ORGANIC LETTERS

2005
Vol. 7, No. 21
4765−4767

Convenient Access to Glutamic Acid Side Chain Homologues Compatible with Solid Phase Peptide Synthesis

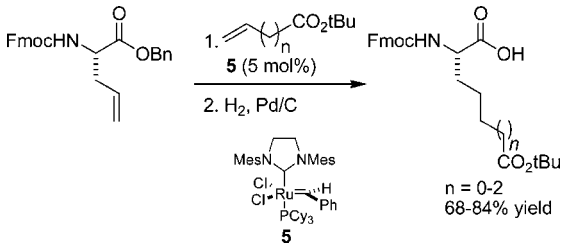
Shannon J. Ryan, Yongda Zhang, and Alan J. Kennan*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

kennan@lamar.colostate.edu

Received August 22, 2005

ABSTRACT



Preparation of several side chain length variants of glutamic acid is achieved via olefin cross metathesis of allyl glycine derivatives. The products are suitably protected for direct use in Fmoc solid-phase peptide synthesis, as demonstrated by successful synthesis of test sequences.

The utility of olefin metathesis reactions in synthetic organic chemistry has been validated across a broad expanse of molecular architectures, from the most simple to the most dauntingly complex.[1] The resulting efficient routes to previously inconvenient targets have facilitated numerous investigations. In the course of peptide molecular recognition studies, we sought to prepare glutamic acid analogues of varying side chain length. Here we report the use of olefin cross metathesis reactions to obtain derivatives having between one and three extra side chain methylenes from a common precursor, and their successful incorporation into peptides via Fmoc solid-phase methods.

The increased flexibility in protein design conferred by unnatural amino acid incorporation has numerous benefits, both structural and functional.[2] We recently undertook an investigation into the impact of side chain length on complex stability in self-assembling peptide systems, particularly with respect to interfacial interactions of glutamic acid−lysine pairs. Although the appropriate lysine analogues are readily commercially available, the glutamic acid variants must be independently synthesized. As such, we explored several alternatives, hoping to identify a route that was sufficiently modular to permit preparation of several different targets, efficient enough to generate multigram quantities, and compatible with standard side chain protecting groups for direct use in solid-phase peptide synthesis.

The use of allyl glycine as a common synthetic intermediate addresses all of these goals. It is easily prepared on gram scale and further elaborated via olefin cross metathesis. The documented use of allyl glycines in diverse metathesis reactions, and the prospects for facile access to suitable coupling partners, further supported the viability of this route.[3]

Allyl glycine was synthesized and N-protected according to methods developed by Myers and co-workers (Scheme

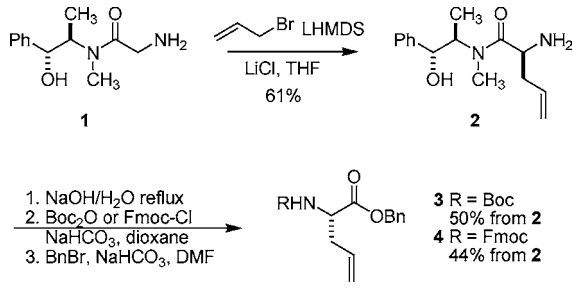
(1) (a) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490−4527. (b) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117−7140. (c) Schrock, R. R.; Hoveyda, A. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4592−4633.

(2) (a) Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 34−66. (b) Hendrickson, T. L.; de Crecy-Lagard, V.; Schimmel, P. *Annu. Rev. Biochem.* **2004**, *73*, 147−176. (c) Bilgiçer, B.; Kumar, K. *J. Chem. Educ.* **2003**, *80*, 1275−1281.

(3) (a) Nolen, E. G.; Kurish, A. J.; Potter, J. M.; Donahue, L. A.; Orlando, M. D. *Org. Lett.* **2005**, *7*, 3383−3386. (b) Boyle, T. P.; Bremner, J. B.; Coates, J. A.; Keller, P. A.; Pyne, S. G. *Tetrahedron* **2005**, *61*, 7271−7276. (c) Nolen, E. G.; Kurish, A. J.; Wong, K. A.; Orlando, M. D. *Tetrahedron Lett.* **2003**, *44*, 2449−2453. (d) Biswas, K.; Coltart, D. M.; Danishefsky, S. J. *Tetrahedron Lett.* **2002**, *43*, 6107−6110. (e) Dondoni, A.; Giovannini, P. P.; Marra, A. J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380−2388. (f) Bremner, J. B.; Keller, P. A.; Pyne, S. G.; Robertson, A. D.; Skelton, B. W.; White, A. H.; Witchard, H. M. *Aust. J. Chem.* **2000**, *53*, 535−540. (g) Biagini, S. C. G.; Gibson, S. E.; Keen, S. P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2485−2499.

10.1021/ol0520222 CCC: $30.25
Published on Web 09/15/2005

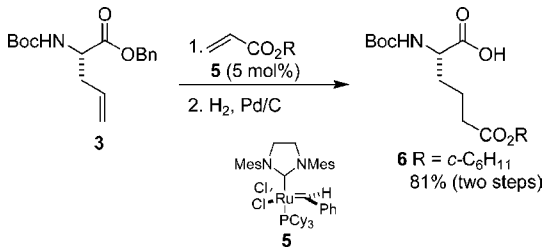
Scheme 1. Synthesis of Allyl Glycine Derivatives



1).[4] Synthesis of pseudoephedrine glycinamide **1** from pseudoephedrine and glycine methyl ester is straightforward. Alkylation, hydrolytic removal of the auxiliary, and protection affords either Boc- or Fmoc-allyl glycine. Benzylation under standard conditions produces either the Boc (**3**) or Fmoc (**4**) substrate for cross metathesis.

Our initial target was homoglutamic acid (HGlu), preferably a derivative compatible with Boc peptide synthesis methods (Scheme 2). Reaction of commercially available

Scheme 2. Synthesis of BocHGlu



cyclohexyl acrylate with **3** in the presence of Grubbs' second generation metathesis catalyst **5**, followed by hydrogenation, produced **6** in good yield (81% over two steps) and high optical purity (methyl ester formed with diazomethane is >99% ee by chiral HPLC).

Having successfully prepared Boc-HGlu with the side chain protected as its cyclohexyl ester (a standard protecting group for Boc peptide synthesis), we undertook its incorporation into test peptide **7** (Figure 1). Although the synthesis

7 Aba-LKALE*KE*LAL-NH_2

8 Aba-KLE*KG-NH_2
9 Aba-KLE**KG-NH_2
10 Aba-KLE***KG-NH_2

11 Ac-AQLE*KE*LQALE*KE*NAQLE*KE*LQALE*KE*LAQ-NH_2

Figure 1. Test peptide sequences, with position of glutamic acid analogues indicated (E* = HGlu, E** = HHGlu, E*** = HHHGlu). All peptides are C-terminally amidated; **7**−**10** contain an N-terminal acetamidobenzoic acid (Aba) group as a spectroscopic label, while **11** has the Aba group attached to the side chain of the underlined lysine and is N-terminally acetylated.

appeared to go smoothly, producing a single dominant peak in the crude HPLC, analysis of the isolated major product revealed that its molecular weight was 180 mass units too high (MW_{obs} = 1495.9, MW_{calc} = 1315.6).[5] This is consistent with bis-alkylation of HGlu-derived acylium ions by the anisole scavenger used during cleavage with anhydrous HF (Figure 2), a result similar to known side reactions of glutamic acid residues.[6]

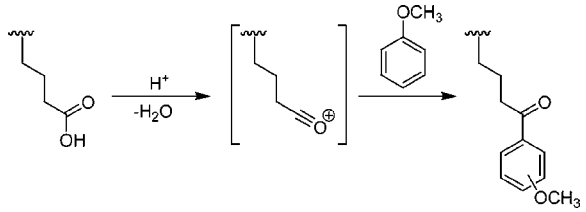
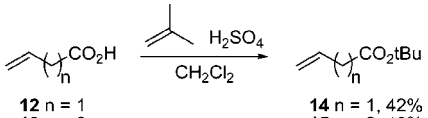


Figure 2. Possible explanation for unsuccessful Boc synthesis. Acylium ion formation and capture with anisole produces a peptide whose molecular weight is 90 mass units higher per alkylation.

It is not clear why conditions that routinely produce only minor if any glutamic acid alkylation should favor near complete modification of HGlu. The cleavage vessel had warmed to room temperature during HF removal, and a repeat experiment using an ice bath throughout did improve matters, affording the desired product as the major peak. However, there remained significant contamination from two other peaks with identical masses consistent with mono-alkylation (MW_{obs} = 1405.73).[5] As a result, the Boc synthesis strategy was jettisoned in favor of an Fmoc one.

The preferred acid side chain protection for Fmoc synthesis is the *tert*-butyl rather than cyclohexyl ester. Thus HGlu preparation requires commercially available *tert*-butyl acrylate as a metathesis coupling partner. To synthesize higher homologues having two (HHGlu) or three (HHHGlu) methylenes more than glutamic acid, we needed to prepare the longer chain alkenes. Fortunately, both vinyl and allyl acetic acid (**12**, **13**) can be converted to the corresponding esters (**14**, **15**) by reaction with isobutylene and catalytic sulfuric acid in methylene chloride (Scheme 3).[7] Although the yields

Scheme 3. Synthesis of Metathesis Substrates



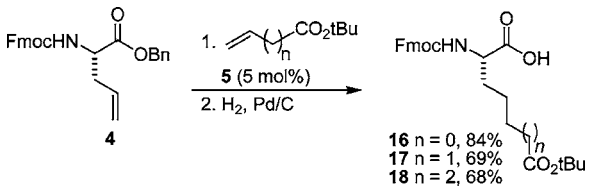
are only moderate, the low-cost starting materials make this a feasible route to multigram quantities of the desired esters.

(4) (a) Myers, A. G.; Schnider, P.; Kwon, S.; Kung, D. W. *J. Org. Chem.* **1999**, *64*, 3322−3327. (b) Myers, A. G.; Gleason, J. L. *Org. Synth.* **1999**, *76*, 57−76. (c) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656−673.

(5) See Supporting Information for details.

With the alkene substrates available, preparation of all three glutamic acid variants by cross metathesis was investigated (Scheme 4). In each case, a reasonable yield of the

Scheme 4. Synthesis of Fmoc Derivatives



chain extended Fmoc acid was obtained, with the side chain acid protected as the *tert*-butyl ester. Each product was optically pure, as judged by chiral HPLC evaluation of the corresponding methyl ester (>99% ee).[5]

To determine the feasibility of peptide synthesis using the new glutamic acid homologues, a second test sequence was used (**8**−**10**, Figure 1). The shorter sequence was designed with only one modified residue, to aid in deciphering possible side products. Fortunately, each synthesis gave the desired peptide as the dominant product in the crude HPLC (Figure 3).[5]

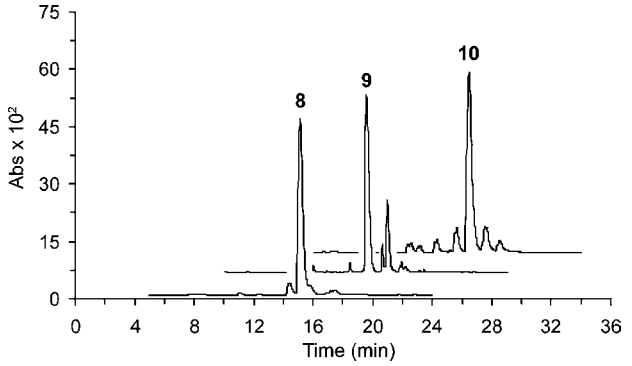


Figure 3. HPLC analysis of crude test peptides **8**−**10** (Figure 1). The major peak in each case is the desired peptide.[5]

Although success with short test peptides was encouraging, we next sought to validate the synthetic HGlu monomer in the context of a more realistically useful sequence, in particular one that incorporated multiple copies of the new amino acid. This sequence (**11**, Figure 1) is derived from GCN4, a natural coiled-coil protein, and contains no fewer than 8 copies of HGlu among its 30 residues. Thus it serves to highlight the feasibility of using these unnatural residues in longer peptides, and to emphasize the need for routes to multigram monomer quantities for some applications.

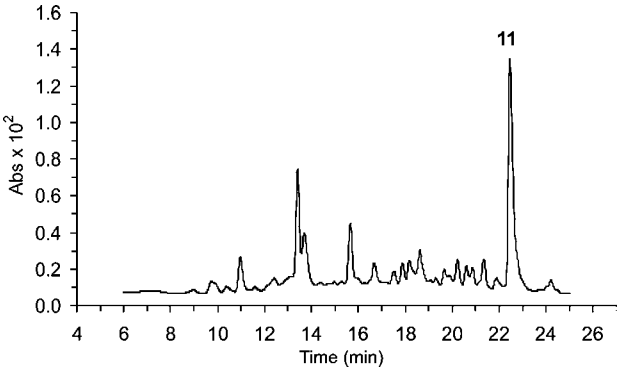


Figure 4. A more challenging application. Crude HPLC trace of the 30 residue peptide **11**, containing 8 copies of the unnatural HGlu residue. The desired peptide is the dominant peak.

As the crude HPLC trace makes clear (Figure 4), even this relatively stringent test of the new methodology was easily passed. The observed dominant major product is the desired peptide.[5]

In summary, we have developed an efficient and modular route to chain-extended glutamic acid analogues which are suitably protected for direct use in Fmoc solid phase peptide synthesis. The derivatives can be prepared on multigram scale, allowing incorporation of even multiple copies into relatively long model peptides. These results pave the way for facile investigation of side chain length effects in designed peptide systems.

Acknowledgment. This work was supported by an NSF CAREER award to A.J.K. (CHE-0239275).

Supporting Information Available: Detailed experimental procedures and peptide characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0520222

(6) Miranda, L. P.; Jones, A.; Meutermans, W. D. F.; Alewood, P. F. *J. Am. Chem. Soc.* **1998**, *120*, 1410−1420.
(7) Anderson, G. W.; Callahan, F. M. *J. Am. Chem. Soc.* **1960**, *82*, 3359.