Tetrahedron Letters 54 (2013) 3940–3943

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Synthesis of β-D-GalNAc(4,6-diS)(1–4)[α-L-Fuc(2,4-diS)(1–3)]-β-D-GlcA, a novel trisaccharide unit of chondroitin sulfate with a fucose branch

Jun-ichi Tamura [a,b,*], Haruna Tanaka [b], Ayumi Nakamura [b], Naoko Takeda [a]

[a] Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Koyamacho-Minami 4-101, Tottori 680-8552, Japan
[b] Department of Regional Environment, Faculty of Regional Sciences, Tottori University, Koyamacho-Minami 4-101, Tottori 680-8551, Japan

ARTICLE INFO

Article history:
Received 1 April 2013
Revised 8 May 2013
Accepted 13 May 2013
Available online 24 May 2013

Keywords:
Chondroitin sulfate
Glycosaminoglycan
Sea cucumber
Glycosylation

ABSTRACT

Novel glycosaminoglycans isolated from the body wall of sea cucumbers consist of chondroitin sulfates like polysaccharides. We have synthesized a representative repeating trisaccharide composed of a chondroitin backbone having a fucose (Fuc) branch at *O*-3 of glucuronic acid (GlcA), β-D-GalNAc(4,6-diS)(1–4)[α-L-Fuc(2,4-diS)(1–3)]-β-D-GlcA, in a stereocontrolled manner. Regiospecific sulfation at *O*-4,6 and *O*-2,4 of the *N*-acetylgalactosamine (GalNAc) and Fuc residues, respectively, was successfully achieved.

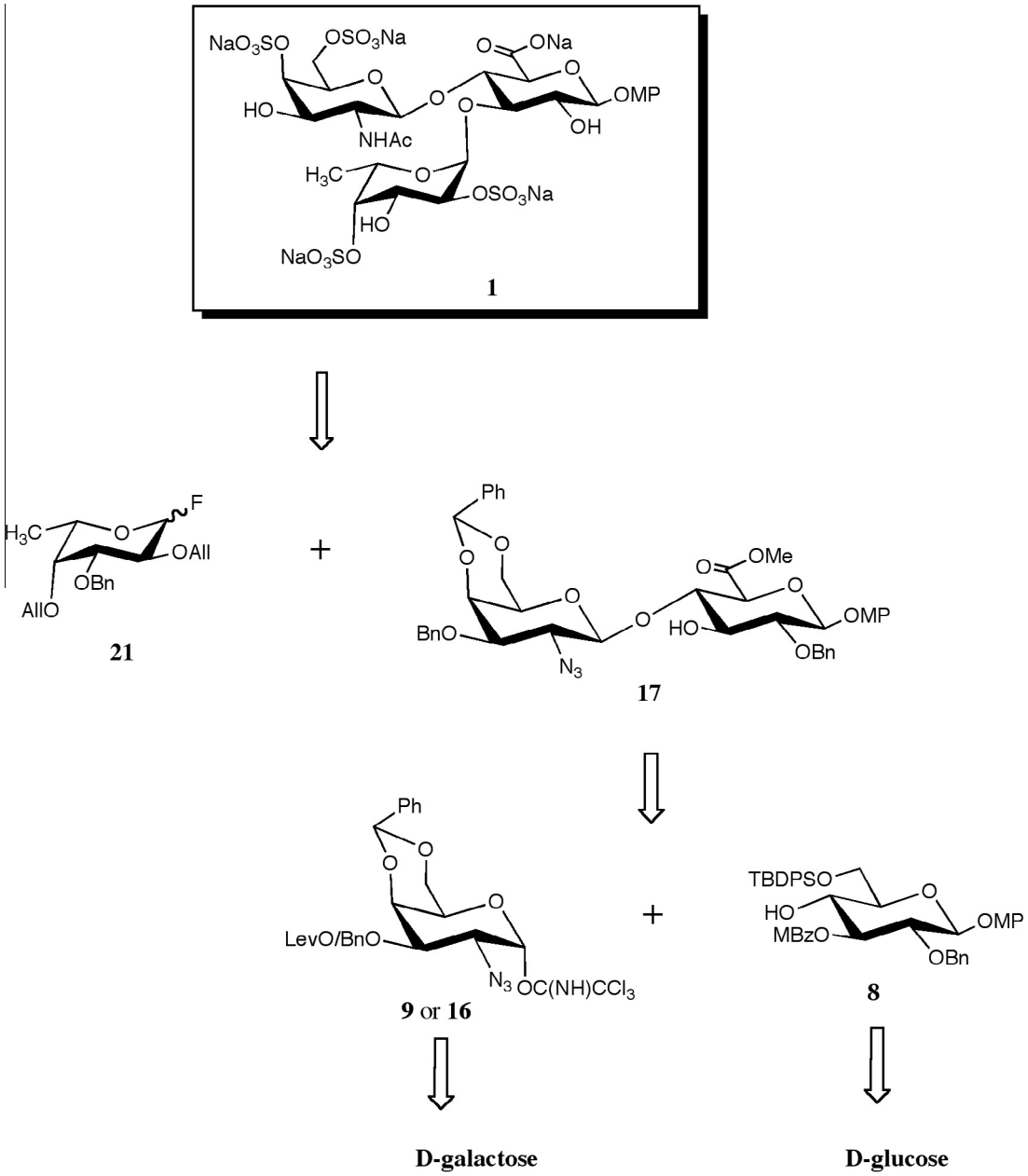
Chondroitin sulfates (CSs), important glycosaminoglycans, have numerous biological activities represented by neuronal growth control.[1,2] Many animals produce CSs as linear polysaccharides composed of a repeating disaccharide unit, -3)-β-D-GalNAc(1-4)-β-D-GlcA(1-, some hydroxyl groups of which are often sulfated. A unique fucosyl chondroitin sulfate (FCS) has been isolated from sea cucumber having a fucose branch at *O*-3 of the GlcA moiety with a variety of sulfation patterns at GalNAc and Fuc residues.[3] Di- or trimeric fucose branches have also been described.[4] FCS inhibits thrombin by activating heparin cofactor II, and also activates factor XII.[5] FCS shows anticoagulant activities even when delivered orally.[6] This remarkable activity is completely different from heparin which is injected intravenously. Medicines having anticoagulant activities are available but can have significant side effects. The sulfation patterns and the role of the fucosyl residues of FCS might be closely related to its biological activities. This prompted us to synthesize the repeating unit of FCS in order to elucidate the relationship between its biological activities and facile structure so as to develop an alternative type of medicine. Here we describe the first synthesis of the FCS trisaccharide, β-D-GalNAc(4,6-diS)(1–4)[α-L-Fuc(2,4-diS)(1–3)]-β-D-GlcA (**1**).

Based on the retrosynthetic analysis depicted in Scheme 1, we planned the fucosylation of a suitably protected chondroitin disaccharide precursor. Subsequent sulfation at specific positions was expected to yield the target compound **1**.

The glycosyl acceptor for obtaining the disaccharide was synthesized as shown in Scheme 2. First, the known diol (**2**)[7] was mono-acetylated with regulated amounts of reagents. The yield was sufficient (73%), but no regioselectivity was shown and the regioisomeric monoacetates (**3a** and **3b**) could not be separated. We tried to benzylate the mixture at the residual positions. To our surprise, a sole compound (**4**) benzylated only at *O*-2 was obtained in 97% yield. We rationalized that the result was due to the basicity of Ag_2O which moved the acetyl group to *O*-3 followed by regioselective benzylation at *O*-2. Similar migration was not observed in the case of the methylbenzoyl (MBz) analogue. The acetate (**4**) was successfully converted to methylbenzoate (**6**) via **5** in 85 and 97% yield, respectively. The benzylidene acetal of **6** was removed in 81% yield and the primary alcohol was selectively protected with TBDPS to yield the glycosyl acceptor (**8**) in 87% yield. Galactosyl imidate (**9**)[8] was stereoselectively coupled with **8** in the presence of $BF_3{\cdot}OEt_2$ in toluene at −50 °C to afford the desired disaccharide (**10**) in 40% yield (Scheme 2). The levulinoyl group of **10** was removed with $H_2NNH_2{\cdot}AcOH$ and the liberated *O*-3 was benzylated with BnBr in the presence of Ag_2O to give **12** in 98 and 79% yield, respectively. Alternatively, we also adopted galactosyl imidate (**16**) masked with a benzyl group at *O*-3 which was obtained from the corresponding hemiacetal (**15**)[9] in 83% yield

* Corresponding author. Tel./fax: +81 857 31 5108.
E-mail address: jtamura@rstu.jp (J. Tamura).

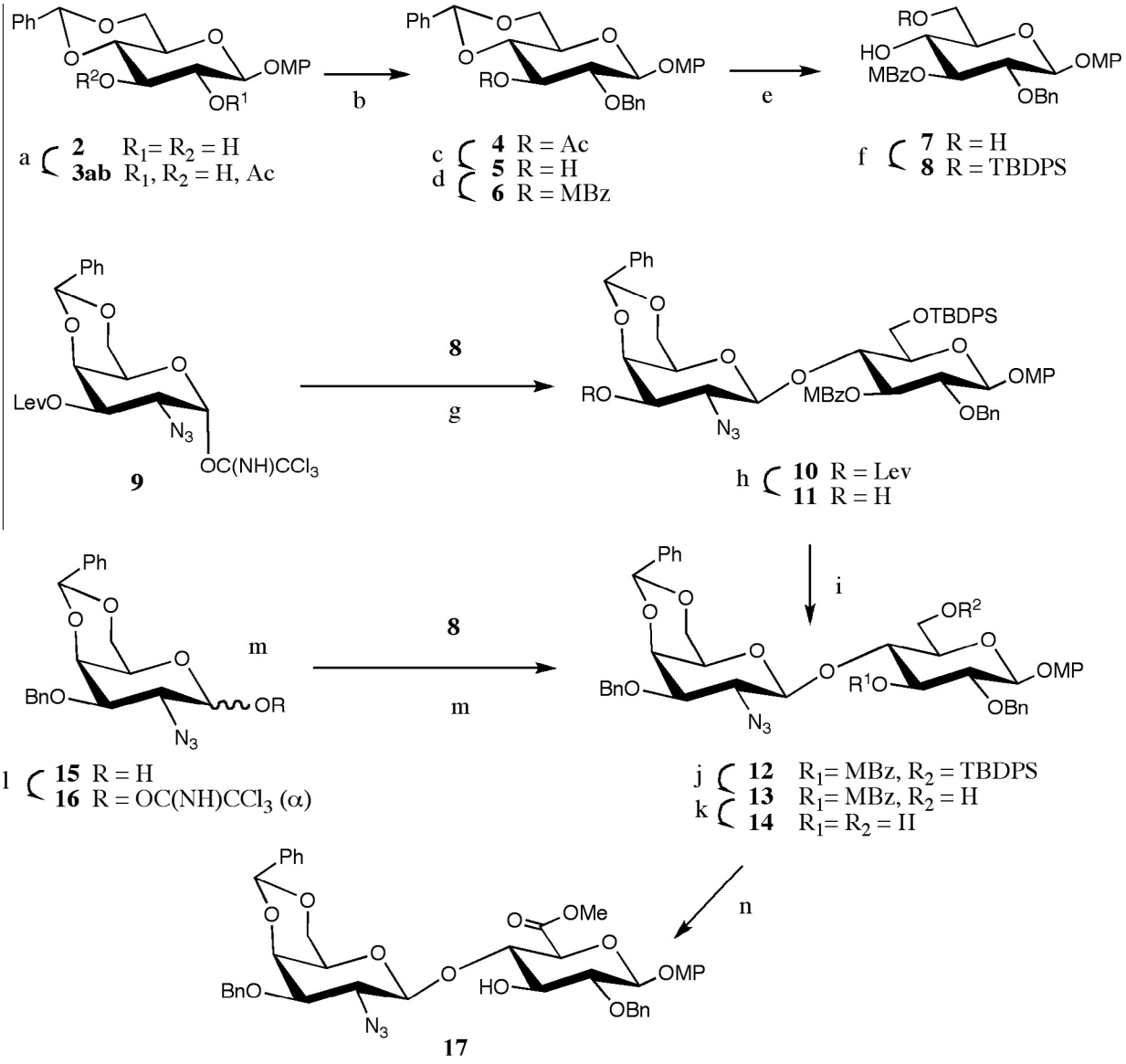
0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.05.064



Scheme 1. Retrosynthetic analysis for the target trisaccharide **1**. Abbreviations: MP, $-C_6H_4OMe(p)$; Bn, $-CH_2Ph$; All, $-CH_2CH{=}CH_2$; TBDPS, $-Si(tert\text{-}Bu)Ph_2$; MBz, $-C(=O)C_6H_4Me(p)$; Lev, $-C(=O)C_2H_4C(=O)Me$.

with complete α-selectivity. The same acceptor (**8**) was coupled with **16** under similar reaction conditions as above. The β-linked disaccharide (**12**) was stereoselectively obtained in 46% yield. The first author has already reported an optimized coupling of **9** and 2-*O*-MBz analogue of **8** in higher yield (69%).[10] The lower coupling yields in this study might be due to the differences of the protecting groups, but should be rationalized. TBDPS and methylbenzoyl groups of **12** were removed stepwise via **13** in 97% and 86% yields, respectively, to give a diol (**14**) (Scheme 2). Regioselective oxidation with TEMPO and NaClO and subsequent $TMSCHN_2$ treatment afforded a methyl ester (**17**) in 86% yield (two steps).

Fucosyl fluoride (**21**) was synthesized from **18**[11] which was converted to the allyl ether (**19**) with AllBr and NaH in 89% yield and the product was subjected to acetolysis. The obtained acetate was easily hydrolyzed at work-up to give **20** in 63% yield. The hemiacetal was converted to the corresponding fluoride (**21**) in 85% yield as an anomeric mixture (Scheme 3). The *OH*-3 at the GlcA moiety was coupled with **21** in the presence of $LiClO_4$ and CsF in CH_2Cl_2.[12] The reaction afforded the desired trisaccharide (**22**) in 63% yield with complete α-selectivity. Corresponding fucosyl imidate and thioglycoside could not give the desired glycoside at all. Allyl ethers of **22** were converted to the corresponding vinyl ethers in the presence of iridium



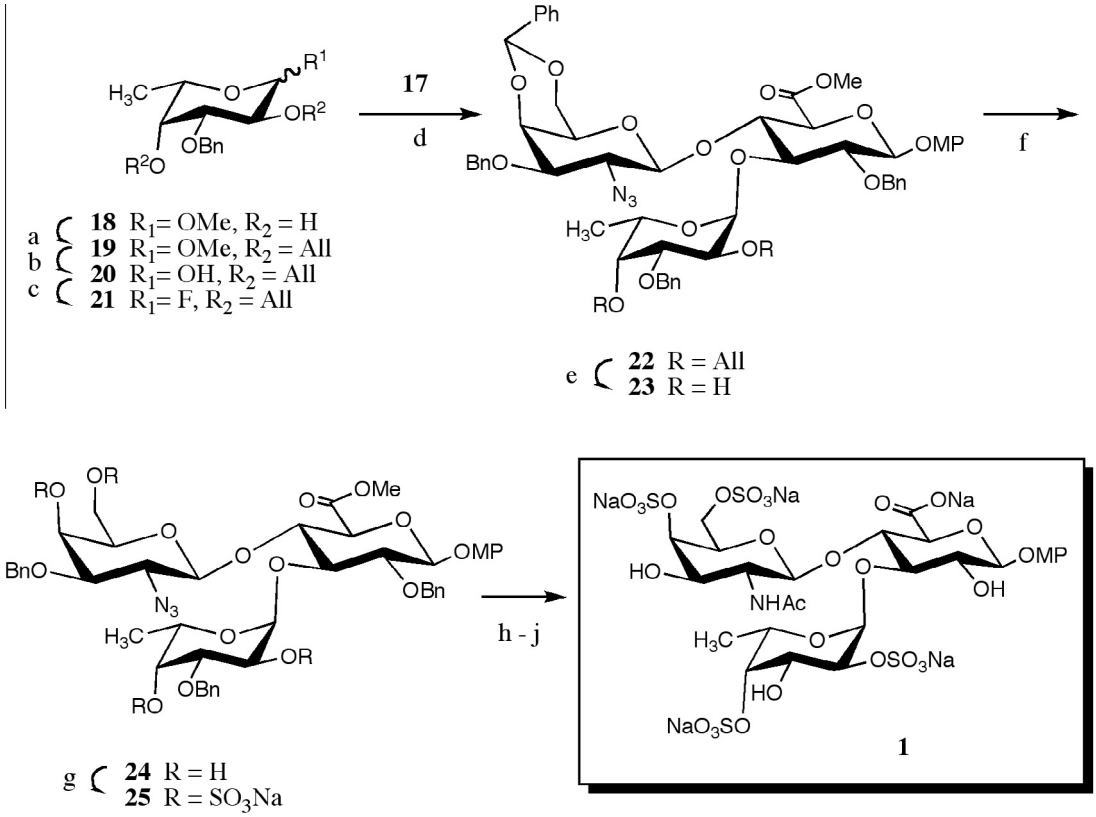
Scheme 2. Synthesis of the glycosyl acceptor **17** and the glycosylation toward the disaccharide moiety. Reagents and conditions: (a) AcCl, pyridine, 73%; (b) BnBr, Ag_2O, KI, DMF, 0 °C–rt, 97%; (c) Et_3N, MeOH, H_2O, 85%; (d) MBzCl, pyridine, 97%; (e) camphorsulfonic acid, CH_2Cl_2–MeOH, 81%; (f) TBDPSCl, imidazole, DMF, 87%; (g) $BF_3{\cdot}OEt_2$, toluene, −50 °C, 40%; (h) $H_2NNH_2{\cdot}AcOH$, toluene–EtOH, 98%; (i) BnBr, Ag_2O, KI, DMF, 0 °C–rt, 79%; (j) *n*-Bu_4NF, THF, 97%; (k) NaOMe, MeOH, 86%; (l) CCl_3CN, DBU, CH_2Cl_2, 0 °C–rt, 83%; (m) $BF_3{\cdot}OEt_2$, toluene, −78 °C, 46%; (n) TEMPO, NaClO, *n*-Bu_4NBr, NaBr, $NaHCO_3$, EtOAc–H_2O, then $TMSCHN_2$, toluene–MeOH, 86%.

complex and subsequent acidic hydrolysis gave **23** in 71% yield. Further acidic hydrolysis afforded **24** in 54% yield. The obtained tetraol was fully sulfated with $SO_3{\cdot}NMe_3$ to give **25** in an almost quantitative yield. Three benzyl ethers were removed by hydrogenolysis in the presence of Pd/C in aq EtOH. The azide was simultaneously reduced to amine which was subsequently acetylated with Ac_2O. $Pd(OH)_2$-catalyzed hydrogenolysis completely reduced the aglycon (4-methoxyphenyl) to a 4-methoxycyclohexyl group. A catalytic amount of AcOH in the presence of Pd/C cleaved the fucosyl residue from the CS backbone. Final saponification of the product and gel-permeation afforded the target compound **1** in 29% yield (from **25**). The final compound was fully characterized by ^1H NMR and ESI-MS.[13]

In summary, we synthesized a fucose-branched chondroitin sulfate trisaccharide, β-D-GalNAc(4,6-diS)(1–4)[α-L-Fuc(2,4-diS)(1–3)]-β-D-GlcA, for the first time. The stepwise coupling of each monosaccharide moiety was achieved with complete stereocontrol.

Acknowledgments

We thank Mrs. Mayumi Ikenari (Research Center for Bioscience and Technology, Division of Instrumental Analysis, Tottori University) for performing the ESI-MS measurement. This study was supported in part by a Grant-in-aid for Scientific Research in Innovative Areas 23110003, MEXT, Japan.



Scheme 3. Coupling of the fucosyl residue and the conversion to the target compound. Reagents and conditions: (a) AllBr, NaH, DMSO, 89%; (b) Ac_2O, AcOH, TFA, 0 °C–rt, 63%; (c) DAST, CH_2Cl_2, −40 °C rt, 85%.; (d) $LiClO_4$, CsF, CH_2Cl_2, −40 °C–rt, 63%; (e) $[Ir(COD)(PPh_2Me)_2]PF_6$, THF, then CSA, CH_2Cl_2–MeOH, 71%; (f) CSA, CH_2Cl_2–MeOH, 54%; (g) $SO_3{\cdot}NMe_3$, DMF, 60 °C, quant; (h) Pd/C, H_2, aq EtOH; (i) Ac_2O, Et_3N, aq EtOH; (j) aq NaOH, 29% (three steps).

References and notes

1. Wang, H.; Katagiri, Y.; McCann, T. E.; Unsworth, E.; Goldsmith, P.; Yu, Z.-X.; Fei, T.; Santiago, L.; Mills, E. M.; Wang, Y.; Symes, A.; Geller, H. M. *J. Cancer Sci.* **2008**, *121*, 3083.
2. Mikami, T.; Yasunaga, D.; Kitagawa, H. *J. Biol. Chem.* **2009**, *284*, 4494.
3. (a) Yoshida, K.; Minami, Y.; Nemoto, H.; Numata, K.; Yamanaka, E. *Tetrahedron Lett.* **1992**, *33*, 4959; (b) Mourão, P. A. S.; Pereira, M. S.; Pavão, M. S. G.; Mulloy, B.; Tollefsen, D. M.; Mowinckel, M.-C.; Abildgaard, U. *J. Biol. Chem.* **1996**, *271*, 23973; (c) Fonseca, R. J. C.; Oliveira, S.-N. M. C. G.; Pomin, V. H.; Mecawi, A. S.; Araujo, I. G.; Mourão, P. A. S. *Thromb. Haemost.* **2010**, *103*, 994.
4. (a) Vieira, R. P.; Mourão, P. A. S. *J. Biol. Chem.* **1988**, *263*, 18176; (b) Kariya, Y.; Watabe, S.; Hashimoto, K.; Yoshida, K. *J. Biol. Chem.* **1990**, *265*, 5081; (c) Kariya, Y.; Watabe, S.; Kyogashima, M.; Ishihara, M.; Ishii, T. *Carbohydr. Res.* **1997**, *297*, 273.
5. Fonseca, R. J. C.; Santos, G. R. C.; Mourão, P. A. S. *Thromb. Haemost.* **2009**, *102*, 829.
6. Fonseca, R. J. C.; Mourão, P. A. S. *Thromb. Haemost.* **2006**, *96*, 822.
7. Slaghek, T.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1992**, *33*, 4971.
8. Goto, F.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 619.
9. Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4697.
10. Tamura, J.; Neumann, K. W.; Ogawa, T. *Liebigs Ann.* **1996**, 1239.
11. (a) Flowers, H. M. *Carbohydr. Res.* **1982**, *99*, 170; (b) Yang, G.; Kong, F.; Zhou, S. *Carbohydr. Res.* **1991**, *211*, 179.
12. Böhm, G.; Waldmann, H. *Tetrahedron Lett.* **1995**, *36*, 3843.
13. ^{1}H NMR assignments were confirmed by two-dimensional HH COSY experiments using BRUKER ADVANCE II 600 MHz spectrometers with *tert*-BuOH as an internal standard (1.23 ppm) in D_2O. As an example of signal assignments, 1^{III} stands for a proton at C-1 of sugar residue III. Positions of the protons in the aglycon are depicted in Scheme 1. Compound **1**: $[\alpha]_D$ −36 (*c* 0.29, H_2O); ^{1}H NMR: δ 7.09–7.07 (m, 2H, Ph), 6.97–6.95 (m, 2H, Ph), 5.69 (d, 1H, $J_{1,2}$ = 3.54 Hz, H-1^{III}), 5.07 (s, 1H, H-4^{III}), 5.05 (m, 1H, H-5^{III}), 5.02 (d, 1H, $J_{1,2}$ = 7.26 Hz, H-1^{I}), 4.72 (d, 1H, $J_{3,4}$ = 2.52 Hz, H-4^{II}), 4.55 (d, 1H, $J_{1,2}$ = 8.34 Hz, H-1^{II}), 4.50 (dd, 1H, $J_{2,3}$ = 10.08 Hz, H-2^{III}), 4.29 (dd, 1H, $J_{5,6a}$ = 4.50 Hz, J_{gem} = 10.68 Hz, H-6^{II}a), 4.20 (dd, 1H, $J_{5,6b}$ = 7.44 Hz, H-6^{II}b), 4.13 (bd, 1H, J = 9.90 Hz, H-3^{III}), 4.08 (bt, 1H, J = 8.28 Hz, H-4^{I}), 4.04 (d, 1H, $J_{4,5}$ = 9.12 Hz, H-5^{I}), 4.00 (bt, 1H, J = 6.12 Hz, H-5^{II}), 3.94 (dd, 1H, $J_{2,3}$ = 10.86 Hz, H-2^{II}), 3.90 (m, 2H, H-2^{I}, 3^{I}), 3.83 (dd, 1H, H-3^{II}), 3.80 (s, 3H, OMe), 2.02 (s, 3H, NAc), 1.37 (d, 1H, $J_{5,6}$ = 6.48 Hz, H-6^{III}). ESI-MS: *m*/*z* calcd for $C_{27}H_{34}NO_{29}S_4Na_6$, 1101.95; found, 1101.94 [M+Na^+].