Tetrahedron Letters 49 (2008) 5629–5631

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of methionine- and norleucine-derived phosphinopeptides

Radek Liboska, Jan Pícha, Ivona Hančlová, Miloš Buděšínský, Miloslav Šanda, Jiří Jiráček *

Institute of Organic Chemistry and Biochemistry, v.v.i., Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 2 June 2008
Revised 30 June 2008
Accepted 9 July 2008
Available online 12 July 2008

ABSTRACT

We present herein a straightforward synthesis of *N*-Fmoc-protected synthons derived from a phosphinic analogue of methionine. These precursors were used successfully for the solid-phase synthesis of methionine-mimic phosphinopeptides using BOP-catalyzed coupling without protection of the phosphoryl moiety. We also prepared a new type of pseudopeptide derived from a phosphinic analogue of norleucine with a –PO(OH)–CH_2–COOR moiety.

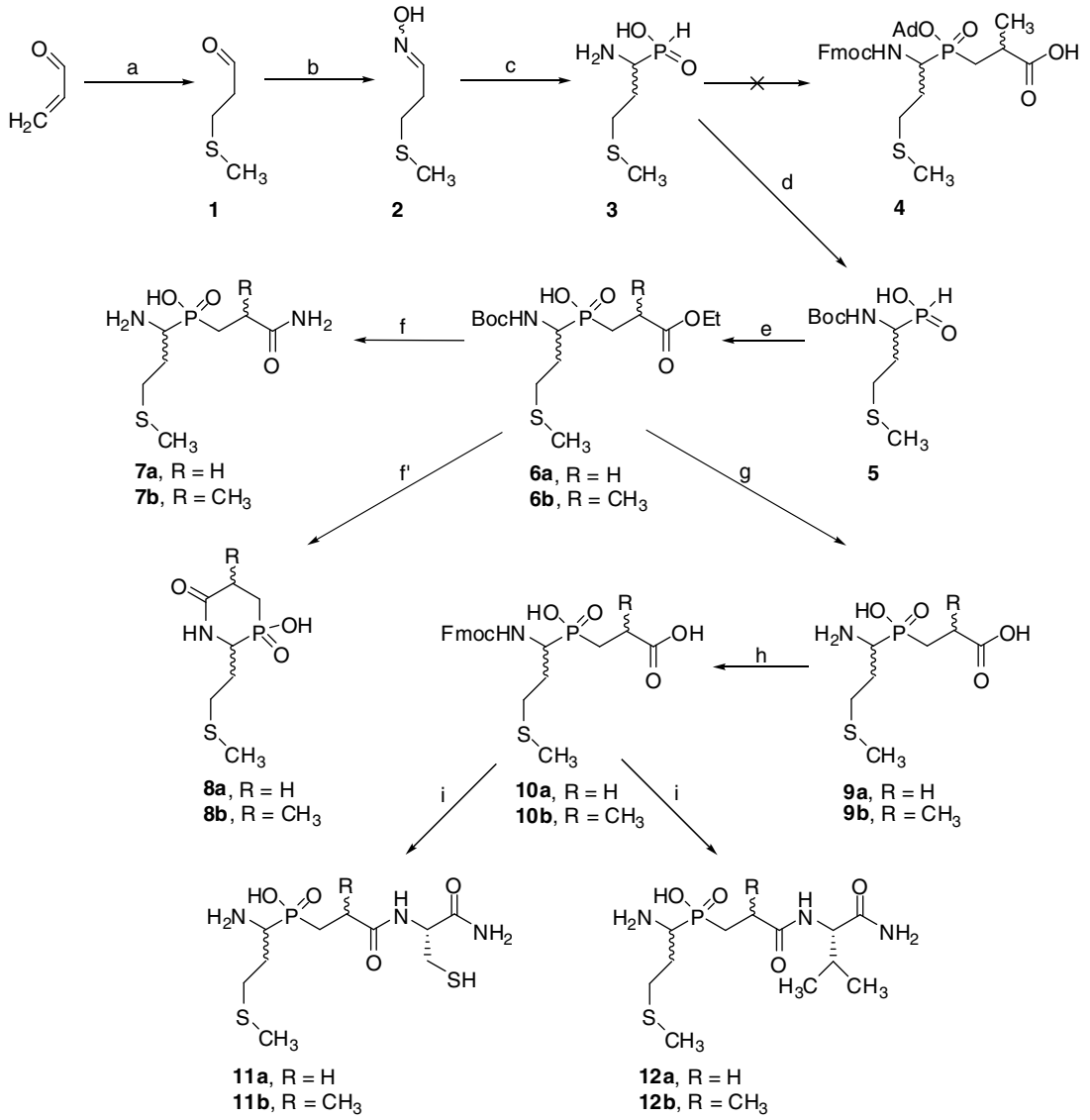
Phosphinopeptides[1,2] (or phosphinic pseudopeptides) have found important applications in biology and medicine, primarily as inhibitors of metalloenzymes.[3,4] The replacement of a hydrolyzable peptide bond by a non-hydrolyzable phosphinate moiety mimics the hypothetical transition state of the native substrate during hydrolysis and may result in a potent inhibition of the respective peptidases.[5]

First, we present an efficient synthesis of a novel type of pseudopeptides of formulas Met-ψ[PO(OH)CH_2]-Ala(Gly), Met-ψ[PO(OH)CH_2]-Ala(Gly)-Val, and Met-ψ[PO(OH)CH_2]-Ala(Gly)-Cys that mimic the sequences Met-Ala(Gly), Met-Ala(Gly)-Val, and Met-Ala(Gly)-Cys, but contain the phosphinate analogue of methionine at the N-terminus (Scheme 1). Second, we demonstrate the preparation of a new type of pseudopeptides of formula Nle-ψ[PO(OH)]-Gly derived from the phosphinate analogue of norleucine (Scheme 2). Both types of compounds represent potential inhibitors of methionine or leucine aminopeptidases. The proposed pseudopeptide sequences should fulfill the structural requirements of methionine aminopeptidases with Met or Nle at the P_1 position and a small aliphatic residue, such as Ala or Gly, at the P'_1 position. The C-terminal Val and Cys at the P'_2 position of the methionine-derived phosphinates were chosen based on our recent results with statin pseudopeptides as inhibitors of methionine aminopeptidases.[6]

The synthesis of the methionine-derived phosphinates (Scheme 1) began with the addition of methanethiol to acrolein[7] followed by reaction of the aldehyde **1** with hydroxylamine hydrochloride in pyridine to give oxime **2** in good yield. The phosphinate intermediate **3** was prepared according to Zhukov et al.[8] by refluxing oxime **2** with anhydrous hypophosphorus acid in dry methanol under an argon atmosphere. The previously published[8] procedures for the isolation of phosphinic acid **3**, such as crystallization from 2-propanol or chromatography (on cellulose or Dowex), afforded rather poor yields (about 30–35%). We were unable to prepare synthon **4,** which contains a phosphinyl moiety protected by an adamantyl group (Ad). However, similar compounds were used previously for the solid-phase synthesis of phosphinic pseudopeptides.[9,10] The preparation of compound **4** failed due to low yields in the adamantylation step and especially in the final catalytic hydrogenation. This difficulty was probably caused by the hydrogenation-poisoning effect of the sulfur atom present in our compounds. Therefore, we returned to phosphinic acid **3** and instead of further difficult purification attempts, we neutralized the redundant phosphorus acid and its phosphoric acid oxidation product with sodium carbonate, partially removed the methanol in vacuo, and protected the amino group of crude compound **3** with di-*tert*-butyl dicarbonate in aqueous sodium carbonate/1,4-dioxane mixture. The *N*-Boc-protected product was extracted from the acidified (pH ~ 2) reaction mixture, and column chromatography on silica gel gave a good yield of compound **5**. P–C bond formation was performed according to Georgiadis et al.[10] via silylation of **5** with chlorotrimethylsilane in dichloromethane/DIPEA followed by addition of ethyl acrylate or ethyl methacrylate to give compounds **6a** (R = H) or **6b** (R = CH_3), respectively. Extraction of these compounds into ethyl acetate after careful acidification and final silica gel chromatography afforded pure compounds **6a** and **6b** in good yields. Unprotected C-terminal amides **7a** and **7b** were prepared from **6a** and **6b**, respectively, using a 20% (w/w) solution of ammonia in anhydrous ethanol heated to 55 °C for 48 h in a sealed container. Alternatively, the use of aqueous ammonia yielded mainly the free acid. The solution was evaporated to dryness in vacuo and the *N*-Boc-protecting group was cleaved with TFA (100%, rt, 4 h). Finally, compounds **7a** and **7b** were purified by RP-HPLC (Phenomenex Luna C-18). If the N-terminal Boc group was removed prior to the ammonolysis of the C-terminal ethyl ester, the lactams **8a** and **8b** were formed. Similar reactivity was observed previously by Yiotakis et al.[9] The unprotected phosphinic amino acids **9a** and **9b** were prepared in two steps; alkaline hydrolysis (4 M aq NaOH) of the ethyl esters **6a** or **6b** was followed, after

* Corresponding author. Tel.: +420 220183441; fax: +420 220183571.
E-mail address: jiracek@uochb.cas.cz (J. Jiráček).

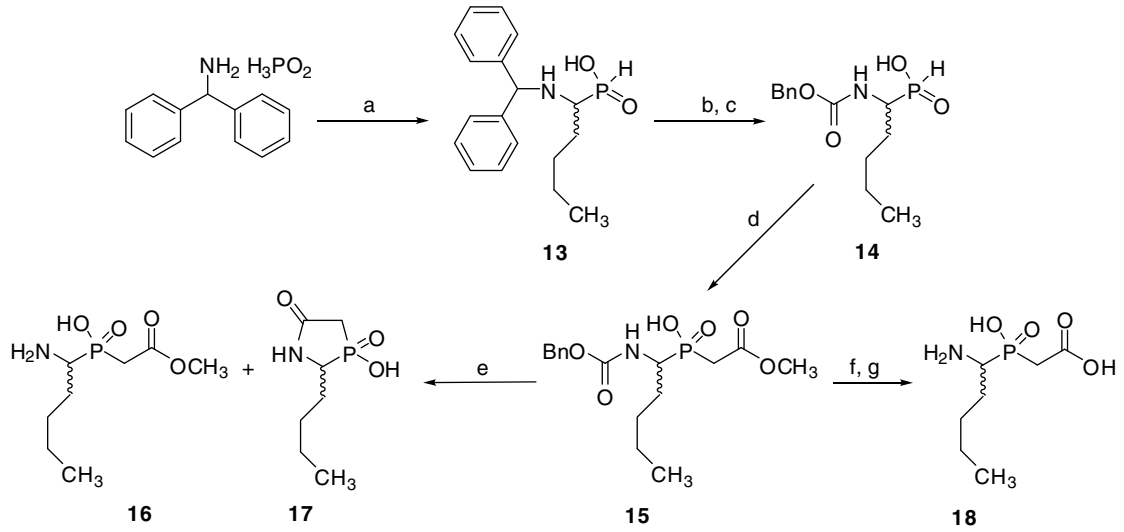
0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.07.062



Scheme 1. Reagents, conditions, and yields: (a) MeSH, 0 °C (41%); (b) $NH_2OH{\cdot}HCl$, pyridine, 14 h rt (80%); (c) anhydrous H_3PO_2 (1 M) in methanol, reflux for 6 h; (d) Boc_2O, Na_2CO_3, water/1,4-dioxane, 0 °C for 1 h then 2 h rt (79%, calculated from **2**); (e) ethyl acrylate, or ethyl methacrylate, TMSCl, DIPEA, rt overnight R = H (88%), R = CH_3 (82%); (f) (i) NH_3/EtOH, 20% (w/w), 55 °C, 48 h; (ii) TFA, 100%, 4 h rt, R = H (46%, after HPLC), R = CH_3 (42% after HPLC); (f′) (i) TFA (100%), 24 h rt; (ii) NH_4OH (25%), 55 °C, 16 h, or aq NaOH (4 M), 4 h rt. The yields of products **8a** and **8b** have not been determined; (g) (i) aq NaOH (4 M), rt overnight; (ii) TFA (100%), 4 h rt, R = H (78%), R = CH_3 (74%); (h) FmocOSu, Na_2CO_3, water/dioxane, 3 h rt R = H, CH_3 (28–92%); (i) (i) Cys(Trt)-Rink Amide AM resin or Val-Rink Amide AM resin (1 equiv), **10a** or **10b** (1.5 equiv), BOP (3 equiv)/DIPEA (5 equiv) in DMF, rt overnight and 6 h; (ii) piperidine/DMF; (iii) TFA (95%), ethanedithiol, water, triisopropylsilane, 2 h, rt (**11a** 21%, **11b** 25%, **12a** 23%, **12b** 30%, yields are given for compounds purified by RP-HPLC as mixtures of diastereoisomers).

removal of sodium ions using Dowex 50W (H^+), by treatment with TFA (100%, rt, 4 h). The products **9a** and **9b** were purified using silica gel chromatography. In order to prepare *N*-Fmoc-protected synthons **10a** and **10b**, convenient precursors for the solid-phase synthesis of phosphinic pseudopeptides, compounds **9a** and **9b** were treated with 9-fluorenylmethyl *N*-succinimidyl carbonate (FmocOSu) in aqueous sodium carbonate/1,4-dioxane. After completion of the reactions (monitored by TLC) and removal of the dioxane, the reaction mixture was acidified with 1 M HCl, and the product was taken up into ethyl acetate and purified by silica gel chromatography. The irreproducible yields of compounds **10a** and **10b** were due to the very low solubility of the products in the available chromatography eluents. As an example, the NMR spectral data for compounds **10** are given.[11] We applied the techniques of solid-phase synthesis for the preparation of pseudopeptides **11** and **12**. The C-terminal valine and cysteine residues were attached to Rink Amide AM resin using an Fmoc/HBTU/DIPEA strategy.[12] The subsequent coupling of the phosphinic synthons **10a** or **10b** was achieved with BOP/DIPEA reagents according to Raguin et al.[13] After the N-terminal deprotection, the pseudopeptides **11** and **12** were cleaved from the resin with a mixture of TFA and scavengers, and purified by RP-HPLC in low yields.

In the second part of our study, we prepared a new type of phosphinate with a –PO(OH)–CH_2–COOR moiety. Similar compounds were previously prepared by Allen et al.[14] The above-described difficulties with methionine-mimic phosphinic pseudopeptides led us to abandon the methionine side chain and to introduce the analogous norleucine-derived side chain.[15] (1-Benzhydrylamino)pentylphosphinic acid **13** was synthesized by heating valeraldehyde and diphenylmethylamine hypophosphite in THF according to the procedure of Baylis et al.[16] The phosphinic acid **13** was converted to (*R*,*S*)-1-aminopentylphosphonic acid by refluxing with concentrated hydrobromic acid followed by treatment with propylene oxide. Reaction of the free phosphinic acid



Scheme 2. Reagents, conditions, and yields: (a) valeraldehyde, THF, reflux for 2 h (75%); (b) 48% HBr, reflux for 8 h, then propylene oxide, ethanol (69%); (c) benzyl chloroformate, Na_2CO_3, water and dioxane, 0 °C 2 h then rt overnight (93%); (d) TMSCl, TEA, methyl bromoacetate, rt overnight (80%); (e) $HCOO^-NH_4^+$, 10% Pd/C, methanol, rt overnight (20% for **16**); (f) NaOH, methanol and water, rt overnight (79%); (g) $HCOO^-NH_4^+$, 10% Pd/C, methanol, rt overnight (48%).

with benzyl chloroformate afforded the Z-protected derivative **14**, which gave intermediate **15** upon Arbuzov reaction[14] with methyl bromoacetate. For reference, the NMR spectral data for compound **15** are provided.[11] The analogous Arbuzov reaction was also performed with the methionine analogue of **14** but this attempt was unsuccessful, probably due to the presence of a sulfur atom. Catalytic transfer hydrogenation[17] of **15** afforded the desired methyl ester **16** along with a considerable amount of cyclic by-product **17**. However, alkaline hydrolysis of **15** followed by catalytic transfer hydrogenation afforded target compound **18**.

The synthesis of methionine-derived phosphinic pseudopeptides is difficult due to the presence of a side-chain sulfur atom. However, phosphinopeptides and phosphonopeptides can be prepared by BOP-catalyzed coupling using N-protected synthons without protection of the phosphoryl moiety. We successfully used this strategy for the synthesis of several methionine-derived phosphinic pseudopeptides as potential inhibitors of aminopeptidases. We also prepared a new type of pseudopeptide derived from the phosphinic analogue of norleucine with a –PO(OH)–CH_2–COOR moiety. The target compounds **7**, **9**, **11**, **12**, **16**, and **18** will be tested for their inhibitory activities toward leucine and methionine aminopeptidases.

Acknowledgments

This project was supported by Grant 203/06/1405 (to J.J.) from the Grant Agency of the Czech Republic, by the Chemical Genetics Consortium No. LC060777 of the Ministry of Education, Youth and Sports of the Czech Republic (to J.J.) and by Research Project Z4 055 0506 of the Academy of Sciences of the Czech Republic.

References and notes

1. Kafarski, P.; Lejczak, B. In *Aminophosphinic and Aminophosphonic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; Synthesis of Phosphono- and Phosphinopeptides; John Wiley & Sons Ltd: Chichester, 2000; pp 173–203.
2. Kafarski, P.; Lejczak, B. In *Aminophosphinic and Aminophosphonic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; The Biological Activity of Phosphono- and Phosphinopeptides; John Wiley & Sons Ltd: Chichester, 2000; pp 407–442.
3. Dive, V.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Beau, F.; Cuniasse, P.; Yiotakis, A. *Cell. Mol. Life Sci.* **2004**, *61*, 2010.
4. Yiotakis, A.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Dive, V. *Curr. Org. Chem.* **2004**, *8*, 1135.
5. Collinsova, M.; Jiracek, J. *Curr. Med. Chem.* **2000**, *7*, 629.
6. Mitra, S.; Dygas-Holz, A. M.; Jiracek, J.; Zertova, M.; Zakova, L.; Holz, R. C. *Anal. Biochem.* **2006**, *357*, 43.
7. Catch, J. R.; Cook, A. M.; Graham, A. R.; Heilbron, I. *J. Chem. Soc.* **1947**, 1609.
8. Zhukov, Y. N.; Khomutov, A. R.; Osipova, T. I.; Khomutov, R. M. *Russ. Chem. Bull.* **1999**, *48*, 1348.
9. Yiotakis, A.; Vassiliou, S.; Jiracek, J.; Dive, V. *J. Org. Chem.* **1996**, *61*, 6601.
10. Georgiadis, D.; Matziari, M.; Yiotakis, A. *Tetrahedron* **2001**, *57*, 3471.
11. *Compound* **10a**: ^{1}H NMR (600 MHz, DMSO): 1.79 (2H, m, P–CH_2); 1.79 and 1.97 (2× 1H, 2× m, C–CH_2–C); 2.04 (3H, s, S–CH_3); 2.40 and 2.46 (2× 1H, 2× m, CH_2–CO); 2.38 and 2.55 (2× 1H, 2× m, S–CH_2); 3.81 (1H, m, CH–P); 4.23 (1H, dd, *J* = 7.2 and 7.0, CH(Fmoc)); 4.31 (1H, dd, *J* = 10.6 and 7.2, O–CHa); 4.38 (1H, dd, *J* = 10.6 and 7.0, O–CHb); 7.32 (1H, m, arom.H); 7.34 (1H, m, arom.H); 7.43 (2H, m, arom.H); 7.64 (1H, d, *J* = 9.4, NH); 7.73 (1H, m, arom.H); 7.74 (1H, m, arom.H); 7.90 (2H, m, arom.H). ^{13}C NMR (150.9 MHz, DMSO): 14.83 (S–CH_3); 21.99 (d, *J*(C,P) = 90.3, P–CH_2); 26.63 (d, *J*(C,P) = 2.8, *CH_2–CO);* 27.15 (d, *J*(C,P) = 2.3, C–CH_2–C); 30.25 (d, *J*(C,P) = 13.1, S–CH_2); 47.02 (>CH– (Fmoc)); 49.13 (d, *J*(C,P) = 106.0, P–CH); 65.83 (O–CH_2); 120.40(2C), 125.48, 125.55, 127.31, 127.35, 127.92, 127.94, 141.00, 141.01, 143.94 and 144.15 (12 arom.C); 156.59 (d, *J*(C,P) = 3.8, O–CO–N); 173.90 (d, *J*(C,P) = 15.9, COOH). *Compound* **10b**: ^{1}H NMR (600 MHz, DMSO): 1.17 (3H, d, *J* = 7.0, CH_3); 1.56 and 2.04 (2× 1H, 2× m, P–CH_2); 1.78 and 1.96 (2× 1H, 2× m, C–CH_2–C); 2.02 (3H, s, S–CH_3); 2.38 and 2.53 (2× 1H, 2× m, S–CH_2); 2.67 (1H, m, CH–CO); 3.78 (1H, m, CH–P); 4.22 (1H, t, *J* = 7.1, CH(Fmoc)); 4.30 (1H, dd, *J* = 10.6 and 7.1, O–CHa); 4.33 (1H, dd, *J* = 10.6 and 7.1, O–CHb); 7.31 (1H, m, arom.H); 7.32 (1H, m, arom.H); 7.41 (2H, m, arom.H); 7.63 (1H, d, *J* = 9.4, NH); 7.71 (2H, m, arom.H); 7.89 (2H, m, arom.H). ^{13}C NMR (150.9 MHz, DMSO): 14.80 (S–CH_3); 18.73 (d, *J*(C,P) = 5.8, C–CH_3); 27.16 (d, *J*(C,P) = 2.7, C–CH_2–C); 29.73 (d, *J*(C,P) = 89.2, P–CH_2); 30.22 (d, *J*(C,P) = 2.9, S–CH_2); 33.31 (d, *J*(C,P) = 3.3, *C*H–CO); 46.94 (>CH– (Fmoc)); 49.86 (d, *J*(C,P) = 105.6, P–CH); 65.83 (O–CH_2); 120.36(2C), 125.47, 125.51, 127.27, 127.30, 127.88(2C), 140.96(2C), 143.94 and 144.11 (12 arom.C); 156.43 (d, *J*(C,P) = 3.8, O–CO–N); 176.89 (d, *J*(C,P) = 12.0, COOH). *Compound* **15**: ^{1}H NMR (600 MHz, $CDCl_3$): 0.87 (3H, t, *J* = 7.0, CH_3); 1.28 and 1.34 (2× 1H, 2× m, CH_2); 1.34 and 1.44 (2× 1H, 2× m, CH_2); 1.54 and 1.84 (2× 1H, 2× m, CH_2); 4.13 (1H, m, CH–P); 2.98 (1H, dd, *J* = 14.8 and 16.8, P–CHa); 3.01 (1H, dd, *J* = 14.8 and 15.8, P–CHb); 3.67 (3H, s, $COOCH_3$); 5.09 and 5.14 (2× 1H, 2× d, *J* = 12.3, O–CH_2); 5.39 (1H, d, *J* = 10.2, NH); 7.30–7.35 (5H, m, C_6H_5); 10.33 (1H,br s, P–OH). ^{13}C NMR (150.9 MHz, CDCl3): 13.84 (CH_3); 22.15 (CH_2); 27.68 (CH_2); 27.84 (d, *J*(C,P) = 11.9, CH_2); 34.94 (d, *J*(C,P) = 82.1, P–CH_2); 50.08 (d, *J*(C,P) = 111.7, P–CH); 52.63 (OCH_3); 67.19 (O–CH_2); 127.97(2C), 128.16, 128.48(2C) and 136.14 (5 arom.C); 156.35 (d, *J*(C,P) = 5.9, O–CO–N); 166.56 (d, *J*(C,P) = 4.4, $COOCH_3$).
12. Fields, G. B.; Noble, R. L. *Int. J. Pept. Prot. Res.* **1990**, *35*, 161.
13. Raguin, O.; Fournie-Zaluski, M. C.; Romieu, A.; Pelegrin, A.; Chatelet, F.; Pelaprat, D.; Barbet, J.; Roques, B. P.; Gruaz-Guyon, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 4058.
14. Allen, M. C.; Fuhrer, W.; Tuck, B.; Wade, R.; Wood, J. M. *J. Med. Chem.* **1989**, *32*, 1652.
15. Picha, J.; Budesinsky, M.; Sanda, M.; Jiracek, J. *Tetrahedron Lett.* **2008**, *49*, 4366.
16. Baylis, E. K.; Campbell, C. D.; Dingwall, J. G. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2845.
17. Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929.