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An Efficient, Scalable Synthesis of the Molecular Transporter Octaarginine via a Segment Doubling Strategy†

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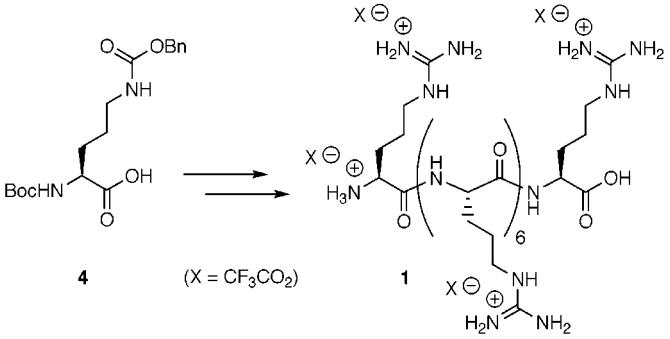
ABSTRACT



Short oligomers of arginine function as remarkably efficient molecular transporters of drugs and probe molecules into cells and tissue. Currently, these compounds are prepared on resin through a unidirectional solid-phase synthesis. To extend the utility of these compounds for therapeutic and research applications, a scalable solution-phase synthesis of Arg_8 (1) has been developed on the basis of a segment doubling strategy that proceeds in 13 steps and 28% overall yield from 4, including a novel one-step perdeprotection–perguanidinylation reaction.

While considerable structural diversity is found among drugs and probe molecules, the physical properties of most of these agents with intracellular targets are limited to a narrow log P range to ensure solubility in the polar extracellular milieu and passive diffusion through the nonpolar lipid bilayer of the cell. Agents falling outside of this range must often be tuned through reiterative analogue synthesis to achieve the optimum balance of water solubility and passive membrane transport. A promising new approach directed at improving or enabling the cellular uptake of drugs or drug candidates possessing a wider range of physical properties involves the use of peptide-based molecular transporters to carry these agents actively into cells.[1−7] Representative of this approach,

† During review a concern was raised about our original preference for the use of "bidirectional" to describe this strategy. It is noted in reviews (see Poss, C. S.; Schreiber, S. L. *Acc. Chem. Res.* **1994**, *27*, 9−17 and Magnuson, S. R. *Tetrahedron* **1995**, *51*, 2167−2213) that both "simultaneous" and "sequential bidirectional" syntheses are possible and that the second is of lesser importance because such a strategy putatively offers no step-saving advantage over a linear (unidirectional) synthesis. However, this restricted definition did not anticipate the work described herein as it **is** a sequential bidirectional synthesis **and** it **does** offer significant step savings. The term "segment doubling" was introduced as an alternative name for this strategy. Abbreviations: Boc = *tert*-butoxycarbonyl; Z = benzyloxycarbonyl; Fmoc = 9-fluorenylmethoxycarbonyl; Mtr = 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; NMM = *N*-methylmorpholine; DMAP = 4-(dimethylamino)pyridine; RP-HPLC = reverse phase high performance liquid chromatography; TFA = trifluoroacetic acid.

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we have recently shown that homooligomers (7−9 mers) of L-arginine upon conjugation to various probe molecules (e.g., fluorescein) or drugs (e.g., cyclosporin A (CsA)) provide highly water-soluble conjugates that rapidly enter cells (e.g., human Jurkat).[1,2] In addition, drug conjugates of these arginine transporters have been shown to exhibit novel and significant penetration into human skin and to release their drug cargo in targeted T cells.[8]

The enormous potential of arginine-based molecular transporters is limited for several applications only by their availability and cost. Such homooligopeptides are usually prepared using solid-phase peptide synthesis.[1,2,9−11] Although this approach is readily automated and allows for the synthesis and purification of long peptides, it suffers drawbacks including high cost, limited scalability, and the need for resin attachment and cleavage. In contrast, solution-phase synthesis avoids the scale and cost restrictions of resins (the cost of the resin-based synthesis is more than an order of magnitude greater than that of the solution-phase synthesis) and, in the particular case of certain oligomers, can be conducted using a step-saving segment doubling strategy (Figure 1).[12]

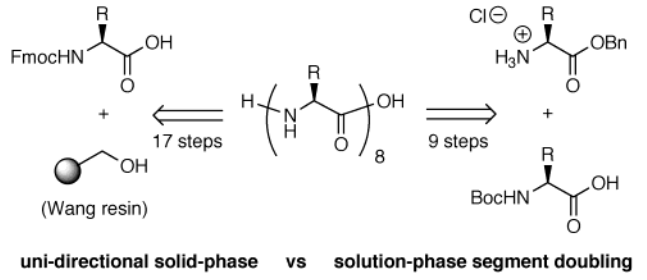

Figure 1. Step count comparison between solid-phase and solution-phase segment doubling strategies.

Illustrative of the latter point, the unidirectional synthesis of an octamer employing solid-phase synthesis requires 17 steps (one coupling and deprotection step for each added monomer and one resin cleavage step), whereas a solution-phase segment doubling synthesis[13−15] of the same octamer would require only 9 steps (three coupling and six deprotection steps). In the specific case of arginine-based peptides, solution-phase synthesis offers the additional advantage of avoiding expensive protecting groups for the guanidinium subunit (e.g., Mtr,[16] Pmc,[17] and Pbf[18]) required in solid-phase synthesis. We report now the first segment doubling synthesis of the arginine oligomer **1** that is both cost-effective and scalable.

Our first strategy directed at the synthesis of **1** involved coupling *arginine* monomers (Figure 2). Of the commercially

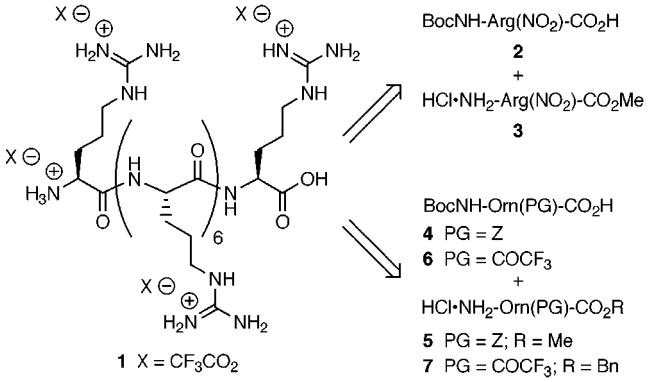

Figure 2. Retrosynthetic analysis.

available arginine monomers that contain differentially protected functionalities, FmocNH-Arg(Pbf)-CO_2Me and BocNH-Arg(NO_2)-CO_2Me, we chose to utilize the latter because of the ease of Boc deprotection in solution and the stability of the intermediate amine salts (which are easy to handle and less susceptible to intramolecular lactamization). The Boc group can be deprotected using either HCl or TFA,[19] the methyl ester of the carboxyl terminus is base-labile (NaOH),[20] and the nitro protecting group of the guanidine can be removed by hydrogenation.[21] Isobutyl chloroformate was used for coupling commercially available BocNH-Arg-(NO_2)-CO_2H (**2**) and HCl·NH_2-Arg(NO_2)-CO_2Me (**3**) as the reagent can be removed in vacuo and has been used to couple

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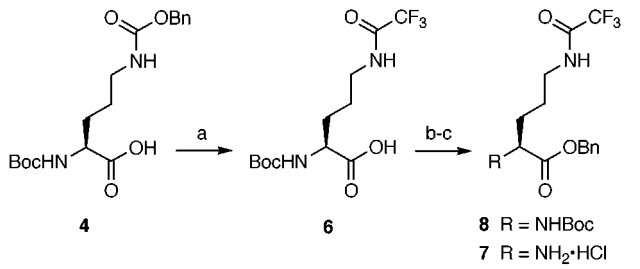
protonated arginine.[22] Unfortunately, low yields of the dimer were obtained (~30%) and all attempts to deprotect the methyl ester of the dimer unexpectedly resulted in decomposition. This strategy was set aside therefore in favor of a more attractive plan based on the use of *ornithine* monomers.

The conversion of oligoamines into oligoguanidines using a perguanidinylation reaction has been previously demonstrated by us (guanidinylated peptoids)[1] and others (guanidinoglycosides).[23,24] As such, the *arginine* octamer **1** could in principle be prepared from an *ornithine* octamer through a late stage perguanidinylation reaction. Orthogonally protected ornithine monomers that are commercially available include BocNH-Orn(Z)-CO_2H (**4**) and HCl·NH_2-Orn(Z)-CO_2-Me (**5**). Thus the orthogonal protecting group strategy for ornithine utilized an acid-labile Boc group on the α-amine, a hydrogenation-labile Z group on the δ-amine, and a base-labile methyl ester on the carboxyl terminus (Figure 2). This strategy yielded promising results at the outset (initial couplings and subsequent deprotections). However, the Z-protected ornithine tetramers proved to have limited solubility in organic solvents, representing a significant problem for scale-up procedures.

To improve the solubilities of the ornithine oligomers, a new protection strategy was required. Previous experience in our group had demonstrated that trifluoroacetamide-protected oligoamines were readily soluble in ethyl acetate. Thus, our synthesis was revised to incorporate the base-labile trifluoroacetamide protecting group on the δ-amine of ornithine. In addition to α-amine Boc protection, the remaining orthogonal protecting group was a hydrogenation-labile benzyl ester on the carboxyl terminus. The requisite ornithine monomers needed to pursue the synthesis of **1**, BocNH-Orn-($COCF_3$)-CO_2H (**6**) and HCl·NH_2-Orn($COCF_3$)-CO_2Me (**7**), were prepared from **4** (Scheme 1). Protecting group interconversion of the Z group of **4** to the corresponding trifluoroacetamide of **6** was accomplished in quantitative yield by hydrogenation followed by treatment with ethyl trifluoroacetate. Esterification of **6** was accomplished using a known procedure[25] by treatment with benzyl chloroformate and DMAP (20 mol %) to give **8** in quantitative yield. Finally, removal of the Boc group with HCl gave acid **7** in 98% yield.

Scheme 1. Monomer Preparation[a]



[a] Conditions: (a) (i) Pd/C, MeOH, H_2; (ii) EtO_2CCF_3, Et_3N (>99%). (b) (i) ClC(O)OBn, NMM, THF −15 °C; (ii) DMAP (>99%). (c) HCl, EtOAc (98%).

Our revised synthesis was then initiated by the coupling of acid **6** with amine **7** using isobutyl chloroformate for activation of **6** and NMM as a base (Scheme 2). This reaction proceeded smoothly to give the fully protected ornithine dimer **9** in 97% yield with sufficient purity after extractive workup to be utilized directly in subsequent reactions. Ornithine dimer **9** was divided into two equal portions. The first part was hydrogenated, giving **11** in quantitative yield, while the second part was treated with HCl, giving the amine hydrochloride salt **10** in 83% yield. Both compounds were of sufficient purity after workup to be utilized directly in the subsequent coupling.

The ornithine dimers **10** and **11** were subsequently coupled with isobutyl chloroformate and NMM and upon extractive workup and purification through a short plug of silica gel gave the ornithine tetramer **12** in 83% yield. As anticipated, **12** was readily soluble in ethyl acetate on a multigram (4 g) scale. The fully protected tetramer **12** was then divided into two equal portions, and each was subjected to the appropriate conditions for the preparation of the free acid **14** and the amine hydrochloride salt **13**, respectively. Coupling **13** and **14** in the usual fashion (isobutyl chloroformate and NMM) proceeded smoothly to give the fully protected ornithine octamer **15** in 83% yield and in sufficient purity to be utilized in subsequent reactions. At this point, attempts to convert the trifluoroacetamides into guanidines via a two-step procedure, involving the initial deprotection of the trifluoroacetamides, gave significant quantities of lactamization at the carboxyl terminus (involving condensation of the free amine onto the benzyl ester). Thus, deprotection of the benzyl ester was deemed necessary before proceeding with the synthesis. Therefore, **15** was hydrogenated to remove the benzyl ester, giving the free acid **16** in quantitative yield.

At this stage, we envisioned that a novel perdeprotection−perguanidinylation process could be achieved in one operation to yield perguanylated **18**. Since aqueous sodium carbonate has previously been utilized to effect the deprotection of trifluoroacetamides[26] and also as one of the reagents in the guanidinylation of amines,[1,27] we reasoned that it would be possible to perform both processes in one operation. This hypothesis proved to have merit, as treatment of the octaornithine derivative **16** (Scheme 3) with sodium carbonate and pyrazole-1-carboxamidine hydrochloride (**17**) in aqueous methanol gave the octaarginine derivative **18** in 51% isolated yield after purification by RP-HPLC (99+% purity, with no observed unreacted amine products observed by MS) and lyophilization. Significantly, eight trifluoroacetamides were converted to eight guanidines in one step (16 transformations overall) under mild conditions. Attempts

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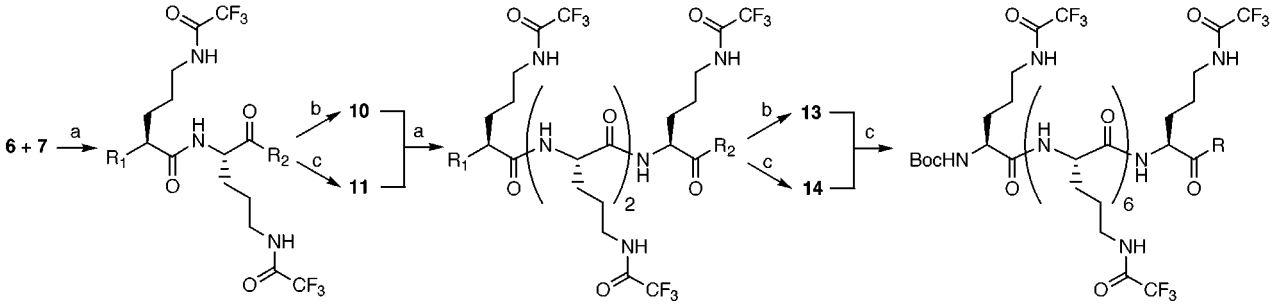
Scheme 2. Segment Doubling Assembly of Ornithine Octamer[a]



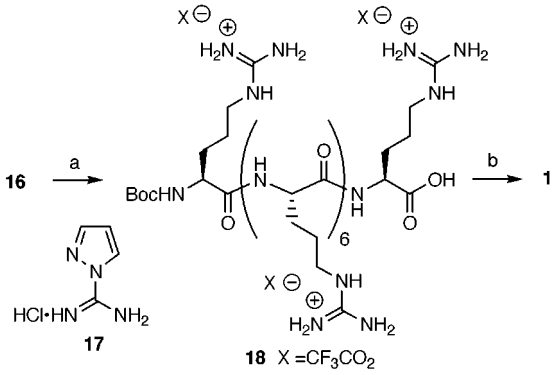
9 R_1 = NHBoc; R_2 = OBn (97%)
10 R_1 = NH_2•HCl; R_2 = OBn (83%)
11 R_1 = NHBoc; R_2 = OH (> 99%)
12 R_1 = NHBoc; R_2 = OBn (83%)
13 R_1 = NH_2•HCl; R_2 = OBn (> 99%)
14 R_1 = NHBoc; R_2 = OH (> 99%)

[a] Conditions: (a) (i) acid, *i*-BuOCOCl, NMM, THF, DMF; (ii) amine, NMM. (b) HCl, EtOAc or dioxane. (c) H_2, Pd/C, MeOH.

to use cyanamide to effect the perguanidinylation were unsuccessful.[28] Finally, the synthesis was completed by treatment of **18** with TFA, which gave the desired octaarginine product **1** in quantitative yield. Octaarginine **1** was identical in all respects to an authentic sample prepared using Fmoc-based solid-phase synthesis.

In summary, a solution-phase synthesis of the novel peptide molecular transporter, octaarginine **1**, was completed in 10 steps and 29% overall yield from protected ornithine monomers **6** and **7.** The choice of appropriate orthogonal protecting groups proved essential to the success of this synthesis, and the segment doubling strategy provided a substantial improvement in the preparation of arginine homooligomers such as **1** in terms of both cost (>10-fold lower) and scalability compared to the previously reported synthesis utilizing a solid-phase strategy.[2] The mild conversion of trifluoroacetamides directly to guanidines allows one to consider a trifluoroacetamide as a masked guanidine, thus avoiding the need to use expensive guanidine protecting groups normally required in peptide synthesis. Overall, this work lays the foundation for the efficient preparation of greater quantities of **1** to further in vitro, in vivo, and clinical studies (in progress) of conjugates of **1** with drugs, drug candidates, and molecular probes.

Scheme 3. Perguanidinylation to Yield **1**[a]



[a] Conditions: (a) **17**, Na_2CO_3, aq. MeOH, 55 °C (51%). (b) TFA (>99%).

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Supporting Information Available: Full experimental details and characterization data for compounds **1**, **6**−**16**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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