated in solution by using ^{31}P NMR spectroscopy to monitor the standard coupling of a nucleoside 3′-phosphoramidite with suitable model alcohols and azides.

Introduction

In the search for selective artificial receptors, we recently described the synthesis and conformational properties of CyPLOS (cyclic phosphate-linked oligosaccharides), which is a novel class of phosphodiester-linked oligosaccharide analogues (**1a**–**c**, Figure 1).[1]



1a (*n* = 1)
1b (*n* = 2)
1c (*n* = 3)

Figure 1. Chemical structures of CyPLOS **1a**–**c** (Ph = phenyl).

Amphiphilic macrocycles with a remarkable propensity toward aggregation were then obtained by attaching long tentacles with different lipophilicity, particularly *n*-undecyl or tetraethylene glycol (TEG) chains, to the CyPLOS backbone (**2**–**4**, Figure 2).[2] Initial studies on the ionophoric activity of amphiphilic CyPLOS[3] showed **4** to be the most effective compound in the series **2**–**4**; this compound was able to completely discharge a pH gradient across liposomial membranes in less than 20 min at 2% ionophore concentration. This property was strictly correlated to the presence of TEG chains, with **4** being much more active than **3**, and tetra-alkylated derivative **2** being almost completely inactive. Subsequent optimization of the design of amphiphilic CyPLOS produced the fluorescent analogue **5**; the insertion of dansyl units at the extremities of the TEG tentacles resulted in a more active ion transporter, and also provided deeper insights into its mechanism of action.[4]

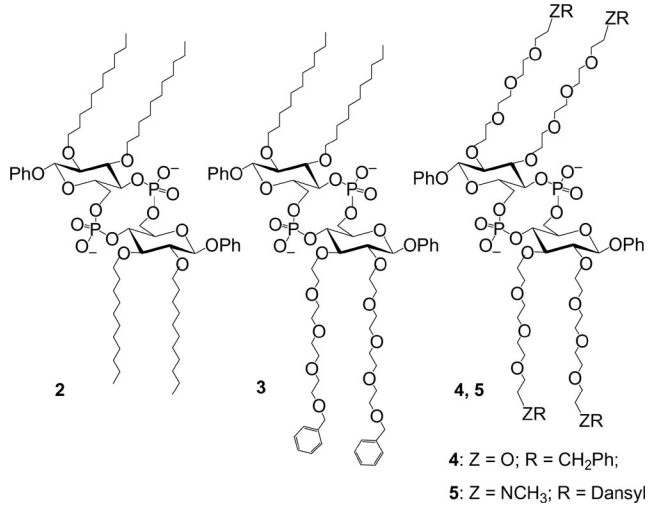


Figure 2. Chemical structures of amphiphilic CyPLOS **2**–**5**.

The dansyl moieties in **5** were introduced at the level of the fully protected monosaccharide, which was synthesized from the TEG-azide, after reduction to the amine and coupling with dansyl chloride (**II**, **III** and **IV**, respectively, Figure 3). With the aim of introducing different reporter groups to the CyPLOS tentacles, which play a major role in self-aggregation and ion transport activity, we realized that the set of accessible CyPLOS analogues following this strategy was limited. Once inserted in the monosaccharide building blocks, most labels, in fact, do not survive the reaction conditions necessary to convert them into the target macrocycle. Therefore, a general approach to the synthesis of diversely end-functionalized analogues – starting from a

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201001057.

unique precursor, in a flexible post-synthetic functionalization protocol – would be highly desirable. In this perspective, terminal azido moieties, if kept intact until the final step of the synthesis, can be used as useful, convertible groups for post-synthetic decoration of CyPLOS.

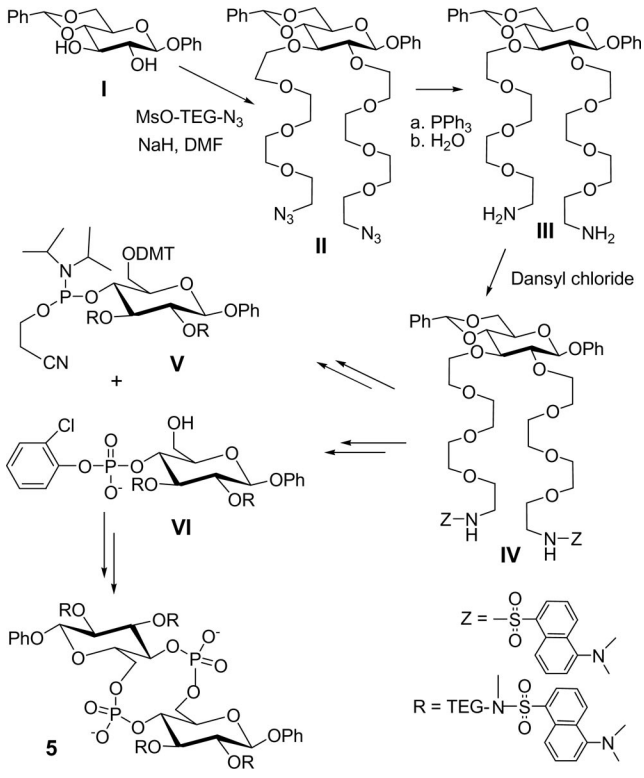


Figure 3. Synthetic scheme for the preparation of dansyl-labeled CyPLOS **5** developed previously.[4] Ms = mesyl; DMT = 4,4′-dimethoxytrityl group.

Many recent works have emphasized the variety of applications of azido moieties in organic chemistry[5] as chemically stable groups that are easily amenable to useful transformations, such as the Staudinger reaction[6–8] and copper-catalyzed 1,3-dipolar cycloaddition, known as "click chemistry".[9–11] Both reactions are extensively exploited in the synthesis of bioconjugates because they involve mild reaction conditions and simple work-up procedures, and generally give high yields.

Encouraged by the increasing use of azido groups as versatile chemical handles for easy synthetic access to various conjugates of multifunctional biopolymers, such as peptides[12] and oligonucleotides,[13–22] we revisited our original design so that this chemical entity could be kept on the CyPLOS backbone. Azido groups – if inserted at the extremities of the tentacles of these macrocycles – would allow post-synthetic condensation with labels that would enable the properties of these artificial ionophores to be studied. We envisioned that the incorporation of a spin label into the CyPLOS tentacle should provide a useful tool that could be used to investigate interactions within phospholipid bilayers by means of ESR spectroscopy. Therefore, starting from azido-functionalized CyPLOS **A**, derivatives **B**, which incorporate one 4-carboxy-2,2,6,6-tetramethyl-1-piperidinoxy (4-carboxy TEMPO) residue,[23] were designed (Figure 4). However, before undertaking the synthesis of CyPLOS **A**, it was necessary to first ascertain whether phosphoramidite-based coupling protocols, which are required to build the backbone of the target molecule, could be applied without significantly interfering with the azido functions. Here we describe coupling experiments, based on standard phosphoramidite protocols, between a nucleoside 3′-phosphoramidite and TEG alcohols or TEG azides, monitored by ^{31}P NMR spectroscopy. On the basis of the obtained results, the convergent synthesis of CyPLOS derivatives **A** and **B** has been successfully realized.

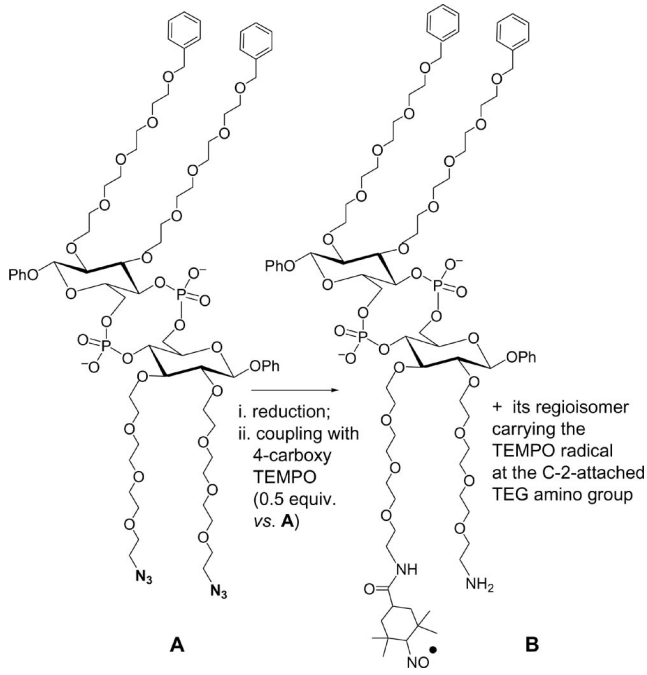


Figure 4. Conversion of azido-functionalized CyPLOS **A** into spin-labeled **B** – obtained as a mixture of regioisomers – through azide reduction followed by coupling with 4-carboxy TEMPO. Reagents and conditions: (i) (a) Ph_3P, THF, room temp., 12 h; (b) H_2O, room temp., 48 h (quant.); (ii) 4-carboxy-TEMPO, DIPEA, DCC, room temp.,12 h (67%).

Results and Discussion

The problem of compatibility of azido groups with standard phosphoramidite chemistry clearly emerged in several studies on oligonucleotide conjugates.[15–22] This aspect was first studied by Sekine et al., who concluded that nucleosides carrying azido groups cannot be adopted in phosphoramidite-based oligonucleotide synthesis because of the interfering Staudinger reaction that takes place between azides and P^{III} derivatives.[24] In their experiment, 2-azido-2′-deoxyadenosine was treated with 1.5 equiv. of diethyl, *N*,*N*-diisopropylphosphoramidite in *N*,*N*-dimethylformamide (DMF)/CD_3CN (9:1, v/v). The progress of the reaction, which was monitored by ^{31}P NMR spectroscopic analysis of the reaction mixture, showed initial formation of the phosphazide intermediate of the Staudinger reaction, which

then converted into the iminophosphorane within approximately two hours. These results indicate that azido alcohols cannot be transformed into the corresponding azido phosphoramidites by treatment with phosphitylating reagents, because of the significant nucleophilicity of P^{III} towards azides. This limitation was circumvented by using H-phosphonate chemistry for the oligonucleotide chain assembly.[15,16,24]

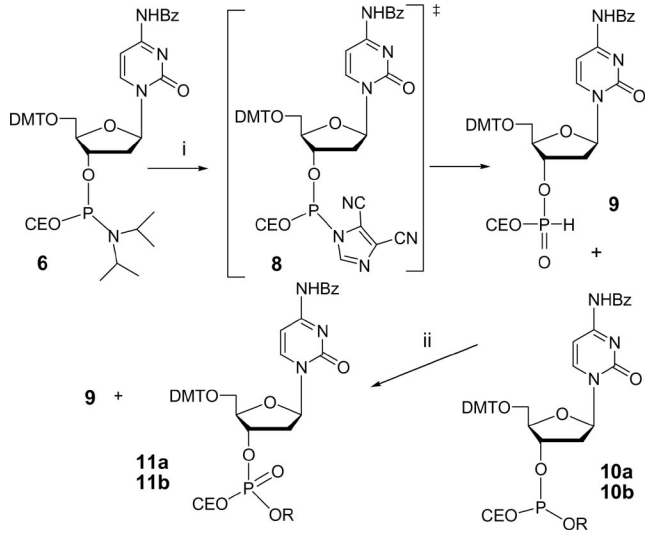
In a more recent paper, van Delft et al. prepared a 2′-*O*-(3-azidopropyl) adenosine derivative that was subsequently converted into the corresponding phosphoramidite nucleoside.[19] This building block was isolated, albeit in extremely low yields, but could not be incorporated at the 5′-end of an oligonucleotide still anchored to the solid support and, when dissolved in acetonitrile, was found to decompose within two weeks. This degradation was not investigated in detail, but the Staudinger-type condensation of the P^{III}-containing moiety with the azido function can reasonably account for the increased lability of the phosphoramidite nucleoside in solution. To avoid this detrimental side reaction, the azido function was introduced by a time-consuming post-elongation synthetic protocol in solution.[16,19] Alternatively, 5′-azido-oligonucleotides were prepared through 5′-iodination,[13] or by use of bromoalkyl building blocks. The latter were inserted into an oligonucleotide sequence[21] or at the 5′-end,[15,17] by using an automated solid-phase synthesis protocol. Both synthetic ways were followed by treatment with sodium azide. Interestingly, Lonnberg et al. introduced the azido function into an oligonucleotide sequence by exploiting a 4′-(azidomethyl)thymidine-3′-*H*-phosphonate building block; chain elongation was then realized by using either H-phosphonate or phosphoramidite chemistry without observing the Staudinger reaction.[18] Indeed, the experiments carried out in the group of van Delft can be interpreted by considering that the competition for a preformed phosphoramidite, which is dissolved in acetonitrile in the presence of an acidic catalyst, by an azido function in solution and a hydroxyl group in the solid phase, completely favors the homogeneous reaction over the heterogeneous condensation.[19] In line with this interpretation, parallel experiments show that if both the azido and the hydroxyl functions are in the solid phase, the support-bound azides do not disturb the phosphoramidite coupling, and phosphite triester bonds are realized smoothly.[18,22] To the best of our knowledge, the concomitant presence *in solution* of an alcohol and an azide, both competing for the condensation with an activated phosphoramidite, was not investigated in detail. This issue was briefly reported in a recent paper, in which the conversion of a 4-azido-4-deoxy-D-galactoside into 4-deoxy-D-*erythro*-hexos-3-ulose by reaction with a phosphoramidite reagent and tetrazole as a catalyst, was described.[25] In all cases, the ready formation of the phosphite derivative of the 4-azido sugar was observed to take place in less than 5 min and – only in the presence of unhindered phosphoramidites – an intramolecular Staudinger reaction occurred as a subsequent step, requiring 5–12 h to go to completion. On the other hand, it was also noted that, in the case of dibenzyl *N*,*N*-diisopropyl-phosphoramidite, coupling with the sugar hydroxyl group gave a stable derivative that did not undergo further conversion, probably due to the bulkiness of the substituents at the P^{III} atom.

For a clear elucidation of the effective potential of azido-functionalized building blocks in phosphoramidite chemistry protocols, here required for the synthesis of **A**-type compounds, we first studied a simple model system. For this purpose, commercially available 5′-*O*-DMT-N^4-benzoyl-2′-deoxycytidine-3′-phosphoramidite (**6**) was reacted under the standard phosphoramidite coupling procedure, by using 0.25 M 4,5-dicyanoimidazole (DCI) in anhydrous CH_3CN as the activator, with azido alcohol N_3-TEG-OH (**7a**; Table 1). This substrate, which was designed for insertion into CyPLOS **A**, was prepared from tetraethylene glycol as described previously.[4]

Table 1. Chemical structures of the TEG-derivatives tested in the coupling with 2′-deoxycytidine-3′-phosphoramidite **6**.

Entry	Chemical structure
7a	$HO(CH_2CH_2O)_3CH_2CH_2N_3$
7b	$HO(CH_2CH_2O)_3CH_2CH_2ODMT$
7c	$N_3(CH_2CH_2O)_3CH_2CH_2N_3$

To obtain suitable reference compounds, the nucleoside phosphoramidite **6** was also reacted under the same conditions with related TEG derivatives carrying only one primary alcohol (DMTO-TEG-OH, **7b**) or azido group (N_3-TEG-N_3, **7c**). In parallel, the same experiment with **7c** was repeated in the absence of activators. The coupling, which was carried out in an NMR tube with $CDCl_3$ as a co-solvent in the presence of activated molecular sieves (4 Å), was monitored by recording a set of ^{31}P NMR spectra at dif-



Scheme 1. General scheme for the coupling of **6** with alcohols **7a** (N_3-TEG-OH) or **7b** (DMTO-TEG-OH). Reagents and conditions: (i) **7a** or **7b** (1.2 equiv.), DCI (600 μL, 0.25 M in $CH_3CN/CDCl_3$, 5:1); (ii) *t*BuOOH (50 μL, 5.5 M in decane). Bz = benzoyl; CE = 2-cyanoethyl; DMT = 4,4′-dimethoxytrityl; R for **10a**, **11a** = $-(CH_2CH_2O)_3CH_2CH_2N_3$; R for **10b**, **11b** = $-(CH_2CH_2O)_3CH_2CH_2ODMT$.

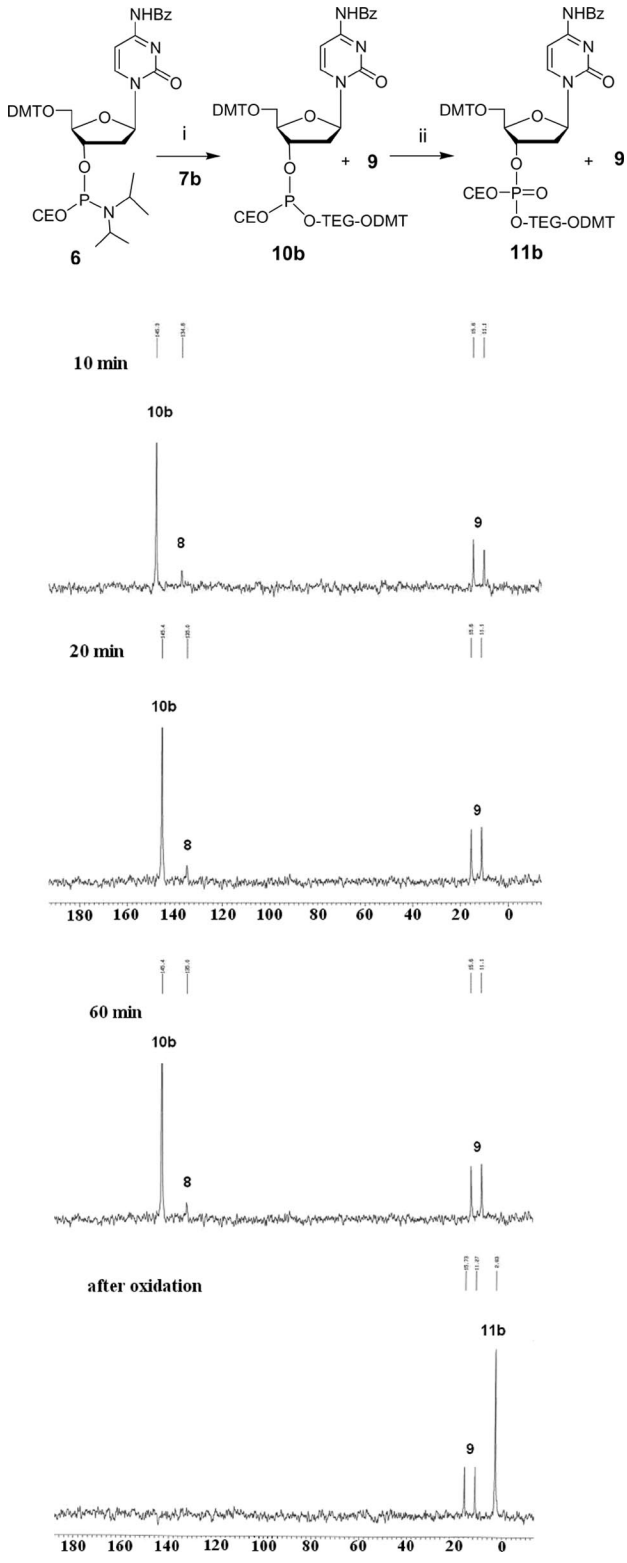
ferent times. Subsequently, a large excess of the oxidizing agent *tert*-butyl hydroperoxide (*t*BuOOH) was added to the reaction mixtures. The general reaction for coupling phosphoramidite **6** with alcohols is depicted in Scheme 1.

As expected, in the reaction with **7b** (Scheme 2), the ready formation (less than 10 min) of the phosphite triester **10b** was observed (^{31}P NMR: δ = 145 ppm); this was obtained through the transient activated phosphoramidite **8** (^{31}P NMR: δ = 135 ppm) along with traces of H-phosphonate diester **9** (due to advantageous moisture in the NMR tube) accounting for the two signals at δ = 15 and 11 ppm (identified as a doublet with J_{HP} = 729 Hz). This reaction mixture, monitored over a period of 6 h, did not show further spontaneous transformations. As expected, upon oxidation the phosphite triester **10b** was rapidly (within less than 5 min) converted into phosphate triester **11b**, which gave rise to a single ^{1}H NMR signal at δ = 2.8 ppm, whereas the H-phosphonate derivative was essentially unaltered.[26]

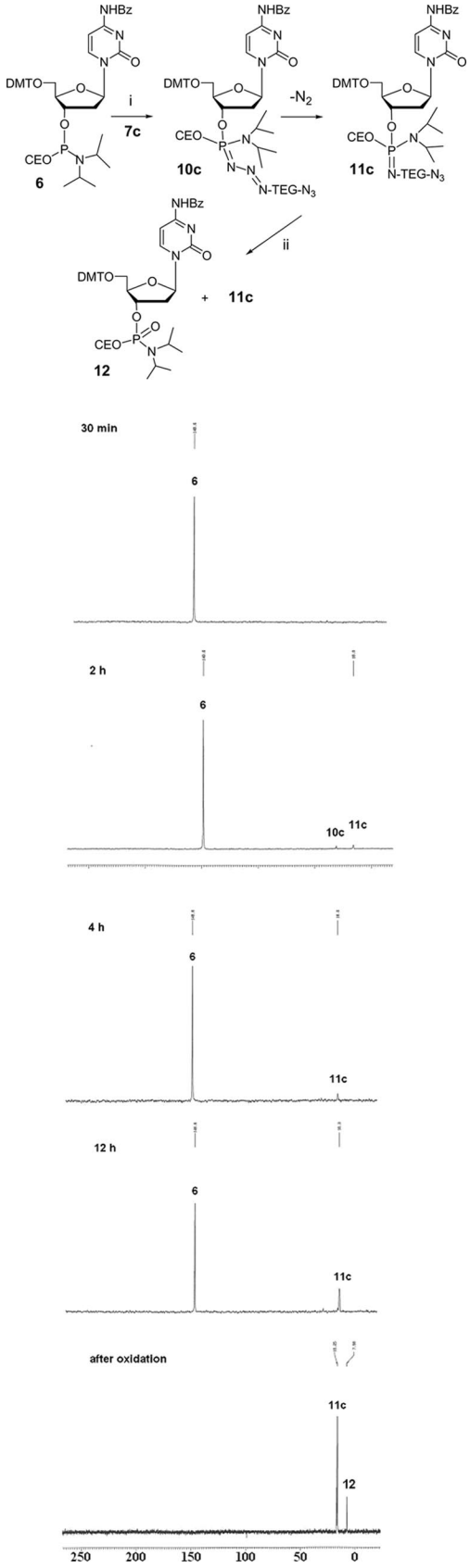
Mixing of **6** and **7c** (Scheme 3) gave, as expected, different results. In the absence of an acidic activator, the ^{31}P NMR spectrum showed a unique signal due to phosphoramidite **6**, and only after 2 h were two new, small signals (δ = 31 and 16 ppm) observed; after 4 h, the signal at δ = 31 ppm disappeared, and no further changes were observed, even not after 12 h. In analogy with the assignments given by the group of Sekine, the signal at δ = 31 ppm was attributed to the Staudinger phosphazide intermediate **10c**. The signal at δ = 16 ppm may be due to the iminophosphorane-type adduct **11c**, which was obtained as a stable derivative upon loss of a nitrogen molecule.[27]

After oxidation, the signal at δ = 153 ppm disappeared and a new signal at δ = 8 ppm emerged that was attributable to phosphoramidate **12**, obtained from **6**, and the signal at approximately δ = 16 ppm (due to **11c**) increased. The Staudinger adduct **11c** was also found in the coupling between **6** and **7c** in the presence of DCI (Scheme 4), but this reaction proved to be much slower than the corresponding reaction carried out without DCI. In fact, after 4 h, the only detectable event was the conversion of **6**, through the reactive intermediate **8**, into H-phosphonate **9**, with the signals at δ = 153 ppm (due to **6**) and at δ = 135 ppm (due to **8**) still prevailing. After a further 16 h reaction time, **6** almost completely degraded to **9**. Only two new, small signals emerged: the first (δ = 13 ppm) was attributed to **11c**, whereas the second (broad resonance at δ = 0 ppm), could be due to the phosphate diester, generated by hydrolysis of **11c**.[28]

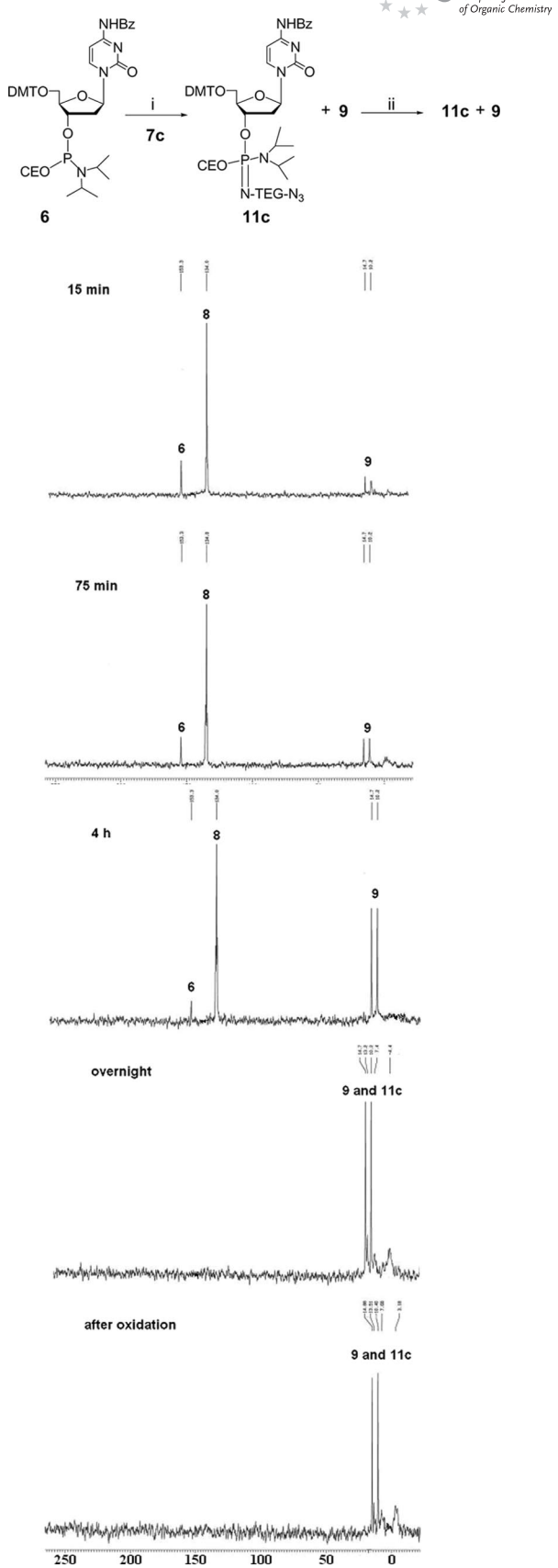
The reaction route observed in the coupling of **6** with azido alcohol **7a** (Scheme 5) was indeed very similar to that observed in the coupling with **7b**. Within less than 10 min, the signal attributable to **6** (δ = 153 ppm) disappeared, and new signals emerged at δ = 144 (prevailing, trialkyl phosphite adduct **10a**), 15, and 10 ppm (minor signals, H-phosphonate derivative **9**). Changes in the ^{31}P NMR spectrum were observed only after 4 h, when a new, very small signal was apparent at δ = 13 ppm due either to adduct **13**, generated by nucleophilic attack of **10a** on residual **7a**, or alternatively to the cyclic derivative generated by intramolecular Staudinger reaction of azido-funcionalized phosphite triester **10a**; after oxidation, the signal at δ = 144 ppm disap-



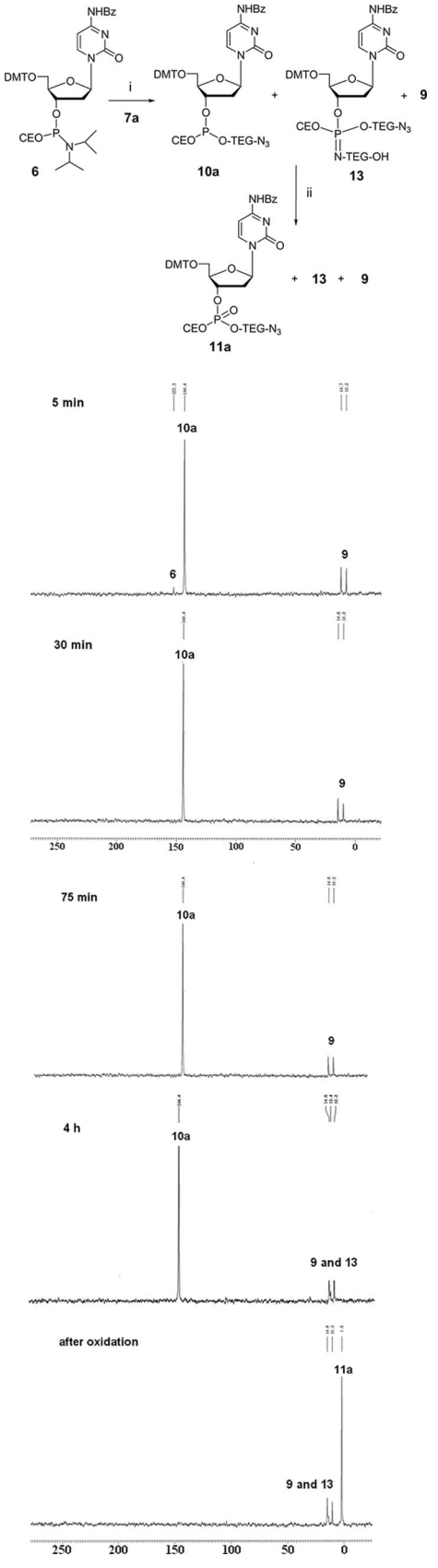
Scheme 2. Reaction scheme and ^{31}P NMR spectra recorded during the coupling of nucleoside **6** with alcohol **7b**: Reagents and conditions: (i) **7b** (1.2 equiv.), DCI (0.25 M in $CH_3CN/CDCl_3$, 5:1); (ii) *t*BuOOH (5.5 M in decane).



Scheme 3. Reaction scheme and ^{31}P NMR spectra recorded during the coupling of nucleoside **6** with TEG-azide **7c** in the absence of activator. Reagents and conditions: (i) **7c** (1.2 equiv.), $CH_3CN/CDCl_3$, 5:1; (ii) *t*BuOOH (5.5 M in decane).



Scheme 4. Reaction scheme and ^{31}P NMR spectra recorded during the coupling of **6** with TEG azide **7c** in the presence of activator. Reagents and conditions: (i) **7c** (1.2 equiv.), DCI (0.25 M in $CH_3CN/CDCl_3$, 5:1); (ii) *t*BuOOH (5.5 M in decane).



Scheme 5. Reaction scheme and ^{31}P NMR spectra for the coupling of nucleoside **6** with azido alcohol **7a**: Reagents and conditions: (i) **7a** (1.2 equiv.), DCI (0.25 M in $CH_3CN/CDCl_3$, 5:1); (ii) *t*BuOOH (5.5 M in decane).

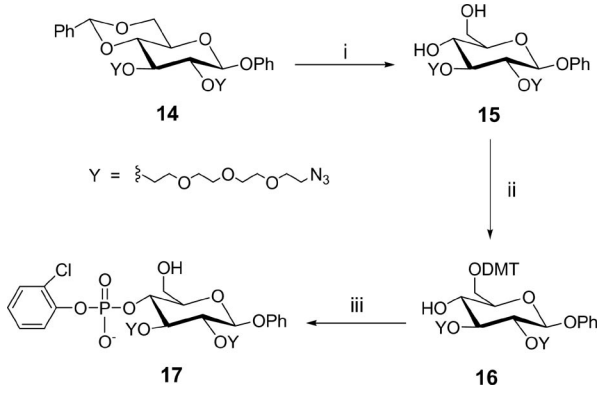
peared, a large new signal emerged at δ = 1.9 ppm, attributable to phosphate triester **11a**, while the resonances at 15, 13 and 10 ppm remained unaltered. The identities of the stable compounds, before and after oxidation, were confirmed by comparison of the ^{31}P NMR spectroscopic data with literature data[24] and by MS and IR experiments. Notably, the band at 2212 cm^{-1} in the IR spectrum of the reaction mixture of **6** and **7a**, which is diagnostic of the azide moiety, did not change either in frequency or relative intensity with time, or upon oxidative treatment. For this reaction, compound **11a** was the only product isolated after column chromatography; it was obtained in 75% yield for the two steps and was characterized by ^1H, ^{13}C, ^{31}P NMR and ESI-MS analyses.

The results described above are consistent with the following interpretation: the coupling of an azido alcohol with an activated phosphoramidite compound under the standard conditions used in oligonucleotide synthesis protocols (fast reaction times, typically requiring 5–30 min) leads to an azido phosphite triester derivative, which is then rapidly converted into a stable azido phosphate triester by oxidation in situ. Only the prolonged contact of a P^{III} compound with the azido alcohol gives also, as minor reaction products, classical Staudinger adducts, which finally collapse into iminophosphorane-type adducts.

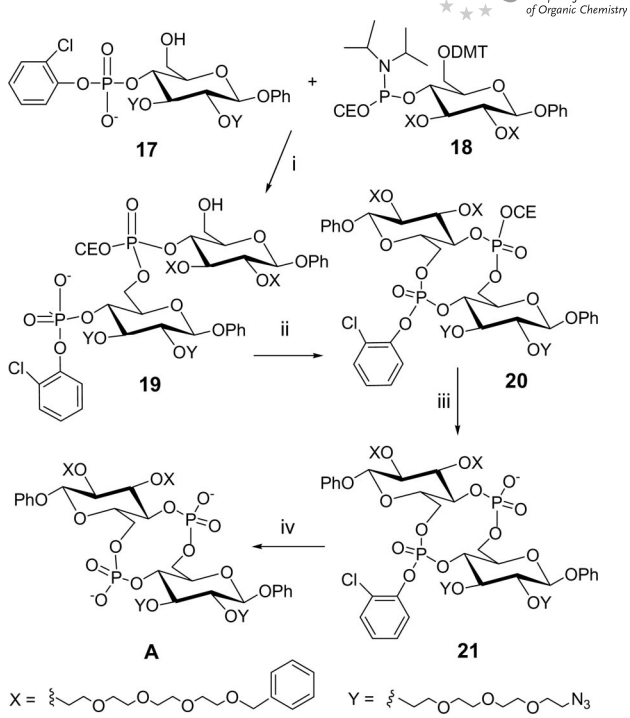
These data are in agreement with the results of Sekine and co-workers, and can be easily rationalized by taking into account that the P^{III} atom in a phosphoramidite reagent behaves differently if it is in the presence of an acidic promoter. Indeed, two different reaction pathways have to be taken into account due to the peculiar behavior of the P–N linkage: when the phosphoramidite reagent is employed under acidic conditions, fast protonation of the nitrogen atom occurs, with the adjacent P^{III} center becoming dramatically more electrophilic. Rapid attack by available nucleophiles then follows, with the most reactive nucleophiles being preferred.[29] On the other hand, under neutral conditions, the nucleophilic character of P^{III} prevails and the only effective mechanism is the nucleophilic attack of phosphoramidites to azides, giving stable iminophosphoranes. Therefore, it can be concluded that a phosphoramidite derivative can be efficiently reacted with an azido alcohol to finally give an azido phosphotriester compound, provided that the coupling reaction is fast (less than 30 min) and that the P^{III} adduct is rapidly oxidized to the corresponding P^V derivative.

Encouraged by these results, we then focused on our original target; the synthesis of an **A**-type compound as a readily convertible CyPLOS derivative. In particular, the synthesis of linear dimer **19** was first realized by coupling two differently substituted monosaccharides: the known **18**,[2] carrying benzyl end-capped TEG tentacles, and the novel TEG-azido-derivatized glucoside **17**. This compound was synthesized in four steps starting from **14**,[4] involving first benzylidene removal leading to **15**, installation of the DMT group on the 6-hydroxyl position to give **16**, followed by a two-step, one-pot reaction for the insertion of a phosphorylated moiety at the 4-hydroxyl group and successive

DMT removal, affording **17** (Scheme 6). Standard phosphoramidite coupling of monosaccharides **17** and **18** gave the desired dimer **19** as a unique compound, as determined by TLC monitoring of the reaction mixture; the compound was isolated after column chromatography in good yields (75% isolated yield for three steps). Cyclization of linear dimer **19** was then carried out by applying a phosphotriester methodology; using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) as the condensing agent in association with 4-(dimethylamino)pyridine (DMAP) as a nucleophilic catalyst, under high dilution conditions (ca. 10^{-3} M), fully protected **20** was recovered with 75% yield.



Scheme 6. Preparation of phosphorylated building block **17**: Reagents and conditions: (i) TFA/CH_2Cl_2/H_2O (1:10:0.5), 4 h, 0 °C (quant.); (ii) DMTCl, Py, 12 h, room temp. (97%); (iii) (a) 2-chlorophenyldichlorophosphate, triethylamine (TEA), triazole, Py, 3 h, room temp.; (b) on-column DMT removal (92% for two steps).



Scheme 7. Synthetic scheme for the preparation of cyclic dimer **A**: Reagents and conditions: (i) (a) DCI (0.25 M in CH_3CN), room temp., 2 h; (b) *t*ButOOH (5.5 M in decane), room temp., 30 min; (c) on-column detritylation (74% for the three steps); (ii) DMAP, MSNT, Py, room temp., 12 h (75%); (iii) piperidine, 70 °C, 12 h (quant.); (iv) satd. aq. LiOH/dioxane (5:1), 50 °C, 12 h (96%).

Subsequent phosphate deprotection reactions, involving first 2-cyanoethyl removal by treatment with piperidine, followed by reaction of **21** with a LiOH solution in dioxane/water (1:5, v/v) at 50 °C to cleave the 2-chlorophenyl group, gave target compound **A** in 94% yield for the two steps (Scheme 7). Following the described procedure, **A** was prepared in four steps and 53% yield on average from building blocks **17** and **18**, the former compound was obtained in four steps with 88% yield from glucoside **14**. For its conversion into spin-labeled derivatives **B** (Figure 4), the azido groups in **A** were first reduced to amines by treatment with triphenylphosphane and water. The post-synthetic condensation with 0.5 equiv. of 4-carboxy-TEMPO,[23] realized using *N*,*N*-dicyclohexylcarbodiimide (DCC) as the condensing agent, yielded **B**, which was obtained as a mixture of regioisomers. These compounds were isolated in 67% yield after chromatography on a Sephadex G25 column and characterized by MS analysis. As expected, the ^1H and ^{13}C NMR spectra of **B** showed very large and unresolved signals that did not allow a definitive characterization of the molecular structure but did confirm its radical nature.

All the intermediates were purified by column chromatography and characterized by ^1H, ^{13}C (and ^{31}P, where present) NMR spectrometry and by MS analysis. For cyclic dimer **A**, in analogy with the previously synthesized congeners, inspection of the ^1H and ^{31}P NMR spectra suggested the presence of strongly self-aggregated systems in $CDCl_3$, with typical chemical shift anisotropy and line broadening. This was not observed, however, when the sample was dissolved in CD_3OD, in which a unique, sharp signal was observed for the two phosphorus atoms, which is consistent with a fully symmetric, well-solvated system. Analysis of the ^{31}P NMR signals of **A** upon varying its concentration in $CDCl_3$ gave deeper insights into the behavior of this macrocycle in solution. In detail, when **A** was analyzed as 10 mM samples in $CDCl_3$ (161.98 MHz, 298 K), a very broad signal, dispersed over a 10 ppm region, was observed in the ^{31}P NMR spectrum. This signal, which was unaltered in the temperature interval 288–338 K, was significantly sharpened only at a concentration of 0.7 mM, to finally give a sharp signal at 0.2 mM, thus allowing a critical aggregation concentration (CAC) for this molecule of approximately 0.5 mM to be determined (see the Supporting Information). Compared to the CAC value of 6–8 mM evaluated for its congener **4**,[2] the prepared macrocycle **A** shows a much higher tendency to self-aggregation in $CDCl_3$. These observations could be attributed to different end-effects: the tentacles carrying the azides are probably better able to interdigitate with adjacent molecules with respect to the more hindered, fully benzyl-capped tentacles in **4**, thus producing larger aggregates (in this respect, compare also the differing behavior of **4** vs. **3**, with **3** having lower, 0.3–0.4 mM, CAC values than **4**). This hypothesis may be corroborated by a detailed microstructural charac-

terization study on this novel macrocycle; the results will be compared to those obtained in previous investigations with **2**–**5**.[4,30]

Further experiments, involving an ESR-based study of the mobility and mechanism of action of macrocycles **B** within phospholipids bilayers, as well as the preparation of a set of differently end-modified CyPLOS analogues starting from convertible compound **A**, are in progress in our laboratories and will be reported in due course.

Conclusions

In this work we describe the synthesis of novel CyPLOS derivative **A**, which is functionalized with terminal azido moieties that can be used for post-synthetic derivatization with various labels by exploiting either "click chemistry" or the Staudinger reaction. As a valuable example, its conversion into a spin-labeled derivative, which is of interest for ESR-based investigations into the mechanism of action and ionophoric activity of CyPLOS analogues, has been described. The linear precursor **19**, obtained by phosphoramidite-based coupling between monosaccharides **17** and **18**, was obtained in good yields (75% isolated yield for three steps). The finding that azides did not detectably interfere in the coupling steps under standard conditions thus demonstrates the compatibility of azido alcohols in the phosphoramidite chemistry approach. Before undertaking this synthesis, the feasibility of the design of **A** was preliminarily proven in dedicated coupling studies. The obtained results demonstrate that a reactive phosphoramidite derivative can be profitably condensed with an azido alcohol in a standard phosphoramidite protocol in solution to give the desired, stable phosphodiester adduct, provided that the reaction is fast and that the phosphite triester is immediately oxidized in situ to the more stable phosphate triester. These data corroborate the analogous results obtained by Morvan et al.,[22] for reactions carried out in the solid phase, thus expanding the repertoire of reactions available to obtain functionalized phosphodiester-linked scaffolds.

Experimental Section

General Methods: TLC analyses were carried out on silica gel plates from Merck (60, F254). Reaction products on TLC plates were visualized under UV light and then by treatment with a 10% $Ce(SO_4)_2/H_2SO_4$ aqueous solution. For column chromatography, silica gel from Merck (Kieselgel 40, 0.063–0.200 mm) was used. HPLC analyses were performed with a Beckman System Gold instrument equipped with a UV detector module 166 and a Shimadzu Chromatopac C-R6A integrator. All the synthesized compounds were found to be more than 98% pure, as assessed by HPLC analysis on a Nucleosil 100–5 C18 Supelco analytical column (250 × 4.6 mm, 5 μm), eluted with a linear gradient from 0 to 100% in 30 min of CH_3CN in H_2O, flow = 0.8 mL/min, detection at λ = 264 nm. For the ESI MS analyses, a Waters Micromass ZQ instrument equipped with an electrospray source was used in the positive and/or negative mode. MALDI TOF mass spectrometric analyses were performed with a PerSeptive Biosystems Voyager–De Pro MALDI mass spectrometer operating in the Linear mode using 2,5-dihydroxybenzoic acid as the matrix. NMR spectra were recorded with Bruker WM-400 or Varian Inova 500 spectrometers. All the chemical shifts are expressed in ppm with respect to the residual solvent signal; *J* values are in Hz. The following abbreviations are used for the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = double doublet. ^{31}P NMR spectra were recorded with 85% H_3PO_4 as the external reference.

General Procedure for the Coupling of 5′-*O*-DMT-*N*^{4}-benzoyl-2′-deoxycytidine-3′-phosphoramidite 6 with 7a, 7b, or 7c in the Presence of Activator: Nucleoside **6** (40 mg, 0.05 mmol) and compound **7a**, **7b**, or **7c** (1.2 equiv.), were treated with a 0.25 M DCI solution in anhydrous CH_3CN (500 μL). The coupling was performed in a standard NMR tube with the addition of 100 μL $CDCl_3$ and activated molecular sieves (4 Å). ^{31}P NMR spectra were recorded at fixed times; after 4 h, a 5.5 M *t*BuOOH solution in decane (50 μL) was added to the reaction mixtures.

Procedure for the Coupling of 5′-*O*-DMT-*N*^{4}-benzoyl-2′-deoxycytidine-3′-phosphoramidite (6) with 7c in the Absence of an Activator: Nucleoside **6** (40 mg, 0.05 mmol) and compound **7c** (1.2 equiv.) were both dissolved in anhydrous CH_3CN (500 μL) in an NMR tube with the addition of $CDCl_3$ (100 μL) and activated molecular sieves (4 Å). ^{31}P NMR spectra were recorded at fixed times; after 4 h, a 5.5 M *t*BuOOH solution in decane (50 μL) was added to the reaction mixture.

5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-*N*^{4}-benzoyl-2′-deoxycytidinyl 3′-*O*-{2-Cyanoethyl2-[2-(2-azidoethoxy)diethoxy]ethyl} Phosphate (11a): The coupling mixture of nucleoside **6** and **7a** described above was evaporated to dryness and then purified by column chromatography ($CHCl_3/CH_3OH$, 1 to 5% with a few drops of TEA) to give pure **11a** (35 mg, 0.037 mmol) in 75% yield as the only product. Oil, R_f = 0.40 [$CHCl_3/CH_3OH$, 95:5 (v/v)]. ^1H NMR ($CDCl_3$, 500 MHz, mixture of two diastereomers): δ = 8.31 (br., 1 H, 6-H), 7.67–6.82 (m, 19 H, ArH and 5-H), 6.27 (br., 1 H, 1′-H), 5.18 (br., 1 H, 3′-H), 4.41–4.20 (m, 4 H, TEG-CH_2-O-P and O-CH_2CH$_2$CN), 3.98 (br., 1 H, 4′-H), 3.80 (s, 6 H, 2 × OCH_3 of DMT), 3.73–3.63 (m, 12 H, 3 × O-CH_2CH_2-O), 3.54 (m, 1 H, 5′-H_a), 3.41–3.37 (m, 3 H, CH_2-N_3 and 5′-H_b), 2.89–2.84 (m, 1 H, 2′-H_a), 2.81 (t, *J* = 6.0, 5.5 Hz, 2 H, O-CH$_2$CH_2CN), 2.57–2.51 (m, 1 H, 2′-H_b) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 168.9 (CO), 162.0, 158.7, 143.8, 134.9, 133.1, 130.0, 128.9, 128.0, 127.4, 113.3 (10 × ArC), 117.5 (CN), 88.1 (C-1′), 87.0 (quaternary C of DMT), 81.3 (C-4′), 70.5, 70.3, 69.9, 69.7 (O-*C*H$_2$-TEG and C-3′), 62.5, 62.0 (C-5′ and O*C*H$_2$CH$_2$CN), 55.1 (2 × O*C*H$_3$ of DMT), 50.6 (2 × TEG-*C*H$_2$N$_3$), 40.3 (C-2′), 19.5 (OCH$_2$*C*H$_2$CN) ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ = –2.03 ppm.

Preparation of the Monosaccharide Building Block 17. Synthesis of Phenyl 2,3-Di-*O*-{2-[2-(2-azidoethoxy)diethoxy]ethyl}-β-D-glucopyranoside (15): Phenyl 2,3-di-*O*-[(2-azidoethoxy)diethoxyethyl]-4,6-*O*-benzylidene-β-D-glucopyranoside (**14**; 665 mg, 0.90 mmol, 1 equiv.) was treated with a TFA/CH_2Cl_2/H_2O (1:10:0.5, v/v/v, 5 mL) solution at 0 °C. After 4 h, the reaction mixture was diluted with CH_2Cl_2, the resulting solution was washed twice with water and then concentrated under reduced pressure. The crude material was purified by column chromatography ($CHCl_3/CH_3OH$, 5 to 15%) to give pure **15** (592 mg, 0.90 mmol) in almost quantitative yield. Oil, R_f = 0.50 ($CHCl_3/CH_3OH$, 9:1). ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.30–7.00 (m, 5 H, ArH), 4.96 (d, *J* = 6.5 Hz, 1 H, 1-H), 4.16–4.05 [m, 2 H, (-CH_a-O-C-2) and (-CH_a-O-C-3)], 3.93–3.82 [m, 4 H, (-CH_b-O-C-2), (-CH_b-O-C-3), 4-H and 6-H_a], 3.75 (m, 1 H, 6-H_b), 3.68–3.54 (m, 25 H, 12 × O-CH_2-TEG and 3-H), 3.45 (m, 1 H, 5-

H), 3.39–3.35 [m, 5 H, 2-H and 2× (-CH_2-N_3)] ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 157.0, 129.5, 122.6, 116.4 (ArH), 101.1 (C-1), 85.8 (C-2), 82.0 (C-5), 75.3 (C-3), 72.3, 71.9 [(CH_2-O-C-2) and (CH_2-O-C-3)], 70.8, 70.7, 70.5, 70.3, 70.2, 69.9 [12× (O-CH_2-TEG) and C-4], 62.8 (C-6), 50.5 [2× (-CH_2-N_3)] ppm. MALDI (+ve): calcd. for $C_{28}H_{46}N_6O_{12}$: 658.32; found: 681.58 [M + Na^+]. HRMS (MALDI-TOF): calcd. for $C_{28}H_{46}N_6O_{12}Na$: 681.3071; found: 681.3109.

Phenyl 2,3-Di-*O*-{2-[2-(2-azidoethoxy)diethoxy]ethyl}-6-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-glucopyranoside (16): Compound **15** (592 mg, 0.90 mmol, 1 equiv.), dissolved in anhydrous pyridine (2.2 mL), was treated with DMTCl (365 mg, 1.1 mmol, 1.2 equiv.). The reaction mixture was stirred at room temperature overnight then diluted with CH_3OH and concentrated under reduced pressure. The crude material was purified on a silica gel column (CH_2Cl_2/CH_3OH, 1 to 5% with a few drops of pyridine) to give pure **16** (580 mg, 0.88 mmol) in 97% yield. Glassy compound; R_f = 0.7 ($CHCl_3$/CH_3OH, 98:2, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.48–6.70 (m, 18 H, ArH), 4.97 (d, J = 7.4 Hz, 1 H, 1-H), 4.17–3.84 [m, 5 H, (-CH_2-O-C-2), (-CH_2-O-C-3) and 4-H], 3.75 [s, 6 H, 2× (OCH_3 of DMT)], 3.73–3.50 [m, 31 H, 14× (O-CH_2-TEG), 6-H_a, 5-H, 3-H], 3.45 (m, 1 H, 2-H), 3.37 (m, 1 H, 6-H_b) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 158.2, 157.3, 145.0, 136.1, 132.7, 130.1, 129.4, 128.2, 127.6, 126.5, 123.6, 122.4, 116.8, 112.9 (ArC), 101.2 (C-1), 86.3 (quaternary C of DMT), 85.8 (C-2), 82.1 (C-5), 75.3 (C-3), 72.4, 71.9 [(CH_2-O-C-2) and (CH_2-O-C-3)], 70.5, 70.4, 70.1, 69.9 [12× (O-CH_2-TEG) and (C-4)], 63.4 (C-6), 55.1 [2× (OCH_3 of DMT)], 50.6 [2× (TEG-C$H_2$$N_3$)] ppm. MALDI (+ve): calcd. for $C_{49}H_{64}N_6O_{14}$: 960.45; found: 983.91 [M + Na^+], 682.25 [M – DMT + Na^+], 698.31 [M – DMT + K^+]. HRMS (MALDI-TOF): calcd. for $C_{49}H_{64}N_6O_{14}Na$: 983.4378; found: 983.4407.

Phenyl 2,3-Di-*O*-{2-[2-(2-azidoethoxy)diethoxy]ethyl}-6-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-glucopyranoside-4-*O*-(2-chlorophenyl)-phosphate Triethylammonium Salt (17): (2-Chlorophenyl) dichlorophosphate (710 μL, 4.4 mmol, 5 equiv.) was added dropwise to a stirred solution of **16** (580 mg, 0.88 mmol, 1 equiv.), 1,2,4-triazole (300 mg, 7.0 mmol, 8 equiv.), and triethylamine (1.0 mL, 7.0 mmol, 8 equiv.) in anhydrous pyridine (12 mL) at 0 °C. The mixture was warmed to room temperature and, after 3 h, the reaction mixture was concentrated under reduced pressure. The crude material was diluted with $CHCl_3$, transferred into a separatory funnel and washed three times with water, then concentrated under reduced pressure and purified by column chromatography (CH_2Cl_2/CH_3OH, 1 to 10% with a few drops of TFA), to afford pure **17** (688 mg, 0.81 mmol) in 92% yield as an oil. R_f = 0.3 (CH_2Cl_2/CH_3OH, 95:5, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.40–6.98 (m, 9 H, ArH), 4.89 (br. s, 1 H, 1-H), 4.04–3.43 [br. m, 20 H, 14× (O-CH_2-TEG), 4-H, 6-H_2, 5-H, 3-H, 2-H], 3.34 [m, 4 H, 2× (-CH_2-N_3)] ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 157.2, 130.0, 129.4, 127.5, 124.4, 122.6, 121.9, 116.8, 109.9 (ArC), 101.5 (C-1), 81.8 (C-5), 79.2 (C-3), 72.3, 71.8 [(CH_2-O-C-2) and (CH_2-O-C-3)], 70.4, 70.1, 69.9 [12× (O-CH_2-TEG) and (C-4)], 60.3 (C-6), 50.7 [2× (TEG-C$H_2$$N_3$)] ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ = –7.29 (br.) ppm. ESI-MS (+ve): calcd. for $C_{34}H_{50}ClN_6O_{15}P$: 848.27; found: 872.59 [M + Na^+], 888.72 [M + K^+]. HRMS (MALDI-TOF): calcd. for $C_{34}H_{50}ClN_6O_{15}PNa$: 871.2658; found: 871.2701.

Synthesis of Linear Dimer 19: Derivative **17** (60 mg, 0.070 mmol, 1 equiv.) and known[2] **18** (108 mg, 0.084 mmol, 1.2 equiv.), which were previously dried by repeated coevaporation with anhydrous CH_3CN and kept under reduced pressure, were treated with a 0.25 M DCI solution in anhydrous CH_3CN (2.0 mL). The reaction was stirred at room temperature and monitored by TLC (CH_2Cl_2/CH_3OH, 95:5). After 2.0 h, 5.5 M *t*BuOOH solution in *n*-decane (300 μL) was added to the mixture, which was stirred at room temperature. After 30 min the reaction mixture was diluted with CH_2Cl_2 and purified by column chromatography (CH_2Cl_2/CH_3OH, 1 to 10% with a few drops of TFA) to afford pure **19** (91 mg, 0.052 mmol) in 74% yield as an oil. R_f = 0.4 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.45–6.89 (m, 24 H, ArH), 4.85 and 4.77 (m, 2× 1 H, 1-H and 1′-H), 4.47 [br. s, 4 H, 2× (CH_2Bn)], 3.97–3.03 [m, 78 H, 16× (CH_2CH_2OTEG), 2× (4-H), 2× (6-H_2), 2× (5-H), 2× (3-H), 2× (2-H) and (O-CH_2CH_2CN)], 2.60 [t, 2 H, (O-CH_2CH_2CN)] ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ = –1.5, –2.5, –6.87 ppm. ESI-MS (+ve): calcd. for $C_{79}H_{113}ClN_7O_{31}P_2$: 1751.65; found: 1774.56 [M + Na^+], 1791.69 [M + K^+], 1854.68 [M + Et_3N^+]. HRMS (MALDI-TOF): calcd. for $C_{79}H_{112}ClN_7O_{31}P_2Na$: 1774.6464; found: 1774.6509.

Synthesis of Cyclic Dimer 20: Derivative **19** (35 mg, 0.020 mmol, 1 equiv.), which was previously dried by repeated coevaporation with anhydrous pyridine, DMAP (3 mg, 0.020 mmol, 1 equiv.), and MSNT (177 mg, 0.6 mmol, 30 equiv.) were dissolved in anhydrous pyridine (20 mL) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure, dissolved in ethyl acetate, transferred into a separatory funnel and washed three times with water. The organic phase was concentrated under reduced pressure and purified by column chromatography (CH_2Cl_2/CH_3OH, 1 to 5%) to afford pure **20** (25 mg, 0.014 mmol) in 75% yield as an oil. R_f = 0.5 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR ($CDCl_3$, 500 MHz; a mixture of two diastereomers in a 1:0.6 ratio): δ = 7.48–6.80 (m, 24 H, ArH), 5.05, 4.90 (2×d, J = 8.0 and 5.5 Hz, 2 H, 1-H and 1′-H, minor stereoisomer), 5.00 and 4.94 (2× d, J = 7.5 and 7.5 Hz, 2 H, 1-H and 1′-H, major stereoisomer), 4.53 [br. s, 4 H, 2× (CH_2Bn)], 4.41–3.27 [m, 76 H, 16× (CH_2CH_2OTEG), 2× (4-H), 2× (6-H_2), 2× (5-H), 2× (3-H) and 2× (2-H)], 2.78–2.69 [m, 4 H, (CH_2CH_2CN)] ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ = –3.0 and –10.7 (major stereoisomer), –5.3 and –9.7 (minor stereoisomer) ppm. ESI-MS (+ve): calcd. for $C_{79}H_{111}ClN_7O_{30}P_2$: 1734.65; found: 1758.62 [M + Na^+], 1772.55 [M + K^+]. HRMS (MALDI-TOF): calcd. for $C_{79}H_{110}ClN_7O_{30}P_2Na$: 1756.6359; found: 1756.6397.

Synthesis of 21: Compound **20** (25 mg, 0.014 mmol, 1 equiv.), which was coevaporated several times with anhydrous pyridine and then dried under reduced pressure, was treated with piperidine (500 μL) and the resulting mixture was stirred overnight at 70 °C. The reaction was stopped by removal of the solvent in vacuo and the crude material was then purified by column chromatography (CH_2Cl_2/CH_3OH, 1 to 10%) to afford pure **21** (23 mg, 0.014 mmol) in an almost quantitative yield as an oil. R_f = 0.2 (CH_2Cl_2/CH_3OH, 95:5). ^{1}H NMR ($CDCl_3$, 500 MHz, piperidinium salt): δ = 7.37–6.85 (m, 24 H, ArH), 4.99–4.84 (m, 2 H, 1-H and 1′-H), 4.54 [br. s, 4 H, 2× (CH_2Bn)], 4.17–2.99 [m, 76 H, 16× (CH_2CH_2OTEG), 2× (4-H), 2× (6-H_2), 2× (5-H), 2× (3-H) and 2× (2-H)] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.9, 152.8, 152.0, 144.6, 129.5, 128.3, 127.6, 116.8 (ArC), 101.1 (C-1), 99.5 (C-1′), 81.1 (C-5 and C-5′), 80.3 (C-3 and C-3′), 75.0, 73.1, 72.0, 69.9, 68.5 [16× (CH_2CH_2OTEG), 2× (CH_2Ph), and 2× (C-4)], 65.5 (C-6 and C-6′), 50.9 [2× (TEG-C$H_2$$N_3$)] ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ = –2.9 and –6.9 ppm. ESI-MS (+ve): calcd. for $C_{76}H_{107}ClN_6O_{30}P_2$: 1681.62; found: 1704.53 [M + Na^+]. HRMS (MALDI-TOF): calcd. for $C_{76}H_{107}ClN_6O_{30}P_2Na$: 1705.6093; found: 1705.6126.

Synthesis of A: Compound **21** (23 mg, 0.014 mmol, 1 equiv.), dissolved in dioxane (200 μL), was treated with satd. aq. LiOH

(1.0 mL) and the resulting mixture was stirred overnight at 70 °C. The reaction mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2, transferred into a separatory funnel and washed three times with water. The organic phase was concentrated under reduced pressure and purified by column chromatography (CH_2Cl_2/CH_3OH, 0 to 15%) to give pure cyclic dimer **A** (21 mg, 0.014 mmol) in 96% yield as an oil. R_f = 0.5 (CH_2Cl_2/CH_3OH, 9:1). ^1H NMR ($CDCl_3$, 500 MHz, lithium salt, broadened signals): δ = 7.33–6.87 (m, 20 H, ArH), 4.97–4.85 (m, 2 H, 1-H and 1′-H), 4.55 [br. s, 4 H, 2× (CH_2Bn)], 4.10–3.05 [m, 76 H, 16× (CH_2CH_2OTEG), 2× (4-H), 2× (6-H_2), 2× (5-H), 2× (3-H) and 2× (2-H)] ppm. ^1H NMR (CD_3OD, 500 MHz, lithium salt, sharp signals): δ = 7.32–6.98 (m, 20 H, ArH), 5.09 (d, J = 7.5 Hz, 2 H, 1-H and 1′-H), 4.57–4.50 [m, 4 H, 2× (CH_2-Ph)], 4.12–3.91 [m, 14 H, 2× (6-H_2), 2× (4-H) and 4× (CH_2-CH_2-O-sugar)], 3.71–3.46 [m, 56 H, 2× (CH_2-O-CH_2Ph), (O-CH_2-CH_2-OTEG), 2× (3-H) and 2× (5-H)], 3.42–3.33 [m, 6 H, 2× (2-H) and 2× (CH_2-N_3)] ppm. ^{13}C NMR (CD_3OD, 125 MHz): δ = 156.8, 131.0, 130.1, 129.9, 129.5, 129.4, 124.2, 118.6, 118.3 (ArC), 102.0 (C-1 and C-1′), 84.1, 83.7 (C-5 and C-5′), 74.6 [2× (O-CH_2-Ph)], 74.0, 73.9 (C-4 and C-4′), 72.3, 72.1, 71.6, 71.3, 71.1 (C-2 and C-2′, C-3 and C-3′, O-CH_2-CH_2-OTEG), 69.4 [4× (CH_2-CH_2-O-sugar)], 68.1 (C-6 and C-6′), 52.3 [2× (O-CH_2-N_3)] ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz, 10 mM): δ = ca. –3 (br.) ppm. ^{31}P NMR ($CDCl_3$, 161.98 MHz, 0.2 mM): δ = 0.23 (sharp) ppm. ^{31}P NMR (CD_3OD, 161.98 MHz, 10 mM): δ = 1.6 (sharp) ppm. ESI-MS (+ve): calcd. for $C_{70}H_{104}N_6O_{30}P_2$: 1570.62; found: 1594.46 [M + Na^+]. HRMS (MALDI-TOF): calcd. for $C_{70}H_{104}N_6O_{30}P_2Na$: 1593.6170; found: 1593.6280.

Synthesis of B (Mixture of Regioisomers): CyPLOS **A** (10 mg, 0.006 mmol, 1 equiv.) was dissolved in anhydrous THF (50 μL). Triphenylphosphane (4 mg, 0.015 mmol, 2.5 equiv.) was added to the solution and the reaction was stirred overnight at room temperature. Water (1.0 mL) was added and the resulting system was stirred for 48 h. The reaction mixture was then treated with dilute aq. HCl solution and exhaustively washed with diethyl ether to remove the impurities. The aqueous phase was neutralized and then extracted with $CHCl_3$/CH_3OH (9:1) to furnish the desired, pure diamino-functionalized CyPLOS (9 mg, 0.006 mmol) as an oil [R_f = 0.4 (*n*-butanol/acetic acid/water, 60:15:25)]. This compound (9 mg, 0.006 mmol, 1 equiv.), previously dried by repeated coevaporations with anhydrous benzene and kept several hours under high vacuum, was dissolved in anhydrous CH_2Cl_2 (100 μL). DIPEA (5 μL, 0.03 mmol, 5 equiv.), DCC (3 mg, 0.015 mmol, 2.5 equiv.), and TEMPO-carboxylic acid (0.5 mg, 0.003 mmol, 0.5 equiv.) were sequentially added and the resulting mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure, diluted with diethyl ether and washed twice with distilled water. The aqueous phase was concentrated under reduced pressure, redissolved in H_2O and purified on a Sephadex G25 column (H_2O/EtOH, 1:1). From UV measurements, the fractions absorbing at λ = 264 nm were collected and evaporated to dryness, yielding target compounds **B** (7 mg, 0.004 mmol) with 67% yield as an oil. R_f = 0.4 [*n*-butanol/acetic acid/water, 60:15:25]. ^1H NMR (CD_3OD, 500 MHz, broad, unresolved signals): δ = 7.40–6.90 (m, 20 H, ArH), 5.07 (d, under the HDO signal, 1-H and 1′-H), 4.58 [br., 4 H, 2× (CH_2-Ph)], 4.06 [m, 8 H, 4× (CH_2-CH_2-O-sugar)], 3.66–3.16 [m, 62 H, 2× (CH_2-O-CH_2Ph), 12× (O-CH_2-CH_2-OTEG), 2× (3-H), 2× (4-H), 2× (6-H_2) and 2× (5-H)], 3.33–3.24 [m, 4 H, 2× (2-H)], 3.02 [br., 4 H, TEG-CH_2NH_2], 1.98–1.60 [m, 7 H, (CH_2 and CH protons of TEMPO)], 1.38–1.05 [m, 12 H, (CH_3 of TEMPO)] ppm. ESI-MS (–ve): calcd. for $C_{80}H_{126}N_3O_{32}P_2$: 1702.76; found: 850.15 [M – $2H^+$]$^{2-}$.

Supporting Information (see footnote on the first page of this article): ^{31}P NMR spectra ($CDCl_3$, 161.98 MHz, 298 K) of macrocycle **A** recorded at different concentrations for the determination of the CAC value.

Acknowledgments

We thank the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) for grants in support of this investigation (PRIN2008) and Centro di Metodologie Chimico-Fisiche (CIMCF), Università di Napoli "Federico II" for providing MS and NMR facilities.

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Received: July 26, 2010
Published Online: January 11, 2011

