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Realisation of Small Molecule Libraries based on Frameworks Distantly Related to Natural Products

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The availability of high-quality screening compounds is of paramount importance for the discovery of innovative new medicines. Natural product (NP) frameworks can inspire the design of productive compound libraries. Here, we describe the design and synthesis of four compound libraries based on scaffolds that have broad NP-like features, but that are only distantly related to specific NPs. The optimisation of syntheses of the scaffolds using [5+2] cycloaddition chemistry is detailed, together with methods to yield exemplar decorated screening compounds. In each case, a library was nominated for production, leading to a total of >2900 screening compounds that augmented the Joint European Compound Library of the European Lead Factory.

Introduction

The design of compound libraries that target diverse biologically-relevant chemical space is a major challenge in chemical biology and medicinal chemistry. In recent years, a limited synthetic toolkit has dominated molecular discovery,[1-2] tending to reinforce the uneven and unsystematic exploration of small molecule frameworks.[3-4] Historically, about one third of approved small molecule drugs have been inspired by natural products (NPs).[5] NPs are necessarily biologically relevant because they arise *via* the evolution of metabolic pathways to provide specific benefits to host organisms.[6-7] NPs are structurally distinctive,[8] and often contain high fractions of sp^3 hybridised carbons, a feature that has also been shown to correlate favourably with the successful translation of clinical candidates.[9] NPs have inspired the design of productive small molecule compound libraries; for instance, biology-oriented synthesis exploits scaffolds that are related to NP frameworks, inspiring the design of productive screening libraries.[10-11]

Recently we described a 'top down' synthetic approach in which a few complex bridged intermediates were transformed into many diverse sp^3-rich molecular scaffolds. The approach yielded small molecule scaffolds that retain local structural features of NPs, yet that are distantly related to specific NP frameworks.[12] Crucially, biological relevance was demonstrated through discovery of fragment hits, based on exemplar scaffolds, for three epigenetic protein targets. It was thereby shown that scaffolds that are only distantly related to specific NPs can facilitate the identification of new biologically-relevant chemical space.

We have now translated four libraries based on scaffolds accessible using our 'top down' approach into the European Lead Factory (ELF). ELF is a collaborative, public-private partnership that aims to generate novel and innovative lead molecules for drug discovery and chemical biology.[13] Here, we describe the development of practical routes for the synthesis of the four scaffolds, and methods for scaffold decoration to yield exemplar screening compounds. We were often able to extend the scope of our established chemistry, enabling, for example, access to derivatives with complementary substitution patterns.[12] In total, the four libraries comprised >2900 screening compounds that augmented the Joint European Compound Library (JECL) of the ELF.

Results and discussion

Synthesis of the scaffold precursors

We harnessed our recently developed 'top down' synthetic approach in which many scaffolds were derived from a small number of complex intermediates.[12] It was envisaged that four compound libraries would be generated based on the scaffolds **3**-**6**, which, in turn, would be derived from the cycloadducts **1** and **2** (Figure 1). The cycloadducts **1** and **2** would be prepared using intramolecular [5+2] cycloaddition reactions.[15-16] Scaffolds **3** and **4** would be prepared *via* manipulation of parent ring-systems of **1** and **2**, respectively by a

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formal ring-expansion (→**3**) and annulation (→**4**). In contrast, scaffolds **5** and **6** would be prepared by functionalisation of the parent cycloadducts **1** and **2**.

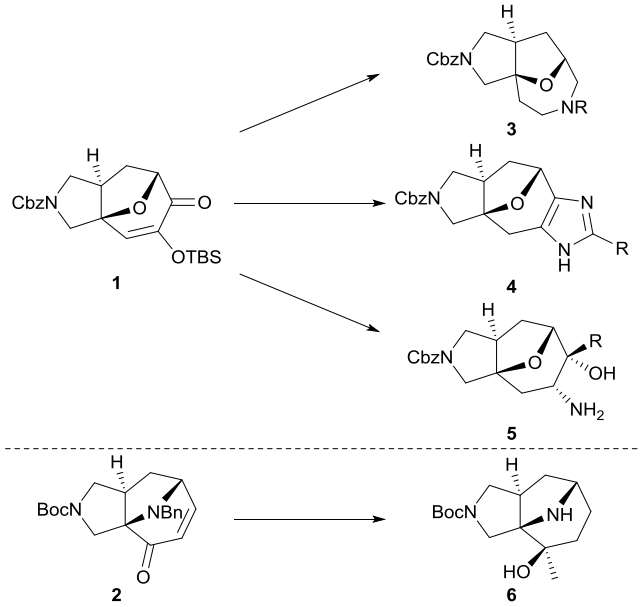
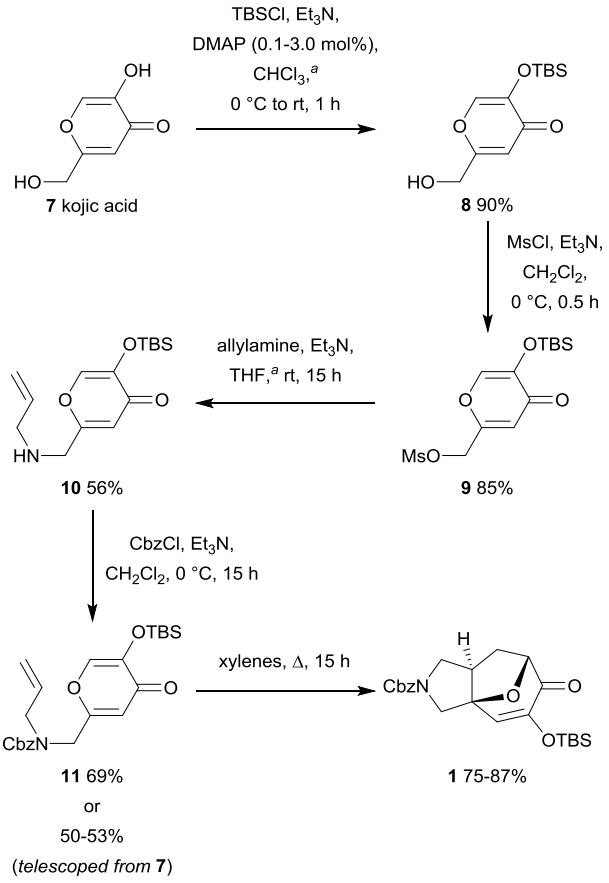


Fig. 1. Scaffolds **3**-**6** selected for translation into compound libraries. The scaffolds were derived from the cycloadducts **1** and **2**.

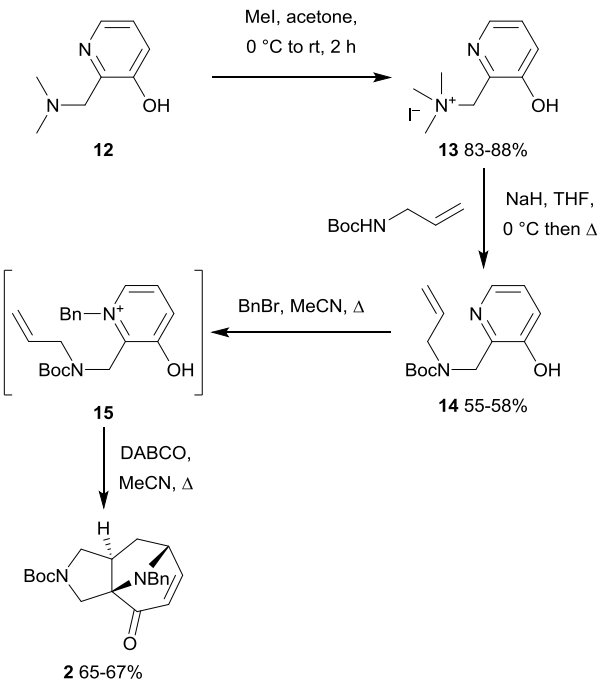
To prepare the oxygen-bridged cycloadduct **1**, we exploited a silyl transfer-induced [5+2] cycloaddition of 3-*tert*-butyldimethylsilyloxy 4*H*-pyran-4-one **11**, drawing inspiration from the pioneering work of Mascareñas.[15] Cycloadduct **1** was prepared in 4 steps starting from commercially available kojic acid **7** (Scheme 1). The pyranone hydroxyl group of kojic acid was initially silylated (→**8**); mesylation of the remaining alcohol, followed by displacement with allylamine furnished amine **10**. Purification of amine **10** was challenging, which impacted significantly on its isolated yield. Subsequent protection of amine **10** using benzyl chloroformate gave the cycloaddition precursor **11** in 30% overall yield (over 4 steps from **7**).

To scale-up the production of compound **11** we developed a telescoped procedure. In this sequence, no purification other than washing and extraction were carried out until **11** was purified by column chromatography; using this approach, compound **11** was prepared in 53% overall yield over 4 steps starting from 70.4 mmol of kojic acid. Scale-up of the optimised route enabled preparation of 76.7 g (0.18 mol) of **11** in a comparable 50% yield. Finally, silyl transfer-induced [5+2] cycloaddition of **11** was achieved by heating **11** in xylenes at 155 °C for 15 h, furnishing the cycloadduct **1** in good yield (from 37.0 mmol **11**: 87%; from 0.18 mol **11**: 75%).

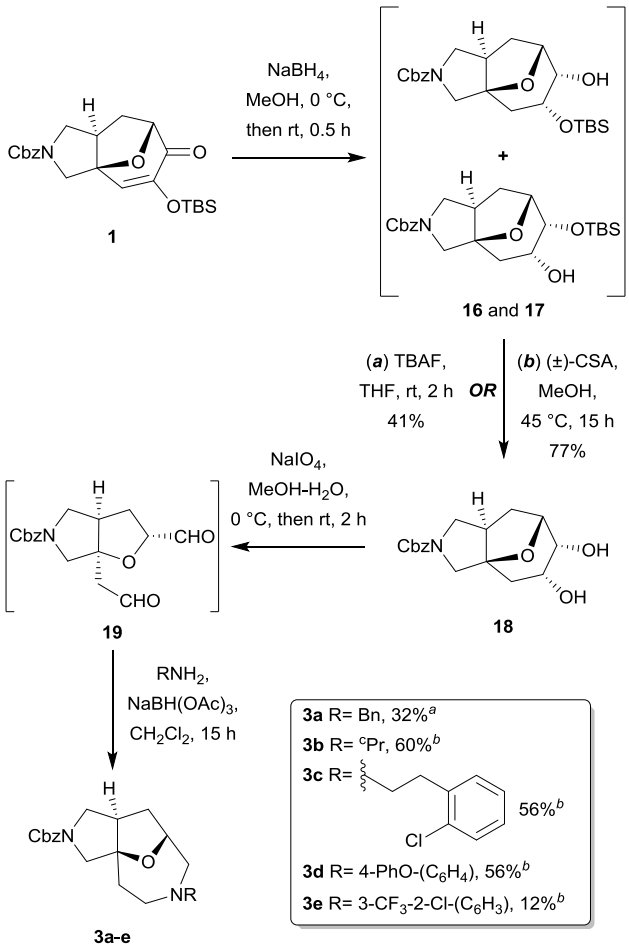


Scheme 1. Preparation of the cycloadduct **1**. [a]CH_2Cl_2 used in the telescoped procedure.

To prepare the nitrogen-bridged cycloadduct **2**, we employed an intramolecular [5+2] cycloaddition of a 3-oxidopyridinium ylid (Scheme 2).[17] Starting with commercially available 2-(dimethylaminomethyl)-3-hydroxypyridine **12**, the amine was quaternised by reaction with methyl iodide. The resulting quaternary ammonium salt **13** was substituted using a nitrogen-centred anion generated from *N*-Boc allylamine and sodium hydride, to give the cycloaddition precursor **14**. Subsequent *N*-benzylation of the pyridine nitrogen (→**15**), followed by treatment with DABCO, provided a one-pot route to the cycloadduct **2** in 31-32% overall yield (over 4 steps from **12**).



Scheme 2. Preparation of the cycloadduct **2**.



Scheme 3. Synthesis of the scaffolds **3**. [a]2 steps from **18**. [b]4 step telescoped procedure from **1**.

Synthesis and decoration of Cbz-protected scaffolds 3

The scaffolds **3** were derived from the cycloadduct **1** using a formal ring-expansion approach (Scheme 3). Cycloadduct **1** was reduced using $NaBH_4$ giving a 40:60 regioisomeric mixture‡ of TBS-protected products **16** and **17**, presumably resulting from partial migration of the silyl group (see Figure 2 for details). Initially, TBAF was used to deprotect the mixture of **16** and **17**; however, separation from tetrabutylammonium salts was challenging using flash chromatography, and the diol **18** was isolated in only 41% yield. Purification following deprotection with (±)-camphorsulfonic acid (CSA) was more straightforward, and the diol **18** was isolated in 77% yield. The diol **18** was cleaved using $NaIO_4$ to give the intermediate dialdehyde **19**, which was subsequently subjected to double reductive amination to afford protected diamine **3a** in 32% yield (over 2 steps from **18**). In large-scale preparations of the scaffolds **3b-e**, we exploited a four-step telescoped sequence which avoided flash chromatography until the final step**.** In the reductive amination step, the aliphatic amine substrate was varied to give the scaffolds **3b** and **c** in 60% and 56% yield respectively over 4 steps. Anilines could also be exploited, enabling the preparation of scaffolds **3d** and **e** in 56% and 12% yield respectively over 4 steps.

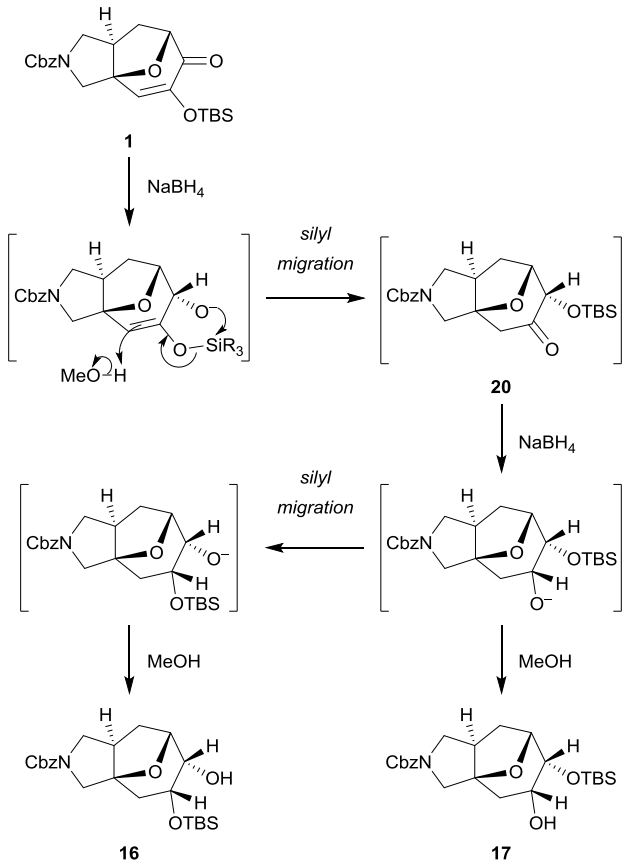
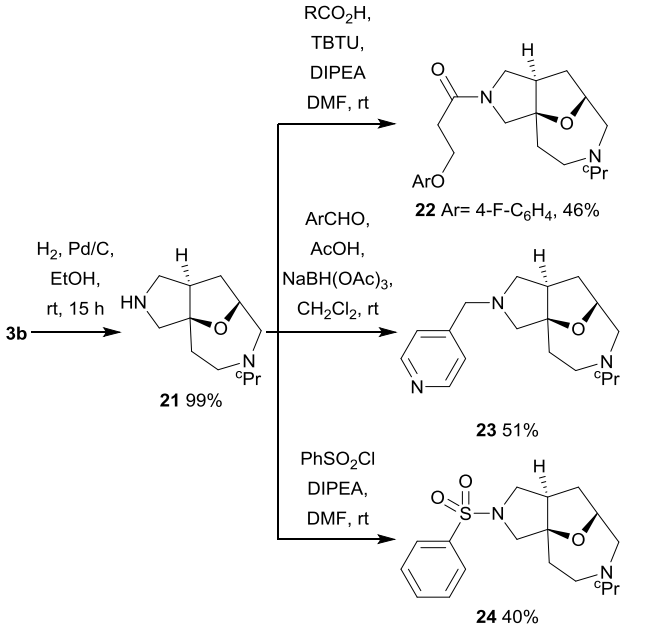


Fig. 2. Proposed mechanism for the formation of protected diols **16** and **17**.

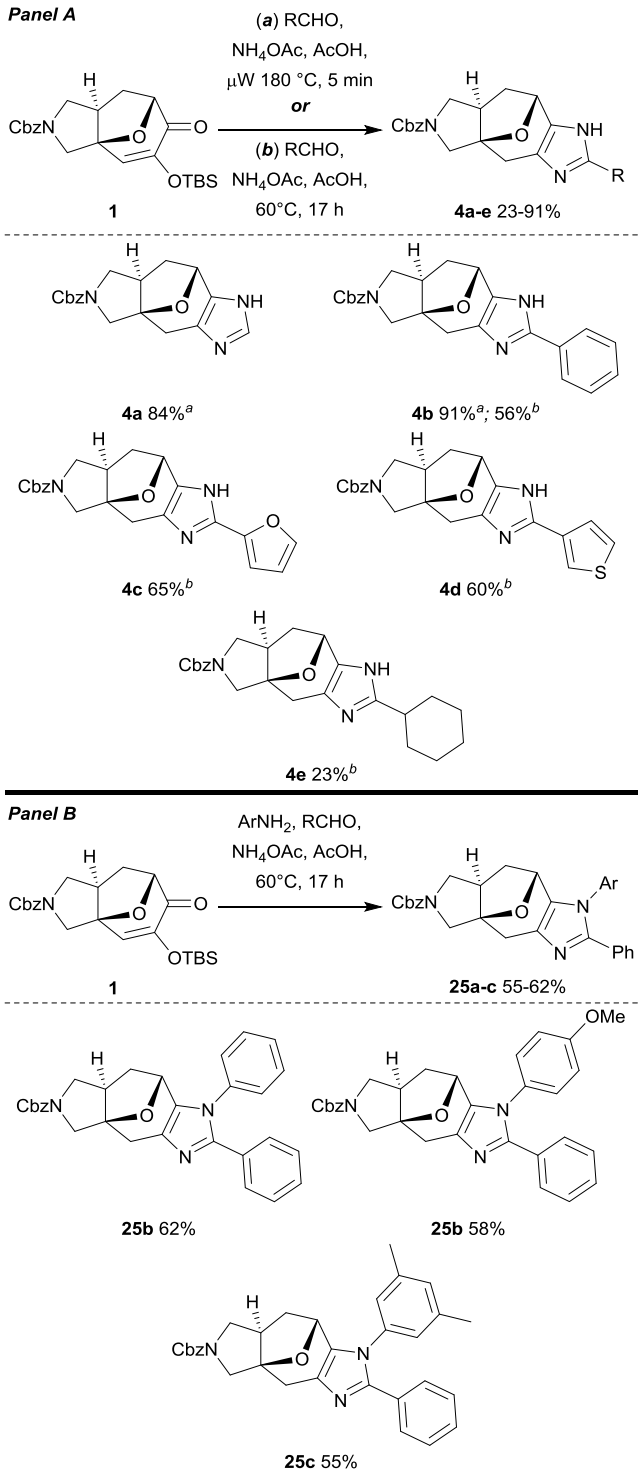
To demonstrate the utility of scaffolds **3** for the preparation of screening compounds, we deprotected the Cbz-protected amine of **3b**, and decorated the corresponding free amine of **21** (Scheme 4). Exemplar reactions exploited included amide formation (→**22**), reductive amination (→**23**) and sulfonamide formation (→**24**). These experiments determined which methods would be subsequently suitable for preparing large numbers of screening compounds from this scaffold.



Scheme 4. Preparation of exemplar screening compounds from scaffold **3b**.

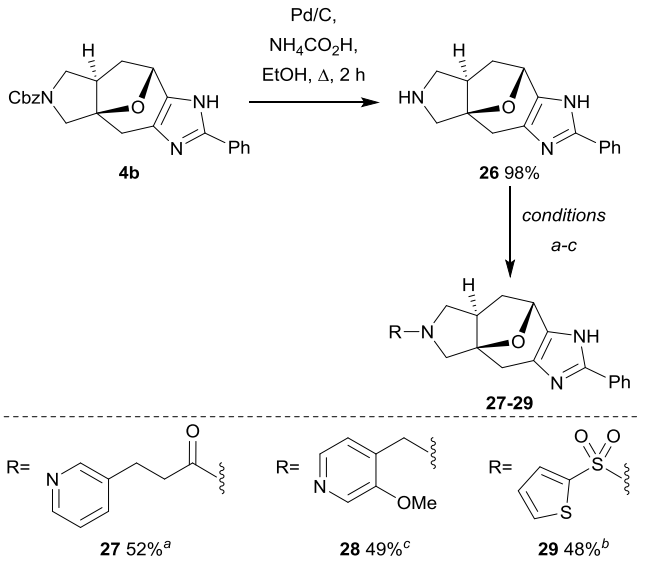
Synthesis and decoration of imidazole-containing scaffolds 4

The scaffolds **4** were prepared directly from the cycloadduct **1** *via* a modified Debus-Radziszewski imidazole synthesis (Scheme 5, Panel A).[18] Heating the cycloadduct **1** with an aldehyde and NH_4OAc in AcOH enabled a one-pot annulation process to furnish imidazoles.[19] Initial conditions focused on the use of microwave conditions (5 min, 180 °C) to prepare imidazoles (→ **4a** and **4b**). However, to enable scale-up, we subsequently investigated conventional heating (60 °C, 17 h). Many non-enolisable aldehydes (e.g. R= H; Ar) typically gave the corresponding imidazoles in high yield (56-91%), although cyclohexanecarboxaldehyde gave the scaffold **4e** in just 23% yield. We also demonstrated that it is possible to prepare *N*-substituted imidazoles **25** regioselectively by exploiting an aniline in place of NH_4OAc (Scheme 5, Panel B).[18]



Scheme 5. Preparation of the imidazole-containing scaffolds **4** (*Panel A*) and **25** (*Panel B*). [a]Conditions *a* used. [b]Conditions *b* used.

Hydrogenation of the Cbz-protected imidazole **4b** gave the free amine **26**. Decoration was accomplished (Scheme 6) by amide formation (→**27**), reductive amination (→**28**) and sulfonylation (→**29**).



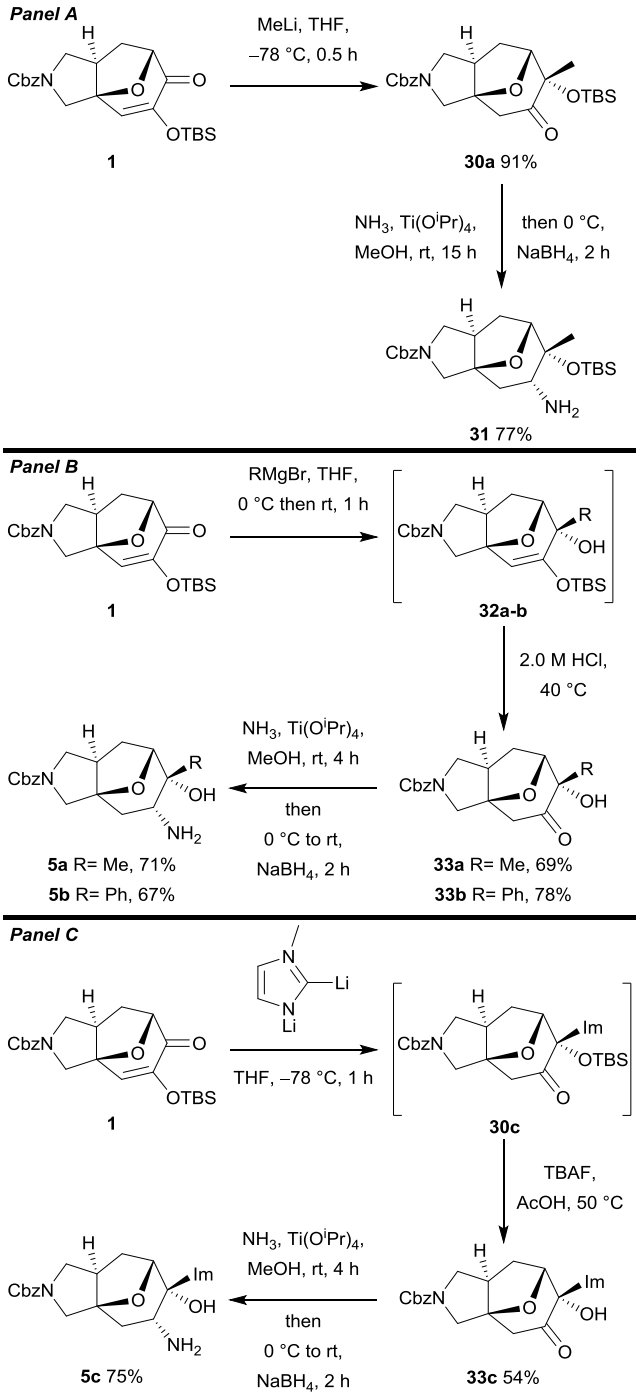
Scheme 6. Decoration of the imidazole-containing scaffold **4b**. **Conditions:** [a]TBTU, RCO_2H, DIPEA, DMF. [b]ArCHO, AcOH, $NMe_4BH(OAc)_3$, DMF, rt; [c]$ArSO_2Cl$, DIPEA, DMF, rt.

Synthesis and decoration of the scaffolds 5

Reaction of the cycloadduct **1** with MeLi gave the TBS-protected α-hydroxy ketone **30a** in 91% yield as a single diastereomer whose relative configuration was determined by NOESY analysis (Scheme 7, Panel A). Here, addition of the nucleophile was followed by silyl migration. Presumably the high diastereoselectivity stems from preferential approach of the nucleophile from the less hindered face of the bridged ketone **1** (Figure 3, Panel A). Treatment of **30a** with methanolic ammonia in the presence of $Ti(O^iPr)_4$, followed by addition of $NaBH_4$, gave the protected 1,2-amino alcohol **31** which was isolated in 77% yield as a single diastereomer.[20]

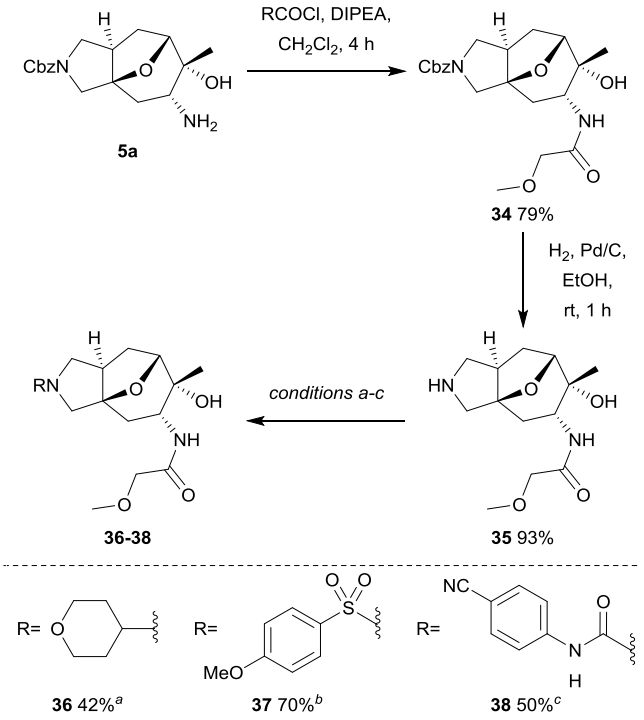
On a larger scale, we elected to substitute the organolithium reagent with Grignard reagents, allowing the development of a one-pot addition-deprotection sequence (Scheme 7, Panel B). Accordingly, addition of alternative Grignard reagents (MeMgBr or PhMgBr) to **1** gave the silyl enol ethers **32**, without triggering subsequent silyl migration.[15] Following completion of the reaction, the silyl enol ethers **32** were deprotected directly by heating the crude reaction mixture with dilute HCl, giving rapid access to the α-hydroxy ketones **33a** and **33b** in 69% and 78% yield respectively. Finally, reductive amination of ketones **33a** and **33b** gave the 1,2-aminoalchohols **5a** and **5b** in 71% and 67% yield respectively.

The reaction was also compatible with more polar nucleophiles, for instance, with the 1-methyl-1H-imidazol-2-yl lithium carbanion, which was generated by treating the corresponding imidazole with *n*-BuLi (Scheme 7, Panel C). The resulting TBS-protected tertiary alcohol was deprotected using TBAF to give α-hydroxy ketone **30c**, which could then be subjected to the previously described reductive amination protocol to furnish the highly functionalised scaffold **5c**.



Scheme 7. Preparation of the scaffolds **5a-c** bearing a 1,2-aminoalcohol functionality. *Panel A:* The initial synthetic route to prepare TBS-protected scaffold **31**. *Panel B:* The optimised synthetic route to prepare scaffolds **5a-b**. *Panel C:* Preparation of imidazole-containing functionalised scaffold **5c**. Im= 1-methyl-1*H*-imidazol-2-yl.

To prepare exemplar screening compounds (Scheme 8), the primary amine of scaffold **5a** was decorated to give the amide **34**. Subsequent hydrogenolysis of **34** removed the Cbz protecting group to furnish the free amine **35**. The amine **35** was subsequently decorated using reductive amination (→**36**), sulfonamide formation (→**37**), and urea formation (→**38**).



Scheme 8. Decoration of scaffold **5a**. **Conditions:** [a]Tetrahydro-4*H*-pyran-4-one, AcOH, $NaBH(OAc)_3$, DMA, rt, 24 h; [b]$ArSO_2Cl$, $NaHCO_3$, DMA, rt, 2.5 h; [c]ArNCO, $NaHCO_3$, DMA, rt, 16 h.

Synthesis and decoration of *N*-bridged scaffold 6

Initially, the cycloadduct **2** was treated with MeLi to give the alcohol **39** in 61% yield (Scheme 9); here, a single diastereomer was isolated by flash chromatography from a 3:1 mixture of diastereomers (as judged by analysis of the crude reaction product by 500 MHz ^{1}H NMR spectroscopy). The relative configuration of the OH-bearing stereocentre was confirmed by X-ray crystallographic analysis of the derivative **40** (Figure 3, Panel C). In direct contrast to the organolithium addition to the cycloadduct **1** (Scheme 8), a strong preference for addition of the organometallic reagent from the rear face (as drawn) was observed (Figure 3, Panel B). The reversed diastereoselectivity may be attributed to the presence of the *N*-benzyl substituent on the bridgehead nitrogen which may hinder approach of the nucleophile from the top face (Figure 2, Panel B). Notably, the procedure for the preparation of the alcohol **39** was suitable for scale-up without further optimisation (64% overall yield from **2** on a 46.4 mmol scale). Subsequent hydrogenation under acid conditions both cleaved the *N*-benzyl protecting group and reduced the alkene to afford the scaffold **6** in 75-95% yield. Finally, decoration at the bridgehead nitrogen was demonstrated using amide formation (→**40**), reductive amination (→**41**), and urea formation (→**42**).

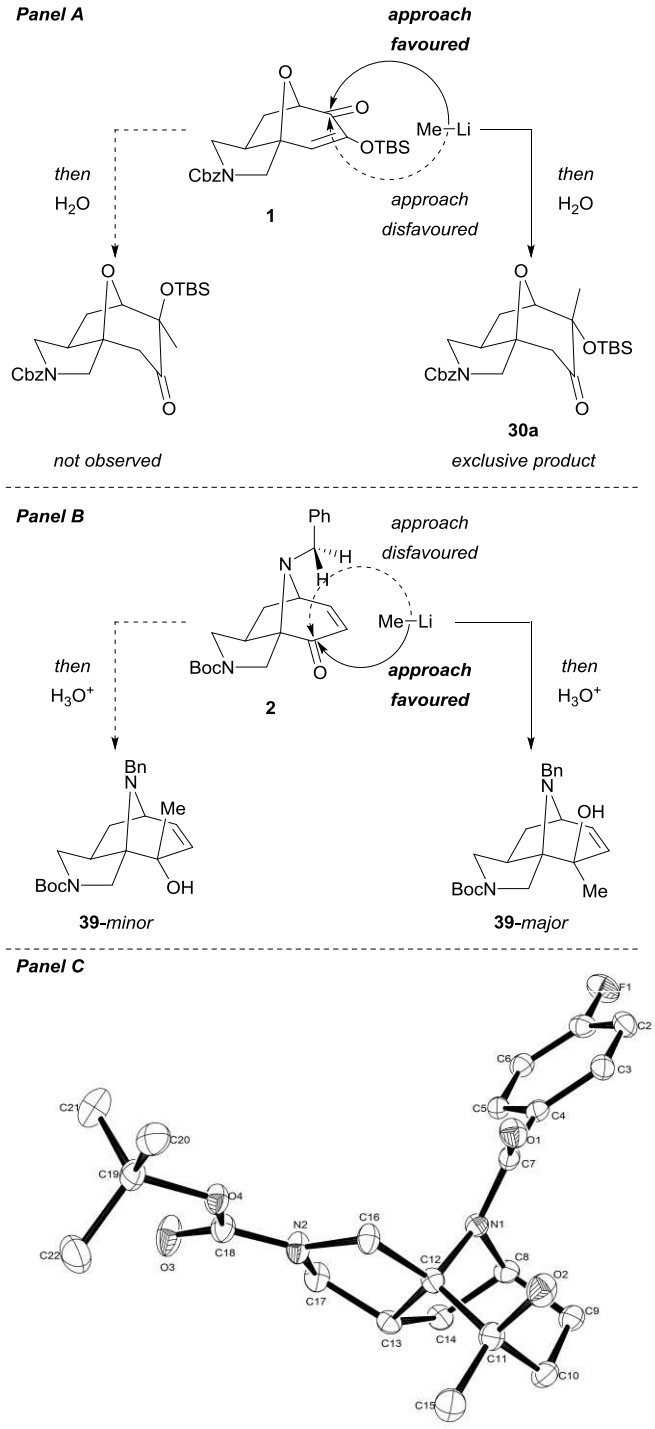
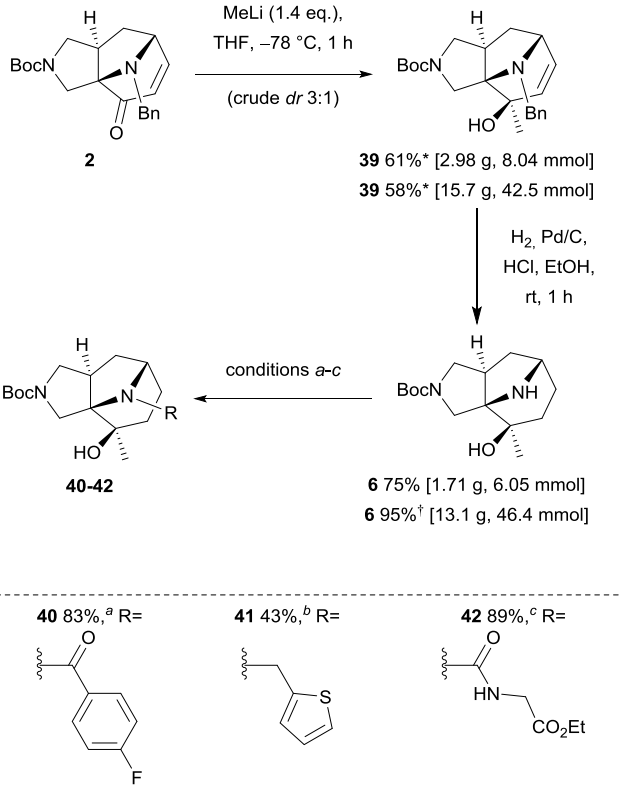


Fig. 3. Diastereoselectivity of the nucleophilic addition of MeLi to compounds **1** and **2**. *Panel A:* Mechanistic rationale for the observed preference for nucleophilic addition of MeLi from the top face (as drawn) of ketone **1**. *Panel B:* Mechanistic rationale for the observed preference for nucleophilic addition of MeLi from the bottom face (as drawn) of ketone **2**. *Panel C:* A crystal structure of compound **40** (CCDC: 1577645).



Scheme 9. Preparation of the scaffold **6**, and decoration of the bridgehead nitrogen to derive compounds **40**-**42**. Conditions: [a]ROCl, DIPEA, CH_2Cl_2, 16 h; [b]ArCHO, $NaBH(OAc)_3$, CH_2Cl_2, rt; [c]ArNCO, DIPEA, CH_2Cl_2, rt, 16 h. *Isolated from the crude reaction product as a single diastereomer. †Prepared from 48.6 mmol compound **39**.

Design and production of libraries based on the scaffolds 3-6

Libraries of screening compounds based on the scaffolds **3**-**6** were produced with properties suitable for early stage drug discovery (Figure 4). The decorations exploited were guided by the successful methods validated in this study, and included *N*-acylations, *N*-alkylations, sulfonamide formations, and urea formations. For each library, the level of success in final decoration step – leading to purified screening compounds – was high (**3**: 751 compounds prepared, 87% success rate; **4**: 594 compounds prepared, 94% success rate; **5**: 1082 compounds prepared, 88% success rate; **6**: 476 compounds prepared, 76% success rate)**.** In total, 2903 final compounds were prepared, highlighting the value of the developed synthetic approaches in augmenting the Joint European Compound Library (JECL) of the European Lead Factory with high-quality, biologically relevant[12] small molecules.

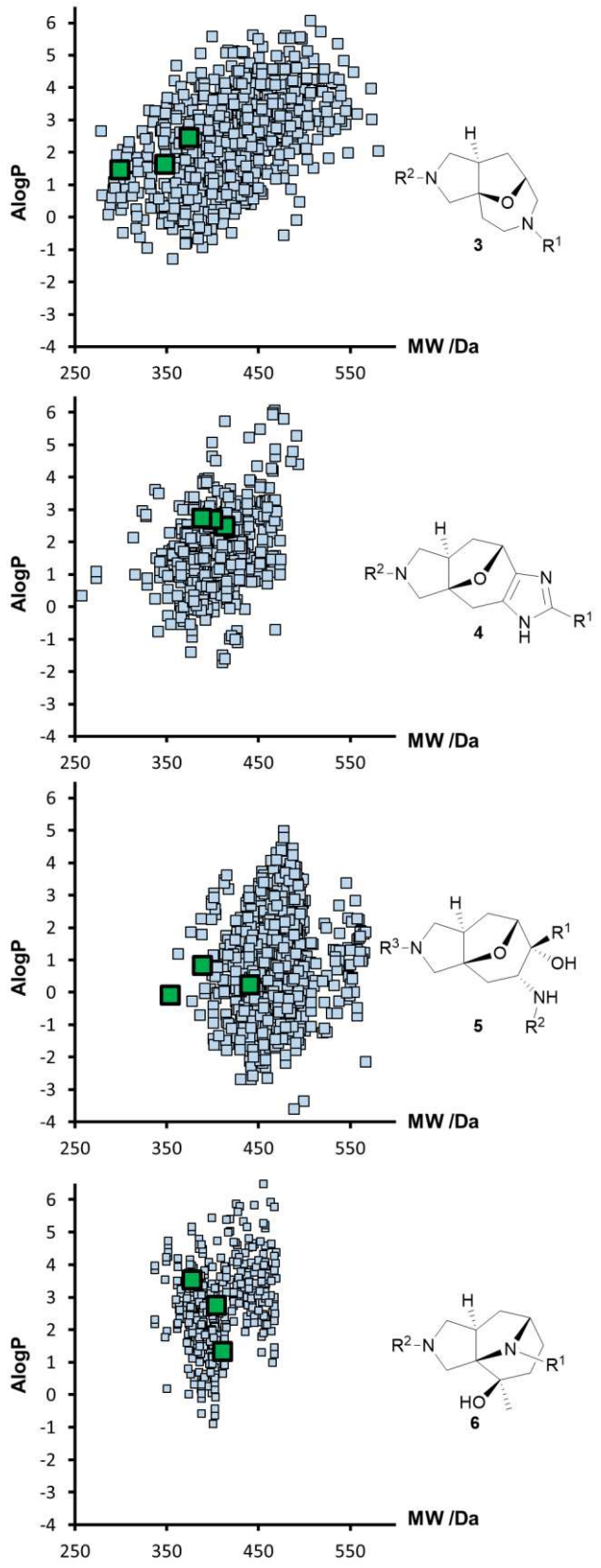


Fig. 4. Molecular properties analysis of produced compound libraries (pale blue squares) and exemplar compounds described in this paper (large, green squares).

Conclusion

Efficient syntheses of four distinct, *sp*^{3}-rich, natural product-like[12] scaffolds were developed from two common cycloadducts. The robust and scalable synthetic methods enabled the scaffolds **3**-**6** to be prepared on a scale that was suitable for the production of large numbers of screening compounds. Ultimately, >2900 medicinally relevant screening compounds were prepared that enhanced the ELF Joint European Compound Library. The structural complexity of the molecular scaffolds is remarkable, and contrasts starkly with those typically explored in early stage drug discovery.[9] The hits identified from screening such compounds would therefore provide highly distinctive starting points for drug discovery programmes.

Conflicts of interest

This research was performed as part of the European Lead Factory (ELF) initiative, whose mission is to provide high quality starting points for early stage drug discovery.

Acknowledgements

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‡The identity of the major regioisomer was not determined.

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