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Solid-Phase and Solution-Phase Syntheses of Oligomeric Guanidines Bearing Peptide Side Chains

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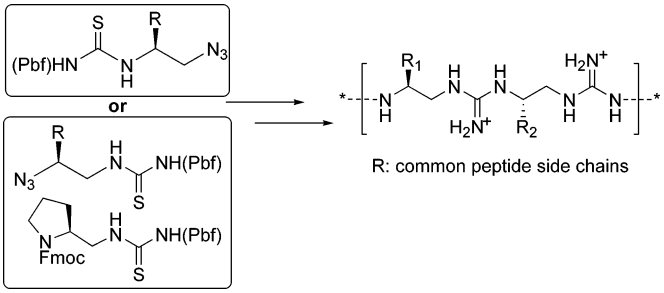

Synthetic strategies for preparing *N*,*N*′-bridged oligomeric guanidines bearing peptide side chains both on solid support and in solution are presented. Monomers are prepared from common α-amino acids and therefore contain conventionally protected peptide side chains. The side chains include alkyl, aromatic, hydroxyl, amino, carboxylic acid, and amide functional groups. Oligomer elongation utilizes acid-sensitive sulfonyl activated thiourea through the formation of carbodiimide intermediate. With proper preparation of monomers, synthesis of oligomer can be performed in two directions (equivalent to N to C terminal or C to N terminal in a peptide sequence) with excellent efficiency.

Introduction

Peptides are an important class of compounds for biomedical studies, such as inhibitors or agonists of protein function. Synthetic peptide libraries are also the first examples used to demonstrate the power of combinatorial chemistry.[1−3] However, for therapeutic development, peptides are often unfavorable. One main issue is the generally poor bioavailability of peptides due to facile degradation by enzymes and/or inadequate cell membrane permeability. To overcome the drawbacks of peptides, the development of peptidomimetics based on oligomers with alternative unnatural backbones while preserving the common side chains has attracted much attention in recent years (Figure 1). Many different types of unnatural bio-oligomers have been studied in connection with peptidomimetics and are the subjects of recent reviews.[4−9] Examples of representative unnatural bio-oligomers include β-peptides,[10] peptoids,[11] ureapeptoids,[12] oligocarbamates,[13] oligoureas,[14−17] azatides,[18] and aza-β3-

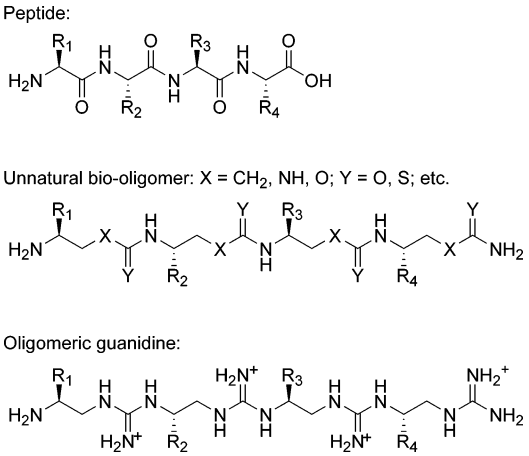

FIGURE 1. Oligomeric structures of a peptide, some unnatural bio-oligomers, and an oligomeric guanidine.

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peptides.[19] However, there are limitations to the current unnatural oligomeric backbones. For example, if a highly hydrophobic peptide sequence is to be mimicked, compounds built on most of the current unnatural backbones will likely suffer from very poor aqueous solubility and/or severe random aggregation in aqueous solution, which is of concern for library screening of inhibitors.[20] We sought to synthesize unnatural oligomers built on guanidine backbones (Figure 1), which may offer a significant advantage in terms of aqueous solubility due to the high pK_a of alkyl- or aryl-substituted guanidine units.[21] In addition, because of the charged guanidine group under physiological conditions, oligomeric guanidines may possess unique properties for application in biomedical research. For example, these oligomers may be used in gene therapy to deliver oligonuceotides with negatively charged backbones. In designing protein ligands, the oligomer backbone may interact with target proteins by electrostatic interactions and/or hydrogen bonds. Oligomeric guanidines have also been shown to be resistant to certain enzyme degradations.[22] All of these properties make oligomeric guanidines a very important complement to the existing unnatural bio-oligomers.

In contrast to a large number of synthetic routes for variously substituted single guanidine units, synthetic methods for oligomeric guanidines are of very limited choice. For example, the *N*,*N*′-disubstituted guanidine moiety has been incorporated in oligomeric form to replace the diphosphate backbone in various forms of oligonucleotides.[23−26] Oligomeric guanidine synthesis has also been demonstrated by two other groups, but with substantial limitations on yields, difficulties in deprotection, or limited choices of side chains.[27−29] Despite those reports, none of the above examples include guanidine oligomers that contain common peptide side chains. In addition, conditions similar to those that work well for replacing the phosphate backbones in oligonucleotides with guanidines did not produce desirable results in the synthesis of peptidomimetics on guanidine backbones.[28] In a previous communication, we have successfully demonstrated the first efficient solid-phase synthesis of *N*,*N*′-bridged oligomeric guanidines bearing simple peptide like side chains (Figure 2).[30] Our method utilized Pbf-activated thiourea for oligomer elongation and azide as a mask for the backbone amine (Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl). The direction of synthesis (direction A in Figure 2) corresponds to the N-terminal to C-terminal direction in a peptide sequence. In this paper, we present detailed synthetic studies of two new sequences of oligomeric guanidine (**1** and **2** in Figure 2) that expand the side-chain functional groups to include neutral hydrophilic, acidic, basic, as well as secondary amine groups. In addition, the preparation of monomers has been improved. The syntheses of **1** and **2** are accomplished both on solid support and in solution, but with different directions for oligomer elongation (direction A for **1** and direction B for **2**, Figure 2). Synthesis direction B is important for the successful preparation of **2** that contains a secondary-amine monomer derived from proline. The advantages and limitations in monomer and oligomer syntheses are discussed.

Results and Discussion

Direction A, Synthesis of 1. The overall synthetic strategy is identical to that described in our previous communication for solid-phase synthesis,[30] with some modifications in the procedure to make it more efficient as will be discussed below. The required monomers for the synthesis of **1** were prepared as shown in Scheme 1. The Fmoc-protected amino alcohols **4** were from commercial sources or converted from the corresponding Fmoc-protected amino acids **3** by reacting first with isobutyl chloroformate, followed by reduction with $NaBH_4$.[31] Fmoc-protected amino alcohols were transformed into azide compounds **5** under Mitsunobu conditions in good yields as described by Boeijen et al.[17] The Fmoc groups in **5** were removed, and the resulting free amino groups were reacted with Pbf-NCS[32] to form monomers **6**. This route is improved from our previously reported method of monomer synthesis where the formation of Pbf-thiourea was accomplished in two steps involving the use of strong nucleophilic conditions (an-

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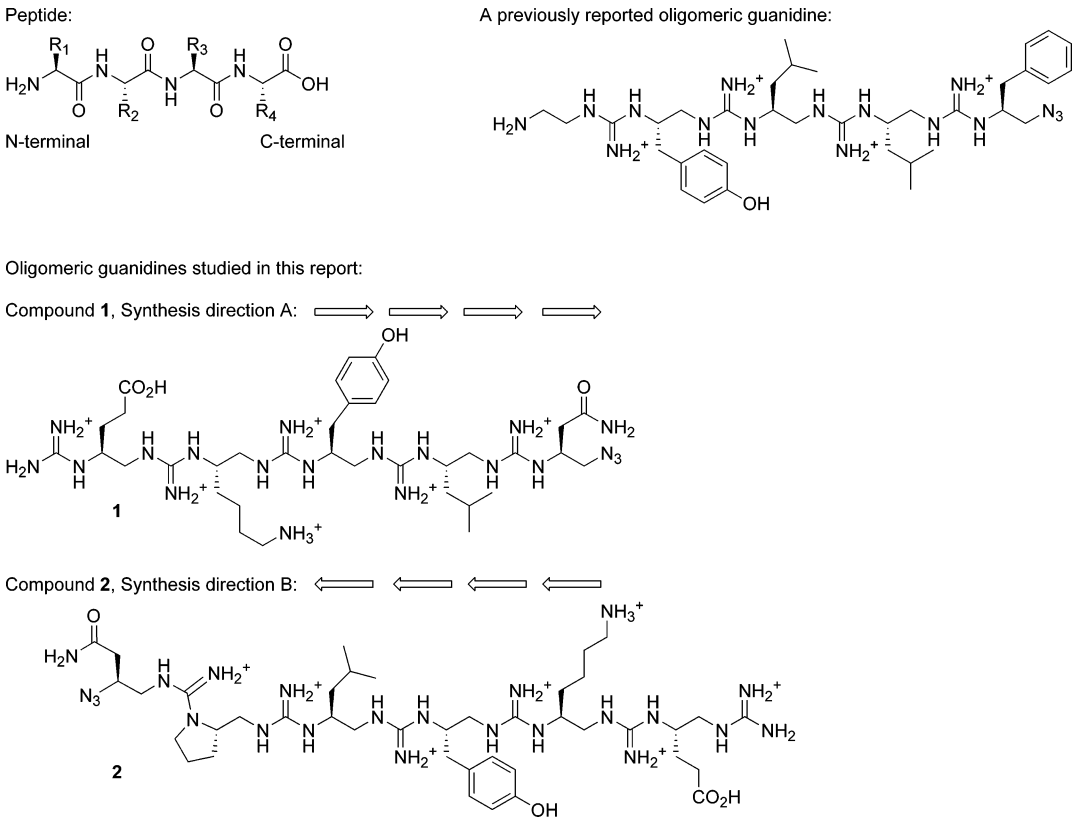
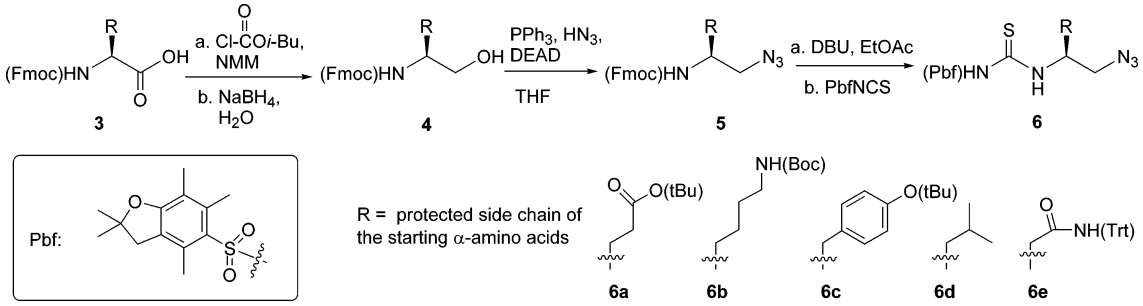


FIGURE 2. Previously reported oligomeric guanidine bearing peptide side chains and compounds (**1** and **2**) studied in this paper. Synthesis directions A (equivalent to N to C terminal in a peptide) and B (equivalent to C to N terminal in a peptide) are defined as shown.

SCHEME 1. Preparation of Monomer 6 for Synthesis of Oligomers in Direction A



ionic form of Pbf-NH_2).[30] During this transformation, the protocol for Fmoc removal is also worth noting. In conventional solid-phase synthesis, the Fmoc group can be removed by 20% piperidine in DMF. However, using similar conditions for monomer synthesis in solution was inconvenient because the excess piperidine required to deprotect the Fmoc group was itself difficult to be separated from the resulting amine product. In large-scale monomer synthesis, the same problem was found for TBAF in THF, which we used in our previous report.[30] Application of resin-bound piperidine to remove the Fmoc group is costly and also inefficient in our hands. We found that DBU was a good choice to remove Fmoc in solution-phase synthesis,[33,34] especially using ethyl acetate as the solvent. Only 1.5 equiv of DBU was required, and Fmoc removal was finished in minutes. The small excess of DBU can be washed away by water. The dibenzofulvene released from the Fmoc group stayed in the organic solution. The resulting amine was used directly in the preparation of monomer **6** without further purification. Apparently, the dibenzofulvene did not form a significant amount of adduct with the liberated amine to interfere with the reaction and was easily separated from the final products **6**.

Synthesis of oligomer **1** in direction A either on solid support or in the solution phase is shown in Scheme 2. In the solid-phase protocol, Rink amide resin was reacted with monomer **6a** to form the first guanidine unit, which is different from our earlier investigation that used the modified Wang resin to yield an amine in the final product. All of the guanidinylation steps on the solid support employed 5 equiv of monomers **6** in anhydrous

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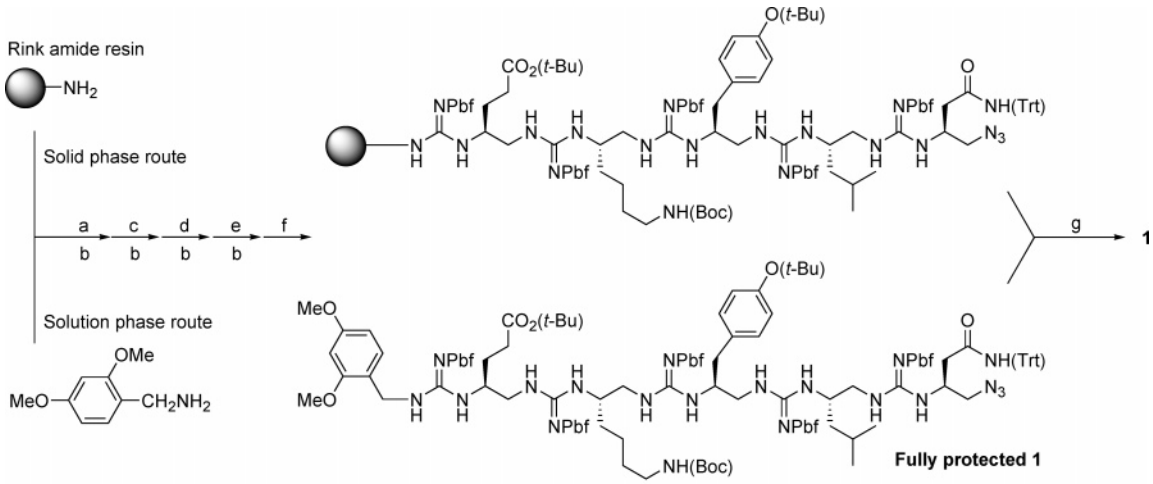
SCHEME 2. Synthesis of 1 in Direction A on Solid Support or in Solution



a) **6a**, EDC; b) $SnCl_2$:PhSH:DIPEA; c) **6b**, EDC; d) **6c**, EDC; e) **6d**, EDC; f) **6e**, EDC; g) TFA:TIS:H_2O.

CH_2Cl_2 in the presence of EDC. The reaction was allowed to proceed at room temperature for 12 h. After each elongation, the azido group at the terminal was reduced to amine using the $SnCl_2$/PhSH/DIPEA complex in anhydrous THF.[35−37] Cycles of guanidinylation and azide reduction were performed. Cleavage of the final product in salt form was achieved using TFA with TIS (triisopropylsilane) and water as scavengers. The overall isolated yield of **1** as TFA salt after HPLC purification was 66%, representing an average of >90% yield for each oligomer elongation cycle.

The anchor for the solution-phase synthesis of **1** was 2,4-dimethoxybenzylamine (DMB-NH_2), which provided an amino group for guanidinylation with **6a**. The DMB group can be removed in the final step with other acid-labile protecting groups when exposed to TFA as shown in Scheme 2. The guanidinylated intermediate after each elongation step was purified by silica gel chromatography, eluting with ethyl acetate and hexanes. Even though such purification required extra work when compared to the solid phase route, the advantage is that only 1 equiv of monomer **6** was required in solution-phase synthesis and that the total reaction time was also shorter (4 h reaction for each guanidinylation step). The azido group was also reduced with the $SnCl_2$/PhSH/DIPEA complex in anhydrous THF, and the excess reducing agent was removed by washing with 2 M NaOH.[37] Oligomer **1** in fully protected form (Scheme 2) prior to TFA treatment was obtained in 60% overall isolated yield, which is similar to the solid-phase protocol for the final product despite the need for extra purification of intermediates. The final product **1** as TFA salt was obtained by cleaving all protecting groups in one step with TFA/TIS/H_2O, followed by ether wash. The yield was 91% from protected **1** (HPLC purity 97%).

Direction B, Synthesis of 2. The commonly accepted mechanism for the activation of thiourea derivatives with EDC during guanidine synthesis involves the transformation of the thiourea moiety into carbodiimide.[28] In the preparation of monomers of Pbf-thiourea **6**, as shown in Scheme 1, compounds derived from most common amino acids can provide a primary amine after Fmoc removal. The subsequent reaction with Pbf-NCS will lead to Pbf-thiourea that can later be transformed into carbodiimide by EDC during oligomer synthesis. However, when proline is used for monomer synthesis, removal of Fmoc will produce a secondary amine, which cannot later be used for guanidine synthesis through activated thiourea followed by carbodiimide formation.[38] To develop a synthetic route compatible to the monomer derived from proline, one may envision that intermediate **5** (Scheme 1) can provide a primary amine after azide reduction. This primary amine can then serve to prepare Pbf-thiourea and be used in oligomer synthesis. In this strategy, the amino group used for Pbf-thiourea formation is derived from the carboxyl end of the starting amino acid, which is "reversed" as compared to monomers **6**. As a consequence, synthesis of oligomers compatible to proline-based monomer will elongate from a different direction (direction B in Figure 2).

If the azido function in compound **5** is turned into Pbf-thiourea, we now face the question of whether to keep or replace the Fmoc protection group of the remaining amine. The reason is that during oligomer elongation, the nucleophilic nitrogen atom of the FmocNH moiety may react with the formed carbodiimide intermediate to produce undesirable cyclic product.[28,39] While this competing side reaction is not a concern for proline-based monomer due to Fmoc-protected secondary amine, other linear monomers may suffer. To probe the protection needed for the amino group, we prepared ethylenediamine-based monomers (glycine equivalent) bearing different protecting groups and tested guanidinylation reactions on solid support (Scheme 3).

We first tested the Fmoc-protected monomer **8**, which was synthesized by reaction of mono-Fmoc-protected ethylenediamine with Pbf-NCS. The trityl resin-bound

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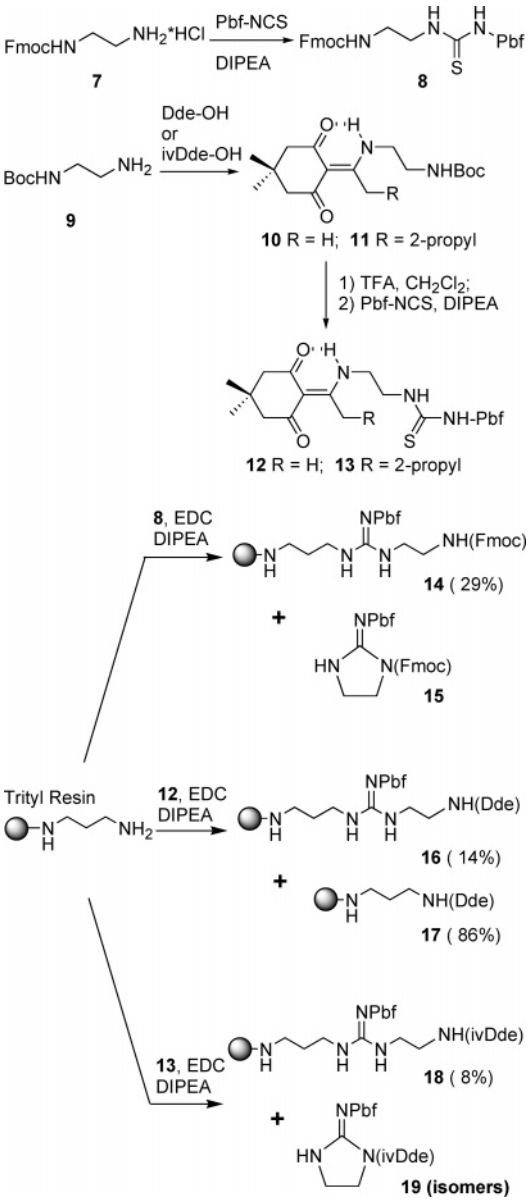
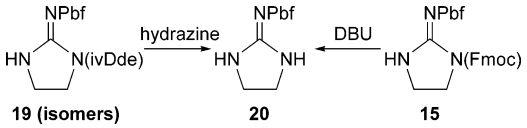
SCHEME 3. Probing Monomers with Different N-Protections during Guanidinylation



1,3-diaminopropane was used to test the guanidinylation reaction. The guanidinylation reaction was carried out with 5 equiv of **8** and EDC in CH_2Cl_2 at room temperature overnight. As expected, in the reaction solution, the majority (>90%) of **8** was cyclized into **15**. Guanidinylation only took place on 29% of resin-bound propylenediamine (Scheme 3), while the rest of the resin-bound amine remained unchanged (see the Experimental Section).

We then chose 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) to replace Fmoc as the amino protecting group. The Dde group is widely used in solid-phase peptide synthesis to orthogonally protect primary amine and can be easily removed with 2% hydrazine in DMF.[40] Both the vinylogous amide moiety and the steric hindrance reduce the nucleophilic property of the Dde-protected amine. We hoped that the Dde will also reduce the intramolecular cyclic guanidine formation. The synthesis of Pbf-thiourea-activated Dde-protected ethylenediamine **12** is shown in Scheme 3. The mono-Boc protected ethylenediamine was reacted with DdeOH to form Boc- and Dde-doubly protected ethylenediamine **10**. After Boc removal, reaction with Pbf-NCS gave the desired monomer **12** for testing. During the guanidinylation test with trityl-resin bound 1,3-diaminopropane (Scheme 3), it was found that no cyclic guanidine was formed in the reaction solution. However, the Dde migration to the resin-bound 1,3-diaminopropane was a severe problem, which masked ~86% of the amino group to be guanidinylated. It is known that Dde could undergo both intra- and intermolecular N to N migration in peptide synthesis, resulting in the scrambling of the lysine group within a peptide chain.[41] However, it was unexpected that the intermolecular migration was so severe in the solid-phase guanidinylation test. Usually, such migration in peptide synthesis can be overcome by replacing the Dde with the more steric ivDde (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) group.[42] Therefore, we prepared ivDde-protected monomer **13** as shown in Scheme 3. In the guanidinylation test, no ivDde migration was found. Unfortunately, intramolecular cyclic guanidine formation of **19** was serious, and only 8% of guanidinylation occurred. It is plausible that, compared to Dde, the much higher steric strain in ivDde could favor the intramolecular cyclization for releasing the strain. The cyclization product **19** was found as two isomers in equilibrium. LC−MS analysis showed two peaks in the ratio of 4:1 with identical mass. These two isomers can be separated by preparative reverse phase HPLC and can also reestablish equilibrium with each other in solution. To confirm that **19** is ivDde-protected cyclic guanidine, the mixture of isomers **19** was treated with 2% hydrazine in DMF to remove the ivDde. N-Pbf cyclic guanidine **20** was formed as shown in Scheme 4. Compound **20** thus prepared is identical to the sample obtained from **15** by removal of the Fmoc protecting group.

SCHEME 4. Confirmation of Cyclic Guanidine Products



Given the difficulties in using Fmoc, Dde, or ivDde for the protection of backbone primary amines during guanidinylation reaction, we decided to prepare monomers similar to those used in direction A synthesis where an azide group is used as amine mask. This requires us to convert the Fmoc protected amino group to an azido group in the monomer. Fortunately, direct conversion of an amine to azide using triflyl azide is possible, and this transformation can be achieved efficiently and consistently with Cu^{2+} catalysis, as demonstrated initially in carbohydrate chemistry.[43] This Cu^{2+}-catalyzed conversion

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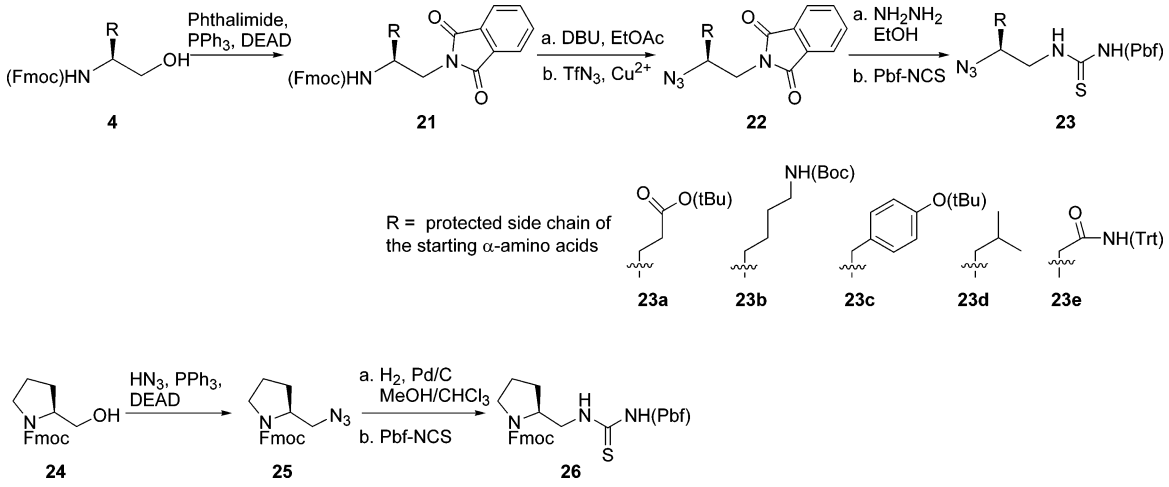
SCHEME 5. Preparation of Monomers for Synthesis of Oligomers in Direction B



retains the stereochemistry of the amino group to be transformed and has been adapted to peptide synthesis.[44,45] Based on this conversion, we successfully prepared azide masked monomers for Direction B oligomer synthesis as shown in Scheme 5. First, amination of Fmoc-protected amino alcohol **4** with phthalimide proceeded smoothly under the Mitsunobu conditions as reported by the Burgess group[16] to give **21** in 83−94% yield. After the Fmoc was removed by DBU in ethyl acetate as performed for compounds **5**, the resulting amines from **21** were converted to azide using the condition reported by the Wong group.[43] The azido compounds **22** were obtained in 72−88% yield when we used 3-fold of triflyl azide in the reaction instead of the 2-fold as reported by the Wong group. Hydrazinolysis of **22** and reaction with Pbf-NCS gave the desired monomers **23** in ∼90% yield. For proline-based monomer, the Fmoc-protected amino alcohol **24** was first turned into azide **25**. Then the azide was reduced and converted to Pbf-thiourea. Monomer **26** retained the Fmoc protecting group.

To investigate the efficiency of monomer **26** in oligomer synthesis, it was tested with a small amount of Fmoc-protected Rink amide resin, and the Fmoc-removal/guanidinylation cycle was performed four times. Cleavage of the product from the resin gave crude **27** (shown in Figure 3), which was subjected to LC−MS analysis. The result (Figure 3) indicated that monomer **26** can efficiently afford the desired oligomer **27** with no major side reactions.

With the monomers **23** and **26** in hand, synthesis of oligomer **2** in direction B, as shown in Scheme 6, was straightforward both on the solid support and in solution, using conditions very similar to those in the direction A synthesis of oligomer **1**. The exceptions in conditions are that after guanidinylation with the proline-based monomer **26**, the Fmoc protection was removed using either piperidine in DMF (solid phase) or DBU in ethyl acetate (solution phase). Again, similar to the direction A synthesis of **1**, high yield was achieved in either the solid-phase or the solution-phase protocol of Direction B synthesis. The final overall isolated yield of **2** as TFA salt

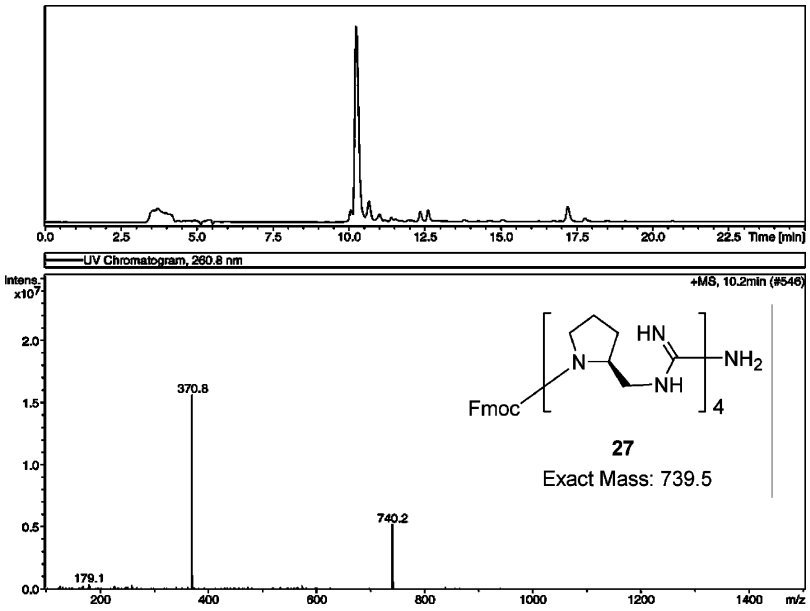
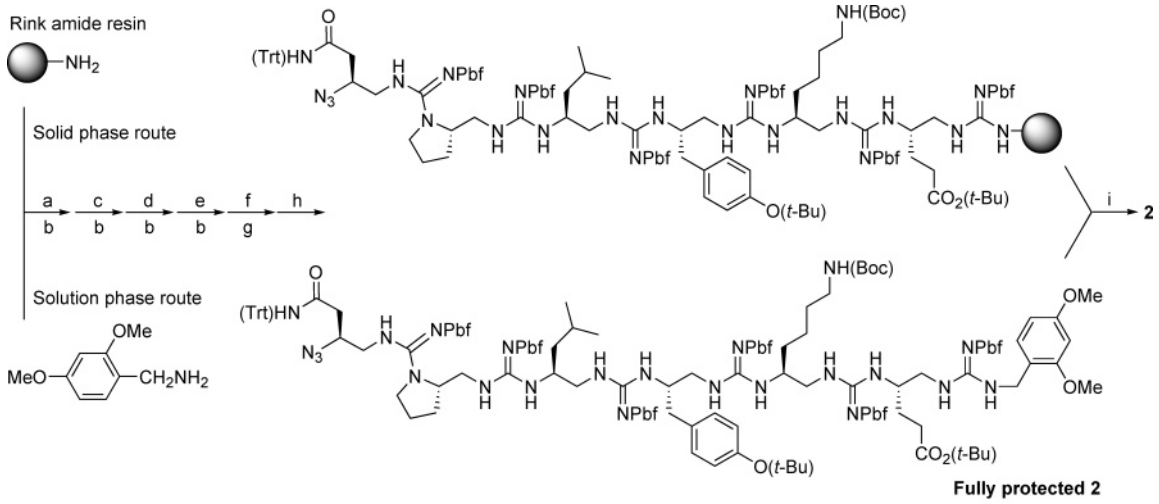


FIGURE 3. LC−MS analysis of crude oligomer **27** with four units of proline-based monomer **26**. Top: LC trace monitored at 260 nm. Bottom: MS spectrum (*m/z*) of the main peak at 10.2 min, showing $(M + H)^+$ of **27** at 740.2 and $(M + 2H)^{2+}$ at 370.8.

SCHEME 6. Synthesis of 2 in Direction B on Solid Support or in Solution



a) **23a**, EDC; b) $SnCl_2$:PhSH:DIPEA; c) **23b**, EDC; d) **23c**, EDC; e) **23d**, EDC; f) **26**, EDC; g) 20% piperidine/DMF (solid phase) or DBU, EtOAc (solution phase); h) **23e**, EDC; i) TFA:TIS:H_2O.

from solid phase synthesis was 60%, representing >90% yield in each guanidinylation and deprotection cycle. In solution synthesis, the yield of fully protected **2** prior to TFA treatment was 56%, also indicating >90% yield in each elongation step despite the required chromatographic purification after each guanidinylation reaction.

Conclusions

Two sequences of oligomeric guanidines bearing peptide side chains (**1** and **2**) were synthesized efficiently either on solid support or in solution. Monomers were derived from common α-amino acids, containing a Pbf-thiourea moiety for elongation of the guanidine backbone and an azide moiety as the mask of backbone primary amine. Oligomer chain elongation can be performed in two opposite directions (Figure 2), one of which is compatible to monomers containing backbone secondary amines, such as the proline-based monomer. In the two guanidine oligomers **1** and **2** reported here, the side chains include most common peptide side chain functionalities. The wide range of side chain compatibility will be a significant advantage of our synthetic strategy for the generation of chemical libraries of short oligomeric guanidines, which may be implemented conveniently using our solid-phase protocols.

Experimental Section

Monomer 6 for Direction A Synthesis. General Procedure. Fmoc-protected amino azide **5** (2.0 mmol) was dissolved in ethyl acetate (80 mL), and DBU (0.457 mL, 3.0 mmol) was added. The mixture was stirred at room temperature for 5−20 min until the Fmoc was completely removed as monitored by TLC. The organic solution was washed with 60 mL of H_2O and saturated NaCl and then dried over anhydrous Na_2SO_4. The solution was filtered, and Pbf-NCS (2.0 mmol) was added dropwise at room temperature. After 2 h of stirring, the mixture was purified by silica gel chromatography eluting with EtOAc/hexanes, and monomer **6** was obtained as a white solid.

Glutamate-Based Monomer 6a. This monomer was synthesized according to the general procedure on 1.6 mmol scale. Yield: 714 mg (85%). Mp: 43 °C. R_f = 0.29 (EtOAc/hexanes, 1/4). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.42 (s, 9H), 1.47 (s, 6H), 1.65−1.73 (m, 1H), 1.87−2.05 (m, 1H), 2.06−2.11 (m, 5H), 2.52 (s, 3H), 2.59 (s, 3H), 2.99 (s, 2H), 3.41−3.49 (m, 2H), 4.47−4.54 (m, 1H), 7.77 (d, J = 8.5 Hz, 1H), 8.46 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.4, 17.5, 19.2, 27.1, 28.0, 28.4, 31.3, 42.8, 53.5, 54.0, 80.7, 87.5, 118.8, 125.9, 126.6, 134.4, 140.0, 160.9, 171.5, 178.2. HRMS (ESI-TOF): *m/z* calcd 526.2152 $(M + H)^+$, found 526.2160; calcd 548.1972 $(M + Na)^+$, found 548.1969.

Solid-Phase Synthesis of Oligomer 1. A 100 mg sample of Rink amide MBHA resin (theoretical loading: 0.64 mmol/g) in a polypropylene column was swelled in 5 mL of DMF for 30 min. The Fmoc group was removed by the treatment of resin with 5 mL of 20% piperidine in DMF for 10 min twice. Guanidinylation was performed with 5 equiv of monomer **6a** (168 mg, 0.32 mmol) in 4 mL of anhydrous CH_2Cl_2 in the presence of DIPEA (0.111 mL, 0.64 mmol) and EDC (62 mg, 0.32 mmol). The reaction was allowed to proceed for 12 h at rt. After being washed with CH_2Cl_2, the resin was capped with acetic anhydride (0.061 mL, 0.64 mmol) and DIPEA (0.223 mL, 1.28 mmol) in 5 mL of CH_2Cl_2 for 20 min. After washing with CH_2Cl_2 and THF, a fresh solution of $SnCl_2$/PhSH/DIPEA (0.32 mmol:1.28 mmol:1.60 mmol or 61 mg:0.13 mL:0.28 mL) in anhydrous THF (3 mL) was added at room temperature and the mixture was rotated for 40 min. This reduction was repeated once. The resin was then washed with 6 mL of THF five times. The loading was determined to be 0.51 mmol/g by monitoring Fmoc release after capping a small amount of resin with FmocOSu overnight. The final yield of **1** was based on this loading. The above monoguanidinylated resin was subjected to four more cycles of guanidinylation with, respectively, 0.255 mmol of monomer **6b** (145 mg), **6c** (143 mg), **6d** (116 mg), and **6e** (177 mg) and azide reduction as described above except that no reduction was performed for the last monomer **6e**. The above resin-bound-protected **1** was cleaved by treatment with 7 mL of TFA/TIS/H_2O (94:3:3) overnight. After solvent removal, the residue was washed with ether. The residue was then dissolved in 0.1% TFA water and acetonitrile. The product was purified by reversed-phase HPLC using a ZOBAX extended-C18 column (Agilent) with 0.1% TFA water and acetonitrile as solvents. Lyophilization gave 51 mg of product **1** as TFA salt (66%). ^{1}H NMR (500 MHz, D_2O): δ = 0.87 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), 1.22−1.32 (m, 1H), 1.33−1.99 (m, 10H), 2.16−2.80 (m, 4H), 2.90−4.20 (m, 19H), 6.86 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H).

(44) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781−783.
(45) Rijkers, D. T. S.; van Vugt, H. H. R.; Jacobs, H. J. F.; Liskamp, R. M. J. *Tetrahedron Lett.* **2002**, *43*, 3657−3660.

MS (ESI-ion trap): *m/z* 416.3 $(M + 2H)^{2+}$, 831.2 $(M + H)^+$, 945.1 $(M + H + TFA)^+$.

Solution-Phase Synthesis of Oligomer 1. 2,4-Dimethoxybenzylamine (0.035 mL, 0.225 mmol) and monomer **6a** (118 mg, 0.225 mmol) were dissolved in 10 mL of anhydrous CH_2Cl_2. EDC (43 mg, 0.225 mmol) and DIPEA (0.08 mL, 0.45 mmol) in 5 mL of anhydrous CH_2Cl_2 were added. The solution was stirred at rt for 4 h. After the solvent was removed, the residue was purified by silica gel chromatography eluting with EtOAc/hexanes to give the monoguanidinylated compound. The monoguanidinylated compound was subjected to reduction with a fresh solution of $SnCl_2$/PhSH/DIPEA (0.45 mmol:1.8 mmol:2.25 mmol or 86 mg:0.18 mL:0.39 mL) in anhydrous THF (4 mL). The clear yellow solution was stirred at rt for 1 h under N_2 atmosphere. After the solvent was removed, the residue was dissolved in 20 mL of CH_2Cl_2, washed with 20 mL of 2 N NaOH twice and 20 mL of saturated NaCl, and dried over Na_2SO_4. The above reduced monoguanidinylated compound was subjected to four more cycles of guanidinylation with, respectively, 0.225 mmol of monomer **6b** (128 mg), **6c** (126 mg), **6d** (102 mg), and **6e** (157 mg) and azide reduction as described above except that no reduction was performed for the last monomer **6e**. After the solvent was removed, the residue was purified by silica gel chromatography eluting with 3% methanol in CH_2Cl_2 to give 362 mg of protected **1** as a white solid (60%). The protected **1** was treated with 10 mL of TFA/TIS/H_2O (94:3:3) overnight. After solvent removal, the residue was washed with ether. The residue was then dissolved in 0.1% TFA water and acetonitrile and its purity checked by LC−MS: purity 97% based on UV absorbance at 230 nm. Lyophilization gave 192 mg of product **1** as TFA salt (91%).

***N*-[2-(Fmoc-amino)ethyl]-*N*′-Pbf-thiourea 8.** Mono-Fmoc-protected ethylenediamine hydrochloride **7** (610 mg, 1.92 mmol) was suspended in anhydrous CH_2Cl_2 (30 mL). DIPEA (0.40 mL, 2.30 mmol) was added at room temperature. After the solution became clear, Pbf-NCS (1.92 mmol) in 10 mL of anhydrous CH_2Cl_2 was added dropwise. After the mixture was stirred at room temperature for 2 h, the solvent was removed under reduced pressure. The crude product was purified on silica gel eluting with MeOH/CH_2Cl_2, and monomer **8** was obtained as a white solid (968 mg, 85%). Mp: 88 °C dec. R_f = 0.58 (MeOH/CH_2Cl_2, 5%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 (s, 6H), 2.05 (s, 3H), 2.39 (s, 3H), 2.56 (s, 3H), 2.84 (s, 2H), 3.26 (m, 2H), 3.73 (m, 2H), 4.21 (m, 1H), 4.45 (d, J = 6.5 Hz, 2H), 4.88 (m, 1H), 7.30−7.33 (m, 2H), 7.38−7.41 (m, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H), 8.12 (m, 1H), 8.39 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.4, 17.5, 19.2, 28.4, 40.0, 42.6, 45.1, 47.1, 66.6, 87.5, 118.8, 119.9, 125.0, 125.7, 126.6, 127.1, 127.7, 134.4, 140.1, 141.3, 143.7, 156.5, 160.9, 179.2. HRMS (ESI-TOF): *m/z* calcd 594.2091 $(M + H)^+$, found 594.2088.

Guanidinylation of Resin-Bound 1,3-Diaminopropane with 8. A 400 mg sample of trityl chloride resin (theoretical loading: 1.20 mmol/g) in a polypropylene column was swelled in 5 mL of CH_2Cl_2. 1,3-Diaminopropane (2.0 mL, 24.0 mmol) in 6 mL of CH_2Cl_2 was added to the resin suspension. The mixture was rotated for 2 h and then quenched with 2 mL of methanol. The resin was washed with CH_2Cl_2 to give the resin-bound 1,3-diaminopropane (loading 1.02 mmol/g, as determined by derivatization with Fmoc-OSu and monitoring the Fmoc release). A 100 mg sample of resin-bound 1,3-diaminopropane in 3 mL of anhydrous CH_2Cl_2 was mixed with **8** (302 mg, 0.51 mmol) and DIPEA (89 μL, 0.51 mmol). Then EDC (98 mg, 0.51 mmol) and DIPEA (89 μL, 0.51 mmol) in 2 mL of anhydrous CH_2Cl_2 were added. The mixture was rotated at rt overnight. The resin was filtered and washed with CH_2Cl_2. The filtrates were combined. The resin was further reacted with Fmoc-OSu (172 mg, 0.51 mmol) and DIPEA (0.18 mL, 1.02 mmol) in CH_2Cl_2 overnight to cap the unreacted resin-bound 1,3-diaminopropane. The resin was subsequently treated with TFA/H_2O/TIS (94:3:3) for 2 h. After the solvent was removed, the residue was subjected to LC−MS analysis. The relative UV (260 nm) intensities of the Fmoc moiety in the released products corresponding to capped 1,3-diaminopropane and resin-bound **14** were determined to be in a 2.4:1 ratio, indicating the degree of guanidinylation at ∼29%. The combinations of filtration were subjected to silica gel chromatography to obtain a white solid **15** in 94% yield (240 mg). Mp: 104 °C dec. R_f = 0.49 (MeOH/CH_2Cl_2, 3%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.40 (s, 6H), 2.02 (s, 3H), 2.53 (s, 3H), 2.60 (s, 3H), 2.83 (s, 2H), 3.67 (d, J = 8.3 Hz, 2H), 3.92 (d, J = 8.3 Hz, 2H), 4.24 (t, J = 7.5 Hz, 1H), 4.41 (d, J = 7.5 Hz, 2H), 7.10 (t, J = 7.5 Hz, 2H), 7.35 (t, J = 7.5 Hz, 2H), 7.54 (d, J = 7.5 Hz, 2H), 7.66 (s, 1H), 7.72 (d, J = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.4, 17.9, 19.3, 28.5, 39.7, 43.0, 43.7, 46.4, 66.3, 86.4, 117.5, 119.8, 124.7, 125.4, 127.0, 127.7, 131.4, 133.0, 139.0, 141.1, 143.3, 151.3, 152.9, 159.2. HRMS (ESI-TOF): *m/z* calcd 560.2219 $(M + H)^+$, found 560.2208.

***N*-[2-(Dde-amino)ethyl]-*N*′-Pbf-thiourea 12.** DdeOH (0.276 g, 1.51 mmol) in 10 mL of anhydrous CH_2Cl_2 was added into a solution of mono-Boc-protected ethylenediamine **9** (0.20 mL, 1.26 mmol) in 10 mL of anhydrous CH_2Cl_2. After 8 h of stirring at room temperature under N_2, the solvent was removed and the residue was purified by silica gel chromatography. *N*-Boc-*N*′-Dde-ethylenediamine **10** was obtained as a white solid (0.376 g, 92%). Mp: 123 °C. R_f = 0.25 (MeOH/CH_2Cl_2, 1/20). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.99 (s, 6H), 1.40 (s, 9H), 2.32 (s, 4H), 2.53 (s, 3H), 3.32−3.33 (m, 2H), 3.53−3.54 (m, 2H), 5.06 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.8, 28.18, 28.26, 30.0, 40.1, 43.0, 52.8, 79.9, 108.1, 155.8, 174.1, 198.1. HRMS (ESI-TOF): *m/z* calcd 325.2127 $(M + H)^+$, found 325.2130. The diprotected amine **10** (0.310 g, 0.96 mmol) was treated with 5 mL of TFA/CH_2Cl_2 (1:1) for 15 min, and then the solvent was removed under vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (20 mL), and DIPEA was added dropwise to neutralize the remaining trace amount of TFA. Subsequently, DIPEA (0.26 mL, 1.50 mmol) and Pbf-NCS (0.96 mmol) in 5 mL of CH_2Cl_2 was added dropwise. After 2 h of stirring at rt, the product was purified by silica gel chromatography eluting with MeOH/CH_2Cl_2. Compound **12** was obtained as a white solid (0.462 g, 90%). Mp: 77 °C dec. R_f = 0.27 (MeOH/CH_2Cl_2, 1/20). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.01 (s, 6H), 1.47 (s, 6H), 2.10 (s, 3H), 2.35 (s, 4H), 2.46 (s, 3H), 2.53 (s, 3H), 2.55 (s, 3H), 2.97 (s, 2H), 3.64 (m, 2H), 3.80 (m, 2H), 8.26 (t, J = 5.5 Hz, 1H), 8.89 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.5, 17.5, 17.8, 19.2, 28.2, 28.5, 30.0, 41.1, 42.8, 44.3, 52.7, 87.5, 108.2, 118.6, 125.7, 126.7, 134.5, 140.2, 161.0, 174.3, 179.7. HRMS (ESI-TOF): *m/z* calcd 536.2247 $(M + H)^+$, found 536.2251.

Guanidinylation of Resin-Bound 1,3-Diaminopropane with 12. The guanidinylation step was similar to those performed with **8**, but instead using 0.51 mmol of **12**. The resin was filtered and washed with CH_2Cl_2 and then was treated with TFA/H_2O/TIS (94:3:3) for 2 h and filtered. After the solvent was removed, the residue was subjected to LC−MS analysis. The relative UV (230 nm) intensities of the released products corresponding to resin-bound **16** and **17** were obtained. The ratio indicated that the degree of guanidinylation was 14% of resin-bound 1,3-diaminopropane. In a separate experiment, 50 mg of **12** (0.093 mmol) was mixed with EDC (18 mg, 0.093 mmol) and DIPEA (0.064 mL, 0.186 mmol) in 10 mL of anhydrous CH_2Cl_2 and stirred at rt overnight. No cyclization product was found in the solution.

***N*-[2-(ivDde-amino)ethyl]-*N*′-Pbf-thiourea 13.** To a solution of mono-Boc-protected ethylenediamine **9** (0.20 mL, 1.26 mmol) in 30 mL of ethanol was added ivDdeOH (0.552 mL, 2.52 mmol). After being refluxed overnight under N_2, the solution was removed and the residue was purified by silica gel chromatography eluting with MeOH/CH_2Cl_2. Diprotected amine **11** was obtained as an oil (0.401 g, 87%). R_f = 0.59 (MeOH/CH_2Cl_2, 5%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.86 (d, J = 7.0 Hz, 6H), 0.91 (s, 6H), 1.33 (s, 9H), 1.83 (m, 1H), 2.24 (s, 4H), 2.90 (br, 2H), 3.26 (m, 2H), 3.51 (m, 2H), 5.34 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.3, 28.0, 28.1, 28.8, 29.6,

36.9, 40.2, 43.1, 52.8, 79.5, 107.0, 155.7, 176.9. HRMS (ESI-TOF): *m/z* calcd 367.2597 $(M + H)^+$, found 367.2591. Compound **11** (0.151 g, 0.412 mmol) was treated with 3 mL of TFA/CH_2Cl_2 (1:1) for 15 min, and the solvent was removed under vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (10 mL), and DIPEA was added dropwise to neutralize the remaining trace amount of TFA. Subsequently, DIPEA (0.14 mL, 0.824 mmol) and Pbf-NCS (0.412 mmol) in CH_2Cl_2 (3 mL) was added dropwise. After 4 h of stirring at rt, the product was purified by silica gel chromatography eluting with EtOAc/hexanes. compound **13** was obtained as a white solid (0.209 g, 88%). Mp: 45 °C dec. $R_f = 0.60$ ($MeOH/CH_2Cl_2$, 3%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.94$ (d, $J = 6.9$ Hz, 6H), 1.01 (s, 6H), 1.46 (s, 6H), 1.84−1.95 (m, 1H), 2.12 (s, 3H), 2.36 (s, 4H), 2.46 (s, 3H), 2.53 (s, 3H), 2.96−3.00 (m, 4H), 3.64−3.70 (m, 2H), 3.76−3.82 (m, 2H), 8.27 (t, $J = 6.0$ Hz, 1H), 8.93 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.4$, 17.5, 19.2, 22.4, 28.1, 28.5, 29.0, 29.8, 37.0, 41.3, 42.4, 44.4, 52.9, 87.4, 107.4, 118.6, 125.6, 126.8, 134.5, 140.1, 160.9, 177.4, 179.7. HRMS (ESI-TOF): *m/z* calcd 578.2722 $(M + H)^+$, found 578.2730.

Guanidinylation of Resin-Bound 1,3-Diaminopropane with 13. The guanidinylation step was similar to those performed with **8**, but instead using 0.51 mmol of **13**. The resin was filtered and washed with CH_2Cl_2. The filtrates were combined. A small amount of the resin was cleaved with TFA/H_2O/TIS (94:3:3) for 2 h. After the solvent was removed, the residue was subjected to LC−MS analysis, which indicated the presence of guanidinylated product corresponding to the release of resin-bound **18**. No ivDde modified 1,3-ethylenediamine was detected, indicating the absence of intermolecular migration of the ivDde group. Then the remaining resin was exposed to 2% hydrazine in DMF for 30 min to remove ivDde in resin-bound **18**. After being washed with DMF and CH_2Cl_2, the resin was reacted with Fmoc-OSu (172 mg, 0.51 mmol) and DIPEA (0.18 mL, 1.02 mmol) in CH_2Cl_2 overnight to cap the unreacted resin-bound 1,3-diaminopropane and the free terminal amine of resin-bound guanidine product. The Fmoc-capped resin was treated with TFA/H_2O/TIS (94:3:3) for 2 h. After the solvent was removed, the residue was subjected to LC−MS analysis. The relative UV (260 nm) intensities of mono Fmoc-protected 1,3-diaminopropane and the derivative from resin-bound **18** were obtained to indicate the degree of guanidinylation at 8%. The combined filtrates were subjected to silica gel chromatography to obtain a white solid **19** as isomers in 85% yield (235 mg). $R_f = 0.27$ ($MeOH/CH_2Cl_2$, 3%). HRMS (ESI-TOF): *m/z* calcd 544.2845 $(M + H)^+$, found 544.2840.

***N*-Pbf-*N*′,*N*″-ethyleneguanidine 20.** Compound **20** can be formed from isomers of **19** or from **15**. (a) A 200 mg portion of **19** (0.368 mmol) was added to 20 mL of 2% hydrazine in DMF. The solution was stirred at rt for 2 h. The solvent was removed, and the residue was washed with water. After silica gel chromatography eluting with ethyl acetate/hexanes, a light yellow solid **20** was obtained (112 mg, 90%). (b) A 200 mg portion of **15** (0.358 mmol) was dissolved in 30 mL of ethyl acetate, and DBU (0.107 mL, 0.716 mmol) was added. The solution was stirred at rt for 15 min and washed with 20 mL of water. After silica gel chromatography purification eluting with ethyl acetate/hexanes, a light yellow solid **20** was obtained (115 mg, 95%). Mp: 214 °C. $R_f = 0.32$ (EtOAc). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 1.45$ (s, 6H), 2.08 (s, 3H), 2.49 (s, 3H), 2.54 (s, 3H), 2.94 (s, 2H), 3.51 (s, 4H), 6.57 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.4$, 17.7, 19.2, 28.5, 41.8, 43.2, 86.4, 117.5, 124.6, 132.2, 132.5, 138.5, 158.8, 160.5. HRMS (ESI-TOF): *m/z* calcd 338.1538 $(M + H)^+$, found 338.1544.

***N*-Fmoc-*N*′-phthaloyl Derivatives 21. General Procedure.** Under a nitrogen atmosphere, DEAD (0.315 mL, 2.0 mmol) was added dropwise to a cooled (0 °C) solution of PPh_3 (525 mg, 2.0 mmol) in anhydrous THF (30 mL). Subsequently, phthalimide (294 mg, 2.0 mmol) in anhydrous THF was added dropwise with stirring. An Fmoc-protected amino alcohol **4** (2.0 mmol) was added in one portion. The reaction mixture was stirred overnight at rt and was concentrated under vacuum. After silica gel chromatography eluting with EtOAc/hexane, compound **21** was obtained as a white solid.

Glutamate-Based Derivative 21a. This compound was synthesized according to the general procedure on a 1.88 mmol scale. Yield: 903 mg (89%). Mp: 64 °C. $R_f = 0.71$ (EtOAc/hexanes, 1/1). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 1.48$ (s, 9H), 1.83 (m, 1H), 1.93 (m, 1H), 2.39−2.42 (m, 2H), 3.80 (d, $J = 6.5$ Hz, 2H), 4.01 (m, 1H), 4.11 (m, 2H), 4.24−4.25 (m, 1H), 5.37 (d, $J = 9.0$ Hz, 1H), 7.24−7.30 (m, 2H), 7.37−7.38 (m, 2H), 7.50−7.51 (m, 2H), 7.55−7.57 (m, 2H),7.72−7.76 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 27.7$, 27.9, 31.9, 41.8, 47.0, 50.6, 66.6, 80.4, 119.7, 123.16, 123.20, 125.0, 125.1, 126.8, 127.4, 131.7, 133.8, 133.9, 140.95, 141.02, 143.6, 144.0, 156.1, 168.3, 172.3. HRMS (ESI-TOF): *m/z* calcd 541.2339 $(M + H)^+$, found 541.2332; calcd 563.2158 $(M + Na)^+$, found 563.2153.

Azido *N*-Phthaloyl Derivative 22. General Procedure. The azide transfer reaction utilized the reported method for carbohydrates in the presence of Cu^{2+}.[43] Triflyl azide preparation: A solution of sodium azide (1.158 g, 17.81 mmol) was dissolved in distilled H_2O (2.9 mL) with CH_2Cl_2 (4.9 mL) and cooled on an ice bath. Triflyl anhydride (1.018 g, 3.6 mmol) was added slowly over 5 min while stirring continued for 2 h. The mixture was placed in a separatory funnel, and the CH_2Cl_2 phase was collected. The aqueous portion was extracted with 3 mL of CH_2Cl_2 twice. The organic fractions, containing the triflyl azide, were pooled, washed once with saturated Na_2CO_3, and used without further purification. Compound **21** (1.2 mmol) was dissolved in ethyl acetate (30 mL), and DBU (0.229 mL, 1.5 mmol) was added. The mixture was stirred at room temperature for about 10 min until Fmoc was completely removed as indicated by TLC. The solution was washed with 40 mL of H_2O. The solvent was removed, and the residue was combined with $CuSO_4$ pentahydrate (1.84 mg), distilled H_2O (3.92 mL), and CH_3OH (11.8 mL). The trifle azide in CH_2Cl_2 was added, and the mixture was stirred at rt overnight. Subsequently, the solvent was removed, and the residue was purified by silica gel chromatography eluting with EtOAc/hexanes to give compound **22**.

Glutamate-Based Derivative 22a. This compound was synthesized according to the general procedure on 1.2 mmol scale. Yield: 313 mg (80%). Colorless oil. $R_f = 0.51$ (EtOAc/hexanes, 1/2). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 1.41$ (s, 9H), 1.70−1.77 (m, 1H), 1.87−1.94 (m, 1H), 2.33−2.45 (m, 2H), 3.67−3.70 (m, 1H), 3.75−3.83 (m, 2H), 7.70−7.71 (m, 2H), 7.82−7.84 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 27.4$, 27.9, 31.6, 41.1, 59.8, 80.7, 123.4, 131.7, 134.1, 167.9, 171.5. HRMS (ESI-TOF): *m/z* calcd 345.1563 $(M + H)^+$, found 345.1566; calcd 367.1382 $(M + Na)^+$, found 367.1383.

Monomer 23 for Direction B Synthesis. General Procedure. A flask with reflux condenser was charged with compound **22** (1.0 mmol), followed by 40 mL of EtOH and N_2H_4 hydrate (0.49 mL, 10 mmol). The mixture was refluxed overnight under N_2 atmosphere, cooled, and concentrated. The residue was dissolved in CH_2Cl_2 (30 mL), washed with 20 mL of water twice and saturated NaCl once, and dried over Na_2SO_4. The solution was filtered, and Pbf-NCS (1.0 mmol) in 10 mL of CH_2Cl_2 was added dropwise at room temperature. After 2 h of stirring, the solvent was removed under vacuum and the residue was purified by silica gel chromatography eluting with EtOAc/hexanes to give monomer **23**.

Glutamate-Based Monomer 23a. This monomer was synthesized according to the general procedure on 0.90 mmol scale. Yield: 435 mg (92%). White solid. Mp: 32 °C dec. $R_f = 0.46$ (EtOAc/hexanes, 1/2). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 1.41$ (s, 9H), 1.45 (s, 3H), 1.46 (s, 3H), 1.62−1.74 (m, 2H), 2.09 (s, 3H), 2.28−2.31 (m, 2H), 2.50 (s, 3H), 2.57 (s, 3H), 2.97 (s, 2H), 3.40−3.46 (m, 1H), 3.65−3.70 (m, 1H), 3.76−3.81 (m, 1H), 8.08 (t, $J = 5.5$ Hz, 1H), 8.58 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.4$, 17.5, 19.1, 26.9, 28.0, 28.4, 31.3, 42.8, 48.4, 60.2, 80.8, 87.4, 118.7, 125.8, 126.6, 134.4, 140.0, 160.9, 171.5,

178.8. HRMS (ESI-TOF): *m/z* calcd 526.2152 $(M + H)^+$, found 526.2150; calcd 548.1972 $(M + Na)^+$, found 548.1978.

Proline-Based Monomer 26. Fmoc-prolinol **24** (0.4169 g, 1.29 mmol) was converted into Fmoc-protected azide **25** (11, 0.385 g, 86%) according to the reported procedure.[17] Compound **25** (0.351 g, 1.01 mmol) was dissolved in 20 mL of methanol/$CHCl_3$ (50:1). To this solution was added a catalytic amount of 10% palladium on carbon. The mixture was stirred at rt under a hydrogen balloon overnight. The solution was filtered, and the solvent was removed. The resulting HCl salt was dissolved in anhydrous CH_2Cl_2, and DIPEA (0.35 mL, 2.02 mmol) was added, followed by Pbf-NCS (1.01 mmol) in 10 mL of CH_2Cl_2. After 3 h of stirring at rt, the product was purified by flash chromatography eluting with 30% ethyl acetate in dichloromethane to obtain the monomer **26** as a white solid (512 mg, 80%). Mp: 55 °C dec. $R_f = 0.40$ ($MeOH/CH_2Cl_2$, 1/50). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.45 (s, 6H), 1.53−1.87 (m, 4H), 2.08 (s, 3H), 2.48 (m, 3H), 2.57 (s, 3H), 2.92 (s, 2H), 3.06−3.41 (m, 3H), 3.65−3.94 (m, 2H), 4.25 (m, 1H), 4.39−4.61 (m, 2H), 7.32 (m, 2H), 7.41 (m, 2H), 7.60 (m, 2H), 7.767 (m, 2H), 8.27 (br, 1H), 8.40 (br, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.4, 17.5, 19.2, 28.4, 28.8, 42.8, 46.8, 47.2, 48.8, 57.2, 67.3, 87.3, 118.5, 119.9, 124.5, 125.1, 125.6, 127.0, 127.7, 134.4, 140.0, 141.3, 143.9, 155.5, 160.7, 178.9. HRMS (ESI-TOF): *m/z* calcd 634.2409 $(M + H)^+$, found 634.2401.

Solid-Phase Synthesis of Oligomer 2. Using 100 mg of Rink amide MBHA resin, the solid-phase procedure was very similar to those for oligomer **1**, except that initial loading of **23a** was determined to be 0.55 mmol/g and subsequent cycles of guanidinylation and reduction was done with 0.275 mmol of monomers **23b** (156 mg), **23c** (154 mg) and **23d** (125 mg). The resin was then guanidinylated with the proline-based monomer **26** (174 mg, 0.275 mmol) in 4 mL of anhydrous CH_2Cl_2 in the presence of DIPEA (0.096 mL, 0.55 mmol) and EDC (53 mg, 0.275 mmol). The reaction was allowed to proceed for 12 h at rt. The Fmoc group was removed by treatment with 6 mL of 20% piperidine in DMF for 15 min twice. After being washed with DMF and CH_2Cl_2, the resin was guanidinylated with **23e** (191 mg, 0.275 mmol) in 4 mL of anhydrous CH_2Cl_2 in the presence of DIPEA (0.096 mL, 0.55 mmol) and EDC (53 mg, 0.275 mmol) for 24 h at rt to ensure reaction completion with the proline secondary amine. The resulting resin-bound **2** was cleaved by treatment with 7 mL of TFA/TIS/H_2O (94:3:3) overnight. After solvent removal, the residue was washed with ether. The residue was dissolved in 0.1% TFA water and acetonitrile and purified by reversed-phase HPLC using a ZOBAX Extended-C18 column (Agilent) with 0.1% TFA water and acetonitrile as solvents. Lyophilization gave 58 mg of product **2** as the TFA salt (60%). ^1H NMR (500 MHz, D_2O): δ = 0.87 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), 1.22−1.31 (m, 1H), 1.32−2.20 (m, 14H), 2.35−2.71 (m, 4H), 2.79−4.28 (m, 24H), 6.86 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H). MS (ESI-ion trap): *m/z* 478.8 $(M + 2H)^{2+}$.

Solution-Phase Synthesis of Oligomer 2. The procedure was very similar to those used for oligomer **1** starting with 0.225 mmol of DMB-NH_2 and monomer **23a**, followed by further reaction cycles with **23b** (128 mg), **23c** (126 mg), and **23d** (102 mg). The resulting intermediate was guanidinylated with **26** (143 mg, 0.225 mmol) and purified. Then the Fmoc group was removed by the treatment with DBU (0.035 mL, 0.225 mmol) in 20 mL of ethyl acetate. After the solution was washed with water (15 mL) and saturated NaCl (20 mL) and dried over Na_2SO_4, the solvent was removed and the residue was dissolved in 10 mL of anhydrous CH_2Cl_2. Final guanidinylation was carried out with monomer **23e** (157 mg, 0.225 mmol) in 2 mL of anhydrous CH_2Cl_2 and DIPEA (0.08 mL, 0.45 mmol) and EDC (43 mg, 0.225 mmol) in 2 mL of anhydrous CH_2Cl_2 at rt for 12 h to ensure completion of the reaction with secondary amine. After the solvent was removed, the residue was purified by silica gel chromatography eluting with 3% methanol in CH_2Cl_2 to give 387 mg of fully protected **2** as a white solid (56%). LC-MS: purity >99% based on UV absorbance at 230 nm. MS (ESI-ion trap): *m/z* 1537.2 $(M + 2H)^{2+}$, 1025.4 $(M + 3H)^{3+}$. The protected **2** was treated with 10 mL of TFA/TIS/H_2O (94:3:3) overnight. After solvent removal, the residue was washed with ether. The residue was dissolved in 0.1% TFA water and acetonitrile and checked by LC−MS: purity 93% based on UV absorbance at 230 nm. Lyophilization gave 214 mg of product **2** as TFA salt (90%).

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Supporting Information Available: Characterization data for new compounds not listed in the Experimental Section and ^{13}C NMR spectra of nonoligomer compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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