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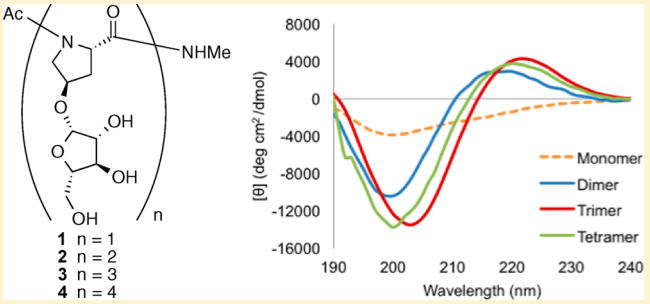
Synthesis of Oligomers of β-L-Arabinofuranosides of (4*R*)-4-Hydroxy-L-proline Relevant to the Mugwort Pollen Allergen, Art v 1

Ning Xie† and Carol M. Taylor*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, United States

Supporting Information

ABSTRACT: An efficient, convergent solution phase synthesis of monomer, dimer, trimer and tetramer of the β-L-arabinofuranosylated hydroxyproline (β-L-Araf-Hyp) glycocluster is described. This motif constitutes the carbohydrate-specific epitope of Art v 1, the major allergen of mugwort pollen. While a single monomeric unit was proposed at the outset, poor yields for the seemingly trivial steps of end-capping to replace protecting groups with *N*-terminal acetamides and *C*-terminal methyl amides led to the introduction of *N*-terminal, central and *C*-terminal β-L-Araf-Hyp building blocks. Dimer **2** was obtained in 60% yield by coupling of two monomers, followed by hydrogenolysis of benzyl ether protecting groups. Trimer **3** was obtained in 35% yield via a [2 + 1] coupling and tetramer **4** in 15% yield via a [2 + 2] fragment condensation. Circular dichroism spectra show that monomer **1** displays no organized structure, whereas compounds **2**−**4** show a strong negative band at 200 nm and a weak positive band at ∼220 mn, as is characteristic of the polyproline II helix.



INTRODUCTION

Pollen from *Artemisia vulgaris*, mugwort, is a major contributor to hay fever in Europe and North America. The major allergen is a heterogeneous glycoprotein known as Art v 1.[1,2] In a study involving 100 pediatric mugwort-allergic patients, 79% of the patients reacted with natural Art v 1, but only 39% showed reactivity with a recombinant allergen, signifying the role of post-translational modifications in allergenicity.[3] Leonard et al. identified a novel motif containing up to four contiguous, β-linked arabinofuranosides of hydroxyproline (β-L-Araf-Hyp) that showed significant binding to IgE from the serum of allergic patients. Moreover, this prolyl domain facilitates protein folding[4] and influences the conformation of the globular domain bearing other epitopes.[5,6]

There are 21 proline residues in the 53-residue *C*-terminal domain of Art v 1. Over 75% of the proline residues are hydroxylated, and 16−17 of these are adorned with β-L-arabinofuranosides. The glycoprotein was isolated in limited quantities. Alkaline hydrolysis of the protein leads to complex mixtures of amino acids, including the β-L-Araf-Hyp residue. The heterogeneous nature of this digest means it is unrealistic to isolate even miniscule amounts of pure amino acids and oligopeptides, thus providing an opportunity for chemical synthesis. In order to determine the minimal allergenic binding motif, and for potential downstream development of diagnostic tools, we set ourselves the target molecules **1**−**4** (Figure 1), representing monomer, dimer, trimer and tetramer of the β-L-Araf-Hyp moiety.

We define a glycocluster as an array of carbohydrate groups that are present in close proximity as a result of primary sequence or backbone conformation. The Art v 1 protein presents an example of the former, viz. a contiguous

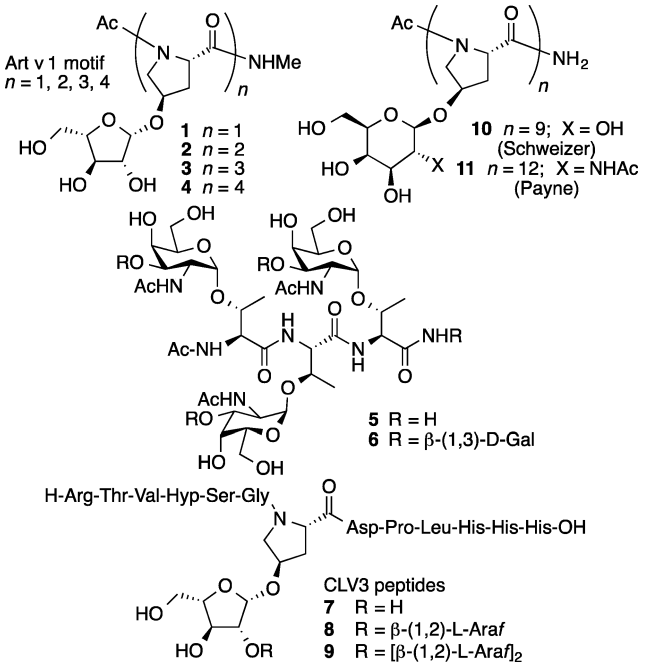


Figure 1. Relevant glycopeptide target molecules.

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glycocluster where two, three or four sequential β-L-arabinosylated hydroxyproline residues occur. The chemical synthesis of contiguous glycoclusters has been largely concerned with mucin-type motifs,[7] dystroglycan[8] and tumor antigens (e.g., **5** and **6**).[9,10] Most precedents for glycocluster assembly therefore relate to glycosylated serine and threonine residues, not hydroxyproline. Preformed glycosylated residues are linked via peptide bond formation. For example, Kunz and co-workers had used ethyldimethylaminopropyl carbodiimide hydrochloride (EDC) with hydroxybenzotriazole (HOBt) in their synthesis of MUC1 core glycopeptides.[11] During the assembly of the T_N and TF tumor-associated antigens, Danishefsky and co-workers employed 2-isobutoxy-1-isobutoxycarbonyl-1,2- dihydroquinoline (IIDQ) which worked well in the case of the T_N antigen, viz. compound **5**.[9]

The TF antigen presented more sterically demanding couplings, requiring 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo-[4,5b]pyridinium hexafluorophosphate 3-oxide (HATU)[12,13] and 1-hydroxy-7-azabenzotriazole (HOAt) to produce **6**.[14] Live, Barany and co-workers used glycosylated threonine building blocks in solid phase peptide synthesis (SPPS), either activated as pentafluorophenyl esters (in combination with HOBt and diisopropylethylamine in DMF) or acids activated by 2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU),[15] HOBt and diisopropylethylamine in *N*-methylpyrrolidone (NMP).[7,8]

Peptide coupling[16−18] to proline residues is naturally slower than to primary amino acids due to steric hindrance. The pyrrolidine nitrogen of (4*R*)-hydroxyproline (Hyp) is less nucleophilic than that of unsubstituted proline (Pro) due to the electronegative oxygen. Because electron withdrawal and steric hindrance are exacerbated upon glycosylation, forging the prolyl peptide bonds in the Art v 1 oligomers is a considerable challenge for synthesis. On the upside, proline carboxyl components are not susceptible to Cα-epimerization during peptide bond formation, widening the scope for selecting coupling reagents.

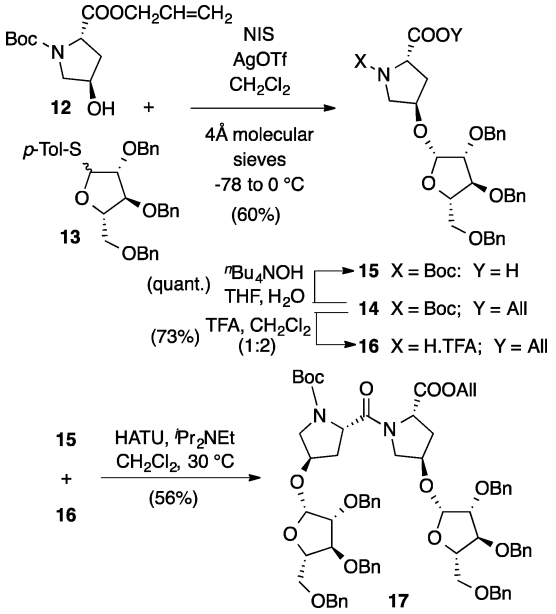
The CLAVATA3 peptide **9** (Figure 1), regulator of stem cell signaling in *Arabidopsis*, contains a single glycosylated Hyp residue at the center of a 13-residue peptide. In their recent SPPS of compounds **7**−**9**, Kaeothip et al. performed all couplings including, and following, the glycosylated Hyp residue manually.[19] While 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium hexafluorophosphate 3-oxide (HBTU) was used for earlier couplings, (1-cyano-2-ethoxy-2-oxo-ethylideneaminooxy)-dimethylaminomorpholinouronium hexafluorophospate (COMU) was used for the glycosylated residue and beyond. Coupling times varied depending on the steric demands of the nucleophile: 2 h for regular amino acids, 4 h for secondary amines and 16 h in the case of glycosylated building blocks. They employed 3 equiv of their Fmoc-protected glycosylated building block and isolated compounds **7**−**9** in 31−37% yield based on initial loading of the resin.

Two solid phase syntheses of oligomeric hydroxyproline glycosides have been reported. Schweizer and co-workers employed tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) for the assembly of a nonamer of (β-D-Gal)-Hyp, compound **10**.[20] The Fmoc-[β-(1,4)-D-Gal$(OAc)_4$]Hyp-OH building block was prepared in one step from commercially available D-galactose pentaacetate and Fmoc-Hyp-OH. The synthesis was conducted on 30 μmol scale, using 3 equiv of building block in each cycle; no yield was reported. Payne and co-workers prepared dodecapeptide **11** among a series of potential antifreeze peptides.[21] Their Fmoc-[β-(1,4)-D-GalNAc-$(OAc)_3$]Hyp-OH building block was prepared in seven steps and 25% overall yield from *N*-acetylgalactosamine. They used only 1.2 equiv of this building block in on-resin couplings mediated by HATU and diisopropylethylamine with extended reaction times (20 h vs 1 h for regular residues). To their credit, a 15% yield of glycopeptide **11** was isolated. On this background we embarked on the synthesis of the Art v 1 β-L-Araf-Hyp oligomers.

RESULTS AND DISCUSSION

In 2010 we reported the construction of the β-glycosidic linkage in monomer **14** and the formation of diglycodipeptide[22] **17** (Scheme 1).[23] The synthesis of the *cis*-1,2-glycosidic linkage

Scheme 1. Synthesis of the (β-Araf)Hyp Monomer and Dimer[23]



presented a considerable challenge, and ultimately conditions were identified that yielded **14** in 60% yield with a 4:1 ratio of anomers that could be separated by flash chromatography. In the 2010 paper, other strategies were discussed to direct the formation of the β-glycoside including conformationally restricted glycosyl donors[24,25] and intramolecular aglycone delivery.[26−29] An updated review and references to these approaches was provided by Kaetothip et al.[19]

The longest linear sequence, from L-arabinose, to produce monomer **14** is 7 steps and was achieved 28% overall yield. In order to optimize peptide coupling conditions in solution it was desirable to carefully monitor reactions and quantify and characterize compounds at each step. Moreover, given the number of residues relative to targets **10** and **11**, solid phase synthesis did not seem advantageous. Deprotection of the amine and carboxyl functionalities of **14** in parallel and coupling of the two resulting monomers afforded the dimer **17**. In our original communication the allyl ester was removed from **14** via Pd^0-mediated transfer to morpholine. In ongoing studies, impurities from this reaction were found difficult to eliminate. Alkaline hydrolysis of the allyl ester was more satisfactory

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(Scheme 1) and an updated procedure is provided for the production of **17**.

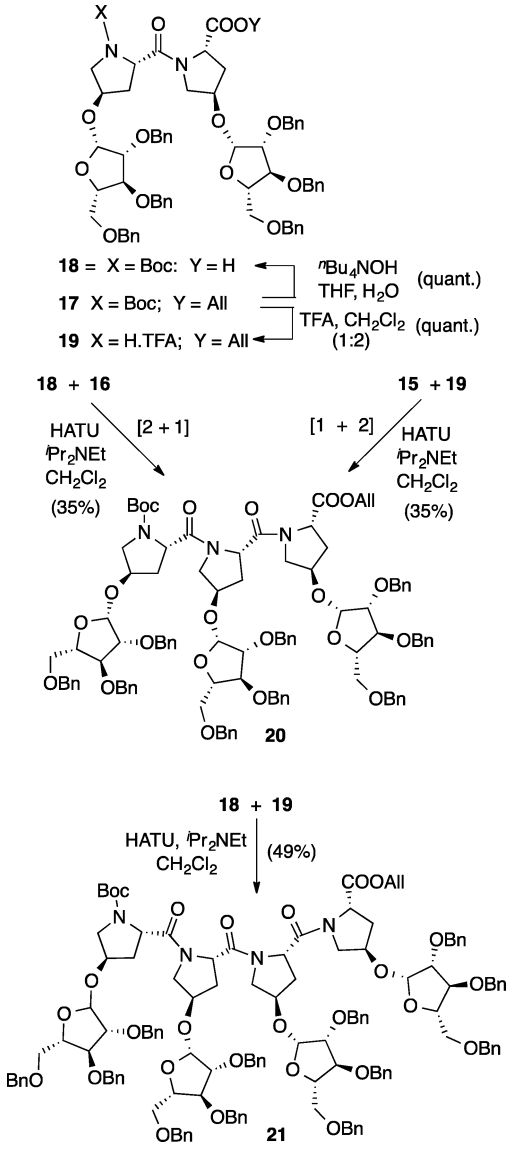
At the dimerization level, coupling conditions were investigated thoroughly. Bromo-*tris*-pyrrolidino-phosphonium-hexafluorphosphate (PyBrOP),[30] tetramethylfluoroformamidinium hexafluorophosphate (TFFH)[31] and HATU were studied, on the basis of the track record of these reagents in challenging couplings.[32] The first two of those reagents activate the carboxylic acid as a putative acyl halide, a bromide in the case of PyBrOP and a fluoride in the case of TFFH. Reaction mixtures were complex and there was considerable unreacted starting material, affording an 11% yield of **17** in the case of PyBrOP and 18−26% yields in the case of TFFH. HATU was the clear leader. Gentle heating of the reaction mixture was also advantageous.

To prepare a triglycotripeptide, two convergent approaches were possible: a [2 + 1] coupling or a [1 + 2] coupling, the former placing the greater steric burden in the carboxyl component and the latter in the amino component (Scheme 2). In practice, the two strategies both gave yields of ∼35%. In principle, three approaches were possible to the tetraglycotetrapeptide: [3 + 1], [2 + 2] and [1 + 3]. However, the increased value of the trimer relative to dimer made the [2 + 2] strategy the obvious choice.

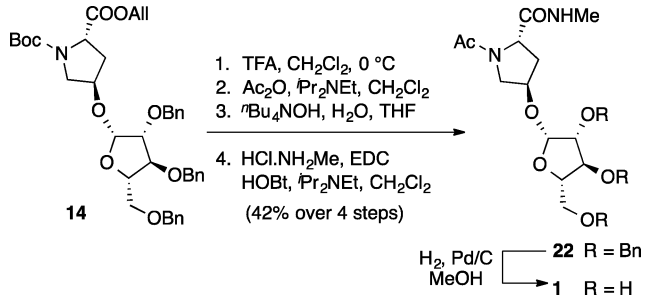
Glycopeptide assembly is generally conducted with protection of the carbohydrate hydroxyl groups as acetate esters.[33−37] This is largely due to the minimal number of steps required to produce these building blocks from peracetylated monosaccarides. Following glycopeptide assembly, the peracetylated compound is purified, treated with NaOMe/MeOH to effect cleavage of the acetate esters, and purified again. In the current context, the benzyl ethers were required to afford good stereoselectivity during glycosylation. The hydrolysis and acetylation of compound **14** was considered, in order to follow the general protocol. However, it turned out that the benzyl ethers could be cleaved at an advanced stage (vide infra) and that minimal purification was required at this final step. We therefore decided not to invoke additional protecting group manipulations in order to follow the norm.

The downstream utility of the oligoglycopeptides produced in this study depend on the feasibility of ligating them to other species, carrier proteins, fluorophores, etc. In the first instance, we sought simply to replace the *N*- and *C*-terminal protecting groups with amides to mimic the extended peptide backbone. These end-capped oligomers will be used for initial structural and biological studies. The seemingly trivial manipulations for end-capping the monomer to produce **22** are shown in Scheme 3. The benzyl ethers were cleaved by standard hydrogenolysis to give the monomer **1**.

Scheme 2. Trimer and Tetramer Assembly

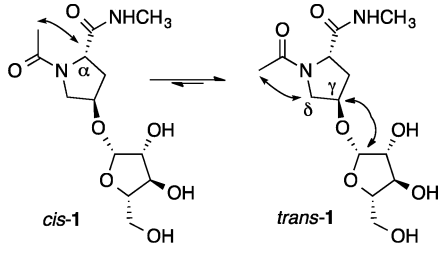


Scheme 3. End-Capping of Monomer



The ^{1}H and ^{13}C NMR spectra of compound **1** (CD_3OD, 400 MHz) were fully assigned on the basis of 2D experiments. On the time scale of the ^{1}H NMR acquisition, a 4:1 ratio of species was observed, reflecting *cis*−*trans* isomerization about the prolyl amide bond (Scheme 4). The NOESY spectrum showed a correlation between the acetamide CH_3 signal (δ 2.08 ppm)

Scheme 4. *cis*−*trans* Isomerism about the Prolyl Amide Bond of 1 with NOE Correlations Illustrated by Double-Headed Arrows



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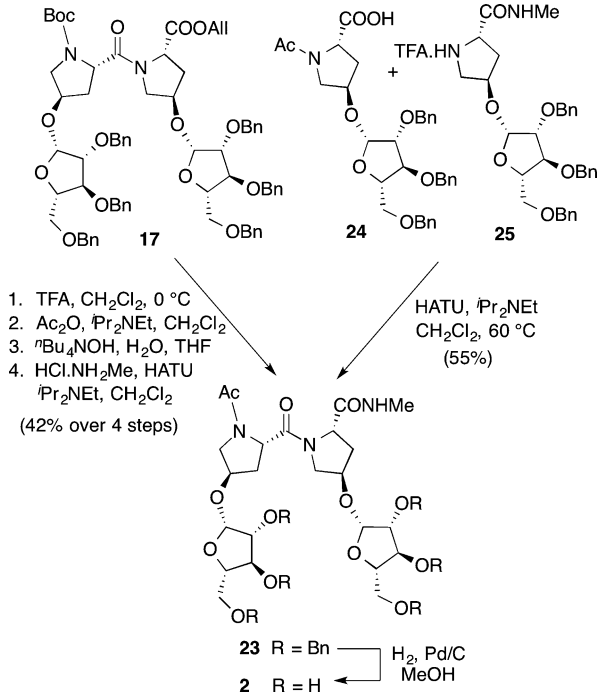
and the Hδ signal (δ 3.73 ppm) of the major species. Thus, the major species in solution adopts the *trans* conformation about the central amide bond (Scheme 4). A correlation is observed between signals corresponding to the acetamide CH_3 (δ 1.93 ppm) and Hα (δ 4.52 ppm) of the minor *cis* conformation An NOE was also observed between the signals corresponding to H1 of the arabinose (δ 4.99 ppm) and Hγ of the Hyp (δ 4.46 ppm).

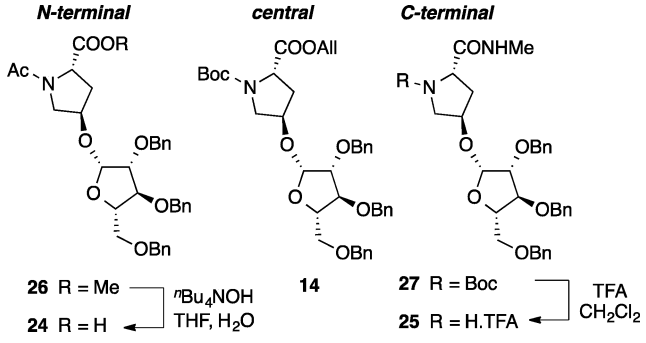
In the case of end-capping the dimer, the formation of the methyl amide proceeded in much lower yield using EDC/HOBt; better results were obtained with HATU, again with an overall yield of 42% for the end-capping manipulations to give **23** (Scheme 5). The end-capped trimer was obtained in only

Scheme 5. Linear and Convergent Approaches to End-Capped Dimer



18% yield and no end-capped tetramer was isolated. At this juncture, our strategy was revised to include end-caps prior to peptide coupling. Thus, two new building blocks were prepared (Scheme 6). Methyl ester **26** and *N*-Boc-protected **27** were fully characterized; each was converted to the requisite free acid or amine respectively prior to peptide coupling. This approach was more convergent and decreased the number of linear steps

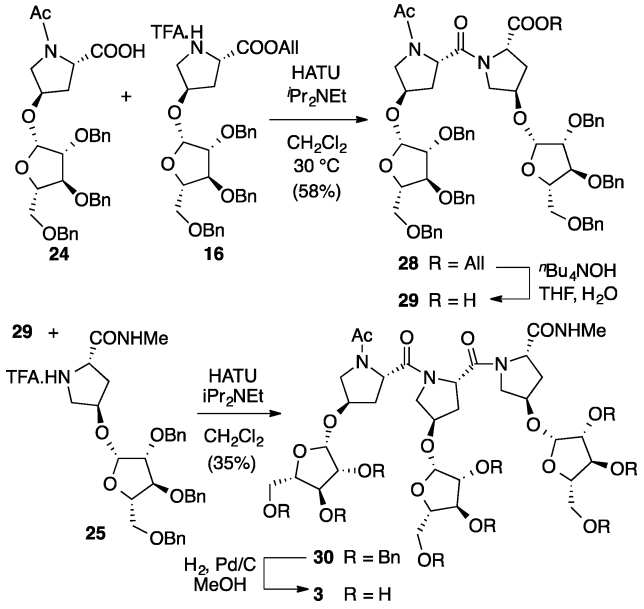
Scheme 6. Three Building Blocks for Oligomer Assembly



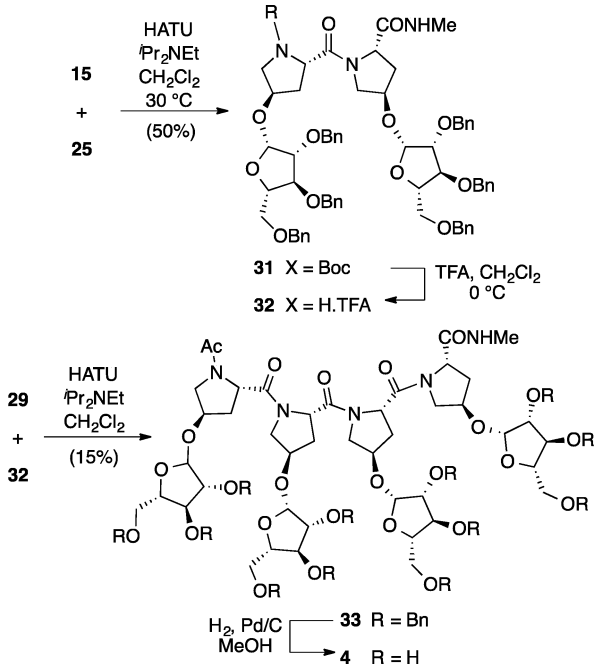
by four, in the assembly of each oligomer, as illustrated in Scheme 5 for the dimer **23**.

Assembly of the trimer and tetramer are illustrated in Schemes 7 and 8 respectively. Since the functionality in each

Scheme 7. Convergent Trimer Assembly



Scheme 8. Convergent Tetramer Assembly



building block is the same, no new signals appeared in the ^1H NMR spectrum to provide evidence for the elongation of the peptide. Integration of the signals due to the *C*-terminal $NHCH_3$ ($\sim\delta$ 2.75 ppm) group and H1, the anomeric protons of the arabinose moieties ($\sim\delta$ 5.00 ppm) gave a ratio that showed congruence with the number of residues incorporated (see Supporting Information). With the addition of each residue, the number of conformational isomers possible (4 for dimer **2**, 8 for trimer **3** and 16 for tetramer **4**) and the

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molecular mass of the peptides made it increasingly difficult to acquire meaningful ^{13}C NMR spectra.

Global debenzylation of all end-capped compounds was carried out under 1 atm of hydrogen in the presence of palladium on carbon. For the larger oligomers, high catalyst loadings were necessary. Trace impurities were removed by reverse extraction: the highly hydrophilic peptides were dissolved in water and washed with dichloromethane. The aqueous layer was then lyophilized to afford glycopeptides **1**−**4**. Both ^{1}H and ^{13}C NMR spectra were simplified dramatically following debenzylation and so we elected to characterize the final oligomers but not the perbenzylated intermediates that adopted a multitude of conformations. Chemical shifts were in agreement with those reported by Leonard et al. for the Art v 1 protein.[2] For the full length heterogeneous protein, they described an "average" structure that distinguished three sets of signals for β-L-Araf-Hyp in the ^{1}H NMR spectrum but a single ^{13}C NMR signal at δ 100.9 ppm for C1. Likewise, the $^{1}H-^{13}C$ HSQC experiment for monomer **1** showed a correlation between the H1 signal and a ^{13}C signal at 101.2 ppm. The anomeric carbon of β-arabinosides falls in the 100−105 ppm range, whereas for α-arabinosides the signal is further downfield.[36]

Natural hydroxyproline-rich glycoproteins have been shown to adopt a polyproline II (PPII) conformation, e.g., the GP1 protein of *Chlamydomonas reinhardtii*[38] and a cell wall protein from carrot root.[39] It was therefore not surprising that compounds **10** and **11** gave rise to stable PPII structures. Schweizer reported an increase in stability of **10** relative to its nonglycosylated counterpart,[20] and subsequent studies have attributed this increase in stability to stabilizing interactions between water and the carbohydrate backbone.[40] On the other hand, compound **11** did not show enhanced thermal stability compared to the nonglycosylated oligomer.[21] Interestingly, the peptides containing contiguous GalNAc residues (e.g., **11**) demonstrated an additional positive signal in their CD spectra below 200 nm.[21]

Circular dichroism spectra of the synthetic glycopeptides **1**−**4** were recorded in the far-ultraviolet region of the spectrum (190−240 nm) (Figure 2). As expected, the monomer is largely unordered. However, the diglycodipeptide Ac-[[β-L-Araf-Hyp)]$_2$-NHMe (**2**) displayed both a positive band (λ_{max} = 220 nm, [θ] = 2905 deg cm^2 dmol^{-1}) and a strong negative band (λ_{min} = 199 nm, [θ] = −10423 deg cm^2 dmol^{-1}) that is characteristic of the PPII conformation.[41] This is significant as previous studies have shown that at least three Pro residues are required for formation of the PPII helix.[42] It is tempting to suggest that the added bulk of the sugars might be contributing to their heightened structure. The CD spectra of both trimer and tetramer showed typical PPII-type helical structure. The relative band strength (ρ) is the ratio of the maximum positive ellipticity to the maximum negative ellipticity.[43] Pysh attributed the increase or decrease in ρ to conformational differences or changes to solvent and carbonyl backbone interactions.[44] A decreasing ρ value corresponds to an increasing solvent-carbonyl interaction. The relative band strength of our dimer, trimer, and tetramer are 0.28, 0.30, and 0.27 respectively. The variation is probably attributable to experimental error. These ρ-values are also in concordance with the ρ-value of the galactosylated hydroxyproline nonamer **5** reported by Owens (ρ = 0.29).[20]

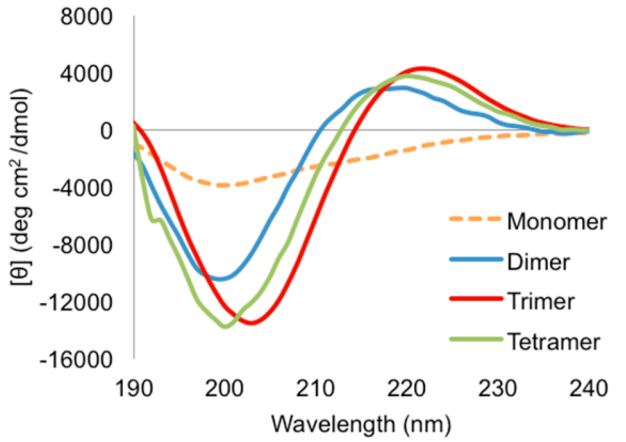

Figure 2. CD spectra of compounds **1** (monomer), **2** (dimer), **3** (trimer) and **4** (tetramer).

CONCLUSIONS

In summary, we have synthesized oligomers of the β-L-Araf-Hyp motif that occurs in the mugwort pollen allergen, Art v 1. This was extremely challenging and demonstrates that there are still problems to be addressed in the synthesis of complex peptides. NMR was used to characterize the monomer and dimer, but has limitations in revealing secondary structure due to the lack of amide protons along the backbone. Circular dichroism revealed distinct PPII character, even at the dimer level, indicating that glycosylation promotes and stabilizes helix formation. Ongoing studies will investigate the interaction of compounds **1**−**4** and derived compounds with biological systems to probe the molecular basis for the interaction of this glycocluster with relevant antibodies.

EXPERIMENTAL SECTION

General Note. The NMR spectra of these oligoprolines are complex. Signals in square brackets, [], refer to resolved signals of minor conformations arising from restricted rotation about the prolyl peptide bonds. ^{13}C NMR signals grouped together in braces, {}, are all the resonances ascribed to a type of ^{13}C nucleus, e.g., the Cβ of the proline residues in their various conformations. Where no such parentheses appear, this indicates either predominantly a single conformation, or signals for different conformations that could not be distinguished.

Acid 15. A 40% aqueous solution of tetrabutylammonium hydroxide (2.2 mL, 876 mg, 3.3 mmol, 3.0 equiv) was added to a solution of compound **14**[23] (766 mg, 1.1 mmol, 1.0 equiv) in THF (9 mL). The mixture was stirred at rt for 1.5 h. The solvent was evaporated and the residue dissolved in EtOAc (45 mL) and washed with 1 M HCl (50 mL). The aqueous layer was back extracted with EtOAc (25 mL). The organic layers were combined, filtered through $MgSO_4$, and concentrated to give acid **15** (quantitative) that was used in subsequent reactions without further purification: R_f 0.33(10:1 CH_2Cl_2−MeOH).

Amine 16. Trifluoroacetic acid (3.3 mL) was added to a solution of compound **14** (789 mg, 1.2 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and concentrated. The residue was purified by flash column chromatography, eluting with EtOAc and then flushing with 4:1 CH_2Cl_2−MeOH to give **16** as a light brown oil (581 mg, 72%): R_f 0.40 (2:1 EtOAc−hexanes).

Diglycodipeptide 17. Acid **15** (489 mg, 0.77 mmol, 1.2 equiv) and amine **16** (443 mg, 0.64 mmol, 1.0 equiv) were suspended in dry CH_2Cl_2 (14 mL) and the mixture was cooled to 0 °C. Diisopropylethylamine (405 μL, 305 mg, 2.3 mmol, 3.7 equiv) and HATU (380 mg, 1.0 mmol, 1.5 equiv) were added successively. The reaction was heated to 30 °C while stirring under N_2 overnight. The

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mixture was diluted with CH_2Cl_2 to a total volume of 70 mL, washed with 1 M HCl (2 × 40 mL), sat'd aq. $NaHCO_3$ (40 mL), and brine (40 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash column chromatography, eluting with 1.5:1 hexanes−EtOAc → 1.5:1 EtOAc−hexanes to give compound **17** as a light oil (431 mg, 56%). ^1H and ^{13}C NMR data were in agreement with those reported previously.[23]

Acid 18. A 40% aq. solution of tetrabutylammonium hydroxide (296 μL, 160 mg, 0.45 mmol, 3.0 equiv) was added to a solution of compound **17** (180 mg, 0.15 mmol, 1.0 equiv) in THF (3 mL). The mixture was stirred at rt under N_2 for 2 h. The solvent was evaporated, the residue dissolved in EtOAc (20 mL) and washed with 1 M HCl (15 mL). The aqueous layer was back-extracted with EtOAc (3 × 10 mL). The organic layers were combined, filtered through $MgSO_4$ and concentrated. The crude acid **18** was obtained in quantitative yield and submitted to the subsequent reactions without further purification: R_f 0.31 (10:1 CH_2Cl_2/MeOH).

Triglycotripeptide 20. Acid **18** (81 mg, 0.07 mmol, 1.0 equiv) and amine **16** (48 mg, 0.07 mmol, 1.0 equiv) and were suspended in dry CH_2Cl_2 (3 mL). Diisopropylethylamine (37 μL, 30 mg, 0.21 mmol, 3.0 equiv) and HATU (40 mg, 0.1 mmol, 1.5 equiv) were added successively. The mixture was stirred for 21 h at rt under N_2. The mixture was diluted with CH_2Cl_2 to a total volume of 25 mL, washed with 1 M HCl (2 × 20 mL), sat'd aq. $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was filtered through $MgSO_4$, concentrated and the residue purified by flash column chromatography, eluting with 1.5:1.0 hexanes−EtOAc → 1.5:1 EtOAc−hexanes →2:1 EtOAc−hexanes to give compound **20** as a light oil (42 mg, 35%): R_f 0.62 (2:1 EtOAc/Hex); $[\alpha]_D^{25}$ +42.6 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 [1.33] (s, 9H), 1.75−1.80 (m, 1H), 1.90−2.00 (m, 1H), 2.04−2.34 (m, 4H), 3.06−3.67 (m, 8H), 3.84−4.13 (m, 12H), 4.38−4.72 (m, 27H), 4.84 (d, *J* = 4.2 Hz, 0.5H), 4.87 (d, *J* = 4.1 Hz, 0.5H), 4.92 (d, *J* = 3.8, 0.5H), 5.09 (d, *J* = 2.8 Hz, 0.5H), 5.15 (d, *J* = 2.1 Hz, 0.5H), 5.16 (d, *J* = 4.0 Hz, 0.5H), 5.19−5.30 (m, 2H), 5.81−5.91 (m, 1H), 7.28−7.33 (m, 45H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.5, {34.9, 35.1, 35.5, 35.7, 36.0, 36.3 (Pro Cβ)}, {50.0, 50.1, 50.5, 50.7, 51.6, 51.9 (Pro Cδ)}, {56.6, 56.8, 56.9, 57.0, 57.9, 58.1 (Pro Cα)}, 65.7, {72.0, 72.2, 72.3, 73.1, 73.3 (Araf C5)}, {73.6, 73.7, 74.5 (Pro Cγ)}, {79.6, 79.7, 79.9, 80.3, 82.6, 82.8, 83.1, 83.3, 83.8, 84.1 (Araf C2,C3,C4; Boc 4 °C)}, {98.2, 98.6, 98.9, 101.0 (Araf C1)}, {118.4, 127.8, 128.0, 128.1, 128.3, 128.4 (Ar CH)}, 131.8, {137.5, 137.9, 138.0, 138.2 (Ar 4 °C)}, 154.2 [153.8], 170.8, 170.8, 171.0, 171.1, 171.4, 171.5; HRMS (ESI) calcd for $C_{101}H_{112}N_3O_{21}Na$ $(M + Na)^+$ 1726.7759, obsd 1726.7750.

Amine 19. Trifluoroacetic acid (1.5 mL) was added to a solution of diglycodipeptide **17** (168 mg, 0.14 mmol, 1 equiv) in dry CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and concentrated to give compound **19** in quantitative yield, which was used in subsequent steps without further purification: R_f 0.59 (9:1 CH_2Cl_2/MeOH).

Tetraglycotetrapeptide 21. Acid **18** (145 mg, 0.13 mmol, 1 equiv) and amine **19** (170 mg, 0.14 mmol, 1.1 equiv) were suspended in dry CH_2Cl_2 (5 mL). HATU (50 mg, 0.13 mmol, 1.0 equiv) and $^iPr_2NEt$ (110 μL, 82 mg, 0.63 mmol, 5.0 equiv) were added successively. The mixture was stirred for 18 h under N_2. Upon completion, the solvent was evaporated and the residue diluted with EtOAc (30 mL), washed with 1 M HCl (30 mL), sat'd aq. $NaHCO_3$ (30 mL), and brine (30 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 1.5:1 hexanes−EtOAc → 1:1 hexanes−EtOAc → 1:1.5 hexanes−EtOAc → 1:2 hexanes−EtOAc to give compound **21** as a light oil (138 mg, 49%): R_f 0.80 (2:1 EtOAc/Hex); $[\alpha]_D^{25}$ +42.2 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 [1.26] (s, 9H), 1.70−2.30 (m, 8H), 3.08−3.66 (m, 16H), 3.76−4.20 (m, 15H), 4.23−4.67 (m, 31H), 4.76−5.10 (m, 4H), 5.18 (d, *J* = 10.4 Hz, 1H), 5.27 (d, *J* = 17.3 Hz, 1H), 5.78−5.88 (m, 1H), 7.26−7.31 (m, 60H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.5 [29.7], {34.8, 35.0, 35.1, 35.3, 35.4, 35.9, 36.2 (Pro Cβ)}, [50.0, 50.1, 50.2, 50.4 50.5, 50.8, 51.6, 52.1 (Pro Cδ)}, {56.7, 56.8, 57.0, 57.1, 57.2, 57.9 (Pro Cα)}, 65.8, {72.4, 72.5, 72.6, 72.8, 72.9. 73.4, 73.6 (Araf C5)}, {73.8, 74.0, 74.1, 74.3 (Pro Cγ)}, {79.8, 79.9, 80.3, 80.4, 80.5, 80.6, 82.9, 83.1, 83.4, 83.7, 84.1, 84.5 (Araf C2,3,4; Boc 4 °C)}, {98.0, 98.1, 98.3, 98.6, 98.9, 99.2, 99.4, 100.8 (Araf C1)}, 118.5 [118.6], {127.9, 128.2, 128.3, 128.4, 128.6, 128.7, 128.9 (Ar CH)}, 131.8, {137.8, 138.0, 138.1, 138.3, 138.5 (Ar 4 °C)}, 154.2 [153.9], 170.4, 170.5, 170.7, 170.9, 171.4; HRMS (ESI) calcd for $C_{131}H_{146}N_4O_{27}Na$ $(M + Na)^+$ 2242.0072, obsd 2241.9979.

Ac-Hyp-OMe. Dicyclohexylcarbodiimide (119 mg, 0.58 mmol, 1.0 equiv) and DMAP (18 mg, 0.15 mmol, 0.25 equiv) were added sequentially to a suspension of Ac-Hyp-OH (100 mg, 0.58 mmol, 1.0 equiv) in dry MeOH (2 mL) and CH_2Cl_2 (2 mL). The mixture was stirred overnight at rt under N_2. The solvent was evaporated and the residue triturated with CH_2Cl_2 and filtered to remove dicyclohexylurea. The filtrate was concentrated and purified by flash column chromatography, eluting with CH_2Cl_2−MeOH (14:1 → 10:1) to give Ac-Hyp-OMe as an amorphous solid (71 mg, 66%): R_f 0.33 (10:1 CH_2Cl_2/MeOH); $[\alpha]_D^{25}$ −89.9 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 2.03−2.10 [2.15−2.23] (m, 1H), 2.07 [1.96] (s, 3H), 2.26−2.32 [2.41−2.47] (m, 1H), 3.51 (d, *J* = 11.2 Hz, 1H), 3.72 [3.77] (s, 3H), 3.74−3.79 (m, 1H), 4.52−4.57 [4.44−4.47] (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2 [21.6], 38.0 [39.7], 52.3 [52.7], 55.9 [54.5], 57.5 [58.8], 70.1 [68.5], 170.0 [170.7], 173.0 [172.7]; HRMS (ESI) calcd for $C_8H_{14}NO_4$ $(M + H)^+$ 188.0917, obsd 188.0919.

Compound 26. A solution of glycosyl donor **13**[23] (342 mg, 0.65 mmol, 1.0 equiv) and Ac-Hyp-OMe (124 mg, 0.66 mmol, 1.0 equiv) in dry CH_2Cl_2 (40 mL) was stirred with activated, powdered 4 Å molecular sieves (1.0 g) under N_2 for ∼30 min at rt. The suspension was cooled to −78 °C (acetone/dry ice) and then NIS (231 mg, 1.0 mmol, 1.5 equiv) and AgOTf (83 mg, 0.32 mmol, 0.5 equiv) were added. The reaction was gradually warmed to 0 °C over 1.5 h. The reaction was quenched by the addition of Et_3N (2 mL) and filtered. The filtrate was diluted with EtOAc (50 mL) and washed with 10% aqueous $Na_2S_2O_3$ (50 mL) and brine (50 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue, determined to be a 3:1 β:α ratio by NMR, was purified by column chromatography, eluting with 3:1 hexanes−EtOAc to afford **26** as a mixture of anomers (317 mg, 83%): R_f 0.34 (8:1 EtOAc/Hex); $[\alpha]_D^{25}$ +39.2 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 2.02−2.08 [2.10−2.17] (m, 1H), 2.03 [1.84] (s, 3H), 2.31−2.40 (m, 1H), 3.41 (dd, *J* = 10.6, 3.6 Hz, 1H), 3.49−3.52 (m, 2H), 3.71 [3.75] (s, 3H), 3.72−3.74 (m, 1H), 4.07−4.14 (m, 3H), 4.29−4.43 (m, 1H), 4.48−4.73 (m, 7H), 4.90 [4.98] (d, *J* = 3.6 [4.0] Hz, 1H), 7.27−7.36 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3 [21.5], 36.0 [38.0], 52.3 [52.7], 52.8 [50.6], 57.5 [58.8], 71.9 [72.2], 72.5 [72.4], 72.7 [73.1], 73.4 [73.3], 76.3, 80.1, 82.4, 84.2 [83.9], 100.5 [99.1], 127.8, 127.9, 128.0, 128.1 (2C), 128.4, 128.5, 128.6, 137.6, 137.8, 137.9, 138.1 (2C), 169.3, 172.7 [172.6]; HRMS (ESI) calcd for $C_{34}H_{40}NO_8$ $(M + H)^+$ 590.2748, obsd 590.2758.

Acid 24. A 40% aq. solution of tetrabutylammonium hydroxide (401 μL, 160 mg, 0.62 mmol, 3.0 equiv) was added to a solution of compound **26** (121 mg, 0.21 mmol, 1.0 equiv) in dry THF (4 mL) was and stirred at rt under N_2 for 1.5 h. The solvent was evaporated and the residue dissolved in EtOAc (25 mL), washed with 1 M HCl (25 mL). The aqueous layer was back-extracted with EtOAc (10 mL). The organic layers were combined, filtered through $MgSO_4$ and concentrated. The acid **24** was obtained in quantitative yield and used in subsequent steps without further purification: R_f 0.19 (9:1 CH_2Cl_2/MeOH).

Compound 27. HATU (50 mg, 0.13 mmol, 1.5 equiv) and triethylamine (62 μL, 45 mg, 0.45 mmol, 5 equiv) were added to a solution of acid **15** (56 mg, 0.09 mmol, 1 equiv) and methylamine hydrochloride (12 mg, 0.18 mmol, 2 equiv) in acetonitrile under an atmosphere of N_2. The mixture was stirred for 18 h and the solvent evaporated. The residue was diluted with EtOAc (30 mL), washed with 1 M HCl (30 mL) and aq. $NaHCO_3$ (30 mL), filtered through $MgSO_4$, and concentrated. The residue was purified by flash column chromatography, eluting with 8:1 EtOAc/Hex to give compound **27** as a light oil (40 mg, 70%): R_f 0.32 (8:1 EtOAc/Hex); $[\alpha]_D^{25}$ +19.6 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 [1.38] (s, 9H), 2.08 (br s, 1H), 2.43 [2.32] (br s, 1H), 2.78 [2.77] (s, 3H), 3.43−3.47

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[3.75−3.78] (m, 2H), 3..52 (app d, J = 3.3 Hz, 2H), 4.06−4.31 (m, 5H), 4.50−4.70 (m, 6H), 4.97 (s, 1H), 6.57 [5.74] (s, 1H), 7.26−7.36 (m, 15H); ^{13}C NMR (100 MHz) δ 26.3 [26.2], 28.5 [28.4], 35.1 [37.8], 51.6 [51.3], 58.9 [60.1], 72.3, 72.4, 73.3, 75.4 [73.7], 80.1, 80.6, 83.0 [82.9], 84.0 [83.8], 99.7 [98.8], 127.7, 127.8, 127.9, 128.1, 128.2, 128.4, 128.5, 128.6, 137.6, 138.0, 138.2, 155.6 [154.5], 172.2 [173.0]; HRMS (ESI) calcd for $C_{37}H_{46}N_2O_8$ $(M + H)^+$ 647.3327, obsd 647.3323.

Amine 25. Trifluoroacetic acid (1.6 mL) was added to a solution of compound **27** (199 mg, 0.31 mmol, 1 equiv) in dry CH_2Cl_2 (5 mL) at 0 °C under an atmosphere of N_2. The mixture was stirred for 3 h at 0 °C and concentrated. The free amine **25** was submitted to subsequent reactions without further purification: R_f 0.42 (9:1 CH_2Cl_2−MeOH).

End-Capped Perbenzylated Monomer 22. Diisopropylethylamine (19 μL, 14 mg, 0.11 mmol, 1.1 equiv), EDC (21 mg, 0.11 mmol, 1.1 equiv) and HOBt (17 mg, 0.13 mmol, 1.3 equiv) were added sequentially to a suspension of acid **24** (56 mg, 0.10 mmol, 1.0 equiv) and methylamine hydrochloride (8 mg, 0.10 mmol, 1.0 equiv) in dry CH_2Cl_2 (3 mL) at 0 °C under N_2. The ice bath was removed and the mixture left to stir overnight, diluted with CH_2Cl_2 (25 mL) and washed with 1 M HCl (25 mL), sat'd aq. $NaHCO_3$ (25 mL), and brine (25 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 19:1 CH_2Cl_2−MeOH to give compound **22** (35 mg, 61%) as an oil: R_f 0.49 (10:1 CH_2Cl_2/MeOH); $[\alpha]_D^{25}$ 20.6 (c 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 [1.84] (s, 3H), 2.04−2.07 (m, 1H), 2.53 (dt, J = 13.0, 5.1 Hz, 1H), 2.72 (2.77) (d, J = 4.8 Hz, 3H), 3.36 (3.43) (dd, J = 11.6 (12.7), 4.6 (4.1) Hz, 1H), 3.53−3.56 (m, 2H), 3.61 (dd, J = 10.7, 5.8 Hz, 1H), 4.07−4.11 (m, 3H), 4.39−4.44 (app. p, J = 5.4 Hz, 1H), 4.48−4.73 (m, 7H), 4.94 (4.99) (d, J = 3.8 Hz, 1H), 7.26−7.35 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5, 26.2, 34.2, 52.9, 58.37, 72.1, 72.5, 72.7, 73.3, 76.3, 80.1, 82.7, 84.2, 100.3, 127.7, 127.9, 128.0, 128.1, 128.4, 128.5, 128.5, 137.6, 137.9, 138.1, 170.5, 171.4; HRMS (ESI) calcd for $C_{34}H_{41}N_2O_7$ $(M + H)^+$ 589.2908, obsd 589.2913.

End-Capped Monomer 1. Palladium on carbon (10% w/w, 45 mg) was added to a solution of compound **22** (35 mg, 0.06 mmol) in MeOH (2 mL). The suspension was stirred under an atmosphere of H_2 gas for 18 h. The mixture was filtered through Celite and concentrated to give the compound **1** as an amorphous solid (19 mg, quantitative): $[\alpha]_D^{25}$ +25.6 (c 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.08 [1.93] (s, 3H), 2.03−2.10 [2.13−2.19] (m, 1H), 2.47−2.53 [2.59−2.64] (m, 1H), 2.73 [2.77] (s, 3H), 3.34 (s, 1H), 3.56 (dd, J = 11.6, 7.1 Hz, 1H), 3.68−3.78 (m, 3H), 3.85−3.91 (m, 1H), 3.96 (dd, J = 7.8, 4.6 Hz, 1H), 4.41 [4.51] (t, J = 8.0 [7.7] Hz, 1H), 4.45−4.47 (m, 1H), 4.99 [4.95] (d, J = 4.6 [4.5] Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 21.0 [20.2], 25.0 [25.1], 36.5 [38.4], 53.5 [51.7], 59.0 [60.1], 63.9 [63.8], 75.0 [74.4], 76.4, 77.2, 83.0, 101.2 [100.7], 171.2 [171.6], 173.7 [173.5]; HRMS (ESI) calcd for $C_{13}H_{23}N_2O_7$ $(M + H)^+$ 319.1500, obsd 319.1486.

End-Capped Diglycodipeptide 2. Acid **24** (94 mg, 0.14 mmol, 1 equiv) and amine **25** (104 mg, 0.14 mmol, 1 equiv) were dissolved in dry CH_2Cl_2 and stirred under N_2. HATU (93 mg, 0.21 mmol, 1.5 equiv) was added and the reaction stirred for 15 min, after which ^{i}Pr$_2$NEt (137 μL, 102 mg, 0.79 mmol, 5.5 equiv) was added to the mixture. The reaction was warmed to 30 °C and stirred for 18 h. The mixture was diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl (25 mL) and brine (25 mL), filtered through $MgSO_4$, and concentrated. The residue was purified by flash chromatography, eluting with 14:1 CH_2Cl_2−MeOH to give the dimer **23** as a cloudy oil (86 mg, 55%): R_f 0.49 (9:1 CH_2Cl_2/MeOH).

Palladium on carbon (20 mg, 10% w/w) was added to a portion of compound **23** (19 mg, 0.018 mmol) in MeOH (1 mL). The suspension was stirred under an atmosphere of H_2 gas for 18 h, then the mixture was filtered through Celite washing well with MeOH, and concentrated. The residue was dissolved in H_2O (10 mL) and washed with CH_2Cl_2 (3 × 10 mL) to remove organic impurities. The aqueous layer was lyophilized to give the fully deprotected dimer **2** (10 mg, quantitative) as an amorphous solid: $[\alpha]_D^{25}$ +30.8 (c 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.00−2.11 (m, 2H), 2.06 (s, 3H), 2.44−2.49 (m, 1H), 2.58−2.63 (m, 1H), 2.73 (s, 3H), 3.55−3.61 (m, 2H), 3.69−3.78 (m, 7H), 3.89−3.99 (m, 4H), 4.12 (d, J = 11.1 Hz, 1H), 4.46 (t, J = 8.1 Hz, 1H), 4.52 (br s, 1H), 4.76 (t, J = 8.1 Hz, 1H), 5.01 (d, J = 4.2 Hz, 1H), 5.04 (d, J = 4.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 20.8, 25.0, 35.6, 36.1, 52.4, 53.5, 56.9, 59.4, 74.9, 75.2, 76.2, 76.7, 77.3, 77.4, 83.0, 83.1, 100.9, 101.0, 170.7, 171.9, 173.4; HRMS (ESI+) calcd for $C_{23}H_{38}N_3O_{13}$ $(M + H)^+$ 564.2399, obsd 564.2390.

Triglycotripeptide 3. Acid **24** (157 mg, 0.27 mmol, 1 equiv) and amine **16** (184 mg, 0.27 mmol, 1 equiv) were dissolved in dry CH_2Cl_2 (7 mL) and stirred under N_2. HATU (156 mg, 0..41 mmol, 1.5 equiv) was added and the reaction stirred for 15 min, after which ^{i}Pr$_2$NEt (174 mg, 233 μL, 1.35 mmol, 5 equiv) was added to the mixture. The mixture was stirred for 19 h. The mixture was diluted with EtOAc (40 mL), washed with 1 M HCl (25 mL) and brine (25 mL), filtered through $MgSO_4$, and concentrated. The residue was purified by flash chromatography, eluting with 14:1 CH_2Cl_2−MeOH to give the dimer **28** as a cloudy oil (176 mg, 58%): R_f 0.37 (4:1 EtOAc−hexanes).

A 40% aq. solution of tetrabutylammonium hydroxide (305 μL, 121 mg, 0.47 mmol, 3 equiv) was added dropwise to a suspension of compound **28** (176 mg, 0.16 mmol, 1 equiv) in THF (5 mL) at 0 °C. The reaction was stirred, warming to rt over 1.5 h. The solvent was evaporated and the residue diluted with EtOAc (30 mL) and washed with 1 M HCl (30 mL). The aqueous layer was back-extracted with EtOAc (2 × 15 mL). The combined organic layers were filtered through $MgSO_4$ and concentrated to give the free acid **29** in quantitative yield: R_f 0.46 (10:1 CH_2Cl_2−MeOH).

The dipeptide acid **29** (156 mg, 0.14 mmol, 1.0 equiv) and amine **25** (94 mg, 0.14 mmol, 1.0 equiv) were dissolved in dry CH_2Cl_2 (5 mL) and stirred under N_2. HATU (82 mg, 0.22 mmol, 1.5 equiv) was added and the mixture stirred for 15 min, after which ^{i}Pr$_2$NEt (124 μL, 92 mg, 0.71 mmol, 5.0 equiv) was added. The mixture was stirred at rt for 21 h, diluted with EtOAc (25 mL), washed with 1 M HCl (25 mL), sat'd $NaHCO_3$ (25 mL) and brine (25 mL), filtered through $MgSO_4$ and concentrated. The residue was purified by flash column chromatography, eluting with 19:1 CH_2Cl_2/MeOH to give the protected trimer **30** as a light oil (81 mg, 35%): R_f 0.50 (10:1 CH_2Cl_2−MeOH).

Palladium on carbon (100 mg, 10% w/w) was added to a compound **30** (11 mg, 6.8 μmol) in MeOH (1.5 mL). The suspension was stirred under an atmosphere of H_2 for 24 h, filtered through Celite, washing well with MeOH, and concentrated. The residue was dissolved in H_2O (10 mL) and washed with CH_2Cl_2 (3 × 10 mL) to remove organic impurities. The aqueous layer was lyophilized to give the fully deprotected trimer **3** (5.5 mg, quantitative) as an amorphous solid: $[\alpha]_D^{25}$ −17.4 (c 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.01−2.17 (m, 3H), 2.11 (s, 3H), 2.47−2.66 (m, 3H), 2.75 (s, 3H), 3.58−3.66 (m, 3H), 3.69−3.78 (m, 10H), 3.86−4.01 (m, 6H), 4.15 (d, J = 11.5 Hz, 1H), 4.26 (d, J = 11.1 Hz, 1H), 4.48 (t, J = 8.5 Hz, 2H), 4.53 (br s, 2H), 4.60 (br s, 1H), 4.79 (t, J = 8.3 Hz, 1H), 5.02 (d, J = 4.4 Hz, 1H), 5.04 (app. t, J = 4.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 24.1, 28.6, 37.5, 37.7, 38.5, 55.4, 55.5, 56.4, 59.6, 60.2, 62.4, 63.2, 65.9, 66.0, 72.5, 74.4, 77.1, 77.2, 78.8, 78.9, 79.0, 79.3, 84.6, 84.7, 102.7, 102.8, 102.9, 173.8, 174.6, 174.8, 175.5, 176.5; HRMS (ESI+) calcd for $C_{33}H_{52}N_4O_{19}$ $(M + H)^+$ 809.3299, obsd 809.3314.

Tetraglycotetrapeptide 4. Acid **15** (155 mg, 0.24 mmol, 1.0 equiv) and amine **25** (161 mg, 0.24 mmol, 1.0 equiv) were suspended in dry CH_2Cl_2 (5 mL) and stirred under N_2. HATU (112 mg, 0.29 mmol, 1.2 equiv) and ^{i}Pr$_2$NEt (213 μL, 158 mg, 1.2 mmol, 5 equiv) were added sequentially. The mixture was stirred for 24 h. The mixture was diluted with EtOAc (50 mL) and washed with 5% citric acid (50 mL) and brine (50 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography eluting with 28:1 CH_2Cl_2−MeOH to give compound **31** as a light oil (138 mg, 49%). Trifluoroacetic acid (0.75 mL) was added to the residue suspended in CH_2Cl_2 (1.5 mL) at 0 °C. The mixture was stirred for 3 h at 0 °C and the solvent was evaporated to give the free amine **32** in quantitative yield: R_f 0.33 (10:1 CH_2Cl_2−MeOH).

Acid **29** (47 mg, 0.04 mmol, 1.0 equiv) and amine **32** (71 mg, 0.06 mmol, 1.4 equiv) were dissolved in dry CH_2Cl_2 (1.75 mL) and stirred

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under N_2. HATU (17 mg, 0.04 mmol, 1.0 equiv) was added and the reaction stirred for 15 min, after which diisopropylethylamine (124 μL, 92 mg, 0.16 mmol, 4.0 equiv) was added. The mixture was stirred at rt for 20 h, diluted with EtOAc (25 mL), washed with 1 M HCl (25 mL) and brine (25 mL), filtered through $MgSO_4$ and concentrated. The residue was purified by flash column chromatography, eluting with 19:1 CH_2Cl_2−MeOH to give the protected tetramer **33** as a light oil (14 mg, 15%): R_f 0.42 (9:1 CH_2Cl_2−MeOH).

Palladium on carbon (25 mg, 10% w/w) was added to a solution of compound **33** (3 mg, 1.4 μmol) in MeOH (0.6 mL). The suspension was stirred under an atmosphere of H_2 gas for 24 h. The mixture was filtered through Celite, washing well with MeOH, and concentrated. The residue was dissolved in H_2O (10 mL) and washed with CH_2Cl_2 (3 × 10 mL) to remove organic impurities. The aqueous layer was lyophilized to give the fully deprotected tetramer **4** (1,5 mg, quantitative) as an amorphous solid: $[\alpha]_D^{25}$ +60.8 (*c* 0.075, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.04−2.19 (m, 4H), 2.09 (s, 3H), 2.47−2.67 (m, 4H), 2.76 (s, 3H), 3.59−3.65 (m, 4H), 3.73−3.79 (m, 12H), 3.86−4.01 (m, 8H), 4.15 (app d, *J* = 11.5 Hz, 3H), 4.50 (t, *J* = 8.0 Hz, 4H), 4.56 (br s, 4H), 5.04 (d, *J* = 4.5 Hz, 2H), 5.04 (d, *J* = 4.3 Hz, 4H); HRMS (ESI+) calcd for $C_{43}H_{67}N_5O_{25}Na$ $(M + Na)^+$ 1076.4017, obsd 1076.3992.

CD Spectroscopy. Compounds **1**−**4** were each lyophilized for 24 h prior to dilution to a concentration of 0.4 mM with purified water. The pH of the samples were measured at rt and found to be 6.80, 7.38, 8.79, and 9.48 for compounds **1**, **2**, **3**, and **4** respectively. For analysis, 175 μL of the sample was loaded into a quartz cell with a path length of 0.1 cm. The CD spectra were recorded at a scan rate of 20 nm per min, data pitch of 1.0 nm, and bandwidth of 2.0 nm. The accumulation of three scans was averaged for each sample, after which a blank of the solvent was subtracted. The CD signal was converted to molar ellipticity per mean residue ([θ]) and the data was smoothed by Savitzky−Golay algorithm. For Ac-([β-L-Araf]Hyp)$_2$-NHMe (**2**), positive band (λ_{max} = 220 nm, [θ] = 2905 deg cm^2 dmol^{-1}) and a negative band (λ_{min} = 199 nm, [θ] = −10423 deg cm^2 dmol^{-1}); trimer Ac-([β-L-Araf]Hyp)$_3$-NHMe (**3**), positive band at 222 nm ([θ] = 4207 deg cm^2 dmol^{-1}) and a negative maxima at 203 nm ([θ] = −13352 deg cm^2 dmol^{-1}); and Ac-([β-L-Araf]Hyp)$_4$-NHMe (**4**), positive band at 220 nm ([θ] = 3704 deg cm^2 dmol^{-1}) and a negative maxima at 200 nm ([θ] = −13816 deg cm^2 dmol^{-1}).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Selected NMR spectra are provided for compounds **20**, **21**, Ac-Hyp-OMe, **26**, **27**, **22**, **1**, **2**, **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: cmtaylor@lsu.edu.

Present Address

†TumorEnd LLC, Baton Rouge, Louisiana 70808, United States.

Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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