Report

Method for the Parallel Preparation of the Aspartic Protease Isostere: Hydroxyethylamino Amides

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Method for the Parallel Preparation of the Aspartic Protease Isostere: Hydroxyethylamino Amides

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The use of parallel synthetic sequences to prepare focused chemical libraries directed toward a specific protein family has become a standard approach for hit generation in drug discovery. When leveraged by a suitably broad synthetic method, this approach becomes highly valuable, because a hit to any member of that protein family may be identified, and the speed in which analogues may be prepared in a subsequent hit to lead program is substantially increased. As part of our continuing efforts toward general synthetic methods for the parallel preparation of directed libraries of compounds, we were interested in synthetic routes to prepare aspartic protease inhibitors. The aspartyl protease family[1] includes β-secretase,[2] cathepsins,[3] renin,[4] plasmepsin,[5] and HIV protease.[6] A number of native and enzyme−inhibitor crystal structures have been solved for these aspartic proteases. A key structural element in most inhibitors is a hydroxyl or hydroxyl-like moiety that binds to the catalytically active aspartic acids in the enzyme active site. The work of nearly three decades in the development of mechanism pathway inhibitors for aspartic proteases has resulted in the identification of a number of transition-state inhibitor classes (Figure 1). These molecules replace the dipeptide subunit that contains the scissile amide bond (P_1-P_1' residues) (Figure 1). Two of the most useful of these are the hydroxyethylene and hydroxyethylamine units; the latter isostere has been employed extensively in agents for the treatment of AIDS and is currently used in clinical practice.[7] Our results toward the development of a synthetically robust and operationally simple solid-phase route to hydroxyethylamino amides[8] are described herein.

We reasoned that the hydroxyethylamino subunit could be easily derived from a resin-bound amidomethylene epoxide central intermediate. Our initial efforts centered on the direct alkylation of acylated Rink resin with epichlorohydrin using the successive amide alkylation procedure of Houghten.[9] Rink resin was acylated with 3-chlorobenzoyl chloride utilizing standard procedures to provide secondary amide **1** (Scheme 1). The Houghten procedure uses 12 successive cycles of exposure to lithium *tert*-butoxide, followed by quenching with electrophile. With our system, exposure of amide **1** to lithium *tert*-butoxide, followed by quenching with epichlorohydrin, provided the alkylated

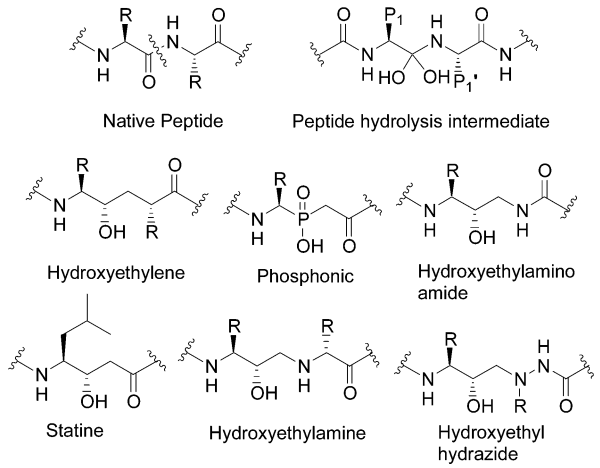
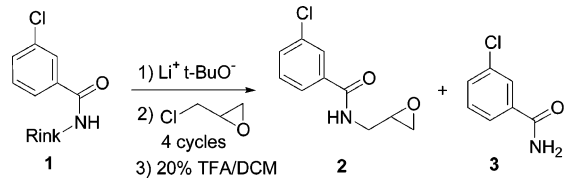


Figure 1. Transition-state isostere dipeptide mimetics.

Scheme 1. Alkylation of Rink Resin



amide **2** along with unreacted amide **3** after cleavage with 20% TFA/DCM. We found that the conversion to **2** stopped after four cycles, with the best conditions providing a 60:40 ratio of **2** to **3**. Various other bases (LDA, NaH, LHMDS) were investigated for the deprotonation of resin **1** as well as other electrophilic replacements for epichlorohydrin; despite these efforts, we were never able to realize higher conversions to epoxyamide **2**.

To achieve higher alkylation rates of our benzhydrylamine resin, we next investigated the conversion of Rink resin to an alloc-protected derivative, anticipating improved alkylation of a carbamate nitrogen. Allyl pyrocarbonate[10] was allowed to react with unprotected Rink resin to prepare resin **4** (Scheme 2).

We exposed resin **4** to lithium *tert*-butoxide in THF and then to epichlorohydrin in DMSO. In this case, the extent of alkylation could not be evaluated until the resin was deprotected and acylated, which led to smooth and reproducible cleavage from the solid support. In the event, removal of the alloc group by exposure to tetrakis(triphenylphosphine)palladium (0) in the presence of excess phenylsilane gave the versatile secondary amine resin **5** (Scheme 2). Acylation of resin **5** with 4-benzylbenzoic acid in the presence of 2-chloro-1,3-dimethylimidazolinium chloride and DIEA provided benzhydrylamide resin **6**. Exposure of **6** to 20% trifluoroacetic acid in dichloromethane allowed for evaluation of the alloc installation and subsequent alkylation of the alloc-carbamate nitrogen of **4**. We were delighted to find a 47% isolated yield of **7** on the basis of initial Rink resin loading after reversed-phase purification.[11] The amount of unalkylated amide **8** was estimated from HPLC analysis to be <5%.

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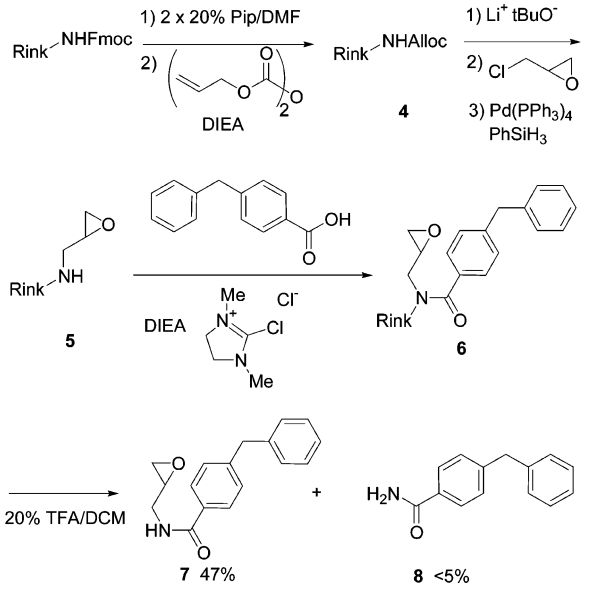
Scheme 2. Alkylation of Alloc-Protected Rink Resin

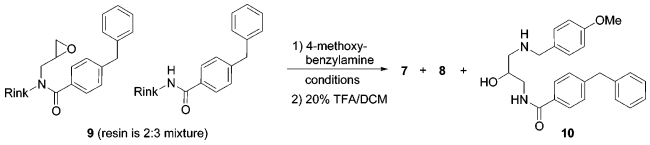


With an effective route to monoalkylated resin-bound amines in hand, we turned our attention to the amine epoxide opening of **6**, which was critical to our approach for the parallel synthesis of hydroxyethylamino amides.

Resin-based epoxides have been exploited in a number of useful synthetic transformations.[12] Resin-based epoxide-opening with amines has been performed thermally, but usually requires an extreme excess of the amine, long reaction times, or elevated temperatures.[13] Instead, we chose to investigate milder room-temperature conditions with Lewis acids. Our initial efforts with $Yb(OTf)_3$[14] were promising because we were able to obtain the desired hydroxyethylamino amides after acid cleavage from support. However, purified gravimetric yields were typically around 1−5%, which would require a substantial amount of resin per reaction to meet our mass goals for this chemistry. HPLC/MS analysis of the $Yb(OTf)_3$–promoted epoxide-opening reaction solution revealed that a significant portion of the amide was cleaved from resin during the epoxide-opening step.

We performed a brief survey of Lewis acids to minimize this premature cleavage of amide **6** while maintaining acceptable rates for the epoxide opening of **6**. We chose resin **9** (Table 1) for this study because it contained both the *N*-epoxymethyl and the *N*−*H* amides (resin **9** was obtained as outlined in Scheme 1). Exposure of **9** to 20% TFA/DCM yielded a 39:61 mixture of **7**/**8**, as determined by integration of the total absorption chromatogram. The presence of a constant amount of primary amide **8** provided a convenient internal standard for our Lewis acid study. The reactions were conducted in the presence of 11 equiv of 4-methoxybenzylamine for 15 h under the conditions listed in Table 1. The reaction solutions were analyzed by HPLC/MS to establish a qualitative determination of premature amide cleavage. We were surprised to find that all conditions, even purely thermal conditions (Table 1, entries 10,11,13), resulted in detectable amounts of premature cleavage of either epoxide **7** or hydroxyethylamino amide **10**; however, we never detected any premature cleavage of primary amide **8** with any of these

Table 1. Lewis Acid Study of the Epoxide Opening of **9**



entry	conditions[a] solvent		rel % conv[b]	rel Lewis acid cleavage stability[c]
1	$Yb(OTf)_3$	DCE	68	55
2	$LiClO_4$	CH_3CN	84	79
3	$MgCl_2$	CH_3CN	91	62
4	$Mg(ClO_4)_2$	CH_3CN	16	85
5	$Zn(ClO_4)_2$	CH_3CN	9	63
6	LiOTf	CH_3CN	60	82
7	$Zn(OTf)_2$	CH_3CN	7	76
8	TMSOTf	DCE	20	60
9	$ZnCl_2$	CH_3CN	3	88
10	none	NMP	2	88
11	80 °C	NMP	88	35
12	10 equiv DIEA	NMP	9	20
13	none	DCE	3	90
14	10 equiv DIEA	DCE	64	21

[a] 1 equiv Lewis acid, 15 h; room temp unless otherwise noted. [b] Area from PDA of LC/MS trace 190−350 nM relative % conv = (**10**/(**7** + **10**)) × 100. [c] Area from PDA of LC/MS trace 190−350 nM; relative Lewis acid cleavage stability =(((**7** + **10**)/(**7** + **8** + **10**))/0.39) × 100.

conditions, which confirmed our ability to use this compound as an internal standard to calculate a relative measure of cleavage stability (Table 1). After cleavage with 20% TFA/DCM, the percent conversion and relative Lewis acid cleavage stability was calculated.[15]

The relative cleavage stability and relative percent conversion provided us with a qualitative means to evaluate each Lewis acid for its ability to deliver good isolated yields of **10**. As our earlier work had shown, $Yb(OTf)_3$ was quite effective at epoxide opening, but a significant portion of the amide was lost due to poor cleavage stability (Table 1, entry 1). $MgCl_2$ gave even better conversion to hydroxyethylamino amide **10**, but again, cleavage stability was an issue (entry 3). Although $ZnCl_2$ (entry 9) had been shown to be effective for resin-based epoxide opening with alcohols,[16] for our system, we observed only trace amounts of product **10**. In the absence of Lewis acid (Table 1, entries 10 and 13), the conversions were significantly less effective. Although the application of purely thermal conditions (entry 11) provided a higher conversion to **10**, a significant portion of premature amide cleavage was observed. In the presence of DIEA, high conversion was observed in DCE (entry 14), but again, cleavage stability was an issue. In the case of perchlorate salts (entries 2, 4, and 5), the counterion played a significant role in the success of the Lewis acid. From our studies, $LiClO_4$[17] (entry 2) was clearly superior to all other Lewis acids investigated. Next, we evaluated $LiClO_4$ in a variety of solvents and found that toluene, DCE, and DCM gave the best overall conversion to **10**, while NMP, DMF, and methyl *tert*-butyl ether gave significantly poorer conversion results.

With acceptable epoxide-opening conditions identified, we subsequently found with resin **6** that 24−30 h of exposure to $LiClO_4$ was necessary to give >95% conversion to **10**.

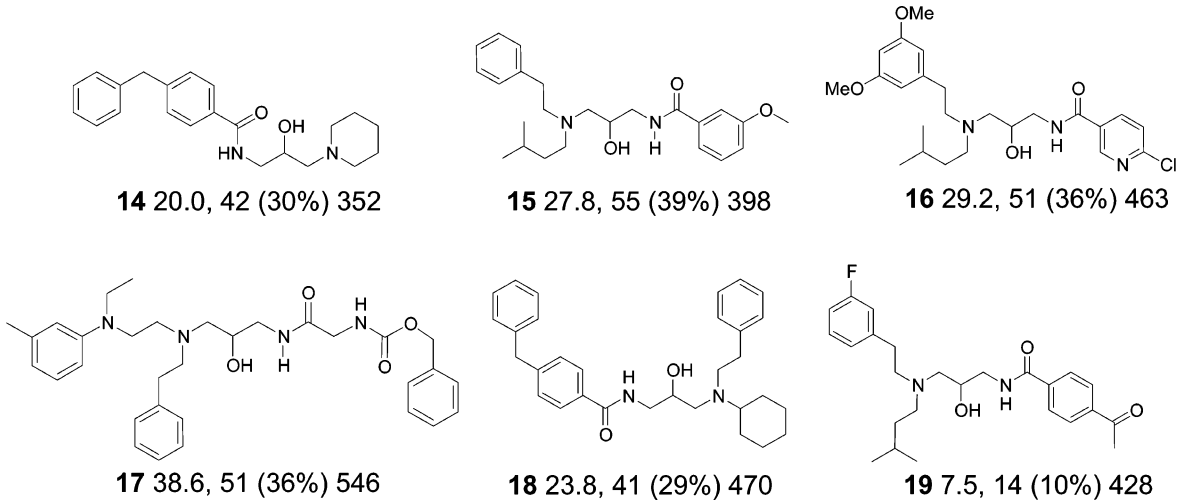
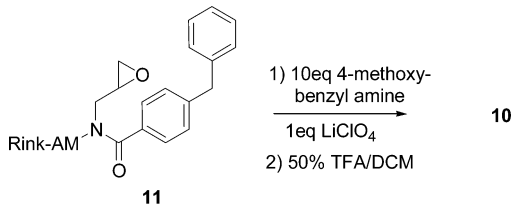
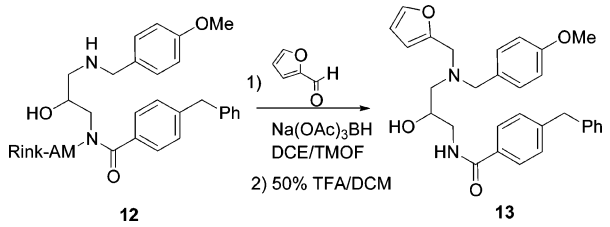


Figure 2. Representative examples with isolated milligrams, micromols (isolated yields), and molecular weights.

Scheme 3. Switch to Rink-AM Resin



Scheme 4. Reductive Amination of **12**



Under these longer reaction times, we were disappointed to find a 12% isolated yield of **10** based on initial resin loading (25% based on isolated yield of **7**).[18] This low yield was mainly due to the premature cleavage of the Rink amide linkage during the extended $LiClO_4$-assisted epoxide-opening step.

We next evaluated the same reaction sequence on the more acid stable Rink−AM resin **11** (Scheme 3) and were encouraged to find a 37% isolated yield of **10** which represents a factor of 3 increase over Rink resin after reversed-phase purification (86% based on isolated yield of **7** from Rink−AM resin). Although some premature cleavage of the resin amide was occurring, we were satisfied with the overall isolated yields and aware that extended reaction times for the epoxide-opening step reduced recoveries of **10**.

Reductive amination of resin **12** followed by 50% TFA/DCM and RP-HPLC purification provided 1,3-diamino-propan-2-ol **13** in 26% yield (Scheme 4). This eight-step sequence on solid support has been applied to a range of 1° amines, 2° amines, aldehydes, and carboxylic acids with isolated yields ranging from 0 to 50%. Amines with low nucleophilicity (i.e., electron-neutral or -deficient anilines) are the only class we have identified that result in little or no desired products. To date, ∼230 compounds have been prepared by this approach in a parallel fashion to define the scope and yields for this new method.

Figure 2 shows some examples of the types of compounds prepared by this method, including molecular weight and yield after reversed-phase purification.

In conclusion, a solid-phase method has been developed to generate hydroxyethylamino amides, which are known aspartyl protease inhibitors. We have performed the eight-step sequence in parallel in as few as 6 days and obtained the resulting hydroxyethylamino amides in good yield without the need for rigorous exclusion of water or oxygen. We have developed a method for the monoalkylation of amine functionalized resins which could prove quite general. The aspartyl protease isosteric compounds are prepared from very large reagent pools: carboxylic acids, 1° amines, 2° amines, and aldehydes, which results in synthetic access to a sizable and diverse set of hydroxyethylamino amides.

Supporting Information Available. Experimental procedures and spectroscopic characterization for compounds **7**, **13**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The relative Lewis acid cleavage stability was calculated as follows: From the total absorption chromatogram, the sum of the area of epoxide **7** and hydroxyethylamino amide **10** was divided by the sum of the area of compounds **7**, **8**, and **10,** then corrected for the initial ratio of **7** to **8** (Table 1 footnote *c*).

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(18) In the selection of conditions for the identification of an optimal Lewis Acid, we chose a reaction time, which left some starting epoxide **7**. This allowed us to obtain a meaningful percent conversion for each set of conditions studied.

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