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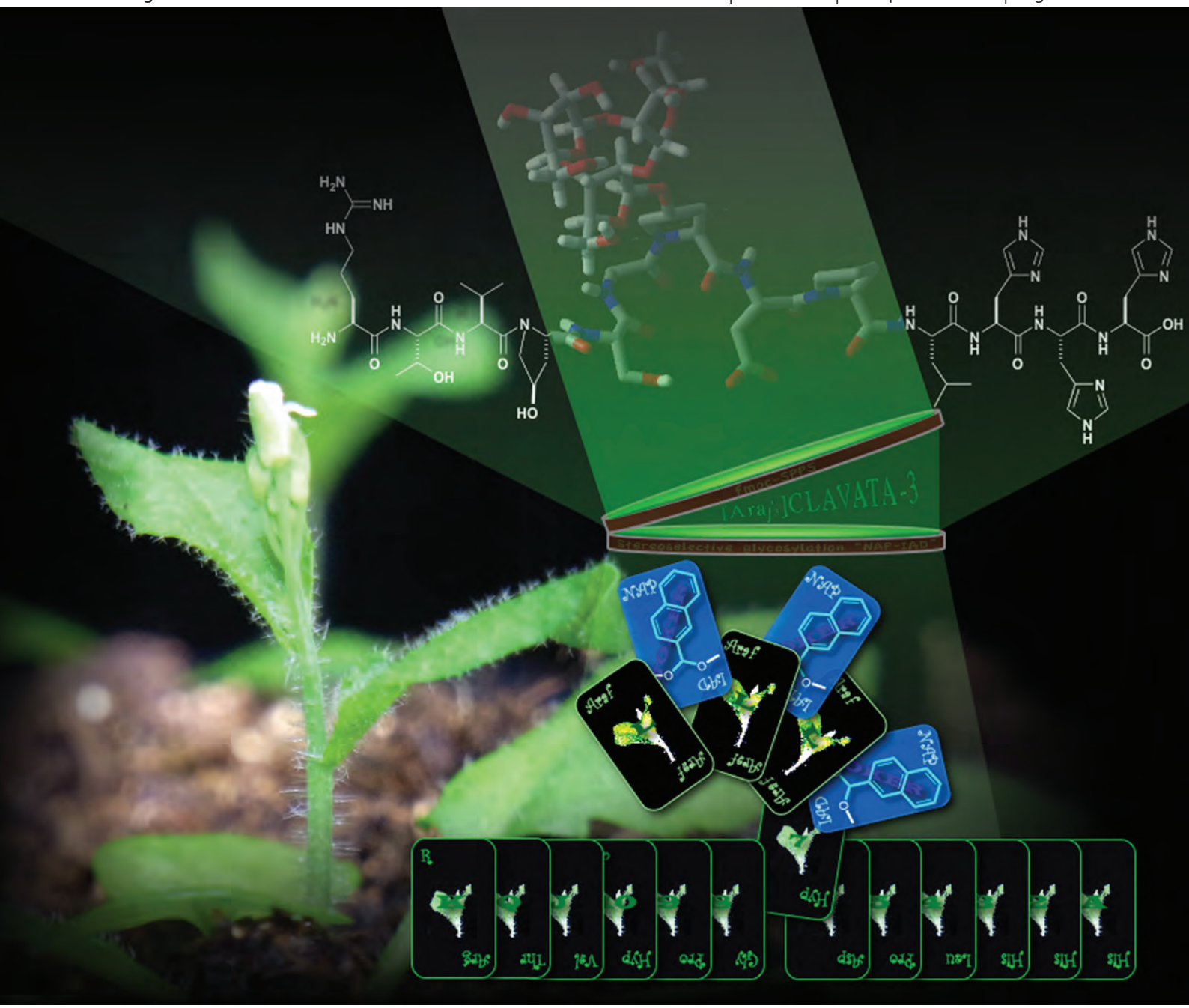

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Stereoselective synthesis of *Arabidopsis* CLAVATA3 (CLV3) glycopeptide, unique protein post-translational modifications of secreted peptide hormone in plant†

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The unique hydroxylproline (Hyp)-linked *O*-glycan modification is a common process in hydroxyproline-rich glycoproteins (HRGPs). The modification occurs through post-translational hydroxylation at 4-position of proline residues some of which are followed by *O*-glycosylation at the resulting Hyp which is also found in some secreted peptide hormones such as CLAVATA3 (CLV3) of *Arabidopsis thaliana* plants. An active mature CLV3 is a tridecapeptide linked to β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→2)-β-L-Ara*f* at a Hyp residue in the center of the peptide sequence such as Arg-Thr-Val-Hyp-Ser-Gly-Hyp(L-Araf_n)-Asp-Pro-Leu-His-His-His (n = 3). We report here the synthesis of the secreted and modified CLV3 glycopeptide with all glycoforms (Araf_{0-3}CLV3) of *A. thaliana* plants. A highly stereoselective β-arabinofuranosylation of Hyp derivatives as the key step of the synthesis of CLV3 glycopeptide was achieved by NAP ether-mediated IAD, which was effectively applied to the synthesis of oligoarabinosylated hydroxylproline [Hyp(L-Araf_{1-3})] derivatives. Fmoc-solid phase peptide synthesis was carried out using COMU as the coupling reagent for the introduction of [Hyp(L-Araf_{0-3})] derivatives as well as further elongation to the CLV3 glycopeptides.

Introduction

Posttranslational modifications regulate the function of proteins in various ways. One of the most prominent among them is glycosylation,[1] which occurs by the combined action of a variety of glycosyltransferases and glycosidases. Whereas the structures of glycoprotein glycans are diverse, most of them can be categorized into two major classes, *N*- and *O*-linked glycans. They comprise oligosaccharides linked to the side chain of asparagine (Asn) or serine (Ser)/threonine (Thr) residue, respectively. In the plant kingdom, a unique hydroxylproline (Hyp)-linked *O*-glycan modification process has been found. It occurs through posttranslational hydroxylation and subsequent glycosylation at 4-position of specific proline residues. This modification is widely distributed in hydroxyproline-rich glycoproteins (HRGPs)[2] such as extensins, proline-rich proteins and arabinogalactan proteins. More recently, secreted peptide hormones,[3] including CLAVATA3 (CLV3),[4] CLE22,[5] and PSY1,[6] have been identified from several plants which carry Hyp modified by oligo-arabinofuranodides (Ara*f*).

CLV3, initially formed as a preproprotein of 96 amino acids, is secreted from stem cells and regulates cell homeostasis in the shoot apical meristem of *Arabidopsis*.[7] As the ligand of CLV1 (a leucine-rich repeat transmembrane receptor serine threonine kinase[8]), CLV3 is responsible for regulating the stem-cell signaling pathway, possibly by the formation of a complex with CLV1 and CLV2 (a similar protein to CLV1 lacking the kinase domain[9]).

In 2009, Ohyama *et al.* reported the structure of mature CLV3 of *Arabidopsis thaliana* plants, which was shown to be a 13 amino-acid oligoarabinofuranosylated glycopeptide (Araf_3CLV3), Arg-Thr-Val-Hyp-Ser-Gly-Hyp(L-Araf_n)-Asp-Pro-Leu-His-His-His (n = 3) (Fig. 1).[10] The trisaccharide [β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→4)-β-*O*-Hyp] linked to the Hyp consists of consecutive β-linked arabinofuranoside linkages. Because of its unique structure and interesting biological activity, we deemed this glycopeptide to be an attractive target.

In order to synthesize CLV3, a problem associated with the construction of β-L-arabinofuranoside (β-L-Ara*f*) was foreseen. The difficulty derives from its 1,2-*cis*, non-axial nature, precluding the use of conventional methodologies in *O*-glycoside synthesis.

In the context of the construction of β-D-Ara*f* linkages found in mycobacterial arabinan, various concepts have been tested. Most notably, Lowary[11] and Kim[12] reported elegant approaches

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†Electronic supplementary information (ESI) available: NMR spectra of synthetic compounds in the Experimental section and HPLC charts and the results of tandem MS/MS analysis of **1–4**. See DOI: 10.1039/c3ob41212a

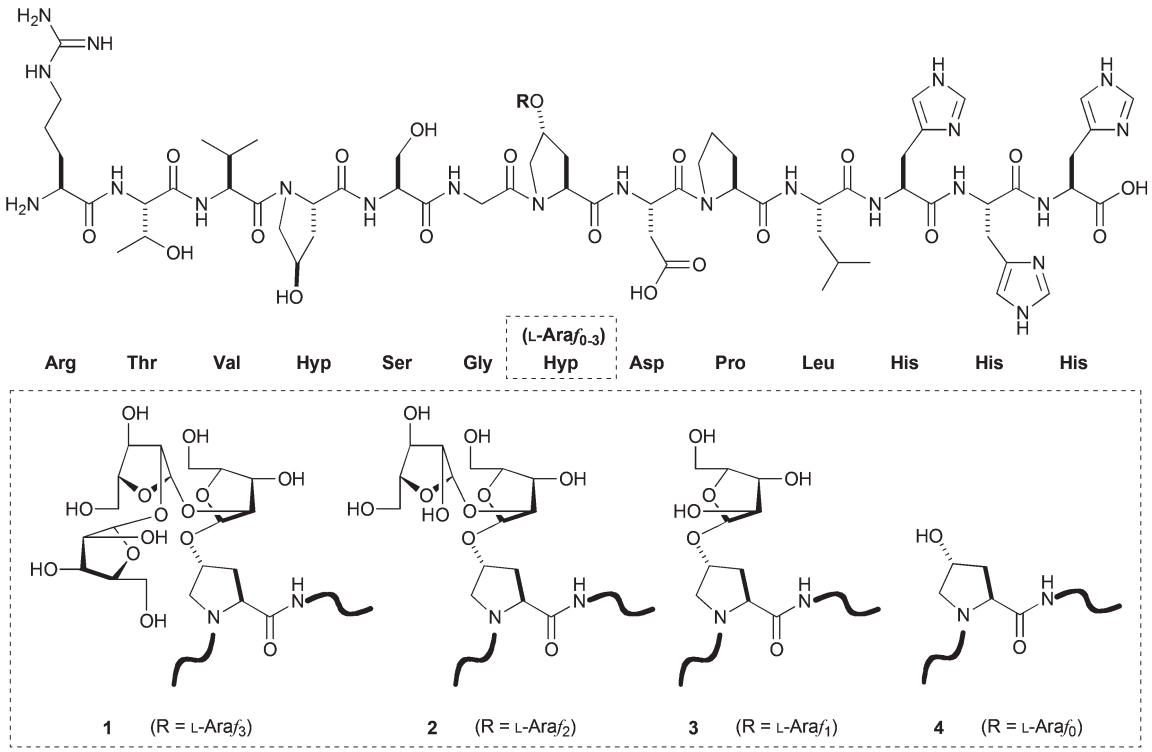


Fig. 1 Post-translationally modified CLV3 (glyco)peptide **1–4**.

based on S_N2-type displacement of α-triflates derived from 2,3-anhydro-[11] or carboxybenzyl-substituted (CB-substituted)[12] donors. Alternatively, certain cyclic protections have proven beneficial in enhancing the β-selectivity, possibly due to their property to bring conformational constraints to arabinofuranosyl donors. For instance, 3,5-*O*-di-*t*-butylsilylene-protected (DTBS-protected) donors, reported by Boons[13] and others,[14–18] were shown to give substantial β-selectivity, while our study indicated the suitability of 3,5-*O*-tetra-*i*-propyldisiloxanylidene-protection (TIPDS-protection) for this purpose.[14]

Recently, an attempt to construct the hydroxylproline-linked β-L-Ara*f* structure of Art v 1, the major allergen of mugwort pollen,[19] by using donors protected by a 3,5-*O*-di-*t*-butylsilylene (DTBS) group was reported by Xie and Taylor.[20] However, both the yield and the selectivity were reported to be low.

In order to realize the exclusive formation of β-Ara*f* linkage, approaches based on intramolecular aglycon delivery (IAD) have been investigated.[21] To this end, 2-*O*-*p*-methoxybenzyl (PMB)[22,23] or 2-*O*-(2-naphthyl)methyl (NAP)[24] and 5-*O*-NAP[25] equipped donors were examined, which were applied to the synthesis of mycobacterial arabinans.[22,25] Recently, Matsubayashi *et al.* reported the stereoselective synthesis of CLV3 glycopeptide using PMB-mediated IAD as the key reaction.[23]

In this report, we describe our own effort to stereoselectively synthesize β-L-Ara*f* linked hydroxyproline through NAP-ether mediated intramolecular aglycon delivery (IAD). Elongation of the arabinan chain gave β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→4)-β-*O*-Hyp (Araf_2Hyp) and β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→2)-β-L-Ara*f*-(1→4)-β-*O*-Hyp (Araf_3Hyp). Finally, the synthesis of CLV3 (glyco)peptides was achieved by Fmoc-based solid phase peptide synthesis using Ara$f_{0–3}$Hyp derivatives.[26]

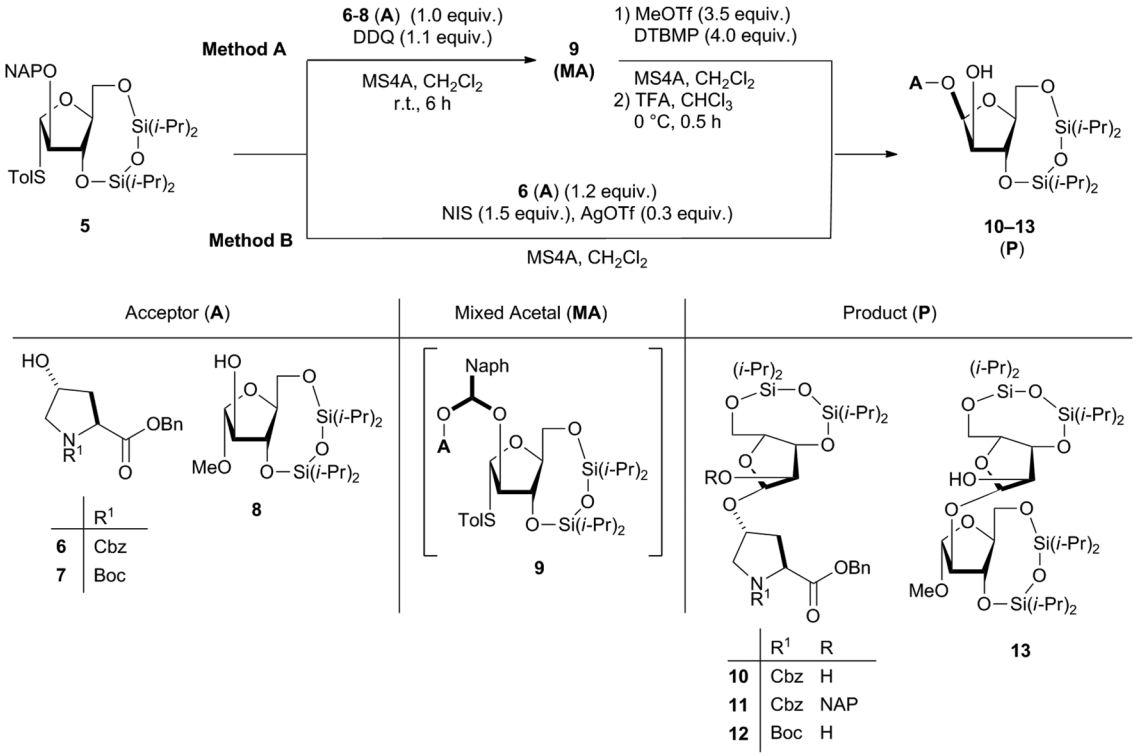
Results and discussion

In the beginning, we examined the suitability of TIPDS-controlled β-L-Ara*f* formation[14] to achieve our goal. Thus, the reaction of the donor **5**[27] with *N*-benzyloxycarbonyl (Cbz)-protected Hyp-OBn **6**[28] was conducted under our standard conditions to give the disaccharide **11**, but in a stereorandom manner (α : β = 1 : 1.3, Table 1, entry 6).

Anomeric configuration of the α- and β-isomers was determined by $J_{H1–H2}$ (1.5 and 4.0 Hz) and δ(C1) (105.1 and 98.4 ppm) values.[29] Since the products were obtained as mixtures of rotamers, rigorous confirmation of the structure was made after removal of the carbamate moiety.[30,31]

Given the limited success obtained by direct glycosylation, we turned our attention to the IAD approach,[24,25] which was examined by using the same donor (**5**)–acceptor (**6**) combination. As depicted in Fig. 2, formation of the mixed acetal **9** proceeded cleanly (87%) in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), whereas subsequent IAD (MeOTf–DTBMP[32]) at room temperature was slow giving β-L-arabinofuranosylhydroxylproline **10** in 37% yield (Table 1, entry 1), with recovery of Cbz-Hyp-OBn and hydrolysis product corresponding to the glycosyl donor **5**. The same IAD at a

Table 1 Examination of β-L-arabinofuranosylation



Entry	Method	Acceptor	MA/%	Temp./time	Product	Yield[a] /%	α : β
1	**A**	**6**	87	r.t./120 h	**10**	37 (32)	β only
2	**A**	**6**	—	40 °C/48 h	**10**	81 (70)	β only
3	**A**	**6**	—	40 °C/48 h[a]	**10**	67[b] (58)	β only
4	**A**	**7**	84	40 °C/48 h	**12**	67 (56)	β only
5	**A**	**8**	92	40 °C/48 h	**13**	79 (72)	β only
6	**B**	**6**	—	−40 °C–r.t./15 min	**11**	76	1 : 1.3

[a] Two step yield in parentheses. [b] The reaction carried out in the presence of 5 equiv. of TTMSS. Method A: intramolecular aglycon delivery; B: intermolecular glycosylation.

slightly elevated temperature (40 °C) pleasingly gave a satisfactory yield (81%) of **10**.

In our previous study of 1,2-*cis* hexopyranoside synthesis, it was found that the naphthylmethyl group was preserved in IAD, when an appropriate hydride donor, in particular tris(trimethylsilyl)silane (TTMSS),[24] was included. However, the conditions that re-generate NAP ether were not found in the IAD of **9**, solely giving **10**, even in the presence of TTMSS (entry 3). Various types of hydride transfer reagents such as dicyclohexylisobutylamine,[33] cycloheptatriene,[34] and poly(methyl-hydrosiloxane)[35] were examined; however, the formation of NAP ether product was not observed. *N-tert*-Butyloxycarbonyl-protected (Boc-protected) Hyp acceptor **7**[36] also gave the desired β-L-arabinofuranosides **12**, but in somewhat lower yield, because of partial cleavage of the Boc group during work-up under acidic conditions (entry 4).

Before conducting elongation of β-D-Ara*f* linkages, the formation of β-L-Ara*f*-(1→2)-β-L-Ara*f* was tested using methyl arabinofuranoside **8**[38] as a model acceptor, which was reacted with the glycosyl donor **5**. Again, the IAD approach was successful, giving the disaccharide **13** in good yield (72%) with complete β-stereoselectivity (entry 5).

Accordingly, a further plan was made to apply the NAP-IAD for the construction of mono-, di- and tri-arabinosylated hydroxyproline **21**, **23**, and **25** (Scheme 1). Treatment of a mixture of the L-Ara*f* donor **5** and Ara*f*$_1$Hyp derivative **10** with DDQ afforded the corresponding mixed acetal **15** in 58% yield. Interestingly, the formation of the same mixed acetal was revealed to be much more facile when a combination of 2-*O*-NAP ether **β-11**[39,40] and the 2-*O*-unprotected donor **14**[27] was employed. This combination cleanly gave **15** (87%), which was subjected to IAD to give Ara*f*$_2$Hyp derivative **16**[30,31] in 73% yield from **5**.

We speculate that the reactivity of the hydroxyl group of the Ara*f*$_2$Hyp derivative **16** is attenuated due to the steric hindrance caused by a *cis*-oriented bulky aglycon. On the other hand, the methylenic carbon of the NAP ether may well be more accessible, because it is two bonds apart from the

furanoside ring. Further introduction of an Ara*f* residue was also conducted by using the 2-*O*-unprotected donor **14**. Namely, compound **16** was once converted to the 2-*O*-NAP ether **17**,[39] treatment of which with the thioglycoside **14** and DDQ gave the mixed acetal **18** in good yield. As we hoped, subsequent IAD afforded a desired Araf_3Hyp derivative **19**[30,31] in 77% yield, successfully completing the construction of all three β-glycosidic linkages.

The thus obtained Araf_{1-3}Hyp derivatives **10**, **16**, and **19** were converted to per-*O*-acetylated derivatives **20**, **22**, and **24** by deprotection of the TIPDS group[40] followed by acetylation. Subsequent hydrogenolysis and Fmoc protection gave Fmoc-Hyp (per-*O*-acetyl-Araf_{1-3}) **21**, **23**, and **25**. With the oligoarabinosylated hydroxylproline derivative in hand, synthesis of the glycopeptides (Araf_{0-3}CLV3) was planned and it was executed by the Fmoc-solid phase peptide synthesis strategy.[26]

Namely, starting from the hexapeptide Fmoc-Asp(*t*Bu)-Pro-Leu-His(Trt)-His(Trt)-His(Trt), immobilized as a trityl ester on NovaSyn®TGT alcohol resin **27**, which was prepared from the monomer **26**, the glycopeptides (Araf_{0-3}CLV3) were synthesized as shown in Scheme 2. Throughout the chain elongation, (1-cyano-2-ethoxy-2-oxo-ethylideneaminooxy)-dimethylamino-morpholinouronium hexa-fluorophosphate (COMU),[41] a non-explosive alternative to the classic benzotriazole coupling reagents, was used as an activating agent, which was expected to reduce the incidence of N-terminal masking during coupling steps.

Introduction of Fmoc-Hyp **28** or arabinosylated Fmoc-Hyp (**21**, **23**, **25**) to the hexapeptide on resin **27** was carried out successfully to give (glyco)heptapeptides (**29–32**), monitoring of which was done by MALDI TOF-MASS analysis after cleavage of small aliquots of resins. A subsequent chain elongation was conducted manually, giving the expected glycosylated tridecapeptides (**33–36**). Acidic cleavage from resin and deacetylation completed the synthesis of Araf_{0-3}CLV3 (**1–4**). Spectral data of synthetic CLV3s were all identical with those of reported compounds.[23]

These glycopeptides were converted to resin-immobilized forms,[42] which were useful in identifying novel lectins that recognize CLV3 or other HRGPs.

Conclusions

We report here the synthesis of the secreted and modified CLV3 glycopeptide (Araf_{0-3}CLV3, **1–4**) of *A. thaliana* plants. Highly stereoselective β-arabinofuranosylation of Hyp derivatives (**6**, **7**) as the key step of the synthesis of **1–3** was achieved by NAP ether-mediated IAD, which was effectively applied to the synthesis of oligoarabinosylated hydroxylproline derivatives (**21**, **23**, **25**). Fmoc-solid phase peptide synthesis was carried out using COMU as the coupling reagent for the introduction of Fmoc-Hyp(per-*O*-acetyl-Araf_{1-3}) (**21**, **23**, **25**) and Fmoc-Hyp(*t*Bu) as well as further elongation to the CLV3 (glyco)tridecapeptide derivatives (**34–36**). These compounds will be useful in finding new CLV3 binding lectins.

Experimental section

General procedures

Column chromatography was performed on silica gel 60 (EM Science, 40–100 mesh). Reactions were monitored by thin-layer chromatography (TLC) using Kieselgel 60 F254 (EM Science), and compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 was freshly obtained from the Glass Contour solvent dispensing system. AgOTf was co-evaporated with toluene (2–3 times) and dried *in vacuo* for 2–3 h directly prior to application. Molecular sieves (4 Å) were activated at 200 °C for 2–3 h under vacuum prior to application. All reactions were carried out under an argon atmosphere. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Advance 500 spectrometer with a cryoprobe. ^{1}H NMR spectra were recorded in $CDCl_3$ and referenced to TMS at 0.00 ppm and H_2O at 4.79 ppm, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at 77.00 ppm. Assignments were made by standard gCOSY and gHSQC. Optical rotations were measured with a Jasco DIP-370 polarimeter. MALDI-TOF mass spectra were recorded using a Bruker Autoflex Speed spectrometer with 2-hydroxy-5-

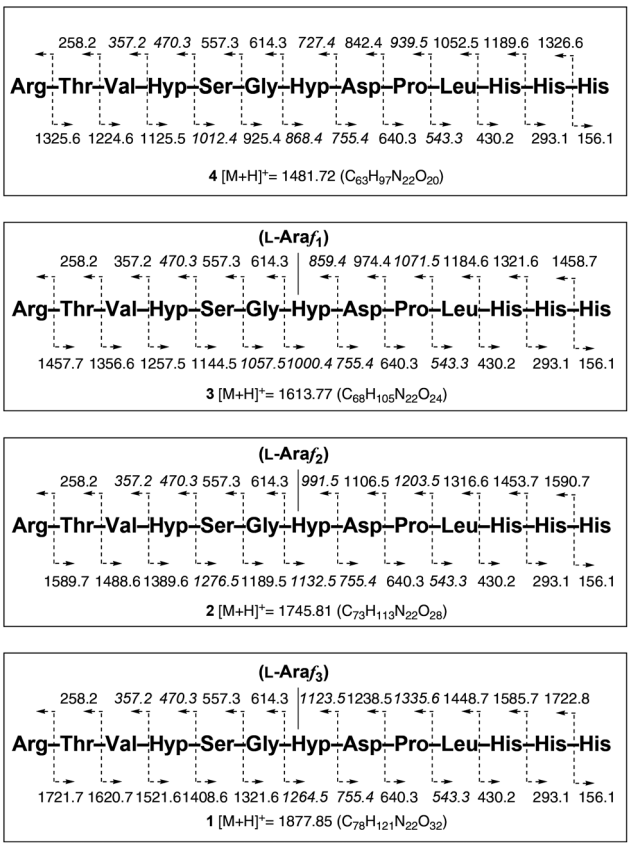
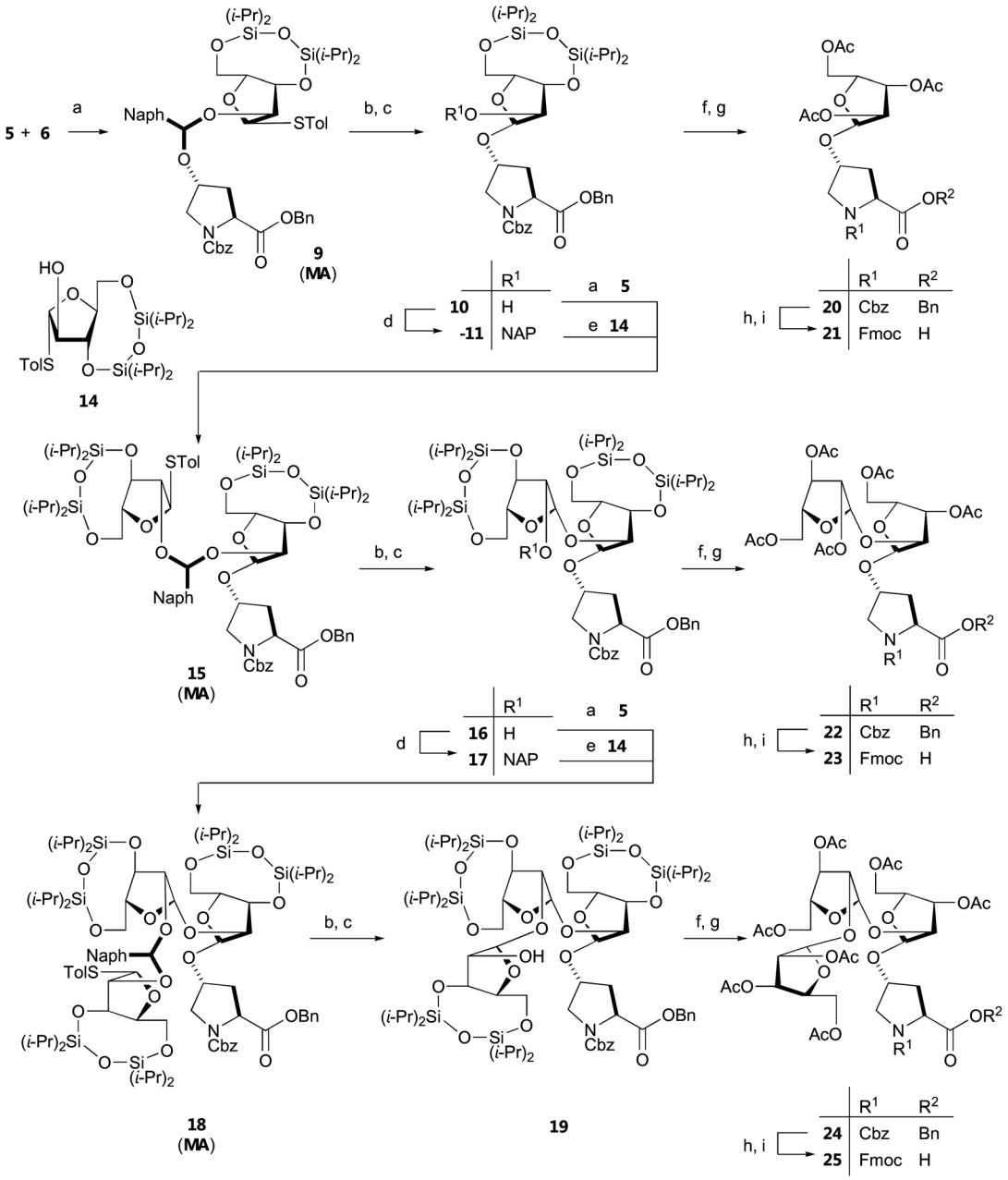


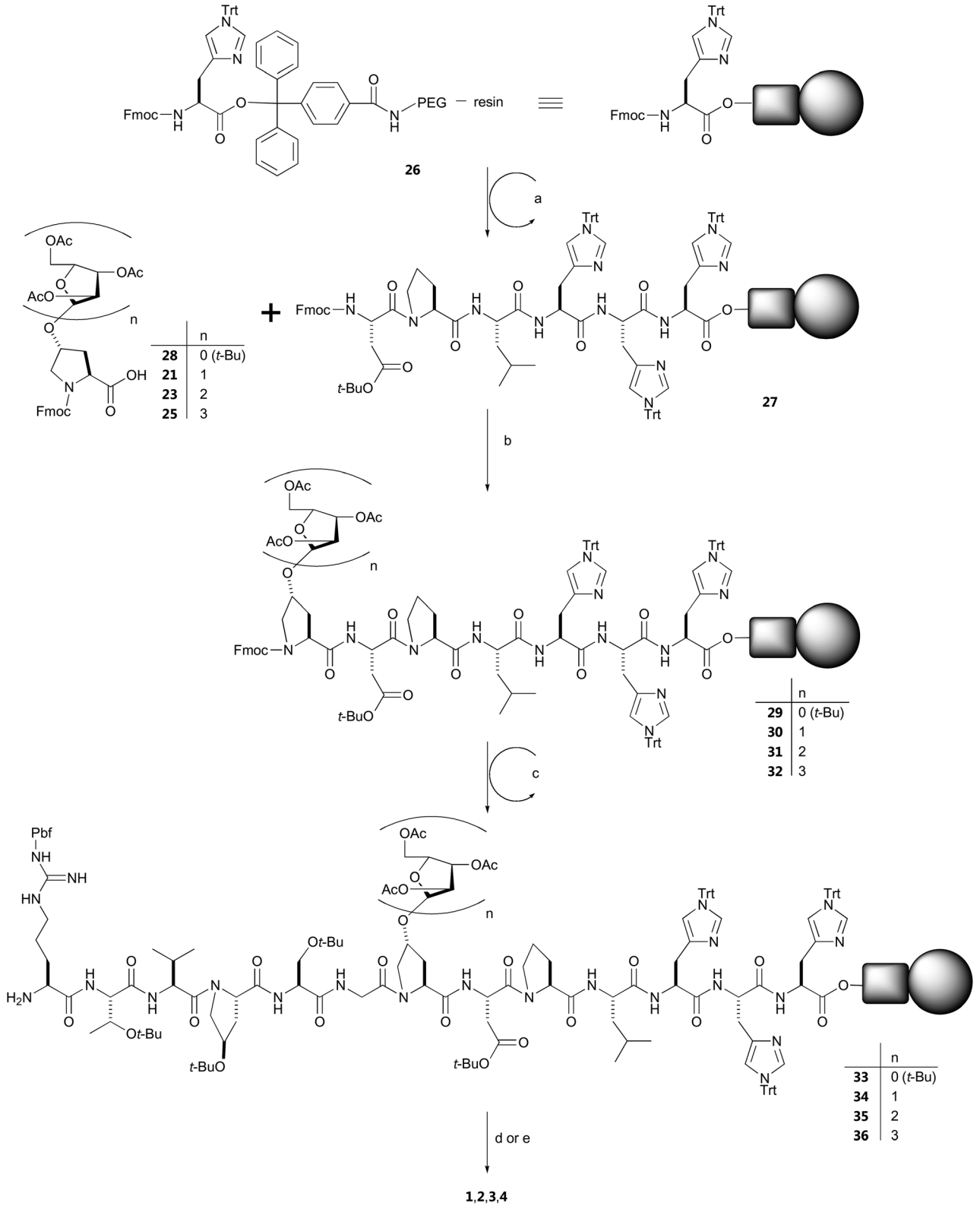
Fig. 2 Fragment ions observed by tandem MS/MS analysis of synthetic [Araf_n]-CLV3 (n = 0–3) (**1–4**). Numbers on the upper and lower sides of 13 (glycosyl)-amino acid sequences are b- and y-ions, respectively. Ions in italic letters were also detected though at a small intensity. See also ESI.†



Scheme 1 Synthesis of oligoarabinosylated hydroxylproline derivative. *Reagents and conditions*: (a) **5** (1.0 equiv.), DDQ (1.1–2.0 equiv.), MS4A, CH_2Cl_2, r.t., 87% (**9** for 6 h), 58% (**15** for 24 h), trace (**18** for 24 h); (b) MeOTf (3.5 equiv.), DTBMP (4.0 equiv.), MS4A, CH_2Cl_2, 40 °C, 48 h; (c) 10% TFA in $CHCl_3$, 0 °C, 0.5 h, 81% for (**10** from **9**), 84% for (**16** from **15**), 77% (**19** from **18**); (d) NAPBr, NaH, TBAI, DMF, −20 °C, 6 h, 82% (**13**), 79% (**17**); (e) **14** (1.0 equiv.), DDQ (1.1 equiv.) MS4A, CH_2Cl_2, r.t., 16 h, 87% (**15**), 67% (**18**); (f) TBAF, pyridine–THF, 0 °C, 1 h; (g) Ac_2O, pyridine, r.t., 3 h, 89% (**20** from **10**), 81% (**22** from **16**), 87% (**24** from **19**); (h) H_2, $Pd(OH)_2$, EtOAc–EtOH (2 : 1), r.t., 16 h; (i) FmocCl, DIPEA, CH_2Cl_2, r.t., 5 h, 81% (**21** from **20**), 74% (**23** from **22**), 81% (**25** from **24**).

methoxybenzoic acid as the matrix. HRMS determinations were performed by the use of a JEOL AccuTOF JMS-T700LCK mass spectrometer with CF_3CO_2Na as the internal standard. Tandem MS/MS was analyzed using the AB Sciex 4800 Plus MALDI TOF/TOF mass spectrometer at the Support Unit for Bio-material Analysis in Research Resources Center, RIKEN Brain Science Institute. All other reagents were purchased from Wako Pure Chemical Industries Ltd, Kanto Chemical Co., Inc., Tokyo Chemical Industry Co., Ltd, and Sigma-Aldrich Co.

4-Methylphenyl 3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-1-thio-α-L-arabinofuranoside (14). To a solution of 4-methylphenyl 1-thio-α-L-arabinofuranoside[37] (3.20 g, 12.4 mmol) in pyridine (30 mL) at 0 °C was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (4.39 mL, 13.7 mmol) dropwise. After stirring for 3 h at 0 °C, the reaction mixture was gradually warmed up to room temperature and stirred for 2 h. Upon completion, the mixture was quenched with saturated $NaHCO_3$ and extracted with $CHCl_3$. The combined organic phase was



Scheme 2 Synthesis of CLV3 glycopeptide. *Reagents and conditions*: (a) see the experimental procedure; (b) i. piperidine, DMF, room temperature, 10 min; ii. Fmoc-Hyp(L-Araf_{0-3}) (3.0 equiv.), COMU (3.0 equiv.), DIPEA (6.0 equiv.), room temperature, 16 h, see also the Experimental section; (c) i. piperidine, DMF, room temperature, 10 min; ii. Fmoc-AA (5.0 equiv.), COMU (5.0 equiv.), DIPEA (10 equiv.), room temperature, 2–4 h. The completion of two step reactions (Fmoc cleavage and coupling) had been estimated by MALDI-TOF MASS of the sample after micro-cleavage. See also the Experimental section; (d) i. TFA–TIPS–H_2O (190 : 5 : 5), 1 h; ii. NeOMe, MeOH, room temperature, 2 h, 37% (**3**), 31% (**2**) 34% (**1**); (e) TFA–TIPS–H_2O (190 : 5 : 5), 1 h, 30% (**4**). The yields were calculated based on the initial loading capacity of the used NovaSyn® TGT alcohol resin (0.02 mmol g^{-1}).

washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (4.48 g, 72%). Analytical data for **14**: R_f = 0.50 (EtOAc–hexane, 1.5/8.5, v/v); $[\alpha]_D^{27}$ −122.39° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94–1.17 (m, 28H, TIPDS), 2.32 (s, 3H, CH_3), 2.43 (d, 1H, *J* = 4.5 Hz, –OH), 3.92–3.95 (m, 1H, 4-H), 3.97–3.98 (m, 2H, 5-Ha, 5-Hb), 4.16–4.21 (m, 2H, 2-H, 3-H), 5.24 (d, 1H, $J_{1,2}$ = 5.0 Hz, 1-H), 7.09 (d, 2H, *J* = 8.5 Hz, aromatic), 7.39 (d, 2H, *J* = 8.5 Hz, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 13.0, 13.3, 13.7, 17.20, 17.26, 17.30, 17.5, 17.6, 21.3, 61.4, 76.4, 80.6, 81.9, 91.3, 129.9, 130.9, 132.0, 137.6; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{24}H_{42}NaO_5SSi_2$ 521.2189, found 521.2204.

4-Methylphenyl 2-*O*-(2-naphthyl)methyl-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-1-thio-α-L-arabinofuranoside (5). To a solution of **14** (2.50 g, 4.8 mmol) in DMF (20 mL) at 0 °C was added 2-(bromomethyl)naphthalene (NAPBr) (1.27 g, 5.7 mmol) and NaH (60% dispersion in oil, 0.23 g, 5.7 mmol) portionwise. The reaction mixture was stirred at 0 °C for 5 h. Upon completion, the reaction was diluted with EtOAc, poured into ice-water, stirred until cessation of H_2 evolution, and then extracted with EtOAc. The combined organic phase was washed with sat. NH_4Cl, and dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (2.60 g, 84%). Analytical data for **2**: R_f = 0.47 (EtOAc–hexane, 1.0/9.0, v/v); $[\alpha]_D^{27}$ −39.19° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94–1.14 (m, 28H, TIPDS), 2.28 (s, 3H, CH_3), 3.69–4.02 (m, 3H, 4-H, 5-Ha, 5-Hb), 4.09 (dd, 1H, $J_{2,3}$ = 4.5 Hz, 2-H), 4.36 (dd, 1H, $J_{3,4}$ = 7.5 Hz, 3-H), 4.81 (d, 1H, *J* = 12.0 Hz, CH_2Ar), 4.87 (d, 1H, *J* = 12.0 Hz, CH_2Ar), 5.44 (d, 1H, $J_{1,2}$ = 4.5 Hz, 1-H), 7.04 (d, 2H, *J* = 8.0 Hz, aromatic), 7.35 (d, 2H, *J* = 8.0 Hz, aromatic), 7.44 (m, 3H, aromatic), 7.78 (m, 4H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 13.0, 13.3, 13.7, 17.2, 17.3, 17.5, 17.8, 21.2, 61.4, 73.0, 76.2, 77.5, 80.3, 89.3, 89.9, 126.0, 126.1, 126.2, 126.8, 127.9, 128.1, 128.2, 129.78, 129.81, 131.4, 131.7, 133.2, 133.4, 135.3, 137.4; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{35}H_{50}NaO_5SSi_2$ 661.2815, found 661.2795.

***N*-Benzyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (10).** DDQ (1.84 g, 8.10 mmol) was added to a mixture of Z-Hyp-OBn[28] (**6**) (2.0 g, 5.63 mmol), glycosyl donor (**5**) (4.31 g, 6.76 mmol), and freshly activated molecular sieves (4 Å, 12 g) in dry CH_2Cl_2 (30 mL) at room temperature. The reaction mixture was stirred for 16 h under an argon atmosphere. The reaction mixture was quenched with aqueous ascorbate buffer, and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$ and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford mixed acetal (4.86 g, 87%); MALDI-TOF MS: $[M + Na]^+$ calcd for $C_{55}H_{69}NNaO_{10}SSi_2$ 1014.4079, found 1014.6565. A mixture of the mixed acetal (2.2 g, 2.21 mmol), DTBMP (1.81 g, 8.87 nmol) and freshly activated molecular sieves (4 Å, 10 g) in dry CH_2Cl_2 (110 mL) was stirred under an argon atmosphere at room temperature for 30 min. MeOTf (0.87 mL, 7.76 mmol) was then added to the mixture and the mixture was stirred for 48 h at 40 °C. Upon completion the reaction mixture was cooled down, quenched with Et_3N, diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo*. The crude residue was dissolved in $CHCl_3$ (30 mL) and cooled at 0 °C. TFA (3 mL) was added and stirred for 30 min at the same temperature. The reaction mixture was diluted with $CHCl_3$ and washed successively with H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (1.40 g, 81%, β-only as the rotamer mixture). Analytical data for **10**: R_f = 0.48 (EtOAc–hexane, 4.0/6.0, v/v); $[\alpha]_D^{26}$ 47.59° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 1.01–1.09 (m, 28H, TIPDS), 2.11–2.20 (m, 2H, –OH, Hyp-β-Ha), 2.48–2.53 (2.39–2.44) (m, 1H, Hyp-β-Hb), 3.66–3.81 (3.56) (m, 4H, 4-H, 5-Ha, Hyp-δ-H), 3.90–3.96 (dd, 1H, *J* = 1.5, 9.5 Hz, 5-Hb), 4.06–4.12 (m, 1H, 2-H), 4.18 (dd, 1H, *J* = 6.5, 7.5 Hz, 3-H), 4.37–4.40 (m, 1H, Hyp-γ-H), 4.46 (4.53) (t, 1H, *J* = 7.5 Hz, Hyp-α-H), 4.92 (4.88) (d, 1H, *J* = 5.0 Hz, 1-H), 4.98 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.02 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.06 (d, H, *J* = 12.5 Hz, CH_2Ph), 5.15 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 7.15–7.40 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 12.6, 12.9, 13.4, 13.6, 17.1, 17.2, 17.50, 17.53, 17.57, 17.64, 36.2 (37.8), 52.4 (51.9), 58.4 (58.0), 65.0, 67.0 (67.2), 67.5, 75.6 (78.0), 77.9 (78.0), 78.2 (78.3), 81.95 (81.99), 100.1 ($^1J_{C1-H1}$ = 172.1 Hz) (100.0), 128.02, 128.03, 128.18, 128.22, 128.24, 128.27, 128.52, 128.57, 128.66, 128.70, 135.4 (135.7), 136.6 (136.5), 154.4 (154.9), 172.4 (172.2); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{48}H_{63}NNaO_{10}SSi_2$ 661.3888, found 661.3903.

***N*-Benzyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2-naphthyl)methyl-L-arabinofuranosyl]oxy-L-proline benzyl ester (11).** A mixture of a thioglycoside (**5**) (250.0 mg, 0.39 mmol), Z-Hyp-OBn (**6**) (115.0 mg, 0.32 mmol) and freshly activated molecular sieves (4 Å, 750 mg) in CH_2Cl_2 (10.0 mL) was stirred under argon at room temperature for 30 min. After the mixture was cooled to −40 °C, *N*-iodosuccinimide (NIS) (132.0 mg, 0.58 mmol) followed by silver trifluoromethanesulfonate (AgOTf) (30.0 mg, 0.11 mmol) were added. The reaction mixture was warmed slowly to room temperature and stirred for 15 min. The reaction was quenched by the addition of Et_3N. The suspension was diluted with $CHCl_3$ and filtered through a pad of Celite; the filtrate was washed successively with 10% $Na_2S_2SO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give a residue. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the corresponding oligosaccharide (265 mg, 76%, α/β ratio 1.3 : 1). Analytical data for the rotamer mixture of β-**11**: R_f = 0.36 (EtOAc–hexane, 3.0/7.0, v/v);

$[\alpha]_D^{26}$ 64.79° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 0.92–1.11 (m, 28H, TIPDS), 2.08–2.12 (m, 1H, *J* = 5.5 Hz, Hyp-β-Ha), 2.42–2.53 (m, 1H, Hyp-β-Hb), 3.62–3.68 (dd, 2H, *J* = 3.5, 11.5 Hz, Hyp-δ-H_2), 3.73–3.82 (m, 2H, 5-Ha, 5Hb), 3.88–3.94 (m, 1H, 4-H), 3.97 (dd, 1H, *J* = 4.5, 7.5 Hz, 2-H), 4.22–4.27 (m, 1H, Hyp-γ-H), 4.52 (dd, 1H, *J* = 6.0, 8.0 Hz, 3-H), 4.64–4.71 (t, 1H, *J* = 7.0 Hz, Hyp-α-H), 4.79 (4.73), (d, 1H, *J* = 4.0 Hz, 1-H), 4.89 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 4.98 (s, 2H, CH_2Ph), 5.02 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.14 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.20 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 7.16–7.80 (m, 17H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 12.6, 13.0, 13.4, 13.6, 17.17, 17.19, 17.22, 17.29, 17.56, 17.60, 17.72, 37.7 (36.7)*, 51.7 (51.4), 58.4 (58.1), 66.3, 67.0 (67.1), 67.35 (67.43), 72.6 (72.9), 73.9, 75.0, 77.4, 82.0 (81.9), 84.25 (84.28), 98.4 ($^1J_{C1-H1}$ = 169.7 Hz) (98.9), 125.9, 126.0, 126.1, 126.2, 126.28, 126.34, 126.7, 126.9, 127.85, 127.87, 127.90, 128.00, 128.04, 128.06, 128.09, 128.19, 128.22, 128.24, 128.32, 128.35, 128.41, 128.48, 128.51, 128.65, 128.69, 133.2, 133.33, 135.34, 135.45, 135.51, 135.7, 136.4, 136.6, 154.5 (154.6), 172.6 (172.4); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{48}H_{63}NNaO_{10}Si_2$ 892.3888, found 892.3903.

Rotamer mixture of α-**11**: R_f = 0.51 (EtOAc–hexane, 3.0/7.0, v/v); $[\alpha]_D^{26}$ −39.19° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 0.89–1.12 (m, 28H, TIPDS), 1.89–1.98 (m, 1H, Hyp-β-Ha), 2.11–2.18 (m, 1H, Hyp-β-Hb), 3.65–3.89 (m, 4H, 5-Ha, 5-Hb, Hyp-δ-H), 3.73–3.82 (m, 2H, 5-Ha, 5-Hb), 3.93–3.95 (m, 1H, 4-H), 3.98 (dd, 1H, *J* = 1.5, 7.0 Hz, 2-H), 4.19–4.31 (m, 2H, 3-H, Hyp-γ-H), 4.46 (4.36) (t, 1H, *J* = 7.5 Hz, Hyp-α-H), 4.71 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.79 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.97 (4.94) (d, 1H, *J* = 1.5 Hz, 1-H), 4.98–5.03 (m, 2H, CH_2Ph), 5.14 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.20 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 7.19–7.79 (m, 17H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 12.7, 13.0, 13.3, 13.72, 13.74, 17.17, 17.24, 17.3, 17.5, 17.6, 35.5 (36.3), 53.1 (53.6), 57.8 (58.1), 61.2, 67.1 (67.0), 67.30 (67.28), 73.13 (73.06), 74.4, 75.4, 75.8, 80.60 (80.63), 89.6 (89.7), 105.1 ($^1J_{C1-H1}$ = 170.0 Hz) (105.0), 125.9, 126.1, 126.3, 127.8, 128.0, 128.11, 128.13, 128.28, 128.34, 128.45, 128.50, 128.54, 128.56, 128.61, 128.7, 133.4 (133.2), 135.3 (135.4), 135.5 (135.8), 136.7 (136.6), 155.1 (154.3), 172.5 (172.3); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{48}H_{63}NNaO_{10}Si_2$ 892.3888, found 892.3900.

***N*-*tert*-Butyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (12).** DDQ (0.51 g, 2.24 mmol) was added to a mixture of Boc-Hyp-OBn (**7**)[36] (0.50, 1.55 mmol), glycosyl donor (**5**) (1.19 g, 1.86 mmol), and freshly activated molecular sieves (4 Å, 3.5 g) in dry CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was stirred for 16 h under an argon atmosphere. The reaction mixture was quenched with aqueous ascorbate buffer, and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$ and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford mixed acetal (1.25 g, 84%); MALDI-TOF MS: $[M + Na]^+$ calcd for $C_{52}H_{71}NNaO_{10}SSi_2$ 980.4235, found 980.5001. A mixture of the mixed acetal (0.64 g, 0.67 mmol), DTBMP (0.54 g, 2.67 mmol) and freshly activated molecular sieves (4 Å, 2 g) in dry CH_2Cl_2 (33 mL) was stirred under an argon atmosphere at room temperature for 30 min. MeOTf (0.26 mL, 2.34 mmol) was then added to the mixture and the mixture was stirred for 48 h at 40 °C. Upon completion the reaction mixture was cooled down, quenched with Et_3N, diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo*. The crude residue was dissolved in $CHCl_3$ (10 mL) and cooled at 0 °C. TFA (1 mL) was added and stirred for 30 min at the same temperature. The reaction mixture was diluted with $CHCl_3$ and washed successively with H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (0.32 g, 67%, β-only as the rotamer mixture). Analytical data for **12**: R_f = 0.48 (EtOAc–hexane, 4.0/6.0, v/v); $[\alpha]_D^{24}$ 52.60° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 0.91–1.09 (m, 28H, TIPDS), 1.34 (1.45) (s, 9H, C(CH_3)$_3$), 2.10 (m, 1H, Hyp-β-Ha), 2.28 (2.24) (d, 1H, *J* = 10.0 Hz, –OH), 2.46–2.51 (2.34–2.38) (m, 1H, Hyp-β-Hb), 3.59–3.66 (3.45) (m, 2H, Hyp-δ-H), 3.75–3.83 (m, 2H, 4-H, 5-Ha), 3.92–3.95 (m, 1H, *J* = 8.0 Hz, 5-Hb), 4.10 (dd, 1H, *J* = 4.5, 7.5 Hz, 2-H), 4.19 (dd, 1H, *J* = 6.0, 7.5 Hz, 3-H), 4.34–4.37 (m, 2H, Hyp-α-H, Hyp-γ-H), 4.93 (4.89) (d, 1H, *J* = 5.0 Hz, 1-H), 5.13 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 5.18 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 7.35 (m, 5H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 12.6, 12.9, 13.4, 13.6, 28.3, 28.5, 36.8 (37.7), 51.8 (51.9), 65.2 (66.9), 75.7 (75.9), 77.9 (78.0), 78.4, 80.56 (80.56), 81.9 (82.0), 100.1 (100.0), 128.1 (128.3), 128.4 (128.5), 128.7 (128.6), 135.5 (135.75), 153.83 (154.4), 172.8 (172.4); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{34}H_{57}NNaO_{10}Si_2$ 718.3419, found 718.3408.

Methyl 3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranoside (13). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (80.5 mg, 0.35 mmol) was added to a mixture of glycosyl acceptor[38] (**8**) (100.0 mg, 0.24 mmol), glycosyl donor (**5**) (188.5 mg, 0.28 mmol), and freshly activated molecular sieves (4 Å, 300 mg) in dry CH_2Cl_2 (5 mL) at room temperature. The reaction mixture was stirred for 16 h under an argon atmosphere. The reaction mixture was quenched with aqueous ascorbate buffer, and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$ and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford corresponding mixed acetal (230.0 g, 92%); MALDI-TOF MS: $[M + Na]^+$ calcd for $C_{53}H_{86}NaO_{11}SSi_4$ 1065.4866, found 1065.5035. A mixture of the mixed acetal (150 mg, 0.14 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (118.0 mg, 0.57 mmol) and freshly activated

molecular sieves (4 Å, 750 mg) in dry CH_2Cl_2 (7.5 mL) was stirred under an argon atmosphere at room temperature for 30 min. Methyl trifluoromethanesulfonate (MeOTf) (57 μL, 0.50 mmol) was then added to the mixture and the mixture was stirred for 48 h at 40 °C. Upon completion the reaction mixture was cooled down, quenched with triethylamine (Et_3N), diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo*. The crude residue was dissolved in $CHCl_3$ (2 mL) and cooled at 0 °C. Trifluoroacetic acid (TFA) (0.2 mL) was added and stirred for 30 min at the same temperature. The reaction mixture was diluted with $CHCl_3$ and washed successively with H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (88.7 mg, 79%, β-only). Analytical data for **13**: R_f = 0.50 (EtOAc–hexane, 2.0/8.0, v/v); $[\alpha]_D^{27}$ 197.99° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01–1.09 (m, 28H, TIPDS), 2.18 (d, 1H, J = 10.5 Hz, –OH), 3.37 (s, 3H, $-OCH_3$), 3.77 (dd, 1H, J = 10.0, 10.5 Hz, 5′-Ha), 3.87–3.93 (m, 3H, 4-H, 4′-H, 5′-Hb), 3.97–4.02 (m, 2H, 5-Ha, 5-Hb), 4.11–4.16 (m, 1H, 2′-H), 4.20 (dd, 1H, J = 6.0, 7.0 Hz, 3′-H), 4.24 (dd, 1H, J = 2.0, 6.0 Hz, 2-H), 4.28 (dd, 1H, J = 6.5, 7.0 Hz, 3-H), 4.80 (d, 1H, J = 2.0 Hz, 1-H), 5.02 (d, 1H, J = 5.0 Hz, 1′-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.7, 12.8, 13.1, 13.2, 13.4, 13.5, 13.6, 17.09, 17.16, 17.31, 17.34, 17.41, 17.53, 17.57, 17.61, 17.69, 17.76, 30.5, 55.37, 61.9, 66.1, 75.7, 79.4, 80.1, 81.3, 82.7, 86.0, 99.3, 106.2; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{35}H_{72}NaO_{11}Si_4$ 803.4049, found 803.4015.

***N*-Benzyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (16).** DDQ (0.41 g, 1.82 mmol) was added to a mixture of glycosyl acceptor **10** (0.91 g, 1.82 mmol), glycosyl donor **5** (1.27 g, 1.52 mmol), and freshly activated molecular sieves (4 Å, 3.5 g) in dry CH_2Cl_2 (20 mL) at room temperature. The reaction mixture was stirred for 16 h under an argon atmosphere. The reaction mixture was quenched with aqueous ascorbate buffer, and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$ and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford mixed acetal **15** (1.81 g, 87%). **15**: MALDI-TOF MS: $[M + Na]^+$ calcd for $C_{72}H_{103}NNaO_{15}SSi_4$ 1388.6023, found 1388.8651. A mixture of the mixed acetal **15** (1.81 g, 1.32 mmol), DTBMP (1.08 g, 5.30 mmol) and freshly activated molecular sieves (4 Å, 10 g) in dry CH_2Cl_2 (67 mL) was stirred under an argon atmosphere at room temperature for 30 min. MeOTf (0.52 mL, 4.63 mmol) was then added to the mixture and the mixture was stirred for 48 h at 40 °C. Upon completion the reaction mixture was cooled down, quenched with Et_3N, diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo*. The crude residue was dissolved in $CHCl_3$ (20 mL) and cooled at 0 °C. TFA (2 mL) was added and kept stirred *in vacuo* for 30 min at the same temperature. The reaction mixture was diluted with $CHCl_3$ washed successively with H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (1.23 g, 84%, β-only as the rotamer mixture). Analytical data for **16**: R_f = 0.48 (EtOAc–hexane, 3.0/7.0, v/v); $[\alpha]_D^{26}$ 73.60° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 0.93–1.11 (m, 56H, 2 × TIPDS), 2.09–2.14 (m, 1H, Hyp-β-Ha), 2.37–2.42 (m, 1H, Hyp-β-Hb), 2.47 (2.50) (d, 1H, J = 10.0 Hz, –OH), 3.51–3.59 (m, 1H, J = 12.0 Hz, Hyp-δ-H), 3.66–3.99 (m, 7H, 4-H, 5-Ha, 5-Hb, 4′-H, 5′-a, 5′-Hb, Hyp-δ-H), 4.01–4.06 (m, 1H, 2′-H), 4.18 (dd, 1H, J = 6.5, 7.0 Hz, 3′-H), 4.23 (dd, 1H, J = 4.5, 8.0 Hz, 2-H), 4.31–4.35 (m, 1H, Hyp-γ-H), 4.44 (dd, 1H, J = 6.5, 7.5 Hz, 3-H), 4.52 (t, 1H, J = 7.5 Hz, Hyp-α-H), 4.74 (4.71) (d, 1H, J = 5.0 Hz, 1′-H), 4.98 (4.93) (d, 1H, J = 4.5 Hz, 1-H), 4.98–5.24 (m, 4H, 2 × CH_2Ph), 7.15–7.39 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 12.6, 12.98, 13.01, 13.4, 13.5, 16.98, 17.06, 17.18, 17.23, 17.33, 17.44, 17.49, 17.53, 17.65, 17.71, 37.7 (36.7), 51.3 (51.2), 58.0 (58.2), 65.9 (65.8), 66.2 (66.1), 67.0 (67.1), 67.5, 73.8, 75.3, 76.7 (76.5), 79.04 (78.97), 80.1 (79.9), 80.7 (80.9), 82.1 (82.0), 82.72 (82.67), 97.0 ($^1J_{C1-H1}$ = 172.2 Hz) (98.0), 99.5 ($^1J_{C1-H1}$ = 170.4 Hz) (99.7), 127.97 (128.00), 128.2, 128.3, 128.5, 128.7 (128.6), 135.5 (135.7), 136.5 (136.6), 154.7 (154.9), 172.6 (172.3); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{54}H_{89}NNaO_{15}SSi_4$ 1126.5207; found 1126.5173.

***N*-Benzyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2-*O*-(2-naphthyl)methyl-β-L-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (17).** To a stirred solution of compound **16** (1.60 g, 1.44 mmol) in dry DMF (20 mL) at −20 °C were added tetrabutylammonium iodide (TBAI) (80 mg, 0.22 mmol), NaH (60% dispersion in mineral oil, 70 mg, 1.74 mmol), and NAPBr (0.38 g, 1.74 mmol). The reaction mixture was stirred for 16 h at the same temperature. Upon completion, the reaction was diluted with EtOAc, poured into ice-water, stirred until cessation of H_2 evolution, and then extracted with EtOAc. The combined organic phase was washed with sat. NH_4Cl, and dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (1.42 g, 79%, rotamer mixture). Analytical data for rotamer mixture of **17**: R_f = 0.54 (EtOAc–hexane, 3.0/7.0, v/v); $[\alpha]_D^{26}$ 76.20° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (major rotamer) 0.87–1.11 (m, 56H, 2 × TIPDS), 2.07 (m, 1H, Hyp-β-Ha), 2.17–2.29 (m, 1H, Hyp-β-Hb), 3.54–4.02 (m, 9H, 2-H, 4-H, 5-Ha, 5-Hb, 4′-H, 5′-Ha, 5′-Hb, Hyp-δ-H), 4.21 (dd, 1H, J = 4.0, 8.0 Hz, 2′-H), 4.38 (t, 1H, J = 7.5 Hz, Hyp-γ-H), 4.55 (m, 2H, 3-H, 3′-H), 4.75 (t, J = 6.0 Hz, Hyp-α-H), 4.84–5.14 (m, 8H, 1-H, 1′-H, 3 × CH_2Ph), 7.06–7.77 (m, 17H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (rotamer mixture) 12.56, 12.59, 12.9, 13.0, 13.4, 13.5, 13.6, 17.01, 17.07,

17.09, 17.13, 17.19, 17.23, 17.29, 17.44, 17.48, 17.52, 17.59, 17.65, 17.76, 36.7, 37.7, 51.1, 51.8, 57.9, 58.2, 65.7, 66.5, 66.6, 66.8, 67.0, 67.4, 71.1, 71.2, 73.6, 74.3, 76.3, 76.4, 77.4, 78.2, 78.3, 78.37, 78.40, 81.8, 81.9, 82.9, 85.2, 85.4, 96.7, 96.8, 97.3, 97.4, 125.49, 125.57, 125.62, 125.8, 125.9, 126.0, 127.65, 127.71, 128.0, 128.1, 128.16, 128.18, 128.28, 128.31, 128.34, 128.4, 128.5, 128.6, 132.9, 133.0, 133.37, 133.40, 135.44, 135.7, 136.0, 136.1, 138.5, 154.2, 154.7, 172.2, 172.3 ppm; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{65}H_{97}NNaO_{15}Si_4$ 1266.5833, found 1266.5866.

***N*-Benzyloxycarbonyl-4*R*-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl-(1→2)-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (19).** DDQ (114 mg, 0.50 mmol) was added to a mixture of glycosyl acceptor **17** (250 mg, 0.50 mmol), glycosyl donor **14** (520 mg, 0.42 mmol), and freshly activated molecular sieves (4 Å, 450 mg) in dry CH_2Cl_2 (7 mL) at room temperature. The reaction mixture was stirred for 16 h under an argon atmosphere. The reaction mixture was quenched with aqueous ascorbate buffer, and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$, and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford mixed acetal **18** (487 mg, 67%). **18**: MALDI-TOF MS: $[M + Na]^+$ calcd for $C_{89}H_{137}NNaO_{20}SSi_6$ 1764.6025, found 1764.8685. A mixture of the mixed acetal **18** (450 mg, 0.25 mmol), DTBMP (209 mg, 1.02 mmol) and freshly activated molecular sieves (4 Å, 1.5 g) in dry CH_2Cl_2 (15 mL) was stirred under an argon atmosphere at room temperature for 30 min. MeOTf (101 μL, 0.89 mmol) was then added to the mixture and the mixture was stirred for 48 h at 40 °C. Upon completion the reaction mixture was cooled down, quenched with Et_3N, diluted with $CHCl_3$, and filtered through a pad of Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo*. The crude residue was dissolved in $CHCl_3$ (10.0 mL) and cooled at 0 °C. TFA (1.0 mL) was added and stirred for 30 min at the same temperature. The reaction mixture was diluted with $CHCl_3$ washed successively with H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (290 mg, 77%, β-only as the rotamer mixture). Analytical data for **19**: R_f = 0.54 (EtOAc–hexane, 3.0/7.0, v/v); $[\alpha]_D^{27}$ 90.00° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (major rotamer) 0.91–1.10 (m, 84H, 3 × TIPDS), 2.11–2.15 (m, 1H, Hyp-β-Ha), 2.37–2.42 (m, 2H, –OH, Hyp-β-Hb), 3.62–4.08 (m, 14H, 4-H, 5-Ha, 5-Hb, 2′-H, 3′-H, 4′-H, 5′-H, 5b′-H, 2″-H, 4″-H, 5a″-H, 5b″-H, Hyp-δ-H), 4.18 (m, 2H, 2-H, 3″-H), 4.33–4.36 (m, 1H, Hyp-γ-H), 4.42–4.54 (m, 2H, 3-H, Hyp-α-H), 4.78 (d, 1H, $J_{1'',2''}$ = 4.00 Hz, 1″-H), 4.88 (d, 1H, $J_{1',2'}$ = 4.5 Hz, 1′-H), 4.95–5.01 (m, 3H, 1-H, CH_2Ph), 5.10–5.23 (m, 2H, CH_2Ph), 7.19–7.36 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (rotamer mixture) 12.6, 12.67, 12.72, 12.9, 13.0, 13.1, 13.3, 13.4, 13.5, 13.6, 17.0, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 17.7, 17.8, 37.66, 37.75, 50.88, 51.5, 57.9, 58.2, 65.76, 65.79, 66.4, 66.5, 66.6, 67.0, 67.2, 67.4, 67.5, 73.5, 74.6, 76.5, 77.9, 78.0, 78.1, 78.2, 79.1, 79.2, 80.4, 80.5, 81.9, 81.95, 81.96, 82.00, 82.5, 82. 6, 83.06, 83.08, 95.9, 96.0, 965, 97.5, 99.1, 99.3, 128.0, 128.1, 128.19, 128.24, 128.3, 128.45, 128.51, 128.6, 128.7, 135.6, 135.7, 136.4, 136.7, 154.4, 154.8, 172.4, 172.7; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{71}H_{123}NNaO_{20}Si_6$ 1500.7152, found 1500.7155.

***N*-Benzyloxycarbonyl-4*R*-[2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (20).** To a solution of **10** (0.65 g, 0.89 mmol) in pyridine–THF (1 : 4, 5 mL) at 0 °C was added a solution of tetrabutylammonium fluoride (1.78 mL, 1.78 mmol, 1 M in THF). After being stirred for 1 h at 0 °C, $CaCO_3$ (0.74 g), DOWEX® 50WX2 (2.20 g, used as supplied), and MeOH (5.2 mL) were added.[40] The suspension was stirred at room temperature for 1 h. All insoluble materials were removed by filtration through a pad of Celite, and then filter Celite was washed with MeOH thoroughly. The combined filtrates were concentrated *in vacuo* to give the crude product. The crude product was subjected to the next step without further purification. The crude product was dissolved in pyridine (8 mL), cooled to 0 °C, and treated with acetic anhydride (4 mL). The reaction mixture was stirred overnight at room temperature. Upon completion, the reaction was diluted with $CHCl_3$ and washed thoroughly with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (0.48 g, 89%, rotamer mixture). Analytical data for **20**: R_f = 0.75 (EtOAc–$CHCl_3$, 6.0/4.0, v/v); $[\alpha]_D^{26}$ 37.19° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 1.94 (2.06) (s, 6H, 2 × OAc), 1.98 (2.01) (s, 3H, OAc), 2.09–2.12 (m, 1H, Hyp-β-Ha), 2.47–2.53 (2.42–2.45) (m, 1H, Hyp-β-Hb), 3.53–3.61 (m, 2H, Hyp-δ-H), 4.06–4.10 (m, 1H, 4-H), 4.20 (4.23) (dd, 1H, *J* = 5.5, 11.5 Hz, 5-Ha), 4.31–4.35 (m, 2H, 5-Hb, Hyp-γ-H), 4.49 (4.44) (t, 1H, *J* = 8.0 Hz, Hyp-α-H), 4.94 (4.90) (dd, 1H, *J* = 4.5, 6.5 Hz, 2-H), 514 (4.99) (s, 2H, CH_2Ph), 5.15 (5.01) (d, 1H, *J* = 12.0 Hz, CH_2Ph), 5.21 (5.05) (d, 1H, *J* = 12.0 Hz, CH_2Ph), 5.28 (dd, 1H, *J* = 6.5, 7.5 Hz, 3-H), 5.32 (5.31) (d, 1H, *J* = 4.5 Hz, 1-H), 7.19–7.38 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 20.09 (20.06), 20.6 (20.5), 36.4 (37.3), 51.1 (51.9), 57.7 (58.0), 65.13 (65.05), 66.8 (66.7), 67.2 (67.0), 75.47 (75.39), 75.58 (75.54), 76.7, 76.9, 78.7 (78.8), 99.4 (98.7), 127.76, 127.87, 127.94, 128.0, 128.1, 128.2, 128.29, 128.34, 128.4, 135.2 (135.5), 136.2 (136.3), 154.8 (154.0), 169.96 (169.90), 170.03 (170.00), 170.3, 172.2 (171.9); ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{31}H_{35}NaO_{12}$ 636.2057, found 636.2083.

***N*-9-Fluorenylmethyloxycarbonyl-4*R*-[2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]oxy-L-proline (21).** Compound **20** (0.48 g, 0.78 mmol) was dissolved in a mixture of EtOAc–MeOH (1 : 1, 8 mL) and 20% $Pd(OH)_2$ (0.48 g) was added. The reaction mixture was stirred under an atmosphere of H_2 for 8 h. Upon completion, the catalyst was filtered through a pad of Celite,

and the filtrate was concentrated under reduced pressure to give a crude amine product. The crude product was then subjected to the next step without further purification. 9-Fluorenylmethyloxycarbonyl chloride (FmocCl) (0.23 g, 0.86 mmol) was added to a stirred solution of the crude amine and *N*,*N*-diisopropylethylamine (DIPEA) (150 μL, 0.86 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 5 h, after which it was diluted with $CHCl_3$ and washed with brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–hexane gradient elution) to afford the title compound as a colorless syrup (0.34 g, 71%, rotamer mixture). Analytical data for **21**: R_f = 0.62 (MeOH–$CHCl_3$, 1.0/9.0, v/v); $[\alpha]_D^{26}$ 21.60° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 1.94 (2.31) (s, 3H, OAc), 2.09 (s, 6H, 2 × OAc), 2.25–2.30 (2.16–2.20) (m, 1H, Hyp-β-Ha), 2.45–2.56 (m, 1H, Hyp-β-Hb), 3.55–3.62 (m, 2H, Hyp-δ-H), 4.08–4.15 (m, 1H, 4-H), 4.21–4.40 (m, 7H, 5-Ha, 5-Hb, Hyp-α-H, Hyp-γ-H, C*H*Fmoc, OCH_2Fmoc), 4.94 (dd, 1H, *J* = 5.5, 10.0 Hz, 2-H), 5.26–5.31 (m, 1H, 3-H), 5.34 (d, 1H, *J* = 4.5 Hz, 1-H), 7.24–7.75 (m, 8H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) 14.4, 20.6 (20.5), 21.01 (20.96), 36.2 (37.7), 47.3, 51.4 (52.2), 58.3, 60.7, 65.3 (65.5), 68.3 (67.9), 75.74 (75.78), 75.85 (75.80), 77.4, 79.1 (79.0), 99.3 (99.9), 120.2, 125.3, 127.3, 128.0, 141.5, 144.0 (143.9), 144.2, 156.1 (154.5), 170.36, 170.40, 170.45, 170.9, 171.0; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{31}H_{33}NNaO_{12}$ 634.1900, found 634.1917.

4*R*-β-L-Arabinofuranosyloxy-L-proline (deprotected derivative of NAP-IAD product 10). To a solution of **20** (40.0 mg, 65.2 μmol) in methanol (2 mL) at 0 °C was added a 0.1 M NaOH solution (2 mL). The reaction mixture was stirred for 4 h at the same temperature and then neutralized with Dowex (H^+), filtered, and concentrated *in vacuo*. The crude residue was dissolved in MeOH–H_2O–HOAc (3 mL/1 mL/0.1 mL) and 20% $Pd(OH)_2$ (15 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 15 h. The catalyst was then filtered-off, washed with methanol and H_2O, and the filtrate was concentrated under reduced pressure. The residue was purified by Sep-pek C-18 cartridge (H_2O–methanol gradient elution). Fractions containing the product were collected and concentrated *in vacuo* to afford the title compound (13.5 mg, 79% in two steps). Analytical data for the title compound: ^{1}H NMR (500 MHz, D_2O): δ 2.22–2.33 (m, 1H, Hyp-β-Ha), 2.69–2.74 (m, 1H, Hyp-β-Hb), 3.50 (dd, 1H, *J* = 4.0, 12.5 Hz, Hyp-δ-Ha), 3.58 (d, 1H, *J* = 12.5 Hz, Hyp-δ-Hb), 3.66 (dd, 1H, *J* = 6.5, 12.0 Hz, 5-Ha), 3.81 (dd, 1H, *J* = 5.0, 12.5 Hz, 5-Hb), 3.87–3.91 (m, 1H, 4-H), 4.04 (dd, 1H, *J* = 8.0 Hz, 3-H), 4.14 (dd, 1H, *J* = 4.5, 8.0 Hz, 2-H), 4.55 (dd, 1H, *J* = 8.5 Hz, Hyp-α-H), 4.70 (m, 1H, Hyp-γ-H), 5.13 (d, 1H, *J* = 4.5 Hz, 1-H); ^{13}C NMR (75 MHz, D_2O): δ 36.41, 51.63, 59.55, 63.36, 74.62, 76.66, 76.70, 82.47, 100.48, 172.97; ESI-TOF MS: $[M + Na - H]^+$ calc for $C_{10}H_{17}NNaO_7$ 286.09, found 286.14; ESI-TOF HRMS: $[M + Na - H]^+$ calc for $C_{10}H_{17}NNaO_7$ 286.0903, found 286.0908.

***N*-Benzyloxycarbonyl-4*R*-[2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]oxy-L-proline benzyl ester (22).** To a solution of **16** (0.69 g, 0.62 mmol) in pyridine–THF (1 : 4, 10 mL) at 0 °C was added a solution of tetrabutylammonium fluoride (2.5 mL, 2.50 mmol, 1 M in THF). After stirring for 3 h at 0 °C, $CaCO_3$ (0.51 g), DOWEX 50WX2 (1.54 g, used as supplied), and MeOH (4 mL) were added. The suspension was stirred at room temperature for 1 h. All insoluble materials were removed by filtration through a pad of Celite, and then filter Celite was washed with MeOH thoroughly. The combined filtrates were concentrated *in vacuo* to give the crude product. The crude product was subjected to the next step without further purification. The crude product was dissolved in pyridine (10 mL), cooled to 0 °C, and treated with acetic anhydride (5 mL). The reaction mixture was allowed to stir overnight at room temperature. Upon completion, the reaction was diluted with $CHCl_3$ and washed thoroughly with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–$CHCl_3$ gradient elution) to afford the title compound as a colorless syrup (0.42 g, 81%, rotamer mixture). Analytical data for **22**: R_f = 0.61 (EtOAc–$CHCl_3$, 6.0/4.0, v/v); $[\alpha]_D^{26}$ 59.40° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (major rotamer) 2.05 (s, 3H, OAc), 2.07 (s, 9H, 3 × OAc), 2.10 (s, 3H, OAc), 2.19 (m, 1H, Hyp-β-Ha), 2.53 (m, 1H, Hyp-β-Hb), 3.65–3.70 (m, 2H, Hyp-δ-H_2), 4.02–4.06 (m, 1H, 2-H), 4.10–4.20 (m, 3H, 4-H, 5-Ha, 5′-Ha), 4.34–4.04 (m, 4H, 4′-H, 5-Hb, 5′-Hb, Hyp-γ-H), 4.50 (t, 1H, *J* = 8.0 Hz, Hyp-α-H), 4.93–5.30 (m, 9H, 1-H, 2-H, 3-H, 1′-H, 3′-H, 2 × CH_2Ph), 7.18–7.37 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (rotamer mixture) 20.3, 20.4, 20.6, 20.65, 20.68, 20.69, 36.1, 37.1, 51.1, 57.7, 58.0, 65.5, 65.69, 65.73, 66.9, 67.1, 67.2, 74.7, 75.6, 75.7, 75.8, 76.3, 76.4, 76.7, 76.8, 77.4, 77.6, 77.7, 79.1, 79.2, 79.3, 79.5, 98.6, 98.7, 98.8, 99.2, 127.78, 127.85, 127.95, 127.97, 128.04, 128.08, 128.20, 128.25, 128.31, 128.41, 128.47, 128.49, 135.3, 135.5, 136.3, 136.4, 154.2, 154.6, 169.91, 169.95, 170.03, 170.06, 170.33, 170.38, 170.42, 170.44, 172.1, 172.4; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{40}H_{47}NNaO_{18}$ 852.2691, found 852.2667.

***N*-9-Fluorenylmethyloxycarbonyl-4*R*-[2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]-oxy-L-proline (23).** Compound **22** (0.67 g, 0.80 mmol) was dissolved in a mixture of EtOAc–MeOH (1 : 1, 10 mL) and 20% $Pd(OH)_2$ (0.50 g) was added. The reaction mixture was stirred under an atmosphere of H_2 for 16 h. Upon completion, the catalyst was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to give a crude amine product. The crude product was then subjected to the next step without further purification. FmocCl (0.32 g, 1.21 mmol) was added to a stirred solution of the crude amine and DIPEA (215 μL, 1.21 mmol) in dry CH_2CL_2 (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 7 h, after which it was diluted with $CHCl_3$ and washed with brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (MeOH–$CHCl_3$ gradient elution) to afford the title compound

as a colorless syrup (0.49 g, 74%, rotamer mixture). Analytical data for **23**: R_f = 0.68 (MeOH–$CHCl_3$, 1.0/9.0, v/v); $[\alpha]_D^{26}$ 63.20° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (separated chemical shifts for minor rotamer in parentheses) major 2.03 (s, 3H, OAc), 2.06 (s, 9H, 3 × OAc), 2.08 (s, 3H, OAc), 2.29 (m, 1H, Hyp-β-Ha), 2.43–2.58 (m, 1H, Hyp-β-Hb), 3.64–3.71 (m, 2H, Hyp-δ-H_2), 4.01–4.07 (m, 1H, H-4), 4.08–4.43 (m, 10H, 2-H, 5-Ha, 5-Hb, 4′-H, 5′-Ha, 5′-Hb, Hyp-γ-H, C*H*Fmoc, OCH_2Fmoc), 4.55 (t, 1H, *J* = 7.5 Hz, Hyp-α-H), 4.94 (dd, 1H, *J* = 5.5, 11.0 Hz, 2′-H), 5.08 (d, 1H, *J* = 4.5 Hz, 1-H), 5.12 (dd, 1H, *J* = 5.5, 6.0 Hz, 3-H), 5.24 (d, 1H, *J* = 4.5 Hz, 1′-H), 5.29 (dd, 1H, *J* = 6.5 Hz, H-3′), 7.14–7.75 (m, 8H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.5, 20.66, 20.70, 20.74, 20.77, 36.0, 37.4, 47.10, 47.11, 51.1, 51.5, 57.4, 57.9, 65.6, 65.7, 65.8, 67.8, 68.0, 74.7, 75.7, 75.9, 76.4, 76.5, 76.8, 77.4, 77.7, 79.2, 79.3, 79.4, 98.58, 98.64, 99.1, 99.3, 119.9, 120.0, 125.1, 125.2, 125.3, 127.0, 127.1, 127.6, 127.7, 128.1, 129.0, 141.2, 141.3, 143.88, 143.91, 144.0, 154.7, 155.2, 170.0, 170.1, 170.2, 170.48, 170.53, 170.66, 170.70, 170.8, 175.3, 176.4; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{40}H_{45}NNaO_{18}$ 850.2534, found 850.2509.

4*R*-[β-L-Arabinofuranosyl-(1→2)-β-L-arabinofuranosyl]oxy-L-proline (deprotected compound of NAP-IAD product 16). To a solution of **22** (40.0 mg, 48.2 μmol) in MeOH (2.0 mL) at 0 °C was added a 0.1 M NaOH solution (2.0 mL). The reaction mixture was stirred for 3 h at the same temperature and then neutralized with Dowex (H^+), filtered, and concentrated *in vacuo*. The crude residue was dissolved in MeOH–H_2O–HOAc (3 mL/1 mL/0.1 mL) and 20% $Pd(OH)_2$ (15 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 15 h. The catalyst was then filtered-off, washed with MeOH and H_2O, and the filtrate was concentrated under reduced pressure. The residue was purified by Sep-Pak C-18 cartridge (H_2O–MeOH gradient elution). Fractions containing the product were collected and concentrated *in vacuo* to afford the fully deprotected disaccharide-linked Hyp (14.1 mg, 74% in two steps). Analytical data for the title compound: $[\alpha]_D^{24}$ 47.59° (c 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 1.95–2.01 (m, 1H, Hyp-β-Ha), 2.43–2.48 (m, 1H, Hyp-β-Hb), 3.21–3.31 (m, 2H, Hyp-δ-H_2), 3.41–3.48 (m, 2H, 5-Ha, 5′-Ha), 3.56–3.58 (m, 2H, 5-Hb, 5′-Hb), 3.65–3.70 (m, 2H, 4-H, 4′-H), 3.87–3.95 (m, 3H, 3-H, 2-H, 3′-H), 4.05 (dd, 1H, *J* = 4.5, 8.5 Hz, 2′-H), 4.19 (t, 1H, *J* = 8.5 Hz, Hyp-α-H), 4.44 (m, 1H, Hyp-γ-H), 4.84 (d, 1H, *J* = 4.5 Hz, 1-H), 5.05 (d, 1H, *J* = 4.5 Hz, 1′-H); ^{13}C NMR (125 MHz, D_2O): δ 36.7, 51.7, 60.2, 63.3 (×2), 73.0, 74.3, 76.9, 77.3, 80.9, 82.1, 82.8, 99.2, 100.8, 174.0; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{15}H_{25}NNaO_{11}$ 418.1325, found 418.1310.

***N*-Benzyloxycarbonyl-*trans*-4-hydroxy-4-*O*-[2,3,5-tri-*O*-acteyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]-L-proline benzyl ester (24).** To a solution of **19** (865 mg, 0.58 mmol) in pyridine–THF (1 : 4, 20 mL) at 0 °C was added a solution of tetrabutylammonium fluoride (3.48 mL, 3.48 mmol, 1 M in THF). After stirring for 3 h at 0 °C, $CaCO_3$ (725 mg), DOWEX 50WX2 (2.17 g, used as supplied), and MeOH (8 mL) were added. The suspension was stirred at room temperature for 1 h. All insoluble materials were removed by filtration through a pad of Celite, and then filter Celite was washed with MeOH thoroughly. The combined filtrates were concentrated *in vacuo* to give the crude product. The crude product was subjected to the next step without further purification. The crude product was dissolved in pyridine (10 mL), cooled to 0 °C, and treated with acetic anhydride (5 mL). The reaction mixture was allowed to stir overnight at room temperature. Upon completion, the reaction was diluted with $CHCl_3$ and washed thoroughly with H_2O, 1 N HCl, saturated $NaHCO_3$ and brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc–$CHCl_3$ gradient elution) to afford the title compound as a colorless syrup (532 mg, 87%, rotamer mixture). Analytical data for **24**: R_f = 0.50 (EtOAc–$CHCl_3$, 6.0/4.0, v/v); $[\alpha]_D^{27}$ 95.79° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ major isomer 2.02–2.12 (s, 21H, 7 × OAc), 2.22 (m, 1H, Hyp-β-Ha), 2.55–2.70 (m, 1H, Hyp-β-Hb), 3.51–3.72 (m, 2H, Hyp-δ-H2), 3.95–4.13 (m, 5H, 2H, 5a-H, 5b-H, 5a′-H, 5b′-H), 4.20–4.45 (m, 7H, 2′-H, 4′-H, 4″-H, 5a″-H, 5b″-H), 4.48 (m, 1H, Hyp-γ-H), 4.60 (*t*, 1H, *J* = 7.5 Hz, Hyp-α-H), 4.97–5.30 (m, 12H, 1′-H, 1″-H, 2″-H, 3-H, 3′-H, 3″-H, 2 × CH_2Ph), 5.32 (d, 1H, *J* = 4.5 Hz, 1-H), 7.19–7.36 (m, 10H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (rotamer mixture) 20.55, 20.57, 20.85, 20.89, 20.92, 20.95, 20.97, 21.01, 36.37, 37.40, 51.3, 51.6, 58.0, 58.2, 65.5, 65.6, 66.2, 66.3, 66.41, 66.43, 67.0, 67.2, 67.36, 67.42, 74.3, 74.9, 75.8, 76.65, 76.68, 76.8, 77.3, 77.4, 77.7, 77.8, 79.2, 79.3, 79.9, 80.55, 80.58, 97.5, 97.6, 97.7, 97.8, 98.4, 98.5, 127.7, 128.0, 128.25, 128.27, 128.30, 128.4, 128.5, 128.6, 128.70, 128.74, 135.6, 135.8, 136.6, 136.7, 154.5, 154.8, 169.9, 170.4, 170.5, 170.6, 170.7, 170.76, 170.82, 170.9, 172.7; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{49}H_{59}NNaO_{24}$ 1068.3324, found 1068.3352.

***N*-9-Fluorenylmethyloxycarbonyl-4*R*-[2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl-(1→2)-2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl]oxy-L-proline (25).** Compound **24** (575 mg, 0.55 mmol) was dissolved in a mixture of EtOAc–MeOH (1 : 1, 5 mL) and 20% $Pd(OH)_2$ (350 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 8 h. Upon completion, the catalyst was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to give a crude amine product. The crude product was then subjected to the next step without further purification. FmocCl (259 mg, mmol) was added to a stirred solution of the crude amine and DIPEA (190 μL, 0.66 mmol) in dry CH_2Cl_2 (7 mL) at 0 °C. The reaction mixture was stirred at room temperature for 5 h, after which it was diluted with $CHCl_3$ and washed with brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (MeOH–$CHCl_3$ gradient elution) to afford the title compound as a colorless syrup (465 mg, 81%, rotamer mixture). Analytical data for **25**: R_f = 0.75 (MeOH–$CHCl_3$, 1.0/9.0, v/v); $[\alpha]_D^{26}$ 75.20° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ major isomer 1.78–2.11 (m, 21H, 7 × OAc), 2.30 (m, 1H, Hyp-β-Ha), 2.61–2.85 (m, 1H, Hyp-β-Hb), 3.67–3.72 (m, 2H, Hyp-δ-H_2), 3.94–4.14 (m, 6H, 2H, 5a-H, 5b-H, 5a′-H, 5b′-H, OC*H*Fmoc), 4.21–4.53 (m, 13H, 2′-H, 4-H, 4′-H, 4″-H, 5a″-H, 5b″-H, OCH_2Ph, Hyp-γ-H), OCH_2Fmoc, 4.67

(t, 1H, J = 7.5 Hz, Hyp-α-H), 4.93 (dd, 1H, J = 4.0, 7.0 Hz, 2″-H), 5.00–5.06 (m, 2H, 1′-H, 3-H), 5.12–5.20 (m, 3H, 1″-H, 3′-H, 3″-H), 5.34 (d, 1H, J = 4.5 Hz, 1-H), 7.13–7.76 (m, 7H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ (rotamer mixture) 20.45, 20.49, 20.6, 20.71, 20.73, 20.9, 21.5, 36.3, 37.6, 47.18, 47.21, 51.4, 51.8, 57.5, 58.0, 65.4, 65.5, 66.2, 66.3, 67.9, 68.0, 74.4, 75.2, 75.3, 75.5, 76.4, 76.5, 76.7, 77.1, 77.4, 78.9, 79.2, 79.5, 79.7, 80.4, 80.5, 97.4, 97.6, 97.7, 97.8, 98.6, 119.9, 120.0, 120.1, 125.07, 125.11, 125.2, 125.38, 127.12, 127.17, 127.21, 127.6, 127.75, 127.84, 128.3, 129.1, 137.9, 141.2, 141.26, 141.31, 141.4, 143.6, 143.9, 144.1, 144.4, 154.7, 155.2, 169.88, 169.92, 170.3, 170.4, 170.6, 170.7, 170.78, 170.83, 170.9, 171.0, 175.9, 177.0; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{49}H_{57}NNaO_{24}$ 1066.3168, found 1066.3140.

4*R*-[β-L-Arabinofuranosyl-(1→2)-β-L-arabinofuranosyl-(1→2)-β-L-arabinofuranosyl]oxy-L-proline (deprotected compound of NAP-IAD product 19). To a solution of **24** (30.0 mg, 28.7 μmol) in MeOH (2.0 mL) at 0 °C was added 0.1 M NaOH solution (2.0 mL). The reaction mixture was stirred for 3 h at the same temperature, and then neutralized with Dowex (H^+), filtered, and concentrated *in vacuo*. The crude residue was dissolved in MeOH–H_2O–HOAc (3 mL/1 mL/0.1 mL) and 20% $Pd(OH)_2$ (15 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 15 h. The catalyst was then filtered-off, washed with MeOH and H_2O, and the filtrate was concentrated under reduced pressure. The residue was purified by Sep-Pak C-18 cartridge (H_2O–MeOH gradient elution). Fractions containing the product were collected and concentrated *in vacuo* to afford the fully deprotected trisaccharide-linked Hyp (10.7 mg, 71% in two steps). Analytical data for deprotected compound of 24: $[\alpha]_D^{24}$ 77.79° (c 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 2.17–2.33 (m, 1H, Hyp-β-Ha), 2.67–2.72 (m, 1H, Hyp-β-Hb), 3.48–3.53 (m, 2H, Hyp-δ-H_2), 3.68–3.75 (m, 3H, 5-Ha, 5′-Ha, 5″-Ha), 3.81–3.84 (m, 3H, 5-Hb, 5′-Hb, 5″-Hb), 3.91–3.99 (m, 3H, 4-H, 4′-H, 4″-H), 4.14–4.20 (m, 3H, 2″-H, 3′-H, 3″-H), 4.26–4.33 (m, 4H, 2-H, 3-H, 2′-H, Hyp-α-H), 4.66 (s, 1H, Hyp-γ-H), 5.13 (d, 1H, J = 4.0 Hz, 1″-H), 5.27 (d, 1H, J = 4.0 Hz, 1-H), 5.29 (d, 1H, J = 4.0 Hz, 1′-H); ^{13}C NMR (75 MHz, D_2O): δ 36.2, 51.1, 60.1, 62.3, 62.6, 63.0, 72.5, 72.6, 74.1, 76.7, 77.1, 80.6, 80.7, 81.7, 82.0, 82.3, 98.0, 99.0, 100.1, 174.2; ESI-TOF HRMS: $[M + Na]^+$ calcd for $C_{20}H_{33}NNaO_{15}$ 550.1748, found 550.1762.

General procedure for (glyco)peptide synthesis

Peptide chain assembly was starting with Fmoc-His(Trt) carrying as a trityl ester on NovaSyn®TGT alcohol resin **26** (loading capacity 0.2 mmol g^{-1}). The first five amino acids were introduced in an automatic peptide synthesizer (Life Technologies ABI433A Peptide Synthesizer) employing the N^{α}-Fmoc-based approach by the research resources center in RIKEN Brain Science Institute. 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) were employed as coupling reagents in an automatic peptide synthesizer. To the hexapeptide Fmoc-Asp(*t*Bu)-Pro-Leu-His(Trt)-His(Trt)-His(Trt) carrying as a trityl ester on NovaSyn®TGT alcohol resin **27**, the rest of the amino acids were added manually. The hexapeptide-bounded resin (Fmoc-Asp(*t*Bu)-Pro-Val-His(Trt)-His(Trt)-His(Trt)-Novasyn-TGT alcohol resin, 100.0 mg, 0.02 mmol) was placed in an SPPS reaction vessel. Fmoc removal was achieved with 20% piperidine in DMF (0.5 mL) for 10 min. The resin was washed with DMF (2 × 3 mL), CH_2Cl_2 (2 × 1 mL) and then DMF (2 × 1 mL). Fmoc-amino acid (5.0 equiv., 0.1 mmol) or Fmoc-glycoamino acid (3.0 equiv., 0.06 mmol), 1-[(1-(cyano-2-ethoxy-2-oxo-ethylideneaminooxy)dimethylaminomorpholino)]uronium hexafluorophosphate (COMU) (1 equiv. to amino acid) and *N*,*N*-diisopropylethylamine (DIPEA) (2 equiv. to amino acid) was mixed in DMF (0.5 mL) and activated for 1–2 min before being added to the resin. The reaction mixture was then agitated slowly for 1 min and allowed to couple for 2 h (4 h for secondary amine and 16 h for glycoamino acids **1–3**). The reaction was monitored by a microcleavage method and then analyzed by MALDI-TOF MS. Upon completion, the resin was washed with DMF and deprotected by 20% piperidine in DMF for 10 min. It was then washed again with DMF, CH_2Cl_2 and DMF, and then coupled with the next amino acid. Coupling and deprotection were repeated until the desired tridecapeptide (Fmoc-Arg(Pbf)-Thr(*t*Bu)-Hyp(*t*Bu)-Val-Ser(*t*Bu)-Gly-Hyp-(L-Araf_{0-3})-Asp(*t*Bu)-Pro-Val-His(Trt)-His(Trt)-His(Trt)-Novasyn-TGT alcohol resin) was obtained. Peptide was cleaved from the resin by treatment with a cocktail of 95% TFA, 2.5% water and 2.5% TIPS (1 mL) for 1 h. Each cleaved peptide resin was then washed extensively with a fresh cleavage cocktail (2 × 1 mL). The peptide was precipitated by the addition of cold diethyl ether at least 10 times the initial cocktail volume. The precipitate was filtered and washed with cold diethyl ether. Precipitated peptide was dissolved in 0.1% TFA in water and then lyophilized. The lyophilized *O*-acetylated glycopeptides were then dissolved in MeOH for removal of *O*-acetyl groups. The pH of the MeOH solution was adjusted to ~10 (as detected by wet litmus paper) by adding 0.1 M NaOMe in MeOH. The reaction was monitored by MALDI-TOF mass spectrometry and found to be complete after 2 h. Powder CO_2 (dry ice) was then added carefully to reach a pH of 6. The crude product was purified by semi-preparative reverse phase HPLC followed by lyophilization of the appropriate fractions. The purity of Araf_{0-3}CLV3 (**1–4**) was shown by ^{1}H NMR spectra as well as a HPLC chart obtained by using a semi-micro reverse-phase HPLC system equipped with a photo diode array detector (Waters Alliance e2695 and 2475 at 220 nm) with a gradient of 5–5–50–50% acetonitrile (containing 0.1% TFA) for 0–5–30–35 min respectively, at a flow rate of 500 μL min^{-1} through InertSustain® C18 column [3 μm (3.0 × 150), GL Science Inc.].[10]

H-Arg-Thr-Val-Hyp-Ser-Gly-Hyp-Asp-Pro-Leu-His-His-His-OH (4). Compound **4** was obtained by the general procedure for peptide synthesis in 30% yield (8.9 mg) as an amorphous white solid. Analytical data for peptide **4** was in good agreement with those reported previously.[23] Analytical data for **4**: ^{1}H NMR (500 MHz, D_2O): δ 0.83 (d, 3H, J = 6.0 Hz, Leu-δ-H_3), 0.90 (d, 3H, J = 6.5 Hz, Leu-δ-H_3), 0.93 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 0.97 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 1.21 (d, 3H, J = 6.0 Hz, Thr-γ-H_3), 1.39–1.42 (m, 1H, Leu-γ-H), 1.52–1.58 (m, 2H, Leu-β-H_2),

1.63–1.68 (m, 2H, Arg-γ-H_2), 1.88–2.13 (m, 8H Arg-β-H_2, Val-β-H, 2 × Hyp-β-Ha, Pro-β-Ha, Pro-γ-H_2), 2.24–2.33 (m, 2H, Hyp-β-Ha, Pro-β-Hb), 2.36–2.41 (m, 1H, Hyp-β-Hb), 2.77–2.81 (m, 1H, Asp-β-Ha), 2.91–2.96 (dd, 1H, J = 8.0, 17.0 Hz, Asp-β-Hb), 3.13–3.34 (m, 8H, Arg-δ-H_2, 3 × His-β-H_2), 3.63–3.95 (m, 8H, 2 × Hyp-δ-H_2, Pro-δ-H_2, Ser-β-H_2), 4.04–4.24 (m, 3H, Arg-α-H, Leu-α-H, Gly-α-Ha), 4.21–4.24 (m, 1H, Gly-α-Hb), 4.36–4.39 (m, 1H, Pro-α-H), 4.42–4.53 (m, 4H, Hyp-α-H, Ser-α-H, Thr-α-H, Hyp-α-H), 4.58–4.73 (m, 4H, 3 × His-α-H, Hyp-α-H), 4.95 (t, 1H, J = 6.5 Hz, Asp-α–H), 7.27–7.31 (m, 4H, His-aromatic), 8.62–8.65 (m, 6H, His-aromatic); MALDI-TOF MS: $[M + H]^+$ calcd for $C_{63}H_{97}N_{22}O_{20}$ 1481.7249, found 1481.8541. See also Fig. 2 and ESI.†

H-Arg-Thr-Val-Hyp-Ser-Gly-Hyp[Ara*f*]-Asp-Pro-Leu-His-His-His-OH (3). Compound **3** was obtained by the general procedure for the peptide synthesis in 37% yield (12.0 mg) as an amorphous white solid. Analytical data for **3**: ^{1}H NMR (500 MHz, D_2O): δ 0.83 (d, 3H, J = 6.0 Hz, Leu-δ-H_3), 0.90 (d, 3H, J = 6.5 Hz, Leu-δ-H_3), 0.93 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 0.98 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 1.21 (d, 3H, J = 6.0 Hz, Thr-γ-H_3), 1.38–1.42 (m, 1H, Leu-γ-H), 1.50–1.58 (m, 2H, Leu-β-H_2), 1.60–1.68 (m, 2H, Arg-γ-H_2), 1.88–2.12 (m, 8H, Arg-β-H_2, Val-β-H, 2 × Hyp-β-Ha, Pro-β-Ha, Pro-γ-H_2), 2.25–2.28 (m, 1H, Pro-β-Hb), 2.37–2.41 (m, 1H, Hyp-β-Ha), 2.36–2.41 (m, 1H, Hyp-β-Hb), 2.76–2.79 (m, 1H, Asp-β-Ha), 2.91–2.96 (m, 1H, Asp-β-Hb), 3.12–3.34 (m, 8H, Arg-δ-H_2, 3 × His-β-H_2), 3.62 (dd, 1H, J = 7.5, 12.0 Hz, 5a-H), 3.75–3.79 (m, 3H, 5b-H, Hyp-δ-H_2), 3.86–3.91 (m, 7H, 4-H, Hyp-δ-H_2, Pro-δ-H_2, Ser-β-H_2), 4.05 (dd, 1H, J = 6.5, 8.0 Hz, 3-H), 4.06–4.16 (m, 3H, 2-H, Arg-α-H, Gly-α-Ha), 4.19–4.20 (m, 1H, Leu-α-H, Gly-α-Hb), 4.36–4.39 (m, 1H, Pro-α-H), 4.43–4.53 (m, 4H, Hyp-α-H, Ser-α-H, Thr-α-H, Hyp-α-H), 4.59–4.71 (m, 4H, 3 × His-α-H, Hyp-α-H), 4.96 (t, 1H, J = 6.5 Hz, Asp-α–H), 5.14 (d, 1H, J = 4.5 Hz, 1-H), 7.27–7.31 (m, 4H, His-aromatic), 8.62–8.65 (m, 6H, His-aromatic); MALDI-TOF MS calcd for $C_{68}H_{105}N_{22}O_{24}$ $[M + H]^+$: 1613.7672, found 1613.7910. See also Tables 2, Fig. 2 and ESI.†

H-Arg-Thr-Val-Hyp-Ser-Gly-Hyp[Ara*f*$_2$]-Asp-Pro-Leu-His-His-His-OH (2). Compound **2** was obtained by the general procedure for the peptide synthesis in 31% yield (10.8 mg) as an amorphous white solid. Analytical data for **2**: ^{1}H NMR (500 MHz, D_2O): δ 0.84 (d, 3H, J = 6.0 Hz, Leu-δ-H_3), 0.90 (d, 3H, J = 6.5 Hz, Leu-δ-H_3), 0.93 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 0.98 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 1.21 (d, 3H, J = 6.0 Hz, Thr-γ-H_3), 1.36–1.41 (m, 1H, Leu-γ-H), 1.50–1.58 (m, 2H, Leu-β-H_2), 1.60–1.68 (m, 2H, Arg-γ-H_2), 1.88–2.12 (m, 8H Arg-β-H_2, Val-β-H, 2 × Hyp-β-Ha, Pro-β-Ha, Pro-γ-H_2), 2.24–2.30 (m, 1H, Pro-β-Hb), 2.37–2.42 (m, 1H, Hyp-β-Ha), 2.48–2.49 (m, 1H, Hyp-β-Hb), 2.73–2.77 (m, 1H, Asp-β-Ha), 2.88–2.94 (m, 1H, Asp-β-Hb), 3.11–3.31 (m, 8H, Arg-δ-H_2, 3 × His-β-H_2), 3.63 (dd, 1H, J = 7.0, 12.0 Hz, 5a-H), 3.69 (dd, 1H, J = 7.0, 12.0 Hz, 5a′-H), 3.75–3.86 (m, 4H, 5b-H, 5b′-H, Hyp-δ-H_2), 3.86–3.93 (m, 8H, 4-H, 4′-H, Hyp-δ-H_2, Pro-δ-H_2, Ser-β-H_2), 4.01–4.16 (m, 6H, 2′-H, 3-H, 3′-H Arg-α-H, Gly-α-Ha), 4.20–4.23 (m, 2H, Leu-α-H, Gly-α-Hb), 4.29 (dd, 1H, J = 4.5, 8.0 Hz, 2-H), 4.37–4.40 (m, 1H, Pro-α-H), 4.42–4.53 (m, 4H, Hyp-α-H, Ser-α-H, Thr-α-H, Hyp-α-H), 4.59–4.69 (m, 4H, 3 × His-α-H, Hyp-α-H), 4.94 (t, 1H, J = 6.5 Hz, Asp-α–H), 5.01 (d, 1H, J = 4.5 Hz, 1′-H), 5.31 (d, 1H, J = 4.5 Hz, 1-H), 7.27–7.31 (m, 4H, His-aromatic), 8.62–8.65 (m, 6H, His-aromatic); MALDI-TOF MS: $[M + H]^+$ calcd for $C_{73}H_{113}N_{22}O_{28}$ 1745.8095, found 1745.6676. See also Table 2, Fig. 2 and ESI.†

Table 2 Selected NMR chemical shifts and $^3J_{H1-H2}$ spin-couplings of saccharide signals of synthetic [Araf_n]CLV3 (n = 1–3) (**1–3**)

H, C/ppm (^3J Hz)	[Araf_1]CLV3 (**3**)	[Araf_2]CLV3 (**2**)	[Araf_3]CLV3 (**1**)
1-H ($^3J_{H1-H2}$)	5.14 (4.5)	5.31 (4.5)	5.33 (4.5)
C1	*100.4*	*98.5*	*98.4*
2-H	4.16	4.29	4.34
3-H	4.05	4.15	4.15
4-H	3.90	3.92	3.92
5-Ha	3.66	3.63	3.72
5-Hb	3.81	3.79	3.84
1′-H ($^3J_{H1'-H2'}$)		5.01 (4.0)	5.13 (4.0)
C1′		*100.1*	*98.0*
2′-H		4.13	4.29
3′-H		4.11	4.23
4′-H		3.90	4.01
5′-Ha		3.69	3.74
5′-Hb		3.80	3.85
1″-H ($^3J_{H1''-H2''}$)			4.95 (4.5)
C1″			*100.2*
2″-H			4.11
3″-H			4.13
4″-H			3.91
5″-Ha			3.69
5″-Hb			3.85

H-Arg-Thr-Val-Hyp-Ser-Gly-Hyp[Ara*f*$_3$]-Asp-Pro-Leu-His-His-His-OH (1). Compound **1** was obtained by the general procedure for the peptide synthesis in 34% yield (12.8 mg) as an amorphous white solid. Analytical data for peptide **1** was in good agreement with those reported previously.[23] Analytical data for **1**: ^{1}H NMR (500 MHz, D_2O): δ 0.84 (d, 3H, J = 6.0 Hz, Leu-δ-H_3), 0.90 (d, 3H, J = 6.5 Hz, Leu-δ-H_3), 0.93 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 0.98 (d, 3H, J = 6.0 Hz, Val-γ-H_3), 1.21 (d, 3H, J = 6.0 Hz, Thr-γ-H_3), 1.35–1.41 (m, 1H, Leu-γ-H), 1.48–1.57 (m, 2H, Leu-β-H_2), 1.60–1.67 (m, 2H, Arg-γ-H_2), 1.88–2.13 (m, 8H, Arg-β-H_2, Val-β-H, 2 × Hyp-β-Ha, Pro-β-Ha, Pro-γ-H_2), 2.23–2.29 (m, 1H, Pro-β-Hb), 2.38–2.41(m, 1H, Hyp-β-Ha), 2.45–2.49 (m, 1H, Hyp-β-Hb), 2.74–2.79 (m, 1H, Asp-β-Ha), 2.89–2.94 (m, 1H, Asp-β-Hb), 3.11–3.31 (m, 8H, Arg-δ-H_2, 3 × His-β-H_2), 3.62 (dd, 1H, J = 7.0, 12.0 Hz, 5a-H), 3.69–3.74 (m, 2H, 5a′-H, 5a″-H), 3.77–3.86 (m, 5H, 5b-H, 5b′-H, 5″b-H, Hyp-δ-H_2), 3.88–4.03 (m, 9H, 4-H, 4′-H, 4″-H, Hyp-δ-H_2, Pro-δ-H_2, Ser-β-H_2), 4.09–4.16 (m, 6H, 2″-H, 3-H, 3″-H, Arg-α-H, Gly-α-H_2), 4.21–4.25 (m, 3H, 3′-H, Leu-α-H, Gly-α-Hb), 4.30 (dd, 1H, J = 4.5, 8.0 Hz, 2′-H), 4.35 (dd, 1H, J = 4.5, 8.0 Hz, 2-H), 4.36–4.39 (m, 1H, Pro-α-H), 4.42–4.53 (m, 5H, 2″-H, Hyp-α-H, Ser-α-H, Thr-α-H, Hyp-α-H), 4.59–4.70 (m, 4H, 3 × His-α-H, Hyp-α-H), 4.94 (t, 1H, J = 6.5 Hz, Asp-α–H), 4.98 (d, 1H, J = 4.5 Hz, 1″-H), 5.14 (d, 1H, J = 4.5 Hz, 1′-H), 5.34 (d, 1H, J = 4.5 Hz, 1-H), 7.27–7.31 (m, 4H, His-aromatic), 8.62–8.65 (m, 6H, His-aromatic); MALDI-TOF MS: $[M + H]^+$ calcd for $C_{78}H_{121}N_{22}NaO_{32}$ 1877.8517, found 1877.7329. See also Table 2, Fig. 2 and ESI.†

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