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Site-Selective Esterifications of Polyol β-Hydroxyamides and Applications to Serine-Selective Glycopeptide Modifications

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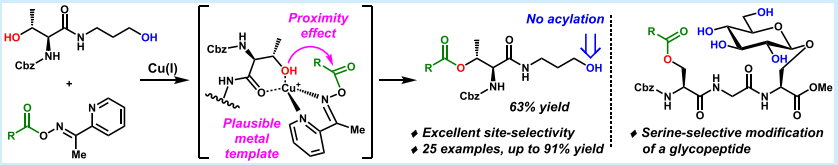
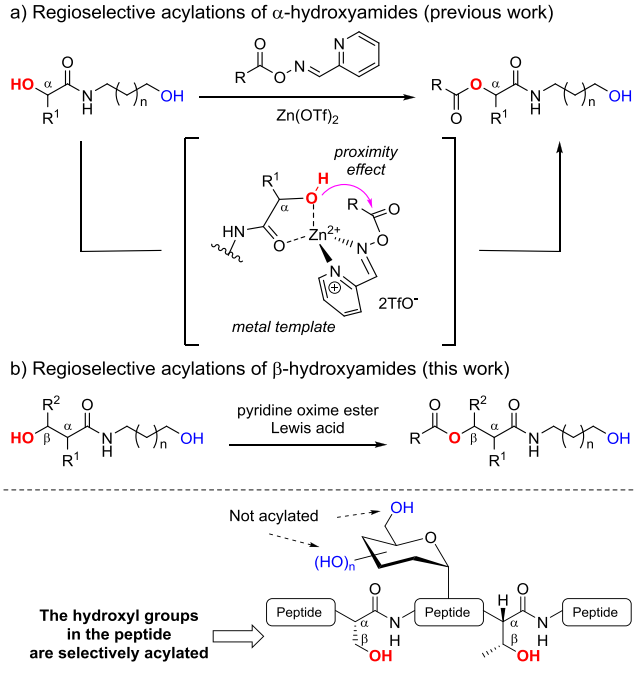
Supporting Information



ABSTRACT: The site-selective acylations of β-hydroxyamides in the presence of other hydroxyl groups are described. Central to the success of this modification is the metal-template-driven acylation using pyridine ketoxime esters as acylating reagents in combination with CuOTf. This strategy enables β-hydroxyl groups to be site-selectively acylated in various derivatives, including sterically hindered secondary β-alcohol. The utility of this methodology is showcased by the serine-selective modification of a glycopeptide with unprotected sugar.

Peptide-based drugs have attracted significant attention because they exhibit therapeutic specificities and efficacies similar to those of protein biologics.[1] Recent representative examples of peptide-based drugs are the glycopeptides used for mucin antitumor vaccines, which induce specific immune responses by inhibiting protein-binding events.[2] To further advance research into glycopeptide-based drugs, the development of useful chemical modification methods that impart new functionalities onto existing glycopeptides is essential. The hydroxyl groups of the side chains of serine and threonine can be used as targets when modifying peptides.[3−5] In the case of glycopeptides in which the attached sugars play important roles in peptide/cell interactions, the selective functionalization of the hydroxyl groups of Ser or Thr residues in the presence of the sugar hydroxyl groups is problematic. Therefore, the development of novel site-selective modification reactions for the Ser or Thr residues in unprotected glycopeptides is highly important for the development of glycopeptides as pharmaceuticals. We also reported a site-selective acylation of the α-hydroxyamides in polyols using pyridine aldoxime esters as acylating reagents (Scheme 1a).[6−10] In this system, a catalytic amount of $Zn(OTf)_2$ was used to moderately activate the pyridine aldoxime ester. The resulting metal complex attracted an α-hydroxyamide to its Lewis basic site, resulting in the formation of a metal template for the facile acylation of α-hydroxyamide in preference to other alcohols.[11] Based on this precedent, the application of this method for the selective acylation of the hydroxyl group at the β-carbon to an amide carbonyl group was pursued.[12] The target hydroxyl groups are very attractive for chemical modification as they are equivalent to the hydroxyl groups on the side chains of Ser and Thr. Compared to α-hydroxyamides that favor the formation of five-membered metal complexes, β-hydroxyamides require the formation of the more flexible six-membered ring with a Lewis acid, which results in the slower formation of the metal template. Furthermore, the differences in the steric and electronic environments of the target hydroxyl group in a β-hydroxyamide and its competitors

Scheme 1. Site-Selective Acylations by a Metal Template Strategy



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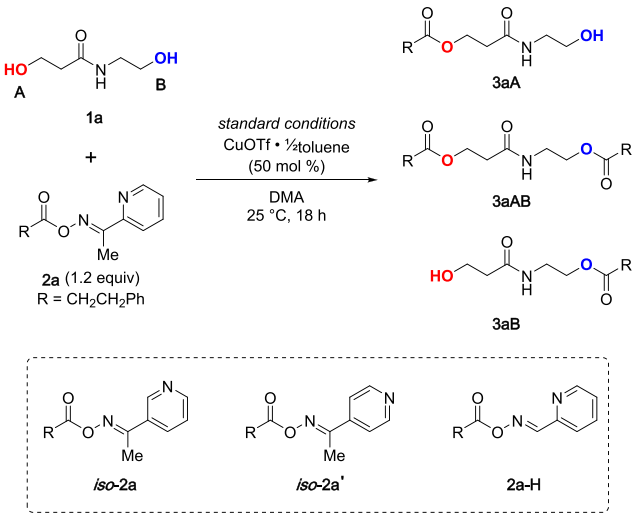
are smaller than those of an α-hydroxyamide, which is problematic from the perspective of distinguishing the target from the other potential reactants. Herein, we report the regioselective acylations of polyols with various β-hydroxyamide structures, including the serine-selective modification of a glycopeptide (Scheme 1b).

To optimize the site-selective acylation reaction conditions, β-hydroxy-(*N*-ethyl-2-hydroxy)propionamide (**1a**) was selected as a model substrate (Table 1). Initially, the acylation of **1a** was attempted using the best conditions for the selective monoacylation of an α-hydroxyamide, which resulted in insufficient yield and selectivity for the desired product (**3aA**: 44%, **3aB** + **3aAB**: 7%).[13] After considerable efforts, excellent yield and good regioselectivity were obtained with CuOTf·1/2 toluene as the Lewis acid catalyst and 2-pyridyl ketoxime ester (**2a**) in *N*,*N*-dimethylacetamide (entry 1). This reaction provided **3aA** in only 53% yield when performed using 20 mol % of CuOTf·1/2 toluene (entry 2), and the reaction did not proceed in the absence of CuOTf·1/2 toluene (entry 3). Various metal salts, such as $Cu(OTf)_2$ and $Zn(OTf)_2$, were next examined, which revealed that CuOTf·1/2 toluene was more efficient than the other catalysts (entries 4 and 5). The reaction afforded lower yields when the reaction time was reduced to 3 h (entry 6). Notably, the use of methyl 2-pyridyl ketoxime provided higher yields and site-selectivities than the corresponding 3-pyridyl or 4-pyridyl derivatives, indicating that the bidentate nature of **2a** was crucial in this reaction (entries 7 and 8). The higher reactivity of **2a** compared to that of 2-pyridyl aldoxime (**2a-H**) is attributable to the methyl group in **2a**, which facilitates the approach of the acyl group to the target hydroxyl group in the transition state due to steric repulsion, thereby enabling acylation (entry 9). No significant improvement in yield was observed when the concentration of **1a** was increased (entry 10). The screening of various coordinating solvents revealed that DMA was most favorable for this reaction (entries 11−13). The addition of water reduced the yield significantly (entry 14), and the yield and selectivity did not change significantly when a larger equivalence of **2a** was used (entry 15). In turn, conventional conditions using the corresponding acyl chloride in the presence of pyridine afforded **3aA**, **3aB**, and **3aAB** in comparable yields in a nonselective reaction, highlighting that the reactivities of two hydroxyl groups in **2a** were intrinsically equal but could be controlled in a desirable fashion in our system (entry 16).

Table 1. Optimization Studies[a]



entry	variations from standard conditions	yield (%)[b] 3aA	3aAB	3aB
1	none	86	5	-
2	CuOTf·1/2 toluene (20 mol %)	53	0	-
3	without CuOTf·1/2 toluene	0	0	-
4	$Cu(OTf)_2$ instead of CuOTf·1/2 toluene	44	5	-
5	$Zn(OTf)_2$ instead of CuOTf·1/2 toluene	39	24	-
6	3 h reaction time	50	0	-
7	*iso*-**2a** instead of **2a**	34	10	8
8	*iso*-**2a**′ instead of **2a**	38	17	11
9	**2a-H** instead of **2a**	50	0	0
10	[**1a**] = 0.4 M instead of 0.2 M	76	5	-
11	DME as the solvent	42	10	-
12	CH_3CN as the solvent	69	8	-
13	DMF as the solvent	69	8	-
14	10% H_2O/DMA as the solvent	26	3	-
15	**2a** (2 equiv)	68	6	-
16	conventional conditions: acyl chloride (1.0 equiv), pyridine (1.5 equiv), and DMA as solvent	19	18	15

[a]Standard reaction conditions: a mixture of **1a** (0.2 mmol), **2a** (0.24 mmol), and CuOTf·1/2 toluene (0.1 mmol) in *N*,*N*-dimethylacetamide (DMA) was stirred at 25 °C for 18 h. [b]Determined by ^{1}H NMR spectroscopy of the crude products.

With the optimized reaction conditions in hand, we next examined the scope of the reaction (Scheme 2). β-Hydroxyamides bearing single hydroxyl groups on alkyl chains of different length provided the corresponding monoacylated products **3aA**−**3cA** in good yields and excellent regioselectivities. The reaction of **1d**, bearing a methyl group at the nitrogen of the amide, afforded product **3da** in 64% yield. Various amino acid derivatives (**1e**−**1h**) were compatible with this reaction, with the corresponding monoacylated products **3eA**−**3hA** obtained in 65%−78% yields. These results show that the phenolic hydroxyl group in **1h** and the β-hydroxyester in **1g** are sufficiently less reactive in this system compared to the β-hydroxyamides (products **3gA** and **3hA**). Surprisingly, using our strategy, sterically hindered secondary alcohols could be acylated in preference to primary alcohols with much higher reactivities to give **3iA** and **3jA**. Furthermore, the selective monoacylations of Cbz-serine and Cbz-threonine derivatives **1k** and **1l** bearing bulky substituents at their α-positions were selectively acylated; the β-hydroxyl groups reacted selectively even in sterically hindered environments to afford the desired monoacylated products **3kA** and **3lA**. Likewise, our method could be applied to dipeptide **1m** to provide the monoacylated product **3 mA** with the hydroxyl group at the *C*-terminal intact.

Encouraged by these results, we decided to further explore the pyridine ketoxime ester substrate range (Scheme 3). Benzoyl and acetyl groups were also selectively introduced onto the β-hydroxyl group in amide **1b**, to afford products **4aA** and **5aA** in high yields, respectively. Acylation with the bulky pivaloyl ester donor **2d** gave product **6aA**, although in relatively low yield. The reaction proceeded smoothly even when an acyl donor bearing a terminal C−C double bond or the nucleophilic C2−C3 double bond of indole was present in the molecule, to give **7aA** and **8aA** as the target products. Subsequently, the introduction of amino acid derivatives onto a target hydroxyl group was examined using this method. When the reaction was carried out using acyl donors **2g**−**2k** derived from amino acids, the desired monoacylated products **9aA**−**13aA** were successfully obtained

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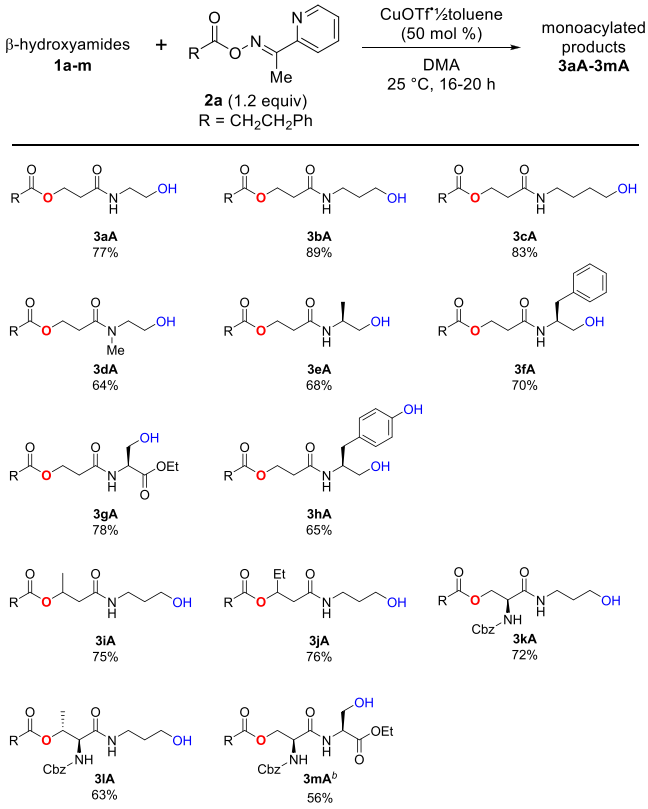
Scheme 2. β-Hydroxyamide Substrate Range for Site-Selective Acylations[a]

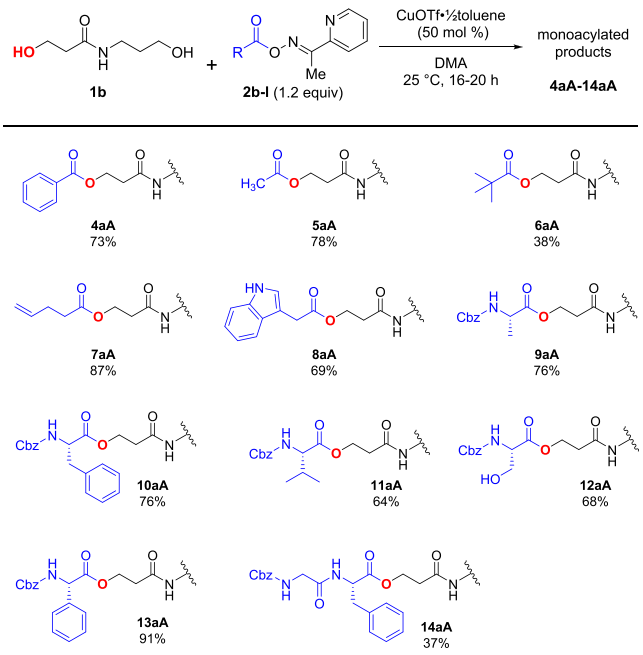


[a]Standard reaction conditions: A mixture of **1** (0.2 mmol), **2a** (0.24 mmol), and CuOTf·1/2 toluene (0.1 mmol) in DMA was stirred at 25 °C for 16−20 h. Yields are for the isolated products.[14] [b]The reaction was performed at 40 °C.

in 64%−91% yield, without affecting the optical purity. Furthermore, the introduction of a dipeptide into **1b** was examined. The C-terminus of a peptide is susceptible to racemization through the formation of oxazolones during the activation of the terminal carboxylic acid. Fortunately, no racemization occurred during the preparation of oxime ester **2l**, and subsequent regioselective reaction with **1a** afforded dipeptide ester **14aA** with 99% ee.

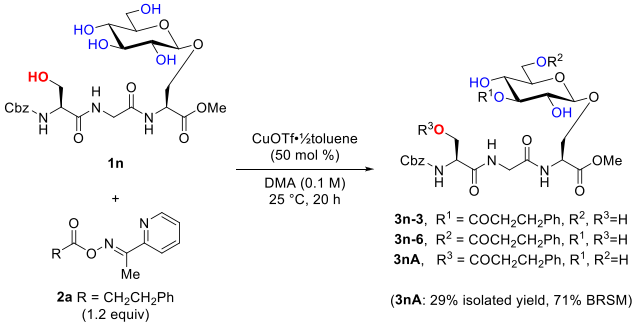
To demonstrate the applicability of our methodology to the regioselective modifications of glycopeptides, glycopeptide **1n** was prepared as a model substrate bearing four competitive hydroxyl groups for acylation.[13] The β-*O*-glucosylated serine residue in a peptide, adopted in **1n**, is a biologically important structure found in the epidermal growth factor domains and notch receptors of various serum proteins.[15] The optimized conditions in our system feature the use of highly polar DMA as the solvent, which is beneficial for dissolving hydrophilic compounds such as **1n**, thereby avoiding solubility issues in these reactions (Scheme 4). The acylation of **1n** under these slightly modified conditions successfully afforded a mixture of products. The reaction solution was analyzed by LC-MS to ensure that all acylated products could be detected. The extracted ion chromatogram (XIC) for the monoacylated product ($[M + Na]^+$ m/z 714.2481) revealed the presence of three regioisomers (Figure 1). Subsequent preparative HPLC followed by 2D NMR spectroscopy enabled the three constituents to be assigned as **3n-3**, **3n-6**, and **3nA**.[13] The

Scheme 3. Pyridine Ketoxime Ester Substrate Range for Site-Selective Acylations[a]



[a]Standard reaction conditions: A mixture of **1b** (0.2 mmol), **2** (0.24 mmol), and CuOTf·1/2 toluene (0.1 mmol) in DMA was stirred at 25 °C for 16−20 h. Yields are for the isolated products.[14]

Scheme 4. Serine OH Selective Acylation of Glycopeptide 1n



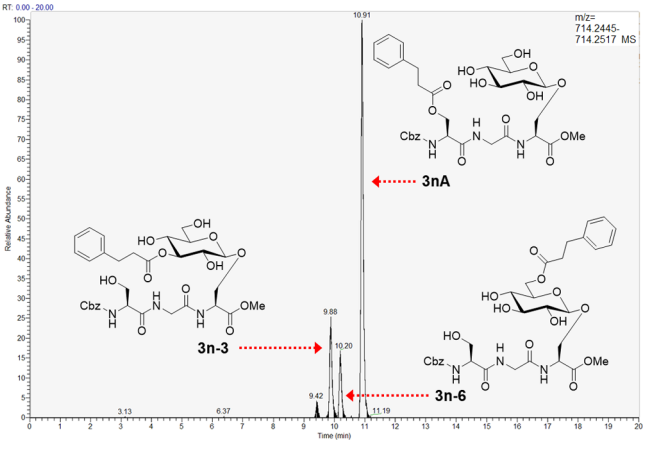


Figure 1. Extracted ion chromatogram (XIC) of monoacylated products ($[M + Na]^+$ m/z 714.2481).

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relative proportions of these compounds were directly obtained from the relative HPLC peak areas at λ = 210 nm (**3n-3**:**3n-6**:**3nA** = 16:7:77; Figure 2, top). The selectivity for the serine-

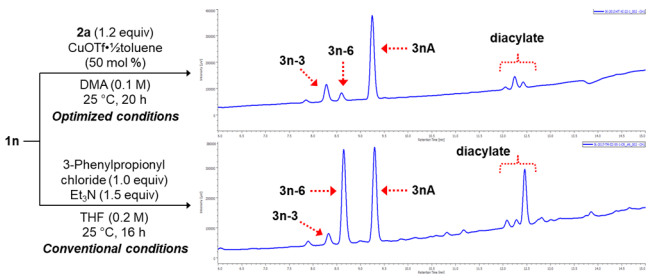

Figure 2. Reversed-phase HPLC traces for the (top) optimized and (bottom) conventional conditions for the acylation of glycopeptide **1n**.

modified product **3nA** was much higher under these conditions than under conventional conditions using 3-phenylpropionyl chloride as the acylating agent (Figure 2, bottom, **3n-3**:**3n-6**:**3nA** = 6:47:47). Furthermore, the formation of diacylated products was efficiently suppressed in our system compared with the products formed under conventional conditions, as confirmed by the XIC. It should also be noted that the most abundant byproduct in our procedure was **3n-3**, in which the 3-OH of the glucose in **1n** is acylated, while it was **3n-6** under conventional conditions. These contrasting results are possibly ascribable to the proximity between the β-hydroxyl group and 3-OH in the transition state involving the metal template. We speculate that intramolecular hydrogen bonding interactions between the glucose and the peptide in **1n** arrange the two hydroxyl groups in proximal positions. Finally, **3nA** was successfully obtained in 29% isolated yield (71% yield based on recovered starting material **1n**).

In conclusion, a site-selective acylation methodology was developed for β-hydroxyamides using CuOTf and pyridine ketoxime esters as acylating agents. This reaction system afforded monoacylated products with good regioselectivities. In addition, this protocol facilitated the introduction of an acyl group onto the serine OH in a glycopeptide bearing an unprotected glucose with good site selectivity. Further applications of this metal-template strategy are currently being explored in our group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02809.

Complementary results, experimental procedures, and spectroscopic data of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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