Glycopeptide Synthesis Using *O*-Pentafluorophenyluronium Salts as Novel Condensing Reagents

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Abstract. Pentafluorophenyluronium salts and related coupling reagents for the solid-phase synthesis of peptides and glycopeptides have been developed and employed in the synthesis of a glycopeptide sequence from the cell adhesion molecule E-CAD 1.

In the field of peptide synthesis pentafluorophenyl esters of amino acids have been reported to be very efficient [1] in amide bond formation. Methods based on *in situ* formation of these activated carboxylic compounds have not been described so far.

We report here on the synthesis and application of several pentafluorophenol-based *in situ*-activation reagents. Among these are bis(tetramethylene)pentafluorophenoxyformamidinium hexafluorophosphate (PfPyU) **1**, *O*-pentafluorophenyl-1,1:3,3-tetramethyluronium hexafluorophosphate (PfTU) **2**, 2,4,6-tris(pentafluorophenyloxy)-1,3,5-triazine (TPfT) **3** and pentafluorophenyl-

PfPyU **1**

PfOP **4**

PfTU **2**

PfPyU **3**

oxy-tris(dimethylamino)phosphonium hexafluorophosphate (PfOP) **4** [2].
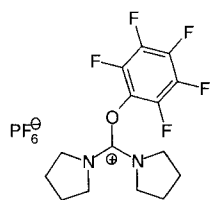
The synthesis of the *O*-pentafluorophenyluronium reagent PfPyU **1** was accomplished by reacting phosgene with pyrrolidine to yield 1,1'-carbonyldipyrrolidine **5** (74%). Upon further treatment of **5** with phosgene and subsequent anion exchange bis(tetramethylene)chloroformamidinium hexafluorophosphate **6** was obtained in a yield of 77%. Compound **6** itself is able to activate carboxylic compounds for reactions with amines, but due to over-activation in form of the resulting acyl chloride racemisation (when using amino acids) or formation of ketens may occur. Therefore, **6** was treated with potassium pentafluorophenolate in dry acetonitrile to give PfPyU **1** in a yield of 90% (scheme 1).

This compound proved to be an efficient *in situ* coupling reagent in peptide synthesis. In an analogous manner the reagent PfTU **2** was synthesised starting from commercially available *N,N,N',N'*-tetramethylurea **7** in a yield of 85% (scheme 2).

The triazine-type reagent TPfT **3** [3] was obtained in a yield of 67% by reacting 2,4,6-trichloro-1,3,5-triazine **9** with potassium pentafluorophenolate (scheme 3).

The oxophosphoniumreagent PfOP **4** was prepared in a yield of 69% by reaction of pentafluorophenol with tris(dimethylamino)phosphine **10** in the presence of tetrachloromethane and subsequent anion exchange [4] (scheme 4).
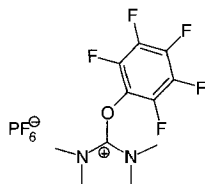
These four reagents were first investigated in several peptide coupling reactions in solution. In these comparative studies, the uronium salt-based reagents **1** and
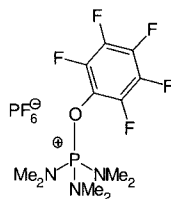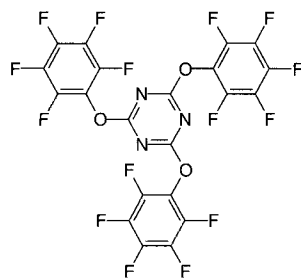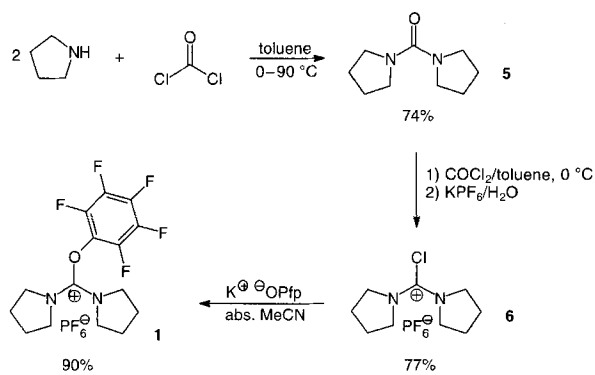
Scheme 1

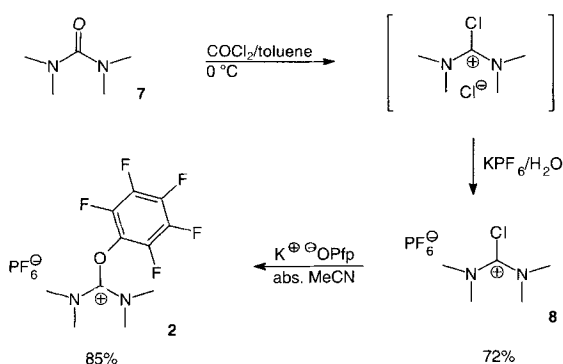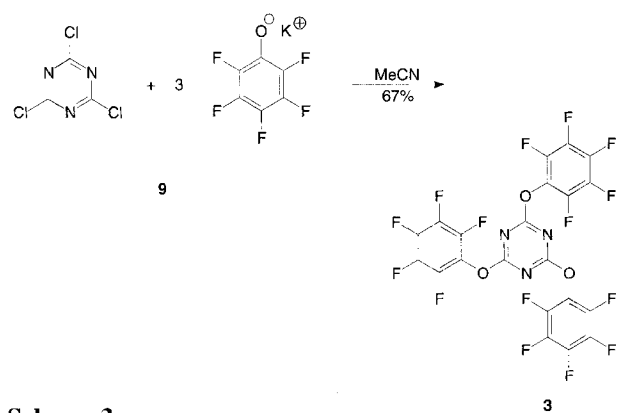**Scheme 2**

Scheme 3

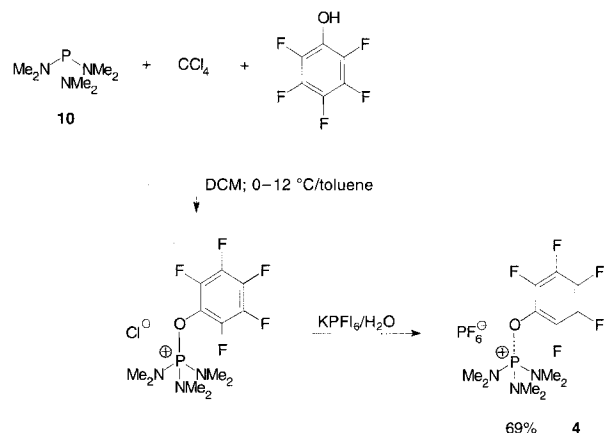**Scheme 4**

2 proved to be highly efficient in forming dipeptides of sterically hindered amino acids and in forming pentafluorophenyl esters. The triazine-based reagent **3** did not show this extreme reactivity. Nevertheless, it pro-
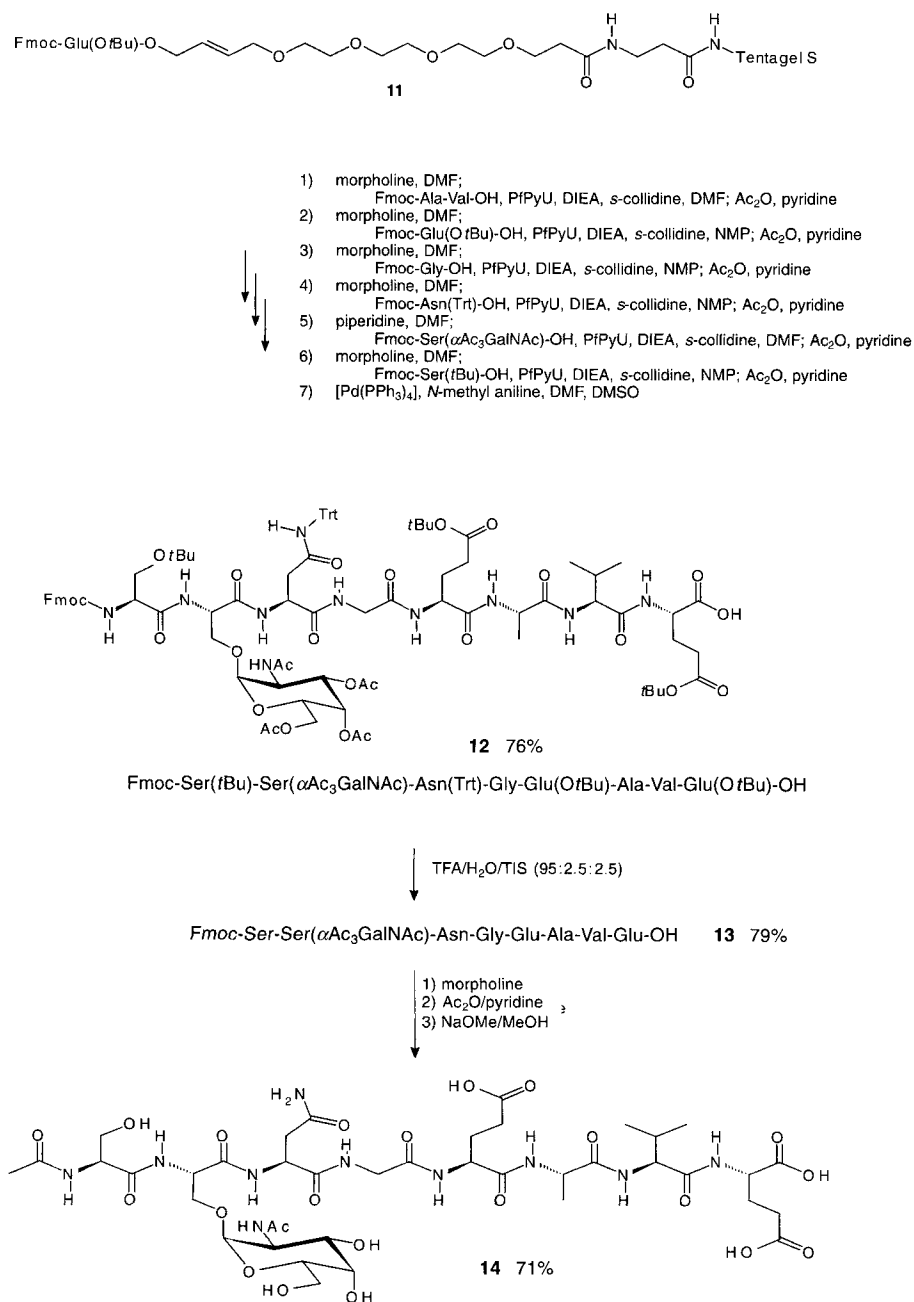
moted the reactions smoothly. It is noteworthy, that this reagent is soluble even in very unpolar solvents. This property is useful if reactions have to be carried out in solvent systems with low polarity. The phosphonium salt-based reagent **4** promoted the reaction as well, but its reactivity in no case was comparable with that of the reagents **1** or **2**.

The reagent PfPyU **1** was applied in the successful synthesis of a glycosylated octapeptide sequence derived from the β-turn forming homophilic recognition domain of mouse epithelial cadherin 1 (E-CAD 1) [5]. The cadherins constitute a family of cell surface glycoproteins involved in Ca^{2+}-dependent cell adhesion processes. Apart from their importance for the morphogenesis of cells and the formation of cell tissues, they are considered to be decisively involved in processes of tumors suppression [6], because their down-regulation in transformed cell lines results in the development of invasive properties [7]. The specificity of cadherins is both homophilic and homotypic. In the course of our investigations towards the synthesis of the complete recognition domain, we synthesised the glycopeptide Ac-SS(αGalNAc)NGEAVE-OH **14** (scheme 5) which contains the T_N-antigen structure occuring on carcinoma cells together with the T-antigen and the sialylated derivatives [8].

The synthesis was carried out using a Tentagel® resin [9] which was preloaded with the starting amino acid-anchoring group conjugate Fmoc-Glu(O*t*Bu)-HYCRON **11** [10]. The Fmoc-group was removed with morpholine in *N*,*N*-dimethylformamide. The allylic anchor remains unaffected under these conditions. Couplings were performed with a five-fold excess (based on the amount of resin-linked starting amino acid [11]) of each the amino acid and the coupling reagent **1** and with a tenfold excess of a mixture of bases (five equivalents of *sym*-collidine and five equivalents of *N*,*N*-diisopro-

Fmoc-Glu(OtBu)-O...

11

1) morpholine, DMF;
   Fmoc-Ala-Val-OH, PfPyU, DIEA, s-collidine, DMF; Ac₂O, pyridine
2) morpholine, DMF;
   Fmoc-Glu(OtBu)-OH, PfPyU, DIEA, s-collidine, NMP; Ac₂O, pyridine
3) morpholine, DMF;
   Fmoc-Gly-OH, PfPyU, DIEA, s-collidine, NMP; Ac₂O, pyridine
4) morpholine, DMF;
   Fmoc-Asn(Trt)-OH, PfPyU, DIEA, s-collidine, NMP; Ac₂O, pyridine
5) piperidine, DMF;
   Fmoc-Ser(αAc₃GalNAc)-OH, PfPyU, DIEA, s-collidine, DMF; Ac₂O, pyridine
6) morpholine, DMF;
   Fmoc-Ser(tBu)-OH, PfPyU, DIEA, s-collidine, NMP; Ac₂O, pyridine
7) [Pd(PPh₃)₄], N-methyl aniline, DMF, DMSO

12 76%

Fmoc-Ser(tBu)-Ser(αAc₃GalNAc)-Asn(Trt)-Gly-Glu(OtBu)-Ala-Val-Glu(OtBu)-OH

TFA/H₂O/TIS (95:2.5:2.5)

Fmoc-Ser-Ser(αAc₃GalNAc)-Asn-Gly-Glu-Ala-Val-Glu-OH   **13 79%**

1) morpholine
2) Ac₂O/pyridine
3) NaOMe/MeOH

14 71%

Scheme 5

pylethylamine) in N,N-dimethylformamide or N-methyl-pyrrolidone. Since a β-sheet forming sequence was to be synthesised, most couplings were repeated once in order to prevent failure sequences. A deviation from this route was carried out while coupling the glycosyl amino acid. This building block was used only in a three-fold excess.

Unchanged amino groups were acetylated after each coupling step. After completion of the solid-phase synthesis, the glycopeptide was released from the solid support by a palladium(0)-catalysed allyl transfer reac-

tion with N-methylaniline as a nucleophile irreversibly trapping the allylic moiety. N-methylaniline also leaves the Fmoc-group intact [12]. The crude protected peptide was then purified by chromatography and gel-permeation. It could be used for fragment condensation reactions. This is a benefit from using the allylic anchoring principle [13], because protected fragments can be obtained very reliably without affecting any protecting group or other sensitive structure. From the protected peptide **12** obtained in a yield of 76%, all acid-labile protecting groups were cleaved off by applica-

tion of trifluoroacetic acid in the presence of water and triisopropylsilane [14]. Purification of the resulting glycopeptide by RP-HPLC yielded 79% of **13** after lyophilisation. The glycopeptide **13** was dissolved in morpholine in order to remove the remaining Fmoc-group, subsequently fully acetylated with acetic anhydride and pyridine and finally subjected to removal of the O-acetyl group by application of sodium methanolate in methanol [15]. The resulting crude glycopeptide **14** was then purified by RP-HPLC and isolated in a yield of 71% as an extremely polar compound. The identity of all glycopeptide derivatives was confirmed by NMR-spectroscopy and MALDI-TOF mass spectrometry.

The efficiency of the novel condensing reagent PfPyU **1** is demonstrated by the high yield (76%, related to **11**) of the glycopeptide **12** obtained after 13 synthetic steps. The average yield of each reaction is 98%. In rating this result it should be considered that the second reaction on solid-phase (scheme 5) consists of a coupling of a dipeptide containing C-terminal valine. In addition, the synthesised sequence is a part of an antiparallel β-sheet and, therefore, prone to backfolding. We assume that the high reactivity achieved by activation of amino acids with PfPyU **1** is to be traced back to the O-aminoacyl uronium salt as the N-acylating intermediate, since isolated Fmoc amino acid pentafluorophenyl esters showed five to six times lower reactivity in analogous coupling reactions [10b]. A further effect promoting the condensation reaction might originate from the tetraalkyl urea which is formed directly at the resin-linked peptide and, thus, can break secondary structures, e.g. back-folding in a β-sheet.

Experimental

General methods

NMR spectra were recorded on Bruker AC-200, AM-400 or AMX-400 machines. Mass spectra were recorded on a Micromass Tofspec E (MALDI-TOF-MS) or on a Finnigan MAT 95 (FD-MS). Melting points were obtained on a Büchi apparatus (Dr. Tottoli) and are uncorrected. Optical rotations were determined using a Perkin Elmer 241 polarimeter. Silica gel for chromatography (0.063–0.200 mm) was obtained from Baker. When double coupling steps during the solid phase synthesis were carried out, this was done with half of the amount of the first coupling for five hours, unless stated otherwise; this is annotated by "dc". After each reaction on the solid-phase six washing steps with 20 ml N,N-dimethylformamide were carried out to prepare the resin for the next reaction. Analytical HPLC was performed using a Knauer K1000 low pressure gradient pump and a Knauer diode array detector 2062; column size: 250 × 4 mm; 1 ml/min. Preparative HPLC was performed on a Knauer high pressure gradient system using two K500 pumps and a variable wavelength monitor (model 287.00); column size 250 × 20 mm; 20 ml/min. Column A: Eurospher 100-C8; column B: Vydac Protein & Peptide C18; gradient PA: acetonitrile/water 1:99 (t = 0 min) – 100:0 (t = 60 min); gradient AA: acetonitrile/water 1:99 (t = 0 min) – 100:0 (42 min); gradient AB: acetonitrile/water 1:99 (t = 0 min) – 1:99 (t = 2 min) – 20:80 (t = 30 min) – 100:0 (t = 42 min); all HPLC solvents were containing 0.1% trifluoroacetic acid.

1,1'-Carbonyldipyrrolidine (5)

A solution of phosgene (20%) in toluene (100 ml = 0.2 mol) was added dropwise to a solution of pyrrolidine (60 g, 0.84 mol, freshly distilled from calcium hydride) in 100 ml of toluene at a temperature of 0 °C. After complete addition the reaction mixture was heated to 90 °C for two hours. Potassium hydroxide (5.6 g, 0.1 mol) was added as a saturated methanolic solution, and the heating was continued for another two hours. After cooling to room temperature, the suspension was filtered, and the filtrate washed three times with 5% aqueous sodium hydroxide. The organic layers were dried over magnesium sulfate and evaporated in vacuo. The residue was purified either by chromatography on silica gel applying a mixture of petroleum ether and ethyl acetate or by distillation in vacuo through a short column. A water-clear liquid was obtained. Yield 25.0 g (74%), after chromatography; b.p. 115–120 °C (0.2 torr), 106 °C (0.17 torr); R_f = 0.32 (EE); $[\alpha]_D^{25}$ = 1.5077, $[\alpha]_D^{26}$ = 1.5071, $[\alpha]_D^{27}$ = 1.5066.

Scale-up: 250 ml 20% phosgene in toluene (= 0.5 mol), 150 g (2.1 mol) pyrrolidine, 250 ml toluene. Yield 62.21 g (74%), by distillation. – ^{1}H NMR (200 MHz, CDCl$_3$): δ/ppm = 3.3 (t, 8H, 4 α-CH$_2$, $J_{\alpha,\beta}$ = 6.35 Hz); 1.75 (tt, 8H, 4 β-CH$_2$, $J_{\beta,\alpha}$ = 6.35 Hz). C$_9$H$_{16}$N$_2$O (168.23).

Bis(tetramethylene)chloroformamidinium Hexafluorophosphate [PyClU] (6)

To a solution of 1,1'-carbonyldipyrrolidine (5) (60.56 g, 0.36 mol) in 120 ml of toluene 242.2 ml of a 20% solution of phosgene in toluene (0.48 mol) was added under vigorous stirring. After 30 min the excess of phosgene was removed in vacuo and the solution was concentrated to 1/3 of its volume. Under vigorous stirring the remainder was poured into 1 l of diethyl ether. The precipitate was collected and again stirred with 1 l of diethyl ether. Once again the precipitate was collected and dissolved in water. To this solution a saturated aqueous solution of 66.75 g (0.36 mol) of potassium hexafluorophosphate was added slowly and under vigorous stirring. The precipitate was filtered off and dried in vacuo. This solid was then dissolved in dry acetone, the solution filtered, and to the filtrate an excess of diethyl ether was added slowly. The precipitating colourless needles were filtered off and dried in vacuo. Yield 91.75 g (77%); m.p. 149.5–150.5 °C. – ^{1}H NMR (200 MHz, MeCN-d$_3$): δ/ppm = 3.76 (t, 8H, 4 α-CH$_2$, $J_{\alpha,\beta}$ = 6.80 Hz); 1.98 (tt, 8H, 4 β-CH$_2$, $J_{\beta,\alpha}$ = 6.80 Hz). – ^{13}C NMR (100.6 MHz, MeCN-d$_3$): δ/ppm = 153.22 (N–C–N); 55.56 (C$^\alpha$); 26.03 (C$^\beta$).

C$_9$H$_{16}$N$_2$PF$_6$Cl calcd.: C 32.50 H 4.85 N 8.40 Cl 10.66
(332.65)   found: C 32.54 H 5.02 N 8.32 Cl 10.08.

Bis(tetramethylene)pentafluorophenoxyformamidinium Hexafluorophosphate [PfPyU] (1)

Pentafluorophenol (1.842 g, 10 mmol) and 0.563 g (10 mmol) of potassium hydroxide were dissolved in water (40 ml) and lyophilised after five minutes of stirring. The obtained dry potassium pentafluorphenolate (1.00 g, 4.5 mmol) was stirred at room temperature with 1.45 g (4.5 mmol) bis(tetramethylene)-chloroformamidinium hexafluorophosphate (6) in 15 ml of dry acetonitrile. After 2.5 hours, the precipitated potassium chloride was filtered off over Hyflo, and the filtrate was concentrated *in vacuo*. This solution was then treated with dry diethyl ether. In the course of five minutes colourless lamella precipitated. These were collected and dried *in vacuo*. Yield 1.942 g (90%); *m.p.* 184–185 °C. – ^{1}H NMR (400 MHz, MeCN-d_3): δ/ppm = 3.61 (t, 8H, 4 α-CH$_2$, $J_{\alpha,\beta}$ = 6.60 Hz); 1.90 (tt, 8H, 4 β-CH$_2$, $J_{\beta,\alpha}$ = 6.60 Hz). – ^{13}C NMR (100.6 MHz, MeCN-d_3): δ/ppm = 155.39 (N–C–N); 141.95 + 139.52 (C3, C5, $J_{C,F}$ = 246 Hz); 140.91 + 138.41 (C2, C6, $J_{C,F}$ = 251 Hz); 128.3 (C4); 51.84 (C$^\alpha$); 25.83 (C$^\beta$).
$C_{15}H_{16}N_2OPF_{11}$ calcd.: C 37.51 H 3.36 N 5.83
(480.26)   found: C 37.49 H 3.41 N 5.92.

Bis(dimethyl)chloroformamidinium Hexafluorophosphate (8)

Tetramethylurea 7 (10 g, 86 mmol) was dissolved in 100 ml of toluene. A solution (50 ml) of phosgene in toluene (20%, 100 mmol) was added at 0 °C. The mixture was stirred until the evolution of gas had deceased. A colorless precipitate was formed. The excess of phosgene was removed *in vacuo* and the precipitate filtered off. After washing with diethylether, the precipitate was dissolved in water. To this solution a saturated aqueous solution of potassium hexafluorophosphate (15.83 g, 86 mmol) was added slowly under vigorous stirring. The precipitate was filtered off and dried *in vacuo*. This solid was then dissolved in dry acetone and the solution filtered. To the filtrate an excess of diethyl ether was added slowly. The precipitating colourless needles were filtered off and dried *in vacuo*. The substance was used without further characterisation. Yield 17.33 g (72%); *m.p.* 86–88 °C.
$C_5H_{12}N_2PF_6Cl$ (280.59).

O-Pentafluorophenyl-1,1:3,3-tetramethyluronium Hexafluorophosphate [PfTU] (2)

Dry potassium pentafluorophenolate (1.00 g, 4.5 mmol) and bis(dimethyl)chloroform-amidinium hexafluorophosphate (8) (1.36 g, 4.5 mmol) in 15 ml of dry acetonitrile were stirred at room temperature. After 3.5 hours the precipitated potassium chloride was filtered off over Hyflo and the filtrate was concentrated *in vacuo*. This solution was then stirred with dry diethyl ether. In the course of five minutes an oil precipitated which crystallised upon addition of more diethyl ether under vigorous stirring. Yield 1.646 g (85%); *m.p.* 155–158 °C. –
^{1}H NMR (200 MHz, MeCN-d_3): δ/ppm = 3.10 (s, 12H, 4CH$_3$). – ^{13}C NMR (50.3 MHz, MeCN-d_3): δ/ppm = 161.37 (N–C–N); 143.45 + 138.44 (C3, C5, $J_{C,F}$ = 251 Hz); 142.06 + 136.99 (C2, C6, $J_{C,F}$ = 255 Hz); 128.31 (C4); 41.11 (CH$_3$).
$C_{11}H_{12}N_2OPF_{11}$ calcd.: C 30.86 H 2.82 N 6.54
(428.18)   found: C 30.06 H 2.95 N 6.55.

2,4,6-Tris(pentafluorophenyloxy)-1,3,5-triazine [TPfT] (3)

2,4,6-Trichloro-1,3,5-triazine (9) (0.22 g, 1.0 mmol) and dry potassium pentafluorophenolate (0.66 g, 3.0 mmol) were stirred over night in 20 ml of dry acetonitrile. The reaction was monitored by FD mass spectrometry [disappearance of peaks at 330.9 (2,4-dichloro-6-pentafluorophenyloxy-1,3,5-triazine) and 479.1 (2-chloro-4,6-bis(pentafluoro-phenyloxy)-1,3,5-triazine)]. The filtrate was thoroughly extracted with *n*-hexane. Evaporation of the *n*-hexane solution gave a colourless residue, which was dried *in vacuo*. Yield 0.42 g (67%); *m.p.* 142–145 °C. – FD-MS (5 kV, 10 mA/min): *m/z* = 627.4 (M$^+$). – ^{13}C NMR (50.3 MHz, DMSO-d_6): δ/ppm = 172.74 (N–C–N); 143.00 + 138.28 (C3, C5, $J_{C,F}$ = 255 Hz); 140.56 + 135.47 (C2, C6, $J_{C,F}$ = 256 Hz); 125.56 (C4).
$C_{21}N_3O_3F_{15}$ calcd.: C 40.21 N 6.70
(627.23)   found: C 39.73 N 6.65.

Pentafluorophenyloxy-tris(dimethylamino)phosphonium Hexafluorophosphate [PfOP] (4)

Tris(dimethylamino)phosphine 10 (1.63 g, 10 mmol) and tetrachloromethane (1.53 g, 10 mmol) were added at 0 °C to a solution of pentafluorophenol (1.84 g, 10 mmol) in 20 ml of dichloromethane. The solution was stirred for seven hours while raising the temperature from 0 °C to 12 °C. The solvent was evporated *in vacuo*. The residue was suspended in water, and a saturated aqueous solution of potassium hexafluorophosphate (1.84 g, 10 mmol) was added. The mixture was vigorously stirred for an hour and then filtered. The residue was washed with water and with diethyl ether prior to recrystallisation twice from acetonitrile/diethyl ether. The obtained colourless needles were dried *in vacuo*. Yield 3.41 g (69%); *m.p.* 155 °C. – ^{1}H NMR (200 MHz, MeCN-d_3): δ/ppm = 2.82 (s, 9H, CH$_3$ ^{a}); 2.77 (s, 9H, CH$_3$ ^{b}). – ^{13}C NMR (50.3 MHz, MeCN-d_3): δ/ppm = 144.69 + 139.91 (C1, $J_{C,P}$ = 240 Hz); 143.68 + 138.65 (C3, C5, $J_{C,F}$ = 252 Hz); 141.96 + 136.91 (C2, C6, $J_{C,F}$ = 254 Hz); 125.29 (C4); 37.71, 37.62 (CH$_3^a$), CH$_3$ ^{b}).
$C_{12}H_{18}N_3OP_2F_{11}$ calcd.: C 29.34 H 3.69 N 8.55
(491.22)   found: C 29.32 H 3.71 N 8.44.

N-(9H-Fluoren-9-yl)-methoxycarbonyl-O-tert.-butyl-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-desoxy-α-D-galacto-pyranosyl)-L-seryl-N⁴-trityl-L-asparaginyl-glycyl-γ-tert.-butyl-L-glutamyl-L-alanyl-L-valyl-γ-tert.-butyl-L-glutamic acid[Fmoc-S(tBu)S(αAc₃GalNAc)N(Trt)GE(OtBu)AVE(OtBu)-OH] (12)

Fmoc-E(OtBu)-HYCRON-βA-TG S NH$_2$ 11 (2.082 g, 0.20 mmol of glutamic acid absolute) [16] was pre-swollen for 60 min in *N,N*-dimethylformamide. After filtration the Fmoc-group was cleaved off by addition of 10 ml morpholine and 10 ml *N,N*-dimethylformamide in the course of two hours.

Protocol for the subsequent coupling reactions:

Fmoc-AV-OH (0.409 g, 1.0 mmol), *N,N*-diisopropylethyl-amine (0.17 ml, 1.0 mmol), *sym.*-collidine (0.13 ml, 1.0 mmol) and PfPyU 1 (0.480 g, 1.0 mmol) in *N,N*-dimethylformamide (20 ml), over night; 60 min capping with acetic anhydride (3 ml) and pyridine (9 ml); 96 min Fmoc-group cleavage with morpholine (10 ml) and *N,N*-dimethylformamide (10 ml).

Fmoc-E(O*t*Bu)-OH (0.425 g, 1.0 mmol), *N,N*-diisopropyl-ethylamine (0.17 ml, 1.0 mmol), *sym.*-collidine (0.13 ml, 1.0 mmol) and PfPyU **1** (0.480 g, 1.0 mmol) in *N*-methyl-pyrrolid-2-one (20 ml), over night, dc; 60 min capping with acetic anhydride (3 ml) and pyridine (9 ml); 90 min Fmoc-group cleavage with morpholine (10 ml) and *N,N*-dimethyl-formamide (10 ml).

Fmoc-G-OH (0.297 g, 1.0 mmol), *N,N*-diisopropylethylamine (0.17 ml, 1.0 mmol), *sym.*-collidine (0.13 ml, 1.0 mmol) and PfPyU **1** (0.480 g, 1.0 mmol) in *N*-methylpyrrolid-2-one (20 ml), over night, dc; 35 min capping with acetic anhydride (3 ml) and pyridine (9 ml); 90 min Fmoc-group cleavage with morpholine (10 ml) and *N,N*-dimethylformamide (10 ml).

Fmoc-N(Trt)-OH (0.596 g, 1.0 mmol), *N,N*-diisopropyl-ethylamine (0.17 ml, 1.0 mmol), *sym.*-collidine (0.13 ml, 1.0 mmol) and PfPyU **1** (0.480 g, 1.0 mmol) in *N*-methyl-pyrrolid-2-one (20 ml), over night, dc; 45 min capping with acetic anhydride (3 ml) and pyridine (9 ml); 180 min Fmoc-group cleavage with piperidine (10 ml) and *N,N*-dimethyl-formamide (10 ml).

Fmoc-S(αAc₃GalNAc)-OH [17] (0.394 g, 0.6 mmol), *N,N*-diisopropylethylamine (0.10 ml, 0.6 mmol), *sym.*-collidine (0.08 ml, 0.6 mmol) and PfPyU **1** (0.288 g, 0.6 mmol) in *N,N*-dimethylformamide (20 ml), over night, dc with 0.6 mmol over night; 90 min capping with acetic anhydride (3 ml) and pyridine (9 ml); 300 min Fmoc-group cleavage with mor-pholine (10 ml) and *N,N*-dimethylformamide (10 ml).

Fmoc-S(*t*Bu)-OH (0.384 g, 1.0 mmol), *N,N*-diisopropyl-ethylamine (0.17 ml, 1.0 mmol), *sym.*-collidine (0.13 ml, 1.0 mmol) and PfPyU **1** (0.480 g, 1.0 mmol) in *N*-methylpyrrolid-2-one (20 ml), over night, dc over night; 120 min capping with acetic anhydride (3ml) and pyridine (9 ml).

Detachment from the resin: To the resin a solution of 1 ml of *N*-methyl aniline, 7 ml of DMSO and 7 ml of DMF was added and the suspension was degassed *in vacuo*. Under argon atmosphere a catalytic amount of tetrakis(triphenylphosphi-ne)palladium(0) was added and the mixture was shaken in darkness for three days. The resin was filtered off and washed six times with 20 ml of DMF and, subsequently, six times with 20 ml of dichloromethane. The combined filtrates were concentrated *in vacuo*, and the residue was purified by chromatography on silica gel with a gradient chloroform to chloroform/methanol (1:1). The isolated crude product was subjected to gel permeation chromatography (Sephadex LH-20, chloroform/methanol 1:1) and preparative RP-HPLC (column A, grad. PA). After lyophilisation a colourless product was obtained. Yield 270 mg (76% on the basis of resin-linked starting amino acid): $[\alpha]_D^{24}$ = 21.85 (c = 0.22, CH₂Cl₂); R_T: 40.40 min (column A, grad. AA). C₉₀H₁₁₆N₁₀O₂₆ (1753.9).
MALDI-TOF-MS (cca, pos): *m/z* = 1776.6 [M+Na]⁺; 1792.3 [M+K]⁺; 1798.5 [M+2Na-H]⁺; 1814.5 [M+K+Na-H]⁺; 1676.7 [M+Na-ketene-acetamide]⁺; 1692.8 [M+K-ketene-acet-amide]⁺. – ¹H NMR (400 MHz, DMSO-d₆, ¹H,¹H-COSY): δ/ppm = 8.69 (s, 1H, NH^{ω}-N); 8.41 (s_b, 1H, NH); 8.19 (d, 1H, NH, *J* = 7.83 Hz); 8.10 (d, 1H, NH, *J* = 7.43 Hz); 8.02 (d, 2H, 2NH, *J* = 7.43 Hz); 7.87 (d, 2H, H4-, H5-Fmoc, $J_{H4,H3} = J_{H5,H6}$ = 7.43 Hz); 7.75 (s_b, 1H, NH); 7.71 (d, 2H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H8,H7}$ = 7.43 Hz); 7.48 (d, 1H, NH-urethane, $J_{NH,S-\alpha}$ = 8.61 Hz); 7.40 (m, 3H, NH, H3-, H6-Fmoc); 7.37–7.15 (m, 19H, H2-, H7-Fmoc, H2-, H3-, H4-, H5-, H6-, H2'-, H3'-,

H4'-, H5'- H6'-, H1"-, H2"-, H3"-, H4"-, H5"-, H6"-Trt, 2NH); 5.26 (s, 1H, H4-Gal); 4.96 (d, 1H, H3-Gal, $J_{H3,H2}$ = 14.19 Hz); 4.85 (d, 1H, H1-Gal, $J_{H1,H2}$ = 3.13 Hz); 4.70–4.63 (m, 1H, Nᵅ); 4.34–3.91 (m, 13H, Vᵅ, Aᵅ, 2Eᵅ, 2Sᵅ, H2-, H5-, H6-Gal, CH₂-Fmoc, H9-Fmoc); 3.78–3.62 (m, 2H, Gᵅ); 3.50–3.30 (m, 4H, 2S^β); 2.71–2.68 (m, 1H, N^{βa}); 2.54–2.40 (m, 1H, N^{β b}); 2.25–2.14 (m, 4H, 2E^γ); 2.07 (s, 3H, CH₃–AcNH); 1.98–1.94 (m, 1H, V^β); 1.92 (s, 3H, CH₃–Ac); 1.87–1.50 (m, 10H, 2E^{βa}, 2E^{βb}), 2CH₃–Ac); 1.36 (s, 18H, 6CH₃–*t*Bu-Ester); 1.33–1.10 (m, 3H, A^β); 1.07 (s, 9H, 3CH₃–*t*Bu-Ether); 0.84–0.79 (m, 6H, V^γ). – ¹³C NMR (100.6 MHz, DMSO-d₆): δ/ppm = 172.34, 172.02, 171.49, 171.17, 170.55, 170.42, 169.84, 169.75, 169.73, 169.68, 169.51, 168.80, 168.64, 168.41 (2E^α-CO, 2E^γ-CO, N^α-CO, N^β-CO, 4CO-Ac, V-CO, A-CO, 2S–CO, G-CO); 155.86 (CO-Urethan); 144.54 (*C_{ipso}*-Trt); 143.79, 143.65 (C4a-, C4b-Fmoc); 140.62 (C8a-, C8b-Fmoc); 128.43 (*C_{meta}*-Trt); 127.52, 127.35 (C3-, C6-Fmoc); 126.94 (C2-, C7-Fmoc); 126.25 (*C_{ortho}*-Trt); 125.19, 125.14 (C1-, C8-Fmoc); 119.97 (C4-, C5-Fmoc); 97.68 (C1-α-Gal); 79.53 (*C_{quart}*-*t*Bu-Ester, *C_{quart}*-Trt); 72.85 (*C_{quart}*-*t*Bu-Ether); 69.37 (S); 68.72 (C3-Gal); 67.67 (C4-Gal); 67.09 (CH₂-Fmoc); 66.15 (C5-Gal); 61.83 (C6-Gal); 61.70 (S); 61.39 (Vᵅ); 55.83, 53.40 (2Sᵅ); 51.69, 51.20 (2 Eᵅ); 50.21 (Nᵅ); 48.63 (C2-Gal); 46.59 (C9-Fmoc, Aᵅ); 42.03 (Gᵅ); 38.88 (N^β); 30.97 (V^β); 28.89, 28.46 (2E^β); 27.65 (6 CH₃–*t*Bu-Ester); 27.21 (2E^γ); 27.11 (3CH₃–*t*Bu-Ether); 22.47, 21.95, 21.88, 20.28 (4CH₃–Ac); 19.04 (A^β); 18.08 (V^{γ a}); 17.51 (V^{γ b}).

N-(9H-Fluoren-9-yl)-methoxycarbonyl-L-seryl-O-(2-aceta-mido-3,4,6-tri-O-acetyl-2-desoxy- α-D-galacto-pyranosyl)-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic acid [Fmoc-SS(αAc₃GalNAc)NGEAVE-OH] (**13**)

Fmoc-S(*t*Bu)S(*a*Ac₃GalNAc)N(Trt)GE(O*t*Bu)AVE(O*t*Bu)-OH (**12**) (11.6 mg, 6.61 µmol) was dissolved in 20 ml of tri-fluoroacetic acid and 0.5 ml of water. Triisopropylsilane (0.5 ml) was added. After stirring over night, the solution was evaporated *in vacuo*. The residue was purified by RP-HPLC (column A, grad. PA). After lyophilisation a colourless solid was recovered. Yield 7.0 mg (79%): $[a]_D^{24}$ = –6.37 (c = 0.70, H₂O); R_T: 22.20 min (Säule A, Grad. AA). – C₅₉H₇₈N₁₀O₂₆ (1343.32). – MALDI-TOF-MS (cca, pos): *m/z* = 1367.3 [M+Na]⁺; 1383.3 [M+K]⁺. – ¹H NMR (400 MHz, DMSO-d₆; ¹H,¹H-TOCSY): δ/ppm = 8.30 (d, 1H, S_{term}^{NH}, $J_{NH,S-a}$ = 8.22 Hz); 8.14–8.07 (m, 3H, S_2^{NH}, N^{NH}, S_{glyco}^{NH}); 8.02 (d, 1H, A^{NH}, $J_{NH,A-a}$ = 6.75 Hz); 7.95 (d, 1H, S_1^{NH}, $J_{NH,E-a}$ = 6.75 Hz); 7.88 (d, 2H, H4-, H5-Fmoc, $J_{H4,H3} = J_{H5,H6}$ = 7.33 Hz); 7.72 (d, 3H, H1-, H8-Fmoc, V^{NH}, $J_{H1,H2} = J_{H8,H7} = J_{NH,V-\alpha}$= 7.92 Hz); 7.56 (d, 1H, GalNAc^{NH}, $J_{NH,H2}$ = 9.10 Hz); 7.42–7.39 (m, 4H, H2-, H7-Fmoc, G^{NH}, N^{NH2-a}); 7.34–7.30 (m, 2H H3-, H6-Fmoc); 6.91 (s_b, 1H, N^{NH2-b}); 5.27 (d, 1H, H4-Gal, $J_{H4,H3}$ = 2.7 Hz); 4.97 (dd, 1H, H3-Gal, $J_{H3,H4}$ = 2.9 Hz, $J_{H3,H2}$ = 12.0 Hz); 4.81 (d, 1H, H1-Gal, $J_{H1,H2}$ = 3.2 Hz); 4.57–4.54 (m, 1H, S_7^{α}); 4.53–4.49 (m, 1H, N ^a); 4.32–4.28 (m, 1H, A^a); 4.27–4.22, (m, 2H, H2-, H5-Gal); 4.20–4.18 (m, 5H, CH₂-Fmoc, V^a, 2E^a); 4.15–4.14 (m, 2H, S_8^{α}, H9-Fmoc); 4.05–4.00 (m, 1H, H6^a-Gal); 3.96–3.91 (m, 1H, H6^b-Gal); 3.79–3.73 (m, 2H, 2S^{ba}); 3.64–3.56 (m, 4H, 2S^{bb}, G^a); 2.58–2.41 (m, N^b); 2.31–2.19 (m, 4H, 2E^g); 2.07 (s, 3H, CH₃–NAc); 1.96–1.90 (m, 1H, V^b); 1.91, 1.88, 1.81 (3s, 3×3H, 3CH₃–OAc);

1.80–1.73 (m, 4H, $2E^b$); 1.19 (d, 3H, A^b, $J_{A-\beta,A-\alpha}$ = 7.04 Hz); 0.84, 0.82 (2d, 6H,$V^{\gamma a}$, $V^{\gamma b}$, $J_{V-\gamma V-\beta}$ = 6.60 Hz).

N-Acetyl-L-seryl-O-(2-acetamido-2-desoxy-α–D-galacto-pyranosyl)-L-seryl-L-asparaginyl- glycyl-L-glutamyl-L-ala-nyl-L-valyl-L-glutamic acid [Ac-SS(aGalNAc)NGEAVE-OH] (14)

Fmoc-SS(αAc$_3$GalNAc)NGEAVE-OH (13) (7.0 mg, 5.2 µmol) was dissolved in 1 ml of morpholine and stirred for 75 min at room temperature. Water (10 ml) was added, and the solution was lyophilised. The residue was dissolved in 1.5 ml of pyridine, and 0.5 ml of acetic anhydride were added. After stirring over night the solution was evaporated *in vacuo,* and the residue was dissolved in water. After lyophilisation the residue was dissolved in methanol and a solution of sodium methanolate (14 mg) in 20 ml of methanol was added dropwise until a wet pH indication paper displayed a pH of 9. After 290 min the mixture was neutralised with acetic acid, evaporated *in vacuo* and the residue purified by RP-HPLC (column B, grad. AB). After lyophilisation a colourless product was obtained. Yield 3.8 mg (71%); $[\alpha]_D^{24}$ = –5.11 (c = 0.22, H$_2$O); R_T 4.85 min (column B, grad. AB). – C$_{40}$H$_{64}$N$_{10}$O$_{22}$ (1036.99). – MALDI-TOF-MS (dhb, pos): m/z = 1059.4 [M+Na]$^+$; 1075.5 [M+K]$^+$; 1081.5 [M+2Na-H]$^+$; 1097.4 [M+K+Na-H]$^+$; 1103.5 [M+3Na-2H]$^+$; 1119.5 [M+K+2Na-2]$^+$; 1125.3 [M+4Na-3H]$^+$. – ^{1}H NMR (400 MHz, DMSO-d$_6$): δ/ppm= 8.11–7.94 (m, 7H, S_7^{NH}, N^{NH}, G^{NH}, 2×E^{NH}, A^{NH}, V^{NH}); 7.73 (d, 1H, S_7^{NH}, $J_{NH,V-a}$ = 8.80 Hz); 7.44 (s$_b$, 1H, N^{NH2-a}); 7.29 (d, 1H, GalNAc^{NH}, $J_{NH,H2}$ = 7.54 Hz); 6.95 (s$_b$, 1H, N^{NH2-b}); 4.59–4.28 (m, 8H, $2S^\alpha$, N^α, $2E^\alpha$, A^α, V^α, H1-Gal); 4.19–4.10 (m, 2H, H2-, H5-Gal); 4.08–4.00 (m, 1H, H4-Gal); 3.72–3.37 (m, 14H, G^α, H3-, H6-Gal, $2S^\beta$, 5OH); 2.56–2.43 (m, 2H, N^β); 2.28–2.19 (m, 2H, E^γ); 2.07–1.92 (m, 2H, $E^{\beta a}$, V^β); 1.87, 1.85 (2s, 2× 3H, 2CH$_3$–NAc); 1.76–1.69 (m, 1H, $E^{\beta b}$); 1.18 (d, 3H, A^b, $J_{A-b,A-a}$ = 7.05 Hz); 0.85–0.81 (m, 6H, $2V^\gamma$).

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