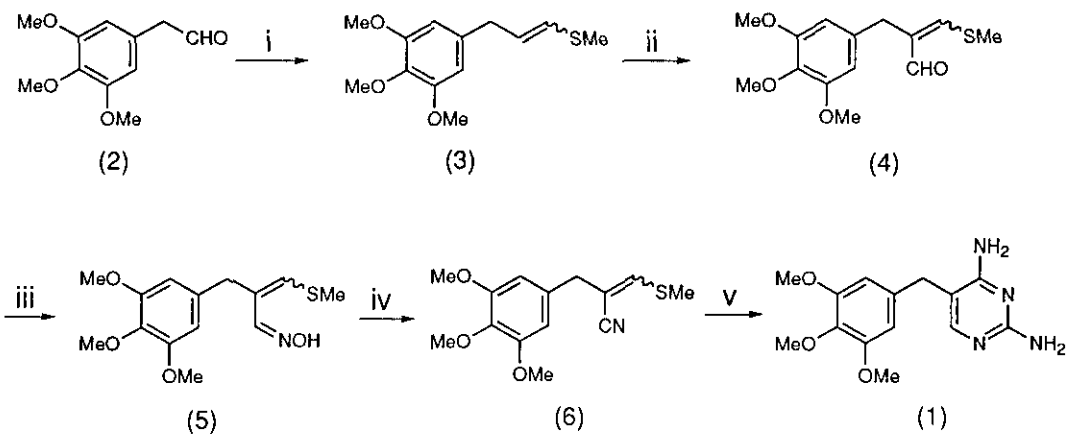
# A NEW ROUTE TO ANTIBACTERIAL TRIMETHOPRIM

Kenichi Harada, Tominari Choshi, Eiichi Sugino,
Kohichi Sato, and Satoshi Hibino*

Faculty of Pharmacy & Pharmaceutical Sciences,
Fukuyama University
Fukuyama, Hiroshima 729-02, Japan

**Abstract**---3-Methylthio-2-(3',4',5'-trimethoxybenzyl)acrylonitrile (**6**) derived from 3,4,5-trimethoxyphenylacetaldehyde (**2**) in a four step sequence was utilized as a new three carbon unit of trimethoprim (**1**).

Trimethoprim, 2,4-diamino-5-(3',4',5'-trimethoxybenzyl)pyrimidine (**1**) is a potent and selective inhibitor of bacterial dihydrofolate, the enzyme responsible for the NADPH-dependent reduction of 7,8-dihydrofolate.[1a-c] It is used solely, or in combination with sulfamethoxazole, to treat a wide range of bacterial infections in humans.[1d,e] Since 1962, a variety of synthetic routes to trimethoprim (**1**) and its analogue for the structure activity relationships have appeared.[2-5] Of synthetic strategies, the condensation of either the cinnamonitriles[2] or the enaminonitriles[3] with guanidine is well known as the most widely applicable method offering direct entry into the 2,4-diaminopyrimidine nucleus.




**Scheme 1.** i. $Ph_3P^+CH_2SMe$ $Cl^-$, *n*-BuLi, 0°C, ii : DMF, $POCl_3$, iii · $NH_2OH$ · HCl, AcONa, iv : $Ac_2O$, reflux, v · guanidine · HCl, EtONa, reflux

We aimed a synthesis of trimethoprim (**1**) using 3-methylthio-2-(3',4',5'-trimethoxybenzyl)acrylonitrile (**6**) as a new three carbon unit of 2,4-diaminopyrimidine ring. For the preparation of the new precursor (**6**), we started from 3,4,5-trimethoxyphenylacetaldehyde (**2**).[6] Wittig reaction of **2**[7] with methylthiomethylenetriphenylphosphorane gave a *E/Z* mixture of the vinyl sulfide (**3**) (99.5%). Subsequent treatment of **3** with freshly prepared Vilsmeier reagent at room temperature afforded the 3-methylthioacrolein (**4**) (73.6%) in a similar result as a previous work.[7,8] The reaction of **4** with hydroxylamine at ambient temperature followed by heating in acetic anhydride provided the desired acrylonitrile (**6**), a new precursor, in 93.5% yield from **4**. Finally, the condensation of the acrylonitrile (**6**) with excess guanidine in refluxing ethanol gave trimethoprim (**1**) in 90.4% yield (**Scheme 1**), whose nmr spectrum and physical data were identical with those in the literature.[2d] Thus, the methylthio group of the precursor (**6**) worked well as a leaving group to form a 2,4-diaminopyrimidine ring. Although, our

sequence was more lengthy than that of the previous reports,[2-5] the overall yield was 61.9%, whose result was almost same in the comparison with that of the recent improved method (61% overall yield).[2d]

## EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (Ir) spectra were measured with a Shimadzu FTIR-8500 spectrophotometer. Proton nuclear magnetic resonance ($^1$H-Nmr) spectra were taken with JEOL PMX60Si and JEOL-A400 spectrometers with $SiMe_4$ as an internal standard. Mass (Ms) spectra were recorded on a Shimadzu 9020DF spectrometer at 70eV on a direct inlet system. Silica gel $60F_{254}$ (60-100 mesh, Merck Art. 7734) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography.

**1-Methylthio-3-(3',4',5'-trimethoxyphenyl)propene** (**3**): A solution of 3,4,5-trimethoxyphenylacetaldehyde (**2**) (530 mg, 2.52 mmol) in anhyd. THF (5 ml) was added dropwise to a solution of methylthiomethylenetriphenylphosphorane [prepared from methylthiomethyltriphenylphosphonium chloride (1.08 g, 3.03 mmol) in anhyd. THF (15 ml) and *n*-BuLi (1.85 ml, 1.64 M in hexane solution, 3.03 mmol) under cooling with ice] under $N_2$ atmosphere at the same temperature. After stirring at room temperature for 14 h, the mixture was worked up with an aqueous $NH_4Cl$ (saturated) solution and then extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc-hexane (3:7, v/v) as an eluent to give a 1:1 mixture of the *E*- and *Z*-vinyl sulfide (**3**) (541 mg, 99.5%) as an oil, bp 167-

169°C/1.8 torr. $^1$H-Nmr ($CDCl_3$): δ2.23(1.5H, s, $SC\underline{H}_3$ × 1/2), 2.30(1.5H, s, $SC\underline{H}_3$ × 1/2), 3.32 and 3.42(2H, br dd, J=2 and 6 Hz, $C\underline{H}_2Ph$), 3.82(9H, s, $OC\underline{H}_3$ × 3), 5.33-6.30(1H, m, $CH_2$-C<u>H</u>=CH-), 6.38(2H, d, J=2 Hz, $C_2$,-H and $C_6$,-H), 7.28(1H, br m, CH=C<u>H</u>-$SCH_3$). Ms *m/z*: 254($M^+$). *Anal.* Calcd for $C_{13}H_{18}O_3S$: C, 61.39; H, 7.13. Found: C, 61.50; H, 7.33.

**3-Methylthio-2-(3',4',5'-trimethoxybenzyl)acrolein** (**4**): A solution of the vinyl sulfide (**3**) (110 mg, 0.443 mmol) was added to a solution of Vilsmeier reagent [prepared from DMF (0.41 ml) and $POCl_3$ (0.47 ml, 0.52 mmol) at room temperature]. After stirring at ambient temperature for 14 h, the reaction mixture was poured into the ice-water. The mixture was basified with 10% aqueous NaOH solution and then extracted with EtOAc. The EtOAc layer was washed with brine, dried over $Na_2SO_4$ and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc-hexane (1:9, v/v) as an eluent to give the aldehyde (**4**) (92 mg, 73.6%) as an oily product, bp 214-215°C/2 torr. Ir(Neat): 1660cm$^{-1}$(CHO). $^1$H-Nmr($CDCl_3$): δ2.50 (3H, s, $SC\underline{H}_3$), 3.50(2H, s, $C\underline{H}_2Ph$), 3.80(9H, s, $OC\underline{H}_3$ × 3), 6.43(2H, s, $C_2$,-H and $C_6$,-H), 7.23(1H, s, =C<u>H</u>-$SCH_3$), 9.23(1H, s, C<u>H</u>O). Ms *m/z*: 282($M^+$). *Anal. Calcd for* $C_{14}H_{18}O_4S$: *C, 59.55; H, 6.43. Found: C, 59.48; H, 6.53.*

**3-Methylthio-2-(3',4',5'-trimethoxybenzyl)acrolein oxime** (**5**): A mixture of the acrolein (**4**) (70 mg, 0.248 mmol), $NH_2OH \cdot HCl$ (19 mg, 0.273 mmol) and AcONa (40.6 mg, 0.298 mmol) in EtOH (5 ml) was stirred at room temperature for 14 h. After removal of solvent, the water was added to the residue. The mixture was extracted with $CHCl_3$, whose organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated. The residue was purified by

column chromatography (silica gel, 10 g) using EtOAc-hexane (3:7, v/v) as an eluent to give the oxime (**5**) (73.7 mg, 100%), mp 113-114°C ($Et_2O$-hexane). $^1$H-Nmr ($CDCl_3$): δ2.37(3H, s, $SCH_3$), 3.60(2H, s, $CH_2Ph$), 3.78(9H, s, $OCH_3$ × 3), 6.42(1H, s, =CH-$SCH_3$), 6.45(2H, s, $C_{2'}$-H and $C_{6'}$-H), 7.63(1H, s, -CH=N). Ms *m/z*: 297($M^+$). *Anal.* Calcd for $C_{14}H_{19}NO_4S$: C, 56.55; H, 6.44; N, 4.71. Found: C, 56.70; H, 6.55; N, 4.60.

**3-Methylthio-2-(3',4',5'-trimethoxybenzyl)acrylonitrile** (**6**): A mixture of the oxime (**5**) (140 mg, 0.47 mmol) in $Ac_2O$ (3 ml, 31.8 mmol) was refluxed for 2 h. After cooling at ambient temperature, the water was added to the $Ac_2O$ solution and the mixture was extracted with $Et_2O$. The $Et_2O$ layer was washed with 10% $K_2CO_3$ solution and brine, which was dried over $Na_2SO_4$ and concentrated. The residue was purified by column chromatography (silica gel, 10 g) using EtOAc-hexane (4:6, v/v) as an eluent to give the nitrile (**6**) (123 mg, 93.5%) as an oil, bp 142-144°C/2 torr. Ir(Neat): 2206$cm^{-1}$(CN). $^1$H-Nmr($CDCl_3$): δ2.47 (3H, s, $SCH_3$), 3.45(2H, s, $CH_2Ph$), 3.83(9H, s, $OCH_3$ × 3), 6.47(2H, s, $C_{2'}$-H and $C_{6'}$-H), 7.05(1H, s, =CH-$SCH_3$). Ms *m/z*: 279($M^+$). *Anal.* Calcd for $C_{14}H_{17}NO_3S$: C, 60.19; H, 6.13; N, 5.01. Found: C, 60.33; H, 6.31; N, 4.95.

**2,4-Diamino-5-(3',4',5'-trimethoxybenzyl)pyrimidine (Trimethoprim)** (**1**): A mixture of guanidine·*HCl* (1.03 g, 10.74 *mmol*) *and NaOEt [freshly prepared* from Na (247 mg, 10.74 mmol) and abs. EtOH (5 ml)] was stirred at ambient temperature for 20 min. A solution of the nitrile (**6**) (100 mg, 0.358 mmol) in abs. EtOH (5 ml) was added to the stirred mixture under cooling with ice-water, which was heated under reflux for 12 h. After removal of

solvent, the residue was purified by column chromatography (Sephadex LH-20) using MeOH to give the crude product. The crude product was recrystallized from aqueous EtOH to give trimethoprim (**1**) (94 mg, 90.4%), mp 198-198.5°C (lit.,[2d] mp 199-200°C). Ir(KBr): 3471cm$^{-1}$($NH_2$). $^1$H-Nmr($CDCl_3$, 400 MHz): δ3.53 (2H, s, $C\underline{H}_2Ph$), 3.62(3H, s, $OC\underline{H}_3$), 3.72(6H, s, $OC\underline{H}_3$ × 2), 5.63, 6.03(2H × 2, s × 2, $N\underline{H}_2$ × 2, exchangeable with $D_2O$), 6.54(2H, s, $C_2$,-H and $C_6$,-H), 7.52(1H, s, $C_6$-H). Ms *m/z*: 290($M^+$).

## REFERENCES AND NOTES

1. (a) S. R. M. Bushby and G. H. Hitchings, *Br. J. Pharmacol. Chemother.*, 1968, **33**, 72; (b) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, 1965, **1**, 126; (c) G. H. Hitchings, *Angew. Chem., Int. Ed. Ing.*, 1989, **28**, 879; (d) J. A. Montgomery and J. R. Piper, in "Folate Antagonists as Therapeutic Agents", Vol. 1, ed. by F. M. Sirotnak, J. J. Burchall, W. W. Ensminger, and J. A. Montgomery, Academic Press, Orlando, Florida, 1984; (e) R. H. Rubin and M. N. Swartz, *New Engl. J. Med.*, 1980, **303**, 426.

2. (a) P. Stenbuck and H. M. Hood, *U. S. Patent* 30495544 (1962) (*Chem. Abstr.*, 1963, **58**, 1478c); (b) P. Stenbuck, R. Baltzly, and H. M. Hood, *J. Org. Chem.*, 1963, **28**, 1983; (c) M. Hoffer, E. Grunberg, M. Mitrovic, and A. Brossi, *J. Med. Chem.*, 1971, **14**, 462; (d) P. S. Manchand, P. Rosen, P. S. Belica, G. V. Oliva, A. V. Perrotta, and H. S. Wong, *J. Org. Chem.*, 1992, **57**, 3531.

3. (a) I. Kompis and A. Wick, *Helv. Chim. Acta*, 1977, **60**, 3025; (b) I. Kompis, R. Then, E. Boehni, G. Rey-Bellet, G. Zanetti, and M. Montavon, *Eur. J. Med. Chem.*, 1980, **15**, 17; (c) M. Calas, A. Barbier, L. Giral, B.

Balmayer, and E. Despaux, *ibid.*, 1982, **17**, 497.

4. (a) B. Roth, E. A. Falco., G. H. Hitchings, and S. R. M. Bushby, *J. Med. Pharm. Chem.*, 1962, **5**, 1103; (b) B. Roth, E. Aig, K. Lane, and B. S. Rauckman, *J. Med. Chem.*, 1980, **23**, 535; (c) B. Roth, J. Z. Strelitz, and B. S. Rauckman, *ibid.*, 1980, **23**, 379.
5. N. Ple, A. Turck, P. Martin, S. Barbey, and G. Quéguiner, *Tetrahedron Lett.*, 1993, **34**, 1605.
6. A. I. Scott, F. McCapra, R. L. Buchanan, and D. W. Young, *Tetrahedron*, 1965, **21**, 3605.
7. (a) S. Kano, Y. Yuasa, S. Shibuya, and S. Hibino, *Heterocycles*, 1982, **19**, 1079; (b) S. Hibino, K. Nomi, Y. Shintani, E. Sugino, H. Fujioka, S. Kadowaki, and M. Otagiri, *Drug Design and Delivary*, 1989, **5**, 49.
8. The acrolein (**4**) showed a behavior of a single like compound in the nmr spectrum.

Received, 10th January, 1994