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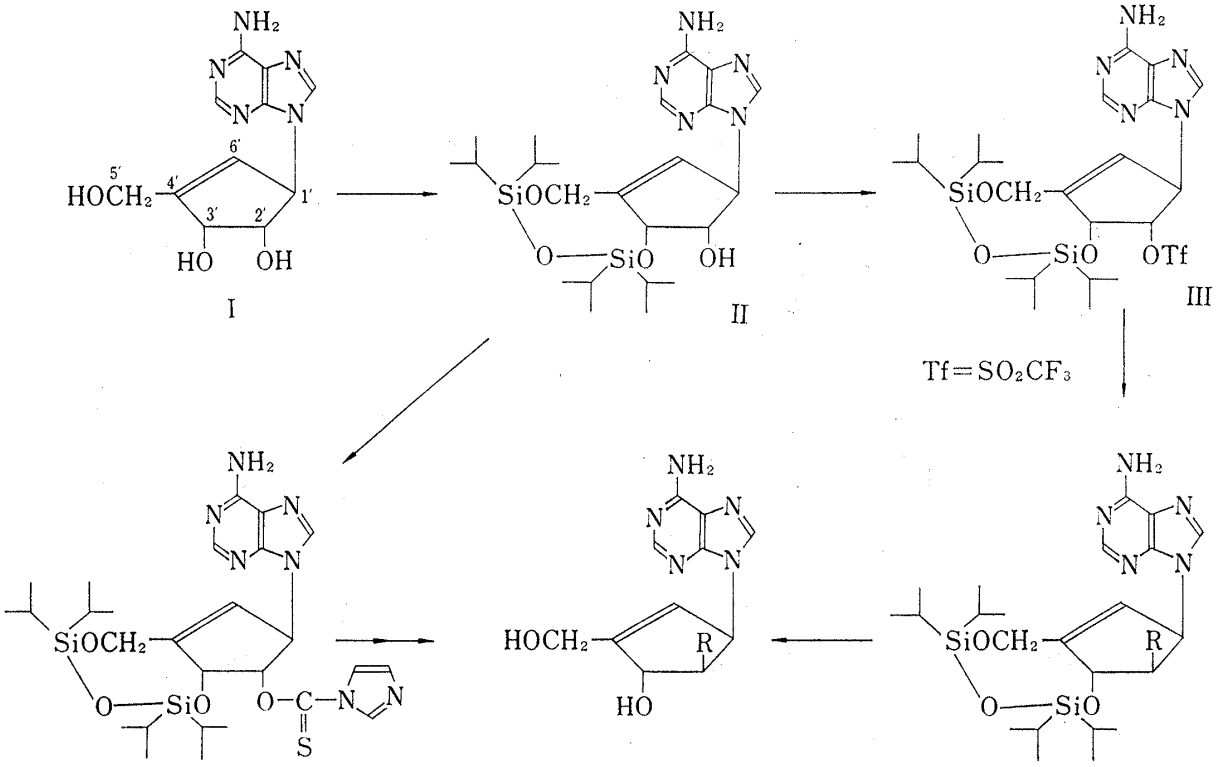
Synthesis of 2′(*R*)-Substituted Neplanocin A's (Nucleosides and Nucleotides. XXXVII[1])

Neplanocin A (I) was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to give 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)neplanocin A (II), which was converted to the 2′-*O*-trifluoromethanesulfonyl derivative (III). Nucleophilic substitution of III with a number of nucleophiles (AcO^-, AcS^-, N_3^-, Cl^-, Br^-, I^-) in hexamethylphosphoric triamide afforded the respective 2′(*R*)-substituted derivatives in high yield. The halogenated derivatives were reduced with tri-*n*-butyltin hydride to the 2′-deoxy compound. 2′-O-Thiocarbonylimidazoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)neplanocin A was also reduced to the 2′-deoxy derivative. The deprotection of the bifunctional silyl group with tetra-*n*-butylammonium fluoride afforded 2′(*R*)-AcO, -AcS, $-N_3$, -Cl, -Br, -I, and 2′-deoxy neplanocin A's, respectively. Physical data of these compounds including nuclear magnetic resonance, mass spectrum, and circular dichroism were given.

Keywords——neplanocin A; nucleoside antibiotic; nucleophilic substitution; triflate; tetra-*n*-butylammonium fluoride; NMR; MS; CD; protecting group

Neplanocin A (I) was isolated as a component of neplanocins from *Actinoplanacea ampullariella sp.*, and showed a marked antitumor activity.[2] The structure of neplanocin A, 1-hydroxymethyl-3(*R*)-(adenin-9-yl)-4(*S*),5(*R*)-dihydroxycyclopent-1-ene, has been confirmed by nuclear magnetic resonance (NMR), mass spectrum (MS), ultraviolet (UV) and X-ray crystallography.[3] There has been considerable interests in the synthesis of 2′-modified nucleosides, stemming primarily from the antitumor or antiviral activities shown by



I II III

XI

XII : R=OAc XVII : R=Cl
XIII: R=SAc XVIII: R=Br
XIV: R=OH XIX : R=I
XV : R=N_3 XX : R=H
XVI: R=NH_2

IV : R=OAc VIII: R=Br
V : R=SAc IX : R=I
VI : R=N_3 X : R=H
VII: R=Cl

Chart 1

TABLE I. Physical Properties of 2′(*R*)-Substituted Neplanocin A's

Compd. No.	mp (°C)	Formula	Analysis(%) Calcd (Found)				Chemical shifts (δ)			MS (*m/e*)	CD$[\theta]_{252}$
			C	H	N	X	H-1′ ($J_{1'2'}$)	H-2′	H-6′		
XII	195—197	$C_{13}H_{15}N_5O_4$	51.14 (51.12	4.95 4.98	22.94 22.74)		5.64 (d) (7Hz)	5.18 (dd)	5.80 (bs)	305(M^+) 245(M^+-AcOH) 136(B+2)	−11900
						X=S					
XIII	165—167	$C_{13}H_{15}N_5O_3S\cdot 1/2H_2O$	47.26 (47.18	4.88 4.94	21.20 21.58	9.70 9.61)	5.61 (d) (8Hz)	4.12 (dd)	5.80 (bs)	320(M^+-1) 246(M^+-SAc) 136(B+2)	−41800
XIV	239—240.5	$C_{11}H_{13}N_5O_3\cdot 1/3H_2O$	49.13 (49.01	5.00 5.01	26.04 25.97)		5.52 (d) (8Hz)	4.14 (dd)	5.72 (bs)	263(M^+) 136(B+2)	−9700
XV	231—233(dec.)	$C_{11}H_{12}N_8O_2$	45.83 (45.86	4.20 4.25	38.87 38.66)		5.64 (d) (8Hz)	4.27 (dd)	5.77 (bs)	288(M^+) 246(M^+-N_3) 136(B+2)	−19900
XVI	—	—	—	—	—	—	5.46 (d) (8Hz)	3.47 (dd)	5.71 (bs)	262(M^+) 136(B+2)	−10000
						X=Cl					
XVII	233—235(dec.)	$C_{11}H_{12}ClN_5O_2$	46.90 (46.88	4.29 4.32	24.86 24.79	12.59 12.51)	5.74 (d) (8Hz)	4.54 (dd)	5.86 (bs)	283,281(M^+) 246(M^+-Cl) 136(B+2)	−11000
						X=Br					
XVIII	224—226(dec.)	$C_{11}H_{12}BrN_5O_2$	40.50 (40.57	3.71 3.63	21.47 21.26	24.50 24.24)	5.70 (d) (8Hz)	4.60 (dd)	5.86 (bs)	327,325(M^+) 246(M^+-Br) 136(B+2)	−13000
						X=I					
XIX	212—215(dec.)	$C_{11}H_{12}IN_5O_2$	35.40 (35.45	3.24 3.28	18.77 19.13	34.01 33.02)	5.59 (d) (8Hz)	4.59 (dd)	5.84 (bs)	373(M^+) 246(M^+-I) 136(B+2)	−19300
XX	231—234	$C_{11}H_{13}N_5O_2$	53.43 (53.43	5.30 5.25	28.33 28.04)		5.64 (m)	2.2—2.4 (m)	5.75 (bs)	247(M^+) 136(B+2)	−6900

arabinofuranosyl-cytosine (ara C) and -adenine (ara A).[4] It is well expected that the 2′-deoxy analog or 2′(*R*)-substituted derivatives of I may exhibit better chemotherapeutic indices than the original compound. For this purpose we have developed a synthetic route of the 2′(*R*)-substituted neplanocin A's.

Compound I was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[5] in dimethylformamide to give a simultaneously protected derivative at the 3′- and 5′-hydroxyls, 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)neplanocin A (II), in 82% yield. In NMR spectrum of II, the 2′-hydroxyl proton appeared as doublet at δ 3.59 (disappears with addition of D_2O) and the 2′-proton appeared as triple doublet at δ 4.32 (collapsed to double doublet on addition of D_2O) which showed the protection had occurred between 5′- and 3′-hydroxyls. The X-ray analysis also confirmed[6] the structure of II. Treatment of II with trifluoromethanesulfonyl chloride and one equivalent of 4-dimethylaminopyridine in pyridine gave the 2′-*O*-triflate (III) in 89% yield. Nucleophilic displacement of III with a number of nucleophiles (NaOAc, KSAc, LiN_3, LiCl, LiBr, and LiI) were successful in hexamethylphosphoric triamide at room temperature for 1—20 hr to give the respective products (IV—IX). Deprotection of the bifunctional silyl group of IV and V with tetra-*n*-butylammonium fluoride in tetrahydrofuran proceeded at room temperature to afford XII and XIII, respectively. Deacetylation of XII gave ara-neplanocin A (XIV), an analog of ara A. De-silylation of VI followed by bubbling of H_2S into the aqueous solution of XV gave ninhydrin-positive 2′(*R*)-amino derivative (XVI), isolated as the amorphous acetate.

The reduction of VIII with tri-*n*-butyltin hydride in the presence of catalytic amount of azo-bis-isobutyronitrile in benzene afforded the 2′-deoxy derivative (X, MS 489 (M^+), mp 149—151°) in 90% yield, In NMR spectrum of X, the 2′-protons appeared at δ 2.3—2.6 as octet, respectively. Compound X was prepared by an alternate route. Treatment of II with N,N′-thiocarbonyldiimidazole in refluxing 1,2-dichloroethane afforded a 2′-*O*-thiocarbonyl-imidazoyl derivative (XI). Reduction of XI with tri-*n*-butyltin hydride gave X in 61% isolated yield. Deprotection of X afforded 2′-deoxyneplanocin A (XX). The deprotection of VII—IX with tetra-*n*-butylammonium fluoride afforded the products (XVII—XIX), respectively, without affecting the *trans*-halohydrin system in these compounds.

The physical properites of the 2′(*R*)-substituted neplanocin A's prepared in this study are summarized in Table I. It should be noted that the bulkiness of the 2′-substituents affects strongly the molecular ellipticities, which may be based on the preference of anti-conformation around the "glycosyl" bond by the bulkier 2′(*R*)-substituents. In NMR spectra, coupling constants of the 1′-protons ($J_{1',2'}$=8 Hz) of these derivatives also reflected the (*R*)-configuration at the 2′-position, as compared with the $J_{1',2'}$ (5 Hz) of I.

Adenosine and other purine nucleosides could also be transformed to the varieties of 2′-modified 2′-deoxyarabinosylpurines by the similar procedure. The method described here has an advantage for the practical preparation of 2′-substituted nucleosides from ribonucleosides in general. The full details of the synthetic studies and results of the biological activities of these derivatives will be reported separately.

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Studies on Peptides C,[1,2] Chemical Synthesis of Crystalline Ribonuclease A

Improved chemical synthesis of bovine pancreatic ribonuclease (RNase) A was achieved by applying a new deprotecting procedure with trifluoromethanesulfonic acid-thioanisole in combination with a modified air oxidation procedure with glutathione for the disulfide formation. After purifications by affinity chromatography followed by ion-exchange chromatography, a protein with the full enzymatic activity was obtained and subsequently crystallized from 95% ethanol according to Kunitz. A totally synthetic enzyme with full RNase A activity was thus obtained in a crystalline form for the first time.

Keywords——total synthesis of RNase A; trifluoromethanesulfonic acid-thioanisole deprotection; glutathione-mediated air oxidation; affinity chromatographic purification; crystals of synthetic RNase A

Recently, in a preliminary communication,[3] followed by a series of six papers,[4] we reported the chemical synthesis of a protein with the full enzymatic activity of bovine pancreatic ribonuclease (RNase) A (Fig. 1). We wish to report that we succeded in crystallizing the fully active synthetic enzyme. Improvement in yield at the final step of the synthesis was achieved by applying a new deprotecting procedure with trifluoromethanesulfonic acid (TFMSA)-thioanisole[5] in combination with a modified oxidation procedure using glutathione for the disulfide formation.[6]

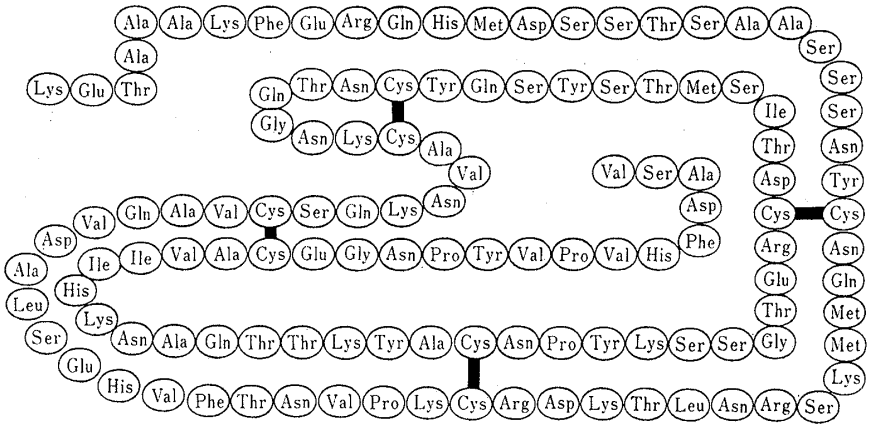

Fig. 1. Structure of Bovine Pancreatic Ribonuclease A

The protected RNase A was treated three times with 1 M TFMSA-thioanisole in TFA (0°, 60 min, each), instead of methanesulfonic acid,[7] in the presence of *m*-cresol to remove all of protecting groups employed (total of 33 groups; benzyloxycarbonyl from Lys, benzyl