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Cyclic Phosphate-Linked Oligosaccharides: Synthesis and Conformational Behavior of Novel Cyclic Oligosaccharide Analogues

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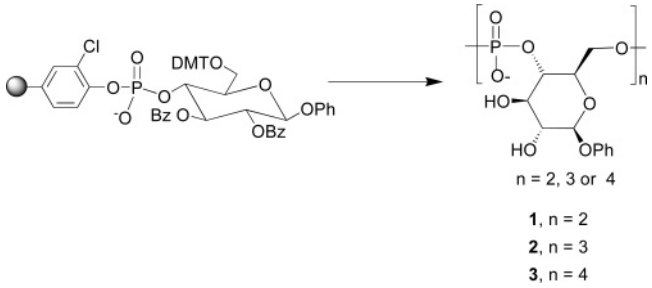

CyPLOS (cyclic phosphate-linked oligosaccharides), that is, novel cyclic oligosaccharide surrogates, consisting of two, three, and four phenyl-β-D-glucopyranoside units, 4,6-linked through stable phosphodiester bonds, were prepared by a straightforward and efficient solid-phase protocol. The assembly of the linear precursors was achieved by standard phosphoramidite chemistry on an automated DNA synthesizer, using a suitably protected 4-phosphoramidite derivative of D-glucose as the building block. For the crucial cyclization step a phosphotriester methodology was exploited, followed by a mild basic treatment releasing the desired cyclic molecules in solution in a highly pure form. The cyclic dimer and trimer were also independently prepared by classical solution synthesis, basically following the same approach. The solution structural preferences of the cyclic dimer and trimer, obtained by detailed NMR analysis, are also reported.

Introduction

The synthesis of macrocyclic compounds is one of the most fascinating problems in organic chemistry.[1] Large cyclic motifs are found in a wide number of naturally occurring compounds with structurally diverse backbones, including peptide and oligosaccharide cores, and are also useful synthetic scaffolds in the design of artificial hosts for selective recognition and catalysis. Due to their unique ability to bind hydrophobic molecules within their cavity while dissolved in polar solvents, cyclodextrins, that is, cyclic oligosaccharides with a toroidal shape composed of 6, 7, or 8 α-(1,4)-linked glucopyranose residues, have attracted widespread interest in supramolecular chemistry.[2] In addition to being ideal hosts for a plethora of guest molecules or ions, cyclodextrins are readily available at low cost, are not toxic, are nonimmunogenic, and exhibit an excellent pharmacokinetic profile.[3] For these reasons, such oligosaccharides have found important applications in many

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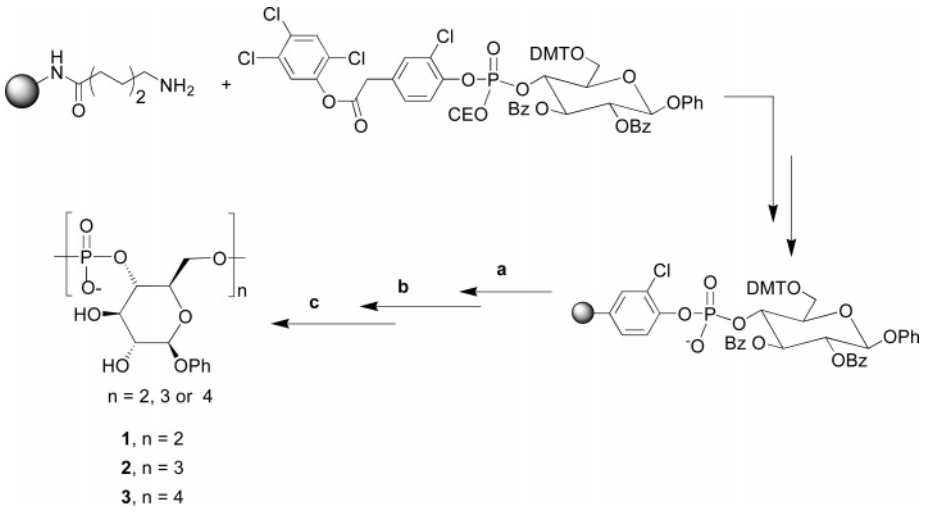
SCHEME 1. General Procedure for the Solid-Phase Synthesis of Cyclic Oligomers 1, 2, and 3[a]



[a] a: Chain elongation and final DMT removal. b: Cyclization. c: Detachment from the support and deprotection.

different fields as molecular reactors,[4] drug delivery systems,[5] enzyme mimics,[6] electrode surface modifiers,[7] and chromatographic materials,[8] just to mention a few. Indeed, cyclodextrins are exploited as pharmaceutical tools mainly to improve drug bioavailability, but also in cosmetics, in tissues, and in food and environmental chemistry.[9]

Various chemically modified cyclodextrin derivatives, cyclodextrin polymers, and cyclodextrin conjugates with drugs, peptides, or differently functionalized molecules have been designed and evaluated.[10] A fine-tuning of the properties and complexation capabilities of cyclodextrins can be achieved by selectively modifying their oligosaccharide backbone.[11] Organic synthesis offers easy access to the construction of modified cyclodextrins displaying the desired functional groups in specific positions on the cyclic molecule.[12] A de novo chemical synthesis approach is particularly useful when incorporation in the cycle of unnatural monosaccharides or of alternative inter-residue linkages is desired.[13] In the search for cyclic oligosaccharides more stable to acidic and enzymatic hydrolysis, β-1,6-linked cycloglucopyranosides, with sulfur replacing oxygen atoms in the glycosidic bridges, were recently synthesized.[13a] Sugar amino acids, that is, carbohydrates bearing both an amino and a carboxyl group, have been also designed as suitable monomers for the construction of modified cyclic oligosaccharides connected through an amide in lieu of glycosidic bonds.[13b−d] These molecules can be useful scaffolds to elicit predictable secondary structures when inserted in peptide backbones or, in the form of cyclic homo-oligomers, to produce new analogues of natural biopolymers, of interest as both peptidomimetics and cyclodextrin mimics.[13e−h] Other interesting examples of modified cyclic oligosaccharides have been described by Vasella and coworkers, who prepared analogues of α-, β- and γ-cyclodextrins including a buta-1,3-diyne-1,4-diyl or a 1,2,3-triazole moiety connecting the saccharidic monomers.[13i,j] Cyclic oligomers formed by butadiyne-linked carbohydrate residues were also shown to easily accommodate a nucleoside.[13k]

Aiming at cyclic oligosaccharide analogues containing glucose units connected through chemically and enzymatically stable bonds, alternative to the natural 1,4-*O*-glycosidic linkages, we here report the synthesis of new cyclic structures we have named CyPLOS (cyclic phosphate-linked oligosaccharides), containing two, three, and four phenyl-β-D-glucopyranoside residues, 4,6-linked through phosphodiester bonds (**1**, **2**, and **3**, Scheme 1), as well as the investigation of their conformational behavior by NMR. Phosphate bridges, chosen as linkers between two or more saccharidic moieties in a cyclic arrangement, may

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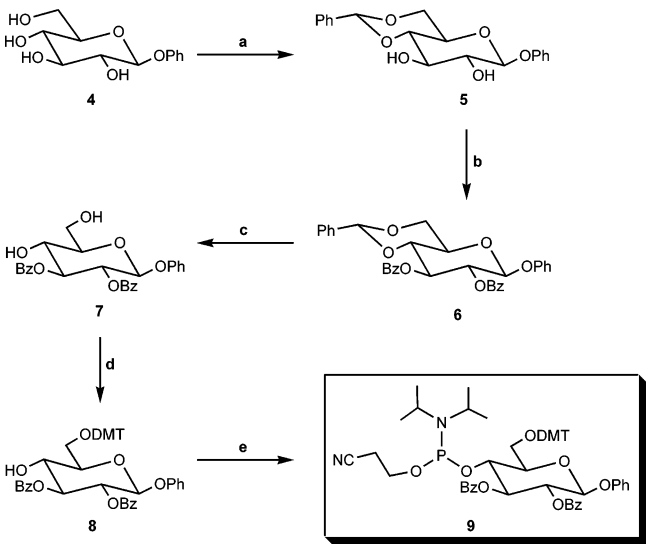
SCHEME 2. Synthesis of Building Block 9[a]



[a] a: Benzaldehyde dimethylacetal, PTSA cat., DMF, 12 h, 50 °C (97%). b: BzCl, pyridine, 12 h, room temperature (quantitative yields). c: I_2, Et_3SiH cat., CH_3OH, 12 h, room temperature (91%). d: DMTCl, pyridine, 12 h, room temperature (95%). e: 2-Cyanoethyl-*N*,*N*-diisopropylaminochlorophosphoramidite, DIEA, CH_2Cl_2, 1 h, room temperature (96%).

provide interesting features: (i) these bonds are chemically stable in a large pH range; (ii) they are naturally negatively charged, which could result in efficient cation binding; (iii) phosphate diesters can be easily and efficiently obtained through straightforward and high-fidelity reactions, well-optimized in oligonucleotide synthesis. These advantages have been clearly recognized by Nicolaou and co-workers, who first proposed phosphate-linked carbohydrate analogues, termed "carbonucleotoids", describing the synthesis of a linear tetramer by classical solution methods starting from glucose pentaacetate.[14]

The here reported synthetic scheme exploits a versatile approach, based on standard phosphoramidite chemistry for the assembly of the sugar−phosphate backbone (Scheme 1). The key intermediate in our strategy is the stable 4-phosphoramidite derivative of D-glucose **9** (Scheme 2). The 4,4′-dimethoxytriphenylmethyl (DMT) group is used for the transient protection of the 6-OH moiety; the phenyl group blocks the anomeric position and allows UV monitoring during the final purification step. Phosphoramidite **9** is exploited for the elongation of the oligosaccharide chain on the solid matrix in an automated DNA synthesizer. After cyclization, performed following phosphotriester procedures,[15] the cyclic molecule is detached from the solid support by a simple oximate treatment and obtained in a very pure form. The solution synthesis of the target cyclic molecules has been achieved following essentially the same synthetic scheme.

Results and Discussion

Synthesis of Building Block 9. Phosphoramidite **9** was prepared as summarized in Scheme 2. This building block contains all the functional groups (DMT as the transient protecting group for the primary hydroxyl and the 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite moiety on a secondary hydroxyl group) typically installed on nucleotide monomers used in standard automated oligonucleotide synthesis. Glucopyranoside **9** was obtained in five steps and with 80% overall yields. Commercially available phenyl-β-D-glucopyranoside **4** was first converted into 4,6-*O*-benzylidene derivative **5**[16] by a reaction with benzaldehyde dimethylacetal in the presence of catalytic amounts of *p*-toluenesulfonic acid (PTSA) and then treated with an excess of benzoyl chloride in pyridine to give **6**.[16] The successive addition of I_2 in CH_3OH in the presence of triethylsilane (Et_3SiH) allowed the selective removal of the benzylidene protecting group, and the primary hydroxyl group of diol **7**[17] was then protected by a reaction with 4,4′-dimethoxytriphenylmethylchloride (DMTCl) in pyridine. Compound **8** was subsequently phosphitylated by addition of 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite and diisopropylethylamine (DIEA), leading to the desired **9**. The synthetic route to phosphoramidite **9** is essentially similar to the one previously adopted for the preparation of the 4-phosphoramidite derivative of methyl-α-D-glucopyranoside **10** (Figure 1), recently described for the synthesis of glycoconjugate oligonucleotides, tethering sugar−phosphate residues.[18]

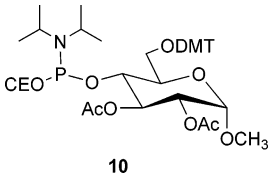


FIGURE 1. Structure of the methyl-α-D-glucopyranoside building block previously used for the conjugation of oligonucleotides with glucose−phosphate tails.[18]

The modifications introduced in building block **9** with respect to **10** led to significant improvements. On one hand, benzoyl was chosen as the semipermanent protecting group for the secondary hydroxyl functions because acetyl groups were found to be not completely stable to the Et_3N treatments, required to remove the 2-cyanoethyl phosphate protecting groups (Montesarchio et al., unpublished results). On the other hand, the UV−visible phenyl group inserted at the anomeric position proved to be useful for the final purification of the target cyclic molecules.

Solid-Phase Synthesis of CyPLOS 1, 2, and 3. With monomer **9** in hand, we first carried out on the solid phase both the elongation of the saccharidic chains and the cyclization, essentially following a procedure already described and optimized for the preparation of cyclic oligonucleotides.[15] The major advantage of this strategy resides in the possibility of obtaining the desired compounds with a high purity, which is intrinsically a nontrivial problem when the synthetic targets are macrocycles. The basic idea is to attach the first monomeric unit to the solid support through a 2-chlorophenylphosphodiester bridge, which, only if converted upon cyclization into a 2-chlorophenylphosphotriester linkage, can be cleaved by the final oximate treatment, selectively releasing the cyclic phosphodiester-linked molecule in solution. Following this strategy, unreacted linear chains or polymers, presumably formed in the solid phase as a

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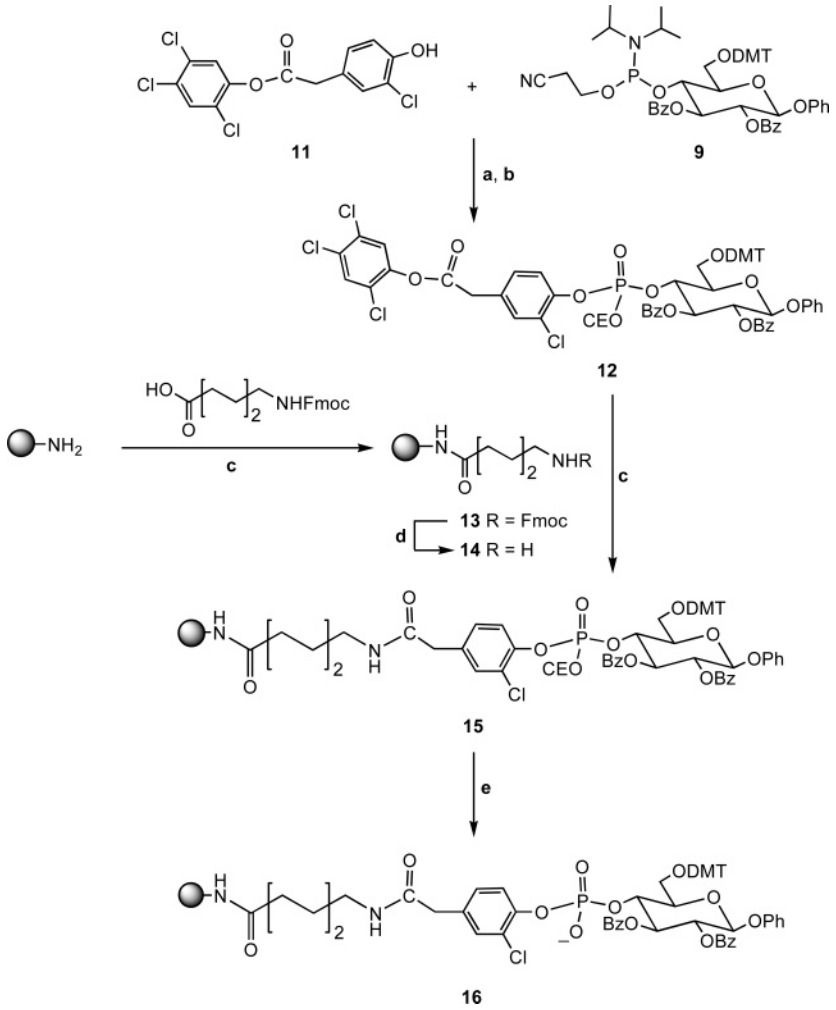
SCHEME 3. Synthesis of Functionalized Solid Support 16[a]



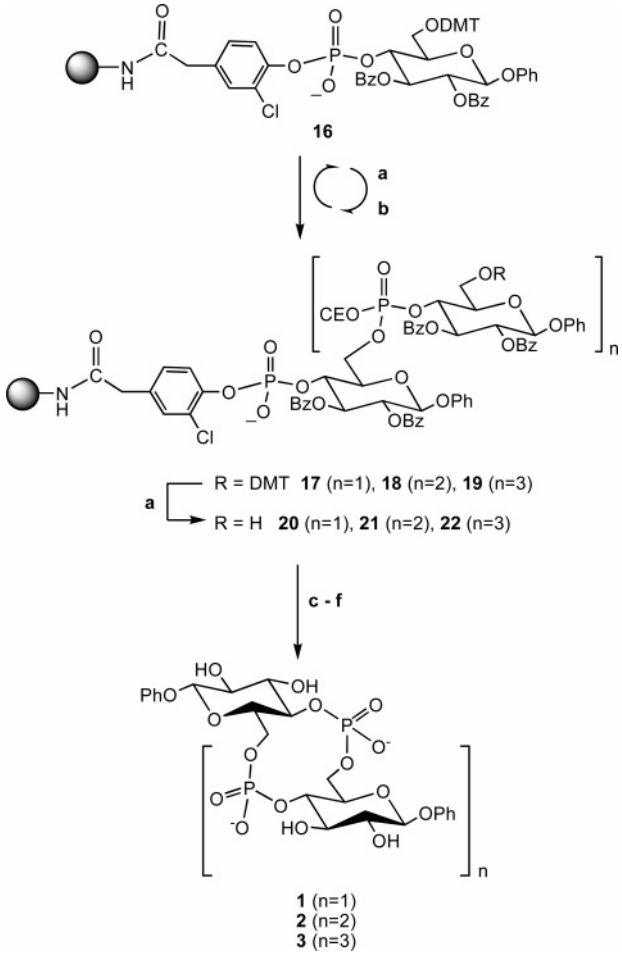
[a] a: Tetrazole 0.45 M in CH_3CN, 4 Å molecular sieves, 1 h, room temperature. b: *t*-BuOOH in decane, 10 min, room temperature. c: DCC, HOBt in CH_2Cl_2/DMF, 12 h, room temperature. d: Piperidine 20% in DMF, 3 × 10 min, room temperature. e: Pyridine/Et_3N (1:1, v/v), 3 × 2 h, room temperature.

result of interstrand condensations, are not affected by oximate and, therefore, are not detached from the support. As the solid support, a Tentagel amino resin (Tentagel-NH_2) was exploited, first derivatized with the spacer 6-aminohexanoic acid, in the form of a N-Fmoc (fluorenylmethoxycarbonyl)-protected derivative, to give **13**, which was then deprotected at the primary amino function, yielding **14** (Scheme 3). The subsequent step was the insertion on the solid support of the bifunctional linker 3-chloro-4-hydroxyphenylacetic acid, bearing the *o*-chlorophenol moiety that generates the desired *o*-chlorophenylphosphodiester linkage when reacted with phosphoramidite **9**. To obtain a homogeneous functionalization of the solid support with the first monomer, the linker, in the form of activated 2,4,5-trichlorophenyl ester **11**[15] was coupled in solution with building block **9** in the presence of tetrazole. The successive oxidation of the phosphite to phosphate triester function gave adduct **12**, which was then reacted with support **14** in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt). The loading of the resulting support **15** was always in the range of 0.13−0.17 mmol/g, as evaluated by spectroscopic measurements of the DMT cation released upon acidic treatment from weighed amounts of the dry resin.

Support **15** was then treated with Et_3N/pyridine, until complete conversion of the phosphotriester into the phosphodiester linkage, as monitored by recording ^{31}P NMR spectra of the resin suspended in $CDCl_3$. Typically, upon 2-cyanoethyl removal from the phosphate triester function on resin **15**, the two signals at about −5 ppm were replaced, in resin **16**, by a single signal at −2.4 ppm. The assembly of the linear oligomers, including final DMT removal, was carried out by a standard phosphoramidite protocol on an automated DNA synthesizer, using phosphoramidite **9** as the building block (Scheme 4).

In a typical experiment, 100 mg of the functionalized support **16** were used in a 5-μmol scale synthesis, including, for each synthetic cycle, the following steps: detritylation, coupling, oxidation, and capping. Incorporation of one, two, or three glucopyranose−phosphate units afforded functionalized resins **17**, **18**, and **19**, respectively, from which the DMT group was then removed. The desired circularization was achieved by the addition of 1-mesitylensulfonyl-3-nitro-1,2,4-triazole (MSNT) in pyridine to give solid support **20**, **21**, or **22**, respectively, following a classical phosphotriester protocol. After treatment with Et_3N/pyridine (1:1, v/v), the selective detachment from the support of the cyclic molecule was then realized by a reaction with 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate (0.2 M solution in dioxane/H_2O, 1:1, v/v). The eluate was successively treated with concentrated aq ammonia for complete hydroxyls deprotection. The concentrated mixture was then

SCHEME 4. Solid-Phase Synthesis of Cyclic Oligomers 1, 2, and 3[a]



[a] a: DCA 2% in CH_2Cl_2, 10 min, room temperature. b: (i) Coupling with **9**; (ii) oxidation; (iii) capping. c: MSNT, pyridine, 12 h, room temperature. d: Et_3N/pyridine (1:1, v/v), 3 h, room temperature. e: 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate 0.2 M in H_2O/dioxane (1:1, v/v), 12 h, room temperature. f: NH_4OH (17 M), 6 h, 55 °C.

applied to a Sephadex G25 column, which yielded the desired compound in pure form, not requiring further purification, as evaluated by HPLC and NMR analysis (see, for example, the HPLC profile for crude cyclic tetramer **3**, as detached from the solid support, in Figure 2). The dimer **1** in the amount of 4 mg, 2 mg of trimer **2**, and 1 mg of tetramer **3** could be on average isolated for each preparation, with overall yields ranging from about 40% for the dimer to 5% for the tetramer. As expected, a marked decrease in cyclization yields was observed upon increasing the length of the oligomer, in agreement with previous findings on cyclic oligonucleotides.[15,19] Purified cyclic molecules **1**, **2**, and **3** were fully characterized by ESI-MS and NMR spectroscopy.

Solution Synthesis of CyPLOS 1 and 2. To further confirm their structures, **1** and **2** were also independently synthesized by solution methods that may facilitate the preparation of large amounts of the desired products. To this purpose, 4-*O*-(2-chlorophenyl)phosphate glucopyranoside **23** (Scheme 5) was prepared and used as the starting material in connection with **9**

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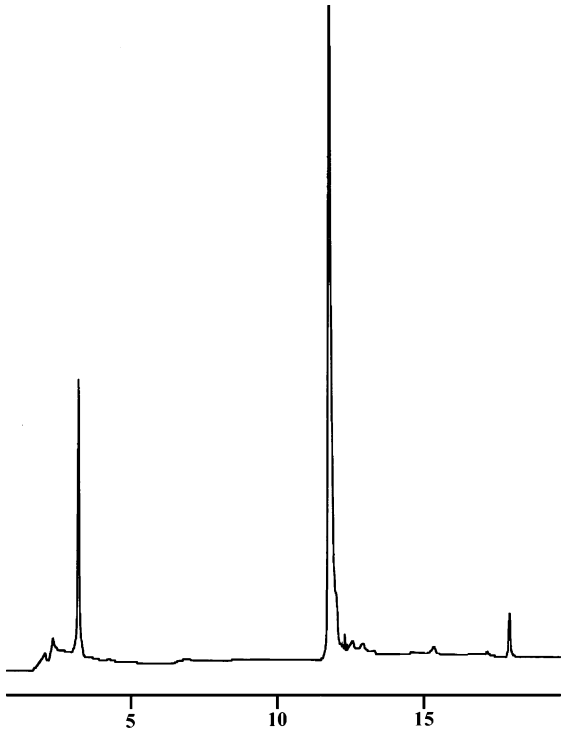
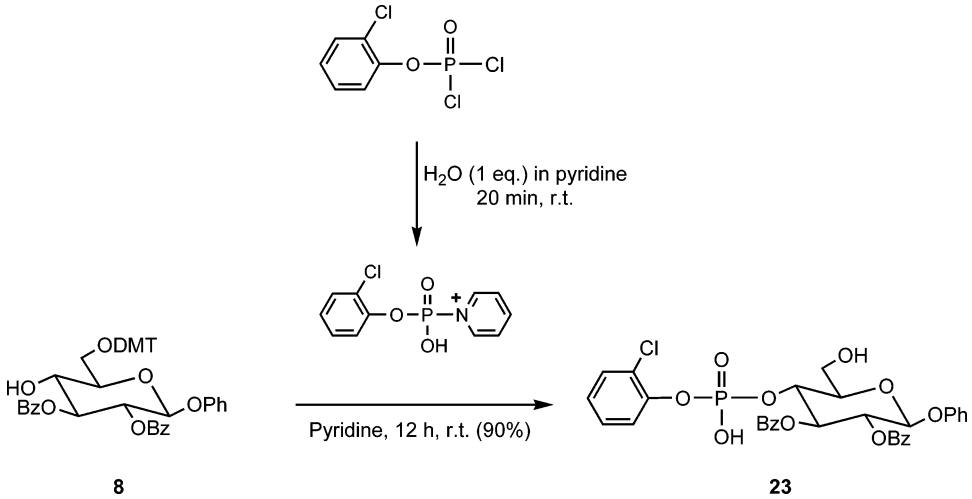


FIGURE 2. HPLC profile of crude cyclic tetramer **3**, as released from the solid support (conditions as reported in Supporting Information). The peak at 12.5 min, collected and analyzed by ESI-MS, was identified as the target macrocyclic compound.

in phosphoramidite solution synthesis protocols. Building block **23** was obtained in a one-pot reaction in 90% yields starting from derivative **8**, which was phosphorylated by treatment with 2-chlorophenylphosphoryl pyridinium chloride. This intermediate was also designed as the model compound to verify the stability of the linkage connecting the 4-phosphoester group of the first monomer to the 3-chloro-4-hydroxyphenylacetic linker on solid-support **16** when exposed to the reaction conditions required for the phosphoramidite-based chain elongation protocol. Therefore, compound **23** was subjected to prolonged treatments (12 h, room temperature) with the deblock [2% dichloroacetic acid (DCA) in CH_2Cl_2], the oxidation [0.02 M I_2 in pyridine/H_2O/tetrahydrofuran (THF), 7:2:1, v/v/v], and the capping solution [acetic anhydride (Ac_2O)/pyridine, 1:1, v/v)] routinely used in oligonucleotide assembly protocols. In no case, degradation products, even in traces, were found, as evaluated by TLC, HPLC, and NMR analysis of the mixtures.

The synthesis of the linear oligomers was accomplished as described in Scheme 6. Solution coupling of glucopyranoside **23** with phosphoramidite **9**, promoted by standard activator solution (0.45 M tetrazole in anhydrous CH_3CN), followed by the addition of 5.5 M anhydrous *t*-ButOOH in decane, for the phosphite to phosphate oxidation, led to a mixture of DMT-protected dimer **24** and deprotected linear dimer **25**. After complete DMT removal, obtained compound **25** was subjected to the cyclization procedure, carried out at 1×10^{-3} M concentration in anhydrous pyridine by the addition of MSNT. Protected cyclic dimer **26** could be isolated in 71% yields. Selective deprotection of the phosphodiester linkages was achieved by treatment with Et_3N/pyridine (1:1, v/v), followed by the reaction with a 0.2 M solution of 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate in dioxane/H_2O (1:1, v/v),

SCHEME 5. Synthesis of Derivative 23



8 **23**

allowing, respectively, the 2-cyanoethyl and 2-chlorophenyl group removal.

A final aq ammonia treatment ensured the complete hydrolysis of the benzoic ester functions, thus affording target compound **1**, which was isolated in 73% yields after a Sephadex G25 column.

A mixture of DMT-protected linear trimer **27** and DMT-free linear trimer **28** was achieved by coupling linear dimer **25** with phosphoramidite **9**, following standard procedures. Trimer **28** (1×10^{-3} M concentration in anhydrous pyridine) was then cyclized by the addition of MSNT. Analogously to **26**, fully protected cyclic trimer **29**, obtained in 56% yields, was next subjected to the successive Et_3N/pyridine, benzaldoximate, and aq ammonia treatments to remove the phosphate and hydroxyl protecting groups, respectively, yielding **2** in 75% yields after a Sephadex G25 column.

All the intermediates and the final compounds were fully characterized by ESI-MS and NMR spectroscopy. The deprotected cyclic molecules obtained by solution synthesis were found to be identical to the ones previously prepared following the solid-phase strategy, as confirmed by HPLC coelution experiments and NMR data comparison.

NMR Analysis and Structural Studies. Macrocycle **1**, **2**, and **3** were converted into the corresponding triethylammonium salts by cation exchange on a DOWEX (Et_3NH^+ form) resin to have homogeneous samples. ^{1}H NMR spectra of all the three compounds were recorded in DMSO-d_6 and D_2O.

As far as **1** is concerned, the ^{1}H, ^{13}C, and ^{31}P NMR spectra, both in D_2O and DMSO-d_6, show a unique signal for each kind of nucleus, as if in the presence of a single glucopyranoside unit. This can be attributed to the high internal symmetry of **1** (2-fold symmetry) and further confirms the cyclic structure of the synthesized molecule. ^{1}H NMR spectra of **1** were recorded in the concentration range 0.1−2.0 mM. No significant changes were observed in the shape or distribution of the ^{1}H NMR resonances, thus allowing to exclude aggregation phenomena under these conditions. Proton (700 MHz, $T = 298$ K), carbon (175 MHz, $T = 298$ K), and phosphorus (202 MHz, $T = 298$ K) assignments were obtained through an in-depth analysis of one-dimensional (1D) ^{1}H and ^{31}P NMR spectra and two-dimensional (2D) PE-COSY, TOCSY, HSQC, and HMBC NMR spectra.

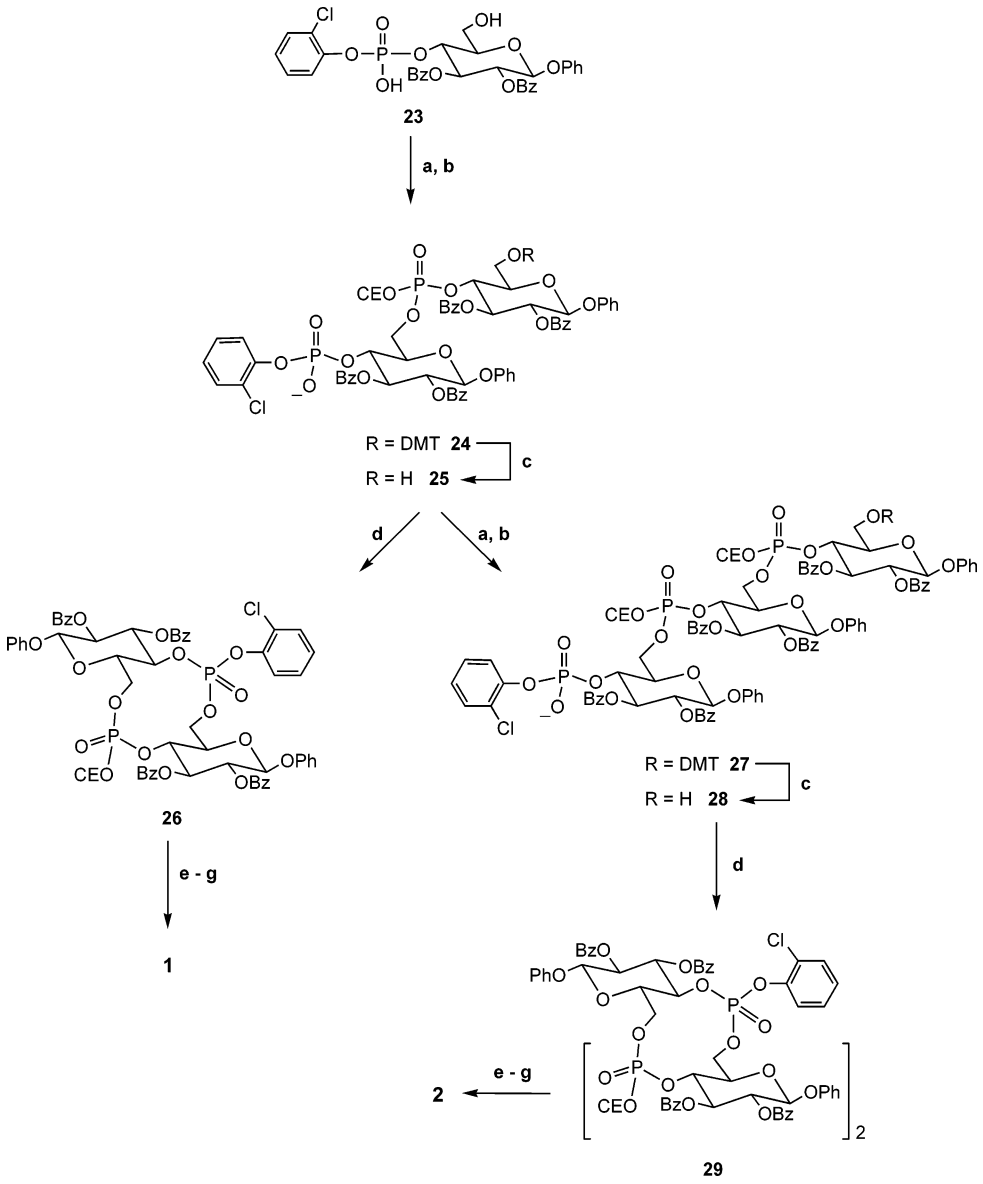
The ^{1}H NMR spectra in DMSO-d_6 and D_2O were very similar (Figure 3). Particularly, all the signals were characterized by the same multiplicity and coupling constants, indicating that **1** adopts the same conformation in both solvents. The 1D proton spectrum of **1** (700 MHz, D_2O, $T = 298$ K) showed the presence of only seven signals attributable to nonexchangeable protons in the region between 3.2 and 5.5 ppm, at δ_H 4.93 (d, H1), 3.22 (t, H2), 3.43 (t, H3), 3.93 (q, H4), 3.50 (br d, H5), 3.86 (br d, $H6_a$), and 3.70 (br d, $H6_b$).

DMSO-d_6 generally allows the observation of exchangeable protons and is useful to get insight into the hydrogen-bonding network and, therefore, into the conformational preferences of the molecule.[20] Particularly, we analyzed three parameters of the two OH signals reported in Table 1: (i) the chemical shift values; (ii) the multiplicity and vicinal coupling constants ($^3J_{CH,OH}$); and (iii) the temperature coefficients ($\Delta\delta/\Delta T$, in ppb $°C^{-1}$). A first inspection of the spectrum clearly showed a notable difference in the chemical environment experienced by OH-3 and OH-2. In fact, the signal attributed to OH-3, resonating at 7.90 ppm (298 K), showed a relevant downfield shift (ca. 2 ppm) with respect to OH-2 and appeared as a broad singlet, as if in the presence of slow exchanges on the NMR time scale. In addition, it exhibited a low-temperature coefficient (−1.4), indicating a very limited exposure to the solvent. On the contrary, the signal attributed to OH-2 resonated at 5.27 ppm (298 K) as a well-resolved doublet with a vicinal coupling constant of 5.1 Hz, which according to a simplified Karplus equation accounts for a free rotation of the hydroxyl group around the C−O bond. A large temperature coefficient was found for this proton (−8.9), which is consistent with intense solvation effects. Being well-accepted that hydrogen-bonded protons are significantly deshielded, it can be concluded that OH-3 is tightly involved in such interactions, whereas OH-2 is not affected.

Altogether, these data agreed with the absence of mutual H-bonding interactions between OH-3 and OH-2, typically observed in cyclodextrins.[20a,b] On the other hand, taking into account the spatial proximity between OH-3 and the negatively charged oxygen atom of the 4-phosphate group, it can be hypothesized that strong intramolecular H-bonding interactions exist between these two groups.

(20) (a) Bernet, B.; Vasella, A. *Helv. Chim. Acta* **2000**, *83*, 995−1021. (b) Bernet, B.; Vasella, A. *Helv. Chim. Acta* **2000**, *83*, 2055−2071. (c) Ivarsson, I.; Sandström, C.; Sandström, A.; Kenne, L. *J. Chem. Soc., Perkin Trans. 2* **2000**, *2*, 2147−2152.

SCHEME 6. Solution Synthesis of Cyclic Oligomers 1 and 2[a]



[a] a: Coupling with **9**. b: Oxidation and capping. c: DCA 2% in CH_2Cl_2, 10 min, room temperature. d: MSNT, pyridine, 12 h, room temperature. e: Et_3N/pyridine (1:1, v/v), 3 h, room temperature. f: 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate 0.2 M in H_2O/dioxane (1:1, v/v), 12 h, room temperature. g: NH_4OH (17 M), 6 h, 55 °C.

2D NOESY experiments allowed confirmation of the assignments and the ability to gain structural information. Essentially all NOEs were between 1,3-diaxial protons within the sugar (Figure 4), thus suggesting that the β-D-glucopyranoside moieties assume a classical $^4C_1$ chair conformation and are not distorted. This observation was also confirmed by the analysis of the proton coupling constants (9 Hz) measured for the sugar ring protons (H1, H2, H3, H4, and H5).

Relevant structural information relative to the central 12-membered ring can be derived from the 1D ^{1}H NMR spectrum and particularly from the analysis of the multiplicity and coupling constants of the H4, $H6_a$, and $H6_b$ (for their couplings with P) and H5 (for its couplings with $H6_a$ and $H6_b$) resonances. All the signals attributed to the H5, $H6_a$, and $H6_b$ protons appear as broad doublets. In particular, H5 exhibits a coupling constant of 9 Hz (due to the coupling with H4), and $H6_a$ and $H6_b$ show a geminal coupling constant of 12 Hz. This suggests that both the dihedral angles H5−C5−C6−$H6_a$ and H5−C5−C6−$H6_b$ are close to 90°. Analogously, the dihedral angles $H6_a$−C6−O6−P and $H6_b$−C6−O6−P do not sensibly deviate from 90° as well. On the other hand, the quartet multiplicity of H4 is justified by scalar couplings exhibiting the same J values (9 Hz) with H3, H5, and P. Unfortunately, for $^3J_{HP} = 9$ Hz, four real roots are obtained when solving the Karplus equation, not allowing the retrieval of any certain structural information.

Three-dimensional structures of **1**, satisfying all the found NOEs and dihedral angle constraints ($^3J_{HH}$ and $^3J_{HP}$), were built by restrained simulated annealing calculations. An initial structure of **1** was constructed and minimized to eliminate any possible source of initial bias in the conformation. Restrained simulations were carried out in vacuo for 150 ps at 1000 K using the consistent valence force field, as implemented in the Discover software (Accelrys, San Diego, USA). Thereafter, the temperature was decreased stepwise to 250 K. The final step

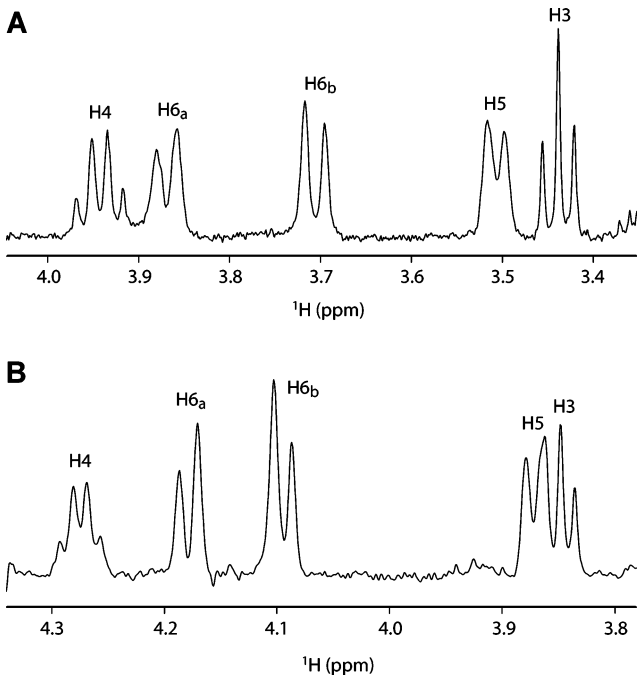


FIGURE 3. Selected region of ^{1}H NMR spectrum (700 MHz, T = 298 K) of dimer **1** in DMSO-d_6 (panel A) and in D_2O (panel B).

TABLE 1. Chemical Shifts, Vicinal Coupling Constants, and Temperature Coefficients for the Hydroxyl Protons in Cyclic Compounds 1 and 2[a]

	HO−C (2)			HO−C (3)		
compounds	δ (OH) ppm	multiplicity, J (H, OH), Hz	$\Delta\delta/\Delta T$ ppb, °C	δ (OH) ppm	multiplicity	$\Delta\delta/\Delta T$ ppb, °C
1	5.32	doublet, 5.1	−8.90	7.90	broad singlet	−1.4
2	5.36	doublet, 5.1	−6.80	7.90	broad singlet	−3.0

[a] Dissolved in DMSO-d_6 (400 MHz, 298 K).

was to energy-minimize and refine the structures obtained by using the steepest descent and the quasi-Newton−Raphson (VA09A) algorithms. Out of 50 structures generated, 20 structures with the lowest energies were selected. It was possible to obtain an excellent superimposition of the 20 structures with root mean square distance (RMSD) values of 0.65 ± 0.38 for heavy atoms. The lack of violations and the low RMSD value suggest that the minimized conformation of **1**, reported in Figure 5, is consistent with the experimentally determined restraints.

Even if no H bond was a priori imposed during the structural calculations, the obtained structure for **1** is characterized by hydrogen bonds between the OH-3 and the adjacent negatively charged oxygen atom of the 4-phosphate group of each subunit, in perfect agreement with the results of the temperature-dependence study carried out on the hydroxyl protons.

The overall structure of **1** appears to be quite planar, with the two sugar moieties, as expected, in the $^4C_1$ chair conformation and the phosphate groups pointing outward. The central 12-membered ring appears to be too small to host cations in the cavity, thus forming inclusion complexes. However, the two phosphate groups pointing toward the same side of the molecule can create a binding site for positively charged groups. In this conformation, the distances between the nonbridged oxygen atoms of the two phosphate groups are in the range 7.1−7.7 Å.

When examining cyclic trimer **2**, on the other hand, the nature of the solvent was found to sensibly affect the conformational

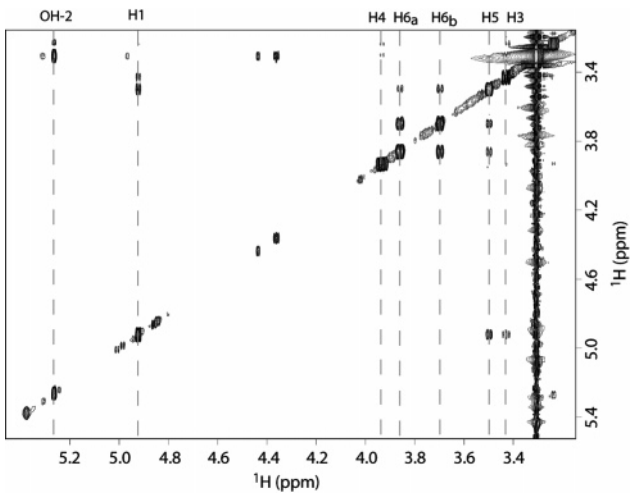


FIGURE 4. Expanded region of 2D NOESY (700 MHz, T = 298 K, mt = 500 ms) of dimer **1** in DMSO-d_6.

behavior of the molecule. In fact, dissolved in D_2O at 25 °C, the ^{1}H NMR spectrum of **2** is very similar to the one of dimer **1**, with three magnetically equivalent glucopyranoside residues (3-fold symmetry). On the contrary, when dissolved in DMSO-d_6 for the analysis of the hydroxyl protons, all the resonances were not resolved and appeared very broadened, as if in the presence of slow equilibriums on the NMR time scale, and no real improvement was obtained by heating the system up to 100 °C. However, it was possible to analyze the signals attributed to OH-2 and OH-3, which at 298 K resonated at 5.32 and 7.90 ppm and showed temperature coefficients of −6.8 and −3.0 ppb °C^{-1}, respectively (see Table 1), basically respecting the same trend observed in cyclic dimer **1**, which suggests again the presence of intramolecular H bonds exclusively involving the OH-3 groups.

Because the spectrum of **2**, when acquired in D_2O, is characterized by well-resolved and sharp resonances, this solvent was used for the structural characterization. A selected region of 1D proton NMR spectrum of **2** (700 MHz, T = 298 K) is reported in Figure 6.

Analyzing both the $^3J_{HH}$ and $^3J_{HP}$ coupling constants, we found that, analogously to **1**, all the β-D-glucopyranoside moieties in **2** are in a $^4C_1$ chair conformation, because all the sugar ring H1, H2, H3, H4, and H5 protons show $^3J_{HH}$ = 9 Hz. This observation was further inferred by the analysis of the 2D NOESY spectrum, in which essentially only the NOEs among the 1,3 diaxial protons were observed (Figure 7).

The multiplicity and coupling constants of H4, H6$_a$, and H6$_b$ (for their couplings with P) and H5 (for its couplings with H6$_a$ and H6$_b$) were analyzed as well. As in **1**, H4 is a quartet due to the coupling with H3, H5, and P (J = 9 Hz). Again, $^3J_{HP}$ = 9 Hz yields four real roots when solving the Karplus equation,[21] not allowing to retrieve any certain structural information. Similarly, both H6$_a$ and H6$_b$ show $^3J_{HP}$ = 6 Hz, which does not allow any restraint in the structural calculation. H5 is characterized, on the other hand, by coupling constants of 6 and 2 Hz, respectively, with H6$_a$ and H6$_b$. Also in this case, $^3J_{HH}$ = 6 Hz could not be used to guide structural calculation. Even if the number of restraints available to characterize the central 18-membered ring was very limited, we attempted to

(21) Lankhorst, P. P.; Haasnoot, C. A. G.; Erkelens, C.; Altona, C. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1387−1405.

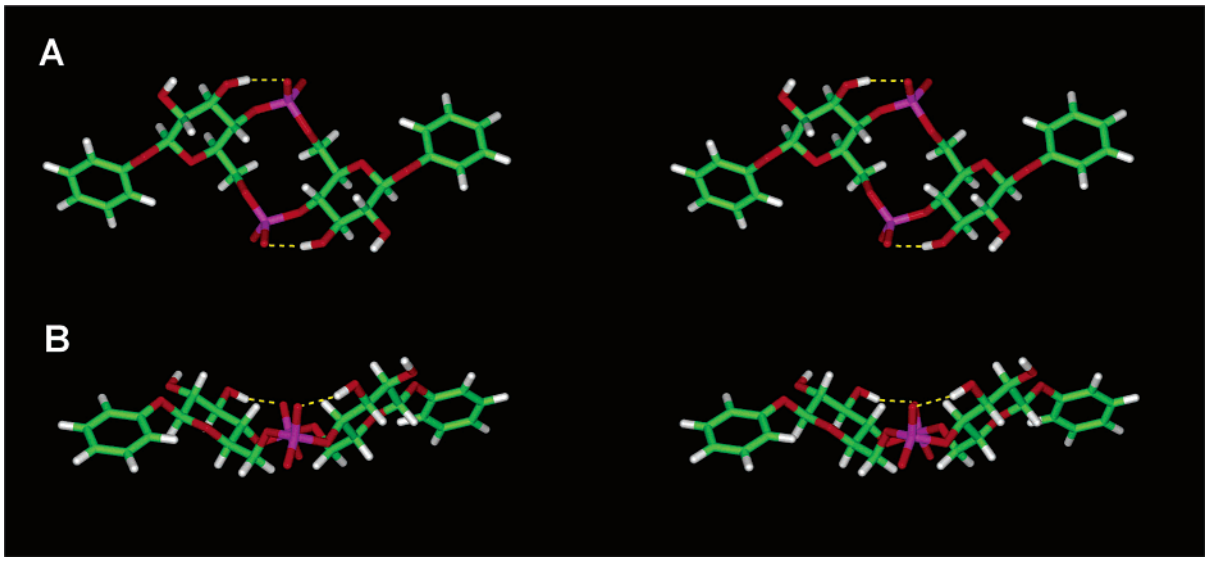


FIGURE 5. Top (**A**) and side (**B**) stereoview representations of the best NMR structure of dimer **1**. The molecule is depicted in colored "stick" form (carbons, green; oxygens, red; hydrogens, white; phosphorus, magenta). Hydrogen bonds are depicted with yellow dashed lines.

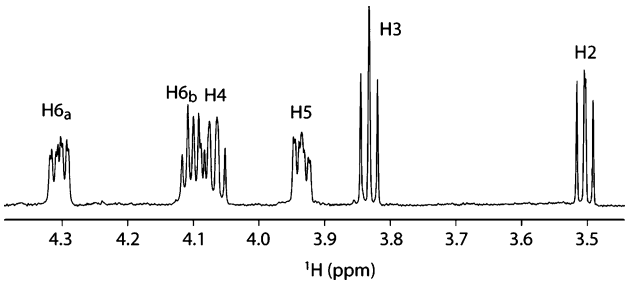


FIGURE 6. Selected region of ^{1}H NMR spectrum (700 MHz, T = 298 K) of trimer **2** in D_2O.

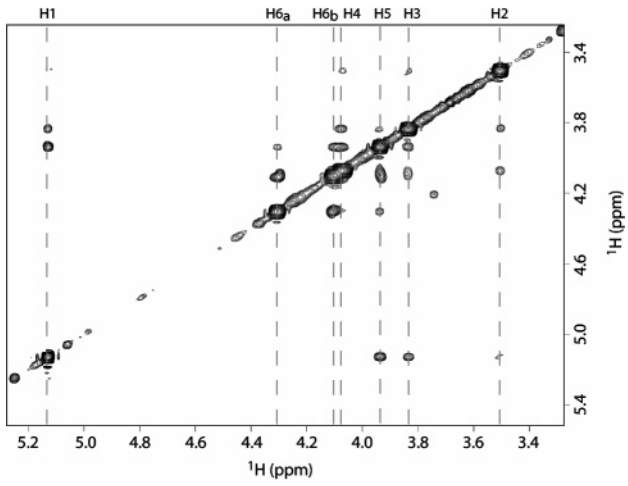


FIGURE 7. Expanded region of 2D NOESY (700 MHz, T = 298 K, mt = 500 ms) of trimer **2** in D_2O.

carry out a restrained structural calculation study for **2**. As expected, this led to an inextricable mixture of isoenergetic conformers, which could not be clustered into any well-defined family. This indicates that, even if strong H-bonding interactions between the OH-3 and the adjacent phosphate group are expected, this cyclic molecule is not sufficiently rigidified to adopt a single, preferential conformation in solution under the studied experimental conditions.

^{1}H NMR analysis could not be carried out on cyclic tetramer **3**, which, in all the investigated solvents (D_2O, DMSO-d_6, and CF_3CD_2OD), exhibited very complex spectra. These were characterized by severely overlapping and broadened signals, whose relative intensities were temperature-dependent, suggesting that **3** exists as a number of different families of conformers in slow equilibrium on the NMR time scale. No benefit in terms of resolution could be obtained by either heating the system (up to 373 K in DMSO-d_6) or cooling it (down to 263 K in CF_3CD_2OD).

Conclusions

A straightforward solid-phase protocol for the synthesis of CyPLOS, a novel class of cyclic oligosaccharide mimics, containing two, three, and four phenyl-β-D-glucopyranoside units, 4,6-linked through stable phosphodiester bonds, has been here described. A suitable building block (**9**) has been devised, which was obtained in five easy and high-yielding steps from commercially available phenyl-β-D-glucopyranoside. Phosphoramidite derivative **9** was first derivatized to allow incorporation into the solid support through a 2-chlorophenylphosphodiester bridge, essentially following the synthetic strategy previously developed for the preparation of cyclic oligonucleotides.[15] Chain elongation was carried out on an automated DNA synthesizer by standard and high-fidelity phosphoramidite chemistry, with **9** as the addition monomer, and a classical phosphotriester protocol was exploited for the cyclization procedure. Selective release in solution of the target cyclic molecules could be achieved using mild basic conditions, under which linear, unreacted oligomers or even polymeric species, formed on the solid phase as side-products during the cyclization procedure, were not detached. After a simple gel filtration chromatography, cyclic dimer **1**, trimer **2**, and tetramer **3** were isolated in overall yields ranging from 40% in the case of the dimer to 5% for the tetramer. Solution synthesis of **1** and **2** provided independently prepared samples, which proved to be identical to those assembled on a solid support, as ascertained by HPLC, NMR, and ESI-MS analysis. Both solution and solid-phase synthetic strategies, based on the same well-known and optimized chemistry, proved to be simple and reliable. In addition, the

reported protocols can be in principle suitably and easily modified so as to obtain all the specific backbone modifications typically introduced in the ribose−phosphate chain of oligonucleotides, as phosphorothioate, methylphosphonate, or phosphoramidate linkages, just to mention a few. Furthermore, the repertoire of the accessible cyclic phosphodiester-linked oligosaccharides (CyPLOS) can be significantly expanded by ad hoc modulating the nature, ring size, stereochemistry, and derivatizations of the saccharidic building block chosen. In this frame, we are currently studying further functionalizations of the hydroxyl groups to obtain oligomers with a higher diversity in terms of structure and function.

A detailed structural study has been carried out by NMR analysis. Using $^1H-^1H$ and $^1H-^{31}P$ vicinal scalar couplings and NOE data, as well as mechanic and dynamic calculations, we concluded that cyclic dimer **1**, having a 2-fold symmetry, adopts a cradle-like preferential conformation in solution, identical both in D_2O and in DMSO, stabilized by strong hydrogen-bonding interactions between the negatively charged phosphate groups and the adjacent OH-3. Cyclic trimer **2** showed a solvent-dependent conformational behavior. Particularly, in D_2O it exhibited a very flexible structure, while in DMSO a number of different families of conformers, in slow equilibrium on the NMR time scale, were present in a large temperature range. In the case of cyclic tetramer **3**, in all the investigated solvents, many different conformers in slow equilibrium could be observed, not allowing a detailed structural analysis.

It could be envisaged, for the largest macrocycles, a cation-dependent conformational behavior, with the metal coordinated to the internal phosphate diester groups placed at definite distances by the pyranoside rings. The potential of these cyclic oligosaccharide analogues as novel artificial receptors, naturally carrying one negative charge per residue in a large pH range, will be investigated in their ability to selectively bind different metal cations.

The here-reported synthetic strategy is highly versatile, allowing in principle to prepare a variety of cyclic oligosaccharide analogues with a high degree of intrinsic chemical diversity, in which the single monomers are connected through stable phosphate bonds or related derivatives. This issue is of the utmost importance in the search for new synthetic molecular receptors, potentially able to specifically bind metal cations or, more generally, positively charged molecules, in the frame of bio-organic and supramolecular chemistry-related applications.

Experimental Section

Synthesis of Phenyl-6-*O*-(4,4′-dimethoxytriphenylmethyl)-2,3-di-*O*-benzoyl-β-D-glucopyranoside-4-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite, 9. Synthesis of 5. Phenyl-β-D-glucopyranoside **4** (3.0 g, 12 mmol, 1 equiv) was dissolved in 25 mL of anhydrous *N*,*N*-dimethylformamide (DMF). PTSA (110 mg, 0.6 mmol, 0.05 equiv) and, dropwise, benzaldehyde dimethylacetal (4 mL, 26 mmol, 2.2 equiv) were sequentially added to the stirred mixture, which was left overnight at 50 °C. The crude was then diluted with ethyl acetate (AcOEt), transferred into a separatory funnel, washed three times with water, concentrated under reduced pressure, and purified by column chromatography. Eluting the column with $CHCl_3$, containing growing amounts of CH_3OH (from 1 to 5%) gave pure **5** (4.0 g, 11.6 mmol) with 97% yield.

5: mp [from ethanol (EtOH)/acetone] 191−193 °C (lit.[16] 194−195 °C). R_f = 0.4 ($CHCl_3/CH_3OH$, 98/2, v/v). ^1H NMR ($CDCl_3$, 200 MHz): δ 7.52−7.00 (complex signals, 10H, aromatic protons), 5.54 (s, 1H, Ph−C*H*), 5.02 (d, *J* = 7.5 Hz, 1H, H-1), 3.36 (dd, *J* = 6.6 and 10 Hz, 1H, H-3), 3.96−3.54 (overlapped signals, 5H, H-2, H-4, H-5, and H_2-6). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 156.7, 129.5, 129.3, 128.3, 126.9, 126.2, 123.2, and 116.8 (aromatic carbons); 101.9 (Ph-*C*H); 101.0 (C-1); 80.2 (C-5); 74.2 (C-3); 73.1 (C-2); 68.5 (C-4); 66.4 (C-6). ESI-MS (positive ions): *m*/*z* calcd for $C_{19}H_{20}O_6$, 344.126; found, 367.19 (M + Na^+), 383.25 (M + K^+). HRMS (MALDI-TOF): *m*/*z* calcd for $C_{19}H_{20}O_6Na$, 367.1158; found, 367.1209 (M + Na^+).

Synthesis of 6. Compound **5** (2.0 g, 5.8 mmol, 1 equiv), dissolved in anhydrous pyridine (10 mL), was reacted with benzoyl chloride (3.4 mL, 29 mmol, 5 equiv). The mixture was left under stirring at room temperature overnight and then concentrated under reduced pressure. The crude was then diluted with $CHCl_3$, transferred into a separatory funnel, and washed three times with a saturated $NaHCO_3$ aqueous solution and then twice with water. The organic phase, dried over anhydrous Na_2SO_4 and filtered, was then concentrated under reduced pressure, furnishing pure **6** (3.2 g, 5.8 mmol) in an almost quantitative yield.

6: mp (from AcOEt/diethyl ether) 199−200 °C (lit.[16] 203 °C). R_f = 0.6 (benzene). ^1H NMR ($CDCl_3$, 200 MHz): δ 8.25−7.03 (complex signals, 20H, aromatic protons), 5.97 (t, *J* = 9.2 and 9.2 Hz, 1H, H-3), 5.85 (apparent t, *J* = 7.8 and 9.2 Hz, 1H, H-2), 5.61 (s, 1H, Ph-C*H*), 5.48 (d, *J* = 7.5 Hz, 1H, H-1), 4.50 (m, 1H, H-5), 4.11 (t, *J* = 9.2 and 9.0 Hz, 1H, H-4), 3.93 (m, 2H, H_2-6). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 165.4 and 165.0 (2 CO); 156.7, 136.6, 134.3, 133.1, 133.0, 131.2, 130.3, 129.6, 129.4, 128.9, 128.7, 128.2, 128.0, 126.0, 123.2, and 117.0 (aromatic carbons); 101.3 (Ph-*C*H); 99.9 (C-1); 78.3 (C-5); 72.1 (C-3); 71.9 (C-2); 68.4 (C-4); 66.5 (C-6). ESI-MS (positive ions): *m*/*z* calcd for $C_{33}H_{28}O_8$, 552.178; found, 575.17 (M + Na^+), 591.15 (M + K^+). HRMS (MALDI-TOF): *m*/*z* calcd for $C_{33}H_{28}O_8Na$, 575.1682; found, 575.1721 (M + Na^+).

Synthesis of 7. Compound **6** (3.2 g, 5.8 mmol, 1 equiv), dissolved in anhydrous CH_3OH (40 mL), was reacted with I_2 (640 mg, 2.6 mmol, 0.45 equiv) and Et_3SiH (45 μL, 0.29 mmol, 0.05 equiv). The reaction mixture, left at room temperature overnight under stirring, was then diluted with AcOEt, and the organic phase was washed twice with a saturated $NaHCO_3$ aqueous solution and then twice with sodium thiosulfate. The organic phase, dried over anhydrous Na_2SO_4, was filtered and concentrated under reduced pressure and then purified by column chromatography. Eluting the column with $CHCl_3$, containing growing amounts of CH_3OH (from 0 to 10%), pure **7** (2.5 g, 5.3 mmol) was recovered in 91% yield.

7: mp (from acetone/*n*-hexane) 84−86 °C (lit.[17] 87 °C). R_f = 0.3 [$CHCl_3/CH_3OH$, 97/3, (v/v)]. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.99−6.93 (complex signals, 15H, aromatic protons), 5.70 (dd, *J* = 8.0 and 9.6 Hz, 1H, H-2), 5.49 (apparent t, *J* = 9.2 and 9.6 Hz, 1H, H-3), 5.34 (d, *J* = 7.8 Hz, 1H, H-1), 4.11−3.73 (overlapped signals, 4H, H-4, H-5 and H_2-6). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 167.6 and 165.2 (2 CO); 156.9, 133.7, 133.3, 130.0, 129.7, 129.6, 129.1, 128.5, 128.4, 123.2, and 116.8 (aromatic carbons); 99.2 (C-1); 77.2 (C-5); 76.1 (C-3); 71.1 (C-2); 69.9 (C-4); 62.2 (C-6). ESI-MS (positive ions): *m*/*z* calcd for $C_{26}H_{24}O_8$, 464.147; found, 487.14 (M + Na^+), 503.15 (M + K^+). HRMS (MALDI-TOF): *m*/*z* calcd for $C_{26}H_{24}O_8Na$, 487.1369; found, 487.1407 (M + Na^+).

Synthesis of 8. Compound **7** (2.0 g, 4.3 mmol, 1 equiv), dissolved in anhydrous pyridine (15 mL), was reacted with DMTCl (1.9 g, 5.6 mmol, 1.3 equiv). The reaction mixture, left at room temperature overnight under stirring, was then diluted with CH_3OH and concentrated under reduced pressure. The crude was next purified on a silica gel column, eluted with cyclohexane containing growing amounts of AcOEt (from 15 to 30%) in the presence of a few drops of pyridine, affording pure **8** (3.1 g, 4.1 mmol) in 95% yield.

8: glassy solid; mp >90 °C dec. R_f = 0.4 [cyclohexane/AcOEt, 7/3 (v/v)]. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.03−6.82 (complex signals, 28H, aromatic protons), 5.74 (dd, *J* = 8.1 and 9.0 Hz, 1H, H-2), 5.52 (apparent t, *J* = 9.3 and 9.3 Hz, 1H, H-3), 5.29 (d, *J* = 7.8 Hz, 1H, H-1), 4.02 (m, 1H, H-4), 3.81 (s, 6H, 2 OCH_3 of DMT

group), 3.76 (m, 1H, H-5), 3.56 (m, 2H, H_2-6). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.1 and 165.2 (2 CO); 158.4, 157.0, 144.5, 135.6, 133.4, 133.1, 129.9, 129.9, 129.7, 129.4, 129.1, 129.0, 128.8, 128.3, 128.2, 128.0, 127.8, 126.8, 123.0, 117.1, and 113.1 (aromatic carbons); 99.3 (C-1); 86.5 (quaternary carbon of DMT group); 77.1 (C-5); 74.9 (C-3); 71.2 (C-2); 70.8 (C-4); 63.4 (C-6); 55.1 (OCH_3 of DMT group). ESI-MS (positive ions): *m/z* calcd for $C_{47}H_{42}O_{10}$, 766.278; found, 767.39 ($M + H^+$), 789.25 ($M + Na^+$), 805.49 ($M + K^+$). HRMS (MALDI-TOF): *m/z* calcd for $C_{47}H_{42}O_{10}Na$, 789.2676; found, 789.2711 ($M + Na^+$).

Synthesis of 9. To a solution of compound **8** (500 mg, 0.65 mmol, 1 equiv), dissolved in anhydrous CH_2Cl_2 (4 mL), DIEA (335 μL, 1.95 mmol, 3 equiv) and 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite (230 μL, 1.04 mmol, 1.6 equiv) were sequentially added under stirring at room temperature. After 30 min, the reaction mixture was diluted with AcOEt, transferred into a separatory funnel, and washed three times with a saturated aqueous NaCl solution and then twice with water. The organic phase was dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude was then chromatographed on a silica gel column, eluted with cyclohexane containing growing amounts of AcOEt (from 25 to 30%) in the presence of a few drops of triethylamine, furnishing the desired compound **9** (610 mg, 0.63 mmol) in 96% yield.

9: oil; R_f = 0.5 [cyclohexane/AcOEt, 7/3 (v/v)]. ^{1}H NMR ($CDCl_3$, 400 MHz; as a diastereomeric mixture): δ 7.99−6.75 (complex signals, 56H, aromatic protons), 5.71 (overlapped signals, 4H, 2 H-2 and 2 H-3), 5.34 (complex signal, 2H, 2 H-1), 4.10 (complex signal, 2H, 2 H-4), 4.00−3.85 (complex signals, 4H, 2 OCH_2CH_2CN), 3.78 and 3.77 (2 s, 12H, 2 OCH_3 of DMT groups), 3.66 (complex signals, 2H, 2 H-5), 3.45−3.17 (complex signals, 8H, 2 H_2-6 and 2 OCH_2CH_2CN), 2.24 and 2.01 (complex signals, 2H each, $N[CH(CH_3)_2]_2$), 0.92, 0.90, 0.85, and 0.83 (complex signals, 24H, $N[CH(CH_3)_2]_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.6 and 165.2 (2 CO); 158.3, 157.1, 144.8, 136.0, 135.8, 133.0, 130.1, 130.0, 129.7, 129.5, 129.3, 128.2, 128.1, 127.6, 126.6, 122.8, and 112.9 (aromatic carbons); 117.1 (CN); 99.4 and 99.2 (2 C-1); 86.1 and 86.0 (2 quaternary carbons of 2 DMT groups); 75.8, 75.2, 74.6, 71.9, 71.8, and 71.1 (C-2, C-3, C-4, and C-5); 63.4 and 62.7 (2 C-6); 57.8 and 57.6 (OCH_2CH_2CN); 55.1 (OCH_3 of DMT group); 43.0 and 42.8 ($N[CH(CH_3)_2]_2$); 24.2 ($N[CH(CH_3)_2]_2$); 19.7 and 19.2 (OCH_2CH_2CN). ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ 154.2 and 153.0. ESI-MS (positive ions): *m/z* calcd for $C_{56}H_{59}N_2O_{11}P$, 966.385; found, 967.19 ($M + H^+$), 989.31 ($M + Na^+$), 1005.42 ($M + K^+$). HRMS (MALDI-TOF): *m/z* calcd for $C_{56}H_{60}N_2O_{11}P$, 967.3935; found, 967.3948 ($M + H^+$).

Functionalization of the Solid Support with Phosphoramidite 9. Synthesis of Derivative 12. Compound **9** (192 mg, 0.2 mmol, 1.2 equiv), previously coevaporated several times with anhydrous CH_3CN, was dissolved in a 0.45 M tetrazole solution in CH_3CN (1.2 mL) and reacted with 3-chloro-4-hydroxy-phenylacetic acid 2,4,5-trichloro-phenylester (**11**, 60 mg, 0.17 mmol, 1 equiv), dissolved in anhydrous CH_2Cl_2 (0.5 mL), in the presence of activated 4 Å molecular sieves. The reaction mixture was left at room temperature under stirring for 1 h and monitored by TLC, using $CHCl_3$ as the eluent. Successively, a 5.5 M *tert*-butylhydroperoxide (*t*-BuOOH) solution in decane (200 μL, 1.7 mmol, 10 equiv) was added to the reaction mixture, which was left for an additional 10 min under stirring. This was then diluted with CH_2Cl_2, transferred into a separatory funnel, and washed twice with a saturated NaCl solution. The organic phase, dried over anhydrous Na_2SO_4, was filtered, concentrated under reduced pressure, and crystallized from *n*-hexane. Isolated compound **12** (168 mg, 0.13 mmol, corresponding to 76% yield) was used as such, without further purification.

12: ^{31}P NMR ($CDCl_3$, 161.98 MHz; mixture of diastereomers): δ −5.2 and −5.6. ESI-MS (positive ions): *m/z* calcd for $C_{64}H_{52}Cl_4NO_{15}P$, 1245.183; found, 1247.132 ($M + H^+$), 1269.373 ($M + Na^+$).

Synthesis of Support 13. Resin TentaGel-NH_2 (300 mg, 0.29 mmol/g, 0.087 mmol, 1 equiv) was treated with N-Fmoc-6-amino-hexanoic acid (61 mg, 0.17 mmol, 2 equiv), HOBt (23 mg, 0.17 mmol, 2 equiv), previously coevaporated several times with anhydrous pyridine, and DCC (35 mg, 0.17 mmol, 2 equiv) in a CH_2Cl_2/DMF, 7/1 (v/v), solution. The reaction mixture was left at room temperature overnight under stirring. Solid support **13** was then exhaustively washed with DMF, CH_2Cl_2, CH_3OH, and CH_3CN and then dried under reduced pressure. The incorporation of the 6-aminohexanoic acid spacer was monitored by spectrophotometric Fmoc test (λ = 301 nm, ϵ = 7800 $cm^{-1}M^{-1}$) after a basic treatment (20% piperidine in DMF) on a dried and weighed amount of the resin. The functionalization was 0.20 mmol/g on average, corresponding to 69% yield. Unreacted amino groups were masked by a standard capping procedure (Ac_2O/pyridine, 1/1, v/v, rt, 2 h).

Synthesis of support 16. For Fmoc removal from support **13** (300 mg), a standard 20% piperidine treatment in DMF was carried out (3 × 2 mL, 10 min each). Resulting support **14** was left in contact overnight with derivative **12** (190 mg, 0.15 mmol, 2.5 equiv), DCC (31 mg, 0.15 mmol, 2.5 equiv), and HOBt (23 mg, 0.15 mmol, 2.5 equiv), previously dried by repeated coevaporations with anhydrous pyridine, in 2.5 mL of CH_2Cl_2/DMF, 4/1 (v/v), under stirring at room temperature. Successively, the support was exhaustively washed with DMF, CH_2Cl_2, CH_3OH, and CH_3CN and then dried under reduced pressure.

The functionalization of support **15** was calculated by spectroscopic measurements at λ = 498 nm (ϵ = 71 700 $cm^{-1}M^{-1}$) of the DMT cation, released by acidic treatment ($HClO_4$/EtOH, 3/2, v/v) from dried and weighed amounts of the resin. An average functionalization of 0.15 mmol/g was obtained, corresponding to a 75% incorporation yield of derivative **12**. Resin **15** was then treated with Et_3N/pyridine (1/1, v/v, 3 treatments of 2 h each) to remove the 2-cyanoethyl group. After exhaustive washings, the resulting support **16** was dried, then suspended in $CDCl_3$ and analyzed by ^{31}P NMR, which confirmed the total conversion of the phosphotriester into phosphodiester groups.

Solid support 16. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ −2.4.

Solid-Phase Synthesis of CyPLOS 1, 2, and 3. Support **16** (100 mg, 0.015 mequiv), previously washed with anhydrous CH_2Cl_2, was treated at room temperature under stirring with 2% DCA in CH_2Cl_2 (4 × 1 mL, 3 min each). Successively, the support, after exhaustive washings with CH_2Cl_2 and anhydrous CH_3CN, was reacted with phosphoramidite **9** (50 mg), previously dried by repeated coevaporations with CH_3CN, in anhydrous CH_3CN (300 μL) and a 0.45 M tetrazole solution in anhydrous CH_3CN (600 μL). After 45 min, under stirring at room temperature, the support was washed several times with CH_3CN and then treated with a 0.02 M I_2 solution (1 mL) in pyridine/H_2O/THF (1/2/7, v/v) for 30 min under stirring. The solid support, repeatedly washed with CH_3CN, was then dried under reduced pressure. The efficiency of the incorporation of the second glycosidic residue onto solid support **17** was determined by measuring the absorbance at λ = 498 nm of the DMT cation (ϵ = 71 700 $cm^{-1}M^{-1}$) released upon acidic treatment [$HClO_4$/EtOH, 3/2 (v/v)] of a dried and weighed amount of the resin. The functionalization was in all cases 0.15 mmol/g, accounting for an almost quantitative incorporation of the second monomer onto the solid support. After a standard capping treatment with Ac_2O/pyridine (1/1, v/v, 30 min), support **17** was subjected to the final detritylation, followed by repeated washings with CH_2Cl_2 and CH_3CN. Resulting support **20** was then reacted at rt under stirring with MSNT (50 mg, 0.17 mequiv), dissolved in anhydrous pyridine (500 μL, 2 × 3 h, followed by an overnight treatment). The obtained support was then repeatedly washed with pyridine and CH_3CN and successively treated with Et_3N/pyridine (2 mL, 1/1, v/v) for 3 h under stirring, specifically to remove the 2-cyanoethyl protecting group. The cyclic dimer was detached from the solid support by an overnight treatment with a 0.2 M 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate solution (1 mL) in H_2O/dioxane (1/1, v/v). The filtered eluate and two washings with

H_2O (1 mL each) of the support, once combined and concentrated under reduced pressure, were treated with a concentrated aq ammonia solution (5 mL) at 55 °C for 6 h. The obtained crude was finally purified by gel filtration chromatography on a G25 Sephadex column eluted with H_2O/EtOH, 4/1 (v/v). The obtained fractions were monitored by spectrophotometric measurements at $\lambda = 264$ nm. Pure cyclic dimer **1** (4 mg, 0.0062 mmol, 42% overall yields) was isolated, characterized by spectroscopic means, and found to be identical to the compound synthesized in solution as a control.

Starting from functionalized support **17** (100 mg, 0.015 mmol), for the synthesis of **2** and **3**, the above-described synthetic cycle was reiterated, once and twice, respectively, including the following steps: (i) detritylation; (ii) coupling with phosphoramidite **9** (50 mg), dissolved in anhydrous CH_3CN (300 μL) and a 0.45 M tetrazole solution in anhydrous CH_3CN (600 μL); (iii) oxidation; and (iv) capping. Obtained supports **18** and **19** were then respectively reacted with 2% DCA in CH_2Cl_2 (4 × 1 mL, 3 min each), giving detritylated supports **21** and **22**. Solid-phase cyclization was then realized by treating **21** or **22** with MSNT (50 mg, 0.17 mmol) dissolved in anhydrous pyridine (500 μL, 2 × 3 h, followed by an overnight treatment). All the successive steps for the final deprotection and detachment from the solid support of the cyclic oligomers were carried out as reported above for cyclic dimer **1**. Gel filtration chromatography of the final mixtures on a G25 Sephadex column eluted with H_2O/EtOH, 4/1 (v/v), yielded 2 mg (14% overall yield) and 1 mg (5% overall yield), respectively, of the pure compounds, which were identified as the desired cyclic molecules **2** and **3**.

1: white amorphous solid. Retention time (conditions as specified in the Supporting Information) = 10.2 min; $R_f = 0.15$ [$CHCl_3$/CH_3OH, 9/1 (v/v)]; $[\alpha]^{25}_D = -17.8°$; $\lambda_{max} = 264$ nm ($\epsilon = 780$ $cm^{-1}M^{-1}$). ^1H NMR (D_2O, 500 MHz; as triethylammonium salt): δ 7.45−7.19 (complex signals, 5H, aromatic protons), 5.22 (d, $J = 8.0$ Hz, 1H, H-1), 4.32 (m, 1H, H-4), 4.22 (br d, $J = 11.6$ Hz, 1H, H-6_a), 4.15 (br d, $J = 11.6$ Hz, 1H, H-6_b), 3.90 (overlapped signals, 2H, H-5 and H-3), 3.70 (t, $J = 8.0$ and 8.0 Hz, 1H, H-2), 3.22 (q, 6H, CH_2 triethylammonium ion), 1.30 (t, 9H, CH_3 triethylammonium ion). ^1H NMR (DMSO-d_6, 400 MHz, 298 K): relevant signals at δ 7.90 (br s, 1H, OH-3); 5.32 (d, $J = 5.1$ Hz, 1H, OH-2). ^{13}C NMR (D_2O, 125 MHz): δ 159.9, 133.1, 126.2, 119.3 (aromatic carbons); 102.7 (C-1); 78.2 (C-3); 75.7 (C-2); 75.5 (C-5); 75.0 (C-4); 66.2 (C-6). ^{31}P NMR (D_2O, 161.98 MHz): δ 3.1. ESI-MS (negative ions): *m*/*z* calcd for $C_{24}H_{30}O_{16}P_2$, 636.101; found, 635.25 $(M - H)^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{24}H_{29}O_{16}P_2$, 635.0929; found, 635.0885 $(M - H)^-$.

2: white amorphous solid. Retention time (conditions as specified in the Supporting Information) = 11.8 min; $R_f = 0.10$ [$CHCl_3$/CH_3OH, 8/2 (v/v)]; $[\alpha]^{25}_D = -22.3°$; $\lambda_{max} = 264$ nm ($\epsilon = 1030$ $cm^{-1}M^{-1}$). ^1H NMR (D_2O, 500 MHz; as triethylammonium salt): δ 7.45−7.18 (complex signals, 5H, aromatic protons), 5.18 (d, $J = 8.0$ Hz, 1H, H-1), 4.35 (m, 1H, H-6_a), 4.13 (overlapped signals, 2H, H-6_b and H-4), 3.98 (m, 1H, H-5), 3.88 (apparent t, $J = 9.5$ and 8.5 Hz, 1H, H-3), 3.55 (apparent t, $J = 8.5$ and 8.0 Hz, 1H, H-2), 3.22 (q, 6H, CH_2 triethylammonium ion), 1.30 (t, 9H, CH_3 triethylammonium ion). ^1H NMR (DMSO-d_6, 400 MHz, 298 K): relevant signals at δ 7.90 (br s, 1H, OH-3), 5.36 (d, $J = 5.1$ Hz, 1H, OH-2). ^{13}C NMR (D_2O, 100 MHz): δ 157.6, 130.2, 123.4, and 116.7 (aromatic carbons); 102.3 (C-1); 77.6 (C-3); 76.3 (C-4); 75.9 (C-5); 74.9 (C-2); 67.2 (C-6). ^{31}P NMR (D_2O, 161.98 MHz): δ 2.9. ESI-MS (negative ions): *m*/*z* calcd for $C_{36}H_{45}O_{24}P_3$, 954.151; found, 953.34 $(M - H)^-$, 476.21 $(M - 2H)^{2-}$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{36}H_{44}O_{24}P_3$, 953.1434; found, 953.1397 $(M - H)^-$.

3: white amorphous solid. Retention time (conditions as specified in the Supporting Information) = 12.5 min; $\lambda_{max} = 264$ nm ($\epsilon = 1430$ $cm^{-1}M^{-1}$). ^1H NMR (D_2O, 400 MHz): 7.51−7.22 (complex signals, 5H, aromatic protons), 5.31 (d, $J = 7.5$ Hz, 1H, H-1), 4.55−3.40 (complex region, broadened signals, H-2, H-3, H-4, H-5, and H_2-6). ^1H NMR (DMSO-d_6, 373 K, 400 MHz): 7.32−7.01 (complex signals, 5H, aromatic protons), 4.88 (d, $J = 6.9$ Hz, 1H, H-1), 3.82 (m, 1H, H-4), 3.68 (m, 2H, H_2-6), 3.54 (overlapped signals, 2H, H-5 and H-3), 3.36 (m, 1H, H-2). At 295 K, another set of signals (the major ones were centered at 5.44 and 5.35 ppm, respectively) were present, attributed to the OH-2 protons, which further broadened at higher temperatures and completely disappeared at 343 K. ^{31}P (D_2O, 161.98 MHz): δ 2.6 ppm. ESI-MS (negative ions): *m*/*z* calcd for $C_{48}H_{60}O_{32}P_4$, 1272.202; found, from combination of multiple charged ions, 1272.80.

Solution Synthesis of CyPLOS 1 and 2. Synthesis of derivative 23. 2-Chlorophenyl-dichlorophosphate (530 μL, 3.3 mmol, $d = 1.52$ g/mL, 5 equiv) was dissolved in anhydrous pyridine (2.5 mL) and left under stirring at room temperature for 10 min. Successively, H_2O (54 μL, 3 mmol) was added to the mixture and left under stirring for an additional 10 min. Finally, derivative **8** (500 mg, 0.66 mmol, 1 equiv), previously dried by repeated coevaporations with anhydrous CH_3CN and dried under reduced pressure, was dissolved in anhydrous pyridine (2 mL) and then added to the mixture containing 2-chlorophenylphosphoryl pyridinium chloride. The reaction was left under stirring at room temperature overnight and monitored by TLC using the eluent systems $CHCl_3$/CH_3OH, 9/1 (v/v), and $CHCl_3$/CH_3OH, 98/2 (v/v). The crude was then purified by silica gel column, eluted first with AcOEt to recover the unreacted starting material and then with a mixture AcOEt/CH_3OH, 8/2 (v/v). The fractions containing pure **23**, collected and concentrated under reduced pressure, gave 385 mg (0.59 mmol, 90% yield) of the desired compound.

23: white amorphous solid. $R_f = 0.3$ [$CHCl_3$/CH_3OH 9/1, (v/v)]. ^1H NMR (CD_3OD, 400 MHz; as pyridinium salt): δ 7.87−6.71 (complex signals, 19H, aromatic protons), 5.89 (dd, $J = 8.9$ and 9.2 Hz, 1H, H-2), 5.52 (overlapped signals, 2H, H-1 and H-3), 4.10−3.93 (overlapped signals, 3H, H-5 and H_2-6). H-4 resonance is submerged under the residual HDO solvent signal. ^{13}C NMR (CD_3OD, 50 MHz): δ 167.3 and 166.8 (2 CO); 158.5, 134.4, 133.9, 130.6, 129.4, 128.9, 128.3, 124.8, 124.0, 122.1, and 117.8 (aromatic C); 100.1 (C-1); 77.0 (C-2); 75.3 (C-3); 73.9 (C-5); 72.8 (C-4); 61.2 (C-6). ^{31}P NMR (CD_3OD, 161.98 MHz): δ −2.5. ESI-MS (negative ions): *m*/*z* calcd for $C_{32}H_{28}O_{11}PCl$, 654.106; found, 653.20 $(M - H)^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{32}H_{27}O_{11}PCl$, 653.0978; found, 653.0962 $(M - H)^-$.

Synthesis of Linear Precursor 25. Derivative **23** (100 mg, 0.15 mmol, 1 equiv) and compound **9** (177 mg, 0.18 mmol, 1.2 eq), previously dried by repeated coevaporations with anhydrous CH_3CN and kept under reduced pressure, were reacted with a 0.45 M tetrazole solution in anhydrous CH_3CN (4 mL, 15 mmol, 10 equiv). The reaction was left under stirring at room temperature and monitored by TLC in the eluent system $CHCl_3$/CH_3OH, 9/1 (v/v). After 2 h, a 5.5 M *t*-BuOOH solution in decane (1 mL) was added to the mixture and left under stirring at room temperature. After 30 min, TLC monitoring of the reaction in $CHCl_3$/CH_3OH, 9/1 (v/v), showed the formation of two products, one at $R_f = 0.55$ (identified as **24**) and the other one at $R_f = 0.45$ (identified as **25**). The concentrated crude was then purified by silica gel column chromatography eluted with $CHCl_3$/CH_3OH, 98/2 (v/v), containing a few drops of pyridine, giving DMT-protected linear dimer **24** (46 mg, 0.03 mmol, 20% yield) and detritylated linear dimer **25** (110 mg, 0.09 mmol, 60% yield).

24: white amorphous solid. $R_f = 0.55$ [$CHCl_3$/CH_3OH, 9/1 (v/v)]. ^1H NMR ($CDCl_3$, 400 MHz; as pyridinium salt): δ 8.04−6.55 (complex signals, 47H, aromatic protons); 5.88 (dd, 1H, H-3 A residue); 5.64 (overlapped signals, 2H, H-2 A residue and H-3 B residue); 5.41 (dd, 1H, H-2 B residue); 5.35 (d, $J = 7.6$ Hz, 1H, H-1 A residue); 5.03 (d, $J = 8.0$ Hz, 1H, H-1 B residue); 4.72 (m, 1H, H-4 B residue); 4.41 (m, 2H, OCH_2CH_2CN); 3.93−3.42 (overlapped signals, 7H, H-4 A residue, 2 H-5 and 2 H_2-6); 3.74 (s, 6H, 2 OCH_3 of the DMT group); 2.59 (t, 2H, OCH_2CH_2CN). ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ −0.5 and −3.7. ESI-MS (negative ions): *m*/*z* calcd for $C_{82}H_{72}NO_{23}P_2Cl$, 1535.366; found,

1534.61 (M − H)$^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{82}H_{71}NO_{23}P_2Cl$, 1534.3579; found, 1534.3544 (M − H)$^-$.

25: white amorphous solid. R_f = 0.45 [$CHCl_3/CH_3OH$, 9/1 (v/v)]. ^{1}H NMR (CD_3OD, 400 MHz; as pyridinium salt): δ 8.02−6.79 (complex signals, 34H, aromatic protons); 5.92 (dd, J = 9.6 and 9.2 Hz, 1H, H-3 A residue); 5.74 (dd, J = 9.6 and 9.2 Hz, 1H, H-3 B residue); 5.59 (dd, J = 8.4 and 9.2 Hz, 1H, H-2 A residue); 5.51 (d, J = 8.0 Hz, 1H, H-1 A residue); 5.43 (dd, J = 8.0 and 9.6 Hz, 1H, H-2 B residue); 5.26 (d, J = 8.0 Hz, 1H, H-1 B residue); 4.70 (m, 1H, H-6_a signal of B residue); 4.60 (m, 1H, H-4 B residue); 4.27 (m, 1H, H-6_b signal of B residue); 4.15 (m, 2H, OC*H*$_2$CH$_2$CN); 4.02−3.90 (overlapped signals, 3H, H-5 and H_2-6 of A residue); 3.79 (m, 1H, H-5 B residue); 2.74 (m, 2H, OCH$_2$C*H*$_2$CN). H-4 of A residue is submerged by the residual HDO signal in CD_3OD. ^{13}C NMR (CD_3OD, 100 MHz): δ 167.8, 167.7, 167.2, and 167.1 (CO); 159.1, 158.8, 150.3, 145.8, 139.2, 135.2, 135.1, 135.0, 134.4, 131.6, 131.3, 131.0, 131.1, 130.9, 130.4, 130.1, 130.0, 129.4, 128.9, 126.1, 125.2, 124.9, 124.6, 122.9, 118.9, and 118.5 (aromatic C); 117.1 (OCH$_2$CH$_2$*C*N); 100.7 (C-1 A residue and B residue); 76.8 (C-5 A residue); 76.1 (C-5 B residue); 75.7, 75.6, and 75.3 (C-3 A residue, C-3 B residue, C-4 A residue); 74.2 (C-2 B residue); 73.9 (C-2 A residue and C-4 B residue); 69.6 (C-6 B residue); 64.9 (O*C*H$_2$CH$_2$CN); 61.8 (C-6 OH A residue); 19.8 and 18.9 (OCH$_2$*C*H$_2$CN). ^{31}P NMR (CD_3OD, 161.98 MHz): δ −0.7 and −3.7. ESI-MS (negative ions): *m*/*z* calcd for $C_{61}H_{54}NO_{21}P_2Cl$, 1233.235; found, 1232.53 (M − H)$^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{61}H_{53}NO_{21}P_2Cl$, 1232.2272; found, 1232.2240 (M − H)$^-$.

Synthesis of Cyclic Dimer 1. Derivative **25** (38 mg, 0.031 mmol, 1 equiv), previously dried by coevaporations with anhydrous pyridine, and MSNT (183 mg, 0.62 mmol, 20 equiv) were dissolved in anhydrous pyridine (30 mL) and left under stirring overnight. The reaction, monitored by TLC in $CHCl_3/CH_3OH$, 9/1 (v/v), showed the complete conversion of derivative **25** into a compound with a higher R_f. The reaction mixture, taken to dryness under reduced pressure, was then purified by silica TLC plates, developed in the eluent system $CHCl_3/CH_3OH$, 9/1 (v/v), which afforded pure **26** (27 mg, 0.022 mmol) with 71% yield. This compound, coevaporated several times with anhydrous pyridine and dried under reduced pressure, was then treated with Et_3N/pyridine (4 mL, 1/1, v/v, 3 h, rt) to selectively remove the 2-cyanoethyl group. The reaction, monitored by TLC in $CHCl_3/CH_3OH$, 9/1 (v/v), was quenched by in vacuo removal of the solvent. The crude, after several coevaporations with anhydrous pyridine, was then treated with a 0.2 M 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate solution (1 mL) in H_2O/dioxane (1/1, v/v). The reaction mixture was then left overnight under stirring at room temperature. Taken to dryness, the crude was then treated with concentrated NH_4OH (1 mL, 6 h, 55 °C). The concentrated mixture was then purified by gel filtration chromatography on a Sephadex G25 column, eluted with H_2O/EtOH, 4/1 (v/v). Fractions containing the cyclic dimer, monitored by spectrophotometric measurements at λ = 264 nm, were collected and concentrated, affording 10 mg (0.016 mmol, 73% yield) of pure **1**.

26: white amorphous solid. R_f = 0.8 [$CHCl_3/CH_3OH$, 9/1 (v/v)]. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.14−6.62 (complex signals, 34H, aromatic protons), 6.03 (dd, J = 9.2 and 9.2 Hz, 1H, H-3 A residue), 5.95 (dd, J = 7.6 and 9.2 Hz, 1H, H-2 A residue), 5.83 (dd, J = 9.2 and 9.2 Hz, 1H, H-3 B residue), 5.70 (dd, J = 8.0 and 9.2 Hz, 1H, H-2 B residue), 5.46 (d, J = 7.6 Hz, 1H, H-1 A residue), 5.41 (m, 1H, H-4 A residue), 5.07 (m, 1H, H-4 B residue), 5.01 (d, J = 8.0 Hz, 1H, H-1 B residue), 4.75 (m, 1H, H-6_a A residue), 4.50 (m, 1H, H-6_a B residue), 4.37 (d, J = 11.6 Hz, 1H, H-6_b A residue), 4.27 (d, J = 11.2 Hz, 1H, H-6_b B residue), 4.04 (m, 1H, H-5 A residue), 3.96 and 3.36 (2 m, 1H each, OC*H*$_2$CH$_2$CN), 3.76 (m, 1H, H-5 B residue), 2.30 (m, 2H, OCH$_2$C*H*$_2$CN). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.8, 165.5, 165.4, and 165.3 (CO); 156.9, 156.8, 133.9, 133.7, 133.6, 133.0, 131.1, 130.3, 130.2, 130.1, 130.0, 129.8, 129.6, 129.5, 129.2 129.1, 129.0, 128.9, 128.7, 128.6, 128.1, 128.0, 127.6, 127.2, 123.9, 123.8, 122.1, 117.9, and 117.1 (aromatic carbons); 116.1 (OCH$_2$CH$_2$*C*N); 99.9 and 99.8 (2 C-1); 73.8, 73.7, 73.5, 73.4, 72.7, 72.5, 71.4, and 71.0 (2 C-2, 2 C-3, 2 C-4 and 2 C-5); 66.1 and 65.6 (2 C-6); 62.0 and 61.0 (O*C*H$_2$CH$_2$CN); 18.8 and 18.7 (OCH$_2$*C*H$_2$CN). ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ −2.3 and −7.5. ESI-MS (positive ions): *m*/*z* calcd for $C_{61}H_{52}NO_{20}P_2Cl$, 1215.225; found, 1216.71 (M + H$^+$), 1238.75 (M + Na$^+$), 1254.88 (M + K$^+$). HRMS (MALDI-TOF): *m*/*z* calcd for $C_{61}H_{52}NO_{20}P_2ClNa$, 1238.2144; found, 1238.2178 (M + Na$^+$).

Solution Synthesis of Cyclic Trimer 2. Synthesis of linear precursor 28. Derivative **25** (50 mg, 0.04 mmol, 1 equiv) and derivative **9** (47 mg, 0.05 mmol, 1.2 equiv), dried by coevaporations with anhydrous CH_3CN, were dissolved in 2 mL (10 equiv) of a 0.45 M tetrazole solution in anhydrous CH_3CN and left under stirring at room temperature. After 2 h, 500 μL of a 5.5 M *t*-BuOOH solution in decane were added to the reaction mixture and left under stirring at room temperature. TLC analysis in $CHCl_3/CH_3OH$ (9/1, v/v) showed the formation of two new products, respectively, at R_f = 0.4 and R_f = 0.3, identified as linear trimer **27** and the corresponding detritylated derivative **28**. The mixture was then purified by TLC plates, developed using the solvent system $CHCl_3/CH_3OH$, 9/1 (v/v), containing a few drops of pyridine, giving detritylated linear trimer **28** (25 mg, 0.014 mmol, 35% yields) and linear trimer **27**, retaining the DMT group (6 mg, 0.003 mmol, 8% yields).

27: white amorphous solid. R_f = 0.7 [$CHCl_3/CH_3OH$, 9/1 (v/v)]. ^{1}H NMR (CD_3OD, 400 MHz; as pyridinium salt): δ 8.12−6.75 (complex signals, 62H, aromatic protons), 6.13 (dd, J = 9.0 and 10.0 Hz, 1H, H-3 A residue), 5.87 (dd, J = 9.5 and 10.0 Hz, 1H, H-3 B residue), 5.71 (overlapped signals, 2H, H-3 and H-1 C residue), 5.64 (dd, J = 9.5 and 10.0 Hz, 1H, H-2 A residue), 5.59 (dd, J = 9.5 and 8.0 Hz, 1H, H-2 B residue), 5.48 (dd, J = 9.5 and 8.0 Hz, 1H, H-2 C residue), 5.40 (d, J = 8.0 Hz, 1H, H-1 B residue), 5.16 (d, J = 7.5 Hz, 1H, H-1 A residue), 4.74 (m, 1H, H-4 A residue), 4.65 (m, 1H, H-4 B residue), 4.57 (m, 1H, H-4 C residue), 4.29 (m, 1H, H-5 B residue), 4.21 (complex signals, 2H, H-5 A residue and H-5 C residue), 4.12−4.01 (complex signals, 4H, 2 OC*H*$_2$CH$_2$CN), 3.99−3.43 (overlapped signals, 6H, 3 × H_2-6), 3.79 (s, 6H, OCH_3 protons DMT group), 2.78−2.60 (complex signals, 4H, 2 OCH$_2$C*H*$_2$CN). ^{13}C NMR (CD_3OD, 100 MHz): δ 165.8, 165.1, and 165.0 (CO); 158.3, 149.6, 133.5, 133.1, 133.0, 131.2, 130.2, 129.7, 129.5, 129.0, 128.6, 128.3, 127.7, 123.7, 123.4, 123.1, 118.1, and 113.1 (aromatic C); 117.2 (OCH$_2$CH$_2$*C*N); 99.1, 99.0, and 98.9 (C-1); 74.6, 74.0, 73.7, 73.6, 73.5, 73.1, 72.7, 71.8, 71.5, and 71.2 (C-2, C-3, C-4, and C-5); 63.0, 62.9, 62.8, and 62.4 (C-6 and O*C*H$_2$CH$_2$CN); 55.1 (OCH_3); 18.9 and 18.9 (OCH$_2$*C*H$_2$CN). ^{31}P NMR (CD_3OD, 161.98 MHz): δ −1.0, −1.2, −3.6. MALDI-TOF MS (negative ions): *m*/*z* calcd for $C_{111}H_{98}N_2O_{33}P_3Cl$, 2114.495; found, 2114.1 (M − H)$^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{111}H_{97}N_2O_{33}P_3Cl$, 2113.4873; found, 2113.4844 (M − H)$^-$.

28: white amorphous solid. R_f = 0.6 [$CHCl_3/CH_3OH$, 9/1 (v/v)]. ^{1}H NMR (CD_3OD, 400 MHz; as pyridinium salt): δ 8.07−6.70 (complex signals, 49H, aromatic protons), 6.12 (dd, J = 9.0 and 10.0 Hz, 1H, H-3 A residue), 5.91 (dd, J = 9.5 and 9.0 Hz, 1H, H-3 B residue), 5.81 (dd, J = 9.5 and 9.0 Hz Hz, 1H, H-3 C residue), 5.69 (d, J = 8.0 Hz, 1H, H-1 C residue), 5.61 (overlapped signals, 2H, H-2 A residue and H-2 C residue), 5.51 (dd, J = 8.5 and 9.0 Hz, 1H, H-2 B residue), 5.46 (d, J = 8.0 Hz, 1H, H-1 B residue), 5.35 (d, J = 7.5 Hz, 1H, H-1 A residue), 4.94 (m, 1H, H-4 A residue), 4.77 (m, 1H, H-4 B residue), 4.65 (m, 1H, H-4 C residue), 4.35 (overlapped signals, 2H, H-5 and H-6_a B residue), 4.22−4.14 (complex signals, 7H, H-6_b B residue, H_2-6 C residue and 2 OC*H*$_2$CH$_2$CN), 4.09 (m, 1H, H-5 A residue), 4.04 (m, 1H, H-6_a A residue), 3.98 (m, 1H, H-5 C residue), 3.89 (m, 1H, H-6_b A residue), 2.80−2.67 (complex signals, 4H, 2 OCH$_2$C*H*$_2$CN). ^{13}C NMR (CD_3OD, 100 MHz): δ 168.0, 167.9, 167.6, 167.5, 167.2, and 167.1 (CO); 159.1, 159.0, 158.7, 158.6, 155.0, 151.5, 150.6, 150.7, 135.3, 135.2, 135.1, 135.0, 134.9, 134.3, 131.7, 131.6, 131.3, 131.2, 131.1, 131.0, 130.9, 130.4, 130.1, 130.0, 129.9, 129.4, 128.8,

125.1, 124.9, 124.5, 122.9, 119.2, 119.0, 118.8, 118.6, 118.5, and 118.2 (aromatic C); 117.2 and 116.9 (OCH_2CH_2CN); 101.0 (C-1 B residue); 100.7 (C-1 A residue); 100.4 (C-1 C residue); 76.7 (C-5 B residue); 76.3 (C-5 A residue); 73.9 (C-5 C residue); 75.9, 75.8, 75.7, 75.2, 75.1, 74.4, 74.2, 73.6, and 70.1 (C-2, C-3, and C-4 for A, B, and C residues); 68.1 (C-6 B residue); 64.9 (overlapped C-6 C residue and OCH_2CH_2CN); 61.8 (C-6 OH A residue); 20.4 and 20.4 (OCH_2CH_2CN). ^{31}P NMR (CD_3OD, 161.98 MHz): δ −1.6, −3.2, and −5.6. ESI-MS (negative ions): *m*/*z* calcd for $C_{90}H_{80}N_2O_{31}P_3Cl$, 1812.365; found, 1812.42 $(M - H)^-$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{90}H_{79}N_2O_{31}P_3Cl$, 1811.3566; found, 1811.3523 $(M - H)^-$.

Synthesis of Cyclic Trimer 2. Derivative **28** (18 mg, 0.010 mmol, 1 equiv), previously dried by coevaporations with anhydrous pyridine, and MSNT (60 mg, 0.21 mmol, 20 equiv) were dissolved in anhydrous pyridine (10 mL) and left under stirring at room temperature overnight. The reaction mixture was next concentrated under reduced pressure and then purified by TLC plates, developed in $CHCl_3/CH_3OH$, 93/7 (v/v). The band at $R_f = 0.9$ furnished fully protected cyclic trimer **29** (10 mg, 0.0056 mmol, 56% yields) as a pure compound. Successively, treatment with a 1/1 (v/v) Et_3N/pyridine solution (4 mL) for 3 h at room temperature allowed the removal of the 2-cyanoethyl group from the phosphodiester linkages. The reaction was quenched by concentrating the mixture under reduced pressure. The crude, dried by coevaporations with anhydrous pyridine, was then treated with a 0.2 M 1,1,3,3-tetramethylguanidinium 2-nitrobenzaldoximate solution (1 mL) in H_2O/dioxane (1/1, v/v). The reaction mixture was left at room temperature overnight and, after removal of the solvent in vacuo, treated with concentrated NH_4OH (1 mL, 6 h, 55 °C). The reaction mixture, taken to dryness and redissolved in H_2O, was then purified by gel filtration chromatography on a Sephadex G25 column eluted with H_2O/EtOH, 4/1 (v/v). Fractions containing the cyclic trimer, monitored by spectrophotometric measurements at $\lambda = 264$ nm, were collected and concentrated, affording 4 mg (0.0042 mmol, 75% yield) of pure **2**.

29: white amorphous solid. $R_f = 0.9$ [$CHCl_3/CH_3OH$, 93/7 (v/v)]. ^{31}P NMR ($CDCl_3$, 161.98 MHz): δ 0.4, −0.9, −1.3, −2.3, −5.8, −6.7. ESI-MS (positive ions): *m*/*z* calcd for $C_{90}H_{78}N_2O_{30}P_3Cl$, 1794.354; found, 1817.72 $(M + Na^+)$, 1834.68 $(M + K^+)$. HRMS (MALDI-TOF): *m*/*z* calcd for $C_{90}H_{78}N_2O_{30}P_3ClNa$, 1817.3439; found, 1817.3471 $(M + Na^+)$.

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Supporting Information Available: Materials and methods, nuclear magnetic resonance methods, and structure calculation methods. Full scale ^1H NMR in D_2O and in DMSO-d_6 for compounds **1** and **2**, ^{31}P NMR of compounds **1**, **2**, and **3**, and NMR data for compounds **8**, **9**, **12**, **16**, and **23**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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