Bioorganic & Medicinal Chemistry 22 (2014) 2090–2101

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Expanded potential of seleno-carbohydrates as a molecular tool for X-ray structural determination of a carbohydrate–protein complex with single/multi-wavelength anomalous dispersion phasing

Tatsuya Suzuki [a,b,†], Hisayoshi Makyio [c,†], Hiromune Ando [a,b,*], Naoko Komura [a,b], Masanori Menjo [a], Yusuke Yamada [c], Akihiro Imamura [a], Hideharu Ishida [a], Soichi Wakatsuki [c,d,e], Ryuichi Kato [c,*], Makoto Kiso [a,b]

[a] Department of Applied Bioorganic Chemistry, Gifu University, 1-1 Yanagido, Gifu-shi, Gifu 501-1193, Japan
[b] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida Ushinomiya-cho, Sakyo-ku, Kyoto 606-8501, Japan
[c] Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK), 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan
[d] Photon Science, SLAC Natl. Accelerator Laboratory Structure Science, 2575 Sand Hill Road, MS 69, Menlo Park, CA 94025-7015, USA
[e] Department of Structural Biology, Stanford University, Beckman Center B105, 279 Campus Drive, Stanford, CA 94305-5126, USA

ARTICLE INFO

Article history:
Received 4 February 2014
Accepted 18 February 2014
Available online 25 February 2014

Keywords:
Seleno-carbohydrate
Carbohydrate–protein complex
X-ray crystallography
SAD/MAD phasing
Lectin

ABSTRACT

Seleno-lactoses have been successfully synthesized as candidates for mimicking carbohydrate ligands for human galectin-9 N-terminal carbohydrate recognition domain (NCRD). Selenium was introduced into the mono- or di-saccharides using *p*-methylselenobenzoic anhydride (Tol_2Se) as a novel selenating reagent. The TolSe-substituted monosaccharides were converted into selenoglycosyl donors or acceptors, which were reacted with coupling partners to afford seleno-lactoses. The seleno-lactoses were converted to the target compounds. The structure of human galectin-9 NCRD co-crystallized with 6-MeSe-lactose was determined with single/multi-wavelength anomalous dispersion (SAD/MAD) phasing and was similar to that of the co-crystal with natural lactose.

1. Introduction

Since Wrede first reported the synthesis of selenium-containing carbohydrates (seleno-carbohydrates),[1] various seleno-carbohydrates have been synthesized by a number of research groups.[2] With the exception of seleno-glycoside synthetic intermediates[2a-j] and selenylsulfide-linked glycoproteins found as metabolites in the liver or urine and recently synthesized by Davis and co-workers,[3] the seleno-carbohydrates synthesized to date are unnatural. Seleno-carbohydrates are expected to function as useful mimics of biologically significant carbohydrates because of the inherent chemical and physical properties of selenium. However, their use in biological studies has been limited since the pioneering work on the inhibition of glycosidase published by Pinto's research group.[4]

Because selenium has a spin of 1/2 (^{77}Se) and exhibits anomalous dispersion characteristics in response to X-ray irradiation, selenium-containing molecules are likely to have great potential for structural analysis of biomolecules by NMR[5] and X-ray spectroscopy. In particular, the anomalous dispersion characteristics have been successfully used in single-wavelength anomalous dispersion (SAD) and multi-wavelength anomalous dispersion (MAD) phasing methods for X-ray crystallography. In protein X-ray crystallography, methionine is replaced with selenomethionine (SeMet) by a recombinant technique, which allows the structural analysis of the SeMet-labeled proteins by the SAD/MAD phasing method. This technique only requires crystals of SeMet-labeled protein for structural determination whilst the conventional method such as multiple isomorphous replacement (MIR) requires more crystals, which are native and heavy atom (Hg etc.)-soaked crystals. Therefore, SAD/MAD methods are widely used and have allowed a dramatic increase in the number of protein structures solved.[6] However, the production of the sufficient quantities of SeMet-labeled proteins for X-ray crystallography remains a challenge, mainly due to the low expression level of the labeled proteins and the high production costs in insect or mammalian cell expression systems. Structural changes or destabilization of

* Corresponding authors. Tel.: +81 58 239 3452; fax: +81 293 3452 (H.A.).
E-mail address: hando@gifu-u.ac.jp (H. Ando).
† Both authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bmc.2014.02.023
0968-0896/

proteins caused by SeMet labeling also limits the application of SAD/MAD.

However, if seleno-carbohydrates serve as a mimic of a ligand for the protein in carbohydrate–protein complexes, the three dimensional structure of the carbohydrate–protein complex could be determined by the SAD/MAD phasing method without a SeMet-labeled protein. Recently, it was demonstrated SAD/MAD phasing using methyl seleno-glycosides of monosaccharides as ligand mimetics.[7] However, further investigation using more than seleno-monosaccharide has not been carried out, probably because of the synthetic difficulties in the seleno-derivatization of carbohydrates. Determining high resolution three-dimensional structures of carbohydrate–protein complexes is invaluable for understanding biological processes that are mediated through carbohydrate-protein interactions on the cell membrane, including cell adhesion, cell proliferation, cell differentiation, and pathogen-host cell interactions. It is also useful for developing drugs based on the biological functions of carbohydrates. Therefore, in this study, to complete the proof-of-concept demonstration of SAD/MAD phasing with seleno-carbohydrates, we synthesized various seleno-lactose derivatives as lactose mimics through a facile and mild selenation method developed by our research group, co-crystallized the seleno-lactose derivatives with a carbohydrate-binding protein and determined the protein structure by using the SAD/MAD phasing method.

2. Results and discussion

2.1. Synthesis of seleno-lactoses

Of the possible seleno-lactose analogues, seleno-glycoside analogues **1** and **2**, and methylseleno (MeSe)-substituted analogues **3** and **4** were selected as lactose mimics (Fig. 1). Based on our X-ray crystallography results for a synthesized glycan–protein complex,[8] the 2-(trimethylsilyl)ethyl group was chosen as the aglycon of seleno-lactoses **2**–**4**. X-ray analysis of these seleno-lactoses co-crystallized with a lactose-binding protein will provide information about whether the binding site allows differences in the van der Waals radius (O 1.52 Å, Se 1.90 Å) and the angle of the glycosidic bond (C—O—C 112°, C—Se—C 96°) between native lactose and seleno-lactoses.

We have previously reported a facile, mild method for the synthesis of seleno-glycosides using potassium *p*-methylselenobenzoate **5** as the key selenation reagent (Scheme 1).[9] Afterward, we noticed that this reagent was frequently contaminated with byproducts, such as diselenide **6**, which were produced during its preparation. Because of its reactivity, complete purification of **5** is not possible at large scales. Impurities in **5** produced several inseparable byproducts during selenation. To solve this problem, we envisioned the use of *p*-methylselenobenzoic anhydride (Tol_2Se) **7**[10] as the synthetic equivalent of the acyl selenolate anion, in light of the known stability of **7**. It was anticipated that reactive anhydride **7** would readily react with a nucleophile to generate a highly reactive acyl selenolate anion in situ, which would subsequently react with the sugar electrophile to produce a seleno-carbohydrate. According to the modified protocol reported by Ishihara et al.,[10] Tol_2Se (**7**) could be obtained on a large scale as pure crystals (Scheme 2).

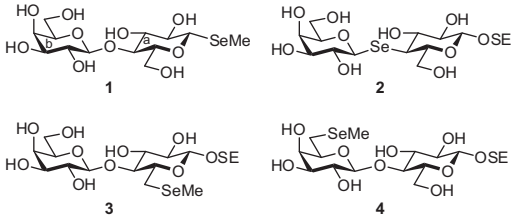
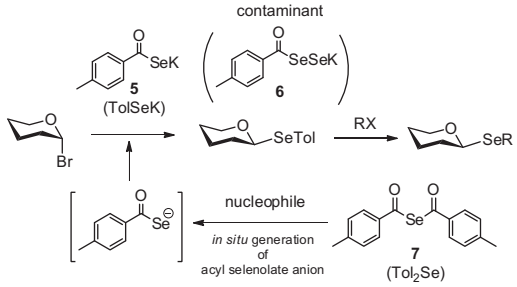


Figure 1. Structures of seleno-lactoses synthesized in this study. SE = 2-(trimethylsilyl)ethyl.

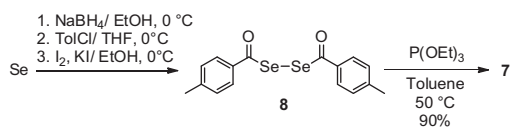


Scheme 1. Reported method for the introduction of selenium into a carbohydrate with TolSeK **5** and the strategy for using Tol_2Se **7** as a $TolSe^-$ equivalent. Tol = *p*-methylbenzoyl.

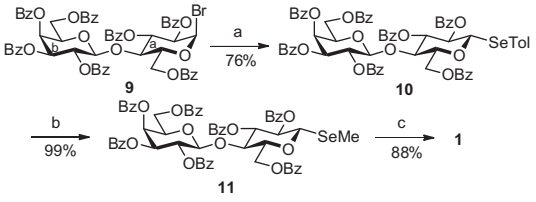
Screening identified piperidine (1.0 equiv) and *N*,*N*-diisopropylethylamine (DIEA; 1.0 equiv) as the conditions that activated Tol_2Se (**7**) (1.0 equiv) most effectively to afford the toluylselenylated sugar. For this system, morpholine could also be used as a nucleophile. However, when DIEA was replaced with an inorganic base such as Cs_2CO_3, the reaction yield decreased considerably. Presumably, this is because of the instability of the cesium acyl selenolate generated from **7**. This agrees well with the recently reported result that trialkylamines such as DIEA can form a more stable salt with acylselenolate anions than metals can.[11] This method was first used in the synthesis of 1-methylseleno-lactoside **1** (Scheme 3). Therefore, the anomeric center of 1-bromo-lactose derivative **9**[12] underwent substitution with a toluylselenyl (TolSe) group by reaction with Tol_2Se (**7**) in the presence of piperidine and DIEA in DMF, giving 1-seleno-lactoside **10** in 76% yield. Next, the TolSe group in compound **10** was converted into a methylselenyl (MeSe) group via the in situ reaction of the glycosyl selenolate anion with MeI in the presence of Cs_2CO_3, following a previously reported method,[9] to afford compound **11** in high yield. Full deprotection of **11** under Zemplén conditions delivered 1-methylseleno-lactoside **1**.

Scheme 4 summarizes the synthesis of seleno-lactose **2**. To construct the intra-residual seleno-glycoside in target compound **2**, toluylseleno-galactoside **13** was prepared by the reaction of 1-bromo-galactose tetraacetate **12** with **7**. Then, seleno-glycoside **15** was synthesized by the previously reported reaction of compounds **13** and **14**.[9] Compound **15** was then de-O-acetylated to give **2**.

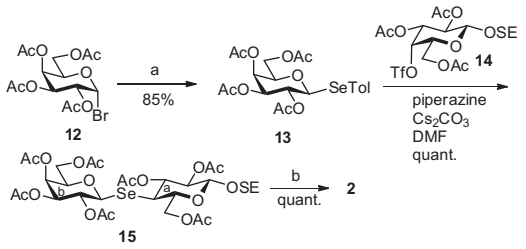
Taking advantage of the facile, mild toluylselenation with Tol_2Se (**7**), 6-methylseleno-glucosyl acceptor **23**, which is the key intermediate for the synthesis of target compound **3**, could also be derived from known 4,6-benzylidene-glucoside **16** (Scheme 5).[13] To boost the reactivity of the C-4 hydroxyl group for glycosylation, the 2,3-diol in **16** was converted into *p*-methoxybenzyl ethers, giving compound **17**. Following acid hydrolysis of the benzylidene acetal of **17**, the C-6 hydroxyl group was selectively substituted with bromine by treatment with CBr_4 and Ph_3P to afford compound **19**. Next, the substitution reaction of 6-bromo-glucose **19**



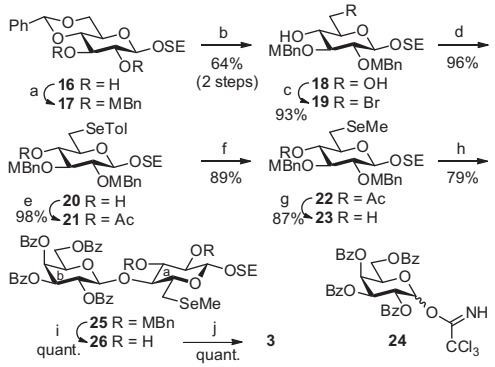
Scheme 2. Preparation of Tol_2Se **7**.



Scheme 3. Synthesis of seleno-lactose **1**. Reagents and conditions: (a) **7**, piperidine, DIEA/DMF, rt, 76%; (b) MeI, $MeNHNH_2$, Cs_2CO_3/DMF, rt, 99%; (c) NaOMe, MeOH/THF (2:1), rt, 88%. Bz = benzoyl, DIEA = *N*,*N*-diisopropylethylamine.



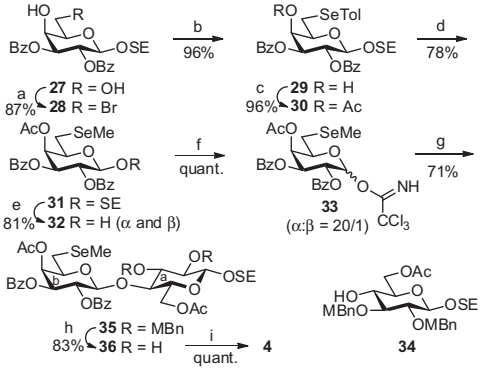
Scheme 4. Synthesis of seleno-lactose **2**. Reagents and conditions: (a) **7**, piperidine, DIEA/DMF, rt, 85%; (b) NaOMe, MeOH/THF (2:1), rt, quant.



Scheme 5. Synthesis of seleno-lactose **3**. Reagents and conditions: (a) *p*-methoxybenzyl chloride, NaH/DMF, rt; (b) TFA/CH_2Cl_2, −20 °C, 64% (2 steps); (c) CBr_4, PPh_3/Pyr, 65 °C, 93%; (d) **7**, piperidine, DIEA/DMF, 60 °C, 96%; (e) Ac_2O/Pyr, rt, 98%; (f) MeI, $MeNHNH_2$, Cs_2CO_3/DMF, rt, 89%; (g) NaOMe/MeOH, rt, 87%; (h) **24**, TMSOTf, 4 Å MS/CH_2Cl_2, 0 °C, 79%; (i) TFA/CH_2Cl_2, −20 °C, quant.; (j) NaOMe, MeOH/THF (2:1), sonication, quant. MBn = *p*-methoxybenzyl, TMSOTf = trimethylsilyl trifluoromethanesulfonate, MS = molecular sieves, TFA = trifluoroacetic acid.

with Tol_2Se (**7**) in the presence of piperidine and DIEA at 70 °C in DMF afforded toluylseleno-glucose **20** in 96% yield. Acetylation of the C-4 hydroxyl group in **20**, followed by conversion of the TolSe group into a MeSe group gave **22** in 89% yield. The acetyl group was deprotected, producing **23** in high yield (75% from **20**). The 6-MeSe-glucosyl acceptor was subjected to glycosylation with galactosyl imidate donor **24**.[14] Equimolar amounts of **23** and **24** were reacted in CH_2Cl_2 in the presence of TMSOTf as a catalyst.[15] When this reaction was performed at 0 °C, the best yield of disaccharide **25** was 79%. The MBn protecting groups were quantitatively removed from the hydroxyl groups with trifluoroacetic acid in CH_2Cl_2 at −20 °C without affecting the MeSe group. Compound **3** was obtained by subsequent de-*O*-benzoylation.

The synthesis of 6′-methylseleno-lactose **4** was achieved by the glycosylation of the C-4 hydroxyl group of a glycosyl acceptor with the 6-MeSe-galactosyl donor (Scheme 6). The 4,6-diol derivative of galactoside **27**[16] was converted into 6-MeSe derivative **31** in relatively high yields via a similar route to that for compound **20** (63% over 4 steps). Acidic treatment of compound **31** afforded



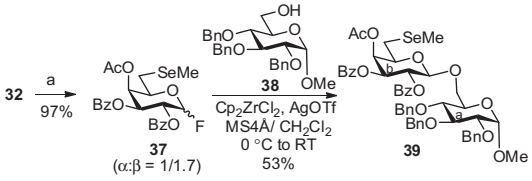
Scheme 6. Synthesis of seleno-lactose **4**. Reagents and conditions: (a) CBr_4, PPh_3/Pyr, 65 °C, 87%; (b) **7**, piperidine, DIEA/DMF, 100 °C, 96%; (c) Ac_2O/Pyr, rt, 96%; (d) MeI, $MeNHNH_2$, Cs_2CO_3/DMF, rt, 78%; (e) TFA/CH_2Cl_2, 0 °C, 81%; (f) CCl_3CN, DBU/CH_2Cl_2, 0 °C, quant.; (g) **34**, TMSOTf, 4 Å MS/CH_2Cl_2, −40 °C, 71%; (h) TFA/CH_2Cl_2, −20 °C, 83%; (i) NaOMe, MeOH/THF (2:1), sonication, quant.

hemiacetal **32**, which was functionalized as a glycosyl imidate donor to give **33**. Based on the synthesis of **3**, the 2,3-di-MBn derivative of glucoside **34** was prepared from compound **17** as a coupling partner for **33**.[17] Examination of the glycosylation of **34** with **33** promoted by TMSOTf revealed that −40 °C was the optimal reaction temperature, although it took a longer time to reach completion (51 h). This reaction produced disaccharide **35** in 71% yield.

We also examined the glycosidation of fluoride donor **37** by using a common promoter system, Cp_2ZrCl_2 (2.5 equiv) and AgOTf (5.0 equiv)[18] (Scheme 7). Even with an acid-resistant, highly nucleophilic glycosyl acceptor **38**, the glycosidation reaction only began to proceed at room temperature and took a long time (24 h), providing glycosylated product **39** in 53% yield. Because these reaction conditions probably affect the MBn groups of glycosyl acceptor **33**, fluoride **37** was not used for the synthesis of **35**. Using the procedure for the full deprotection of **25** on seleno-lactose derivative **35** produced target compound **4** in high yield.

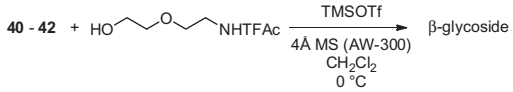
The glycosidations of glycosyl donors **33** and **37** indicated that the MeSe group at the C-6 position may affect the reactivity of the compounds. To confirm this, glycosylation reactions using lactosyl imidate donors **40**,[19] **41**, and **42** were performed.[20] All glycosylations were carried out in CH_2Cl_2 at 0 °C in the presence of a catalytic amount of TMSOTf. The results clearly indicate that the reactivity of 6-MeSe-lactosyl donor **41** was substantially lower than that of **40** and **42** (Table 1). Thus, lactosyl donor **41** required four-fold the equivalents of TMSOTf and around eleven-fold longer than 6′-MeSe-lactosyl donor **42** to complete the glycosylation. Comparing entries 1 and 3 indicates that the MeSe group reduced the reaction rate, probably because of the coordination of the highly nucleophilic selenium and the Lewis acid.

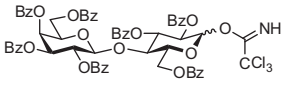
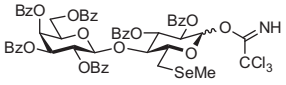
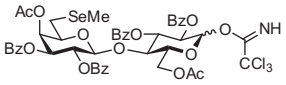
In the case of 6-MeSe-lactosyl donor **41**, the coordination may induce a Se···O interaction,[21] which renders the glycosyl donor much less reactive. A similar deactivation may have occurred during the glycosidation of donors **33** and **37** (Scheme 8). Although the



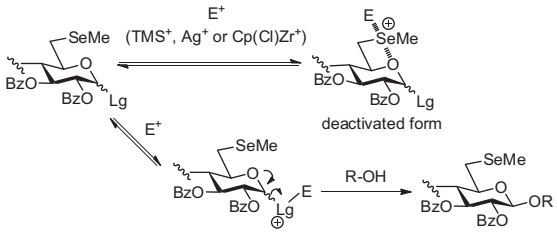
Scheme 7. Glycosidation of 6-MeSe-galactosyl fluoride donor **37**. Reagents and conditions: (a) DAST/CH_2Cl_2, 0 °C, 97%. DAST = diethylaminosulfur trifluoride.

Table 1
Comparison of the glycosylations using lactosyl **40**, 6-MeSe-lactosyl **41**, and 6′-MeSe-lactsoyl **42** imidates



Entry	Donor	Equiv of TMSOTf	Time	Product (% yield)
1	 **40** (α:β= 20/1)	0.1	30 min	**43** (97)
2	 **41** (α:β= 20/1)	0.4	8 h	**44** (66)
3	 **42** (α:β= 20/1)	0.1	45 min	**45** (90)

TFAc = trifluoroacetyl.



Scheme 8. Proposed mechanism for the diminished reactivity of the 6-MeSe-glycosyl donor based on the results in this study. Lg = leaving group.

presence of the MeSe group affected the reactivity of the glycosyl donor, seleno-lactoses **1** to **4** were obtained.

2.2. Structure of human galectin-9 N-terminal carbohydrate recognition domain with seleno-lactose

We attempted to use seleno-lactoses **1** to **4** to solve the structure of human galectin-9 N-terminal carbohydrate recognition domain (NCRD).[22] Some crystal structures of human galectin-9 NCRD with various carbohydrates, including lactose, have been solved by X-ray crystallography.[23] First, we attempted to co-crystallize human galectin-9 NCRD with seleno-lactoses **1** to **4**. Crystals of human galectin-9 NCRD with seleno-lactoses **1**–**3** were obtained under different conditions; however, seleno-lactose **4** crystals were not obtained. Although anomalous selenium signals were observed from the crystals of human galectin-9 NCRD with seleno-lactoses **1** to **3** in the X-ray absorption fine structure (XAFS) experiments, we could not obtain crystals with seleno-lactoses **1** and **2** with sufficient quality for phase determination. In the seleno-lactose **1** crystal, not all the selenium positions could be correctly identified in the asymmetric unit. The affinity of seleno-lactose **1** for human galectin-9 NCRD may be slightly weaker than that of the other seleno-lactoses. It was difficult for the phase determination and model building of human galectin-9 NCRD with seleno-lactose **2** because of the slight low resolution (2.7–2.8 Å). However, the structure of human galectin-9 co-crystallized with 6-MeSe-lactose **3** was successfully determined at a higher resolution (1.4 Å).

Good anomalous signals were obtained from all the crystals by XAFS before the diffraction data were collected. The structure of human galectin-9 NCRD with 6-MeSe-lactose **3** was solved by the SAD method and also by the MAD method (data not shown). In this case, the SAD method was sufficient to determine the structure of galectin-9 with 6-MeSe-lactose **3**. Therefore, the structural details described hereafter are from the SAD data.

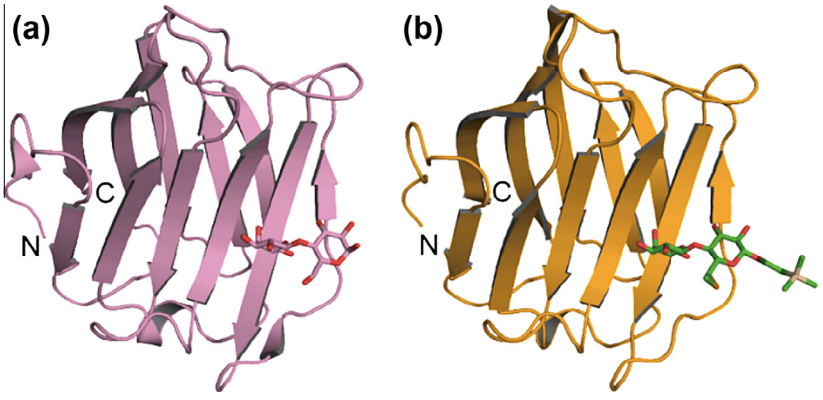


Figure 2. Comparison of overall structure of human galectin-9 NCRD with α-lactose and seleno-lactose **3**. (a) Cartoon representation of human galectin-9 NCRD with α-lactose (PDB_id: 2EAK), shown in pink. α-Lactose is represented as a stick structure. (b) Cartoon representation of human galectin-9 NCRD with 6-MeSe-lactose **3**, shown in orange. Carbon atoms in 6-MeSe-lactose **3** are shown in green in the stick structure (PDB id: 3WLU).

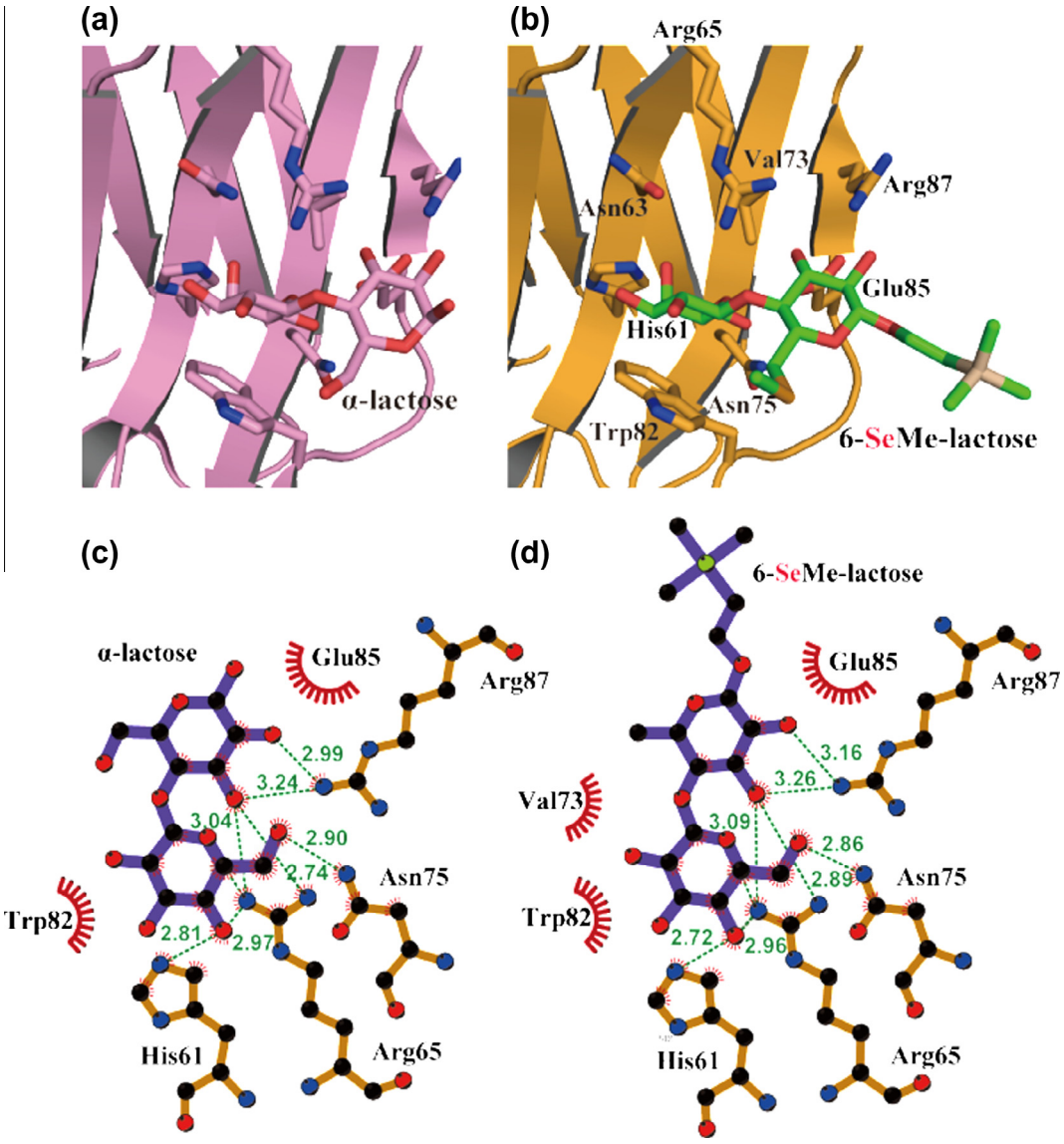


Figure 3. Comparison of the binding site of human galectin-9 NCRD with 6-MeSe-lactose **3** and α-lactose. (a) Cartoon representation of the α-lactose binding site of human galectin-9 NCRD, shown in pink. α-Lactose and residues that interacted with α-lactose are shown as stick structures. (b) Cartoon representation of 6-MeSe-lactose binding site of human galectin-9 NCRD. 6-MeSe-lactose and residues that interact with 6-MeSe-lactose are shown as stick structures. The colors of galectin-9 and 6-MeSe-lactose are the same as in Figure 2. (c) Schematic diagram of α-lactose created in Ligplot. Green dotted lines indicate hydrogen bonds between α-lactose and the galectin-9 NCRD residues. Lengths of the hydrogen bonds are shown in green (Å). (d) Schematic diagram of 6-MeSe-lactose created in Ligplot.

Four molecules of galectin-9 with 6-MeSe-lactose **3** are present in an asymmetric unit. There are no significant differences between the four galectin-9 structures with 6-MeSe-lactose **3** in the asymmetric unit. The RMSD values obtained by secondary structure matching were between 0.303 and 0.548 Å. The positions of the anomalous signals at high occupancies were consistent with the positions of the selenium atoms on 6-MeSe-lactose **3** bound to human galectin-9 NCRD. The selenium atom in seleno-lactose **3** has multiple conformations in the binding forms. The details of the structure determination are described in the experimental section and the refinement statistics are presented in the Supplementary Material.

The overall structure of human galectin-9 NCRD with 6-MeSe-lactose **3** (PDB_ID: 3WLU) is almost same as the structure of human galectin-9 NCRD with α-lactose (PDB_ID: 2EAK) (Fig. 2). 6-MeSe-lactose **3** is located on the same α-lactose binding site in the structure of human galectin-9 NCRD. The difference in RMSD values between the structures obtained by secondary structure matching is less than 0.617 Å. There are no major distortions of the residues on the ligand binding site of human galectin-9 NCRD between 6-MeSe-lactose **3** and the α-lactose binding forms (Fig. 3). The same residues form the hydrogen bonds to the sugar chain backbone in both structures. The relative positions of the protein to the ligand are very similar in both structures.

3. Conclusions

We have shown that Tol_2Se (**7**) functions as a synthetic equivalent of the toluylselenolate anion, which enabled the efficient incorporation of selenium at the electrophilic site of carbohydrate derivatives. The considerable advantage of selenation with **7** over the widely used method with RSeSeR and $NaBH_4$ is the compatibility with ester groups. Although the 6-MeSe glycosyl donors showed moderate reactivity in the coupling reaction, the compatibility of the seleno-carbohydrate units with conventional chemistry for carbohydrate synthesis allowed four types of seleno-lactose (**1**–**4**) to be synthesized.

Our case study of the structural determination of a carbohydrate–protein complex has extended the potential of seleno-carbohydrate as molecular tool for SAD/MAD phasing. Because the proteins do not need to be modified with SeMet, this phasing method does not interfere with protein production and does not alter the structural and chemical properties of the protein. Furthermore, the correct ligand configuration can be confirmed by detecting the anomalous signal from the selenium atoms incorporated in the seleno-carbohydrates, even if the collected data is diffracted at low resolution. This might be useful for drug design based on carbohydrate-protein recognition. However, since the proper positioning of selenium atom in the carbohydrate residue cannot be predicted in every case of co-crystallization with protein, the creation of a library of seleno-carbohydrates is necessary for analyzing the structures of unknown carbohydrate-binding proteins. Therefore, we are currently assembling a diverse collection of seleno-carbohydrates by using the facile selenation method developed in this study.

4. Experimental section

4.1. General procedures

All reactions were performed in round-bottom flask fitted with balloon filled with argon, otherwise specified. Transfer of air and moisture sensitive liquids were performed via cannula under a positive pressure of argon. When necessary, reaction mixtures were sonicated with AS ONE US CLEANER USD-4R by following the reported procedure.[24] TLC analysis was performed on Merck TLC (silica gel $60F_{254}$ on glass plate). Compounds were visualized by exposure to UV light (254 nm) or by spraying either with H_2SO_4 solution in EtOH (10%) or with Ninhydrine Spray which was purchased from Wako Pure Chemical Industries Ltd, followed by heating. Flash column chromatography on silica gel (Fuji Silysia, 80 mesh and 300 mesh) or size exclusion chromatography on Sephadex (Pharmacia LH-20) was performed with the solvent systems (v/v) specified. Quantity of silica gel was usually estimated as 100 to 150-fold weight of sample to be charged. Dichloromethane, *N*,*N*-dimethylformamide (DMF), methanol, tetrahydrofuran (THF) and toluene as reaction media were purchased from Wako Pure Chemical Industries Ltd, dried over 3 Å or 4 Å molecular sieves and used without purification. When necessary, solvents were degassed prior to use by sonication under reduced presseure for 20 min, followed by bubbling argon through the solvents for 30 min. Molecular sieves were purchased from Wako Chemical Inc. and dried at 300 °C for 2 h in muffle prior to use. ^{1}H, ^{13}C and ^{77}Se NMR spectra were recorded with JEOL JNM-ECA400, JNM-ECA500, JNM-ECA600 and Bruker Avance III 500 spectrometers. ^{1}H NMR chemical shifts are expressed in ppm (δ) relative to the signal of Me_4Si as an internal standard. ^{13}C NMR chemical shifts are expressed in ppm (δ) relative to the signal of the solvent as a standard. ^{77}Se NMR chemical shifts are expressed in ppm (δ) relative to the external standard. High-resolution mass spectrometry (HRMS) was performed with a Bruker Daltonics micrOTOF (ESI-TOF) mass spectrometer. Specific rotations were measured with a Horiba SEPA-300 high-sensitivity polarimeter.

4.1.1. *p*-Methylselenobenzoic anhydride (7)

Triethylphosphite (8.7 mL, 50 mmol) was added dropwise to a solution of **8** (19.9 g, 50 mmol) in toluene (250 mL) at rt. The mixture was stirred for 2 h at rt (completion of the reaction was confirmed by TLC analysis; CH_2Cl_2/hexane, 1:2), then the reaction mixture was evaporated. The residue was dissolved in Et_2O and filtered through Celite. Combined filtrate and washings were directly crystallized from Et_2O/hexane to give **7** (14.3 g, 90%). Spectroscopic data (^{1}H, ^{13}C, ^{77}Se NMR) of compound **7** were identical to those reported;[10] HRMS: *m*/*z* $C_{16}H_{14}O_2SeNa^+$: 341.0051 $[M+Na]^+$; found: 341.0051.

4.1.2. *p*-Methylbenzoyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-seleno-β-D-glucopyranoside (10)

Compound **9** (298 mg, 264 μmol) in degassed DMF (2.7 mL) was added dropwise to a solution of **7** (101 mg, 318 μmol), *N*,*N*-diisopropylethylamine (48 μL, 279 μmol) and piperidine (30 μL, 302 μmol) in degassed DMF (2.7 mL) as bubbled with argon gas. The mixture was stirred for 40 min at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 2:3), then the reaction mixture was diluted with EtOAc and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:30) to give **10** (250 mg, 76%). $[\alpha]_D$ = +46.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$):δ 8.02–7.13 (m, 39H, Ar), 5.92 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.9 Hz, H-2^a), 5.79–5.71 (m, 4H, H-1^a, H-3^a, H-2^b, H-4^b), 5.35 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3^b), 4.86 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1^b), 4.57 (dd, 1H, J_{gem} = 12.4 Hz, $J_{5,6a}$ = 1.6 Hz, H-6a^a), 4.50 (dd, 1H, $J_{5,6b}$ = 4.2 Hz, H-6b^a), 4.24 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4^a), 4.02 (m, 1H, H-5^a), 3.88 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5^b), 3.75–3.68 (m, 2H, H-6a^b, H-6b^b), 2.34 (s, 3H, Ar*CH*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.7, 165.8, 165.6, 165.4, 165.4, 165.3, 165.2, 164.8, 145.3, 135.5, 133.5, 133.4, 133.3, 133.3, 133.2, 133.1, 130.0, 129.9, 129.8, 129.8, 129.7, 129.6, 129.5, 129.5, 128.9, 128.9, 128.7, 128.6, 128.6, 128.5, 128.5, 128.3, 128.2, 127.6, 101.0, 79.9, 78.5, 75.7, 74.2, 71.8, 71.3, 70.7, 69.8, 67.5, 62.6, 61.1, 21.7; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 626.2; HRMS: *m*/*z* calcd for $C_{69}H_{56}O_{18}SeNa^+$: 1275.2524 $[M+Na]^+$; found: 1275.2524.

4.1.3. Methyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-seleno-β-D-glucopyranoside (11)

Methyl hydrazine (2.5 μL, 64 μmol) was added to a solution of **10** (40 mg, 32 μmol), cesium carbonate (21 mg, 48 μmol) and methyl iodide (4.0 μL, 64 μmol) in degassed DMF (1.8 mL) in ice bath as bubbled with argon gas. The mixture was stirred for 40 min at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:10), then the reaction mixture was diluted with EtOAc and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:25) to give **11** (37 mg, 99%). $[\alpha]_D$ = +47.3° (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02–7.13 (m, 35H, Ph), 5.80 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3^a), 5.74–5.70 (m, 2H, H-2^b, H-4^b), 5.53 (t, 1H, $J_{1,2}$ = 9.8 Hz, H-2^a), 5.38 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3^b), 4.89–4.85 (m, 2H, H-1^a, H-1^b), 4.60 (dd, 1H, J_{gem} = 12.3 Hz, $J_{5,6a}$ = 1.5 Hz, H-6a^a), 4.49 (dd, 1H, $J_{5,6b}$ = 4.3 Hz, H-6b^a), 4.25 (t, 1H, $J_{4,5}$ = 9.6 Hz, H-4^a), 3.91 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.8 Hz, H-5^b), 3.86 (m, 1H, H-5^a), 3.79–3.70 (m, 2H, H-6a^b, H-6b^b), 2.34 (s, 3H, SeMe); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.8, 166.5, 166.4, 165.4, 165.3, 165.2, 164.8, 133.5, 133.3, 133.3, 133.3, 133.2, 133.1, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.4, 129.1, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.2, 100.9, 78.1, 77.2, 75.8, 73.8, 71.8, 71.3, 70.9, 69.9, 67.5, 62.5, 61.0, 29.6, 2.4; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 211.2; HRMS: *m*/*z* calcd for $C_{62}H_{52}O_{17}SeNa^+$: 1171.2262 $[M+Na]^+$; found: 1171.2262.

4.1.4. Methyl (β-D-galactopyranosyl)-(1→4)-1-seleno-β-D-glucopyranoside (1)

Sodium methoxide (28% in MeOH, 8 mg, 39 μmol) was added to a solution of **11** (45 mg, 39 μmol) in THF (430 μL) and MeOH (870 μL). The reaction mixture was stirred for 4 days (completion

of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH/H_2O, 5:4:1). Then the reaction mixture was concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O, 8:5:1) and Sephadex LH-20 (MeOH/H_2O, 8:1) to give **1** (15 mg, 88%). $[\alpha]_D = -1.0°$ (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 4.75 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1^a), 4.48 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1^b), 4.00–3.70 (m, 10H, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b), 3.57 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2^b), 3.50 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2^a), 2.15 (s, 3H, SeMe); ^{13}C NMR (125 MHz, D_2O): δ 104.3, 81.4, 79.6, 77.1, 76.8, 74.0, 73.6, 72.4, 70.0, 62.5, 61.7 3.2; ^{77}Se NMR (94 MHz, D_2O): δ 183.4; HRMS: *m*/*z* calcd for $C_{13}H_{24}O_{10}SeNa^+$: 443.0427 [*M*+Na]$^+$; found: 443.0429.

4.1.5. *p*-Methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-β-D-galactopyranoside (13)

12 (90 mg, 220 μmol) in degassed DMF (2.2 mL) was added dropwise to a solution of **7** (84 mg, 264 μmol), *N*,*N*-diisopropylethylamine (45 μL, 264 μmol) and piperidine (25 μL, 253 μmol) in degassed DMF (2.2 mL) as bubbled with argon gas. The mixture was stirred for 1.5 h at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 2:3), then the reaction mixture was diluted with EtOAc and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (acetone/hexane, 1:5) to give **13** (99 mg, 85%). Spectroscopic data (^{1}H, ^{13}C, ^{77}Se NMR) of compound **13** were identical to those reported[9]; HRMS: *m*/*z* calcd for $C_{22}H_{26}O_{10}SeNa^+$: 553.0583 [M+Na]$^+$; found: 553.0583.

4.1.6. 2-(Trimethylsilyl)ethyl Se-(β-D-galactopyranosyl)-(1→4)-4-dexoy-4-seleno-β-D-glucopyranoside (2)

Sodium methoxide (28% in MeOH, 8 mg, 44 μmol) was added to a solution of **15** (35 mg, 44 μmol) in THF (490 μL) and MeOH (980 μL). The reaction mixture was stirred for 20 h (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 2:1). Then the reaction mixture was neutralized with Dowex-50 (H^+) and filtered through cotton, and removed resin was washed with MeOH. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH) to give **2** (22 mg, quant.). $[\alpha]_D = -41.7°$ (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.73 (d, 1H, $J_{1,2}$ = 10.8 Hz, H-1^b), 4.25 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1^a), 4.08–3.43 (m, 12H, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, $TMSCH_2CH_2$), 3.17 (t, 1H, $J_{2,3}$ = 8.0 Hz, H-2^a), 2.92 (t, 1H, $J_{2,3}$ = 10.8 Hz, H-2^b), 1.05-0.91 (m, 2H, $TMSCH_2$), 0.00 (s, 9H, TMS); ^{13}C NMR (125 MHz, CD_3OD): δ 104.4, 83.0, 82.8, 79.0, 77.2, 76.8, 76.7, 72.7, 71.6, 68.9, 64.9, 63.7, 45.1, 20.0, −0.5; ^{77}Se NMR (94 MHz, CD_3OD): δ 288.0; HRMS: *m*/*z* calcd for $C_{17}H_{34}O_{10}SeNa^+$: 529.0979 [*M*+Na]$^+$; found: 529.0979.

4.1.7. 2-(Trimethylsilyl)ethyl 2,3-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (18)

NaH (60% in oil, 220 mg, 5.49 mmol) was added to a solution of **16** (674 mg, 1.83 mmol) in DMF at 0 °C. The mixture was stirred for 30 min, after which *p*-methoxybenzyl chloride (632 μL, 4.03 mmol) was added at 0 °C. Stirring was continued for 22 h at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:2), the reaction mixture was quenched by addition of saturated aqueous NH_4Cl at 0 °C and the residual solvent was removed by coevaporation with toluene. The residue was dissolved in EtOAc and the solution was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:10) to give the crude product **17**. The crude product **17** was pumped in high vacuum for 12 h. Then the crude product **17** was dissolved in AcOH (16 mL) and H_2O (4 mL) at rt. The reaction mixture was stirred for 6 h at 50 °C (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:2), then coevaporation with toluene. The residue was dissolved in EtOAc and the solution was washed with water, saturated aqueous Na_2CO_3, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:2) to give **18** (608 mg, 64% in 2 steps). $[\alpha]_D = -27.5°$ (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31–6.86 (m, 8H, Ar), 4.90–4.87 (2 d, 2H, J_{gem} = 11.6 Hz, ArCH_2), 4.67 (d, 1H, ArCH_2), 4.59 (d, 1H, ArCH_2), 4.44 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.00 (m, 1H, $TMSCH_2CH_2$), 3.86 (m, 1H, H-6a), 3.80 (s, 3H, $ArOCH_3$), 3.79 (s, 3H, $ArOCH_3$), 3.74 (m, 1H, H-6b), 3.63 (m, 1H, $TMSCH_2CH_2$), 3.51–3.48 (near t, 1H, $J_{3,4} = J_{4,5}$ = 8.9 Hz, $J_{4,OH}$ = 2.1 Hz, H-4), 3.40 (t, 1H, $J_{2,3}$ = 8.9 Hz, H-3), 3.36 (t, 1H, H-2), 3.32–3.29 (m, 1H, H-5), 2.40 (d, 1H, OH), 2.15 (t, 1H, $J_{6,OH}$ = 6.2 Hz, OH), 1.07–1.04 (m, 2H, $TMSCH_2$), 0.04 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.3, 159.2, 130.6, 130.5, 129.8, 129.6, 114.0, 113.8, 103.4, 83.4, 81.6, 74.8, 74.7, 74.3, 70.4, 67.8, 62.7, 55.2, 18.6, −1.5; HRMS: *m*/*z* calcd for $C_{27}H_{40}O_8SiNa^+$: 543.2390 [*M*+Na]$^+$; found: 543.2390.

4.1.8. 2-(Trimethylsilyl)ethyl 6-bromo-6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (19)

Carbontetrabromide (114 mg, 345 μmol) and triphenylphosphine (89 mg, 340 μmol) were added to a solution of **18** (117 mg, 225 μmol) in pyridine (2.3 mL) in ice bath, and stirred for 10 min. Then reaction mixture was warmed to 65 °C. Stirring was continued for 1 h (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:1). Then the reaction mixture was quenched by addition of MeOH and the residual solvent was removed by coevaporation with toluene. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:20) to give **19** (122 mg, 93%). $[\alpha]_D = -25.4°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–6.86 (m, 8 H Ar), 4.89 (2 d, 2H, ArCH_2), 4.66 (d, 1H, ArCH_2), 4.56 (d, 1H, ArCH_2), 4.43 (d, 1H, $J_{1,2}$ = 6.8 Hz, H-1), 4.03 (m, 1H, $TMSCH_2CH_2$), 3.79 (s, 6H, $ArOCH_3$), 3.71–3.64 (m, 2H, H-6a, $TMSCH_2CH_2$), 3.45 (m, 1H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.42–3.36 (m, 4H, H-2, H-3, H-4, H-5), 2.24 (s, 1H, OH), 1.10–1.04 (m, 2H, $TMSCH_2$), 0.04 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 159.2, 130.5, 130.4, 129.8, 129.6, 114.0, 113.8, 103.1, 83.1, 81.5, 74.7, 74.2, 71.8, 67.5, 55.2, 32.6, 18.4, −1.5; HRMS: *m*/*z* calcd for $C_{27}H_{39}BrO_7SiNa^+$: 605.1546 [*M*+Na]$^+$; found: 605.1546.

4.1.9. 2-(Trimethylsilyl)ethyl 6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-(*p*-methylbenzoylseleno)-β-D-glucopyranoside (20)

7 (702 mg, 2.21 mmol), *N*,*N*-diisopropylethylamine (377 μL, 2.22 mmol) and piperidine (220 μL, 2.22 mmol) were added to a solution of **19** (1.03 g, 1.77 mmol) in degassed DMF (18 mL) at rt as bubbled with argon gas. Then reaction mixture was warmed to 60 °C. Stirring was continued for 45 min (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:5), then the reaction mixture was diluted with EtOAc, and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) and silica gel (EtOAc/toluene, 1:20) to give **20** (1.19 g, 96%). $[\alpha]_D = -8.9°$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.82–6.85 (m, 12H, Ar), 4.87–4.82 (m, 2H, ArCH_2), 4.70–4.65 (m, 2H, ArCH_2), 4.41 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 3.97 (m, 1H, $TMSCH_2CH_2$), 3.79 (2s, 6H, $ArOCH_3$), 3.62 (m, 1H, $TMSCH_2CH_2$), 3.52 (dd, 1H, J_{gem} = 13.1 Hz, $J_{5,6a}$ = 2.7 Hz, H-6a), 3.48 (m, 1H, H-5), 3.44–3.42 (m, 2H, H-3, H-4), 3.39 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.35 (t, 1H, $J_{2,3}$ = 8.9 Hz, H-2), 2.97 (d, 1H, $J_{4,OH}$ = 2.8 Hz, OH), 2.40 (s, 3H, ArCH_3), 1.06–1.03 (m, 2H, $TMSCH_2$), 0.03 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 195.8, 159.2, 159.2, 144.8, 136.2, 130.7, 130.7, 129.8, 129.6, 129.4, 127.4, 113.9, 113.7, 103.0, 83.0, 81.6, 75.0, 74.3, 73.1, 67.5, 55.2, 27.4, 21.7, 18.5, −1.4; ^{77}Se NMR

(113 MHz, $CDCl_3$): δ 485.9; HRMS: m/z calcd for $C_{36}H_{46}O_9SeSiNa^+$: 725.2019 $[M+Na]^+$; found: 725.2024.

4.1.10. 2-(Trimethylsilyl)ethyl 4-*O*-acetyl-6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-(*p*-methylbenzoylseleno)-β-D-glucopyranoside (21)

Acetic anhydride (7.6 mL, 8.06 mmol) was added to a solution of **20** (1.13 g, 1.61 mmol) in pyridine (16.1 mL) in ice bath. The mixture was stirred for 17 h at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene 1:5), then reaction mixture was quenched by addition of MeOH and the residual solvent was removed by coevaporation with toluene. The residue was dissolved in EtOAc, and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:30) to give **21** (1.17 g, 98%). $[\alpha]_D = -4.7°$ (c 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78–6.83 (m, 12 H Ar), 4.91 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 4.84 (d, 1H, $J_{gem} = 10.3$ Hz, ArCH_2), 4.72 (d, 1H, ArCH_2), 4.63 (d, 1H, $J_{gem} = 11.0$ -Hz, ArCH_2), 4.54 (d, 1H, ArCH_2), 4.37 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 3.97 (m, 1H, TMSCH$_2$CH_2), 3.78 (s, 6H, ArOCH_3), 3.66–3.62 (m, 1H, TMSCH$_2$CH_2), 3.54 (t, 1H, $J_{2,3} = 8.9$ Hz, H-3), 3.48–3.43 (m, 2H, H-2, H-5), 3.38 (dd, 1H, $J_{gem} = 13.1$ Hz, $J_{5,6a} = 2.8$ Hz, H-6a), 3.05 (dd, 1H, $J_{5,6b} = 9.6$ Hz, H-6b), 2.38 (s, 3H, ArCH_3), 2.05 (s, 3H, Ac), 1.06–1.01 (m, 2H, TMSCH_2), 0.26 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 193.5, 170.0, 159.2, 159.1, 144.6, 136.2, 130.6, 129.8, 129.4, 127.2, 113.7, 102.9, 81.9, 81.1, 74.6, 74.5, 73.8, 73.6, 67.4, 55.2, 26.8, 21.7, 21.0, 18.4, −1.4; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 499.1; HRMS: m/z calcd for $C_{37}H_{48}O_9SeSiNa^+$: 767.2131 $[M+Na]^+$; found: 767.2130.

4.1.11. 2-(Trimethylsilyl)ethyl 4-*O*-acetyl-6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-methylseleno-β-D-glucopyranoside (22)

Cesium carbonate (157 mg, 480 μmol), methyl iodide (28.3 μL, 456 μmol) and methyl hydrazine (24.0 μL, 456 μmol) were added to a solution of **21** (68 mg, 91 μmol) in degassed DMF (1.8 mL) at rt as bubbled with argon gas. The mixture was stirred for 25 min at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:5). Then the reaction mixture was diluted with EtOAc, and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:5) to give **22** (52 mg, 89%). $[\alpha]_D = -10.6°$ (c 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.28–6.84 (m, 8 H Ar), 4.87–4.82 (m, 2H, H-4, ArCH_2), 4.73 (d, 1H, ArCH_2), 4.64 (d, 1H, ArCH_2), 4.54 (d, 1H, ArCH_2), 4.41 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 4.01 (m, 1H, TMSCH$_2$CH_2), 3.80 (s, 3H, ArOCH_3), 3.79 (s, 3H, ArOCH_3), 3.67 (m, 1H, TMSCH$_2$CH_2), 3.48 (m, 3H, H-2, H-3, H-5), 2.64 (dd, 1H, $J_{gem} = 13.1$ Hz, $J_{5,6a} = 8.9$ Hz, H-6a), 2.58 (dd, 1H, $J_{5,6b} = 2.7$ Hz, H-6b), 2.06 (s, 3H, SeMe), 1.95 (s, 3H, Ac), 1.06 (t, 2H, TMSCH_2), 0.04 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.8, 159.2, 159.1, 130.6, 130.0, 129.4, 113.7, 113.7, 103.0, 81.9, 81.1, 75.1, 74.6, 74.5, 73.9, 67.4, 55.2, 26.3, 21.0, 18.5, 5.7, −1.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 76.9; HRMS: m/z calcd for $C_{30}H_{44}O_8SeSiNa^+$: 663.1863 $[M+Na]^+$; found: 663.1868.

4.1.12. 2-(Trimethylsilyl)ethyl 6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-methylseleno-β-D-glucopyranoside (23)

A catalytic amount of sodium methoxide (28% in MeOH) was added to a solution of **22** (45 mg, 69 μmol) in MeOH (1.4 mL). The mixture was stirred for 44 h at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:5). Then the reaction mixture was neutralized with Dowex-50 (H^+), the mixture was filtered through cotton, and removed resin was washed with MeOH. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:6) to give **23** (36 mg, 87%). $[\alpha]_D = -29.7°$ (c 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31–6.87 (m, 8H, Ar), 4.89 (2 d, 2H, ArCH_2), 4.66 (d, 1H, ArCH_2), 4.56 (d, 1H, ArCH_2), 4.41 (d, 1H, $J_{1,2} = 7.4$ Hz, H-1), 4.00 (m, 1H, TMSCH$_2$CH_2), 3.80 (s, 6H, ArOCH_3), 3.65 (m, 1H, TMSCH$_2$CH_2), 3.43–3.35 (m, 4H, H-2, H-3, H-4, H-5), 2.93 (dd, 1H, $J_{gem} = 13.2$ Hz, $J_{5,6a} = 2.9$ Hz, H-6a), 2.69 (dd, 1H, $J_{5,6b} = 8.2$ Hz, H-6b), 2.16 (s, 1H, OH), 2.07 (s, 3H, SeMe), 1.06 (t, 2H, TMSCH_2), 0.04 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.4, 159.2, 130.6, 129.8, 129.6, 114.0, 113.8, 103.1, 83.3, 81.8, 76.2, 74.7, 74.2, 73.3, 67.4, 55.2, 26.9, 18.5, 5.6, −1.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 63.3; HRMS: m/z calcd for $C_{30}H_{44}O_8SeSiNa^+$: 621.1757 $[M+Na]^+$; found: 621.1757.

4.1.13. 2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-6-deoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-methylseleno-β-D-glucopyranoside (25)

Molecular sieves (MS4Å, 148 mg) were added to a solution of **24** (58 mg, 78 μmol) and **23** (46 mg, 77 μmol) in CH_2Cl_2 (1.5 mL). The suspension was stirred for 2 h at rt. Then TMSOTf (1.5 μL, 7.7 μmol) was added to the solution at 0 °C. The mixture was stirred for 14 h at 0 °C (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:8). The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (500 μL) then filtered through Celite, and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) to give **25** (71 mg, 79%). $[\alpha]_D = +36.0°$ (c 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02–6.75 (m, 28H, Ar), 5.91 (d, 1H, $J_{3,4} = 3.4$ Hz, H-4^b), 5.78 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.4$ Hz, H-2^b), 5.53 (dd, 1H, H-3^b), 5.15 (d, 1H, H-1^b), 4.93 (s, 2H, ArCH_2), 4.82 (d, 1H, $J_{gem} = 10.7$ Hz, ArCH_2), 4.61 (d, 1H, ArCH_2), 4.36–4.33 (m, 2H, H-1^a, H-6a^b), 4.26 (dd, 1H, $J_{gem} = 11.2$ Hz, $J_{5,6b} = 7.8$ Hz, H-6b^b), 4.03 (t, 1H, H-5^b), 3.92 (m, 1H, TMSCH$_2$CH_2), 3.78 (m, 3H, ArOCH_3), 3.68–3.55 (m, 6H, H-3^a, H-4^a, ArOCH_3, TMSCH$_2$CH_2), 3.45–3.35 (m, 2H, H-2^a, H-5^a), 2.83 (dd, 1H, $J_{gem} = 12.7$ Hz, $J_{5,6a} = 2.7$ Hz, H-6a^a), 2.47 (dd, 1H, $J_{5,6b} = 9.1$ Hz, H-6b^a), 1.86 (s, 3H, SeMe), 1.04-0.98 (m, 2H, TMSCH_2), 0.01 (s, 9H, TMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 165.5, 165.4, 165.1, 159.2, 158.8, 133.5, 133.5, 133.3, 133.1, 131.1, 130.6, 129.9, 129.8, 129.7, 129.7, 129.7, 129.4, 129.0, 129.0, 128.7, 128.6, 128.4, 128.3, 113.7, 113.6, 102.9, 101.4, 82.3, 81.9, 81.8, 75.4, 74.5, 74.3, 71.8, 71.4, 70.6, 67.8, 67.3, 61.3, 55.2, 55.1, 26.5, 18.5, 5.3, 0.0, −1.5; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 66.8; HRMS: m/z calcd for $C_{62}H_{68}O_{16}SeSiNa^+$: 1199.3334 $[M+Na]^+$; found: 1199.3334.

4.1.14. 2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-6-deoxy-6-methylseleno-β-D-glucopyranoside (26)

Trifluoroacetic acid (290 μL) was added to a solution of **25** (52 mg, 44 μmol) in CH_2Cl_2 (580 μL) at −20 °C, and the mixture was stirred for 75 min at −20 °C (completion of the reaction was confirmed by TLC analysis; MeOH/$CHCl_3$, 1:10). The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (3 mL) and extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 3:8) to give **26** (41 mg, quant.). $[\alpha]_D = +78.4°$ (c 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.23 (m, 20H, Ph), 6.00 (d, 1H, $J_{3,4} = 3.1$ Hz, H-4^b), 5.87 (dd, 1H, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 10.4$ Hz, H-2^b), 5.63 (dd, 1H, H-3^b), 4.99 (d, 1H, H-1^b), 4.64 (dd, 1H, $J_{gem} = 11.5$ Hz, $J_{5,6a} = 4.4$ Hz, H-6a^b), 4.53–4.45 (m, 2H, H-5^b, H-6b^b), 4.36 (s, 1H, OH), 4.32 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1^a), 3.92 (m, 1H, TMSCH$_2$CH_2), 3.73 (t, 1H, $J_{2,3} = J_{3,4} = 8.3$ Hz, H-3^a), 3.61 (m, 1H, TMSCH$_2$CH_2), 3.56–3.49 (m, 2H, H-4^a, H-5^a), 3.41 (t, 1H,

H-2[a]), 2.58 (dd, 1H, J_{gem} = 12.7 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a[a]), 2.49–2.45 (m, 2H, H-6b[a], OH), 1.69 (s, 3H, SeMe), 1.06-0.94 (m, 2H, TMSCH_2), 0.00 (s, 9H, TMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.1, 165.4, 165.0, 133.8, 133.7, 133.4, 133.4, 130.0, 129.8, 129.7, 129.1, 128.7, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 102.3, 101.6, 85.7, 74.7, 74.7, 73.7, 72.3, 71.5, 69.6, 68.0, 67.2, 62.4, 29.7, 25.9, 18.2, 5.0, 0.0, −1.5; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 61.1; HRMS: *m*/*z* calcd for $C_{46}H_{52}O_{14}SeSiNa^+$: 959.2184 [*M*+Na]$^+$; found: 959.2184.

4.1.15. 2-(Trimethylsilyl)ethyl (β-D-galactopyranosyl)-(1→4)-6-deoxy-6-methylseleno-β-D-glucopyranoside (3)

Sodium methoxide (28% in MeOH, 7 mg, 38 μmol) was added to a solution of **26** (36 mg, 39 μmol) in THF (420 μL) and MeOH (840 μL). The reaction mixture was sonicated for 5.5 h (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH/H_2O, 5:4:1). Then the reaction mixture was neutralized with Dowex-50 (H^+) and filtered through cotton, and the removed resin was washed with MeOH. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:7) and Sephadex LH-20 (MeOH) to give **3** (20 mg, quant.). $[\alpha]_D$ = +3.5° (*c* 0.4, MeOH); ^{1}H NMR (500 MHz, CD_3 OD): δ 4.34 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1[b]), 4.29 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1[a]), 3.95 (m, 1H, TMSCH$_2$CH_2), 3.82 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4[b]), 3.78 (dd, 1H, J_{gem} = 11.5 Hz, $J_{5,6a}$ = 7.6 Hz, H-6a[b]), 3.71–3.65 (m, 2H, H-6b[b], TMSCH$_2$CH_2), 3.60–3.51 (m, 3H, H-5[a], H-2[b], H-5[b]), 3.50–3.43 (m, 3H, H-3[a], H-4[a], H-3[b]), 3.23 (t, 1H, H-2[a]), 3.18 (dd, 1H, J_{gem} = 13.0 Hz, $J_{5,6a}$ = 2.5 Hz, H-6a[a]), 2.79 (dd, 1H, $J_{5,6b}$ = 8.1 Hz, H-6b[a]), 2.06 (s, 1H, SeMe), 1.02 (m, 2H, TMSCH_2), 0.0 (s, 9H, TMS); ^{13}C NMR (125 MHz, CD_3OD): δ 105.5, 103.5, 84.6, 77.1, 77.0, 76.3, 74.9, 74.8, 72.5, 70.2, 68.0, 62.5, 27.5, 19.1, 5.2, −1.4; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 56.5; HRMS: *m*/*z* calcd for $C_{18}H_{36}O_{10}SeSiNa^+$: 543.1135 [*M*+Na]$^+$; found: 543.1135.

4.1.16. 2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-bromo-6-deoxy-β-D-galactopyranoside (28)

Carbontetrabromide (262 mg, 790 μmol) and triphenylphosphine (310 mg, 1.18 mmol) were added to a solution of **27** (384 mg, 787 μmol) in pyridine (5.3 mL) in ice bath, and stirred for 10 min. Then the reaction mixture was warmed to 65 °C. Stirring was continued for 1 h (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:5), then the reaction mixture was quenched by addition of MeOH, and the residual solvent was removed by coevaporation with toluene. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:20) to give **28** (379 mg, 87%). $[\alpha]_D$ = +63.8° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99–7.36 (m, 10H, Ph), 5.69 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 5.32 (dd, 1H, $J_{3,4}$ = 3.2 Hz, H-3), 4.72 (d, 1H, H-1), 4.47 (dd, 1H, $J_{4,OH}$ = 6.0 Hz, H-4), 4.07 (m, 1H, TMSCH$_2$CH_2), 3.93 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5), 3.69–3.57 (m, 3H, H-6a, H-6b, TMSCH$_2$CH_2), 2.25 (d, 1H, OH), 1.00-0.81 (m, 2H, TMSCH_2), −0.07 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 165.3, 133.5, 133.1, 129.9, 129.7 128.9, 128.5, 128.3, 100.8, 74.6, 74.3, 69.5, 67.6, 67.3, 28.9, 17.9, −1.5; HRMS: *m*/*z* calcd for $C_{25}H_{31}BrO_7SiNa^+$: 573.0915 [*M*+Na]$^+$; found:573.0912.

4.1.17. 2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-deoxy-6-(*p*-methylbenzoylseleno)-β-D-galactopyranoside (29)

7 (4.12 g, 13.0 mmol), *N*,*N*-diisopropylethylamine (2.2 mL, 13.0 mmol) and piperidine (1.3 mL, 13.0 mmol) were added to a solution of **28** (5.61 g, 10.2 mmol) in degassed DMF (67 mL) at rt as bubbled with argon gas. Then mixture was warmed to 100 °C and stirred for 45 min (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:2). The reaction mixture was diluted with EtOAc, and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:15) to give **29** (6.52 g, 96%). $[\alpha]_D$ = +36.8° (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99–7.47 (m, 14H, Ar), 5.72 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.30 (dd, 1H, $J_{3,4}$ = 3.2 Hz, H-3), 4.70 (d, 1H, H-1), 4.36 (dd, 1H, $J_{4,OH}$ = 6.0 Hz, H-4), 4.06 (m, 1H, TMSCH$_2$CH_2), 3.85 (t, 1H, $J_{5,6a}$ = -$J_{5,6b}$ = 7.3 Hz, H-5), 3.63 (m, 1H, TMSCH$_2$CH_2), 3.50–3.39 (m, 2H, H-6a, H-6b), 2.59 (d, 1H, OH), 2.41 (s, 3H, ArCH_3), 1.00–0.84 (m, 2H, TMSCH_2), −0.07 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.3, 165.9, 165.3, 145.1, 136.0, 133.3, 133.0, 129.9, 129.7, 129.5, 129.1, 129.0, 128.4, 128.2, 127.4, 100.7, 74.5, 69.7, 68.4, 67.4, 24.5, 21.7, 17.9, −1.5; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 530.7; HRMS: *m*/*z* calcd for $C_{33}H_{38}O_8SeSiNa^+$: 693.1393 [*M*+Na]$^+$; found: 693.1390.

4.1.18. 2-(Trimethylsilyl)ethyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-(*p*-methylbenzoylseleno)-β-D-galactopyranoside (30)

Acetic anhydride (3.9 mL, 41.3 mmol) was added to a solution of **29** (5.48 g, 8.18 mmol) in pyridine (40.9 mL) in ice bath. The reaction mixture was stirred for 25 h at rt (completion of the reaction was confirmed by TLC analysis, EtOAc/hexane, 1:3), which was then quenched by addition of MeOH, and the residual solvent was removed by coevaporation with toluene. The residue was dissolved in EtOAc and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:8) to give **30** (5.60 g, 96%). $[\alpha]_D$ = +30.8° (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97–7.25 (m, 14H, Ar), 5.76 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 5.67 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 5.40 (dd, 1H, H-3), 4.73 (d, 1H, H-1), 4.07 (m, 1H, TMSCH$_2$CH_2), 3.97 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 7.3 Hz, H-5), 3.64 (m, 1H, TMSCH$_2$CH_2), 3.36–3.25 (m, 2H, H-6a, H-6b), 2.41 (s, 3H, ArCH_3), 2.16 (s, 3H, Ac), 1.02–0.85 (m, 2H, TMSCH_2), −0.07 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.0, 170.1, 165.5, 165.2, 145.0, 135.9, 133.2, 133.0, 129.7, 129.6, 129.5, 129.0, 128.3, 128.2, 127.3, 100.7, 73.2, 72.1, 69.6, 69.1, 67.7, 60.3, 24.4, 21.7, 20.6, 17.9, 14.1, −1.5; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 495.6; HRMS: *m*/*z* calcd for $C_{35}H_{40}O_9SeSiNa^+$: 735.1504 [*M*+Na]$^+$; found: 735.1504.

4.1.19. 2-(Trimethylsilyl)ethyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-β-D-galactopyranoside (31)

Cesium carbonate (289 mg, 885 μmol), methyl iodide (53.9 μL, 865 μmol) and methyl hydrazine (34.2 μL, 650 μmol) were added to a solution of **30** (308 mg, 432 μmol) in degassed DMF (8.7 mL) at rt as bubbled with argon gas. The mixture was stirred for 2 h at rt, while monitoring the reaction by TLC analysis (EtOAc/toluene, 1:10). Then another amount of methyl iodide (53.9 μL, 865 μmol) and methyl hydrazine (34.2 μL, 650 μmol) were added to the reaction mixture and then bubbling with argon gas was stopped. Stirring was continued for another 15h, then the reaction mixture was diluted with EtOAc, and the solution was washed with 2_M aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:3) to give **31** (206 mg, 78%). $[\alpha]_D$ = +31.2° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98–7.33 (m, 10H, Ph), 5.69–5.64 (m, 2H, H-4, H-2), 5.41 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 4.74 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.06 (m, 1H, TMSCH$_2$CH_2), 3.98 (m, 1H, $J_{5,6a}$ = 7.8 Hz, $J_{5,6b}$ = 6.0 Hz, H-5), 3.64 (m, 1H, TMSCH$_2$CH_2), 2.84 (dd, 1H, J_{gem} = 12.8 Hz, H-6a), 2.60 (dd, 1H, H-6b), 2.14 (s, 3H, Ac), 2.09 (s, 3H, SeMe), 1.02–0.85 (m, 2H, TMSCH_2), −0.07 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 165.6, 165.2, 133.3, 133.1, 132.3, 129.7, 129.6, 129.0, 128.4, 128.4, 128.3, 100.8, 74.6, 72.1, 69.6, 69.3, 67.7, 24.6, 20.6, 17.9, 5.6, −1.5; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 73.2; HRMS: *m*/*z* calcd for $C_{28}H_{36}O_8SeSiNa^+$: 631.1237 [*M*+Na]$^+$; found: 631.1237.

4.1.20. 4-*O*-Acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-D-galactopyranose (32)

Trifluoroacetic acid (2.1 mL) was added to a solution of **31** (190 mg, 312 μmol) in CH_2Cl_2 (4.1 mL) at 0 °C. The mixture was stirred for 2 h at rt (completion of the reaction was confirmed by TLC analysis; MeOH/$CHCl_3$, 1:50), then the reaction mixture was coevaporation with toluene. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:20) to give **32** (128 mg, 81%): α-isomer; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00–7.35 (m, 10H, Ph), 5.88 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 5.75–5.71 (m, 2H, H-1, H-4), 5.58 (dd, 1H, $J_{1,2}$ = 3.4 Hz, H-2), 4.54 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5), 3.36 (br s, 1H, OH), 2.74 (dd, 1H, J_{gem} = 13.1 Hz, H-6a), 2.58 (dd, 1H, H-6b), 2.16 (s, 3H, Ac), 2.05 (s, 3H, SeMe); ^{77}Se NMR (113 MHz, $CDCl_3$):δ 64.4; HRMS: *m*/*z* calcd for $C_{23}H_{24}O_8SeNa^+$: 531.0534 [*M*+Na]$^+$; found: 531.0534.

4.1.21. 4-*O*-Acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-D-galactopyranosyl trichloroacetimidate (33)

Trichloroacetonitrile (70.8 μL, 706 μmol) and DBU (3.2 μL, 210 μmol) were added to a solution of **32** (36 mg, 71 μmol) in CH_2Cl_2 (1.4 mL) at 0 °C. The mixture was stirred for 70 min at 0 °C (completion of the reaction was confirmed by TLC analysis; MeOH/$CHCl_3$, 1:15). The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:2) to give **33** (46 mg, quant., α/β = 20/1). α-isomer; $[\alpha]_D$ = +75.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (s, 1H, NH), 8.00–7.26 (m, 10H, Ph), 6.76 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1), 5.92–5.82 (m, H-2, H-3, H-4), 4.52 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5), 2.76 (dd, 1H, J_{gem} = 12.8 Hz, H-6a) 2.58 (dd, 1H, H-6b), 2.19 (s, 3H, Ac), 2.03 (s, 3H, SeMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 165.7, 165.5, 160.6, 133.5, 133.4, 129.8, 129.6, 129.0, 128.8, 128.5, 128.4, 93.8, 90.9, 72.5, 69.5, 68.7, 67.6, 24.0, 20.6, 5.3, 0.0; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 71.3; HRMS: *m*/*z* calcd for $C_{25}H_{24}Cl_3O_8SeNa^+$: 673.9631 [*M*+Na]$^+$; found: 673.9630.

4.1.22. 2-(Trimethylsilyl)ethyl (4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (35)

Molecular sieves (MS4Å, 206 mg) were added to a solution of **33** (48 mg, 74 μmol) and **34** (61 mg, 109 μmol) in CH_2Cl_2 (1.5 mL). The suspension was stirred for 1 h at rt. Then TMSOTf (1.4 μL, 7.3 μmol) was added to the solution at −40 °C. The reaction mixture was stirred for 48 h at −40 °C, after which TMSOTf (1.4 μL, 7.3 μmol) was added to the solution at −40 °C. Stirring was continued for another 3 h at −40 °C (completion of the reaction was confirmed by TLC analysis; EtOAc/toluene, 1:4). The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (500 μL) then filtered through Celite, and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) and silica gel (EtOAc/toluene, 1:8) to give **35** (55 mg, 71%). $[\alpha]_D$ = +40.3° (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98–6.80 (m, 18H, Ar), 5.71 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4^b), 5.65 (d, 1H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 10.3 Hz, H-2^b), 5.35 (dd, 1H, H-3^b), 4.94 (d, 1H, J_{gem} = 10.3 Hz, ArCH_2), 4.91 (d, 1H, H-1^b), 4.83 (d, 1H, ArCH_2), 4.81 (d, 1H, ArCH_2), 4.62 (d, 1H, ArCH_2), 4.32–4.30 (m, 2H, H-1^a, H-6a^a), 4.07 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, H-6b^a), 3.89 (m, 1H, TMSCH$_2$CH_2), 3.84–3.74 (m, 8H, H-4^a, H-5^b, ArOCH_3), 3.61–3.53 (m, 2H, H-3^a, TMSCH$_2$CH_2), 3.39 (m, 1H, H-5^a), 3.45 (t, 1H, H-2^a), 2.58 (dd, 1H, J_{gem} = 13.1 Hz, $J_{5,6a}$ = 6.2 Hz, H-6a^b), 2.49 (dd, 1H, $J_{5,6b}$ = 7.6 Hz, H-6b^b), 2.12 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.87 (s, 3H, SeMe), 0.99 (t, 2H, TMSCH_2), 0.00 (s, 9H, TMS); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.4, 169.9, 165.4, 165.1, 159.1, 158.9, 133.4, 133.3, 131.2, 130.5, 129.8, 129.7, 129.6, 128.9, 128.9, 128.8, 128.5, 128.4, 113.7, 113.5, 102.9, 100.6, 82.2, 81.5, 77.3, 74.6, 74.4, 72.3, 72.1, 70.2, 68.6, 67.5, 62.4, 55.3, 55.2, 29.7, 23.9, 20.7, 20.6, 18.4, 5.5, 1.0, 0.0, −1.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 64.8; HRMS: *m*/*z* calcd for $C_{52}H_{64}O_{16}SeSiNa^+$: 1075.3021 [*M*+Na]$^+$; found: 1075.3021.

4.1.23. 2-(Trimethylsilyl)ethyl (4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-β-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-β-D-glucopyranoside (36)

Trifluoroacetic acid (350 μL) was added to a solution of **35** (55 mg, 52 μmol) in CH_2Cl_2 (700 μL) at −20 °C, and the mixture was stirred for 2.5 h at −20 °C (completion of the reaction was confirmed by TLC analysis; MeOH/$CHCl_3$, 1:15). The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (3 mL) and extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 3:8) to give **36** (35 mg, 83%). $[\alpha]_D$ = +43.8° (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95–7.34 (m, 10H, Ph), 5.72–7.68 (m, 2H, H-2^b, H-4^b), 5.43 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3^b), 4.91 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1^b), 4.32 (d, 1H, OH), 4.28 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1^a), 4.17 (dd, 1H, J_{gem} = 12.0 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a^a), 4.02 (m, 1H, H-5^b), 3.95–3.88 (m, 2H, H-6b^a, TMSCH$_2$CH_2), 3.75 (m, 1H, H-2^a), 3.61–3.54 (m, 2H, H-3^a, TMSCH$_2$CH_2), 3.49 (m, 1H, H-5^a), 3.41 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.6 Hz, H-4^a), 2.79 (dd, 1H, J_{gem} = 13.1 Hz, $J_{5,6a}$ = 8.3 Hz, H-6a^b), 2.66 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6b^b), 2.57 (br s, 1H, OH), 2.17 (s, 3H, Ac), 1.69 (s, 3H, SeMe), 1.77 (s, 3H, Ac), 1.04-0.89 (m, 2H, TMSCH_2CH$_2$), −0.01 (s, 9H, TMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0 169.9, 165.4, 165.1, 133.5, 133.4, 129.7, 129.6, 129.0, 128.7, 128.6, 128.5, 128.4, 128.2, 101.7, 101.6, 80.9, 75.1, 73.5, 73.3, 71.9, 71.6, 69.4, 68.9, 67.4, 62.2, 24.7, 20.5, 20.4, 18.1, 5.6, −1.5; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 61.1; HRMS: *m*/*z* calcd for $C_{46}H_{52}O_{14}SeSiNa^+$: 835.1876 [*M*+Na]$^+$; found: 835.1876.

4.1.24. 2-(Trimethylsilyl)ethyl (6-deoxy-6-methylseleno-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (4)

Sodium methoxide (28% in MeOH, 9 mg, 48 μmol) was added to a solution of **36** (39 mg, 48 μmol) in THF (530 μL) and MeOH (1.1 mL). The reaction mixture was sonicated for 3.5 h (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH/H_2O, 5:4:1). Then the reaction mixture was neutralized with Dowex-50 (H^+) and filtered through cotton, and removed resin was washed with MeOH. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH) to give **4** (24.8 mg, quant.). $[\alpha]_D$ = −6.7° (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.38 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1^b), 4.30 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1^a), 3.99 (m, 1H, TMSCH$_2$CH_2), 3.95 (d, 1H, $J_{3,4}$ = 2.6 Hz, H-4^b), 3.91 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5,6a}$ = 5.6 Hz, H-6a^a), 3.84 (dd, 1H, $J_{5,6b}$ = 4.2 Hz, H-6b^a), 3.70 (t, 1H, H-5^b), 3.63 (m, 1H, TMSCH$_2$CH_2), 3.58–3.51 (m, 4H, H-3^a, H-4^a, H-2^b, H-3^b), 3.41 (m, 1H, H-5^a), 3.23 (t, 1H, H-2^a), 2.83–2.76 (m, 2H, H-6a^b, H-6b^b), 2.04 (s, 1H, SeMe), 1.09–0.94 (m, 2H, TMSCH_2), 0.0 (s, 9H, TMS); ^{13}C NMR (125 MHz, CD_3OD): δ 105.1, 103.7, 80.9, 76.5, 76.3, 76.1, 74.9, 72.2, 70.8, 68.2, 62.0, 25.5, 19.1, 4.5, −1.4; ^{77}Se NMR (94 MHz, $CDCl_3$): δ 51.8; HRMS: *m*/*z* calcd for $C_{18}H_{36}O_{10}SeSiNa^+$: 543.1135 [*M*+Na]$^+$; found: 543.1135.

4.1.25. 4-*O*-Acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-D-galactopyranosyl fluoride (37)

N,*N*-diethylaminosulfur trifluoride (39.6 μL, 300 μmol) was added to a solution of **32** (101 mg, 199 μmol) in CH_2Cl_2 (2.0 mL) at 0 °C, and the mixture was stirred for 30 min at 0 °C (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:2), then the reaction mixture was diluted with $CHCl_3$, and the solution

was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/toluene, 1:2) to give **37** (99 mg, 97%, α/β = 1/1.7). α-isomer; $[\alpha]_D$ = +113.5° (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01–7.36 (m, 10H, Ph), 5.98 (dd, 1H, $J_{1,2}$ = 2.8 Hz, $J_{1,F}$ = 53.2 Hz, H-1), 5.86–5.81 (m, 2H, H-3, H-4), 5.63 (m, 1H, $J_{2,F}$ = 23.8 Hz, H-2), 4.47 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 7.7 Hz, H-5), 2.77 (dd, 1H, J_{gem} = 12.8 Hz, H-6a) 2.61 (dd, 1H, H-6b), 2.18 (s, 3H, Ac), 2.08 (s, 3H, SeMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 165.9, 165.4, 133.4, 129.9, 129.6, 129.0, 128.7, 128.5, 128.4, 105.8, 103.5, 71.9, 69.2, 68.3, 68.1, 68.0, 23.8, 20.6, 5.4; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 64.8; HRMS: *m*/*z* calcd for $C_{23}H_{23}FO_8SeNa^+$: 533.0485 $[M+Na]^+$; found: 533.0482; β-isomer; $[\alpha]_D$ = +74.4° (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99–7.35 (m, 10 H Ph), 5.81–5.73 (m, 2H, H-2, H-4), 5.60–5.45 (m, 2H, $J_{1,F}$ = 52.7 Hz, H-1, H-3), 4.10 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5), 2.87 (dd, 1H, J_{gem} = 13.3 Hz, H-6a), 2.66 (dd, 1H, H-6b), 2.17 (s, 3H, Ac), 2.09 (s, 3H, SeMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 165.4, 165.1, 133.5, 129.8, 129.6, 128.8, 128.6, 128.5, 128.4, 108.2, 106.1, 74.9, 71.0, 70.9, 69.6, 69.3, 68.3, 23.9, 20.6, 5.7; ^{77}Se NMR (75 MHz, $CDCl_3$): δ 71.3; HRMS: *m*/*z* calcd for $C_{23}H_{23}FO_8SeNa^+$: 533.0485 $[M+Na]^+$; found: 533.0485.

4.1.26. Methyl (4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-6-methylseleno-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (39)

Molecular sieves (MS4Å, 106 mg) were added to a solution of **37** (52 mg, 102 μmol) and **38** (47 mg, 102 μmol) in CH_2Cl_2 (2 mL). The suspension was stirred for 1 h at rt. Then Cp_2ZrCl_2 (74 mg, 254 μmol) and AgOTf (131 mg, 510 μmol) were added to the mixture at 0 °C. Stirring was continued for 24 h at rt (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 2:3). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) to give **39** (52 mg, 53%). $[\alpha]_D$ = +44.6° (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88–7.06 (m, 25H, Ph), 5.72 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.3 Hz, H-2^b), 5.66 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4^b), 5.41 (dd, 1H, H-3^b), 4.88 (d, 2H, J_{gem} = 10.9 Hz, PhC*H*$_2$), 4.74–4.67 (m 3H, H-1^b, PhC*H*$_2$), 4.58 (d, 1H, PhC*H*$_2$), 4.47 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1^a), 4.32 (d, 1H, PhC*H*$_2$), 4.19 (m, 1H, H-6a^a), 3.92–3.87 (m, 2H, H-3^a, H-5^b), 3.75–3.71 (m, 2H, H-5^a, H-6b^a), 3.43–3.36 (m, 2H, H-2^a, H-4^a), 3.20 (s, 3H, OMe), 2.81 (dd, 1H, J_{gem} = 13.2 Hz, $J_{5,6a}$ = 8.0 Hz, H-6a^b), 2.58 (dd, 1H, $J_{5,6b}$ = 5.7 Hz, H-6b^b), 2.16 (s, 3H, Ac), 2.06 (s, 3H, SeMe); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 165.6, 165.1, 138.8, 133.3, 133.1, 128.4, 128.3, 128.4, 128.1, 127.9, 127.6, 127.5, 101,6, 97.9, 81.9, 79.8, 77.4, 75.5, 74.7, 74.7, 73.3, 71.9, 69.4, 69.3, 68.5, 54.9, 24.5, 20.6, 5.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 72.6; HRMS: *m*/*z* calcd for $C_{51}H_{54}O_{13}SeNa^+$: 977.2622, $[M+Na]^+$; found: 977.2627.

4.2. Protein expression, purification and crystallization

The human galectin-9 NCRD was expressed as a glutathione S-transferase (GST) fusion protein and purified as described.[23] 5 mg/ml of human galectin-9 NCRD was mixed with seleno-lactose **1** to **4** (10 mM) before crystallization. The crystallization was manually performed by using grid screening based on the crystallization condition of human or mouse galectin-9 NCRD with lactose (pdb_id:2EAK and 2D6M).[23] Finally, the crystals of human galectin-9 NCRD with seleno-lactoses **1**, **2** and **3** were obtained under the optimized condition (**1**. 20% w/v PEG 3350 and 8% v/v Tacsimate pH 8.0; **2.** 20% w/v PEG 3350, 0.2 M Sodium Iodide and 100 mM Tris-HCl pH 8.0; **3.** 10% w/v PEG 6000 and 100 mM Tris–HCl pH 7.5, respectively) at 289 K for 2 or 3 days.

4.3. Data collection and structure determination of co-cystal with seleno-lactose (3)

The crystals were soaked in the cryo protectant buffer (10% w/v PEG6000, 100 mM Tris–HCl pH 7.5 and 20% glycerol) in a minute and flash-cooled in liquid nitrogen before data collection. The diffraction data of crystals are collected at BL-17A beamline on Photon Factory of KEK (Tsukuba, Japan). The best data was collected at 0.97889 Å for SAD phasing method. The data was processed by XDS package.[25] The scaling was performed with pointless and aimless in CCP4 suite.[26] The maximum resolution is determined by a correlation factor CC (1/2).[27] The structure of human galectin-9 NCRD was solved using Autosol in phenix software.[28] After Autosol, 562 residues were built and *R* and R_{free} was 0.20 and 0.21, respectively. Further refinement was performed using Coot,[29] manually. Finally, deposited structure was refined using Refmac[30] with TLS option and no NCS restrains. Seleno-lactose **3** coordinate and cif files were made using Jligand and phenix.elbow.[31] The geometry of refined structure was validated with MolProbity.[32] The statistics of data collection and refinements were described in the Supplementary Material.

4.4. Structure analysis of human galectin-9 with seleno-lactose (3)

RMSD value was calculated by SUPERPOSE in CCP4 suite.[26] Hydrogen bonds between human galectin-9 NCRD and seleno-lactose **3** were determined using Ligplot+[33] and CONTACT programs.[26] All figures of crystal structures were prepared using Pymol.[34]

Acknowledgements

The iCeMS is supported by World Premier International Research Center Initiative (WPI), MEXT, Japan. This work was financially supported in part by MEXT of Japan (a Grant-in-Aid for Scientific Research (B) No. 22380067 to M.K., and a Grant-in-Aid for Young Scientists (A) No. 23688014 and Grant-in-Aid on Innovative Areas No. 24110505, Deciphering sugar chain-based signals regulating integrative neuronal functions to H.A.). We thank Ms. Kiyoko Ito (Gifu Univ.) for providing technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.02.023.

References and notes

1. Wrede, F. Z. *Physiol. Chem.* **1921**, *115*, 284.
2. Recent reports on the use of seleno-carbohydrates as synthetic intermediates; (a) Yamago, S.; Kokubo, K.; Murakami, H.; Mino, Y.; Hara, O.; Yoshida, J.-I. *Tetrahedron Lett.* **1998**, *39*, 7905; (b) Jiaang, W.-T.; Chang, M.-Y.; Tseng, P.-H.; Chen, S.-T. *Tetrahedron Lett.* **2000**, *41*, 3127; (c) Yamago, S.; Yamada, T.; Hara, O.; Ito, H.; Mino, Y.; Yoshida, J.-I. *Org. Lett.* **2001**, *3*, 3867; (d) Ikeda, K.; Sugiyama, Y.; Tanaka, K.; Sato, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2309; (e) Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 828; (f) Mukherjee, C.; Tiwari, P.; Misra, A. K. *Tetrahedron Lett.* **2006**, *47*, 441; (g) Tiwari, P.; Misra, A. K. *Tetrahedron Lett.* **2006**, *47*, 2345; (h) Valerio, S.; Iadonisi, A.; Adinolfi, A.; Ravidà, A. *J. Org. Chem.* **2007**, *72*, 6097; (i) Di Bussolo, V.; Fiasella, A.; Balzano, F.; Barretta, G. U.; Crotti, P. *J. Org. Chem.* **2010**, *75*, 4284; (j) Kumar, A. A.; Illyes, T. Z.; Kover, K. E.; Szilagyi, L. *Carbohydr. Res.* **2012**, *360*, 8; Selenocarbohydrates as mimetics of carbohydrates; (k) Czernecki, S.; Randriamandimby, D. *J. Carbohydr. Chem.* **1996**, *15*, 183; (l) Sivapriya, K.; Suguna, P.; Chandrasekaran, S. *Tetrahedron Lett.* **2007**, *48*, 2091; (m) Abdo, M.; Knapp, S. *J. Am. Chem. Soc.* **2008**, *130*, 9234; (n) Kim, E. J.; Love, D. C.; Darout, E.; Abdo, M.; Rempel, B.; Withers, S. G.; Rablen, P. R.; Hanover, J. A.; Knapp, S. *Bioorg. Med. Chem.* **2010**, *18*, 7058; (o) Fournière, V.; Cumpstey, I. *Tetrahedron Lett.* **2010**, *51*, 2127; (p) Cumpstey, I.; Ramstadius, C.; Akhtar, T.; Goldstein, I. J.; Winter, H. C. *Eur. J. Org. Chem.* **2010**, 1951; (q) Braga, H. C.; Stefani, H. A.; Paixão, M. W.; Santos, F. W.; Lüdtke, D. S. *Tetrahedron* **2010**, *66*, 3441; (r) Affeldt, R. F.; Braga, H. C.; Baldassari, L. L.; Lüdtke, D. S. *Tetrahedron* **2012**, *68*, 10470.

3. Boutureira, O.; Bernardes, G. J. L.; Fernández-González, M.; Anthony, D. C.; Davis, B. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 1432.
4. (a) Mehta, S.; Andrews, J. S.; Johnson, B. D.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **1995**, *117*, 9783; (b) Szczepina, M. G.; Johnston, B. D.; Yuan, Y.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 12458; (c) Liu, H.; Pinto, B. M. *J. Org. Chem.* **2005**, *70*, 753; (d) Liu, H.; Nasi, R.; Jayakanthan, K.; Sim, L.; Heipel, H.; Rose, D. R.; Pinto, B. M. *J. Org. Chem.* **2007**, *72*, 6562.
5. (a) Gettins, P.; Wardlaw, S. A. *J. Biol. Chem.* **1991**, *266*, 3422; (b) House, K. L.; Dunlap, R. B.; Odom, J. D.; Wu, Z. P.; Hilvert, D. *J. Am. Chem. Soc.* **1992**, *114*, 8573; (c) Mobli, M.; Morgenstern, D.; King, G. F.; Alewood, P. F.; Muttenthaler, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 11952; (d) Schaefer, S. A.; Dong, M.; Rubenstein, R. P.; Wilkie, W. A.; Bahnson, B. J.; Thorpe, C.; Rozovsky, S. *J. Mol. Biol.* **2013**, *425*, 222.
6. (a) Taylor, G. *Acta. Crystallogr.* **2003**, *D59*, 1881; (b) Hendrickson, W. A.; Teeter, M. M. *Nature* **1981**, *290*, 107; (c) Hendrickson, W. A.; Pahler, A.; Smith, J. L.; Satow, Y.; Merritt, E. A.; Phizackerley, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2190; (d) Yang, W.; Hendrickson, W. A.; Crouch, R. J.; Satow, Y. *Science* **1990**, *249*, 1398; (e) Du, Q.; Carrasco, N.; Teplova, M.; Wilds, C. J.; Egli, M.; Huang, Z. *J. Am. Chem. Soc.* **2002**, *124*, 24.
7. (a) Buts, L.; Loris, R.; De Genst, E.; Oscarson, S.; Lahmann, M.; Messens, J.; Brosens, E.; Wyns, L.; De Greve, H.; Bouckaert, J. *Acta. Crystallogr.* **2003**, *D59*, 1012; (b) Kostlánová, N.; Mitchell, E. P.; Lortat-Jacob, H.; Oscarson, S.; Lahmann, M.; Gilboa-Garber, N.; Chambat, G.; Wimmerová, M.; Imberty, A. *J. Biol. Chem.* **2005**, *280*, 27839.
8. (a) Fotinou, C.; Emsley, P.; Black, I.; Ando, H.; Ishida, H.; Kiso, M.; Sinha, K. A.; Fairweather, N. F.; Isaacs, N. W. *J. Biol. Chem.* **2001**, *276*, 32274; (b) Attrill, H.; Imamura, A.; Sharma, R. S.; Kiso, M.; Crocker, P. R.; van Aalten, D. M. F. *J. Biol. Chem.* **2006**, *281*, 32774; (c) Stenmark, P.; Dupuy, J.; Imamura, A.; Kiso, M.; Stevens, R. C. *PLoS Pathog.* **2008**, *4*, e1000129; (d) Neu, U.; Hengel, H.; Blaum, B. S.; Schowalter, R. M.; Macejak, D.; Gilbert, M.; Wakarchuk, W. W.; Imamura, A.; Ando, H.; Kiso, M.; Arnberg, N.; Garcea, R. L.; Peters, T.; Buck, C. B.; Stehle, T. *PLoS Pathog.* **2012**, *8*, e1002738.
9. (a) Kawai, Y.; Ando, H.; Ozeki, H.; Koketsu, M.; Ishihara, H. *Org. Lett.* **2005**, *5*, 4653; (b) Nanami, M.; Ando, H.; Kawai, Y.; Koketsu, M.; Ishihara, H. *Tetrahedron Lett.* **2007**, *48*, 1113.
10. Ishihara, H.; Sato, S.; Hirabayashi, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3007.
11. Surabhi, P.; Wu, X.; Hu, L. *Tetrahedron Lett.* **2006**, *47*, 4609.
12. Lichtenthaler, F. W.; Kaji, E.; Weprek, S. *J. Org. Chem.* **1985**, *50*, 3505.
13. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G.; Dahmen, J.; Noori, G.; Stenvall, K. *J. Org. Chem.* **1988**, *53*, 5629.
14. Rio, S.; Beau, J. M.; Jacquinet, J. C. *Carbohydr. Res.* **1991**, *219*, 71.
15. (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731; (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212.
16. Zhang, Z.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 2394.
17. See the Supplementary Material for the experimental details of the synthesis of glycosyl acceptor **34**.
18. Suzuki, K.; Maeta, H.; Suzuki, T.; Matsumoto, T. *Tetrahedron Lett.* **1989**, *30*, 6879.
19. Experimental details for the synthesis of compounds **41** and **42** and their glycosidation reactions are reported in the Supplementary Material.
20. Boons, G. J. P. H.; van Delft, F. L.; van der Klein, P. A. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1992**, *48*, 885.
21. (a) Kurose, N.; Takahashi, T.; Koizumi, T. *J. Org. Chem.* **1996**, *61*, 2932; (b) Mukherjee, A. J.; Zade, S. S.; Singh, H. B.; Sunoj, R. B. *Chem. Rev.* **2010**, *110*, 4357.
22. Hirabayashi, J.; Kasai, K. *Glycobiology* **1993**, *3*, 297.
23. (a) Nagae, M.; Nishi, N.; Murata, T.; Usui, T.; Nakamura, T.; Wakatsuki, S.; Kato, R. *J. Biol. Chem.* **2006**, *281*, 35884; (b) Nagae, M.; Nishi, N.; Nakamura-Tsuruta, S.; Hirabayashi, J.; Wakatsuki, S.; Kato, R. *J. Mol. Biol.* **2008**, *375*, 119.
24. Deng, S.; Gangadharmath, U.; Chang, C.-W. T. *J. Org. Chem.* **2006**, *71*, 5179.
25. Kabsch, W. *Acta. Cryst.* **2010**, *D66*, 125.
26. Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. *Acta. Crystallogr.* **2011**, *D67*, 235.
27. Karplus, P. A.; Diederichs, K. *Science* **2012**, *336*, 1030.
28. Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. *Acta. Crystallogr.* **2010**, *D66*, 213.
29. Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. *Acta. Crystallogr.* **2010**, *D66*, 486.
30. Murshudov, G. N.; Skubák, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.; Nicholls, R. A.; Winn, M. D.; Long, F.; Vagin, A. A. *Acta. Crystallogr.* **2011**, *D67*, 355.
31. (a) Lebedev, A. A.; Young, P.; Isupov, M. N.; Moroz, O. V.; Vagin, A. A.; Murshudov, G. N. *Acta Crystallogr. D Biol. Crystallogr.* **2012**, *68*, 431; (b) Moriarty, N. W.; Grosse-Kunstleve, R. W.; Adams, P. D. *Acta. Crystalloge.* **2009**, *D65*, 1074.
32. Chen, V. B.; Arendall, W. B., 3rd; Headd, J. J.; Keedy, D. A.; Immormino, R. M.; Kapral, G. J.; Murray, L. W.; Richardson, J. S.; Richardson, D. C. *Acta. Crystallogr.* **2010**, *D66*, 12.
33. Laskowski, R. A.; Swindells, M. B. *J. Chem. Inf. Model.* **2011**, *51*, 2778.
34. The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.