Special Issue: Peptoids – Part 1

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Phosphoramitoids—A submonomer approach to sequence defined N-substituted phosphoramidate polymers

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**Abstract**

There is a growing interest in new methods to generate bio-inspired, chemically diverse, sequence-defined synthetic polymers. Solid-phase submonomer approaches offer facile access to these types of materials, since they take advantage of readily available synthons. Submonomer approaches to date have been applied to peptidomimetics with oligo-amide backbones. Here we extend the approach to a phosphorous-containing backbone, where N-substituted phosphoramidate oligomers are constructed from a set of amine submonomers, diphenyl H-phosphonate, and cyclohexane diol. The key chemical steps in chain elongation are a chain extension reaction based on H-phosphonate (P III) chemistry, and a side chain attachment step based on the Atherton-Todd reaction. Cheap, stable chemical reagents are used without heating, all reaction times are 30 minutes or less and open to air, and no main-chain protecting groups are required. Phosphoramitoid tetramers and pentamers displaying a variety of side chain functionalities were synthesized by a three-step solid-phase submonomer method, typically with >85% crude purities.

**KEYWORDS**

Atherton-Todd reaction, H-phosphonate chemistry, N-substituted phosphoramidates, sequence defined polymer, submonomer approach

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**1 | INTRODUCTION**

The synthesis of discrete monodisperse polymers of exact sequence remains one of the most challenging frontiers of polymer chemistry and biomimetic nanoscience research. A variety of approaches have been developed that link together chemically diverse amino acid-like building blocks in high yields.\(^1\) However, the advancement of the sequence-defined polymer field has been slowed by the need to simultaneously solve several key synthetic challenges.\(^2\) Most importantly, the coupling yield for the iterative addition of each monomer needs to be nearly quantitative in order to achieve significant molecular weights. Furthermore, the chemical building blocks must be chemically diverse and be readily available at low cost. In order to be widely adopted and automated, the needed reagents should be nonhazardous, not air- or water-sensitive, and the chemical reactions should occur at or near room temperature on a time scale of minutes. Additionally, there must be ways to unambiguously purify and characterize the polymers to determine their structure, sequence, macromolecular shape, molecular weight, and other properties.

The solid-phase submonomer method was first established in 1992 as an efficient route to sequence-defined peptoid oligomers, or N-(substituted)glycines.\(^3\) The method solves most of the synthetic challenges mentioned above, enabling the rapid synthesis of relatively long peptoid chains (up to ~50mer) using any of >200 readily available primary amines building blocks at each position.\(^4\) A solid-phase submonomer route to azapeptides was also developed.\(^5\) Peptoids of low molecular weight have been found to act as specific ligands for receptor binding and offer great promise as small-molecule drug mimics.\(^6\) Higher molecular weight peptoids have been used to create protein-mimetic polymers with defined sequences that can fold into precise nanostructures, or mimic simple homopolymers and diblock copolymers.\(^7\) In all cases, the sequence control allows the molecular structure (and subsequent function) to be precisely tuned. Thus, the ability to program sequence information into synthetic polymers holds great potential, yet there are few polymer classes that match the synthetic efficiency of peptoids.

We sought to extend the submonomer approach to oligomers with DNA-like backbones.
The solid-phase synthesis of DNA oligomers has been well established over many years. The most well-developed approaches, which use DMT-phosphoramidite or H-phosphonate monomer units, have inspired a number of non-natural DNA-mimetic oligomers with phosphodiester or other phosphorus-containing backbones, where suitable monomers are coupled to the growing oligomer. In one case, a series of N-substituted DMT hydroxymethylpyrrolidinol phosphoramidites were used for introducing new functionalities into chemically diverse phosphodiester-linked oligomer libraries. In another case special diol compounds contained side-chains imitating amino acid side-groups were synthesized, converted into phosphoramidite monomers and used to prepare libraries of oligomers with a phosphodiester backbone for scanning against biological targets. Oligomers carrying multiple chromophore units linked by phosphodiester backbones have been reported for use as optical labels in biological systems and for the synthesis of oligomers carrying DNA-intercalating compounds.

Sequence-defined non-natural polyphosphates were also assembled using iterative phosphoramidite protocols on a solid support using three monomers in high yields. Short phosphoramidate mixtures have been prepared by traditional monomer-based approaches in which diols were converted into their corresponding DMT-H-phosphonates. These were used to generate up to H-phosphonate trimers and the H-phosphonate diesters were converted into N-substituted phosphoramidates by reaction with a wide range of primary and secondary amines.

DNA synthesis has evolved rapidly since the first dinucleotide synthesis utilizing H-phosphonate chemistry by Michelson and Todd in 1955. The solid-phase synthesis of DNA oligomers has been well established over many years and the most well-developed approaches use DMT-phosphoramidite or H-phosphonate monomer units. Using H-phosphonate monomers DNA oligomers containing N-substituted phosphoramidate linkages have been prepared. The DNA sequence was assembled on a solid support using H-phosphonate monomer units without oxidation between monomer additions, since H-phosphonate dimers are stable to mild acidic conditions used to remove the DMTr group. All the H-phosphonate dimers were oxidized at the conclusion of the synthesis by reaction with an amine in carbontetrachloride. Phosphoramidites were utilized to synthesize dimer blocks with stereo-defined phosphoramidate linkages. These were conversion to phosphoramidite and incorporated into oligomers with alternating negative and positive back-bone charges. The oligomers are stable to mild base and acid. Hydrolysis of N-substituted phosphoramidate oligomers with 88% aqueous formic acid (95 °C for 30 minutes) afforded the corresponding phosphodiester linked oligomers. The chirality of N-substituted phosphoramidate linkages were shown to affect the hybridization properties of the oligomers.

Although these prior approaches resulted in useful polymers, they are limited to generating oligomers of low diversity, due to the need to prepare a set of fully protected monomers. This is very laborious and costly, and has limited these approaches to simple oligomers.

When considering a new submonomer synthesis of a phosphorus-containing sequence-defined polymer, we looked for ways to tap into the vast pool of primary amine synthons, inspired by the efficiency of peptoid submonomer synthesis. We settled upon a backbone based on repeating N-substituted phosphoramidate units, since H-phosphonate diesters have been shown to react readily with primary amines in the Atherton-Todd phosphoramidation reaction. This reaction has been used to make (nonoligomeric) N-substituted phosphoramidates in high yields in solution and on a solid support. To generate a H-phosphonate diester on a solid support we envisioned first introducing a H-phosphonate monoester by transesterification on a hydroxyl-bearing solid support using cheap, commercially available diphenyl H-phosphonate. Because the phenyl ester is not very reactive toward alcohols we aimed to hydrolyze the resin-bound phenyl H-phosphonate intermediate to the free H-phosphonate monoester. Activation of this resin-bound H-phosphonate monoester with a suitable coupling reagent, such as PyBOP, in the presence of a diol would generate a H-phosphonate diester and regenerate a terminal hydroxy group. The phosphoramidation step would introduce the side chain, resulting in two chiral isomers of phosphorus which do not exchange.

Other synthetic strategies based on resin-bound H-phosphonate diesters have been employed with limited success. These intermediates can be converted into alpha-amino phosphonates and alpha-hydroxy phosphonates in variable yields. The methods described are not readily adapted to oligomers and are limited by the use of exotic and reactive reagents making it difficult to control the synthesis outcome.

Here, resin bound H-phosphonate diesters are extended to the synthesis of N-substituted phosphoramidate oligomers. Using a novel solid-phase submonomer approach based on H-phosphonate chemistry, amines are incorporated into the oligomer backbone using easy to handle, commercially available reagents to create chemically diverse tetramers and pentamers in excellent yield.

EXPERIMENTAL PROCEDURES

All chemicals were purchased from Aldrich, except for PyBOP which was purchased from Chem-Impex. Note: carbon tetrachloride (CCl4) is toxic to the liver. Handle with care in a hood. Pentachloroethane, hexachloroethane and bromotrichloromethane may substitute for carbon tetrachloride, but are also toxic; handle with care in a hood.

Preparation of HO-Linker-Rink-Polystyrene supports.

2.1 HO-linker1-rink-PS

The synthesis was conducted with Rink amide polystyrene resin (1 mmol; 5 g; substitution level 0.2 mmol/g). The bromoacetylation reaction was performed by the addition of bromoacetic acid (1M; 1.95 g in 12 mL DMF), followed by displacement with 3-aminopropanol (1M; 2.8 mL in 12 mL DMF), and on a solid support. To generate a H-phosphonate diester on a solid support we envisioned first introducing a H-phosphonate monoester by transesterification on a hydroxyl-bearing solid support using cheap, commercially available diphenyl H-phosphonate. Because the phenyl ester is not very reactive toward alcohols we aimed to hydrolyze the resin-bound phenyl H-phosphonate intermediate to the free H-phosphonate monoester. Activation of this resin-bound H-phosphonate monoester with a suitable coupling reagent, such as PyBOP, in the presence of a diol would generate a H-phosphonate diester and regenerate a terminal hydroxy group. The phosphoramidation step would introduce the side chain, resulting in two chiral isomers of phosphorus which do not exchange.

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(3 mL of a 1M solution in water) in 17 mL DMF with agitation for 3 hours at 25 °C to give HO-Linker1-Rink-PS.

### 2.2 | HO-linker2-rink-PS

The synthesis was conducted with Rink amide polystyrene resin (0.5 mmol; 2.5 g; substitution level 0.2 mmol/g). After the second bromoacetylation step the displacement reaction was performed with 2-mercaptoethanol (0.5 mL) in 14 mL DMF containing 1 mL DIPEA to give HO-linker2-rink-PS.

Experiments were performed on HO-linker1-rink-amide-polystyrene or HO-linker2-rink-amide-polystyrene supports as noted. Substitution 0.2 mmole/g. The Rink linker is acid labile releasing the phosphoramitoid as the terminal carboxamide by exposure to 5% TFA/DCM for 10 minutes. After removal of solvents under a gentle stream of air the released products were solubilized with water/acetonitrile (ACN; 1:1 v/v) and analyzed by HPLC or UPLC.

Phosphoramitoid synthesis cycle (40 μmole scale). All steps performed at room temperature.

**Step 1: Phosphonylation.**
1. 1M (PhO)_2PHO in 2 mL 20% NMI/DMF for 30 minutes. Followed by
2. hydrolysis in water/TEA/DMF (2 mL; 1:1:8 v/v) for 30 minutes.

**Step 2: Diol condensation.**
1. 1.0M trans-1,4-cyclohexanediol and 0.5M PyBOP in 2 mL 20% NMI/DMF for 30 minutes

**Step 3: Phosphoramidation.**
1. 1M amine in 2 mL DMF/CCl₄ (9:1 v/v) for 30 minutes.

Steps 1-3 were repeated until the desired oligomer was assembled. After chain elongation, all chains were cleaved from the support with 5% TFA/DCM for 10 minutes.

### 2.3 | Analysis

Synthesis products were analyzed by reversed-phase HPLC using a Varian ProStar 210 Analytical HPLC system with diode array detector and a Vydac C18 5 μm 150 × 4.6 mm with a Vydac C18 Guard Cartridge: 5%-95% gradient at 1.0 mL/minute over 20 minutes at 60 °C; solvent A: water +0.1% (v/v) TFA, solvent B: acetonitrile +0.095% (v/v) TFA.

HPLC chromatograms are provided in supporting information.

UPLC/MS were run on a Waters Acquity UPLC system with Acquity Diode.

Array UV detector and Waters SQD2 mass spectrometer and a Waters Acquity UPLC BEH300 C4, 300 Å pore size, 1.7 μm particle size, 2.1 mm × 100 mm with ACQUITY UPLC BEH300 C4 VanGuard pre-column, 1.7 μm, 2.1 mm × 5 mm; 20%-80% gradient at 0.8 mL/minute over 6.8 minutes at 60 °C; solvent A: water +0.1% (v/v) TFA, solvent B: acetonitrile +0.095% (v/v) TFA. UPLC chromatograms are available in the supporting information. The mass spectra were collected in ESI+ mode.

HPLC/MS were run on an Agilent 1100 series HPLC and Bruker Daltonics microTofQ mass spectrometer. The system is configured with a Vydac C18 column model 218TP5415 5 μm particle size, 300 Å pore size, 4.6 mm × 150 mm. The mass spectra were collected in ESI+ mode.

### 3 | RESULTS AND DISCUSSION

N-substituted phosphoramidate oligomers are made using a submonomer synthesis cycle as shown in Scheme 1, consisting of three steps starting from a resin-bound hydroxyl group: (a) phosphorylation, (b) diol condensation, and (3) phosphoramidation.

SCHEME 1  Solid-phase submonomer synthesis cycle to generate N-substituted phosphoramidates (or phosphoramiitoids). The solid-phase synthesis resin is denoted as a gray ball.
3.1 | Optimization of individual steps

We first optimized the individual steps of the cycle separately. To facilitate rapid analysis of the reaction products, we performed these exploratory conditions on two resin-bound linkers that incorporated a UV tag and a terminal primary hydroxyl group, attached to a standard polystyrene solid support via a Rink amide linker (see supporting information). The Rink moiety can be cleaved under mild acidic conditions (5% trifluoroacetic acid [TFA]/dichloromethane [DCM]) allowing us to cleave off and analyze the products and intermediates by HPLC and mass spectrometry.

3.1.1 | Phosphonylation step

Procedures developed for solution phase synthesis of nucleoside H-phosphonate esters were adapted to solid supported synthesis.[21] Transesterification of hydroxy-terminated linker 1 with commercially available phosphonylating reagent (diphenyl H-phosphonate) in pyridine generated the phenyl H-phosphonate diester in high yield. This was readily hydrolyzed with mild aqueous base (water in triethylamine [TEA]/pyridine) to generate the free H-phosphonate mono-ester 2 in 80% yield. See Figure 1.

3.1.2 | Diol condensation step

To optimize this step, we first used a simple primary alcohol, 2-phenylethanol, as a test compound. Wada et al. tested a large number of peptide coupling agents for their ability to activate nucleoside H-phosphonates in their reaction with an alcohol.[22] They found that benzo tri azol -1-yl-oxy tri pyrrolidinophosphonium hexafluorophospho phate (PyBOP) was the most potent activator that produced the H-phosphonate diester in high yield. They further demonstrated that PyBOP does not react with hydroxy groups or the exocyclic amino groups of nucleosides. Activation of phosphate esters on solid support has usually been inefficient.[23] But when we activated a solid supported H-phosphonate with PyBOP in the presence of phenethyl alcohol in pyridine it rapidly produced the corresponding H-phosphonate diester 3 in 90% yield as shown in Figure 1.

To extend the scheme to the synthesis of phosphoramitoid polymers a diol is needed in this step to regenerate the starting terminal hydroxyl group. Ethylene glycol was not suitable because coupling under basic conditions resulted in the cyclative cleavage of the phosphonate unit to leave the starting hydroxyl moiety.[24] To overcome this limitation we turned to trans-1,4-cyclohexanediol, a sterically constrained diol. HO-Linker1-Rink-Amide-PS 5 was first phosphonylated, and when solid supported H-phosphonate 6 was activated with PyBOP in the presence of trans-1,4-cyclohexanediol in pyridine it rapidly produced the corresponding H-phosphonate diester 7 in 81% yield as shown in Figure 2.

3.1.3 | Phosphoramidation step

In solution, H-phosphonate diesters are readily oxidized with amines in the presence of CCl₄ (Atherton-Todd phosphoramidation)
to give the \( N \)-substituted phosphoramidate in high yield.\[^{20}\] We showed that a solid supported H-phosphonate diester underwent phosphoramidation with phenethylamine in \( \text{CCl}_4 \)/pyridine to give the \( N \)-phenethyl phosphoramidate 4 in 65% yield as shown in Figure 1.

### 3.2 Solid-phase synthesis of \( N \)-substituted phosphoramidate oligomers

Using these optimized conditions, we next performed the steps in sequence to generate sequence-defined phosphoramitoid tetramers and pentamers from a variety of amine submonomers. We ran all
reactions without special precautions at room temperature, open to air, and by sequentially adding reagents without preactivation or other limitations. The H-phosphonate monoester is a stable moiety that was readily introduced using diphenyl phosphonate, a stable liquid, in pyridine.\(^{[21]}\) It was readily activated with an inexpensive coupling reagent PyBOP, and in the presence of unprotected trans-1,4-cyclohexanediol the corresponding H-phosphonate diester was generated in high yield.\(^{[23]}\) Phosphoramidation to the phosphoramidate under Atherton-Todd conditions proceeded in high yield.\(^{[20]}\) The method is in general robust and not sensitive to moisture. Each cycle consists of three high yield steps performed at room temperature using inexpensive commercially available reagents.

### 3.3 | Monomer addition cycle

**Step 1: Phosphonylation.**

This step consists of two parts: (a) phosphorylation of a terminal hydroxyl group with diphenyl H-phosphonate, followed by (b) phenyl ester hydrolysis

- a. 1M \((\text{PhO})_2\text{PO}\) in 20% N-methylimidazole (NMI)/dimethylformamide (DMF) for 30 minutes, followed by
- b. Hydrolysis in water/TEA/DMF (1:1:8 v/v) for 30 minutes.

**Step 2: Diol condensation.**

1.0M trans-1,4-cyclohexanediol and 0.5M PyBOP in 20% NMI/DMF for 30 minutes

**Step 3: Phosphoramidation to incorporate amine into the chain.**

1M amine in DMF/CCl\(_4\) (9:1) for 30 minutes

This three-step cycle was used to synthesize phosphoramitoids \(8-13\) containing 3, 4, or 5 different amines per chain (Figure 3). All compounds were cleaved from the resin with 5% TFA/DCM for 10 minutes prior to analysis. All compounds were analyzed by HPLC and mass spectrometry. Several of the phosphoramitoids were obtained in greater than 55% crude purity (Table 1). The UPLC chromatograms of \(8b\) and \(12\) as crude products are shown in Figure 4. All other analytical data are provided as supporting information (Supporting Information Figures S2-S13).

Phosphoramitoid pentamer \(8b\) was synthesized with a crude purity of 85% that would correspond to an average coupling yield of 96.5% per cycle. The phosphoramidation generates chiral phosphoramidate linkages. Thus, phosphoramitoid \(8b\) should contain 32 different stereo isomers that display slightly different properties as seen by the broad elution chromatograms in HPLC and UPLC. The chiral phosphoramidate linkages of phosphoramitoids may affect the properties, such as protein binding and oligomer organization. High synthesis yields are achievable with the optimized synthesis cycle and compound \(12\), a 4-mer, was synthesized with a crude purity of 78% that would correspond to an average coupling yield of 94% per cycle. UPLC analyses of phosphoramitoid pentamer \(8b\) (a) and tetramer \(12\) (b) are shown in Figure 4.

Phosphoramitoids are in general stable to the acidic conditions needed to cleave the Rink linker (5% TFA in DCM). However, when phosphoramitoid \(10\) it was exposed to 95% aq. TFA to deprotect the t-butyl ester the deprotected phosphoramitoid \(11\) was only detected in low yield and significant degradation was observed. To make carboxyl containing phosphoramitoids, alternative carboxyl protection schemes are required.

Initially, all reactions were run in pyridine under strictly anhydrous conditions. Phosphoramitoid \(8a\) was synthesized in 55% crude purity when pyridine was used as solvent. Subsequent experiments demonstrated that using DMF containing 20% NMI as solvent resulted in improved yields in each of the three steps. When phosphoramitoid

### Table 1: Phosphoramitoid trimers, tetramers, and pentamers synthesized in this study

<table>
<thead>
<tr>
<th>Compound</th>
<th>General synthesis solvent</th>
<th>Oligomer length</th>
<th>Crude purity (%)</th>
<th>MW (Calc)</th>
<th>MW (Found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>Pyridine</td>
<td>5</td>
<td>55(^a)</td>
<td>1676.8</td>
<td>1677.0(^c)</td>
</tr>
<tr>
<td>8b</td>
<td>DMF/NMI</td>
<td>5</td>
<td>85(^b)</td>
<td>1715.8</td>
<td>1716.0(^d)</td>
</tr>
<tr>
<td>9</td>
<td>DMF/NMI</td>
<td>4</td>
<td>37(^b)</td>
<td>1409.5</td>
<td>1410.0(^d)</td>
</tr>
<tr>
<td>10</td>
<td>DMF/NMI</td>
<td>4</td>
<td>55(^b)</td>
<td>1456.6</td>
<td>1457.0(^d)</td>
</tr>
<tr>
<td>11</td>
<td>DMF/NMI</td>
<td>4</td>
<td>15(^b)</td>
<td>1400.5</td>
<td>1400.0(^d)</td>
</tr>
<tr>
<td>12</td>
<td>DMF/NMI</td>
<td>4</td>
<td>78(^b)</td>
<td>1495.6</td>
<td>1496.0(^d)</td>
</tr>
<tr>
<td>13</td>
<td>DMF/NMI</td>
<td>3</td>
<td>63(^b)</td>
<td>1182.3</td>
<td>1183.0(^d)</td>
</tr>
</tbody>
</table>

\(^a\) As determined by HPLC.
\(^b\) As determined by UPLC.
\(^c\) As determined by ESI-MS (Agilent/Bruker).
\(^d\) As determined by ESI-MS (Waters).

**FIGURE 4** HPLC analysis of (A) phosphoramitoid pentamer \(8b\) and (B) tetramer \(12\). Starred peak indicates desired product as determined by electrospray mass spectrometry.
8 was resynthesized using DMF/NMI it was synthesized in 85% crude purity (phosphoramitoid 8b). Attempts to use N-methyl-2-pyrrolidone (NMP)/NMI as solvent resulted in reduced yields in each of the three steps.

Amines are commercially available in large numbers making it possible to introduce great diversity into phosphoramitoids. In the examples reported here, 10 different amines of diverse chemical structure were successfully incorporated. In principle, only a small excess of amine; however, in practice we used a 1M solution of amine. The intermediate phosphoryl chloride reacts faster with amines than water, so the phosphoramidation reaction itself is not sensitive to small amounts of water in the reaction mixture. In this study we incorporated seven different amines and observed consistently high yields in the phosphorylation steps.

Peptoid synthesis is limited to primary amines. The phosphoramitoid synthesis method can be used to incorporate a secondary amine into a phosphoramitoid. Using the standard phosphoramitoid synthesis cycle and the addition of triethylamine during step 3: Phosphoramidation we synthesized a 3-mer phosphoramitoid, compound 13, incorporating morpholine, a secondary amine, with a crude purity of 63%. The structure of compound 13 is shown in Figure 3. Other analytical data is provided in Table 1 and as supporting information (Figure S13).

The synthesis cycle is robust using stable reagents. The synthesis system is self-drying. In the phosphorylation step any moisture is removed by hydrolysis of excess diphenyl H-phosphonate generating inert phenyl H-phosphonate. In the diol condensation step moisture is removed by hydrolysis of an excess of the PyBOP activated H-phosphonate, regenerating the solid supported H-phosphonate monoster, which in turn can be reactivated by excess PyBOP. All the reagents are robust and stable in solution, opening up the potential for automation.

We envision further developments to the submonomer method described here. Mono-protected diols allow for changes in the distance and nature of the connecting diol. Ethylene glycol and other similar diols can be incorporated into the backbone of phosphoramitoids if they are mono-protected. For example, Fmoc-O-ethyl-OH could be used but would require a fourth Fmoc deprotection step (20% TEA in pyridine or DMF).

The present synthesis of phosphoramitoids generates two phosphorus stereoisomers during each cycle. It may be possible in the future to control the stereo chemistry of the phosphoramidate linkages by using a mono-protected trans-1,4-cyclohexanediol that incorporates a diastereopure O-oxazaphospholidine H-phosphonate moiety, one for each of the desired stereoisomers. This strategy of DMT diastereopure O-oxazaphospholidine H-phosphonate nucleoside monomers has been used successfully in the synthesis of stereo-defined phosphorothioate DNA oligomers.\textsuperscript{[29]}

The H-phosphonate diester is a versatile synthetic intermediate and a number of interesting reactions are known to proceed in high yields.\textsuperscript{[30]} Besides amines, other monomers can be used to generate oligomers with interesting phosphorus-derived backbones. Imines react with H-phosphonate diesters to generate aminomethylphosphonates;\textsuperscript{[25]} aldehydes react to make hydroxymethylphosphonates;\textsuperscript{[24]} and N-methoxyoxypyridinium tosylate reacts to make 2-pyridylphosphonates.\textsuperscript{[31]}

Oxidation with 4-[(2-cyanoethyl)sulfanyl]morpholine-3,5-dione generates phosphorothioates;\textsuperscript{[32]} with N-[(phenyl)sulfanyl]phthalimide phosphorothiotriesters\textsuperscript{[33]} are generated; iodine/water oxidation generates phosphates;\textsuperscript{[34]} with alcohols phosphorothiester are produced\textsuperscript{[35]}

4 | CONCLUSION

We have implemented a novel submonomer approach for the synthesis of pentamer phosphoramitoids in 85% crude purity. This new sequence-defined oligomer contains a backbone of N-substituted phosphoramidates, displaying unique side-chain groups. The synthesis scheme is robust, uses readily available building blocks, and has short reaction times at room temperature that are insensitive to moisture. The monomer addition cycle proceeds without the use of main chain protecting groups. The scope of the reaction has been extended beyond primary amines to secondary amine monomers. A set of nine diverse primary amines and one secondary amine were incorporated into phosphoramitoids in good to excellent yields.

The solid-phase submonomer method is an efficient route to new sequence-defined N-substituted phosphoramide oligomers with coupling yields that are near-quantitative. The amine building blocks are chemically diverse and are readily available at low cost. The needed reagents are nonhazardous, not air- or water-sensitive, and the chemical reactions occur at or near room temperature on a time scale of minutes. Phosphoramidite oligomers are readily purified to determine their structure, sequence, molecular weight, and other properties. Short phosphoramitoids could prove useful in drug discovery as peptidomimetics with improved pharmacological properties. Longer phosphoramitoids could be useful in the design of nanostructures for molecular recognition and catalysis.

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REFERENCES

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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