Total Synthesis of Dephostatin, a Novel Protein Tyrosine Phosphatase Inhibitor

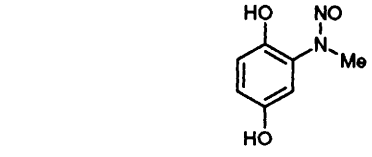
Takumi Watanabe,[a] Tomio Takeuchi,[a] Masami Otsuka[b] and Kazuo Umezawa*[c]

[a] *Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan*
[b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*
[c] *Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan*

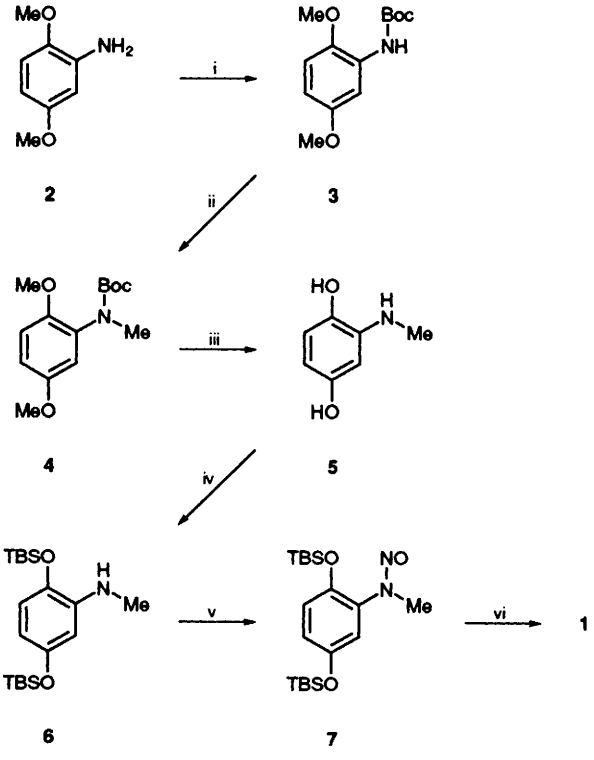
The first synthesis of dephostatin, conducted by use of a silyl protective group removable with HF–NaF buffer without damaging the labile *N*-nitrosamino group, is described.

Protein tyrosine phosphatase has been implicated in the regulation of the cell cycle[1] and activation of lymphocytes.[2] Recently, a novel protein tyrosine phosphatase inhibitor, dephostatin **1**, was isolated from the culture filtrate of *Streptomyces* sp. MJ742-NF5.[3,4] Dephostatin and its derivatives are expected to be useful biochemical and therapeutic agents.

In spite of its potential utility, sufficient amounts of dephostatin are difficult to obtain from the natural source because of its low production by the microorganism and lability to air, heat, light, solvents and acidic conditions. Therefore, a practical chemical synthesis is needed. Herein the first synthesis of dephostatin is described.



Dephostatin 1



Scheme 1 *Reagents and conditions*: i, $(Boc)_2O$ (1.2 equiv.), TEA (1.2 equiv.), 1,4-dioxane, H_2O, 25 °C, 24 h: 83% yield; ii, NaH (1.2 equiv.), DMF, 0 to 25 °C, 1 h then MeI (5 equiv.), −23 to 0 °C, 3 h: 98% yield; iii, BBr_3 (3 equiv.), CH_2Cl_2, −78 to 25 °C, 7 h; iv, TBSCl (2.5 equiv.), imidazole (5 equiv.), DMF, −23 to 25 °C, 24 h: 41% yield for two steps; v, $NaNO_2$ (1 equiv.), 1 mol dm^{-3} HCl (excess), THF, 0 °C, 3 h: 81% yield; vi, HF–NaF buffer (pH 4.98, excess), 25 °C, 24 h: 66% yield

As commercially available 2,5-dimethoxyaniline **2** appeared to be the most suitable starting material, the amino group of **2** was protected as *tert*-butyl carbamate **3** (83%). Treatment of **3** with NaH (1.2 equiv.) in DMF followed by addition of MeI (excess) gave the *N*-methylated product **4** in 98% yield.

The *tert*-butyloxycarbonyl and two methyl ether groups of **4** were simultaneously removed with BBr_3 (3 equiv.) in CH_2Cl_2 at room temp. After completion of the reaction, the reaction mixture was poured onto ice-water; and the aqueous layer was neutralized with $NaHCO_3$, washed with $CHCl_3$, and concentrated *in vacuo* to give a crude mixture containing the deprotected product **5**. Crude **5** was used for the following reaction without further purification because of its intrinsic lability.

Attempts to direct the *N*-nitrosation of **5** with HNO_2 generated from $NaNO_2$ and 1 mol dm^{-3} HCl[5] failed to give **1** due to the unexpected side reactions, presumably polymerization and *C*-nitrosation. Instability of **5**, being an obstacle for this reaction, seems to arise from the hydroquinone structure. Therefore, temporal protection of the hydroxy groups was necessary for the subsequent *N*-nitrosation, and so we employed *tert*-butyldimethylsilyl (TBS) ether, which is known to be removed selectively under mild conditions.[6]

Thus, treatment of crude **5** with TBSCl (2.5 equiv.) in the presence of imidazole (5 equiv.) gave **6** in 41% overall yield from **4**. Intermediate **6** was stable under the standard conditions of *N*-nitrosation; exposure of **6** to 1 mol dm^{-3} HCl (excess) and $NaNO_2$ (1 equiv.) in H_2O–THF provided *N*-nitrosamine **7** in 81% yield.

Deprotection of **7** was examined under various mild conditions. Desilylation of **7** with tetrabutylammonium fluoride (TBAF), in fact, proceeded smoothly. However, the resulting **1** after TBAF treatment was an intractable mixture with unknown impurity. This problem was successfully resolved by the method of Cook *et al.*[7] Addition of HF–NaF buffer (pH 4.98) to **7** in THF afforded dephostatin **1** as a pure form (light-brown powder) in 66% yield from **7**.† The overall yield was 18% based on the starting material **2** (six steps). Synthetic **1** was identical to the authentic sample in all respects (mp, IR, ^1H NMR and ^{13}C NMR spectroscopy, FABMS and TLC), and gave satisfactory FAB HRMS data (found: *m/z* MH^+, 169.0627 $C_7H_9N_2O_3$ requires 169.0613), which could not be obtained from the natural material. Synthetic **1** also showed tyrosine phosphatase inhibitory activity indistinguishable from that of the natural material.

We thank Dr M. Imoto, Keio University and Dr H. Naganawa, Dr D. Ikeda, and Mr R. Sawa, Institute of Microbial Chemistry, for valuable suggestions.

Received, 18th October 1993; Com. 3/06211B

Footnote

† An alternative route to **1** *via* *N*-methyl-*N*-nitroso-2,5-dimethoxyaniline derived from **4** was unsuccessful due to the difficulty of selective demethylation without destroying the *N*-nitroso moiety.

References

1 K. L. Gould, S. Moreno, N. K. Tonks and P. Nurse, *Science*, 1990, **250**, 1573.
2 G. A. Koretzky, J. Picus, T. Schultz and A. Weiss, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 2037.
3 M. Imoto, H. Kakeya, T. Sawa, C. Hayashi, M. Hamada, T. Takeuchi and K. Umezawa, *J. Antibiot.*, 1993, **46**, 1342.
4 H. Kakeya, M. Imoto, Y. Takahashi, H. Naganawa, T. Takeuchi and K. Umezawa, *J. Antibiot.*, 1993, **46**, 1716.
5 W. W. Hartman and L. J. Roll, *Org. Synth.*, 1943, **II**, 460.
6 T. W. Greene and P. G. M. Wuts, *Protective Group in Organic Synthesis*, John Wiley & Sons, New York, 2nd edn., 1991, pp. 77, 161.
7 P. M. Kendall, J. V. Johnson and C. E. Cook, *J. Org. Chem.*, 1979, **44**, 1421.