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Synthesis of the Endothelin Converting Enzyme Inhibitor Phosphoramidon

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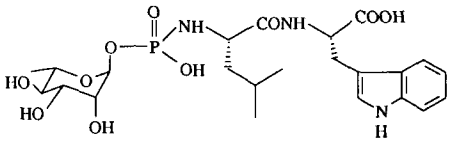
Abstract : Phosphoramidon, an endothelin converting enzyme inhibitor, is synthesized by coupling of a rhamnose derivative with a dichlorophosphate and a suitably protected dipeptide.

Phosphoramidon 1, N-α-L-(rhamnopyranosyloxyhydroxyphosphinyl)-L-leucin-L-tryptophan, was isolated in 1972 by Umezawa and co-workers[1] from a strain of *Streptomyces tanashiensis*. Soon afterwards, it was described as an inhibitor of thermolysin, a metalloendopeptidase isolated from the culture filtrate of *Bacillus thermoproteolyticus*.[2,3]



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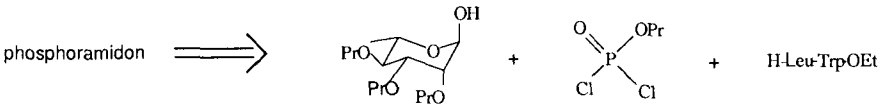
After isolation and characterization of endothelin-1 (ET-1) in 1988 by Yanagisawa and coworkers,[4] a metalloprotease, endothelin converting enzyme (ECE), has been proposed to be responsible for the formation of ET-1 from its precursor big endothelin-1 (big ET-1) by selective cleavage of a Trp^{21}-Val^{22} bond. *In vitro*, big ET-1 has a vasoconstrictor effect a hundred times weaker than that of ET-1, suggesting that the bioconversion of big ET-1 is essential for the pathophysiological significance of ET-1.[5]

Matsumura and coworkers[6] reported in 1990 that phosphoramidon specifically decreased the big ET-1 induced hypertensive effect when administered i.v. to anaesthetized rats, without affecting the hypertension induced by ET-1. Subsequently, phosphoramidon was proposed to be a potent inhibitor of ECE.[7]

As part of an ongoing project aimed to discover potent and selective inhibitors of ECE[8], we were faced with the preparation of analogs of phosphoramidon. Thus, we thought that the synthetic pathway used for the preparation of these original compounds could also be useful for the synthesis of the reference substance.

Synthetic studies have been performed by Umezawa in 1975[3] on phosphoramidon analogs (His instead of Trp) : basically, α-L- rhamnopyranosyl-1-phosphate tributylammonium was reacted with N,N'-carbonyl diimidazole and then with the sodium salt of L-leucyl-L-histidine. After several purifications, the desired compound was obtained with 6% yield. Curiously, no attempt is reported with L-leucyl-L-tryptophan.

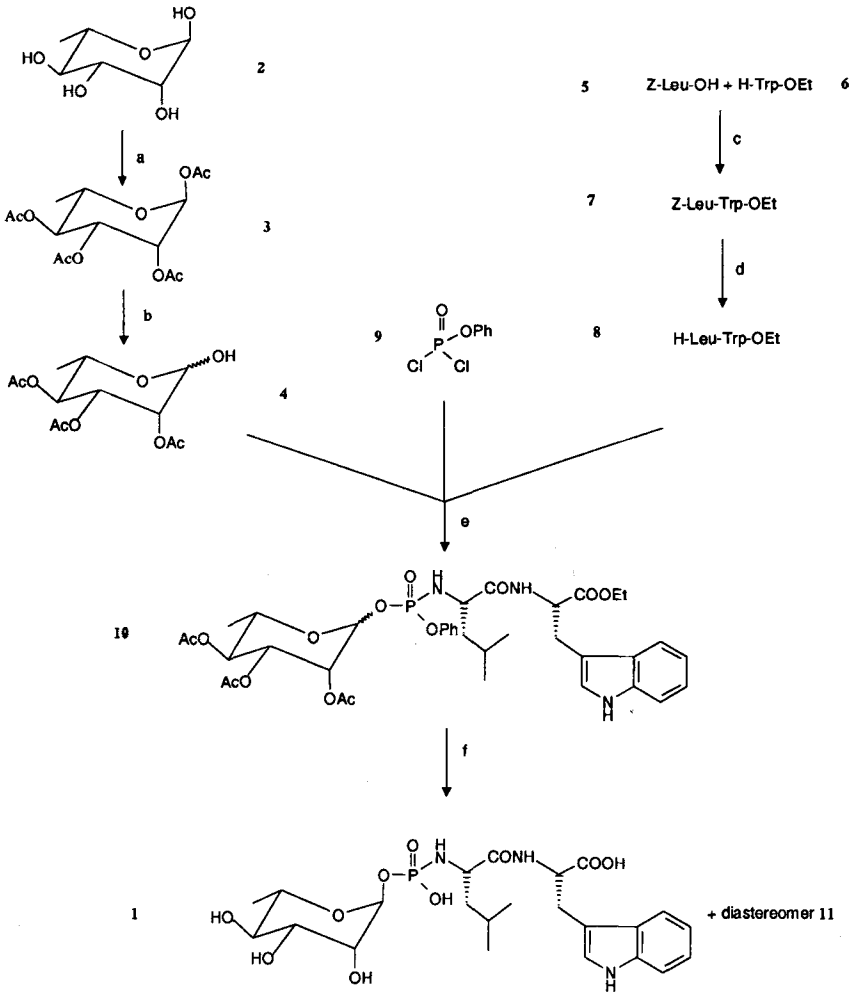
From these preliminary studies, it appeared that the starting synthons could be: an appropriately protected rhamnose, a dichlorophosphate, and finally the carboxylic ester of the dipeptide (scheme 1 : Pr = protective group).



scheme 1

It is known that the P^{IV}-N bond is not stable at acidic pH[9]. The last step (hydrolysis of the five ester functions) had to be performed under alkaline conditions. Thus the four hydroxyl groups of

commercial rhamnose **2** (Sigma) were first protected as acetates (Ac_2O-pyridine, 72h, 5°C, 98%) (scheme 2).



Reagents: (a) Ac_2O, pyridine; (b) NH_3, THF-methanol; (c) DCC, HOBT, DMF; (d) H_2, Pd/C 10%, EtOH; (e) Et_3N, $CHCl_3$; (f) NaOH, THF.

scheme 2

At this stage, the two anomeric forms were detected in a non attributed ratio of 77-23.[10] Selective deprotection of the anomeric hydroxyl group of compound **3** was performed by bubbling ammonia into a THF-methanol solution of the tetraacetate.[11] After silica gel chromatography (CH_2Cl_2-AcOEt 95 : 5), the desired compound **4** was obtained as a white solid (mp 90°) in 76% yield. The ratio α/β of the anomeric forms was found to be 5 : 95, determined by measuring the coupling constant between the anomeric and the equatorial C-3 protons.[11]

Meanwhile, the dipeptide moiety was obtained by coupling Z-Leu-OH **5** with H-Trp-OEt **6** under classical peptidic conditions (HOBT, DCC, Et_3N, DMF, 0° then room temperature 48h), affording **7**, after trituration with ether, as a pale yellow solid (mp 128°) in 84% yield. The benzyloxy group was then

removed by hydrogenolysis (cat 10% Pd/C, EtOH, overnight, room temperature and pressure) to give dipeptide **8**.

The key reaction was performed by coupling rhamnose triacetate **4** with phenyldichlorophosphate (Et_3N, $CHCl_3$, 0°, 4h), followed by addition of the dipeptide in chloroform to afford a crude oil that was purified on a silica gel column (CH_2Cl_2-EtOH 98 : 2) to provide 22% of the desired, totally protected phosphoramidon **10**.

Complete deprotection (hydrolysis of the five esters functions) was then carried out by reacting **10** in THF with 5 equivalents of 1N sodium hydroxide at 0°. After warming and stirring at room temperature for 48 hours, the organic solvent was removed, and the crude aqueous phase was lyophilised. Purification by HPLC (RP18, H_2O) afforded phosphoramidon **1** (27 %) as well as its diastereoisomer **11** (15 %) both as their disodium salts.[12]

^1H NMR, IR and MS (FAB) data of **1** were identical with those of a commercial sample of phosphoramidon (Sigma) and with data described in the literature.[1] Moreover, HPLC retention times were identical, and coinjection of both synthetic and commercial samples in four different chromatographic systems resulted in only one single peak. Finally $[\alpha]_D$ values were in the same range.[12] The diastereomer **11** presented the same spectroscopic data as natural phosphoramidon; however, HPLC retention time was higher and its $[\alpha]_D$ value lower than for phosphoramidon.

To ensure the validity of our work, commercial and synthetic phosphoramidon as well as diastereomer **11** were tested in isolated perfused rat kidneys, where phosphoramidon is known to inhibit the conversion of big ET-1 into ET-1.[13]
The three compounds were studied in the concentration range 10^{-7}-10^{-5} M ; comparable IC_{50} values were obtained as illustrated in Table 1.

Table 1. Inhibition of Conversion of big-ET-1 to ET-1 in rat kidney

	commercial **1**	synthetic **1**	**11**
IC_{50} (μM)	0.70	0.13	2.3

Conclusion : In spite of the present lack of optimizations, in particular of the key coupling step, this reaction pathway constitutes the first synthesis of phosphoramidon. Optimization of this route is currently under investigation as well as its application to the preparation of novel analogues of phosphoramidon.

Acknowledgments : The authors wish to thank Dr. J.P. Volland and his group for the analytical and spectral data.

References and notes :

1. Umezawa, S.; Tatsuta, K.; Izawa, O.; Tsuchiya, T. *Tetrahedron Lett.* **1972**, *43*, 97-100.
2. Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *J. Antibiotics*, **1972**, *26*, 621-623.
3. Komiyama, T.; Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H.; Fujimoto, K.; Umezawa, S. *Arch.Biochem. Biophys.* **1975**, *171*, 727-731.
4. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. *Nature. (London)*, **1988**, *332*, 411-415.
5. Kimura, S.; Kasuya, Y.; Sawamura, T.; Shinmi, O.; Sugita, Y.; Yanagisawa, M.; Goto, K.; Masaki, T. *J. Cardiovascular Pharmacol.* **1989**, *13 (Suppl. 5)*, S5-S7.
6. Matsumura, Y.; Hisaki, K.; Takaoka, M.; Morimoto, S. *Eur. J. Pharmacol.* **1990**, *185*, 103-106.
7. Matsumura, Y.; Ikegawa, R.; Tsukahara Y.; Takaoka M.; Morimoto, S. *FEBS Lett.* **1990**, *272*, 166-170.
8. De Nanteuil, G.; Rémond, G.; Verbeuren, T.; French Patent n° 93.07927.

9. Rahil, J.; Haake, P. *J. Am. Chem. Soc.* **1981**, *103*, 1723-1734.
10. Chatterjee, A.K.; MacDonald, D.L. *Carbohyd. Res.* **1968**, *6*, 253-254.
11. Fiandor, J.; Garcia-Lopez, M.T.; De Las Heras, F.G.; Mendez-Castrillon, P.P. *Synthesis*, **1985**, 1121-1123.
12. spectral data : **compound 3** : IR (nujol, cm^{-1}) : 1751; ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 6.0 (d)-5.85 (s) (1H), 5.5-5.1 (m, 3H), 4.0-3.65 (m, 1H), 2.25-2.0 (m, 12H), 1.3 (d)-1.25 (d) (3H).
Elemental analysis (%) : calc : C :50.60, H : 6.07, found : C : 50.34, H : 5.84
compound 4 : IR (nujol, cm^{-1}) : 3425, 1745-1726; ^1H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.4 (dd, 1H), 5.3 (m, 1H), 5.15 (d, 1H), 5.10 (t, 1H), 4.15 (m, 1H), 4.10 (d, 1H), 2.2-2.0 (3s, 9H), 1.25 (s, 3H).
compound 7 : IR (nujol, cm^{-1}) : 3365, 3329, 3300, 1728, 1680, 1645, 1535; ^1H NMR (200 MHz, $CDCl_3$, δ ppm) : 8.0 (m, 1H), 7.6-7.0 (m, 9H), 6.9 (m, 1H), 6.5 (m, 1H), 5.1 (m, 1H), 5.0 (broad s, 2H), 4.90 (m, 1H), 4.20 (m, 3H), 3.30 (d, 2H), 1.60 (m, 3H), 1.20 (t, 3H), 0.9 (d, 6H).
Elemental analysis (%) : calc : C : 67.62, H : 6.94, N : 8.76, found : C : 67.84, H : 6.94, N : 8.80.
compound 8: IR (nujol, cm^{-1}) : 3311, 1739, 1662, 1516; ^1H NMR (200 MHz, $CDCl_3$, δ ppm) : 8.25 (s, 1H), 7.60 (d, 1H), 7.55 (d, 1H), 7.35 (d, 1H), 7.25-7.05 (m, 2H), 7.05 (d,1H), 4.90 (m,1H), 4.10 (m, 2H), 3.40 (m, 3H), 1.70 (m, 3H), 1.40 (s, 2H), 1.25 (t, 3H), 0.9 (t, 6H).
compound 10 : IR (nujol, cm^{-1}) : 3300, 1751, 1664, 1220; ^1H NMR (300 MHz, $CDCl_3$, δppm) : 8.4 (m, 1H), 7.5 (m, 1H), 7.4-6.9 (m, 9H), 6.8 (m, 1H), 5.4-4.7 (m, 5H), 4.2-3.3 (m, 6H), 2.1. (s, 9H), 1.9-1.3 (m, 3H), 1.2 (t + d, 6H), 0.8 (d, 6H).
compound 11 : IR (nujol, cm^{-1}) : 3370, 2723, 1600; ^1H NMR (300 MHz, D_2O, δ ppm) 7.7 (d, 1H), 7.5 (d, 1H), 7.25 (m,3H), 5.0 (m, 1H), 4.6 (m, 1H), 3.9 (d, 1H), 3.7-3.2 (m, 6H), 1.55 (m, 1H), 1.4-1.0 (m, 5H), 0.8 (d, 6H).
mass spectroscopy : FAB ± : m/z = 587
compound 1 : IR (nujol, cm-1) : 3700-3100, 1650-1599, 1219, 1082; ^1H NMR (300 MHz, D_2O, δ ppm) : 7.7 (d, 1H), 7.45 (d, 1H), 7.2 (m, 3H), 5.25 (dd, 1H); 4.50 (m, 1H), 3.80 (m, 3H), 3.2 (dd, 1H), 2.90 (m, 3H), 1.6 (m, 1H), 1.25 (d, 3H), 1.20-0.90 (m, 2H), 0.80 (2d, 6H).
mass spectroscopy : FAB + : M + H]+ = 588, M + Na]+ = 610
FAB - : M - H]- = 586, M - Na]- = 564
$[\alpha]_D^{20}$ = -30 (c = 0.96, H_2O)
commercial phosphoramidon : IR (nujol, cm-1) : 3298-2600, 1650, 1597; ^1H NMR (300 MHz, D_2O, δ ppm) : 7.7 (d, 1H), 7.45 (d, 1H), 7.2 (m, 3H), 5.25 (dd, 1H); 4.50 (m, 1H), 3.80 (m, 3H), 3.2 (dd, 1H), 2.90 (m, 3H), 1.6 (m, 1H), 1.25 (d, 3H), 1.20-0.90 (m, 2H), 0.80 (2d, 6H).
$[\alpha]_D^{20}$ = -35 (c = 0.95, H_2O)
13. Verbeuren, T.J.; Mennecier, P.; Merceron, D.; Simonet, S.; De Nanteuil, G.; Vincent, M.; Laubie, M. *J. Cardiovascular Pharmacol.* **1993**, *22 (suppl. 8)*, S81-S84.

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