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Orthogonal Synthesis of "Easy-to-Read" Information-Containing Polymers Using Phosphoramidite and Radical Coupling Steps

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Supporting Information

ABSTRACT: A new orthogonal solid-phase iterative strategy is proposed for the synthesis of sequence-coded polymers. This approach relies on the use of two successive chemoselective steps: (*i*) phosphoramidite coupling, and (*ii*) radical−radical coupling. These repeated steps can be performed using two different types of building blocks, i.e. a phosphoramidite monomer that also contains an alkyl bromide and a hydroxy-functionalized nitroxide. The phosphoramidite and the hydroxy group are reacted in step (*i*), thus leading to a phosphite that is oxidized *in situ* into a phosphate bond. The alkyl bromide is activated by copper bromide in step (*ii*) to afford a carbon-centered radical that is spin-trapped *in situ* by the nitroxide. The iterative repetition of these steps allow synthesis of uniform polymers, as evidenced by high-resolution electrospray mass spectrometry. Moreover, binary information could be easily implemented in the polymers using different types of phosphoramidite monomers in step (*i*). Interestingly, it was found that the formed information-containing polymers are very easy to sequence by tandem mass spectrometry due to the presence of easily cleavable alkoxyamine bonds formed in step (*ii*).

For about two years, the design of non-natural information-containing macromolecules has become an important research trend in synthetic polymer chemistry.[1] Indeed, this new type of polymer allows information storage at the molecular level using a controlled sequence of monomers and could be therefore useful for applications in the fields of data storage and anticounterfeiting technologies.[1c] For example, digital information can be stored in such polymers using two monomers intentionally defined as 0− and 1− bits. The sequence-coded information is usually "written" using a solid-phase iterative strategy[2] and "read" by a sequencing methodology,[3] such as tandem mass spectrometry (MS/MS). For example, our group has recently reported the synthesis of binary-coded poly(triazole amide)s, poly(alkoxyamine amide)s, and poly(phosphodiester)s.[4] The latter class of polymers was prepared using solid-phase phosphoramidite protocols that have been developed for oligonucleotide synthesis.[5] Initially described by Caruthers,[6] this coupling chemistry relies on the reaction of a hydroxy group with a phosphoramidite, followed by the oxidation of the resulting phosphite into a phosphate linkage. This chemistry can be used for the preparation of non-natural sequence-defined polymers using non-nucleoside phosphoramidite monomers,[7] and in particular for the synthesis of information containing polymers.[4c] For instance, our group has shown that long polymer chains containing more than 100 coded monomers can be prepared using automated phosphoramidite chemistry,[4d] thus opening the possibility to write about a decabyte of ASCII-coded information on a single polymer chain.

Although very promising, this approach still suffers from two drawbacks. First, standard phosphoramidite protocols utilize monomers in which the reactive hydroxy group is protected, and therefore a deprotection step is needed after each monomer coupling. Furthermore, long sequence-coded poly(phosphodiester)s are not trivial to sequence using routine mass spectrometers because they undergo relatively complex MS/MS fragmentation patterns. These polymers are more difficult to "read" than other information-containing macromolecules. For instance, it has been observed that poly(alkoxyamine amide)s are extremely easy to sequence by MS/MS because they contain weak alkoxyamine bonds that lead to highly predictable MS/MS fragments.[4b] Based on these observations, we report herein a strategy that simplifies both the synthesis and the sequencing of sequence-coded poly(phosphodiester)s. As shown in Figure 1, this approach is an orthogonal iterative process that relies on the use of two different types of building blocks. It has been shown that the use of protecting-group-free coupling steps may greatly simplify the iterative synthesis of sequence-defined polymers.[8] For example, Zuckermann and co-workers have reported the synthesis of uniform peptoids using an elegant submonomer approach.[9] Related examples have been also described by our group and others.[10]

The iterative strategy investigated in the present work relies on the use of two successive chemoselective steps, namely phosphoramidite coupling and nitroxide radical coupling. The latter step is the reaction of a carbon-centered radical with a nitroxide to afford an alkoxyamine bond.[11] As shown in Figure 1, two different types of monomers are needed to perform these steps: a bromo-functional phosphoramidite monomer and

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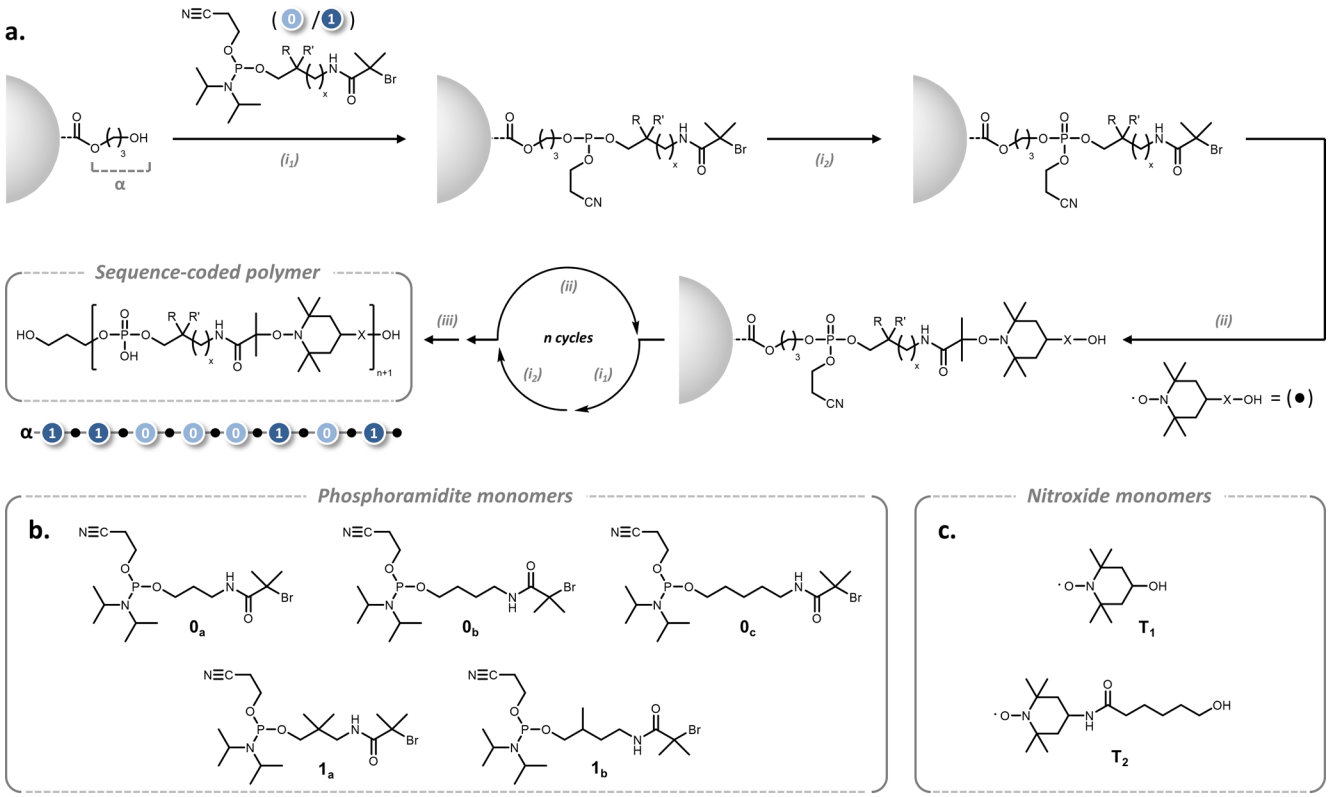

Figure 1. (a) Strategy studied for the synthesis of sequence-coded polymers. Experimental conditions: (i_1) phosphoramidite coupling: rt, AcCN, tetrazole; (i_2) oxidation: rt, I_2, 2,6-lutidine, THF/H_2O; (*ii*) radical−radical coupling: CuBr, Me_6TREN, DMSO; (*iii*) cleavage: piperidine, AcCN, rt, then $MeNH_2$, NH_4OH, H_2O, rt. (b−c) Molecular structures of the monomers used in this work.

Table 1. HR-ESI-MS Individual Characterization of the Sequence-Coded Oligomers Studied in This Work

	sequence	mass	m/z_{th}	m/z_{exp}
1	α-$0_aT_10_aT_10_aT_10_aT_10_aT_1$ [a]	1967.0	490.7458 [b]	490.7465
2	α-$0_aT_20_aT_20_aT_2$	1549.9	515.6196 [c]	515.6187
3	α-$0_aT_20_aT_20_aT_20_aT_2$	2041.2	509.2819 [d]	509.2814
4	α-$1_aT_20_aT_20_aT_21_aT_2$	2097.2	523.2975 [d]	523.2977
5	α-$(1_aT_2)_2(0_aT_2)_31_aT_20_aT_21_aT_2$	4118.4	514.0417 [e]	514.0429
6	α-$0_bT_20_bT_20_bT_20_bT_2$	2097.2	523.2975 [d]	523.2970
7	α-$0_bT_20_bT_21_bT_21_bT_2$	2125.3	530.3053 [d]	530.3060
8	α-$0_bT_21_bT_2(0_bT_2)_2(1_bT_2)_21_bT_20_bT_2$	4174.4	521.0496 [e]	521.0495
9	α-$0_cT_20_cT_20_cT_20_cT_2$	2153.3	537.3132 [d]	537.3123

[a]Although the expected mass was found, the ESI spectrum of this oligomer indicated a marked polydispersity. [b]$[M - 2H]^{2-}$. [c]$[M - 3H]^{3-}$. [d]$[M - 4H]^{4-}$. [e]$[M - 8H]^{8-}$, measured at isotopic maximum.

a hydroxy-functional nitroxide. The phosphoramidite is first reacted with a hydroxy group immobilized on a solid support (step (i_1) in Figure 1a) to afford a phosphite linkage, which is immediately oxidized into a phosphate bond (step (i_2) in Figure 1a). As previously shown by Matyjaszewski, Das and co-workers in another context, these steps are chemoselective and do not affect the alkyl bromide moiety.[12] The resulting bromo-functional resin is then activated by copper bromide (step (*ii*) in Figure 1a) to form a carbon-centered radical that is reacted with the nitroxide. The hydroxy group of the nitroxide does not need to be protected in that step because the radical coupling reaction is chemoselective.[11b] The obtained hydroxy-functional resin can then be involved in a new cycle of steps (i_1), (i_2), (*ii*). Thus, the repetition of phosphoramidite and radical coupling steps allows protecting-group-free synthesis of uniform polymers.[13]

This orthogonal iterative concept was tested with different types of monomers as shown in Figure 1b−c. Bromo-functional phosphoramidite monomers containing alkyl spacers of different length (i.e., *n*-propyl, *n*-butyl, and *n*-pentyl) were investigated in this work ($\mathbf{0}_{a\text{-}c}$). In addition, monomers with methyl side chains ($\mathbf{1}_{a\text{-}b}$) were also synthesized (Scheme S1) and tested. It was evidenced with other types of polymers that simple building blocks with proton and methyl side chains are sufficient to implement an MS/MS readable molecular binary code in a polymer (i.e., $H/CH_3 = 0/1$).[4b] Thus, the monomer pairs $\mathbf{0}_a/\mathbf{1}_a$ and $\mathbf{0}_b/\mathbf{1}_b$ were considered herein as molecular bits, although as discussed in a recent publication,[1c] other monomer-based alphabets could also be useful for the design of information-containing macromolecules.

The first attempt to synthesize a uniform polymer via this orthogonal approach was made using $\mathbf{0}_a$ and 2,2,6,6-tetramethyl-4-hydroxypiperidinyloxy (4-hydroxy-TEMPO, $\mathbf{T}_1$,

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Figure 1c) as building blocks (Table 1, entry 1). Although the targeted polymer was detected by electrospray ionization mass spectrometry (ESI-MS), a marked polydispersity was also observed (Figure S1). These results are probably due to the secondary alcohol of $\mathbf{T}_1$ that leads to incomplete yields in step (i_1). It should be noted that, in an iterative solid-phase strategy, uniform sequence-defined polymers can only be obtained if the yields of all steps are near-quantitative.[2] In order to bypass this problem, a nitroxide containing a primary alcohol group was synthesized ($\mathbf{T}_2$ in Figure 1c). The synthesis of uniform homopolymers was tested using building-block combinations $\mathbf{0}_a/\mathbf{T}_2$, $\mathbf{0}_b/\mathbf{T}_2$, and $\mathbf{0}_c/\mathbf{T}_2$ (Table 1, entries 2, 3, 6, and 9). In all cases, the targeted monodisperse polymers were detected by ESI-MS (Table 1, Figure 2 and Figures S2–S4). For example,

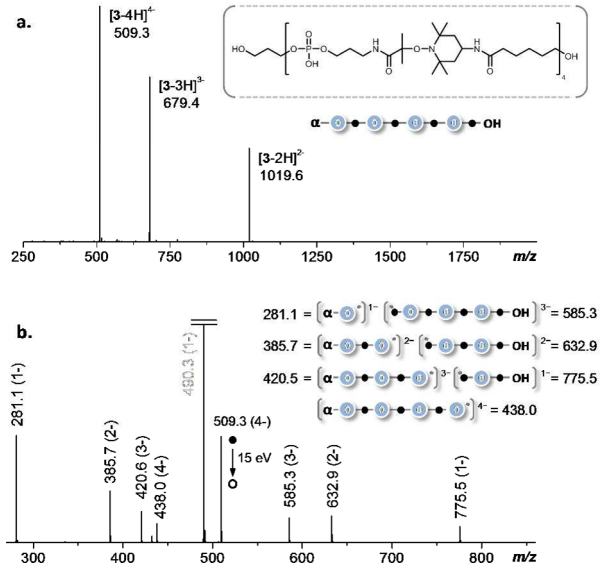

Figure 2. (a) Negative mode ESI-MS spectrum of a homopolymer constructed with the building-blocks $\mathbf{0}_a/\mathbf{T}_2$ (Table 1, entry 3). (b) MS/MS sequencing of the same polymer obtained after collisional activation of the $[M - 4H]^{4-}$ precursor at m/z 509.3. The peak noted in light gray corresponds to a secondary internal fragment. See Scheme S2 for symbols used to describe product ions.

Figure 2a shows a negative mode MS spectrum obtained for a homopolymer constructed with the building-blocks $\mathbf{0}_a/\mathbf{T}_2$ (Table 1, entry 3). In this spectrum, the targeted uniform poly(alkoxyamine phosphodiester) can be clearly observed in different deprotonation states ranging from 2− to 4−. These results confirm that the primary alcohol of $\mathbf{T}_2$ leads to near-quantitative yields in step (*i*). In addition, they indicate that the use of tertiary carbon-centered radicals in step (*ii*) does not lead to significant H-transfer side reactions at room temperature. This observation is in agreement with existing literature on the topic.[4b,11a,14] It is also interesting to note that the copper/ligand catalyst used in step (*ii*) does not lead to strand breaks as observed for DNA.[15]

The homopolymers were also examined by MS/MS (Figure 2 and Figures S2–S4) and NMR (Figure S5). Figure 2b shows the MS/MS spectrum of homopolymer **3**. Two series of complementary fragments were detected, arising from the homolytic cleavage of each C–ON alkoxyamine bond. While the same dissociation pattern was observed for any $[M - zH]^{z-}$ precursor ion, collision-induced dissociation data collected from fully ionized molecules, in which all phosphate groups are deprotonated (e.g., $[M - 4H]^{4-}$ in Figure 2), are probably the most practical species for MS/MS sequencing. Indeed, such polyanions exhibiting one negative charge per monomer lead to two MS/MS fragmentation series, in which each fragment exhibits a single charge signature, with z increasing with the number of monomers (Figure 2b). Thus, the resulting peaks can be easily interpreted and correlated to the primary structure of the polymer. In contrast, partially ionized oligomers, which contain randomly deprotonated phosphate groups, lead to fragments that have different ionization states and therefore to more complex MS/MS spectra (Figure S6). Similar results were obtained with all other homopolymers (Figures S2–S4).

Based on these promising results, the synthesis of binary-coded polymers was investigated using monomer combinations $\mathbf{0}_a/\mathbf{1}_a/\mathbf{T}_2$ and $\mathbf{0}_b/\mathbf{1}_b/\mathbf{T}_2$ (Table 1, entries 4, 5, 7, and 8). In all cases, digital sequences could be efficiently synthesized, as evidenced by uniform ESI-MS spectra (Figures S7–S9) and NMR (Figure S10). Figure 3a shows for example the ESI-MS spectrum of a copolymer containing the sequence α-$1_aT_21_aT_20_aT_20_aT_20_aT_21_aT_20_aT_21_aT_2$ (Table 1, entry 5), which could be detected as deprotonated species with z ranging from 3− to 8−. Interestingly, for the two studied binary alphabets, similar reaction conditions were used for the two coded building blocks **0**/**1** without leading to marked polydispersity. This is an interesting advantage over other types of information-containing macromolecules,[4b] in which different bits may lead to different yields. Furthermore, all the digitally encoded copolymers could be efficiently sequenced by MS/MS (Figures S7–S9). For instance, Figure 3c shows the MS/MS fragmentation pattern obtained for copolymer **5**. This spectrum was obtained by collision-induced dissociation of the [M –

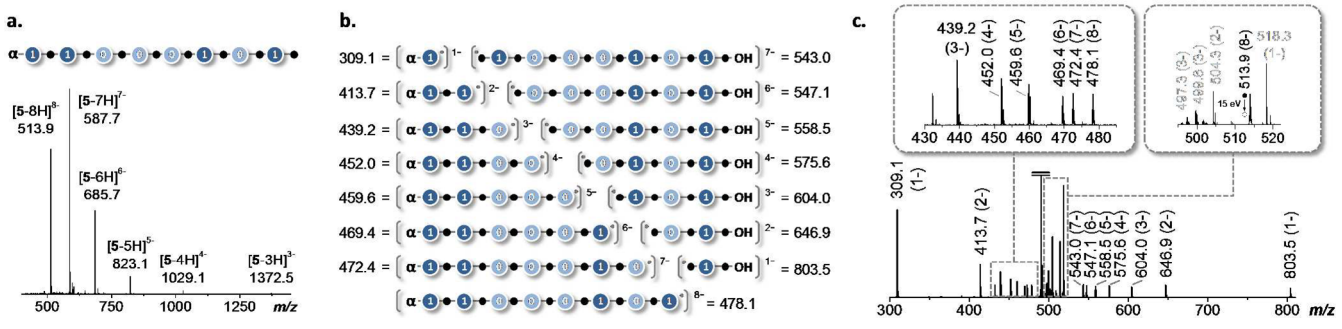

Figure 3. (a) Negative mode ESI-MS characterization of a coded copolymer constructed with the building-blocks $\mathbf{0}_a/\mathbf{1}_a/\mathbf{T}_2$ (Table 1, entry 5). (b) Chain fragmentations observed for this copolymer in MS/MS analysis (c) MS/MS sequencing of the same copolymer obtained after collisional activation of the m/z 513.9 precursor $[M - 8H]^{8-}$. The peaks listed in light gray correspond to secondary fragments.

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$8H]^{8-}$ precursor ion. All theoretical fragments (Figure 3b) were experimentally observed (Figure 3c) and correlated to the binary sequence of the polymer. Indeed, upon activation of the precursor ion, homolytic cleavage of all alkoxyamine bonds occurred in a competitive manner. Each fragment hence contained one piece of different length of the original sequence, linked to one or the other end group. As a result, based on the mass of the different building blocks, their connectivity in the original chain can be unambiguously determined from m/z values measured for all fragments (Figure S11).

In summary, we report here a facile orthogonal method for the synthesis of digitally encoded polymers. This method allows preparation of uniform polymers using fast and easy coupling steps. Moreover, the formed poly(alkoxyamine phosphodiester)s can be sequenced by MS/MS in a much easier fashion than other poly(phosphodiester)s such as DNA. These results further highlight the advances of synthetic polymers for storing information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b06222.

Full experimental part: detailed experimental procedures; Supplementary Figures S1−S11 and Schemes S1−S2 (PDF)

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Notes

The authors declare no competing financial interest.

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