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PAPER

A two-directional strategy for the diversity-oriented synthesis of macrocyclic scaffolds†

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Macrocyclic compounds represent a structural class with exceptional potential for biological activity; however, they have historically been underrepresented in screening collections and synthetic libraries. In this article we report the development of a highly step-efficient strategy for the diversity-oriented synthesis of complex macrocyclic architectures, using a modular approach based on the two-directional synthesis of bifunctional linear precursors and their subsequent combination in a two-directional macrocyclisation process. In this proof of principle study, the synthesis of 14 such compounds was achieved. Cheminformatic analysis of the compounds produced suggests that they reside in biologically relevant regions of chemical space and the compounds were screened for activity against two cancer cell lines.

Introduction

Diversity-oriented synthesis (DOS) aims to produce compound collections that incorporate high degrees of structural diversity.[1] These synthetic campaigns can provide a source of compounds for use in screening experiments that attempt to discover novel biologically active entities.[2] The libraries produced by DOS find particular utility in unbiased screening experiments, such as phenotypic assays, where the precise nature of the biological target is undefined. Experiments of this type give the potential for the discovery of novel biological targets or modes of action, as well as novel agents for targets that are already well understood.[3]

The potential for a given molecule to interact with biological systems, and so exhibit biological activity, is intrinsically related to the structure of that molecule.[4] The macromolecules that make up living systems represent 3D arrangements of functional groups and potential binding regions that can interplay with complementary regions and functionality displayed around small molecules. The positions and orientations of these groups around a small molecule determine the nature of the binding interactions that molecule is capable of achieving. Therefore, the molecular structure, and consequentially the molecular shape, of a small molecule are fundamental to determining the biological activity of that molecule.[5] The presence of multiple structural classes and therefore a high degree of structural diversity within a library leads to an increased likelihood of achieving broad ranging biological activity across that library.

There are a number of ways that structural diversity can be incorporated into a compound collection; however, variation of the *molecular scaffold* is widely thought to be the most important. The scaffold (or skeleton) of a molecule is usually considered to be the combination of rigidifying elements, such as ring architectures, that comprise the core structure of the molecule.[6] As such, varying the molecular scaffold has a far greater influence on the overall shape of molecules than altering more peripheral structural characteristics such as the attached functional groups. Incorporating a high degree of scaffold diversity into a library allows that library to span a comparatively large area of chemical space.

Compounds containing macrocyclic ring structures (a ring size of 12 atoms or above) are capable of extremely potent biological activity and specificity. This can be clearly illustrated by the exquisite biological activity of many macrocyclic natural products that has been harnessed by medicinal chemists to provide chemotherapy for a broad range of conditions. Currently macrocyclic structures account for over 100 approved drugs covering examples of antibiotic, immunosuppressant and anticancer chemotherapeutics.[7]

This biological activity has been attributed to the ability of macrocyclic compounds to represent a compromise between structural pre-organization and conformational flexibility.[8] Their cyclic structure means they have less conformational freedom than an equivalent acyclic compound and so suffer a correspondingly smaller entropic penalty upon binding to a receptor. However, unlike smaller cyclic systems, they are not completely

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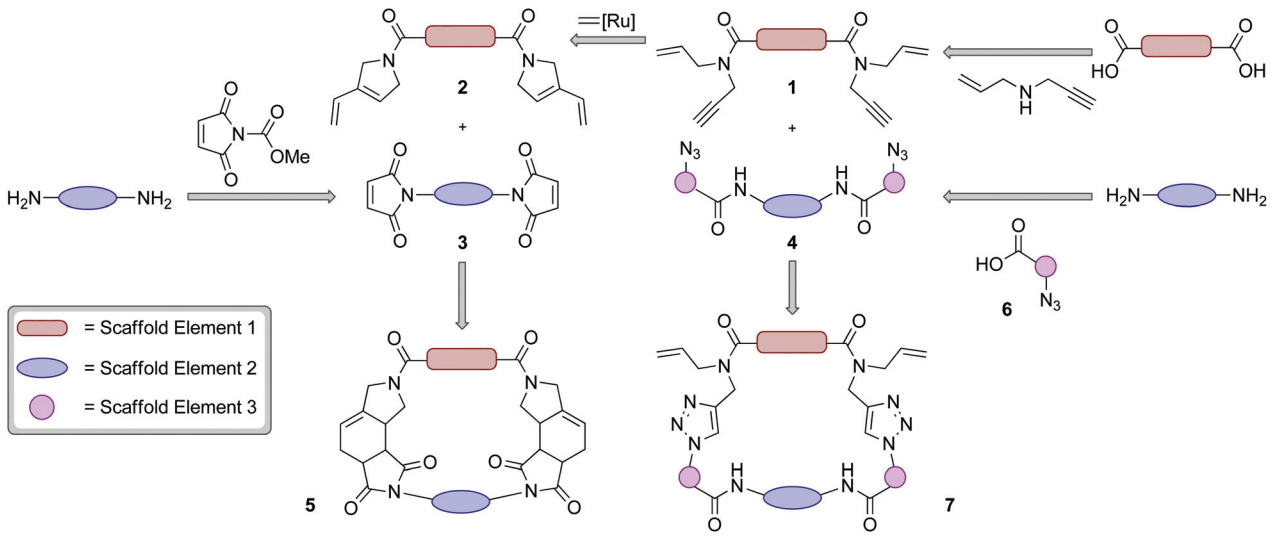


Fig. 1 Two-directional strategy for macrocyclisation used in the DOS.

rigid and so potentially have the ability to mould to a surface to achieve optimal binding. In addition to this, the comparatively large size of macrocyclic compounds gives the possibility for binding across extended surfaces that are difficult to access with more traditional small molecules.[9] For these reasons macrocycles can be thought to occupy something of a middle ground between (rule of 5 compliant) small molecules and biological treatments. They can be capable of the desirable pharmacokinetic properties associated with small molecules in terms of oral bioavailability and cell permeability, but also allow for the possibility of modulating difficult targets usually attenuated by biological treatments. However, despite this unique activity, they are widely thought to be underrepresented in screening collections.[8,10] This apparent deficiency is normally attributed to the synthetic difficulties associated with the synthesis of macrocycles: the necessity for high dilution conditions, and the substrate dependant nature of the macrocyclisation step, being poorly compatible with library synthesis. However, this is beginning to change and recent years have seen several DOS campaigns targeted at macrocyclic structures.[11]

Herein, we report a proof of principle study on the development of a novel strategy for the DOS of complex macrocyclic compounds. This strategy involves the initial two-directional synthesis of symmetrical 'linear' precursors bearing reactive functionality at two positions.[12] Linear precursors containing mutually complementary functionality are then able to react together in a two-directional macrocyclisation process to give the desired molecular architectures.[13] This strategy allows a complexity generating reaction to be performed at two positions on a linear species while concurrently creating a macrocyclic scaffold, allowing an extremely broad scope for the degree of complexity that can be produced in a single synthetic step. The nature of the strategy also means that the linker units between the reacting groups can be regarded as 'scaffold elements' with regards to the eventual macrocyclic structure. Combining appropriately functionalised scaffold elements is then able to lead directly to distinct macrocyclic ring sizes and scaffolds. This strategy has the potential to provide extremely efficient access to a range of macrocyclic scaffolds because the combinatorial variation of scaffold elements can lead to the generation of a unique scaffold from each macrocyclisation process. Fig. 1 shows an overview of the two-directional strategy.

Results and discussion

The two reactions utilized to accomplish the two-directional macrocyclisation process were: the Diels–Alder reaction and the copper-catalysed azide–alkyne cycloaddition (CuAAC).[14] The Diels–Alder reaction is a complexity-generating reaction, capable of creating a cyclohexene ring and up to four stereocentres. The CuAAC was chosen for its broad substrate tolerance and because the resulting triazoles are regarded as biologically relevant species with the ability to act as peptidomimetic structural elements.[15]

In order to create a convergent strategy for the macrocyclisations, one of the linear precursors was based on bis-enyne amides (**1**), which are easily accessible from the corresponding, commercially available, dicarboxylic acids (scaffold element 1). These bis-enyne amides are suitable for use immediately in the CuAAC reaction and can be transformed into the requisite conjugated 1,3-dienes (**2**) for the Diels–Alder reactions by ring-closing enyne metathesis (RCEYM). The complementary reacting partners: bis-maleimides (**3**) and bis-azides (**4**) were then obtained from commercially available diamines (scaffold element 2). Maleimides were chosen as dienophiles due to their high reactivity and for the plane of symmetry through the alkene, which circumvents this regioselectivity issue. The Diels–Alder reaction between bis-dienes **2** and bis-maleimides **3** then gave macrocycles of type **5**, all of which were obtained as racemates. The bis-azide species used were bis-amides, synthesised from the linear diamines and azido-acids (**6**). The direct synthesis of bis-azides from the corresponding diamines was avoided as these species would have unacceptably high nitrogen to carbon ratios.[16] The use of azido-acids to 'cap' the diamines and provide the desired azide functionality, also introduced a third

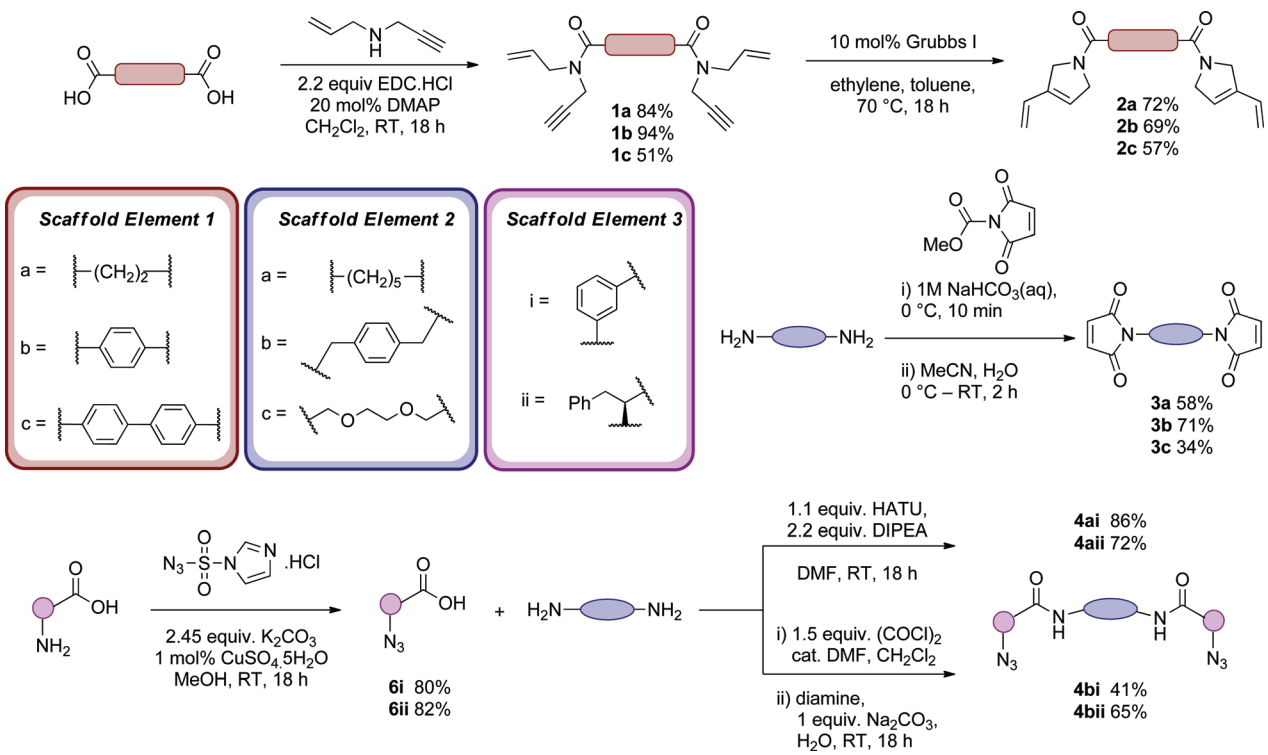
scaffold element (scaffold element 3) into the linear precursors. The reaction between bis-enyne amides **1** and bis-azides **4**, gave macrocycles of type **7**. It would also have been possible to add a fourth scaffold element to the strategy by varying the choice of amine used to synthesise **1**; however, for this proof of principle study, work focused solely on the use of allylpropargylamine. Both of the two-directional macrocyclisations chosen left alkene groups in the final macrocycles, which could potentially be used as handles for further reactivity.

The linear precursors were all obtained in one or two steps from commercially available materials; three bis-enyne amides (**1a–c**) were synthesised from the corresponding dicarboxylic acids by coupling with allylpropargylamine using EDC·HCl and DMAP mediated conditions (Scheme 1). Treatment of **1a–c** with 10 mol% Grubbs' first generation catalyst under an ethylene atmosphere then furnished the desired bis-dienes (**2a–c**).[17] Three bis-maleimides (**3a–c**) were obtained by treatment of the linear diamines with *N*-methoxycarbonyl maleimide.[18] Azido-acids **6a** and **6b** were synthesised using the diazo-transfer agent developed by Stick *et al.*,[19] then coupled to two diamines: 1,5-diaminopentane and *p*-xylenediamine (scaffold element 2a and b) to give four bis-azides. When 1,5-diaminopentane was used, coupling was achieved using HATU to facilitate the reaction, giving **4ai** and **4aii**. When *p*-xylenediamine was used, all peptide-coupling reagents proved ineffective, and it was necessary to first convert the azido-acids to the corresponding acid chlorides with oxalyl chloride to achieve the synthesis of **4bi** and **4bii**.

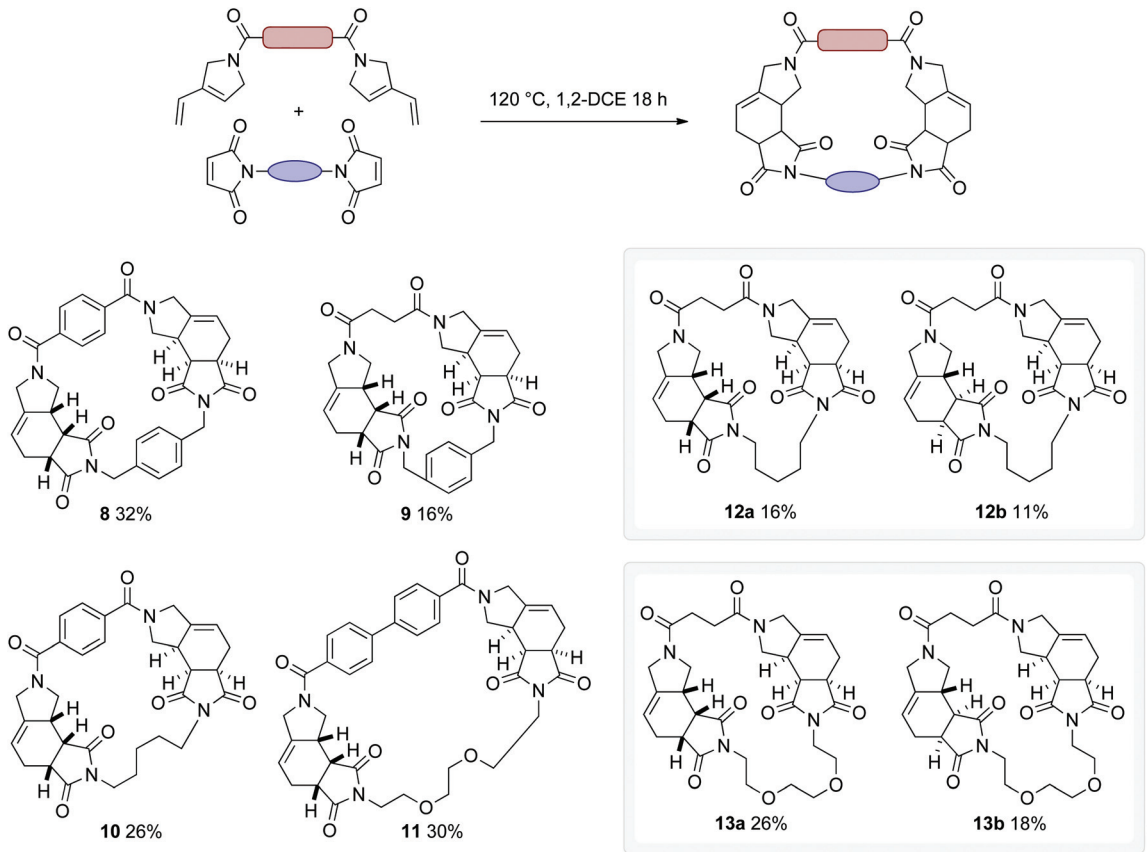
The two-directional Diels–Alder macrocyclisation proved successful; creating extremely complex molecular architectures containing six stereocentres in a single transformation from 'flat' achiral precursors. The reactions were carried out in 1,2-dichloroethane, which was chosen as the solvent because it provided good solubility properties for both the bis-dienes and bis-maleimides. It was found to be necessary to heat the reactions to 120 °C to achieve macrocyclisation, and the reactions were carried out in sealed tubes with heating for 18 hours, at concentrations of around 10 mM. Under these conditions six macrocyclisations were achieved. For four examples (compounds **8–11**), a single diastereomer of the macrocyclic product was isolated; for the other two examples (**12** and **13**), two diastereomers were isolated (Scheme 2).[20] The products were obtained in unoptimised isolated yields (11–33%) after flash chromatography and HPLC purification to remove starting materials and non-cyclised materials.

In the cases where a single diastereomer was obtained, it was always found to be the result of the Diels–Alder reaction occurring in the *endo* orientation at both reaction sites. This was determined by NOESY spectroscopy. For the two examples where two diastereomeric products were obtained, the additional product was the result of one Diels–Alder process proceeding *via* an *endo*-transition state and the other *via* an *exo*-transition state.

NMR experiments were unable to determine whether the Diels–Alder reactions took place on the same face or the opposite face of the dienes, making assignment of relative stereochemistry between the two 5–6–5 ring systems difficult.[21] However, for compound **8** an X-ray crystal structure was obtained (Scheme 3) which confirmed the stereochemistry at each of the stereocentres and so showed that, in this case, the reactions had occurred on opposite faces of the bis-diene.[22] In the absence of further information, the rest of the Diels–Alder products (**9–13**) are drawn with analogous stereochemistry;



Scheme 1 Preparation of linear precursors.



Scheme 2 Two-directional Diels–Alder macrocyclisation products; diastereomeric pairs are outlined. Compounds **9–13** are drawn with analogous relative stereochemistry to compound **8**; however, for these compounds it is not certain which *endo–endo* or *endo–exo* isomer was isolated.

however, for these cases either of the *endo–endo* products could be present.

Compound **8** was also used to illustrate the potential for the further elaboration of these compounds. In order to provide enough material for experimentation, the synthesis of this compound was scaled up to 1.6 mmol scale to provide 270 mg of the Diels–Alder product.[23] The alkene groups resulting from the Diels–Alder cyclisations give the potential for a multitude of synthetic transformations to be performed after the macrocyclisation step; for example, hydrogenation and dihydroxylation (Scheme 3). Hydrogenation with hydrogen gas and Pd/C proceeded smoothly to give fully saturated compound **14** in 50% yield. The hydrogenation of alkenes within cyclic systems can cause large changes in the shape of a molecule and so alter the biological interactions it is capable of achieving.[24] In this case, it also appeared to affect the conformational flexibility of the macrocycle with NMR suggesting that **14** exists as a number of interconverting conformers at ambient temperature. The dihydroxylation of **8** proved more problematic; due, in the most part, to difficulties in separating and isolating the products of the reaction. After some optimisation, a 23% yield of **15** was achieved using eight equivalents of NMO and HPLC purification of the crude reaction mixture.

A range of conditions were trialled for the CuAAC macrocyclisations, including both CuI and $CuSO_4$/ascorbic acid based protocols. The $CuSO_4$/ascorbic acid based systems proved ineffective, possibly due to the low solubility of the substrates in the $^tBuOH/H_2O$ mixtures that the reactions are usually performed in. Using CuI and DIPEA in THF provided a viable system for the macrocyclisations; however, the solubility of the substrates again proved troublesome and so the reactions were only performed with the most soluble bis-enyne, which was found to be **1b**. The reactions were carried out at 20–30 mM concentration at 80 °C in sealed tubes and gave the desired products **16–19** in unoptimised 7–23% isolated yields (Scheme 4).[25]

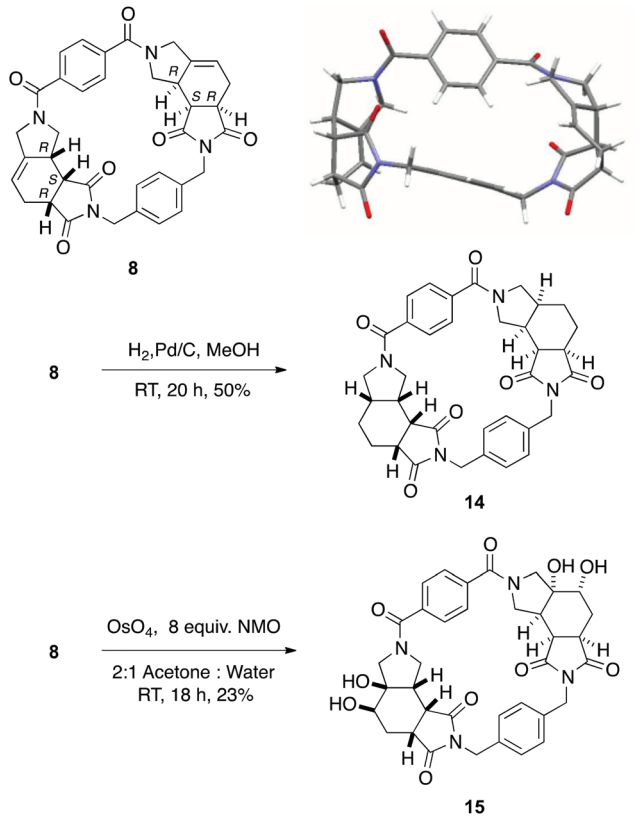
Between the Diels–Alder and CuAAC macroyclisations 14 complex macrocyclic products were produced, providing a clear indication of the validity of this approach to DOS. The compounds were produced in three to five synthetic steps from commercially available materials, and in most cases, a single step from simple, easily accessible, linear precursors. This approach therefore represents an effective strategy for the rapid generation of molecular complexity. Nine macrocyclic ring sizes are represented within the compound collection: 21, 22, 23, 24, 27, 28, 30, 31 and 32; indicating coverage of a relatively large area of macrocyclic chemical space.

While, to some degree, the presence of this range of ring sizes is, in itself, indicative of the diversity of the compounds, we sought to further explore and quantify the diversity and chemical space coverage achieved by these compounds using computational analysis. The diversity of the compounds synthesized was evaluated by calculating their 2D molecular descriptors

(as implemented in MOE),[26] followed by principal component analysis (PCA) of the values obtained.[27] Also included in the analysis was a collection of 656 drugs (red dots) randomly selected from the Drugbank[28] database and the 95 compounds in Drugbank that contain a macrocyclic ring (and molecular weight < 1000 Da) (blue triangles). The inclusion of these additional compound sets in the analysis allows the comparison of the chemical space covered by the collections and also gives some context to the positions of the library compounds in chemical space with respect to known biologically active regions. Fig. 2 shows a scatter plot of the three compound collections.[29] The first thing that is apparent from this scatter plot is that the library compounds (black dots) cover a relatively large area of chemical space given the small number of compounds synthesised, suggesting the successful incorporation of a high degree of molecular diversity into the synthesis.

This is particularly impressive given the highly step-economical nature of the synthesis. In terms of the positions of the library compounds with respect to the other compound collections it appears that the library compounds occupy an area removed from the majority of the randomly selected drug compounds and from the two main areas of concentration of the macrocyclic drug compounds. However, there is clearly still significant overlap with both compound collections suggesting that the compounds produced in this campaign do have potentially biologically relevant physical properties.

This computational assessment is useful, in terms of providing a visually accessible representation of the abstract concepts of molecular diversity and chemical space; however, a more



Scheme 3 X-ray crystal structure of **8** proving the relative stereochemistry; post-macrocyclisation modifications of **8**.

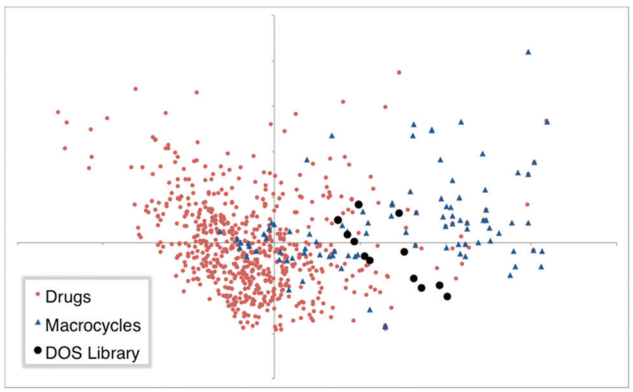
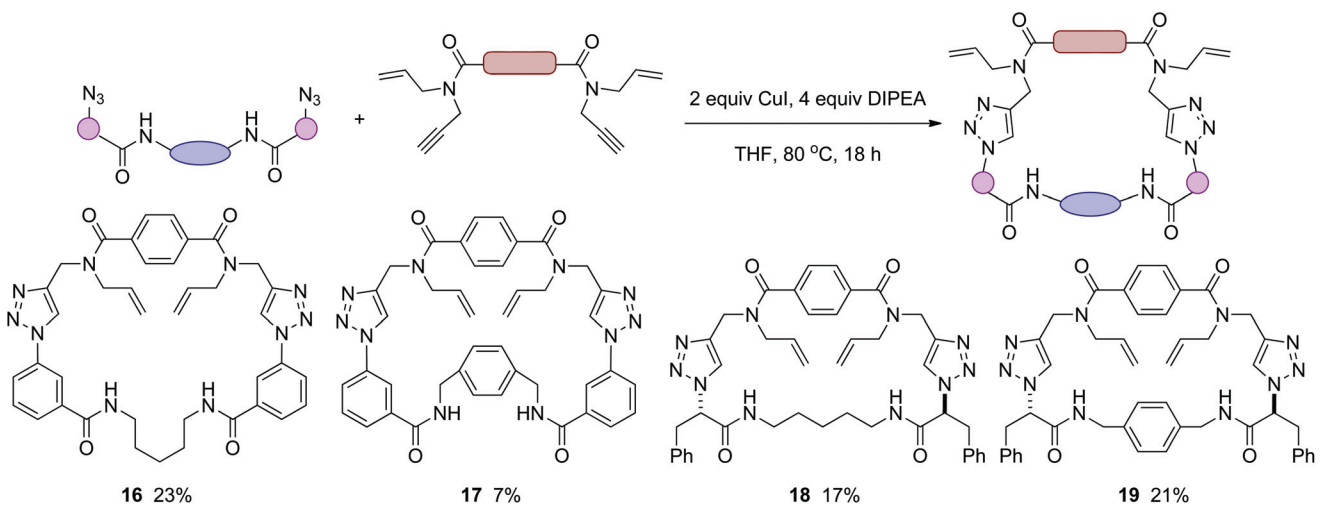


Fig. 2 Computational assessment of the position of the macrocycles in chemical space calculated using 2D molecular descriptors and principle component analysis.



Scheme 4 Two-directional CuAAC macrocyclisation.

stringent test of the usefulness, and functional diversity, of a compound collection such as this is the biological evaluation of the compounds produced. With this in mind, the library members were tested for antiproliferative activity against two cancer cell lines.

All of the macrocycles synthesised were tested for their ability to inhibit the proliferation of an osteosarcoma cell line (U2OS) and a human lung adenocarcinoma epithelial cell line (A549) using a sulforhodamine B (SRB) assay (see ESI†). Two of the library compounds were then found to inhibit the growth of one or both cell lines. Compound **9** was found to inhibit the proliferation of the U2OS cell line with an IC_{50} of 141 μM, and compound **19** was found to inhibit the proliferation of both the cell lines with IC_{50} values of 131 μM (U2OS) and 125 μM (A549).[30]

While the IC_{50} values obtained for compounds **9** and **19** represent only moderate potency, it should be stressed that these compounds are 'starting points' for biologically active scaffolds that can be optimised with various appendages. As such, the discovery of two hits from a library of 14 compounds represents a successful screening campaign. Additionally, both compounds are based around novel macrocyclic scaffolds, implying the discovery of new areas of bioactive chemical space. The bioactivity of these compounds also provides further support for the contention that varying the molecular scaffolds across a library is important in terms of achieving functional diversity. As a result of the synthetic strategy employed, both compounds have essentially all of their functional groups in common with several other library members suggesting that it must be the molecular scaffold that is leading to the activity observed for these compounds.

Conclusions

In summary, we have reported a strategy for the DOS of complex macrocyclic compounds using a two-directional synthesis approach involving the modular synthesis of linear precursors and their subsequent combination to form macrocyclic architectures. The approach was used to rapidly synthesise 14 macrocyclic compounds including examples of nine ring sizes, in three to five synthetic steps from commercially available materials. Cheminformatic analysis of the compounds indicates that they occupy biologically relevant positions in chemical space, and two of the 14 compounds were found to exhibit antiproliferative activity against cancer cell lines, further implying potential biological utility. Studies are ongoing to test these compounds against a wider range of biological targets, and to apply this synthetic strategy to the synthesis of a larger library of compounds.

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20 In all of the Diels–Alder cyclisations there was evidence for the formation of additional cyclic and acyclic products by LCMS and HPLC but only the compounds described were isolated in any significant quantity.
21 An additional difficulty in determining the structures of the compounds by NMR was that a number of the products proved conformationally dynamic on the NMR timescale resulting in complex spectra. A number of VT experiments were run; however, they did little to improve the spectra and so for these Diels–Alder reactions the structures were assigned based on the complex room temperature spectra.
22 Crystal data for **8**: Molecular formula $C_{36}H_{32}N_4O_6{\cdot}2CHCl_3$, M = 855.39, monoclinic, a = 15.1710(2) Å, b = 17.9068(3) Å, c = 140740(2) Å, α = 90°, β = 90.417(1)°, γ = 90°, V = 3823.3(1) Å^3, T = 180(2) K, space group $P2(1)/c$, Z = 4, 38 105 reflections measured, 8725 independent reflections (R_{int} = 0.0688). The final R_1 values were 0.0559 ($I > 2\sigma(I)$). The final w$R(F^2)$ values were 0.1152 ($I > 2\sigma(I)$). The final R_1 values were 0.0956 (all data). The final w$R(F^2)$ values were 0.1322 (all data).
23 This scale represents 10 times the library scale where the reactions were carried out on 0.156 mmol scale. The yield of this scaled up process remained in line with the library reaction at 32%.
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25 Attempts were then made to apply the alternative ruthenium-catalysed azide–alkyne cycloaddition to this macrocylisation. However, the reaction did not prove suitable with no productive reaction occurring in any case. The reason for this intractability is not clear but it is possible that the ruthenium can become complexed between the alkyne and alkene groups of the enyne amides, leading to catalytically inactive species as observed for 1,5-diynes in B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Lia and V. V. Fokin, *J. Am. Chem. Soc.*, 2008, **130**, 8923–2930.
26 *Molecular Operating Environment (MOE*), 2011.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2011. Further details of this analysis are given in the accompanying ESI.†
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29 It should be noted that there are only 12 'black dots' on the plot to represent the 14 library compounds, this is because the 2D molecular descriptors chosen do not distinguish stereoisomers (or any other 3D information) and so the two diastereomeric pairs (**12a** and **12b** and **13a** and **13b**) represent a single point each.
30 At present the mode of action of these active compounds is unknown. The compounds were screened in an assay for their ability to arrest cells in mitosis; however, this assay suggested that none of the compounds have this ability and that **19** appears to induce apoptosis. See ESI† for further information.