CHRYSCANDIN†, A NOVEL PEPTIDYL NUCLEOSIDE ANTIBIOTIC

II. STRUCTURE DETERMINATION AND SYNTHESIS

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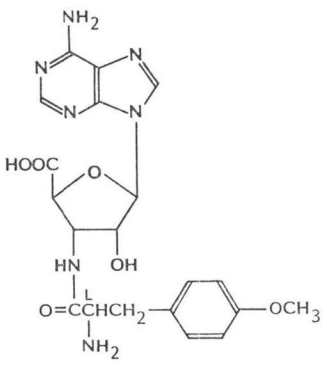
The structure of chryscandin, a novel antifungal antibiotic, produced by *Chrysosporium pannorum* No. 4629 was deduced to be **1** from spectroscopic and chemical evidences. In order to confirm the structure and to determine the absolute configuration, the total synthesis of chryscandin was performed. D-Xylose was transformed into **10** in ten steps. After β-glycosidation of silylated benzoyl adenine with **10**, the resulting **11** was converted into **4**, which was identical with the product obtained from chryscandin by alkaline hydrolysis. From the key intermediate **13**, chryscandin was synthesized *via* peptide formation followed by removal of the protecting groups. Chryscandin (**1**) is the first naturally occurring nucleoside antibiotic possessing a 3-aminoribofuranuronic acid in the molecule.

Chryscandin is a new antifungal antibiotic produced by *Chrysosporium pannorum* No. 4629. Its taxonomy, fermentation, isolation and characterization were described in the preceding paper[1]. In the previous communication[2], we reported the structure and total synthesis of chryscandin. The present paper deals with a full account of that work.

Structure of Chryscandin

Chryscandin (**1**) is a water-soluble, amphoteric substance, mp 215~233°C (dec), $[\alpha]_D^{22}$ +34° (*c* 1.0, 1 N HCl). The molecular formula ($C_{20}H_{23}N_7O_6$) of **1** was established by elemental analysis and FD mass spectrum[1]. The UV, IR and ^{1}H NMR spectra are shown in Figs. 1, 2 and 3, respectively. The presence of the adenine nucleus in the chryscandin molecule was suggested by the following spectroscopic data, *i.e.* the UV absorption at 260 nm (ε 32,500) and the two proton signals at δ 8.18 (s) and 8.46 (s) in the ^{1}H NMR spectrum, together with five carbon signals at δ 150.9 (s), 147.9 (s), 145.7 (d), 141.3 (d) and 118.7 (s) in the ^{13}C NMR spectrum. In addition, the presence of one each of carboxylic acid and amide function was indicated from the IR (KBr) absorptions at 1720 (sh), 1695 and 1665



Chryscandin (**1**)

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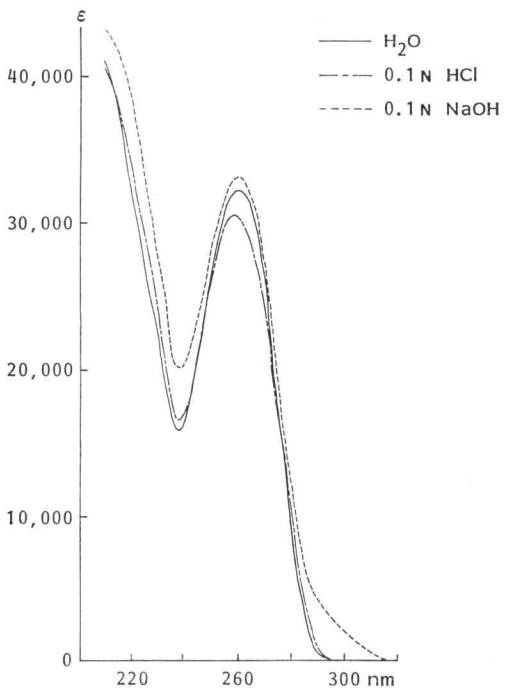
Fig. 1. UV spectra of chryscandin.



cm^{-1}, and two carbon signals at δ 168.3 (s) and 171.2 (s) in the ^{13}C NMR spectrum. In order to cleave the amide bond, acid hydrolysis of **1** with 6 N HCl was conducted at 110°C for 12 hours to yield *O*-methyl-L-tyrosine and adenine. The signals attributed to *O*-methyl-L-tyrosine in the ^{1}H NMR spectrum of chryscandin were observed at δ 2.95 (1H, dd, J=7 and 14 Hz), 3.10 (1H, dd, J=6 and 14 Hz), 3.73 (3H, s), 4.10 (1H, dd, J=7 and 6 Hz), 6.90 (2H, d, J=8.5 Hz) and 7.23 (2H, d, J=8.5 Hz). Further investigation of the ^{1}H NMR spectrum of **1** was done with the aid of a double resonance technique. The signal at δ 6.12 (1H, d, J=2 Hz) probably attributed to an anomeric proton[3] was found to have a coupling to the multiplet signal at δ 4.70, and the multiplet signal at δ 4.78 coupled to both doublets at δ 9.01 (1H, J=8 Hz, NH) and at δ 4.37 (1H, J=7 Hz). These results suggested the presence of a sugar amino acid moiety in **1**.

Acetylation of chryscandin with Ac_2O in pyridine and successive treatment of the solution with MeOH, gave the tetraacetyl methyl ester derivative (**2**) together with the triacetyl methyl ester (**3**) as a minor product. Analysis of the ^{1}H NMR spectrum of **2** using a double resonance technique (Table 2) enabled us to assign all the proton signals as shown in Table 1 and to reveal the structure (**2**). Thus, the coupling constants $J_{1',2'}$=2, $J_{2',3'}$=5 and $J_{3',4'}$=6 Hz are determined. Analogy of these values with those of puromycin[4] assisted assignment of the relative stereochemistry of the amino sugar moiety as shown in the structure of **2**. Based on the chemical structure (**2**), natural chryscandin is supposed

Fig. 2. IR spectrum of dihydrochloride of chryscandin (KBr).

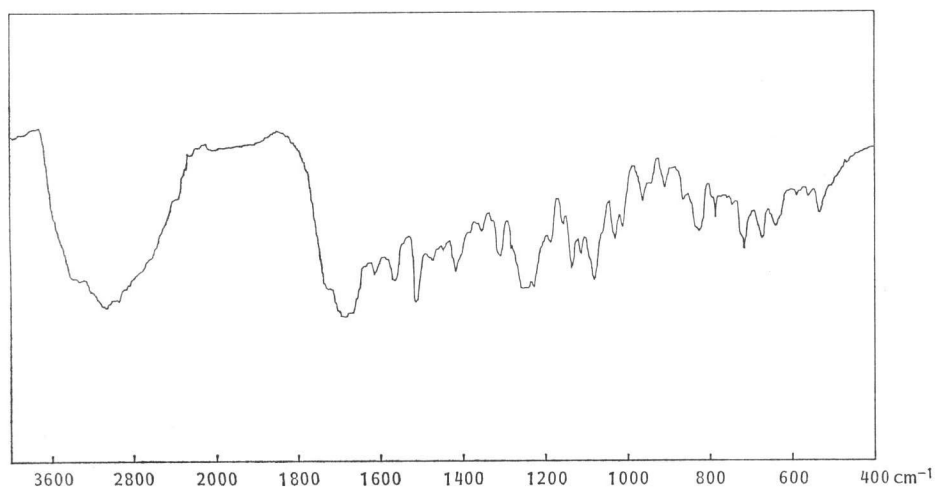


Fig. 3. ^{1}H NMR spectrum of chryscandin (270 MHz, DMSO-d_6).

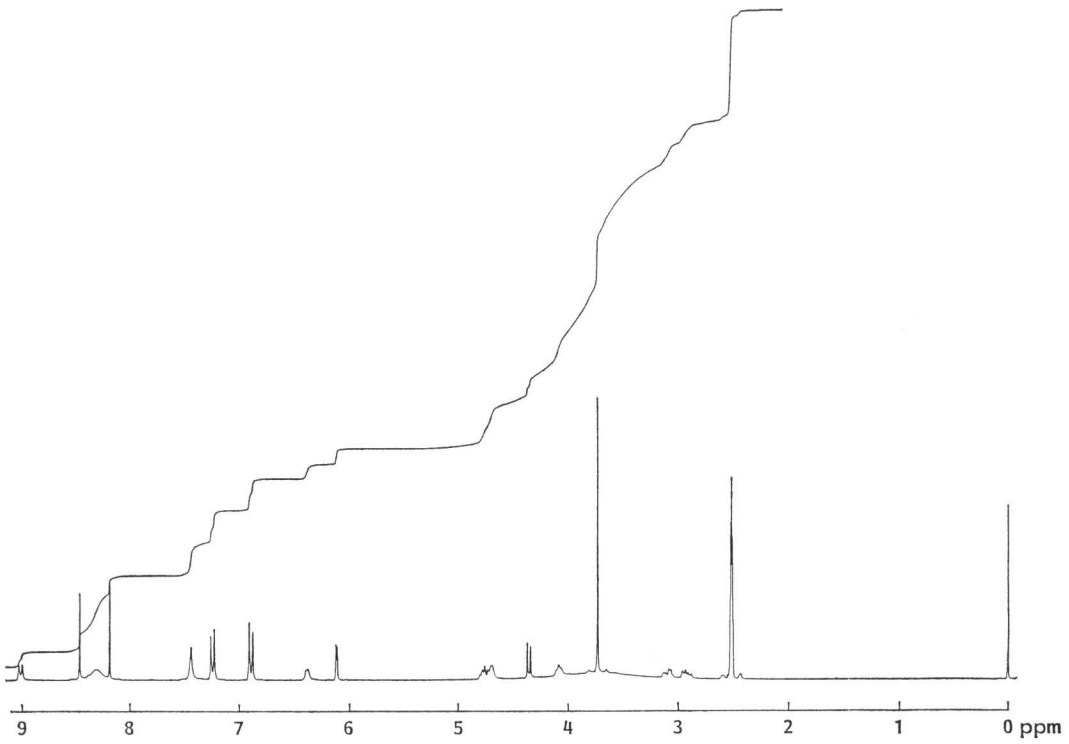
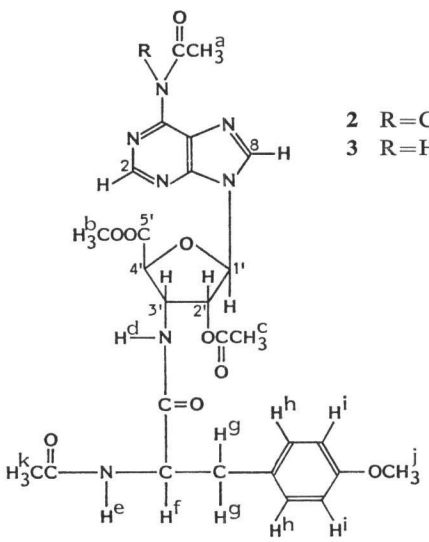


Table 1. ^{1}H NMR spectral data of **2** (100 MHz, $CDCl_3$).



2 R=$COCH_3$[a]
3 R=H

Proton	δ	Multiplicity	J (Hz)
2-H	8.75	s	
8-H	8.92	s	
1′-H	6.30	d	2
2′-H	5.76	dd	2, 5
3′-H	5.09	m	
4′-H	4.41	d	6
H[a](6H)	2.35	s	
H[b](3H)	3.72	s	
H[c](3H)	2.13	s	
H[d]	7.25	d	7.5
H[e]	6.50	d	7.5
H[f]	4.65	dt	7.5, 7.5
H[g](2H)	2.95	d	7.5
H[h](2H)	7.15	d	8
H[i](2H)	6.83	d	8
H[j](3H)	3.76	s	
H[k](3H)	1.96	s	

dt=doublet of triplet.

to have the structure **1**. The carbon signals observed in the ^{13}C NMR spectrum of chryscandin are consistent with the structure **1** (Fig. 4). Further corroboration on the structure of **1** was obtained by a chemical degradation. Thus, alkaline hydrolysis of **1** caused peptidyl bond cleavage to furnish **4** and *O*-methyl-L-tyrosine. Spectroscopic data of **4** are satisfied with the structure shown (see Ex-

Fig. 4. Assignments of ^{13}C NMR spectrum of chryscandin (DMSO-d_6, 67.8 MHz).
*, **, ***: interchangeable assignments.

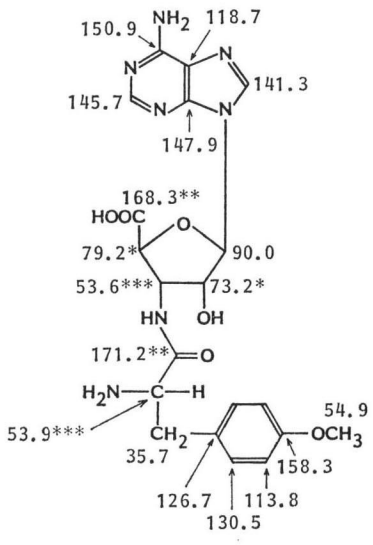


Table 2. Decoupling experiment of ^1H NMR of **2**.

Irradiated H	Observed H	Response
H^g	H^f	dt → d (J=7.5 Hz)
H^e	H^f	dt → t (J=7.5 Hz)
H^f	H^g	d → s
	H^e	d → s
1′-H	2′-H	dd → d (J=5 Hz)
2′-H	1′-H	d → s
	3′-H	m → dd (J=6 and 7.5 Hz)
3′-H	2′-H	dd → d (J=2 Hz)
	4′-H	d → s
4′-H	3′-H	m → dd (J=5 and 7.5 Hz)

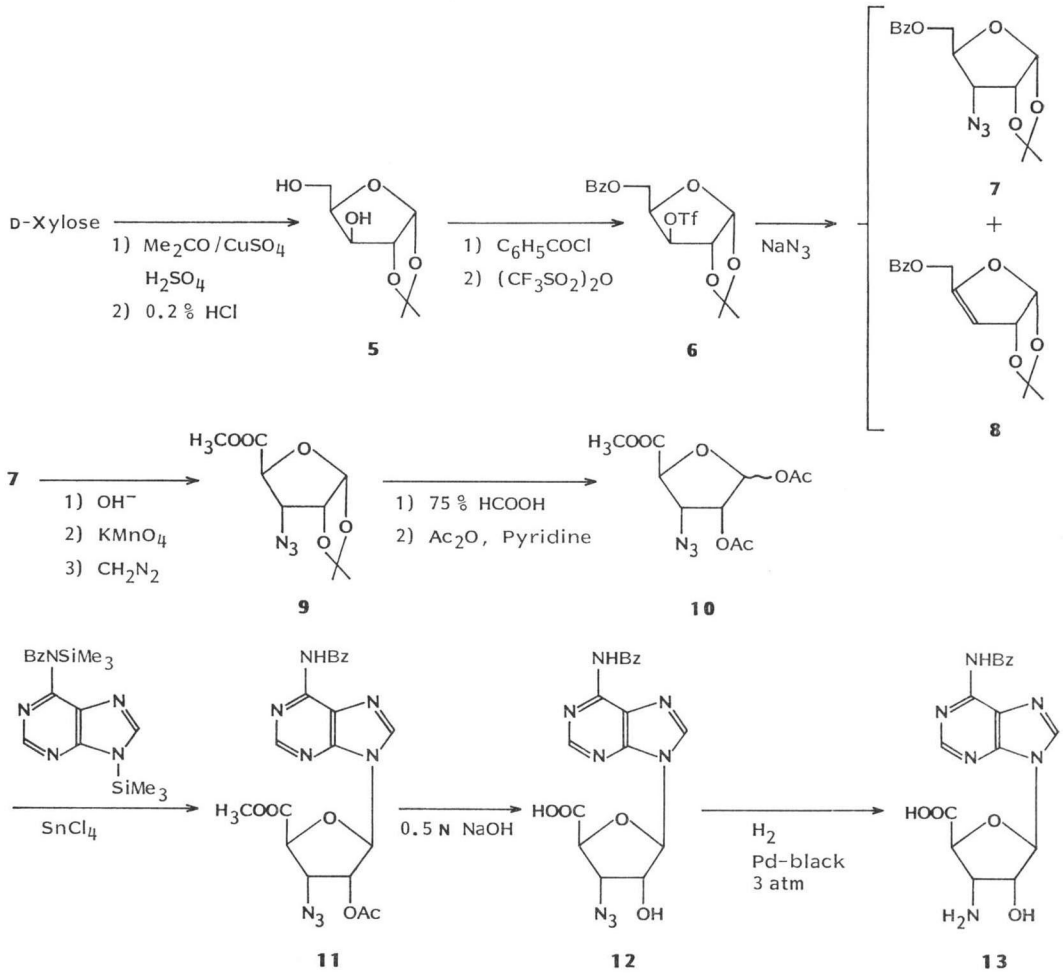
perimental). Therefore, the structure **1** is assigned to chryscandin. Confirmation of the deduced structure and determination of the absolute configurations of chryscandin were achieved by the total synthesis as described in the following section.

Synthesis of Chryscandin

The synthetic course conducted is summarized in the Scheme 1. The 5-hydroxyl group of the diol acetonide **5**[5], prepared from D-xylose by the standard method, was selectively protected by benzoylation with benzoyl chloride (1.3 equiv) in pyridine, and then the 3β-OH group was acylated by triflic anhydride to give **6**. Reaction of **6** with sodium azide in refluxing ethanol for 40 hours afforded two reaction products (96%) almost in the same ratio, one of which was the desired 3α-azide **7** resulting from a SN_2 reaction. The other was the 3,4-eliminated alkene **8**[6] which was separated from **7** by column chromatography. Prior to glycosidation, oxidation at C-5 into the carboxyl function was intended. Thus, after **7** was hydrolyzed with 1 N NaOH - MeOH, the resulting alcohol was oxidized with potassium permanganate to the carboxylic acid, which was followed by treatment with diazomethane to give the methyl ester **9** in satisfactory yield. The 1,2-isopropylidene group of **9** was cleaved with 75% formic acid, and then the diol was acetylated resulting in the desired diacetate **10** in 84% yield. ^1H NMR spectrum of **10** indicated that the β-anomer at C-1 was exclusively formed.

The next stage of the synthesis was the glycosidation of the adenine nucleus with **10**. Stereocontrol to β-orientation was the aim through neighbouring participation of the 2′-α-acetoxy function in **10**. This was performed according to the procedure modified by LICHTENTHALER *et al.*[7], utilizing the fully silylated N^6-benzoyladenine prepared *in situ* and stannic chloride as the FRIEDEL-CRAFTS catalyst. The reaction was highly stereoselective and the isolated product was only the 9-β-nucleoside **11** in 57% yield. Next, the reduction of the azide group to the amino function was effected after the ester groups of **11** were hydrolyzed with 0.5 N NaOH to **12**. Then, catalytic hydrogenation of **12** over palladium-black at 3 atm furnished the N^6-benzoylated adenine aminonucleoside **13**. The yield of **13** from **11** was 73%. Debenzoylation of **13** with *n*-butylamine in refluxing methanol afforded 3-amino-1-(6-amino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic acid, which was all identical with the compound **4** derived from chryscandin by alkaline hydrolysis. From this, the stereochemistry of the deduced structure and the absolute configurations of chryscandin were unequivocally defined.

Scheme 1. Synthetic route to chryscandin.

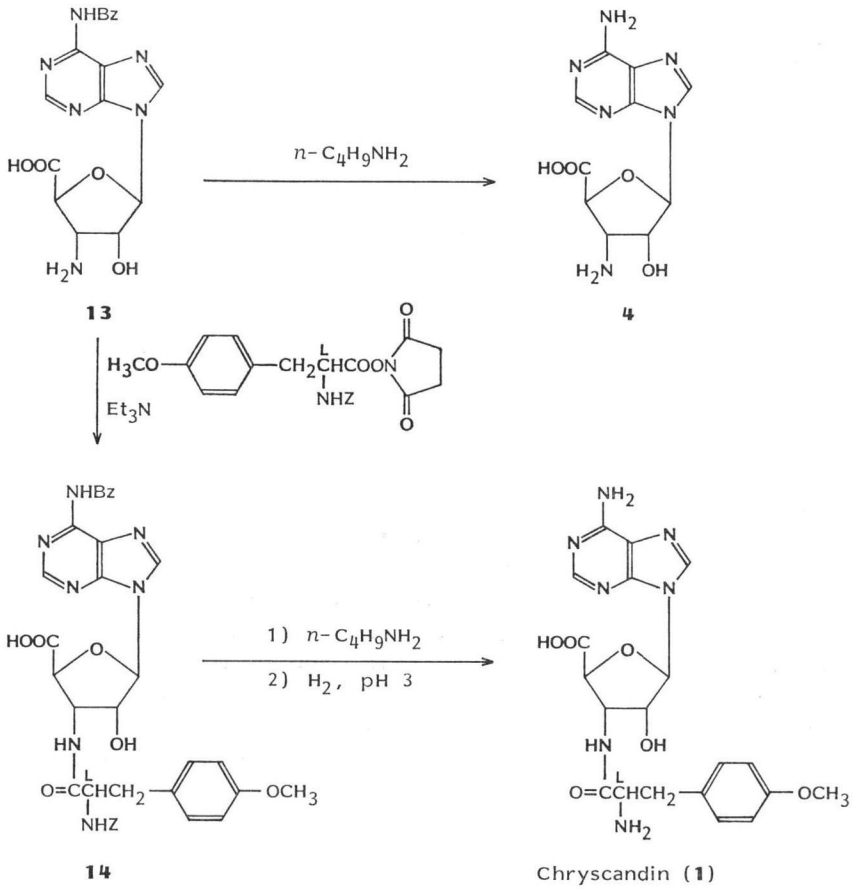


Finally, the synthesis of chryscandin from the key intermediate **13**, was achieved as follows. Condensation of **13** with Z-*O*-methyl-L-tyrosine-ONSu* was effected by the conventional method to yield the peptide **14** in 56% yield. The protecting groups in **14** were cleaved successively by treatment with *n*-butylamine (78%), followed by catalytic hydrogenation over palladium-black at 3~3.5 atm and pH 3. The crude product was purified by column chromatography of Diaion HP-20 to provide the pure compound: 1-(6-amino-9*H*-purin-9-yl)-1,3-dideoxy-3-(*O*-methyl-L-tyrosylamino)-β-D-ribofuranuronic acid (**1**), which was crystallized from 0.1 N HCl as the 2HCl salt (51% yield). This synthetic product was shown to be identical with the natural chryscandin in all respects: synthetic $[\alpha]_D^{20}$ +33° (*c* 1.0, 1 N HCl); natural, $[\alpha]_D^{22}$ +34° (*c* 1.0, 1 N HCl).

In conclusion, the achievement of total synthesis of chryscandin confirmed the above deduced structure and established the absolute configuration of **1** as well. Chryscandin was found to be the first naturally occurring nucleoside possessing a 3-aminoribofuranuronic acid in the molecule. The synthetic route to **1** could serve for the synthesis of analogues related to chryscandin for more detailed biological testing.

* Z-: Benzyloxycarbonyl-, -ONSu: succinimido-oxy-.

Scheme 1. (Continued)



Experimental

Melting points were measured with a Yanagimoto microscope hot-stage apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-140 automatic polarimeter. IR spectra were recorded with Jasco IRA-2 and Jasco A-102 grating spectrophotometer. UV spectra were recorded by a Hitachi model 220A spectrophotometer. ^{1}H NMR spectra were recorded using Jeol PMX-60, Jeol PS-100 and Jeol FX-270 spectrophotometers. Mass spectra were determined with a Jeol JMS-D-300 mass spectrometer.

Acid Hydrolysis of Chryscandin (**1**)

A suspension of **1** (300 mg) in 6 N HCl (20 ml) was refluxed for 12 hours at 110°C. The resulting solution was evaporated *in vacuo*. The residue was dissolved in H_2O and adjusted to pH 7.0 with 1 N NaOH. Then, the solution was applied on a column of Diaion HP-20 (200 ml). The column was washed with H_2O (400 ml) and eluted with 30% aq MeOH (200 ml). The solvent was evaporated *in vacuo*. The residue was chromatographed on a cellulose column (100 ml) and eluted with 80% aq acetonitrile. The first fractions containing a ninhydrin positive and weak UV absorbing compound were combined and the solvent was evaporated *in vacuo* to give *O*-methyl-L-tyrosine (30 mg). $[\alpha]_D^{20}$ −8° (*c* 1.0, 1 N HCl); IR (Nujol) 3400, 3650~2250 (br), 1675 and 1615 cm^{-1}; ^{1}H NMR (D_2O - DCl) δ 3.20 (2H, d, J=6 Hz), 3.80 (3H, s), 4.33 (1H, t, J=6 Hz), 6.93 (2H, d, J=8 Hz) and 7.20 (2H, d, J=8 Hz). The second strong UV absorbing fractions were combined and the solvent was evaporated *in vacuo*

to give adenine (33 mg). UV $\lambda_{max}^{H_2O}$ nm(ε) 207 (23,100) and 260 (13,300); IR (Nujol) 3450, 3300, 2700, 1670 and 1600 cm^{-1}; ^1H NMR (D_2O) δ (ppm) 8.20 (1H, s) and 8.32 (1H, s).

Tri-*N*-acetyl Mono-*O*-acetyl Monomethyl Ester (**2**) and Di-*N*-acetyl Mono-*O*-acetyl Monomethyl Ester (**3**)

A suspension of **1** (20 mg) in acetic anhydride (1 ml) and pyridine (3 ml) was stirred at room temp for 2 days. MeOH (10 ml) was added to the cooled reaction mixture and the resulting solution was concentrated *in vacuo*. To the residue was added H_2O and the mixture was extracted with $CHCl_3$ (10 ml×3). The combined extract was washed with 1 N HCl and saturated brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by preparative TLC on silica gel with $CHCl_3$-MeOH (5: 1) to afford **2** (8 mg) and **3** (4 mg).

Compound **2**: IR ($CHCl_3$) 1775, 1722, 1660, 1605, 1580, 1515 and 1375 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.96 (3H, s), 2.13 (3H, s), 2.35 (6H, s), 2.95 (2H, d, J=7.5 Hz), 3.72 (3H, s), 3.76 (3H, s), 4.41 (1H, d, J=6 Hz), 4.65 (1H, dt, J=7.5 and 7.5 Hz), 5.09 (1H, m), 5.76 (dd, J=2 and 5 Hz), 6.30 (1H, d, J=2 Hz), 6.50 (1H, d, J=7.5 Hz), 6.83 (2H, d, J=8 Hz), 7.15 (2H, d, J=8 Hz), 7.25 (1H, d, J=7.5), 8.75 (1H, s) and 8.92 (1H, s); FD mass m/z 597 (M^+-42).

Compound **3**: IR ($CHCl_3$) 1750, 1690, 1660, 1610, 1590, 1510 and 1378 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.96 (3H, s), 2.15 (3H, s), 2.60 (3H, s), 2.96 (2H, d, J=8 Hz), 3.72 (3H, s), 3.76 (3H, s), 4.42 (1H, d, J=8 Hz), 4.60 (1H, m), 5.10 (1H, m), 5.75 (1H, m), 6.20 (2H, m), 6.83 (2H, d, J=8 Hz), 7.16 (2H, d, J=8 Hz), 8.50 (1H, s) and 8.75 (1H, s); FD mass m/z 597 (M^+).

Alkaline Hydrolysis of Chryscandin (**1**)

A solution of **1** (100 mg) in 1 N NaOH in MeOH (10 ml) was refluxed for 20 hours. To the cooled solution was added H_2O (50 ml) and the MeOH was evaporated under reduced pressure. The resulting aq solution was adjusted to pH 7 with 1 N HCl and applied on a column of activated carbon (20 ml). The column was washed with H_2O (40 ml) and eluted with 50% aq MeOH. The eluate was evaporated *in vacuo*. The residue was chromatographed on a cellulose column and eluted with 80% aq acetonitrile. The first ninhydrin positive and weak UV absorbing fractions were evaporated *in vacuo* to give a powder of *O*-methyl-L-tyrosine (21 mg). The second ninhydrin positive and strong UV absorbing fractions were evaporated *in vacuo* to give a powder of 1-(6-amino-9*H*-purin-9-yl)-1,3-dideoxy-3-amino-β-D-ribofuranuronic acid (**4**) (35 mg).

Compound **4**: $[\alpha]_D^{20}$ −28° (*c* 0.25, 1 N HCl); IR (Nujol) 3480, 3600~2100 (br), 1655, 1600 and 1575 cm^{-1}; ^1H NMR (D_2O - DCl) δ 5.09 (1H, d, J=7 Hz), 5.17 (1H, dd, J=2 and 6 Hz), 6.40 (1H, d, J=2 Hz), 8.43 (1H, s) and 8.52 (1H, s); FD mass m/z 281 (M^++1).

Anal Calcd for $C_{10}H_{12}N_6O_4 \cdot H_2O$: C 40.27, H 4.73, N 28.18.
Found: C 39.98, H 4.62, N 28.21.

5-*O*-Benzoyl-1,2-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-xylofuranose (**6**)

To a stirred solution of pyridine (3.3 ml) in CH_2Cl_2 (240 ml) was added dropwise a solution of $(CF_3SO_2)_2O$ (6.08 ml) in CH_2Cl_2 (60 ml) and then a solution of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (8.82 g) in CH_2Cl_2 (40 ml) at −10°C. The resulting solution was stirred at −10°C for 30 minutes, and then poured into 5% $NaHCO_3$-crushed ice (600 ml). After the mixture was stirred for 30 minutes, the CH_2Cl_2 layer was separated, and the aq layer was extracted with CH_2Cl_2. The extracts were combined, washed with 1 N HCl, saturated $NaHCO_3$, saturated brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was recrystallized from MeOH to give **6** (9.3 g): mp 79~80°C; IR 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (3H, s), 1.54 (3H, s), 4.38~4.56 (3H, m), 4.80 (1H, d, J=3.6 Hz), 5.34 (1H, d, J=2.8 Hz), 6.05 (1H, d, J=3.6 Hz), 7.26~7.64 (3H, m) and 7.98~8.08 (2H, m).

Anal Calcd for $C_{16}H_{17}O_8SF_3$: C 45.07, H 4.02, S 7.52, F 13.37.
Found: C 45.21, H 3.92, S 7.43, F 13.15.

3-Azido-5-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (**7**)

To a solution of **6** (8.0 g) in abs EtOH (250 ml) was added NaN_3 (15 g). The resulting solution was refluxed for 40 hours. EtOH was removed *in vacuo* from the resultant solution. The residue was dissolved in H_2O (300 ml) and extracted with $CHCl_3$ (300 ml×2). The extracts were combined,

washed with saturated brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column and eluted with *n*-hexane - EtOAc (9: 1). The first fraction, containing the by-product, was evaporated *in vacuo* to give 2.3 g of **8** (48.9%): IR (Nujol) 1740, 1730 and 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (3H, s), 1.50 (3H, s), 4.85 (2H, s), 5.28 (2H, m), 6.11 (1H, m), 7.26~7.67 (3H, m) and 7.92~8.10 (2H, m): EI mass *m/z* 276 (M^+). The second fraction, containing the compound sought, was evaporated to give 2.8 g of **7** (47.0%): IR (Nujol) 2130 and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (3H, s), 1.61 (3H, s), 3.43 (1H, dd, J=4 and 8 Hz), 4.26~4.66 (2H, m), 4.79 (1H, t, J=4 Hz), 5.85 (1H, d, J=4 Hz), 7.26~7.62 (3H, m) and 7.99~8.14 (2H, m): EI mass *m/z* 319 (M^+).

3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose

To a stirred solution of **7** (4.95 g) in MeOH (50 ml) was added, dropwise, 1 N NaOH (50 ml). The solution was stirred for 1 hour and concentrated to a volume of 50 ml *in vacuo* and extracted with $CHCl_3$ (50 ml×2). The extracts were combined, washed with saturated brine (50 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* to give 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (3.4 g) as an oil: IR ($CHCl_3$) 3600, 3000, 2840 and 2120 cm^{-1}; ^1H NMR ($CDCl_3$ - D_2O) δ 1.37 (3H, s), 1.57 (3H, s), 3.40~4.30 (3H, m), 4.72 (1H, t, J=3.5 Hz) and 5.92 (1H, d, J=3.5 Hz): EI mass *m/z* 215 (M^+).

3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranuronic Acid

To a stirred suspension of 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (3.34 g) in a solution of KOH (5.5 g) in H_2O (200 ml) was added dropwise a solution of potassium permanganate (6.8 g) in H_2O (200 ml) over 2 hours interval. The resulting suspension was stirred at ambient temp for 1 hour. The precipitated manganese dioxide was removed by filtration and the filtrate was decolorized with $NaHSO_3$ and then acidified with 1 N HCl. The solution was extracted with EtOAc (100 ml×3). The extract was washed with saturated brine (100 ml) and dired over $MgSO_4$. The solvent was removed *in vacuo* and the residue was recrystallized from Et_2O - *n*-hexane to give 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranuronic acid (2.93 g); mp 56~58°C; IR ($CHCl_3$) 3600~2400 (br), 2990, 2820, 2130 and 1720 cm^{-1}; ^1H NMR ($CDCl_3$) 1.37 (3H, s), 1.57 (3H, s), 3.72 (1H, dd, J=3.5 and 9 Hz), 4.60 (1H, d, J=9 Hz), 4.73 (1H, t, J=3.5 Hz), 5.92 (1H, d, J=3.5 Hz) and 9.13 (1H, s).

Anal Calcd for $C_8H_{11}N_3O_5$: C 41.92, H 4.84, N 18.34.
Found: C 41.78, H 4.69, N 18.21.

Methyl 3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranuronate (**9**)

A solution of 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranuronic acid (2.7 g) in MeOH (50 ml) was treated with excess CH_2N_2 in ether. After the reaction was completed, the solvent was evaporated *in vacuo*. The residue was chromatographed on a silica gel column and eluted with $CHCl_3$. The fractions, containing the desired compound, were combined and the solvent was evaporated *in vacuo* to give **9** (2.34 g) as colorless oil: $[\alpha]_D^{22}$ +116° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 2990, 2130 and 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (3H, s), 1.57 (3H, s), 3.70 (1H, dd, J=3.5 and 9 Hz), 3.83 (3H, s), 4.55 (1H, d, J=9 Hz), 4.73 (1H, t, J=3.5 Hz) and 5.88 (1H, d, J=3.5 Hz); EI mass *m/z* 243 (M^+).

Methyl 1,2-Di-*O*-acetyl-3-azido-3-deoxy-D-ribofuranuronate (**10**)

The compound **9** (2.29 g) was added to 75% HCOOH (200 ml). The mixture was heated at 50°C for 2 hours, and then evaporated *in vacuo* to dryness. The residue was dissolved in a mixture of acetic anhydride (12 ml) and pyridine (20 ml), and allowed to stand at room temp for 2 hours. The resultant mixture was poured onto crushed ice and extracted with $CHCl_3$ (100 ml×3). The extracts were combined, washed with 1 N HCl, saturated $NaHCO_3$ and saturated brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column and eluted with *n*-hexane - EtOAc (7: 3). The fractions containing the object compound were combined and the solvent was evaporated *in vacuo* to give 2.27 g of **9** (84%) as syrup: IR ($CHCl_3$) 3030, 2950, 2130 and 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.07 (3H, s), 2.17 (3H, s), 3.82 (3H, s), 4.20~4.65 (2H, m), 5.33 (1H, d, J=4 Hz) and 6.17 (1H, s); EI mass *m/z* 287 (M^+).

Methyl 2-*O*-Acetyl-3-azido-1-(6-benzoylamino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronate (**11**)

A suspension of N^6-benzoyladenine (552 mg) in hexamethyldisilazane (10 ml) and chlorotrimethylsilane (0.6 ml) was refluxed for 3 hours. Excess hexamethyldisilazane was removed *in vacuo* from the resultant solution. To the residue, dissolved in 1,2-dichloroethane (10 ml), were added a solution of **10** (500 ml) in 1,2-dichloroethane (5 ml) and $SnCl_4$ (0.5 ml). The mixture was stirred for 4 hours at 60~70°C and then poured onto crushed ice. The resultant mixture was extracted with $CHCl_3$ (20 ml×3). The extracts were combined, washed with saturated $NaHCO_3$ and saturated brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column and eluted with $CHCl_3$ - MeOH (93: 7). The fractions, containing the objective compound, were combined and the solvent was evaporated *in vacuo* to give **11** (450 mg) as syrup: $[\alpha]_D^{22}$ +13° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3120, 3000, 2130, 1750 and 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13 (3H, s), 3.80 (3H, s), 4.60 (1H, d, *J*=5 Hz), 4.87 (1H, t, *J*=5 Hz), 5.84 (1H, t, *J*=5 Hz), 6.29 (1H, d, *J*=5 Hz), 7.20~7.61 (3H, m), 7.70~8.12 (2H, m), 8.42 (1H, s), 8.71 (1H, s) and 8.97 (1H, s); EI mass *m/z* 466 (M^+).

3-Azido-1-(6-benzoylamino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic Acid (**12**)

11 (280 mg) was dissolved in 0.5 N NaOH (20 ml) and the solution was stirred for 30 minutes at room temp. This solution which contained **12** was used for the next step without purification.

3-Amino-1-(6-benzoylamino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic Acid (**13**)

To the whole reaction mixture which contained **12** was added 1 N HCl to adjust to pH 2. The mixture was hydrogenated under medium pressure (3.0~3.5 atm) over Pd-black (50 mg) at room temp for 1 hour. The catalyst was removed by filtration and the filtrate was adjusted to pH 7.0 with 1 N NaOH. The resulting solution was applied to a column of Diaion HP-20 and the column was washed with H_2O (40 ml) and then eluted with 50% aq MeOH (60 ml). The solvent was evaporated *in vacuo* and the residue was recrystallized from H_2O to give **13** (170 mg): mp 220~225°C (dec); $[\alpha]_D^{22}$ −20° (*c* 1.0, 1 N HCl); IR (Nujol) 3600~2200 (br), 1685, 1640 and 1620 cm^{-1}; ^1H NMR (D_2O - DCl) δ 5.23 (1H, dd, *J*=2 and 6 Hz), 6.57 (1H, d, *J*=2 Hz), 7.30~7.80 (3H, m), 7.80~8.20 (2H, m), 8.97 (1H, s) and 9.05 (1H, s).

Anal Calcd for $C_{17}H_{16}N_6O_5 \cdot H_2O$: C 50.74, H 4.51, N 20.89.
Found: C 50.56, H 4.35, N 20.66.

3-Amino-1-(6-amino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic Acid (**4**) from **13**

A suspension of **13** (50 mg) in a mixture of MeOH (5 ml) and *n*-butylamine (2.5 ml) was refluxed for 1 hour. The solvent was evaporated *in vacuo*, and the residue was subjected to column chromatography on a cellulose column and eluted with 80% aq acetonitrile. The fraction containing the object compound were combined and the solvent was evaporated *in vacuo* to give 3-amino-1-(6-amino-9*H*-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic acid (**4**) (21 mg): $[\alpha]_D^{20}$ −28° (*c* 0.25, 1 N HCl); IR (Nujol) 1655, 1600 and 1575 cm^{-1}; ^1H NMR (D_2O - DCl) δ 5.09 (1H, d, *J*=7 Hz), 5.17 (1H, dd, *J*=2 and 6 Hz), 6.40 (1H, d, *J*=2 Hz), 8.43 (1H, s) and 8.52 (1H, s); FD mass *m/z* 281 (M^++1).

Anal Calcd for $C_{10}H_{22}N_6O_4 \cdot H_2O$: C 40.27, H 4.73, N 28.18.
Found: C 40.12, H 4.68, N 28.11.

1-(6-Benzoylamino-9*H*-purin-9-yl)-3-[(*N*-benzyloxycarbonyl-*O*-methyl-L-tyrosyl)amino]-1,3-dideoxy-β-D-ribofuranuronic Acid (**14**)

To a stirred solution of *N*-benzyloxycarbonyl-*O*-methyl-L-tyrosine (181 mg) and *N*-hydroxysuccinimide (64 mg) in dioxane (10 ml) was added *N*,*N*′-dicyclohexylcarbodiimide (114 mg) under cooling in an ice bath. The mixture was stirred overnight at room temp. The suspension was filtered and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in THF (5 ml) and the solution was added to the solution of **13** (150 mg) and triethylamine (0.08 ml) in H_2O (5 ml). The mixture was stirred for a day at room temp. THF was evaporated *in vacuo* and the residual aq solution was adjusted to pH 2 with 1 N HCl and extracted with EtOAc (30 ml×3). The extracts were combined, washed with saturated brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was subjected to a column chromatography on silica gel and eluted with $CHCl_3$ - MeOH

(8: 2). The fractions containing the desired compound were combined and the solvent was evaporated *in vacuo* to give **14** (150 mg) as syrup: $[\alpha]_D^{22}$ −23° (*c* 1.0, $CHCl_3$ - MeOH, 1: 1); IR (Nujol) 3700~2100 (br), 1700, 1675, 1640 and 1610 cm^{-1}; ^1H NMR (DMSO-d_6 - D_2O) δ 3.63 (3H, s), 4.07~4.7 (4H, m), 4.87 (2H, m), 6.23 (1H, d, *J*=2 Hz), 6.70 (2H, d, *J*=8 Hz), 6.97~7.75 (10H, m), 7.80~8.10 (2H, m), 8.63 (1H, s) and 9.40 (1H, s); FD mass *m/z* 696 (M^++1).

1-(6-Amino-9*H*-purin-9-yl)-3-[(*N*-benzyloxycarbonyl-*O*-methyl-L-tyrosyl)amino]-1,3-dideoxy-β-D-ribofuranuronic Acid

A suspension of **14** (300 mg) in a mixture of MeOH (20 ml) and *n*-butylamine (10 ml) was heated under reflux for 1 hour. The mixture was evaporated *in vacuo*. The residue was subjected to a column chromatography on silica gel and eluted with $CHCl_3$ - MeOH (7: 3). The fraction containing the object compound were combined and the solvent was evaporated *in vacuo* to give 1-(6-amino-9*H*-purin-9-yl)-3-[(*N*-benzyloxycarbonyl-*O*-methyl-L-tyrosyl)amino]-1,3-dideoxy-β-D-ribofuranuronic acid (200 mg): $[\alpha]_D^{22}$ +2° (*c* 1.0, $CHCl_3$ - MeOH, 1: 1); IR (Nujol) 3650~2450, 1685, 1650, 1635 and 1610 cm^{-1}; ^1H NMR ($CDCl_3$ - CD_3OD, 1: 1) δ 2.98 (2H, d, *J*=7 Hz), 3.70 (3H, s), 4.94 (2H, s), 6.08 (1H, d, *J*=2 Hz), 6.70 (2H, d, *J*=8 Hz), 7.07 (2H, d, *J*=8 Hz), 7.20 (5H, s), 8.13 (1H, s) and 8.80 (1H, s); FD mass *m/z* 592 (M^++1).

Chryscandin (**1**)

A suspension of 1-(6-amino-9*H*-purin-9-yl)-3-[(*N*-benzyloxycarbonyl-*O*-methyl-L-tyrosyl)amino]-1,3-dideoxy-β-D-ribofuranuronic acid (150 mg) in H_2O (200 ml) was adjusted to pH 3 with 1 N HCl. The resulting mixture was hydrogenated under medium pressure (3.0~3.5 atm) over Pd-black (30 mg) for 3 hours. The catalyst was removed by filtration and the filtrate was adjusted to pH 7 with 1 N NaOH. The resulting mixture was concentrated *in vacuo* to a volumn of 30 ml. The concentrate was applied on a column of Diaion HP-20 (50 ml) and the column was washed with H_2O (100 ml) and then eluted with MeOH - H_2O (3: 7) (100 ml). The solvent was evaporated *in vacuo* and the residue was crystallized from 0.1 N HCl to give **1** (60 mg): mp 215~230°C (dec); $[\alpha]_D^{20}$ +33° (*c* 1.0, 1 N HCl); IR (KBr) 3600~2200, 1720 (sh), 1695, 1665, 1630, 1610 and 1560 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.95 (1H, dd, *J*=7 and 14 Hz), 3.10 (1H, dd, *J*=6 and 14 Hz), 3.73 (3H, s), 4.10 (1H, dd, *J*=7 and 6 Hz), 4.37 (1H, d, *J*=7 Hz), 4.70 (1H, m), 4.78 (1H, m), 6.12 (1H, d, *J*=2 Hz), 6.39 (1H, br), 6.90 (2H, d, *J*=8.5 Hz), 7.23 (2H, d, *J*=8.5 Hz), 7.44 (2H, br s), 8.18 (1H, s), 8.30 (3H, br), 8.46 (1H, s) and 9.01 (1H, d, *J*=8 Hz).

Anal Calcd for $C_{20}H_{23}N_7O_6 \cdot 2HCl \cdot H_2O$: C 43.80, H 4.96, N 17.88, Cl 12.93.
Found: C 44.06, H 5.03, N 18.12, Cl 12.64.

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