ORGANIC
LETTERS

2013
Vol. 15, No. 22
5870–5873

Soluble and Reusable Poly(norbornene) Supports with High Loading Capacities for Peptide Synthesis

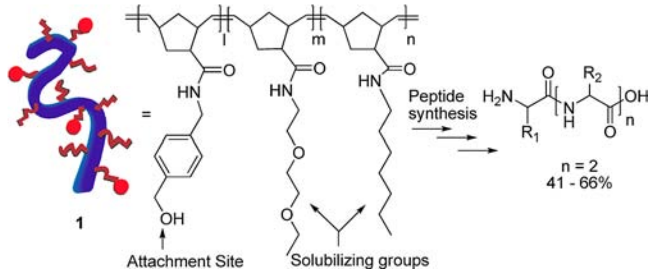
Nimmashetti Naganna and Nandita Madhavan*

Department of Chemistry, Indian Institute of Technology Madras, Chennai - 600036,
Tamil Nadu, India

nanditam@iitm.ac.in

Received October 11, 2013

ABSTRACT



Poly(norbornene) supports comprising solubilizing ethylene glycol units and multiple amino acid attachment sites have been developed for peptide synthesis. A variety of amino acids have been efficiently loaded (0.6−1.1 mmol/g) onto the support in high yields (83−98%). Several tripeptides have been synthesized in moderate-to-good overall yields (41−66%) using only 1.2 equiv of coupling reagents/amino acids, and the support could be efficiently recycled up to 3 times.

The growing number of oligopeptide-derived therapeutic agents, and biomaterials, necessitates the development of efficient methods for peptide synthesis. Solid phase peptide synthesis (SPPS), where the growing peptide is attached to a resin, is extremely convenient for laboratory-scale peptide synthesis, as it facilitates peptide recovery via filtration.[1] However, the amino acids attached to insoluble SPPS supports are less reactive and excess coupling reagents are typically required to ensure completion of reactions. Liquid phase peptide synthesis (LPPS), which employs soluble supports for attaching amino acids, provides an attractive alternative to SPPS, as the amino acid reactivity is not compromised and the growing peptide can be readily isolated by precipitation or extraction.[2] LPPS has been achieved using fluorous,[3] polymeric,[2] hydrophobic,[4] and ionic liquid supports.[5] Poly(ethylene glycol) supports[2b,6] are among the most efficient polymeric LPPS supports, as they provide a localized polar environment for solubilizing and improving the reactivity of the growing peptides. Despite the versatility of PEG supports their widespread use for peptide synthesis is limited by their low loading capacities (0.1−0.5 mmol/g). Polymeric LPPS supports derived from poly(styrene) and other hydrophilic polymers typically have higher loading capacities than PEG, but are not as efficient for peptide synthesis due to their lower solubility.[2c,7] Herein, we present poly(norbornene) **1**

---

(1) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149. (b) Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press Inc.: New York, 2000. (c) Martin, F. G.; Albericio, F. *Chim. Oggi* **2008**, *26*, 29.

(2) (a) Bayer, E.; Mutter, M. *Nature* **1972**, *237*, 512. (b) Pillai, V. R.; Mutter, M.; Bayer, E.; Gatfield, I. *J. Org. Chem.* **1980**, *45*, 5364. (c) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489.

(3) (a) Mizuno, M.; Goto, K.; Miura, T.; Hosaka, D.; Inazu, T. *Chem. Commun.* **2003**, 972. (b) Zhang, W. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 784. (c) Mizuno, M.; Goto, K.; Miura, T.; Inazu, T. *QSAR Comb. Sci.* **2006**, *25*, 742. (d) Fustero, S.; Sancho, A. G.; Chiva, G.; Sanz-Cervera, J. F.; del Pozo, C.; Aceña, J. L. *J. Org. Chem.* **2006**, *71*, 3299.

(4) (a) Chiba, K.; Kono, Y.; Kim, S.; Nishimoto, K.; Kitano, Y.; Tada, M. *Chem. Commun.* **2002**, 1766. (b) Okada, Y.; Suzuki, H.; Nakae, T.; Fujita, S.; Abe, H.; Nagano, K.; Yamada, T.; Ebata, N.; Kim, S.; Chiba, K. *J. Org. Chem.* **2013**, *78*, 320. (c) Takahashi, D.; Yano, T.; Fukui, T. *Org. Lett.* **2012**, *14*, 4514. (d) Takahashi, D.; Inomata, T. *Pept. Sci.* **2013**, *49*, 83.

(5) (a) Miao, W.; Chan, T. H. *Acc. Chem. Res.* **2006**, *39*, 897. (b) Miao, W.; Chan, T.-H. *J. Org. Chem.* **2005**, *70*, 3251. (c) He, X.; Chan, T. H. *Org. Lett.* **2007**, *9*, 2681.

(6) (a) Zhang, C.-X.; Tong, H.-B.; Yan, C.-G. *J. Comb. Chem.* **2007**, *9*, 924. (b) Fischer, P. M.; Zheleva, D. I. *J. Pept. Sci.* **2002**, *8*, 529. (c) Janda, K. D.; Han, H. *Methods Enzymol.* **1996**, *267*, 234.

10.1021/ol4029435

comprising multiple solubilizing diethylene glycol groups as well as attachment sites as efficient polymeric LPPS supports with high loading capacities (Figure 1).

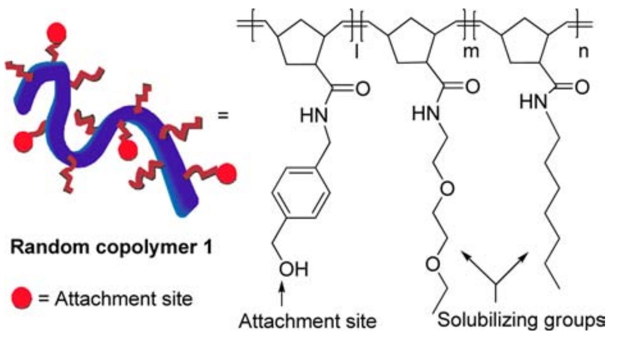
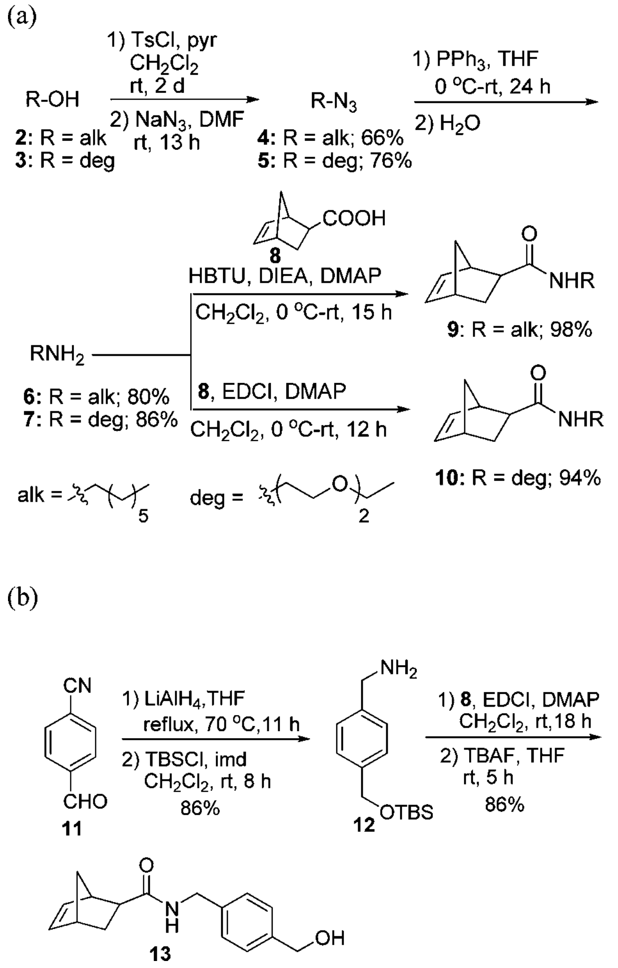


Figure 1. Poly(norbornene) LPPS support.

Poly(norbornene) was chosen as the support, as its highly controlled synthesis *via* ring-opening metathesis polymerization (ROMP) affords polymers with relatively uniform molecular weight distributions.[8] Alkyl and oligoether chains have been incorporated to increase support solubility and spacing the attachment sites apart. The synthesis of support **1** can be envisioned by the random copolymerization of the requisite monomers. Such an approach provides a modular support for peptide synthesis wherein the properties of the support can be tuned by varying the monomer ratios during ROMP.

The monomers containing the heptyl and diethylene glycol solubilizing groups were synthesized starting from the alcohols **2** and **3**, respectively (Scheme 1a). The alcohols were tosylated and converted to the corresponding azides **4** and **5** using sodium azide and DMF. Treatment of the azides with triphenyl phosphine in THF, followed by an aqueous workup, gave the corresponding amines **6** and **7**. The alkyl amine **6** was coupled with norbornene-*exo*-acid **8** in the presence of *N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), *N*diisopropylethylamine (DIEA) and *N*,*N*-dimethyaminopyridine (DMAP) to give alkyl monomer **9** in 98% yield. The oligoether amine **7** was reacted with acid **8** in the presence of EDCI and DMAP to give the diethylene glycol monomer **10** in 94% yield. The monomer containing the attachment site was synthesized starting from commercially available *p*-cyano benzaldehyde **11** as shown in Scheme 1b. Compound **11** was reduced using lithium aluminum hydride in THF, and the hydroxy group was protected using *tert*-butyldimethylsilyl (TBS) chloride and imidazole to afford the amine **12** in 86% yield. The amine **12** was coupled with norbornene-exo-acid **8** using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and DMAP and subsequently deprotected to afford the monomer **13** containing the attachment site in 86% yield.

Scheme 1. Synthesis of Monomers



Poly(norbornene) support **1** was synthesized by adding a Grubbs third generation initiator to a solution of monomers **9**, **10**, and **13** (eq 1).[9] Polymers **1a**−**e** with varying *l*, *m*, and *n* ratios and polymer lengths were synthesized (Table 1). The yields for lower molecular weight polymers **1a** and **b** were less due to their higher solubility in solvents used to precipitate the polymers. In all other cases, the polymers were obtained in excellent yields. The loading capacities of the supports were determined using ^{1}H NMR spectroscopy in the presence of TCE as a standard, as the protons corresponding to the benzylic position of the attachment site were distinctly seen in the NMR spectrum.[10] The loading capacities (1.3−2.6 mmol/g) of the polymers were

---

(7) (a) Narita, M.; Hirata, M.; Kusano, K.; Itsuno, S.; Ue, M.; Okawara, M. *Pept. Chem.* **1980**, *17th*, 107. (b) Meszynska, A.; Badi, N.; Boerner, H. G.; Lutz, J.-F. *Chem. Commun.* **2012**, *48*, 3887.

(8) (a) Choi, T. L.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 1743. (b) Barrett, A. G. M.; Hopkins, B. T.; Köbberling, J. *Chem. Rev.* **2002**, *102*, 3301. (c) Dickerson, T. J.; Reed, N. N.; Janda, K. D. *Chem. Rev.* **2002**, *102*, 3325. (d) Harned, A. M.; Mukherjee, S.; Flynn, D. L.; Hanson, P. R. *Org. Lett.* **2003**, *5*, 15. (e) Harned, A. M.; Zhang, M.; Vedantham, P.; Mukherjee, S.; Herpel, R. H.; Flynn, D. L.; Hanson, P. R. *Aldrichimica Acta* **2005**, *38*, 3. (f) Lee, B. S.; Mahajan, S.; Clapham, B.; Janda, K. D. *J. Org. Chem.* **2004**, *69*, 3319. (g) Harned, A. M.; Probst, D. A.; Hanson, P. R. The Use of Olefin Metathesis in Combinatorial Chemistry: Supported and Chromatography-Free Syntheses. In *Handbook of Metathesis: Catalyst Development*; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2008, doi: 10.1002/9783527619481.ch25.

(9) Grubbs first generation initiator was also surveyed for ROMP. However, the *l*:*m*:*n* ratios of the resultant polymers varied significantly due to different rates of polymerization for the three monomers.

comparable to those seen for SPPS supports. The solubility of supports **1c**−**e** in solvents used for peptide synthesis as well as the ease of their recovery from solution was investigated. Polymer **1e** (entry 5, Table 1) with an *l*, *m*, *n* ratio of 1:1:2 and a loading capacity of 1.35 mmol/g was found to be the optimal support for peptide synthesis, as it was readily soluble in dichloromethane/THF/DMF and could be easily recovered from solution via precipitation with ether.

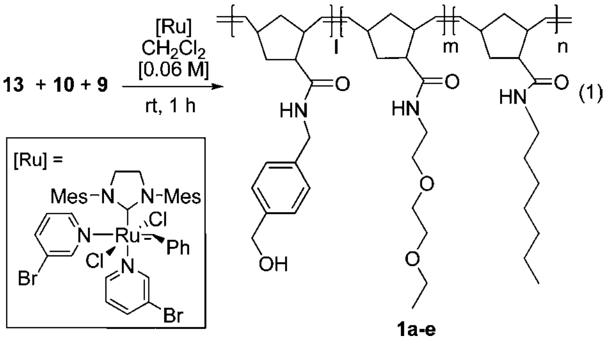
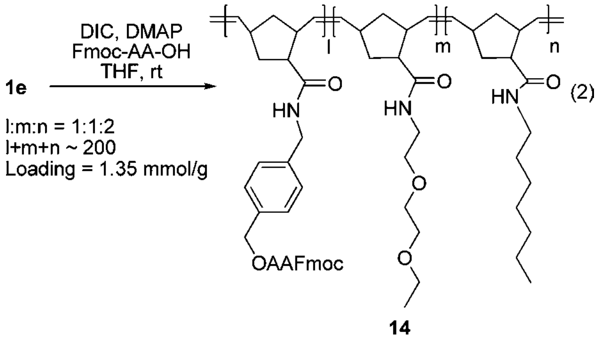


Table 1. Supports Synthesized by ROMP

	1	*x* (**13**:**10**:**9**)[a]	yield (%)	loading[b] (mmol/g)	*l*:*m*:*n*[b]
1	**1a**	31(1:1:1)	36	1.5	1:0.6:0.5
2	**1b**	41(1:1:1)	42	2.6	1:0.6:0.4
3	**1c**	54 (1:1:1)	95	1.6	1:1:1.1
4	**1d**	66 (1:1:1)	95	1.7	1:1.1:1.1
5	**1e**	50 (1:1:2)	95	1.35	1:1:2

[a] *x* = equivalents with respect to [Ru]. [b] Determined by ^{1}H NMR.

The first approach to obtain supports containing amino acids involved attachment of amino acids to support **1e** using diisopropyl carbodiimide (DIC) and DMAP in THF (eq 2). Using this approach, a variety of polar, nonpolar, acidic, and basic amino acids could be attached in excellent yield to support **1e** (Table 2). The solubility of the support facilitated efficient loading of amino acids onto support **1e**, using only a slight excess of Fmoc amino acids (1.2−1.5 equiv). Furthermore, the loading of amino acids onto the polymer support could be determined without cleaving the amino acids, using ^{1}H NMR spectroscopy.[10]



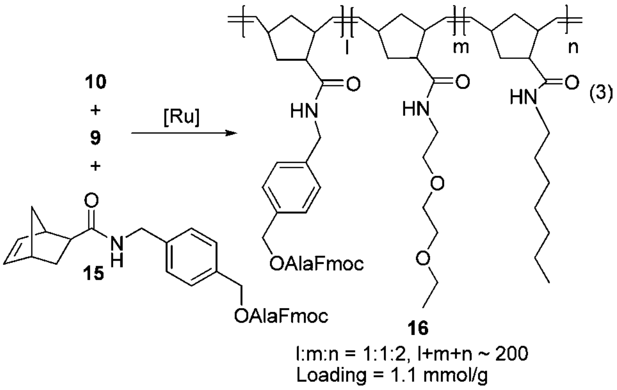
A second approach involves ROMP of monomer **15** containing an amino acid along with monomers **9** and **10** using the before mentioned optimal monomer ratios (eq 3).

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

	amino acid (AA)	time (h)	yield (%)	loading[a] (mmol/g)
1	**14a**: Ala	3	87	1.1
2	**14b**: Phe	6	96	0.9
3	**14c**: Leu	2	92	0.92
4	**14d**: Pro	4	96	0.96
5	**14e**: Met	3	98	1.1
6	**14f**: Tyr	3	95	0.87
7	**14g**: Asp(O^{t}Bu)	3	97	0.84
8	**14h**: Lys (Boc)	3	93	0.77
9	**14i**: His (Trt)	6	83	0.6
10	**14j**: Arg (Pbf)[c]	6	98	0.75

[a] Determined using ^{1}H NMR. [c] Pbf = (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-.

Such an approach, while not as versatile as the first approach, ensures that there are no free hydroxyl groups in the support. The polymer **16** containing FmocAla was obtained in 95% yield using this approach. The overall yield for obtaining **16** from monomer **13** is 86%, which is slightly higher than that for obtaining **14a** (83%).



As a proof of concept, alanine attached support **16** was used to synthesize several tripeptides as shown in Scheme 2 and Table 3. The polymer **16** was treated with a solution of 20% piperidine in DMF to remove Fmoc. The resultant polymer with the free amine was isolated as a precipitate with hexane and ether. The amine was coupled with Fmoc protected amino acid (AA$_1$) using 1-[bis(dimethylamino)-methylene]-6-chloro-1*H*-benzotriazolium hexafluorophosphate 3-oxide (HCTU) and DIEA in dichloromethane to give the dipeptide, which was isolated by precipitation with diethyl ether. The precipitate was dissolved in DMF and reprecipitated with diethyl ether multiple times to ensure removal of byproducts and excess coupling reagents. The dipeptide was subjected to the deprotection and coupling steps as described above to gain polymer attached tripeptides. After obtaining the requisite peptides, the Fmoc protecting group was removed and the peptides were cleaved from the support *via* base mediated hydrolysis. The supernatant contained the peptides in their salt form, while support **1e** was recovered as a precipitate in 92−98% yield. Neutralization

---

(10) See Supporting Information for details.

Scheme 2. Peptide Synthesis on Support

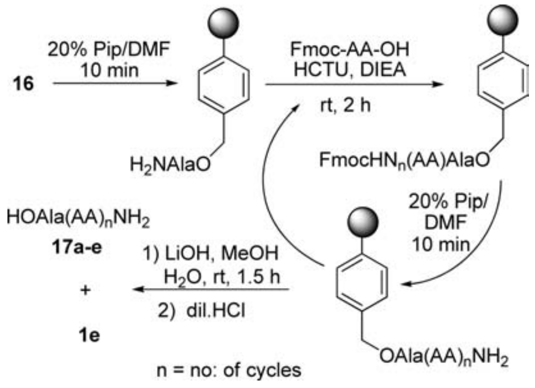


Table 3. Synthesis of Peptides Using Support **16**

	peptide **17**	isolated yield[a] (%)
1	**17a:** H_2N-TyrPheAla-OH	46
2	**17b**: H_2N-PheMetAla-OH	41
3	**17c**: H_2N-ProPheAla-OH	52
4	**17d**: H_2N-TrpLeuAla-OH	66
5	**17e**: H_2N-LeuPheAla-OH	43

[a] Isolated using semipreparative RP-HPLC.

of the supernatant with dil. HCl afforded peptides **17a**−**e**. Since we wished to probe the reactivity of amino acids on the support, only 1.2 equiv of Fmoc-amino acids were used for the coupling reactions. Crude HPLC and NMR of the tripeptides were relatively clean and indicated formation of a few byproducts in relatively small amounts.[10,11] Pure tripeptides were isolated by semipreparative reversed phase HPLC in 41−66% yields (Table 3), which is notable considering the fact that we were not using an excess of amino acids or coupling reagents. We believe that the lower isolated yields could be due to the higher reactivity of amino acids on our supports, which could result in the formation of diketopiperazines during deprotection of the dipeptide.

The support **1e** could be recovered in high yield and purity after peptide synthesis. Alanine was loaded onto **1e** using eq 2 to give support **16**, which was used for synthesizing tripeptide **17e** following Scheme 2. The isolated yields for **17e** did not vary significantly from the first cycle (Table 4), indicating that amino acid attachment and peptide synthesis were equally efficient with the recovered polymer.

Table 4. Reuse of Support for Synthesis of Peptide **17e**

	support	cycle	isolated yield[a] (%)
1	**16**	1	43
2	**16**[b]	2	44
3	**16**[b]	3	41

[a] Isolated using semipreparative RP-HPLC. [b] Synthesized from recycled polymer **1e**.

In conclusion, soluble poly(norbornene) supports with high loading capacities of attachment sites (> 1 mmol/g) have been developed for peptide synthesis. The support contains solubilizing ethylene glycol and alkyl units which improves its efficiency for peptide synthesis. A variety of amino acids have been efficiently loaded (0.6−1.1 mmol/g) onto the support in excellent yields (83−98%). Several tripeptides have been synthesized in good to moderate yields (41−66%) without using excess amino acids or coupling reagents. Lastly, the cleaved support could be readily isolated in high purity and recycled up to 2 times to synthesize tripeptides. Efforts are currently underway to improve the efficiency of peptide synthesis using our poly(norbornene) supports *via* modified linkers and attachment sites, for obtaining more complex oligopeptides.

Acknowledgment. This research was supported by CSIR (No. 01(2333)/09/EMR-II), New Delhi, India. N.N acknowledges CSIR, India for his research fellowship.

Supporting Information Available. Detailed experimental procedures, characterization of compounds, HPLC traces of crude tripeptides. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

---

[11] The yield for H_2N-LeuPheAla-OH prior to HPLC (determined using ^{1}H NMR spectroscopy in the presence of TCE as a standard) is 73%, which is comparable to the overall yield (77%) for tripeptide GlyValGly-Fmoc synthesized on the mPEG LPPS supports using 2 equiv of amino acids.[6a]