Total Synthesis of α-*C*-Mannosyltryptophan, a Naturally Occurring *C*-Glycosyl Amino Acid[1]

Toshio Nishikawa, Miyuki Ishikawa, Kyoko Wada, Minoru Isobe*

Laboratory of Organic Chemistry, School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Fax+81 (52) 7894111; E-mail: isobem@agr.nagoya-u.ac.jp

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

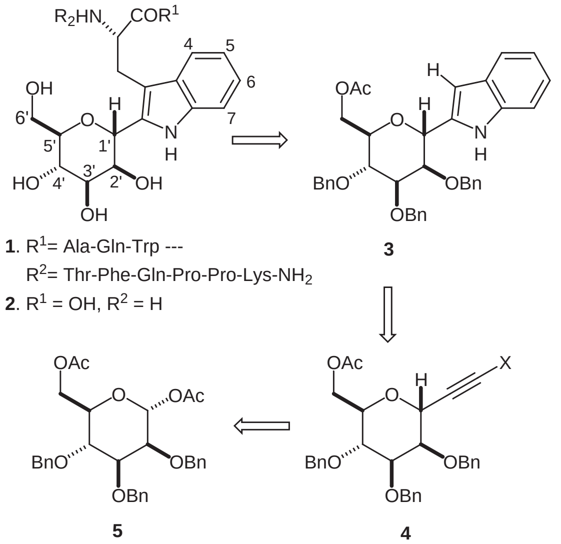
Abstract: A stereocontrolled synthesis of α-*C*-mannosyltryptophan, a new type of glycosyl amino acid, was achieved by $Sc(ClO_4)_3$ mediated coupling between α-*C*-mannosylindole and L-serine-derived 2-aziridinecarboxylate as a key step.

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 12β[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 urine[11] 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]

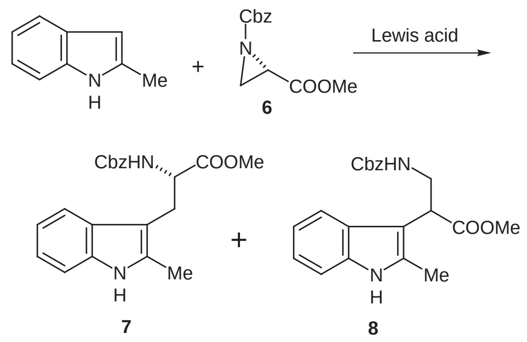
In our previous paper, a dehydrotryptophan intermediate was synthesized from *C*-mannosylindole **3**, which was prepared from D-mannose derivative **5** through ethynylmannose **4** by stereoselective α-*C*-glycosidation[14] with tinacetylene and Castro indole synthesis as the key steps (Scheme 1).[15] However, hydrogenation of the dehydrotryptophan 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 Kozikowski[17] and then Bennani.[18]



Scheme 1

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 $Zn(OTf)_2$ 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 $Sc(OTf)_3$ 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 $Sc(OTf)_3$ was different from those used in literature, which might affect the selectivity. In spite of changing the sources of reagent $Sc(OTf)_3$ from various suppliers,[22] and prepared by procedures,[23] no significant improvement was observed with the regioselectivity. After many experiments, however, we found that

$Sc(ClO_4)_3$[24] instead of $Sc(OTf)_3$ 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

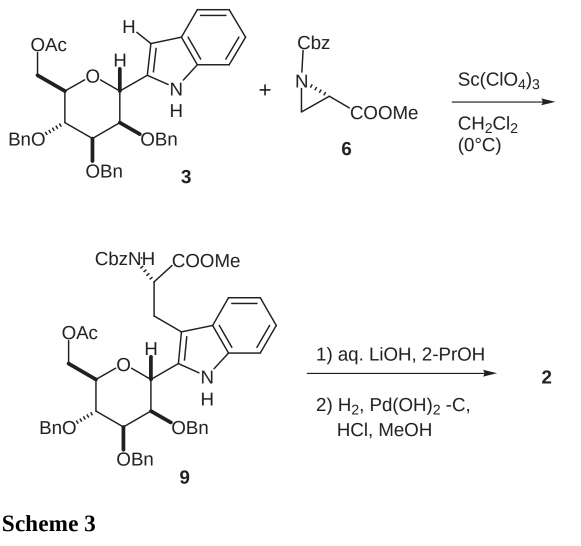
Table Coupling reaction between 2-methyindole and the aziridine **6**

entry	conditions [a]			yield	ratio (**7**:**8**)[b]
	Lewis acid (equiv)	solvent	temp.		
1	$Zn(OTf)_2$ (2)	$CHCl_3$	80 °C	69%	10:1
2	$Sc(OTf)_3$ (1)	CH_2Cl_2	0	50	3:1-3:2
3	$Sc(ClO_4)_3$ (1)	CH_2Cl_2	0	80	10:1

[a] All reactions were carried out using 2 equiv of 2-methyindole and 1 equiv of aziridine **6**

[b] The ratios were determined from the integration values of ^{1}H-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 $Sc(ClO_4)_3$ 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 $Sc(OTf)_3$.[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 $75C_{18}$-OPN, nacalai tesque) to afford **2**[28] in 50% yield. ^{1}H- and ^{13}C-NM R 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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(21) A very similar reaction of 2-methylindole with benzylester of **6** was reported to give the corresponding product to **7** exclusively in 66% yield,[18] in which $Sc(OTf)_3$ purchased from Aldrich was used. Personal communication from Dr. Y. L. Bennani.
(22) We have checked commercially available $Sc(OTf)_3$ from Aldrich, TCI and Taiheiyo-Kinzoku Corporation.
(23) (a) Prof. Murai et al. reported that selectivity of *endo-*/*exo-*cyclization of a hydroxyepoxide strongly depended on different preparation of $La(OTf)_3$. Personal communication from Dr. K. Fujiwara. For related studies, see: Fujiwara, K.; Mishima, H.; Amano, A.; Tokiwano, T.; Murai, A. *Tetrahedron Lett.* **1998**, *39*, 393. (b) For preparation of $Sc(OTf)_3$, see: Kobayashi, S.; Hachiya, I.; Araki, M.; Ishitani, H. *Tetrahedron Lett.* **1993**, *34*, 3755.
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(25) ^{1}H NMR (400 MHz, $CDCl_3$) 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, $COOCH_3$), 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, N*H*-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, $C_6H_5 \times 4$ & H-5), 7.64 (1H, d, *J* = 7.5 Hz, H-4), 8.18 (1H, s, N*H* of indole).
(26) Scope and limitation of $Sc(ClO_4)_3$ 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) ^{1}H NMR (600 MHz, D_2O) 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). ^{13}C NMR (150 MHz, D_2O) 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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