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On the mechanism of *p*-methoxybenzylidene assisted intramolecular aglycon delivery

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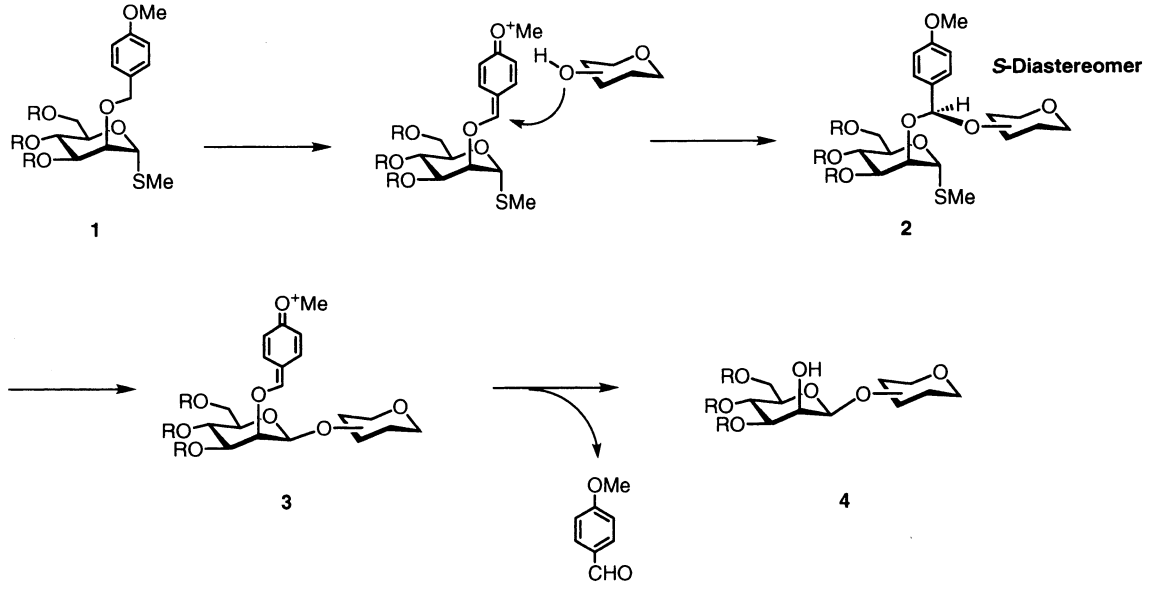
Abstract—*p*-Methoxybenzylidene-assisted intramolecular aglycon delivery was developed in this laboratory and has been demonstrated to be highly efficient and versatile particularly for the synthesis of complex glycoprotein-related glycans. It was revealed that the reaction course of IAD can be clearly monitored by performing the reaction in an NMR tube. Our results suggest that the nonhydrolytic pathway that converts metastable intermediate **3** to the product is functional. Comparative experiments in the presence and absence of water in the reaction media afforded supporting evidence eliminating the possibility of fortuitous contamination of water under standard IAD conditions.

1. Introduction

Among various approaches to achieve the stereocontrolled formation of β-manno glycoside,[1] the one called intramolecular aglycon delivery (IAD) is particularly attractive, because exclusive formation of the correct stereoisomer can be expected.[2–4] In this approach, aglycon is first connected to the axially oriented C-2 hydroxy group of the mannosyl donor, by a certain type of tether.

p-Methoxybenzylidene-assisted IAD using donor **1** (Scheme 1) was developed in this laboratory[4–6] and has



Scheme 1. Presumed reaction pathway of *p*-methoxybenzylidene-assisted intramolecular aglycon delivery.

Keywords: intramolecular aglycon delivery; glycoprotein; oligosaccharides; stereoselective; glycosylation.

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been demonstrated to be highly efficient and versatile particularly for the synthesis of complex glycoprotein-related oligosaccharides. This reaction takes advantage of the facile formation of mixed acetal **2** under oxidative conditions.[7] Subsequent activation of the mannose anomeric position triggers IAD to give β-glycoside **4**.

This method has several attractive features; (1) various methods for the introduction of *O*-*p*-methoxybenzyl (PMB) group have been established, (2) the conditions required for the formation of the mixed acetal are mild and near neutral, which may well be compatible with the presence of various types of protecting groups, and (3) compared to other IAD systems developed previously, more efficient charge delocalization and therefore clean IAD by a mesomerically stabilized transition state can be expected in this system, due to the assistance of an electron donating *p*-methoxyphenyl group.

In particular, IAD using 4,6-*O*-cyclohexylidene-protected thioglycoside **5a** proved to be a highly efficient donor. For instance, reaction with glucosamine-derived acceptor **6** afforded 83% yield of **7** which corresponds to the core disaccharide (Manβ1→4GlcNAc) of an Asn-linked glycan chain[6] (Scheme 2). The synthesis of complex-type undecasaccharide **8** was achieved using this reaction.[8] Quite interestingly, the formation of the mixed acetal **2** was revealed to be a remarkably stereoselective process and the absolute configuration of the acetalic carbon was assigned *S* (Scheme 1).[9]

On the other hand, analysis of the assumed reaction pathway (Scheme 1) gave rise to a simple question. Immediate product after successful glycoside bond formation would most likely be a quinonemethide (or benzyl cation) like species **3**, which eventually collapses to the end-product **4** and *p*-anisaldehyde most understandably after aqueous work-up.

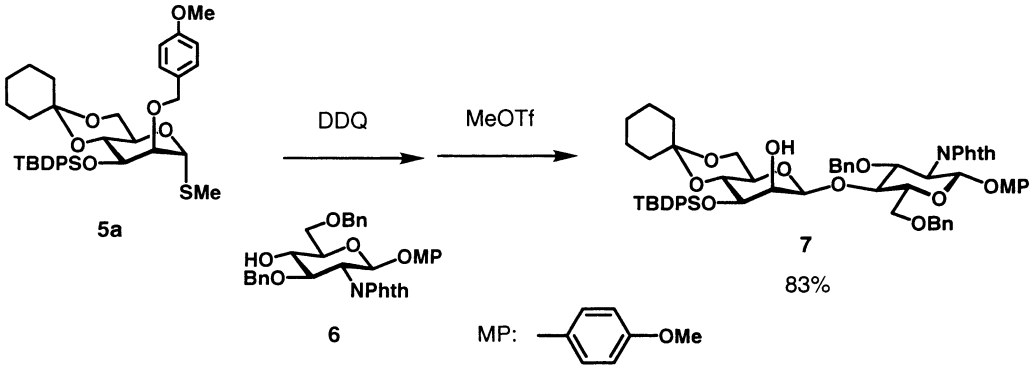
Such an assumption seems to be potentially hazardous. With the accumulation of a highly reactive species like **3**, stability of an oligosaccharide that includes various functional groups would be a subject of concern. In order to see if **3** is actually produced in substantial concentration during the course of IAD, the progress of the reaction was monitored by ^{1}H-NMR. For this purpose, fructose-derived **9a**[10] and 4,6-*O*-cyclohexylidene-protected thiomannoside **5a** were used as glycosyl acceptor (aglycon) and donor, respectively (Scheme 3). Since **9a** is devoid of the anomeric proton, analysis of ^{1}H-NMR was expected to be greatly simplified.

2. Results and discussion

Previously reported **5a**[6] was prepared in a modified manner as depicted in Scheme 4. Thus, methyl 1-thio-α-D-mannopyranoside (**14**) was treated with cyclohexanone dimethylacetal and camphorsulfonic acid (CSA) to provide 4,6-*O*-cyclohexylidene-protected **15**. Subsequent transformation to bis-acetal **16** was effected by *p*-anisaldehyde dimethylacetal and pyridinium *p*-toluenesulfonate (PPTS), which was followed by chemo- and regioselective ring opening with diisobutyl aluminum hydride (DIBAL-H)[11] in toluene to afford 2-*O*-PMB derivative **17** as the major product,[12] together with a small amount of corresponding regioisomer in a ratio of 20:1. Subsequent silylation under standard conditions gave **5a**.

The formation of the mixed acetal **10a** from **5a** and **9a** (1.3 equiv.) was performed under our standard conditions (1.25 equiv. DDQ, MS 4 A, CH_2Cl_2, r.t., 130 min). After reductive work-up[13] and purification by size exclusion chromatography on Bio Beads SX-3 (Bio Rad) eluted with toluene, mixed acetal **10a** [δ_H 5.85 (acetal CH), 5.44 (H-1_{Man}) was obtained in a quantitative yield (Scheme 3). Subsequent IAD was performed in an NMR tube in the presence of MeOTf[14] and 2,6-di-*tert*-butyl-4-methylpyridine (3.3 equiv. each) and powdered molecular sieves 4 A in $CDCl_3$ at 35°C. As seen from Fig. 1, the progress of the reaction can be clearly monitored by ^{1}H-NMR. The most diagnostic was the decrease of the signals of mixed acetal CH (δ 5.85) and H-1_{Man} (δ 5.44).

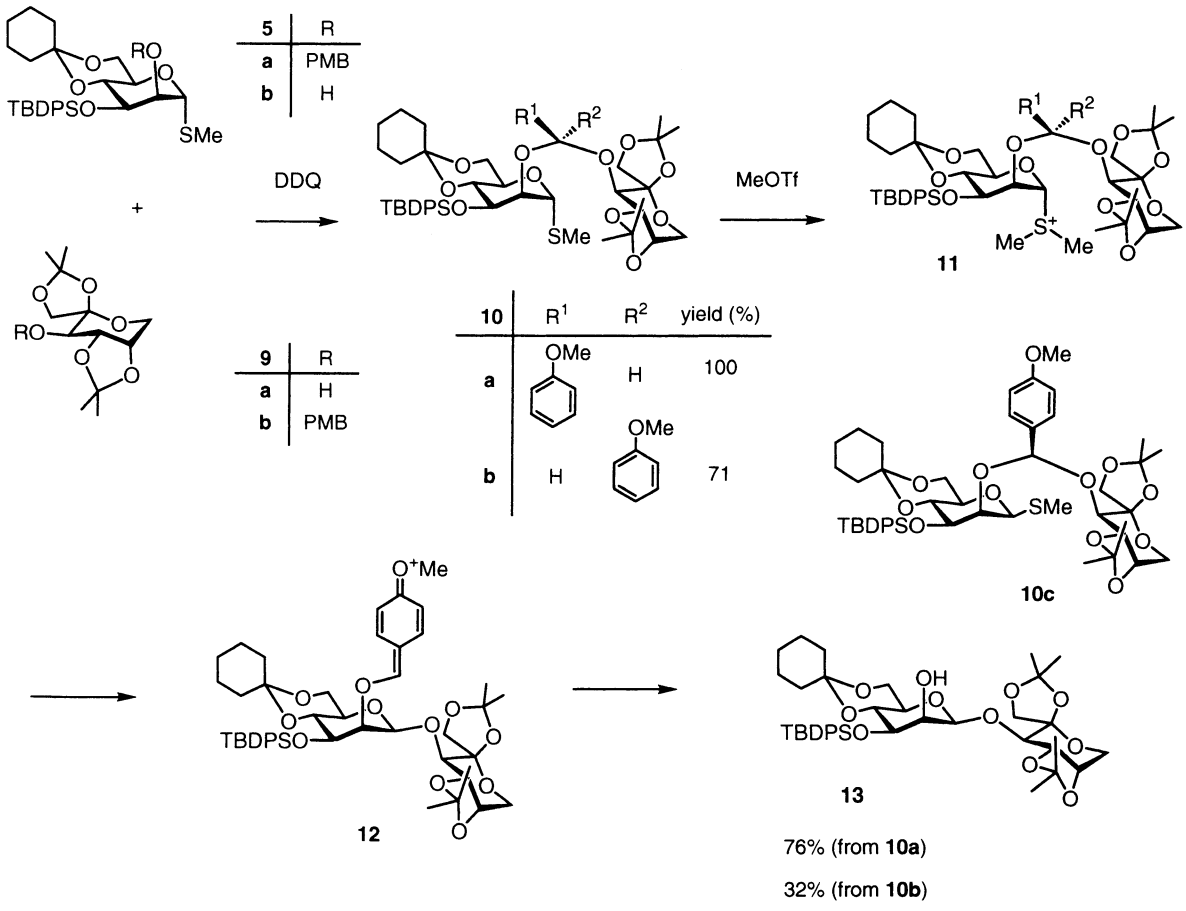
A new set of signals (δ 5.77 and 5.40) was observed at the



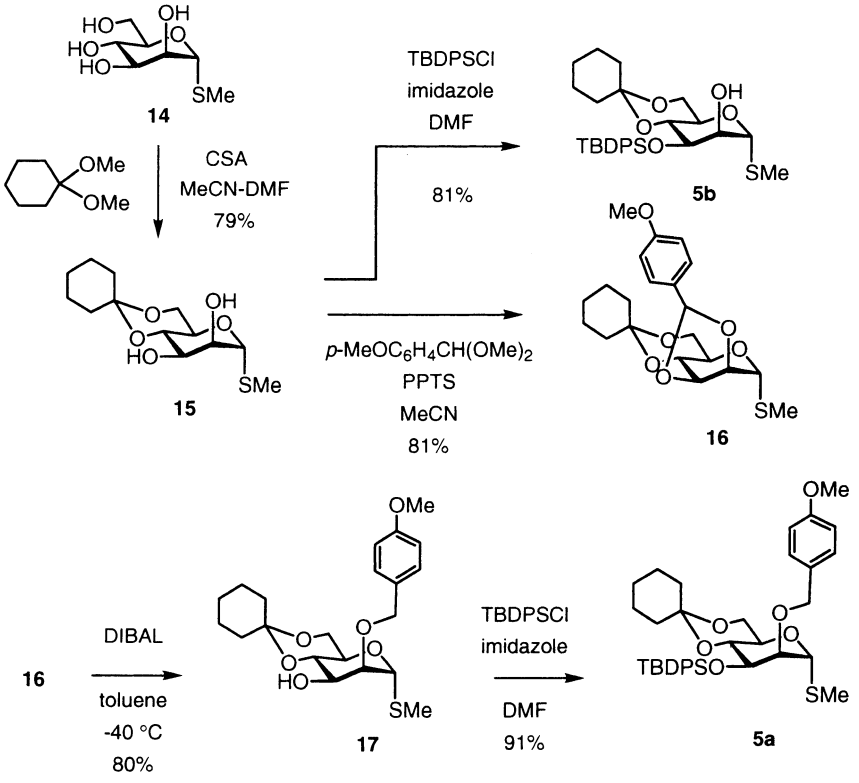
NeuAcα2→3**Gal**β1→4**GlcNAc**β1→2**Man**α1→6
Manβ1→4**GlcNAc**β1→4**GlcNAc**
NeuAcα2→3**Gal**β1→4**GlcNAc**β1→2**Man**α1→3

8

Scheme 2. Typical results of β-mannosylation.



Scheme 3. Formation of Manβ1→3Fru (**13**) by intramolecular aglycon delivery.



Scheme 4. Preparation of the mannosyl donor.

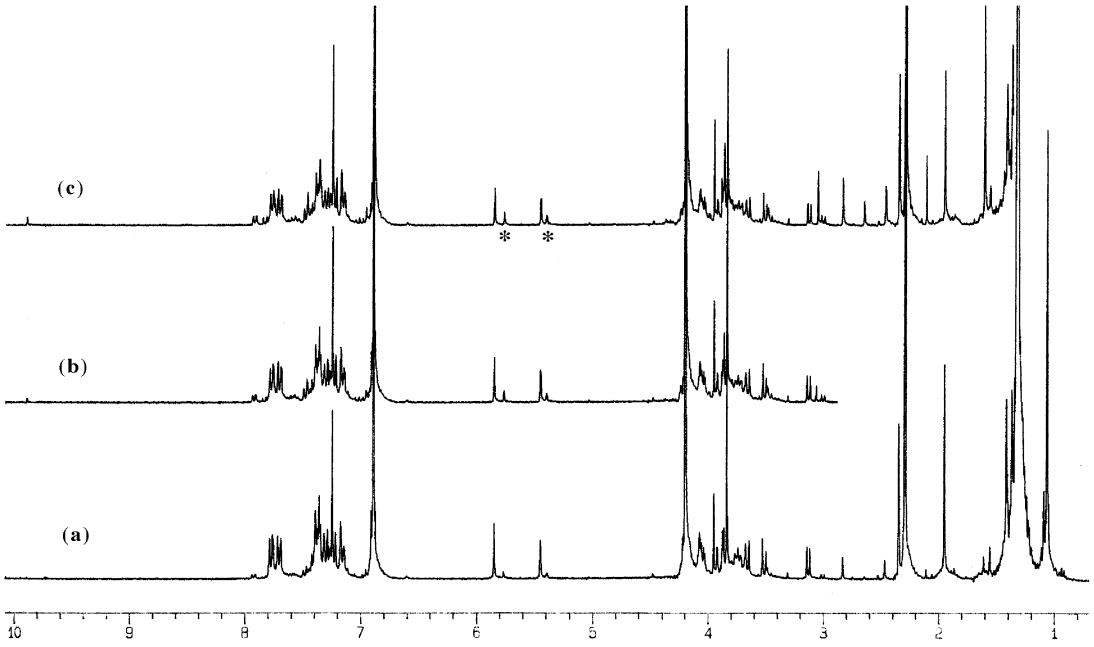


Figure 1. Starting from **10a**, the reaction course of IAD was followed by ^{1}H-NMR ($CDCl_3$, 270 MHz, 35°C): (a), 5 min; (b), 10 min; (c), 20 min.

early stage of the reaction (see asterisks). The transient species observed here may be assignable as either cationic intermediate **11** or β-thioglycoside **10c** derived from anomerization of **10a**, possibly via **11**. Since the anomeric proton of pyranosides carrying a positively charged substituent at *C*-1 is expected to appear at lower field compared to the parent neutral species,[15] the latter possibility would be more likely. The stereochemical scrambling of the acetalic carbon was initially conceived. However, this possibility was excluded by a comparative experiment using diastereomeric acetal **10b** (vide infra).

After 160 min, it was observed that the product **13** having liberated 2-OH was produced quite cleanly together with an equimolar amount of *p*-anisaldehyde (Fig. 2). Therefore, the formation of the end-product **13** does not seem to require aqueous work-up. With a closer look at the NMR spectrum, it was noticed that H-2_{Man} (δ 3.58) of **13** present in the reaction mixture had a signal broadening, compared to the same signal of the purified material. It probably means that the product **13** had a C-2 hydroxy group deuterated (at least partially), suggesting the intermediacy of an oxy-radical-like species which abstracts deuterium from the solvent $CDCl_3$. The same reaction was performed in a preparative scale (78 μmol) in CH_2Cl_2 (7 ml) to afford **13** in 76% yield based on **5a**.

In contrast, the DDQ-mediated reaction of 2-*O*-unprotected thiomannoside **5b** (prepared from **15**, see Scheme 4) and 3-*O*-PMB-protected fructose derivative **9b** afforded diastereomeric mixed acetal **10b** [δ_H 5.90 (acetal CH), 4.66 (H-1_{Man})] as the major isomer (~10:1). NMR experiment (see Fig. 1 and **3**) as well as preparative scale reaction revealed several interesting aspects of IAD. Firstly, configurational rigidity of the acetal carbon was retained with no indication of the stereochemical scrambling between **10a,b** throughout the course of IAD. It is to be noted that the transiently observed species (see asterisks) derived from **10a** (Fig. 1) and **10b** (Fig. 3) were clearly distinct from **10b** and **10a**, respectively. Therefore, the possibility of the interconversion between two diastereomers, **10a** and **10b**, during IAD can be excluded. Secondly, the reaction of *R*-configured **10b** turned out to be less efficient, in terms of both reaction kinetics and efficiency. Namely, IAD of **10b** was ca. 50% completion after 220 min, while **10a** was completely consumed after 160 min (Fig. 3). After 24 h, product **13** was isolated in 32% yield which was substantially lower than that obtained from **10a** (76%).

Since it was previously observed that 3-*O*-TMS-protected donor **18** gave cyclic acetal **21** as the β-mannoside product (Scheme 5), it is quite probable that IAD of mixed acetal **19** first gives metastable cationic product **20**, which is immediately quenched intramolecularly to give **21** (instead of **22**). On the other hand, in the case of reaction using **5a**, *C*-3 oxygen of **12** is probably not competent for intramolecular participation, because of the steric hindrance of the TBDPS group.

Of course the possibility of the contamination of water in the reaction mixture cannot be readily excluded. In fact, addition of base (2,6-di-*tert*-butyl-4-methylpyridine) seem to be an absolute requirement for successful IAD. Its absence resulted in the rapid decomposition of the mixed acetal to afford a complex mixture. Therefore, under our experimental conditions, a trace amount of water may be present and generates acidic species (e.g. trifric acid). Compared to conventional glycosylation, IAD might well be more resistant to moisture because of its intramolecular nature. It can be argued that contamination of water may simply help quenching the initial product **3** into **4** without affecting the efficiency of the aglycon transfer step. However, in a separate experiment using glucosamine-derived **6** as an acceptor, it was demonstrated that the efficiency of IAD is not totally insensitive to the presence of water (Scheme 6). The presence of 1.6 and 10 equiv. of

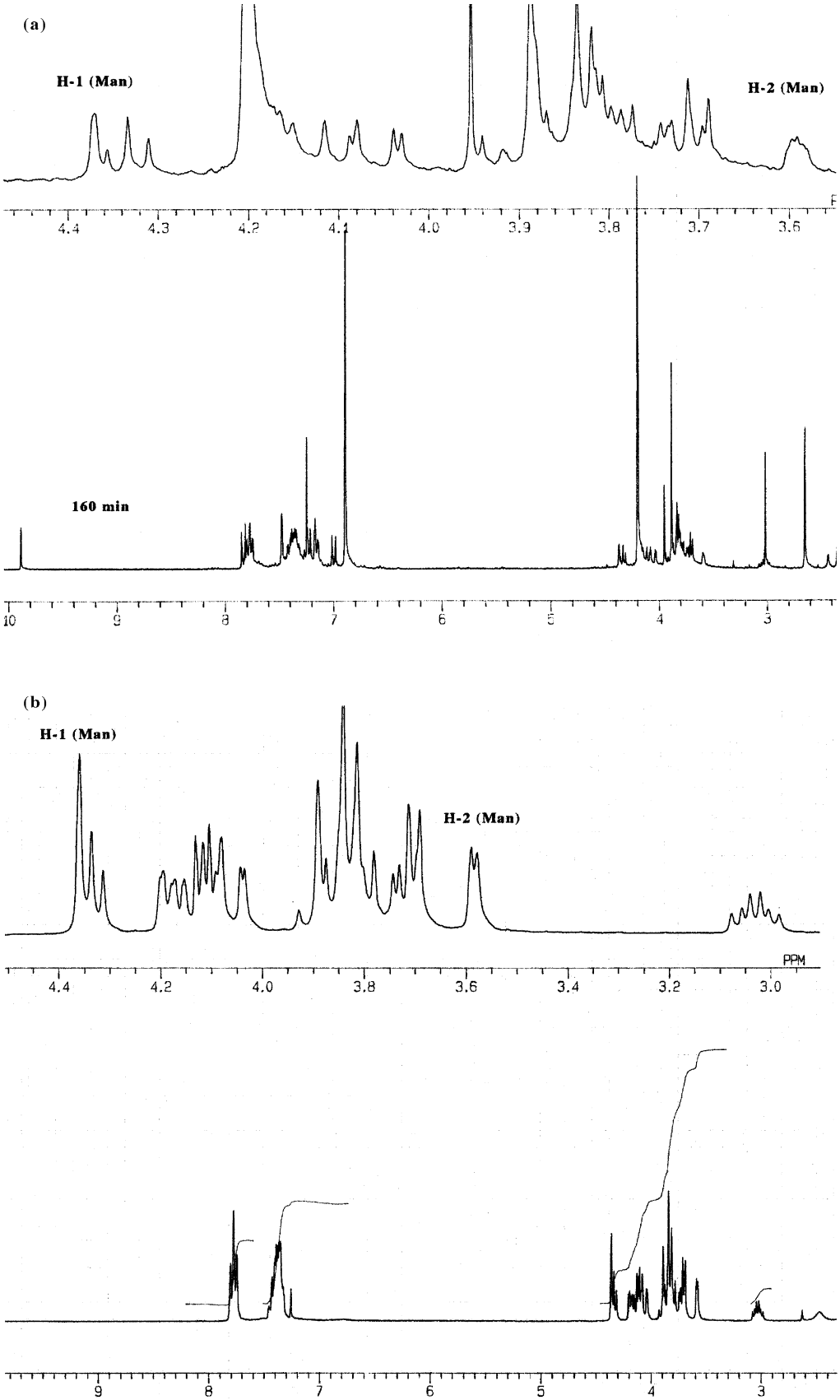


Figure 2. IAD was nearly complete after 160 min at 35°C. Comparison of ^{1}H-NMR ($CDCl_3$, 270 MHz): (a), reaction mixture (35°C); (b), purified product **13** (room temperature).

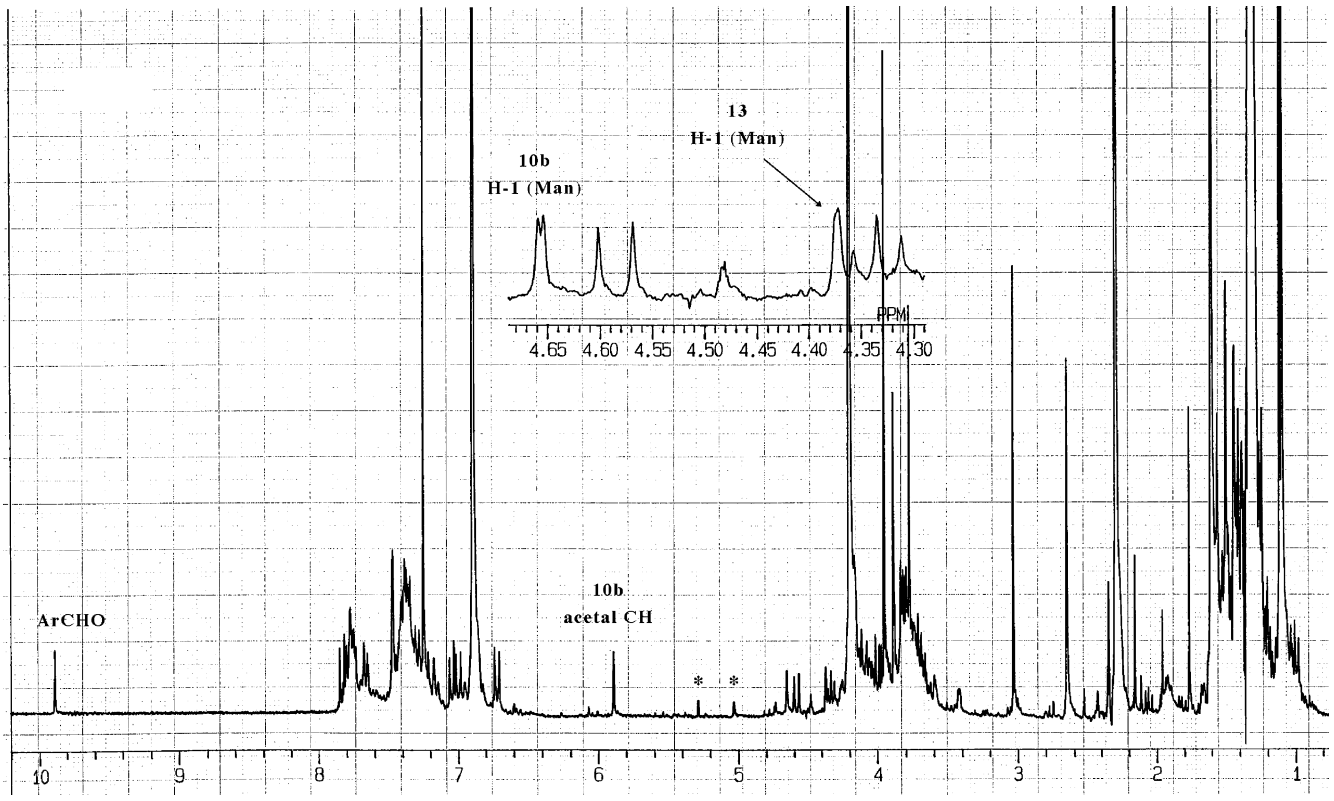
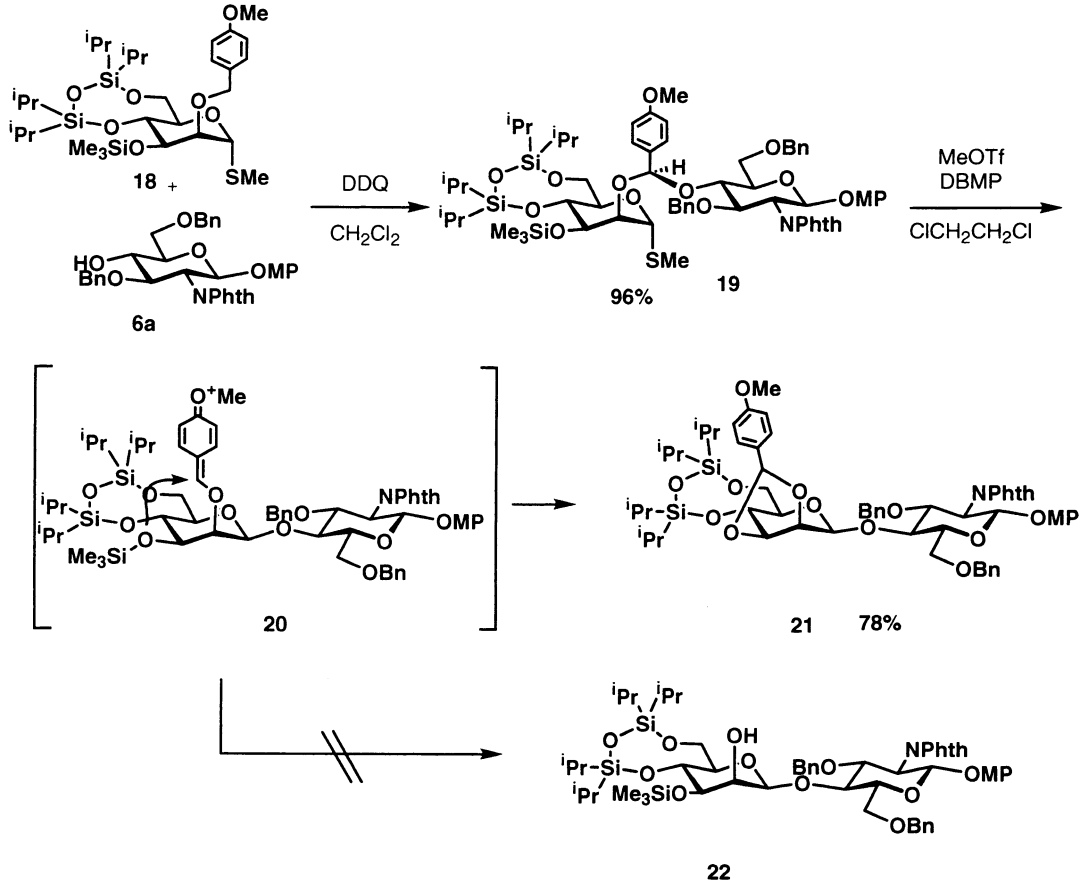
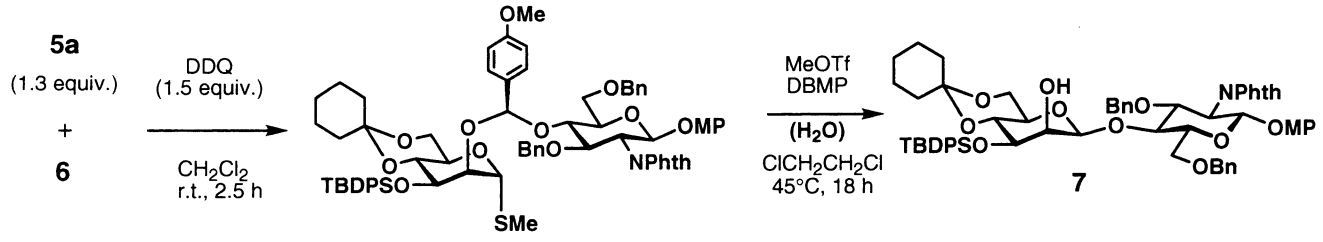


Figure 3. [1]H-NMR profile of IAD starting from diastereomeric mixed acetal **10b** (35°C, 220 min).



Scheme 5. Formation of cyclic acetal containing β-mannoside from the 3-*O*-TMS-protected donor **18**.



H_2O (equiv.)	Yield (%)
0	83
1.6	54
10	17

Scheme 6. Intramolecular aglycon delivery under water containing conditions.

H_2O results in the formation of the IAD product **7** in 54% and 17% yield, respectively, while the same product was obtained in 83% yield under anhydrous conditions. It is highly unlikely that a stoichiometric amount (but less than 1.6 equiv.) of water constantly exists in the reaction mixture of IAD. NMR analysis (Fig. 3) suggested the intermediacy of an oxy-radical species (vide supra). The initial formation of quinonemethide-like **12** may well be a reasonable premise. However, its lifetime seems to be rather short, being undetectable by NMR, and the subsequent pathway is triggered immediately.[16]

Taken together, results presented here suggest that a non-hydrolytic pathway, which leads **3** to **4**, is functional in IAD, although its precise nature is not clear at this moment.

3. Experimental

3.1. General methods

Starting materials and reagents were purchased from standard vendors and used without purification unless otherwise noted. All reactions sensitive to air or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Anhydrous solvents were purchased from Kanto Chemical Co. (Tokyo, Japan). Molecular sieves were purchased from Nacalai Tesque (Kyoto, Japan) and activated by heating at 180°C under vacuum prior to use. Analytical thin-layer chromatography (TLC) was developed on E. Merck Silica Gel 60 F_{256} plates (0.25 mm thickness). Column chromatography was performed on E. Merck Silica Gel 60 (60–230 mesh or 130–400 mesh). Melting points were determined with a Büchi 510 melting point apparatus and uncorrected. Optical rotations were measured with a JASCO DIP 370 polarimeter at ambient temperature (20±3°C). NMR spectra were obtained on a JEOL EX-270, EX-400, or α-400 spectrometer at ambient temperature unless otherwise noted. ^{1}H chemical shifts are in parts per million (δ) adjusted to Me_4Si at 0 ppm as an internal reference. ^{13}C chemical shifts were referenced with $CDCl_3$ at 77.0 ppm or with CD_3OD at 49.0 ppm.

3.1.1. Methyl 4,6-O-cyclohexylidene-1-thio-α-D-mannopyranoside (15). Methyl 1-thiomannoside **14** (5.481 g, 26.1 mmol) was dissolved in MeCN–DMF (1:2, 150 ml) containing camphorsulfonic acid (0.303 g, 1.3 mmol), cyclohexanone dimethylacetal (7.8 ml, 52 mmol) and Drierite (5 g) and the mixture stirred for 1.5 h and exposed to vacuum (~10 mmHg) to remove methanol. After being stirred for an additional 0.5 h, the reaction was quenched with triethylamine and filtered through Celite. The filtrate was evaporated in vacuo and the residue was chromatographed over silica gel (hexane–AcOEt 3:1) to afford 6.01 g (79%) of the title compound as a crystalline solid; mp 98–100°C; $[\alpha]_D$=+182.6 (c 1.0, $CHCl_3$); ^{1}H-NMR (CD_3OD, 400 MHz) δ 5.11 (1 H, s, H-1), 4.04 (1 H, t, J=9.5 Hz, H-6), 3.94 (1 H, d, J=3.9 Hz), 3.90 (1 H, d, J=9.5 and 4.4 Hz, H-6′), 3.84 (1 H, t, J=10.1 Hz, H-4), 3.75–3.68 (2 H, m, H-3,5), 2.10 (3 H, s, SMe); ^{13}C-NMR (CD_3OD, 100 MHz) δ 101.2 (cyclohexylidene), 88.8 (C-1), 74.0, 71.7, 70.4, 66.6. 62.5, 39.2, 29.0, 23.8, 23.6, 13.8 (MeS). Anal. Calcd for $C_{13}H_{22}O_5S$: C, 53.77; H, 7.64; S, 11.04. Found: C, 53.44; H, 7.44; S, 10.61.

3.1.2. Methyl 4,6-O-cyclohexylidene-2,3-O-*p*-methoxybenzylidene-1-thio-α-D-mannopyranoside (16). A solution of compound **15** (5.00 g, 17.2 mmol), *p*-anisaldehyde dimethylacetal (3.8 ml, 22 mmol) and PPTS (0.28 g, 1.1 mmol) in MeCN (45 ml) was stirred at room temperature for 40 min. The mixture was quenched with Et_3N and evaporated in vacuo. The residue was coevaporated with toluene and chormatographed over silica gel (hexane–AcOEt 15:1) to afford 5.13 g (81%) of the title compound as a mixture (ca. 6:5) of diastereomers; ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.44 (d, J=8.8 Hz), 7.36 (d, J=8.8 Hz), 6.91 (d, J=8.8 Hz), 6.89 (d, J=8.8 Hz), 6.18, 5.91, 5.52 and 5.46 (s, acetal CH and H-1), 4.5–3.7 (m, 6 H), 3.82 and 3.81 (s, OMe), 2.13 and 2.11 (s, SMe), 2.0–1.3 (m, 10 H, cyclohexylidene). Anal. Calcd for $C_{21}H_{28}O_6S$: C, 61.74; H, 6.91. Found: C, 61.62; H, 6.94.

3.1.3. Methyl 4,6-O-cyclohexylidene-2-O-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (17). To a stirred solution of compound **16** (280 mg, 0.685 mmol) in toluene (6 ml) was added DIBAL (1.01 M toluene solution, 2.0 ml, 2.0 mmol) at −40°C. The mixture was stirred at the same temperature for 30 min and quenched with an aqueous solution of Rochelle salt (6 ml). The mixture was stirred at room

temperature for 1 h and extracted with AcOEt. The organic layer was successively washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. Purification by silica gel column chromatography (hexane–AcOEt 10:1) afforded 224 mg (80%) of **17**.

$[\alpha]_D$=+88.1 (c 0.96, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.26 (2 H, d, J=8.3 Hz, Ar), 6.88 (2 H, d, J=8.3 Hz, Ar), 5.18 (1 H, s, H-1), 4.64 and 4.50 (each 1H, d, J=11.5 Hz), 3.79 (3 H, s, OMe), 4.00–3.70 (6 H, m), 2.06 (3 H, s, SMe); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 159.5, 129.7, 129.3, 113.9, 100.0 (cyclohexylidene), 83.7, 79.4, 72.7, 71.4, 69.3, 64.9, 61.4, 55.3, 38.0, 28.0, 25.6, 22.8, 22.6, 13.7. Anal. Calcd for $C_{21}H_{30}O_6S$: C, 61.44; H, 7.37; S, 7.81. Found: C, 60.97; H, 7.07; S, 7.78.

3.1.4. Methyl 4,6-O-cyclohexylidene-2-O-*p*-methoxybenzyl-3-O-*tert*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (5a). To a solution of compound **17** (0.52 g, 1.27 mmol) in acetonitrile containing imidazole (256 mg, 3.76 mmol) was added *t*-$BuPh_2SiCl$ (0.66 ml, 2.5 mmol) at 0°C. After being stirred at room temperature for 2 h, the reaction was cooled down to 0°C and quenched with MeOH. The mixture was diluted with AcOEt, washed successively with water and brine, dried (Na_2SO_4) and evaporated in vacuo. The residue was chromatographed over silica gel (hexane–AcOEt 10:1) to afford 0.75 g (91%) of the title compound; $[\alpha]_D$=+5.8 (c 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.78 (4 H, m, Ar), 7.37 (6 H, m, Ar), 7.3–7.5 (6 H, Ar), 7.10 (2 H, d, J=8.5 Hz, Ar), 6.79 (2 H, d, J=8.5 Hz, Ar) 4.82 (1 H, s, H-1), 4.45 (1 H, d, J=11.5 Hz, ArCH_2), 4.22 (1 H, d, J=11.5 Hz, ArCH_2), 4.20 (2 H, bs), 4.19 (1 H, d, J=11.6 Hz, ArCH_2), 3.9–3.7 (3 H, m), 3.77 (3 H, s, OMe), 3.22 (1 H, bs), 1.88 (3 H, s, SMe), 1.12 (9 H, s, *t*-Bu); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 158.9, 136.4, 136.1, 135.8, 134.5, 133.3, 130.2, 129.6, 129.4, 129.2, 127.8, 127.6, 127.4, 127.2, 113.5, 99.8 (cyclohexylidene), 84.7 (C-1), 79.8, 72.9, 71.5, 70.6, 65.8, 61.5 (CH_2), 55.2, 38.1 (CH_2), 28.0 (CH_2), 27.0 (Me_3C), 25.7 (CH_2), 22.7 (CH_2), 22.5 (CH_2), 19.4 (Me_3C), 13.7 (MeS). Anal. Calcd for $C_{37}H_{48}O_6SSi$: C, 68.48; H, 7.46. Found: C, 68.14; H, 7.50.

3.1.5. Methyl 4,6-O-cyclohexylidene-3-O-*tert*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (5b). Compound **15** (111 mg, 0.385 mmol) was dissolved in DMF (2 ml) containing imidazole (50 mg, 0.73 mmol). *t*-$BuPh_2SiCl$ (0.16 ml, 0.62 mmol) was added and the mixture was stirred at room temperature for 20 h. Resulting mixture was diluted with ether, washed with water and brine, successively, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane–AcOEt 8:1) to afford 165 mg (81%) of the title compound; $[\alpha]_D$=+12.1 (c 0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 5.04 (1 H, s, H-1), 4.18–4.02 (2 H, m), 3.95–3.68 (3 H, m), 3.43 (1 H, d, J=2.6 Hz, H-2), 1.96 (3 H, s, SMe), 1.11 (9 H, s, *t*-Bu); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 136.1, 135.7, 133.5, 132.6, 130.1, 129.8, 127.9, 127.7 and 127.5 (Ar), 99.9 (cyclohexylidene), 85.1 (C-1), 72.4, 71.4, 70.5, 64.9, 61.3 (C-6), 38.0 (CH_2), 27.7 (CH_2), 26.9 (Me_3C), 25.6 (CH_2), 22.5 (CH_2), 22.3 (CH_2), 19.2 (Me_3C), 13.3 (SMe). Anal. Calcd for $C_{29}H_{40}O_5SSi$: C, 65.87; H, 7.62. Found: C, 65.95; H, 7.62.

3.1.6. 1,2;4,5-Di-O-isopropylidene-3-O-*p*-methoxybenzyl-α-D-fructopyranose (9b). To a solution of compound **9a** (602 mg, 2.31 mmol) in DMF (10 ml) was added NaH (60%, 130 mg, 3.3 mmol) under ice-water cooling and stirred for 10 min. *p*-Methoxybenzyl chloride (0.44 ml, 3.0 mmol) was added dropwise and the mixture was stirred at 0°C to room temperature for 2 h. The reaction was quenched with MeOH (~0.1 ml), diluted with ether, washed with water and brine, successively, dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed over silica gel (hexane–AcOEt 3:1) to afford 858 mg (98%) of the title compound; $[\alpha]_D$=−82.3 (c, 0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.28 and 6.80 (each 2 H, d, J=8.8 Hz, Ar), 4.88 and 4.60 (each 1H, d, J=11.6 Hz, CH_2Ar), 4.37 (1 H, dd, J=7.1 and 5.6 Hz, H-4), 4.21 (1 H, dd, J=5.6 and 2.7 Hz, H-5), 4.13 (1 H, dd, J=13.4 and 2.7 Hz, H-6), 4.02 (1 H, d, J=8.4 Hz, H-1), 3.99 (1 H, d, J=13.4 Hz, H-6′), 3.85 (1 H, d, J=8.4 Hz, H-1′), 3.80 (3 H, s, OMe), 3.48 (1 H, d, J=7.1 Hz, H-3), 1.55, 1.49, 1.40, and 1.39 (each 3 H, s, isopropylidene); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 158.9, 130.1, 129.2, 113.5, 112.0, 108.9, 104.3, 77.8, 74.5, 73.8, 72.7, 71.8, 60.2, 55.2, 28.3, 27.0, 26.3, 26.1. Anal. Calcd for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.07; H, 7.47.

3.1.7. Formation of mixed acetal (10a). A mixture of compounds **5a** (156 mg, 0.24 mmol) and **9a** (81 mg, 0.31 mmol) and molecular sieves 4 A (0.25 g) in CH_2Cl_2 (5 ml) was stirred at ice-water temperature. To the mixture was added DDQ (68 mg, 0.30 mmol) with a positive flush of nitrogen and stirring continued at 0°C for 5 min and at room temperature for 130 min. The reaction was quenched with an aqueous solution of ascorbic acid (0.7%)–citric acid (1.3%)–NaOH (0.9%) (10 ml). The resulting lemon-yellow mixture was diluted with AcOEt and filtered through Celite. The filtrate was washed successively with aqueous $NaHCO_3$ and brine, and combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by size exclusion chromatography with Bio Beads S-X3 (toluene) to afford 220 mg (quantitative) of compound **10a**; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.85–7.65 (4 H, m, Ar), 7.50–7.10 (8 H, m, Ar), 6.90 (2 H, m, J=8.6 Hz, Ar), 5.85 (1 H, s, acetal CH), 5.44 (1 H, d, J=1.0 Hz, H-1), 3.84 (3 H, s, OMe), 3.67 and 3.52 (each 1 H, d, J=8.6 Hz, H-1_{Fru}), 3.13 (1 H, d, J=6.6 Hz, H-3_{Fru}), 1.95 (3 H, s, SMe), 1.42, 1.37, 1.33 and 1.31 (each 3 H, s, isopropylidene), 1.07 (9 H, s, *t*-Bu); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 160.2, 136.3, 136.1, 134.3, 134.1, 130.5, 129.4, 129.0, 128.9, 127.6, 127.2, 125.3, 113.4, 111.8, 109.0, 104.8, 104.1, 99.8, 87.0, 80.4, 78.1, 73.7, 71.4 (CH_2), 71.3, 70.3, 65.8, 61.5 (CH_2), 60.0 (CH_2), 55.3, 37.8 (CH_2), 28.0, 27.6 (CH_2), 27.0, 26.7, 26.4, 26.1 (CH_2), 25.6, 22.5, 22.4, 21.4 (Me_3C), 19.6, 13.4 (SMe).

3.2. O-(4,6-O-Cyclohexylidene-2-O-*p*-methoxybenzyl-3-O-*tert*-butyldiphenylsilyl-β-D-mannopyranosyl)-(1→3)-1,2;4,5-di-O-isopropylidene-α-D-fructopyranose (13)

3.2.1. Preparative reaction. A mixture of mixed acetal **10a** (70.5 mg, 0.0777 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (55 mg, 0.27 mmol) and molecular sieves 4 A (0.2 g) in 1,2-dichloroethane (7 ml) was stirred at 0°C. A solution of methyl trifluoromethanesulfonate (1 M in CCl_4, 0.27 ml,

0.27 mmol) was added and stirring continued at 35°C. After 18 h, the mixture was diluted with AcOEt–ice water, quenched with Et_3N (~50 μl), and filtered through Celite. The filtrate was washed with aqueous $NaHCO_3$ and brine, successively, dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed over silica gel (hexane–AcOEt 3:1) to afford 43.3 mg (76%) of the title compound; $[\alpha]_D$=−46.7 (c 1.02, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.7–7.8 (4 H, m, Ar), 7.3–7.5 (6 H, m, Ar), 4.36 (1 H, s, H-1_{Man}), 4.34 (1 H, t, *J*=6 Hz, H-4_{Fru}), 4.19 (1 H, dd, *J*=6.3 and 1.9 Hz, H-5_{Fru}), 4.06 (1 H, dd, *J*=13.2 and 2.2 Hz, H-6_{Fru}), 3.70 (1 H, d, *J*=6.4 Hz, H-3_{Fru}), 3.58 (1 H, d, *J*=2.9 Hz, H-2_{Man}), 3.03 (1 H, ddd, *J*=10.0, 10.0 and 5.4 Hz, H-5_{Man}), 1.45, 1.41, 1.30 and 1.24 (each 3 H, s, isopropylidene), 1.09 (9 H, s, *t*-Bu); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 136.1, 135.8, 133.7, 132.9, 129.8, 129.6, 127.6, 127.3, 111.2, 109.1, 103.6, 101.1 (C-1_{Man}, $^1J_{C-H}$=160 Hz), 99.9, 76.4, 74.9, 73.3, 73.1, 72.6 (CH_2), 71.0, 69.7, 68.3, 61.3 (CH_2), 61.2 (CH_2), 38.0 (CH_2), 27.8 (CH_2), 27.3, 26.9 (*Me*$_3$C), 26.4, 26.2, 25.7, 25.6 (CH_2), 22.6 (CH_2), 22.4 (CH_2), 19.3 (Me$_3$*C*). Anal. Calcd for $C_{40}H_{56}O_{11}Si$: C, 64.84; H, 7.62. Found: C, 64.64; H, 7.64.

3.2.2. NMR experiment. Mixed acetal **10a** (10.8 mg, 0.012 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (9 mg, 0.04 mmol) was dissolved in $CDCl_3$ (ca. 0.6 ml, contains TMS) and transferred to an NMR tube. Pre-activated molecular sieves 4 A (20 mg) were added followed by MeOTf (1 M in CCl_4, 40 μl, 0.04 mmol). The mixture was shaken for 10 min and left at 35°C. The progress of the reaction was followed by ^{1}H-NMR (JEOL EX-270, Figs. 1 and 2).

3.2.3. From 5b and 9b: via diastereomeric mixed acetal 10b. A mixture of DDQ (39 mg, 0.17 mmol) and molecular sieves 4 A (0.15 g) in CH_2Cl_2 (1 ml) was stirred under ice-water cooling. Compounds **5b** (60.3 mg, 0.114 mmol) and **9b** (58.0 mg, 0.152 mmol) were added as a solution in CH_2Cl_2 (1.5 ml) and the whole was stirred at room temperature for 70 min. The mixture was worked up as described for **10a** and purified by a column of Bio Beads S-X3 (toluene) to afford the mixed acetal (74.0 mg, 71%), which consists of **10b** as the major diastereomer (~10:1). The mixed acetal (74.0 mg, 0.0816 mmol) was dissolved in $CDCl_3$ (0.37 ml), 50 μl of which was transferred to an NMR tube and diluted with $CDCl_3$ into ca. 0.6 ml. The solution was treated with MeOTf (1 M, 40 μl, 0.04 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (9 mg, 0.04 mmol) at 35°C (Fig. 3). The rest of the mixed acetal was subjected to IAD (35°C, 24 h) in a standard manner using MeOTf (1 M, 0.26 ml, 0.26 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (59 mg, 0.29 mmol). After 24 h, both mixtures were combined and worked up as described above. Successive purification by Bio Beads SX-3 (toluene) and preparative TLC (hexane–AcOEt 2:1) afforded 19.1 mg (32 %) of compound **13**.

3.3. *p*-Methoxyphenyl O-(4,6-O-cyclohexylidene-2-O-*p*-methoxybenzyl-3-O-*tert*-butyldiphenylsilyl-β-D-manno-pyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthal-imido-β-D-glucopyranoside (7)

3.3.1. Under anhydrous conditions. To an ice-water cold mixture of DDQ (48 mg, 0.21 mmol) and molecular sieves 4 A (0.2 g) in CH_2Cl_2 (1 ml) was added a solution of compounds **5a** (120.8 mg, 0.186 mmol) and **6** (81.6 mg, 0.137 mmol) as a solution in CH_2Cl_2 (3 ml in total). The mixture was stirred at 0°C (15 min) and at room temperature for 130 min and worked up as described for **10a**. The organic layer was dried over $MgSO_4$, evaporated in vacuo and exposed to high vacuum for 2 h to afford crudely mixed acetal **23**, which was used directly for IAD. To a flask containing molecular sieves 4 A (0.4 g), was added a mixture of **23** and 2,6-di-*tert*-butyl-4-methylpyridine (98 mg, 0.48 mmol) as a solution in $ClCH_2CH_2Cl$ (8 ml in total) and then cooled to ice-water temperature. A solution of MeOTf in CCl_4 (1 M, 0.48 ml, 0.48 mmol) was added and the mixture was stirred at 0°C to room temperature for 1 h and at 45°C for 20 h. The reaction was quenched under ice-water cooling with Et_3N (~100 μl) and diluted with AcOEt. Aqueous $NaHCO_3$ was added and the two-phase mixture was filtered through Celite. The filtrate was washed with water and brine, successively, dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed over silica gel (hexane–AcOEt 5:2) to afford 122.0 mg (83%) of the title compound; $[\alpha]_D$=+40.7 (c 1.8, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.9–6.6 (28 H, Ar), 5.55 (1 H, d, *J*=8.3 Hz, H-1_{GN}), 4.82, 4.56, 4.45 and 4.25 (each 1 H, d, *J*=12 Hz, benzyl), 4.36 (1 H, s, H-1_{Man}), 4.03 (1 H, m), 4.35 (1 H, d, *J*=3.9 Hz, H-2_{Man}), 3.98 (1 H, t, *J*=9.3 Hz), 3.76 (1 H, dd, *J*=10.7, 5.4 Hz), 3.68 (3 H, s, OMe), 3.68 (1 H, m), 3.64–3.52 (3 H, m), 3.43–3.38 (2 H, m), 2.87 (1 H, dd, *J*=10, 10, 5 Hz, H-5_{Man}), 2.61 (1 H, br, OH), 2.0–1.3 (10 H, m, cyclohexyl), 1.11 (9 H, s, *t*-Bu); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 100.2 (cyclohexylidene), 99.8 ($^1J_{CH}$=159 Hz), 97.7 ($^1J_{CH}$=165 Hz), 78.7, 74.6, 73.4 (CH_2), 73.1, 71.3, 69.8, 67.9 (CH_2), 67.7, 61.2 (CH_2), 60.3 (CH_2), 55.5, 37.9 (CH_2), 27.7 (CH_2), 26.8 (*Me*$_3$C), 25.6 (CH_2), 22.6 (CH_2), 22.3 (CH_2), 19.2 (Me$_3$*C*). Anal. Calcd for $C_{63}H_{69}NO_{13}Si$: C, 70.30; H, 6.46; N, 1.30. Found: C, 70.06; H, 6.54; N, 1.31.

3.3.2. In the presence of water. Mixed acetal **23** was prepared from 29.6 mg (0.046 mmol) of **5a** and 20.5 mg (0.034 mmol) of **6** using 12 mg (0.053 mmol) of DDQ and molecular sieves 4 A (0.15 g) as described above and dissolved in $ClCH_2CH_2Cl$ (3 ml) containing 2,6-di-*tert*-butyl-4-methylpyridine (24 mg, 0.12 mmol). MeOTf (0.12 mmol) and H_2O (1.0 μl, 0.06 mmol) was added and the mixture was stirred at 45°C for 20 h. Subsequent work-up and purification by silica gel column chromatography afforded 20.1 mg (54%) of compound **7** together with 6.8 mg (33%) of recovered **6**.

The same reaction was performed using 47.9 mg (0.074 mmol) of **5a**, 32.3 mg (0.054 mmol) of **6**, 19 mg (0.084 mmol) of DDQ, 0.19 mmol of MeOTf and 39 mg (0.19 mmol) of 2,6-di-*tert*-butyl-4-methylpyridine in the presence of 10 equiv. (10 μl, 0.55 mmol) of H_2O to give 9.8 mg (17%) of compound **7**.

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16. Attempted quenching of **12** by performing IAD in the presence of an excess amount (7 equiv.) of triethylsilane was not successful in giving the regular product **13** (60% yield).