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

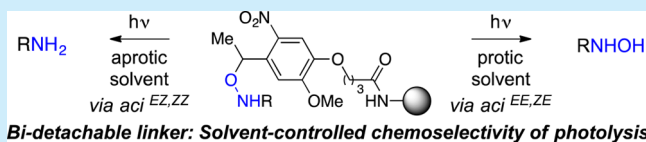
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S Supporting Information

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-phase² combinatorial chemistry³ 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 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*-nitroveratryl group⁷ 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 (PEGA₈₀₀) afforded the

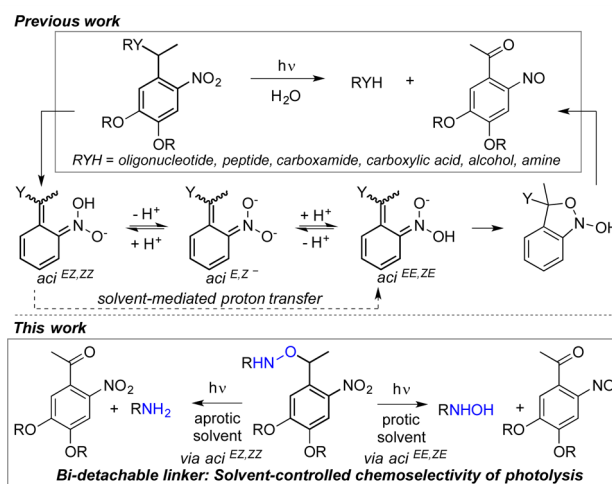


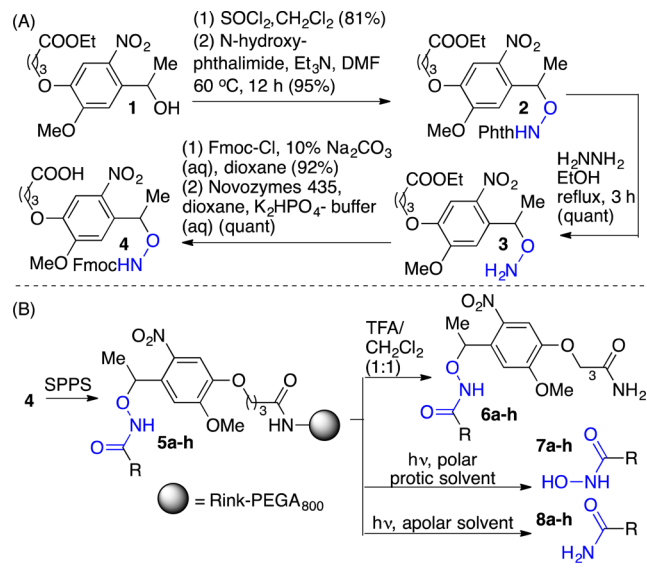
Figure 1. 4,5-Dialkoxy-2-nitrobenzyl moiety in photolabile linkers for solid-phase synthesis.

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 solvent⁹ 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



64 photolysis of **6a** on the level of final product formation. The
 65 photoreaction was studied by photolyzing aliquots of the resin
 66 **6a** in various solvents and determining the product distribution
 67 via HPLC analysis. Because the solvent also influences the
 68 swelling and solvation properties of the support, the obtained
 69 results are merely qualitative. While this technique did not
 70 allow us to quantify the amount of products formed, it did
 71 provide an expedient method to determine the relative
 72 photoproduct formation. Selected product yield profiles are
 73 listed in [Table 1](#) (for a comprehensive list consult the
 74 [Supporting Information \(SI\)](#)). It is evident that the solvent
 75 has a strong influence on the product ratio of the reaction and
 76 some general conclusions may be drawn. Polar solvents favor
 77 formation of the hydroxamic acid product **7a**, while apolar
 78 solvents mainly give the carboxamide product **8a**. In particular,
 79 the polar fluorinated alcohol, hexafluoroisopropanol (HFIP),
 80 with a high hydrogen-bond-donating ability led to hydroxamic
 81 acid product **7a** with high selectivity. Apolar solvents favor
 82 formation of the carboxamide product **8a** over hydroxamate
 83 product **7a**. Notably, when using mesitylene, carboxamide
 84 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 ₂ O (1:4)	67:33
C	H ₂ O	60:40
D	HFIP	98:2
E	mesitylene/HFIP (1:1)	98:2

^aPhotolytic cleavage was carried out for 0.5 h with an LED UV-lamp (360 nm). ^bProduct distribution was determined by RP-HPLC.

The effect of Lewis acid catalysis on the photoreaction of **5a** 85
 has also been investigated ([SI](#)). The qualitative studies showed 86
 that a wide range of Lewis acids favor the formation of the 87
 hydroxamate product. The most efficacious Lewis acid was 88
 found to be BF₃, giving high selectivity toward formation of the 89
 hydroxamate product **8a**. 90

It is well-known that *o*-nitroveratryl compounds upon 91
 irradiation undergo a Norrish Type II β -hydrogen abstraction 92
 to give the biradical intermediate **11**,¹² which after photo- 93
 isomerization forms the *E,Z*-**12** and *Z,Z*-**12** isomers ([Figure 2](#)). 94
 From there it can again undergo isomerization to the *E-aci*- 95
 nitro forms **14^{EE}** and **14^{ZE}**. Measurements of *aci*-nitro transients 96
 have confirmed the presence of **13⁻** as an intermediate between 97
12 and **14**,¹¹ and direct isomerization of **12** to **14** by rotation 98
 about the C=N bond has been excluded.^{13,14} Also, the 99
 conversion of **12** to **14** via direct proton shift between the two 100
 oxygen atoms of the *aci*-nitro group (without participation of a 101
 water molecule) seems unlikely.^{15,11} The activation barrier 102
 computed by our density functional theory calculations yielded 103
 a barrier of 123 kJ mol⁻¹ for this direct proton shift in the 104
 species **12** derived from **9**, where R¹ = Ph, R² = Me. 105
 Furthermore, inclusion of a single water molecule to mediate 106
 the shift of this proton was computed to lower the activation 107
 barrier by at least 81 kJ mol⁻¹ (see [SI](#) for computational 108
 details). The presence of additional water molecules in bulk 109
 solution should lower the activation barrier for water-mediated 110
 proton exchange even further.¹⁵ Taking this solvation effect 111
 into account, water-mediated proton exchange (or proton 112
 transfer mediated by other protic solvents) via the anion **13** can 113
 be assumed to be the most likely path between the nitronic acid 114
 isomers **12** and **14**, with an activation barrier of only a few kJ 115
 mol⁻¹. Based on this discussion, we assume that the equilibrium 116
 between the two possible protonation sites on the nitronic acid 117

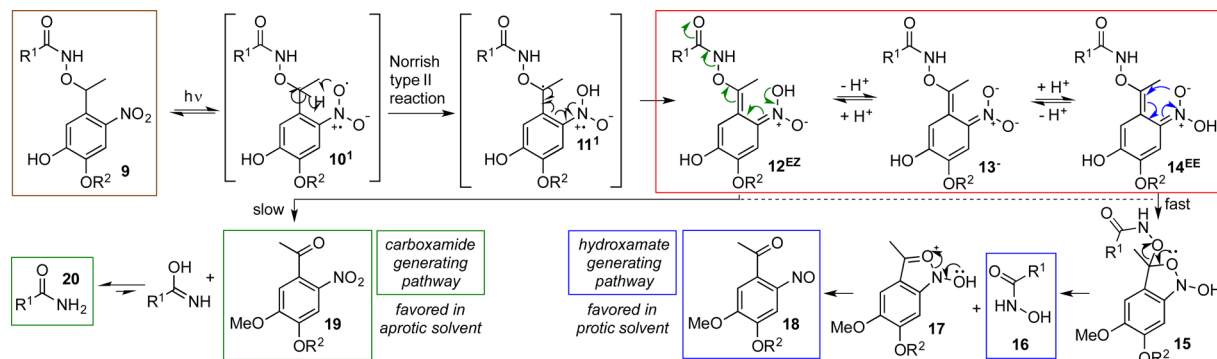


Figure 2. Proposed mechanism for the photolytic degradation of hydroxamate-functionalized *o*-nitroveratryl derivatives. For simplicity, only the *E*-isomers with regard to the =C-OR group are shown.

118 function is established on the ns time scale in polar protic
119 solutions.

120 It is generally assumed that the decay of the *aci*-nitro forms is
121 the rate-limiting step in the photoisomerization of *o*-nitrobenzyl
122 derivatives and that cyclization to form intermediate **15**
123 proceeds only from the neutral *aci*-tautomer **14**.^{11–13} Under
124 conditions where interconversion between the two *aci*-nitro
125 forms is efficient, we expect the “normal” hydroxamic acid
126 product forming pathway (**14** → **15** → **16**) to be fast.
127 However, in aprotic solvent where ionization to **13**[−] does not
128 occur, the *Z-aci*-nitro species **12** give rise to a N–O bond
129 fragmentation pathway, which generates the amide and
130 nitroketone products **19** and **20**. The proposed mechanism is
131 depicted in Figure 2.

132 To further investigate the photolysis of hydroxamate-
133 functionalized *o*-nitroveratryl compounds, we synthesized **21**
134 (SI) as a model compound and studied the photolysis in
135 solution (Figure 3). Hereby we were able to identify the nature

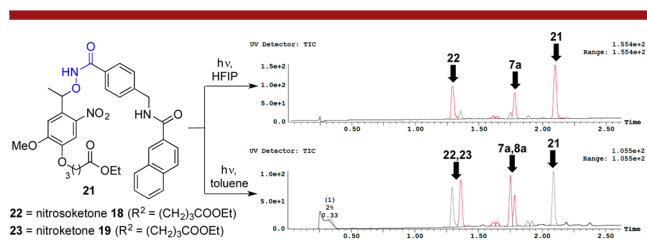


Figure 3. Study of product distribution for the photolytic degradation of **21** in HFIP and toluene, respectively.

136 of byproducts formed in the photolysis of a hydroxamate-
137 functionalized *o*-nitroveratryl compound. Furthermore, solution
138 phase photolysis experiments provide the opportunity to study
139 the photolysis without influences from swelling and solvation
140 properties of the solid support. Photolysis of **21** was carried out
141 in a broad range of solvents (see SI). The low solubility of **21**
142 did not allow an investigation of irradiation experiments in
143 mesitylene and saturated hydrocarbon solvents. In polar
144 solvents and in acidic solutions (CH₃CN, HFIP, 1% TFA in
145 MeOH) the hydroxamic acid product formation is the only
146 observed pathway, while the apolar solvent toluene gave a
147 mixture of hydroxamic acid **7a** and carboxamide **8a** in a ratio of
148 1:1. Two examples of our results with **21** are shown in Figure 3.
149 Each peak in the chromatograms is characterized and identified
150 by UPLC-MS. In agreement with our proposed mechanism, we
151 observed from these experiments that the major byproduct
152 formed in polar solvents was *o*-nitrosobenzaldehyde **22**, with
153 only minor impurities of **23**, while *o*-nitrosobenzaldehyde **22**
154 and *o*-nitrobenzaldehyde **23** were formed in a ratio of ~1:1 in
155 toluene. The absence of other peaks in the chromatograms
156 indicates that no other side reactions had occurred. Confident
157 with the photolysis strategy, we employed the hydroxylamine
158 linker **4** for the parallel synthesis of a library of putatively
159 HDAC inhibitors¹⁶ (Table 2 and SI). A Rink linker was
160 positioned between the support and the photolinker unit to
161 optimize and verify attachment chemistry of linker **4** on the
162 solid support. After incubating the supports **5a–e** with TFA/
163 CH₂Cl₂ (1:1) for 2 h, one major peak corresponding to
164 cleavage of the Rink linker was generally observed (**6a–e**),
165 indicating high efficiency of the attachment chemistry of **4** and
166 high stability of the photolabile unit toward TFA deprotection
167 conditions normally used in standard peptide synthesis
168 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 : > 95 8a : > 95	7a : 59 8a : 58
B		6b : 92 7b : > 95 8b : 90	7b : 61 8b : 53
C		6c : > 95 7c : > 95 8c : > 95	7c : 55 8c : 46
D		6d : 95 7d : > 95 8d : > 95	7d : 56 8d : 49
E		6e : 94 7e : 94 8e : > 95	7e : 47 8e : 35
F		7f : > 95 8f : 93 7g : > 95 8g : 94	7f : 54 8f : 40
G		7g : > 95 8g : 94 7h : 94 8h : > 95	7g : 48 8g : 46
H		7h : 94 8h : > 95	7h : 63 8h : 51

^aPhotolytic cleavage was carried out for 2 h with an LED UV-lamp (360 nm). ^bPurity was determined by RP-HPLC.

mg of resin suspended in appropriate solvent by irradiating for 169
0.5–3 h at rt with 365 nm light using an LED UV-lamp. We 170
showed the possibility of selectively cleaving these compounds 171
to give the hydroxamate and the carboxamide products, 172
respectively. Selected examples of cleavage of a variety of 173
compounds are presented in Table 2 (for a more elaborate 174
study on cleavage of the full compound library, see SI). From 175
Table 2 it can be concluded that the developed solid-phase 176
methodology is very robust and applicable to a range of both 177
aromatic and aliphatic hydroxamates. The liberated products 178
were recovered in high purity (90–95%) and satisfactory yields 179
(35–63%). 180

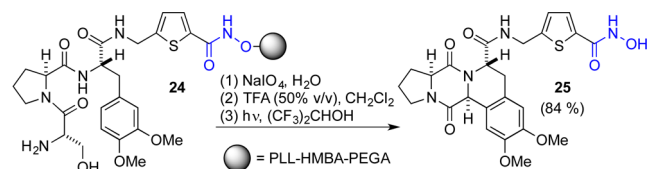
While the linker **4** has been shown to be stable toward both 181
acidic and basic condition, we investigated the utility of the 182
linker for the synthesis of acid- and base-labile substrates. Both 183
hydroxamate functionalized amino acid derivatives containing 184
Boc- (**7h**) and Fmoc- (**7g**) protected α -amino groups, Trt- 185
protected amide (**7g**), and Pbf-protected guanidinium (**7h**) side 186
chain groups were successfully released, demonstrating the 187
extraordinary protecting group compatibility of this linker resin. 188

To further demonstrate the synthetic potential of the linker 189
for the generation of more complex structures, we investigated 190
the use of linker **4** for the synthesis of a derivative of a known 191
diketopiperazine (DKP) hydroxamic acid HDAC inhibitor.¹⁷ 192

Massive efforts in solid-phase synthesis have strived for the 193
development of synthesis methodology, which systematically 194
generates natural product-like compounds of high spatial 195
complexity. In this context a current limitation is the difficulties 196
faced in the synthesis of acid and base sensitive scaffolds, 197
including racemization-prone structures. To demonstrate the 198
use of linker **4** for the generation of the hydroxamate- 199
functionalized DKP derivative **25**, a serine-terminated oligo- 200
meric peptide sequence **24** was assembled on a hydroxylamine- 201
functionalized photolabile support by standard SPPS protocols. 202
Exposing the resin **24** to classical periodate oxidation 203

204 conditions generated the corresponding aldehyde, and
 205 subsequent TFA treatment mediated the *N*-acyliminium
 206 cyclization. Rewardingly, photolytic release gave the hydrox-
 207 amate-functionalized DKP-derivative **25** in high purity (Scheme
 208 2).

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



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

226 ■ ASSOCIATED CONTENT

227 ● Supporting Information

228 The Supporting Information is available free of charge on the
 229 ACS Publications website at DOI: [10.1021/acs.orglett.7b01386](https://doi.org/10.1021/acs.orglett.7b01386).

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

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239 Notes

240 The authors declare no competing financial interest.

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