Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2441–2446

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF A PHOSTONE GLYCOMIMETIC OF THE ENDOTHELIN CONVERTING ENZYME INHIBITOR PHOSPHORAMIDON‡

Stephen Hanessian* and Olivier Rogel

Department of Chemistry, Université de Montréal, P.O. Box 6128, Station Centre-ville, Montréal, QC, CANADA H3C 3J7

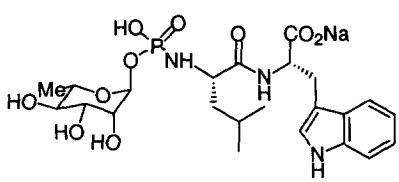
Received 7 June 1999; accepted 15 July 1999

Abstract: The phostone analog of phosphoramidon, an inhibitor of endothelin converting enzyme, was synthesized from L-rhamnose. Coupling of the cyclic phosphonic acid with the dipeptide H-Leu-Trp-OMe gave, after deprotection and purification by reverse-phase HPLC, the desired phostone which exhibited an IC_{50} of 5.05 ± 2.7 μM.

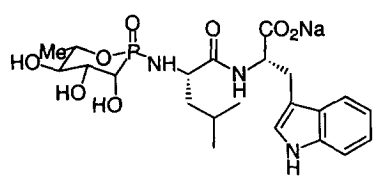
The naturally occurring peptide endothelin has been recognized as a potent vasoconstrictor that seriously affects the pathophysiology of cardiovascular, renal, and respiratory disease states.[1] Since its isolation over two decades ago,[2] three distinct genes have been identified that express the peptides ET-1, ET-2 and ET-3.[3] The metalloprotease endothelin converting enzyme (ECE) plays an important role in the biosynthesis of these peptides.[2] It effects a selective cleavage of the Trp^{21}-Val^{22} bond of the so-called big ET-1 to produce ET-1. Since big ET-1 is a much weaker vasoconstrictor in vitro, compared to ET-1, it is generally accepted that the formation of the latter by enzymatic cleavage is the cause of the observed pathophysiological effects.

The amino acid phosphoramidate derivative phosphoramidon **1** (Figure 1), produced by Actinomyces and initially reported as an inhibitor of thermolysin,[4] was also found to inhibit the hypertensive effect induced by big ET-1.[5] The design and synthesis of selective inhibitors of ECE based on the structure of phosphoramidon[6] and other non-peptidic structures[7] have been areas of great interest in medicinal chemistry. Inhibitors of ECE have also been isolated from fermentation[8] and marine[9] sources.

Figure 1. Phosphoramidon and its phostone analog



Phosphoramidon, 1



Phostone variant, 2

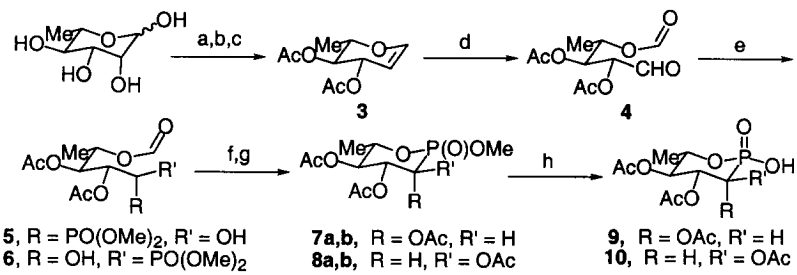
‡Dedicated to the memory of Professor Larry Weiler (University of British Columbia) – a scholar and a friend.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00401-1

As part of an on-going project aimed[10] at the synthesis of cyclic phosphonates (phostones),[11-13] we report on the synthesis of a novel structural prototype **2** related to phosphoramidon[14] (Figure 1). The L-Leu-L-Trp peptide portion was maintained as with the natural product, but the phosphoramidate unit was modified by including it within the L-rhamnose core in the form of the corresponding phostone. A notable functional difference between the natural product **1** and the prototype **2**, is the lack of the acidic phosphate group in the latter. The synthesis and biological activity of phosphonamidates have been areas of interest for some time.[15]

The readily available L-rhamnal derivative[16] **3** was cleaved by ozonolysis to produce the corresponding lyxose ester **4**. Treatment of **4** under the conditions of the Abramov reaction[17] in the presence of trimethylphosphite in glacial acetic acid[12] led to a mixture of epimeric α-hydroxy phosphonates **5** and **6**.[18] The mixture was subjected to ring closure with sodium methoxide to give the corresponding cyclic phosphonate esters **7** and **8** after reacetylation. Cleavage of the methyl phosphonates with trimethylsilyl bromide was selective to afford the corresponding epimeric cyclic phosphonic acids **9** and **10** as an inseparable mixture.

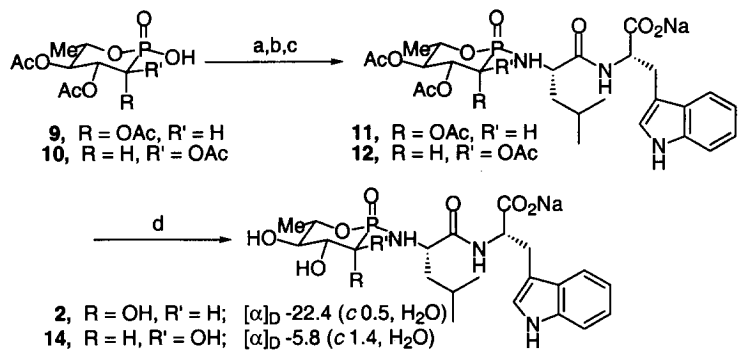
Scheme 1



(a) Ac_2O, DMAP cat., pyridine, 20 h, 98%; (b) HBr/AcOH; (c) Zn, AcONa.3 H_2O, AcOH/H_2O, 55% 2 steps; (d) O_3, MeOH, -78 °C; (e) $P(OMe)_3$, AcOH, 70% 2 steps; (f) MeONa, MeOH; (g) Ac_2O, $BF_3.Et_2O$, 70% 2 steps; (h) TMSBr, THF, 95%.

Coupling of phosphonic acids with peptides and amino acids has been done using a variety of methods.[15] In our case, we found it most practical to couple the L-Leu-Trp-OMe unit starting with the phosphonyl chlorides[19] corresponding to **9** and **10** in the presence of triethylamine (Scheme 2). Fortunately, the cyclic phosphoramidates **11** and **12** were separable by column chromatography. They were subjected to hydrolysis with 1N sodium methoxide[14] to afford the corresponding sodium carboxylates as crude products. Purification by reverse-phase HPLC afforded the desired **2** and its epimer **13** as pure amorphous solids based on their NMR spectra (^1H, ^{13}C, ^{31}P). An IC_{50} of 5.05 ± 2.7 μM was determined for compound **2** in the conversion big ET-1 to ET-1, which is approximately 10 times weaker than phosphoramidon itself.[14]

Scheme 2



(a) $(COCl)_2$, DMF cat. CH_2Cl_2; (b) Et_3N, CH_2Cl_2; (c) H-Leu-Trp-OMe, THF, 45% 3 steps; (d) NaOH 1N, THF, 2 days, 65% .

In conclusion, we have explored the importance of the phosphoramidate group in phosphoramidon, by replacing it with a non-acidic variant as in compound **2**. The weaker activity of **2** could be due to the absence of the acidic phosphate group as well as the spacial characteristics of the anomeric phosphoramidate group in the natural product.

Experimental section

General. Unless otherwise noted, all starting materials and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed on 230-240 mesh silica gel. Thin-layer chromatography (TLC) was performed on glass plates coated with 0.02 mm layer of silica gel 60 F-254. All solvents were dried, distilled freshly before use and stored dry by common procedures. Mass spectra were recorded using electron ionization (EI) at 70 eV or by fast atom bombardment (FAB) techniques with an error magnitude $\sigma < 0.67–3.00$ ppm. Optical rotations were measured at 25 °C at the sodium line.

2,3-Di-*O*-acetyl-5-deoxy-1-C-dimethylphosphonyl-4-*O*-formyl-L-lyxose/L-xylose (5 and 6). A stream of O_3/O_2 was passed into a cooled (-78 °C) solution of 3,4-di-*O*-acetyl-L-rhamnal[16] (2 g, 9.34 mmol) in CH_2Cl_2 (20 mL) until the color turned blue (15 min). The solution was purged with N_2 (10 min) and evaporated to give a foam. The product was suspended in glacial acetic acid (20 mL) and trimethyl phosphite (3 equiv. 3.3 mL) was added. The reaction was stirred at r.t. overnight, concentrated and the residue was purified by flash chromatography (AcOEt/hexanes, 8/2) to give an inseparable mixture of **5** and **6** as a syrup (2.3 g, 70%, ratio *S/R* 2/1). MS(FAB) *m/e* 357 (M^+), 297, 154, 137, 107; HMRS calcd for $C_{12}H_{22}O_{10}P$ (M^+): 357.09506, found 357.09610.

2,3,4-Tri-*O*-acetyl-L-rhamno-(2*R/S*)-methoxy-1,2λ^5-oxaphosphorinan-2-one (7a,b), and 2,3,4-tri-*O*-acetyl-L-gluco-(2*R/S*)-methoxy-1,2λ^5-oxaphosphorinan-2-one (8a,b). The mixture of **5** and **6** (500 mg, 1.4 mmol) was dissolved in dry methanol (10 mL) and a few drops of sodium methoxide (25% in methanol) were added with stirring. After 2 h, the pH was made neutral by addition of Amberlite IR-120 (H^+), the solid filtered, and the filtrate was evaporated to give a white foam. The residue was suspended in Ac_2O (20 mL), cooled in an ice bath and $BF_3.Et_2O$ (100 μL) was added gradually. After being stirred overnight, the solution was concentrated, the residue was dissolved in AcOEt (50 mL), washed with 5% $NaHCO_3$ (20 mL), HCl 1N (20 mL), and brine (20 mL). The organic layer was dried, filtered, concentrated and the residue was purified by flash chromatography (AcOEt/hexanes, 1/1) to give an inseparable mixture of **7a,b** and **8a,b** as a syrup (330 mg, 70%). MS(FAB) *m/e* 339 (M^+), 296, 237, 177, 137, 55; HMRS calcd for $C_{12}H_{20}O_9P$ (M^+): 339.08450, found 339.08430.

2,3,4-Tri-*O*-acetyl-L-rhamno-1,2λ^5-oxaphosphorinan-2-one (9), and 2,3,4-tri-*O*-acetyl-L-gluco-1,2λ^5-oxaphosphorinan-2-one (10). A mixture of **7a,b** and **8a,b** (220 mg, 0.65 mmol) was dissolved in THF (10 mL) and trimethylsilyl bromide (5 equiv. 430 μL) was added. The solution was stirred overnight at r.t. with protection from atmospheric moisture. Water (200 μL) was added and the solution was stirred for 30 min. After concentration, the residue was purified by precipitation (AcOEt/pentane, 1/20) to give a mixture of **9** and **10** as an amorphous white powder (200 mg, 95%). MS(FAB) *m/e* 325 (M^+), 307, 154, 89, 77; HMRS calcd for $C_{11}H_{18}O_9P$ (M^+): 325.06885, found 325.06970.

2,3,4-Tri-*O*-acetyl-L-rhamno-(Leu-Trp-OMe)-1,2λ^5-oxaphosphi-nan-2-one (11), and 2,3,4-Tri-*O*-acetyl-L-talo-(Leu-Trp-OMe)-1,2λ^5-oxaphosphinan-2-one (12). A solution of **9** and **10** (100 mg, 0.31 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C, and dry dimethylformamide (0.1 equiv. 10 μL) and oxalyl chloride (3 equiv. 100 μL) were added under nitrogen. The mixture was stirred for 1 h at 0 °C and dry benzene (2 mL) was added. After concentration, the residue was diluted in CH_2Cl_2 (10 mL) and treated with Et_3N (5 equiv. 220 μL). After 5 min at r. t., H-Leu-Trp-OMe (3 equiv. 300 mg) was introduced and the mixture was stirred for 2 h. Flash chromatography (AcOEt/hexanes, 1/1) gave **11** (60 mg) and **12** (30 mg) for an overall yield of 45%. MS(FAB) *m/e* 638 (M^+), 265, 201, 154, 130, 86; HRMS calcd for $C_{29}H_{41}N_3O_{11}P$ (M^+): 638.24786, found 638.25000.

L-Rhamno-(Leu-Trp-ONa)-1,2λ^5-oxaphosphinan-2-one (2). To a cooled solution of **11** (20 mg, 0.031 mmol) in THF was added NaOH 1 N (5 equiv. 160 μL). The mixture was stirred at r.t. for 2 days. After evaporation, the mixture was purified by HPLC (reverse-phase C-18, H_2O/MeCN,

98/2) and lyophilized to give **2** (10 mg, 65%); $[\alpha]_D$ -22.4° (*c* 0.5, H_2O); mp 144–146 °C (dec.); MS(FAB) *m/e* 520 (M^+); HRMS calcd for $C_{22}H_{31}N_3NaO_8P$ (M^+): 519.17482 found 519.18055.

L-Gluco-(Leu-Trp-ONa)-1,2λ^5-oxaphosphinan-2-one (13). To a cooled solution of **12** (20 mg, 0.031 mmol) in THF was added NaOH 1 N (5 equiv. 160 μL). The mixture was stirred at r.t. for 2 days. After evaporation, the mixture was purified by HPLC (reverse-phase, H_2O/MeCN, 98/2) and lyophilised to give **13** (10 mg, 65%); $[\alpha]_D$ -5.8° (*c* 1.4, H_2O); mp 120–122 °C (dec.); MS(FAB) *m/e* 520 (M^+); HRMS calcd for $C_{22}H_{31}N_3NaO_8P$ (M^+): 519.17482 found 519.18055; ^{1}H NMR (400 MHz, D_2O, ppm) δ : 7.72 (d, 1H, *J* = 7.8 Hz), 7.50 (d, 1H, *J* = 7.8 Hz), 7.26–7.16 (m, 3H), 4.56 (dd, 1H, *J* = 4.9 and 8.0 Hz), 3.96 (t, 1H, *J* = 8.4 Hz), 3.83 (t, 1H, *J* = 7.3 Hz), 3.65–3.58 (m, 2H), 3.42 (dd, 1H, *J* = 5.0 and 14.7 Hz), 3.20 (dd, 1H, *J* = 8.2 and 14.6 Hz), 1.56–1.47 (m, 1H), 1.40–1.08 (m, 2H), 1.26 (d, 3H, *J* = 6.4 Hz), 0.99-0.92 (m, 1H), 0.81 (d, 3H, *J* = 6.6 Hz), 0.76 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, D_2O, ppm) δ : 180.2, 178.3, 136.7, 124.9, 122.4, 119.8, 119.4, 112.4, 110.9, 74.19 (d, *J* = 7.5 Hz), 70.5, 70.2 (d, *J* = 144.7 Hz), 67.8, 43.8 (d, *J* = 2.7 Hz), 28.2, 24.4, 23.1, 21.6, 19.65 (d, *J* = 8.6 Hz); ^{31}P NMR (162 MHz, D_2O, ppm) δ : 21.1.

Acknowledgments: We thank NSERCC for financial assistance through the Medicinal Chemistry Chair Program. We thank Laboratoires Servier, Suresnes, France for the enzyme inhibition tests.

References and Notes

1. Turner, A. J.; Murphy, L. J. *Biochem. Pharmacol.* **1996**, *51*, 91. Azarani, A.; Boileau, G.; Crine, P. *Biochem. J.* **1998**, *333*, 439.
2. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. *Nature (London)* **1988**, *332*, 411.
3. Rubanyi, G. M.; Polokoff, M. A. *Pharmaceuticals Rev.* **1994**, *46*, 325.
4. Umezawa, S.; Tatsuta, K.; Izawa, O.; Tsuchiya, T. *Tetrahedron Lett.* **1972**, 97. Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 621. Matsumura, Y.; Hisaki, K.; Takaoka, M.; Morimoto, S. *Eur. J. Pharmacol.* **1990**, *185*, 103.
5. Veubeuren, T. J.; Mennecier, P.; Merceron, D.; Simonet, S.; De Nanteuil, G.; Vincent, M.; Laubie, M. *J. Cardiovascular Pharmacol.* **1993**, *22 (Suppl. 8)*, S81. McMahon, E. G.; Palomo, M. A.; Moore, W. M.; McDonald, J. F.; Stern, M. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 703. Sawamura, T.; Kasuya, Y.; Matsushita, Y.; Suzuki, N.; Shinmi, O.; Kishi, N.; Sugita, Y.; Yanagisawa, M.; Goto, K.; Masaki, T.; Kimura, S. *Biochem. Biophys Res. Commun.* **1991**, *174*, 779.
6. McKittrick, B. A.; Stamford, A. W.; Weng, X.; Ma, K.; Chackalamannil, S.; Czarniecki, M.; Cleven, R. M.; Fawzi, A. B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1629.
7. Wallace, E. M.; Moliterni, J. A.; Moskal, M. A.; Neubert, A. D.; Marcopoulos, N.; Stamford, L. B.; Trapani, A. J.; Savage, P.; Chou, M.; Jeng, A. Y. *J. Med. Chem.* **1998**, *41*, 1513. Ahn, K.;

Sisneros, A. M.; Herman, S. B.; Pan, S. M.; Hupe, D.; Lee, C.; Nikam, S.; Cheng, X-M.; Doherty, A. M.; Schroeder, R. L.; Haleen, S. J.; Kaw, S.; Emoto, N.; Yanagisawa, M. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 184. Bihovsky, R.; Levinson, B. L.; Loewi, R. C.; Erhardt, P. W.; Polokoff, M. A. *J. Med. Chem.* **1995**, *38*, 2119.

8. Tsurumi, Y.; Fujie, K.; Nishikawa, M.; Kiyoto, S.; Okuhara, M. *J. Antibiot.* **1995**, *48*, 169. Tsurumi, Y.; Ohhata, N.; Iwamoto, T.; Shigematsu, N.; Sakamoto, K.; Nishikawa, M.; Kiyoto, S. *J. Antibiot.* **1995**, *47*, 619.
9. Takaishi, S.; Tuchiya, N.; Sato, A.; Negishi, T.; Takamatsu, Y.; Matsushita, Y.; Watanabe, T.; Iijima, Y.; Haruyama, H.; Kinoshita, T.; Tanaka, M.; Kodama, K. *J. Antibiot.* **1998**, *51*, 805.
10. Hanessian, S.; Galéotti, N.; Rosen, P.; Oliva, G.; Babu, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2763.
11. Cremer, S. E.; Sommese, A. G.; Rodriguez, O. *Phosphorus, Sulfur, and Silicon* **1993**, *75*, 107. Yokomatsu, T.; Shioya, Y.; Iwasawa, H.; Shibuya, S. *Heterocycles* **1997**, *46*, 463. Brel, V. K. *Synthesis* **1998**, 710. Wroblewski, A. E. *Liebigs Ann. Chem.* **1986**, 1854.
12. Darrow, J. W.; Drueckhammer, D. G. *J. Org. Chem.* **1994**, *59*, 2976.
13. Harvey, T. C.; Simiand, C.; Weiler, L.; Withers, S. G. *J. Org. Chem.* **1997**, *62*, 6722.
14. For a synthesis of phosphoramidon, see: De Nanteuil, G.; Benoist, A.; Rémond, G.; Descombes, J-J.; Barou, V.; Verbeuren, T. J. *Tetrahedron Lett.* **1995**, *36*, 1435.
15. Musiol, H-J.; Grams, F.; Rudolph-Böhner, S.; Moroder, L. *J. Org. Chem.* **1994**, *59*, 6144. Malachowski, W. P.; Coward, J. K. *J. Org. Chem.* **1994**, *59*, 7625. Elhaddadi, M.; Jacquier, R.; Petrus, C.; Petrus, F. *Phosphorus, Sulfur, and Silicon* **1991**, *63*, 255. Bartlett, P. A.; Sampson, N. S. *J. Org. Chem.* **1988**, *53*, 4500. Elliot, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. *J. Med. Chem.* **1985**, *28*, 1208. Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654. Hariharan, M.; Chaberek, S; Martell, A. E. *Synth. Commun.* **1973**, *3*, 375.
16. El Khadem, H. S.; Swartz, D. L.; Nelson, J. K.; Berry, L. A. *Carbohydr. Res.* **1977**, *58*, 230.
17. Abramov, V. S. *Zh. Obshch. Khim.*, **1957**, *22*, 647.
18. For the synthesis of α-hydroxy phosphonate derivatives in the amino acid series, see: Berkowitz, D. B.; Egger, M.; Shen, Q.; Shoemaker, R. K. *J. Org. Chem.* **1996**, *61*, 4666.
19. Smith III, A. B.; Hirschmann, R.; Yager, K.M.; Taylor, C. M.; Witherington, J.; Sprengeler, P. A.; Philips, B. W.; Moore, W. *J. Am. Chem. Soc.* **1997**, *119*, 8177. Smith III, A. B.; Hirschmann, R.; Yager, K. M.; Taylor, C. M.; Moore, W.; Sprengeler, P. A.; Witherington, J.; Phillips, B. W. *J. Am. Chem. Soc.* **1995**, *117*, 6370.