JOC*Note*

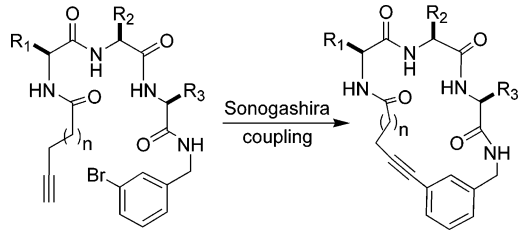
Synthesis of Conformationally Constrained Cyclic Peptides Using an Intramolecular Sonogashira Coupling†

V. Balraju,‡,§ D. Srinivasa Reddy,‡
Mariappan Periasamy,§ and Javed Iqbal*,‡

Discovery Research, Dr. Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad, 500 049, A.P., India, and School of Chemistry, University of Hyderabad, Central University P.O., Gachibowli, Hyderabad 500 046, A.P., India

javediqbaldrf@hotmail.com

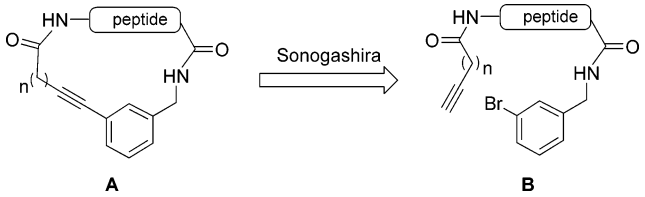
Received July 8, 2005



Small peptides having a 3-bromobenzyl group at the *C*-termini and *n*-alkynoyl group at the *N*-termini undergo a smooth copper-free intramolecular Sonogashira coupling reaction to afford the corresponding cyclic peptides in moderate yields. Scope and limitations of this macrocyclization is demonstrated with di-, tri-, and tetrapeptides.

The enhanced bioavailability, biological specificity, and reduced conformational flexibility of cyclic peptides make them attractive leads in modern drug discovery.[1] Several methods are known for cyclic peptide synthesis, including disulfide bond formation,[2] ring-closing metathesis,[3] S_NAr coupling,[4] Heck coupling,[5] and free-radical reactions.[6] Cyclization of peptides introduces an extra element of constraint (e.g., double bond, aromatic ring, etc.), which confers conformational restriction to the peptide leading to an entropically advantageous mode of binding to the target protein.[7,8] In connection with a project on peptidomimetics,[9] we are interested in developing novel and efficient methods for macrocyclizations to furnish cyclic peptides with various linkers. We envisaged the introduction of a rigid linker with an aryl alkynyl moiety in macrocycle **A** from the corresponding precursor **B** through $sp-sp^2$ bond formation using a Pd-catalyzed intramolecular Sonogashira coupling (Scheme 1).

SCHEME 1



The Sonogashira macrocyclization on resin-bound peptide appears to be the only known example for the synthesis of cyclic peptides using this reaction.[10] This reaction generally is cocatalyzed by copper(I) and uses an amine as a base and a phosphine as a ligand for

* Corresponding author. Phone: +91-40-2304-5439. Fax: +91-40-2304-5438.

† DRL Publication No. 497.

‡ Dr. Reddy's Laboratories Ltd..

§ University of Hyderabad.

(1) (a) Goodman, M.; Seonggu, R. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M. E., Ed.; Wiley: New York, 1995; Vol. 1. (b) Thompson, L. A.; Ellman, J. A. *Chem. Rev*. **1996**, *96*, 555. (c) Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Biol*. **1998**, *2*, 439. (d) Wiley, R. A.; Rich, D. H. *Med. Res. Rev*. **1993**, *13*, 327. (e) Freidinger, R. M. *Curr. Opin. Chem. Biol*. **1999**, *3*, 395. (f) Aube, J. In *Advances in Amino Acid Mimetics and Peptidomimetics*; Abell, A., Ed.; JAI Press: Greenwich, CT, 1997; Vol. 1, pp 193−232. (g) MacDonald, M.; Aube, J. *Curr. Org. Chem.* **2001**, *5*, 417−438. (h) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789. (i) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826. (j) Rose, G. D.; Gierasch, L. M.; Smith, J. A. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic: Orlando, FL, 1985; Vol. 37, pp 1−109. (k) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (l) Hruby, V. J. *Nat. Rev. Drug Discovery* **2002**, *1*, 847. (m) Suat Kee, K.; Jois, S. D. S. *Curr. Pharm. Des.* **2003**, *9*, 1209−1224.

(2) (a) Janecka, A.; Zubrzycka, M.; Janecki, T. *J. Pept. Res.* **2001**, *58*, 91. (b) Eichler, J.; Houghten, R. A. *Protein Pept. Lett.* **1997**, *4*, 157−164. (c) Hargittai, B.; Barany G. *Methods Enzymol*. **1997**, *289*, 198−221.

(3) (a) Prabhakaran, E. N.; Rajesh, V.; Dubey, S.; Iqbal, J. *Tetrahedron Lett.* **2001**, *42*, 339. (b) Prabhakaran, E. N.; Rao, I. N.; Boruah, A.; Iqbal, J. *J. Org. Chem.* **2002**, *67*, 8247. (c) Nandy, J. P.; Prabhakaran, E. N.; Kumar, S. K.; Kunwar, A. C.; Iqbal, J. *J. Org. Chem*. **2003**, *68*, 1679. (d) Creighton, C. J.; Reitz, A. B. *Org. Lett.* **2001**, *3*, 893. (e) Beal, L. M.; Liu, B.; Chu, W. H.; Moeller, K. D. *Tetrahedron* **2000**, *56*, 10113. (f) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J., Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638. (g) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606.

(4) (a) Feng, Y. B.; Pattarawarapan, M.; Wang, Z. C.; Burgess, K. *Org. Lett.* **1999**, *1*, 121. (b) Feng, Y. B.; Wang, Z. C.; Jin, S.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 10768.

(5) Reddy, P. R.; Balraju, V.; Madhavn, G. R.; Banerji, B.; Iqbal, J. *Tetrahedron Lett*. **2003**, *44*, 353.

(6) Balraju, V.; Reddy, D. S.; Periasamy, M.; Iqbal, J. *Tetrahedron Lett*. **2005**, *46*, 5207.

(7) (a) Davies, J. S. *J. Pept. Sci*. **2003**, *9*, 471−501. (b) Li, P.; Roller, P. P.; Xu, J. *Curr. Org. Chem.* **2002**, *6*, 411−440. (c) Wipf, P. *Chem. Rev*. **1995**, *95*, 2115. (d) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev*. **1997**, *97*, 2243. (e) Webster, K. L.; Maude, A. B.; O'Donnell, M. E.; Mehrotra, A. P.; Gani, D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1673. (f) Dyker, H.; ScherKenbeck, J.; Gondol, D.; Goehrt, A.; Harder, A. *J. Org. Chem.* **2001**, *66*, 3760. (g) Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1223. (h) Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502.

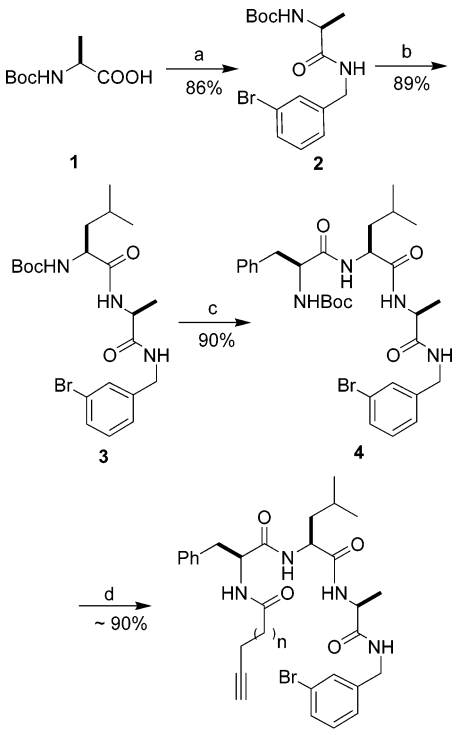
(8) See references using solid-phase synthesis: (a) Ishida, H.; Qi, Z.; Sokabe, M.; Donowaki, K.; Inoue, Y. *J. Org. Chem.* **2001**, *66*, 2978. (b) Wittmann, V.; Seeberger, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 4348. (c) Lee, Y.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 303. (d) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441 and references therein.

(9) Previous references from our laboratory on peptidomimetics: (a) Banerji, B.; Mallesham, B.; Kiran kumar, S.; Kunwar, A. C.; Iqbal, J. *Tetrahedron Lett*. **2002**, *43*, 6479. (b) Banerji, B.; Bhattacharya, M.; Madhu, B. R.; Das, S. K.; Iqbal, J. *Tetrahedron Lett*. **2002**, *43*, 6473. (c) Saha, B.; Das, D.; Banerji, B.; Iqbal, J. *Tetrahedron Lett*. **2002**, *43*, 6467. (d) Sastry, T. V. R. S.; Banerji, B.; Kirankumar, S.; Kunwar, A. C.; Das, J.; Nandy, J. P.; Iqbal, J. *Tetrahedron Lett*. **2002**, *43*, 7621. (e) Saha, B.; Nandy, J. P.; Shukla, S.; Siddiqui, I.; Iqbal, J. *J. Org. Chem.* **2002**, *67*, 7858. (f) Boruah, A.; Rao, I. N.; Nandy, J. P.; Kumar, S. K.; Kunwar, A. C.; Iqbal, J. *J. Org. Chem.* **2003**, *68*, 5006. (g) Rao, I. N.; Boruah, A.; Kumar, S. K.; Kunwar, A. C.; Devi, A. S.; Vyas, K.; Ravikumar, K.; Iqbal, J. *J. Org. Chem.* **2004**, *69*, 2181.

(10) Spivey, A. C.; McKendrick, J.; Srikaran, R. *J. Org. Chem.* **2003**, *68*, 1843−1851.

10.1021/jo051412z CCC: $30.25
Published on Web 10/08/2005

SCHEME 2[a]



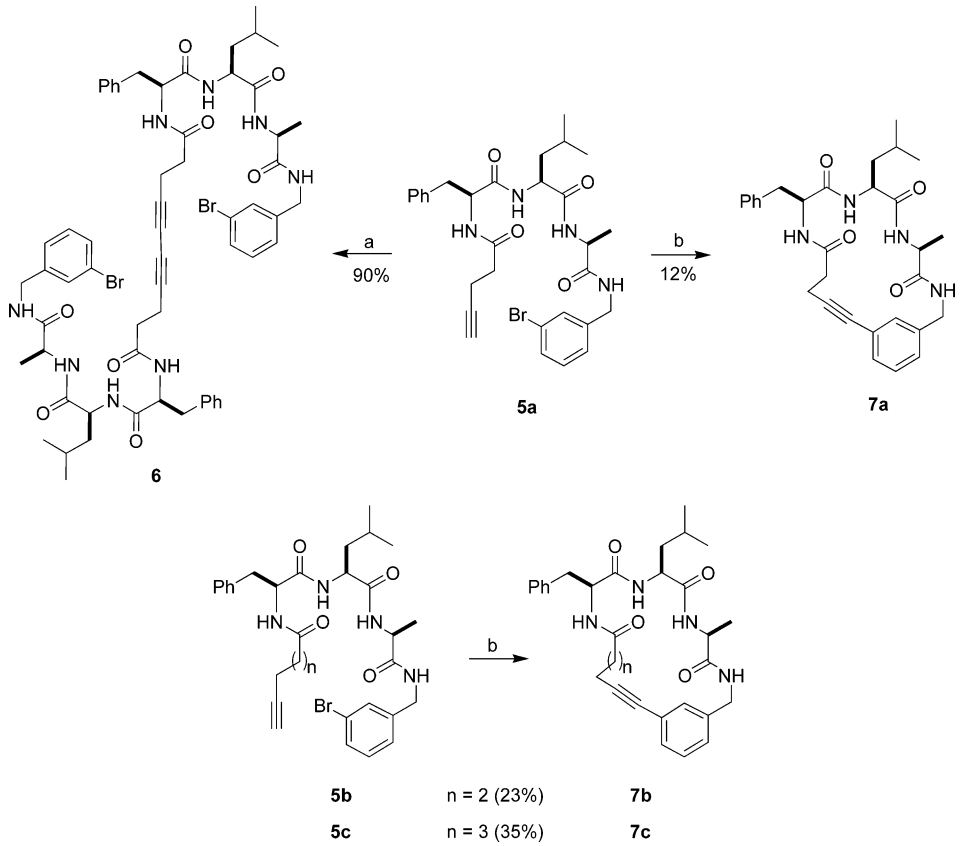
[a] Conditions: (a) 3-bromobenzylamine, Et_3N, HOBt, EDC, DCM; (b) i. TFA/DCM, ii. *N*-Boc-Leu-OH, Et_3N, HOBt, EDC, DCM; (c) i. TFA/DCM, ii. *N*-Boc-Phe-OH, Et_3N, HOBt, EDC, DCM; (d) i. TFA/DCM, ii. *n*-alkynoic acid, Et_3N, HOBt, EDC, DCM.

palladium.[11] This Note describes our preliminary studies on the copper-free Sonogashira reaction on acyclic peptide **B**. The synthesis of precursors based on **B** for Sonogashira coupling are summarized in Scheme 2.

Thus, the *N*-Boc-Ala-OH **1** was reacted with 3-bromobenzylamine to give compound **2** using standard solution-phase peptide coupling ((EDC, HOBt, Et_3N, DCM). The Boc deprotection (TFA/DCM) on **2**, followed by coupling with *N*-Boc-Leu-OH, furnished the peptide **3** in 89% yield. Removal of the Boc on **3**, followed by coupling with *N*-Boc-Phe-OH, gave tripeptide **4**. Finally, deprotection of **4**, followed by coupling with readily available *n*-alkynoic acids, gave desired acyclic precursors **5a**−**c** in excellent yields.

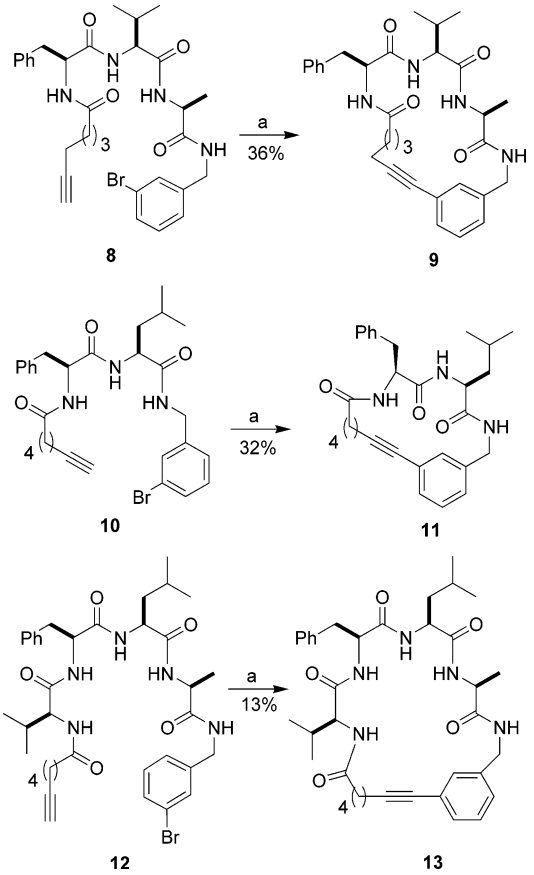
Initially, the acyclic peptide **5a** was subjected to a variety of known Sonogashira conditions, which resulted in the isolation of dimer **6** as the sole product. The formation of compound **6** can be explained by Glaser-type oxidative dimerization of the alkyne, which is one of the side reactions commonly encountered in Sonogashira reactions.[12] However, reacting **5a** under the copper-free Sonogashira coupling conditions[12,13] involving a bulky, electron-rich phosphine ligand [$Pd(OAc)_2$, (*o*-tolyl)$_3$P, and EtN(i-Pr)$_2$] in acetonitrile at 110 °C resulted in formation of the desired cyclic peptide **7a** in 12% isolated yield. We then subjected **5b** and **5c** to copper-free Sonogashira coupling conditions, and to our delight, both reactions afforded the corresponding cyclic peptides **7b** and **7c**, respectively, in moderate yields (Scheme 3). It is interesting to note that increase in the chain length between the

SCHEME 3[a]



[a] Conditions: (a) $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN + DMF, room temperature, 15 h. (b) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, EtN(*i*-Pr)$_2$, CH_3CN, 110 °C, 15 h.

SCHEME 4[a]



[a] Conditions: (a) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, EtN(*i*-Pr)$_2$, CH_3CN, 110 °C, 15 h.

alkyne and the amide bond resulted in a higher isolated yield of the cyclic peptide. Thus, the peptide incorporating 6-heptynoic acid afforded compound **7c** in 35% isolated yield compared to the 12% isolated yield of the cyclic peptide incorporating 4-pentynoic acid.[14] All the cyclic peptides were purified on silica gel column chromatography and characterized using various spectral techniques (see Experimental Section).

Having demonstrated that reacting the acyclic precursors **5** under the copper-free intramolecular conditions gave the corresponding cyclic peptides **7**, we extended the scope of this reaction to other acyclic peptide precursors by varying the amino acids and the size of the acyclic peptide. The change at the $i + 1$ amino acid residue from leucine to valine (compound **8**) gave desired cyclization in 36% yield to furnish cyclic peptide **9**. Similarly, dipeptide **10** underwent smooth cylization to furnish **11** in 32% yield. However, the tetrapeptide **12** gave a poor yield of the corresponding cyclic peptide **13** (Scheme 4).[14] These preliminary studies indicate that the yields of the cyclization of these peptides under the copper-free Sonogashira conditions used are dependent upon both the number of amino acid residues and the chain length of the incorporated *n*-alkynoic acids.

In conclusion, we have demonstrated that copper-free Sonogashira coupling conditions can be used for the macrocyclization of di-, tri-, and tetrapeptides to produce cyclic peptides with rigid linkers leading to useful peptidomimetics. To the best of our knowledge, this is the first report of copper-free intramolecular Sonogashira macrocyclization for the synthesis of cyclic peptides. These cyclic peptides may prove to be useful in understanding the utility of constrained structures in the search for novel lead molecules.

Experimental Section

Typical Procedure for Sonogashira Macrocyclization for 7c. $Pd(OAc)_2$ (72 mg, 0.32 mmol) and (*o*-tolyl)$_3$P (195 mg, 0.64 mmol) were added to warm HPLC-grade acetonitrile (1.2 L) and refluxed at 110 °C for 30 min. Then acylic peptide **5c** (500 mg, 0.80 mmol) was added in single portion, and the reaction was continued for 15 min at the same temperature. Finally, *N*-ethyldiisopropylamine (0.7 mL, 4 mmol) was added. After 15 h, the reaction mixture was filtered through a pad of Celite and washed with hot acetonitrile (100 mL). The filtrate was concentrated, and the product was isolated as a white solid (243 mg, 35%) after flash column chromatography on (230−400) silica gel using CH_2Cl_2/MeOH (98:2) as eluent. mp 289−290 °C; $[\alpha]_D$ +6.80 (*c* 0.5, DMSO); IR (KBr), 3315, 2929, 1652, 1590, 1524, 1467 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.29 (m, 2H), 8.19 (t, J = 5.37 Hz, 1H), 7.28−7.14 (m, 10H), 4.50 (dd, J_1 = 7.25 Hz, J_2 = 16.12 Hz, 1H), 4.41−4.36 (m, 2H), 4.20−4.16 (m, 1H), 4.05 (dd, J_1 = 4.84 Hz, J_2 = 16.12 Hz, 1H), 3.12 (dd, J_1 = 4.57 Hz, J_2 = 13.97 Hz, 1H), 2.80 (dd, J_1 = 10.21 Hz, J_2 = 13.97 Hz, 1H), 2.38 (t, J = 6.98 Hz, 2H), 2.19−2.14 (m, 1H), 2.03−1.98 (m, 1H), 1.73−1.44 (m, 7H), 1.26 (d, J = 7.25 Hz, 3H), 0.84 (dd, J_1 = 6.18 Hz, J_2 = 14.24 Hz, 6H); ^{13}C NMR (DMSO-d_6, 50 MHz) 172.5, 172.4, 171.6, 170.6, 139.7, 138.3, 137.9, 134.6, 129.1, 128.9 (2C), 128.8, 128.1 (2C), 127.9, 126.2, 126.1, 123.5, 54.3, 50.2, 49.1, 41.8, 41.0, 35.8, 34.9, 27.9, 24.6, 23.8, 23.2, 21.6, 18.6, 16.9; ESMS *m/z* 545.5 (M^+, 100%); HRMS calcd for $C_{32}H_{41}N_4O_4$ 545.3127, found 545.3115.

Acknowledgment. We thank Dr. Reddy's Laboratories Ltd. for financial support and the analytical department for their help in recording spectral data. V.B. thanks Dr. Santanu Maitra for his help and cooperation.

Supporting Information Available: Experimental procedures, characterization data, and copies of NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051412Z

(11) (a) Sonogashira, K. In *Metal-Catalyzed Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: NewYork, 1998. (b) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467. (c) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46. (d) Rossi, R.; Carpita, A.; Bellina, F. *Org. Prep. Proced. Int.* **1995**, 129. (e) Tykwinski, R. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1566.

(12) Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem., Int. Ed.* **2000**, *39*, 2632.

(13) (a) Rossi, R.; Carpita, A.; Bigelli, C. *Tetrahedron Lett.* **1985**, *26*, 523. (b) Alami, M.; Ferri, F.; Linstrumelle, G. *Tetrahedron Lett.* **1993**, *34*, 6403. (c) Liu, Q.; Burton, D. J. *Tetrahedron Lett.* **1997**, *38*, 4371. (d) Hundertmark, T.; Littke, A. F.; Buchwald, S. L.; Fu, G. C. *Org. Lett.* **2000**, *2*, 1729. (e) Liao, Y.; Fathi, R.; Reitman, M.; Zhang, Y.; Yang, Z. *Tetrahedron Lett.* **2001**, *42*, 1815. (f) Dai, W.-M.; Wu, A. *Tetrahedron Lett.* **2001**, *42*, 81. (g) Elangovan, A.; Wang, Y.-H.; Ho, T.-I. *Org. Lett.* **2003**, *5*, 1841. (h) Liang, B.; Dai, M.; Chen, J.; Yang, Z. *J. Org. Chem.* **2005**, *70*, 391.

(14) All the new compounds are characterized on the basis of spectral data. See Supporting Information for details.