JOC The Journal of Organic Chemistry

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α-Azido Acids in Solid-Phase Peptide Synthesis: Compatibility with Fmoc Chemistry and an Alternative Approach to the Solid Phase Synthesis of Daptomycin Analogs

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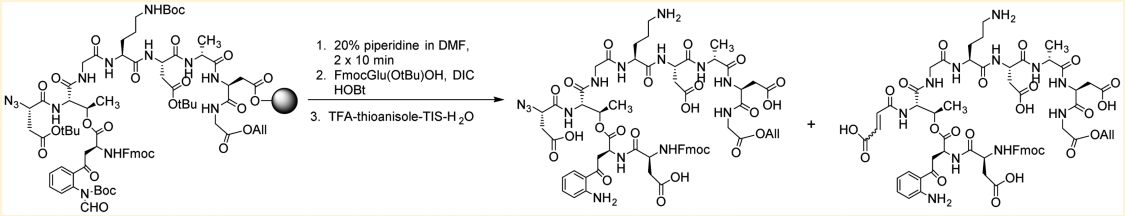
Supporting Information



ABSTRACT: α-Azido acids have been used in solid phase peptide synthesis (SPPS) for almost 20 years. Here we report that peptides bearing an *N*-terminal α-azidoaspartate residue undergo elimination of an azide ion when treated with reagents that are commonly used for removing the Fmoc group during SPPS. We also report an alternative solid-phase route to the synthesis of an analog of daptomycin that uses a reduced number of α-azido amino acids and without elimination of an azide ion.

α-Azido acids have been used for solid phase peptide synthesis (SPPS) since 1997.[1] They have been shown to be particularly effective for achieving hindered couplings[1,2] and for reducing the formation of diketopiperazines during SPPS.[3] They have also been used for solid phase Staudinger ligations,[4] and for solution phase synthesis of large peptidomimetics via alkyne−azide ligation.[5] They are readily prepared in a single step without racemization from α-NH_2-, α-COOH-unprotected amino acids using diazotransfer reagents and often do not require chromatographic purification.[3,6−9] Reduction of the α-azido group to an α-amino group is readily achieved under mild conditions with phosphines or DTT/DIPEA.[1,3]

Very recently, we[10] and Lam et al.[11] reported the total synthesis of daptomycin (Dap, Figure 1), a cyclic lipodepsipeptide antibiotic that is used clinically for treating serious infections caused by Gram-positive bacteria.[12a−d] We also reported the synthesis of several Dap analogs, most notably, DapE12W13 (Figure 1), which does not contain Kynurenine (Kyn) or the synthetically challenging (2*S*,3*R*)-3-methylglutamate residue, yet exhibits in vitro biological activity approaching that of Dap.[10] Both our and Lam et al's syntheses used one or more α-azido acids as key building blocks. The α-azido group was employed as an α-amino protecting group due to its apparent orthogonality to Fmoc chemistry. Here we report that the orthogonality of α-azido acids to Fmoc chemistry is not universal in that peptides bearing α-azidoaspartate undergo loss of an azide ion when treated with piperidine and other reagents that are used for removing the Fmoc group during SPPS. We also report an alternative approach to the solid-phase synthesis of Dap analogs that uses a reduced number of α-azido acids without loss of an azide ion.

Key intermediates in our previously reported synthesis of Dap and DapE12W13 were peptides **1** and **2** (Scheme 1) which contained an α-azidoasparate residue. In our reported synthesis of Dap and DapE12W13,[10] the azido group in peptides **1** and **2** was reduced to the amine and then D-Asn2 and Trp1 were installed using α-azido acids, followed by attachment of the decanoyl tail to give peptides **3** and **4** (Scheme 1, route A). The synthesis was completed using Fmoc SPPS and an on-resin cyclization. Although this route was successful, we had also considered an alternative route to Dap and its analogs (Scheme 1, route B). This involved preparing the macrocyclic portion (peptides **5** and **6**) from peptides **1** and **2** before reduction of the azido group. After macrocyclization, the azido group in peptides **5** and **6** would then be reduced, D-Asn2 and Trp1 would be installed using Fmoc SPPS, and the decanoyl tail would be attached to give peptides **7** and **8**. Simultaneous global deprotection of the side chains and cleavage from the resin would yield Dap and DapE12W13. The advantage of route B over route A is that only a single α-azido acid is required in route B as opposed to three α-azido acids that were employed in route A and, in route B, the cyclization would be carried out in the absence of the decanoyl tail which we anticipated might make the cyclization step more efficient.

Prior to attempting to prepare Dap using the approach outlined in route B we attempted to prepare a Dap analog, DapE12, using this approach. Before proceeding beyond peptide **1**, a small quantity of peptide **1** was subjected to TFA−thioanisole−TIS−H_2O and the resulting crude peptide was analyzed by HPLC and HRMS (Scheme 2). A single major

Received: December 23, 2015
Published: March 3, 2016

DOI: 10.1021/acs.joc.5b02882
J. Org. Chem. 2016, 81, 2624−2628

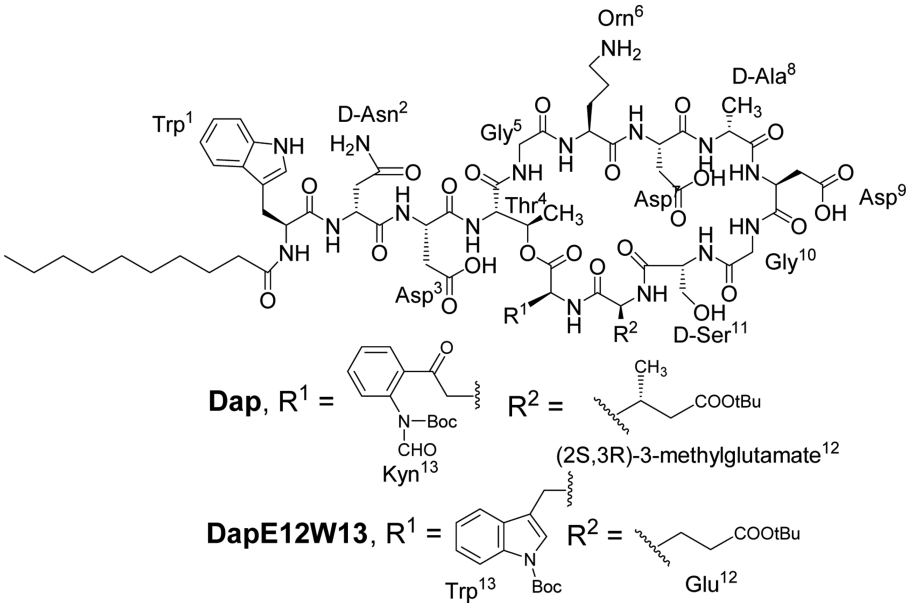
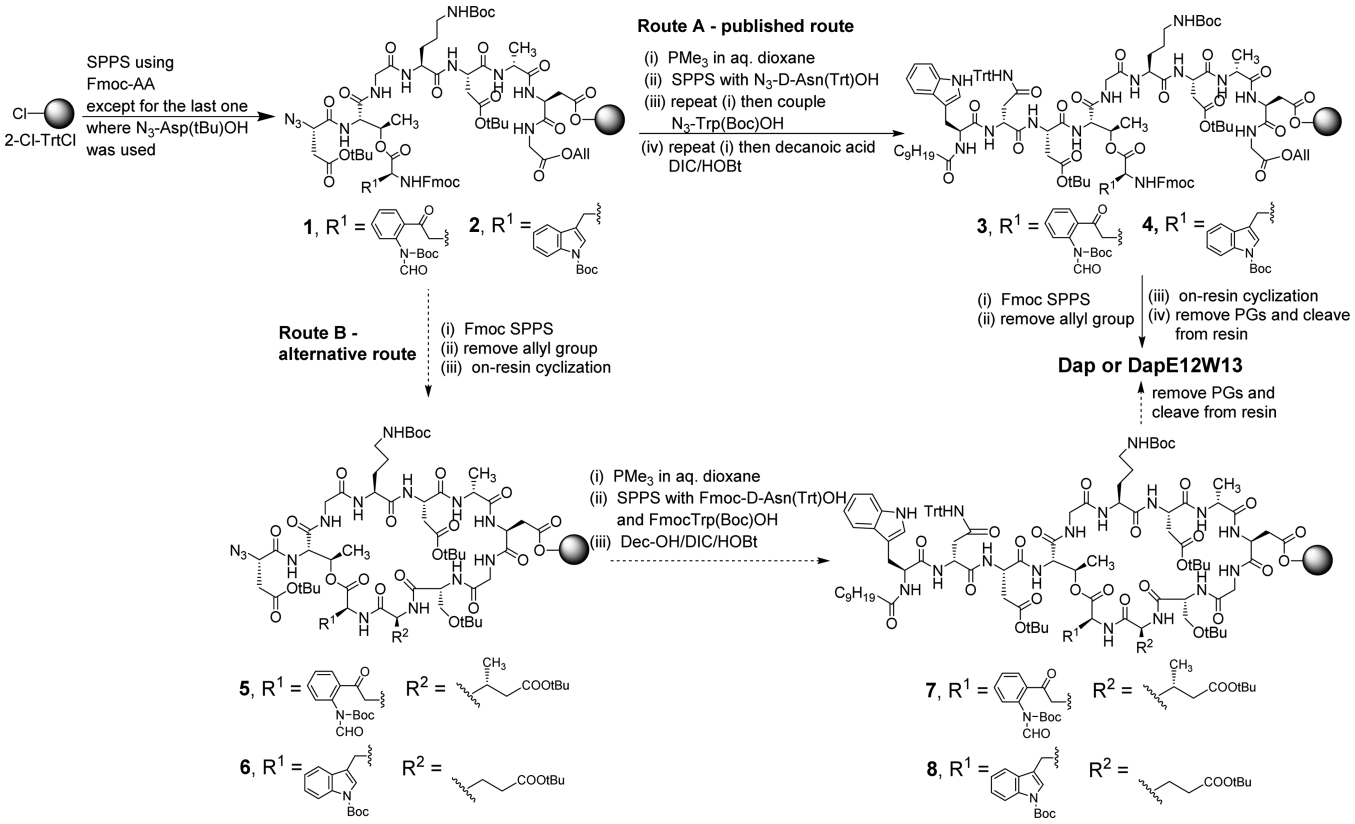


Figure 1. Structure of daptomycin and DapE12W13.

Scheme 1. Approaches to the Synthesis of Daptomycin and DapE12W13

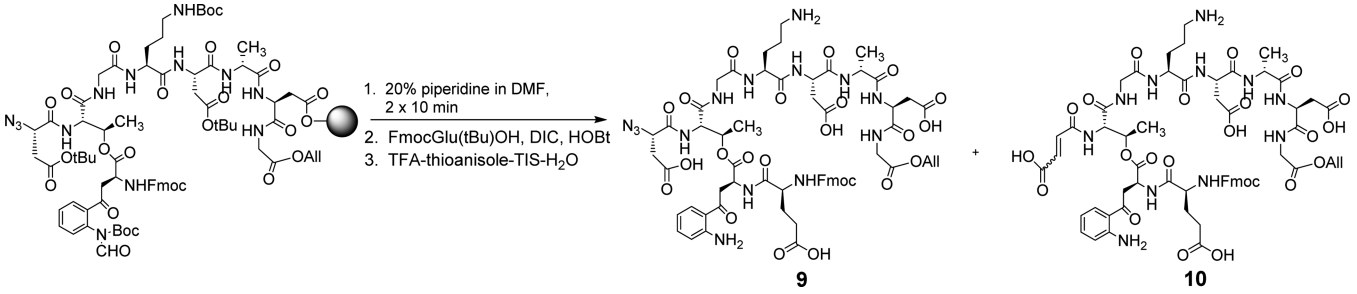


peak corresponding to N_3Asp-Thr(FmocKyn(Boc,CHO))-Gly-Orn-Asp-D-Ala-Asp-GlyOAll (**11**; see Figures S1 and S2 in the Supporting Information (SI)) was evident. Peptide **1** was then subjected to 20% piperidine in DMF (2 × 10 min) to remove the Fmoc group from the Kyn residue, and then FmocGlu-(OtBu)OH was installed using DIC/HOBt. Before continuing, a small amount of the resin was subjected to TFA−thioanisole−TIS−H_2O, the peptide precipitated in ether, and the resulting crude material was analyzed by HPLC. Two major peaks were evident in the HPLC chromatogram (see Figure S3 in the SI). HRESI$^+$MS analysis of the crude mixture revealed these peaks corresponded to the desired peptide **9** (Scheme 2) and the dehydropeptide **10** (Scheme 2) (see Figure S4 in the SI). We reasoned that peptide **10** was formed by the elimination of the azide ion during the piperidine treatment.

The appearance of dehydropeptide **10** prompted us to examine the stability of other α-azido acids to piperidine. A series of resin-bound peptides were prepared (**12**−**17**, Scheme

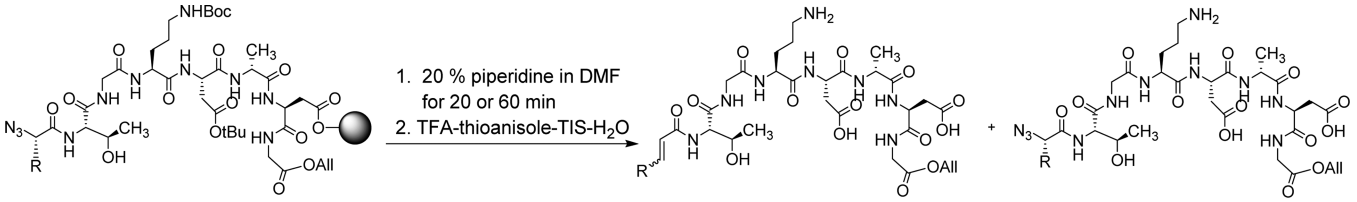
DOI: 10.1021/acs.joc.5b02882
J. Org. Chem. 2016, 81, 2624−2628

Scheme 2. Major Products Obtained after Subjecting Peptide 1 to 20% Piperidine in DMF (2 × 10 min) Followed by Coupling of FmocGluOH and Cleavage from the Resin[a]



[a]The relative amounts of the *E* and *Z* isomers in peptide **10** were not determined.

Scheme 3. Reaction of Peptides 12−17 with 20% Piperidine in DMF



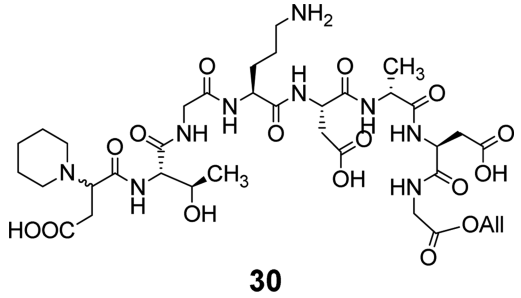
12, R = $CH_2COOtBu$ (Asp)
13, R = $CH_2CH_2COOtBu$ (Glu)
14, R = $CH_2CONHTrt$ (Asn)
15, R = CH_3 (Ala)
16, R = CH_2Ph (Phe)
17, R = CH_2Indole (Trp)

18, R = COOH
19, R = CH_2COOH
20, R = $CONH_2$
21, R = H
22, R = Ph
23, R = Indole

24, R = CH_2COOH (Asp)
25, R = CH_2CH_2COOH (Glu)
26, R = CH_2CONH_2 (Asn)
27, R = CH_3 (Ala)
28, R = CH_2Ph (Phe)
29, R = CH_2Indole (Trp)

3) containing *N*-terminal α-azido acids that we anticipated might be prone to deprotonation and elimination of the azide ion. These peptides were subjected to 20% piperidine in DMF for 20 or 60 min. The products were cleaved from the support, precipitated in ether, and analyzed as described above. As expected, after treating peptide **12**, which has an α-azido asparate residue, with 20% piperidine for 20 min, the dehydro product, **18**, and the α-azidopeptide, **24**, were the major products (see Figures S7 and S8 in the SI). After treatment of peptide **12** with 20% piperidine for 60 min, a single major peak was present in the HPLC chromatogram (see Figure S7 in the SI). HRESI$^+$MS analysis of the crude peptide mixture indicated that it consisted almost exclusively of peptide **18** along with a small amount of piperidine adduct **30** (Figure 2) (see Figure S8 in the SI).[13]

Only peptides **25−29** were obtained after treating peptides **13−17** with 20% piperidine for 20 or 60 min (see Figures S9−S28 in the SI). This indicates that it is the relatively greater acidity of the β-protons in the α-azidoaspartate residue that promotes the elimination reaction. A peptide bearing an α-azidoaspartate residue, very similar in structure to peptide **1**, was subjected to 20% piperidine in DMF several times during Lam et al.'s synthesis of Dap.[11] Although Lam et al. did not mention the formation of an azide elimination product(s), our results suggest that elimination of azide must have occurred to a considerable extent if standard Fmoc deprotection conditions (20% piperidine, 2 × 10 min) were employed.[14]



30

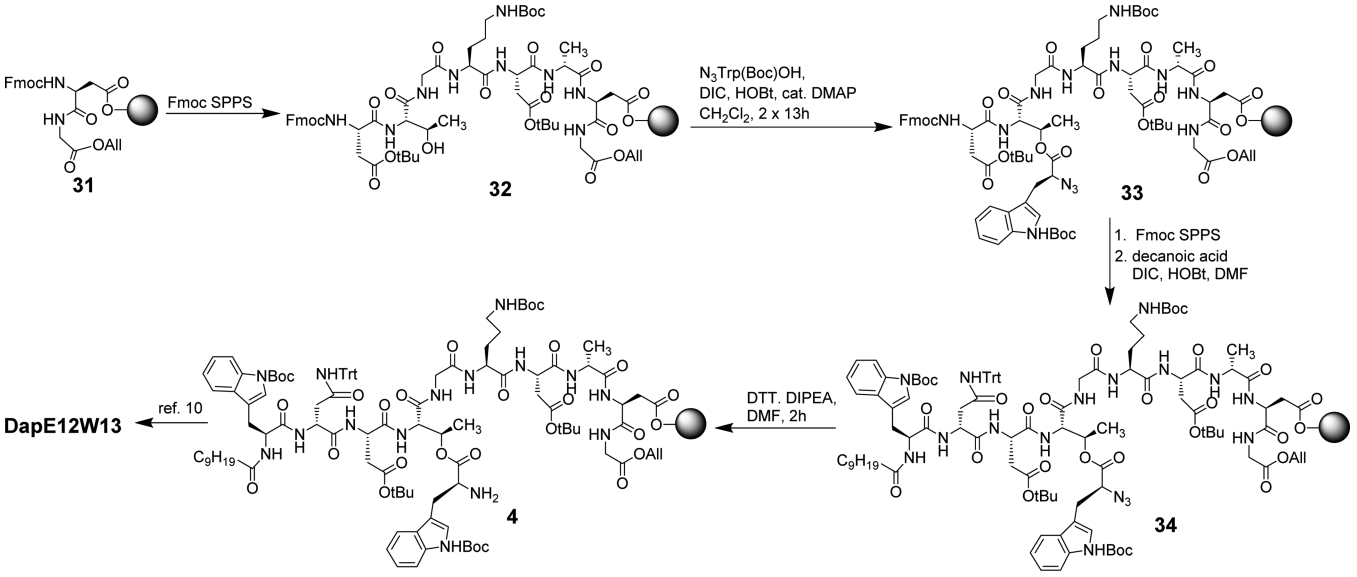
Figure 2. Piperidine adduct **30**.

To determine if other conditions that have been used to remove the Fmoc group during SPPS also lead to elimination of azide in peptides containing α-azidoaspartate, peptide **12** was treated with 2% DBU in DMF, 20% 2-methylpiperidine in DMF, or TBAF in THF. Treatment of peptide **12** with 2% DBU in DMF for 10 min resulted in complete conversion to peptide **18**. Treatment of peptide **12** with 20% 2-methylpiperidine in DMF for 20 min resulted in some formation of peptide **18** though peptide **24** was the major product. Treatment of peptide **12** with 20% 2-methylpiperidine in DMF for 60 min resulted in peptide **18** being the dominant product though peptide **24** was also still present. Treatment of peptide **12** with 1.1 equiv of TBAF in THF for 10 min resulted in some formation of peptide **18** though peptide **24** was the major product. Treatment of peptide **12** with 1.1 equiv of TBAF in THF for 30 min resulted in the formation of an almost equal mixture of peptides **18** and **24**. Treating peptide **12** with 50% morpholine in DMF for 1 h resulted in only a small amount of peptide **18** though the amount of peptide **18** increased to almost 30% after a 3 h treatment.

Since peptide **1** undergoes elimination of the azide ion upon removal of the Fmoc group, then route B outlined in Scheme 1 is not a practical route for the synthesis of Dap or Dap analogs containing an Asp residue at position 3. As an Asp residue at position 3 has been found to be important for biological activity,[15] then it is crucial that Dap analogs contain Asp at this

DOI: 10.1021/acs.joc.5b02882
J. Org. Chem. 2016, 81, 2624−2628

Scheme 4. A New Route to DapE12W13



position. However, as N_3Trp is stable to Fmoc deprotection conditions, we envisioned another approach to the synthesis of Dap analogs that employs only a single azido acid as illustrated in Scheme 4 for the synthesis of DapE12W13. This involved first preparing peptide **32** from dipeptide **31**[10] using standard Fmoc SPPS followed by the installation of N_3Trp(Boc)OH at position 13 to give peptide **33**. This was followed by the incorporation of residues 1 and 2 using Fmoc SPPS, utilizing 2-methylpiperidine (2-MP) for Fmoc removal, and then attachment of the decanoyl tail to give peptide **34**. Reduction of the azido group in **34** with DTT/DIPEA in DMF gave peptide **4**. The synthesis of Dap-E12W13 was then completed as previously described.[10] The DapE12W13 prepared in this manner exhibited identical biological activity as DapE12W13 prepared by our previous route (MIC of 1 μg/mL against *Bacillus subtilis* 1046 and 3 μg/mL against *B. subtilis* PY79 in the presence of 5 mM $CaCl_2$).[10] This new route to Dap analogs should be applicable to the synthesis of any Dap analogs that do not contain an Asp residue at position 13.[16]

In summary, we have shown that the orthogonality of α-azido acids to Fmoc chemistry is not universal. Peptides bearing α-azidoaspartate readily undergo azide elimination in the presence of piperidine and other reagents that have been used for removing Fmoc groups during SPPS. Although this side reaction was undesirable from the standpoint of Dap synthesis, it does provide a simple route to peptides containing dehydroaspartate, a residue that is found in some peptide natural products, such as the potent micotubule inhibitors, phomopsins A and B.[17,18] We also have developed an alternative solid-phase route to Dap analogs that requires only a single α-azido acid as demonstrated by the synthesis of DapE12W13. The synthesis of other Dap analogs using this new route is currently in progress and will be reported in due course.

EXPERIMENTAL SECTION

General. 2-Chlorotrityl chloride resin, all Fmoc amino acids, and coupling reagents used for peptide synthesis were purchased from commercial sources. The following amino acids were used for peptide synthesis: FmocAsp(tBu)OH, FmocAspOallyl, FmocGlyOH, Fmoc-Glu(tBu)OH, FmocTrp(Boc)OH, Fmoc-D-Ser(tBu)OH, Fmoc-Orn-(Boc)OH, FmocThrOH, Fmoc-D-AlaOH, Fmoc-D-Asn(Trt)OH. N_3-D-Asp(tBu)OH, N_3-Glu(tBu)OH, N_3-D-Asn(Trt)OH, N_3AlaOH, N_3PheOH, and N_3Trp(Boc)OH were prepared according to literature procedures.[3,5−8] ACS grade *N,N′*-dimethylformamide (DMF), 1,4-dioxane, 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. All peptide syntheses were performed manually using a rotary shaker for agitation.[19] Peptide **1** was prepared as previously described.[10] Peptides **12**−**17** were prepared starting from dipeptide **31**[10] using standard Fmoc SPPS (DIC/HOBt for couplings, 20% piperidine in DMF for Fmoc removal) and the corresponding azido acids for the *N*-terminal residues. Reversed-phase C18 columns (a 10 μM, 250 mm × 4.6 mm or 10 μM, 250 mm × 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. High resolution positive ion electrospray (ESI) mass spectra were obtained using an Orbitrap mass spectrometer. 1:1 CH_3CN/H_2O + 0.2% formic acid was used as a solvent. The biological activity of DapE12W13 was determined as previously described.[10]

Synthesis of DapE12W13. The Fmoc group in peptide **31**[10] (prepared from 33 mg of 2-chlorotrityl chloride (2-ClTrtCl) resin, substitution = 1.5 mmol/g, 50 μmoles), and in all subsequent peptides up to the synthesis of peptide **32**, was removed with 20% piperidine in DMF (1.5 mL, 1 × 5 min, 1 × 15 min). The resin was washed with DMF (3 × 3 min) and DCM (3 × 3 min) after each deprotection. All Fmoc amino acids were introduced using standard SPPS employing Fmoc amino acids (4 equiv), diisopropyl carbodiimide (DIC, 4 equiv), and HOBt (4 equiv) in DMF (1.5 mL) and 3−4 h coupling times. After each coupling the resin was washed with DMF (3 × 3 min) and DCM (3 × 3 min). The depsi bond in peptide **33** was made by activating N_3Trp(Boc)OH (10 equiv) with DIC (5 equiv) in dry DCM (2 mL) for 30 min. The precipitated urea byproduct was removed by filtration, and the filtrate was mixed with peptide **32** followed by addition of 4-dimethylaminopyridine (DMAP, 0.1 equiv). The reaction mixture was agitated for 13 h, then filtered, and washed with DMF (3 × 3 min) and DCM (3 × 3 min). This procedure was repeated using N_3Trp(Boc)OH (5 equiv), DIC (2.5 equiv), and DMAP (0.05 equiv) for another 13 h to give peptide **33**. The Fmoc group in peptide **33**, and in all subsequent peptides, was deprotected using 20% 2-methylpiperidine (2-MP) in DMF (1.5 mL, 3 × 10 min) followed by the usual washing procedure. The decanoyl tail was introduced using decanoic acid (4 equiv), and DIC (4 equiv)/HOBt (4 equiv) in DMF (1.5 mL) agitating for 13 h followed by the usual washing procedure. The azido group in peptide **34** was reduced using

DOI: 10.1021/acs.joc.5b02882
J. Org. Chem. 2016, 81, 2624−2628

2 M DTT/1 M DIPEA in DMF for 2 h.[1] The mixture was filtered, and the resin washed with DMF (4 × 5 mL) and DCM (4 × 5 mL). The allyl group of Gly10 was removed using 1,3-dimethylbarbituric acid (DMBA, 10 equiv) and cat. $Pd(PPh_3)_4$ (0.2 equiv) in DMF/DCM (1.5 mL, 1:3) for 1 h. The resin was washed with DCM (3 × 3 min), a 1.0% solution of sodium diethyldithiocarbamate trihydrate in DMF (3 × 3 min) to remove excess Pd catalyst, and then DCM (3 × 3 min) and DMF (3 × 3 min). Cyclization of peptide **35** to peptide **36** was performed using PyAOP (5 equiv), HOAt (5 equiv), and 2,4,6-collidine (10 equiv) in DMF/DCM (1:1 v/v/) (2 × 1.5 h). Crude DapE12W13 was obtained by treating peptide **36** 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 additional cleavage cocktail. The combined filtrates were concentrated one-fourth the original volume using a stream of N_2 gas. The peptide was precipitated by addition of cold ether (3 mL). The precipitated peptide was collected by centrifugation and washed with cold ether (2×). Pure DapE12W13 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 DapE12W13 were collected, concentrated by high vacuum, and lyophilized to give pure DapE12W13 as a white powder (1.9 mg, 2.3% yield based on resin loading). HRMS (ESI^+) calcd for $C_{72}H_{100}N_{17}O_{25}$ 1602.70708 $[M + H]^+$; found 1602.70941. See Figures S29 and S30 in the SI for the analytical HPLC chromatogram and mass spectral data for purified DapE12W13.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02882.

HPLC chromatograms and HRMS data for peptides **1** and **12**−**17** before and after being subjected to piperidine removal conditions. Analytical HPLC chromatogram and mass spectral data for DapE12W13 (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support of this work via a Discovery Grant (Grant No. 155283-2012) to S.D.T. We also thank the University of Waterloo for financial support.

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(13) No attempts were made to separate peptide **30** from peptide **18**.
(14) 20% piperidine was used for Fmoc deprotection during Lam et al.'s synthesis of Dap though deprotection times were not reported.
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(16) Although our studies have shown that only N_3Asp undergoes azide elimination, it is possible that this side reaction could also occur with peptides bearing α-azido acids that were not examined here (such as Kyn) or if peptides bearing terminal azido groups are subjected to piperidine for periods of time that are longer than those used in this study (>1 h). Hence, we recommend caution when subjecting peptides bearing any α-azido acid to piperidine especially for prolonged periods of time.
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