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Convergent Synthesis of an Inner Core GPI of Sperm CD52

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Abstract—An inner core of the GPI anchor of sperm CD52 antigens was synthesized by a highly convergent process using specially modified inositol, glucosamine and phospholipid as key building blocks. This paper also presents a new and efficient procedure to prepare 1,2,6-differentiated derivatives of inositol for GPI syntheses.

CD52 antigens, which are expressed on virtually all human lymphocytes[1] and sperm cells,[2] belong to a very unique class of glycoproteins that are anchored to plasma membranes by glycosylphosphatidylinositols (GPIs). CD52 antigens play a fundamental role in the recognition process of the immune system and the specific interactions between eggs and sperms.[1,2]

Despite the fact that GPI-anchoring is very common in the eukaryotic world,[3] it is fairly difficult to obtain homogeneous GPIs and GPI-anchored glycoproteins from nature. Thus, chemical synthesis of GPIs has been a major research focus in the past decade.[4–9] However, there is no report yet about the synthesis of CD52 GPIs, though a glycopeptide fragment of CD52 antigens has been recently synthesized by solid-phase method.[10] This paper describes the first chemical synthesis of a short GPI anchor (**1**) of sperm CD52.

As indicated by our retrosynthetic analysis (Fig. 1), the convergent assembly of **1** would require three key building blocks **2**, **3**, and **4**, of which the specially modified D-*myo*-inositol **3** was critical.

During the past decades, numerous methods have been designed for the synthesis of optically pure derivatives of inositol.[11] However, most methods utilized only one resolved enantiomer of a racemic intermediate, which has severely affected the overall synthetic efficiency. To solve this problem, we designed a new method (Scheme 1) for a key intermediate **5** in the synthesis of **3**, which made use of both enantiomers of a partially protected derivative, **6**, of inositol.

The preparation and resolution of **6** were achieved by a reported method[1b] that could afford both enantiomers in excellent yields and optical purity. The allylation of (+)-**6** by means of Bu_2SnO and allyl bromide gave a 6-allyl product **7** in good yields (Scheme 1a) and only a small amount (5%) of the 1-allyl isomer. This regioselectivity is proposed to result from steric effect, as the 1-position of the tin complex of **6** (Fig. 2) would be more sterically hindered than the 6-position. An experimental support for this rationale is that when a bulkier reagent, such as *p*-methoxybenzyl chloride (MBnCl), was utilized for the reaction, only 6-alkylation was observed. Then, the free hydroxyl group in **7** was protected by an MBn group to give **8**. When **8** was treated by HCl in $MeOH/CH_2Cl_2$ for a short period (10 min), the more strained *trans* ketal was selectively cleaved to offer **9** (60%). This reaction should be monitored closely, as the remaining ketal in **9** could also be removed

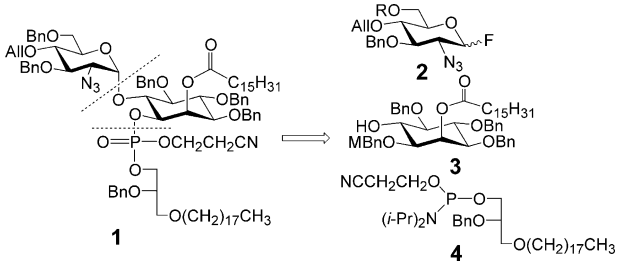

Figure 1. Retrosynthetic analysis.

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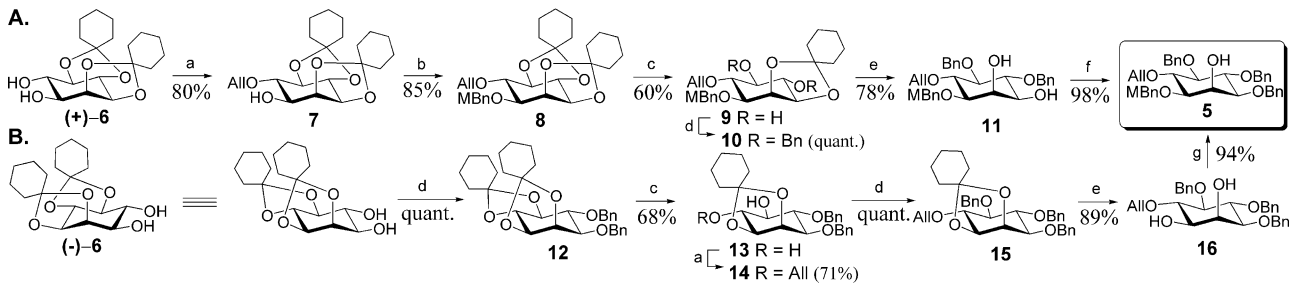


Scheme 1. Reagents and conditions: (a) (i) Bu_2SnO, toluene, reflux, 2.5 h; (ii) AllBr, DMF, rt, 16 h; (b) MBnCl, NaH, DMF, rt, 3 h; (c) AcCl, MeOH–CH_2Cl_2, rt, 10 min; (d) BnBr, NaH, DMF, rt, 3 h; (e) AcCl, MeOH–CH_2Cl_2, rt, 2 h; (f) (i) Bu_2SnO, toluene, reflux, 2.5 h; (ii) BnBr, DMF, rt, 15 h; (g) (i) Bu_2SnO, toluene, reflux, 2.5 h; (ii) MBnCl, DMF, rt, 16 h.

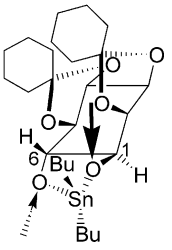
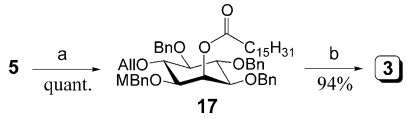
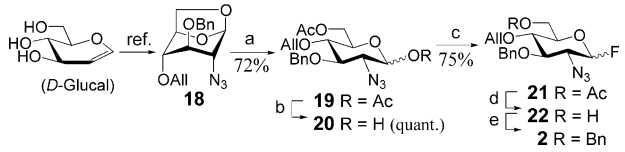


Figure 2. The tin complex involved in the selective allylation of (+)-**6**.



Scheme 2. Reagents and conditions: (a) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2, rt, overnight; (b) $PdCl_2$, NaOAc, AcOH, THF, rt, 18 h.



Scheme 3. Reagents and conditions: (a) Ac_2O–AcOH (1:1), H_2SO_4 (cat), 0 °C, 15 min; (b) $BnNH_2$, Et_2O, rt, 2 h; (c) DAST, CH_2Cl_2, 0 °C, 30 min; (d) NaOMe, MeOH, rt, 2 h; (e) BnBr, NaH, DMF, rt, 3 h.

on prolonged exposure to acid. Thereafter, **9** was benzylated to produce **10**. The ketal in **10** was finally deblocked by HCl/MeOH (2 h), and the resulting diol **11** was regioselectively benzylated in the presence of Bu_2SnO to afford the key derivative **5**. Only the 3-benzylation was observed in this reaction. Thus, **5** was effectively prepared on a multigram scale from (+)-**6** in six separate steps and a 32% overall yield.

To further improve the overall yield of **5** from inositol, we studied the feasibility to make use of the remaining enantiomer of **6**. In fact, as inositol itself is symmetric and the temporary chiral property of **6** was created by derivatization, if we introduce new protecting groups to (−)-**6** (in replacement of the exiting ones) by a sequence reverse to that of Scheme 1a, it is possible to derive the same synthetic target from both enantiomers.

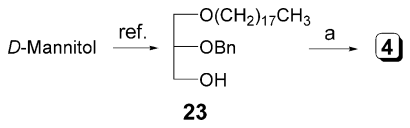
Therefore, we designed another synthetic procedure for **5** starting from (−)-**6** (Scheme 1b). The reactions used herein were similar to those used in the former procedure. First, (−)-**6** was benzylated to produce **12**. Then, its *trans* ketal was selectively cleaved under an acidic condition described above to give a diol **13** (68%). Regioselective allylation of **13** was achieved again under the influence of Bu_2SnO to afford **14** (71%). Then, benzylation of **14**, removal of the ketal in the resulting **15** and selective methoxybenzylation of **16** in the presence of Bu_2SnO finally offered **5** in an excellent yield (84%). Thus, **5** was prepared from (−)-**6** in 6 separate steps and a 42% overall yield.

While **5** contains a free 2-hydroxyl to which various functional or protecting groups can be attached, its 1- and 6-positions are respectively protected by MBn and All that can be selectively removed by CAN/H_2O and $PdCl_2$/HOAc without affecting benzyl groups at other positions. Therefore, once **5** was obtained, it was quite straightforward to transform **5** to **3**. 2-Acylation of **5** using DCC as a condensation reagent and then selective deallylation of the product **17** afforded **3** in an excellent yield (Scheme 2).

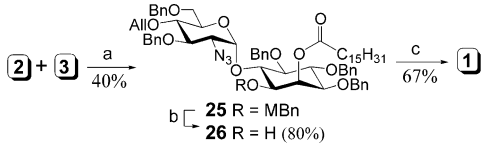
The glycosyl donor **2,** having its amino group protected as an azido group, was then prepared from D-glucal by a procedure shown in Scheme 3. D-Glucal was readily transformed to **18** by a reported method.[12] Selective breaking of the 5-membered ring in **18** by a mixture of acetic acid and acetic anhydride (1:1) containing a trace amount of concentrated sulfuric acid gave the diacetate **19** ($\alpha/\beta = 3.5:1$), which was selectively deprotected by benzylamine to yield a hemiacetal **20**. Treatment of **20** with DAST afforded the glycosyl fluoride **21** ($\alpha/\beta = 1:6$). Finally, **2** was obtained by replacing the 6-acetyl group in **21** for a benzyl group in two steps using conventional protocols.

The phospholipid block **4** was prepared according to Scheme 4. First, D-mannitol was converted to **23** by a reported procedure.[13] Then, phosphorylation of **23** by **24** afforded **4**. It has a defined *R*-configuration. Compound **4** was proved during chromatography to be unstable on a silica gel column. Thus, crude **4** was directly used for the next step.

The glycosylaction of **3** by **2** was achieved in anhydrous Et_2O using dichlorohafnocene and silver perchlorate as



Scheme 4. Reagents and conditions: (a) $(NCCH_2CH_2O)P(NPr^i_2)_2$ (**24**), diisopropylaminium tetrazolide, CH_2Cl_2–MeCN, rt, 2 h.



Scheme 5. Reagents and conditions: (a) Cp_2HfCl_2, AgOTf, MS 4Å, Et_2O, −15 °C to rt, overnight; (b) CAN, H_2O–MeCN (1:9), rt, 2 h; (c) **4**, tetrazole, MS 3Å, CH_2Cl_2–MeCN, rt, 6 h; O_2, light.

a promoter (Scheme 5), and a mixture of the expected product **25** and its β-anomer (4:3) was obtained in 70% yield. The anomers were easily separable on a silica gel column. Meanwhile, it was interesting to notice that the same reaction carried out in CH_2Cl_2 gave a mixture in favor of the β-isomer (α/β = 4:5). After removal of the MBn in **25** by oxidation with CAN, **26** was obtained in a good yield (80%). Finally, phosphorylation of **26** by **4** in the presence of tetrazole proceeded very smoothly to give a product (67% yield after column chromatography) that was projected to be a phosphite. However, the ^{31}P NMR (δ 8.71, 7.35) and MS spectra clearly indicated that the product was a diastereoisomeric mixture of the expected final product **1**[14] that might result from the oxidation of the phosphite by oxygen on the column, which was previously observed by Frier[15] as well.

In conclusion, this paper described a highly convergent synthesis of a GPI anchor (**1**) of sperm CD52. Since the 4-position of its glucosamine residue, which is protected by an allyl group, can be selectively exposed, it will be possible to further modify the glycan for the preparation of intact CD52 GPI anchors. In the meantime, this paper also presented a new and effective method to prepare an enantiomerically pure derivative (**5**) of D-*myo*-inositol with its 1,2,6-positions well differentiated. Because the inositol residue in many GPI anchors has its 2-position modified by various lipid chains, **5** can be widely useful to the synthesis of other GPI anchors. The synthesis of **5** is highlighted by making use of both enantiomers of an intermediate. Enantiomers of inositol derivatives were previously employed by Chen[11a] and Fraser-Reid[5a] in their works, but these studies, like ours, used both enantiomers of a certain intermediate in different ways and for different purposes.

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14. **5**: $[\alpha]_D = -8°$ (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.36 (m, 17H), 6.92 (d, $J = 8.5$ Hz, 2H), 5.96–6.08 (m, 1H), 5.32 (dd, $J = 17.3$, 1.6 Hz, 1H), 5.19 (d, $J = 10.4$ Hz, 1H), 4.85–4.94 (m, 4H), 4.73 (s, 2H), 4.68 (d, $J = 11.4$ Hz, 1H), 4.64 (d, $J = 11.4$ Hz, 1H), 4.42 (m, 2H), 4.19 (br, 1H), 3.98 (t, $J = 9.5$ Hz, 1H), 3.85 (m, 1H), 3.84 (s, 3H), 3.42 (t, $J = 9.3$ Hz, 1H), 3.39 (dd, $J = 9.6$, 2.5 Hz, 1H), 3.33 (dd, $J = 9.6$, 2.5 Hz, 1H), 2.48 (s, 1H). (−)-**6**: [α]−16.5° (*c* 1.0, $CHCl_3$); Lit.:[11a] −16° (*c* 1.0, $CHCl_3$). **1**: ^1H NMR (600 MHz, $CDCl_3$) δ 7.12–7.37 (m, 30H), 5.76 (m, 1H), 5.60 (bs, 1H), 5.40 (d, $J = 3.6$ Hz, 1H), 5.10 (d, $J = 17.4$ Hz, 1H), 5.04 (d, $J = 10.2$ Hz, 1H), 4.89 (d, $J = 11.4$ Hz, 1H), 4.72 (d, $J = 11.4$ Hz, 1H), 4.69 (m, 2H), 4.60–4.64 (m, 3H), 4.50–4.54 (m, 3H), 4.44 (d, $J = 11.2$ Hz, 1H), 4.39 (d, $J = 12.6$ Hz, 1H), 3.30–4.22 (m, 19H), 3.17 (br, 1H), 3.13 (t, $J = 9.0$ Hz, 1H), 2.94 (d, $J = 9.6$ Hz, 1H), 2.63 (t, $J = 6.0$ Hz, 2H), 2.26 (m, 2H), 1.56 (m, 4H), 1.01–1.18 (m, 54H), 0.91 (m, 6H); ^{31}P NMR ($CDCl_3$) δ 8.71, 7.35; FABMS: calcd for $C_{97}H_{137}N_4O_{16}P$, 1645.0. Found, 1684.1 ($M + K^+$), 1668.0 ($M + Na^+$), 1640.1 ($M - N_2 + Na^+$), and 1549.1 ($M - N_2 - Bn + Na^+$).
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