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Synthesis and properties of 2-deoxy-2-fluoromannosyl phosphate derivatives

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ABSTRACT

2-Deoxy-2-fluoromannosyl phosphotriester and phosphodiester derivatives were synthesized and they were demonstrated to be more stable than the 2-OH compounds under acidic conditions. 2-Fluoro substituted analog of α-mannopyranosyl 1-phosphodiester **20** was found to be significantly stable at pH 1, where 2-OH compound **21** was gradually hydrolyzed. In addition, 2-F analogs of α-mannopyranosyl 1-phosphotriesters also was found to be stable under both Brønsted and Lewis acidic conditions. These results strongly suggested that the 2-F substitution is useful for both the synthesis of mannosyl phosphodiester derivatives and improvement of their chemical stability.

1. Introduction

The α-glycosyl phosphate unit, which has a phosphodiester linkage between the anomeric carbon in one sugar and a carbon atom in another sugar, is known as a constituent of capsular polysaccharides (CPS) in pathogenic bacteria such as *Neisseria meningitidis* and *Streptococcus pneumoniae*,[1] and the glycocalyx of parasitic protozoans such as *Leishmania* and *Trypanosoma*.[2] These units are considered to be important components in biological phenomena including immunological responses and infection.[3] Furthermore, there have been some reports in which some phosphoglycans have shown vaccine activity, and vaccines based on them have been developed.[4]

Therefore, stable provision of pure compounds containing the α-glycosyl phosphate structure is useful for both the elucidation of their functions and the development of α-glycosyl phosphate-based vaccines and drugs. However, this remains a challenge for both biologists and chemists, because sugar chains in living systems have structural heterogeneity and are present in very small quantities; furthermore, their structures and their synthetic intermediates are chemically unstable in some cases.[5] Therefore, chemical modification has been spotlighted as a method for the stable supply of pure compounds containing the α-glycosyl phosphate structure.[6] In this study, we focus on 2-deoxy-2-fluoromannopyranosyl 1-phosphate derivatives as glycosyl phosphate analogs.

Derivatives of α-mannopyranosyl 1-phosphate can be seen in the glycocalyx of *Leishmania*[1a] and *Hansenula capsulata* Y-1842 exophosphomannan,[7] and there are some derivatives containing α-2-*N*-acetylmannosamine 1-phosphate units in CPS in *Neisseria meningitidis* (Fig. 1).[1a] In this study, we chose the Man-α-(1 → 6)-*P*-Man structure as a synthetic target in the model compounds, poly-(mannopyranosyl 1-phosphates), in *Hansenula capsulata* Y-1842 exophosphomannan.

A fluorine atom is electronically equivalent to a hydroxyl group. Indeed, 2-deoxy-2-(^{18}F)fluoro-D-glucose is widely used as a glucose analog for positron emission tomography (PET),[8] and fluorinated sugars have been reported as sugar analogs that serve as substrates or inactivators for glycosidases, glycosyltransferases, and other enzymes.[9] Furthermore, a fluorine atom shows strong electron withdrawing properties, and it can be expected to be advantageous for the stabilization of α-glycosyl phosphate structures and their synthetic intermediates. Some glycosyl 1-P structures and their synthetic intermediates are degraded mainly through elimination of the phosphorous containing groups at the anomeric position and generation of the glycosyl oxocarbenium ion.[5,10] A fluorine atom at the 2-position of a pyranose can destabilize such a cation and make α-glycosyl phosphate structures and their intermediates harder to chemically degrade.

Therefore, we firstly aimed to synthesize mannopyranosyl 1-

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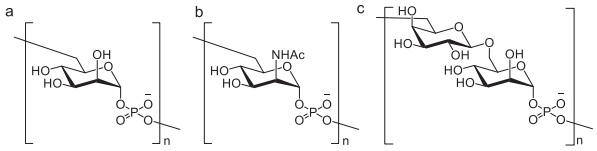


Fig. 1. α-mannopyranosyl 1-phosphate repeating units seen in (a) *Hansenula capsulata* Y-1842 exophosphomannan (b) CPS from *Neisseria meningitidis* (c) *Leishmania* glycocalyx.

diethylphosphate and 2-deoxy-2-fluoromannopyranosyl 1-diethylphosphate derivatives as analogs of the synthetic intermediates using the phosphoramidite method, which is one of the most commonly used synthetic methods of synthesizing α-glycosyl phosphate derivatives.[1a] Secondly, we synthesized α-glycosyl phosphate derivatives containing an α-Man-(1-PO_4^--6)-Man structure. Furthermore, the chemical stabilities of mannopyranosyl and 2-deoxy-2-fluoromannopyranosyl derivatives were compared.

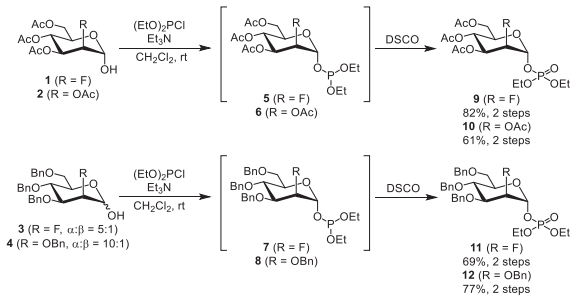
2. Results and discussion

2.1. Synthesis of phosphotriester analogs

Compounds **1**–**4** were known compounds and their synthetic procedures are described in the literature.[11] The anomeric hydroxyl group of each compound was phosphitylated using diethyl chlorophosphite to afford compounds **5**–**8**. All compounds were subsequently oxidized with (+)-(8,8-dichlorocamphorylsulfonyl) oxaziridine (DCSO) and then mannosyl α-1-diethylphosphate derivatives **9**–**12** were obtained in good yields (82%, 61%, 69%, 77% respectively).[12] Incidentally, the β anomer was not observed in these reactions except in the synthesis of compound **12**, in which the β anomer was isolated in 21% yield (Scheme 1).

2.2. Stability of mannosyl phosphotriesters under acidic conditions

A glycosyl phosphotriester structure is an intermediate in the synthesis of glycosyl phosphate derivatives in the phosphoramidite method. One of the most serious concerns regarding these reactions is the instability of the phosphotriester intermediates under acidic conditions, such as in deprotection. Therefore, the stability of mannosyl phosphotriesters **9**–**12** in dichloromethane containing 3% (v/v) dichloroacetic acid (DCA), which is prescribed for the removal of trityl type protecting groups,[13] was evaluated (Table 1, left column). In these experiments, the reaction time was 24 h, and the unreacted phosphotriester was recovered by silica gel column chromatography. Most large quantities of 2-F and 2-OAc compounds **9**–**11** remained intact under the conditions and 69–79% of them were recovered. On the other hand, 2-OBn compound **12** was slowly degraded (almost completely disappeared in 24 h by TLC monitoring, see SI) and nothing was recovered after the reaction. The major degraded products were hydrolyzed product **4** and anomeric dichloro acetate. By comparing the results for **9**–**11** and **12**, it is clearly demonstrated that electron withdrawing 2-F and 2-OAc substituents stabilized the mannosyl phosphotriesters in the presence of dichloroacetic acid.



Scheme 1. Synthesis of mannopyranosyl 1-diethylphosphate and 2-deoxy-2-fluoromannopyranosyl 1-diethylphosphate derivatives.

Next, the stability of these compounds under Lewis acidic conditions was also investigated (Table 1, right column). In these experiments, dichloromethane containing 0.1 M of $BF_3{\cdot}Et_2O$ was used to achieve Lewis acidic conditions. Under the conditions, the 2-*O*-benzyl mannosyl phosphotriester **12** was completely degraded in 1 h (see SI). Furthermore, 2-*O*-acetyl one **10** was slowly degraded, and nothing was recovered after 24 h of exposure to the acidic solution. On the other hand, 2-fluoro mannosyl phosphotriesters **9** and **11** were observed to be intact even after 24 h, and 83% and 71% of **9** and **11**, respectively, were recovered. From these results, it was shown that introducing a fluoro group instead of an OAc or OBn groups at the 2-position of mannopyranose is much effective for stabilization of mannosyl phosphotriesters under both Brønsted and Lewis acidic conditions.

2.3. Synthesis of mannosyl phosphates

Next, we synthesized compounds containing the Man-α-(1 → 6)-*P*-Man structure as analogs of *Hansenula capsulata* Y-1842 exophosphomannan. Firstly, mannose derivatives containing a hydroxyl group on their anomeric carbon atom, **13** and **14**, were prepared. Compound **13** was synthesized from a known acetyl-protected compound (see SI),[11a] and **14** was prepared by a procedure described in the literature.[14] Compounds **13** and **14** were phosphitylated using 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to afford **15** and **16** in 86% and 73% yields, respectively. The 6-hydroxy compound of thiomannoside **17** was prepared by slight modification of a procedure described in the literature.[15] In the condensation reaction of phosphoramidite monomer **16** with alcohol **17**, the reaction did not proceed efficiently and over 80% of unreacted **17** was recovered when 1-*H*-tetrazole was used as an acidic activator. These condensation reactions proceeded well when 5-ethylthio-1-*H*-tetrazole (ETT) was used as a more acidic activator,[16] and subsequently oxidation using *tert*-butyl hydroperoxide[17] afforded Man-α-(1 → 6)-*P*-Man precursors **18** and **19** in good yield as shown in Scheme 2. The cyanoethyl groups on compounds **18** and **19** were removed with triethylamine and the benzoyl groups were subsequently removed in the presence of ammonia to afford the ammonium salts of the Man-α-(1 → 6)-*P*-Man derivatives. These compounds were purified by reversed phase HPLC (RP-HPLC) and were finally transformed to the sodium salts on ion-exchange resin to afford compounds **20** and **21** (41% and 26% yields, respectively) (see Scheme 3).

2.4. Stability of Man-α-(1 → 6)-P-Man structures under acidic conditions

To evaluate the properties of α-2-deoxy-2-fluoromannosyl 1-phosphate **20** and α-mannosyl 1-phosphate **21**, these compounds were exposed to aqueous solutions at pH 7.0, 5.0, and 1.0 and the degradation was monitored by RP-HPLC. At pH 7.0 and 5.0, no degradation was observed with compounds **20** and **21**. On the other hand, degradation of 2-OH compound **21** was observed at pH 1.0 at 37 °C and it was almost completely decomposed after 48 h (Fig. 2). In contrast, 2-F derivative **20** was found to be completely stable under the same acidic conditions. These results clearly indicated that the 2-F substituent stabilized not only mannosyl

Table 1
Recovery ratio of mannosyl phosphotriesters after treatment with DCA or $BF_3{\cdot}Et_2O$ over 24 h.

Compound	3% (v/v) DCA /CH_2Cl_2 at rt	0.1 M $BF_3{\cdot}Et_2O/CH_2Cl_2$-$Et_3SiH$ (9:1, v/v) at 0°°C
9	76%	83%
10	79%	0%
11	69%	71%
12	0%	0%

phosphotriesters under both Brønsted and Lewis acidic conditions.

phosphotriesters under acidic conditions but also mannosyl phosphodiesters even at a pH of 1.0, which represents the most acidic conditions in living systems such as in the stomach.

3. Conclusion

In this study, 2-deoxy-2-fluoromannosyl phosphotriester and phosphodiester derivatives were synthesized and their stability under acidic conditions was investigated. Phosphotriester derivatives **9** and **11** and phosphodiester derivative **20** were demonstrated to be more stable under acidic conditions than the corresponding mannosyl phosphate derivatives. These results strongly suggested that the 2-F substitution is useful for stabilizing both mannosyl phosphotriester derivatives and their synthetic intermediates. The properties of these analog molecules of Man-α-(1 → 6)-P-Man, including their biological activities are currently being investigated.

4. Experimental section

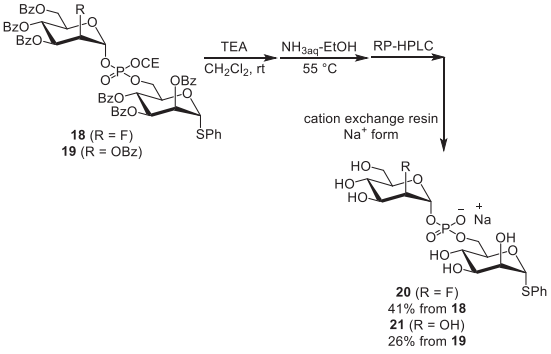
4.1. General information

All reactions were carried out under an argon atmosphere. ^1H NMR spectra were obtained at 300 MHz on a Varian MERCURY 300 spectrometer or at 400 MHz on a JEOL Lambda 400 spectrometer with tetramethylsilane (TMS) as an internal standard (δ 0.0) in $CDCl_3$ and with trimethylsilyl propanoic acid (TSP) as an external standard (δ 0.0) in D_2O. ^{13}C NMR spectra were obtained at 75.5 MHz on a Varian MERCURY 300 spectrometer with $CDCl_3$ as an internal standard (δ 77.0) in $CDCl_3$, or at 100.8 MHz on a JEOL Lambda 400 spectrometer with TPS as an external standard (δ 0.0) in D_2O. ^{31}P NMR spectra were obtained at 121.5 MHz on a Varian MERCURY 300 spectrometer or at 162.0 MHz on a JEOL Lambda 400 spectrometer with 85% H_3PO_4 (δ 0.0) as an external standard. ^{19}F NMR spectra were obtained at 376.3 MHz on a JEOL Lambda 400 spectrometer with trifluoroacetic acid as an external standard (δ -76.55). Mass spectra were recorded on a Voyager System 4327 (Applied Biosystem) or a 910-MS FTMS system (Varian). Analytical TLC was performed on Merck Kieselgel 60-F254 plates. Silica gel column chromatography was carried out using Silica gel 60N (63–210 μm or 40–50 μm) unless otherwise noted. Reversed phase HPLC was carried out using a μBondasphere 5 μm C18, 100 Å, 3.9 mm × 150 mm (Waters) with a gradient of 0–20% acetonitrile in 0.1 M triethylammonium acetate buffer at 25 °C at a rate of 0.5 mL/min, or SunFire 5 μm C18, 100 Å, 19 mm × 150 mm (Waters) with a gradient of 0–40% acetonitrile in 0.1 M triethylammonium acetate buffer at 25 °C at a rate of 2.5 mL/min. Organic solvents were purified and dried by the appropriate procedure. all the new compounds were not crystalized and obtained as oil or amorphous solid, and so melting temperatures were not measured.

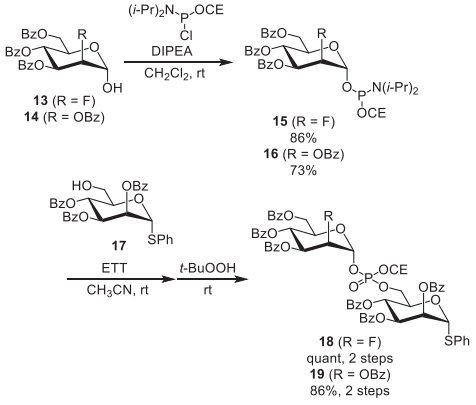
4.2. Elucidation of stability of Man-α-(1 → 6)-P-Man structures under acidic conditions

To evaluate the stability of **20** or **21** pH 1.0, to a 2.0 mM aqueous solution of compound **20** or **21** (50 μL), an acidic aqueous solution containing 0.2 M hydrochloride and 0.2 M potassium chloride (50 μL) was added. The solution was maintained at 37 °C on a thermal cycler. After 1, 4.45, 8, 19, 30, and 48 h, 5 μL was transferred out of the solution and diluted with 100 μL of 0.1 M triethylammonium acetate buffer containing 30 nmol of benzamide. The mixture was analyzed by RP-HPLC, and the ratio of intact mannosyl phosphodiester **20** or **21** was calculated based on area ratio between benzamide and **20** or **21**.

To evaluate the stability of **20** or **21** pH 7.0 and 5.0, the same procedure was used in the following solutions; pH 7.0: 0.1 M phosphate buffer (NaH_2PO_4-Na_2HPO_4); pH 5.0: citric-phosphate buffer (citric acid- Na_2HPO_4).



Scheme 3. Synthesis of Man-α-(1 → 6)-P-Man derivatives **20** and **21.**



Scheme 2. Synthesis of Man-α-(1 → 6)-P-Man precursors **18** and **19.**

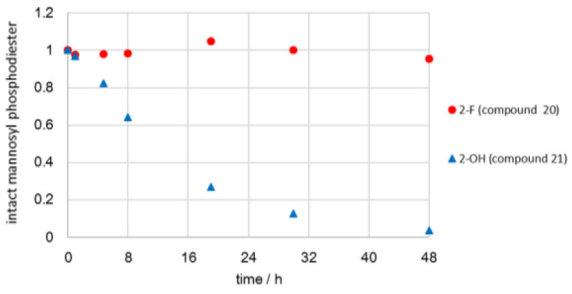


Fig. 2. Stability of 2-deoxy-2-fluoro mannosyl phosphate **20** and mannosyl phosphate **21** in acidic aqueous solution (pH 1.0, 37 °C).

4.3. Elucidation of stability of mannosyl phosphotriesters under Brønsted acidic conditions

Compound **9**, **10**, **11**, and **12** (0.12 mmol) were dissolved in dichloromethane (12 mL) respectively. To each solution, dichloroacetic acid (36 μL), and the reactions were monitored by TLC. After 24 h, each reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated, and compound **9**, **10**, **11**, and **12** were recovered by silica gel column chromatography.

4.4. Elucidation of stability of mannosyl phosphotriesters under Lewis acidic conditions

Compound **9**, **10**, **11**, and **12** (0.1 mmol) were dissolved in a mixed solvent of dichloromethane and triethylsilane (10:1, v/v, 1.0 mL) respectively. Each solution was cooled to 0 °C, BF_3-Et_2O (17 μL) was added, and the reactions were monitored by TLC. After 24 h, each reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated, and compound **9**, **10**, **11**, and **12** were recovered by silica gel column chromatography.

4.5. Compound *9*

Compound **1** (0.266 g, 0.86 mmol) was dissolved in dichloromethane (8.6 mL), and the solution was stirred and cooled to 0 °C. Triethylamine (0.48 mL, 3.84 mmol) and diethyl chlorophosphite (0.38 mL, 1.92 mmol) were added to the solution, and the mixture was warmed to room temperature. After 2.5 h, the solution containing compound **5** was cooled to 0 °C again, and then (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (0.769 g, 2.58 mmol) was added. The solution was warmed to room temperature, stirred for 4 h, and then diluted with chloroform (10 mL). The solution was washed with a saturated aqueous solution of $NaHCO_3$ (20 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (1:1, v/v)) to afford **9** as a colorless solid (0.331 g, 0.74 mmol, 87%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.79 (1H, dt, J = 2.4, 7.5 Hz, H-1), 5.39 (1H, t, J = 10.1 Hz, H-3), 5.27 (1H, m, H-4), 4.82 (td, J = 2.1, 48.9 Hz, H-2), 4.30–4.08 (7H, m, H-5, H-6, POCH_2CH$_3$), 2.09 (3H, s, OAc), 2.07 (3H, s, OAc), 2.04 (3H, s, OAc), 1.36 (6H, tt, J = 6.9, 1.5 Hz, POCH$_2$CH_3) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −2.9.

MALDI-TOF MS: *m/z* calcd for $C_{16}H_{26}FNaO_{11}P$ $[M+Na]^+$ 467.11; found 466.98.

4.6. Compound *10*

Compound **2** (0.188 g, 0.54 mmol) was dissolved in dichloromethane (5.4 mL), and the solution was stirred and cooled to 0 °C. Triethylamine (0.28 mL, 2.2 mmol) and diethyl chlorophosphite (0.16 mL, 1.1 mmol) were added to the solution, and the mixture was warmed to room temperature. After 3 h, more diethyl chlorophosphite (0.16 mL, 1.1 mmol) was added and the mixture was stirred for 3 h. The solution containing compound **6** was cooled to 0 °C again and then (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (0.480 g, 1.61 mmol) was added. The solution was warmed to room temperature, stirred for 1 h, and then diluted with chloroform (10 mL). The solution was washed twice with a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (1:1, v/v)) to afford **10** as a pale-yellow oil (0.1596 g, 0.33 mmol, 61%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.63 (1H, dt, J = 1.5, 6.9 Hz, H-1), 5.40–5.30 (3H, m, H-2, H-3, H-4), 4.31 (1H, dd, J = 12.3, 4.8 Hz, H-6a), 4.24–4.09 (6H, m, H-5, H-6b, POCH_2CH$_3$), 2.18 (3H, s, OAc), 2.10 (3H, s, OAc), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc), 1.38 (6H, tt, J = 6.9, 1.2 Hz, POCH$_2$CH_3) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −3.0.

4.7. Compound *11*

Compound **3** (1.2 g, 2.7 mmol) was dissolved in dichloromethane (27 mL), and the solution was stirred and cooled to 0 °C. Triethylamine (1.35 mL, 10.8 mmol) and diethyl chlorophosphite (1.08 mL, 5.4 mmol) were added to the solution, and the mixture was warmed to room temperature. After 20.5 h, the solution containing compound **7** was cooled to 0 °C again, and then (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (2.4 g, 8.1 mmol) was added. The solution was warmed to room temperature, stirred for 1 h, and then diluted with chloroform (300 mL). The solution was washed with a saturated aqueous solution of $NaHCO_3$ (50 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (17:3, v/v)) to afford **11** as a pale-yellow oil (1.11 g, 1.89 mmol, 69%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.17 (m, 15H, Ph) 5.79 (1H, dt, J = 1.8, 7.5 Hz, H-1), 4.89–4.47 (7H, m, H-2, PhCH_2), 4.12–3.67 (9H, m, H-3, H-4, H-5, H-6, POCH_2CH$_3$), 1.30–1.24 (6H, m, POCH$_2$CH_3) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −2.8.

MALDI-TOF MS: *m/z* calcd for $C_{31}H_{38}FNaO_8P$ $[M+Na]^+$ 611.22; found 611.30.

4.8. Compound *12*

Compound **4** (1.4 g, 2.6 mmol) was dissolved in dichloromethane (26 mL), and the solution was stirred and cooled to 0 °C. Triethylamine (1.3 mL, 10.4 mmol) and diethyl chlorophosphite (1.04 mL, 5.2 mmol) were added to the solution, and the mixture was warmed to room temperature. After 33 h, more diethyl chlorophosphite (0.52 mL, 2.6 mmol) was added and the mixture was stirred for 3 h. The solution containing compound **8** was cooled to 0 °C again, and then (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (2.30 g, 7.8 mmol) was added. The solution was warmed to room temperature, stirred for 12 h, and then diluted with chloroform. The solution was washed twice with a saturated aqueous solution of $NaHCO_3$ and twice with phosphate buffer (pH 7). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (3:7, v/v)) to afford **12** as a colorless solid (1.2818 g, 1.90 mmol, 73%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.18 (m, 20H, Ph), 5.73 (1H, dd, J = 6.3, 2.1 Hz, H-1), 4.90 (1H, d, J = 10.8, PhCH_2) 4.74 (2H, s, PhCH_2), 4.67–4.48 (5H, m, PhCH_2), 4.10–3.72 (10H, m, H-2, H-3, H-4, H-5, H-6, POCH_2CH$_3$), 1.27–1.21 (6H, m, POCH$_2$CH_3) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −2.6.

MALDI-TOF MS: *m/z* calcd for $C_{38}H_{45}NaO_9P$ $[M+Na]^+$ 699.27; found 699.16.

4.9. Compound *15*

Compound **13** (98.9 mg, 0.2 mmol) was coevaporated with pyridine and toluene, and then dissolved in dichloromethane (2.0 mL). The solution was stirred and cooled to 0 °C, and then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (90 μL, 0.4 mmol) and *N*,*N*-diisopropylethylamine (140 μL, 0.6 mmol) were added. The mixture was warmed to room temperature, and ethanol (10 mL) was added after 2.5 h. The solution was diluted with chloroform (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL × 2). The organic layer was dried over Na_2SO_4,

filtered, and concentrated. The crude product was purified by alumina column chromatography (hexane-ethyl acetate (9:1, v/v)) to afford **15** as a colorless solid (116 mg, 0.17 mmol, 86%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06–7.92 (m, 6H, Ph), 7.58–7.33 (m, 9H, Ph), 5.93 (q, J = 9.6 Hz, H-4), 5.80–5.61 (m, 1H, H-3), 5.58–4.49 (m, 1H, H-1), 5.07–4.80 (m, 1H, H-2), 4.66–4.42 (3H, m, H-5, H-6), 3.94–3.66 (4H, m, OCH_2CH_2CN, $OCH(CH_3)_2$), 2.69, 2.54 (2H, 2t, J = 6.3 Hz OCH_2CH_2CN, diastereomers), 1.32–1.17 (12H, m, $OCH(CH_3)_2$).

^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 165.8, 165.5, 165.4, 165.1, 165.0, 133.2, 132.9, 132.8, 129.6, 129.5, 129.4, 128.6, 128.2, 128.1, 117.3, 92.7, 92.4, 92.3, 92.0, 88.9, 88.8, 88.6, 86.4, 86.2, 70.5, 70.2, 69.6, 69.2, 66.3, 66.1, 62.9, 62.8, 58.8, 58.7, 58.6, 58.5, 43.6, 43.5, 43.4, 43.3, 24.4, 24.3, 24.2, 20.1, 20.0, 19.9.

^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 153.4, 149.6.

4.10. Compound **16**

Compound **14** (0.179 g, 0.3 mmol) was coevaporated with pyridine and toluene, and then dissolved in dichloromethane (3.0 mL). The solution was stirred and cooled to 0 °C, and then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (134 μL, 0.6 mmol) and *N*,*N*-diisopropylethylamine (204 μL, 0.9 mmol) were added. The mixture was warmed to room temperature, and ethanol (10 mL) was added after 2.5 h. The solution was diluted with chloroform (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by alumina column chromatography (hexane-ethyl acetate (9:1, v/v)) to afford **16** as a colorless solid (0.1737 g, 0.22 mmol, 69%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13–7.82 (m, 8H, Ph), 7.61–7.24 (m, 12H, Ph), 6.23–6.13 (m, H-4), 5.98–5.93 (m, 1H, H-3), 5.68–5.50 (m, 2H, H-1, H-2), 4.76–4.45 (m, 3H, H-5, H-6), 4.14–3.71 (4H, m, OCH_2CH_2CN, $OCH(CH_3)_2$), 2.75, 2.61 (2H, dt, t, J = 6.3, 2.7, 6.3 Hz, OCH_2CH_2CN, diastereomers), 1.36–1.21 (12H, m, $OCH(CH_3)_2$) ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 166.0, 165.5, 165.4, 165.3, 133.4, 133.1, 133.0, 129.7, 129.6, 129.1, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 117.5, 117.4, 93.0, 92.9, 92.7, 92.6, 71.3, 71.1, 71.0, 69.9, 69.8, 69.7, 69.4, 66.7, 66.4, 62.7, 62.6, 59.1, 59.0, 58.8, 58.7, 43.8, 43.7, 43.6, 24.5, 20.3, 20.2.^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 152.7, 150.1.

4.11. Compound **18**

Compound **15** (0.734 g, 1.1 mmol) and compound **17** (0.757 g, 1.3 mmol) were coevaporated with acetonitrile, and then dissolved in acetonitrile (2.0 mL). To this solution, a solution of 5-ethylthio-1*H*-tetrazole (1.4 g, 11 mmol) in acetonitrile (10 mL) was added and the mixture was stirred for 10 min *tert*-butyl hydroperoxide (2.1 mL, 11 mmol) was added to the solution after cooling to 0 °C, and then the mixture was warmed to room temperature. After 50 min, the solution was diluted with ethyl acetate (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (3:2, v/v)) to afford **18** as a colorless solid (1.10 g, 0.92 mmol, 85%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10–7.81 (m, 12H, Ph), 7.60–7.20 (m, 24H, Ph), 6.06–5.57 (m, 7H, H-1, H-2, H-3, H-4, H-1′, H-3′, H-4’), 5.17–4.81 (m, 2H, H-2′), 4.61–4.10 (m, 7H), 2.66, 2.53 (2H, t, q, J = 6.3, 6.3 Hz, OCH_2CH_2CN, diastereomers) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −3.1, −3.9.

ESI MS: *m/z* calcd for $C_{63}H_{54}FNO_{18}PS$ $[M+H]^+$ 1194.28; found 1194.28.

4.12. Compound **19**

Compound **16** (0.956 g, 1.2 mmol) and compound **17** (0.818 g, 1.4 mmol) were coevaporated with acetonitrile, and then dissolved in acetonitrile (2.0 mL). To this solution, a solution of 5-ethylthio-1*H*-tetrazole (0.818 g, 1.4 mmol) in acetonitrile (10 mL) was added and the mixture was stirred for 5 min *tert*-Butyl hydroperoxide (2.25 mL, 12 mmol) was added to the solution after cooling to 0 °C, and then the mixture was warmed to room temperature. After 25 min, the solution was diluted with ethyl acetate (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (3:2, v/v)) to afford **19** as a colorless solid (1.23 g, 0.949 mmol, 77%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14–7.77 (m, 14H, Ph), 7.60–7.17 (m, 28H, Ph), 6.22–5.76 (m, 8H), 5.00–4.90 (br, 1H), 4.76–4.15 (m, 7H), 2.71, 2.61 (2H, t, J = 6.3 Hz, m) ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ −3.1, −3.6 MALDI-TOF MS: *m/z* calcd for $C_{70}H_{58}NNaO_{20}PS$ $[M+Na]^+$ 1318.29; found 1318.50.

4.13. Compound **20**

Compound **18** (0.259 g, 0.2 mmol) was dissolved in a mixed solvent of dichloromethane and triethylamine (5:7, v/v, 0.48 mL) and stirred for 16 h. The mixture was concentrated and dissolved in ethanol (6.0 mL). To the solution, 25% aqueous ammonia (20 mL) was added and the mixture was stirred for 5 h at 55 °C. The solution was cooled to room temperature and ammonia was removed under reduced pressure. The aqueous solution was washed with diethyl ether (10 mL × 5) and concentrated. Triethylammonium hydrogencarbonate buffer (1.0 M) was added to the mixture, which was and then concentrated again. The crude product was lyophilized, and then one tenth of it was purified by RP-HPLC. The purified product was converted to the sodium salt by addition of Dowex® 50 W X8 (Na^+ form) to afford **20** (2.7 mg, 5.3 μmol, 27%).

^{1}H NMR (D_2O, 400 MHz) δ 7.62–7.60 (2H, m), 7.46–7.41 (3H, m), 5.54 (1H, t, J = 7.2 Hz), 5.48 (1H, s), 4.82–4.68 (0.5H, s), 4.35–4.33 (1H, m), 4.24–4.23 (1H, q, J = 1.6 Hz), 4.16–4.07 (2H, m), 3.99–3.85 (2H, m), 3.80–3.68 (1H, m, 5H).

^{31}P NMR (D_2O, 162.0 MHz) δ −1.3.

^{13}C NMR (D_2O, 100.8 MHz) δ 135.6, 134.8, 132.1, 131.2, 96.0, 95.7, 93.2, 93.1, 91.4, 91.3, 91.0, 76.2, 75.0, 74.9, 74.0, 73.6, 71.8, 71.6, 69.3, 68.7, 67.5, 67.4, 62.7.

^{19}F NMR (D_2O, 376.3 MHz) −(204.8–205.1).

MALDI-TOF MS: *m/z* calcd for $C_{18}H_{25}FO_{12}PS$ $[M]^-$ 515.08; found 515.11.

4.14. Compound **21**

Compound **19** (0.139 g, 0.2 mmol) was dissolved in a mixed solvent of dichloromethane and triethylamine (5:7, v/v, 0.48 mL) and stirred for 16 h. The mixture was concentrated and dissolved in ethanol (6.0 mL). To the solution, 25% aqueous ammonia (20 mL) was added and the mixture was stirred for 5 h at 55 °C. The solution was cooled to room temperature and ammonia was removed under reduced pressure. The aqueous solution was washed with diethyl ether (10 mL × 5) and concentrated. Triethylammonium hydrogencarbonate buffer (1.0 M) was added to the mixture, which was and then concentrated again. The crude product was lyophilized, and then one tenth of it was purified by RP-HPLC. The purified product was converted to the sodium salt by addition of Dowex® 50 W X8 (Na^+ form) to afford **21** (4.2 mg, 8.1 μmol, 41%).

^{1}H NMR (D_2O 400 MHz) δ 7.65–7.62 (2H, m), 7.49–7.42 (3H, m), 5.51 (1H, d, J = 1.2 Hz), 5.42 (1H, dd, J = 2.0, 7.6 Hz), 4.38–4.34 (1H,

m), 4.26, (1H, q, 1.6 Hz), 4.14 (2H, dd, J = 4.0, 6.0 Hz), 3.99 (1H, t, J = 2.4 Hz), 3.92–3.68 (7H, m).

(2H, m), 3.99–3.85 (2H, m), 3.80–3.68 (1H, m, 5H).

^{31}P NMR (D_2O, 162.0 MHz) δ −1.0.

^{13}C NMR (D_2O, 100.8 MHz) δ 135.7, 134.9, 132.2, 131.2, 98.9, 98.8, 91.1, 76.4, 75.1, 75.0, 74.1, 73.7, 73.2, 73.1, 72.6, 69.3, 68.9, 67.4, 67.3, 63.3.

MALDI-TOF MS: m/z calcd for $C_{18}H_{26}O_{13}PS$ $[M]^-$ 513.08; found 513.08.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2017.06.015.

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