Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2339–2344

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

HIGHER ORDER IMINODIACETIC ACID LIBRARIES FOR PROBING PROTEIN–PROTEIN INTERACTIONS

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Received 15 June 1998; accepted 25 June 1998

Abstract: Higher order iminodiacetic acid diamide trimer (560 compounds) and tetramer libraries (1260 compounds) are described and were assembled in a convergent multistep solution-phase synthesis for use in studying protein–protein interactions.

Ligand-induced receptor and protein dimerization or oligomerization has emerged as a general mechanism of signal transduction[1–10] and important therapeutic applications may arise from either the development of antagonists or agonists of such protein dimerization.[11] Our interest in studying such events has led us to explore the potential of utilizing a common approach to the discovery of ligand binding antagonists and their dimerization/oligomerization conversion to agonists, mimicking an endogenous ligand (Figure 1).[12] We have disclosed two approaches that entail the dimerization linkage of iminodiacetic acid diamides[13] using symmetrical dicarboxylic acids[14] or the olefin metathesis reaction to join and randomize the length of the linking tether.[15] In the latter case, we have also disclosed the two-fold dimerization[11,16] linkage of iminodiacetic acid diamides to provide higher order libraries containing up to eight variable groups and the accompanying powerful technique of deletion synthesis deconvolution[16] to identify lead compounds derived from unsymmetrical dimerization combinations. Unlike the linear divergent synthesis of libraries which is characteristic of solid-phase synthesis, the convergent dimerizations are especially suited for solution-phase synthesis and would be precluded by typical solid-phase techniques where the combining components are on mutually exclusive solid phases. Herein, we provide a summary of related efforts on the preparation of such higher order libraries that rely on a coupling of iminodiacetic acid diamides with tricarboxylic acids or sequential couplings with dicarboxylic acids.

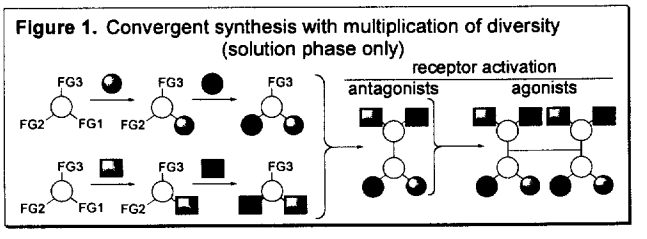

Figure 1. Convergent synthesis with multiplication of diversity (solution phase only)

Trimerization of Iminodiacetic Acid Diamides. Complementary to the approach of assembling symmetrical iminodiacetic acid diamides dimers through a final *N*-acylation with symmetrical dicarboxylic acids,[14] a library of 560 symmetrical trimers was assembled in a 8 × 10 × 7 matrix with the final diversification

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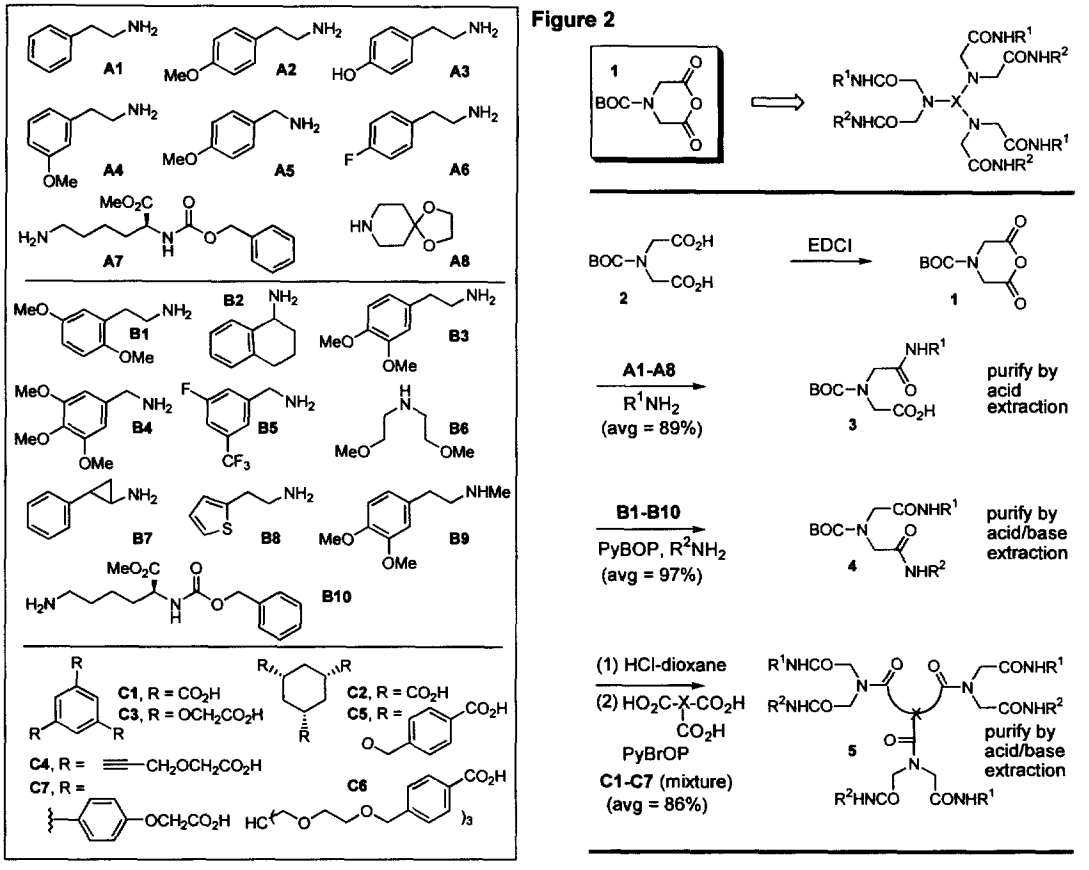
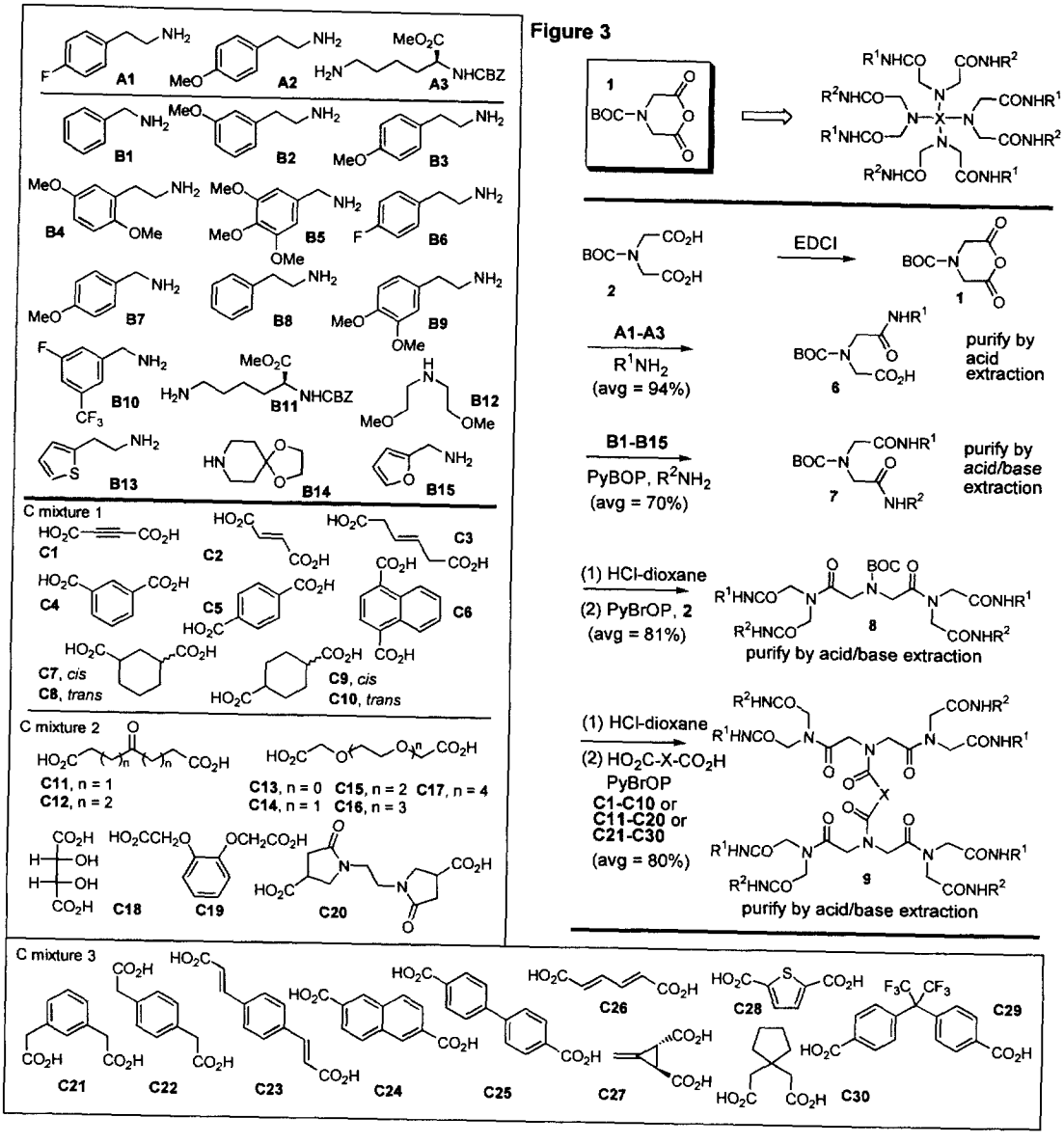


being conducted with the **C1–C7** mixture to provide a mixture of 7 compounds containing variations only in the linking tricarboxylic acid domain (Figure 2). For the 3-step synthesis, this utilized 8 amines (**A1–A8**), 10 amines (**B1–B10**), and 7 tricarboxylic acids (**C1–C7**) in 168 reactions to produce 80 sublibraries each containing 7 compounds. The library screening is conducted with small mixtures of 7 compounds, deconvolution by resynthesis of the 7 individual components of the final mixtures from archived samples of the 80 immediate precursors **4** is straightforward, and the mixtures differ only in the structure of the linking tricarboxylic acid. The preceding or simultaneous examination of the intermediate *N*-BOC iminodiacetic acid diamides permits, in principle, the identification of competitive binders (antagonists) that upon trimerization might function as agonists of ligand-induced protein homodimerization or trimerization.

Reaction of *N*-BOC-iminodiacetic acid with EDCI (1.0 equiv, DMF, 25 °C, 1 h) and subsequent treatment with **A1–A8** (1 equiv, DMF, 25 °C, 70–99%) was conducted on a 20 mmol scale to provide 6–10 g of each of the 8 monoamides **3** in superb yields (avg = 89%). Simply washing the crude product diluted with

EtOAc with 10% aqueous HCl and saturated aqueous NaCl served to remove unreacted amine, EDCI and its byproducts to provide the pure monoamides (≥95% pure). Each monoamide was divided into 10 portions and treated with the 10 amines **B1–B10** (1.5 equiv) and PyBOP (1–1.1 equiv, 3 equiv *i*-Pr_2NEt, DMF, 25 °C, 16 h, 44–100%) to afford the 80 diamides **4** which were purified by sequential 10% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl extractions. Each reaction was conducted on a 1.5 mmole scale (*ca.* 0.5 g of monoamide) providing 1.6–0.35 g of **4** (avg = 97%) in ≥95% purity independent of the reaction efficiency. The final step in the preparation of the library of 560 compounds entailed 80 coupling reactions of each iminodiacetic acid diamide (**A1B1–A8B10**) with an equimolar mixture of the 7 tricarboxylic acids (**C1–C7**) producing 80 sublibraries of 7 compounds. This was accomplished by acid-catalyzed deprotection of **4** (0.15 mmol, 4 M HCl–dioxane, 25 °C, 3 h) followed by coupling (0.15 mmol PyBrOP, 1.5 mmol *i*-Pr_2NEt, DMF, 25 °C, 16 h, 19–100%) of the crude amine hydrochloride with an equimolar mixture of the tricarboxylic acids **C1–C7** (0.005 mmol each, 0.035 mmol total, 0.7 equiv). The secondary amine was used in excess and the reactions were run for an extended time period to drive the couplings to completion insuring a near equimolar generation of each compound. Purification by sequential extractions with 10% aqueous HCl (3×), saturated aqueous $NaHCO_3$ (2×), and saturated aqueous NaCl provided the final mixtures (avg = 86%, 10–34 mg) free of contaminant starting materials, reagents and their byproducts, and any partially coupled free carboxylic acid (≥95% pure). Matrix characterization of the 80 sublibraries by MS and ^1H NMR confirmed the constitution of the mixtures and a comparison of the sublibrary **A1B3C1–C7** with a reconstituted mixture prepared by combining an equimolar mixture of the individual components established its integrity.

Sequential Dimerizations of Iminodiacetic Acid Diamides. An additional approach that complements our disclosure of a two-fold dimerization of iminodiacetic acid diamides via the olefin metathesis reaction that ultimately incorporates eight variable groups and randomizes the length of the linking tether adding a ninth degree of diversification,[16] entails their two-fold dimerization by sequential coupling with appropriately functionalized dicarboxylic acids. This is illustrated with the preparation of the tetramers **9** derived first from dimerization with *N*-BOC-iminodiacetic acid (**2**) to provide **8** which, following *N*-BOC deprotection, sets up the second dimerization conducted with a range of dicarboxylic acids (Figure 3). In addition to the eight variable groups incorporated into the iminodiacetic acid diamides, a ninth diversification is incorporated with the second set of linking dicarboxylic acids. A library of 1260 compounds was prepared in a format of 126 mixtures of 10 compounds each. Thus, 42 individual iminodiacetic acid diamides **7** were prepared in a 3×14[17] format on a scale analogous to that described for **3** and **4** (Figure 2). Deprotection of **7** (4 M HCl–dioxane, 25 °C, 4 h) followed by the individual dimerization coupling of each amine hydrochloride with *N*-BOC-iminodiacetic acid (1.0 equiv **2**, 3 equiv PyBrOP, 9 equiv *i*-Pr_2NEt, DMF, 25 °C, 16 h, 31–100%) provided **8**. Conducting the reaction with excess amine hydrochloride (1.5 equiv/3 molar equiv) and stoichiometry limiting dicarboxylic



acid insured complete dimerization coupling and the excess starting amine, residual carboxylic acid, reagents, and reagent byproducts were removed by acid/base extraction providing the 42 pure individual dimers **8** (≥95% pure, avg yield = 81%). These individual 42 iminodiacetic acid diamide dimers (1.5 equiv/3 molar equiv), which themselves constitute an important sublibrary for antagonist screening, were deprotected (4 M HCl–dioxane, 25 °C, 4 h) and subsequently coupled (3 equiv PyBrOP, 9 equiv *i*-Pr_2NEt, DMF, 25 °C, 16 h, 10–97%, avg = 80%) with the 30 dicarboxylic acids **C1–C30** in mixture groupings of 10 (**C1–C10**, **C11–C20**, and **C21–**

C30) producing 126 mixtures each containing 10 compounds with mixture variations only in the last linking dicarboxylic acid domain. Thus, the library preparation and screening are conducted with small mixtures of 10 compounds, and deconvolution by resynthesis of the 10 individual components of a final mixture is straightforward from archived samples of the 42 immediate precursors **8**. Matrix full characterization of the 42 dimers **8**, full characterization of representative individual tetramers **9**, and MS characterization of the **A2B5C1–C10**, **A2B5C11–C20**, and **A2B2C21–C30** sublibraries confirmed the presence of all 10 components in the final mixtures.

Conclusions. Complementary to our use of the olefin metathesis reaction to assemble higher order iminodiacetic acid diamide libraries,[11,16] their trimerization or sequential dimerizations with tricarboxylic acids and dicarboxylic acids, respectively, provide a powerful approach to the convergent synthesis of combinatorial libraries applicable to the discovery of antagonists of ligand-induced protein–protein dimerization/oligomerization and their conversion to potential agonists. Such studies are in progress and will be disclosed in due course.

Acknowledgments. We gratefully acknowledge the financial support of the National Institutes of Health (CA78045), Johnson and Johnson, The Skaggs Institute for Chemical Biology, and the award of a National Defense Science and Engineering predoctoral fellowship (JG).

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17. The use of three **A** amines additionally as **B** amines (**B2**, **B6**, and **B11**) in the 3 × 15 format produces three duplicate samples (i.e., 3 × 14 format, **A1B2** = **A2B6**, **A1B11** = **A3B6**, and **A2B11** = **A3B2**) which were not prepared twice, only once. This duplication was deliberate and was based on prior testing results in targets under investigation that identified their presence in lead structures.