The Novel Glycoprotein Structure; C-Mannosyl Tryptophan

タンパク質修飾の新しい形；C-結合型糖ペプチド

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Abstract

Glycosylation is one of the most important post- or co-translational modifications of proteins, which affects the biological activities of the parent proteins by influencing the higher-order structure. This modification has been classified into two subtypes: namely *N*-linked type and *O*-linked type. Recently, a highly novel variant of glycoproteins that incorporate a *C*-glycosylated amino acid was identified in various proteins. The total synthesis of one such *C*-glycosyl amino acid, namely, *C*²- α -D-*C*-mannosylpyranosyl-L-tryptophan and related peptides was successfully achieved. The mannose and tryptophan moieties were connected via a ring opening of benzyl-protected 1,2-anhydro-mannose by a lithiated indole derivative. After the functional group conversion and deprotection steps, the glyco-amino acid was synthesized in a concise and stereoselective manner, in high overall yields. Furthermore, intermediate azide acid can serve as a useful building block for peptide elongation. The stereoisomer, *C*²- α -D-*C*-glucosylpyranosyl-L-tryptophan was synthesized in a similar way.

We describe here the synthesis of *C*-Man-Trp including by other groups and the possibility of application to clinical methods.

要 約

糖鎖修飾はタンパク質の翻訳後修飾のもっとも大切なかたちのひとつであり、タンパク質の高次構造を変化させることにより活性を調節する。タンパク質糖鎖修飾は*N*-結合型と*O*-結合型に分類されてきた。しかしながら最近、新規タンパク質修飾型としてさまざまなタンパク質に*C*-結合型糖鎖が見出されている。新規に見出された*C*-マンノシルトリプトファンの合成に成功した。マンノースとトリプトファンはベンジル基で保護した1,2-アンヒドロマンノースとリチオ化したインドールとの反応により、結合させた。官能基変換と脱保護反応を経て*C*-マンノシルトリプトファンを短工程、高収率で合成できた。また、その構造を含むペプチドや立体異性体の合成にも成功している。我々の合成を中心に他のグループによる合成、臨床への応用の可能性について述べる。

A. Introduction

Post- or co-translational modifications of proteins, such as phosphorylation, ubiquitination, and disulfide bond formation, are involved in many important biological events (1). One of the most common and widespread post-translational modifications of proteins is glycosylation. The carbohydrate moiety of glycoproteins has been known to exert influence upon the properties of the parent proteins in various ways, such as enhancing their thermal stability, protecting against proteolysis, influencing the protein conformation, and modifying the protein's physicochemical properties, which include solubility, electrical charge, mass, and viscosity of the solution (2, 3). Characteristic carbohydrates, which are present in cell adhesion molecules, tumor-associated antigens, viral or bacterial invasion targets, and blood group determinants, are most typically

A. はじめに

生体内で機能するタンパク質はDNAに貯えられている遺伝情報をもとに合成されるが、翻訳後すぐに機能を発揮することとはまれであり、ジスルフィド結合の形成、リン酸化、ユビキチン化などの翻訳後修飾を受けて成熟した形として発現し、その機能を発揮するものも多い(1)。グリコシル化反応はそのような翻訳後修飾の典型的なかたちのひとつであるが、他の修飾型に比べてかさ高いことからタンパク質のコンフォメーションを変えたり、ペプチダーゼからの安定性を向上させたりする働きも持つ(2,3)。タンパク質を修飾する糖鎖の形としてはおおまか

involved in the binding process of molecular recognition systems in the form of glycoproteins.

Although other linkages have been reported, in most cases, protein glycosylation can be classified into two major groups: i) *O*-glycosylation, where an *N*-acetylgalactosamine residue is covalently attached to the hydroxyl group of either serine or threonine of the protein, and ii) *N*-glycosylation, where a glycan chain is linked via a glycosylamido linkage to an asparagine side chain (4,5). The latter is further classified into subtypes as high mannose-type, complex-type, and hybrid-type.

However, in 1994, a novel class of glycoprotein structural type (Figure 1) was identified in human RNase Us, where a mannose residue is connected to tryptophan via a C-glycosidic linkage (6-9). The primary structure of glycosylated peptide **2** was determined by Edman degradation, and by mass and NMR spectroscopy. Peptides from different enzymatic digests, which contained Trp-7, were found ESI-MS to have a mass, which is a 162 Da higher than expected for a non-modified peptide. In addition, the loss of 120 Da, which is a characteristic feature of low molecular weight C-glycopyranoside. ¹H-NMR analysis revealed that all protons of the indole side chain were observed, except for the one at position 2.

Now, it is possible to determine the C-mannosylated tryptophan from Edman degradation. Although phenylthiohydantoin-Man-Trp was initially reported that the major derivative eluted shortly after Phenylthiohydantoin-Tyr, further experiment revealed that in most of the time, it was co-eluted. This problem could be solved by adequate choice of buffer composition and batches of column material (10). Another problems are the low yield and reproducibility of phenylthiohydantoin-Man-Trp during Edman degradation.

Although the function of C-glycosylated tryptophan is still unclear, additional examples of C-mannosylated proteins are continually discovered from several sources, including recombinant human IL-12 expressed in Chinese hamster ovary cells(11), thrombospondin type 1 repeat (12, 13), and terminal components of complement (C6, C7, C8 and C9)(14). Most strikingly, properdin (15), a positive regulator of complement, contains 20 tryptophan residues, in which as many as 17 are

には *N*-結合型糖鎖と *O*-結合型糖鎖の 2 つにわけられてきた。*N*-結合型糖鎖はアスパラギン側鎖アミド基を介して糖鎖とタンパク質が結合しており、*O*-結合型では糖鎖とタンパク質が側鎖水酸基を介して結合している。*N*-結合型糖鎖はさらに high-mannose タイプ、complex タイプ、hybrid タイプにわけられ、また *O*-結合型糖鎖は Core 1 から Core 8 まで類別される (4,5)。

しかしながら 1994 年、Hofsteenge らによりマンノースアノマー位とトリプトファンが炭素結合を介して結合した新規修飾型がヒト RNase より発見された(6-9)(Figure 1)。これは RNase を Edman 分解した後のヘキサペプチドを NMR と MS により分析した結果明らかになったものである。ESI-MS では RNase の Trp-7 において 162 Da の分子量の増加が観測された。さらに C-グリコシドで特徴的にあらわれるマススペクトルでの 120 Da のフラグメンテーションが観測されることより *O*-グリコシドではなく C-グリコシドであると推測された。NMR においてインドールの 2 位の水素以外はすべて観測できることからトリプトファンのインドール 2 位でマンノースと結合していると推測された。

現在では Edman 分解により C-マンノシル化されたトリプトファンの存在を知ることできる。一般的な条件では phenylthiohydantoin-(C2-Man-Trp) と phenylthiohydantoin-Tyr の分離は難しいが、ある緩衝液条件下、またはカラムの担体を選択すると phenylthiohydantoin-(C2-Man-Trp) は phenylthiohydantoin-Tyr のすぐ後に溶出される (10)。またマンノシル化されたトリプトファンの phenylthiohydantoin への収率は他のアミノ酸に比べて若干低く、再現性が十分でないこともこの修飾型を発見しにくくしている原因と思われる。

当初、この修飾型は非常に特異な構造と考えられていたが、CHO 細胞で発現させたヒト IL 12 (11) や thrombospondin type 1 repeat (12,13) や補体系 (14)、properdin (15)、またヒト尿中に見い出されている(16)。なかでも properdin においては 20 個のトリプトファンのうち、実に 17 個がマンノシル化されてい

- 1 R₁ = H, R₂ = OH
2 R₁ = Phe-Thr, R₂ = Ala-Gln-Trp
3 R₁ = Ala-Gln-Trp-NH₂

Figure 1. The structures of mannosylated tryptophan **1** and its related peptides **2** and **3**.

mannosylated. Also mannosylated tryptophan **1** was identified in human urine (16). Moreover, mannosyl-tryptophan **1** was found, not only in higher vertebra, but also in marine ascidians (17, 18).

Subsequent studies have revealed that C-mannosylation involves the attachment of a mannose residue to the indole moiety of *Trp*-Xaa-Xaa-Trp (Xaa is any amino acid; glycosylated tryptophan is italicized) as a consensus recognition site (19). Activated donor dolichyl-phosphate mannose is the precursor in the biosynthetic pathway (20), and C-mannosyltransferase activity is detected in organisms ranging from *C. elegans* to humans. Presently, a total of 49 C-mannosylated tryptophan moieties have been identified, derived from 11 different proteins.

Rapidly growing interest in understanding the molecular mechanisms of biological events involving glycoproteins has resulted in intense attention to protein glycosylation in the last decade. Since glycosylated proteins are not readily available in the homogenous form by gene technological methods, chemical syntheses of precisely defined model glycopeptides are especially important as a valuable tool (21, 22, 23).

We have recently succeeded in the first total synthesis of C-mannosyl tryptophan in a concise stereocontrolled manner (24, 25). There are two other examples of C-mannosylated tryptophan **1** (16, 26, 27). Herein, we review mannosylated tryptophan science mainly focusing on the synthesis of mannosylated tryptophan **1**.

B. Synthesis by RIKEN Group (24, 25)

We planned a synthetic route to not only synthesize **1** but also providing biological probes including its labeled compounds and derivatives. Important points for the successful

る。また脊椎動物だけではなく、昆虫、海綿にも存在することが明らかになっており、自然界に広く存在していることが予想されている (17,18)。

また、タンパク質側における C-マンノース修飾に必要な認識部位は両末端にトリプトファンを両端に含むテトラペプチドであることも明らかになっている(19)。また、生合成経路においての前駆体はドリコールリン酸マンノースである(20)。C-マンノシル化は転移酵素により触媒されており、この酵素活性は *C. elegans* からヒトにいたるまで幅広く認められる。現在まで 11 の分泌型タンパク質に 49 個の C-マンノシル化されたトリプトファンが見い出されている。

糖ペプチドの化学合成は N-結合型糖鎖、O-結合型糖鎖を中心にさまざまに検討されている (21,22,23) が、我々はこの新規 C-結合型糖アミノ酸；C-マンノシルトリプトファン **1**, およびそれを含むペプチド **2,3** の初の立体選択的合成に成功した(24,25)。

これまでにマンノシルトリプトファンの合成は他に 2 例知られている(16,26,27)。以下、我々の合成法を中心に述べたい。

B. 理研グループ (24,25)

生化学的プローブとしても使用することが可能であることを考慮に入れて短行程、高収率であり、異性体合成、ラベル化合

Scheme 1. Synthetic strategies for **1**. PG = protecting group.

synthesis of **1** are: i) construction of a C-C bond between the 2-position of the indole ring of tryptophan and the anomeric carbon of mannose, and ii) installation of the asymmetric carbon of amino acid (Scheme 1). Although there are several well-established methods for the synthesis of C-glycoside, our plan was to directly incorporate the indole ring at the anomeric carbon of mannose. We expected that the coupling between C-2 lithiated indole derivatives and 1,2-anhydro-mannose **4** (28), which is easily obtained from D-mannose in 6 steps, would result in the direct incorporation of the C-1 linked mannose onto the 2-position of indole. Furthermore, because epoxide ring openings by organometallic reagent are known to proceed via an S_N2 pathway, the product was expected have an α -configuration. In fact, some studies have demonstrated that nucleophilic attacks on 1,2-anhydro- β -D-mannopyranoses by organometallic reagents gave α -C-glycosides (29). *N*-Arylsulfonated indoles **5** have been known to be amenable to direct metallation at the 2-position. Subsequent quenching with an electrophile provides an easy access to 2-substituted indole derivatives (30).

Initially, lithiated **5a** was tested as a model substrate (Table I). Contrary to our expectation, the reaction resulted in a mixture of two compounds, which were difficult to separate by silica gel column chromatography (31). However, after removal of the benzyl group and subsequent acetylation, compounds **6a** and **7a** were readily separated using silica gel column chromatography. Compound **8**, which was derived from **6a**, was confirmed as the α -linked product with $^1\text{C}_4$ conformation of the mannopyranoside ring; conversely, **7a** corresponded to **9**, which were assigned as the β -isomer with $^4\text{C}_1$ conformation (Figure 2).

Using NMR studies, Vliegenthaldt *et al.* has also reported that the mannopyranosyl ring of glycopeptide **2** adopted $^1\text{C}_4$

物合成ができるように合成計画をたてた。合成化学的に鍵となるのはいかにマンノースとインドールをアノマー位不斉炭素の立体化学を制御してつなげるか、どのようにアミノ酸不斉炭素を構築するかという点である (Scheme 1)。

まず、インドールとマンノースの炭素結合はマンノースから6行程で容易に得られる1,2-アンヒドロマンノース **4** (28)とリチオ化したインドール **5** をルイス酸性条件下反応させて連結させることとした。エポキシドは通常有機金属試薬とS_N2反応で反応することが知られている。1,2-アンヒドリド糖においても有機金属試薬とS_N2反応した付加体を与えることが知られており(29)、窒素原子を電子吸引性のスルホニルアミド基で保護したインドール誘導体の2位をリチオ化して反応させればアノマー位において望む立体配置の付加体が得られると予想された(30)。まずインドールのスルホニルアミド体 **5a** のリチオ体を1,2-アンヒドリド糖 **4** と反応させた。BF₃·OEt₂ 存在下、反応は進行したが、驚いたことに α 体 **6a** と β 体 **7a** の両方の付加体が得られた(31)。(水酸基の保護基がベンジル基である場合、シリカゲルカラムクロマトグラフィーで異性体を分離することができなかったのもので、脱保護後、アセチル化してそれぞれを単離した。) また、 α 体 **8** に関しては $^1\text{H-NMR}$ のアノマー位のカップリング定数と NOE 実験よりピラン環が反転した $^1\text{C}_4$ 配座をとって

Table I. Stereoselectivities of the reactions between epoxide **4** and lithiated indole **5**.

entry	6	R ₁	R ₂	Yield(%)	products	α : β
1	a	H	SO ₂ Ph	39	6a/7a	69:31
2	b	H	Boc	49	6b/7b	34:66
3	c	CH ₃	SO ₂ Ph	39	6c/7c	87:13
4	d	CH ₃	Boc	56	6d/7d	17:83
5	e	CH ₂ CH ₃	SO ₂ Ph	38	6e/7e	>95:5
6	f	CH ₂ OTBS	SO ₂ Ph	50	6f/7f	>95:5
7	g	CH ₂ OTBS	Boc	17	6g/7g	77:23
8	h	CH ₂ CH ₂ OTBS	SO ₂ Ph	50	6h/7h	>95:5

Figure 2. Conformation analyses of **8** and **9** using ^1H -NMR spectroscopy.

conformation (6–9). This unusual conformation can be attributed to two reasons: i) for *C*-glycoside, the absence of the anomeric effect, which is a dominant stereoelectronic factor for *O*-glycosides, and 2) the bulkiness of the indole moiety, which prefers the equatorial conformation.

Further systematic investigations have revealed that the stereoselectivity of epoxide opening is strongly dependent on the nature of the substituents on the nitrogen, as well as the 3-position of the indole ring. Representative results are shown in Table I. When sulfonamide was used as a nitrogen-protecting group and the substituent at the 3-position of indole was larger than methyl, the reactions were highly stereoselective, in favor of the α products (Table I, entries 1, 3, 5, 6, and 8). On the other hand, when Boc was used as a protecting group of nitrogen, the β product was obtained as a major product (Table I, entries 2, 4, and 7).

From these results, this epoxide ring opening appears to proceed through a mixed $\text{S}_{\text{N}}1/\text{S}_{\text{N}}2$ mechanism (Scheme 2). The significant lack of stereospecificity can be explained as the Lewis acid pre-activating epoxide **4** into oxocarbenium-type intermediate **11**, which is subsequently captured by an electrophile in a non-stereospecific manner. However, at this time, we were unable to rationalize the substituent effects on stereoselectivity.

In an attempt to complete the synthesis of **1**, we undertook the seemingly straightforward reaction between aziridine **13** and indole **12** to introduce the asymmetric center of amino

ることが明らかになった (Figure 2)。これはペプチド中でのマンノシルトリプトファンコンフォメーションも $^4\text{C}_1$ 配座をとらず、実際にはさまざまなコンフォメーションをとっていることと一致する(6–9)。これは*C*-グリコシドに*O*-グリコシドに見られるアノマー効果が存在しないこと、またインドール環がかさ高いためにエクアトリアル方向を向く方が立体的に有利であるためである。

種々のインドール誘導体 **5** について立体選択性を検討したところ、窒素原子の保護基がスルホニル基である場合、インドール β 位の置換基がかさ高くなるにつれて α 選択性が向上し、逆に Boc 基にすると β 選択性が増すことが分かった (Table I)。本反応の立体化学を含めた詳細な反応機構についてはいまだ不明であるが、期待した $\text{S}_{\text{N}}2$ 反応ではなく、ルイス酸によりまず、エポキシドが開環して生じたオキソカルベニウムイオン **11** がインドールリチオ体と反応する $\text{S}_{\text{N}}1$ 反応、あるいは $\text{S}_{\text{N}}1$ 反応と $\text{S}_{\text{N}}2$ 反応の両方のメカニズムで進行していると推測される (Scheme 2)。

Scheme 2. Mechanisms to explain the stereochemistries of the reactions between **1,2**-anhydromannose **4** and lithiated indole derivatives **5**.

Scheme 3. Reagents and conditions: i) BnBr , Bu_4NI , NaH , DMF , r.t., overnight, 87%; ii) 10% NaOH aq., EtOH , reflux, overnight, 80%.

acid (Figure 2, route A). However, in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ (32), the reaction did not proceed at all. Although the use of $\text{Sc}(\text{OTf})_3$ as an efficient catalyst for this type of reaction was recently reported by Bennani(33), our yields were extremely low when applied to substrate 14 (Scheme 3; Later, this problem was solved by the Nagoya group.).

Since the formation of the C-C bond between mannose and tryptophan was successful with 5f, we turned our attention to the synthesis of the amino acid through one-carbon homologation (Figure 2, route B). Since various chiral glycine enolates have been developed, our strategy involved the reaction between enolates 15–18 (34–37) and bromide 14. Following the protection of the 2-hydroxy group as a benzyl ether, the TBS group was removed under acidic conditions, and the resulting alcohol was converted to compound 14 using NBS- PPh_3 (Scheme 4). However, all four chiral glycine enolates 15–18 did not result in the desired products, presumably because of the steric hindrance of substrate 14.

Since following the indole-mannose coupling with the introduction of the amino acid moiety proved to be highly chal-

ここで、インドール窒素保護基を脱保護した化合物 12 にアジリジン 13 をルイス酸性条件下反応させればアミノ酸部が導入できるように思われた (Figure 2, route A)。しかしながら、12 に対しての 13 の反応性が乏しく、簡単な基質に関して報告されている文献 (32,33) に記載されている条件では反応させることができなかった (Scheme 3)。(名古屋大グループは後にこの問題点を解決している。)

アミノ酸部を導入するために次にブロミド 14 を調製し、さまざまなキラルグリシンエノレート等価体 15–18 (34–37) と反応させ、アミノ酸部を導入することとした (Figure 2, route B)。ブロミド 14 はインドール誘導体 5f と 1,2-アンヒドロマンノース 4 を反応させた後、シリル保護基を脱保護し、水酸基を変換させて合成した (Scheme 4)。このブロミド 14 とさまざまなキラル

Scheme 4. Reagents and conditions: i) BnBr , Bu_4NI , NaH , DMF , r.t., overnight, 85%; ii) $\text{TsOH} \cdot \text{H}_2\text{O}$, MeOH , r.t., 5h, 90%; iii) NBS , PPh_3 , CH_2CH_2 , quant.

Scheme 5. Reagents and conditions: i) 3-methyl-3-oxetanmethanol, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl, DMAP, DMF, r.t., overnight, quant.; ii) H_2 , 10% Pd-C, MeOH, 2h; iii) TfN_3 , DMAP, CH_3CN , r.t., overnight, 79% (2 steps); iv) $BF_3 \cdot OEt_2$ (0.25 equiv.), CH_2Cl_2 , 34%; v) $PhSO_2Cl$, BuLi, THF, $-78^\circ C$ – r.t., overnight, 90%; vi) BuLi, $BF_3 \cdot OEt_2$, THF, $-78^\circ C$, 18%, 6i/7i = 55:45 (from 1H NMR analysis).

Scheme 6. Reagents and conditions: i) BuLi, THF, $-78^\circ C$, 78%; ii) BuLi, $BF_3 \cdot OEt_2$, THF, $-78^\circ C$, 6j (10%) and 7j (8%).

lenging, our third approach involved a coupling reaction between mannose and a fully constructed and masked tryptophan, specifically **5i** (Scheme 6) and **5j** (Scheme 7)(Figure 2, route C). In the case of **5i**, azide, which is stable under strongly basic conditions, was chosen as the latent amino group. After deprotection of the carbobenzyloxyl group under catalytic hydrogenation conditions, the amino group was transformed to the azide through a diazo transfer reaction, using TfN_3 , with retention of configuration of the chiral carbon (38). Cyclic 2,6,7-trioxabicyclo[2,2,2]octane orthoester was employed as the protecting group for the carboxylic acid because of its stability under strongly basic conditions, such as BuLi, and facile deprotection under mild Brönsted acidic conditions. Following the procedures of Corey (39), orthoester **5i** was prepared from 3-methyl-3-oxetanmethanol ester **20**. Alternately, in the case of compound **5j**, the amino and the acid groups were protected as the bis-lactim ether ring. As shown in Scheme 6, compound **5j**

グリシンエノレート等価体 **15–18** とを反応させたが、やはり化合物 **14** の反応性が乏しく目的物はほとんど得られなかった。以上の結果から、付加した後にインドール側鎖に置換基を導入するのはおそらく立体的な要因から、困難であることがわかった。

そこでアミノ酸不斉炭素部をマスクした形で導入した基質 **5i**, **5j** を用いて付加反応を行うことにした (Figure 2, route C)。ここでアミノ基とカルボン酸は BuLi を使用する強塩基性条件下安定である保護基で保護しなければならない。ここでふたつの保護のパターンが考えられた。ひとつはアミノ基をアジド基として保護、一方、カルボン酸を環状オルトエステルとして保護す

Scheme 7. Reagents and conditions: i) N_3Tf , DMAP, CH_3CN , r.t., overnight, then TBSCl, imidazole, DMF, r.t., overnight, 82% (2 steps); ii) PhSO_2Cl , BuLi, THF, -78°C -r.t., overnight, 75%; iii) BuLi, $\text{BF}_3\cdot\text{OEt}_2$, THF, 63%, $6\mathbf{k}/7\mathbf{k} = 95:5$; iv) BnBr, Bu₄NI, NaH, THF, 0°C -r.t., overnight, 92%; v) TsOH $\cdot\text{H}_2\text{O}$, MeOH, r.t., overnight, 91%; vi) idosobenzene diacetate, TEMPO, CH_3CN , H_2O , r.t., 3 h, 97%; vii) 10% NaOH, EtOH, reflux, overnight, 68%; viii) H_2 , 20% $\text{Pd}(\text{OH})_2/\text{C}$, EtOH, dioxane, H_2O , 67%.

was prepared from sulfonamide-protected indolyl bromide **21** and Schöllkopf's chiral bis-lactim ether **15**. Although the coupling between protected tryptophan derivatives **5i** and **5j** and mannose epoxide **4** afforded the desired properly functionalized precursors of the target molecule, the yields and stereoselectivities were unsatisfactory.

The successful strategy (Figure 2, route D) involved indole derivative **5k** as a latent tryptophan moiety, in which the bulky acyclic substituent at the 3-position of the indole ring and sulfonamide as the protection group of the indole nitrogen would enhance α -selectivity. As shown in Scheme 7, the straightforward synthesis of **5k** from commercially available (L)-tryptophanol **22** resulted in high yields. After the amino group was converted to an azide using TfN_3 , the hydroxyl group was protected as *tert*-butyldimethylsilyl (TBS) ether. The indole ring was protected as the benzenesulfonamide using benzenesulfonyl chloride and BuLi to give **5k** in 61% yield (from **22**). Subsequent coupling with 1,2-anhydro-mannose **4** proceeded with high selectivity (95:5) and satisfactory efficiency (63% yield) to afford **6k**, along with a small amount of stereoisomer **7k**. Benzylolation and subsequent removal of the TBS ether under

るパターン **5i** である (Scheme 5)。Cbz-トリプトファン **19** を出発物質としてアミノ基からアジド基への変換は不斉炭素の立体化学を保持したまま TfN_3 を用いてのジアゾ転移反応 (38) により行った。環状オルトエステルは Corey らの手法にしたがって、3-methyl-3-oxetanmethanol エステル **20** より合成できる (39)。もうひとつはアミノ基とカルボン酸をbis-lactim エーテルとして保護するパターン **5j** である (Scheme 6)。これらの基質 **5i**, **5j** を 1,2-アンヒドロマンノース **4** と反応させたが、両方とも収率、アノマー位の立体化学の選択性ともに低かった。インドール β 位にかさ高い環を持つので反応性が低下したためであると考えられる。

最終的には以下に述べる方法で合成することに成功した (Figure 2, route D, Scheme 7)。すなわち、アミノ基をアジド基として保護し、カルボン酸は還元して水酸基シリルエーテルとし

Scheme 8. *Reagents and conditions:* i) TFFH, $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, CH_2Cl_2 , H_2O , 90%; ii) PMe_3 , THF, H_2O , Fmoc-Thr-OH, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl, HOBT, CH_2Cl_2 , HOBT, CH_2Cl_2 , 84%; iii) piperidine, DMF, Z-Phe-OH, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl, HOBT, CH_2Cl_2 , 72%; iv) H_2 , 20% $\text{Pd}(\text{OH})_2/\text{C}$, MeOH, H_2O , THF, AcOH, 57%.

acidic conditions afforded **23** in 91% yield. With expectations that the strong electron withdrawing nature of the sulfonamide group would protect the indole group from oxidation, attempts to directly oxidize alcohol **23** to carboxylic acid **24** were carried out using typical oxidants. A combination of TEMPO and iodosobenzene diacetate (**40**) was found to afford carboxylic acid **24** directly from **23** in excellent yields. Usually, the indole group is sensitive to the oxidation conditions, electron withdrawing sulfonyl group protected the indole group by deducing the electron density.

A two-step deprotection from **25** was accomplished with concurrent reduction of the azide group. Final purification was performed using reverse-phase silica gel column chromatography ($\text{H}_2\text{O}/\text{MeOH}$ 4:1) or size exclusion column chromatography (Bio-Gel P-2 gel, extra fine, $\text{H}_2\text{O}/\text{MeOH}$ 9:1). ^1H -NMR data of synthetically derived **1** (in D_2O ; $^3J_{1,2} = 8.1$ Hz) clearly showed that the mannose ring generally adopts the $^1\text{C}_4$ conformation, with the tryptophan moiety in the equatorial position; the NMR data is in good agreement with those reported for mannosyl-tryptophan containing peptides.

With attainment of homogeneous synthetic **1**, we consequently undertook the synthesis of hexapeptide **2**, which corresponds to residues 5–10 of Human RNase Us. As shown in Scheme 8, intermediate **25** was proposed as the building block for C-mannosyl tryptophan. Azide acids, which do not form oxazolones, can be used for peptide synthesis without likelihood of racemization (41). The peptide bond formation between azide acid **25** and tripeptide **26** was performed using tetramethylfluoroformidium hexafluorophosphate (TFFH) mediated *in situ* acid fluoride formation protocol to provide tetrapeptide **27** in 90% yield. After selective reduction of the azide group using PMe_3 , subsequent coupling reactions with Fmoc-Thr-OH and Z-Phe-OH were suc-

て保護した。アミノ基を TfN_3 を用いてアジド基へ変換し、インドール窒素の保護基は α 選択性を高くするために表 I で得られた結果からスルホニル基で保護することとした。このインドール誘導体 **5k** と 1,2-アンヒドロ糖 **4** との反応は収率よく進行し、期待した通りに α 体 **6k** を優先的に生じた。2 位の水酸基をベンジルエーテルとして保護し、シリル基を脱保護して生じた水酸基を一気に TEMPO-BAIB (**40**) によりカルボン酸 **24** へと酸化した。一般にインドールは酸化反応などに敏感であるが、強い電子吸引性の SO_2Ph 基がインドール環の電子密度を低下させているために安定であった。最後に SO_2Ph 基をアルカリ性条件で脱保護し、接触水素添加条件でベンジル基を脱保護するとともにアジド基を還元し、目的物である C-マンノシルトリプトファン **1** を得ることができた。C-マンノシルトリプトファンは ^1H -NMR で重水中アノマー位の水素の結合定数 J が 8.1 Hz であることから、 $^1\text{C}_4$ 配座を優先してとることが分かった。

また、中間体アジド酸 **25** は C-マンノシルトリプトファンを含むペプチド合成に有用な中間体となる。アジド基はカルボン酸が活性化されたときに隣接基関与しないのでラセミ化が問題とならない利点がある(41)。アジド酸 **25** と保護したトリペプチド **26** とのアミド結合形成反応は TFFH (tetramethylfluoroformidium hexafluorophosphate)(42) を用いてフッ化物を経ることにより収率

Scheme 9. *Reagents and conditions:* i) Fmoc-OSu, NaHCO₃, DME, H₂O, 67%; ii) 31, MeOH, 94%.

cessfully carried out to afford **28** in good yields. The final deprotection of compound **28** was performed under mild catalytic hydrogenation conditions using Pd(OH)₂/C to yield product **2**, which was purified by gel filtration column chromatography (Sephadex LH-20, MeOH).

A more concise approach to glycopeptide synthesis is based upon minimal protection strategy, in which only the side chain carboxylic acid and amino groups are protected. Because of the large differences in reactivities between amino and hydroxyl groups, protection of the hydroxyl groups of the sugar moiety may be omitted. We turned our attention to a novel amide formation protocol that is compatible with hydroxyl groups, which features 4-(4,6-dimethoxyl-1,3,5-triazine-2-yl)-4-methyl-

90 %で行った (Scheme 8)。その後、アジド基をトリメチルホスフィンにより選択的に還元し、さらにペプチドを Fmoc 法により伸長し、最後に接触還元により、最終脱保護を行い、最初に同定された human RNase Us の部分構造 **2** を合成した。また C-マンノシルトリプトファン **1** のアミノ基を Fmoc 基で保護した化合物 **29** はトリアジン誘導体 **31** (43,44) を用いると厳密な反応条件の設定を行わなくとも水酸基を無保護のままアミド結合を形成できる (Scheme 9)。この方法を用いて、認識部位である

Scheme 10. *Reagents and conditions:* i) BuLi, BF₃•OEt₂, THF, **34** (10%) and **35** (2%); ii) BnBr, Bu₄NI, NaH, THF, 0°C, r.t., overnight, 77%; iii) TsOH•H₂O, MeOH, r.t., overnight, 99%; iv) idosobenzene diacetate, TEMPO, CH₃CN, H₂O, r.t., 3 h, 85%; v) 10% NaOH, EtOH, reflux, overnight, 56%; v) H₂, 20% Pd(OH)₂/C, EtOH, dioxane, H₂O, 65%.

morfolinium chloride **31** (42) (Scheme 9). Compound **31** was reported to be effective for amide bond formation in protic solvents (43, 44). The amino group of mannosyl tryptophan was protected with Fmoc in the usual manner in 68% yield. By this method, the recognition sequence tetrapeptide with mannose **32** was synthesized. The advantage of this procedure is that the carboxylic acid can be used directly without previous activation.

Using similar procedures as described above, glucosyl tryptophan **36**, which is a stereoisomer of mannosyl tryptophan, was synthesized (Scheme 10).

C. Identification, Synthesis, and Immunoassay by Kyowa-medex. Co. and Marine Biotechnology Institute Co.

C-Mannosyltryptophan **1** was discovered in human urine after HPLC analysis of various biological samples. This compound was then synthesized and antibodies against it were produced.

After preprocessing, purification was repeated using a Sephadex G-25 super fine column, a positive ion-exchange resin Sepharose fast-flow column, a negative ion-exchange resin DEAE-Sepharose column and a reverse phase column. From 500 L of urine, 5 mg of **1** was obtained. At a measurement wavelength of 350 nm, there was a favorable linear correlation between fluorescence intensity and concentration of **1**, and it was found that **1** could be quantified using *N*-methylserotonin as an

テトラペプチドにマンノースが付加した化合物 **32** を合成した。

また、*C*-Man-Trp と同様の戦略により立体異性体である *C*-グルコシルマンノース **36** についても合成することができた (Scheme 10)。

本合成は類縁体、ラベル化合物などの合成も可能である柔軟性のある合成ルートである。

C. 協和メディックス・海洋バイオテクノロジーグループ(16)

彼等は HPLC で生体試料中の種々の化合物を分析中、ヒト尿中に **1** が存在することを見出した。彼等は **1** を合成するとともにそれに対する抗体を作成した。

尿からは前処理後、セファデックス G-25 スーパーファインカラム、陽イオン交換樹脂セファロースファーストフローカラム、陰イオン交換樹脂 DEAE-セファセルカラム、逆相カラムクロマトグラフィーによる精製を繰り返し、500 l の尿から 5 mg のマンノシルトリプトファン **1** を得た。測定波長 350 nm において **1** の蛍光強度と濃度は良好な直線関係を示し、*N*-メチルセロトニンを内部標準として **1** を定量することが可能であること

Scheme 11. i) LDA, THF, toluene, -78 °C, 30 min, 47%; ii) LAH, THF, 0 °C, 30 min, 75%; iii) TsOH·H₂O, toluene, 1 h, 58%, diastereomer ratio 1.4:1; iv) 50% NaOH, EtOH, 100 °C, 1h, 76%; v) **40**, Na₂CO₃, CH₂Cl₂, r.t., 1.5 h, 37%; vi) Al-Hg, THF, 55 °C, 0.5 h, 90%, diastereomer ratio 1.6:1; vii) ZCl, Et₃N, CHCl₃, toluene, -20 °C, 15 min, 95%; viii) LiOH·H₂O, dichloroethane, H₂O, r.t., 16 h, 40%; ix) H₂, 20% Pd(OH)₂/C, EtOH, 60 °C, 11h, 62%.

internal standard. With this method, the level of **1** in healthy men and women aged up to 90 years has been measured at 76.3 and 74.5 ng/ml, respectively, while that in patients with nephropathy has been measured at about 850 ng/ml. In addition, the level of **1** in the spinal fluid of healthy individuals has been measured at 130.4 ng/ml, while that of patients with spinocerebellar degeneration has been measured higher at 400 ng/ml and patients with metastatic brain tumor 823 ng/ml. Furthermore, the level of **1** in the amniotic fluid of healthy individuals has been measured at 353 ng/ml, but that of patients with pregnancy toxemia has been measured much higher at 1411 ng/ml.

With regard to synthesis, lithioindole **5a** is first added to lactone **37**, which is derived from mannose. After reducing the resulting lactol **38**, cyclo-dehydration is conducted under acidic conditions (Scheme 11). The hemiacetal of lactol **38** can be also directly reduced using $\text{Et}_3\text{SiH}\cdot\text{BF}_3\cdot\text{OEt}_2$, but the yield is low (16%). The nitrogen-protecting group of indole is deprotected under basic conditions. An amino acid moiety is introduced using **40** in the presence of Na_2CO_3 . As the amino group is protected as an oxime, **1** is obtained through a sequence of reduction of the oxime by Al amalgam, protection of the amino group by the benzyloxycarbonyl group, deprotection of the carboxylate ester, and finally deprotection based on catalytic hydrogenation. Numerous points need to be improved, such as stereoselectivity of cyclization in an acidic environment, stereoselectivity of oxime reduction, hydrolysis of the ester of the carboxylate protecting group, and racemization of the asymmetric carbon atoms in the amino acid region.

Synthesized **1** (strictly speaking, a mixture of isomers at asymmetric carbon atoms in the amino acid moiety) was bound to either KLH (keyhole limpet hemocyanin) or BSA (bovine serum albumin) using EDC. Mice were immunized against these haptens to produce antibodies. Then, hybridoma was produced using antibody-producing cells and mouse myeloma cells (P3U1). After cloning, purified antibodies were obtained from the mice. These antibodies were used to measure the levels of **1** in urine and serum samples by ELISA. The results showed that, regardless of age or gender, the amount of **1** excretion was comparable, thus suggesting that it could be used as a new glomerular filtration indicator instead of creatinine clearance or inulin clearance. The present study is very interesting because mannosyltryptophan **1** was not only synthesized, but its practical application was also suggested.

D. Synthesis by Nagoya Group (26, 27)

Isobe's group has designed and developed their original C-alkynylation method, and applied the methodology to the natural product synthesis such as Okadaic acid (45). They reported the synthesis of C-mannosyl-tryptophan **1** through their original alkyne C-glycosylation method and indole construction by use of palladium chemistry (26). The alkyne **42** reacts

を見出した。その方法を用いることにより新生児から90歳までの健常人では男性76.3 ng/ml、女性74.5 ng/mlの**1**が尿中に存在するのに対し、腎臓疾患患者では約850 ng/mlの**1**が存在することが確かめられた。また、髄液中では健常人で130.4 ng/mlで存在するのに対し、脊髄小脳変性患者では400 ng/ml、転移性脳腫瘍患者では823 ng/mlの高値を示した。また、羊水中においては健常人では**1**が353 ng/mlで含まれるのに対し、妊娠中毒症患者では1411 ng/mlで含まれている。

合成はまず、マンノースから誘導されるラクトン**37**にリチオ化したインドール**5a**を付加させ、生じたラクトール**38**を還元した後、酸性条件で脱水環化させている(Scheme 11)。ラクトール**38**を $\text{Et}_3\text{SiH}\cdot\text{BF}_3\cdot\text{OEt}_2$ により還元することもできるが収率が低い(16%)。インドールの窒素保護基をアルカリ性条件で脱保護し、 Na_2CO_3 存在下**40**を用いてアミノ酸部を導入している。アミノ基はオキシムとして保護しているが、オキシムのAlアマルガムによる還元、ベンジルオキシカルボニル基によるアミノ基の保護、カルボン酸エステルの脱保護を経て、最終的に接触水素添加により、脱保護を行い、**1**を得ている。しかしながら酸性条件における環化反応の立体選択性、オキシムの還元の立体選択性、またカルボン酸保護基のエステル加水分解のさいにアミノ酸部不斉炭素のラセミ化が見られる点など改善すべき点が多い。

彼等は合成した**1**(厳密にはアミノ酸不斉炭素に関して両異性体の混合物)をKLH(キーホールリンペットヘモンシアニン)、あるいはBSA(ウシ血清アルブミン)にEDCを用いて結合させた。これらのハプテンをマウスに免疫し、抗体を産生させた。この抗体を産生細胞とマウスミエロマ細胞P3U1からハイブリドマを作成した。クローニングを行い、精製抗体をマウスから取得した。これら抗体を用いてELISA法により尿中、血清中の**1**の存在量を測定した。その結果、老若男女を問わず、**1**の排出量がある一定の限局した値をとり、腎糸球体濾過量をはかる指標であるイヌリンクリアランス法あるいはクレアチンクリアランス法にかわる新しい指標となる可能性を提示している。マンノシルトリプトファン**1**の合成だけでなく、実際に臨床にも応用できる可能性を示した点で非常に興味深い。

D. 名古屋大グループ(26, 27)

名古屋大学磯部グループでは以前から糖にアルキン誘導体をアノマー位に α 選択的に導入する優れた方法を開発しており、その手法をOkadaic acidに代表されるさまざまな天然物合成に精力的に応用してきた(45)。この手法を基盤としてC-マンノシルトリプトファン**1**の合成を行っている。

彼等はまずはじめに下マンノース誘導体**41**とシリルアセチレン**42**をTMSOTf存在下反応させてC-グリコシドを α 立体選

Scheme 12. i) **42**, TMSOTf, CH₂Cl₂; ii) TBAF, aq. THF, 91% (2 steps); iii) *o*-PhINHTs, Pd(OAc)₂, Ph₃P, CuI, *n*-butylamine, THF, 96%; iv) CuI, Et₃N, DMF, 80%; v) TBAF, THF, vi) POCl₃, DMF; aq. KOH; vii) Ac₂O, pyridine, 88% (3 steps); viii) AcHNCH(COOH)COOEt, Ac₂O, pyridine, 66%; ix) H₂, Pd-C, MeOH, 64%.

with mannose derivative **41** in the presence of TMSOTf to give the adduct. After desilylation, the compound **43** was obtained with complete α -selectivity. The indole ring was installed by use of Sonogashira's conditions (**46**) and Castro indole synthesis (**47**). After deprotection of tosyl group, aldehyde **46** was synthesized by Vilsmeier reaction. Then, aldehyde **46** was condensed with acetamide malonate monoethylester to give olefin **47** and hydrogenation of olefin **47** to give inseparable diastereomeric mixtures at asymmetric center **48**.

Then they turned their attention to the use of aziridine **13** to install the asymmetric carbon center (**27**). Boron etherate and lanthanide triflate is reported to be an effective Lewis acid for the aziridine opening (**32**, **33**). However, these Lewis acids did not give satisfactory results. Then, they investigated fully about the suppliers and preparation method of lanthanide triflate. Finally, they found that Sc(ClO₄)₃ was the effective Lewis acid for the reaction between **13** and **49** (**48**). After three-step deprotection procedure, the target molecule **1** was obtained.

Furthermore, they attempted synthesis of glucosyl-tryptophan (**49**). They commenced with the synthesis by preparation of compound **51**. Iodoacetylene **51** was coupled with (L)-

tryptophan (**13**) to give the adduct (**46**). Castro reaction (**47**) by indole ring was obtained. After deprotection of tosyl group, aldehyde **46** was synthesized by Vilsmeier reaction. Then, aldehyde **46** was condensed with acetamide malonate monoethylester to give olefin **47** and hydrogenation of olefin **47** to give inseparable diastereomeric mixtures at asymmetric center **48**.

そこで彼等はアジリジン **13** を用いてルイス酸条件下アミノ酸部を導入することを試みた(Scheme 13)。インドールの β 位にアジリジン **13** を付加させる反応において当初は BF₃·OEt₂ が用いられていたが (**32**)、その後、ランタノイドトリフラートが有効であることが報告されている (**33**)。しかしながら、これらの試薬では **49** に対して十分な結果を得ることができず、彼等は様々なランタノイドトリフラートの購入先、調整法について詳細に検討し、Sc(ClO₄)₃ が最良の結果を与えることを見出した (**48**)。3 段階の脱保護反応を行い、マンノシルトリプトファン **1** を得ている (**27**)。

さらにグルコース誘導体合成を行うべく (**49**)、糖アセチレン誘導体 **51** から Jackson らの手法 (**50**) により (L)-セリンから誘導した亜鉛-銅試薬 **52** を用いてあらかじめアミノ酸不斉炭素を導

Scheme 13. i) TBAF, THF, ii) $\text{Sc}(\text{ClO}_4)_3$, CH_2Cl_2 , 0 °C, 66%; iii) aq. LiOH, *i*-PrOH, 62%; iv) H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, HCl, MeOH, 50%.

Scheme 14. i) THF, -78 - 4 °C, 12 h, 43%; ii) $\text{Pd}(\text{OAc})_2$, PPh_3 , *n*-Bu₄NCl, Na₂CO₃, DMF, 100 °C, 24 h, 89% (R = Ts).

serine derived zinc-copper reagent **52**, which was developed by Jackson (50). They made an attempt to annulate indole by Larock's procedure using *o*-iodoaniline **54**. Unfortunately, the product **55** was the *iso*-tryptophan, not the desired tryptophan. Although Larock suggested the regioselectivity of this annulation was controlled by steric balance between two substituents of acetylene to afford a product having a sterically hindered substituent at the 2-position of indole in high selectivity (51, 52, 53), this is the first example of complete reverse regioselectivity in Larock's heteroannulation.

E. Conclusion

The report described the various tissues surrounding synthesis of *C*-mannosyltryptophan. At present, even with the GlycoSuite Database (54), *C*-bound sugar chains are recognized as one of the categories and are being more widely accepted. Mannosylated peptides are also known to improve resistance

入し、Larock らのインドール合成法 (51) により環化することを試みた (Scheme 14)。Larock は環化反応の位置選択性がアセチレンの置換基により変化することを見出している (51, 52, 53) が、しかしながら非常に興味深いことに得られたものは環化反応において位置選択性が完全に逆転した *iso*-インドール体 **55** であった。インドール窒素の保護基 R が環化の収率に大きく影響を及ぼすことも興味深い。

E. 結 論

以上、*C*-マンノシルトリプトファンの合成を中心について述べた。現在は GlycoSuite database (54) にも *C*-結合型糖鎖はカテゴリーのひとつとして認知されるに至っており、市民権を得つつあるように見える。今後の研究の発展が望まれる。また、マンノシル化されたペプチドは chymotrypsin, carboxypeptidases

against chymotrypsin, carboxypeptidase A, carboxypeptidase Y or Aminopeptidase M (10), but the biological significance of C-bound sugar chains has yet to be elucidated. In any case, future research is needed to ascertain whether the sequences recognized by mannosylated peptides resemble WS Box (WSXWS), which is common to cytokine Class I receptors, and whether mannosylated peptides are universal to membrane proteins because their correlation to certain diseases has been documented (55).

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