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Synthesis of a Molecular Mimic of the Glc_1Man_9 Oligoside as Potential Inhibitor of Calnexin Binding to ΔF508 CFTR Protein

Slim Cherif,[a] Michael R. Leach,[b] David B. Williams[b] and Claude Monneret[a,*]

[a]*UMR 176 CNRS-Institut Curie, Section de Recherche, 26 rue d'Ulm, 75248 Paris Cedex 05, France*
[b]*Department of Biochemistry, University of Toronto, Toronto, Canada M5S 1A8*

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Abstract—Deletion of phenylalanine at position 508 of the CFTR protein is associated with a severe form of cystic fibrosis. Biosynthetic arrest of the misfolded ΔF508 CFTR protein in the endoplasmic reticulum is due to prolonged interaction with protein chaperones. In order to overcome this retention and thereby restore the delivery of the protein to the plasma membrane, a molecular mimic of the glycoprotein oligoside moiety has been designed and synthesized. Ability of this mimic to inhibit the binding of the natural $Glc_1Man_9GlcNAc$ oligoside to calnexin has been measured.

The autosomal recessive human genetic disorder cystic fibrosis (CF) is caused by the loss or dysfunction of a plasma membrane Cl^- channel known as the CF transmembrane conductance regulator (CFTR).[1] Among the over eight hundred mutations that have been detected, deletion of a phenylalanine at position 508 (>90% of CF patients) is associated with a severe form of the disease.[2] The ΔF508 mutation is hypothesized to cause 'misfolding' of the nascent chain and prevents delivery of the cystic fibrosis transmembrane conductance regulator to the plasma membrane.[3] Several data suggest that at least three chaperones, Hsc70,[4] Hsp90,[5] and calnexin,[6] participate in CFTR biogenesis. Both chaperones form transient complexes with nascent, immature CFTR molecules. Because restoration of processing can be partially obtained by incubation of cells between 25 and 29 °C[7,8] and, since the ΔF508 mutation does not substantially decrease the chloride channel activity of CFTR,[9] any circumvention of the misprocessing of ΔF508 CFTR provides an attractive therapeutic strategy. For example, drugs which influence the interaction of molecular chaperones with their substrates[10] have already been investigated with some success.

Any attempt to overcome the biosynthetic arrest of ΔF508 CFTR protein requires knowledge of the mechanism that causes this retention.[11] Newly synthesized glycoproteins bind to certain molecular chaperones only subsequent to the processing of the carbohydrate units (a triglucosyl sequence) to the monoglucosylated state. Binding studies to calnexin[12] and to calreticulin[13] have indicated that a monoglucosylated minimum structure like $Glc_1Man_5GlcNAc$ with its α 1→6 branch point, **A**–**F**, as underlined in Figure 1, is required for relevant chaperone binding.

En route towards this oligosaccharide, we already reported[14] the synthesis of disaccharide **A**–**B** and trisaccharide **A**–**B**–**C** moieties and, more recently, the synthesis of the methyl glycoside of the tetrasaccharide **A**–**B**–**C**–**D**.[15]

It remained to determine whether a molecule containing the determinant disaccharide, Glc-Man **A**–**B** and the two terminal mannoses **G** and **H** with the same orientation in space as in the parent structure, is still a relevant ligand for calnexin binding. To progress in this

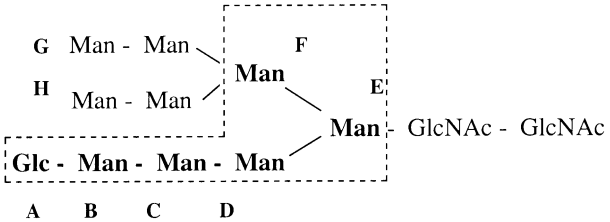

Figure 1. Oligoside core of CFTR.

*Corresponding author. Tel.: +33-1-4234-6655 fax: +33-1-4234-6631; e-mail: claude.monneret@curie.fr

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knowledge, we have designed the molecular mimic **1** of the oligoside core of CFTR including these different elements separated by linkers. Simultaneously, we postulated that, by introducing carbamate linkages instead of glycosidic bonds, higher affinity between such a molecular mimic and the protein chaperone could be achieved by improving hydrophobicity of the molecule, and better stability could be observed (Fig. 2).

To obtain the disaccharide determinant, a glucose-mannose-containing disaccharide selectively deprotected at the anomeric position was first prepared. Thus the per-*O*-benzoyl-D-mannose **2a** was converted into the chloride derivative **3a** which, in turn, led to the *p*-methoxybenzyl glycoside **4** under Koenigs–Knorr conditions (AgOTf, *sym*-collidine, 62%). It must be noticed that, under the same conditions, the corresponding *O*-acetylated mannose compound **2b** and thereby the chloride derivative **3b** led to the 1,2-*ortho*-ester **5** exclusively (Scheme 1).

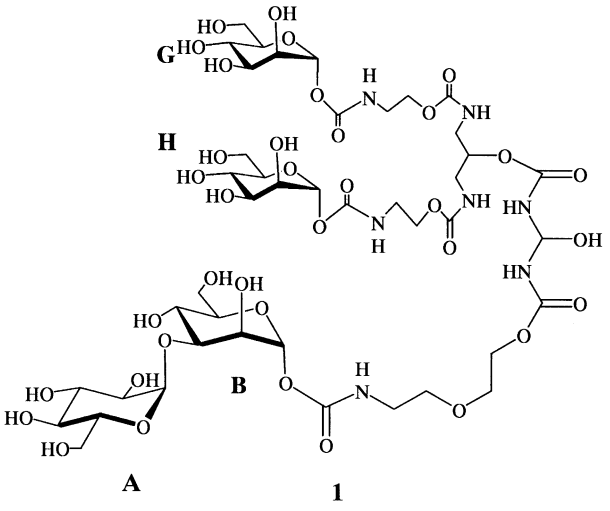


Figure 2. Target molecule.

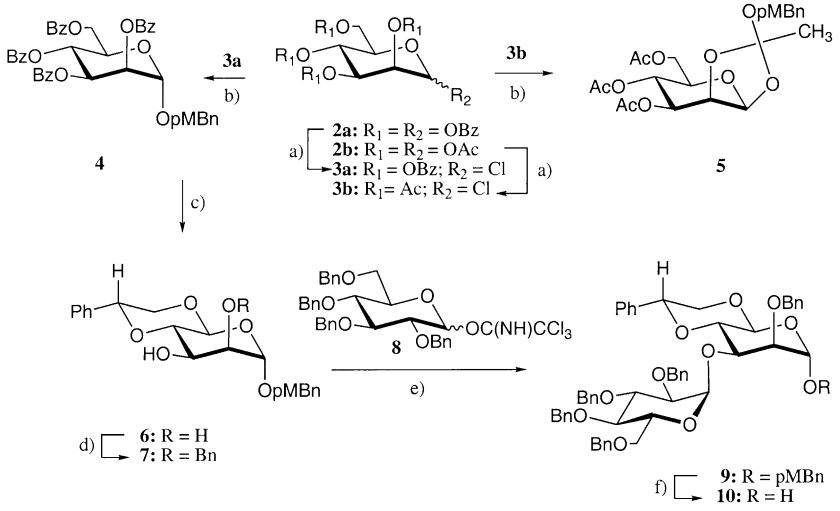
Conversion of **4** into the mannosyl acceptor **7** was conveniently achieved after debenzoylation (NaOMe–MeOH), monobenzylidenation (HBF_4, 42%),[16] and regioselective benzylation of **6** (55% yield) under phase transfer catalysis[17] (BnBr, NaOH, Bu_4NHSO_4). Condensation of **7** with per-*O*-benzyl glucose trichloroacetimidate[18] **8** (TMSOTf, dioxane, rt) afforded, with high stereoselectivity (>95:5), the disaccharide **9**, isolated in 71% yield. Next, removal of the *p*-methoxybenzyl group led to **10** (42% yield) which can be activated at the anomeric position to subsequently afford linear tri or tetrasaccharide **A**–**B**–**C** or **A**–**B**–**C**–**D** identical to those previously reported.[14,15]

However, subsequent progress in the present synthesis involved the conversion of **9** into the corresponding per-*O*-acetylated compound **11** by hydrogenolysis (Pd/C 10%, MeOH, quantitative) and pyridine acetylation. After selective anomeric deprotection, compound **12** was activated as the phenylcarbonate **13**[19] before coupling with 2-(2-aminoethoxy)ethanol to afford **14** which was subsequently transformed into the activated species **15** (Scheme 2).

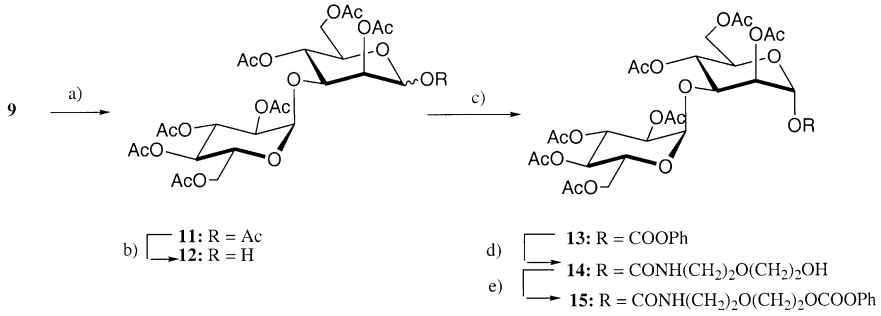
On the other hand, the di-mannose fragment **19** was readily prepared (Scheme 3) from per-*O*-acetylated D-mannose **16**. Thus, **16** was regioselectively deacetylated (H_2N-NH_2, AcOH, 65%) at the anomeric carbon to afford **17**, which was activated as the 4-nitrophenylcarbonate (90%) **18** and condensed with 2-(2-aminoethoxy)ethanol in the presence of Et_3N (90%).

The next step involved formation of the 4-nitrophenylcarbonate of **19** followed by condensation with diaminopropanol to give **21**[20] (74% yield) which, in turn, was once again, activated and condensed with mono-Boc-1,3-diaminopropanol leading to **23** (59%) and to **24**,[21] after deprotection under acidic conditions.

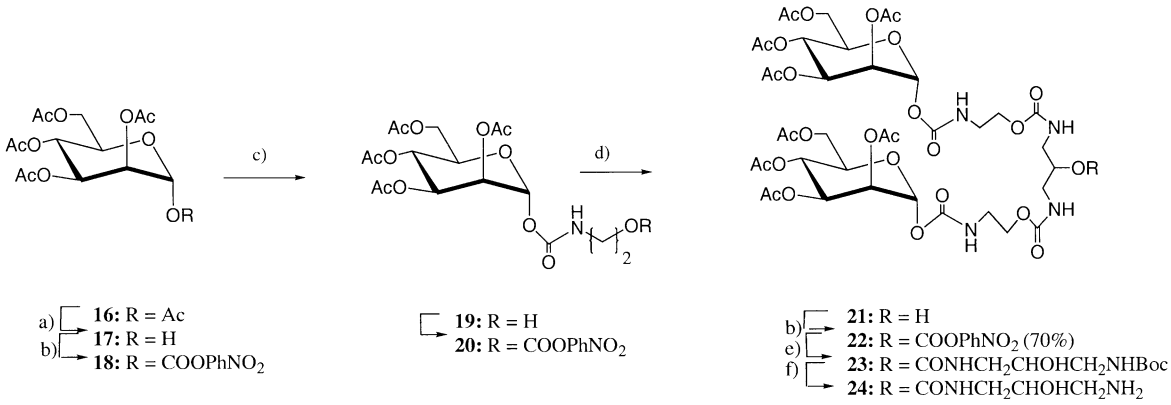
Synthesis of **1** [HRMS: (Maldi-TOF, DHB, $EtOH{\cdot}H_2O$, TFA 0.1%). Calculated: *m*/*z* 1314.4327 (M+ Na+).



Scheme 1. (a) α,α-Dichloromethyl methylether (5 equiv), $ZnCl_2$–Et_2O (1 M in Et_2O) (0.1 equiv), CH_2Cl_2, 16 h, 87%; (b) *p*-MBnOH (4 equiv), CF_3SO_3Ag (1.3 equiv), sym-collidine (0.8 equiv), CH_2Cl_2, 4 Å, molecular sieves, 15 h, rt, 62% for **4** and 85% for **5**; (c) NaOMe–MeOH, rt, 24 h, then benzylidene dimethylacetal (1.1 equiv), HBF_4 in Et_2O (1 equiv), DMF, 24 h, 42%; (d) BnBr, aq NaOH, Bu_4NHSO_4, CH_2Cl_2, 55%; (e) TMSOTf, dioxane, **8** (2 equiv), rt, 30 min, 71%; (f) CAN, CH_3CN–H_2O (4:1), 0 ° to rt, 42%.



Scheme 2. (a) H_2, Pd/C, rt, 5 h; Ac_2O, pyridine, 15 h, 80%; (b) $H_2N–NH_2$, DMF, 16 h, 50 °C, 52%; (c) phenyl chloroformate (2.5 equiv), pyridine (2.5 equiv), CH_2Cl_2, 3 h, rt, 79%; (d) 2-(2-aminoethoxy)ethanol (1.5 equiv), Et_3N, CH_2Cl_2, 15 h rt, 74%; (e) 4-nitrophenyl chloroformate (2.5 equiv), pyridine (2.5 equiv), CH_2Cl_2, 0 °C to rt, 15 h, 79%.



Scheme 3. (a) $H_2N–NH_2$, AcOH, THF, DMF, 15 min, 50 °C, 65%; (b) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, 5 h, rt, 85%; (c) 2-(2-aminoethoxy)ethanol, Et_3N, CH_2Cl_2, 15 h, rt, 90%; (d) 1,3-diaminopropanol (0.5 equiv), Et_3N, 15 h, rt, 74%; (e) Mono-boc-1,3-diaminopropanol, DMAP, THF, 15 h, 50 °C, 59%; (f) 1 M HCl, EtOAc, 4.5 h, rt, 62%.

Found: 1314.4306) was conveniently achieved through condensation of fragment **15** with fragment **24** followed by deacylation under Zemplen conditions (37% overall yield for the last two steps).

Molecular mimic **1** was tested for its ability to inhibit the binding of [^{3}H] $Glc_1Man_9GlcNAc_2$ to GST-fused calnexin[22] immobilized on glutathione-agarose. Compared to the Glc α (1→3) Man disaccharide, compound **1** was a less potent inhibitor, since concentrations producing 50% inhibition were about 300 μM for compound **1** and 160 μM for the disaccharide. That the linear tetrasaccharide Glc_1Man_3 (**A**–**B**–**C**–**D** in Fig. 1) is several hundred-fold more potent that the disaccharide itself suggests that the linear disposal of the four sugar units is more relevant for binding inhibition than the additional terminal mannose residues that mimic the other arms of the $Glc_1Man_9GlcNAc_2$ oligoside. However, among the different hypotheses which cannot be excluded to explain this relatively low activity, are the nature of the linkers and scaffold and the length of the linkers. Therefore, experiments are going on to synthesize other molecular mimics of the oligoside part of a high-mannose type glycoprotein.

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19. Compound **13**: syrup; $[\alpha]_D^{22}$ +34.5° (*c* 1, chloroform); MS (FAB) 779.05 ($M+Na^+$), 794.9 ($M+K^+$); ^{1}H NMR: δ 2.01,

2.05, 2.06, 2.07, 2.10, 2.11, 2.29 (7s, 7×3H, OAc), 4.05–4.31 (m, 6H, 3-H, 5′-H, 6′-H, 6′-H), 4.34 (dd, 1H, $J=9.5$, $J'=3.5$ Hz), 4.76 (dd, 1H, $J=3.5$ Hz), 5.01 (dd, 1H, $J=J'=9.5$ Hz), 5.30–5.45 (m, 4H), 6.07 (d, 1H, $J=1.8$ Hz, 1-H), 7.18–7.44 (m, 5H, Ar).

20. Compound **21**: syrup; $[\alpha]_D^{22}$ +46° (*c* 1, chloroform); MS (FAB) 1013.39 ($M+H^+$), 1035.3 ($M+Na^+$); (found C: 44.86; H, 5.51; N, 6.10). $C_{39}H_{56}N_4O_{27}$ requires C, 46.25; H, 5.57; N, 6.20; ^{1}H NMR: δ 1.82 (broad s, 1H, OH), 2.01, 2.05, 2.1, 2.18 (4s, 8×3H, OAc), 3.28 (m, 4H, CH_2NH), 3.48 (m, 4H, CH_2OCO), 4.06–4.16 (m, 4H, 5-H, 6′-H), 5.68 (m, 4H, NH), 6.00 (s, 1H, 1-H).

21. Compound **24**: syrup; $[\alpha]_D^{22}$ +34° (*c* 1, chloroform); MS (FAB) 1129.37 ($M+H^+$), 1151.38 ($M+Na^+$); HMRS *m*/*z* 1151.3662. calcd 1151.3615; ^{1}H NMR: δ 2.00, 2.05, 2.09, 2.18 (4s, 8×3H, OAc), 4.77 (broad s, 1H, NH), 6.00 (s, 1H, 1-H), 6.20–6.70 (m, 10H).

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