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Solvent-Controlled Chemoselectivity in the Photolytic Release of Hydroxamic Acids and Carboxamides from Solid Support

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Supporting Information

ABSTRACT: The synthetic utility and theoretical basis of a photolabile hydroxylamine-linker are presented. The developed protocols enable the efficient synthesis and chemoselective photolytic release of either hydroxamates or carboxamides from solid support. The bidetachable mode of the linker unit is uniquely dependent on the solvent. Hydroxamic acids are obtained by performing photolysis in protic solvents, whereas photolysis in aprotic solvents enables the selective release of carboxamides.

Hydroxamic acids have been the source of much biochemical interest in recent years. Therefore, the use of solid-phase combinatorial chemistry for high-throughput generation of structurally diverse hydroxamic acids is highly relevant. Although hydroxamic acids may be obtained by direct cleavage of resin-bound esters with hydroxylamine derivatives, this strategy requires an excess of hydroxylamine and/or addition of base which complicates postcleavage workup. Several approaches involving resin-bound hydroxylamine linkers have been reported. However, these hydroxamate linkages suffer from only being cleavable under acidic conditions, which limits the range of chemical transformations applicable to the solid-phase synthesis of structurally diverse hydroxamic acids. Therefore, other cleavage principles are necessary in order to provide complex molecules assembled through a diverse range of chemical reactivity. A linker system that can be cleaved under photolytic conditions may be considered truly orthogonal in this context. Furthermore, photolytic cleavage offers a mild method of cleavage which is particularly attractive for the direct release of screening compounds into biological screens without contamination by cleavage reagents.

We now wish to report a complete study on a photolabile linker based on the o-nitroveratraldehyde group capable of releasing hydroxamates upon UV irradiation. Uniquely, this linker unit may function as a “bidetachable” system. By simply varying the reaction solvent, the photolysis can be controlled to provide either C−O or C−N bond cleavage, which allows for controlled release of the hydroxamate or carboxamide, respectively (Figure 1). This strategy may introduce further diversity into target molecules and compound libraries. Linker 4 was readily prepared in a few high-yielding steps (Scheme 1) before being explored as a hydroxamate-releasing linker. A N-[[(1H-benzo triazol-1-yl)(dimethylamino)methylene]-N-methylmethylaminium tetrafluoroborate N-oxide (TBTU)-mediated coupling of 4 to a Rink linker attached to the commercially available amino-functionalized support (PEGA400) afforded the hydroxylamine-functionalized photolabile support. Using standard TBTU-mediated peptide coupling reactions, derivative 5a was synthesized as a simple and easily monitorable model system. Photolytic cleavage was carried out on resin suspended in H2O/MeOH (4:1) by irradiating for 30 min at rt with 365 nm light using an LED UV-lamp. Analysis of the released material via RP-UPLC, however, showed release of two products: the hydroxamate 7a and the carboxamide 8a resulting from C−O and N−O cleavage, respectively, in a 3:4 ratio.

The nature of the solvent and the acidity of the solution have been demonstrated to have pronounced effects on the kinetics and equilibrium position of aci-nitro compounds (Figure 2). We first explored the solvent effects in the

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Figure 1. 4,5-Dialkoxy-2-nitrobenzyl moiety in photolabile linkers for solid-phase synthesis.
photolysis of 6a on the level of final product formation. The photoreaction was studied by photolyzing aliquots of the resin 6a in various solvents and determining the product distribution via HPLC analysis. Because the solvent also influences the swelling and solvation properties of the support, the obtained results are merely qualitative. While this technique did not allow us to quantify the amount of products formed, it did provide an expedient method to determine the relative photoproduction yield profiles. Selected product yield profiles are listed in Table 1 (for a comprehensive list consult the Supporting Information (SI)). It is evident that the solvent has a strong influence on the product ratio of the reaction and some general conclusions may be drawn. Polar solvents favor formation of the hydroxamic acid product 7a, while apolar solvents mainly give the carboxamide product 8a. In particular, the polar fluorinated alcohol, hexafluoroisopropanol (HFIP), with a high hydrogen-bond-donating ability led to hydroxamic acid product 7a with high selectivity. Apolar solvents favor formation of the carboxamide product 8a over hydroxamate product 7a. Notably, when using mesitylene, carboxamide product 8a was formed exclusively.

Table 1. Relative Product Yields for Photolysis of 5a at 360 nm in Various Solvents

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>product 7a:8a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mesitylene</td>
<td>0:100</td>
</tr>
<tr>
<td>B</td>
<td>MeOH/H2O (1:4)</td>
<td>67:33</td>
</tr>
<tr>
<td>C</td>
<td>H2O</td>
<td>60:40</td>
</tr>
<tr>
<td>D</td>
<td>HFIP</td>
<td>98:2</td>
</tr>
<tr>
<td>E</td>
<td>mesitylene/HFIP (1:1)</td>
<td>98:2</td>
</tr>
</tbody>
</table>

*Photolytic cleavage was carried out for 0.5 h with an LED UV-lamp (360 nm). *Product distribution was determined by RP-HPLC.

The effect of Lewis acid catalysis on the photoreaction of 5a has also been investigated (SI). The qualitative studies showed that a wide range of Lewis acids favor the formation of the hydroxamate product. The most efficacious Lewis acid was found to be BF3, giving high selectivity toward formation of the hydroxamate product 8a.

It is well-known that o-nitroveratryl compounds upon irradiation undergo a Norrish Type II β-hydrogen abstraction to give the biradical intermediate 11,12 which after photostabilization forms the E,Z-12 and Z,Z-12 isomers (Figure 2). From there it can again undergo isomerization to the E-ace-nitro forms 14EE and 14EE. Measurements of ace-nitro transients have confirmed the presence of 13 as an intermediate between 12 and 14,13 and direct isomerization of 12 to 14 by rotation about the C==N bond has been excluded.13,14 Also, the conversion of 12 to 14 via direct proton shift between the two oxygen atoms of the ace-nitro group (without participation of a water molecule) seems unlikely.15 The activation barrier computed by our density functional theory calculations yielded a barrier of 123 kJ mol−1 for this direct proton shift in the species 12 derived from 9, where R1 = Ph, R2 = Me.15 Furthermore, inclusion of a single water molecule to mediate the shift of this proton was computed to lower the activation barrier by at least 81 kJ mol−1 (see SI for computational details). The presence of additional water molecules in bulk solution should lower the activation barrier for water-mediated proton exchange even further.15 Taking this solvation effect into account, water-mediated proton exchange (or proton transfer mediated by other protic solvents) via the anion 13 can be assumed to be the most likely path between the nitronic acid isomers 12 and 14, with an activation barrier of only a few kJ mol−1. Based on this discussion, we assume that the equilibrium between the two possible protonation sites on the nitronic acid 11...
function is established on the ns time scale in polar protic solutions.

It is generally assumed that the decay of the aci-nitro forms is the rate-limiting step in the photoisomerization of o-nitrobenzyl derivatives and that cyclization to form intermediate 15 proceeds only from the neutral aci-tautomers. Under conditions where interconversion between the two aci-nitro forms is efficient, we expect the "normal" hydroxamic acid product forming pathway (14 → 15 → 16) to be fast. However, in an aprotic solvent where ionization to 13− does not occur, the Z-aci-nitro species 12 give rise to a N−O bond fragmentation pathway, which generates the amide and nitroketone products 19 and 20. The proposed mechanism is depicted in Figure 2.

To further investigate the photolysis of hydroxamate-functionalized o-nitroveratryl compounds, we synthesized 21 (SI) as a model compound and studied the photolysis in solution (Figure 3). Hereby we were able to identify the nature of byproducts formed in the photolysis of a hydroxamate-functionalized o-nitroveratryl compound. Furthermore, solution phase photolysis experiments provide the opportunity to study the photolysis without influences from swelling and solvation properties of the solid support. Photolysis of 21 was carried out in a broad range of solvents (see SI). The low solubility of 21 did not allow an investigation of irradiation experiments in mesitylene and saturated hydrocarbon solvents. In polar solvents and in acidic solutions (CH3CN, HFIP, 1% TFA in MeOH) the hydroxamic acid product formation is the only observed pathway, while the apolar solvent toluene gave a mixture of hydroxamic acid 7a and carboxamide 8a in a ratio of 1:1. Two examples of our results with 21 are shown in Figure 3.

Each peak in the chromatograms is characterized and identified by UPLC-MS. In accordance with our proposed mechanism, we observed from these experiments that the major byproduct formed in polar solvents was o-nitrosobenzaldehyde 22, with only minor impurities of 23, while o-nitrosobenzaldehyde 24 and o-nitrobenzaldehyde 23 were formed in a ratio of ∼1:1 in toluene. The absence of other peaks in the chromatograms indicates that no other side reactions had occurred. Confident with the photolysis strategy, we employed the hydroxylamine linker 4 for the parallel synthesis of a library of putatively HDAC inhibitors (Table 2 and SI). A Rink linker was positioned between the support and the photolinker unit to optimize and verify attachment chemistry of linker 4 on the solid support. After incubating the supports 5a−e with TFA/CH2Cl2 (1:1) for 2 h, one major peak corresponding to cleavage of the Rink linker was generally observed (6a−e), indicating high efficiency of the attachment chemistry of 4 and high stability of the photolabile unit toward TFA deprotection conditions normally used in standard peptide synthesis procedures. Photolytic cleavage was carried out on 30−100 mg of resin suspended in appropriate solvent by irradiating for 0.5−3 h at rt with 365 nm light using an LED UV-lamp. We showed the possibility of selectively cleaving these compounds to give the hydroxamate and the carboxamide products, respectively. Selected examples of cleavage of a variety of compounds are presented in Table 2 (for a more elaborate study on cleavage of the full compound library, see SI). From Table 2 it can be concluded that the developed solid-phase methodology is very robust and applicable to a range of both aromatic and aliphatic hydroxamates. The liberated products were recovered in high purity (90−95%) and satisfactory yields (35−63%).

While the linker 4 has been shown to be stable toward both acidic and basic condition, we investigated the utility of the linker for the synthesis of acid- and base-labile substrates. Both hydroxamate functionalized amino acid derivatives containing Boc- (7h) and Fmoc- (7g) protected α-amino groups, Trt-protected amide (7g), and Pbf-protected guanidinium (7h) side chain groups were successfully released, demonstrating the extraordinary protecting group compatibility of this linker resin.

To further demonstrate the synthetic potential of the linker for the generation of more complex structures, we investigated the use of linker 4 for the synthesis of a derivative of a known diketopiperazin (Dkp) hydroxamic acid HDAC inhibitor. Massive efforts in solid-phase synthesis have strived for the development of synthesis methodology, which systematically generates natural product-like compounds of high spatial complexity. In this context a current limitation is the difficulties faced in the synthesis of acid and base sensitive scaffolds, including racemization-prone structures. To demonstrate the use of linker 4 for the generation of the hydroxamate-functionalized Dkp derivative 25, a serine-terminated oligomeric peptide sequence 24 was assembled on a hydroxylamine-functionalized photolabile support by standard SPPS protocols. Exposing the resin 24 to classical periodate oxidation

Table 2. Synthesis and Photolytic Release of Hydroxamates 7a−h and Carboxamides 8a−h

<table>
<thead>
<tr>
<th>entry (a)</th>
<th>substrate</th>
<th>purity (%) (b)</th>
<th>yield (%)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>6a</td>
<td>80</td>
<td>7a: 59</td>
</tr>
<tr>
<td>B</td>
<td>7a</td>
<td>&gt;95</td>
<td>8a: 58</td>
</tr>
<tr>
<td></td>
<td>7b</td>
<td>&gt;95</td>
<td>8b: 53</td>
</tr>
<tr>
<td></td>
<td>7c</td>
<td>&gt;95</td>
<td>8c: 46</td>
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<td></td>
<td>7d</td>
<td>&gt;95</td>
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<td></td>
<td>7e</td>
<td>94</td>
<td>7f: 54</td>
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<td></td>
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<td>94</td>
<td>7h: 63</td>
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<tr>
<td></td>
<td>7j</td>
<td>95</td>
<td>8h: 51</td>
</tr>
</tbody>
</table>

(a) Photolytic cleavage was carried out for 2 h with an LED UV-lamp (360 nm). (b) Purity was determined by RP-HPLC.

Figure 3. Study of product distribution for the photolytic degradation of 21 in HFIP and toluene, respectively.
Conditions generated the corresponding aldehyde, and subsequent TFA treatment mediated the N-acyliminium cyclization. Rewardingly, photolytic release gave the hydroxamate-functionalized DKP-derivative 25 in high purity (Scheme 2).

Scheme 2. Synthesis of a Hydroxamate-Functionalized Fused Natural Product-like DKP Derivative (25)

In summary, we have developed a photolabile hydroxylamine linker for the synthesis of hydroxamic acids on solid support. The synthesis strategy shows excellent compatibility with a range of structurally diverse compounds. The linker is compatible with most commonly used protecting groups for SPPS and remains intact throughout the multistep synthesis. Products are ultimately released from the solid support in high purity using light. In addition, this linker unit may also function in a bidetachable mode, enabling the release of the corresponding carboxamides when photolysis is performed in an aprotic solvent. Based on results from density functional theory calculations, the present paper provides evidence of the mechanism allowing for the control and selection between these two competing reaction pathways. Finally, we have demonstrated the use of the linker for the generation of a pharmacologically relevant hydroxamate-functionalized natural product-like DKP derivative in high purity.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01386.

Experimental details; RP-HPLC, RP-UPLC, MS, 1H and 13C NMR data; computational details (PDF)

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Notes

The authors declare no competing financial interest.

**REFERENCES**


