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Synthesis of Small Glycopeptides by Decarboxylative Condensation and Insight into the Reaction Mechanism

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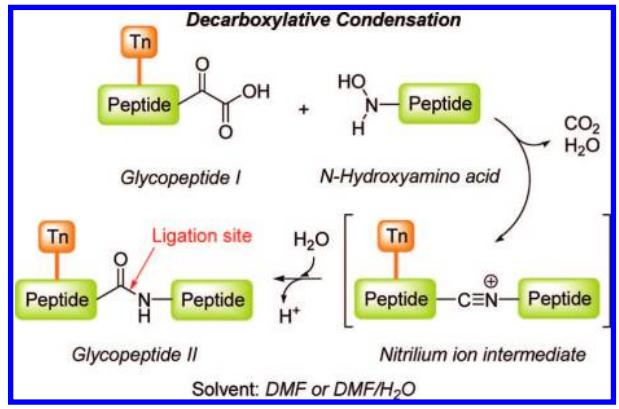

The chemical synthesis of homogeneous glycoproteins and glycopeptides facilitates progress toward understanding the functional role of carbohydrates attached to proteins and is important in the preparation of glycopeptide-based therapeutics. A series of protected and unprotected glycosyl dipeptides, glycopeptide I, which contained the α-ketoacid moiety at the C-terminus, were synthesized and ligated with a series of *O-tert*-butyl-protected *N*-hydroxylamino acids to afford *O-tert*-butyl-protected glycosyl tripeptides, glycopeptide II. The reactions were carried out under both anhydrous and aqueous conditions at neutral pH to produce glycopeptide products in yields ranging from 15% to 86% depending on the amino acids present at the ligation junction. The best yields were obtained when both the α-ketoacid and the *N*-hydroxylamino acid contained medium-sized side chains. In addition to the expected tripeptide product, 2,5-substituted oxazoles were isolated when *O-tert*-butyl protected *N*-hydroxylamines of glycine were employed in the reaction. The formation of the oxazole is believed to result from an intramolecular cyclization of the *O-tert*-butyl ester on a nitrilium ion intermediate followed by aromatization. A decarboxylative condensation between O^{18}-labeled phenyl pyruvic acid and *N*-hydroxyphenethylamine oxalate salt resulted in amide products lacking the O^{18}-label, providing further support for the nitrilium ion in the reaction pathway.

Introduction

Significant efforts have been made to access homogeneous glycoproteins and glycopeptides over the past two decades.[1] Chemical synthesis has the potential to fulfill this need, which facilitates progress toward understanding the functional role of carbohydrates attached to a particular protein[2] and for the preparation of glycopeptide- or glycoconjugate-based therapeutics.[3,4] Currently, solid-phase peptide synthesis methods[5] are considered practical for the preparation of peptides containing ≤40 amino acids.[6] If larger peptides are needed or if chemoenzymatically derived glycopeptides are to be joined, chemical methods for coupling glycopeptide fragments are frequently required. The current standard reactions for joining two peptide segments together to create a longer native peptide have overwhelmingly been the native chemical ligation (NCL)[7] and more recently the application of Staudinger reaction[8] in the form

(1) Review: Nilsson, B. L.; Soellner, M. B.; Raines, R. T. *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 91–118.

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of the traceless Staudinger ligation.[9] The reactivity of thioesters used in the NCL provides practical ways to access full-length proteins and glycoproteins. The potential of these powerful techniques was recently demonstrated in the synthesis of a HIV protease covalent dimer[7t] containing 203 amino acids in the sequence. While each of these reactions has enjoyed great success, each reaction has intrinsic limitations: the former has an absolute reliance on a cysteine residue, present in <1.7% of all residues in globular proteins,[7h,10] at the ligation site, and the latter encounters issues of solubility of the phosphinothiol reagent in aqueous media[9f] and is most effective when one of the two residues at the ligation junction is glycine.[9e,f] To bypass the cysteine specific limitation of the NCL, a variety of innovative auxiliaries have been developed.[7c,f−i,k,l,o,r,s] While auxiliary-based efforts can deliver full-length peptides or glycopeptides, they often require a less hindered amino acid residues at the ligation junction for efficient ligation. In the second phase of auxiliary development, a new approach consisting of a glycopeptide segment with the sugar bearing acetamidomethyl-thio-handle[11] at its C-2 position has been effective in delivering longer O-linked and N-linked glycopeptides and glycoproteins. The latter works by way of the thio-auxiliary in the form of a sugar-assisted ligation. In the pursuit of more general chemistry for glycopeptide synthesis, thiol-auxiliary-free cysteine-free coupling protocols have been developed. In this regard, amide ligation by decarboxylative condensation between a hydroxylamine and a peptide bearing an α-ketoacid moiety at C-terminus,[12] metal using silver chloride (AgCl) and metal-free (tris(2-carboxyethyl)phosphine, TCEP) mediated coupling,[13] and small peptide and thioacid-2,4-dinitrobenzenesulfonamide couplings[14] represent the latest alternatives to cysteine-based and auxiliary-based ligation protocols. Although the AgCl-assisted phenolic ester directed amide coupling (PEDAC-AgCl) or PEDAC-TCEP[13] has been a reliable protocol for the synthesis of complex glycan-containing polypeptides, it is not without limitation and an issue of racemization has been noted. Moreover, compatibility of thioacid-2,4-dinitrobenzenesulfonamide couplings[14] has not been extended to the context of glycopeptide synthesis, and chemoselectivity is an issue. Emerging chemistry that is capable of linking two glycopeptide segments should in principle (a) avoid amino-acid-specific limitations at the ligation junction, (b) be water-tolerant, (c) be devoid of restricted access to peptide thioesters,[7u] and (d) possess high chemoselectivity.

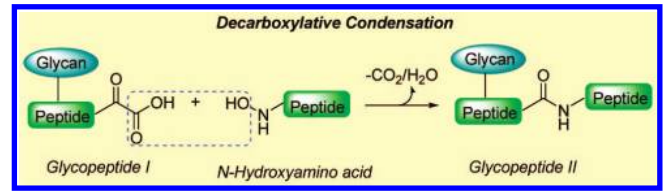

FIGURE 1. General concept for decarboxylative condensation.

In this paper we describe the ligation of glycopeptide fragments by way of the decarboxylative condensation reaction, an amide ligation reported by Bode.[12a−c] We were intrigued by this reaction because of its independence from thiol capture methods, lack of requirement for external reagents, "traceless" nature, water tolerance, and reported chemoselectivity. We envisioned that this unique condensation chemistry would prove ideal for ligating water-soluble, unprotected, and pH-sensitive glycopeptides. To demonstrate the chemistry, we prepared glycosyl dipeptides with C-terminal α-ketoacid moieties and a series of amino acids containing *N*-hydroxylamino moieties. The two components were used to study the amino acid requirements at the ligation junction and evaluate the chemoselectivity in the presence of unprotected glycans. The general concept for the ligation is illustrated in Figure 1.

Results and Discussion

To explore both the compatibility of the decarboxylative condensation with glycans and systematically explore the amino acid side chain requirement of the ligation junction a series of key intermediates Ac_3GalNAc-α-*O*-Thr-Gly-C_2O_3H **1**, Ac_3GalNAc-

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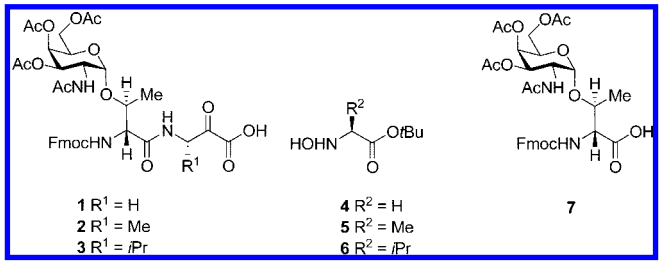
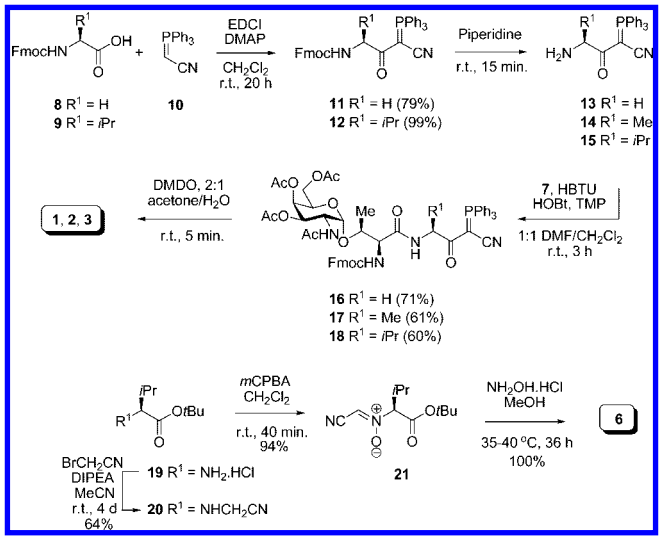


FIGURE 2. Key intermediates for amide ligation.

SCHEME 1. Synthesis of Glycosyl Dipeptide α-Ketoacids 1, 2, and 3 and Valine Hydroxyl Amine 6[a]



[a] Compounds **1**−**3** and **13**−**15** formed quantitatively and were used as crude materials without further purification.

α-*O*-Thr-Ala-C_2O_3H **2**, and Ac_3GalNAc-α-*O*-Thr-Val-C_2O_3H **3** were prepared. To explore the side chain requirement of *N*-hydroxyamine components, *N*-hydroxyglycine *tert*-butyl ester (**4**),[12a,15] *N*-hydroxyalanine *tert*-butyl ester (**5**),[15] and *N*-hydroxyvaline *tert*-butyl ester (**6**) were also prepared (Figure 2).

Glycosyl dipeptides **1**−**3** bearing C-terminal α-ketoacid moieties were prepared by the reaction of a known glycosylamino acid building block **7**[16] (Figure 2) with glycine cyanophosphorane **13**, alanine cyanophosphorane **14**,[17] and valine cyanophosphorane **15**, respectively (Scheme 1). Hitherto unknown **13** and **15** were prepared by the same reaction conditions employed for the synthesis of **14**.[17] Commercially available Fmoc-protected glycine **8** was coupled with (cyanomethylene)triphenylphosphorane **10**[18] in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) to afford Fmoc-protected glycine cyanophosphorane **11** in 79% yield. The Fmoc group of **11** was subsequently removed by treatment with piperidine[19] at ambient temperature to furnish **13** as a crude material. Similarly, valine cyanoketophosphorane **15** was synthesized quantitatively by the condensation of **9** and **10**, via **12**, by the same reaction conditions (Scheme 1). Without further purification, cyanophosphoranes **13**−**15** having residual piperidine were coupled with **7** in the presence of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyuronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), and 2,4,6-trimethylpyridine (TMP)[20] in DMF−CH_2Cl_2 (1:1) to generate cyano ketophosphorane derivatives **16**, **17**, and **18** in 71%, 61%, and 60% yields, respectively. During the HBTU condensation, a piperidine adduct **A**, Supporting Information, was isolated in 25% yield in each case as the byproduct. Cyano ketophosphorane derivatives **16**, **17**, and **18** were oxidized by dimethyldioxirane (DMDO) in acetone−H_2O[21,22] to generate α-ketoacids **1**, **2**, and **3** in quantitative yield, respectively (Scheme 1).

Having obtained the key α-ketoacid components **1**, **2**, and **3**, we next prepared *N*-hydroxylamines **4**,[12a,15] **5**,[15] and **6**. *N*-Hydroxylamines **4** and **5** were prepared by the reported literature protocols.[12a,15] *N*-Hydroxyvaline derivative **6** was unknown and was synthesized from valine *tert*-butyl ester **19**. Protected valine was alkylated with 2-bromoacetonitrile[12a,15] to afford **20** in 64% yield. Compound **20** was quantitatively converted to **6**, via its nitrone derivative **21** by oxidation with *m*CPBA followed by aminolysis in the presence of hydroxylamine hydrochloride (Scheme 1).

With α-ketoacids **1**, **2**, and **3** in hand, we condensed each of them separately with appropriate *N*-hydroxyamino acid **4**, **5**, or **6** in anhydrous DMF or 5:1 DMF−water at 35−40 °C (Scheme 2). The results of decarboxylative condensations are summarized in Table 1. Compound **1** with a glycine residue at the C-terminus was reacted with a *N*-hydroxyglycine ester **4** in anhydrous DMF at 40 °C to generate glycosyl tripeptide **22** and an oxazole byproduct **23** in 41% and 22% yields, respectively (entry 1; Table 1). Similarly, 45% and 22% yields, respectively, of **22** and **23** were isolated from the reaction between **1** and **4** at 40 °C in 5:1 DMF−water (entry 2; Table 1). Next, a slightly bulkier *N*-hydroxyalanine ester **5** was reacted with **1** in both anhydrous and aqueous DMF under the general reaction condition and glycosyl tripeptide **24** was obtained in 40% and 23% yields, respectively after 25 h (entries 3 and 4; Table 1). To our surprise no oxazole byproduct formed under both anhydrous and aqueous conditions. Next, we wished to employ this mild reaction condition in a relatively hindered junction such as Ala−Gly, Ala−Ala, or Gly−Val to ensure influence of the size of amino acid side chain on the outcome of ligation. Therefore, α-ketoacid **2** bearing an alanine residue at the C-terminus was treated with *N*-hydroxyglycine ester **4** in anhydrous DMF at 42 °C, and we isolated glycopeptide **25** and an oxazole byproduct **26** in 43% and 23% yields, respectively, in 6 h. In an effort to improve yield, the latter reaction was carried out at room temperature, and after 24 h, both **25** and **26** were isolated in 39% and 29% yields, respectively. Similarly, in 5:1 DMF−water, reaction of α-ketoacid **2** with **4** provided **25** and **26** in 46% and 23% yields, respectively, after 48 h (entries 5, 6 and 7; Table 1). At this point we were curious to know the outcome of ligation at the "Ala−Ala" junction. Hence, α-ketoacid **2** was condensed with *N*-hydroxylamine **5** in both anhydrous and aqueous DMF to furnish 86% and 54% yields in 38 and 48 h, respectively (entries 8 and 9; Table 1). Interestingly, no oxazole byproduct formed

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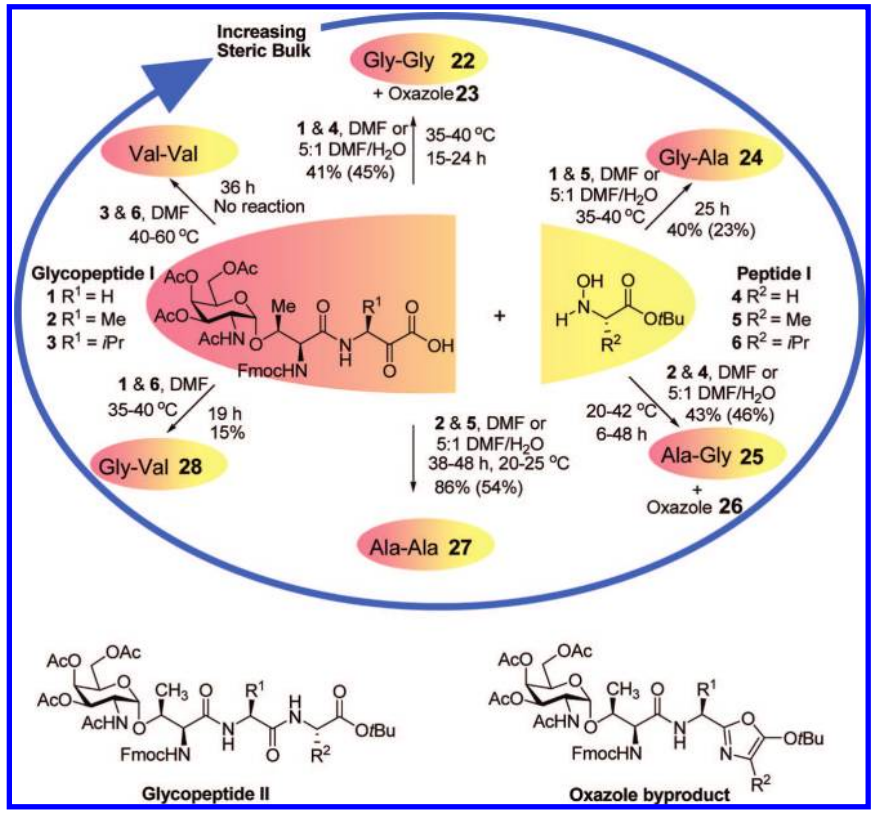
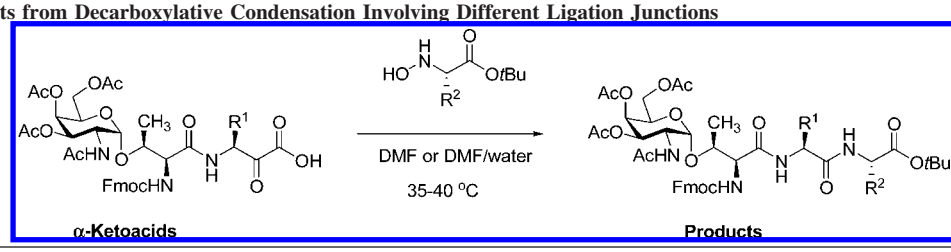
SCHEME 2. Decarboxylative Condensation Involving Protected Glycosyl Dipeptide α-Ketoacids and *N*-Hydroxyamino Acids[a]



[a] Yields in parenthesis are from aqueous conditions.

TABLE 1. Products from Decarboxylative Condensation Involving Different Ligation Junctions



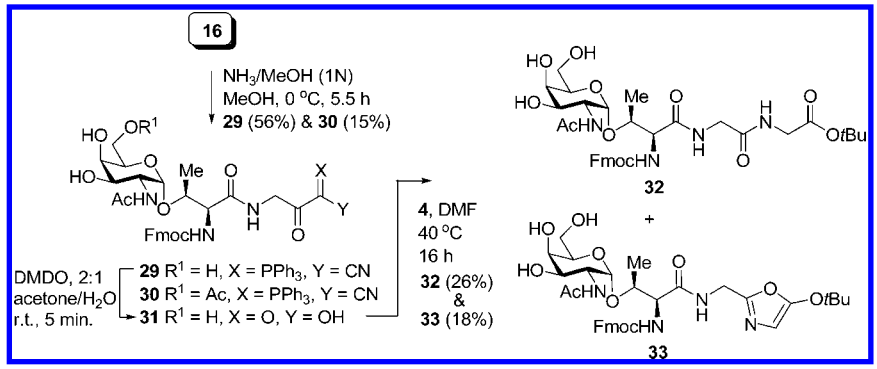
entry	α-ketoacids	*N*-hydroxyamines	reaction conditions	products yields (%)[h]	byproducts yields (%)[h]
1	$R^1 = H^a$	$R^2 = H^a$	DMF, 24 h[d]	**22** (41)	**23** (22)
2	$R^1 = H^a$	$R^2 = H^a$	DMF/H_2O (5:1), 15 h[d]	**22** (45)	**23** (22)
3	$R^1 = H^a$	$R^2 = Me^b$	DMF, 25 h[d]	**24** (40)	
4	$R^1 = H^a$	$R^2 = Me^b$	DMF/H_2O (5:1), 25 h[d]	**24** (23)	
5	$R^1 = Me^b$	$R^2 = H^a$	DMF,6 h[e]	**25** (43)	**26** (23)
6	$R^1 = Me^b$	$R^2 = H^a$	DMF, 24 h[f]	**25** (39)	**26** (29)
7	$R^1 = Me^b$	$R^2 = H^a$	DMF/H_2O (5:1), 48 h[f]	**25** (46)	**26** (23)
8	$R^1 = Me^b$	$R^2 = Me^b$	DMF, 38 h[f]	**27** (86)	
9	$R^1 = Me^b$	$R^2 = Me^b$	DMF/H_2O (5:1), 48 h[d]	**27** (54)	
10	$R^1 = H^a$	$R^2 = iPr^c$	DMF, 19 h[d]	**28** (15)	
11	$R^1 = iPr^c$	$R^2 = iPr^c$	DMF, 36 h[g]		

[a] Glycine. [b] Alanine. [c] Valine. [d] 35−40 °C. [e] 40−42 °C. [f] 20−25 °C. [g] 40−60 °C. [h] Isolated yield.

in either medium. Condensation of **1** with bulkier *N*-hydroxyvaline ester **6** was low yielding, and 15% yield of the desired glycosyl tripeptide **28** was isolated in anhydrous DMF after 19 h. Again, we did not observe oxazole formation in this case. In general we isolated the expected glycosyl tripeptides in moderate to high yields from the decarboxylative condensation with protected glycosyl dipeptide α-ketoacids with the exception of the ligation involving glycosyl α-keto acid **3** and valine hydroxylamine **6** for "Val−Val" junction (entry 11; Table 1, Scheme 2) at the ligation site. In the latter case, reaction did not proceed after 36 h under the general reaction condition employed. Our efforts to initiate reaction by raising temperature or by using *p*-toluenesulfonic acid (TsOH) as a catalyst did not help.

With a notion to validate the chemoselectivity of this efficient protocol for amide bond formation, next acetyl groups of **16** were deprotected in the presence of ammonia (1 N) in anhydrous MeOH at 0 °C (Scheme 3) to provide the desired trihydroxy derivative **29** in 56% yield accompanied by a small amount of partially deprotected 6-*O*-acetyl derivative **30** (15% yield).

SCHEME 3. Synthesis of Deprotected Glycopeptide and Chemoselective Ligation[a]



[a] Conversion of **29** to **31** was quantitative, and the reaction mixture was concentrated and used without further purification. For decarboxylative condensation of **31** with **4** under aqueous condition (5:1 DMF/H_2O, 40 °C, 24 h), only **32** could be isolated (25% yield).

Compound **29** was converted to α-keto acid **31** with DMDO in quantitative yield. α-Ketoacid **31** was subjected to decarboxylative condensation in the presence of **4** in anyhydrous DMF to deliver the glycosyl tripeptide **32** and an oxazole byproduct **33** in 26% and 18% yields, respectively. Condensation of **31** with **4** was also carried out in 5:1 DMF−water, and the desired glycosyl tripeptide **32** was isolated in 25% yield. Oxazole byproduct **33** in the latter case formed in low quantity, and isolation was difficult due to paucity of the materials (Scheme 3).

Compounds **6**, **11**, **12**, **16**−**18**, **20**−**30**, **32**, and **33** were characterized by ^{1}H NMR, ^{1}H−^{1}H gCOSY, ^{13}C NMR and HRMS. DEPT experiments were carried out on a few of the compounds to verify the number of methylene groups present in the molecules.

Appearance of isolated singlets in ^{1}H NMR of **23**, **26**, and **33** at $\delta = 6.1$, 6.13, and 6.24 ppm and carbons at $\delta = 107.59$, 107.41, and 108.93 ppm, respectively, in ^{13}C NMR were characteristics of the oxazole byproducts. The assignment of the H-4 attached to the oxazole moiety was unambiguously determined by carrying out the gHMQC NMR experiment on **33**. The characteristic region of the gHMQC of **33** showing the correlation between C-4 and H-4 of the oxazole moiety is depicted in Figure 3.

On the basis of these results it is clear that hindered C-terminal amino acids are much less reactive than the moderately sized amino acids. Our outcome of ligations at Gly−Ala and Ala−Ala junctions are consistent with those known in the literature.[7o,r,11]

Next, we revisited the reported mechanism of the decarboxylative condensation to rationalize the formation of the oxazoles (**23**, **26**, or **33**). Two pathways, A and B, have been previously proposed to arrive at amide products (Scheme 4).[12a] Common to each pathway is the initial formation of a hemiaminal. In pathway A, the hemiaminal loses a molecule of CO_2 and water to provide an imidic acid which undergoes tautomerisation to afford the desired glycosyl tripeptides (dotted arrows; Scheme 4).

A mechanism arising from an intermediate along this pathway leading to oxazoles is not readily apparent. A second plausible mechanism for amide formation is illustrated along pathway B. In this pathway the hemiaminal loses a molecule of water to afford an (*E*/*Z*) mixture of nitrones. Loss of molecule of CO_2 and water from the (*E*) nitrone would be expected to give rise to a reactive nitrilium ion. The nitrilium species is in the right setting to rapidly cyclize via attack of the *tert*-butyl ester

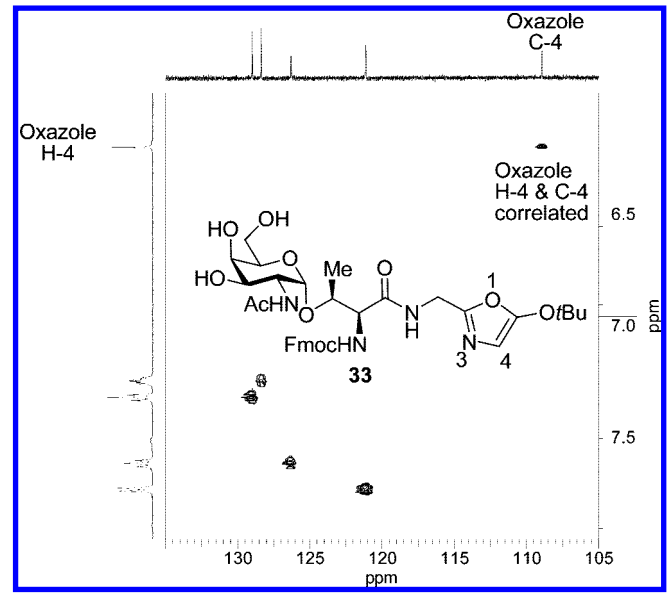
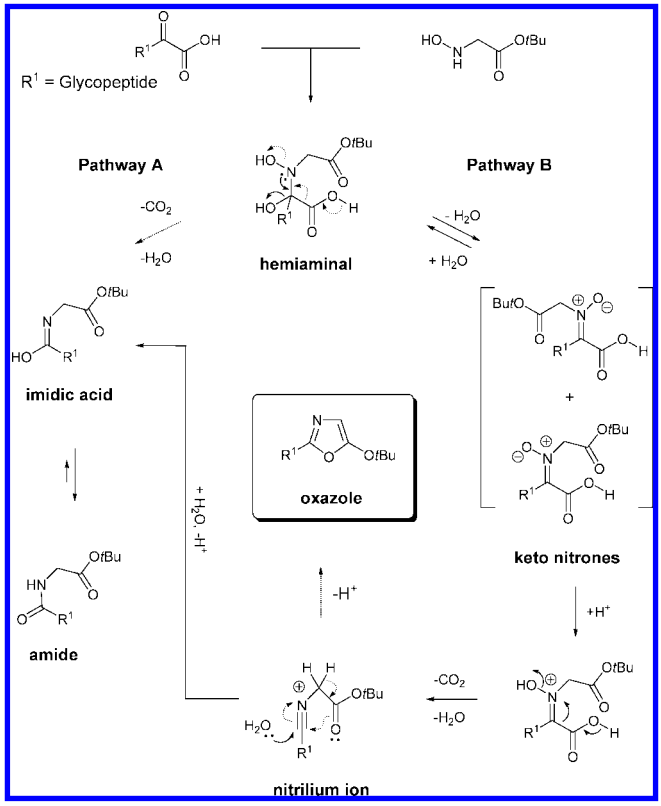


FIGURE 3. Characteristic region of gHMQC of oxazole byproduct **33**.

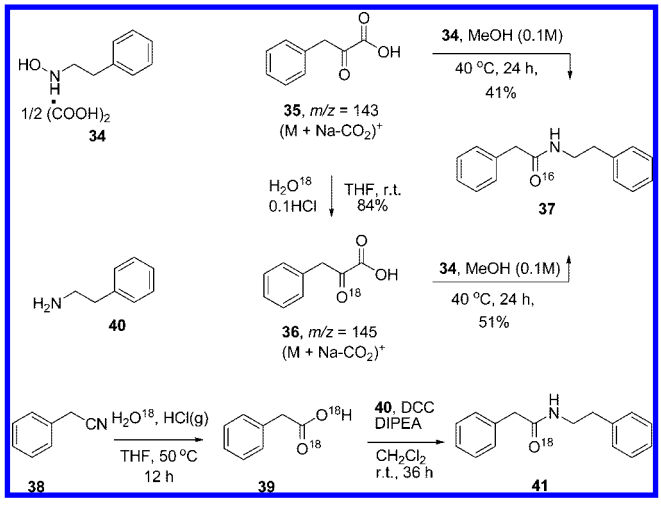
carbonyl followed by aromatization to provide an oxazole byproduct (dotted arrows; Scheme 4). The nitrilium ion could also undergo addition of a water molecule to generate the imidic acid of pathway A. Previous studies using various nucleophiles failed to trap the speculated nitrilium intermediate suggesting pathway A was the operative mechanism.[12a] Isolation of oxazoles suggests that a nitrilium ion might be the key intermediate in the decarboxylative ligation (vida infra). As it appears, oxazole byproduct formed readily in the case of Gly−Gly or Ala−Gly ligation junction. Exclusive isolation of glycosyl tripeptide in the case of ligation between Ala−Ala and Gly−Val suggests a role of steric bulk at the hydroxylamine bearing amino acid. It is apparent that formation of an oxazole byproduct is favored via a nitrilium ion intermediate when the hydroxylamine residue at the ligation site is Gly. In addition to this observation couplings were most effective when either amino acid at the ligation junction lacked β-branching.

To further distinguish between path A and B, a model study was performed involving decarboxylative condensation of unlabeled and O^{18}-labeled phenyl pyruvic acids **35** and **36**, respectively, with *N*-hydroxyphenethylamine oxalate salt **34**.[12a] In doing so, we synthesized *N*-hydroxyphenethylamine oxalate salt **34** (Scheme 5) under the reaction conditions analogous to the synthesis of **11**. Commercially available phenyl pyruvic acid

SCHEME 4. Proposed Mechanisms Leading to Amides and Oxazoles in the Decarboxylative Condensation



SCHEME 5. Isotope Labeling Experiment with O^{18}-Labeled Phenylpyruvic Acid[a]



[a] Compounds **39** and **41** were crude materials and no purification was carried out.

35 was used as an α-ketoacid. Compound **35** was first labeled with O^{18}-oxygen in the presence of H_2O^{18} and 0.1 N HCl in anhydrous THF to obtain **36** in 84% yield.[23] Both **35** and **36** showed mass fragments of $m/z = 143.1$ $[M + Na - CO_2]^+$ and $m/z = 145.1$ $[M + Na - CO_2]^+$, respectively, with a loss of one molecule of CO_2. Compound **35** was subjected to decarboxylative condensation in the presence of **34** in MeOH to afford amide **37** [$m/z = 240.3$ $(M + H)^+$] in 41% yield (Scheme 5). Next, O^{18}-labeled **36** was reacted with hydroxylamine **34** under the same reaction conditions.

(23) Byrn, M.; Calvin, M. *J. Am. Chem. Soc.* **1966**, *88*, 1916–1922.

To our surprise, amide **37** [$m/z = 240.2$ $(M + H)^+$] was obtained with complete loss of label in 51% yield. Initially we thought that loss of O^{18}-oxygen in **37**[12a] could be either the result of an exchange with H_2O^{16} or a problem of detection by the mass spectrometer used. We verified the result by carrying out an alternative synthesis where commercially available benzyl cyanide **38** was hydrolyzed in a mixture of H_2O^{18} and anhydrous THF in the presence of HCl.[24] The labeled carboxylic acid **39** [$m/z = 163$ $(M + Na)^+$] was condensed with commercially available phenethylamine **40** in the presence of dicyclohexylcarbodiimide (DCC), and the crude product **41** (Scheme 5) was analyzed by mass spectrometry.

Compound **41** showed a peak at $m/z = 242.4$ $[M + H]^+$ indicating complete retention of O^{18}-oxygen. The result suggests that the O^{18}-labeled amide is fairly stable once formed and does not exchange with O^{16} in the mass spectrometry employed and amide **37** loses its labeling during the amidation reaction.

Loss of labeling in **37** supports pathway B as the operative mechanism for the decarboxylative condensation of *N*-hydroxylamines and α-ketoacids.

In summary, the importance of this synthetic strategy for the ligation of glycopeptide fragments stems from the fact that the ligation proceeds under neutral aqueous conditions and occurs at low molar concentrations. Decarboxylative ligation is proven to be chemoselective in the presence of free hydroxyls. It is now clear that conditions such as these would be useful for joining unprotected glycopeptides that have been prepared by chemoenzymatic methods. These studies are the first to take advantage of the decarboxylative condensation to produce glycopeptides. Isolation of oxazoles and isotope labeling experiment on a model system provide reasonable evidence for a nitrilium ion as the key intermediate in the decarboxylative condensation. Work is in progress to explore its use in the synthesis of glycopeptides of interest.

Experimental Section

General Methods. Amino acids and other fine chemicals were purchased from commercial suppliers and were used without further purification. All solvents used for reactions were dried following the standard procedures.[25] Thin-layer chromatography (TLC, silica gel 60, f_{254}) were performed in distilled solvents as specified and visualized under UV light or by charring in the presence of 5% H_2SO_4/MeOH. Flash column chromatography was performed on silica gel (230−400 mesh) column using solvents as received. ^1H NMR were recorded on either a 400 or a 600 MHz spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 using residual $CHCl_3$, CH_3OH, or DMSO as internal references, respectively. ^{13}C NMR were recorded on either a 100.56 or a 150.83 MHz in $CDCl_3$, CD_3OD, or DMSO-d_6 using the triplet centered at δ77.273 for $CDCl_3$, septet centered at δ 49.0 for CD_3OD, or septet centered at δ 39.5 for DMSO-d_6 as internal reference, respectively. ^{31}P NMR was recorded on a 161.9 MHz spectrometer using either $CDCl_3$ or CD_3OD as solvent. $^1H-^1H$ gCOSY and $^1H-^{13}C$ gHMQC NMR were performed on a 600 MHz spectrometer. Melting points of all crystalline solids were determined using a capillary tube and are uncorrected. High resolution mass spectrometry (HRMS) were performed on a TOF mass spectrometer.

Fmoc-Protected Glycine Cyanophosphorane 11. To a well-stirred solution of Fmoc-protected glycine **8** (1.00 g, 3.36 mmol),

(24) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 4609–4614.

(25) Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 5th ed.; Butterworth-Heinemann: New York, 2003; pp 80−388.

(26) Murray, R. W.; Singh, M. *Organic Synthesis*; Wiley: New York, 1998; Collect. Vol. IX; p 288; *Org. Synth.* **1997**, *74*, 91.

DMAP (0.04 g, 0.33 mmol), and EDCI (0.84 g, 4.37 mmol) in CH_2Cl_2 (50 mL) was added (cyanomethylene)triphenylphophorane **10** (1.19 g, 3.53 mmol) at ambient temperature under N_2 atmosphere. The resulting solution was stirred at ambient temperature. The reaction was monitored by TLC and appeared to stop after 20 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed successively with water (30 mL), saturated $NaHCO_3$ (2 × 30 mL) and water (30 mL). Combined organic phases were dried (anhydrous Na_2SO_4) and filtered, and the filtrate was concentrated to dryness under reduced pressure. Purification of the crude material by silica gel flash column chromatography (10 × 5.5 cm) with 3:3:14 and then 1:1:2 acetone/$CHCl_3$/hexanes afforded the desired product **11** as a fluffy mass, highly hygroscopic in nature: yield 1.51 g (79%); silica gel TLC $R_f = 0.45$ (1.5:1 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.16 (t, 1H, $J = 7.2$ Hz, Fmoc CH), 4.34 (d, 2H, $J = 7.2$ Hz, Fmoc CH_2), 4.42 (d, 2H, $J = 4.2$ Hz, α-CH_2), 5.61 (br.s, 1H, NH), 7.25 (t, 2H, $J = 7.2$ Hz, aromatic), 7.35 (t, 2H, $J = 7.8$ Hz, aromatic), 7.53 (m, 6H, aromatic), 7.58 (m, 8H, aromatic), 7.64 (m, 3H, aromatic), 7.71 (d, 2H, $J = 7.8$ Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 47.2, 47.7 (CH_2), 66.8 (CH_2), 119.9, 122.1, 122.7, 125.2, 127.0, 127.6, 129.3, 129.4, 133.5, 133.53, 133.56, 133.6, 141.2, 144.0, 156.1 (C═O), 189.9 (C═O); ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 20.9 (s, PPh_3); mass spectrum (HRMS), $m/z = 603.1805$ $(M + Na)^+$ ($C_{37}H_{29}N_2NaO_3P$ requires 603.1814).

Fmoc-Protected Valine Cyanophosphorane 12. Fmoc-protected valine **9** (1.1 g, 3.24 mmol) was reacted with (cyanomethylene)triphenylphophorane **10** in the presence of EDCI (0.807 g, 4.21 mmol) and DMAP (0.04 g, 0.32 mmol) to furnish valine cyanophosphorane derivative **12** in 20 h, following the procedure described for compound **11**. Purification of the crude material by silica gel flash column chromatography (10 × 5.5 cm) with 1:1:7.1 acetone/$CHCl_3$/hexanes produced **5** as a fluffy white solid: yield 2.0 g (99%); $R_f = 0.55$ (1.5:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, 3H, $J = 6.8$ Hz, Val-CH_3), 1.07 (d, 3H, $J = 6.8$ Hz, Val-CH_3), 2.43 (m, 1H, Val-CH), 4.22 (t, 1H, $J = 7.2$ Hz, Fmoc CH), 4.34 (m, 2H, Fmoc CH_2), 4.91 (dd, 1H, $J = 4.0$, 8.8 Hz, Val-α-CH), 5.60 (d, 1H, $J = 8.8$ Hz, NH), 7.27 (t, 2H, $J = 7.2$ Hz, aromatic), 7.37 (t, 2H, $J = 7.6$ Hz, aromatic), 7.48−7.64 (m, 17H, aromatic), 7.75 (d, 2H, $J = 7.6$ Hz, aromatic); ^{13}C NMR (100.56 MHz, $CDCl_3$) δ 16.9, 20.2, 32.2, 47.4, 61.0, 61.1, 120.0, 122.4, 123.3, 125.4, 127.2, 127.7, 129.3, 129.4, 133.44, 133.47, 133.6, 133.7, 141.38, 141.40, 156.4, 194.0; ^{31}P NMR (80.95 MHz, $CDCl_3$) δ 21.3 (s, PPh_3); mass spectrum (ESI-MS), $m/z = 623.3$ $(M + H)^+$ ($C_{40}H_{36}N_2O_3P$ requires 623.2); mass spectrum (HRMS), $m/z = 645.2289$ $(M + Na)^+$ ($C_{40}H_{35}N_2O_3PNa$ requires 645.2283).

Glycine Cyanophosphorane 13 and Valine Cyanophosphorane 15. Compound **11** (2.04 g, 3.52 mmol) was taken in anhydrous piperidine (10 mL), and the resulting solution was stirred at ambient temperature. The reaction was monitored by TLC and appeared to stop within 15 min. Excess piperidine was removed under reduced pressure, and the crude material **13** thus obtained was coevaporated with anhydrous CH_2Cl_2 and triethylamine. The residue was dried in high vacuum overnight before being used in the next step without further purification. Crude dried material was off-white in color: yield (quantitative); silica gel TLC $R_f = 0.41$ (1:9 MeOH/$CHCl_3$); mass spectrum (ESI-MS), $m/z = 359.1$ $(M + H)^+$ ($C_{22}H_{20}N_2OP$ requires 359.1). Similarly, Fomoc-protected valine cyanophosphorane **12** (0.15 g, 0.24 mmol) was reacted with neat piperidine to generate valine cyanophosphorane **15** in quantitative yield and was used in the next step without further purification. Crude dried material was off-white in color: silica gel TLC $R_f = 0.44$ (1:9 MeOH/CH_2Cl_2); mass spectrum (ESI-MS), $m/z = 401.5$ $(M + H])^+$($C_{25}H_{26}N_2OP$ requires 401.17).

Glycodipeptide Cyanophosphorane Analog 16 and Glycosylamino Acid-Piperidine Byproduct A. N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonine **7** (0.8 g, 1.19 mmol), HBTU (0.9 g, 2.38 mmol), and HOBt (0.32 g, 2.38 mmol) were taken together and dried for 0.5 h in high vacuum before addition of 1:1 anhydrous DMF and CH_2Cl_2 (15 mL), and the resulting mixture was stirred for 15 min at ambient temperature. Glycine cyanophosphorane derivative **13** (0.85 g, 2.38 mmol) in CH_2Cl_2 (5 mL) was added dropwise, followed by addition of TMP (174 μL, 1.31 mmol) under N_2 atmosphere, and stirring was continued at ambient temperature. The reaction was monitored by TLC and appeared complete within 3 h. The reaction mixture was diluted with CH_2Cl_2 (ca. 60 mL) and successively washed with cold water (30 mL), 1% citric acid (30 mL), cold $NaHCO_3$ (30 mL), and water (30 mL). Each aqueous fraction was back extracted with CH_2Cl_2 (40 mL), the combined organic phases were dried (anhydrous Na_2SO_4) and filtered, and the filtrate was concentrated to dryness under reduced pressure. The crude material thus obtained was purified by silica gel flash column chromatography (10 × 5.5 cm). Elution with 0.6:0.6:1.2:7.6 MeOH/acetone/$CHCl_3$/hexanes generated desired product **16** as a colorless fluffy mass: yield 0.85 g (71%); silica gel TLC $R_f = 0.3$ (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.2 (d, 3H, 6.6 Hz, Thr-CH_3), 1.72 (s, 3H, CH_3CO), 1.84 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 4.03 (m, 2H, H-6/H-6′), 4.21 (m, 4H, Thr-α-CH, β-CH, H-5 and Fmoc CH), 4.38 (m, 3H, Fmoc CH_2 and Gly-CH), 4.52 (m, 2H, H-2 and Gly-CH), 4.94 (d, 1H, $J = 3.6$ Hz, H-1), 5.02 (dd, 1H, $J = 3.0$, 11.4 Hz, H-3), 5.33 (s, 1H, H-4), 5.92 (d, 1H, $J = 7.8$ Hz, Fmoc NH), 6.68 (d, 1H, $J = 9.6$ Hz, NHAc), 7.03 (br. s, 1H, Gly-NH), 7.26 (t, 2H, $J = 7.8$ Hz, aromatic), 7.36 (m, 2H, aromatic), 7.56 (m, 14H, aromatic), 7.65 (m, 3H, aromatic), 7.73 (d, 2H, $J = 7.2$ Hz, aromatic); ^{13}C NMR (100.56 MHz, $CDCl_3$) δ 20.6, 20.71, 20.77, 22.8, 46.3 (CH_2), 46.4, 47.0, 47.1, 47.5, 58.1, 62.1 (CH_2), 67.1 (CH_2), 67.2, 67.4, 68.5, 76.6, 77.4, 99.3 (C-1), 119.9, 120.2, 120.4, 121.5, 122.5, 125.2, 127.1, 127.7, 129.4, 129.5, 129.4, 129.5, 133.5, 133.6, 133.7, 141.2, 143.7, 143.8, 156.5 (C═O), 169.1 (C═O), 170.4 (3 × C═O), 170.5 (C═O), 171.0 (C═O), 189.5, 189.5; ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 20.6 (s, PPh_3); mass spectrum (HRMS), $m/z = 1033.3407$ $(M + Na)^+$ ($C_{55}H_{55}N_4NaO_{13}$ requires 1033.3401). When the reaction for compound **16** was repeated on a 1.18 g scale, a byproduct **A** (0.328 g, 25%) was isolated as a white fluffy mass after purification as described above: silica gel TLC $R_f = 0.31$ (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.36 (d, 3H, $J = 6.0$ Hz, Thr-CH_3), 1.39−1.67 (m, 6H, piperidine Hs), 1.98 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.14 (s, 3H, CH_3CO), 3.43 (m, 3H, piperidine Hs), 3.62 (m, 1H, piperidine H), 3.86 (m, 1H, β-CH), 4.06 (m, 2H, Fmoc CH_2), 4.23 (dd, 2H, $J = 7.2$, 12.6 Hz, H-5 and Fmoc CH), 4.35 (dd, 1H, $J = 7.2$, 10.2 Hz, H-6), 4.41 (dd, 1H, $J = 7.2$, 10.2 Hz, H-6′), 4.58 (ddd, 1H, $J = 3.0$, 9.0, 9.0 Hz, H-2), 4.68 (dd, 1H, $J = 1.8$, 9.6 Hz, Thr-α-CH), 4.74 (d, 1H, $J = 3.0$ Hz, H-1), 5.06 (dd, 1H, $J = 3.0$, 11.4 Hz, H-3), 5.4 (d, 1H, $J = 2.4$ Hz, H-4), 5.92 (d, 1H, $J = 9.0$ Hz, Fmoc NH), 6.39 (d, 1H, $J = 9.6$ Hz, N*H*Ac), 7.31 (dd, 2H, $J = 7.8$, 15.6 Hz, aromatic), 7.38 (d, 2H, $J = 7.2$, 13.8 Hz, aromatic), 7.63 (d, 2H, $J = 7.8$ Hz, aromatic), 7.75 (d, 2H, $J = 7.2$ Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 18.8 (Thr-CH_3), 20.7, 20.8, 20.84, 23.1, 24.2 (CH_2), 25.6 (CH_2), 26.7 (CH_2), 43.5 (CH_2), 46.8 (CH_2), 47.1, 47.2, 55.1, 62.3 (CH_2), 67.4 (CH_2), 67.5, 67.6, 69.2, 78.2, 101.3 (C-1), 120.0, 125.2, 125.3, 127.2, 127.7, 129.3, 129.4, 133.6, 133.65, 141.3, 143.8, 143.8, 156.8 (C═O), 168.2 (C═O), 170.4 (2 × C═O), 170.7 (C═O), 170.8 (C═O); mass spectrum (HRMS), $m/z = 760.3043$ $(M + Na)^+$ ($C_{38}H_{47}N_3NaO_{12}$ requires 760.3057).

Glycodipeptide Cyanophosphorane Analog 17. Glycosylamino acid **7** (0.15 g, 0.22 mmol) was reacted with alanine cyanophosphorane derivative **14** (0.13 g, 0.34 mmol) in the presence of HBTU (0.178 g, 0.469 mmol), HOBt (0.071 g, 0.464 mmol), and TMP (33 μL, 0.246 mmol) in 3:7 anhydrous DMF/CH_2Cl_2 (9 mL) to produce compound **17** as colorless amorphous solid, following the procedure described for compound **16**. The reaction appeared complete within 3.5 h. The crude material was purified by silica gel flash column chromatography (7 × 4.5 cm) using 0.5:0.5:1:8 and then 1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes to afford **17** as a

white amorphous solid: yield 0.14 g (61%); silica gel TLC R_f = 0.31 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.16 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.50 (d, 3H, J = 7.2 Hz, Ala-CH_3), 1.81 (s, 3H, CH_3CO), 1.85 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 4.03 (d, 2H, J = 5.4 Hz, H-6 and H-6′), 4.13−4.22 (m, 4H, Thr-α-CH, β-CH, H-5 and Fmoc CH), 4.36 (m, 2H, Fmoc CH_2), 4.52 (m, 1H, H-2), 4.84 (dd, 1H, J = 3.0, 11.4 Hz, H-3), 4.86 (d, 1H, J = 3.6 Hz, H-1), 5.13 (m, 1H, Ala-α-CH), 5.28 (d, 1H, J = 2.4 Hz, H-4), 5.83 (d, 1H, J = 7.2 Hz, Fmoc NH), 6.81 (d, 1H, J = 9.6 Hz, N*H*Ac), 7.10 (d, 1H, J = 6.6 Hz, Ala-NH), 7.27 (m, 2H, aromatic), 7.36 (t, 2H, J = 7.2 Hz, aromatic), 7.55 (m, 14H, aromatic), 7.64 (t, 3H, J = 6.6 Hz, aromatic), 7.73 (d, 2H, J = 7.8 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.5, 19.8, 20.6, 20.6, 20.7, 22.8, 47.0 (d, J_{CP} = 2.9 Hz), 51.0 (d, J_{CP} = 9.3 Hz), 57.2, 62.1, 66.9, 67.2, 67.2, 68.6, 75.5, 98.5 (C-1), 119.9, 119.91, 122.0 (d, J_{CP} = 93.2 Hz), 125.1, 127.0, 127.0, 127.6, 129.2 (d, J_{CP} = 12.9 Hz), 133.5 (d, J_{CP} = 2.6 Hz), 133.5 (d, J_{CP} = 10.4 Hz), 141.2, 141.2, 143.6, 143.8, 156.2 (C═O), 167.8 (C═O), 170.1 (C═O), 170.3 (C═O), 170.4 (C═O), 170.7 (C═O), 193.9 (d, J_{CP} = 3.6 Hz, C═O); ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 21.1 (s, PPh_3); mass spectrum (HRMS), m/z = 1047.3565 $(M + Na)^+$ ($C_{56}H_{57}NaN_4O_{13}P$ requires 1047.3557).

Glycodipeptide Cyanophosphorane Analogue 18. Glycosylamino acid **7** (0.1 g, 0.149 mmol) was reacted with crude valine cyanophosphorane derivative **15** (0.96 g, 0.24 mmol) in the presence of HBTU (0.0.85 g, 0.223 mmol) and HOBt (0.030 g, 0.223 mmol) in 1:1 anhydrous DMF/CH_2Cl_2 (5 mL) to produce a crude mixture, following the procedure described for compound **16**. The reaction was monitored by TLC and appeared complete within 2 h. The crude material was purified by silica gel flash column chromatography (7 × 3 cm) with 0.5:0.5:1:8 and then 0.7:0.7:1.4:7.2 MeOH/acetone/$CHCl_3$/hexanes to generate **18** as colorless fluffy mass: yield 0.95 g (60%); silica gel TLC R_f = 0.35 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.78 (d, 3H, J = 6.6 Hz, Val-CH_3), 1.06 (d, 3H, J = 6.6 Hz, Val-CH_3), 1.23 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.92 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.18 (s, 3H, CH_3CO), 2.51 (m, 1H, Val-CH), 4.05−4.11 (m, 3H, Thr-β-CH, H-6 and H-6′), 4.19 (t, 1H, J = 6.6 Hz, H-5), 4.24 (t, 1H, J = 7.2 Hz, Fmoc CH), 4.30 (dd, 1H, J = 1.8, 7.8 Hz, Thr-α-CH), 4.40 (m, 2H, Fmoc CH_2), 4.51 (ddd, 1H, J = 3.6, 10.8, 10.8 Hz, H-2), 4.74 (d, 1H, J = 3.0 Hz, H-1), 4.82 (dd, 1H, J = 3.0, 11.4 Hz, H-3), 5.11 (dd, 1H, J = 3.6, 7.0 Hz, Val-α-CH), 5.31 (d, 1H, J = 2.4 Hz, H-4), 5.84 (d, 1H, J = 7.8 Hz, Fmoc NH), 6.59 (d, 1H, J = 10.2 Hz, N*H*Ac), 6.66 (d, 1H, J = 8.4 Hz, Val-NH), 7.33 (m, 2H, aromatic), 7.40 (m, 2H, aromatic), 7.55 (m, 6H, aromatic), 7.59−7.68 (m, 11H, aromatic), 7.76 (d, 2H, J = 7.8 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.7, 17.3, 20.6, 20.8, 20.9, 20.99, 23.2, 32.4, 47.1, 47.3, 57.9, 59.6, 59.6, 62.4, 67.1, 67.5, 67.6, 69.2, 76.7, 99.5, 120.1, 120.2, 122.1, 122.7, 125.4, 125.4, 129.4, 129.4, 133.6, 133.7, 133.8, 133.9, 141.4, 143.9, 144.0, 156.5 (C═O), 169.1 (C═O), 170.4 (C═O), 170.4 (C═O), 170.6 (C═O), 171.0 (2 × C═O), 193.5, 193.5; ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 21.0 (s, PPh_3); mass spectrum (HRMS), m/z = 1075.3815 $(M + Na)^+$ ($C_{58}H_{61}N_4NaO_{13}P$ requires 1075.3870).

***N*-Cyanomethylvaline *tert*-Butyl Ester (20).** To a well-stirred suspension of valine *tert*-butyl ester hydrochloride **19** (0.5 g, 2.38 mmol) in anhydrous acetonitrile (10 mL) was added DIPEA (0.87 mL, 5.25 mmol) dropwise over a period of 15 min, and the resulting solution was stirred under N_2 atmosphere for 5 min. Bromoacetonitrile (0.17 mL, 2.38 mmol) was added dropwise over a period of 15 min, and stirring was continued. The reaction was monitored by TLC and appeared to stop after 3 days. Excess solvent was evaporated to dryness under reduced pressure to get a crude material which was dissolved in CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$. Aqueous layer was back-extracted with CH_2Cl_2 (2 × 50 mL). Combined organic layers were washed with brine (1 × 50 mL), dried (anhydrous Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure to get the crude residue. Purification of the crude residue with silica gel flash chromatography (8 × 3.5 cm) with 1:9 EtOAc/hexanes generated **20** as a white amorphous solid: yield 0.325 g (64%); silica gel TLC R_f = 0.37 (1:4 EtOAc/hexanes); mp 25.5−26 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (d, 3H, J = 7.2 Hz, Val-CH_3), 0.93 (d, 3H, J = 6.6 Hz, Val-CH_3), 1.45 (s, 9H, *t*Bu), 1.85 (br.s, 1H, NH), 1.93 (m, 1H, Val-CH), 2.97(d, 1H, J = 4.2 Hz, Val-α-CH), 3.50 (dd, 2H, J = 17.4, 33.0 Hz, CH_2CN); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 17.8, 19.3, 28.2 (3 × CH_3 of *t*Bu), 31.8, 36.9, 66.6, 82.1, 82.1, 117.9 (CN), 173.0 (C═O); mass spectrum (HRMS), m/z = 235.1423 $(M + Na)^+$ ($C_{11}H_{20}NaN_2O_2$ requires 235.1422).

***N*-Cyanomethyl *N*-oxide Valine *tert*-Butyl Ester 21.** To a well-stirred solution of compound **20** (0.284 g, 1.34 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added *m*CPBA (70−75%, 0.627 g, 2.72 mmol) in six portions at 5 min intervals. The resulting solution was allowed to stir at ambient temperature. Completion of the reaction was detected by TLC and appeared complete within 30 min. Sodium thiosulfate $Na_2S_2O_3 \cdot 5H_2O$ (0.664 g, 2.68 mmol) dissolved in water (2.9 mL) was added followed by addition of saturated $NaHCO_3$ (7.2 mL) at 0 °C, and the resulting solution was stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (60 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3 × 100 mL), and the organic layers were washed with brine (100 mL), dried (anhydrous Na_2SO_4), and filtered. The filtrate was concentrated to dryness under reduced pressure. The crude material thus obtained was purified by silica gel flash column chromatography (9 × 4.6 cm) with 3:7 EtOAc/hexanes to yield **21** as white amorphous solid: yield 0.285 g (94%); silica gel TLC R_f = 0.28 (1:4 EtOAc/hexanes); mp 60−61 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.01 (d, 3H, J = 6.6 Hz, Val-CH_3), 1.03 (d, 3H, J = 7.2 Hz, Val-CH_3), 1.48 (s, 9H, *t*Bu), 2.44 (m, 1H, Val-CH), 4.17 (d, 1H, J = 10.2 Hz, Val-α-CH), 6.97 (s, 1H, CHCN); ^{13}C NMR (100.56 MHz, $CDCl_3$) δ 18.7, 18.9, 27.9 (3 × CH_3 of *t*Bu), 31.1, 84.6, 86.0, 107.5 (*C*HCN), 112.2 (CN), 164.9 (C═O); mass spectrum (HRMS), m/z = 249.1216 $(M + Na)^+$ ($C_{11}H_{18}NaN_2O_3$ requires 249.1215).

***N*-Hydroxyvaline *tert*-Butyl Ester 6.** To a well-stirred solution of compound **21** (0.266 g, 1.18 mmol) in MeOH (30 mL) was added hydroxylamine hydrochloride (0.409 g, 5.88 mmol), and the resulting solution was stirred at 35−40 °C. The reaction was monitored by TLC and appeared complete after 36 h. The reaction mixture was allowed to attain room temperature, diluted with CH_2Cl_2 (30 mL), and stirred for 5 min. Saturated $NaHCO_3$ (70 mL) was added, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried (anhydrous Na_2SO_4), and filtered. The filtrate was concentrated to dryness under reduced pressure to provide the crude material. To the above crude material was added oxalic acid (0.212 g, 2.36 mmol) in MeOH (3.5 mL), and the resulting solution was triturated with hexanes. The liquid containing solids was centrifuged to render **6** as a white powder: yield 0.276 g (100%); silica gel TLC R_f = 0.28 (3:7 EtOAc/hexanes); mp 66−67 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.87 (d, 3H, J = 6.6 Hz, Val-CH_3), 0.94 (d, 3 H, J = 6.6 Hz, Val-CH_3), 1.43 (s, 9H, *t*Bu), 1.87 (m, 1H, Val-CH), 3.28 (d, 1H, J = 6.6 Hz, Val-α-CH); ^{13}C NMR (100.56 MHz, DMSO-d_6) δ 18.5, 19.6, 27.8, 27.9, 70.9, 81.2 (α-CH), 161.8 ($C{=}O_{oxalate}$), 170.6 (C═O); mass spectrum (HRMS), m/z = 212.1261 $(M + Na)^+$ ($C_9H_{19}NaNO_3$ requires 212.1263).

Glycotripeptide *tert*-Butyl Ester 22 and Glycopeptide-Derived Oxazole Byproduct 23. Cyanophosphorane **16** (0.08 g, 0.0791 mmol) was first converted to α-ketoacid **1** in quantitative yield with DMDO (4 mL in acetone, approximately 2 equiv) in acetone (0.5 mL) and water (200 μL). Crude material **1** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxy glycine *tert*-butyl ester·oxalate salt **4** (0.037 g, 0.158 mmol) in anhydrous DMF (2 mL) at 40 °C to furnish glycotripeptide **22** after 24 h, following the general procedure described in Supporting Information Part-I. Crude material having two new UV active spots was separated by silica

gel flash column chromatography (10 × 3 cm). Elution with 1:1:2 acetone/$CHCl_3$/hexanes and then 0.75:0.75:1.5:7 MeOH/acetone/$CHCl_3$/hexanes yielded desired product **22** as a white fluffy mass: yield 0.027 g (41%); silica gel TLC R_f = 0.26 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.88 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.44 (s, 9H, *t*Bu), 1.9 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 3.89 (t, 2H, J = 3.6 Hz, CH_2COO*t*Bu), 3.93 (dd, 1H, J = 4.2, 16.8 Hz, Gly-CH), 4.06 (m, 3H, H-6, H-6′ and Gly-CH), 4.23 (dd, 2H, J = 7.2, 12.5 Hz, Fmoc CH and H-5), 4.28 (m, 2H, Thr-α-CH and β-CH), 4.44 (m, 2H, Fmoc CH_2), 4.54 (m, 1H, H-2), 5.09 (m, 2H, H-1 and H-3), 5.36 (br.s, 1H, H-4), 5.96 (d, 1H, J = 7.8 Hz, Fmoc NH), 6.48 (br.s, 1H, *NH*CH_2CO_2*t*Bu), 6.9 (d, 1H, J = 9 Hz, N*H*Ac), 7.1 (br.s, 1H, *NH*CH_2-), 7.3 (t, 2H, J = 7.2 Hz, aromatic), 7.38 (t, 2H, J = 7.2 Hz, aromatic), 7.59 (d, 2H, J = 7.8 Hz, aromatic), 7.74 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.9 (Thr-CH_3), 20.9, 20.9, 21.0, 23.0, 28.2 (3 × CH_3 of *t*Bu), 29.9, 42.2 (CH_2), 42.7 (CH_2), 47.4, 47.9, 58.2, 62.3 (CH_2), 67.3 (CH_2), 67.4, 67.5, 68.3, 75.8, 83.1, 99.1 (C-1), 120.2, 125.3, 127.3, 127.4, 128.0, 128.4, 129.2, 141.5, 141.5, 143.86, 143.88, 156.5 (C═O), 168.6 (C═O), 168.7 (C═O), 169.8 (C═O), 170.6 (2 × C═O), 171.0 (C═O), 171.1 (C═O); mass spectrum (HRMS), m/z = 863.3309 (M + Na)$^+$ ($C_{41}H_{52}N_4NaO_{15}$ requires 863.3327). Also produced was a byproduct **23** as a colorless amorphous solid: yield 0.014 g (22%); silica gel TLC R_f = 0.34 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.19 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.36 (s, 9H, *t*Bu), 1.8 (s, 3H, CH_3CO), 1.99 (s, 6H, 2 × CH_3CO), 2.14 (s, 3H, CH_3CO), 4.06 (d, 2H, J = 6.6 Hz, H-6 and H-6′), 4.24 (m, 2H, Fmoc CH and H-5), 4.29 (m, 1H, Thr-α-CH), 4.35 (m, 2H, Gly-CH and Thr-β-CH), 4.46 (m, 2H, Fmoc CH_2), 4.56 (m, 2H, H-2 and Gly-CH), 5.13 (d, 1H, J = 3.6 Hz, H-1), 5.16 (dd, 1H, J = 3.0, 11.4 Hz, H-3), 5.35 (br.s, 1H, H-4), 6.02 (d, 1H, J = 7.2 Hz, Fmoc NH), 6.1 (s, 1H, oxazole H-4), 7.07 (br.s, 1H, *NH*CH_2-), 7.30 (m, 3H, NH and aromatic), 7.38 (m, 2H, aromatic), 7.61 (t, 2H, J = 7.2 Hz, aromatic), 7.74 (t, 2H, J = 7.8 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 14.3, 16.9, 20.9, 20.9, 21.0, 22.8, 28.2 (3 × CH_3 of *t*Bu), 29.9, 37.4, 47.4, 47.9, 58.0, 62.2, 67.23, 67.26, 67.4, 68.2, 75.6, 99.0 (C-1), 107.6 (oxazole C-4), 120.2, 125.3, 127.3, 127.3, 127.9, 128.0, 141.5, 141.5, 143.8, 143.9, 153.2, 156.5 (C═O), 157.2, 169.5 (C═O), 170.6 (C═O), 170.7 (C═O), 170.9 (2 × C═O); mass spectrum (HRMS), m/z = 845.3204 (M + Na)$^+$ ($C_{41}H_{50}N_4NaO_{14}$ requires 845.3221).

Glycotripeptide *tert*-Butyl Ester 24. Cyanophosphorane **16** (0.08 g, 0.0791 mmol) was first converted to α-ketoacid **1** in quantitative yield with DMDO (4 mL in acetone, approximately 2 equiv) in acetone (0.5 mL) and water (200 μL). Crude material **1** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxyalanine *tert*-butyl ester·oxalate salt **5** (0.030 g, 0.118 mmol) in anhydrous DMF (2 mL) at 40 °C to furnish glycotripeptide **24** after 25 h, following the general procedure described in Supporting Information Part-I. The crude material was purified by silica gel flash column chromatography (10 × 3 cm). Elution with 0.5:0.5:1:8 and then 0.7:0.7:1.4:7.2 MeOH/acetone/$CHCl_3$/hexanes yielded desired product **24** as a colorless fluffy mass: yield 0.027 g (40%); silica gel TLC R_f = 0.3 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.21 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.38 (d, 3H, J = 6.6 Hz, Ala-CH_3), 1.46 (s, 9H, *t*Bu), 1.92 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 3.95 (m, 1H, Gly-CH), 4.08 (m, 3H, Gly-CH, H-6 and H-6′), 4.22−4.33 (m, 4H, H-5, Fmoc CH, Thr-β-CH and Thr-α-CH), 4.35 (4.47 (m, 3H, Fmoc CH_2 and Ala-CH), 4.58 (m, 1H, H-2), 5.14 (d, 1H, J = 3.0 Hz, H-1), 5.15 (dd, 1H, J = 3.0, 11.4 Hz, H-3), 5.40 (br.s, 1H, H-4), 5.96 (d, 1H, J = 7.2 Hz, Fmoc NH), 6.63 (br.s, 1H, Ala-NH), 7.07 (d, 1H, J = 9.0 Hz, N*H*Ac), 7.21 (br.s, 1H, Gly-NH), 7.32 (t, 2H, J = 6.0 Hz, aromatic), 7.40 (t, 2H, J = 6.6 Hz, aromatic), 7.61 (d, 2H, J = 7.2 Hz, aromatic), 7.76 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.78, 18.44, 20.91, 23.08, 28.08, 28.15, 29.93, 42.79, 47.37, 47.93, 49.31, 58.01, 62.31, 67.37, 67.51, 68.32, 75.78, 82.80, 99.11 (C-1), 120.27, 125.29, 125.34, 127.36, 128.02, 141.54, 143.87, 143.96, 156.46 (C═O), 167.94 (C═O), 169.58 (C═O), 170.69 (2 × C═O), 170.87 (C═O), 171.13 (C═O), 171.97 (C═O); mass spectrum (HRMS), m/z = 877.3519 (M + Na)$^+$ ($C_{42}H_{54}N_4NaO_{15}$ requires 877.3483).

Glycotripeptide *tert*-Butyl Ester 25 and Glycopeptide-Derived Oxazole Byproduct 26. Cyanophosphorane **17** (0.053 g, 0.0517 mmol) was first converted to α-ketoacid **2** in quantitative yield with DMDO (4 mL, approximately 2 equiv) in acetone (2 mL) and water (250 μL). Crude material **2** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxy glycine *tert*-butyl ester·oxalate salt **4** (0.015 g, 0.0620 mmol) in anhydrous DMF (1.5 mL) at 40−42 °C to afford glycotripeptide **25** and **26** after 6 h, following the general procedure described in Supporting Information Part-I. Crude material was purified by silica gel flash column chromatography (10 × 3 cm). Elution with 1.5:98.5 MeOH/CH_2Cl_2 yielded desired product **25** as white amorphous solid: yield 0.019 g (43%); silica gel TLC R_f = 0.22 (2.5:97.5 MeOH/CH_2Cl_2, run twice); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.15 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.41 (d, 3H, J = 7.2 Hz, Ala-CH_3), 1.44 (s, 9H, *t*Bu), 1.98 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.997 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 3.91 (t, 2H, J = 4.8 Hz, Gly-CH_2), 4.05 (d, 2H, J = 6.6 Hz, H-6 and H-6′), 4.17 (dd, 1H, J = 3.6, 6.6 Hz, Thr-β-CH), 4.20 (t, 2H, J = 6.6 Hz, H-5 and Fmoc CH), 4.28 (dd, 1H, J = 3.6, 7.2 Hz, Thr-α-CH), 4.38 (d, 2H, J = 7.2 Hz, Fmoc CH_2), 4.56 (m, 2H, H-2 and Ala-CH), 5.07 (dd, 1H, J = 3.0, 12.0 Hz, H-3), 5.16 (d, 1H, J = 3.0 Hz, H-1), 5.36 (d, 1H, J = 2.4 Hz, H-4), 5.93 (d, 1H, J = 7.2 Hz, Fmoc NH), 6.54 (t, 1H, J = 4.2 Hz, Gly-NH), 7.13 (d, 1H, J = 6.6 Hz, Ala-NH), 7.17 (d, 1H, J = 8.4 Hz, N*H*Ac), 7.29 (t, 2H, J = 7.2 Hz, aromatic), 7.38 (t, 2H, J = 7.2 Hz, aromatic), 7.58 (d, 2H, J = 7.2 Hz, aromatic), 7.74 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.5, 20.0, 20.88, 20.97, 20.99, 23.2, 28.2 (3 × CH_3 of *t*Bu), 29.9, 42.4, 47.3, 47.8, 49.2, 57.4, 62.2, 67.2, 67.4, 67.45, 68.2, 75.1, 83.0, 98.8 (C-1), 120.2, 120.2, 125.3, 125.3, 127.3, 127.9, 141.5, 143.8, 143.9, 156.2 (C═O), 168.5 (C═O), 168.7 (C═O), 170.6 (2 × C═O), 170.8 (C═O), 171.0 (C═O), 172.3 (C═O); mass spectrum (HRMS), m/z = 877.3501 (M + Na)$^+$ ($C_{42}H_{54}NaN_4O_{15}$ requires 877.3483) and **26** as white amorphous solid: yield 0.010 g (23%); R_f = 0.24 (2.5:97.5 MeOH-CH_2Cl_2, run twice); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.15 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.37 (s, 9H, *t*Bu), 1.49 (d, 3H, J = 6.6 Hz, Ala-CH_3), 1.87 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 2.14 (s, 3H, CH_3CO), 4.06 (d, 2H, J = 6.6 Hz, H-6 and H-6′), 4.22 (m, 3H, H-5, Thr-β-CH and Fmoc CH), 4.28 (dd, 1H, J = 3.0, 6.6 Hz, Thr-α-CH), 4.39 (dd, 2H, J = 3.0, 6.6 Hz, Fmoc CH_2), 4.63 (m, 1H, H-2), 5.04 (m, 1H, Ala-CH), 5.18 (dd, 1H, J = 3.0, 12.0 Hz, H-3), 5.21 (d, 1H, J = 3.0 Hz, 1H; H-1), 5.35 (d, 1H, J = 2.4 Hz, H-4), 5.84 (d, 1H, J = 6.6 Hz, Fmoc NH), 6.13 (s, 1H, oxazole H-4), 7.26 (br.s, 1H, Ala-NH), 7.30 (m, 2H, aromatic), 7.38 (t, 2H, J = 7.2 Hz, aromatic), 7.59 (t, 3H, J = 7.8 Hz, N*H*Ac and aromatic), 7.74 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (100.56 MHz, $CDCl_3$) δ 16.2, 20.7, 20.9, 20.98, 21.0, 22.9, 28.2 (3 × CH_3 of *t*Bu), 29.9, 44.8, 47.3, 47.8, 57.0, 62.2, 67.1, 67.3, 67.5, 68.3, 74.4, 84.9, 98.2 (C-1), 107.4 (oxazole C-4), 120.2, 120.2, 125.3, 125.3, 127.3, 127.9, 141.5, 143.8, 144.0, 156.1, 156.7 (C═O), 157.0, 168.6 (C═O), 170.6 (C═O), 170.7 (C═O), 170.7 (C═O), 170.9 (C═O); mass spectrum (HRMS), m/z = 859.3375 (M + Na)$^+$ ($C_{42}H_{52}NaN_4O_{14}$ requires 859.3378).

Glycotripeptide *tert*-Butyl Ester 27. Cyanophosphorane **17** (0.056 g, 0.0546 mmol) was first converted to α-ketoacid **2** in quantitative yield with DMDO (4 mL in acetone, approximately 2 equiv) in acetone (2 mL) and water (250 μL). Crude material **2** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxyalanine *tert*-butyl ester·oxalate salt **5** (0.016 g, 0.0656 mmol) in anhydrous DMF (1.5 mL) at 35−40 °C to produce glycotripeptide **27** after 38 h as the sole product, following the general procedure described in Supporting Information Part-I. Crude material was purified by silica gel flash column chromatography (6 × 3.5 cm). Elution with 0.5:

0.5:1:8 MeOH/acetone/$CHCl_3$/hexanes yielded desired product **27** as white amorphous solid: yield 0.041 g (86%); silica gel TLC R_f = 0.21 (1:1:1:7 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.17 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.40 (d, 3H, J = 7.2 Hz, Ala-CH_3), 1.45 (s, 12H, *t*Bu and Ala-CH_3), 2.00 (s, 3H, CH_3CO), 2.02 (s, 6H, CH_3CO), 2.15 (s, 3H, CH_3CO), 4.08 (d, 2H, J = 6.6 Hz, H-6 and H-6'), 4.23 (m, 3H, H-5, Thr-β-CH and Fmoc CH), 4.32 (dd, 1H, J = 3.0, 6.6 Hz, Thr-α-CH), 4.38 (m, 1H, Ala-CH), 4.40 (d, 2H, J = 7.2 Hz, Fmoc CH_2), 4.55 (m, 1H, Ala-CH), 4.60 (m, 1H, H-2), 5.11 (dd, 1H, J = 3.0, 12.0 Hz, H-3), 5.17 (d, 1H, J = 3.0 Hz, H-1), 5.40 (br.s, 1H, H-4), 5.99 (d, 1H, J = 6.6 Hz, Fmoc NH), 6.63 (d, 1H, J = 6.6 Hz, Ala-NH), 7.25 (d, 1H, J = 6.6 Hz, Ala-NH), 7.30 (m, 3H, NHAc and aromatic), 7.40 (t, 2H, J = 7.2 Hz, aromatic), 7.60 (d, 2H, J = 7.2 Hz, aromatic), 7.76 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.3, 18.2 (Ala-CH_3), 20.1, 20.8, 20.9, 23.1, 28.1 (3 × CH_3 of *t*Bu), 29.9, 47.3, 47.8, 49.1, 49.3, 57.2, 62.2, 67.2, 67.4, 67.4, 68.2, 74.73, 82.5, 98.6 (C-1), 120.2, 120.22, 125.3, 125.33, 127.3, 127.9, 141.47, 141.48, 143.8, 143.9, 156.1 (C═O), 168.3 (C═O), 170.6 (C═O), 170.6 (C═O), 170.8 (C═O), 171.0 (C═O), 171.8 (C═O), 171.8 (C═O); mass spectrum (HRMS), m/z = 891.3650 $(M + Na)^+$ ($C_{43}H_{56}NaN_4O_{15}$ requires 891.3640).

Glycotripeptide *tert*-Butyl Ester 28. Cyanophosphorane **16** (0.08 g, 0.0791 mmol) was first converted to α-ketoacid **1** in quantitative yield with DMDO (4 mL in acetone, approximately 2 equiv) in acetone (0.5 mL) and water (200 μL). Crude material **1** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxyvaline *tert*-butyl ester·oxalate salt **6** (0.033 g, 0.118 mmol) in anhydrous DMF (2 mL) at 40 °C to generate glycotripeptide **28** after 19 h, following the general procedure described in Supporting Information Part-I. Crude material was purified by silica gel flash column chromatography (6.5 × 3 cm). Elution with 0.4:0.4:0.8:8.4 and then 0.6:0.6:1.2:7.6 MeOH/acetone/$CHCl_3$/hexanes yielded desired product **28** as a colorless fluffy mass: yield 0.011 g (15%); silica gel TLC R_f = 0.35 (1:1:2:6 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.93 (t, 6H, J = 7.2 Hz, 2 × Val-CH_3), 1.21 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.47 (s, 9H, *t*Bu), 1.93 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.16 (s, 4H, CH_3CO and Val-CH), 3.97 (dd, 1H, J = 4.2, 16.8 Hz, Gly-CH), 4.08 (d, 2H, J = 6.0 Hz, H-6 and H-6′), 4.13 (dd, 1H, J = 4.2, 16.8 Hz, Gly-CH), 4.25 (m, 2H, Fmoc CH and H-5), 4.31 (d, 2H, J = 6.0 Hz, Thr-α-CH and Thr-β-CH), 4.37 (dd, 1H, J = 4.2, 7.8 Hz, Val-α-CH), 4.45 (m, 2H, Fmoc CH_2), 4.59 (m, 1H, H-2), 5.12 (d, 1H, J = 3.0 Hz, H-1), 5.14 (dd, 1H, J = 2.4, 12.0 Hz, H-3), 5.41 (br.s, 1H, H-4), 5.85 (d, 1H, J = 6.6 Hz, Fmoc NH), 6.40 (d, 1H, J = 8.4 Hz, Val-NH), 7.01 (d, 1H, J = 8.4 Hz, N*H*Ac), 7.14 (br.s, 1H, Gly-NH), 7.33 (t, 2H, J = 7.2 Hz, aromatic), 7.41 (t, 2H, J = 7.2 Hz, aromatic), 7.62 (d, 2H, J = 7.2 Hz, aromatic), 7.77 (d, 2H, J = 7.8 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 16.7, 18.0, 18.9, 20.9, 21.0, 22.9, 23.1, 28.3, 29.9, 31.5, 42.9, 47.41, 47.9, 58.0, 58.1, 62.3, 67.4, 67.5, 68.3, 75.6, 82.9, 99.0 (C-1), 120.2, 120.29, 125.3, 125.3, 127.4, 128.0, 141.6, 143.8, 143.9, 156.4 (C═O), 168.3 (C═O), 169.5 (C═O), 170.7 (2 × C═O), 170.74 (C═O), 170.8 (C═O), 171.1 (C═O); mass spectrum (HRMS), m/z = 905.3792 $(M + Na)^+$ ($C_{45}H_{62}NaN_4O_{15}$ requires 905.3796).

Glycodipeptide Cyanophosphorane Analog 29 and 6-*O*-Acetylglycodipeptide Cyanophosphorane Analog 30. Starting material **16** (0.25 g, 0.247 mmol) was dissolved in anhydrous MeOH (8.0 mL), and the temperature was lowered to 0 °C. Ammonia in MeOH (2.1 mL, 7 N) was added dropwise under N_2 atmosphere. The resulting solution was stirred at 0 °C. Reaction was monitored by TLC and appeared to stop after 5.5 h. Solvent was removed under reduced pressure, and the crude material thus obtained was purified by silica gel flash column chromatography (6.0 × 3.0 cm) with 1:1:2:6 (300 mL) and then 1.5:1.5:3:4 (200 mL) MeOH/acetone/$CHCl_3$/hexanes to yield **29** and **30**, respectively, as white amorphous solids. For compound **29**: yield 123 mg (56%); silica gel TLC R_f = 0.26 (1.5:1.5:3:4 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.15 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.94 (s, 3H, CH_3CONH), 2.80 (br. hump, 1H, OH), 3.69 (m, 1H, H-3), 3.75 (m, 1H, H-6), 3.80 (t, 1H, J = 4.8 Hz, H-5), 3.87 (dd, 1H, J = 4.8, 11.4 Hz, H-6′), 3.90 (br.s, 1H, H-4), 4.11 (m, 1H, H-2), 4.17 (m, 2H, Fmoc CH and Thr-β-CH), 4.20 (d, 1H, J = 8.4 Hz, Thr-α-CH), 4.36 (d, 2H, J = 7.2 Hz, Fmoc CH_2), 4.41 (dd, 2H, J = 3.6, 12.6 Hz, Gly-CH_2), 4.88 (d, 2H, J = 3.0 Hz, H-1 and OH), 5.75 (d, 1H, J = 6.0 Hz, Fmoc NH), 7.10 (br.s, 1H, Gly-NH), 7.25 (t, 2H, J = 7.2 Hz, aromatic), 7.34 (ddd, 2H, J = 3.0, 6.0, 6.0 Hz, aromatic), 7.47 (d, 1H, J = 6.6 Hz, N*H*Ac), 7.54 (m, 14H, aromatic), 7.71 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 17.4, 22.4, 29.3, 29.7, 46.2, 46.3, 47.1, 47.2, 48.1, 50.8, 67.2, 69.4, 69.7, 70.6, 75.7, 99.1 (C-1), 119.9, 120.5, 120.6, 121.7, 122.3, 125.2, 127.2, 127.7, 129.4, 129.5, 133.6, 133.7, 133.73, 141.2, 141.3, 143.7, 143.9,156.6 (C═O), 169.8 (C═O), 173.4 (2 × C═O), 189.5, 189.5; ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 20.7 (s, PPh_3); mass spectrum (HRMS), m/z = 907.3056 $(M + Na)^+$ ($C_{49}H_{49}N_4NaO_{10}P$ requires 907.3084). For compound **30**: yield 34 mg (15%); R_f = 0.44 (1.5:1.5:3:4 MeOH/acetone/$CHCl_3$/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.20 (d, 3H, J = 6.6 Hz, Thr-CH_3), 2.00 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.10 (br. hump, 1H, OH), 3.72 (dd, 1H, J = 2.4, 10.2 Hz, H-3), 3.81 (br.s, 1H, H-4), 3.97 (t, 1H, J = 5.4 Hz, H-5), 4.08 (m, 2H, H-2 and Thr-β-CH), 4.19 (t, 1H, J = 6.6 Hz, Fmoc CH), 4.22−4.29 (m, 3H, Thr-α-CH, H-6 and H-6′), 4.37 (d, 2H, J = 6.6 Hz, Fmoc CH_2), 4.40 (d, 2H, J = 4.2 Hz, Gly-CH_2), 4.78 (d, 1H, J = 3.6 Hz, H-1), 5.09 (br. hump, 1H, OH), 5.71 (d, 1H, J = 9.0 Hz, Fmoc NH), 7.05 (br.s, 1H, Gly-NH), 7.25 (m, 2H, aromatic), 7.35 (m, 2H, aromatic), 7.46 (d, 1H, J = 7.2 Hz, NHAc), 7.55 (m, 14H, aromatic), 7.64 (m, 3H, aromatic), 7.72 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$) δ 18.0, 21.0, 22.8, 29.9, 46.3, 46.4, 47.3, 51.6, 58.4, 64.2, 67.5, 68.4, 68.5, 71.2, 99.9 (C-1), 120.1, 120.2, 120.5, 120.6, 121.8, 122.5, 125.2, 127.3, 127.31, 127.9, 129.6, 129.7, 133.7, 133.74, 133.8, 133.81, 133.9, 133.92, 141.4, 141.5, 143.8, 144.0, 156.7 (C═O), 170.5 (C═O), 170.9 (C═O), 174.7 (C═O), 189.1, 189.1; ^{31}P NMR (161.9 MHz, $CDCl_3$) δ 20.7 (s, PPh_3); mass spectrum (HRMS), m/z = 949.3192 $(M + Na)^+$ ($C_{51}H_{51}N_4NaO_{11}$ requires 949.3190).

Glycotripeptide *tert*-Butyl Ester 32 and Glycopeptide-Derived Oxazole Byproduct 33. Cyanophosphorane **29** (0.07 g, 0.0791 mmol) was first converted to α-ketoacid **31** in quantitative yield with DMDO (4 mL in acetone, approximately 2 equiv) in acetone (0.5 mL) and water (200 μL). Crude material **31** was dried in high vacuum for 0.5 h and reacted with *N*-hydroxy glycine *tert*-butyl ester·oxalate salt **4** (0.037 g, 0.158 mmol) in anhydrous DMF (2 mL) at 40 °C to produce glycotripeptide **32** and an oxazole byproduct **33** after 16 h, following the general procedure described in Supporting Information Part-I. Purification of the crude material by silica gel flash column chromatography (6.0 × 3.0 cm) with 1:1:2:6 and then 1.5:1.5:3:4 MeOH/Acetone/$CHCl_3$/hexanes generated **32** as colorless glassy solid: yield 15 mg (26%); ^{1}H NMR (600 MHz, CD_3OD) δ 1.19 (d, 3H, J = 6.0 Hz, Thr-CH_3), 1.40 (s, 9H, *t*Bu), 1.92 (s, 3H, CH_3CO), 3.67 (m, 3H, H-3 and two other protons), 3.79 (dd, 2H, J = 17.4, 46.8 Hz, Gly-CH_2), 3.84 (t, 2H, J = 3.6 Hz), 3.87 (d, 1H, J = 3.6 Hz, H-4), 4.15 (dd, 1H, J = 4.2, 11.4 Hz, H-2), 4.19 (d, 2H, J = 2.4 Hz), 4.21 (t, 1H, J = 6.0 Hz, H-5), 4.24 (dd, 1H, J = 1.8, 6.0 Hz), 4.41 (dd, 1H, J = 6.6, 10.8 Hz, H-6), 4.51 (dd, 1H, J = 6.6, 10.8 Hz, H-6′), 4.87 (s, 1H, H-1), 7.28 (m, 2H, aromatic), 7.35 (m, 2H, aromatic), 7.65 (t, 2H, J = 7.8 Hz, aromatic), 7.76 (d, 2H, J = 7.2 Hz, aromatic); ^{13}C NMR (150.83 MHz, CD_3OD) δ 19.1, 23.2, 28.4 (3 × CH_3 of *t*Bu), 42.8, 43.3, 51.6, 60.7, 62.8, 68.0, 70.3, 70.5, 73.0, 77.1, 83.1, 100.1 (C-1), 121.1, 121.1, 126.3, 126.33, 128.3, 128.38, 128.97, 128.99, 142.8, 145.2, 145.4, 159.2 (C═O), 170.6 (C═O), 171.8 (C═O), 173.1 (C═O), 174.3 (C═O); mass spectrum (HRMS), m/z = 737.2963 $(M + Na)^+$ ($C_{35}H_{46}N_4NaO_{12}$ requires 737.3010). Also produced was **33** as off-white amorphous solid: yield 10 mg (18%); ^{1}H NMR (600 MHz, CD_3OD) δ 1.20 (d, 3H, J = 6.6 Hz, Thr-CH_3), 1.32 (s, 9H, *t*Bu), 1.89 (s, 3H, NHCOC*H*$_3$), 3.68 (m, 3H), 3.85 (m, 2H), 4.16 (dd, 1H, J = 3.6, 10.8 Hz, H-2), 4.22 (m, 3H,

H-5 and two other protons), 4.33 (dd, 2H, $J = 16.8$, 30.0 Hz, Gly-CH_2), 4.40 (dd, 1H, $J = 6.0$, 10.8 Hz, H-6), 4.51 (dd, 1H, $J = 6.6$, 10.8 Hz, H-6′), 4.87 (s, 1H, H-1), 6.24 (s, 1H, oxazole H-4), 7.28 (m, 2H, aromatic), 7.36 (t, 2H, $J = 7.2$ Hz, aromatic), 7.66 (t, 2H, $J = 7.8$ Hz, aromatic), 7.77 (d, 2H, $J = 7.2$ Hz, aromatic); ^{13}C NMR (150.83 MHz, CD_3OD) δ 19.2, 23.1, 28.5, 37.8, 51.6, 60.6, 62.8, 68.0, 70.4, 70.5, 73.0, 77.6, 101.0 (C-1), 108.9 (oxazole C-4), 121.1, 126.3, 126.33, 128.3, 128.4, 128.9, 129.0, 142.8, 145.2, 145.5, 159.1 (C═O), 173.0 (C═O), 174.3 (C═O); mass spectrum (HRMS), $m/z = 719.2860$ $(M + Na)^+$ ($C_{35}H_{44}N_4NaO_{11}$ requires 719.2904).

[O^{18}] 3-Phenyl-2-oxopropanoic Acid (36). To a vacuum-dried phenylpyruvic acid **35** (0.1 g, 0.609 mmol) under N_2 atmosphere was added 0.5 mL of 0.1 N HCl in anhydrous THF and H_2O^{18} (1.0 g, 49.95 mmol). The mixture was stirred at room temperature, and the O^{18}-exchange was complete in 15 min (monitored by ESI-MS). The mixture was concentrated under reduced pressure in a N_2-flushed rotary evaporator and the residual solvent was removed in high vacuum to afford **36** as a light yellow amorphous solid (0.087 g, 84%). ^1H NMR (400 MHz, DMSO-d_6) δ 6.40 (s, 1H, *H*C═C-OH), 7.24 (t, 1H, $J = 7.2$ Hz, aromatic), 7.34 (t, 3H, $J = 7.6$ Hz, aromatic), 7.75 (d, 2H, $J = 7.6$ Hz, aromatic), 9.26 (br.s, 1H, HC═CO*H*), 13.21 (br.s, 1H, COOH); ^{13}C NMR (100.57 MHz, DMSO-d_6) δ 109.5 (*C*═*C*-OH)) 127.2 (*C* = C-OH), 128.3, 129.3, 135.0, 141.9, 166.4 (C═O); mass spectrum (ESI-MS), phenylpyruvic acid was decarboxylated at 320 °C to give 2-phenylethanal, $m/z = 145.1$ $(M + Na)^+$ (C_8H_8ONa requires 145.05).

***N*-(2-Phenylethyl)phenylacetamide (37).** A vacuum-dried mixture of O^{18}-labeled phenylpyruvic acid **36** (0.063 g, 0.370 mmol) and phenethyl hydroxyl amine oxalate salt **34** (0.081 g, 0.444 mmol) was dissolved in anhydrous MeOH (8 mL). The resulting mixture was stirred at 35−40 °C under N_2 atmosphere. After 18 h, an aliquot of the reaction mixture was analyzed by mass spectroscopy (ESI-MS). Excess solvent was removed under reduced pressure. The crude material was purified by silica gel flash column chromatography (9 × 3.5 cm) using 1:99 MeOH/CH_2Cl_2 to furnish **37** as a light yellow solid; yield 0.045 g (51%). Similarly, compound **35** (0.076 g, 0.457 mmol) was converted to amide **37** under the same reaction conditions in 41% yield. The ^1H and ^{13}C NMR of **37** from either route were found identical to those reported in the literature (ref Bode et al.).

[O^{18}] *N*-(2-Phenylethyl)phenylacetamide (41). To a stirred solution of benzyl cyanide **38** (55 μL, 0.477 mmol) in anhydrous THF (0.5 mL) was added H_2O^{18} (0.478 g, 23.9 mmol) dropwise under N_2 atmosphere. HCl (g) was bubbled into the reaction mixture for 10 min. The resulting mixture was refluxed at 50 °C for 12 h under N_2 atmosphere. The desired O^{18}-labeled phenylacetic acid was detected by ESI-MS [$m/z = 163.15$ $(M + Na)^+$]. The mixture was concentrated under reduced pressure in a N_2-flushed rotary evaporator. The residual solvent was removed in high vacuum to afford the crude product **39** (0.070 g) which was used in the next step without further purification. To a well-stirred solution of **39** (0.070 g) in anhydrous CH_2Cl_2 (2 mL) was added freshly distilled dicyclohexylcarbodiimide (0.095 g, 0.460 mmol), phenethylamine **40** (0.060 mL, 0.476 mmol) and DIPEA (0.16 mL, 0.968 mmol). The resulting mixture was stirred at room temperature for 36 h under N_2 atmosphere. ESI-MS of the reaction mixture revealed labeled amide **41** with $m/z = 242.4$ $(M + H)^+$ ($C_{16}H_{18}N_{18}O$ requires 242.14).

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Supporting Information Available: General procedure for DMDO oxidation, Copies of ^1H, ^{13}C and ^{31}P NMR for compounds **11**, **12**, **16**−**18**, **29**, and **30**; ^1H and ^{13}C NMR for compounds **6**, **20**, **21**, **22**−**28**, **32**, **33**, **36**, and byproduct **A**; and mass spectra (ESI-MS) for **36**, **37**, **39**, and **41**; and $^1H-^1H$ gCOSY NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

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