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Fragment Condensation on Solid-Phase in the Synthesis of an Amphiphilic Glycopeptide from the Homophilic Recognition Domain of Epithelial Cadherin 1

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Abstract: The lipo-glycopeptide *6* containing the homophilic recognition motif of mouse epithelial cadherin 1 was synthesised via a fragment condensation on a solid phase linked peptide using an allylic anchor and a pentafluorophenol-based coupling reagent.

The cadherins, which are found on every tissue-forming cell type[1a], constitute a family of about 30 cell surface glycoproteins involved in cell adhesion processes[2], cell morphogenesis[1], cell targeting[3] and contact inhibition[4]. Their down regulation in tumor cells leads to the acquisition of invasiveness[5], an observation suggesting that cadherins are *in vivo* tumor suppressing molecules[6]. The specificity of cadherins is both homophilic and homotypic and thought to be mediated by a homophilic domain in the first out of five extracellular repeating units. The interaction is induced by the dimerisation of two cadherin molecules on one cell, and the binding of this dimer to analogous dimers on other cells of the same type[7]. The recognition domain exposes the tripeptide portion His-Ala-Val on a β-sheet surface loop[8]. The synthesis of the loop structure with a lipophilic tail and a glycosylation was of interest for biological investigations.

We have synthesised a glycododecapeptide amphiphile using a convergent solid-phase approach with a very simple but nevertheless versatile hydrophobic tail group introduced by Kunitake *et al.*[9] For the convenient synthesis of the protected fragments the allylic HYCRON anchor[10] and the PfPyU coupling reagent[11] were used. First, the *N*-terminal tetrapeptide with the lipophilic tail group was synthesised. The loading of the polymer was carried out using the allylic HYCRON anchor conjugate *1* of Fmoc-Val-OH and β-alanyl-polystyrene according to a method described earlier[10]. The initial load of *1* was 0.77 mmol Val/g resin[12]. The second amino acid was coupled as the Boc-amino acid in order to minimise the formation of diketopiperazine on the stage of the resin-linked dipeptide[10]. The resulting polymer bound tetrapeptide was then reacted with the lipophilic carboxylic acid derivative *2* obtained according to the method of Fields *et al.*[9b] with minor alterations. The protected lipotetrapeptide fragment *3* was detached from the resin by palladium(0)-catalysed allyl transfer to morpholine in DMF/DMSO (1:1). The desired product[13] was obtained in a yield[14] of 20% by extraction with cold acetonitrile since all impurities and the catalyst were soluble in that solvent (scheme 1). The synthesis of the resin-linked glycopeptide octapeptide fragment Fmoc-S(*t*Bu)S(αAc$_3$GalNAc)N(Trt)GE(O*t*Bu)AVE(O*t*Bu)-HYCRON *4* was carried out according to the method described earlier[11c]. The initial load of the resin was 0.17 mmol Glu/g resin[15]. The carbohydrate moiety was introduced as the preformed glycosyl amino acid (Fmoc-Ser(αAc$_3$GalNAc)-OH)[16]. After each coupling, unreacted amino groups were capped with a (1:3)-mixture of Ac$_2$O/pyridine. The terminal Fmoc-group was cleaved with morpholine in DMF. The lipotetrapeptide fragment *3* was suspended in DMSO/DMF and added to the glycooctapeptide resin in a 1.7-fold excess together with one equivalent of each *N*,*N*-diisopropylethylamine, *sym*-collidine and PfPyU[11]. The mixture was shaken for 11 days. The resin was

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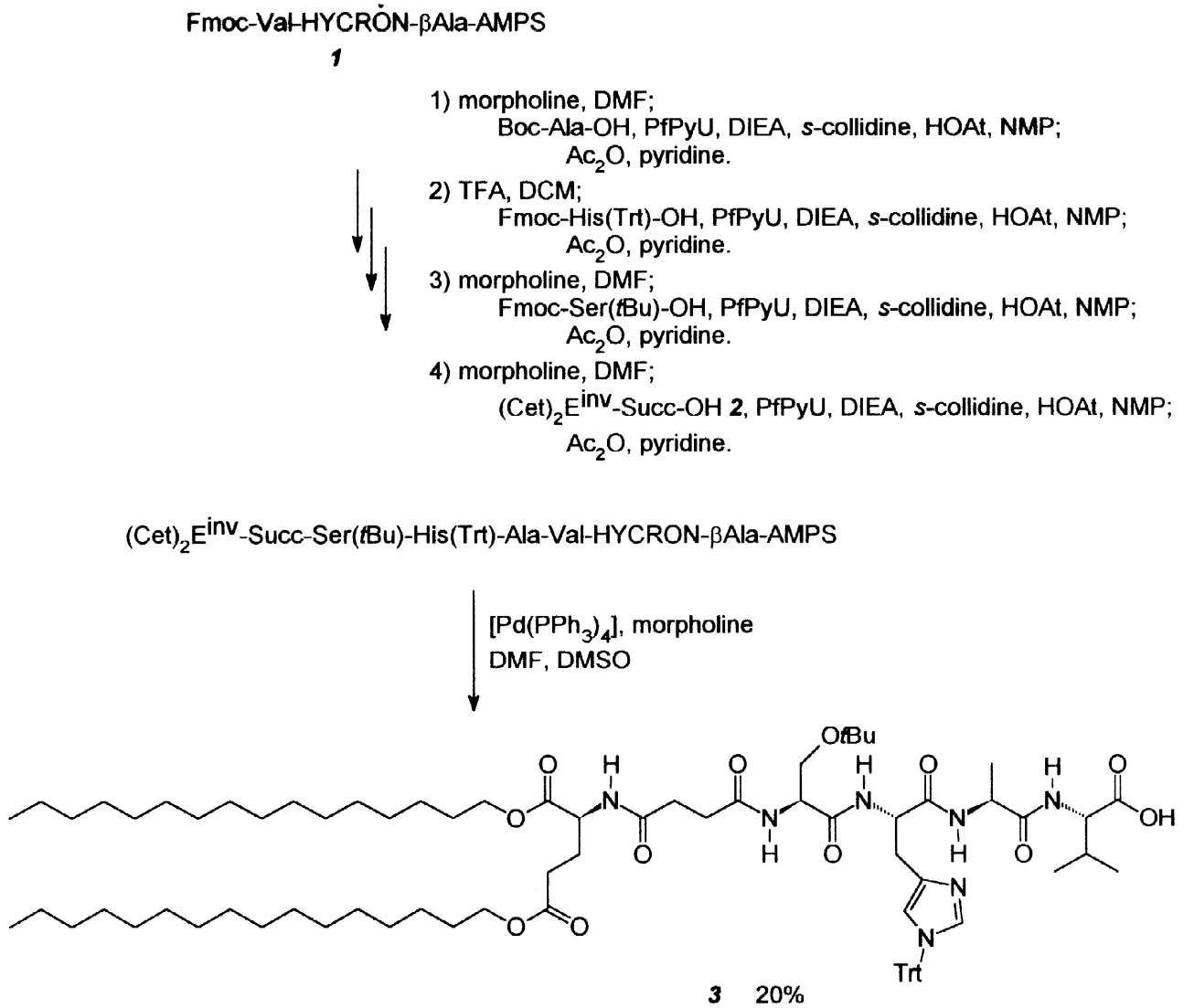
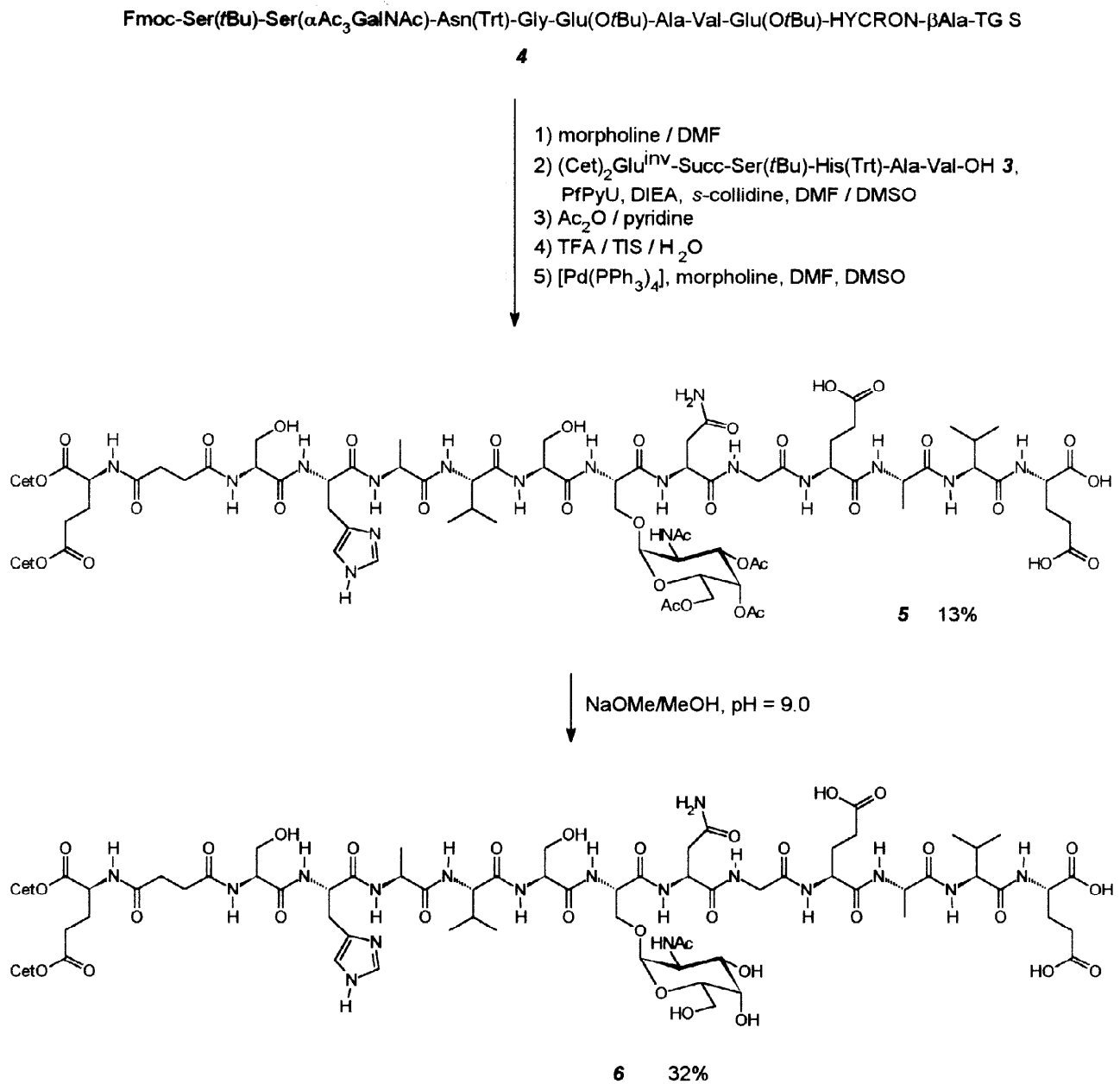


Scheme 1

washed thoroughly with DMSO and then subjected to the capping reaction. Afterwards, all acid-labile protecting groups were cleaved off from the resin-bound lipo-glycopeptide applying trifluoroacetic acid, water and triisopropylsilane (95:2.5:2.5) according to a method described by Pearson *et al.*[17] The lipo-glycopeptide was detached from the resin using catalytic amounts of tetrakis(triphenylphosphine) palladium(0) and morpholine as the allyl trapping nucleophile[10]. The cleavage solution was then filtered and concentrated *in vacuo*. The residue was suspended in acetonitrile and water was added. The precipitate was collected and dissolved in trifluoroacetic acid. This solution was subjected to preparative RP-HPLC (Vydac Protein C4). After purification the almost insoluble product *5* was obtained as a colorless solid in a yield[14] of 13%[18]. The *O*-acetyl groups of the carbohydrate moiety were cleaved off by Zemplén transesterification[19] in DMSO/methanol (1:4) at pH 9.0 to furnish the deprotected lipo-glycopeptide *6* in a yield of 32% after purification by RP-HPLC[20] (scheme 2). The low yield is due to the insufficient discrimination between the acetyl esters at the carbohydrate portion and the glutaminic acid cetyl ester.



Scheme 2

The purity of all products was determined by RP-HPLC. The glycopeptides were characterised by NMR spectroscopy and MALDI-TOF mass spectrometry. The biological evaluation of cadherin-derived glycopeptides is under investigation and will be described elsewhere.

The synthesis outlines the versatility of the allylic anchoring system HYCRON which can be used to generate protected peptide fragments for block condensation reactions as well as it may faciliate the work-up procedure by cleaving off most of the protecting groups from the peptide still bound to the resin. The results described also illustrate the efficiency of PfPyU as condensing reagent even in the coupling of peptides to solid-phase linked amino components.

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[12] Amino acid analyses were carried out by the Orpegen Pharma GmbH, Heidelberg (Germany) who supported this work with generous donations of amino acid derivatives.

[13] Analytical data of *3*: MALDI-TOF (cca, pos): m/z = 1389.8 $[M+H]^+$, 1411.8 $[M+Na]^+$; $[\alpha]_D^{24}$: 4.96 (c = 0.37, $CHCl_3$); R_T: 26.87 min (Eurospher 100-C8, 250x4, 1 ml/min, 1% MeCN (t = 0 min) - 100% MeCN (t = 42 min), 0.1% TFA).

[14] The yield is based on the amount of resin-linked starting amino acid.

[15] The resin used was Fmoc-Glu(O*t*Bu)-HYCRON-βAla-Tentagel S NH_2 (see lit. 11b).

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[18] Analytical data of *5*: MALDI-TOF (cca, pos): m/z = 2216.2 $[M+Na]^+$, 2232.8 $[M+K]^+$; $[\alpha]_D^{24}$: 6.19 (c = 0.77, DMSO-d_6); R_T: 39.27 min (Vydac Protein C4, 250x4, 1 ml/min), 1% MeCN (t = 0 min) - 100% MeCN (t = 42 min), 0.1% TFA).

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[20] Analytical data of *6*: ^1H NMR (400 MHz, TFA-d_1), δ/ppm: 8.47 (H-Im^2), 7.30 (H-Im^4), 2.54 (CH_2-Succ); 1.63 (C1-Cet); 0.98-0.91 (V^γ); 0.76-0.73 (CH_3-Cet); ^{13}C NMR (100.6 MHz, TFA-d_1, DEPT), δ/ppm: 47.57 (G^α), 32.32 (N^β), 14.58 (C16-Cet); MALDI-TOF (cca, pos): m/z = 2090.3 $[M+Na]^+$, 2106.0 $[M+K]^+$, 2112.1 $[M+2Na-H]^+$; $[\alpha]_D^{24}$: -11.09 (c = 0.40, TFA); R_t: 38.97 min (Vydac Protein C4, 250x4, 1 ml/min), 1% MeCN (t = 0 min) - 100% MeCN (t = 42 min), 0.1% TFA).