ORGANIC LETTERS

2005
Vol. 7, No. 13
2535−2538

Small-Molecule Diversity Using a Skeletal Transformation Strategy

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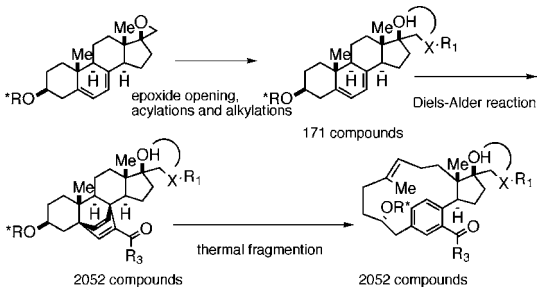
Received February 27, 2005 *(Revised Manuscript Received May 13, 2005)*

ABSTRACT



We describe a short synthetic sequence resulting in >4000 skeletally diverse small molecules that have three distinct skeletal frameworks among other unique structural features. The sequence entails skeletal transformations pioneered by Winterfeldt and co-workers. We use epoxide ring openings and subsequent functionalizations that provide, e.g., spirocyclic oxazolidines, Lewis acid catalyzed Diels–Alder reactions of a steroid-derived diene that afford stable and isolable ring B adducts, and subsequent retro-Diels–Alder fragmentations that yield 14-membered paracyclophanes.

We initiated the research described in this paper with the premise that small molecules having stereochemical and skeletal diversity, and functionalities that enable the attachment of appendages, will be well suited for efforts aimed at probing cell circuitry with small molecules.[1,2] The first property intuitively favors a greater variation of assay outcomes than would be obtained with a collection of more similar compounds,[3] and the second property should facilitate efforts to optimize properties of small moecules identified in small-molecule screens.

The reported research illustrates a step toward these long-range goals. We have developed a short synthetic pathway leading to small molecules having three types of carbon skeletons among other unique structural features. A steroidal diene is first converted to a bridged polycycle and then to a fused bicycle containing 5- and 14-membered rings, the latter containing a paracyclophane.

The pathway relies on a key discovery, made by Winterfeldt and co-workers,[4,5] of a Diels−Alder and retro-Diels−Alder reaction sequence of a steroidal diene (a derivative of ergosterol 3-acetate). We modified and adapted the chemistry that underlies this sequence to the steroidal diene epoxide **2**, whose functionality enabled both an appending process (via ring opening of the epoxide) and attachment to high-capacity macrobeads.[6] The starting compound **2** was synthesized from commercially available dehydroisoandrosterone 3-acetate **1** by a reported method[4,5] followed by Corey−Chaykovsky epoxidation (Figure 1).[7] Compound **2** was then

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10.1021/ol0504345 CCC: $30.25 © 2005 American Chemical Society
Published on Web 05/26/2005

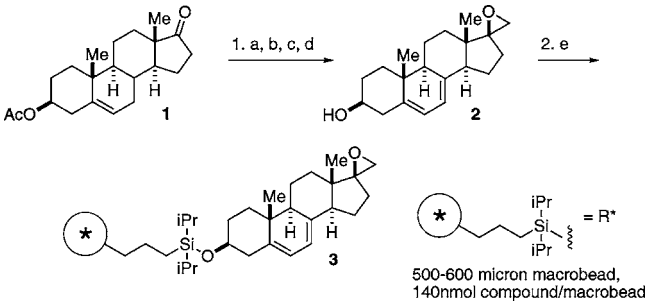

Figure 1. Synthesis of a steroidal epoxide and loading onto macrobeads: (a) 1,3-dibromo-5,5-dimethylhydantoin, AIBN, cyclohexane; (b) TBAF, THF; (c) KOH, *t*-BuOH, H_2O; (d) $(Me)_3SI$, NaH, DMSO; (e) silyl-functionalized macrobeads, TfOH, 2,6-lutidine, CH_2Cl_2.

loaded onto macrobeads (500−600 mm diameter) via a silyl ether linkage to give macrobead-containing **3** at a loading level of 140 nmol per bead (0.80 mmol/g) as determined by a UV (280 nm)-based standard curve.[6] The DOS pathway emanating from **3** is outlined in Figure 2. It entails epoxide

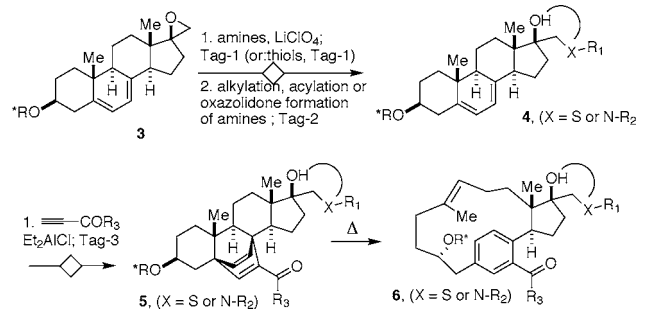

Figure 2. Split-pool synthesis of small molecules having three types of carbon skeletons (and a spirocyclic oxazolidone skeleton in the case of functionalization reactions involving the amino alcohol moiety and chloroformate reagents; symbolized by the half circle).

ring openings, funtionalizations of the amino alcohols via N-acylation or spirocyclic oxazolidone formation, intermolecular Diels−Alder reactions, and transannular retro-Diels−Alder reactions. We made no assumptions about which of the resulting skeletons would be of greater interest in subsequent small-molecule screens, and we therefore devised an overall process that yielded roughly equal numbers of each type of carbon skeleton. We note, however, that compounds related to the end products of our sequence have been reported as screening hits in recent studies elsewhere.[8]

Optimization of both the epoxide-opening reactions and the subsequent amine or amino alcohol acylations was accomplished on macrobeads using **3**.[9] When **3** was immobilized on macrobeads, we found that thermal reactions with ynones led to domino Diels−Alder/retro-Diels−Alder reactions leading directly to paracyclophanes. Since we are interested in using the ring-B bicyclic Diels−Alder adducts in small-molecule screens, we required punctuated reactions—conditions that permit the formation on macrobeads and isolation of the intermediate Diels−Alder adducts. We reasoned that Lewis acids might preferentially accelerate the Diels−Alder reactions over the retro-Diels−Alder reactions. Using ergosterol acetate as a model substrate, we screened for Lewis acids that enhance the Diels−Alder reactions with ynones first in solution phase and found that several, including boron trifluoride diethyl etherate, tin(IV) chloride, titanium(IV) chloride, and diethylaluminum chloride, accelerate the reactions, yielding the Diels−Alder adducts at room temperature, without promoting the retro-Diels−Alder reaction. These catalyzed reactions also yielded a single regio- and diastereoisomer (cf. **7** in Figure 3).[10]

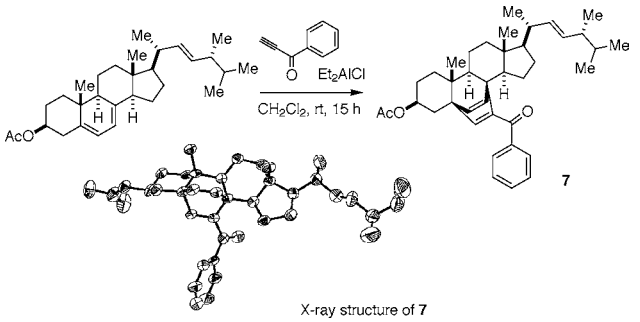

Figure 3. Lewis acid catalysts enable an effective application of the Winterfeldt reaction to diversity synthesis since they permit an efficient and stereoselective synthesis on macrobeads and isolation of the intermediate B-ring bicycle, as exemplified by compound **7**. Such compounds can be used both in small-molecule screens and as substrates for a paracyclophane-forming reaction.

When the catalyzed reactions were performed with substrates attached to macrobeads, all Lewis acids tested with the exception of diethylaluminum chloride resulted in low yields due to cleavage of the silyl ether linkage. Diethylaluminum chloride led to efficient Diels−Alder reactions in 4 h at room temperature with no observable cleavage of the supported substrates or products. The resulting adducts were converted to the paracylcophanes efficiently upon heating the dry macrobeads (in the absence of solvent) at 110 °C for 1.5 h. Heating dry macrobeads, which proved to be robust under these conditions, obviated the need of washing and drying the beads after the last step in the library synthesis.

Candidate nucleophiles, electrophiles, and dienophiles were selected on the basis of their overall participation in

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(9) See the Supporting Information.

(10) CCDC-260317 (**7**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033

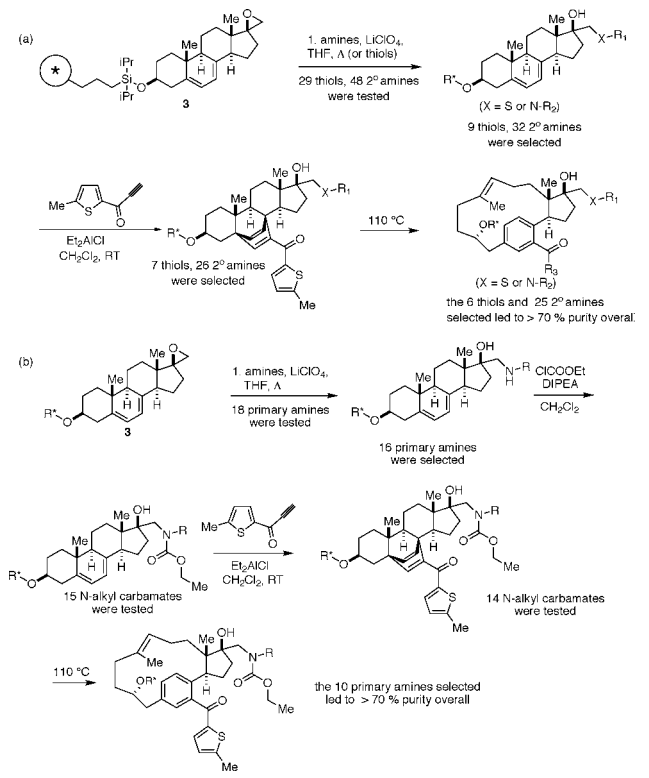

Figure 4. Flowchart showing the testing phase of reagents, catalysts, and substrates and their criteria for selection in the split-pool synthesis: (a) secondary amines and thiols; (b) primary amines.

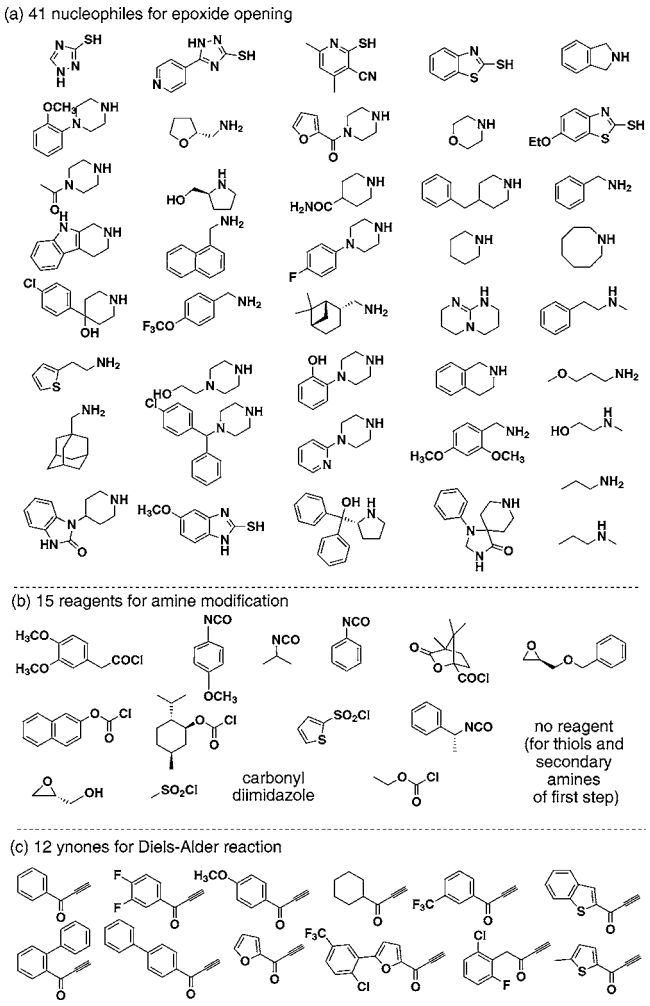

Figure 5. Reagents and substrates selected for the split-pool synthesis: (a) nucleophiles; (b) electrophiles; (c) dienophiles.

all reactions leading to the final paracyclophanes (Figure 4). Substrates were selected for the library synthesis that yielded the paracyclophanes in >70% purity.

For the epoxide-opening step, 95 nucleophiles (29 thiols, 48 secondary amines, and 18 primary amines) were screened with **3**, 41 of which (6 thiols, 25 secondary amines, and 10 primary amines) were selected for the library synthesis (Figure 5a).

For the subsequent acylation or alkylation (with epoxides) step, 14 (in addition to one skip step, Figure 5b) out of 38 reagents were selected. Finally, 12 out of 19 ynones (Figure 5c) were selected as substrates for the Diels−Alder reactions. The 41 nucleophiles, 15 amine modification reagents, and 12 ynones theoretically can produce a maximum of 4275 distinct compounds.

Approximately 25 650 macrobeads, theoretically capable of yielding an average of six copies of each compound, were divided into 31 portions of 150 macrobeads (for reactions with thiols or secondary amines) and 10 portions of 2100 macrobeads (for reactions with primary amines). Each pool of macrobeads was reacted with the above nucleophiles and then encoded with chloroaromatic diazoketone tags.[11] The 10 portions of macrobeads that had been reacted with primary amines were pooled and split into 14 portions. Each of these portions was acylated and encoded with the second set of tag reagents. The 31 portions of macrobeads that had been reacted with thiols or secondary amines were pooled and encoded in one vessel. After combining all 25 650 macrobeads, 1026 were put aside as the first part of the library (Figure 6).

The remaining macrobeads (24 624 theoretical) were divided into 12 portions for Diels−Alder reactions with ynones. After the cycloaddition and third encoding step, all of the [4 + 2] cycloaddition products were combined. Half of these (12 384 theoretical) were put aside and constitute the second part of library. The retro-Diels−Alder reaction was performed on the remainder of the macrobeads to yield the third part of the library.

We synthesized in large scale on macrobeads and fully characterized 19 demonstration compounds representing the different reaction pathways in the library.[9] We also performed extensive quality control tests for purity of products and

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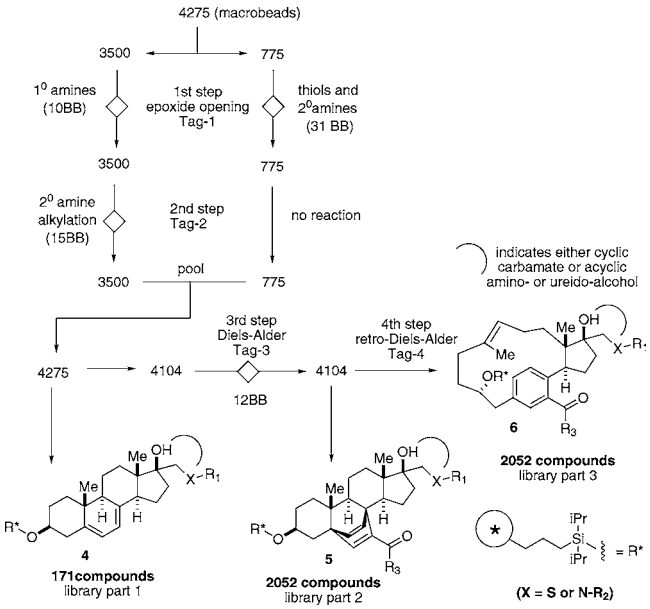

Figure 6. Flowchart of the encoded, split-pool synthesis.

tagging efficiency at each step of the pathway.[9] For each reaction, three beads were randomly selected, cleaved, and analyzed. The purity of the cleaved compounds was determined by LC−MS, and tags, oxidatively cleaved from the macrobeads, were analyzed by GC−MS. One hundred twenty-three macrobeads (3 macrobeads × 41) were analyzed after the first step. The average of purities estimated by the UV−vis spectra was 86%, and GC−MS analyses confirmed the integrity of the tags.

In the same way, three macrobeads were randomly selected from each collection of 15 reactions after the second step. The average purity was 78%, and 43 structures of the 45 were successfully inferred by GC decoding. These 43 compounds gave the expected molecular ion peaks using LC−MS. The structures of the remaining two were inferred by their respective masses, determined by LC−MS. After the Diels−Alder reaction and third tagging step, the average purity of 36 products (3 macrobeads × 12 reactions) was 71%.

The structures of 34 compounds were inferred using GC decoding, and LC−MS gave the expected molecular ion peaks. The structures of the remaining two products were inferred by LC−MS alone. After the last retro-Diels−Alder reaction, 32 macrobeads were randomly selected for cleavage and product analysis. The average purity of the 32 products was 62%. Of these 32 compounds, 29 structures were inferred by GC decoding, while the remaining three were inferred by LC−MS. Over 90% of the structures were inferred successfully using GC decoding, and all structures were inferred by the combined use of LC−MS and GC decoding. The purity of all library members was considered sufficient for inclusion in ongoing small-molecule screens.

Achieving high degrees of skeletal diversity in DOS pathways is a challenging problem. In this paper, we explored a new strategy involving skeletal transformations. A key feature of the pathway is the effective use of intramolecular reactions following intermolecular appending processes (including one that alters the skeleton). Small molecules derived from this research are being formatted for small-molecule screening at the Broad Institute Chemical Biology Program[12] and will be available to researchers through Investigator-Initiated and Disease Biology Screening Programs sponsored by the NCI's Initiative for Chemical Genetics. All results from these studies will be made available via the Web-accessible public database ChemBank.[2]

Acknowledgment. We thank Alexander M. Taylor for helpful suggestions. We are grateful to the National Institute of General Medical Sciences and the National Cancer Institute (Initiative for Chemical Genetics) for support of this research. S.L.S. is an Investigator at the Howard Hughes Medical Institute in the Department of Chemistry and Chemical Biology, Harvard University.

Supporting Information Available: Experimental procedures and characterization data for all compounds and crystal information files (CIF) for compound **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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