First Total Synthesis of Mer-N5075A, A New HIV-I Protease Inhibitor from *Streptomyces Chromofuscus*

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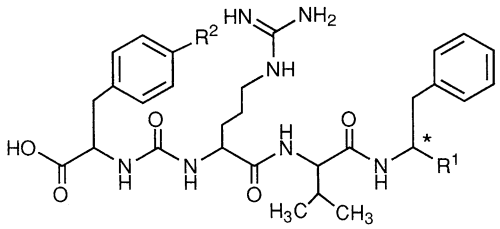
(Received December 25, 1996)

The first total synthesis of Mer-N5075A (**1**), a new potential HIV-I protease inhibitor produced from *Streptomyces chromofuscus*, was achieved. The synthetic method is available for Mer-N5075A analogues such as α-MAPI (**2**), GE20372 A (**4**) and other chemically modified compounds.

Mer-N5075A (**1**) is an anomalous tetrapeptide having potent human immunodeficiency virus type I (HIV-I) protease inhibition and is isolated from *Streptomyces chromofuscus* Mer-N5075 which is collected in Okinawa Prefecture, Japan.[1] Mer-N5075A belongs to the MAPI group[2] of compounds **2** and **3** having microbial alkaline protease inhibition. Recently, Stefanelli *et al.* isolated novel HIV-I protease inhibitors, GE20372 A (**4**) and B (**5**)[3] which are structually related to **1**, **2**, and **3**. These tetrapeptides (**1**-**5**) show the structural characteristics having terminal carboxyl and alcohol or aldehyde groups and an ureido bond, and their synthesis has not yet been reported. We have been interested in the synthesis and bioactivities of naturally occuring peptides comprising anomalous parts, and have already reported on the synthesis of azinomycin B.[4] In continuation of our synthetic and biological studies on naturally occuring bioactive peptide, we focused our attention on **1** and its analogues. It is reported[1] that α-MAPI (**2**) showed much more strong HIV-I protease inhibition. The terminal hydroxymethyl group of **1** is thought to be converted to a compound having more potent activity such as α-MAPI and other analogus compound, thus leading to the development of effective therapeutic agents for the treatment of AIDS. This background prompted us to synthesize **1** and their analogues.

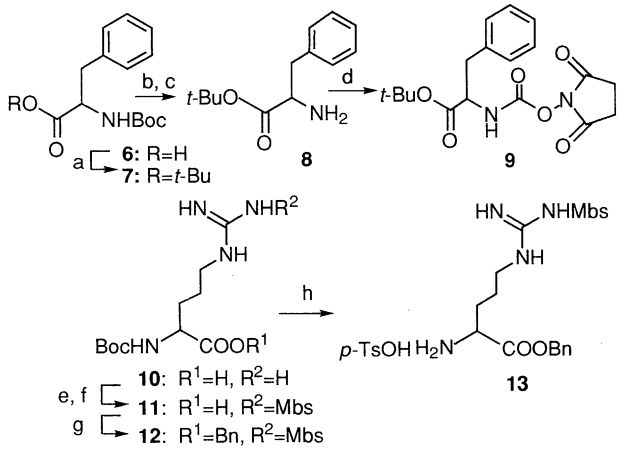
In this paper, we describe the first synthesis of **1** and the related compound **21** which is an important intermediate to convert **1** to other analogues.



	Compound	R^1	R^2	Config. at *
1	Mer-N5075A	CH_2OH	H	S
2	α-MAPI	CHO	H	S
3	β-MAPI	CHO	H	R
4	GE20372A	CHO	OH	S
5	GE20372B	CHO	OH	R

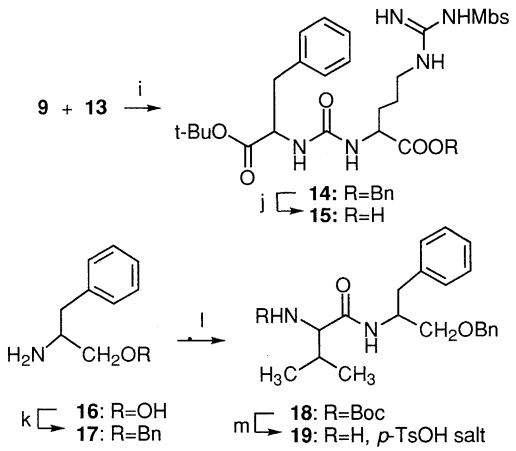
Compound **1** consists of three ordinal amino acids, L-phenylalanine (Phe), L-arginine (Arg), L-valine (Val) and (*S*)-phenylalaninol (PheAla). Our synthetic strategy for **1** is to connect two protected dipeptides **15** and **19** made of the protected Arg and Phe, and of Val and PheAla, respectively, to give protected tetrapeptide **20**. N,N'-Disuccinimidylcarbonate (DSC) which was developed by us[5] as an activating reagent was used to introduce the ureido group and DMAP-catalyzed esterification method developed by us[6] was used to synthesize *t*-butyl ester **7**.

Dipeptide **15** was synthesized as follows. Compound **6** was esterified by treatment with di-*tert*-butyl dicarbonate in the presence of DMAP in *tert*-BuOH giving a *tert*-butyl ester **7** in 92% yield, followed by the selective deprotection of the Boc group of **7** in the presence of *tert*-butyl ester according to the procedure described[7] by Goodacre *et al.*. Thus, treatment of **7** with *p*-toluenesulfonic acid (*p*-TsOH) gave the *p*-TsOH salt in 77% yield. The salt was then passed through anion-exchange resin (Amberlyst A-21) yielding amine **8** in 83% yield. This was converted to the activated ester **9** by treatment with DSC in 86% yield. The primary amino group of Boc-L-arginine (**10**) was protected by the *p*-methoxybenzenesulfonyl (Mbs) group using Mbs chloride followed by purification as a cyclohexylamine salt, then neutralization by citric acid afforded the acid **11** in 82% total yield from **10**. Esterification of **11** by benzyl alcohol in the presence of EDCI (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide) and DMAP gave the ester **12** in 61% yield, subsequent deprotection of the Boc group of **12** with *p*-TsOH gave the salt **13**, which was connected with the active ester **9** in



a) di-*t*-butyl dicarbonate, DMAP, *t*-BuOH, rt, 92%; b) *p*-TsOH, ether-EtOH, 0 ℃→ rt, 77%; c) Amberlyst A-21, EtOH, 83%; d) DSC, MeCN, rt, 86%; e 1) MbsCl , 4N NaOH, aq. acetone, 2) cyclohexylamine, 0 °C, 92%; f) 10% citric acid, 89%; g) benzyl alcohol, EDCI, DMAP, THF, rt, 61%; h) *p*-TsOH, ether, 0 ℃→rt.

Scheme 1.

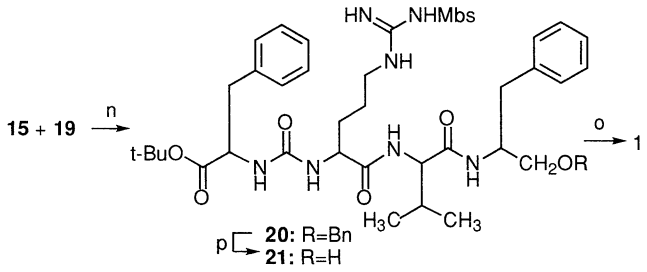


i) NMM, CH_2Cl_2, rt, 70% from **12**; j) H_2/Pd-C, EtOH, rt, 96%; k) benzyl bromide, NaH, DMF, 0 ℃→rt, quant.; l) Boc-L-valine, EDCI, CH_2Cl_2, 0 ℃→rt, 73%; m) *p*-TsOH, ether, 0 ℃→rt, quant.

Scheme 2.

the presence of NMM[8] to afford the dipeptide **14** in 70% total yield from **12** (Scheme 1 and 2).

Debenzylation of **14** by catalytic hydrogenation with Pd-C in ethanol gave the acid **15** in 96% yield. Dipeptide **19** was synthesized as shown in Scheme 2. (*S*)-Phenylalaninol (**16**) was benzylated with benzyl bromide and NaH to give the ether **17**, quantitatively. Connection of the ether **17** with Boc-L-valine using EDCI afforded the dipeptide **18** in 73% yield. Deprotection of the Boc group of **18** afforded the salt **19** quantitatively. Condensation of **19** with the dipeptide **15** in the presence of EDCI, HOBt, and NMM afforded successfully the protective tetrapeptide **20** in 95% yield. The Mbs, *tert*-butyl, and benzyl groups were removed at the same time by treatment with methanesulfonic acid[9] to afford Mer-N5075 A (**1**)[10] in 60% yield as shown in Scheme 3. ^1H and ^{13}C NMR spectra of



n) EDCI, HOBt, NMM, THF-CH_2Cl_2, 0 ℃→rt , 95%; o) 1) CH_3SO_3H, anisole, THF, rt, 2) Amberlite IRA-410, H_2O, 60%; p) H_2/Pd-C, AcOH, rt, 95%.

Scheme 3.

synthetic Mer-N5075A were quite identical with those of the natural compound described in the literature.[1] After purification of **1** by column chromatography followed by Sephadex LH-20,[11] **1** showed the optical rotation value of $[\alpha]_D^{27}$ -24.4° (c=0.32, AcOH), which is close to the reported one ($[\alpha]_D^{28}$ -27.6°, *c*=0.11, AcOH). In order to synthesize α-MAPI (**2**), debenzylation of the protective tetrapeptide **20** was examined. Debenzylation did not occurred under the conditions of catalytic hydrogenation by Pd-C in EtOH nor of the hydrogen transfer using Pd-black and Pd-C in EtOH. However, debenzylation of **20** by catalytic hydrogenation with Pd-C in AcOH afforded the alcohol **21** successfully in 80% yield. We are now investigating the conversion of the alcohol **21** to α-MAPI (**2**) and other various analogues.

In conclusion, the first synthesis of a new HIV-I protease inhibitor, Mer-N5075 A, was achieved. Each reaction involved in this procedure is employable in a large scale preparation and applicable to the synthesis of its analogues. The work on synthesis of α-MAPI and other analogues to obtain more potential HIV-I protease inhibitors is now in progress.

References and Notes

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10 Synthetic Mer-N5075A shows *Rf*=0.15 (BuOH/AcOH=5/1, Silica gel); 0.56 (MeOH/AcOH=100/1, Silica gel), mp 182 °C; HRFAB-ms: Calcd for $C_{30}H_{44}N_7O_6$ 598.3353 $(M+H)^+$; Found 598.3387.
11 Column chromatography was performed on silica gel using n-BuOH/AcOH=5/1 and Sephadex LH-20 using EtOH as eluates.