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Investigation of the specificity of an α-L-arabinofuranosidase using C-2 and C-5 modified α-L-arabinofuranosides

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Abstract—The synthesis of three novel glycosyl donors presenting the same scaffold as α-L-arabinofuranose but modified at the C-2 or C-5 positions has been achieved. Furthermore, chemoenzymatic syntheses using the α-L-arabinofuranosidase AbfD3 and these unnatural furanosides were investigated. The use of the novel *p*-nitrophenyl furanoside donors revealed that AbfD3 can perform transglycosylation with the C-5 deoxygenated donor but not with the C-2 modified one. These results emphasize the vital role for OH-2 in AbfD3 substrate recognition.
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1. Introduction

Biocatalytic methods are useful additions to the classical sugar chemist's toolbox.[1] Among these tools, retaining O-glycosidases are carbohydrate-acting enzymes that perform catalysis through a two-step mechanism that involves enzyme glycosylation, followed by nucleophile-promoted deglycosylation. When used for synthetic applications, O-glycosidases often display only moderate regioselectivity and conversion yields. Nevertheless, they are generally considered to be useful because they are readily available and catalytically versatile.[2–5] The efficiency of O-glycosidases is emphasized by the fact that some can accept unnatural substrates, displaying modifications of the sugar moiety and/or a variety of aglycone groups.[6–8] However, to the best of our knowledge, apart from the synthesis of fructooligosaccharides by fructosidases, very few examples of O-glycosidase-catalyzed syntheses of furanose-containing oligosaccharides have been published.[9–12]

In previous studies, an α-L-arabinofuranosidase of bacterial origin (AbfD3) has been successfully employed for the synthesis of novel alkyl-glycosides[13] and furanose-containing disaccharides.[14,15] In the case of disaccharide synthesis, it was shown that AbfD3 can catalyze the self-condensation of both *p*-nitrophenyl α-L-arabinofuranoside and *p*-nitrophenyl β-D-galactofuranoside. Similarly, using these *p*-nitrophenyl furanosides as donors and β-D-xylosides as acceptors mixed disaccharides could be obtained. Depending on the donor/acceptor ratio, the reactions occurred with variable degrees of regioselectivity towards both (1→2)- and (1→3)-linkages, which reflects the previously observed bond specificity of AbfD3 operating in hydrolytic mode.[16] Other authors have previously suggested that there is a causal relationship between the bond specificity of an O-glycosidase working in the hydrolytic mode and regiospecificity during transglycosylation.[17] More recently, we demonstrated that AbfD3 can also catalyze syntheses involving galactofuranosyl analogues

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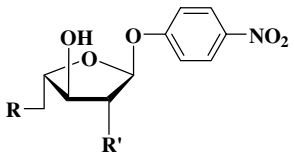


		R	R'
*p*NP α-L-Ara*f*		OH	OH
*p*NP 5-F-α-L-Ara*f*	**6**	F	OH
*p*NP 5-deoxy-α-L-Ara*f*	**10**	H	OH
*p*NP 2-deoxy-α-L-Ara*f*	**15**	OH	H

Figure 1. *p*-Nitrophenyl activated C-2- and C-5-modified α-L-arabinofuranosides.

modified at the C-6 position, that is, *p*-nitrophenyl β-D-fucofuranoside and *p*-nitrophenyl-6-deoxy-6-fluoro-β-D-galactofuranoside. These reactions were also characterized by diverse regioselectivity in favor of the β-(1→2) linkage.[18] Encouraged by these results, it appears useful to pursue this approach and to aim at a better control of regioselectivity through the use of tailor-made substrates.

In this work we have attempted to manipulate the regioselectivity of AbfD3-catalyzed transglycosylation reactions by synthesizing a series of unnatural donors that display structural modifications at their C-2 or C-5 positions. We have thus synthesized the three donors, *p*-nitrophenyl 5-deoxy-5-fluoro-α-L-arabinofuranoside **6**, *p*-nitrophenyl 5-deoxy-α-L-arabinofuranoside **10** and *p*-nitrophenyl 2-deoxy-α-L-arabinofuranoside **15** (Fig. 1). Following synthesis, these glycosides were tested to determine if these deoxygenated analogues could be recognized as substrates in AbfD3-catalyzed reactions.

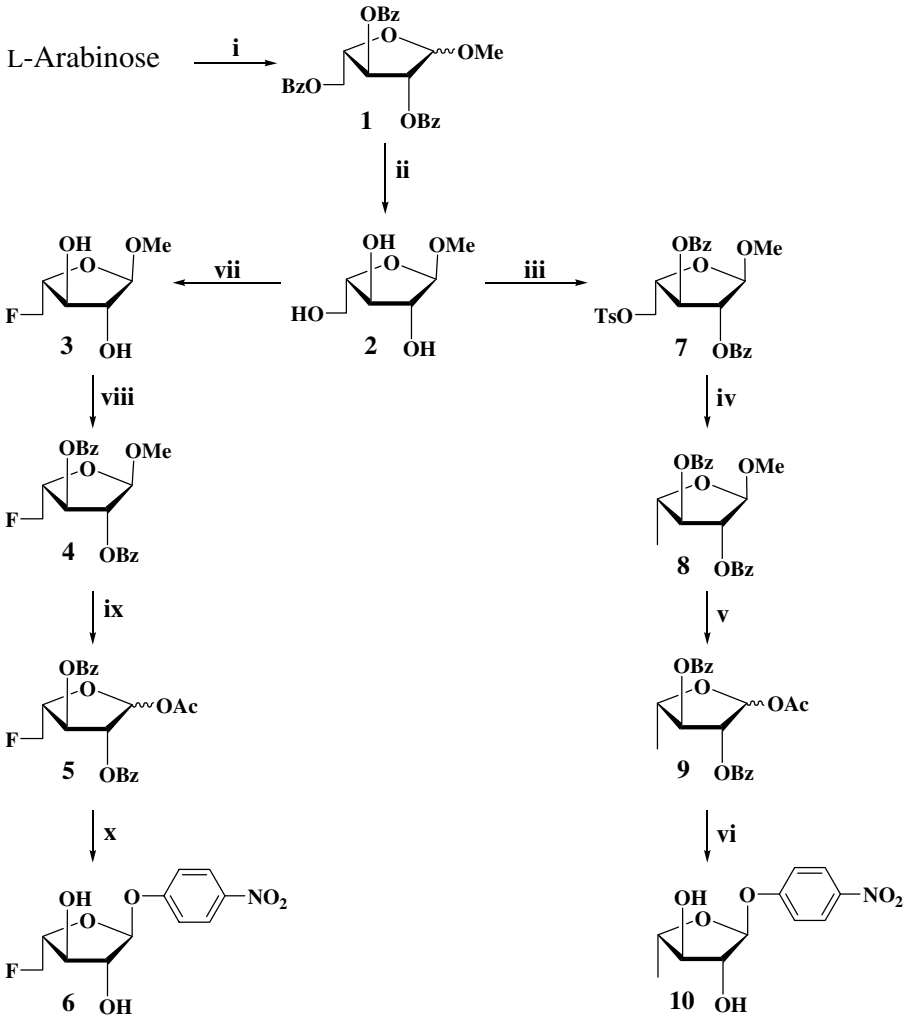
2. Results and discussion

2.1. Synthesis of α-L-arabinofuranoside analogues

Our first attempts to synthesize the desired activated donors **6** and **10** starting from an anomeric mixture of methyl L-arabinofuranoside failed in the first step. Indeed, as previously observed on a galactofuranose derivative,[19] differentiation between the primary and secondary hydroxyl groups of methyl L-arabinofuranoside by tritylation or tosylation was extremely tricky and led to very poor product yields. Consequently, methyl α-L-arabinofuranoside **2** was used as a common building block for the syntheses of both targets (Scheme 1). First, a multi-gram scale synthesis of per-O-benzoylated methyl L-arabinofuranoside **1** was performed using a conventional method.[15] Next, using a previously reported protocol,[20] the α-anomer of **1** was quantitatively crystallized from ethanol leading to **2** after Zemplén transesterification. Gratifyingly, methyl α-L-arabinofuranoside **2** was clearly identified on the basis of NMR spectroscopy in accordance with the literature data.[21] The singlet signal for H-1 was found at δ 4.8 ppm in the ^{1}H NMR spectrum while the C-1 resonated at δ 108.6 ppm, typical values for α-L-arabinofuranosides.[22]

The preparation of the fluorinated target **6** required a free primary hydroxyl group, which was first differentiated from the secondary ones according to a conventional tritylation–benzoylation protocol.[23] However, the fluorination step was inefficient and led to a poor overall yield (14%). Therefore, we obtained the 5-monofluorinated compound **3** via an alternative route through direct substitution of the 5-hydroxyl group using the well-known fluorinating agent diethylaminosulfur trifluoride (DAST) on the unprotected sugar **2**. Using previously reported procedures,[24,25] the treatment of **2** with an excess of DAST at 0 °C afforded **3** in a moderate, but largely improved (48%) yield as the only α-anomer. Unfortunately, attempts to use less than a sixfold excess of DAST afforded an inextricable mixture. Difficulties associated with the introduction of fluorine at a primary position in pentofuranoses have already been encountered by others.[26] Indeed, upon DAST treatment a methoxy shift from C-1 to C-5 of methyl β-D-ribofuranosides has been observed. Moreover, diverse side reactions often occur when fluorine is introduced into sugar derivatives via nucleophilic substitution using DAST reagent. In particular, anhydro compounds and cyclic sulfites (during work-up) are generated when unprotected sugars are used.[27] In our case, the formation of the fluorinated compound **3** was accompanied by the generation of a degradation compound that was observed in the final fractions after chromatographic purification. Incorporation of fluorine in **3** was confirmed by NMR data that were coherent with reported values.[28] In the ^{1}H NMR spectrum of **3**, the resonances for each of the diastereotopic hydrogens on C-5 appeared as a doublet of a doublet of a doublet, with $^3J_{H,F}$ magnitudes of 47.2 and 47.5 Hz. Furthermore, in the ^{13}C NMR spectrum a doublet signal, characterized by a high coupling constant ($^1J_{C,F} =$ 186.7 Hz), was found for C-5.

Subsequently, compound **5** was synthesized by benzoylation of **3** leading to the precursor **4**, which was subjected to acetolysis. The overall yield for the two steps was 79%. Finally, **5** was used as a donor for the glycosylation of *p*-nitrophenol. This reaction was promoted by tin(IV) chloride,[29,30] leading to a moderate (50%) yield of the desired arabinofuranoside **6** after Zemplén deacylation. The formation of the reducing arabinofuranosyl derivative during the course of the reaction led us to suspect that the acidic conditions were responsible for partial degradation of the donor **5** and/or the product **6**. Therefore, the less aggressive promoter, boron trifluoride–triethylamine (prepared in situ),[31]

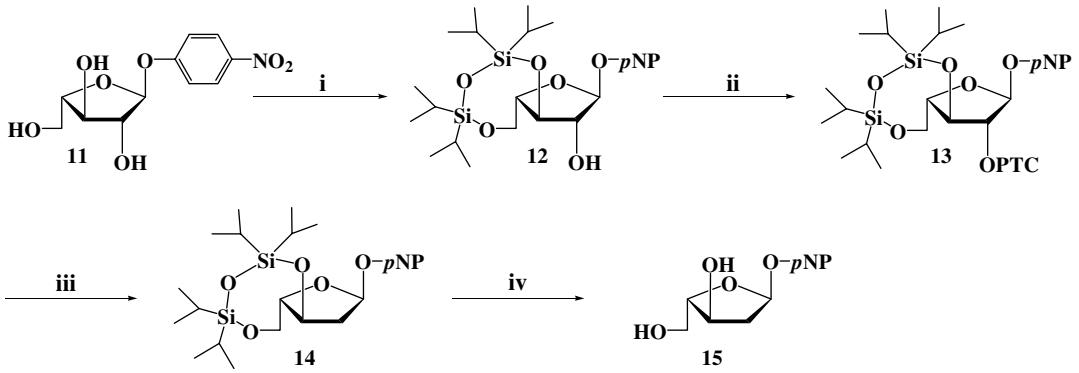


Scheme 1. Reagents and conditions: (i) (a) AcCl–MeOH, 3 h, rt, (b) BzCl, pyridine, 2 h, rt (100%); (ii) (a) cryst. EtOH, (b) NaOMe–MeOH, 3 h, rt (50%); (iii) (a) TsCl, DMAP, pyridine, overnight, rt, (b) BzCl, pyridine, overnight, rt (79%); (iv) $NaBH_4$, DMF, 6 h, 65 °C (55%); (v) Ac_2O, H_2SO_4, CH_2Cl_2, overnight, rt (95%); (vi) (a) *p*-nitrophenol, $BF_3{\cdot}OEt_2$, Et_3N, CH_2Cl_2, 3 h, rt, (b) NaOMe–MeOH, 3 h, rt (55%); (vii) DAST, CH_2Cl_2, 1 h, 0 °C to rt (48%); (viii) BzCl, pyridine, overnight, rt (80%); (ix) Ac_2O, H_2SO_4, CH_2Cl_2, overnight, rt (99%); (x) (a) *p*-nitrophenol, $BF_3{\cdot}OEt_2$, Et_3N, CH_2Cl_2, 3 h, rt, (b) NaOMe–MeOH, 3 h, rt (59%).

was used to obtain the *p*-nitrophenyl 5-deoxy-5-fluoro-α-L-arabinofuranoside donor **6**, after deprotection, with a significantly improved yield (59%) and excellent α-stereoselectivity.

The deoxygenated donor **10** was prepared through selective tosylation of the primary hydroxyl group in **2**, followed by benzoylation to afford the known furanoside **7**[32] in good yield (79% for the two steps). Sodium borohydride reduction of **7** afforded the deoxygenated arabinofuranoside **8** with a moderate 55% yield. In this case, sodium borohydride reduction was preferred to the usually more convenient lithium aluminum hydride reduction of partially benzylated furanosides,[33,34] due to the greater tolerance of sodium borohydride towards benzoyl participating groups. Acetolysis of **8** led to the production of the donor **9**, followed by a one-pot *p*-nitrophenyl glycosidation reaction and final Zemplén deacylation. This sequence afforded pure *p*-nitrophenyl 5-deoxy-α-L-arabinofuranoside **10** in 52% yield for the last three steps. The coupling constants observed between H-1 and H-2 ($^3J_{1,2} = 1.8$ Hz for **6** and 1.3 Hz for **10**) and the C-1 signals in the ^{13}C NMR spectra of **6** and **10** (at δ 107.7 ppm and δ 105.4 ppm, respectively) allowed us to ascertain the α configuration of these novel glycosyl donors.

For many years, chemical 2-deoxygenation of pentofuranoses has been used with variable success to study ribonucleosides. Considerable improvements to the deoxygenation of carbohydrate-type compounds have been achieved thanks to the Barton and McCombie radical deoxygenation.[35] Reductive deoxygenation of phenyl thionocarbonate from unhindered secondary alcohols usually proceeds smoothly using tri-*n*-butyltin hydride and a free radical initiator. As illustrated in



Scheme 2. Reagents and conditions: (i) $TIPSCl_2$, pyridine, 1 h, rt (85%); (ii) PTCCl, overnight, rt (100%); (iii) *n*-Bu_3SnH, AIBN, toluene, overnight, 80 °C (63%); (iv) TBAF–THF, overnight, rt (95%).

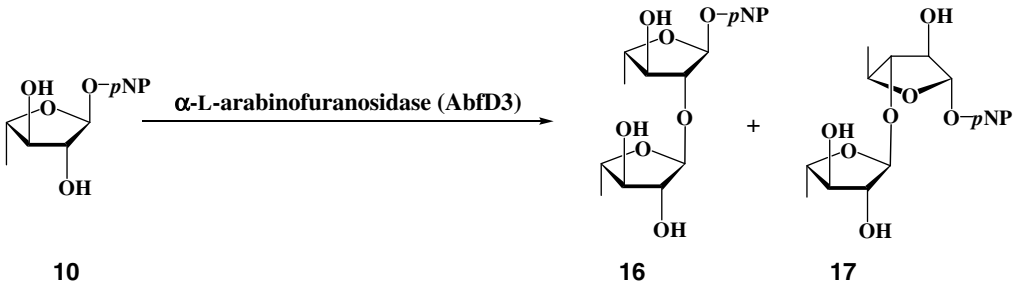
Scheme 2, the initial synthesis of *p*-nitrophenyl 2-deoxy-α-L-*erythro*-pentofuranoside, **15**, involved simultaneous protection of the 3- and 5-positions of the known *p*-nitrophenyl α-L-arabinofuranoside **11**[36,37] by selective silylation with 1,3-dichloro-1,1,3,3-tetra-isopropyldisiloxane in pyridine to give **12** with the expected reported 85% yield.[38] Phenoxythiocarbonyl chloride (PTC-Cl) was shown to quantitatively convert **12** into the phenyl thionocarbonate derivative **13**. Standard reduction using 1.5 equiv of tri-*n*-butyltin hydride and 0.2 equiv of AIBN,[39,40] followed by TBAF desilylation afforded the novel compound **15** in good yield. The very important upfield shift of the C-2 of the 2-deoxygenated arabinofuranoside from 92.2 (for **13**) to 42.5 ppm (for **15**) was consistent with such a chemical modification.[21]

2.2. Chemoenzymatic synthesis of furanose-containing disaccharides

To test the suitability of modified arabinofuranosyl donors for AbfD3-catalyzed synthesis, compounds **6**, **10**, and **15** were incubated with AbfD3 (12 IU) at 60 °C for short periods. After the incubation, high performance thin-layer chromatography (HPTLC) was used to detect transglycosylation products. Using modified arabinofuranoside **10**, the HPTLC chromatogram revealed that after 30 min, concomitant to *p*-nitrophenol release, two novel species were formed, although a large amount of non-reacted substrate remained. These products were isolated as previously described[18] and identified by NMR spectroscopy.

Analysis of ^{13}C NMR data for the major product, **16**, revealed a downfield shift of the C-2^{I} of the reducing 5-deoxy-L-arabinofuranoside from 81.9 (for **10**) to 88.06 ppm (for **16**), as well as weak upfield shifts ($\Delta\delta = -1.4$ ppm and -1.9 ppm) for the signals of the neighboring carbons C-1^{I} and C-3^{I}. Moreover, an intense three-bond coupling between C-2^{I} (88.06 ppm) and H-1^{II} (5.06 ppm) in the ^{1}H–^{13}C (HMBC) spectrum allowed the unambiguous identification of **16** as a (1→2)-linked disaccharide. Finally, the small coupling constant observed between H-1^{II} and H-2^{II} ($J_{1^{II}2^{II}} = 1.2$ Hz) clearly indicated that the glycosidic bond in **16** exhibited α-stereochemistry (Scheme 3). On the basis of similar data analysis, the minor product **17** was identified as a α-(1→3) disaccharide.

This result was rather expected because the analogously modified D-galactofuranoside derivative, *p*-nitrophenyl β-D-fucofuranoside, also led to the synthesis of α-(1→2)- and α-(1→3)-linked disaccharides.[18] Moreover, according to HPTLC analysis of the AbfD3-catalyzed condensation of *p*-nitrophenyl-5-deoxy-α-L-arabinofuranoside **10**, the regioselectivity of the transglycosylation appeared to be low (**16**/**17**, 1.5:1) as previously observed using the galactofuranosyl derivative



Scheme 3. Structures of the disaccharides, **16** and **17**, which were obtained from the AbfD3-catalyzed condensation of **10**.

reduced at the C-6 position, that is *p*-nitrophenyl-β-D-fucofuranoside.[18]

In a similar way, the furanosyl donor **6** was incubated with AbfD3 and the course of the reaction was monitored by HPTLC. However, while the major product appeared to be *p*-nitrophenol, traces of two inseparable other products (suspected to be disaccharides due to the HPTLC profile) could be detected attesting that hydrolysis of compound **6** largely overwhelmed the autocondensation pathway. Overall, these results suggest that both C-5 deoxygenated α-L-arabinofuranosides are good substrates for hydrolysis but rather poor ones for the transglycosylation process. Indeed, incubation of the furanoside **10** in the described conditions led to very poor yield (<5%) of the isolated disaccharides.

Finally, when the 2-deoxy analogue **15** was incubated with AbfD3 in the same conditions, HPTLC analysis failed to reveal any reaction products. Moreover, the free sugar could not be detected by charring the plate with a general acidic reagent, confirming that AbfD3 is not active towards this analogue. As suspected, this result indicates that the presence of the OH-2 group in the donor sugar is crucial for substrate recognition by AbfD3.

3. Conclusion

This study clearly demonstrates that besides cleavage, the AbfD3 furanosidase is able to catalyze transglycosylation reactions with C-5 modified *p*-nitrophenyl arabinofuranosides and confirmed that AbfD3 possesses the ability to synthesize both α-(1→2)- and α-(1→3)-linked regioisomeric disaccharides from the C-5 deoxygenated substrate **10**. The preparation of three original arabinofuranosyl donors, *p*-nitrophenyl 5-deoxy-α-L-arabinofuranoside, *p*-nitrophenyl 5-deoxy-5-fluoro α-L-arabinofuranoside, and *p*-nitrophenyl 2-deoxy-α-L-*erythro*-pentofuranoside, has allowed us to demonstrate that the presence of OH-5 is not required for AbfD3 substrate recognition. On the other hand, the present data also underline the importance of OH-2 for substrate recognition. This is coherent with structural data available for other GH-51 arabinofuranosidases[10,41] that indicate that OH-2 is involved in at least two interactions, one with the catalytic nucleophile and the other with the adjacent Asn residue. Consequently, this present state of affairs precludes the use of a C-2 deoxygenation approach for the synthesis of (1→5)-linked oligosaccharides.

Finally, like many other transglycosylating O-glycosidases, hydrolysis by AbfD3 is favored with respect to transglycosylation. Further development of this strategy aiming at reducing water activity is now underway.

4. Experimental

4.1. General methods

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Melting points were determined on a Reichert microscope and are uncorrected. Thin-layer chromatography (TLC) analyses were conducted on E. Merck 60 F_{254} Silica Gel non-activated plates and compounds were revealed using a 5% solution of H_2SO_4 in EtOH followed by heating. High performance TLC (HPTLC) experiments were performed on a CAMAG TLC SCANNER 3, designed for densitometric measurement of thin-layer chromatograms up to 200 × 200 mm in size and controlled by the newly designed software WINCATS. TLC (HPTLC) analyses were carried out with detection by UV absorption (300 nm) and when necessary charring with 5% solution of H_2SO_4 in EtOH. For column chromatography, Geduran Si 60 (40–63 μm) Silica Gel was used. All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy. Preparative TLC was performed using 0.25 mm Silica Gel 60 plates (E. Merck). ^{1}H, ^{13}C, HMQC, and COSY NMR spectra were recorded on a Brüker ARX 400 spectrometer at 400 MHz for ^{1}H, 100 MHz for ^{13}C, and 376 MHz for ^{19}F analyses. 1D and 2D spectra of the disaccharides were all recorded on a Brüker ARX 500 spectrometer at 500 MHz for ^{1}H and 126 MHz for ^{13}C. $CDCl_3$ was employed as an NMR solvent for protected sugars and D_2O or CD_3OD for the free sugars. Chemical shifts are given in δ-units (ppm) measured downfield from Me_4Si. Disaccharide-associated carbons and protons are designated with the superscript 'I' for the reducing residue and 'II' for the non-reducing residue. The HRMS were measured at the CRMPO (Rennes, France) with an MS/MS ZabSpec TOF Micromass using *m*-nitrobenzylic alcohol as a matrix and accelerated cesium ions for ionization.

4.2. Methyl α-L-arabinofuranoside (2)[21]

Compound **1**[42] (10 g, 67 mmol) was re-crystallized from ethanol to give methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside **1α** with 50% maximal yield as a white solid. Compound **1α** (30 g, 80 mmol) was then added to a 1 N MeONa solution in MeOH (200 mL). After stirring for 3 h at room temperature, the reaction mixture was neutralized by adding glacial HOAc and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (CH_2Cl_2–MeOH, 4:1) to yield **2** (13 g, 100%) as a white solid (CH_2Cl_2–MeOH, 4:1, $R_f = 0.28$); mp 143 °C; $[\alpha]_D^{20}$ −7.2 (*c* 1, MeOH); ^{1}H NMR (D_2O, 400 MHz): δ 4.81 (d, 1H, H-1, $J_{1,2} = 1.5$ Hz), 4.21–4.13 (m, 2H, H-2, H-4, $J_{2,3} =$ 3,3 Hz, $J_{3,4} = 5.9$ Hz, $J_{4,5} = 3.6$ Hz, $J_{4,5'} = 5.6$ Hz),

4.05 (dd, 1H, H-3), 3.92 (dd, 1H, H-5, $J_{5,5'} = 12.2$ Hz), 3.25 (s, 1H, OCH_3); ^{13}C NMR (D_2O, 100 MHz): δ 108.6 (C-1), 84.2 (C-4), 80.9 (C-2), 76.7 (C-3), 61.5 (C-5), 55.2 (OCH_3). ESIMS *m/z* calcd for $[C_6H_{12}O_5]Na^+$: 187.0582, found: 187.0587.

4.3. Methyl 5-deoxy-5-fluoro-α-L-arabinofuranoside (3)

A solution of **2** (700 mg, 4.8 mmol) in dry CH_2Cl_2 (20 mL) was prepared, then DAST (3.6 mL, 29 mmol) was added dropwise at 0 °C. After warming to room temperature and stirring for 1 h, MeOH (10 mL) was added at 0 °C. The mixture was concentrated and purified by flash chromatography (petroleum ether–EtOAc, 2:3) to give **3** (340 mg, 48%) as a white solid (petroleum ether–EtOAc, 3:2, $R_f = 0.42$); mp 136 °C; $[\alpha]_D^{20}$ +27.4 (*c* 1, MeOH); ^1H NMR (D_2O, 400 MHz): δ 4.82 (s, 1H, H-1), 4.63 (ddd, 1H, $J_{4,5} = 4.1$ Hz, $J_{5,5'} = 10.7$ Hz, $J_{5,F} = 47.5$ Hz, H-5), 4.43 (ddd, 1H, $J_{4,5'} = 5.1$ Hz, $J_{5',F} = 47.2$ Hz, H-5′), 4.05 (dtd, 1H, $J_{3,4} = 5.9$ Hz, $J_{4,F} = 24.6$ Hz, H-4), 3.93 (dd, 1H, $J_{1,2} = 1.5$ Hz, H-2), 3.87 (dd, 1H, H-3), 3.25 (s, 3H, OCH_3); ^{13}C NMR (D_2O, 100 MHz): δ 108.8 (C-1), 82.6 (C-5, $J_{5,F} =$ 186.7 Hz), 82.6 (C-4, $J_{4,F} = 18.5$ Hz), 80.8 (C-2), 75.7 (C-3, $J_{3,F} = 7.2$ Hz), 55.3 (OCH_3); ^{19}F NMR (D_2O, 376 MHz): δ −239 (F-5). ESIMS *m/z* calcd for $[C_6H_{11}O_4F]Na^+$: 189.0539, found: 189.0540.

4.4. Methyl 2,3-di-*O*-benzoyl-5-deoxy-5-fluoro-α-L-arabinofuranoside (4)

Following the preparation of a solution of **3** (125 mg, 0.75 mmol) in dry pyridine (8 mL), benzoyl chloride (350 μL, 3 mmol) was added dropwise at 0 °C. After stirring overnight at room temperature the reaction mixture was partitioned between CH_2Cl_2 (30 mL) and water (5 mL) and then decanted. The organic layer was washed with 1 N aqueous HCl (5 × 5 mL), satd aq $NaHCO_3$ (5 × 5 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **4** (225 mg, 80%) as a colorless oil (petroleum ether–EtOAc, 3:2, $R_f = 0.7$); $[\alpha]_D^{20}$ +11.2 (*c* 1, $CDCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.30 (m, 10H, H_{arom}), 5.45 (s, 1H, H-2), 5.36 (d, 1H, $J_{3,4} =$ 5.1 Hz, H-3), 5.11 (s, 1H, H-1), 4.81 (d, 1H, $J_{4,5} =$ 3.3 Hz, $J_{5,F} = 47.2$ Hz, H-5), 4.70 (d, 1H, $J_{4,5'} = 3.3$ Hz, $J_{5',F} = 46.7$ Hz, H-5′), 4.36 (dtd, 1H, $J_{4,F} = 25.3$ Hz, H-4), 3.41 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.3–165.8 (CO), 134.0–128.9 (C_{arom}), 107.4 (C-1), 82.6 (C-4, $J_{4,F} = 18.5$ Hz), 82.5 (C-5, $J_{5,F} = 174.3$ Hz), 81.8 (C-2), 77.3 (C-3), 55.5 (OCH_3); ^{19}F NMR (D_2O, 376 MHz): δ −230 (F-5). ESIMS *m/z* calcd for $[C_{20}H_{19}O_6F]Na^+$: 397.1063, found: 397.1059.

4.5. 1-*O*-Acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-fluoro-α,β-L-arabinofuranose (5)

A solution of **4** (150 mg, 0.4 mmol) in dry CH_2Cl_2 (4 mL) was prepared and acetic anhydride (151 μL, 1.6 mmol) was added, followed by a catalytic amount of H_2SO_4. After stirring overnight at room temperature, the reaction mixture was neutralized by Et_3N (3 mL) at 0 °C and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (20 mL), washed (3 × 5 mL of water), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 6:4) to yield **5** (160 mg, 99%) as a colorless oil (petroleum ether–EtOAc, 8:2, $R_f = 0.43$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.30 (m, 10H, H_{arom}). α-Anomer: 6.39 (s, 1H, H-1), 5.59 (s, 1H, H-2), 5.45 (d, 1H, $J_{3,4} = 4.1$ Hz, H-3), 4.72 (dddd, 2H, $J_{4,5} = 3.8$ Hz, $J_{4,5'} = 2.8$ Hz, $J_{5,F} = 47.2$ Hz, $J_{5',F} = 46.5$ Hz, H-5, H-5′), 4.48 (dtd, 1H, $J_{4,F} = 25.4$ Hz, H-4), 2.20 (s, 3H, OAc). β-Anomer: 6.53 (d, 1H, H-1, $J_{1,2} = 4.6$ Hz), 5.81 (dd, 1H, $J_{2,3} = 7.1$ Hz, $J_{3,4} = 6.1$ Hz, H-3), 5.60 (dd, 1H, H-2), 4.71 (m, 2H, $J_{5,F} = 47.2$ Hz, $J_{5',F} = 47.2$ Hz, H-5, H-5′), 4.30 (m, 1H, $J_{4,F} = 22.9$ Hz, H-4), 2.10 (s, 3H, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6–155.5 (CO), 135.0–128.3 (C_{arom}). α-Anomer: 99.8 (C-1), 84.7 (C-4, $J_{4,F} = 19.2$ Hz), 82.0 (C-5, $J_{5,F} =$ 175.1 Hz), 80.9 (C-2), 76.9 (C-3, $J_{3,F} = 6.9$ Hz), 21.5 (OAc). β-Anomer: 93.6 (C-1), 82.9 (C-5, $J_{5,F} =$ 175 Hz), 81.0 (C-4, $J_{4,F} = 19.2$ Hz), 76.1 (C-2), 74.5 (C-3, $J_{3,F} = 6.9$ Hz), 21.45 (OAc); ^{19}F NMR (D_2O, 376 MHz): δ −229 (F-5 β); −231 (F-5 α). ESIMS *m/z* calcd for $[C_{21}H_{19}O_7F]Na^+$: 425.1013, found: 425.1009.

4.6. *p*-Nitrophenyl 5-deoxy-5-fluoro-α-L-arabinofuranoside (6)

To a solution of **5** (100 mg, 0.25 mmol) in CH_2Cl_2 (3 mL), $BF_3{\cdot}OEt_2$ (79 μL, 0.62 mmol) then Et_3N (18 μL, 0.13 mmol) were added at 0 °C. After stirring 10 min at 0 °C, *para*-nitrophenol (42 mg, 0.3 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. Water (15 mL) was added and the mixture was diluted with CH_2Cl_2 (20 mL). After decantation, the organic layer was washed with 1 N aqueous HCl (5 × 10 mL), satd aq $NaHCO_3$ (5 × 10 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The crude residue was dissolved in 1 N MeONa–MeOH solution and then stirred during 3 h at room temperature. After neutralization by glacial HOAc and concentration under reduced pressure, the resulting crude product was purified by column chromatography (CH_2Cl_2–MeOH, 9:1) to yield **6** (40 mg, 59% (two steps)) as a white solid (CH_2Cl_2–MeOH, 9:1, $R_f = 0.43$); mp 132 °C; $[\alpha]_D^{20}$ −9.5 (*c* 1, MeOH); ^1H NMR (CD_3OD, 400 MHz): δ 8.21 (d, 2H, $J = 9.2$ Hz,

H-3′, H-5′), 7.20 (d, 2H, $J = 9.4$ Hz, H-2′, H-4′), 5.67 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1), 4.55 (dddd, 2H, $J_{4,5} = 2.8$ Hz, $J_{4,5'} = 4.8$ Hz, $J_{5,5'} = 10.4$ Hz, $J_{5,F} = 51.4$ Hz, $J_{5',F} = 51.4$ Hz, H-5, H-5′), 4.32 (m, 1H, $J_{2,3} = 4.3$ Hz, H-2), 4.16 (dddd, 1H, $J_{3,4} = 6.9$ Hz, $J_{4,F} = 24.3$ Hz, H-4), 4.12 (dd, 1H, H-3); ^{13}C NMR (CD_3OD, 100 MHz): δ 164.9 (C-1′), 143.2 (C-4′), 126.7 (C-3′, C-5′), 117.7 (C-2′, C-6′), 107.7 (C-1), 84.6 (C-4, $J_{4,F} = 19.1$ Hz), 83.6 (C-2), 83.3 (C-5, $J_{5,F} = 170.2$ Hz), 77.3 (C-3, $J_{3,F} = 6.9$ Hz); ^{19}F NMR (D_2O, 376 MHz): δ −232 (F-5). ESIMS *m*/*z* calcd for $[C_{11}H_{12}NO_6F]Na^+$: 296.0546, found: 296.0543. Anal. Calcd for $C_{11}H_{12}NO_6F$: C, 48.36; H, 4.43. Found: C, 48.39; H, 4.52.

4.7. Methyl 2,3-di-*O*-benzoyl-5-*O*-tosyl-α-L-arabinofuranoside (7)

Tosyl chloride (2.6 g, 13.7 mmol) and DMAP were added successively (560 mg, 4.6 mmol) to a solution of **2** (1.5 g, 9.1 mmol) in pyridine (50 mL). The reaction mixture was stirred overnight at room temperature. Water was added (30 mL) and the mixture was diluted with CH_2Cl_2 (50 mL). After decantation, the organic layer was washed with 1 N aqueous HCl (5 × 20 mL), satd aq $NaHCO_3$ (5 × 20 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The crude product (2.55 g) was dissolved in pyridine (80 mL) and then benzoyl chloride (4 mL, 32 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature. Water was added (30 mL) and the mixture was diluted with CH_2Cl_2 (50 mL). After decantation, the organic layer was washed with 1 N aqueous HCl (5 × 20 mL), satd aq $NaHCO_3$ (5 × 20 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 3:2) to yield **7** (3.8 g, 79% (two steps)) as a white solid (petroleum ether–EtOAc, 3:2, $R_f = 0.61$); mp 141 °C, lit.[32] mp 150 °C; $[\alpha]_D^{20}$ −32.4 (*c* 1, $CHCl_3$), lit.[32] $[\alpha]_D^{20}$ −30.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20–7.30 (m, 14H, H_{arom}Bz + Ts), 5.50 (d, 1H, $J_{1,2} = 1.0$ Hz, H-2), 5.32 (d, 1H, $J_{3,4} = 4.6$ Hz, H-3), 4.45 (d, 2H, $J_{4,5} = J_{4,5'} = 8.4$ Hz, H-5, H-5′), 4.40 (dd, 1H, H-4), 3.50 (s, 3H, OMe), 2.40 (s, 3H, CH_3Ts); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6–165.3 (COBz), 134.2–125.2 (C_{arom}), 107.9 (C-1), 82.1 (C-2), 81.8 (C-4), 78.5 (C-3), 69.7 (C-5), 55.8 (OMe), 22.2 (CH_3Ts). ESIMS *m*/*z* calcd for $[C_{27}H_{26}O_9S]Na^+$: 549.1195, found: 549.1195.

4.8. Methyl 2,3-di-*O*-benzoyl-5-deoxy-α-L-arabinofuranoside (8)

A solution of **7** was prepared (3.2 g, 6.1 mmol) in DMF (60 mL), then $NaBH_4$ (1.2 g, 30.4 mmol) was added progressively. The reaction mixture was stirred 6 h at 65 °C. After neutralization with glacial HOAc, the mixture was diluted with CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$ (5 × 20 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **8** (1.2 g, 55%) as a white solid (petroleum ether–EtOAc, 9:1, $R_f = 0.32$); mp 165 °C; $[\alpha]_D^{20}$ −12.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.40 (m, 10H, H_{arom}), 5.48 (d, 1H, $J_{1,2} = 1.5$ Hz, H-2), 5.22 (ddd, 1H, $J_{2,3} = 2.0$ Hz, $J_{3,4} = 5.3$ Hz, H-3), 5.10 (s, 1H, H-1), 4.39 (q, 1H, $J_{4,5} = 6.4$ Hz, H-4), 3.50 (s, 3H, OMe), 1.58 (d, 3H, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6–165.3 (CO), 134.2–125.2 (C_{arom}), 113.2 (C-1), 87.3 (C-2), 86.2 (C-3), 79.5 (C-4), 55.1 (OMe), 19.9 (C-5). ESIMS *m*/*z* calcd for $[C_{20}H_{20}O_6]Na^+$: 379.1158, found: 379.1154.

4.9. 1-*O*-Acetyl-2,3-di-*O*-benzoyl-5-deoxy-α,β-L-arabinofuranose (9)

To a solution of **8** (620 mg, 1.74 mmol) in dry CH_2Cl_2 (20 mL), acetic anhydride (660 μL, 7 mmol) was added followed by a catalytic amount of H_2SO_4. After stirring overnight at room temperature, the reaction mixture was neutralized by Et_3N (20 mL) at 0 °C and concentrated under reduced pressure. The crude product was diluted by CH_2Cl_2 (40 mL), washed (3 × 20 mL of water) and dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **9** (635 mg, 95%) as a colorless oil (petroleum ether–EtOAc, 9:1, $R_f = 0.25$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.30–7.50 (m, 10H, H_{arom}); α-Anomer: 6.51 (s, 1H, H-1), 5.74 (d, 1H, $J_{1,2} = 1.3$ Hz, H-2), 5.38 (ddd, 1H, $J_{2,3} = 2.5$ Hz, $J_{3,4} = 4.6$ Hz, H-3), 4.64 (ddd, 1H, $J_{4,5} = 6.4$ Hz, H-4), 2.25 (s, 3H, OAc), 1.68 (d, 3H, H-5). β-Anomer: 6.69 (d, 1H, $J_{1,2} = 4.6$ Hz, H-1), 5.84 (dd, 1H, $J_{2,3} = 6.4$ Hz, H-2), 5.75 (dd, 1H, $J_{3,4} = 5.1$ Hz, H-3), 4.46 (ddd, 1H, $J_{4,5} = 6.6$ Hz, H-4), 2.28 (s, 3H, OAc), 1.73 (d, 3H, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6–155.5 (COBz), 135.0–125.4 (C_{arom}). α-Anomer: 99.8 (C-1), 81.8 (C-2), 81.7 (C-4), 81.6 (C-3), 21.5 (OAc), 19.5 (C-5). β-Anomer: 95.0 (C-1), 81.0 (C-2), 80.6 (C-4), 80.4 (C-3), 21.6 (OAc), 21.5 (C-5). ESIMS *m*/*z* calcd for $[C_{21}H_{20}O_7]Na^+$: 407.1107, found: 407.1107.

4.10. *p*-Nitrophenyl 5-deoxy-α-L-arabinofuranoside (10)

To a solution of **9** (455 mg, 1.18 mmol) in CH_2Cl_2 (6 mL), $BF_3{\cdot}OEt_2$ (374 μL, 2.95 mmol) then Et_3N (84 μL, 0.6 mmol) were added at 0 °C. After stirring 10 min at 0 °C, *p*-nitrophenol (200 mg, 1.42 mmol) was added and the reaction mixture was stirred during 3 h at room temperature. Water (30 mL) was added and the mixture was diluted with CH_2Cl_2 (50 mL). After decantation, the organic layer was washed with 1 N

aqueous HCl (5 × 20 mL), satd aq $NaHCO_3$ (5 × 20 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The crude residue was dissolved in 1 N MeONa–MeOH solution and stirred during 3 h at room temperature. After neutralization by glacial HOAc and concentration under reduced pressure, the resulting crude product was purified by column chromatography (CH_2Cl_2–MeOH, 9:1) to yield **10** (165 mg, 55% (two steps)) as a beige solid (CH_2Cl_2–MeOH, 9:1, $R_f = 0.43$); mp 139 °C; $[\alpha]_D^{20}$ −29.2 (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 8.30 (d, 2H, $J = 7.4$ Hz, H-3′, H-5′), 7.20 (d, 2H, $J = 7.4$ Hz, H-2′, H-4′), 5.90 (s, 1H, H-1), 4.43 (dd, 1H, $J_{1,2} = 1.3$ Hz, $J_{2,3} = 3.6$ Hz, H-2), 4.23 (q, 1H, $J_{3,4} = 6.1$ Hz, $J_{4,5} = 6.4$ Hz, H-4), 3.91 (dd, 1H, H-3), 1.45 (d, 3H, H-5); ^{13}C NMR (CD_3OD, 100 MHz): δ 164.5 (C-1′), 143.3 (C-4′), 126.5 (C-3′, C-5′), 116.9 (C-2′, C-6′), 105.4 (C-1), 81.9 (C-2), 81.7 (C-3), 81.3 (C-4), 26.4 (C-5). ESIMS *m*/*z* calcd for $[C_{11}H_{13}NO_6]Na^+$: 278.0641, found: 278.0639. Anal. Calcd for $C_{11}H_{13}NO_6$: C, 51.77; H, 5.13. Found: C, 51.91; H, 5.22.

4.11. *p*-Nitrophenyl 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane)-α-L-arabinofuranoside (12)

A solution of **11**[37,38] (170 mg, 0.62 mmol) in pyridine (6 mL) was prepared and $TIPSCl_2$ (395 μL, 1.24 mmol) was added. The reaction mixture was stirred overnight at room temperature. Water was added (20 mL) and then the mixture was diluted with CH_2Cl_2 (20 mL). After decantation, the organic layer was washed with 1 N aqueous HCl (5 × 10 mL), satd aq $NaHCO_3$ (5 × 10 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **12** (270 mg, 85%) as a brown oil (petroleum ether–EtOAc, 9:1, $R_f = 0.32$); $[\alpha]_D^{20}$ −65.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (d, 2H, $J = 7.4$ Hz, H-3′, H-5′), 7.10 (d, 2H, $J = 7.4$ Hz, H-2′, H-4′), 5.70 (s, 1H, H-1), 4.61 (dd, 1H, $J_{1,2} = 1.1$ Hz, $J_{2,3} = 3.3$ Hz, H-2), 4.23 (dd, 1H, $J_{3,4} = 4.4$ Hz, H-3), 3.99–3.91 (m, 2H, H-5), 3.88–3.93 (m, 1H, H-4), 3.11 (s, 1H, –OH), 1.31–1.26 (m, 4H, CH*i*-Pr), 1.07–1.02 (m, 24H, CH_3*i*-Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.7 (C-1′), 143.8 (C-4′), 126.5 (C-3′, C-5′), 116.9 (C-2′, C-6′), 104.1 (C-1), 82.2 (C-2), 74.3 (C-4), 72.5 (C-3), 62.3 (C-5), 16.1 (CH*i*-Pr), 12.9 (CH_3*i*-Pr). ESIMS *m*/*z* calcd for $[C_{23}H_{39}NO_8Si_2]Na^+$: 536.2112, found: 536.2117.

4.12. *p*-Nitrophenyl 2-*O*-phenylthionocarbonate-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane)-α-L-arabinofuranoside (13)

Phenylthionocarbonate chloride (355 μL, 2.1 mmol) and DMAP (250 mg, 2.1 mmol) were added successively to a solution of **12** (480 mg, 0.9 mmol) in pyridine (10 mL). The reaction mixture was stirred overnight at room temperature. Water was added (10 mL) and then the mixture was diluted with CH_2Cl_2 (25 mL). After decantation, the organic layer was washed with 1 N aqueous HCl (5 × 10 mL), satd aq $NaHCO_3$ (5 × 10 mL), dried on $MgSO_4$, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **13** (584 mg, 100%) as a yellow oil (petroleum ether–EtOAc, 9:1, $R_f = 0.68$); $[\alpha]_D^{20}$ +25.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (d, 2H, $J = 7.3$ Hz, H-3′, H-5′), 7.35 (m, 5H, H_{arom} PTC), 7.26 (d, 2H, $J = 7.3$ Hz, H-2′, H-4′), 6.25 (s, 1H, H-1), 5.93 (dd, 1H, $J_{1,2} = 1.1$ Hz, $J_{2,3} = 5.21$ Hz, H-2), 4.61 (dd, 1H, $J_{3,4} = 4.5$ Hz, H-3), 3.99–3.94 (m, 3H, H-5, H-4), 1.38–1.32 (m, 4H, CH*i*-Pr), 1.06–1.01 (m, 24H, CH_3*i*-Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 192.3 (C=S), 164.9 (C-1′), 152.8 (C_{ipso} PTC), 149.4 (C-4′), 126.5 (C-3′, C-5′), 122.5 (C_{arom} PTC), 116.9 (C-2′, C-6′), 99.5 (C-1), 92.2 (C-2), 74.5 (C-4), 73.8 (C-3), 64.3 (C-5), 18.1 (CH*i*-Pr), 15.9 (CH_3*i*-Pr). ESIMS *m*/*z* calcd for $[C_{30}H_{43}NO_9SSi_2]Na^+$: 672.2095, found: 672.2090.

4.13. *p*-Nitrophenyl 2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane)-α-L-arabinofuranoside (14)

A solution of **13** was prepared (590 mg, 0.91 mmol) in toluene (30 mL), then *n*-Bu_3SnH (282 μL, 1.37 mmol) and AIBN (32 mg, 0.19 mmol) were added successively. The reaction mixture was stirred overnight at 80 °C and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether–EtOAc, 9:1) to yield **14** (584 mg, 63%) as a brown oil (petroleum ether–EtOAc, 9:1, $R_f = 0.39$); $[\alpha]_D^{20}$ +48.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.25 (d, 2H, $J = 7.3$ Hz, H-3′, H-5′), 7.18 (d, 2H, $J = 7.4$ Hz, H-2′, H-4′), 6.30 (s, 1H, H-1), 4.48 (ddd, 1H, $J_{2a,3} = 6.7$ Hz, $J_{2b,3} = 2.1$ Hz, $J_{3,4} = 3.2$ Hz, H-3), 4.06–4.01 (m, 2H, H-5), 3.56–3.51 (m, 1H, H-4), 2.45 (dddd, 2H, $J_{1,2a} = 1.3$ Hz, $J_{1,2b} = 4.5$ Hz, H-2), 1.29–1.23 (m, 4H, CH*i*-Pr), 1.16–1.12 (m, 24H, CH_3*i*-Pr); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.6 (C-1′), 142.9 (C-4′), 126.5 (C-3′, C-5′), 116.9 (C-2′, C-6′), 101.6 (C-1), 75.2 (C-4), 64.7 (C-3), 60.3 (C-5), 45.1 (C-2), 18.2 (CH*i*-Pr), 13.9 (CH_3*i*-Pr). ESIMS *m*/*z* calcd for $[C_{23}H_{39}NO_7Si_2]Na^+$: 520.2163, found: 520.2166.

4.14. *p*-Nitrophenyl 2-deoxy-α-L-*erythro*-pentofuranoside (15)

Compound **14** (100 mg, 0.20 mmol) was added to a solution of TBAF 1 M in THF (10 mL). The reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure. The resulting crude product was purified by column chromatography

(CH_2Cl_2–MeOH, 20:1) to yield **15** (23 mg, 88%) as a yellow solid (CH_2Cl_2–MeOH, 20:1, $R_f = 0.35$); mp 123 °C; $[\alpha]_D^{20}$ +79.4 (*c* 1, acetone); ^{1}H NMR (D_2O, 400 MHz): δ 8.15 (d, 2H, $J = 7.3$ Hz, H-3′, H-5′), 7.11 (d, 2H, $J = 7.4$ Hz, H-2′, H-4′), 6.16 (s, 1H, H-1), 4.21 (ddd, 1H, $J_{2a,3} = 6.3$ Hz, $J_{2b,3} = 2.9$ Hz, $J_{3,4} = 3.7$ Hz, H-3), 3.99–3.95 (m, 1H, H-4), 3.77–3.71 (m, 2H, H-5), 2.24 (dddd, 2H, $J_{1,2a} = 1.1$ Hz, $J_{1,2b} = 4.7$ Hz, H-2); ^{13}C NMR (D_2O, 100 MHz): δ 161.4 (C-1′), 142.9 (C-4′), 126.4 (C-3′, C-5′), 117.6 (C-2′, C-6′), 102.6 (C-1), 86.2 (C-4), 71.4 (C-3), 63.1 (C-5), 42.5 (C-2). Anal. Calcd for $C_{11}H_{13}NO_6$: C, 51.77; H, 5.13. Found: C, 51.84; H, 5.23.

4.15. Autocondensation reactions

A 5 mM aqueous solution of **6** or **10** (1.4 mL) was incubated with 12 IU AbfD3 with magnetic stirring for 1 h at 60 °C. Reactions were quenched by enzyme denaturation at 100 °C for 10 min. After lyophilization, the residue was dissolved in water (0.1 mL) and the autocondensation products were purified by preparative TLC using 4:1 CH_2Cl_2–MeOH as the mobile phase. The desired products were detected by UV absorption (254 nm), collected from plates and extracted with 1:1 CH_2Cl_2–MeOH (4 mL). After filtration and freeze-drying, the isolated disaccharides **16** and **17** were characterized by NMR and high resolution mass spectrometry.

4.16. *p*-Nitrophenyl 5-deoxy-α-L-arabinofuranosyl-(1→2)-5-deoxy-α-L-arabinofuranoside (16)

(~1 mg accumulated from a few preparative plates) HPTLC (CH_2Cl_2–MeOH, 4:1) $R_f = 0.46$; ^{1}H NMR (D_2O, 500.13 MHz): δ 8.17 (d, 2H, $J = 9.3$ Hz, H-3′, H-5′), 7.11 (d, 2H, $J = 9.3$ Hz, H-2′, H-6′), 5.81 (d, 1H, $J_{1a,2a} = 1.7$ Hz, H-1a), 5.06 (d, 1H, $J_{1b,2b} = 1.2$ Hz, H-1b), 4.24 (dd, 1H, $J_{2a,3a} = 4.9$ Hz, H-2a), 4.09 (dd, 1H, $J_{4a,5a} = 6.4$ Hz, $J_{3a,4a} = 7.5$ Hz, H-4a), 4.03 (dd, 1H, $J_{2b,3b} = 3.9$ Hz, H-2b), 3.92 (t, 1H, $J_{4b,5b} = 6.5$ Hz, H-4b), 3.86 (dd, 1H, H-3a), 3.60 (dd, 1H, $J_{3b,4b} = 6.7$ Hz, H-3b), 1.25 (d, 3H, H-5a), 1,07 (d, 3H, H-5b); ^{13}C NMR (D_2O, 125.76): δ 161.20 (C-1′), 142.10 (C-4′), 126.10 (C-3′, C-5′), 116.43 (C-2′, C-6′), 107.63 (C-1b), 103.99 (C-1a), 88.06 (C-2a), 81.80 (C-2b), 81.58 (C-3b), 79.78 (C-3a), 79.37 (C-4b), 79.08 (C-4a), 17.23 (C-5b), 16.93 (C-5a); HRMS (ESI^+) for $C_{16}H_{21}NO_9Na$: *m/z* $[M + Na]^+$ calcd 394.1114, found: 394.1116.

4.17. *p*-Nitrophenyl 5-deoxy-α-L-arabinofuranosyl-(1→3)-5-deoxy-α-L-arabinofuranoside (17)

(~1 mg accumulated from a few preparative plates) HPTLC (CH_2Cl_2–MeOH, 4:1) $R_f = 0.49$; ^{1}H NMR (D_2O, 500.13 MHz): δ 8.17 (d, 2H, $J = 9.2$ Hz, H-3′, H-5′), 7.12 (d, 2H, $J = 9.3$ Hz, H-2′, H-6′), 5.78 (s, 1H, H-1a), 5.08 (s, 1H, H-1b), 4.44 (dd, 1H, $J_{1a,2a}$ <1 Hz, $J_{2a,3a} = 2.1$ Hz, H-2a), 4.20 (t, 1H, $J_{4a,5a} = 6.5$ Hz, H-4a), 4.02 (dd, 1H, $J_{1b,2b}$ <1 Hz, $J_{2b,3b} = 4.4$ Hz, H-2b), 3.98 (t, 1H, $J_{4b,5b} = 6.3$ Hz, H-4b), 3.81 (dd, 1H, $J_{3a,4a} = 5.2$ Hz, H-3a), 3.65 (dd, 1H, $J_{3b,4b} = 6.7$ Hz, H-3b), 1.29 (d, 3H, H-5a), 1.25 (d, 3H, H-5b); ^{13}C NMR (D_2O, 125.76): δ 161.30 (C-1′), 142.20 (C-4′), 126.05 (C-3′, C-5′), 116.68 (C-2′, C-6′), 106.81 (C-1b), 105.15 (C-1a), 86.93 (C-3a), 81.60 (C-2b, C-3b), 79.98 (C-2a), 79.27 (C-4a, C-4b), 17.59 (C-5a, C-5b); HRMS (ESI^+) for $C_{16}H_{21}NO_9Na$: *m/z* $[M+Na]^+$ calcd 394.1114, found: 394.1117.

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