Introduction

Library realization of small molecules by split-pool diversity-oriented synthesis (DOS) plays a key role in the chemical genetic approach to the study of cellular events. We plan to use these libraries of small molecules as molecular ‘circuit breakers’ as a method to dissect and understand complex biological systems. In order to increase the opportunities for discovering new and important biology by using small molecules, we propose to increase the amount of skeletal diversity found within individual collections of molecules. We speculate that using this strategy will be a more efficient method of identifying new small molecule ligands for proteins. Our first experiments are based on the use of carbohydrate and steroidal scaffolds as starting points for generating skeletal diversity. All of the chemistries presented were explored on polystyrene macrobeads which are amenable to our "one-bead, one-stock solution approach to chemical genetics".

Once hits are identified in biological assays, we will require a method of resynthesizing molecules on a larger scale for more detailed analysis (e.g. Titration curves). We have initiated research into using our alkylsilyl-tethered linker on a new solid-phase platform, where chemistry previously performed on the macrobeads can now be run on a larger scale. The support we are investigating is known as a Lantern™. The advantage of this approach is that many molecules can be made on a larger scale in parallel without using large quantities of beads which are fragile and cumbersome.

Pathway development for carbohydrate library

Carbohydrates are one of the major and general constituents of cells, playing a key role in many cellular events including differentiation of cells, metastasis, inflammation, and cell-cell adhesion. From a structural point of view, carbohydrates (monosaccharides) are highly attractive elements for DOS because of the many structural variants which allow for construction of a large collection of diverse oligosaccharides. Our experiments toward carbohydrate-based libraries are focusing on a conformational rearrangement using the monosaccharide, 1,6-anhydro-D-glucose (1), which starts in the $^{1}C_{4}$ conformation.

2-Amino-4-azido-2,4-deoxy-1,6-anhydro-β-glucose (2), prepared in 5 steps, was loaded onto the
macrobeads 4 through a urea functionality (1 mmol/g, **Scheme 1**). After acetylation of its 3-hydroxy group, the 5-membered bridge on the pyranose ring was cleaved by nucleophilic attack of a phenylthio group (TMSSPh, ZnI₂, Bu₄NI) to give 7. This cleavage is accompanied by a ring flip of the pyranose ring to ⁴C₁ conformer to achieve a skeletal rearrangement of the carbohydrate scaffold on solid phase. Since 1-phenylthio and 6-hydroxy functionalities generated can be further derivatized based on glycosylation chemistries either with carbohydrate or non-carbohydrate building blocks, a large collection of oligosaccharide compounds becomes accessible by this transformation. On the other hand, the 4-azide group in 6 was chemospecifically transformed into triazole (8) and amide (9) functionalities. Optimization of these reaction conditions is currently underway toward completion of the pathway reaction development of this carbohydrate library.

**Pathway development for steroidal library**

A second but distinct example of increasing the skeletal diversity of small molecule libraries has been developed using a steroid-based ring system as the starting point. Steroids are one of many classes of isoprenoids, and are extensively found in plants, animals, and fungi. Many steroidal compounds are known to have biological activities as hormones and vitamins as well as anti-inflammatory drugs. Use of steroidal compounds as a starting material for small compound library is, therefore, expected to lead us to a discovery of specific compounds which interact with biological targets such as proteins in cells. With respect to chemical structure, the steroidal skeleton is complex and rigid which conforms to the structural requirement for small molecules used in chemical genetics studies (see above). Furthermore, the steroid has a small molecular weight (MW is 300-400 generally), and is thus suitable for addition of building blocks to introduce
building block diversity. These features prompted us to use a steroid as a starting material for a small molecule library. Using a steroidal scaffold, we have developed a series of chemo- and stereoselective reactions that result in a skeletally-diverse collection of molecules.

At first we prepared the starting compound 11 from commercially available dehydroepiandrosterone 3-acetate (10) as shown in Scheme 2. The secondary alcohol 11 thus prepared was loaded onto macrobeads 12 by silyl ether formation to give a resin-bound 5,7-diene-17,20-epoxysteroid 13 at 140 nmol/bead (0.80 mmol/g) of loading level which is satisfactorily high for our current chemical genetics research.

Our library synthetic route is shown in Scheme 3. The first step is an epoxide opening using LiClO₄ by various nucleophiles including secondary amines 14. This building block addition step introduces new substituents at the 17-position, which is known to give diverse and distinct biological activities to steroids. Besides secondary amines, thiols and primary amines can also be used for the epoxide opening. When primary amines are used, resulting in secondary amines, they can be further derivatized by acylation to give further building block diversity (data not shown).

The epoxide-opened steroid 15 was next modified by Diels-Alder reaction using Et₂AlCl with yrones 16 possessing diverse substituents. The reaction took place with high regio- and stereoselectivities to add an additional 1,4-cyclohexadiene ring system efficiently onto 15. This transformation allows us to increase diversity by the addition of building blocks but also change the basic skeleton of the steroid backbone into a nonsteroidal skeleton. The 1,4-cyclohexadiene function of 17 provides further opportunity to change the scaffold. When 17 was heated without any solvent or reagents, retro-Diels-Alder reaction proceeded smoothly to give the 14-membered ansa-ring system 18. Although no building block was added by this reaction, this step gives another set of compounds 18, which is accompanied by building blocks, and having totally different scaffold from those of 15 or 17. These two-step sequential transformations, Diels-Alder reaction followed by retro-Diels-Alder reaction, drastically modify the scaffold of starting steroid 15. These successive scaffold transformations (15→17→18) after (or with) building blocks addition (13→15) is a powerful method to introduce scaffold diversity with building block diversity in the library. Furthermore, the scaffold transforming process occurs without any net increase of molecular weight which should be suitable for library synthesis directed toward lead discovery because the yielded compounds are of medium molecular weight (~600), and offer room for further
derivatization. When intermediates (15 and 17) are kept from each step and included in the final library for biological assays, we feel this will be a truly diverse library.

**Scheme 3**

Alkylsilyl-tethered Lanterns as a new support for resynthesis in chemical genetics studies

The research described above has been carried out on polystyrene macrobeads (500-600 µm) which were developed specifically for the diversity-oriented synthesis of libraries of compounds to be used in one-bead, one-stock solution chemical genetics. The resynthesis of larger quantities of molecules discovered to be biologically interesting will enable us to collect more precise data on the numerous screens in our future research. To have this option in our chemical genetics studies, functionalization of Lanterns™ 19 (Mimotopes), which have a grafted-polystyrene surface, has been achieved, resulting in a system that possesses 40 µmol of Si/unit (Scheme 4). Bromination of Lanterns 19 was carried out with Br₂ (40.0 µmol) and Tl(OAc)₃ (20 mol% to Br₂). The reaction proceeded in nearly quantitative yield to give the regiospecifically brominated Lanterns 20, which were homologated with silylated alkylborane 21 by Suzuki reaction. Because we envision using this platform for all future studies, it is important to be able to perform these chemistries on a scale relevant to a large, systematic research program aimed at dissecting complex biological systems. We have successfully performed these reactions to give 2000 units of Lanterns 22 which have 34.62 µmol of silicon on each unit (86.6% yield).

Loading of alcohols on 22 was investigated next. We found that the standard procedure for macrobeads can be applied for
Lanterns 22. Surprisingly, however, the loading of primary and secondary alcohols (24 and 25) proceeds in >90% yield even with stoichiometric amount of alcohols (Figure 1). The yield fell into 72% with 1 eq of phenol 26, indicating excess reagent is required for less reactive phenols.

Some basic reactions were demonstrated to evaluate the reactivity of small molecules on the Lanterns thus prepared (Scheme 5). Many types of important reactions including simple reaction (acylation, 27→28) to complexity-generating multi-component coupling reaction (Ugi followed by intramolecular Diels-Alder reaction, 29→30) were found to proceed successfully. An asymmetric inverse electron demand hetero-Diels-Alder reaction was also realized (data not shown), showing that Lanterns are suitable support for generation of enantiomerically pure material as well.

Cleavage of the silyl ethers were effected with a premixed cocktail of HF-pyr/pyr/THF (1:1:18) to release small molecules from Lanterns. The reaction is complete within 2 h for 28 to generate a primary alcohol in quantitative yield. For the silyl ether of secondary alcohol, longer reaction time (6 h) was generally required to complete the cleavage. In all cases, small molecules were isolated in a pure state after quenching excess HF by TMSOMe followed by concentration of the reaction mixture. The alkysilyl-tethered Lanterns are thus a useful platform for resynthesis of lead compounds from our chemical genetics studies.

References

Pathway Development for Library Realization on Carbohydrate and Steroidal Scaffolds by Diversity-Oriented Organic Synthesis

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A second but distinct example of increasing the skeletal diversity of small molecule libraries has been developed using a steroid-based ring system as the starting point. Starting from resin-bound 5,7-diene-17,20-epoxysteroid 13, which was prepared from dehydroisoandrosterone 10 in 5 steps, the epoxy group was opened by secondary amine building blocks 14 to introduce diversity at 17-position. Besides secondary amines, primary amines and thiols can be also used for the epoxide opening. Secondary amines generated by the addition of primary amines are further derivatized by acylation. The epoxide-opened steroid, 15, was next modified by Diels-Alder reaction with yrones 16 possessing diverse substituents. The reaction took place with high regio- and stereoselectivities to add an additional 1,4-cyclohexadiene ring system efficiently onto 15. This transformation thus allows us to increase diversity by the addition of building blocks but also change the basic skeleton of the steroid backbone. The 1,4-cyclohexadiene function of 17 provides further opportunity to change the scaffold. When 17 was heated, a retro-Diels-Alder reaction proceeded smoothly to give the 14-membered ansa-ring system 18. These two-step sequential transformations, Diels-Alder reaction followed by retro-Diels-Alder reaction, drastically modify the scaffold of the starting steroid. When intermediates are kept from each step and included in the final library for biological assays, we feel this will be a truly diverse library.

Once hits are identified in biological assays, we require a method of resynthesizing molecules on a larger scale for more detailed analysis (e.g. Titration curves). For the resynthesis of larger quantities of molecules discovered to be biologically interesting, functionalization of Lanterns™ (Mimotopes), which have a grafted-poly styrene surface, has been achieved, resulting in a system that possesses 35 μmol of Si/unit. Preliminary experiments using several reactions showed that the alkylsilyl-tethered Lanterns are also useful resins for generation of small molecules.