Synthesis of Fluorinated Analogues of Tumor-Associated Carbohydrate and Glycopeptide Antigens

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Abstract: Partial structures of tumor-associated mucin glycoproteins are interesting target structures for the development of selective anticancer vaccines. To probe the effect of fluorination on the immunological and metabolic properties of mucin glycopeptides, six novel fluorinated glycosyl–threonine conjugates have been synthesized. The synthesis of the orthogonally protected glycosyl amino acids was achieved using microwave irradiation in key fluorination and glycosylation steps. The 2′-deoxy-2′-fluoro- and 6′-deoxy-6′-fluoro-T antigen building blocks were applied to the synthesis of analogues of MUC1 tandem repeat-glycopeptide antigens via SPPS.

Key words: fluorinated carbohydrates, glycopeptide antigens, MUC1, solid-phase synthesis, microwave-assisted glycosylations

Cell-surface glycoconjugates have received much attention in recent years owing to their crucial role in mediating important cellular recognition processes.[1] For example, the potential of mucin glycopeptide antigens, resulting from aberrant glycosylation of tumor cells,[2] as selective anticancer vaccines has been demonstrated in mouse models.[3] However, the immunogenicity of the tumor-associated carbohydrate antigens is low, and these structures are readily metabolized by glycosidases. Both factors oppose the development of efficient synthetic anticancer vaccines. An interesting approach to overcome these limitations is the use of hydrolytically stable glycopeptide mimics for synthetic tumor-associated antigen analogues.

Fluorinated carbohydrate derivatives have emerged as powerful tools for providing insight into the specificity of carbohydrate binding to a variety of proteins.[4] Fluorosugars often retain much of the reactivity of natural saccharides, with particular reference to polarity and structural integrity. However, the presence of fluorine atoms close to the anomeric center modulates the reactivity of the glycosidic bond and stabilizes it against hydrolysis.[5] Despite examples of antibody–antigen complexes with fluorinated saccharides,[6] the use of such compounds in the development of carbohydrate-based vaccines has not been studied. Thus, besides a number of methods for the synthesis of fluorinated mono- and oligosaccharides,[6,7] including mucin-like core structures,[8] only few examples of fluorinated glycosyl amino acids can be found in the literature.[5c,9]

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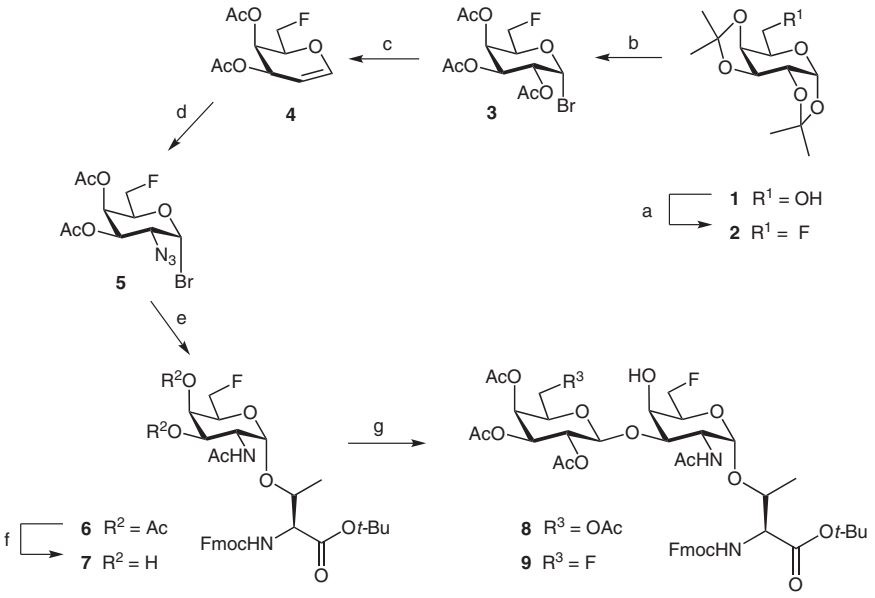
We here describe the synthesis of a first series of deoxyfluoro analogues of the tumor-associated T_N, T, and 2,6-sialyl-T antigens. The assembly of the fluorinated glycosyl amino acids was accomplished by stepwise extension of the saccharide chain.[10] The synthesis of the 6-deoxy-6-fluoro-T_N antigen analogue **6** started from the known precursor **2**[11] for which a convenient and scalable microwave-assisted fluorination procedure was developed (Scheme 1). Thus, reacting 1,2:3,4-di-*O*-isopropylidene galactose (**1**) with (diethylamino)sulfur trifluoride (DAST) in the presence of 2,6-collidine[11a] under microwave irradiation (100 W[12]) led to the desired product **2** in 84% yield within one hour. By contrast, the corresponding thermal reaction of **1** with DAST only furnished **2** in a markedly reduced yield (45%, 2.5 h). Conversion of **2** into galactosyl bromide **3**[5b,13] and subsequent reductive elimination of the 1-bromo and 2-acetoxy groups afforded the 6-deoxy-6*F*-galactal (**4**, 63% over 4 steps). Azidonitration[14] and installation of the anomeric bromide furnished galactosyl donor **5** for $Ag_2CO_3/AgClO_4$-promoted conjugation[15] to Fmoc-Thr-O*t*-Bu.[16] Under these conditions predominant formation of the desired α-configured conjugate was observed. Finally, reductive acetylation and de-O-acetylation under Zemplén conditions at pH 8.5[17] yielded T_N analogue **7** in 35% over three steps.

Compound **7** was then subjected to a selective 3-β-galactosylation with known αAc_4Gal-trichloroacetimidate donor[18] to provide the 6-deoxy-6-fluoro-T antigen analogue **8** in 42%. The corresponding 6,6′-difluoro-analogue **9** was also obtained from acceptor **7**. In this case, however, best results of the desired disaccharide were obtained using 6′-fluoro-galactosyl donor **3** and $Hg(CN)_2$ as promoter in a microwave-assisted glycosylation reaction[19] (Scheme 1). Thus, under microwave irradiation (80 °C, 4 h, 100 W) clean conversion to the β-configured disaccharide **9** (73%, β-anomer) was observed, while conventional heating mainly led to decomposition of the donor. It is remarkable, that the corresponding galactosylation using the nonfluorinated galactosyl bromide proceeded smoothly at room temperature[10] without microwave support.

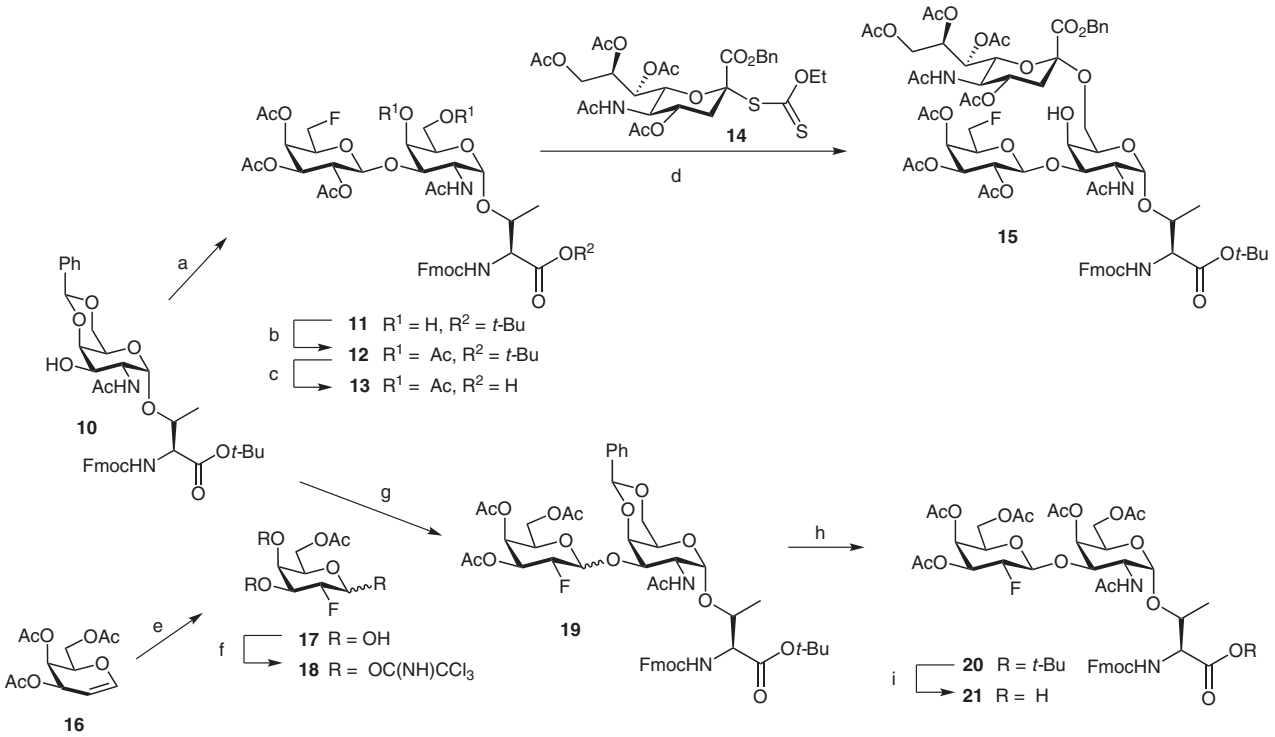
In a similar manner, the 6′-deoxy-6′-fluoro-T antigen analogue **11** was prepared in an excellent yield by microwave-assisted 3-β-galactosylation of Fmoc-Thr(α4,6-*O*-Bzn-GalNAc)-O*t*-Bu (**10**)[20] with **3** (Scheme 2). Again, the desired β-anomer was obtained without significant formation of orthoester or α-configured byproducts. Subsequent



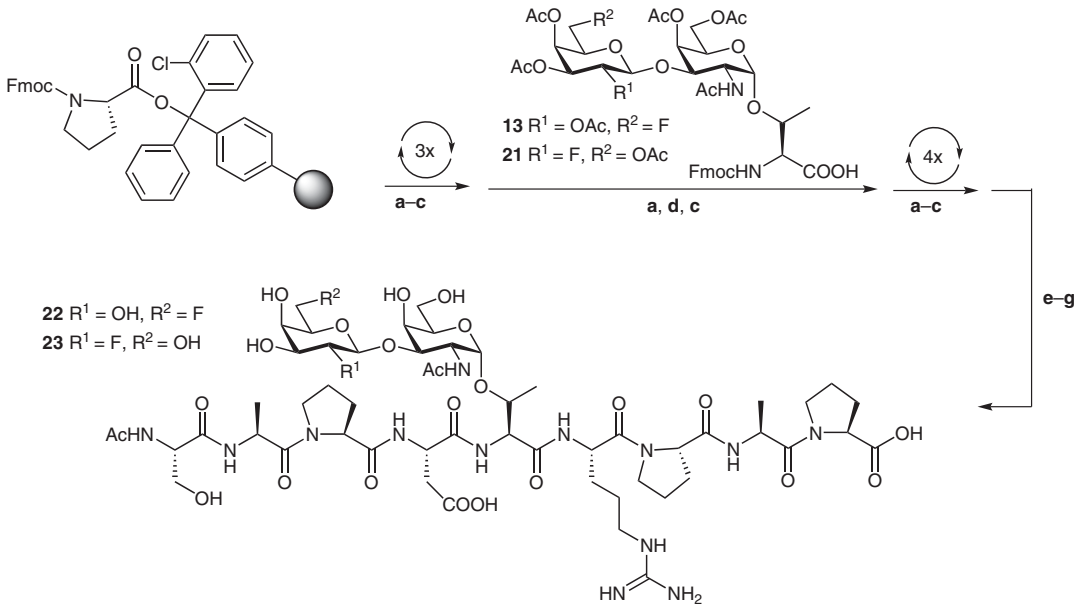
Scheme 1 *Reagents and conditions*: (a) i. DAST, 2,6-collidine, MW 100 W, 80 °C, 1 h, 84%; b) i. AcOH, reflux, then Ac_2O–pyridine (1:2), 99%; ii. HBr/AcOH, CH_2Cl_2, 0 °C to r.t., 71%; (c) Zn, *N*-methylimidazole, EtOAc, reflux, 89%; (d) i. CAN, NaN_3, MeCN, –18 °C, 47%; ii. LiBr, MeCN, 67%; (e) i. Fmoc-Thr-O*t*-Bu, Ag_2CO_3/$AgClO_4$, MS 4 Å, toluene–CH_2Cl_2 (1:1), 0 °C to r.t., 59% (α-anomer); ii. Zn, THF–Ac_2O–AcOH (3:2:1), 75%; (f) NaOMe (cat.), MeOH, pH 8.5, 78%; (g) αAc_4Gal-trichloroacetimidate, TMSOTf (cat.), MS 4 Å, CH_2Cl_2, –20 °C to r.t., 54% (**8**, β-anomer); **3**, $Hg(CN)_2$, $MeNO_2$–CH_2Cl_2 (3:2), MS 4 Å, MW 100 W, 80 °C, 4 h, 73% (**9**, β-anomer).

cleavage of the benzylidene acetal furnished disaccharide **11** (72% over 2 steps), which after regio- and stereoselective sialylation reaction at 6-OH with sialyl xanthate **14**[21] furnished fluorinated α2,6-sialyl-T antigen analogue **15** in 42% yield. Besides, **11** was converted into glycopeptide building block **13** after acetylation and liberation of the carboxy group (79%, 2 steps).

Antigen analogues with fluoro substituents in 2-position of the galactose moiety are particularly interesting, as the



Scheme 2 *Reagents and conditions*: (a) i. **3**, $Hg(CN)_2$, $MeNO_2$–CH_2Cl_2 (3:2), MS 4 Å, MW 100 W, 80 °C, 1 h, 98% (β-anomer); ii. I_2, MeOH, reflux, 73%; (b) Ac_2O–pyridine (1:2), 60 °C, 81%; (c) TFA–anisole (10:1), CH_2Cl_2, 97%; (d) MeSBr, AgOTf, MS 4 Å, MeCN–CH_2Cl_2 (2:1), –40 °C to r.t., 43% (α-anomer); (e) Selectfluor, $MeNO_2$–H_2O (4:1), MW 100 W, 108 °C, 2 min, 66%; (f) CCl_3CN, DBU, CH_2Cl_2, 71%; (g) TMSOTf, MS 4 Å, CH_2Cl_2, 70% (α/β = 2:1); (h) i. I_2, MeOH, 60 °C; ii. Ac_2O–pyridine (1:2), 70% over 2 steps; (i) TFA–anisole (10:1), CH_2Cl_2, 88%.



Scheme 3 *Reagents and conditions*: (a) piperidine–NMP (20%); (b) Fmoc-AA-OH (10 equiv), HBTU, HOBt, DIPEA, NMP; (c) Ac_2O–DIPEA–HOBt (4:1:0.12); (d) **13**/**21**, HATU, HOAt, NMM, NMP, 9 h; (e) AcOH–CF_3CH_2OH–H_2O (1:1:8), r.t., 1 h; (f) TFA–*i*-Pr_3SiH–H_2O (15:0.9:0.9), r.t., 2 h; (g) NaOMe, MeOH, 0 °C to r.t., 24 h, 41% (**22**) and 18% (**23**).

stabilizing influence of the fluorine atom on the rate of hydrolysis will be maximal. As a consequence, 2-deoxy-2-fluoroglycosides represent powerful mechanism-based inhibitors for retaining β-glycosidases.[22] Following published literature reports on 2-deoxy-2-fluoro-glycosylations,[9a,b,23] we relied upon the use of known trichloroacetimidate derivative **18**[9b] for the preparation of the 2′-deoxy-2′-fluoro-T antigen derivative **20** (Scheme 2). The latter can be conveniently prepared by electrophilic fluorination of 3,4,6-tri-*O*-acetylgalactal **16** with Selectfluor.[24] Again, microwave irradiation proved beneficial with regard to reaction time. Hence, 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluorogalactose (**17**) was obtained in 66% yield within minutes at 108 °C (100 W) as compared to 63% yield after 6.5 hours of reflux without microwave support. Compound **17** was then transformed into **18** using DBU in CH_2Cl_2 (71%). In the subsequent glycosylation, formation of the α-anomer of **19** was favored (α/β, 2:1), due to electronic effects and the nonparticipating character of the fluorine substituent. However, chromatographic separation of the desired β-anomer was possible after exchange of the benzylidene acetal with acetyl groups (β-**20**,[25] 16% over 3 steps). Acidolytic cleavage of the *tert*-butyl ester finally provided analogue **21** (88%) for glycopeptide synthesis.

Both T-antigen analogues **13** and **21** were incorporated into a MUC1 tandem repeat-peptide sequence comprising the immunodominant PDTRP epitope[26] (SAPDTRPAP) by automated solid-phase peptide synthesis[27] (SPPS) according to Fmoc strategy.[28] Using the commercial PS-2-CT polystyrene resin[29] equipped with the trityl linker and preloaded with Fmoc-proline, coupling of the amino acids was achieved by HBTU/HOBt[30] and DIPEA in NMP (Scheme 3). The fluorinated T antigen–threonine building blocks **13** and **21** were coupled over a period of 9 hours using HATU/HOAt[31] and NMM for activation. After Fmoc deprotection of the *N*-terminal Ser residue, the nonapeptides were acetylated on-bead and detached from the resin by treatment with HOAc/CF_3CH_2OH. Complete deprotection using a sequence of acidolysis and de-O-acetylation furnished the desired glycopeptides **22**[32] and **23** in overall yields of 41% and 18% (based on the loaded resin) after HPLC purification.

In summary, we have prepared a series of novel orthogonally protected glycosyl amino acids with one or two fluoro substituents within the glycan portion for solid-phase glycopeptide synthesis. By incorporating these molecules into mucin peptide sequences, as shown for compounds **13** and **21**, tumor-associated glycopeptide antigen analogues were obtained. These compounds are now available for investigations of their metabolic stability and their immunological properties.

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(19) **Typical Experimental Procedure**
A solution of acceptor **7** (150 mg, 0.25 mmol) in anhyd $MeNO_2$–CH_2Cl_2 (3:2, 4 mL) was stirred with $Hg(CN)_2$ (125 mg, 0.49 mmol) and activated pulverized MS 4 Å (200 mg) for 30 min under argon. A solution of donor **3** (185 mg, 0.49 mmol) in anhyd $MeNO_2$–CH_2Cl_2 (3:2, 4 mL) was added, and the reaction mixture was irradiated in a microwave reactor for 5 h (80 °C, 100 W), diluted with CH_2Cl_2 (20 mL), and filtered through Hyflo Supercel. The filtrate was washed with sat. aq $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography on silica gel (cyclohexane–EtOAc, 5:1) afforded **9** as a colorless, amorphous solid (162 mg, 73%); R_f = 0.53 (cyclohexane–EtOAc, 5:1); $[\alpha]_D^{23}$ 39.42 (*c* 1, $CHCl_3$).

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(25) **Compound β-20**
$[\alpha]_D^{23}$ = 55.74 (*c* 1, $CHCl_3$); t_R = 14.6 min [Perfectsil C18, grad.: MeCN–H_2O, (50:50) → (90:10), 30 min → (100:0), 10 min]. ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 5.43 (d, 1 H, 4-H, $J_{3,4}$ = 2.0 Hz), 5.36–5.32 (m, 1 H, 4′-H), 5.10–5.00 (m, 1 H, 3′-H), 4.87 (d, 1 H, 1-H, $J_{1,2}$ = 3.5 Hz), 4.65 (dt, 1 H, 2-H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.6 Hz), 4.60–4.37 (m, 4 H, 1′-H {4.57}, 2′-H, CH_2 (Fmoc) {4.52}), 4.29–4.08 (m, 6 H, T^{α} {4.23}, T^{β} {4.16}, 6a-H {4.11}, 5′-H {4.13}, 6a′-H {4.21}, 9-H (Fmoc) {4.22}), 4.03–3.84 (m, 3 H, 5-H, 6b-H, 6b′-H), 4.03–3.94 (m, 1 H, 3′-H), 3.79 (dd, 1 H, 3-H, $J_{3,4}$ = 3.2 Hz, $J_{2,3}$ = 11.0 Hz), 2.12 [s, 3 H, CH_3(Ac)], 2.10 [s, 3 H, CH_3(Ac)], 2.03 [s, 9 H, 3 × CH_3(Ac)], 1.97 [s, 3 H, CH_3(NHAc)], 1.44 [s, 9 H, CH_3(*t*-Bu)], 1.28 (d, 3 H, T^{γ}, $J_{T\gamma,T\beta}$ = 6.1 Hz) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, BB, HMQC): δ = 101.9 (d, C1′, $J_{C1',F}$ = 23.5 Hz), 100.3 (C1), 87.8 (d, C2′, $J_{C2',F}$ = 186.2 Hz), 83.2 [Cq(*t*-Bu)], 77.5 (C3), 77.2 (T^{β}), 70.8 (d, C3′, $J_{C3',F}$ = 19.1 Hz), 70.5 (C5), 69.0 (C4), 68.1 (C5′), 67.2 (d, C4′, $J_{C4',F}$ = 8.0 Hz,), 66.8 (CH_2-Fmoc), 63.2 (C6′), 60.8 (C6) 59.0 (T^{α}), 48.0 (C2), 47.2 (CH-Fmoc), 28.0 [CH_3(*t*-Bu)], 23.0 [CH_3(NHAc)], 20.8, 20.7, 20.6, 20.5, 20.5 [CH_3(Ac)], 18.5 (T^{γ}) ppm. ESI-MS (pos. ion mode): *m/z* calcd for $C_{47}H_{59}FN_2NaO_{19}$: 997.36; found: 997.34 $[M + Na]^+$.

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(32) **Compound 22**
$[\alpha]_D^{25}$ = –97.61 (*c* 1, $CHCl_3$); t_R = 21.2 min [Luna C18, grad.: MeCN–H_2O + 0.1% TFA, (5:95) → (50:50), 80 min → (100:0), 20 min]. ^{1}H NMR (400 MHz, DMSO-d_6, COSY): δ = 8.25 (d, 1 H, R^{NH}, $J_{NH,\alpha}$ = 7.2 Hz), 8.21 (d, 1 H, D^{NH}, $J_{NH,\alpha}$ = 8.7 Hz), 8.10 (d, 1 H, A^{NH}, $J_{NH,\alpha}$ = 7.3 Hz) 8.00 (d, 1 H, A^{NH}, $J_{NH,\alpha}$ = 7.3 Hz), 7.92 (d, 1 H, S^{NH}, $J_{NH,\alpha}$ = 8.0 Hz), 7.61–7.53 (m, 2 H, T^{NH} {7.56}, R^{Gua} {7.58}), 6.93 (d, 1 H, NH-GalNAc, $J_{NH,2}$ = 8.7 Hz), 4.69–4.62 (m, 2 H, 1-H {4.67}, D^{α} {4.65}), 4.58 (m, 1 H, 6′a-H), 4.54-4.43 (m, 3 H, A^{α} {4.51}, R^{α} {4.47}, 6′b-H {4.47}), 4.43–4.36 (m, 2 H, A^{α}

{4.40}, T^{α} {4.39}), 4.36–4.29 (m, 2 H, 2 × P^{α} {4.33}, {4.31}), 4.28–4.23 (m, 2 H, S^{α} {4.26}, 1′-H {4,24}), 4.20 (dd, 1 H, P^{α}, $J_{\alpha,\beta a}$ = 4.4 Hz, $J_{\alpha,\beta b}$ = 8.7 Hz), 4.17–4.12 (m, 1 H, 2-H), 4.11–4.08 (m, 1 H, T^{β}), 3.89–3.85 (m, 1 H, 4-H), 3.76–3.60 (m, 3 H, 5′-H {3.69}, 5-H {3.65}, 4′-H {3.63}), 3.61–3.38 (m, 11 H, S^{β} {3.52}, 3 × P^{δ} {3.54}, 6a-H {3.43}, 6b-H {3.41}, 3-H {3.57}), 3.35–3.28 (m, 2 H, 2′-H {3.52}, 4′-H {3.30}), 3.15–3.01 (m, 2 H, R^{δ}), 2.75 (dd, 1 H, $D^{\beta a}$, $J_{\beta a,\alpha}$ = 6.4 Hz, $J_{\beta a,\beta b}$ = 16.5 Hz), 2.49 (m, 1 H, $D^{\beta b}$, under DMSO-d_6), 2.18–2.09 (m, 1 H, $P^{\beta a}$), 2.07–1.97 (m, 2 H, 2 × $P^{\beta a}$, {2.06}, {2.01}), 1.94–1.74 [m, 15 H, 6 × P^{γ} {1.91}, {1.88}, {1.83}, 3 × $P^{\beta b}$ {1.84}, {1.81}, {1.78}, 2 × CH_3 (NHAc)], 1.73–1.65 (m, 1 H, $R^{\beta a}$), 1.59–1.42 (m, 3 H, R_{β}, {1.52}, $R_{\beta}{}^{b}$ {1.49}), 1.19 (d, 3 H, A^{β}, $J_{\alpha,\beta}$ = 7.4 Hz), 1.17 (d, 3 H, A^{β}, $J_{\alpha,\beta}$ = 7.4 Hz), 1.10 (d, 3 H, T^{γ}, $J_{\beta,\gamma}$ = 6.4 Hz) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, BB, HMQC): δ = 173.3, 171.9, 171.8, 171.0, 170.8, 170.5, 170.5, 169.7, 169.7, 169.6, 169.4 (12 × C=O), 156.8 (C=N), 104.8 (C1′), 98.6 (C1), 83.3 (d, C6′, $J_{C6',F}$ = 166.9 Hz), 78.8 (C3), 75.0 (T^{β}), 73.1 (d, C5′, $J_{C5',F}$ = 19.1 Hz), 72.5 (C3′), 71.3 (C5), 70.5 (C2′), 68.0 (d, C4′, $J_{C4',F}$ = 6.5 Hz), 67.8 (C4), 61.8 (S^{β}), 60.6 (C6), 59.5, 59.0, 58.5 (3 × P^{α}), 55.8 (T^{α}), 55.0 (S^{α}), 50.1 (R^{α}), 49.3 (D^{α}), 48.0 (C2), 46.7, 46.5, 46.3, 46.3 (2 × A^{α}, 3 × P^{δ}), 40.5 (R^{δ}), 35.9 (D^{β}), 29.1, 28.8, 28.6 (3 × P^{β}), 28.3 (R^{β}), 24.8 (R^{γ}), 24.6, 24.4, 24.3 (3 × P^{γ}), 23.1, 22.6 [2 × CH_3(NHAc)], 18.3 (T^{γ}) 17.1, 16.6 (2 × A^{β}) ppm. ESI-MS (pos. ion mode): *m/z* calcd for $C_{54}H_{87}FN_{13}O_{24}$: 1320.59; found: 1320.60 $[M + H]^+$.