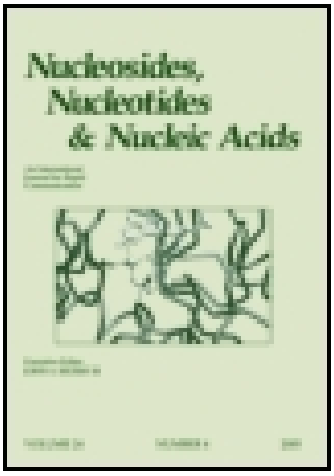

Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Reaction of 2-Deoxy-2-C-(3-bromoacetoxypropyl)-α-D-arabinofuranosides with Oligonucleotide[1]

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Published online: 24 Sep 2006.

To cite this article: S. Mori , A. Katoh , H. Takenaka , H. Tanaka , H. Kaji , T. Samejima & O. Mitsunobu (1994) Reaction of 2-Deoxy-2-C-(3-bromoacetoxypropyl)-α-D-arabinofuranosides with Oligonucleotide[1] , Nucleosides and Nucleotides, 13:10, 2081-2104, DOI: 10.1080/15257779408013209

To link to this article: http://dx.doi.org/10.1080/15257779408013209

NUCLEOSIDES & NUCLEOTIDES, 13(10), 2081-2104 (1994)

REACTION OF 2-DEOXY-2-C-(3-BROMOACETOXYPROPYL)-α-D-ARABINOFURANOSIDES WITH OLIGONUCLEOTIDE[1)]

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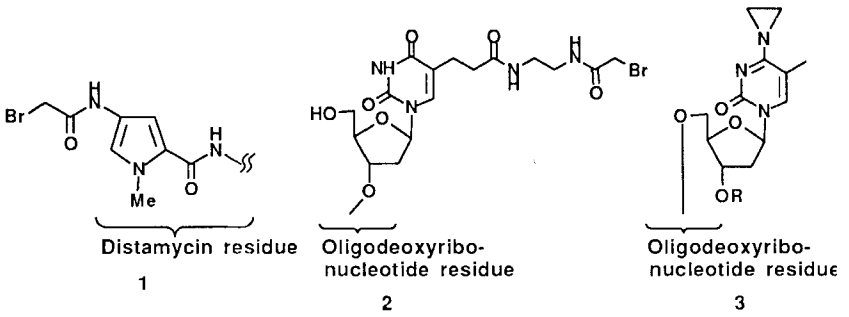
Abstract: Reaction of methyl 2-deoxy-2-C-(3-bromoacetoxypropyl)-α-D-arabinofuranosides, prepared from methyl 2,3-anhydro-α-D-ribofuranoside, with oligodeoxyribonucleotide (21mer) in acetonitrile-H_2O (pH 7) and subsequent treatment with piperidine resulted in the cleavage of the nucleotide chain at the position G, A, and C.

Sequence-specific modification and cleavage reaction of nucleic acids have been expected as very powerful tools in the elucidation of many fundamental problems associated with functions of nucleic acids. In order to modify and cleave nucleic acids at the specific position, the reagent must have both a reaction site and a recognition site in a single molecule.[3)] For the design of the recognition site, intercalater-DNA complexes,[4)] protein-DNA binding motifs,[5)] and Watson-Crick fashion toward complementary, single-stranded nucleic acids, or via triple-helix formation toward double-stranded DNA[3c,6)] would be possible and reliable model. As reaction sites, three types of cleaving pattern would be possible;[7)] radical initiated cleavage of sugar moiety,[8)] electrophilic modification of a base,[7,9-12)] and hydrolytic cleavage of phosphate diester bond.[13)]

This paper is dedicated to Professor Morio Ikehara on the occasion of his 70th birthday.

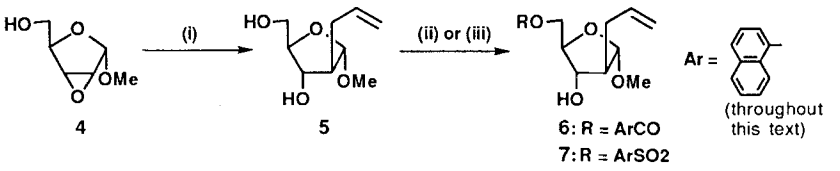


Scheme 1

Although electrophilic modification of a base would be attractive for the preparation of nondiffusible modifying and cleaving reagents, only few groups capable of alkylating heterocycles have been utilized in sequence specific cleaving reagents. Thus, Dervan and coworkers have extensively studied the reaction of DNA with bromoacetamido group bound to distamycin (**1**)[10a] and oligonucleotide (**2**) (Scheme 1).[7,10b] Shaw *et al.* have reported the reaction of aziridinyl group bound to oligonucleotide (**3**) with DNA (Scheme 1).[11a] DNA interstrand cross-linking reactions by bifunctional alkylating reagents have also been reported.[14]

Main factors which control the reactivity and the selectivity in alkylating reaction of nucleic acids involve i) intrinsic electrophilicity of alkylating reagent used and its accessibility to target molecules, ii) hard-soft relationship of reagent-substrate combination, and iii) the nature of nucleophilic sites in nucleic acids. The net reactivity of the nucleophilic sites in nucleic acids depends on both steric hindrance and electrostatic potentials associated with phosphate groups and bases themselves.[9] Therefore, in order to obtain definite information relating to the design of effective modifying and cleaving reagents for nucleic acids, a systematic study of the reaction of various alkylating reagents with oligonucleotides would be necessary.

From the synthetic point of view, it is desirable to prepare a variety of reactive molecules from a single intermediate by simple operation. We have recently reported that the reaction of methyl 2,3-anhydro-α-D-ribofuranoside (**4**) with allylmagnesium chloride selectively gave methyl 2-deoxy-2-C-allyl-α-D-arabinofranoside(**5**) (Scheme 2).[15] It would be anticipated that the allylic side chain can be converted into a variety of reaction sites



4 5 6: R = ArCO 7: R = ArSO2

Reagents and conditions: (i) Mg, THF, then CH_2=$CHCH_2Cl$, THF, -10 °C→rt, 1d: 83%. (ii) To **6**; 1-Naphthalenecarboxylic acid, DEAD-TPP, toluene, 0 °C→rt, 3.5h: 86%. (iii) To **7**; 1-Naphthalenesulfonyl chloride, Py, 0 °C→rt, 24 h: 77%.

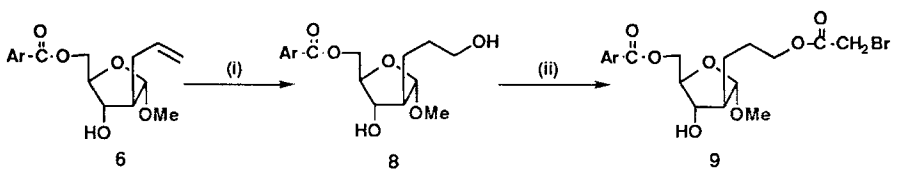
Scheme 2

amenable to react with bases involved in nucleic acids, whereas the hydroxyl groups at the 3- and/or 5-positions could be utilized for introducing recognition site.[15] In order to materiarize this possibility, the preparation of arabinofuranoside derivatives bearing bromoacetoxy group and bromoacetamido group and their reaction with oligonucleotide have been investigated.

As the protecting group of the hydroxyl group at the 5-position of arabinofuranoside **5**, 1-naphthoyl group and 1-naphthalenesulfonyl group were used because they could be expected to function as not only protecting group but also intercalater for double stranded DNA.[16] Thus, the reaction of **5** with 1-naphthalenecarboxylic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (TPP) selectively afforded 5-O-(1-naphthoyl)-2-deoxy-2-C-allyl-α-D-arabinofuranoside (**6**) in 86% yield.[17] The reaction of **5** with 1-naphthalenesulfonyl chloride gave 5-O-(1-naphthalenesulfonyl)-2-deoxy-2-C-allyl-α-D-arabinofuranoside (**7**) in 77% yield (Scheme 2).

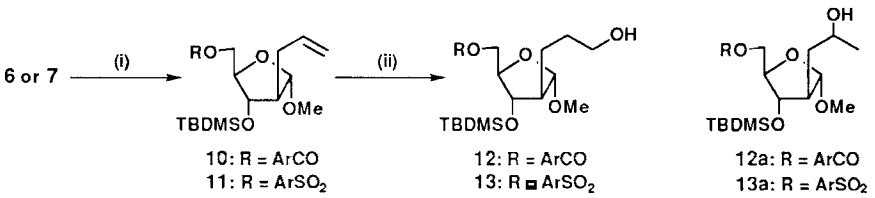
Hydroboration (disiamylborane)-oxidation of **6** gave methyl 5-O-(1-naphthoyl)-2-deoxy-2-C-(3-hydroxypropyl)-α-D-arabinofuranoside (**8**) in 54% yield.[18] The resulting prim, sec-diol **8** reacted with bromoacetic acid, DEAD, and TPP in toluene to exclusively give methyl 2-deoxy-2-C-(3-bromoacetoxypropyl)-α-D-arabinofuranoside (**9**) in 53% yield (Scheme 3).

3-O-t-Butyldimethylsilyl (TBDMS) derivative of **9** was also prepared. Thus, the hydroxyl group of **6** was protected by t-butyldimethylsilyl group by standard procedure to give the desired product **10** in 84% yield.[19] Hydroboration-oxidation of **10** gave the corresponding 2-deoxy-2-C-(3-hydroxypropyl)-α-D-arabinofuranoside **12** and secondary alcohol **12a** in 66%



Reagents and conditions: (i) $(Sia)_2BH$, ether, 0 °C, 4 h, then H_2O_2, NaOHaq, 0 °C→rt, 1 h: 54%. (ii) $BrCH_2COOH$, DEAD-TPP, 0 °C→rt, 2 h: 53%.

Scheme 3

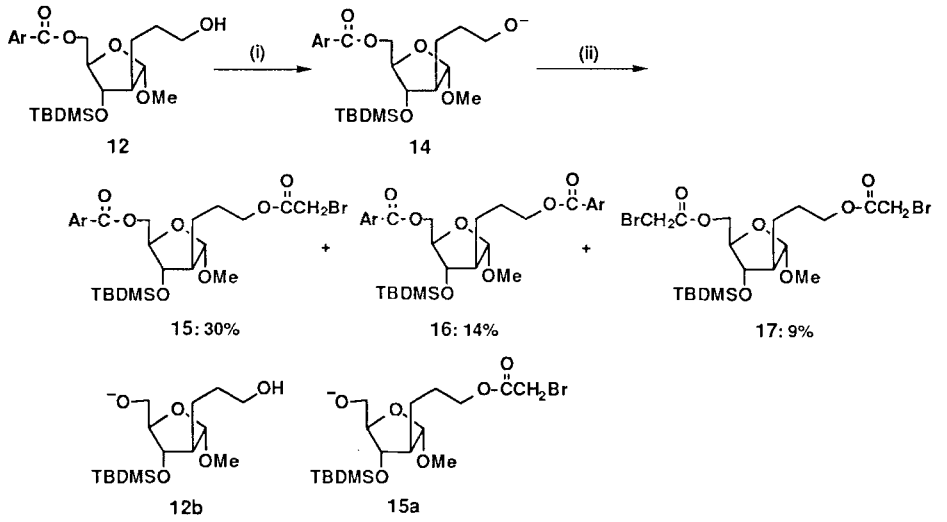


Reagents and conditions: For **6**: (i) TBDMS-Cl, imidazole, Py, 3 d: 84%. (ii) $(Sia)_2BH$, ether, 0 °C, 4.5 h, then H_2O_2, NaOHaq, 0 °C→rt, 16 h: 66%. For **7**: (i) TBDMS-Cl, imidazole, Py, 3 d: 70%. (ii) $BH_3 \cdot SMe_2$, ether, 0 °C→rt, 11 h, then H_2O_2, NaOHaq, 0 °C→rt, 3 h: 82%.

Scheme 4

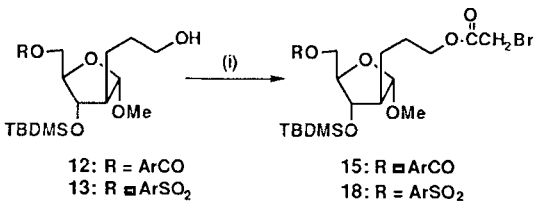
and 7% yields, respectively (Scheme 4). By the similar way, 5-O-(1-naphthalenesulfonyl) derivative **13** was prepared in 57% overall yield from **7** via **11** (Scheme 4). Hydroboration-oxidation of **11** also gave a small amount of a side product which was assumed to be secondary alcohol **13a** but not fully characterized.

Treatment of **12** with KH, followed by the reaction with bromoacetyl bromide afforded the expected 5-O-(1-naphthoyl)-2-C-(3-bromoacetoxypropyl)-α-D-arabinofuranoside **15** in 30% yield with concomitant formation of undesirable dinaphthoyl derivative **16** and bis(bromoacetyl) derivative **17** in 14% and 9% yields, respectively (Scheme 5). The formation of **16** could be explained by the reaction of the oxido anion **14** with unreacted **12** and/or desired product **15**. The oxido anions **12b** and/or **15a** thus formed in turn reacted with bromoacetyl bromide giving **17** (Scheme 5).



Reagents and conditions: (i) KH, THF, 0 °C, 1.5 h. (ii) $BrCH_2COBr$. 0 °C, 1.5 h.

Scheme 5

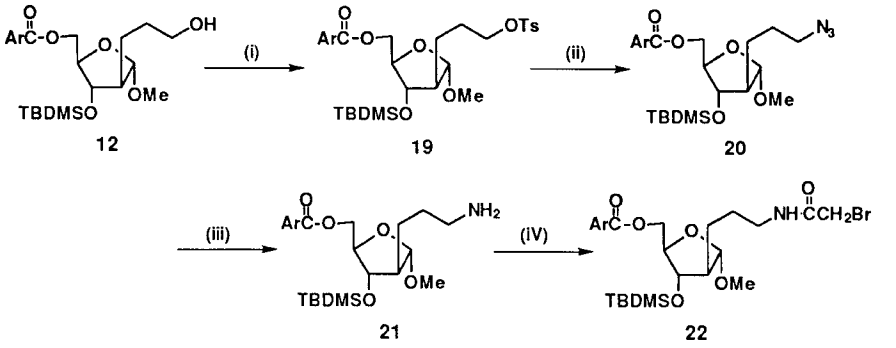


Reagents and conditions: For **12**: (i) $BrCH_2COOH$, DEAD-TPP, 0 °C→rt, 1 d: 47%. For **13**: (i) KH, THF, 0 °C, 1.5 h, then $BrCH_2COBr$, THF, 0 °C→rt, 1 h: 78%.

Scheme 6

The reaction of **12** with bromoacetic acid in the presence of DEAD and TPP gave the expected **15** in 47% yield (Scheme 6). Although the yield of **15** was not still high, no attempt was made to prepare **15** by silylation of **9** because undesirable side reaction might be conceivable to take place at the bromoacetyl moiety under the required reaction conditions.

Contrary to 5-O-(1-naphthoyl) derivative **12**, the successive reactions of 5-O-(1-naphthalenesulfonyl) derivative **13** with KH and bromoacetyl bromide gave the desired 2-C-(3-bromoacetoxypropyl) derivative **18** in 78% yield (Scheme 6).



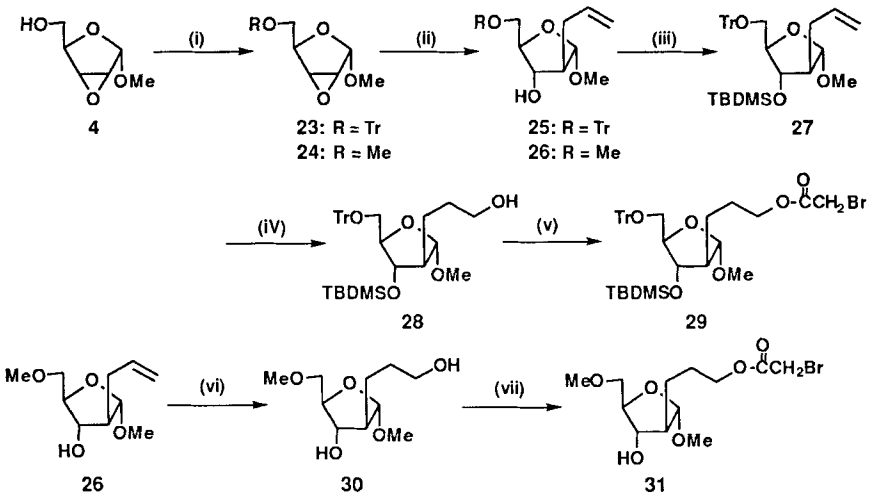
Reagents and conditions: (i) TsCl, Py, 0 °C→rt. 5.5 h: 64%. (ii) NaN_3, DMF, rt, 1 d: 90%. (iii) $NaBH_4$, $C_{16}H_{33}P(C_4H_9)_3Br$, toluene-$H_2O$, 40 °C~50 °C, 48 h: 37%. (iv) $BrCH_2COOH$, DCC, THF, 0 °C, 3 h: 60 %.

Scheme 7

In order to compare the reactivity of bromoacetoxy group with bromoacetamido group, bromoacetamido derivative **22** was prepared. Thus, 2-C-(3-hydroxypropyl)furanoside **12** was converted into the corresponding azide **20** in 58% overall yield from **12** via tosylate **19**. Reduction of the azide **20** by $NaBH_4$ under phase transfer conditions afforded the expected 2-deoxy-2-C-(3-aminopropyl)arabinofuranoside **21** in 37% yield.[20] The condensation of **21** with bromoacetic acid by dicyclohexylcarbodiimide (DCC) afforded the expected **22** in 60% yield (Scheme 7).

In order to obtain the information of the effect of the bulkiness of the substituent at the 5-position, 5-O-tritylarabinofuranoside **29** and 5-O-methyl-arabinofuranoside derivatives **31** were prepared. 2,3-Anhydroribofuranoside **4** successively reacted with trityl chloride, allylmagnesium chloride, and t-butyldimethylsilyl chloride to give the fully protected 2-deoxy-2-C-allyl-α-D-arabinofuranoside **27** in 58% overall yield. Hydroboration-oxidation and subsequent reaction with bromoacetic acid, DEAD, and TPP gave the bromoacetyl derivative **29** in 36% yield (Scheme 8).

2,3-Anhydroribofuranoside **4** reacted with methyl iodide in the presence of Ag_2O,[19a,b,21] followed by the reaction with allylmagnesium chloride to afford 5-O-methyl-2-deoxy-2-C-allyl-α-D-arabinofranoside **26** in 65% yield. Compound **26** was converted into desired bromoacetate **31** in 31% yield by the same procedure used in the preparation of **29** (Scheme 8).



Reagents and conditions: (i) To **23**: TrCl, Py, rt, 1 d: 79%. To **24**: MeI, Ag_2O, CH_3CN, rt, 64 h: 90%. (ii) For **23**: Mg, THF, then CH_2=$CHCH_2Cl$, THF, -10 °C→rt, 1 d: quant. For **24**: Mg, THF, then CH_2=$CHCH_2Cl$, THF, -10 °C→rt, 21.5 h: 72%. (iii) TBDMS-Cl, imidazole, Py, rt, 3 d: 84%. (iv) BH_3·SMe_2, THF, 0 °C→rt, 1 d, then H_2O_2, NaOHaq, 0 °C→rt, 7 h: 65%. (v) $BrCH_2COOH$, DEAD-TPP, toluene-THF, 0 °C→rt, 3.25 h: 36%. (vi) BH_3·SMe_2, THF, -10 °C→rt, 3.5 h, then H_2O_2, NaOHaq, 0 °C, 1 h: 25%. (vii) $BrCH_2COOH$, DEAD-TPP, toluene, -10 °C→rt, 2 h: 31%.

Scheme 8

Nucleotide cleavage activity of bromoacetyl derivatives prepared was investigated with synthesized 21mer, 5'-d-GATAAGCTTGAATTCATGGCC-3' (**32**). Thus, the oligonucleotide **32** was labeled with ^{32}P on 5'-end by the reaction with [γ-^{32}P]ATP and T4 polynucleotide kinase. In the alkylation reaction, 5 pmol of the labeled oligomer in 50 µl of phosphate buffer (50 mM; pH 7) was made by estimating 50% of the original oligonucleotide was recovered in the labeling experiment (see Experimental). A solution (50 µl) of the labeled oligomer was mixed with a solution (50 µl) of alkylating reagent in CH_3CN (20 µM or 2000 µM concentration) and incubated at 37 °C for 3 days, followed by piperidine treatment (90 °C, 30 min).[22] The ^{32}P end-labeled products resulting from the cleavage reaction were separated by polyacrylamide gel electrophoresis (lanes 1-17).[23] The gel electrophoresis was also performed before piperidine treatment (lanes 18-24). The autoradiogram is shown in Figure 1. In control, no cleavage was observed in the absence of the bromoacetyl derivatives (lanes 1 and 24).

Maxam-Gilbert G reaction[24] was performed with the non-labeled oligonucleotide as well as with the 5'-end-labeled 21mer. In the experiment using non-labeled 21mer, the 5'-end labelling was carried out with [γ-^{32}P]ATP and T4 polynucleotide kinase after piperidine treatment and analyzed by gel electrophoresis. Thus, the fragments resulting from G reaction lack 3'-phosphate groups because of 3'-phosphatase activity of the kinase,[25] and therefore migrate slower (lane 17) than those obtained in pre-labeled experiment (lane 2).

The product analysis carried out before piperidine treatment indicates that no nucleotide cleavage occurred at this stage (lanes 19-24). However the appearance of slower migrating bands (lanes 19, 21, and 22) than the original 21mer suggests significant alkylation took place in the reaction with bromoacetoxy derivatives used. Although the structures of the reaction products have not yet been elucidated, alkylated cationic species would be most probable. Since the slower migrating bands disappeared after piperidine treatment, modification and subsequent reaction with piperidine are essential for the nucleotide chain cleavage.

Comparison of lanes 3-16 with lane 17 indicates that no reaction causing the removal of 3'-phosphate group from cleavage products occurred.

As shown in Fig 1, cleavage took place not only at the position of G but also at A and C with reactivity order G > A > C, irrespective of the structure of bromoacetoxy derivatives. With respect to alkylating reagents, methyl bromoacetate was found to be more reactive than sugar esters (lanes 3-12 vs. lane 15), while bromoacetamido derivative **22** is less reactive than the corresponding bromoacetoxy derivative **15** (lanes 5, 6 vs. lanes 13, 14). Same results were obtained in the reactions of the 21mer with methyl bromoacetate (lane 15) and bromoacetamide (lane 16).

Although, in the present study, no significant difference was observed in the reactivity by changing the substituent group at the 5-position of sugar moeity, further works involving the reaction with double-stranded oligonucleotides would be necessary to evaluate the effects of the substituents introduced in the sugar moiety.

It is noteworthy that, in contrast to the reaction with dimethyl sulfate, bromoacetoxy derivatives examined in the present study can react with not only G, but also A, and C. This feature suggests that the bromoacetoxy derivatives would be converted into sequence specific G-, A-, or C-cleaving reagent by introducing an appropriate recognition site.

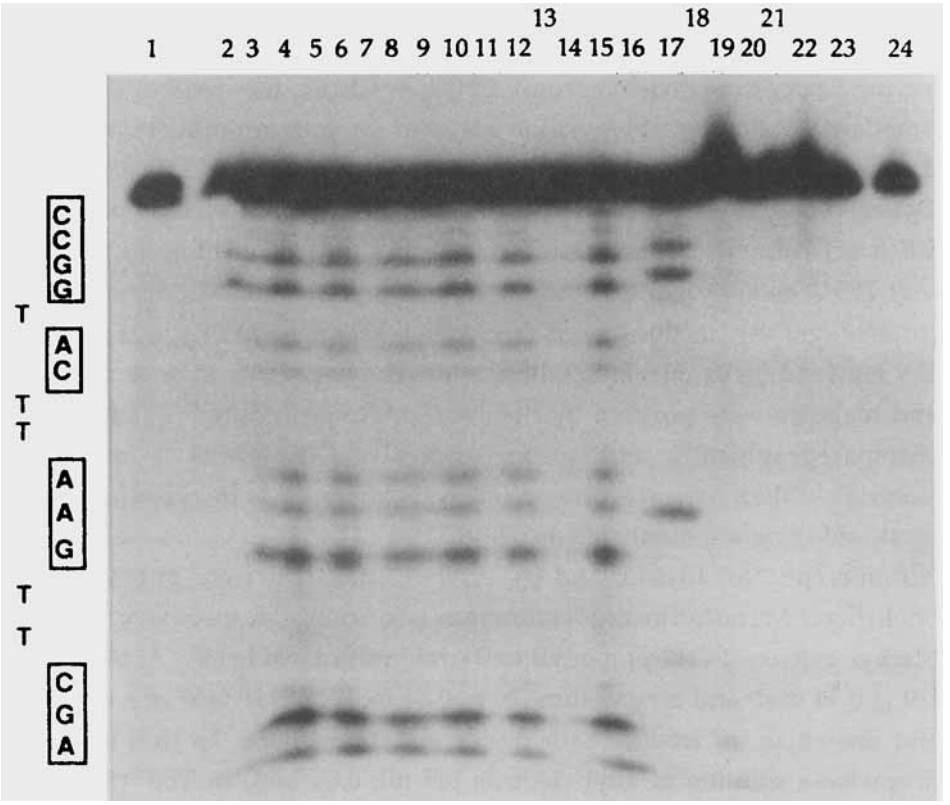


Fig. 1. Autoradiogram of polyacrylamide gel of the products from the reaction of 5'-d-^{32}P-GATAAGCTTGAATTCATGGCC-3' (**32**) with various bromoacetyl derivatives at 37 °C for 3 d. Lanes 1 and 24; control. Lanes 1-17; the reaction products were treated with piperidine (90 °C, 30 min). Lanes 18-24; without piperidine treatment. Lane 2: Maxam-Gilbert G-reaction. Lanes 3 and 4; **18** (20 μM and 2000 μM), Lanes 5 and 6; **15** (20 μM and 2000 μM). Lanes 7 and 8; **9** (20 μM and 2000 μM). Lanes 9 and 10; **31** (20 μM and 2000 μM). Lanes 11 and 12; **29** (20 μM and 2000 μM). Lanes 13 and 14; **22** (20 μM and 2000 μM). Lane 15; $BrCH_2$-CO_2Me (23 μM). Lane 16; $BrCH_2$-$CONH_2$ (20 μM). Lane 17; Maxam-Gilbert G-reaction (post-label; see text). Lanes 18 and 19; **9** (20 μM and 2000 μM). Lane 20 and 21; **31** (20 μM and 2000 μM). Lane 22; $BrCH_2$-CO_2Me (20 μM). Lane 23; $BrCH_2$-$CONH_2$ (20 μM).

EXPERIMENTAL

General Methods. NMR spectra were recorded on JEOL-GX270 spectrometer. Unless otherwise stated, spectra were measured in $CDCl_3$ and are reported in ppm (δ value) relative to Me_4Si as the internal standard. For the compounds having t-butyldimethylsilyl group, $CHCl_3$ or CH_2Cl_2 was used as the internal standard. Following abbreviation are used for spin multiplicity; s = singlet, d = doublet, t = triplet, q = qualtet, sx = sextet, br = broad, m = multiplet. Optical rotations were measured on JASCO-DIP-370 photoelectric polarimeter. Silica gel column chromatography were performed on Merck Kieselgel 60 (Art 7734) or Wakogel C-300. All reactions were monitored by thin-layer chromatography on silica gel plates (Merck Kieselgel 60 PF_{254} Art 7749) with UV light and/or ca. 50% H_2SO_4-heat as developing agent. In general, solvents and reagents were purified by the appropriate procedure.[26] Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials unless otherwise stated. The substituents at the position 2 of the furanoside ring are numbered as C(2)-C(1')-C(2')-C(3'). Polynucleotide kinase (10 units/μl; No. 194645) and [γ-^{32}P]ATP (PB 10218) were purchased from Boehringer Mannheim and Amersham Life Science, respectively.

Methyl 2-deoxy-2-C-(2-propenyl)-α-D-arabinofuranoside (5). A mixture of **4** (20 g, 0.14 mol) and magnesium (20 g, 0.84 mol) in THF (450 ml) was stirred and cooled in an ice-salt bath under Ar atmosphere. To this was added dropwise a solution of allyl chloride (69 ml, 0.84 mol) in THF (50 ml) and the mixture was allowed to warm to room temperature. After the mixture had been stirred for 1 d, the reaction was quenched by the addition of saturated aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 (x 4; the organic phase = solution A) and concentrated under reduced pressure. Dichloromethane was added to the residue and undissolved salts were filtered off. The filtrate was combined to the solution A, dried ($MgSO_4$), and concentrated under reduced pressure. Silica gel column chromatography (ethyl acetate) gave **5** (22 g, 83%) as yellow syrup. NMR: 1.83 (dd, ^1H, $J_{5a,5\text{-}OH}$ = 6.59 Hz, $J_{5b,5\text{-}OH}$ = 5.61 Hz; 5-OH), 2.11-2.13 (m, 3H, J = 0.66 Hz, J = 3.63 Hz; 2-H and 1'-H), 2.23 (d, 1H, $J_{3,3\text{-}OH}$ = 8.57 Hz; 3-OH), 3.31 (s, 3H; CH_3), 3.65 (ddd, 1H, $J_{4,5a}$ = 4.95 Hz, $J_{5a,5b}$ = 11.54 Hz; 5-Ha), 3.72-3.83 (m, 1H; 5-Hb), 3.79 (ddd, 1H, $J_{3,4}$ = 5.28 Hz; 3-H), 3.93 (td, 1H, $J_{4,5b}$ = 3.63 Hz,; 4-H), 4.65 (br d, 1H, $J_{1,2}$ = 3.63 Hz; 1-H), 5.00-5.08 (m, 2H; 3'-H), 5.75 (ddt, 1H, J = 6.60 Hz, J = 10.22 Hz, J = 14.18 Hz; 2'-H).

Methyl 2-deoxy-5-O-(1-naphthoyl)-2-C-(2-propenyl)-α-D-arabinofuranoside (6). A solution of TPP (6.27 g, 23.9 mmol) in toluene (10 ml) was added dropwise to a solution of DEAD (4.16 g, 23.9 mmol) in toluene (10 ml) at 0 °C under N_2 and stirred for 45 min. To the solution was added a solution of **5** (3.00 g, 15.9 mmol) and 1-naphthalenecarboxylic acid (3.02 g, 17.5 mmol) in THF (20 ml) at 0 °C and stirred for 3.5 h during which time the temperature was allowed to warm to room temperature. The mixture was concentrated under reduced pressure and the residue was chromatographed (hexane-ethyl acetate = 3 : 1) to give **6** (syrup, 4.69 g, 86%). $[\alpha]_D^{25}$ +54.4° (c 1.05, $CHCl_3$). NMR: 2.16-2.26 (m, 3H; 2-H and 1'-H), 2.46 (d, 1H, $J_{3,3\text{-OH}}$ = 8.25 Hz; 3-OH), 3.42 (s, 3H; CH_3), 3.89 (ddd, 1H, $J_{2,3}$ = 2.64 Hz, 3-H), 4.30 (ddd, 1H, $J_{3,4}$ = 5.28 Hz; 4-H), 4.59 (d, 1H, $J_{4,5a}$ = 4.95 Hz, 5-Ha), 4.60 (d, 1H, $J_{4,5b}$ = 4.95 Hz; 5-Hb), 4.78 (d, 1H, $J_{1,2}$ = 0.66 Hz; 1-H), 5.06-5.16 (m, 2H; 3'-H), 5.79 (ddt, 1H, J = 6.60 Hz, J = 9.90 Hz, J = 17.8 Hz; 2'-H), 7.47-8.92 (m, 7H; aromatic-H).

Methyl 2-deoxy-5-O-(1-naphthalenesulfonyl)-2-C-(2-propenyl)-α-D-arabinofuranoside (7). To a solution of **5** (1.0 g, 5.3 mmol) in pyridine (5 ml) was added a solution of 1-naphthalenesulfonyl chloride (1.8 g, 8.0 mmol) in pyridine (7 ml) at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 24 h, followed by the addition of water, and extracted with CH_2Cl_2 (x 3). Drying ($CuSO_4$) followed by concentration and purification by column chromatography (hexane-ethyl acetate = 2 : 1) gave **7** (syrup, 1.5 g, 77%). NMR (C_6D_6): 1.58 [d, 1H, $J_{3,3\text{-OH}}$ = 6.27 Hz; 3-OH (in $CDCl_3$; 2.15 ppm, d, $J_{3,3\text{-OH}}$ = 8.24 Hz)], 1.79 [m, 2H; 1'-Ha and 1'-Hb), 2.01 (dddd, 1H, $J_{1,2}$ = 2.31 Hz, $J_{2,3}$ = 6.59 Hz, $J_{2,1'a}$ = 4.62 Hz, $J_{2,1'b}$ = 8.52 Hz; 2-H), 2.95 (s, 3H; CH_3), 3.51 (q, 1H, $J_{3,4}$ = 6.27 Hz; 3-H), 3.80 (ddd, 1H, $J_{4,5a}$ = 3.30 Hz, $J_{4,5b}$ = 4.29 Hz: 4-H), 3.94 (dd, 1H, $J_{5a,5b}$ = 11.2 Hz; 5-Ha), 4.04 (dd, 1H; 5-Hb), 4.18 (d, 1H; 1-H), 4.85-4.93 (m, 2H, 3'-H), 5.51 (ddt, 1H, $J_{2'3'a}$ = 17.1 Hz, $J_{2',3'b}$ = 10.6 Hz, $J_{1'a,2'}$ = $J_{1'b,2'}$ = 6.60 Hz; 2'-H), 6.85-8.99 (m, 7H; aromatic-H).

Methyl 2-deoxy-2-C-(3-hydroxypropyl)-5-O-(1-naphthoyl)-α-D-arabinofuranoside (8). To a stirred solution of 2-methyl-2-butene (1.55 mmol) in ether (1.81 ml) was slowly added borane-dimethyl sulfide complex (2 M in ether; 3.36 ml, 6.72 mmol) under N_2 at 0 °C and stirred for 4 h. To the reaction mixture was added a solution of **6** (1.00 g, 2.92 mmol) in ether (3 ml). After being stirred for 4.5 h, aqueous NaOH (1 M; 1 ml) and excess 35% H_2O_2 were sequentially added to the reaction mixture and the cooling bath was removed. Stirring was continued at room temperature for 1 h and the reaction was quenched by the addition of aqueous sodium thiosulfate (10%). The organic

phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed (hexane-ethyl acetate = 1 : 5) to give **8** (syrup; 0.86 g, 54%). NMR: 1.48-1.66 (m, 4H; 1'-H and 2'-H), 2.16 (ddt, 1H, $J_{1,2}$ = 1.98 Hz, $J_{2,3}$ = 4.29 Hz, $J_{2,1'}$ = 7.26 Hz; 2-H), 2.86 (d, 1H, $J_{3,3\text{-OH}}$ = 6.60 Hz; 3-OH), 3.42 (s, 3H; CH_3), 3.59 (t, 2H, $J_{2',3'}$ = 5.94), 3.84 (ddd, 1H, $J_{3,4}$ = 5.61 Hz; 3-H), 4.27 (dt, 1H, $J_{4,5}$ = 4.62 Hz; 4-H), 4.61 (d, 1H; 5-H), 4.75 (d, 1H; 1-H), 7.47-8.91 (m, 7H; aromatic-H).

Methyl 2-C-(3-bromoacetoxypropyl)-2-deoxy-5-O-(1-naphthoyl)-α-D-arabinofuranoside (9). A solution of DEAD (0.26 g, 1.50 mmol) in toluene (1 ml) was slowly added to a stirred solution of TPP (0.43 g, 1.63 mmol) in toluene (1 ml) at 0 °C under N_2 and stirred for 1.5 h. To this solution was sequentially added **8** (0.45 g, 1.25 mmol) in THF (2 ml) and bromoacetic acid (0.21 g, 1.20 mmol). The reaction mixture was slowly allowed to warm to room temperature, stirred for 2 h, evaporated, and chromatographed (benzene-ethyl acetate = 4 : 1) to give **9** (syrup; 0.32 g, 53%). NMR (ref = $CHCl_3$ = 7.26 ppm): 1.49-1.79 (m, 4H, 1'-H and 2'-H), 2.12 (ddt, 1H, $J_{1,2}$ = 1.98 Hz, $J_{2,1'}$ = 7.26 Hz; 2-H), 2.58 (d, 1=H, $J_{3,3\text{-OH}}$ = 8.24 Hz; 3-OH), 3.42 (s, 3H; CH_3), 3.77 (s, 2H; CH_2Br), 3.83 (ddd, 1H, $J_{2,3}$ = 4.29 Hz, $J_{3,4}$ = 5.28 Hz; 3-H), 4.07 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H), 4.26 (dt, 1H, $J_{3,4}$ = 5.28 Hz, $J_{4,5}$ = 4.29 Hz; 4-H), 4.61 (d, 2H; 5-H), 4.75 (d, 1H; 1-H), 7.47-8.90 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthoyl)-2-C-(2-propenyl)-α-D-arabinofuranoside (10). A solution of t-butyldimethylsilyl chloride (3.1 g, 20.6 mmol) in pyridine (30 ml) was slowly added to a solution of **6** (4.7 g, 13.7 mmol) and imidazole (2.34 g, 34.3 mmol) in pyridine (30 ml) under N_2 with stirring. After the mixture had been stirred for 3 d, the reaction was quenched with water. The resulting mixture was extracted with dichloromethane. The organic extract was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The trace of pyridine remained was removed by coevaporation with toluene (x 2). Column chromatography (hexane-ethyl acetate = 10 : 1) of the residue gave **10** (syrup; 5.25 g, 84%). NMR (ref = CH_2Cl_2 = 5.25 ppm): 0.02 (s, 6H; CH_3-Si), 0.84 [s, 9H; $(CH_3)_3$C-Si], 2.04-2.42 (m, 3H; 2-H and 1'-H), 3.36 (s, 3H; CH_3O), 3.85 (dd, 1H, $J_{2,3}$ = 4.62 ppm, $J_{3,4}$ = 7.26 ppm; 3-H), 4.19 (ddd, 1H, $J_{4,5a}$ = 2.64 Hz, $J_{4,5b}$ = 5.28 Hz; 4-H), 4.41 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.63 (dd, 1H; 5-Hb), 4.67 (s, 1H; 1-H), 4.98-5.05 (m, 2H, 3'-H), 5.74 (ddt, 1H, J = 6.60 Hz, J = 9.90 Hz, J = 17.8 Hz; 2'-H), 7.43-8.90 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthalenesulfonyl)-2-C-(2-propenyl)-α-D-arabinofuranoside (11). A solution of t-butyldimethylsilyl chloride (0.8 g, 5.3 mmol) in pyridine (10 ml) was slowly added to a solution of **7** (1.0 g, 2.6 mmol) and imidazole (0.44 g, 6.5 mmol) in pyridine (5 ml) under N_2 with stirring. After the mixture had been stirred for 3 d, the reaction was quenched with water. The resulting mixture was extracted with dichloromethane. The organic extract was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Column chromatography (hexane-ethyl acetate = 10 : 1) of the residue gave **11** (syrup; 0.85 g, 70%). NMR (ref = CH_2Cl_2 = 5.25 ppm): -0.17 (s, 3H; CH_3-Si), -0.095 (s, 3H; CH_3-Si), 0.715 [s, 9H; $(CH_3)_3$C-Si], 1.78 (m, 1H; 1'-Ha), 1.91 (dtd, 1H, $J_{2,3}$ = 4.95 Hz, $J_{2,1'a}$ = 9.56 Hz, $J_{2,1'b}$ = 4.95 Hz; 2-H), 2.11 (tddd, 1H, $J_{1'a,1'b}$ = 9.23 Hz, $J_{1',3'a}$ = 2.64 Hz, $J_{1',3'b}$ = 1.32 Hz; 1'-Hb), 3.07 (s, 3H; CH_3-O), 3.57 (dd, 1H, $J_{3,4}$ = 6.92 Hz; 3-H), 3.77 (ddd, 1H, $J_{4,5a}$ = 2.64 Hz, $J_{4,5b}$ =4.29 Hz), 4.04 (dd, 1H, $J_{5a,5b}$ = 11.2 Hz; 5-Ha), 4.09 (d, 1H; 1-H), 4.25 (dd, 1H; 5-Hb), 4.89-4.97 (m, 2H; 3'-H), 5.58 (dddd, 1H, $J_{2'3'a}$ = 18.1 Hz, $J_{2',3'b}$ = 9.56 Hz, $J_{2'1'a}$ = 7.58 Hz, $J_{2',1'b}$ = 6.27 Hz; 2'-H), 7.48-8.62 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-C-(3-hydroxypropyl)-5-O-(1-naphthoyl)-α-D-arabinofuranoside (12). To a stirred solution of 2-methyl-2-butene (0.59 ml, 6.57 mmol) in ether (0.8 ml) was slowly added a solution of borane-dimethyl sulfide complex in ether (2M, 1.65 ml, 3.29 mmol) at 0 °C under N_2. After being stirred for 3 h at this temperature, a solution of **10** (11.1 g, 2.19 mmol) in ether (3 ml) was added and stirred for 4.5 h. To this mixture was sequentially added aqueous NaOH (1M, 1 ml) and 35% H_2O_2. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. After the reaction had been quenched with aqueous sodium thiosulfate (10%), the resulting mixture was extracted with dichloromethane, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed (hexane-ethyl acetate = 3 : 2) to give **12** (0.57 g, 66%) and methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-C-(2-hydroxy-propyl)-5-O-(1-naphthoyl)-a-D-arabinofuranoside (**12a**: 0.06 g, 7%). NMR (ref = $CHCl_3$ = 7.26): 0.03 [s, 6H; $(CH_3)_2$Si], 0.84 [s, 9H; $(CH_3)_3$C-Si], 1.30 (t, 1H, $J_{3',3'\text{-OH}}$ = 5.28 Hz; 3'-OH), 1.42-1.72 (m, 4H; 1'-H and 2'-H), 2.06-2.15 (m, 1H; 2-H), 3.37 (s, 3H; CH_3O), 3.63 (dt, 2H, $J_{2',3'}$ =5.95; 3'-H), 3.85 (dd, 1H, $J_{2,3}$ = 5.61 Hz $J_{3,4}$ = 7.26 Hz; 3-H), 4.21 (ddd, 1H, $J_{4,5a}$ = 5.28, $J_{4,5b}$ = 2.31; 4-H), 4.44 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.68 (dd, 1H; 5-Hb), 4.70 (s, 1H; 1-H), 7.48-8.94 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-(3-hydroxypropyl)-5-O-(1-naphthalenesulfonyl)-α-D-arabinofuranoside (13). To a stirred ice cold solution

of **11** (0.3 g, 0.6 mmol) in THF (1.3 ml) was slowly added a solution of borane-dimethyl sulfide complex in ether (2 M; 0.8 ml) under N_2. The solution was allowed to warm to room temperature and stirred for 11 h. Hydrogen peroxide (35%; 0.16 ml, 1.8 mmol) and aqueous NaOH were added to the solution and stirred for 3 h. The reaction was quenched by the addition of aqueous sodium thiosulfate (10%). The resulting mixture was extracted with CH_2Cl_2 (x 3). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The products were separated by column chromatography (hexane-ethyl acetate = 3 : 1) to give the desired **13** (syrup; 0.36 g, 82%) and secondary alcohol **13a** (sirup; 7.5 mg, 1.7%). NMR (C_6D_6): 0.0246 (s, 3H; CH_3-Si), 0.0783 (s, 3H; CH_3-Si), 0.920 [s, 9H; $(CH_3)_3$C-Si], 1.32-1.64 (m, 4H, J = 2.31 Hz, 5.94 Hz, 14.2 Hz; 1'-Ha, 1'-Hb, and 2'-H), 2.15 (dddd, 1H, $J_{1,2}$ = 3.30 Hz, $J_{2,3}$ =5.94 Hz, $J_{2,1'a}$ = 4.95 Hz, $J_{2,1'b}$ = 10.6 Hz; 2-H), 3.11 (s, 3H; CH_3O), 3.34 (m, 1H; 3'-H) 3.80 (dd, 1H, $J_{3,4}$ = 6.93 Hz; 3-H), 3.97 (ddd. 1H, $J_{4,5a}$ = 2.31 Hz, $J_{4,5b}$ = 3.96 Hz; 4-H), 4.12 (dd, 1H, $J_{5a,5b}$ = 11.2 Hz, 5-Ha), 4.18 (d, 1H; 1-H) 4.33 (dd, 1H; 5-Hb); 6.95-9.12 (m, 7H; aromatic-H).

Methyl 2-C-(3-bromoacetoxypropyl)-3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthoyl)-α-D-arabinofuranoside (15). a) Reaction via oxido anion **14**: Potassium hydride (35% dispersion in mineral oil; 0.29 g, 2.5 mmol; washed twice with hexane and then with THF) was suspended in THF (1 ml) and stirred at 0 °C under Ar. To this mixture was slowly added a solution of **12** (0.31 g, 0.66 mmol) in THF (2 ml). After being stirred for 1.5 h, a solution of bromoacetyl bromide (0.11 ml, 1.26 mmol) was slowly added to the mixture and stirred at 0 °C for 1.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), evaporated, and column chromatographed (hexane-ethyl acetate = 5 : 1) to give **15** (0.12 g, 30%), **16** (0.06 g, 14%), and **17** (0.03 g, 9%). NMR: Compound **15** (ref = $CHCl_3$ = 7.26 ppm); 0.07 (s, 3H; CH_3-Si), 0.08 (s, 3H; CH_3-Si), 0.89 [s, 9H; $(CH_3)_3$C-Si], 1.38-1.81 (m, 4H; 1'-H and 2'-H), 2.04-2.13 (m, 1H; 2-H), 3.42 (s, 3H; CH_3O-), 3.80 (s, 2H; $BrCH_2$), 3.84 (dd, 1H, $J_{2,3}$ = 5.94 Hz; 3-H), 4.16 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H), 4.21 (ddd, 1H, $J_{3,4}$ = 7.28 Hz, $J_{4,5a}$ = 5.28 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.44 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.69 (d, 1H, J1,2 = 2.97 Hz; 1-H), 4.70 (dd, 1H; 5-Hb), 7.49-8.93 (m, 7H; aromatic-H). Compound **16** (ref = CH_2Cl_2 = 5.25 ppm); 0.00 (s, 3H; CH_3-Si), 0.02 (s, 3H; CH_3-Si), 0.81 [s, 9H; $(CH_3)_3$C-Si], 1.45-1.98 (m, 4H; 1'-H and 2'-H), 2.11 (m, 1H; 2-H), 3.39 (s, 3H; CH_3O-), 3.83, (dd, 1H, $J_{2,3}$ = 5.94 Hz; 3-H), 4.17 (ddd, 1H, $J_{3,4}$ = 7.59 Hz, $J_{4,5a}$ = 5.28 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.35 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H),

4.40 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.65 (dd, 1H; 5-Hb), 4.71 (d, 1H, $J_{1,2}$ = 2.97 Hz; 1-H). Compound **17** (ref = CH_2Cl_2 = 5.25 ppm); 0.06 (s, 3H; CH_3-Si), 0.08 (s, 3H; CH_3-Si), 0.88 [s, 9H; $(CH_3)_3$C-Si], 1.40-1.87 (m, 4H; 1'-H and 2'-H), 2.04 (m, 1H; 2-H), 3.39 (s, 3H; CH_3O), 3.74 (dd, 1H, $J_{2,3}$ = 5.94 Hz, $J_{3,4}$ = 7.26 Hz; 3-H), 3.83 (s, 2H; $BrCH_2$), 3.88 (d, 2H; BrCH2), 4.03 (ddd, 1H, $J_{4,5a}$ = 4.95 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.19 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.21 (t, 2H, $J_{2',3'}$ = 6.60 Hz; 3'-H), 4.46 (dd, 1H, 5-Hb), 4.63 (d, 1H, $J_{1,2}$ = 3.30 Hz; 1-H).

b) Reaction of **12** with bromoacetic acid, DEAD, and TPP: A solution of DEAD (0.15 g, 0.89 mmol) in toluene (3 ml) was added to a solution of TPP (0.23 g, 0.89 mmol) in toluene (2 ml) at 0 °C under N_2 and stirred for 2.5 h. To this was added sequentially solutions of **12** (0.28 g, 0.59 mmol) in THF (3 ml) and bromoacetic acid (0.11 g, 0.77 mmol) in toluene (3 ml) and the resulting solution was allowed to warm to room temperature. After being stirred for 1 d, the mixture was concentrated under reduced pressure and column chromatographed (hexane-ethyl acetate = 3 : 1) to afford **15** (0.17 g, 47%).

Methyl 2-C-(3-bromoacetoxypropyl)-3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthalenesulfonyl)-α-D-arabinofuranoside (18). Potassium hydride (35% in mineral oil; 0.14 g, 1.2 mmol) was successively washed with hexane (x 2) and THF (x 1) and suspended in THF (2 ml). To this mixture was added **13** (0.19 g, 0.37 mmol) and stirred at 0 °C for 30 min under N_2. A solution of bromoacetyl bromide (0.11 g, 0.56 mmol) in THF (0.5 ml) was slowly added to the mixture. After being stirred for 1 h, reaction was quenched by the addition of saturated aqueous NH_4Cl. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (x 3). The combined organic phase was dried ($MgSO_4$), concentrated, and column chromatographed (hexane-ethyl acetate = 3 : 1) to afford **18** (syrup: 0.18 g, 78%). NMR (ref = $CHCl_3$ = 7.26 ppm): -0.105 (s, 3H; CH_3-Si), -0.0362 (s, 3H; CH_3-Si), 0.768 [s, 9H; $(CH_3)_3$C-Si], 1.04-1.64 (m, 4H; 1'-H and 2'-H), 1.84 (m, 1H; 2-H), 3.12 (s, 3H; CH_3-O), 3.59 (dd, 1H, $J_{2,3}$ = 6.27 Hz, $J_{3,4}$ = 7.25 Hz; 3-H), 3.80 (ddd, 1H, $J_{4,5a}$ = 2.31 Hz, $J_{4,5b}$ = 3.96 Hz; 4-H), 3.82 (s, 2H; CH_2Br), 4.05 (d, 1H, $J_{1,2}$ = 3.30 Hz; 1-H), 4.09 (dd, 1H, $J_{5a,5b}$ = 11.5 Hz ; 5-Ha), 4.11 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H), 4.31 (dd, 1H; 5-Hb), 7.54-8.66 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthoyl)-2-C-(3-tosyloxypropyl)-α-D-arabinofuranoside (19). A solution of p-tosyl chloride (0.44 g, 2.28 mmol) in pyridine (3 ml) was slowly added to a solution of **12** (0.72 g, 1.52 mmol) in pyridine at 0 °C under N_2. The mixture was allowed to warm

to room temperature and stirred for 5.5 h. The reaction was quenched by the addition of water and the mixture was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), concentrated, and column chromatographed (hexane-ethyl acetate = 3 : 1) to give **19** (syrup, 0.61 g, 64%). $[\alpha]_D^{27}$ +37.2° (c 0.79, $CHCl_3$). NMR (ref = $CHCl_3$ = 7.26 ppm): 0.03 (s, 3H; CH_3-Si), 0.05 (s, 3H; CH_3-Si), 0.86 [s, 9H; $(CH_3)_3$C-Si], 1.34-1.82 (m, 4H; 1'-H and 2'-H), 1.95-2.05 (m, 1H; 2-H), 2.43 (s, 3H; $C\underline{H}_3$-C_6H_4-), 3.38 (s, 3H; CH_3-O), 3.79 (dd, 1H; $J_{2,3}$ = 5.94 Hz; 3-H), 4.01 (m, 2H; 3'-H), 4.16 (ddd, 1H, $J_{3,4}$ = 7.28 Hz, $J_{4,5a}$ = 5.28 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.40 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.62 (d, 1H, $J_{1,2}$ = 3.30 Hz; 1-H), 4.67 (dd, 1H, 1-H), 7.32 (d, 2H, J = 8.24 Hz; tosyl-H), 7.77 (d, 2H; tosyl-H), 7.49-8.92 (m, 7H; naphthyl-H).

Methyl 2-C-(3-azidopropyl)-3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthoyl)-α-D-arabinofuranoside (20). To a stirred solution of sodium azide (0.14 g, 2.14 mmol) and 15-crown-5-ether (0.09 ml, 0.14 mmol) in DMF (1.5 ml) was slowly added a solution of **19** (0.43 g, 0.68 mmol) in DMF (1.5 ml) at room temperature under Ar. After being stirred for 1 d, the reaction was quenched by the addition of water. The mixture was extracted with ether. The organic layer was dried ($MgSO_4$), evaporated under reduced pressure, and column chromatographed (hexane-ethyl acetate = 10 : 1) to give **20** (syrup, 0.31 g, 90%). $[\alpha]_D^{26}$ +44.9° (c 0.99, $CHCl_3$). IR (liquid film on KBr): 2130 cm^{-1}; N_3. NMR (ref = $CHCl_3$ = 7.26 ppm): 0.08 [s, 6H; $(CH_3)_2$Si], 0.89 [s, 9H; $(CH_3)_3$C-Si], 1.40-1.74 (m, 4H; 1'-H and 2'-H), 2.03-2.12 (m, 1H; 2-H), 3.26 (m, 2H; 3'-H), 3.42 (s, 3H; CH_3O), 3.84 (dd, 1H; $J_{2,3}$ = 5.94 Hz, $J_{3,4}$ = 7.28 Hz; 3-H), 4.20 (ddd, 1H, $J_{4,5a}$ = 5.28 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.44 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; H-5a), 4.69 (d, 1H, $J_{1,2}$ = 3.30 Hz; 1-H), 4.69 (dd, 1H; H-5b), 7.49-8.90 (m, 7H; aromatic-H).

Methyl 2-C-(3-aminopropyl)-3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-(1-naphthalenesulfonyl)-α-D-arabinofuranoside (21). To a two-phase solution of **20** (0.15 g, 0.30 mmol) and hexadecyltributylphosphonium bromide (0.015 g, 0.03 mmol) in toluene (0.5 ml)-water (0.03 ml) was added a solution of $NaBH_4$ (0.06 g, 1.65 mmol) in water (0.07 ml) at room temperature with vigorous stirring. After being stirred for 1.5 h at this temperature, the mixture was stirred at 40 °C-50 °C for 48 h with vigorous stirring, and then extracted with CH_2Cl_2 (x 3). The combined organic layer was dried ($MgSO_4$), evaporated, and column chromatographed (hexane-ethyl acetate = 4 : 1) to give **21** (syrup, 0.052 g, 37%). NMR (ref = $CHCl_3$ = 7.25 ppm): 0.0499 (s, 3H; CH_3-Si), 0.0661 (s, 3H; CH_3-Si), 0.874 [s, 9H, $(CH_3)_3$C-Si], 1.55-1.48 (m, 4H; 1'-H and 2'-H), 2.00-2.09 (m, 1H; 2-H), 2.72 (dt, 2H, $J_{2',3'}$ = 7.23 Hz, $J_{3',NH2}$ = 7.23 Hz; 3'-H), 3.41 (s, 3H;

CH_3O), 3.56 (br s, 2H; NH_2), 3.82 (dd, 1H, $J_{2,3}$ = 6.32 Hz, $J_{3,4}$ = 7.23 Hz; 3-H), 4.18 (ddd, 1H, $J_{4,5a}$ = 2.26 Hz, $J_{4,5b}$ = 4.97 Hz; 4-H), 4.44 (dd, 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.66 (br s, 1H; 1-H), 4.69 (dd, 1H; 5-Hb), 7.48-8.89 (m, 7H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-C-[(3-bromoacetamido)propyl]-5-O-(1-naphthoyl)-α-D-arabinofuranoside (22). To an ice-cold solution of **21** (0.04 g, 0.08 mmol) in THF (2 ml) was added a solution of DCC (0.021 g, 0.1 mmol) and bromoacetic acid (0.014 g, 0.1 mmol) in THF (3 ml) and stirred for 3 h at this temperature. Water was added and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic layer was dried ($MgSO_4$), evaporated, and column chromatographed ($CHCl_3$-ethyl acetate = 10 : 1) to give **22** (syrup; 0.03 g, 60%). NMR (ref = $CHCl_3$ = 7.25 ppm): 0.0575 (s, 3H; CH_3-Si), 0.0624 (s, 3H; CH_3-Si), 0.873 [s, 9H; $(CH_3)_3$C-Si], 1.52-1.72 (m, 4H; 1'-H and 2'-H), 2.01-2.10 (m, 1H; 2-H), 3.26 (q, 2H, $J_{2',3'}$ = $J_{3',NH2}$ =3.26 Hz; 3'-H), 3.41 (s, 2H; CH_3-O), 3.82 (dd, 1H, $J_{2,3}$ = 5.94 Hz, $J_{3,4}$ = 7.58 Hz; 3-H), 3.85 (s, 2H; Br-CH_2), 4.19 (ddd, 1H, $J_{4,5a}$ = 5.28 Hz, $J_{4,5b}$ = 2.31 Hz; 4-H), 4.42 (dd; 1H, $J_{5a,5b}$ = 12.2 Hz; 5-Ha), 4.68 (dd, 1H; 5-Hb), 4.68 (d, 1H; $J_{1,2}$ = 2.97 Hz; 1H), 6.48 (br s, 1H; NH). 7.48-8.91 (m, 7H; aromatic-H).

Methyl 2,3-anhydro-5-O-trityl-α-D-ribofuranoside (23). A solution of **4** (1.8 g, 12 mmol) and trityl chloride (5.2 g, 19 mmol) in pyridine (20 ml) was stirred at room temperature for 1 d. Water was added and the resulting mixture was extracted with CH_2Cl_2 (x 4). The combined organic layer was dried ($MgSO_4$), evaporated, and column chromatographed (hexane-ethyl acetate = 5 : 1) to afford **23** as white crystals which were recrystallized from hexane; 4.8 g, 79%, mp 125 °C. NMR: 3.13 (dd, 1H, $J_{4,5a}$ = 2.97 Hz, $J_{5a,5b}$ = 10.22 Hz; H-5a), 3.38 (dd, 1H, $J_{4,5b}$ = 4.29 Hz; H-5b), 3.56 (s, 3H; CH_3O), 3.65 (d, 1H, $J_{2,3}$ = 2.97 Hz; 3-H), 3.85 (dd, 1H; $J_{1,2}$ = 0.66 Hz; 2-H), 4.38 (dd, 1H; 4-H), 5.36 (br s, 1H; 1-H), 7.23-7.43 (m, 15H; aromatic-H).

Methyl 2-deoxy-2-C-(2-propenyl)-5-O-trityl-α-D-arabinofuranoside (25). A mixture of **23** (3.6 g, 9.3 mg) and Mg (2.4 g, 93 mmol) in THF (30 ml) was cooled in an ice-salt bath, and magnetically stirred under Ar. To this was added slowly a solution of 2-propenyl chloride (7.6 ml, 93 mmol) in THF (20 ml) and then the mixture was allowed to room temperature. After being stirred for 1 d, ice-water and saturated aqueous NH_4Cl was added and then extracted with CH_2Cl_2 (x 4). The combined organic layer was dried ($MgSO_4$), evaporated to give **25** as a yellow syrup (4.03 g, quantitative yield) which was used in the next step without purification. NMR: 2.01-2.21 (m, 3H; 2-H, 1'-Ha, and 1'-Hb), 2.42 (d, 1H, $J_{3,OH}$ = 7.91 Hz; 3-OH), 3.19 (d, 1H, $J_{4,5a}$ = 5.28

Hz, $J_{5a,5b}$ = 9.89 Hz; H-5a), 3.34 (d, 1H, $J_{4,5b}$ = 5.28 Hz; H-5b), 3.67 (s, 3H; CH_3O), 3.74 (ddd, 1H, $J_{2,3}$ = 2.97 Hz, $J_{3,4}$ = 4.95 Hz; 3-H), 4.09 (q, 1H; 4-H), 4.71 (d, 1H, $J_{1,2}$ = 0.66 Hz; 1-H), 5.00 (m, 2H; 3'-Ha and 3'-Hb), 5.76 (ddt, 1H, J = 6.60 Hz, J = 13.19 Hz, J = 17.15 Hz; 2'-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-C-(2-propenyl)-5-O-trityl-α-D-arabinofuranoside (27). To a solution of **25** (4.3 g, 10 mmol) and imidazole (1.7 g, 2.5 mmol) in pyridine (60 ml) was added a solution of t-butyldimethylsilyl chloride (2.3 g, 15 mmol) in pyridine at room temperature. After being stirred at room temperature for 3 d, saturated aqueous $NaHCO_3$ was added and extracted with CH_2Cl_2 (x 4). The combined organic layer was dried ($MgSO_4$), evaporated, and column chromatographed (hexane-ethyl acetate = 20 : 1) to give **27** (syrup, 4.6 g, 84%). NMR (the Si-CH_3 signal resonated at lower field was taken as 0 ppm): 0.00 (s, 3H; CH_3-Si), 0.21 (s, 3H; CH_3-Si), 1.01 [s, 9H; $(CH_3)_3$C-Si], 2.35-2.65 (m, 3H; 2-H, H-1'a, and H-1'b), 3.30 (dd, 1H, $J_{4,5a}$ = 5.28 Hz, $J_{5a,5b}$ = 10.22 Hz; H-5a), 3.63 (dd, 1H, $J_{4,5b}$ = 2.64 Hz; H-5b), 3.71 (s, 3H; CH_3O-), 4.08 (dd, 1H, $J_{2,3}$ = 4.61 Hz, $J_{3,4}$ = 6.93 Hz; H-3), 4.30 (ddd, 1H; H-4), 5.01 (d, 1H, $J_{1,2}$ = 2.31 Hz; 1-H), 6.09 (m, 1H; 2'-H), 7.46-7.78 (m, 15H; aromatic-H).

Methyl 3-O-(t-butyldimethylsilyl)-2-deoxy-2-C-(3-hydroxypropyl)-5-O-trityl-α-D-arabinofuranoside (28). To an ice-cold solution of **27** (1.1 g, 2.0 mmol) in THF (7 ml) was slowly added a solution of borane-dimethylsulfide complex in THF (2 M, 3 ml, 6 mmol) under Ar with stirring. After being stirred at room temperature for 1 d, aqueous NaOH (3M, 2 ml) and 35% H_2O_2 (0.5 ml) was sequentially added and stirred for 7 h. The reaction was quenched by the addition of 10% aqueous sodium thiosulfate. The resulting mixture was extracted with $CHCl_3$ (x 4). The combined organic layer was dried ($MgSO_4$), evaporated, and column chromatographed (hexane-ethyl acetate = 7 : 2) to give **28** (0.73 g, 65%). NMR (ref = CH_2Cl_2 = 5.25 ppm): -0.33 (s, 3H; CH_3-Si), -0.11 (s, 3H, CH_3-Si), 0.69 [s, 9H; $(CH_3)_3$C-Si], 1.29 (t, 1H, $J_{3',OH}$ = 5.61 Hz; 3-OH), 1.40-1.65 (m, 4H; 1'-H and 2'-H), 1.97 (m, 1H; 2-H), 2.97 (dd, 1H, $J_{4,5a}$ = 5.28 Hz, $J_{5a,5b}$ = 10.22 Hz; H-5a), 3.30 (dd, 1H, $J_{4,5b}$ = 2.31 Hz; 5-Hb), 3.41 (s, 3H; CH_3O), 3.62 (q, 2H, $J_{2',3'}$ = 5.28 Hz; H-3'), 3.71 (dd, 1h, $J_{3,4}$ = 7.25 Hz; 3-H), 3.96 (ddd, 1H; 4-H), 4.69 (d, 1H, $J_{1,2}$ = 2.97 Hz; 1-H), 7.14-7.45 (m, 15H; aromatic-H).

Methyl 2-C-(3-bromoacetoxypropyl)-3-O-(t-butyldimethylsilyl)-2-deoxy-5-O-trityl-α-D-arabinofuranoside (29). A solution of DEAD (0.16 g, 0.91 mmol) in toluene (1 ml) was slowly added to a solution of TPP (0.28 g, 1.08 mmol) in toluene (1 ml) at 0 °C under Ar and stirred for 75 min. To this solution

were sequentially added a solution of **28** (0.32 g, 0.57 mmol) in THF (2 ml) and a solution of bromoacetic acid (0.11 g, 0.80 mmol) in toluene (1 ml). After the reaction solution was allowed to warm to room temperature and stirred for 3.25 h, the solvent was removed under reduced pressure. The residue was separated by column chromatography (hexane-ethyl acetate = 10 : 1) to afford **29** (syrup, 0.14 g, 36%). NMR (ref = CH_2Cl_2 = 5.25 ppm): -0.33 (s, 3H; CH_3-Si), -0.12 (s, 3H; CH_3-Si), 0.69 [s, 9H; $(CH_3)_3C$-Si], 1.33-1.80 (m, 4H; 1'-H, 2'-H), 1.91-2.01 (m, 1H; 2-H), 2.97 (dd, 1H, $J_{4,5a}$ = 5.28 Hz, $J_{5a,5b}$ = 10.2 Hz; 5-Ha), 3.31 (dd, 1H, $J_{4,5b}$ = 2.31 Hz; H-5b), 3.41 (s, 3H; CH_3O), 3.70 (dd, 1H, $J_{2,3}$ = 5.94 Hz, $J_{3,4}$ = 7.26 Hz; 3-H), 3.76 (s, 2H; $BrCH_2$),3.96 (ddd, 1H; 4-H), 4.15 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H), 4.68 (d, 1H, $J_{1,2}$ = 3.30 Hz; 1-H), 7.15-7.45 (m, 15H; aromatic-H).

Methyl 2,3-anhydro-5-O-methyl-α-D-ribofuranoside (24). To a stirred mixture of **4** (2.0 g, 14 mmol) and Ag_2O (7.5 g, 32 mmol) in acetonitrile (20 ml) was added a solution of methyl iodide (2.1 ml, 32 mmol) in acetonitrile (5 ml) at room temperature. After being stirred for 64 h, water was added and the precipitate was filtered off. The organic solution was separated and the aqueous phase was extracted with CH_2Cl_2 (x 3). The combined organic phase was dried ($MgSO_4$), concentrated, and column chromatographed (hexane-ethyl acetate = 1 : 2) to give **24** (syrup, 2.0 g, 90%). $[\alpha]_D^{25}$ +12.1° (c 1.11, $CHCl_3$). NMR: 3.36 (s, 3H; CH_3O), 3.49 (s, 3H; CH_3O), 3.48 (d, 2H, $J_{4,5}$ = 3.63 Hz; H-5), 3.64 (d, 1H, $J_{2,3}$ = 2.97 Hz, 3-H), 3.70 (dd, 1H, $J_{1,2}$ = 0.33 Hz; 2-H), 4.31 (t, 1H; 4-H), 5.11 (d, 1H; 1-H).

Methyl 2-deoxy-5-O-methyl-2-C-(2-propenyl)-α-D-arabinofuranoside (26). To a mixture of **24** (2.0 g, 13 mmol) and Mg (1.5 g, 62 mmol) in THF (20 ml) was slowly added a solution of allyl chloride (5.1 ml, 62.4 mmol) in THF (20 ml) at -10 °C (ice-salt bath) under N_2. The mixture was allowed slowly to warm to room temperature, and stirring was continued at room temperature for 21.5 h. The reaction was quenched with saturated aqueous NH_4Cl solution. The mixture was extracted with CH_2Cl_2 (x 3). The combined organic phase was dried ($MgSO_4$), concentrated, and column chromatographed (hexane-ethyl acetate = 1 : 2) to give **26** (syrup, 1.8 g, 72%). $[\alpha]_D^{26}$ +82.9° (c 1.02, $CHCl_3$). NMR (C_6D_6) 2.01 (tdt, 1H, $J_{1'a,1'b}$ = 6.92 Hz, $J_{2,1'a}$ = 8.90 Hz; 1'-H), 2.05 (d, 1H, $J_{3,3\text{-OH}}$ = 7.58; 3-OH), 2.11 (qt, 1H; 1'-Hb), 2.25 (dddd, 1H, $J_{1,2}$ = 1.98 Hz, $J_{2,3}$ = 4.29 Hz, $J_{2,1'b}$ = 6.92 Hz; 2-H), 3.06 (s, 3H; CH_3O), 3.17 (s, 3H; CH_3O), 3.36 (dd, 1H, $J_{4,5a}$ = 4.62 Hz, $J_{5a,5b}$ = 10.2 Hz; 5-Ha), 3.41 (dd, 1H, $J_{4,5b}$ = 4.62 Hz; 5-Hb), 3.77 (ddd, 1H, $J_{3,4}$ = 5.61 Hz; 3-H), 4.13 (dt, 1H; 4-H), 4.63 (d, 1H; 1-H), 4.96 (ddt, 1H,

$J_{1'a,3'a}$ = $J_{1'b,3'a}$ = 0.99 Hz, $J_{3'a,3'b}$ = 17.1 Hz, $J_{2',3'a}$ = 10.2 Hz; 3'-Ha), 5.01 (dq, 1H, $J_{1'a,3'b}$ = $J_{1'b,3'b}$ = $J_{2',3'b}$ = 1.65 Hz; 3'-Hb), 5.69 (ddt, 1H, $J_{1'a,2'}$ = $J_{1'b,2'}$ = 6.60 Hz; 2'-H).

Methyl 2-deoxy-2-C-(3-hydroxypropyl)-5-O-methyl-α-D-arabinofuranoside (30). To a solution of **26** (0.40 g, 2 mmol) in THF (5 ml) was slowly added a solution of borane-dimethylsulfide complex in ether (2 M, 3 ml, 6 mmol) at -10 °C. Stirring was continued for 3.5 h and then allowed to warm to room temperature. The mixture was recooled to 0 °C, and aqueous NaOH solution (3M, 0.8 ml) and 35% H_2O_2 (0.51 ml, 6 mmol) were added. After being stirred for 1 h, reaction was quenched with aqueous sodium thiosulfate. The resulting mixture was extracted with CH_2Cl_2 (x 3). The combined organic phase was dried ($MgSO_4$), concentrated, and column chromatographed (ethyl acetate) to give **30** (syrup, 0.11g, 25%). NMR ($CDCl_3$): 1.49-1.58 (m, 2H; 1'-H), 1.63-1.74 (m, 2H; 2'-H), 2.10 (tdd, 1H, $J_{1,2}$ = 1.98 Hz, $J_{2,3}$ = 3.96 Hz, $J_{2,1'}$ = 7.58 Hz; 2-H), 3.39 (s, 3H; CH_3O), 3.41 (s, 3H; CH_3O), 3.54 (dd, 1H, $J_{4,5a}$ = 5.61 Hz, $J_{5a,5b}$ = 10.2 Hz; 5-Ha), 3.58 (dd, 1H, $J_{4,5b}$ = 4.29 Hz; 5-Hb), 3.69 (t, 2H, $J_{2',3'}$ = 6.27 Hz; 3'-H), 3.73 (br-dd, 1H, $J_{2,3}$ = 3.96 Hz, $J_{3,4}$ = 5.61 Hz; 3-H), 4.04 (ddd, 1H; 4-H), 4.72 (d, 1H; 1-H).

Methyl 2-C-(3-bromoacetoxypropyl)-2-deoxy-5-O-methyl-α-D-arabinofuranoside (31). A solution of DEAD (0.05 ml, 0.30 mmol) in toluene (0.5 ml) was added to a solution of TPP (0.08 g, 0.30 mmol) in toluene (0.5 ml) cooled in an ice-salt bath and stirred for 1 h. To this was added a solution of **30** (0.043 g, 0.20 mmol) and bromoacetic acid (0.04 g, 0.30 mmol) in THF (1 ml) and the resulting solution was allowed to warm to room temperature. After being stirred for 2 h, the solution was evaporated, followed by column chromatography (benzene-ethyl acetate = 1 : 1) to give **31** (syrup, 0.021 g, 31%). NMR: 1.51-1.56 (m, 2H; 1'-H), 1.73-1.84 (m, 2H; 2'-H), 2.07 (tdd, 1H, $J_{1,2}$ = 1.65 Hz, $J_{2,3}$ = 3.96 Hz, $J_{2,1'}$ = 7.91 Hz; 2-H), 3.42 (s, 3H; CH_3O), 3.56 (d, 2H, $J_{4,5}$ = 5.28 Hz; 5-H), 3.73 (dd, 1H, $J_{3,4}$ = 5.28 Hz, 3-H), 3.84 (s, 2H; $BrCH_2$), 3.89 (s, 3H, CH_3O), 4.04 (q, 1H; 4-H), 4.73 (d, 1H; 1-H).

End labelling of 5'-d-GATAAGCTTGAATTCATGGCC-3' (32). A mixture of 2 μl of the 21mer (100 pmol/μl), 10μl of ATP (10 pmol/μl), 5 μl of [γ-^{32}P]ATP (2 pmol/μl), 1 μl of T4 polynucleotide kinase, and 2 μl 10× kinase buffer was incubated at 37 °C for 1 h. The solution was subjected to 7 M urea/17% polyacrylamide gel electrophoresis. The location of the labeled 21mer band was detected by autoradiography. The gel containing the labeled 21mer was cut out and was soaked in H_2O (500 μl) at room temperature (overnight).

The mixture was centrifuged and supernatant was collected. The remained gel segments were again immersed in H_2O and centrifuged (200 μl ×2). The precipitated gel had almost no radio activity by measurement using GM-counter. The combined supernatant was applied to Oligo-Pak® column (MilliGen/Biosearch). After the column was washed with H_2O (10 ml), the labeled 21mer was successively eluted with 20% CH_3CN in H_2O (1 ml ×2) and 100% CH_3CN (1 ml ×1). The combined eluant was concentrated to dryness. By preliminary measurement of radio activity of each aliquot using GM-counter through microtube, the overall yield for labeling experiment of the oligomer was estimated to be about 50%. The residue was dissolved in H_2O (1000 μl), divided into 20 Eppendorf tubes (1.5 ml), and again concentrated to dryness. The each residue was dissolved in 50 μl of phosphate buffer (50 mM; pH 7). By the estimation of the yield, each microtube contains 5 pmol of the 5'-end labeled 21mer.

Oligonucleotide cleavage assay. To 50 μl of a solution of a cleaving reagent (20 μM or 2000 μM) in acetonitrile was added 50 μl of the 5'-end-labeled 21mer (**32**) in phosphate buffer (pH 7) and incubated at 37 °C for 3 d or at 60 °C for 6 h. Chloroform (100 μl) was added to the solution and centrifuged. The aqueous phase was separated, washed with butanol (50 μl x 2), and evaporated at room temperature under reduced pressure. The residue was incubated with 10% piperidine in H_2O (100 μl) at 90 °C for 30 min. After evaporation, the residue was dissolved in H_2O (10 μl) and loaded onto 17% polyacrylamide gel [acryl amide/N,N'-methylenebis(acrylamide) = 19 : 1 w/w] containing 7 M urea. The gel was subjected to electrophoresis in a buffer (tris-boric acid) and exposed to Fuji RX X-ray film.

Maxam-Gilbert G-reaction (post-label). One hundred μl of 10 μM solution of the 21mer (**32**) in buffer containing 50 mM sodium cacodylate (pH 7.0) and 1 mM EDTA (pH 8.0) was incubated with 5 μl of 3% dimethyl sulfate in EtOH at room temperature for 20 min and quenched by the addition of "stop solution" (50 μl) containing 1.5 M sodium acetate (pH 7.0) and 1 M 2-mercaptoethanol. To the solution was added 4M sodium acetate (pH 5.2; 15 μl) and ethanol (-20 °C; 340 μl). The mixture was allowed to stand at -80 °C for 30 min and centrifuged at 15000 rpm for 30 min at -15 °C. The supernatant was removed and the pellet was rinsed with 70% aqueous ethanol (-20 °C, 100 μl), centrifuged, and dried at room temperature under vacuum. The dried pellet was dissolved in 10% piperidine-H_2O (100 μl) and incubated at 90 °C for 30 min. The solution was evaporated at room temperature under

vacuum. 5'-^{32}P end-labeling was accomplished with T4 polynucleotide kinase and [γ-^{32}P]ATP. Gel electrophoresis was performed as described above.

Acknowledgment. This work was supported in part by Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan, and a grant from the Research Institute of Aoyama Gakuin University.

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Received February 17, 1994
Accepted May 30, 1994