ChemPubSoc Europe

DOI: 10.1002/ejoc.201601148

EurJOC European Journal of Organic Chemistry

Communication

Hydroxyamine Amidation

Native Serine Peptide Assembly – Scope and Utility

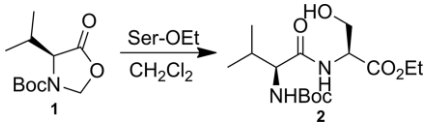
Michael C. Pirrung*[a] and Ryan S. Schreihans[a]

Abstract: This work develops serine peptide assembly (SPA), which complements and contrasts with classic native chemical ligation (NCL). Advances in reagentless peptide-bond formation have been applied to serine (and serine models) and a range of *C*-terminal amino acids, including bulky residues that are not amenable to NCL. The particular appeal of SPA is preparative-scale segment condensations with zero racemization risk and favorable process mass intensity (PMI). Mechanistic studies support a previously proposed reaction pathway via an initial transesterification step. An understanding of the factors favoring this pathway relies on hard–soft acid–base theory, according to which mildly activated esters with the largest carbonyl positive charge are most reactive with hydroxyamines. Novel *C*-terminal activators have been discovered that enhance reactivity and give harmless byproducts.

Introduction

We recently introduced a method for reagentless amide bond formation with a focused set of amines, those bearing nearby hydroxy groups.[1] Their carboxyl reaction partners were esters mildly activated for acyl transfer by strain or inductive effects or both. We suggested that the pathway for these reactions involves initial transesterification of the alcohol and ensuing rearrangement to give the more stable hydroxyamide. Envisioning the application of this technology to preparative peptide segment condensations at *N*-terminal serine residues, we aim here to expand the scope to diverse activated acyl derivatives, including native amino acids with various mildly activated esters. This work provides further support for the transesterification/rearrangement pathway that mimics native chemical ligation (NCL) and has uncovered readily introduced, superior *C*-terminal activating groups.

Our earlier work was limited to *N*-Boc-valine. The valine carboxylate was activated as a cyanomethyl ester or oxazolidinone (**1**, Scheme 1). Nonetheless, successful amide formation with this amino acid derivative (with its bulky α-substituent) far surpasses the capabilities of NCL, which is the sulfur relative of SPA.[2] In NCL, valine is tolerated at the *C*-terminus only when using a selenoester with selenocysteine.[3] Assemblies were performed in relatively nonpolar media at ambient temperature for extended periods or more rapidly with microwave heating.

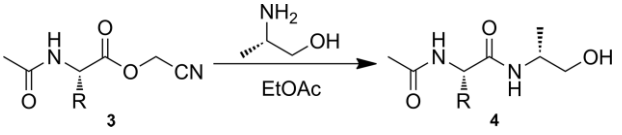


Scheme 1. Initial peptide assembly with serine.

Results and Discussion

To study a reaction partner that better represents the *C*-terminus of a peptide segment, we replaced the carbamate *N*-protection from the earlier study with an *N*-acetyl group. Particularly, *N*-acyl amino acids (like peptide *C*-termini) are vulnerable to racemization when the carboxylate is activated with conventional coupling agents, because of competing oxazolone formation that labilizes the α-hydrogen of the *C*-terminal residue. In examining the relative reactivity of different native amino acids, L-alaninol was used as a simple serine surrogate. Each of the known acetyl amino acids in Table 1 was converted to its cyanomethyl (CM) ester **3** in 80–95 % yield under the conditions reported earlier: 1 equiv. Cs_2CO_3, 1.5 equiv. $BrCH_2CN$, ambient temperature, 1.5–8 h. These esters were allowed to react with a 50 % excess amount of alaninol, either at ambient temperature or with microwave heating at 100 °C, in 1 M solutions. Ethyl

Table 1. Peptide-bond formation with *N*-acetylamino cyanomethyl esters.



Entry	Ac-AA	Time [h][a]	Yield [%]	Time [min][b]	Yield [%]
a	Val	72	78	90	78
b	Leu	72	38	90	53
c	Phe	48	76	90	76
d	Met	48	90	90	91
e	Pro	72	64	90	54
f	Gly	72	72	90	73
g	(Trt)Asn	24	79	ND[c]	ND
h	(Bn)Cys	48	78	ND	ND

[a] Ambient temperature. [b] Microwave heating. [c] ND: not determined.

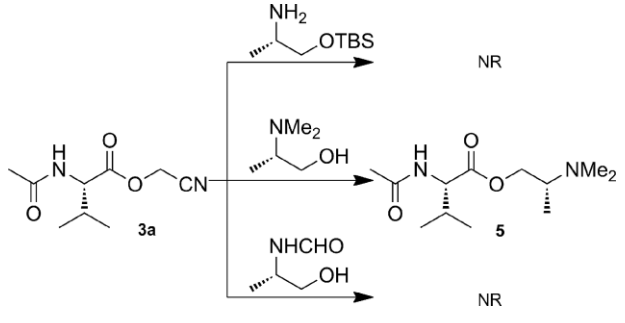
[a] *Department of Chemistry, University of California, Riverside, CA 92521 USA*
E-mail: Michael.pirrung@ucr.edu

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201601148.

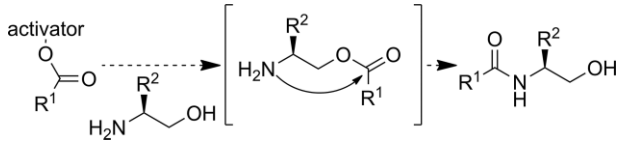
acetate proved a useful solvent for these reactions, dissolving polar reactants that are insoluble in the hydrocarbons that were preferred earlier, but still providing a relatively nonpolar medium that gives higher conversions. The results are summarized in Table 1.

Table 1 shows that significant variation in the *C*-terminal amino acid is very well-tolerated. Leucine is a slower reactant, which can be explained on the basis of *syn*-pentane interactions or other remote steric effects.[4] Successful reaction with proline is notable, as Pro (like Val) is not useful in NCL.[5] Though some advancements have been made in this area,[6] *C*-terminal bulk is still a significant problem for NCL. No racemization (less than 0.1 %) was observed in any of these reactions, as established by detailed spectroscopic examination of the products in comparison with the peptide diastereomer generated with D-alaninol.

We also aimed to provide greater support for the mechanism postulated earlier; that is, an initial transesterification, enhanced by internal H-bonding between the basic amine and the hydroxyl group, followed by a rearrangement (via transacylation) to produce the hydroxyamide. Structural variations were made to the amino alcohol to examine this issue. As Table 1 shows, when Ac-Val-OCM reacts with alaninol under ambient conditions, the formation of **4a** is complete in 72 h. When *N*,*N*-dimethylalaninol is substituted, the transesterification product **5** is formed in 89 % yield, also in 72 h. When alaninol *N*-formamide is used, there is no reaction. When alaninol *tert*-butyldimethylsilyl ether is used, there is no reaction (Scheme 2). We showed earlier that alaninol *tert*-butyldimethylsilyl ether does not react with a γ-lactone, demonstrating the essential nature of the alcohol in these amide-forming reactions.[1] The current results show that transesterification is kinetically competent as the initial step in the overall process, that direct acylation is not a favorable reaction pathway, and that transesterification requires a nearby basic nitrogen. All of these data support the mechanism discussed above, as explicitly detailed in Scheme 3.



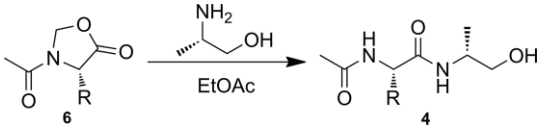
Scheme 2. Results supporting the transesterification/rearrangement pathway.



Scheme 3. The transesterification/rearrangement mechanism.

The *N*-acetyl oxazolidinones **6** of several native amino acids were also investigated. These are prepared from the *N*-acetyl amino acids in 55–84 % yield by treatment with *p*TsA and paraformaldehyde in toluene for 3–12 h. As Table 2 shows, the trends observed in the CM ester study hold here, the Leu derivative again being lower-yielding.

Table 2. Peptide-bond formation with *N*-acetyl oxazolidinones.



6 4

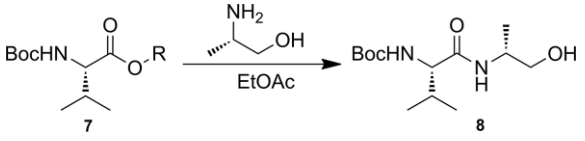
Entry	Ac-AA	Time [h][a]	Yield [%]	Time [min][b]	Yield [%]
a	Val	72	81	90	78
b	Leu	72	41	90	58
c	Phe	48	78	90	74
d	Met	36	82	90	76

[a] Ambient. [b] Microwave heating.

When extending these reactions of **3a** or **6a** to SPA with ethyl serinate, a reactant more representative of a peptide *N*-terminus, yields were only moderate. This stimulated the search for superior *C*-terminal mildly activated esters that could be readily generated from peptides. Boc-Val was used as a test amino acid as it maintains the steric demand uniquely tolerated in hydroxy amine amidation while enabling the rapid synthesis of a wide range of esters using multiple conventional methods.

This study was performed by treating valine esters **7** with alaninol under our standard ambient reaction conditions and observing the time for complete consumption of starting material to form **8**. The results in Table 3 show interesting features. Although CM esters have been used in transesterification reactions in the past,[7] they are among the slowest of all investigated reactive esters. A significant rate gain is observed with fluorinated esters, the best being the vinyl esters. The fastest, (methyl trifluorocrotonate)yl (entry m), is prepared by conjugate addition[8] of the carboxylate to ethyl trifluorobutynoate. Hexa-

Table 3. Boc-Val-ester reactivity under ambient conditions.

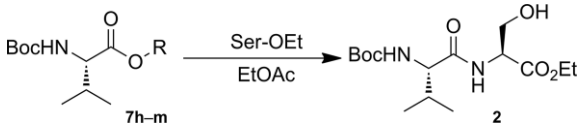


7 8

Entry	R	Time [h]
a	CH_2SCH_3	84
b	CH_2CCl_3	80
c	CH_2CH_2Cl	76
d	CH_2CN	70
e	CH_2CHCl_2	54
f	CH_2SOCH_3	50
g	$CH_2SO_2CH_3$	48
h	CH_2CF_3	32
i	$CH_2CF_2CF_3$	28
j	$CH(CF_3)_2$	18
k	$C{=}CH_2[C(CF_3)_2CF_2CF_3]$	18
l	$(MeO_2C)C{=}CH(CO_2Me)$	15
m	$(CF_3)C{=}CH(CO_2Me)$	8

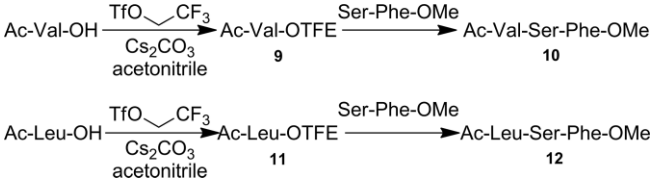
fluoroisopropyl (HIP) and trifluoroethyl (TFE) esters also have good reactivity. Some other Boc-Val esters (structures in the Supporting Information) show essentially no reaction, despite a reasonable expectation of good reactivity based on their group electronegativities (vide infra).

Preparative yields for the six most reactive esters **7** for SPA with ethyl serinate (Scheme 4) were determined under the standard reaction conditions. Additional products that diminished the isolated yields of Boc-Val-Ser-OEt (**2**) were observed with the three vinyl esters, but the HIP and TFE esters gave yields greater than 92 % in 20–32 h under ambient conditions. Experiments to establish the reaction pathway for amide formation were performed with **7j** to parallel those with **3a** (Scheme 2), with the same outcome: the transesterification product is formed from *N*,*N*-dimethylalaninol in 94 % yield in the same reaction time, which means that this step is kinetically competent, and no other alaninol analogs react.



Scheme 4. Preparation of **2** from the six most reactive esters **7**.

Identification of these superior esters prompted development of methods for mild ester formation from free *C*-termini by using *N*-Ac-Val as a model. In Sn2 reactions with the carboxylate as the nucleophile, commercially available trifluoroethyl triflate proved about as reactive in forming TFE esters as the bromoacetonitrile used to make CM esters, providing the ester in essentially quantitative crude yield in a few hours. However, the TFE ester is more desirable, because it is more reactive and its byproduct is volatile and nontoxic (Scheme 5). Like CM esters, mildly activated TFE esters are formed without generating a reactive acylating agent from the carboxyl group, which eliminates all concerns about racemization via oxazolone formation. These reactions have been performed on scales up to 1 g of TFE ester **9**. It is stable upon storage and has proved resistant to racemization even with microwave heating in ethyl acetate under reaction conditions in the absence of a hydroxyamine. We have generally observed that these mildly activated esters can be used in 20–50 % excess and that the unreacted starting material can be recovered after the reaction and reused.



Scheme 5. Model tripeptide syntheses using SPA of TFE esters.

We applied serine peptide assembly to two simple examples. On treatment of **9** with seryl-phenylalanine ester, tripeptide **10** forms in 76 % yield in 36 h (room temp.). Changing the CM ester to the TFE ester mitigated the earlier difficulty with leucine, as treatment of **11** with seryl-phenylalanine ester forms tripeptide **12** in 66 % yield (48 h, room temp.).

As many chemists would judge nitrogen as intrinsically more nucleophilic than oxygen, the mechanistic pathway inferred for SPA is counterintuitive. Likewise, it could be said that sulfur is more nucleophilic than either, hence its utility in NCL. However, it is known that oxy anions are more nucleophilic than amide anions, as shown by Brønsted linear free energy relationships in substitution reactions (on benzyl chloride).[9] The accessibility of the oxy anion is also generally better, owing to intrinsically lower pK_As for alcohols than for amines, and specifically in these reactants based on the internal basic amine.

In considering these results, we aimed to develop structure–reactivity correlations that would enable understanding and prediction of ester reactivity in SPA (see tables in the Supporting Information). Group electronegativity data is available for some of the ester groups used, but it shows poor agreement with reactivity. For example, the ethynyl and cyano groups have the same group electronegativity (3.3),[10] but propargyl esters are unreactive whereas cyanomethyl esters react fairly well. Other correlations were based on electronic structure calculations using the PM3 semiempirical method. The LUMO energies and carbonyl electrostatic charges were examined for acetyl derivatives of ester groups from Table 3, as well as other commonly used active esters. The best predictor of the observed reactivity (with alaninol) was the electrostatic charge on the carbonyl carbon atom. We interpret this preference as the tendency of the hard nucleophile alkoxide to transesterify faster with harder carbonyls.

Of the metrics that have been proposed to evaluate the sustainability of chemical processes, a prominent measure is process mass intensity (PMI).[11] This is the ratio of the mass of all materials entering a reaction (excluding aqueous solvents) to the mass of final product, the best possible PMI being 1. For the preparation of tripeptides **10** and **12** by SPA, the two-step PMI is approximately 5. This is a relatively low value and contrasts with higher PMI values observed for conventional peptide couplings that include condensing agents and additives to suppress racemizations that are unnecessary in SPA.

Conclusion

This work complements the substantial achievements in ligations at Ser and Thr residues with unprotected polypeptides in dilute aqueous media.[12]

Experimental Section

Ac-Val-OTFE (9): Cesium carbonate (1.0 mmol, 325.3 mg) was added to a solution of N-Ac-Val (1.0 mmol, 217.3 mg) in acetonitrile (10 mL). This solution was stirred at room temperature for 15 min, and then 2,2,2-trifluoroethyl trifluoromethanesulfonate (1.5 mmol, 348.2 mg) was added in one portion. The reaction mixture was stirred at room temperature for 3 h and filtered through Celite. The filtrate was concentrated in vacuo and purified with silica gel flash chromatography using ethyl acetate as eluent to yield 228 mg (95 %) of the title compound as a colorless oil.

Ac-Val-Ser-Phe-OMe (10): Ac-Val-OTFE (0.15 mmol, 36.6 mg) was added to a solution of Ser-Phe-OMe (0.13 mmol, 33.6 mg) in ethyl

acetate (0.13 mL). The reaction mixture was stirred at room temperature for 36 h and concentrated in vacuo. The residue was purified by silica gel chromatography using 10 % methanol in dichloromethane as eluent to yield 48.7 mg (76 %) of the title compound as a white solid. M.p. 202 °C (decomposes).

Acknowledgments

This work was partially supported by grants from National Science Foundation (NSF) (CHE 1362737) and the National Institutes of Health (NIH) (AI106597). We are grateful to Kian Sedighi and Jody Gotoc for technical assistance.

Keywords: Amides · Amino acids · Acylation · Nucleophilic substitution · Transesterification

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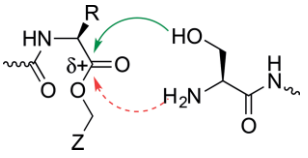
Received: September 15, 2016
Published Online: ■

Hydroxyamine Amidation

M. C. Pirrung, R. S. Schreihans* 1–5

Native Serine Peptide Assembly – Scope and Utility



Surprisingly, mildly activated esters of α-amino acids prefer to react with amino alcohol nucleophiles to first transesterify, guided by a favorable hard–hard interaction of the alcohol nucleophile and carbonyl positive charge, and then undergo a dyotropic-like rearrangement to form serine peptide bonds.

DOI: 10.1002/ejoc.201601148