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Solid-Phase Synthesis of a Glycopeptide from the Homophilic Recognition Domain of Epithelial Cadherin 1 Using a *O*-Pentafluorophenyluronium Salt

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Abstract: The β-turn forming glycopeptide *6* from the homophilic recognition domain of mouse epithelial cadherin 1 carrying a T_N-antigen side chain was synthesised on solid phase using an allylic anchor and the new coupling reagent *N,N-N',N'*-bis(tetramethylene)-*O*-pentafluorophenyluronium hexafluorophosphate *3*.

The cadherins constitute a family of about 30 cell surface glycoproteins decisively involved in Ca^{2+}-dependent adhesion of cells. They are regulating molecules important for the morphogenesis of cells and found on every tissue-forming cell type[1]. Down regulation of cadherins in tumor cells leads to the acquisition of invasiveness[2]. These observations suggest that cadherins are *in vivo* turmor suppressing molecules[3]. The specificity of cadherins is homophilic (binding between two identical molecules) and homotypic (binding between the same cell type). The bindings are thought to be mediated by a homophilic domain in the first out of five extracellular repeating units. This domain mainly consists of a β-turn sequence exposing the tripeptide portion His-Ala-Val[4]. The loop sequence around this peptide is of interest for interdisciplinary investigations of cell adhesion and metastasis.

Our first attempts to synthesise the E-CAD1 sequence SHAVSSNGEAVE *1* carried out on solid phase and using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[5] as the coupling reagent gave only small amounts of the target compound although TBTU was proven efficient in analogous syntheses of mucin-type glycopeptides[6]. The β-sheet forming sequence obviously is strongly prone to backfolding, and a particularly efficient activation of the amino acids is required for successful coupling reactions.

Moreover, diketopiperazine formation on the stage of the resin-linked dipeptide should be minimised. In previous syntheses this was achieved using the allylic HYCRON anchor, which is stable to bases and acids, by changing the temporary amino protection from Fmoc to Boc on the dipeptide level[6]. Because the acid-labile *tert.*-butyl ester is required for sidechain protection of the C-terminal glutamic acid, this switch in the protecting group strategy can not be applied to the synthesis of the target E-CAD1 sequence *1*. To achieve an efficient solid-phase synthesis of a glycopeptide of sequence *1* containing α-GalNAc at serine within the turn structure, we decided to couple the dipeptide Fmoc-Ala-Val-OH directly to resin-loaded glutamic acid ester *2* after removal of the Fmoc group. The loading of the polymer was carried out with the allylic HYCRON anchor conjugate of Fmoc-Glu(O*t*Bu)-OH and β-alanyl-Tentagel S[7] according to a method described earlier[6]. The initial load was 0.1 mmol Glu/g resin[8]. Because the coupling of a dipeptide is often accompanied by epimerisation of the carboxylic component, and couplings of valine always are sterically demanding, a mild but very efficient carboxylic activation is required also for this step.

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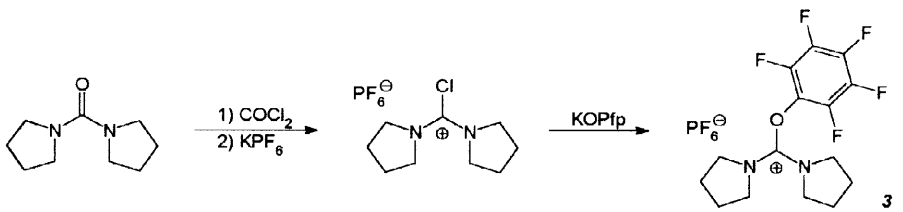
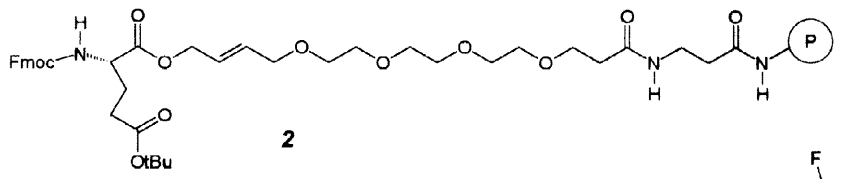
To solve these problems, we have introduced *N,N-N',N'*-bis(tetramethylene)-*O*-pentafluorophenyluronium hexafluorophosphate (PfPyU) ***3*** and analogous *N,N'*-tetraalkyl derivatives[9] as coupling reagents. The synthesis of PfPyU is easily achieved by treatment of 1,1'-carbonyldipyrrolidine with phosgene, subsequent anion exchange and final reaction with potassium pentafluorophenolate (Scheme 1).



Scheme 1

In comparative coupling reactions of Fmoc-Ala-OH to H-Val-O*t*Bu, PfPyU showed an 8 times higher reactivity than TBTU (completion of the reaction within 25 min) and was of equal reactivity (completion within 3 min) as *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[10], which is more difficult to synthesise. Because activation by PfPyU ***3*** resulted in higher reactivity than that of isolated pentafluorophenyl esters of amino acids (about 6 times faster formation of Fmoc-Ala-Val-O*t*Bu)[11], the *O*-acyl uronium intermediates are more likely the reactive species than the esters formed *in situ*. Pentafluorophenol in contrast to benzotriazol-based hydroxyl compounds lacks α-nucleophilicity and therefore reacts more slowly with the *O*-acyl uronium intermediate. As a consequence, during the coupling process the *N,N'*-tetraalkyl urea is set free at the growing peptide chain . Thus it can exhibit a promoting hydrogen bridge-breaking effect and suppress the formation of secondary structures of the resin-linked peptide chain.

During the solid-phase synthesis (Scheme 2) the Fmoc group was cleaved off with morpholine/DMF. All couplings were carried out using a threefold excess of Fmoc-Ala-Val-OH (first coupling) or Fmoc-protected amino acid, PfPyU ***3***, diisopropylethylamine and *sym*-collidine in *N*-methylpyrrolidone. The carbohydrate was introduced as preformed glycosyl amino acid (Fmoc-Ser(αAc_3GalNAc)-OH). After each coupling, unchanged amino groups were capped with a (1:3)-mixture of Ac_2O/pyridine. The protected glycopeptide was detached from the resin by palladium(0)-catalysed allyl transfer to *N*-methylaniline in DMSO/DMF (1:1). The irreversible trapping of the allyl moiety leaves the Fmoc group intact[12]. The glycopeptide ***4*** was isolated in a yield of 55%[13] after 22 steps and purification by GPC on Sephadex LH-20. Acid labile protecting groups were removed from ***4*** using trifluoroacetic acid, water and triisopropylsilane (95:2.5:2.5)[14]. After purification by RP-HPLC (Eurospher 100-C8) the product ***5*** was obtained almost quantitatively. The *O*-acetyl groups of the carbohydrate were cleaved off by Zemplén transesterification[15] at pH 9.5 to furnish the deprotected glycopeptide ***6***[16] in a yield of 57% after purification by RP-HPLC (Eurospher 100-C8). At a pH of 8.5, the Fmoc-protected glycopeptide 7 was obtained from ***5*** in a yield of 86% after purification by RP-HPLC. The purity of all glycopeptides was determined by RP-HPLC and they were characterised by NMR spectroscopy and MALDI-TOF mass spectrometry. Their analytical data confirmed that the strong carboxylic activation by PfPyU ***3*** proceeded without detectable racemisation and allowed an efficient synthesis of the β-sheet forming glycopeptide which contains demanding peptide bonds, e.g. between valine and the sidechain protected glutamic acid or between *O*-glycosyl serine and the N^{α}-trityl asparagine (see compound ***4***).



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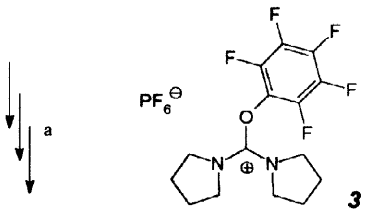
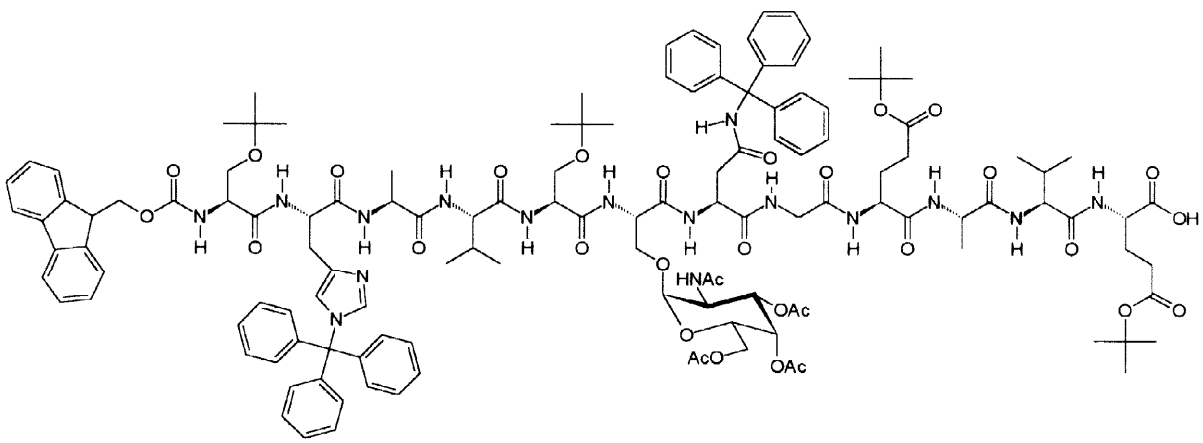


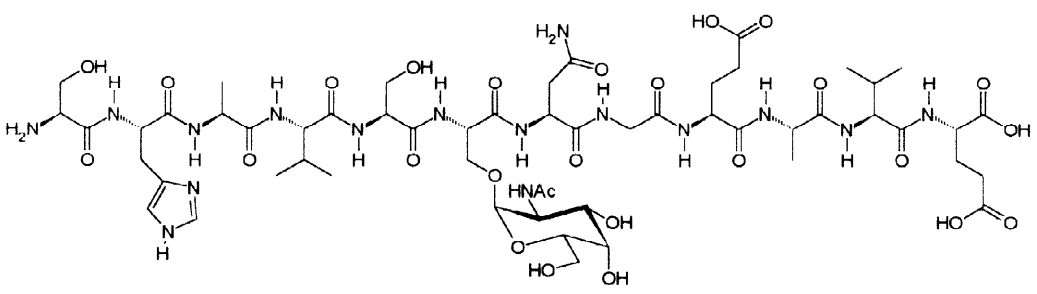
3

Fmoc-S(*t*Bu)H(Trt)AVS(*t*Bu)S(αAc$_3$GalNAc)N(Trt)GE(O*t*Bu)AVE(O*t*Bu)-HYCRON-βA-Ⓟ



Fmoc-S(*t*Bu)H(Trt)AVS(*t*Bu)S(αAc$_3$GalNAc)N(Trt)GE(O*t*Bu)AVE(O*t*Bu)-OH ***4***

Fmoc-SHAVSS(αAc$_3$GalNAc)NGEAVE-OH ***5***



SHAVSS(αGalNAc)NGEAVE-OH ***6***

Fmoc-SHAVSS(αGalNAc)NGEAVE-OH ***7***

Scheme 2: **a) morpholine/DMF; 3 eq. Fmoc-Xaa-OH, PfPyU *3*, *sym*-collidine, *i*Pr$_2$NEt, NMP; b) cat. [Pd^0(PPh$_3$)$_4$], *N*-methylaniline, DMF/DMSO (1/1); c) TFA/H$_2$O/TIS (95/2.5/2.5); d) NaOMe/MeOH, pH = 9.5; e) NaOMe/MeOH, pH = 8.5. Ⓟ = Tentagel S.**

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16 Analytical data of *6*: ^{1}H NMR (400 MHz, D_2O, ^{1}H,^{1}H COSY), δ/ppm: 8.62 (H-Im^2); 7.30 (H-Im^4); 4.88 (H1-Gal); 3.72 (G$^\alpha$); 2.44 (E$^\gamma$); 1.35 (A$^\beta$); 0.93 (V$^\gamma$); ^{13}C NMR (100.6 MHz, D_2O, DEPT), δ/ppm: 115.54 (H-Im^{C4}); 95.98 (C1-Gal); 69.49 (C5-Gal); 64.99 (C6-Gal); 40.73 (G$^\alpha$); 34.27 (N$^\beta$); 28.52, 28.32 (2x V$^\beta$); 28.24 (E$^\gamma$); 20.24 (CH_3-NAc); MALDI-TOF (dhb, pos): m/z = 1389.8 $[M+H]^+$, 1412.5 $[M+Na]^+$, 1186.6 [M+H-GalNAc]$^+$, 1211.2 [M+Na-GalNAc]$^+$; $[\alpha]_D^{24}$: -14.01 (c = 0.81, H_2O); R_T: 12.79 min (Eurospher 100-C8, 250x4, 1 ml/min, 1% MeCN (t = 0 min) - 100% MeCN (t = 42 min), 0.1% TFA).