First efficient synthesis of α-MAPI

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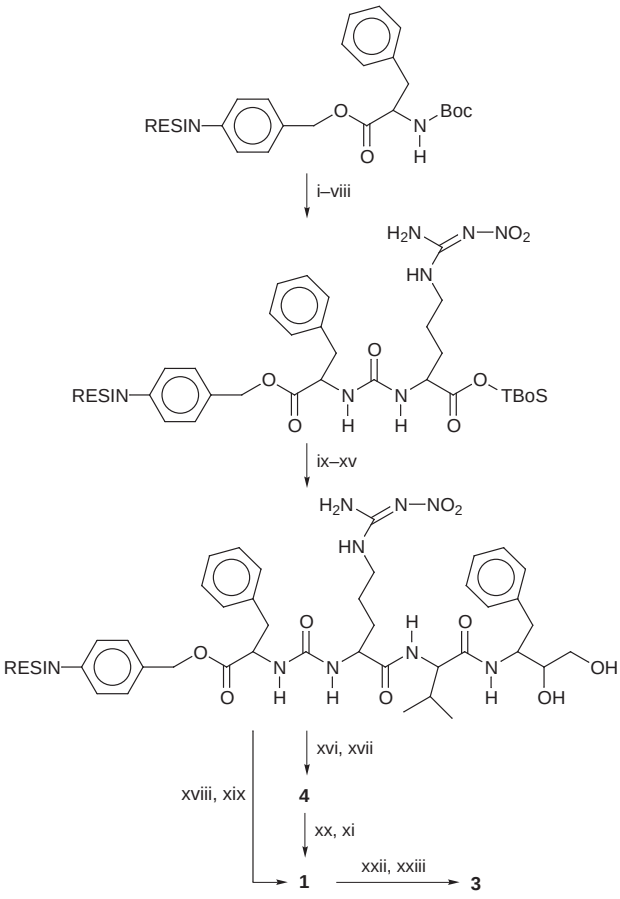
α-MAPI 1 and its analogues have been synthesised using tri-*tert*- butoxysilyl protected amino acids in conjunction with a solid-phase N→C assembly; the terminal aldehyde group of the peptide is generated from a C-modified amino acid containing a *gem*-diol.

MAPI (*microbial alkaline proteinase inhibitor*) is a mixture of three compounds, α-, β- and γ-MAPI possessing similar activity which are produced by *streptomyces nigrescens* WT-27.[1–3] The unique features of these peptides are that they contain terminal carboxy and aldehyde groups and a ureido (–NH–CO–NH–) function. Furthermore, α-MAPI **1** has been shown to inhibit HIV-I protease.[4] Recently, a closely related family of tetrapeptides **3**, **5** and **6** which inhibit HIV-protease has been reported.[5–7] It is hoped that these compounds may ultimately lead to the development of effective anti-proteolytic drugs for the treatment of AIDS. There is no report on the synthesis of **1** and **2**. Therefore, the synthesis of these compounds and their analogues is of paramount importance.



Scheme 1 *Reagents and conditions*: i, CH_2Cl_2 (2 × 1 min); ii, 50% TFA–CH_2Cl_2 (5 and 25 min); iii, CH_2Cl_2 (4 × 1 min); iv, CDI (78 mg, 0.48 mmol), CH_2Cl_2, 30 min; v, repeat iii; vi, remove 2–3 mg resin for ninhydrin assay; vii, Arg–TBos (223 mg, 0.48 mmol), CH_2Cl_2, 120 min; viii, repeat iii; ix, 25% TFA–CH_2Cl_2 (2 × 5 min); x, repeat iii; xi, Val-TBos (172 mg, 0.48 mmol); BOP–HOBt–DIPEA (1 1 : 1 : 3 equiv.), CH_2Cl_2, 120 min; xii, repeat iii, xiii, repeat ix, x; xiv, Phe-diol (87 mg)–BOP–HOBt–DIPEA (1 : 1 : 1 : 3 equiv.), DMF, 120 min; xv, DMF (2 × 1 min), CH_2Cl_2 (2 × 1 min); xvi, HF; xvii, RP-HPLC; xviii, $NaIO_4$, MeOH, 90 min; xix, repeat xv–xvii; xx, $NaIO_4$, MeOH–H_2O (80%, 2 ml), 45 min; xxi, repeat xvii; xxii, $NaBH_4$, MeOH, 30 min, AcOH; xxiii, repeat xvii. The solvent used was 10 ml throughout for 200 mg of resin.

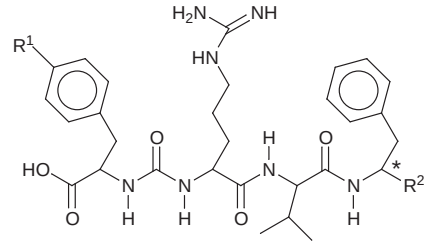


Table 1 Configuration data for **1**–**6**

Compound	R^1	R^2	Config. at *
1 α-MAPI	H	CHO	*S*
2 β-MAPI	H	CHO	*R*
3 Mer-N5075A	H	CH_2OH	*S*
4 α-MAPI-diol	H	$CHOHCH_2OH$	*S*
5 GE20372A	OH	CHO	*S*
6 GE20372B	OH	CHO	*R*

Our recently reported[8] new approach for the assembly of peptides on solid-phase from N→C direction was extended for the synthesis of α-MAPI **1** and its analogues **3** and **4** as illustrated in Scheme 1.

Boc-Phe-Merrifield resin was treated with TFA to remove the Boc group. The resin was thoroughly washed (CH_2Cl_2) and incubated with *N*,*N*′-carbonyldiimidazole (CDI). The reaction was monitored by ninhydrin assay.[9] Arginine-(N^G-NO_2)-tri-*tert*-butoxysilyl ester[8] (TBos)† was added and the mixture was shaken for 2 h. The removal of tri-*tert*-butoxysilyl group was accomplished in quantitative yield with 25% TFA. The peptide chain was further elongated by coupling valine-tri-*tert*-butoxysilyl ester followed by the deprotection of the ester group as before. Finally, the resulting peptidyl-resin was coupled to phenylalanine diol[10] in DMF to give the required peptidyl-resin. The peptide was then cleaved from the resin using liquid HF[11] and after preparative RP-HPLC followed by lyophilisation gave α-MAPI-diol **4** in 75% yield.§ Analytical and spectral data are in agreement with the assigned structure.¶

The diol **4** was smoothly oxidized with $NaIO_4$ to give α-MAPI **1**. The extent of reaction completeness was monitored by RP-HPLC and mass spectrometry. The oxidation was complete in 45 min. RP-HPLC purification afforded α-MAPI **1** in 84% yield§ and gave the expected spectral data.¶ The oxidation of the diol was also carried out on the solid-phase to give the aldehyde **1** in good yield.

α-MAPI **1** was treated with $NaBH_4$ and gave after purification the expected alcohol **3** in 64% yield.¶ Further work in this area is continuing.

Notes and References

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‡ All amino acids described in this work are of L-configuration. TBos refers to the tri-*tert*-butoxysilyl group.

§ Analytical and preparative reversed-phase HPLC (RP-HLPC) experiments were performed on a Gilson 715 instrument equipped with a multi-wave length detector (Applied Biosystems 759A) and two slave 306 pumps. Retention times are given for gradient elution using the following conditions: Column, Vydac C_{18} (10 μm, 0.46 and 2.2 × 25 cm); eluent A, 0.1% (v/v) TFA in H_2O; eluent B, 0.1% (v/v) TFA in acetonitrile, gradient, 0% B over 2 min, 0–80% B over 32 min; flow rate 1 ml min^{-1} (analytical) and 10 ml min^{-1} (preparative); absorbance, 216 nm. Molecular weight determinations were carried out by electrospray (ES) Micromass Quattro II mass spectrometer.

¶ All compounds reported herein are white solids and exhibited satisfactory analytical and spectral data. *Selected data* for α-MAPI-diol **4**: single peak, retention time, 16.5 min HPLC; ESMS, *m/z* 628 $[M + H]^+$. *Selected data* for α-MAPI **1**: single peak, retention time, 16.8 min HPLC; mp 211–213 °C (decomp.) (lit.,[2] 204–205 °C); $[\alpha]_D^{24}$ 22.2 (*c* 0.9, AcOH) (lit.,[2] −18); ESMS, *m/z* 596 $[M + H]^+$ and 614 $[M + H_2O]^+$, hemi-acetal; δ_H (360 MHz, $[^2H_7]$DMF) 0.79 (d, 3H, *J* 6.7 CH–C*H*$_3$, Val), 0.81 (d, 3H, *J* 6.9, CH-C*H*$_3$, Val), 1.5–1.75 (m, 4H, CH–C*H*$_2$–C*H*$_2$, Arg), 2.08 (h, 1H, *J* 6.55, C*H*$[CH_3]_2$, Val), 3.0–3.35 (m, 4*H*, 2 × C*H*$_2$, Phe), 3.6 (2H, obscured by solvent, –CH_2–NH, Arg), 4.3–4.6 (m, 4H, 4 × methines), 6.5 (d, 1H, *J* 8), 6.7 (d, 1H, *J* 7.8), 7.2 (m, 10H, 2 × C_6H_5, Phe), 7.55 (br), 7.85 (d, 1H, *J* 8.6), 8.5 (d, 1H, *J* 7.1), 9.58 (s, 1H, C*H*O). *Selected data* for alcohol **3**: single peak, retention time, 16.8 min HPLC; mp 155 °C, softens and 180–182 °C, (decomp. lit.,[5] 182 °C); $[\alpha]_D^{24}$ −24.0 (*c* 0.5, AcOH) (lit.,[5] –24.4); ESMS, *m/z* 598 $[M + H]^+$; ^1H NMR (360 MHz) and ^{13}C NMR (90 MHz) gave expected chemical shift values.

1 S. Murao and T. Watanabe, *Agric. Biol. Chem.*, 1977, **41**, 1313.
2 T. Watanabe, K. Fukuhara and S. Murao, *Tetrahedron Lett.*, 1979, 625.
3 T. Shin-Watanabe, K. Fukuhara and S. Murao, *Tetrahedron*, 1982, **38**, 1775.
4 R. Kaneto, H. Chiba, K. Dobashi, I. Kojima, K. Sasaki, N. Shibamoto, H. Nishida, R. Okamoto, H. Akagawa and S. J. Mizuno, *J. Antibiot.*, 1993, **46**, 1622.
5 Y. Konda, Y. Takahashi, H. Mita, K. Takeda and Y. Harigaya, *Chem. Lett.*, 1997, 345.
6 E. Sarubbi, P. F. Seneci, M. R. Angelastro, N. P. Peet, M. Denaro and K. Islam, *FEBS Lett.*, 1993, **319**, 253.
7 S. Stefanelli, E. Cavaletti, E. Sarubbi, L. Ragg, L. Colombo and E. Selva, *J. Antibiot.*, 1995, 48, 332.
8 R. P. Sharma, D. A. Jones, R. J. Broadbridge, D. L. Corina and M. Akhtar, in *Innovation and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids, Biological and Biomedical Applications*, ed. R. Epton, Mayflower Worldwide Ltd., Birmingham, UK, 1994, p. 353; R. P. Sharma, D. A. Jones, R. J. Broadbridge, D. L. Corina and M. Akhtar, in *Peptides: Chemistry, Structure and Biology*, ed. R. S. Hodges and J. A. Smith, Leiden, ESCOM, 1994, 127; R. P. Sharma, R. J. Broadbridge, D. A. Jones, D. L. Corina and M. Akhtar, in *Peptides: Biology and Chemistry*, ed. G. Lu and J. P. Tam, Leiden, ESCOM, 1995, p. 31.
9 V. K. Sarin, S. B. H. Kent, J. P. Tam and R. B. Merrifield, *Anal. Biochem.*, 1981, **117**, 147.
10 R. P. Sharma, M. G. Gore and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1979, 875.
11 J. P. Tam, W. F. Heath and R. B. Merrifield, *J. Am. Chem. Soc.*, 1983, **105**, 6442.

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