Total Synthesis of Novel Subclass of Glyco-amino Acid Structure Motif: *C*^{2}-α-L-*C*-Mannosylpyranosyl-L-tryptophan

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It is now widely recognized that attachment of carbohydrates is one of the most important posttranslational protein modifications affecting biological activities by way of controlling higher order structure, stability, immunogenicity, and carbohydrate−protein interaction.[1] In most cases, protein glycosylation can be classified into two major subtypes: *O*-glycosylation, where an *N*-acetylgalactosamine residue is linked to the hydroxyl group of either serine or threonine, and *N*-glycosylation, where a glycan chain is linked via a glycosylamido linkage to an asparagine residue.[2] However, in 1994, a new class of glycoprotein structural motif **1** (Figure 1) was identified in human RNase, where a mannose residue is connected to tryptophan via a *C*-glycosidic linkage.[3a−e] Further investigations have revealed that the consensus recognition site for *C*-glycosylation is *Trp*-Aaa-Aaa-Trp (glycosylation site indicated as italic).[3d] More recently, the same structural motif was found in recombinant human IL-12, implying that this posttranslational modification might be more widespread in various proteins containing the sequence *Trp*-Aaa-Aaa-Trp.[3f] Herein we report the total stereocontrolled synthesis of this novel type of glyco-amino acid, *C*^{2}-α-L-*C*-mannosylpyranosyl-L-tryptophan **2**, and preliminary results on the synthesis of this glycopeptide, which constitute the partial structure of human RNase.[4]

N−Arylsulfonated indoles have been known to be amenable to direct metalation at the 2-position, which on subsequent quenching with an electrophile provides an easy access to 2-substituted indole derivatives.[5] We have recently reported the direct incorporation of C-1 linked mannose onto the 2-position of indole by using C-2 lithiated indole derivatives and 1,2-anhydro-mannose[6] **3** in the presence of BF_3·OEt_2.[4a] Further systematic investigation revealed that the strereoselectivity of this transformation strongly depends on the protecting group and substituent on the indole. Representative results are shown in Table 1. When sulfonamide was used as a protecting group for the indole nitrogen, acyclic C-2 substituents larger than methyl uniformly gave a higher degree of stereoselectivity in favor of the α products (entries 5, 6, 8, and 9). Encouraged by these results, we designed the indole derivative **4j** as a latent tryptophan moiety of the target molecule.

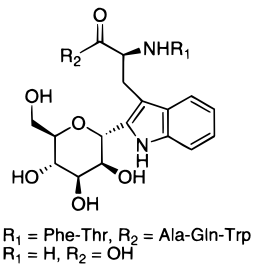
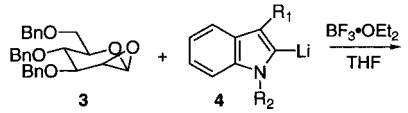
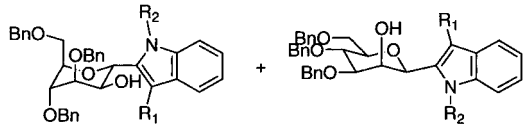
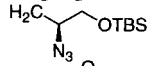
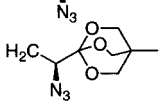
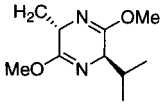


Figure 1.

Table 1. Stereochemistry of Epoxide Opening Reaction by Lithiated Indole Derivatives

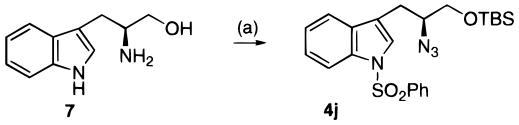




entry	4	R_1	R_2	yield (%)	products	α/β [a]
1[b]	**a**	H	SO_2Ph	39	**5a/6a**	69:31
2[b]	**b**	H	Boc	49	**5b/6b**	34:66
3[b]	**c**	CH_3	SO_2Ph	39	**5c/6c**	87:13
4[b]	**d**	CH_3	Boc	56	**5d/6d**	17:83
5	**e**	CH_2CH_3	SO_2Ph	38	**5e/6e**	>95/5
6	**f**	CH_2OTBS	SO_2Ph	50	**5f/6f**	>95/5
7	**g**	CH_2OTBS	Boc	17	**5g/6g**	77:23
8	**h**	CH_2CH_2OTBS	SO_2Ph	50	**5h/6h**	>95/5
9	**i**	CH_2CH_2OMOM	SO_2Ph	47	**5i/6i**	>95/5
10	**j**		SO_2Ph	63	**5j/6j**	95:5
11	**k**		SO_2Ph	18	**5k/6k**	55:45
12	**l**		SO_2Ph	18	**5l/6l**	56:44

[a] Calculated based on isolated yield except entry **2** and **10**, where the ratios were determined by ^{1}H NMR. [b] Taken from ref 4a

Scheme 1



The synthesis of **4j** was straightforward and high yielding as depicted in Scheme 1. Commercially available (L)-(−)-tryptophanol **7** was converted to the corresponding azide by treatment of TfN_3,[7] and the hydroxyl group was protected as a *tert*-butyldimethylsilyl (TBS) ether. The indole ring was further protected as a benzenesulfonamide by the action of benzenesulfonyl chloride and *n*-BuLi to give **4j** in 61% overall yield from **7**. Subsequent coupling with 1,2-anhydro-mannose **3** proceeded in a satisfactory manner, in terms of both selectively (95:5) and reaction efficiency

(1) Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319. Varki, A. *Glycobiology* **1993**, *3*, 97. Giannis, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 178. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.

(2) For recent synthetic studies on glycopeptides, see; Meinjohanns, E.; Meldal, M.; Paulsen, H.; Dwek, R. A.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 549. Elofsson, M.; Salvador, L. A.; Kihlberg, J. *Tetrahedron* **1997**, *53*, 369. Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317 and references therein.

(3) (a) Hofsteenge, J.; Müller, D. R.; Beer, T.; Löffler, A.; Richter, W. J.; Vliegenthart, J. F. G. *Biochemistry* **1994**, *33*, 13524. (b) Beer, T.; Vliegenthart, J. F. G.; Löffler, A.; Hofsteenge, J. *Biochemistry* **1995**, *34*, 11785. (c) Löffler, A.; Doucey, M.-A.; Jansson, A. M.; Müller, D. R.; Beer, T.; Hess, D.; Meldal, M.; Richter, W. J.; Vliegenthart, J. F. G.; Hofsteenge, J. *Biochemistry* **1996**, *35*, 12005. (d) Kreig, J.; Hartmann, S.; Vicentini, A.; Gläsner, W.; Hess, D.; Hofsteenge, J. *Mol. Biol. Cell*. **1998**, *9*, 301. (e) Vliegenthart, J. F. G.; Casset, F. *Curr. Opin. Struct. Biol.* **1998**, *8*, 565. (f) Doucey, M.-A.; Hess, D.; Blommers, M. J. J.; Hofsteenge, J. *Glycobiology* **1999**, *9*, 435.

(4) Previous synthetic studies: (a) Manabe, S.; Ito, Y.; Ogawa, T. *Chem. Lett.* **1998**, 919. (b) Nishikawa, T.; Ishikawa, M.; Isobe, M. *SYNLETT* **1999**, 123 (the synthesis of proctected **2** as a diastereomeric mixture is reported). (c) Hassan, H. H. A. M.; Bredenkamp, M. W. XIXth International Carbohydrate Symposium, San Diego, August, 1998, Abstract, BP 295.

(5) Sundberg, R. J.; Russell. H. F. *J. Org. Chem.* **1973**, *38*, 3324.

(6) Du, Y.; Kong, F. *J. Carbohydr. Chem.* **1995**, *14*, 341.

(7) Vasella, A.; Witzig, C.; Chiara, J.-L.; Martin-Lomas, M. *Helv. Chim. Acta*. **1991**, *74*, 2073.

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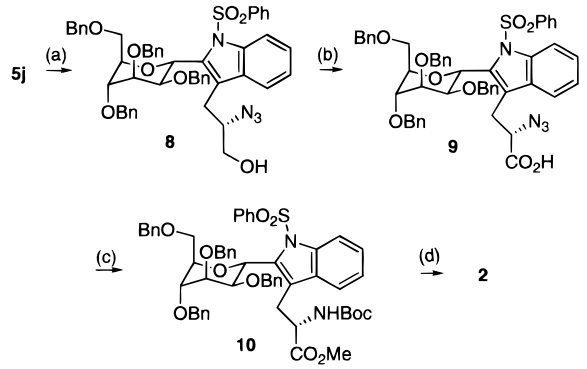
(63% yield) to afford **5j** together with a small amount of the stereoisomer **6j** (entry 10). On the other hand, the masked tryptophan derivatives **4k**[8] and **4l**[9] also gave corresponding coupled products, which can be seen as properly functionalized precursors of the target molecule. However, in these cases, both yields and stereoselectivities were far from satisfactory (entries 11, and 12).

Compound **5j** was transformed into **8**, which was oxidized to carboxylic acid **9** by TEMPO−iodosobenzene diacetate combination[10,11] in an excellent yield (Scheme 2). Protection of the

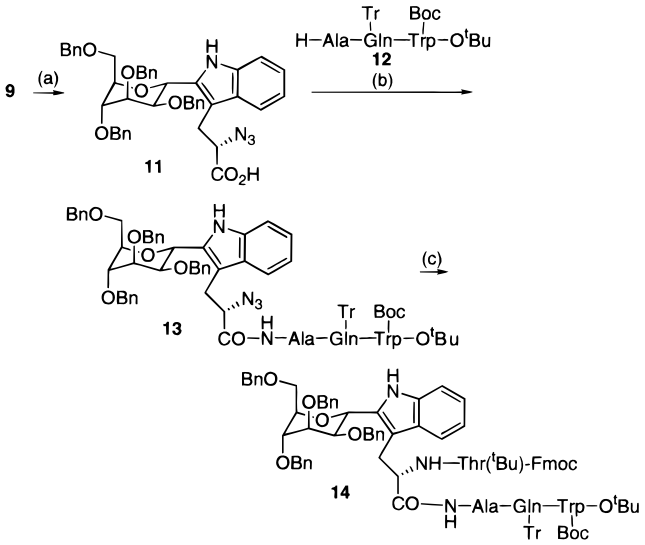
Scheme 2



carboxylic acid as a methyl ester was followed by phosphine-mediated azide reduction to afford the amine that was isolated as Boc-protected **10,** following the protocol of Ariza et al.[12] Debenzylation was accomplished under standard conditions, followed by acidic cleavage of the Boc group. Final deprotection of the methyl ester and sulfonamide groups was performed under alkaline hydrolytic conditions. After purification by gel filtration, the desired product **2** was obtained as a sodium salt in 87% yield. Compound **2** thus obtained gave fully assignable ^{1}H NMR, and ^{13}C NMR spectra that compared well with those reported for the hexapeptide **1**.[13]

In contrast to the usually observed $^4C_1$ conformation, the mannose moiety in peptide **1** was reported to adopt a $^1C_4$-like chair conformation.[3a−c] With rigorously defined synthetic **2** in hand, ^{1}H NMR analysis clearly revealed that the mannosylated tryptophan adopts the $^1C_4$-like conformation with an equatorially oriented tryptophan moiety ($^3J_{1,2}$ = 8.1 Hz), presumably because of its bulkiness as well as the absence of the anomeric effect.

Scheme 3



With the use of azide acids in "racemization free" peptide synthesis in mind,[14] peptide elongation was commenced from **9** as follows. Removal of the indole ring protection of compound **9** was achieved with 10% NaOH aqueous in EtOH to give compound **11** in 72% yield (Scheme 3). The coupling reaction of acid **11** and tripeptide **12**[15] proceeded in 90% yield in the presence of tetramethylfluoroformidium hexafluorophosphate (TFFH).[16] Thus obtained, **13** showed no indication of epimerization within the detection limit of 500 MHz ^{1}H NMR. After reduction of the azide group in the mannose-linked tetrapeptide by PMe_3, the coupling reaction with Fmoc-Thr(*t*-Bu)-OH was successfully performed to afford **14** in good yield. Adequacy of the present strategy for further peptide elongation both in solution phase and solid phase is quite obvious.[17]

In summary, a total synthesis of *C*-linked amino acid **2** was achieved in a concise manner. Our strategy may well be readily applicable to the incorporation of ^{13}C- or ^{15}N- labels using appropriately labeled tryptophan precursors. Furthermore, the pentapeptide sequence **14** was prepared using **11** as a glyco-amino acid unit. Investigation on further elongation of the peptide chain for conformational analysis of peptides containing **2** is underway.

Acknowledgment. This work was supported by the Science and Technology Agency of the Japanese Government through Special Researcher's Basic Science Program at RIKEN (S.M.). We thank Ms. A. Takahashi for technical assistance. We thank Dr. J. Uzawa for recording and measuring the NMR spectra. We thank Dr. Chihara and his staff for elemental analysis.

Supporting Information Available: Experimental details and ^{1}H NMR spectra of compound **4j**, **5j**, **8**, **9**, **10**, **11**, **12**, **13**, and **14** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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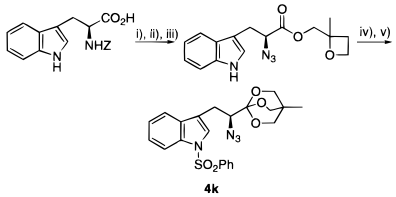
(8) **4k** was prepared from (*S*)-(−)-(*Z*)-Trp in five steps: (i) 3-methyl-3-oxetanmethanol, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl, DMAP, DMF, quant., (ii) H_2, 10% Pd−C, MeOH, (iii) TfN_3, DMAP, CH_3CN, 79% in 2 steps, (iv) BF_3·OEt_2 (0.25 equiv), CH_2Cl_2, 34% (starting material was recovered in 18% yield.), (v) $PhSO_2Cl$, *n*-BuLi, THF, 90%.



For rearrangement of oxetan ester to 2,6,7-trioxabicyclo[2, 2, 2]octane ortho ester, see; Corey, E. J.; Raju, N. *Tetrahedon Lett.* **1983**, *24*, 5571.

(9) Liu, R.; Zhang, P.; Gan, T.; Cook, J. M. *J. Org. Chem.* **1997**, *62*, 7447. Schöllkopf, U.; Groth, U.; Deng, C. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 798.

(10) Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293.

(11) The compound **5k** was converted to the corresponding carboxylic acid **9** quantitatively in two steps ((i) 1 M HCl, THF, (ii) 1 M LiOH, aqueous MeOH,) and was identical with **9** derived from alcohol **5j**.

(12) Ariza, X.; Urpí, F.; Viladomat, C.; Vilarrasa, J. *Tetrahedron Lett.* **1998**, *39*, 9101.

(13) ^{1}H NMR (600 MHz, D_2O, *t*-BuOH as an internal standard; *t*-Bu was adjusted 1.23 ppm) δ 7.73 (d, J = 7.7 Hz, 1H, H-4), 7.52 (d, J = 7.7 Hz, 1H, H-7), 7.30 (t, J = 7.3 Hz, 1H, H-6), 7.20 (t, J = 7.3 Hz, 1H, H-5), 5.16 (d, J = 8.1 Hz, 1H, H-1′), 4.42 (dd, J = 8.1, 3.3 Hz, 1H, H-2′) 4.25 (dd, J = 12.5, 8.8 Hz, 1H, H-6′), 4.11 (dd, J = 3.3, 3.3 Hz, 1H, H-3′), 4.01 (dd, J = 9.2, 5.1 Hz, 1H, H-α), 3.94 (dd, J = 4.0, 3.3 Hz, 1H, H-4′), 3.88 (ddd, J = 3.3, 3.3, 8.8 Hz, 1H, H-5′) 3.72 (dd, J = 3.3, 12.5 Hz, 1H, H-6′), 3.55 (dd, J = 5.1, 15.3 Hz, 1H, H-*β*), 3.35 (dd, J = 15.3, 9.2 Hz, 1H, H-*β*); ^{13}C NMR (150 MHz, D_2O) δ 175.9 (C), 137.5 (C), 134.8 (C), 128.5 (C), 124.4 (CH, C-6), 121.3 (CH, C-5), 120.2 (CH, C-4), 113.3 (CH, C-7), 109.8 (C), 80.5 (CH, C-5′), 71.9 (CH, C-3′), 70.4 (CH, C-4′), 69.1 (CH, C-2′), 67.4 (CH, C-1′), 60.4 (CH_2, C-6′), 56.6 (CH, C-α), 27.3 (CH_2, C-*β*).

(14) Peptide synthesis using azido acids on solid phase has been reported to proceed with a negligible degree of racemization: Meldal, M.; Juliano, M. A.; Jansson, A. M. *Tetrahedron Lett.* **1997**, *38*, 2531.

(15) For preparation of **12**, see Supporting Information.

(16) Carpino, L. A.; E-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401.

(17) Further incorporation of a Phe residue was successfully performed to give hexapeptide corresponding to **1** in a fully protected form. However, the final deprotection turned out to be problematic, presumably due to the instability of mannosylated tryptophan portion under acidic conditions required for the complete removal of *t*-Bu and Boc groups. This problem would be solved by making slight modification of amino acid protection strategy (Fmoc/benzyl), which is currently under investigation.