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TOTAL SYNTHESIS OF NUCLEOSIDE ANTIBIOTIC, ASCAMYCIN

Makoto Ubukata and Kiyoshi Isono*

Antibiotics Laboratory
RIKEN (The Institute of Physical and Chemical Research)
Wako-shi, Saitama 351-01, Japan

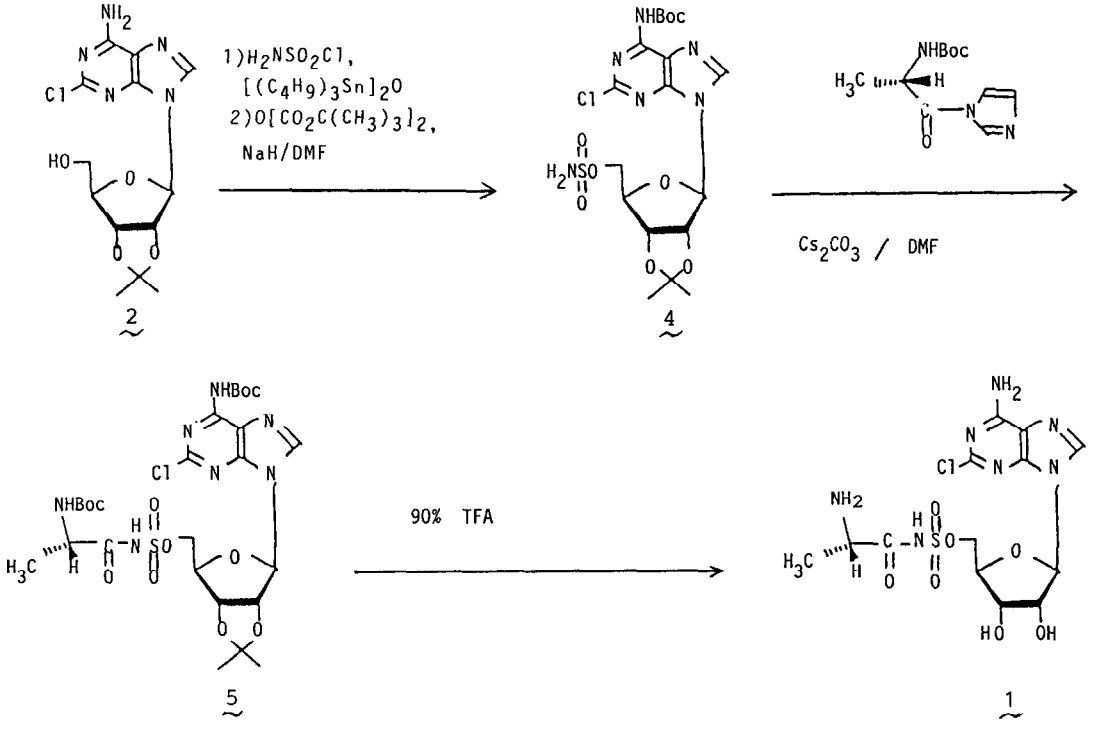
Summary: Synthesis of ascamycin was achieved by the condensation of N^6-t-butyloxycarbonyl-2-chloro-9-(2',3'-O-isopropylidene-5'-O-sulfamoyl-β-D-ribofuranosyl)adenine(**4**) with t-butyloxycarbonyl-L-alanylimidazole in the presence of Cs_2CO_3 in DMF as a key step.

A nucleoside antibiotic, ascamycin (2-chloro-9-[5'-O-(N-L-alanyl)sulfamoyl-β-D-ribofuranosyl]adenine) (**1**) has a selective antibacterial activity against plant pathogenic Xanthomonas species, while dealanylascamycin shows a broad antibacterial activity.[1] We have shown[2] that both ascamycin and dealanylascamycin inhibit strongly protein synthesis in vitro but the antibacterial activity of ascamycin is masked by the alanyl group which interferes with membrane transport in bacteria. Xanthomonas citri is susceptible to ascamycin by virtue of ascamycin hydrolyzing enzyme (Xc-aminopeptidase) which exists on the cell surface.[3] Other bacteria which lack the enzyme are not susceptible to ascamycin. To provide biological probes related to this antibiotic, we attempted the total synthesis of ascamycin, which lead to the preparation of amino acid analogs potentially more selective for both prokaryotic and eukaryotic cells.

2-Chloroadenosine was prepared from 2,6-dichloropurine and β-D-ribofuranose 1,2,3,5-tetraacetate by the method described previously.[4] Treatment of 2',3'-O-isopropylidene acetal (**2**) of 2-chloroadenosine with 8 eq. of sulfamoyl chloride in the presence of 3.25 eq. of bis(tri-n-butyltin)oxide in 1,4-dioxane (2 hrs., reflux) gave 2-chloro-9-(2',3'-O-isopropylidene-5'-O-sulfamoyl-β-D-ribofuranosyl)adenine[5] (**3**) in 82 % yield.

The acid laibile protective groups were chosen because of the instability of ascamycin on prolonged storing in alkaline, acidic and even neutral media at room temperature. Treatment of **3** with 1.1 eq. of di-t-butyl dicarbonate in the presence of 5.5 eq. of NaH (2 hrs., -20°C -> 0°C) gave **4** in 80 % yield. Aminoacylation[6] of **4** with 1.5 eq. of t-butyloxycarbonyl-L-alanylimidazole in the presence of 1 eq. of cesium carbonate gave **5** in 86 % yield. Deprotection of **5** with 90 % TFA (1 hr., 0°C -> r.t.) gave ascamycin in 79 % yield after separation by HPLC(column: Senshu Pak ODS-H, solvent: 30 % MeOH). The synthetic ascamycin was indistinguishable from natural ascamycin in ^1H NMR, SIMS, IR, UV, CD, and antibacterial activity against Xanthomonas citri, and toxicity against Balb 3T3 cell in culture. Optical yield of the alanyl moiety was 88 % e.e..[7]

Synthesis and biological activity of the amino acid analogs of ascamycin will be reported elsewhere.



RFERENCES AND NOTES

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3. H. Osada and K. Isono Biochem. J. **233**, 459 (1985).
4. J. A. Montgomery and K. Hewson J. Heterocycl. Chem. **1**, 213 (1964).
5. This compound was prepared previously by sulfamoyl chloride treatment in the presence of NaH in 40 % yield; G. R. Gough, D. M. Nobbs, J. C. Middleton, F. Penglis-Caredes and M. H. Maguire J. Med. Chem. **21**, 520 (1978).
6. Aminoacylation of **3** with 1.5 eq. of t-butyloxycarbonyl-L-alanylimidazole in the presence of 1 eq. of Cs_2CO_3 (8hrs.,-20°C -> r.t.) gave regioselectively 2-chloro-9-[2',3'-O-isopropylidene-5'-O-(N-t-butyloxycarbonyl-L-alanyl)sulfamoyl-β-D-ribofuranosyl] adenine, which was converted into ascamycin, in 16 % yield. On the other hand, aminoacylation of **3** in the presence of NaH gave regioselectively N^6-(N-t-butyloxy-carbonyl-L-alanyl)-2-chloro-9-(2',3'-O-isopropylidene-5'-O-sulfamoyl-β-D-ribofuranosyl) adenine.
7. Aminoacylation of **4** in the presence of NaH insted of Cs_2CO_3 resulted in considerable racemization of the alanyl moiety (70 % e.e.); M. Ubukata, H. Osada and K. Isono Nucleic Acids Research., Symposium Series, **16**, 81 (1985). Optical yield was determined by the following procedure. i) Hydrolysis of **1** with 0.5 N HCl. ii) HCl/MeOH. iii) 3,5-dinitrobenzoyl chloride. iv) HPLC analysis (column; Sumipax OA-1000, solvent; hexane-dichloroethane-EtOH, 20 : 2 : 3, UV; 254 nm).

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