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Oligomeric guanidine synthesis assisted by TFA-sensitive arylsulfonylthiourea

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Abstract—Through arylsulfonyl activation of thiourea, efficient synthesis of oligomeric guanidines can be achieved in either solution or solid-phase. Incorporation of TFA-sensitive arylsulfonyl groups, such as Pbf, during the synthesis greatly simplifies deprotection procedures for obtaining the final oligomeric guanidine products. © 2003 Elsevier Science Ltd. All rights reserved.

Structure-based design of high-affinity multivalent protein ligands has attracted much attention recently.[1–8] We have successfully demonstrated up to one million-fold enhancement in inhibitory power of structurally-complementary penta- or decavalent ligands to the receptor-binding process of cholera toxin or the closely related *E. coli* heat-labile enterotoxin over the corresponding monovalent ligands.[5,7,8] In those systems, very long and flexible linkers were employed to achieve complementarity between the effective dimension of the ligand and the binding site distribution of the protein targets. Our recent success[7,8] in obtaining high-resolution crystal structures of the target protein bound to the multivalent ligands has opened up new opportunities in designing novel multivalent ligands. Through a structure-based approach, incorporating functional groups into the linker portion of multivalent ligands offers more possibilities to improve affinities and specificities. However, the current linker backbone is not an adequate choice due to its already low solubility in aqueous solutions.[7] It is therefore very desirable if highly water-soluble backbones are available for multivalent ligand synthesis, to which many functional groups may be attached to provide favorable interactions to the protein target's surface. In this regard, oligomeric structures bearing peptide-like side chains and built on repeating N,N'-substituted guanidine units are good candidates.

For the oligomeric structures of peptides and the many other types of unnatural oligomers aimed at mimicking peptides, efficient solution or solid-phase synthetic methodologies have been widely documented and reviewed.[9–14] In contrast, to the best of our knowledge, there is no example of general synthesis of oligomeric guanidines presenting peptide-like side chains. Although in special cases layered symmetrical aromatic oligomeric guanidines have also been synthesized,[15] general solid-phase and solution oligomeric guanidine syntheses have been reported mainly from two research groups. The most successful examples are the oligomeric deoxynucleic and ribonucleic guanidines that have been extensively studied by the Bruice group.[16–25] The most efficient solid-phase method for this class of oligomeric guanidines employed alkoxycarbonyl activated thiourea for guanidinylation at the elongation step.[20,23] In those methods, the non-TFA-cleavable protection on all the guanidine groups required additional handling after solid-phase synthesis, which may not be desirable for incorporation of peptide-mimetic oligomeric guanidines. In addition, those procedures relied on the use of toxic mercury compound to promote guanidinylation.[20] The Anslyn group also investigated various solid-phase oligomeric guanidine synthesis methods.[26] Unfortunately, only benzoyl activated thiourea gave significant yields of oligomeric products and an additional deprotection step under harsh conditions was also required.[26] Furthermore, none of the oligomeric products contained peptide-like side chains.[26] It is clear that alternative and highly efficient general syntheses of oligomeric guanidi-

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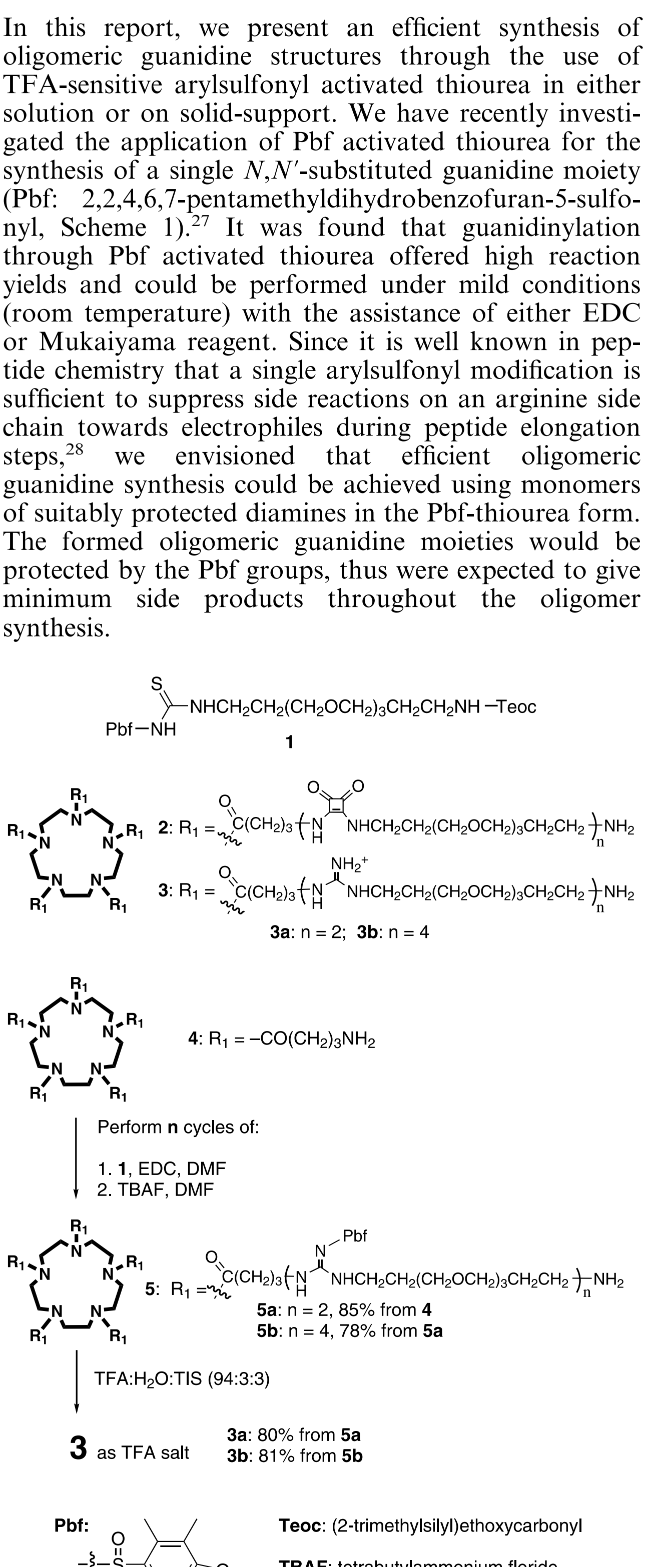
nes will be a valuable tool for access to compounds that are important both for general peptido-mimetics and for our structure-based multivalent ligand design as mentioned earlier.

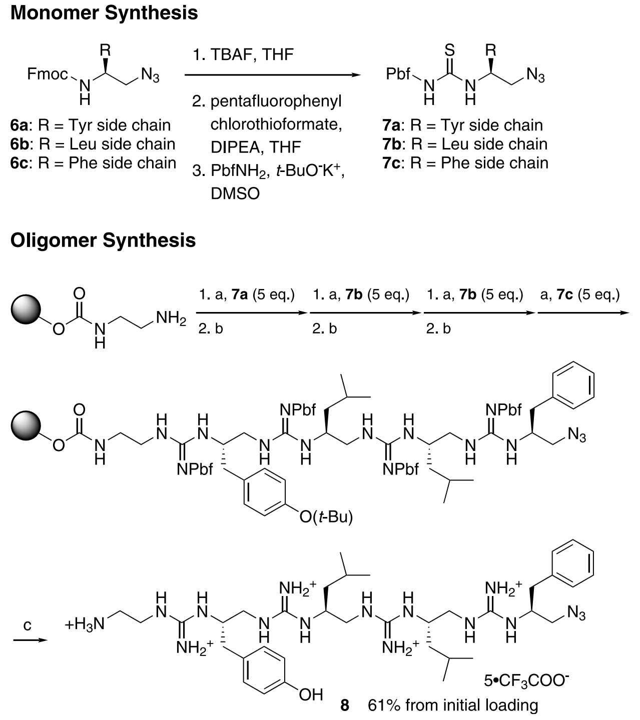
In this report, we present an efficient synthesis of oligomeric guanidine structures through the use of TFA-sensitive arylsulfonyl activated thiourea in either solution or on solid-support. We have recently investigated the application of Pbf activated thiourea for the synthesis of a single N,N'-substituted guanidine moiety (Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, Scheme 1).[27] It was found that guanidinylation through Pbf activated thiourea offered high reaction yields and could be performed under mild conditions (room temperature) with the assistance of either EDC or Mukaiyama reagent. Since it is well known in peptide chemistry that a single arylsulfonyl modification is sufficient to suppress side reactions on an arginine side chain towards electrophiles during peptide elongation steps,[28] we envisioned that efficient oligomeric guanidine synthesis could be achieved using monomers of suitably protected diamines in the Pbf-thiourea form. The formed oligomeric guanidine moieties would be protected by the Pbf groups, thus were expected to give minimum side products throughout the oligomer synthesis.



Scheme 1. Solution synthesis of oligomeric guanidine linked core-linker assembly for multivalent ligands.

We first attempted solution synthesis of oligomeric guanidines using Teoc ((2-trimethylsilyl)ethoxycarbonyl) protected monomer **1**.[29] As shown in Scheme 1, we aimed to synthesize the oligomeric guanidine linked core-linker assemblies **3**, which are similar to squarate linked molecule **2** reported in our previous multivalent ligand systems.[5] Because there were five primary amines in the pentacyclen-based core[5] **4** at the start of synthesis, each guanidinylation step allowed for attachment of five monomers. This resulted in a substantial increase in molecular weights during the elongation step going from starting material to the product. It therefore enabled convenient product purification using size-exclusion chromatography.[30,31] A complete cycle of oligomeric chain formation consisted of two simple reactions: i) an EDC promoted guanidinylation in 17 h with 12 equiv. of monomer **1** (2.4 equiv. per reaction site) in DMF at room temperature; and ii) deprotection with tetrabutylammonium floride (TBAF) in DMF for 1 h at room temperature. After each guanidinylation step, the product was purified by size-exclusion chromatography in DMF. As shown in Scheme 1, very high yields of oligomeric guanidines were obtained in Pbf protected form. With two cycles of chain formation, **5a** was obtained in 85% isolated yield from the starting core **4**. With two more cycles of reaction, **5b** with 20 Pbf-protected guanidine units was obtained from **5a** in 78% isolated yield. Final removal of the Pbf moiety was again very effectively achieved by treating **5** with a mixture of TFA:H_2O:TIS (94:3:3, TIS: triisopropylsilane) at room temperature for 2–4 h. In the case of deprotecting **5b** to **3b**, all 20 Pbf groups were cleanly removed using this procedure and the final product **3b** was isolated in 81% yield (as TFA salts) after HPLC purification.

Of course, the current oligomeric guanidine based multivalent backbones do not include any functional side chains for potential interactions with target proteins. We can imagine one possible solution that if a short oligomeric guanidine bearing peptide-like side chains is pre-synthesized, it can then replace one or more of the linker units in **2** or **3** using the proven squarate-based coupling method.[5] Therefore, we turned our attention to find solid-phase synthetic conditions for the general synthesis of oligomeric guanidines bearing peptide-like side chains. A solid-phase approach is shown in Scheme 2, where the Pbf-thiourea monomer bearing an azide as a mask of amine was used for oligomer elongation. Reduction of the azide on the support following literature reported procedure[32–34] provided a terminal amine for further elongation. All monomers were prepared from commercially available and side chain protected amino alcohols. The amino alcohols were first turned into the azides **6a**–**c**.[35] After Fmoc removal, all monomers were synthesized following previously reported procedures to form the desired Pbf-thiourea moiety.[27] Before the synthesis of a sequence with multiple different side chains, we first investigated the conditions for solid-phase guanidinylation and azide reduction using monomer **7a** bearing a tyrosine-like



Scheme 2. Solid-phase synthesis of oligomeric guanidine. *Reagents and conditions*: (a) 5 equiv. EDC, 10 equiv. DIPEA, DMF, rt, 2–16 h; (b) 5 equiv. $SnCl_2$:PhSH:DIPEA (1:4:5), THF, rt, 1 h; (c) TFA:H_2O:TIS (94:3:3), rt, 2 h.

side chain. Up to four cycles of guanidinylation and azide reduction were performed on solid-support at room temperature (data not shown), with variations in reaction time as well as the molar excess of reagents relative to resin-bound materials. Product formation was monitored by LC–MS analysis after cleavage. After checking guanidinylation and reduction efficiencies, a minimum reaction time for each step was established. It turned out that the guanidinylation can be accomplished in 2 h at room temperature with 5 equiv. of monomer **7a**. Azide reduction was completed in 1 h.

With this information in hand, we then synthesized an oligomeric guanidine version of a naturally occurring hydrophobic peptide sequence, β-lactorphin Tyr-Leu-Leu-Phe, which was shown to improve arterial function in an animal model.[36] For convenience, we first added a glycine mimic to our oligomeric guanidine sequence by reacting *p*-nitrophenyl carbonate Wang resin with excess amounts of ethylenediamine. Then cycles of guanidinylation and azide reduction were performed with monomers **7a**–**c**. While the time for guanidinylation was 2–3 h with **7a** and **7c**, reactions with **7b** were left overnight (16 and 9 h) for convenience. Final cleavage with concomitant Pbf removal was achieved with a mixture of TFA, water and TIS in 2 h at room temperature. The crude product was washed with ether, and then purified by HPLC to afford the final oligomeric guanidine product **8** as its penta-TFA salt in 61% overall yield based on initial loading of the Wang resin. This suggested that each individual oligomer elongation and reduction cycle achieved ~90% yield. It is also important to note that during HPLC purification of the final oligomer **8**, only a small amount (< 5%) of truncated but azide-reduced tri-guanidine oligomer was found. No shorter truncation was detected. This suggests that when longer oligomer is synthesized, a 2-h coupling step will not be optimal and there is room for further improvements.

Our solid-phase method represents an improved procedure over the well studied protocol developed by the Bruice group for oligomeric ribonucleic guanidines. Concomitant removal of guanidine protection (the Pbf group) in the final cleavage step is one advantage. The avoidance of using mercury salts and the associated extra step for mercury removal[20,23] simplifies our reaction procedures. Compared to the solid-phase procedures reported by the Anslyn group,[26] our method offered much improved final isolated yields for oligomeric guanidines with facile deprotection steps. In addition, we have achieved in solid-phase synthesis the preparation of oligomeric guanidines bearing peptide-like side chains.

In summary, we have shown efficient syntheses of oligomeric guanidines both in solution and on solid support using TFA-sensitive arylsulfonyl activated thiourea as monomers. The presented synthetic routes offer high yields and mild reaction conditions, and are good complements to existing methodologies. Examples described in this report also demonstrate the access to highly water soluble backbones for designing novel multivalent protein ligands and to potential oligomeric guanidine based peptido-mimetics.

Acknowledgements

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29. All compounds were characterized with satisfactory spectroscopic data. For the final oligomric guanidine products, the characterizations are listed below: Compound **3a** (20 mg, 80% from **5a**), ^{1}H NMR (D_2O): δ 3.60–3.38 (m, 140H), 3.11–3.06 (t, 40H, $J=6.7$ Hz), 2.93–2.89 (t, 10H, $J=7.3$ Hz), 2.50–2.30 (br, 10H), 1.80–1.62 (br, 50H); ESI-mass m/z: 442.9 $(M+H)^{7+}$, 387.6 $(M+H)^{8+}$, 344.7 $(M+H)^{9+}$, 310.3 $(M+H)^{10+}$, 282.2 $(M+H)^{11+}$, 258.7 $(M+H)^{12+}$, 238.9 $(M+H)^{13+}$, 221.9 $(M+H)^{14+}$; HPLC purity >95%. Compound **3b** (55 mg, 81% from **5b**), ^{1}H NMR (D_2O): δ 3.60–3.33 (m, 260H), 3.13–3.09 (t, 80H, $J=6.5$ Hz), 3.02–2.91 (t, 10H, $J=7.0$ Hz), 2.58–2.26 (br, 10H), 1.82–1.61 (m, 90H); ESI-mass m/z: 427.6 $(M+H)^{13+}$, 397.1 $(M+H)^{14+}$, 370.7 $(M+H)^{15+}$, 347.6 $(M+H)^{16+}$, 327.3 $(M+H)^{17+}$, 309.1 $(M+H)^{18+}$, 292.9 $(M+H)^{19+}$, 278.3 $(M+H)^{20+}$, 265.1 $(M+H)^{21+}$, 253.0 $(M+H)^{22+}$, 242.1 $(M+H)^{23+}$; HPLC purity >95%. Compound **8** (33 mg, 61% from initial resin loading), ^{1}H NMR (D_2O): δ 7.36–6.99 (m, 5H), 6.92 (d, 2H, $J=8.8$ Hz), 6.64 (d, 2H, $J=8.3$ Hz), 3.87–3.60 (br, 2H), 3.59–2.08 (m, 18H), 1.53–0.93 (br, 6H), 0.93–0.33 (m, 12H); ESI-mass m/z: 735.4 $(M+H)^+$; HPLC purity >95%.
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