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

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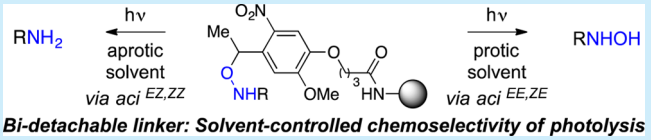
Supporting Information

**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.[1] Therefore, the use of solid-phase[2] combinatorial chemistry[3] 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,[4] 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.[5] 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.[6] Futhermore, 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*-nitroveratryl group[7] 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)[8] before being explored as a hydroxamate-releasing linker. A *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU)-mediated coupling of **4** to a Rink linker attached to the commercially available amino-functionalized support ($PEGA_{800}$) 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 $H_2O$/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.

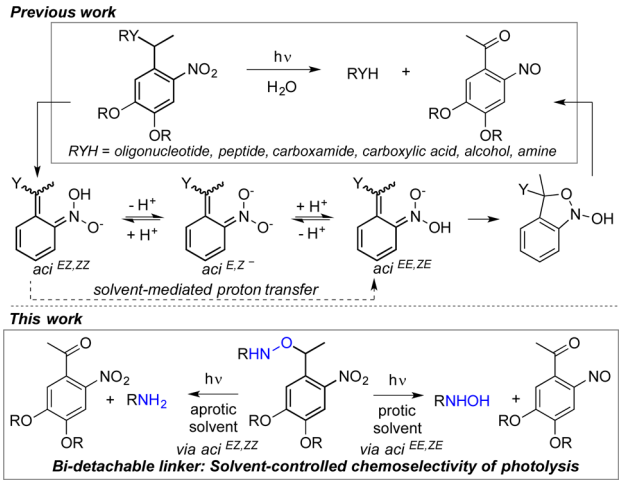



**Figure 1.** 4,5-Dialkoxy-2-nitrobenzyl moiety in photolabile linkers for solid-phase synthesis.

The nature of the solvent[9] and the acidity of the solution[10,11] have been demonstrated to have pronounced effect on the kinetics and equilibrium position of *aci*-nitro compounds (Figure 2). We first explored the solvent effects in the

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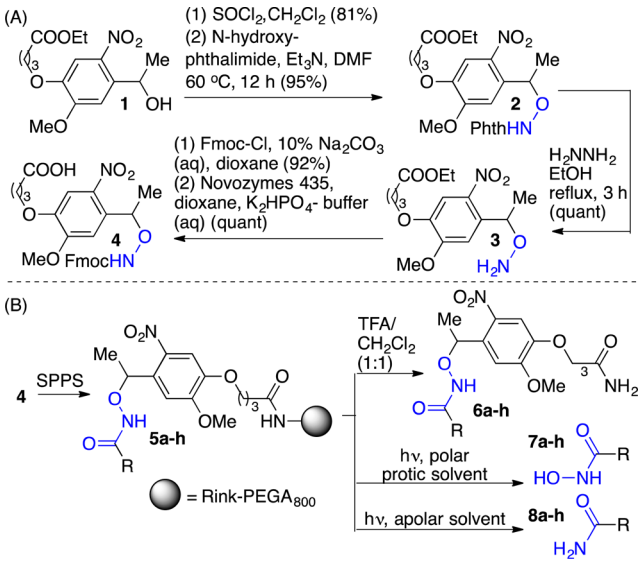
**Scheme 1. (A) Synthesis of Fmoc-Protected Hydroxylamine-Functionalized Carboxylic Acid Linker (4); (B) Application of Linker 4 in Solid-Phase Peptide Synthesis (SPPS) for the Photolytic Release of Peptide Hydroxamic Acids and Carboxamides**




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 photoproduct formation. 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**

| entry[a] | solvent | product **7a**:**8a**[b] |
|---|---|---|
| A | mesitylene | 0:100 |
| B | MeOH/$H_2O$ (1:4) | 67:33 |
| C | $H_2O$ | 60:40 |
| D | HFIP | 98:2 |
| E | mesitylene/HFIP (1:1) | 98:2 |

[a]Photolytic cleavage was carried out for 0.5 h with an LED UV-lamp (360 nm). [b]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 $BF_3$, 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 $\beta$-hydrogen abstraction to give the biradical intermediate **11**,[12] which after photoisomerization forms the *E,Z*-**12** and *Z,Z*-**12** isomers (Figure 2). From there it can again undergo isomerization to the *E-aci*-nitro forms $\mathbf{14}^{EE}$ and $\mathbf{14}^{ZE}$. Measurements of *aci*-nitro transients have confirmed the presence of $\mathbf{13}^-$ as an intermediate between **12** and **14**,[11] 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 *aci*-nitro group (without participation of a water molecule) seems unlikely.[15,11] 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 $R^1$ = Ph, $R^2$ = Me. 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

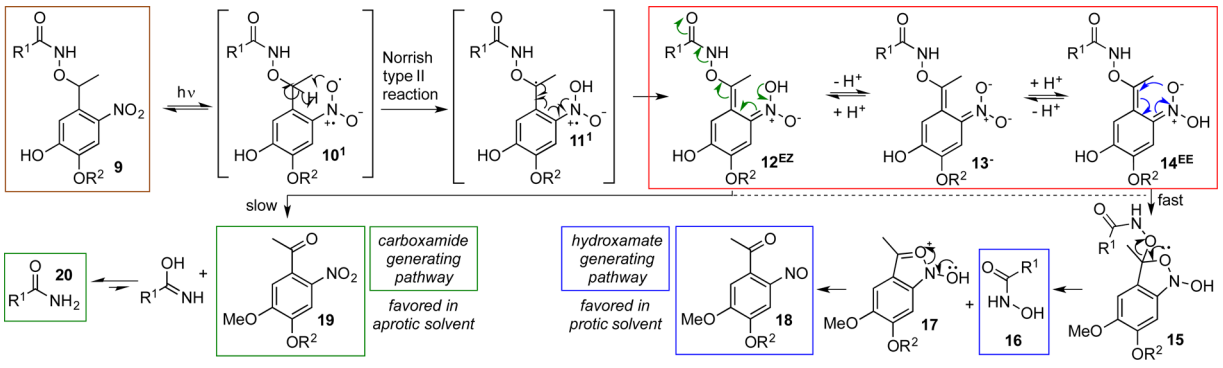



**Figure 2.** Proposed mechanism for the photolytic degradation of hydroxamate-functionalized *o*-nitroveratryl derivatives. For simplicity, only the *E*-isomers with regard to the =C–OR group are shown.



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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*-tautomer **14**.[11−13] 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 aprotic solvent where ionization to $\mathbf{13}^-$ 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

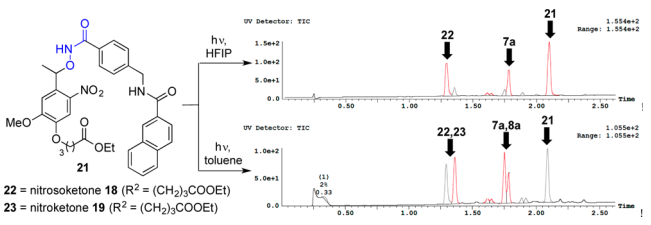
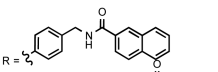
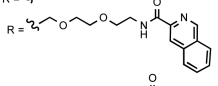
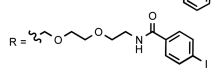
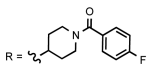
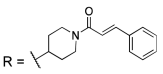
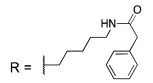
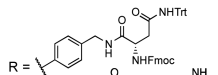
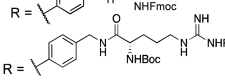



**Figure 3.** Study of product distribution for the photolytic degradation of **21** in HFIP and toluene, respectively.

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 ($CH_3CN$, 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 agreement 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 **22** 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[16] (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/$CH_2Cl_2$ (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

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

| entry[a] | substrate | purity (%)[b] | yield (%) |
|---|---|---|---|
| A |  | **6a**: 80 | **7a**: 59 |
| | | **7a**: > 95 | **8a**: 58 |
| | | **8a**: > 95 | |
| B |  | **6b**: 92 | **7b**: 61 |
| | | **7b**: > 95 | **8b**: 53 |
| | | **8b**: 90 | |
| C |  | **6c**: > 95 | **7c**: 55 |
| | | **7c**: > 95 | **8c**: 46 |
| | | **8c**: > 95 | |
| D |  | **6d**: 95 | **7d**: 56 |
| | | **7d**: > 95 | **8d**: 49 |
| | | **8d**: > 95 | |
| E |  | **6e**: 94 | **7e**: 47 |
| | | **7e**: 94 | **8e**: 35 |
| | | **8e**: > 95 | |
| F |  | **7f**: > 95 | **7f**: 54 |
| | | **8f**: 93 | **8f**: 40 |
| G |  | **7g**: > 95 | **7g**: 48 |
| | | **8g**: 94 | **8g**: 46 |
| H |  | **7h**: 94 | **7h**: 63 |
| | | **8h**: > 95 | **8h**: 51 |

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

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 $\alpha$-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 diketopiperazine (DKP) hydroxamic acid HDAC inhibitor.[17]

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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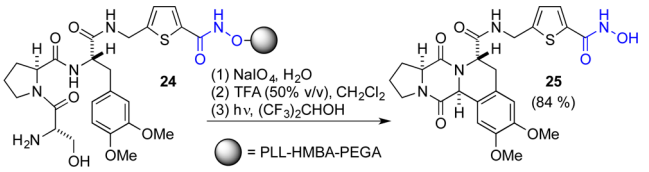
conditions generated the corresponding aldehyde, and subsequent TFA treatment mediated the *N*-acyliminium cyclization. Rewardingly, photolytic release gave the hydroxamate-functionalized DKP-derivative **25** in high purity (Scheme 2).

**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, $^{1}$H and $^{13}$C NMR data; computational details (PDF)

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### Notes

The authors declare no competing financial interest.

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