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SYNTHESIS OF (+)-BIOTIN DERIVATIVES AS HIV-1 PROTEASE INHIBITORS

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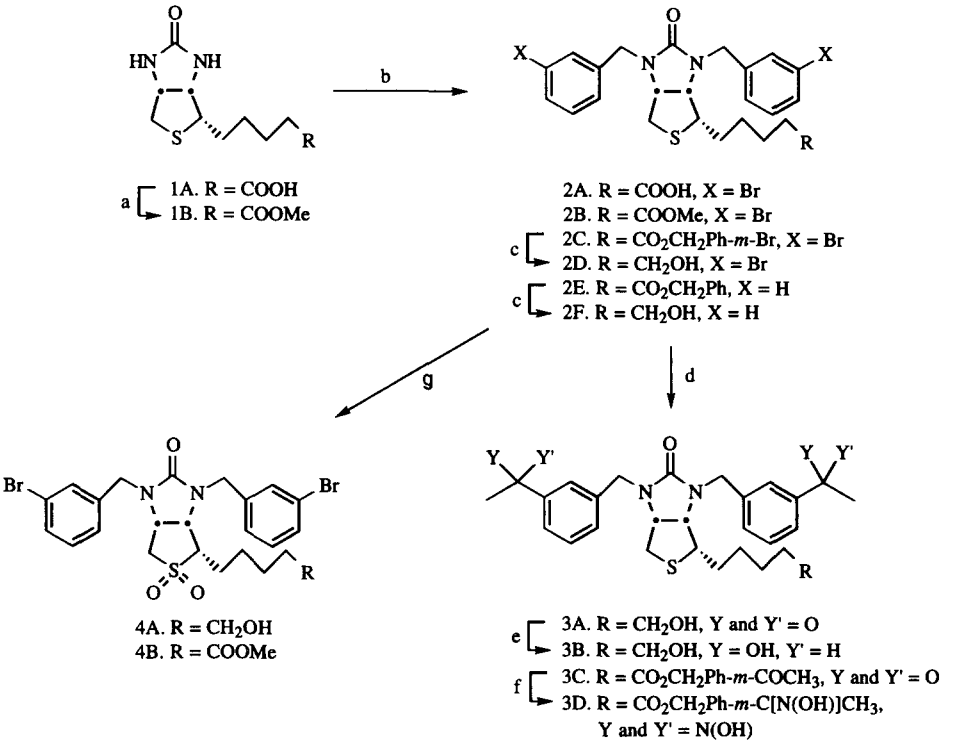
Abstract: Several bis-*N*-alkylated (+)-biotin derivatives were synthesized and evaluated for activities against HIV-1 protease. The most potent inhibitor, **2D**, synthesized in two steps from (+)-biotin, has K_i of 0.50 μM and antiviral IC_{90} of 7 μM. The (+)-biotin analogs in general have good translations from enzymatic K_i to antiviral cell assay IC_{90}.

Human immunodeficiency virus (HIV) is the causative agent of Acquired Immunodeficiency Syndromes (AIDS).[1] Inhibition of the virally encoded proteinase (HIV protease) is recognized as an important therapeutic strategy for the treatment of AIDS.[2] Recently, HIV protease inhibitors had been approved for AIDS chemotherapy in combination with reverse transcriptase inhibitors.[3] HIV-1 protease is a homo dimeric aspartic protease with a C2 axis of symmetry.[4] X-ray structures indicate that the backbone N-H of Ile 50 and Ile 50' in the flaps of the enzyme are hydrogen bonded to inhibitors through a tetra-coordinated structural water molecule.[4] We had recently rationally designed and synthesized a series of potent nonpeptide seven-membered ring cyclic ureas that incorporated this structural water in a preorganized scaffold.[5] Other nonpeptide inhibitors that can displace the structural water had also been reported.[6] One of nature's tightest complexes between a macromolecule and a small molecule is the streptavidin-biotin complex. The X-ray structure of this complex revealed the importance of the urea oxygen of (+)-biotin as a superior hydrogen-bond acceptor.[7] The excellent hydrogen-bond accepting ability of urea was one reason for the incorporation of this functional group in our potent cyclic urea series.[5] It occurred to us that (+)-biotin, comprised of two *cis*-fused five-membered rings with the important urea group, can be used as a scaffold for synthesizing HIV-1 protease inhibitors. In addition, modelling suggested that the (+)-biotin derivatives can fit in the binding site of the HIV-1 protease, and the sulfur atom in (+)-biotin may form a weak hydrogen bond[8] with the aspartate residues of the enzyme (Asp 25 and Asp 25'). (+)-Biotin is also a commercially available natural product with relatively low toxicity and easy to be modified to give the desired derivatives. We report here our preliminary results of synthesis and biological activities of the new derivatives of (+)-biotin.

Since our previous work in cyclic urea revealed that *meta*-substituted benzyl on the urea nitrogen gave good inhibitors,[9] we decided to explore *meta*-substituted benzyl groups on the urea nitrogen of (+)-biotin. The synthesis of (+)-biotin derivatives is outlined in Scheme 1.

Scheme 1



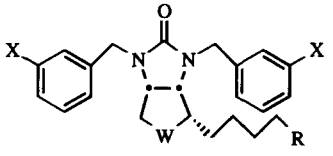
a. MeOH, *conc*. H_2SO_4, 100%; b. *m*-Br-PhCH$_2$Br or PhCH$_2$Br, NaH, DMF, 40-95%; c. LiAlH(OMe)$_3$, 75-91%; d. (for 2D to 3A, and 2C to 3C), 1-ethoxy-1-(trimethylstannyl)-ethylene, Pd(PPh$_3$)$_4$, THF, and then 1N HCl, 40-54%; e. $NaBH_4$, MeOH, 75%; f. NH_2OH•HCl, pyridine, 100%; g. (for 2D to 4A, and 2B to 4B), *OXONE*, aq. MeOH, 76-95%.

(+)-Biotin **1A** was esterified with acidic methanol to form the corresponding ester **1B**. Alkylation of **1A** and **1B** could be readily accomplished by reaction with 3-bromobenzyl bromide or benzyl bromide in the presence of NaH in DMF to give **2A**, **2B**, **2C**, and **2E**, respectively. Compounds **2D** and **2F** were obtained by the reductions of **2C** and **2E** with LiAlH(OMe)$_3$, respectively. Stille coupling reaction of **2D** and **2C** with 1-ethoxy-1-(trimethylstannyl)-ethylene worked well to give **3A** and **3C**, respectively, after hydrolysis with 1N HCl. Compound **3A** could be reduced with $NaBH_4$ to give an alcohol **3B**, and **3C** could be converted to **3D** by treatment with NH_2OH•HCl in pyridine. The compounds **2D** and **2B** were oxidized with *OXONE* (potassium peroxymonosulfate) in aqueous methanol at room temperature to produce sulfones **4A** and **4B**, respectively.

Enzymatic K_i and antiviral cell RNA-IC$_{90}$ values were measured as described in literature.[6] K_i is the inhibition constant. RNA-IC$_{90}$ is the concentration of inhibitor resulting in 90% inhibition of viral RNA production in HIV-1 infected MT-2 cells. The biological results of (+)-biotin derivatives are summarized in Table 1. All our (+)-biotin derivatives show activity against HIV-1 protease. Compounds **2B** and **2D** have essentially identical inhibition constants with K_i values of 0.58 μM and 0.50 μM, respectively, and they are 4-

fold more potent than **2A**. Replacement of bromide in **2D** with hydrogen significantly decreased binding and **2F** is 20 fold less active than **2D**, indicating that the presence of bulky and hydrophobic group such as bromide is important. Increasing the size of the R-group from methyl ester in **2B** to *m*-bromobenzyl ester in **2C** retains the same activity. Unfortunately, introduction of bulkier and more hydrophilic groups did not increase the potency, and compounds **3A** to **3D** are all less active than **2D** or **2C**. Although sulfones are better hydrogen bond acceptors than sulfides, the parent (+)-biotin derivatives **2D** and **2B** are more active than the sulfone analogs **4A** and **4B**, respectively. The precise reasons for this observation are not known. The (+)-biotin sulfone analogs could be too tall to fit into the active site of HIV-1 protease. The (+)-biotin analogs in general have good translations from K_i to RNA-IC_{90}. Half of the analogs shown in Table 1 lost only one order of magnitude in translation. The binding affinity of (+)-biotin analogs to HIV-1 protease could be increased when an additional P_1' fragment is introduced into the current parent structure of (+)-biotin derivatives.

Table 1



#	X	R	W	K_i (μM)	IC_{90} (μM)
2A	Br	CO_2H	S	2.2	34
2B	Br	CO_2Me	S	0.58	44
2C	Br	CO_2CH_2Ph-*m*-Br	S	0.68	45
2D	**Br**	**CH_2OH**	**S**	**0.5**	**7**
2F	H	CH_2OH	S	10.3	46
3A	COMe	CH_2OH	S	3.7	26
3B	$CH(OH)CH_3$	CH_2OH	S	1.9	> 98
3C	$COCH_3$	CO_2CH_2Ph-*m*-$COCH_3$	S	3.4	12.5
3D	$C[N(OH)]CH_3$	CO_2CH_2Ph-*m*-$C[N(OH)]CH_3$	S	2.7	67
4A	Br	CH_2OH	SO_2	6.9	80
4B	Br	CO_2Me	SO_2	4.1	> 80

In conclusion, we have designed and synthesized a variety of (+)-biotin derivatives and demonstrated that they are a new class of HIV-1 protease inhibitors with good translations from K_i to antiviral IC_{90}. The most potent inhibitor in this class compounds, **2D**, has a K_i value of 0.50 μM and an IC_{90} value of 7 μM.

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