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A Novel Linkage for the Solid-Phase Synthesis of Hydroxamic Acids

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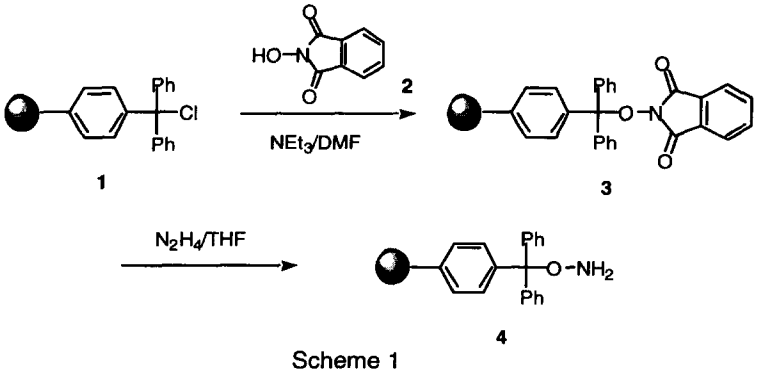
Abstract: A novel linkage for the solid-phase synthesis of hydroxamic acids is described and its facile application for the solid phase synthesis of peptidyl, succinyl and urea-type hydroxamic acids is illustrated. Cleavage is induced under mild acidic conditions by treatment with formic acid in THF, providing hydroxamic acids in high purity and fair to good yields. © 1997 Elsevier Science Ltd.

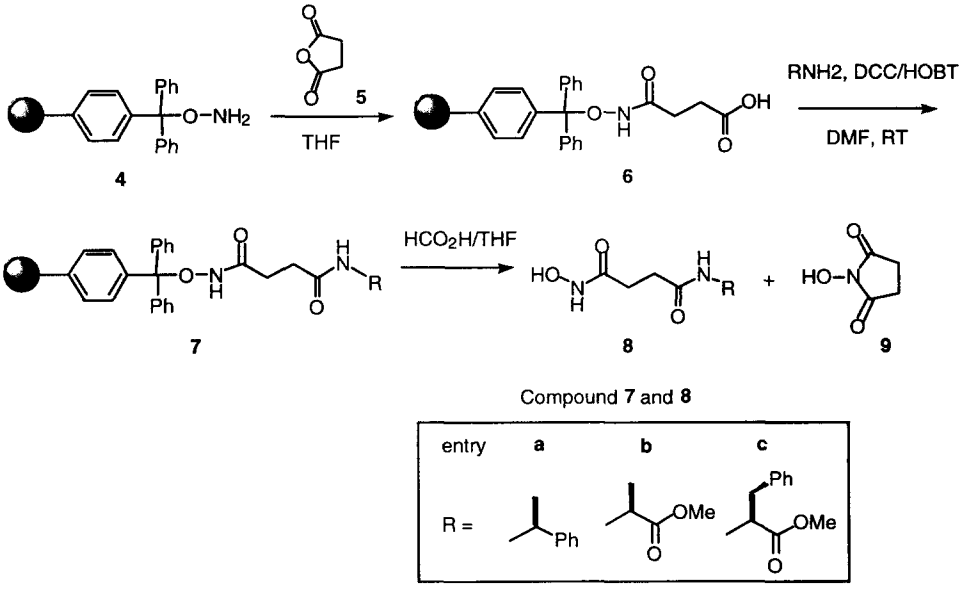
Naturally occurring hydroxamic acids have been isolated from various microbial sources a century ago and exert biological effects due to their high affinity towards metal ion.[1] They have resurged recently since their identification as potent matrix metalloprotease (MMP) inhibitors. Several pseudopeptide hydroxamic acids have been advanced into human clinical trails for the treatment of arthritis, corneal ulceration, and cancers.[2] As a part of our ongoing interest into the design and synthesis of these inhibitors we have been developing an effective route to the generation of hydroxamic acids on solid support.[3]

Here we present the preparation of a novel hydroxylamine resin **4** using trityl chloride resin as the base matrix as well as its application to the synthesis of various hydroxamic acids. The synthesis of the hydroxylamine resin **4** is summarized in Scheme 1. N-Hydroxyphthalimide **2** was used as a source of the hydroxylamine reacting with trityl chloride resin **1** in the presence of triethylamine to obtain the N-hydroxyphthalimide derivative **3**. The formation of **3** was confirmed by the infra-red spectroscopy (ν_{max} 1792 and 1743 cm^{-1}). This intermediate is cleanly transformed to the desired hydroxylamine resin **4** by treatment with hydrazine at room temperature for 12 h.[4]



Scheme 1

In order to establish the utility of the modified resin the synthesis of succinyl amides which have been reported as potent inhibitors of MMPs and related proteases was chosen as a synthetic test (Scheme 2). Treatment of resin **4** with succinic anhydride **5** in THF at 60°C for 6 h or room temperature for 16 h provided the acids **6**, confirmed by infra-red spectroscopy (ν_{max} 1712 cm^{-1}). Resin bound acid **6** was coupled to a 5-fold excess of L-phenylethylamine or L-amino acid ester hydrochloride/triethylamine using DCC/HOBt to give the resin bound succinyl amides **7**. The FT-IR of resin **7b** exhibited a clear NH stretch at 3297 cm^{-1} as well as ester and amide stretching vibrations at 1743 and 1653 cm^{-1}, respectively. Cleavage of the product from the resin was readily achieved by HCO_2H/THF (1:3, 1 h), providing the amides **8** in high purity (> 84%) and fair to good yields (50 - 78%).[5] The cleavage cocktail also contained N-hydroxy-succinimide **9** in varying amounts (usually 10 - 15%), as determined by HPLC and ^1H NMR (Figure 1). We noticed that the yield obtained in some cases varied which might be due to the formation of a cross-linked product generated upon cleavage of hydroxylamine from the resin by the generated free acid. This also accounts, at least in part, for the relatively high amount of generated N-hydroxy-succinimide **9**.



Scheme 2

Treatment of the resin **4** with substituted succinic anhydrides **10**, subsequent coupling of the thus formed acids with L-phenylethylamine or amino acid ester hydrochlorides/triethylamine in DMF and cleavage from the resin as described above gave the hydroxamic acids **11** and **12** as a 1:1 mixture (Scheme 3) in 48 - 78% yield. Purities of the crude compounds **11a-c** were typically in excess of 78% based on HPLC and ^1H NMR. The alkylidenesuccinic anhydride **10d** provided the amide **11d** as the only product (yield: 48%), however it exhibited a purity of only 65%. Again, the products **11/12** were contaminated by 10 - 15% of the corresponding N-hydroxy-succinimide.

We have also been able to synthesize a wide variety of peptidic and peptidomimetic hydrox-amic acids using the described linkage and known synthesis procedures (Scheme 4).

The peptides **13** and **14** were constructed on the resin **4** using standard Fmoc protocols. Cleavage from the resin employing HCO_2H/THF (1:3, 1 h) gave the desired hydroxamic acids as the only peptidic products in 79 and 62% yield, respectively.[6] Resin **4** can also be manipulated using isocyanates providing urea-type compounds[7] such as **15** (purity: 87%; yield 82%) or using standard procedures for submonomer synthesis[8] yielding **16** (purity: 75%; yield 62%). All the compounds gave single hydroxamic acid components ($FeCl_3$ test) which were readily purified by flash column chromatography.

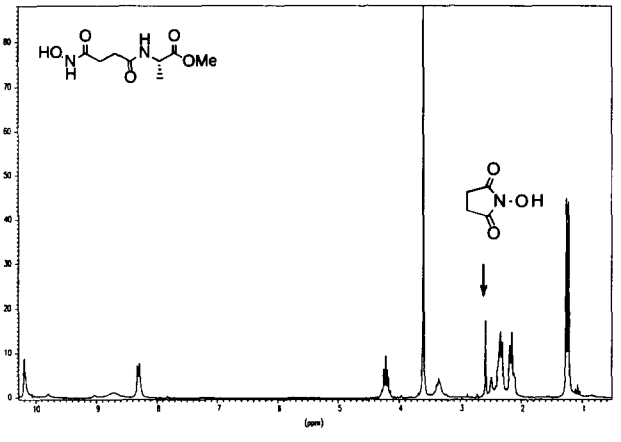
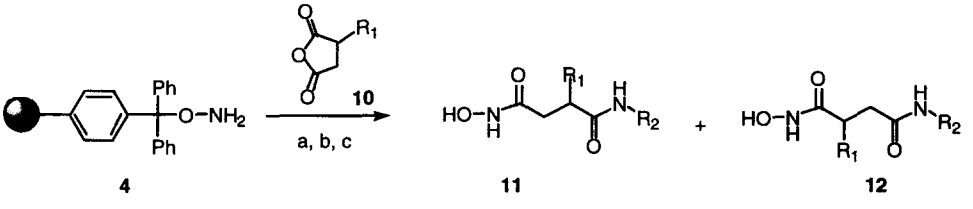
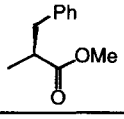
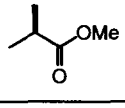


Figure 1. Crude ^{1}H NMR of succinamide 8b (DMSO-d_6, 200 MHz)



Compound 10, 11, 12

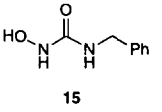
entry	a	b	c	d
R_1 =	Me	Ph	Bn	
R_2 =				

Reagents and conditions: a) 60 °C, 6 h, THF; b) (S)-phenylethylamine or amino acid eater hydrochloride/ triethylamine, HOBt, DCC (5-fold excess), DMF, 6 h; c) HCO_2H/THF (1:3), 1 h

Scheme 3

HO-NH-Leu-Pro-Z **13**

HO-NH-Val-Phe-Gly-Z **14**



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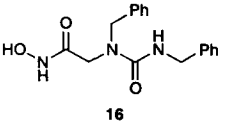

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Scheme 4

In summary, we have developed a novel linkage for the generation of hydroxamic acids on solid support. Efforts towards the synthesis of heterocyclic hydroxamic acids and the application of this linkage to the production of a variety of combinatorial libraries for biological screening are under way.

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References and notes:

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3. During the course of this work methods for the synthesis of hydroxamic acids on solid support have been published: a) Floyd, C. D.; Lewis, C. N.; Patel, S. R. and Whittaker, M. *Tetrahedron Lett.* **1996**, *37*, 8045. b) Richter, L. S.; Desai, M. C. *Tetrahedron Lett.* **1997**, *38*, 321. c) Mellor, S.L.; McGuire, C.; Chan, W.C. *Tetrahedron Lett.* **1997**, *38*, 3311.
4. To a solution of N-hydroxyphthalimide (0.8 g, 5 mmol) and triethylamine (0.5 g, 5 mmol) in 8 mL dry DMF was added trityl chloride resin (1.0 g, 1 mmol/g loading, Novabiochem). After 12 h the resin was collected by filtration, washed successively with DMF, water, THF, MeOH and diethylether and then dried in vacuo. This resin was suspended in THF (10 mL) and hydrazine hydrat (1 mL) was added. After 12 h the resin was collected, washed with DMF, 5 M ammonium hydroxid solution, water, THF, MeOH and diethylether and then dried in vacuo. Loading >= 0.9 mmol/g. FT-IR (3: vmax (KBr) 1792, 1743, 1667, and 1602 cm-1; 4: vmax (KBr) 1653, and 1597 cm-1) revealed the absence of any carbonyl substituent confirming the hydrazinolysis of phthalimide. The loadings of 3 and 4 were determined by weight recovery of N-hydroxyphthalimide (upon cleavage) and phthalylhydrazide by-product, respectively. They correlated well with the observed yields of products.
5. All compounds were characterized by the usual spectroscopic techniques (^{1}H NMR, ^{13}C NMR and MS). Compound **8b**: ^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.23 (d, J = 7.2 Hz, 3H, CH_3); 2.07 - 2.43 (m, 4H, CH_2); 3.60 (s, 3H, OCH_3); 4.22 (qn, J = 7.2 Hz 1H, CH); 8.27 (bd, ≈1H, NH); 10.20 (s, ≈1H, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 16.83 (CH_3); 27.54, 30.11 ($2CH_2$); 47.35 (CH); 51.54 (OCH_3); 168.18, 170.92, 172.94 (3C=O). MS (CI, NH_3): m/z (%) = 219 (10) [M^+ + 1], 203 (29), 186 (78), 104 (96), 44 (100). Reactions of polymer bound materials were monitored by FT-IR. In addition, 15% by weight of each dry resin-bound intermediate was retained and the products resulted upon cleavage from the solid support were characterized by NMR and MS.
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