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Synthesis of Non-Natural Sequence-Encoded Polymers Using Phosphoramidite Chemistry

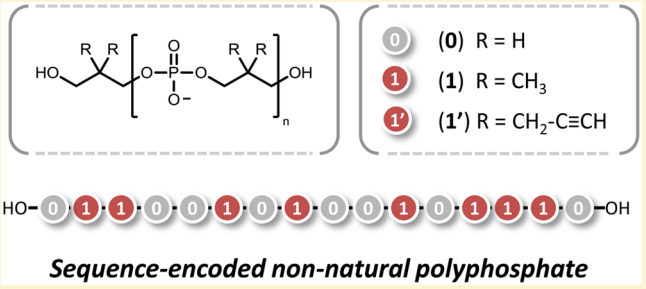
Abdelaziz Al Ouahabi,[†] Laurence Charles,[‡] and Jean-François Lutz*[,†]

[†]Precision Macromolecular Chemistry, Institut Charles Sadron, UPR-22 CNRS, BP 84047, 23 rue du Loess, 67034 Strasbourg Cedex 2, France

[‡]Aix-Marseille Université, CNRS, Institute of Radical Chemistry, UMR 7273, 13397 Marseille Cedex 20, France

Supporting Information

ABSTRACT: Sequence-defined non-natural polyphosphates were prepared using iterative phosphoramidite protocols on a polystyrene solid support. Three monomers were used in this work: 2-cyanoethyl (3-dimethoxytrityloxy-propyl) diisopropyl-phosphoramidite (**0**), 2-cyanoethyl (3-dimethoxytrityloxy-2,2-dimethyl-propyl) diisopropylphosphoramidite (**1**), and 2-cyanoethyl (3-dimethoxytrityloxy-2,2-dipropargyl-propyl) diisopropylphosphoramidite (**1′**). Phosphoramidite coupling steps allowed rapid synthesis of homopolymers and copolymers. In particular, the comonomers (**0**, **1**), (**0**, **1′**), and (**1**, **1′**) were used to synthesize sequence-encoded copolymers. It was found that long encoded sequences could be easily built using phosphoramidite chemistry. ESI-HRMS, MALDI-HRMS, NMR, and size exclusion chromatography analyses indicated the formation of monodisperse polymers with controlled comonomer sequences. The polymers obtained with the comonomers (**0**, **1′**) and (**1**, **1′**) were also modified by copper-catalyzed azide−alkyne cycloaddition with a model azide compound, namely 11-azido-3,6,9-trioxaundecan-1-amine. ^{1}H and ^{13}C NMR analysis evidenced quantitative modification of the alkyne side-chains of the monodisperse copolymers. Thus, the molecular structure of the coding monomer units can be easily varied after polymerization. Altogether, the present results open up interesting avenues for the design of information-containing macromolecules.



INTRODUCTION

Sequence-controlled polymers have recently gained increasing attention in chemical sciences.[1,2] Indeed, the precise adjustment of monomer units in polymer chains opens up interesting perspectives for controlling molecular, supramolecular and macroscopic properties of polymer materials.[3−10] Thus, many new concepts for monomer sequence-regulation have been introduced during the past few years.[11−13] For example, strategies for controlling comonomer sequences in classical polymerization approaches such as chain-growth[14−19] and step-growth[20] polymerizations have been described. Yet, polymers prepared using these strategies exhibit chain-length polydispersity.[21] In order to design polymers with perfectly controlled primary structures, other polymerization tools have to be developed.[22,23] For instance, solid-phase iterative chemistry, which is a multistep-growth polymerization process, allows design of monodisperse sequence-defined polymers.[1] In such strategies, monomers are sequentially attached one-by-one to a growing chain, which is immobilized on a cross-linked or soluble support. These methods are efficient but are limited by the yields and duration of the coupling steps. Thus, various approaches have been recently proposed to simplify multistep growth processes.[24−30] In addition, biochemical methodologies, which have been developed and optimized for bio-oligomer synthesis, can be adapted for the preparation of non-natural sequence-defined polymers.[1] For example, Fmoc-based protocols that were initially introduced for solid-phase peptide chemistry have been extensively studied for the preparation of synthetic oligomers.[31−33] On the other hand, the phosphoramidite coupling reaction used in oligonucleotide synthesis has been barely tested in synthetic polymer science.[34] This method has, however, valuable advantages for preparing sequence-defined polymers. In this strategy, a phosphate linkage is obtained by reaction of a phosphoramidite with a hydroxy group, followed by the oxidation of the resulting phosphite into a phosphate. Using AB-type monomers containing a phosphoramidite function and a dimethoxytrityl (DMT)-protected hydroxy function, a controlled multistep growth polymerization process can be executed on a solid support. The iterative synthesis involves typically a three-step cycle: phosphoramidite coupling, phosphite oxidation, and DMT deprotection. Despite these steps and the use of protecting groups, this chemistry is extremely fast and efficient because it has been optimized by decades of biochemistry research.[35] Typically, near-quantitative yields can be obtained for each cycle within a few minutes. With the help of automated synthesizers, it is nowadays possible to synthesize sequence-

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defined polynucleotides containing more than a hundred residues using this approach.[36,37] Thus, phosphoramidite coupling seems very appealing for the preparation of other polymers than nucleic acids. A wide variety of non-nucleoside phosphoramidite monomers has been reported in the literature. However, these monomers are used, in general, for oligonucleotide modification rather than for preparing synthetic polymers.[38−43] Ganesan and co-worker have reported the synthesis of peptidomimetic polyphosphates using phosphoramidite chemistry.[44] Häner and co-workers have described the solid-phase phosphoramidite synthesis of oligopyrenotides.[45,46] These authors have also studied in details the folding and self-assembly properties of these non-natural oligophosphates. In a recent publication, Sleiman and co-workers have described the synthesis of polymer-DNA biohybrids using phosphoramidite chemistry.[47] In particular, alternating, periodic, and block amphiphilic segments were prepared in this work. More recently, the same group has described the relevance of these biohybrid structures for the precise construction of 3D assemblies.[48]

In the present work, phosphoramidite chemistry was used for the preparation of non-natural sequence-defined polyphosphates (Figure 1). In particular, this methodology was

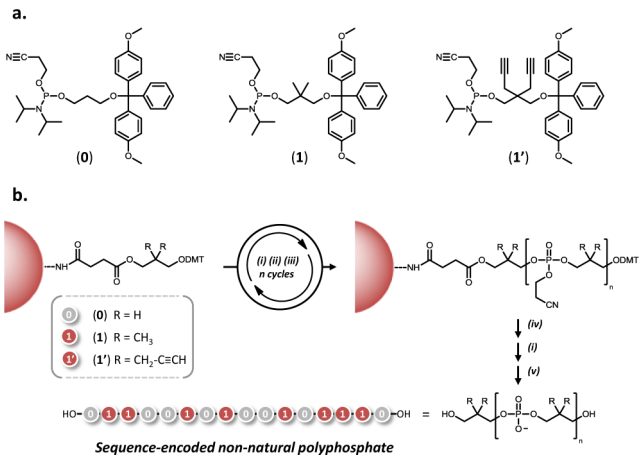

Figure 1. General strategy for the synthesis of sequence-defined non-natural polyphosphates. (a) Molecular structures of the comonomers. (b) Iterative polymer synthesis on a solid support (red bead). Experimental conditions: (i) DMT deprotection: CCl_3−COOH, CH_2Cl_2; (ii) coupling step: RT, AcCN, tetrazole; (iii) oxidation: RT, I_2, H_2O/Pyridine/THF; (iv) cyanoethyl deprotection: piperidine, AcCN; (v) cleavage: NH_3, H_2O, dioxane.

investigated for the synthesis of information-containing macromolecules.[49] In recent publications,[50] our group has shown that digital information can be encrypted in synthetic polymers using monomers intentionally defined as 0 and 1 bit. In addition, analytical methods for reading sequence-encoded information have been described.[51,52] Thus, this novel type of macromolecules opens up interesting perspectives for applications in the fields of data storage and molecular identification.[49] As shown in Figure 1, three phosphoramidite monomers were prepared in this work. With the exception of phosphoramidite and DMT-protected hydroxy groups, it is important to note that these monomers do not share any structural feature with phosphoramidite nucleosides. In order to implement a molecular code in the polymer chains, two monomers containing a propyl (2-cyanoethyl (3-dimethoxytrityloxy-propyl) diisopropylphosphoramidite, **0**) and a 2,2-dimethyl-propyl (2-cyanoethyl (3-dimethoxytrityloxy-2,2-dimethyl-propyl) diisopropylphosphoramidite, **1**) spacer were synthesized. Additionally, a monomer containing a 2,2-dipropargyl-propyl spacer was designed (2-cyanoethyl (3- dimethoxytrityloxy-2,2-dipropargyl-propyl) diisopropylphosphoramidite, **1′**). Such a monomer allows postpolymerization modification of the polymers using different chemistries, e.g., azide−alkyne Huisgen cycloaddition, Sonogashira or Castro-Stephens coupling.[53,54]

METHODS

General. 2-Cyanoethyl diisopropylchlorophosphoramidite (95%, Alfa Aesar), 4,4′-dimethoxytriphenylmethyl chloride (≥97.0%, Sigma-Aldrich), *N*,*N*-diisopropylethylamine (DIPEA, 99%, Alfa Aesar), propargyl bromide (solution 80% w/w in toluene, Alfa Aesar), tetrakis(acetonitrile)copper(I) tetrafluoroborate (97%, Sigma-Aldrich), 4-(dimethylamino)pyridine (DMAP, 99%, Sigma-Aldrich), *N*,*N*′-dicyclohexylcarbodiimide, (99%, DCC, Alfa Aesar), and amino-polystyrene resin (1.4 mmol·g^{-1}, Novabiochem) were used as purchased. Anhydrous dichloromethane, pyridine, and acetonitrile were purchased from Aldrich. Anhydrous THF was obtained using a dry solvent station GT S100. The 3% trichloroacetic acid solution in dichloromethane has been prepared from trichloroacetic acid purchased from Sigma-Aldrich. 11-Azido-3,6,9-trioxaundecan-1-amine was synthesized as previously reported.[50] The synthesis of d-**1′** was adapted from the literature.[55] D-**0** and **0** were synthesized according to reported procedures.[56] All air-sensitive reactions have been carried out under argon atmosphere. The phosphoramidite compounds have been kept in the freezer at −18 °C. Oligomer syntheses were performed in an equipped argon/vacuum solid-phase extraction glass tube (12 mL with frit 3) and stirred with an IKA HS 260 Basic shaker.

Instrumentation. ^{1}H NMR spectra were recorded (in D_2O for polymers or in acetone and $CDCl_3$ for monomers) on a Bruker Avance 400 MHz spectrometer. ^{13}C NMR spectra were recorded at 100.6 MHz and ^{31}P NMR spectra were recorded at 161.92 MHz and were externally referenced to 85% phosphoric acid. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) operated in the positive mode. The capillary voltage was set at +5500 V and the cone voltage at +75 V. Samples were diluted in a methanolic solution of ammonium acetate (3 mM) and injected into the ESI source at a 5 μL·min^{-1} flow rate using a syringe pump. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) experiments were performed on a Synapt G2 HDMS mass spectrometer (Waters, Manchester, U.K.) equipped with a laser emitting at 355 nm. MALDI samples were prepared by mixing the matrix (2,5-dihydroxybenzoic acid in methanol) and the analyte (in methanol containing 3 mM ammonium acetate) in a 1000:1 matrix-to-analyte molar ratio. A 1 μL aliquot of this binary mixture was deposited on the sample plate and allowed to air dry. Both instruments allowed accurate mass measurements thanks to the high-resolution capability of the orthogonal acceleration time-of-flight (oa-TOF) mass analyzer operated in the reflectron mode. Internal calibration of the oa-TOF was performed with poly(ethylene oxide) oligomers adducted with ammonium in ESI-MS experiments and with red phosphorus aggregates in MALDI-MS experiments. The number-average molar mass (M_n) and the polydispersity index (M_w/M_n = PDI) were determined using a DIONEX HPLC system, Ultimate 3000 (degasser, pump, autosampler) equipped with 4 Shodex OH-pak columns 30 cm (802.5HQ, 804HQ, 806HQ, 807HQ), a guard column (separation range: 300−100 000 000 g·mol^{-1}), 1 diode array UV detector (Dionex), 1 differential refractometer OPTILAB rEX (Wyatt Tech.), and 1 light scattering detector DAWN HELEOS II (Wyatt Tech.). The measurements were performed at 30 °C in water containing 40% acetonitrile (ACN) and 0.1 M of $NaNO_3$ at a flow rate of 0.5 mL·min^{-1}.

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Synthesis of Monomers. *Synthesis of D-1.* 2,2-Dimethyl-1,3-propandiol d-**1** (0.7 g, 6.7 mmol) was co-evaporated with 5 mL of anhydrous pyridine in order to eliminate residual water present in the diol. Afterward, 8 mL of pyridine and 12 mL of anhydrous THF were introduced, and the diol was reacted with 4,4-dimethoxytrityl chloride (DMTrCl) (2.27 g, 6.7 mmol). The DMTrCl was added in three equal portions at a rate of one portion every hour. After the three additions, the mixture was stirred at room temperature for 2 h. The reaction was stopped with the addition of 5 mL of methanol, and the mixture was evaporated to dryness. The residue was dissolved in ethyl acetate (30 mL) and washed with 5% sodium bicarbonate ice-cold solution. The aqueous layer was extracted with 30 mL of ethyl acetate. The combined organic layers were washed with water and brine, dried with anhydrous Na_2SO_4, and evaporated. The resulting product was chromatographed on silica gel (20% ethyl acetate in cyclohexane with a 1% triethylamine) yielding 2.3 g of D-**1** (85%) as a colorless oil. ^{1}H NMR [$(CD_3)_2CO$, δ, ppm]: 7.50−7.20 (m, 9H), 6.88−6.86 (m, 4H), 3.77 (s, 6H), 3.43 (m, 2H), 2.94 (s, 2H), 0.91 (s, 6H)

Synthesis of D-1′. 2,2-Dipropargyl-1,3-propandiol d-**1′** (1.5 g, 9.8 mmol) was reacted with four portions of DMTrCl (4 × 0.83 g, 9.8 mmol) in 15 mL of pyridine and 30 mL of THF as described above. The reaction was stirred for 40 min between each addition. The resulting product was chromatographed on silica gel (20% ethyl acetate in cyclohexane with a 1% triethylamine) yielding 4.19 g of D-**1′** (94%) as a colorless oil. ^{1}H NMR [$(CD_3)_2CO$, δ, ppm]: 7.49−7.20 (m, 9H), 6.88−6.86 (m, 4H), 3.77 (s, 6H), 3.63 (s, 2H), 3.20 (s, 2H), 2.40−2.39 (d, 4H), 2.30 (t, 2H)

Synthesis of 1. D-**1** (2.03 g, 5 mmol) was co-evaporated with 8 mL of anhydrous dichloromethane and dried under vacuum over 30 min, and then 0.1 g of molecular sieves 3 Å were added. Ten mL of anhydrous dichloromethane and DIPEA (3.6 mL, 20 mmol) were added successively under argon, and the system was cooled with an ice−water bath. *O*-2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphor-amidite (1.2 g, 5.1 mmol) was added dropwise with continuous stirring. The reaction flask was then allowed to warm to room temperature and stirred for 1 h. The mixture was evaporated to dryness and dissolved in ethyl acetate, and then chromatographed directly on silica gel using hexane/ethyl acetate (3:2) + 1% triethylamine as eluent yielding 2.7 g of monomer **1** (92%) as a foam. ^{31}P NMR [$CDCl_3$, δ, ppm]: 148.4. ^{1}H NMR [$(CD_3)_2CO$, δ, ppm]: 7.49−7.17 (m, 9H), 6.86−6.89 (m, 4H), 3.78 (s, 6H), 3.75−3.77 (m, 2H), 3.57−3.61 (m, 3H), 3.43−3.47 (m, 1H), 2.91−2.98 (m, 2H), 2.67 (t, 2H), 1.13−1.19 (dd, 12H), 0.93−0.96 (d, 6H). ^{13}C NMR [$(CD_3)_2CO$, δ, ppm]: 159.51, 146.63, 137.29, 131.06, 129.12, 128.46, 127.39, 118.91, 113.76, 86.31, 70.65, 69.09, 59.43, 55.48, 43.79, 37.56, 27.53, 24.95, 20.85

Synthesis of 1′. D-**1′** (4.47 g, 9.8 mmol) was reacted with *O*-2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite (2.5 g, 10 mmol) and DIPEA (11 mL, 60 mmol) as described above. The resulting product was purified by chromatography on silica gel (hexane/ethyl acetate (7:3) + 1% triethylamine) yielding 5.4 g of monomer **1′** (84%) as a colorless liquid-foam. ^{31}P NMR [$CDCl_3$, δ, ppm]: 147.4. ^{1}H NMR [$(CD_3)_2CO$, δ, ppm]: 7.19−7.51 (m, 9H), 6.87−6.89 (m, 4H), 3.78 (s, 6H), 3.75−3.77 (m, 3H), 3.59−3.67 (m, 3H), 3.21−3.23 (m, 2H), 2.69 (t, 2H), 2.35−2.46 (m, 6H), 1.13−1.19 (dd, 12H). ^{13}C NMR [$(CD_3)_2CO$, δ, ppm]: 159.60, 146.31, 136.94, 131.13, 129.13, 128.52, 127.48, 118.87, 113.83, 86.81, 81.09, 72.59, 65.62, 64.28, 59.47, 55.50, 43.87, 43.26, 27.53, 24.97, 20.84.

Resin Functionalization. *Preparation of Succinates h-0, h-1, and h-1′.* In a 25 mL round-bottom flask, D-**0**, D-**1**, and D-**1′** (1 mmol) were co-evaporated with 4 mL of pyridine, dried under vacuum for 30 min, and reacted with 1.5 equiv of succinic anhydride and 1.5 equiv of DMAP in 4 mL of anhydrous pyridine at 35 °C for 5h. The reaction was quenched with methanol, and the solvent was concentrated. The resulting oil was diluted with ethyl acetate (20 mL) and washed with water, 0.1 M sodium phosphate (pH 5.0), and again with water. The organic phase was dried with MgSO4, and the solvent was evaporated to dryness to give a colorless oil.

Resin modification. The succinate h-**0**, h-**1**, or h-**1′** (0.73 mmol) was reacted with 0.4 g of amino-polystyrene resin (1.4 mmol·g^{-1}), 0.26 mmol of DMAP, and 2.26 mmol of DCC in 5 mL of anhydrous dichloromethane following a previously reported procedure.[57] The unreacted amino groups were capped with acetic anhydride in pyridine (1:5 v/v). The loading of the support was measured by the removal of the DMT group of an aliquot and analyzing its absorbance at 504 nm to find a loading of about 0.8 mmol·g^{-1}.

Solid-Phase Synthesis of the Oligomers. The couplings were carried out under vacuum-argon in rigorously dry conditions using a homemade peptide synthesis reaction vessel equipped with a fritted glass plate and a stopcock. The general procedure is the following one: 1 mol equiv of polystyrene support R-**0** or R-**1′** (0.8 mmol·g^{-1}) was introduced in the reaction vessel, treated with a 3% trichloroacetic acid solution in dichloromethane and washed with a solution of 2% pyridine in acetonitrile and again with pure acetonitrile. After a fast swelling in dichloromethane, the appropriate phosphoramidite (1.5 equiv) dissolved in anhydrous acetonitrile (0.1 M) and tetrazole (4 equiv, 0.45 M solution in acetonitrile) were added under argon atmosphere. The reactor was shaken at room temperature for 15 min. Afterward, the solution was removed, and the polystyrene support was washed with acetonitrile, and the resulting phosphite-triester was oxidized for 2 min to phosphate-triester with a 0.1 M iodine solution (I_2 in water/pyridine/THF 2/20/80). The excess of iodine was removed by acetonitrile and dichloromethane washes. The three steps (i.e., DMT-deprotection, phosphoramidite coupling and iodine oxidation) were iteratively repeated until the formation of the desired polymer. Finally, deprotection of the cyanoethyl phosphate protecting group was performed using a 10% piperidine solution in acetonitrile (5 min), the terminal DMT-protecting group was deprotected using the 3% trichloroacetic acid solution, and the polymers were cleaved from the resin using 30% aqueous ammonia solution/dioxane (1:1 v/v) for 12 h at 45 °C. The ammonia mixture was filtered, and the flask was left for 5 min under argon bubbling in order to eliminate the excess of gaseous ammonia. After solvent evaporation and drying under vacuum, the resulting products were characterized by NMR ^{1}H, ^{13}C, ^{31}P, SEC, and ESI-MS.

CuAAC Functionalization of the Polymers. *Functionalization of Sequence-Encoded Oligomer 1′1′1′1′1′1′1′1′ in Solution.* The sequence-encoded oligomer **1′1′1′1′1′1′1′1′** (16 μmol) was mixed with 11-azido-3,6,9-trioxaundecan-1-amine (0.5 mmol, 32 equiv) in 4 mL of water under argon. A 2 mL freshly prepared solution containing $Cu(MeCN)_4$,BF_4/ligand (THPTA) (1:1) in a mixture of water and acetonitrile (1:1, 3.2 equiv) was then introduced.[58,59] The mixture was stirred at room temperature overnight. The reaction was diluted with water (10 mL) and dialyzed.

Functionalization of Sequence-Encoded Oligomer 1′11′111′11′ on the Resin. To a polystyrene-resin loaded with the deprotected sequence-encoded oligomer **1′11′111′11′** (0.03 mmol) were added (under argon) 11-azido-3,6,9-trioxaundecan-1-amine (0.5 mmol, 16 equiv), 4 mL of water, and 1 mL of the previously described solution containing $Cu(MeCN)_4$, BF_4, (THPTA), water, and acetonitrile. The mixture was shaken at room temperature overnight. The solution was removed by filtration, and the resin was washed with acetonitrile, water, and acetone. Finally, the resulting polymer was cleaved from the resin using 30% aqueous ammonia/dioxane (1:1 v/v) for 12 h at 45 °C. The ammonia mixture was filtered, and the flask was left for 5 min under argon bubbling in order to eliminate the excess of gaseous ammonia. After evaporation of the solvents and drying under vacuum, the resulting products were characterized by NMR ^{1}H, ^{13}C, ^{31}P, and SEC.

■ RESULTS AND DISCUSSION

The synthesis of the phosphoramidite monomers is shown in Scheme 1. They were obtained in two steps from the corresponding 1,3-diol d-**0**, d-**1**, and d-**1′**. First, the 1,3-propanediol derivatives were monoprotected by dimethoxytrityl (DMT). The reaction with 1 mol equiv of dimethoxytrityl chloride (DMTrCl) in the presence of pyridine yielded the DMT-derivatives D-**0**, D-**1**, and D-**1′** in relatively high yield. DMTrCl was added gradually in order to minimize dimer formation. The resulting DMT-derivatives were reacted with

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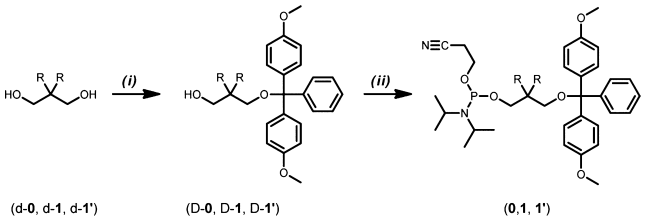
Scheme 1. Synthesis of the Phosphoramidite Monomers 0 (R = H), 1 (R = CH_3), and 1′ (R = CH_2−C≡C)[a]

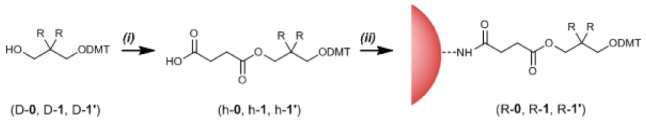


[a](i) DMTrCl, DIPEA, CH_2Cl_2; (ii) $(iPr)_2NP(Cl)OCH_2CH_2CN$, DIPEA, CH_2Cl_2.

chloro-*N*,*N*-diisopropylamino-*O*-2-cyanoethoxyphosphine in anhydrous conditions to yield the corresponding phosphoramidites. The use of molecular sieves in this step led to quantitative yields. The synthesis of monomer **1′** required first the preparation of diethyldipropargylmalonate followed by its reduction using $LiAlH_4$ to afford the 2,2-dipropargylpropan-1,3-diol derivative, which was afterward converted into **1′** as described above.

The phosphoramidite monomers **0**, **1**, and **1′** were then investigated for the synthesis of sequence-defined copolymers on a solid support. Phosphoramidite oligonucleotide chemistry is often performed on porous glass beads.[60,61] However, such solid supports do not swell in the reaction solvent, and large pore sizes are required to synthesize long sequence-defined polynucleotides. As mentioned above, the non-natural monomers **0**, **1**, and **1′** have little in common with phosphoramidite nucleosides. Thus, more conventional cross-linked polystyrene supports were used to synthesize the polymers in the present work. These hydrophobic supports are useful for moisture-sensitive phosphoramidite chemistry. The functionalization of the polystyrene solid supports is shown in Scheme 2. The

Scheme 2. Resin Functionalization (R = H, CH_3 or CH_2−C≡C)[a]



[a](i) Succinic anhydride, DMAP, pyridine; (ii) DCC, CH_2Cl_2.

DMT-monoprotected hydroxy-functional precursors D-**0**, D-**1**, and D-**1′** were first reacted with succinic anhydride. The resulting hemisuccinyl esters h-**0**, h-**1**, and h-**1′** were afterward coupled to amino-functional polystyrene beads to generate the corresponding DMT-functionalized resins R-**0**, R-**1**, and R-**1′**. The formed succinyl linker can be easily cleaved under basic conditions, e.g., using an aqueous ammonia solution. After modification, a typical resin loading of about 0.8 mmol·g^{-1} was obtained. The successful preparation of the three different types of resins R-**0**, R-**1**, and R-**1′** implies that coded sequences can be started by any of the three monomers **0**, **1**, and **1′**.

In order to test the viability of our approach, model homopolymers of the monomer **0** were first prepared on the modified resin R-**0** (samples **H1**−**H3** in Table 1). These polymers were prepared following the experimental conditions shown in Figure 1. The resin was first detritylated using a solution of trichloroacetic acid in dichloromethane, washed, and swelled. Afterward, coupling with **0** was performed using the commercial activator 1H-tetrazole. The formed phosphite bond was then oxidized to phosphate by adding a 0.1 M iodine solution. Detrytilation/coupling/oxidation cycles were repeated until a desired chain length was reached. After the multistep growth process, the 2-cyanoethyl phosphate protecting groups were removed by treatment with a piperidine/acetonitrile solution. Finally, the terminal DMT protecting group was removed, and the polymers were cleaved from the resin using an ammonia solution. It should be noted that this final procedure employing three steps is not standard. In conventional oligonucleotide chemistry, two steps are usually performed: the DMT group is first removed, and afterward both phosphate deprotection and cleavage are performed in a single step in the presence of ammonia. However, the final polymer has usually to be purified by HPLC. The three-step strategy shown in Figure 1, in which cyanoethyl groups are removed before polymer cleavage, allows an easier purification of the polymer while it is still attached to the resin.

The formed homopolymers were characterized by electrospray ionization high-resolution mass spectrometry (ESI-HR-MS), matrix-assisted laser desorption/ionization high-resolution mass spectrometry (MALDI-HR-MS), and size exclusion chromatography (SEC) in water/acetonitrile as well as ^1H and ^{13}C NMR. ^{31}P NMR was also used to follow the oxidation of the trivalent phosphorus to the pentavalent state and to monitor phosphate deprotection. Figure 2 shows SEC and ESI-MS data measured for different homopolymers. All these analyses account for the formation of monodisperse non-natural polyphosphodiesters. SEC chromatograms suggest an excellent control of chain-length on resin R-**0** (Figure 2a). The number-average molecular weights M_n and the molecular weight distribution M_w/M_n of the different homopolymers are listed in Table 1. In all cases, the measured experimental M_n values were found to be in very good agreement with the theoretical values. Moreover, monomodal peaks and narrow molecular weight distributions were observed for all samples. It should be however noted that monodisperse samples never lead to polydispersity index values of 1 in SEC. Due to axial dispersion and other peak broadening effects, apparent values above 1 are usually observed.[62] This aspect is particularly true in the case of charged polyelectrolytes that are never trivial to analyze by SEC. This assumption was confirmed by MS measurements, which showed the formation of monodisperse polymers with defined molecular weights. Accurate mass measurements were performed using ammonium acetate to promote the formation of protonated molecules. Although both ESI-MS and MALDI-MS allowed homopolymer detection, ESI-MS led to cleaner spectra (Figure 2b). Indeed, MALDI-MS measurements of these polyacids were complicated by proton exchanges with sodium impurities coming from the matrix (Figure S1). The quality of MS analysis also depended on the chain-length of the analyzed polymers. For example, **H3** was detected by MALDI-MS but not by ESI-MS. Nevertheless, the expected species were observed in all cases. The formation of pure homopolymers was further confirmed by NMR analysis (Figure S2). It should be specified that all polymers were synthesized without capping step and using only 1.5 equiv of phosphoramidites in coupling steps. These results clearly show the efficiency of phosphoramidite chemistry for preparing non-natural polymers.

Afterward, the synthesis of coded polymers using comonomers (**0**, **1**) was investigated on R-**0**. Table 1 shows the characterization of these copolymers (samples **C1**−**C4**). At

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Table 1. Characterization of Polyphosphodiester Homopolymers (H) and Copolymers (C) Synthesized by Solid-Phase Chemistry

	sequence	yield [%][a]	M_n [g·mol^{-1}][b]	M_w/M_n[b]	m/z_{th}	m/z_{exp}
H1	00000	≥98	650	1.04	629.0925 $[M + H]^+$	629.0916[c] $[M + H]^+$
						629.0923[d] $[M + H]^+$
H2	00000000	≥98	1040	1.03	522.0622 $[M + 2H]^{2+}$	522.0610[c] $[M + 2H]^{2+}$
					1043.1171 $[M + H]^+$	1043.1155[d] $[M + H]^+$
H3	0000000000000000	≥95	1850	1.01	2147.1827 $[M + H]^+$	2147.1779[d] $[M + H]^+$
C1	01010	≥98	730	1.05	685.1551 $[M + H]^+$	685.1564[c] $[M + H]^+$
						685.1544[d] $[M + H]^+$
C2	0100101001010010	≥95	2000	1.07	2315.3705 $[M + H]^+$	–[e]
C3	0100100010000100	≥98	1850	1.07	2259.3079 $[M + H]^+$	2259.3050[d] $[M + H]^+$
C4	00100010000100000010000000	≥87	2950	1.08	3363.3734 $[M + H]^+$	–[e]
H1′	1′1′1′1′1′1′1″1′	≥98	–[f]	–[f]	826.1874 $[M + 2H]^{2+}$	826.1861[c] $[M + 2H]^{2+}$
					1651.3675 $[M + H]^+$	1651.3652[d] $[M + H]^+$
C1′	1′01′001′01′	≥98	–	–	674.1248 $[M + 2H]^{2+}$	674.1248[c] $[M + 2H]^{2+}$
					1347.2428 $[M + H]^+$	1347.2392[d] $[M + H]^+$
C2″	1′11′111′11′	≥98	1799	1.02	730.1874 $[M + 2H]^{2+}$	730.1863[c] $[M + 2H]^{2+}$

[a]Percentage of the total isolated polymer. [b]Measured by SEC in H_2O/ACN. [c]Measured by ESI-HR-MS. [d]Measured by MALDI-HR-MS. [e]Not detected. [f]This polymer precipitates in the SEC solvent mixture.

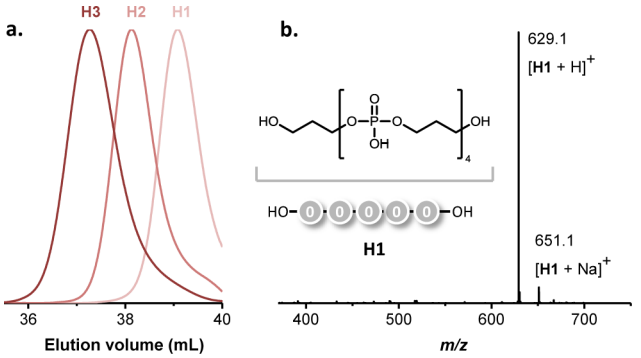

Figure 2. Characterization of homopolymers of **0**. (a) Size exclusion chromatography recorded in ACN/H_20 for homopolymers of different chain length (Table 1, entries **H1**–**H3**). (b) ESI mass spectrum recorded for the homopolymer **H1**.

first, a model alternating pentamer **C1** was prepared in order to optimize all iterative steps with comonomers **0** and **1**. SEC (Table 1 and Figure S3), ESI-HR-MS (Table 1), MALDI-HR-MS (Table 1), and NMR (Figure S4) confirmed the formation of a monodisperse sequence-defined oligomer. Based on these encouraging results, longer sequences were prepared on R-**0**. Copolymers **C2** and **C3** have a similar chain length (i.e., 16 monomer units) but contain different sequences. SEC analysis (Table 1) evidenced the formation of polymers of controlled molecular weight. As expected, sample **C2** that contains six units of **1** eluted at lower elution volume than **C3**, which contains only four (Figure S2). This controlled comonomer composition was further confirmed by the integration of methyl groups signals in ^{1}H NMR (Figure S5). The synthesis of a coded 24-mer **C4** was also achieved in this work (Table 1 and Figure S6). This particular chain length is certainly not the upper limit of our approach. Since all polymers shown in this work were prepared manually, longer sequences were not investigated. Nevertheless, based on existing literature knowledge about sequence-defined oligonucleotides,[36,37] it is almost certain that longer non-natural sequences are achievable.

The synthesis of copolymers using comonomers (**0**, **1′**) was then studied. As mentioned in the introduction, monomer **1′** is interesting because it allows synthesis of homopolymers and copolymers that can be postmodified after polymerization. Thus, it enables to bypass the use of complex functional monomers. A homopolymer of **1′** (sample **H1′** in Table 1) and a coded copolymer of **1′** and **0** (sample **C1′** in Table 1) were prepared on resin R-**1′**. The presence of terminal alkynes in the final polymers was confirmed by ^{1}H and ^{13}C NMR analysis (Figure S7). In addition, ESI-MS analysis confirmed formation of the expected macromolecules. The modification of polymers **H1′** and **C1′** by copper-catalyzed azide–alkyne cycloaddition (CuAAC) was then investigated. 11-Azido-3,6,9-trioxaundecan-1-amine was used as a model organic azide in these reactions. This model compound is interesting because it allows synthesis of modified polyelectrolytes bearing hydrophilic oligo(ethylene glycol) side chains terminated by primary amine groups. CuAAC was performed in water in the presence of $Cu(MeCN)_4$, BF_4, and THPTA.[58,59] For both **H1′** and **C1′**, a quantitative conversion of the terminal alkyne functions was evidenced by ^{1}H and ^{13}C NMR (Figure S7). In ^{13}C NMR, the alkyne peaks at 72 ppm fully vanished after CuAAC and were replaced by two new peaks at 125 and 143 ppm that correspond to the secondary and tertiary carbon of the newly formed triazole ring, respectively. Quantitative polymer modification was further confirmed by ^{1}H NMR analysis. Indeed, the peaks due to the alkyne protons at 2.44–2.46 ppm disappeared after CuAAC. In addition, new peaks due to the triazole ring proton, to the methylene protons of the oligo(ethylene glycol) spacer in alpha of the triazole and to the repeat units of oligo(ethylene glycol) appeared in the spectra of the modified polymers at 7.99, 4.54–4.58, and 3.60–3.63 ppm, respectively. Thus, NMR clearly confirmed the PEG functionalization of **H1′** and **C1′**. A change in the physical appearance of these polymers was also observed, i.e., varying from a solid white powder before functionalization to a viscous oil after oligo(ethylene glycol) modification. The CuAAC postmodification has been tested on the support R-**1′** (i.e., after deprotection but before cleavage) and on a cleaved deprotected polymer. In both cases, near-quantitative yields were obtained. However, resin-supported modification allowed a better removal of CuAAC catalyst and reactants.

The monomer alphabets (**0**, **1**) and (**0**, **1′**) used in this paper were, of course, arbitrarily chosen. The iterative synthesis and

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the postmodification concept studied in the present work can be potentially extended to other types of comonomers. For instance, during the evaluation of this article, one reviewer asked if the synthesis of copolymers based on comonomers (**1**, **1′**) was feasible. Other comonomer combinations and sequences are indeed attainable. As a proof of concept, Table 1 shows the characterization of copolymer **C2′** that contain the sequence **1′11′111′11′**. This copolymer was synthesized and cleaved from resin R-**1′**. SEC and ESI-HR-MS confirmed the formation of a monodisperse sequence-defined oligomer (Table 1). Moreover, this copolymer could be easily post-modified by CuAAC as described in the previous paragraph. NMR analysis (Figure 3) indicated quantitative side-chain modification.

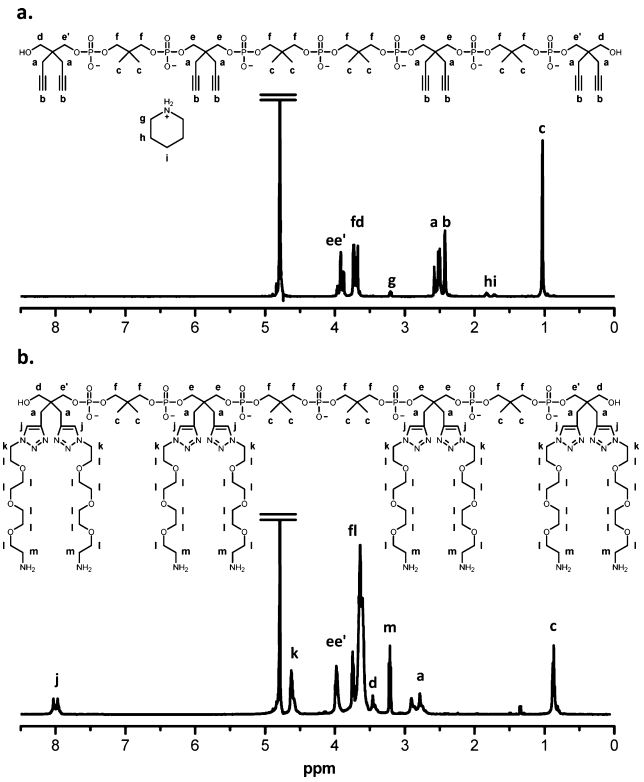

Figure 3. ^{1}H NMR spectra recorded in D_2O for copolymer **C2′** before (a) and after (b) modification by copper-catalyzed azide−alkyne cycloaddition with 11-azido-3,6,9-trioxaundecan-1-amine.

CONCLUSION

In summary, phosphoramidite chemistry was found to be a rapid and convenient method for the synthesis of non-natural sequence-encoded polyphosphates. This approach allowed synthesis of near-monodisperse homopolymers and sequence-encoded copolymers. SEC, ESI-MS, and ^{1}H, ^{13}C, and ^{31}P NMR confirmed the formation of macromolecules with precisely controlled chain lengths (i.e., from pentamer to 24-mer) and primary structures. It was also shown that these polymers can be modified after their synthesis. Using a phosphoramidite monomer bearing terminal alkyne functions, it was possible to prepare coded macromolecules that can be modified by CuAAC. As a proof of concept, the PEGylation of sequence-defined polyphosphates was reported.

These results indicate that phosphoramidite chemistry is a valid option for the preparation of synthetic polymers. Very interestingly, experimental protocols (e.g., HPLC purification, automated synthesis) that have been optimized among past years for the synthesis of oligonucleotides can also be utilized for the synthesis of non-natural monodisperse polymers. Thus, it is very realistic to imagine that long sequence-defined polymers can be synthesized using phosphoramidite chemistry, although slightly different experimental conditions than those studied herein (e.g., higher monomer excess, capping steps) might be required for reaching longer chain-lengths.

In addition, the coded primary structure of these new polyphosphates can be most probably analyzed using DNA sequencing technologies. For instance, such water-soluble non-natural macromolecules are interesting analytes for protein-based nanopore sequencing.[52] Since their side chains can be easily modified, their molecular structure can be possibly adapted for optimal pore readability. Hence, the results of this article open up interesting perspectives for the emerging field of information-containing macromolecules.

ASSOCIATED CONTENT

Supporting Information

Supplementary Figures S1−S7. This material is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*jflutz@unistra.fr

Notes

The authors declare no competing financial interest.

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