SELECTIVE PROTECTION OF THE PRIMARY HYDROXYL GROUPS OF OXETANOCIN A AND CONFORMATIONAL ANALYSIS OF *O*-PROTECTED OXETANOCIN A[1)]

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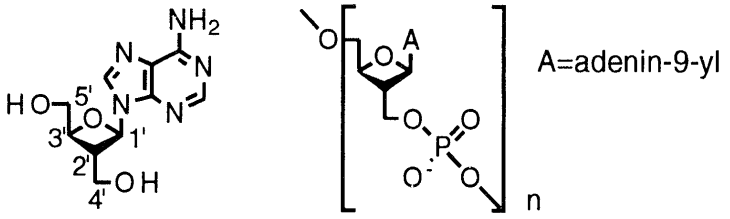
One of the two primary hydroxyl groups of oxetanocin A was protected selectively with acetyl and *o*-nitrobenzyl groups using enzymatic and photochemical reactions, respectively. On the basis of ^{1}H-NMR spectroscopy and NOE experiment, 4'-*O*-protected oxetanocin A was found to take syn conformation in an aprotic solvent irrespective of the kind of protecting group owing to an intramolecular hydrogen bond.

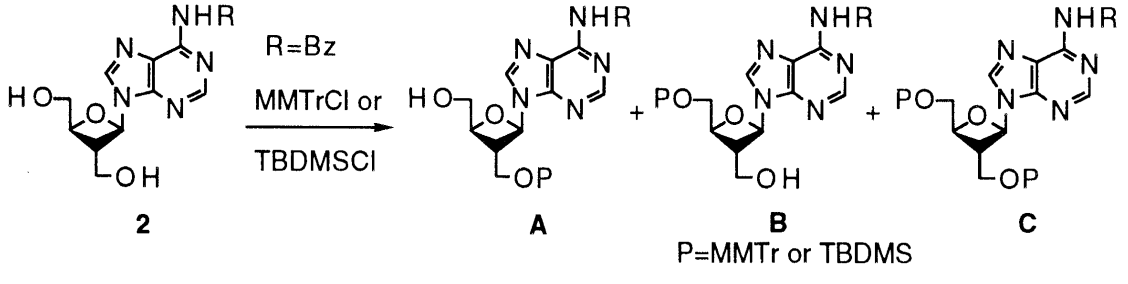
KEY WORDS oxetanocin A; selective protection; lipase; photochemical reaction; conformation; hydrogen bond

Recently, Eschenmoser reported the synthesis of oligonucleotide constituted with hexose nucleosides and found that, compared with normal DNA, its double strand structure (homo-DNA) involves stronger complexation between the strands, a much longer helix pitch, and pairing rules that differ from the Watson-Crick rules.[2)] Oxetanocin A (**1**),[3)] a constitutional isomer of 2'-deoxyadenosine and isolated from Bacillus megaterium, is a highly modified nucleoside bearing an oxetane ring as the sugar moiety and shows antiviral activity.[4c)] We have been interested in the structure of oligonucleotide (so-called nor-DNA) containing **1** and examined the selective protection of two primary hydroxyl groups required for the synthesis of the corresponding nucleotides. In this paper, we report the selective protection of the hydroxyl group of **1** and the conformational analysis of *O*-protected oxetanocin A.



oxetanocin A (1) nor-DNA

First, the hydroxyl groups of *N*-benzoyloxetanocin A (**2**) were protected with monomethoxytrityl chloride (MMTrCl) under ordinary conditions to give mono *O*-protected (**A** and **B**) and di-*O*-protected oxetanocin A (**C**) in 20, 21, and 15% yields, respectively. Silylation of **2** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of 2-isopropylimidazole was also carried out to afford three products (**A**, **B**, and **C**) in respective yields of 19, 26, and 24%.[5)] In both cases, the regioselectivity was low. Since we could not distinguish two hydroxyl groups of **1** chemically, we intended to protect the hydroxyl groups using enzymatic reaction.[6)] Thus, di-*O*-acetylated oxetanocin A (**3**)[7)] was treated with lipase type XIII[8)] in 10% acetone-phosphate buffer (pH 7.0) at 37 ℃ for 3 d to give 4'-*O*-acetyloxetanocin A (**4**)[9)] as a major product. Other commercially available lipases such as lipase MY or PS did not catalyze the deacetylation. The selective protection was also achieved by



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photochemical reaction.[10] Thus, **1** was treated with two equivalent amounts of *o*-nitrobenzyl bromide in the presence of NaH in DMF to afford di-*O*-*o*-nitrobenzylated oxetanocin A (**5**)[11] in 49% yield, which was irradiated by 350 nm rays at room temperature for 5 min to give selectively 4'-*O*-*o*-nitrobenzyloxetanocin A (**6**)[12] in 76% yield. The structure of **6** was determined by its transformation to 5'-*O*-acetyloxetanocin A (**8**). Thus, **6** was acetylated in the usual manner to give the di-protected oxetanocin A (**7**). Compound **7** was irradiated again with 350 nm rays at room temperature for 30 min to give **8**.[13] Survival of the *o*-nitrobenzyl group at 4'-*O*-position on the irradiation of **5** may be attributable to the effect of the adenine ring.

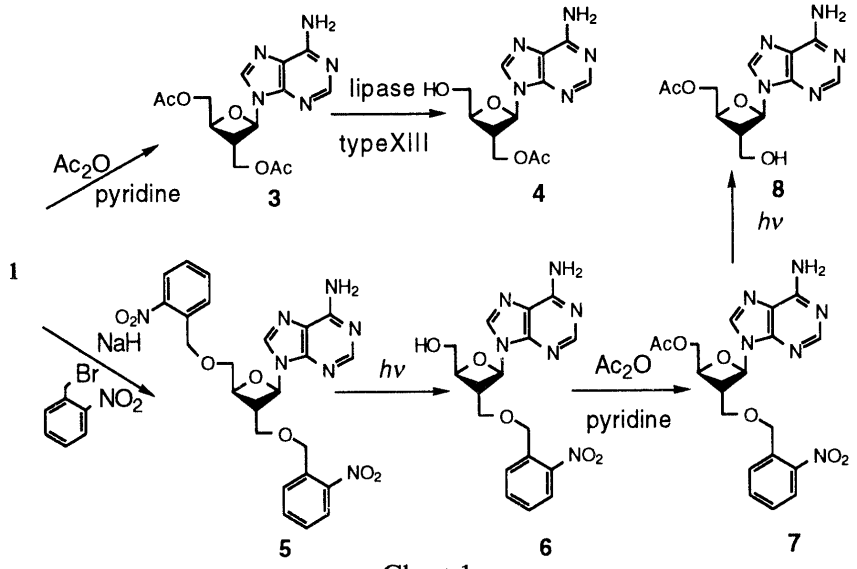
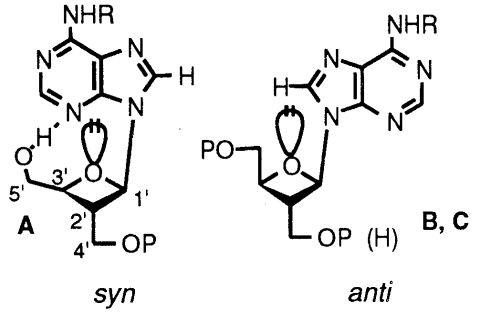


Chart 1

The structures including their syn and anti conformations of **A**, **B**, and **C** were determined by ^{1}H-NMR spectra (in $CDCl_3$). Thus, the 2'-H of **A** was observed at lower field whereas the 8-H[14] was observed at higher field, compared with those of **B** and **C** (Table 1). This phenomenon would be attributable to the syn and anti conformations. Due to its intramolecular hydrogen bond between 5'-OH and 3-nitrogen, **A** would be assumed to take the syn conformation in an aprotic solvent such as chloroform. Therefore, the 2'-H was observed at lower field owing to the current effect of adenine ring while 8-H was observed at higher field because of the disappearance of lone pair effect of oxetane ring. The syn conformation of **A** was also confirmed by its NOE experiment. Thus, in $CDCl_3$ solution 5.8% NOE effect was observed between 8-H and 1'-H of **4**. However, the effect

Table 1. ^{1}H-NMR Spectra ($CDCl_3$) of *O*-Protected Oxetanocin A



Protecting group (P)		Chemical shift ($CDCl_3$, ppm) 2'-H	8-H	2-H
MMTr	**A**	4.19	8.16	8.81
(R=Bz)	**B**	3.66	8.44	8.77
	C	3.78	8.49	8.74
TBDMS	**A**	4.22	8.18	8.86
(R=Bz)	**B**	3.65	8.63	8.80
	C	3.69	8.74	8.82
Ac	**A** (**4**)	4.40	7.88	8.39
(R=H)	**B** (**8**)	3.55	8.20	8.34
	C (**3**)	3.97	8.20	8.36

disappeared in CD_3OD. Since **1** has been determined to be anti conformation in its solid state by X-ray crystallographic analysis,[4b] it is probable that **1** takes the same conformation in a protic solvent. In general, anti conformation is more stable than syn conformer in common nucleosides or nucleotides. However, it is reported that some modified nucleosides such as 8-substituted purine nucleosides take syn conformation because of the steric interaction between the 8-substituent and the

furanose sugar moiety.[15] It is noteworthy that an intramolecular hydrogen bond orientates the syn and anti conformation of nucleosides. The synthesis of oligonucleotides using protected oxetanocin A (**4**, **6**, and **8**) is in progress.[16]

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3) Numbering shown in the structure is different from the previously reported one[4] and is tentatively used for convenience' sake.

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7) **3**: mp 136-138 °C (hexane-ethyl acetate). 500 MHz ^{1}H-NMR ($CDCl_3$) δ: 2.12, 2.17 (each 3H, s, Me x 2), 3.97 (1H, dddd, *J*=7.50, 6.25, 5.25, 5.00 Hz, 2'-H), 4.33 (1H, dd, *J*=12.00, 5.00 Hz, 4'-H), 4.42 (1H, dd, *J*=12.00, 5.25 Hz, 4'-H'), 4.50 (1H, dd, *J*=13.00, 3.50 Hz, 5'-H), 4.53 (1H, dd, *J*=13.00, 4.10 Hz, 5'-H'), 4.80 (1H, ddd, *J*=7.50, 4.10, 3.50 Hz, 3'-H), 5.80 (2H, s, NH_2), 6.46 (1H, d, *J*=6.25 Hz, 1'-H), 8.20 (1H, s, 8-H), 8.36 (1H, s, 2-H).

8) Lipase type XIII was purchased from Sigma.

9) **4** (33%, recovery of **3**; 27%): mp 168-169 °C (chloroform-methanol). 500 MHz ^{1}H-NMR ($CDCl_3$) δ: 2.09 (3H, s, Me), 3.78 (1H, br d, *J*=13.75 Hz, 5'-H), 4.12 (1H, d, *J*=13.75 Hz, 5'-H'), 4.24 (1H, dd, *J*=11.25, 3.75 Hz, 4'-H), 4.40 (1H, dddd, *J*=7.50, 6.25, 5.50, 3.75 Hz, 2'-H), 4.43 (1H, dd, *J*=11.25, 5.50 Hz, 4'-H'), 4.72 (1H, d, *J*=7.50 Hz, 3'-H), 5.78 (2H, s, NH_2), 6.32 (1H, d, *J*=6.25 Hz, 1'-H), 6.41-6.56 (1H, br s, OH), 7.88 (1H, s, 8-H), 8.39 (1H, s, 2-H).

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11) **5**: mp 200-201 °C (hexane-ethyl acetate). 500 MHz ^{1}H-NMR ($CDCl_3$) δ: 2.22 (2H, s, NH_2), 3.72 (1H, dd, *J*=13.50, 2.00 Hz, 5'-H), 3.79 (1H, dd, *J*=12.25, 4.75 Hz, 4'-H), 3.83 (1H, dd, *J*=12.25, 5.25 Hz, 4'-H'), 3.96 (1H, dddd, *J*=7.25, 6.00, 5.25, 4.75 Hz, 2'-H), 4.02 (1H, dd, *J*=13.50, 1.50 Hz, 5'-H'), 4.75 (1H, ddd, *J*=7.25, 2.00, 1.50 Hz, 3'-H), 5.31-5.60, 5.74-6.02 (each 2H, br s, 2 x C$\underline{H}_2$-Ph), 6.39 (1H, d, *J*=6.00 Hz, 1'-H), 7.37-8.17 (8H, m, Ph x 2), 8.05 (1H, s, 8-H), 8.34 (1H, s, 2-H).

12) **6**: mp 180-182 °C (ethyl acetate). 500 MHz ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 2.90 (2H, s, NH_2), 3.73 (1H, dd, *J*=13.00, 2.25 Hz, 5'-H), 3.79 (1H, dd, *J*=11.75, 4.75 Hz, 4'-H), 3.82 (1H, dd, *J*=11.75, 4.75 Hz, 4'-H'), 3.90 (1H, ddt, *J*=7.00, 6.50, 4.75 Hz, 2'-H), 4.03 (1H, dd, *J*=13.00, 2.00 Hz, 5'-H'), 4.76 (1H, ddd, *J*=7.25, 2.25, 2.00 Hz, 3'-H), 5.17 (2H, s, C$\underline{H}_2$-Ph), 6.43 (1H, d, *J*=6.50 Hz, 1'-H), 7.42-8.13 (4H, m, Ph), 8.28 (1H, s, 8-H), 8.34 (1H, s, 2-H).

13) **8** (92%): mp 158-159 °C (chloroform-methanol). 500 MHz ^{1}H-NMR ($CDCl_3$) δ: 1.93-2.08 (1H, br s, OH), 2.09 (3H, s, Me), 3.55 (1H, dddd, *J*=7.25, 7.00, 5.50, 4.00 Hz, 2'-H), 3.92 (1H, dd, *J*=11.50, 4.00 Hz, 4'-H), 4.06 (1H, dd, *J*=11.50, 7.25 Hz, 4'-H'), 4.37 (1H, dd, *J*=12.75, 4.50 Hz, 5'-H), 4.46 (1H, dd, *J*=12.75, 2.75 Hz, 5'-H'), 4.86 (1H, ddd, *J*=7.00, 4.50, 2.75 Hz, 3'-H), 5.64 (2H, s, NH_2), 6.46 (1H, d, *J*=5.50 Hz, 1'-H), 8.20 (1H, s, 8-H), 8.34 (1H, s, 2-H).

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