Chem. Pharm. Bull.
34(3)1089—1093(1986)

Modification of the Cysteamine Side Chain of Thienamycin. I[1]

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(Received August 22, 1985)

A new type of thienamycin derivative (**2**) having a monothioacetal side chain at the C-2 position was prepared by means of a replacement reaction of protected thienamycin sulfoxide (**13**) with the monothiohemiacetal (**16**) liberated *in situ* by the retro-Michael reaction of the masked thiol (**12**) with 1,8-diazabicyclo[5.4.0]undec-7-ene.

Keywords——thienamycin; monothiohemiacetal; masked thiol; protective group; carbapenem antibiotics

In the preceding paper,[2] we reported the Pummerer reaction of the thienamycin model compound (**3**) for the preparation of the *S*-α-methoxythienamycin derivative (**2**). In the model studies, the preparation of **2** by use of the Pummerer reaction was found to be difficult, because the additive-type Pummerer reaction proceeded predominantly, instead of the normal reaction. Here we wish to describe a facile method for the synthesis of **2**.

1 (thienamycin): R=H
2: R=OMe

3: R=H
4: R=OMe

Chart 1

Recently, a group at Sanraku-Ocean[3] reported a replacement reaction of the C-2 side chain of carbapenem antibiotics. In the case of the preparation of **2**, however, this method requires the synthesis of a monothiohemiacetal[4] such as **16** as a nucleophile, but the isolation of this substance is likely to be difficult owing to its instability. Thus, we searched for a convenient method to prepare the desired compound (**2**) containing a monothioacetal moiety.

Jung and co-workers[5] reported that the thiol group of cysteamine derivatives can be protected as Michael adducts by the use of β-nitrostyrene, and the original thiols can be regenerated under mild alkaline conditions. From their study, it seemed possible that the Michael adducts might be used for the replacement reaction instead of the thiols.

First, a model study was carried out as shown in Chart 2. We investigated the replacement reaction of the sulfone (**5**)[6] with the masked thiol (**6**) in order to ascertain whether the masked compound (**6**) could be used as a nucleophile precursor instead of a thiol. When the sulfone (**5**) was treated with the masked thiol (**6**)[7] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[8] as a base, the desired compound (**7**) was isolated in 63% yield after purification by preparative thin-layer chromatography (preparative TLC).

This preliminary study showed that benzyl mercaptan, which was split off *via* a retro-Michael reaction of the masked thiol (**6**) in the reaction system, reacted with **5** as a nucleophile. The success of the model reaction prompted us to prepare a masked thiol (**12**) having a monothioacetal moiety. The methoxy group of **12** was thought to have been introduced by the Pummerer reaction of **10**. The dimethyl group of the starting material (**10**) was introduced for the regiospecific Pummerer rearrangement.

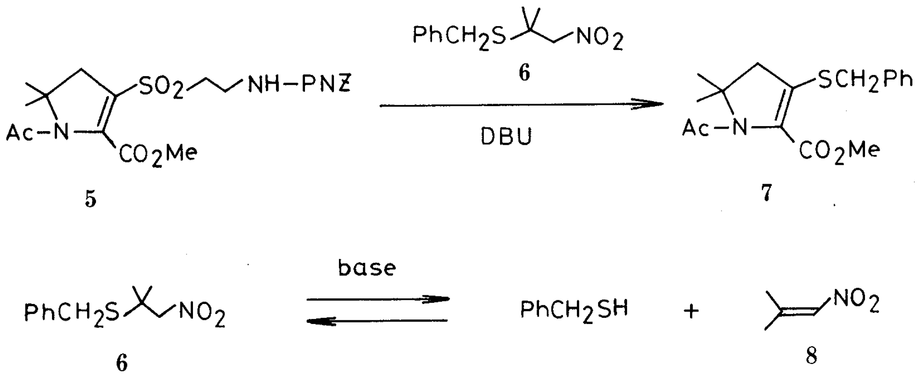

Chart 2

A solution of **9**[9] in $CHCl_3$ was treated with 2-methyl-1-nitropropene (**8**)[10] in the presence of a catalytic amount of triethylamine to give the Michael adduct (**10**) in 90% yield. Then the Pummerer reaction[11] for the introduction of a methoxy group was studied. Reaction of **10** with 1 eq of *N*-chlorosuccinimide (NCS) in deuteriochloroform was monitored by nuclear magnetic resonance (NMR) spectroscopy. Within 10 min, the disappearance of a singlet at δ 2.94 due to NCS and a triplet at δ 2.74 assigned to the S–CH_2 protons was observed. The formation of an unstable *S*-α-chloro derivative (**11**) was confirmed by the appearance of a triplet at δ 5.30 assigned to the methine proton of S–CH–Cl. Treatment of the sulfide (**10**) with NCS in CH_2Cl_2 followed by a replacement reaction using MeOH in the presence of Ag_2O afforded the desired masked thiol (**12**) in 54% yield. The structure of **12** was established by the NMR spectrum, infrared (IR) spectrum, mass spectrum (MS), and elemental analysis. The NMR spectrum of **12** showed the presence of a singlet at δ 3.41 assigned to the methoxy group and a triplet at δ 4.80 due to the methine proton of S–CH–OMe. Upon treatment of **5** with 1.2 eq of the masked thiol (**12**) and 1.2 eq of DBU in *N*,*N*-dimethylformamide (DMF) for 2 h at room temperature, the desired compound (**4**) was obtained in 44% yield after silica gel column chromatography with a 42% recovery of the starting material (**5**).

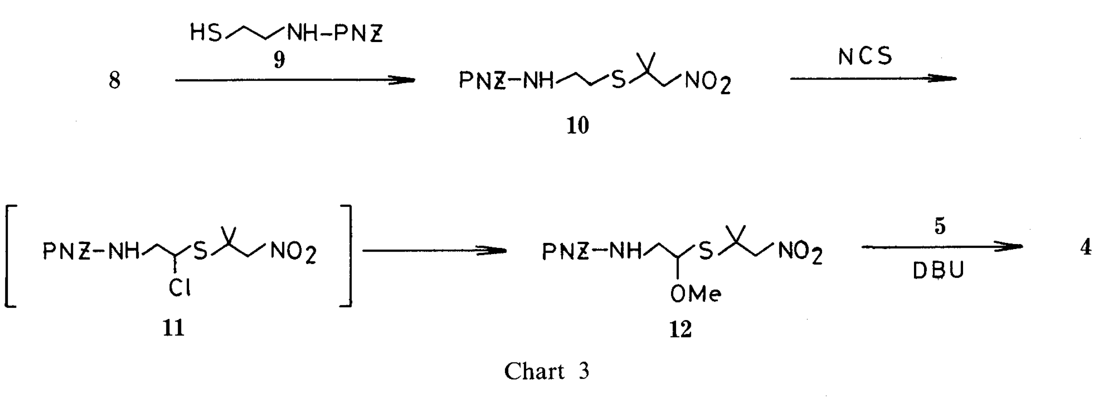

Chart 3

On the basis of the model reaction, the application of this methodology for the synthesis of **2** was then undertaken. The replacement reaction of protected thienamycin sulfoxide (**13**) with 1.2 eq of the masked thiol (**12**) in the presence of 1.2 eq of DBU in DMF at $-50\,^{\circ}C$ for 15 min provided the desired compound (**14**) in 28% yield along with the disulfide (**15**) after purification by high-performance liquid chromatography (HPLC). The by-product (**15**) was presumed to be formed by condensation[12] of the thiol (**16**) liberated from masked thiol (**12**) with the split sulfenic acid (**17**). The poor yield of the target compound (**14**) was attributable to the consumption of the thiol (**16**). When 2 eq of masked thiol (**12**) was used in this reaction, the yield of **14** was improved to 47%. Finally, catalytic hydrogenation of **14** in the presence of PtO_2, according to the method of Schmitt *et al.*,[13] afforded the target compound (**2**) in 36% yield after purification by column chromatography on XAD-2.

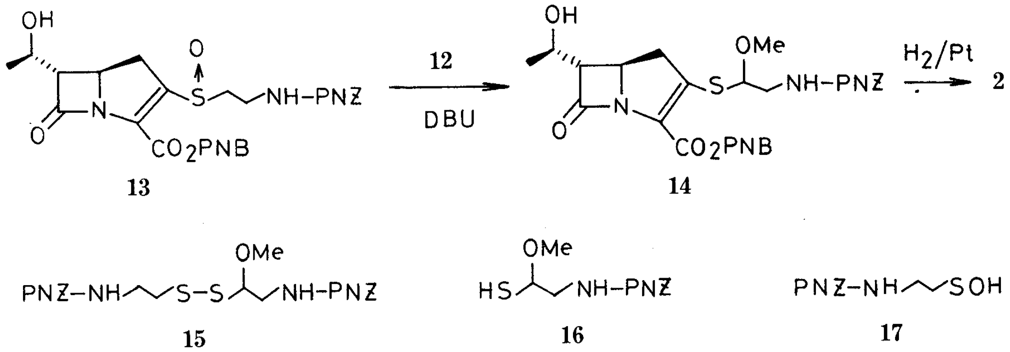

Chart 4

In summary, a new type of thienamycin derivative (**2**) having a monothioacetal side chain at the C-2 position was prepared by the replacement reaction of protected thienamycin sulfoxide (**13**) with the masked thiol (**12**). 2-Methyl-1-nitropropene (**8**) has been shown to be a very useful protective group for thiol compounds whose isolation or preparation is troublesome owing to their instability. This new method should be widely applicable for modifying the C-2 side chain of carbapenem antibiotics. The antimicrobial activity and the susceptibility to dehydropeptidase-1 of **2** and related compounds will be discussed in a forthcoming publication.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus, and are uncorrected. IR spectra were recorded on a Hitachi 260-30 infrared spectrophotometer. ^{1}H-NMR spectra were obtained on a Hitachi R-40 (90 MHz) or a Varian XL-200 (200 MHz) spectrometer. MS were recorded on a JEOL JMS-01SG-2 mass spectrometer. Preparative TLC was performed by using Merck Silica gel 60 F_{254} plates. HPLC purification was performed on a Waters ALC/GPC Model 201.

2-Benzylthio-2-methylnitropropane (6)——A mixture of nitromethane (2.28 g, 37.5 mmol), benzyl mercaptan (3.72 g, 30 mmol), acetone (2.30 g, 40 mmol), and piperidine (0.26 g, 3 mmol) was heated under reflux for 15 h. After cooling, the reaction mixture was chromatographed on silica gel (100 g) with benzene–hexane (1 : 1) to give **6** (4.12 g, 49%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (6H, s, $2\times CH_3$), 3.80 (2H, s, SCH_2Ar), 4.41 (2H, s, CH_2NO_2), 7.30 (5H, s, aromatic-H).

1-Acetyl-3-benzylthio-5,5-dimethyl-2-methoxycarbonyl-2-pyrroline (7)——DBU (18 mg, 0.12 mmol) was added to a stirred solution of **5** (48 mg, 0.1 mmol) and **6** (26 mg, 0.12 mmol) in acetonitrile (2 ml) and dimethyl sulfoxide (0.5 ml). After being stirred for 1.5 h at room temperature, the reaction mixture was diluted with AcOEt, washed with 10% citric acid and water, dried over $MgSO_4$ and concentrated. The residue was fractionated by preparative TLC with benzene–AcOEt (1 : 1) to give **7** (20 mg, 63%) as a colorless oil, which crystallized on standing in a refrigerator, mp 80—85 °C. IR (KBr): 1705, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, s, $2\times CH_3$), 1.98 (3H, s, CH_3CO–), 2.57 (2H, s, C_4–H_2), 3.80 (3H, s, CO_2CH_3), 3.98 (2H, s, SCH_2Ar), 7.27 (5H, s, aromatic-H). *Anal.* Calcd for $C_{17}H_{21}NO_3S$: C, 63.92; H, 6.63; N, 4.39. Found: C, 63.69; H, 6.54; N, 4.44.

2-Methyl-1-nitro-2-[2-(*p*-nitrobenzyloxycarbonylamino)ethylthio]propane (10)——A solution of **8** (0.58 g, 5.7 mmol), 2-(*p*-nitrobenzyloxycarbonylamino)ethanethiol (**9**) (1.20 g, 4.7 mmol), and triethylamine (0.05 g, 0.5 mmol) in $CHCl_3$ (10 ml) was stirred for 24 h at room temperature under argon. The mixture was diluted with $CHCl_3$, washed with 10% citric acid and water, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (25 g) using benzene–AcOEt (10 : 1) to give **10** (1.51 g, 90%) as a colorless oil, which crystallized on standing at room temperature, mp 46—47 °C. IR (KBr): 3455, 1725, 1605 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.46 (6H, s, $2\times CH_3$), 2.74 (2H, t, $J=6$ Hz, SCH_2), 3.38 (2H, q, $J=6$ Hz, NCH_2), 4.48 (2H, s, CH_2NO_2), 5.17 (2H, s, NCO_2CH_2Ar), 5.59 (1H, br s, NH), 7.47 (2H, d, $J=9$ Hz, aromatic-H), 8.15 (2H, d, $J=9$ Hz, aromatic-H). *Anal.* Calcd for $C_{14}H_{19}N_3O_6S$: C, 47.05; H, 5.36; N, 11.76. Found: C, 46.80; H, 5.32; N, 11.42.

1-Nitro-2-methyl-2-[1-methoxy-2-(*p*-nitrobenzyloxycarbonylamino)ethylthio]propane (12)——NCS (75 mg, 0.55 mmol) was added to a stirred solution of **10** (179 mg, 0.5 mmol) in CH_2Cl_2 (3 ml) under ice-cooling. The mixture was stirred for 0.5 h at the same temperature, then MeOH (0.2 ml) and Ag_2O (115 mg, 0.5 mmol) were added and the whole was allowed to warm to room temperature. Stirring was continued for 2 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel (8 g) with benzene–AcOEt (5 : 1) to give **12** (104 mg, 54%) as a colorless powder, mp 83—84 °C. IR (KBr): 3345, 1720 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.53 (6H, s, $2\times CH_3$), 3.41 (3H, s, OCH_3), 3.52 (2H, t, $J=6$ Hz, NCH_2), 4.57 (2H, s, CH_2NO_2), 4.80 (1H, t, $J=6$ Hz, S–CH–OMe), 5.23 (2H, s, NCO_2CH_2Ar), 5.40 (1H, br s, NH), 7.53 (2H, d, $J=9$ Hz, aromatic-H), 8.21 (2H, d, $J=9$ Hz, aromatic-H). *Anal.* Calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.45. Found: C, 46.51; H, 5.36; N, 10.78.

1-Acetyl-5,5-dimethyl-2-methoxycarbonyl-3-[1-methoxy-2-(*p*-nitrobenzyloxycarbonylamino)ethylthio]-2-pyrroline (4)——DBU (18 mg, 0.1 mmol) was added to a stirred solution of **5** (48 mg, 0.1 mmol) and **12** (46 mg, 0.12 mmol) in DMF (1 ml). After being stirred for 2 h at room temperature, the reaction mixture was diluted with AcOEt, washed with 5% $NaHCO_3$, water, 0.5 N HCl, and water, then dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (4 g) using benzene–AcOEt (2 : 1) to give **4** (21 mg, 44%) and the starting material (**5**) (20 mg, 42%). The NMR spectrum of **4** was identical with that of an authentic sample.

***p*-Nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(1-methoxy-2-*p*-nitrobenzyloxycarbonylaminoethylthio)-carbapen-2-em-3-carboxylate (14)**——DBU (30 mg, 0.2 mmol) was added to a solution of **13** (60 mg, 0.1 mmol) and **12** (77 mg, 0.2 mmol) in DMF (2 ml) at −50 °C under argon. After being stirred for 15 min at the same temperature, the reaction mixture was diluted with AcOEt, washed with 0.5 N HCl and water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by HPLC [Nucleosil $5C_{18}$ column 20 mm × 30 cm, acetonitrile–water (2 : 1)] to give **14** (29 mg, 47%) and **15** (20 mg, 37%).

14: Colorless powder, mp 74—77 °C (dec.). IR (KBr): 1775, 1720 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.37 (3H, d, $J=6$ Hz, CH_3), 3.0—3.3 (2H, m, C_1-H_2), 3.3—3.7 (3H, m, C_6-H, NCH_2), 3.49 (3H, s, OCH_3), 4.2—4.4 (2H, m, C_5-H, C_8-H), 4.87 (1H, dd, $J=6$, 8 Hz, S–CH–OCH_3), 5.25 (2H, s, NCO_2CH_2Ar), 5.28 and 5.56 (2H, ABq, $J=14$ Hz, CO_2CH_2Ar), 7.55 (2H, d, $J=9$ Hz, aromatic-H), 7.70 (2H, d, $J=9$ Hz, aromatic-H), 8.26 (4H, d, $J=9$ Hz, aromatic-H). FD-MS *m/e*: 616 (M^+). *Anal.* Calcd for $C_{27}H_{28}N_4O_{11}S\cdot 1/2H_2O$: C, 51.83; H, 4.67; N, 8.96. Found: C, 52.13; H, 4.55; N, 9.06.

15: Oil. IR ($CHCl_3$): 3460, 1725 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 2.35—2.95 (2H, m, SCH_2), 3.47 (3H, s, OCH_3), 3.50—3.75 (4H, m, $2\times NCH_2$), 4.46 (1H, t, $J=6$ Hz, S–CH–OCH_3), 5.23 (4H, s, $2\times NCO_2CH_2Ar$), 7.54 (4H, d, $J=9$ Hz, aromatic-H), 8.24 (4H, d, $J=9$ Hz, aromatic-H). FD-MS *m/e*: 541 (M^++1), 287, 253. *Anal.* Calcd for $C_{21}H_{24}N_4O_9S_2\cdot 1/2H_2O$: C, 45.89; H, 4.59; N, 10.20. Found: C, 46.15; H, 4.38; N, 10.00.

(5*R*,6*S*)-2-(2-Amino-1-methoxyethylthio)-6-[(*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylic Acid (2)——A mixture of **14** (80 mg, 0.13 mmol) and PtO_2 (106 mg) in tetrahydrofuran (THF) (13 ml), EtOH (5.3 ml), deionized water (5.3 ml), dioxane (1.3 ml), and 0.1 M MOPS buffer (pH 7.1, 5.3 ml) was subjected to catalytic hydrogenation under 4 atm. for 1 h at room temperature. The catalyst was filtered off and washed with water. The filtrate and washings were combined and washed with Et_2O. The separated aqueous layer was lyophilized to give a yellowish powder, which was applied to an XAD-2 column (18 mm × 21 cm) which was eluted successively with water and water–THF (95 : 5). The fractions having ultraviolet (UV) absorption at 296 nm were combined and lyophilized to give **2** (14 mg, 36%) as a colorless powder. IR (KBr): 1760, 1620, 1590 cm^{-1}. ^1H-NMR (D_2O) δ: 1.27 (3H, d, $J=6$ Hz, CH_3), 3.51 and 3.53 (3H, each s, OCH_3), 4.16—4.30 (2H, m, C_5-H, C_8-H), 4.80 (HOD). UV $\lambda_{\max}^{H_2O}$ nm: 296.

Acknowledgment We wish to express our gratitude to Dr. B. G. Christensen for his helpful suggestions and discussions relating to this work.

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1) This work was presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985.

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