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Soluble Non-Cross-Linked Poly(norbornene) Supports for Peptide Synthesis with Minimal Reagents

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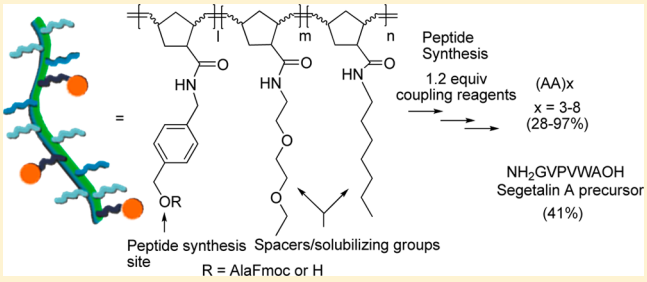
Supporting Information

ABSTRACT: Solid-phase peptide synthesis has been an attractive method for synthesizing peptides because it is quick and can be automated. The heterogeneous reaction medium in solid-phase peptide synthesis necessitates the use of large equivalents of reagents to drive the reactions to completion. Peptide synthesis using soluble, yet isolable, supports is an attractive alternative to solid-phase peptide synthesis. Reported herein is a soluble poly(norbornene)-derived support containing multiple attachment sites for high loading capacities and solubilizing oligoether/alkyl groups. The Ala-attached support has been used to synthesize tri- to octapeptides in 28 to 97% yields using only 1.2 equiv of amino acids and coupling reagents. The acyclic hexapeptide precursor to natural product segatalin A was synthesized in 41% yield on the support using one-eighth of the equivalents of coupling reagents compared to that in reported procedures. The support could be recovered in up to 98% yield after peptide synthesis, and the recovered support was utilized to synthesize tri- and tetrapeptides that contain amino acids other than Ala at the C-terminus in ca. 80% yields.



INTRODUCTION

Peptides have been used as therapeutic agents, and their self-assembly is being widely exploited for the development of interesting materials. There is a growing need for effective methods of synthesizing peptides. Peptides were originally synthesized in solution, where chromatographic separation was typically required after each coupling step.[1] Synthesis of large peptides in solution was extremely challenging due to their lower solubility in the reaction medium. Peptide synthesis has become less time-consuming since the development of solid-phase peptide synthesis (SPPS) by Merrifield.[2] In SPPS, the peptide is grown on an insoluble resin, whereas the reagents are solubilized in the reaction medium.[3−5] The growing peptide can be readily recovered by filtration in SPPS, which prevents the need for time-consuming chromatographic purification after each coupling step. However, the reaction medium is heterogeneous in SPPS, which diminishes the reactivity of the supported amino acids or peptides. Typically, excess of coupling reagents and amino acids are added to drive the coupling reactions to completion. Liquid-phase peptide synthesis (LPPS), which utilizes soluble supports for attaching peptides, is an attractive alternative because it provides a homogeneous peptide synthesis medium.[6−9] Fluorous supports, where peptides can be separated using simple extraction or fluorous phase columns, have been used in LPPS.[10−13] However, they have not been explored for the synthesis of large peptides, and, additionally, the use of fluorous solvents and supports is not very cost-effective. Ionic-liquid supports, where the reagents can be separated via phase separation, have emerged as an attractive alternative to fluorous supports.[14−19] Hydrophobic supports that can be isolated from the reaction medium via precipitation have offered good peptide loading capacities and have been efficiently used for synthesis of peptides.[20−24] Recently, the group-assisted purification (GAP) approach has been used to synthesize peptides.[25] In this approach, a group that can be readily isolated as a precipitate is attached at one end of the growing peptide. Soluble polymers that can be readily separated from the reaction medium by precipitation are the closest LPPS analogues to SPPS supports.[8,26,27] Poly(ethylene glycol) (PEG) supports have been found to be efficient supports for peptide synthesis because they provide a localized polar environment to the growing peptide, which improves its solubility in the reaction medium.[28−32] Loading capacities of PEG polymers normally vary from 0.1 to 0.5 mmol/g, since each PEG polymer has only one site for attaching peptides. We have recently developed soluble poly(norbornene)-derived supports that are reusable and have loading capacities of 0.6−1.1 mmol/g. As a proof of concept, an alanine-attached support was used for the synthesis of tripeptides in moderate yields of 41−66%.[33]

Herein, we optimize the efficiency of alanine-attached poly(norbornene) support **1** for synthesis of tripeptides (97% yield) and expand its scope for synthesizing larger oligopeptides (Figure 1). Support **1** has also been used for synthesizing the linear precursor to natural product segetalin A in 41% overall

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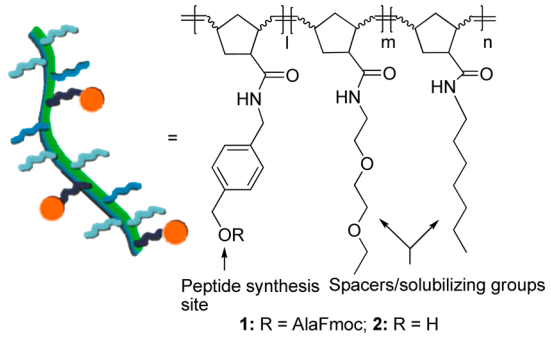
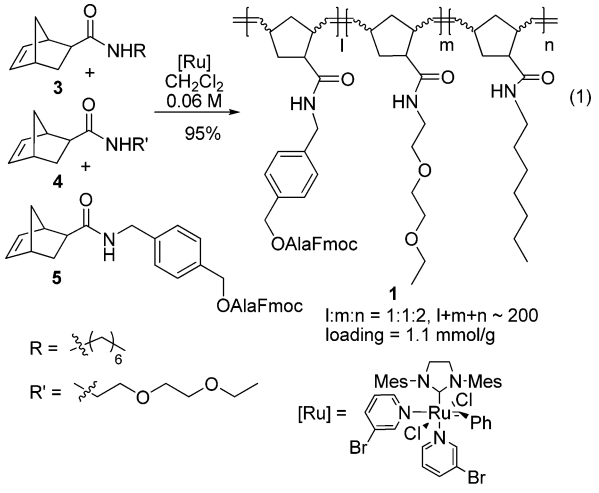


Figure 1. Supports for peptide synthesis.

yield. Our supports utilize only 1.2 equiv of amino acids and coupling reagents, which is one-eighth lower than reported procedures for the synthesis of the segetalin A precursor. The versatility of our support has also been improved for synthesizing peptides other than those that contain alanine at the C-terminus. As a proof of concept, tri- and tetrapeptides have been synthesized using support **2** in 82 and 78% yields, respectively. Although the supports have been efficiently used for the synthesis of a variety of peptides, their use for longer oligopeptides remains to be tested.

RESULTS AND DISCUSSION

The alanine-attached support was synthesized starting from the corresponding norbornene monomers **3**–**5** (eq 1).[33] Alanine



was loaded onto the monomer prior to polymerization in order to ensure complete loading of Ala on the attachment site. Ala-attached polymer **1** could be readily recovered after the polymerization reaction as a precipitate with diethyl ether. The ^{1}H NMR spectrum of polymer **1**, recorded in the presence of a known amount of 1,1,2,2-tetrachloroethane (TCE), indicated that 1 g of polymer **1** contained 1.1 mmol of attachment sites. Such high loading capacities are not typically observed in PEG-based soluble supports. Support **1** was found to be highly soluble in organic solvents such as dicholoromethane, THF, and DMF. Attempts made to synthesize tripeptides using support **1** resulted in moderate yields of 41–66%.[33] Therefore, we wished to improve the utility of these supports with high loading capacities for peptide synthesis.

Support **1** was highly soluble in the reaction medium (Figure 2a) and could be readily precipitated using diethyl ether

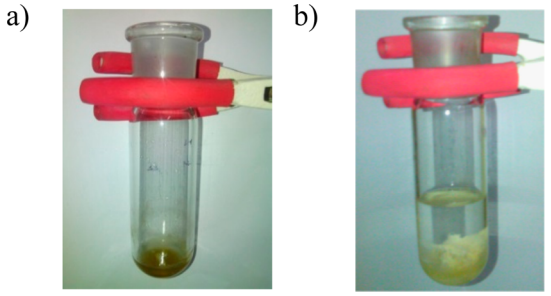
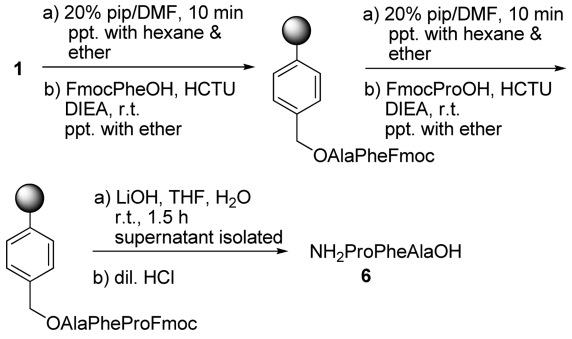


Figure 2. Pictures of a (a) homogeneous reaction mixture and (b) precipitate of peptide-attached polymer obtained after adding diethyl ether.

(Figure 2b). Furthermore, the pure support could be isolated in 92–98% yield after removal of tripeptides, indicating that the loss of support did not account for the lower yields of tripeptides.

In order to ensure that the coupling reactions were going to completion during peptide synthesis, their reaction times were optimized for the synthesis of tripeptide **6** using support **1** (Scheme 1). A slight increase in yields from 38 to 43% was

Scheme 1. Tripeptide Synthesis



observed when the coupling reaction time was changed from 1 to 2 h (Table 1). However, a further increase in reaction time

Table 1. Optimization of Reaction Time for Coupling Reactions

no.	time[a] (h)	yield of **6** (%)[b]
1	1	38
2	2	43
3	4	42

[a]Reaction time for coupling steps in Scheme 1. [b]Isolated by RP-HPLC.

to 4 h did not significantly change the yield. Therefore, we concluded that reaction times longer than 2–3 h did not improve the yields for peptide synthesis.

The lower yields for tripeptides could be attributed to the formation of the diketopiperazine (DKP) after deprotection of the dipeptide. The higher reactivity of the amino acids attached to our soluble supports in comparison to those attached to insoluble resins would presumably facilitate DKP formation. In order to exploit the reactivity of amino acids on our support and to prevent diketopiperazine formation, we used a combination approach for peptide synthesis.[34,35] In this approach, Fmoc-protected dipeptides **11** were synthesized in

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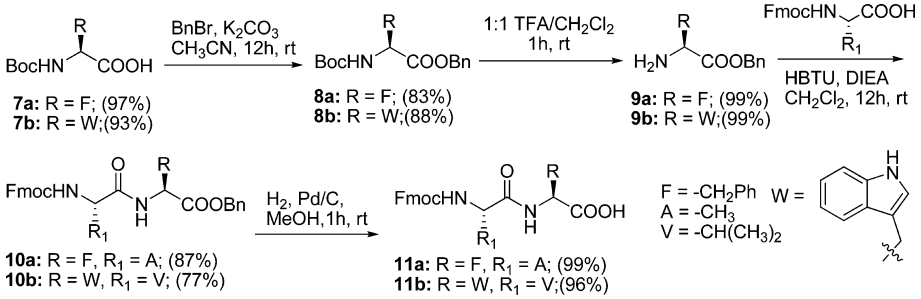
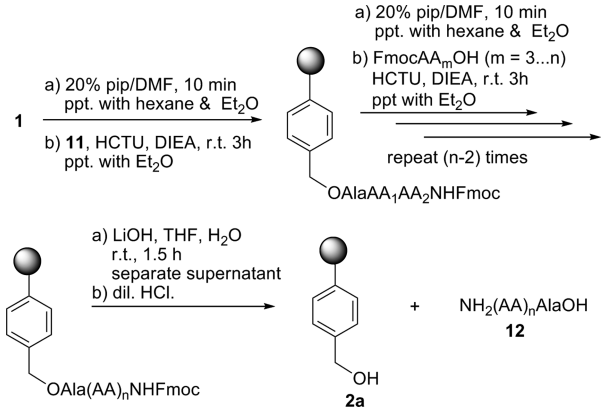
Scheme 2. Synthesis of Dipeptide 11



solution, as shown in Scheme 2, prior to peptide synthesis on support **1**.

Peptide synthesis was carried out using the combination approach as shown in Scheme 3. Support **1** was treated with

Scheme 3. Combination Approach for Peptide Synthesis



20% piperidine in DMF to remove the Fmoc protecting group. Hexane and diethyl ether were sequentially added to precipitate the support containing the free amine. Dipeptides **11** were loaded onto the support containing the free amine in the presence of HCTU and base. The resultant tripeptide-attached support was readily obtained as a precipitate upon addition of diethyl ether. The tripeptide was cleaved from the resin via base hydrolysis and could be readily recovered from the supernatant. It is to be noted that base hydrolysis might not be compatible with residues such as Ser, Thr, and Cys. The combination approach circumvented DKP formation, and the tripeptide was obtained in 97% yield (Table 2, entry 1). This approach was expanded to synthesize tetra- to octapeptides (entries 2–10, Table 2) in good to excellent yields. In all cases, the reaction medium was homogeneous, and the peptide-attached support could be readily isolated after each step. As before, free hydroxyl-containing support **2** could be isolated in 92–98% yield, with high purity. The HPLC profile of the crude reaction mixture in all cases showed the desired peptide as the major product. A representative profile of crude octapeptide **12h** is shown in Figure 3.

Table 2. Peptides Synthesized following Scheme 3

no.	peptide **12**	yield (%)[a]
1	**12a**: AlaPheAla	97
2	**12b**: ProAlaPheAla	82
3	**12c**: IleAlaPheAla	79
4	**12d**: PheProAlaPheAla	65
5	**12e**: ProMetAlaPheAla	60
6	**12f**: ProValAlaPheAla	62
7	**12g**: GlyProValValTrpAla	44
8	**12h**: PheProGlyMetAlaAlaPheAla	33
9	**12i**: IleProMetValGlyAlaPheAla	31
10	**12j**: PheGlyProAlaIleAlaPheAla	28

[a]Isolated using RP-HPLC.

The combination approach was also used for synthesizing the linear precursor, **13**, to natural product segetalin A (Scheme 4). Segetalin A **14**, a cyclic hexapeptide isolated from the seeds of *Vaccaria segetalis*, has been found to possess estrogen-like activity.[36,37] To date, there are two reports describing the synthesis of this peptide using FmocAlaSasrin or FmocGly-Sasrin supports.[38,39] The peptide couplings were carried out on these SPPS resins using up to 10 equiv of coupling reagents and amino acids. Support **1** was used in the presence of only 1.2 equiv of coupling reagents/amino acids to obtain 41% of segetalin precursor **13** (after isolation by RP-HPLC). Hence, support **1** could be an attractive alternative to the Sasrin resin for synthesizing peptides such as **13**, as it would minimize the amounts of unused amino acids and coupling reagents typically seen with SPPS. The HPLC profile of crude hexapeptide **13** showed mainly the peak corresponding to peptide **13**. The absence of smaller peptides in the HPLC profile indicated that the coupling reactions were going to completion without a large excess of amino acids and coupling reagents (Figure 4).

One can envision expanding the scope of the current approach for synthesizing peptides containing other amino acids at the C-terminus by polymerizing the monomer attached with the requisite amino acid-attached support following eq 1. However, we followed a more versatile route involving the synthesis of polymer **2b** containing the free hydroxyl group, followed by the attachment of the requisite amino acid (Scheme 5). Support **2b** can be readily obtained from the corresponding monomers, **3**, **4**, and **15**. We used this approach, as we were previously successful in efficiently loading a variety of amino acids onto support **2b** to give loaded support **16**.[33] Support **16** was soluble in solvents such as THF and DMF. Upon using support **16** for peptide synthesis, we observed that the solubility of the support was reduced drastically after the first deprotection step (Scheme 5). Even in the case of the alanine-loaded support, which we believed would be analogous to support **1**, we did not notice an improvement in the support's solubility.

We initially attributed the lower solubility of the supports to the presence of unreacted hydroxyl groups at the attachment sites during peptide synthesis. However, the high loading capacities of amino acid-attached supports **16** indicated that the number of unreacted hydroxyl groups was minimal.[33] We

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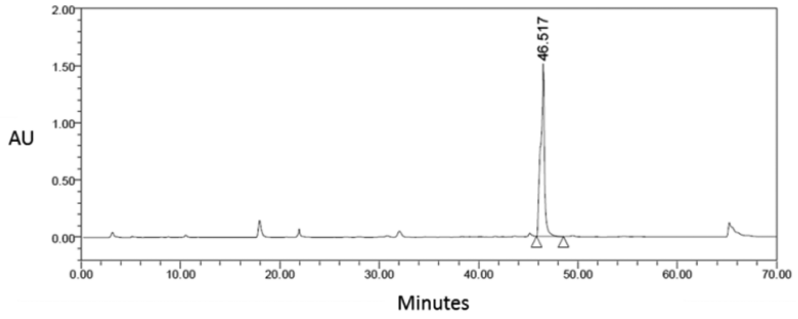
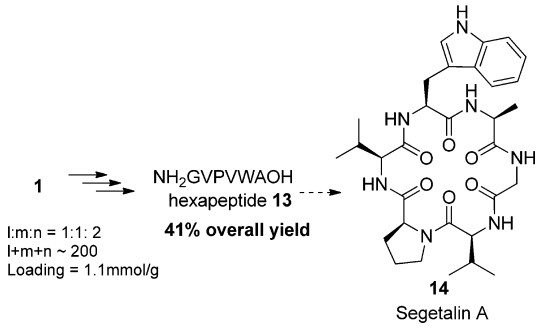


Figure 3. HPLC of crude **12h** obtained after cleavage from support **1**.

Scheme 4. Synthesis of Segetalin A Precursor



currently believe that the superior solubility of support **1** could be due to an optimal spacing of the attachment sites in this polymer (Figure 5a). The presence of a bulky Fmoc-Ala group during polymerization would ensure that the attachment sites are spaced regularly in support **1**. This would prevent clustering of side chains (Figure 5b), which could contribute to a marked difference in the solubility properties of the polymer. This hypothesis is also corroborated by the fact that recovered support **2a** is soluble in solvents such as dichloromethane, THF, and DMF. Furthermore, support **2a** is also soluble in the reaction medium used for the synthesis of peptides that contain Ala at the C-terminus.[33,40]

Therefore, we attempted to use the recovered support **2a** instead of support **2b** for synthesizing peptides containing amino acids other than Ala at the C-terminus. As a proof of concept, phenyl alanine and methionine were loaded onto support **2a** following Scheme 5 (Table 3), and the corresponding loaded support was used for peptide synthesis following Scheme 3. The support was found to be soluble in the reaction medium and was used for synthesizing tri- and tetrapeptides in 82 and 78% yields, respectively (Table 3).

CONCLUSIONS

A soluble poly(norbornene)-derived support containing multiple attachment sites loaded with alanine and solubilizing oligoether/alkyl groups has been efficiently optimized to synthesize peptides without using an excess of coupling reagents. The synthesis of peptides including larger octapeptides could be carried out in a homogeneous reaction medium, and the support could be efficiently recovered via precipitation. Tri- to octapeptides were obtained in 28 to 97% yields on the support using only 1.2 equiv of amino acids and coupling reagents. The HPLC traces of the crude peptides indicated that peptide coupling reactions proceeded to completion, even with the lower equivalents of amino acids. The efficiency of the support was illustrated for synthesizing the acyclic hexapeptide precursor to natural product segatalin A. The acyclic hexapeptide was obtained in 41% yield using only one-eighth of the amount of coupling reagents used in previously reported approaches with insoluble resins. Peptides that contain amino acids other than Ala at the C-terminus were synthesized using the recovered deprotected poly(norbornene) support. Phenyl- and methionine-containing peptides were synthesized in 82 and 78% yields, respectively, using the recovered deprotected poly(norbornene) support.

EXPERIMENTAL SECTION

General Methods. All air-sensitive reactions were performed under an inert atmosphere of nitrogen. Unless stated otherwise, all reagents for synthesis were purchased from commercially available suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from sodium benzophenoneketyl; *N*,*N*-diisopropylethylamine (DIEA), dichloromethane, and piperidine were distilled from calcium hydride; *N*,*N*-dimethylformamide (DMF), 20% piperidine−DMF, and *N*,*N*-diisopropylcarbodiimide (DIC) were dried over 4 Å molecular sieves. All dry solvents were stored over 4 Å molecular sieves prior to use.

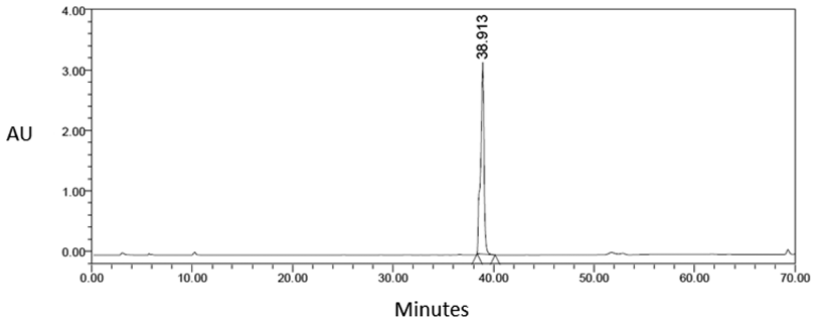


Figure 4. HPLC of crude **13** obtained after cleavage from support **1**.

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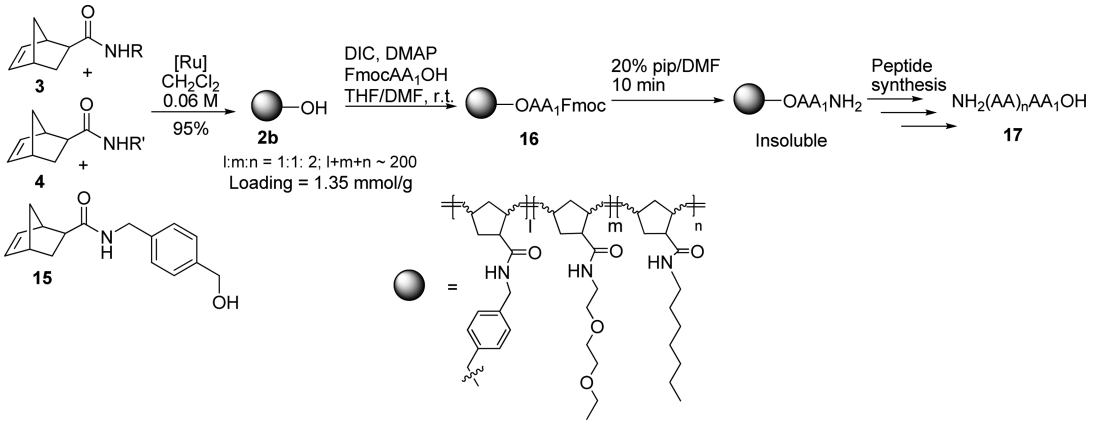
Scheme 5. Synthesis and Utility of Support 2b



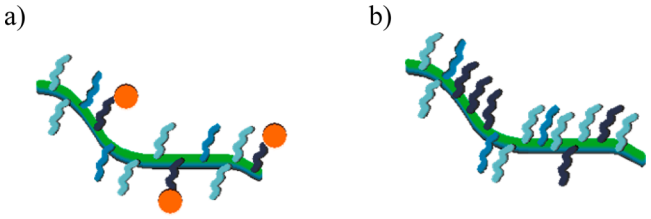


Figure 5. Schematic representation of supports (a) **1** and (b) **2b**.

Table 3. Peptides Synthesized Using Support 2a

no.	support **16**	loading (mmol/g)	peptide **17**	yield (%)[a]
1	**16a**: AA_1 = F	0.9	**17a**: AFF	82
2	**16b**: AA_1 = M	1.1	**17b**: IAFM	78

[a]Isolated using RP-HPLC.

Analytical thin-layer chromatography (TLC) was performed on MERCK precoated silica gel 60 F_{254}TLC plates. Eluting solvents are reported as volume percents. Compounds were visualized using UV light, ninhydrin, and iodine stains. Flash column chromatography was performed using silica gel (100−200 mesh). All 1D and 2D NMR spectra were recorded using $CDCl_3$, CD_3OD, D_2O, or DMSO-d_6 as solvent. The NMR spectra were referenced using residual solvent peaks as the standard. Chemical shifts are denoted in parts per million (δ), and coupling constants (J) are reported in hertz (Hz). The spin multiplicities are reported as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), quintet (quint), apparent quintet (app. quint.), and multiplet (m).

High-resolution mass spectra (HRMS) were recorded using the ESI technique with a Q-TOF mass analyzer. All IR spectra were recorded in the form of a KBr pellet for solids or as thin films in chloroform for liquids. IR spectra peaks are reported in wavenumbers (cm^{-1}) as strong (s), medium (m), weak (w), and broad (br).

Semi-preparative RP-HPLC was carried out using water (0.1% TFA) and acetonitrile (0.1% TFA) as the mobile phase and a C 18, 5 μm, 10 × 250 mm column as the stationary phase. Peptides were injected at a concentration of 10 mg/mL, and a flow rate of 4.1 mL/min was used for semi-preparative RP-HPLC. Peptide elution was monitored at 254 nm.

Boc-L-Phe-OH (7a). Boc anhydride (4.10 mL, 0.02 mol, 1.5 equiv) was added to the stirred solution of L-Phe-OH (2.00 g, 0.01 mol, 1 equiv) and sodium bicarbonate (2.00 g, 0.02 mol, 2 equiv) in 1:1 water/dioxane (20 mL) at 0 °C. The reaction was warmed to RT and allowed to stir for 12 h, following which dioxane was concentrated in vacuo. The residual aqueous solution was washed with ethyl acetate (2 × 30 mL), acidified with dilute HCl (5%), and extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give Boc-L-Phe-OH in 3.1 g (97%) as white solid. TLC R_f = 0.25 (50% ethyl acetate/hexane). The NMR data matched with the reported spectra in the literature.[41]

Boc-L-Phe-O-Bn (8a). Benzyl bromide (2.10 mL, 0.018 mol, 1.5 equiv) was added to a solution of Boc-phenyl alanine **7** (3.20 g, 0.012 mol, 1 equiv) and potassium carbonate (3.32 g, 0.024 mol, 2 equiv) in MeCN (40 mL) at RT. The reaction was allowed to stir for 12 h. Subsequently, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography (5% ethyl acetate/hexane) afforded compound **8a** in 3.5 g (83%) as pale yellow solid. TLC R_f = 0.2 (7% ethyl acetate/hexane). The NMR data matched with the reported spectra in the literature.[42]

Fmoc-L-Ala-L-Phe-OBn (10a). TFA (4.0 mL, 0.04 mol, 10 equiv) was added dropwise over a period of 10 min (using syringe) to a solution of Boc-L-Phe-O-Bn **8a** (1.50 g, 0.004 mol, 1 equiv) in DCM (4 mL) at 0 °C. The reaction mixture was allowed to stir for 1 h at RT. Excess TFA was removed in vacuo, and the residue was dissolved in water (5 mL) and lyophilized to give 1.5 g (99%) of compound **9a** as a white solid. Compound **9a** was used in the next step without further purification. TLC R_f = 0.2 (10% ethyl acetate/hexane). IR (KBr pellet): ν = 3442 (m), 3180 (m), 2940 (m), 1743 (s), 1657 (s), 1604 (s), 1529 (s), 1207 (s), 1146 (s), 704 (m) cm^{-1}; HRMS (ESI^+): calcd. for $C_{16}H_{18}NO_2$ (MH^+), 256.1338; found, 256.1343.

To a solution of Fmoc-L-Ala-OH (1.26 g, 0.004 mol, 1 equiv) in DCM (30 mL) at 0 °C were added HBTU (1.87 g, 0.0049 mol, 1.2 equiv), amine **9a** (1.56 g, 0.004 mol, 1 equiv), and DIEA (2.1 mL, 0.012 mol, 3 equiv). The reaction mixture was warmed to room temperature and allowed to stir for 12 h. The reaction mixture was diluted with DCM (30 mL) and washed with aqueous $NaHCO_3$ (2 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (gradient: 15−20% ethyl acetate/hexane) afforded 1.97 g (87%) of peptide **10a** as a white solid. TLC R_f = 0.2 (20% ethyl acetate/hexane). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.78 (d, J = 7.6 Hz, 2H; $H_{Ar(Fmoc)}$), 7.58 (d, J = 6.8 Hz, 2H; $H_{Ar(Fmoc)}$), 7.45−7.27 (9H; $H_{Ar(Fmoc)}$ and H_{Ar}), 7.2−7.14 (m, 3H; H_{Ar}), 7.02−6.95 (m, 2H; H_{Ar}), 6.35 (d, J = 6.4 Hz, 1H; N*H*), 5.3−5.24 (m, 1H; N*H*), 5.14 (q, J = 12 Hz, 2H; CH_2), 4.89 (dd, J = 12.8 Hz, 6 Hz, 1H; CH_{Phe}), 4.37 (app. quint., 2H; $CH_{2(Fmoc)}$), 4.25−4.18 (2H; $CH_{(Fmoc)}$ and $CH_{(Ala)}$), 3.2−3.05 (m, 2H; $CH_{2(Phe)}$), 1.34 (d, J = 6 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (100 MHz $CDCl_3$, 25 °C): δ 171.9, 171.2, 156, 143.9, 141.5, 135.6, 135.1, 129.4, 128.8, 128.7, 127.9, 127.3, 127.2, 125.2, 120.1, 67.5, 53.3, 50.5, 47.3, 37.9, 29.8, 18.7; IR (KBr pellet): ν = 3293 (s), 3074 (m), 2927 (m), 1719 (s), 1686 (s), 1649 (s), 1544 (s), 1450 (s), 1250 (s), 1044 (m), 742 (s), 695 (s) cm^{-1}; HRMS (ESI^+): calcd. for $C_{34}H_{33}N_2O_5$ (MH^+), 549.2389; found, 549.2372.

Fmoc-L-Ala-L-Phe-OH (11a). To a solution of dipeptide **10a** (100 mg, 0.18 mmol, 1 equiv) in MeOH (5 mL) was added Pd/C (23 mg, 10 mol %) at RT. The reaction was allowed to stir in an atmosphere of

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hydrogen (balloon) over 1 h, following which it was filtered over Celite. The Celite was washed multiple times with MeOH, and the combined filtrate was concentrated in vacuo to give 81 mg (99%) of dipeptide **11a** as a white solid. TLC R_f = 0.2 (5% MeOH/DCM). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ 8 (d, J = 7.5 Hz, 1H; NH), 7.89 (d, J = 8 Hz, 2H; $H_{Ar(Fmoc)}$), 7.72 (t, J = 7.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.47 (d, J = 7.5 Hz, 1H; NH), 7.41 (t, J = 7.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.32 (t, J = 7.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.27−7.13 (5H; $H_{Ar(Fmoc)}$ and H_{Ar}), 4.39 (app. quint., 1H; CH_{Phe}), 4.27−4.16 (3H; $CH_{2(Fmoc)}$ and $CH_{(Fmoc)}$), 4.06 (app. quint., J = 7 Hz, 1H; $CH_{(Ala)}$), 3.04 (dd, J = 14 Hz, 5 Hz, 1H; $CH_aCH_{b(Phe)}$), 2.91 (dd, J = 14 Hz, 8 5 Hz, 1H; $CH_aCH_{b\ (Phe)}$), 1.18 (d, J = 7.5 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 172.7, 172.4, 155.6, 143.9, 140.7, 137.5, 129.2, 128.1, 127.6, 127.1, 126.3, 125.31, 125.28, 120.1, 65.6, 53.4, 49.9, 46.6, 36.6, 18.2; IR (KBr pellet): ν = 3305 (s), 3063 (m), 2925 (s), 1742 (m), 1690 (s), 1661 (s), 1611 (s), 1537 (s), 1450 (s), 1256 (s), 739 (s), 698 (s) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{27}H_{27}N_2O_5$ (MH$^+$), 459.1920; found, 459.1929.

Boc-L-Trp-OH (7b). Boc anhydride (3.37 mL, 0.015 mol, 1.5 equiv) was added to a stirred solution of L-Trp-OH (2 g, 0.009 mol, 1 equiv) and sodium bicarbonate (1.64 g, 0.019 mol, 2 equiv) in 1:1 water/dioxane (20 mL) at 0 °C. The reaction was warmed to RT and allowed to stir for 13 h, following which dioxane was concentrated in vacuo. The residual aqueous solution was washed with ethyl acetate (2 × 30 mL), acidified with dilute HCl (5%), and extracted with ethyl acetate (2 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give Boc-L-Trp-OH **7b** in 2.7 g (93%) as a white solid. TLC R_f = 0.2 (50% ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 8.1 (bs, 1H; $NH_{Ar(Trp)}$), 7.60 (d, J = 7.6 Hz, 1H; H_{Ar}), 7.36 (d, J = 8 Hz, 1H; H_{Ar} bs, 1H; H_{Ar}), 7.21 (t, J = 7.6 Hz, 1H; H_{Ar}), 7.12 (t, J = 7.6 Hz, 1H; H_{Ar}), 7.02 (bs, 1H; H_{Ar}), 5.04 (d, J = 6.8 Hz, 1H; NH_{Trp}), 4.65 (bs 1H; CH_{Trp}), 3.33 (bs 2H; $CH_{2(Trp)}$), 1.5−1.2 (9.3H; $CH_{3(Boc)}$ and rotamer); ^{13}C NMR (125 MHz $CDCl_3$, 25 °C): δ 176.3, 155.8, 136.3, 127.9, 123.2, 122.3, 119.8, 118.9, 111.4, 110.1, 80.4, 54.4, 28.5, 27.7; IR (thin film): ν = 3376 (br), 2927 (m), 1721 (s), 1651 (s), 1404 (s), 1247 (m), 1164 (s), 1053 (m), 744 (s), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{16}H_{20}N_2O_4Na$ (MNa$^+$), 327.1321; found, 327.1310.

Boc-L-Trp-O-Bn (8b). Benzyl bromide (0.87 mL, 0.0074 mol, 1.5 equiv) was added to a solution of Boc-tryptophan **7b** (1.5 g, 0.005 mol, 1 equiv) and potassium carbonate (1.35 g, 0.01 mol, 2 equiv) in MeCN (25 mL) at RT. The reaction was allowed to stir for 12 h. Subsequently, the reaction mixture was diluted with ethyl acetate (35 mL) and washed with water (2 × 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography (10% ethyl acetate/hexane) afforded compound **8b** in 1.7 g (88%) as a white solid. TLC R_f = 0.5 (20% ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 8.02 (s, 1H; $NH_{Ar(Trp)}$), 7.55 (d, J = 7.6 Hz, 1H; H_{Ar}), 7.39−7.30 (4H; H_{Ar}), 7.25−7.22 (2H; H_{Ar}), 7.19 (t, J = 7.2 Hz, 1H; H_{Ar}), 7.11 (t, J = 7.2 Hz, 1H; H_{Ar}), 6.81 (s, 1H; H_{Ar}), 5.15−5.03 (3H; CH_2 and $NH_{(Trp)}$), 4.8−4.6 (m, 1H; $CH_{(Trp)}$), 3.29 (app. d, 2H; $CH_{2(Trp)}$), 1.42 (bs, 9H; $CH_{3(Boc)}$); ^{13}C NMR (100 MHz $CDCl_3$, 25 °C): δ 172.3, 155.4, 136.2, 135.5, 128.7, 128.6, 128.5, 127.8, 123.0, 122.3, 119.8, 118.9, 111.3, 110.2, 80, 67.2, 54.5, 28.5, 28.1; IR (KBr pellet): ν = 3352 (s), 2970 (m), 1727 (s), 1690 (s), 1518 (s), 1454 (m), 1227 (s), 1168 (s), 749 (s) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{23}H_{26}N_2O_4Na$ (MNa$^+$), 417.1790; found, 417.1800.

TFA.H$_2$N-Trp-O-Bn (9b). TFA (1.8 mL, 0.024 mol, 10 equiv) was added dropwise over a period of 10 min (using syringe) to a solution of Boc-L-Trp-O-Bn **8b** (0.94 g, 0.0024 mol, 1 equiv) in DCM (1.8 mL) at 0 °C. The reaction mixture was allowed to stir for 1 h at RT. Excess TFA was removed in vacuo, and the residue was dissolved in water (4 mL) and lyophilized to give 0.89 g of compound **9b** (99%) as a brown solid. Compound **9b** was used in the next step without further purification. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ 11.12 (s, 1H; $NH_{Ar(Trp)}$), 8.56 (s, 3H; NH_3), 7.50 (d, J = 7.5 Hz, 1H; H_{Ar}), 7.40 (d, J = 8 Hz, 1H; H_{Ar}), 7.38−7.3 (3H; H_{Ar}), 7.24−7.16 (3H; H_{Ar}), 7.11 (t, J = 7 Hz, 1H; H_{Ar}), 7.01 (t, J = 7.5 Hz, 1H; H_{Ar}), 5.15 (d, J = 12.5 Hz, 1H; Ph−CH_aCH_b), 5.06 (d, J = 12.5 Hz, 1H; Ph−CH_aCH_b), 4.34 (t, J = 6.5 Hz, 1H; CH_{Trp}), 3.35−3.24 (m, 2H; $CH_{2(Trp)}$); ^{13}C NMR (125 MHz DMSO-d_6, 25 °C): δ 169.4, 136.3, 134.9, 128.4, 128.3, 128.1, 126.9, 124.9, 121.2, 118.7, 118.0, 111.6, 106.4, 67.1, 52.8, 26.4; IR (KBr pellet): ν = 3436 (br), 2926 (m), 1735 (m), 1671 (s), 1518 (m), 1423 (m), 1206 (s), 1135 (s), 747 (s), 593 (m), 528 (m) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{18}H_{19}N_2O_2$ (MH$^+$), 295.1447; found, 295.1461.

Fmoc-L-Val-L-Trp-OBn (10b). To a solution of Fmoc-L-Val-OH (0.44 g, 0.0013 mol, 1 equiv) in DCM (15 mL) at 0 °C were added HCTU (0.64 g, 0.0015, 1.2 equiv), amine **9b** (0.51 g, 0.0013 mol, 1 equiv), and DIEA (0.66 mL, 0.0039 mol, 3 equiv). The reaction mixture was warmed to room temperature and allowed to stir for 12 h. The reaction mixture was diluted with DCM (30 mL) and washed with aqueous $NaHCO_3$ (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography to afforded 0.62 g (77%) of peptide **10b** as a white solid. TLC R_f = 0.1 (DCM). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 10.85 (s, 1H; $NH_{Ar(Trp)}$), 8.42 (d, J = 7.2 Hz, 1H, NH), 7.88 (d, J = 7.6 Hz, 2H; $H_{Ar(Fmoc)}$), 7.74 (t, J = 6.8 Hz, 2H; $H_{Ar(Fmoc)}$), 7.49 (d, J = 7.6 Hz, 1H; NH), 7.41 (t, J = 7.2 Hz, 2H; $H_{Ar(Fmoc)}$), 7.36−7.22 (8H; $H_{Ar(Fmoc)}$ and $H_{Ar(Trp)}$), 7.18−7.12 (3H; H_{Ar}), 7.06 (t, J = 7.2 Hz, 1H; $H_{Ar(Trp)}$), 6.97 (t, J = 7.2 Hz, 1H; $H_{Ar(Trp)}$), 4.99 (dd, J = 31.6 Hz, J = 12.4 Hz, 2H; CH_2), 4.6 (app. quint., 1H; $CH_{(Trp)}$), 4.34−4.15 (3H; $CH_{2(Fmoc)}$ and $CH_{(Fmoc)}$), 3.95 (app. t, 1H; $CH_{(Val)}$), 3.23−3.0 (m, 2H; $CH_{2(Trp)}$), 2.0−1.89 (m, 1H; -$(CH_3)_2$−$CH_{(Val)}$), 0.88−0.78 (6H; $CH_{3(Val)}$); ^{13}C NMR (100 MHz DMSO-d_6, 25 °C): δ 171.6, 171.3, 156.0, 143.9, 143.7, 140.7, 136.1, 135.6, 128.2, 127.9, 127.7, 127.6, 127.0, 125.3, 123.7, 120.9, 120.0, 118.4, 117.9, 111.4, 109.2, 65.9, 65.6, 59.7, 53.2, 46.7, 30.5, 27.0, 19.0, 18.1; IR (KBr pellet): ν = 3410 (s), 3299 (s), 2924 (s), 2854 (s), 1734 (s), 1692 (s), 1653 (s), 1534 (s), 1455 (m), 1290 (m), 1244 (s), 1029 (m), 739 (s) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{38}H_{37}N_3O_5Na$ (MNa$^+$), 638.2631; found, 638.2617.

Fmoc-L-Val-L-Trp-OH (11b). To a solution of dipeptide **10b** (500 mg, 0.8 mmol, 1 equiv) in MeOH (22 mL) was added Pd/C (100 mg, 10 mol %) at RT. The reaction was allowed to stir in an atmosphere of hydrogen (balloon) over 1 h, following which it was filtered over Celite. The Celite was washed multiple times with MeOH, and the combined filtrates were concentrated in vacuo to give 402 mg (96%) of dipeptide **11b** as a white solid. TLC R_f = 0.2 (5% MeOH/DCM). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ 10.8 (s, $NH_{Ar(Trp)}$), 8.17 (d, J = 7.5 Hz, 1H; NH), 7.88 (d, J = 7.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.74 (t, J = 8.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.52 (d, J = 8 Hz, 1H; NH), 7.40 (t, J = 7.5 Hz, 2H; $H_{Ar(Fmoc)}$), 7.38−7.26 (4H; $H_{Ar(Fmoc)}$ and H_{Ar}), 7.18−7.13 (m, 1H; $H_{Ar(Trp)}$), 7.04 (t, J = 7 Hz, 1H; $H_{Ar(Trp)}$), 6.96 (t, J = 7.5 Hz, 1H; $H_{Ar(Trp)}$), 4.49 (app. q, 1H; CH_{Trp}), 4.33−4.17 (3H; $CH_{2(Fmoc)}$ and $CH_{(Fmoc)}$), 3.95−3.88 (m, 1H; $CH_{(Val)}$), 3.2−3.12 (m, 1H; $CH_aCH_{b(Trp)}$), 3.04 (dd, J = 14.5 Hz, 8.5 Hz, 1H; $CH_aCH_{b\ (Trp)}$), 1.96 (septet, J = 7 Hz, 1H; $CH_{(Val)}$), 0.83 (t, J = 6 Hz, 6H; $CH_{3(Val)}$); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 173.2, 171.2, 156.0, 144, 143.8, 140.7, 136.0, 127.6, 127.2, 127.1, 125.4, 123.6, 120.9, 120.1, 118.3, 118.1, 111.3, 109.7, 65.7, 59.9, 52.9, 46.7, 30.5, 27.1, 19.1, 18.2; IR (KBr pellet): ν = 3410 (br), 3319 (br), 2927 (m), 1731 (m), 1645 (s), 1541 (s), 1452 (m), 1241 (s), 1106 (m), 1030 (m), 739 (s), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{31}H_{31}N_3O_5Na$ (MNa$^+$), 548.2161; found, 548.2140.

General Procedure for Determination of Loading Capacities. The loading capacities of supports **1**, **2**, and **16** were determined by recording their ^{1}H NMR spectra in the presence of a known amount of TCE. The integration of the peak at δ 6.95 ppm, corresponding to TCE, was compared with the peak at δ 7.38 ppm, corresponding to the Fmoc protons, for support **1**; δ 4.44 ppm, corresponding to the benzylic protons, for support **2a**; δ 7.37 ppm, corresponding to the Fmoc protons, for support **16a**; and δ 7.39 ppm, corresponding to the Fmoc protons, for support **16b**.

General Procedure for Peptide Synthesis on Support 1. Polymer **1** (70 mg, 0.077 mmol, 1 equiv) was treated with a solution of 20% piperidine in DMF (0.7 mL) for 10 min. Subsequently, hexane (5 mL × 3) was added to the reaction mixture. The supernatant hexane was partially removed each time, following which diethyl ether (8 mL)

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was added. The suspension was centrifuged, and the supernatant was decanted to afford the support containing the free amine as a precipitate, which was dried in vacuo. A solution of Fmoc-AA_1-OH or AA_2-AA_1-OH dipeptide (1.2 equiv) and HCTU (1.2 equiv) in CH_2Cl_2 (1.5 mL) and DMF (0.5 mL) were added to the polymer containing the free amine, DIEA (3 equiv) was added to the mixture, and the reaction was allowed to stir for 3 h. The reaction mixture was concentrated to 1 mL, and diethyl ether (10 mL) was added to it. The suspension was centrifuged, and the supernatant was decanted to afford the support-containing tripeptide as a precipitate. The precipitate was dissolved in DMF (0.5 mL) and reprecipitated with diethyl ether (10 mL × 3). The precipitate was dried in vacuo,and the deprotection/coupling steps were repeated (with a single amino acid) as described above to obtain the peptide with free amine-attached to the support. Finally, the peptide was cleaved from the support using LiOH·H_2O (4 equiv) in THF for 1 h. The supernatant contained the peptides in their salt form, whereas support **2a** was recovered as a precipitate. The lithium salt of peptide was acidified with dilute HCl, and the aqueous solution was lyophilized to give the crude peptide, which was purified by semipreparative RP-HPLC.

HCl·H_2N-Pro-Phe-Ala-OH (6). The spectral data is as previously reported in the literature.[33]

HCl·H_2N-Ala-Phe-Ala-OH (12a). ^{1}H NMR (400 MHz, MeOH-d_4, 25 °C): δ 7.33−7.17 (5H; H_{Ar}), 4.7 (dd, J = 9.6, 4.8 Hz, 1H; CH_{Phe}), 4.38 (q, J = 7.2 Hz, 1H; CH_{Ala}), 3.84 (q, J = 6.8 Hz, 1H; CH_{Ala}), 3.22 (dd, J = 14.4, 4.8 Hz, 1H; $CH_aH_{b(Phe)}$), 2.9 (dd, J = 14.4, 9.6 Hz, 1H; $CH_aH_{b(Phe)}$), 1.48 (d, J = 6.8 Hz, 3H; $CH_{3(Ala)}$), 1.41 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (100 MHz, MeOH-d_4, 25 °C): 175.6, 173.0, 170.9, 138.3, 130.3, 129.5, 127.8, 56.0, 50.0, 38.8, 17.7; IR (KBr pellet): ν = 3432 (br), 2927 (m), 1658 (s), 1552 (m), 1198 (s), 1139 (s), 661 (w), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{15}H_{22}N_3O_4$ (MH^+), 308.1610; found, 308.1624.

HCl·H_2N-Pro-Ala-Phe-Ala-OH (12b). ^{1}H NMR (500 MHz, D_2O, 25 °C): δ 7.4−7.27 (5H; H_{Ar}), 4.62 (dd, J = 8.5, 6.5 Hz, 1H; CH_{Phe}), 4.39−4.28 (3H; $CH_{(Ala)}$ and $CH_{(Pro)}$), 3.48−3.35 (m, 2H; $CH_{2(Pro)}$), 3.16 (dd, J = 14, 6.5 Hz, 1H; $CH_aCH_{b(Phe)}$), 3.05 (dd, J = 14 Hz, 8 Hz, 1H; $CH_aCH_{b(Phe}$), 2.45−2.36 (m, 1H; $CH_{(Pro)}$), 2.1−1.92 (3H; $CH_{(Pro)}$), 1.38 (d, J = 7 Hz, 3H; $CH_{3(Ala)}$), 1.34 (d, J = 7 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): 176.2, 174.1, 172.3, 169.2, 136.1, 129.3, 128.7, 127.1, 59.4, 54.6, 49.8, 48.7, 46.5, 37.0, 29.7, 23.7, 16.44, 16.37; IR (KBr pellet): ν = 3418 (br), 3358 (br), 3281 (br), 2923 (s), 1626 (s), 1448 (m), 1381 (m),1020 (s), 796 (s), 595 (w), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{20}H_{29}N_4O_5$ (MH^+), 405.2138; found, 405.2151.

HCl·H_2N-Ile-Ala-Phe-Ala-OH (12c). ^{1}H NMR (500 MHz, D_2O, 25 °C): δ 7.4−7.27 (5H; H_{Ar}), 4.59 (t, J = 7.5 Hz, 1H; CH_{Phe}), 4.38 (q, J = 7.5 Hz, 1H; CH_{Ala}), 4.3 (q, J = 7.5 Hz, 1H; CH_{Ala}), 3.81 (d, J = 6 Hz, 1H; $CH_{(Ile)}$), 3.16 (dd, J = 14, 6.5 Hz, 1H; $CH_aCH_{b(Phe}$), 3.03 (dd, J = 14, 8.5 Hz, 1H; $CH_aCH_{b(Phe}$), 1.97−1.87 (1H; $CH_{(Ile)}$), 1.5−1.41 (m, 1H; $CH_aCH_{b(Ile)}$), 1.37 (d, J = 7.5 Hz, 3H; $CH_{3(Ala)}$), 1.34 (d, J = 7 Hz, 3H; $CH_{3(Ala)}$), 1.22−1.13 (m, 1H; $CH_aCH_{b(Ile)}$), 0.96−0.88 (6H; $CH_{3(Ile)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): 176.6, 173.9, 172.2, 168.7, 136.2, 129.2, 128.7, 127.1, 57.5, 54.8, 49.3, 49.0, 37.0, 36.2, 23.9, 16.7, 16.5, 13.9, 10.4; IR (KBr pellet): ν = 3427 (br), 3296 (br), 2923 (s), 1634 (s), 1452 (s), 1029 (m), 600 (w), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{21}H_{33}N_4O_5$ (MH^+), 421.2451; found, 421.2462.

HCl·H_2N-Phe-Pro-Ala-Phe-Ala-OH (12d). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 7.5−7.25 (10H; H_{Ar}), 4.63 (t, J = 5.6 Hz, 1H; CH_{Phe}), 4.56 (t, J = 5.6 Hz, 1H; CH_{Phe}), 4.45 (t, J = 5.6 Hz, 1H; CH_{Pro}), 4.37−4.22 (2H; CH_{Ala}), 3.8−3.7 (1H; CH_{Pro}), 3.45−3.3 (2H; $CH_{(Pro)}$ and $CH_aCH_{b(Phe)}$), 3.2−3.0 (3H; $CH_{(Phe)}$), 2.3−2.2 (m, 1H; $CH_{(Pro)}$), 2.02−1.95 (2H; $CH_{2(Pro)}$), 1.85−1.75 (m, 1H; $CH_{(Pro)}$), 1.4−1.3 (6H; $CH_{3(Ala)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): δ 176.4, 174.5, 173.1, 172.2, 167.7, 136.1, 133.4, 129.7, 129.32, 129.28, 129.2, 128.7, 128.1, 127.1, 60.4, 54.5, 53.2, 49.6, 49.0, 47.9, 37.0, 35.6, 29.3, 24.7, 16.5; IR (KBr pellet): ν = 3441 (br), 1644 (s), 1454 (s), 1200 (m), 857 (s), 746 (w), 564 (m) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{29}H_{38}N_5O_6$ (MH^+), 552.2822; found, 552.2816.

HCl·H_2N-Pro-Met-Ala-Phe-Ala-OH (12e). ^{1}H NMR (500 MHz, D_2O, 25 °C): δ 7.4−7.26 (5H; H_{Ar}), 4.6 (t, J = 6.5 Hz, 1H; CH_{Phe}), 4.49−4.4.39 (2H; CH_{Met} and CH_{Pro}), 4.34 (q, J = 7.5 Hz, 1H; CH_{Ala}), 4.29 (q, J = 7 Hz, 1H; CH_{Ala}) 3.5−3.33 (m,2H; $CH_{2(Pro)}$), 3.16 (dd, J = 14, 6.5, 1H; $CH_aH_{b(Phe)}$), 3.04 (dd, J = 14, 8 Hz, 1H; $CH_aH_{b(Phe)}$), 2.6−2.42 (3H; $SCH_{2(Met)}$ and $CHaCH_{b(Pro)}$), 2.15−1.9 (8H; $CH_{3(Met)}$, $CH_{2(Met)}$, $CH_{2(Pro)}$ and $CHaCH_{b(Pro)}$), 1.39 (d, J = 7.5 Hz, 3H; $CH_{3(Ala)}$), 1.33 (d, J = 7.5 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): δ 176.2, 174.1, 172.6, 172.3, 169.6, 136.2, 129.3, 128.7, 127.1, 59.5, 54.6, 52.9, 49.5, 48.7, 46.5, 37.0, 30.3, 29.7, 29.1, 23.7, 16.6, 16.3, 14.1; IR (KBr pellet): ν = 3426 (br), 2925 (m), 1631 (s), 1541 (m), 1451 (m), 1063 (br), 626 (w) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{25}H_{38}N_5O_6S$ (MH^+), 536.2543; found, 536.2543.

HCl·H_2N-Pro-Val-Ala-Phe-Ala-OH (12f). ^{1}H NMR (500 MHz, D_2O, 25 °C): δ 7.4−7.25 (5H; H_{Ar}), 4.59 (t, J = 6.5 Hz, 1H; CH_{Phe}), 4.44 (dd, J = 8.5, 6.5 Hz, 1H; CH_{Pro}), 4.33−4.27 (2H; CH_{Ala}), 4.1 (d, J = 7.5 Hz, 1H; CH_{Val}), 3.5−3.35(m, 2H; $CH_{2(Pro)}$), 3.14 (dd, J = 14, 6.5 Hz, 1H; $CH_aCH_{b(Phe)}$), 3.05 (dd, J = 14, 8 Hz, 1H; $CH_aCH_{b(Phe)}$), 2.52−2.4 (m, 1H; $CH_{(Pro)}$), 2.13−1.95 (m, 4H; $CH_{(Pro)}$ and $CH_{(Val)}$), 1.37 (d, J = 7 Hz, 3H; $CH_{3(Ala)}$), 1.33 (d, J = 7 Hz, 3H; $CH_{3(Ala)}$), 0.92 (dd, J = 11.5, 7 Hz, 6H; $CH_{3(Val)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): δ 176.4, 174.2, 172.6, 172.2, 169.6, 136.1, 129.2, 128.7, 127.1, 59.7, 59.4, 54.7, 49.4, 48.9, 46.5, 37.0, 30.03, 29.85, 23.7, 18.3, 17.6, 16.6, 16.5; IR (KBr pellet): ν = 3423 (br), 3307 (br), 2925 (m), 1636 (s), 1543 (m), 1556 (s), 1203 (s), 1138 (s), 1054 (m), 670 (w), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{25}H_{38}N_5O_6$ (MH^+), 504.2822; found, 504.2828.

HCl·H_2N-Gly-Pro-Val-Val-Trp-Ala-OH (12g). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 7.68 (d, J = 7.6 Hz, 1H; $H_{Ar(Trp)}$), 7.5 (d, J = 8.4 Hz, 1H; $H_{Ar(Trp)}$), 7.28−7.22 (2H; $H_{Ar(Trp)}$), 7.17 (t, J = 7.6 Hz, 1H; $H_{Ar(Trp)}$), 4.51 (dd, J = 8.8, 4.8 Hz, 1H; CH_{Pro}), 4.29 (q, J = 7.2 Hz, 1H; CH_{Ala}), 4.14−4.07 (m, 1H; CH_{Val}), 4.04−3.97 (3H; CH_{Val} and $CH_{2(Gly)}$), 3.6−3.54 (2H; CH_{Pro}), 3.31 (dd, J = 14.4, 6.4 Hz, 1H; $CH_aCH_{b(Trp)}$), 3.21 (dd, J = 14.4, 8 Hz, 1H; $CH_aCH_{b(Trp)}$), 2.35−2.2 (m, 1H; CH_{Pro}), 2.05−1.85 (5H; CH_{Val} and CH_{Pro}), 1.34 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 0.94−0.84 (9H; $CH_{3(Val)}$), 0.71 (d, J = 6.8 Hz, 3H; $CH_{3(Val)}$); ^{13}C NMR (125 MHz, D_2O, 25 °C): δ 176.1, 173.8, 173.2, 172.7, 172.6, 165.5, 136.2, 126.8, 124.5, 121.9, 119.3, 118.2, 111.9, 108.7, 60.1, 59.8, 59.2, 54.0, 46.9, 40.4, 30.3, 29.9, 29.6, 27.1, 24.2, 18.25, 18.18, 18.0, 17.6, 16.5; IR (KBr pellet): ν = 3416 (br), 1643 (s), 1442 (m), 1200 (s), 1139 (s), 720 (m), 509 (s), cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{31}H_{46}N_7O_7$ (MH^+), 628.3459; found, 628.3440.

HCl·H_2N-Phe-Pro-Gly-Met-Ala-Ala-Phe-Ala-OH (12h). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 7.5−7.24 (10H; H_{Ar}), 4.63−4.57 (2H; CH_{Phe}), 4.53−4.45 (2H; CH_{Met} and CH_{Pro}), 4.37−4.2 (3H; CH_{Ala}), 4.06−3.94 (2H; $CH_{2(Gly)}$), 3.82−3.75 (1H; CH_{Pro}), 3.35−3.4 (1H; NCH_{Pro}), 3.37−3.29 (1H; $CH_aCH_{b(Met)}$), 3.2−2.88 (5H; $CH_aCH_{b(Met)}$ and $CH_{2(Phe)}$), 2.74−2.68 (3H; $CH_{3(Met)}$), 2.4−1.9 (6H; $CH_{2(Met)}$ and $CH_{2(Pro)}$), 1.39 (d, J = 8 Hz, 3H; $CH_{3(Ala)}$); 1.34 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 1.3 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$); IR (KBr pellet): ν = 3431 (br), 2927 (m), 1658 (s), 1538 (s), 1455 (m), 1388 (m), 1195 (m), 1143 (m), 1055 (m), 700 (m) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{39}H_{54}N_8O_9SK$ (MK^+), 849.3372; found, 849.3375

HCl·H_2N-Ile-Pro-Met-Val-Gly-Ala-Phe-Ala-OH (12i). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 7.44−7.25 (5H; H_{Ar}), 4.67−4.63 (m, 1H; CH_{Phe}), 4.6−4.47 (2H; CH_{Met} and CH_{Pro}), 4.37 (q, J = 7.2 Hz, 1H; CH_{Ala}), 4.32−4.22 (2H; CH_{Ala} and CH_{Val}), 4.18 (d, J = 7.2 Hz, 1H; CH_{Ile}), 3.95 (d, J = 3.2 Hz, 2H; $CH_{2(Gly)}$), 3.85−3.77 (1H; CH_{Pro}), 3.72−3.6 (2H; CH_{Pro}), 3.22 (dd, J = 14.4, 6.4 Hz, 1H; $CH_aCH_{b(Phe)}$), 3.1−2.89 (3H; $CH_aCH_{b(Phe)}$ and $CH_{2(Met)}$), 2.73 (3H; $CH_{3(Met)}$), 2.4−1.88 (9H; CH_{Val}, CH_{Ile}, $CH_{2(Met)}$ and $CH_{2(Pro)}$), 1.6−1.5 (1H; CH_{Ile}), 1.4 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 1.29 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 1.26−1.2 (1H; CH_{Ile}), 1.12 (d, J = 7.2 Hz, 3H; $CH_{3(Val)}$), 1.03−0.9 (9H; $CH_{3(Val)}$ and $CH_{3(Ile)}$); IR (KBr pellet): ν = 3423 (br), 3295 (br), 2928 (m), 1636 (s), 1533 (s), 1453 (m), 1199 (m), 1138 (m), 1025 (m) cm^{-1}; HRMS (ESI$^+$): calcd. for $C_{38}H_{62}N_8O_9SCl$ (MH^+), 841.4049; found, 841.4044

HCl·H_2N-Phe-Gly-Pro-Ala-Ile-Ala-Phe-Ala-OH (12j). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 7.52−7.3 (10H; H_{Ar}), 4.65(t, J = 6.8 Hz,1H; CH_{Phe}), 4.56−4.3 (4H; CH_{Ala} and CH_{Pro}), 4.25−4.0 (3H; $CH_{2(Gly)}$ and CH_{Ile}), 3.72−3.6 (m, 2H; $CH_{2(Pro)}$), 3.4−3.0 (4H; $CH_{2(Phe)}$), 2.4−2.20 (m, 1H; $CH_aH_{b(Pro)}$), 2.15−1.8 (4H; $CH_aH_{b(Pro)}$,

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$CH_{2(Pro)}$ and CH_{Ile}), 1.6−1.4 (6H; $CH_{3(Ala)}$), 1.36 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 1.29−1.17 (m, 1H; CH_{Ile}), 1.0−0.8 (6H; $CH_{3(Ile)}$); IR (KBr pellet): ν = 3428 (br), 3289 (br), 1630 (s), 1545 (s), 1453 (s), 1202 (s), 1137 (m), 1023 (w), 700 (w), cm^{-1}; HRMS (ESI^+): calcd. for $C_{40}H_{57}N_8O_9$ (MH^+), 793.4249; found, 793.4246.

HCl·H_2N-Gly-Val-Pro-Val-Trp-Ala-OH (13). ^1H NMR (500 MHz, D_2O + CD_3CN, 25 °C): δ 7.51 (d, J = 8 Hz, 1H; $H_{Ar(Trp)}$), 7.37 (d, J = 8.5 Hz, 1H; $H_{Ar(Trp)}$), 7.15−7.06 (2H; $H_{Ar(Trp)}$), 7.03 (t, J = 7.5 Hz, 1H; $H_{Ar(Trp)}$), 4.59 (t, J = 6.5 Hz, 1H; CH_{Trp}), 4.33 (d, J = 7 Hz, 1H; CH_{Val}), 4.26−4.18 (2H; CH_{Ala} and CH_{Pro}), 3.88 (d, J = 7 Hz, 1H; CH_{Val}), 3.76−3.62 (3H; $CH_{2(Gly)}$ and CH_{Pro}), 3.5−3.42 (m, 1H; CH_{Pro}), 3.15 (d, J = 6.5 Hz, 2H; $CH_{2(Trp)}$), 2.0−1.7 (8H; CH_{Pro}, CH_{Val} and solvent), 1.3−1.22 (m, 1H; $CH_{(Pro)}$), 1.18 (d, J = 7.5 Hz, 3H; $CH_{3(Ala)}$), 0.87 (d, J = 6.5 Hz, 3H; $CH_{3(Val)}$), 0.84−0.74 (9H; $CH_{3(Val)}$); ^{13}C NMR (125 MHz, D_2O + CD_3CN, 25 °C): δ 172.9, 171.7, 170.7, 165.9, 135.6, 126.4, 123.6, 121.1, 117.6, 111.0, 107.9, 59.4, 58.9, 56.3, 52.6, 47.7, 47.4, 39.5, 29.4, 29.2, 28.3, 26.3, 24.0, 17.6, 16.8, 16.5, 15.7; IR (KBr pellet): ν = 3429 (br), 1683 (s), 1631 (s), 1202 (s), 1135 (s), 1021 (s), cm^{-1}; HRMS (ESI^+):calcd. for $C_{31}H_{46}N_7O_7$ (MH^+), 628.3459; found, 628.3439.

General Procedure for Attachment of Amino Acids to Support 2a. *N*,*N*-Diisopropylcarbodiimide (31.2 μL, 0.2 mmol, 3 equiv) and DMAP (2.4 mg, 0.02 mmol, 0.3 equiv) were added to a solution of support **2** (50 mg, 0.067 mmol, 1 equiv) and Fmoc-AA-OH (1.2 equiv) in DMF or THF. The reaction was allowed to stir at room temperature for 4−6 h. Precipitation with diethyl ether, followed by centrifugation, gave amino acid-attached polymer **16**.

Fmoc-L-Phe-OH Supported Polymer (16a). The general procedure for attachment of amino acids to support **2** was followed. DMF (0.5 mL) was used, and the reaction was allowed to proceed for 6 h to afford 72 mg (96%) of **16a**. Loading was determined to be 0.91 mmol/g. The spectra data is as reported in the literature[33]

Fmoc-L-Met-OH Supported Polymer (16b). The general procedure for attachment of amino acids to support **2** was followed. THF (1.5 mL) was used, and the reaction was allowed to proceed for 4 h to afford 72 mg (96%) of **16b**. Loading was determined to be 1.1 mmol/g. ^1H NMR (400 MHz, DMSO-d_6, 25 °C): δ 8.15 (1H; NH_{att}), 7.86 (2.6H; $H_{Ar(Fmoc)}$), 7.8−7.5 (4.6H; $NH_{alk,\ deg}$ and $H_{Ar(Fmoc)}$), 7.4 (1.8H; $H_{Ar(Fmoc)}$), 7.35−7.1 (5H; $H_{Ar(Fmoc)}$ and $H_{Ar(att)}$), 5.4−4.9 (9.1H; CH = CH and NH_{Met}), 4.4−4 (5H; $ArCH_2NH$, $CH_{(Fmoc),}$ $CH_{2,(Fmoc)}$ and CH_{Met}), 3.6−2.7 (68.7H; $CH_{2(alk,\ deg,\ and\ att)}$, $CH_{(nb)}$ and solvent), 2.7−2.1 (20.7H; CH_{nb} and solvent), 2.1−1.6 (12.7 H; $CH_{(nb)}$, $CH_{2(Met)}$ and $CH_{3(Met)}$), 1.6−0.9 (32.8H; $CH_{2(alk)}$, $CH_{3(deg)}$ and $CH_{(nb)}$), 0.82 (6.9H; $CH_{3(alk)}$).

HCl·H_2N-Ala-Phe-Phe-OH (17a). ^1H NMR (400 MHz, D_2O, 25 °C): δ 7.4−7.2 (10H; H_{Ar}), 4.65−4.57 (m, 2H; CH_{Phe}), 3.98 (q, J = 7.2 Hz, 1H; CH_{Ala}), 3.20 (dd, J = 14, 5.6 Hz, 1H; CH_{Phe}), 3.1−2.9 (m, 3H; $CH_{(Phe)}$), 1.43 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$); ^{13}C NMR (100 MHz, D_2O, 25 °C): δ 174.3, 172.1, 170.1, 136.4, 136.0, 129.2, 129.1, 128.73, 128.67, 127.2, 127.1, 55.0, 54.1, 48.8, 37.1, 36.8, 16.5; IR (KBr pellet): ν = 3378 (m), 3226 (m), 3078 (m), 3022 (m), 1727 (s), 1643 (s), 1517 (s), 1447 (s), 1202 (s), 1135 (s), 696 (w); HRMS (ESI^+): calcd. for $C_{21}H_{26}N_3O_4$ (MH^+), 384.1923; found, 384.1927.

HCl·H_2N-Ile-Ala-Phe-Met-OH (17b). ^1H NMR (400 MHz, D_2O, 25 °C): δ 7.4−7.2 (m, 5H; H_{Ar}), 4.62 (t, J = 7.2 Hz, 1H; CH_{Phe}), 4.48 (dd, J = 9.2, 4.4 Hz, 1H; CH_{Met}), 4.39 (q, J = 7.2 Hz, 1H; CH_{Ala}), 3.83 (d, J = 5.6 Hz, 1H; CH_{Ile}), 3.14 (dd, J = 13.6 Hz, 7.2 Hz, 1H; $CH_aCH_{b(Phe)}$), 3.06 (dd, J = 13.6 Hz, 8 Hz, 1H; $CH_aCH_{b(Phe)}$), 3.2−3.02 (m, 2H; $CH_{2(Phe)}$), 2.6−2.4 (2H; $SCH_{2(Met)}$), 2.2−2.05 (4H; $CH_{3(Met)}$ and $CH_aH_{b(Met)}$), 2.02−1.9 (m, 2H; $CH_aH_{b(Met)}$ and $CH_{(Ile)}$), 1.53−1.42 (m, 1H; $CH_aH_{b(Ile)}$), 1.35 (d, J = 7.2 Hz, 3H; $CH_{3(Ala)}$), 1.25−1.15 (m, 1H; $CH_aH_{b(Ile)}$), 1−0.86 (m, 6H; $CH_{(Ile)}$); ^{13}C NMR (100 MHz, D_2O, 25 °C): δ 174.9, 173.8, 172.6, 168.8, 136.2, 129.2, 128.8, 127.2, 57.6, 55.0, 51.8, 49.4, 37.0, 36.3, 30.1, 29.3, 24.0, 16.8, 14.1, 14.0, 10.5; IR (KBr pellet): ν = 3431 (br), 2916 (m), 1637 (s), 1388 (m), 1024 (s), 796 (w) cm^{-1}; HRMS (ESI^+): calcd. for $C_{23}H_{37}N_4O_5S$ (MH^+), 481.2485; found, 481.2480.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral data of compounds and peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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