Skeletal diversity construction via a branching synthetic strategy†

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A branching synthetic strategy was used to efficiently generate structurally diverse scaffolds, which span a broad area of chemical descriptor space, and their biological activity against MRSA was demonstrated.

Small molecule modulation of protein gene products (chemical genetics) is a powerful approach for the study of biological systems,[1] and is complementary to nucleic acid based approaches (such as siRNA) that target the gene locus or mRNA. In order to find a selective small molecule modulator of any protein function a structurally diverse compound collection is required. Natural products are structurally diverse;[2] however, there are many disadvantages with using extracts in chemical genetic experiments (e.g. limited availability, bioactive constituent identification, and complex analogue synthesis). These problems have led to a complementary approach of *synthesizing* structurally diverse small molecules directly and efficiently, an approach known as diversity–oriented synthesis (DOS).[3] Whereas compound collections of a common scaffold decorated with diverse building blocks have been synthesized efficiently,[4] there have been limited examples of the synthesis of small molecules with a high degree of skeletal diversity.[5] Nature synthesizes many diverse molecular frameworks using a divergent synthetic strategy from the basic 'two–carbon' starting unit acetyl CoA (Fig. 1).[6] Herein, we report the use of a fluorous-tagged diazoacetate (**1**) as a basic 'two–carbon' starting unit in divergent reaction pathways to synthesize drug-like and natural product-like compounds in just 2-4 synthetic steps.

The fluorous-tagged diazoacetate (**1**) was identified as an attractive DOS starting unit for two key reasons. Firstly, the reactive diazo functionality permits a wide range of complexity–generating, C-C bond–forming reactions,[7] which can be used to generate a wide range of scaffolds with versatile functionality that can be diversified further. The diazoacetate functionality can be nucleophilic and/or electrophilic under controllable conditions allowing ultimate mechanistic flexibility. This versatility makes diazoacetate **1** a more powerful starting unit than simple acetate esters. The diversity of scaffold–forming reactions possible with diazoacetates leads to skeletally–diverse products. Secondly, polyfluorocarbon tag technology enables solution phase

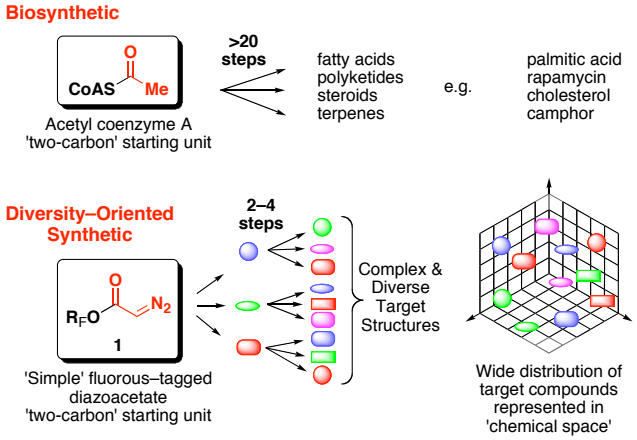

Fig. 1 The biosynthesis of many natural products originates from the 'two–carbon' starting unit acetal CoA in divergent biosynthetic pathways. In our complementary diversity–oriented synthesis approach the fluorous–tagged α-diazoacetate 'two-carbon' unit **1** was used in divergent reaction pathways to yield drug-like and natural product-like small molecules. Coenzyme A and the fluorous tag act as handles with which the synthetic units can be manipulated.[6]

combinatorial synthesis with the generic purification of product from reagents by fluorous solid–phase extraction (SPE), reverse fluorous SPE or liquid-liquid extraction.[8] A reliable, multigram scale synthesis of the diazoacetate (**1**) was achieved and it was stable to chromatography and could be stored for months without significant decomposition.

In the first step of the diversity–oriented synthesis, **1** was exploited in three general divergent reaction pathways: (*i*) three–membered ring formation (shown in Scheme 1 and 2); (*ii*) 1,3-dipolar cycloadditions (b and d, Scheme 2); and (*iii*) α-deprotonation and subsequent quenching with an electrophile and carbenoid formation (c, Scheme 2). The second steps of the synthesis involves complexity-generating reactions to diversify the molecular frameworks further. For example, as illustrated in Scheme 1, step 1 involved cyclopropanation of benzene to give cycloheptatriene **3**, via **2**.[9] Treatment of **3** with primary amines gives ecgonine analogues (**5**, cf. cocaine scaffold);[10] alternatively, heating **3** with dienophiles forms polycarbocyclic adducts (**6**). Alkynes react with **1** to yield cyclopropenes **4**, which were rearranged to furans (not shown) or used as dienophiles to give *cis*-fused [4.1.0] ring systems (**7**) with cyclopentadiene.[7] Further divergent reaction pathways included: uncatalyzed 1,3-dipolar cycloadditions with electron–deficient alkenes to give 2-pyrazolines (b, Scheme 2), three-component ylide-mediated cycloadditions to form 2,5-*trans*-substituted pyrrolidines (d, Scheme 2),[11] and 1,3-keto ester formation (**8** and **9**; c, Scheme 2).[7]

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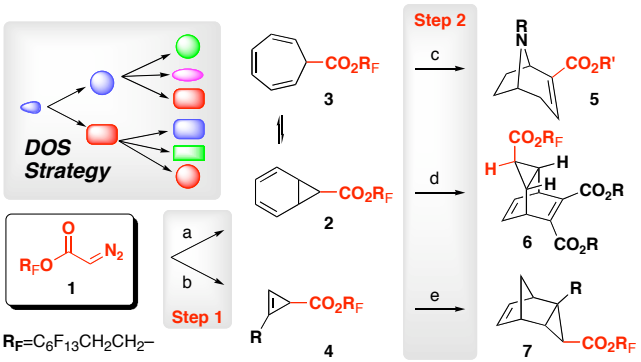

Scheme 1 Example of diversity–oriented synthesis with fluorous–tagged diazoacetate (**1**). (a) C_6H_6, $Rh_2(O_2CCF_3)_4$, 70%; (b) RCCH, $Rh_2(OAc)_4$, [BuCCH, 57%]; (c) RNH_2, NaOH then MeOH, H_2SO_4, [$MeNH_2$, 35%]; (d) dienophile [dimethyl acetylenedicarboxylate, 59%]; (e) C_5H_6, 92%.

The β-dicarbonyl compounds were exploited to generate diverse heterocyclic products, such as coumarins and amino pyrimidinones. A wide range of naturally–occurring and synthetic coumarins and pyrimidine derivatives are known to be pharmacologically active, such as the anticoagulant warfarin and batzelladine alkaloids. These molecular frameworks are considered to be privileged scaffolds and are therefore desirable to include in a structurally diverse compound collection for use in chemical genetic screens. The dihydropyrimidine derivatives were further modified by reaction with a range of 3-formylchromones to form unusual pyrimido[1,2-a]pyrimidines.[12] Fluorous-tagged ester products were varyed divergently by ester hydrolysis, transesterification, ester reduction and transamidation; thereby, both carbons of the 'two-carbon' starting unit were diversified structurally.

The divergent chemistry of the fluorous–tagged diazoacetate described above was exploited in the diversity–oriented synthesis of 223 small molecules that have 30 discrete molecular frameworks among other unique structural features. The library was made using parallel synthetic techniques leading to 2-15 mg of each final product (molecular weight range 140-614). All library members were assessed for their identity and quality, and purified if necessary by recrystallization, chromatography or extraction to ensure >90% purity of final products (^{1}H NMR, HPLC and LCMS).[13] Full characterization of 20 demonstration compounds representing each divergent reaction pathway was also undertaken.

In order to assess the degree of overall diversity obtained in our diversity–oriented synthesis we compared the diversity of our library to the chemical space spanned by 'benchmark collections': (1) known pharmacologically active small molecules (MDL Drug Data Report database)[14] and (2) a focused library (conventional combinatorial chemistry).[15] A visual representation of the diversity of the collections in 'chemical space' is depicted in Figure 2.

Perhaps the biggest challenge for synthetic chemists involved in diversity–oriented synthesis is to achieve efficiently, high levels of skeletal diversity and complexity in order to explore biologically–relevant regions of chemical space. We have presented a new strategy of starting from a fluorous–tagged 'two–carbon' (diazoacetate) unit and using divergent, complexity–generating reaction pathways to create maximum skeletal diversity in final products. A library of 223 small molecules was synthesized, which have 30 distinct molecular frameworks. Significantly, the physicochemical and topological diversity of the compounds synthesized compared favourably with databases of known drugs, which include pharmacologically active synthetic small molecules and natural products. Phenotypic screening experiments showed that a high number of the compounds, with diverse scaffolds, modulate the growth of pathogenic strains of methicillin resistant *Staphylococcus aureus* (MRSA).[16] We will report a full account of our screening experiments and the discovery of new antibacterials with novel modes of action in due course.

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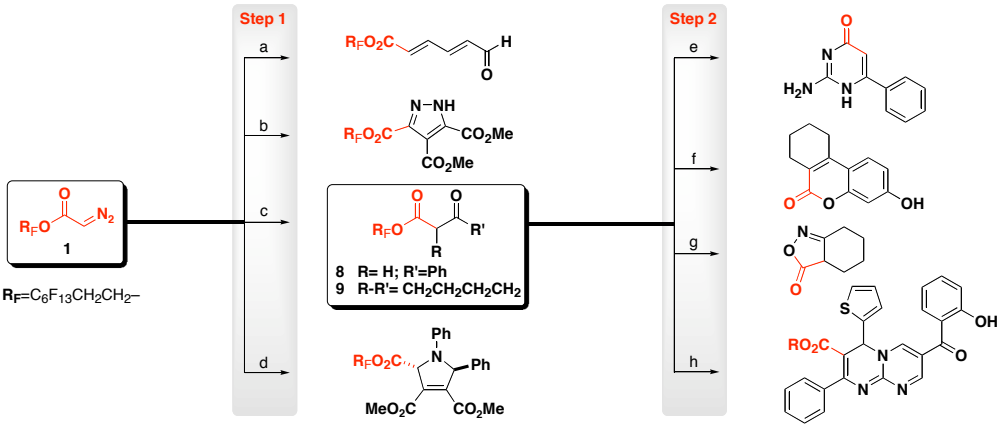
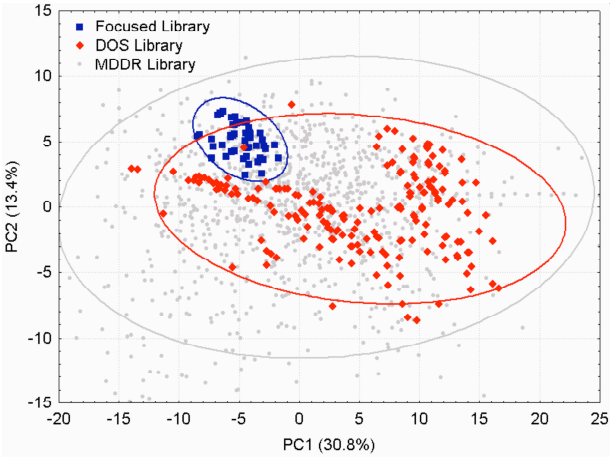


Scheme 2 Divergent reaction pathways lead to skeletal diversity. (a) $Rh_2(OAc)_4$, furan, then I_2, 60% (91%). (b) DMAD, 84% (88%). (c) LDA, RCOR', then $Rh_2(OAc)_4$; **8**: 49% (90%); **9**: 68% (97%). (d) PhCHO, $PhNH_2$, then DMAD, $Rh_2(OAc)_4$, d.r.=20:1, 51% (80%). (e) Guanidine carbonate 62% (96%). (f) Resorcinol, H_2SO_4, 74% (95%). (g) NH_2OH, 77% (89%). (h) Thiophene-2-carboxaldehyde, guanidine carbonate, then 3-formylchromone, 43% (98%). Yields and purity (in brackets) of the product example following generic purification using (reverse) fluorous SPE or precipitation shown. Purity determined by HPLC, LCMS or ^{1}H NMR. DMAD = dimethyl acetylenedicarboxylate.



Library	STDDEV (PC1)	STDDEV (PC2)	STDDEV (PC3)	Average 'Chemical Space' occupied per compound
Focused	4.90	0.46	0.31	0.70
DOS	13.18	1.05	0.81	11.25
MDDR	17.31	1.44	1.13	28.28

Fig. 2 Visual representation of the diversity of different chemical collections in physicochemical and topological space using MOE descriptors followed by principal component analysis (PCA). The DOS library synthesized in this paper is depicted in small diamonds. For comparison, a focused library (small squares) and the MDL Drug Data Repository (small grey dots) are depicted. Library diversity can be described as the standard deviation of properties in this PCA space, normalized to a per-compound-basis. Normalization to give a value of 100% for the most diverse library (MDDR) gives values of 40% for the DOS library and 3% for the focused library. The DOS library spans a large part of chemical space, illustrating the value of our diversity-oriented synthesis approach to deliver diverse products.

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16 *Staphylococcus aureus* strain MRSA-15 (common pathogenic strain in UK hospitals) were used for inhibition of proliferation phenotypic experiments. Compounds modulated growth over a range of concentrations 100 μM concentration (29%), 50 μM (6%), 25 μM (4%), 10 μM (2%). The most active compounds were found to have Minimal Inhibitory Concentrations (MIC) of 3.56 and 6.05 μg/ml.