Rapid access to reverse turn peptidomimetics by a 3C-Ugi reaction on 3,4-dihydroisoquinoline.

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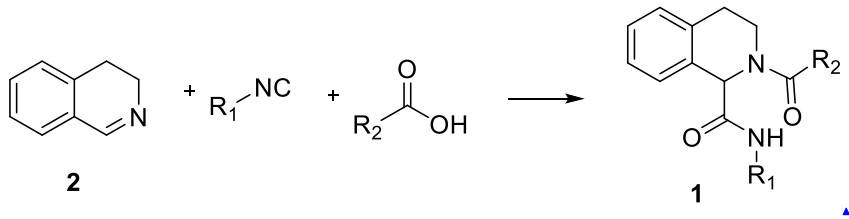
Introduction

In the last decades the field of peptidomimetics has been thoroughly investigated as a promising access to novel and efficient therapeutic agents.[1] Many strategies toward the reproduction of the most relevant secondary structures have been proposed. Reverse turns have been identified as a main target in the relevant PPI (protein-protein interactions) process.[2] In particular, γ-turns and β-turns have been studied both as a recognition motif and a β-sheet inducer toward more complex structures. A common way to obtain the desired geometry of a secondary structure mimics is to design rigid scaffolds able to correctly arrange the functional residues in the space.[3] Anyway, the exploitation of constrained peptidomimetics in medicinal chemistry is often limited for the fact that long and inefficient synthetic sequences are often required. With this regards, multicomponent reactions[4] can be very effective in the synthesis of complex and highly functionalized scaffolds in few steps and often in high yields. ~~Despite this fact, few~~Recently some example of multicomponent approaches to the synthesis of potential peptidomimetics have been reported,[5] mainly based on the Ugi reaction.[6,7] The Ugi reaction is a well-known and exploited four component reaction including the use of an amine, an aldehyde, a carboxylic acid and an isocyanide to produce an amino acid derivative. Following these observations, accordingly to our experience in the preparation of peptidomimetics,[8] we decided to design the synthesis of a reverse turn mimic easily achievable by a multicomponent approach based on tetrahydroisoquinoline scaffolds. Tetrahydroisoquinoline is present in a large number of natural compounds and pharmacologically active molecules.[9] The great structural diversity of this class of molecules is often due to the presence of many different substituents and functional groups on the piperidine ring. These heterocyclic scaffolds are most often prepared by cyclization procedures (e.g. Pictet-Spengler[10] and Bischler-Napieralskij[11] reactions) toward the formation of the piperidine ring with the concomitant introduction of substituent in the key C1 position. Further elaborations of these structures allow the preparation of a

variety of derivatives. Besides the interest for their biological activities, that spread from antimalarials to opioid mimetics, tetrahydroisoquinolines have been proposed as efficient peptide secondary structure inducers in the field of peptidomimetics due to their rigid polycyclic structures. Following these observations we become interested in the design of new peptidomimetics **1** based on the 1-carboxy-tetrahydroisoquinoline scaffold by means of a~~n~~ ~~—~~three component Ugi multicomponent reaction (Scheme 1) on the preformed imine ~~. The Ugi reaction is a well known and exploited four component reaction including the use of an amine, an aldehyde, a carboxylic acid and an isocyanide. The first step of the reaction is the formation of an imine; for this reason it is possible to perform Ugi reactions on preformed imines, thus working in a three component system. In our strategy, a multicomponent Ugi 3 component reaction on the~~ 3,4-dihydroisoquinoline **2** ~~was selected as a rapid access to peptidomimetics **1**~~.



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Scheme 1. Ugi 3CR for the synthesis of peptidomimetics **1**

Results and discussion

The starting material **2** could be easily obtained by a Bischler-Napieralski cyclization of the related formamide precursor. We first tested the reaction of **2** with acetic acid and benzylisocyanide in different solvents and temperature for 24h to afford **3** (Table 1).

Table 1. optimization of the reaction conditions for the preparation of **3**

entry	Solvent	T (°C)	Conversion (%)[a]
1	DCM	25	44
2	DCM	60	58
3	THF	25	31
4	THF	60	34
5	MeOH	25	84
6	MeOH	60	79

7	acetonitrile	25	51
8	acetonitrile	60	54
9	toluene	25	23
10	toluene	60	27

[a] conversions were measured by GC/MS analysis or by ^{1}H-NMR on the crude.

The use of DCM and acetonitrile produced a modest 58% and 54% yield, whereas with toluene and THF only a 23-34% yield could be observed. The best results were achieved when using methanol at room temperature (84%). Increasing the temperature did not provided any improvement. By using these reaction conditions, we prepared a small combinatorial library of target compounds with different components. Three alkylisocyanide were used, namely benzylisocyanide, cyclohexylisocyanide and terbutylisocyanide. In order to better test the reverse turn capability of the final products, ethylisocyanoacetate was also used as a mimic of a glycine residue. For the acidic component, we used two protected amino acids, *N*-acetylglycine and (L)-*N*-Cbz-alanine. Moreover, 1-pentenoic acid was selected for its easy removal from the final product, thus allowing the possibility to further decorate the tetrahydroisoquinoline nitrogen. Finally chloroacetic acid was employed in order to explore the possibility to obtain a fused diketopiperazine ringby a post-cyclization of the Ugi adduct (Scheme 2). The isolated final products are listed in Figure 1.

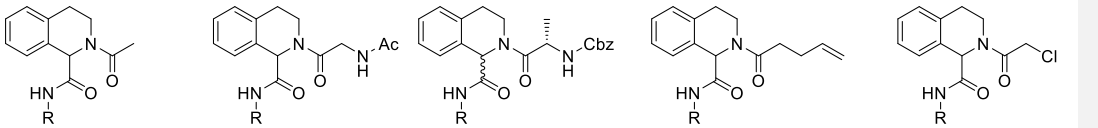

Figure 1. List of prepared compounds. Yields after purification are reported in parenthesis.

In most of the cases the reactions proceeded smoothly and after 24-48h the solvent was evaporated and the crude purified by column chromatoghrapy. In few cases, a solid precipitated out or could be easily recovered after treatment of the crude with a methanol/hexane mixture. All products could be isolated in moderate to good yields. When (L)-Cbz-alanine was used, a 1:1 diastereoisomeric mixture was obtained. The two diastereoisomers could be efficiently separated by column chromatography. To explore the possibility to obtain a diketopiperazine~~18-21~~ derivative, compound ~~14-17~~12 was further reacted with a base to promote the intramolecular cyclization (Scheme 2).

Commento [AR1]: In entrambi i casi (con Bn e CH2COOEt)? O c'e' qualche tipo di induzione dovuta a ingombro sterico?

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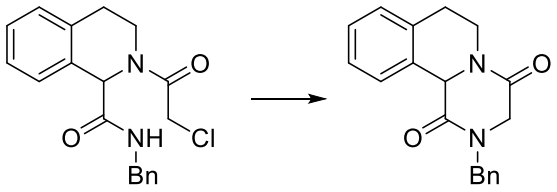
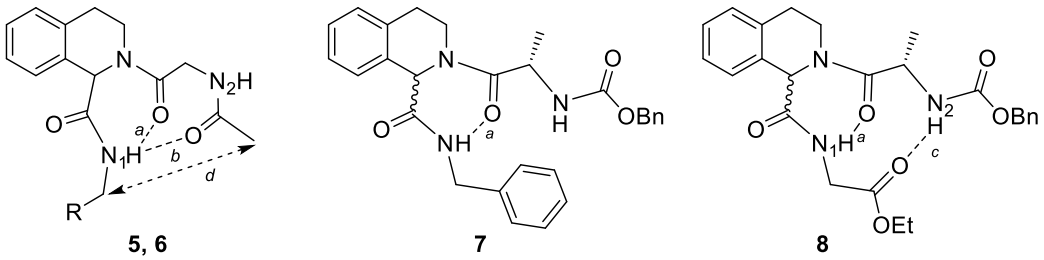


12 **16**

Scheme 2. Intramolecular cyclization for the synthesis of diketopiperazine ~~**18**~~ ~~**2**~~**16**~~**1**~~

The use of strong bases (NaH or *t*-BuOK) yielded a mixture of inseparable products. Methanolic NaOH afforded the product in a 12% conversion yield (measured from GC-MS and ^{1}H-NMR on the crude), whereas with cesium carbonate in acetonitrile conversion was 16% (as measured on the crude). In any case, due to the presence of a great number of by-products, we were not able to isolate the pure product.

We then investigated the ability to adopt a reverse turn-like conformation for compounds **5**-~~**9**~~-**8** by means of computational tools. A conformational analysis was performed with the use of a Monte Carlo search and a Molecular Mechanics energy minimization. [12] Reverse turns are stabilized by intramolecular hydrogen bonds and according to the H-bonds pattern, γ (7 membered H-bond ring) and β (10 membered H-bond ring) turns can be defined. In addition, for the β-turn, a measure of the distance d < 7Å is a favorable condition (see Figure 3). We could identify three most common hydrogen bonds in our models: the *a* and *b* hydrogen bond patterns, respectively related to a γ and β-turn, and the H-bond *c*. Interestingly this last pattern is found in anti-parallel β-sheet motifs.[13] These structures have raised great attention in the last years for their possible implications in protein aggregation diseases.[14]



5, 6 **7** **8**

Figure 3. Definition of the geometrical parameters for the reverse-turn evaluation of compounds **5**-~~**9**~~**8**

Detailed analysis of the results are reported in table 3. All the scaffolds are able to adopt a classic γ-turn like conformation through the presence of the *a* H-bond. The presence of a *N*-acetylglycine residue (compounds **5**~~-~~ and **7**) promoted the formation of a β-turn, in particular for **5** (81% of conformers with $d < 7$Å); in all cases, the analysis of the dihedral angles established the presence of a type II β-turn. As expected, for ~~**8**~~ **7** and ~~**9**~~ **8** some differences between the two diastereoisomers are observed: for the (*S,S)*-isomer there is a prevalence for type *a* bond, while in the (*R,S)*-isomer some attitudes towards the β-turn conformation is also revealed. Notably the presence of the *N*-Cbz-alanine in compound **9** promoted the formation of the H-bond *c*.

Table 3. Results from conformational analysis. Results are reported as percentage of conformers meeting the indicated requirement. Only conformers within the 6 kcal/mol were considered

Compound	conf.	*a* bond (%)	*b* bond (%)	*c* bond (%)	*d* < 7Å (%)
5	58	16	40	n.a.	81
6	39	31	36	n.a.	54
~~**7**~~	~~21~~	~~43~~	~~10~~	~~n.a.~~	~~48~~
(*S,S*)-~~**8**~~**7**	69	23	1	n.a.	n.a.
(*R,S*)-~~**8**~~**7**	21	76	0	n.a.	n.a.
(*S,S*)-~~**9**~~**8**	33	45	9	33	n.a.
(*R,S*)-~~**9**~~**8**	25	88	0	16	n.a.

In order to confirm the presence of the computationally predicted H-bonds, variable temperature NMR experiments were performed on compounds~~s~~ **5** and on one of the two diastereoisomers of ~~**9**~~**8**. For compound **5** values of $\Delta\delta/\Delta T$ corresponding to -7.7 ppb for N_1H and -6.3 ppb for N_2H were measured. Similarly for ~~**9**~~ **8** the detected values of $\Delta\delta/\Delta T$ were -6.1 ppb for N_1H and -5.4 ppb for N_2H. According to literature,[15] these values are in agreement with the presence of intramolecular hydrogen bonds, thus supporting the presence of stabilized secondary structures. Figure 4 shows the structures of **5**, (*S,S*)-~~**9**~~ **8** and (*R,S*)-~~**9**~~ **8** as obtained from calculations.

Commento [AR2]: Non sono sicura si possa scrivere cosi'..

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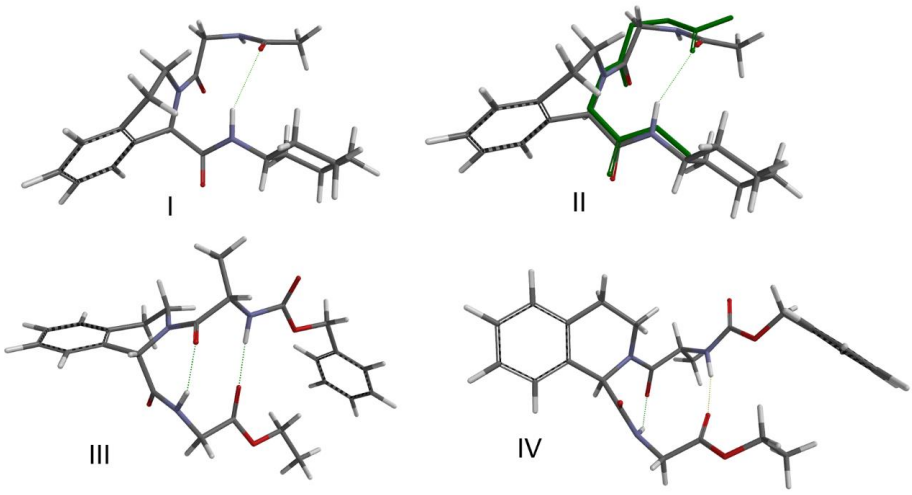

Figure 4. Compound **5** (I) and its superimposition with a type II β-turn model (II). Structures of (*S*,*S*)-~~9~~ **8** (III) and (*R*,*S*)-~~9~~ **8** (IV).

Commento [AR3]: Qui non fai superimposition?

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Conclusions

In this work we reported the use of a three component Ugi reaction as an effective tool for the rapid creation of peptidomimetic scaffolds based on the tetrahydroisoquinoline moiety. By this approach we could prepare a combinatorial library of derivatives with different components. For some of them a computational aided investigation on the conformational properties was performed thus predicting their ability to mimic some classical reverse-turn structures. Variable temperature NMR studies finally supported the presence of intramolecular hydrogen bond patterns. According to the different residues present on the tetrahydroisoquinoline scaffold, a γ-turn, β-turn or an antiparallel β-sheet could be recognized.

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