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Synthesis and Antiviral Activities of the Major Metabolites of the HIV Protease Inhibitor ABT-378 (Lopinavir)

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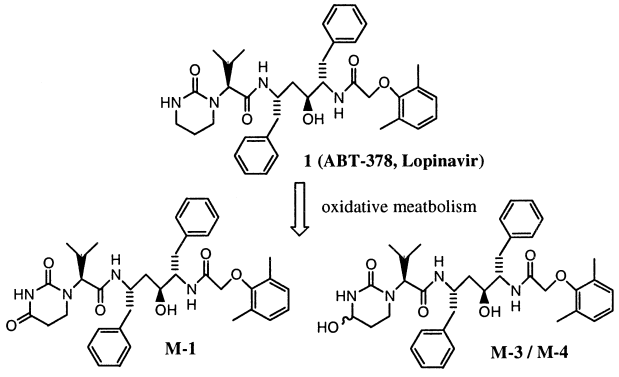
Abstract—The HIV protease inhibitor ABT-378 (Lopinavir) is metabolized rapidly and extensively by CYP-3A4 catalyzed oxidation. Three of the major metabolites identified were synthesized and their antiviral (HIV) activities determined.

The HIV protease inhibitor ABT-378[1] (Lopinavir) is the antiviral component of Kaletra™ (approved by FDA in September, 2000), the latest approved HIV protease inhibitor for the treatment of human immunodeficiency virus (HIV) infection. ABT-378 is a highly potent inhibitor of HIV protease ($K_i = 1.3$–28 pM) against wild-type and mutant HIV protease.[1,2] ABT-378 (**1**) was metabolized very extensively and rapidly by liver microsomes from mouse, rat, dog, monkey, and humans. The predominant site of oxidative metabolism was the cyclic urea moiety of ABT-378.[3] In all five species, the major metabolites (out of **M-1**–**M-12**) were **M-1**, **M-3**, and **M-4** (Scheme 1); **M-3** and **M-4** are the epimeric C-4 hydroxy products of oxidation in the cyclic urea moiety and **M-1** is the C-4 oxo product. They were characterized by mass and NMR spectroscopy. The very small quantities of these metabolites isolated precluded the determination of their antiviral activities. Thus, the synthesis of these major metabolites was undertaken to provide materials both to confirm the reported structures and to determine their antiviral activities.

The synthesis of ABT-378 has been reported.[4] A retrosynthetic analysis of the synthesis is shown in Scheme 2. The synthesis of the core diamino compound **3** has also been reported.[5,6] We envisioned that the synthesis of **M-1** and **M-3**/**M-4** could be accomplished by the synthesis of the key carboxylic acid **5**. Coupling of known intermediates **3**, **4**, and then with **5** would provide **M-1**, which upon reduction would provide **M-3**/**M-4**.

Michael addition of the *t*-butyl ester of L-valine to acrylic acid methyl ester provided **6** (94%). Hydrolysis of the diester **6** by aqueous lithium hydroxide provided the corresponding mono-acid, which was reacted with disuccinimidocarbonate to give the active ester (Scheme 3). Aminolysis of the succinimidyl ester with concentrated ammonium hydroxide provided the amide **7** (68%, three steps). Refluxing amide **7** with carbonyldiimidazole in THF provided the cyclic oxo-urea **8** in 61% yield. Trifluoroacetic acid hydrolysis of the *t*-butyl ester provided the key intermediate acid **5** in 95% yield.



Scheme 1. ABT-378 and its three major metabolites.

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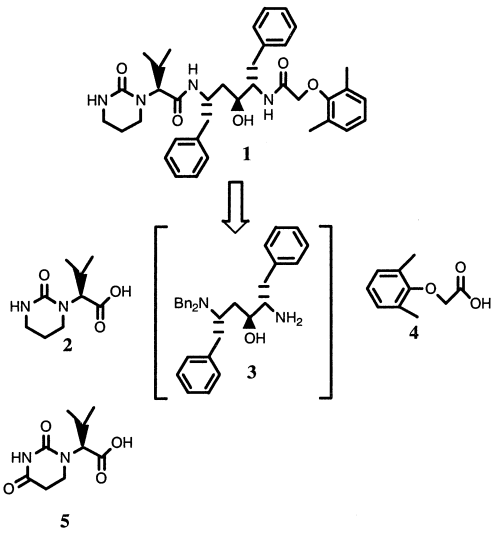
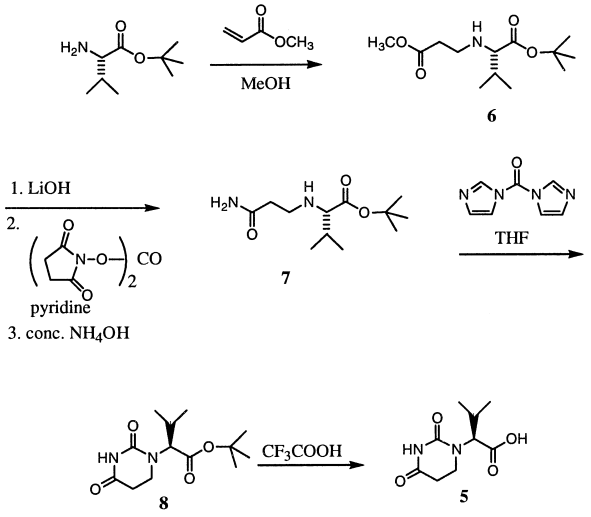


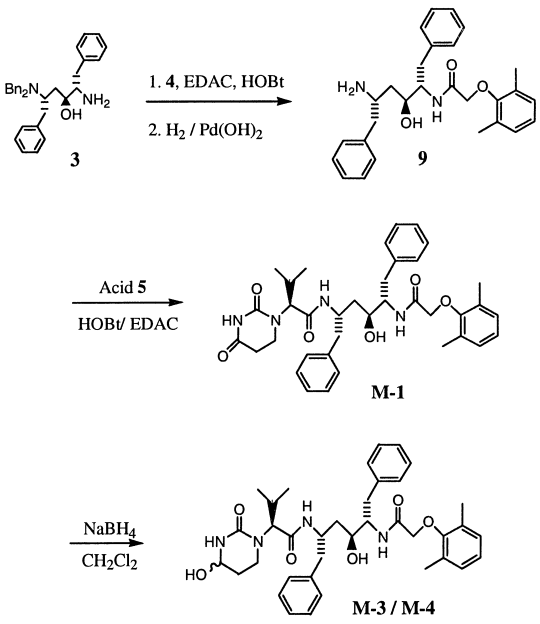
Scheme 2. Retrosynthetic scheme of ABT-378 and the key intermediates **3**, **4**, and **5** necessary for the synthesis of **M-1** and **M-3**/**M-4**.



Scheme 3. Synthesis of acid **5**.

Coupling of the known intermediate **3** from the synthesis of ABT-378,[4] with acid **4** using a standard peptide coupling procedure (EDAC, HOBt),[7] followed by hydrogenolysis provided amine **9** (85%). Coupling of amine **9** with acid **5** gave **M-1** in 82% yield (Scheme 4). Reduction of **M-1** with sodium borohydride in methylene chloride provided an inseparable epimeric mixture (~1:1) of **M-3**/**M-4** in 61% yield.[8] The NMR, mass spectroscopy, and HPLC retention times of the synthetic materials were identical to that isolated from metabolism studies. The biological activities of **M-1**, **M-3**/**M-4** in comparison with ABT-378 are shown in Table 1.

In summary, a concise synthesis of the major metabolites of the potent second generation HIV protease inhibitor ABT-378 (Lopinavir) was developed. It allowed the confirmation of the structures of the metabolites and the determination of the biological properties of these metabolites.



Scheme 4. Synthesis of **M-1** and **M-3**/**M-4**.

Table 1. Inhibition of HIV protease and antiviral activities in MT-4 cells

Compounds	K_i (HIV protease), pM	EC_{50}[a] (μM)
1 (ABT-378)	1.3	0.104
M-1	0.7	1.413
M-3/M-4	1.2	2.31

[a]In the presence of 50% human serum.

Acknowledgements

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8. All compounds reported have mass, NMR, and analytical data consistent with the structures assigned.