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Solid-phase synthesis of 3,7-disubstituted perhydro-1,4-diazepine-2,5-diones from amino acids and β-amino acids

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Abstract—We report here the synthesis of 3,7-disubstituted perhydro-1,4-diazepine-2,5-diones from amino acids and β-amino acids using a backbone amide linker (BAL) as a *cis*-configuration inductor. We have optimized each steps of the synthesis on solid support using Mimotopes crowns.

One of the major goals in medicinal chemistry is the identification of high affinity and activity ligands to receptors and enzymes. To address a growing demand of new compounds, several programs in medicinal chemistry exploit combinatorial methods[1] by using simultaneous or parallel synthesis of libraries. The use of solid-phase synthesis for the generation of diverse libraries of small organic compounds which can be used as conformationally restricted dipeptide surrogates or lead compounds have been extensively employed in the drug discovery process.[2,3] We focused our efforts toward the solid-phase synthesis of perhydro-1,4-diazepine-2,5-diones structure. Previous reports suggest that these heterocyclic derivatives could offer a useful structure for the discovery of new active compounds and various supported synthesis routes were described to design 3,7-disubstituted perhydro-1,4-diazepine-2,5-diones[4,5] (Fig. 1).

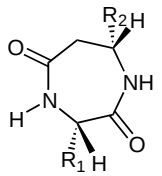

Figure 1. 3,7-Disubstituted perhydro-1,4-diazepine-2,5 diones.

In order to focus on the synthesis of libraries, we decided to develop a new preparation of these compounds by using as starting material commercially available amino acids and their β-homologated analogs which can be prepared by an Arndt–Eistert reaction. The seven-membered heterocyclic compounds lacking the fused aromatic nucleus are difficult to prepare. However, it has been shown that the presence of a secondary amide which adopt a *cis*-configuration can favor the formation of cyclic compounds such as diketopiperazines.[6] Thus, we choose a backbone amide linker (BAL)[7] to anchor the growing compound to the solid support and the Mimotope's Multipin method[8] which is beneficial to rapidly explore a large range of reaction parameters in parallel experiments. As the purification of resin-anchored compound is not possible, the conversion ratio of each intermediate step is critical for the purity of the target compound.

To quickly optimize the reaction conditions (solvent, temperature, reaction time…) of each synthesis step, we used a spacer especially designed to evaluate the conversion percent by a single LC/MS analysis. This model system was applied to the synthesis of 4-aminoproline analogs.[9] In our case, the internal probe carries a mimic of the BAL linker, incorporating a carboxybenzaldehyde moiety. This linker allows reductive amination and, unlike BAL linker, is stable to acidic treatment. The first target to illustrate the strategy was compound **5** (Scheme 1). As a model, we synthesized the 3-benzyl-perhydro-1,4-diazepine-2,5-dione from β-Ala and Phe residues.

Keywords: diazepine; mimotopes crowns; backbone amide linker.

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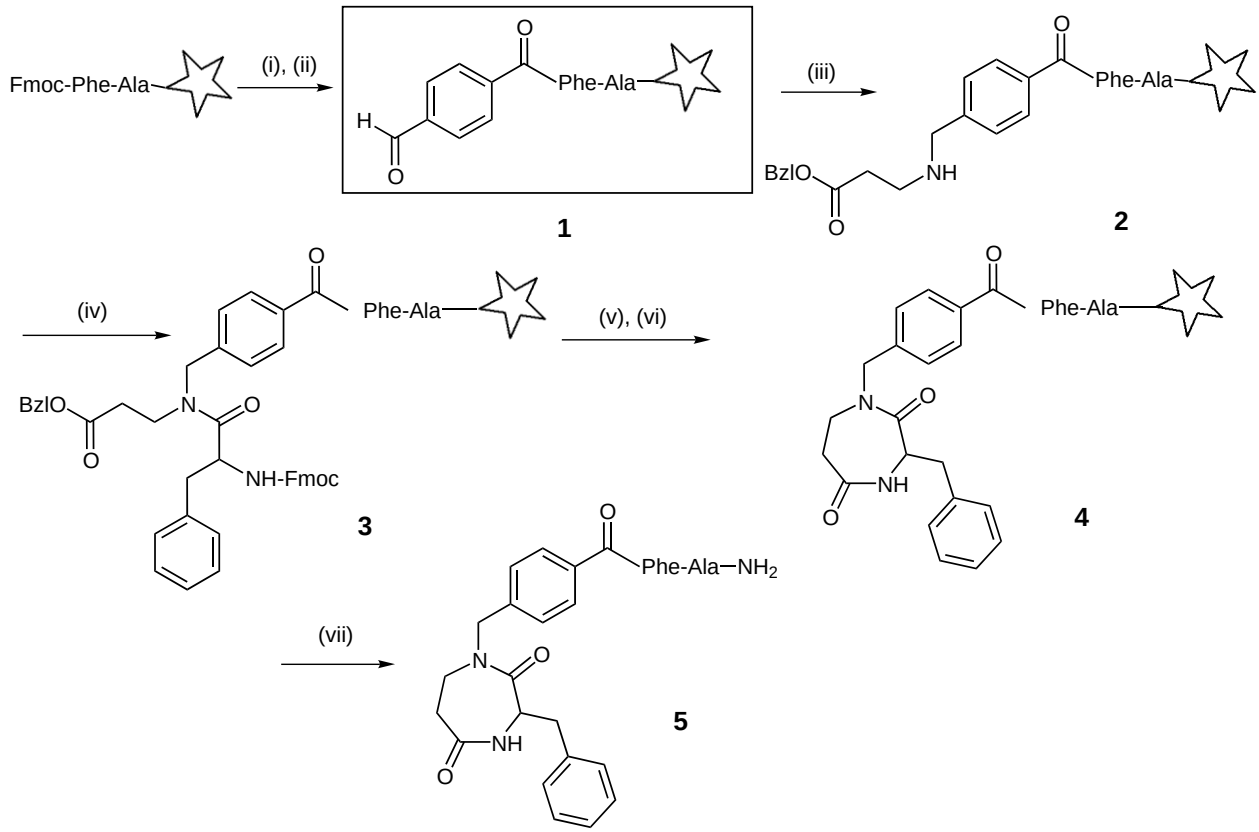


Scheme 1. (i) 20% piperidine/DMF, 20 min; (ii) HOOC-C_6H_5-CHO/HBTU/DIEA; (iii) H-β-Ala-OBzl/$NaBH_3CN$/1% AcOH/DMF, 60°C, 3 h; (iv) FmocPhe-OH/DIC, DCM–DMF (50/50); (v) 20% piperidine/DMF; (vi) DBU/DMSO, 60°C, 3 h; (vii) TFA/H_2O (50:50).

In the initial experiments, we have used Synphase™ rink-amide PS crowns,[10] with a loading of 1.7 μmol to obtain the probe linker OHC-C_6H_5-CO-Phe-Ala-crown (**1**). Fmoc-Ala-OH and Fmoc-Phe-OH were coupled for 2 h (at a 200 mM concentration) with DIC/HOBt in DMF. Deprotection steps were performed with a piperidine/DMF solution (20/80) for 30 min. Different reagents were tried for the 4-carboxybenzaldehyde coupling: BOP/DIEA/DMF; HBTU/DIEA/DMF; DIC/HOBt/DMF, at a 200 mM concentration for 1 or 3 h. After TFA cleavage (TFA/DCM, 1:1) of H-Phe-Ala-crown and OHC-C_6H_5-CO-Phe-Ala-crown, the percentage conversion[11] coupling was calculated. The most effective condition was 200 mM HBTU in the presence of 200 mM DIEA in DMF at rt for 3 h.

The linker was then subjected to reductive amination (step iii) with H-β-Ala-OBzl in the presence of $NaBH_3CN$ to yield **2**. Five parameters were varied simultaneously: solvent (DMF, MeOH), additive (1% AcOH), temperature (25°C, 60°C), $NaBH_3CN$ concentration (0.1 M, 1 M) and H-β-Ala-OBzl concentration (0.5 M, 1 M). As previously, TFA cleavages were simultaneous performed and relative conversion determined. The best conversion (94%) was obtained by treatment with 1% AcOH/DMF, 0.5 M H-β-Ala-OBzl, 0.1 M $NaBH_3CN$ for 2 h at 60°C.

We then coupled Fmoc-Phe-OH to **2** to obtain **3** (step iv). To determine the most efficient coupling conditions for acylating the sterically hindered secondary amine, we simultaneously examined various coupling protocols, i.e. solvent (DMF; DCM; DCM–DMF (5/5), DCM–DMF (9/1), reagent [(HATU/DIEA; Brop/DIEA; TFFH; NCA; DIC (symmetrical anhydride)]. As previously described by Jensen et al.[7] high yields for acylation were obtained using HATU/DIEA/DCM–DMF (9/1) or DIC/DCM–DMF (9/1) coupling protocols. In both cases the reaction was run overnight at rt and the conversion was almost 98%.

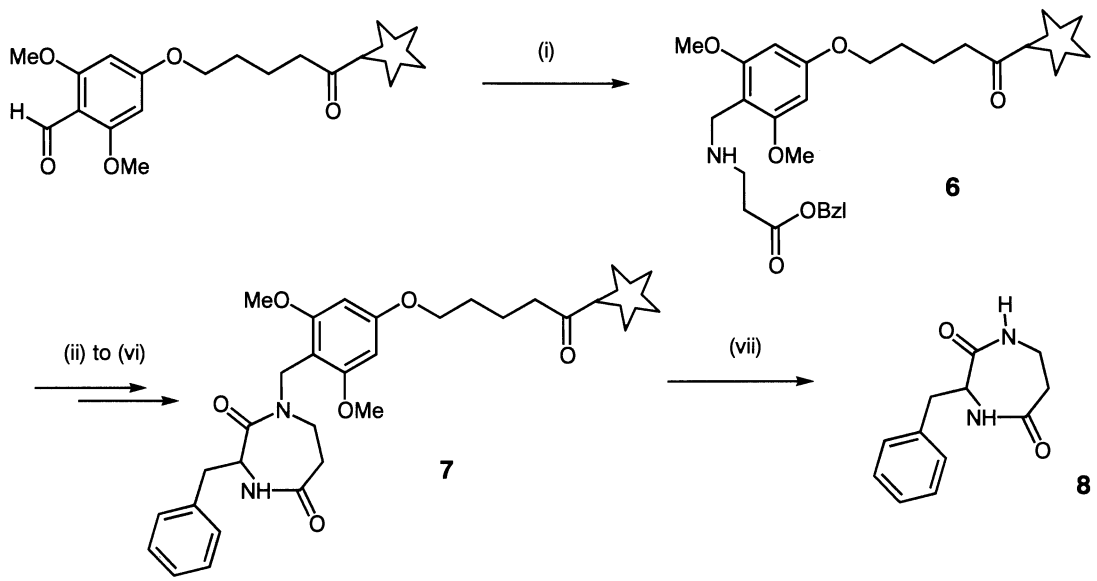
We then tried two different strategies for cyclization which is one of the critical step of the synthesis. One is based on a coupling procedure between the free amine and the free carboxylic acid and the other one on nucleophilic substitution of the free amine on the benzyl ester.

The coupling strategy was performed by KOTMS[12] treatment in various solvents (THF, DMF, DMF/DCM) or NaOH treatment in H_2O/MeOH (1/4) followed by coupling (BOP, HBTU, DIC/HOBT, HATU). However, we observed epimerization of the phenylalanine residue during basic treatment (NaOH or KOTMS).

Table 1. Cyclization step via various experimental conditions

Entry	Base	Solvent	Temp. (°C)	T_R (%)[a]		
				8.26[b] (%)	7.99[c] (%)	Others[d] (%)
1	DBU	DMSO	20	5	89	6
2	DBU	DMSO	60	0	94	6
3	DBU	DMF	60	0	92	8
4	Pyr	DMSO	20	80	15	5
5	Pyr	DMSO	60	57	34	9
6	Pyr	DMF	60	56	38	6
7	NEt_3	DMSO	20	86	0	14
8	NEt_3	DMF	20	85	0	15

[a] Percentage calculated from reverse-phase HPLC spectra at 220 nM.
[b] Fmoc deprotected intermediate.
[c] Cyclized compound.
[d] Non-identified impurities.



Scheme 2. Preparation of 3-benzyl perhydro-1,4-diazepine-2,5 dione using secondary amine forming (SAF) derivatized Synphase™ crowns; steps (i) to (vii) are the same as those previously described in Scheme 1.

In the nucleophilic substitution strategy, the Fmoc protecting group removed by a DMF/piperidine solution (80:20) followed by subsequent cyclization generated the diazepine **4** attached to the handle. Various basic treatments (Et_3N, DBU, pyridine), temperatures (20°C, 60°C) and solvents (DMF, DMSO) were tested to displace the benzyl ester (Table 1). Following TFA cleavage, the products were analyzed by HPLC and LC/MS and relative conversion rates determined. The optimal conditions for performing the cyclization step were DBU in DMSO at 60°C.

The final acidolytic cleavage (TFA/DCM, 1:1) released the free diazepine **5** with a 94% purity.

After optimization of each step, we transferred our strategy on SAF-PS-crown.[13] Optimum conditions, previously determined were applied to the preparation of compound **8** (Scheme 2). Reductive amination with H-β-Ala-OBzl on the SAF linker lead to compound **6**. After acylation with Fmoc-Phe-OH and removing of the Fmoc protecting group, the compound was cyclized with DBU in DMSO at 60°C to lead to **7**. The final compound **8** was obtained after acidolytic cleavage with a 97% purity (LC/MS determination).

In conclusion, we have developed a strategy to obtain diazepinones in high purity using the BAL linker which probably favored the seven-membered-ring system formation by adopting a *cis*-configuration. Diversity on the diazepinone core could be introduced by using various aminoacids and their β-homologated analogues. The application of this strategy for generating a library of diazepinones on SAF-PS-crowns is under investigation.

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11. It is based on HPLC peak areas at 214 and 254 nm of H-Phe-Ala-NH_2, OHC-C_6H_5-CO-Phe-Ala-NH_2 (and potential impurities).
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13. SAF (secondary amide forming handle) is the given name for the BAL linker on the Multipin method (SP-PS-I-SAF, loading 28 μmol, available from Mimotopes).