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Solid- and Solution-Phase Synthesis of Highly-Substituted-Pyrrolidine Libraries

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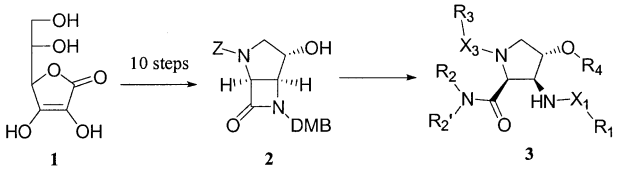
Abstract—Starting from a complex bicyclic β-lactam scaffold we have demonstrated the possible production of libraries of a new class of drug-like, highly substituted pyrrolidines. The choice of the type of substitution was made by optimizing various synthetic routes. The selection of each compound is the result of a filtration of a large virtual combinatorial chemical space, using simple criteria. The access to these complex pyrrolidines needed only four to six synthetic steps. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

The pyrrolidine chemical entity is found in a large number of natural and synthetic biologically active compounds. They are used in the treatment of cancer, obesity, fungal and viral infections as well as of hyperglycemia.[1]

For the synthesis of large combinatorial libraries based on heterocyclic scaffolds, a method of choice is the linear assembly of components followed by cyclisation. This condensation method was also successfully applied to the synthesis of substituted pyrrolidines.[2] Hereby, we report an alternative approach based on the use of the complex pyrrolidino-azetidinone **2** as starting material. This was available in large scale from a medicinal chemistry program in the field of antibacterial research. Following standard chemical manipulations, the four-member ring of the complex bicyclic azetidinone was opened to give access to unusually highly substituted pyrrolidines **3** (Scheme 1).

The initial bicyclic azetidinone **2** was prepared in 10 steps from ascorbic acid **1**, following our well established large scale synthesis using a classical ketene-imine cycloaddition reaction.[3–5] The starting bicyclic-β-lactam **2** has several properties that make it ideal for the use as a scaffold in combinatorial chemistry for the production of libraries containing highly diverse molecules with presumably drug-like character. (A) It has a low molecular weight, which allows the introduction of a large variety of building blocks without violating the empirical values widely accepted for oral absorption.[6] (B) It already contains four potential points of diversification for the introduction of a large variety of building blocks, namely the two nitrogen atoms, the alcohol function and as a key element, the opening of the β-lactam ring with nucleophiles. (C) Finally and very importantly, scaffold **2** has the advantage that it can be attached on solid phase at its various functional groups. We took full advantage of these aspects, for the efficient production of large libraries of type **3** by a variety of synthetic strategies (Scheme 1).



Scheme 1. Synthesis of the bicyclic β-lactam scaffold **2**, used for the production of a library of highly substituted pyrrolidines **3**.

Chemistry

The targeted substitution pattern of the desired pyrrolidine libraries will obviously influence not only the

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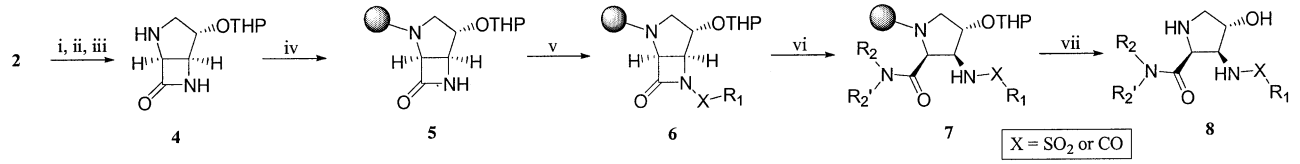


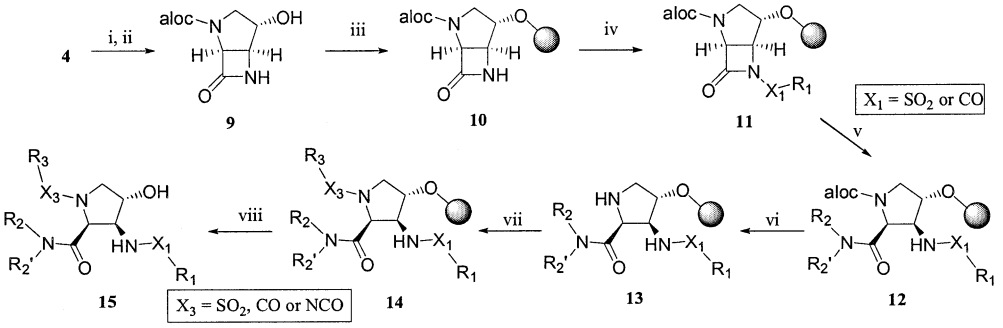
Scheme 2. (i) $K_2O_8S_2$, CH_3CN, H_2O, $NaHCO_3$, pH 4.5, 80 °C, 5 h (82%); (ii) 3,4-dihydro-2*H*-pyran (1.6 equiv), TsOH, (0.02 equiv), THF, rt, 12 h (87%); (iii) H_2, Pd/C (10%) (0.03 equiv), EtOAc, rt, 4 h (81%); (iv) TCP-resin, *N*-ethyldiisopropylamine, rt, 15 h; (v) R_1-X-Cl (5 equiv), Et_3N (8 equiv), DMAP (2 equiv), CH_2Cl_2, rt, 6 h; (vi) $R_2R_2'NH$ (8 equiv), Et_3N (2 equiv), CH_2Cl_2, rt, 15 h; (vii) 20% TFA, 10% MeOH, CH_2Cl_2, rt, 2 h.

choice of the attachment point to the resin, but also the sequence of protecting group manipulations. In a first approach the secondary alcohol function of **2** was protected with a tetrahydropyran-2-yl-group (THP).[7] The 3,4-dimethoxy-benzyl- (DMB) protective group of the β-lactam nitrogen was oxidatively removed,[5] followed by the removal of the benzyloxycarbonyl- (Z) protecting group on the pyrrolidine nitrogen. The latter protecting group was removed under hydrogenation conditions. The modified scaffold **4** was attached to the solid phase, using a commercially available tritylchloride polystyrene (TCP) resin. The first reaction, which was carried out on solid support, was the introduction of the primary diversity vector. The β-lactam nitrogen of **5** was acylated or sulfonated using acid-chlorides or sulfonyl-chlorides, respectively, to yield the intermediates **6**. This reaction not only allows the first introduction of building blocks, but also at the same time facilitates the key step, the opening of the β-lactam ring with amines. Due to the large variety of acid chlorides, sulfonyl chlorides and amines available, we decided to generate a library of 800 compounds, which was designed to offer maximal diversity from a structural point of view (see below). Therefore the pyrrolidines **7**, after the ring-opening with the amines, were cleaved off the resin, with simultaneous cleavage of the THP group, to yield a library of *N*-unsubstituted-hydroxy-pyrrolidines **8**.

Using this route (Scheme 2), the production of libraries of single, purified compounds (>10 mg of each) was achieved using the IRORI MicroKans™ in connection with the IRORI AutoSort™.

In a second approach, the scaffold **2** was attached via the alcohol function to a solid support in order to introduce further diversity at the pyrrolidine nitrogen, whilst the compounds still remain on the solid phase. The initial scaffold **2** had therefore to be pre-modified again. The DMB protecting group was removed and the benzyloxycarbonyl protecting group on the pyrrolidine nitrogen was replaced by an allyloxycarbonyl (aloc) group. The latter protecting group can be easily removed on solid phase with tetrakis-(triphenyl-phosphine)-palladium. The deprotection of the THP protected alcohol was carried out by polymer-supported PPTS, to yield the scaffold **9** in good yield and purity. The alcohol function was attached to the resin via a 3,4-dihydro-2*H*-pyran-2-ylmethoxy-methyl-linker in the presence of TsOH at room temperature. The standard literature procedure with PPTS was not successful on this particular scaffold.[8]

Then the β-lactam-ring of **10** was activated by the introduction of the first diversity vector, followed by the opening of the intermediates **11** with amines to yield disubstituted pyrrolidines **12** in a similar fashion as shown in Scheme 2 (steps v–vi). To be able to introduce a third vector of diversity, it was necessary to initially deprotect the pyrrolidine nitrogen. This was carried out efficiently by the use of tetrakis-(triphenylphosphine) palladium as catalyst in presence of an allyl scavenger. In our case the most successful reagent to prevent the re-protection of the nitrogen by the allyl group was found to be the borane dimethylamine complex.[9] The use of other standard allyl scavengers such as dimedone or typically more then 0.05 equivalents of the palladium catalyst were not successful and led to the allylation of the pyrrolidine nitrogen.[10] The free amino function of **13**, was the ideal intermediate to construct a very large variety of libraries using acid chlorides, sulphonyl chlorides, isocyanates and chloroformates to yield the corresponding amides, sulfonamides, ureas and carbamates



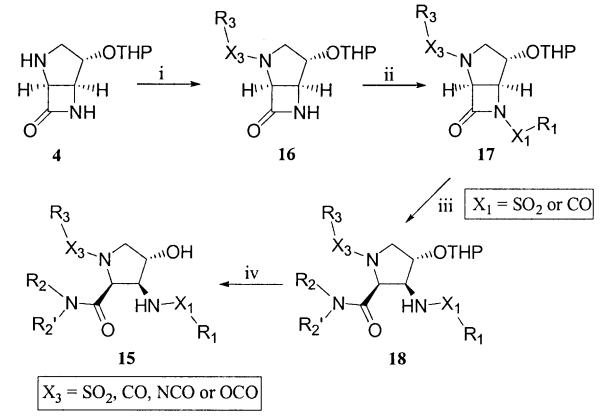
Scheme 3. (i) AlocCl (1.1 equiv), pyridine (2.5 equiv), CH_2Cl_2, −15 °C to rt, 1 h (93%); (ii) PPTS-polymer bound (1 equiv), EtOH, 60 °C, 10 h (80%); (iii) DHP resin, TsOH (3 equiv), rt, 15 h; (iv) R_1-X_1-Cl (5 equiv), Et_3N (8 equiv), DMAP (2 equiv), CH_2Cl_2, rt, 6 h; (v) $R_2R_2'NH$ (8 equiv), Et_3N (2 equiv), CH_2Cl_2, rt, 15 h; (vi) $Pd(PPh_3)_4$ (0.05 equiv), $Me_2NH{\cdot}BH_3$ (20 equiv), CH_2Cl_2, rt, 1 h; (vii) R_3COCl, R_3SO_2Cl or R_3-N=C=O (5 equiv), Et_3N (8 equiv), CH_2Cl_2, rt, 2 h; (viii) 20% TFA, 10% MeOH, CH_2Cl_2.

14, respectively. After an easy cleavage from the resin using TFA/methanol in dichloromethane, the final crude products **15** were obtained.

The synthesis of a small library of highly substituted pyrrolidines was carried out successfully as outlined in Scheme 3. However the synthesis strategy for the production of a large library of pyrrolidines **15** had to be changed, because the loading of the THP-resin with the scaffold **9** was inefficient. A maximum loading of only 0.6 mmol/g was achieved under several different loading conditions. This was the reason why it was not possible to carry out the production of this second library using the IRORI Autosort™ technology, since the IRORI MicroKans™, were too small to yield sufficient product after cleavage from the resin. Production of the library using a larger amount of resin per compound would have been no more cost effective.

Therefore, it was decided to carry out the library production of the pyrrolidines **15** by a solution-phase strategy. The introduction of the diversity vectors was reversed and a set of 12 different scaffolds **16** was produced. This was achieved by reacting the bicyclic β-lactam **4** with a variety of acid chlorides, sulfonyl chlorides, chloroformates or isocyanates to yield the substituted pyrroldines **16** in good yield and purity without further purification. Each of the 12 precursors was then divided into 96 tubes and the established chemistry was again applied in solution, using a Büchi Syncore™ shaker.

The 12 different scaffolds **16** were reacted with a variety of acid chlorides and sulfonyl chlorides to introduce the second diversity vector and to facilitate the subsequent β-lactam ring-opening with primary and secondary amines to yield the THP protected pyrrolidines **18**. The alcohol function of the pyrrolidine was then deprotected with catalytic amounts of PPTS in ethanol to yield the highly substituted pyrrolidines **15** (Scheme 4). The 12 scaffolds **16** where combined with eight different building blocks on the β-lactam-nitrogen and 12 different amines for the opening of the β-lactam. In this way a library of approx. 1200 compounds was produced with a crude purity of 50–90%. All compounds were purified by preparative HPLC[11] to yield single compounds in 5–20 mg quantities with >95% purity. All final products were analyzed by HPLC methods employing MS, UV and ELS detection to verify the correct mass and to determine the purity. Some compounds were also analyzed by ^{1}H and ^{13}C NMR to demonstrate further proof of structure.



Scheme 4. (i) R_3SO_2Cl, R_3COCl, R_3OOCCl or R_3-N=C=O (1.05 equiv), pyridine (2.5 equiv), CH_2Cl_2, −50 °C, rt, 15 h (70–97%); (ii) R_1-X_1-Cl (1.1 equiv), Et_3N (1.1 equiv), DMAP (0.1 equiv), DMF, rt, 4 h; (iii) $R_2R_2'NH$ (1.1 equiv), Et_3N (2 equiv), CH_2Cl_2, rt, 15 h; (iv) PPTS (0.1 equiv), EtOH, 60 °C, 15 h.

Selection of Building Blocks

The choice of the compounds to be synthesized within the library was made to complement the chemical diversity of our in-house historical depository with new drug-like substances. In a first step, the scope and limitations of the chemistry possible around the bicycle β-lactam scaffold was established. Based on this knowledge, a first selection of building blocks was made using a database of commercially available reagents and a database of the in-house compound depository. With the building blocks chosen, a virtual library of more then 10,000 molecules was created, before the compounds were actually synthesized. For this purpose an in-house chemical fragment assembly program was used. As we limited ourselves to 1000 compounds per library, the selection of the compounds to be synthesized was crucial.

By applying a set of four filters, we chose the 'best' building blocks to be used in the actual library production. For this virtual screening process, the applied filters included the rule-of-five[6] for the prediction of the bioavailability, a structural drug-likeness prediction,[12] a descriptor for pharmacophore similarity (CATS)[12,13] and a structural diversity analysis.

The drug-likeness prediction is based on a comparison of the molecules with 2-D structural fragments obtained from known drugs.[12] In the CATS program, atoms have been assigned to five different groups: H-donor, -acceptor, potentially positive or negative charges or lipophilic groups. Then they are pairwise compared with pharmacophore patterns of known drugs.[12,13] For the structural diversity analysis, a program was used, which transformed 2-D structures into fingerprints. The resulting bit strings were then compared

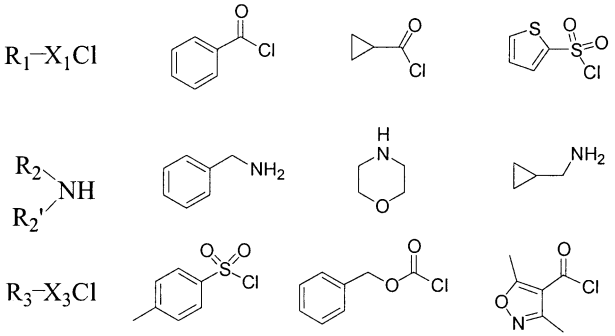


Figure 1. Selection of building blocks used in the production of highly substituted pyrrolidines.

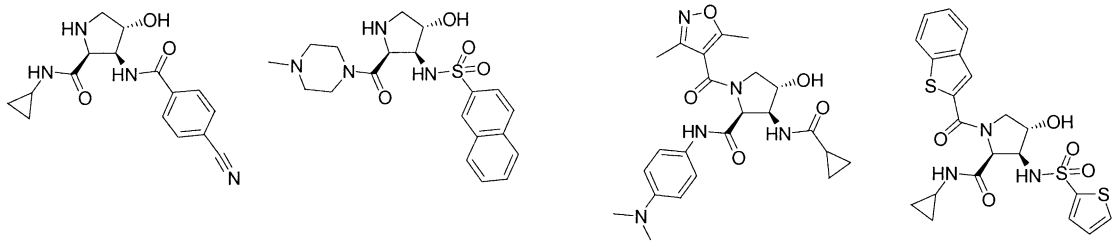


Figure 2. Representative examples of produced and purified, highly-substituted pyrrolidines.

with those of the compounds of the Roche corporate depository, using the Tanimoto index.[12] It was observed that generally all final products **15** showed high drug-like characters and reasonable diversity to the in-house depository. Figure 1 shows some building blocks, which gave for the final products **15** optimal values in all four parameters as described above. These building blocks were integrated with priority among others in the realization of the library (Fig. 2).

Conclusion

In summary, we have demonstrated the attractiveness of a highly complex bicyclic-β-lactam as a starting material for the synthesis of large combinatorial libraries. The initial scaffold could easily be diversified and the resulting library yielded drug-like compounds. Although the purity of the final product was usually good, they were all purified by preparative HPLC to give 5–20 mg of each compound in >95% purity. The resulting highly substituted pyrrolidines are now available for general screening in the search for new lead molecules in future pharmaceutical research programs.

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References and Notes

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