Total Synthesis of α -C-Mannosyltryptophan, a Naturally Occurring C-Glycosyl Amino Acid¹

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Dedicated to Professor Ryoji Noyori in recognition of his significant contributions to the art of organic synthesis

Abstract: A stereocontrolled synthesis of α -C-mannosyltryptophan, a new type of glycosyl amino acid, was achieved by $\text{Sc}(\text{ClO}_4)_3$ mediated coupling between α -C-mannosylindole and L-serine-derived 2-aziridinecarboxylate as a key step.

Key words: C-glycoside, aziridine, scandium perchlorate, natural product, total synthesis

Carbohydrate parts of glycoprotein and glycolipid have been found to play an important role in a variety of biological events such as cell-cell recognition, immune response, stabilization of protein, local conformational change of protein backbone, etc.² Most of the carbohydrate moiety are linked to protein through *N*-glycosidic bond with asparagine and *O*-glycosidic bond with serine or threonine.^{3,4} However, in 1994, Trp 7 of ribonuclease 2 (RNase 2) isolated from human urine was found to be α -C-mannosylated at 2 position of the indole (**1** in Scheme 1).⁵ This is the first example of naturally occurring C-glycosyl amino acid found in protein, although many synthetic C-glycosyl amino acids have been reported.⁶ This post-translational modification is catalyzed by microsome-associated enzyme,⁷ "C-mannosyltransferase", which recognized an amino acid sequence Trp-x-x-Trp to glycosylate the first tryptophan of this motif.⁸ These studies imply that this linkage is more common than expected in the early stage of this study. In fact, C-mannosylated protein was also found in recombinant interleukin 12 β ⁹ and later in human complement system.¹⁰ Furthermore C-mannosyltransferase activity was detected in many organisms such as mammals, birds, amphibians and fish.¹⁰ On the other hand, monomeric α -C-mannosyltryptophan (**2**) was isolated not only from human urine¹¹ but also from marine organisms.¹²

We have studied the synthesis of α -C-mannosyltryptophan and its analogs in order to investigate the generality and distribution in nature and to elucidate the biological functions of this new type of sugar chain. Herein we describe an efficient stereocontrolled synthesis of α -C-mannosyltryptophan (**2**).¹³

In our previous paper, a dehydrotryptophan intermediate was synthesized from C-mannosylindole **3**, which was prepared from D-mannose derivative **5** through ethynylmannose **4** by stereoselective α -C-glycosidation¹⁴ with tinacetylene and Castro indole synthesis as the key steps (Scheme 1).¹⁵ However, hydrogenation of the dehydro-

Scheme 1

tryptophan gave a diastereomeric mixture, whose absolute stereochemistry has not been determined.¹⁶ In order to synthesize an α -C-mannosyltryptophan with the definite absolute configuration, we would exploit a coupling reaction between indole and chiral aziridine-2-carboxylate, originally developed by Kozikowski¹⁷ and then Benani.¹⁸

Before going on the synthesis of **2**, we would examine the coupling of 2-methylindole as a model substrate with L-serine-derived methyl *N*-Cbz-2-aziridinecarboxylate (**6**)¹⁹ under the conditions reported^{17,18} (Scheme 2). Representative results are shown in the Table. With $\text{Zn}(\text{OTf})_2$ as a promoter, 2-methyltryptophan **7** was obtained in moderate yield with high selectivity (entry 1).²⁰ On the other hand, the same reaction in the presence of $\text{Sc}(\text{OTf})_3$ gave a 3:1-3:2 mixture of the desired product **7** and the regioisomer **8** formed by opening of the aziridine at the more substituted position (entry 2).²¹ We were concerned whether the quality of $\text{Sc}(\text{OTf})_3$ was different from those used in literature, which might affect the selectivity. In spite of changing the sources of reagent $\text{Sc}(\text{OTf})_3$ from various suppliers,²² and prepared by procedures,²³ no significant improvement was observed with the regioselectivity. After many experiments, however, we found that

$\text{Sc}(\text{ClO}_4)_3$ ²⁴ instead of $\text{Sc}(\text{OTf})_3$ was superior Lewis acid (entry 3). That is, the reaction proceeded at 0 °C to give the desired product **7** in good yield and high selectivity.

Scheme 2

Table Coupling reaction between 2-methylindole and the aziridine **6**

entry	conditions ^a			yield	ratio (7:8) ^b
	Lewis acid (equiv)	solvent	temp.		
1	$\text{Zn}(\text{OTf})_2$ (2)	CHCl_3	80 °C	69%	10:1
2	$\text{Sc}(\text{OTf})_3$ (1)	CH_2Cl_2	0	50	3:1-3:2
3	$\text{Sc}(\text{ClO}_4)_3$ (1)	CH_2Cl_2	0	80	10:1

^a) All reactions were carried out using 2 equiv of 2-methylindole and 1 equiv of aziridine **6**

^b) The ratios were determined from the integration values of ^1H -NMR.

Utilizing the best conditions found in the above model experiments, α -C-mannosylindole **3** was coupled with the aziridine **6** in the presence of $\text{Sc}(\text{ClO}_4)_3$ to provide a fully protected α -C-mannosyltryptophan **9**^{25,26} in 66% yield exclusively (Scheme 3). On the other hand, the same reaction gave a mixture (3:1) of **9** and its regioisomer in case of the presence of $\text{Sc}(\text{OTf})_3$.²⁷

Scheme 3

Finally, two step deprotections were carried out; alkaline hydrolysis of esters (62%) followed by hydrogenolysis of benzyl groups. The resulting hydrochloride salt was purified by a reversed phase column chromatography (Cosmosil 75C₁₈-OPN, nacalai tesque) to afford **2**²⁸ in 50% yield. ^1H - and ^{13}C -NMR spectra of synthetic **2** were in good agreement with those of literature.^{11a}

In summary, an efficient total synthesis of **2** was achieved in 10 steps from commercially available α -methyl-D-mannoside.²⁹ This route should provide a practical route to **2** and its analogs for the future biological studies.³⁰

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- (22) We have checked commercially available Sc(OTf)₃ from Aldrich, TCI and Taiheiyo-Kinzoku Corporation.
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- (25) ¹H NMR (400 MHz, CDCl₃) δ 1.89 (3H, s, OAc), 3.15 (1H, dd, *J* = 14.5, 10.5 Hz, H- β), 3.37 (1H, dd *J* = 14.5, 4.5 Hz, H- β), 3.55 (1H, br d, *J* = 3 Hz, H-4'), 3.75 (3H, s, COOCH₃), 3.84 (1H, br t, *J* = 3 Hz, H-3'), 3.86 (1H, dd, *J* = 9, 2.5 Hz, H-2'), 4.04 (1H, d, *J* = 13 Hz, CH_AH_BPh), 4.05 (1H, dd, *J* = 12, 4 Hz, H-6'), 4.12 (1H, d, *J* = 13 Hz, CH_AH_BPh), 4.17 (1H, br dd, *J* = 9, 4 Hz, H-5'), 4.34 (1H, d, *J* = 12 Hz, CH_CH_DPh), 4.46 (1H, d, *J* = 12 Hz, CH_CH_DPh), 4.56 (1H, d, *J* = 12 Hz, CH_EH_FPh), 4.65 (1H, br dt, *J* = 10.5, 4.5 Hz, H- α), 4.73 (1H, d, *J* = 12 Hz, CH_EH_FPh), 4.78 (1H, dd, *J* = 12, 9 Hz, H-6'), 4.87 (1H, d, *J* = 12 Hz, CH_GH_HPh), 4.99 (1H, d, *J* = 12 Hz, CH_GH_HPh), 5.20 (1H, d, *J* = 9 Hz, H-1'), 6.37 (1H, d, *J* = 5 Hz, NH-Cbz), 6.70 (1H, d, *J* = 7.5 Hz, H-7), 7.01 (1H, t, *J* = 7.5 Hz, H-6), 7.09-7.39 (21H, m, C₆H₅ $\times$ 4 & H-5), 7.64 (1H, d, *J* = 7.5 Hz, H-4), 8.18 (1H, s, NH of indole).
- (26) Scope and limitation of Sc(CIO₄)₃ as a promoter in this type of tryptophan synthesis will be reported elsewhere.
- (27) The coupling between C-mannosylindole **3** and the aziridine **6** in the presence of Zn(OTf)₂ as a Lewis acid did not proceed, while the aziridine **3** was decomposed under the condition.
- (28) ¹H NMR (600 MHz, D₂O) δ 3.36 (1H, dd, *J* = 15, 9 Hz, H- β), 3.57 (1H, dd, *J* = 15, 5 Hz, H- β), 3.74 (1H, dd, *J* = 12, 3 Hz, H-6'), 3.90 (1H, dt, *J* = 9, 3 Hz, H-5'), 3.96 (1H, dd, *J* = 5, 3 Hz, H-4'), 4.03 (1H, dd, *J* = 9, 5 Hz, H- α), 4.13 (1H, dd, *J* = 5, 3 Hz, H-3'), 4.27 (1H, dd, *J* = 12, 9 Hz, H-6'), 4.44 (1H, dd, *J* = 8, 3 Hz, H-2'), 5.18 (1H, d, *J* = 8 Hz, H-1'), 7.22 (1H, t, *J* = 8 Hz, H-5), 7.32 (1H, t, *J* = 8 Hz, H-6), 7.54 (1H, d, *J* = 8 Hz, H-7), 7.75 (1H, d, *J* = 8 Hz, H-4). ¹³C NMR (150 MHz, D₂O) δ 28.7, 58.0, 61.8, 68.9, 70.5, 71.7, 73.3, 81.8, 111.1, 114.7, 121.5, 122.7, 125.7, 129.9, 136.2, 138.8, 177.2.
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- (30) Preparation of a monoclonal antibody against α -C-mannosyltryptophan is currently underway in collaboration with Dr. Hofsteenge's group.

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