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Hydroxymethyl Salicylaldehyde Auxiliary for a Glycine-Dependent Amide-Forming Ligation

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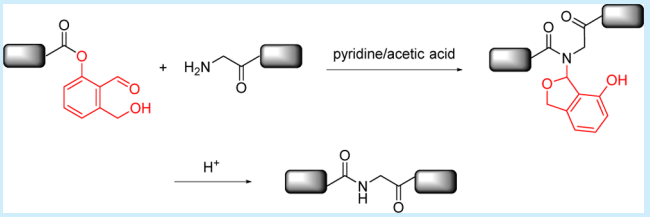
Supporting Information

ABSTRACT: A new amide-forming ligation that requires a glycine or a primary amine at the linkage site is described herein. The distinguishing feature of this ligation is its reliance on an *O*-hydroxymethyl salicylaldehyde ester at the *C*-terminus which allows, via an *N*,*O*-acetal intermediate, the formation of a native peptide bond.



With their unique biological properties and their ability to target protein−protein interactions, peptides are receiving a great deal of interest within the pharmaceutical industry as potential therapeutic agents.[1,2] Naturally, this has led to a greater focus on the development of new synthetic methodologies for generating peptides and proteins of increasing size and complexity. The most remarkable breakthrough in the field of peptide synthesis was made by Merrifield in 1963, with the "solid-phase-peptide-synthesis" (SPPS) concept.[3] Today, SPPS still plays a major role in the preparation of peptides, but this technique also suffers from its extreme linearity, making the synthesis of long peptides (40−50 amino acid residues) very difficult. Therefore, in order to gain access to much larger peptides and proteins, chemoselective ligation protocols were further developed throughout the 1990s. The Native Chemical Ligation (NCL), developed by Kent and co-workers, proved to be a very powerful method for the synthesis of complex peptides and proteins,[4,5] and despite many other recent contributions,[6−10] NCL still represents the state-of-the-art technique in the field of peptide ligation. However, a major drawback of NCL is its reliance on cysteine at the ligation site. This is particularly problematic considering the occurrence of cysteine in natural proteins is only 1.1%. Even though desulfurization reactions could overcome this limitation,[11] there is still a need for diversification of ligation strategies in order to meet the synthetic challenge for generating peptides and proteins with increasing complexity.

More recently, Li and co-workers developed a new serine/threonine dependent approach based on the functionalization of peptides at their *C*-terminus with a salicylaldehyde auxiliary.[12,13] This methodology, which is an extension of the work by Tam et al.,[14] caught our attention due to its selective linkage (serine and threonine are abundant residues in proteins), leading to the formation of a native peptide bond (Figure 1a).

We believed that the field of peptide research would benefit from a new chemical ligation method which utilizes a different linkage site from those that have already been reported in the literature.[4−10] Moreover, because several literature examples have already demonstrated the robustness and generality of the

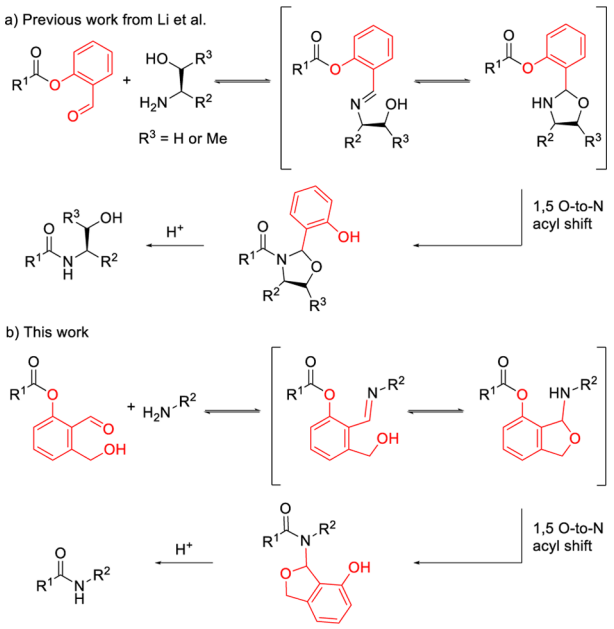


Figure 1. *C*-Terminal auxiliary promoted ligations.

salicylaldehyde auxiliary mediated ligation,[12,13] we decided to develop a method that relies on the same concept. Thus, we envisaged building a new auxiliary by transferring the CH_2-OH moiety from the *N*-terminal Ser/Thr side chain onto the *C*-terminal salicylaldehyde. This new auxiliary should deliver a ligation method independent from the presence of an *N*-terminal Ser/Thr at the linkage site (Figure 1b). The ligation would follow a similar mechanism with first, imine formation followed by cyclization with the auxiliary hydroxyl group, and subsequent *O*-to-*N* acyl shift via a 6-membered ring intermediate. Finally, the *N*,*O*-acetal intermediate should be cleaved under acidic

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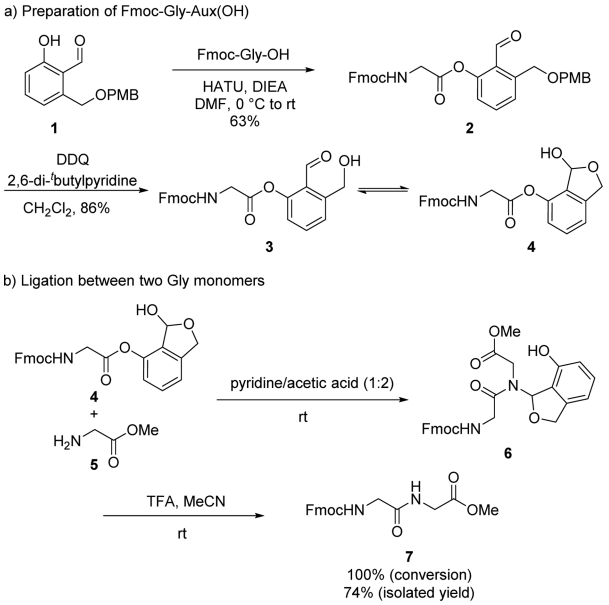
Scheme 1. Synthesis of Fmoc-Gly-Gly-OMe



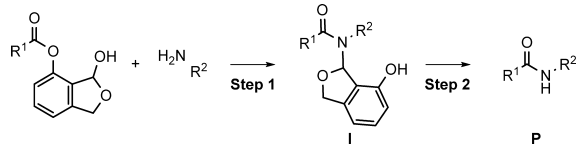
conditions to afford the ligation product with a native peptide bond at the linkage site. Herein, we describe our efforts toward the development of this auxiliary as a new tool for the synthesis of peptides.

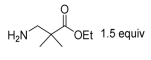
To validate our starting hypothesis, a ligation between two glycine monomers was investigated (Scheme 1). Auxiliary **1**[15] was coupled to Fmoc-Gly using HATU in DMF to give ester **2**. Oxidative cleavage of the PMB ether was carried out with DDQ. It is worth noting that without 2,6-di-*tert*-butylpyridine, partial degradation of the product was observed.[16] Our choice to install a PMB was driven by the need to have a protecting group with cleavage conditions compatible with those used with the more common protecting groups found in peptide chemistry. NMR analysis confirmed the formation of the hemiacetal intermediate **4** after deprotection (Scheme 1a). Following the isolation of **4**, a ligation was attempted with Gly-OMe (**5**). A mixture of pyridine/acetic acid (1:2) proved to be the best solvent system to promote the formation of intermediate **6**.[17] The crude mixture was then subjected to acidic treatment and the desired dipeptide **7** was isolated in excellent yield over two steps (Scheme 1b).

In order to demonstrate the scope and limitations of this method, sterically demanding amino acids were investigated (Table 1). It is well established that bulky residues at the *C*-terminal amino acids are a limiting factor for ligation reactions.[18] Therefore, it was decided to test a range of α- and β-branched amino esters, bearing the *o*-hydroxymethyl salicylaldehyde auxiliary at their *C*-terminus, for ligation with Gly-OMe.[19] Even though β-branched amino acids, for instance, proline and valine, significantly affected the rate of the ligation, all reactions (Table 1, entries 1−6) showed high conversions, and after cleavage of the auxiliary, the desired dipeptides were isolated in good yield and excellent enantiomeric purity.[20]

However, a limitation regarding *N*-terminal amino acids was observed: when the ligation was carried out with Ala-OBz or Phe-OBz (Table 1, entries 7 and 8), formation of the desired intermediate was unsuccessful. Mild heating resulted mainly in hydrolysis of the ester but also in its direct aminolysis. In order to better understand this limitation and to check if the imine intermediate was formed, the reaction mixture was treated with the reducing agent $NaCNBH_3$. This led to the formation of the

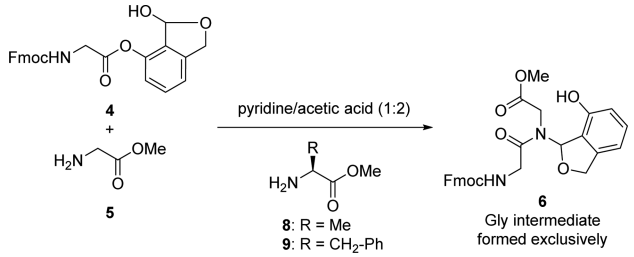
Table 1. Scope and Limitations of the Ligation between Monomers



entry	R^1	$R^{2\,a}$ (1.2 equiv)	I[b], % (t, h)	yield P[c], % (*ee*, %)[d]
1	Fmoc-Ala	Gly-OMe	100 (17)	47 (99.75)
2	Fmoc-Phe	Gly-OMe	100 (17)	55 (99.70)
3	Fmoc-Leu	Gly-OMe	100 (18)	81 (100.00)
4	Fmoc-Val	Gly-OMe	100 (40)	74 (100.00)
5	Fmoc-Pro	Gly-OMe	80 (40)	66 (100.00)
6	Fmoc-Gly	Gly-OH	100 (22)	59
7	Fmoc-Gly	Phe-OBz	no reaction	
8	Fmoc-Gly	Ala-OBz	no reaction	
9	Fmoc-Gly	 1.5 equiv	90 (36)	66

Conditions: All reactions were performed at room temperature, 20 mM. Step 1: pyridine/acetic acid (1:2). Step 2: TFA (20 equiv) in MeCN. [a]The HCl salt form was used. [b]Intermediate: conversion was checked by LC−MS and was calculated on the basis of consumption of AA-Aux(OH). [c]Product: yield corresponds to the isolated product after chromatography purification. [d]The *ee* was obtained by chiral HPLC by comparison between Fmoc-L-AA-Gly-OMe and Fmoc-D-AA-Gly-OMe retention times.

Scheme 2. Chemoselectivity Studies between Gly and Phe/Ala



corresponding amine showing that the imine formation did occur but that the following imine capture or migration failed due to the presence of sterically demanding *N*-terminal amino acids.[21] We actually believe that the *O*-to-*N* acyl shift is responsible for disrupting the ligation, as it proceeds through an energetically unfavorable 6-membered ring intermediate. In this context, the ligation was also tested with an *N*-terminal β-amino-acid and proved to be successful (Table 1, entry 9), demonstrating again

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Table 2. Scope and Limitation of the Ligation Using Unprotected Side-Chain Residues

entry	R^1	$R^{2\,a}$ (equiv)	I^b, % (t, h)	yield P^c, % (*de*, %)[d]
1	Fmoc-Gly	Ser-Phe-OEt (1.5)	100 (72)	30
2	Fmoc-Gly	Gly-Arg-OMe (1.2)	NA	64
3	Fmoc-Gly	Gly-Trp-OH (1.2)	100 (16)	56 (100)
4	Fmoc-Gly	Gly-Leu-Tyr-OH (1.2)	100 (16)	59 (100)
5	Fmoc-Phe	Gly-Leu-Tyr-OH (1.2)	100 (60)	72 (100)
6	Fmoc-Phe	Gly-Lys-OH (1.5)	8:3 mixture (*N*-Terminal Gly / Lys side chain)[e]	
7	Fmoc-Tyr (O^tBu)	Gly-Glu-OH (2)	80 (60)	50[f] (96.5)

Conditions: All reactions were performed at room temperature, 20 mM. Step 1: pyridine/acetic acid (1:2). Step 2: TFA (20 equiv) in MeCN. [a]The HCl salt form was used. [b]Intermediate: conversion was checked by LC−MS and was calculated on the basis of consumption of peptide-Aux(OH). [c]Product: yield corresponds to the isolated product after chromatography purification. [d]The *de* was obtained by chiral HPLC. [e]The ratio of regioisomers was determined by LC−MS. [f]Cleavage of the auxiliary was carried out in neat TFA and led to the ^{t}Bu-deprotected peptide.

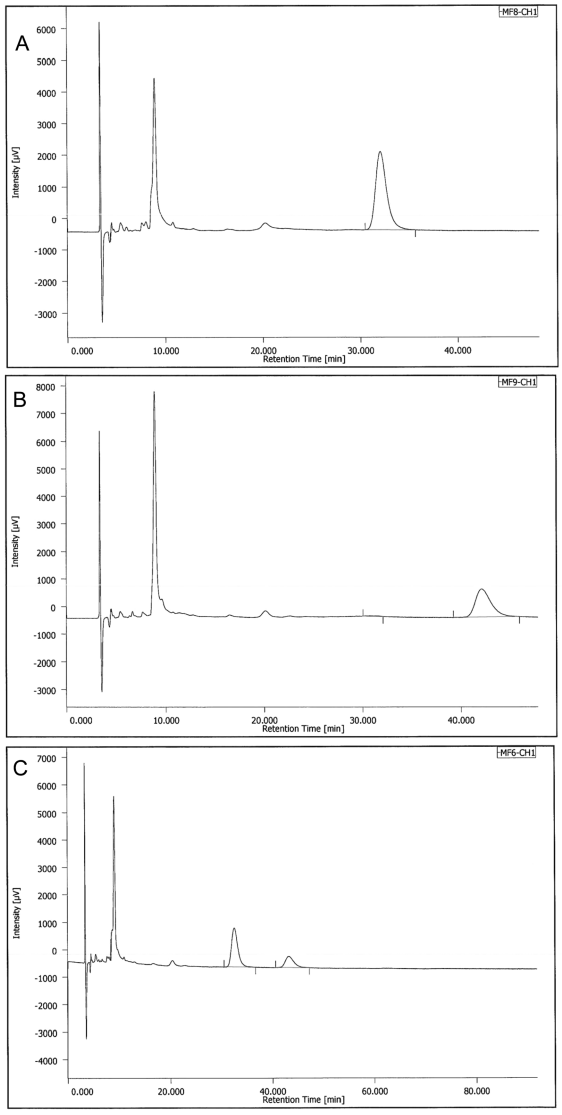

Figure 2. Epimerization determination between the ligation reaction of Fmoc-L-Phe-Aux(OH)/Fmoc-D-Phe-Aux(OH) and Gly-Leu-Tyr-OH. (A) Chiral HPLC profile of crude ligation products using Fmoc-L-Phe-Aux(OH). (B) Chiral HPLC profile of crude ligation products using Fmoc-D-Phe-Aux(OH). (C) Co-injection of crude ligation products.

that steric hindrance at the α-position is a key factor of this method.

In order to showcase the difference in reactivity of glycine versus alanine or phenylalanine at the *N*-terminus, a competition experiment was carried out (Scheme 2). Although in the presence of other potential ligation partners, only Gly-OMe readily reacted with **4** to form the corresponding intermediate with 100% conversion.[22] With these experiments, the *o*-hydroxymethyl salicylaldehyde auxiliary has demonstrated that it can offer a new linkage site for the synthesis of a target peptide and also provides a chemoselective method in a mixture of peptides. Furthermore, even though a ligation reaction with *N*-terminal serine was successful (Table 2, entry 1) the chemoselectivity of this ligation toward Gly-OMe in the presence of Ser-OMe was demonstrated in a similar competition experiment whereby, upon completion, the formation of a majority of the glycine intermediate **6** was observed (glycine intermediate/serine intermediates 9:1).[22]

The synthesis of tri-, tetra-, penta-, and decapeptides from unprotected or partially protected smaller units was then

Table 3. Scope and Limitation of the Ligation between Peptidic Fragments

entry	R^1	R^{2a} (equiv)	I^b (%) (time, h)	yield P^c (%) (de, %)[d]
1	Boc-Phe-Phe-Gly	Gly-His-OH (2)	100 (18)	64[e]
2	Boc-Phe-Phe-Gly	Gly-Ser-OH (2)	100 (48)	48[e] (100)
3	Boc-Phe-Phe-Gly	Gly-Ile-Ser-Thr-Pro-Val-Ile-Phe-OH (1.5)	100 (60)	66[e]
4	Boc-Phe-Thr(O^tBu)-Phe	Gly-Trp-OH (2)	100 (60)	46[f] (100)

Conditions: All reactions were performed at room temperature, 20 mM. Step 1: pyridine/acetic acid (1:2). Step 2: TFA (40 equiv) in MeCN. [a]The HCl salt form was used. [b]Intermediate: conversion was checked by LC−MS and was calculated on the basis of consumption of peptide-Aux(OH). [c]Product: yield corresponds to the isolated product after chromatography purification. [d]The *de* was obtained by chiral HPLC. [e]Cleavage of the auxiliary led to the Boc-deprotected peptide. [f]Cleavage of the auxiliary was carried out in HCl in dioxane (4 M) and led to the fully deprotected peptide.

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investigated (Tables 2 and 3). To our delight, peptide fragments with unprotected serine, glutamine, arginine, histidine, and tryptophan were tolerated. However, as expected, unprotected lysine residue competed with the *N*-terminus of glycine and led to the formation of two regioisomers (Table 2, entry 6). For reasons that are unclear to us, the product corresponding to the ligation with the *N*-terminal glycine proved to be the major one compared to the product formed by the ligation with the amino group of the lysine side-chain.

Tripeptides bearing the *C*-terminal auxiliary were also prepared and ligated successfully with various peptides. Boc- and *tert*-butyl-protected peptides (Table 3) could be deprotected during the cleavage of the auxiliary, giving rise to a straightforward synthesis of fully deprotected peptides in good yields. It is noteworthy that the synthesis of a decapeptide (Table 3, entry 3) was also achieved in good yield.

The reported peptides (Table 1, entries 1−5, and Table 2, entries 1−5 and 7, and Table 3, entries 1−4) were isolated in excellent diastereoisomeric purity.[23] Epimerization was also further investigated with the ligation between Fmoc-L-Phe-Aux(OH) and Gly-Leu-Tyr-OH that was compared to the ligation with Fmoc-D-Phe-Aux(OH) (Figure 2). During this experiment, epimerization was not detected.[24]

In conclusion, a new amide-forming ligation has been reported. This strategy, which requires a glycine or a primary amine at the linkage site, enables the use of unprotected side-chain peptide fragments (excepted lysine). At this stage, the auxiliary has to be installed after resin cleavage but, as demonstrated for the salicylaldehyde auxiliary, SPPS synthesis could be envisaged.[25] In the near future, we aim to demonstrate the usefulness of this method with the synthesis of longer and/or cyclic peptides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02350.

Experimental details, spectral and analytical data, and ^{1}H NMR and ^{13}C NMR spectra for new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(17) See the Supporting Information, section 2.4, for characterization of the intermediate.

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(19) All *C*-terminal 6-(hydroxymethyl)salicylaldehyde esters were prepared according to Scheme 1. Chiral HPLC of Fmoc-Phe-Aux(OPMB) proved that the auxiliary can be installed racemization free; see the Supporting Information, section 2.

(20) The *ee* was obtained by chiral HPLC by comparison between Fmoc-L-AA-GlyOMe and Fmoc-D-AA-GlyOMe retention times; see the Supporting Information, section 2.4.

(21) The reactions were followed by LC−MS; see the Supporting Information, section 3.

(22) The competition experiments were followed by LC−MS; see the Supporting Information, section 4.

(23) For all synthesized peptides (expect Table 2, entries 1 and 2, Table 3, entries 1 and 3), chiral HPLC was performed to determine the *ee*/*de*. Otherwise (Table 2, entries 1 and 2, and Table 3, entries 1 and 3), NMR analysis showed the presence of one diastereoisomer.

(24) See the Supporting Information, section 2.4.

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