Synthetic Studies of Pyridomycin. II.[1] Synthesis of a Model Twelve-membered Ring Compound Related to Pyridomycin

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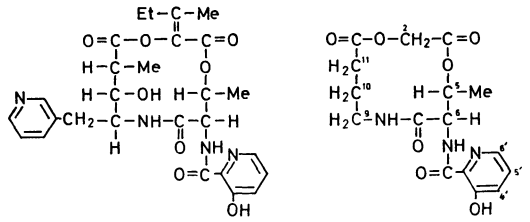
A prototypic compound of pyridomycin, 6-(3-hydroxypicolinoylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (**11**), was synthesized. 4-(Benzyloxycarbonylamino)butyric acid was coupled with *t*-butyl glycolate in the presence of DCCI and pyridine to afford *t*-butyl [4-(benzyloxycarbonylamino)butyryloxy] acetate (**4**). De-*t*-butylation of **4** followed by condensation with *N*-*t*-butoxycarbonyl-L-threonine benzyl ester by the DCCI method gave *O*-[{4-(benzyloxycarbonylamino)butyryloxy}acetyl]-*N*-*t*-butoxy-carbonyl-L-threonine benzyl ester (**6**). Deprotection of **6** followed by cyclization with Woodward's reagent K afforded 6-(*t*-butoxycarbonylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (21% yield), which was transformed into the title compound **11**.

Pyridomycin is an antimycobacterial antibiotic[2] produced by *Streptomyces pyridomyceticus*.[3] The structure of pyridomycin (**1**) was determined by means of X-ray analysis[4] and chemical degradation.[5] The most striking characteristic of the structure is its novel heterocyclododecane system containing unique exocyclic *s*-butylidene and 3-pyridylmethyl side chains.



1 **11**

Chart 1.

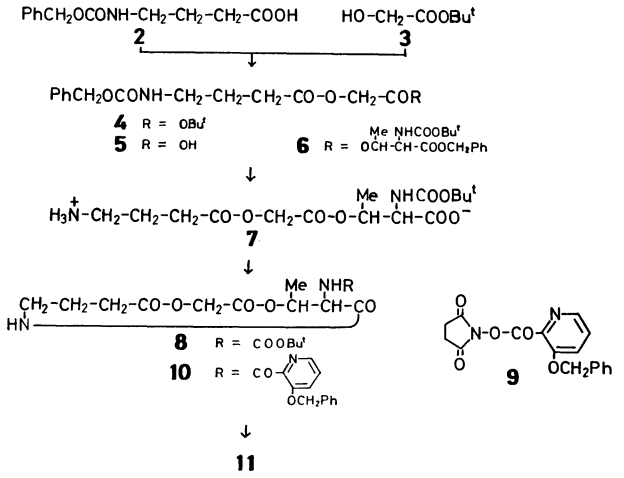


Chart 2.

During the course of studies on the total synthesis of pyridomycin, the natural (—)-4-amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic acid, which is a building block containing the 3-pyridylmethyl side chain of the pyridomycin ring system, was synthesized.[1] So far no attempt seems to have been made for the preparation of this new type of medium ring system present in pyridomycin. We therefore tried to synthesize a prototype in which, except for the moiety of L-threonine, the structure was made simpler than that of the natural antibiotic. This paper deals with the synthesis of 6-(3-hydroxypicolinoylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (**11**) whose cyclic structure is composed of *N*-(3-hydroxypicolinoyl)-L-threonine, 4-aminobutyric acid, and glycolic acid (Chart 1). In the synthesis of **11**, we adopted the method of cyclization by amide bond formation. The pathway is most commonly used for the synthesis of peptolides and peptide lactones. The synthetic scheme is shown in Chart 2.

4-(Benzyloxycarbonylamino)butyric acid (**2**) was prepared by the Schotten-Baumann reaction of 4-aminobutyric acid with benzyl chloroformate. *t*-Butyl glycolate (**3**)[6] was prepared by selective saponification of *t*-butyl acetoxyacetate[6] obtained by *t*-butylation of acetoxyacetic acid[7] with 2-methylpropene in the usual way. Condensation of **2** with **3** was carried out in the presence of dicyclohexylcarbodiimide (DCCI) and pyridine in THF to afford *t*-butyl [4-(benzyloxycarbonylamino)butyryloxy]acetate (**4**) in 64.5% yield after being subjected to silica gel chromatography. Treatment of **4** with trifluoroacetic acid gave [4-(benzyloxycarbonylamino)butyryloxy]acetic acid (**5**) in 96.7% yield. *N*-*t*-Butoxycarbonyl-L-threonine benzyl ester[8] was acylated with **5** in THF using DCCI and pyridine to afford the protected open-chain intermediate (**6**) in 90.4% yield after silica gel chromatography. Deprotection of **6** by hydrogenolysis over palladium black gave the linear system **7** with terminal amino and carboxy group in 99.5% yield. Cyclization of **7** was performed with *N*-ethyl-5-phenylisoxazolium-3'-sulfonate (Woodward's reagent K)[9] according to the procedure of Bláha and Rudinger,[10] and of Gisin and Merrifield[11] to afford the intramolecular cyclization product, 6-(*t*-butoxycarbonylamino)-5-methyl-8-aza-1,4-dioxacyclodecane-3,7-12-trione (**8**) as colorless needles in 21.1% yield. The structure of **8** was confirmed by PMR analysis and molecular weight determination by mass spectrum. The cyclization of **7** which was carried out with large excess of DCCI by the method of Vogler *et al.*,[12] or with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in dichloromethane at 10^{-2}—10^{-3} M concentration of **7** by the method of Sheehan and Ledis,[13] yielding the cyclized product **8** in lower yields (4—8.3%). In all the cyclization reactions abovementioned, no appreciable amount of intermolecular

cyclization products could be isolated.

De-*t*-butoxycarbonylation of **8** followed by *N*-acylation with *N*-(3-benzyloxypicolinoyloxy)succinimide (**9**) gave the 3-benzyloxypicolinamide derivative **10** in 85.2% yield. Removal of the benzyl group of **10** by hydrogenolysis over palladium black afforded the pyridomycin analog **11** as colorless plates in 80.7% yield. The structure was confirmed by elemental and PMR analyses.

Experimental

Melting points were determined on a micro hot-stage and are uncorrected. IR spectra were taken on a Hitachi 225 spectrophotometer and PMR spectra on a Varian A-60D spectrometer using TMS as an internal standard. Specific rotations were determined with a Zeiss Photoelectric Polarimeter. TLC and column chromatography were performed on Wakogel B-5 and C-200, respectively. Concentration was carried out below 40 °C under reduced pressure.

*4-(Benzyloxycarbonylamino)butyric Acid (**2**).* *N*-Benzyloxycarbonylation of 4-aminobutyric acid was carried out with benzyl chloroformate and 2M NaOH solution under the Schotten-Baumann conditions to afford **2** as colorless needles in 64.9% yield: Mp 63—64 °C (ether-petroleum ether).

Found: C, 60.46; H, 6.25; N, 5.89%. Calcd for $C_{12}H_{15}NO_4$: C, 60.75%; H, 6.37; N, 5.90%.

t-*Butyl Glycolate (**3**).*[6] Liquid 2-methylpropene (86 ml) was added to a solution of acetoxyacetic acid[7] (16.7 g) in dichloromethane (141 ml) containing concd H_2SO_4 (1.41 ml) at −30 °C under cooling. The stoppered flask was allowed to stand at room temperature for 3 days. The usual work-up followed by distillation afforded a fraction of *t*-butyl acetoxyacetate boiling at 59—62 °C/5 Torr; yield 19.0 g (77.2%), n_D^{19} 1.4131 (lit,[6] bp 79—81 °C/12 Torr n_D^{20} 1.4142); TLC [silica gel, petroleum ether–diisopropyl ether (3 : 1)] of the sample showed a single spot detected by H_2SO_4. A solution of the ester (12.106 g, 69.52 mmol) in acetone (35 ml) was cooled to 0 °C and 2M NaOH solution (38.2 ml) was added slowly under stirring. After the addition of NaOH, stirring was continued at 0 °C for 1 h until the reaction mixture showed a constant pH value (*ca.* 9). The mixture was then neutralized with 5% citric acid and concentrated in order to remove acetone. The residue was extracted four times with ether, the combined ethereal extracts were washed with saturated $NaHCO_3$ and saturated NaCl solutions, dried, and immediately evaporated to give a colorless syrup of **3** (3.908 g, 42.6%). The fresh sample of **3** was homogeneous on TLC with petroleum ether–diisopropyl ether (3 : 1). However, when a fresh sample was stored in a desiccator overnight, it showed on TLC a new spot with lower R_f-value in addition to the original spot of the fresh sample. Although the sample of **3** could be distilled at 40—57 °C/7—14 Torr [lit,[6] bp 56 °C/12 Torr], all of the distillates were not homogeneous on TLC.

t-*Butyl [4-(Benzyloxycarbonylamino)butyryloxy]acetate (**4**).* A solution of **2** (7.017 g, 29.6 mmol) in dry THF (15.5 ml) was added slowly dropwise to a stirred solution of the freshly prepared sample of **3** (3.908 g, 29.6 mmol), DCCI (6.782 g, 32.9 mmol) and pyridine(2.45 ml, 31.2 mmol) in dry THF (11.6 ml), cooled at 0 °C. Stirring was continued at 0 °C for 1 h and then at room temperature for 3 days. The precipitate of *N,N'*-dicyclohexylurea (DCU) was filtered off and the filtrate was treated with acetic acid (*ca.* 0.25 ml) for 1 h. The resulting DCU was removed and the filtrate was diluted with ethyl acetate and washed with saturated $NaHCO_3$ and saturated NaCl solutions. The dried solution was evaporated to afford yellow syrup (8.533 g), which was chromatographed on silica gel (341 g) with benzene–ethyl acetate (2 : 1) to give **4** (6.701 g, 64.5%) as colorless crystals: Mp 63.5—64.5 °C from ethyl acetate); δ($CDCl_3$) 1.48(s, 9H, OBu^t), *ca.* 1.9 (m, 2H, H-10*), *ca.* 2.5(m, 2H, H-11), 3.34(dd, 2H, H-9, $J_{9,10}=J_{9,NH}=6.8$ Hz), 4.57(s, 2H, H-2), 5.20(s, 2H, OCH$_2$-Ph), *ca.* 5.1 (br, 1H, NH), and 7.35(s, 5H, Ph).

Found: C, 61.63; H, 7.20; N, 3.79%. Calcd for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99%.

*[4-(Benzyloxycarbonylamino)butyryloxy]acetic Acid (**5**).* A mixture of **4** (5.200 g, 14.8 mmol) and trifluoroacetic acid (15.9 ml) was kept at room temperature for 40 min and then evaporated. The residual syrup was coevaporated with ether repeatedly and dried over NaOH pellets in a desiccator to afford a crystalline solid, which was recrystallized from ethyl acetate to yield the first crop of **5** (3.418 g, 78.2%), mp 78—79.5 °C. From the mother liquor the second and third crops were obtained, the total yield amounting to 96.7%: δ($CDCl_3$) 1.92(m, 2H, H-10), 2.53(t, 2H, H-11, J=7 Hz), 3.38(m, 2H, H-9), 4.72(s, 2H, H-2), 5.21(s, 2H, OCH$_2$Ph), 5.36(br, 1H, NH), 7.48(s, 5H, Ph), and 8.0(br, 1H, COOH).

O-[{*4-(Benzyloxycarbonylamino)butyryloxy}acetyl*]-N-t-*butoxycarbonyl*-L-*threonine Benzyl Ester (**6**).* A solution of **5** (1.405 g, 4.78 mmol) in THF (3.0 ml) was added slowly to a stirred solution of *N*-*t*-butoxycarbonyl-L-threonine benzyl ester[8] (1.486 g, 4.78 mmol), DCCI (1.183 g, 5.735 mmol), and pyridine (0.387 ml, 4.78 mmol) in THF (7.8 ml) at 0 °C. Stirring was continued at 0 °C for 10 min and then at room temperature for 3 days. Work-up of the reaction mixture in the same way as in the preparation of **4** afforded a yellow syrup of crude product. The product (3.103 g) was chromatographed on silica gel (175 g) with hexane–ethyl acetate(2 : 1) to give **6** (2.532 g, 90.4%). A sample of this syrup (107 mg) was chromatographed again on silica gel (5 g) with the solvent system to afford the analytical sample (84 mg) as a colorless syrup: $[\alpha]_D^{15}$ +17°(*c*, 1.0, $CHCl_3$); δ($CDCl_3$) 1.38(d, 3H, 5-Me, *J*=6.5 Hz), 1.47(s, 9H, *t*-Bu), 1.90(m, 2H, H-10), 2.50 (m, 2H, H-11), 3.30(m, 2H, H-9, $J_{9,10}=J_{9,NH}=6$ Hz), 4.35 and 4.53 (each d, H-2, $J_{gem}=16$ Hz), 4.4—4.67(m, 1H, H-6), 5.15 and 5.20(each s, 4H, OCH$_2$Ph), 5.1—5.3(m, 2H, NH), 5.53 (dq, 1H, H-5, $J_{5,6}=3$ Hz), and 7.40(s, 10H, Ph).

Found: C, 61.51; H, 6.57; N, 4.62%. Calcd for $C_{30}H_{38}N_2O_{10}$: C, 61.42; H, 6.53; N, 4.78%.

O-[*(4-Aminobutyryloxy)acetyl*]-N-t-*butoxycarbonyl*-L-*threonine (**7**).* A solution of **6** (150 mg) in methanol (5.3 ml) was stirred with palladium black under bubbling with hydrogen gas for 30 min. The filtered solution was evaporated and then coevaporated with hexane to afford **7** as a colorless glassy solid showing a single spot (ninhydrin and H_2SO_4 reagents) on TLC (silica gel) with 1-butanol–acetic acid–water (4 : 1 : 1): Yield, 92.1 mg (99.5%); $\nu_{max}^{CHCl_3}$ 3430 (NH), 2800—2500(NH_3^+), 1735 (ester), 1700(amide I), 1610(COO^-), and 1490 cm^{-1}(amide II); δ($CDCl_3$) 1.35(d, 3H, 5-Me, *J*= 6.5 Hz), 1.46(s, 9H, *t*-Bu), 2.10(m, 2H, H-10), 2.67(m, 2H, H-11), 3.17(m, 2H, H-9), 4.30(m, 1H, H-6), 4.68(s, 2H, H-2), and 5.46—5.80(m, 2H, H-5 and NH).

*6-(t-Butoxycarbonylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (**8**).* Compound **7** (368 mg, 1.02 mmol) was activated in DMF (35 ml) with Woodward's reagent K[9] (273 mg (purity 95%), 1.02 mmol) at 0—10 °C for 3.5 h, according to the procedure of Bláha and Rudinger[10] and Gisin and Merrifield.[11] The resulting turbid solution

* Numbering of the protons corresponds to that of compound **11** in Chart 1.

was diluted with DMF (25 ml) and dichloromethane (1.02 l). Triethylamino (0.143 ml, 1.02 mmol) was added to the solution, and the mixture was stirred at room temperature for 60 h. The clear solution was evaporated and the residue was extracted with ethyl acetate. The extract was washed twice with water and the aqueous layers were extracted with ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried, and evaporated to afford a yellow syrup (356 mg). The syrup was chromatographed on silica gel (36 g) with hexane-acetone (2 : 1) to give **8** (74.2 mg, 21.1%) as colorless needles. Recrystallization from ethyl acetate afforded the analytical sample: Mp 216—217 °C(sub.); $[\alpha]_D^{14}$ −5° (*c* 1.0, $CHCl_3$); ν_{max}^{KBr} 1757, 1737 (lactone), 1715(urethane), 1654(amide I), and 1515 cm^{-1} (amide II); $\delta(CDCl_3)$, 1.26(d, 3H, 5-Me, J=6.5 Hz), 1.42 (s, 9H, *t*-Bu), *ca.* 2.0(m, 2H, H-10), *ca.* 2.5(m, 2H, H-9), 2.8—4.0(m, 2H, H-11), 4.20(dd, 1H, H-6, $J_{5,6}$=5 Hz, $J_{6,NH}$ =8 Hz), 4.50 and 4.80(each d, 2H, H-2, J_{gem}=15 Hz), 5.27 (m, 1H, H-5) and *ca.* 6.1(br, NH); molecular ion at *m/e* 344.162(calcd, 344.1583).

Found: C, 52.14; H, 6.98; N, 8.09%. Calcd for $C_{15}H_{24}N_2O_7$: C, 52.32; H, 7.03; N, 8.14%.

*N-(3-Benzyloxypicolinoyloxy)succinimide (**9**).* 3-Benzyloxypicolinic acid hydrochloride sesquihydrate[14] (500 mg, 1.71 mmol) was suspended in a mixture of ethyl acetate (7.3 ml) and triethylamine (0.25 ml) and stirred at room temperature for 1 h. The crystals of triethylamine hydrochloride were filtered off and washed with ethyl acetate. *N*-Hydroxysuccinimide (210 mg, 1.82 mmol) and DCCI (370 mg, 1.82 mmol) were added to the combined filtrates. The mixture was stirred at room temperature for 2 h, and then kept at 0 °C for 0.5 h. The resulting crystalline product (a mixture of **9** and DCU) was collected by filtration and extracted with DMF (10 ml) under stirring at room temperature for 1 h. After removal of undissolved DCU, the DMF solution was evaporated to give a crystalline solid (528 mg). The solid was again dissolved in DMF (6.9 ml) at 50 °C in order to remove DCU and the filtered solution was evaporated. The residual crude crystals of **9** (443 mg, 79.6%) were recrystallized from a mixture of DMF (3.2 ml) and ethyl acetate (68 ml) to afford a practically pure sample of **9** (348 mg, 62.3%), mp 179—183 °C. The sample was again recrystallized from the same solvent system to give an analytical sample, mp 181—183 °C.

Found: C, 62.35; H, 4.51; N, 8.49%. Calcd for $C_{17}H_{14}N_2O_5$: C, 62.57; H, 4.32; N, 8.59%.

*6-(3-Benzyloxypicolinoylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (**10**).* A sample of **9** (20.0 mg, 0.0581 mmol) was dissolved in trifluoroacetic acid (0.1 ml). The solution was kept at room temperature for 5 min and then evaporated to afford a colorless glassy solid (20.8 mg) of the trifluoroacetate of the amino peptide dilactone. 3-Benzyloxypicolinic acid active ester **9** (19.0 mg, 0.0581 mmol) was added to a solution of the trifluoroacetate(20.8 mg) in DMF (0.25 ml). Triethylamine was then added until the solution became neutral (pH 7), and the mixture was allowed to stand at 37 °C for 4 days. The reaction mixture was evaporated and dissolved in ethyl acetate. The solution was washed with water and saturated NaCl solution, dried and evaporated. The residual pale-yellow syrup (40 mg) was chromatographed on silica gel (3 g) with benzene-acetone (1 : 1) to afford a colorless syrup of **10** (22.5 mg, 85.2%); $\delta(CDCl_3)$, 1.28(d, 3H, 5-Me, J=6.8 Hz), *ca.* 2.1(m, 2H, H-10), *ca.* 2.5(m, 2H, H-9), 3.0—4.0 (m, 2H, H-11), 4.56 and 4.82(each d, 2H, H-2, J_{gem}=15 Hz), 4.77(dd, 1H, H-6, $J_{6,NH}$=8 Hz, $J_{5,6}$=5 Hz), 5.27(s, 2H, OCH_2Ph), 5.42(m, 1H, H-5), *ca.* 6.2(m, 1H, NH), 7.39 (s, 5H, Ph), *ca.* 7.5(m, 2H, picolinyl H-4′, 5′), 8.3(m, 1H, picolinyl H-6′), and 8.45(m, 1H, NH).

*6-(3-Hydroxypicolinoylamino)-5-methyl-8-aza-1,4-dioxacyclododecane-3,7,12-trione (**11**).* A solution of **10** (22.4 mg) in methanol(3 ml) was stirred with palladium black for 10 min under bubbling with hydrogen gas. Acetone was added to the reaction mixture, the catalysts were filtered off, and the filtrate was evaporated to give **11** (14.3 mg, 80.7%). TLC (silica gel, benzene-acetone(1 : 1)) of the sample showed a single spot (R_f 0.68) detected by means of $FeCl_3$ and H_2SO_4. The sample was recrystallized from acetone to afford the pure sample: Mp 253—255 °C; $[\alpha]_D^{26}$ +9° (*c* 0.45, $CHCl_3$); δ ($CDCl_3$), 1.36(d, 3H, 5-Me, J=6.8 Hz), *ca.* 2.0(m, 2H, H-10), *ca.* 2.5(m, 2H, H-9), *ca.* 3.8(m, 2H, H-11), 4.57 and 4.96(each d, 2H, J_{gem}=15 Hz), 4.60(dd, 1H, H-6, $J_{5.6}$=5 Hz, $J_{6,NH}$ =8 Hz), 5.42(m, 1H, H-5), *ca.* 6.0(br, 1H, NH), *ca.* 7.4(m, picolinyl H-4′,5′), 8.2(m, 1H, H-6′), and 8.7(m, 1H, NH).

Found: C, 52.82; H, 5.31; N, 11.61%. Calcd for $C_{16}H_{19}N_3O_7$: C, 52.60, H, 5.24; N, 11.50%.

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