SYNTHESIS OF WATER SOLUBLE DERIVATIVES OF MILBEMYCIN D

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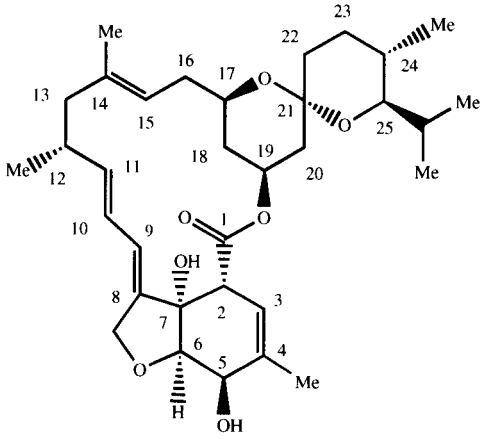
Abstract - Water Soluble 5-*O*-(*S*-glutathionylthio)cysteinyl-milbemycin D was successfully synthesized by using 3-nitro-2-pyridinesulfenyl (Npys) reagent through esterification of a Boc-cysteine derivative to a hydroxyl group at the C-5 position of milbemycin D, activation of the conventional *S*-protecting group, and asymmetrical disulfide bond formation.

Milbemycin D is one of a family of 16-membered ring macrolides isolated from *Streptomyces hygroscopicus* subspecies *aureolacrimosus* [1] and exhibits very potent acaricidal and insecticidal activities.[2] Numerous efforts have been made to develop naturally occurring compounds or semisynthetic derivatives as insecticides and anthelmintics. However, these compounds and derivatives were found to be so lipophilic that they dissolve in ether, and migration of the Δ^3-double bond to Δ^2-isomers was a major obstacle under basic conditions.[3]



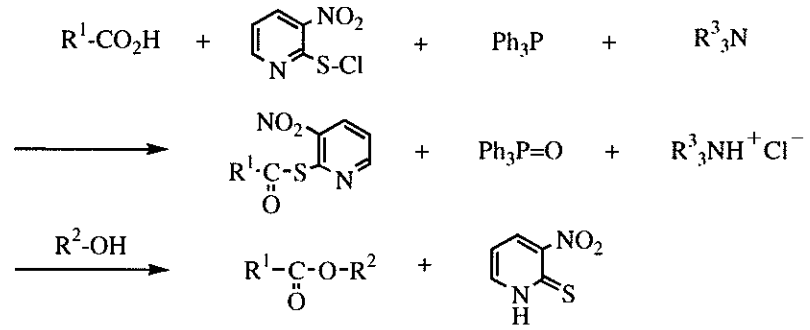
Milbemycin D(**MD**)

Thus, developing water soluble derivatives was tried to understand water solubility-bioavailability relationships. Several amino acids were successfully

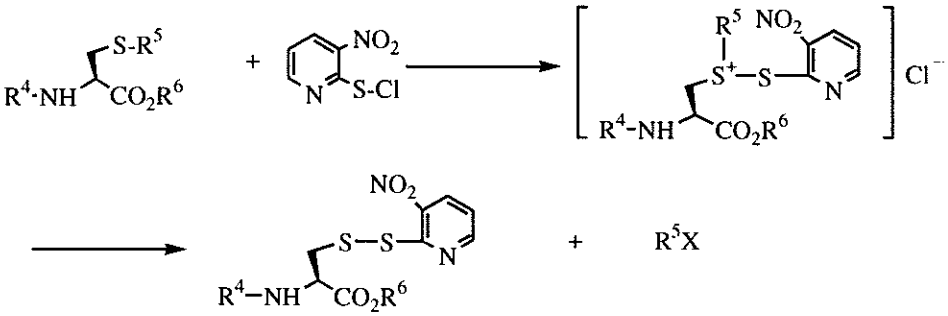
esterified to the hydroxyl group at C-5 position of milbemycin D by the esterification method *via* 3-nitro-2-pyridinesulfenyl(Npys)-thiolester that had been used to produce cephalosporanic acid esters without migration of the Δ^3-double bond.[4] However, unfortunately, these esters were found to be insoluble in water even though they were converted into hydrochlorides of the free amino form and further study was made to develop derivatives with more functional groups. In this paper, we describe the synthesis of the water soluble cysteine derivative of milbemycin D by using 3-nitro-2-pyridinesulfenyl (Npys) reagent.

3 Types of Reaction by Npys Reagent

Reaction I Npys-thiolester formation and esterification



Reaction II Activation of the conventional *S*-protecting group of cysteine by conversion into Npys group



Reaction III Asymmetrical disulfide bond formation

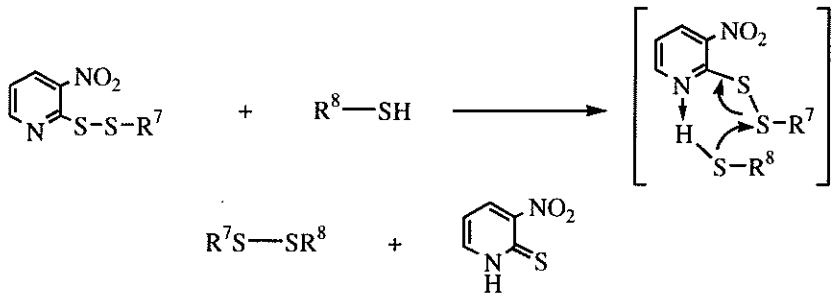
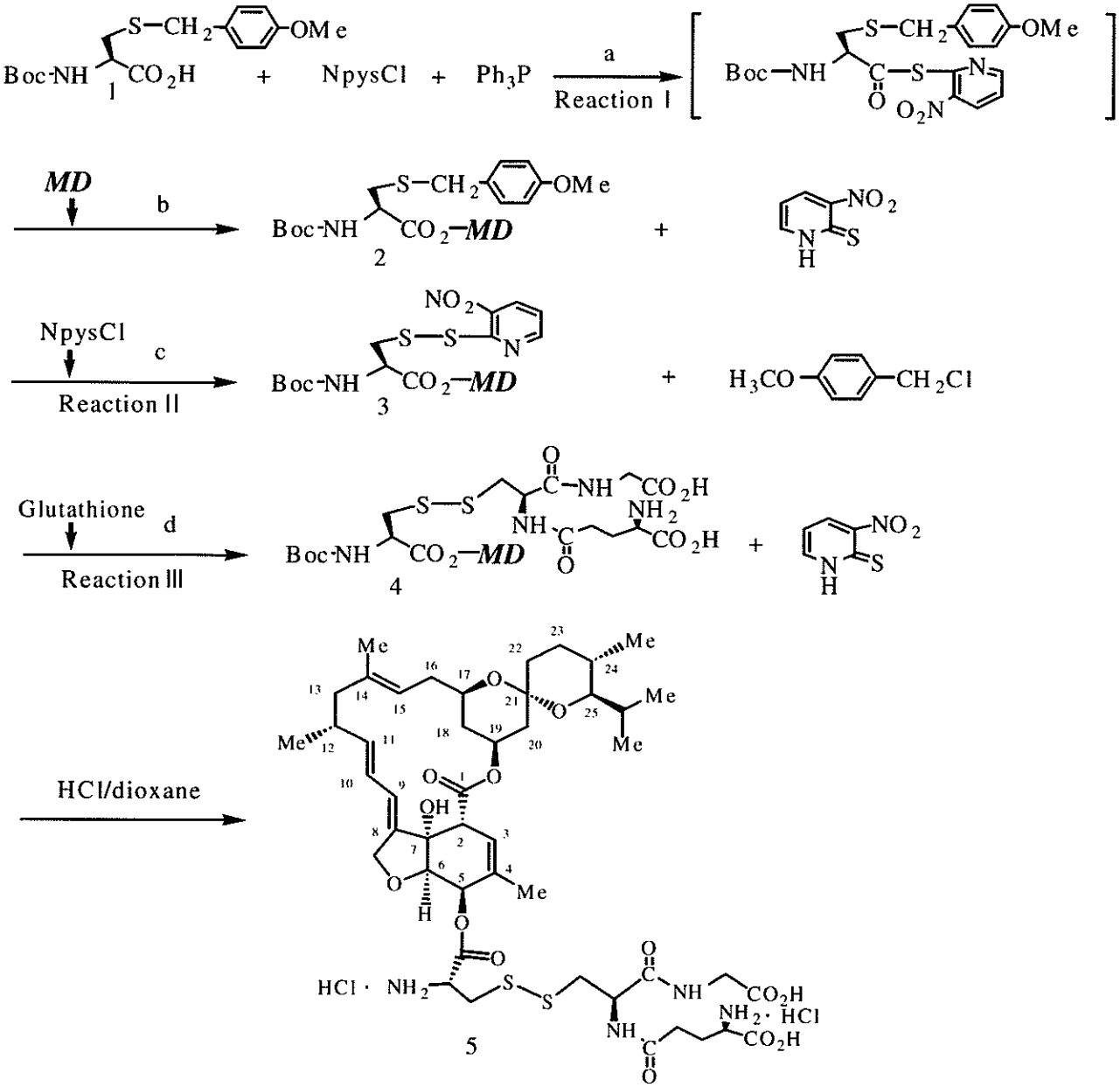


Figure1

Npys chloride was was found to be extraordinary stable and was the first isolated solid among the nitrogen-containing heterocyclic sulfenyl halides.[5] It is useful not only as a protecting group[6] for amino, hydroxyl, and thiol functions but also as a

reagent for organic reactions. Three types of reaction of the Npys reagent are shown in Figure 1: Npys-thiolester formation, activation of conventional *S*-protecting group, and asymmetrical disulfide bond formation.

N-tert-Butyloxycarbonyl-*S*-(*p*-methoxybenzyl)-L-cysteine(Boc-Cys(*p*-MeOBzl)-OH) was converted into Npys-thiolester by reaction I using equivalent amounts of each component and allowed to react with milbemycin D (MD) at 40 ℃ for 3 h. Esterified milbemycin D (compound (**2**), mp 94-98 ℃, $[\alpha]_D^{22}$-2.9° (*c*=1, MeOH)) was obtained in 57% without migration of Δ^3-double bond, which was confirmed[7]



a) Mixed at 0 ℃ and stirred for 30 min at rt; b) 3h at 40 ℃, silica gel chromatography with AcOEt/n-hexane(35:65); c) 30 min at rt in CH_2Cl_2, silica gel chromatography with AcOEt/n-hexane(1:1); d) Overnight at rt in MeOH/H_2O(8:2), gel filtration with Sephadex LH-20 in MeOH.

Scheme 1

by ^{1}H NMR of 5-H and 6-H: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56(1H, m, 5-H), 4.06(1H, d, J=6.0 Hz, 6-H). Conversion of the *S-p*-methoxybenzyl protecting group into the Npys group by reaction II was carried out in CH_2Cl_2, and compound (**3**) (mp124-126 ℃, $[\alpha]_D^{22}$-2.6° (c=1, MeOH)) was obtained (90%). Reaction III between compound (**3**) and glutathione carried out in MeOH/water(8:2) at room temperature overnight gave compound (**4**) with an asymmetrical disulfide bond (65%, mp 165-168 ℃, $[\alpha]_D^{22}$-48.5° (c=1, MeOH)).[9] Deprotection of the Boc-group by 4N HCl/dioxane gave the dihydrochloride of 5-*O*-(*S*-glutathionylthio)-cysteinylmilbemycin D(compound (**5**), mp148-150 ℃, $[\alpha]_D^{22}$-88.0° (c=1, MeOH)). Compounds (**1**, **2**, **3**, and **4**) were not soluble in water. But, surprisingly, it was found that compound (**5**) was soluble in water and showed biological activity against *N. brasiliensis*. Furthermore, derivatives with both a free amino group and a carboxyl group, such as the hydrochloride of 5-*O*-aspartylmilbemycin D (mp115-120 ℃, $[\alpha]_D^{22}$-64.9° (c=1, MeOH)), were also found to be soluble in water.[10] Their bioavailabilities and biological activities will be reported elsewhere.

In summary, we have shown that 3 types of reaction by Npys reagent were successfully applied to the synthesis of the Cys-containing derivative of milbemycin D and derivatives with both a free amino group and a carboxyl group were water soluble.

REFERENCES AND NOTES

1. H. Mishima, M. Kurabayashi, C. Tamura, S. Sato, H. Kuwano, A. Sato, and A. Aoki, Abstract Papers 18th Symp. Chem. Natural Products, Kyoto, 1974, pp. 309-316.
2. H. Mishima, M. Kurabayashi, C. Tamura, S. Sato, H. Kuwano, A. Saito, and A. Aoki, *Tetrahedron Lett.*, 1975, 711. Y. Takiguchi, H. Mishima, M. Okuda, M. Terao, A. Aoki, and R. Fukuda, *J. Antibiotics*, 1980, **33**, 1120.
3. J.V. Pivnichny, J-S. K. Shim, L.A. Zimmerman, *J. Pharm. Sci.*, 1983, **72**, 1447. J. V. Pivnichny, B. H. Arison, F. A. Preiser, J-S. K. Shim, and H. Mrozik, *J. Agric. Food Chem,* 1988, **36**, 826.
4. R. Matsueda, *Chem. Lett.*, 1978, 979.
5. R. Matsueda and K. Aiba, *Chem. Lett.*, 1978, 951.
6. R. Matsueda and R. Walter, *Int. J. Peptide Protein Res.*, 1980, **16**, 392.
7. Coupling constant between 5-H and 6-H for natural products and migrated ones is 5.0-6.0 and 1.0-1.5, respectively.[8]
8. S. Naito, T. Nanba, Y. Owatari, Y. Nakada, S. Muramatsu, and J. Ide, *J. Antibiot.*, 1994, **47**, 233.
9. *Anal.* Calcd for $C_{51}H_{76}N_4O_{16}S_2.3H_2O$: C, 54.72; H, 7.38; N, 5.01; S, 5.73. Found: C, 54.49; H, 7.19; N, 5.26; S, 6.02. MS *m/z* 1065 (M^+) $C_{51}H_{76}N_4O_{16}S_2$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (1H, m, 5-H), 4.06 (1H, d, J=5.9 Hz, 6-H).
10. A. Terada, S. Naruto, R. Matsueda, and S. Higashida, *Japanese Patent Kokai*, 1985, 60-184085 (*Chem. Abstr.*, **104**, 207055).

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