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# Total Synthesis of Flutimide, A Novel Endonuclease Inhibitor of Influenza Virus

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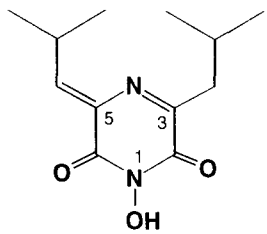
**Abstract:** Flutimide is a completely substituted 1-N-hydroxy-2,6-diketo-Δ3-piperazine isolated from a new species of *Delitschia cofertaspora*. It selectively inhibits influenza virus A endonuclease activity without affecting any viral transcription activity. Total synthesis of flutimide starting from L-leucine has been described.

Influenza, an acute contagious respiratory disease is caused by influenza viruses A and B.[1] Influenza is characterized by a severe pulmonary infection which periodically causes epidemics in the elderly and other high risk populations. Influenza virus is a negative strand RNA virus with a segmented genome.[1] The replication of influenza virus is initiated when capped and methylated RNA, obtained by cap-dependent endonucleolytic cleavage of the host cell RNA polymerase II transcripts, is used to prime viral mRNA synthesis. Cap-dependent cleavage appears to be unique to influenza viruses and is thus an attractive target for the development of a selective anti-viral agent.[2]




**FLUTIMIDE (1)**

Flutimide (**1**)[3] is a novel natural product discovered in our laboratories using a cap-dependent influenza A endonuclease screen. This compound was isolated from a new species of *Delitschia confertaspora*,[4] a fungus collected from dung of a dassie in Namibia. Flutimide selectively inhibits influenza A endonuclease activity with an $IC_{50}$ of ~3μM.[5] The initial structural elucidation of flutimide[3] was accomplished by NMR and MS techniques. However, unequivocal distinction between two structural (2,5-diketo *vs* 2,6-diketo) possibilities could not be easily made on the basis of only spectroscopic data and was finally resolved in favor of **1** by hydrogenation and hydrolysis reactions.[3] due to the presence of contiguous quaternary carbons and/or hetero-atoms. In order to verify the structure and to have an adequate supply of material, a total synthesis of flutimide was undertaken. In this paper, an eight-step total synthesis of flutimide from L-leucine is described.

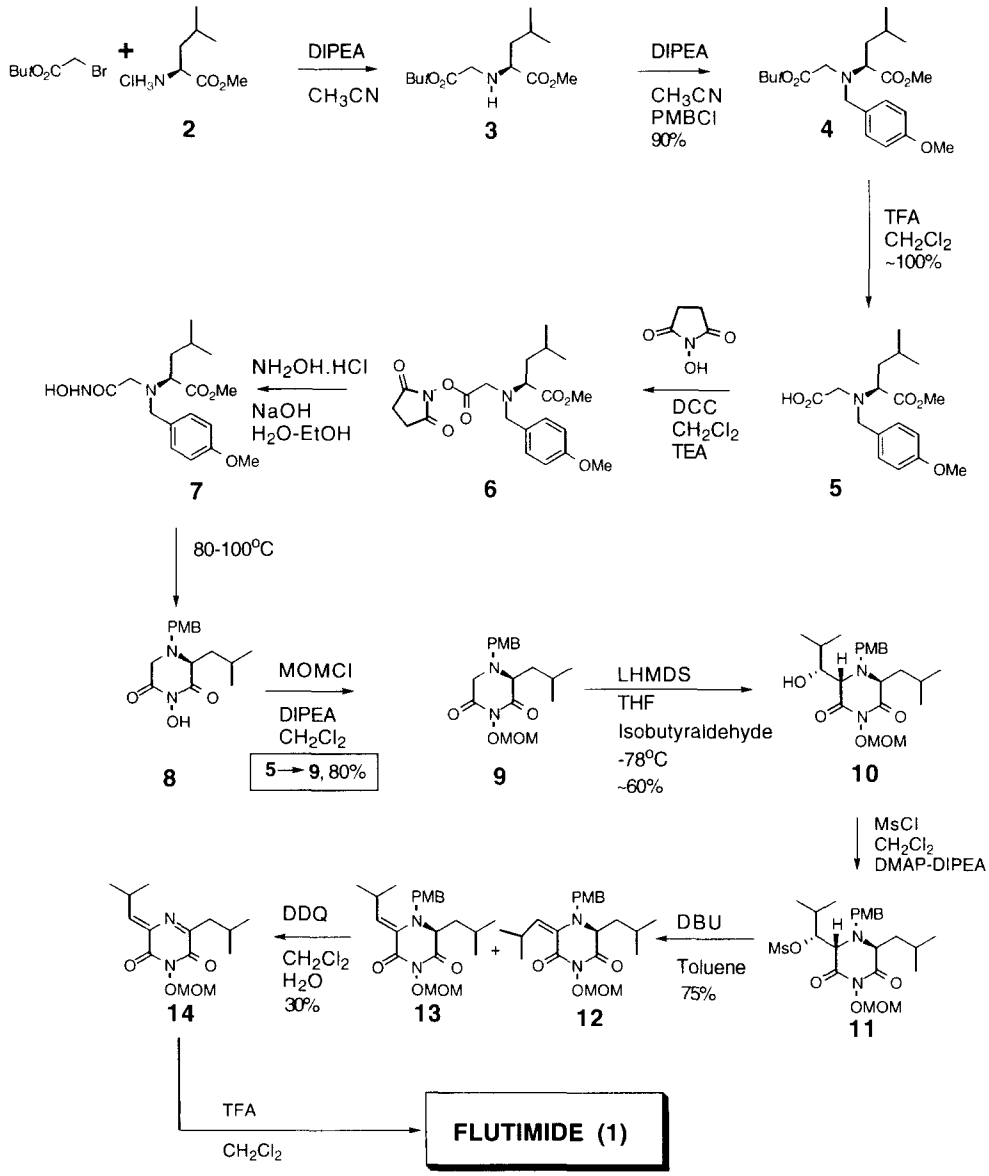
The structure of flutimide (**1**) contains several unusual and reactive group such as N-hydroxy-imide and exocyclic enamine. The enamine is probably stabilized by extended conjugation within the 2,6-diketopiperazine. Retrosynthetic analysis of flutimide reveals the following key steps: (a) a cyclization step to make N-hydroxy-2,6-diketopiperazine; (b) preparation of the exocyclic enamine; (c) preparation of the endocyclic C=N in the presence of enamine; (d) selection of a proper protecting group for the N-hydroxy group which can be cleaved with TFA (flutimide had previously been shown to be stable to TFA).

An exocyclic enamine could be introduced either before or after cyclization to the 2,6-diketopiperazine. It was decided to introduce this group after cyclization; this not only avoids excessive chemical operations in the presence of this sensitive group but also gives the possibility for easy SAR development. As enamines containing a free NH group are extremely unstable, the formation of C=N and deprotection was chosen as the penultimate step.The *p*-methoxybenzyl (pmb) group was selected as the protecting group since its cleavage and oxidation to C=N could be achieved simultaneously with DDQ. Methoxymethyl (MOM) ether was found to be most suitable for protection of the N-hydroxy group as it was readily put on and subsequently cleaved by TFA . The synthesis of flutimide (**1**) is presented in scheme 1.

N-Alkylation of (S)-Leu-OMe.HCl (**2**) with *tert*-butyl-bromomethyl acetate in acetonitrile using diisopropylethyl amine gave[6] **3** which was subsequently reacted with *p*-methoxybenzyl chloride in the same reaction vessel without any work-up to give compound **4** in 90% overall yield. Cleavage of the *tert*-butyl ester with trifluoroacetic acid gave acid **5** which was activated with N-hydroxysuccinimide to give an active ester **6**. Reaction of **6** with neutralized hydroxylamine in aqueous ethanol gave **7** which was cyclized *in situ* to give **8** by heating at 80-100°C. The N-hydroxy group of **8** was protected as the MOM ether using methoxymethyl chloride in methylene chloride and diisopropylethyl amine to give **9** in an overall (**5**→**9**) yield of 80%. Aldol condensation of **9** with isobutyraldehyde using lithium hexamethyldisilazide (LHDMS) gave ~60% yield of **10** as a major isomer.[7] The stereochemistry of **10** was determined by NOE measurement and from the elimination product (*vide infra*). Reaction of **10** with methanesulfonyl chloride (MsCl) at -23°C gave mesylate **11** in almost quantitative yield. Elimination of the mesylate turned out to be rather difficult; conditions which were most suitable for this reaction are as follows: DBU (3 eq) was added to a cooled (0°C) solution of mesylate in toluene and the solution was allowed to warm slowly to room temperature. The reaction was generally complete in less than 2 hrs and gave ~75 % yield[7] of the isomeric (*E/Z*) products **12** and **13** in a ratio of ~1 : 2.5. The olefin geometry of **12** and **13** was assigned on the basis of NOE measurements (irradiation of the olefinic proton doublet of **12** at δ 6.84 showed enhancement to one of the two doublets (δ 4.00 ) of the benzylic protons). DDQ oxidation of **13** in a mixture of $CH_2Cl_2$-$H_2O$ (2:1) gave[7] oxidized product **14** in ~30% yield. Deprotection of the MOM ether of **14** with TFA in $CH_2Cl_2$ gave almost quantitative yield of **1** which proved to be identical to natural flutimide as determined by NMR, MS and HPLC and biological properties.

Small scale (low mg) purification of synthetic flutimide could be easily accomplished by reversed phase HPLC but injection of larger quantity of material resulted in extremely low recovery. The synthesis of flutimide reported here is amenable to analog development. We are in the process of synthesizing such analogs and the SAR of the series will be reported in due course.

## SCHEME 1: SYNTHESIS OF FLUTIMIDE (1)



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4. Pelaez, F.; Polishook, J. D.; Valldosera, M.; Guarro, J. *Mycotocin* **1994**, *L*, 115.
5. See Tomassini, J.; Lingham, R. B. *et al* (a manuscript under preparation) for the details of biological properties.
6. All compounds gave satisfactory spectroscopic data and elemental analysis. NMR data of a few important compounds are described in the footnote 7.
7. **10**. $[\alpha]_D^{25}$ -24.2 (c, 0.54, $CH_3OH$), $^1H$ NMR ($C_6D_6$): δ 0.52 (3H, d, J = 6.5 Hz), 0.74 (3H, d, J = 7.0 Hz), 0.88 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 7.0 Hz), 1.41 (2H, t, J = 7.5 Hz), 1.73 (1H, nonet, J = 6.5 Hz), 2.10 (1H, doublet of heptets, J = 7.0, 3.5 Hz), 3.19 (1H, d, J = 13 Hz), 3.25 (3H, s), 3.49 (3H, s), 3.58 (1H, d, J = 13 Hz), 3.62 (1H, d, J = 7.5 Hz), 3.72 (1H, t, J = 7.5 Hz), 3.91 (1H, brdd, J = 7.0, 2.5 Hz),4.92 (1H, d, J = 7.5 Hz), 5.02 (1H, d, J = 7.5 Hz), 6.66 (2H, d, J = 8.5 Hz), 6.98 (2H, d, J = 9.0 Hz); **12**: E-isomer: $[\alpha]_D^{25}$ +76 (c, 0.25, $CH_3OH$), $^1H$ NMR ($CDCl_3$): δ 0.81 (3H, d, J = 6.4 Hz), 0.83 (3H, d, J = 6.4 Hz), 0.93 (3H, d, J = 6.4 Hz), 0.98 (3H, d, J = 6.4 Hz), 1.41 (1H, ddd, J = 14, 7.0, 7.0 Hz), 1.50 (1H, ddd, J = 14, 7.0, 7.0 Hz), 1.74 (1H, nonet, J = 6.4 Hz), 3.58 (1H, m), 3.63 (3H, s), 3.65 (1H, t, J = 8.0 Hz), 3.78 (1H, d, J = 12.8 Hz), 3.79 (3H, s), 4.00 (1H, d, J = 13.2 Hz), 5.00 (2H, ABq, J = 7.2 Hz), 5.44 (1H, d, J = 10 Hz), 6.84 (2H, d, J = 8.8 Hz), 7.15 (2H, d, J = 8.4 Hz); **13**: Z-isomer: $[\alpha]_D^{25}$ +37.6 (c, 0.21, $CH_3OH$); $^1H$ NMR ($CDCl_3$): δ 0.63 (3H, d, J = 6.4 Hz), 0.84 (3H, d, J = 6.8 Hz), 0.99 (3H, d, J = 6.8 Hz), 1.03 (3H, d, J = 6.8 Hz), 1.29 (1H, ddd, J = 14, 9.6, 4.4 Hz), 1.49 (1H, ddd, J = 13.6, 10.8, 4.4 Hz), 1.77 (1H, m), 3.11 (1H, m), 3.55 (1H, dd, J = 11.2, 4.4 Hz), 3.62 (3H, s), 3.78 (1H, d, J = 12.8 Hz), 3.79 (3H, s), 3.81 (2H, s), 4.95 (2H, ABq, J = 7.2 Hz), 6.71 (1H, d, J = 11.2 Hz), 6.86 (2H, d, J = 8.4 Hz), 7.20 (2H, d, J = 8.8 Hz); **14**: $^1H$ NMR ($CDCl_3$): δ 0.99 (3H, d, J = 6.8 Hz), 1.13 (3H, d, J = 6.4 Hz), 2.19 (1H, heptet, J = 6.4 Hz), 2.64 (1H, d, J = 6.8 Hz), 3.54 (1H, m), 3.66 (3H, s), 5.08 (2H, s), 7.13 (1H, d, J = 10.4 Hz); $^{13}C$ NMR ($CDCl_3$): 21.85 (2 x $CH_3$), 22.56 (2 x $CH_3$), 26.41 (CH), 27.41 (CH), 41.81 ($CH_2$), 58.44 ($OCH_3$), 100.66 ($OCH_2O$), 133.89 (C), 155.29 (C), 157.47 (C), 158.39 (CH), 159.83 (C).

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