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Block synthesis of blood group tetrasaccharides B (types 1, 3 and 4)

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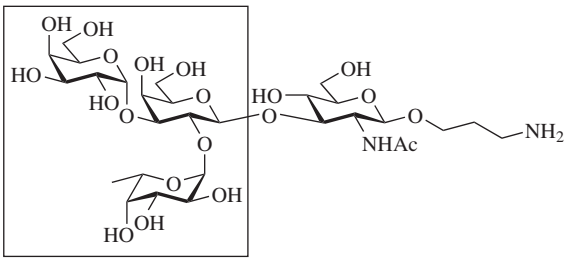
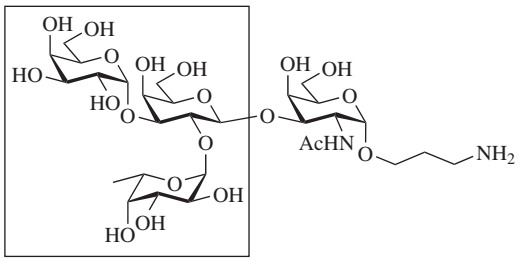
3-Aminopropyl glycosides of the tetrasaccharides Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ (B type 1), Galα1-3(Fucα1-2)-Galβ1-3GalNAcα (B type 3), and Galα1-3(Fucα1-2)Galβ1-3GalNAcβ (B type 4) were synthesised using acetylated Galα1-3-(Fucα1-2)Gal trichloroacetimidate **4** as a glycosyl donor at the key stage.

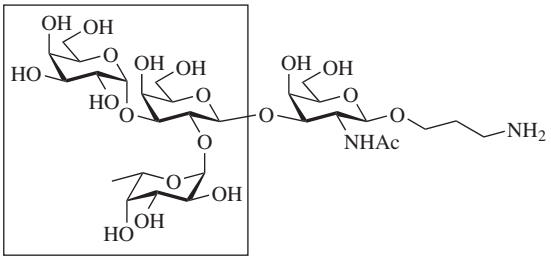
Originally discovered on red cells, the blood group ABO(H) antigens are secreted in saliva and expressed in many human tissues, especially, on endothelial and epithelial cells.[1] They are responsible for hemolytic reactions in blood transfusion and acute rejection in bone marrow and organ transplantation. Their expression varies during cell differentiation, maturation and malignant transformations in cancer and cardio-vascular diseases.[2] Fundamental studies in glycobiology and, moreover, the solution of problems in practical hematology and transplantology require oligosaccharides determining the blood group antigen specificity in amounts that cannot be obtained from natural sources. Thus, the access to pure blood group oligosaccharides fully relies on



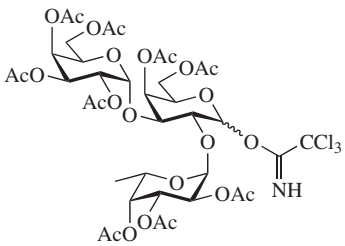
1 B (type 1)



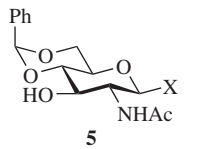
2 B (type 3)



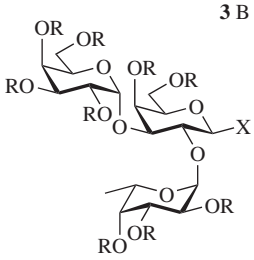
3 B (type 4)



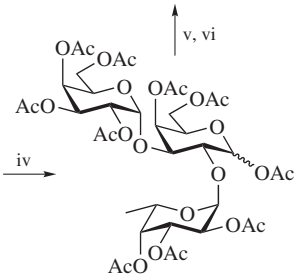
4



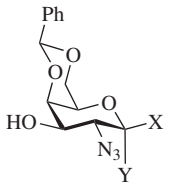
5
X = $O(CH_2)_3NHCOCF_3$



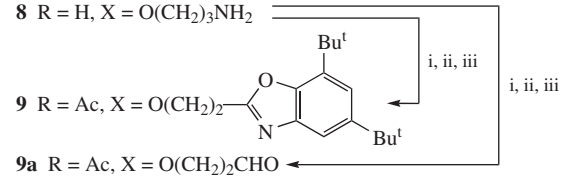
8 R = H, X = $O(CH_2)_3NH_2$



9 iv → **10**

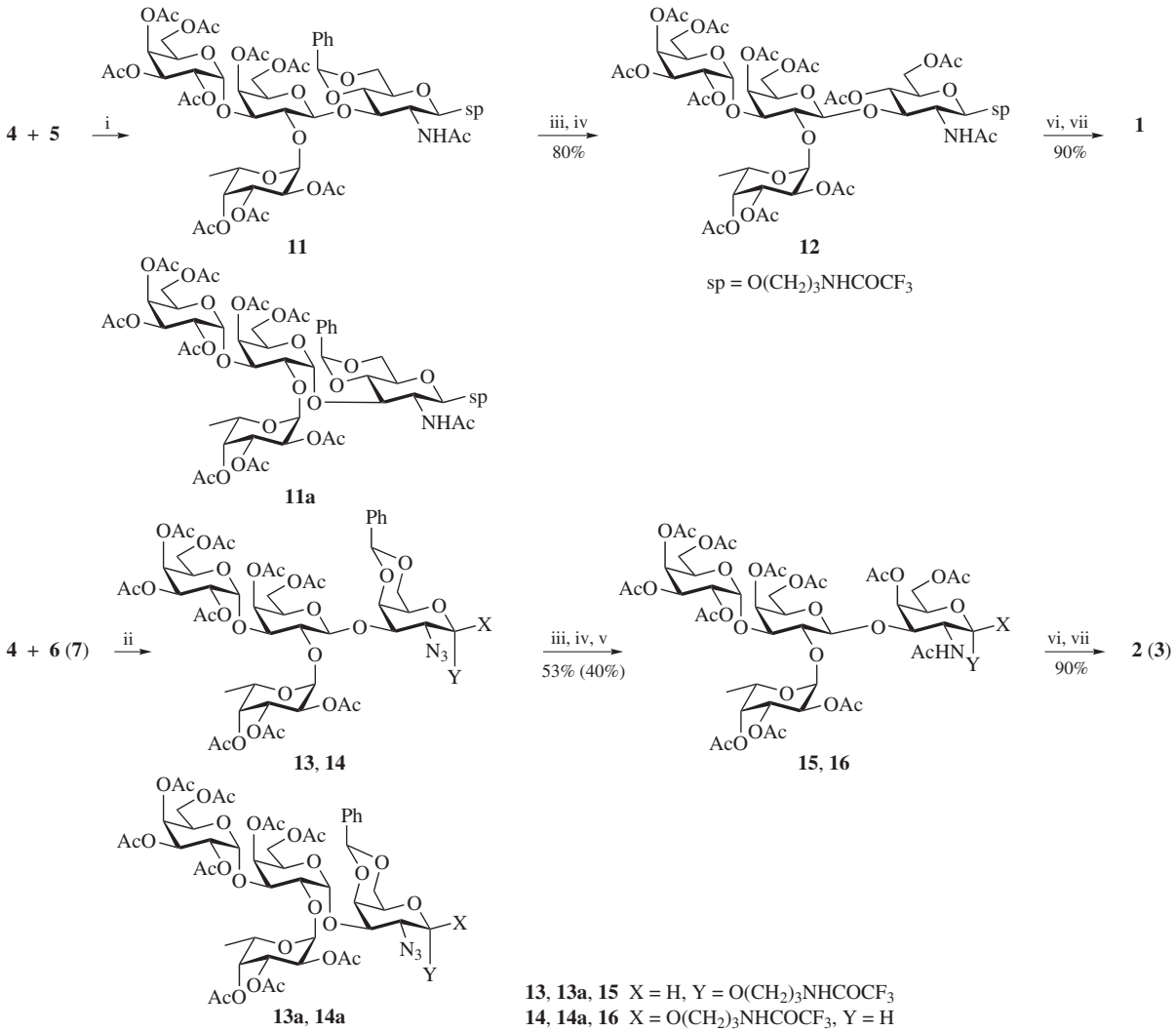


6 X = H, Y = $O(CH_2)_3NHCOCF_3$
7 X = $O(CH_2)_3NHCOCF_3$, Y = H



9 R = Ac, X = $O(CH_2)_2$–
9a R = Ac, X = $O(CH_2)_2CHO$

Scheme 1 Synthesis of trichloroacetimidates of acetylated trisaccharide B. *Reagents and conditions*: i, 3,5-di-*tert*-butyl-1,2-benzoquinone, MeOH; ii, $H_2C_2O_4$; iii, Ac_2O/Py; iv, AcOH–Ac_2O–AcONa, 100 °C; v, N_2H_4·AcOH/DMF, 50 °C; vi, Cl_3CCN, DBU, CH_2Cl_2, –20 °C → room temperature.



Scheme 2 Synthesis of aminopropyl glycosides of B tetrasaccharides (types 1, 3 and 4). *Reagents and conditions*: i, TMSOTf, MeCN–CH_2Cl_2 (1:1), MS 4 Å; ii, TMSOTf, MeCN, MS 4 Å; iii, 80% AcOH, 80 °C; iv, Ac_2O/Py; v, Ph_3P, THF/H_2O, Ac_2O/Py; vi, MeONa, MeOH; vii, NaOH, H_2O.

chemical[3–8] and chemoenzymatic[9,10] syntheses. Here, we report a chemical block-synthetic approach to obtain blood group B tetrasaccharides of various types, which allows us to minimize the number of synthetic steps.

The methodology of stepwise elongation of the carbohydrate chain starting from the reducing end is most often used in the synthesis of blood group tetrasaccharides.[3–7] Only in one paper[8] it is reported that the disaccharide glycosyl donor has been used for the synthesis of blood group tetrasaccharide A (type 3). Blood group tetrasaccharides B **1**–**3**† contain the same structural fragment, trisaccharide B. This allows one to use the block scheme '3 + 1' in their synthesis, where '3' is a glycosyl donor, in particular, acetylated glycosyl trichloroacetimidate of trisaccharide **4** and '1' is the corresponding glucosamine (**5**[11]) or galactosamine (**6**[12] or **7**[12]) glycosyl acceptor. As far as we know, 2,3-branched oligosaccharides have not been used as glycosyl donors for synthesis of 1,2-*trans*-glycosides.

Aminopropyl glycoside **8**[13] has been selected as a precursor for the preparation of the glycoside donor, and the method of aminopropyl spacer elimination published previously[14] has been optimized (Scheme 1). In the cited paper,[14] aldehyde **9a** was obtained in a low total yield (41%) by derivatization of aminopropyl glycoside **5** with 3,5-di-*tert*-butyl-1,2-benzoquinone[15] (BQ), treatment of the resulting azomethine with oxalic acid dihydrate, and acetylation. We have changed the ratio of reagents in azomethine synthesis [**8**:BQ, 1:1.5 (1:1[14])] and increased the pH value upon acidification from pH 2[14] to pH 4, thus affording benzoxazole derivative **9** in 88% yield. The structure of compound **9** was confirmed by mass spectrometry [*m*/*z* 1124 (M^+ + H), 1146 (M^+ + Na)] and ^{1}H NMR spectroscopy [two siglets at δ 1.44 (9H) and 1.49 (9H) related to *tert*-butyl groups and a multiplet at δ 7.2–7.5 (2H) of aromatic protons] data. Base-catalyzed acetolysis/acetylation[14] of benzoxazole **9** gave peracetylated trisaccharide **10** in 90% yield. Then, selective 1-*O*-deacetylation of derivative **10** with hydrazine acetate[16] and treating with trichloroacetonitrile in the presence of 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) gave glycosyl trichloroacetimidate **4**, which was isolated by column chromatography on silica gel [elution: hexane–ethyl acetate (1:1), 1% triethylamine] as a mixture of α/β anomers in 80% yield.

Glycosylation of derivatives **5**, **6**, and **7** (Scheme 2) with trichloroacetimidate **4** was the key stage in tetrasaccharide synthesis. The glycosylation was carried out in acetonitrile (or its mixture with dichloromethane) at room temperature using a two-fold excess of the glycosyl acceptor and trimethylsilyl triflate (0.1 equiv.) as the reaction promoter.

Tetrasaccharides **11**, **13**, and **14** were isolated by column chromatography on silica gel in 52, 60, and 55% yields, respectively. The β-configuration of generated glycosidic bond for compounds **11**, **13**, and **14** was confirmed by ^{1}H NMR spectroscopy including spin decoupling and COSY experiments.‡ α-Isomers of tetrasaccharides type 1 (**11a**) and type 4 (**14a**) were isolated in 4 and 10% yields, respectively. We were not able to isolate α-isomer of tetrasaccharide type 3 present in the mixture in

negligible amount. The high stereoselectivity of glycosylation can be explained primarily by the glycosyl donor structure. The bulky substituent at C-2, acetylated fucose, and conformational rigidity of the molecule due to the presence of two monosaccharide residues in adjacent positions (Fuc at C-2 and Gal at C-3) hinder the nucleophilic attack from α-side, thus resulting in the formation of β-glycosides. Additionally, the use of acetonitrile as the solvent upon glycosylation also affects positively the reaction stereochemistry.[17]

The target aminopropyl glycosides of tetrasaccharides **1**–**3** were obtained by conventional deprotection methods (Scheme 2). The protected oligosaccharides were isolated by column chromatography on silica gel, whereas aminopropyl glycosides **1**–**3** were purified by ion-exchange chromatography on DOWEX (H^+) cationite. The structure of the compounds synthesized was confirmed by ^{1}H NMR (COSY) spectroscopy and mass spectrometry.

Thus, the suggested block scheme is advantageous for the synthesis of blood group B tetrasaccharides. We suppose to use this approach in the further syntheses of the more complex blood group oligosaccharides, in particular, biantennary structures, containing two fragments of B trisaccharide in one molecule.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2009.05.013.

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† This paper describes the synthesis of three tetrasaccharides, in which B-trisaccharide is linked to the hydroxyl group at C-3 of glucosamine (type 1) or galactosamine (type 3 and type 4) *via* the β-glycosidic bond. Synthesis of tetrasaccharide B (type 2), where B-trisaccharide is linked to the 4-OH group of glucosamine, will be described elsewhere.

Spectral characteristics for oligosaccharides. ^{1}H NMR spectra were recorded at 30 °C on Bruker WM 500 MHz and Bruker WM 600 MHz instruments. Mass spectra (MALDI-TOF) were recorded on a VISION 2000 mass spectrometer. Optical rotations were measured on a Perkin Elmer 341 polarimeter at 25 °C.

Symbols of monosaccharide residues for trisaccharides: a – α/β-Gal (reducing end), b – α-Gal, c – α-Fuc, for tetrasaccharides: a – α/β-GalNAc or β-GlcNAc, b – β-Gal, c – α-Gal, d – α-Fuc.

1: ^{1}H NMR (selected data, 600 MHz, 40 °C, D_2O) δ: 1.37 (d, 3H, H-6d, $J_{5,6}$ 6.6 Hz), 2.05–2.12 (m, 2H, CCH_2C), 2.21 (s, 3H, Ac), 3.19–3.23 (m, 2H, NHCH_2), 4.47–4.50 (m, 1H, H-5d), 4.56 (d, 1H, H-1a, $J_{1,2}$ 8.4 Hz), 4.84 (d, 1H, H-1b, $J_{1,2}$ 7.8 Hz), 5.36 (d, 1H, H-1c, $J_{1,2}$ 5.0 Hz), 5.37 (d, 1H, H-1d, $J_{1,2}$ 4.6 Hz). MS, *m*/*z*: 749 (M^+ + H), 771 (M^+ + Na), 787 (M^+ + K). $[\alpha]_D$ –33.2 (*c* 1, H_2O).

2: ^{1}H NMR (selected data, 500 MHz, D_2O) δ: 1.20 (d, 3H, H-6d, $J_{5,6}$ 6.4 Hz), 1.95–2.02 (m, 2H, CCH_2C), 2.06 (s, 3H, Ac), 3.04–3.15 (m, 2H, NHCH_2), 3.49–3.56 (m, 1H, OC*H*H), 4.70 (d, 1H, H-1b, $J_{1,2}$ 7.4 Hz), 4.90 (d, 1H, H-1a, $J_{1,2}$ 3.0 Hz), 5.25 (d, 1H, H-1c, $J_{1,2}$ 2.6 Hz), 5.27 (d, 1H, H-1d, $J_{1,2}$ 4.2 Hz). MS, *m*/*z*: 749 (M^+ + H), 771 (M^+ + Na), 787 (M^+ + K). $[\alpha]_D$ +81.0 (*c* 0.5, H_2O).

3: ^{1}H NMR (selected data, 600 MHz, D_2O) δ: 1.24 (d, 3H, H-6d, $J_{5,6}$ 6.4 Hz), 1.92–2.02 (m, 2H, CCH_2C), 2.09 (s, 3H, Ac), 3.10–3.13 (m, 2H, NCH_2), 4.36 (d, 1H, H-1a, $J_{1,2}$ 7.9 Hz), 4.72 (d, 1H, H-1b, $J_{1,2}$ 7.6 Hz), 5.27 (d, 1H, H-1c, $J_{1,2}$ 3.1 Hz), 5.29 (d, 1H, H-1d, $J_{1,2}$ 4.1 Hz). MS, *m*/*z*: 749 (M^+ + H), 771 (M^+ + Na), 787 (M^+ + K). $[\alpha]_D$ +5.0 (*c* 0.5, H_2O).

‡ **11**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.30 (d, 3H, H-6d, $J_{5,6}$ 6.4 Hz), 1.90, 1.95, 1.98, 2.01, 2.06, 2.08, 2.14, 2.16, 2.17, 2.19 (10s, 10×3H, 10Ac), 3.14–3.18 (m, 1H, H-2a), 3.17–3.25 (m, 1H, NHC*H*H), 3.43–3.50 (m, 1H, OC*H*H), 3.56 (dd, 1H, H-2b, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 10.0 Hz), 3.59–3.64 (m, 1H, H-5a), 3.63–3.70 (m, 1H, NHCH*H*), 3.70 (dd, 1H, H-3b, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.8 Hz), 3.78–3.87 (m, 3H, H-3a, H-4a, H-5a), 3.88–3.92 (m, 1H, H-5b), 3.95–4.04 (m, 2H, H-6′c, OCH*H*), 4.05–4.13 (m, 2H, H-6′a, H-6′b), 4.28–4.36 (m, 2H, H-6c, H-6b), 4.37 (d, 1H, H-1a, $J_{1,2}$ 8.5 Hz), 4.45–4.49 (m, 1H, H-5c), 4.53 (d, 1H, H-1b, $J_{1,2}$ 7.7 Hz), 4.57–4.62 (m, 1H, H-5d), 4.98–5.12 (m, 2H, H-2d, H-3d), 5.24 (dd, 1H, H-4b, $J_{3,4}$ 2.8 Hz, $J_{4,5}$ < 1 Hz), 5.30 (dd ≈ br. s, 1H, H-4d), 5.34 (d, 1H, H-1c, $J_{1,2}$ 3.8 Hz), 5.36 (dd, 1H, H-2c, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.9 Hz), 5.45 (dd, 1H, H-3c, $J_{2,3}$ 10.9 Hz, $J_{4,3}$ 3.2 Hz), 5.46 (d, 1H, H-1d, $J_{1,2}$ 2.9 Hz), 5.52 (s, 1H, PhC*H*), 5.58 (dd, 1H, H-4c, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ < 1 Hz), 7.02 (d, 1H, N*H*Ac, $J_{NH,2}$ 7.6 Hz), 7.35–7.48 (m, 5H, Ph), 7.91–7.96 [m, 1H, NHC(O)CF_3].

13: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.98 (d, 3H, H-6d, $J_{5,6}$ 6.6 Hz), 1.93, 1.99, 2.00, 2.04, 2.08, 2.09, 2.12, 2.14, 2.15 (9s, 9×3H, 9Ac), 3.49–3.65 (m, 3H, NHCH_2, OC*H*H), 3.70–3.74 (m, 1H, H-5a), 3.80–3.85 (m, 2H, H-2b, H-5b), 3.90–3.97 (m, 2H, H-3b, OCH*H*), 4.06–4.29 (m, 8H, H-2a, H-3a, H-6a, H-6′a, H-6b, H-6′b, H-6c, H-6′c), 4.39 (dd, 1H, H-4a, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ < 1 Hz), 4.41–4.45 (m, 1H, H-5c), 4.45–4.51 (m, 1H, H-5d), 4.83 (d, 1H, H-1b, $J_{1,2}$ 7.2 Hz), 5.11 (d, 1H, H-1a, $J_{1,2}$ 3.4 Hz), 5.19–5.26 (m, 3H, H-2d, H-3d, H-4d), 5.30 (dd, 1H, H-2c, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.9 Hz), 5.36 (d, 1H, H-1c, $J_{1,2}$ 3.6 Hz), 5.38 (dd, 1H, H-3c, $J_{2,3}$ 10.9 Hz, $J_{3,4}$ 3.0 Hz), 5.47 (dd ≈ br. s, 1H, H-4b), 5.58 (dd, 1H, H-4c, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ < 1 Hz), 5.63 (s, 1H, C*H*Ph), 5.65 (d, 1H, H-1d, $J_{1,2}$ 3.4 Hz), 6.89–6.93 [m, 1H, NHC(O)CF_3], 7.31–7.58 (m, 5H, Ph). MS, *m*/*z*: 1317 (M^+ + Na), 1333 (M^+ + K). $[\alpha]_D$ +75.2 (*c* 1, $CHCl_3$).

14: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.96 (d, 3H, H-6d, $J_{5,6}$ 6.4 Hz), 1.91–1.97 (m, 2H, CCH_2C), 1.92, 1.98, 1.99, 2.05, 2.06, 2.07, 2.10, 2.14, 2.15 (9s, 9×3H, 9Ac), 3.43–3.45 (m, 1H, H-5a), 3.46–3.62 (m, 2H, NHCH_2), 3.69 (dd, 1H, H-3a, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.4 Hz), 3.71–3.75 (m, 1H, OC*H*H), 3.76–3.81 (m, 2H, H-2b, H-5b), 3.90 (dd, 1H, H-3b, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.0 Hz), 4.01–4.06 (m, 1H, OCH*H*), 4.07–4.19 (m, 6H, H-2a, H-6′a, H-6b, H-6′b, H-6c, H-6′c), 4.25 (dd, H-4a, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ < 1 Hz), 4.28–4.32 (m, 1H, H-6a), 4.38–4.47 (m, 3H, H-1a, H-5c, H-5d), 5.01 (d, 1H, H-1b, $J_{1,2}$ 7.4 Hz), 5.18 (dd, 1H, H-3d, $J_{2,3}$ 10.9 Hz, $J_{3,4}$ 3.2 Hz), 5.22 (dd, 1H, H-4d, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ < 1 Hz), 5.25 (dd, 1H, H-2d, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.9 Hz), 5.28 (dd, 1H, H-2c, $J_{1,2}$ 3.2 Hz, $J_{2,3}$ 10.7 Hz), 5.34 (d, 1H, H-1c, $J_{1,2}$ 3.2 Hz), 5.37 (dd, 1H, H-3c, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 3.1 Hz), 5.43 (dd, 1H, H-4b, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ < 1 Hz), 5.57 (dd, 1H, H-4c, $J_{3,4}$ 3.1 Hz, $J_{4,5}$ < 1 Hz), 5.60 (s, 1H, C*H*Ph), 5.64 (d, 1H, H-1d, $J_{1,2}$ 3.6 Hz), 7.02–7.08 [m, 1H, NHC(O)CF_3], 7.33–7.54 (m, 5H, Ph). MS, *m*/*z*: 1317 (M^+ + Na), 1333 (M^+ + K). $[\alpha]_D$ +1.2 (*c* 1, $CHCl_3$).

For spectral characteristics of compounds **4**, **9**, **10**-α, **10**-β, **12**, **15** and **16**, see Online Supplementary Materials.