Two-Step Synthesis of Complex Artificial Macrocyclic Compounds

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Abstract

The design and synthesis of head-to-tail linked artificial macrocycles using the Ugi-reaction has been developed. This synthetic approach of just two steps is unprecedented, short, efficient and works over a wide range of medium (8–11) and macrocyclic (12) loop sizes. The substrate scope and functional group tolerance is exceptional. Using this approach, we have synthesized 39 novel macrocycles by two or even one single synthetic operation. The properties of our macrocycles are discussed with respect to their potential to bind to biological targets that are not druggable by conventional, drug-like compounds. As an application of these artificial macrocycles we highlight potent p53-MDM2 antagonism.

TOC image

Short path, large rings: An unprecedented short and diverse 2-step synthesis is described towards artificial 8–19-membered macrocycles. Artificial macrocycles are useful tools to address difficult biological targets. An example of a designed macrocycle to antagonize the protein–protein interaction p53–MDM2 is given.

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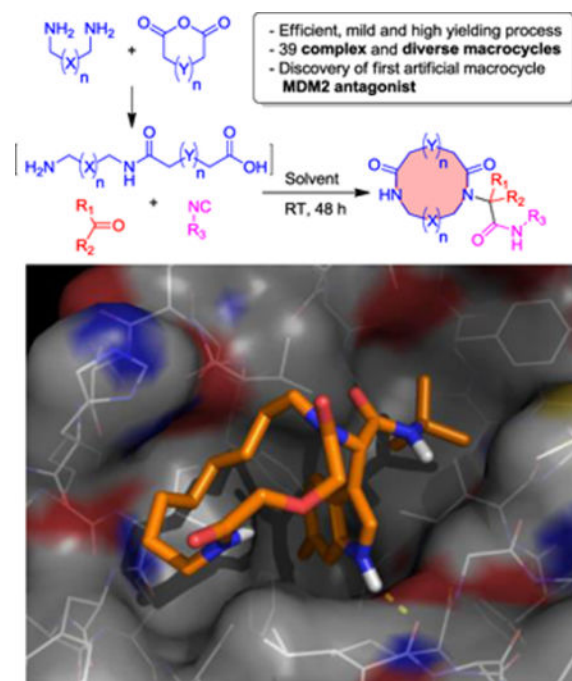
Keywords

drug discovery; macrocycles; protein–protein interactions; synthetic methods; Ugi reaction

Artificial non peptide derived macrocycles are a rather rare and neglected compound class, presumably due to their complex sequential synthesis.[1] Moreover, they have generally not been classified as orally bioavailable and drug-like, until recent advancements in their synthesis and development.[2] However, macrocycles have huge potential in targeting modern postgenomic targets which are difficult to address by small molecules, such as protein–protein interactions (PPI) that are currently a therapeutic domain mostly covered by antibodies.[3–5] As opposed to their natural twins, artificial macrocycles promise to provide better control over synthesizability and their physicochemical properties resulting in drug-like properties. However, very few synthetic methods allow for convergent and fast access to a large macrocyclic chemical space, while not jeopardizing chemical diversity.[6–10]

Owing to increasing interest in macrocycles, many efforts have been made to identify new synthetic methods for their preparation.[11–12] To date, the majority of these methods have focused on sequential multi-step peptide synthesis, followed by stapling *via* disulfide bridges, cysteine-based cross linkers, RCM, click chemistry, etc.[13] These strategies are not suitable for the elaboration of medium-sized rings or macrocycles with non-natural (non amino acid) side chains and additional ring heteroatoms. Therefore, we introduce here a general synthesis concept for the fast assembly of macrocycles of different size and shape, side-chain and functional-group content. We envisioned a linker moiety using a simple and versatile chemistry. The linker moiety is decorated with orthogonal α,ω-functional groups which can be macrocyclized by another diverse chemistry. Recently, we published an example of this general concept, where we synthesized the linker motive by employing Ugi

tetrazole chemistry, followed by a macrocyclization using a second Passerini- or Ugi-multicomponent reaction.[14–15] Here, we wanted to create a manifold of artificial macrocycles by an even shorter sequence involving an initial linear diversification, followed by an exponential diversification step of macrocyclization using Ugi MCR, resulting in an overall 2-step synthesis of complex macrocycles (Figure 1, Scheme 1).

A core feature of our strategy was the use of simple, predictable reactions between diamines, cyclic anhydrides, aldehydes and isocyanides, all of which are starting materials of wide availability. The first step involved the ring opening of cyclic carboxylic acid anhydrides with symmetrical diamines to yield α,ω-amino carboxylic acids. For unambiguous reactivity, we first reacted the mono-Boc protected diamines to yield α,ω-N-Boc amino carboxylic acids, however we soon abandoned this strategy. In order to introduce more flexibility, avoid the use of the halogenated solvent $CHCl_3$ and reduce the number of steps we envisioned performing the direct ring opening reaction of cyclic anhydrides with unprotected alkyl diamines. With this we have synthesized a 36-membered library of terminal amino acids of different chain lengths through the ring opening reaction from commercially available alkyl diamines and cyclic anhydrides in good to excellent yields (see SI_Table 1). Next, we optimized the conditions for the Ugi-macroringclosure of macrocycles (SI_Table 2). The optimized conditions were 1.0 equiv. of **3**, 1.0 equiv. of aldehyde/ketone **4**, 1.0 equiv. of isocyanide **5** in 0.01M solution of MeOH at room temperature for 24 to 48 h. With this optimized condition in hand, we studied the scope of macrocyclization. We have synthesized a small library of 39, 8-to 19-membered medium to macrocycles in 22 to 75% yields (Table 1, Scheme 2 and **6ad**). Different lengths of terminal amino acids including simple methylene chains, heteroatoms, small rings and peripheral groups furnished the macrocycles in good yields. Heteroatoms in a ring, e.g. *N*-methylated macrocycles are interesting as they can increase hydrophilicity. Phenyl, potentially atropisomeric biphenyl and naphthalene can be conveniently included as ring fragments. Unprotected orthogonally functionalized oxo components such as 3-hydroxybenzaldehyde, terephthalaldehyde and 2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetaldehyde were tolerated under the reaction conditions and furnished the target macrocycles (**6l**, **6s** and **6ai**) in 72%, 43% and 29% yields, respectively. These functional groups can be potentially further elaborated into different products. Similarly, to aldehydes, ketones also worked well and could be incorporated as side chains of different macrocycles in good to excellent yields. The universality of this macrocyclization reaction was further supported by using various functionalized isocyanides derived from aliphatic, aromatic, benzylic, amino acids and heterocycles. Fascinatingly, all these isocyanides reacted very smoothly and resulted in a variety of macrocycles in good yields. The ability of the designed macrocyclization to address complexity was assessed by using tylosin as an oxo component. We have synthesized a tylosin based macrocycle **6al** in good yield (53%) with 8:1 ratio of diastereomers that were separated by chromatography. This example nicely underscores the mildness of the procedure being compatible with free hydroxyl groups, α,β-unsaturated ketones, esters, and acetales. Another complex macrocycle is **6am** which can be accessed in just two steps, based on a glycosyl isocyanide.[16] Chirality control was introduced in macrocycle 6w, derived from tartaric acid. Our examples indicate that more elaborated substitution patterns and chiral centers can also be employed and lead to complex

macrocycles, which are valuable and otherwise difficult to prepare, including spiro cycles, phenyl, biphenyls, naphthalene, pyridine, tylosin and glycosylated macrocycle.

In some cases, we faced problems in isolating the α,ω-amino acids due to their high polarity (SI_Table 1 red examples). In these cases, we removed the THF solvent of the anhydride ring opening reaction and reacted the crude α,ω-amino acids with the oxo and isocyanide components in the Ugi reaction. Surprisingly, the one pot reaction also produced the desired products (**6e, 6k, 6r, 6u, 6v, 6y, 6aa, 6ae and 6ak**) in good to moderate yields (Table 1). For a number of the macrocycles we were able to grow crystals and solve the 3D structures. Interestingly in several of the structures the exocyclic amide group is bent back over the macrocycle to form an intramolecular hydrogen bonding with the macrocycles amide group (SI_Figure 2). It has been shown that similar exocyclic amide group arrangements provide structural rigidity within cyclic peptidomimetics and promote the creation of a stabilizing intramolecular hydrogen bonding network.[17] This exocyclic control element can also contribute to increased membrane permeability by allowing the macrocycles to switch between membrane and aqueous solution optimized conformations reminiscent of a chameleonic behavior.[18] Thus, intramolecular hydrogen bonds can contribute to the flexibility of the macrocycles to allows them to adapt to their environment, thereby combining aqueous and lipo solubility, cell permeability, and efficient target binding.[19]

Whitty and coworkers recently analyzed multiple X-ray structures of natural product macrocycles in their protein receptors and proposed specific guidelines for the design of synthetic macrocycle libraries with structural and physicochemical features likely to favor strong binding to protein targets as well as good bioavailability.[20] Thus, we compare the calculated values based on the 38 herein described artificial macrocycles with Whitty's dataset (SI_Table 3). Macrocyclic heavy atoms (HA) were categorized as (i) 'ring atoms', (ii) 'peripheral atoms', small groups such as methyl, carbonyl, hydroxyl and halogens that consist of a single HA appending the ring, and (iii) 'substituent atoms', comprising larger structures connected to the ring. Structural diversity of all three regions is important for binding to the receptor. All the regions of our artificial macrocycles are within or close to the range of Whitty's design rules. The balance of polar to non-polar atoms that is important to ensure adequate PSA, is critical for good aqueous solubility. cLogP, MW and other parameters also compare favorably with the dataset of Whitty (SI_Table 4). In summary, our artificial macrocycles fall well into Whitty's' design criteria for macrocyclic libraries in most properties.

Macrocycles possess a larger surface area than small molecules and have been proposed to be a suitable molecular class to antagonize protein-protein interactions (PPIs). Specifically, loops and their binding partners represent new and promising PPIs for the development of macrocyclic and constrained peptide inhibitors.[21] Based on our previous knowledge of p53-MDM2 antagonists and as an application of our macrocycles we have designed 15-membered molecule **6ad** which should mimic the hot-spot triad (F^{19}, W^{23} and L^{26}) of p53 interacting with the negative regulator protein MDM2 (see Figure 2).[22] We tested the binding of **6ad** towards MDM2 by 2D HSQC-NMR and fluorescence polarization and found a binding constant K_i of 2.25 μM (see SI_Table 5). In accordance with the peak shifts of MDM2 and the modelling we speculate that the macrocycle binds into the L^{26} pocket, the 6-

chloro indole moiety into the W^{23} and the tert-butyl group into the F^{19} pocket of the triad binding site. To the best of our knowledge this is the first artificial macrocycle shown to bind to the MDM2 receptor. Stapled peptides bind also to MDM2 but their staple is oriented to a binding site adjacent to the hot-spot.[23] Future reporting will be done on detailed SAR of our artificial macrocycles binding to MDM2 and other protein protein interactions.

In summary, we have developed a general method that can quickly and accurately convert simple, broadly available, small molecule building blocks into macrocycles via a one-pot Ugi-reaction. This robust and operationally simple 2-step, air- and moisture-tolerant procedure is a valuable addition to MCR chemistry and expands its unique scaffold diversity by giving access to 8–19 membered rings. Recent research suggests that passive cell permeability in cyclic peptides and non peptidic macrocycles sharply decreases at a MW of approximately 1000 Da.[24] However, the macrocyclic space of compounds less than 1000 Da remains largely unexplored and represents an interesting opportunity to chart new territories of drug discovery. The herein described unprecedented short 2-step synthesis of artificial macrocycles with 4 points of diversity covers this interesting chemical space and provides a fast entry to test binding hypothesis towards difficult intracellular targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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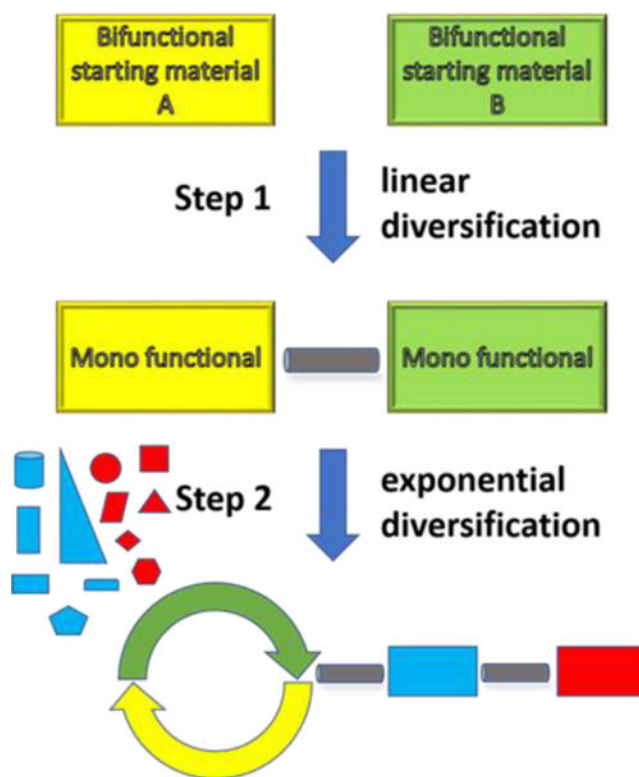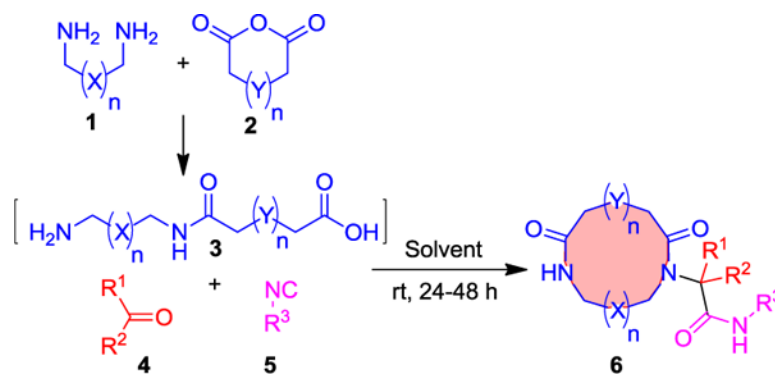
Figure 1.
Synthetic concept of rapid generation of macrocycles.
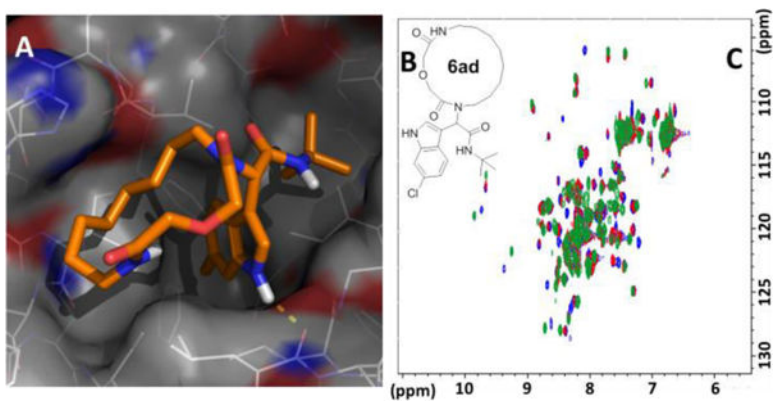
Figure 2.
Macrocycle **6ad** binding to MDM2 receptor (details SI). **A**: Modelling of **6ad** into MDM2 receptor (PDB ID 1YCR); **B**: 2D structure of **6ad**; **C**: Superimposed ^{1}H-^{15}N HSQC NMR spectra ^{15}N-labelled MDM2 titrated with **6ad**: blue - reference MDM2 spectrum, red – 4:1 (MDM2:**6ad**) titration step, green – 1:5 (MDM2:**6ad**; overtitration).
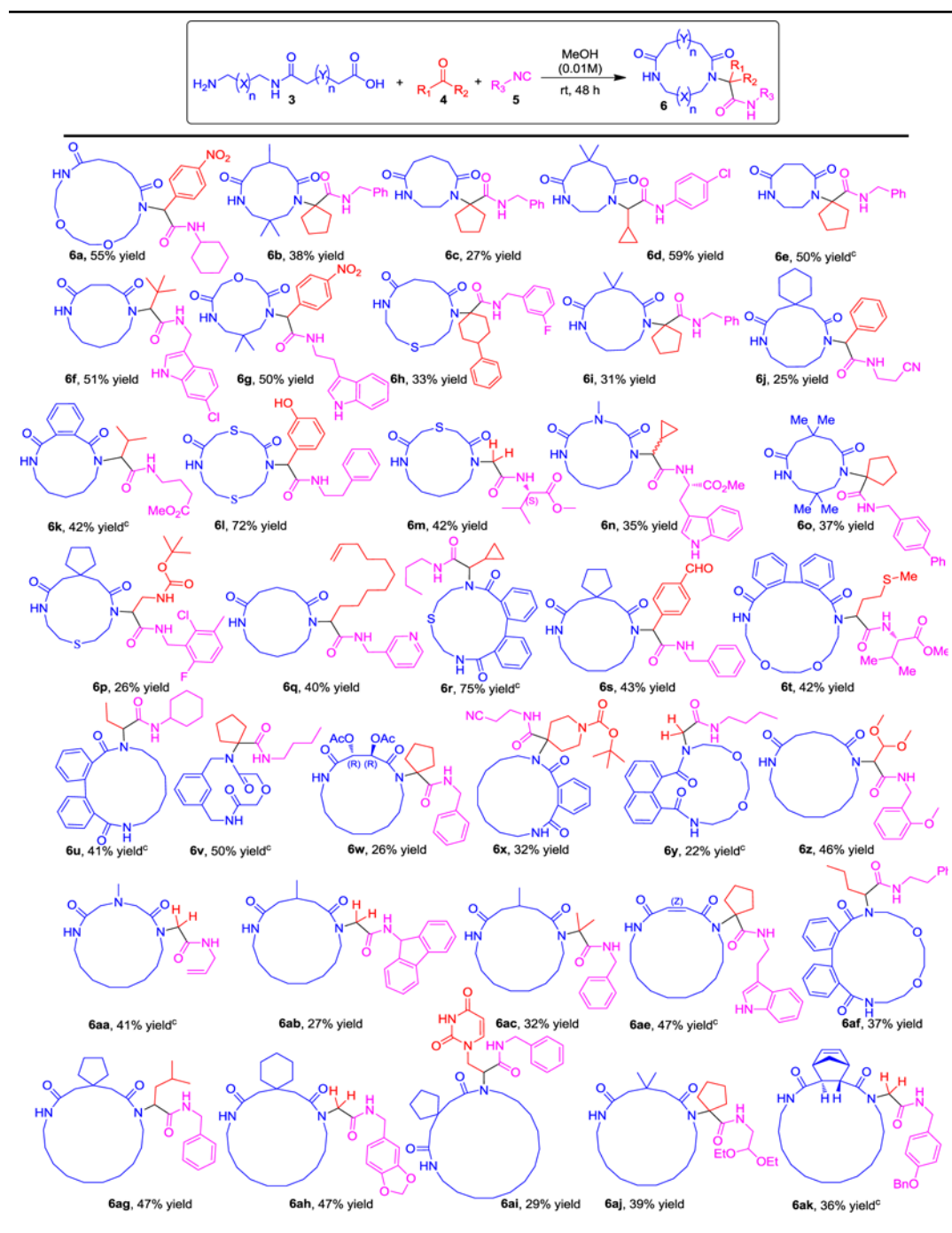
Scheme 1.
Direct 2-step macrocyclization.
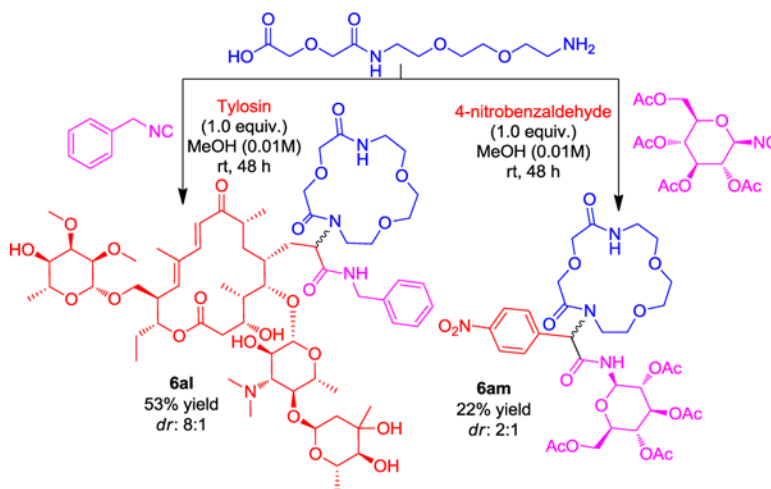
Scheme 2.
Synthesis of Tylosin and sugar-based macrocycles (**6al** and **6am**).

Table 1

Substrate scope of the macrocyclization.[a,b]

[c]Reaction performed in a cascade one-pot manner (ring opening followed by macrocyclization, see SI procedure C).