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# From Solution-Phase Studies to Solid-Phase Synthesis: A New Indole Based Scaffold for Combinatorial Chemistry

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*Abstract:* The core structure of a natural product was selected as scaffold for combinatorial library synthesis. The key step for the construction of the 3,9-diazabicyclo[3.3.1]non-6-ene core is a novel Dakin-West/Pictet-Spengler reaction sequence. Route selection for library synthesis was determined by solution-phase experiments. The solid-phase synthesis was developed based on the synthesis worked out in solution. A number of resins and linkers were studied to obtain the best loading and cleavage conditions. Potential target scaffolds using tryptophan, histidine and phenylalanine as building blocks were investigated. These efforts led to the development of a synthesis protocol for a tetracyclic scaffold incorporating tryptophan, useful for the preparation of a combinatorial library.

**Keywords:** Combinatorial chemistry · Dakin-West · Natural products · Pictet-Spengler · Tryptophan

With the emergence of automated solution and solid-phase synthesis, combinatorial chemistry has become an essential tool in the discovery of new therapeutic agents. Since the first synthesis of peptide libraries, a large number of non-peptidic, particularly heterocyclic, libraries have been published [1]. In the process of identifying new scaffolds, a possible approach is to gain inspiration from the pool of diverse natural products [2]. We turned our interest to polycyclic molecules with defined 3D architecture and restricted number of rotatable bonds. With these considerations in mind, our attention was attracted by the Saframycin, Safracin, Renieramycin and Ecteinascidin families (Fig. 1) which show powerful antiproliferative and antitumor properties making them very attractive targets [3]. All of the compounds of these families are characterized by a common 3,9-diazabicyclo[3.3.1]non-6-ene core structural element.

Recently Myers and Lanman reported a very elegant solid-phase synthesis of a small series of analogues of (–)-Saframycin A [4]. Herein, we would like to report our efforts for the solution and solid-phase syntheses of polycyclic compounds bearing a common 3,9-diazabicyclo[3.3.1]non-6-ene core [5]. Our approach consists of a simple linear construction of the molecule prior to cyclization *via* an intramolecular Pictet-Spengler reaction. In particular this route enables the introduction of a quaternary carbon center with defined stereochemistry. The key step of the synthesis is a novel sequential Dakin-West/Pictet-Spengler reaction.

## Solution-phase Synthesis Strategy

First, a linear dipeptide was synthesized in solution. The first N-α-Boc protected amino acid required an aromatic side chain for the final Pictet-Spengler type cyclization. Four amino acids (3,4-dimethoxy-L-phenyl-

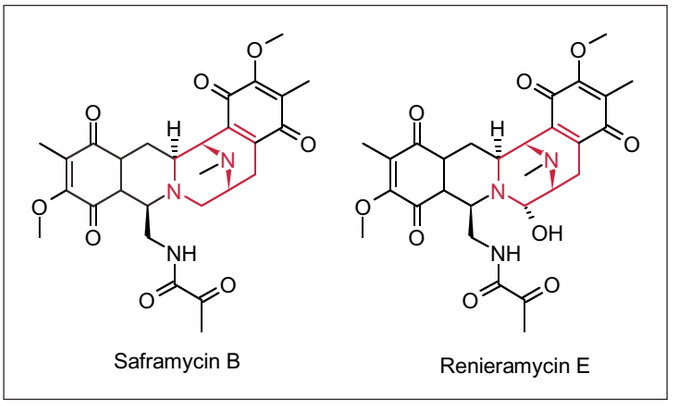


Fig. 1. Examples of structures from Saframycin and Renieramycin families: the 3,9-diazabicyclo[3.3.1]non-6-ene core is outlined in red

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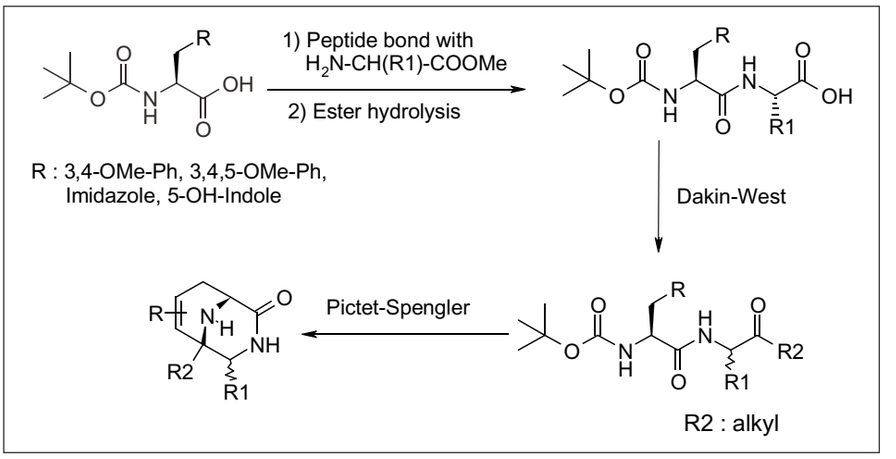
alanine, 3,4,5-trimethoxy-L-phenylalanine, L-histidine and 5-hydroxy-L-tryptophan) were chosen for position one. After coupling with the second amino acid methyl ester, the free carboxylic acid was generated by hydrolysis. Then, the carboxylic function was transformed to the corresponding alkyl ketone under Dakin-West conditions [6] affording the required ketopeptide prior to cyclization (Scheme 1). This linear setup would allow combinatorial synthesis of the dipeptide unit followed by further diversification in the Dakin-West step using different anhydrides.

The described strategy was applied for the synthesis of phenylalanine, histidine and tryptophan derivatives. Initially we focused on phenylalanine derivatives. The acyclic precursors **5** and **6** were obtained in good yields. It is noteworthy that during the Dakin-West reaction epimerization occurred at the C-α position. An acid-catalyzed Pictet-Spengler type cyclization [7] was investigated using different conditions: TFA/DCM, HCl/MeOH and *p*-TsOH. However, no traces of the expected bicyclic compound were obtained (Scheme 2).

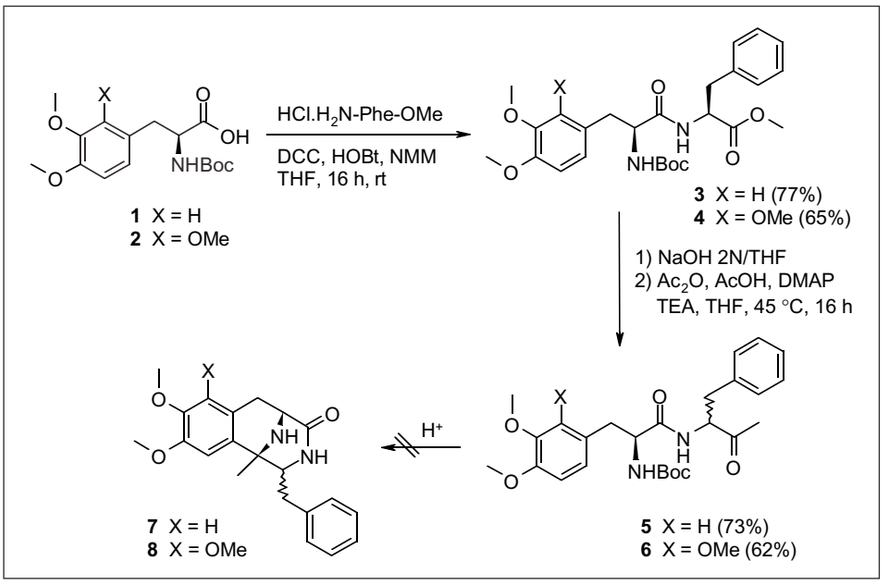
Many examples of Pictet-Spengler cyclizations between an aldehyde and a L-DOPA scaffold are known in the literature but to our knowledge no analogous transformations between a ketone and a free amino group have been reported. Apparently the methoxyphenyl derivative is not reactive enough to allow cyclization of the generated intermediate imine. If compound **6** was treated with *p*-TsOH in refluxing toluene, pyrazinone **11** was obtained in 40% isolated yield. The pyrazinone formation is most likely the result of an oxidation of the intermediate imine **9**. When acetylating this intermediate in basic media N-acetylation of the corresponding enamine to give **12** was observed. Sodium borohydride reduction resulted in the formation of reduced compound **10**, which was obtained as a major diastereoisomer (Scheme 3).

Analogous results were observed when starting from histidine. Under acidic conditions, the acyclic precursor **15** did not form the bicycle **16**. Imine formation was put in evidence by acetylation of the crude mixture to afford the acylated enamine **17** as major compound (Scheme 4).

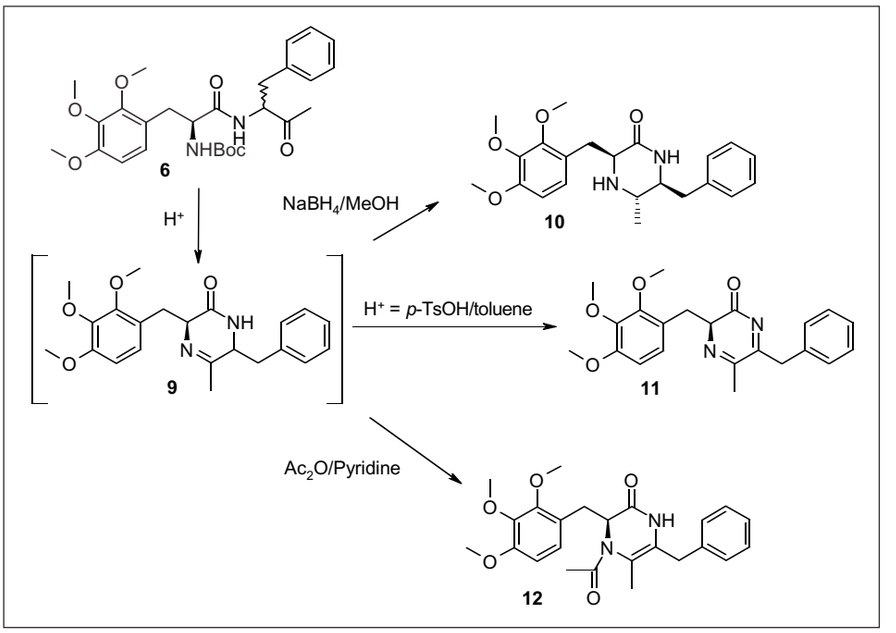
Finally, we turned our efforts towards tryptophan derivatives. Starting from 5-hydroxytryptophan dipeptide **19** was obtained in 63% yield after coupling of **18** and phenylalanine methylester followed by subsequent ester hydrolysis. Then the carboxylic acid function was transformed into a methyl ketone through a Dakin-West reaction. Partial racemization at the C-α stereocenter was observed under the applied




Scheme 1. General solution-phase route



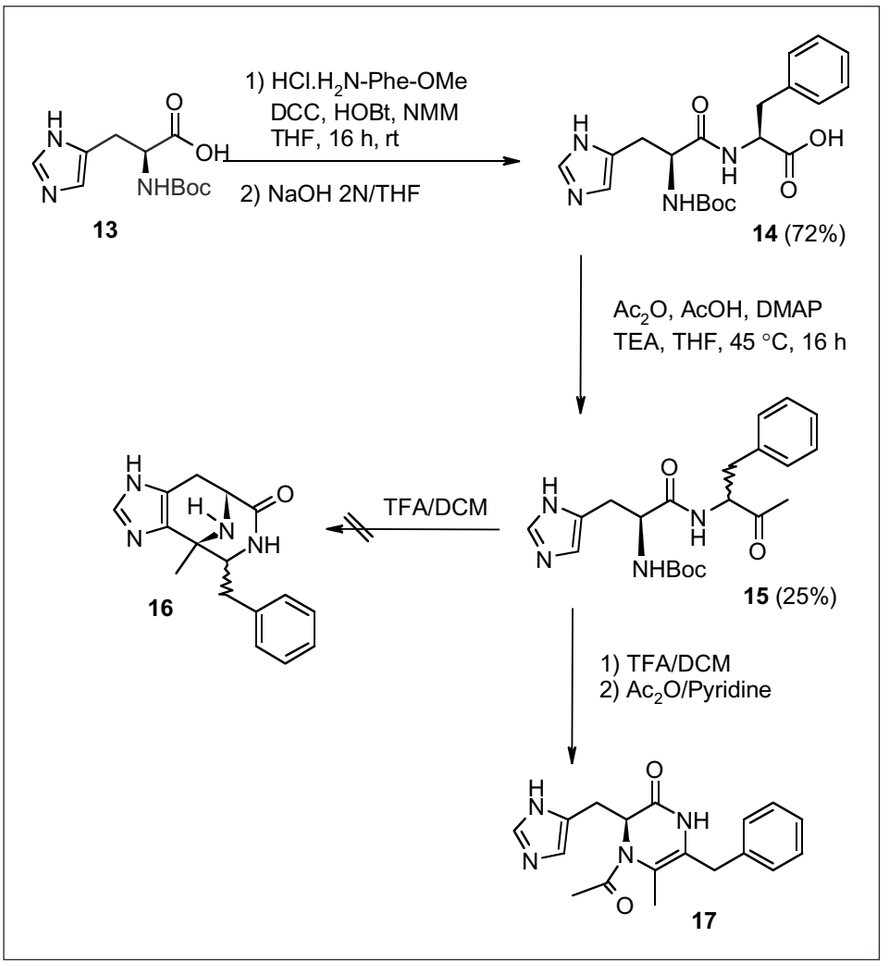

Scheme 2. L-Phenylalanine derivatives synthesis




Scheme 3. Evidence of the imine formation

reaction conditions. The methyl ketone derivative **20** was isolated as a mixture of two diastereoisomers (57:43) in 77% yield. The Boc group was cleaved under acidic conditions (20% TFA in DCM), generating a free amino group, which immediately resulted in the formation of the cyclic imine with the methyl ketone followed by an intramolecular Pictet-Spengler reaction. The 3,9-diazabicyclo[3.3.1]non-6-en-2-one derivative **21** (100% conversion) was isolated in 62% yield (Scheme 5). The quaternary stereo center generated during the cyclization is controlled by the asymmetric center of L-tryptophan. Target structure **21** was obtained as a mixture of diastereoisomers (*cis*-**21**/*trans*-**21** 57:43) which were separated by flash chromatography on silica gel [8]. The relative configuration between the methyl and the benzyl group of the diastereoisomers was determined by modified $^1$H-$^1$H ROESY experiments [9] on a BRUKER DPX 400 MHz. For the *cis* diastereoisomer, NOE effects were observed between ($CH_2^{16}$)-$NH^5$ and $CH_3^3$-$NH^5$ where as for the *trans* diastereoisomer, NOE effects were observed between ($CH^{11}$)-$NH^5$ and $CH_3^3$-$NH^5$.

This solution synthesis has shown that the planed bicyclic scaffold is accessible by the application of an intramolecular Pictet-Spengler strategy. The indole system was identified to be the preferred target structure for further investigations on solid-phase.

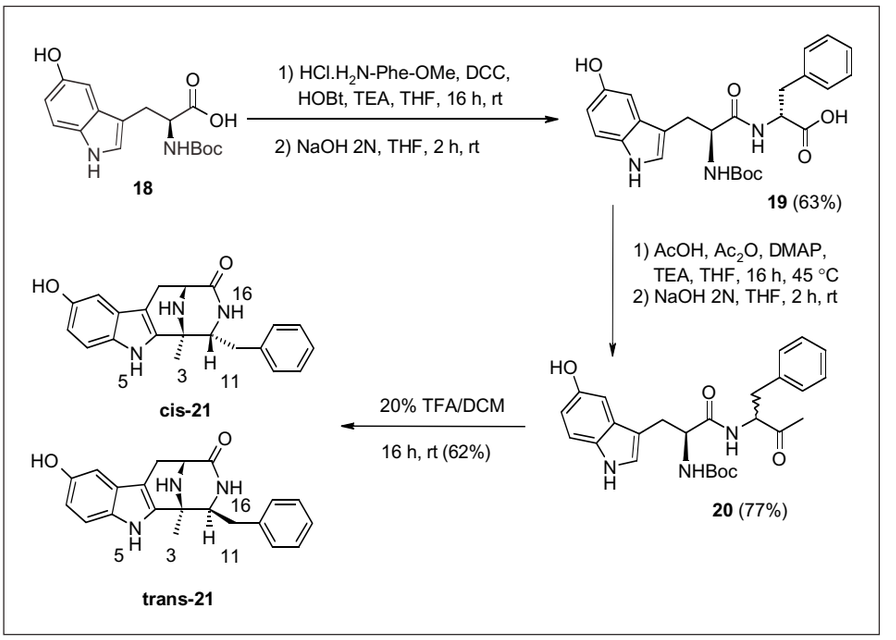



Scheme 4. Synthesis using histidine as building block

## Solid-phase Synthesis Strategy

Solid phase synthesis involves a cyclization under acidic conditions and therefore required an acid-stable linker. Mohan and co-workers reported a serine-based carbamate linker for the attachment of phenolic compounds stable to TFA and organic bases [10]. The cleavage mechanism is based on the removal of the TIPS protecting group of a serine side chain by treatment with 1M TBAF in THF. The formed alkoxy anion attacks intramoleculary the urethane bond, which results in the release of the phenolic compound. For this study, Mohan has used a BocNH-Trp(OH)-OMe template (Scheme 6).

In our experiments, aminomethyl polystyrene D-Lanterns from Mimotopes [11] were used. According to Mohan, we immobilized the BocNH-Trp(OH)-OMe template on the solid phase. Our synthetic plan required methyl ester hydrolysis. Under standard conditions, significant loss of BocNH-Trp(OH)-OH due to linker cleavage was observed. No selective hydrolysis reaction conditions could be found. Therefore, we



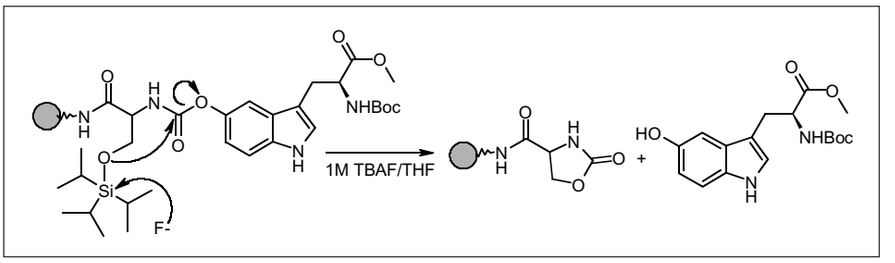

Scheme 5. Solution synthesis of 3,9-diazabicyclo[3.3.1]non-6-en-2-one scaffold

decided to replace the methyl ester by an allyl ester function. In this context, we realized a new efficient synthesis of the Mohan linker by using a preformed protected template prepared in solution prior to the anchorage on the solid support. Esterification of amino acid derivative **18** was realized under conditions reported by Albericio and co-workers [12] with a mixture of allyl bromide and diisopropyl ethylamine in acetonitrile. After aqueous work-up and extraction, compound **22** was directly isolated by precipitation from hexanes in 63% yield. *p*-Nitrophenylcarbonate **23** was generated by treating **22** with *p*-nitrophenyl chloroformate (66% yield after flash chromatography). The other part of the linker was introduced *via* peptide bond formation with BocNH-Ser(OTIPS)-OH. After Boc deprotection, template **24** was immobilised on the solid-phase *via* an urethane bond linkage (Scheme 7). Cleavage of the immobilised scaffold revealed a loading of around 50% after mass quantification. These low loading Lanterns were used to check the feasibility of a solid-phase synthesis.

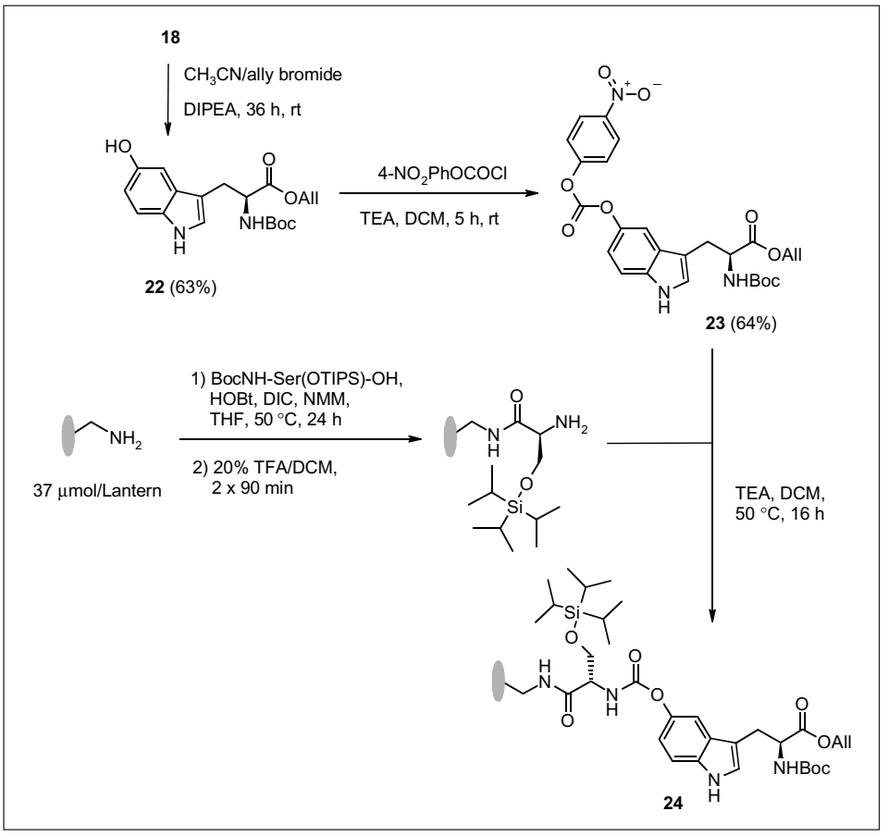
Solid-phase synthesis of the 3,9-diazabicyclo[3.3.1]non-6-en-2-one scaffold was initiated by palladium-catalyzed cleavage [13] of the allyl group from starting Lanterns **24**. Then, phenylalanine allyl ester was coupled under standard conditions and the allyl group was subsequently removed to afford the free carboxylic acid **25**. Completion of the reaction was monitored by cleavage of cut-off Lantern's loops with 1M TBAF/THF and analysis by LC/MS. The modified Dakin-West reaction required significantly prolonged reaction time compared to the solution-phase pendant to go to completion. Cyclization of **26** with 20% TFA/DCM at RT did not lead to any of the expected scaffolds. However, complete cyclization was observed with 20% TFA in DCE at 50 °C for 16 h (Scheme 8).

Cleavage experiments with 1M TBAF in THF solution were very efficient, however LC/MS analysis of the samples were sometimes tedious due to the presence of high concentrations of tetrabutylammonium salts which saturated the mass detector. From our initial experiments, we knew that linker cleavage was observed when inorganic bases were used. Applying similar conditions, effective cleavage of the final scaffold was achieved with a cocktail solution containing 2N NaOH/$H_2O$/THF (2:8:10) for 2 h at rt. Target compound **4** was recovered in an overall yield of 35% (after 6 steps, based on 100% loading) with a purity of 90% after aqueous work-up. NMR and MS confirmed the structure of **21**.

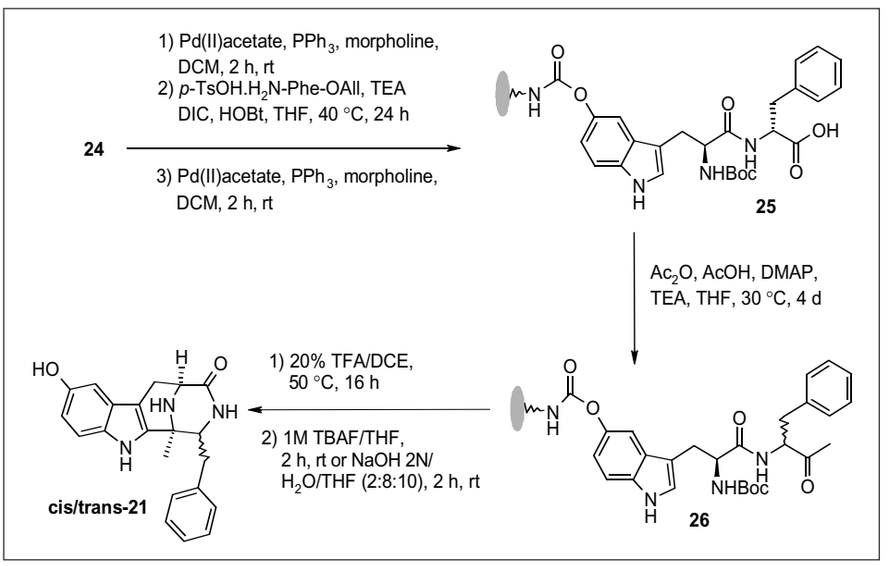
A first outlook of possible diversification (acylation and alkylation) on the



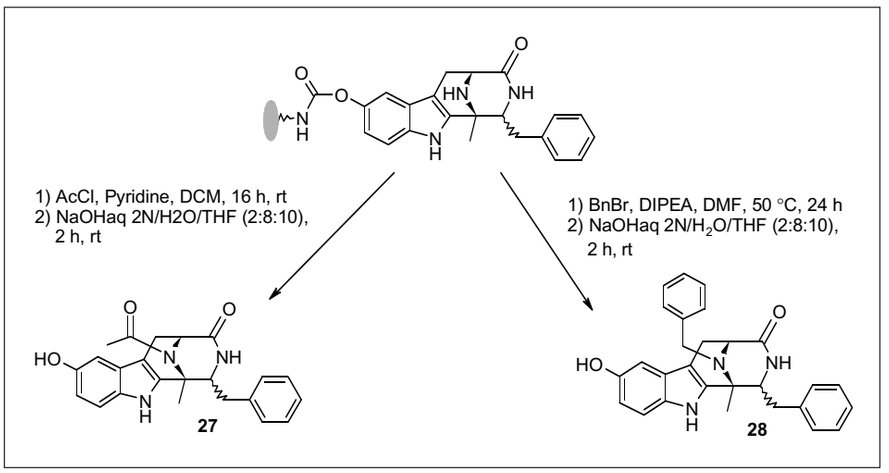

Scheme 6. Serine-based linker developed by Mohan




Scheme 7. Template synthesis and loading



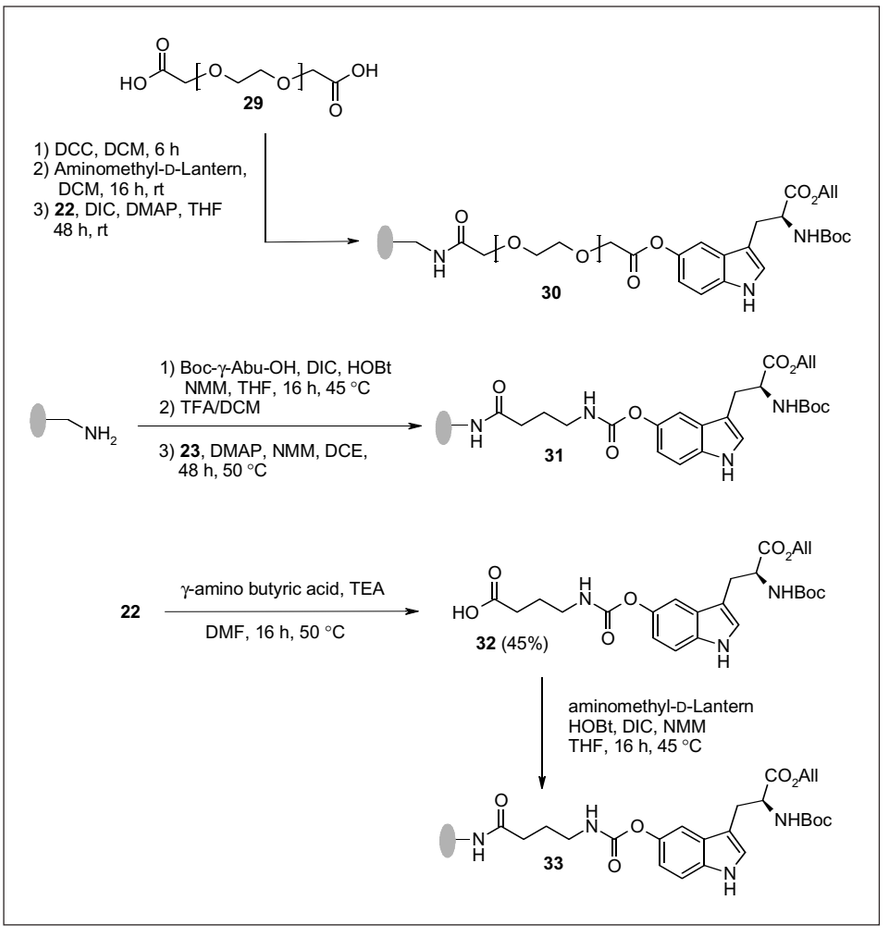

Scheme 8. Solid-phase synthesis of the 3,9-diazabicyclo[3.3.1]non-6-en-2-one scaffold




Scheme 9. Functionalization of the immobilized scaffold

bridged nitrogen was explored (Scheme 9). After cleavage, samples were analyzed by LC/MS which confirmed the mass of the expected materials with purities around 80% for **27** and **28**.

Due to the low loading, further investigations on the attachment of the template to the solid-phase were carried out. From our initial experiments, we knew that the aromatic urethane was cleaved under aqueous basic conditions. These cleavage conditions were compatible with the synthesis scheme. Therefore we decided to use a linker which is cleavable under aqueous basic conditions. Different spacers for the immobilization were reviewed. A small PEG chain was immobilised on the solid-phase by reaction of the anhydride of **29** and aminomethyl polystyrene D-Lanterns. Then, the template **22** was loaded to the resin *via* ester formation to afford resin **30**. A pre-loaded and direct-loaded [14] γ-aminobutyric linker was also investigated. For the direct loading pathway, the resin was reacted with N-Boc-γ-aminobutyric acid first and after Boc deprotection the template was attached by addition of **23** to give the direct loaded resin **31**. In the pre-loading approach, the linker–template adduct **32** was formed in solution by reaction of **23** with γ-aminobutyric acid before its attachment to the Lantern *via* peptide bond formation to afford pre-loaded resin **33** (Scheme 10). The four Lanterns were subjected to the cleavage conditions ($NaOH/H_2O/THF$) and the amount of the released BocNH-Trp(OH)-OH was analyzed by HPLC. The results are summarized in Table 1, resin **33** showed a double loading compared to resins **24**, **30** and **31**. Mass quantification after cleavage from **33** gave a 105% mass recovery of material calculated from the theoretical 37 μmol/Lantern loading.




Scheme 10. Linker syntheses

Table 1. HPLC quantification of released compound.

| Resin | 24 | 30 | 31 | 33 |
|---|---|---|---|---|
| **HPLC Area For Boc-Trp(OH)-OH (x $10^6$)** | 1.56 | 1.73 | 1.98 | 4.24 |

Attaching a preformed linker–template adduct resulted in high loaded Lanterns. These Lanterns were used for the synthesis as previously described. The cyclization was realized with 20% TFA in DCE at 50 °C for 16 h. After cleavage, the expected bicycle was formed, however a more apolar by-product (27%) was present in the mixture reaction and its structure was elucidated as the pyrazinone derivative **34** (Scheme 11).

To suppress this by-product, many cyclization cocktails were investigated (Table 2). The best selectivity was found with 20% TFA in toluene at 80 °C under argon but the pyrazinone formation could not be suppressed completely.

If the methyl ketone was replaced by a benzyl ketone, less of the pyrazinone by-product was formed. After cyclization, alkylation of the nitrogen with benzyl bromide and cleavage, the expected compound was obtained as the major compound **35** (42%) (non-alkylated material 8%, pyrazinone 3%) (Scheme 12).

To avoid pyrazinone formation, we considered switching from polystyrene to polyamide backbone solid-support. The loading of the polyamide Lantern (20 µmole/Lantern) is lower compared to the polystyrene analogue (37 µmole/Lantern). The preformed linker-template (methyl ketone) was loaded to the polyamide Lanterns. After cyclization and cleavage, **21** was the only compound detectable by HPLC without any trace of pyrazinone by-product (Scheme 13).

In conclusion, we have realized solution-phase and solid-phase syntheses of diazabicyclo[3.3.1]nonenone compounds, where the core structure is an attractive scaffold for combinatorial synthesis. A first study showed the feasibility of the synthesis on solid-phase but in low yield. The linker issue was solved. However, with higher loading the formation of a pyrazinone by-product was observed. The switch from polystyrene to polyamide support allowed the elimination of the by-product formation and the diazabicyclo[3.3.1] nonenone derivative was obtained in high purity by solid-phase synthesis. Currently, outlook of the diversification and possible modification of the scaffold on solid-phase are under investigation.

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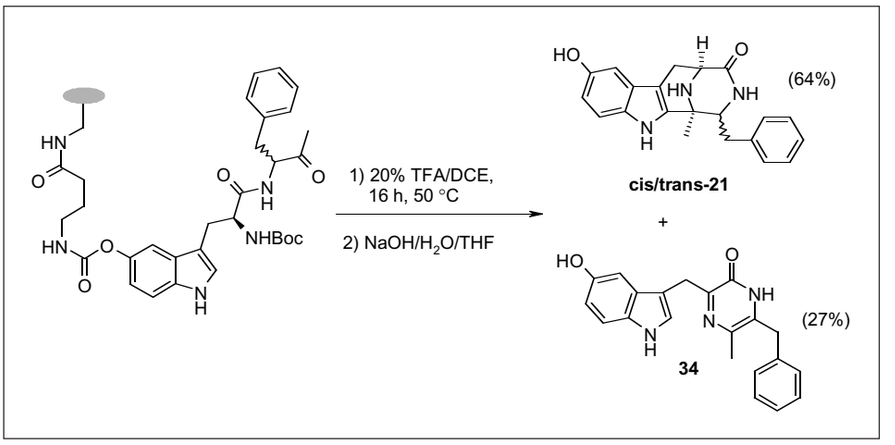
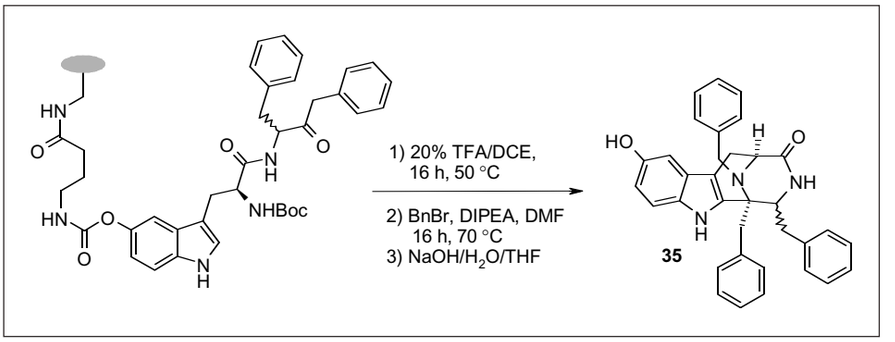


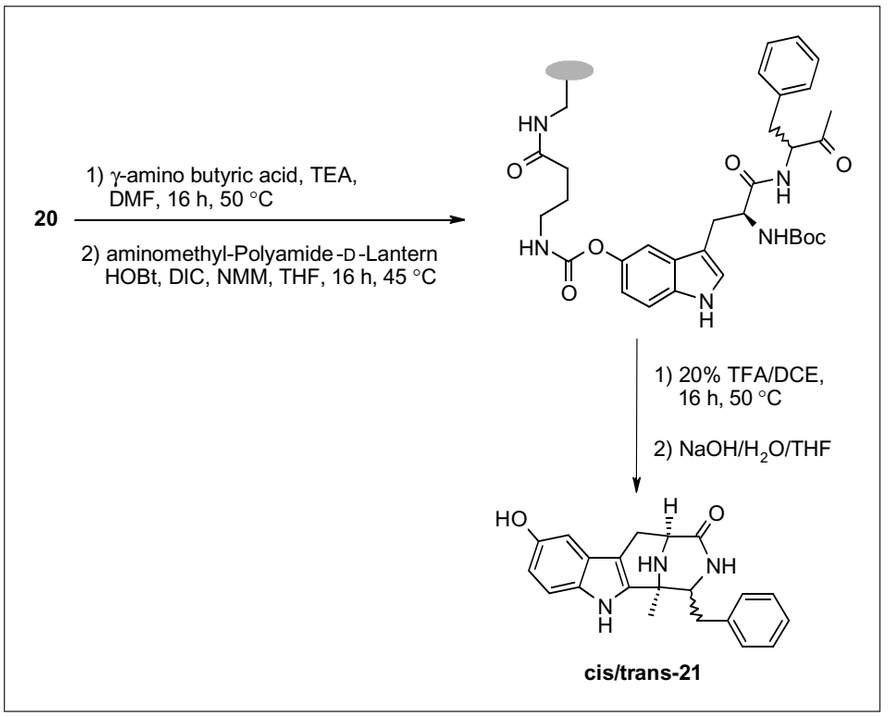

Scheme 11. Cyclization on full loaded resin

Table 2. Cyclisation solution used and relative amount of the different materials.

| Assay | Solution | Atm | T [°C] | Free amine | Bicycle | Pyrazinone |
|---|---|---|---|---|---|---|
| **1** | 20% TFA/DCE | air | 50 °C | 0% | 64% | 27% |
| **2** | 20% TFA/DCE | Ar | 50 °C | 0% | 69% | 20% |
| **3** | 50% TFA/DCE | Ar | 50 °C | 0% | 71% | 18% |
| **4** | 20% TFA/Toluene | Ar | 50 °C | 0% | 71% | 22% |
| **5** | 50% TFA/Toluene | Ar | 50 °C | 0% | 73% | 22% |
| **6** | 20% TFA/Toluene | Ar | 80 °C | 0% | 76% | 20% |
| **7** | 20% TFA/dioxane | Ar | 50 °C | 60% | 0% | 30% |
| **8** | 20% TFA/MeOH | Ar | 50 °C | 70% | 0% | 13% |
| **9** | 20% TFA/DMF | Ar | 50 °C | 72% | 0% | 9% |
| **10** | 20% TFA/$CH_3CN$ | Ar | 50 °C | 0% | 36% | 30% |
| **11** | 20% TFA/Cyclohexane | Ar | 50 °C | 0% | 53% | 23% |
| **12** | 5% TFA/DCE | Ar | 50 °C | 0% | 48% | 28% |




Scheme 12. Cyclization and alkylation of benzyl ketone derivative




Scheme 13. Synthesis on the polyamide Lanterns

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For the *cis* diastereoisomer $^1$H NMR (400 MHz, [D$_6$]DMSO, 26°C) δ = 1.44 (*s*, 3H, C*H*$_3$); 2.67 (*d*, $^2J$(H,H) = 15.04 Hz, 1H, IndC*H*H-); 2.84 (*dd*, $^2J$(H,H) = 15.04 Hz, $^3J$(H,H) = 4.30 Hz, 1H, IndCH*H*-); 2.95 (*dd*, $^2J$(H,H) = 13.97 Hz, $^3J$(H,H) = 13.97 Hz, 1H, C*H*HPh); 3.12 (*dd*, $^2J$(H,H) = 13.97 Hz, $^3J$(H,H) = 4.83 Hz, 1H, CH*H*-Ph); 3.58 (*m*, 1H, CC*H*NHCO); 3.73 (*m*, 1H, C*H*NH); 6.97 (*m*, 1H, N*H*CO); 6.55 (*dd,* $^3J$(H,H) = 8.60 Hz, $^4J$(H,H) = 2.14 Hz, 1H, Ar*H*); 6.66 (*d*, $^4J$(H,H) = 2.14 Hz, 1H, Ar*H*); 7.08 (*d*, $^3J$(H,H) = 8.60 Hz, 1H, Ar*H*); 7.22 (*t*, $^3J$(H,H) = 7.52 Hz, 1H, *PhH*); 7.24 (*d*, $^3J$(H,H) = 7.52 Hz, 2H, Ph*H*); 7.32 (*t*, $^3J$(H,H) = 7.52 Hz, 2H, Ph*H*); 8.55 (*s*, 1H, O*H*), 10.55 (*s*, 1H, Indole N*H*). MS (negative electrospray): *m/z* (%): 346 (100) [M-H]$^-$, 460 (25) [M+CF$_3$COO$^-$], 693 (5) [2M-H]$^-$.
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