Toward Synthesis of Novel C-glycoprotein from Human RNase; Unexpected Stereochemistry of Epoxide Opening Reaction by Organolithium Reagents in the Presence of Lewis Acid

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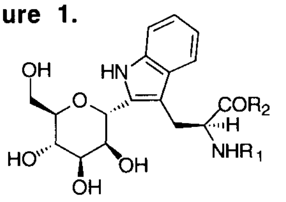
A nucleophilic attack reaction to 1,2-anhydro-β-D-mannnopyranose by indole derived organolithium reagents in the presence of BF_3•OEt_2 afforded isomers of C-aryl glycoside corresponds to the basic skeleton of C-linked mannnosyl tryptophan, which is recently identified in human RNase.

Recent investigations have revealed the importance of glycoconjugate carbohydrate chains, which are involved in a myriad of biologically significant functions.[1] In spite of being highly diverse in structures, they can be classified systematically according to common structural motifs.[2] For instance, N-glycans are linked to proteins via a glycosylamido linkage to aspargine residues, while another major subgroup, O-glycans are linked to hydroxyl group of serine or threonine residue. However, a new class of glycopeptide **1** (Figure 1) was identified in human RNase, where a mannose residue is connected to tryptophan via C-glycosidic linkage.[3] Mannose portion adopts $^1C_4$ conformation due to the steric hindrance of tryptophan group at C-1 position.

Figure 1.



1 R_1 = Phe-Thr, R_2 =Ala-Gln-Trp
2 R_1 =H, R_2 = OH

In the course of our project on the synthesis of glycoprotein-related molecules,[4] we are interested in the synthesis of this novel glycoprotein structural motif. There are a number of well established strategies for formation C-glycoside.[5] However, most of them have been developed for the introduction of relatively simple C_1~C_3 carbon units, and effective strategy for the direct incorporation of larger aglycon is yet to be established. The nucleophilic reaction of organometallic compounds to epoxides is one of the most well documented reactions in synthetic chemistry. Since nucleophilic ring opening of epoxide generally proceeds with S_N2 pathway, coupling of 1,2-anhydro-mannose **3** with C-2 metalated indole would seem to be the most logical choice for the stereocontrolled synthesis of the backbone structure of tryptophan substituted mannose **2**.[6]

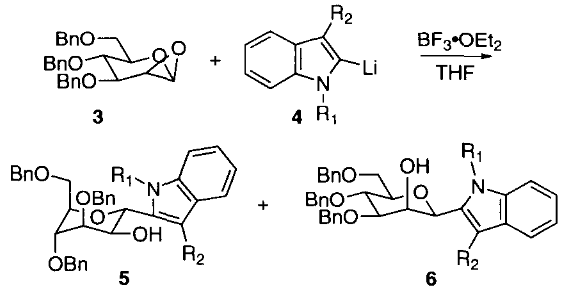


Table 1.

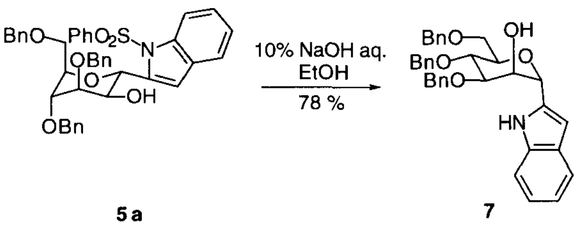
entry	4	R_1	R_2	yield/%	products (ratio)[a]
1	**4a**	SO_2Ph	H	39	**5a** / **6a** (69: 31)
2	**4b**	SO_2Ph	Me	39	**5b** / **6b** (87: 13)
3	**4c**	Boc	H	49	**5c** / **6c** (34: 66)[b]
4	**4d**	Boc	Me	56	**5d** / **6d** (17: 83)

[a]Calculated based on isolated yield except entry 3.
[b]Determined by ^1H-NMR.

According this scenario, we examined the reaction between **3**[7] and lithiated sulfonamide protected indole **4a** in the presence of BF_3•OEt_2. The reaction gave products, **5a** and **6a**, in a ratio of 69:31. Since the anomeric effect[8] is not a dominant stereoelectronic factor in C-aryl glycoside, the compound **5a** predominantly adopts $^1C_4$ conformer ($^3J_{1,2}$ = 7.6 Hz) due to the steric bulkiness of N-protected indole. The stereochemical assignments of **5** and **6** were rigorously confirmed by NOE experiment mainly between H-1 and H-5.[9]

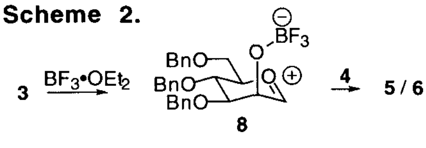
After removal of benzensulfonyl group by aqueous 10% NaOH (Scheme 1), the conformation of the compound **7** became the $^4C_1$ conformation ($^3J_{1,2}$ ~ 0 Hz).[10]

Scheme 1.



Significant loss of stereospecificity of this epoxide ring opening reaction might be explained by assuming that Lewis acid pre-activates the epoxide into oxocarbenium ion-like intermediate **8**, which subsequently captures **4a** in a stereorandom manner (Scheme 2). This stereochemical bias was revealed to be quite sensitive to minor structural modification of the indole unit (Table 1). When methyl substituted indole derivative **4b** was used, the stereoselectivity was increased to 87:13 in favor of **5b**. On the other hand, reactions using closely related Boc protected indole derivatives **4c** and **4d**[11] afforded β-substituted **6c** and **6d** as major products, respectively.[12]

Scheme 2.



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Typical experimental procedure; To a solution of 1-(benzenesulfonyl)-3-methyl-indole (82.8 mg, 0.215 mmol) in THF (6 ml), *t*-BuLi (0.13 ml, 1.53 M pentane solution, 0.20 mmol) was added at -78 °C under Ar atmosphere. After 30 min, to a solution of epoxide (62.0 mg, 0.143 mmol) in THF (1 ml), the lithioindole **4b** was transferred via cannula, then $BF_3 \cdot Et_2O$ (25 µl, 0.20 mmol) was dropped. After stirring the mixture at -78 °C for 5 h, Et_3N (100 µl) was added to neutralize the mixture, and then saturated $NaHCO_3$ aq. was added. The aqueous phase was extracted with EtOAc three times, then combined organic layers were washed with brine. After drying over Na_2SO_4, the solvent was evaporated. The crude material was purified by preparative TLC to afford **5b** (38.4 mg, 34%), and **6b** (5.9 mg, 5.0%).

Although the yields of indole C-mannosides (**5a**-**5d**) are modest, their multi-gram supply can be now assured, because all precursors (**3**, **4a**-**4d**) are readily available in large quantities. Further transformation into **1** is under current investigation.

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Reference and Notes

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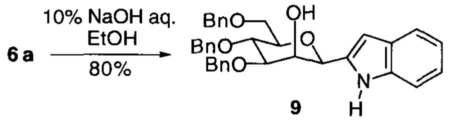
9 **5a**, ^{1}HNMR (270 MHz, $CDCl_3$) δ = 8.10 (1H, d, *J* = 8.3 Hz), 7.87 (2H, d, *J* = 8.3 Hz), 7.5-7.2 (21H, m), 6.74 (1H, s), 5.73 (1H, d, *J* = 7.3 Hz), 4.66 (1H, d, *J* = 11.5 Hz), 4.62 (2H, s), 4.57 (1H, d, *J* = 11.5 Hz), 4.51 (1H, d, *J* = 11.9 Hz), 4.46 (1H, d, *J* = 11.9 Hz), 4.33 (1H, m), 4.0-3.9 (3H, m), 3.84 (1H, dd, *J* = 5.9 Hz, 10.2 Hz), 3.57 (1H, dd, *J* = 5.6 Hz, 10.2 Hz), 2.52 (1H, d, *J* = 7.9 Hz). **6a**, ^{1}HNMR (270 MHz, $CDCl_3$) δ = 8.02 (1H, d, *J* = 8.2 Hz), 7.82 (2H, d, *J* = 8.6 Hz), 7.5-7.2 (22H, m), 5.23 (1H, s), 4.92 (1H, d, *J* = 10.9 Hz), 4.82 (1H, d, *J* = 11.6 Hz), 4.72 (1H, d, *J* = 11.6 Hz), 4.6-4.5 (2H, m), 4.61 (1H, d, *J* = 11.9 Hz), 4.49 (1H, d, *J* = 11.9 Hz), 4.0-3.7 (5H, m), 2.39 (1H, d, *J* = 3.6 Hz). **7**, ^{1}HNMR (270 MHz, $CDCl_3$) δ = 8.51 (1H, bs), 7.58 (1H, d, *J* = 7.9 Hz), 7.5-7.1 (19H, m), 6.03 (1H, s), 5.25 (1H, s), 4.80 (1H, d, *J* = 11.6 Hz), 4.79 (1H, d, *J* = 11.2 Hz), 4.72 (1H, d, *J* = 11.6 Hz), 4.59 (1H, d, *J* = 11.9 Hz), 4.55 (1H, d, *J* = 11.9 Hz), 4.50 (1H, d, *J* = 11.2 Hz), 4.46 (1H, m), 3.90 (1H, dd, *J* = 3.2, 8.6 Hz), 3.80 (1H, dd, *J* = 8.9, 8.9 Hz), 3.8-3.7 (2H, m), 3.56 (1H, m), 2.78 (1H, d, *J* = 2.3 Hz).

10 The possibility of epimerization at C-1 position during hydrolysis of sulfonamide is excluded, because **9** was obtained from **6a** in 80% yield under the same conditions (10% NaOH aq., EtOH).

Scheme 3.



11 For generation of **4a**; M. M. Cooper, G. J. Hignett, and J. A. Joule, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 3008. **4c**; I. Hasan, E. R. Marinelli, L-C. C. Lin, F. W. Fowler, and A. B. Levy, *J. Org. Chem.*, **46**, 157 (1981). **4d**; This compound is generated from corresponding bromide by halogen-metal exchange by BuLi. The synthesis of bromide; R. Liu, P. Zhang, T. Gan, and J. M. Cook, *J. Org. Chem.*, **62**, 7447 (1997).

12 The products **5a** and **6a** were not interconvertible in the presence of **4a** (2.4 equiv.) at -40 °C in THF for 5 h.