

博士論文

論文題目 Sodium glucose co-transporter 2 (SGLT2)
阻害作用に基づく新規糖尿病治療薬
TA-1887の創出に関する研究

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略語表

Ac (acetyl)

AGE (advanced glycation end product)

APCI (atmospheric-pressure chemical ionization)

aq. (aqueous)

Ar (aryl)

AUC_{0-inf} (area under the plasma concentration-time curve from time zero extrapolated to infinite time)

BA (bioavailability)

BG (blood glucose)

Bn (benzyl)

Boc (*t*-butoxycarbonyl)

BSA (*N,O*-bis(trimethylsilyl)acetamide)

Bu (butyl)

calcd (calculated)

cat. (catalyst)

CL_{tot} (total plasma clearance)

C_{max} (observed maximum plasma concentration)

dba (dibenzylideneacetone)

DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone)

DMAP (*N,N*-dimethyl-4-aminopyridine)

DMF (*N,N*-dimethylformamide)

DMSO (dimethyl sulfoxide)

Et (ethyl)

EWG (electron withdrawing group)

F(%) (bioavailability(%))

Het (heteroaromatic)

HF-KK mice (high-fat diet-fed KK mice)

HPLC (high-performance liquid chromatography)

hSGLT (human sodium glucose co-transporter)

IR (infrared)

i.v. (intravenous)

Me (methyl)

n (*normal*)

NMR (nuclear magnetic resonance)

o (*ortho*)

p (*para*)

Pd/C (palladium on carbon)

PK (pharmacokinetic)

p.o. (per os)

QOL (quality of life)
rt (room temperature)
rUGE (rat urinary glucose excretion)
SD rat (Sprague glucos rat)
S.E.M. (standard error of the mean)
SGLT (sodium glucose co-transporter)
 t (*tertiary*)
 $t_{1/2}$ (terminal phase half-life)
TFA (trifluoroacetic acid)
THF (tetrahydrofuran)
 t_{\max} (time of the observed maximum plasma concentration)
TMSOTf (trimethylsilyl trifluoromethanesulfonate)
UGE (urinary glucose excretion)
 $V_{d_{ss}}$ (volume of distribution at steady-state)

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第 1 章 序論

第 1 節 2 型糖尿病治療薬の課題

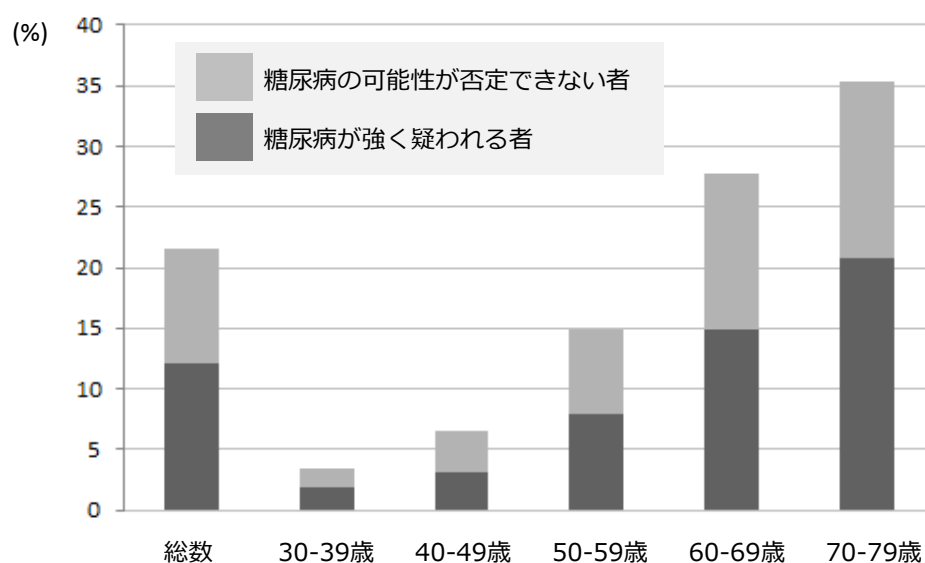
2 型糖尿病とは、インスリン作用不足による慢性の高血糖状態を主徴とする代謝症候群で、慢性的に続く高血糖や代謝異常は、網膜・腎の細小血管症および全身の動脈硬化症を起こし、これを進展させる。さらに、神経障害、白内障などの合併症は、患者の生活の質（QOL）を著しく低下させる。そのため、合併症の発症や進展を阻止し、QOL を維持するためには良好な血糖コントロールの維持が必要となる¹⁾。

人類は進化の過程で長い飢餓時代を経て、エネルギーの基となる血糖を高める（維持する）手段を多く備えたと考えられる。例えばグルカゴン、アドレナリン、糖質コルチコイドなどの仕組みが挙げられ、本研究のターゲットである sodium glucose co-transporter（SGLT）もその一つと考えられる。しかし、わずかここ数十年間での急激な食生活の変化や車社会化などの影響でカロリー過多となり、2 型糖尿病患者が増加してきた²⁾。平成 25 年国民健康・栄養調査報告（厚生労働省）によると、国民の 5 人に 1 人以上が糖尿病、あるいはその予備軍と考えられている（図表 1－1）³⁾。

【図表 1－1】

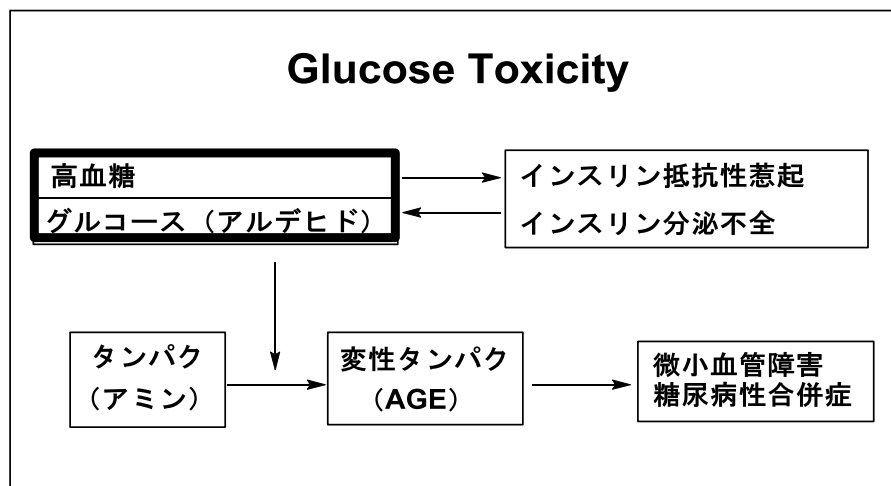
国内における2型糖尿病患者の割合

国民の5人に1人以上が糖尿病かその予備群



【図表 1－2】

糖毒性の概念



AGE: advanced glycation end product

唯一の血糖を下げるホルモンがインスリンで、その働きにより血中の余分なグルコースは主として筋肉と脂肪細胞に取り込まれる。そのため、インスリンは糖尿病治療に用いられてはいるが、体重増加を生じやすい。

糖毒性とは、生体にとって本来重要な栄養素の一つであるグルコースが過剰になった場合、逆に毒物として作用してしまうという概念である（図表 1－2）⁴⁾。高血糖状態が続くとインスリン抵抗性、インスリン分泌不全が増悪し、これがさらに血糖を高めてしまう悪循環に陥る。また、グルコースのアルデヒドはタンパク質のアミノ基と反応し、不溶性の変性タンパク（AGE：advanced glycation end product）を生成する。この AGE の沈着が微小血管障害をきたし、糖尿病性合併症の一因となっている。

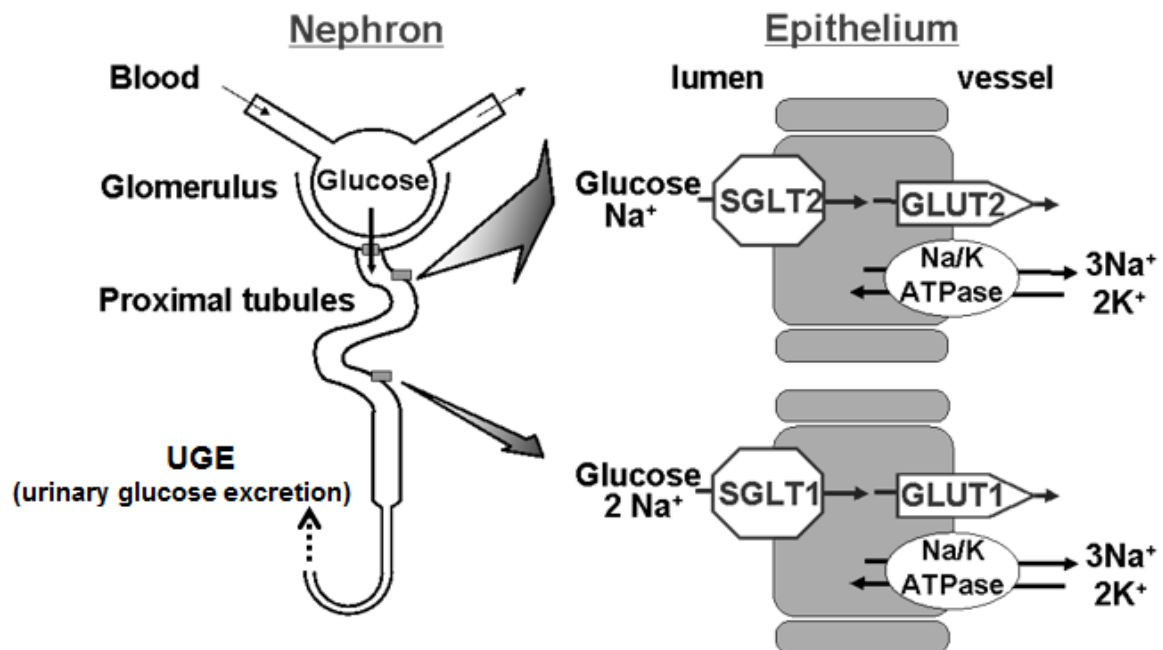
2 型糖尿病患者の治療目標は、血糖値を正常レベルに厳格に管理することにある。しかしながら、2 型糖尿病は上述のように進行性の性質を有し、既存の血糖降下薬で治療目標を達成するのは難しく⁵⁾、2010 年現在でも良好な血糖管理に至るのは 35%程度の患者に限られている⁶⁾。また、既存の血糖降下薬は、インスリン抵抗性改善薬あるいはインスリン分泌促進薬がそのほとんどであり、これらはインスリンに依存したメカニズムを有し、低血糖、膵β細胞の疲弊、体重増加といった副作用が課題となっている⁷⁾。従って、新規なメカニズム、特にインスリンに依存しない治療薬の開発が望まれている。

第 2 節 SGLT2 阻害薬について

血中のグルコースは、腎糸球体で濾過され、その大部分（約 90%）が近位尿細管上皮細胞に存在する sodium glucose co-transporter 2（SGLT2）によって、残りが SGLT1 によって血中に再吸収される（図表 1－3）⁸⁾。正常血糖の場合、濾過された全てのグルコースが尿細管腔から血中に再吸収されるが、高血糖時には再吸収が飽和し尿糖排泄（UGE：urinary glucose excretion）が血糖値に応じて増加する⁹⁾。なお、SGLT2 は腎特異的に発現しているのに対し、SGLT1 は小腸、心臓、気管などにも存在しており¹⁰⁾、ヒト SGLT1（hSGLT1）の遺伝子変異を有する場合、小腸での糖吸収不全に伴う重篤な下痢や脱水症状がみられる¹¹⁾。一方、ヒト SGLT2（hSGLT2）の遺伝子変異については、腎性糖尿病の症状を呈するものの、血糖値は正常であり健康に問題はないことが報告されている¹²⁾。従って、hSGLT2 阻害のコンセプト自体に基づく毒性・副作用は、特に重篤ではないであろうと予想できる。

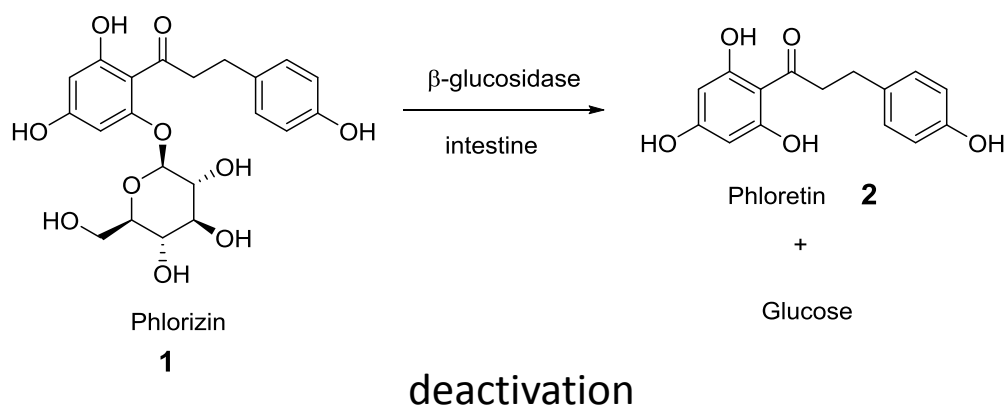
【図表 1－3】

腎臓におけるSGLTによる糖再吸収



【図表 1－4】

β-グルコシダーゼによるフロリジンの加水分解



- ✓ β-Glucosidase hydrolyzes phlorizin into its aglycon (phloretin) and glucose in the intestine.

リンゴやナシなどの樹皮から得られる天然配糖体 phlorizin (1) は SGLT 阻害作用を有し、本化合物を動物に皮下あるいは静脈内投与した場合、UGE を認めることが報告されている¹³⁾。一方、経口投与した場合は、吸収過程で腸管に存在するβ-グルコシダーゼにより C-O グルコシド結合が加水分解を受けて phloretin (2) とグルコースに分解されるため SGLT 阻害作用が消失し、UGE を促進することが出来ない (図表 1－4)¹⁴⁾。

辻原らは、化合物 1 を端緒化合物とした構造変換によりβ-グルコシダーゼに対する抵抗性を付与し、世界初となる経口投与でも有効性を発揮する hSGLT2 阻害薬 T-1095 (3b) を見出した (図表 1－5)¹⁵⁾。化合物 3b はプロドラッグで、経口投与の後に小腸から吸収、肝臓でのエステラーゼによって活性本体 3a に変換され腎近位尿細管の SGLT2 を阻害する。その結果、UGE を促進し、各種糖尿病モデルでインスリン非依存的に血糖降下作用を発揮することが確認された¹⁶⁾。

以上より、選択的な hSGLT2 阻害薬は、インスリン非依的なメカニズムに基づく 2 型糖尿病治療薬として魅力的な創薬アプローチと考えた^{17a)}。

研究当時の代表的な経口 hSGLT2 阻害薬としては、2 つのケミカルクラスに大別された (図表 1 - 5) ¹⁷⁾。

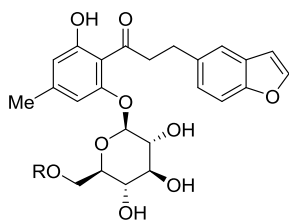
最初に見出されたケミカルクラスは、前述の T-1095 (**3b**) や sergliflozin (**4b**) ¹⁸⁾ に代表されるフェノール-*O*-グルコシド誘導体 (以下、*O*-グルコシド) である。化合物 **3b** の臨床試験では期待通り UGE の促進にともなう血糖降下作用が確認されたが、一方で代謝安定性の問題も判明した。

次に見出されたのが、特許化合物 **5** ¹⁹⁾ や、dapagliflozin (**6**) ²⁰⁾、あるいは筆者らのグループによる canagliflozin (**7**) ²¹⁾ に代表されるアリール-*C*-グルコシド誘導体 (以下、*C*-グルコシド) である。これらは *O*-グルコシドと比べて生体内における代謝安定性に優れ、経口投与においても強力な薬効を示す ¹⁷⁾。現在では、臨床試験を経て化合物 **6** や **7** をはじめ *C*-グルコシド数剤が各国で上市、承認あるいは承認申請されるに至っている。

【図表 1 - 5】

代表的な経口 hSGLT2 阻害薬の構造

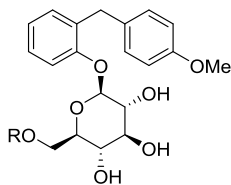
Phenol-*O*-glucosides



3a: R = H (T-1095A)

3b: R = CO₂Me (T-1095)

Mitsubishi Tanabe

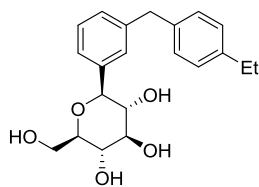


4a: R = H (Sergliflozin-A)

4b: R = CO₂Et (Sergliflozin)

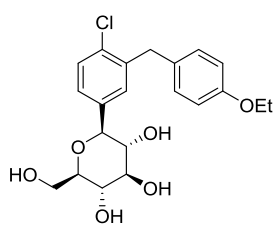
Kissei

Aryl-*C*-glucosides



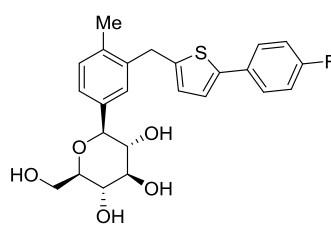
5

Bristol-Myers Squibb



6: Dapagliflozin

Bristol-Myers Squibb



7: Canagliflozin

Mitsubishi Tanabe

N-glucosides

Not reported

ところで、創薬化学（メディシナルケミストリー）の研究においては、先行化合物と異なる基本骨格を有する化合物を保有することが望ましい。その理由としては、将来的に先行化合物に新たなリスクが発じた場合に備え、それらを回避する可能性を少しでも高めておくことが挙げられる。例えば、先行化合物における長期連続投与での毒性発現、薬効不足、関連する他者特許の出現、スケールアップ合成時のトラブル、結晶化を含む製剤化時のトラブルなどのリスクが挙げられ、これらは基本骨格に由来する場合も多いためである。

我々のグループでは既に化合物 **7** に代表される *C*-グルコシドの化合物探索を実施していた背景の下、筆者は **hSGLT2** 阻害薬の新たなケミカルクラスとして *N*-グルコシド型化合物を適用できるのではないかと期待し、経口投与で有効かつ選択的 **hSGLT2** 阻害薬の探索に着手した。

第 2 章 アニリン-*N*-グルコシドに関する研究

第 1 節 *N*-グルコシドの起点化合物の創出と課題

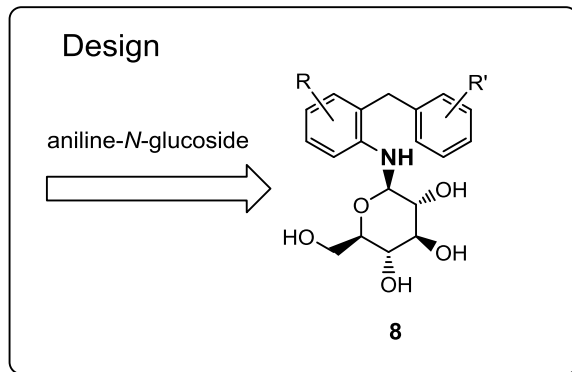
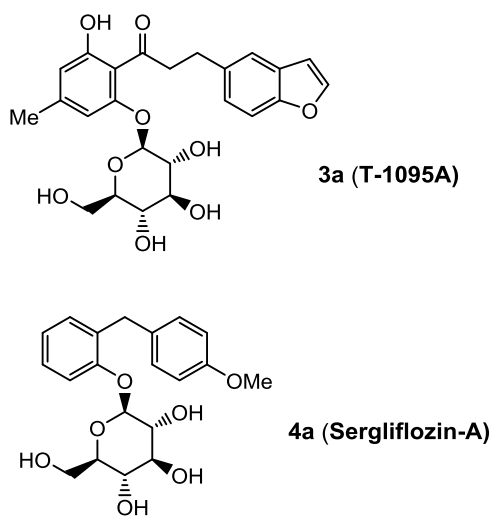
まず初めに、当時知られていた化合物 **3a** や **4a** のような *O*-グルコシドを参考にして、*N*-グルコシド型化合物の可能性を調べる目的で酸素原子を窒素原子に置き換えたアニリン-*N*-グルコシド **8** をデザインした（図表 2 - 1）。

【図表 2 - 1】

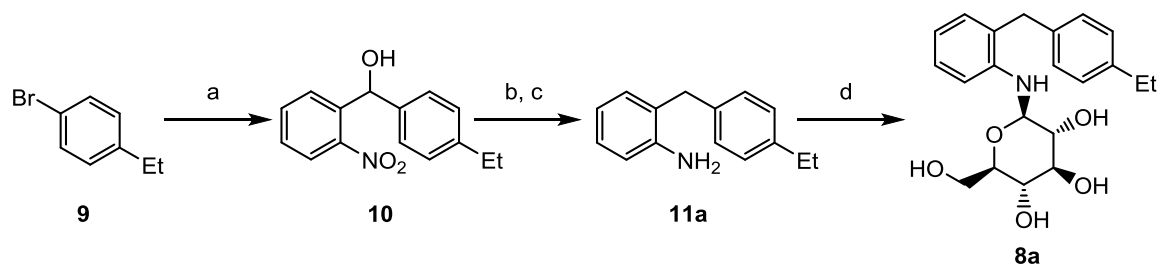
アニリン-*N*-グルコシドのデザイン

Design :

Replacement of the phenol aglycon with aniline



【図表 2 - 2】



Synthesis of aniline-*N*-glucosides **8a**. Reagents and conditions: (a) *n*-BuLi, THF, -78°C , then *o*-nitrobenzaldehyde, THF, -78°C to 0°C (27%); (b) H_2 , Pd/C (10%), EtOH, rt (100%); (c) Et_3SiH , $\text{BF}_3\cdot\text{Et}_2\text{O}$, MeCN, -78°C to rt (75%); (d) D-(+)-glucose, NH_4Cl , MeOH, reflux (56%).

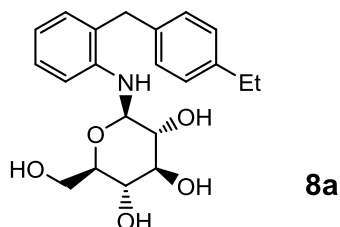
まず化合物 **8a** の合成を行った (図表 2 - 2)。原料となる 4-ブロモエチルベンゼン **9** を *n*-ブチルリチウム (*n*-BuLi) で処理後、2-ニトロベンズアルデヒドとの反応により *o*-ニトロジフェニルカルビノール **10** を 27%の収率で得た。次いで、化合物 **10** のニトロ基を水素気流下で Pd/C を用いた接触還元によりアミノ基にほぼ定量的に変換し、さらに水酸基をトリエチルシラン (Et_3SiH) と三フッ化ホウ素・ジエチルエーテル錯体 ($\text{BF}_3\cdot\text{Et}_2\text{O}$) の組み合わせで加水素分解還元することでアグリコン **11a** を 75%の収率で得た。アニリン **11a** を、D-グルコースとともに触媒量の弱酸を加えてメタノール中で加熱還流後、結晶化により精製することで中程度の収率 (56%) で目的のβ-*N*-グルコシド化合物 **8a** を得た²²⁾。なお、化合物 **8a** のアノマー位の立体構造は、 ^1H NMR におけるアノマー位 C-H と隣接する C-H との間のカップリング定数からβ配置と決定した ($J = 8.4\text{ Hz}$ 、DMSO- d_6 中 D_2O 交換時)。

アニリン-*N*-グルコシドではαβ間の平衡が存在することが報告されているが²³⁾、本化合物の場合、アノマー効果で有利なα体に対し、アグリコン部分の嵩高さの影響で熱力学的により安定なβ体が優先して得られたものと考えている。

次に化合物 **8a** の生物活性評価を行った (図表 2 - 3)。hSGLT2 阻害作用²⁴⁾においては、臨床試験化合物である T-1095 (**3b**) の活性本体である *O*-グルコシド **3a** に匹敵する強力な活性が確認された (**8a**: $\text{IC}_{50} = 3.9\text{ nM}$ 、**3a**: $\text{IC}_{50} = 5.2\text{ nM}$)。

更に、本化合物の *in vivo* での効果として、Sprague-Dawley (SD) ラットを用いて経口投与 (30 mg/kg) での UGE (rUGE) の促進作用を評価した。その結果、化合物 **8a** は高い *in vitro* 活性を示すにもかかわらず rUGE の促進作用をほとんど示さなかった (**8a**: rUGE = 93 mg/day、30 mg/kg、p.o.)。

アニリン-*N*-グルコシド **8a** の薬理プロファイル



run	Compd.	hSGLT2 ^a IC ₅₀ (nM)	rUGE ^b (mg/day)
1	8a	3.9	93 ± 27
2	3a (T-1095A)	5.2	-
3	3b (T-1095)	-	422 ± 76

^a These data were obtained by a single determination performed in duplicate.

^b Each compound was orally administered at a dose of 30 mg/kg to male Sprague-Dawley (SD) rats. Rat urinary glucose excretion (rUGE) data over 24 h were normalized per 200 g body weight. Values are expressed as mean ± S.E.M. (n=3).

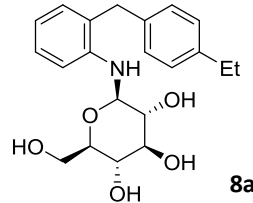
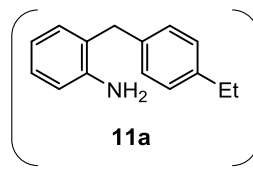
✓ Despite its excellent hSGLT2 inhibitory activity, **8a** showed poor rUGE effect.

化合物 **8a** のラット pharmacokinetic 試験 (PK 試験) (10 mg/kg、p.o.) を実施したところ、**8a** の血中暴露は低く (AUC_{0-inf} = 0.23 µg·h/mL, BA = 17%、10 mg/kg、p.o.)、加水分解を受けて生成したアグリコン **11a** が血中に観察された (図表 2 - 4)。従って、in vivo 作用の増強のためには、C-N グルコシド結合の加水分解の抑制を指向した構造修飾による血中暴露改善が必要であると考えた。

加水分解の原因を探るべく、化合物 **8a** の溶液中での安定性を確認した。その結果、酸性水溶液中 (0.5 N、HCl、37 °C) で速やかに加水分解を受け、アグリコン **11a** が生成することが判明した。従って化合物 **8a** が生体内においても胃酸等による分解を受けている可能性が想定された²⁵⁾。また、*O*-グルコシドと同様に、β-グルコシダーゼによる代謝反応もその原因として推察された。即ち、*O*-グルコシドのβ-グルコシダーゼによる加水分解のメカニズムとしては、グルコシダーゼ上のカルボン酸からのアノマー位酸素原子へのプロトネーションによるアシストが考えられていることから (図表 2 - 5)²⁶⁾、アニリンの窒素原子においても同様にカルボン酸のプロトネーションを受け、加水分解が促進される可能性が考えられた。

【図表 2 - 4】

化合物8aのラット薬物動態

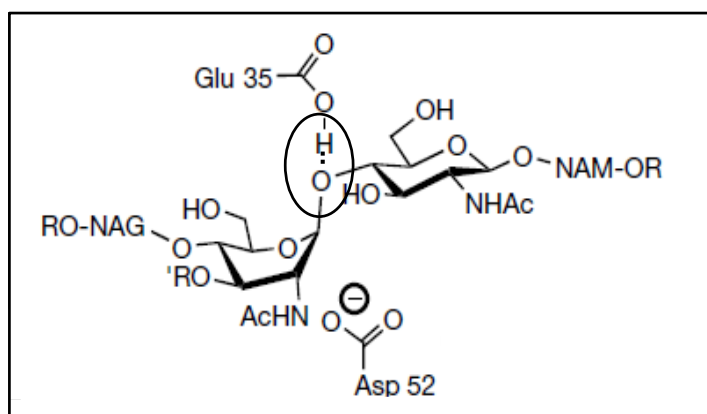
 8a	Dose (mg/kg; i.v.)	3
	$t_{1/2}$ (h)	0.58
	AUC _{0-inf} (μg·h/mL)	0.41
	CL _{tot} (L/h/kg)	7.4
	Vd _{ss} (L/kg)	4.5
 11a	Dose (mg/kg; p.o.)	10
	$t_{1/2}$ (h)	0.43
	t_{max} (h)	0.25
	C _{max} (μg/mL)	0.43
	AUC _{0-inf} (μg·h/mL)	0.23
	F (%)	17

Values are the mean of two rats.

- ✓ Pharmacokinetic results of **8a** showed poor profiles such as high clearance and low bioavailability.
- ✓ Aglycon **11a** was observed in the serum.

【図表 2 - 5】

β-グルコシダーゼによる加水分解メカニズム



- ✓ Protonation on the anomeric oxygen atom is speculated to induce the hydrolysis of glucosidic linkage in O-glucoside.

これらのことから、血中暴露を向上するべく、胃酸や β -グルコシダーゼ等による加水分解を抑制することを基本方針とし、そのきっかけになりうると推察されるアノマー位窒素原子へのプロトネーションを可能な限り低減する構造展開を行うことにした。

第 2 節 アニリン-*N*-グルコシドの初期展開

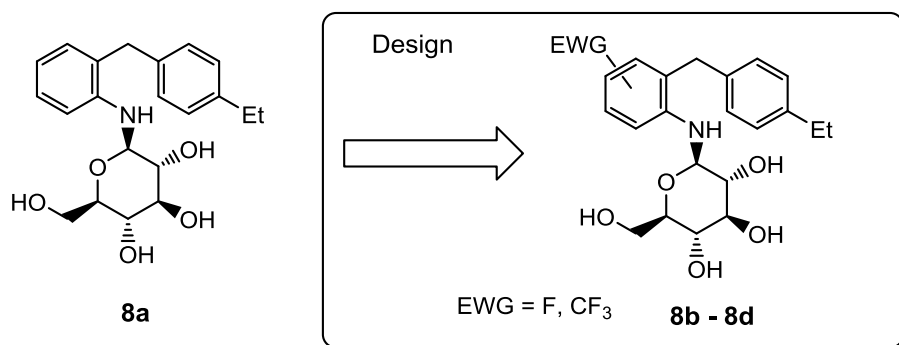
アニリン-*N*-グルコシドの更なる最適化にあたり、アニリン窒素原子へのプロトネーションを抑制するべく、化合物 **8a** のベンゼン環に電子吸引性置換基 (electron withdrawing group : EWG) を導入し、窒素原子の塩基性を低減するデザインを行った (図表 2 - 6)。

【図表 2 - 6】

アニリン-*N*-グルコシド最適化のデザイン

Strategy :

Introduction of electron-withdrawing substituent(s) on the aniline ring to diminish protonation on the nitrogen atom

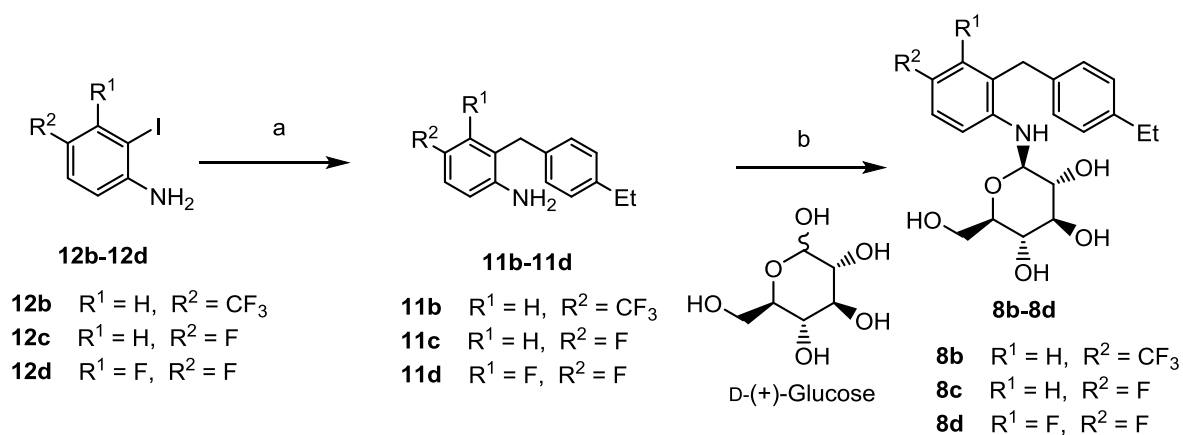


具体的には、化合物 **8a** のベンゼン環上 R^2 にトリフルオロメチル基導入した化合物 **8b**、フッ素原子を R^2 に導入した化合物 **8c**、あるいは R^1 と R^2 にフッ素原子を 2 つ導入した化合物 **8d** の検討を行った（図表 2－7）。

アグリコン **11b-11d** は、対応する 2-ヨードアニリン **12b-12d**²⁷⁾と（4-エチルベンジル）亜鉛ブロミドからパラジウム触媒を用いた根岸カップリング反応により中程度の収率で得ることができた（**11b**: 62%、**11c**: 64%、**11d**: 49%）。

アニリン **11b-11d** の D-グルコースとの縮合反応では、電子吸引基の影響で収率は低下するものの、化合物 **11a** の場合と同様に β -N-グルコシド化合物 **8b-8d** を得ることができた（**8b**: 26%、**8c**: 23%、**8d**: 23%）。なお、化合物 **8b-8d** のアノマー位の立体構造は、¹H NMR におけるアノマー位 C-H と隣接する C-H との間のカップリング定数から β 配置と決定した（**8b**: $J = 8.4$ Hz、**8c**: $J = 8.4$ Hz、**8d**: $J = 8.5$ Hz、DMSO- d_6 中 D₂O 交換時）。

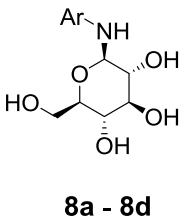
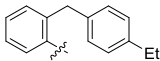
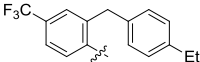
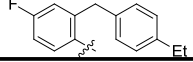
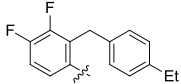
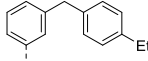
【図表 2－7】



Synthesis of aniline-N-glucosides **8b-8d**. Reagents and conditions: (a) 4-Ethylbenzylzinc bromide, Pd₂(dba)₃, tri(2-furyl)phosphine, DMF–THF, rt (**11b** 62%, **11c** 64%, **11d** 49%); (b) D-(+)-glucose, NH₄Cl, MeOH, reflux (**8b** 26%, **8c** 23%, **8d** 23%).

【図表 2 - 8】

アニリン-*N*-グルコシド **8a-8d** のプロファイル

	run	Compd.	Ar	hSGLT2 ^a IC ₅₀ (nM)	rUGE ^b (mg/day)
	1	8a		3.9	93 ± 27
	2	8b		750	-
	3	8c		5.1	-
	4	8d		2.9	439 ± 94
	5	3a (T-1095A)		5.2	-
	6	3b (T-1095)		-	422 ± 76
	7	5 (C-glucoside)		5.1	1485 ± 201

^a These data were obtained by a single determination performed in duplicate.

^b Each compound was orally administered at a dose of 30 mg/kg to male Sprague–Dawley (SD) rats. UGE data over 24 h were normalized per 200 g body weight. Values are expressed as mean ± S.E.M. (n=3).

- ✓ 3,4-Difluoroaniline-*N*-glucoside **8d** was identified as a potent and orally active hSGLT2 inhibitor comparable to **3b**.

まず、化合物 **8b-8d** の hSGLT2 阻害活性評価を行ったところ、4-トリフルオロメチル体 **8b** では起点化合物 **8a** と比べて作用が大きく減弱した (**8b**: IC₅₀ = 750 nM、**8a**: IC₅₀ = 3.9 nM) (図表 2 - 8)。一方、4-フルオロ体 **8c** および 3,4-ジフルオロ体 **8d** では **8a** に匹敵する強力な hSGLT2 阻害活性を有することを見出した (**8c**: IC₅₀ = 5.1 nM、**8d**: IC₅₀ = 2.9 nM)。

次に、最も強力な活性を示した化合物 **8d** の *in vivo* 試験として rUGE 促進作用を検討した。その結果、期待通り起点化合物 **8a** と比較して 4 倍以上に増強され、その作用は臨床試験化合物 **3b** に匹敵することを見出した (**8d**: rUGE = 439 mg/day、**8a**: rUGE = 93 mg/day、**3b**: rUGE = 422 mg/day、30 mg/kg、p.o.)。

ところでこの頃、hSGLT2 阻害剤の新たなケミカルクラスとして *C*-グルコシド化合物が特許公開された¹⁹⁾。それらの化合物の中から代表化合物 **5** のプロファイルを検討したところ、化合物 **8d** に匹敵する hSGLT2 阻害作用を有した (**5**: IC₅₀ = 5.1 nM、**8d**: IC₅₀ = 2.9 nM) (図表 2-8)。更には、化合物 **5** の C-C グルコシド結合は生体内においても安定で、非常に強力な in vivo 作用を示すことが判明した (rUGE = 1485 mg/day、30 mg/kg、p.o.)。筆者が研究する *N*-グルコシドにおいても大幅な in vivo 作用の増強が望まれた。

一方、アニリン-*N*-グルコシドでは、窒素原子へのプロトネーションを完全に回避することは原理的に不可能であることから、加水分解の抑制に基づく更なる血中暴露改善は難しいと考え、この時点で検討を中止することとした。

第 3 章 ヘテロアリール-*N*-グルコシドに関する研究

第 1 節 ヘテロアリール-*N*-グルコシドのデザインと合成

C-グルコシドに匹敵する強力な *in vivo* 作用を有する化合物の創出を目指し、窒素原子へのプロトネーションを完全に回避するべく新規な *N*-グルコシドのデザインを行った。即ち、アノマー位窒素原子を芳香環内に組み込んだ種々のヘテロアリール-*N*-グルコシド化合物 **13** の探索を計画した（図表 3-1）。

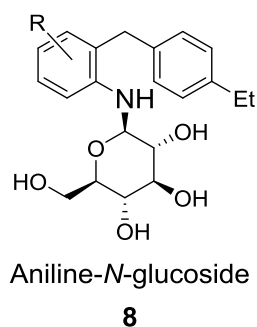
【図表 3-1】

ヘテロアリール-*N*-グルコシド**13**のデザイン

Strategy :

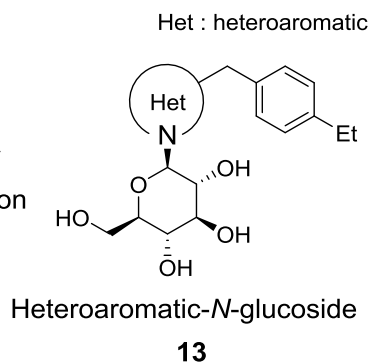
Incorporation of the anomeric nitrogen atom into heteroaromatic systems to avoid protonation

N-glucoside



Design

Avoidance of protonation
on nitrogen atom



まず、含窒素ヘテロアリアル環型の各種アグリコン **14a-14e** の合成を行った（図表 3-2）。

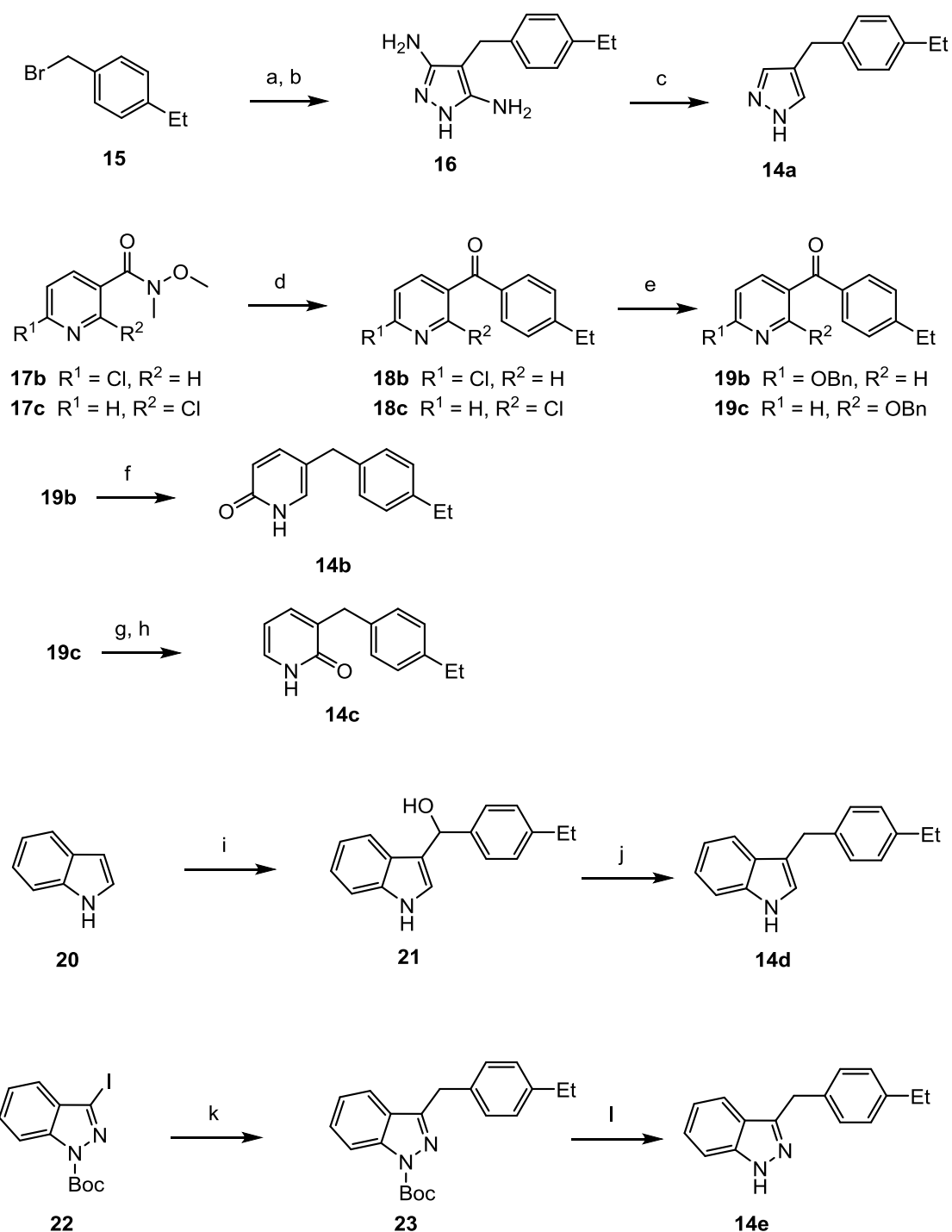
ピラゾール型アグリコン **14a** は Echevarría らの手法を参考にして合成した²⁸⁾。即ちマロノニトリルに対して 4-エチルベンジルブロミド **15** によるアルキル化（35%）の後、ヒドラジン付加による環化反応でジアミノピラゾール **16** とし（69%）、次いで 2 つのアミノ基を同時に脱アミノ化することでピラゾール体 **14a** を得た（37%）。

ピリドン型アグリコン **14b** および **14c** は対応するケトン **19b** および **19c** より合成した。既知の Weinreb アミド **17b**²⁹⁾ および **17c**³⁰⁾ に対して 4-エチルフェニルマグネシウムブロミドを反応させてケトン **18b** (72%) および **18c** (78%) とし、次いでナトリウムベンジルオキシドを反応させることでベンジルオキシ体 **19b** (77%) および **19c** (41%) を得た。ケトン **19b** はヒドラジンおよび水酸化カリウムを用いた Wolff-Kishner 還元条件により目的のピリドン **14b** に導いた (44%)。ケトン **19c** を水素化ほう素ナトリウム (NaBH_4) によるアルコールへの還元後、水素気流下で Pd/C を用いた接触還元に付すことで目的のピリドン **14c** を得た (27%、two steps)。

インドール型アグリコン **14d** の合成では、まず Zhou らの手法³¹⁾を参考にして、インドール **20** および 4-エチルベンズアルデヒドからアルコール **21** を得た (16%)。次いで水酸基を Et_3SiH と $\text{BF}_3 \cdot \text{Et}_2\text{O}$ の組み合わせで加水素分解還元することで目的のインドール **14d** に導いた (77%)。

インダゾール型アグリコン **14e** は、Collot の手法³²⁾により得た 1-*t*-ブトキシカルボニル-3-ヨードインダゾール **22** を原料とし、(4-エチルベンジル) 亜鉛ブロミドとのパラジウム触媒を用いた根岸カップリング反応により側鎖導入を行い (70%)、次いで脱 *t*-ブトキシカルボニル化反応により合成した (61%)。

【图表 3 - 2】

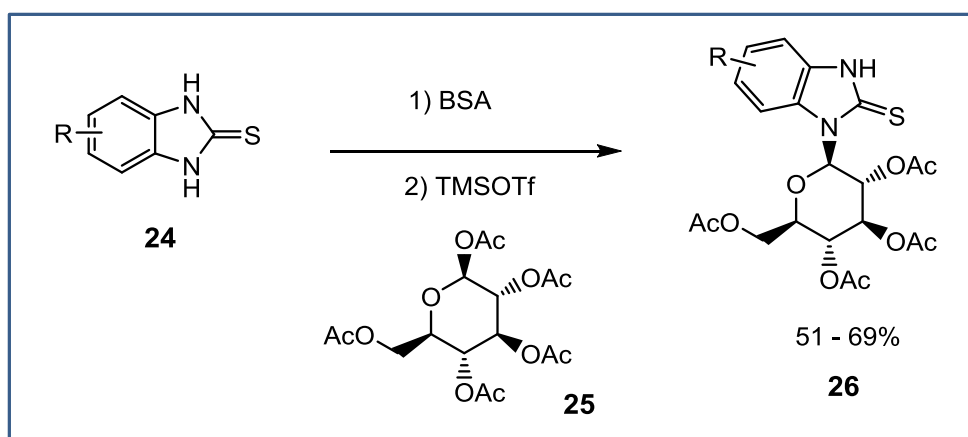


Synthesis of aglycons **14a–14e**. Reagents and conditions: (a) malononitrile, K_2CO_3 , $n\text{Bu}_4\text{NBr}$, toluene, rt (35%); (b) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, EtOH, reflux (69%); (c) H_3PO_2 aq., NaNO_2 , 5 °C to rt (37%); (d) 4-ethylphenylmagnesium bromide, THF, 0 °C (**18b** 72%, **18c** 78%); (e) benzylalcohol, NaH, DMF, rt (–90 °C for **19b**) (**19b** 77%, **19c** 41%); (f) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, KOH, ethylene glycol, 190 °C (44%); (g) NaBH_4 , EtOH, rt; (h) H_2 , Pd/C (10%), conc. HCl, MeOH, rt (27%, two steps); (i) 4-ethylbenzaldehyde, NaOH, MeOH, rt (16%); (j) Et_3SiH , $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , –78 °C to 0 °C (77%); (k) 4-ethylbenzylzinc bromide, $\text{Pd}_2(\text{dba})_3$, tri(2-furyl)phosphine, DMF–THF, 0 °C to rt (70%); (l) MeONa, MeOH, rt (61%).

含窒素ヘテロアリアル環に対する直接的な *N*-グルコシド化の手法として、これまでにベンズイミダゾールチオン **24** を基質とした反応例が知られていた (図表 3-3) ³³⁾。本法はアグリコン **24** を *N,O*-ビス (トリメチルシリル) アセトアミド (BSA) を用いて活性化し、糖部分として 1,2,3,4,6-ペンタ-*O*-アセチル-β-D-グルコピラノース **25** をトリメチルシリルトリフルオロメタンスルホナート (TMSOTf) 存在下で反応させることで、目的とするカップリング体 **26** を得るものである。ただし、本条件のベンズイミダゾールチオン以外のヘテロアリアル環への *N*-グルコシド化の報告はなく、筆者は種々ヘテロ環への適用拡大を試みた。

【図表 3-3】

ヘテロアリアル-*N*-グルコシドの合成例

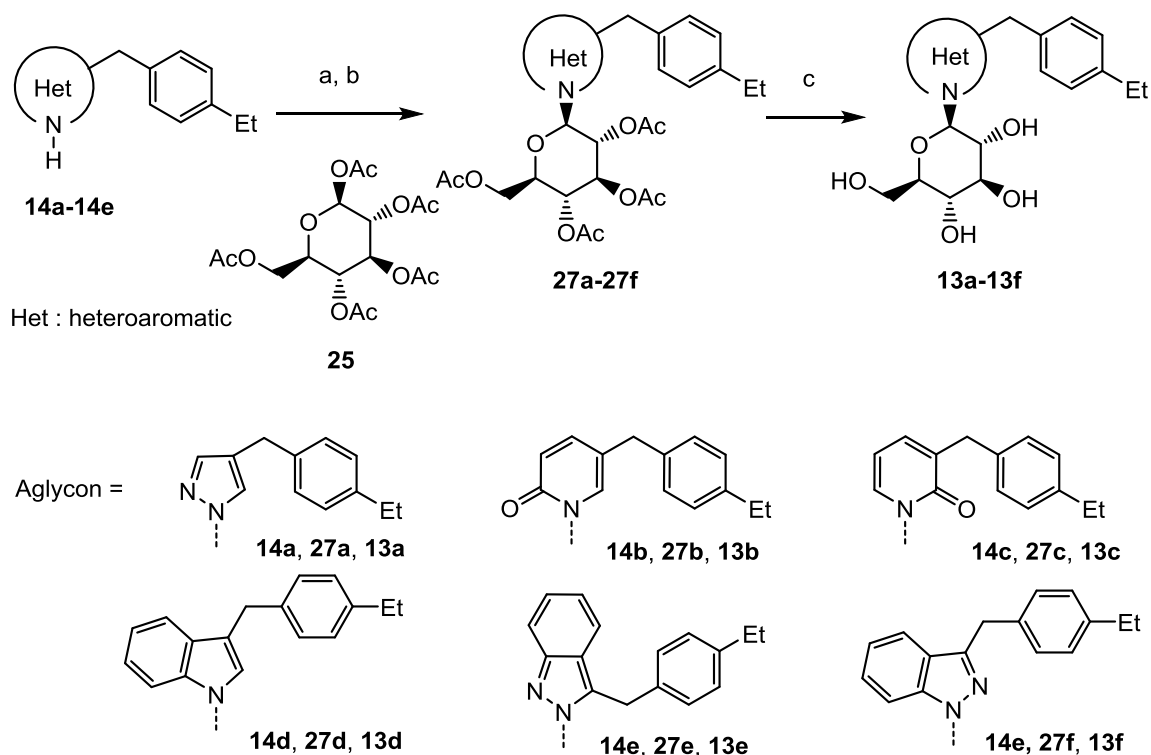


- ✓ A patent literature reports that benzimidazole-2-thiones **24** are silylated by *N,O*-bis(trimethylsilyl)acetamide (BSA) followed by coupling with pentaacetylglucose **25** in the presence of trimethylsilyl triflate (TMSOTf) to give *N*-glucosides **26**.

本法を用いた *N*-グルコシド化検討の結果、ヘテロアリール型アグリコン **14a-14e** および化合物 **25** から低～中程度の収率ではあるが対応する *N*-グルコシド体 **27a-27f** を取得した (**27a**: 44%、**27b**: 37%、**27c**: 4%、**27d**: 17%、**27e+27f**: >47%) (図表 3-4)。本手法が多様な含窒素ヘテロアリール環に応用可能であることを示すことができた。

次いで、化合物 **27a-27f** のグルコース部分のアセチル保護基を除去することで目的の β -*N*-グルコシド化合物 **13a-13f** に導いた (**13a**: 78%、**13b**: 82%、**13c**: 48%、**13d**: 48%) (**13e** 42% and **13f** 5%, three steps)。なお、化合物 **13a-13f** のアノマー位の立体構造は、¹H NMR におけるアノマー位 C-H と隣接する C-H との間のカップリング定数から β 配置と決定した (**13a**: $J = 9.2$ Hz、**13b,c**: $J = 9.3$ Hz、**13d**: $J = 9.0$ Hz、**13e**: $J = 8.8$ Hz、**13f**: $J = 9.0$ Hz、DMSO-*d*₆ 中)。

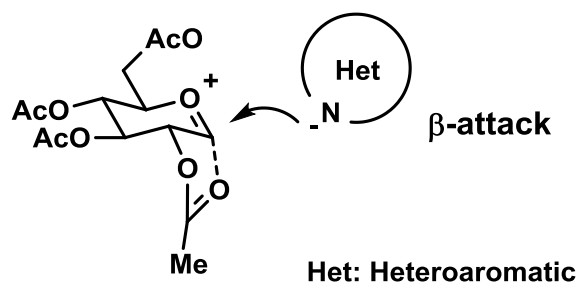
【図表 3-4】



Synthesis of heteroaromatic-*N*-glucosides **13a-13f**. Reagents and conditions: (a) *N,O*-bis(trimethylsilyl)acetamide, MeCN, 60 °C; (b) **25**, trimethylsilyl triflate, 1,2-dichloroethane, 80 °C (**27a** 44%, **27b** 37%, **27c** 4%, **27d** 17%, two steps); (c) NaOMe, MeOH, rt (**13a** 78%, **13b** 82%, **13c** 48%, **13d** 48%) (**13e** 42% and **13f** 5% based on **14e**, respectively).

詳細には未検討であるが、いずれの化合物においても α 体の単離には至っていない。グルコース 2 位のアセトキシ基による隣接基関与³⁴⁾によって α 面がブロックされた結果、アグリコンによる β 面からの攻撃が優先し、望みの β 体が主生成物として得られたものと考えている（図表 3－5）。

【図表 3－5】

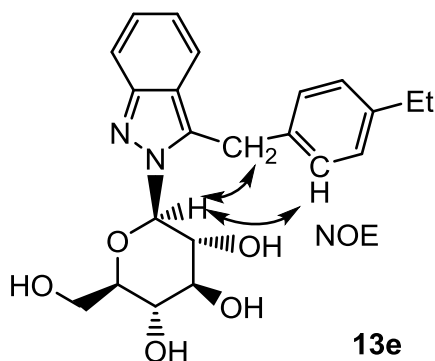


Neighboring group participation in glucosylation

なお、ピリドン-*N*-グルコシド **13b** および **13c** においては、IR スペクトル解析によってカルボニル吸収がそれぞれ 1667 cm^{-1} 、 1650 cm^{-1} に観察されたことから *N*-グルコシドであることを確認した³⁵⁾。

インダゾール型アグリコン **14e** とグルコース誘導体 **25** のカップリング反応では、主生成物の 2-グルコシド体 **27e** とともに少量の 1-グルコシド体 **27f** がカラム分離不能の混合物として得られ、アセチル基の脱保護後に目的の *N*-グルコシド **13e** および **13f** を結晶化により分離した (**13e**: 42%、**13f**: 5% based on **14e**)。化合物 **13e** の ^1H NMR において図表 3－6 に示すようなアノマー位プロトンとベンジル位およびフェニル基上プロトンとの間に NOE を観察した。

【図表 3－6】



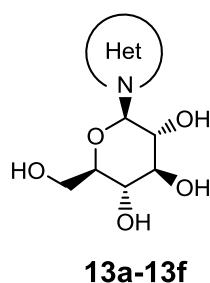
第 2 節 ヘテロアリアル-*N*-グルコシドのプロファイル

化合物 **13a-13f** の hSGLT2 阻害活性評価を行った (図表 3-7)。ピラゾール体 **13a** では中程度の活性であった ($IC_{50} = 381 \text{ nM}$)。また、ピリドン体においては、化合物 **13b** が中程度の活性を示したのに比べて化合物 **13c** では約 40 倍の活性低下がみられた (**13b**: $IC_{50} = 163 \text{ nM}$ 、**13c**: $IC_{50} = 6671 \text{ nM}$)。ピリドン体 **13c** における 2-カルボニル基は、グルコース部位と側鎖ベンジル基の位置関係を極めて不利に導いたと考えられる。一方、インドール体 **13d** においては、起点化合物であるアニリン-*N*-グルコシド **8a** と同等の強力な活性を有することが判明した (**13d**: $IC_{50} = 7.1 \text{ nM}$ 、**8a**: $IC_{50} = 3.9 \text{ nM}$)。インダゾール体においては、2-グルコシド体 **13e** が弱い活性であったのに比べて、1-グルコシド体 **13f** では約 16 倍の活性向上がみられた (**13e**: $IC_{50} = 1098 \text{ nM}$ 、**13f**: $IC_{50} = 69 \text{ nM}$)。これまでに文献報告がなされている *C*-グルコシドの場合と同様に、グルコース部位と側鎖ベンジル基の位置関係としては 1,3-タイプの組み合わせが 1,2-の場合よりも活性向上する結果となった。

次に、強力な hSGLT2 阻害作用が見出されたインドール-*N*-グルコシド **13d** について、*in vivo* 試験として rUGE の促進作用を評価した。その結果、同等の hSGLT2 阻害活性を有する起点化合物 **8a** と比較して 20 倍増強され、その作用は *C*-グルコシド化合物 **5** に匹敵することが判明した (**13d**: rUGE = 1830 mg/day、**8a**: rUGE = 93 mg/day、**5**: rUGE = 1485 mg/day、30 mg/kg、p.o.)。

【図表 3 - 7】

ヘテロアリール-*N*-グルコシド**13a-13f**のプロファイル



run	Compd.	Het N-	hSGLT2 ^a IC ₅₀ (nM)	rUGE ^b (mg/day)
1	13a		381	-
2	13b		163	-
3	13c		6671	-
4	13d		7.1	1830 ± 75
5	13e		1098	-
6	13f		69	-
7	8a (aniline- <i>N</i> -glucoside)		3.9	93 ± 27
8	5 (C-glucoside)		5.1	1485 ± 201

^a These data were obtained by a single determination performed in duplicate.

^b Each compound was orally administered at a dose of 30 mg/kg to male Sprague-Dawley (SD) rats.

UGE data over 24 h were normalized per 200 g body weight. Values are expressed as mean ± S.E.M. (n=3).

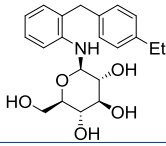
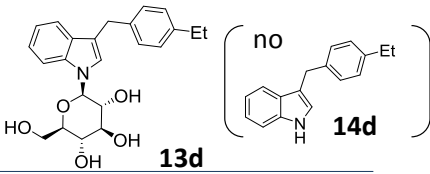
✓ Compound **13d** possessing an indole core structure was identified to show good in vitro and in vivo activity comparable to C-glucoside **5**.

ラット PK 試験においては、インドール体 **13d** はアニリン体 **8a** と比較して顕著に血中暴露が改善され (**13d**: AUC_{0-inf} = 17 µg · h/mL, BA = 59%、**8a**: AUC_{0-inf} = 0.23 µg · h/mL, BA = 17%、10 mg/kg、p.o.)、rUGE 促進作用の大幅な増強を支持する結果となった (図表 3 - 8)。この際、化合物 **13d** が加水分解を受けた場合に生じるインドールアグリコン **14d** は血中に観察されなかった。更に、第 2 章 (第 1 節) にてアニリン化合物 **8a** が速やかな加水分解を受けた酸性条件 (0.5 N、HCl、37 °C) において化合物 **13d** が安定であることも判明した。これらのことから、アニリン化合物 **8a** と比べてインドール化合物 **13d** の in vivo における PK および rUGE 促進作用のプロファイルの大幅な改善は、C-N グルコシド結合の加水分解に対する優れた安定性が寄与しているものと推察された。

これらの結果をもとに、インドール-*N*-グルコシド **13d** をリード化合物として、周辺置換基の最適化研究により更なる薬効増強を目指すこととした。

化合物8aと13dのラット動態比較

Pharmacokinetic parameters of **8a** and **13d** in SD rats

Compound	 8a	 13d
Dose (mg/kg; i.v.)	3	3
$t_{1/2}$ (h)	0.58	3.0
AUC_{0-inf} ($\mu\text{g}\cdot\text{h/mL}$)	0.41	8.7
CL_{tot} (L/h/kg)	7.4	0.35
Vd_{ss} (L/kg)	4.5	1.3
Dose (mg/kg; p.o.)	10	10
$t_{1/2}$ (h)	0.43	2.9
t_{max} (h)	0.25	0.5
C_{max} ($\mu\text{g/mL}$)	0.43	2.0
AUC_{0-inf} ($\mu\text{g}\cdot\text{h/mL}$)	0.23	17
F (%)	17	59

Values are the mean of two rats.

- ✓ Large enhancement of in vivo potency of **13d** was clearly supported by the good pharmacokinetic results. We attribute these enhanced in vivo profiles of **13d** in part to the inherent stability of the C-N glucosidic bond against hydrolysis.

第 4 章 インドール-*N*-グルコシドの最適化研究

第 1 節 インドール-*N*-グルコシドの合成

インドール-*N*-グルコシド **13d** をリード化合物とした周辺置換基の最適化研究において、インドール環 4 位およびベンジル側鎖上のパラ位の置換基の変換を行った。

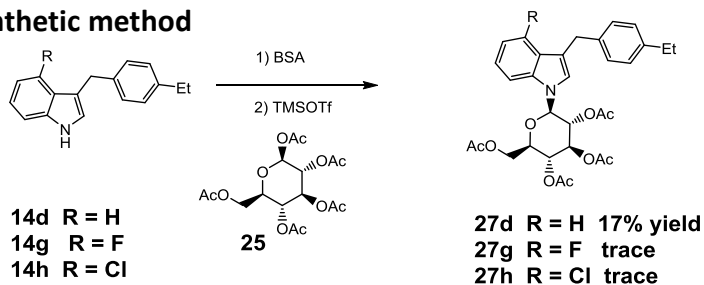
まずインドール環 4 位にフッ素原子 (**14g**) あるいは塩素原子 (**14h**) のような電子吸引基を有するアグリコンに対して、化合物 **13d** を合成したグルコシド化反応 (BSA、化合物 **25** および TMSOTf) を検討したところ、目的とするグルコシド化合物 **27g** および **27h** を痕跡量しか得ることができなかった (図表 4-1)。

そこで新たな手法として、予めグルコース部分を導入した共通中間体 **30** を取得し、後半工程にて Friedel-Crafts 反応や Grignard 反応でインドール 3 位へのベンジル側鎖導入を行う合成戦略を立てた。

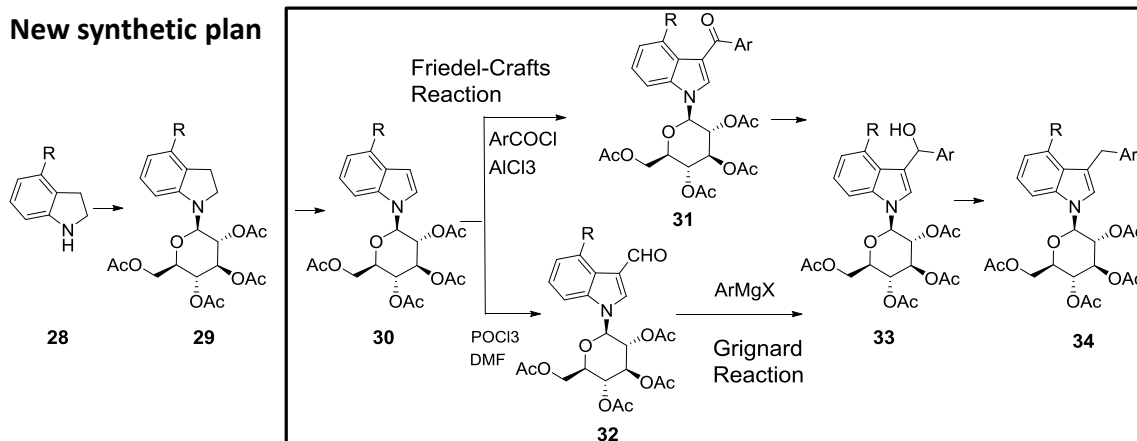
【図表 4-1】

インドール-*N*-グルコシドの合成計画

Previous synthetic method



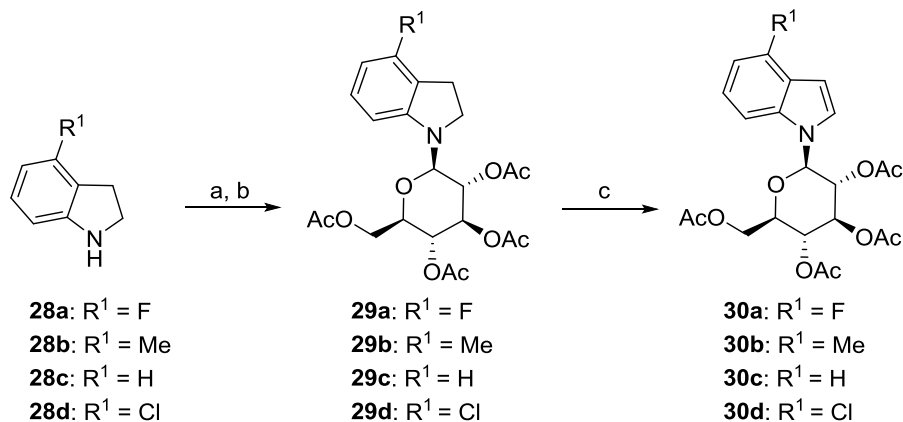
New synthetic plan



まず、Messaoudi らの方法³⁶⁾を参考にして、4 位にフッ素原子あるいは塩素原子などを
含むインドール-*N*-グルコシド **30a-30d** を合成した (図表 4-2)。

即ち、求核性の高いインドリン **28a-28d** を原料としてグルコースと加熱攪拌することで
対応するインドリン-β-*N*-グルコシド体へと導いた後、結晶性のよいアセチル保護体
29a-29d として中程度以上の収率で単離した (**29a**: 62%、**29b**: 37%、**29c**: 49%、**29d**: 61%、
two steps)。立体選択性については、第 2 章 (第 1 節) のアニリン-*N*-グルコシドの場合と
同様に、アノマー効果で有利な α 体に対し、アグリコン部分の嵩高さの影響で熱力学的に
より安定な β 体が優先して得られたものと考えている。次いでインドリン-*N*-グルコシド
29a-29d を 2,3-ジクロロ-5,6-ジシアノ-1,4-ベンゾキノン (DDQ) を用いた酸化反応により、
高収率でインドール-*N*-グルコシド **30a-30d** へと導き、側鎖導入を行う共通中間体とした
(**30a**: 89%、**30b**: 94%、**30c**: 86%、**30d**: 93%)。なお、各化合物のアノマー位の立体構造
は、¹H NMR におけるアノマー位 C-H と隣接する C-H との間のカップリング定数から β
配置と決定した (**29a-29d**: *J* = 9.3 Hz、**30a-30d**: *J* = 9.0 Hz、DMSO-*d*₆ 中)。

【図表 4-2】



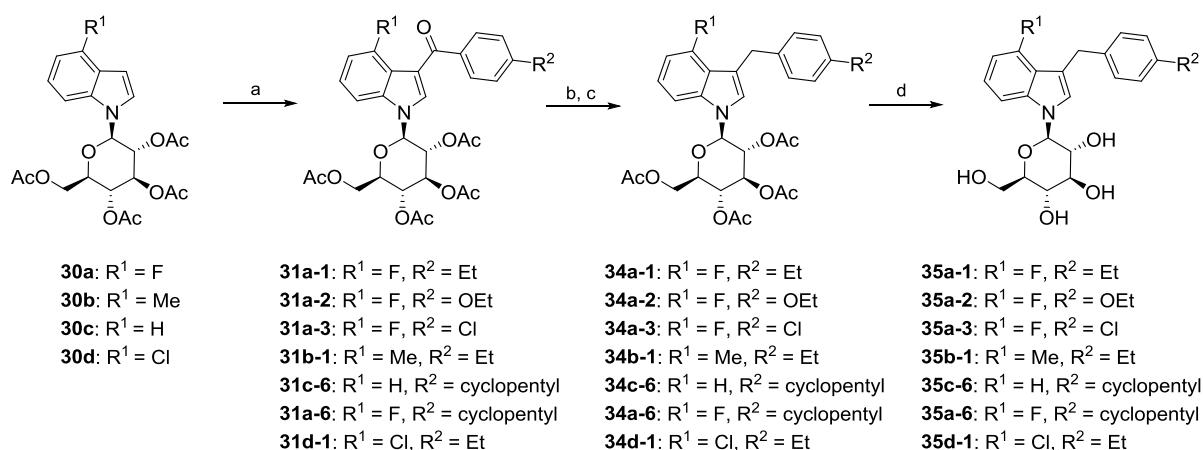
Synthesis of indole-*N*-glucosides **30a-30d**. Reagents and Conditions: (a) D-(+)-glucose, EtOH-water, reflux; (b) Ac₂O, pyridine, cat. DMAP, CHCl₃, rt (**29a** 62%, **29b** 37%, **29c** 49%, **29d** 61%, two steps); (c) DDQ, 1,4-dioxane-water, rt (**30a** 89%, **30b** 94%, **30c** 86%, **30d** 93%, two steps).

インドール環 3 位への側鎖導入法として、初めに Friedel-Crafts 反応を利用した手法を検討した (図表 4-3)。インドール環 3 位へのベンゾイルクロリドの Friedel-Crafts 反応は一般的ではあるが、配糖体 **30** では 4 つのアセチル保護基へのルイス酸の配位が想定され、アシル化剤である酸クロリドの活性化への影響が懸念された。実際、化合物 **30a** および 4-エチルベンゾイルクロリド (1.3 当量) に対して 1~2 当量の塩化アルミニウム (III) (AlCl_3) をルイス酸として用いた場合、アシル化反応の進行は不完全で原料 **30a** は残存する結果となった。そこで AlCl_3 の 5 当量への増量を試みたところ、原料 **30a** は完全に消費され、98%の収率で目的の 3-ベンゾイルインドール **31a-1** を与えることを見出した。配糖体 **30** の 4 つのアセチル保護基への配位により、過剰量のルイス酸が必要になったものと考えている。同様にインドール **30a-30d** を原料として 3-ベンゾイルインドール **31** を得た (**31a-2**: 99%、**31a-3**: 42%、**31b-1**: 83%、**31c-6**: 100%、**31a-6**: 100%、**31d-1**: 95%)³⁷⁾。

次いで、化合物 **31** のケトンを塩化セリウム (III) (CeCl_3) 共存下で NaBH_4 によってアルコールに還元後³⁸⁾、引き続き水酸基を Et_3SiH と $\text{BF}_3 \cdot \text{Et}_2\text{O}$ の組み合わせで加水素分解還元することで 3-ベンジルインドール **34** に高収率で導いた (**34a-1**: 92%、**34a-2**: 83%、**34a-3**: 87%、**34b-1**: 83%、**34c-6**: 82%、**34a-6**: 78%、**34d-1**: 80%、two steps)。最後にテトラアセチル体 **34** を脱保護することで、目的のインドール-*N*-グルコシド誘導体 **35** を得た

(**35a-1**: 99%、**35a-2**: 100%、**35a-3**: 81%、**35b-1**: 98%、**35c-6**: 98%、**35a-6**: 96%、**35d-1**: 100%)。

【図表 4-3】

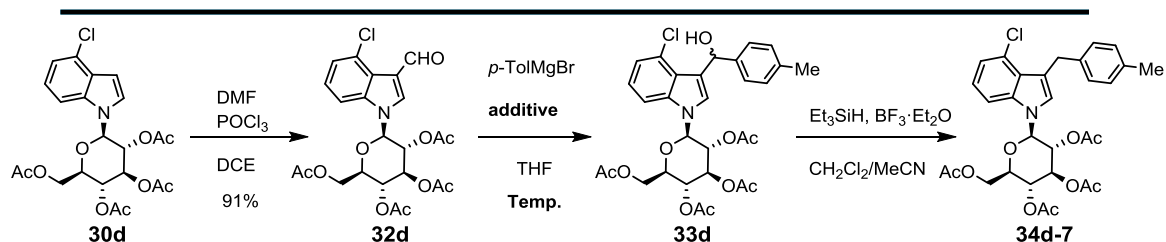


Synthesis of indole-*N*-glucosides **34**. Reagents and conditions: (a) ArCOCl , AlCl_3 (5 eq.), CH_2Cl_2 , 0 °C (**31a-1** 98%, **31a-2** 99%, **31a-3** 42%, **31b-1** 83%, **31c-6** 100%, **31a-6** 100%, **31d-1** 95%); (b) NaBH_4 , $\text{CeCl}_3 \cdot 5\text{H}_2\text{O}$, EtOH-THF , 0 °C; (c) Et_3SiH , $\text{BF}_3 \cdot \text{OEt}_2$, $\text{MeCN-CH}_2\text{Cl}_2$, -10 °C (**34a-1** 92%, **34a-2** 83%, **34a-3** 87%, **34b-1** 83%, **34c-6** 82%, **34a-6** 78%, **34d-1** 80%, two steps); (d) cat NaOMe , MeOH-THF , rt (**35a-1** 99%, **35a-2** 100%, **35a-3** 81%, **35b-1** 98%, **35c-6** 98%, **35a-6** 96%, **35d-1** 100%)

鎖導入の別法として、共通中間体 **30** からホルミル体 **32** へと導き、次いで Grignard 反応を用いた側鎖導入を検討した（図表 4－4）。具体的には共通中間体 **30d** を出発原料として化合物 **34d-7** を合成する場合をモデルケースとして反応条件を精査した。この場合、Grignard 試薬と反応可能な 4 つのアセチル基共存下でのインドール環 3 位ホルミル基への選択的な反応が課題と考えられた。

【図表 4－4】

Grignard 反応条件の最適化



run	p-TolMgBr (eq.)	additive	Temp. (°C)	33d ^a	34d-7 (yield) ^b
1	1.2	-	-78	trace	-
2	3.0	-	-78	major	83%
3	1.2	MgBr ₂ ·Et ₂ O (3.0)	-78	major	61%
4	1.2	BF ₃ ·Et ₂ O (3.0)	-78	trace	-
5	1.2	ZnCl ₂ (3.0)	-78	trace	-
6	1.2	-	0	major	63%
7	2.0	-	0	major	92%

^a Judged by TLC analysis ^b Isolated yields after column purification are shown.

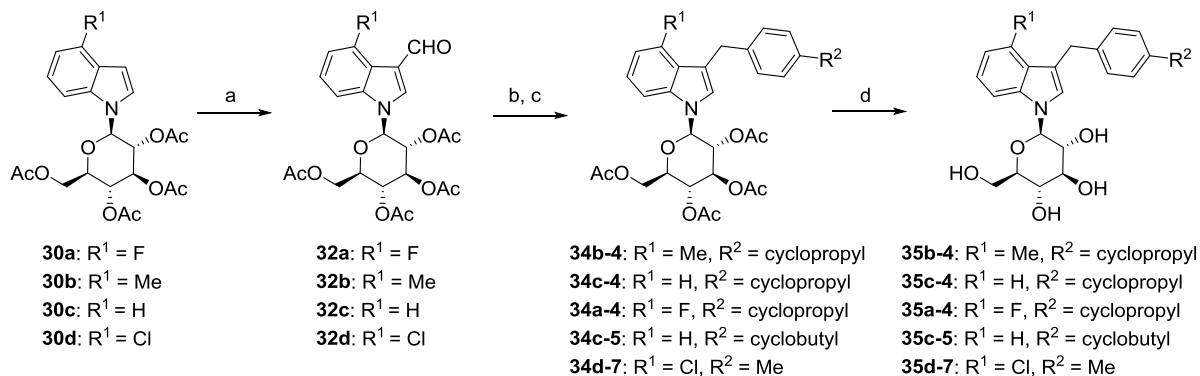
- ✓ Although 3-formylindole **32d** had four acetyl groups, desired coupling product **33d** was obtained in high yield by using 2 eq. of aryl magnesium bromide at 0 °C.

まず、化合物 **30d** は Vilsmeier 反応により 91%の高収率でホルミル体 **32d** へと導いた。Grignard 反応については、まず低温下（-78℃）にて小過剰（1.2 当量）の(*p*-メチルフェニル)マグネシウム ブロミドのアルデヒド **32d** との反応を試みた。その結果、目的とするカルビノール中間体 **33d** は得られなかった（run 1）。Grignard 試薬がアセチル部位でのエノール化やキレーションに費やされ、所望のホルミル基への反応に至っていないのではないかと考えた。まず Grignard 試薬を 3 当量に増量して反応を検討したところ、カルビノール **33d** の Et₃SiH と BF₃·Et₂O の組み合わせによる還元を経て 83%収率で目的物 **34d-7** を得ることが出来た（run 2）。一方、マグネシウムがアセチル基への配位に費やされている可

能性を考え、Grignard 試薬を小過剰（1.2 当量）のままで additive の検討を行った。その結果、二臭化マグネシウム・ジエチルエーテル錯体 ($\text{MgBr}_2 \cdot \text{Et}_2\text{O}$) を加えた場合 **34d-7** が 61% で得られ、additive を加えない場合 (run 1) と比べて収率の明らかな向上が観察された (run 3)。なお、 $\text{BF}_3 \cdot \text{Et}_2\text{O}$ や塩化亜鉛では改善はみられなかった (run 4、5)。次に、アセチル部位への作用よりも先にホルミル基への反応が進行することを期待し、0℃ に昇温して反応を試みた。その結果、小過剰（1.2 当量）の Grignard 試薬の場合でも 63% 収率で目的物 **34d-7** が得られることが判明した (run 6)。この場合に副生物の生成が微量であったことから、Grignard 試薬を 2 当量に増量する検討を行ったところ、望みの反応がほぼ完結して 92% 収率で **34d-7** を得ることが出来た (run 7)。

本条件を適用し³⁹⁾、種々目的のインドール-*N*-グルコシド誘導体 **34** を取得した (**34b-4**: 85%、**34c-4**: 97%、**34a-4**: 81%、**34c-5**: 73%、**34d-7**: 92%)。化合物 **34** はアセチル保護基を除去により目的のインドール-*N*-グルコシド誘導体 **35** に導いた (**35b-4**: 85%、**35c-4**: 99%、**35a-4**: 93%、**35c-5**: 95%、**35d-7**: 85%) (図表 4 - 5)。

【図表 4 - 5】



Synthesis of indole-*N*-glucosides **35**. Reagents and conditions: (a) DMF, POCl_3 , 1,2-dichloroethane, 70 °C (**32a** 81%, **32b** 71%, **32c** 75%, **32d** 91%); (b) ArMgBr , THF, 0 °C; (c) Et_3SiH , $\text{BF}_3 \cdot \text{OEt}_2$, $\text{MeCN}-\text{CH}_2\text{Cl}_2$, -10 °C (**34b-4** 85%, **34c-4** 97%, **34a-4** 81%, **34c-5** 73%, **34d-7** 92%, two steps); (d) cat. NaOMe , $\text{MeOH}-\text{THF}$, rt (**35b-4** 85%, **35c-4** 99%, **35a-4** 93%, **35c-5** 95%, **35d-7** 85%).


第 2 節 インドール-*N*-グルコシドのプロファイル

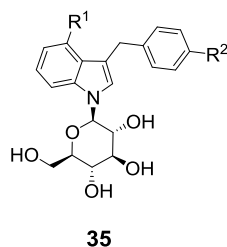
インドール-*N*-グルコシドの R¹ および R² 置換基の生物活性への影響を評価した (図表 4-6)。

R¹ 置換基の比較では、R¹ = H (**13d**) と比較して、R¹ = F (**35a-1**)、Cl (**35d-1**)、R¹ = Me (**35b-1**) はいずれも強力な hSGLT2 阻害活性を有した (**35a-1**: IC₅₀ = 5.2 nM、**35d-1**: IC₅₀ = 2.9 nM、**35b-1**: IC₅₀ = 1.1 nM、**13d**: IC₅₀ = 7.1 nM)。rUGE 促進作用においては R¹ = F (**35a-1**) と R¹ = Cl (**35d-1**) が R¹ = H (**13d**) よりも強い作用を示した (**35a-1**: rUGE = 2937 mg/day、**35d-1**: rUGE = 2185 mg/day、**13d**: rUGE = 1830 mg/day、30 mg/kg、p.o.)。これら 2 化合物について hSGLT1 阻害活性を測定したところ R¹ = F (**35a-1**) の場合の方が R¹ = Cl (**35d-1**) よりも高い hSGLT2 への選択性を有することが示唆された (**35a-1**: hSGLT1 IC₅₀ = 210 nM, hSGLT1/hSGLT2 = 40、**35d-1**: hSGLT1 IC₅₀ = 49 nM, hSGLT1/hSGLT2 = 16.9)。

一方、R² 置換基の比較では、R¹ = F の場合に R² = Et (**35a-1**) と R² = EtO (**35a-2**) が同等の hSGLT2 阻害活性および rUGE 促進作用を示したのに対し、R² = Cl (**35a-3**) は hSGLT2 阻害活性が低下した (**35a-1**: IC₅₀ = 5.2 nM, rUGE = 2937 mg/day、**35a-2**: IC₅₀ = 4.8 nM, rUGE = 2683 mg/day、**35a-3**: IC₅₀ = 18 nM (rUGE は 30 mg/kg、p.o.))。また R¹ = Cl の場合に R² = Et (**35d-1**) では R² = Me (**35d-7**) と比較して hSGLT2 阻害作用は同等であるものの rUGE 促進作用が大きく増強されることが判明した (**35d-1**: IC₅₀ = 2.9 nM, rUGE = 2185 mg/day、**35d-7**: IC₅₀ = 2.4 nM, rUGE = 1300 mg/day (rUGE は 30 mg/kg、p.o.))。一方、R¹ = Me の場合に R² = Et (**35b-1**) を R² = cyclopropyl (**35b-4**) に置き換えることで hSGLT2 阻害作用は同等であるものの rUGE 促進作用が大きく増強されることが判明した (**35b-1**: IC₅₀ = 1.1 nM, rUGE = 1664 mg/day、**35b-4**: IC₅₀ = 1.6 nM, rUGE = 2830 mg/day (rUGE は 30 mg/kg、p.o.))。In vitro および in vivo 作用の双方において、R² 置換基として cyclopropyl 基を有する化合物 **35b-4** が強力な作用を示したことから、次に、他の環状アルキル置換基による影響を精査することとした。

インドール-*N*-グルコシド**35**のプロファイル①

run	Cpd.	R ¹	R ²	hSGLT2 ^a IC ₅₀ (nM)	rUGE ^b (mg/day)	hSGLT1 ^a IC ₅₀ (nM)	selectivity ^c
1	35a-1	F	Et	5.2	2937 ± 106	210	40
2	35a-2	F	OEt	4.8	2683 ± 83		
3	35a-3	F	Cl	18	-		
4	35d-1	Cl	Et	2.9	2185 ± 85	49	16.9
5	35d-7	Cl	Me	2.4	1300 ± 188		
6	35b-1	Me	Et	1.1	1664 ± 63		
7	35b-4	Me		1.6	2830 ± 150	22	13.8
8	13d	H	Et	7.1	1830 ± 75		



^a These data were obtained by a single determination performed in duplicate.

^b Each compound was orally administered at a dose of 30 mg/kg to male Sprague-Dawley (SD) rats.

UGE data over 24 h were normalized per 200 g body weight. Values are expressed as mean ± S.E.M. (n=3).

^c Values are shown by hSGLT1(IC₅₀)/hSGLT2(IC₅₀).

R² 置換基として環状アルキル基のによる生物活性の評価結果を図表 4 - 7 に示す。

R² = cyclopropyl 基の場合には、R¹ = H (**35c-4**) と R¹ = F (**35a-4**) のいずれにおいても *C*-グルコシド **5** を凌ぐ強力な in vitro および in vivo 作用を有した (**35c-4**: IC₅₀ = 2.2 nM, rUGE = 2435 mg/day、**35a-4**: IC₅₀ = 1.4 nM, rUGE = 2502 mg/day、**5**: IC₅₀ = 5.1 nM, rUGE = 1485 mg/day (rUGE は 30 mg/kg、p.o.))。R² = cyclobutyl 基 (R¹ = H: **35c-5**) あるいは R² = cyclopentyl 基 (R¹ = H: **35c-6**、R¹ = F: **35a-6**) の場合は、いずれも hSGLT2 阻害作用が減弱する結果となった (**35c-5**: IC₅₀ = 11 nM、**35c-6**: IC₅₀ = 93 nM、**35a-6**: IC₅₀ = 24 nM)。






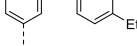
次に、in vitro および in vivo 作用の双方で強力な作用を有した R² = cyclopropyl 基の化合物 **35b-4** および **35a-4** について hSGLT1 阻害作用を評価した (図表 4 - 6、4 - 7)。その結果、R¹ = F (**35a-4**) の方が R¹ = Me (**35b-4**) より高い hSGLT2 選択性を有した (**35a-4**: hSGLT1 IC₅₀ = 230 nM, hSGLT1/hSGLT2 = 160、**35b-4**: hSGLT1 IC₅₀ = 22 nM, hSGLT1/hSGLT2 = 13.8)。

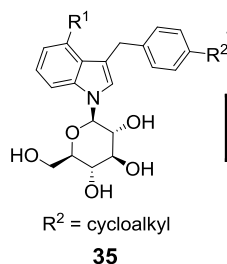
更に、化合物 **35a-4** については、あらゆる組織に存在する促通拡散型グルコーストランスポーターGLUT1⁴⁰⁾への作用についても検討を行った。本トランスポーターを強く阻害した場合、脳をはじめとする生体機能への影響が懸念されるが、化合物 **35a-4** においてはGLUT1 の阻害作用は認められず、本作用に基づく副作用リスクの低い化合物であることが示唆された (**35a-4**: GLUT1 IC₅₀ > 10,000 nM)。

以上の結果より、選択的かつ強力な hSGLT2 阻害作用、および経口投与でも C-グルコシド **5** を凌ぐ薬効 (rUGE 促進作用) を有する化合物 **35a-4** を臨床試験候補化合物 (TA-1887/JNJ-39933673) として選抜した。

【図表 4 - 7】

インドール-N-グルコシド**35**のプロファイル②

run	Cpd.	R ¹	R ²	hSGLT2 ^a IC ₅₀ (nM)	rUGE ^b (mg/day)	hSGLT1 ^a IC ₅₀ (nM)	Selectivity ^{c,d}
1	35c-4	H		2.2	2435 ± 150		
2	35a-4	F		1.4	2502 ± 75	230	160
3	35c-5	H		11	-		
4	35c-6	H		93	-		
5	35a-6	F		24	-		
6	5			5.1	1485 ± 201		



^a These data were obtained by a single determination performed in duplicate.

^b Each compound was orally administered at a dose of 30 mg/kg to male Sprague-Dawley (SD) rats. UGE data over 24 h were normalized per 200 g body weight. Values are expressed as mean ± S.E.M. (n=3).

^c Values are shown by hSGLT1(IC₅₀)/hSGLT2(IC₅₀).

^d In case of **35a-4**, inhibitory activity against GLUT1 is IC₅₀ > 10000 nM.

- ✓ Indole-N-glucoside **35a-4** is a highly potent and selective inhibitor of hSGLT2 and remarkably increases rUGE, which is comparable to that of aryl-C-glucoside **5**.
- ✓ Compound **35a-4** was selected as a clinical candidate (TA-1887/JNJ-39933673).

第 5 章 TA-1887 (35a-4) のプロファイル

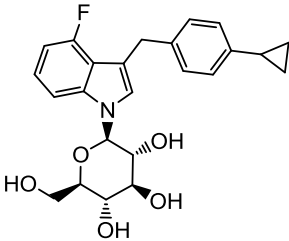
インドール-*N*-グルコシド化合物 **35a-4** のラット PK 試験の結果を図表 5-1 に示す。

化合物 **35a-4** は、インドールリード化合物 **13d** (図表 3-8) と比較して血中暴露の改善が確認され (e.g. **35a-4**: $AUC_{0-inf} = 28 \mu\text{g} \cdot \text{h/mL}$, BA = 78%、**13d**: $AUC_{0-inf} = 17 \mu\text{g} \cdot \text{h/mL}$, BA = 59%、10 mg/kg、p.o.)、rUGE 促進作用の増強を支持する結果であった。なお、化合物 **35a-4** が加水分解を受けた場合に生じるインドールアグリコンは血中に観察されず、また第 2 章 (第 1 節) でアニリン化合物 **8a** が速やかな加水分解を受けた酸性条件 (0.5 N、HCl、37 °C) において安定であることから、C-N グルコシド結合の加水分解に対する優れた安定性が確認された⁴¹⁾。リード化合物 **13d** 同様に C-N グルコシド結合の物理化学的および代謝的安定性の向上が、優れた血中暴露や強力な経口活性に寄与したと推察している。

【図表 5-1】

35a-4のラット薬物動態

Pharmacokinetic parameters of **35a-4** in SD rats

 35a-4	Dose (mg/kg)	3	10
	route	i.v.	p.o.
	$t_{1/2}$ (h)	3.9	3.9
	t_{max} (h)		1.5
	C_{max} ($\mu\text{g/mL}$)		2.7
	AUC_{0-inf} ($\mu\text{g} \cdot \text{h/mL}$)	11	28
	CL_{tot} (L/h/kg)	0.28	
	Vd_{ss} (L/kg)	1.1	
	F (%)		78

Values are the mean of two rats.

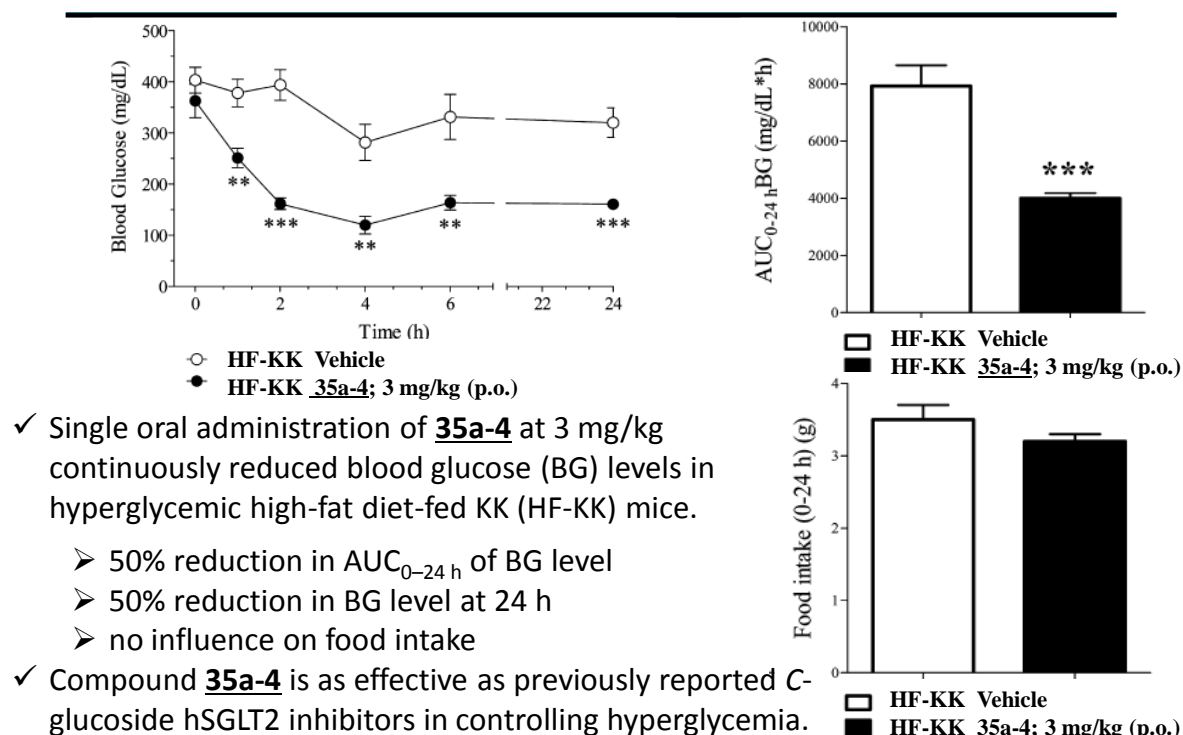
- ✓ We suggest that **35a-4** induces large rUGE through continuous suppression of renal glucose reuptake, which is supported by excellent pharmacokinetic properties as well as high potency of SGLT2 inhibition.
- ✓ No aglycon was observed in the pharmacokinetic studies of **35a-4** in rats.

次に、糖尿病モデル動物である HF-KK マウス (hyperglycemic high-fat diet-fed KK mice) における血糖降下作用を評価した (図表 5-2)。インドール-*N*-グルコシド化合物 **35a-4** を 3 mg/kg の投与量で経口投与したところ、摂餌量に影響することなく持続的な血糖降下作用を示した。血中グルコース (BG) 濃度の AUC_{0-24h} は媒体群より 50%低減し、24 時間後においても媒体群の 50%低減を維持し続けていることが判明した。なお、この実験時とは異なるが、化合物 **35a-4** (1 mg/kg) をマウスに経口投与した際の予備的な血中薬物濃度測定において、投与後 24 時間後においても血中薬物濃度の持続が確認されていることから、このような血糖降下作用の持続は妥当なものと考えている。

これらのことから、化合物 **35a-4** の強力な糖尿病治療薬としての可能性を示すことが出来た。

【図表 5-2】

35a-4のHF-KKマウスにおける血糖降下作用



- ✓ Single oral administration of **35a-4** at 3 mg/kg continuously reduced blood glucose (BG) levels in hyperglycemic high-fat diet-fed KK (HF-KK) mice.
 - 50% reduction in AUC_{0-24h} of BG level
 - 50% reduction in BG level at 24 h
 - no influence on food intake
- ✓ Compound **35a-4** is as effective as previously reported C-glucoside hSGLT2 inhibitors in controlling hyperglycemia.

Data are expressed as the mean ± SEM (N = 6): **P < 0.01, ***P < 0.001 vs. vehicle.

第 6 章 総括

2 型糖尿病患者の治療目標は、血糖値を正常レベルに厳格に管理することにある。しかしながら現在でも多くの患者で血糖管理目標を達成するには至っておらず、既存の血糖降下薬とは異なる新規なメカニズム、特にインスリン状態に依存しない治療薬の開発が望まれていた。その中で、hSGLT2 阻害薬は UGE を促進するメカニズムでインスリン非依存的に血糖降下作用を発揮することから注目を集め、精力的な研究開発が進められていた。

筆者は、世界的に研究が行われていた *O*-グルコシド、あるいは *C*-グルコシドとは異なり、新たなケミカルクラスとなる *N*-グルコシド型化合物に着目した。hSGLT2 選択的かつ経口投与で強力な薬効を示す化合物の探索研究を行い、臨床試験化合物 **35a-4** (TA-1887/JNJ-39933673) を創出することに成功した (図表 6-1)。

第 2 章では、アニリン-*N*-グルコシドの探索において、強力な hSGLT2 阻害作用を有する化合物 **8a** を見出した。血中暴露改善のために加水分解への抵抗性を獲得するべく、化合物 **8a** の窒素原子上のプロトネーションを抑制する方針を立て、アニリンのフェニル基上に電子吸引基の導入を行った。その結果、臨床試験化合物である T-1095 (**3b**) に匹敵する rUGE 促進作用を有する化合物 **8d** を見出した。

第 3 章では、更に強力な *in vivo* 薬効を目指して、窒素原子へのプロトネーションを完全に回避する方針を立て、ヘテロアリール-*N*-グルコシドの探索を行った。その結果、*C*-グルコシド化合物に匹敵する強力な rUGE 促進作用を有するインドール-*N*-グルコシド **13d** を見出した。合成にあたっては、含窒素ヘテロアリール環の直接的な *N*-グルコシド化の手法として、BSA および TMSOTf を用いた活性化法を適用し、既に報告されているベンズイミダゾールチオン以外の多様な含窒素ヘテロアリール環にも適用可能であることを示した。

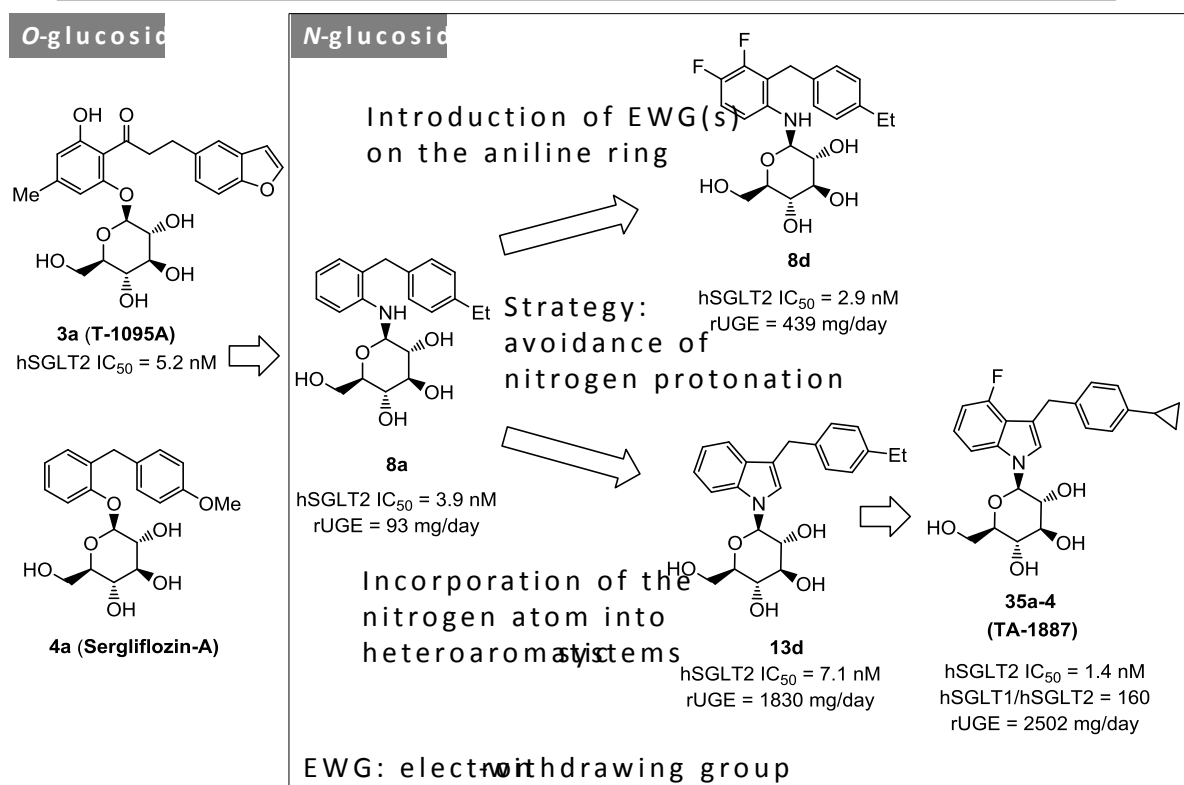
第 4 章では、インドール-*N*-グルコシド **13d** をリード化合物とした周辺置換基の最適化研究を行った。構造活性相関検討の結果、選択的かつ強力な hSGLT2 阻害作用、および *C*-グルコシドに匹敵する rUGE 促進作用を有する化合物 **35a-4** を臨床試験候補化合物 (TA-1887/JNJ-39933673) として選抜した。合成にあたっては、インドール-*N*-グルコシド中間体 **30** のインドール環 3 位へのベンジル基導入に際して、4 つのアセチル基で保護されたグルコース部共存下で、それらと相互作用しうる試薬を用いるにもかかわらず、一般

性の高い簡便な手法を見出した。即ち、 AlCl_3 を用いた Friedel-Crafts 反応により 3-ベンゾイルインドール **31** に導く手法、あるいは Grignard 試薬を用いた 3-ホルミルインドール **32** のホルミル基への選択的反応によりカルビール **33** に導く手法を経て高収率で目的物 **34** を取得した。

第 5 章では、インドール-*N*-グルコシド **35a-4** がリード化合物 **13d** よりも更に優れた薬物動態プロファイルを有し、糖尿病モデル動物における持続的な血糖降下作用を確認した。これらのことから、化合物 **35a-4** の強力な 2 型糖尿病治療薬としての可能性を示した。

【図表 6 - 1】

構造展開のまとめ



本研究により、hSGLT2 阻害薬として強力な薬効を発揮するアリール-*C*-グルコシドに匹敵する新規なケミカルクラスとして、良好な薬物動態及び薬効プロファイルを有するインドール-*N*-グルコシドを見出すことが出来た。最近では、他のグループからグルコース部分をキシロースに置き換えた hSGLT1 および hSGLT2 の dual 阻害剤（インドール-*N*-キシ

ロシド)に関する報告⁴²⁾や、インドール環 3 位の置換基を修飾した hSGLT1 選択的阻害剤 (インドール-*N*-グルコシド) の報告がなされ⁴³⁾、インドール-*N*-グリコシド化合物が hSGLT 阻害薬の基本骨格として適用されつつある。

さらに今回の研究結果は、インドールを含む各種ヘテロアリアル-*N*-グルコシドが、アリアル-*C*-グルコシドの生物学的等価体として適用できる可能性を示唆している。例えば、アリアル-*C*-グルコシドの中には様々な生物活性を有する化合物が報告されていることから⁴⁴⁾、それらをリード化合物とした薬剤開発において、ヘテロアリアル-*N*-グルコシドが新たな構造展開のオプションとして適用されることが期待できる。

実験の部

Chemistry

All reactions were carried out under inert gas or with CaCl_2 tube and reaction mixtures were stirred magnetically. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise noted. Reaction products were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F254) and were visualized using UV light or 5% phosphomolybdic acid in 95% EtOH. Silica gel column chromatography was performed on Silica gel BW-300 (Fuji Silicia) or NH-silica gel Chromatorex (Fuji Silicia), using the solvent systems (volume ratios) indicated below. NMR spectra were collected on JEOL JNM-ECX400P and Varian UNITY INOVA500 spectrometers. Chemical shifts were given in parts per million (ppm) downfield from internal reference tetramethylsilane standard; coupling constants (J value) were given in hertz (Hz). Melting points were measured by BÜCHI Melting Point B-545 and were uncorrected. APCI-MS spectra were obtained on Finnigan MAT SSQ7000C or ThermoQuest LCQ Advantage eluting with 10 mM $\text{AcONH}_4/\text{MeOH}$. GC-MS spectra were measured on Shimadzu GCMS-QP2010. Analytical HPLC spectra were reported using Agilent 1100 with a UV detector measuring absorbance at 210 nm.

2-(4-Ethylphenylmethyl)-*N*-(β -D-glucopyranosyl)aniline (**8a**).

A solution of 1-bromo-4-ethylbenzene **9** (6.43 g, 34.74 mmol) in tetrahydrofuran (50 mL) was cooled to -78 °C under argon atmosphere, and thereto was added dropwise *n*-butyl lithium (2.6 M hexane solution, 14.0 mL, 36.4 mmol). The mixture was stirred at the same temperature for 30 minutes, and the reaction solution was added dropwise to a solution of *o*-nitrobenzaldehyde (5.0 g, 33.09 mmol) in tetrahydrofuran (50 mL) at -78 °C. The mixture was stirred at the same temperature for 30 minutes, and warmed to 0 °C over a period of 1 hour. To the mixture was added an aqueous ammonium chloride solution, and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 10:1) to give (4-ethylphenyl)(2-nitrophenyl)methanol **10** (2.26g, 27%) as a colorless oil. APCI-Mass *m/z* 275 (M+NH₄). ¹H NMR (CDCl₃) δ 1.21 (t, *J* = 7.6 Hz, 3H), 2.63 (q, *J* = 7.7 Hz, 2H), 2.71 (d, *J* = 4.5 Hz, 1H), 6.41 (d, *J* = 4.4 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 1H).

To a stirred solution of (4-ethylphenyl)(2-nitrophenyl)methanol **10** (1.85 g, 7.19 mmol) in ethanol (74 mL) was added wet palladium-carbon (10%) (370 mg). The mixture was stirred at room temperature under hydrogen atmosphere for 4 hours. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The resultant residue was dissolved in acetonitrile (45 mL), and thereto was added dropwise boron trifluoride diethyl etherate (1.71 mL, 13.46 mmol) at -30 °C. Triethylsilane (2.15 mL, 13.46 mmol) was added at -30 °C, and the mixture was stirred at the same temperature for 1 hour. The mixture was warmed to 0 °C over a period of 30 minutes, and was further stirred at room temperature for 1.5 hour. To the mixture was added a saturated aqueous sodium hydrogen carbonate solution, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 24:1) to give 2-(4-ethylphenylmethyl)aniline **11a** (1.07 g, 75%) as a colorless oil. APCI-Mass *m/z* 212 (M+H). ¹H NMR (CDCl₃) δ 1.21 (t, *J* = 7.7 Hz, 3H), 2.61 (q, *J* = 7.7 Hz, 2H), 3.51 (s, 2H), 3.87 (s, 2H), 6.66 (d, *J* = 7.7 Hz, 1H), 6.76 (t, *J* = 7.5 Hz, 1H), 7.04–7.13 (m, 6H).

To a stirred solution of 2-(4-Ethylphenylmethyl)aniline **11a** (500 mg, 2.37 mmol) in methanol (5 mL) were added D-(+)-glucose (516 mg, 2.84 mmol) and ammonium chloride (25 mg, 0.474

mmol), and the mixture was heated under reflux for 2 hours. Methanol was evaporated under reduced pressure, and thereto was added water. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was evaporated. The resultant residue was purified by silica gel column chromatography (chloroform : methanol = 40:1 – 20:1) to give 2-(4-phenylmethyl)-*N*-(β -D-glucopyranosyl)aniline **8a** (495 mg, 56%) as colorless crystals. APCI-Mass m/z 374 (M+H). ^1H NMR (DMSO- d_6) δ 1.16 (t, $I = 7.7$ Hz, 3H), 2.56 (q, $J = 7.6$ Hz, 2H), 3.07–3.34 (m, 4H), 3.37–3.48 (m, 1H), 3.62–3.70 (m, 1H), 3.81 (s, 2H), 4.27 (t, $J = 7.3$ Hz, 1H)(changed to δ 4.31 (d, $J = 8.4$ Hz, 1H) when treated with D₂O.), 4.45 (t, $J = 5.8$ Hz, 1H), 4.91 (d, $J = 5.1$ Hz, 1H), 4.99 (d, $J = 4.4$ Hz, 1H), 5.01 (d, $J = 5.1$ Hz, 1H), 5.22 (d, $J = 6.4$ Hz, 1H), 6.62 (t, $J = 7.3$ Hz, 1H), 6.75 (d, $J = 7.7$ Hz, 1H), 6.86 (d, $J = 7.5$ Hz, 1H), 7.03 (t, $J = 7.7$ Hz, 1H), 7.12 (s, 4 H).

2-(4-Ethylphenylmethyl)-4-trifluoromethyl-*N*-(β -D-glucopyranosyl)aniline (**8b**).

A mixture of zinc powder (817 mg, 12.5 mmol) and 1,2-dibromoethane (0.044 mL, 0.511 mmol) in dimethylformamide (3 mL) was stirred with heating at 70 °C for 10 minutes. The reaction solution was cooled to room temperature, and thereto was added chlorotrimethylsilane (0.050 mL, 0.394 mmol), and further stirred for 30 minutes. To the mixture was added dropwise a solution of 4-ethylbenzyl bromide (1.99 g, 10 mmol) in dimethylformamide (10 mL) at 0 °C over a period of 2 hours. The mixture was stirred at the same temperature for 2 hours. The resultant mixture was poured to the mixture of tris(dibenzylideneacetone)palladium(0) (140 mg, 0.25 mmol), tri(2-furyl)phosphine (120 mg, 0.5 mmol) and 4-amino-3-iodobenzo trifluoride **12b** (1.44 g, 5 mmol) in tetrahydrofuran (30 mL), and the mixture was stirred overnight at room temperature under argon atmosphere. The reaction mixture was poured into water, and was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 9:1) to give 2-(4-ethylphenylmethyl)-4-trifluoromethylaniline **11b** (866 mg, 62%) as a colorless oil. APCI-Mass m/z 280 (M+H). ^1H NMR (CDCl₃) δ 1.22 (t, $J = 7.5$ Hz, 3H), 2.62 (q, $J = 7.5$ Hz, 2H), 3.79 (s, 2H), 3.89 (s, 2H), 6.67 (d, $J = 8.8$ Hz, 1H), 7.07 (d, $J = 8.4$ Hz, 2H), 7.13 (d, $J = 8.4$ Hz, 2H), 7.3–7.35 (br, 2H).

8b was prepared in a manner similar to **8a** from the corresponding aniline **11b** (26.3%): APCI-Mass m/z 442 (M+H). ^1H NMR (DMSO- d_6) δ 1.16 (t, $J = 7.5$ Hz, 3H), 2.57 (q, $J = 7.5$ Hz, 2H), 3.09–3.18 (m, 1H), 3.22–3.32 (m, 3H), 3.38–3.48 (m, 1H), 3.63–3.71 (m, 1H), 3.85 (d, $J =$

15.9 Hz, 1H), 3.93 (d, $J = 15.9$ Hz, 1H), 4.38 (t, $J = 7.3$ Hz, 1H) (changed to δ 4.41 (d, $J = 8.4$ Hz, 1H) when treated with D₂O.), 4.46 (t, $J = 5.9$ Hz, 1H), 4.94 (d, $J = 5.1$ Hz, 1H), 5.03 (d, $J = 4.4$ Hz, 1H), 5.04 (d, $J = 5.5$ Hz, 1H), 5.87 (d, $J = 6.6$ Hz, 1H), 6.86 (d, $J = 8.6$ Hz, 1H), 7.12 (s, 1H), 7.15 (s, 4H), 7.35 (d, $J = 8.6$ Hz, 1H),

2-(4-Ethylphenylmethyl)-4-fluoro-*N*-(β -D-glucopyranosyl)aniline (8c)

3,4-Difluoro-2-(4-ethylphenylmethyl)-*N*-(β -D-glucopyranosyl)aniline (8d)

11c, **8c**, **11d** and **8d** were prepared in a manner similar to **8b** from the corresponding 2-iodoaniline **12**²⁷⁾.

11c (64.1%): APCI-Mass m/z 230 (M+H). ¹H NMR (CDCl₃) δ 1.22 (t, $J = 7.5$ Hz, 3H), 2.62 (q, $J = 7.5$ Hz, 2H), 3.39 (s, 2H), 3.83 (s, 2H), 6.56–6.62 (m, 1H), 6.77 (d, $J = 8.6$ Hz, 1H), 6.8 (dd, $J = 8.2$ and 4.0 Hz, 1H), 7.08 (d, $J = 8.2$ Hz, 2H), 7.14 (d, $J = 8.2$ Hz, 2H).

8c (23.3%): APCI-Mass m/z 392 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.16 (t, $J = 7.5$ Hz, 3H), 2.57 (q, $J = 7.5$ Hz, 2H), 3.08–3.14 (m, 1H), 3.17–3.28 (m, 3H), 3.39–3.45 (m, 1H), 3.64–3.69 (m, 1H), 3.79 (d, $J = 15.7$ Hz, 1H), 3.85 (d, $J = 15.6$ Hz, 1H), 4.23 (t, $J = 7.6$ Hz, 1H) (changed to δ 4.24 (d, $J = 8.4$ Hz, 1H) when treated with D₂O), 4.45 (t, $J = 5.8$ Hz, 1H), 4.91 (d, $J = 5.3$ Hz, 1H), 4.98 (d, $J = 3.5$ Hz, 1H), 5.04 (d, $J = 5$ Hz, 1H), 5.2 (d, $J = 6.6$ Hz, 1H), 6.66 (dd, $J = 9.8$ and 3.0 Hz, 1H), 6.74 (dd, $J = 9.0$ and 5.1 Hz, 1H), 6.86 (td, $J = 8.6$ and 2.9 Hz, 1H), 7.15 (s, 4H).

11d (48.9%): APCI-Mass m/z 247 (M+H). ¹H NMR (CDCl₃) δ 1.21 (t, $J = 7.5$ Hz, 3H), 2.61 (q, $J = 7.5$ Hz, 2H), 3.45 (s, 2H), 3.94 (s, 2H), 6.34 (ddd, $J = 8.9$, 4.0 and 2.0 Hz, 1H), 6.87 (dt, $J = 10.1$ and 8.8 Hz, 1H), 7.11 (s, 4H),

8d (23%): APCI-Mass m/z 410 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.14 (t, $J = 7.6$ Hz, 3H), 2.54 (q, $J = 7.7$ Hz, 2H), 3.08–3.12 (m, 1H), 3.17–3.27 (m, 3H), 3.39–3.44 (m, 1H), 3.65 (d, $J = 11.9$ Hz, 1H), 3.86 (d, $J = 15.9$ Hz, 1H), 4.03 (d, $J = 16$ Hz, 1H), 4.23 (t, $J = 7.7$ Hz, 1H) (changed to δ 4.23 (d, $J = 8.5$ Hz, 1H) when treated with D₂O), 4.43–4.50 (br, 1H), 4.89–5.02 (br, 3H), 5.46 (d, $J = 6.4$ Hz, 1H), 6.56 (dd, $J = 9.2$ and 2.9 Hz, 1H), 7.07–7.16 (m, 5H).

4-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)pyrazole (**13a**).

A suspension of 4-ethylbenzyl bromide **15** (10.0 g, 50.2 mmol), malononitrile (6.64 g, 101 mmol), potassium carbonate (6.94 g, 50.2 mmol) and tetra-*n*-butylammonium bromide (648 mg, 2.01 mmol) in toluene (100 mL) was vigorously stirred at room temperature for 17 hours. The reaction mixture was poured into water, and was extracted with ethyl acetate twice. The organic layer was washed successively with water and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 6:1) to give 2-(4-ethylphenylmethyl)malononitrile (3.28 g, 35.4%) as a colorless solid. A solution of 2-(4-ethylphenylmethyl)malononitrile (3.27 g, 17.7 mmol) and hydrazine hydrate (0.86 mL, 17.7 mmol) in ethanol (35 mL) was heated under reflux for 4 hours. Hydrazine hydrate (0.43 mL, 8.86 mmol) was further added thereto and the mixture was further heated under reflux for 4 hours. The reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The resultant residue was crystallized from ethyl acetate–diethyl ether to give 3,5-diamino-4-(4-ethylphenylmethyl)pyrazole **16** (2.63 g, 68.6%) as pale pink powder. APCI-Mass m/z 217 ($M+H$). 1H NMR (DMSO- d_6) δ 1.13 (t, $I = 7.7$ Hz, 3H), 2.53 (q, $J = 7.6$ Hz, 2H), 3.44 (s, 2H), 4.21 (br, 4H), 7.04 (d, $J = 8.1$ Hz, 2 H), 7.11 (d, $J = 8.1$ Hz, 2 H), 9.95 (br, 1H).

3,5-Diamino-4-(4-ethylphenylmethyl)pyrazole **16** (1.30 g, 6.01 mmol) was added to a 50% aqueous phosphoric acid solution (19 mL, 183 mmol), and thereto was further added water (10 mL). The stirred mixture was cooled to 0 °C, and thereto was added dropwise an aqueous solution (4 mL) of sodium nitrite (912 mg, 13.2 mmol) over 3 minutes. The mixture was stirred at the same temperature for 30 minutes, and further stirred at room temperature for 4 hours. The reaction mixture was cooled again to 0 °C, and 10% aqueous sodium hydroxide solution was added thereto to adjust pH to 7. The mixture was extracted with ethyl acetate, washed successively with water and brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (chloroform : methanol = 100:0 – 90:10) to give 4-(4-ethylphenylmethyl)pyrazole **14a** (414 mg, 37%) as a pale brown solid. APCI-Mass m/z 187 ($M+H$). 1H NMR (DMSO- d_6) δ 1.14 (t, $J = 7.7$ Hz, 3H), 2.55 (q, $J = 7.6$ Hz, 2H), 3.72 (s, 2H), 7.1 (s, 4H), 7.3 (br, 1H), 7.46 (br, 1H), 12.54 (br, 1H).

To a solution of 4-(4-ethylphenylmethyl)pyrazole **14a** (495 mg, 2.66 mmol) in acetonitrile (2.0 mL) was added *N,O*-bis(trimethylsilyl)acetamide (>75%) (1.05 mL, ca. 3.19 mmol), and the

mixture was stirred under heating at 60 °C for 2.5 hours under argon atmosphere. The reaction mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. The resultant residue was dissolved in dichloroethane (7.0 mL), and added thereto were molecular sieve 4A powder (500 mg), 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranose **25** (1.04 g, 2.66 mmol) and trimethylsilyl trifluoromethanesulfonate (0.51 mL, 2.82 mmol). The mixture was stirred under heating at 80 °C for 3 hours under argon atmosphere. The reaction mixture was cooled to room temperature, and insoluble materials were filtered off. Subsequently, the filtrate was poured into a saturated aqueous sodium hydrogen carbonate solution. The mixture was extracted twice with dichloromethane, and the organic layer was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 80:20 – 50:50) to give 4-(4-ethylphenylmethyl)-1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)pyrazole **27a** (610 mg, 44%) as a colorless solid. APCI-Mass m/z 517 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.15 (t, J = 7.7 Hz, 3H), 1.75 (s, 3H), 1.94 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.55 (q, J = 7.7 Hz, 2H), 3.71 (s, 2H), 4.02 (dd, J = 12.2 and 2.1 Hz, 1H), 4.12 (dd, J = 12.5 and 5.5 Hz, 1H), 4.21–4.24 (m, 1H), 5.06 (t, J = 9.8 Hz, 1H), 5.44 (t, J = 9.6 Hz, 1H), 5.53 (t, J = 9.5 Hz, 1H), 5.86 (d, J = 9.3 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 7.36 (s, 1H), 7.68 (s, 1H).

To a stirred solution of 4-(4-ethylphenylmethyl)-1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)pyrazole **27a** (604 mg, 1.17 mmol) in methanol (6 mL) was added sodium methoxide (28% methanol solution, 1 drop). After being stirred at room temperature for 30 minutes, the solvent was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (chloroform : methanol = 100:0 – 80:20) to give 4-(4-ethylphenylmethyl)-1-(β -D-glucopyranosyl)pyrazole **13a** (319 mg, 78.3%) as colorless viscous oil. APCI-Mass m/z 433 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.15 (t, J = 7.7 Hz, 3H), 2.55 (q, J = 7.7 Hz, 2H), 3.11–3.16 (m, 1H), 3.27–3.34 (m, 2H), 3.37–3.42 (m, 1H), 3.63–3.73 (m, 2H), 3.71 (s, 2H), 4.45 (t, J = 5.8 Hz, 1H), 5.01 (d, J = 5.5 Hz, 1H), 5.07 (d, J = 9.2 Hz, 1H), 5.08 (d, J = 4.8 Hz, 1H), 5.1 (d, J = 4.8 Hz, 1H), 7.11 (d, J = 8 Hz, 2H), 7.14 (d, J = 8 Hz, 2H), 7.32 (s, 1H), 7.6 (s, 1H).

5-(4- Ethylphenylmethyl)-1-(β -D-glucopyranosyl)pyridin-2(1H)-one (**13b**).

A solution of the *N*-methoxy-*N*-methyl-6-chloronicotineamide **17b**²⁹⁾ (4.2 g, 20.93 mmol) in tetrahydrofuran (40 mL) was cooled to 0 °C, and thereto was added dropwise 4-ethylphenylmagnesium bromide (0.5 M tetrahydrofuran solution, 55 mL, 27.5 mmol). The

mixture was stirred at 0 °C for 4 hours, and then at the room temperature for 10 minutes. The reaction mixture was cooled again to 0 °C, and added thereto was 10% aqueous hydrochloric acid. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 20:1) to give 6-chloro-3-pyridyl 4-ethylphenyl ketone **18b** (3.68 g, 72.2%) as colorless crystals. APCI-Mass m/z 246/248 (M+H). ^1H NMR (DMSO- d_6) δ 1.23 (t, J = 7.5 Hz, 3H), 2.72 (q, J = 7.7 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.2 Hz, 2H), 8.15 (dd, J = 8.4 and 2.6 Hz, 1H), 8.7 (d, J = 2.3 Hz, 1H).

To a stirred solution of 6-chloro-3-pyridyl 4-ethylphenyl ketone **18b** (1.68 g, 6.84 mmol) in *N,N*-dimethylformamide (20 mL) were added benzylalcohol (815 mg, 7.54 mmol) and 60% sodium hydride (275 mg, 6.88 mmol). The mixture was stirred at room temperature for 6 hours, and then at 90 °C for 1 hour. The reaction mixture was cooled to room temperature, and the mixture was quenched with water. The mixture was extracted with ethyl acetate. The organic layer was washed with water and subsequently with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 100:0 – 95:5) to give 6-benzyloxy-3-pyridyl 4-ethylphenyl ketone **19b** (1.68 g, 77.3%) as colorless oil. APCI-Mass m/z 318 (M+H). ^1H NMR (DMSO- d_6) δ 1.23 (t, J = 7.7 Hz, 3H), 2.72 (q, J = 7.6 Hz, 2H), 5.47 (s, 2H), 7.04 (d, J = 8.7 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.4 (t, J = 6.9 Hz, 2H), 7.42 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 7.1 Hz, 2H), 7.7 (d, J = 8.2 Hz, 2H), 8.09 (dd, J = 8.7 and 2.4 Hz, 1H), 8.55 (d, J = 2.2 Hz, 1H).

To a stirred solution of 6-benzyloxy-3-pyridyl 4-ethylphenyl ketone (865 mg, 2.73 mmol) in ethylene glycol (8.5 mL) were added hydrazine hydrate (0.44 mL, 8.99 mmol) and potassium hydroxide (550 mg, 9.8 mmol). The mixture was stirred under heating at 190 °C for 8 hours. The reaction mixture was cooled to room temperature, and water was added thereto. The mixture was extracted with ethyl acetate. The organic layer was washed with water three times and subsequently with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 100:0 – 0:100) to give 3-(4-ethylphenylmethyl)-6-hydroxypyridine **14b** (256 mg, 44.2%) as colorless powder. APCI-Mass m/z 214 (M+H). ^1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.7 Hz, 3H), 2.55 (q, J = 7.5 Hz, 2H), 3.6 (s, 2H), 6.25 (d, J = 9.3 Hz, 1H), 7.12 (d, J = 9.3 Hz, 4H), 7.18 (d, J = 2.1 Hz, 1H), 7.25 (dd, J = 9.5 and 2.6 Hz, 1H), 11.38 (br, 1H).

27b and **13b** were prepared in a manner similar to **13a** from **14b**.

27b (37.1%): APCI-Mass m/z 544 (M+H). ^1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.6 Hz, 3H), 1.82 (s, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.55 (q, J = 7.4 Hz, 2H), 3.6 (d, J = 14.9 Hz, 1H), 3.64 (d, J = 14.9 Hz, 1H), 4.07 (dd, J = 12.2 and 5.6 Hz, 1H), 4.12 (dd, J = 12.5 and 2.6 Hz, 1H), 4.3–4.33 (m, 1H), 5.24 (t, J = 9.8 Hz, 1H), 5.47 (t, J = 9.3 Hz, 1H), 5.55 (t, J = 9.5 Hz, 1H), 6.3 (d, J = 9.5 Hz, 1H), 6.39 (d, J = 9.2 Hz, 1H), 7.12 (s, 4H), 7.16 (dd, J = 9.5 and 2.4 Hz, 1H), 7.87 (s, 1H).

13b (81.9%): APCI-Mass m/z 376 (M+H). ^1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.5 Hz, 3H), 2.55 (q, J = 7.6 Hz, 2H), 3.16–3.21 (m, 1H), 3.31–3.38 (m, 2H), 3.41–3.52 (m, 2H), 3.66 (s, 2H), 3.66–3.72 (m, 1H), 4.57 (t, J = 5.9 Hz, 1H), 5.07 (d, J = 5.6 Hz, 1H), 5.18 (d, J = 5.9 Hz, 1H), 5.2 (d, J = 4.5 Hz, 1H), 5.74 (d, J = 9.3 Hz, 1H), 6.33 (d, J = 9.5 Hz, 1H), 7.12 (d, J = 8.3 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H), 7.2 (dd, J = 9.5 and 2.5 Hz, 1H), 7.56 (d, J = 1.9 Hz, 1H).

3-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)pyridin-2(1H)-one (13c).

18c and **19c** were prepared in a manner similar to **19b** from **17c**³⁰.

18c (78%): APCI-Mass m/z 246/248 (M+H). ^1H NMR (DMSO- d_6) δ 1.21 (t, J = 7.5 Hz, 3H), 2.71 (q, J = 7.7 Hz, 2H), 7.42 (d, J = 8.2 Hz, 2H), 7.61 (dd, J = 7.5 and 4.8 Hz, 1H), 7.68 (d, J = 8.3 Hz, 2H), 8.03 (dd, J = 7.5 and 1.9 Hz, 1H), 8.61 (dd, J = 5.0 and 1.9 Hz, 1H).

19c (41%): APCI-Mass m/z 318 (M+H). ^1H NMR (DMSO- d_6) δ 1.21 (t, J = 7.6 Hz, 3H), 2.69 (q, J = 7.6 Hz, 2H), 5.34 (s, 2H), 7.02–7.05 (m, 2H), 7.17–7.21 (m, 4H), 7.35 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 7.86 (dd, J = 7.2 and 1.8 Hz, 1H), 8.37 (dd, J = 5.0 and 1.8 Hz, 1H).

To a stirred solution of 2-benzyloxy-3-pyridyl 4-ethylphenyl ketone **19c** (1.69 g, 5.32 mmol) in ethanol (15 mL) was added sodium borohydride (403 mg, 10.65 mmol), and the mixture was stirred at room temperature for 3 hours. The solvent was evaporated under reduced pressure, and to the resultant residue was added ethyl acetate. The mixture was washed with water and successively with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure to give crude 2-benzyloxy-3-pyridyl-4-ethylphenylmethanol as a colorless oil. The resultant residue was dissolved in methanol (10 mL), and thereto were added concentrated hydrochloric acid (1.0 mL) and 10% palladium-carbon (500 mg). The mixture was stirred at

room temperature for 15 hours under hydrogen atmosphere. Insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The resultant residue was dissolved in ethyl acetate, washed with water and successively with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (chloroform : methanol = 100:0 – 97:3) to give 3-(4-ethylphenylmethyl)-2-hydroxypyridine **14c** (307 mg, 27%) as a pale brown solid. APCI-Mass m/z 214 (M+H). ^1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.7 Hz, 3H), 2.55 (q, J = 7.5 Hz, 2H), 3.64 (s, 2H), 6.09 (t, J = 7.3 Hz, 1H), 7.1 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8 Hz, 2H), 7.16 (dd, J = 6.7 and 1.9 Hz, 1H), 7.22 (dd, J = 6.4 and 1.8 Hz, 1H), 11.5 (s, 1H),

27c and **13c** were prepared in a manner similar to **13a** from **14c**.

27c (4.1%): APCI-Mass m/z 544 (M+H). ^1H NMR (DMSO- d_6) δ 1.12 (t, J = 7.6 Hz, 3H), 1.62 (s, 3H), 1.94 (s, 3H), 2 (s, 3H), 2.01 (s, 3H), 2.5 (q, J = 7.7 Hz, 2H), 3.63 (d, J = 14.5 Hz, 1H), 3.69 (d, J = 14.3 Hz, 1H), 4.06 (br, 2H), 4.28–4.31 (m, 1H), 5.2 (t, J = 9.8 Hz, 1H), 5.32 (t, J = 9.5 Hz, 1H), 5.51 (t, J = 9.6 Hz, 1H), 6.21 (t, J = 6.9 Hz, 1H), 6.42 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 8 Hz, 2H), 7.12 (d, J = 8 Hz, 2H), 7.23 (d, J = 5.5 Hz, 1H), 7.7 (d, J = 5.5 Hz, 1H).

13c (48.2%): APCI-Mass m/z 376 (M+H). ^1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.7 Hz, 3H), 2.55 (q, J = 7.6 Hz, 2H), 3.14–3.19 (m, 1H), 3.29 (s, 2H), 3.38–3.46 (m, 2H), 3.65 (d, J = 14.9 Hz, 1H), 3.65–3.70 (m, 1H), 3.69 (d, J = 14.8 Hz, 1H), 4.54 (t, J = 5.9 Hz, 1H), 5.05 (d, J = 5.6 Hz, 1H), 5.18 (d, J = 5.8 Hz, 1H), 5.18 (d, J = 4.7 Hz, 1H), 5.8 (d, J = 9.3 Hz, 1H), 6.18 (t, J = 6.9 Hz, 1H), 7.1 (d, J = 8 Hz, 2H), 7.15 (d, J = 7.9 Hz, 2H), 7.14–7.16 (d, J = 6.9 Hz, 1H), 7.49 (dd, J = 6.9 and 1.6 Hz, 1H).

3-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)indole (**13d**)

To a solution of indole **20** (6.00 g, 51.21 mmol) in methanol (60 mL) were added sodium hydroxide (2.25 g, 56.34 mmol) and 4-ethylbenzaldehyde (7.56 g, 56.34 mmol), and the mixture was stirred at room temperature for 3 days under argon atmosphere. Water was added, and methanol was evaporated under reduced pressure. The resultant mixture was extracted with diethyl ether. The organic layer was washed with water and dried over magnesium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 98:2 – 70:30) to give 4-ethylphenyl-(1H-indol-3-yl)methanol **21** (2.10 g, 16.3%) as a colorless solid. APCI-Mass m/z

234 (M+H-H₂O). ¹H NMR (CDCl₃) δ 1.23 (t, *J* = 7.7 Hz, 3H), 2.64 (q, *J* = 7.5 Hz, 2H), 5.56 (s, 1H), 6.91 (d, *J* = 2.6 Hz, 1H), 7.07 (t, *J* = 7 Hz, 1H), 7.16 (t, *J* = 8 Hz, 1H), 7.17 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 1H), 8.01 (s, 1H).

To a stirred solution of 4-ethylphenyl-(1H-indol-3-yl)methanol **21** (2.1 g, 8.36 mmol) in dichloromethane (60 mL) were added triethylsilane (3.99 mL, 25 mmol) and boron trifluoride diethyl etherate (3.17 mL, 25 mmol) at -78 °C under argon atmosphere. The reaction mixture was gradually allowed to warm to 0 °C. To the mixture was added a saturated aqueous sodium hydrogen carbonate solution, and the mixture was extracted with dichloromethane. The organic layer was dried over magnesium sulfate, concentrated under reduced pressure and purified by silica gel column chromatography (hexane : ethyl acetate = 98:2 – 85:15) to give 3-(4-ethylphenylmethyl)-1H-indole **14d** (1.52g, 77.3%) as colorless crystals. APCI-Mass *m/z* 236 (M+H). ¹H NMR (CDCl₃) δ 1.21 (t, *J* = 7.5 Hz, 3H), 2.61 (q, *J* = 7.7 Hz, 2H), 4.08 (s, 2H), 6.9 (d, *J* = 2.4 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.1 (d, *J* = 8.2 Hz, 2H), 7.17 (t, *J* = 8.1 Hz, 1H), 7.2 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.9 (s, 1H).

27d and **13d** were prepared in a manner similar to **13a** from **14d**.

27d (17.1%): APCI-Mass *m/z* 583 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.13 (t, *J* = 7.5 Hz, 3H), 1.62 (s, 3H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.53 (q, *J* = 7.5 Hz, 2H), 3.95 (s, 2H), 4.08 (dd, *J* = 12.2 and 1.9 Hz, 1H), 4.13 (dd, *J* = 12.5 and 5.5 Hz, 1H), 4.29 (m, 1H), 5.21 (t, *J* = 9.6 Hz, 1H), 5.52 (t, *J* = 9.3 Hz, 1H), 5.56 (t, *J* = 9.5 Hz, 1H), 6.15 (d, *J* = 8.7 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 7.08 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 7.7 Hz, 1H), 7.25 (s, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H).

13d (48%): APCI-Mass *m/z* 415 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.14 (t, *J* = 7.5 Hz, 3H), 2.54 (q, *J* = 7.5 Hz, 2H), 3.20–3.48 (m, 4H), 3.63–3.73 (m, 2H), 3.97 (s, 2H), 4.51 (t, *J* = 5.5 Hz, 1H), 5.07 (d, *J* = 5.1 Hz, 1H), 5.15 (d, *J* = 5.0 Hz, 1H), 5.16 (d, *J* = 5.9 Hz, 1H), 5.36 (d, *J* = 9.0 Hz, 1H), 6.99 (t, *J* = 7.3 Hz, 1H), 7.08–7.12 (m, 3H), 7.21 (d, *J* = 7.9 Hz, 2H), 7.24 (s, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 1H).

3-(4-Ethylphenylmethyl)-2-(β-D-glucopyranosyl)-2H-indazole (13e)

3-(4-Ethylphenylmethyl)-1-(β-D-glucopyranosyl)-1H-indazole (13f)

A mixture of zinc powder (712 mg, 10.89 mmol) and dibromoethane (0.04 mL, 0.045 mmol) in *N,N*-dimethylformamide (2.5 mL) were stirred under heating at 70 °C for 10 minutes under argon atmosphere. The reaction mixture was cooled to room temperature, and chlorotrimethylsilane (0.04 mL, 0.343 mmol) was added thereto, and the mixture was stirred at room temperature for 30 minutes. To the activated zinc was added dropwise a solution of 4-ethylbenzyl bromide **15** (1.74 g, 8.72 mmol) in *N,N*-dimethylformamide (10 mL) at 0 °C over a period of 2 hours. Subsequently, the mixture was stirred at 0 °C for 2 hours(: mixture A).

A mixture of tris(dibenzylideneacetone)dipalladium (0) (167 mg, 0.29 mmol) and tri(2-furyl)phosphine (135 mg, 0.581 mmol) in tetrahydrofuran (20 mL) was stirred at room temperature for 5 minutes under argon atmosphere. Thereto were added 1-*t*-butoxycarbonyl-3-iodo-1H-indazole³²) (2.0 g, 5.81 mmol) and the above mixture A at 0 °C, and the mixture was stirred at room temperature for 5 hours. The reaction mixture was poured into water and extracted with diethyl ether. The organic layer was washed with water and dried over magnesium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 100:0 – 92:8) to give 1-*t*-butoxycarbonyl-3-(4-ethylphenylmethyl)-1H-indazole **23** (1.37 g, 70.2%) as a colorless syrup. APCI-Mass *m/z* 337 (M+H). ¹H NMR (CDCl₃) δ 1.2 (t, *J* = 7.5 Hz, 3H), 1.74 (s, 9H), 2.6 (q, *J* = 7.5 Hz, 2H), 4.33 (s, 2H), 7.1 (d, *J* = 8.2 Hz, 2H), 7.16 (t, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8 Hz, 1H), 7.44 (t, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 1H).

To a stirred solution of 1-*t*-butoxycarbonyl-3-(4-ethylphenylmethyl)-1H-indazole **23** (1.35 g, 4 mmol) in methanol (15 mL) was added 28% sodium methoxide solution (methanol solution, 1.0 mL), and the mixture was stirred at room temperature for 1 hour. Aqueous citric acid solution was added, and the mixture was extracted with ethyl acetate. The organic layer was washed successively with water and brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure, and the resultant residue was crystallized from hexane to give the desired 3-(4-ethylphenylmethyl)-1H-indazole **14e** (800 mg, 61.2%) as colorless crystals. APCI-Mass *m/z* 237 (M+H). ¹H NMR (CDCl₃) δ 1.21 (t, *J* = 7.7 Hz, 3H), 2.6 (q, *J* = 7.5 Hz, 2H), 4.32 (s, 2H), 7.07 (t, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.1 Hz, 2H), 7.33 (t, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 9.92 (br, 1H).

To a solution of 3-(4-ethylphenylmethyl)-1H-indazole **14e** (150 mg, 0.637 mmol) in acetonitrile (5.0 mL) was added *N,O*-bis(trimethylsilyl)acetamide(>75%) (225mg, ca. 0.828 mmol), and the mixture was stirred under heating at 60 °C for 3 hours under argon atmosphere. The reaction mixture was cooled to room temperature, and the solvent was evaporated under

reduced pressure. The resultant residue was dissolved in dichloroethane (5.0 mL), and added thereto were 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranose **25** (373 mg, 0.955 mmol) and trimethylsilyl trifluoromethanesulfonate (0.16 mL, 0.89 mmol). The mixture was stirred under heating at 80 °C for 3 hours under argon atmosphere. The reaction mixture was cooled to room temperature. Methanol (5 mL) and sodium methoxide (28% methanol solution, 3 mL) were added to the reaction mixture. After being stirred at room temperature for 30 minutes, aqueous ammonium chloride solution was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (chloroform : methanol = 100:0 – 88:12) to give a mixture of 3-(4-Ethylphenylmethyl)-2-(β -D-glucopyranosyl)indazole **13e** and 3-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)indazole **13f**. The mixture was crystallized from chloroform–diethyl ether to give 3-(4-Ethylphenylmethyl)-2-(β -D-glucopyranosyl)indazole **13e** (106 mg, 42%, 2 steps). The mother liquid was concentrated under reduced pressure, and the resultant residue was crystallized from diethyl ether to give 3-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)indazole **13f** (12 mg, 4.7%, 2 steps).

13e: APCI-Mass m/z 399 (M+H). ^1H NMR (DMSO- d_6) δ 1.13 (t, J = 7.5 Hz, 3H), 2.54 (q, J = 7.5 Hz, 2H), 3.25–3.29 (m, 1H), 3.36–3.45 (m, 3H), 3.54–3.57 (m, 1H), 4.15–4.20 (m, 1H), 4.17 (d, J = 14.6 Hz, 1H), 4.19 (d, J = 14.4 Hz, 1H), 4.19 (t, J = 6.1 Hz, 1H), 5.05 (d, J = 5.1 Hz, 1H), 5.11 (d, J = 5.3 Hz, 1H), 5.13 (d, J = 5.8 Hz, 1H), 5.61 (d, J = 8.8 Hz, 1H), 6.94 (t, J = 8.2 Hz, 1H), 7.09 (d, J = 7.9 Hz, 2H), 7.21 (t, J = 8.7 Hz, 1H), 7.23 (d, J = 7.7 Hz, 2H), 7.54 (d, J = 9.5 Hz, 2H)

13f: APCI-Mass m/z 399 (M+H). ^1H NMR (DMSO- d_6) δ 1.13 (t, J = 7.5 Hz, 3H), 2.54 (q, J = 7.5 Hz, 2H), 3.23–3.27 (m, 1H), 3.40–3.48 (m, 3H), 3.66–3.69 (m, 1H), 4.05–4.10 (m, 1H), 4.21 (d, J = 15.3 Hz, 1H), 4.24 (d, J = 15.1 Hz, 1H), 4.52 (t, J = 5.8 Hz, 1H), 5.06 (d, J = 5.6 Hz, 1H), 5.06 (d, J = 4.3 Hz, 1H), 5.08 (d, J = 5.1 Hz, 1H), 5.56 (d, J = 9 Hz, 1H), 7.08 (t, J = 7.6 Hz, 1H), 7.11 (d, J = 8 Hz, 2H), 7.24 (d, J = 7.9 Hz, 2H), 7.36 (t, J = 7.4 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.66 (d, J = 8.5 Hz, 1H).

3-(4-Ethylphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole (35a-1).

A mixture of 4-fluoroindoline **28a** (27.4 g, 200 mmol) and D-glucose (28.8 g, 160 mmol) in ethanol (550 mL)–water (55 mL) was refluxed for 20 hours under argon atmosphere, and to the mixture was added D-glucose (18.0 g, 99.9 mmol) followed by being refluxed for 18 hours. The solvent was evaporated under reduced pressure, and the resultant residue was dissolved in brine (300 mL). The mixture was extracted with ethyl acetate–tetrahydrofuran (2:1, 900 mL) twice and the with ethyl acetate (600 mL). After being dried over magnesium sulfate, the organic layer was treated with activated carbon. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The resultant residue was dissolved in chloroform (600 mL), and thereto were added acetic anhydride (94.4 mL, 999 mmol), pyridine (80.8 mL, 999 mmol) and 4-(dimethylamino)pyridine (1.22 g, 9.99 mmol). After the mixture was stirred at room temperature for 4 days, the solvent was evaporated under reduced pressure. The resultant residue was dissolved in ethyl acetate (2000 mL), and the solution was washed with successively 10% aqueous copper(II) sulfate solution (150 mL) 3 times, 10% aqueous citric acid solution (100 mL), water (150 mL) and saturated aqueous sodium hydrogen carbonate solution (150 mL). After being dried over magnesium sulfate with activated carbon, the insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residual solid was recrystallized from ethanol (340 mL) to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindoline **29a** (57.8 g, 61.8%) as pale yellow crystals: mp 129–131 °C. HPLC 98.8% (t_R = 3.05 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN/20 mM phosphate buffer (pH 6.5) (55/45)). APCI-Mass m/z 468 (M+H). ^1H NMR (DMSO- d_6) δ 1.93 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.83 (ddd, J = 15.5, 10.5 and 10.3 Hz, 1H), 2.99–3.05 (m, 1H), 3.49–3.57 (m, 2H), 3.95–3.99 (m, 1H), 4.07–4.11 (m, 2H), 4.95 (t, J = 9.5 Hz, 1H), 5.15 (t, J = 9.4 Hz, 1H), 5.42 (t, J = 9.6 Hz, 1H), 5.49 (d, J = 9.3 Hz, 1H), 6.48 (t, J = 8.6 Hz, 1H), 6.60 (d, J = 8.0 Hz, 1H), 7.05–7.10 (m, 1H).

A mixture of 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindoline **29a** (56.0 g, 120 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (29.9 g, 132 mmol) in 1,4-dioxane (560 mL)–water (28 mL) was stirred at room temperature for 3 days, and then the solvent was evaporated under reduced pressure. The resultant residue was basified with aqueous sodium hydrogen carbonate solution, and the mixture was extracted with ethyl acetate (400 mL) 4 times. The combined organic layer was washed with water (100 mL) and brine (100 mL). After being dried over magnesium sulfate, the mixture was treated with activated carbon. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residual

solid was recrystallized from ethanol (600 mL) to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindole **30a** (49.9 g, 89.3%) as a colorless crystals: mp 137–139 °C. HPLC 98.8% (t_R = 2.83 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (55/45)). APCI-Mass m/z 483 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.64 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 4.10 (dd, J = 12.4 and 2.7 Hz, 1H), 4.14 (dd, J = 12.4 and 5.2 Hz, 1H), 4.31 (ddd, J = 10.0, 5.2 and 2.7 Hz, 1H), 5.25 (t, J = 9.7 Hz, 1H), 5.53 (t, J = 9.5 Hz, 1H), 5.61 (t, J = 9.3 Hz, 1H), 6.22 (d, J = 9.0 Hz, 1H), 6.58 (d, J = 3.4 Hz, 1H), 6.88 (dd, J = 10.8 and 7.9 Hz, 1H), 7.19 (td, J = 8.1 and 5.3 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 3.4 Hz, 1H).

To a stirred solution of 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindole **30a** (301 mg, 0.647 mmol) and 4-ethylbenzoyl chloride (0.124 mL, 0.841 mmol) in dichloromethane (12 mL) was added aluminum chloride (431 mg, 3.23 mmol) at 0 °C. After being stirred at the same temperature for 1 hour, the resulting mixture was poured into ice–water (15 mL), and extracted with chloroform (40 mL) twice. The combined organic layer was washed with water (10 mL) and saturated aqueous sodium hydrogen carbonate solution (15 mL), and dried over magnesium sulfate. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane : ethyl acetate = 90:10 – 55:45) to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindol-3-yl 4-ethylphenyl ketone **31a-1** (378 mg, 97.8%) as colorless foam: APCI-Mass m/z 598 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.25 (t, J = 7.5 Hz, 3H), 1.69 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.73 (q, J = 7.5 Hz, 2H), 4.07–4.12 (m, 2H), 4.27–4.30 (m, 1H), 5.31 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.6 Hz, 1H), 5.77 (t, J = 9.3 Hz, 1H), 6.34 (d, J = 9.0 Hz, 1H), 7.03 (dd, J = 10.8 and 8.0 Hz, 1H), 7.38 (td, J = 8.2 and 5.1 Hz, 1H), 7.41 (d, J = 7.9 Hz, 2H), 7.63 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 8.2 Hz, 2H), 8.16 (s, 1H).

To a stirred solution of the above obtained 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindol-3-yl 4-ethylphenyl ketone **31a-1** (375 mg, 0.627 mmol) in ethyl alcohol (4 mL)–tetrahydrofuran (8 mL) were added cerium(III) chloride heptahydrate (701 mg, 1.88 mmol) and sodium borohydride (71.2 mg, 1.88 mmol) at 0 °C. After being stirred at the same temperature for 1 hour, to the mixture was added a 0.5 N aqueous hydrochloric acid solution, and the mixture was extracted with ethyl acetate (40 mL) twice. The combined organic layer was washed with a saturated aqueous sodium hydrogen carbonate solution (12 mL) and dried over magnesium sulfate. The insoluble materials were filtered off, and the

filtrate was evaporated under reduced pressure to give crude [1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindol-3-yl]-4-ethylphenylmethanol, which was used in the subsequent step without further purification. To a stirred solution of the above compound in acetonitrile (8 mL)–dichloromethane (4 mL) were added triethylsilane (0.501 mL, 3.14 mmol) and boron trifluoride diethyl etherate (0.398 mL, 3.14 mmol) at $-10\text{ }^{\circ}\text{C}$ under argon atmosphere. After being stirred at the same temperature for 10 minutes, to the mixture was added a saturated aqueous sodium hydrogen carbonate solution (15 mL), and the organic solvent was evaporated under reduced pressure. The resultant residue was extracted with ethyl acetate (40 mL) twice, and the combined organic layer was dried over magnesium sulfate. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure to give crude 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-3-(4-ethylphenylmethyl)-4-fluoroindole **34a-1**, which was partially deacetylated. This crude compound was dissolved in chloroform (11 mL), and to the mixture were added successively pyridine (0.152 mL, 1.88 mmol), acetic anhydride (0.178 mL, 1.88 mmol) and 4-(dimethylamino)pyridine (7.7 mg, 0.627 mmol). After being stirred at room temperature for 1 hour, the solvent was evaporated under reduced pressure. The resultant residue was dissolved in ethyl acetate (40 mL), and the mixture was washed with a 10% aqueous copper(II) sulfate solution (5 mL) twice and a saturated aqueous sodium hydrogen carbonate solution (15 mL), and dried over magnesium sulfate. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residual solid was triturated with ethyl alcohol (9 mL) under heating to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-3-(4-ethylphenylmethyl)-4-fluoroindole **34a-1** (335 mg, 91.6%) as colorless crystals: mp 188–189 $^{\circ}\text{C}$. HPLC 98.2% (t_{R} = 8.32 min, L-column ODS (5 μm particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (70/30)). APCI-Mass m/z 601 ($\text{M}+\text{NH}_4$). ^1H NMR (DMSO- d_6) δ 1.14 (t, J = 7.6 Hz, 3H), 1.63 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.54 (q, J = 7.5 Hz, 2H), 4.02 (s, 2H), 4.09 (dd, J = 12.4 and 2.4 Hz, 1H), 4.13 (dd, J = 12.4 and 5.4 Hz, 1H), 4.29 (ddd, J = 9.9, 5.2 and 2.7 Hz, 1H), 5.23 (t, J = 9.6 Hz, 1H), 5.49–5.56 (m, 2H), 6.15 (d, J = 8.5 Hz, 1H), 6.77 (dd, J = 10.9 and 7.9 Hz, 1H), 7.09 (s, 4H), 7.14 (td, J = 8.0 and 5.3 Hz, 1H), 7.24 (s, 1H), 7.46 (d, J = 8.2 Hz, 1H).

The above obtained 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-3-(4-ethylphenylmethyl)-4-fluoroindole **34a-1** (321 mg, 0.549 mmol) was dissolved in methanol (3 mL)–tetrahydrofuran (6 mL), and thereto was added sodium methoxide (28% methanol solution, 1 drop). After being stirred at room temperature for 3 hours, the reaction solvent was evaporated under reduced pressure. The resultant residue was purified by silica gel column chromatography (chloroform : methanol =

100:0 – 90:10) to give 3-(4-ethylphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole **35a-1** (226 mg, 99.2%) as colorless foam: HPLC 99.3% (t_R = 11.06 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (40/60)). APCI-Mass m/z 433 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.14 (t, J = 7.6 Hz, 3H), 2.54 (q, J = 7.6 Hz, 2H), 3.21–3.27 (m, 1H), 3.35–3.48 (m, 3H), 3.62–3.70 (m, 2H), 4.04 (s, 2H), 4.54 (t, J = 5.6 Hz, 1H), 5.10 (d, J = 5.3 Hz, 1H), 5.18 (d, J = 4.9 Hz, 1H), 5.21 (d, J = 5.9 Hz, 1H), 5.37 (d, J = 9.2 Hz, 1H), 6.74 (dd, J = 11.3 and 7.6 Hz, 1H), 7.03–7.08 (m, 1H), 7.09 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 7.22 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H).

3-(4-Ethoxyphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole (35a-2)

3-(4-Chlorophenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole (35a-3)

3-(4-Ethylphenylmethyl)-1-(β -D-glucopyranosyl)-4-methylindole (35b-1)

3-(4-Cyclopentylphenylmethyl)-1-(β -D-glucopyranosyl)indole (35c-6)

3-(4-Cyclopentylphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole (35a-6)

4-Chloro-3-(4-ethylphenylmethyl)-1-(β -D-glucopyranosyl)indole (35d-1)

29b, **30b**, **29c**, **30c**, **29d** and **30d** were prepared in a manner similar to **30a** from the corresponding indoline **28**. **31a-2**, **34a-2**, **35a-2**, **31a-3**, **34a-3**, **35a-3**, **31b-1**, **34b-1**, **35b-1**, **31c-6**, **34c-6**, **35c-6**, **31a-6**, **34a-6**, **35a-6**, **31d-1**, **34d-1** and **35d-1** were prepared in a manner similar to **35a-1** from the corresponding benzoyl chloride³⁷⁾ and indole **30**.

31a-2 (99.1%): APCI-Mass m/z 614 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.38 (t, J = 6.9 Hz, 3H), 1.68 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 4.11 (d, J = 4.0 Hz, 2H), 4.16 (q, J = 7.0 Hz, 2H), 4.28–4.31 (m, 1H), 5.30 (t, J = 9.8 Hz, 1H), 5.54 (t, J = 9.6 Hz, 1H), 5.76 (t, J = 9.3 Hz, 1H), 6.34 (d, J = 9.0 Hz, 1H), 7.01 (dd, J = 10.6 and 8.0 Hz, 1H), 7.07 (d, J = 8.7 Hz, 2H), 7.36 (td, J = 8.1 and 4.9 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.83 (d, J = 8.8 Hz, 2H), 8.14 (s, 1H).

34a-2 (82.8%): mp 146–148 °C. HPLC 96.4% (t_R = 8.59 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (65/35)). APCI-Mass m/z 617 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.29 (t, J = 7.0 Hz, 3H), 1.64 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 3.96 (q, J = 7.1 Hz, 2H), 3.98 (s, 2H), 4.09 (dd, J = 12.4 and 2.6 Hz, 1H), 4.13 (dd, J = 12.4 and 5.4 Hz, 1H), 4.28 (ddd, J = 9.9, 5.2 and 2.7 Hz, 1H), 5.22 (t, J = 9.5 Hz, 1H), 5.48–5.56 (m, 2H), 6.14 (d, J = 8.5 Hz, 1H), 6.77 (dd, J = 10.8 and 7.7 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 7.14 (td, J = 8.0 and 5.3 Hz, 1H), 7.21 (s, 1H), 7.46 (d, J = 8.2 Hz, 1H).

35a-2 (100%): HPLC 99.2% (t_R = 6.84 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (40/60)). APCI-Mass m/z 449 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.29 (t, J = 7.0 Hz, 3H), 3.21–3.27 (m, 1H), 3.35–3.48 (m, 3H), 3.65 (td, J = 9.2 and 5.5 Hz, 2H), 3.96 (q, J = 7.0 Hz, 2H), 4.01 (s, 2H), 4.53 (t, J = 5.6 Hz, 1H), 5.10 (d, J = 5.3 Hz, 1H), 5.17 (d, J = 5.1 Hz, 1H), 5.21 (d, J = 5.7 Hz, 1H), 5.36 (d, J = 9.0 Hz, 1H), 6.74 (dd, J = 11.2 and 7.7 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 7.06 (td, J = 8.1 and 5.2 Hz, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.19 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H).

31a-3 (41.8%): APCI-Mass m/z 604/606 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.68 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 4.10 (d, J = 4.0 Hz, 2H), 4.29 (dt, J = 10.0 and 4.2 Hz, 1H), 5.29 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.6 Hz, 1H), 5.76 (t, J = 9.3 Hz, 1H), 6.34 (d, J = 9.0 Hz, 1H), 7.05 (dd, J = 10.8 and 8.1 Hz, 1H), 7.39 (td, J = 8.1 and 4.9 Hz, 1H), 7.63 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H), 8.21 (s, 1H).

34a-3 (87.2%): mp 177–178 °C. HPLC 97.2% (t_R = 10.74 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (65/35)). APCI-Mass m/z 607/609 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.63 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 4.06 (s, 2H), 4.09 (dd, J = 12.5 and 2.6 Hz, 1H), 4.13 (dd, J = 12.5 and 5.3 Hz, 1H), 4.29 (ddd, J = 9.6, 5.4 and 2.7 Hz, 1H), 5.23 (t, J = 9.5 Hz, 1H), 5.49–5.56 (m, 2H), 6.16 (d, J = 8.5 Hz, 1H), 6.78 (dd, J = 11.0 and 7.9 Hz, 1H), 7.15 (td, J = 8.0 and 5.1 Hz, 1H), 7.20 (d, J = 8.5 Hz, 2H), 7.29 (s, 1H), 7.31 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.3 Hz, 1H).

35a-3 (81.3%): mp 112–116 °C. HPLC 99.9% (t_R = 8.56 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (40/60)). APCI-Mass m/z 422/424 (M+H). ¹H NMR (DMSO-*d*₆) δ 3.24 (td, J = 8.9 and 5.2 Hz, 1H), 3.39 (td, J = 8.9 and 5.3 Hz, 1H), 3.43–3.47 (m, 2H), 3.65–3.69 (m, 2H), 4.08 (s, 2H), 4.54 (t, J = 5.5 Hz, 1H), 5.10 (d, J = 5.3 Hz, 1H), 5.18 (d, J = 5.9 Hz, 1H), 5.22 (d, J = 5.8 Hz, 1H), 5.38 (d, J = 9.2 Hz, 1H), 6.74 (dd, J = 11.1 and 7.9 Hz, 1H), 7.08 (td, J = 8.1 and 5.3 Hz, 1H), 7.25–7.33 (m, 5H), 7.37 (d, J = 8.3 Hz, 1H). Anal. Calcd for C₂₁H₂₁ClFNO₅: C, 59.79; H, 5.02; Cl, 8.40; F, 4.50; N, 3.32. Found: C, 59.44; H, 4.99; Cl, 8.29; F, 4.81; N, 3.30.

29b (36.8%): mp 163–172 °C. APCI-Mass m/z 464 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.93 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.11 (s, 3H), 2.68–2.75 (m, 1H), 2.87–2.93 (m, 1H), 3.41–3.51 (m, 2H), 3.93–3.97 (m, 1H), 4.08 (d, J = 10.0 Hz, 2H), 4.93 (t, J = 9.3 Hz, 1H), 5.14 (t, J = 9.4 Hz, 1H), 5.42 (t, J = 9.5 Hz), 5.44 (d, J = 9.3 Hz, 1H), 6.50 (d, J = 7.4 Hz, 1H), 6.56 (d, J

= 8.0 Hz, 1H), 6.93 (t, J = 7.7 Hz, 1H).

30b (93.8%): mp 156–157 °C. APCI-Mass m/z 479 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.64 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.45 (s, 3H), 4.07 (dd, J = 12.4 and 2.4 Hz, 1H), 4.12 (dd, J = 12.4 and 5.4 Hz, 1H), 4.30 (ddd, J = 10.0, 5.4 and 2.4 Hz, 1H), 5.21 (t, J = 9.7 Hz, 1H), 5.54 (t, J = 9.5 Hz, 1H), 5.61 (t, J = 9.3 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 6.53 (d, J = 3.4 Hz, 1H), 6.88 (d, J = 7.2 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 7.43 (d, J = 3.4 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H).

31b-1 (83.3%): APCI-Mass m/z 594 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.25 (t, J = 7.6 Hz, 3H), 1.70 (s, 3H), 1.95 (s, 3H), 1.97 (s, 3H), 2.08 (s, 3H), 2.51 (s, 3H), 2.73 (q, J = 7.5 Hz, 2H), 4.07 (d, J = 4.2 Hz, 2H), 4.28 (dt, J = 10.0 and 4.3 Hz, 1H), 5.27 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.6 Hz, 1H), 5.75 (t, J = 9.3 Hz, 1H), 6.31 (d, J = 9.0 Hz, 1H), 7.04 (d, J = 7.4 Hz, 1H), 7.27 (t, J = 7.7 Hz, 1H), 7.40 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.3 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.99 (s, 1H).

34b-1 (82.5%): mp 182–184 °C. HPLC 94.8% (t_R = 9.55 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (70/30)). APCI-Mass m/z 580 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.15 (t, J = 7.5 Hz, 3H), 1.67 (s, 3H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.38 (s, 3H), 2.55 (q, J = 7.7 Hz, 2H), 4.08 (dd, J = 12.3 and 2.3 Hz, 1H), 4.13 (dd, J = 12.1 and 5.6 Hz, 1H), 4.13 (s, 2H), 4.29 (ddd, J = 9.9, 5.4 and 2.4 Hz, 1H), 5.20 (t, J = 9.6 Hz, 1H), 5.50–5.57 (m, 2H), 6.15 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 7.2 Hz, 1H), 7.01 (d, J = 8.0 Hz, 2H), 7.04 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.13 (s, 1H), 7.44 (d, J = 8.3 Hz, 1H).

35b-1 (98.1%): HPLC 99.6% (t_R = 7.51 min, L-column ODS (5 μ m particle size, 4.6 \times 150 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (45/55)). APCI-Mass m/z 412 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.15 (t, J = 7.6 Hz, 3H), 2.41 (s, 3H), 2.56 (q, J = 7.5 Hz, 2H), 3.23 (td, J = 8.9 and 5.2 Hz, 1H), 3.37–3.47 (m, 3H), 3.64–3.69 (m, 2H), 4.16 (s, 2H), 4.51 (t, J = 5.3 Hz, 1H), 5.06 (d, J = 5.1 Hz, 1H), 5.13–5.15 (m, 2H), 5.34 (d, J = 9.0 Hz, 1H), 6.70 (d, J = 7.0 Hz, 1H), 6.97 (t, J = 7.7 Hz, 1H), 7.07–7.12 (m, 5H), 7.34 (d, J = 8.3 Hz, 1H).

29c (49.2%): mp 106–108 °C. APCI-Mass m/z 450 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.92 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.78–2.86 (m, 1H), 2.91–2.96 (m, 1H), 3.40–3.49 (m, 2H), 3.93–3.97 (m, 1H), 4.07–4.11 (m, 2H), 4.93 (t, J = 9.5 Hz, 1H), 5.15 (t, J = 9.4 Hz, 1H), 5.42 (t, J = 9.5 Hz, 1H), 5.48 (d, J = 9.3 Hz, 1H), 6.67 (t, J = 7.2 Hz, 1H), 6.74 (d, J = 7.9 Hz, 1H), 7.03 (t, J = 7.7 Hz, 1H), 7.05 (d, J = 7.1 Hz, 1H).

30c (85.6%): mp 136–138 °C. APCI-Mass m/z 465 (M+NH₄), 448 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.63 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 4.09 (dd, J = 12.4 and 2.1 Hz, 1H), 4.13 (dd, J = 12.3 and 5.5 Hz, 1H), 4.31 (ddd, J = 9.7, 5.4 and 2.4 Hz, 1H), 5.23 (t, J = 9.7 Hz, 1H), 5.54 (t, J = 9.5 Hz, 1H), 5.61 (t, J = 9.2 Hz, 1H), 6.22 (d, J = 9.0 Hz, 1H), 6.51 (d, J = 3.2 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 3.2 Hz, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.64 (d, J = 8.6 Hz, 1H).

31c-6 (100%): APCI-Mass m/z 620 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.57–1.72 (m, 4H), 1.67 (s, 3H), 1.79–1.87 (m, 2H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.05–2.13 (m, 2H), 3.10 (m, 1H), 4.08–4.14 (m, 2H), 4.29 (m, 1H), 5.33 (t, J = 9.8 Hz, 1H), 5.54 (t, J = 9.6 Hz, 1H), 5.85 (t, J = 9.3 Hz, 1H), 6.35 (d, J = 9.2 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 8.5 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 8.28 (s, 1H).

34c-6 (82.3%): mp 163–165 °C. HPLC 99.1% (t_R = 3.35 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (75/25)). APCI-Mass m/z 623 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.42–1.52 (m, 2H), 1.58–1.66 (m, 2H), 1.62 (s, 3H), 1.69–1.78 (m, 2H), 1.92–2.00 (m, 2H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.89 (m, 1H), 3.95 (s, 2H), 4.06–4.17 (m, 2H), 4.29 (m, 1H), 5.21 (t, J = 9.6 Hz, 1H), 5.52 (t, J = 9.4 Hz, 1H), 5.56 (t, J = 9.2 Hz, 1H), 6.15 (d, J = 8.7 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 7.10–7.19 (m, 5H), 7.25 (s, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H).

35c-6 (98.0%): HPLC 100% (t_R = 3.10 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (50/50)). APCI-Mass m/z 438 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.45–1.53 (m, 2H), 1.57–1.66 (m, 2H), 1.70–1.76 (m, 2H), 1.92–2.00 (m, 2H), 2.90 (m, 1H), 3.20–3.47 (m, 4H), 3.64–3.74 (m, 2H), 3.97 (s, 2H), 4.51 (t, J = 5.5 Hz, 1H), 5.07 (d, J = 5.1 Hz, 1H), 5.15 (d, J = 5.0 Hz, 1H), 5.16 (d, J = 5.9 Hz, 1H), 5.36 (d, J = 9.2 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.13 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.25 (s, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H).

31a-6 (100%): APCI-Mass m/z 638 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.69 (s, 3H), 1.55–1.72 (m, 4H), 1.77–1.85 (m, 2H), 1.97 (s, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.04–2.12 (m, 2H), 3.09 (quintet, J = 8.8 Hz, 1H), 4.10 (m, 2H), 4.28 (m, 1H), 5.31 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.5 Hz, 1H), 5.78 (t, J = 9.4 Hz, 1H), 6.34 (d, J = 9.2 Hz, 1H), 7.03 (dd, J = 10.7 and 8.3 Hz, 1H), 7.38 (td, J = 8.0 and 4.7 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 8.2 Hz, 2H),

8.18 (s, 1H).

34a-6 (78.1%): HPLC 99.8% (t_R = 3.41 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (75/25)). APCI-Mass m/z 641 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.40–1.50 (m, 2H), 1.55–1.65 (m, 2H), 1.63 (s, 3H), 1.70–1.80 (m, 2H), 1.80–1.95 (m, 2H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.90 (quintet, J = 7.5 Hz, 1H), 4.01 (s, 2H), 4.11 (m, 2H), 4.28 (m, 1H), 5.23 (t, J = 9.6 Hz, 1H), 5.51 (t, J = 9.3 Hz, 1H), 5.55 (t, J = 9.2 Hz, 1H), 6.15 (d, J = 8.7 Hz, 1H), 6.78 (dd, J = 11.0 and 7.9 Hz, 1H), 7.09 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 7.15 (dd, J = 8.2 and 5.3 Hz, 1H), 7.26 (s, 1H), 7.46 (d, J = 8.3 Hz, 1H).

35a-6 (96.1%): HPLC 99.6% (t_R = 3.99 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (50/50)). APCI-Mass m/z 473 (M+NH₄), 456 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.47 (m, 2H), 1.62 (m, 2H), 1.73 (m, 2H), 1.95 (m, 2H), 2.91 (m, 1H), 3.20–3.50 (m, 4H), 3.66 (m, 2H), 4.04 (s, 2H), 4.53 (t, J = 5.5 Hz, 1H), 5.09 (d, J = 5.3 Hz, 1H), 5.16 (d, J = 5.0 Hz, 1H), 5.21 (d, J = 5.8 Hz, 1H), 5.37 (d, J = 9.0 Hz, 1H), 6.75 (dd, J = 11.0 and 7.9 Hz, 1H), 7.07 (td, J = 7.9 and 5.5 Hz, 1H), 7.13 (d, J = 8.1 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 7.24 (s, 1H), 7.36 (d, J = 8.3 Hz, 1H).

29d (60.7%): mp 141–143 °C. APCI-Mass m/z 484/486 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.93 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.81–2.89 (m, 1H), 2.97–3.04 (m, 1H), 3.50–3.57 (m, 2H), 3.95–3.99 (m, 1H), 4.07–4.12 (m, 2H), 4.96 (t, J = 9.6 Hz, 1H), 5.16 (t, J = 9.3 Hz, 1H), 5.42 (t, J = 9.5 Hz, 1H), 5.49 (d, J = 9.3 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 7.9 Hz, 1H).

30d (92.9%): mp 126–127 °C. APCI-Mass m/z 499/501 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.65 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 4.08–4.16 (m, 2H), 4.28–4.32 (m, 1H), 5.26 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.5 Hz, 1H), 5.62 (t, J = 9.3 Hz, 1H), 6.23 (d, J = 9.2 Hz, 1H), 6.56 (d, J = 3.4 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 3.5 Hz, 1H), 7.67 (d, J = 8.2 Hz, 1H).

31d-1 (95.2%): APCI-Mass m/z 614/616 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.24 (t, J = 7.5 Hz, 3H), 1.70 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.72 (q, J = 7.7 Hz, 2H), 4.10 (d, J = 4.2 Hz, 2H), 4.27–4.31 (m, 1H), 5.29 (t, J = 9.8 Hz, 1H), 5.53 (t, J = 9.6 Hz, 1H), 5.73 (t, J = 9.3 Hz, 1H), 6.33 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.39 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.5 Hz, 1H), 8.11 (s, 1H).

34d-1 (80.2%): APCI-Mass m/z 617/619 ($M+NH_4$). 1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.6 Hz, 3H), 1.65 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.55 (q, J = 7.7 Hz, 2H), 4.08–4.15 (m, 2H), 4.19 (d, J = 3.1 Hz, 2H), 4.26–4.30 (m, 1H), 5.24 (t, J = 9.6 Hz, 1H), 5.50 (t, J = 9.4 Hz, 1H), 5.55 (t, J = 9.2 Hz, 1H), 6.17 (d, J = 8.8 Hz, 1H), 7.04–7.10 (m, 5H), 7.16 (t, J = 7.9 Hz, 1H), 7.27 (s, 1H), 7.64 (d, J = 8.3 Hz, 1H).

35d-1 (99.5%): APCI-Mass m/z 432/434 ($M+H$). 1H NMR (DMSO- d_6) δ 1.15 (t, J = 7.5 Hz, 3H), 2.55 (q, J = 7.7 Hz, 2H), 3.21–3.47 (m, 4H), 3.62–3.70 (m, 2H), 4.23 (s, 2H), 4.53 (t, J = 5.5 Hz, 1H), 5.09 (d, J = 5.3 Hz, 1H), 5.16 (d, J = 5.0 Hz, 1H), 5.20 (d, J = 5.9 Hz, 1H), 5.40 (d, J = 9.0 Hz, 1H), 7.02 (d, J = 7.5 Hz, 1H), 7.08–7.15 (m, 5H), 7.24 (s, 1H), 7.53 (d, J = 8.2 Hz, 1H).

3-(4-Cyclopropylphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole (35a-4).

1-(2,3,4,6-tetra-*O*-Acetyl- β -D-glucopyranosyl)-4-fluoroindole **30a** (54.6 g, 117 mmol) and *N,N*-dimethylformamide (54.5 mL, 704 mmol) were dissolved in 1,2-dichloroethane (550 mL), and to the mixture was added dropwise phosphorus(III) oxychloride (32.8 mL, 352 mmol) at room temperature. The mixture was stirred at 70 °C for 1 hour, and to the mixture was added water (600 mL) under ice–water cooling. The resultant mixture was extracted with ethyl acetate (1000 and 700 mL), and the combined organic layer was washed with saturated aqueous sodium hydrogen carbonate solution (200 mL) twice and brine (200 mL), and dried over magnesium sulfate with activated carbon. The insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residual solid was recrystallized from ethanol (300 mL) to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindole-3-carboxyaldehyde **32a** (47.0 g, 81.4%) as almost colorless crystals: mp 187–189 °C. HPLC 94.7% (t_R = 3.26 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (45/55)). APCI-Mass m/z 494 ($M+H$). 1H NMR (DMSO- d_6) δ 1.64 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 4.12 (dd, J = 12.4 and 2.5 Hz, 1H), 4.17 (dd, J = 12.4 and 5.5 Hz, 1H), 4.33 (ddd, J = 10.0, 5.5 and 2.5 Hz, 1H), 5.32 (t, J = 9.8 Hz, 1H), 5.56 (t, J = 9.6 Hz, 1H), 5.66 (t, J = 9.3 Hz, 1H), 6.36 (d, J = 9.0 Hz, 1H), 7.11 (dd, J = 10.6 and 8.0 Hz, 1H), 7.38 (td, J = 8.1 and 5.1 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 8.53 (s, 1H), 10.0 (d, J = 2.9 Hz, 1H).

To a vigorously stirred suspension of magnesium turnings (4.26 g, 175 mmol, dried by heating under reduced pressure) in tetrahydrofuran (80 mL) was added dropwise a solution of 1,2-dibromoethane (0.02 mL, 0.232 mmol) and 1-bromo-4-cyclopropylbenzene⁴⁵⁾ (34.5 g, 175 mmol) in tetrahydrofuran (20 mL) over 45 minutes at room temperature under argon atmosphere.

The mixture was stirred at room temperature for 30 minutes to give a corresponding Grignard reagent. To a solution of 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-fluoroindole-3-carboxyaldehyde **32a** (43.2 g, 87.5 mmol) in tetrahydrofuran (860 mL) was added dropwise the previous Grignard reagent over 30 minutes under ice–water cooling (internal temperature; 6–8 °C) under argon atmosphere. After stirred at the same temperature for 20 minutes, the mixture was quenched with saturated aqueous ammonium chloride solution (1000 mL), and extracted with ethyl acetate (1000 and 800 mL). The combined organic layer was washed with brine (400 mL) and dried over magnesium sulfate. The insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The resultant residue and triethylsilane (21 mL, 131 mmol) were dissolved in dichloromethane (220 mL)–acetonitrile (440 mL), and to the mixture was added dropwise boron trifluoride diethyl etherate (13 mL, 105 mmol) over 10 minutes under ice–water cooling under argon atmosphere. The resultant mixture was stirred at the same temperature for 30 minutes, and to the mixture was added saturated aqueous sodium hydrogen carbonate solution (800 mL). The organic solvent was evaporated under reduced pressure, and the resultant residue was extracted with ethyl acetate (1000 and 800 mL). After being dried over magnesium sulfate, the solution was treated with activated carbon. The insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The residual solid was triturated with ethanol (600 mL) under heating to give 1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)-3-(4-cyclopropylphenylmethyl)-4-fluoroindole **34a-4** (42.1 g, 80.8%) as colorless crystals: mp 186–189 °C. IR (Nujol) 1741, 1627, 1579, 1546, 1516, 1497, 1459, 1376 cm⁻¹. HPLC 98.6% (*t*_R = 3.18 min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (70/30)). APCI-Mass *m/z* 613 (M+NH₄), 596 (M+H). ¹H NMR (DMSO-*d*₆) δ 0.60 (ddd, *J* = 6.3, 4.6 and 4.3 Hz, 2H), 0.88 (ddd, *J* = 8.2, 6.3 and 4.2 Hz, 2H), 1.63 (s, 3H), 1.81–1.87 (m, 1H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 4.00 (s, 2H), 4.09 (dd, *J* = 12.3 and 2.7 Hz, 1H), 4.13 (dd, *J* = 12.6 and 5.2 Hz, 1H), 4.28 (ddd, *J* = 10.0, 5.1 and 2.9 Hz, 1H), 5.22 (t, *J* = 9.6 Hz, 1H), 5.49–5.56 (m, 2H), 6.15 (d, *J* = 8.7 Hz, 1H), 6.77 (dd, *J* = 10.9 and 7.9 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 7.14 (td, *J* = 7.9 and 5.3 Hz, 1H), 7.23 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 1H). Anal. Calcd for C₂₂H₃₄FN₂O₉: C, 64.53; H, 5.75; F, 3.19; N, 2.35. Found: C, 64.57; H, 5.63; F, 3.08; N, 2.36.

1-(2,3,4,6-tetra-*O*-Acetyl- β -D-glucopyranosyl)-3-(4-cyclopropylphenylmethyl)-4-fluoroindole **34a-4** (40.5 g, 68.0 mmol) was dissolved in methanol (200 mL)–tetrahydrofuran (400 mL), and to the mixture was added sodium methoxide (28% methanol solution, 0.394 mL, 2.04 mmol). After being stirred at room temperature for 3 hours under argon atmosphere, the solvent was evaporated

under reduced pressure. The resultant residue was dissolved in ethyl acetate (2000 mL), and the solution was washed with brine (200 mL) twice. After being dried over magnesium sulfate, the solution was treated with activated carbon. The insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The resultant residue was dissolved in ethanol (150 mL) under heating, and to the mixture were added water (300 mL) and a seed crystals at room temperature. The mixture was stirred at room temperature for 19 hours, and the precipitates were collected by filtration and washed with ethanol–water (1:4) to give 3-(4-cyclopropylphenylmethyl)-4-fluoro-1-(β -D-glucopyranosyl)indole **35a-4** (26.9 g, 92.5%) as colorless crystals: mp 107–110 °C. IR (Nujol) 3525, 3313, 1625, 1582, 1555, 1517, 1495, 1457 cm^{-1} . HPLC 99.1% (t_R = 2.67 min, L-column ODS (3 μm particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (45/55)). APCI-Mass m/z 445 ($\text{M}+\text{NH}_4$). ^1H NMR ($\text{DMSO}-d_6$) δ 0.60 (ddd, J = 6.3, 4.7 and 4.3 Hz, 2H), 0.87–0.90 (m, 2H), 1.82–1.87 (m, 1H), 3.24 (td, J = 8.9 and 5.6 Hz, 1H), 3.36–3.41 (m, 1H), 3.43–3.46 (m, 2H), 3.63–3.69 (m, 2H), 4.02 (s, 2H), 4.53 (t, J = 5.5 Hz, 1H), 5.09 (d, J = 5.3 Hz, 1H), 5.15 (d, J = 5.0 Hz, 1H), 5.20 (d, J = 5.8 Hz, 1H), 5.37 (d, J = 9.0 Hz, 1H), 6.74 (dd, J = 11.2 and 7.9 Hz, 1H), 6.96 (d, J = 8.0 Hz, 2H), 7.07 (td, J = 8.0 and 5.4 Hz, 1H), 7.13 (d, J = 7.9 Hz, 2H), 7.21 (s, 1H), 7.35 (d, J = 8.3 Hz, 1H). Anal. Calcd for $\text{C}_{24}\text{H}_{26}\text{FNO}_5 \cdot 0.7 \text{H}_2\text{O}$: C, 65.50; H, 6.28; F, 4.32; N, 3.18. Found: C, 65.53; H, 6.16; F, 4.12; N, 3.13.

3-(4-Cyclopropylphenylmethyl)-1-(β -D-glucopyranosyl)-4-methylindole (35b-4)

3-(4-Cyclopropylphenylmethyl)-1-(β -D-glucopyranosyl)indole (35c-4)

3-(4-Cyclobutylphenylmethyl)-1-(β -D-glucopyranosyl)indole (35c-5)

4-Chloro-3-(4-methylphenylmethyl)-1-(β -D-glucopyranosyl)indole (35d-7)

32b, **32c** and **32d** were prepared in a manner similar to **32a** from the corresponding indole **30**. **34b-4**, **35b-4**, **34c-4**, **35c-4**, **34c-5**, **35c-5**, **34d-7** and **35d-7** were prepared in a manner similar to **35a-4** from the corresponding aryl Grignard reagents⁴⁶⁾ and indole-3-carboxyaldehyde **32**.

32b (71.2%): mp 170–172 °C. APCI-Mass m/z 490 ($\text{M}+\text{H}$). ^1H NMR (CDCl_3) δ 1.69 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.83 (s, 3H), 4.00–4.07 (m, 1H), 4.17 (dd, J = 12.7 and 2.1 Hz, 1H), 4.32 (dd, J = 12.5 and 5.0 Hz, 1H), 5.30 (t, J = 9.7 Hz, 1H), 5.40–5.55 (m, 2H), 5.65 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 7.3 Hz, 1H), 7.25 (t, J = 8.2 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H), 7.97 (s, 1H), 10.1 (s, 1H).

34b-4 (85.1%): mp 191–193 °C. HPLC 98.7% (t_R = 3.83 min, Sumipax ODS D-210SLP (3 μm

particle size, 4.6 × 50 mm), 0.05% TFA in MeCN / 0.05% TFA in water (70/30)). APCI-Mass m/z 592 (M+H). ^1H NMR (DMSO- d_6) δ 0.55–0.65 (m, 2H), 0.85–0.95 (m, 2H), 1.67 (s, 3H), 1.80–1.90 (m, 1H), 1.95 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.37 (s, 3H), 4.05–4.17 (m, 4H), 4.25–4.35 (m, 1H), 5.19 (t, J = 9.7 Hz, 1H), 5.50–5.60 (m, 2H), 6.15 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 7.3 Hz, 1H), 6.95 (s, 4H), 7.03 (t, J = 8.2 Hz, 1H), 7.11 (s, 1H), 7.44 (d, J = 8.2 Hz, 1H).

35b-4 (84.8%): mp 116–119 °C. HPLC 99.9% (t_R = 2.04 min, Sumipax ODS D-210SLP (3 μm particle size, 4.6 × 50 mm), 0.05% TFA in MeCN / 0.05% TFA in water (50/50)). APCI-Mass m/z 424 (M+H). ^1H NMR (DMSO- d_6) δ 0.60 (dt, J = 6.0 and 5.1 Hz, 2H), 0.90 (ddd, J = 8.5, 6.4 and 4.2 Hz, 2H), 1.85 (ddd, J = 13.3, 8.5 and 5.1 Hz, 1H), 2.40 (s, 3H), 3.24 (td, J = 8.8 and 5.4 Hz, 1H), 3.37–3.40 (m, 3H), 3.65–3.71 (m, 2H), 4.14 (s, 2H), 4.57 (t, J = 5.1 Hz, 1H), 5.08 (d, J = 5.5 Hz, 1H), 5.13 (d, J = 6.2 Hz, 1H), 5.15 (d, J = 5.8 Hz, 1H), 5.34 (d, J = 9.1 Hz, 1H), 6.71 (d, J = 7.3 Hz, 1H), 6.98 (d, J = 8.2 Hz, 2H), 6.99 (t, J = 6.0 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 7.09 (s, 1H), 7.33 (d, J = 8.2 Hz, 1H).

32c (74.9%): APCI-Mass m/z 476 (M+H). ^1H NMR (DMSO- d_6) δ 1.63 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 4.10–4.20 (m, 2H), 4.40 (m, 1H), 5.30 (t, J = 9.8 Hz, 1H), 5.57 (t, J = 9.5 Hz, 1H), 5.66 (t, J = 9.3 Hz, 1H), 6.34 (d, J = 9.0 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 8.51 (s, 1H), 9.96 (s, 1H).

34c-4 (96.8%): mp 184–186 °C. APCI-Mass m/z 595 (M+NH₄), 578 (M+H). ^1H NMR (DMSO- d_6) δ 0.57–0.61 (m, 2H), 0.86–0.90 (m, 2H), 1.62 (s, 3H), 1.81–1.87 (m, 1H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 3.94 (s, 2H), 4.08 (dd, J = 12.7 and 1.9 Hz, 1H), 4.13 (dd, J = 12.4 and 5.6 Hz, 1H), 4.29 (ddd, J = 10.0, 5.8 and 2.2 Hz, 1H), 5.21 (t, J = 9.6 Hz, 1H), 5.51 (t, J = 9.3 Hz, 1H), 5.56 (t, J = 9.1 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 2H), 7.01 (t, J = 9.5 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.16 (t, J = 7.5 Hz, 1H), 7.23 (s, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H).

35c-4 (99.0%): HPLC 97.8% (t_R = 3.28 min, L-column ODS (3 μm particle size, 4.6 × 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (40/60)). APCI-Mass m/z 427 (M+NH₄), 410 (M+H). ^1H NMR (DMSO- d_6) δ 0.60 (dt, J = 6.2 and 4.7 Hz, 2H), 0.88 (ddd, J = 8.3, 6.3 and 4.2 Hz, 2H), 1.84 (ddd, J = 13.2, 8.4 and 5.1 Hz, 1H), 3.24 (td, J = 8.9 and 5.2 Hz, 1H), 3.37–3.44 (m, 3H), 3.65–3.71 (m, 2H), 3.95 (s, 2H), 4.51 (t, J = 5.5 Hz, 1H), 5.07 (d, J = 5.3 Hz, 1H), 5.14 (d, J = 5.0 Hz, 1H), 5.16 (d, J = 5.9 Hz, 1H), 5.36 (d, J = 9.2 Hz, 1H), 6.96 (d, J = 8.0 Hz, 2H), 6.98 (t, J = 7.7 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 7.9 Hz, 2H), 7.23 (s, 1H), 7.42 (d, J = 8.0 Hz,

1H), 7.49 (d, $J = 8.2$ Hz, 1H).

34c-5 (72.5%): mp 174–177 °C. HPLC 99.7% ($t_R = 4.09$ min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (70/30)). APCI-Mass m/z 609 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.62 (s, 3H), 1.73–1.82 (m, 1H), 1.96 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 1.88–2.06 (m, 3H), 2.18–2.27 (m, 2H), 3.44 (quintet, $J = 8.7$ Hz, 1H), 3.96 (s, 2H), 4.07–4.17 (m, 2H), 4.29 (m, 1H), 5.21 (t, $J = 9.5$ Hz, 1H), 5.52 (t, $J = 9.3$ Hz, 1H), 5.56 (t, $J = 9.2$ Hz, 1H), 6.15 (d, $J = 8.7$ Hz, 1H), 7.01 (t, $J = 7.5$ Hz, 1H), 7.10 (d, $J = 7.9$ Hz, 2H), 7.16 (d, $J = 7.9$ Hz, 2H), 7.17 (t, $J = 7.9$ Hz, 1H), 7.25 (s, 1H), 7.41 (d, $J = 7.9$ Hz, 1H), 7.60 (d, $J = 8.2$ Hz, 1H).

35c-5 (95.0%): HPLC 100% ($t_R = 3.64$ min, L-column ODS (3 μ m particle size, 4.6 \times 50 mm), MeCN / 20 mM phosphate buffer (pH 6.5) (45/55)). APCI-Mass m/z 441 (M+NH₄), 424 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.75–1.83 (m, 1H), 1.90–2.00 (m, 1H), 2.00–2.10 (m, 2H), 2.20–2.30 (m, 2H), 3.20–3.50 (m, 6H), 3.68 (m, 2H), 3.97 (s, 2H), 4.51 (t, $J = 5.6$ Hz, 1H), 5.07 (d, $J = 5.1$ Hz, 1H), 5.15 (d, $J = 5.0$ Hz, 1H), 5.16 (d, $J = 5.9$ Hz, 1H), 5.36 (d, $J = 9.0$ Hz, 1H), 6.99 (t, $J = 7.5$ Hz, 1H), 7.11 (m, 1H), 7.12 (d, $J = 8.2$ Hz, 2H), 7.22 (d, $J = 8.2$ Hz, 2H), 7.24 (s, 1H), 7.43 (d, $J = 7.9$ Hz, 1H), 7.49 (d, $J = 8.3$ Hz, 1H).

32d (90.8%): APCI-Mass m/z 527/529 (M+NH₄). ¹H NMR (DMSO-*d*₆) δ 1.64 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.05 (s, 3H), 4.09–4.19 (m, 2H), 4.30 (m, 1H), 5.34 (t, $J = 9.8$ Hz, 1H), 5.54 (t, $J = 9.5$ Hz, 1H), 5.70 (t, $J = 9.3$ Hz, 1H), 6.37 (d, $J = 9.0$ Hz, 1H), 7.35–7.42 (m, 2H), 7.82 (d, $J = 7.5$ Hz, 1H), 8.54 (s, 1H), 10.51 (s, 1H).

34d-7 (91.8%): APCI-Mass m/z 586/588 (M+H). ¹H NMR (DMSO-*d*₆) δ 1.65 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.25 (s, 3H), 4.10–4.12 (m, 2H), 4.18 (d, $J = 4.17$ Hz, 2H), 4.27–4.28 (m, 1H), 5.23 (t, $J = 9.6$ Hz, 1H), 5.50 (t, $J = 9.3$ Hz, 1H), 5.54 (t, $J = 9.1$ Hz, 1H), 6.16 (d, $J = 8.7$ Hz, 1H), 7.02–7.07 (m, 5H), 7.16 (t, $J = 7.9$ Hz, 1H), 7.24 (s, 1H), 7.64 (d, $J = 8.3$ Hz, 1H).

35d-7 (85.4%): APCI-Mass m/z 418/420 (M+H). ¹H NMR (DMSO-*d*₆) δ 2.25 (s, 3H), 3.21–3.25 (m, 1H), 3.32–3.39 (m, 1H), 3.43–3.47 (m, 2H), 3.61–3.69 (m, 2H), 4.22 (s, 2H), 4.53 (t, $J = 5.5$ Hz, 1H), 5.01 (d, $J = 5.3$ Hz, 1H), 5.15 (d, $J = 5.0$ Hz, 1H), 5.20 (d, $J = 5.8$ Hz, 1H), 5.39 (d, $J = 9.2$ Hz, 1H), 7.06–7.12 (m, 5H), 7.21 (s, 1H), 7.53 (d, $J = 8.2$ Hz, 1H).

Pharmacology

In Vitro hSGLT1 and hSGLT2 Assays (Sodium-Dependent Glucose Uptake in CHO Cells Expressing hSGLT1 and hSGLT2.²⁴⁾

Parental Chinese hamster ovary-K (CHOK) cells expressing hSGLT1 and hSGLT2 were used in these experiments. For the uptake assay, cells were seeded into 24-well plates, and were post-confluent on the day of assay.

Cells were rinsed one time with 400 μ L Assay Buffer (137 mM NaCl, 5 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 50 mM HEPES, 20 mM Tris Base, pH 7.4), and were pre-incubated with the solutions of compounds (250 μ L) for 10 min at 37°C. The transport reaction was initiated by addition of 50 μ L alpha methyl-D-glucopyranoside (AMG) / ^{14}C -AMG solution (16.7 μCi ; final concentration, 0.3 mM for CHOK-SGLT1 and 0.5 mM for CHOK-SGLT2, respectively) and incubated for 120 min at 37°C. After the incubation, the AMG uptake was halted by aspiration of the incubation mixture followed by immediate washing three times with PBS. The cells were solubilized in 0.3 N NaOH of 300 μ L and the radioactivity associated with the cells was monitored by a liquid scintillation counter (Quantsmart™ (Packard, Boston, MA, USA)). Inhibitory concentration of 50% (IC_{50}) was calculated by nonlinear least squares analysis using a four-parameter logistic model (Prism version 4; GraphPad Software, San Diego, CA, USA).

In Vitro GLUT1 Assay (2-Deoxy-Glucose (2-DG) Uptake in L6 Myoblast Cells.⁴⁰⁾

The rat skeletal muscle cell line, L6 (JCRB9081), was obtained from Health Science Research Resources Bank (HSRRB, Osaka, Japan). L6 myoblast cells were maintained in Dulbecco's modified Eagle's medium containing 5.6 mM glucose supplemented with 10% FBS. Cells were seeded in 24-well plates at a density of 3.0×10^5 cells / well and cultured for 24 hours in an atmosphere of 5% CO_2 at 37°C before the experiment. Prior to the transport experiment, cells were rinsed twice with KRPH buffer (pH 7.4, 150 mM NaCl, 5 mM KCl, 1.25 mM MgSO_4 , 1.25 mM CaCl_2 , 10 mM HEPES, 2.9 mM Na_2HPO_4), and were pre-incubated with compounds (250 μ L) for 5 min at room temperature. The transport reaction was initiated by addition of 50 μ L ^3H -2-deoxy-glucose solution (0.625 μCi ; final concentration, 750 μM) and incubated for 15 min at room temperature. After the incubation, the 2-DG uptake was halted by aspiration of the incubation mixture. Cells were immediately washed three times with ice-cold PBS and were solubilized in 300 μ L of 0.3 N NaOH. The radioactivity associated with the cells was determined by a liquid scintillation counter (Quantsmart™ (Packard, Boston, MA, USA)).

Rat Urinary Glucose Excretion (rUGE) Study.

Male Sprague-Dawley (SD) rats aged 4-5 weeks were obtained from Japan SLC (Shizuoka, Japan) and were used for experiments at 6 weeks of age after acclimation period. The animals were divided into experimental groups matched for body weight ($n = 2-3$). The compounds were prepared in vehicles as suspension or solution. UGE studies were performed after two-day acclimation period in metabolic cages. The compounds or vehicle were orally administered at a dose of 30 mg/kg in 0.2% CMC/0.2% Tween 80. Urine samples were collected for 24 hours using metabolic cages to measure urinary glucose excretion. Urine glucose contents were determined by an enzymatic assay kit (UGLU-L, Serotec, Hokkaido, Japan) and normalized per 200 g body weight. All animals were allowed free access to a standard pellet diet (CRF1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water.

Blood Glucose-Lowering Study in Hyperglycemic High-Fat Diet-Fed KK (HF-KK) mice (Single Oral Dosing Study).

Male KK/Ta Jcl mice aged 9 weeks were obtained from CLEA Japan Inc. (Tokyo, Japan) and kept on a standard diet (CRF-1; 5.7% (w/w) fat, 3.59 kcal/g, Oriental Yeast Co., Ltd., Tokyo, Japan), 20-week-old mice were fed with a high-fat diet (60 kcal%, Research Diets, Inc., New Brunswick, NJ) for 4 weeks. The experiment was carried out at the age of 24 weeks. Male C57BL/6N mice aged 11 weeks were obtained from Charles River Laboratories Japan, INC. (Yokohama, Japan) and were also used in this study. The animals were divided into experimental groups matched for body weight and blood glucose levels, which were measured in the fed state on the day of the experiment.

The compounds (3 mg/kg) or vehicle (0.2% CMC/0.2% Tween 80) were orally administered at a volume of 10 mL/kg. The blood samples were collected from the tail vein before and at 1, 2, 4, 6 and 24 hr after the administration.

The blood glucose level was determined using commercially available kits based on the glucose oxidase method (Glucose CII-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). Data are expressed as means \pm SEM. Area under the curve for blood glucose levels ($AUC_{0-24hr}BG$) was calculated by the trapezoidal rule. Differences between groups were analyzed by repeated measurement ANOVA followed by Student's *t*-test (EXSAS, Arm Systex Co. Ltd.). Probabilities less than 1% ($P < 0.01$) were considered to be statistically significant.

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主論文目録

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