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α-Azido Esters in Depsipeptide Synthesis: C−O Bond Cleavage during Azido Group Reduction

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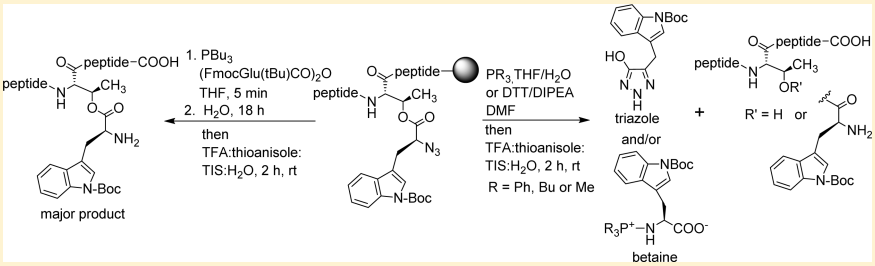
Supporting Information



ABSTRACT: α-Azido esters, when treated with dithiothreitol (DTT)/diisopropylethylamine (DIPEA), undergo both azido group reduction to give α-amino esters and C−O bond cleavage to give triazoles. The extent of triazole formation depends upon leaving group ability. Some C−O bond cleavage via triazole formation was also found to occur when a resin-bound peptide, which contained a terminal α-azido ester group, was treated with DTT/DIPEA. C−O bond cleavage also took place when this peptide was treated with PPh_3, PBu_3, or PMe_3; however, in these cases, C−O bond cleavage occurred via either triazole formation and/or hydrolysis of the ester bond in the iminophosphorane intermediate to give betaines. The mechanism that dominated for C−O bond cleavage depended upon the phosphine that was used for azido group reduction. C−O bond cleavage during reduction of the azido group in the peptide was minimized by performing the reduction with PBu_3 in the presence of a symmetric anhydride derived from an amino acid in dry THF followed by the addition of water. Surprisingly, these conditions provided the amine as the major product, while the expected amide was formed as a minor product. These conditions were employed in an improved synthesis of an analogue of the cyclic lipodepsipeptide antibiotic daptomycin.

INTRODUCTION

The synthesis of complex or challenging peptides often requires the use of α-amino protecting groups beyond the traditional Fmoc or Boc groups that are commonly employed in peptide synthesis. One alternative α-amino protecting group with a number of attractive features is the azido group. First, most peptides bearing α-azido groups are stable to the basic conditions that are used to remove Fmoc groups[1] and to the acidic the conditions that are used to remove acid-labile groups such as Boc-protected amines and *t*-butyl esters. Hence, they provide an additional orthogonal dimension which is useful for the synthesis of complex targets.[2,3] Second, they minimize side reactions, such as diketopiperazine formation, that can occur using standard Fmoc chemistry.[4] Third, they are useful for hindered couplings.[5,6] Fourth, in comparison to Fmoc or Boc groups, the azido group is atom-economical, and α-azido acids are readily prepared from α-amino acids using diazo transfer reagents and often do not require chromatographic purification.[4,7−10] Finally, the azido group is reduced to the corresponding amino group usually in good to excellent yield under very mild conditions using phosphines in aqueous mixtures of organic solvents (Staudinger reaction) or with dithiols in the presence of a mild organic base.[4,5,11−13]

We have recently reported employing α-azido acids in two different solid phase syntheses of analogues of the cyclic depsipeptide antibiotic daptomycin, as outlined in Scheme 1, which shows the synthesis of the daptomycin analogue, Dap-E12-W13.[1,2] Route A, from peptide **1**, was developed first and uses three azido acids to arrive at peptide **3**. After each azido acid was incorporated into the peptide, the azido group was reduced to the amine using PMe_3 in dioxane/H_2O. However, as most α-azido acids are not commercially available, it was desirable to reduce the number of azido acids used in the synthesis. Hence, route B was developed which employs just one azido acid that is incorporated into the peptide as an α-azido ester (peptides **2** and **4**). In route B, we chose to reduce the azido group in peptide **4** with DTT/DIPEA, which are less expensive and have longer shelf lives than PMe_3. Route A required the use of PMe_3 because DTT/DIPEA were found to cause some removal of the Fmoc group.[1]

During our studies on the utility of α-azido acids in peptide synthesis, we recently discovered a previously unnoticed variant of the Staudinger reaction on α-azido esters in which phosphazides, generated from the reaction of trialkyl phosphines (PMe_3, PBu_3, or $POct_3$) with phenyl esters of α-azido acids in THF/H_2O, undergo an intramolecular cyclization to provide 5-substituted 2*H*-1,2,3-triazol-4-ols **7** in good to

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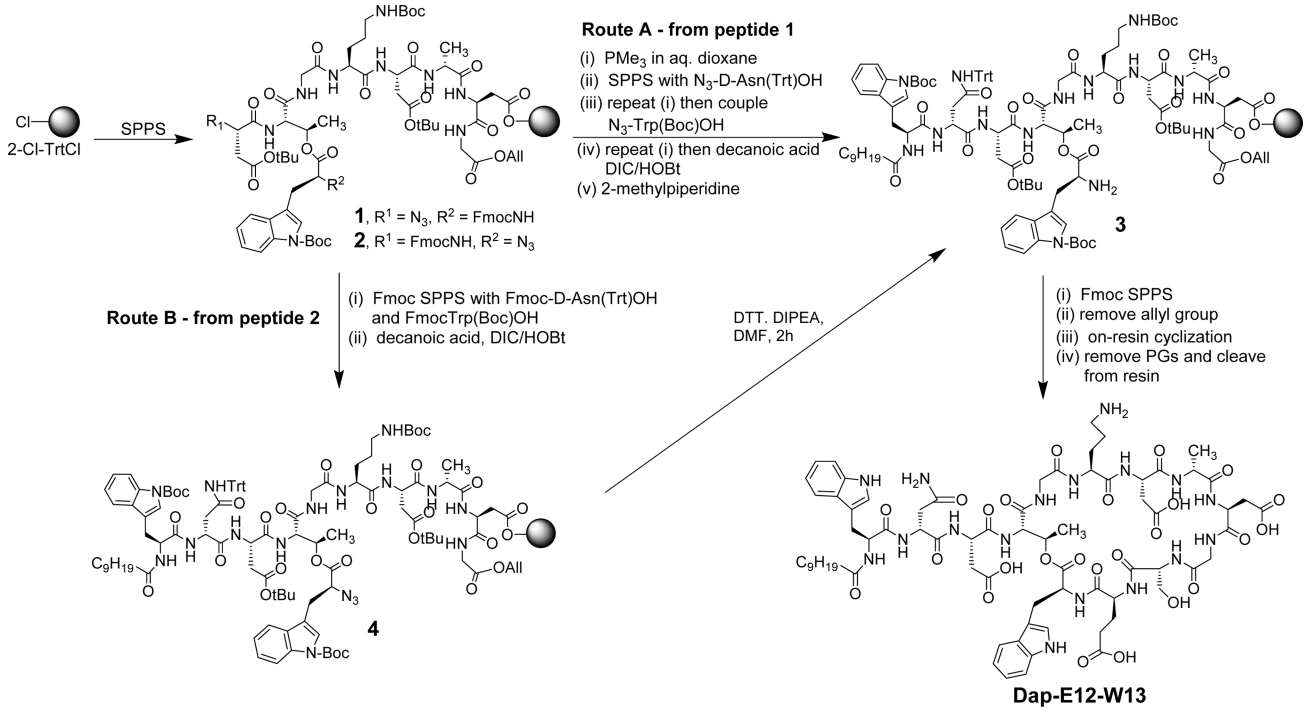
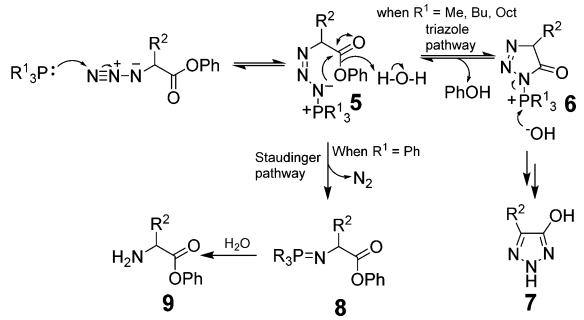
Scheme 1. Two Different Approaches to the Synthesis of Daptomycin Analogues



excellent yields (Scheme 2).[14] Triazole formation is most likely due to cyclization of the phosphazide **5** to give intermediate **6**

Scheme 2. Synthesis of Triazoles from Phenyl Esters of α-Azido Acids Using Trialkyl Phosphines



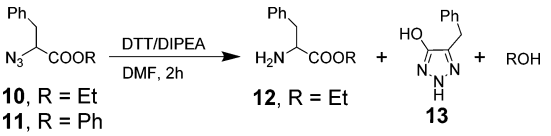
(triazole pathway) before it can lose nitrogen and give the iminophosphorane **8** (Staudinger pathway). Hydrolysis of **6** followed by protonation and tautomerization gives the triazoles. Ethyl esters gave an approximately 1:1 mixture of the triazole and Staudinger product, suggesting that the rate-determining step for triazole formation is loss of the leaving group. Surprisingly, PPh_3 gave only the Staudinger products and no triazoles.

Here, we report that C−O bond cleavage via triazole formation occurs when α-azido esters, including peptide **4**, are treated with DTT/DIPEA. We also report that C−O bond cleavage occurs when peptide **4** is treated with phosphines including PPh_3; however, C−O bond cleavage can occur by two different mechanisms, namely, via triazole formation or hydrolysis of the ester bond in the iminophosphorane intermediate. What mechanism dominates for C−O bond cleavage depends upon the phosphine (PMe_3, PBu_3, or PPh_3) used for azido group reduction. C−O bond cleavage during reduction of the azido group in peptide **4** was minimized by performing the reduction with PBu_3 in the presence of a symmetric anhydride derived from FmocGlu(OtBu)OH in dry THF followed by the addition of water. Surprisingly, these conditions provided amine **3** as the major product, while the expected amide was formed as a minor product. These conditions were employed in an improved synthesis of Dap-E12-W13.

RESULTS AND DISCUSSION

We first examined whether triazole formation occurs when α-azido esters are treated with DTT/DIPEA by subjecting model esters **10** and **11** to 4 equiv of DTT and 2 equiv of DIPEA in DMF for 2 h and analyzing the crude reaction mixture by HPLC (Scheme 3). Ethyl ester **10** gave a mixture of amine **12**

Scheme 3. Products Formed upon Treatment of Esters 10 and 11 with DTT/DIPEA



and triazole **13**, and phenyl ester **11** gave, almost exclusively, triazole **13** which was isolated in an 83% yield. This suggests that the rate-determining step for triazole formation is loss of the leaving group. These product distribution patterns are very similar to those we found when these esters were subjected to alkyl phosphines.[14]

The mechanism for the reduction of an azido group with a dithiol has not been studied in detail. Cartwright et al. proposed two possible mechanisms, one of which involves attack of the thiolate on the terminal (γ) nitrogen of the azido group to give intermediate **14** (Scheme 4).[11,15] Disulfide formation followed by loss of nitrogen from intermediate **15** gives the amine **12**. Triazole formation may occur by cyclization of intermediate **16**

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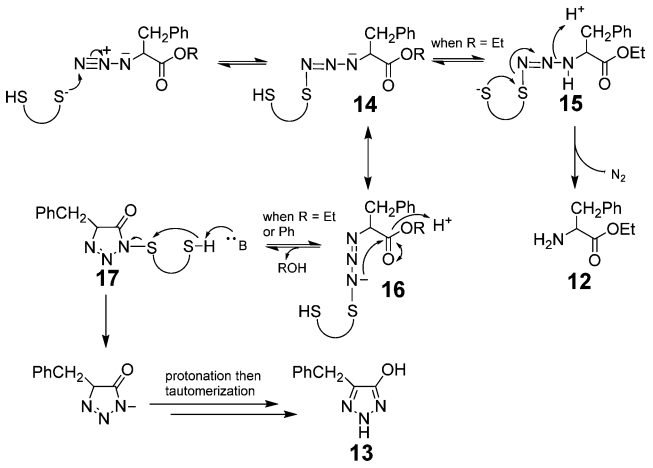
Scheme 4. Possible Mechanism for the Formation of Triazole 13 from α-Azido Esters Using Dithiols under Basic Conditions



to give triazolone **17** which, after disulfide formation, tautomerizes to give triazole **13**.[16]

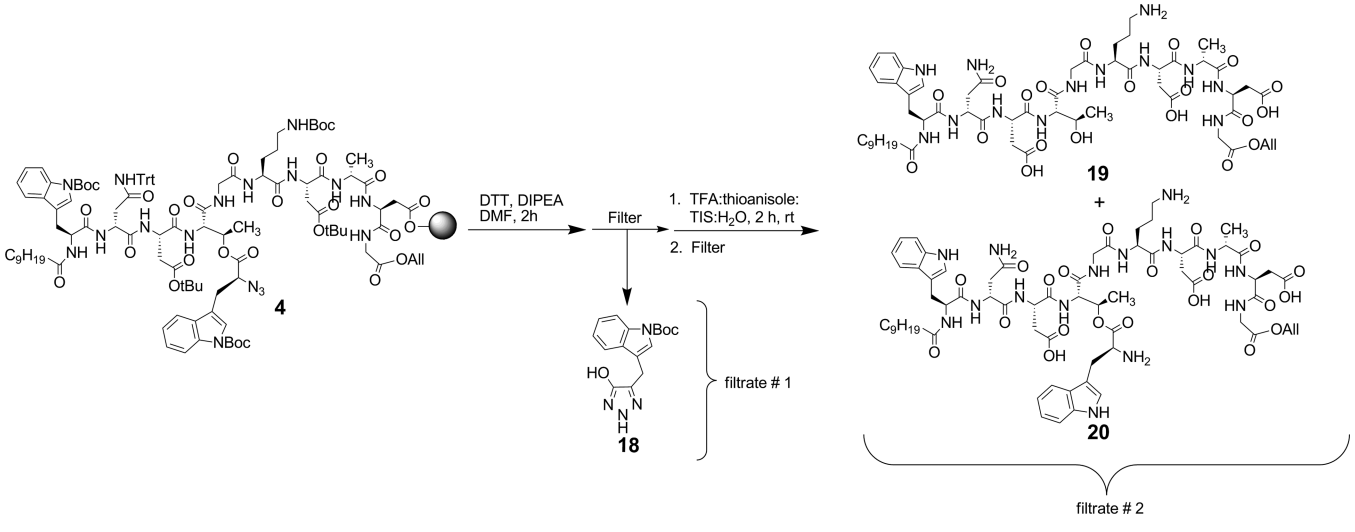
The above results with ethyl ester **10** prompted us to examine whether triazole formation also occurs when peptide **4** is subjected to DTT/DIPEA. Thus, peptide **4** was subjected to DTT/DIPEA in DMF for 2 h and then filtered (filtrate #1, Scheme 5). The resin was then treated with TFA/thioanisole/TIS/H_2O and filtered (filtrate #2, Scheme 5). The HPLC chromatogram from the first filtrate exhibited one major peak, which MS analysis revealed to be triazole **18**. The HPLC chromatogram from the second filtrate exhibited two major peaks of almost equal intensity, which MS analysis revealed to be peptide **19**, resulting from C−O bond cleavage, and the amine peptide **20**. These findings indicate that some C−O bond cleavage does indeed occur during reduction of this azido group with DTT/DIPEA. This result most likely accounts for the lower yield that we obtained for Dap-E12-W13 using route B (2.3%, Scheme 1) in comparison to route A (3.7%).

In order to increase the yield of dap analogues via route B (Scheme 1), it was necessary to minimize C−O bond cleavage during reduction of the azido group in peptide **4**. Therefore, we decided to use PPh_3 in THF/H_2O to reduce the azido group in **4**, as our previous studies with α-azido phenyl and ethyl esters revealed that this reagent gives the Staudinger products and no triazole.[14,17] Thus, peptide **4** was subjected to 6 equiv of PPh_3 in THF/H_2O for 15 h and then filtered (filtrate #1, Scheme 6). The resin was then treated with TFA/thioanisole/TIS/H_2O and filtered again (filtrate #2, Scheme 6). The filtrates from both steps were subjected to HPLC, and the resulting products were analyzed by MS. Surprisingly, filtrate #2 contained peptides **19** and **20** in an approximately 1:1 ratio. However, the filtrate from the first step did not contain triazole **18**. Instead, HRMS analysis indicated that filtrate #1 contained a compound whose structure which we initially assigned to be betaine **21** (Scheme 6). To confirm the structure of **21**, we prepared this compound by reacting PPh_3 with N_3Trp(Boc)-OH in dry ether.[18] This resulted in the formation of a precipitate which upon filtration yielded a white solid. When subjected to HPLC, the precipitate eluted in a single peak that had a retention time identical to that of compound **21**. The HRMS of this solid was also consistent with this compound being betaine **21**. However, the ^{31}P NMR spectrum of this solid in THF exhibited a peak at −39.7 ppm, indicating that, in this solvent, it existed almost exclusively as the cyclic trigonal bipyramidal phosphorane **24** (Scheme 7) and not the betaine, which would be expected to have a ^{31}P NMR chemical shift greater than 20 ppm.[18−20] In contrast, the ^{31}P NMR spectrum in THF/10% H_2O exhibited a large broad peak at 2 ppm, suggesting that compound **24** is in equilibrium with betaine **21** in this solvent.

The above studies with PPh_3 reveal that phosphazide **25** does not cyclize but instead loses N_2 to give iminophosphorane **28** (Scheme 8). However, the C−O bond in iminophosphorane **28** is susceptible to hydrolysis, and ester hydrolysis competes effectively with hydrolysis of the phosphorane moiety (Scheme 8).

Our previous studies on ethyl ester **10** suggested that C−O bond cleavage via triazole formation would occur if peptide **4** was subjected to PMe_3 or PBu_3 in THF/H_2O. However, our results with peptide **4** and PPh_3 indicate that C−O bond cleavage can also occur via hydrolysis of the ester bond in the iminophosphorane intermediate. To determine if this is also the case with PMe_3 and PBu_3, peptide **4** was subjected to 3 equiv of PMe_3 or PBu_3 in THF/H_2O (1 h for PMe_3 and 16 h for PBu_3),

Scheme 5. Products Formed upon Treatment of Peptide 4 with DTT/DIPEA



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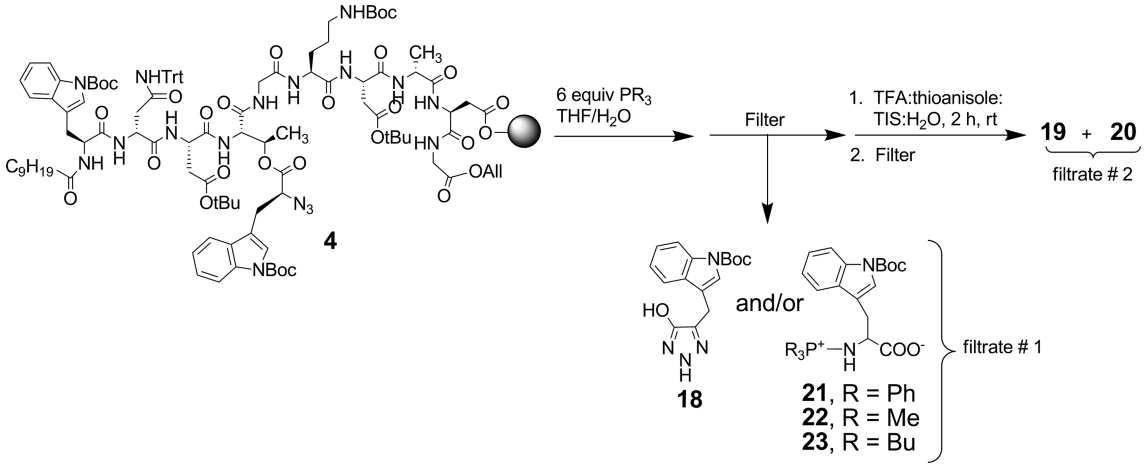
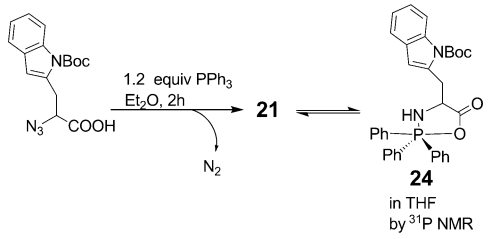
Scheme 6. Products Formed upon Treatment of Peptide 4 with Phosphines in THF/H_2O



Scheme 7. Products Formed upon Reaction of N_3Trp(Boc)OH with PPh_3



and the reaction was analyzed as described above for PPh_3. With PMe_3, the first filtrate contained triazole **18** and, to a lesser extent, betaine **22**[21] (Scheme 6). The second filtrate contained peptide **19** as the major product, and only a relatively small amount of peptide **20** was formed. Thus, with PMe_3, C−O bond cleavage occurs by both triazole formation from phosphazide **26** (Scheme 8) and hydrolysis of the ester in iminophosphorane **29** (Scheme 8).[22] With PBu_3, the filtrate after the first step contained betaine **23**[21] but no triazole **18**. In the second filtrate, peptide **19** was the major product and only a relatively small amount of peptide **20** was formed. Therefore, with PBu_3, loss of N_2 from phosphazide **27** is faster than triazole formation, and hydrolysis of the ester moiety in the iminophosphorane **30** is faster than phosphorane hydrolysis.

Interestingly, tripeptide **31** exhibited different reactivity patterns compared to those of peptide **4** when subjected to phosphines in THF/H_2O (Scheme 9). Subjecting **31** to 1.2 equiv of PMe_3 or PBu_3 in THF/H_2O for 1−3 h gave an approximately equal mixture of triazole **18** and peptides **32** and **33** as determined by HPLC of the crude reaction mixture. Subjecting **31** to 1.2 equiv of PPh_3 in THF/H_2O for 20 h gave only amine **32**. After 20 h, this reaction was not complete, as the iminophosphorane derived from peptide **31** and PPh_3 was evident in the HPLC chromatogram. Thus, peptide **31** exhibits a product distribution fairly similar to that found with ester **10** with all of the phosphines.[14] Betaines **21**−**23** were not formed in any of the reactions, indicating that the ester moiety in the iminophosphoranes derived from peptide **31** is not as susceptible to hydrolysis as the analogous ester in the iminophosphoranes (**28**−**30** in Scheme 8) derived from peptide **4**.

It is not clear to us why the reactivity patterns (in terms of the relative proportions of C−O bond cleavage, azide reduction, and triazole and betaine products) are so different between peptide **4** and ester **10** and peptide **31** when treated with phosphines in THF/H_2O. The greater susceptibility of the ester moiety in iminophosphoranes **28**−**30** than of the analogous ester in the iminophosphorane derived from ethyl ester **10** and peptide **31** may result from iminophosphoranes **28**−**30** adopting a conformation that allows a nearby proton to act as a general acid and promote ester hydrolysis. It is also possible that iminophosphoranes **28**−**30** may adopt a conformation that results in the iminophosphorane abstracting a proton from the amide nitrogen of the adjacent glycine, which could promote ester cleavage through intramolecular attack of the amide oxygen. Phosphazides **25** and **27** may also adopt a conformation that prevents these phosphazides from cyclizing, and hence triazoles are not formed when peptide **4** is treated with PBu_3 or PPh_3. Phosphazide **26** may adopt a similar conformation; however, the smaller size of the trimethylphosphine moiety may still allow for cyclization to occur.

The ester moiety in phosphoranes **28**−**30** (Scheme 8) appears to be fairly susceptible to hydrolysis in comparison to the ester moiety in peptide **4**, which does not undergo any detectable hydrolysis after 24 h in THF/H_2O, as determined by HPLC. Scheme 10 shows two possible mechanisms for the hydrolysis of these esters. The phosphorane nitrogen may attack the carbonyl carbon followed by loss of the threonine-derived peptides to give the three-membered ring intermediate **34**, which then hydrolyzes to give the betaines (pathway a in Scheme 10). Alternatively, direct attack of water on the carbonyl carbon may occur, and the resulting tetrahedral intermediate may be stabilized by the iminophosphorane group possibly by forming a pentacoordinate intermediate such as **35** (pathway b in Scheme 10). It is also possible that the iminophosphorane nitrogen or the γ-nitrogen of the initially formed phosphazide acts as general base by deprotonation of a water molecule and concomitant attack of the resulting activated water molecule on the carbonyl group. Our data do not allow us to distinguish between these two mechanisms or rule out any others.

In order to avoid C−O bond cleavage, we attempted the coupling of an activated form of FmocGlu(OtBu)OH with the iminophosphorane derived from peptide **4**. Lundquist and Pelletier have reported the formation of peptide bonds in the solid phase by reacting resin-bound peptides having an N-terminal azido group with PMe_3 in the presence of succinimidyl

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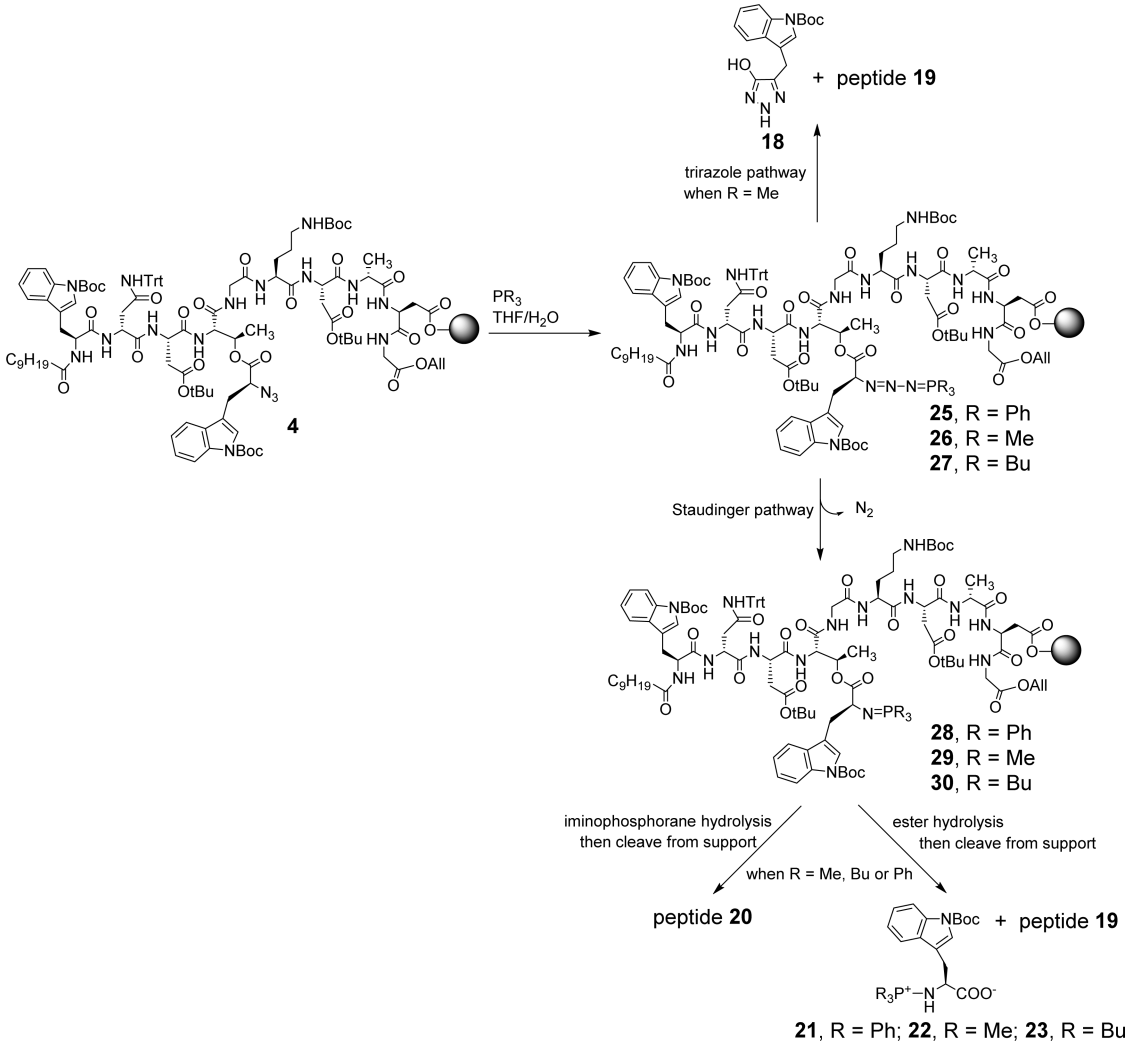
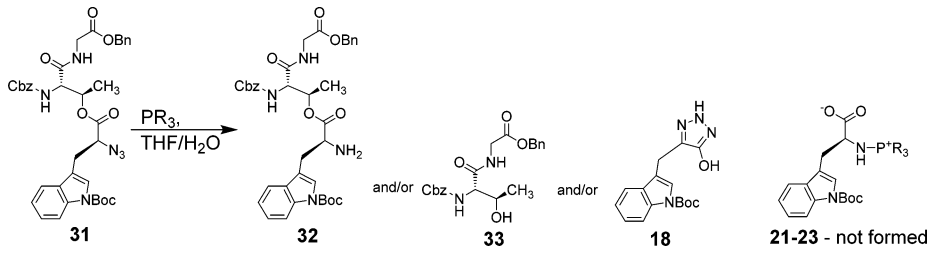
Scheme 8. Pathways for C−O Bond Cleavage When Peptide 4 Is Treated with Phosphines in THF/H_2O



Scheme 9. Products Formed upon Treatment of Tripeptide 31 with Phosphines in THF/H_2O



esters of Fmoc amino acids in dry dioxane.[4] We attempted this procedure on peptide **4** with FmocGlu(OtBu)OSu; however, this resulted in the formation of mainly iminophosphorane **29**. Malkinson et al. conjugated an amino acid to a resin-bound glycosyl azide by reacting the azide with the Fmoc amino acid in the presence of HOBt, DIC, and PBu_3 in dry THF.[23] Applying these conditions to the coupling of FmocGlu(OtBu)-OH to peptide **4** resulted in a complex mixture of products which included the desired coupled product (**36** in Scheme 11), as well as peptides **19** and **20**. Surprisingly, when this procedure was performed in the presence of 5% water, the reduced peptide **20** was the major product and considerably less C−O cleavage product (peptide **19**) and coupled product **36** were formed. Further experimentation revealed that the amount of peptide **19** could be reduced even further by reacting peptide **4** with 5 equiv of the symmetric anhydride of FmocGlu(OtBu)OH in the presence of 3 equiv of PBu_3 for 5 min in dry THF, followed by the addition of about 5% water and stirring for 18 h. However, very little of the desired product **36** was formed. Instead, the reduced peptide **20** was almost the sole product, as determined by HPLC analysis of the crude reaction mixture after cleavage from the support (Scheme 11). It was found that reacting peptide **4** with 5 equiv of the symmetric anhydride of FmocGlu(OtBu)OH in the presence of 3 equiv of PBu_3 in dry THF for 30 min or 5 h, followed by the addition of water and stirring for 18 h, resulted in a more complex mixture of products and an increase in the amount of peptide **19**.

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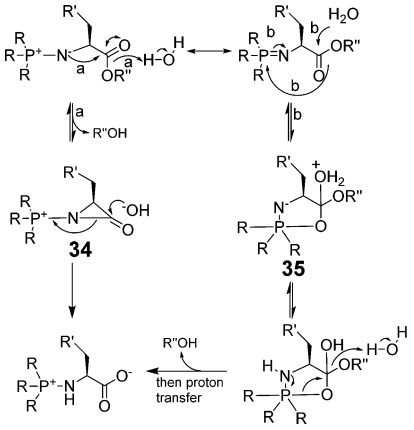
Scheme 10. Possible Mechanisms for the Hydrolysis of the Ester Moiety in Iminophosphoranes 28−30



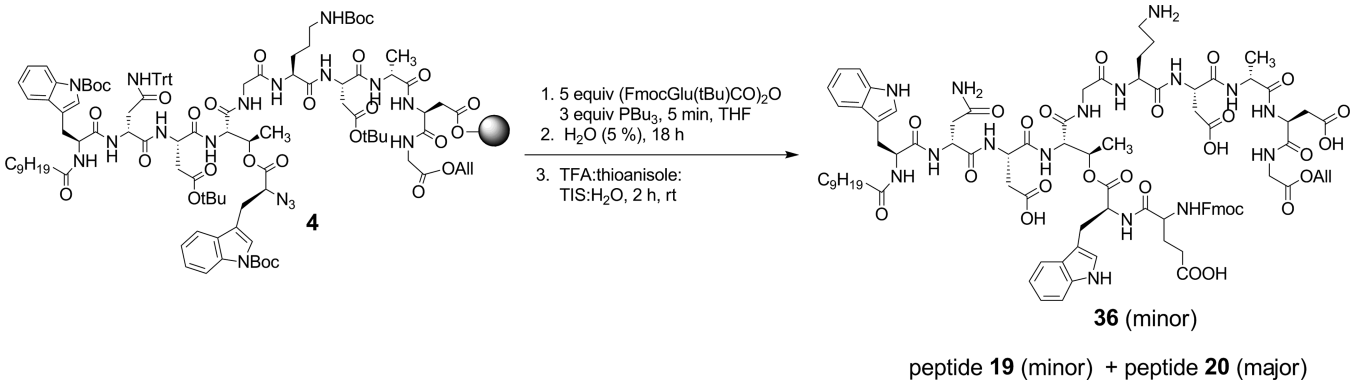
Peptide **31** exhibited very different reactivity patterns compared to those of peptide **4** when subjected to the conditions outlined in Scheme 11. HPLC and MS analysis of the reaction mixture revealed that the coupled product **37** was by far the dominant peptide produced (Scheme 12). A small amount of peptides **32** and **38** was also formed. We did not detect any products resulting from C−O bond cleavage (betaine **23**, triazole **18**, or peptide **33**). This was not entirely unexpected since we had already found that the reactivity patterns exhibited by resin-bound peptide **4** (Schemes 7 and 8) can be very different from those exhibited by simpler substrates such as ester **10** or peptide **31** in solution (Schemes 3 and 9).

The reason behind the preferential formation of peptide **20** over peptides **19** and **36** under the conditions described in Scheme 11 is not entirely clear. Nevertheless, in Scheme 13, we present a mechanism that might explain this phenomenon. In this mechanism, peptide **4** reacts with PBu_3 in dry THF to give phosphazide **27**. The latter can undergo loss of N_2 to give iminophosphorane **30** (pathway A), which can produce C−O bond cleavage products **19** and **23** or peptide **20**. It is possible that phosphazide **27** can also react with the excess anhydride to give intermediate **39**, which would be in equilibrium with intermediate **40** (pathway B). Addition of water results in protonation of **39** to give *N*-acyl triazenes **41** and **42**.[24] It is possible that **42** hydrolyzes to give N_2 and the reduced peptide **20**, even though studies on *N*-acyl-1,3-dialkyltriazenes have shown that hydrolysis is not the dominant pathway by which these compounds fragment in aqueous solution. Instead, in aqueous solution, they readily undergo $N^{\alpha}-N^{\beta}$ fragmentation to give the corresponding diazonium salts and amides.[25,26] We are not aware of any detailed studies on the hydrolysis of *N*-acyl-3-alkyltriazenes, although it appears that these species also readily fragment in aqueous solution to give the respective diazonium salts and amides.[24,27] Moreover, we were unable to detect *N*-acyl triazene **43** (Scheme 14) when tripeptide **31** was subjected to the conditions outlined in Schemes 11 and 12. However, a small amount of hydrazone **38** was detected, which suggests that a small amount of diazonium salt **45** was formed in this reaction as hydrazone **38** was most likely produced by PBu_3 reduction of **45** (Scheme 14).[28] It is possible that **45** was produced by fragmentation of the *N*-acyl triazene **43** even though we were unable to detect any FmocGlu(OtBu)NH_2 (**44**). It is also possible that **45** was produced by fragmentation of the initially formed phosphazide.[29]

Although the mechanism outlined in Scheme 13 has yet to be supported by the detection of any of the proposed intermediates, it is possible that the resin-bound peptide may adopt a conformation that promotes the hydrolysis of **41** analogous to how a specific conformation in intermediate **30** may promote the hydrolysis of the ester group in the phosphorane moiety to give betaine **23** (Schemes 7 and 8).

Having developed a procedure for reducing the azido group in peptide **4**, we applied it to the synthesis of Dap-E12-W13 (Scheme 15). After the azido group in peptide **4** was reduced using the procedure outlined in Scheme 11 to give mainly peptide **3**, standard Fmoc SPPS (DIC/HOBt) was employed to incorporate Glu12 and D-Ser11, and then the alloc group was removed using our standard conditions to give peptide **46**.[1] In our previous syntheses of Dap-E12-W13, cyclization of peptide **46** to peptide **47** was achieved using PyBOP/HOAt/DIPEA in DMF/DCM/NMP (3:2:2 (v/v)) containing 1% Triton X-100 (magic mixture) for 2 × 13 h.[2] However, the cyclization efficiency was only 40%. We have now found that the cyclization can be achieved with 90% efficiency (by HPLC) in just 2 × 1.5 h by using PyAOP/HOAt/2,4,6-collidine in DMF containing 1% Triton X-100. The peptide was cleaved from the resin, and side chain protecting groups were removed using standard conditions. The overall yield of Dap-E12-W13 was 10.3%. The Dap-E12-W13 prepared in this manner exhibited biological activity identical to that of Dap-E12-W13 prepared by our previous routes (MIC of 1 μg/mL against

Scheme 11. Products Formed upon Reaction of Peptide 4 with an Excess of the Symmetric Anhydride of FmocGlu(OtBu)OH in the Presence of Excess PBu_3 in Dry THF for 5 min Followed by the Addition of Water and Stirring for 18 h



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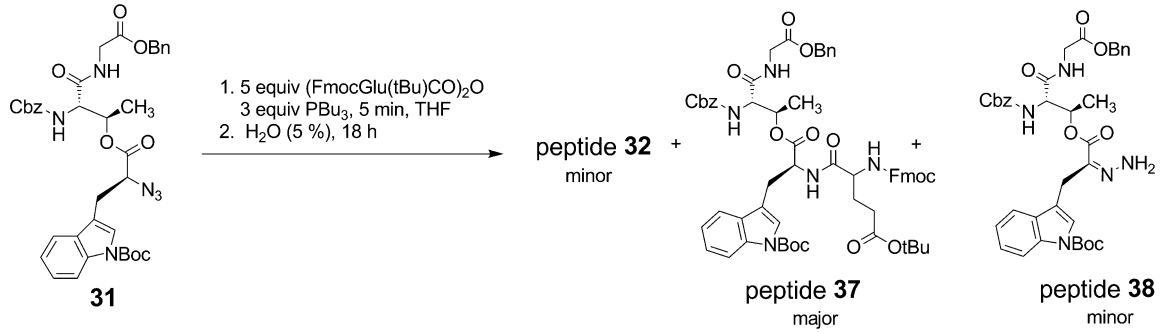
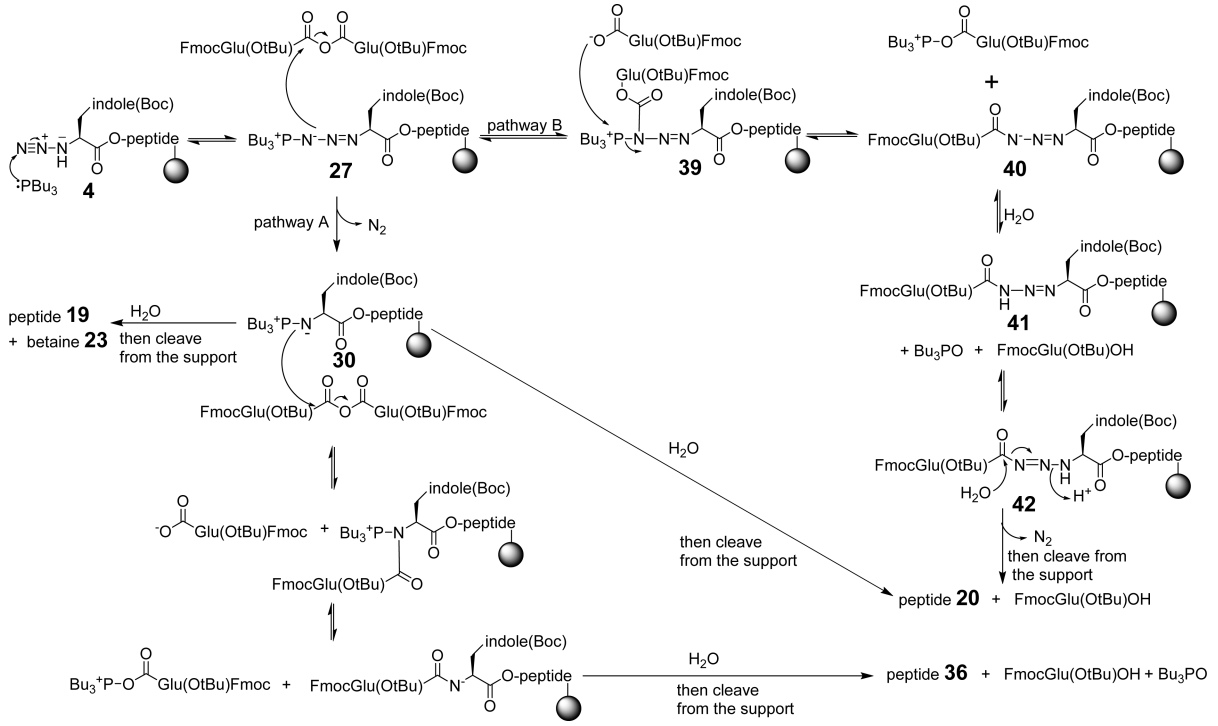
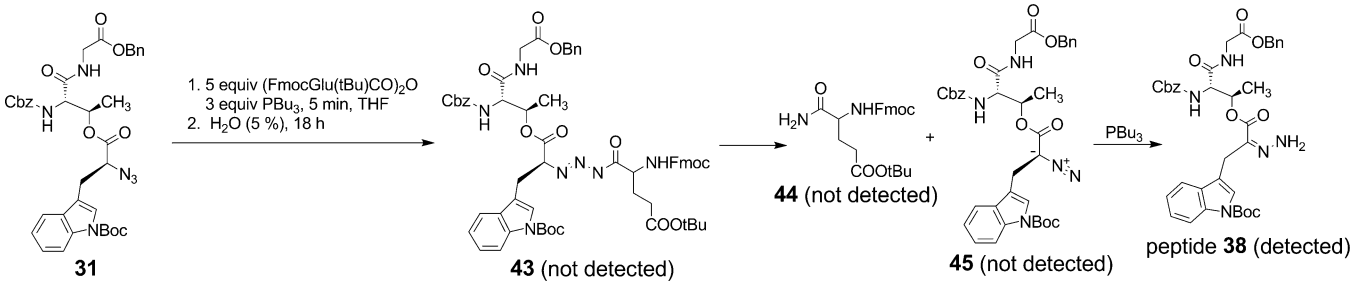
Scheme 12. Products Formed upon Reaction of Peptide 31 with an Excess of the Symmetric Anhydride of FmocGlu(OtBu)OH in the Presence of Excess PBu_3 in Dry THF for 5 min Followed by the Addition of Water and Stirring for 18 h



Scheme 13. Possible Mechanism for the Formation of Peptide 20



Scheme 14. Possible Route to Peptide 38 during the Reaction of Peptide 31 under the Conditions Outlined in Schemes 11 and 12



Bacillus subtilis 1046 and 3 μg/mL against *B. subtilis* PY79 in the presence of 5 mM $CaCl_2$).[1,2]

SUMMARY AND CONCLUSIONS

We have shown that α-azido esters, when treated with DTT/DIPEA, can undergo C−O bond cleavage to give triazoles, analogous to when α-azido esters are treated with alkyl phosphines. The extent of triazole formation depends upon leaving group ability. Some C−O bond cleavage and formation of triazole **18** occurred when peptide **4** was treated with DTT/DIPEA. In contrast to the simple α-azido ester **10**, which gave only amine **12** when treated with PPh_3 in THF/H_2O, peptide **4** gave a mixture of the desired amine product **20** as well as the undesired C−O cleavage product **19** and betaine **21** when

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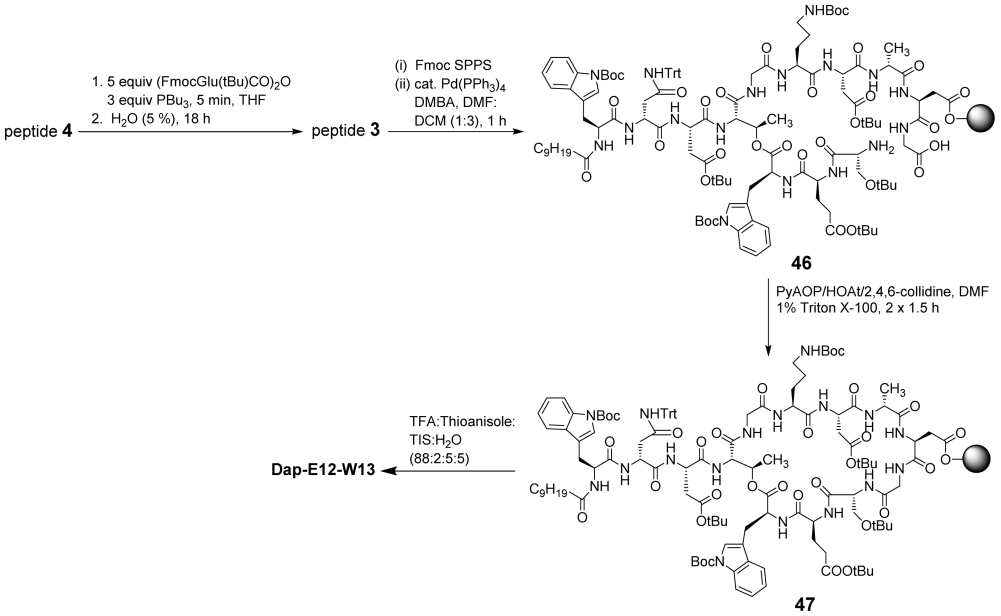
Scheme 15. Modified Route to Dap-E12-W13



treated with PPh_3 in THF/H_2O. Peptides **19** and **20**, betaines **22** and **23**, and/or triazole **18** were formed when peptide **4** was treated with PMe_3 and PBu_3. Overall, these results indicate that one must exercise caution when azido esters are employed in depsipeptide synthesis.

It was found that the azido group in peptide **4** could be efficiently reduced to the desired amino peptide **3** by reacting peptide **4** with an excess of the symmetric anhydride of FmocGlu(OtBu)OH in the presence of excess PBu_3 for 5 min in dry THF, followed by the addition of about 5% water and stirring for 18 h. By applying this new reduction procedure, as well as an improved cyclization protocol, to the synthesis of the daptomycin analogue, Dap-E12-W13, we achieved a much higher yield in comparison to our previously reported approaches.

EXPERIMENTAL SECTION

General. 2-Chlorotrityl chloride resin, all Fmoc amino acids, and coupling reagents used for peptide synthesis were purchased from commercial sources. N_3Trp(Boc)OH was prepared according to a literature procedure.[30] ACS grade DMF, THF, piperidine, TFA, TIS, thioanisole, and 2-methylpiperidine (2-MP) were purchased from commercial suppliers and used without further purification. CH_2Cl_2 (DCM) was distilled from calcium hydride under nitrogen. Peptide **4** was prepared as previously described.[1] Peptide syntheses and studies with peptide **4** were performed manually using a rotary shaker for agitation.[31] Reversed-phase C18 columns (10 μM, 250 × 4.6 mm, or 10 μM, 250 × 20 mm) were used for analytical and semipreparative HPLC at flow rates of 1.0 mL/min for analytical HPLC and 10 mL/min for semipreparative HPLC. The HPLC was equipped with a UV detector. All HPLC chromatograms (see the Supporting Information) were obtained using a linear gradient of 10% ACN/90% H_2O (0.1% TFA) to 90% ACN/10% H_2O (0.1%TFA) over 50 min, with the detector set to 280 nm unless stated otherwise. High-resolution positive ion electrospray (ESI^+HRMS) mass spectra were obtained using an orbitrap mass spectrometer. 1:1 MeOH/H_2O + 0.1% formic acid was used as a solvent. The biological activity of Dap-E12-W13 was determined as previously described.[2]

^{1}H NMR and *J*-modulated spin−echo (*J*-MOD) ^{13}C NMR spectra were recorded on a 300 MHz NMR instrument. Chemical shifts (δ) for ^{1}H NMR spectra run in $CDCl_3$ are reported in parts per million relative to the internal standard tetramethylsilane (TMS). Chemical shifts (δ) for ^{1}H NMR spectra run in CD_3OD are reported in parts per million relative to residual solvent protons (δ 3.30). For ^{13}C NMR spectra run in $CDCl_3$, chemical shifts are reported in parts per million relative to the solvent residual carbon (δ 77.0 for central peak). For ^{13}C NMR spectra run in CD_3OD, chemical shifts are reported in parts per million relative to the CD_3OD residual carbon (δ 49.0 for the central peak). All ^{31}P NMR spectra are referenced to H_3PO_3 (external standard).

General Procedure for the Reduction of Esters 10 and 11 with DTT/DIPEA. To a solution of ester **10** or **11** in DMF (0.1 mmol in 1 mL dry DMF) was added a solution of DTT (0.4 mmol) and DIPEA (0.2 mmol) in dry DMF (0.5 mL). The mixture was stirred for 2 h and then analyzed by analytical HPLC (linear gradient of 5% ACN/95% H_2O (0.1% TFA) to 100% ACN over 45 min, λ = 230 nm, see Figures S1 and S2 in the Supporting Information). Triazole **13**, from the reaction of phenyl ester **11**, was purified by preparative HPLC (linear gradient of 15% ACN/85% H_2O (0.1% TFA) to 25% ACN/75% H_2O (0.1% TFA) over 60 min, t_r = 55 min). Yield = 14.5 mg (83%, white solid). The ^{1}H NMR spectrum of compound **13** obtained in this manner was identical to that of compound **13** obtained previously by reduction of ester **11** using PMe_3 in THF/H_2O:[14] ^{1}H NMR (CD_3OD) δ 7.17−7.31 (5H, m), 3.95 (2H, s); ^{13}C NMR (CD_3OD) δ 140.4, 129.4, 127.2, 30.0; HRMS (*m*/*z*) $[M + H]^+$ calcd for $C_9H_{10}ON_3$ 176.08184; found 176.08185.

Reaction of Peptide 4 with DTT/DIPEA. To peptide **4** (0.025 mmol) was added a solution of DTT (2M) and DIPEA (1M) in dry DMF (2 mL containing 0.616 g of DTT and 0.348 mL of DIPEA). The mixture was stirred at rt for 2 h and filtered. The filtrate was analyzed by analytical HPLC and HRMS (see Figure S5 in the Supporting Information). The resin was washed with DMF (3×) and DCM (3×) and then subjected to TFA/thioanisole/TIS/H_2O (88:2:5:5, 2 mL). The mixture was filtered, and the resin was rinsed with an additional cleavage cocktail. The combined filtrates were concentrated to one-fourth the original volume using a stream of N_2 gas. The peptide was precipitated by adding cold ether (3 mL). The precipitated peptide (white precipitate) was collected by centrifugation and washed with cold ether (2×) and then analyzed by analytical HPLC and HRMS (see Figure S6 in the Supporting Information).

Reaction of Peptide 4 with Phosphines in THF/H_2O. To peptide **4** (0.025 mmol) was added a solution of PPh_3 (6 equiv) or PMe_3 or PBu_3 (3 equiv) in 2 mL of THF/H_2O (8:2 v/v). The mixture was agitated for 1 h for Me_3P and 13 h for Bu_3P and Ph_3P and then filtered and washed with DMF and DCM. The filtrate was analyzed by analytical HPLC and HRMS (see Figures S7, S16, and S18 in the

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Supporting Information). The resin was treated with TFA/thioanisole/TIS/H_2O (88:2:5:5, 2 mL). The mixture was filtered, and the resin was rinsed with an additional cleavage cocktail. The combined filtrates were concentrated to one-fourth the original volume using a stream of N_2 gas. The peptide was precipitated by adding cold ether (3 mL). The precipitated peptide (white precipitate) was collected by centrifugation and washed with cold ether (2×). The peptide was analyzed by analytical HPLC and HRMS (see Figures S8, S17, and S19 in the Supporting Information).

Reaction of N_3Trp(Boc)OH with PPh_3. To a solution of N_3Trp(Boc)OH (0.200 g, 0.61 mmol) in dry ether (4 mL) was added a solution of PPh_3 (0.159g, 0.61 mmol) in dry ether (2 mL). The mixture was stirred at rt for 2 h and filtered. The filter cake was washed with ether and dried under high vacuum, yielding a white solid (0.102 g, 30%). The analytical HPLC chromatogram of this precipitate shows one major peak with t_r = 36.6 min as well as well as a few very minor peaks due to PPh_3 and $POPh_3$ (see Figure S9 in the Supporting Information): ^{1}H NMR ($CDCl_3$) δ 8.10 (1H, d, J = 8.4 Hz), 7.60 (1H, d, J = 7.4 Hz), 7.47 (1H, s), 7.19−7.40 (19H, m), 4.17 (1H, ddd, J = 10.6, 9.0, 3.2 Hz), 3.15 (1H, dd, J = 14.8, 3.2 Hz), 3.14 (1H, dd, J = 14.8, 9.0 Hz), 1.63 (9H, s); ^{13}C NMR ($CDCl_3$) δ 172.3, 149.4, 135.4, 134.4, 133.6 (d, J = 19.5 Hz), 131.4 (d, J = 10.8 Hz), 131, 130.4, 128.3 (d, J = 14.1 Hz), 124.5, 124.0, 122.5, 119.2, 116.8, 115.2, 83.5, 56.2, 30.0, 28.1; ^{31}P NMR ($CDCl_3$) δ −10.9, −2.8 (PPh_3), 31.7 ($POPh_3$), 51.2; ^{31}P NMR (THF) δ −39.4, −0.4 (PPh_3), 23.8 ($POPh_3$); ^{31}P NMR (THF/10% H_2O) δ 2.05, 27.5 ($POPh_3$); HRMS (m/z) $[M + H]^+$ calcd for $C_{34}H_{34}O_4N_2P$ 565.22507; found 565.22446. The HPLC chromatogram of a mixture of this precipitate with compound **21**/**24** isolated from filtrate #1 in the reaction of peptide **4** with PPh_3 in THF/H_2O shows a single peak, suggesting that these two compounds are identical (see Figure S10 in the Supporting Information).

Reaction of Peptide 4 with Excess PBu_3 in the Presence of the Symmetric Anhydride of FmocGlu(OtBu)OH and Water. The symmetric anhydride of FmocGlu(OtBu)OH was prepared by stirring FmocGlu(OtBu)OH (0.25 mmol) and DIC (0.125 mmol) in dry THF (2 mL) for 1 h at rt.[32] The mixture was filtered. The filtrate was added to peptide **4** (0.025 mmol), followed by Bu_3P (3 equiv), and agitated for 5 min. H_2O (0.1 mL) was added, and the mixture was agitated for 18 h. The resin was washed with DMF (3×) and DCM (3×) and then treated with TFA/thioanisole/TIS/H_2O (88:2:5:5, 2 mL). The mixture was filtered, and the resin was rinsed with an additional cleavage cocktail. The combined filtrates were concentrated to one-fourth the original volume using a stream of N_2 gas. The peptide was precipitated by adding cold ether (3 mL). The precipitated peptide (white precipitate) was collected by centrifugation and washed with cold ether (2×). The peptide was analyzed by analytical HPLC (see Figure S20 in the Supporting Information).

Peptide 31. To a solution of N_3Trp(Boc)OH (0.660 g, 2.00 mmol) in dichloromethane (5 mL) was added diisopropylcarbodiimide (DIC, 0.154 mL, 1.00 mmol). The mixture was stirred for 1 h. ZThrGlyOBn[33] (0.4 g, 1.0 mmol) and DMAP (0.012 g, 0.1 mmol) were added, and the mixture was stirred for 24 h. The mixture was filtered, and the filtrate wsa dissolved in Et_2O and washed with 0.25 M NaOH, 0.1 M HCl, and saturated brine. The organic layer was dried (Na_2SO_4) and concentrated, giving a yellow oil. The oil was subjected to flash chromatography (30% EtOAc/70% hexane) which provided peptide **31** as a white foam (0.606 g, 85% yield): ^{1}H NMR ($CDCl_3$) δ 8.13 (1H, d, J = 7.9 Hz), 7.51−7.55 (2H, m), 6.87−7.32 (12H, m), 6.89 (1H, t, J = 5.3 Hz), 5.68 (1H, d, J = 8.5 Hz), 4.48−5.51 (1H, m), 5.13 (2H, s), 5.11 (2H, s), 4.48 (1H, d, J = 5.3 Hz), 4.23 (1H, t, J = 6.4 Hz), 3.99 (2H, br s), 3.23 (1H, dd, J = 5.3, 14.8 Hz), 3.09 (1H, dd, J = 8.5, 14.8 Hz), 1.65 (9H, s), 1.23 (3H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 169.2, 168.8, 168.7, 156.3, 149.4, 135.8, 135.3, 134.9, 129.9, 128.5, 128.2, 128.0, 124.6, 124.3, 122.5, 118.6, 115.3, 114.8, 83.7, 71.9, 67.4, 67.2, 61.7, 57.7, 41.3, 28.1, 27.1, 15.9; HRMS (m/z) $[M + H]^+$ calcd for $C_{37}H_{41}O_9N_6$ 713.29295; found 713.29287.

Reaction of Peptide 31 with Phosphines in THF/H_2O. To a solution of peptide **31** (0.02 g, 0.028 mmol) in THF (0.124 mL) and H_2O (0.010 mL) was added a 1 M solution of PMe_3, PBu_3, or PPh_3 in THF (0.056 mL, 1.2 equiv). The mixture was stirred for 1 h for the Me_3P and Bu_3P reactions and 20 h for Ph_3P reaction and then analyzed by analytical HPLC (linear gradient of 5% ACN/95% H_2O (0.1% TFA) to 100% ACN over 60 min, λ = 230 nm; see Figures S23−S25 in the Supporting Information) and LRMS.

Reaction of Peptide 31 with Excess PBu_3 in the Presence of an Excess Amount of the Symmetric Anhydride of FmocGlu-(OtBu)OH and Water. A solution of the symmetric anhydride of FmocGlu(OtBu)OH in dry THF (2 mL, 5 equiv) was added to peptide **31** (0.023 g, 0.030 mmol) followed by 3 equiv of Bu_3P and stirred for 5 min. H_2O (0.1 mL) was added, and the mixture was stirred for 18 h. An aliquot was withdrawn from the reaction mixture, diluted in CH_3CN/H_2O (1:1), and then analyzed by analytical HPLC (λ = 230 nm; see Figure S26 in the Supporting Information).

Synthesis of Dap-E12-W13 Starting from Peptide 4. To peptide **4** (0.025 mmol) was added a solution of 5 equiv of the symmetric anhydride of FmocGlu(OtBu)OH in THF (0.5 mL) followed by Bu_3P (3 equiv). The mixture was agitated for 5 min, and H_2O (0.1 mL) was added and the mixture agitated for 18 h. The mixture was filtered and washed with DMF (3×) and DCM (3×). FmocGlu(OtBu)OH and Fmoc-D-Ser(OtBu)OH were incorporated using standard Fmoc SPPS techniques employing DIC/HOBt as coupling agents (3.5 h) and 20% 2-methylpiperidine/DMF (3 × 10 min) for Fmoc removal. The alloc group was removed using DMBA (dimethylbarbituric acid, 10 equiv) in the presence of a catalytic amount of $Pd(PPh_3)_4$ in DCM/DMF (3:1 v/v) for 1 h. After filtration, the resin was washed with DCM (3×) and a 1.0% solution of sodium diethyldithiocarbamate trihydrate in DMF (3×) to remove excess Pd catalyst and then DCM (3×) and DMF (3×). On-resin cyclization was performed using PyAOP/HOAt/2,4,6-collidine (5/5/10 equiv) in the presence of 1% Triton X-100 in DMF (2 mL) (2 × 1.5 h). Crude Dap-E12-W13 was obtained by treating peptide **47** with a solution of TFA/thioanisole/TIS/H_2O (88:2:5:5, 2 mL) for 2 h. The mixture was filtered, and the resin was rinsed with an additional cleavage cocktail. The combined filtrates were concentrated to one-fourth the original volume using a stream of N_2 gas. The peptide was precipitated by adding cold ether (3 mL). The precipitated peptide was collected by centrifugation and washed with cold ether (2×). Pure Dap-E12-W13 was obtained using semipreparative HPLC employing a linear gradient of 35:65 CH_3CN/H_2O (0.1% TFA) to 43:57 CH_3CN/H_2O (0.1% TFA) over 60 min. Fractions containing Dap-E12-W13 were collected, concentrated by high vacuum, and lyophilized to give pure Dap-E12-W13 as a white powder (4.3 mg, 10.7% yield based on resin loading): HRMS (ESI$^+$) calcd for $C_{72}H_{100}N_{17}O_{25}$ 1602.70708 $[M + H]^+$; found 1602.70525. See Figures S27 and S28 in the Supporting Information for the analytical HPLC chromatogram and mass spectral data of purified Dap-E12-W13.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02309.

Analytical HPLC chromatograms, ^{1}H, ^{13}C, and ^{31}P NMR spectra, and HRMS data (PDF)

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Notes

The authors declare no competing financial interest.

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(15) The other mechanism proposed by Cartwright et al. involved attack at the α-nitrogen.

(16) There are many possible variations on the mechanism for triazole formation shown in Scheme 4, including one that involves attack at the α-nitrogen.

(17) We had previously avoided using PPh_3 for reducing the azido groups as we found that the reaction proceeded considerably slower in comparison to PMe_3.

(18) Gololobov and co-workers reported that the reaction of triphenylphosphine with azidoacetic acid in dry THF yielded a precipitate that exhibited a ^{31}P NMR chemical shift of 25.2 ppm in $CDCl_3$, which they attributed to the betaine, Ph_3P^+-$NHCH_2COO^-$. See: Gololobov, Y. G.; Gusar, N. I.; Chaus, M. P. *Tetrahedron* **1985**, *41*, 793−799. Earlier, these workers had reported that the reaction of 2-azidobutanoic acid or N_3ValOH with PPh_3 in THF or benzene also gave the corresponding betaines after recrystallization from acetonitrile; however, the ^{31}P NMR chemical shift of these compound in DMF was −28.4 and −37 ppm, respectively, indicating that these compounds exist as the corresponding cyclic trigonal bipyramidal phosphoranes in DMF. See: Chaus, M. P.; Gusar, N. I.; Gololobov, Y. G. *Zh. Obshch. Khim.* **1982**, *52*, 24−30.

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(21) We have chosen to show compounds **22** and **23** as betaines for simplicity sake, even though it is possible that they could be in equilibrium with their corresponding cyclic trigonal bipyramidal phosphoranes.

(22) We previously reported that when α-azido ester **10** was subjected to PMe_3 in dry THF for 30 min followed by reaction with water for 2 h, only the reduced amine **12** was formed and no C−O bond cleavage occurred (see ref 14). This suggested another potential way to avoid C−O bond cleavage when reducing the azido group in peptide **4**. However, the fact that the ester bonds in the iminophosphoranes **28**−**30** are susceptible to hydrolysis indicates that this stepwise approach to the reduction of the azido group in peptide **4** would still result in the formation of peptide **19** and betaines **21**−**23**.

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