

Angular 型 5-6-5 縮合ヘテロ環の合成研究および

経口吸収性を改善した次世代型メラトニン受容体作動薬の創製

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## 緒言

現在臨床で使用されている低分子医薬品の多くが、その分子内にヘテロ環を有している。とりわけ近年の創薬化学では極めて高い頻度でヘテロ環が採用されており、2020年にFDAに承認された34個の低分子薬のうち31個がヘテロ環誘導体である。ヘテロ環は、分子に適度な極性を付与する、標的タンパク質と水素結合する、環上の置換基の向きを規定するなど、分子がその機能を発揮するための重要な役割を担っており、医薬品の活性、安全性、薬物動態に大きな影響を与える。このように、ヘテロ環の探索は優れた医薬品の創出において非常に重要な要素である。

縮合ヘテロ環は、単環と比較して水素結合や置換基の向きがさらに固定されていることを特徴としており、中でも三つのヘテロ環が縮合した三環性縮合ヘテロ環は、活性、安全性、薬物動態の改善効果という点で特に注目されている。さらには、その特徴的な化学構造から新たなケミカルスペースを開拓することが可能であり、新規ターゲットや新規作用機序の発見も期待される。

ラメルテオンは著者が所属する武田薬品工業で創製されたメラトニン受容体 ( $MT_1$  および  $MT_2$ ) アゴニストで、<sup>1</sup> 不眠症治療薬として 2005 年に日本、2010 年に米国にて承認された。ラメルテオンは、天然リガンドであるメラトニンをもとにデザインされ、新規三環性縮合ヘテロ環であるインデノ[5,4-*b*]フラン環を母核として有する (Figure 1)。インデノ[5,4-*b*]フラン環は、メラトニンのインドール環をインダン環に変換しさらにフラン環を縮合させた構造を持ち、3環が湾曲上に縮環した angular 型 5-6-5 縮合ヘテロ環に分類される。本骨格の発見により活性向上、オフターゲット選択性向上、代謝安定性向上が達成され、その結果としてメラトニンの種々プロファイルが改善し、ラメルテオンの上市につながった。これは、angular 型 5-6-5 縮合ヘテロ環の優れた有用性を示す好例である。

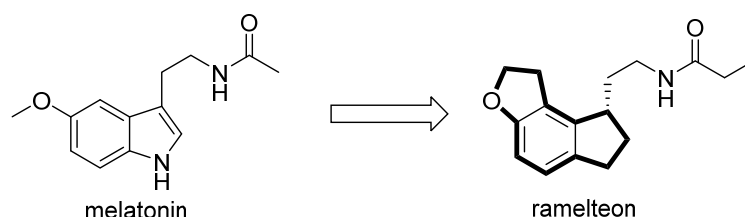
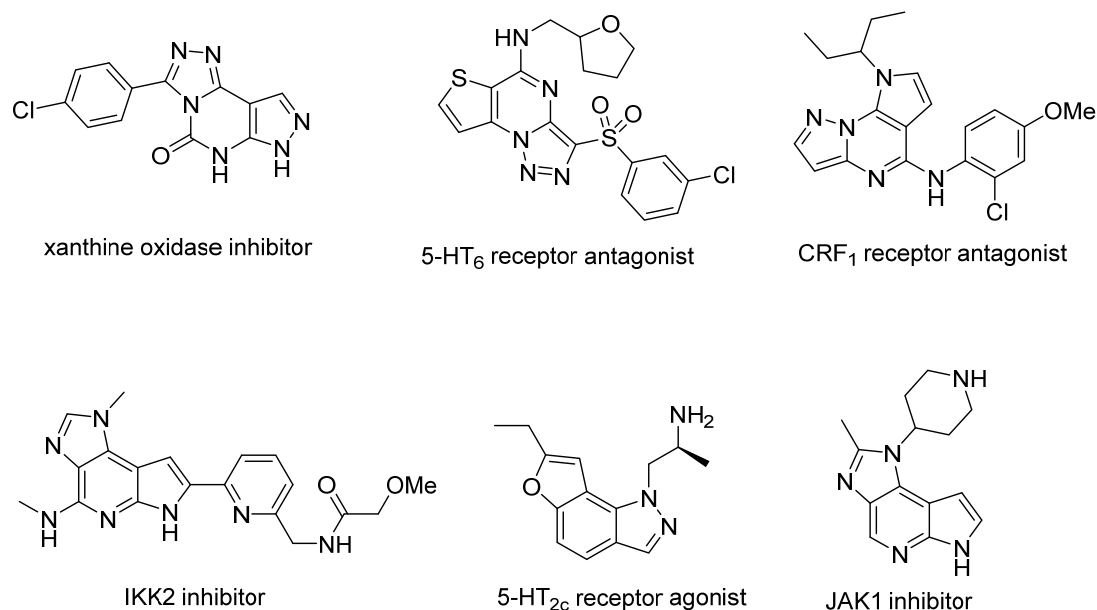


Figure 1. Discovery of ramelteon.

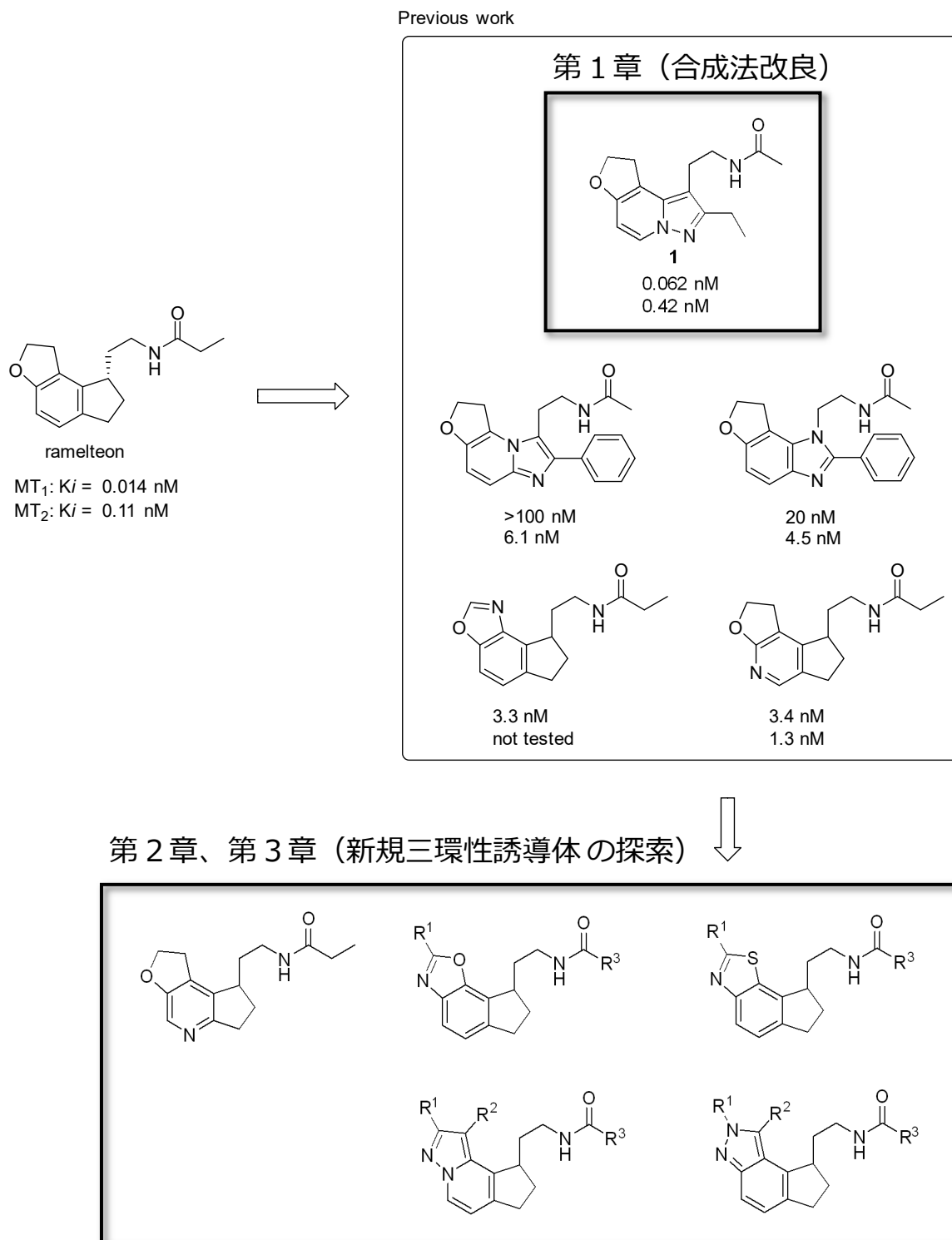
そのほか、angular 型 5-6-5 縮合ヘテロ環を有する種々の生理活性物質として、キサンチンオキシダーゼ阻害剤、<sup>2</sup> セロトニン 6 (5-HT<sub>6</sub>) 受容体アンタゴニスト、<sup>3</sup> コルチコトロピン放出因子 (CRF<sub>1</sub>) 受容体アンタゴニスト、<sup>4</sup> IκB キナーゼ 2 (IKK2) 阻害剤、<sup>5</sup> セロトニン 2C (5-HT<sub>2c</sub>) 受容体アゴニスト、<sup>6</sup> ヤヌスキナーゼ 1 (JAK1) 阻害剤<sup>7</sup> などが報告されている (Figure 2)。これらの化合物はいずれも、二環性誘導体と比較して活性増強や標的外分子に対する選択性の向上が達成されている。このように、angular 型 5-6-5 縮合ヘテロ環は生理活性物質のプロファイルを改善しうる極めて有用な化学構造であり、その合成研究は医薬品化学において非常に価値が高い。



**Figure 2.** Chemical structures of biologically active angularly fused 5-6-5 heterocyclic derivatives.

このような背景のもと著者は、angular 型 5-6-5 縮合ヘテロ環を母核として有する MT<sub>1</sub>/MT<sub>2</sub> アゴニストの合成研究に着手した。MT<sub>1</sub>/MT<sub>2</sub> アゴニストは、睡眠誘発作用を発揮するだけでなく、<sup>8</sup> うつ、<sup>9</sup> 不安、<sup>10</sup> 癌、<sup>11</sup> パーキンソン病<sup>12</sup> の治療薬としての可能性も秘めている。一方で、不眠症治療薬として承認された唯一の MT<sub>1</sub>/MT<sub>2</sub> アゴニストであるラメルテオンはヒトでの経口吸収性が 2%未満であることが臨床試験において示されている。<sup>13</sup> さらに、経口吸収性の個人差が極めて大きく (C<sub>max</sub> および AUC が最大数十倍の差)、<sup>13</sup> 血中濃度が高い患者では昼間に眠気が残ってしまうこともある。個人差を改善する方法としては、経口吸収性の向上が最も効果的であることから、経口吸収性の向上した MT<sub>1</sub>/MT<sub>2</sub> アゴニストの創製を目指すこととした。また、angular 型 5-6-5 縮合ヘテロ環は医薬品開発において有用な化学構造であるので、その効率的合成法を構築することにより様々な創薬ターゲットへの応用も期待できる。

経口吸収性の向上を目的に、**Figure 3** に示すような angular 型 5-6-5 縮合ヘテロ環誘導体を著者らはこれまで合成してきているが、<sup>1,14</sup> その多くが活性が大きく減弱する結果となっている。第一章では、その中でも唯一有望な化合物として見出されたフロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **1** について、その合成法の改良を述べる。第二章および第三章では、angular 型 5-6-5 縮合ヘテロ環誘導体のさらなる探索について述べる。

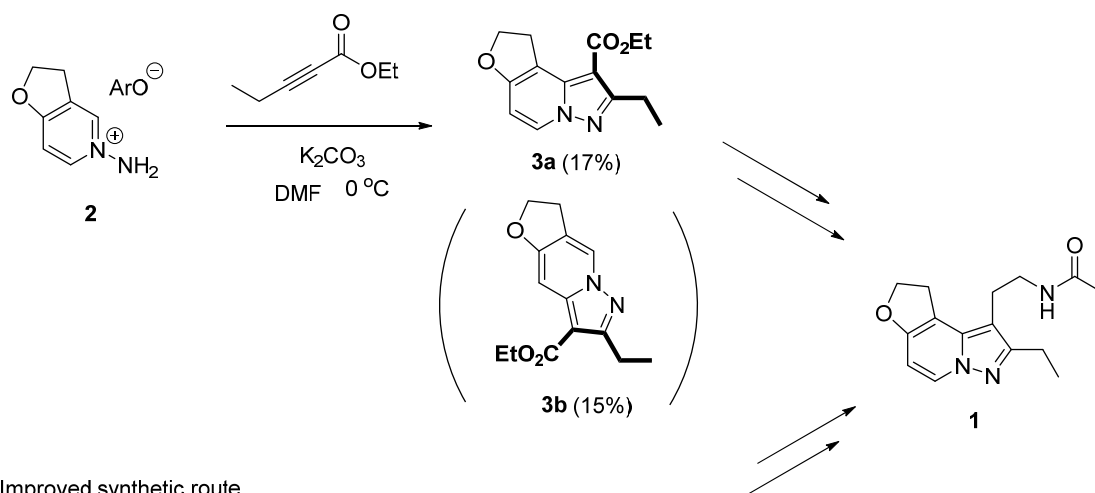


**Figure 3.** Previous and this work on MT<sub>1</sub>/MT<sub>2</sub> agonist with angular fused 5-6-5 heterocycles.

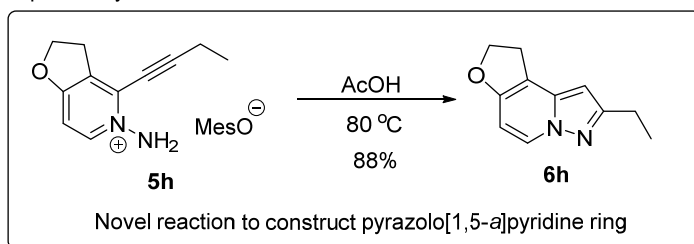
## 1) フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体の合成研究

著者らは、経口吸収性改善が期待できる MT<sub>1</sub>/MT<sub>2</sub> アゴニストとして、フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **1** を既に報告している (Scheme 1)。<sup>14a</sup> しかしながら、ピラゾロ[1,5-*a*]ピリジン環構築の工程に課題があり、スケールアップ合成に適していない。そこで、ピラゾロ[1,5-*a*]ピリジン環構築のための新規反応開発に着手した。その結果、*N*-アミノ-2-アルキニルピリジン誘導体 **5h** を酢酸中で加熱することでピラゾロ[1,5-*a*]ピリジン **6h** が効率よく合成できることを見出した。本反応の発見により、フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **1** の合成における総収率の大幅な向上を達成した。さらに、本反応は基質一般性が高く、多種多様なピラゾロ[1,5-*a*]ピリジン誘導体を高収率で合成することに成功した。これらの詳細について第一章で述べる。

Our previous synthetic route



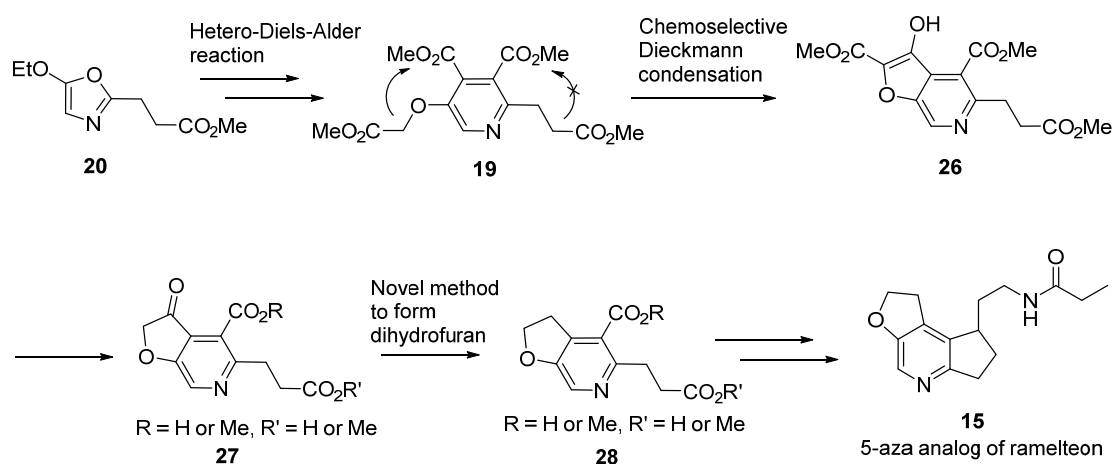
Improved synthetic route



**Scheme 1.** Improvement of synthetic route to compound **1**.

## 2) シクロペンタ[*b*]フロ[3,2-*d*]ピリジン誘導体の合成研究

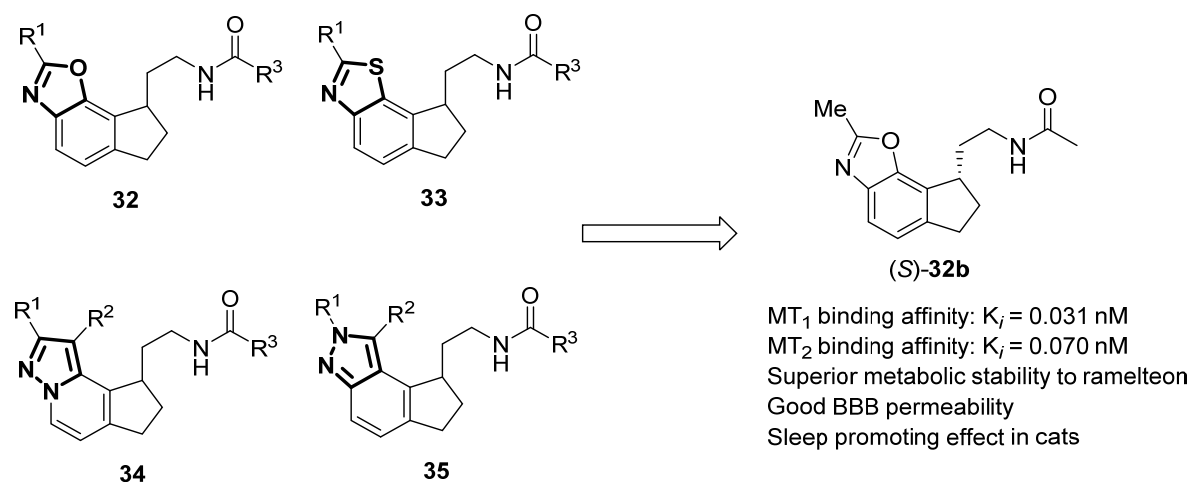
ラメルテオンは、ヒトでの経口吸収性が 2%未満であることが臨床試験において示されており、主な原因は酸化代謝による初回通過効果であると推察されている。そこで、酸化代謝に対する安定性を向上した MT<sub>1</sub>/MT<sub>2</sub> アゴニストの創製を目指し、シクロペンタ[*b*]フロ[3,2-*d*]ピリジンを母核として有する 5-アザラメルテオン **15** をデザインした (Scheme 2)。**15** は、ラメルテオンと比較して脂溶性が低減しているため、代謝安定性の向上が期待できる。一方で、シクロペンタ[*b*]フロ[3,2-*d*]ピリジン環は新規のヘテロ環であり、その合成法は知られていない。**15** の合成は、ヘテロ Diels–Alder 反応とそれに続く官能基選択的 Dieckmann 縮合反応を応用することにより達成した。また、その検討の中で、水素添加反応のみで一挙にオキソフラン体 **27** からジヒドロフラン体 **28** へと変換する新たな方法を見出した。最後に、5-アザラメルテオン **15** の MT<sub>1</sub>/MT<sub>2</sub> 結合親和性の測定を実施した。これらの詳細について第二章で述べる。



Scheme 2. Synthesis of 5-aza analog of ramelteon.

### 3) ラメルテオンのジヒドロフラン環をアゾール環に変換した新規三環性誘導体の合成研究

ラメルテオンのインダン環への窒素原子導入が  $MT_1/MT_2$  結合親和性の減弱をもたらすことが、著者らのこれまでの報告および前章にて示された。そこで、結合親和性を減弱させることなく代謝安定性を向上させることを目的として、ラメルテオンのジヒドロフラン環をアゾール環に変換した新規三環性誘導体の合成研究を計画した (Figure 4)。デザインしたインデノ[5,4-*d*][1,3]オキサゾール誘導体 (32)、インデノ[5,4-*d*][1,3]チアゾール誘導体 (33)、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 (34)、シクロペンタ[*e*]インダゾール誘導体 (35) は、脂溶性低減による代謝安定性向上だけでなく、アゾール環が  $MT_1/MT_2$  と水素結合することによる強力な結合親和性が期待できる。いずれの骨格も合成法が知られておらず、各骨格の合成ルートを新たに構築した。 $MT_1/MT_2$  結合親和性を測定した結果、母核および側鎖 ( $R^1$  基、 $R^2$  基) の構造活性相関情報が得られ、 $MT_1/MT_2$  リガンドの合成研究に新たな知見を与えた。 $MT_1/MT_2$  結合親和性およびヒト肝ミクロソーム中における代謝安定性を指標に構造最適化を行ったところ、強力な  $MT_1/MT_2$  結合親和性と優れた代謝安定性を併せ持つ (S)-32b を見出した。(S)-32b は  $MT_1/MT_2$  に対してフルアゴニストとして作用し、ラットにおいて良好な脳内移行性を示すとともに、ネコを用いた睡眠評価モデルにおいて睡眠誘発作用を示した。これらの詳細について第三章で述べる。



**Figure 4.** Discovery of potent and orally bioavailable melatonin receptor agonist (S)-32b.

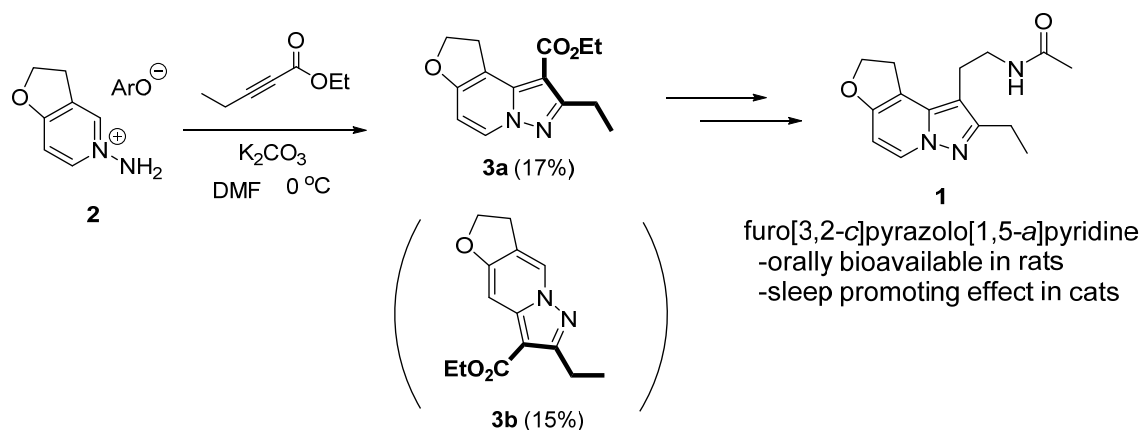
## 第1章

### フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体の合成研究

#### 第1節 ピラゾロ[1,5-*a*]ピリジン環の新規合成法の開発

著者らは、Angular 型 5-6-5 縮合ヘテロ環を有する MT<sub>1</sub>/MT<sub>2</sub> アゴニストとしてフロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **1** を既に報告している (Scheme 3)。<sup>14a</sup> **1** は MT<sub>1</sub> および MT<sub>2</sub> にフルアゴニストとして作用し、ラットにおいて良好な経口吸収性を示すとともにネコへの経口投与により睡眠導入作用を示したことから、次世代の MT<sub>1</sub>/MT<sub>2</sub> アゴニストとして有望な化合物である。一方で、著者らが既に報告した合成ルートは、ピラゾロ[1,5-*a*]ピリジン環構築の際の収率が低いだけでなく (**3a**、17%収率)、除去が困難な異性体 **3b** を生成するため、スケールアップ合成に適していない。臨床試験に向けた種々の評価にはより多くの化合物量が必要になるため、合成ルートの改良は必要不可欠である。このような背景のもと著者は、ピラゾロ[1,5-*a*]ピリジン環を効率良く構築する新規反応の探索に着手した。

ピラゾロ[1,5-*a*]ピリジン環構築は、最も一般的な方法として *N*-アミノピリジン誘導体とアルケン又はアルキン誘導体による分子間[3+2]環化付加反応が知られている。<sup>15</sup> しかしながら、非対称な *N*-アミノピリジン誘導体からの合成では、Scheme 3 で示すように位置異性体が非選択的に生成する。<sup>14a,15b,15e</sup>

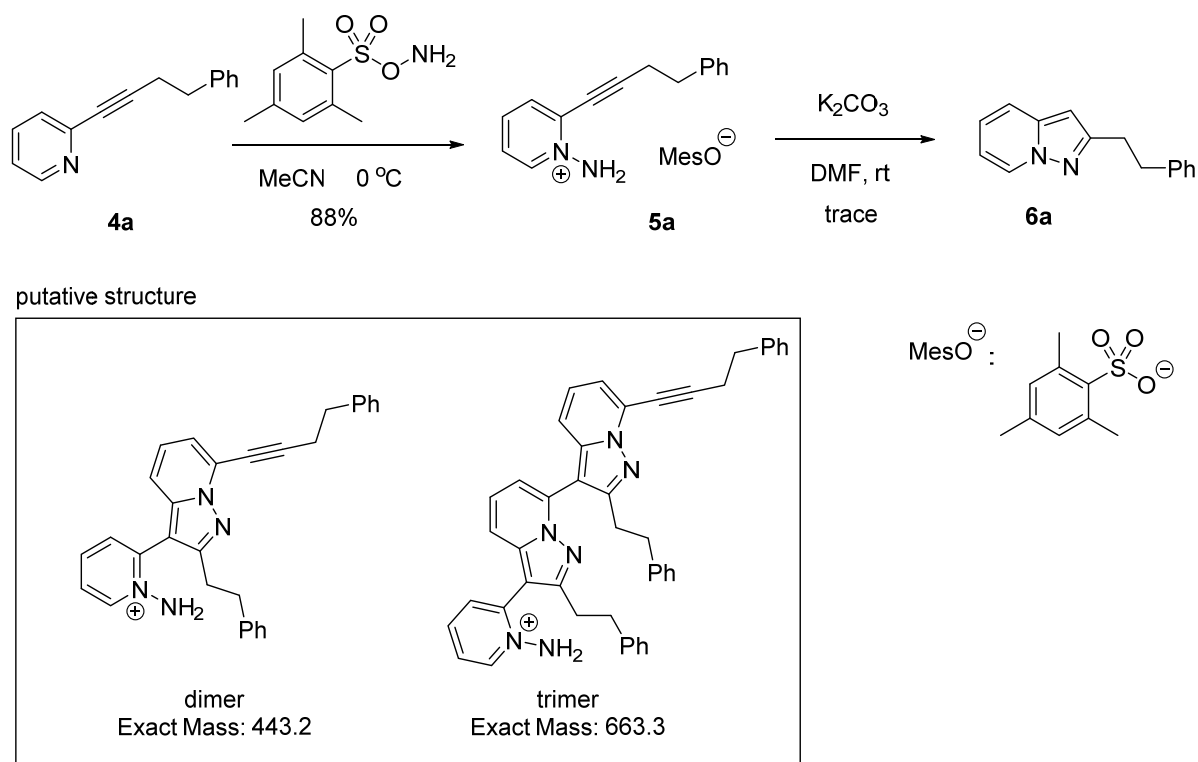


Scheme 3. Melatonin receptor agonist **1** and its synthetic route.

[*Tetrahedron Lett.* **2013**, 54, 2199–2202, Scheme 1 with modifications]

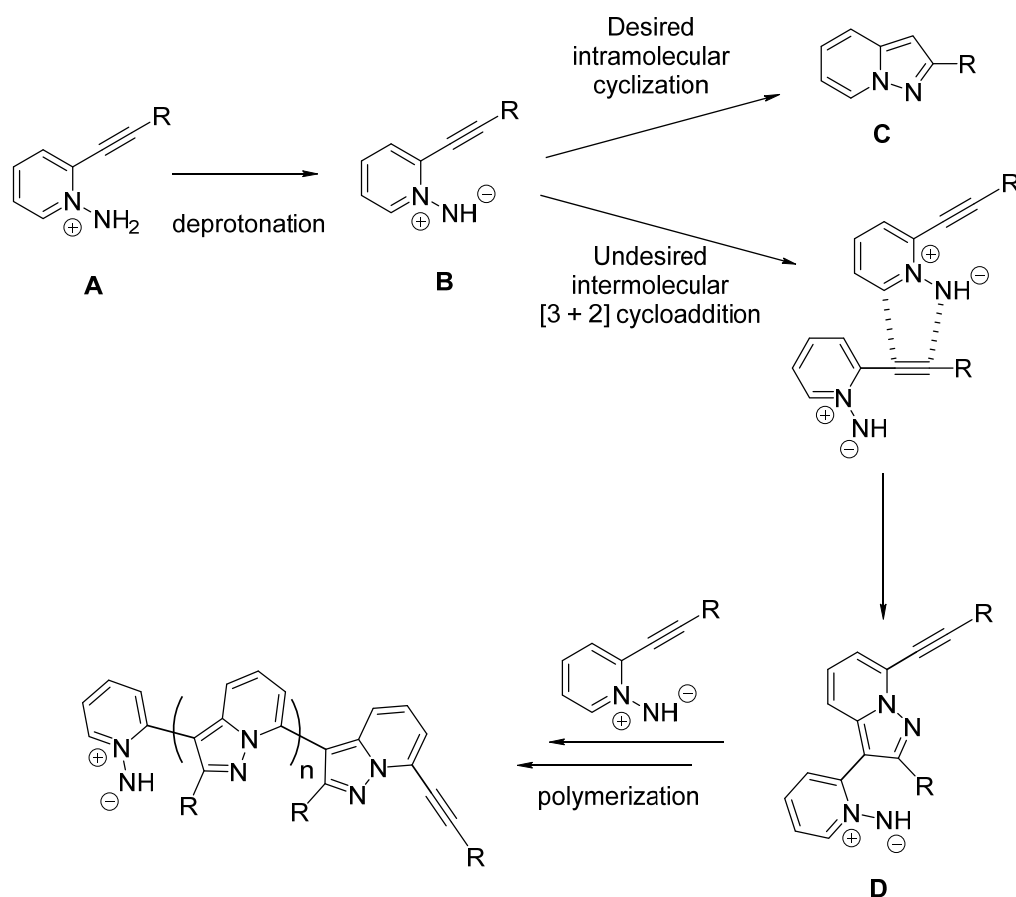
そこで著者は、位置異性体を生成しない分子内環化反応に着目した。分子内環化反応は様々な種類の反応が報告されているものの、その多くが基質一般性に乏しく、<sup>16-19</sup> 化合物 **1** の合成に適用できない。一方で、比較的汎用性の高い合成法として *N*-アミノ-2-アルキニルピリジ

ン誘導体を用いた分子内環化反応が Tsuchiya らに報告されており、<sup>20</sup> 種々の生理活性物質の合成に応用されている。しかしながら、いずれの例においても収率が低い結果となっており、<sup>21</sup> 未だ改良の余地がある。実際に、モデル基質 **5a** (2-アルキニルピリジン **4a** を文献既知の方法<sup>20</sup> でアミノ化することにより得た) を用いて Tsuchiya らの報告と同じ条件でピラゾロ[1,5-*a*]ピリジン誘導体の合成を試みたところ (Scheme 4)、複雑な混合物を与えるのみで、目的とする[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン **6a** は痕跡量でしか得られなかった。



**Scheme 4.** Intramolecular cyclization with known method

LCMS 分析の結果、反応混合物中にアミノピリジン **5a** の二量体および三量体と思われる分子イオンピークを検出した (Found: *m/z* 443.3, 663.5)。推定構造を Scheme 4 に示す (Exact Mass: 443.2, 663.3)。二量体の生成は、脱プロトン化により生成した 1,3-dipole **B** と別分子のアルキンによる分子間[3+2]環化付加反応が進行した結果であると考えられる (Scheme 5)。さらに、二量体 **D** は依然として[3+2]環化付加反応の基質となっているので、[3+2]環化付加反応が引き続き進行することで、多量体が形成されると予想される。実際に、三量体の分子イオンピークが検出され、また多量体とみられる不溶物が反応系中に多くみられた。すでに報告されたいくつかの例<sup>21</sup> における収率の低さも、同様にこの副反応によるものと推察される。



**Scheme 5.** Plausible reaction pathway through 1,3-dipole **B**.

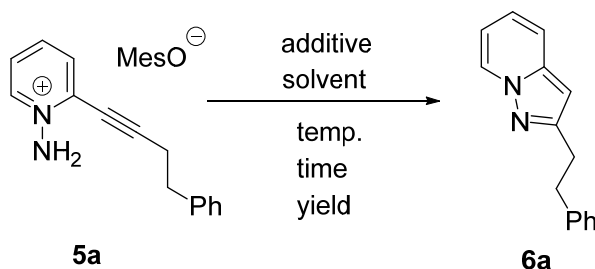
[*Tetrahedron Lett.* **2013**, 54, 2199–2202, **Scheme 2** with modifications]

以上の考察を元に、ピラゾロ[1,5-*a*]ピリジン誘導体を効率的に得るためには、分子間[3+2]環化付加反応の抑制が鍵であると考えた。分子間反応の抑制を目的とした新たな反応条件の検討結果を **Table 1** に示す。まず初めに、低濃度で反応を進行させることによる分子間反応の抑制を狙った。溶媒量の増量により濃度を低くする手法は直接的なアプローチであるものの、スケールアップが難しくなることや環境への負荷が高くなるため、別の方法を試みた。すなわち、中性リン酸緩衝液を用いることで収率の改善を狙った。塩基性条件下では脱プロトン化が一挙に進行し全ての基質が 1,3-dipole となる一方で、中性緩衝液中では 1,3-dipole が低濃度で維持される。基質の濃度が低いほど分子間反応が抑制されるため、分子内環化反応が優先的に進行すると考えられる。実際にエタノールと中性リン酸緩衝液 (pH 7.3) の混合液中で反応を実施したところ目的物 **6a** が 49%の収率で得られ、大幅な収率の改善が見られた (entry 1)。しかしながら、緩衝液条件下においても依然として副生成物 **D** の生成が確認された。そこで、1,3-dipole の生成しない反応条件を検討することによりさらなる収率向上を目指した。1,3-dipole はアミノピリジンの脱プロトン化により生成するため、脱プロトン化しない反応条件として、添加剤無しの条件 (entry 2) および酸性条件 (entries 3 and 4) にて反応を実施した。しかしながらこれらの条件下では全く反応は進行せず、原料を回収するのみであった。次に、添加剤を加えず 80°Cで加熱したところ、目的とする分子内環化反応が進行し **6a** が 63%の収

率で得られた (entry 5)。1,3-dipole を生成させないという狙い通り、本反応条件下では二量体の生成が確認されなかった。さらに、酢酸を溶媒として用いたところ、**6a** が 94% という極めて優れた収率で得られた (entry 6)。塩基による活性化を経ることなく、*N*-アミノ-2-アルキニルピリジン誘導体からピラゾロ[1,5-*a*]ピリジン誘導体を得た初めての例である。

**Table 1.** Exploration of intramolecular cyclization reaction conditions.

[*Tetrahedron Lett.* **2013**, 54, 2199–2202, **Table 1** with modifications]



entry	additive	solvent	temp.	time (hr)	yield of <b>6a</b> (%)
1	Phosphate buffer (pH 7.3)	EtOH	r.t.	24	49
2	None	DMF	r.t.	24	no reaction
3	None	AcOH	r.t.	24	no reaction
4	H <sub>2</sub> SO <sub>4</sub> (1.0 eq.)	DMF	r.t.	24	no reaction
5	None	DMF	80 °C	24	63
6	None	AcOH	80 °C	24	94

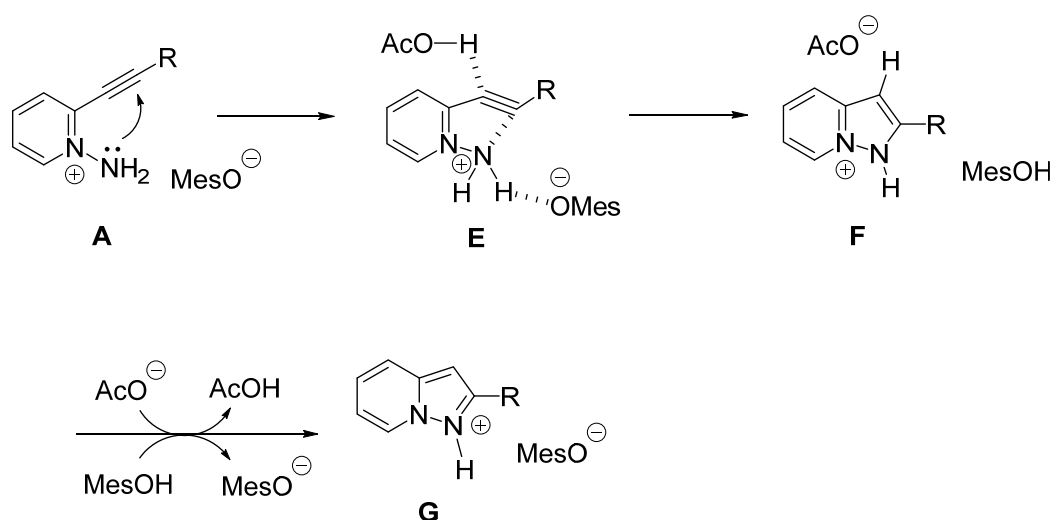
次にピリジン環上の R<sup>1</sup> 置換基およびアルキン末端の R<sup>2</sup> 置換基の一般性について検討した (Table 2)。ピリジン環の C5 位へ電子求引基である塩素基を導入したところ、無置換体 **6a** と同様に高い収率で化合物 **6b** が得られた (entry 1)。また、電子供与基であるメトキシ基を導入した場合においても高い収率で化合物 **6c** が得られた (entry 2)。ピリジン環の C3 位および C6 位にメチル基を導入した基質 (化合物 **6d** および **6e**) においても高い収率で目的物が得られた (entries 3 and 4)。次に、R<sup>2</sup> に *tert*-ブチル及びフェニル基を導入することでアルキン末端の一般性を検討したところ (化合物 **6f** および **6g**)、同様に高い収率で目的物が得られた (entries 5 and 6)。最後に縮合ピリジンを用いて検討したところ、高収率で化合物 **6h** が得られた。これらの結果から、本反応の開発により多種多様なピラゾロ[1,5-*a*]ピリジン誘導体を高収率かつ容易に合成できることが示された。

**Table 2.** Scope and limitation of substrates.

[*Tetrahedron Lett.* **2013**, 54, 2199–2202, **Table 2** with modifications]

	<b>4b-h</b>	<b>5b-h</b>	<b>6b-h</b>	
entry	yield of <b>5</b> (%)		yield of <b>6</b> (%)	
1	 <b>5b</b>	95	 <b>6b</b>	91
2	 <b>5c</b>	80	 <b>6c</b>	91
3	 <b>5d</b>	64	 <b>6d</b>	88
4	 <b>5e</b>	89	 <b>6e</b>	92
5	 <b>5f</b>	74	 <b>6f</b>	97
6	 <b>5g</b>	82	 <b>6g</b>	90
7	 <b>5h</b>	80	 <b>6h</b>	88

推定される反応機構を **Scheme 6** に示す。本反応は活性化剤として塩基を使用していないため、*N*-アミノピリジンの脱プロトン化が進行せず 1,3-dipole を生成しない。したがって、反応の開始はアミノ基の孤立電子対によるアルキン部位への攻撃であると考えられる。酢酸及びメシチレンスルホン酸アニオンが、それぞれ酸および塩基として働くことで効率よく環化反応が進行し (**E**)、ピラゾロ[1,5-*a*]ピリジンのプロトン化体 **F** が得られる。DMF 中にて反応を行った際に収率が中程度であったのは (**Table 1**, entry 6)、アルキンが円滑にプロトン化されず副反応が進行したためと考えられる。続いて酢酸アニオンとメシチレンスルホン酸がプロトン交換し、ピラゾロ[1,5-*a*]ピリジンのメシチレンスルホン酸塩 **G** が得られる。この生成物は反応系を酸性にするため、酸性条件下不安定な基質を用いる際には中性の緩衝液条件下 (**Table 1**, entry 1) で反応を行うのが望ましいと考えられる。



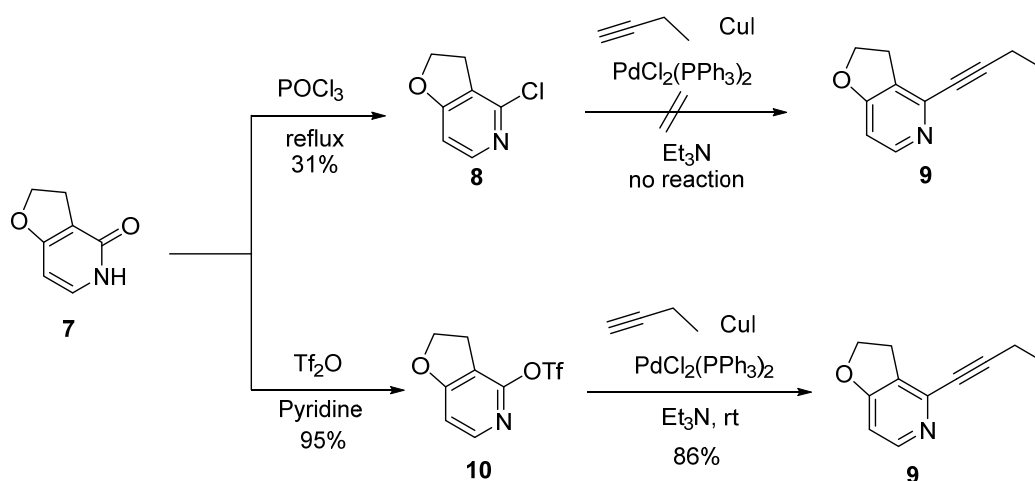
**Scheme 6.** Plausible reaction mechanism.

[*Tetrahedron Lett.* **2013**, 54, 2199–2202, **Scheme 3** with modifications]

以上述べてきたように、*N*-アミノ-2-アルキニルピリジン誘導体を酢酸中 80°Cでの加熱することで、ピラゾロ[1,5-*a*]ピリジンを効率良く合成することに成功した。また、本反応は基質一般性が高く、多種多様なピラゾロ[1,5-*a*]ピリジン誘導体を高収率かつ容易に合成できるため、ピラゾロ[1,5-*a*]ピリジン環を有する様々な生理活性物質の合成法として極めて有用である。

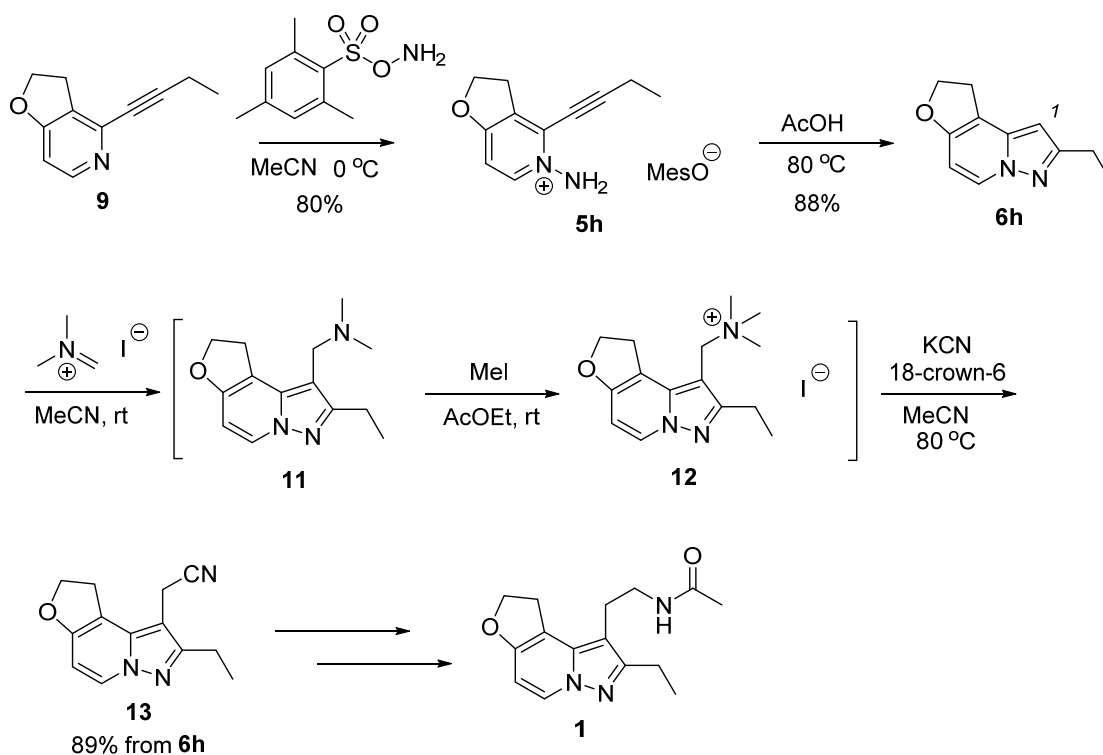
## 第2節 フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン誘導体合成への応用

前節において、高収率かつ異性体を生成することなくピラゾロ[1,5-*a*]ピリジン環を得る合成法を見出したので、続いて MT<sub>1</sub>/MT<sub>2</sub> アゴニスト **1** の合成ルートの改良に着手した。2-アルキニルピリジン誘導体 **9** の合成を **Scheme 7** に示す。まず初めに、既知化合物であるピリドン **7**<sup>22</sup> をオキシ塩化リンを用いてクロロ化したところ、クロロピリジン **8** が 31%の収率で得られた。この時、ジヒドロフラン環が開環したと思われる副生成物が検出された。続いて菌頭カップリングにてアルキニル基の導入を試みたが、通常の条件下では全く反応が進行しなかった。そこで、脱離基をより脱離能の高いトリフラート基に変えて再度菌頭カップリングを試みた。トリフルオロメタンスルホン酸無水物を用いたピリドン **7** のトリフラート化は効率良く進行し、トリフラート体 **10** を 95%の収率で得た。本基質を用いた場合には菌頭カップリングが問題なく進行し、2-アルキニルピリジン **9** を 86%の収率で得ることに成功した。



**Scheme 7.** Synthesis of 2-alkynyl pyridine

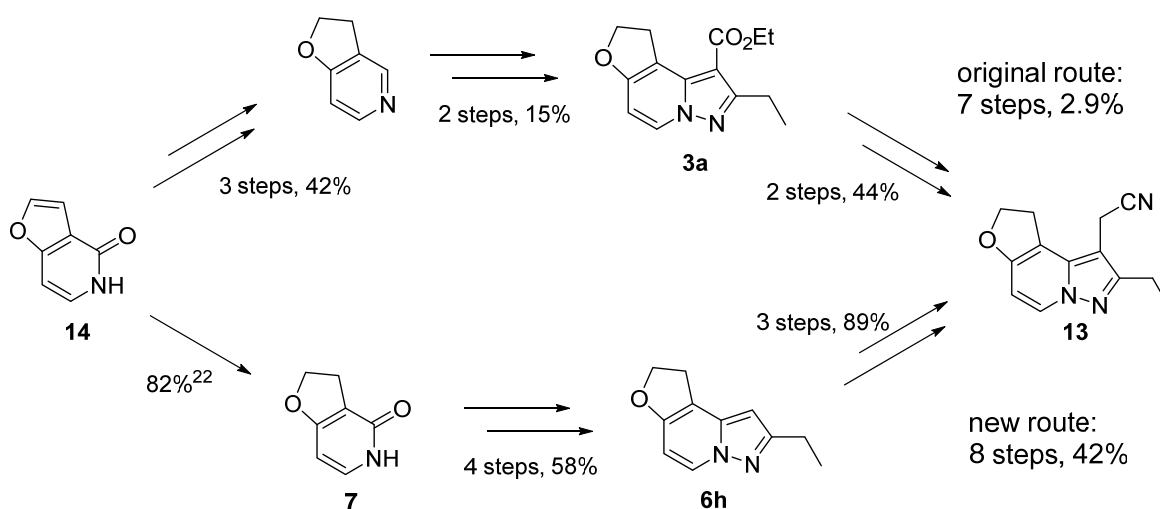
次に、化合物 **1** の既知合成中間体であるニトリル体 **13** の合成を **Scheme 8** に示す。2-アルキニルピリジン **9** をアミノ化剤<sup>23</sup> と反応させ、分子内環化反応の基質である *N*-アミノ-2-アルキニルピリジン **5h** を 80%の収率で得た後に酢酸溶媒中加熱することで、フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン **6h** が高い収率で得られた (88%収率)。フロ[3,2-*c*]ピラゾロ[1,5-*a*]ピリジン環の C1 位への置換基導入は、既知の方法<sup>24</sup> を参考に達成した。すなわち、Eschenmoser 塩で処理することでジメチルアミノメチル基を導入し、続いてアミノ基をメチル化することで脱離基に変換し、最後にシアノ基を求核置換反応させることで、既知中間体 **13**<sup>14a</sup> を高い収率で得た (3 工程 89%収率)。



**Scheme 8.** Synthesis of known intermediate **13** using novel intramolecular cyclization reaction.

[*Tetrahedron Lett.* **2013**, 54, 2199–2202, **Scheme 4** with modifications]

共通中間体 **14** から **13** までの総収率の比較を **Scheme 9** に示す。今回見出した新しい合成ルートでは、以前の合成ルート<sup>14a</sup>と比較して大幅に総収率が改善され (2.9% → 42%)、かつ位置異性体の生成を回避している。本合成ルートを開発した結果、より多くの化合物量を効率的に合成することが可能となった。



**Scheme 9.** Comparison with original synthetic route

## 第2章

### シクロペンタ[b]フロ[3,2-*d*]ピリジン誘導体の合成研究

#### 第1節 経口吸収性の改善を目指したドラッグデザイン

武田薬品工業で見出したラメルテオンは、ヒトでの経口吸収性が2%未満であることを緒言にて述べた。ヒトにおけるラメルテオンの放射性同位体および代謝物の解析から、経口吸収性が2%未満である主な原因は酸化代謝による初回通過効果であると推察されている。<sup>13</sup> このような背景から、著者は、酸化代謝に対する安定性を向上した MT<sub>1</sub>/MT<sub>2</sub> アゴニストの創製を目指すこととした。

代謝安定性を高める戦略としては、大きく二つのグループに分けられる。一つは代謝的に不安定な部位の修飾・変換であり、もう一つは分子全体の脂溶性の低減である。<sup>25</sup> 前者は代謝を防ぐシンプルかつ強力なアプローチであるが、化合物が複数の部位で代謝される場合には複数箇所の修飾・変換が必要であり、あまり効率的ではない。後者は、汎用的な方法であるものの、主活性の減弱を伴うケースが多い。ラメルテオンはジヒドロフラン環、シクロペントタン環及びアミド鎖などの複数の部位で代謝されることが報告されているため (Figure 5)、<sup>13</sup> 代謝的部位の修飾・変換ではなく、分子全体の脂溶性を低減させることによる代謝安定性向上を目指した。

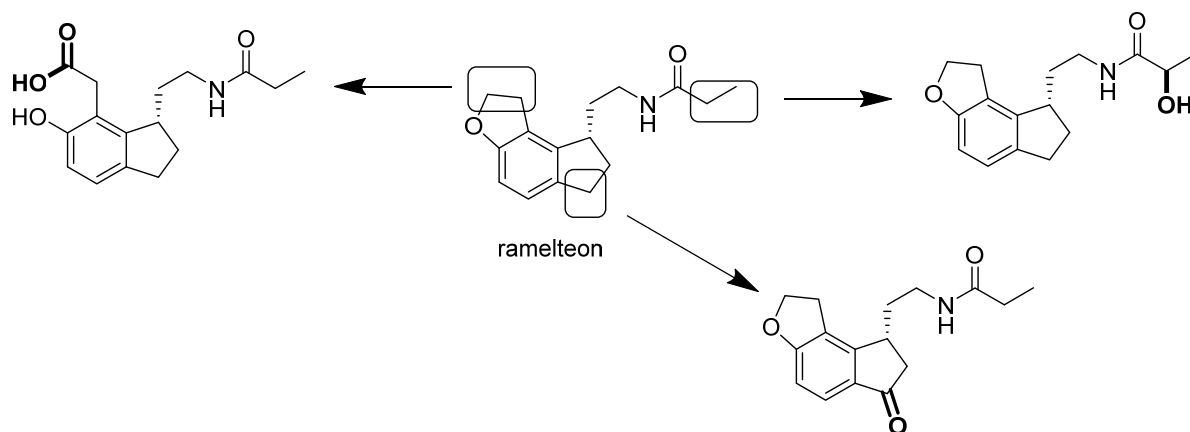
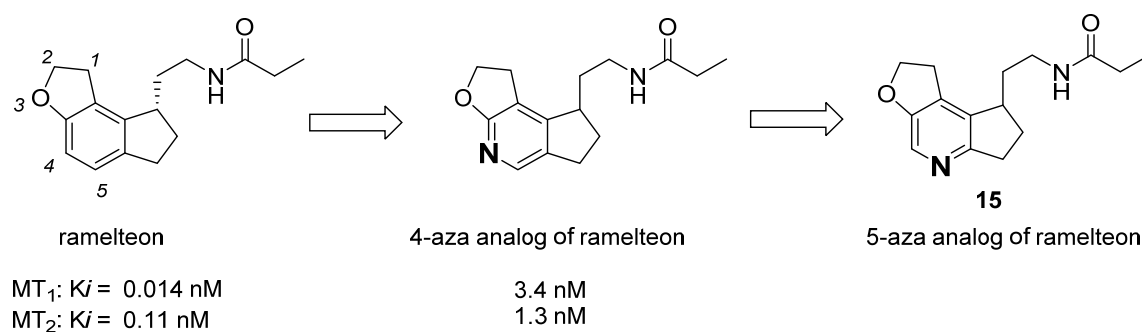


Figure 5. Metabolic pathway of ramelteon in human.

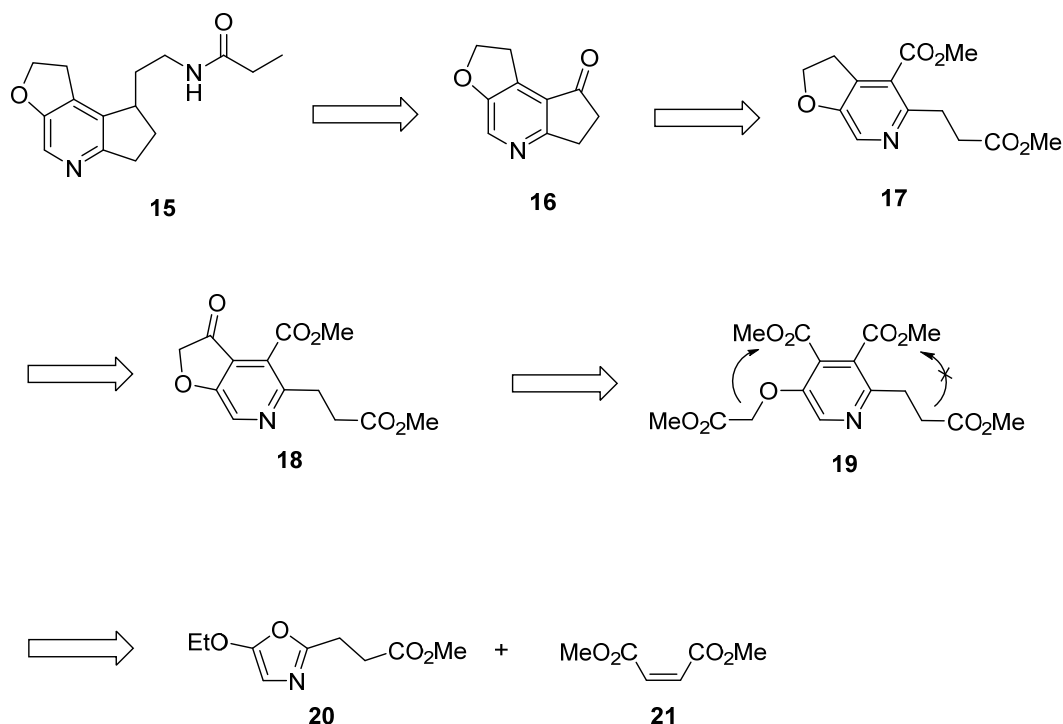
著者らは、ラメルテオンの母核であるインデノ[5,4-*b*]フラン環の C4 位の炭素原子を窒素原子に変換した 4-アザラメルテオンを既に報告している。ラメルテオンと同様にジヒドロフラン環とシクロペンタン環が中心六員環に対して **angular** 型に縮環しており、強力な活性が期待できる。しかしながら、活性の減弱が認められており、C4 位への窒素原子導入は許容されないことがわかった。そこで、続いて C5 位に窒素原子を導入した誘導体の活性を見極めるため、新規シクロペンタ[*b*]フロ[3,2-*d*]ピリジンを母核として有する 5-アザラメルテオン **15** をデザインした (Figure 6)。



**Figure 6.** Chemical structures of ramelteon, 4-aza analog of ramelteon, and 5-aza analog of ramelteon **15**

## 第2節 5-アザラメルテオンの合成および MT<sub>1</sub>/MT<sub>2</sub> 結合親和性

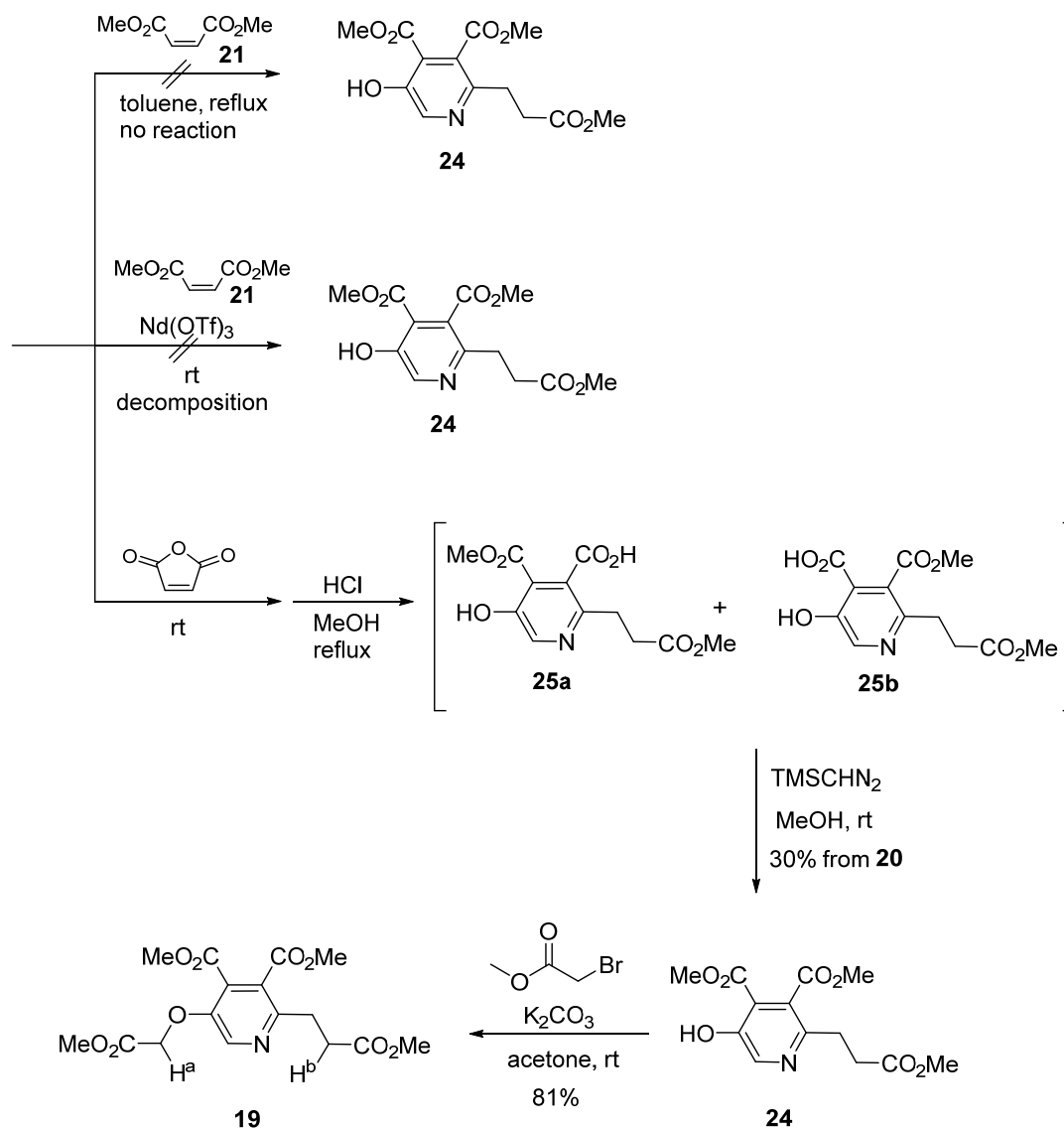
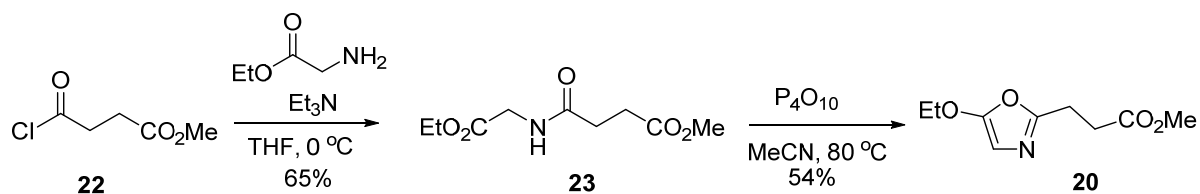
シクロペンタ[*b*]フロ[3,2-*d*]ピリジン環は新規のヘテロ環であり、その合成法は知られていない。そこで、逆合成解析を実施した (Scheme 10)。5-アザラメルテオン **15** のアミド側鎖の導入は、ラメルテオンの合成における既存の方法に従い、<sup>1</sup> 三環性ケトン体 **16** から容易に合成可能であると考えた。ラメルテオンの合成では、Friedel-Crafts 反応によりシクロペンタノン構造を構築したが、**15** は中心環としてピリジンを有するため、本骨格の合成には適応できないと予想した。そこで、堅実な方法として、ジエステル体 **17** の Dieckmann 縮合とそれに続く脱炭酸反応によりシクロペンタノン構造を構築することを計画した。フロ[2,3-*c*]ピリジンのジヒドロフラン環は、オキソフラン体 **18** から還元反応を含む工程を経て合成可能であり、本オキソフラン構造は、シクロペンタノン構造と同じく、Dieckmann 縮合とそれに続く脱炭酸反応を利用することで構築可能であることが知られている。<sup>26</sup> したがって、テトラエステル体 **19** より三環性ケトン体 **16** が合成可能であると考えた。しかしながら、二つの Dieckmann 縮合を同時に実施した後にオキソフラン構造の還元反応を実施した場合には、オキソフラン構造と同様にシクロペンタノン構造も還元される可能性が高い。したがって、官能基選択的に Dieckmann 縮合を進行させジヒドロフラン環を優先的に構築することが、三環性ケトン体 **16** の合成のための鍵反応となる。テトラエステル体 **19** は、オキサゾール誘導体 **20** とマレイン酸ジメチル **21** とのヘテロ Diels-Alder 反応により構築可能な、多置換型ピリジン誘導体を經由して合成可能であると考えた。<sup>27</sup>



**Scheme 10.** Retrosynthetic analysis of 5-aza analog of ramelteon **15**.

[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Scheme 1** with modifications]

官能基選択的 Dieckmann 縮合の基質である **19** までの合成を **Scheme 11** に示す。まず初めに、4-クロロ-4-オキソブタン酸メチル (**22**) をグリシンエチルエステルと縮合させアミド体 **23** を調製し (65% yield)、続いて五酸化二リンを用いて脱水環化させることでオキサゾール誘導体 **20** を得た (54% yield)。オキサゾール誘導体 **20** およびマレイン酸ジメチル **21** を用いてヘテロ Diels-Alder 反応を試みたが、既存の条件下<sup>27</sup> では、反応が進行しない、もしくは複雑な混合物を与えるのみであった。一方で、無水マレイン酸との反応条件<sup>28</sup> を試みたところ、ヘテロ Diels-Alder 反応は円滑に進行し、ピリジンカルボン酸の混合物 **25a** および **25b** を得た。フェノキシ基を反応させずにカルボン酸のみをメチル化するため、トリメチルシリルジアゾメタンを用いた。その結果、カルボン酸のみが選択的にメチル化され、トリエステル体 **24** を得ることに成功した (**20** からの総収率: 30%)。続いて、ブromo酢酸メチルを用いてフェノキシ基をアルキル化することで **19** を 81%の収率で得た。

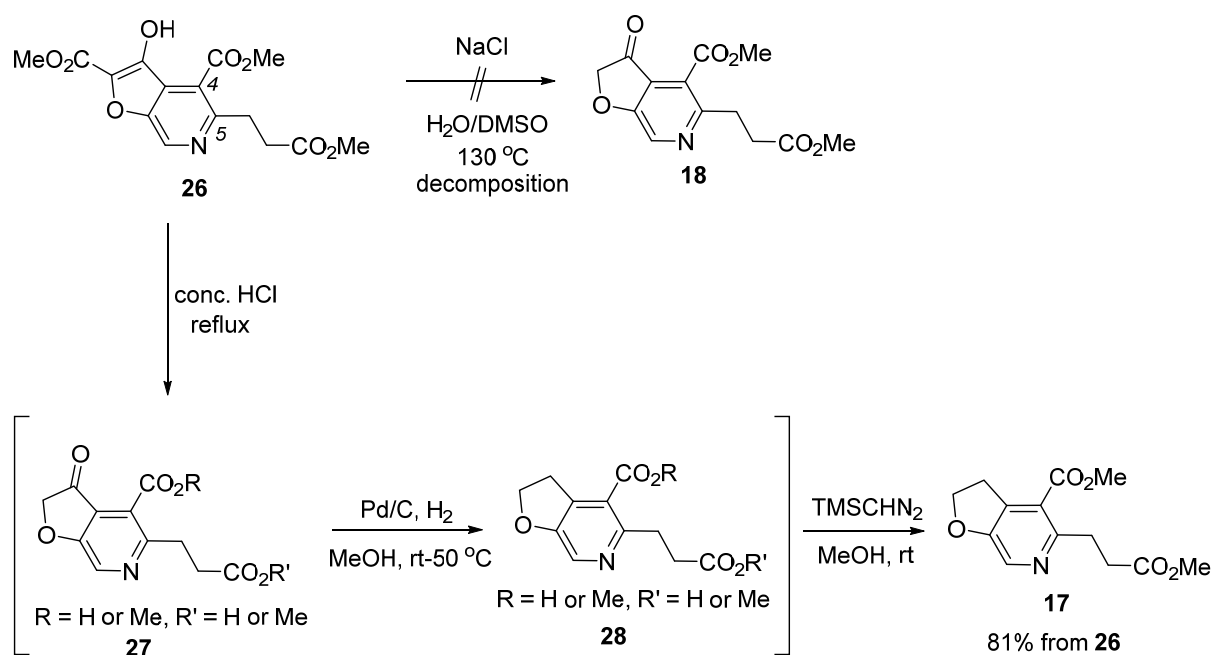


**Scheme 11.** Synthesis of substrate for chemoselective Dieckmann condensation.

[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Scheme 2** with modifications]

[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Scheme 3** with modifications]

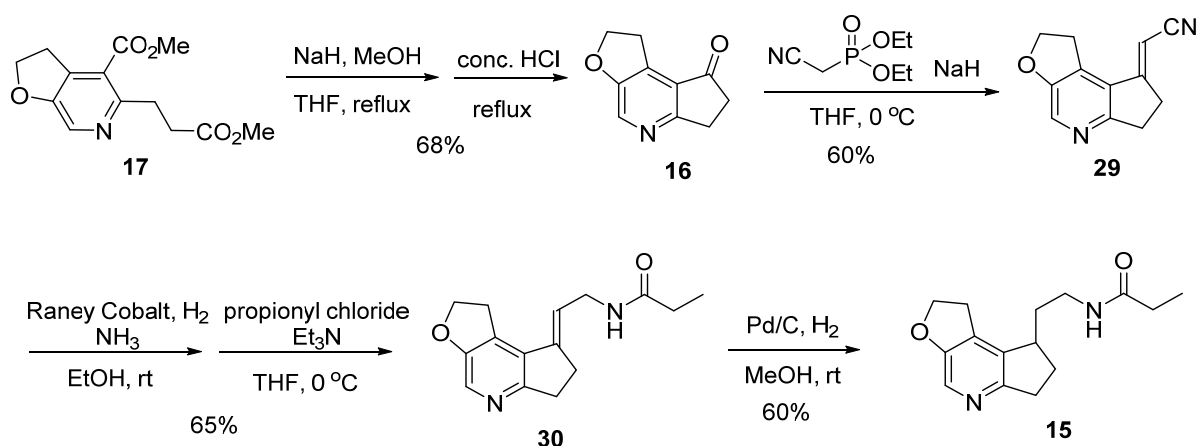
次に、ジヒドロフラン環への変換を実施した (**Scheme 13**)。まず初めに、C4 位および C5 位エステル基の加水分解を避けるため、Krapcho 脱炭酸反応<sup>30</sup>を試みた。しかしながら、この条件では複雑な混合物を与えるのみであった。そこで、濃塩酸中で脱炭酸を実施したところ、C4 位および C5 位エステル基が部分的に加水分解されたものの、脱炭酸反応は円滑に進行した (**26**→**27**)。オキソフラン環のジヒドロフラン環への変換は、ヒドリド還元、<sup>26a</sup> 脱水、<sup>26a</sup> 水素添加<sup>26b</sup>と、いくつかの工程を必要とすることが報告されているが、鋭意検討した結果、**27**に対してパラジウム触媒を用いた水素添加反応を行うことで一挙にジヒドロフラン環へと変換可能であることを見出した (**27**→**28**)。続いて、トリメチルシリルジアゾメタンを用いてカルボン酸をメチル化し、**17**を良好な収率 (**26**からの総収率：81%)で与えた。



**Scheme 13.** Transformation to dihydrofuran ring.

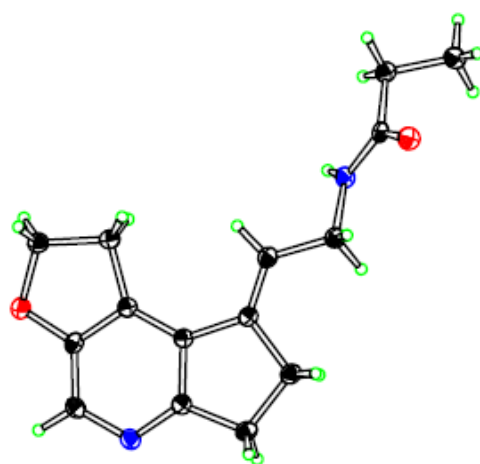
[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Scheme 4** with modifications]

シクロペンタ[*b*]フロ[3,2-*d*]ピリジン環の構築およびアミド側鎖の導入を **Scheme 14** に示す。一般的な条件<sup>29</sup> を用いて二回目の Dieckmann 縮合反応を実施した後に、脱炭酸条件に付することで、シクロペンタ[*b*]フロ[3,2-*d*]ピリジン環が構築され、三環性ケトン体 **16** が 68% の収率で得られた。続いて、既存の方法<sup>1</sup> に従ってアミド側鎖を導入した。シアノメチルホスホン酸ジエチルを用いた Horner–Wadsworth–Emmons 反応によりニトリル体 **29** を得た後、ニトリル基をラネーコバルトを用いた水素添加反応で還元し、続いてプロピオニルクロリドによりアシル化することで、不飽和アミド体 **30** を得た。**30** の X 線単結晶構造解析により、新規三環性シクロペンタ[*b*]フロ[3,2-*d*]ピリジン環の化学構造を確認した (**Figure 7**)。最後に、水素添加反応により二重結合を還元し、標的化合物である **15** の合成を達成した。



**Scheme 14.** Formation of cyclopenta[*b*]furo[3,2-*d*]pyridine ring and synthesis of 5-aza analog of ramelteon **15**.

[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Scheme 4** with modifications]



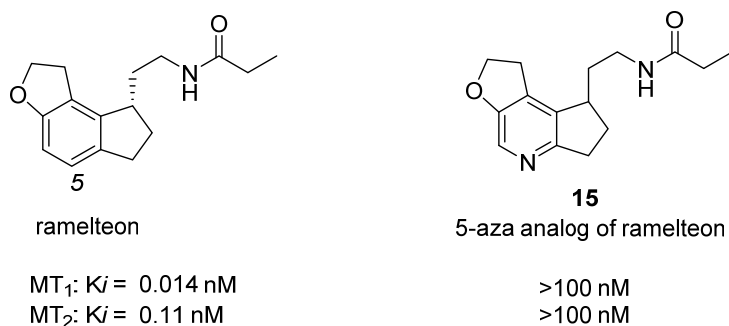
**Figure 7.** Single-crystal X-ray structure of compound **30**.

Black: carbon, green: hydrogen, red: oxygen, blue: nitrogen.

[*Tetrahedron Lett.* **2014**, 55, 4014–4016, **Figure 2** with modifications]

MT<sub>1</sub>/MT<sub>2</sub>に対する結合親和性を測定した結果、本化合物は MT<sub>1</sub>/MT<sub>2</sub> に対してわずかな結合親和性を示すのみで (MT<sub>1</sub>:  $K_i = >100$  nM, MT<sub>2</sub>:  $K_i = >100$  nM)、ラメルテオンと比較して大幅な結合親和性の低下が見られた (**Figure 8**)。ラメルテオンはインデノ[5,4-*b*]フラン環の C5 位部分がメラトニン受容体と疎水性相互作用することで強力な親和性を発現しており、C5 位への極性基導入により疎水性相互作用を失ったことが結合親和性低下の原因であると考えられる。

以上述べてきたように、ヘテロ Diels–Alder 反応とそれに続く官能基選択的 Dieckmann 縮合反応により、新規シクロペンタ[*b*]フロ[3,2-*d*]ピリジンを母核として有する 5-アザラメルテオン **15** の合成を達成した。さらに、水素添加反応のみで一挙にオキソフランからジヒドロフランへと変換する方法を見出した。



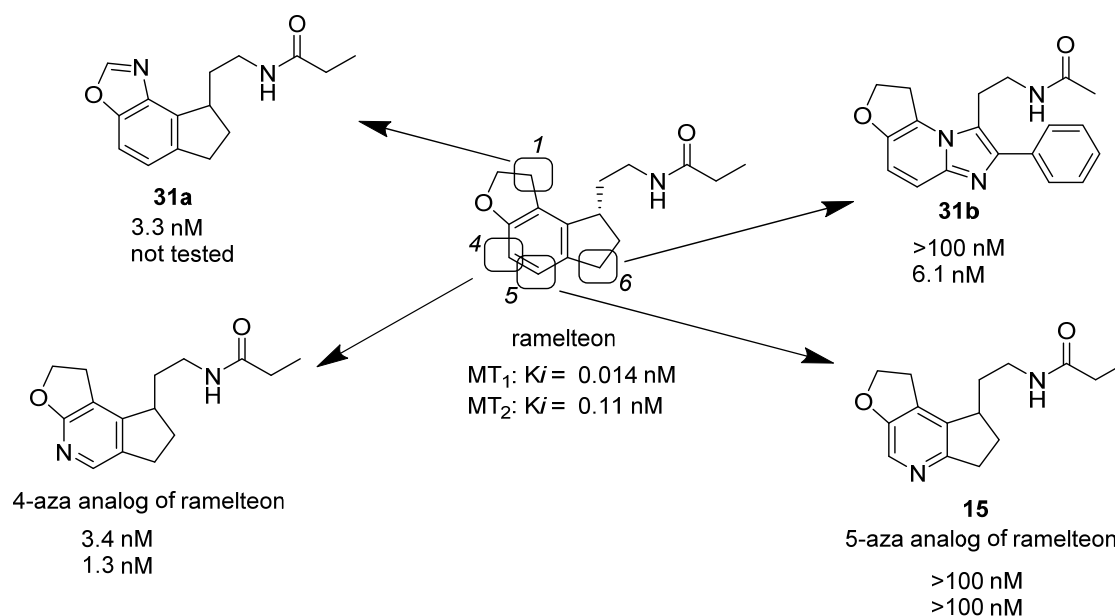
**Figure 8.** Binding affinities for human MT<sub>1</sub> and MT<sub>2</sub> receptors.

### 第3章

## ラメルテオンのジヒドロフラン環をアゾール環に変換した新規三環性誘導体の合成研究

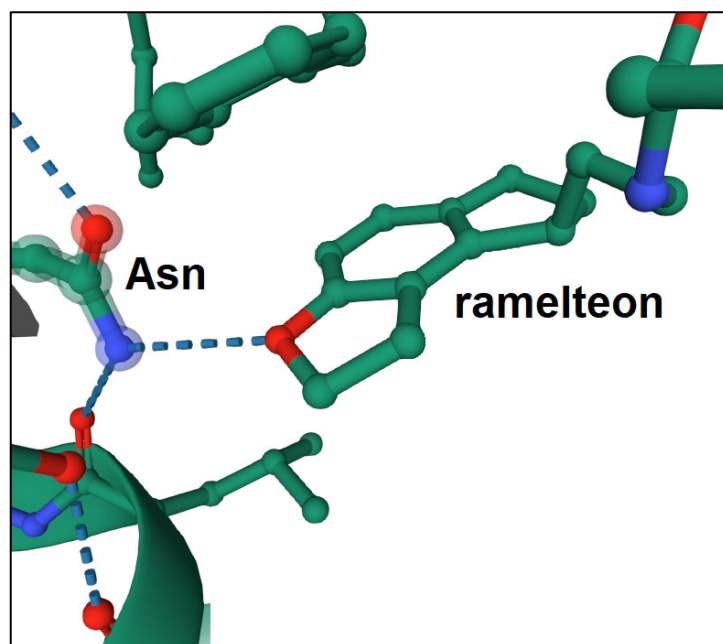
### 第1節 経口吸収性と MT<sub>1</sub>/MT<sub>2</sub> 結合親和性の両立を目指したドラッグデザイン

これまでに得られた母核の構造活性相関を **Figure 9** に示す。前章にて、代謝安定性を向上した MT<sub>1</sub>/MT<sub>2</sub> アゴニストの創製を目指し、ラメルテオンの母核であるインデノ[5,4-*b*]フラン環の C5 位を窒素原子に変換した 5-アザラメルテオン **15** を合成した。しかしながら、**15** の MT<sub>1</sub>/MT<sub>2</sub> に対する結合親和性は、ラメルテオンと比較して大きく減弱する結果となった。また、著者らは、インデノ[5,4-*b*]フラン環の C1 位、C4 位、C6 位に窒素原子を導入した種々の三環性誘導体についても報告しており (**31a**、4-アザラメルテオン、**31b**)、いずれもラメルテオンと比較して MT<sub>1</sub>/MT<sub>2</sub> への結合親和性が減弱している。<sup>1,14a</sup> このように、インデノ[5,4-*b*]フラン環の疎水部への極性基導入は、結合親和性を減弱させることがわかった。ラメルテオンの脂溶性部位は、MT<sub>1</sub>/MT<sub>2</sub> と疎水性相互作用することで強力な親和性発現に寄与していると考えられる。



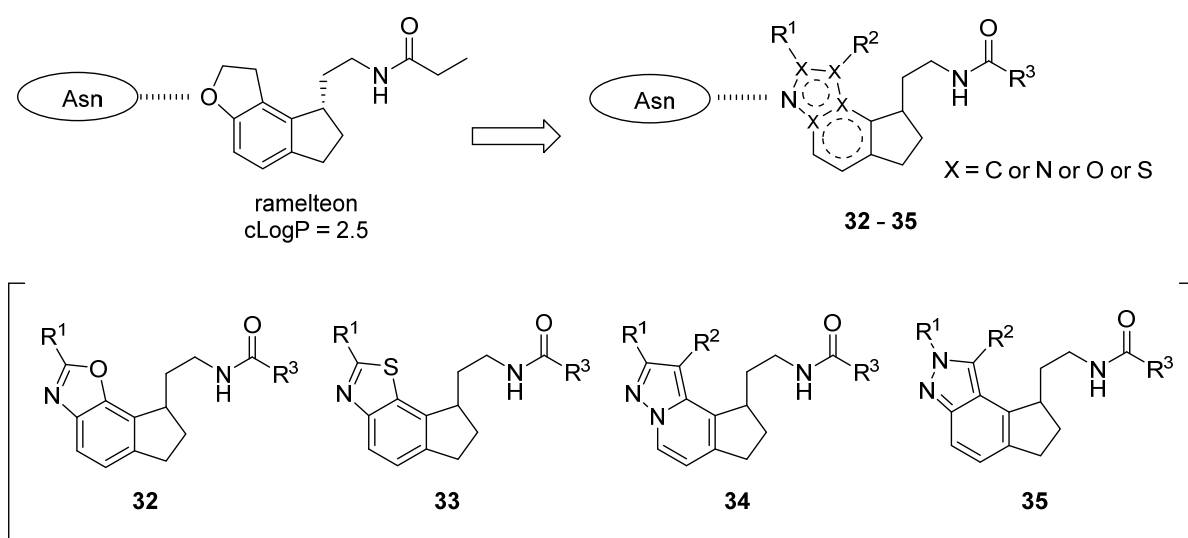
**Figure 9.** Structure-activity relationship on central tricyclic core.

近年、MT<sub>1</sub>およびMT<sub>2</sub>とラメルテオンとの複合体の X 線結晶構造解析により、ジヒドロフラン環の酸素原子が MT<sub>1</sub>およびMT<sub>2</sub>のアスパラギン (MT<sub>1</sub>: N162, MT<sub>2</sub>: N175) と水素結合していることが明らかとなった (**Figure 10**: for MT<sub>2</sub>/ramelteon)。<sup>31</sup> また、これまでに武田薬品工業で行われた MT<sub>1</sub>/MT<sub>2</sub> リガンドの合成研究の結果から、ジヒドロフラン環の酸素原子が強力な結合親和性を発揮するために重要であることが示唆されている。<sup>1,14,32</sup> これらのことから、極性基として作用しているジヒドロフラン環と同様の様式で MT<sub>1</sub>/MT<sub>2</sub> と水素結合できれば、より極性の高いヘテロ環であっても高い結合親和性を維持できると考えた。



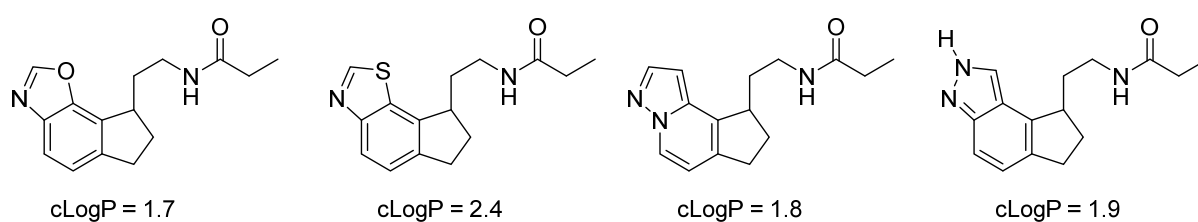
**Figure 10.** X-ray crystal structure of MT<sub>2</sub> in complex with ramelteon  
[PDB, 6ME9]

このような背景のもと著者は、結合親和性を減弱させることなく脂溶性を低減させることを目的として、ジヒドロフラン環の酸素原子を窒素原子へと変換したより極性の高い三環性骨格の合成研究を計画した (**Figure 11**)。具体的には、オキサゾール (**32**)、チアゾール (**33**)、ピラゾール (**34** および **35**) のようなアゾール環を有する新規三環性誘導体をデザインした。ラメルテオンと同様の置換基における脂溶性を計算したところ (**Figure 12**,  $c\text{LogP} = 1.7\text{-}2.4$ )、ラメルテオン ( $c\text{LogP} = 2.5$ ) よりも低い結果となったことから、脂溶性の低減による代謝安定性の向上、さらにはヒトでの経口吸収性向上が期待できる。また、アゾール環上の窒素原子が水素結合受容基として機能することで、強力な  $\text{MT}_1/\text{MT}_2$  結合親和性を示すと考えられる。



**Figure 11.** Design of novel tricyclic derivatives.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Figure 2** with modifications]



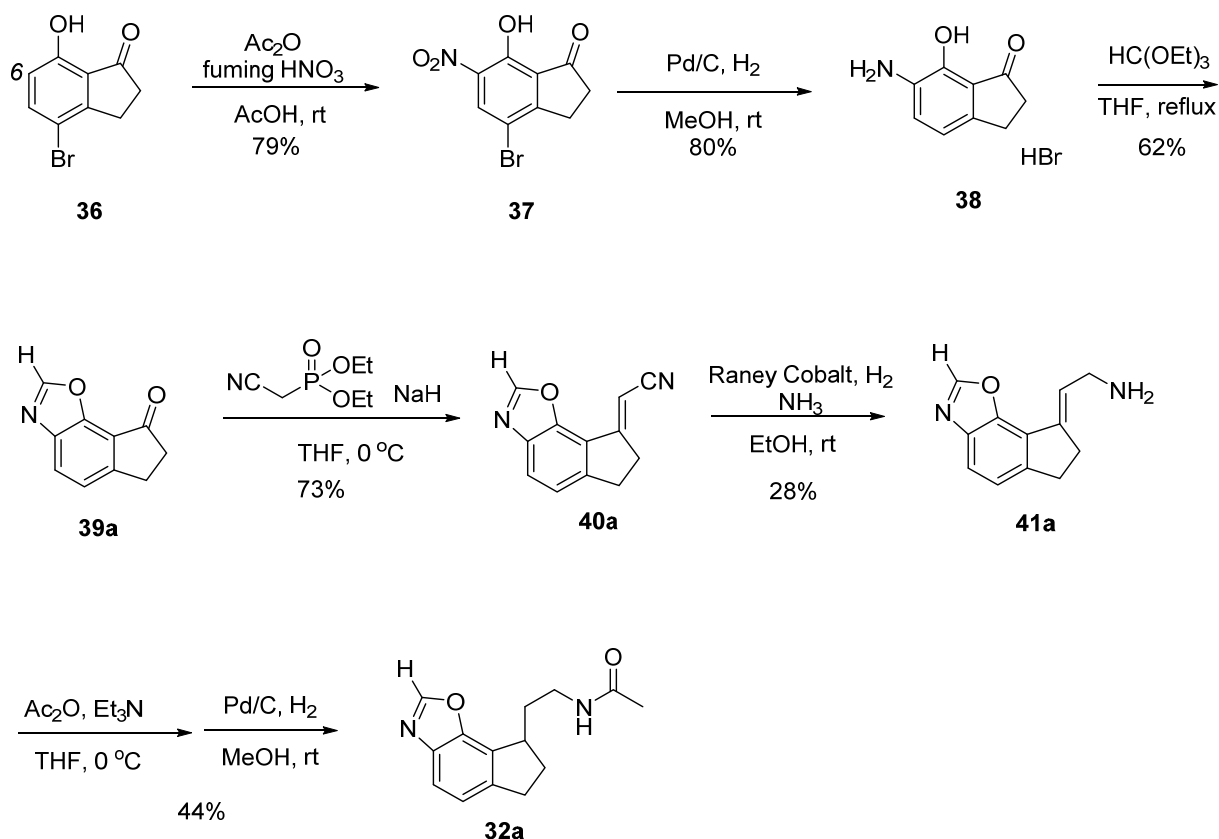
**Figure 12.**  $c\text{LogP}$  values of representative compounds.

## 第2節 デザインした新規三環性誘導体の合成

デザインしたインデノ[5,4-*d*][1,3]オキサゾール誘導体 (**32**)、インデノ[5,4-*d*][1,3]チアゾール誘導体 (**33**)、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 (**34**)、シクロペンタ[*e*]インダゾール誘導体 (**35**) の合成に着手した。いずれの骨格も合成法が知られておらず、新たに合成ルートを構築する必要がある。

### 第1項 インデノ[5,4-*d*][1,3]オキサゾール誘導体の合成

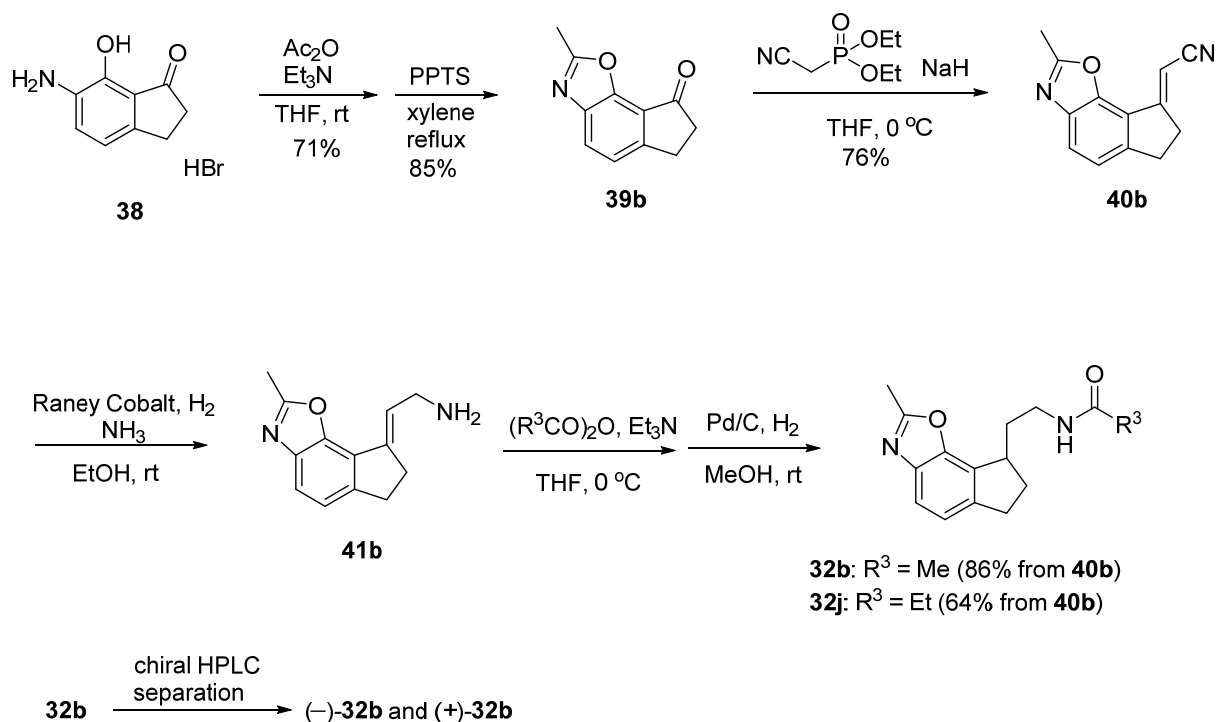
インデノ[5,4-*d*][1,3]オキサゾール誘導体 **32a** ( $R^1 = H$ ) の合成を **Scheme 15** に示す。まず初めに文献既知の 4-ブromo-7-ヒドロキシインダン-1-オン (**36**)<sup>33</sup> を発煙硝酸によりニトロ化した。**36** は C4 位にブromo基を有しているために選択的に C6 位がニトロ化され、ニトロ体 **37** が 79%の収率で得られた。**37** のニトロ基及びブromo基を同時に水素添加反応により還元し、アニリン **38** を臭化水素酸塩として得た。**38** をオルトギ酸トリエチルの存在下 THF 中で加熱還流することで、三環性ケトン体 **39a** が 62%の収率で得られた。続いて、第二章と同様の方法にてアミド側鎖を導入し、インデノ[5,4-*d*][1,3]オキサゾール誘導体 **32a** を得ることに成功した。



**Scheme 15.** Synthesis of indeno[5,4-*d*][1,3]oxazole derivative **32a**.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 1** with modifications]

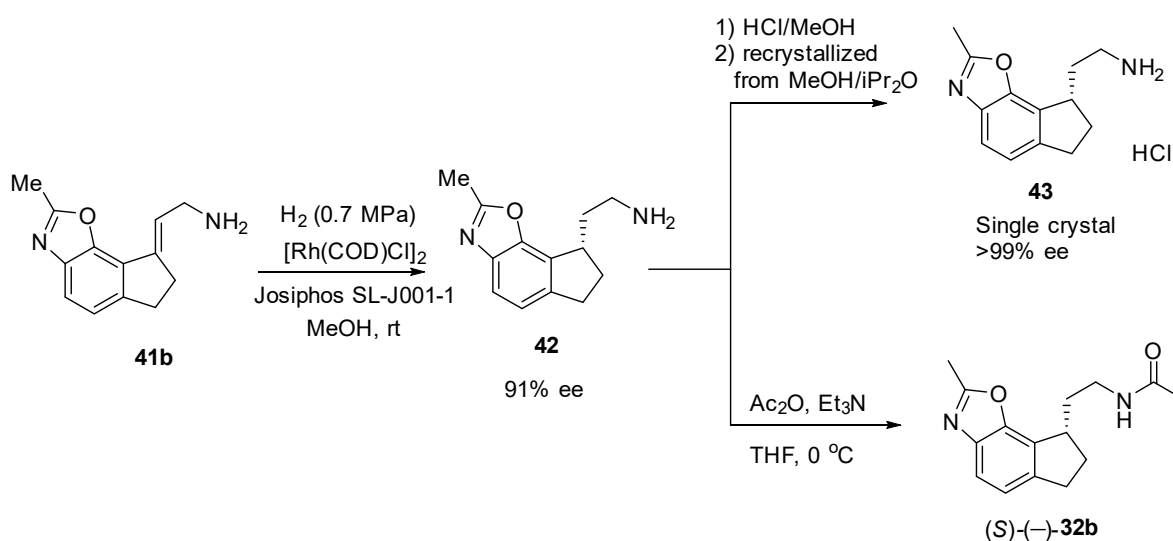
次に、インデノ[5,4-*d*][1,3]オキサゾール誘導体 **32b** ( $R^1 = \text{Me}$ ,  $R^3 = \text{Me}$ ) および **32j** ( $R^1 = \text{Me}$ ,  $R^3 = \text{Et}$ ) の合成を **Scheme 16** に示す。**38** を無水酢酸でアセチル化 (71%収率) した後に、ピリジニウム *p*-トルエンスルホン酸の存在下キシレン中で加熱還流することでオキサゾール環が形成され (85%収率)、 $R^1$  にメチル基を有する三環性ケトン体 **39b** を得ることができた。先ほどと同様にしてアミド側鎖を導入し、インデノ[5,4-*d*][1,3]オキサゾール誘導体 **32b** および **32j** を得た。さらに、キラルカラム用いて **32b** を光学分割し、光学活性体 (–)-**32b** および (+)-**32b** が得られた。



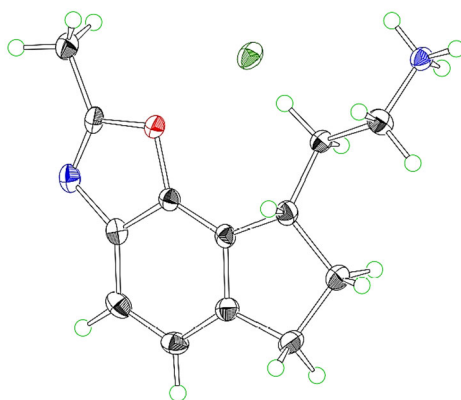
**Scheme 16.** Synthesis of 2-methylindeno[5,4-*d*][1,3]oxazole derivatives and chiral separation of compound **32b**.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 1** with modifications]

光学活性体 (–)-**32b** の絶対立体配置決定について **Scheme 17** に示す。アリルアミン **41b** を、類似基質での例<sup>34</sup> と同様の方法で不斉水素化反応に付すことで、光学活性なアミン **42** を 91% のエナンチオマー過剰率で得た。続いて、塩化水素のメタノール溶液を用いて塩酸塩 **43** とした後に再結晶を試みたところ、エナンチオマー過剰率が>99%に向上し、さらに単結晶が得られた。得られた単結晶を用いて X 線結晶構造解析を実施したところ、*S* 体であることが判明した (**Figure 13**)。光学活性なアミン **42** を無水酢酸を用いてアセチル化し、キラルカラムによる分析を実施したところ、光学活性体 (–)-**32b** の保持時間と一致した。したがって、(–)-**32b** は *S* 体であることが判明した (以降、(*S*)-**32b** と記載する)。



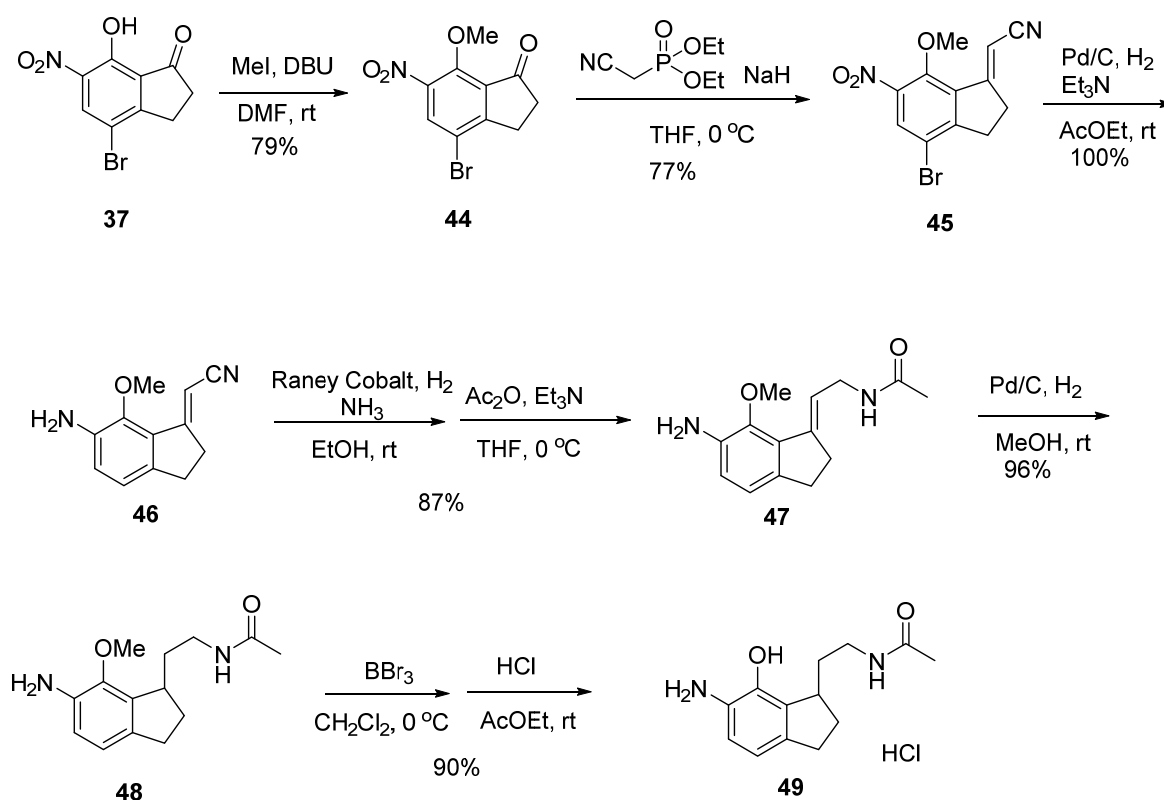
**Scheme 17.** Determination of absolute configuration of (–)-**32b**



**Figure 13.** Single-crystal X-ray structure of compound of **43**.

Black: carbon, light green: hydrogen, red: oxygen, blue: nitrogen, green: chloride

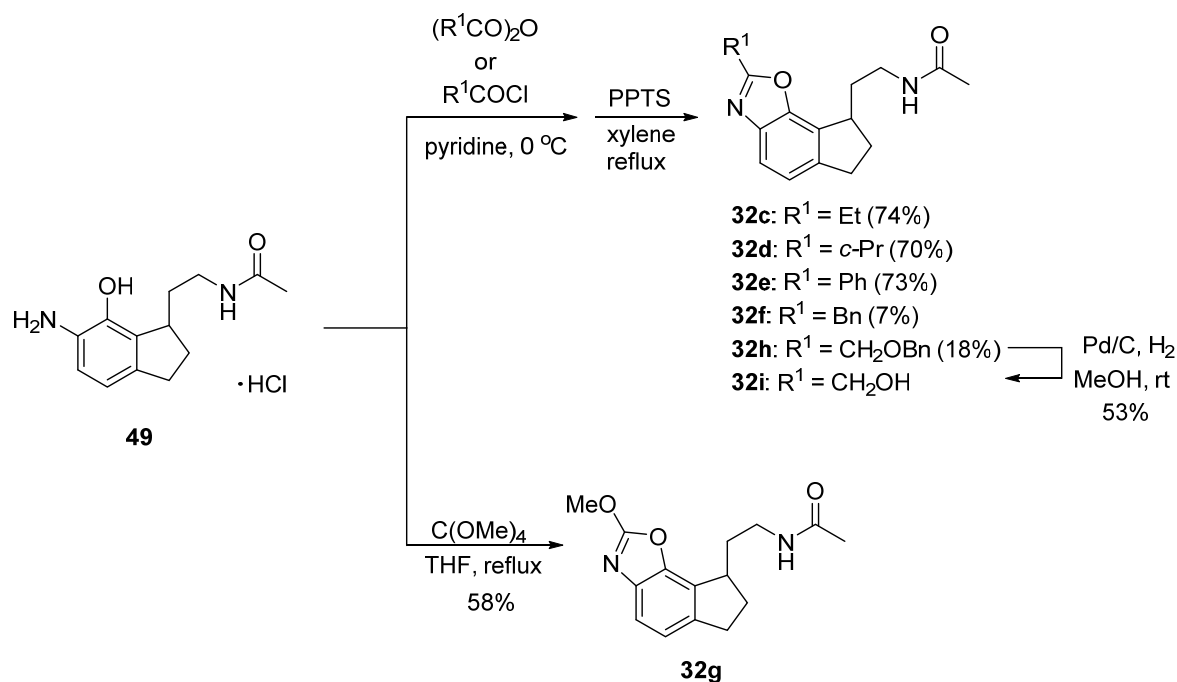
次に、種々の  $R^1$  置換基を効率的に探索するため、最終工程でオキサゾール環を形成する新たな合成ルートの構築に着手した (Scheme 18 および Scheme 19)。鍵中間体であるアミノフェノール **49** の合成を Scheme 18 に示す。まず初めに、すでに合成している中間体 **37** に、これまでと同様の方法でアミド側鎖を導入した。その際、フェノキシ基が無保護であると Horner–Wadsworth–Emmons 反応が進行しなかったため、メチル基で保護した **44** に対してアミド側鎖を導入した。また、不飽和ニトリルの構造変換の過程でニトロ基およびブロモ基の還元も実施し、メトキシアニリン体 **48** を効率よく得た。続いて、三臭化ホウ素によりメチル基を除去し、鍵中間体 **49** を合成することに成功した。



**Scheme 18.** Synthesis of compound **49** for diversity-oriented synthesis of indeno[5,4-*d*][1,3]oxazole derivatives.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 2** with modifications]

鍵中間体 **49** を用いた種々オキサゾール環の合成を **Scheme 19** 示す。化合物 **32c–f** および **32h** のオキサゾール環は、アシル化とそれに続くピリジニウム *p*-トルエンスルホン酸を用いた環化反応によって形成された (74–18%収率)。**32h** のベンジル保護基を水素添加反応で脱保護して、ヒドロキシメチル体 **32i** を得た。メトキシ体 **32g** は、テトラメトキシメタン存在下、THF 中で **49** を還流することで得られた (58%収率)。

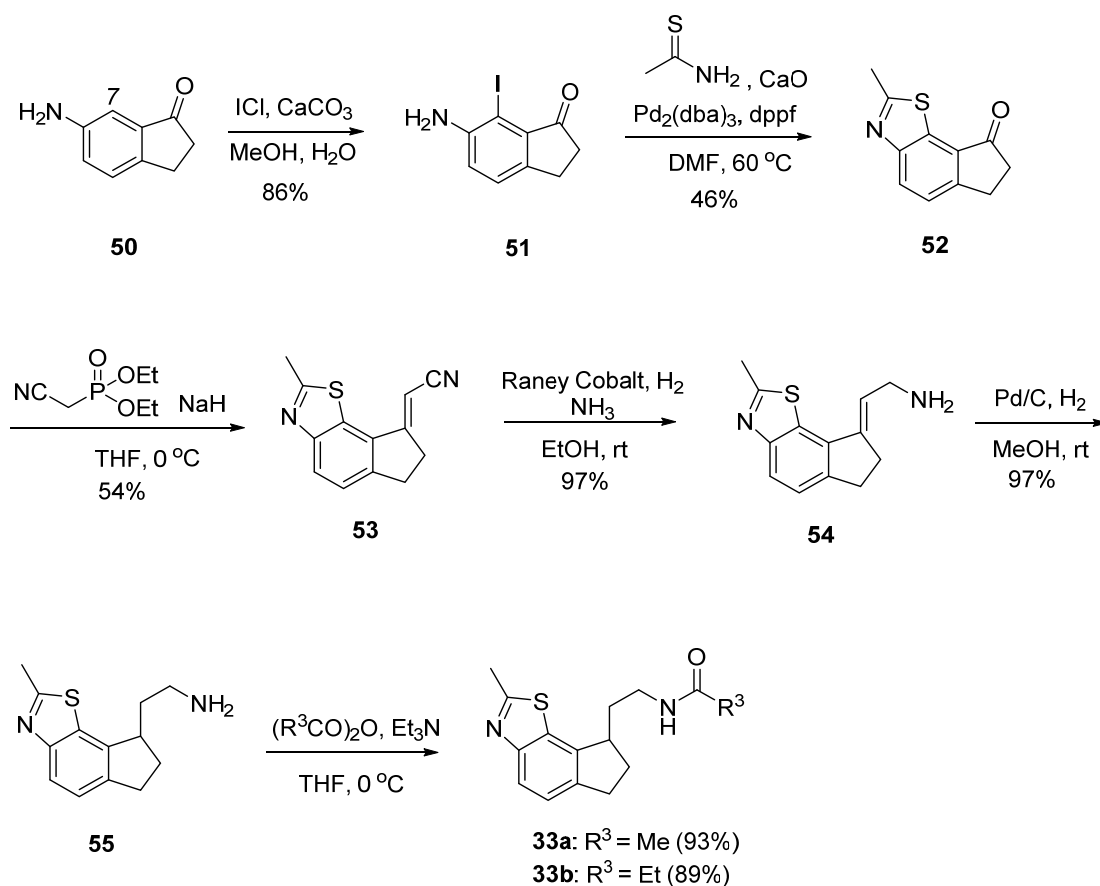


**Scheme 19.** Diversity-oriented synthesis of indeno[5,4-*d*][1,3]oxazole derivatives.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 2** with modifications]

## 第2項 インデノ[5,4-*d*][1,3]チアゾール誘導体の合成

インデノ[5,4-*d*][1,3]チアゾール誘導体 **33a** および **33b** の合成を **Scheme 20** に示す。文献既知の 6-アミノインダン (**50**)<sup>35</sup> を一塩化ヨウ素でヨウ素化したところ、望む C7 位が位置選択的にヨウ素化され、化合物 **51** が 86%の収率で得られた。選択的にヨウ素化ができたことで、インデノ[5,4-*d*][1,3]チアゾール環を構築するために必要な置換基が効率良く導入できた。チアゾール環は、パラジウム触媒を用いた文献既知の方法で構築した。<sup>36</sup> 得られた三環性ケトン体 **52** に、これまでと同様の方法でアミド側鎖を導入し、インデノ[5,4-*d*][1,3]チアゾール誘導体 **33a** および **33b** を得ることに成功した。

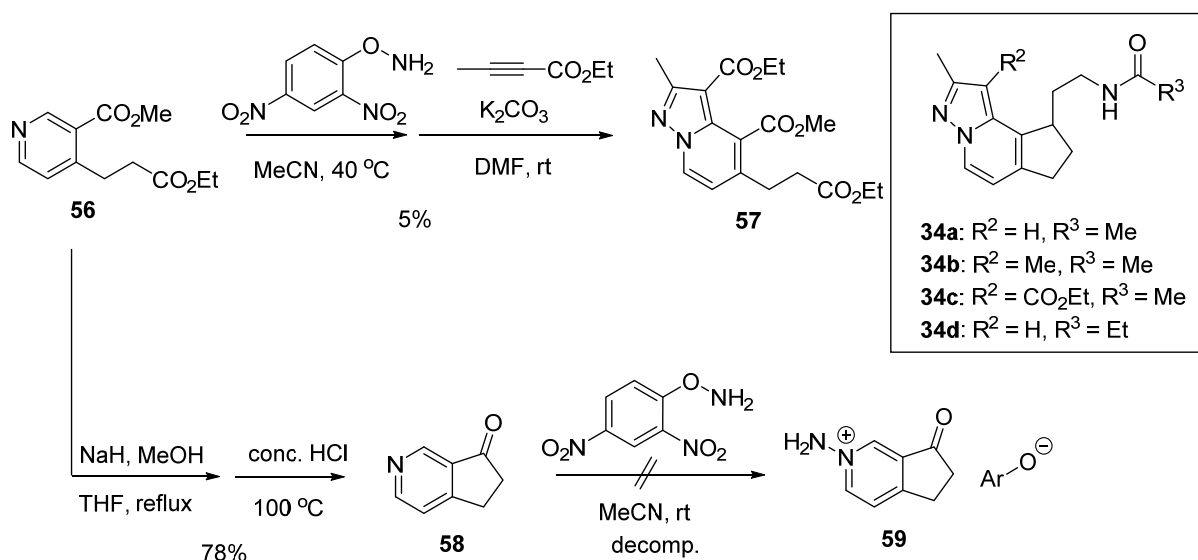


**Scheme 20.** Synthesis of indeno[5,4-*d*][1,3]thiazole derivatives.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 3** with modifications]

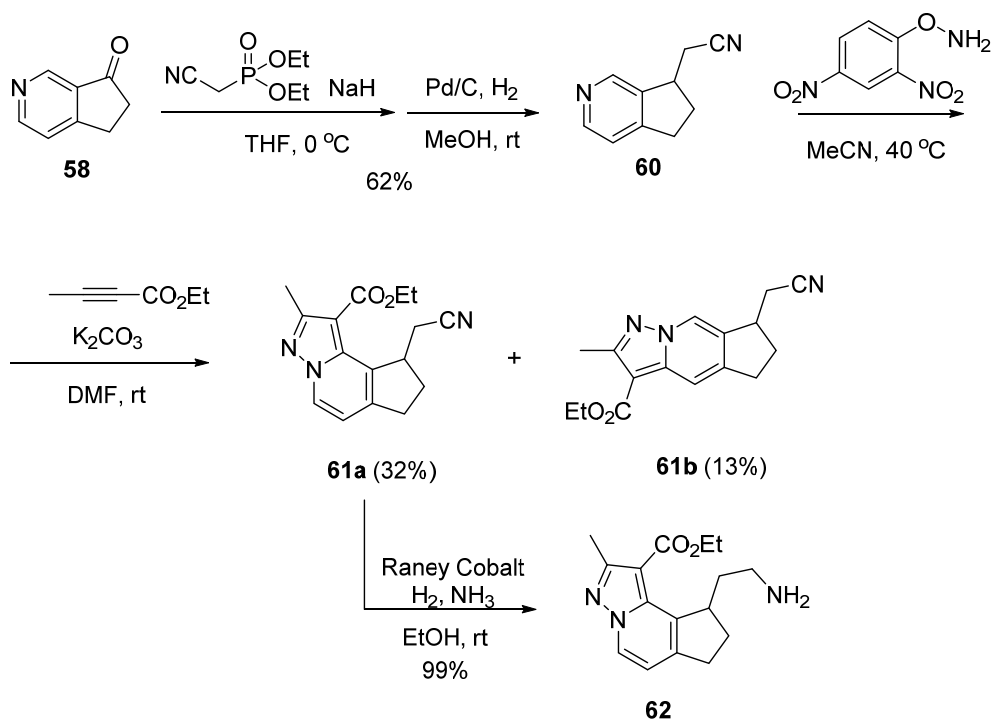
### 第3項 シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体の合成

文献既知のピリジン誘導体 **56**<sup>37</sup> を出発原料として選択し、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **34a-d** の合成に着手した (Scheme 21-23)。まず初めに、一般的なピラゾロ[1,5-*a*]ピリジン環構築の方法として知られる *N*-アミノピリジンとアルキンとの[3+2]環化付加反応<sup>15</sup>を試みた。しかしながら、目的とするピラゾロ[1,5-*a*]ピリジン誘導体 **57** は 5%の収率でしか得られなかった。**56** はピリジン環上に電子求引基であるエステル基を有しているため、ピリジンの求核力が低くアミノ化剤と効率良く反応できなかったことが低収率の原因と考えられる。そこで、シクロペンタン環の構築とアミド側鎖の導入を先に進めることで、ピリジン環上に電子求引基を有さない中間体への誘導を試みた。**56** の Dieckmann 縮合とそれに続く脱炭酸によりシクロペンタン環が構築され、ケトン体 **58** が得られた。**58** もピリジン環上に電子求引基を有しており、**56** と同様にピリジンのアミノ化が効率良く進行しなかった。



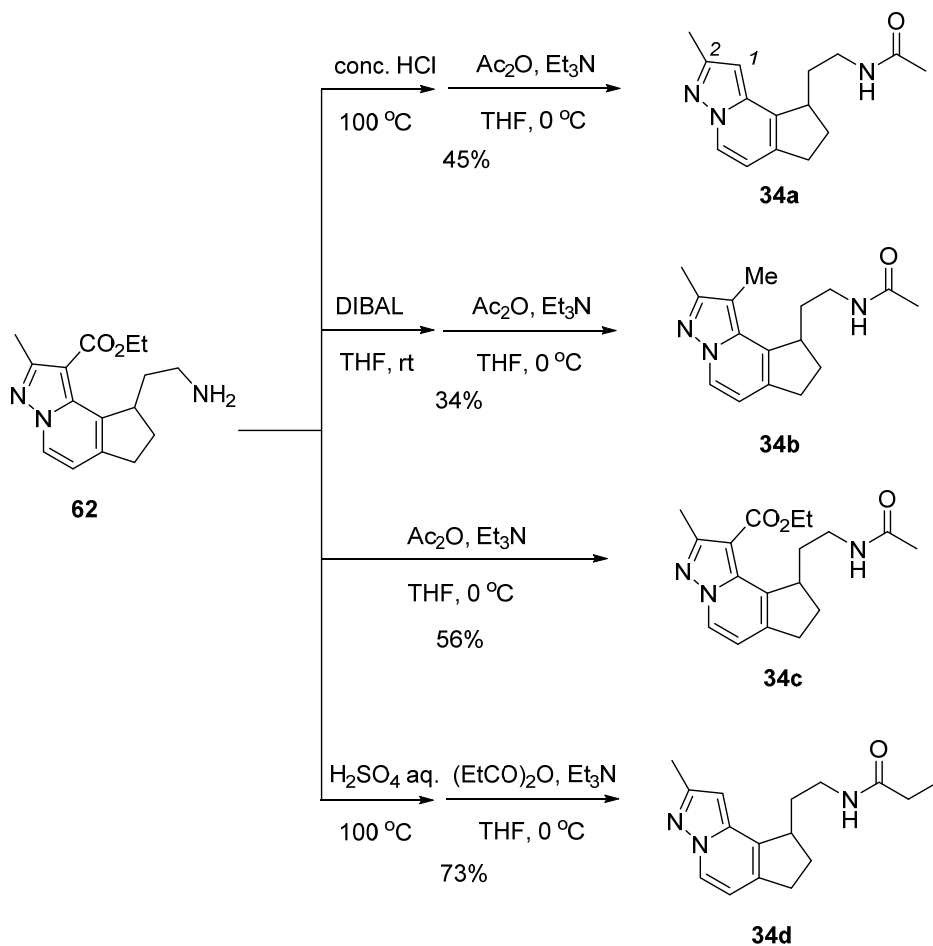
**Scheme 21.** 1,3-Dipolar cycloaddition reaction of electron-deficient pyridines.

続いて、Horner–Wadsworth–Emmons 反応とそれに続く水素添加反応により、ニトリル **60** を得た (Scheme 22)。 **60** はピリジン環上に電子求引基を有していないため、円滑なアミノ化反応が期待できる。実際に **60** にアミノ化剤を反応させたところ、アミノ化は円滑に進行し、引き続きアルキンとの環化付加反応を実施した。その結果、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン骨格の構築に成功し、**61a** が 32%の収率で得られた。同時に位置異性体である **61b** が生成し、第一章で述べたように、非対称なピリジンから位置異性体が生成した。次に、**61a** のニトリル残基をラネーコバルトによる水素添加反応で還元してアミン体 **62** を得た。



**Scheme 22.** Formation of cyclopenta[*c*]pyrazolo[1,5-*a*]pyridine ring.  
[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 4** with modifications]

最後に、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **34a–d** の合成を **Scheme 23** に示す。**62** のエステル基を酸性条件下で加水分解および脱炭酸した後に、アシル化することで、2-メチル体 **34a** および **34d** を得た。水素化ジイソブチルアルミニウムでエステル基をメチル基に還元した後に、アセチル化することで、1,2-ジメチル体 **34b** が得られた。エステル基を変換せずにアセチル化することで **34c** が得られた。

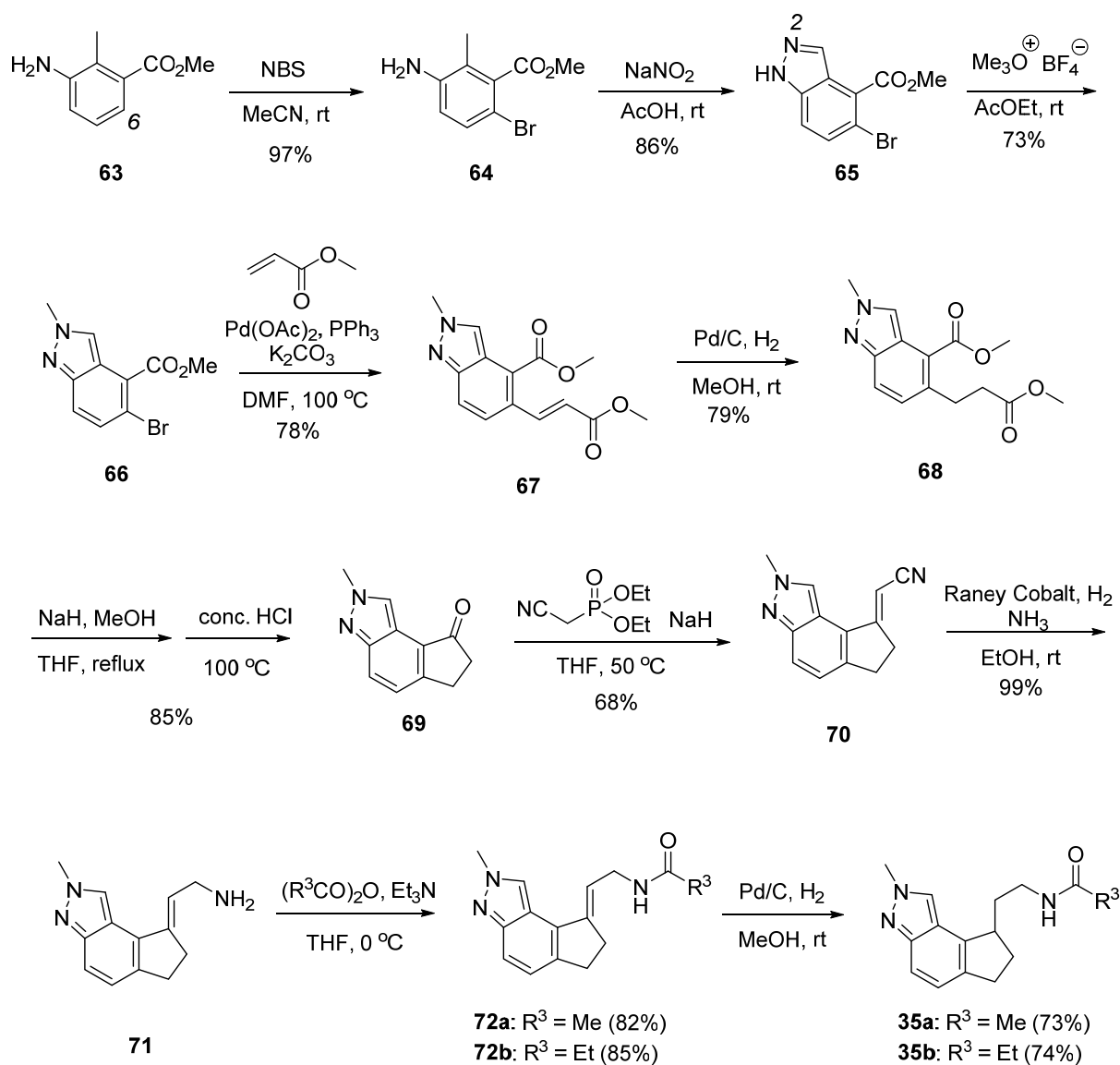


**Scheme 23.** Synthesis of cyclopenta[*c*]pyrazolo[1,5-*a*]pyridine derivatives.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Scheme 4** with modifications]

#### 第4項 シクロペンタ[e]インダゾール誘導体の合成

シクロペンタ[e]インダゾール誘導体 **35a** および **35b** の合成を **Scheme 24** に示す。市販の 3-アミノ-2-メチル安息香酸メチル (**63**) を *N*-ブロモスクシンイミドを用いてブロモ化したところ、C6 位が位置選択的にブロモ化され、化合物 **64** が 97%の収率で得られた。選択的にブロモ化ができたことで、シクロペンタ[e]インダゾール環を構築するために必要な置換基が効率良く導入できた。**64** を酢酸中亜硝酸ナトリウムで処理することでインダゾール環を形成し、**65** を 86%の収率で得た。インダゾールの N2 位窒素原子を選択的にメチル化することが知られているテトラフルオロホウ酸トリメチルオキシニウム (Meerwein 試薬)<sup>38</sup> を用いてメチル化することで、化合物 **66** が 73%の収率で得られた。シクロペンタン環を構築するために、アクリル酸メチルを Heck 反応により導入して、**67** を得た。続いて、水素添加反応、Dieckmann 縮合、脱炭酸を経て、シクロペンタ[e]インダゾール環が構築され、三環性ケトン体 **69** が得られた。これまでと同様の方法でアミド側鎖を導入し、シクロペンタ[e]インダゾール誘導体 **35a** および **35b** を得ることに成功した。



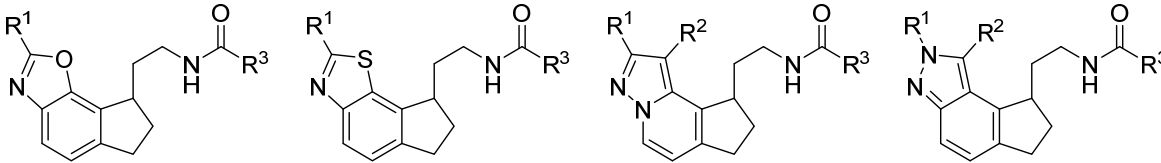
**Scheme 24.** Synthesis of cyclopenta[*e*]indazole derivatives.

[*J. Med. Chem.* **2021**, *64*, 3059–3074, **Scheme 5** with modifications]

### 第3節 MT<sub>1</sub>/MT<sub>2</sub> 結合親和性および構造活性相関

合成した新規三環式誘導体の MT<sub>1</sub>/MT<sub>2</sub> に対する結合親和性を **Table 3** に示す。

**Table 3.** Binding affinities for human MT<sub>1</sub> and MT<sub>2</sub> receptors.  
[*J. Med. Chem.* **2021**, 64, 3059–3074, **Table 1** with modifications]

					
<b>32a-g, i-j</b>		<b>33a-b</b>		<b>34a-d</b>	
				<b>35a-b</b>	
compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	<i>K<sub>i</sub></i> <sup>a</sup> (nM)	
				MT <sub>1</sub>	MT <sub>2</sub>
melatonin				0.24 ± 0.06 <sup>b</sup>	0.21 ± 0.03 <sup>b</sup>
<b>32a</b>	H		Me	0.45 ± 0.17	0.073 ± 0.026
<b>32b</b>	Me		Me	0.067 ± 0.014	0.13 ± 0.02
<b>32c</b>	Et		Me	0.22 ± 0.04	0.16 ± 0.03
<b>32d</b>	<i>c</i> Pr		Me	0.39 ± 0.06	0.15 ± 0.03
<b>32e</b>	Ph		Me	1.3 ± 0.4	0.23 ± 0.11
<b>32f</b>	Bn		Me	4.4 ± 0.5	0.12 ± 0.01
<b>32g</b>	OMe		Me	0.45 ± 0.07	0.15 ± 0.03
<b>32i</b>	CH <sub>2</sub> OH		Me	1.2 ± 0.5	1.1 ± 0.5
<b>32j</b>	Me		Et	0.067 ± 0.033	0.088 ± 0.046
<b>33a</b>	Me		Me	0.037 ± 0.007	0.086 ± 0.014
<b>33b</b>	Me		Et	0.027 ± 0.003	0.058 ± 0.010
<b>34a</b>	Me	H	Me	0.20 ± 0.02	1.4 ± 0.3
<b>34b</b>	Me	Me	Me	9.3 ± 0.3	n.d. <sup>c</sup>
<b>34c</b>	Me	CO <sub>2</sub> Et	Me	n.d. <sup>c</sup>	n.d. <sup>c</sup>
<b>34d</b>	Me	H	Et	0.17 ± 0.02	0.95 ± 0.28
<b>35a</b>	Me	H	Me	0.35 ± 0.08	0.75 ± 0.22
<b>35b</b>	Me	H	Et	0.30 ± 0.09	0.35 ± 0.08

<sup>a</sup>Data are presented as mean values with the standard deviation from a single experiment in triplicate.

<sup>b</sup>Data are presented as mean values with the standard deviation from 6 independent experiments in more than triplicate. <sup>c</sup>Not determined; indicates <50% inhibition at a concentration of 100 nM.

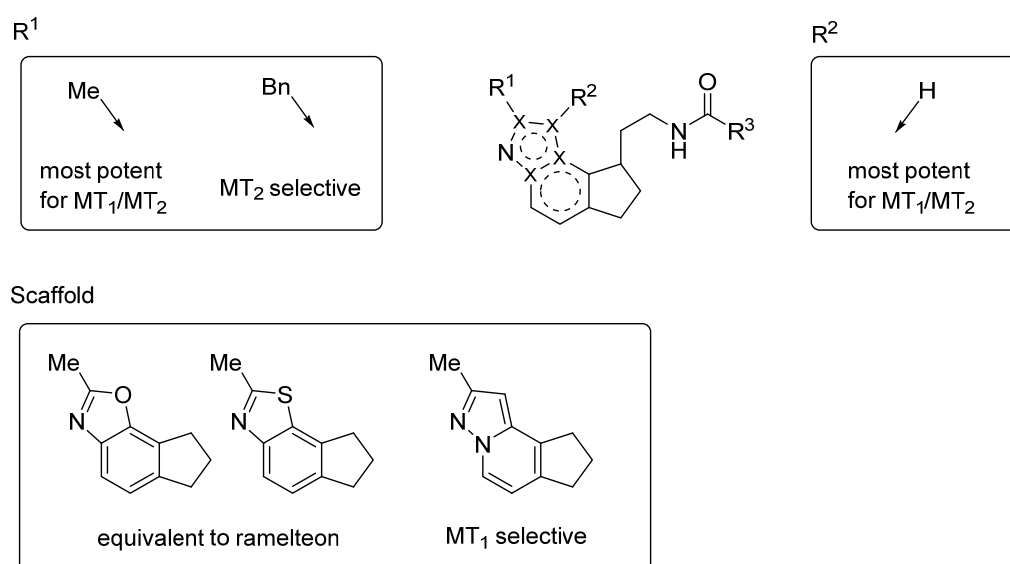
まず初めに、インデノ[5,4-*d*][1,3]オキサゾール誘導体の構造活性相関について述べる。ラメルテオンの母核であるインデノ[5,4-*b*]フラン環のジヒドロフラン環をオキサゾール環に変換した **32a** の結合親和性 ( $R^1 = H$ ; MT<sub>1</sub>,  $K_i = 0.45$  nM; MT<sub>2</sub>,  $K_i = 0.073$  nM) は、天然リガンドであるメラトニンと同等 (MT<sub>1</sub>,  $K_i = 0.24$  nM; MT<sub>2</sub>,  $K_i = 0.21$  nM) の強力な値を示した。この結果は、狙い通りオキサゾール環上の窒素原子が水素結合受容基として機能したことを示唆している。インデノ[5,4-*d*][1,3]オキサゾール誘導体は  $R^1$  置換基の探索が容易に実施できるため (Scheme 20 の合成法参照)、より強力な結合親和性を目指し、 $R^1$  置換基の最適化に着手した。メチル基を導入することにより (**32b**)、MT<sub>2</sub> 結合親和性がわずかに減弱したものの、MT<sub>1</sub> 結合親和性は約 7 倍増強された (MT<sub>1</sub>,  $K_i = 0.067$  nM; MT<sub>2</sub>,  $K_i = 0.13$  nM)。エチル基 (**32c**)、シクロプロピル基 (**32d**)、フェニル基 (**32e**)、ベンジル基 (**32f**) と、より嵩高い置換基を導入したところ、置換基の嵩高さに応じて MT<sub>1</sub> 結合親和性が減弱した。対照的に、嵩高い置換基の導入は MT<sub>2</sub> 結合親和性にはほとんど影響を与えなかった。その結果、MT<sub>2</sub> 選択的リガンド **32f** ( $R^1 = Bn$ ; MT<sub>1</sub>/MT<sub>2</sub> = 37) が見出された。電子供与基のメトキシ基を有する化合物 **32g** がエチル体 **32c** と同様の結合親和性を示したことから、ベンゾオキサゾール環の電子密度は MT<sub>1</sub>/MT<sub>2</sub> 結合親和性に影響を及ぼさないと考えられる。親水性基であるヒドロキシメチル基を有する化合物 **32i** は、MT<sub>1</sub> および MT<sub>2</sub> の両方に対して結合親和性が減弱した (MT<sub>1</sub>,  $K_i = 1.2$  nM; MT<sub>2</sub>,  $K_i = 1.1$  nM)。  $R^1$  置換基に相当する部位の結合ポケットは疎水性であることが X 線結晶構造解析から明らかになっており、<sup>31</sup> 今回得られた構造活性相関はその結果と一致している。以上述べてきた結果から、MT<sub>1</sub> および MT<sub>2</sub> の両方に対して強力な結合親和性を示す  $R^1$  置換基としては、メチル基が最適であると結論付けた。アミド側鎖に関しては、プロパンアミド基が最も強力な結合親和性を発揮することを以前に報告している。<sup>1</sup> プロパンアミド基に変換した **32j** は、アセトアミド体の **32b** に対してわずかな MT<sub>2</sub> 結合親和性向上がみられた (MT<sub>1</sub>,  $K_i = 0.067$  nM; MT<sub>2</sub>,  $K_i = 0.088$  nM)。

$R^1$  置換基にメチル基を有するインデノ[5,4-*d*][1,3]チアゾール誘導体 **33a** は、インデノ[5,4-*d*][1,3]オキサゾール誘導体の **32b** と比較してわずかに結合親和性が向上した (MT<sub>1</sub>,  $K_i = 0.037$  nM; MT<sub>2</sub>,  $K_i = 0.086$  nM)。オキサゾール環と同様にチアゾール環も効果的に水素結合受容基として機能したと考えられる。プロパンアミド体に変換した **33b** も同様に、強力な結合親和性を示した (MT<sub>1</sub>,  $K_i = 0.027$  nM; MT<sub>2</sub>,  $K_i = 0.058$  nM)。

$R^1$  置換基にメチル基を有するシクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 **34a** ( $R^1 = Me$ ,  $R^2 = H$ ) は、メラトニンと同等の MT<sub>1</sub> 結合親和性を示したが、MT<sub>2</sub> 結合親和性はやや減弱した (MT<sub>1</sub>,  $K_i = 0.20$  nM; MT<sub>2</sub>,  $K_i = 1.4$  nM)。この結果は、さらなる三環性骨格の探索により MT<sub>1</sub> 選択的リガンドが見出される可能性を示唆している。本母核は、 $R^2$  置換基の探索を可能にしておき、結合親和性の向上を目指してメチル基 (**34b**) およびエステル基 (**34c**) を導入した。しかしながら、いずれの化合物も MT<sub>1</sub>/MT<sub>2</sub> 結合親和性が著しく低下した。X 線結晶構造解析の結果から、バリン残基 (MT<sub>1</sub>: V191, MT<sub>2</sub>: V204) がラメルテオンのジヒドロフラン環の近接位置に位置することが明らかになっており、<sup>31</sup>  $R^2$  置換基はバリン残基と立体反発していると考えられる。これまでに得られた構造活性相関情報を元にデザインされた、シクロペンタ[*e*]インダゾール誘導体 **35a** ( $R^1 = Me$ ,  $R^2 = H$ ) は、メラトニンと同等の結合親和性を示した (MT<sub>1</sub>,

$K_i = 0.35$  nM; MT<sub>2</sub>,  $K_i = 0.75$  nM)。シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジンおよびシクロペンタ[*e*]インダゾール誘導体においても、これまでと同様にピラゾール環が水素結合受容基として機能したと考えられる。プロパンアミド基に変換した **34d** および **35b** は、アセトアミド体の **34a** および **35a** に対してそれぞれわずかな MT<sub>1</sub>/MT<sub>2</sub> 結合親和性向上がみられた。

新たに得られた構造活性相関情報についてまとめる (Figure 14)。デザインした新規三環性誘導体はいずれも強力な MT<sub>1</sub>/MT<sub>2</sub> 結合親和性を示し、ジヒドロフラン環の酸素原子を窒素原子へと変換したとしても MT<sub>1</sub>/MT<sub>2</sub> に対する結合親和性を維持できることを明らかにした。中でも、インデノ[5,4-*d*][1,3]オキサゾール誘導体およびインデノ[5,4-*d*][1,3]チアゾール誘導体が特に強い MT<sub>1</sub>/MT<sub>2</sub> 結合親和性を示した。また、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体は MT<sub>1</sub> 選択性を示し、さらなる三環性骨格の探索による MT<sub>1</sub> 選択的リガンド創出の可能性を示唆した。R<sup>1</sup> 置換基としてはメチル基が最も強い MT<sub>1</sub>/MT<sub>2</sub> 結合親和性を示し、嵩高いベンジル基が MT<sub>2</sub> 選択性を示した。R<sup>2</sup> 置換基は置換基導入を許容せず、水素原子が最適であった。



**Figure 14.** Summary of structure-activity relationship.

#### 第4節 代謝安定性の評価と精査化合物の選択

各三環性誘導体から結合親和性の強い代表的な化合物を選択した。それらの cLogP 値、代謝安定性及びラット経口吸収性を **Table 4** に示す。cLogP 値は Daylight Chemical Information Systems, Inc.のソフトウェアを用いて算出し、代謝安定性はヒト及びラット肝ミクロソーム中における *in vitro* クリアランスを測定することにより評価した。クリアランス値が低いほど代謝安定性が良いと判断できる。

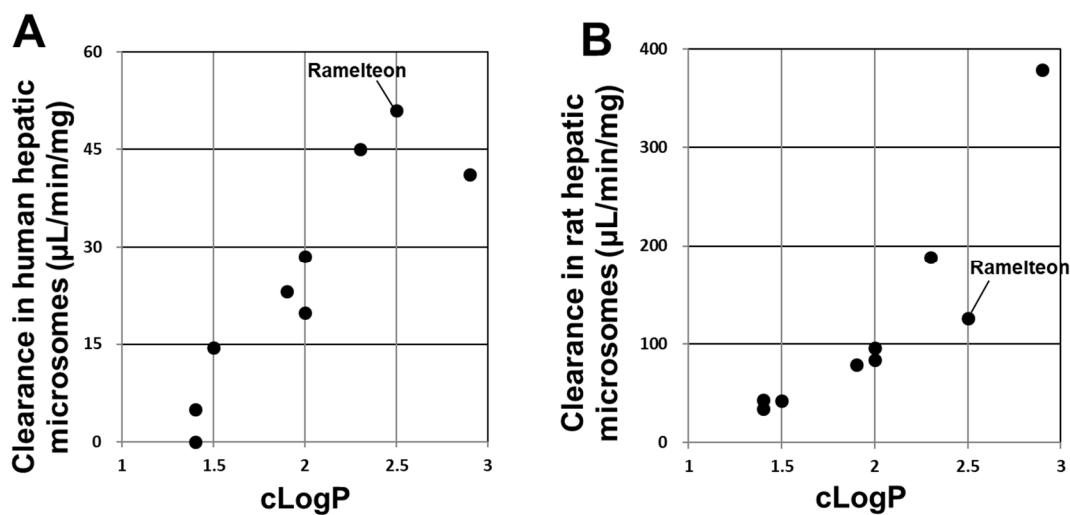
ラメルテオンはアミド側鎖にプロパンアミド基を有するので、ラメルテオン (cLogP=2.5) よりも脂溶性の低いプロパンアミド誘導体 ( $R^3 = \text{Et}$ ; **32j**, cLogP = 2.0; **34d**, cLogP = 2.0; **35b**, cLogP = 1.9) とラメルテオンの代謝安定性を比較した。ラメルテオンのヒト肝ミクロソーム中におけるクリアランスが 51  $\mu\text{L}/\text{min}/\text{mg}$  であったのに対して、脂溶性が低下したこれらの化合物はクリアランスの低下が見られ (**32j**, 29  $\mu\text{L}/\text{min}/\text{mg}$ ; **34d**, 20  $\mu\text{L}/\text{min}/\text{mg}$ ; **35b**, 23  $\mu\text{L}/\text{min}/\text{mg}$ )、代謝安定性の向上が見られた。一方で、ラメルテオンよりも脂溶性の高いインデノ[5,4-*d*][1,3]チアゾール誘導体 **33b** (cLogP = 2.9) はラメルテオンと同等のクリアランスであった (41  $\mu\text{L}/\text{min}/\text{mg}$ )。また、プロパンアミドよりも脂溶性の低いアセトアミド誘導体 **32b**, **34a**, **35a** は、対応するプロパンアミド誘導体 **32j**, **34d**, **35b** よりも代謝的に安定であった (**32b**, 0  $\mu\text{L}/\text{min}/\text{mg}$ ; **34a**, 14  $\mu\text{L}/\text{min}/\text{mg}$ ; **35a**, 5  $\mu\text{L}/\text{min}/\text{mg}$ )。Figure 15A は、cLogP に対してヒト肝ミクロソーム中のクリアランス値をプロットした散布図である。cLogP とクリアランスの間に相関があることが明らかであり、代謝安定性向上のため脂溶性を低減させた戦略が有効であったことを示している。また、ラット肝ミクロソーム中においても、ヒト肝ミクロソームと同様に cLogP が低い化合物ほどクリアランスが低くなるという結果が得られた (**Table 4** and **Figure 15B**)。

**Table 4.** Values for cLogP, in vitro clearance in human and rat hepatic microsomes, and oral bioavailability in rats of selected compounds.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Table 2** with modifications]

compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	cLogP	microsomal clearance ( $\mu\text{L}/\text{min}/\text{mg}$ )		$F^a$ (%)
					human	rat	
ramelteon				2.5	51	126	$8.5 \pm 2.0^b$
<b>32b</b>	Me		Me	1.4	0	34	$18.5 \pm 5.3$
<b>32j</b>	Me		Et	2.0	29	96	$6.8 \pm 2.0$
<b>33a</b>	Me		Me	2.3	45	188	$4.8 \pm 1.7$
<b>33b</b>	Me		Et	2.9	41	379	$3.2 \pm 1.0$
<b>34a</b>	Me	H	Me	1.5	14	42	$14.3 \pm 1.8$
<b>34d</b>	Me	H	Et	2.0	20	84	
<b>35a</b>	Me	H	Me	1.4	5	44	$20.9 \pm 9.0$
<b>35b</b>	Me	H	Et	1.9	23	79	$13.7 \pm 5.6$

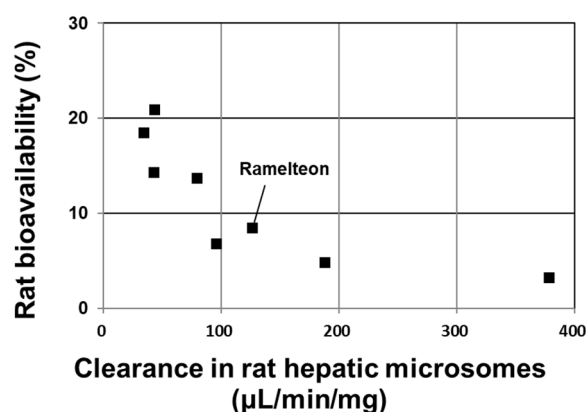
<sup>a</sup>Oral bioavailability after cassette dosing at 0.1 mg/kg (iv) and 1 mg/kg (po). <sup>b</sup>Test compound was administered discretely at 1 mg/kg (iv) and 1 mg/kg (po).



**Figure 15.** Plot of clearance in human (A) or rat (B) hepatic microsomes against cLogP.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Figure 3** with modifications]

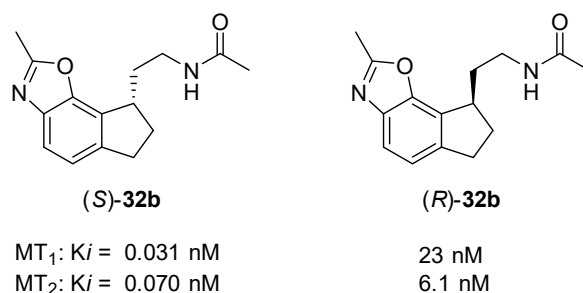
続いて、ラットにおける経口吸収性を測定した。ラメルテオンの経口吸収性が 8.5% であるのに対して、良好なクリアランス値を示した **32b**, **34a**, **35a** はラメルテオンよりも優れた経口吸収性を示した (**32b**, 18.5%; **34a**, 14.3%; **35a**, 20.9%)。Figure 16 は、ラット肝ミクロソーム中のクリアランス値に対してラット経口吸収性をプロットした散布図であり、クリアランス値と経口吸収性に明らかに相関がみられる。これは、代謝安定性が向上することで経口吸収性が改善することを示唆している。したがって、ヒト肝ミクロソーム中において優れた代謝安定性を有する化合物は、ヒトにおいて良好な経口吸収性を示すと予想される。インデノ[5,4-*d*][1,3]オキサゾール誘導体 **32b** は、ヒト肝ミクロソームにおいて代謝安定性が優れているばかりでなく (0  $\mu\text{L}/\text{min}/\text{mg}$ )、非常に強力な結合親和性 (MT<sub>1</sub>,  $K_i = 0.067$  nM; MT<sub>2</sub>,  $K_i = 0.13$  nM) を示すことから、薬理的評価などのさらなる評価を実施する精査化合物として選択した。



**Figure 16.** Plot of rat oral bioavailability against clearance in rat hepatic microsomes.

[*J. Med. Chem.* **2021**, 64, 3059–3074, **Figure 4** with modifications]

**32b** はラセミ体であるので、活性本体であるユートマーを決定するために光学活性体の MT<sub>1</sub>/MT<sub>2</sub> 結合親和性を測定した (Figure 17)。*S* 体である (*S*)-**32b** はラセミ体のおよそ 2 倍の結合親和性を示し (MT<sub>1</sub>,  $K_i = 0.031$  nM; MT<sub>2</sub>,  $K_i = 0.070$  nM)、ラメルテオン (MT<sub>1</sub>,  $K_i = 0.014$  nM; MT<sub>2</sub>,  $K_i = 0.11$  nM) に匹敵する強力な結合親和性であった。一方で、*R* 体である (*R*)-**32b** の結合親和性は非常に弱かった (MT<sub>1</sub>,  $K_i = 23$  nM; MT<sub>2</sub>,  $K_i = 6.1$  nM)。この結果から、(*S*)-**32b** がユートマーであることが判明した。(*S*)-**32b** はまた、ヒト肝ミクロソーム中での代謝安定性 (0  $\mu\text{L}/\text{min}/\text{mg}$ ) およびラット経口吸収性 ( $F = 27.0\%$ ) はラセミ体 **32b** と同等であることが確認された。



**Figure 17.** Binding affinities of (*S*)-**32b** and (*R*)-**32b**

## 第5節 (S)-32b の脳内移行性評価および薬理評価

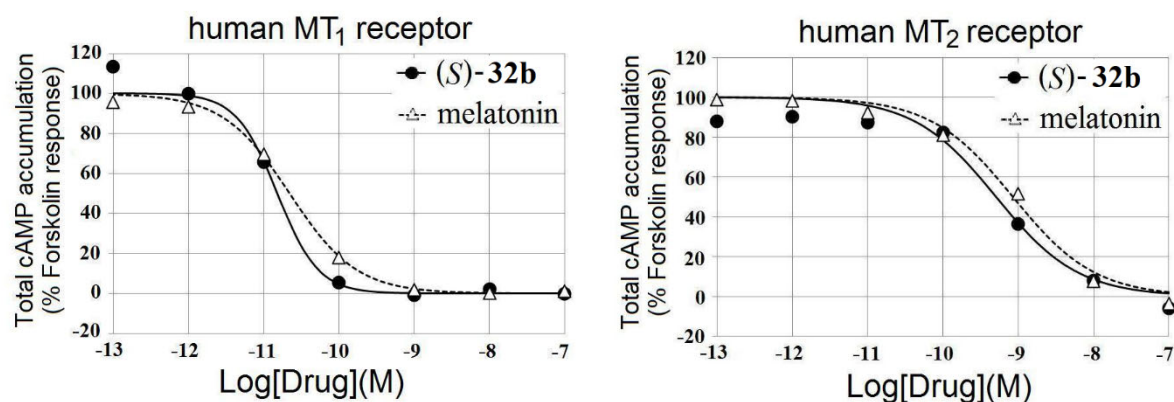
MT<sub>1</sub>/MT<sub>2</sub> アゴニストが睡眠誘発作用を示すためには、脳内でその作用を発揮する必要がある。そのためには、末梢血液中の薬物が血液脳関門 (Blood-brain barrier, BBB) を通過し、脳内へと移行しなければならない。そこで、経口投与後のラットにおける脳/血漿濃度比 (B/P 比) を計測することにより (S)-32b の脳内移行性を評価した (Table 5)。脳内濃度は血漿中濃度の約半分 (B/P 比 = 0.48) であったことから、(S)-32b はラメルテオン (B/P 比 = 1.1) と同様に良好な脳内移行性を示した。

**Table 5.** Brain-to-plasma concentration ratio (B/P ratio) 60 min after oral administration of ramelteon and (S)-32b to rats.

[*J. Med. Chem.* **2021**, 64, 3059–3074, Table 3 with modifications]

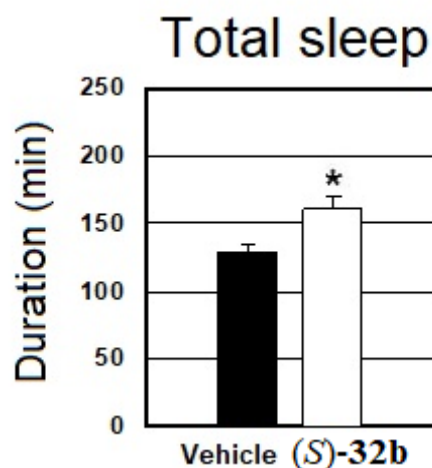
compd	dose (mg/kg)	brain (ng/g)	plasma (ng/mL)	B/P ratio
ramelteon	1.0	17	15	1.1
(S)-32b	0.1	1.4	2.9	0.48

また、化合物が睡眠誘発作用を発揮するためには、MT<sub>1</sub>/MT<sub>2</sub> に結合するだけでなく、MT<sub>1</sub>/MT<sub>2</sub> を活性化することが必要不可欠である。そこで、(S)-32b のアゴニスト活性を、ヒト MT<sub>1</sub>/MT<sub>2</sub> 発現 CHO 細胞における cAMP 産生を指標に評価した (Figure 18)。陽性コントロールのメラトニンがフォルスコリン刺激による cAMP の産生を濃度依存的に阻害する (MT<sub>1</sub>, EC<sub>50</sub> = 0.022 nM; MT<sub>2</sub>, EC<sub>50</sub> = 0.85 nM) のに対し、(S)-32b もメラトニンと同様のフルアゴニスト活性を示した (MT<sub>1</sub>, EC<sub>50</sub> = 0.015 nM; MT<sub>2</sub>, EC<sub>50</sub> = 0.51 nM)。



**Figure 18.** Functional analysis of melatonin and (*S*)-**32b** on forskolin-stimulated cAMP formation in CHO cells expressing either human MT<sub>1</sub> or human MT<sub>2</sub> receptor. The 100% value was the mean cAMP production with 2  $\mu$ mol/L forskolin. The 0% value was that with 1  $\mu$ mol/L melatonin and 2  $\mu$ mol/L forskolin. Data were expressed as mean of percentage forskolin response at each receptor. The EC<sub>50</sub> values were estimated by a 2-parameter nonlinear logistic regression analysis. The EC<sub>50</sub> values for melatonin are 0.022 nM (MT<sub>1</sub>, 95 % confidence interval = 0.019–0.025 nM) and 0.85 nM (MT<sub>2</sub>, 95 % confidence interval = 0.65–1.1 nM). Those for (*S*)-**32b** are 0.015 nM (MT<sub>1</sub>, 95 % confidence interval = 0.012–0.023) and 0.51 nM (MT<sub>2</sub>, 95 % confidence interval = 0.33–0.77). [*J. Med. Chem.* **2021**, 64, 3059–3074, **Figure 5** with modifications]

以上の結果から (S)-32b は動物において睡眠誘発作用を示し得ると考え、文献既知のネコを用いた睡眠評価モデルを用いて評価した。<sup>39</sup> 評価方法としては、経口投与後 8 時間の総睡眠時間を記録し、vehicle 群と比較した。その結果、(S)-32b の 10 mg/kg 経口投与により、vehicle 群と比較して総睡眠時間が有意に延長した (Figure 19)。経口投与後のネコにおける (S)-32b の睡眠誘発作用を確認することができた。



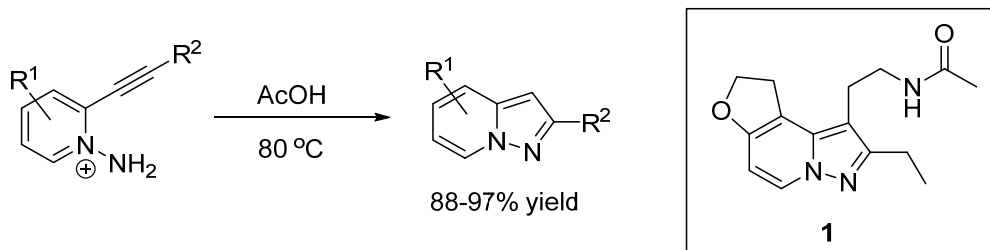
**Figure 19.** Effects of (S)-32b on total sleep time in freely moving cats. Seven cats were treated with (S)-32b (10 mg/kg, p.o.) or vehicle. Data were expressed as the mean of total time sleep during 8 h following the oral administration with standard error of mean. \* $p < 0.05$  (versus the vehicle-treated control by ANOVA). [J. Med. Chem. 2021, 64, 3059–3074, Figure 6 with modifications]

以上述べてきたように、経口吸収性向上と強力な  $MT_1/MT_2$  結合親和性の両立を目指した新規三環性誘導体の探索を行った。 $MT_1/MT_2$  結合親和性およびヒト肝ミクロソーム中における代謝安定性を指標に最適化を実施したところ、強力な  $MT_1/MT_2$  結合親和性 ( $MT_1$ ,  $K_i = 0.031$  nM;  $MT_2$ ,  $K_i = 0.070$  nM) と優れた代謝安定性 ( $0 \mu\text{L}/\text{min}/\text{mg}$ ) を併せ持つ (S)-32b を見出すことに成功した。今回合成した一連の化合物群が、ラット肝ミクロソーム中におけるクリアランスとラット経口吸収性に明らかな相関を示したことから、ヒト肝ミクロソーム中において優れた代謝安定性を示す化合物はヒトにおいて良好な経口吸収性を示すと考えられる。すなわち、ラメルテオン ( $51 \mu\text{L}/\text{min}/\text{mg}$ ) と比較して優れた代謝安定性を示す (S)-32b はヒトでの良好な経口吸収性が期待できる。さらに、(S)-32b は  $MT_1/MT_2$  に対してフルアゴニストとして作用し、ラットにおいて良好な脳内移行性を示すとともに、ネコを用いた睡眠評価モデルにおいて睡眠誘発作用を示した。これらの結果より、(S)-32b は臨床試験に向けたさらなる検討を実施中である。

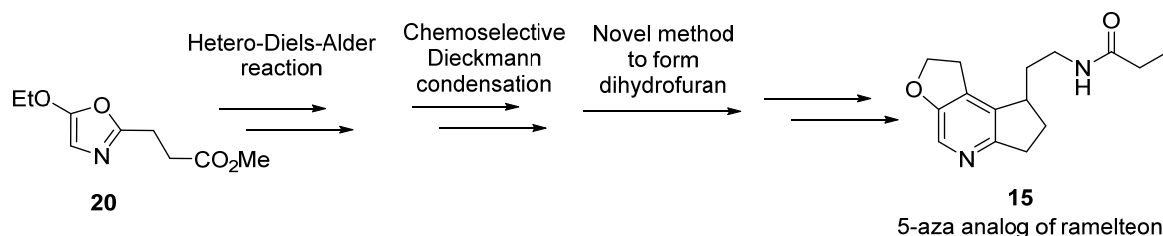
## 結語

著者は、母核に **angular** 型 5-6-5 縮合ヘテロ環を有するメラトニン受容体作動薬の合成研究を実施した。本研究で得られた知見を以下にまとめる。

1. MT<sub>1</sub>/MT<sub>2</sub> アゴニスト **1** の合成法を改良するため、課題となっているピラゾロ[1,5-*a*]ピリジン環の新規合成法を探索した。その結果、*N*-アミノ-2-アルキニルピリジン誘導体を酢酸中 80°C で加熱することにより、ピラゾロ[1,5-*a*]ピリジンが効率よく合成できることを見出した。本反応は基質一般性が高く、ピラゾロ[1,5-*a*]ピリジン環を有する様々な生理活性物質の合成法として極めて有用である。また、本反応を利用して、MT<sub>1</sub>/MT<sub>2</sub> アゴニスト **1** の新たな合成ルートを構築した。今回見出した新しい合成ルートでは、以前の合成ルートと比較して共通中間体 **14** から **13** までの総収率が大幅に改善された (2.9%→42%)。  
(第 1 章)

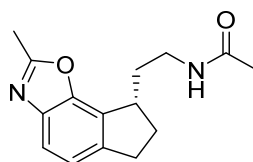


2. 経口吸収性を向上した MT<sub>1</sub>/MT<sub>2</sub> アゴニストの創製を目指し、新規シクロペンタ[*b*]フロ[3,2-*d*]ピリジンを母核として有する 5-アザラメルテオン **15** をデザインした。**15** の合成は、ヘテロ Diels-Alder 反応とそれに続く官能基選択的 Dieckmann 縮合反応により達成した。また、水素添加反応のみで一挙にオキソフラン体 **27** からジヒドロフラン体 **28** へと変換する新たな変換方法を見出した。**15** はラメルテオンと比較して MT<sub>1</sub>/MT<sub>2</sub> 結合親和性の大幅な低下が見られ、ラメルテオンのベンゼン環上への窒素原子導入が結合親和性に極めて大きな影響を与えることを見出した。(第 2 章)



3. 経口吸収性の向上と強力な結合親和性の両立を目指した MT<sub>1</sub>/MT<sub>2</sub> アゴニストとして、インデノ[5,4-*d*][1,3]オキサゾール誘導体 (**32**)、インデノ[5,4-*d*][1,3]チアゾール誘導体 (**33**)、シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体 (**34**)、シクロペンタ[*e*]インダゾール誘導体 (**35**) をデザインし、それらの合成を達成した。これらの誘導体は大幅な親和性の減弱

をもたらさず、特にインデノ[5,4-*d*][1,3]オキサゾール誘導体およびインデノ[5,4-*d*][1,3]チアゾール誘導体はラメルテオンに匹敵する強い MT<sub>1</sub>/MT<sub>2</sub> 結合親和性を示すことを明らかにした。シクロペンタ[*c*]ピラゾロ[1,5-*a*]ピリジン誘導体は MT<sub>1</sub> 選択性を示し、さらなる三環性骨格の探索による MT<sub>1</sub> 選択的リガンド創出の可能性を示唆した。MT<sub>1</sub>/MT<sub>2</sub> 結合親和性およびヒト肝ミクロソーム中における代謝安定性を指標に最適化を実施したところ、強力な MT<sub>1</sub>/MT<sub>2</sub> 結合親和性 (MT<sub>1</sub>,  $K_i = 0.031$  nM; MT<sub>2</sub>,  $K_i = 0.070$  nM) と優れた代謝安定性 (0  $\mu$ L/min/mg) を併せ持つ (S)-**32b** を見出すことに成功した。ラメルテオン (51  $\mu$ L/min/mg) と比較して優れた代謝安定性を示す (S)-**32b** はヒトでの良好な経口吸収性が期待できる。さらに、(S)-**32b** は MT<sub>1</sub>/MT<sub>2</sub> に対してフルアゴニストとして作用し、ラットにおいて良好な脳内移行性を示すとともに、ネコを用いた睡眠評価モデルにおいて睡眠誘発作用を示した。これらの結果より、(S)-**32b** は臨床試験に向けたさらなる検討を実施中である。(第3章)



(S)-**32b**

MT<sub>1</sub> binding affinity:  $K_i = 0.031$  nM  
 MT<sub>2</sub> binding affinity:  $K_i = 0.070$  nM  
 Superior metabolic stability to ramelteon  
 Good BBB permeability  
 Sleep promoting effect in cats

以上述べたように、著者は種々の angular 型 5-6-5 縮合ヘテロ環の合成法を新たに開発した。本研究で見出した合成法を用いることで、様々な創薬ターゲットへの応用が期待される。また、angular 型 5-6-5 縮合ヘテロ環を母核として有する MT<sub>1</sub>/MT<sub>2</sub> アゴニストの合成研究の結果、経口吸収性を改善した次世代型 MT<sub>1</sub>/MT<sub>2</sub> アゴニスト (S)-**32b** の創製に成功した。(S)-**32b** は、不眠症治療薬だけでなく様々な疾患への応用が可能と考えられ、医療に貢献する化合物として今後の展開に期待したい。

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## Experimental Section

Melting points were determined on a Buchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance ( $^1\text{H}$ -NMR) spectra were recorded on Varian Gemini-200 (200 MHz), Varian Gemini-300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as  $\delta$  values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; brs, broad singlet; m, multiplet. Coupling constants ( $J$  values) are given in hertz (Hz). LCMS analysis ( $\text{ESI}^+$ ) was performed on Waters 2795 separations module (L-column2 ODS (3.0 x50 mm I.D., CERI, Japan); 0.05% TFA in ultrapure water/acetonitrile gradient; UV detection 220 nm or 254 nm) and MS spectra were recorded using a Waters ZQ2000 with electrospray ionization. Optical rotations were determined on a JASCO P-1030 polarimeter. The purities (>95%) of all compounds tested in biological systems were established by HPLC analyses or elemental analyses. HPLC was performed on a Shimadzu LC-VP instrument, equipped with CAPCELL PAK C18 UG120 S-3  $\mu\text{m}$ ,  $2.0 \times 50$  mm column with a 4 min linear gradient from 90/10 to 5/95 and subsequently with a 1.5 min isocratic elution 5/95 A/B, where A =  $\text{H}_2\text{O}$ -0.1%TFA, B =  $\text{CH}_3\text{CN}$ -0.1%TFA, at a flow rate of 0.5  $\mu\text{L}/\text{min}$ , with UV detection at 220 and 254 nm. Element analyses were carried out by Takeda Analytical Laboratories and Discovery Research Laboratories, and the results were within 0.4% of theoretical values unless otherwise noted. Chromatographic purification was carried out on thin layer chromatography (TLC silica gel 60 F<sub>254</sub>, Merck) or on silica gel columns [(Merck Kieselgel 60, 70–230 mesh or 230–400 mesh, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh)] or on Purif-Pack (SI 60  $\mu\text{M}$  or NH 60  $\mu\text{M}$ , Shoko Scientific Co., Ltd.). Reagents and solvents were obtained from commercial sources and used without further purification. All animal experiments in this paper were conducted in accordance with *principles and guidelines on animal experimentation of Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd.*

### Chapter 1

#### **1-Amino-2-(4-phenylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (5a)**

A suspension of 2-bromopyridine (1.58 g, 10.0 mmol), 4-phenyl-1-butyne (1.56 g, 12.0 mmol), bis(triphenylphosphine)palladium(II) dichloride (702 mg, 1.00 mmol) and copper iodide (190 mg, 1.00 mmol) in triethylamine (20 mL) was stirred at room temperature for 2 hr. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded 2-(4-phenylbut-1-yn-1-yl)pyridine **4a** (1.41 g, yield 68%) as pale yellow oil. Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 2-(4-phenylbut-1-yn-1-yl)pyridine **4a** (104 mg, 0.500 mmol) in acetonitrile (2.5 ml) at 0 °C. After 1 hr, diethyl ether was

added and the resulting solid was collected by filtration to afford **5a** (186 mg, yield 88 %) as colorless solid. MS (ESI):  $m/z$  223  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  2.23 (s, 3H), 2.68 (s, 6H), 2.85–3.05 (m, 4H), 6.81 (s, 2H), 7.18–7.38 (m, 5H), 7.57 (dd,  $J$  = 8.0, 1.6 Hz, 1H), 7.62–7.72 (m, 1H), 7.83 (t,  $J$  = 8.0 Hz, 1H), 8.45 (brs, 2H), 9.46 (d,  $J$  = 6.0 Hz, 1H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  140.2, 139.08, 139.05, 138.2, 137.10, 137.05, 130.7, 130.6, 130.1, 128.8, 128.4, 127.1, 126.0, 111.2, 70.8, 33.4, 23.1, 22.1, 20.8.

### 1-Amino-5-chloro-2-(4-phenylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (**5b**)

A suspension of 2-bromo-5-chloropyridine (674 mg, 3.50 mmol), 4-phenyl-1-butyne (547 mg, 4.20 mmol), bis(triphenylphosphine)palladium(II) dichloride (246 mg, 0.350 mmol) and copper iodide (66.7 mg, 0.350 mmol) in triethylamine (7 mL) was stirred at room temperature for 30 min. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded 5-chloro-2-(4-phenylbut-1-yn-1-yl)pyridine **4b** (749 mg, yield 89%) as pale yellow oil. Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 5-chloro-2-(4-phenylbut-1-yn-1-yl)pyridine **4b** (121 mg, 0.500 mmol) in acetonitrile (2.5 ml) at 0 °C. After being stirred for 1.5 hr at room temperature, the reaction mixture was concentrated in vacuo and diethyl ether was added. The resulting solid was collected by filtration to afford **5b** (218 mg, yield 95%) as colorless solid. MS (ESI):  $m/z$  257  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  ppm 2.22 (s, 3H), 2.68 (s, 6H), 2.92 (s, 4H), 6.80 (s, 2H), 7.17–7.25 (m, 3H), 7.28–7.36 (m, 2H), 7.47 (d,  $J$  = 8.8 Hz, 1H), 7.72 (dd,  $J$  = 8.8, 1.9 Hz, 1H), 8.78 (brs, 2H), 9.56 (d,  $J$  = 1.9 Hz, 1H).  $^{13}C$  NMR ( $DMSO-d_6$ )  $\delta$  142.6, 139.7, 138.3, 137.3, 136.3, 135.9, 132.0, 131.3, 129.8, 129.3, 128.5, 128.4, 126.5, 111.2, 70.5, 32.8, 22.7, 21.6, 20.3.

### 1-Amino-5-methoxy-2-(4-phenylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (**5c**)

A suspension of 2-bromo-5-methoxypyridine (940 mg, 5.00 mmol), 4-phenyl-1-butyne (1.30 g, 10.0 mmol), bis(triphenylphosphine)palladium(II) dichloride (351 mg, 0.500 mmol) and copper iodide (95.2 mg, 0.500 mmol) in triethylamine (10 mL) was stirred at room temperature for 2 hr. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded 5-methoxy-2-(4-phenylbut-1-yn-1-yl)pyridine **4c** (1.08 g, yield 91%) as pale yellow oil. Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 5-methoxy-2-(4-phenylbut-1-yn-1-yl)pyridine **4c** (119 mg, 0.500 mmol) in acetonitrile (1.0 ml) at 0 °C. After 1 hr, the reaction mixture was concentrated in vacuo and diethyl ether was added. The solvent was removed by decantation and the resulting precipitate was dried in vacuo to afford **5c** (182 mg, yield 80%) as yellow amorphous. MS (ESI):  $m/z$  253  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  ppm 2.23 (s, 3H), 2.69 (s, 6H), 2.84–3.02 (m, 4H), 4.08 (s, 3H), 6.83 (s, 2H), 7.20–7.28 (m, 3H), 7.30–7.39 (m, 3H), 7.44 (d,  $J$  = 9.0 Hz, 1H), 9.34 (d,  $J$  = 2.7 Hz, 1H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  157.5, 139.8, 139.0, 138.4, 137.1, 130.6, 129.7, 128.9, 128.4, 127.2, 125.7, 124.7, 123.0, 108.9, 70.2, 58.4, 33.6, 23.0, 22.0, 20.8.

#### 1-Amino-3-methyl-2-(4-phenylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (5d)

A suspension of 2-bromo-3-methylpyridine (860 mg, 5.00 mmol), 4-phenyl-1-butyne (976 mg, 7.50 mmol), bis(triphenylphosphine)palladium(II) dichloride (176 mg, 0.250 mmol) and copper iodide (47.6 mg, 0.250 mmol) in triethylamine (10 mL) was stirred at 60 °C for 1 hr. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded 3-methyl-2-(4-phenylbut-1-yn-1-yl)pyridine **4d** (1.03 g, yield 93%) as brown oil. Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 3-methyl-2-(4-phenylbut-1-yn-1-yl)pyridine **4d** (111 mg, 0.500 mmol) in acetonitrile (1.0 ml) at 0 °C. After 1.5 hr, the reaction mixture was concentrated in vacuo and diethyl ether was added. The solvent was removed by decantation and the resulting precipitate was dried in vacuo to afford **5d** (141 mg, yield 64%) as pale yellow solid. MS (ESI):  $m/z$  237  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  ppm 2.21 (s, 3H), 2.31 (s, 3H), 2.65 (s, 6H), 2.98 (s, 4H), 6.79 (s, 2H), 7.16–7.36 (m, 5H), 7.53 (dd,  $J$  = 8.0, 6.4 Hz, 1H), 7.67 (d,  $J$  = 8.0 Hz, 1H), 9.23 (d,  $J$  = 6.4 Hz, 1H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  140.7, 140.0, 139.0, 138.2, 137.6, 137.1, 136.5, 130.6, 130.4, 128.8, 128.4, 127.1, 124.8, 114.7, 69.9, 33.5, 23.1, 22.1, 20.8, 19.7.

#### 1-Amino-6-methyl-2-(4-phenylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (5e)

A suspension of 2-bromo-6-methylpyridine (860 mg, 5.00 mmol), 4-phenyl-1-butyne (976 mg, 7.50 mmol), bis(triphenylphosphine)palladium(II) dichloride (176 mg, 0.250 mmol) and copper iodide (47.6 mg, 0.250 mmol) in triethylamine (10 mL) was stirred at room temperature for 2.5 hr. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded 6-methyl-2-(4-phenylbut-1-yn-1-yl)pyridine **4e** (967 mg, yield 87%) as yellow oil. Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 6-methyl-2-(4-phenylbut-1-yn-1-yl)pyridine **4e** (111 mg, 0.500 mmol) in acetonitrile (1.0 ml) at 0 °C. After 1.5 hr, the reaction mixture was concentrated in vacuo and diethyl ether was added. The resulting solid was collected by filtration to afford **5e** (195 mg, yield 89%) as colorless solid. MS (ESI):  $m/z$  237  $M^+$ .  $^1H$  NMR ( $DMSO-d_6$ )  $\delta$  ppm 2.16 (s, 3H), 2.49 (s, 6H), 2.73 (s, 3H), 2.88–3.09 (m, 4H), 6.73 (s, 2H), 7.17–7.39 (m, 5H), 7.66 (brs, 2H), 7.84–7.94 (m, 2H), 8.13 (t,  $J$  = 8.0 Hz, 1H).  $^{13}C$  NMR ( $DMSO-d_6$ )  $\delta$  152.2, 142.7, 139.8, 139.5, 136.2, 135.8, 133.4, 129.8, 129.0, 128.5, 128.4, 128.0, 126.5, 109.2, 71.4, 32.8, 22.7, 21.5, 20.2, 19.5.

#### 1-Amino-2-(3,3-dimethylbut-1-yn-1-yl)pyridinium 2,4,6-trimethylbenzenesulfonate (5f)

Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of 2-(3,3-dimethylbut-1-yn-1-yl)pyridine **4f**<sup>20</sup> (79.6 mg, 0.500 mmol) in acetonitrile (1.0 ml) at 0 °C. After 1.5 hr, the reaction mixture was concentrated in vacuo and diethyl ether was added. The resulting solid was

collected by filtration to afford **5f** (138 mg, yield 74%) as colorless solid. MS (ESI):  $m/z$  175  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  ppm 1.39 (s, 9H), 2.23 (s, 3H), 2.68 (s, 6H), 6.81 (s, 2H), 7.63–7.77 (m, 2H), 7.85–7.97 (m, 1H), 9.49 (d,  $J$  = 6.4 Hz, 1H).

### 1-Amino-2-(phenylethynyl)pyridinium 2,4,6-trimethylbenzenesulfonate (**5g**)<sup>20</sup>

Wet *O*-(mesitylsulfonyl)hydroxylamine (431 mg, 1.00 mmol) was added to a solution of 2-(phenylethynyl)pyridine **4g**<sup>20</sup> (179 mg, 1.00 mmol) in acetonitrile (5.0 ml) at 0 °C. After 1 hr, diethyl ether was added. The resulting solid was collected by filtration to afford **5g** (323 mg, yield 82%) as colorless solid. MS (ESI):  $m/z$  195  $M^+$ .  $^1H$  NMR ( $CDCl_3$ )  $\delta$  ppm 2.20 (s, 3H), 2.66 (s, 6H), 6.78 (s, 2H), 7.39–7.44 (m, 2H), 7.47–7.55 (m, 1H), 7.61–7.66 (m, 2H), 7.71–7.76 (m, 1H), 7.80 (dd,  $J$  = 8.1, 1.7 Hz, 1H), 7.91–7.96 (m, 1H), 8.73 (brs, 2H), 9.59 (d,  $J$  = 6.4 Hz, 1H).

### 5-Amino-4-(but-1-yn-1-yl)-2,3-dihydrofuro[3,2-*c*]pyridinium 2,4,6-trimethylbenzenesulfonate (**5h**)

Wet *O*-(mesitylsulfonyl)hydroxylamine (215 mg, 1.00 mmol) was added to a solution of **9** (87 mg, 0.500 mmol) in  $CH_3CN$  (2.5 ml) at 0 °C. After 30 min,  $Et_2O$  was added and the resulting solid was collected by filtration to afford **5h** (156 mg, yield 80%) as colorless solid. MS (ESI):  $m/z$  189  $M^+$ .  $^1H$  NMR ( $DMSO-d_6$ )  $\delta$  ppm 1.24 (t,  $J$  = 7.4 Hz, 3H), 2.15 (s, 3H), 2.48 (s, 6H), 2.67 (q,  $J$  = 7.4 Hz, 2H), 3.37 (t,  $J$  = 8.8 Hz, 2H), 4.95 (t,  $J$  = 8.8 Hz, 2H), 6.72 (s, 2H), 7.31 (d,  $J$  = 7.1 Hz, 1H), 7.50 (brs, 2H), 8.57 (d,  $J$  = 7.1 Hz, 1H).  $^{13}C$  NMR ( $DMSO-d_6$ )  $\delta$  168.8, 145.2, 142.8, 136.1, 135.8, 132.7, 132.1, 129.8, 112.3, 106.4, 76.1, 68.9, 27.5, 22.7, 20.2, 13.1, 12.7.

### 2-(2-Phenylethyl)pyrazolo[1,5-*a*]pyridine (**6a**)

A solution of **5a** (169 mg, 0.400 mmol) in AcOH (2.0 mL) was stirred for 24 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6a** (83.8 mg, yield 94%) as colorless solid. Mp 68–70 °C (recrystallized from hexane). MS (ESI):  $m/z$  223 ( $M+H$ )<sup>+</sup>.  $^1H$  NMR ( $CDCl_3$ )  $\delta$  3.00–3.22 (m, 4H), 6.27 (s, 1H), 6.63–6.71 (m, 1H), 6.99–7.09 (m, 1H), 7.15–7.34 (m, 5H), 7.41 (d,  $J$  = 8.8 Hz, 1H), 8.38 (d,  $J$  = 7.1 Hz, 1H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  155.5, 141.7, 141.0, 128.44, 128.40, 128.2, 126.0, 123.2, 117.5, 111.0, 95.3, 36.0, 30.5. Anal. Calcd for  $C_{15}H_{14}N_2$ : C, 81.05; H, 6.35; N, 12.60. Found: C, 81.17; H, 6.52; N, 12.66.

### 6-Chloro-2-(2-phenylethyl)pyrazolo[1,5-*a*]pyridine (**6b**)

A solution of **5b** (182 mg, 0.400 mmol) in AcOH (2.0 mL) was stirred for 24 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo.

The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6b** (93.1 mg, yield 91%) as colorless solid. Mp 89–91 °C (recrystallized from hexane). MS (ESI):  $m/z$  257 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.99–3.21 (m, 4H), 6.28 (s, 1H), 7.03 (dd,  $J$  = 9.5, 1.8 Hz, 1H), 7.10–7.45 (m, 6H), 8.38–8.45 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 156.1, 141.5, 139.3, 128.4, 126.4, 126.1, 124.7, 119.0, 117.6, 96.3, 35.8, 30.4. Anal. Calcd for C<sub>15</sub>H<sub>13</sub>N<sub>2</sub>Cl: C, 70.18; H, 5.10; N, 10.91. Found: C, 70.13; H, 5.09; N, 10.88.

#### 6-Methoxy-2-(2-phenylethyl)pyrazolo[1,5-*a*]pyridine (**6c**)

A solution of **5c** (175 mg, 0.387 mmol) in AcOH (1.9 mL) was stirred for 72 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6c** (88.5 mg, yield 91%) as colorless oil. MS (ESI):  $m/z$  253 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.96–3.18 (m, 4H), 3.81 (s, 3H), 6.21 (s, 1H), 6.88 (dd,  $J$  = 9.7, 2.1 Hz, 1H), 7.15–7.35 (m, 6H), 8.01 (d,  $J$  = 2.1 Hz, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 154.4, 148.6, 141.8, 137.3, 128.43, 128.37, 125.9, 118.5, 117.3, 110.8, 95.1, 56.0, 36.1, 30.5.

#### 4-Methyl-2-(2-phenylethyl)pyrazolo[1,5-*a*]pyridine (**6d**)

A solution of **5d** (133 mg, 0.305 mmol) in AcOH (1.5 mL) was stirred for 3 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6d** (63.5 mg, yield 88%) as colorless oil. MS (ESI):  $m/z$  237 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.41 (s, 3H), 3.01–3.21 (m, 4H), 6.26 (s, 1H), 6.53–6.65 (m, 1H), 6.79–6.88 (m, 1H), 7.14–7.34 (m, 5H), 8.08–8.36 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 155.0, 142.1, 141.8, 128.43, 128.40, 127.2, 126.0, 125.9, 122.0, 111.0, 94.2, 36.1, 30.6, 18.2. Anal. Calcd for C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>•0.2H<sub>2</sub>O: C, 80.10; H, 6.89; N, 11.68. Found: C, 80.00; H, 6.84; N, 11.79.

#### 7-Methyl-2-(2-phenylethyl)pyrazolo[1,5-*a*]pyridine (**6e**)

A solution of **5e** (178 mg, 0.408 mmol) in AcOH (2.0 mL) was stirred for 3 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6e** (89.0 mg, yield 92%) as colorless oil. MS (ESI):  $m/z$  237 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.73 (s, 3H), 3.04–3.14 (m, 2H), 3.15–3.25 (m, 2H), 6.32 (s, 1H), 6.51–6.56 (m, 1H), 7.00 (dd,  $J$  = 8.7, 6.8 Hz, 1H), 7.15–7.37 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 155.0, 141.9, 141.4, 137.8, 128.5, 128.4, 125.9, 123.1, 115.0, 110.2, 95.5, 36.1, 30.5, 18.0. Anal. Calcd for C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>•0.1H<sub>2</sub>O: C, 80.71; H, 6.86; N, 11.76. Found: C, 80.79; H, 6.88; N, 11.93.

### 2-(1,1-Dimethylethyl)pyrazolo[1,5-*a*]pyridine (**6f**)<sup>15c</sup>

A solution of **5f** (123 mg, 0.328 mmol) in AcOH (1.6 mL) was stirred for 30 min at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6f** (55.2 mg, yield 97%) as colorless oil. MS (ESI): *m/z* 175 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.42 (s, 9H), 6.33 (s, 1H), 6.59–6.67 (m, 1H), 6.96–7.08 (m, 1H), 7.36–7.46 (m, 1H), 8.36–8.43 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 165.0, 140.8, 128.4, 122.9, 117.4, 110.7, 92.8, 32.4, 30.7.

### 2-Phenylpyrazolo[1,5-*a*]pyridine (**6g**)<sup>20</sup>

A solution of **5g**<sup>20</sup> (150 mg, 0.380 mmol) in AcOH (1.9 mL) was stirred for 36 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6g** (66.4 mg, yield 90%) as colorless solid. MS (ESI): *m/z* 195 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.74 (td, *J* = 6.8, 1.2 Hz, 1H), 6.80 (s, 1H), 7.06–7.13 (m, 1H), 7.33–7.54 (m, 4H), 7.88–8.04 (m, 2H), 8.45–8.50 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 153.6, 141.7, 133.2, 128.8, 128.5, 128.4, 126.5, 123.5, 117.9, 111.7, 93.7.

### 2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine (**6h**)

A solution of **5h** (140 mg, 0.360 mmol) in AcOH (1.8 mL) was stirred for 2 hr at 80 °C. The reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane) to afford **6h** (59.6 mg, yield 88%) as colorless solid. Mp 51–53 °C (recrystallized from hexane). MS (ESI): *m/z* 189 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.35 (t, *J* = 7.5 Hz, 3H), 2.82 (q, *J* = 7.5 Hz, 2H), 3.28 (t, *J* = 9.1 Hz, 2H), 4.72 (t, *J* = 9.1 Hz, 2H), 5.98 (s, 1H), 6.40 (d, *J* = 7.4 Hz, 1H), 8.18 (d, *J* = 7.4 Hz, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 159.2, 156.0, 140.0, 128.6, 109.0, 98.9, 90.6, 72.3, 28.6, 21.9, 13.9. Anal. Calcd for C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O: C, 70.19; H, 6.43; N, 14.88. Found: C, 70.17; H, 6.27; N, 14.84.

### 2,3-Dihydrofuro[3,2-*c*]pyridin-4-yl trifluoromethanesulfonate (**10**)

To a stirred suspension of **7**<sup>22</sup> (4.60 g, 33.5 mmol) in pyridine (90 mL) was added trifluoromethane sulfonic anhydride (6.76 mL, 40.2 mmol) at 0 °C. After 10 min, H<sub>2</sub>O (90 mL) was added, and the mixture was concentrated in vacuo. The residue was extracted with ethyl acetate, washed with 1 M hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded **10** (8.60 g, yield 95%) as colorless solid. MS (ESI): *m/z* 270 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.34 (t, *J* = 8.9 Hz, 2H), 4.77 (t, *J* = 8.9 Hz, 2H), 6.79 (d, *J* = 5.5 Hz, 1H), 8.07 (d, *J* = 5.5 Hz,

1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 171.3, 152.7, 148.7, 118.5 (q, *J* = 320.5 Hz), 114.4, 107.0, 73.1, 26.0. Calcd for C<sub>8</sub>H<sub>6</sub>NF<sub>3</sub>O<sub>4</sub>S: C, 35.69; H, 2.25; N, 5.20. Found: C, 35.79; H, 2.32; N, 5.20.

#### 4-But-1-yn-1-yl-2,3-dihydrofuro[3,2-*c*]pyridine (**9**)

After bubbling 1-butyne (2.2 g, 40.7 mmol) gas into triethylamine (30 mL), **10** (4.00 g, 14.9 mmol), bis(triphenylphosphine)palladium(II) dichloride (1.05 g, 1.49 mmol) and CuI (284 mg, 1.49 mmol) was added. After being stirred at room temperature for 6 hr, the reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded **9** (2.21 g, yield 86%) as pale yellow solid. Mp 59–61 °C (recrystallized from ethyl acetate/hexane). MS (ESI): *m/z* 174 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.26 (t, *J* = 7.5 Hz, 3H), 2.47 (q, *J* = 7.5 Hz, 2H), 3.27 (t, *J* = 8.8 Hz, 2H), 4.65 (t, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 5.5 Hz, 1H), 8.23 (d, *J* = 5.5 Hz, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 166.6, 150.0, 140.2, 126.3, 104.8, 95.3, 77.8, 72.0, 27.9, 13.7, 13.1. Anal. Calcd for C<sub>11</sub>H<sub>11</sub>NO: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.26; H, 6.32; N, 8.04.

#### (2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)acetonitrile (**13**)<sup>14</sup>

To a stirred suspension of Eschenmoser's salt (182 mg, 0.982 mmol) in acetonitrile (4.1 mL) was added a solution of **6h** (154 mg, 0.818 mmol) in acetonitrile (4.1 mL) at 0 °C. After completion of addition, the reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was dissolved in ethyl acetate (4.1 mL) and iodomethane (50.9 μL, 0.818 mmol) was added at room temperature. After 1 hr, iodomethane (50.9 μL, 0.818 mmol) was added. After 2 hr, the resulting solid was collected by filtration. To a stirred suspension of the obtained solid in acetonitrile (4.1 mL) was added potassium cyanide (80.1 mg, 1.23 mmol) and 18-crown-6 (43.2 mg, 0.164 mmol). After being stirred for 3 hr at 80 °C, the reaction mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded **13** (165 mg, yield 89%) as colorless solid. MS (ESI): *m/z* 228 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.36 (t, *J* = 7.6 Hz, 3H), 2.80 (q, *J* = 7.6 Hz, 2H), 3.56 (t, *J* = 9.1 Hz, 2H), 3.73 (s, 2H), 4.77 (t, *J* = 9.1 Hz, 2H), 6.45 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 156.9, 156.6, 138.4, 129.2, 118.6, 108.0, 99.7, 92.9, 72.4, 28.1, 20.0, 13.6, 12.3. Anal. Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>O: C, 68.70; H, 5.77; N, 18.49. Found: C, 68.84; H, 5.74; N, 18.61.

## Chapter 2

#### Methyl 4-[(2-ethoxy-2-oxoethyl)amino]-4-oxobutanoate (**23**)

Triethylamine (9.99 mL, 71.6 mmol) and methyl 4-chloro-4-oxobutyrates (5.39 g, 35.8 mmol) was added to a suspension of glycine ethyl ester hydrochloride (5.00 g, 35.8 mmol) in THF (300 mL) at 0 °C. After the mixture was stirred at room temperature for 30 minutes, water and EtOAc were added to the mixture. The organic layer was washed with saturated aqueous NaHCO<sub>3</sub> and brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo, and the residue was recrystallized from a mixture of EtOAc and hexane to afford **23** (5.05 g, 65%) as solid. Mp 61–62 °C (recrystallized from EtOAc and hexane). MS (ESI): *m/z* 218 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.29 (3H, t, *J* = 7.0 Hz), 2.56 (2H, t, *J* = 6.2 Hz), 2.69 (2H, t, *J* = 6.2 Hz), 3.69 (3H, s), 4.03 (2H, d, *J* = 4.9 Hz), 4.21 (2H, q, *J* = 7.0 Hz), 6.17 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.1, 29.2, 30.7, 41.5, 51.9, 61.5, 169.9, 171.5, 173.3. Anal. Calcd for C<sub>9</sub>H<sub>15</sub>NO<sub>5</sub>: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.80; H, 6.95; N, 6.39.

### Methyl 3-(5-ethoxy-1,3-oxazol-2-yl)propanoate (**20**)

A solution of **23** (630 mg, 2.90 mmol) in MeCN (1.7 mL) was added to a suspension of P<sub>4</sub>O<sub>10</sub> (1.65 g, 5.80 mmol) in MeCN (7.0 mL) at 80 °C. After 4 hr. The reaction mixture was concentrated in vacuo, and AcOEt and ice was added. The aqueous phase was neutralized with saturated aqueous NaHCO<sub>3</sub> and the organic phase was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by silica gel column chromatography to yield **20** (309 mg, 54%) as oil. MS (ESI): *m/z* 200 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.41 (3 H, t, *J* = 7.2 Hz), 2.72–2.80 (2 H, m), 2.92–3.01 (2 H, m), 3.70 (3H, s), 4.08 (2 H, q, *J* = 7.2 Hz), 5.95 (1 H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.5, 23.5, 30.5, 51.9, 68.0, 99.1, 153.6, 159.5, 172.5.

### Dimethyl 5-hydroxy-2-(3-methoxy-3-oxopropyl)pyridine-3,4-dicarboxylate (**24**)

A mixture of **20** (7.00 g, 35.1 mmol) and maleic anhydride (3.44 g, 35.1 mmol) was stirred at room temperature for 2 hr, then 10% methanolic hydrogen chloride (100 mL) added and the resultant mixture was refluxed for 15 hr. After cooling to room temperature, the solvent was removed in vacuo. The residue was purified by silica gel column chromatography. The purified compounds was diluted with MeOH (180 mL), and a solution of TMSCHN<sub>2</sub> in Et<sub>2</sub>O (2M, 34.0 mL) was added dropwise at room temperature. After being stirred for 30 min, the reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography to afford **24** (3.10 g, 30%) as solid. Mp 66–67 °C (recrystallized from EtOAc and diisopropyl ether). MS (ESI): *m/z* 298 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.72–2.82 (2H, m), 2.96–3.04 (2H, m), 3.67 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 8.47 (1H, s), 10.23 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 29.6, 32.7, 51.7, 52.9, 53.6, 115.1, 126.6, 142.6, 147.0, 153.3, 167.8, 168.0, 173.3. Anal. Calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>7</sub>: C, 52.53; H, 5.09; N, 4.71. Found: C, 52.51; H, 4.94; N, 4.67.

### Dimethyl 5-(2-methoxy-2-oxoethoxy)-2-(3-methoxy-3-oxopropyl)pyridine-3,4-dicarboxylate (**19**)

Methyl bromoacetate (3.26 mL, 34.4 mmol) was added to a mixture of **24** (5.10 g, 17.2 mmol) and K<sub>2</sub>CO<sub>3</sub> (12.3 g, 69.0 mmol) in acetone (100 mL) at room temperature. After being stirred at room

temperature for 30 minutes, the reaction mixture was concentrated in vacuo. The residue was diluted with H<sub>2</sub>O (100 mL), extracted with EtOAc, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **19** (5.14 g, 81%) as solid. Mp 47–48 °C (recrystallized from diethyl ether and hexane). MS (ESI): *m/z* 370 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm 2.79 (2H, t, *J* = 7.3 Hz), 3.25 (2H, t, *J* = 7.3 Hz), 3.67 (3H, s), 3.80 (3H, s), 3.90 (3H, s), 3.92 (3H, s), 4.75 (2H, s), 8.30 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 30.3, 32.5, 51.6, 52.5, 52.90, 52.95, 67.0, 125.5, 131.3, 137.8, 148.9, 152.6, 165.0, 166.1, 168.1, 173.4. Anal. Calcd for C<sub>16</sub>H<sub>19</sub>NO<sub>9</sub>: C, 52.03; H, 5.19; N, 3.79. Found: C, 52.02; H, 5.15; N, 3.75.

### Dimethyl 3-hydroxy-5-(3-methoxy-3-oxopropyl)furo[2,3-*c*]pyridine-2,4-dicarboxylate (**26**)

To a solution of **19** (92.3 mg, 0.250 mmol) in THF (2.5 ml) was added a solution of NaOMe in methanol (28%, 53.1 mg, 0.275 mmol) at room temperature. After 30 min, the mixture was neutralized with 1N HCl (0.300 mL) and extracted with EtOAc twice. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by column chromatography to afford **26** (74.0 mg, 0.219 mmol, 88 %) as solid. Mp 117–118 °C (recrystallized from EtOAc and diisopropyl ether). MS (ESI): *m/z* 338 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.86 (2H, t, *J* = 7.3 Hz), 3.51 (2H, t, *J* = 7.3 Hz), 3.68 (3H, s), 4.03 (3H, s), 4.11 (3H, s), 8.93 (1H, s), 10.13 (1H, brs). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 31.8, 33.0, 51.7, 52.4, 53.7, 118.6, 127.2, 130.3, 138.1, 146.0, 148.0, 154.4, 160.5, 169.3, 173.5. Anal. Calcd for C<sub>15</sub>H<sub>15</sub>NO<sub>8</sub>: C, 53.42; H, 4.48; N, 4.15. Found: C, 53.44; H, 4.41; N, 4.14.

### Methyl 5-(3-methoxy-3-oxopropyl)-2,3-dihydrofuro[2,3-*c*]pyridine-4-carboxylate (**17**)

After a mixture of **26** (3.10 g, 9.19 mmol) and conc. HCl (31 mL) was stirred at reflux for 1 hr, the mixture was concentrated in vacuo. The residue was dissolved in MeOH (60 mL) and the mixture was stirred in the presence of 10% Pd/C (0.600 g, containing 50% water) at room temperature for 3 hr under hydrogen atmosphere. After the catalyst was removed by filtration, the filtrate was concentrated in vacuo. The residue was dissolved in MeOH (60 mL) and the mixture was stirred in the presence of 10% Pd/C (1.00 g, containing 50% water) at 50 °C for 4 hr under hydrogen atmosphere. After the catalyst was removed by filtration, the filtrate was concentrated in vacuo. The residue was dissolved in MeOH (60 mL) and a solution of TMSCHN<sub>2</sub> in Et<sub>2</sub>O (2M, 9.20 mL) was added dropwise at 0 °C. After 30 minutes, the reaction mixture was quenched with saturated aqueous sodium hydrogencarbonate and extracted with EtOAc. The extract was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford **17** (1.98 g, 81%) as solid. Mp 80–81 °C (recrystallized from EtOAc). MS (ESI): *m/z* 266 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.78 (2H, t, *J* = 7.4 Hz), 3.33 (2H, t, *J* = 7.4 Hz), 3.43 (2H, t, *J* = 8.8 Hz), 3.66 (3H, s), 3.92 (3H, s), 4.62 (2H, t, *J* = 8.8 Hz), 8.13 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 30.9, 31.0, 33.1, 51.5, 52.1, 71.7, 123.1, 132.5, 138.8, 151.8, 155.8, 166.8, 173.8. Anal. Calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>5</sub>: C, 58.86; H, 5.70; N, 5.28. Found: C, 58.81; H, 5.67; N, 5.24.

### **1,2,6,7-Tetrahydro-8*H*-cyclopenta[*b*]furo[3,2-*d*]pyridin-8-one (16)**

A solution of **17** (1.98 g, 7.46 mmol) in THF (80 mL) was added to a suspension of 65% NaH (in mineral oil, 1.38 g, 37.4 mmol) in THF (10 mL) at room temperature. After addition of MeOH (0.2 mL), the resultant mixture was stirred at reflux for 10 minutes. The reaction mixture was cooled to 0 °C, and conc. HCl (40 mL) was added dropwise to the mixture at 0 °C. After removing THF in vacuo, the residue was stirred at reflux for 20 minutes. After cooling to 0 °C, the mixture was alkalified with 8N NaOH and extracted with EtOAc. The extract was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **16** (886 mg, 68%) as solid. Mp 148–149 °C (recrystallized from EtOAc and hexane). MS (ESI):  $m/z$  176 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.75–2.82 (2H, m), 3.17–3.23 (2H, m), 3.49 (2H, t,  $J$  = 8.9 Hz), 4.71 (2H, t,  $J$  = 8.9 Hz), 8.31 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 28.26, 28.30, 36.8, 72.5, 127.2, 134.1, 136.8, 157.5, 166.1, 205.6. Anal. Calcd for C<sub>10</sub>H<sub>9</sub>NO<sub>2</sub>: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.61; H, 5.18; N, 8.03.

### **(2*E*)-1,2,6,7-Tetrahydro-8*H*-cyclopenta[*b*]furo[3,2-*d*]pyridin-8-ylideneacetonitrile (29)**

After 65% NaH (in mineral oil, 137 mg, 3.71 mmol) was added to a solution of diethyl cyanomethylphosphonate (757 mg, 4.27 mmol) in THF (20 mL) at 0 °C, the resultant mixture was stirred at room temperature for 30 minutes. The resultant mixture was added dropwise to a solution of **16** (500 mg, 2.85 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 10 minutes. After addition of saturated aqueous sodium hydrogencarbonate, the resultant mixture was extracted with EtOAc. The extract was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was recrystallized from EtOAc to afford **29** (337 mg, 60%) as solid. Mp 231–233 °C (recrystallized from EtOAc). MS (ESI):  $m/z$  199 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.13–3.17 (4H, m), 3.33 (2H, t,  $J$  = 8.8 Hz), 4.72 (2H, t,  $J$  = 8.8 Hz), 5.48–5.52 (1H, m), 8.14 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 28.7, 30.3, 31.2, 71.7, 90.6, 117.2, 128.9, 131.6, 134.2, 157.1, 161.5, 164.8. Anal. Calcd for C<sub>12</sub>H<sub>10</sub>NO<sub>2</sub>: C, 72.71; H, 5.08; N, 14.13. Found: C, 72.44; H, 5.03; N, 14.01.

### ***N*-[(2*E*)-2-(1,2,6,7-Tetrahydro-8*H*-cyclopenta[*b*]furo[3,2-*d*]pyridin-8-ylidene)ethyl]propanamide (30)**

A solution of **29** (230 mg, 1.16 mmol) and Raney cobalt (2.3 g, washed with ethanol before use) in 0.67 M NH<sub>3</sub> methanol solution (18 mL) was stirred at room temperature for 2 hr under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and triethylamine (167 μL, 1.20 mmol) and propionyl chloride (104 μL, 1.20 mmol) was added at 0 °C. After 10 min, saturated aqueous sodium hydrogencarbonate was added. The mixture was extracted with EtOAc, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by NH silica gel column chromatography and recrystallized from a mixture of chloroform and hexane to afford **30** (195 mg, 65%) as solid. Mp 163–

164 °C (recrystallized from chloroform and hexane). MS (ESI):  $m/z$  259 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.19 (3H, t,  $J$  = 7.7 Hz), 2.24 (2H, q,  $J$  = 7.7 Hz), 2.80–2.89 (2H, m), 3.01–3.09 (2H, m), 3.29 (2H, t,  $J$  = 8.8 Hz), 4.02–4.09 (2H, m), 4.65 (2H, t,  $J$  = 8.8 Hz), 5.56 (1H, brs), 5.71–5.80 (1H, m), 7.96 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  9.8, 26.7, 28.9, 29.7, 31.2, 38.5, 71.5, 120.4, 130.0, 130.4, 131.1, 143.5, 156.8, 159.2, 173.6. Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.58; H, 6.99; N, 10.85.

### Crystal data for 30

CCDC 909597 for compound **30** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via <http://www.ccdc.cam.ac.uk/structures>. Crystal size, 0.80 x 0.08 x 0.01 mm; colorless, needle; monoclinic, space group  $P2_1/n$ ,  $a$  = 4.979(2) Å,  $b$  = 17.976(5) Å,  $c$  = 14.337(4) Å,  $\alpha$  =  $\gamma$  = 90°,  $\beta$  = 92.25(2)°,  $V$  = 1282(2) Å<sup>3</sup>,  $Z$  = 4,  $D_x$  = 1.338 g/cm<sup>3</sup>,  $T$  = 100 K,  $\mu$  = 0.723 mm<sup>-1</sup>,  $\lambda$  = 1.5419 Å,  $R_1$  = 0.050,  $wR_2$  = 0.119. All measurements were made on a Rigaku R-Axis RAPID diffractometer using graphite monochromated Cu-K $\alpha$  radiation. The structure was solved by direct methods with SIR92<sup>40</sup> and was refined using full-matrix least-squares on  $F^2$  with SHELXL-97.<sup>41</sup> All non-H atoms were refined with anisotropic displacement parameters.

### *N*-[2-(1,6,7,8-Tetrahydro-2*H*-cyclopenta[*b*]furo[3,2-*d*]pyridin-8-yl)ethyl]propanamide (**15**)

A mixture of **30** (65.0 mg, 0.252 mmol) and 10% Pd/C (13 mg, containing 50% water) in MeOH (1.3 mL) was stirred at 50 °C for 6 hr under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **15** (39.5 mg, 60%) as oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.15 (3H, t,  $J$  = 7.7 Hz), 1.59–1.73 (1H, m), 1.77–1.90 (1H, m), 1.98–2.11 (1H, m), 2.19 (2H, q,  $J$  = 7.7 Hz), 2.27–2.39 (1H, m), 2.78–3.39 (7H, m), 4.48–4.66 (2H, m), 5.48 (1H, brs), 7.91 (1H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  9.9, 28.2, 29.8, 29.9, 32.2, 33.5, 37.7, 40.1, 71.3, 129.0, 132.4, 136.1, 156.6, 157.1, 173.8. HRMS (ESI) calcd for C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: 261.1598. Found: 261.1580 (M+H)<sup>+</sup>.

## Chapter 3

### *N*-[2-(7,8-Dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]acetamide (**32a**)

A mixture of **40a** (210 mg, 1.07 mmol) and Raney cobalt (2 g, washed with water and ethanol before use) in 0.67 M ammonia ethanol solution (12 mL) was stirred at room temperature for 5 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by NH silica gel column chromatography to afford 2-(6,7-Dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-ylidene)ethanamine (**41a**, 60.2 mg, 28%). To a stirred solution of 2-(6,7-Dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-ylidene)ethanamine (30.0 mg, 0.148 mmol) in tetrahydrofuran

(1 mL) was added triethylamine (31.0  $\mu$ L, 0.222 mmol) and acetic anhydride (16.8  $\mu$ L, 0.178 mmol) at 0 °C, and the mixture was stirred for 15 min. Saturated aqueous sodium hydrogencarbonate was added, and the mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. The residue was purified by silica gel column chromatography. A mixture of the obtained compound and 10% palladium on carbon (10 mg, containing 50% water) in methanol (1 mL) was stirred at room temperature for 1 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by thin layer chromatography afforded **32a** (16.0 mg, 44%) as solid. Totally 12% from **32a**. Mp 101–103 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  245 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.74–1.97 (2H, m), 1.99 (3H, s), 2.21–2.36 (1H, m), 2.39–2.54 (1H, m), 2.93–3.18 (2H, m), 3.27–3.41 (1H, m), 3.42–3.61 (2H, m), 5.56 (1H, brs), 7.23 (1H, d,  $J$  = 8.0 Hz), 7.59 (1H, d,  $J$  = 8.0 Hz), 8.03 (1H, s). Anal. Calcd for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.69; H, 6.60; N, 11.26.

#### ***N*-[2-(2-Methyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]acetamide (**32b**)**

A mixture of **40b** (1.34 g, 6.37 mmol) and Raney cobalt (13 g, washed with water and ethanol before use) in 0.67 M ammonia ethanol solution (63 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in tetrahydrofuran (64 mL), and triethylamine (1.33 mL, 9.56 mmol) and acetic anhydride (723  $\mu$ L, 7.64 mmol) was added at 0 °C. After 15 min, saturated aqueous sodium hydrogencarbonate was added, and the mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. The residue was purified by silica gel column chromatography. A mixture of the obtained compound and 10% palladium on carbon (600 mg, containing 50% water) in methanol (60 mL) was stirred at room temperature for 12 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **32b** (1.42 g, 86%) as solid. Mp 93–95 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  259 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.69–1.96 (2H, m), 1.99 (3H, s), 2.23–2.50 (2H, m), 2.63 (3H, s), 2.89–3.15 (2H, m), 3.28–3.56 (3H, m), 5.54 (1H, brs), 7.15 (1H, d,  $J$  = 8.0 Hz), 7.44 (1H, d,  $J$  = 8.0 Hz). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.77; H, 6.97; N, 10.95.

#### **Chiral Resolution of **32b****

The racemic **32b** (768 mg, 3.00 mmol) was fractionated by high performance liquid chromatography (instrument: Prep LC 2000 (manufactured by Nihon Waters K.K.), column: CHIRALPAK AD (50 mmID  $\times$  500 mmL, manufactured by Daicel Chemical Industries, Ltd.), mobile phase: hexane/ethanol/diethylamine=90/10/0.1, flow rate: 60 mL/min, column temperature: 30°C, sample concentration: 1.02 mg/mL, injection weight: 31 mg) to afford (*S*)-**32b** (retention time: 21.1 min, 381 mg, 99.9% ee) and (*R*)-**32b** (retention time: 24.6 min, 381 mg, 99.7% ee). Enantiomer excess (ee) was measured by high performance liquid chromatography (column: CHIRALPAK AD (4.6 mmID  $\times$  250

mmL, manufactured by Daicel Chemical Industries, Ltd.), mobile phase: hexane/ethanol/diethylamine=90/10/0.1, flow rate: 0.5 mL/min, column temperature: 30°C, sample concentration: 0.65 mg/mL (hexane/ethanol), injection volume: 10 µL).

**(S)-N-[2-(2-Methyl-7,8-dihydro-6H-indeno[5,4-d][1,3]oxazol-8-yl)ethyl]acetamide ((S)-32b)**

A solid.  $[\alpha]_D^{20}$ :  $-53.4^\circ$  (c 0.50, methanol). Mp 111–113 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  259 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.69–1.96 (2H, m), 1.98 (3H, s), 2.23–2.50 (2H, m), 2.63 (3H, s), 2.89–3.15 (2H, m), 3.28–3.56 (3H, m), 5.56 (1H, brs), 7.15 (1H, d,  $J$  = 8.0 Hz), 7.44 (1H, d,  $J$  = 8.0 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  14.6, 23.4, 31.9, 32.4, 34.1, 38.2, 41.0, 117.6, 120.41, 128.15, 140.56, 141.55, 147.56, 162.84, 169.97. Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.53; H, 7.01; N, 10.96.

**(R)-N-[2-(2-Methyl-7,8-dihydro-6H-indeno[5,4-d][1,3]oxazol-8-yl)ethyl]acetamide ((R)-32b)**

A solid.  $[\alpha]_D^{20}$ :  $+50.7^\circ$  (c 0.51, methanol). Mp 111–113 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  259 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.69–1.96 (2H, m), 1.99 (3H, s), 2.23–2.50 (2H, m), 2.63 (3H, s), 2.89–3.15 (2H, m), 3.28–3.56 (3H, m), 5.54 (1H, brs), 7.15 (1H, d,  $J$  = 8.0 Hz), 7.44 (1H, d,  $J$  = 8.0 Hz). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: C, 69.74; H, 7.02; N, 10.84. Found: C, 69.61; H, 7.01; N, 10.89.

**N-[2-(2-Ethyl-7,8-dihydro-6H-indeno[5,4-d][1,3]oxazol-8-yl)ethyl]acetamide (32c)**

To a stirred solution of **49** (100 mg, 0.369 mmol) in pyridine (4 mL) was added propionic anhydride (52.1 µL, 0.406 mmol) at 0 °C, and the mixture was stirred for 15 min. Water was added, and the mixture was concentrated in vacuo. Purification by silica gel column chromatography afforded *N*-{3-[2-(Acetylamino)ethyl]-4-hydroxy-2,3-dihydro-1*H*-inden-5-yl}propanamide (94.5 mg, 88%). A mixture of *N*-{3-[2-(Acetylamino)ethyl]-4-hydroxy-2,3-dihydro-1*H*-inden-5-yl}propanamide (88.5 mg, 0.305 mmol) and pyridinium *p*-toluenesulfonate (15.3 mg, 0.061 mmol) in xylene (3.1 mL) was stirred at reflux for 2.5 h. The solvent was concentrated in vacuo, and the residue was purified by silica gel column chromatography to afford the title compound **32c** (69.8 mg, 84%) as solid. Totally 74% from **49**. Mp 76–78 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  273 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.46 (3H, t,  $J$  = 7.7 Hz), 1.71–1.96 (2H, m), 1.98 (3H, s), 2.20–2.34 (1H, m), 2.36–2.51 (1H, m), 2.96 (2H, q,  $J$  = 7.7 Hz), 2.98–3.15 (2H, m), 3.28–3.41 (1H, m), 3.42–3.57 (2H, m), 5.54 (1H, brs), 7.15 (1H, d,  $J$  = 8.0 Hz), 7.46 (1H, d,  $J$  = 8.0 Hz). Anal. Calcd for C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.25; H, 7.35; N, 10.33.

**N-[2-(2-Cyclopropyl-7,8-dihydro-6H-indeno[5,4-d][1,3]oxazol-8-yl)ethyl]acetamide (32d)**

By a similar procedure to that used for **32c**, **32d** (70%) was prepared from **49** and cyclopropylcarbonyl chloride as solid. Mp 92–95 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  285 (M+H)<sup>+</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.10–1.20 (2H, m), 1.21–1.29 (2H, m), 1.69–1.93 (2H, m), 1.98 (3H, s), 2.11–2.30 (2H, m), 2.31–2.49 (1H, m), 2.84–3.16 (2H, m), 3.25–3.57 (3H, m), 5.73 (1H, brs), 7.11 (1H, d, *J* = 8.0 Hz), 7.38 (1H, d, *J* = 8.0 Hz). Anal. Calcd for C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.69; H, 7.11; N, 9.79.

***N*-[2-(2-Phenyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]acetamide (32e)**

By a similar procedure to that used for **32c**, **32e** (73%) was prepared from **49** and benzoyl chloride as solid. Mp 124–126 °C (recrystallized from ethyl acetate/diisopropyl ether). MS (ESI): *m/z* 321 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.76–1.97 (2H, m), 1.99 (3H, s), 2.31–2.57 (2H, m), 2.92–3.18 (2H, m), 3.37–3.66 (3H, m), 5.59 (1H, brs), 7.21 (1H, d, *J* = 8.0 Hz), 7.50–7.60 (4H, m), 8.20–8.27 (2H, m). Anal. Calcd for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 74.98; H, 6.29; N, 8.74. Found: C, 74.86; H, 6.26; N, 8.83.

***N*-[2-(2-Benzyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]acetamide (32f)**

By a similar procedure to that used for **32c**, **32f** (7%) was prepared from **49** and phenylacetyl chloride as solid. MS (ESI): *m/z* 335 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.73–1.91 (2H, m), 1.93 (3H, s), 2.07–2.21 (1H, m), 2.35–2.49 (1H, m), 2.87–3.14 (2H, m), 3.14–3.30 (1H, m), 3.37–3.52 (2H, m), 4.27 (2H, s), 5.45 (1H, brs), 7.15 (1H, d, *J* = 8.0 Hz), 7.26–7.40 (5H, m), 7.47 (1H, d, *J* = 8.0 Hz).

***N*-[2-(2-Methoxy-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]acetamide (32g)**

A mixture of **49** (100 mg, 0.369 mmol) and tetramethoxymethane (151 mg, 1.11 mmol) in tetrahydrofuran (3.7 mL) was stirred at reflux for 2 h. The reaction mixture was diluted with ethyl acetate and saturated aqueous sodium hydrogencarbonate. The mixture was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Purification by silica gel column chromatography afforded **32g** (58.4 mg, 58%) as a solid. Mp 126–128 °C (recrystallized from ethyl acetate). MS (ESI): *m/z* 275 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.72–1.94 (2H, m), 1.98 (3H, s), 2.13–2.29 (1H, m), 2.31–2.48 (1H, m), 2.85–3.12 (2H, m), 3.23–3.37 (1H, m), 3.37–3.55 (2H, m), 4.21 (3H, s), 5.57 (1H, brs), 7.09 (1H, d, *J* = 8.0 Hz), 7.29 (1H, d, *J* = 8.0 Hz). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.56; H, 6.48; N, 10.22.

***N*-(2-{2-[(Benzyloxy)methyl]-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl}ethyl)acetamide (32h)**

By a similar procedure to that used for **32c**, **32h** (18%) was prepared from **49** and (benzyloxy)acetyl chloride as solid. MS (ESI): *m/z* 365 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.75–1.94 (2H, m), 1.95 (3H, s), 2.20–2.34 (1H, m), 2.37–2.51 (1H, m), 2.93–3.15 (2H, m), 3.24–3.38 (1H, m), 3.41–3.58 (2H, m), 4.70 (2H, s), 4.78 (2H, s), 5.62 (1H, brs), 7.21 (1H, d, *J* = 8.0 Hz), 7.28–7.42 (5H, m), 7.54 (1H, d, *J* = 8.0 Hz).

***N*-(2-[2-(Hydroxymethyl)-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl]ethyl)acetamide (32i)**

A mixture of **32h** (50.0 mg, 0.131 mmol) and 10% palladium on carbon (100 mg, containing 50% water) in methanol (1 mL) was stirred at 50 °C for 24 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography and recrystallization (ethyl acetate/hexane) afforded **32i** (19.0 mg, 53%) as solid. Mp 132–134 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  275 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.80–1.97 (2H, m), 1.99 (3H, s), 2.08–2.25 (1H, m), 2.36–2.51 (1H, m), 2.91–3.16 (2H, m), 3.33–3.61 (4H, m), 4.90 (2H, d,  $J$  = 5.5 Hz), 5.57 (1H, brs), 7.19 (1H, d,  $J$  = 8.0 Hz), 7.50 (1H, d,  $J$  = 8.0 Hz). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.54; H, 6.63; N, 10.11.

#### ***N*-[2-(2-Methyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]oxazol-8-yl)ethyl]propionamide (32j)**

A mixture of **41b** (21.0 mg, 0.0999 mmol) and Raney cobalt (200 mg, washed with water and ethanol before use) in 0.6 M ammonia ethanol solution (1 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in tetrahydrofuran (1 mL), and triethylamine (18.1 μL, 0.13 mmol) and propionic anhydride (15.4 μL, 0.12 mmol) was added at 0 °C. After 15 min, saturated aqueous sodium hydrogencarbonate was added, and the mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. The residue was purified by silica gel column chromatography. A mixture of the obtained compound and 10% palladium on carbon (4.0 mg, containing 50% water) in methanol (1 mL) was stirred at room temperature for 20 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **32j** (17.5 mg, 64%) as solid. Mp 111–113 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  273 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.16 (3H, t,  $J$  = 7.6 Hz), 1.70–1.98 (2H, m), 2.15–2.51 (4H, m), 2.63 (3H, s), 2.88–3.15 (2H, m), 3.28–3.56 (3H, m), 5.54 (1H, brs), 7.14 (1H, d,  $J$  = 7.7 Hz), 7.44 (1H, d,  $J$  = 7.7 Hz). Anal. Calcd for C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.56; H, 7.39; N, 10.28. Found: C, 70.14; H, 7.28; N, 10.23.

#### ***N*-[2-(2-Methyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]thiazol-8-yl)ethyl]acetamide (33a)**

To a stirred solution of **55** (1.66 g, 7.14 mmol) in tetrahydrofuran (70 mL) was added triethylamine (2.00 mL, 14.3 mmol) and acetic anhydride (810 μL, 8.57 mmol) at 0 °C. After 1 h, the reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography to afford **33a** (1.83 g, 93%) as solid. Mp 115–116 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  275 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.60–1.80 (1H, m), 1.84–2.06 (4H, m), 2.14–2.30 (1H, m), 2.34–2.51 (1H, m), 2.82 (3H, s), 2.88–3.18 (2H, m), 3.24–3.51 (3H, m), 5.62 (1H, brs), 7.30 (1H, d,  $J$  = 8.2 Hz), 7.76 (1H, d,  $J$  = 8.2 Hz). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>OS: C, 65.66; H, 6.61; N, 10.21. Found: C, 65.59; H, 6.62; N, 10.17.

#### ***N*-[2-(2-Methyl-7,8-dihydro-6*H*-indeno[5,4-*d*][1,3]thiazol-8-yl)ethyl]propionamide (33b)**

To a stirred solution of **55** (100 mg, 0.430 mmol) in tetrahydrofuran (4 mL) was added triethylamine (120  $\mu$ L, 0.861 mmol) and propionic anhydride (66.2  $\mu$ L, 0.516 mmol) at 0 °C. After 1 h, the reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography to afford **33b** (110 mg, 89%) as solid. Mp 122–123 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  289 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.13 (3H, d,  $J$  = 7.6 Hz), 1.67–1.81 (1H, m), 1.88–2.06 (1H, m), 2.09–2.29 (3H, m), 2.33–2.52 (1H, m), 2.82 (3H, s), 2.90–3.19 (2H, m), 3.26–3.51 (3H, m), 5.42 (1H, brs), 7.30 (1H, d,  $J$  = 8.2 Hz), 7.76 (1H, d,  $J$  = 8.2 Hz). Anal. Calcd for C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>OS: C, 66.63; H, 6.99; N, 9.71. Found: C, 66.62; H, 7.02; N, 9.66.

***N*-[2-(2-Methyl-8,9-dihydro-7*H*-cyclopenta[*c*]pyrazolo[1,5-*a*]pyridin-9-yl)ethyl]acetamide (**34a**)**

A mixture of **62** (140 mg, 0.487 mmol) and 12M hydrochloric acid (5 mL) was stirred at 100 °C for 2 days, and the reaction mixture was concentrated in vacuo. The residue was dissolved in tetrahydrofuran (5 mL), and triethylamine (1.36 mL, 9.76 mmol) and acetic anhydride (115  $\mu$ L, 1.22 mmol) was added at 0 °C. After 15 min, the reaction solution was diluted with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography and recrystallization (ethyl acetate/diisopropyl ether) afforded **34a** (56.6 mg, 45%) as solid. Mp 106–107 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  258 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.63–1.78 (1H, m), 1.88–2.03 (4H, m), 2.11–2.26 (1H, m), 2.33–2.45 (1H, m), 2.47 (3H, s), 2.78–3.07 (2H, m), 3.24–3.50 (3H, m), 5.43 (1H, brs), 6.15 (1H, s), 6.57 (1H, d,  $J$  = 7.2 Hz), 8.21 (1H, d,  $J$  = 7.2 Hz). Anal. Calcd for C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O: C, 70.01; H, 7.44; N, 16.33. Found: C, 69.67; H, 7.43; N, 16.38.

***N*-[2-(1,2-Dimethyl-8,9-dihydro-7*H*-cyclopenta[*c*]pyrazolo[1,5-*a*]pyridin-9-yl)ethyl]acetamide (**34b**)**

To a stirred solution of **62** (75.0 mg, 0.261 mmol) in tetrahydrofuran (2.6 mL) was added 1.5 M diisobutylaluminum hydride in toluene (2.1 mL, 3.2 mmol) at room temperature, and the mixture was stirred for 3 h. Sodium sulfate decahydrate (2.1 g) was added and the mixture was filtered and the filtrate was concentrated in vacuo. To a stirred solution of the residue and triethylamine (54.6  $\mu$ L, 0.392 mmol) in tetrahydrofuran (2.5 mL) was added acetic anhydride (37.0  $\mu$ L, 0.391 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. After adding water (70  $\mu$ L), the mixture was concentrated in vacuo. Purification by silica gel column chromatography and recrystallization (ethyl acetate/diisopropyl ether) afforded **34b** (24.4 mg, yield 34%) as solid. Mp 134–135 °C (recrystallized from ethyl acetate/diisopropyl ether). MS (ESI):  $m/z$  272 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.49–1.68 (1H, m), 1.82–1.97 (4H, m), 2.01–2.14 (1H, m), 2.21–2.34 (4H, m), 2.37 (3H, s), 2.73–2.85 (1H, m), 2.89–3.04 (1H, m), 3.26–3.50 (2H, m), 3.51–3.62 (1H, m), 5.41 (1H, brs), 6.48 (1H, d,  $J$  = 6.9 Hz), 8.12 (1H, d,  $J$  = 6.9 Hz). Anal. Calcd for C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O: C, 70.82; H, 7.80; N, 15.49. Found: C, 70.52; H, 7.92; N, 15.30.

**Ethyl 9-[2-(acetylamino)ethyl]-2-methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridine-1-carboxylate (34c)**

To a stirred solution of **62** (50.0 mg, 0.174 mmol) and triethylamine (36.4  $\mu$ L, 0.261 mmol) in tetrahydrofuran (2 mL) was added acetic anhydride (24.7  $\mu$ L) at 0 °C, and the mixture was stirred at room temperature for 30 min. After adding water (50  $\mu$ L), the mixture was concentrated in vacuo. Purification by silica gel column chromatography and recrystallization (diisopropyl ether) afforded **34c** (32.1 mg, yield 56%) as solid. Mp 101–103 °C (recrystallized from diisopropyl ether). MS (ESI):  $m/z$  330 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.38–1.55 (4H, m), 1.77–1.92 (1H, m), 1.96–2.10 (4H, m), 2.14–2.36 (1H, m), 2.63 (3H, s), 2.82–2.93 (1H, m), 2.97–3.13 (1H, m), 3.25–3.36 (1H, m), 3.36–3.50 (1H, m), 4.16–4.26 (1H, m), 4.29–4.41 (2H, m), 6.81 (1H, d,  $J$  = 6.9 Hz), 7.21 (1H, brs), 8.25 (1H, d,  $J$  = 6.9 Hz). Anal. Calcd for C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.63; H, 7.09; N, 12.41.

**N-[2-(2-Methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridin-9-yl)ethyl]propionamide (34d)**

A mixture of **62** (333 mg, 1.16 mmol) and 40% sulfuric acid (6 mL) was stirred at 100 °C for 2 h. The reaction mixture was cooled to 0 °C, diluted with water, and neutralized with saturated aqueous sodium hydrogen carbonate. The mixture was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo to afford 2-(2-methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridin-9-yl)ethanamine (239 mg, 96%). To a stirred solution of 2-(2-methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridin-9-yl)ethanamine (69.7 mg, 0.324 mmol) and triethylamine (90.3  $\mu$ L, 0.648 mmol) in tetrahydrofuran (3 mL) was added propionic anhydride (49.9  $\mu$ L, 0.389 mmol) at 0 °C. After 15 min, the reaction solution was diluted with saturated aqueous sodium hydrogen carbonate and concentrated in vacuo. Purification by silica gel column chromatography afforded **34d** (66.8 mg, 76%) as solid. Totally 73% from **62**. Mp 100–102 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  272 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.10 (3H, t,  $J$  = 7.6 Hz), 1.62–1.77 (1H, m), 1.88–2.02 (1H, m), 2.07–2.25 (3H, m), 2.32–2.44 (1H, m), 2.46 (3H, s), 2.78–3.05 (2H, m), 3.22–3.48 (3H, m), 5.38 (1H, brs), 6.14 (1H, s), 6.57 (1H, d,  $J$  = 7.0 Hz), 8.20 (1H, d,  $J$  = 7.0 Hz). Anal. Calcd for C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O: C, 70.82; H, 7.80; N, 15.49. Found: C, 70.99; H, 7.90; N, 15.61.

**N-[2-(2-Methyl-2,6,7,8-tetrahydrocyclopenta[e]indazol-8-yl)ethyl]acetamide (35a)**

A mixture of **72a** (311 mg, 1.22 mmol) and 10% palladium on carbon (31 mg, containing 50% water) in methanol (12 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **35a** (229 mg, 73%) as solid. Mp 123–125 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  258 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.64–1.85 (2H, m), 1.90 (3H, s), 2.16–

2.29 (1H, m), 2.32–2.46 (1H, m), 2.82–3.10 (2H, m), 3.24–3.52 (3H, m), 4.20 (3H, s), 5.48 (1H, brs), 7.17 (1H, d,  $J = 8.7$  Hz), 7.52 (1H, d,  $J = 8.7$  Hz), 7.88 (1H, s). Anal. Calcd for  $C_{15}H_{19}N_3O$ : C, 70.01; H, 7.44; N, 16.33. Found: C, 69.73; H, 7.43; N, 16.27.

#### ***N*-[2-(2-Methyl-2,6,7,8-tetrahydrocyclopenta[*e*]indazol-8-yl)ethyl]propionamide (35b)**

A mixture of **72b** (83.0 mg, 1.22 mmol) and 10% palladium on carbon (8 mg, containing 50% water) in methanol (2 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by thin layer chromatography afforded **35b** (45.1 mg, 74%) as solid. Mp 105–107 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  272 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  1.11 (3H, t,  $J = 7.4$  Hz), 1.67–1.82 (1H, m), 1.82–1.97 (1H, m), 2.11 (2H, q,  $J = 7.4$  Hz), 2.17–2.31 (1H, m), 2.33–2.48 (1H, m), 2.84–3.11 (2H, m), 3.28–3.53 (3H, m), 4.21 (3H, s), 5.38 (1H, brs), 7.17 (1H, d,  $J = 8.8$  Hz), 7.52 (1H, d,  $J = 8.8$  Hz), 7.88 (1H, s). Anal. Calcd for  $C_{16}H_{21}N_3O$ : C, 70.82; H, 7.80; N, 15.49. Found: C, 70.54; H, 7.78; N, 15.52.

#### **4-Bromo-7-hydroxy-6-nitroindan-1-one (37)**

To a stirred suspension of **36** (3.06 g, 13.5 mmol) in acetic acid (20 mL) was added a solution of acetic anhydride (1.66 mL, 17.6 mmol) and fuming nitric acid (838  $\mu$ L, 20.2 mmol) in acetic acid (10 mL) at room temperature. After 3 h, the reaction mixture was evaporated in vacuo and the resulting solid was collected by filtration to afford **37** (2.98 g, 79%) as solid. Mp 149–151 °C (recrystallized from methanol). <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  2.83–2.92 (2H, m), 3.08–3.16 (2H, m), 8.50 (1H, s), 10.99 (1H, s). Anal. Calcd for  $C_9H_6BrNO_4$ : C, 39.73; H, 2.22; N, 5.14. Found: C, 39.88; H, 2.40; N, 5.37.

#### **6-Amino-7-hydroxyindan-1-one hydrobromide (38)**

A mixture of **37** (2.90 g, 10.7 mmol) and 10% palladium on carbon (290 mg, containing 50% water) in methanol (53 mL) was stirred at room temperature for 6 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **38** (2.08 g, yield 80%) as solid. <sup>1</sup>H NMR ( $METHANOL-d_4$ )  $\delta$  2.69–2.82 (2H, m), 3.12–3.21 (2H, m), 7.13 (1H, d,  $J = 8.0$  Hz), 7.57 (1H, d,  $J = 8.0$  Hz).

#### **6,7-Dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-one (39a)**

A mixture of **38** (50.0 mg, 0.205 mmol) and triethyl orthoformate (128  $\mu$ L, 0.769 mmol) in tetrahydrofuran (2.5 mL) was stirred at reflux for 0.5 h. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **39a** (21.9 mg, 62%) as solid. Mp 188–190 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  174 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  2.80–2.87

(2H, m), 3.29–3.36 (2H, m), 7.48 (1H, d,  $J = 8.2$  Hz), 8.02 (1H, d,  $J = 8.2$  Hz), 8.19 (1H, s). Anal. Calcd for  $C_{10}H_7NO_2$ : C, 69.36; H, 4.07; N, 8.09. Found: C, 69.04; H, 4.02; N, 8.14.

### 2-Methyl-6,7-dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-one (**39b**)

To a stirred suspension of **38** (800 mg, 3.28 mmol) in tetrahydrofuran (20 mL) was added triethylamine (571  $\mu$ L, 4.10 mmol) and acetic anhydride (387  $\mu$ L, 4.10 mmol) at room temperature. After 1.5 h, saturated aqueous sodium hydrogencarbonate was added. The mixture was extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, filtrated and concentrated in vacuo. Purification by silica gel column chromatography afforded *N*-(4-hydroxy-3-oxo-2,3-dihydro-1*H*-inden-5-yl)acetamide (481 mg, 71%). A mixture of *N*-(4-hydroxy-3-oxo-2,3-dihydro-1*H*-inden-5-yl)acetamide (469 mg, 2.29 mmol) and pyridinium *p*-toluenesulfonate (115 mg, 0.457 mmol) in xylene (23 mL) was stirred at reflux for 2.5 h. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography to afford **39b** (363 mg, 85%) as solid. Mp 106–107 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  188 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  2.71 (3H, s), 2.78–2.85 (2H, m), 3.24–3.33 (2H, m), 7.38 (1H, d,  $J = 8.0$  Hz), 7.86 (1H, d,  $J = 8.0$  Hz).

### (*E*)-(6,7-Dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-ylidene)acetonitrile (**40a**)

To a stirred suspension of 60% sodium hydride (in mineral oil, 73.4 mg, 1.84 mmol) in tetrahydrofuran (8 mL) was added diethyl cyanomethylphosphonate (322  $\mu$ L, 1.99 mmol) at 0 °C, and the mixture was stirred for 15 min. A solution of **39a** (265 mg, 1.53 mmol) in tetrahydrofuran (8 mL) was added, and the mixture was stirred for 30 min. Saturated aqueous ammonium chloride was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **40a** (220 mg, 73%) as solid. The *Z* form was removed by column chromatography. Mp 166–168 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  197 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  3.16–3.37 (4H, m), 6.07 (1H, t,  $J = 2.5$  Hz), 7.36 (1H, d,  $J = 8.2$  Hz), 7.81 (1H, d,  $J = 8.2$  Hz), 8.15 (1H, s). Anal. Calcd for  $C_{12}H_8N_2O$ : C, 73.46; H, 4.11; N, 14.28. Found: C, 73.44; H, 4.05; N, 14.49.

### (*E*)-(2-Methyl-6,7-dihydro-8*H*-indeno[5,4-*d*][1,3]oxazol-8-ylidene)acetonitrile (**40b**)

To a stirred suspension of 60% sodium hydride (in mineral oil, 90.0 mg, 2.24 mmol) in tetrahydrofuran (9 mL) was added diethyl cyanomethylphosphonate (393  $\mu$ L, 2.43 mmol) at 0 °C, and the mixture was stirred for 15 min. A solution of **39b** (350 mg, 1.87 mmol) in tetrahydrofuran (9 mL) was added, and the mixture was stirred for 1 h. Saturated aqueous ammonium chloride was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **40b** (300 mg, 76%) as solid. The *Z* form was removed by column chromatography. Mp 180–

182 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  211 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.70 (3H, s), 3.15–3.31 (4H, m), 6.04 (1H, t,  $J$  = 2.6 Hz), 7.28 (1H, d,  $J$  = 8.0 Hz), 7.67 (1H, d,  $J$  = 8.0 Hz). Anal. Calcd for C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.22; H, 4.75; N, 13.16.

#### (*S*)-2-(2-Methyl-7,8-dihydro-6*H*-indeno[5,4-*d*]oxazol-8-yl)ethan-1-amine hydrochloride (**43**)

Asymmetric hydrogenation of allylamine **41b** using a complex of cyclooctadiene rhodium(I) chloride dimer and (*R*)-1-[(*S*)-2-(diphenylphosphino)ferrocenyl]ethylidicyclohexylphosphine ethanol adduct (Josiphos SL-J001-1) gave **42** with good enantio excess (91% ee, determined by chiral HPLC, SUMICHIRAL OA4700, eluted with n-hexane/ethanol/methanol/trifluoroacetic acid=950/25/25/2 (v/v/v), flow rate: 1.0 mL/min, column temperature: 30°C, 45.1 min: **42**, 48.8 min: enantiomer of **42**). Recrystallization from methanol/isopropyl ether following salt formation gave **43** with excellent enantio excess (>99% ee) as a single crystal.

#### Crystal data for **43**

CCDC 2034940 for compound **43** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via <http://www.ccdc.cam.ac.uk/structures>. Crystal size, 0.80 x 0.40 x 0.30 mm; colorless, prism; orthorhombic, space group  $P2_12_12_1$ ,  $a$  = 5.7189(10) Å,  $b$  = 8.5189(12) Å,  $c$  = 26.785(4) Å,  $\alpha$  =  $\beta$  =  $\gamma$  = 90°,  $V$  = 1304.9(3) Å<sup>3</sup>,  $Z$  = 4,  $D_x$  = 1.286 g/cm<sup>3</sup>,  $T$  = 100 K,  $\mu$  = 2.474 mm<sup>-1</sup>,  $\lambda$  = 1.54184 Å,  $R_1$  = 0.0257,  $wR_2$  = 0.0737,  $S$  = 1.059, Flack Parameter<sup>42</sup> = 0.012(5). All measurements were made on a Rigaku R-Axis RAPID diffractometer using graphite monochromated Cu-K $\alpha$  radiation. The structure was solved by direct methods with SIR92<sup>40</sup> and was refined using full-matrix least-squares on  $F^2$  with SHELXL-2018/3.<sup>41</sup> All non-H atoms were refined with anisotropic displacement parameters.

#### 4-Bromo-7-methoxy-6-nitroindan-1-one (**44**)

To a stirred solution of **37** (8.07 g, 29.7 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (5.33 mL, 35.6 mmol) in *N,N*-dimethylformamide (150 mL) was added iodomethane (18.5 mL, 297 mmol) at room temperature. After 40 h, saturated aqueous sodium hydrogencarbonate was added. The mixture was extracted with diethyl ether, washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **44** (6.70 g, 79%) as solid. Mp 138–139 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  286 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.78–2.86 (2H, m), 3.07–3.15 (2H, m), 4.13 (3H, s), 8.16 (1H, s). Anal. Calcd for C<sub>10</sub>H<sub>8</sub>NO<sub>4</sub>Br: C, 41.98; H, 2.82; N, 4.90. Found: C, 41.98; H, 2.76; N, 4.82.

#### (*E*)-(4-Bromo-7-methoxy-6-nitro-2,3-dihydro-1*H*-inden-1-ylidene)acetonitrile (**45**)

To a stirred suspension of 60% sodium hydride (in mineral oil, 1.03 g, 25.6 mmol) in tetrahydrofuran (100 mL) was added diethyl cyanomethylphosphonate (4.52 mL, 28.0 mmol) at 0 °C, and the mixture

was stirred for 15 min. A solution of **44** (6.67 g, 23.3 mmol) in tetrahydrofuran (50 mL) was added, and the mixture was stirred for 30 min. Saturated aqueous ammonium chloride was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **45** (5.54 g, 77%) as solid. Mp 156–158 °C (recrystallized from ethyl acetate/hexane). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.09–3.25 (4H, m), 3.94 (3H, s), 6.27 (1H, t, *J* = 2.6 Hz), 8.03 (1H, s). Anal. Calcd for C<sub>12</sub>H<sub>9</sub>N<sub>2</sub>O<sub>3</sub>Br: C, 46.63; H, 2.93; N, 9.06. Found: C, 46.66; H, 2.86; N, 9.09.

#### **(*E*)-(6-Amino-7-methoxy-2,3-dihydro-1*H*-inden-1-ylidene)acetonitrile (**46**)**

A mixture of **45** (47.0 mg, 0.152 mmol), triethylamine (22.3 μL, 0.160 mmol), and 10% palladium on carbon (10 mg, containing 50% water) in ethyl acetate (1.5 mL) was stirred at room temperature for 1.5 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **46** (30.4 mg, 100%) as solid. Mp 140–142 °C (recrystallized from ethyl acetate/hexane). MS (ESI): *m/z* 201 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.94–3.01 (2H, m), 3.04–3.11 (2H, m), 3.76 (3H, s), 3.78 (2H, brs), 6.11 (1H, t, *J* = 2.6 Hz), 6.81 (1H, d, *J* = 8.0 Hz), 6.91 (1H, d, *J* = 8.0 Hz). Anal. Calcd for C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.60; H, 6.14; N, 13.94.

#### **(*E*)-*N*-[2-(6-Amino-7-methoxy-2,3-dihydro-1*H*-inden-1-ylidene)ethyl]acetamide (**47**)**

A mixture of **46** (15.2 mg, 0.076 mmol) and Raney cobalt (150 mg, washed with water and ethanol before use) in 1 M ammonia ethanol solution (1 mL) was stirred at room temperature for 2 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue and triethylamine (21.2 μL, 0.152 mmol) was dissolved in tetrahydrofuran (0.9 mL), and a solution of acetic anhydride (7.18 μL, 0.076 mmol) in tetrahydrofuran (0.1 mL) was added at 0 °C. After 15 min, saturated aqueous sodium hydrogencarbonate was added, and the mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. Purification by silica gel column chromatography afforded **47** (16.2 mg, 87%) as solid. Mp 105–107 °C (recrystallized from ethyl acetate/hexane). MS (ESI): *m/z* 247 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.01 (3H, s), 2.70–2.80 (2H, m), 2.85–2.96 (2H, m), 3.75 (3H, s), 4.01–4.09 (2H, m), 5.52 (1H, brs), 6.25–6.33 (1H, m), 6.65 (1H, d, *J* = 8.0 Hz), 6.82 (1H, d, *J* = 8.0 Hz). Anal. Calcd for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>: C, 68.27; H, 7.37; N, 11.37. Found: C, 67.93; H, 7.25; N, 11.10.

#### ***N*-[2-(6-Amino-7-methoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]acetamide (**48**)**

A mixture of **47** (2.62 g, 10.7 mmol) and 10% palladium on carbon (500 mg, containing 50% water) in methanol (50 mL) was stirred at room temperature for 5 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **48** (2.56 g, 96%) as solid. Mp 130–132 °C (recrystallized from ethyl acetate/hexane). MS (ESI): *m/z* 249 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.68–1.93 (3H, m), 1.95 (3H, s), 2.16–2.31 (1H, m), 2.65–2.79 (1H, m), 2.81–2.96 (1H, m),

3.09–3.24 (1H, m), 3.28–3.50 (2H, m), 3.79 (3H, s), 3.91 (2H, brs), 5.71 (1H, brs), 6.60 (1H, d,  $J = 8.0$  Hz), 6.78 (1H, d,  $J = 8.0$  Hz). Anal. Calcd for  $C_{14}H_{20}N_2O_2$ : C, 67.71; H, 8.12; N, 11.28. Found: C, 67.56; H, 8.01; N, 11.27.

#### ***N*-[2-(6-Amino-7-hydroxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]acetamide hydrochloride (**49**)**

To a stirred solution of **48** (2.56 g, 10.3 mmol) in dichloromethane (80 mL) was added a solution of boron tribromide in dichloromethane (1M, 22.7 mL, 22.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 1.5 h. Water was added and the mixture was diluted with ethyl acetate. The extract was washed with saturated aqueous sodium hydrogencarbonate and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was dissolved in dichloromethane (80 mL), and a solution of boron tribromide in dichloromethane (1M, 22.7 mL, 22.7 mmol) was added at 0 °C. The mixture was stirred at room temperature for 1.5 h, and then water was added. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium hydrogencarbonate and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was dissolved in ethyl acetate, and 4M HCl ethyl acetate solution was added at room temperature. The solvent was removed in vacuo to afford **49** (2.51 g, 90%). MS (ESI):  $m/z$  235 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.31–1.46 (1H, m), 1.68–1.86 (3H, m), 1.80 (3H, s), 1.99–2.14 (1H, m), 2.64–2.77 (1H, m), 2.80–2.95 (1H, m), 3.04–3.13 (1H, m), 3.37–3.50 (1H, m), 6.74 (1H, d,  $J = 8.0$  Hz), 7.13 (1H, d,  $J = 8.0$  Hz), 8.09 (1H, brs), 9.87 (3H, brs), 10.14 (1H, brs).

#### **6-Amino-7-iodoindan-1-one (**51**)**

To a stirred solution of **50** (5.00 g, 34.0 mmol) in methanol (200 mL) and water (50 mL) was added calcium carbonate (6.81 g, 68.0 mmol) and iodine(I) chloride (2.22 mL, 44.2 mmol), and the mixture was stirred at room temperature for 2 h. Saturated aqueous sodium thiosulfate was added and concentrated in vacuo. To the mixture was added saturated aqueous sodium hydrogencarbonate. The mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. The residue was washed with methanol and ethyl acetate to afford **51** (7.95 g, 86%) as solid. Mp 183–186 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  274 ( $M+H$ )<sup>+</sup>. <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  2.56–2.66 (2H, m), 2.78–2.87 (2H, m), 5.48 (2H, s), 7.08 (1H, d,  $J = 8.2$  Hz), 7.25 (1H, d,  $J = 8.2$  Hz). Anal. Calcd for  $C_9H_8NOI$ : C, 39.59; H, 2.95; N, 5.13. Found: C, 39.65; H, 2.87; N, 5.07.

#### **2-Methyl-6,7-dihydro-8*H*-indeno[5,4-*d*][1,3]thiazol-8-one (**52**)**

A mixture of **51** (1.00 g, 3.66 mmol), thioacetamide (413 mg, 5.49 mmol), 1,1'-bis(diphenylphosphino)ferrocene (383 mg, 1.46 mmol), calcium oxide (411 mg, 7.32 mmol), and tris(dibenzylideneacetone)dipalladium(0) (335 mg, 0.37 mmol) in *N,N*-dimethylformamide (12 mL) was stirred at 60°C for 1 h. After cooling to room temperature, water was added. The mixture was extracted with ethyl acetate, washed with brine, and concentrated in vacuo. Purification by silica gel

column chromatography and NH silica gel column chromatography afforded **52** (340 mg, 46%) as solid. Mp 163–165 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  204 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.80–2.86 (2H, m), 2.90 (3H, s), 3.27–3.33 (2H, m), 7.54 (1H, d,  $J$  = 8.2 Hz), 8.15 (1H, d,  $J$  = 8.2 Hz). Anal. Calcd for C<sub>11</sub>H<sub>9</sub>NOS: C, 65.00; H, 4.46; N, 6.89. Found: C, 65.00; H, 4.29; N, 6.94.

**(E)-2-(2-Methyl-6,7-dihydro-8H-indeno[5,4-*d*][1,3]thiazol-8-ylidene)acetonitrile (53)**

To a stirred solution of diethyl cyanomethylphosphonate (348 μL, 2.22 mmol) in tetrahydrofuran (6 mL) was added 65% sodium hydride (in mineral oil, 66.0 mg, 1.79 mmol) at 0 °C. After 30 min, a solution of **52** (300 mg, 1.48 mmol) in tetrahydrofuran (6 mL) was added. After 2 h, 65% sodium hydride (in mineral oil, 16.0 mg, 0.43 mmol) and diethyl cyanomethylphosphonate (116 μL, 0.74 mmol) was added. After 30 min, saturated aqueous ammonium chloride was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **53** (181 mg, 54%) as solid. Mp 194–195 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  227 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.91 (3H, s), 3.27 (4H, s), 5.60–5.63 (1H, m), 7.46 (1H, d,  $J$  = 8.2 Hz), 8.01 (1H, d,  $J$  = 8.2 Hz). Anal. Calcd for C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>S: C, 69.00; H, 4.45; N, 12.38. Found: C, 68.76; H, 4.19; N, 12.40.

**(E)-2-(2-Methyl-6,7-dihydro-8H-indeno[5,4-*d*][1,3]thiazol-8-ylidene)ethanamine (54)**

A mixture of **53** (2.50 g, 11.0 mmol) and Raney cobalt (25 g, washed with water and ethanol before use) in 2 M ammonia ethanol solution (110 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **54** (2.47 g, 97%). Mp 145–147 °C (recrystallized from methanol/ethyl acetate). MS (ESI):  $m/z$  231 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 2.79–2.94 (5H, m), 3.10–3.22 (2H, m), 3.57 (2H, d,  $J$  = 6.8 Hz), 5.89–6.04 (1H, m), 7.36 (1H, d,  $J$  = 8.0 Hz), 7.83 (1H, d,  $J$  = 8.0 Hz).

**2-(2-Methyl-7,8-dihydro-6H-indeno[5,4-*d*][1,3]thiazol-8-yl)ethanamine (55)**

A mixture of **54** (2.09 g, 9.07 mmol) and 10% palladium on carbon (100 mg, containing 50% water) in methanol (90 mL) was stirred at room temperature for 16 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **55** (2.04 g, 97%). MS (ESI):  $m/z$  233 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 1.58–1.75 (1H, m), 1.84–2.00 (1H, m), 2.07–2.23 (1H, m), 2.30–2.47 (1H, m), 2.73–2.89 (2H, m), 2.83 (3H, s), 2.89–3.16 (2H, m), 3.33–3.47 (1H, m), 7.30 (1H, d,  $J$  = 8.2 Hz), 7.75 (1H, d,  $J$  = 8.3 Hz).

**3-Ethyl 4-methyl 5-(3-ethoxy-3-oxopropyl)-2-methylpyrazolo[1,5-*a*]pyridine-3,4-dicarboxylate (57)**

A mixture of **56** (1.00 g, 4.21 mmol) and 1-(aminooxy)-2,4-dinitrobenzene (838 mg, 4.21 mmol) in acetonitrile (10 mL) was stirred at 40 °C for 14 h, and then 1-(Aminooxy)-2,4-dinitrobenzene (838 mg,

4.21 mmol) was added. The mixture was stirred at 50°C for 12 h. The solvent was concentrated in vacuo, and the residue was dissolved in *N,N*-dimethylformamide (10 mL). To the mixture, ethyl 2-butynoate (491 mL, 4.21 mmol) and potassium carbonate (2.23 g, 16.1 mmol) were added, and the mixture was stirred at room temperature for 12 h. The reaction solution was diluted with water and extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography and preparative HPLC afforded **57** (70.0 mg, 5%) as oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.24 (3H, t, *J* = 7.1 Hz), 1.37 (3H, t, *J* = 7.1 Hz), 2.62 (3H, s), 2.68 (2H, t, *J* = 7.7 Hz), 3.01 (2H, t, *J* = 7.7 Hz), 3.97 (3H, s), 4.14 (2H, q, *J* = 7.1 Hz), 4.33 (2H, q, *J* = 7.1 Hz), 6.82 (1H, d, *J* = 7.1 Hz), 8.36 (1H, d, *J* = 7.1 Hz).

### 5,6-Dihydro-7*H*-cyclopenta[*c*]pyridin-7-one (**58**)

To a stirred suspension of 60% sodium hydride (in mineral oil, 7.80 g, 200 mmol) in tetrahydrofuran (84 mL) were added a solution of **56** (10.0 g, 42.1 mmol) in tetrahydrofuran (84 mL) and methanol (84 μL) at room temperature, and the mixture was refluxed for 3 h. The reaction mixture was concentrated in vacuo after cooling, and the residue was added portion wise to 12 M hydrochloric acid (42 mL) at 0°C. The mixture was refluxed for 30 min. After cooling, the reaction solution was alkalified with saturated aqueous sodium hydrogen carbonate. The resultant mixture was filtered and extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was washed with hexane to afford **58** (4.39 g, yield 78%) as solid. MS (ESI): *m/z* 134 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.66–2.76 (2H, m), 3.14–3.23 (2H, m), 7.42–7.50 (1H, m), 8.71 (1H, d, *J* = 5.2 Hz), 9.00 (1H, d, *J* = 0.8 Hz).

### 6,7-Dihydro-5*H*-cyclopenta[*c*]pyridin-7-ylacetonitrile (**60**)

To a stirred solution of diethyl cyanomethylphosphonate (306 μL, 1.95 mmol) in tetrahydrofuran (10 mL) was added 60% sodium hydride (in mineral oil, 54.0 mg, 1.35 mmol) at 0°C and the mixture was stirred for 30 min. The mixture was added to a solution of **58** (130 mg, 0.976 mmol) in tetrahydrofuran (5 mL) at 0°C and the mixture was stirred for 15 min. The reaction solution was diluted with saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by silica gel column chromatography. A mixture of the purified compound and 10% palladium on carbon (30 mg, containing 50% water) in methanol (10 mL) was stirred at room temperature for 4 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **60** (95.0 mg, 62%) as solid. MS (ESI): *m/z* 159 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.87–2.03 (1H, m), 2.42–3.14 (5H, m), 3.53–3.66 (1H, m), 7.22 (1H, dd, *J* = 4.9, 0.8 Hz), 8.46 (1H, d, *J* = 4.9 Hz), 8.55 (1H, s).

**Ethyl 9-(cyanomethyl)-2-methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridine-1-carboxylate (61a)**

**and Ethyl 7-(cyanomethyl)-2-methyl-6,7-dihydro-5H-cyclopenta[d]pyrazolo[1,5-a]pyridine-3-carboxylate (61b)**

To a solution of **60** (90.0 mg, 0.569 mmol) in acetonitrile (1.0 mL) was added 1-(aminooxy)-2,4-dinitrobenzene (113 mg, 0.567 mmol), and the mixture was stirred at 40 °C for 12 h. The solvent was concentrated in vacuo, and the residue was dissolved in *N,N*-dimethylformamide (1.0 mL). To the mixture, ethyl 2-butynoate (80.0  $\mu$ L, 0.686 mmol) and potassium carbonate (94.4 mg, 0.683 mmol) were added, and the mixture was stirred at room temperature for 4 h. The reaction solution was diluted with water and extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded less polar **61a** (51.2 mg, 32%) as solid and polar **61b** (20.2 mg, 13%) as solid. **61a**: Mp 136–140 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  284 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.42 (3H, t,  $J$  = 7.2 Hz), 2.18–2.31 (1H, m), 2.42–2.66 (5H, m), 2.76–2.86 (1H, m), 2.90–3.02 (1H, m), 3.20–3.36 (1H, m), 4.36 (2H, q,  $J$  = 7.2 Hz), 4.44–4.56 (1H, m), 6.84 (1H, d,  $J$  = 6.8 Hz), 8.31 (1H, d,  $J$  = 6.8 Hz). Anal. Calcd for C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.68; H, 6.01; N, 14.86. **61b**: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.42 (3H, t,  $J$  = 7.1 Hz), 1.93–2.09 (1H, m), 2.47–2.79 (6H, m), 2.93–3.20 (2H, m), 3.56–3.66 (1H, m), 4.38 (2H, q,  $J$  = 7.1 Hz), 7.91–7.94 (1H, m), 8.36 (1H, s).

**Ethyl 9-(2-aminoethyl)-2-methyl-8,9-dihydro-7H-cyclopenta[c]pyrazolo[1,5-a]pyridine-1-carboxylate (62)**

A mixture of **61a** (145 mg, 0.512 mmol) and Raney cobalt (1.5 g, washed with water and ethanol before use) in 2.0 M ammonia ethanol solution (50 mL) was stirred at room temperature for 14 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **62** (145 mg, 99%) as oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.41 (3H, t,  $J$  = 7.0 Hz), 1.63–2.41 (6H, m), 2.63 (3H, s), 2.80–3.11 (2H, m), 4.14–4.42 (3H, m), 6.78 (1H, d,  $J$  = 6.6 Hz), 8.23 (1H, d,  $J$  = 6.6 Hz). MS (ESI+): 288 (M+H).

**Methyl 3-amino-6-bromo-2-methylbenzoate (64)**

To a stirred solution of **63** (10.0 g, 60.5 mmol) in acetonitrile (50 mL) was added *N*-bromosuccinimide (10.8 g, 60.5 mmol) portion wise over 20 min at 0 °C. After the addition completed, the reaction mixture was concentrated in vacuo and diisopropylether (50 mL) was added. The mixture was filtrated, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **64** as oil (14.4 g, 97%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.08 (3H, s), 3.70 (2H, brs), 3.94 (3H, s), 6.58 (1H, d,  $J$  = 8.5 Hz), 7.18 (1H, d,  $J$  = 8.5 Hz).

**Methyl 5-bromo-1H-indazole-4-carboxylate (65)**

To a stirred solution of **64** (5.44 g, 22.3 mmol) in acetic acid (110 mL) was added a solution of sodium nitrite (1.69 g, 24.5 mmol) in water (11 mL) at room temperature. After 20 h, the organic solvent was concentrated in vacuo. The residual aqueous solution was diluted with saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with ethyl acetate. The extract was washed with saturated brine, dried over anhydrous sodium sulfate, filtrated and evaporated. Purification by NH silica gel column chromatography to afford **65** (4.86 g, yield 86%) as solid. Mp 164–165 °C (recrystallized from ethyl acetate). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.06 (3H, s), 7.48 (1H, dd, *J* = 8.8, 1.1 Hz), 7.63 (1H, d, *J* = 8.8 Hz), 8.26 (1H, d, *J* = 1.1 Hz), 10.59 (1H, brs).

#### **Methyl 5-bromo-2-methyl-2*H*-indazole-4-carboxylate (66)**

To a stirred solution of **65** (4.50 g, 17.6 mmol) in ethyl acetate (176 mL) was added trimethyloxonium tetrafluoroborate (3.38 g, 22.9 mmol) at room temperature. After 2.5 h, the reaction solution was diluted with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **66** (3.42 g, yield 73%) as solid. Mp 103–104 °C (recrystallized from ethyl acetate/hexane). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.01 (3H, s), 4.22 (3H, s), 7.49 (1H, d, *J* = 9.1 Hz), 7.66 (1H, dd, *J* = 9.1, 0.8 Hz), 8.15 (1H, s). Anal. Calcd for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>O<sub>2</sub>Br: C, 44.63; H, 3.37; N, 10.41. Found: C, 44.69; H, 3.30; N, 10.50.

#### **Methyl 5-[3-methoxy-3-oxoprop-1-en-1-yl]-2-methyl-2*H*-indazole-4-carboxylate (67)**

A suspension of **66** (1.65 g, 6.18 mmol), palladium acetate (139 mg, 0.62 mmol), triphenylphosphine (325 mg, 1.24 mmol), potassium carbonate (1.28 g, 9.27 mmol) and methyl acrylate (834 μL, 9.27 mmol) in *N,N*-dimethylformamide (60 mL) was stirred at 100°C for 50 min. After cooling, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **67** (1.33 g, yield 78%) as solid. Mp 232–234 °C (recrystallized from ethyl acetate). MS (ESI): *m/z* 275 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.83 (3H, s), 4.03 (3H, s), 4.25 (3H, s), 6.39 (1H, d, *J* = 15.9 Hz), 7.54 (1H, d, *J* = 9.1 Hz), 7.85 (1H, d, *J* = 9.1 Hz), 8.28 (1H, s), 8.61 (1H, d, *J* = 15.9 Hz). Anal. Calcd for C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.02; H, 5.13; N, 10.26.

#### **Methyl 5-(3-methoxy-3-oxopropyl)-2-methyl-2*H*-indazole-4-carboxylate (68)**

A mixture of **67** (1.02 g, 3.72 mmol) and 10% palladium on carbon (100 mg, containing 50% water) in methanol (40 mL) was stirred at room temperature for 12 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Purification by silica gel column chromatography afforded **68** (813 mg, yield 79%) as oil. MS (ESI): *m/z* 277 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)

$\delta$  2.61–2.74 (2H, m), 3.30–3.42 (2H, m), 3.67 (3H, s), 3.99 (3H, s), 4.23 (3H, s), 7.21 (1H, d,  $J$  = 8.0 Hz), 7.79 (1H, d,  $J$  = 8.0 Hz), 8.20 (1H, s),

### **2-Methyl-6,7-dihydrocyclopenta[*e*]indazol-8(2*H*)-one (69)**

To a stirred suspension of 60% sodium hydride (in mineral oil, 145 mg, 3.62 mmol) in tetrahydrofuran (10 mL) were added a solution of **68** (500 mg, 1.81 mmol) in tetrahydrofuran (10 mL) and methanol (1 drop), and the mixture was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and the resulting solid was added portion wise to 12 M hydrochloric acid (5 mL) heated to 100°C. After 4 h, the reaction solution was neutralized with 8 M aqueous sodium hydroxide after cooling, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **69** (285 mg, yield 85%) as solid. Mp 162–164 °C (recrystallized from ethyl acetate). MS (ESI):  $m/z$  187 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.65–2.80 (2H, m), 3.14–3.25 (2H, m), 4.25 (3H, s), 7.36 (1H, d,  $J$  = 8.9 Hz), 7.95 (1H, d,  $J$  = 8.9 Hz), 8.36 (1H, s). Anal. Calcd for C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.87; H, 5.37; N, 15.16.

### **(*E*)-2-(2-Methyl-6,7-dihydrocyclopenta[*e*]indazol-8(2*H*)-ylidene)acetonitrile (70)**

To a stirred suspension of 60% sodium hydride (in mineral oil, 116 mg, 2.90 mmol) in tetrahydrofuran (8 mL) was added diethyl cyanomethylphosphonate (516  $\mu$ L, 3.19 mmol) at room temperature, and the mixture was stirred for 30 min. A solution of **69** (180 mg, 0.97 mmol) in tetrahydrofuran (2 mL) was added, and the mixture was stirred at 50°C for 30 min. Water was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **70** (138 mg, 68%) as solid. The *Z* form was removed by column chromatography. Mp 189–192 °C (recrystallized from hexane/ethyl acetate). MS (ESI):  $m/z$  210 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.16–3.21 (4H, m), 4.27 (3H, s), 5.53 (1H, s), 7.29 (1H, d,  $J$  = 8.9 Hz), 7.79 (1H, d,  $J$  = 8.9 Hz), 8.00 (1H, s). Anal. Calcd for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.48; H, 5.27; N, 20.16.

### **(*E*)-2-(2-Methyl-6,7-dihydrocyclopenta[*e*]indazol-8(2*H*)-ylidene)ethanamine (71)**

A mixture of **70** (600 mg, 2.87 mmol) and Raney cobalt (6.0 g, washed with water and ethanol before use) in 1.0 M ammonia ethanol solution (28 mL) was stirred at room temperature for 3 h under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford **71** (614 mg, 99%) as solid. MS (ESI):  $m/z$  214 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.77–2.89 (2H, m), 3.01–3.15 (2H, m), 3.55 (2H, d,  $J$  = 6.9 Hz), 4.23 (3H, s), 5.89–6.01 (1H, m), 7.20 (1H, d,  $J$  = 8.8 Hz), 7.57 (1H, d,  $J$  = 8.8 Hz), 8.11 (1H, s).

### **(*E*)-*N*-[2-(2-Methyl-6,7-dihydrocyclopenta[*e*]indazol-8(2*H*)-ylidene)ethyl]acetamide (72a)**

To a stirred solution of **71** (400 mg, 1.88 mmol) in tetrahydrofuran (19 mL) were added triethylamine (287  $\mu$ L, 2.06 mmol) and acetic anhydride (195  $\mu$ L, 2.06 mmol) at 0 °C. After 15 min, saturated aqueous sodium hydrogencarbonate was added, and the mixture was extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. Purification by silica gel column chromatography afforded **72a** (393 mg, 82%) as solid. Mp 200–202 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  256 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.03 (3H, s), 2.81–2.93 (2H, m), 3.05–3.13 (2H, m), 4.06–4.15 (2H, m), 4.23 (3H, s), 5.62 (1H, brs), 5.81–5.91 (1H, m), 7.20 (1H, d,  $J$  = 8.8 Hz), 7.60 (1H, d,  $J$  = 8.8 Hz), 8.06 (1H, s). Anal. Calcd for C<sub>15</sub>H<sub>17</sub>N<sub>3</sub>O: C, 70.56; H, 6.71; N, 16.46. Found: C, 70.20; H, 6.78; N, 16.41.

#### **(E)-N-[2-(2-Methyl-6,7-dihydrocyclopenta[*e*]indazol-8(2*H*)-ylidene)ethyl]propionamide (72b)**

To a stirred solution of **71** (100 mg, 0.469 mmol) in tetrahydrofuran (4.7 mL) were added triethylamine (72.0  $\mu$ L, 0.516 mmol) and propionic anhydride (66.2  $\mu$ L, 0.516 mmol) at 0 °C. After 15 min, saturated aqueous sodium hydrogencarbonate was added and concentrated in vacuo. Purification by silica gel column chromatography afforded **72b** (108 mg, 85%) as solid. Mp 185–187 °C (recrystallized from ethyl acetate/hexane). MS (ESI):  $m/z$  270 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.20 (3H, t,  $J$  = 7.4 Hz), 2.25 (2H, q,  $J$  = 7.4 Hz), 2.81–2.94 (2H, m), 3.04–3.15 (2H, m), 4.05–4.16 (2H, m), 4.23 (3H, s), 5.56 (1H, brs), 5.81–5.92 (1H, m), 7.20 (1H, d,  $J$  = 8.8 Hz), 7.60 (1H, d,  $J$  = 8.8 Hz), 8.07 (1H, s). Anal. Calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O: C, 71.35; H, 7.11; N, 15.60. Found: C, 71.29; H, 7.04; N, 15.69.

### Biological evaluation

#### **Preparation of CHO Membrane for Melatonin Receptor (MT<sub>1</sub> or MT<sub>2</sub>) Binding Assays**

Cell lines stably expressing human MT<sub>1</sub> receptor (hMT<sub>1</sub>-CHO/binding, cell line: TPCCB0117) or human MT<sub>2</sub> receptor (hMT<sub>2</sub>-CHO, cell line: TPCCB0098) were used for the experiments. hMT<sub>1</sub>-CHO/binding cells and hMT<sub>2</sub>-CHO cells were cultured in F-12 Nutrient Mixture (Ham) supplemented with 10% fetal bovine serum (FBS) and 500  $\mu$ g/mL of Geneticin, 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin in a 5% CO<sub>2</sub>/95% air atmosphere. Cells were harvested at confluence in Ca<sup>2+</sup>-Mg<sup>2+</sup>-free phosphate buffered saline (PBS) containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) and collected by centrifugation. Cells were washed with PBS, pelleted, and homogenized in ice-cold 10 mM NaHCO<sub>3</sub> buffer (pH 7.4 at 25 °C) containing 5 mM EDTA and 1x Complete proteinase inhibitor (Roche). Cell homogenates were centrifuged (1,000 xg, 10 min, 4 °C). Supernatant was collected by centrifugation (140,000 xg, 60 min, 4 °C). Pellets were resuspended in ice-cold Tris-HCl buffer (pH 7.4 at 25 °C) containing 1 mM EDTA and 1x Complete proteinase inhibitor and stored at -80 °C until binding assays.

#### **Affinity for the Human MT<sub>1</sub> and MT<sub>2</sub> receptors**

The frozen homogenate was thawed, suspended in ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C), and used for the binding assay. For the assay using CHO cell membrane homogenate, 2 µL of DMSO solution of a test compound was mixed with homogenate and 40 (MT<sub>1</sub>) or 80 pM (MT<sub>2</sub>) 2-[<sup>125</sup>I]iodomelatonin in a total volume of 200 µL and incubated at 25 °C for 150 min. The binding reaction was terminated by rapid filtration using a cell harvester (PerkinElmer) followed by four 300 µL washes with ice-cold incubation buffer. Nonspecific binding was defined in the presence of 1 µM melatonin. GF/C filter plates were dried and radioactivity determined after addition of 25 µL Microscint-0 using a TopCount (PerkinElmer). The 50% inhibitory concentration (IC<sub>50</sub>) was calculated using non-linear regression analysis (a three parameter dose-response curve) in GraphPad Prism software (GraphPad Software). The dissociation constant of the compound for the receptor (*K<sub>i</sub>*) was calculated using the following equation:

$$K_i = IC_{50}/(1 + L/K_d)$$

where *L* and *K<sub>d</sub>* represent the concentration and the affinity constant of 2-[<sup>125</sup>I]melatonin in the binding assay, respectively. The specific binding of 2-[<sup>125</sup>I]melatonin for human MT<sub>1</sub> receptors in hMT<sub>1</sub>-CHO/binding was saturable. The one site specific binding plot in GraphPad Prism software (GraphPad Software) of saturation isotherm using ligand concentrations ranging from 5 to 160 pM revealed affinity binding sites with a *K<sub>d</sub>* value of 33.9 ± 4.2 pM and a *B<sub>max</sub>* of 33.8 ± 1.6 fmol/mg protein (one experiment). Human MT<sub>2</sub> receptors expressed in hMT<sub>2</sub>-CHO showed lower affinities than those of human MT<sub>1</sub> receptors, and its *K<sub>d</sub>* value and *B<sub>max</sub>* were 72.6 ± 9.4 pM and 72.1 ± 4.4 fmol/mg protein (one experiment), respectively determined with ligand concentrations ranging from 5 to 160 pM. Data of melatonin were obtained from 6 experiments in more than triplicate. Data of the other compounds were obtained from a single experiment in triplicate.

### **In Vitro Clearance in Human and Rat Hepatic Microsomes**

Hepatic microsomes from rats and humans were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH<sub>2</sub>PO<sub>4</sub>\_K<sub>2</sub>HPO<sub>4</sub> phosphate buffer (pH 7.4) and 1 µmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L MgCl<sub>2</sub>, 50 mmol/L glucose 6-phosphate, 5 mmol/L β-NADP<sup>+</sup>, and 15 unit/mL glucose 6-phosphate dehydrogenase was added to the incubation mixture with a 10% volume of the reaction mixture to initiate the enzyme reaction. After the addition of the NADPH-generating system, the reaction mixture was incubated at 37 °C for 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. As a control, the mixture without 37 °C incubation was also prepared. Test compound in the reaction mixture was measured by an HPLC system equipped with a UV detector. For

the determinations of the metabolic clearance, chromatograms were analyzed for parent compound disappearance from the reaction mixtures. Data were obtained from a single experiment in duplicate.

### **Oral bioavailability**

Test compounds were administered to male Sprague-Dawley rats (8 weeks old, non-fasted, n=3 or 5). After oral and intravenous administration, blood samples were collected and immediately centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.2% (v/v) formic acid in 10 mmol/L ammonium formate (pH 3) and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

### **Brain-to-Plasma Concentration ratio in Rats**

Ramelteon and compound (*S*)-**32b** was administered to male Sprague-Dawley rats (8 weeks old, non-fasted, n=3). In 60 min after oral administration at 1.0 mg/kg (ramelteon) and 0.1 mg/kg ((*S*)-**32b**), blood and plasma samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The brain samples were homogenized in saline to obtain the brain homogenate. The plasma and brain homogenate samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.2% (v/v) formic acid in 10 mmol/L ammonium formate (pH 3) and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

### **Inhibition of Forskolin-Induced cAMP Formation in CHO Cells Expressing Human Melatonin Receptor (MT<sub>1</sub> or MT<sub>2</sub>)**

For the assay of human MT<sub>1</sub> receptor, we used Chinese Hamster Ovary (CHO) cells expressing human MT<sub>1</sub> receptors. A cell line stably expressing human MT<sub>1</sub> receptors (human MT<sub>1</sub>/CHO-K1 CL3) was selected after transfected with human MT<sub>1</sub> genes to parental CHO cells (Health Science Research Resources Bank, Japan) and cultured in Ham's F-12 supplemented with 10% FBS, 100 units/mL Penicillin/ 100 µg/mL Streptomycin solution and 500 µg/mL G418 at 37°C in 5% CO<sub>2</sub>/95% air atmosphere in humid conditions. For the assay of human MT<sub>2</sub> receptor, we used CHO cells expressing human MT<sub>2</sub> receptors. A cell line stably expressing human MT<sub>2</sub> receptor [human MT<sub>2</sub> /CHO-K1 (PerkinElmer)] was purchased from PerkinElmer and cultured in Ham's F-12 supplemented with 10% FBS, 100 units/mL Penicillin/ 100µg/mL Streptomycin solution and 500 µg/mL G418 at 37°C in 5% CO<sub>2</sub>/95% air atmosphere in humid conditions. The following Buffer A and B were used for cAMP production assay. Buffer A: modified Hanks' balanced salt solution [mHBSS: HBSS without NaHCO<sub>3</sub> containing 5 mmol/L HEPES] containing 0.1% BSA, 100 µmol/L IBMX, 100 µmol/L Ro 20-1724 and 6 µmol/L forskolin. Buffer B: mHBSS containing 0.1% BSA, 100 µmol/L IBMX and 100 µmol/L Ro 20-1724. 384-well plates were added with 10 µL of Buffer B. Melatonin and (*S*)-**32b** were dissolved in

DMSO to make 0.03, 0.3, 3, 30, 300, 3000 and 30000 nmol/L solution and diluted with Buffer A to make 100 times the final desired concentration. Each compound solution was added with 10  $\mu$ L/well. All cell lines were cultured in HAM's F-12 containing 10% FBS and harvested at confluence by Accutase treatment. The cell line of human MT<sub>1</sub> was seeded at  $2 \times 10^4$  cells/well in the plates with 10  $\mu$ L of Buffer B. The cell line of human MT<sub>2</sub> was seeded at  $4 \times 10^4$  cells/well as same as another cell line. After shortly shaking, cells were incubated at room temperature for 30 minutes. Melatonin and (*S*)-**32b** were dissolved in the final reaction solution to make 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 nmol/L solution. We used AlphaScreen cAMP assay kit to measure concentrations of cAMP in cells. After the incubation, 10  $\mu$ L of Buffer B containing 100 units/mL anti cAMP-acceptor beads was added to each well. The reaction was terminated by the addition of 10  $\mu$ L of Buffer B containing 1.5% Tween-20, 106 units/mL biotinylated cAMP and 50  $\mu$ g/mL donor-SA beads. After shaking for 5 minutes, the plates were incubated in the dark at room temperature for more than 6 hours to 18 hours. The AlphaScreen's chemiluminescence of each well was detected by using EnVision plate reader (PerkinElmer). The procedure of cAMP production assay was based on the method of PerkinElmer's AlphaScreen cAMP assay kit protocol with minor modifications. The counts of the AlphaScreen's chemiluminescence were converted to concentrations of cAMP accumulations in the cells by using standard curves estimated by a 4-parameter nonlinear logistic regression analysis. The 100% value was the mean cAMP production with 2  $\mu$ mol/L forskolin. The 0% value was that with 1  $\mu$ mol/L melatonin and 2  $\mu$ mol/L forskolin. The EC<sub>50</sub> values were estimated by a 2-parameter nonlinear logistic regression analysis. Data were expressed as percentage forskolin response at each receptor (mean  $\pm$  S.D.). Data were obtained from a single experiment in quadruplicate.

### Sleep-Promoting Effect of (*S*)-**32b** in Cats

Healthy male and female European Shorthair cats were purchased from Nisseiken Company, Ltd. (Tokyo, Japan) and housed individually in a room maintained at 22-24°C with a 12 h light-dark cycle (light on at 7:00 AM). They were fed once daily (7:00 AM) and water was available *ad libitum*.

Surgery for EEG/EMG recordings was performed as previously described.<sup>39</sup> Cats were placed on a stereotaxic apparatus under general anesthesia. Electrodes for electroencephalogram (EEG) recording were implanted bilaterally in the frontal and parietal cortices and hippocampus according to the cat brain atlas of Snider and Niemer (1961). Stainless steel screws were used as cortical electrodes. The depth bipolar recording electrode consisted of insulated stainless-steel wires (0.3 mm in diameter) except at the tips (0.5 mm). A pair of stainless-steel wires was implanted into the back cervical muscles to record electromyogram (EMG) data.

The cats were allowed to recover from surgery for at least 7 days before habituation to test chamber (an acrylic cage 75×65×40 cm located in a soundproof, electrically shielded room that was maintained under conditions similar to those of the home cage). The animals were connected to the recording equipment via a light flexible cable with a slip-ring connector that permitted them free movement. After we confirmed that animals sleep sufficiently in the experimental room, polysomnographic recordings

were conducted. The behavioral and postural changes of the animals were observed using a video camera continuously throughout the experiment. The EEG-EMG signals were acquired using an electroencephalograph (GE Yokogawa Medical System, Osaka, Japan). These recordings were performed for 8 h after drug administration. Compound (*S*)-**32b** (10 mg/kg) or vehicle was orally administered between 9:30 AM and 10:00 AM. The signals were digitally filtered (EEG, 0.5-30 Hz; EMG, 20-200 Hz) and semi automatically scored in 20 s epochs as wakefulness, slow wave sleep (SWS), or rapid eye movement sleep (REM) by a sleep scoring system (Sleep Sign ver. 2; Kissei Comtec, Matsumoto, Japan). This preliminary scoring was visually inspected and corrected if necessary. Compound (*S*)-**32b** was suspended in 0.5% (w/v) methylcellulose solution. The solution was administered orally to each cat in a gelatin capsule. In the control trial, each cat was given a capsule-containing vehicle. We used a crossover design with 7 cats per group and at least 7 days washout periods. Data were expressed as the total time spent in sleep stages (SWS and REM) during 8 h following the oral administration. The statistical differences between the vehicle-treated group and Compound (*S*)-**32b**-treated group were analyzed using a crossover analysis of variance (ANOVA) followed by a contrast test with significant set at  $p < 0.05$ .

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## 論文目録

### 1. 学位論文の基礎となる報文の著者名、題目、印刷・公表の方法、その時期および該当する章・節

1) Yasutaka Hoashi, Takafumi Takai, Etsuo Kotani, Tatsuki Koike. Synthesis of pyrazolo[1,5-*a*]pyridines by thermal intramolecular cyclization. *Tetrahedron Lett.* **2013**, 54, 2199–2202. [第 1 章]

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