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An improved soluble polynorbornene support for peptide synthesis†

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Soluble polymers that can be readily isolated *via* precipitation and possess multiple amino acid attachment sites are highly attractive for peptide synthesis. Polyethylene glycol supports that solubilize the growing peptide chain and can be readily isolated have been widely used for peptide synthesis. However, a stoichiometric amount of the PEG support is required because each PEG support typically has a single attachment site for peptide synthesis. Reported herein is the development of a polynorbornene support containing multiple attachment sites as well as alkyl and oligoether solubilizing/spacer groups. The attachment site is connected to the polymer backbone through a solubilizing oligoether linker. The support was developed after evaluating the effect of the linker and attachment site-spacing on peptide synthesis using suitably designed polynorbornene supports. The high solubility of the support minimizes the equivalents of reagents used for peptide synthesis. The support has been used to synthesize the natural product Leu^5-enkephalin in 52% overall yield using only 1.2 equivalents of coupling reagents, which is comparable or superior to reported procedures using a large excess of reagents.

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Introduction

Insoluble polymers have been widely used as supports in peptide synthesis so that the growing peptides can be readily isolated by filtration.[1] Synthesis using these heterogeneous supports requires excess reagents to drive the peptide coupling reactions to completion. Soluble, non-crosslinked polymers have been utilized for peptide synthesis as they do not require excess reagents and the growing peptides can be recovered by precipitation.[2–4] Among the soluble polymer supports, polyethylene glycol (PEG) derivatives significantly enhance the solubility and reactivity of the growing peptide chain in the reaction medium.[5–11] However, the loading capacity (*i.e.* mmols of amino acids attached per gram) of the PEG support is low because each support contains only one amino acid attachment site at the end of the polymer chain. Cross-linked resins incorporated with PEG chains have been developed that have excellent loading capacities and improved swelling properties. Examples of such resins include the cross-linked polystyrene resins (Tentagel),[12] completely cross-linked polyethylene glycol (PEGA) resin[13] and polyethylene glycol–polyvinyl methylamine resins (Chem Matrix).[14] Despite, the incorporation of the PEG linkers, these supports require excess reagents (~2–10 equiv.) for peptide synthesis. Non-crosslinked soluble polymer supports (without PEG) containing multiple attachment sites have also been explored for peptide synthesis. However, they show a decrease in solubility after tripeptide synthesis.[15–18]

Our group is interested in developing soluble polystyrene and polynorbornene supports with high loading capacities (comparable to resins) for peptide synthesis.[19–21] Such supports possess the advantages of solid phase peptide synthesis as well as PEG derived soluble supports. Herein, we report the development of a highly efficient second generation polynorbornene support **4** (Fig. 1), where a solubilizing and flexible oligoether linker is incorporated in the support design. The support **4** was designed after determining the effect of linker and spacer/solubilizing groups using supports **2** and **3** that do not contain solubilizing oligoether chains as model systems. The

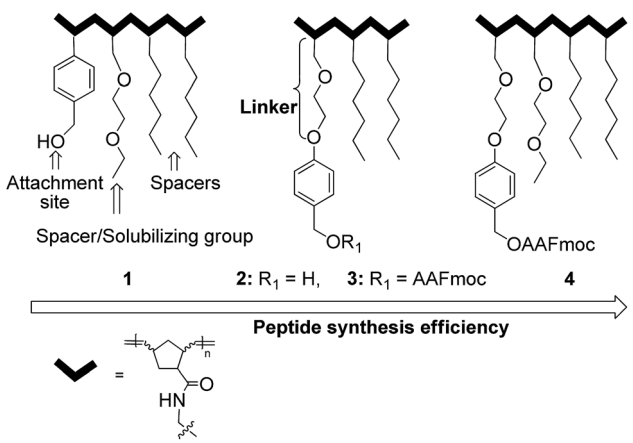

Fig. 1 Poly(norbornene) supports for peptide synthesis.

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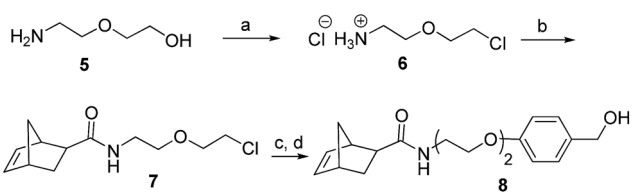
second generation support **4** containing the solubilizing linker as well as oligoether chains was utilized to synthesize the natural product Leu^5-enkephalin in 52% overall yield. The yield obtained with our support using only 1.2 equivalents of coupling reagents is superior or comparable to previously reported procedures with supports that utilize excess coupling reagents (1.5–4.5 equivalents).

Results and discussion

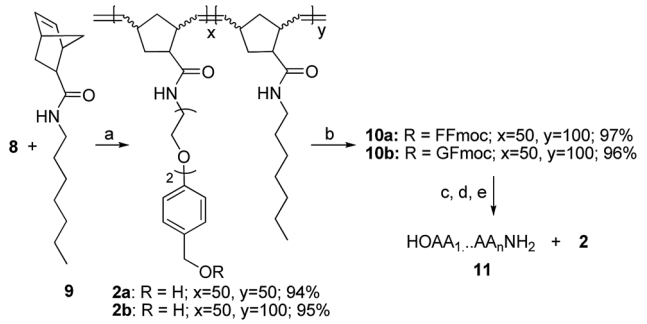
Our previously developed support **1** comprised multiple attachment sites dispersed with alkyl and oligoether chains as spacers/solubilizing groups. Support **1** could not be used for peptide synthesis as it was insoluble in the reaction medium after Fmoc deprotection of the first amino acid attached. We envisioned that incorporating an oligoether linker between the attachment site and the support could serve the dual role of improving support solubility and providing flexibility to the attachment site to access reagents.

At first, we wanted to determine whether the linker had an effect on the support solubility. Therefore the synthesis of support **2** that has no solubilizing oligoether groups was pursued. The alkyl groups were retained to function as spacers. The alkyl monomer was synthesized as described earlier.[19,20] The linker incorporated attachment site was synthesized as shown in Scheme 1. Hydroxyamine **5** was treated with $SOCl_2$ to afford the 2-(2-chloroethoxy) ethanamine **6** in 94% yield. The amine **6** was treated with norbornene-*exo*-acid in the presence of HBTU and DIEA to give amide **7** in 92% yield. The amide **7** was treated with 4-hydroxybenzaldehyde and K_2CO_3 to give the corresponding aldehyde in 96% yield. The monomer **8** was obtained in 90% yield by reducing the aldehyde using sodium borohydride in methanol.

Supports **2** were synthesized by polymerizing monomers **8** and **9** (Scheme 2). The ratio of monomers ($x : y$) as well as the total monomer : initiator ratio *i.e.* $[x + y]$: [Ru] was varied to obtain supports **2a** and **b** with varying proportion of spacers (Table 1). The polymerization reaction was carried out in the presence of Grubbs' third generation initiator. The reaction was terminated by addition of ethyl vinyl ether and the polymers **2** were isolated by precipitation with diethyl ether. The completion of polymerization was confirmed by the absence of signals corresponding to the monomers in the ^{1}H NMR spectra of polymers **2**. The number of attachment sites present per gram of polymer (loading) was determined by recording the ^{1}H NMR



Scheme 1 Synthesis of monomer (a) $SOCl_2$, toluene, 0 °C, rt, 3 h, 94% (b) *exo*-norbornene acid, HBTU, DIEA, DCM, DMAP, rt, 13 h, 92%, (c) 4-hydroxy benzaldehyde, K_2CO_3, DMF, 120 °C, 12 h, 96%, (d) $NaBH_4$, MeOH, 0 °C, rt, 1 h, 90%.



Scheme 2 Synthesis of supports **2** and peptide synthesis on support **2b** (a) Grubbs' third generation catalyst, CH_2Cl_2, rt, 1 h, (b) Fmoc-AA-OH, DIC, DMAP, THF (c) 20% pip/DMF, 10 min, rt (d) Fmoc-AA-OH, HCTU, DIEA, DMF : CH_2Cl_2 (1 : 1), rt (e) LiOH, THF, rt, 1 h.

Table 1 Synthesis of polymer supports **2** and peptide synthesis on support **2b**

2	Loading[a] **2** (mmol g^{-1})	Loading[a] **10** (mmol g^{-1})	**11**	Yield[b] **11** (%)
2a	1.7	n.d	n.d	n.d
2b	1.23	0.88	**11a**:MF	78
2b	1.23	0.94	**11b**:IFG	70

[a] Determined using ^{1}H NMR. [b] Isolated yield after RP-HPLC purification.

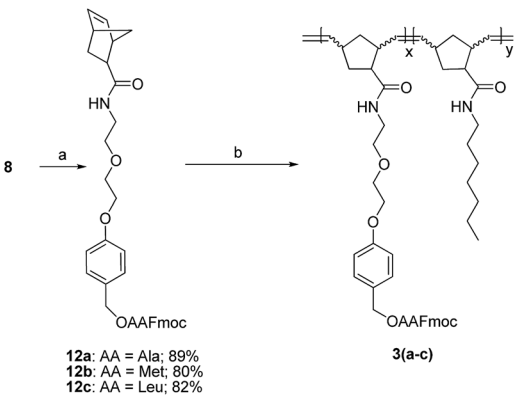
spectra of polymers **2** in the presence of a known amount of 1,1,2,2-tetrachloroethane (TCE). The integration of the peak at $\delta = 6.9$ ppm corresponding to TCE was compared with the peak at $\delta = 4.4$ ppm for the benzylic protons in polymers **2a** and **b** to determine their loading capacities. The integration values of benzylic protons ($\delta = 4.4$), and methyl protons of the alkyl chain ($\delta = 0.83$) were compared to get the $x : y$ ratios.

Support **2a** was found to be insoluble, while support **2b** was found to be soluble in solvents such as DCM or THF. Amino acids were loaded onto these supports using DIC to obtain amino acid attached polymer **10a** and **b** with loading capacities of ~0.9 mmol g^{-1} (Scheme 2 and Table 1). The resulting polymer was treated with 20% piperidine in DMF and stirred for 10 min to deprotect the Fmoc group. The polymer was isolated as a precipitate with diethyl ether and then coupled with another amino acid by using HCTU and DIEA in DCM : DMF (1 : 1) to afford the dipeptides. The deprotection and coupling reactions were repeated to obtain tripeptides. Finally peptides were cleaved from the support using LiOH in THF. Di and tri peptides **11a** and **b** were synthesized in 70 and 78%, respectively using support **2b**. However, a drop in support solubility was observed after each coupling step. It is notable that polymer support **2b** could be used for synthesizing tripeptides in good yields in contrast to the analogous hydroxy support **1** with three monomers that has a higher proportion of solubilizing groups. The improved solubility of support **2b** despite the absence of solubilizing oligoether groups indicates that the linker plays a role in enhancing the solubility of the support.

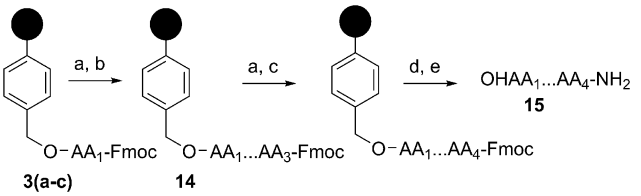
In the past, we had observed that supports derived from monomers preloaded with amino acids were more soluble than support **1** because the bulky Fmoc group on the amino acid ensured uniform spacing of attachment sites.[20] We wished to check if the lower solubility of supports **2** after tripeptide synthesis could be attributed to this clustering of attachment sites. Therefore, we synthesized supports **3** from monomers preloaded with amino acids (Scheme 3). Amino acid attached monomers **12a–c** were synthesized in excellent yields by coupling monomer **8** with the appropriate amino acid in the presence of EDCI. Supports **3a–c** were synthesized in 88–96% yields from amino acid attached monomers **12** and monomer **9** (Table 2). The polymer supports **3(a–c)** had good loading capacities (~0.9 mmol g^{-1}) and were extremely soluble in DCM, THF and DMF. Due to the high solubility of support **3**, we were concerned that diketopiperazine would be readily formed during peptide synthesis. We had previously observed this problem during deprotection of dipeptides on our previously reported soluble supports. To circumvent this problem, we directly attached a dipeptide synthesized in solution to the loaded support **3**.

Peptide synthesis was initiated by deprotecting the Fmoc group of support **3** using 20% piperidine in DMF (Scheme 4). The polymer with the free amine was isolated by precipitation with hexane and ether. The amine was coupled with Fmoc protected dipeptide (Fmoc-AA_2-AA_1-OH) using HCTU and DIEA in dichloromethane. The support was coupled with a dipeptide instead of a single amino acid because highly soluble supports such as ours that maintain the reactivity of the attached amino acids are prone to form diketopiperazines when the dipeptide is deprotected.[22,23] After completion of the reaction, tripeptide attached support **14** was obtained as a precipitate with diethyl ether. The supported tripeptide **14** was dissolved in a few drops of DMF and re-precipitated using diethyl ether multiple times to ensure the removal of excess reagents and by-products. Deprotection and coupling steps were repeated to get the polymer supported tetrapeptide. 1.2 equiv. of coupling reagents and amino acids were used in each coupling step. The peptide **15** was cleaved from the support using base hydrolysis. A variety of tetrapeptides **15a–f** were synthesized from supports **3a–c** in 62–78% yields (Table 3). The effect of the attachment site spacing on the support properties is evidenced by the fact that supports **3** are more efficient than supports **2** despite having no solubilizing groups. When we attempted using support **3** for synthesizing a peptide of biological importance such as Leu-enkephalin[24] we observed that the solubility of the support decreased after tetrapeptide synthesis. We chose Leu-enkephalin **17** as a model system as there are several reports for the synthesis of this peptide using soluble supports.[24–29] The diminished solubility of support **3** indicates that incorporation of solubilizing ether groups is necessary for synthesis of larger peptides.

The studies with supports **2** and **3** illustrated that a support containing the linker, oligoether chains and regularly spaced attachment sites would be desirable for peptide synthesis. Hence, we designed support **4** from pre-loaded monomers that contains the linker as well as solubilizing groups. Support **4** was synthesized in 88% yield (loading = 0.72 mmol g^{-1}) from monomers **12c**, **9** and **16** (Scheme 5). The *x*, *y* and *z* ratios were chosen based on our success with our reported poly(norbornene) supports derived from three monomers.[19,20]



Scheme 3 Synthesis of supports **3** (a) Fmoc-AA-OH, EDCI, DCM, rt. (b) Monomer **9**, Grubbs' third generation catalyst, CH_2Cl_2, rt, 1 h.



Scheme 4 Peptide synthesis on polymer supports **3(a–c)** (a) 20% pip/DMF, 10 min, precipitation with hexane and diethyl ether; (b) FmocAA_2AA_1OH, HCTU, DIEA, rt, 3 h, precipitation with diethyl ether; (c) FmocAAOH, HCTU, DIEA, rt, 3 h, precipitation with diethyl ether; (d) LiOH, THF, H_2O, rt, 1 h, separate supernatant; (e) dil. HCl.

Table 2 Synthesis of polymer supports **3a–c**

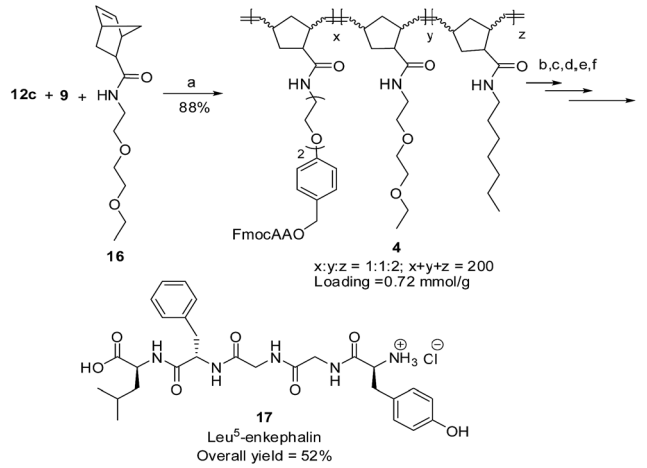
No.	Polymer 3	AA	R_1	n^a (*x* : *y*)	Yield (%)	Loading^b (mmol g^{-1})
1	**3a**	Ala	Alk	50 (1 : 2)	88	0.9
2	**3b**	Met	Alk	50 (1 : 2)	96	0.87
3	**3c**	Leu	Alk	50 (1 : 2)	94	0.88

[a] Equivalents with respect to [Ru]. [b] Determined using ^{1}H NMR.

Table 3 Synthesis of peptides using polymer supports **3a–c**

No.	Polymer	Peptide	Yield[a] (%)
1	**3a**	**15a:** Leu–Ala–Phe–Ala	77
2	**3a**	**15b:** Pro–Val–Trp–Ala	78
3	**3a**	**15c:** Met–Val–Trp–Ala	76
4	**3b**	**15d:** Ala–Gly–Phe–Met	72
5	**3b**	**15e:** Ala–Ser(O^tBu)–Phe–Met	62
6	**3c**	**15f:** Pro–Gly–Phe–Leu	64

[a] Isolated yield after purification using RP-HPLC.



Scheme 5 Synthesis of polymer support **4** and peptide synthesis (a) Grubbs' third generation catalyst, CH_2Cl_2, rt, 1 h; (b) 20% pip/DMF, 10 min, precipitation with hexane and diethyl ether; (c) FmocGly-Phe-OH, HCTU, DIEA, rt, 3 h, precipitation with diethyl ether; (d) FmocAAOH, HCTU, DIEA, rt, 3 h, precipitation with diethyl ether (repeat); (e) LiOH, THF, H_2O, rt, 1.5 h, separate supernatant; (f) dil. HCl.

Leu-enkephalin **17** was synthesized in 52% overall yield using support **4** and following the peptide synthesis approach shown in Scheme 5. The support was soluble during the synthesis in contrast to support **3**. The HPLC profile of the crude pentapeptide mainly showed the peak corresponding to the desired product (Fig. 2).

Comparison of the yields for peptide synthesis using support **4** and previously reported examples[26–28] are shown in Table 4. Support **4** affords Leu-enkephalin in superior yields compared to the ionic liquid and SPPS supports (entries 3 and 4). The yield for synthesis of **17** using support **4** is lower than the fluorous support (entry 2). However, given the fact that the fluorous supports require the use of expensive fluorinated solvents/supports and the synthesis requires 4–4.5 equivalents of coupling reagents/amino acids, we believe our supports are more practically viable.

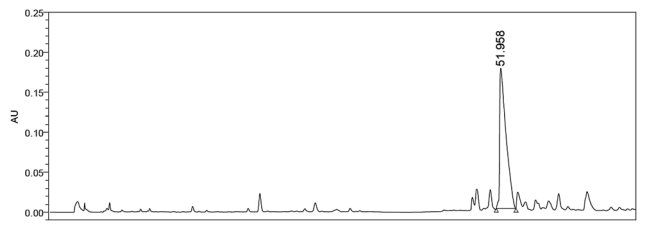


Fig. 2 HPLC chromatogram of crude Leu^5-enkephalin.

Table 4 Comparison of yields for Leu-enkephalin **17** using different supports

No.	Support	Coupling reagents (equiv.)	Yield **17** (%)
1	**4**: polynorbornene	1.2	52[a]
2	Fluorous	4–4.5	70
3	Ionic liquid	1.5–2.5	50
4	SPPS – silyl linker	4	25

[a] Isolated yield after purification using RP-HPLC.

Conclusions

A second generation polynorbornene support **4** that contains a solubilizing oligoether linker between the amino acid attachment site and the polymer backbone has been developed. The linker is found to have a significant effect on the solubility and efficiency of the supports during peptide synthesis. Support **1** containing no linker is found to be insoluble after the first step of peptide synthesis despite containing solubilizing oligoether side chains in contrast with linker containing supports **2** and **3**. Supports **3** formed with preloaded monomers were found to be more efficient than supports **2** and were used for synthesizing tri to tetrapeptides in 62–78% yields using only 1.2 equivalents of coupling reagents/amino acids. The higher efficiency of supports **3** is explained by the easier access of reagents in this support due to the uniform spacing of attachment sites. Presence of bulky Fmoc groups in the pre-loaded monomers prevents crowding of attachment sites on the polymer support. However, support **3** could not be used for synthesizing natural product Leu-enkephalin due to decrease in its solubility after tetrapeptide synthesis. The results from supports **2** and **3** suggested that the solubility as well as spacing of the attachment sites was crucial for peptide synthesis. Therefore, a highly efficient second generation support **4** containing the solubilizing linker as well as spacers was synthesized. The efficiency of the support was demonstrated by synthesizing the natural product Leu^5-enkephalin in 52% overall yield. The yield obtained with our support using only 1.2 equivalents of reagents is superior or comparable to previously reported procedures with either expensive supports or supports that use large excess of reagents.

Acknowledgements

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