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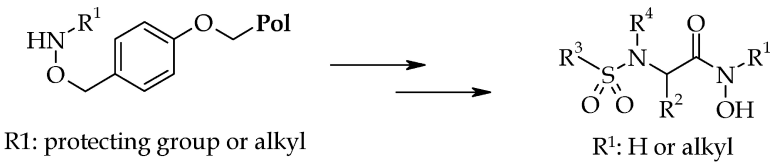

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Polymer-Supported N-Derivatized, O-Linked Hydroxylamine for Concurrent Solid-Phase Synthesis of Diverse *N*-Alkyl and *N*−*H* Hydroxamates

Keith J. Stanger[†] and Viktor Krchňák*[,‡]

Walther Cancer Institute and Department of Chemistry and Biochemistry, 251 Nieuwland Science Center, University of Notre Dame, Notre Dame, Indiana 46556

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We describe parallel/combinatorial, solid-phase, supported synthesis of diverse hydroxamates using a common intermediate, an N-derivatized, O-linked hydroxylamine. The method allows the concurrent synthesis of both *N*-alkyl and *N*−*H* hydroxamates and is compatible with a wide range of chemical transformations. The synthesis of *NH* hydroxamates includes protection of the nitrogen with a 2,4-dimethoxybenzyl group at the stage of polymer-supported benzyloxyamine. The protecting group eliminates side reactions caused by the presence of a free hydroxamate NH group and is simultaneously removed during cleavage of target compounds from the solid support. The chemical route has been thoroughly tested on model compounds with several linkers, and a high yield and purity synthesis of more than 50 hydroxamates, designed to inhibit cell proliferation of breast cancer cell lines, is described.

Introduction

The current prevailing route for solid-phase synthesis of hydroxamates is through immobilized hydroxylamine. Typically, the hydroxylamine derivatives are attached to a solid support via the oxygen (Scheme 1), although attachment through the nitrogen has also been utilized.[1] The most common preparative route to O-immobilized hydroxylamine is by the reaction of *N*-hydroxyphthalimide with Wang or Sasrin resins under Mitsunobu conditions.[2−4] Alternatively, hydroxyl resins can be converted to mesylate resins and reacted with *N*-hydroxyphthalimide,[5] or trityl chloride resins can be directly reacted with either *N*-hydroxyphthalimide[6−8] or *N*-Fmoc hydroxylamine.[9] The resulting polymer-supported hydroxylamines can then be elaborated through acylation with carboxylic acids to give various hydroxamic acids.

However, these current solid-phase approaches are severely limited by the presence of the free hydroxamate nitrogen, which prevents many chemical transformations from being used on the side chain. For example, Boc-protected amino acid-acylated benzyloxyamines, **1**, alkylated with electrophiles and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene resulted in double alkylation, **2**, on the side chain as well as the hydroxamate (Scheme 2). Such side reactions have limited the application of polymer-supported N-acylated benzyloxyamine.[10]

Furthermore, N-alkylation of hydroxamic acids, by reaction with electrophiles[11,12] or through Mitsunobu reaction with alcohols,[13−17] is accompanied by carbonyl O-alkylation (**5**, Scheme 3). Experiments in solution indicate that the alkylation outcome is substrate-dependent. Electrophilic alkylation of free hydroxamic acids resulted in a mixture of carbonyl *O*- and *N*-alkyl products.[11] *O*-alkyl hydroxamates alkylated under Mitsunobu conditions afforded *N*-alkyl derivatives,[16,17] whereas *O*-acylhydroxamates gave a mixture of N- and carbonyl O-alkylkated products.[16] A recent report described the carbonyl *O*-alkyl derivative as the major product in alkylation of *O*-benzylhydroxamates.[13]

To prevent side reactions caused by the NH group of hydroxamates, Ngu and Patel[18] tethered the hydroxylamine via its nitrogen (Scheme 4). First, hydroxylamine was linked to Barany's backbone amide linker, **6**,[19] while the oxygen was protected by either the acid-labile tetrahydropyran group or the acid-stable allyl group.[18] The resulting N,O-derivatized hydroxylamines **7** were protected with a fluorenylmethyloxycarbonyl (Fmoc) group and attached to an aminomethyl polystyrene-type resin by acylation. After cleavage of the Fmoc group, the resin-supported hydroxylamines **8** and **9** were used for the synthesis of hydroxamate-based matrix metalloproteinase (MMP) inhibitors.

In this article, we describe an alternative hydroxylamine-based O-linking strategy with protection of the nitrogen by an acid-labile protecting group which has several advantages over the existing methods. Our approach allows the traditional starting intermediate, polymer-supported benzyloxyamine, to be used for three different synthetic pathways (Scheme 5): (i) synthesis of hydroxamates in which the presence of a hydroxamate NH group is tolerated (route A),

* Corresponding author. Phone: (574) 631-5113. E-mail: vkrchnak@nd.edu.
[†] Walther Cancer Institute.
[‡] Department of Chemistry and Biochemistry.

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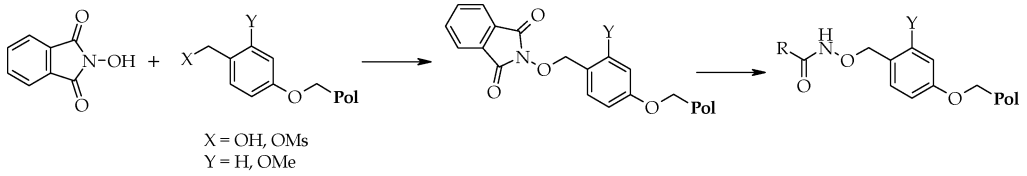
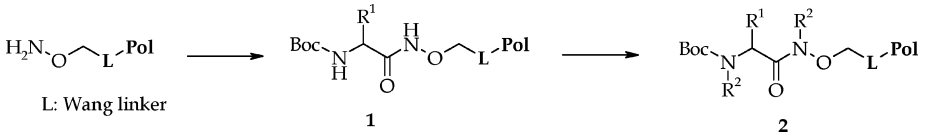
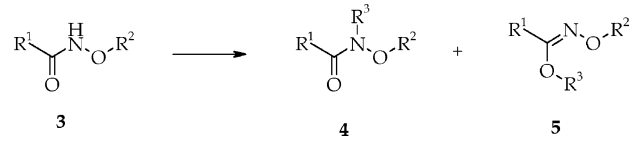
Scheme 1. Solid-Phase Synthesis of Hydroxamic Acids



Scheme 2. Double Alkylation of Boc Amino Acid Hydroxamates[10]



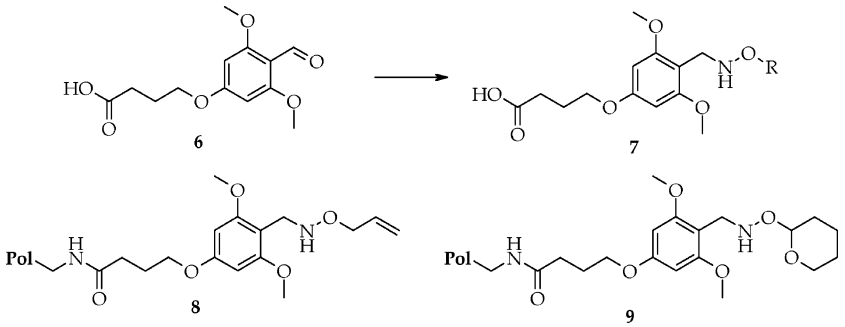
Scheme 3. Competing Carbonyl O- and N-Alkylation of Hydroxamates[11]



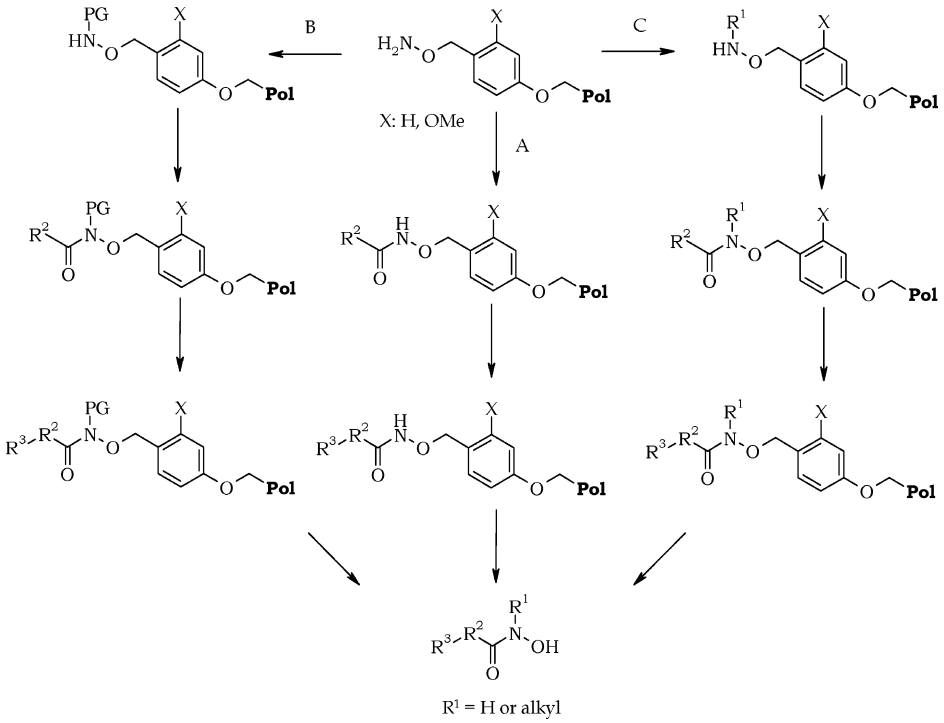
(ii) synthesis of hydroxamates requiring NH protection (route B) because of free NH incompatibility with the chemicals used to perform transformations on the side-chain, and (iii) synthesis of *N*-alkyl hydroxamates (route C).

To demonstrate the utility of this approach, we synthesized a set of hydroxamates derived from a common sulfonamide hydroxamate motif. Such sulfonamide hydroxamates are present in numerous MMP inhibitors, including the stromelysin inhibitor CGS 27023A (Figure 1).[20] Our methodology allowed for the preparation of N-substituted as well as N-unsubstituted compounds from identical solid-supported

Scheme 4. Synthesis of Hydroxamic Acids via N-Linking Strategy[18]



Scheme 5. General Concept of Hydroxamic Acid Synthesis



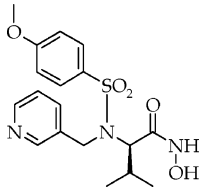
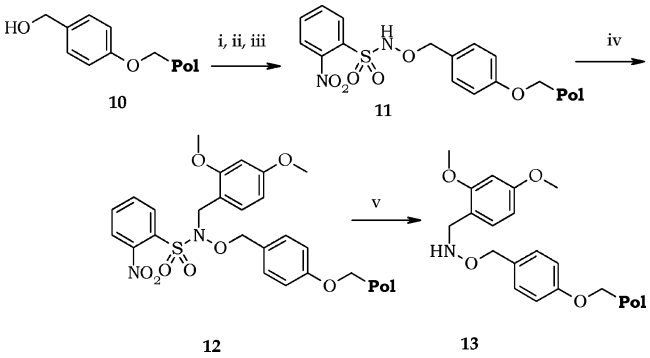


Figure 1. Structure of CGS 27023A.

Scheme 6. Solid-Phase Synthesis of Polymer-Supported *N*-(2,4-Dimethoxybenzyl)benzyloxyamine



Reagents: (i) *N*-hydroxyphthalimide, PPh_3, DIAD, anhydrous THF, 20 °C, overnight; (ii) 5% hydrazine hydrate, THF/MeOH (1:1), 20 °C, overnight; (iii) Nos-Cl, 2,6-lutidine, DCM, 20 °C, overnight; (iv) 2,4-dimethoxybenzyl alcohol, PPh_3, DIAD, anhydrous THF, 20 °C, overnight; (v) 2-mercaptoethanol, DBU, DMF, 20 °C, 30 min.

intermediates. By utilizing a synthetic scheme that allows for common intermediates deep into the synthesis, parallel synthesis of a diverse variety of compounds was facilitated.

Results and Discussion

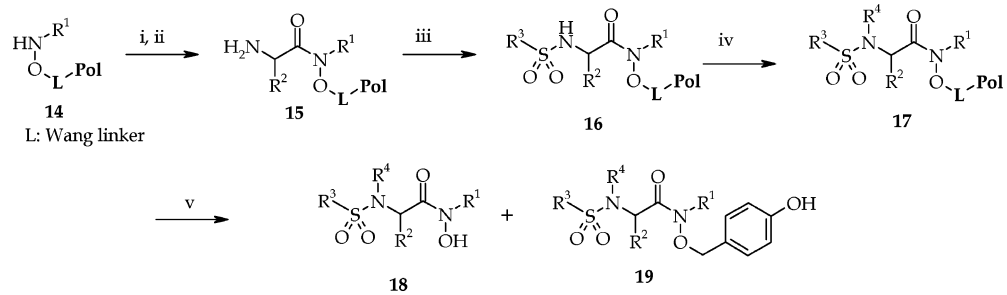
We expanded the most frequently used methodology for the synthesis of hydroxamates by making it compatible with a wider range of chemical transformations to provide easy synthetic access to structurally diverse hydroxamates. In other current methodologies, the presence of the free NH hydroxamate group is problematic, severely limiting compatible chemical transformations. To prevent potential side reactions at the hydroxamate NH group, the nitrogen is protected with an electron-rich 2,4-dimethoxybenzyl group (Scheme 6). This protecting group is acid-labile and removed simultaneously during acid-mediated cleavage of the product from the resin. The synthesis of the key resin-bound intermediate, the N-protected O-linked hydroxylamine **13**, is simple and straightforward and follows the same reaction sequence used for the synthesis of *N*-alkyl hydroxamates reported by us[21] and others;[22,23] however, in this case, the *N*-alkyl group serves to temporarily protect the NH hydroxamate. Coupling of Wang resin **10** with *N*-hydroxyphthalimide, removal of the phthalimide group,[2] and N-sulfonation with 2-nitrobenzenesulfonyl chloride (Nos-Cl) affords resin **11**. The amino group of resin **11** is alkylated under Mitsunobu conditions with 2,4-dimethoxybenzyl alcohol to provide the N-protected species **12**, which is then treated with thiol/base to liberate O-linked, N-protected, polymer-supported hydroxylamine **13**.

To demonstrate the usefulness of the resin-bound intermediate **13**, we synthesized model compounds **18** (Scheme 7), which are related to the known stromelysin inhibitor CGS 27023A.[20] Our targets consisted of both *N*−*H* and *N*-alkyl hydroxamates to document the applicability of the reaction route for those two classes of compounds. The syntheses were carried out on traditional resin beads and Stratosphere Plugs, a modular solid phase support suitable for directed split-and-pool combinatorial synthesis. Both solid supports were derivatized with the Wang linker.[24] Acylation of **14**, where R^1 is the 2,4-dimethoxybenzyl protecting group or an alkyl group, by in situ-prepared symmetrical anhydrides of *N*-Fmoc protected amino acids, followed by removal of the Fmoc group, yielded polymer-supported hydroxamates **15** ready for further chemical transformation of the side chain. The liberated amino group was reacted with sulfonyl chlorides to provide resin-bound sulfonamides **16**. A final Mitsunobu reaction or electrophilic substitution N-alkylated the sulfonamide to yield the polymer-supported target compounds **17**.

Quantitative cleavage of the target compounds from Wang linker and removal of the 2,4-dimethoxybenzyl protecting group required treatment with 90% TFA for 1 h. Typical conditions for cleavage of carboxylic acids, 50% TFA in DCM for 30 min,[25] resulted in incomplete cleavage of hydroxamates from the Wang linker, and the product **18** was contaminated by a side product **19** containing an *O*-(4-hydroxy)benzyl group. This side product is generated by cleavage of Wang linker from the polystyrene-based support.[26] To use milder cleavage conditions, one may consider using the more acid-labile 2,4,6-trimethoxybenzyl protecting group with more acid-labile linkers, such as the 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB) linker.[27]

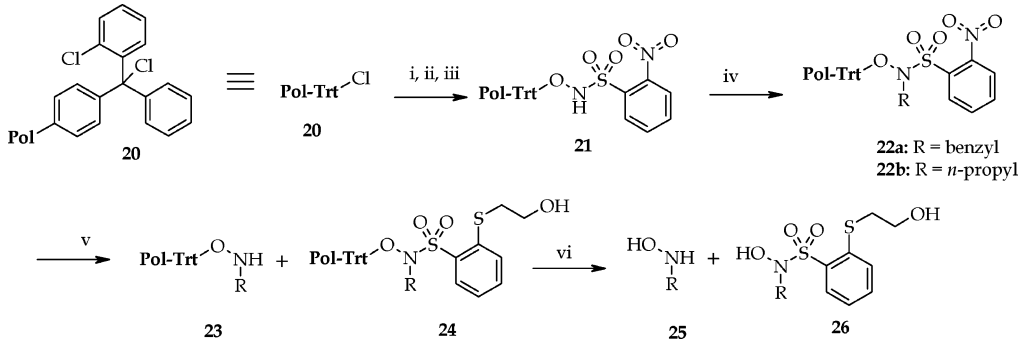
To confirm the necessity of the *N*-(2,4-dimethoxybenzyl) protecting group, direct synthesis of two model compounds with the free hydroxamate was attempted. Model compounds were synthesized using Fmoc-D-valine in the acylation step,

Scheme 7. Solid-Phase Synthesis of Hydroxamates



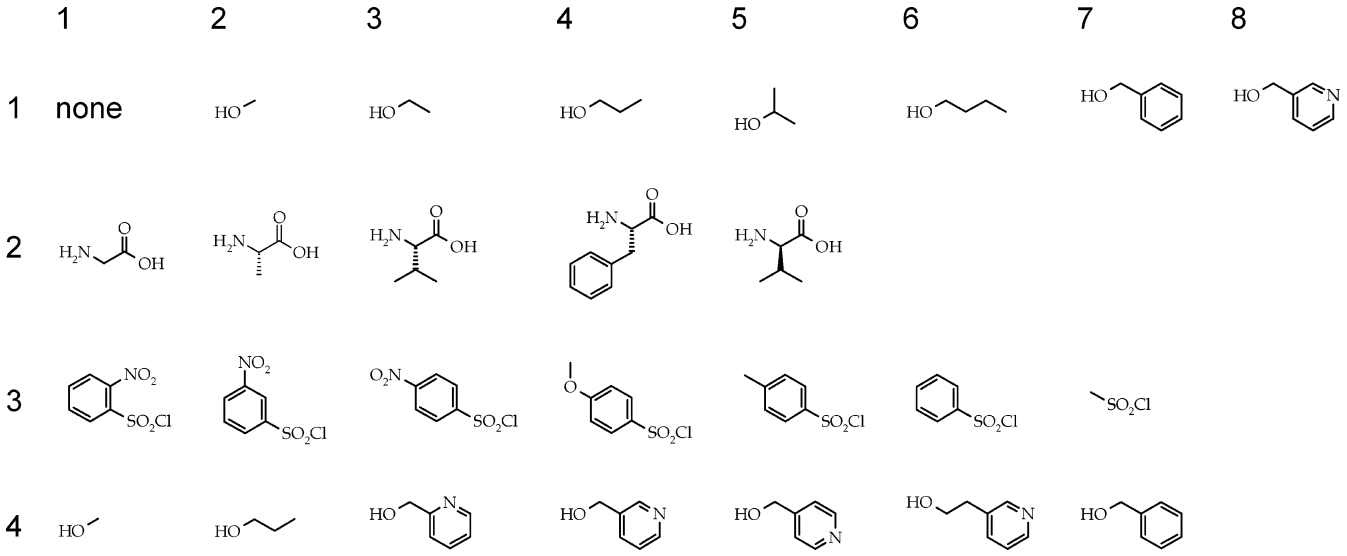
Reagents: (i) Fmoc-amino acid, DIC, DMF; (ii) piperidine, DMF; (iii) sulfonyl chloride, 2,6-lutidine, DCM, 20 °C, overnight; (iv) alcohol, PPh_3, DIAD, anhydrous THF, 20 °C, overnight; (v) TFA in DCM; for concentration and reaction time, see the text.

Scheme 8. Attempted Solid-Phase Synthesis of *N*-Alkyl Hydroxamic Acids



Reagents: (i) *N*-hydroxyphthalimide, DIEA, anhydrous DMF, 20 °C, overnight; (ii) 5% hydrazine hydrate, THF/MeOH (1:1), 20 °C, overnight; (iii) Nos-Cl, 2,6-lutidine, DCM, 20 °C, overnight; (iv) PPh_3, R−OH, DIAD, anhydrous THF, 20 °C, 3 h; (v) 2-mercaptoethanol, DBU, DMF, 20 °C, 30 min, 10% TFA, DCM, 20 °C, 30 min.

Table 1. Structure of Building Blocks Used in Four Diversity Transformation



2-nitrobenzenesulfonyl and 4-methoxybenzenesulfonyl chlorides for introducing the sulfonamides and 2-pyridylmethanol as the alkylating agent. Synthesis proceeded without incident until the final Mitsunobu alkylation. As expected, Mitsunobu conditions alkylated both the side chain and hydroxamate, with the carbonyl O-alkylated product predominating. Evidence for the product was provided by LC/MS data of the cleaved compound.

Attempts to apply our reaction sequence to the acid-labile 2-chlorotrityl linker failed unexpectedly (Scheme 8). 2-Chlorotrityl chloride resin **20** was successfully reacted with *N*-hydroxyphthalimide in the presence of a tertiary amine base according to a previously described procedure.[6−8] The phthaloyl protecting group was cleaved by hydrazine hydrate, and the resulting aminoxy derivative was reacted with Nos-Cl. The amino group of **21** was then alkylated by benzyl alcohol or *n*-propanol under Mitsunobu conditions to produce the resin-bound Nos-protected intermediates **22a** and **b**.[21] Unfortunately, acylation of resin-bound **23** proved very difficult and proceeded in low yields due to the severe steric constraints around the trityl linkage. Problems acylating *N*-methylhydroxylamine on trityl resins have been reported by others.[9] Furthermore, treatment of the Nos intermediate with mercaptoethanol and DBU led to replacement of a nitro group by mercaptoethanol, structures **26a** and **b**, as indicated by mass spectra of the cleaved products from the resin by dilute TFA. The isolated benzyl and *n*-propyl products **26** (semipreparative HPLC) exhibit ^{1}H NMR spectra consistent with that of *N*-hydroxy-*N*-benzyl-2-(2-hydroxyethyl)thiobenzenesulfonamide **26a** and *N*-hydroxy-*N*-propyl-2-(2-hydroxyethyl)thiobenzenesulfonamide **26b**, respectively. The relative ratio of the expected product **25** to **26** was 4:1, as estimated from the NMR spectra of crude cleaved material. The inability to cleanly remove the Nos group because of nucleophilic replacement of the nitro group and problematic acylation of the secondary amino group led us to abandon the trityl linker.

Considering the results described above, we choose to synthesize a library of compounds containing both *N*−*H* and *N*-alkyl hydroxamates using the Wang linker-derivatized support. The synthesis with Wang linker provided crude compounds with very good overall purity when slightly rigorous cleavage conditions, 90% TFA in DCM for 1 h, were used to eliminate the formation of *O*-alkyl species. The structure of our compounds tolerated such conditions without compromising purity.

Our decision to synthesize *N*-alkyl and *N*−*H* hydroxamates based on motif **18** was triggered by an unexpected finding that N-alkylated versions of known sulfonamide hydroxamate MMP inhibitors inhibited proliferation of invasive breast

cancer cells, despite being devoid of any significant MMP inhibitory activity. Compounds were designed to evaluate the effect of structural changes at four positions, $R^1-R,^4$ **18**, on the molecule's ability to inhibit cell proliferation. We included seven alcohols for the introduction of R^1 groups (in addition to the *N*−*H* hydroxamate), five amino acids (R^2 groups), seven sulfonyl chlorides (R^3 groups), and seven alcohols (R^4 groups). Table 1 shows individual building blocks used to introduce structural modifications into the parent sulfonamide hydroxamate **18**. All compounds were fully characterized, and analytical data on individual compounds are available in the Supporting Information. For the sake of simplicity, individual compounds are coded by a four-digit code, each digit corresponding to building block number (Table 1; e.g., compound **2413** was synthesized using methanol, Fmoc-L-phenylalanine, 2-nitrobenzenesulfonyl chloride, and 2-pyridylmethanol, in the first, second, third, and fourth diversity steps, respectively). Synthesized compounds have been tested for inhibition of cell proliferation and cell migration of highly invasive breast cancer cells MDA-MB-231, and detailed results of biological testing will be the subject of a dedicated communication.

Conclusion

A general method for the concurrent synthesis of diverse *N*-alkyl and *N*−*H* hydroxamates on solid phase was developed. This route is particularly useful for the combinatorial/multiple synthesis of compound arrays comprising both N-unsubstituted and N-alkylated hydroxamates. The Nos-derivatized polymer-supported benzyloxyamine **11** serves as a common intermediate for introduction of a permanent *N*-alkyl substituent as well as the 2,4-dimethoxybenzyl protecting group. The method was thoroughly tested and optimized on model compounds. Synthesis on the resin-supported Wang linker required slightly forced cleavage conditions, 90% TFA, to eliminate the *O*-(4-hydroxy)benzyl side product. The chlorotrityl linker is not compatible with this method, because the deprotection of the Nos group by thiols is accompanied by aromatic nucleophilic displacement of the nitro group, and acylation of the resin-bound *N*-alkyl benzyloxyamine is difficult. The optimized protocol on Wang resin was used for the synthesis of more than 50 *N*−*H* and *N*-alkyl hydroxamates designed for an SAR study of inhibition of proliferation of invasive breast cancer cells. The route is straightforward, high yielding, and uses commercially available inexpensive reagents.

Acknowledgment. The work was supported by the Department of Chemistry and Biochemistry, University of Notre Dame, and the Walther Cancer Institute.

Supporting Information Available. Details of experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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