Synthesis of an antifreeze glycoprotein analogue: efficient preparation of sequential glycopeptide polymers†

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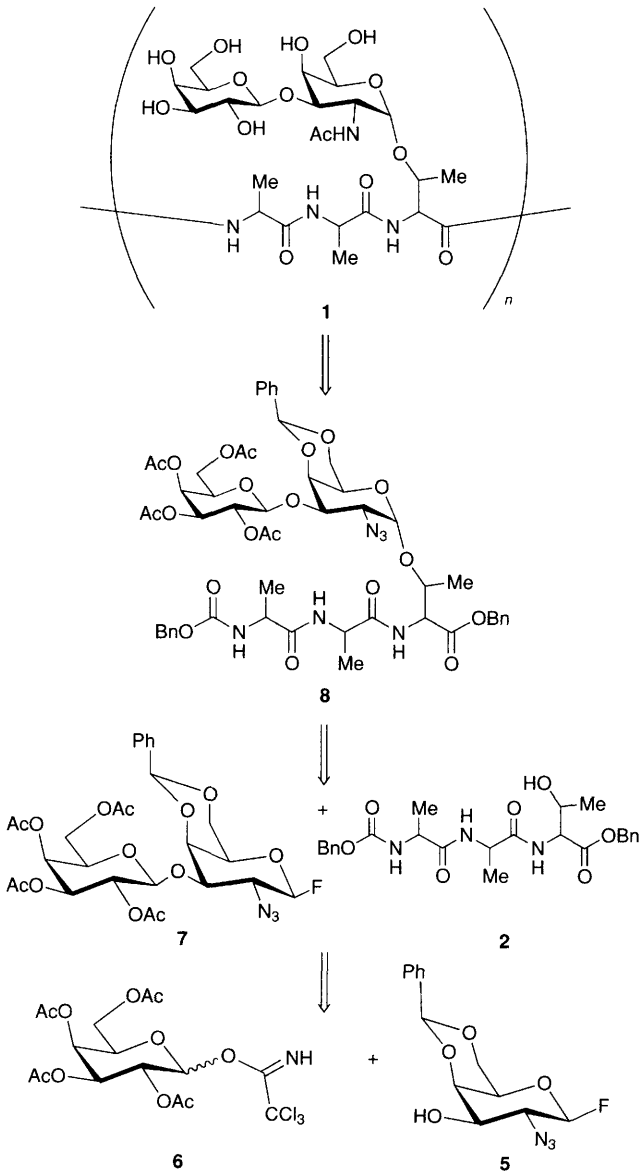
A sequential glycopeptide polymer, antifreeze glycoprotein (AFGP) 1, was efficiently synthesised by simple polymerisation of the repeating glycopeptide unit of AFGP 10 with diphenylphosphoryl azide (DPPA) as a convenient promotor.

Antifreeze glycoprotein (AFGP) in the serum of polar and deep-sea fish is known to depress the freezing point of their blood and help these fish to survive at temperatures below −2 °C. It has been suggested that the depression of the freezing point by AFGP seems not to obey the molar colligative melting point depression law.[1] However, the proposed mechanisms of the antifreeze activity of AFGP are highly speculative,[2] and little evidence has been reported to assist the complete explanation of this phenomenon owing to the difficulties in obtaining a large enough amount of AFGP. Thus, the total synthesis and the characterization of AFGP and its analogues[3] is urgently required.

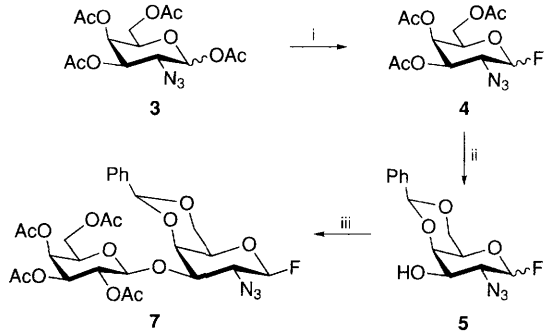
Here we report a facile and efficient synthesis of AFGP as the first attempt to synthesise a macromolecule composed of sequential glycopeptides. AFGPs are sequential polyglycopeptides consisting of a tripeptide repeating unit (Ala-Ala-Thr)$_n$ (n = 4–55) with a disaccharide moiety (Galβ1 → 3GalNAcα1) attached to each threonyl residue. The retrosynthetic analysis of AFGP is shown in Scheme 1. The advantages in this synthetic strategy are that (i) a specific and efficient glycosylation between glycosyl imidate **6** and fluoride **5** is achieved by regulating the reaction temperature to afford a disaccharide derivative **7** and (ii) macromolecule **1** of the sequential glycopeptide [Ala-Ala-(Galβ1 → 3GalNAcα1)Thr] **10**, obtained by the coupling of tripeptide **2** and disaccharide **7**, was successfully synthesized by a simple polymerisation reaction with diphenylphosphoryl azide (DPPA)[4] as an initiator.

Scheme 2 indicates the synthetic route to disaccharide intermediate **7**. Here, we were pleased to find that the known galactosyl imidate **6**[5] can be specifically activated using trimethylsilyl trifluoromethanesulfoate (TMSOTf) at low temperature (−20 °C) and allowed to react with the readily available glycosyl fluoride **5** derived from **3**.[6] The coupling reaction of **6** and **5** proceeded smoothly to give disaccharide **7** in 81% yield.‡§ This suggests that the much higher reactivity of glycosyl imidate **6** compared to glycosyl fluoride **5** facilitates the selective activation under mild conditions using Lewis acids commonly employed as promotors for both glycosyl donors.[5,7]

Next, the glycosyl donor **7** was directly coupled with the readily available tripeptide **2** in the presence of $(C_5H_5)_2ZrCl_2$–

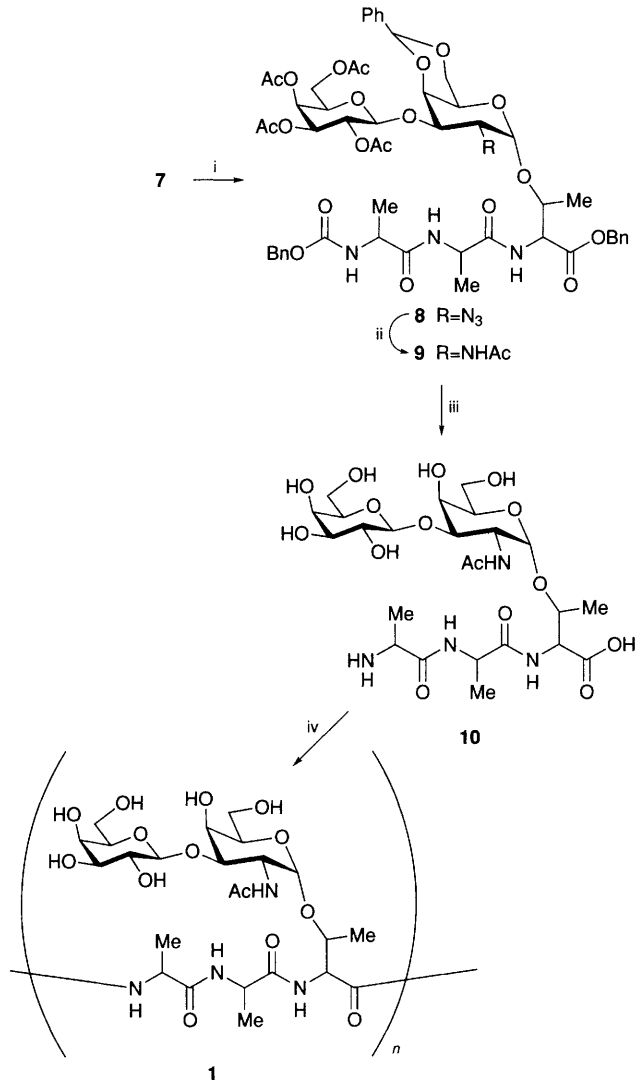


Scheme 1 Retrosynthetic analysis of antifreeze glycoprotein (AFGP)



Scheme 2 *Reagents and conditions*: i, $BnNH_2$ (1.5 equiv.), THF, 2 h, 20 °C, then diethylaminosulfur trifluoride (1.2 equiv.), THF, 3 h, 20 °C, 92% from **3**; ii, NaOMe (0.1 equiv.), THF–MeOH (2:3, *v*/*v*), 2 h, 20 °C, then $C_6H_5CH(OMe)_2$ (3 equiv.), camphorsulfonic acid (0.5 equiv.), DMF, 2 h, 20 °C, 92% from **4**; iii, **6** (1.6 equiv.), **5** (0.1 equiv.), TMSOTf (0.1 equiv.), CH_2Cl_2, 4 Å molecular sieves, 1.5 h, −20 °C, 81%

silver perchlorate (1 : 2) in CH_2Cl_2 according to the method reported by Matsumoto *et al.*[8] to afford α-glycoside **8** in 64% yield (Scheme 3).§ The azido group of the glycopeptide intermediate **8** was then converted into an acetamide group by treatment with a nickel–boride reagent [nickel(II) chloride–sodium borohydride] followed by acetylation in 69% yield.[9] Finally, removal of the protective groups from compound **9** gave the repeating unit of AFGP **10** in 56% yield.§ Polymerisation of compound **10** was performed by employing diphenylphosphoryl azide (DPPA) as an efficient promoter in the presence of triethylamine.§¶ The molecular weight of this artificial glycoprotein was estimated to be 6000–7300 (10–12 repeat units) by gel permeation chromatographic analysis.¶ No side reactions were observed during the polymerisation reaction with DPPA, which is known as a specific activator of the C-terminal position[4] of the peptides. This synthetic strategy can therefore be used for the preparation of sequential polypeptide architectures bearing neutral carbohydrate branches. Although quantitative analysis of biological activity is still under investigation, an AFGP analogue prepared here showed significant antifreeze activity *in vitro*.



Scheme 3 *Reagents and conditions*: i, **2** (3.0 equiv.), $(C_5H_5)_2ZrCl_2$ (2 equiv.), $AgClO_4$ (4 equiv.), CH_2Cl_2, 4 Å molecular sieves, 3 h, −20 to −10 °C, 64%; ii, $NiCl_2{\cdot}6H_2O$ (10 equiv.), $B(OH)_3$ (20 equiv.), $NaBH_4$, EtOH, 1 h, 0 °C, then Ac_2O (excess), EtOH, 20 °C, 2 h, 69% from **8**; iii, NaOMe (0.1 equiv.), THF–MeOH (2 : 3, *v*/*v*), 1 h, 0 °C, then Pd–C, H_2 gas, MeOH, 48 h, 20 °C, 56% from **9**; iv, $Ph_2P(O)N_3$ (1.3 equiv.), Et_3N (2.3 equiv.), Me_2SO, 20 °C, 54 h, 69%

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Footnotes

† A part of this work was presented at the 18th International Carbohydrate Symposium in Milan, Italy, in July 1996.

‡ *Synthesis* of **7**. Compounds **6** (941 mg, 1.91 mmol) and **5** (470 mg, 1.59 mmol) were dissolved in CH_2Cl_2 (5 ml) in the presence of 4 Å molecular sieves (600 mg) and the mixture was stirred at −20 °C under nitrogen atmosphere. After 20 min, a solution of TMSOTf (31 μl, 159 μmol) in CH_2Cl_2 (0.5 ml) was added and stirred for 30 min. Further compound **6** (350 mg, 0.71 mmol) dissolved in CH_2Cl_2 (2 ml) was added to the solution. After 1 h, triethylamine (0.2 ml) was added to the solution to quench the TMSOTf and the residue was dissolved in $CHCl_3$. The mixture was washed with brine and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated, and the residual syrup was chromatographed on silica gel with 4 : 1 (*v*/*v*) toluene–ethyl acetate containing 0.5% of triethylamine as an eluent to give **7** (801 mg, 81%).

§ *Selected data* for **7**: δ_H ($CDCl_3$) 7.55–7.36 (m, 5 H, aromatic), 5.07 (dd, 1 H, *J* 52.5 and 7.5, H-1), 4.80 (d, 1 H, *J* 7.9, H-1′), 4.00–3.91 (m, 2 H, H-2, 6′a), 2.16, 2.07, 2.05 and 1.98 (each s, 3 H, MeCO). For **8**: δ_H ($CDCl_3$) 7.36–7.31 (m, 15 H, aromatic), 6.84 [d, 1 H, *J* 8.09, Thr(NH)], 6.63 [br s, 1 H, ^{2}Ala(NH)], 5.36 [br s, 1 H, ^{1}Ala(NH)], 4.93 (d, 1 H, *J* 3.4, H-1), 4.83 (d, 1 H, *J* 7.9, H-1′), 4.66 (dd, 1 H, *J* 8.8 and 3.0), 2.16, 2.06, 2.03 and 2.00 (each s, 3 H, MeCO), 1.52–1.23 (m, 9 H, Ala-β-Me × 2, Thr-γ-Me). For **10**: δ_H (D_2O) 4.47 (d, 1 H, H-1′, *J* 3.8), 4.37 (d, 1 H, H-1′, *J* 6.8), 4.07 (d, 1 H, Thr-α-CH, *J* 6.4), 2.05 (s, 3 H, MeCO), 1.55–1.20 (m, 9 H, Ala-β-Me × 2, Thr-γ-Me). For **1**: δ_H (D_2O) 4.50–4.38 (br s, 2 H, H-1, H-1′), 4.07–4.00 (br s, 1 H, Thr-α-CH), 2.06 (br s, 3 H, MeCO), 1.66–1.10 (m, 9 H, Ala-β-Me × 2, Thr-γ-Me).

¶ *Polymerization* of **10**. To a solution of **10** (16 mg, 25.5 μmol) in Me_2SO (0.3 ml) was added DPPA (7.15 μl, 33.2 μmol) and triethylamine (8.2 ml, 58.7 μmol). The mixture was stirred at room temperature for 54 h. The precipitate obtained by addition of diethyl ether was collected and dissolved in water (2 ml). The crude product was then purified by chromatography on a Sephadex G-20 column and eluted with water. The polymer fractions were collected and concentrated to give pure **1**. The molecular weight of the product was measured and estimated to be 6000–7300 (10–12 repeating units) by gel permeation chromatography with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186 and 380 K; Shodex Standard P-82) were used as standards].

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