Versatile Soluble Oligomeric Styrene Supports for Peptide Synthesis

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ABSTRACT: Soluble oligomeric styrene supports are reported here with high loading capacities of 1.5–1.6 mmol/g similar to resins used in solid phase peptide synthesis. Oligoether and alkyl chains are incorporated into the scaffold to improve the support solubility and act as spacers between the attachment sites. Amino acids have been attached to the support in 59–85% yields and 0.87–1.3 mmol/g loading. The supports have been used to synthesize tri- to hexapeptides in 38–64% yields using only 2 equivalents of coupling reagents, which is much lower than the amount of reagents typically used in solid phase synthesis. A modular synthetic approach is used to obtain the supports so that any efficient styrene-based attachment site can be readily incorporated into our soluble support scaffold. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2015**, *53*, 2501–2509

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INTRODUCTION Efficient methods for synthesizing peptides are essential due to their widespread use as materials and therapeutic agents. Solid phase peptide synthesis (SPPS), which utilizes insoluble resins to attach the growing peptide chain has been an attractive method for synthesizing peptides. SPPS is extremely efficient as the growing peptide can be readily recovered from the reaction mixture by filtration and purification is carried out only after the peptide is cleaved form the resin.[1] However, the heterogeneous reaction medium necessitates the use of a large excess of coupling reagents (4–5 equivalents) in SPPS. Therefore, soluble polymers with appropriate attachment sites for amino acids have been explored as supports for what is often referred to as liquid phase peptide synthesis (LPPS).[2] These supports are attractive alternatives to resins as they are soluble in the reaction medium and can be readily isolated by precipitation with a suitable solvent. The design requirements for LPPS supports are very different from catalyst/reagent supports. A catalyst support is used multiple times for the same reaction. Soluble catalyst supports are designed such that they are soluble, recoverable and reusable.[3] In the case of peptide synthesis, an amino acid is attached to the support and a peptide is grown on the support using a series of reactions. The LPPS support properties change with the growing length of the peptide. Therefore, these supports are designed such that the support solubility is maintained despite the attachment of polar peptide chains. In LPPS, the most widely used polymeric supports are derived from polyethylene glycol (PEG) as the PEG chains solubilize the growing peptide chains.[2(a),4] PEG supports have been used for the synthesis of a variety of oligopeptides and have also been used for the combinatorial synthesis of peptides. However, the number of attachment sites per gram of polymer (i.e., the loading capacity) of the PEG polymer is typically low (0.1–0.5 mmol/g) as each polymer unit contains only one attachment site at the end of the polymer. SPPS resins on the contrary have loading capacities of ~1 mmol/g on an average, with the exception of few resins that have lower or higher loading capacities.[1(a)] While lower molecular weight hydrophobic,[5] fluorous,[6] and ionic liquid supports[7] have been explored to improve the loading capacities as well as efficiency of LPPS, there are not many examples of soluble polymer supports with high loading capacities for efficient peptide synthesis. Our group has developed soluble poly(norbornene) supports appended with oligoether and alkyl chains that have high loading capacities (0.6–1.1 mmol/g).[8] Poly(styrene) derived LPPS supports have also been reported that possess high loading capacities, but are not as soluble as the polar PEG supports.[9] The advantage in developing styrene based supports is that a large number of styrene monomers containing efficient amino acid attachment sites already exist in the literature because styrene is the most widely used support in SPPS. Recently, more soluble poly(styrene) supports have been developed that have a single amino acid attachment site for each polymer chain, which lowers their loading capacity.[10] Herein, we report a soluble oligomeric styrene support **1** (Fig. 1) appended with oligoether and alkyl chains for peptide synthesis. We have incorporated the oligoether

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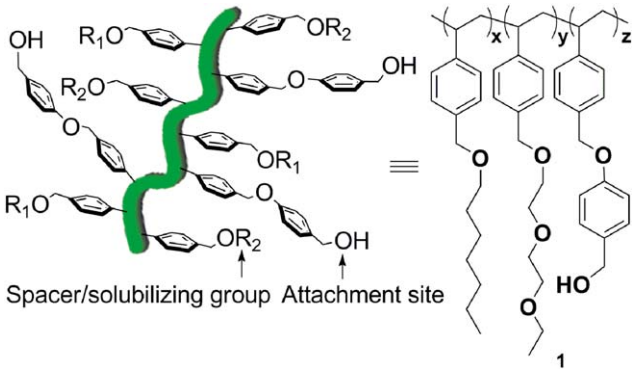


FIGURE 1 Soluble support with multiple attachment sites. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chains in order to provide a polar environment for the growing peptide chains similar to the PEG based supports. The alkyl chains and oligoether chains have also been incorporated to act as spacers between the attachment sites. A modular synthetic route is proposed to access the supports in order to facilitate incorporation of any attachment site into our soluble oligomer scaffold containing the spacer/solubilizing groups. As a proof of concept the benzylic linker (Wang resin type) is used to attach the peptides. This linker is compatible with Fmoc protected amino acids. The utility of the support for peptide synthesis has been demonstrated by loading a variety of amino acids in 59–85% yields and synthesizing hexapeptides in 50% overall yields (using only 2 equiv. of reagents). In contrast to our earlier poly(norbornene) support, these supports are more versatile as they need not be preloaded with amino acids prior to polymerization to improve their solubility. Therefore, peptides with different amino acids at the C-terminus can be readily synthesized using these supports. Given the modular nature of the support synthesis, the utility of the support can be expanded to include other linkers at the attachment site such that protecting groups other than Fmoc can be used for peptide synthesis.

EXPERIMENTAL

General Methods

All air-sensitive reactions were performed under an inert atmosphere of nitrogen with magnetic stirring. Syringe or cannula was used to transfer air-sensitive solvents and solutions. Unless stated otherwise, all the reagents for synthesis were purchased from commercially available suppliers and used without further purification. Tetrahydrofurane (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. Analytical thin-layer chromatography (TLC) was performed on MERCK precoated silica gel 60 F254TLC 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 (200–400 mesh) from Acme chemicals. All 1D and 2D NMR spectra were recorded on Bruker 400 or Bruker 500 spectrometers using $CDCl_3$, or DMSO-d6 as solvents. The NMR spectra were referenced using residual solvent peaks as the standards. 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). The peaks corresponding to the alkyl, diethylene glycol and attachment site monomers are denoted as alk, deg and att, respectively in the ^{1}H NMR data. High-resolution mass spectra (HRMS) were recorded on MICRO-Q-TOF mass spectrometer using the ESI technique. FT-IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. 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 on a Waters HPLC system using water (0.1% TFA) and methanol (0.1% TFA) as the mobile phase and a Sunfire prep C 18, 5 μm, 10 $\times$ 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 with the Waters 2489 UV/visible detector.

1-((heptyloxy)methyl)-4-vinylbenzene (3)

[11]A suspension of NaH (0.178 g, 7.429 mmol, 2.1 equiv.) in THF (2 mL) was slowly added to 1-heptanol (2.014 g, 17.339 mmol, 4.9 equiv.) in THF (4 mL) at 0 °C for 2 h. A solution of *p*-chloromethylstyrene **2** (0.540 g, 3.538 mmol, 1 equiv.) in THF (3 mL) was slowly added to the reaction mixture at °C. The mixture was allowed to reflux for 24 h. Subsequently, water (4 mL) was added and the reaction mixture was extracted with dichloromethane (3 $\times$ 35 mL). The organic layer was concentrated *in vacuo* to afford the crude residue. Purification by column chromatography (hexane) afforded 0.67 g (81%) of ether **3** as colorless oil. TLC R_f = 0.20 (1% ethyl acetate/hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.41 (d, J = 7.6 Hz, 2H; H_{Ar}), 7.32 (d, J = 7.6 Hz, 2H; H_{Ar}), 6.74 (dd, J = 17.2, 10.8 Hz, 1H; CH=CH$_2$), 5.77 (d, J = 17.6 Hz, 1H; CH=CH_2), 5.25 (d, J = 10.8 Hz, 1H; CH=CH_2), 4.51 (s, 2H; PhCH_2O), 3.52–3.44 (m, 2H; CH_2O), 1.69–1.6 (m, 2H; CH_2) 1.44–1.27 (8H; 4CH_2), 0.96–0.88 (m, 3H; CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C): δ = 138.5, 136.9, 136.7, 127.9, 126.4, 126.3, 126.2, 113.7, 72.7, 70.6, 31.9, 29.9, 29.3, 26.3, 22.7, 14.2; **IR** (thin film): 2923 (s), 2856 (s), 1695 (s), 1458 (s), 1373 (s) 1023 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{16}H_{25}O$ (MH^+), 233.1905 found 233.1899.

1-((2-(2-ethoxyethoxy)ethoxy)methyl)-4-vinylbenzene (4)

[11]A suspension of NaH (0.178 g, 7.429 mmol, 2.1 equiv.) in THF (3 mL) was slowly added to diethyleneglycol monoethylether (2.326 g, 17.339 mmol, 4.9 equiv.) in THF (20 mL) at

0 °C for 2 h. A solution of *p*-chloromethylstyrene **2** (0.540 g, 3.538 mmol, 1 equiv.) in THF (3 mL) was slowly added to the reaction mixture at °C. The mixture was allowed to reflux for 24 h. Subsequently, water (4 mL) was added and the reaction mixture was extracted with dichloromethane (3 × 30 mL). The organic layer was concentrated *in vacuo* to afford the crude residue. Purification by column chromatography (10% ethyl acetate/hexane) afforded 0.77 g (87%) of ether **4** as colorless oil. TLC R_f = 0.30 (20% ethyl acetate/ hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.38 (d, J = 8 Hz, 2H; H_{Ar}), 7.29 (d, J = 8 Hz, 2H; H_{Ar}), 6.70 (dd, J = 18, 11.2 Hz, 1H; C*H*═CH_2), 5.73 (d, J = 17.6 Hz, 1H; CH═CH_2), 5.22 (d, J = 11.2 Hz, 1H; CH═CH_2), 4.55 (s, 2H; PhCH_2O), 3.7–3.57 (8H; OCH_2), 3.52 (q, J = 7.2 Hz, 2H; CH_2), 1.21 (t, J = 7.2, 3H; CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C): δ = 138.0, 137.1, 136.7, 128.1, 126.3, 113.8, 73.1, 70.81, 70.75, 69.9, 69.5, 66.8, 15.3; **IR** (thin film): 3065 (bs), 3025 (s) 2921 (s), 2853 (s), 1598 (s), 1491 (s), 1449 (s) 1025 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{15}H_{23}O_3$ (MH^+), 251.1647 found 251.1653.

(4-(4-vinylbenzyloxybenzylalcohol) (5)

To a mixture of 4-hydroxybenzaldehyde (1.190 g, 9.745 mmol, 1.3 equiv.) and anhydrous K_2CO_3 (1.340 g, 9.745 mmol, 1.3 equiv.) in CH_3CN (10 mL) was added *p*-chloromethylstyrene **2** (1.144 g, 7.496 mmol, 1 equiv.) in CH_3CN (10 mL). The mixture was allowed to reflux for 30 h, following which it was cooled to room temperature, filtered and washed with dichloromethane (3 × 20 mL). The filtrate was concentrated *in vacuo* to give a yellow oil, which was purified using column chromatography 10% ethyl acetate/hexane) to yield 1.54 g (86%) of the ether as a white solid. TLC R_f = 0.30 (10% ethyl acetate/ hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 9.89 (s, 1H; C*H*O), 7.84 (d, J = 8.4 Hz, 2H; H_{Ar}), 7.44 (d, J = 8 Hz, 2H; H_{Ar}), 7.38 (d, J = 8.4 Hz, 2H; H_{Ar}), 7.07 (d, J = 8.4 Hz, 2H; H_{Ar}), 6.72 (dd, J = 17.6, 11.2 Hz, 1H; C*H*═CH_2), 5.77 (d, J = 17.6 Hz, 1H; CH═CH_2), 5.27 (d, J = 10.8 Hz, 1H; CH═CH_2), 5.14 (s, 2H; CH_2O); **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C): δ = 191.0, 163.8, 137.8, 136.4, 135.5, 132.2, 130.3, 127.9, 126.7, 115.3, 114.6, 70.2; **IR** (KBr pellet): 2932 (s), 2877 (s), 2853 (s), 2726 (s), 1697 (bs), 1596 (s), 1513(s), 1464 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{16}H_{14}O_2$ Na (MNa^+) 261.0891, found 261.0891.

The above synthesized ether (1.420 g, 5.966 mmol, 1 equiv.) in methanol (25 mL) and THF (7 mL) at 0 °C was added sodium borohydride (0.441 g, 11.932 mmol, 2 equiv.). The reaction mixture was allowed to stir at room temperature for 3h, following which the volatile solvents were removed *in vacuo*. Water (25 mL) was added to the mixture and the resulting suspension was neutralized with 2N HCl. The reaction mixture was extracted with dichloromethane (3 × 50 mL) and washed with water (20 mL). The combined organic layers were dried over sodium sulphate, filtered and concentrated in vacuo to give a white solid. Purification using column chromatography (15% ethyl acetate/hexane) gave 1.37 g (96%) of 4-(4-vinylbenzyloxybenzylalcohol) **5** as a white solid. TLC R_f = 0.40 (20% ethyl acetate/ hexane). **^{1}H NMR**(400) MHz, $CDCl_3$, 25 °C): δ = 7.45-7.37 (4H; H_{Ar}), 7.29 (d, J = 8.4 Hz, 2H; H_{Ar}), 6.75 (d, J = 8.4 Hz, 2H; H_{Ar}), 6.72 (dd, J = 17.6, 10.8 Hz, 1H; C*H*═CH_2), 5.76 (d, J = 18 Hz, 1H; CH═CH_2), 5.25 (d, J = 10.8 Hz, 1H; CH═CH_2), 5.06 (s, 2H; PhCH_2O), 4.62 (s, 2H; PhCH_2OPh); **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C): δ = 158.5, 137.5, 136.6, 136.6, 133.6, 128.8, 127.8, 126.6, 115.1, 114.2, 70.0, 65.2; **IR** (KBr pellet): 3147 (bs), 2979 (s), 2867 (s), 1604 (bs), 1512 (s), 1405 (s) 1244 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{16}H_{17}O_2$ (MH^+) 241.1229, found 241.1230.

General Procedure for Polymerization

A solution containing required amounts of monomers **3**, **4,** and **5** in THF was added to a sealed tube and deoxygenated using a stream of nitrogen gas.[12] A deoxygenated solution of azobisisobutyronitrile (AIBN) in THF was added to this solution and the reaction mixture was allowed to stir at 80 °C for 3 days. The reaction mixture was concentrated *in vacuo* to a minimum volume. Cold hexane (20 mL) was added to obtain the polymer support as a precipitate. The precipitate was dissolved in CH_2Cl_2 (2 mL) and reprecipitated with cold hexane (3 × 20 mL). The precipitate was isolated and dried *in vacuo* to afford the polymer support **1** as a viscous liquid.

General Procedure for Determining Loading Capacity of Support 1 by ^{1}H NMR

The number .of attachment sites present per gram of polymer **1** (loading) was determined by recording the ^{1}H NMR spectrum of polymer **1** in the presence of a known amount of 1,1,2,2-tetrachloroethane (TCE). The integration of the peak at δ = 5.95 ppm for TCE was compared with the peak at δ = 4.9 ppm for the benzylic protons of the attachment site in polymer **1** to determine the number of attachment sites in polymer **1**.

General Procedure for Determining the *x*:*y*:*z* Ration of Supports 1

The *x: y: z* ratio of the polymer **1** was determined using ^{1}H NMR spectroscopy. The integration values of benzylic protons (δ = 4.9 and 4.56–4.20) and methyl protons of the alkyl chain (δ = 0.87) were used to get the *x*: *y*: *z* ratio.

Polymer 1a

AIBN (0.600 mg, 0.003 mmol, 1 equiv.) in THF (0.5 mL) was added to monomer **3** (41.76 mg, 0.18 mmol, 60 equiv.), monomer **4** (45.00 mg, 0.18 mmol, 60 equiv.), and monomer **5** (43.200 mg, 0.180 mmol, 60 equiv.) in THF (1 mL) to afford 92 mg (71%) of polymer **1a**. Loading = 1.5 mmol/g; *x*: *y*: *z* = 1:0.9:1. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.3–6.7 (10.7H; H_{Ar}), 6.7–6.2 (5.5H; H_{Ar}), 4.90 (bs, 2H; PhOCH_2OH), 4.6–4.2 (5.8H; PhCH_2O), 3.7–3.2 (11.7H; $OCH_{2(alk,\ deg)}$), 1.8–1.1 (23.0H; PhC*H*CH_2, $CH_{2(alk,\ deg)}$), 0.87 (bs, 3H; $CH_{3(alk)}$).

Polymer 1b

AIBN (2 mg, 0.012 mmol, 1 equiv.) in THF (0.5 mL) was added to monomer **3** (339.184 mg, 1.462 mmol, 120 equiv.), monomer **4** (365.500 mg, 1.462 mmol, 120 equiv.) and monomer **5** (350.880 mg, 1.462 mmol, 120 equiv.) in THF

(2 mL) to afford 919 mg (87%) of polymer **1b**. Loading = 1.2 mmol/g; x: y: z = 0.8:0.6:1. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.3–6.7 (10.0H; H_{Ar}), 6.7–6.2 (4.9H; H_{Ar}), 4.90 (bs, 2H; PhOCH_2OH), 4.6–4.2 (5.2H; PhCH_2O), 3.7–3.2 (9.2H;OC$H_{2(alk,\ deg)}$), 2.1–1.1 (21.3H; PhCHCH_2, C$H_{2(alk,\ deg)}$), 0.87 (s, 2.6H; C$H_{3(alk)}$).

Polymer 1c

AIBN (0.400 mg, 0.002 mmol, 1 equiv.) in THF (0.5 mL) was added to monomer **3** (111.36 mg, 0.48 mmol, 240 equiv.), monomer **4** (120.00 mg, 0.48 mmol, 240 equiv.) and monomer **5** (57.600 mg, 0.240 mmol, 120 equiv.) in THF (1 mL) to afford 251 mg (87%) of polymer **1c**. Loading = 1.3 mmol/g; *x*: *y*: *z* = 2.2:2.1:1. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.3–6.7 (17.4H; H_{Ar}), 6.7–6.2 (10.6H; H_{Ar}), 4.92 (bs, 2H; PhOCH_2OH), 4.6–4.2 (11.1H; PhCH_2O), 3.7–3.2 (27.5H; OC$H_{2(alk,\ deg)}$), 2.1–1.5 (16.0H; PhCHCH_2,) 1.4–1.1 (m, 35H; C$H_{2(alk,\ deg)}$), 0.88 (s, 7H; C$H_{3(alk)}$).

Polymer 1d

AIBN (1.000 mg, 0.006 mmol, 1 equiv) in THF (0.5 mL) was added to monomer **3** (334.08 mg, 1.44 mmol, 240 equiv.), monomer **4** (540.00 mg, 2.16 mmol, 360 equiv.) and monomer **5** (172.00 mg, 0.72 mmol, 120 equiv.) in THF (2 mL) to afford 794 mg (76%) of polymer **1d**. Loading = 0.8 mmol/g; *x*: *y*: *z* = 1.8:2.6:1. **^{1}H NMR** (400) MHz, $CDCl_3$, 25 °C): δ = 7.3–6.7 (17.8H; H_{Ar}), 6.7–6.2 (10.0H; H_{Ar}), 4.9 (bs, 2H; PhOCH_2OH), 4.6–4.2 (10.1H; PhCH_2O), 3.7–3.2 (28.7H; OC$H_{2(alk,\ deg)}$), 1.9–1.5 (15.5H; PhCHCH_2,) 1.4–1.1 (32.5H; C$H_{2(alk,\ deg)}$), 0.88 (s, 6H; C$H_{3(alk)}$).

Oligomer 1e

AIBN (30.0 mg, 0.182 mmol, 1 equiv.) in THF (0.5 mL) was added to monomer **3** (422.24 mg, 1.82 mmol, 10 equiv.), monomer **4** (455.00 mg, 1.82 mmol, 10 equiv.) and monomer **5** (436.80 mg, 1.82 mmol, 10 equiv.) in THF (2 mL) to afford 1.077 g (82%) of polymer **1e**. Loading = 1.5 mmol/g; *x*: *y*: *z* = 1.3:0.75:1. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.3–6.7 (11.5H; H_{Ar}), 6.7–6.3 (5.8H; H_{Ar}), 4.91 (bs, 2H; PhOCH_2OH), 4.7–4.2 (5.7H; PhCH_2O), 3.7–3.3 (11.1H;OC$H_{2(alk,\ deg)}$), 1.9–1.1 (26.3H; PhCHCH_2, C$H_{2(alk,\ deg)}$), 0.88 (s, 3.9H; C$H_{3(alk)}$).

General Procedure for Synthesis of 6

To a solution of **1e** (1 equiv.) in THF was added the required amino acid (2 equiv.), DMAP (0.2 equiv.), and DIC (2 equiv.). The reaction mixture was allowed to stir for 2 h. The reaction mixture was filtered to remove diisopropylurea. The filtrate was concentrated *in vacuo* to a minimum volume. Cold 20% IPA/hexane (20 mL) was added to obtain the amino acid attached polymer as a precipitate. The precipitate was dissolved in 0.2–0.5 mL of THF and reprecipitated with cold 20% IPA/Hexane (3 × 15 mL) to afford amino acid attached oligomer **6**.

General Procedure for Determination of Amino Acid Loading

The loading capacities of oligomers **6(a–j)** 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 = 5.9 ppm corresponding to TCE was compared with the peak at δ = 7.7 ppm for the Fmoc protons of polymers **6(a–j)**.

6a

Oligomer **1e** (0.800 g, 0.904 mmol), Fmoc-L-Ala-OH (562.288 mg, 1.808 mmol), DMAP (22.057 mg, 0.1808 mmol), DIC (283.955 μL, 1.808 mmol, 2 equiv.), and THF (10 mL) were used for the reaction. 863 mg (81%) of **6a** was obtained. Loading = 1.3 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.73 (bs, 2H; $H_{Ar(Fmoc)}$), 7.57 (bs, 2H; $H_{Ar(Fmoc)}$), 7.36 (bs, 2H; $H_{Ar(Fmoc)}$), 7.2–6.7 (13.8H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.3 (4.9H; H_{Ar}), 5.50 (bs, 0.68H; N*H*), 5.09 (bs, 2H; PhOCH_2OH$_{(Att)}$), 4.9 (bs, 1.9H; PhOC$H_{2(Att)}$), 4.5–4.3 (6.3H; PhCH_2O, C$H_{2(Fmoc)\ and}$ C$H_{(Ala)}$), 4.19 (s, 1.1; C$H_{(Fmoc)}$), 3.7–3.3 (10.5H; OC$H_{2(alk,\ deg)}$), 1.9–1.1 (28.3H; PhCHCH_2, C$H_{3(Ala)\ and\ (deg)\ and}$ C$H_{2(alk)}$), 0.86 (s, 3.2H; C$H_{3(alk)}$).

6b

Oligomer **1e** (350.000 mg, 0.532 mmol), Fmoc-L-Phe-OH (411.768 mg, 1.064 mmol), DMAP (12.932 mg, 0.106 mmol), DIC (167.106 μL, 1.064 mmol) and THF (5 mL) were used for the reaction. 469 mg (85%) of **6b** was obtained. Loading = 1.13 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.74 (s, 2H; $H_{Ar(Fmoc)}$), 7.54 (s, 2H; $H_{Ar(Fmoc)}$), 7.4–6.7 (17.8H; $H_{Ar,\ Ar(Phe)\ and\ Ar(Fmoc)}$), 6.7–6.2 (5.3H; H_{Ar}), 5.36 (bs, 0.7H; N*H*), 5.2–4.7 (3.7H; PhOCH_2OH$_{(Att)\ and}$ PhOC$H_{2(Att)}$), 4.67 (s, 0.9H; C$H_{(Phe)}$), 4.5–4.2 (5.8H; PhCH_2O, C$H_{2(Fmoc)}$), 4.17 (s, 0.9H; C$H_{(Fmoc)}$), 3.7–3.3 (10.6H; OC$H_{2(alk,\ deg)}$), 3.09 (bs, 1.5H; C$H_{2(Phe)}$), 1.8–1.1 (24.9H; PhCHCH_2, C$H_{3(deg)\ and}$ C$H_{2(alk)}$), 0.87 (s, 3.8H; C$H_{3(alk)}$).

6c

Oligomer **1e** (120 mg, 0.180 mmol), Fmoc-L-Ile-OH (127.08 mg, 0.36 mmol), DMAP (4.392 mg, 0.036 mmol), DIC (56.539 μL, 0.360 mmol) and THF (3 mL) were used for the reaction. 126 mg (70%) of **6c** was obtained. Loading = 1.12 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.74 (s, 2H; $H_{Ar(Fmoc)}$), 7.58 (s, 1.8H; $H_{Ar(Fmoc)}$), 7.37 (s, 1.9H; $H_{Ar(Fmoc)}$), 7.3–6.7 (16.1H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.2 (6.6H; H_{Ar}), 5.42(bs, 0.8H; N*H*), 5.2–5.00 (2H; PhOCH_2OH$_{(Att)}$), 4.84 (bs, 2.4H; PhOC$H_{2(Att)}$) 4.5–4.3 (bs, 7.69H; PhCH_2O, C$H_{2(Fmoc)\ and}$ C$H_{(Ile)}$), 4.21 (s, 1.1; C$H_{(Fmoc)}$), 3.7–3.3 (13.4H; OC$H_{2(alk,\ deg)}$), 2.17(bs, 1H; C$H_{(Ile)}$), 1.8–1.1 (30.9H; PhCHCH_2, C$H_{3\ (deg)\ and}$ C$H_{2(alk)}$), 1.0–0.7 (10.7H; C$H_{3(alk)\ and\ (Ile)}$).

6d

Oligomer **1e** (106.000 mg, 0.159 mmol), Fmoc-L-Val-OH (107.929 mg, 0.318 mmol), DMAP (3.782 mg, 0.031 mmol), DIC (49.943 μL, 0.318 mmol) and THF (3 mL) were used for the reaction. 129 mg (82%) of **6d** was obtained. Loading = 1.0 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.73 (s, 2H; $H_{Ar(Fmoc)}$), 7.57 (s, 2H; $H_{Ar(Fmoc)}$), 7.52 (s, 2H; $H_{Ar(Fmoc)}$), 7.3–6.7 (13.4H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.2 (5.6H; H_{Ar}), 5.34(s, 0.81H; N*H*), 5.09 (s, 2H; PhOCH_2OH$_{(Att)}$), 4.83(s, 1.9H; PhOC$H_{2(Att)}$), 4.5–4.3 (7.69H; PhCH_2O, C$H_{2(Fmoc)\ and}$ C$H_{(Val)}$), 4.19 (s, 1.1; C$H_{(Fmoc)}$), 3.7–3.3 (11H; OC$H_{2(alk,\ deg)}$), 1.9–1.1 (28.5H; PhCHCH_2, C$H_{3\ (deg)}$, C$H_{(Val)\ and}$ C$H_{2(alk)}$), 1–0.7 (10.2H; C$H_{3(alk)\ and\ (Val)}$).

6e

Oligomer **1e** (143.000 mg, 0.214 mmol), Fmoc-L-His(Trt)-OH (265.855 mg, 0.429 mmol), DMAP (5.124 mg, 0.042 mmol) and DIC (67.376 μL, 0.429 mmol) and THF (3 mL) were used for the reaction. 186 mg (69%) of **6e** was obtained. Loading = 0.81 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.72 (s, 1.4H; $H_{Ar(Fmoc)}$), 7.60 (s, 1.34H; $H_{Ar(Fmoc)}$), 7.45 (s, 0.8H; N=C$H_{(His)}$), 7.3–6.3 (31H; $H_{Ar,\ Ar(Trt)\ and\ Ar(Fmoc)}$), 5.1–4.1 (9.6H; PhOC$H_2OH_{(Att)}$, PhOC$H_{2(Att,\ alk\ and\ deg)}$, C$H_{(His)}$, C$H_{2(Fmoc)\ and}$ C$H_{(Fmoc)}$), 3.7–3.3 (9.3H; OC$H_{2(alk,\ deg)}$), 3.09 (s, 1.3H; C$H_{2(His)}$), 2.2–1.1 (24.5H; PhCHCH_2, C$H_{3\ (deg)\ and}$ C$H_{2(alk)}$), 1–0.7 (3.7H; C$H_{3(alk)}$).

6f

Oligomer **1e** (112.000 mg, 0.168 mmol), Fmoc-L-Pro-OH (113.359 mg, 0.336 mmol), DMAP (4.099 mg, 0.033 mmol), DIC (52 μL, 0.336 mmol) and THF (3 mL) were used for the reaction. 117 mg (71%) of **6f** was obtained. Loading = 1.02 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.8–7.65 (2H; $H_{Ar(Fmoc)}$), 7.6–7.45 (2.1H; $H_{Ar(Fmoc)}$), 7.4–6.7 (16.6H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.2 (5.13H; H_{Ar}), 5.2–4.9 (1.8H; PhO-CH_2OH$_{(Att)}$), 4.85–4.5 (1.9H; PhOC$H_{2(Att)}$), 4.5–3.8 (7.14H; PhCH_2O, C$H_{2(Fmoc)\ and}$ C$H_{(Pro)}$, C$H_{(Fmoc)}$), 3.7–3.3 (12.3H; OC$H_{2(alk,\ deg)}$), 2.3–1.5 (18.6H; PhCHCH_2, 2C$H_{2(Pro)}$), 1.45–1.1 (16.1H; C$H_{3\ (deg)\ and}$ C$H_{2(alk)\ \&}$), 0.85 (bs, 3.71H; C$H_{3(alk)}$).

6g

Oligomer **1e** (0.800 g, 0.904 mmol), Fmoc-L-Ala-OH (562.288 mg, 1.808 mmol), DMAP (22.057 mg, 0.1808 mmol), DIC (283.955 μL, 1.808 mmol, 2 equiv.) and THF (10 mL) were used for the reaction. 863 mg (81%) of **6g** was obtained. Loading = 1.3 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.73 (bs, 2H; $H_{Ar(Fmoc)}$), 7.57 (bs, 2H; $H_{Ar(Fmoc)}$), 7.36 (bs, 2H; $H_{Ar(Fmoc)}$), 7.2–6.7 (13.8H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.3 (4.9H; H_{Ar}), 5.50 (bs, 0.68H; NH), 5.09 (bs, 2H; PhOCH_2OH$_{(Att)}$), 4.9 (bs, 1.9H; PhOC$H_{2(Att)}$), 4.5–4.3 (6.3H; PhCH_2O, C$H_{2(Fmoc)\ and}$ C$H_{(Ala)}$), 4.19 (s, 1.1; C$H_{(Fmoc)}$), 3.7–3.3 (10.5H; OC$H_{2(alk,\ deg)}$), 1.9–1.1 (28.3H; PhCHCH_2, C$H_{3(Ala)\ and\ (deg)\ and}$ C$H_{2(alk)}$), 0.86 (s, 3.2H; C$H_{3(alk)}$).

6h

Oligomer **1e** (116 mg, 0.174 mmol), Fmoc-L-Gly-OH (103.467 mg, 0.348 mmol), DMAP (4.148 mg, 0.034 mmol), DIC (54.655 μL, 0.348 mmol) and THF (10 mL) were used for the reaction. 141 mg (85%) of **6h** was obtained. Loading = 1.29 mmol/g. **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C): δ = 7.78 (s, 2H; $H_{Ar(Fmoc)}$), 7.74 (s, 2H; $H_{Ar(Fmoc)}$), 7.58 (s, 2H; $H_{Ar(Fmoc)}$), 7.4–6.7 (14.1H; $H_{Ar\ and\ Ar(Fmoc)}$), 6.7–6.3 (5.5H; H_{Ar}), 5.5 (bs, 0.57H; NH), 5.09 (bs, 1.7H; PhOCH_2OH$_{(Att)}$), 4.84 (bs, 2H; PhOC$H_{2(Att)}$) 4.5–4.3 (5.6H; PhCH_2O, C$H_{2(Fmoc)}$), 4.23 (s, 1.2H; C$H_{(Fmoc)}$), 3.95 (s, 1.9H; C$H_{2(Gly)}$), 3.7–3.3 (11.1H; OC$H_{2(alk,\ deg)}$), 1.9–1.1 (27.5H; PhCHCH_2, C$H_{3(deg)\ and}$ C$H_{2(alk)}$), 0.86 (s, 3.6H; C$H_{3(alk)}$).

General Procedure for Tripeptide Synthesis

Oligomer **6** (0.1 g, 0.1 mmol, 1 equiv.) was treated to a solution of 20% piperidine in THF (1 mL) for 10 min. Subsequently cold hexane (5 mL) was added to the reaction mixture. The resulting 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-OH (2 equiv.), HCTU (2 equiv.) in THF (2 mL) was added to the free amine, following which DIEA (3 equiv.) was added and the reaction was allowed to stir for 2 h. The reaction mixture was concentrated to a minimum volume (1 mL) and water (15 mL) was added to it. The suspension was centrifuged and the supernatant was decanted to afford the support containing dipeptide as a precipitate. The precipitate was dissolved in THF (0.5 mL) and reprecipitated with water (2 × 15 mL). The precipitate was washed with methanol (3 × 10 mL) and dried *in vacuo*. The deprotection/coupling steps were repeated as described above to get the Fmoc protected tripeptide attached to the support. The tripeptide was cleaved from the support using 20% TFA in CH_2Cl_2 for 3 h. The reaction mixture was concentrated *in vacuo* to a minimum volume. Methanol was added to reaction mixture to precipitate the oligomer. The supernatant containing the peptide was concentrated and purified by semi-preparative RP-HPLC to give the pure tripeptide.

General Procedure for HPLC Purification and Analysis of Peptides

All peptides were purified by semi preparative RP-HPLC (Reverse Phase High Performance Liquid Chromatography) on a Waters HPLC system with water (0.1% TFA) and methanol (0.1% TFA) as mobile phase solvent. The flow rate used for analytical RP-HPLC and semipreparative RP-HPLC were 1 mL/min and 4.1 mL/min, respectively. Peptide was injected at a concentration of 1.5 mg/mL for analytical HPLC and 10 mg/mL for semipreparative HPLC. The column used for semipreparative HPLC was a Sunfire Prep C18, 5 μm. Peptide elution was monitored at 254 and 220 nm with the Waters 2489 UV/visible detector. The gradient system shown in Supporting Information Table S1 was used to separate the peptides.

Analytical Data for Tripeptides

Fmoc-Phe-Val-Ala-OH (7a)

^{1}H NMR (400 MHz, DMSO-d6, 25 °C): δ = 8.28 (d, J = 7.2 Hz, 1H; NH), 7.90-7.82 (3H; $H_{(Fmoc)}$ and NH), 7.65–7.58 (3H; NH and $H_{(Fmoc)}$), 7.44-7.37 (m, 2H; $H_{(Fmoc)}$), 7.34-7.14 (7H; $H_{(Fmoc)}$ and $H_{Ar(Phe)}$), 4.38-4.13 (6H, C$H_{(Val)}$ C$H_{(Phe)}$ C$H_{(Ala)}$ CHC$H_{2\ (Fmoc)}$), 2.99 (dd, J = 11.2, 3.6 Hz, 1H; C$H_{2(Phe)}$), 2.77 (dd, J = 11.2, 11.2 Hz, 1H; C$H_{2(Phe)}$), 2.04-1.92 (m, 1H; C$H_{(Val)}$), 1.27 (d, J = 7.6, Hz, 3H; C$H_{3(Ala)}$) 0.93–0.82 (6H; C$H_{3(Val)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C): δ = 173.9, 171.3, 170.5, 155.7, 143.71, 143.69, 140.6, 138.1, 129.2, 128.0, 127.6, 127.0, 126.2,125.3, 125.2, 120.0, 65.6, 57.1, 56.0, 47.5, 46.5, 37.3, 31.0, 19.1, 18.0, 17.0; **IR** (KBr pellet): 3434 (bs), 3293 (s), 3064 (s), 2960 (s), 2926 (s), 1694 (bs), 1541 (s), 1448 (s) 1262 (s), 1086 (s), 1041 (s). cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{32}H_{35}N_3O_6Na$ (MNa^+) 580.2424, found 580.2417.

Fmoc-Ala-Phe-Ala-OH (7b)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.20 (d, J = 6 Hz, 1H; N*H*), 7.89 (d, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.71 (t, J = 6.4 Hz, 2H; $H_{(Fmoc)}$), 7.47 (d, J = 7.6 Hz, 1H; N*H*), 7.41 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.33 (t, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.26-7.12 (5H; $H_{Ar(Phe)}$), 4.51 (q, J = 8.8, 1H; C$H_{(Ala)}$), 4.30–4.15 (4H; C$H_{(Ala)}$, C$H_{2(Fmoc)}$ and C$H_{(Phe)}$), 3.98 (t, J = 7.2 **Hz**, 1H ; C$H_{(Fmoc)}$), 3.04 (dd, J = 14, 4 Hz, 1H; C$H_{2(Phe)}$), 2.8 (dd, J = 13.6, 9.2 Hz, 1H; C$H_{2(Phe)}$), 1.27 (d, J = 7.2 Hz, 3H; C$H_{3(Ala)}$), 1.12 (d, J = 7.2 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C): δ = 173.8, 172.1, 170.6, 155.7, 143.8, 140.7, 137.6, 129.2, 127.8, 127.6, 126.1, 125.2, 120.0, 65.6, 53.3, 50.2, 47.5, 46.6, 37.3, 18.0, 17.1; **IR** (KBr pellet): 3388 (bs), 2977 (s), 2933 (s), 1689 (s), 1591 (s), 1405 (s), 1166 (s), 1071 (s), 1025 cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{30}H_{31}N_3O_6Na$ (MNa^+) 552.2105, found 552.2109.

Fmoc-Ala-Pro-Ala-OH (7c)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.09 (d, J = 7.2 Hz, 1H; N*H*), 7.88 (d, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.71 (t, J = 6.4 Hz, 2H; $H_{(Fmoc)}$), 7.55 (d, J = 7.6 Hz, 1H; N*H*), 7.42 (t, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.32 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 4.38-4.11 (6H; C$H_{(Ala),}$C$HCH_{2(Fmoc)}$ C$H_{(Pro)}$), 3.62-3.5 (m, NCH_2), 2.05–1.75 (4H; CH_2), 1.25 (d, J = 7.6 Hz, 3H; C$H_{3(Ala)}$), 1.19 (d, J = 6.8 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C): δ = 174.1, 171.3, 170.8, 143.9, 140.7, 127.6, 127.1, 125.3, 120.1, 65.6, 58.9, 47.9, 47.4, 46.7, 28.9, 24.4, 17.1, 16.8; **IR** (KBr pellet): 3343(bs), 2934 (s), 1691 (s), 1646 (s), 1590 (s), 1542 (s), 1272 (s), 1025 cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{26}H_{29}N_3O_6Na$ (MNa^+) 502.1954, found 502.1955.

Fmoc-Phe-Phe-Phe-OH (7d)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.31 (bs, 1H; N*H*), 8.04 (d, J = 8.4 Hz, 1H; N*H*), 7.87 (d, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.60 (t, J = 7.6 Hz, 2H, $H_{(Fmoc)}$), 7.50 (d, J = 8.8 Hz, 1H; N*H*), 7.40 (t, J =8, 2H; $H_{(Fmoc)}$), 7.33-7.10 (17H, $H_{(Fmoc)}$ and $H_{Ar(Phe)}$), 4.62-4.53 (m, 1H; C$H_{(Phe)}$), 4.50-4.40 (m, 1H; C$H_{(Phe)}$), 4.23-4.04 (4H; C$H_{(Phe)}$, CHCH_2 $_{(Fmoc)}$), 3.11-2.62 (6H; C$H_{2(Phe)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C)**:** δ = 171.2, 171.0, 155.6, 143.8, 143.7, 140.6, 138.1, 137.5, 129.3, 129.1, 128.2, 128.0, 127.6, 127.1, 126.4, 126.23, 126.17, 125.3, 125.2, 120.1, 65.6, 56.0, 53.6, 46.5, 38.5, 37.6, 37.4, 36.7; **IR** (KBr pellet): 3286 (bs), 3054 (s), 2922 (s), 2847 (s), 1688 (bs), 1645, 1542 (s), 1256 (s) cm^{-1}; **HRMS (ESI+):** calcd. for $C_{42}H_{39}N_3O_6Na$ (MNa^+) 704.2737, found 704.2727.

Fmoc-Gly-Val-Phe-OH (7e)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.29 (d, J = 7.6 Hz, 1H; N*H*), 7.89 (d, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.73–7.65 (3H; $H_{(Fmoc)}$ and N*H*), 7.53 (t, J = 6 Hz, 1H; N*H*), 7.42 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.32 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.28–7.15 (5H; $H_{Ar(Phe)}$), 4.44-4.37 (m, 1H; C$H_{(Val)}$), 4.29–4.20 (4H; C$H_{(Phe)}$, CHCH_2 $_{(Fmoc)}$), 3.71–3.59 (m, 2H; C$H_{2(Gly)}$), 3.01 (dd, J = 14, 5.6 Hz, 1H; C$H_{2(Phe)}$), 2.89 (dd, J = 14, 9.2 Hz, 1H; C$H_{2(Phe)}$), 1.98-1.88 (m, 1H; C$H_{(Val)}$), 0.82 (d, J = 6.8 Hz, 3H; C$H_{3(Val)}$) 0.76 (d, J = 6.4 Hz, 3H; C$H_{3(Val)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C)**:** δ = 172.7, 170.8, 168.7, 156.4, 143.8, 140.7, 137.5, 129.0, 128.1, 127.6, 127.1, 126.4, 125.2, 120.1, 65.7, 57.0, 53.4, 46.6, 43.3, 36.6, 30.9, 29.0, 19.1, 17.8; **IR** (KBr pellet): 3372 (bs), 2967 (s), 2931 (s), 1669 (bs), 1591 (s), 1404 (s) 1210 (s), 1053 (s), 1024 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{31}H_{33}N_3O_6Na$ (MNa^+) 566.2262, found 566.2276.

Fmoc-Phe-Ile-Phe-OH (7f)

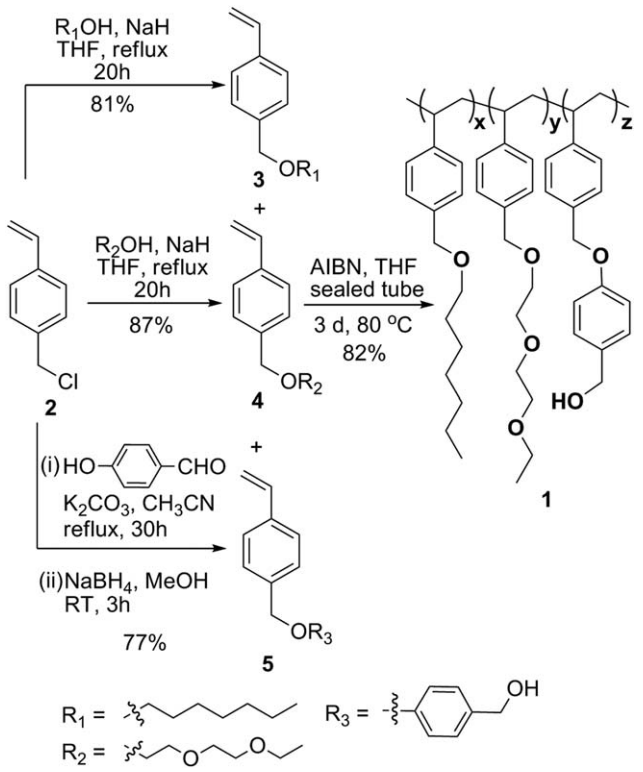
^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.20 (bs, 1H; N*H*), 7.89–7.80 (3H; $H_{(Fmoc)}$ and N*H*), 7.63–7.57 (3H, $H_{(Fmoc)}$ and N*H*), 7.40 (t, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.32-7.12 (12H, $H_{(Fmoc)}$ and $H_{Ar(Phe)}$), 4.45-4.38 (m, 1H; C$H_{(Phe)}$), 4.34-4.20 (2H; C$H_{(Ile)}$ C$H_{(Phe)}$), 4.18-4.0 (3H; CHCH_2 $_{(Fmoc)}$), 3.05 (dd, J = 13.6, 5.2 Hz, 1H; C$H_{2(Phe)}$), 2.95–2.85 (m, 2H; C$H_{2(Phe)}$), 2.73 (dd, J = 11.2, 13.6 Hz, 1H; C$H_{2(Phe)}$), 1.75–1.65 (m, 1H; C$H_{(Ile)}$), 1.45–1.30 (m, 1H; C$H_{2(Ile))}$, 1.09-0.98 (m,1H; C$H_{2(Ile)}$), 0.90-0.76 (6H, CH_3); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C): δ = 171.2, 170.8, 155.7, 143.7, 140.6, 138.2, 137.6, 129.2, 129.0, 128.1, 127.9, 127.6, 127.0, 126.3, 126.1, 125.3, 125.2, 120.0, 65.6, 56.6, 55.9, 53.3, 28.9, 24.0, 15.2, 11.0; **IR** (KBr pellet): 3316 (s), 3274 (s), 3069 (s), 3029 (s), 2963 (s), 2926 (s), 2877 (s), 1714 (s), 1643 (s), 1537 (s), 1450 (s), 1256 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{39}H_{41}N_3O_6$ (MNa^+) 670.2888, found 670.2901.

General Procedure for Hexapeptide Synthesis

Oligomer **6** (0.1 g, 0.1 mmol, 1 equiv.) was treated to a solution of 20% piperidine in DMF (1 mL) for 10 min. Hexane (5 mL) was added and the reaction mixture was allowed to stir for 1 min. The hexane layer was decanted and this process repeated to afford the support containing the free amine as a precipitate, which was dried in vacuo. A solution of Fmoc-AA_1-AA_2-OH[13] (2 equiv.), HCTU (2 equiv.) in THF (2 mL) was added to the free amine, following which DIEA (3 equiv.) was added and the reaction was allowed to stir for 4 h. The reaction mixture was concentrated to a minimum volume (1 mL) and water (15 mL) was added to it. The suspension was centrifuged and the supernatant was decanted to afford the support containing dipeptide as a precipitate. The precipitate was dissolved in THF (0.5 mL) and re-precipitated with water (2 × 15 mL). The precipitate was washed with methanol (3 × 10 mL) and dried in vacuo. The deprotection and coupling steps were repeated with Fmoc-AA-OH as described above to get the Fmoc protected hexapeptide attached to the support. The hexapeptide was cleaved by treating the support with 20% TFA in CH_2Cl_2 for 3 h. The reaction mixture was concentrated in vacuo to a minimum volume and methanol was added to reaction mixture to precipitate the oligomer. The supernatant containing the peptide was concentrated and purified by semi-preparative RP-HPLC to give the pure hexapeptide.

Fmoc-Ala-Gly-Val-Ile-Pro-Phe-OH (7g)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 8.14 (t, J = 6 Hz, 1H; N*H*), 8.01 (d, J = 7.6 Hz, 2H; N*H*), 7.89 (d, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.74–7.68 (2H; N*H*), 7.59 (d, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.41 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.33 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.27–7.17 (5H; $H_{Ar\ and\ (Phe)}$), 4.42–4.17 (7H; C$H_{(Val),\ (Ala),\ (Phe),\ (pro),\ (Ile)\ and}$ CHCH_2 $_{(Fmoc)}$), 4.04 (t, J = 1H;



SCHEME 1 Modular synthetic approach for obtaining support.

C*H*CH$_{2\ (Fmoc)}$), 3.83–3.67 (3H; C$H_{2(Gly)\ and}$ NC$H_{2(pro))}$, 3.55–3.45 (m, 1H; NC$H_{2(pro))}$, 3.02–2.87 (2H; C$H_{2(Phe)}$), 1.98-1.67 (6H; C$H_{(Ile)}$, C$H_{(Val)\ and}$ NC$H_{2(pro)}$), 1.55–1.46 (m, 1H; C$H_{2(Ile))}$, 1.23 (d, J = 6.8 Hz, 3H; C$H_{3(Ala)}$), 1.09–0.98 (m, 1H; C$H_{2(Ile)}$), 0.86–0.70 (12H; C$H_{3(Val)\ and\ (Ile)}$); **^{13}C NMR** (125 MHz, DMSO-*d6*, 25 °C): δ = 172.9 172.8, 171.5, 170.7, 169.9, 168.5, 155.8, 143.9, 143.8, 140.7, 137.3, 129.2,128.2, 127.6, 127.1, 126.5, 125.3, 125.3, 120.1, 65.7, 59.0, 56.9, 54.6, 53.6, 50.2, 47.1, 46.6, 42.1, 36.7, 35.8, 30.8, 29.0, 24.3, 19.0, 18.0, 17.9, 14.9, 10.7; **IR** (KBr pellet): 3403 (bs), 3303 (bs), 2964 (s), 2929 (s), 1649 (bs), 1628, 1526, (s), 1450 (s) 1198 (bs), cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{45}H_{57}N_6O_9$ (M^+) 825.4187, found 825.4169.

TABLE 1 Supports (Supp.) **1a–e** Synthesized for Peptide Synthesis

Supp.	n[a](3:4:5)	Yield (%)	Loading[b] (mmol/g)	x:y:z[b]
1a	60(1:1:1)	71	1.5	1:0.9:1
1b	120(1:1:1)	87	1.2	0.8:0.6:1
1c	120(2:2:1)	87	1.3	2:2.1:1
1d	120(2:3:1)	67	0.8	1.8:2.6:1
1e	10(1:1:1)	82	1.5	1.3:0.8:1

[a] n = equiv. with respect to AIBN.
[b] Determined by ^{1}H NMR.

TABLE 2 Attachment of Amino Acids onto Support **1e**.

No.	AA_1	Yield (%)[a]	Loading (mmol/g)[b]
1.	**6a**: Ala	81	1.3
2	**6b**: Phe	85	1.13
3	**6c**: Ile	70	1.12
4	**6d**: Val	59	1.0
6	**6e**: His (Trt)	69	0.81
7	**6f**: Pro	71	1.02
8	**6g**: Ala	86	1.35
9	**6h**: Gly	85	1.29

Isolated yield
Determined by ^{1}H NMR.

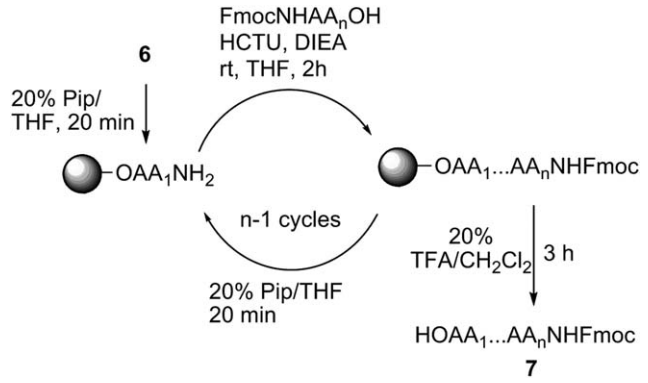
Fmoc-Ala-Gly-Val-Ile-Pro-Ala-OH (7h)

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): (extra solvent peaks seen) δ = 8.19–8.08 (2H; N*H*), 8.00 (d, J = 8 Hz, 1H; N*H*), 7.89 (d, J = 7.6 Hz, 2H; $H_{(Fmoc)}$), 7.74–7.68 (2H; N*H*), 7.59 (d, J = 8 Hz, 2H; $H_{(Fmoc)}$), 7.41 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 7.33 (t, J = 7.2 Hz, 2H; $H_{(Fmoc)}$), 4.34–4.00 (8H; C$H_{(Val),\ (Ala),\ (Phe),\ (Pro),\ (Ile)\ and}$ C*H*C$H_{2\ (Fmoc)}$) 3.83–3.67 (4H; C$H_{2(Gly)\ and}$ NC$H_{2(pro))}$, 1.99–1.71 (6H; C$H_{(Ile)}$, C$H_{(Val)\ and}$ NC$H_{2(pro)}$), 1.54–1.47 (m, 1H; C$H_{2(Ile))}$, 1.28–1.19 (6H; C$H_{3(Ala)}$), 1.09–1.0 (m, 1H; C$H_{2(Ile)}$), 0.88–0.72 (12H; C$H_{3(Val)\ and\ (Ile)}$); **^{13}C NMR** (100 MHz, DMSO-*d6*, 25 °C): δ = 174.0, 172.8, 171.2, 170.7, 169.8, 168.4, 155.5, 143.9, 140.7, 127.6, 127.1, 125.2, 120.1, 65.7, 58.9, 57.0, 54.6, 50.2, 47.4, 47.1, 46.6, 42.1, 35.8, 30.8, 29.0, 24.4, 24.3, 19.0, 17.9, 17.0, 14.8, 10.7; **IR** (KBr pellet): 3216 (bs), 2922 (s), 2508 (s), 1664 (bs), 1454 (s) 1193 (bs) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{39}H_{53}N_6O_9$ (M^+) 749.3874, found 749.3882.

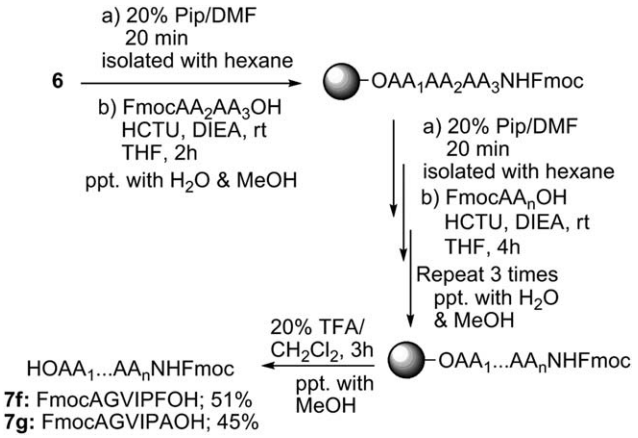
RESULTS AND DISCUSSION

Support Synthesis

The monomers for synthesizing the support could be readily obtained starting from 4-vinylbenzylchloride **2** (Scheme 1). Treatment of the chloride **2** with sodium hydride and the requisite alcohol afforded monomers **3** and **4** in 81 and 87% yields, respectively. Monomer **5** was obtained in 77% yield by treatment of chloride **2** with 4-hydroxy benzaldehyde in the presence of K_2CO_3 and subsequent reduction with sodium borohydride. Supports **1** were obtained from monomers **3**, **4** and **5** via free radical polymerization using AIBN as the initiator. Such a modular synthetic approach was proposed to facilitate easy incorporation of any styrene based attachment site into the soluble polymer backbone. The polymerization was carried out in THF for 3 days at 80 °C in a sealed tube. Initially, polymer supports **1a, b** ($x + y + z$ = 60 and $x + y + z$ = 120) with a 1:1:1 monomer ratio was synthesized (Table 1). However, these polymers were sparingly soluble in organic solvents such as dichloromethane and THF. Increasing the proportion of the solubilizing groups did not improve the solubility of the polymer (**1c** and **d**, Table 1). Oligomer **1e** was found to be the most optimal



SCHEME 2 Peptide synthesis using support **6**.



SCHEME 3 Hexapeptide synthesis using combination approach.

support for peptide synthesis as it was highly soluble in organic solvents and could be recovered using cold hexane or water. The supports were found to be partially soluble in methanol.[14] The loading capacity of supports **1e** could be easily determined using ^{1}H NMR spectroscopy in the presence of 1,1,2,2-tetrachloroethane (TCE) as a standard.

Amino Acid Attachment

Nonpolar, polar and basic amino acids amino acids were loaded onto the supports **1e** using DIC and DMAP in 59–86% yields (eq 1, Table 2). The loaded supports **6** could be readily characterized using ^{1}H NMR spectroscopy as they were soluble in organic solvents, such as dichloromethane, THF, and DMF. The integration values corresponding to TCE was compared with those of the aromatic protons of Fmoc. The loading capacities of the supports **6** (0.87–1.3 mmol/g) were similar to those typically found in the SPPS resins.

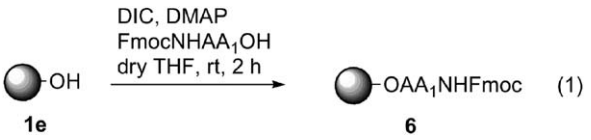


TABLE 3 Tripeptides Synthesized Using the Oligomeric Supports

	6	Peptide 7	Isolated yield[a] (%)
1.	**6g**	**7a**: FmocNHPheValAlaOH	44
2.	**6a**	**7b**: FmocNHAlaPheAlaOH	46
3.	**6g**	**7c**: FmocNHAlaProAlaOH	64
4.	**6b**	**7d**: FmocNHPhePhePheOH	38
5.	**6b**	**7e**: FmocNHGlyValPheOH	58
6.	**6b**	**7f**: FmocNHPheIlePheOH	53

[a] Isolated using semipreparative RP-HPLC.

Peptide Synthesis

The loaded supports **6** were used for synthesizing tripeptides following the route illustrated in Scheme 2. The Fmoc group in support **6** was deprotected using 20% piperidine in DMF. A phase separation was observed when hexane was added after completion of the reaction.[15] The upper layer was removed with a dropper and after three to four washes with hexane, the polymer was obtained as a precipitate. Subsequent treatment with the Fmoc protected second amino acid afforded the supported dipeptide. The supported dipeptide was isolated *via* precipitation with water. The precipitate was sequentially washed with water and methanol to remove excess reagents. One can envision continuing the cycle $(n-1)$ times to obtain the peptide of desired length (n). The support was found to be soluble in the reaction medium during peptide synthesis. Two equivalents of coupling reagents and amino acids were used for each step, which is much lower that what is typically used for SPPS. After completion of synthesis, the peptide **7** was cleaved form the support using 20% TFA. The support was removed as a precipitate in methanol and the supernatant was purified using reversed phase HPLC to obtain the Fmoc protected peptide **7**. A variety of tripeptides **7a–f** were synthesized in 38–64% yields using supports **6(a, b & g)** (Table 3). The yields are good considering the fact that we are only using 2 equivalents of coupling reagents.

Since peptide synthesis is being carried out from the C- to N-terminus and the reactivity of amino acids is maintained in a homogeneous reaction medium, the slightly lower yields could be attributed to diketopiperazine (DKP) formation (after deprotection of the dipeptides). One can envisage improving the yields for peptide synthesis if the DKP formation is prevented or avoided. Therefore, we used a combination approach, where dipeptides synthesized in solution[12] were loaded onto the oligomeric support for synthesizing hexapeptides (Scheme 3). We have observed that such an approach leads to a significant improvement in the yields for peptide synthesis. The protocols used for the deprotection and coupling steps were as described before. The

hexapeptides were purified by RP-HPLC after cleavage from the support. Following the combination approach peptides **7f** and **7g** were obtained from supports **6b** and **6h** in 51 and 45% overall yields, respectively. As before, only 2 equivalents of coupling reagents were used. Despite the fact that hexapeptide synthesis requires six additional steps as compared to the tripeptide synthesis (Table 3), it is notable that we are able to get hexapeptides in ~50% yields.

CONCLUSIONS

In conclusion, oligomeric styrene supports with loading capacities of 1.5–1.6 mmol/g analogs to styrene-based resins used in SPPS have been synthesized using a modular approach. The supports are soluble in organic solvents, such as DCM, THF or DMF and can be readily precipitated using cold hexane or water. Nonpolar, polar, basic, and aromatic amino acids could be loaded on to the supports in 0.87–1.3 mmol/g. The amino acid loading could be determined using NMR spectroscopy without cleaving the amino acid. Tripeptides were synthesized in 38–64% yield using the support, wherein only 2 equivalents of coupling reagents and amino acids were used. A combination approach which prevented DKP formation was used to synthesize larger hexapeptides with improved overall yields (ca. 50%). The synthetic approach for formation of our oligomeric supports is modular. Therefore, one can readily incorporate the efficient styrene monomers with attachment sites used in SPPS resins into our support scaffold to obtain efficient soluble styrene supports. Current efforts are focused on expanding the scope of these supports for peptide synthesis by varying the nature of the attachment sites.

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13 See Supporting Information for solution phase synthesis of dipeptides.

14 We observe a decrease in solubility of the polymers and oligomers when stored over extended periods of time.

15 We believe that we might be losing a small amout of polymer during this step, which might account for the lowering of yield.