An Improved, Scalable Synthesis of bis-Amino Acids

Jae Eun Cheong^{§‡}, Conrad T. Pfeiffer^{§‡}, JD Northrup^{§‡}, Matthew F. L. Parker[†], Christian E. Schafmeister^{§*}

[§]Department of Chemistry, Temple University, 1901 N. 13th Street, Philadelphia, Pennsylvania 19122, United States of America

[†]Department of Radiology & Biomedical Imaging, University of California, San Francisco

[‡]Author's contributed equally

*Corresponding author. Tel.: +1 2152047128; e-mail: meister@temple.edu

Abstract

Trans-4-hydroxy-*L*-proline derived bis-amino acids are chiral, cyclic building blocks that display two alpha-amino acids that are differentiated from each other with protecting groups. They are assembled into spiroligomers – rigid, shape-programmable spirocyclic oligomers that are both stereochemically and functionally diverse. The synthesis presented here focuses on recent improvements that allow for a convenient, large-scale synthesis of twelve stereochemically pure bis-amino acids from inexpensive *trans*-4-hydroxy-*L*-proline. The bis-amino acids differ in stereochemistry as well as the amine protecting group, one of which (*para*-nitrobenzyl carbamate) has not been previously incorporated into bis-amino acids.

Keywords:

Spiroligomer; Bis-amino acids; Amino acids; Peptidomimetics; Diastereomers

1. Introduction

Proteins act as scaffolds to position multiple chemically active groups in three-dimensional space, either inward in the case of enzymes, or outward to interact with other proteins. The fields of peptidomimetics and foldamers seek to mimic the functions of proteins using unnatural building blocks that create specific three-dimensional structures.¹⁻⁵ Common challenges shared by all foldamers is predicting and controlling folding into specific three-dimensional structures.^{2,5-7} To this end, we have developed spiroligomers,⁸ oligomers composed of *trans*-4-hydroxy-*L*-proline derived bis-amino acids⁹⁻¹¹ connected through pairs of

amide bonds to form ladder molecules containing linkages formed by diketopiperazines (DKP) rather than single amide bonds. By forming spirocyclic DKPs, the spiroligomer backbone has no rotatable bonds; therefore, they are inherently rigid molecules with complex and predictable three-dimensional shapes. Functionalization is possible at specific positions on this backbone, which altogether allows for a pre-organized, stereochemically and functionally diverse oligomer that has been utilized for a variety of applications including demonstration of a transesterification catalyst,¹² a proline-aldol catalyst,¹³ an aromatic Claisen rearrangement catalyst,¹⁴ a spiroligomer that binds the protein MDM2,¹⁵ the formation of supramolecular metal binding complexes,¹⁶ the first donor-bridge-acceptor molecule that demonstrated acceleration of electron transfer in water,¹⁷ a metal binding mechanical molecular actuator,¹⁸ and molecular rulers.¹⁹

2. Results and discussion

The goal of this work is to scale up the synthesis of *trans*-4-hydroxy-*L*-proline derived bis-amino acids to make this chemistry more accessible to others, to lower the environmental impact of the synthesis, and to access larger quantities of bis-amino acids in less time. The syntheses presented here demonstrate recent advancements that allow for the large-scale synthesis (~200-250 grams theoretical yield of hydantoin products) of twelve different bis-amino acids, some of which have been previously reported by our group but now have much improved syntheses.^{8-10,20} Increasing the concentration of the reactions and workup procedures, while increasing the scale of the synthesis and purifications, has led to a reduction in the overall volume of organic solvents needed throughout the synthetic effort. Additionally, we have reduced the equivalents of carcinogenic chromium trioxide and toxic potassium cyanide used during synthesis. As described by our group,^{20,21} the overall shape of any spiroligomer is controlled by the stereochemistry of each bis-amino acid subunit, making it important to have access to all stereoisomers. Furthermore, having synthetic access to different, orthogonal protected versions of the bis-amino acids allows more flexibility in the choice of chemistry used to assemble them into functional spiroligomer nanostructures. (e. g. solution phase versus solid phase.)

Scheme 1: Reaction scheme detailing the synthesis of all four stereoisomers of hydantoins. Compound **12** is the enantiomer of **5**, and compound **11** the enantiomer of **6**. (a) Cbz-Cl, NaHCO₃, 1:10 dioxane/water (b) Jones reagent, acetone, 0 °C to rt; (c) Isobutylene, H₂SO₄ (cat.), CH₂Cl₂, 0 °C to rt; (d) (NH₄)₂CO₃, KCN, 1:1 EtOH/water, sealed tube; (e) (i) AcOH/Ac₂O, Reflux; (ii) 2M HCl, reflux

The synthesis begins with the Cbz protection of the pyrrolidine nitrogen of *trans*-4-hydroxy-*L*-proline (**1**) employing Schotten-Baumann conditions,²² as shown in Scheme 1. The Schotten-Baumann conditions allow the pyrrolidine protection in a single step at room temperature. The next step of the synthesis oxidizes the secondary alcohol, **2**, to the corresponding ketone, **3**, via Jones oxidation. We have attempted many other oxidation methods for this step; however, only a method using trichloroisocyanuric acid (TCCA/TEMPO)²³ provided comparable yields. Unfortunately, this reaction also produced cyanuric acid as a side product that could not be removed without extensive chromatography, which led to lower yields in subsequent reactions. Since the other oxidation methods were fruitless, we reduced the number of equivalents of carcinogenic chromium trioxide used during the reaction to 38% of what was previously reported (from 8 equivalents to 3), and compensated for this reduction by increasing the reaction time. The next step of the synthesis is the *tert*-butyl protection of **3** to yield ester **4**.⁹ A Bucherer-Bergs reaction²⁴ is used to form the diastereomeric hydantoins **5** and **6** in a sealed, round bottom flask at room temperature. In this synthesis, the number of equivalents of toxic potassium cyanide is reduced to 70% of what we had previously reported (from 1.5 equivalents to 1.05).

The diastereomers formed from the Bucherer-Bergs reaction, **5** and **6**, were obtained in a combined yield of 63% (5:1 ratio of ss:sr) over four reactions after purification by normal phase flash chromatography with a 0-10% IPA in DCM gradient which was carried out in multiple batches due to volume limits of our

automated flash chromatography system. By switching to an IPA/DCM solvent system, we have improved the separation of an impurity that elutes just prior to the major product peak. Also of note, it is possible to isolate and recycle the DCM and IPA by extractive distillation²⁵, which would be financially and environmentally responsible, should the scale of the purification be further increased in the future.

Scheme 2. Reaction scheme detailing the specific protecting group conversion, and hydrolysis of the hydantoin. Compounds shown in orange are the enantiomers of those shown in black, and those shown in red are the enantiomers of those shown in blue. (a) 5 wt % Pd/C, H₂ (g), THF, Boc₂O; (b) (i) 5 wt % Pd/C, H₂ (g), THF; (ii) Ar_(g), pNZ-Cl, DIPEA; (c) (i) (Boc)₂O, DMAP, THF, rt (ii) 2 M KOH, 0 °C

After separation of the hydantoin diastereomers, the Cbz group attached to the pyrrolidine nitrogen may be converted to a Boc protecting group (Scheme 2, a) or a *p*-nitrobenzyloxycarbonyl protecting group (pNZ, Scheme 2, b.)^{22,26} Synthetic access to multiple protecting groups is advantageous when assembling bis-amino acids into spirologomers, since one protecting group may be more suitable depending on the implemented synthetic method (e. g. solution versus solid phase synthesis.) Once the desired pyrrolidine nitrogen protecting group is installed, each diastereomer is simultaneously Boc protected at both of the imide and amide nitrogens of the hydantoin, followed by the hydrolysis of the hydantoin under mild conditions by addition of 2 M KOH. This hydrolysis reveals the second amino acid at what was originally the position of the alcohol of *trans*-4-hydroxy-*L*-proline. Each bis-amino acid is isolated by precipitation via neutralization of the basic solution, with yields that range from 21.5% for **27** to 91.7% for **25**, with the average being 61% from the Cbz-protected hydantoin **5**, **6**, **11**, or **12**. They are then dried and stored at room temperature in a desiccator until needed. The synthetic steps outlined have

also been applied to *cis*-4-hydroxy-*D*-proline, which was obtained by epimerizing the C-2 acid of *trans*-4-hydroxy-*L*-proline using a published method.^{27,28}

3. Conclusion

We have greatly improved and increased the scale of the synthesis of proline-based bis-amino acid monomers. This synthesis is not only divergent, with all twelve bis-amino acids stemming from a common *trans*-4-hydroxy-*L*-proline feedstock, but it is also more convenient and environmentally friendly than our previous work. Three different protected forms of the bis-amino acid (pNZ, Cbz, and Boc) are described, which increase the versatility of these building blocks for different solid-phase and solution phase syntheses of oligomers.

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Graphical Abstract