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Tandem Mannich/Diels-Alder reactions for the synthesis of indole compound libraries

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A tandem Mannich/Diels-Alder sequence for the synthesis of small-molecule libraries with an indolyl-octahydro-3a,6-epoxy-isoindole core structure is demonstrated in this study. Representative diversification examples based on this scaffold were performed, and a library is being produced within the European Lead Factory (ELF) Consortium.

A large portion of pharmaceutically active compounds and approved drugs,[1] including all small-molecule kinase inhibitors approved by FDA so far,[2, 3] are structurally dependent on heterocyclic scaffolds.[4] The indole core structure is embedded in a plethora of compounds, which exhibit a broad range of biological activities, such as anticancer,[5] antibacterial,[6] anti-inflammatory,[7] anti-HIV,[8] and clearly one of the most intensively studied heterocyclic scaffolds.[9] In fact, indole or fused indole moieties are present in more than 50 FDA-approved small-molecule drugs and countless biologically active compounds currently in clinical or preclinical development.[1] Due to the fact that present drug discovery efforts tend to focus on a limited number of scaffolds, there is a growing interest within the chemical and pharmaceutical communities to develop synthetic approaches towards small-molecule libraries that also incorporate new scaffolds.[10] Properties relating to lipophilicity, fraction of sp^3-hybridised carbon atoms, ratio of chiral/non-chiral centers, and drug-likeness parameters differentiate these newly sought-after scaffolds from most traditional aromatic scaffolds. It is expected that the exploitation of new chemical space represented by these new scaffolds will be associated with novel physico-chemical properties and potentially useful biological effects.[11]

The synthesis of biologically active and structurally diverse small molecule libraries is a current focus of our group.[12-14] As a continuation of our efforts to synthesize indole derivatives,[15] the amine protected compounds **1** with an indolyl-hexahydroepoxyisoindole core was designed. This scaffold has previously been synthesized employing an intramolecular Diels-Alder reaction[16-18] and is virtually unexplored biologically. We envisioned its formation through a convenient tandem Mannich/Diels-Alder reaction sequence. Subsequent cycles of deprotection and functionalization lead to indole compounds **3** with an octahydro-3a,6-epoxyisoindole core and three sites for diversification: one introduced intrinsically by the indole component, in addition to a primary amine and a secondary amine for further decoration (Fig. 1). In the present work, we describe the synthesis of a small-molecule library based on this indolyl-octahydroepoxyisoindole scaffold (**3**), which combines a bicyclic aromatic indole with a tricyclic aliphatic 3a,6-epoxy-isoindole ring, the latter displaying a high Fsp3 value and several chiral centers.

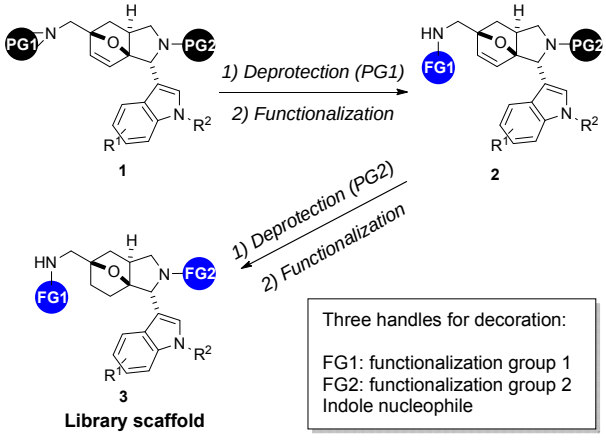

Fig. 1 Production of indole compounds **3** through cycles of deprotection and functionalization.

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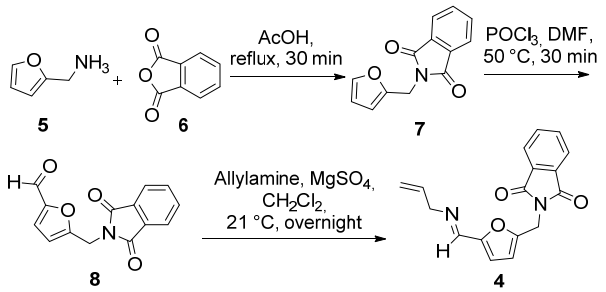
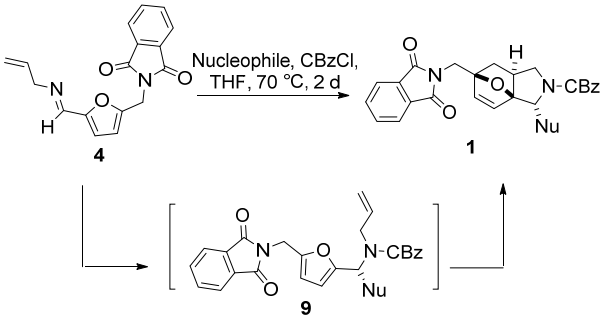


Fig. 2 Synthesis of substrate **4** for the Mannich/Diels-Alder reaction.

Table 1. One-pot Mannich/Diels-Alder Reaction



Entry	Nucleophile[a]	Product **1**	Yield[b]
1	*N*-methylindole	**1a**	59%
2	Indole	**1b**	56%
3	6-fluoroindole	**1c**	62%
4	5-methoxy-indole	**1d**	72%
5	5-fluoroindole	**1e**	60%
6	1,3-dimethoxybenzene	**1f**	-[c]
7	1*H*-pyrrolo[2,3-*b*]pyridine	**1g**	-[c]
8	1*H*-indazole	**1h**	-[c]
9	thiophene	**1i**	-[c]

[a] 4 equiv. of nucleophile was added in the reaction.
[b] Isolated yield after column chromatography. [c] Intractable mixture.

The synthesis of substrate **4** for the Mannich/Diels-Alder reaction was achieved by protection of furfurylamine **5**, followed by formylation using phosphoryl chloride to give aldehyde **8**,[19] which readily reacted with allylamine to afford the Schiff base **4** in high yield (Fig. 2).

In initial experiments with the one-pot Mannich/Diels-Alder reaction sequence, indole was employed as the *C*-nucleophile in THF at 70 °C, together with CBzCl, to give the amino-protected compound **1b** in 56% yield (Entry 1, table 1). This process is easily applicable to other indole nucleophiles, substituted with either electron-withdrawing or electron-donating groups, affording the corresponding tricyclic 3a,6-epoxyisoindole derivatives **1a** and **1c-e** in good yields ranging from 59 to 73%, (Table 1, Entries 2-6). Attempts to use other *C*-nucleophilic aromatic systems, such as 1,3-dimethoxybenzene, 1*H*-pyrrolo[2,3-b]pyridine, 1*H*-indazole, and thiophene, in this one-pot process led to intractable mixtures with little or no trace of Diels-Alder product. The reaction was monitored by C-MS, where Mannich intermediates **9a-e** could be observed, as detected by a significant [M+Na$^+$] peak, while the Diels-Alder products **1a-e** were characterized by a significant [M+H$^+$] peak. The relative stereochemistry was determined by NOESY analysis. Although compounds **1** were obtained as racemates, it is noteworthy to mention the possibility of accessing both enantiomers, through either chiral preparative HPLC or enantioselective Diels-Alder reactions,[20, 21] during lead optimization of identified hits..

Removal of the phthalimido proctecting group of **1a-e** with hydrazine and hydrochloric acid in methanol afforded the corresponding compounds with a primary amine handle, which was subject to a subsequent round of diversification steps.‡ Selected modification examples include sulfonylation with 4-(trifluoromethyl)benzene sulfonyl chloride to give **2a**, TBTU-mediated acylation with cyclopropanecarboxylic acid to give **2b**, urea-formation using phenyl isocyanate to give **2c**, and acylation with cyclohexanecarbonyl chloride to give **2d** (Fig. 3).

Cbz-deprotection of **2a-d** was carried out under reducing condition using 10% Pd/C and a hydrogen atmosphere. However, except in the case of *N*-methyl indole substituted compound **2a**, epimerization at the 3-CH-indole position with varied ratio ranging from 9:1 to 3:2 was observed during the deprotection for compounds **2b-d**. The crude compound was simply isolated by concentration in vacuo and used directly in the next steps, since any attempts to purify it by column chromatography failed probably due to poor stability of the free amines.§

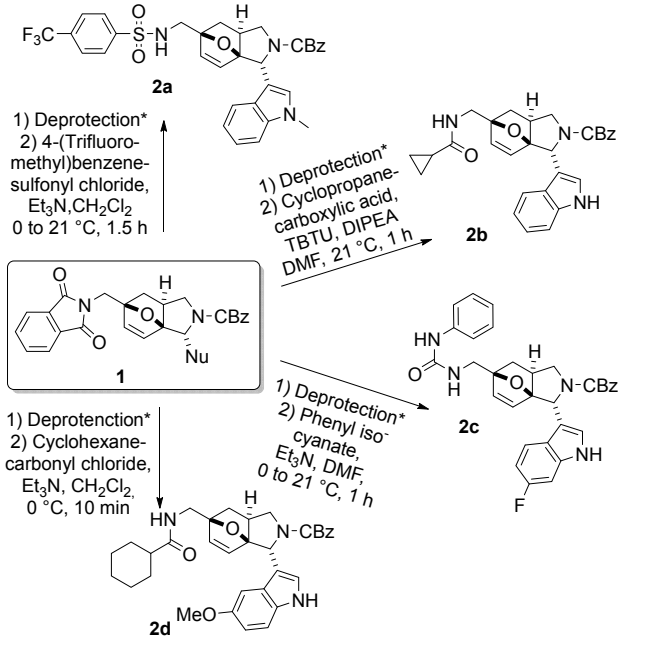


Fig. 3 Examples of functionalized compounds **2** after the first step. * Deprotection condition: hydrazine, MeOH, 21 °C, 1 h.

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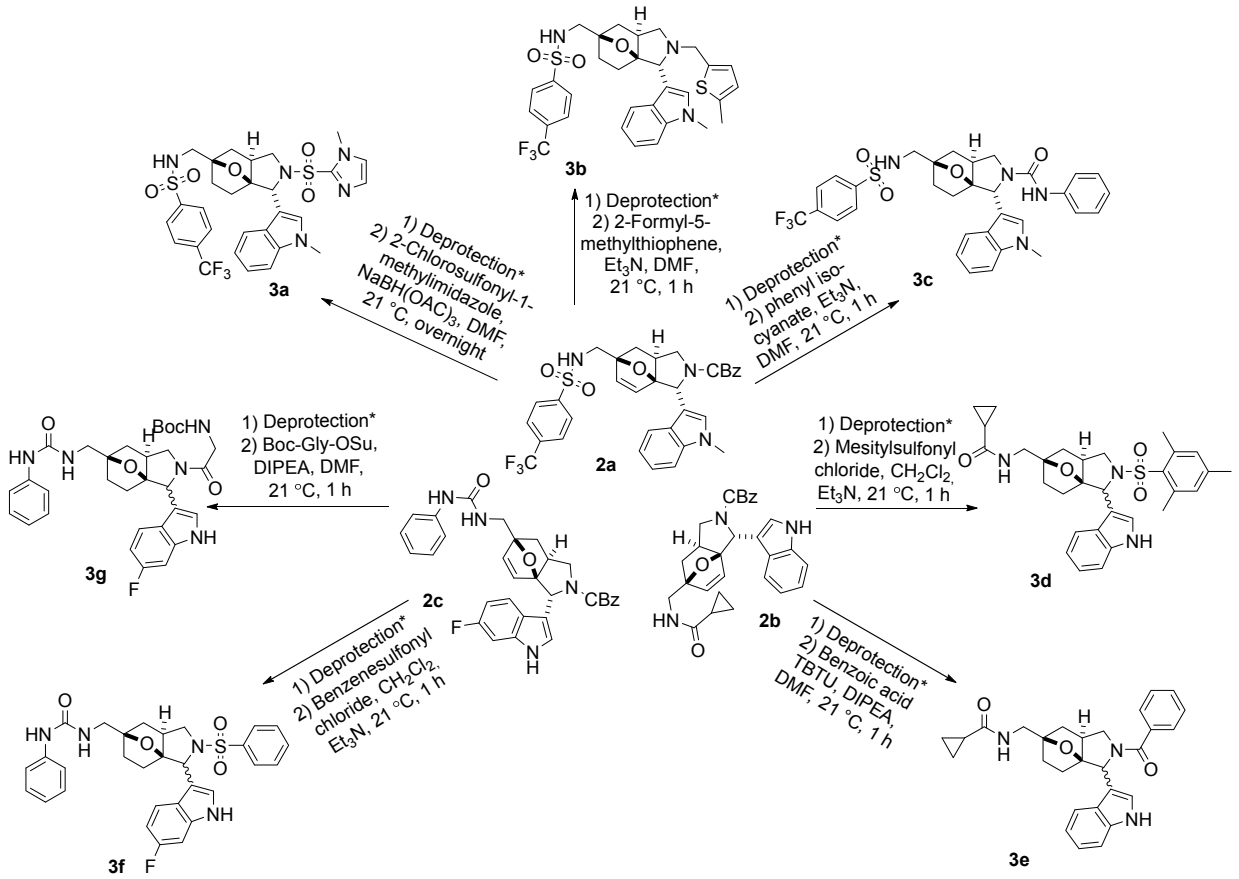

Fig. 4 Examples of validated compounds **3** for library synthesis. * Deprotection condition: 10% Pd/C (10 mol%), H_2, MeOH, DMF, 21 °C, overnight.

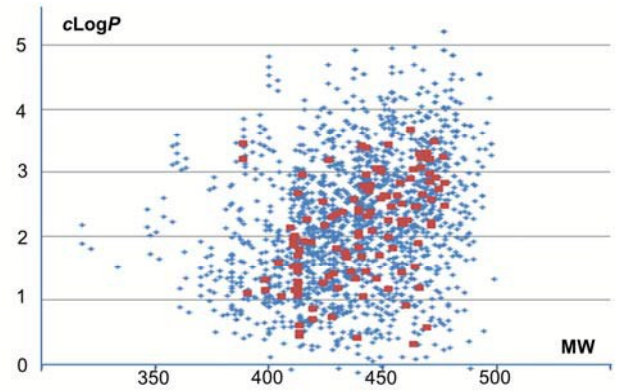

Fig. 5 Physical chemical property analysis of the produced compounds (red dots) vs enumerated compounds in library (blue dots).

The secondary amino handle was then subject to another round of diversification steps. For example, the deprotected product of sulfonamide **2a** was converted to the bissulfonamide **3a**, alkylated compound **3b**, and urea **3c**. The deprotect product of amide **2b** was functionalized to give sulfonylated compound **3d** and acylated compound **3e** through a sulfonylation reaction and a TBTU-coupling reaction, respectively, and the free amine derived from urea compound **2c** was further functionalized to give sulfonylated compound **3f** and amide **3g** (Figure 4).

All of the functionalized compounds **3a-g** were purified by direct preparative HPLC, which underpin the subsequent production of a screening compound library. Based on the steps of phthalimido deprotection, functionalization of primary amine, Cbz deprotection, and functionalization of secondary

amine for the synthesis of compounds with an indolyl-octahydroepoxyisoindole core, a collection of 120 compounds that resemble the structural features of compounds **3a-c** have been produced as a part of a small-molecule screening library under the ELF consortium. All produced compounds and most of the enumerated compounds are compliant with the Linpinski's Rule of Five in terms of *c*LogP values and MW (Figure 5).

Effectively, the production focused on final compounds in *N*-methyl indole series since no epimerization at the 3-CH-indole position occurred during the Cbz-deprotection step. Noteworthy, the Mannich/Diels-Alder reaction was reproducible on a 0.1 mol scale with yields comparable to those shown in Table 1. To expand the library, future productions will involve additional *N*-substituted indoles. A systematic relative configuration assignment method would also be approached with the aim to include both diastereomers from *N*-unsubstituted indoles series in the library, since most of these diastereomers could be separated by preparative LCMS (C18-phase). The goal is to populate new, biologically relevant chemical scaffolds that are not represented in existing screening collections.

Conclusions

An efficient protocol for the rapid assembly of indolyl-hexahydro-3a,6-epoxyisoindole via a tandem Mannich/Diels-Alder synthesis sequence has been developed in the reported study. Diversification of the indolyl-octahydroepoxyisoindole core through amino group functionalization has led to the validation of 120 compounds which will be incorporated in a small-molecule library under the ELF consortium.

Acknowledgements

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Notes and references

‡ The phthalimido deprotection was also tested in $MeNH_2$, although the conversion was initially effective, it was proved to be reversible, and difficult to perform the reaction in reproducible results.

§ An alternative approach to remove the Cbz protection before phthalimido deprotection led to slow reactions in both deprotection steps.

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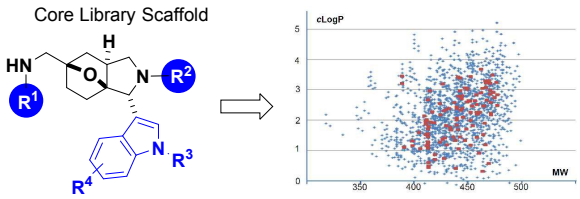

A core scaffold for screening library production was synthesized in just four steps using a tandem Mannich/Diels-Alder sequence.