JOURNAL OF combinatorial CHEMISTRY

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J. Comb. Chem., **2004**, 6 (2), 286-292• DOI: 10.1021/cc020076m • Publication Date (Web): 20 January 2004

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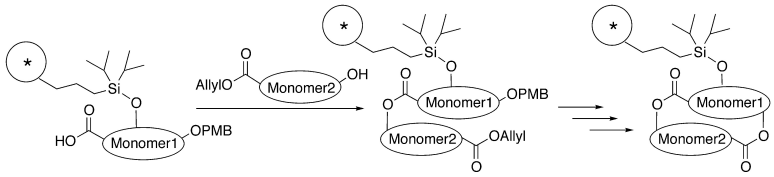

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Macrolactones in Diversity-Oriented Synthesis: Preparation of a Pilot Library and Exploration of Factors Controlling Macrocyclization

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Received August 30, 2002

Introduction

Natural products that modulate protein activity have played key roles in investigations of biological pathways.[1] For example, the natural product α-amantin inhibits RNA polymerase II and was thus used to determine that RNA polymerase II transcribes mRNA, but not tRNA or rRNA. Advances in several areas of science, especially in diversity-oriented synthesis, have enabled a systematic use of small molecules to study biology in this way.[2] In chemical genetics, a small molecule that inhibits the activity of a protein of interest is identified. Cells are treated with this small molecule, and cellular changes are determined. This aids an understanding of the protein's cellular function. This approach allows essential proteins and proteins that are required at multiple points in development to be studied. Because small molecules can rapidly activate or inhibit their protein target, the precise timing of changes can be determined.[2−5]

Macrolides are an attractive class of compounds for chemical genetic studies. A large number of simple macrolides exist that have been shown to affect a wide variety of protein targets.[6] Structural diversity is also a realistic goal for a macrocycle library. For molecules with more than 10 atoms in the ring, multiple low-energy conformations are often available.[7] Changes in the position and nature of substituents can alter the energetics of a ring and make a different conformer the lowest in energy.[8,9]

Consequently, a macrolide library with multiple, varied substituents and with diversity in the position and stereochemistry of those substituents would be attractive. In planning our library, we sought to use monomers other than amino acids because many cyclic peptide libraries have been synthesized,[10] and more backbone variety than provided by amino acids was desired. It was envisioned that the coupling of two hydroxyacids followed by terminal deprotection of the resulting molecule and then cyclization of the linear product would give the desired macrocycle (Figure 4). Since cyclization was considered to be the most difficult step, a ring size known to close readily was necessary (i.e., 13-membered or greater).[11] A library constructed from diverse 5- and 6-carbon hydroxyacids would give rings of the desired 13- and 14-member size. Thus, rapid and efficient solution syntheses of stereochemically and regiochemically diverse hydroxyacids were developed with many monomers available through a common pathway. These compounds were then used to synthesize a model library.

Results and Discussion

We chose to use a linker-and-bead combination developed at ICCB,[12] since it is part of an integrated technology platform well-suited for academic laboratories and for use in chemical genetic screens. Large (500−600-μm) polystyrene beads are first derivatized with an alkylsilyl linker. Activation of the linker as a silyltriflate allows high resin loadings of sterically hindered alcohols, and automated cleavage of the compound from the resin can be performed by treatment with HF-pyridine. Consequently, it was necessary to synthesize protected hydroxyacids containing a free alcohol that could be used to load the compound on the resin. A second hydroxyacid would then be coupled to the one on the resin.

To this end, an efficient synthetic route was developed that produced a variety of protected monomers. The choice of hydroxyacid protecting groups was critical for the monomer syntheses and library construction. The protecting groups must be readily and quantitatively removed on solid phase from a variety of intermediates. Many protecting groups were investigated,[13] and an allyl ester and a 4-methoxybenzyl (PMB) ether proved to be the most appropriate for solid-phase macrolide synthesis. The allyl ester had to be installed at a late stage, since it was incompatible with monomer synthesis. Therefore, we began our monomer synthesis with **1a** (Figure 1), which is available in three steps from sorbic acid and contains a methyl ester and a 4-methoxybenzyl (PMB) ether. The 4-methoxyphenol (PMP) compound, **1b**, was also synthesized.[14] Sharpless asymmetric dihydroxylation directed by the PMP[15] and PMB,[16] aromatic groups, produced diol **2** in high enantiomeric excess. Using the pseudoenantiomer of the Sharpless asymmetric dihydroxylation catalyst, the enantiomer of **2** was also synthesized. From this starting point, all final monomers were synthesized as enantiomeric pairs. Transesterification[17] of **2** followed by silylation generates three products, two monosilylation products and a bis-silylated compound. The desired monosilylated products **3** and **4** were isolated, while the other products were recycled.[18] Diol **2** was also used to build other hydroxyacids. Formation of cyclic sulfite **6** followed by Sn2′ methyl cuprate addition[19] gave stereoselective addition to produce alcohol **7** in good yield. Starting from alcohol **7**, additional diversity is available. Formation of a vinyl ether, Claisen rearrangement, and selective reduction of the resulting aldehyde produced alcohol **8**.

Protecting group changes were then necessary to complete synthesis of the monomers, which would be loaded onto the resin or used in the coupling reaction. Compound **7a** was transesterified to give allyl ester **10**. Silylation and PMB deprotection provided **11**. This same reaction sequence

10.1021/cc020076m CCC: $27.50 © 2004 American Chemical Society
Published on Web 01/20/2004

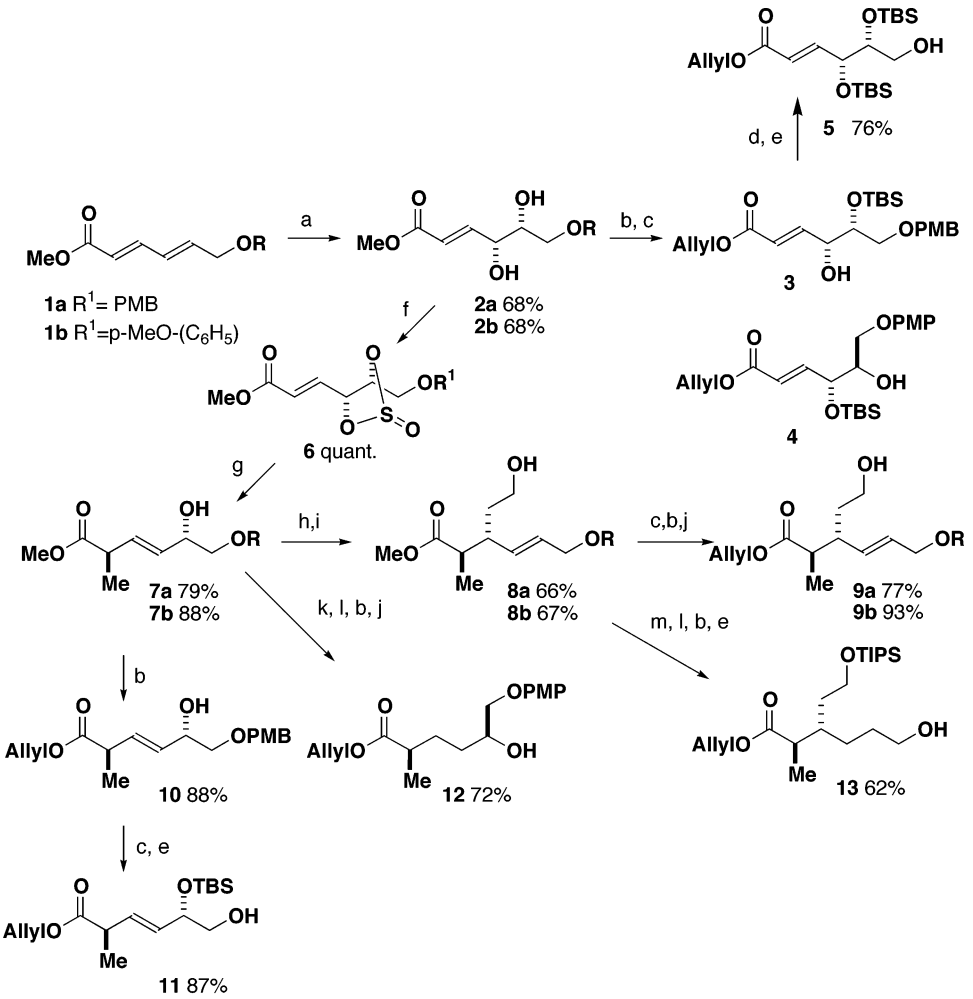


Figure 1. (a) AD mix β, $MeSO_2NH_2$, *t*BuOH/H_2O; (b) $Bu_2SCNSnOSnBu_2OH$, allyl alcohol, toluene; (c) TBDMSCl, imidazole, DMF; (d) TBDMSOTf, 2,6-lutidine, CH_2Cl_2; (e) DDQ, H_2O/CH_2Cl_2; (f) $SOCl_2$, NEt_3, CH_2Cl_2; (g) CuCN, MeLi−LiI, BF_3-Et_2O, THF; (h) i. $Hg(TFA)_2$, butyl vinyl ether; ii. *o*-xylene, △; (i) $NaBH_4$, MeOH; (j) AcOH/THF/H_2O; (k) TESOTf, 2,6-lutidine, CH_2Cl_2; (l) 10% Pd/C, H_2, THF; (m) TIPSOTf, 2,6-lutidine, CH_2Cl_2. The enantiomer of each compound was also synthesized, but for clarity, only one enantiomer is shown in this scheme.

provided **5** from **3**. Synthesis of **9** from **8** required TBDMS protection prior to transesterification to prevent lactonization. The synthesis of **12** and **13** included hydrogenation of the double bond, since an internal allylic ester would be incompatible with deprotection of the terminal allylic group used to protect the carboxylic acid.[20] Though many other interesting hydroxyacids could be envisioned to arise from further transformations carried out on compounds **2**−**13**, we initially chose to focus on these monomers for the synthesis of a small macrocycle library. Six protected hydroxyacids would be loaded onto the solid phase, and 12 alcohols would be coupled to them (Figure 2).

Having completed synthesis of the monomers, solution and solid-phase syntheses of model 14-member macrolide **18** (Figure 3) were investigated in order to compare the efficiency of cyclization in these two environments. Alcohol **10** was protected as the TBS ether or loaded onto resin to produce **14a** and **14b**, respectively. Deprotection of the allyl esters gave acid **15**, and coupling of **15** with alcohol **11** generated **16**. Removal of the PMB and allyl protecting groups gave hydroxyacid **17**. Macrocyclizations are typically performed under dilute conditions in solution, because intermolecular coupling to form higher-order polymers occurs in a concentration-dependent manner. Higher concentrations lead to lower yields of the desired product and greater amounts of larger, polymeric rings. On solid phase, hydroxyacids are in close proximity, and production of higher-order products could be a major side reaction.[21] Yamaguchi conditions were used for macrocyclization because this procedure produced higher yields than other methods in model studies. In solution, macrocyclization of hydroxyacid **17a** at 10 mM gave **18a**[22] in 36% yield. In addition, higher-order structures were isolated that were produced by coupling of two or more hydroxyacids followed by cyclization. We were gratified to find that solid-phase synthesis led to isolation of 40% overall yield of **B3** after cleavage of **18b** from the resin.

Having confirmed that macrocyclization was feasible on solid support, synthesis and analysis of a small library was the next goal. Many macrolide syntheses have been conducted, and the key macrolactonization step was often found to be problematic. For example, in syntheses of erythromycin, cyclization occurred efficiently only when certain protecting groups were used, and the inversion of stereochemistry at a single center inhibited cyclization.[23] For the macrolide library described in this paper, a variety of stereocenters are present.

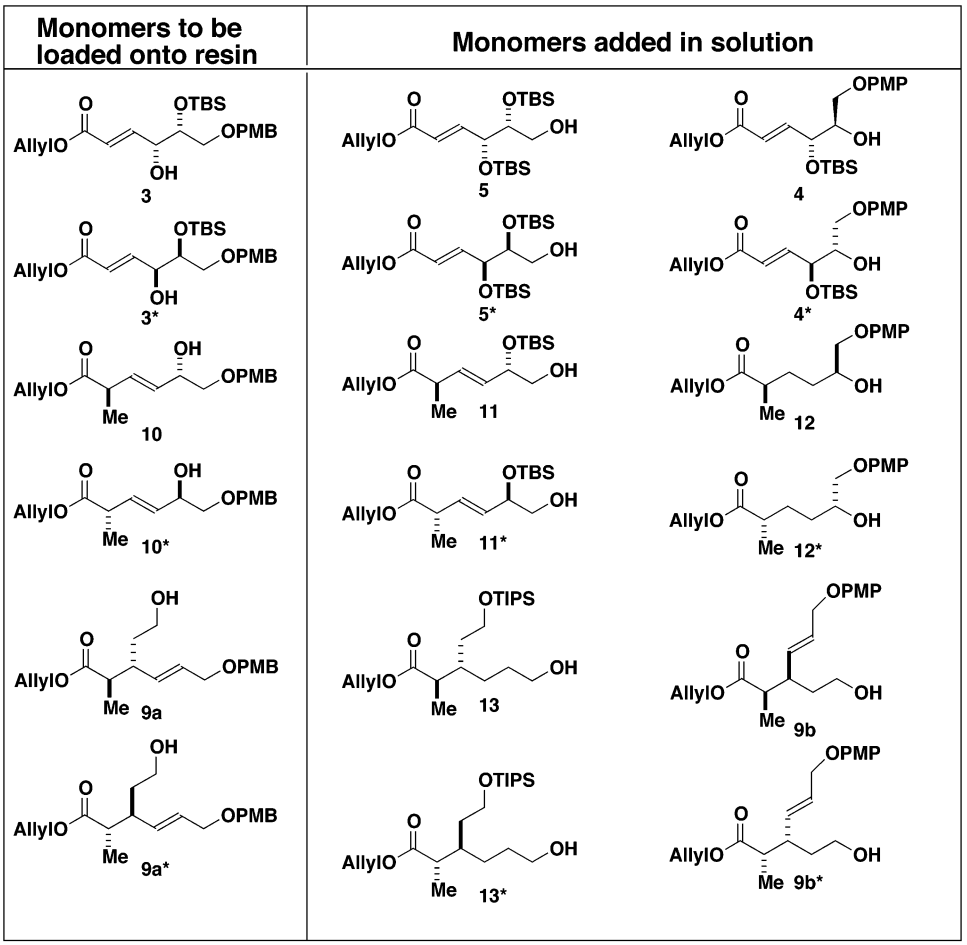


Figure 2.

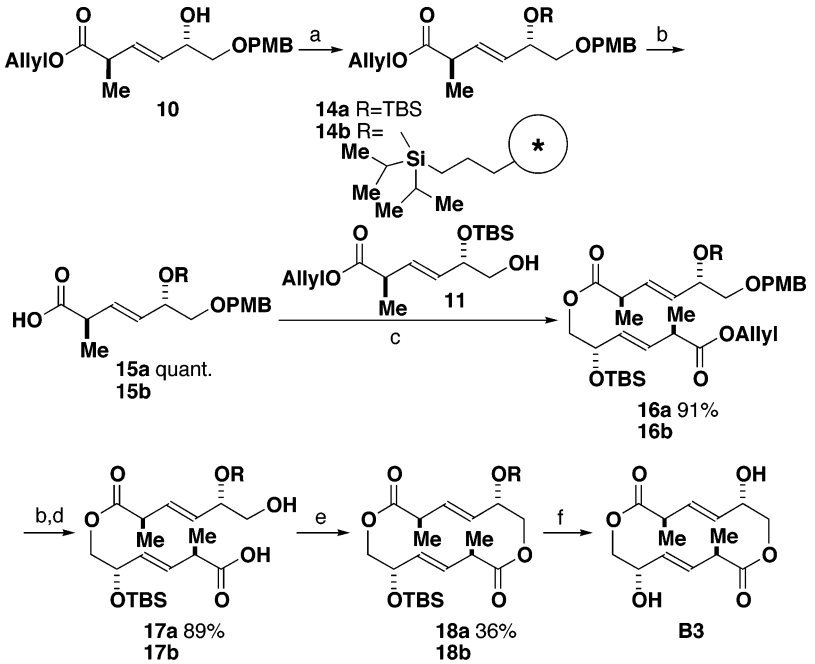


Figure 3. (a) for **14a**, TBDMSCl, imidazole, DMF; for **14b**, silyl triflate resin, 2,6-lutidine, CH_2Cl_2; (b) for **14a**, $Pd(PPh_3)_4$, morpholine, THF; for **14b**, $Pd(PPh_3)_4$, thiosalicylic acid, THF; (c) EDCI, DMAP, CH_2Cl_2; (d) DDQ, H_2O/CH_2Cl_2; (e) (i) 2,4,6 trichlorobenzoyl chloride, $^{i}Pr_2EtN$, THF; (ii) DMAP; (f) HF-pyridine, THF.

Thus, some compounds found in the library may not cyclize efficiently because they contain inhibitory structural features. In difficult cases, reaction between adjacent monomers would be favored over intramolecular reaction, producing more higher-order macrocycles or polymeric hydroxyacid. Hence, it seemed necessary to determine some general rules for

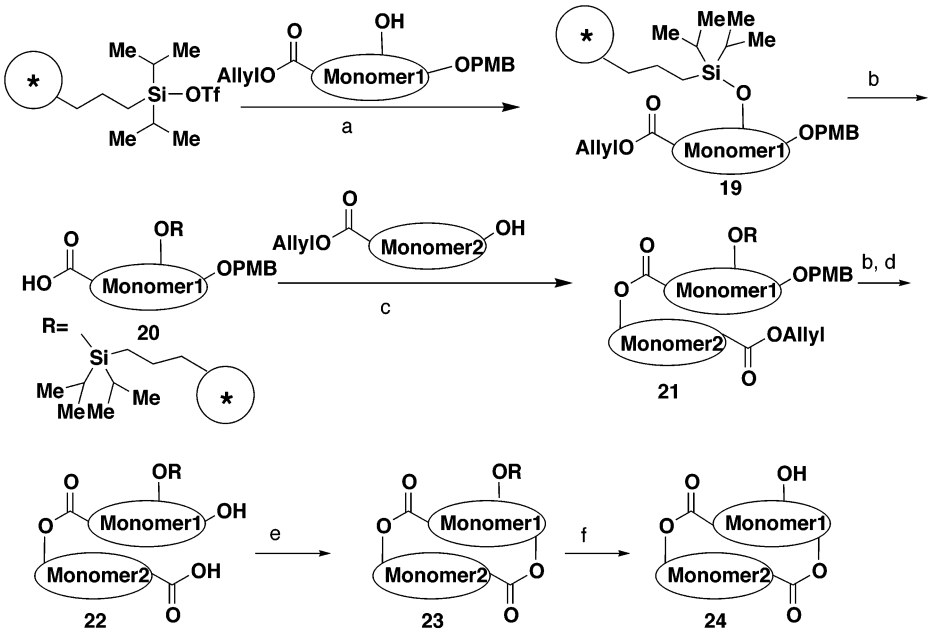


Figure 4. (a) 2,6-lutidine, CH_2Cl_2; (b) $Pd(PPh_3)_4$, thiosalicylic acid, THF; (c) EDCI, DMAP, CH_2Cl_2; (d) DDQ, H_2O/CH_2Cl_2; (e) (i) 2,4,6-trichlorobenzoyl chloride, $^iPr_2EtN$, THF; (ii) DMAP; (f) HF-pyridine, THF.

efficient cyclization. To that end, synthesis of a small library in which the efficiency of cyclization of each member could be assessed by purification was undertaken.

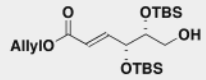
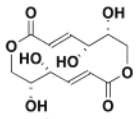
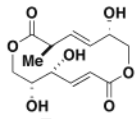
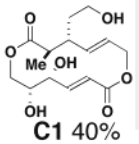
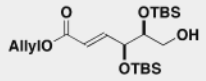
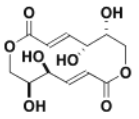
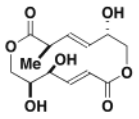
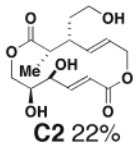
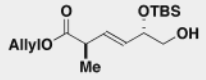
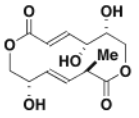
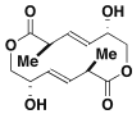
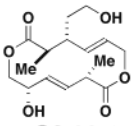
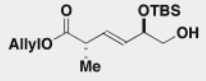
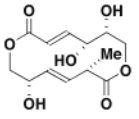
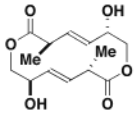
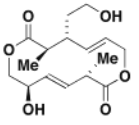
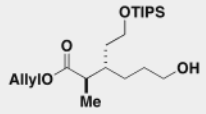
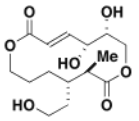
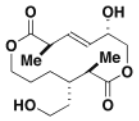
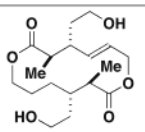
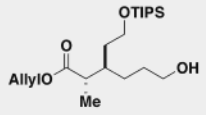
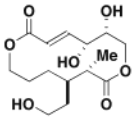
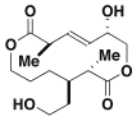
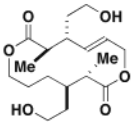
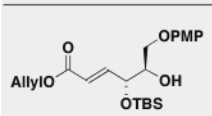
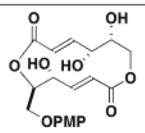
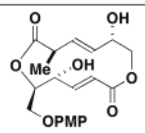
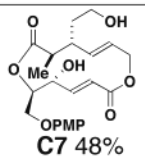
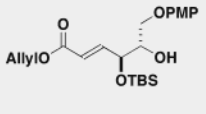
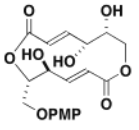
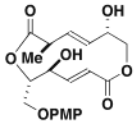
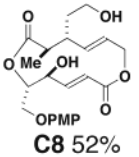
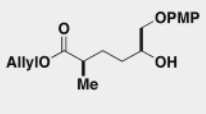
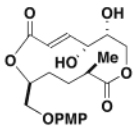
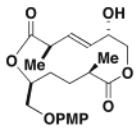
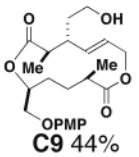
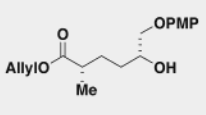
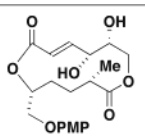
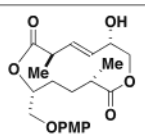
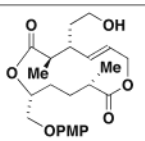
However, before library construction could begin, it was necessary to run several test reactions in order to confirm that the reaction conditions developed for the synthesis of **B3** would work well for other substrates. First, one enantiomer of each alcohol was loaded onto macrobeads. Next, the allyl group was removed, and each resulting acid was coupled to alcohol **11**. Allyl and PMB removal furnished the desired hydroxyacids. In a separate set of experiments, macrobeads were derivatized with compound **10**, and the allyl ester was deprotected. One enantiomer of each alcohol was coupled to this acid, and deprotection was performed. Following each of these test reactions, 4−5 mg of macrobeads was submitted to HF-pyridine cleavage, and the resulting compounds were characterized by ^{1}H NMR. All reactions proceeded with high purity.

Having confirmed that the reaction conditions developed were amenable to multiple substrates, parallel library construction was begun. Library synthesis commenced with loading of the six alcohols (Figure 4) onto macrobeads via the alkylsilyloxy linkage. The allyl esters of **19** were removed to provide carboxylic acid intermediates **20** attached to the macrobeads. All acids on the macrobeads were then coupled to the twelve alcohols shown in Figure 2 to generate **21**. For each compound, the allyl ester was converted to the acid, and the PMB group was removed to give hydroxyacids **22**. Cyclizations of these hydroxyacids were performed under Yamaguchi conditions.[24] The macrocycles were then cleaved from the macrobeads with HF-pyridine to give **24**.

Both enantiomers of the library compounds were synthesized for biological studies, but only one enantiomer was synthesized on a scale that was sufficient for purification and quantification of the desired product. In the solution synthesis of **B3**, dimer and trimer could be separated from the desired product by flash chromatography due to the greater polarity of the higher-order polymers. Accordingly, we hoped that other members of the library could also be purified from undesired polymers by flash chromatography. In most cases, a major spot was detected and isolated. ^{1}H NMR and LC/MS were used to confirm the identity of each compound and to ensure that higher-order polymer was not present. Figure 5 shows the compounds obtained and their isolated yield. Row and column headings indicate the monomers used to generate the product. The northern portion of the molecule, arising from the compound attached to the resin (monomer 1), will be referred to as fragment 1. The southern portion of the molecules, arising from the alcohol added in solution (monomer 2), will be referred to as fragment 2. Compounds **A1**−**12** could not be detected in the mixture cleaved from the resin, indicating that these cyclizations failed to provide any of the desired product.

Some interesting trends can be seen in these results. The monomer attached to the macrobead has a significant influence on cyclization efficiency. When a protected diol was attached to the resin, cyclization was completely inhibited (**A1**−**12**, column 1). This is not surprising, since the compound on the resin would have an −OTBS in close proximity to the $-OSi(^iPr)_2$ resin functionality (see heading of column 1). This steric crowding might limit the ability of the compounds to cyclize. Smaller alcohol protecting groups may solve this problem.

In comparing compounds **B1**−**12** (column 2) and **C1**−**12** (column 3), a similar dominance of the monomer attached to the resin is seen. With a few exceptions, the **C** series provided better yields than the **B** series. We consider two possible explanations for this phenomenon. First, **C1**−**12** were attached to the bead via a primary alcohol with a two-carbon linker between the site of attachment and the backbone of the ring, whereas **B1**−**12** were attached to the resin via a secondary alcohol on the ring backbone. The space between the backbone of the ring and the resin in **C1**−**12** may allow the compound greater flexibility, and this could

Monomer two	Monomer one: 3	Monomer one: 10	Monomer one: 9a
 5	 **A1** 0%	 **B1** 6%	 **C1** 40%
 5*	 **A2** 0%	 **B2** 8%	 **C2** 22%
 11	 **A3** 0%	 **B3** 41%	 **C3** 23%
 11*	 **A4** 0%	 **B4** 34%	 **C4** 29%
 13	 **A5** 0%	 **B5** 43%	 **C5** 50%
 13*	 **A6** 0%	 **B6** 24%	 **C6** 40%
 4	 **A7** 0%	 **B7** 25%	 **C7** 48%
 4*	 **A8** 0%	 **B8** 20%	 **C8** 52%
 12	 **A9** 0%	 **B9** 13%	 **C9** 44%
 12*	 **A10** 0%	 **B10** 22%	 **C10** 46%

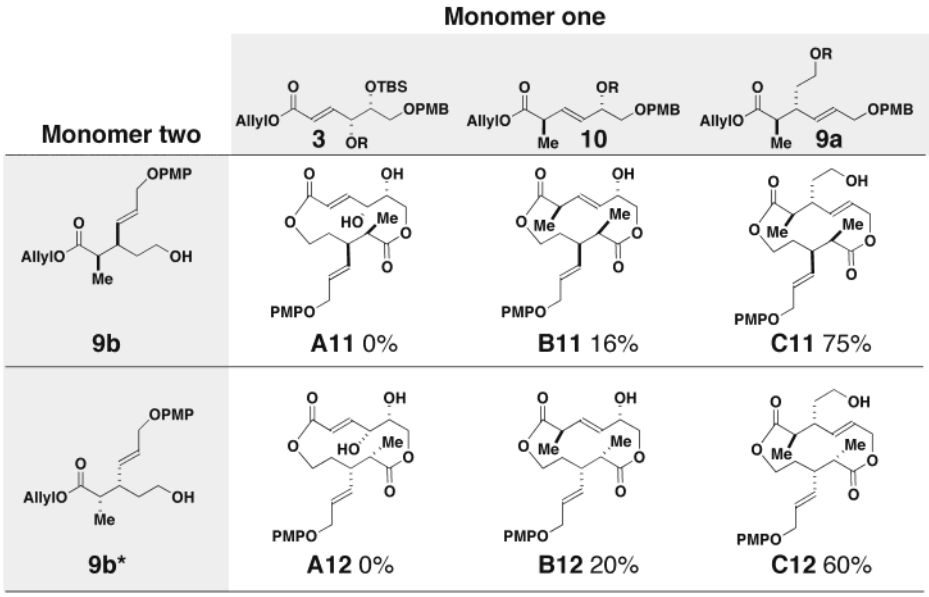


Figure 5. Products and yields of library syntheses. The rows show compounds arising from the monomer 2 shown in the row heading. The columns show compounds arising from the use of the resin in the column headings. Thus, the components used to produce each product can be determined by identifying the compound's row and column headings.

promote the accessibility of conformations capable of cyclizing. Second, the position of the double bond in the rings of **B1**−**12** differs from that found in **C1**−**12**, and this could influence the efficiency of cyclization.

Another striking feature of the results in Figure 5 is that the stereochemistry in fragment 2 does not seem to play a large role in cyclization efficiency. Compounds that arose from coupling of enantiomeric alcohols to the same acid on the macrobeads, such as **C11** and **C12**, have, in fragment 2 of the molecule, the opposite chirality at each stereocenter. However, with a few exceptions, they show similar cyclization yields. We consider two possible explanations for this result. First, though compounds with the opposite chirality in fragment two would present their substituents on opposite faces of the molecule, they have the same hybridization pattern in the ring. Thus, the absolute stereochemistry of substituents may be less important to cyclization than the hybridization pattern of the atoms in the ring. A second possibility is that these molecules with enantiomeric second fragments contain the same steric interactions within fragment 2. Therefore, interactions between the substituents found in fragment 2 may be more important for cyclization than interactions between fragments 1 and 2.

The rules for efficient macrocyclization are fairly complex. However, this study indicates that an understanding of general trends is possible, and further work may be able to shed more light on the issues involved in macrocylization.

Conclusion

Though macrocycles are a class of compounds rich in biological activity, the construction of diverse macrocycle libraries has been challenging, because little is known about what controls macrocyclization efficiency, especially on the solid phase. The work presented here develops methodology for the synthesis of a macrolide library, the members of which are diverse in the position of double bonds and in the nature and stereochemistry of substituents. Other macrolide libraries[10,25] synthesized to date do not contain this level of backbone diversity. In addition, analysis of this library provides information on general trends that can control cyclization on the solid phase. Though further work is necessary to confirm and fully elaborate the factors that control macrocylization on solid phase, this work represents an initial attempt to understand which members of a library of compounds will cyclize efficiently.

Supporting Information Available. Experimental details are available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Even if two compounds have a similar preferred conformation, they may vary in the energetic costs of occupying an alternative conformation. Thus, the free energy penalty for distortion of one of the small molecules upon binding to a protein target will differ from that of the other. Therefore, macrolide libraries may achieve a high level of structural diversity by containing molecules that can achieve different macrocycle conformations.
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(13) For acid protection, methyl esters could not be selectively removed without cleavage of the ester linkage between monomers. A MOM ester was compatible with monomer and macrolide synthesis and had the advantage that it did not preclude the use of allylic alcohols in the library synthesis. However, on solid phase, alcohols with MOM esters did not couple to acids cleanly. For alcohol protection, a 4-methoxyphenyl ether was also investigated, but conditions for its removal on solid-phase cleaved the silyl ether linkage to the bead.
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(20) Alcohols **12**, **9a**, and **9b** can form lactones, but this reaction was slow if acid was rigorously removed and the compounds were stored at −20 °C.
(21) Estimates based on bead swelling properties in various solvents indicate that hydroxyacid would be present at 0.1−0.2 M on the solid phase.
(22) Interestingly, the nuclear magnetic resonance (NMR) of **B2** was unsymmetrical (Figure 3), giving different signals for protons that are chemically equivalent. Nuclear Overhauser effect spectroscopy (NOESY) data indicates that while one alkene proton interacts with the methylene proton, the symmetrical alkene proton interacts with the allylic proton and not with the methylene protons. This indicates that the 14-member macrocycle has an unsymmetrical conformation.
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(24) Cyclization was initially effected by activation with 2,4,6-trichlorobenzoyl chloride in the presence of Hunig's base. After 20 h, additional benzoyl chloride and base were added, followed by DMAP. It had previously been shown that, for the model macrolide, cyclization could occur without DMAP and that DMAP increased the side reaction of coupling between hydroxyacids. Therefore, it was decided that cyclization would first be performed without DMAP to cyclize those rings that could close efficiently without DMAP. Next, a cyclization reaction accelerated by DMAP to close rings that were less efficiently cyclized would be conducted.
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