General Solid-Phase Synthesis Approach To Prepare Mechanism-Based Aspartyl Protease Inhibitor Libraries. Identification of Potent Cathepsin D Inhibitors

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Abstract: A general method has been developed for the expedient solid-phase synthesis of aspartyl protease inhibitors that do not incorporate any amino acids. The synthesis approach was designed to allow the introduction of diverse functionality at *all* four variable sites in inhibitors **1a,b** using readily available precursors. Representative, analytically pure inhibitors were obtained in 45−64% overall yields for the 12-step solid-phase synthesis sequence that includes both stereoselective reduction and carbon−carbon bond forming steps. A library of 204 spatially separate compounds was also prepared and screened against cathepsin D and resulted in the identification of a number of potent small molecule inhibitors ($K_i = 0.7-3$ nM). The described synthesis approach enables the rapid identification of small molecule inhibitors to diverse aspartyl protease targets. The general design principles are also applicable to other enzyme classes.

Introduction

Proteases, which catalyze the hydrolysis of amide bonds in peptides and proteins, play essential roles in numerous biological processes and are important therapeutic targets in a multitude of diseases including cancer, viral, parasitic, and bacterial infections, inflammation, and cardiovascular disease.[1−3] One of the most powerful strategies for designing inhibitors of proteases is to employ a mechanism-based pharmacophore as a key binding element that interacts with the active site of the protease. Pharmacophores have been developed that target each of the four major protease classes, which are defined according to the mechanism of protease-catalyzed amide bond hydrolysis. Examples of mechanism-based pharmacophores used to target proteases include secondary alcohols for aspartyl proteases, ketone and aldehyde carbonyls for cysteine and serine proteases, and hydroxamic acids for zinc metalloproteases.

The synthesis and evaluation of small molecule libraries employing mechanism-based pharmacophores have begun to have a significant impact upon the development of protease inhibitors.[4] These initial efforts demonstrated the utility of designing a solid-phase synthesis about a pharmacophore-based scaffold. However, these syntheses either were based on peptidic libraries[5] or only accessed a portion of the available sites of diversity about the pharmacophore structure. The full potential of these library approaches will only be realized if a full range of diverse functionality can be displayed at *all* variable sites about the mechanism-based pharmacophore. In the first report of a general solid-phase synthesis approach to target the aspartyl proteases, we described a synthesis approach for displaying functionality about a preformed hydroxyethylamine isostere that had a fixed substituent at one of the potential sites of variability.[6] We have successfully used this chemistry to identify low nanomolar small molecule inhibitors of cathepsin D.[7] Since our initial report, other researchers have reported solid-phase synthesis routes to display diverse functionality about mechanism-based pharmacophores to aspartyl proteases[8] and to metalloproteases.[9] Herein we describe the first general synthesis approach to incorporate diverse functionality at *all* variable sites about a secondary alcohol pharmacophore targeting aspartyl proteases. We further demonstrate the utility of this method by the optimization of small molecule inhibitors targeting cathepsin D.

Library Design

The aspartyl proteases are a ubiquitous class of enzymes that play an important role in mammals, plants, fungi, parasites, and

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(2) Dunn, B. M. In *Structure and Function of the Aspartic Proteinases: Genetics, Structures, and Mechanisms*; Plenum Press: New York, 1991; Vol. 306, xviii, 585 pp.

(3) Takahashi, K. In *Aspartic Proteinases: Structure, Function, Biology, and Biomedical Implications*; Plenum Press: New York, 1995.

(4) For recent reviews, see: (a) Whittaker, M. *Curr. Opin. Chem. Biol.* **1998**, *2*, 386-396. (b) Dolle, R. E. *Mol.Diversity* **1997**, *2*, 223−236.

(5) (a) In seminal work in this area, Owens and co-workers incorporated statine isosteres into a peptide library to identify HIV protease inhibitors. Owens, R. A.; Gesellchen, P. D.; Houchins, B. J.; DiMarchi, R. D. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 402−408. (b) Recent examples of peptidic libraries toward matrix metalloproteases are provided in ref 4.

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(8) (a) Wang and co-workers have reported an approach to display functionality about the diamino diol and diamino alcohol cores to target HIV-1 protease. In this approach the P_1 and P_1' side chains are invariant (Figure 1). Wang, G. T.; Li, S.; Wideburg, N.; Krafft, G. A.; Kempf, D. L. *J. Med. Chem.* **1995**, *38*, 2995−3002. (b) Rotella has recently reported preliminary efforts toward a potential approach to introduce P_1 side chain diversity into pharmacophore-based inhibitors by attaching amino alcohols onto solid support. However, methods to display functionality on the P′ side of the inhibitors were not demonstrated and the synthesis proceeded in low yield. Rotella, D. P. *J. Am. Chem. Soc.* **1996**, *118*, 12246−12247.

(9) A number of research groups have reported strategies to prepare inhibitors incorporating the hydroxamic acid pharmacophore motif by the display of functionality upon support-bound hydroxylamine derivatives. Whittaker, M. *Curr. Opin. Chem. Biol.* **1998**, *2*, 386-396.

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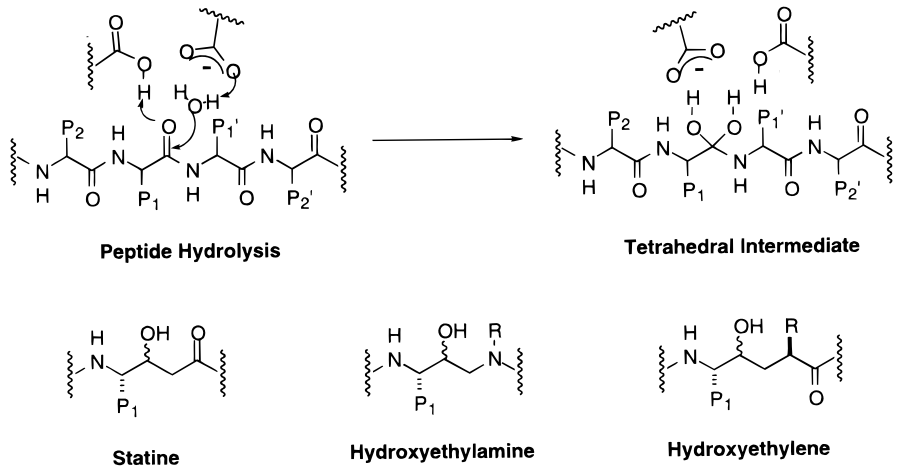

Figure 1. Common isosteres that mimic the tetrahedral intermediate of amide bond hydrolysis as catalyzed by aspartyl proteases.

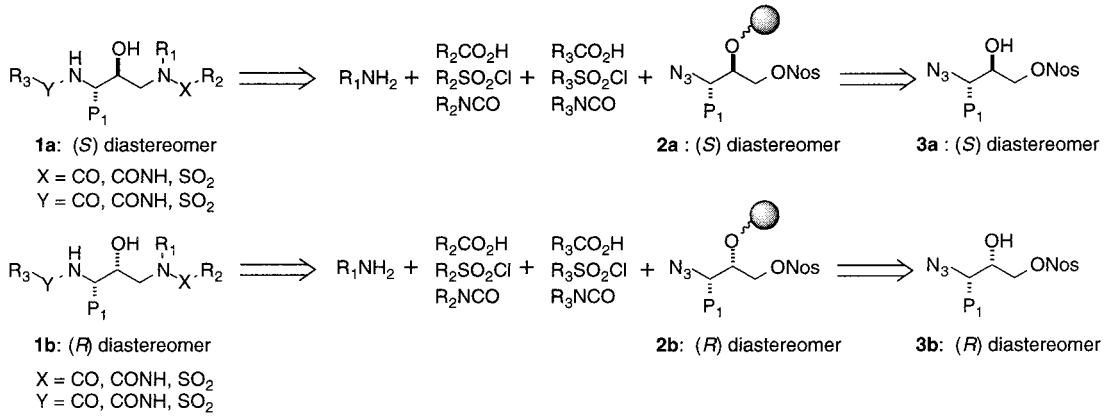

Figure 2. Synthesis strategy to incorporate R_1, R_2, and R_3 functionality.[6]

retroviruses.[2,3] Not surprisingly, aspartyl proteases have been implicated in a number of disease states and serve as important therapeutic targets. Most notably, HIV-1 protease inhibitors have had a huge impact on the treatment of AIDS.[10] Other aspartyl proteases that may prove to be important therapeutic targets include renin for the treatment of hypertension,[11] cathepsin D in breast tumor metastasis,[12,13] plasmepsins I and II in malaria,[14,15] and *Candida albicans* proteases in fungal infection.[16] The aspartyl proteases are endopeptidases that use two aspartic acid residues to catalyze the hydrolysis of amide bonds through a tetrahedral intermediate (Figure 1). Potent inhibitors of the aspartyl proteases have been developed that utilize a secondary alcohol as a stable mimetic of the tetrahedral intermediate. The alcohol is incorporated within isosteres that span the P_1-P_1' region.[17] Common isosteres include statine, hydroxyethylene, and hydroxyethylamine (Figure 1).

To develop a general solid-phase approach to target aspartyl proteases, we chose to display diverse functionality about the hydroxyethylamine isostere (**1a,b**) (Figures 1 and 2). In contrast to the other isosteres, the hydroxyethylamine motif readily allows for the rapid display of diverse functionality at the P_1' site by straightforward amine alkylation. In addition, several approved HIV protease drugs and clinical candidates are based upon this structure.[18] A key feature of our synthesis approach is the attachment of the starting scaffold to the solid support through the secondary hydroxyl group (Figure 2). Linkage through the secondary alcohol is ideal because it is the *only invariant part of the inhibitor structure and allows diversity to be displayed at all variable sites of the inhibitor*. In addition, the linker serves to protect the alcohol functionality throughout the synthesis sequence.

Although our ultimate goal was to develop a synthesis sequence to incorporate diverse functionality at all four sites of the hydroxyethylamine isostere (P_1, R_1, R_2, and R_3), we initially reported a general method to introduce R_1, R_2, and R_3 functionality (Figure 2).[6] The pendant side chains, R_1, R_2, and R_3, were incorporated into inhibitor structures **1a,b**, after linking the scaffolds **3a,b** to the solid support through the invariant secondary alcohol. Both diastereomers were used to access final products **1a** and **1b** because the preferred alcohol stereochemistry, *S* or *R*, respectively, depends on both the targeted aspartyl protease and the overall inhibitor structure. The R_1 functionality was obtained through amine addition into intermediates **2a,b**. Acylation with a variety of acylating agents introduced R_2 functionality. Azide reduction released the primary amine,

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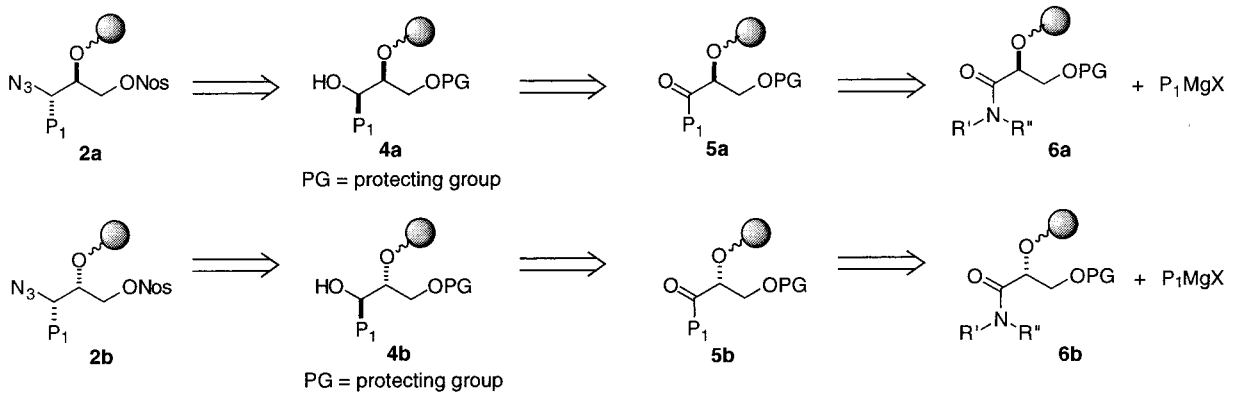


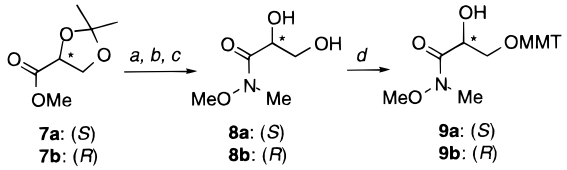
Figure 3. Retrosynthetic strategy of introducing P_1 side chain diversity.

which was treated with a variety of acylating agents for R_3 incorporation. We have synthesized several libraries using this chemistry and have identified potent inhibitors to the aspartyl proteases cathepsin D ($K_i = 9-20$ nM)[7] and plasmepsins I and II.[19]

A general solid-phase synthesis strategy designed to incorporate diverse functionality at the P_1 side chain would allow for rapid identification of potent inhibitors toward any aspartyl protease and would enable us to further optimize inhibitors of cathepsin D and plasmepsins I and II. The importance of exploring P_1 side chain diversity is clearly demonstrated by Agouron's development of their approved HIV-1 protease inhibitor.[20] By replacing the benzyl P_1 side chain with the methylthiophenyl side chain, a 10-fold improvement in potency was observed. To probe the specificity of the P_1 side chain toward aspartyl proteases, a solid-support synthesis of scaffolds **2a,b** with diverse P_1 side chains is clearly preferred over the time-consuming multistep preparation of numerous scaffolds incorporating different P_1 side chains. In our initial synthesis efforts, we delayed developing a general sequence to introduce diverse P_1 side chains due to the difficulty of achieving this goal. A synthesis approach quite different from previously reported solution-phase isostere synthesis efforts would be required due to the necessity of attaching the isostere to the support through the secondary alcohol. In addition, introduction of the P_1 side chain with the natural *S* stereochemistry would require both carbon−carbon bond formation and stereoselective transformations on solid support.

Herein, we report a solid-phase synthesis approach to access diversity at all sites including the P_1 site. We envisioned introduction of the P_1 side chain through a synthesis sequence that would provide the previously employed azide intermediates **2a,b** by functional group transformations upon alcohols **4a,b** (Figure 3). Alcohols **4a** and **4b** would be prepared by chelation or nonchelation controlled reduction of ketones **5a** and **5b**, respectively, to set the appropriate stereochemistry of the P_1 side chain. The P_1 side chain in ketones **5a,b** would be introduced by Grignard addition to corresponding tertiary amides **6a,b**. Since the aspartyl proteases have a hydrophobic P_1 pocket, the synthesis is biased toward use of hydrophobic groups at the P_1 site.[21] Grignard reagents are therefore ideal for the introduction of a large set of diverse hydrophobic side chains at this site.

Scheme 1[a]



[a] (a) LiOH, dioxane/H_2O, 99%. (b) *i*-BuOCOCl, NMM, Me(OMe)NH·HCl, CH_2Cl_2, 99%. (c) 40% Aqueous acetic acid, 85%. (d) MMTCl, DMAP, pyridine, 65%.

Results and Discussion

To prepare tertiary amides **6a**,**b**, appropriate linkage to solid support and a primary alcohol protecting group needed to be selected. For our previously reported synthesis approach we employed the acid-labile tetrahydropyranyl linkage.[6,22] Unfortunately, chelation-controlled reductions of THP-protected α-hydroxyketones proceed with poor diastereoselectivity. In contrast, high diastereoselectivity is observed for reductions of α-hydroxyketones protected as ethers including benzyl and substituted benzyl ethers.[23] Therefore, we elected to employ the acid labile *p*-alkoxybenzyl linker. The mono-*p*-methoxytrityl (MMT) group was selected to protect the primary alcohol, since mild acid treatment would selectively remove the protecting group without any cleavage of material from the resin. In addition, trityl-based protecting groups provide convenient spectrophotometric methods for quantitating loading levels and for confirming complete protecting group cleavage.

To introduce P_1 side chain functionality, we chose to develop a Weinreb amide substrate that would provide a ketone product after Grignard addition. Enantiomeric Weinreb amides **9a** and **9b** were prepared in four steps from the commercially available (*S*)- and (*R*)-methyl esters of isopropylidene glycerate, **7a** and **7b**, respectively (Scheme 1). After saponification of the methyl esters, activation of the resulting acids with isobutyl chloroformate and *N*-methylmorpholine was followed by treatment with *N*,*O*-dimethylhydroxylamine hydrochloride to provide the corresponding Weinreb amides. After isopropylidene deprotection

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(21) Almost every aspartyl protease (e.g., HIV protease, renin, cathepsins D and E, plasmepsins I and II, pepsin, etc.) for which substrate specificities have been performed prefers hydrophobic amino acid side chains at the P_1 position. Two reported exceptions are the yeast aspartic protease 3 and the related mammalian pro-opiomelanocortin converting enzymes that cleave between basic residues to process prohormones. Loh, Y. P.; Cawley, N. X.; Friedman, T. C.; Pu, L. P. In *Aspartic Proteinases: Structure, Function, Biology, and Biomedical Implications*; Takahashi, K., Ed.; Plenum Press: New York, 1995; pp 519−527.

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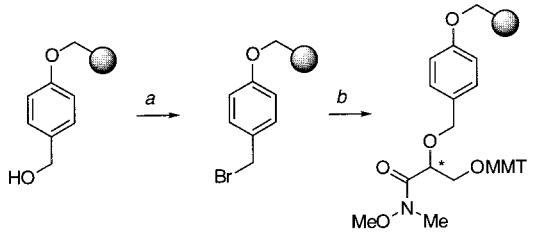
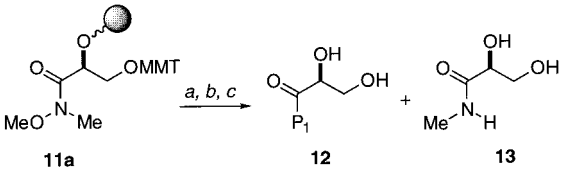
Scheme 2[a]



[a] (a) PPh_3, CBr_4, CH_2Cl_2. (b) **9a** or **9b**, NaH, Bu_4NI, 18-crown-6, THF, 2 h, 45 °C.

Scheme 3[a]



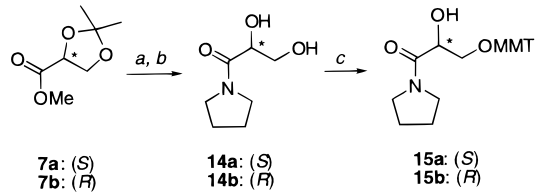
[a] (a) P_1MgX, THF, 0 °C. (b) 1% *p*-TsOH, CH_2Cl_2. (c) TFA, CH_2Cl_2.

with 40% aqueous acetic acid, primary alcohols **8a,b** were protected using 4-anisylchlorodiphenylmethane (MMTCl) and (dimethylamino)pyridine (DMAP). The Weinreb amide products **9a,b** were then attached to the support using (benzyloxy)-benzyl bromide resin **10**, which was readily prepared from commercially available Wang resin by treatment with carbon tetrabromide and triphenylphosphine.[24] An 81% loading efficiency was achieved employing sodium hydride, tetrabutylammonium iodide, and catalytic 18-crown-6 in THF for 2 h at 45 °C (Scheme 2).[25]

Grignard reagents were added to support-bound α-alkoxy Weinreb amide **11a** to introduce diversity at the P_1 site. We initially encountered two difficulties in obtaining clean Grignard addition products. First, the aqueous Grignard workup sequence needed to be modified for solid-phase chemistry. After filtering away the excess Grignard reagent from the support, direct addition of a mild acid solution resulted in some overaddition, presumably due to breakdown of the tetrahedral intermediate before all of the residual Grignard reagent had been quenched. This problem was readily solved by consuming residual Grignard reagent with addition of acetone before the quench step. Second, Grignard addition and subsequent cleavage from the solid support provided the desired ketone **12** contaminated with 7−18% of methyl amide side product **13** depending on the Grignard reagent that was employed (Scheme 3). Side product **13** resulted from competitive N−O bond cleavage of Weinreb amide **11a**.[26]

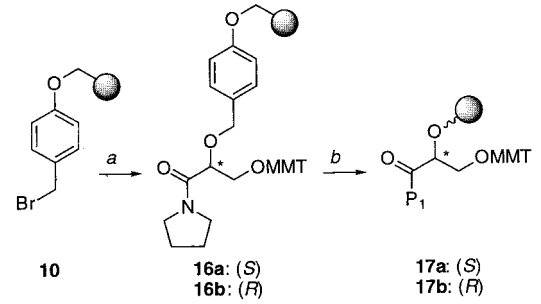
In an effort to eliminate amide side product **13**, we replaced the *N*-methoxy-*N*-methyl (Weinreb) amide with a pyrrolidine amide. Romea and co-workers have recently reported that addition of Grignard reagents to α-alkoxy-substituted pyrrolidine amides cleanly provides ketones with minimal overaddition, in contrast to tertiary amides without α-alkoxy substituents.[27] Presumably, chelation of the α-alkoxy group to the magnesium stabilizes the tetrahedral intermediate. Preparation and loading

Scheme 4[a]



[a] (a) Pyrrolidine, 95%. (b) 40% Aqueous acetic acid, 94%. (c) MMTCl, DMAP, pyridine, 85%.

Scheme 5[a]



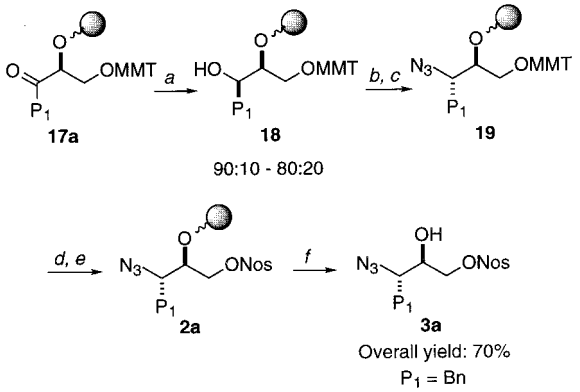
[a] (a) **15a** or **15b**, NaH, Bu_4NI, 18-crown-6, THF, 2 h, 45 °C. (b) P_1MgX, THF, 0 °C.

of pyrrolidine amides **15a,b** are straightforward and can readily be performed on a large scale (Scheme 4). Pyrrolidine amides **15a** and **15b** were prepared in 76% overall yield in three steps from commercially available (*S*)- and (*R*)-methyl esters of isopropylidene glycerate, **7a** and **7b**, respectively. Treatment of **7a,b** with neat pyrrolidine followed by deprotection with 40% aqueous acetic acid provided amides **14a,b**. The only purification in the sequence was performed after protection of the primary alcohol with MMTCl and DMAP in pyridine. Amides **15a,b** were coupled to support as previously described for Weinreb amides **11a,b** in 84% loading efficiency (Scheme 5). Minimal (<2%) racemization[28] was observed in the coupling step as determined by cleavage of amide **16a** from support followed by derivatization with α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPACl) and ^{19}F NMR analysis of the diester product.[29] Addition of Grignard reagents to support-bound amide **16a** and subsequent cleavage from the solid support cleanly provided desired ketone **12** with no overalkylation product observed.

The next step was to set the stereochemistry of the P_1 side chain. We first chose to develop chemistry to access core isostere **1a** (Figure 2), which has the *S* secondary alcohol stereochemistry, since we required this epimer for optimization of our cathepsin D inhibitors (vide infra). Based on our proposed synthesis strategy, isostere **1a** would be derived through the use of chelation controlled reduction of α,*β*-dialkoxy ketone **5a** (Figure 3).[30,31] Zinc borohydride ($Zn(BH_4)_2$) was selected as the reducing agent because the reagent afforded higher diastereoselectivity compared to less oxophilic reducing agents such as lithium aluminum hydride. The support-bound ketone **17a** was reduced with $Zn(BH_4)_2$ in diethyl ether:THF at −20 °C (Scheme 6). To determine reduction diastereoselectivity as well as to ensure that minimal racemization had occurred during the Grignard addition and reduction steps, secondary alcohol

(24) Ngu, K.; Patel, D. V. *Tetrahedron Lett.* **1997**, *38*, 973−976.
(25) Altava, B.; Burgrete, M. I.; Luis, S. V.; Mayoral, J. A. *Tetrahedron* **1994**, *50*, 7535−7542.
(26) Armstrong has previously reported similar side products for Grignard additions on solid support. Dinh, T. Q.; Armstrong, R. W. *Tetrahedron Lett.* **1996**, *37*, 1161−1164.
(27) Martin, R.; Pascual, O.; Romea, P.; Rovira, R.; Urpi, F.; Vilarrasa, J. *Tetrahedron Lett.* **1997**, *38*, 1633−1636.
(28) Extended reaction times can result in 10% racemization.
(29) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(30) Iida, H.; Yamazaki, N.; Kibayashi, C. *J. Org. Chem.* **1986**, *51*, 3769−3771 and references therein.
(31) Pikul, S.; Raczko, J.; Ankner, K.; Jurczak, J. *J. Am. Chem. Soc.* **1987**, *109*, 3981−3987 and references therein.

Scheme 6[a]

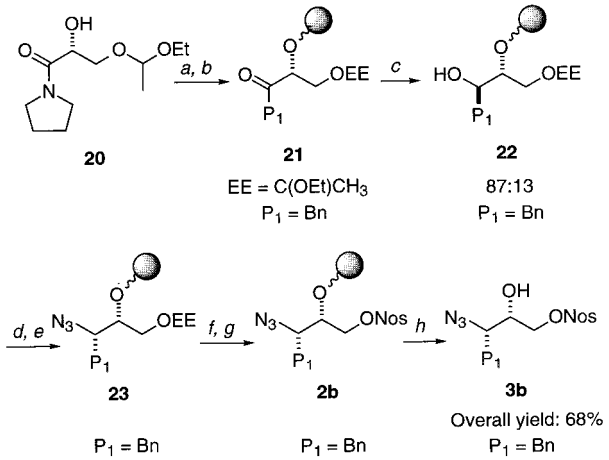


[a] (a) $Zn(BH_4)_2$, Et_2O:THF, −20 °C. (b) NosCl, 4-pyrrolidinopyridine, $CHCl_3$. (c) NaN_3, DMF, 50 °C. (d) 1% *p*-TsOH, CH_2Cl_2. (e) NosCl, pyridine, $CHCl_3$. (f) TFA, CH_2Cl_2.

18 (P_1 = Bn) was cleaved from the support to provide the corresponding triol product, which was converted to the triacetate using acetic anhydride and DMAP.[32] A 90:10 to 85:15 diastereoselectivity range was observed by GC analysis of the triacetate. As expected, chelation-controlled reduction performed on model substrates in solution provided similar levels of diastereoselectivity. $Zn(BH_4)_2$ reductions performed on ketone **17a** with six different P_1 side chains (benzyl, *m*-phenoxybenzyl, *β*-naphthylmethyl, *p*-phenylbenzyl, *p*-bromobenzyl, and isobutyl) provided diastereoselectivities ranging from 90:10 to 80:20. The variability in diastereoselectivity depended primarily on the batch of $Zn(BH_4)_2$ employed.

Conversion of secondary alcohol **18** to azide **19** required considerable optimization. Mitsunobu reactions performed with diphenyl phosphoryl azide, hydrazoic acid, or zinc azide bispyridine complex proceeded poorly or resulted in significant epimerization. We next investigated alcohol activation and displacement. To achieve good conversion to the activated alcohol, it was necessary to employ 4-nitrobenzenesulfonyl chloride with 4-pyrrolidinopyridine as the catalyst and chloroform as the reaction solvent. Poor conversion was observed with alcohol activation to the tosyl or mesyl alcohol, when the reaction was performed in other solvents such as methylene chloride, or when using other catalysts such as DMAP. Rapid precipitation of DMAP salts occluded reaction sites on the resin, which prevented good conversion from being achieved. After primary alcohol activation, displacement with sodium azide was accomplished in *N*,*N*-dimethylformamide at 50 °C. Minimal competitive elimination of the nosyl alcohol (0−<5%) was observed even for P_1 side chains that contained acidic *β*-hydrogens (i.e., P_1 = CH_2Ar). The MMT protecting group was selectively removed using 1% *p*-toluenesulfonic acid (*p*-TsOH) in CH_2Cl_2 (w/v). The primary alcohol was then activated to obtain nosyl alcohol **2a** using 4-nitrobenzenesulfonyl chloride and pyridine in chloroform (Scheme 6). Intermediate **2a** can then be converted to the final inhibitor structures using the previously reported synthesis sequence (Figure 2). To establish the fidelity of the chemistry to this intermediate in the synthesis sequence, nosyl alcohol **3a** (P_1 = Bn) was cleaved from the support and purified by column chromatography in 70% overall yield based on the loading level of support-bound pyrrolidine amide **16a**.[33]

Scheme 7[a]



[a] (a) NaH, resin **10**, Bu_4NI, 18-crown-6, THF, 2 h, 45 °C. (b) BnMgCl, THF, 0 °C. (c) l-selectride, THF, −78 °C to −20 °C. (d) NosCl, 4-pyrrolidinopyridine, $CHCl_3$. (e) NaN_3, DMF, 50 °C. (f) 2% *p*-TsOH, CH_2Cl_2. (g) NosCl, pyridine, $CHCl_3$. (h) TFA, CH_2Cl_2.

To have a completely general synthesis approach targeting aspartyl proteases it was also necessary to access isostere **1b** (Figure 2), which has the *R* secondary alcohol stereochemistry. This isostere is accessible by performing non-chelation-controlled reduction upon ketone precursor **5b** (Figure 3). Reduction of ketone **5b** with hindered reducing agents such as L-selectride should provide **4b** with high diastereoselectivity based upon literature reports of highly diastereoselective reductions of α,*β*-dialkoxy ketones in solution.[30,31,34,35] Unfortunately, L-selectride reduction of ketone **17** (P_1 = Bn) resulted in poor non-chelation-controlled diastereoselectivity (<60:40). Poor diastereoselectivity for L-selectride reductions of related α,*β*-dialkoxyketones in solution was also observed, indicating that our initial findings were not due to a support effect. We therefore investigated the *β*-hydroxyl protecting group strategy. In solution-phase model studies, replacement of the MMT group with the nonchelating but sterically hindered TIPS group also resulted in poor reduction diastereoselectivity. In contrast, 90:10 diastereoselectivity was observed when the less hindered ethoxyethyl (EE) group was employed.

Accordingly, pyrrolidine amide **14b** was treated with ethyl vinyl ether and pyridinium *p*-toluenesulfonate in CH_2Cl_2 to provide amide **20** (Scheme 7). Coupling to support was accomplished using the conditions established to afford support-bound Weinreb amide **11a**. After Grignard addition with benzylmagnesium chloride, the resulting support-bound benzyl ketone **21** was reduced using L-selectride in THF to afford alcohol **22** with 87:13 diastereoselectivity as determined by GC analysis of the triacetate produced upon cleavage of the triol from support followed by acetylation.[36] Support-bound alcohol **22** is converted to the azide precursor **2b** according to the conditions used to prepare **2a**. Although slightly stronger acidic conditions, 2% *p*-TsOH in CH_2Cl_2 (w/v), were required for complete removal of the ethoxyethyl group relative to the MMT group, no cleavage of azide **23** from support was observed under these reaction conditions. Removal of the ethoxyethyl group and activation of the primary alcohol were performed as previously described to obtain the desired nosyl alcohol

(32) Chiral HPLC analysis (P_1 = Bn) showed that 5−6% racemization had occurred to this point in the synthesis.

(33) When Weinreb amide **11a** was employed, a slightly reduced overall yield was observed (65%) in addition to 8.0% of the aforementioned methyl amide side product.

(34) Mori, Y.; Kuhara, M.; Takeuchi, A.; Suzuki, M. *Tetrahedron Lett.* **1988**, *29*, 5419−5422.

(35) Mead, K.; MacDonald, T. L. *J. Org. Chem.* **1985**, *50*, 422−424.

(36) Chelation-controlled reduction of ketone **21** with $Zn(BH_4)_2$ provided the same level of diastereoselectivity that is observed for chelation controlled reduction of the MMT-protected ketone **17**.

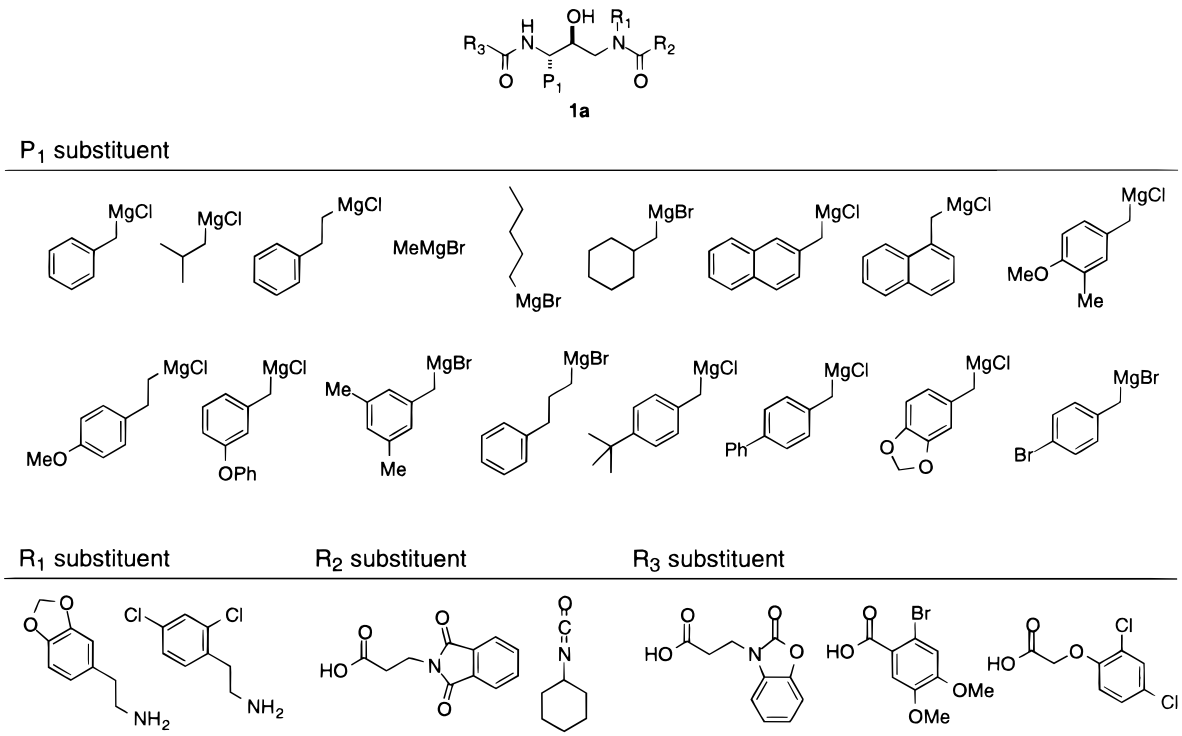


Figure 4. Library components.

intermediate **2b,** which can be employed directly in the previously developed synthesis sequence. To demonstrate a high level of fidelity for the solid-phase chemistry, intermediate **3b** (P_1 = Bn) was cleaved from the support and purified by column chromatography in 68% overall yield based on the initial loading level of pyrrolidine amide **20**.

Library Synthesis and Screening

Using the aforementioned chemistry, a library of 204 compounds with the general structure **1a** was synthesized to identify cathepsin D inhibitors with optimized side chains at the P_1 pocket (Figure 4). The crystal structure of cathepsin D with pepstatin bound in the active site shows a large hydrophobic P_1 pocket that appears able to incorporate groups larger than the benzyl group,[37] which had been used in our initial library syntheses toward cathepsin D.[7] Seventeen different Grignard reagents were chosen on the basis of structural features for cathepsin D inhibition and/or to demonstrate the generality of the chemistry. From our earlier libraries of cathepsin D inhibitors, a representative set of the most active side chains at R_1, R_2, and R_3 were incorporated into the library of 204 compounds.

For the library synthesis, the Weinreb amide **11a** was employed because the pyrrolidine amide system had not yet been developed. Since the methyl amide side product resulting from Grignard addition to the Weinreb amide **11a** (Scheme 3) does not contain either the P_1 or R_3 side chain, the methyl amide side product does not significantly inhibit cathepsin D.

Of the 17 Grignard reagents used in the library, 5 were commercially available and the remaining 12 were readily prepared by one of two methods (Figure 4). The alkyl Grignard reagents were synthesized from gradual addition of the corresponding halide to activated magnesium turnings in diethyl ether.[38] In general, this method did not efficiently provide the more activated, substituted benzylic-type Grignard reagents as precedented in the literature.[39,40] Therefore, we investigated the use of a more THF-soluble source of magnesium, (Mg(anthracene)$(THF)_3$), and lower Grignard concentrations.[39,40] All of the benzylic Grignard reagents were easily prepared from gradual addition of the corresponding halide to the magnesium anthracene THF complex. In solution-phase chemistry, one problem with using Grignard reagents prepared from the magnesium anthracene THF complex is the separation of anthracene byproducts from desired products in the reaction workup. In contrast, in the solid-support workup process, the excess Grignard reagent along with any anthracene byproducts are simply washed away from support-bound products.

After solid-support Grignard addition, a portion of resin for each P_1 side chain was cleaved at ketone **17a** to determine reaction completion. For the 17 Grignard reagents used in library synthesis, the corresponding ketones were cleanly obtained, as determined by NMR, except for 7−18% of methyl amide side product **13** (Scheme 4). Two commercially available Grignard reagents, neopentylmagnesium chloride and (2-methyl-2-phenylpropyl)magnesium chloride, did not provide sufficiently clean addition to Weinreb amide **11a** and were not included in the library synthesis. Conversion of all 17 ketone intermediates to nosyl alcohols **2a** were performed as earlier described. All scaffolds were cleaved at intermediate **2a**, and in every case, the major product was determined by TLC and NMR to be the desired alcohol **3a** (^{1}H NMR spectra supplied in the Supporting Information).

Introduction of R_1, R_2, and R_3 was performed as previously reported (Scheme 8).[7] The amine displacement to incorporate R_1 was performed in parallel in sealed vials with heating in *N*-methylpyrrolidinone (NMP). The remaining reactions to introduce R_2 and R_3 were performed in a spatially separate array

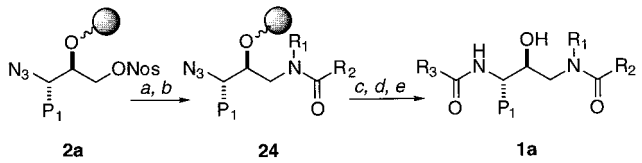
(37) Baldwin, E. T.; Bhat, N.; Gulnik, S.; Hosur, M. V.; Sowder, R. C. I.; Cachau, R. E.; Collins, J.; Silva, A. M.; Erickson, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6796−6800.

(38) Baker, K. V.; Brown, J. M.; Hughes, N.; Skarnulis, A. J.; Sexton, A. *J. Org. Chem.* **1991**, *56*, 698−703.

(39) Gallagher, M. J.; Harvey, S.; Raston, C. L.; Sue, R. E. *J. Chem. Soc., Chem. Commun.* **1988**, 289−290 and references therein.

(40) Harvey, S.; Junk, P. C.; Raston, C. L.; Salem, G. *J. Org. Chem.* **1988**, *53*, 3134−3140 and references therein.

Scheme 8[a]



[a] (a) R_1NH_2, NMP, 80 °C. (b) R_2CO_2H, HOAt, PyBOP, *i*-Pr_2EtN, NMP or R_2NCO, NMP. (c) $SnCl_2$, PhSH, NEt_3, THF. (d) R_3CO_2H, HOAt, PyBOP, *i*-Pr_2EtN, NMP. (e) TFA, CH_2Cl_2.

using a 96-well filter apparatus.[41] After acylation with R_2 functionality, support-bound azide **24** was reduced using tin(II) chloride, thiophenol, and triethylamine in THF. The resulting amine was then acylated with the R_3 carboxylic acids. The library of compounds was resubjected to the coupling conditions for both R_2 and R_3 acylations to ensure complete reactions. The compounds were then cleaved from the resin using TFA:CH_2Cl_2 (1:1).

The library was screened for cathepsin D inhibition at 330, 100, 33, and 10 nM in a high-throughput fluorescence assay.[7,42] Several of the most potent inhibitors were synthesized on larger scale using the α-alkoxy pyrrolidine amide route, purified by chromatography, and characterized by NMR, mass spectrometry, and elemental analysis (Table 1). The overall yields of the scaled-up inhibitors ranged from 45 to 64% for the 12-step solid-phase synthesis sequence and were determined by the mass balance of purified final product compared to the initial loading of starting pyrrolidine amide **16a** (Schemes 5, 6, and 8). Compounds for scale-up were predominantly chosen on the basis of potency in the library screen and to provide SAR. In addition, inhibitor **31** was synthesized on large scale to demonstrate the synthesis generality using alkyl Grignard reagents. Inhibition constants (K_i) were determined for each of these compounds (Table 1).

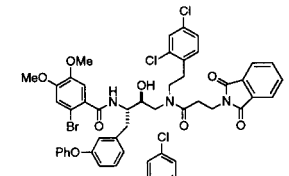
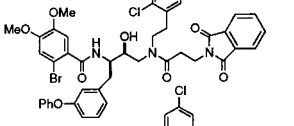
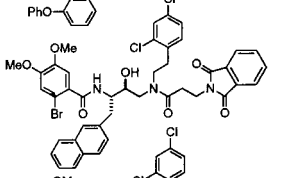
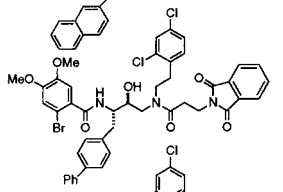
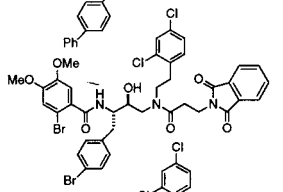
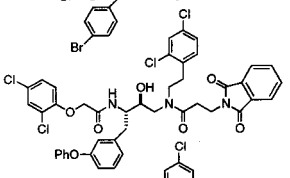
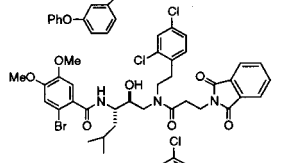
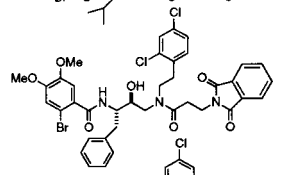
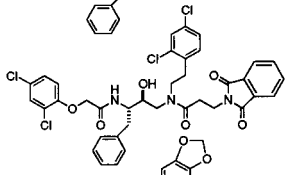
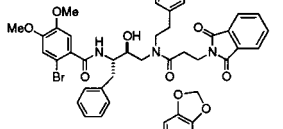
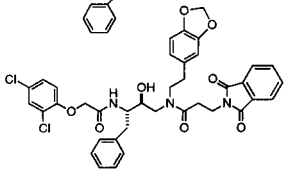
As previously described (Schemes 6 and 7), the reduction step is not completely stereoselective. Therefore, in library screening, the inhibitors were contaminated with minor diastereomeric byproducts that incorporate P_1 side chains with the unnatural *R* stereochemistry. To establish that the minor diastereomers do not contribute to inhibition in library screening, the K_i of **26**, the minor diastereomer of the most potent inhibitor **25** (vide infra), was determined to be >5 μM, clearly demonstrating the importance of side chain stereochemistry for aspartyl protease inhibition.

Several inhibitors that were prepared on large scale had K_i values of <3 nM (Table 1). The most potent compound, **25**, had a K_i of 0.7 nM. In comparison with the most potent compound we had previously reported, the potency has increased greater than 4-fold.[43] For the most potent inhibitors from our library (inhibitors **25**, **27**, and **28**), the larger hydrophobic side chains at P_1 were preferred over the smaller benzyl side chain (inhibitor **32**).

Conclusions

In summary, we have reported a general synthesis strategy that successfully allows for full diversification about the hydroxyethylamine aspartyl protease isostere using a 12-step solid-phase synthesis sequence that includes both stereoselective reduction and carbon−carbon bond forming steps. Using this synthesis approach, a diverse array of hydrophobic functionality

Table 1[a]

Entry Number	Inhibitor	K_i (nM)	Overall Yield (12 steps)
25		0.7 ± 0.3	46%
26		> 5000	—
27		1.3 ± 0.2	48%
28		2.7 ± 0.4	48%
29		5.6 ± 0.6	46%
30		30 ± 4	45%
31		85 ± 15	64%
32		5.2 ± 0.4	—**
33		8.0 ± 0.9	—**
34		5.9 ± 0.3	—**
35		3.0 ± 0.2	—**

[a] (*) The K_i for compound **26** was determined to establish that the diastereomer with the unnatural stereochemistry at the P_1 site does not inhibit the enzyme. Since the minor diastereomer was isolated from a diastereomeric mixture, the yield was not determined. (**) Inhibitors **32**−**35** with benzyl P_1 side chains were synthesized and purified on large scale in previously reported work.[7]

(41) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240−1256.

(42) Krafft, G. A.; Wang, G. T. *Methods Enzymol.* **1994**, *241*, 70−86.

(43) A few of the compounds with the benzyl side chain at P_1 (e.g., inhibitor **35**) were assayed previously.[7] Different batches of cathepsin D isolated from human liver were used in the previous work and the work reported here. This resulted in somewhat different K_i values. For example, inhibitor **35** was determined to have a K_i value of 9 ± 2 nM in previously reported work and was determined to have a K_i value of 3.0 ± 0.2 nM in this study.

can readily be incorporated into the P_1 side chain followed by a wide range of diversity at the three other sites. By performing the synthesis in a library format, several potent cathepsin D inhibitors have been identified with optimized P_1 side chains. We are currently using the synthesis approach to optimize binding and pharmacokinetic properties of inhibitors targeting the malarial proteases, plasmepsins I and II. This synthesis approach demonstrates the utility of designing a general, solid-phase synthesis about a mechanism-based pharmacophore by linking through an essential, invariant portion of the pharmacophore. In due course, we will report an extension of this general design strategy to library synthesis approaches targeting other enzyme classes including the cysteine and serine protease classes.

Experimental Section

General Methods. Materials were obtained from commercial suppliers and employed without further purification unless otherwise stated. Wang resin was purchased from NovaBiochem (San Diego, CA). Sodium borohydride ($NaBH_4$) was purchased from Alfa AESAR (Ward Hill, MA). Anhydrous *N*,*N*-dimethylformamide (DMF) and *N*-methylpyrrolidinone (NMP) were purchased from Aldrich (Milwaukee, WI). The following solvents were distilled under N_2 from specified drying agents: tetrahydrofuran (THF) and diethyl ether (Et_2O) from sodium/benzophenone ketyl, dioxane from sodium, and methylene chloride (CH_2Cl_2) and pyridine from calcium hydride. Chloroform ($CHCl_3$) was passed through basic alumina prior to use. Flash column chromatography was carried out with Merck 60 230−400-mesh silica gel according to the procedure reported by Still.[44] GC analysis was performed on a Hewlett-Packard 5890 Series II gas chromatograph using a Hewlett-Packard Ultra 2 column. Infrared spectra (IR) were recorded with a Perkin-Elmer 1600 Series Fourier transform spectrometer using KBr pellets or NaCl plates, and only partial spectral data is listed. ^{1}H and ^{13}C NMR spectra were recorded using a UCB Bruker AMX-300, AMX-400, AM-400, or DRX-500 spectrometer. ^{19}F NMR spectra were obtained with a UCB Bruker AM-400. All spectra were obtained in $CDCl_3$, and chemical shifts are recorded in parts per million relative to the internal solvent peak. Coupling constants are reported in hertz. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

The following procedures were employed to synthesize both *S* and *R* isomers. Only one example is provided below.

(2*S*)-2,3-*O*-Isopropylidene-*N*-methoxy-*N*-methylpropanamide. To a solution of 10.0 g (62.4 mmol) of the (*S*)-methyl ester of isopropylidene glycerate (**7a**) in 250 mL of dioxane:H_2O (1:1) at 0 °C was added 2.88 g (68.6 mmol) of lithium hydroxide monohydrate. The reaction mixture was stirred for 12 h at 4 °C followed by the addition of 1.0 M citric acid (70 mL) to acidify the reaction solution (pH = 4). The solution was saturated with NaCl and then washed with ethyl acetate (8 × 75 mL). The organic extracts were dried over Na_2SO_4 and concentrated to afford a yellow oil that was carried onto the next step. ^{1}H NMR (300 MHz): δ 1.39 (s, 3H), 1.50 (s, 3H), 4.16 (dd, J = 4.8, 8.8, 1H), 4.27 (dd, J = 7.4, 8.8, 1H), 4.61 (dd, J = 4.8, 7.4, 1H), 10.03 (broad s, 1H).

To a solution of 9.10 g (0.0624 mol) of the corresponding carboxylic acid in 230 mL of CH_2Cl_2 at −11 °C (ethanol−ice bath) was added 14.4 mL (0.131 mol) of *N*-methylmorpholine followed by 8.09 mL (0.0624 mol) of isobutyl chloroformate. The reaction mixture was stirred for 15 min, changing from yellow to dark orange in color. To the reaction solution was added 6.70 g (0.0690 mol) of *N*,*O*-dimethylhydroxylamine hydrochloride at −11 °C. The mixture was stirred for 8 h at −11 °C with warming to room temperature. The reaction solution was poured into vigorously stirring H_2O (150 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 75 mL). The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash chromatography (80:20 hexanes:ethyl acetate, then 67:33) to yield 11.1 g (94% for two steps) of the product as a yellow oil. ^{1}H NMR (300 MHz): δ 1.42 (s, 3H), 1.48 (s, 3H), 3.19 (s, 3H), 3.70 (s, 3H), 4.05 (m, 1H), 4.24 (m, 1H), 4.84 (apparent t, J = 6.6, 1H). ^{13}C NMR (101 MHz): δ 25.62, 25.69, 61.30, 66.73, 73.13, 110.43, 110.76, 171.15.

(2*S*)-2,3-Dihydroxy-*N*-methoxy-*N*-methylpropanamide (8a). A solution of 21.0 g (0.111 mol) of (2*S*)-2,3-*O*-isopropylidene-*N*-methoxy-*N*-methylpropanamide in 500 mL of 40% aqueous acetic acid was stirred at 35 °C for 10 h. The solution was concentrated, and toluene (5 × 50 mL) was added to form an azeotrope with acetic acid during the concentration step. The crude yellow oil was purified by flash chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 14.1 g (85%) of the diol as a colorless oil. IR: 3381, 2942, 1650 cm^{-1}. ^{1}H NMR (300 MHz): δ 3.23 (s, 3H), 3.68 (dd, J = 5.1, 11.5, 1H), 3.70 (s, 3H), 3.84 (dd, J = 3.2, 11.5, 1H), 4.48 (dd, J = 3.2, 5.1, 1H). ^{13}C NMR (101 MHz): δ 32.32, 53.37, 61.30, 64.02, 172.14. Anal. Calcd for $C_5H_{11}N_1O_4$: C, 40.27; H, 7.43; N, 9.39. Found: C, 40.46; H, 7.31; N, 9.15.

(2*S*)-2-Hydroxy-*N*-methoxy-*N*-methyl-3-mono-*p*-methoxytritylpropanamide (9a). To a solution of 13.3 g (43.1 mmol) of 4-anisylchlorodiphenylmethane (MMTCl) in 140 mL of pyridine at room temperature was added a solution of 5.50 g (36.9 mmol) of **8a** in 20 mL of pyridine followed by the addition of 0.225 g (1.84 mmol) of DMAP. The reaction mixture was stirred for 4.5 h, diluted in 150 mL of CH_2Cl_2, washed with 1.0 M citric acid (8 × 200 mL), saturated $NaHCO_3$ (2 × 150 mL), and brine (1 × 150 mL), dried over Na_2SO_4, and concentrated**.** The crude yellow oil was purified by chromatography (80:19:1 hexanes:ethyl acetate:triethylamine, then 67:32:1 hexanes:ethyl acetate:triethylamine) to provide 10.2 g (66%) of **9a** as a white solid. ^{1}H NMR (400 MHz): δ 3.21 (s, 3H), 3.28 (dd, J = 4.1, 9.4, 1H), 3.39 (dd, J = 2.8, 9.4, 1H), 3.44 (s, 3H), 3.72 (d, J = 8.2, 1H), 3.78 (s, 3H), 4.54 (m, 1H), 6.81 (m, 2H), 7.18−7.21 (m, 2H), 7.27−7.33 (m, 6H), 7.44 (m, 4H). ^{13}C NMR (101 MHz): δ 55.18, 60.96, 65.38, 69.09, 85.97, 113.03, 126.83, 127.75, 128.44, 128.45, 130.30, 135.49, 144.21, 158.48. Anal. Calcd for $C_{25}H_{27}N_1O_5$: C, 71.24; H, 6.46; N, 3.32. Found: C, 71.15; H, 6.29; N, 3.37.

(2*S*)-2,3-*O*-Isopropylidene-*N*,*N*-tetramethylenepropanamide. A solution of 14.8 g (0.0924 mol) of **7a** in 9.26 mL (0.111 mol) of pyrrolidine was stirred at room temperature for 30 h. The solution was concentrated to a yellow oil, and the crude product was carried on to the next step without purification. However, the yellow oil can be purified by chromatography (67:33 hexanes:ethyl acetate) to provide the product as a colorless oil. ^{1}H NMR (500 MHz): δ 1.38 (s, 3H), 1.41 (s, 3H), 1.80−1.98 (m, 4H), 3.46 (m, 3H), 3.66 (m, 1H), 4.14 (dd, J = 6.9, 8.3, 1H), 4.28 (dd, J = 6.5, 8.3, 1H), 4.59 (apparent t, J = 6.7, 1H). ^{13}C NMR (125 MHz): δ 23.33, 25.21, 25.33, 25.72, 45.75, 45.89, 65.97, 74.04, 109.92, 167.20. Anal. Calcd for $C_{10}H_{17}N_1O_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.40; H, 8.38; N, 7.09.

(2*S*)-2,3-Dihydroxy-*N*,*N*-tetramethylenepropanamide (14a). A solution of 18.0 g (0.0904 mol) of (2*S*)-2,3-*O*-isopropylidene-*N*,*N*-tetramethylenepropanamide in 300 mL of 40% aqueous acetic acid was stirred with heating to 35 °C for 12 h. The solution was concentrated, and toluene (5 × 50 mL) was added to form an azeotrope with acetic acid during the concentration step. The crude yellow oil was purified by flash chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 13.1 g (89% for two steps) of **14a** as a white solid. ^{1}H NMR (500 MHz): δ 1.78−1.97 (m, 4H), 3.36 (m, 1H), 3.43 (m, 1H), 3.50 (m, 2H), 3.72 (dd, J = 4.7, 11.8, 1H), 3.80 (dd, J = 3.0, 11.8, 1H), 4.46 (m, 1H), 5.94 (broad s, 2H). ^{13}C NMR (125 MHz): δ 23.43, 25.62, 45.86, 45.90, 63.62, 70.66, 170.22. Anal. Calcd for $C_7H_{13}N_1O_3$: C, 52.82; H, 8.23; N, 8.80. Found: C, 52.65; H, 8.00; N, 8.71.

(*R*,*R*)-(+,+)-MTPA Diester.[29] The following procedure was used for small-scale preparation to determine the enantiopurity of starting material pyrrolidine amide **14** and enantiopurity of pyrrolidine amide **14** after cleavage from support. To a solution of 4.0 mg (0.025 mmol) of pyrrolidine amide **14** in 75 μL of CH_2Cl_2 was added 0.20 mL (2.5 mmol) of pyridine followed by 24 μL (0.13 mmol) of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-(+)-MTPACl). The reaction solution was stirred at room temperature for 8 h, at which time 20 μL (0.20 mmol) of 3-(dimethylamino)-1-propylamine was

(44) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

added and was stirred for 5 min to convert excess (*R*)-(+)-MTPACl into basic amide to be removed in acidic workup. The reaction mixture was diluted in ether, washed with cold 1 M HCl, saturated Na_2CO_3, and brine, dried over Na_2SO_4, and concentrated.

Determination of Enantiopurity of Starting Material Pyrrolidine Amide 14a,b. Diastereomer *S,R,R* Prepared from Starting Material (2*S*)-2,3-Dihydroxy-*N*,*N*-tetramethylenepropanamide (14a). ^{1}H NMR (400 MHz): δ 1.85 (m, 2H), 1.96 (m, 2H), 3.27 (s, 3H), 3.37−3.58 (m, 3H), 3.63 (s, 3H), 3.72 (m, 1H), 4.47 (dd, J = 7.8, 11.9, 1H), 4.67 (dd, J = 4.5, 11.9, 1H), 5.44 (dd, J = 4.5, 7.8, 1H), 7.36−7.61 (m, 10H). ^{19}F NMR (327 MHz): δ −9.13, −8.96. ^{19}F NMR integration showed 2.5% of the other diastereomer (*R,R,R*).[45]

Diastereomer *S,R,R* Prepared from 14a after Cleavage from the Support. ^{19}F NMR integration showed 2.8−3.3% of the other diastereomer (*R,R,R*), indicating that <2.0% racemization occurred during the support coupling step.

Diastereomer *R,R,R* prepared from Starting Material (2*R*)-2,3-Dihydroxy-*N*,*N*-tetramethylenepropanamide (14b). ^{1}H NMR (400 MHz): δ 1.85 (m, 2H), 1.95 (m, 2H), 3.35 (s, 3H), 3.33−3.53 (m, 3H), 3.50 (s, 3H), 3.63 (m, 1H), 4.59 (dd, J = 8.1, 12.1, 1H), 4.79 (m, 1H), 5.47 (m, 1H), 7.38−7.59 (m, 10H). ^{19}F NMR (327 MHz): δ −9.72, −8.84. ^{19}F NMR integration showed 1.5% of the other diastereomer (*S,R,R*).

(2*S*)-2-Hydroxy-3-mono-*p*-methoxytrityl-*N*,*N*-tetramethylenepropanamide (15a). To a solution of 28.9 g (93.5 mmol) of MMTCl in 260 mL of pyridine at room temperature was added a solution of 13.5 g (85.0 mmol) of **14a** in 40 mL of pyridine followed by the addition of 0.610 g (5.00 mmol) of DMAP. The reaction mixture was stirred for 7 h, diluted with 300 mL of CH_2Cl_2, washed with 1.0 M citric acid (8 × 400 mL), saturated $NaHCO_3$ (2 × 300 mL), and brine (1 × 300 mL), dried over Na_2SO_4, and concentrated**.** The crude yellow oil was purified by chromatography (80:19:1 hexanes:ethyl acetate:triethylamine, then 67:32:1 hexanes:ethyl acetate:triethylamine) to provide 31.2 g (85%) of **15a** as a white solid. ^{1}H NMR (400 MHz): δ 1.84 (m, 4H), 3.07 (m, 1H), 3.30 (m, 3H), 3.48 (m, 1H), 3.58 (m, 1H), 3.79 (s, 3H), 3.90 (d, J = 7.7, 1H), 4.35 (m, 1H), 6.82 (m, 2H), 7.20−7.23 (m, 2H), 7.26−7.38 (m, 6H), 7.43 (m, 4H). ^{13}C NMR (101 MHz): δ 23.85, 25.93, 45.83, 46.31, 55.20, 65.45, 69.45, 86.29, 110.46, 113.08, 126.91, 127.79, 128.40, 130.29, 135.32, 144.08, 144.12, 158.56, 170.58. Anal. Calcd for $C_{27}H_{29}N_1O_4$: C, 75.15; H, 6.77; N, 3.25. Found: C, 75.26; H, 6.88; N, 3.20.

General Solid-Phase Chemistry Methods. All solid-phase reaction mixtures were stirred at the slowest rate. For the general solid-phase workup procedure, excess reagent and the reaction solution were filtered away from support-bound material using polypropylene cartridges with 70-μm PE frits (Spe-ed Accessories) attached to Teflon stopcocks. Cartridges and stopcocks were purchased from Applied Separations (Allentown, PA). The support-bound material was thoroughly washed with various solvents as described below. If the support-bound material was carried directly on to the next step, the last rinse was the solvent of the next reaction (3×). For each solid-phase reaction in the synthesis of intermediate **2**, a general procedure based on 1.0 g of support-bound material is provided. A specific example with appropriate characterization for each general method is also reported.

***p*-(Benzyloxy)benzyl Bromide Resin (10).**[24] To 3.25 g (2.80 mmol, loading 0.86 mequiv/g) of Wang (*p*-(benzyloxy)benzyl alcohol) resin solvated in 27 mL of CH_2Cl_2 cooled to 0 °C was added 2.32 g (6.99 mmol) of carbon tetrabromide followed by a solution of 1.65 g (6.29 mmol) of triphenylphosphine in 5 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 3 h, washed with CH_2Cl_2 (6 × 30 mL), dried under vacuum, and employed in the coupling step within 48 h of preparation.

Support-Bound Weinreb Amide 11a. To a suspension of 0.22 g (9.0 mmol) of NaH in 20 mL of THF at room temperature was added a solution of 3.8 g (9.0 mmol) of amide **9a** in 10 mL of THF. The suspension was stirred for 50 min followed by the addition of 0.33 g (0.90 mmol) of tetrabutylammonium iodide (Bu_4NI), 0.002 g (0.009 mmol) of 18-crown-6, and lastly 3.2 g of resin **10**. The reaction mixture was stirred at 45 °C for 2 h and then was transferred into one or more polypropylene cartridges with 70-μm PE frits (Spe-ed Accessories) attached to Teflon stopcocks for the workup process. The resin was washed with THF (1 × 30 mL), THF:H_2O (2:1) (3 × 30 mL), THF:H_2O (1:1) (3 × 30 mL), THF:H_2O (1:2) (3 × 30 mL), THF:H_2O (2:1) (3 × 30 mL), THF (1 × 30 mL), DMF (3 × 30 mL), CH_2Cl_2 (5 × 30 mL), and MeOH (2 × 30 mL). The resin was dried under vacuum and stored at −20 °C. The exact loading level of the resin was determined by subjecting a portion of the resin (0.271 g) to MMT group deprotection and the cleavage conditions reported below. The crude material obtained from the cleavage step was purified by flash chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 0.021 g (0.141 mmol) of the corresponding Weinreb amide diol (**8a**) as a colorless oil (0.52 mequiv/g, 81% loading efficiency based on a 0.86 mequiv/g loading level of Wang resin).

Support-Bound Pyrrolidine Amide 16a. To a suspension of 0.650 g (27.1 mmol) of NaH in 65 mL of THF at room temperature was added a solution of 11.7 g (27.1 mmol) of amide **15a** in 30 mL of THF. The suspension was stirred for 50 min followed by the addition of 1.00 g (2.71 mmol) of Bu_4NI, 0.007 g (0.03 mmol) of 18-crown-6, and lastly 9.00 g of resin **10**. The reaction mixture was stirred at the slowest rate at 45 °C for 2 h and then was transferred into one or more polypropylene cartridges with 70-μm PE frits (Spe-ed Accessories) attached to Teflon stopcocks for the workup process. The resin was washed with THF (1 × 30 mL), THF:H_2O (2:1) (3 × 30 mL), THF:H_2O (1:1) (3 × 30 mL), THF:H_2O (1:2) (3 × 30 mL), THF:H_2O (2:1) (3 × 30 mL), THF (1 × 30 mL), DMF (3 × 30 mL), CH_2Cl_2 (5 × 30 mL), and MeOH (2 × 30 mL). The resin was dried under vacuum and stored at −20 °C. The exact loading level of the resin was determined by subjecting a portion of the resin (0.460 g) to MMT group deprotection and the cleavage conditions reported below. The crude material obtained from the cleavage step was purified by flash chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 0.039 g (0.245 mmol) of the corresponding pyrrolidine amide diol (**14a**) as a colorless oil (0.53 mequiv/g, 84% loading efficiency based on a 0.86 mequiv/g loading level of Wang resin).

General Procedure for MMT Group Deprotection and Cleaving Material from the Support.[46] MMT group deprotection was accomplished using 1% (w/v) *p*-toluenesulfonic acid (*p*-TsOH) in CH_2Cl_2, which was prepared by dissolving *p*-TsOH in minimal MeOH followed by dilution with CH_2Cl_2. To resin solvated in minimal CH_2Cl_2 was added 1% *p*-TsOH solution to provide a bright orange solution indicating presence of mono-*p*-methoxytrityl cation. The resin was subjected to 1% *p*-TsOH in CH_2Cl_2 (4 × 5 min) until most of the orange solution had dissipated followed by washes with 3% MeOH in CH_2Cl_2 (3×) and CH_2Cl_2 (5×).

Cleavage of material from the support was performed as follows. To resin solvated in minimal CH_2Cl_2 was added trifluoroacetic acid:CH_2Cl_2 (1:1). The reaction mixture was stirred for 30 min followed by filtration of the solution. The resin was washed with CH_2Cl_2 (5×). The filtrate and washings were combined and immediately concentrated. Toluene was added to form an azetrope with trifluoroacetic acid during the concentration step.

Grignard Addition to Tertiary Amide (17). General Procedure with Grignard Reagent Concentrations of >0.2 M. To 1.0 g of support-bound tertiary amide **6** solvated in 10 mL of THF at 0 °C was added P_1MgX (0.20−0.25 M) (for preparation of Grignard reagents, see Library Synthesis). The reaction mixture was stirred for 10 h at 4 °C. The reaction solution was removed and the resin was washed with THF (1 × 15 mL), acetone (3 × 15 mL), 0.28 M hydrocinnamic acid in THF (3 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). **P_1 = Bn.** To support-bound Weinreb amide **11a** (1.0 g, 0.52 mmol) solvated in 10 mL of THF at 0 °C was added 1.2 mL (2.4 mmol) of 2.0 M benzylmagnesium chloride in THF. The reaction mixture was stirred at 4 °C for 8 h. After filtration of the reaction solution, the resin was washed with THF (1 × 15 mL), acetone (3 × 15 mL), 0.28

(45) The (*S*)-methyl ester of isopropylidene glycerate (**7a**), which was obtained from Aldrich, was deprotected with 40% aqueous acetic acid and treated separately with (*R*)-(+)-MTPACl and (*S*)-(−)-MTPACl to form the *S,R,R* and *S,S,S* diastereomers, respectively. ^{19}F NMR analysis indicated that 2.3% of the (*R*)-methyl ester enantiomer was present.

(46) It should be noted that MMT deprotection and spectrophotometric quantitation of the MMT cation provided a false, high loading level presumably because some MMT material was entrapped in the resin.

M hydrocinnamic acid in THF (3 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion of the support-bound material was deprotected (MMT group) and cleaved (see General Procedure for MMT group deprotection and cleaving material from the support). The crude oil was purified by column chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to provide benzyl ketone **12** as a colorless oil. IR: 3345, 1716, 1454, 1405 cm^{-1}. ^1H NMR (400 MHz): δ 3.84 (d, $J = 16.1$, 1H), 3.90 (d, $J = 16.1$, 1H), 3.94 (d, $J = 3.4$, 2H), 4.31 (t, $J = 3.4$, 1H), 7.19−7.35 (m, 5H). ^{13}C NMR (101 MHz): δ 45.12, 63.47, 77.16, 127.29, 128.75, 129.49, 132.77, 207.87. Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.87; H, 6.83.

(2*S*)-2,3-Dihydroxy-*N*-methylpropanamide (13). ^1H NMR (400 MHz): δ 2.89 (d, $J = 5.0$, 3H), 3.84 (d, $J = 6.4$, 11.3, 1H), 3.94 (t, $J = 3.$, 1H), 4.36 (dd, $J = 3.7$, 6.4, 1H), 7.19−7.35 (m, 5H).

General Procedure with Grignard Reagent Concentrations of <0.2 M. To 1.0 g of support-bound tertiary amide **6** solvated in minimal THF at 0 °C was added 5 equiv of P_1MgX (0.05−0.15 M). The reaction mixture was stirred for 20 h at 4 °C. The reaction solution was removed, and the resin was washed with THF (1 × 15 mL), acetone (3 × 15 mL), 0.28 M hydrocinnamic acid in THF (3 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL).

Zinc Borohydride ($Zn(BH_4)_2$) Preparation.[47] To a suspension of 2.32 g (61.3 mmol) of $NaBH_4$ in 33 mL of Et_2O at room temperature was added 44 mL of a $ZnCl_2$ solution (0.69 M) in Et_2O. The $ZnCl_2$ solution was prepared from recrystallized $ZnCl_2$ which was fused multiple times and heated in Et_2O at reflux for 45 min. The suspension was stirred for 48 h and could be stored at −20 °C for up to 3 weeks.

General Procedure for $Zn(BH_4)_2$ Reduction (18). To 1.0 g of **17a** in 2.5 mL of THF at −20 °C was added 7.5 mL (3.0 mmol) of 0.40 M $Zn(BH_4)_2$ in Et_2O. The reaction mixture was stirred at −20 °C for 20 h and then was allowed to warm to 0 °C over 1.5 h. Excess reagent was filtered away, and the resin was washed with THF (1 × 15 mL), ethanolamine:H_2O:THF (10:2:88 by volume) (3 × 15 mL, 15 min per wash), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion of the support-bound material was deprotected (MMT group) and cleaved to determine product purity and reaction completion. Due to poor resin swelling in Et_2O and the ability for resin to solidify or crystallize at low temperatures, the resin was resubjected to the $Zn(BH_4)_2$ reduction conditions if the first subjection did not provide complete reduction as indicated by the TLC of crude product from cleavage. Crude product can be purified by chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to provide the corresponding triol as a white solid. **P_1 = Bn.** IR: 3355, 2930, 1457 cm^{-1}. ^1H NMR (300 MHz): δ 1.96 (broad s, 3H), 2.75 (dd, $J = 9.3$, 13.8, 1H), 2.95 (dd, $J = 3.9$, 13.8, 1H), 3.66 (ddd, $J = 3.7$, 5.4, 9.1, 1H), 3.82 (dd, $J = 3.7$, 11.3, 1H), 3.88 (dd, $J = 5.4$, 11.3, 1H), 3.97 (ddd, $J = 3.9$, 9.1, 9.3, 1H), 7.22−7.35 (m, 5H). ^{13}C NMR (101 MHz): δ 39.52, 63.23, 73.39, 74.37, 126.68, 128.68, 129.32, 137.78. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 66.11; H, 7.88.

Preparation of Triacetate. Crude triol product is directly employed to form the corresponding triacetate. To a solution of crude triol product (5.0 mg) was added 1 mL of acetic anhydride and DMAP (15 mg, 0.122 mmol). The reaction solution was stirred for 4 h followed by concentration and purification by chromatography using a short plug of silica gel (75:25 hexanes:ethyl acetate). **P_1 = Bn.** ^1H NMR (400 MHz): δ 1.96 (s, 3H), 2.06 (s, 6H), 2.88 (dd, $J = 8.4$, 14.2, 1H), 2.95 (dd, $J = 5.2$, 14.2, 1H), 4.18 (dd, $J = 6.8$, 12.2, 1H), 4.34 (dd, $J = 3.2$, 12.2, 1H), 5.18 (ddd, $J = 3.2$, 4.9, 6.8, 1H), 5.32−5.37 (m, 1H), 7.18−7.31 (m, 5H). GC conditions: 180−220 °C; Ramp: 1 °C/min. Pressure: 15 psi. Retention times (major:minor): 13.2:13.7 min. Integration (major:minor): 90:10.

Determination of Enantiomeric Purity. To determine the enantiomeric purity of the compounds after zinc borohydride reduction, chiral HPLC analysis was performed. The diastereomers of the triacetate derivatives reported above were separated by preparative HPLC. Chiral HPLC of the major diastereomer revealed a 95:5 enantiomer ratio. As previously mentioned, the starting material, (*S*)-methyl ester of isopropylidene glycerate (**7a**), which is obtained from Aldrich, originally contained 2.3% of the enantiomer. Therefore, during our synthesis sequence, 5−6% racemization occurs. HPLC conditions: Chiralcel OD column (Diacel Chemical Industries). Solvent: 99:1 hexanes:2-propanol. Flow Rate: 1.0 mL/min. Retention times (major:minor) 14.2 min:17.2 min. Integration: 95:5 (major:minor).

General Procedure for Secondary Alcohol Activation and Azide Displacement (19). To 1.0 g of support-bound secondary alcohol **18** solvated in 10 mL of $CHCl_3$ was added 1.11 g (7.50 mmol) of 4-pyrrolidinopyridine followed by the addition of 1.11 g (5.00 mmol) of 4-nitrobenzenesulfonyl chloride (NosCl) at room temperature. The reaction mixture was stirred for 9 h. After filtration of excess reagent, the resin was washed with CH_2Cl_2 (5 × 15 mL) and DMF (4 × 15 mL).

To 1.0 g of support-bound secondary nosyl alcohol solvated in 10 mL of DMF was added 0.650 g (10.0 mmol) of sodium azide. The reaction mixture was stirred at 45 °C for 24 h. Following filtration of the reaction solution, the resin was washed with DMF (2 × 15 mL), DMF:H_2O (1:1) (3 × 15 mL), DMF (2 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion of the resin was deprotected (MMT group) and cleaved to determine reaction completion. The crude oil can be purified by column chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to provide the corresponding azide as a colorless oil. **P_1 = Bn.** IR: 3381, 2930, 2109, 1456 cm^{-1}. ^1H NMR (300 MHz): δ 2.95 (dd, $J = 7.9$, 13.6, 1H), 3.07 (dd, $J = 6.2$, 13.6, 1H), 3.57−3.80 (m, 4H), 7.26−7.35 (m, 5H).

General Procedure for Primary Alcohol Activation (2a) and Cleavage of Support-Bound Material To Afford 3a. To 1.0 g of support-bound secondary azide **19** solvated in minimal CH_2Cl_2 was added 10 mL of 1% *p*-TsOH in CH_2Cl_2 (see General Procedure for MMT group deprotection) to provide a bright orange solution indicating the presence of mono-*p*-methoxytrityl cation. The orange solution was filtered away from support-bound material. This step was repeated three times until most of the orange solution had dissipated followed by washes with 3% MeOH in CH_2Cl_2 (3×) and CH_2Cl_2 (5×). To the deprotected support-bound material in 10 mL of $CHCl_3$ was added 0.49 mL (6.0 mmol) of pyridine followed by the addition of 0.89 g (4.0 mmol) NosCl at room temperature. The reaction mixture was stirred for 9 h. After filtration of excess reagents, the resin was washed with CH_2Cl_2 (2 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion (0.360 g) of support-bound nosyl alcohol **2a** was cleaved, and the crude oil was purified by column chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 0.046 g of **3a** (**P_1 = Bn**; 70% yield based on a 0.51 mequiv/g initial loading level of **16a**) as a colorless oil. ^1H NMR (400 MHz): δ 2.25 (d, $J = 6.7$, 1H), 3.03 (m, 2H), 3.56 (dt, $J = 2.7$, 7.5, 1H), 3.79 (m, 1H), 4.13 (dd, $J = 4.7$, 10.3, 1H), 4.21 (dd, $J = 7.0$, 10.3, 1H), 7.21−7.35 (m, 5H), 8.09 (d, $J = 9.0$, 2H), 8.39 (d, $J = 9.0$, 2H).[48]

(2*R*)-3-(1-Ethoxy-1-ethyl)-2-hydroxy-*N*,*N*-tetramethylenepropanamide (20). To a solution of 15.0 g (0.940 mol) of (2*R*)-2,3-dihydroxy-*N*,*N*-tetramethylenepropanamide (**14b**) in 450 mL of CH_2Cl_2 at 0 °C was added 8.51 mL (0.890 mol) of ethyl vinyl ether followed by 3.0 g (0.012 mol) of pyridinium *p*-toluenesulfonate. The reaction solution was stirred at room temperature for 6 h, washed with H_2O (3 × 500 mL), saturated $NaHCO_3$ (3 × 500 mL), and brine (2 × 500 mL), dried over Na_2SO_4, and concentrated. The crude yellow oil was purified by chromatography (80:19:1 hexanes:ethyl acetate:triethylamine, then 67:32:1 hexanes:ethyl acetate:triethylamine) to provide 14.4 g (66%) of **20** as a colorless oil. NMR data are reported for a 1:1 mixture of diastereomers. ^1H NMR (500 MHz): δ 1.06 (t, $J = 7.1$, 3H), 1.07 (t, $J = 7.1$, 3H), 1.17 (d, $J = 9.8$, 3H), 1.18 (d, $J = 9.8$, 3H), 1.72−1.89 (m, 8H) 3.32−3.62 (m, 16 H), 3.72 (d, $J = 7.4$, 1H), 3.73 (d, $J = 7.4$, 1H), 4.27 (m, 2H), 4.62 (q, $J = 5.3$, 1H), 4.63 (q, $J = 5.3$, 1H). ^{13}C NMR (101 MHz): δ 15.01, 19.38, 19.41, 23.66, 23.67, 25.78, 45.91, 45.93, 46.06, 46.08, 60.88, 60.93, 66.54, 66.66, 68.93, 69.19, 99.57, 99.59, 170.33, 170.40. Anal. Calcd for $C_{11}H_{21}N_1O_4$: C, 57.12; H, 9.15; N, 6.06. Found: C, 56.94; H, 8.98; N, 6.05.

Support-Bound Pyrrolidine Amide. Preparation of the support-bound pyrrolidine amide was accomplished using the conditions

(47) Nakata, T.; Tani, Y.; Hatozaki, M.; Oishi, T. *Chem. Pharm. Bull.* **1984**, *32*, 1411−1415.

(48) Liu, G. Ph.D. Dissertation, University of California at Berkeley, Berkeley, CA, 1998.

described to prepare support-bound Weinreb amide **11** and pyrrolidine amide **16**. The exact loading level of the resin was determined by cleaving a portion of support-bound material (0.330 g). Prior to cleavage of the material from support, the ethoxyethyl group was deprotected with 2% *p*-TsOH in CH_2Cl_2 (5 × 5 min) and washed with 3% MeOH in CH_2Cl_2 (3×) and CH_2Cl_2 (5×). The crude material obtained from the TFA cleavage was purified by flash chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 0.030 g of **14b** as a colorless oil (0.57 mequiv/g, 78% loading efficiency based on a 0.86 mequiv/g loading level of Wang resin).

Grignard Addition to Pyrrolidine Amide (21). To the support-bound pyrrolidine amide (1.0 g, 0.57 mmol) solvated in 10 mL of THF at 0 °C was added 1.2 mL (2.4 mmol) of 2.0 M benzylmagnesium chloride in THF. The reaction mixture was stirred at 4 °C for 8 h. After filtration of the reaction solution, the resin was washed with THF (1 × 15 mL), acetone (3 × 15 mL), 0.28 M hydrocinnamic acid in THF (3 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL).

L-Selectride Reduction (22). To 1.0 g of support-bound ketone **21** in 5.0 mL of THF at −75 °C was added 5.0 mL (5.0 mmol) of 1.0 M L-selectride in THF. The reaction mixture was stirred at −75 to −65 °C for 20 h and then was allowed to warm to −20 °C over 3 h. Excess reagent was filtered away, and the resin was washed with THF (1 × 15 mL), 0.28 M hydrocinnamic acid in THF (3 × 15 mL, 15 min per wash), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion of the resin was deprotected (EE group) and cleaved to determine product purity and reaction completion. Due to the tendency for the resin to solidify or crystallize at low temperatures, the resin was resubjected to the L-selectride reduction conditions if the first subjection did not provide complete reduction as indicated by the TLC of crude product from cleavage. Crude product can be purified by (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to provide the corresponding triol as a white solid. **P_1 = Bn.** IR: 3355, 2930, 1457 cm^{-1}. ^1H NMR (300 MHz): δ 2.06 (broad s, 3H), 2.84 (dd, J = 8.2, 13.6, 1H), 2.93 (dd, J = 5.5, 13.6, 1H), 3.59 (m, 1H), 3.74 (dd, J = 4.4, 11.1, 1H), 3.79 (dd, J = 3.7, 11.1, 1H), 3.86 (m, 1H), 7.22−7.34 (m, 5H). ^{13}C NMR (101 MHz): δ 40.05, 64.90, 72.51, 73.61, 126.58, 128.61, 129.40, 137.94.

Corresponding Triacetate. The triacetate was prepared using the aforementioned method. **P_1=Bn.** ^1H NMR (400 MHz): δ 1.99 (s, 3H), 2.02 (s, 3H), 2.13 (s, 3H), 2.86 (d, J = 7.0, 2H), 4.05 (dd, J = 6.8, 11.8, 1H), 4.26 (dd, J = 4.6, 11.8, 1H), 5.19 (ddd, J = 3.7, 4.6, 6.8, 1H), 5.32 (dt, J = 3.7, 7.0, 1H), 7.16−7.30 (m, 5H). GC conditions: 180−220 °C. Ramp: 1 °C/min. Pressure: 15 psi. Retention times (major:minor): 13.1:13.7 min. Integration (major:minor): 13:87.

Procedure for Secondary Alcohol Activation and Azide Displacement (23). To 1.0 g of support-bound secondary alcohol **22** solvated in 10 mL of $CHCl_3$ was added 1.11 g (7.50 mmol) of 4-pyrrolidinopyridine followed by the addition of 1.11 g (5.00 mmol) of 4-nitrobenzenesulfonyl chloride (NosCl) at room temperature. The reaction mixture was stirred for 9 h. After filtration of excess reagent, the resin was washed with CH_2Cl_2 (5 × 15 mL) and DMF (4 × 15 mL).

To 1.0 g of support-bound secondary nosyl alcohol solvated in 10 mL of DMF was added 0.650 g (10.0 mmol) of sodium azide. The reaction mixture was stirred at 45 °C for 24 h. Following filtration of the reaction solution, the resin was washed with DMF (2 × 15 mL), DMF:H_2O (1:1) (3 × 15 mL), DMF (2 × 15 mL), and CH_2Cl_2 (5 × 15 mL).

Procedure for Primary Alcohol Activation (2b) and Cleavage of Support-Bound Material To Afford 3b. To 1.0 g of support-bound secondary azide **23** solvated in minimal CH_2Cl_2 was added 10 mL of 2% *p*-TsOH in CH_2Cl_2 (5 × 5 min) followed by washes with 3% MeOH in CH_2Cl_2 (3×) and CH_2Cl_2 (5×). To the deprotected support-bound material in 10 mL of $CHCl_3$ was added 0.49 mL (6.0 mmol) of pyridine followed by the addition of 0.89 g (4.0 mmol) NosCl at room temperature. The reaction mixture was stirred for 9 h. After filtration of excess reagents, the resin was washed with CH_2Cl_2 (2 × 15 mL), DMF (3 × 15 mL), and CH_2Cl_2 (5 × 15 mL). A portion (0.470 g) of support-bound secondary azide **23** was cleaved, and the crude oil was purified by column chromatography (99:1 CH_2Cl_2:methanol, then 97:3 CH_2Cl_2:methanol) to afford 0.057 g of **3b** (**P_1 = Bn**; 68% yield based on a 0.57 mequiv/g initial loading level of pyrrolidine amide **20** to support) as a colorless oil. ^1H NMR (400 MHz): δ 2.32 (broad s, 1H), 2.80 (dd, J = 8.6, 14.1, 1H), 3.06 (dd, J = 4.3, 14.1, 1H), 3.69 (m, 1H), 3.78 (apparent dt, J = 2.8, 6.5, 1H), 4.18 (dd, J = 6.5, 10.5, 1H), 4.31 (dd, J = 2.8, 10.5, 1H), 7.21−7.33 (m, 5H), 8.11 (d, J = 8.9, 2H), 8.40 (d, J = 8.9, 2H). ^{13}C NMR (101 MHz): δ 36.86, 64.45, 70.76, 72.13, 124.59, 127.19, 128.77, 129.34, 130.44, 136.28, 141.08, 150.90.[49]

Library Synthesis. Grignard Reagent Preparation. The Grignard reagents that were not commercially available were prepared by one of two methods. A sample procedure is provided for each method. To test the efficacy of the preparation, each Grignard reagent was added to (2*S*)-2,3-*O*-isopropylidene-*N*-methoxy-*N*-methylpropanamide and the reaction completion to the corresponding ketone was monitored by TLC. The commercially available reagents included benzylmagnesium chloride, isobutylmagnesium chloride, phenethylmagnesium chloride, methylmagnesium bromide, and *n*-pentylmagnesium bromide.

Preparation from Magnesium Turnings. Magnesium turnings were stirred under N_2 for 48−72 h. To a vigorously stirring suspension of 1.82 g (0.075 mol) activated magnesium turnings in 20 mL of Et_2O was very slowly added dropwise 6.98 mL (0.0500 mol) of cyclohexylmethyl bromide. Cyclohexylmethyl bromide was passed through a short plug of basic alumina prior to use. After the addition of the first 2−3 drops of cyclohexylmethyl bromide, initiation of Grignard reagent formation was observed by exotherm and dark gray color of the reaction solution. The addition was complete after 2 h, and stirring was continued at room temperature for 1 h. The Grignard reagent was filtered away from remaining magnesium into Schlenk apparatus and stored at −20 °C.

Preparation of Magnesium Anthracene THF Complex and Grignard Reagent.[39,40] To a suspension of 6.0 g (0.25 mol) of magnesium turnings and 24.5 g (0.137 mol) of anthracene in 250 mL of THF was added 0.41 mL of ethyl bromide. The reaction mixture was stirred for 28 h at 60 °C. The white-yellow solution changed to green and then orange over the course of the reaction. In an inert atmosphere, the excess reagents were filtered away from desired orange crystals and excess magnesium. The product was washed with THF (2 × 70 mL) and dried under vacuum.

To a suspension of 3.06 g (7.31 mmol) of magnesium anthracene THF complex in 55 mL of THF was very slowly added a solution of 1.24 g (6.09 mmol) of *p*-phenylbenzyl chloride in 5 mL of THF. The solution changed from orange to dark green to gray over the addition period. The reagent was tested and subsequently used in solid-phase reactions.

Synthesis of Intermediates 2a. The library of 204 compounds was synthesized according to the conditions reported above through intermediate **2a**. The support-bound Weinreb amide **11a** that was used for the library synthesis had an initial loading level of 0.28 mmol/g. The resin was split into 17 batches of 0.7 g prior to the Grignard addition. The synthesis was carried out in 50 mL round-bottom flasks through intermediate **2a**. The preparation of the Grignard reagents is listed above. For each of the 17 ketones resulting from the Grignard addition to Weinreb amide **11a**, 45−50 mg of resin was cleaved. The corresponding ketones were obtained in high purity by ^1H NMR, except for 7−18% of methyl amide side product **13**. Approximately 20 mg of resin was cleaved after zinc borohydride reduction and after conversion to the azide **19** to determine reaction completion by TLC. In several cases the support-bound material had to be resubjected to the reaction with NosCl, 4-pyrrolidinopyridine in $CHCl_3$, followed by the azide displacement to obtain azide **19**, due to incomplete reaction. For each resin, 100−125 mg was also cleaved at intermediate **2a**, and in every case, the major product was determined by TLC and ^1H NMR to be the desired compound **3a** (NMR data listed below).

NMR Data for Intermediates 3a. P_1 = benzyl. ^1H NMR (400 MHz): δ 2.25 (d, J = 6.7, 1H), 3.03 (m, 2H), 3.56 (apparent dt, J = 2.7, 7.5, 1H), 3.79 (m, 1H), 4.13 (dd, J = 4.7, 10.3, 1H), 4.21 (dd, J = 7.0, 10.3, 1H), 7.21−7.35 (m, 5H), 8.09 (d, J = 9.0, 2H), 8.39 (d, J = 9.0, 2H).

(49) Kick, E. K. Ph.D. Dissertation, University of California at Berkeley, Berkeley, CA, 1998.

P_1 = Isobutyl. ^{1}H NMR (400 MHz): δ 0.95 (d, J = 3.2, 3H), 0.96 (d, J = 3.2, 3H), 1.40 (m, 1H), 1.66 (m, 1H), 1.77 (m, 1H), 3.37 (m, 1H), 3.87 (m, 1H), 4.16−4.22 (m, 2H), 8.13 (d, J = 8.6, 2H), 8.42 (d, J = 8.6, 2H).

P_1 = Phenethyl. ^{1}H NMR (300 MHz): δ 1.89−2.05 (m, 2H), 2.25 (broad s, 1H), 2.64−2.86 (m, 2H), 3.26 (m, 1H), 3.89−3.93 (m, 1H), 4.15 (m, 2H), 7.16−7.33 (m, 5H), 8.08 (d, J = 9.0, 2H), 8.39 (d, J = 9.0, 2H).

P_1 = Methyl. ^{1}H NMR (400 MHz): δ 1.36 (d, J = 6.7, 3H), 3.60 (m, 1H), 3.77 (m, 1H), 4.17 (d, J = 5.4, 2H), 8.13 (d, J = 8.9, 2H), 8.42 (d, J = 8.9, 2H).

P_1 = *n*-Pentyl. ^{1}H NMR (400 MHz): δ 0.90 (t, J = 6.8, 3H), 1.31−1.45 (m, 4H), 1.63−1.71 (m, 4H), 3.33 (m, 1H), 3.89 (m, 1H), 4.17 (m, 2H), 8.14 (d, J = 8.9, 2H), 8.43 (d, J = 8.9, 2H)

P_1 = Cyclohexylmethyl. ^{1}H NMR (300 MHz): δ 0.86−0.96 (m, 2H), 1.11−1.27 (m, 3H), 1.38−1.47 (m, 2H), 1.56−1.76 (m, 6H), 2.2 (broad s, 1H), 3.38 (m, 1H), 3.86 (m, 1H), 4.12−4.20 (m, 2H), 8.12 (d, J = 9.0, 2H), 8.41 (d, J = 9.0, 2H).

P_1 = β-Naphthylmethyl. ^{1}H NMR (300 MHz): δ 2.2 (broad s, 1H), 3.19 (m, 2H), 3.66 (apparent dt, J = 2.7, 7.5, 1H), 3.80 (m, 1H), 4.11 (dd, J = 4.9, 10.4, 1H), 4.20 (dd, J = 7.0, 10.4, 1H), 7.32 (dd, J = 1.8, 8.3, 1H), 7.48 (m, 2H), 7.68 (apparent s, 1H), 7.77−7.84 (m, 3H), 8.03 (d, J = 9.0, 2H), 8.32 (d, J = 9.0, 2H).

P_1 = α-Naphthylmethyl. ^{1}H NMR (400 MHz): δ 3.46 (dd, J = 7.0, 13.7, 1H), 3.57 (dd, J = 7.8, 13.7, 1H), 3.72 (m, 1H), 3.77 (m, 1H), 4.10 (dd, J = 4.6, 10.4, 1H), 4.25 (dd, J = 7.0, 10.4, 1H), 7.42 (m, 2H), 7.54 (m, 3H), 7.80 (m, 1H), 7.90 (m, 1H), 8.01 (d, J = 8.8, 2H), 8.32 (d, J = 8.8, 2H).

P_1 = 4-Methoxy-3-methylbenzyl. ^{1}H NMR (400 MHz): δ 2.28 (s, 3H), 2.92−2.97 (m, 2H), 3.51 (m, 1H), 3.80 (m, 1H), 3.82 (s, 3H), 4.11 (dd, J = 4.6, 10.3, 1H), 4.19 (dd, J = 7.0, 10.3, 1H), 6.76 (dd, J = 2.1, 8.1, 1H), 6.99 (m, 2H), 8.09 (d, J = 9.0, 2H), 8.40 (d, J = 9.0, 2H).

P_1 = *p*-Methoxyphenethyl. ^{1}H NMR (300 MHz): δ 1.95 (m, 2H), 2.61−2.80 (m, 2H), 3.26 (m, 1H), 3.78 (s, 3H), 3.89 (m, 1H), 4.16 (m, 2H), 6.36 (d, J = 8.6, 2H), 7.09 (d, J = 8.6, 2H), 8.08 (d, J = 9.0, 2H), 8.41 (d, J = 9.0, 2H).

P_1 = *m*-Phenoxybenzyl. ^{1}H NMR (300 MHz): δ 2.99 (m, 2H), 3.54 (m, 1H), 3.81 (m, 1H), 4.12 (dd, J = 4.6, 10.4, 1H), 4.20 (dd, J = 6.7, 10.4, 1H), 6.89−7.01 (m, 5H), 7.11 (m, 1H), 7.28−7.36 (m, 3H), 8.09 (d, J = 8.6, 2H), 8.40 (d, J = 8.6, 2H).

P_1 = 3,5-Dimethylbenzyl. ^{1}H NMR (400 MHz): δ 2.30 (s, 6H), 2.95 (dd, J = 7.4, 13.5, 1H), 2.96 (dd, J = 7.6, 13.5, 1H), 3.54 (ddd, J = 2.6, 7.4, 7.6, 1H), 3.80 (m, 1H), 4.11 (dd, J = 4.7, 10.3, 1H), 4.20 (dd, J = 7.0, 10.3, 1H), 6.82 (s, 2H), 6.90 (s, 1H), 8.09 (d, J = 8.8, 2H), 8.40 (d, J = 8.8, 2H).

P_1 = 3-Benzylpropyl. ^{1}H NMR (400 MHz): δ 1.65 (m, 4H), 2.65 (m, 2H), 3.33 (m, 1H), 3.86 (m, 1H), 4.15 (m, 2H), 7.16−7.31 (m, 5H), 8.11 (d, J = 9.0, 2H), 8.41 (d, J = 9.0, 2H).

P_1 = 4-*tert*-Butylbenzyl. ^{1}H NMR (400 MHz): δ 1.31 (s, 9H), 2.20 (broad s, 1H), 3.00 (m, 2H), 3.53 (apparent dt, J = 2.7, 7.5, 1H), 3.84 (m, 1H), 4.12 (dd, J = 4.5, 10.3, 1H), 4.20 (dd, J = 7.1, 10.3, 1H), 7.14 (d, J = 8.2, 2H), 7.35 (d, J = 8.2, 2H), 8.10 (d, J = 8.9, 2H), 8.41 (d, J = 8.9, 2H).

P_1 = *p*-Phenylbenzyl. ^{1}H NMR (400 MHz): δ 2.25 (broad s, 1H), 3.08 (m, 2H), 3.60 (apparent dt, J = 4.7, 10.2, 1H), 3.87 (m, 1H), 4.14 (dd, J = 4.7, 10.2, 1H), 4.23 (dd, J = 7.0, 10.2, 1H), 7.30 (d, J = 8.0, 2H), 7.36 (m, 1H), 7.45 (m, 2H), 7.54−7.59 (m, 4H), 8.10 (d, J = 8.6, 2H), 8.39 (d, J = 8.6, 2H).

P_1 = 3,4-Methylenedioxybenzyl. ^{1}H NMR (400 MHz): δ 2.19 (broad s, 1H), 2.94 (m, 2H), 3.49 (apparent dt, J = 2.8, 10.2, 1H), 3.81 (m, 1H), 4.11 (dd, J = 4.7, 10.3, 1H), 4.20 (dd, J = 7.0, 10.3, 1H), 5.96 (s, 2H), 6.67 (d, J = 7.6, 1H), 6.69 (s, 1H), 6.76 (d, J = 7.6, 1H), 8.11 (d, J = 8.9, 2H), 8.41 (d, J = 8.9, 2H).

P_1 = *p*-Bromobenzyl. ^{1}H NMR (400 MHz): δ 2.26 (broad s, 1H), 2.93−3.06 (m, 2H), 3.53 (apparent dt, J = 2.8, 7.4, 1H), 3.81 (m, 1H), 4.12 (dd, J = 4.8, 10.3, 1H), 4.21 (dd, J = 6.9, 10.3, 1H), 7.11 (d, J = 8.3, 2H), 7.46 (d, J = 8.3, 2H), 8.10 (d, J = 8.8, 2H), 8.41 (d, J = 8.8, 2H).

Introduction of R_1, R_2, and R_3. Each of the 17 support-bound scaffolds **2a** was split into two 0.2-g batches for amine displacement with 3,4-methylenedioxyphenethylamine and 2,4-dichlorophenethylamine. 3,4-Methylenedioxyphenethylamine was prepared from the commercially available hydrochloride salt by basic extraction with aqueous Na_2CO_3 and ethyl acetate. The reactions were performed with 1.0 M amine in NMP at 80 °C for 36 h in sealed vials. The resins were washed with NMP (3×), THF (2×), CH_2Cl_2 (3×), and ether (1×) and dried under vacuum overnight. The resins were then transferred to a 96-well filter apparatus.[41] Each well had approximately 15 mg of resin, with each P_1R_1 combination placed in six wells for coupling to the R_2 and R_3 side chains. Prior to each R_2 or R_3 coupling the resin was rinsed twice with anhydrous NMP. Incorporation of *N*-phthaloyl-β-alanine was carried out using a stock solution of 0.3 M acid, 0.3 M HATU, and 0.9 M *i*-Pr_2EtN in NMP overnight. Precipitation sometimes occurs with reactions of *N*-phthaloyl-β-alanine acid and PyBOP, so HATU was employed for this coupling. The acylation with (cyclohexyl)isocyanate was carried out with 0.3 M isocyanate in NMP. The resins were washed with NMP (4×), CH_2Cl_2 (3×), and THF (1×). The azide reduction was accomplished using 0.2 M $SnCl_2$, 0.8 M PhSH, and 1.0 M Et_3N in THF for 4 h. The resins were washed with THF:H_2O (1:1), THF (3×), and CH_2Cl_2 (3×). The R_3 acids, 3-(2-benzoxazolin-2-on-3-yl)propionic acid, 2-bromo-4,5-dimethoxybenzoic acid, and 2,4-dichlorophenoxyacetic acid, were coupled using stock solutions of 0.3 M carboxylic acid, 0.3 M PyBOP, 0.3 M HOAt, and 0.9 M *i*Pr_2EtN in NMP overnight. The resins were washed with NMP (4×), THF (2×), and CH_2Cl_2 (3×). The resin was resubjected to the coupling conditions for both R_2 and R_3 acylations to ensure completion of the reaction. The compounds were cleaved from solid support into 2-mL well plates using TFA:CH_2Cl_2 (1:1) for 30 min followed by rinses with CH_2Cl_2 (3×). The combined filtrates were concentrated to dryness using a Jouan 10.10 centrifugation concentrator. Toluene was added to form an azeotrope with TFA during the concentration step. The resulting products were stored at −20 °C. Twenty-five compounds from the library were picked at random and were analyzed by mass spectrometry in a matrix of α-cyano-4-hydroxycinnamic acid on a Perseptive Biosystems MALDI spectrometer. The expected molecular ion peaks were obtained for 25 of the 25 compounds tested.

High-Throughput Cathepsin D Assay. A fluorometric high-throughput assay for activity toward human liver cathepsin D (Calbiochem: San Diego, CA) was performed in 96-well microtiter plates.[42] The peptide substrate (Ac-Glu-Glu(Edans)-Lys-Pro-Ile-Cys-Phe-Phe-Arg-Leu-Gly-Lys(Dabcyl)-Glu-NH_2) used in the assay has been previously reported ($K_m \approx 4$ μM).[37] The assay was performed in DYNATECH Microfluor fluorescence microtiter plates, and readings were taken on a Perkin-Elmer LS-50B with an attached Perkin-Elmer 96-well plate reader. The excitation wavelength was 340 nm. A 340-nm interference filter (Hoya, U-340) for excitation and a 430-nm cutoff filter for emission were used. For the microwell-based assays the substrate concentration was 5 μM and the cathepsin D concentration was 1 nM in a 0.1 M formic acid buffer (pH = 3.7). DMSO (10%) was used to ensure complete dissolution of the inhibitors. The fluorescent unit readings were taken at three time points (5, 10, and 15 min) within the linear region of the substrate cleavage, and the percent activity of the enzyme was determined by comparing the change of fluorescent units (FU) for each well to the average change in FU for six control wells without inhibitor. The library was screened at approximately 330 nM of inhibitor. The concentration was based on the assumption that 50% of the theoretical yield was obtained for each inhibitor. The library was subsequently screened at 100, 33, and 10 nM for active inhibitors.

Synthesis of Potent Inhibitors. Several of the most potent compounds were synthesized on the solid support following the previously described method using the pyrrolidine amide **16a** with a 0.51 mequiv/g initial loading level. Overall yields of the compounds were determined by the mass balance of desired product after column chromatography purification (100:0 CH_2Cl_2:methanol gradually increasing polarity until 97:3 CH_2Cl_2:methanol). The ^{1}H NMR is only listed for the major amide rotamer for each compound.

Inhibitor 25. From 0.338 g of resin was isolated 57 mg of pure inhibitor **25** (46%) after chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (m, 2H), 2.88 (apparent t, J = 7.7, 2H), 3.01 (apparent t, J = 6.9, 2H), 3.24 (m, 1H), 3.47 (m, 2H), 3.83−3.96 (m, 4H), 3.85 (s, 3H), 3.89 (s, 3H), 4.34 (apparent q, J = 8.3, 1H), 4.66 (broad s, 1H),

6.71 (d, $J = 9.2$, 1H), 6.84 (dd, $J = 1.7$, 8.0, 1H), 6.93−7.00 (m, 5H), 7.05 (m, 1H), 7.05 (s, 1H), 7.07 (s, 1H), 7.16 (dd, $J = 2.1$, 8.1, 1H), 7.23−7.30 (m, 3H), 7.34 (d, $J = 2.1$, 1H), 7.71 (m, 2H), 7.83 (m, 2H). Anal. Calcd for $C_{44}H_{40}N_3O_8Cl_2Br_1$: C, 59.41; H, 4.53; N, 4.72. Found: C, 59.22; H, 4.76; N, 4.52.

Inhibitor 26. ^{1}H NMR (400 MHz): δ 2.00 (broad s, 1H), 2.69 (m, 2H), 2.94 (m, 4H), 3.12 (m, 1H), 3.55 (m, 4H), 3.81 (s, 3H), 3.87 (s, 3H), 3.96 (apparent t, $J = 7.2$, 2H), 4.36 (m, 1H), 6.39 (d, $J = 8.3$, 1H), 6.85 (m, 1H), 6.90−6.95 (m, 5H), 7.03−7.09 (m, 2H), 7.11 (s, 1H), 7.14 (dd, $J = 2.0$, 8.1, 1H), 7.22−7.29 (m, 3H), 7.32 (d, $J = 2.0$, 1H), 7.70 (m, 2H), 7.83 (m, 2H). Anal. Calcd for $C_{44}H_{40}N_3O_8Cl_2Br_1$: C, 59.41; H, 4.53; N, 4.72. Found: C, 59.60; H, 4.70; N, 4.59.

Inhibitor 27. From 0.290 g of resin was isolated 48 mg of pure inhibitor **27** (48%) after chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.62 (apparent t, $J = 7.5$, 2H), 2.82 (apparent t, $J = 7.6$, 2H), 3.18−3.25 (m, 3H), 3.40−3.47 (m, 2H), 3.57 (s, 3H), 3.85 (s, 3H), 3.91−3.96 (m, 4H), 4.47 (apparent q, $J = 8.4$, 1H), 4.76 (broad s, 1H), 6.69 (s, 1H), 6.92 (d, $J = 8.2$, 1H), 6.95 (s, 1H), 7.04 (dd, $J = 2.1$, 8.2, 1H), 7.29 (d, $J = 2.1$, 1H), 7.40−7.45 (m, 3H), 7.68 (m, 2H), 7.71−7.80 (m, 6H). Anal. Calcd for $C_{42}H_{38}N_3O_7Cl_2Br_1$: C, 59.52; H, 4.52; N, 4.96. Found: C, 59.63; H, 4.67; N, 4.69.

Inhibitor 28. From 0.314 g of resin was isolated 55 mg of pure inhibitor **28** (48%) after chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (m, 2H), 2.85 (apparent t, $J = 7.3$, 2H), 3.08 (apparent t, $J = 6.7$, 2H), 3.23 (m, 1H), 3.44 (m, 1H), 3.57 (m, 1H), 3.75 (s, 3H), 3.86 (s, 3H), 3.94 (m, 4H), 4.39 (apparent q, $J = 8.3$, 1H), 4.73 (broad s, 1H), 6.78 (d, $J = 9.2$, 1H), 6.93 (s, 1H), 6.97 (s, 1H), 7.02 (d, $J = 8.2$, 1H), 7.10 (dd, $J = 2.1$, 8.2, 1H), 7.30 (d, $J = 2.1$, 1H), 7.36−7.42 (m, 5H), 7.51−7.54 (m, 4H), 7.68 (m, 2H), 7.81 (m, 2H). Anal. Calcd for $C_{44}H_{40}N_3O_7Cl_2Br_1$: C, 60.49; H, 4.62; N, 4.81. Found: C, 60.23; H, 4.86; N, 4.58.

Inhibitor 29. From 0.325 g of resin was isolated 55 mg of pure inhibitor **29** (46%) after chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64 (m, 2H), 2.86 (apparent t, $J = 7.1$, 2H), 2.96 (m, 2H), 3.20 (m, 1H), 3.46 (m, 1H), 3.54 (m, 1H), 3.78 (m, 2H), 3.82 (s, 3H), 3.86 (s, 3H), 3.91 (m, 2H), 4.31 (apparent q, $J = 8.5$, 1H), 4.73 (broad s, 1H), 6.73 (d, $J = 9.3$, 1H), 6.85 (s, 1H), 6.96 (s, 1H), 7.03 (d, $J = 8.3$, 1H), 7.14 (m, 2H), 7.16 (dd, $J = 2.2$, 8.3, 1H), 7.32 (d, $J = 2.2$, 1H), 7.37−7.41 (m, 2H), 7.70 (m, 2H), 7.80 (m, 2H). Anal. Calc′d for $C_{38}H_{35}N_3O_7Cl_2Br_2$: C, 52.08; H, 4.03; N, 4.79. Found: C, 52.28; H, 4.09; N, 4.60.

Inhibitor 30. From 0.307 g of resin was isolated 49 mg of pure inhibitor **30** (45%) after chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (apparent t, $J = 7.2$, 2H), 2.85 (apparent t, $J = 7.6$, 2H), 2.91 (m, 2H), 3.12 (m, 1H), 3.42−3.48 (m, 2H), 3.63 (m, 1H), 3.88 (m, 1H), 3.94 (m, 2H), 4.16 (apparent q, $J = 8.1$, 1H), 4.39 (d, $J = 14.5$, 1H), 4.48 (d, $J = 14.5$, 1H), 4.50 (broad s, 1H), 6.76 (d, $J = 8.8$, 1H), 6.82 (m, 1H), 6.92 (m, 2H), 6.97 (m, 2H), 7.04 (m, 2H), 7.11−7.27 (m, 6H), 7.32 (m, 1H), 7.38 (d, $J = 2.5$, 1H), 7.70 (m, 2H), 7.82 (m, 2H). Anal. Calc′d for $C_{43}H_{37}N_3O_8Cl_4$: C, 60.79; H, 4.39; N, 4.95. Found: C, 61.01; H, 4.40; N, 4.80.

Inhibitor 31. From 0.366 g of resin was isolated 79 mg of pure inhibitor **31** (64%) after chromatography. ^{1}H NMR (500 MHz): δ 0.90 (d, $J = 6.2$, 3H), 0.93 (d, $J = 6.2$, 3H), 1.39 (m, 1H), 1.66 (m, 2H), 2.59−2.71 (m, 2H), 2.89−2.93 (m, 2H), 3.27 (dd, $J = 2.1$, 14.0, 1H), 3.45−3.56 (m, 2H), 3.78−3.83 (m, 2H), 3.85 (s, 3H), 3.86 (s, 3H), 3.89−3.94 (m, 2H), 4.16 (m, 1H), 4.37 (broad s, 1H), 6.59 (d, $J = 9.5$, 1H), 6.97 (s, 1H), 7.10 (d, $J = 8.2$, 1H), 7.13 (dd, $J = 2.0$, 8.2, 1H), 7.16 (s, 1H), 7.30 (d, $J = 2.0$, 1H), 7.67 (m, 2H), 7.78 (m, 2H). Anal. Calc′d for $C_{35}H_{38}N_3O_7Cl_2Br_1$: C, 55.06; H, 5.02; N, 5.50. Found: C, 55.02; H, 5.18; N, 5.50.

Cathepsin D Assay. The cathepsin D assays for the compounds that had been fully characterized were performed in a quartz cuvette with a Perkin-Elmer LS-50B spectrometer (excitation = 336 nM, emission = 490 nM). The same substrate as reported for the high-throughput assay was used. A 0.1 M formic acid buffer (pH = 3.7) was used with a final concentration of 0.5 nM cathepsin D. In a typical assay with a final volume of 600 μL, to 555 μL of buffer is added 20 μL of inhibitor in DMSO, followed by 5 μL of cathepsin D stock (0.05% Triton X-100 in H_2O). After a 3.5-min incubation time at 25 °C, 20 μL of substrate stock (2:1 H_2O:DMSO) is added. The increase of fluorescence intensity was recorded as a function of time. Assays were performed in duplicate or triplicate at five to six inhibitor concentrations for each K_i determination. Data were fitted by nonlinear regression analysis to the equation derived by Williams and Morrison:[50]

$$v = \frac{v_0}{2E_t}\left\{\left[\left(K_i\left(1+\frac{S}{K_m}\right)+I_t-E_t\right)^2+4K_i\left(1+\frac{S}{K_m}\right)E_t\right]^{1/2}-\left[K_i\left(1+\frac{S}{K_m}\right)+I_t-E_t\right]\right\}$$

The K_m for the substrate was determined to be 4 μM by using a Lineweaver−Burke plot (the K_m was previously determined by Krafft to be 5 μM).[37] The variables S, E_t, and I_t are the concentrations of substrate, active enzyme, and inhibitor, respectively.

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Supporting Information Available: ^{1}H NMR spectra of intermediates **3** for each P_1 side chain are provided (18 pages print). See any current masthead page for ordering information and Web access instructions.

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