Tetrahedron Letters 50 (2009) 2787–2789

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Combined solid/solution phase synthesis of large surface area scaffolds derived from aminomethyl-benzoates

Rishi K. Jain, Lun K. Tsou, Andrew D. Hamilton *

Department of Chemistry, Yale University, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 27 February 2009
Revised 21 March 2009
Accepted 23 March 2009
Available online 27 March 2009

ABSTRACT

A set of macrocycles was generated by solid phase synthesis of linear trimers of 5-aminoacyl-3-aminomethyl-benzoates followed by resin cleavage and solution phase macrocyclization. These scaffolds can serve as useful building blocks for molecular recognition studies, especially where differentially functionalized molecular platforms spanning large surface areas are required.

Published by Elsevier Ltd.

Inspired by enzymes, where functional groups in the form of side chain, backbone, and cofactor residues project toward an active site, many have successfully designed artificial receptors orienting recognition elements in a convergent manner to bind small guests.[1] More recently, larger biochemical structures such as proteins,[2] DNA,[3] and carbohydrates[4] have increasingly become targets for artificial receptor design. Generally, strategies employed here differ from small molecule recognition in that artificial receptors contain larger numbers of recognition elements placed in a more divergent orientation. For example, in DNA recognition, Dervan's polyamides[5] project multiple H-bonding groups linked together to form a chain-like compound. Multiple pair-wise interactions with nucleotide bases in the minor groove over a long stretch of molecular surface lead to high affinity and specificity in binding. In the case of carbohydrate-protein interactions, many weak interactions between individual components of synthetic polyvalent-glycosides and polyvalent lectins lead to overall high affinity binding.[6]

Protein and peptide recognition by synthetic receptors require similar design considerations. We, and others have been interested in designing receptors containing multiple charged and hydrophobic,[7] histidine binding,[8] and hydrophobic side-chain binding[9] groups in a divergent manner to interact with protein surfaces with high affinity. Such synthetic agents capable of modulating protein function by targeting the solvent exposed exterior are of great interest as disruptors of protein–protein interactions.

Recently, several such macrocyclic structures of suitable dimensions and functionalities have been reported.[10] Some of these macrocyclic platforms are related to marine natural products and are interesting in terms of conformational rigidity and size.[10] In previous work, we have developed antibody mimetics where the hypervariable loops were mimicked by constrained cyclic peptides and the constant region by a calix[4]arene scaffold.[11a] High affinity receptors for cytochrome c (cyt. c.),[11b] chymotrypsin,[11c] platelet-derived growth factor, and vascular endothelial growth factor[11d] were identified using similar designs. In a further investigation of structure and affinity, a series of tetraphenyl-porphyrin (TPP) based receptors were generated where fluorescence titrations allowed facile measurement and comparison of K_d for a series of related receptors against cyt. c.[11b]

The majority of these receptor designs were of high symmetry and thereby targeted protein surfaces that were similar in surface topology. On further developing this strategy, we sought differentially functionalized receptors with lower symmetry which might bind protein surfaces containing a more complex arrangement of binding domains. Unfortunately, calix[4]arenes[12a,b] and TPPs[12c] do not lend themselves to the facile synthesis of unsymmetrically functionalized receptors. We required conformationally defined macrocycles with dimensions of ~10–20 Å, that could be sequentially functionalized, and could potentially bind to protein surfaces or to other large molecular surfaces in water. While these derivatives show promise as templates for protein recognition, we were interested in the construction of a molecular scaffold in which the majority of the assembly and diversification steps are carried out on the solid phase and do not employ chiral monomers that are prone to racemization.

Based on these requirements, we have designed macrocycle **1**, a cyclic trimer of 5-amino-3-aminomethyl-benzoic acid as a readily functionalized molecular platform. Each aminomethylbenzoate monomer is isosteric with a dipeptide unit, and in a trimeric arrangement gives rise to a 18-membered ring macrocycle comparable to a cyclic hexapeptide. Molecular modeling using the Macromodel[13] conformational search routine (AMBER, in water) indicated that the global minimum of **1** was a C3 symmetric cone conformation as shown in Figure 1.

Initially, we set out to synthesize derivatives of **1** with three identical peripheral attachments as shown in Scheme 1. For the solid support, Sasrin® resin was chosen since the cleavage conditions (1% TFA/CH_2Cl_2) are orthogonal with many protective groups.

* Corresponding author. Tel./fax: +1 203 432 5570.
E-mail address: andrew.hamilton@yale.edu (A.D. Hamilton).

0040-4039/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2009.03.153

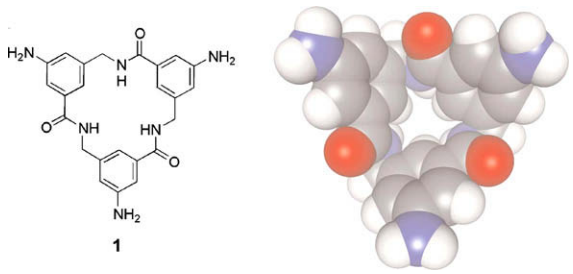
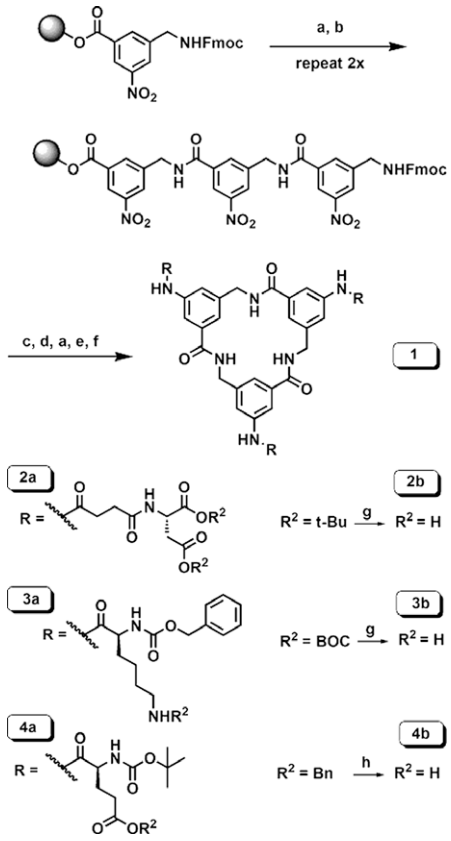


Figure 1. Global minimum structure of a monte carlo conformational search routine (Macromodel, Amber force field, in water) for a cyclic trimer of 3-aminomethyl-5-amino benzoic acid. The structure is *C*-3 symmetric, with the amide NH pointing into the resultant cavity. The dimension described by the edge (distance between two aniline amines, shown in blue) is approximately 11 Å.



Scheme 1. Reagents and conditions: (a) 20% piperidine/DMF (2×) 10 min; (b) Fmoc–Amb(NO_2)–OH (1.5 equiv), PyBOP (1.5 equiv), DIEA(3 equiv), DMF, 1 h; (c) $SnCl_2$ (10 equiv), DMF, 8 h; (d) corresponding protected acids (4 equiv), PyBrOP (4 equiv), DIEA(6 equiv) CH_2Cl_2, 3 h (×2); (e) resin cleavage with 1% TFA/CH_2Cl_2; (f) PyBOP (3 equiv), HOBt (3 equiv) DIEA (5 equiv), DMF (2 mM peptide) 6 h; (g) TFA/CH_2Cl_2/triethylsilane/H_2O (45:45:2.5:2.5) 3 h; (h) H_2, 10% Pd/C, MeOH.

Attachment of the first amino acid (Fmoc–Amb(NO_2)–OH) through an ester group to the resin was carried out with DIC/HOBt/DMF/pyridine, and resin loading was determined from the change in mass of the resin. The linear trimer chain was assembled using standard *N*-Fmoc-based solid phase peptide synthesis conditions (Fmoc-SPPS). Following chain extension, the aryl-nitro groups were reduced to their corresponding arylamine with $SnCl_2$. Acylation of the arylamine using coupling reagents such as DIC/DMAP, DIC/HOBt, BOP/HOBt, and HATU/HOAt, or by addition of pre-activated esters such as *N*-hydroxysuccinimidyl or pentafluorophenyl proved to be sluggish, giving only 5–10% conversion after 12 h. This observation is not unexpected due to the low nucleophilicity of aniline derivatives.

PyBrOP, a coupling reagent typically used for secondary amide formation improved the rate of the reaction, and full conversion was observed after 3 h, using 4 equiv (1.33 equiv/amine) of reagent and acid. Typically, two rounds of coupling, were performed to ensure quantitative conversion. The reaction proceeds cleanly with

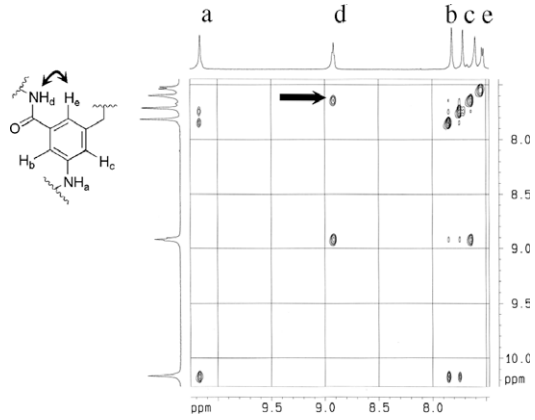
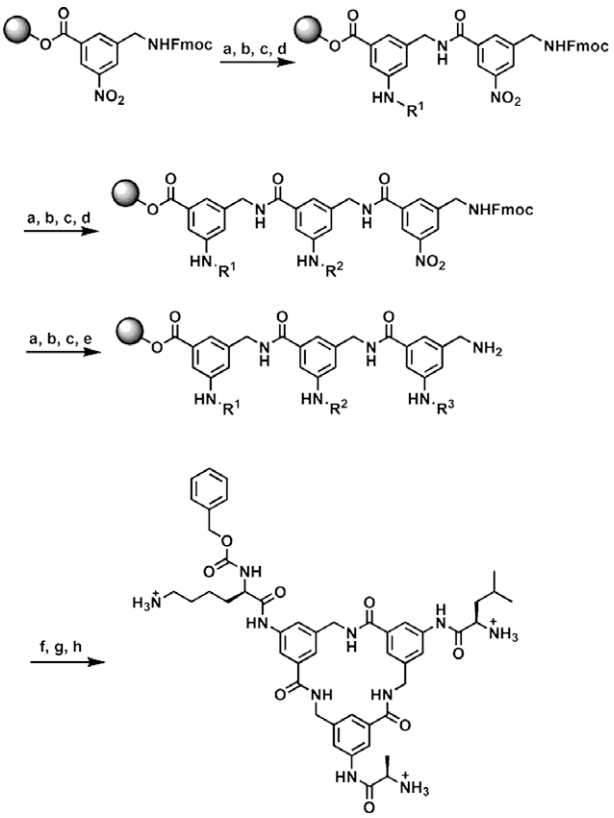


Figure 2. NOESY data of **3a** in DMSO-d_6, 298 K. A structure of the part of the monomeric unit is shown with the protons appropriately labeled. The arrow indicates the H_d–H_e NOE.



Scheme 2. Reagents and conditions: (a) $SnCl_2$ (10 equiv), DMF, 8 h; (b) corresponding protected acids (4 equiv), PyBroP (4 equiv), DIEA(6 equiv) CH_2Cl_2, 12 h (×2); (c) 20% piperidine/DMF (2×) 10 min; (d) Fmoc–Amb(NO_2)–OH (1.5 equiv), PyBOP (1.5 equiv), DIEA(3 equiv), DMF, 1 h; (e) resin cleavage with 1% TFA/CH_2Cl_2; (f) PyBOP (3 equiv), HOBt (3 equiv) DIEA (5 equiv), DMF (2 mM peptide) 6 h; (g) TFA/CH_2Cl_2/triethylsilane/H_2O (45:45:2.5:2.5) 3 h.

no byproducts being observed by HPLC. The cleavage of the functionalized trimer from the resin was effected by 1% TFA/CH_2Cl_2. The crude linear trimer was subjected to macrocyclization conditions in DMF and the product was purified by silica gel chromatography, to give **2a**–**4a**, and after deprotection, **2b**–**4b**. Overall yields were typically 25–30% based on initial resin loading.

A single set of resonances were present in the 1H NMR spectra of **3a**, suggesting the lack of desymmetrized conformers within the NMR time scale. A strong NOE between the macrocyclic backbone amide (H_d) and the inner C–H of the aromatic ring (H_e) can be seen in the NOESY spectrum of **3a** (Fig. 2). H_d and H_b, which are also separated by the same number of atoms, do not show any NOEs. This suggests that the orientation of the amide bond and the inner aryl C–H (H_e) is toward the interior of the macrocycle rather than the 180° rotated alternative that would project the C–H to the exterior. This observation is consistent with the cone conformation found from the molecular modeling studies (Fig. 1).

To further extend this approach to less symmetrical scaffolds, we prepared a macrocycle appended with three different amino acid derivatives on the exterior aniline positions (Scheme 2). Reactions were monitored by HPLC to ensure at least 90% conversion for each solid phase step. Conditions employed in the synthesis of **2**–**4** were used to provide crude **5** at which point HPLC purification was carried out to afford 5 in 15% overall yield over 15 steps. This corresponds to approximately 88% average stepwise yield.

Using the methods described here, it is possible to prepare small arrays of molecular platforms attached with different combinations of functionality. Binding studies of such molecular surfaces with protein targets should reveal interesting structure–affinity relationships.

Acknowledgment

We thank the National Institutes of Health (GM 35208) for financial support of this work.

References and notes

1. (a) Rojas, C. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 5120–5121; (b) Rebek, J., Jr.; Marshall, L.; Wolak, R.; Parris, K.; Killoran, M.; Askew, B.; Nemeth, D.; Islam, N. *J. Am. Chem. Soc.* **1985**, *107*, 7476–7481; (c)For an exhaustive survey of the area: *Comprehensive Supramolecular Chemistry*; Lehn, J.-M., Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: New York, 1996.
2. Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479–2493.
3. Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215–2235.
4. Lundquist, J. L.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
5. Dervan, P. B.; Bürli, R. W. *Curr. Opin. Chem. Biol.* **1999**, *3*, 688–693.
6. Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71–77.
7. (a) Fletcher, S.; Hamilton, A. D. *Curr. Opin. Chem. Biol.* **2005**, *9*, 632–638; (b) Sadowsky, J. D.; Schmitt, M. A.; Lee, H.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966–11968; (c) Schneider, T. L.; Mathew, R. S.; Rice, K. P.; Tamaki, K.; Wood, J. L.; Schepartz, A. *Org. Lett.* **2005**, *7*, 1695–1698; (d) Gradl, S. N.; Felix, J. P.; Isacoff, E. Y.; Garcia, M. L.; Trauner, D. *J. Am. Chem. Soc.* **2003**, *125*, 12668–12669.
8. (a) Fazal, M. A.; Roy, B. C.; Sun, S. G.; Mallik, S.; Rodgers, K. R. *J. Am. Chem. Soc.* **2001**, *123*, 6283–6290; (b) Sun, S.; Fazal, M. A.; Roy, B. C.; Mallik, S. *Org. Lett.* **2000**, *2*, 911–914.
9. Leung, D. K.; Yang, Z. W.; Breslow, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5050–5053.
10. (a) Kang, S.-W.; Gothard, C. M.; Maitra, S.; Wahab, A.-T.; Nowick, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 1486–1487; (b) Ishida, H.; Qi, Z.; Sokabe, M.; Donowaki, K.; Inoue, Y. *J. Org. Chem.* **2001**, *66*, 2978–2989; (c) Pattenden, G.; Thompson, T. *Chem. Commun.* **2001**, *8*, 717–718; (d) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189–8196; (e) Löwik, D. W. P. M.; Lowe, C. R. *Eur. J. Org. Chem.* **2001**, 2825–2839; (f) Somogyi, L.; Haberhauer, G.; Rebek, J., Jr. *Tetrahedron* **2001**, *9*, 1699–1708; (g) Haberhauer, G.; Somogyi, L.; Rebek, J., Jr. *Tetrahedron Lett.* **2000**, *41*, 5013–5016; (h) Rasmussen, P. H.; Rebek, J., Jr. *Tetrahedron Lett.* **1999**, *40*, 3511–3514; (i) Kubik, S. *J. Am. Chem. Soc.* **1999**, *121*, 5846–5855; (j) Mink, D.; Mecozzi, S.; Rebek, J., Jr. *Tetrahedron Lett.* **1998**, *39*, 5709–5712; (k) Ishida, H.; Donowaki, K.; Suga, M.; Shimose, K.; Ohkubo, K. *Tetrahedron Lett.* **1995**, *36*, 8987–8990.
11. (a) Blaskovich, M. A.; Lin, Q.; Delarue, F. L.; Sun, J.; Park, H. S.; Coppola, D.; Hamilton, A. D.; Sebti, S. M. *Nat. Biotechnol.* **2000**, *18*, 1065–1070; (b) Jain, R. K.; Hamilton, A. D. *Org. Lett.* **2000**, *2*, 1721–1723; (c) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8–13; (d) Sun, J.; Wang, D.; Jain, R. K.; Carie, A.; Paquette, S.; Ennis, E.; Blaskovich, M. A.; Baldini, L.; Coppola, D.; Hamilton, A. D.; Sebti, S. M. *Oncogene* **2005**, *24*, 4701–4709.
12. (a) Casnati, A. *Gazz. Chim. Ital.* **1997**, *127*, 637–649; (b) Vanloon, J. D.; Verboom, W.; Reinhoudt, D. N. *Org. Prep. Proc. Int.* **1992**, *24*, 437–462; (c) Wallace, D. M.; Leung, S. H.; Senge, M. O.; Smith, K. M. *J. Org. Chem.* **1993**, *58*, 7245–7257.
13. Mohammadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.