RS ウイルス感染症治療薬の開発を目的とした RSV F タンパク質阻害物質の創薬研究

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山口　亮

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略語表

本論文において以下示す略語及び略号を用いた。

Ac	acetyl
ALRI	acute lower respiratory infection
APCI	atmospheric pressure chemical ionization
Ar	aryl
Boc	*tert*-butoxycarbonyl
Cbz	carbobenzoxy
CDI	1,1'-carbonyldiimidazole
CPE	cytopathic effect
CYP	cytochrome P450
DMA	dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
dppf	1,1'-bis(diphenylphosphino)ferrocene
EC_{50}	half maximal (50%) effective concentration
ELSD	evaporative light-scattering detector
ESI	electrospray ionization
Et	ethyl
EXSY	exchange spectroscopy
F protein	fusion protein
G protein	glycoprotein
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HMDS	hexamethyldisilazane
HPLC	high performance liquid chromatography
HR-MS	high-resolution mass spectrometry
*i*Pr	isopropyl
kb	kilo bases

LCMS	liquid chromatography mass spectrometry
L protein	large protein
MD	molecular dynamics
MDI	metabolism-dependent inhibition
Me	methyl
Ms	methanesulfonyl
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
N protein	nucleoprotein
PAMPA	parallel artificial membrane permeability assay
PDB	Protein Data Bank
Ph	phenyl
P protein	phosphoprotein
Pr	propyl
REMD	replica-exchange molecular dynamics
RSV	respiratory syncytial virus
RNA	ribonucleic acid
SAR	structure–activity relationship
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl

序論

RS ウイルス感染症について

呼吸器合胞体ウイルス（Respiratory Syncytial Virus：RSV）は、幼児期の気管支炎や肺炎の原因としてよく知られており[1]、軽度の風邪様症状から喘息、重度の気管支炎、肺炎などの下気道疾患に至るまで、様々な症状を呈する[2–8]。一般的に、RSV に感染すると 4 ～ 5 日の潜伏期間を経て、発熱、鼻汁、咳などの上気道炎を発症する。約 7 割の症例では上気道炎のみの症状にとどまり数日で回復するが、残りの 3 割では 2 ～ 3 日後に感染が下気道に及び、咳の増強、喘鳴、さらには呼吸困難などの下気道炎（気管支炎、細気管支炎、肺炎）の症状を呈し重症化する。その後下気道炎は回復期に入り、数日～ 1 週間を経過して快方に向かう[9]。

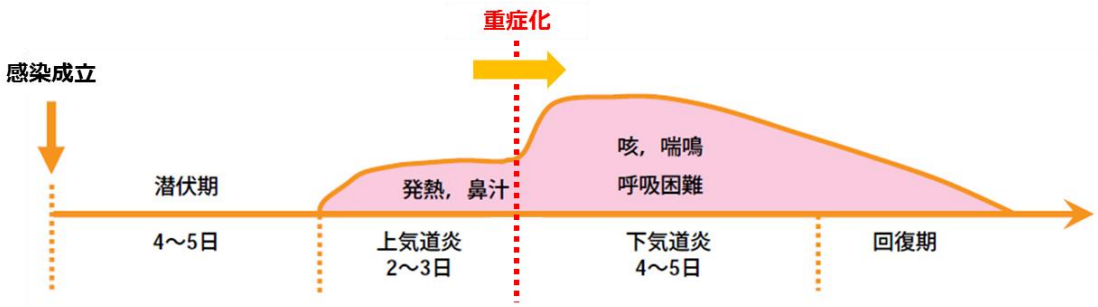


Figure 1. RSV の一般的な臨床経過（文献 9 を一部改変）

RSV は世界中に分布しており、繰り返し感染する。1 歳未満の乳児の半数以上、2 歳未満の乳児のほとんどが少なくとも 1 回は RSV に感染するといわれている[10,11]。5 歳以下の子どものうち、RSV による急性下気道感染症（Acute Lower Respiratory Infection：ALRI）の患者数は推定 3,380 万人で（ALRI の全症例の 22%を占める）、そのうち 340 万人以上が入院を必要とし、年間 66,000～99,000 人が死亡していると報告されている[8]。RSV 感染症関連死亡率は麻疹の死亡率に次いで高く、インフルエンザの死亡率よりも高くなっている[12]。さらに、1 歳未満での重症 RSV 気管支炎の発症は、成人早期にアレルギー性喘息を発症するリスクの増加と関連しており[3]、成人では高齢であることや免疫不全や心肺疾患などの基礎疾患を有することが RSV 感染症の重症化に寄与する高危険因子として報告されている[13]。

現在のところ RSV 感染症に対する有効な治療薬やワクチンはなく、治療法の選択肢は限られており、酸素補充、水分投与、呼吸管理などの支持療法が中心となっている[14–17]。2002 年に日本国内で承認された RSV F タンパク質に対するモノクローナル抗体である palivizumab（Synagis®）は RSV 感染症の小児の入院予防に有効であるが、高額であることから心臓や肺に基礎疾患を有する未熟児や小児のハイリスク患者にしか使用されていない[18,19]。また、米国において承認されているグアニンアナログの ribavirin も RSV に対して抗ウイルス活性を有するが、比較的毒性が強く、臨床的な有効性については議論の的となっている[20,21]。したがって、乳幼児での死亡率が高く、高齢者や免疫不全者での重症化リスクの高い RSV 感染症を迅速に治療するためには、有効で安全かつ安価な新しい経口低分子薬の登場が望まれる。

RSV の構造およびライフサイクルと RSV 感染を制御する標的因子

RSV はニューモウイルス属に属するパラミクソウイルスである。直径約 200 nm から成るエンベロープを有し、表面には細胞への吸着、および膜融合を担う 10～12 nm のスパイクがある（**Figure 2**）。RSV のウイルスゲノムは約 15.2 kb で、マイナス一本鎖 RNA から構成されており、11 個のタンパク質をコードする 10 個の遺伝子がある[22]。また、G タンパク質（glycoprotein: G protein）の性状の差から、RSV は二つのサブグループ（RSV-A と RSV-B）に分類される。

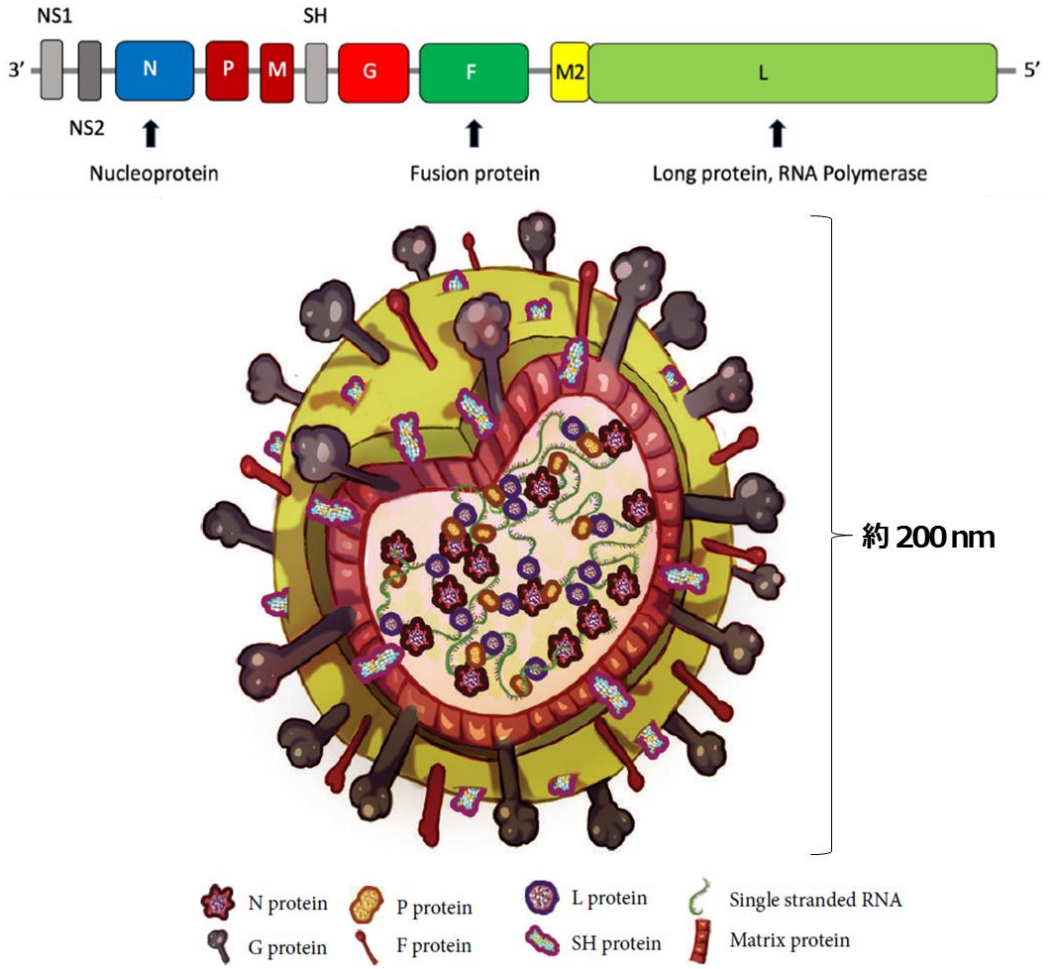


Figure 2. RSV の構造とゲノム構成（文献 22 および文献 23 を一部改変）

Figure 3 に RSV のライフサイクルを示した。RSV は G タンパク質を介して宿主細胞に接着し、F タンパク質（fusion protein: F protein）に構造変化が起こり、それを介してウイルスエンベロープが宿主細胞膜と融合することにより細胞内に侵入する [24,25]。侵入後、L タンパク質（large protein: L protein）と P タンパク質（phosphoprotein: P protein）から成る RNA 依存性 RNA ポリメラーゼが、ゲノム RNA–N タンパク質（nucleoprotein: N protein）複合体を鋳型として転写と複製の両過程を担い [26]、F タンパク質を媒介とした細胞–細胞融合によりウイルスが拡散し合胞体が形成される [27]。RSV 感染を制御するためには、ウイルスの感染・複製に必要なこれらのウイルス因子を阻害する必要がある。

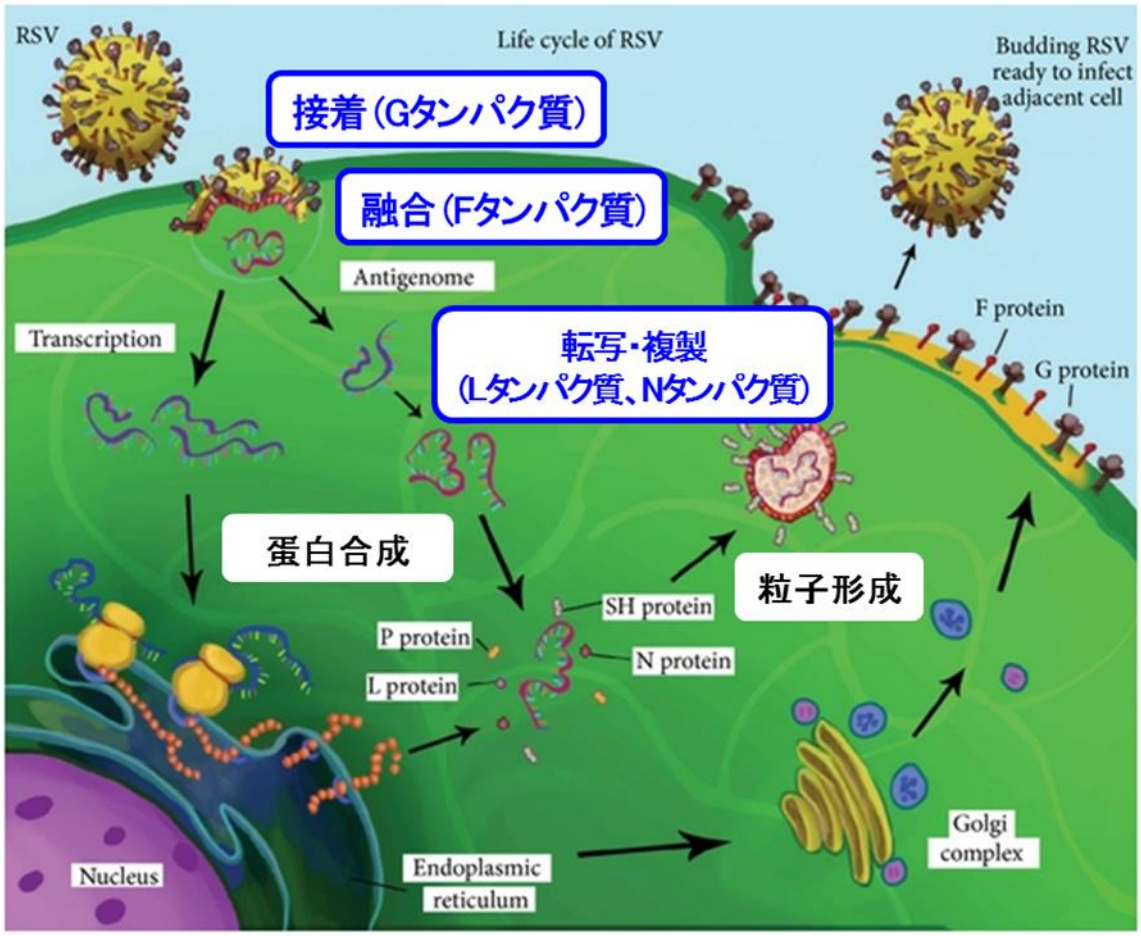


Figure 3. RSV の複製機構（ライフサイクル）（文献 22 を一部改変）

これらの標的因子の中でこれまでに F タンパク質、L タンパク質、および N タンパク質を標的とした低分子 RSV 治療薬の開発が報告されている。その中でも、F タンパク質を標的とした低分子 RSV 治療薬としては presatovir（GS-5806）[28]、JNJ-53718678[29]、RV521[30]、ziresovir（AK0529）[31]、BMS-433771[32]、VP-14637[33]が報告されており、数多くの低分子化合物が臨床試験まで進むなど、活発に創薬研究が展開されている。また、L タンパク質を標的とした治療薬の開発としては lumicitabine（ALS-008176）[34]、PC786[35]が、N タンパク質を標的としたものとしては RSV604[36]、EDP-938[37]が報告されている（**Figure 4**）。一方、G タンパク質は転写・翻訳の際に高頻度で変異を生じることから低分子 RSV 治療薬に適した標的か否かが不明であるため、積極的な創薬研究は行われていない。

これらの低分子 RSV 治療薬の中でも、ウイルス表面にある F タンパク質を標

的とした治療薬は、宿主細胞外においてウイルスとの膜融合を阻害することで、ウイルスライフサイクルの初期過程を制御できるという利点がある。さらに、F タンパク質は G タンパク質とは異なり、転写・翻訳の際の保存性も高い。また、L タンパク質を標的とした場合には宿主細胞の核酸合成酵素阻害作用との選択性の確保が必須であり、ウイルス側にのみ存在する F タンパク質を標的とした治療薬と比較して創薬難易度が上がることが想定される。したがって、F タンパク質の機能である膜融合能を阻害することは、RSV の増殖を抑制するためのもっとも有力な戦略の一つであると考えられる [28–31,38,39]。以上のように、F タンパク質阻害物質は安全で有効な RSV 感染症治療薬になりうることが示唆されている。

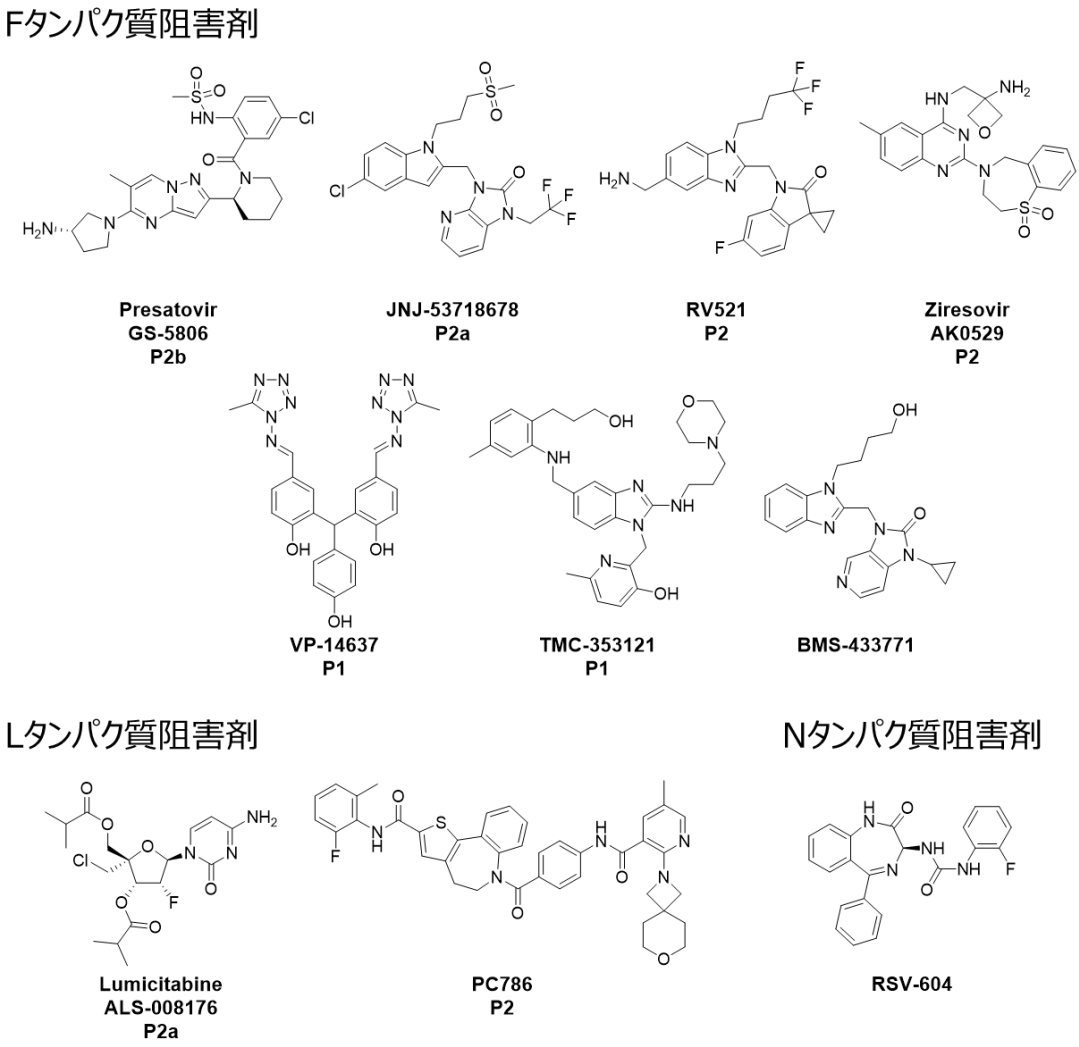


Figure 4. これまで開発されてきた RSV 治療薬

一方、近年になって臨床試験が進んでいる F タンパク質阻害物質において複数の耐性分離株が報告され、F タンパク質阻害物質に対する薬剤耐性化ウイルスの出現の懸念が顕在化してきた。

RSV の薬剤耐性化について

F タンパク質阻害物質は、ウイルスの一点突然変異でも抗ウイルス活性を失うことが多いことが知られている。F タンパク質阻害物質の耐性分離株は、以下の 3 つの領域で耐性変異を引き起こすことがもっとも多い。①融合ペプチド（アミノ酸 140～144）、②システインリッチ領域（アミノ酸 392～401）、③ヘプタドリピート 2（アミノ酸 486～489）である [40]。

既知の変異体のうち、D486N 変異体は presatovir をはじめとする RSV F タンパク質阻害物質の臨床試験で分離されており、VP-14637 や TMC-353121 などの様々な化学構造を有する化合物に対して交差耐性を示している（**Figure 4**）[41,42]。RSV と同じ RNA ウイルスであるインフルエンザウイルス（A/H1N1）では抗インフルエンザ薬タミフルの発売から 12 年後に 98%のウイルスに薬剤耐性化が認められた [43,44]。したがって、D486N などの薬剤耐性変異体や交差耐性変異体の出現は、人類の健康への潜在的な懸念事項となっており [45]、変異株が世界的に広まる前に、これらに対する有効な RSV F タンパク質阻害物質の開発が急務となっている。

本研究の目的と概要

著者は新規 RSV 治療薬を創出すべく、RSV F タンパク質阻害物質の創薬研究を開始した。著者が研究を開始した時点ですでに複数の製薬会社から RSV F タンパク質阻害物質が報告されており、F タンパク質阻害物質の化合物クラスとしては、ピラゾロ[1,5-a]ピリミジンシリーズ [46–49]、ベンズイミダゾールシリーズ [50–52]、ピペラジニルキノリンシリーズ [53]などが知られていた。その中でもピラゾロ[1,5-a]ピリミジン骨格を有する presatovir は、先頭を切って臨床試験が進められており、もっとも良好な抗 RSV 活性を示していた。そこで、著者はピラゾロ[1,5-a]ピリミジンシリーズの化合物を合成展開することで、有効な新規 RSV F タンパク質阻害物質の創出を目指すことにした。

また、近年いくつかの阻害物質についてFタンパク質との共結晶構造がX線構造解析により明らかとなっている。それらの構造情報を利用して、薬剤耐性化の懸念のある耐性変異株に対しても有効性を示す新規RSV Fタンパク質阻害物質を探索することにした。

第1章ではピラゾロ[1,5-a]ピリミジン骨格を有する化合物シリーズを元にデザイン、合成して見出した新規骨格を有する1-メチルアミノプロピル誘導体の創出、および構造活性相関（SAR）研究について述べる。Gilead社は、ピラゾロ[1,5-a]ピリミジン骨格を有するpresatovirのX線単結晶構造情報からピラゾロ[1,5-a]ピリミジン環とアシルピペリジン環が形成する2面角が良好な抗RSV活性を示すのに重要であることを報告している[46]。著者は、presatovirがもつピペリジン環を開環し種々の2面角分布を示す化合物をデザイン、合成し、その2面角分布と抗RSV活性の関係を調査するとともに、より強力な抗RSV活性を有する新規RSV Fタンパク質阻害物質の創出を目指した。その結果、presatovirと類似の2面角分布をもつ1-メチルアミノプロピル誘導体がもっとも良好な活性を示した。その後、Ar位置換基の最適化により、強力な抗RSV活性を示す化合物**20f**（EC_{50}＝0.15 nM）を見出した。また、ピラゾロ[1,5-a]ピリミジン環7位への置換基導入により、7位置換基の許容性を確認し、生物学的・物理化学的プロファイルをさらに改善できる可能性を示した。しかしながら、これら1-メチルアミノプロピル誘導体にはpresatovirと同様に耐性変異株D486Nに対する活性は認められなかった。

第2章では耐性変異株D486N株に有効な新規マクロサイクル化合物**39h**の創出について、マクロサイクル化に至るドラッグデザインとそのSARについて述べる。また、マクロサイクル化合物**39h**が変異株D486Nに有効であった理由についてドッキングシミュレーションを用いて論じる。2015年にBattles（Janssen社）らによってFタンパク質といくつかの阻害物質（ベンズイミダゾールシリーズの化合物）とのX線共結晶構造が取得された[54]。結晶構造情報により、阻害物質とFタンパク質との結合様式や周囲のアミノ酸残基が明らかとなり、耐性変異に関する考察も可能になってきた。著者はこれらのX線共結晶構造情報を用いてピラゾロ[1,5-a]ピリミジン化合物とFタンパク質とのドッキングシミュレーションを実施し、変異株D486Nに対して活性を示さなかった理由を考察した。続いて、分子をマクロサイクル化し固定化することにより、変異株D486N

にも有効な化合物を見出せるのではないかとの仮説を立て、検証した。その結果、野生株だけでなく変異株 D486N にも有効な 15 員環マクロサイクル化合物 **39h**（A2 EC_{50}: 2.0 nM、D486N EC_{50}: 8.1 nM）を見出した。しかしながら、15 員環マクロサイクル化合物 **39h** はアトロプ異性体の混合物であることが、NMR によるアトロプ異性体交換速度測定から判明した。アトロプ異性体混合物の創薬開発は、開発ステージごとにその異性体比を確認する必要があるため、非常に困難であるとされている [55–57]。アトロプ異性体の回避が新たな課題となった。

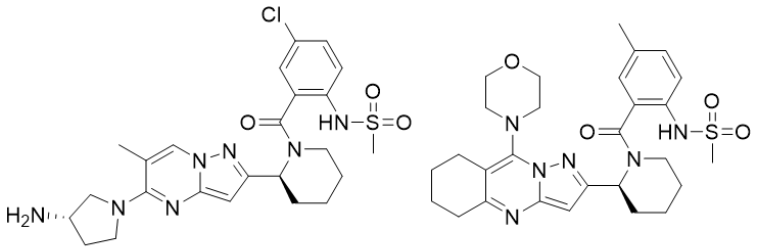
第 3 章ではアトロプ異性体を回避したアミドリンカーを有する新規 16 員環マクロサイクル誘導体 **93b** の創出について、アトロプ異性体を回避するための仮説と、検証におけるリンカー部位（マクロサイクル化の架橋部分）の変換、さらに見出した新規アミドリンカー誘導体の最適化検討結果について述べる。すなわち、15 員環マクロサイクル化合物 **39h** のアトロプ異性体が生じている原因が 15 員環のもつ立体的な制約にあると考え、リンカー部位を 1 炭素伸長することでアトロプ異性体を回避できるとの仮説を立てた。16 員環化合物をデザイン・合成・評価した結果、アトロプ異性体を回避し、野生株および変異株 D486N に対する活性が向上したアミドリンカー化合物 **76c** を見出した。化合物 **76c** の Ar 位置換基およびピラゾロ[1,5-a]ピリミジン環 5 位置換基を最適化することで、さらに抗 RSV 活性を改善したアミドリンカー化合物 **93b** の創出に至った。

本論

第 1 章　抗 RSV 活性を有する新規 1-メチルアミノプロピル誘導体の創出

第 1 節　2 面角分布とドラッグデザイン

新規 RSV F タンパク質阻害物質を創出するにあたり、初めに、当時唯一利用可能であった TMC-353121 と F タンパク質との複合体 X 線結晶情報（2010 年 Johnson & Johnson 社が報告）[58]を用いて、ファーマコフォア仮説を構築し、自社化合物ライブラリの in silico スクリーニングを実施し、化合物を選抜した。選抜した化合物の抗 RSV 活性の評価および周辺誘導体展開を検討したが、活性が維持・向上する化合物を取得することはできなかった。そこで、既知化合物からの誘導体展開によるアプローチを図ることにした。RSV F タンパク質阻害物質であるピラゾロ[1,5-a]ピリミジン骨格を有する化合物シリーズには Gilead 社の presatovir（**1**）や Janssen 社の P3（**2**）が報告されている（**Figure 5**）。ピラゾロ[1,5-a]ピリミジンシリーズは他の化合物シリーズに比べて抗 RSV 活性が高く、また、presatovir の臨床試験がフェーズ 2b の段階にあり、ヒトにおける安全性が確認されている化合物シリーズであることから、本シリーズの化合物を元に誘導体を展開する方針を立てた。



1
Presatovir
RSV EC_{50} = 0.37 nM
Gilead

2
P3
RSV EC_{50} = 0.56 nM
Janssen

Figure 5. ピラゾロ[1,5-a]ピリミジンシリーズ

Presatovir（**1**）とその誘導体の SAR については詳細に検討されており、Gilead 社から報告されている [46]。Gilead 社は、化合物 **1** の X 線単結晶構造情報から、ピラゾロ[1,5-a]ピリミジン環とピペリジン環上のアミド結合平面との間の 2 面角が良好な抗 RSV 活性の発現に重要であることを報告している（**Figure 6**）。すなわち、ピペリジン環による分子の固定化が良好な抗 RSV 活性発現に最適な 2 面角を維持していると結論付けている。また、Gilead 社および Janssen 社が公開しているピラゾロ[1,5-a]ピリミジンシリーズの特許において、いずれもピペリジン環が基本構造としてクレームされていることからもピペリジン環の抗 RSV 活性に対する重要性がうかがえる [47–49]。

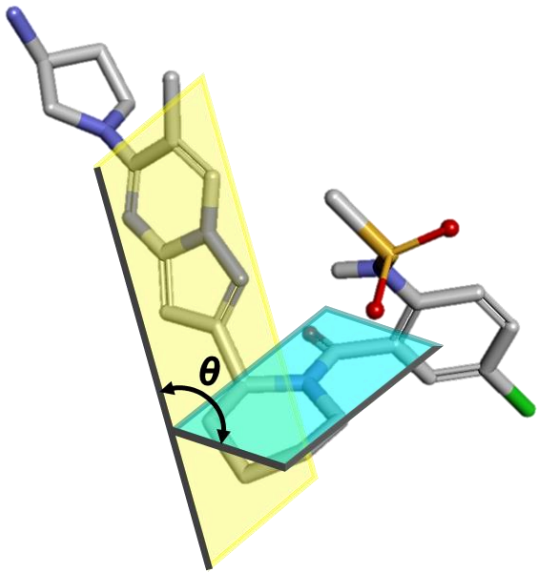


Figure 6. ピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角（θ）

そこで、ピペリジン環の誘起する 2 面角の抗 RSV 活性への影響をより明確にすることを目的として、化合物 **1** がもつピペリジン環を開環し、種々の 2 面角分布を示す化合物をデザイン、合成し、その 2 面角分布と活性との関係を調査することとした。ピペリジン環の代わりにピラゾロ[1,5-a]ピリミジン環の 2 位に非環状鎖を導入した化合物で、当該の 2 面角分布を最適化することができれば、より強力な抗 RSV 活性を有する新規 RSV F タンパク質阻害物質が得られる可能性もあるという仮説を立てた。

まず、ピペリジン環をもつ化合物 **1** のコンフォメーションをレプリカ交換分

子動力学法（replica-exchange molecular dynamics: REMD）[59]により解析し、ピラゾロ[1,5-a]ピリミジン環とアミド平面との間の 2 面角の分布を算出した。REMD とは、分子動力学シミュレーションを同時に複数の温度で実施することにより、化合物のエネルギー状態が極小状態にトラップされることを回避する方法である。REMD を利用することにより、従来の分子動力学法（MD）と比較して、短時間で広範囲のエネルギー状態をサンプリングすることが可能となる。なお、REMD シミュレーションには分子動力学シミュレーションソフトである GROMACS 5.0.4. [60]を用いた。化合物 **1** の 2 面角分布を **Figure 7** に示す。縦軸にはコンフォメーション数を、横軸には 2 面角の角度を表記している。ピペリジン化合物 **1** の場合、2 面角が 82° 付近のコンフォメーションがもっとも多く観測された。

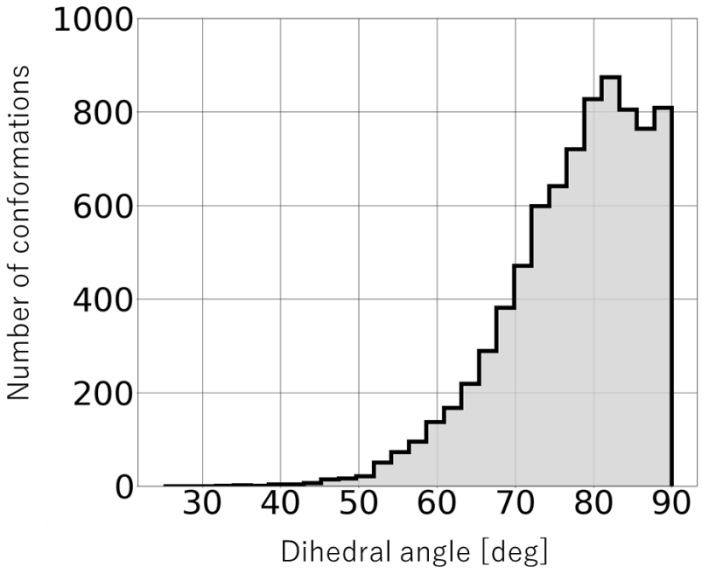


Figure 7. 化合物 **1** の 2 面角分布

続いて、非環状鎖を有する化合物（**3**–**8**）をデザインし、そのピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角分布を、化合物 **1** と同様の手法で解析した。化合物 **1** とそれぞれの非環状鎖化合物（**3**–**8**）の 2 面角分布を重ね合わせた結果を **Figure 8** に示す。灰色に塗りつぶした部分が化合物 **1** の 2 面角分布を、黒色の実線がそれぞれの非環状鎖化合物の 2 面角分布を示している。

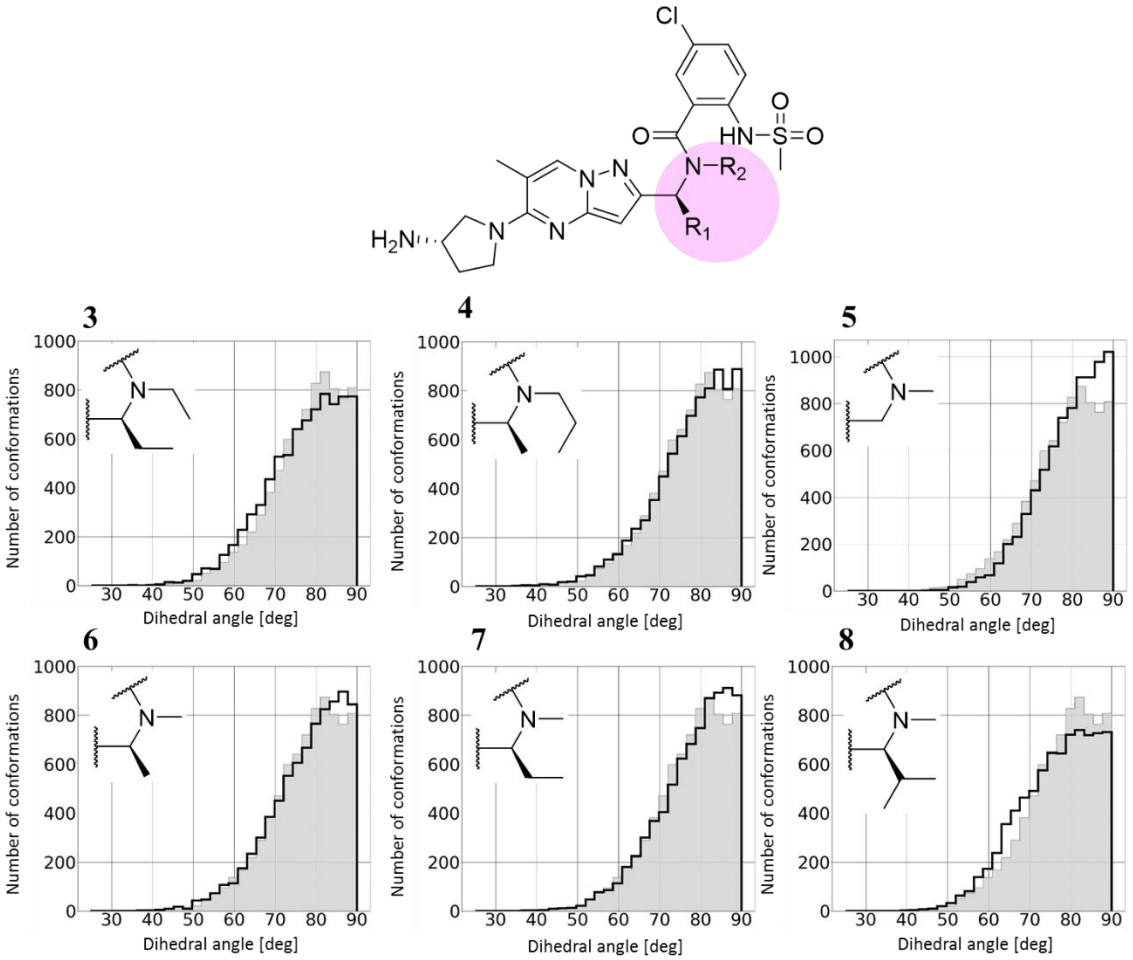


Figure 8. 非環状鎖化合物の 2 面角分布（灰色の塗りつぶしが化合物 **1** の 2 面角分布、黒色の実線が非環状鎖化合物各々の 2 面角分布を示す）

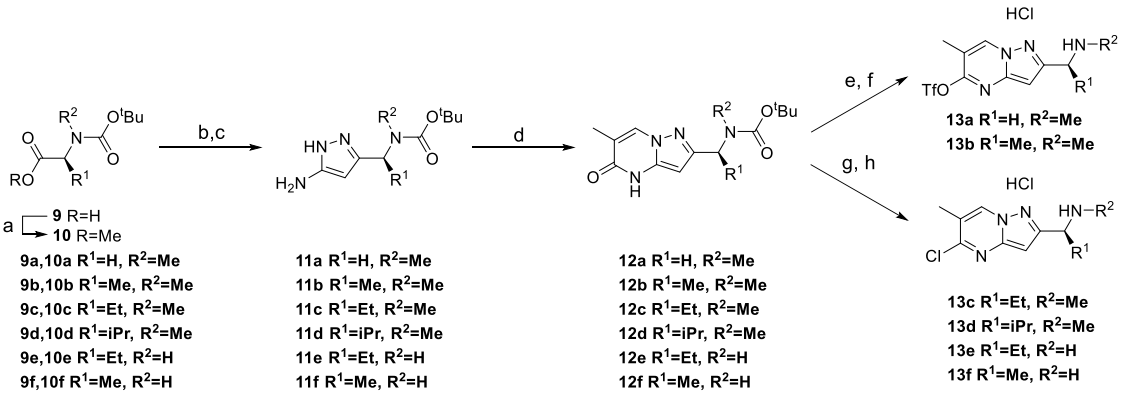
まず、炭素数を変えずにピペリジン環を開いた化合物 **3**、**4** をデザインして、その 2 面角分布について解析した。化合物 **3** では 2 面角が 82° 付近のコンフォメーション数がもっとも多く、化合物 **4** では 84° と 89° 付近に二峰性の 2 面角分布が認められている。化合物 **3**、**4** ともに、化合物 **1** の 2 面角分布と比較するとコンフォメーション数がもっとも多くなるピーク角度も全体的な 2 面角分布のパターンもよく似ている。続いて、R_1 の置換基を無置換、メチル基、エチル基、およびイソプロピル基に変換した化合物（**5**–**8**）の 2 面角分布について解析すると、化合物 **6**、**7** の 2 面角分布のピークは 87° 付近であり、全体的な 2 面角分布は化合物 **1** のものと類似している。一方、R_1 無置換化合物 **5** では 90° 付近

でもっとも安定なコンフォメーションをとり、2 面角も全体的に 90° の方に分布している。また、イソプロピル基を導入した化合物 **8** ではコンフォメーション数がもっとも多くなるピーク角度は化合物 **1** と同様に 82° 付近であるが、全体的な 2 面角分布が化合物 **1** のものとは大きく異なっていた。

これらの結果から、ピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角分布は、R_1 や R_2 の置換基に依存して変化することが示唆された。すなわち、R_1 や R_2 の置換基を変換することで、ピペリジン環を有する化合物 **1** と類似の 2 面角分布を示す化合物や、反対に化合物 **1** とは 2 面角分布が大きく異なる化合物がデザインできることを意味している。化合物の 2 面角分布と抗 RSV 活性との関係を明らかにすることを目的として、非環状鎖をもつ化合物（**3**–**8**）を実際に合成し、抗 RSV 活性を評価することにした。

第 2 節　非環状鎖を有する化合物（**3**–**8**）の合成

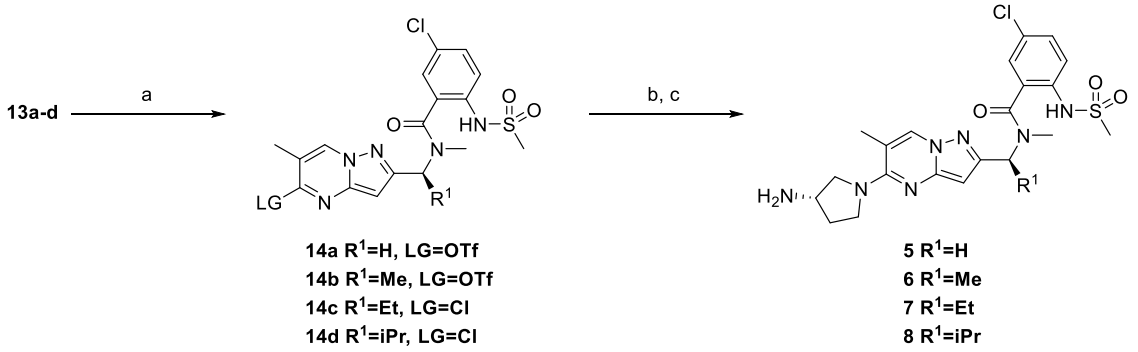
ピラゾロ[1,5-a]ピリミジン環の 2 位に非環状鎖を有する化合物（**3**–**8**）を **Schemes 1**–**3** に示す方法で合成した。ピラゾロ[1,5-a]ピリミジン環中間体 **13a–f** を市販の *tert*-ブチルオキシカルボニル（Boc）基で保護されたアミノ酸 **9a–f** より **Scheme 1** に示す方法で合成した。すなわち、まず、R_1 および R_2 に置換基を有し、Boc 基で保護されたアミノ酸 **9a–f** をメチルエステル化し **10a–f** に変換した。続いて、**10a–f** にアセトニトリルアニオンを求核的に付加させた後、ヒドラジンと作用させることによりアミノピラゾール環を構築し **11a–f** を得た。アミノピラゾール **11a–f** をナトリウムエトキシド存在下、エチル (*E*)-エトキシ-2-メチルアクリレートと反応させることにより、ピラゾロピリミドン **12a–f** を得た。化合物 **12a–b** を無水トリフルオロメタンスルホン酸でトリフラート化した後、Boc 基を除去することにより、ピラゾロ[1,5-a]ピリミジン環中間体 **13a–b** を、また、**12c–f** の Boc 基を脱保護したのちオキシ塩化リンで塩素化することにより、ピラゾロ[1,5-a]ピリミジン環中間体 **13c–f** を得た。



Scheme 1. (a) $TMSCH_2N_2$, toluene, MeOH, r.t.; (b) MeCN, NaHMDS (1.9 M in THF), THF, -78 °C to -50 °C; (c) NH_2NH_2, AcOH, EtOH, r.t., 3 steps 56–96%; (d) ethyl (*E*)-3-ethoxy-2-methyl acrylate, Cs_2CO_3, DMF, 120 °C, or NaOEt (2.94 M in EtOH), EtOH, 90 °C, 22–77%; (e) Tf_2O, pyridine, $CHCl_3$, r.t., 84–99%; (f) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%; (g) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%; (h) $POCl_3$, 110 °C, 98–100%.

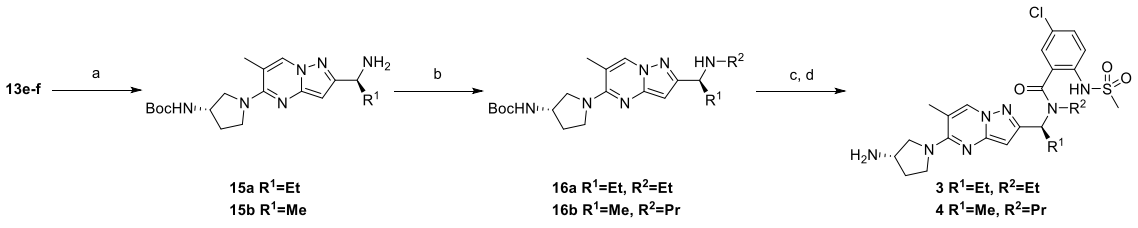
ピラゾロ[1,5-a]ピリミジン環の 2 位に非環状鎖を有する化合物（**5**–**8**）を **Scheme**

2 に示す方法で合成した。すなわち、**13a**–**d** と 5-クロロ-2-（メチルスルホンアミド）安息香酸との縮合反応により得られた **14a**–**d** に対し、塩基性条件下、(*S*)-*tert*-ブチルピロリジン-3-イルカルバメートの求核置換反応によりアミノピロリジンユニットを導入した。最後に、Boc 基を除去することにより目的とする化合物（**5**–**8**）を得た。



Scheme 2. (a) 5-chloro-2-(methylsulfonamido)benzoic acid, HATU, Et_3N, DMF, r.t., 30–76%; (b) (*S*)-*tert*-butylpyrrolidin-3-ylcarbamate, Et_3N, THF, 80 °C, 84–98%; (c) 4 M HCl in dioxane, 1,4-dioxane, r.t. 79–100%.

一方、R_2 にエチル基およびプロピル基を導入した化合物（**3**、**4**）は中間体 **13e**–**f** から **Scheme 3** に示す方法で合成した。すなわち、中間体 **13e**–**f** に対する(*S*)-*tert*-ブチルピロリジン-3-イルカルバメートの求核置換反応によりアミノピロリジンユニットを導入した後、対応するアルキルハライドを用いたアミノ基のモノアルキル化により **16a**–**b** を得た。最後に、**16a**–**b** と 5-クロロ-2-（メチルスルホンアミド）安息香酸との縮合反応後、Boc 基を脱保護することにより、目的化合物（**3**、**4**）を合成した。



Scheme 3. (a) (*S*)-*tert*-butylpyrrolidin-3-ylcarbamate, Et_3N, MeOH, 65 °C, 49–65%; (b) R^2-I, K_2CO_3, DMF, 80 °C; (c) 5-chloro-2-(methylsulfonamido)benzoic acid, HATU, Et_3N, DMF, r.t. 2 steps 15–24%; (d) 4 M HCl in dioxane, 1,4-dioxane, r.t. 100%

第 3 節　非環状鎖を有する化合物（**3**–**8**）の抗 RSV 活性

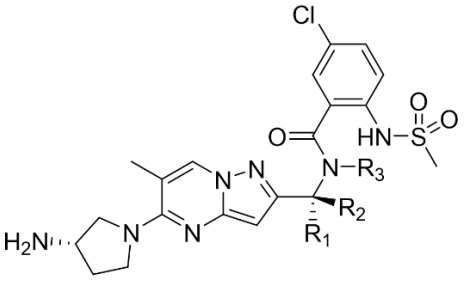
合成した化合物の抗 RSV 活性は、細胞変性効果（cytopathic effect: CPE）抑制法により評価した。CPE 抑制法は、RSV 感染によって誘導される CPE に対する薬剤の影響を評価する方法である。培養した HEp-2 細胞に各試験化合物を添加した後、RSV A2 に感染させ、誘導される CPE に対する化合物の阻害活性を XTT 試薬を用いて評価した。また、化合物が CPE を 50%阻害するのに必要な濃度（EC_{50}）を最小二乗法により算出し、抗 RSV 活性とした。

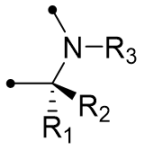
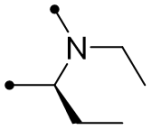
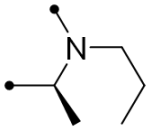
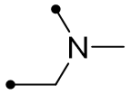
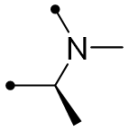
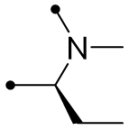
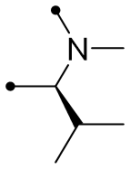
非環状鎖を有する化合物（**3**–**8**）の抗 RSV 活性を **Table 1** に示す。化合物（**3**、**4**、**6**、**7**）は、EC_{50} 値が 4 nM 以下であり、良好な抗 RSV 活性を示した。これらの化合物のピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角分布は、化合物 **1** と類似している（**Figure 8**）。一方、抗 RSV 活性が著しく低下した化合物（**5**、**8**）の 2 面角分布は、化合物 **1** のものとは異なっていた（**Figure 8**）。これらの結果から、ピペリジン環を持たない化合物（**3**–**8**）の抗 RSV 活性とその 2 面角分布がよく相関することが示唆された。また、これらの化合物の中で、1-メチルアミノプロピル部位を有する化合物 **7** はもっとも強力な抗 RSV 活性を示し、EC_{50} 値は化合物 **1** と同程度のサブナノモルレベルであった [46]。

以上の結果から、化合物 **1** のピペリジン環部位はピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角を制御し、RSV F タンパク質と効果的な相互作用を可能にする重要な役割を果たしていることが示唆された。また同時に、ピペリジン環を持たない非環状鎖を有する化合物においても 2 面角分布を適切に制御することで、強力な抗 RSV 活性が得られることを見出した。

さらなる抗 RSV 活性の改善を目指し、得られた 1-メチルアミノプロピル誘導体 **7** をリード化合物として最適化研究を進めることにした。

Table 1. 非環状鎖を有する化合物（**3–8**）の抗 RSV 活性



Compound		Anti-RSV activity EC_{50} (nM)[a]
3		1.8
4		3.8
5		110
6		2.5
7		0.58
8		420

[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

第 4 節　1-メチルアミノプロピル誘導体の合成

リード化合物 **7** の SAR を探索すべく、**Figure 9** に示した Ar 部位およびピラゾロ[1,5-a]ピリミジン環 7 位を変換した誘導体を合成した。Ar 部位を変換した化合物は中間体 **19a** または **19b** と所望の Ar 部位を有するカルボン酸との縮合反応により合成した（**Schemes 5**、**6**）。中間体（**19a**、**19b**）は **Scheme 4** に示す方法で合成した。すなわち、中間体 **13e** に対する(*S*)-*tert*-ブチルピロリジン-3-イルカルバメートまたは(*S*)-ベンジルピロリジン-3-イルカルバメートの求核置換反応によりアミノピロリジンユニットを導入した後、1 級アミンをトリフルオロアセチル基で保護することにより、化合物（**18a**、**18b**）を得た。化合物（**18a**、**18b**）に塩基性条件下、ヨウ化メチルを作用させることによりアミド部位をモノメチル化した後、トリフルオロアセチル基を除去することにより、中間体（**19a**、**19b**）を得た。

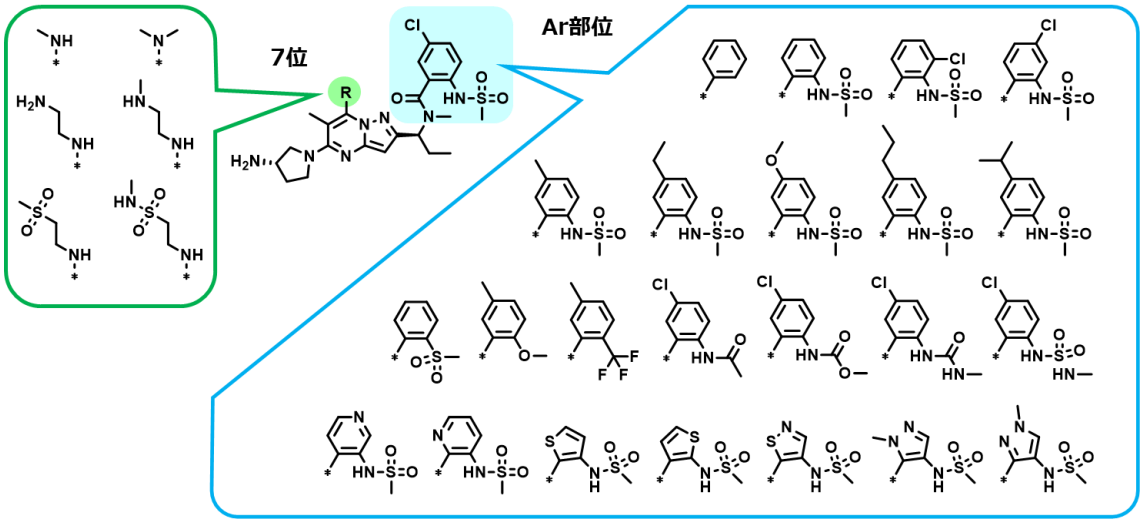
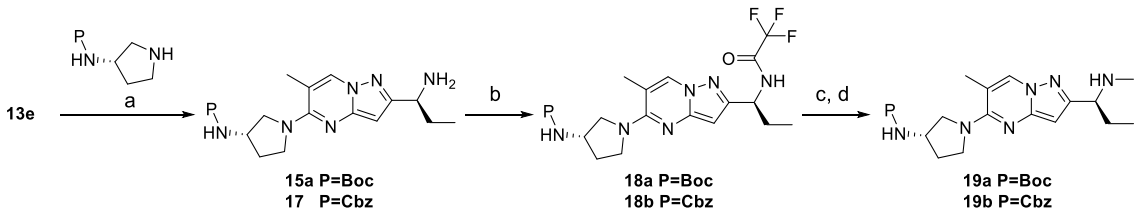
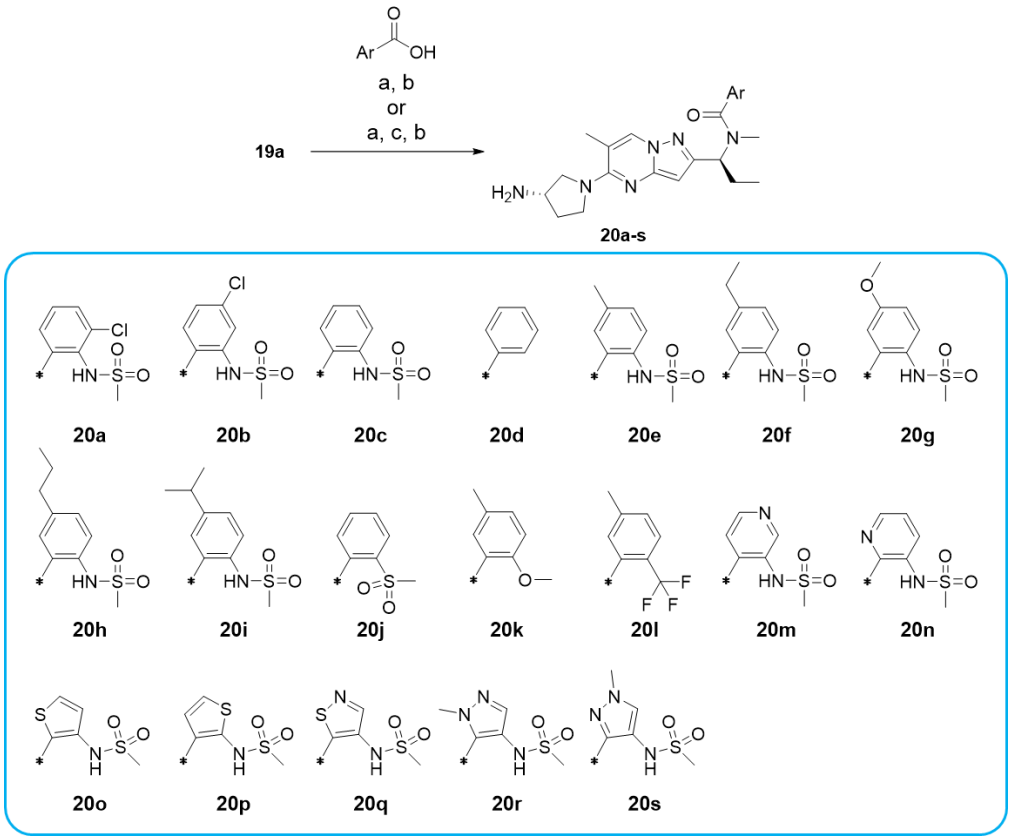


Figure 9. 本節で合成した 1-メチルアミノプロピル誘導体



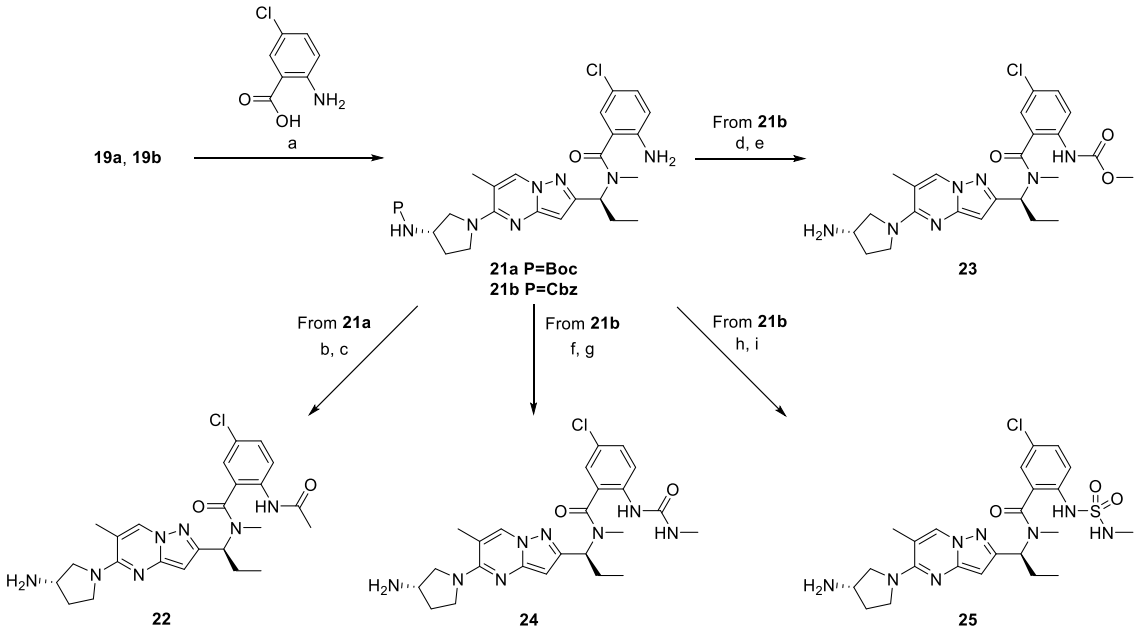
Scheme 4. (a) *tert*-butyl-(*S*)-pyrrolidin-3-ylcarbamate or benzyl (*S*)-pyrrolidin-3-ylcarbamate, Et_3N, MeOH, 70 °C, 65–76%; (b) ethyl trifluoroacetate, Et_3N, MeOH, r.t.; (c) MeI, Cs_2CO_3, DMF, 65 °C; (d) 1M NaOH aq., THF, MeOH, r.t., 3 steps 43–98%.

Ar 部位を変換した化合物 **20a–s** の合成を **Scheme 5** に示す。中間体 **19a** と対応する芳香族カルボン酸パーツとを縮合した後、Boc 基を脱保護することにより、化合物 **20a–s** を合成した。一部の化合物については Boc 基を除去する前に、アミノ基をモノメシル化することにより合成した。



Scheme 5. (a) HATU, Et_3N, DMF, r.t., 35–97%; (b) 4 M HCl in dioxane, 1,4-dioxane, r.t., 69–100%; (c) MsCl, Et_3N, $CHCl_3$, r.t. then NaOEt (2.94 M in EtOH), EtOH, r.t., 43–87%.

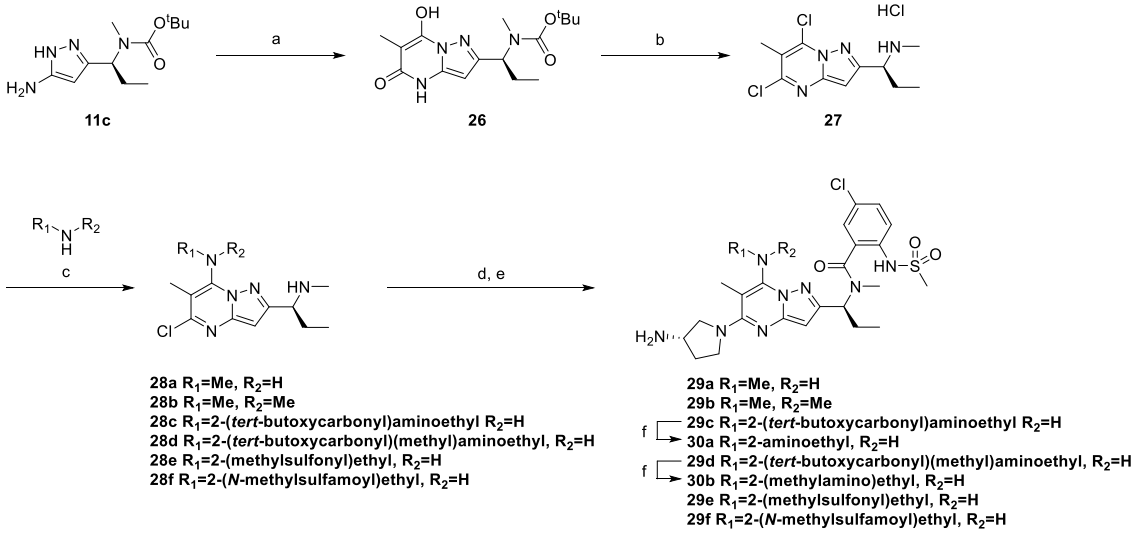
続いて、メチルスルホンアミド部位を変換した化合物 **22**–**25** を **Scheme 6** に示す方法で合成した。中間体（**19a**、**19b**）と 2-アミノ-5-クロロ安息香酸を縮合することにより、アニリン中間体（**21a**、**21b**）に導いた。アニリン中間体 **21a** に塩基性条件下、無水酢酸を作用させることによりアセチル化した後、Boc 基を除去することにより **22** を得た。続いて、アニリン中間体 **21b** に塩基性条件下、クロロギ酸メチルを作用させた後、水素添加反応に付すことにより Cbz 基を脱保護することでカーバメート **23** を得た。同様の手法により、ウレア **24** およびスルホニルウレア **25** を合成した。



Scheme 6. (a) HATU, Et_3N, DMF, r.t., 91–96% (b) Ac_2O, pyridine, $CHCl_3$, r.t. 60%; (c) TFA, $CHCl_3$, r.t., 100%; (d) methyl chloroformate, pyridine, r.t., 40%; (e) H_2, 10% Pd/C, MeOH, r.t., 91%; (f) 4-nitrophenyl chloroformate, pyridine, $CHCl_3$, r.t. then $MeNH_2$, $CHCl_3$, r.t., 81%; (g) H_2, 10% Pd/C, MeOH, r.t., 85%; (h) chlorosulfonyl isocyanate, 2-chloroethanol, Et_3N, $CHCl_3$, r.t. then $MeNH_2$, Et_3N, $CHCl_3$, r.t., 30%; (i) H_2, 10% Pd/C, MeOH, r.t., 92%.

次に、ピラゾロ[1,5-a]ピリミジン環 7 位に置換基を導入した化合物（**29a**、**29b**、**29e**、**29f**、**30a**、**30b**）を **Scheme 7** に示す方法で合成した。すなわち、ナトリウムエトキシド存在下、アミノピラゾール **11c** と 2-メチルマロン酸ジエチルを反

応することにより、ジヒドロピラゾロピリミジン **26** を得た。化合物 **26** にオキシ塩化リンを作用させることにより塩素化し、5 位と 7 位に Cl 基をもつピラゾロ[1,5-a]ピリミジン中間体 **27** を合成した。中間体 **27** に対し、アセトニトリル-水溶媒中、炭酸水素ナトリウム存在下、対応するアミン試薬を作用させることにより、7 位にのみアミンが付加した化合物 **28a–f** を得た。化合物 **28a–f** を 5-クロロ-2-（メチルスルホンアミド）安息香酸と縮合した後、塩基性条件下、マイクロウェーブ 150 °C にて(*S*)-3-アミノピロリジンと作用させることにより、目的化合物（**29a**、**29b**、**29e**、**29f**）および前駆体（**29c**、**29d**）を合成した。化合物（**29c**、**29d**）は Boc 基を除去することにより目的化合物（**30a**、**30b**）に導いた。



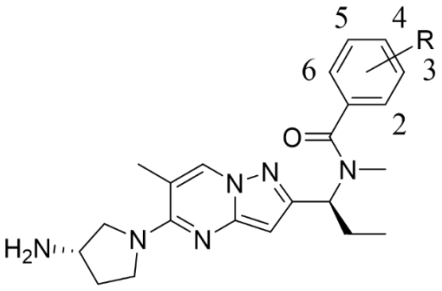
Scheme 7. (a) diethyl 2-methylmalonate, NaOEt (2.94 M EtOH), EtOH, 90 °C, 89%; (b) $POCl_3$, 110 °C, 42%; (c) corresponding amine reagent, $NaHCO_3$, CH_3CN, H_2O, r.t., 27–100%; (d) 5-chloro-2-(methylsulfonamido)benzoic acid, HATU, Et_3N, DMF, r.t., 43–90%; (e) (*S*)-3-aminopyrrolidine, Et_3N, DMF, microwave 150 °C, 29–56%; (f) TFA, $CHCl_3$, r.t., 82–91%.

第 5 節　1-メチルアミノプロピル誘導体の抗 RSV 活性

前節で合成した化合物の抗 RSV 活性は第 3 節と同様に CPE 抑制法により評価し、化合物が CPE を 50%阻害するのに必要な濃度（EC_{50}）を抗 RSV 活性として算出した。

初めに、ピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角に影響を与える可能性のあるベンゼン環上の置換基に注目した。ベンゼン環上の置換基変換についての SAR を **Table 2** に示す。リード化合物 **7** のベンゼン環の 5 位にある塩素原子の必要性の有無と最適な位置を調べるために、異なる位置に塩素原子をもつ化合物（**20a**、**20b**）と、塩素原子をもたない化合物 **20c** を合成した。化合物（**20a**、**20b**）の抗 RSV 活性は、化合物 **7** の抗 RSV 活性に比べて 7 倍以上弱いが、化合物 **20c** は化合物 **7** と同等の活性を示した。また、ベンゼン環 2 位のメチルスルホンアミド基を除去した無置換ベンゼン化合物 **20d** の活性は著しく減弱した。続いて、ベンゼン環の 5 位の置換基の立体的な効果を調べるために、5 位に様々な大きさの置換基をもつ化合物 **20e–i** を合成した。化合物 **7** の塩素原子は、メチル基（**20e**）、エチル基（**20f**）、およびメトキシ基（**20g**）のような比較的小さな置換基に変換可能であったが、プロピル基（**20h**）およびイソプロピル基（**20i**）ような比較的長鎖の置換基や嵩高い置換基への変換は、抗 RSV 活性を 2 桁オーダー減弱させた。ベンゼン環の 5 位に置換基を導入した化合物の中では、化合物 **20f** がもっとも強力な抗 RSV 活性を示した。化合物 **20f** の EC_{50} 値は 0.15 nM であり、化合物 **1** を上回る抗 RSV 活性を示した。化合物 **20f** の 2 面角分布を計算したところ、化合物 **1** の 2 面角分布と類似していることがわかった（**Figure 10**）。

Table 2. ベンゼン環上の置換基変換についての SAR



Compound	R	Anti-RSV activity EC_{50} (nM)[a]
7	2-$NHSO_2Me$, 5-Cl	0.58
20a	2-$NHSO_2Me$, 3-Cl	4.4
20b	2-$NHSO_2Me$, 4-Cl	13
20c	2-$NHSO_2Me$	0.59
20d	2-H	42
20e	2-$NHSO_2Me$, 5-Me	0.33
20f	2-$NHSO_2Me$, 5-Et	0.15
20g	2-$NHSO_2Me$, 5-OMe	0.43
20h	2-$NHSO_2Me$, 5-Pr	39
20i	2-$NHSO_2Me$, 5-*i*Pr	51
20j	2-SO_2Me	1500
20k	2-OMe, 5-Me	3.8
20l	2-CF_3, 5-Me	9.2
22	2-NHCOMe, 5-Cl	0.84
23	2-$NHCO_2Me$, 5-Cl	0.57
24	2-NHCONHMe, 5-Cl	1.0
25	2-$NHSO_2NHMe$, 5-Cl	1.8

[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

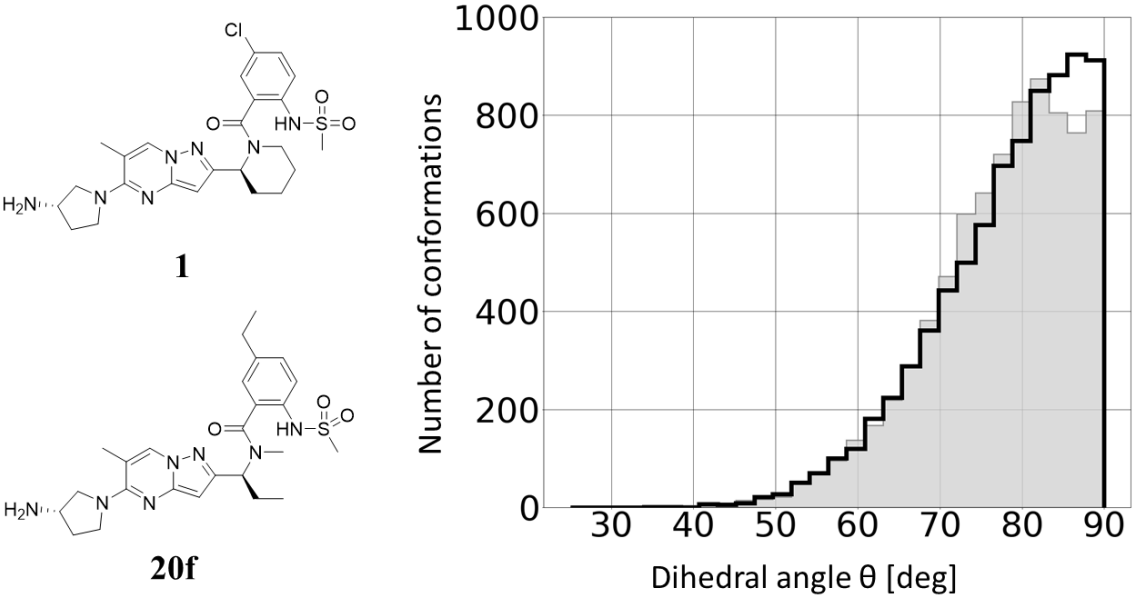


Figure. 10. 化合物 **20f** の 2 面角分布（灰色の塗りつぶしが化合物 **1** の 2 面角分布、黒色の実線が化合物 **20f** の 2 面角分布を示す）

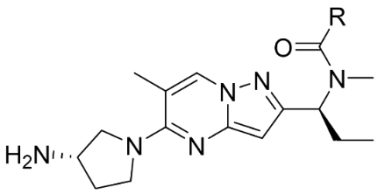
次に、ベンゼン環の 2 位のメチルスルホンアミド基について置換の可否を検証するために、2 位の置換基を変換した 7 つの化合物（**20j–l**、**22**–**25**）を合成した。その結果、メチルスルホン基を導入した化合物 **20j** は、同じ位置にメチルスルホンアミド基を有する化合物 **20c** と比較して、抗 RSV 活性が 4 桁オーダー劇的に低下した。また、メチルスルホンアミド基（**20e**）の代わりにメトキシ基（**20k**）やトリフルオロメチル（**20l**）基で置換した場合にも、抗 RSV 活性はそれぞれ 12 倍および 28 倍減弱した。一方、メチルスルホンアミド基（**7**）の代わりにアセトアミド基（**22**）、メトキシカルボキサミド基（**23**）、メチルウレイド基（**24**）、およびメチルスルホニルウレイド基（**25**）で置換した場合には、EC_{50} 値は 0.57～1.8 nM と活性を維持する傾向が認められた。すなわち、良好な抗 RSV 活性を有する化合物のベンゼン環 2 位の置換基としては、プロトンドナーとプロトンアクセプターの両方の機能を有する置換基が好ましいことが示唆された。

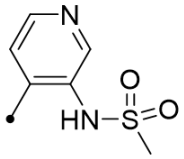
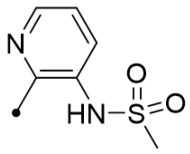
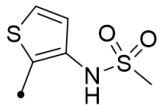
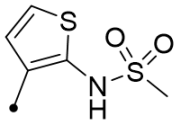
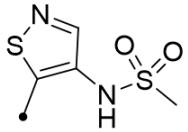
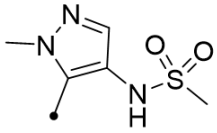
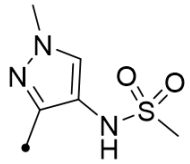
本研究で得られたベンゼン環上の置換基の SAR は、化合物 **1** およびその誘導体に関する先行研究の結果とよく一致していた [46]。しかしながら、ベンゼン環から複素環への変換についてはこれまでほとんど注目されていなかったこと

から、様々なヘテロ 5 員環およびヘテロ 6 員環を有する化合物（**20m–s**）をデザイン・合成し、その抗 RSV 活性への影響を検討した（**Table 3**）。今回合成したすべてのヘテロ環化合物には、良好な抗 RSV 活性発現に重要と考えられるメチルスルホンアミド基を化合物 **20c** のベンゼン環 2 位に対応する位置にそれぞれ導入した。

ピリジン環を有する化合物（**20m**、**20n**）のようなヘテロ 6 員環やチオフェン環を有する化合物（**20o**、**20p**）、チアゾール環を有する化合物 **20q** およびピラゾール環を有する化合物（**20r**、**20s**）のようなヘテロ 5 員環を有する化合物を合成したが、いずれの化合物も抗 RSV 活性が著しく低下した。また、データは示していないがピリジン環化合物（**20m**、**20n**）の窒素原子の位置異性体や、ピラジン環やピリミジン環を有するヘテロ環誘導体も、抗 RSV 活性が著しく低下した。ヘテロ環誘導体の中でもっとも活性が良好であったチオフェン環を有する化合物 **20o** においても EC_{50} 値が 53 nM とベンゼン環化合物 **20c** と比べて 100 倍程度活性が減弱した。以上の結果は、ベンゼン環から複素環への変換が許容されないことを示唆している。

Table 3. ベンゼン環から複素環への変換についての SAR



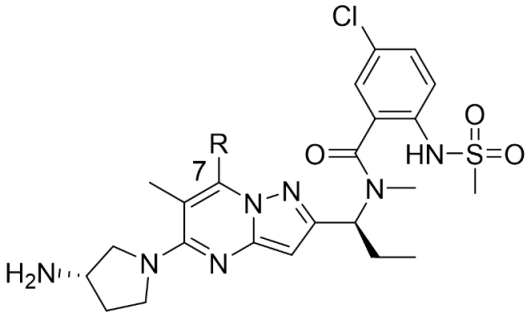
Compound	R	Anti-RSV activity EC_{50} (nM)[a]
20m		900
20n		550
20o		53
20p		110
20q		570
20r		880
20s		860

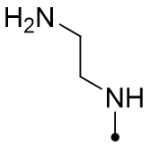
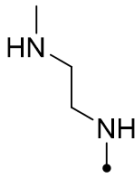
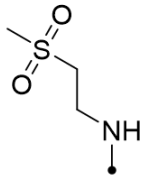
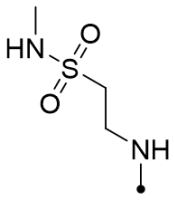
[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

これまで Gilead 社と Janssen 社 はピリミジン環をもつ誘導体に対し、ピラゾロ[1,5-a]ピリミジン環の 7 位に置換基を導入しても、抗 RSV 活性にほとんど影響を与えないことを特許で報告している[47–49]。そこで、本研究で見出したピリミジン環を開環した 1-メチルアミノプロピル誘導体として、ピラゾロ[1,5-a]ピリミジン環 7 位に種々の置換基を導入した誘導体を合成することで、置換基の抗 RSV 活性に及ぼす影響を調査した（**Table 4**）。

その結果、ピラゾロ[1,5-a]ピリミジン環の 7 位にメチルアミノ基を導入した化合物 **29a** およびジメチルアミノ基を導入した化合物 **29b** には、化合物 **7** と比較してそれぞれ 7.8 倍および 21 倍の抗 RSV 活性の減弱が認められた。7 位においては、第 3 級アミン（**29b**）に比較して第 2 級アミン（**29a**）の許容度が高いことが示唆される。一方、エチレンジアミンやメチルエチレンジアミンのような直鎖状のパーツを導入した化合物（**30a**、**30b**）では、化合物 **29a** と比較して 4.2〜4.7 倍抗 RSV 活性が低下した。興味深いことに、ピラゾロ[1,5-a]ピリミジン環の 7 位にメチルスルホンやスルホンアミドを有する化合物（**29e**、**29f**）は強力な抗 RSV 活性を示し、EC_{50} 値は 1 nM 未満であった。これらの結果から、メチルスルホン（**29e**）およびスルホンアミド（**29f**）は、RSV F タンパク質と新たに相互作用を獲得している可能性があることが示唆された。また、1-メチルアミノプロピル誘導体においても、ピラゾロ[1,5-a]ピリミジン環 7 位には比較的大きな極性基が許容されることから、本章でもっとも強力な抗 RSV 活性を有する化合物 **20f** の生物学的および物理化学的特性は、7 位置換体により調整可能であると考えられる。

Table 4. ピラゾロ[1,5-a]ピリミジン環 7 位置換基導入についての SAR



Compound	R	Anti-RSV activity EC_{50} (nM)[a]
29a		4.5
29b		12
30a		19
30b		21
29e		0.75
29f		0.86

[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

続いて、本章で得られた新規骨格を有する 1-メチルアミノプロピル誘導体の耐性変異株 D486N に対する抗 RSV 活性を評価した。化合物の抗 RSV 活性評価には第 3 節と同様に CPE 抑制法を用いた。RSV F タンパク質に D486N 変異をもつ耐性変異株は、化合物 **7** 存在下、野生株 A2 を継代培養することにより取得した。また、この耐性変異株 D486N は遺伝子型解析により、F 遺伝子に他の変異がないことを確認している。

抗 RSV 活性の評価結果を **Table 5** に示す。先行化合物 **1** は論文情報より耐性変異株 D486N に対する活性が、野生株 A2 に対する活性と比較して 1 万倍以上減弱することが分かっている [42]。化合物 **1** と同様に、1-メチルアミノプロピル誘導体（**20k**、**29e**）も耐性変異株 D486N に対して活性を示さなかった。本章における研究で野生株に有効な新規骨格を有する RSV F タンパク質阻害物質は得られたものの、耐性変異株 D486N に対する有効性の獲得には至らなかった。

一方、当時（2015 年）に Battles（Janssen 社）らによって F タンパク質と複数の阻害物質（ベンズイミダゾールシリーズの化合物）との X 線共結晶構造が明らかにされた [54]。この構造解析により阻害物質の結合様式や周囲のアミノ酸残基が視覚化され、耐性変異に関する考察もできるようになった。著者はこれらの X 線共結晶構造情報を利用して、耐性変異株 D486N に有効な化合物を創出するべくさらに研究を進めることとした。

Table 5. ピラゾロ[1,5-a]ピリミジン誘導体の D486N に対する抗 RSV 活性

Compound	Anti-RSV activity	
	A2 EC_{50} (nM)[a]	D486N EC_{50} (nM)[a]
1	0.10 ± 0.07[b]	1193 ± 562[b]
20k	3.8	>1000
29e	0.75	>1000

[a] RSV A2 および D486N に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。EC_{50} 値は 3 つの独立した実験（n = 3）の幾何学的平均を示す。[b] Gilead 社が報告している EC_{50} 値 [42]。

第 6 節　まとめ

本章では先行化合物 **1** の構造情報をもとに、ピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角分布に着目し、様々な 2 面角分布を示す非環状鎖誘導体をデザイン・合成し、2 面角分布と抗 RSV 活性の関係について検証した。その結果、ピペリジン環をもつ化合物 **1** と類似の 2 面角分布を示す非環状鎖誘導体（**3**、**4**、**6**、**7**）は比較的良好な抗 RSV 活性を示す一方で、2 面角分布が化合物 **1** と異なる化合物（**5**、**8**）では活性が大幅に減弱することを見出した。この結果は、ピペリジン環が、ピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角を適切な分布に固定することで、活性発現に重要な役割を担っていることを示唆している。また同時に、ピペリジン環を開環した非環状鎖誘導体においても置換基を選択することで、適切な 2 面角分布をもつ化合物をデザインできることが明らかとなった。以上の検討により、新規骨格を有する RSV F タンパク質阻害物質 1-メチルアミノプロピル誘導体 **7** を見出したことから、化合物 **7** をリード化合物とし、Ar 部位の変換とピラゾロ[1,5-a]ピリミジン環 7 位への置換基導入を検討した。その結果、強力な抗 RSV 活性を有する化合物 **20f** を創出するとともに、ピラゾロ[1,5-a]ピリミジン環 7 位の置換基許容性を確認した。しかしながら、良好な抗 RSV 活性を示す 1-メチルアミノプロピル誘導体 **20k**、**29e** について耐性変異株 D486N に対する活性を評価した結果、先行化合物 **1** と同様に **20k**、**29e** においても耐性変異株 D486N に対する活性は認められなかった。耐性変異株 D486N に有効な化合物を得るためには、X 線共結晶構造情報を用いて阻害物質の結合様式を考察し、新たな戦略のもとに構造展開する必要があると考えた。

第 2 章　耐性変異株 D486N に有効な新規マクロサイクル化合物の創出

第 1 節　化合物 **1** のドッキングシミュレーション

分子ドッキングは、タンパク質とリガンドとの相互作用解析や医薬分子設計などに広く利用されているシミュレーション手法のひとつである。第 1 章で課題点として挙げた、耐性変異株 D486N に対する活性を有する化合物を見出すにあたり、まず、化合物 **1** の野生株 A2 および耐性変異株 D486N に対する結合様式をドッキングシミュレーションにより比較し、それぞれの株に対する結合様式と阻害活性との関係について検証した。Battles（Janssen 社）らによって、タンパク質データバンク（PDB）に報告されている RSV F タンパク質の野生株 A2 と阻害物質との X 線共結晶構造情報 5EA3[54]を用いて、ドッキングシミュレーションを実施した。分子ドッキングシミュレーションには、Discovery Studio 2017 R2[61]の CDOCKER アルゴリズムを使用した。また、耐性変異株 D486N については、野生株 A2 から耐性変異株 D486N への座標補正を手動で行った後、MOE[62]の Amber10 力場を用いてアミノ酸残基の位置を最適化したものを用いた。化合物 **1** と野生株 A2 とのドッキングシミュレーション結果を **Figure 11** に示す。

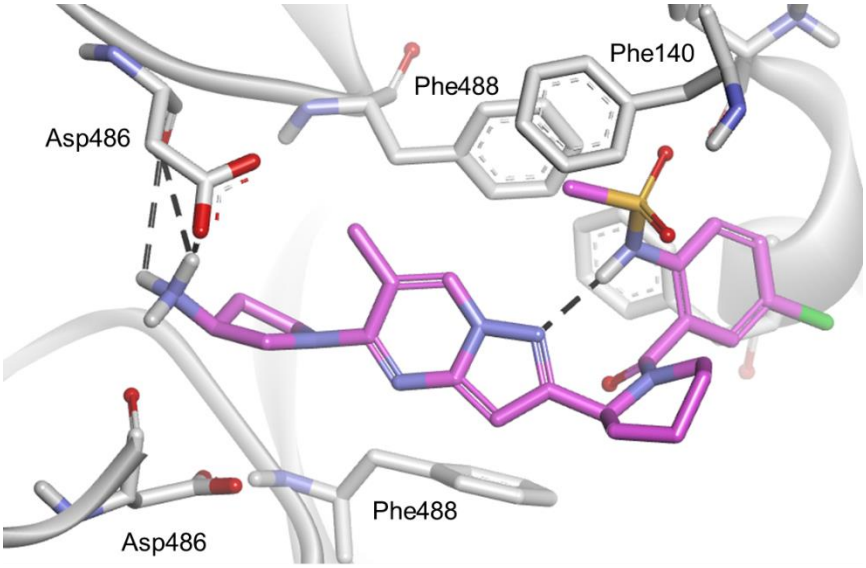


Figure 11. 化合物 **1**（マゼンタ）と野生株 A2（灰色）とのドッキングモデル
黒色破線は水素結合を示す

RSV F タンパク質はホモ 3 量体から成り、宿主細胞膜と融合する際に構造変化する。化合物 **1** は、構造変化前の F タンパク質ホモ 3 量体が形成する中央の空間に結合している。相互作用の特徴としては Asp^{486} のカルボン酸とアミノピロリジンのアミノ基との間の水素結合が挙げられる。また、化合物 **1** は Ar 部位のメチルスルホンアミド基の水素原子とピラゾロ[1,5-a]ピリミジン環 1 位窒素原子との間で分子内水素結合を形成していることが示唆される。この分子内水素結合により分子全体のコンフォメーションが固定化されることで、Phe^{488} や Phe^{140} に囲まれた空間に分子が適切に収っていると考えられる。次に、化合物 **1** と耐性変異株 D486N とのドッキングシミュレーション結果を **Figure 12** に示す。

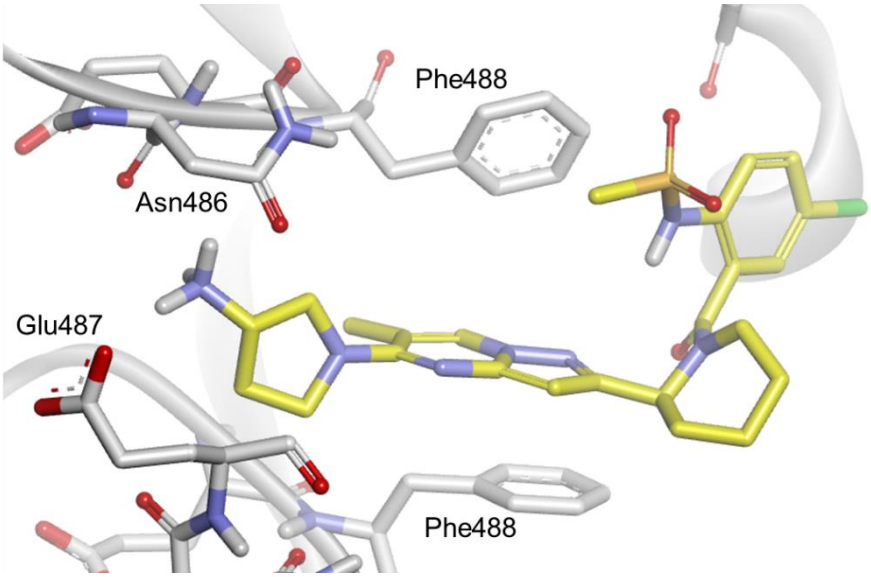


Figure 12. 化合物 **1**（黄色）と耐性変異株 D486N（灰色）とのドッキングモデル

耐性変異株 D486N の場合、486 位のアミノ酸が Asp から Asn に変異しているため、アミノピロリジン部位と Asn^{486} と間で水素結合の形成は認められない。その代わり、さらに奥側にある Glu^{487} のカルボン酸と弱いながらも静電的相互作用をしている可能性が示唆される。また、化合物 **1** の Ar 部位メチルスルホンアミド基の水素原子とピラゾロ[1,5-a]ピリミジン環 1 位窒素原子との間の分子内水素結合も失われている。

Figure 13 に D486N に結合した化合物 **1** と A2 に結合した化合物 **1** を重ね合わせたドッキングシミュレーションの結果を示す。D486N に対する化合物 **1** の結

合ポーズは、A2 に対する結合ボーズに比べてピラゾロ[1,5-a]ピリミジン環が 90° 程度回転している。この回転により Ar 部位メチルスルホンアミド基の水素原子とピラゾロ[1,5-a]ピリミジン環 1 位窒素原子との距離が離れることで、分子内水素結合が形成されない（**Figure 13A**）。

486 位のアミノ酸が Asp から Asn に変異することで、3 つの Asp または Asn で形成されるアミノピロリジン部位周辺の空間が A2 株に比べて D486N 株では狭くなっている。ピラゾロ[1,5-a]ピリミジン環 6 位のメチル基の立体的影響により、D486N 株では 90° 程度回転したコンフォメーションで結合するものと考察した。ドッキングシミュレーションの結果をアミノピロリジン部位側から見ると（**Figure 13B**、灰色が D486N タンパク質、オレンジ色が A2 タンパク質を示す）、Glu^{487} の主鎖カルボニル基と Asp^{486} のカルボキシル基との距離が 4.9Å、Asn^{486} のカルバモイル基との距離が 3.6Å とやや空間が狭くなっていることがわかる。

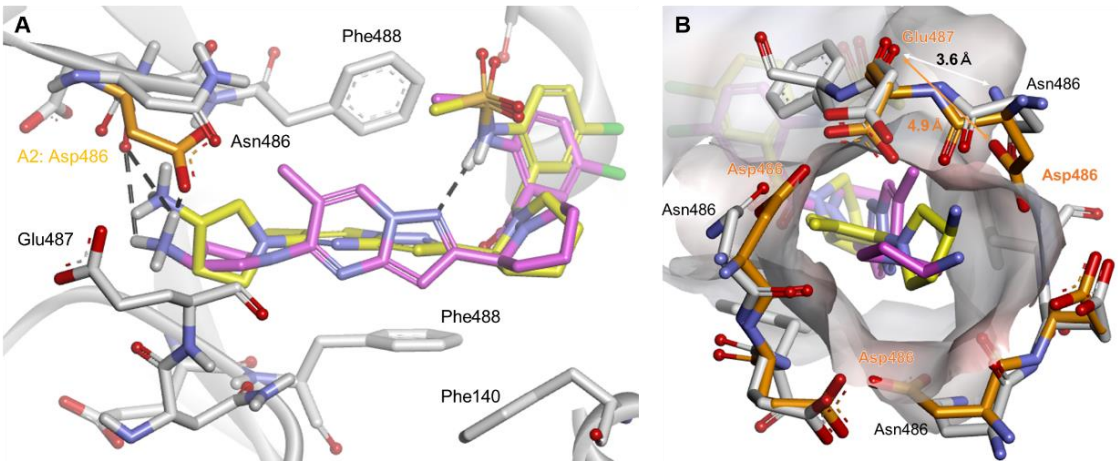


Figure 13. (A) D486N（灰色）に結合した **1**（黄色）と A2（オレンジ）に結合した **1**（マゼンタ）を重ね合わせたドッキングモデル。黒色破線は水素結合を示す。(B)アミノピロリジン部位側から見たドッキングモデル。D486N タンパク質の表面を透明な灰色で示す。

以上、ドッキングシミュレーションによる化合物 **1** の野生株 A2 および耐性変異株 D486N に対する結合様式の比較から、化合物 **1** の耐性変異株 D486N に対する活性が減弱した要因は二つ考えられる。一つは、野生株 A2 と耐性変異株

D486N の化合物 **1** 結合部位周辺の構造変化により Asp^{486} とアミノピロリジンのアミノ基との間の水素結合が失われたこと、もう一つは Ar 部位メチルスルホンアミド基の水素原子とピラゾロ[1,5-a]ピリミジン環 1 位窒素原子との間の分子内水素結合が失われたことである。

第 2 節　ドラッグデザイン（マクロサイクル化）

前節のドッキングシミュレーションによる化合物 **1** と野生株 A2 および耐性変異株 D486N に対する結合様式の相違から、抗 RSV 活性発現に重要な要因の一つとして、分子内水素結合による活性コンフォメーションの固定化が挙げられる。そこで、化合物が耐性変異株 D486N に結合する際に、野生株 A2 に結合する際と同様に活性コンフォメーションを保持したまま結合することができれば、耐性変異株 D486N に対する有効性を示せるものとの仮説を立てた。

A2 タンパク質に結合した際の化合物 **1** の活性コンフォメーションにおいて（**Figure 11**）、ベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環の 7 位は互いに近接していることがわかる。また、第 1 章の誘導体展開からピラゾロ[1,5-a]ピリミジン環の 7 位には比較的に大きな置換基が許容されることを確認している。そこで、**Figure 14** に示すように分子をマクロサイクル化することにより、活性コンフォメーションを固定化する戦略を立案した。すなわち、化合物 **20k** のベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環 7 位を連結したマクロサイクル化合物をデザイン・合成し、上述した仮説を検証するとともに耐性変異株 D486N に有効な化合物の創出を目指すこととした。

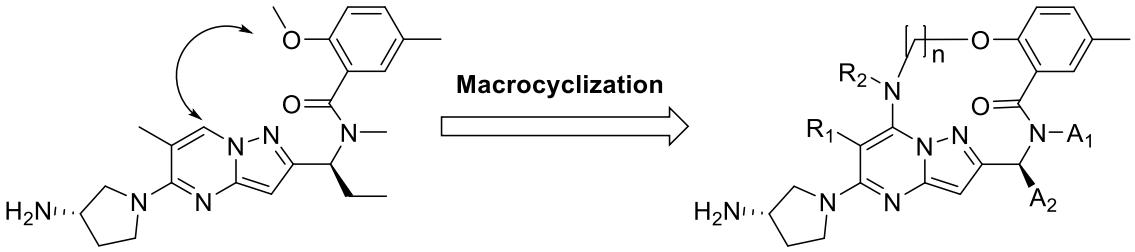


Figure 14. 化合物 **20k** のマクロサイクル化を介したコンフォメーション固定化戦略

第 3 節　マクロサイクル化合物（**39a–h**）の合成

化合物 **20k** のベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環 7 位を連結したマクロサイクル化合物の SAR を検討すべく、**Figure 15** に示す化合物をデザイン・合成した。まず、リンカー部分を含む Ar パーツ（**33a–e**）を **Scheme 8** に示す方法で合成した。すなわち、Boc 基で保護したアミノアルコール（**31a–e**）の水酸基をメシル化した後、塩基性条件下、2-ヒドロキシ-5-メチル安息香酸メチルを作用させることにより、化合物（**32a–e**）を得た。最後に、Boc 基を除去することにより、Ar パーツ（**33a–e**）を合成した。

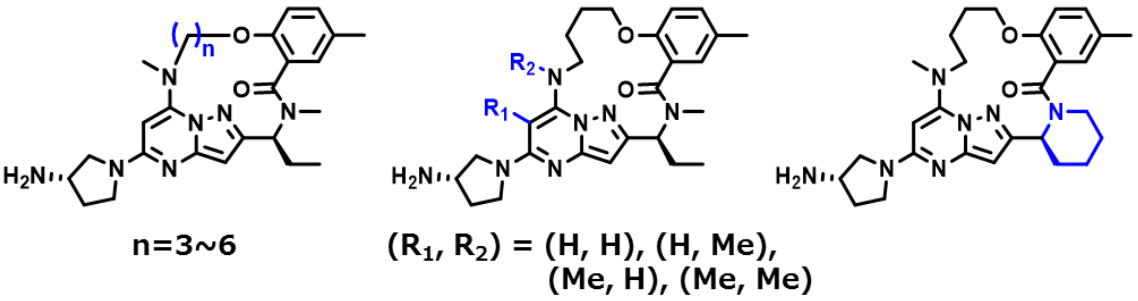
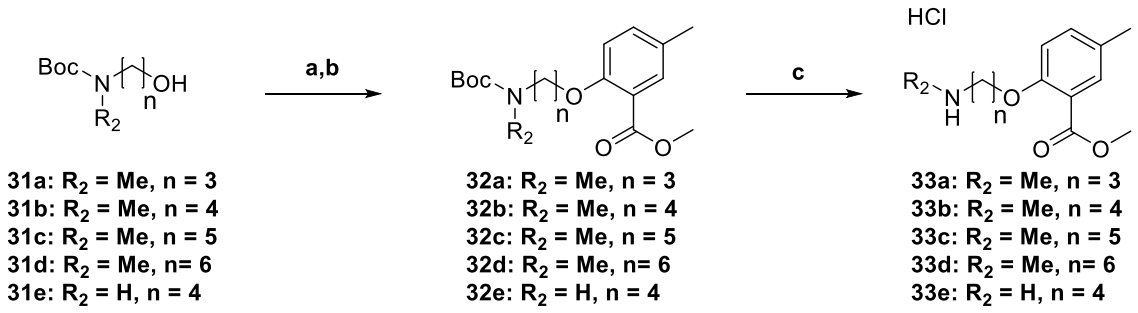


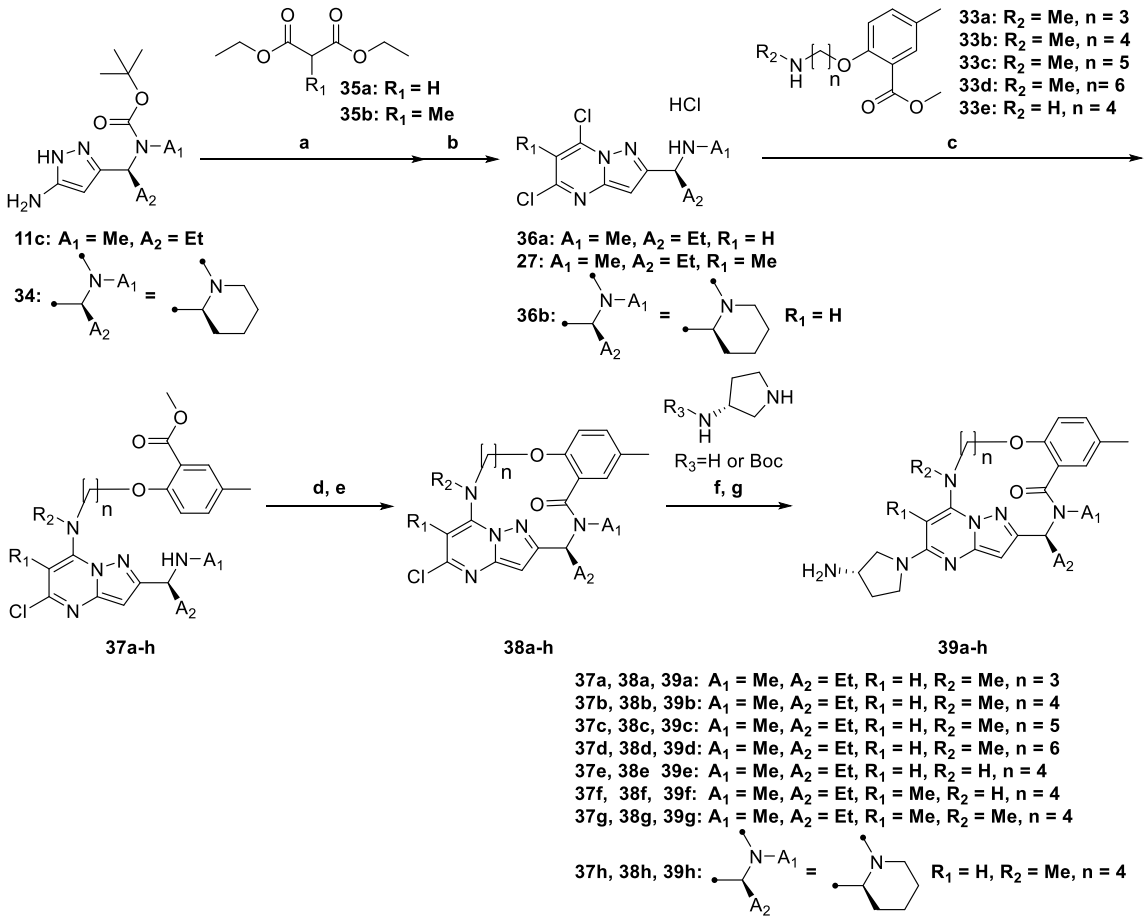
Figure 15. マクロサイクル化合物のデザイン



Scheme 8. (a) MsCl, Et_3N, $CHCl_3$, r.t.; (b) methyl 2-hydroxy-5-methylbenzoate, K_2CO_3, DMF, 90 °C, 2 steps 71–86%; (c) 4 M HCl in dioxane, 1,4-dioxane, r.t., 90–100%.

次に、マクロサイクル化合物（**39a–h**）を **Scheme 9** に示す方法で合成した。すなわち、アミノピラゾール（**11c**、**34**）をナトリウムエトキシド存在下、マロン酸エステル試薬（**35a–b**）と反応させることにより、ピラゾロピリミドン環を構

築した後、オキシ塩化リンを作用させ、Boc 基の脱保護および塩素化を同時に行うことにより、ピラゾロ[1,5-a]ピリミジン中間体（**27**、**36a**–**b**）を得た。中間体（**27**、**36a**–**b**）に塩基存在下、対応する Ar パーツ（**33a**–**e**）をそれぞれ作用させることにより、ピラゾロ[1,5-a]ピリミジン環 7 位に Ar パーツを導入した化合物（**37a**–**h**）を得た。続いて、エステル部位を加水分解した後、高希釈条件下、分子内アミド化反応により、マクロサイクル化合物（**38a**–**h**）を合成した。最後に、塩基性条件下、マイクロウェーブ 150 °C にてアミノピロリジンユニットを導入し、必要に応じて Boc 基を除去することにより、所望のマクロサイクル化合物（**39a**–**h**）を得た。



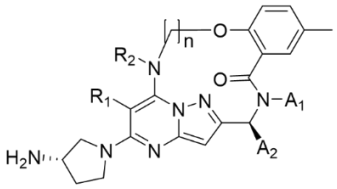
Scheme 9. (a) NaOEt (2.94 M in EtOH), EtOH, 90 °C, 68–100%; (b) $POCl_3$, 90–110 °C, 42–100%; (c) Et_3N, EtOH, 70 °C, 42–100%; (d) 1M NaOH aq., THF, 2-propanol, 65 °C, (e) HATU, Et_3N, DMF [0.02 M], r.t. 2 steps 16–100%; (f) Et_3N, NMP, microwave 150 °C, 27–86%; (g) 4 M HCl in dioxane, 1,4-dioxane, r.t., 98–99%.

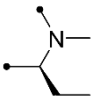
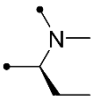
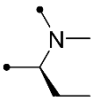
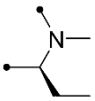
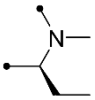
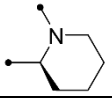
第 4 節　マクロサイクル化合物（**39a–h**）の抗 RSV 活性

前節で合成したマクロサイクル化合物の抗 RSV 活性は、第 1 章と同様に CPE 抑制法により評価し、化合物が CPE を 50%阻害するのに必要な濃度（EC_{50}）を抗 RSV 活性として算出した。RSV F タンパク質に D486N 変異をもつ耐性変異株は、第 1 章で得たピラゾロ[1,5-a]ピリミジン誘導体 **7** 存在下、野生株 A2 を継代培養することにより取得した。また、この耐性変異株 D486N は遺伝子型解析により、F 遺伝子に他の変異がないことを確認している。

マクロサイクル化合物（**39a–h**）の野生株 A2 および耐性変異株 D486N に対する抗 RSV 活性を **Table 6** に示す。まず、化合物 **1** の分子内水素結合により固定化された活性コンフォメーションを模倣したマクロサイクル化合物の最適な環サイズを調べるため、14〜17 員環サイズのマクロサイクル化合物（**39a–d**）を合成した。マクロサイクル化合物の合成経路を簡略化するため、リンカー部分には単純なアルカノールアミンリンカーを選択した。マクロサイクル化合物（**39a–d**）の中で、環サイズが 15 員環の化合物 **39b** がもっとも強力な抗 RSV A2 活性を有しており、非マクロサイクル化合物 **20k** を 5.8 倍上回る活性を示した。さらに、化合物 **39b** は耐性変異株 D486N に対する活性が非マクロサイクル化合物 **1** や **20k** と比べて 2 倍以上改善した。環サイズが 15 員環および 16 員環化合物である化合物（**39b**、**39c**）は、それぞれ比較的良好な抗 RSV A2 活性を示したが、その一方で、14 員環および 17 員環化合物である化合物（**39a**、**39d**）は著しく抗 RSV 活性が低下した。

Table 6. マクロサイクル化合物の抗 RSV 活性



Compound		n	R_1, R_2	A2 EC_{50} (nM)[a]	D486N EC_{50} (nM)[a]
1				0.10 ± 0.07[b]	1193 ± 562[b]
20k				3.8	>1000
39a		3	H, Me	17	>1000
39b		4	H, Me	0.66	440
39c		5	H, Me	6.2	>1000
39d		6	H, Me	54	>1000
39e		4	H, H	61	>1000
39f		4	Me, H	1.4	>1000
39g		4	Me, Me	8.1	>1000
39h		4	H, Me	2.0	8.1

[a] RSV A2 および D486N に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。EC_{50} 値は 3 つの独立した実験（n = 3）の幾何学的平均を示す。[b] Gilead 社が報告している EC_{50} 値 [42]。

良好な抗 RSV 活性を示した 15 員環マクロサイクル化合物 **39b** と A2 タンパク質とのドッキングシミュレーションを実施し、その結合ポーズを確認したところ、化合物 **39b** は化合物 **1** と同様のポーズで結合していることが示唆された（**Figure 16**）。すなわち、化合物 **39b** のアミノピロリジン部位のアミノ基は、A2 タンパク質の Asp^{486} のカルボキシル基と水素結合を形成している。また、化合物 **39b** のベンゼン環とピラゾロ[1,5-a]ピリミジン環は、化合物 **1** と同一平面上に固定化されている。これらの結果から、マクロサイクル化合物 **39b** は A2 タンパク質に結合する際、化合物 **1** の分子内水素結合により拘束された活性コンフォメーションと同様の三次元構造を形成していることが示唆される。

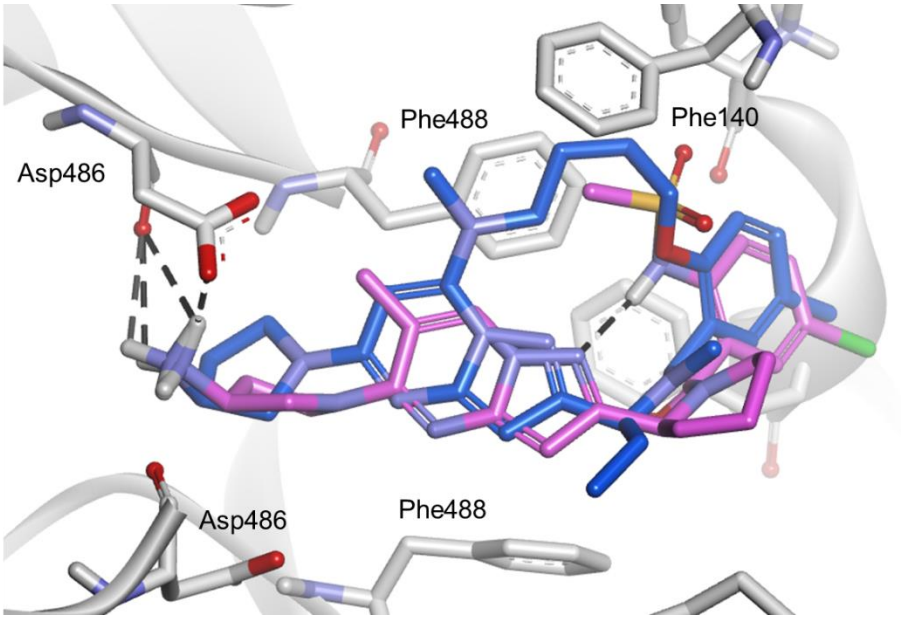


Figure 16. 化合物 **1**（マゼンタ）および化合物 **39b**（青色）の野生株 A2（灰色）に対するドッキングモデル。黒色破線は水素結合を示す。

次に、マクロサイクルのコンフォメーションに影響を与える可能性のあるピラゾロ[1,5-a]ピリミジン環の 6、7 位（R_1、R_2）の置換基変換について、最適な環サイズをもつ 15 員環化合物を用いて検討した。具体的には、化合物 **39b** とは 6、7 位（R_1、R_2）のメチル基有無のパターンが異なる化合物（**39e–g**）を合成し、抗 RSV 活性に対する影響を検討した（**Table 6**）。

その結果、化合物 **39b** の置換基の組み合わせ（R_1＝H、R_2＝Me）がもっとも強力な抗 RSV 活性を示した。一方、逆の組み合わせ（R_1＝Me、R_2＝H）をもつ化合物 **39f** では活性がやや減弱した［EC_{50} 値 : 0.66 nM（**39b**）および 1.4 nM（**39f**）］。また、6、7 位ジメチル化合物 **39g**（R_1＝Me、R_2＝Me）は一桁ナノモルの活性を示し、6、7 位モノメチル化合物（**39b**、**39f**）に比べて多少の活性低下にとどまったが、6、7 位無置換体 **39e**（R_1＝H、R_2＝H）では顕著に活性が低下した［EC_{50} 値：8.1 nM（**39g**）および 61 nM（**39e**）］。

ドッキングシミュレーションより、A2 タンパク質にはマクロサイクル化合物の R_1 および R_2 置換基周辺に疎水性ポケットが存在していることが示唆された。したがって、6、7 位モノメチル化合物（**39b**、**39f**）は効率的にその疎水性ポケットを占有することにより強力な抗 RSV 活性を示す一方で、6、7 位にメチル基をもたない化合物 **39e** は疎水性相互作用を獲得できないために活性が減弱したと考えられる。また、疎水性ポケットを占有するジメチル基をもつ化合物 **39g** で抗 RSV 活性が中程度であった原因としては、二つのメチル基の間の立体的な反発によりマクロサイクル構造が歪むことで、適切なコンフォメーションをとれないためと考察した。

マクロサイクル化合物の環構造をさらに制限することによる抗 RSV 活性に及ぼす影響を確認するため、1-メチルアミノプロピル部位をもつ化合物 **39b** の代わりにピペリジン環を有する化合物 **39h** を合成し、その抗 RSV 活性を評価した（**Table 6**）。その結果、耐性変異株 D486N に対する化合物 **39h** の活性は、非マクロサイクル化合物（**1**、**20k**）と比較して 100 倍以上の劇的な改善が認められた。一方で、野生株 A2 に対する化合物 **39h** の活性は、化合物 **39b** と比較してわずかに減弱した。

耐性変異株 D486N に対して優れた活性を有する化合物 **39h** の結合コンフォメーションを解析するため、ドッキングシミュレーションを実施した（**Figure 17**）。青色が D486N タンパク質に結合した際の **39h** を示し、水色が A2 タンパク質に結合した際の **39h** を示す。

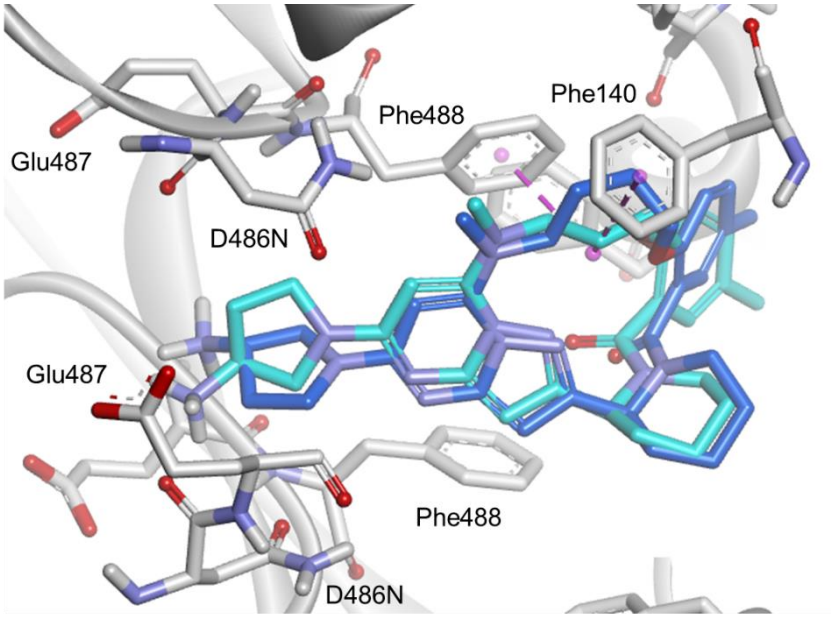


Figure 17. D486N（灰色）に結合した **39h**（青色）と A2 に結合した **39h**（水色）を重ね合わせたドッキングモデル。ピンク色破線は CH-π 相互作用を示す。

マクロサイクル化合物 **39h** は、D486N タンパク質および A2 タンパク質の双方に対して類似の結合ポーズを示しており（**Figure 17**）、本章第 1 節で示した化合物 **1** の結合ポーズとは異なる（**Figure 13A**）。すでに第 1 節で述べたが、D486N タンパク質ではアミノピロリジン部位周辺の空間が A2 株に比べて狭くなっており（**Figure 13B**）、それに伴い化合物 **1** では D486N タンパク質と A2 タンパク質に対する結合ポーズが大きく異なっている。一方、マクロサイクル化合物 **39h** では、D486N タンパク質に結合する場合にも、A2 タンパク質と結合する際の活性コンフォメーションが維持されている。

さらに、化合物 **39h** が耐性変異株 D486N に対する活性が改善している要因として、マクロサイクル化するために導入したリンカー部分が D486N タンパク質と新たな相互作用を獲得していることが考えられる。すなわち、ピラゾロ[1,5-a]ピリミジン環とベンゼン環を架橋する炭化水素鎖が、Phe^{140} や Phe^{488} 残基に挟まれる形で疎水性相互作用することで（**Figure 17**、青色）、D486N に対する活性改善に寄与しているものと考えられる。一方、化合物 **39h** が A2 タンパク質に結合する際の炭化水素鎖の結合ポーズは D486N タンパク質と結合する際とわずかに異なり、CH-π 相互作用する炭化水素鎖と Phe^{140} 残基との距離がやや遠くなっている（**Figure 17**、水色）。したがって、化合物 **39h** の野生株 A2 に対する活性が

改善されなかった要因の一つとしては、この炭化水素リンカー部分の相互作用の違いにあると考えられる。

本章での仮説検証において、マクロサイクル化が、化合物の三次元構造を活性コンフォメーションに固定化することで、D486N タンパク質に対する活性発現に効果的に機能することを見出した。さらに、マクロサイクル化の際に導入した炭化水素リンカー部位が、新たな疎水性相互作用を獲得していることが示唆された。マクロサイクル化による上述の二つの効果により、化合物 **39h** の耐性変異株 D486N に対する活性が大幅に改善したものと考察した。

化合物 **39b** の 1-メチルアミノプロピル部位をピペリジン環に変換したことにより、耐性変異株 D486N に対する活性が著しく向上したが（**Table 6**、**39b** vs **39h**）、ピペリジン環に変換したことによる効果については上述のドッキングシミュレーションの結果からは十分に説明できていない。説明の一つとしては、化合物 **39b** の 1-メチルアミノプロピル部位をピペリジン環に変換し環構造を増やしたことにより、活性コンフォメーションがさらに固定化された可能性が考えられる。もう一つの説明としては、化合物 **39h** のベンゼン環 5 位の置換基の向きが化合物 **39b** のそれよりも、D486N タンパク質との相互作用に適している可能性が挙げられる。すなわち、ピペリジン環導入に伴い、化合物 **39h** のベンゾイル基の可動域が化合物 **39b** に比べて制限されることで、ベンゼン環 5 位の置換基が D486N タンパク質に適した方向に位置している可能性がある。具体的なデータは示していないが、ベンゼン環 5 位の置換基変換は耐性変異株 D486N に対する活性に影響を与えることがわかっている。ベンゼン環 5 位の置換基の役割を詳細に調査するためは化合物 **39h** と D486N タンパク質との X 線共結晶構造解析が必要であるが、現在のところ結晶構造は取得できていない。今後、マクロサイクル化合物と RSV A2 および D486N タンパク質との X 線複合体結晶が取得できれば、化学構造と機能との関係についてさらなる理解が進むものと考えている。

第 5 節　まとめ

本章では耐性変異株 D486N に対する活性改善を目的とし、ドッキングシミュレーションを用いて化合物をデザインした。すなわち、活性コンフォメーションの固定化を狙って 1-メチルアミノプロピル誘導体 **20k** のベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環 7 位を架橋したマクロサイクル化合物をデザイン・合成した。一連のマクロサイクル化合物の中で、15 員環を有する化合物 **39b** は野生株 A2 に対してもっとも高い活性を示し、非マクロサイクル化合物 **20k** と比較して 5.8 倍活性が向上した。さらに、化合物 **39h** は臨床で報告されている薬剤耐性変異株 D486N に対しても強力な活性を示した。著者の知る限り、化合物 **39h** は耐性変異株 D486N に対する有効性が示された、初めての低分子 RSV F タンパク質阻害物質である。また、化合物 **39h** と D486N タンパク質とのドッキングシミュレーションによる解析から、マクロサイクル化により分子構造が活性コンフォメーションに固定化されるとともに、導入した炭化水素リンカー部位が新たに疎水性相互作用を獲得していること示唆された。野生株 A2 のみならず耐性変異株 D486N に対しても強力な抗 RSV 活性を有する **39h** をリード化合物とし、新規 RSV 治療薬創出に向けたさらなる最適化研究へと展開することとした。

第 3 章　アトロプ異性体を回避した新規アミドリンカー誘導体の創出

第 1 節　アトロプ異性体混合物の開発難易度とその回避戦略

第 2 章で見出した 15 員環マクロサイクル化合物 **39h** は野生株 A2 だけでなく耐性変異株 D486N に対しても良好な抗 RSV 活性を示す有望な化合物である。しかしながら、室温下での ^{1}H-NMR 測定の結果、二つの化合物の混合物を示すチャートが得られたことから、アトロプ異性体混合物であることが懸念された。アトロプ異性体はその回転エネルギー障壁と相互変換率に基づいて三つのクラスに分類される（**Figure 18**）[55]。

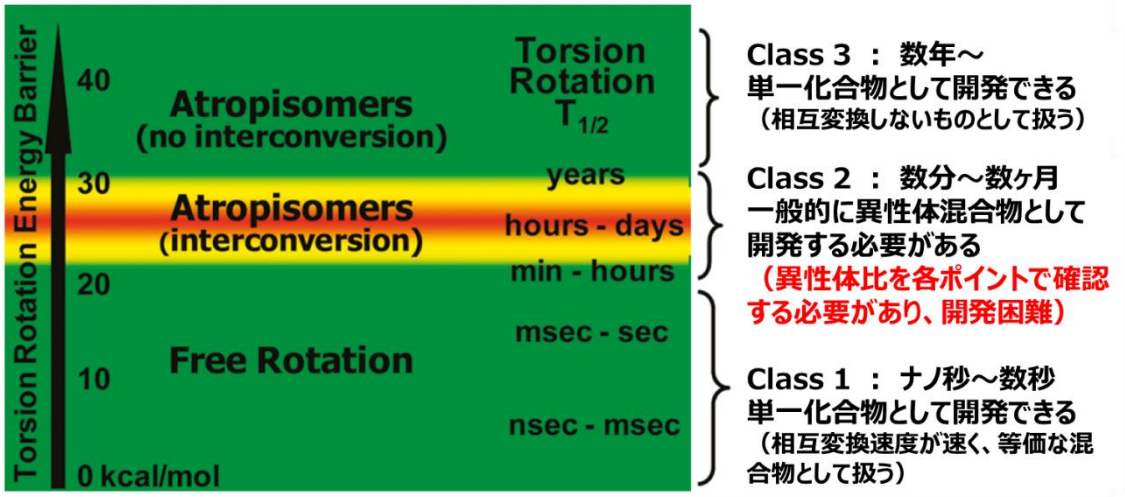


Figure 18. アトロプ異性体の分類（文献 55 を一部改変）

これらアトロプ異性体の分類は医薬品開発の難易度と密接に関連している。アトロプ異性体間の回転半減期が数秒以下の化合物は Class 1（非アトロプ異性体混合物）、数年以上の場合は Class 3 に分類され、どちらのクラスの化合物も単一化合物として扱うことができるので開発上問題はない。一方、アトロプ異性体間の回転半減期が数分から数ヶ月である Class 2 の化合物については、創薬開発の各ステージでその異性体比を随時確認していかなければならないため、非常に開発が困難であるとされている [55–57]。実際に近年では、Class 2 化合物は発売まで至っていない [56,57] 。

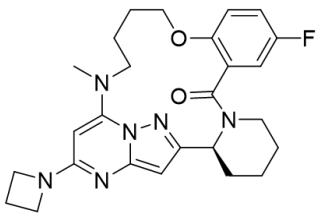
そこで、NMR スペクトルを用いた詳細な検討により、15 員環マクロサイクル

化合物の回転半減期を算出することで、アトロプ異性体混合物クラスの分類を確認することとした。

アトロプ異性体間の回転半減期の算出には、化合物の動的過程を解析するのに有効な二次元 EXSY 法（two-dimensional exchange spectroscopy）を利用した。15 員環マクロサイクル化合物 **39h** は高温測定条件下でやや不安定であったため、類似の構造を有する 15 員環マクロサイクル化合物 **40** を NMR 実験に用いた。化合物 **40** のベンゾイル部位 5 位およびピラゾロ[1,5-a]ピリミジン環 5 位の置換基は化合物 **39h** とは異なるが、マクロサイクル部位から離れた部分の相違であることから、アトロプ異性体間の回転半減期にはほとんど影響しないものと考えている。

15 員環マクロサイクル化合物 **40** の EXSY スペクトルにおいて、室温（25 °C）ではシグナル交換が見られなかったが、高温下（60 °C～90 °C）ではシグナル交換が観測された。各温度における化合物 **40** の相互変換率と回転半減期を EXSY シグナル強度の比から計算し、25 °C での回転半減期を 60 °C、 70 °C、 80 °C、および 90 °C での相互変換率から外挿した。その結果、化合物 **40** の回転半減期は 25 °C で約 5 分であり、Class 2 のアトロプ異性体混合物であることが判明した（**Table 7**）。したがって、類似の構造を有する化合物 **39h** も同様に Class 2 のアトロプ異性体混合物であることが示唆された。

Table 7. 15 員環マクロサイクル化合物 **40** の回転半減期とアトロプ異性体混合物のクラス

Compound	Structure	Ring size	Rotation $t_{1/2}$ (DMSO-d_6, 25 °C)		Atropisomer classification
			main	minor	
40		15	4.92 min[a]	6.30 min[a]	Class 2

[a] 高温時の相互変換率から回転半減期を外挿した。

開発可能な薬剤候補を得るためには化合物 **39h** のさらなる最適化検討を実施し、アトロプ異性体混合物を回避する必要がある。アトロプ異性体混合物の課題をもつ Class 2 化合物を回避するための一般的な戦略としては、次の三つの方法がある。

（1）回転障壁をもつキラル軸周りの相互変換率（回転速度）を高める
（回転半減期が数秒未満の Class 1 への変換）
（2）回転を凍結させる（半減期が数年以上の Class 3 への変換）
（3）回転軸の周りの対称化によりキラル軸を排除する

15 員環マクロサイクル化合物 **40** の場合、ピペリジン環と Ar 部位との間のアミド結合に立体的な回転障害が生じることで、Class 2 のアトロプ異性体混合物を与えているものと推定された。そこで、ピラゾロ[1,5-a]ピリミジン環 7 位とベンゾイル部位 2 位を連結するリンカー部位を最適化することにより、アミド結合の回転速度を改善し、回転半減期が数秒未満の Class 1 化合物に変換する戦略をとることにした。すなわち、15 員環マクロサイクル化合物 **40** の環サイズをさらに大きな 16 員環や 17 員環にすることにより、環全体の立体的な制約が低減され、アミド結合の回転障害が改善されるものと考えた。第 2 章の検討結果から、16 員環マクロサイクル化合物 **39c** は比較的良好な抗 RSV 活性を維持している一方で、17 員環マクロサイクル化合物 **39d** は活性が大きく減弱することが分かっている（**Table 6**）。そこで、16 員環を基本構造としたマクロサイクル化合物のリンカー部位を最適化することにより、Class 2 アトロプ異性体混合物を回避し、抗 RSV 活性を維持または向上する化合物の創出を目指すこととした。

第 2 節　リンカー部位を変換したマクロサイクル化合物の合成

アトロプ異性体混合物の課題を克服することを目的として、マクロサイクル化合物のリンカー部位を変換した化合物をデザイン・合成した（**Figure 19**）。一連の化合物のピラゾロ[1,5-a]ピリミジン環 5 位はアゼチジン、ベンゾイル部位 5 位は F で固定した。マクロサイクル化合物は、ジクロロピラゾロ[1,5-a]ピリミジンパーツ **36b** と対応する各リンカーパーツとを合成の終盤に連結する収束的な合成法により合成した（**Scheme 10**）。

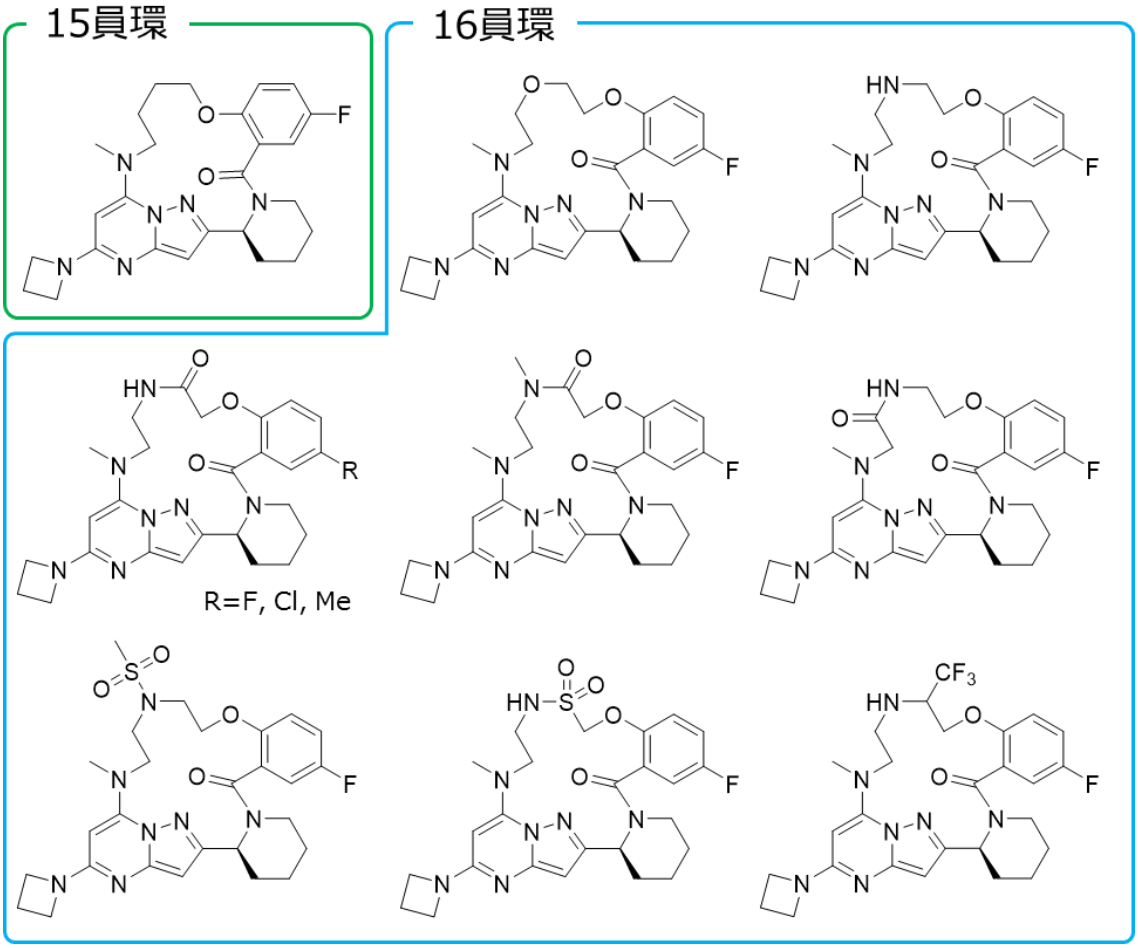
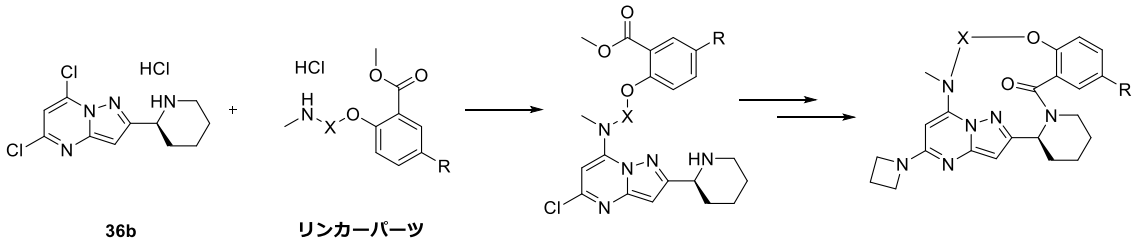
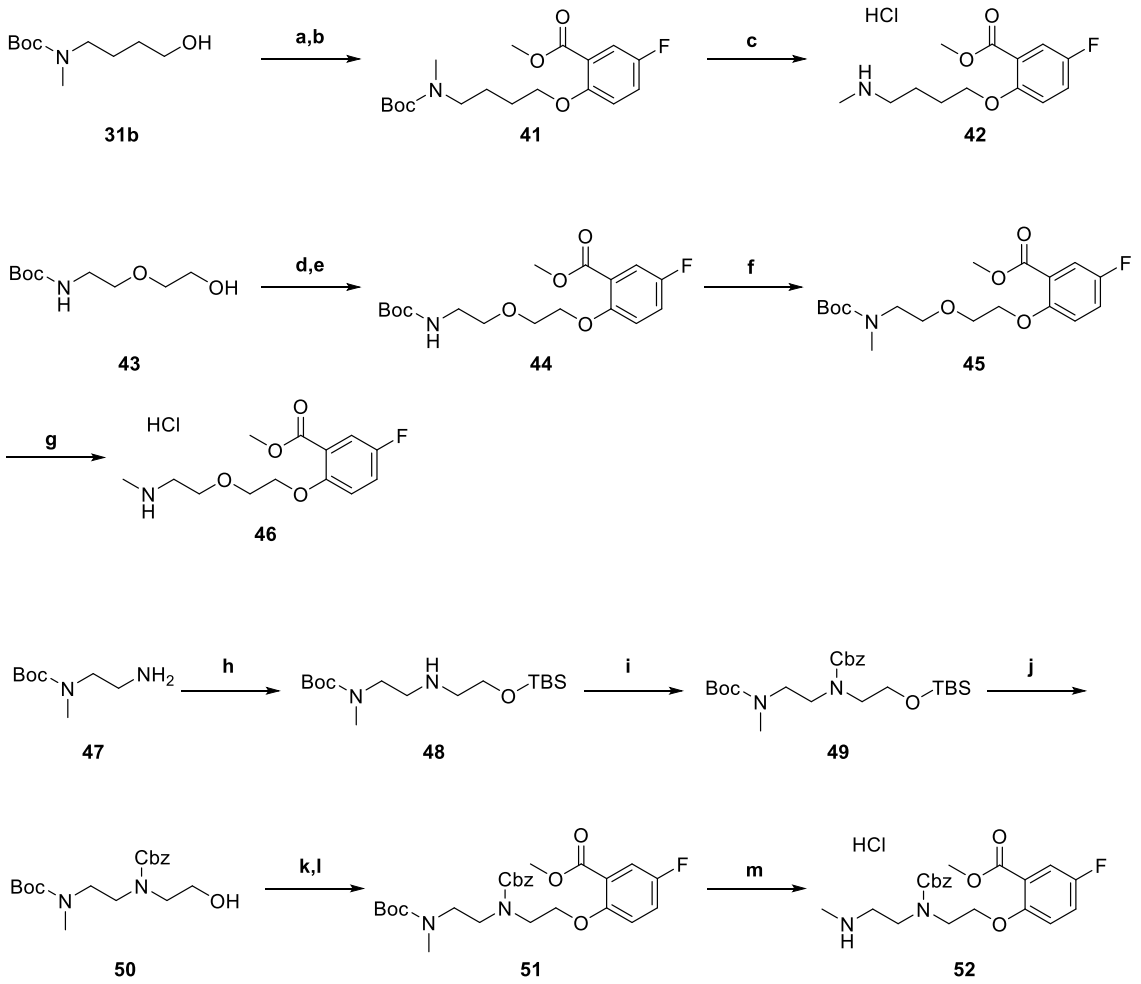


Figure 19.マクロサイクル化合物のデザイン



Scheme 10.

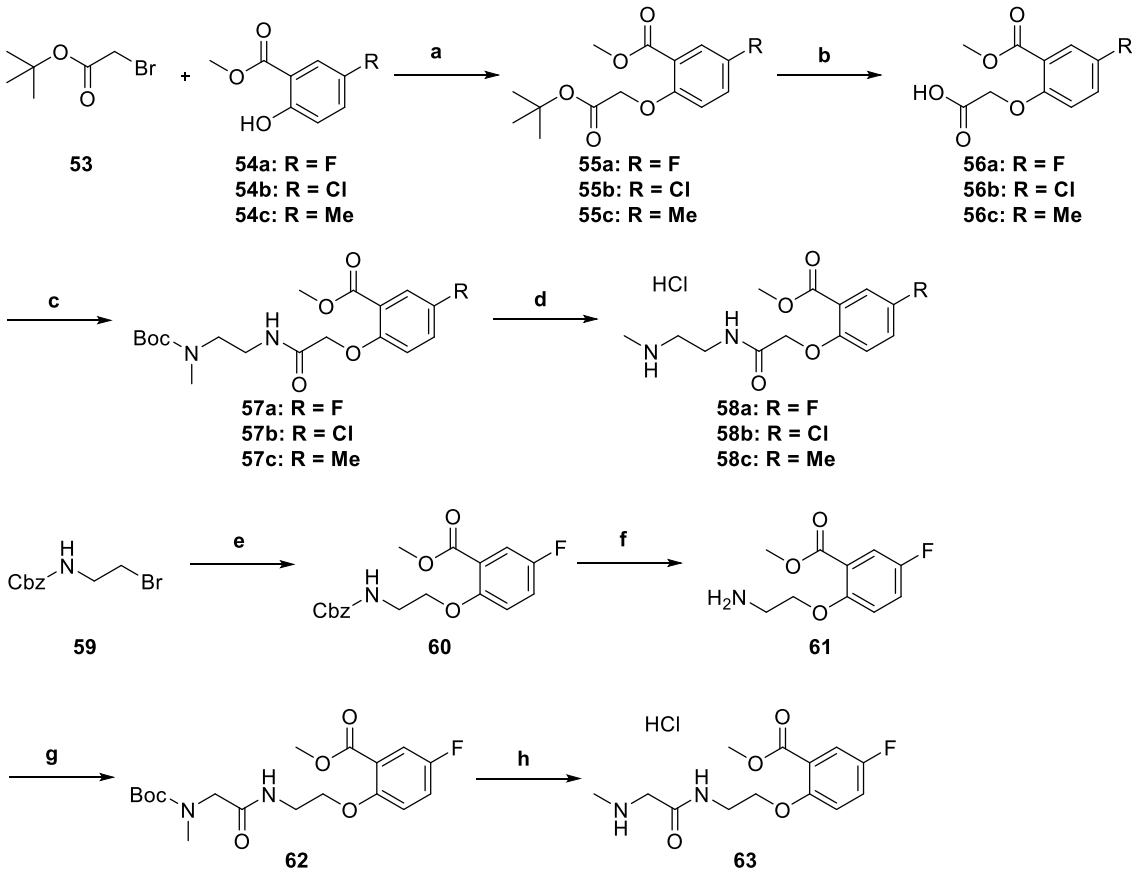
初めに、リンカーパーツを合成した。**Scheme 11** にリンカーパーツ（**42**、**46**、**52**）の合成ルートを示す。アルコール **31b** の *O*-メシル体に対する 5-フルオロ-2-ヒドロキシ安息香酸メチルの付加反応により化合物 **41** とした後、Boc 基を除去することにより、リンカーパーツ **42** を得た。同様に、アルコール **43** の *O*-メシル体に対する 5-フルオロ-2-ヒドロキシ安息香酸メチルの付加反応により化合物 **44** を得た後、ヨウ化メチルと酸化銀を用いて窒素原子をメチル化し、Boc 基を除去することによりリンカーパーツ **46** を得た。アミン **48** は、2-ブロモエトキシ-*tert*-ブチルジメチルシランに対するアミン試薬 **47** の S_N2 反応により調製した。続いて、アミン **48** の第二級アミンを Cbz 基で保護した後、TBAF を用いて TBS 基を除去することによりアルコール **50** を合成した。リンカーパーツ **52** はアルコール **50** からリンカーパーツ **42** の合成法と同様の方法で調製した。



Scheme 11. (a) MsCl, Et_3N, $CHCl_3$, r.t.; (b) methyl 5-fluoro-2-hydroxybenzoate, K_2CO_3, DMF, 90 °C, 2 steps 80%; (c) 4 M HCl in dioxane, 1,4-dioxane, r.t., 79%; (d) MsCl, Et_3N, $CHCl_3$, r.t.; (e) methyl 5-fluoro-2-hydroxybenzoate, K_2CO_3, DMF, 90 °C, 2 steps 75%; (f) MeI, Ag_2O, DMF, 90 °C, 99%; (g) 4 M HCl in dioxane, 1,4-dioxane, r.t., 99%; (h) 2-bromoethoxy-*tert*-butyldimethylsilane, K_2CO_3, MeCN, 80 °C, 69%; (i) benzyl chloroformate, Et_3N, $CHCl_3$, r.t., quant.; (j) TBAF, THF, r.t., 81%; (k) MsCl, Et_3N, $CHCl_3$, r.t.; (l) methyl 5-fluoro-2-hydroxybenzoate, K_2CO_3, DMF, 90 °C, 2 steps 38%; (m) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%.

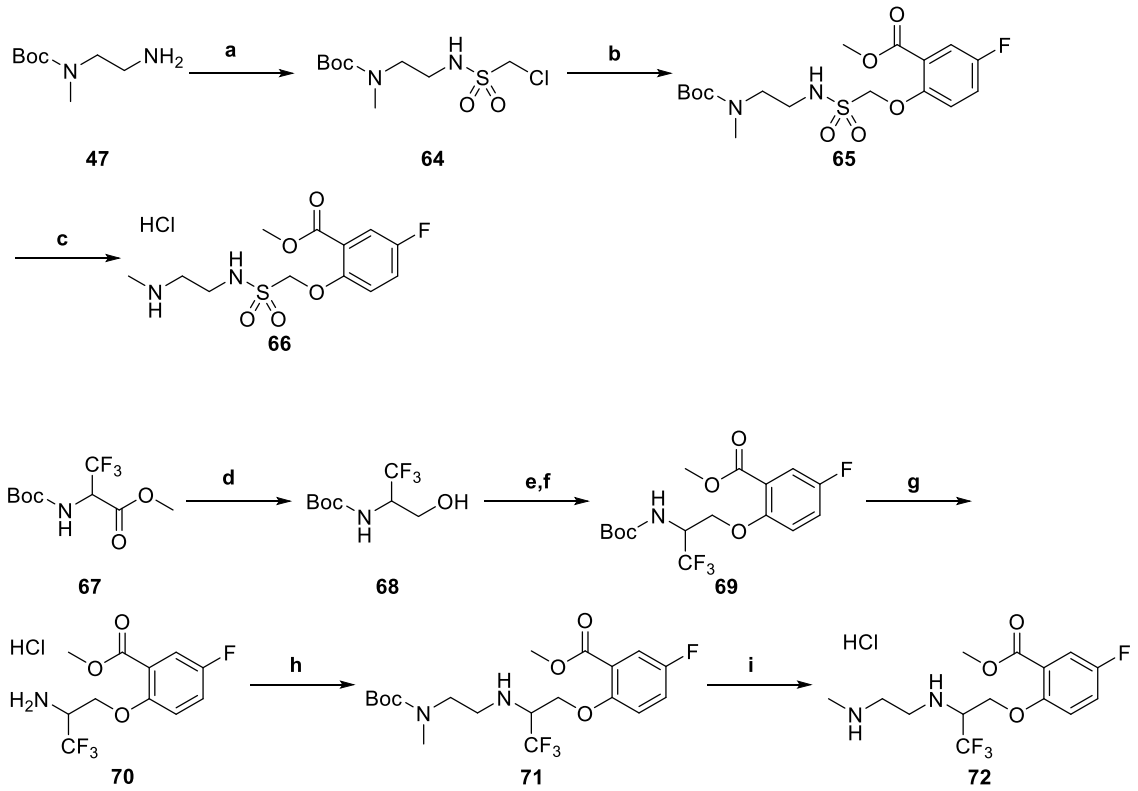
Scheme 12 にアミド結合をもつリンカーパーツ（**58a**–**c**、**63**）の合成ルートを示す。フェノール（**54a**–**c**）を *tert*-ブチル-2-ブロモアセテート **53** でアルキル化した後、*tert*-ブチル基を除去することでカルボン酸（**56a**–**c**）を得た。カルボン酸

（**56a**–**c**）と *tert*-ブチル *N*-（2-アミノエチル）-*N*-メチルカルバメートを縮合した後、Boc 基を除去することによりリンカーパーツ（**58a**–**c**）を得た。アミン中間体 **61** は、5-フルオロ-2-ヒドロキシ安息香酸メチルをベンジル *N*-（2-ブロモエチル）カルバメート **59** でアルキル化し、Cbz 基を脱保護することにより得た。アミン中間体 **61** と 2-［*tert*-ブトキシカルボニル（メチル）アミノ］酢酸との縮合反応と続く Boc 基の脱保護によりリンカーパーツ **63** を合成した。



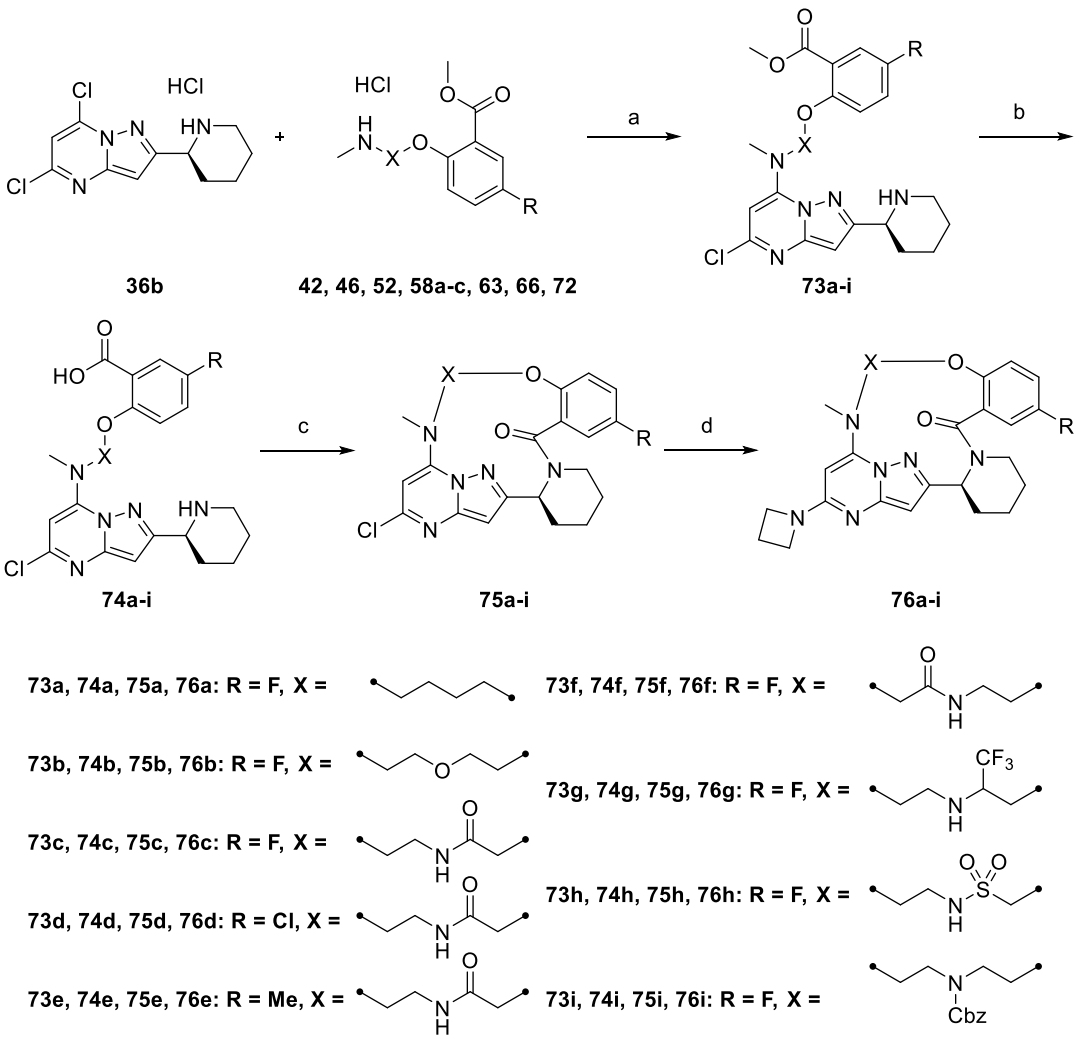
Scheme 12. (a) K_2CO_3, MeCN, 70 °C, 98–100%; (b) TFA, $CHCl_3$, r.t.; (c) *tert*-butyl *N*-(2-aminoethyl)-*N*-methylcarbamate (**47**), HATU, Et_3N, DMF, r.t. 2 steps 67–99%; (d) 4 M HCl in dioxane, 1,4-dioxane, r.t., 91–100%; (e) methyl 5-fluoro-2-hydroxybenzoate, NaH, THF, reflux, 37%; (f) H_2, 10% Pd/C, MeOH, r.t., 100%.; (g) 2-[*tert*-butoxycarbonyl(methyl)amino]acetic acid, HATU, Et_3N, DMF, r.t., 100%; (h) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%.

Scheme 13 にアミド結合のバイオアイソスターをもつリンカーパーツ（**66**、**72**）の合成を示す。*tert*-ブチル *N*-（2-アミノエチル）-*N*-メチルカルバメート **47** をクロロメタンスルホニルクロリドで処理した後、5-フルオロ-2-ヒドロキシ安息香酸メチルの付加反応により化合物 **65** とし、最後に Boc 基を脱保護することによりスルホンアミドリンカーパーツ **66** を得た。化合物 **67** のエステル部位を還元することによりアルコール **68** を得た。アルコール **68** の *O*-メシル体に対する 5-フルオロ-2-ヒドロキシ安息香酸メチルの付加反応により化合物 **69** とした後、Boc 基を除去することによりアミン **70** を得た。*tert*-ブチル *N*-メチル-*N*-（2-オキソエチル）カルバメートとの還元的アミノ化反応により化合物 **71** とした後、Boc 基を除去することによりトリフルオロエチルアミンリンカーパーツ **72** を得た。



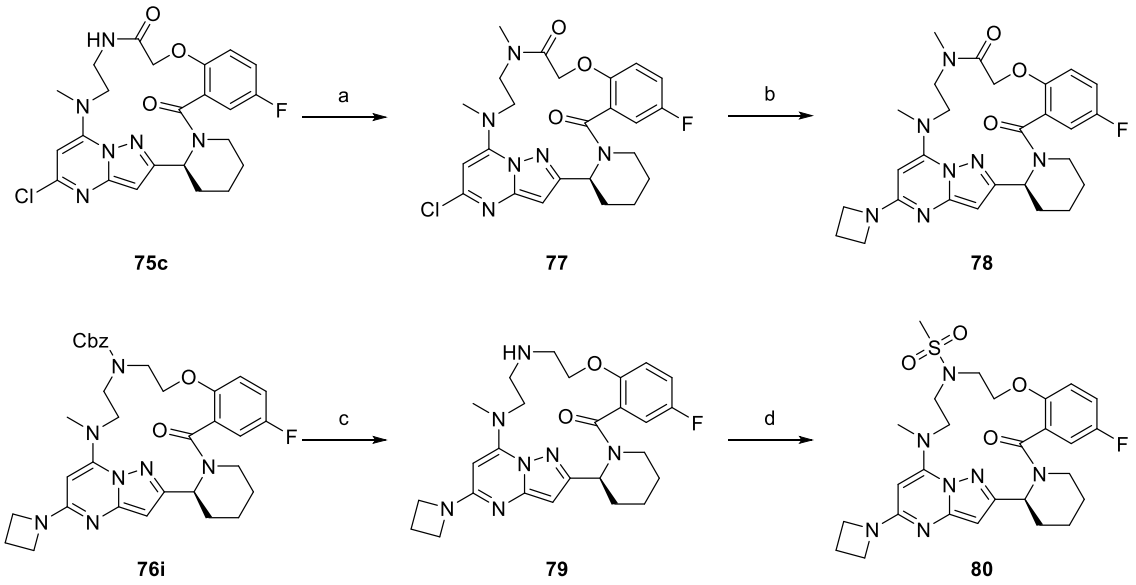
Scheme 13. (a) chloromethanesulfonyl chloride, Et_3N, $CHCl_3$, 0 °C, 87%; (b) methyl 5-fluoro-2-hydroxybenzoate, K_2CO_3, DMF, 80 °C, 10%; (c) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%; (d) $LiBH_4$, THF, r.t., 93%; (e) MsCl, Et_3N, $CHCl_3$, r.t.; (f) methyl 5-fluoro-2-hydroxybenzoate, K_2CO_3, DMF, 90 °C, 2 steps 21%; (g) 4 M HCl in dioxane, 1,4-dioxane, r.t.; (h) *tert*-butyl *N*-methyl-*N*-(2-oxoethyl)carbamate, $NaHB(OAc)_3$, $CHCl_3$, r.t., 2 steps 86%; (i) 4 M HCl in dioxane, 1,4-dioxane, r.t., 100%.

マクロサイクル化合物（**76a–i**）の合成ルートを **Scheme 14** に示す。リンカーパーツ（**42**、**46**、**52**、**58a–c**、**63**、**66**、**72**）を塩基性条件下、ジクロロピラゾロ[1,5-a]ピリミジンパーツ **36b** の 7 位に導入することにより化合物（**73a–i**）を合成した。続いて、エステル部位を加水分解した後、高希釈条件下での分子内アミド化反応によりマクロサイクルを形成した化合物（**75a–i**）を得た。最後に、マイクロウェーブ照射下、塩基性条件下にてアゼチジンをピラゾロ[1,5-a]ピリミジン環 5 位に導入することにより、所望のマクロサイクル化合物（**76a–i**）を合成した。



Scheme 14. (a) Et_3N, EtOH, 70 °C, 56–92%; (b) 1M NaOH aq., THF, 2-propanol, 80 °C; (c) HATU, Et_3N, DMF, r.t., 2 steps 57–100%; (d) azetidine, Et_3N, NMP, microwave 150 °C, 51–100%.

さらに、**Scheme 15** に示すように中間体 **75c** および中間体 **76i** を用いて、マクロサイクル化合物（**78**–**80**）を合成した。すなわち、マクロサイクル化合物 **78** は、中間体 **75c** のアミド部分をメチル化し、ピラゾロ[1,5-a]ピリミジン環 5 位にアゼチジンを導入することで合成した。また、マクロサイクル化合物 **79** は中間体 **76** の Cbz 基を脱保護することにより調製し、マクロサイクル化合物 **80** は化合物 **79** の第二級アミンをメシル化することにより合成した。



Scheme 15. (a) NaH, MeI, DMF, r.t. 100%; (b) azetidine, Et_3N, NMP, microwave 150 °C, 35%; (c) H_2, 10% Pd/C, MeOH, r.t., 75%; (d) MsCl, Et_3N, $CHCl_3$, r.t., 91%.

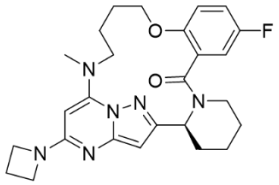
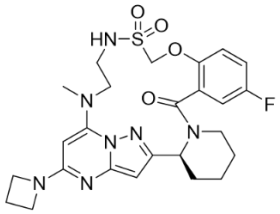
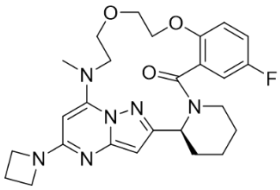
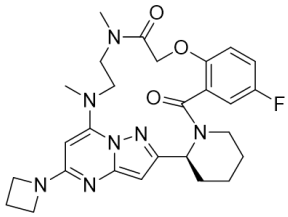
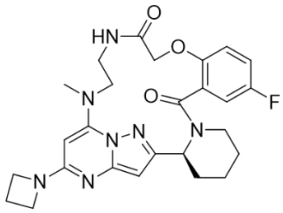
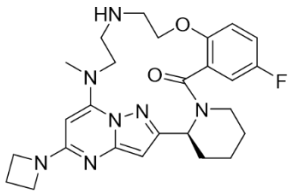
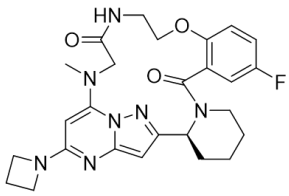
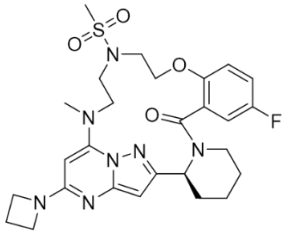
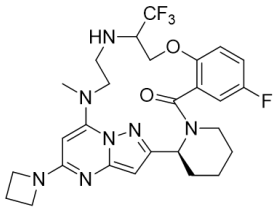
第 3 節　リンカー部位を変換したマクロサイクル化合物の抗 RSV 活性

前節で合成したマクロサイクル化合物の抗 RSV 活性は、前章と同様に CPE 抑制法により評価した。ベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環 7 位を連結する修飾リンカー部位を変換したマクロサイクル化合物（**76a–c、76f–h、78–80**）の野生株 A2 に対する抗 RSV 活性を示す（**Table 8**）。マクロサイクルを 16 員環に拡大することより、アトロプ異性体混合物の課題を解決するとともに、抗 RSV 活性が維持または向上するリンカー部位を探索した。第 2 章までのマクロサイクル化合物の検討では、炭化水素リンカーを有する化合物の SAR 取得にとどまっていたため、本章ではターゲットタンパク質との新たな相互作用獲得を目指して、リンカー部位にプロトンドナーやプロトンアクセプターをもつ極性基を導入し、抗 RSV 活性への効果を検証した。

炭化水素リンカー化合物 **76a** のリンカー部位へ酸素原子の導入は抗 RSV 活性に影響を与えなかった［EC_{50}：17 nM（**76a**）、EC_{50}：17 nM（**76b**）］。一方、リンカー部位に窒素原子を導入した化合物 **79** は、炭化水素リンカー化合物 **76a** と比較して 10 倍以上強い抗 RSV 活性（EC_{50}：1.4 nM）を示した。リンカー部位にアミド基を有する **76c** には、さらに強力な抗 RSV 活性（EC_{50}：0.33 nM）が認められたことから、アミドリンカー化合物 **76c** の類縁体についてさらなる検討を進めることにした。

アミド基を *N*-メチル化した化合物 **78** およびアミド結合の向きを反転させた化合物 **76f** は、アミドリンカー化合物 **76c** と比較して、それぞれ 4.2 倍および 7.6 倍［EC_{50}：1.4 nM（**78**）、2.5 nM（**76f**）］抗 RSV 活性が減弱した。また、アミド基のバイオアイソスターとして知られているトリフルオロエチルアミンを導入した化合物 **76g** では抗 RSV 活性（EC_{50}：21 nM）が著しく低下した。また、スルホンアミド基を導入した化合物 **80**、**76h** についても、化合物 **76c** と比較して抗 RSV 活性が著しく減弱した［EC_{50}：4.6 nM（**80**）、54 nM（**76h**）］。種々のリンカー構造を有する 16 員環マクロサイクル化合物の中で、アミドリンカー化合物 **76c** は 15 員環マクロサイクル化合物 **39h** を上回るもっとも強力な抗 RSV 活性を示した［EC_{50}：2.0 nM（**39h**）］。

Table 8. リンカー部位を変換したマクロサイクル化合物の抗 RSV 活性

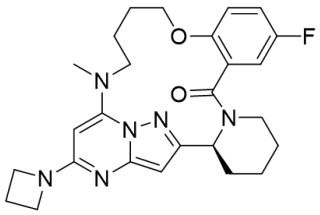
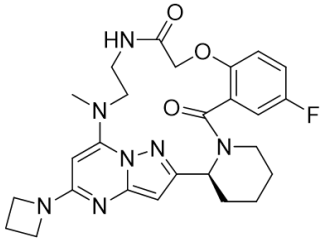
No.	Structure	A2 EC_{50} (nM)[a]	No.	Structure	A2 EC_{50} (nM)[a]
76a		17	**76h**		54
76b		17	**78**		1.4
76c		0.33	**79**		1.4
76f		2.5	**80**		4.6
76g		21			

[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

第 4 節　16 員環アミドリンカー化合物 **76c** のアトロプ異性体クラスの同定

第 3 節のリンカー部位の最適化検討により、抗 RSV 活性が良好な 16 員環化合物 **76c** を見出したことから、続いて、16 員環への環拡大がアトロプ異性体間の相互変換速度に及ぼす影響を検証した。本章第 1 節と同様に二次元 EXSY 法を用いて 16 員環アミドリンカー化合物 **76c** のアトロプ異性体間の回転半減期を算出した（**Table 9**）。その結果、化合物 **76c** は室温（25 °C）で明確なシグナル交換を示し、二つのアトロプ異性体間の回転半減期は約 2 秒であった。したがって、16 員環アミドリンカー化合物 **76c** は Class 1 の非アトロプ異性体混合物に分類される。期待していた通り、マクロサイクルの環のサイズを拡大する戦略は、Class 2 化合物（**40**、15 員環）を Class 1 化合物（**76c**、16 員環）に変換するために有効であることが示された。

Table 9. マクロサイクル化合物 **40** および **76c** の回転半減期とアトロプ異性体混合物のクラス

Compound	Structure	Ring size	Rotation $t_{1/2}$ (DMSO-d_6, 25°C)		Atropisomer classification
			main	minor	
40		15	4.92 min[a]	6.30 min[a]	Class 2
76c		16	1.90 s	2.21 s	Class 1

[a] 高温時の相互変換率から回転半減期を外挿した。

第 5 節　16 員環アミドリンカー化合物 **76c** の周辺誘導体合成

抗 RSV 活性のさらなる向上を図るため、アトロプ異性体混合物の問題のない 16 員環アミドリンカー化合物 **76c** に焦点を当て、最適化検討を実施した。すなわち、化合物 **76c** のピラゾロ[1,5-a]ピリミジン環 5 位およびベンゾイル部位 5 位を変換した誘導体を合成した（**Figure 20**）。

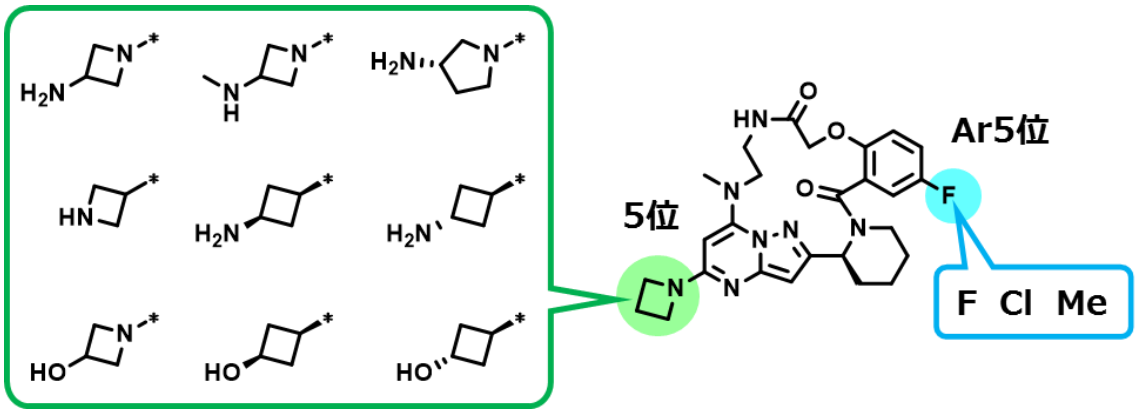
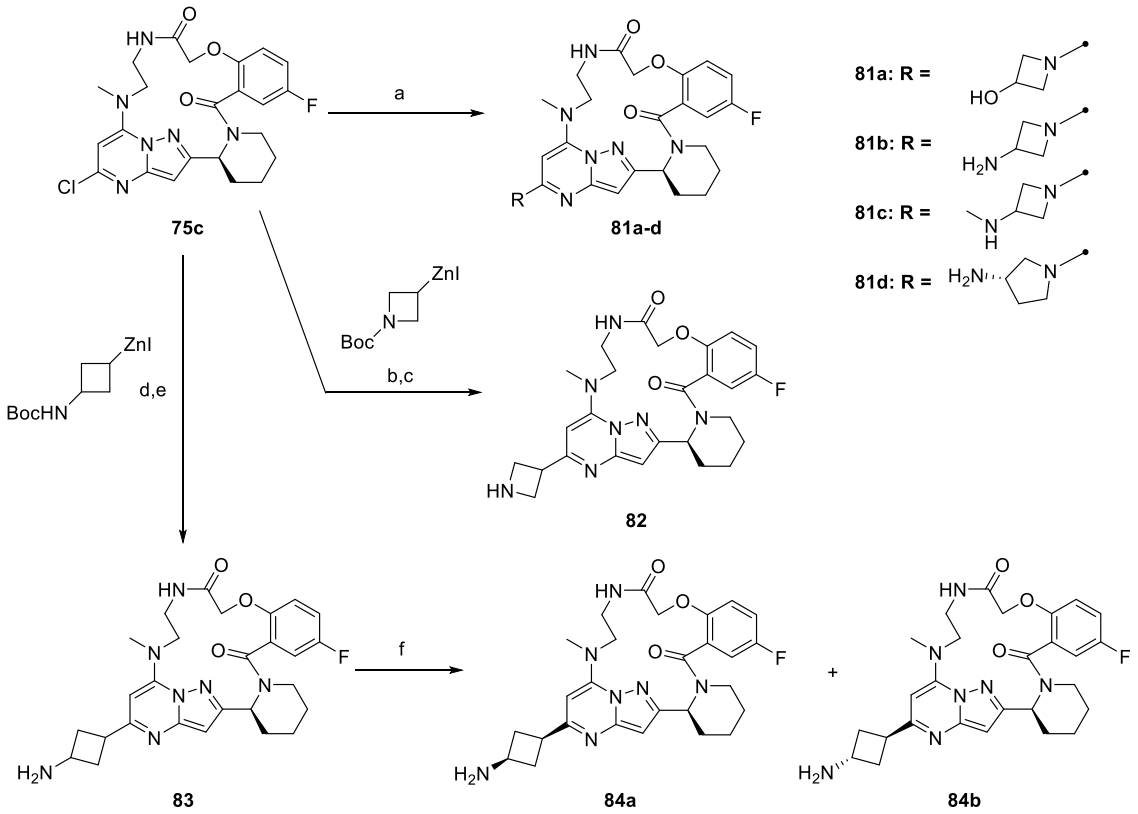


Figure 20. 本節で合成したマクロサイクル化合物

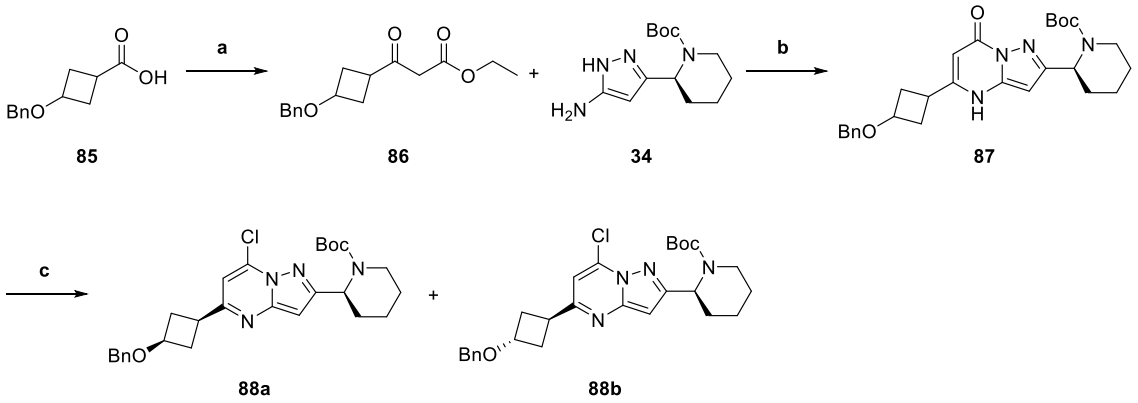
Scheme 16 にピラゾロ[1,5-a]ピリミジン環 5 位置換基を変換したマクロサイクル誘導体（**81a–d**、**82**、**84a–b**）の合成ルートを示す。中間体 **75c** に対し、マイクロウェーブ照射下、塩基性条件にて対応するアミンパーツを導入し、必要に応じて Boc 基を脱保護することにより、所望のマクロサイクル化合物（**81a–d**）を合成した。アゼチジンを導入した化合物 **82** は、別途調製した有機亜鉛試薬を用いた根岸カップリング反応と続く Boc 基の脱保護により中間体 **75c** から合成した。3-アミノシクロブタン化合物（**84a–b**）は、化合物 **82** と同様に調製した有機亜鉛試薬を用いた根岸カップリング反応と続く Boc 基の脱保護によりラセミ混合物 **83** を得た後、キラルカラムを用いて分離精製した。



Scheme 16. (a) amine, Et_3N, NMP, microwave 150 °C, then TFA, $CHCl_3$, r.t., (only for compounds possessing Boc group), 25–86%; (b) CuI, $PdCl_2$(dppf), DMA, 85 °C, 73%; (c) TFA, $CHCl_3$, r.t., 42%; (d) CuI, $PdCl_2$(dppf), DMA, 85 °C; (e) TFA, $CHCl_3$, r.t., 2 steps 44%; (f) chiral colomn separation.

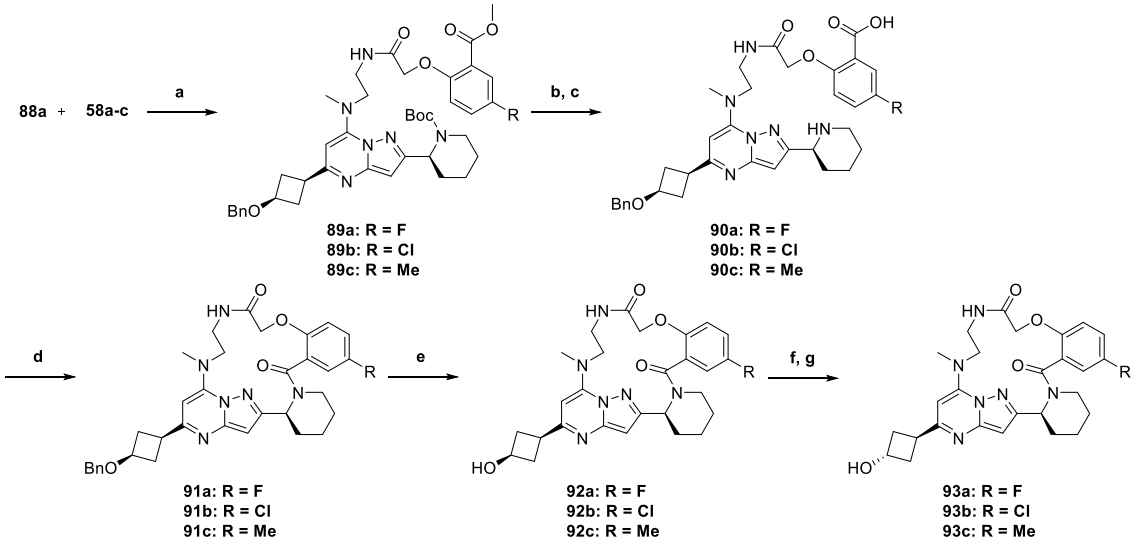
Scheme 17 および **Scheme 18** にピラゾロ[1,5-a]ピリミジン環 5 位に 3-ヒドロキシシクロブタンを導入した化合物（**92a**–**c**、**93a**–**c**）の合成ルートを示す。まず、ピラゾロ[1,5-a]ピリミジン中間体（**88a**–**b**）を合成した（**Scheme 17**）。すなわち、3-ベンジルオキシシクロブタンカルボン酸 **85** とマロン酸モノエチルカリウムを塩化マグネシウムの存在下、1,1'-カルボニルジイミダゾール（CDI）で処理することにより、ケトエステル **86** を得た。ケトエステル **86** とアミノピラゾール中間体 **34** を酢酸中で反応させることにより、ピラゾロピリミドン環を構築した後、酸性条件下で一部 Boc 基の脱離したアミノ基を再度 Boc 基で保護することにより中間体 **87** を得た。中間体 **87** をピリジン溶媒中、オキシ塩化リンおよび 4-(*N*,*N*-

ジメチルアミノ）ピリジン（DMAP）に付すことにより塩素化した後、得られたシクロブタノール部位の *cis*/*trans* 混合物をシリカゲルカラムクロマトグラフィーで分離精製することによりピラゾロ[1,5-a]ピリミジン中間体（**88a**–**b**）を得た。



Scheme 17. (a) potassium 3-ethoxy-3-oxopropanoate, CDI, $MgCl_2$, THF, 65 °C, 45%; (b) AcOH, 100 °C, then, Boc_2O, Et_3N, $CHCl_3$, r.t., 88%; (c) $POCl_3$, DMAP, pyridine, 65 °C, **88a**: 65%, **88b**: 15%.

続いて、ピラゾロ[1,5-a]ピリミジン中間体 **88a** とリンカーパーツ（**58a**–**c**）から **Scheme 18** に示す方法で 3-ヒドロキシシクロブタンを導入した化合物（**92a**–**c**、**93a**–**c**）を合成した。すなわち、中間体 **88a** のピラゾロ[1,5-a]ピリミジン 7 位に対応するリンカーパーツ（**58a**–**c**）をアミノ化反応により導入した後、エステルの加水分解、Boc 基の脱保護によりマクロサイクル環化前駆体（**90a**–**c**）とした。高希釈条件下における分子内アミド化反応の後、ベンジル基を除去することにより所望の *cis* 体（**92a**–**c**）を合成した。また、*trans* 体（**93a**–**c**）は *cis* 体（**92a**–**c**）の水酸基を光延反転することにより合成した。



Scheme 18. (a) Et_3N, NMP, microwave 150 °C, or Et_3N, DMF, 80 °C, 34–88%; (b) 1M NaOH aq., THF, MeOH, r.t. or 60 °C; (c) 4 M HCl in 1,4-dioxane, 1,4-dioxane, r.t.; (d) HATU, Et_3N, DMF [0.02 M], r.t., 3 steps 80–89%; (e) H_2, $Pd(OH)_2/C$, MeOH, 60 °C, or TMSCl, NaI, MeCN, 65 °C; 87–94%; (f) *p*-nitrobenzoic acid, bis(2-methoxyethyl)azodicarboxylate, PPh_3, THF, 60 °C; (g) 1M NaOH aq., THF, r.t., 2 steps 54–96%.

第 6 節　16 員環アミドリンカー化合物 **76c** 周辺誘導体の in vitro 評価

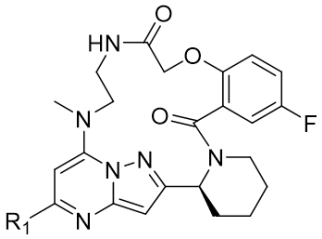
抗 RSV 活性のさらなる向上を図るため、アトロプ異性体混合物の問題のない 16 員環アミドリンカー化合物 **76c** のピラゾロ[1,5-a]ピリミジン環 5 位の最適化検討を実施した。**Table 10** に 5 位置換基変換化合物の野生株 A2 に対する抗 RSV 活性と並行人工膜透過性試験（parallel artificial membrane permeability assay: PAMPA）の結果を示す。

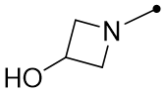
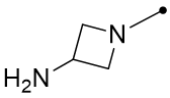
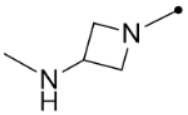
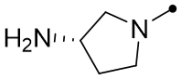
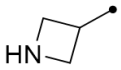
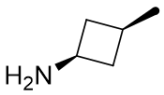
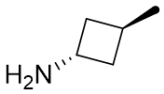
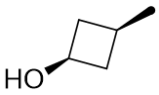
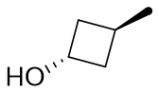
これまでのピラゾロ[1,5-a]ピリミジン誘導体の SAR 研究から、ピラゾロ[1,5-a]ピリミジン環 5 位にアミノピロリジンのような塩基性基を導入すると RSV F タンパク質の Asp^{486} との間に水素結合が形成されるため、野生株 A2 に対する活性が向上することが明らかになっている（e.g. **Figure 11**、**Figure 16**）。これらの SAR は、第 1 章で示した非マクロサイクル誘導体や第 2 章で示した炭化水素リンカーを有する **39h** などのマクロサイクル化合物の SAR においても確認されている。したがって、本章で見出した 16 員環アミドリンカー化合物のピラゾロ[1,5-a]ピリミジン環 5 位についても塩基性官能基などの導入によりアスパラギン酸残基との水素結合を形成させることで、抗 RSV 活性が向上するものと考えた。

アゼチジン環の 3 位に水酸基を有する化合物 **81a** は、アゼチジン化合物 **76c** と同程度の強力な抗 RSV 活性［EC_{50}: 0.33 nM（**76c**）、0.81 nM（**81a**）］を示したのに対し、アゼチジン環の 3 位にアミノ基およびメチルアミノ基を有する化合物（**81b**–**c**）の抗 RSV 活性はいずれも減弱した［EC_{50}: 2.7 nM（**81b**）、7.0 nM（**81c**）］。化合物 **81b** の 3-アミノアゼチジン-1-イル基を（3*S*）-3-アミノピロリジン-1-イル基で置換した化合物 **81d** では抗 RSV 活性にほとんど変化がなかった（**81d**、EC_{50}: 2.1 nM）。化合物 **76c** のアゼチジン環を反転させた化合物 **82** は、化合物 **76c** と比べて 5.2 倍活性が低下した（**82**、EC_{50}: 1.7 nM）。

ピラゾロ[1,5-a]ピリミジン環 5 位に塩基性基を導入した化合物の中で、3-アミノシクロブチル基を有する化合物（*cis* 体 : **84a**、*trans* 体 : **84b**）がもっとも高い活性を示し、EC_{50} 値は 1 nM 前後であった［EC_{50} : 0.87 nM（**84a**）、1.1 nM（**84b**）］。また、化合物（**84a**–**b**）のアミノ基を水酸基に置換した化合物（**92a**、**93a**）も同等の抗 RSV 活性を示した［EC_{50} : 1.1 nM（**92a**）、0.74 nM（**93a**）］。いずれの場合も、*cis*/*trans* 異性体間で抗 RSV 活性に顕著な差は認められなかった。

Table 10. 16 員環アミドリンカー化合物の 5 位置換基（R_1）変換誘導体の SAR



Compound	R_1	Anti-RSV activity EC_{50} (nM)[a] A2	PAMPA pH 6.2 (10^{-6} cm/s)
76c		0.33	80
81a		0.81	8.0
81b		2.7	3.3
81c		7.0	5.9
81d		2.1	0.3
82		1.7	0
84a		0.87	0
84b		1.1	0
92a		1.1	28
93a		0.74	27

[a] RSV A2 に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

以上の検討結果より、これまでのピラゾロ[1,5-a]ピリミジン誘導体の SAR とは異なり 16 員環アミドリンカー誘導体においては、ピラゾロ[1,5-a]ピリミジン環 5 位に塩基性基を導入しても抗 RSV A2 活性は大幅には改善しないことがわかった。また、PAMPA の結果から、ピラゾロ[1,5-a]ピリミジン環 5 位に塩基性官能基を有する一連の化合物（**81b**、**81d**、**82**、**84a**–**b**）の膜透過性は低く、塩基性基の導入は経口投与を目指した薬剤の最適化には不適であることが示唆された。

経口投与可能な新規 RSV F タンパク質阻害物質を得るために、強力な抗 RSV A2 活性と良好な膜透過性を併せもつ有望なリード化合物（**76c**、**93a**）について、Ar 部位 5 位置換基（R_2）の最適化を検討した。**Table 11** に野生株 A2 および耐性変異株 D486N に対する抗 RSV 活性、CYP3A 代謝依存性阻害作用（metabolism-dependent inhibition: MDI）、およびタンパク結合率の結果を示した。

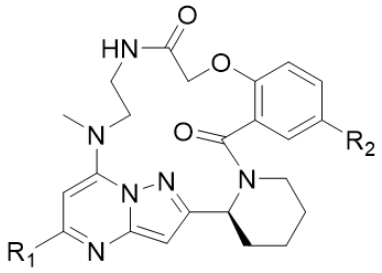
これまでのピラゾロ[1,5-a]ピリミジンシリーズの SAR 研究では、Ar 部位 5 位に塩素またはメチル基を導入した化合物で抗 RSV 活性が良好であることが示されている（第 1 章、第 5 節、**Table 2** 参照）。そこで、化合物 **76c** および **93a** の Ar 部位 5 位を塩素またはメチル基に変換した化合物を合成した。その結果、ピラゾロ[1,5-a]ピリミジンシ環 5 位にアゼチジンを有する化合物の場合、Ar 部位 5 位フッ素の塩素（**76d**）またはメチル基（**76e**）への変換による抗 RSV 活性の改善は認められなかった［EC_{50}：0.33 nM（**76c**）、0.47 nM（**76d**）、0.64 nM（**76e**）］。一方、3-ヒドロキシシクロブチル部位を有する化合物の場合、Ar 部位 5 位のフッ素を塩素（**93b**）に変換することにより、抗 RSV 活性が向上した［EC_{50} 値：0.74 nM（**93a**）、0.27 nM（**93b**）］。また、R_2 置換基としてメチル基を導入した化合物 **93c** はフッ素化合物 **93a** と同等の抗 RSV 活性を示した［EC_{50} 値：0.77 nM（**93c**）］。

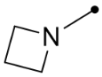
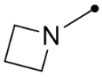
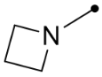
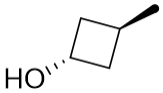
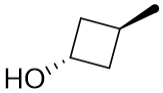
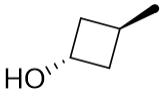
野生株 A2 に対して他の化合物よりも強力な抗 RSV 活性を示した化合物（**76c**, **93b**）は、耐性変異株 D486N 変異体に対しても強力な活性を示し、EC_{50} 値はそれぞれ 3.1 nM および 0.70 nM であった。特に、化合物 **93b** の EC_{50} 値はいずれもサブナノモルの範囲であり、野生株 A2 と耐性変異株 D486N に対する EC_{50} 値の差はわずか 2.6 倍であった。

続いて、一連の化合物（**76a**–**c**、**93a**–**c**）の in vitro ADMET 試験の結果について

述べる。Ar 部位 5 位にメチル基を有する化合物（**76e**、**93c**）は、CYP3A 代謝依存性阻害作用（MDI）を有することが示唆された。また、もっとも良好な抗 RSV 活性を有する化合物 **93b** はヒトおよびマウス血漿中で良好なタンパク質結合率を示した。

Table 11. Ar 部位 5 位置換基（R_2）の最適化

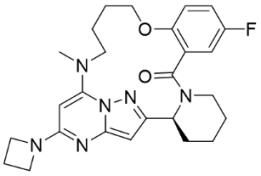
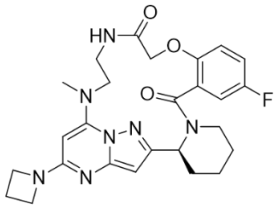
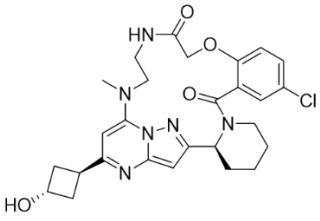


No.	R_1	R_2	EC_{50} (nM)[a] A2	EC_{50} (nM)[a] D486N	CYP3A MDI (%) @10 μM	Protein binding (human/mouse) (%)
76c		F	0.33	3.1	−4.7	87.0/98.0
76d		Cl	0.47	NT	−2.9	NT
76e		Me	0.64	NT	60.1	93.6/96.9
93a		F	0.74	NT	0.4	NT
93b		Cl	0.27	0.70	−1.4	90.7/85.5
93c		Me	0.77	NT	57.0	88.8/77.8

[a] RSV A2 および D486N に感染した HEp-2 細胞を用いて、化合物の CPE 阻害活性を評価し、EC_{50} 値を算出した。

続いて、化合物 **76c** と同様に二次元 EXSY 法を用いて化合物 **93b** のアトロプ異性体間の回転半減期を算出した（**Table 12**）。その結果、化合物 **93b** は室温（25 °C）で明確なシグナル交換を示し、二つのアトロプ異性体間の回転半減期は約 2 秒であった。したがって、化合物 **93b** は化合物 **76c** と同様に Class 1 の非アトロプ異性体混合物に分類される。

Table 12. マクロサイクル化合物 **40**、**76c**、および **93b** の回転半減期とアトロプ異性体混合物のクラス

Compound	Structure	Ring size	Rotation $t_{1/2}$ (DMSO-d_6, 25 °C)		Atropisomer classification
			main	minor	
40		15	4.92 min[a]	6.30 min[a]	Class 2
76c		16	1.90 s	2.21 s	Class 1
93b		16	2.31 s	2.35 s	Class 1

[a] 高温時の相互変換率から回転半減期を外挿した。

第 7 節　16 員環アミドリンカー化合物 **93b** のドッキングシミュレーション

本章第 3 節の検討により、リンカー部位を炭化水素リンカー（**76a**）からアミドリンカー（**76c**）に変換することで大幅に抗 RSV 活性が向上することを見出した。アミドリンカー化合物が良好な活性を示した理由について、A2 タンパク質との結合ポーズから考察を試みた。具体的には、本節ではもっとも良好なプロファイルをもつアミドリンカー化合物 **93b** の A2 タンパク質に対する MD シミュレーションから結合ポーズを検証した。以下に結果および考察について述べる。

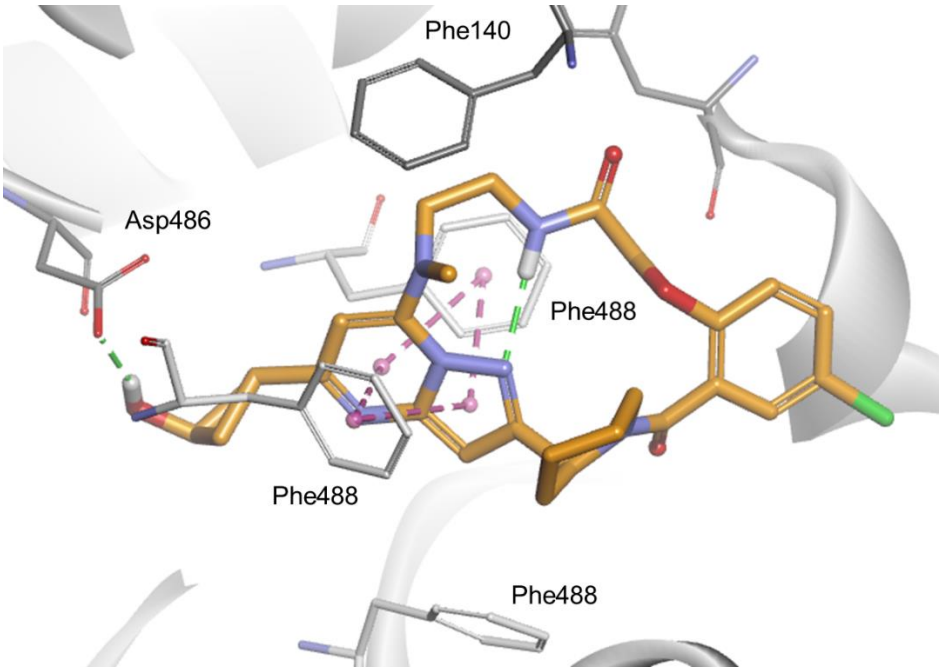


Figure 21. 化合物 **1**（オレンジ）と野生株 A2（灰色）との分子動力学モデル
黄緑色破線は水素結合を示す。ピンク色破線はπ-π相互作用を示す。

RSV A2 F タンパク質（PDB: 5EA3）に対して化合物 **93b** の MD シミュレーションを実施した（**Figure 21**）。化合物 **93b** と A2 タンパク質との間には、三つの特徴的な相互作用が観察された。一つ目は、ピラゾロ[1,5-a]ピリミジン環が F タンパク質三量体の二つの Phe^{488} 残基の間に挟まれており、π-π相互作用を形成している（**Figure 21**、ピンク色の破線）。二つ目は、3-ヒドロキシシクロブチル

基が Asp^{486} のカルボキシル基と水素結合を形成している（**Figure 21**、黄緑色の破線）。三つ目は、ピラゾロ[1,5-a]ピリミジン環の 1 位窒素原子とアミドリンカー部位との間に分子内水素結合が形成されている（**Figure 21**、黄緑色の破線）。この分子内水素結合は、炭化水素リンカーをもつマクロサイクル化合物では観察されていなかった新たな相互作用である。したがって、アミドリンカー化合物が炭化水素リンカー化合物よりも良好な抗 RSV 活性を示した一つの要因として、この分子内水素結合形成によるコンフォメーション固定化の寄与が示唆される。

第 8 節　まとめ

本章では臨床開発が困難な Class 2 アトロプ異性体混合物である 15 員環マクロサイクル化合物を、16 員環マクロサイクル化合物に環拡大することによりアトロプ異性体混合物を回避する戦略を立てた。また、アトロプ異性体混合物の回避と合わせて、良好な抗 RSV 活性を目指してリンカー部位の最適化を検討した。リンカー部位に種々の極性官能基を導入した 16 員環マクロサイクル化合物を合成した結果、強力な抗 RSV 活性を有する 16 員環アミドリンカー誘導体 **76c** を見出した。化合物 **76c** は、NMR 解析からアトロプ異性体間の回転半減期が約 2 秒の Class 1 非アトロプ異性体混合物に分類された。また、アミドリンカー化合物は分子動力学的研究から、ピラゾロ[1,5-a]ピリミジン環 1 位窒素原子とリンカー部位のアミド基との間で分子内水素結合を形成していることが示唆された。この分子内水素結合が抗 RSV 活性の向上に寄与していると考察した。最後にアミドリンカー化合物 **76c** のピラゾロ[1,5-a]ピリミジン環 5 位とベンゼン環 5 位の置換基（R_1、R_2）をそれぞれ最適化した結果、野生株 A2 および耐性変異株 D486N のいずれに対しても強力な抗 RSV 活性を示し、アトロプ異性体混合物を回避したマクロサイクル化合物 **93b** の創出に至った。化合物 **93b** は良好な動態プロファイルを示すことから、新たな RSV 感染症治療薬として優れたポテンシャルを有する。

結論

RSV は世界中に広く分布しており、繰り返し人類に感染し、呼吸器感染症を引き起こす身近な原因ウイルスの一つである。乳幼児、高齢者、および免疫不全者では重症化しやすく、RSV 感染症関連の死亡率はインフルエンザの死亡率よりも高い。現在のところ、心臓や肺に基礎疾患をもつハイリスク小児患者に適応可能な palivizumab（Synagis®）が予防抗体として承認されているのみで、RSV 感染症に対する有効な治療薬やワクチンはないことから、安全かつ有効な新しい RSV 治療薬の開発が望まれている。

RSV F タンパク質はウイルス表面に存在し、ウイルスが宿主細胞に侵入する際に膜融合を引き起こすウイルス因子である。F タンパク質はウイルス側にのみ存在し、ウイルスの感染・増殖の初期段階である宿主細胞への侵入を司る。F タンパク質を標的とした阻害物質は、安全で有効な新規 RSV 感染症治療薬になりうると考え、本研究を開始した。また、今後、薬剤耐性化の懸念のある耐性変異株 D486N に対しても有効性を示す新規 RSV F タンパク質阻害物質の創製を目指した。

第 1 章ではピラゾロ[1,5-a]ピリミジン骨格を有する化合物 **1** を基に新規骨格を有する RSV F タンパク質阻害物質の創出を検討した。化合物 **1** のピラゾロ[1,5-a]ピリミジン環とアミド結合平面との間の 2 面角分布に着目し、様々な 2 面角分布を示す非環状鎖誘導体をデザイン・合成し、2 面角分布と抗 RSV 活性の関係を検証した。その結果、ピペリジン環をもつ化合物 **1** と類似の 2 面角分布を示す非環状鎖誘導体が良好な抗 RSV 活性を有することを見出し、新規骨格をもつ RSV F タンパク質阻害物質 1-メチルアミノプロピル誘導体 **7** を得た。化合物 **7** をリード化合物とし、Ar 部位の最適化およびピラゾロ[1,5-a]ピリミジン環 7 位への置換基導入を検討した結果、強力な抗 RSV 活性を有する化合物 **20f**（EC_{50} = 0.15 nM）を見出した。しかしながら、1-メチルアミノプロピル誘導体は化合物 **1** と同様に耐性変異株 D486N に活性を示さなかった。

第 2 章では耐性変異株 D486N に対する活性改善を目的とし、ドッキングシミュレーションを利用したドラッグデザインを実施した。シミュレーション結果から、1-メチルアミノプロピル誘導体 **20k** のベンゾイル部位 2 位とピラゾロ[1,5-a]ピリミジン環 7 位を架橋することにより活性コンフォメーションを固定化す

ることで、耐性変異株 D486N に対する活性が改善されるという仮説を立て、検証した。その結果、15 員環を有するマクロサイクル化合物 **39h** は耐性変異株 D486N に対しても強力な活性を示した（A2 EC_{50}: 2.0 nM、D486N EC_{50}: 8.1 nM）。耐性変異株 D486N に対する活性が改善した理由について考察するため、ドッキングシミュレーションにより化合物 **39h** と D486N タンパク質との結合様式を解析した結果、マクロサイクル化により分子構造が活性コンフォメーションに固定化され、導入した炭化水素リンカー部位が新たな疎水性相互作用を獲得していることが示唆された。15 員環マクロサイクル化合物 **39h** は耐性変異株 D486N に対して有効であることが示された初めての低分子 RSV F タンパク質阻害物質である。しかしながら、二次元 EXSY 法を用いた NMR 測定の結果、**39h** は Class 2 アトロプ異性体混合物に分類された。アトロプ異性体混合物の創薬開発は、各ステージでその異性体比を確認する必要があることから非常に困難である。アトロプ異性体の回避が新たな課題となった。

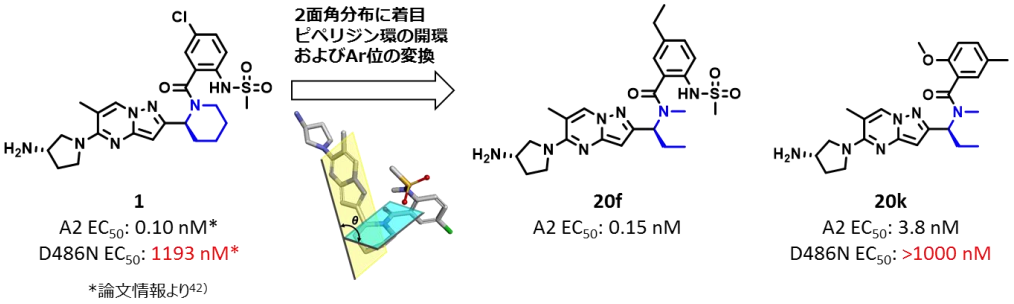
第 3 章では臨床開発が困難な Class 2 アトロプ異性体混合物である 15 員環マクロサイクル化合物を、16 員環マクロサイクル化合物に環拡大することにより、環全体の立体的制約を軽減し、アミド結合の回転障害を改善することで Class 2 アトロプ異性体混合物を回避する戦略を立てた。リンカー部位に種々の極性官能基を導入した 16 員環マクロサイクル化合物をデザイン・合成した結果、強力な抗 RSV 活性をもち、アトロプ異性体混合物を回避した 16 員環アミドリンカー誘導体 **76c** を見出した。化合物 **76c** をリード化合物としてピラゾロ[1,5-a]ピリミジン環 5 位とベンゼン環 5 位の置換基を最適化した結果、野生株 A2 および耐性変異株 D486N のいずれに対しても強力な抗 RSV 活性を示し、アトロプ異性体混合物を回避したマクロサイクル化合物 **93b** に至った。化合物 **93b** は良好な動態プロファイルを有しており、新たな RSV 感染症治療薬の候補化合物である。

本論文は、既知 RSV F タンパク質阻害物質が耐性変異株 D486N に対して無効であるという課題を、ドッキングシミュレーションを利用した精緻なドラッグデザインと合成により克服した初めての報告例である。また、化合物 **93b** を見出すに至るまでの構造活性相関は、今後の RSV F タンパク阻害物質の創薬研究の進展に貢献するものと考えられる。さらに、分子をマクロサイクル化することにより、複数のタンパク質に対して有効な薬剤を創出する合成戦略は、他の標的分子への創薬応用が期待される。マクロサイクル化合物のアトロプ異性体回避

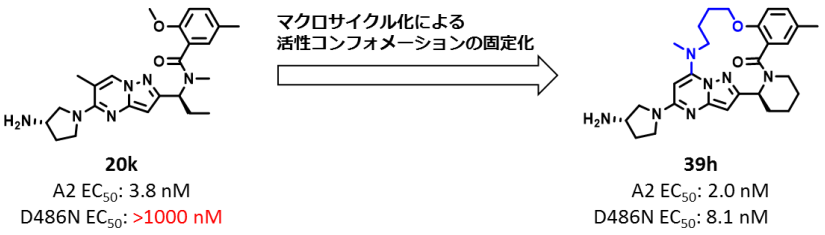
戦略と得られた知見についても、今後の創薬における留意点と解決策を提示するものとなった。

本研究において、野生株だけでなく耐性変異株 D486N に対しても有効な化合物が取得できることを、世界に先駆けて提示するとともに、高活性化合物 **93b** の取得に至った。化合物 **93b** が、薬剤耐性の問題も解決しうる新規 RSV 治療薬となることが期待される。

第 1 章　2 面角分布に着目した 1-メチルアミノプロピル誘導体の創出



第 2 章　耐性変異株 D486N に有効なマクロサイクル化合物の創出



第 3 章　アトロプ異性体混合物を回避した 16 員環アミドリンカー誘導体の創出

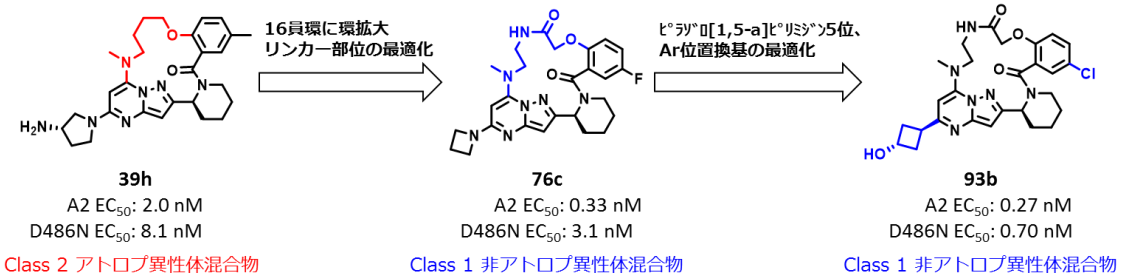


Figure 22. 本研究の概要

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実験の部

Chemistry

All solvents and reagents were purchased from commercial suppliers and used without purification or were prepared according to published procedures. The ^{1}H-NMR and ^{13}C-NMR spectra of compounds synthesized in this study were recorded using a JNM-ECA600, JNM-ECA500 (JEOL Ltd., Tokyo, Japan), or Avance III HD 400 (Bruker Corp., Billerica, MA, USA), and the chemical shifts were expressed in δ (:) values, with tetramethylsilane as the internal standard (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and brs = broad singlet). Two sets of NMR signals were observed because of structural variations of the *cis*- and *trans*-amide rotamers. Mass spectra were recorded on a Micromass Platform LC (Micromass Ltd., Manchester, UK) or Shimadzu LCMS-2010EV (Shimadzu Corp., Kyoto, Japan). High-resolution (HR) mass spectral data were acquired using an LCMS-IT-TOF equipped with an electrospray ionization (ESI)/atmospheric pressure chemical ionization (APCI) dual ion source (Shimadzu Corp.). Intermediates and final compounds were purified using preparative HPLC and an Agilent 1260 Infinity/Agilent 6130 (Agilent Technologies Inc., Santa Clara, CA, USA) or a GX-281, UV/VIS-155, 331 PUMP, 332 PUMP, or SOFTA Model 300S ELSD (Gilson Inc., Middleton, WI, USA) under the following conditions: column, Sunfire prep C18 OBD (5.0 μm, 30 mm × 50 mm) (Waters Corp., Milford, MA, USA), YMC-Actus Triart C18 (5.0 μm, 30 mm × 50 mm) (YMC Co., Ltd., Kyoto, Japan), Xbridge Prep C18 OBD (5.0 μm, 30 mm × 50 mm) (Waters Corp.), or XSelect CSH C18 (5.0 μm, 30 mm × 50 mm) (Waters Corp.); flow, 50 mL/min; linear gradient, 10%−95% acetonitrile in water containing 0.1% formic acid for 7.5−11.5 min; detection wavelength, 254 nm. The purity of the synthesized compounds was determined using an LC-MS system (Agilent 1290 Infinity, Agilent Technologies Inc.) under the following conditions: column, ACQUITY UPLC CSH C18 (1.7 μm, 2.1 × 50 mm) (Waters Corp.); flow, 0.8 mL/min; linear gradient, 20%–99% acetonitrile in water containing 0.1% formic acid in 1.2 min; detection wavelength, 254 nm. All final compounds had a purity of ≥95%.

第 1 章

tert-Butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)ethyl]methylcarbamate (11b)

To a solution of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine (6.0 g, 29.3 mmol) in toluene (18 mL)–methanol (12 mL) was added 2.0 M of trimethylsilyl diazomethane in diethyl ether (22.0 mL, 43.9 mmol) at 0 °C, and the mixture was stirred for 1 h at room temperature. Acetic acid was added to the reaction solution until the solution became clear. The reaction mixture was basified with saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alaninate (6.5 g, 100%) as a colorless oil, which was used for the next reaction without further purification.

A 1.9 M of sodium bis(trimethylsilyl)amide in tetrahydrofuran (68 mL, 88.6 mmol) was added to a solution of acetonitrile (4.6 mL, 88.6 mmol) in tetrahydrofuran (75 mL) at −78 °C. After stirring at −50 °C for 20 min, methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alaninate (6.0 g, 29.5 mmol) in tetrahydrofuran (45 mL) at −78 °C was added to the mixture. After stirring at −50 °C for 1 h, acetic acid (5.2 mL, 91.5 mmol) at −78 °C was added to the mixture. The mixture was poured into saturated aqueous ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to obtain *tert*-butyl (*S*)-(4-cyano-3-oxobutan-2-yl)methylcarbamate (7.2 g, crude) as a brown oil, which was used for the next reaction without further purification.

To a solution of hydrazine monohydrate (4.1 mL, 84.8 mmol), acetic acid (4.9 mL, 84.8 mmol), and ethanol (20 mL) was added *tert*-butyl (*S*)-(4-cyano-3-oxobutan-2-yl)methylcarbamate (6.0 g, 28.3 mmol) in ethanol (20 mL) at 0 °C, and the mixture was stirred for 2 d at room temperature. Then, the reaction mixture was concentrated under reduced pressure and diluted with chloroform. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0%–10% methanol in chloroform) to obtain **11b** (5.8 g, 23.9 mmol, 85%) as a brown gum.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.44 (d, 3H, J = 7.0 Hz), 1.48 (s, 9H), 2.65 (s, 3H),

5.36 (brs, 1H), 5.55 (brs, 1H); MS (ESI/APCI dual) *m/z*: 241 $[M+H]^+$.

***tert*-Butyl [(5-amino-1*H*-pyrazol-3-yl)methyl]methylcarbamate (11a)**

The title compound **11a** was synthesized according to the procedure described for **11b** from **9a** (56% yield).

Brown gum; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.41–1.57 (m, 9H), 2.75–3.01 (m, 3H), 4.26 (brs, 2H), 5.59 (s, 1H); MS (ESI/APCI dual) *m/z*: 227 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)propyl]methylcarbamate (11c)**

The title compound **11c** was synthesized according to the procedure described for **11b** from **9c** (86% yield).

Orange amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.90–1.02 (m, 4H), 1.48 (s, 9H), 1.74–1.95 (m, 2H), 2.63 (s, 3H), 3.62 (brs, 2H), 4.96−5.14 (m, 1H), 5.54 (s, 1H); MS (ESI/APCI dual) *m/z*: 255 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)-2-methylpropyl]methylcarbamate (11d)**

The title compound **11d** was synthesized according to the procedure described for **11b** from **9d** (96% yield).

Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.93 (d, 3H, J = 15.4 Hz), 0.94 (d, 3H, J = 15.4 Hz), 1.46 (brs, 9H), 2.22 (brs, 1H), 2.67 (s, 3H), 3.43–3.77 (m, 2H), 4.52–4.63 (m, 1H), 5.55 (s, 1H); MS (ESI/APCI dual) *m/z*: 269 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)propyl]carbamate (11e)**

The title compound **11e** was synthesized according to the procedure described for **11b** from **9e** (61% yield).

Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 0.95−1.06 (m, 3H), 1.43–1.46 (m, 9H), 1.63−1.77 (m, 1H), 1.83–1.98 (m, 1H), 4.35−4.58 (m, 1H), 4.61–4.90 (m, 1H), 5.49 (m, 1H); MS (ESI/APCI dual) *m/z*: 241 $[M+H]^+$; chiral HPLC, 99% *ee* (CHIRALPAK IC-3 5 μm 4.6 mm × 250 mm; flow, 1 mL/min, 20% ethanol in hexane; detection wavelength, 254 nm), (*S*)-isomer t_R = 6.62 min, (*R*)-isomer t_R = 5.13 min.

***tert*-Butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)ethyl]carbamate (11f)**

The title compound **11f** was synthesized according to the procedure described for **11b** from **9f** (79% yield).

Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.43−1.49 (m, 12H), 4.71–4.81 (m, 1H), 4.85 (brs, 1H), 5.49 (s, 1H), 5.64 (brs, 1H); MS (ESI/APCI dual) *m/z*: 227 $[M+H]^+$.

***tert*-Butyl methyl[(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)ethyl]carbamate (12b)**

To a solution of **11b** (1.7 g, 6.87 mmol) in *N*,*N*-dimethylformamide (10 mL) was added ethyl (*E*)-3-ethoxy-2-methylacrylate (1.6 g, 10.3 mmol) and cesium carbonate (6.7 g, 20.6 mmol). After stirring at 120 °C for 8 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0%–10% methanol in chloroform) to obtain **12b** (1.4 g, 4.41 mmol, 64%) as a brown amorphous.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.49 (s, 9H), 1.51 (d, 3H, J = 7.0 Hz), 2.10 (s, 3H), 2.67 (brs, 3H), 5.28–5.62 (m, 1H), 5.78 (s, 1H), 8.01 (s, 1H), 10.85 (brs, 1H); MS (ESI/APCI dual) *m/z*: 307 $[M+H]^+$.

***tert*-Butyl methyl[(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)methyl]carbamate (12a)**

The title compound **12a** was synthesized according to the procedure described for **12b** from **11a** (24% yield).

Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.39–1.54 (m, 9H), 2.09 (s, 3H), 2.78–2.95 (m, 3H), 4.31–4.51 (m, 2H), 5.72–5.90 (m, 1H), 7.96 (s, 1H), 10.77 (brs, 1H); MS (ESI/APCI dual) *m/z*: 291 $(M-H)^-$.

***tert*-Butyl methyl[(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]carbamate (12c)**

The title compound **12c** was synthesized according to the procedure described for **12b**

from **11c** (65% yield).

Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.92–1.01 (m, 3H), 1.49 (s, 9H), 1.78–1.93 (m, 1H), 1.99–2.13 (m, 4H), 2.67 (s, 3H), 5.01–5.41 (m, 1H), 5.79 (brs, 1H), 8.00 (s, 1H), 10.81 (brs, 1H); MS (ESI/APCI dual) *m/z*: 321 $[M+H]^+$.

tert-Butyl methyl[(1*S*)-2-methyl-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]carbamate (12d)

The title compound **12d** was synthesized according to the procedure described for **12b** from **11d** (77% yield).

Brown powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.91 (d, 3H, J = 6.5 Hz), 0.97 (d, 3H, J = 6.5 Hz), 1.47 (brs, 9H), 2.10 (s, 3H), 2.31–2.45 (m, 1H), 2.71 (s, 3H), 4.64–5.03 (m, 1H), 5.71–5.90 (m, 1H), 8.01 (s, 1H), 10.08–10.27 (m, 1H); MS (ESI/APCI dual) *m/z*: 335 $[M+H]^+$.

tert-Butyl [(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]carbamate (12e)

To a solution of **11e** (8.3 g, 34.5 mmol) in EtOH (120 mL) was added (*E*)-ethyl-3-ethoxy-2-methyl acrylate (7.65 g, 48.4 mmol) and 2.94 M sodium ethoxide in EtOH (47.0 mL, 138.2 mmol), and the mixture stirred at 90 °C for 8 h. The reaction mixture was added acetic acid (11.8 mL, 207.2 mmol), concentrated under reduced pressure and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel colomn chromatography (50%–100% ethyl acetate in hexane) to obtain **12e** (5.8 g, 18.9 mmol, 27%) as pale yellow powder.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 0.88–0.97 (m, 3H), 1.39–1.51 (m, 9H), 1.78 (dt, 1H, J = 13.8, 7.1 Hz), 1.90 (dd, 1H, J = 13.6, 7.0 Hz), 2.07–2.14 (m, 3H), 4.72 (d, 1H, J = 6.2 Hz), 5.06 (d, 1H, J = 7.0 Hz), 5.82 (s, 1H), 7.98 (d, 1H, J = 0.8 Hz), 10.81 (brs, 1H); MS (ESI/APCI dual) *m/z*: 307 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)ethyl]carbamate (12f)**

The title compound **12f** was synthesized according to the procedure described for **12e** from **11f** (22% yield).

Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.50 (d, 3H, J = 6.6 Hz), 2.09 (s, 3H), 4.88 (brs, 1H), 5.06 (brs, 1H), 5.81 (s, 1H), 7.96 (s, 1H), 10.23 (brs, 1H); MS (ESI/APCI dual) *m/z*: 293 $[M+H]^+$.

2-{(1*S*)-1-[{5-Chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (14b)

To a solution of **12b** (717 mg, 2.34 mmol) and pyridine (0.95 mL, 11.7 mmol) in chloroform (8.0 mL) was added trifluoromethanesulfonic anhydride (0.79 mL, 4.68 mmol) at 0 °C, and the mixture was stirred for 3.5 h at room temperature. The reaction mixture was poured into saturated aqueous ammonium chloride and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5%–100% ethyl acetate in hexane) to obtain 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (0.87 g, 1.98 mmol, 84%) as a colorless powder.

To a solution of 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (0.86 g, 1.96 mmol) in 1,4-dioxane (3.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (10 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **13b** (1.1 g, 2.60 mmol, 100%) as a pale yellow oil. This compound was used for the next reaction without further purification.

To a solution of **13b** (0.81 g, 1.96 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (0.59 g, 2.36 mmol) in *N*,*N*-dimethylformamide (10 mL) was added 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (1.1 g, 2.95 mmol) and triethylamine (1.7 mL, 11.8 mmol). After stirring at room temperature for 15 h, the reaction mixture was added to water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure.

The residue was purified by silica gel column chromatography (10%–100% ethyl acetate in hexane) to obtain **14b** (0.71 g, 1.24 mmol, 63%) as a colorless amorphous.
^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.68 (d, 1.4H, J = 7.4 Hz), 1.72 (d, 1.6H, J = 7.4 Hz), 2.39 (s, 1.8H), 2.40 (s, 1.2H), 2.66 (s, 1.6H), 2.80 (s, 1.4H), 2.96 (s, 1.8H), 3.05 (s, 1.2H), 5.00 (q, 0.5H, J = 7.4 Hz), 6.31 (q, 0.5H, J = 7.4 Hz), 6.45 (s, 0.5H), 6.46 (s, 0.5H), 7.28–7.34 (m, 1H), 7.35–7.42 (m, 1H), 7.62 (d, 0.5H, J = 8.7 Hz), 7.71 (d, 0.5H, J = 8.7 Hz), 8.79 (s, 1H), 8.93 (s, 0.5H), 9.10 (s, 0.5H).

2-{[{5-Chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]methyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (14a)

The title compound **14a** was synthesized according to the procedure described for **14b** from **12a** (75% yield).
Pale yellow oil; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 2.36–2.42 (m, 3H), 2.83–3.14 (m, 6H), 4.58 (brs, 0.6H), 5.02 (brs, 1.4H), 6.37–6.51 (m, 1H), 7.15–7.78 (m, 3H), 8.72–8.87 (m, 1H), 9.08 (s, 0.7H), 10.09 (s, 0.3H).

5-Chloro-*N*-[(1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)propyl]-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (14c)

To a solution of **12c** (1.6 g, 5.12 mmol) in 1,4-dioxane (15 mL) was added 4 M hydrogen chloride in 1,4-dioxane (15 mL) and the mixture was stirred for 0.5 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain (*S*)-6-methyl-2-[1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (1.3 g, 5.90 mmol, 100%) as a colorless powder, which was used for the next reaction without further purification.

The mixture of (*S*)-6-methyl-2-[1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (1.3 g, 5.90 mmol) and phosphorus oxychloride (30 mL) was stirred at 100 °C for 3 h. Then, the reaction mixture was concentrated under reduced pressure to obtain **13c** (1.4 g, 5.91 mmol, 100%) as black oil, which was used for the next reaction without further purification.

To a solution of **13c** (1.4 g, 5.91 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (1.8 g, 7.08 mmol) in *N*,*N*-dimethylformamide (15 mL) was added 1-

[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (2.9 g, 7.67 mmol) and triethylamine (6.0 mL, 59.0 mmol). After stirring at room temperature for 2 h, the reaction mixture was added to water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20%–65% ethyl acetate in hexane) to obtain **14c** (0.88 g, 1.88 mmol, 32%) as a colorless amorphous.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 1.07–1.20 (m, 3H), 1.86–2.35 (m, 4H), 2.36–2.44 (m, 3H), 2.60 (s, 1H), 2.78 (s, 2H), 2.96 (s, 1H), 3.04 (s, 2H), 4.69–4.78 (m, 0.5H), 6.03–6.13 (m, 0.5H), 6.40–6.55 (m, 1H), 7.24–7.32 (m, 2H), 7.33–7.43 (m, 1H), 7.56–7.77 (m, 1H), 8.81 (s, 0.5H), 8.92 (s, 0.5H), 9.08 (s, 0.5H), 10.52 (brs, 0.5H); MS (ESI/APCI dual) *m/z*: 470 $[M+H]^+$.

5-Chloro-*N*-[(1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)-2-methylpropyl]-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (14d)

The title compound **14d** was synthesized according to the procedure described for **14c** from **12d** (29% yield).

Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.95–1.34 (m, 6H), 2.35–2.53 (m, 4H), 2.55 (s, 1H), 2.73 (s, 2H), 2.94–3.03 (m, 3H), 4.32–4.44 (m, 0.7H), 5.78–5.90 (m, 0.3H), 6.52–6.63 (m, 1H), 7.17–7.50 (m, 2H), 7.59–7.74 (m, 1H), 8.36–8.49 (m, 0.3H), 8.83 (brs, 0.7H), 9.00 (brs, 0.3H), 10.51–10.81 (m, 0.7H); MS (ESI/APCI dual) *m/z*: 484 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}ethyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide hydrochloride (6)**

To a solution of **14b** (0.70 g, 1.23 mmol) and (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate (0.46 g, 2.46 mmol) in tetrahydrofuran (10 mL) was added triethylamine (0.87 mL, 6.16 mmol). After stirring at 80 °C for 1 h, the reaction mixture was added to water and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography (5%–100% ethyl acetate in hexane) to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (0.73 g, 1.21 mmol, 98%) as a colorless amorphous.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.60 (d, 2H, J = 7.0 Hz), 1.64 (d, 1H, J = 7.0 Hz), 1.86–1.97 (m, 1H), 2.16–2.27 (m, 1H), 2.35–2.39 (m, 3H), 2.63 (s, 1H), 2.81 (s, 2H), 2.95 (s, 1H), 3.03 (s, 2H), 3.50–3.58 (m, 1H), 3.69–3.76 (m, 1H), 3.77–3.85 (m, 1H), 3.90–3.97 (m, 1H), 4.24–4.34 (m, 1H), 4.67 (brs, 1H), 4.88 (q, 0.5H, J = 7.0 Hz), 5.97–6.04 (m, 1H), 6.23 (q, 0.5H, J = 7.0 Hz), 7.27–7.31 (m, 1H), 7.33–7.40 (m, 1H), 7.61 (d, 0.6H, J = 8.7 Hz), 7.69 (d, 0.4H, J = 8.7 Hz), 8.36 (s, 1H), 8.64 (s, 0.5H), 9.40 (s, 0.5H); MS (ESI/APCI dual) *m/z*: 606 $[M+H]^+$.

To a solution of *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (0.69 g, 1.14 mmol) in 1,4-dioxane (6.9 mL) was added 4 M hydrogen chloride in 1,4-dioxane (6.9 mL) and the mixture was stirred for 5 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **6** (0.62 g, 1.14 mmol, 100%) as a colorless powder.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.59 (d, 1.8H, J = 7.0 Hz), 1.64 (d, 1.2H, J = 7.0 Hz), 1.76–1.86 (m, 1H), 2.11–2.22 (m, 1H), 2.37 (s, 1.2H), 2.38 (s, 1.8H), 2.62 (s, 1.2H), 2.81 (s, 1.8H), 2.95 (s, 1.2H), 3.03 (s, 1.8H), 3.46 (dd, 1H, J = 10.9, 4.3 Hz), 3.66–3.78 (m, 2H), 3.88 (dd, 2H, J = 10.3, 4.5 Hz), 4.88 (q, 0.6H, J = 6.6 Hz), 5.98 (s, 0.6H), 5.99 (s, 0.4H), 6.22 (d, 0.4H, J = 7.0 Hz), 7.22–7.32 (m, 1H), 7.32–7.40 (m, 1H), 7.61 (d, 0.6H, J = 8.7 Hz), 7.68 (d, 0.4H, J = 8.7 Hz), 8.34 (s, 0.6H), 8.61 (s, 0.4H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 15.63, 17.66, 29.22, 31.35, 40.05, 40.54, 46.73, 48.73, 52.61, 89.81, 107.96, 126.69, 126.77, 129.67, 129.99, 132.40, 133.49, 134.61, 147.03, 155.29, 155.73, 166.67; HRMS ESI/APCI dual *m/z* calcd for $C_{22}H_{28}ClN_7O_3S$ $[M+H]^+$: 506.1736, found: 506.1711.

_N_-({5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}methyl)-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide hydrochloride (5)

The title compound **5** was synthesized according to the procedure described for **6** from **14a** (80% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 2.02–2.13 (m, 1H), 2.19–2.30 (m, 1H), 2.35 (s, 3H), 2.82 (s, 1.5H), 2.93 (s, 1.5H), 3.00–3.06 (m, 3H), 3.71–3.80 (m, 2H), 3.82–3.90 (m, 2H), 3.90–3.99 (m, 1H), 4.37 (s, 1H), 4.75 (s, 1H), 6.05 (s, 0.5H), 6.10 (s, 0.5H), 7.42–7.56 (m, 3H), 8.33–8.39 (m, 3H), 8.45 (s, 0.5H), 8.50 (s, 0.5H), 9.27 (s, 0.5H), 9.65 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 18.05, 29.64, 37.12, 40.47, 45.00, 47.19, 49.12, 53.04, 90.16, 108.93, 126.41, 127.18, 127.54, 130.17, 133.06, 133.59, 135.07, 147.41, 152.54, 155.67, 167.05; HRMS ESI/APCI dual *m/z* calcd for $C_{21}H_{26}ClN_7O_3S$ $[M+H]^+$: 492.1579, found: 492.1564.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide hydrochloride (7)

The title compound **7** was synthesized according to the procedure described for **6** from **14c** (80% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.91 (brs, 1.5H), 1.03 (t, 1.5H, J = 7.4 Hz), 1.82–2.11 (m, 3H), 2.12–2.30 (m, 2H), 2.35 (s, 1.5H), 2.37 (s, 1.5H), 2.58 (s, 1.5H), 2.72 (brs, 1.5H), 2.97–3.06 (m ,3H), 3.71–3.82 (m, 2H), 3.82–3.90 (m, 2H), 3.90–3.98 (m, 1H), 4.54 (dd, 0.5H, J = 9.9, 5.0 Hz), 5.73 (dd, 0.5H, J = 10.3, 5.4 Hz), 6.13 (s, 1H), 7.36–7.57 (m, 3H), 8.34 (brs, 3H), 8.51 (s, 0.5H), 9.21 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.27, 17.24, 21.98, 28.8, 30.83, 39.63, 40.15, 46.32, 48.31, 52.17, 89.59, 107.62, 126.15, 126.63, 129.23, 129.81, 131.78, 133.55, 134.17, 146.53, 154.51, 154.89, 167.01; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{30}ClN_7O_3S$ $[M+H]^+$: 520.1892, found: 520.1873.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}-2-methylpropyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (8)**

The title compound **8** was synthesized according to the procedure described for **6** from **14d** (70% yield).

Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 0.73–1.10 (m, 6H), 2.00–2.13 (m, 1H), 2.19–2.31 (m, 1H), 2.31–2.40 (m, 3H), 2.60–2.81 (m, 3H), 2.92–3.05 (m, 3H), 3.69–3.99 (m, 5H), 4.09–4.21 (m, 0.5H), 5.42–5.52 (m, 0.5H), 6.13 (s, 0.5H), 6.26 (brs, 0.5H), 7.21–7.61 (m, 3H), 8.27–8.40 (m, 3H), 8.50 (s, 0.5H), 8.99 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 17.53, 19.36, 20.55, 27.56, 29.21, 31.74, 40.75, 46.76, 48.72, 52.59, 57.27, 90.95, 108.27, 126.52, 128.73, 129.58, 130.68, 132.17, 134.60, 135.35, 146.34, 153.92, 155.28, 167.06; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{32}ClN_7O_3S$ $[M+H]^+$: 534.2049, found: 534.2023.

***tert*-Butyl [(3*S*)-1-{2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (15a)**

To a solution of **12e** (5.4 g, 17.6 mmol) in methanol (30 mL) was added 4 M hydrogen chloride in 1,4-dioxane (30 mL) and the mixture was stirred for 2 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain 2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (4.7 g) as a brown powder, which was used for the next reaction without further purification.

The mixture of 2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (4.5 g, 18.5 mmol) and phosphorus oxychloride (85 g, 556 mmol) was stirred at 100 °C for 2 h. Then, the reaction mixture was concentrated under reduced pressure to obtain (1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)propan-1-amine (**13e**) (5.0 g, 19.2 mmol) as a brown amorphous, which was used for the next reaction without further purification.

To a solution of **13e** (4.8 g, 18.4 mmol) in methanol (100 mL) was added (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate (17.1 g, 91.9 mmol) and triethylamine (20 mL, 147 mmol). After stirring at 65 °C for 4 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (50%–100% ethyl acetate in hexane; 20% methanol in chloroform) to obtain **15a** (4.5 g, 12.0 mmol, 65%) as a brown amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 0.88–0.99 (m, 3H), 1.45 (s, 9H), 1.72–1.97 (m, 3H), 2.16–2.25 (m, 1H), 2.33 (s, 3H), 3.53 (dd, 1H, J = 10.7, 3.7 Hz), 3.71 (ddd, 1H, J = 10.7, 7.8, 5.8 Hz), 3.80 (dt, 1H, J = 10.7, 7.4 Hz), 3.88–3.97 (m, 2H), 4.30 (brs, 1H), 4.71 (brs, 1H), 5.91–6.09 (m, 1H), 8.02 (s, 1H); MS (ESI/APCI dual) *m/z*: 375 $[M+H]^+$.

tert-Butyl [(3*S*)-1-{2-[(1*S*)-1-aminoethyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (15b)

The title compound **15b** was synthesized according to the procedure described for **15a** from **12f** (49% yield).

Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 1.41–1.50 (m, 12H), 1.89–1.95 (m, 1H), 2.18–2.24 (m, 1H), 2.33 (m, 3H), 3.53 (dd, 1H, J = 11.4, 3.9 Hz), 3.68–3.74 (m, 1H), 3.77–3.83 (m, 1H), 3.88–3.95 (m, 1H), 4.20 (q, 1H, J = 6.9 Hz), 4.30 (brs, 1H), 4.69 (brs, 1H), 6.02 (s, 1H), 8.02 (s, 1H); MS (ESI/APCI dual) *m/z*: 361 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-ethyl-2-[(methanesulfonyl)amino]benzamide hydrochloride (3)**

To a solution of **15a** (50 mg, 0.134 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added potassium carbonate (46 mg, 0.334 mmol) and iodoethane (0.011 mL, 0.134 mmol). After stirring at 80 °C for 1 h, 5-chloro-2-(methylsulfonamido)benzoic acid (40 mg, 0.160 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (66 mg, 0.174 mmol) and triethylamine (0.093 mL, 0.668 mmol) were added to the reaction mixture. After stirring at room temperature for 16 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(ethyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (**16a**) (13 mg, 0.021 mmol, 15%) as colorless powder.

To a solution of **16a** (16 mg, 0.025 mmol) in 1,4-dioxane (1.0 mL) was added 4 M

hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **3** (15 mg, 0.026 mmol, 100%) as a colorless powder.

^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.80–0.98 (m, 6H), 1.96–2.30 (m, 4H), 2.32–2.40 (m, 3H), 2.51–2.64 (m, 3H), 2.98–3.05 (m, 2H), 3.69–3.97 (m, 5H), 4.47–4.55 (m, 1H), 6.15–6.21 (m, 1H), 7.45–7.56 (m, 3H), 8.21 (brs, 3H), 8.52 (s, 0.5H), 9.20 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.85, 13.44, 17.86, 29.22, 36.84, 40.05, 40.80, 46.70, 48.73, 52.57, 58.64, 90.56, 107.98, 126.35, 127.32, 127.70, 129.49, 129.61, 133.63, 134.55, 146.97, 154.69, 155.59, 161.22; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{32}ClN_7O_3S$ $[M+H]^+$: 534.2049, found: 534.2037.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}ethyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-propylbenzamide hydrochloride (4)

The title compound **4** was synthesized according to the procedure described for **3** from **15b** (24% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.53 (t, 1H, J = 7.2 Hz), 0.77 (t, 2H, J = 7.4 Hz), 1.21–1.54 (m, 4H), 1.57 (d, 2H, J = 7.0 Hz), 1.66 (d, 1H, J = 7.0 Hz), 2.00–2.10 (m, 1H), 2.20–2.30 (m, 1H), 2.34–2.38 (m, 3H), 2.85–2.98 (m, 2H), 3.04 (s, 3H), 3.81–3.96 (m, 5H), 4.74–4.82 (m, 0.7H), 5.73 (brs, 0.3H), 6.13 (s, 1H), 7.43–7.58 (m, 3H), 8.18 (brs, 3H), 8.52 (s, 0.5H), 9.34 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 11.49, 17.04, 17.76, 21.44, 29.22, 40.68, 43.95, 46.72, 48.71, 52.59, 52.71, 89.79, 107.84, 125.19, 126.43, 127.48, 129.53, 129.61, 132.18, 134.35, 146.95, 155.45, 156.56, 166.85; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{32}ClN_7O_3S$ $[M+H]^+$: 534.2049, found: 534.2011.

***tert*-Butyl [(3_S_)-1-{6-methyl-2-[(1_S_)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (19a)**

To a solution of **15a** (4.3 g, 11.4 mmol) in methanol (48 mL) was added ethyl 2,2,2-trifluoroacetate (2.0 g, 14.8 mmol) and triethylamine (2.5 mL, 18.2 mmol). After stirring overnight at room temperature, the reaction mixture was concentrated under reduced

pressure to obtain *tert*-butyl [(3*S*)-1-{6-methyl-2-[(1*S*)-1-(2,2,2-trifluoroacetamido)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (**18a**) (6.5 g, crude) as a pale yellow powder, which was used for the next reaction without further purification.

To a solution of **18a** (5.4 g, 11.5 mmol) in *N*,*N*-dimethylformamide (50 mL) was added iodomethane (2.2 mL, 34.4 mmol) and cesium carbonate (15 g, 45.9 mmol). After stirring at 65 °C for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain *tert*-butyl [(3*S*)-1-(6-methyl-2-{(1*S*)-1-[methyl(trifluoroacetyl)amino]propyl}pyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (5.60 g, crude) as a pale yellow oil, which was used for the next reaction without further purification.

To a solution of *tert*-butyl [(3*S*)-1-(6-methyl-2-{(1*S*)-1-[methyl(trifluoroacetyl)amino]propyl}pyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (5.5g, 11.4 mmol) in tetrahydrofuran (40 mL)–methanol (40 mL) was added 1 M aqueous sodium hydroxide (40 mL). After stirring for 1 h at room temperature, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **19a** (4.3 g, 11.1 mmol, 98%) as a brown amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 0.88 (t, 3H, J = 7.4 Hz), 1.45 (s, 9H), 1.68–1.98 (m, 3H), 2.14–2.25 (m, 1H), 2.28–2.42 (m, 6H), 3.48–3.63 (m, 2H), 3.66–3.85 (m, 2H), 3.87–3.98 (m, 1H), 4.30 (brs, 1H), 4.59–4.78 (m, 1H), 5.91–6.07 (m, 1H), 7.96–8.10 (m, 1H); MS (ESI/APCI dual) *m/z*: 389 $[M+H]^+$.

Benzyl [(3*S*)-1-{2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate

The title compound **17** was synthesized according to the procedure described for **15a** from **12e** (50% yield).

MS (ESI/APCI dual) *m/z*: 409 $[M+H]^+$.

Benzyl [(3*S*)-1-{6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (19b)

The title compound **19b** was synthesized according to the procedure described for **19a** from **17** (43% yield).

Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.82–0.94 (m, 3H), 1.70–1.90 (m, 2H), 1.90–2.01 (m, 1H), 2.17–2.29 (m, 1H), 2.32 (s, 3H), 2.36 (s, 3H), 3.53–3.62 (m, 2H), 3.66–3.87 (m, 2H), 3.88–4.00 (m, 1H), 4.30–4.41 (m, 1H), 4.86–4.97 (m, 1H), 5.12 (s, 2H), 6.03 (s, 1H), 7.28–7.41 (m, 5H), 8.04 (s, 1H); MS (ESI/APCI dual) *m/z*: 423 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide hydrochloride (20a)**

To a solution of **19a** (10 mg, 0.026 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added 3-chloro-2-(methylsulfonamido)benzoic acid (6.4 mg, 0.031 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (13 mg, 0.034 mmol), and triethylamine (0.018 mL, 0.13 mmol). After stirring at room temperature for 3 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[3-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (12 mg, 0.019 mmol, 72%) as a pale yellow powder.

To a solution of *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[3-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (12 mg, 0.019 mmol) in 1,4-dioxane(1.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **20a** (10 mg, 0.019 mmol, 100%) as a colorless powder.

^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.79–1.05 (m, 3H), 1.84–2.30 (m, 4H), 2.33–2.42 (m, 3H), 2.43–2.59 (m, 3H), 2.98–3.17 (m, 3H), 3.68–4.01 (m, 5H), 4.37 (brs, 0.5H), 5.75 (brs, 0.5H), 6.06–6.25 (m, 1H), 7.23–7.67 (m, 3H), 8.17–8.27 (m, 3H), 8.51 (s, 0.5H), 9.43 (brs, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.43, 17.48, 22.81, 29.28, 31.23, 40.05, 42.69, 46.68, 48.77, 52.55, 90.10, 108.14, 125.89, 128.84, 129.39, 130.20,

130.25, 131.90, 134.53, 146.89, 151.11, 154.89, 166.59; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{30}ClN_7O_3S$ $[M+H]^+$: 520.1892, found: 520.1872.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-_N_-methylbenzamide hydrochloride (20c)

The title compound **20c** was synthesized according to the procedure described for **20a** from **19a** (52% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.85–1.06 (m, 3H), 1.86–2.30 (m, 4H), 2.33–2.40 (m, 3H), 2.57 (s, 1.5H), 2.73 (brs, 1.5H), 3.02 (s, 3H), 3.76 (brs, 2H), 3.82–3.89 (m, 2H), 3.90–3.99 (m, 1H), 4.55 (brs, 0.5H), 5.77 (dd, 0.5H, J = 9.7, 5.6 Hz), 6.12 (s, 1H), 7.26–7.39 (m, 2H), 7.41–7.52 (m, 2H), 8.39 (brs, 3H), 8.51 (s, 0.5H), 9.08 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.71, 17.70, 22.43, 29.24, 31.31, 40.47, 46.81, 48.69, 52.63, 58.26, 89.95, 108.04, 125.11, 125.91, 127.07, 127.70, 129.81, 133.33, 134.73, 146.83, 155.15, 155.47, 169.04; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{31}N_7O_3S$ $[M+H]^+$: 486.2282, found: 486.2257.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-_N_-methylbenzamide hydrochloride (20d)

The title compound **20d** was synthesized according to the procedure described for **20a** from **19a** (54% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.75–0.85 (m, 2H), 0.95–1.03 (m, 1H), 1.85–2.13 (m, 3H), 2.20–2.29 (m, 1H), 2.36 (s, 3H), 2.65 (s, 1H), 2.77 (s, 2H), 3.72–3.80 (m, 2H), 3.82–3.88 (m, 2H), 3.91–3.97 (m, 1H), 4.67–4.73 (m, 0.5H), 5.72–5.81 (m, 0.5H), 6.04 (s, 1H), 7.37–7.51 (m, 5H), 8.43 (brs, 3H), 8.51–8.59 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.51, 17.44, 23.49, 29.22, 40.05, 46.72, 48.73, 52.57, 58.40, 89.61, 108.32, 126.61, 128.38, 129.14, 134.65, 136.92, 146.71, 155.11, 155.35, 171.21; HRMS ESI/APCI dual *m/z* calcd for $C_{22}H_{28}N_6O$ $[M+H]^+$: 393.2397, found: 393.2393.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-_N_,5-dimethylbenzamide hydrochloride (20e)

The title compound **20e** was synthesized according to the procedure described for **20a** from **19a** (76% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.86–0.95 (m, 1.5H), 0.99–1.06 (m, 1.5H), 1.84–2.29 (m, 4H), 2.29–2.38 (m, 6H), 2.56 (s, 1.5H), 2.72 (brs, 1.5H), 2.96 (s, 3H), 3.70–4.10 (m, 5H), 4.51–4.58 (m, 0.5H), 5.74–5.80 (m, 0.5H), 6.11 (s, 1H), 7.14 (s, 1H), 7.22–7.29 (m, 1H), 7.31–7.38 (m, 1H), 8.35 (brs, 3H), 8.51 (s, 0.5H), 8.97 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.68, 17.63, 20.31, 22.38, 29.21, 31.20, 40.04, 40.28, 46.74, 48.68, 52.6, 89.92, 107.99, 125.64, 127.10, 130.22, 130.52, 132.59, 134.60, 135.69, 146.82, 155.14, 155.26, 169.05; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{33}N_7O_3S$ $[M+H]^+$: 500.2438, found: 500.2414.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-_N_-methyl-5-propylbenzamide hydrochloride (20h)

The title compound **20h** was synthesized according to the procedure described for **20a** from **19a** (60% yield).

Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 0.81–1.06 (m, 6H), 1.51–1.64 (m, 2H), 1.83–2.31 (m, 6H), 2.32–2.38 (m, 3H), 2.57 (s, 1.5H), 2.72 (brs, 1.5H), 2.98 (s, 3H), 3.45–3.96 (m, 5H), 4.48–4.58 (m, 0.5H), 5.73–5.81 (m, 0.5H), 6.11 (s, 1H), 7.14 (s, 1H), 7.22–7.31 (m, 1H), 7.33–7.41 (m, 1H), 8.19–8.35 (m, 3H), 8.51 (s, 0.5H), 8.95 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.72, 13.53, 17.63, 22.40, 23.92, 29.21, 31.22, 36.32, 40.04, 40.34, 46.71, 48.70, 52.56, 89.94, 107.99, 125.54, 126.54, 127.33, 129.56, 130.80, 133.88, 140.55, 146.96, 154.66, 155.58, 169.15; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{37}N_7O_3S$ $[M+H]^+$: 528.2751, found: 528.2731.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-_N_-methyl-5-(propan-2-yl)benzamide hydrochloride (20i)

The title compound **20i** was synthesized according to the procedure described for **20a** from **19a** (68% yield).

Pale pink amorphous; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.87–0.96 (m, 1.5H), 1.00–1.07 (m, 1.5H), 1.13–1.24 (m, 6H), 1.84–2.30 (m, 4H), 2.34–2.38 (m, 3H), 2.56 (s, 1.5H), 2.72 (brs, 1.5H), 2.86–3.02 (m, 4H), 3.70–4.07 (m, 5H), 4.47–4.56 (m, 0.5H), 5.73–5.81 (m, 0.5H), 6.12 (s, 1H), 7.13–7.24 (m, 1H), 7.29–7.40 (m, 2H), 8.36 (brs, 3H), 8.51 (s, 0.5H), 8.98 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.52, 17.65, 22.44, 23.66, 23.72, 29.23, 31.22, 32.71, 40.04, 40.42, 46.72, 48.70, 52.58, 89.98, 108.01, 124.63, 125.26, 125.72, 127.45, 130.82, 132.51, 134.58, 146.94, 155.14, 155.52, 169.19; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{37}N_7O_3S$ $[M+H]^+$: 528.2751, found: 528.2740.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-(methanesulfonyl)-_N_-methylbenzamide hydrochloride (20j)

The title compound **20j** was synthesized according to the procedure described for **20a** from **19a** (70% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.98–1.11 (m, 3H), 1.85–1.98 (m, 1H), 2.01–2.21 (m, 2H), 2.21–2.29 (m, 1H), 2.31–2.41 (m, 3H), 3.23–3.41 (m, 6H), 3.69–4.08 (m, 5H), 5.68–5.82 (m, 1H), 6.03–6.23 (m, 1H), 7.38–7.64 (m, 1H), 7.64–7.88 (m, 3H), 7.97–8.07 (m, 1H), 8.27–8.41 (m, 3H), 8.50–8.61 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.76, 17.47, 22.24, 24.39, 29.21, 31.72, 45.01, 46.94, 48.72, 52.74, 90.57, 108.19, 127.55, 129.09, 129.80, 133.60, 134.22, 134.84, 136.83, 137.66, 154.84, 155.04, 169.11; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{30}N_6O_3S$ $[M+H]^+$: 471.2173, found: 471.2179.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-methoxy-_N_,5-dimethylbenzamide hydrochloride (20k)

The title compound **20k** was synthesized according to the procedure described for **20a** from **19a** (66% yield).

Colorless amorphous; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 0.72–0.89 (m, 1.5H), 0.92–1.05 (m, 1.5H), 1.77–1.95 (m, 1H), 2.00–2.17 (m, 2H), 2.18–2.31 (m, 4H), 2.31–2.40 (m, 3H), 3.35–3.67 (m, 3H), 3.70–4.05 (m, 8H), 4.41–4.49 (m, 0.5H), 5.74–5.84 (m, 0.5H), 5.88–6.05 (m, 1H), 6.84–7.08 (m, 2H), 7.13–7.23 (m, 1H), 8.43–8.59 (m, 4H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.76, 17.47, 19.86, 23.78, 29.21, 30.05, 46.80, 48.74, 52.64, 55.18, 60.10, 89.78, 108.25, 111.31, 126.56, 129.58, 130.24, 130.30, 134.72, 134.84, 138.28, 152.55, 168.37, 171.74; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{32}N_6O_2$ $[M+H]^+$: 437.2660, found: 437.2639.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-_N_,5-dimethyl-2-(trifluoromethyl)benzamide hydrochloride (20l)

The title compound **20l** was synthesized according to the procedure described for **20a** from **19a** (58% yield).

Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 0.72–1.06 (m, 3H), 1.75–2.30 (m, 4H), 2.31–2.46 (m, 6H), 3.70–3.99 (m, 5H), 4.37–4.43 (m, 0.5H), 5.73–5.81 (m, 0.5H), 5.82–6.13 (m, 1H), 7.18–7.50 (m, 2H), 7.65–7.74 (m, 1H), 8.33–8.47 (m, 3H), 8.50–8.58 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.81, 17.46, 20.70, 22.29, 29.22, 31.25, 46.89, 48.71, 52.71, 58.60, 89.91, 108.14, 121.93, 122.15, 123.02, 124.84, 126.41, 127.58, 128.46, 129.71, 129.91, 134.71, 134.79, 135.52, 142.39, 143.37, 146.45, 154.81, 155.17, 168.11; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{29}F_3N_6O$ $[M+H]^+$: 475.2428, found: 475.2402.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-_N_-methylthiophene-2-carboxamide hydrochloride (20o)

The title compound **20o** was synthesized according to the procedure described for **20a** from **19a** (35% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.88–0.97 (m, 3H), 1.89–2.29 (m, 4H), 2.35 (s, 3H), 2.81 (brs, 3H), 3.06 (brs, 3H), 3.69–3.96 (m, 5H), 6.09 (s, 1H), 7.16 (s, 1H), 7.76 (s, 1H), 8.23 (s, 3H), 8.47 (s, 1H), 10.11 (s, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.63, 17.58, 22.95, 29.24, 35.23, 40.29, 46.68, 48.75, 52.55, 58.16,

90.22, 110.98, 119.30, 122.85, 128.06, 128.18, 134.41, 146.89, 154.71, 155.43, 164.07; HRMS ESI/APCI dual *m/z* calcd for $C_{21}H_{29}N_7O_3S_2$ $[M+H]^+$: 492.1846, found: 492.1808.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-_N_-methylthiophene-3-carboxamide hydrochloride (20p)

The title compound **20p** was synthesized according to the procedure described for **20a** from **19a** (42% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.78–1.02 (m, 3H), 1.84–2.33 (m, 4H), 2.35 (s, 3H), 2.63–2.75 (m, 3H), 3.01 (s, 3H), 3.68–4.17 (m, 5H), 4.61–4.73 (m, 0.5H), 5.65–5.78 (m, 0.5H), 6.05–6.20 (m, 1H), 7.00 (s, 1H), 7.39 (s, 1H), 8.25 (brs, 3H), 8.36–8.55 (m, 1H), 9.87 (brs, 0.5H), 10.62 (brs, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.54, 17.57, 22.86, 29.21, 31.00, 40.04, 40.63, 46.72, 48.70, 52.58, 90.37, 108.01, 120.51, 125.14, 132.95, 134.10, 136.23, 146.72, 152.13, 155.28, 165.59; HRMS ESI/APCI dual *m/z* calcd for $C_{21}H_{29}N_7O_3S_2$ $[M+H]^+$: 492.1846, found: 492.1812.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-_N_,1-dimethyl-1_H_-pyrazole-5-carboxamide hydrochloride (20r)

The title compound **20r** was synthesized according to the procedure described for **20a** from **19a** (52% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.80–0.94 (m, 1.5H), 0.95–1.05 (m, 1.5H), 1.84–2.29 (m, 4H), 2.33–2.37 (m, 3H), 2.63–2.80 (m, 3H), 2.81–2.96 (m, 3H), 3.70–3.80 (m, 5H), 3.81–3.97 (m, 3H), 4.61–4.75 (m, 0.5H), 5.67–5.75 (m, 0.5H), 6.05–6.10 (m, 0.5H), 7.47 (s, 1H), 8.21–8.30 (m, 3.5H), 8.47–8.54 (m, 0.5H), 9.07 (brs, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.70, 17.43, 24.04, 29.21, 30.09, 37.95, 40.02, 40.36, 46.69, 48.70, 52.54, 90.05, 108.17, 116.35, 119.53, 131.63, 134.50, 146.86, 154.46, 155.36, 161.29; HRMS ESI/APCI dual *m/z* calcd for $C_{21}H_{31}N_9O_3S$ $[M+H]^+$: 490.2343, found: 490.2321.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-_N_,1-dimethyl-1_H_-pyrazole-3-carboxamide hydrochloride (20s)

The title compound **20s** was synthesized according to the procedure described for **20a** from **19a** (73% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.86–0.95 (m, 3H), 1.82–2.28 (m, 4H), 2.32 (s, 3H), 2.71 (s, 1.5H), 2.92 (s, 1.5H), 2.96 (s, 3H), 3.68–3.76 (m, 2H), 3.79–3.94 (6H, m), 5.73–5.78 (m, 0.5H), 5.87–5.93 (m, 0.5H), 6.03 (s, 0.5H), 6.07 (s, 0.5H), 7.78–7.82 (m, 1H), 8.31 (brs, 3H), 8.44 (s, 0.5H), 8.49 (s, 0.5H), 9.00 (s, 0.5H), 9.15 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.45, 17.48, 22.43, 29.20, 31.19, 38.93, 40.05, 46.83, 48.73, 52.67, 56.81, 90.12, 108.28, 120.50, 125.79, 134.71, 138.31, 146.35, 155.13, 155.19, 163.51; HRMS ESI/APCI dual *m/z* calcd for $C_{21}H_{31}N_9O_3S$ $[M+H]^+$: 490.2343, found: 490.2316.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide hydrochloride (20b)

To a solution of **19a** (50 mg, 0.13 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added 4-chloroanthranilic acid (27 mg, 0.15 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (64 mg, 0.17 mmol) and triethylamine (0.090 mL, 0.64 mmol). After stirring at room temperature for 3 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl ((*S*)-1-{2-[(*S*)-1-(2-amino-4-chloro-*N*-methylbenzamido)propyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl)carbamate (52 mg, 0.10 mmol, 75%) as a colorless powder.

To a solution of *tert*-butyl ((*S*)-1-{2-[(*S*)-1-(2-amino-4-chloro-*N*-methylbenzamido)propyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl)carbamate (52 mg, 0.10 mmol) in chloroform (2.0 mL) was added triethylamine (0.14 mL, 0.97 mmol) and methanesulfonyl chloride (0.022 mL, 0.29 mmol) at 0 °C. After stirring for 10 min at 0 °C, the reaction mixture was concentrated under reduced pressure. Ethanol (2.0 mL) and 2.94 mol/L sodium ethoxide in ethanol (0.16 mL, 0.48 mmol) were

added to the residue. After stirring at room temperature for 15 min, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[4-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (40 mg, 0.065 mmol, 67%) as a colorless powder.

To a solution of *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[4-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (40 mg, 0.065 mmol) in 1,4-dioxane (1.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then the reaction mixture was concentrated under reduced pressure to obtain **20b** (36 mg, 0.063 mmol, 98%) as a colorless powder.

^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.86–0.92 (m, 1.5H), 1.02 (t, 1.5H, J = 7.2 Hz), 1.86–2.30 (m, 4H), 2.33–2.38 (m, 3H), 2.58 (s, 1.5H), 2.73 (brs, 1.5H), 3.01–3.12 (m, 3H), 3.68–4.15 (m, 5H), 4.56 (dd, 0.5H, J = 9.9, 4.5 Hz), 5.73 (dd, 0.5H, J = 10.1, 5.6 Hz), 6.11 (s, 1H), 7.39 (s, 2H), 7.49 (d, 1H, J = 11.6 Hz), 8.23 (brs, 3H), 8.51 (s, 0.5H), 9.28 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.70, 17.65, 22.40, 29.23, 31.34, 40.05, 40.77, 46.72, 48.70, 52.60, 89.98, 108.03, 124.17, 125.66, 128.83, 129.32, 130.36, 133.88, 134.58, 146.98, 154.96, 155.54, 168.14; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{30}ClN_7O_3S$ $[M+H]^+$: 520.1892, found: 520.1872.

_N_-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-ethyl-2-[(methanesulfonyl)amino]-_N_-methylbenzamide hydrochloride (20f)

The title compound **20f** was synthesized according to the procedure described for **20b** from **19a** (53% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.86–0.95 (m, 1.5H), 1.00–1.06 (m, 1.5H), 1.14–1.22 (m, 3H), 1.84–2.29 (m, 4H), 2.33–2.38 (m, 3H), 2.57 (s, 1.5H), 2.58–2.66 (m, 2H), 2.72 (brs, 1.5H), 2.97 (s, 3H), 3.71–3.99 (m, 5H), 4.50–4.57 (m, 0.5H), 5.74–5.80 (m, 0.5H), 6.12 (s, 1H), 7.16 (brs, 1H), 7.26–7.32 (m, 1H), 7.34–7.40 (m, 1H),

8.35 (brs, 3H), 8.51 (s, 0.5H), 8.98 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.51, 15.41, 17.64, 22.41, 27.37, 29.22, 31.23, 40.05, 40.35, 46.73, 48.69, 52.59, 89.95, 108.00, 125.69, 126.77, 129.02, 130.73, 132.58, 134.57, 141.87, 146.93, 155.15, 155.53, 169.12; HRMS ESI/APCI dual *m/z* calcd for $C_{25}H_{35}N_7O_3S$ $[M+H]^+$: 514.2595, found: 514.2559; chiral HPLC, 99% *ee* (CHIRALCEL OZ-3 5 μm 4.6 mm × 150 mm; flow, 1 mL/min, 60% ethanol in hexane; detection wavelength, 254 nm), (*S*)-isomer t_R = 9.37 min, (*R*)-isomer t_R = 7.31 min.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-5-methoxy-_N_-methylbenzamide hydrochloride (20g)

The title compound **20g** was synthesized according to the procedure described for **20b** from **19a** (30% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.85–0.97 (m, 1.5H), 0.99–1.06 (m, 1.5H), 1.82–2.30 (m, 4H), 2.33–2.38 (m, 3H), 2.56 (s, 1.5H), 2.71 (brs, 1.5H), 2.87–2.99 (m, 3H), 3.72–3.81 (m, 5H), 3.82–3.89 (m, 2H), 3.90–3.98 (m, 1H), 4.47–4.58 (m, 0.5H), 5.73–5.80 (m, 0.5H), 6.09–6.16 (m, 1H), 6.78–6.92 (m, 1H), 6.97–7.06 (m, 1H), 7.31–7.39 (m, 1H), 8.36 (brs, 3H), 8.51 (s, 0.5H), 8.96 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.73, 17.62, 22.47, 29.22, 31.13, 40.05, 40.21, 46.72, 48.69, 52.59, 55.55, 89.99, 108.04, 111.66, 112.38, 115.00, 125.39, 128.76, 134.55, 135.23, 146.93, 155.33, 157.56, 168.62; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{33}N_7O_4S$ $[M+H]^+$: 516.2387, found: 516.2378.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-_N_-methylpyridine-4-carboxamide hydrochloride (20m)

The title compound **20m** was synthesized according to the procedure described for **20b** from **19a** (37% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.84–0.93 (m, 1.5H), 0.97–1.05 (m, 1.5H), 1.86–2.30 (m, 4H), 2.34–2.39 (m, 3H), 2.58 (s, 1.5H), 2.75 (brs, 1.5H), 3.05–3.12 (m, 3H), 3.69–3.99 (m, 5H), 4.44–4.49 (m, 0.5H), 5.70–5.77 (m, 0.5H), 6.12–

6.17 (m, 1H), 7.44–7.50 (m, 1H), 8.27–8.33 (m, 3H), 8.34–8.38 (m, 0.5H), 8.51–8.53 (m, 0.5H), 8.53–8.57 (m, 1H), 8.66–8.72 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.72, 17.61, 22.40, 29.21, 31.12, 34.11, 41.21, 46.84, 48.70, 52.62, 90.09, 108.15, 122.60, 123.47, 134.64, 142.44, 144.43, 146.60, 146.84, 154.54, 155.18, 165.99; HRMS ESI/APCI dual *m/z* calcd for $C_{22}H_{30}N_8O_3S$ $[M+H]^+$: 487.2234, found: 487.2217.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-_N_-methylpyridine-2-carboxamide hydrochloride (20n)

The title compound **20n** was synthesized according to the procedure described for **20b** from **19a** (33% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.88–0.94 (m, 1.5H), 0.98–1.06 (m, 1.5H), 1.87–2.30 (m, 4H), 2.32–2.38 (m, 3H), 2.59 (s, 1.5H), 2.75 (s, 1.5H), 3.06–3.11 (m, 3H), 3.65–3.97 (m, 5H), 4.55–4.61 (m, 0.5H), 5.72–5.80 (m, 0.5H), 6.10–6.16 (m, 1H), 7.46–7.55 (m, 1H), 7.87–7.93 (m, 1H), 8.24 (brs, 3H), 8.36 (s, 0.5H), 8.44–8.49 (m, 1H), 8.52 (s, 0.5H), 9.39 (s, 0.5H), 10.18 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.62, 17.77, 22.40, 26.96, 29.19, 30.86, 40.89, 46.86, 48.74, 52.64, 90.09, 108.07, 124.69, 133.52, 134.70, 145.55, 145.88, 146.62, 147.95, 155.14, 155.36, 167.38; HRMS ESI/APCI dual *m/z* calcd for $C_{22}H_{30}N_8O_3S$ $[M+H]^+$: 487.2234, found: 487.2200.

_N_-[(1_S_)-1-{5-[(3S)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-_N_-methyl-1,2-thiazole-5-carboxamide hydrochloride (20q)

The title compound **20q** was synthesized according to the procedure described for **20b** from **19a** (34% yield).

Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ ppm 0.84–0.91 (m, 1.5H), 0.95–1.01 (m, 1.5H), 1.88–2.31 (m, 4H), 2.35 (s, 3H), 2.73 (s, 1.5H), 2.78 (s, 1.5H), 3.07 (s, 3H), 3.68–3.95 (m, 5H), 4.98–5.04 (m, 0.5H), 5.70–5.76 (m, 0.5H), 6.09–6.14 (m, 1H), 8.21 (brs, 3H), 8.44 (s, 0.5H), 8.52 (s, 0.5H), 8.81 (s, 0.5H), 8.86 (s, 0.5H), 9.62 (s, 0.5H), 9.93 (s, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.63, 17.50, 22.45, 29.22, 31.05, 40.05, 46.72, 48.75, 52.57, 57.66, 90.44, 108.10, 132.16, 134.59, 138.59, 141.04, 146.71,

154.57, 155.31, 163.77; HRMS ESI/APCI dual *m/z* calcd for $C_{20}H_{28}N_8O_3S_2$ $[M+H]^+$: 493.1799, found: 493.1780.

tert-Butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-amino-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (21a)

To a solution of **19a** (50 mg, 0.129 mmol) and 2-amino-5-chloro-benzoic acid (24 mg, 0.142 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added triethylamine (0.090 mL, 0.64 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (59 mg, 0.154 mmol). After stirring at room temperature for 1 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain **21a** (60 mg, 0.117 mmol, 91%) as a colorless amorphous.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.87–1.19 (m, 3H), 1.46 (s, 9H), 1.85–2.30 (m, 4H), 2.35 (s, 3H), 2.83 (brs, 3H), 3.49–3.60 (m, 1H), 3.66–3.86 (m, 2H), 3.88–3.99 (m, 1H), 4.23–4.48 (m, 3H), 4.67 (brs, 1H), 6.07 (s, 1H), 6.58–6.68 (m, 1H), 7.04–7.14 (m, 1H), 7.22–7.30 (m, 1H), 8.04 (s, 1H); MS (ESI/APCI dual) *m/z*: 542 $[M+H]^+$.

Benzyl [(3*S*)-1-(2-{(1*S*)-1-[(2-amino-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (21b)

The title compound **21b** was synthesized according to the procedure described for **21a** from **19b** (96% yield).

Colorless amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.85–1.15 (m, 3H), 1.88–2.29 (m, 4H), 2.32 (s, 3H), 2.70–2.96 (m, 3H), 3.52–3.63 (m, 1H), 3.67–3.87 (m, 2H), 3.89–4.01 (m, 1H), 4.28–4.43 (m, 1H), 4.89–5.01 (m, 1H), 5.12 (s, 2H), 6.06 (s, 1H), 6.58–6.67 (m, 1H), 7.04–7.22 (m, 1.5H), 7.29–7.41 (m, 5.5H), 8.04 (s, 1H); MS (ESI/APCI dual) *m/z*: 576 $[M+H]^+$.

2-Acetamido-*N*-[(1*S*)-1-{5-[(3*S*)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-methylbenzamide hydrochloride (22)

To a solution of **21a** (42 mg, 0.081 mmol) in chloroform (3.0 mL) was added acetic anhydride (0.022 mL, 0.24 mmol) and pyridine (0.033 mL, 0.40 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure

and purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-acetamido-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.045 mmol, 60%) as a colorless amorphous.

To a solution of *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-acetamido-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (24 mg, 0.045 mmol) in chloroform (0.50 mL) was added trifluoroacetic acid (0.50 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **22** (25 mg, 0.052 mmol, 100%) as a colorless amorphous.

^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 0.79–0.99 (m, 1.5H), 1.03–1.13 (m, 1.5H), 1.74–1.83 (m, 1H), 1.89–2.08 (m, 2H), 2.10–2.26 (m, 4H), 2.34–2.42 (m, 3H), 2.73 (s, 1.5H), 2.91 (s, 1.5H), 3.40–3.47 (m, 1H), 3.66–3.79 (m, 2H), 3.85–3.94 (m, 2H), 4.79–4.88 (m, 0.5H), 5.90–5.97 (m, 0.5H), 5.98–6.08 (m, 1H), 7.23 (brs, 0.5H), 7.31–7.37 (m, 1H), 8.01 (brs, 1H), 8.06–8.21 (m, 1H), 8.67 (brs, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.73, 17.64, 22.23, 27.51, 31.03, 33.22, 47.49, 50.34, 52.61, 56.97, 89.77, 107.96, 126.35, 127.94, 129.02, 129.33, 133.10, 133.99, 134.15, 147.35, 154.63, 155.61, 167.37, 168.74; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{30}ClN_7O_2$ $[M+H]^+$: 484.2222, found: 484.2200.

Methyl (2-{[(1*S*)-1-{5-[(3*S*)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl](methyl)carbamoyl}-4-chlorophenyl)carbamate (23)

To a solution of **21b** (60 mg, 0.10 mmol) in pyridine (1.0 mL) was added methyl chloroformate (0.16 mL, 2.1 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10%–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methoxycarbonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.041 mmol, 40%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methoxycarbonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.041 mmol) in methanol (2.1 mL) was added 10% palladium on activated carbon (13 mg). The reaction was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through membrane filter and concentrated under reduced pressure to obtain **23** (19 mg, 0.037 mmol, 91%) as a colorless amorphous.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.79–1.12 (m, 3H), 1.83–2.28 (m, 4H), 2.28–2.38 (m, 3H), 2.67–2.74 (m, 1.5H), 2.77–2.86 (m, 1.5H), 3.56–3.67 (m, 1H), 3.70–3.80 (m, 3H), 3.82–4.06 (m, 4H), 4.67–4.82 (m, 0.5H), 5.72–5.88 (m, 0.5H), 5.91–6.07 (m, 1H), 7.19–7.24 (m, 0.5H), 7.27–7.38 (m, 1H), 7.77–8.23 (m, 2H), 8.54–8.68 (m, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.63, 17.52, 22.23, 29.60, 31.17, 40.05, 46.70, 48.88, 52.07, 52.89, 89.85, 107.94, 126.49, 126.89, 127.52, 128.20, 129.33, 133.51, 134.49, 147.19, 154.33, 154.91, 155.55, 167.57; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{30}ClN_7O_3$ $[M+H]^+$: 500.2171, found: 500.2145.

_N_-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-_N_-methyl-2-[(methylcarbamoyl)amino]benzamide (24)

To a solution of **21b** (50 mg, 0.087 mmol) in chloroform (0.87 mL) was added pyridine (0.028 mL, 0.347 mmol) and 4-nitrophenyl chloroformate (19 mg, 0.095 mmol). After stirring at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure.

To a solution of the residue in chloroform (0.87 mL) was added 2.0 M methylamine / tetrahydrofuran (0.071 mL, 0.694 mmol). After stirring at room temperature for 2 h, the reaction mixture was added to water and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10%–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylcarbamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (44 mg, 0.070 mmol, 81%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-

[(methylcarbamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (44 mg, 0.070 mmol) in methanol (3.5 mL) was added 10% palladium on activated carbon (22 mg). The reaction was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through a membrane filter and concentrated under reduced pressure to obtain **24** (30 mg, 0.060 mmol, 85%) as a colorless amorphous.
^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.82–1.15 (m, 3H), 1.75–1.88 (m, 1H), 1.88–2.23 (m, 3H), 2.32–2.41 (m, 3H), 2.62–2.92 (m, 6H), 3.41–3.53 (m, 1H), 3.67–3.79 (m, 2H), 3.82–3.94 (m, 2H), 4.69–4.85 (m, 0.5H), 4.93–5.07 (m, 0.5H), 5.51–5.64 (m, 0.5H), 5.83–5.94 (m, 0.5H), 5.95–6.10 (m, 1H), 7.10–7.34 (m, 2H), 7.97–8.21 (m, 2H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.79, 17.66, 23.45, 26.02, 31.39, 33.24, 47.49, 50.36, 57.01, 58.84, 89.37, 107.94, 123.00, 124.34, 124.83, 125.91, 126.13, 129.00, 134.15, 147.25, 154.47, 155.43, 155.65, 167.99; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{31}ClN_8O_2$ $[M+H]^+$: 499.2331, found: 499.2306.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-_N_-methyl-2-[(methylsulfamoyl)amino]benzamide (25)

To a solution of chlorosulfonyl isocyanate (0.020 mL, 0.231 mmol) in chloroform (1.2 mL) was added 2-chloroethanol (0.031 mL, 0.462 mmol). After stirring at room temperature for 1 h, the reaction mixture was added triethylamine (0.032 mL, 0.231 mmol) and **21b** (67 mg, 0.115 mmol). After stirring at room temperature for 6 h, the reaction mixture was concentrated under reduced pressure.

To a solution of the residue in chloroform (1.2 mL) was added triethylamine (0.048 mL, 0.346 mmol) and 2.0 M methylamine / tetrahydrofuran (0.46 mL, 0.923 mmol). After stirring at 120 °C under microwave irradiation for 30 min, the reaction mixture was added to water and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10%–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylsulfamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (23 mg, 0.035 mmol, 30%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylsulfamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (22 mg, 0.032 mmol) in methanol (1.6 mL) was added 10% palladium on activated carbon (11 mg). The reaction mixture was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through a membrane filter and concentrated under reduced pressure to obtain **25** (16 mg, 0.030 mmol, 92%) as a colorless amorphous.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 1.00–1.16 (m, 3H), 1.77–2.23 (m, 4H), 2.34–2.40 (m, 3H), 2.60–2.65 (m, 1.5H), 2.71 (s, 2H), 2.75–2.83 (m, 2.5H), 3.39–3.54 (m, 1H), 3.67–3.78 (m, 2H), 3.82–3.95 (m, 2H), 4.60–4.70 (m, 1H), 5.92–6.04 (m, 1H), 7.22–7.25 (m, 0.5H), 7.30–7.39 (m, 1H), 7.63–7.74 (m, 1H), 8.36 (brs, 0.5H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.63, 17.87, 22.35, 28.17, 31.21, 31.97, 47.21, 49.86, 52.90, 55.67, 89.61, 107.84, 122.29, 126.45, 127.28, 129.51, 130.19, 133.57, 134.35, 147.29, 154.47, 155.69, 167.59; HRMS ESI/APCI dual *m/z* calcd for $C_{23}H_{31}ClN_8O_3S$ $[M+H]^+$: 535.2001, found: 535.1982.

***tert*-Butyl [(1*S*)-1-(7-hydroxy-6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]methylcarbamate (26)**

To a solution of **11c** (0.55 g 2.16 mmol) in ethanol (10 mL) was added diethyl 2-methylmalonate (0.55 mL, 3.24 mmol) and 2.94 M sodium ethoxide in ethanol (3.7 mL, 10.81 mmol), and the mixture was stirred at 90 °C for 5 h. The reaction mixture was concentrated under reduced pressure. The residue was acidified with 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was added to diethyl ether, precipitated, and collected to obtain **26** (0.62 g, 1.92 mmol, 89%) as a pale yellow powder.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.90–1.01 (m, 3H), 1.49 (s, 9H), 1.69–1.75 (m, 3H), 1.83–1.97 (m, 1H), 2.10–2.22 (m, 1H), 2.67 (s, 3H), 3.58–3.69 (m, 1H), 4.98–5.28 (m, 1H), 5.73 (brs, 1H), 9.16 (brs, 1H); MS (ESI/APCI dual) *m/z*: 337 $[M+H]^+$.

(1*S*)-1-(5,7-Dichloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)-*N*-methylpropan-1-amine hydrochloride (27)

The mixture of **26** (4.6 g, 13.6 mmol) and phosphorus oxychloride (13 mL, 136.1 mmol) was stirred at 110 °C for 3 h, and the reaction mixture was concentrated under reduced pressure. After purifying by silica gel column chromatography (NH, 1%–5% methanol in chloroform), 4 M hydrogen chloride in ethyl acetate was added to the residue and the mixture was concentrated under reduced pressure to obtain **27** (1.8 g, 5.77 mmol, 42%) as a colorless powder.

^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 0.73–0.87 (m, 3H), 1.94–2.20 (m, 2H), 2.45 (s, 3H), 2.48 (s, 3H), 4.34–4.46 (m, 1H), 7.05 (s, 1H), 9.38 (brs, 2H); MS (ESI/APCI dual) *m/z*: 273 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methyl-7-(methylamino)pyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (29a)**

To a solution of **27** (0.12 g, 0.388 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) was added 9.8 M methylamine /methanol (0.20 mL, 1.94 mmol) and sodium bicarbonate (0.33 g, 3.88 mmol). After stirring at room temperature for 22 h, the reaction mixture was added to 20% aqueous potassium carbonate and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure to obtain 5-chloro-*N*,6-dimethyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (**28a**) (0.10 g, 0.372 mmol, 96%) as a colorless powder.

To a solution of **28a** (97 mg, 0.361 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (99 mg, 0.397 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added triethylamine (0.25 mL, 1.80 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (0.17 g, 0.433 mmol). After stirring at room temperature for 1 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain 5-chloro-*N*-{(1*S*)-1-[5-chloro-6-methyl-7-(methylamino)pyrazolo[1,5-a]pyrimidin-2-yl]propyl}-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (0.13 g, 0.904 mmol, 90%) as a colorless powder.

To a solution of 5-chloro-*N*-{(1*S*)-1-[5-chloro-6-methyl-7-(methylamino)pyrazolo[1,5-

a]pyrimidin-2-yl]propyl}-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (0.12 g, 0.252 mmol) in 1-methyl-2-pyrrolidone (1.0 mL) was added triethylamine (0.35 mL, 2.52 mmol) and (*S*)-pyrrolidin-3-amine (0.11 mL, 1.26 mmol). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was purified by reversed-phase preparative HPLC to obtain **29a** (41 mg, 0.30 mmol, 30%) as a pale yellow powder.
^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.94–1.02 (m, 3H), 1.74–2.19 (m, 4H), 2.23 (s, 3H), 2.80 (s, 3H), 2.97 (s, 3H), 3.21–3.28 (m, 3H), 3.29–3.38 (m, 1H), 3.52–3.81 (m, 4H), 4.49–4.60 (m, 1H), 6.03 (s, 1H), 6.07–6.16 (m, 1H), 7.32–7.42 (m, 2H), 7.52–7.58 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.81, 14.95, 22.85, 27.45, 31.63, 32.72, 40.49, 47.77, 49.96, 52.49, 55.29, 84.19, 90.42, 123.82, 126.23, 127.44, 128.60, 129.12, 133.28, 146.87, 148.18, 154.13, 159.73, 169.12; HRMS ESI/APCI dual *m/z* calcd for $C_{24}H_{33}ClN_8O_3S$ $[M+H]^+$: 549.2158, found: 549.2143.

_N_-[(1_S_)-1-{5-[(3_S_)-3-Aminopyrrolidin-1-yl]-7-(dimethylamino)-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide (29b)

The title compound **29b** was synthesized according to the procedure described for **29a** from **27** (10% yield).
Pink powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.96–1.04 (m, 3H), 1.67–1.77 (m, 1H), 1.93–2.06 (m, 2H), 2.09–2.20 (m, 4H), 2.86–2.95 (m, 6H), 3.11–3.19 (m, 6H), 3.26–3.34 (m, 1H), 3.57–3.70 (m, 4H), 3.74–3.85 (m, 0.3H), 4.58–4.69 (m, 0.7H), 6.05 (s, 1H), 7.24–7.33 (m, 1H), 7.36–7.41 (m, 1H), 7.58–7.63 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.83, 15.57, 22.73, 30.73, 31.87, 40.54, 41.38, 47.59, 50.02, 52.59, 55.49, 58.32, 90.22, 94.88, 124.36, 126.29, 127.12, 128.66, 129.02, 133.45, 148.28, 149.48, 153.91, 159.41, 168.98; HRMS ESI/APCI dual *m/z* calcd for $C_{25}H_{35}ClN_8O_3S$ $[M+H]^+$: 563.2314, found: 563.2274.

_N_-[(1_S_)-1-(5-[(3_S_)-3-Aminopyrrolidin-1-yl]-7-{[2-(methanesulfonyl)ethyl]amino}-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide (29e)

The title compound **29e** was synthesized according to the procedure described for **29a**

from **27** (22% yield).

Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.99 (brs, 3H), 1.40–2.18 (m, 4H), 2.24 (brs, 3H), 2.81 (brs, 3H), 2.97 (brs, 3H), 3.01 (brs, 3H), 3.21–4.09 (m, 9H), 4.53 (brs, 1H), 6.06 (brs, 2H), 7.21–7.45 (m, 2H), 7.49–7.61(m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.83, 14.57, 22.75, 30.67, 31.57, 38.61, 40.54, 40.96, 47.83, 49.92, 52.47, 54.08, 55.21, 86.26, 90.46, 124.04, 126.29, 127.22, 128.64, 129.00, 133.39, 146.29, 147.31, 154.39, 159.25, 169.08; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{37}ClN_8O_5S_2$ $[M+H]^+$: 641.2090, found: 641.2060.

_N_-[(1_S_)-1-(5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methyl-7-{[2-(methylsulfamoyl)ethyl]amino}pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide (29f)

The title compound **29f** was synthesized according to the procedure described for **29a** from **27** (27% yield).

Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.98 (t, 3H, J = 7.0 Hz), 1.70–1.82 (m, 1H), 1.94–2.06 (m, 2H), 2.08–2.19 (m, 4H), 2.21 (brs, 3H), 2.82 (brs, 6H), 2.97 (s, 3H), 3.26–3.80 (m, 7H), 3.98 (q, 2H, J = 6.6 Hz), 4.53 (t, 1H, J = 7.2 Hz), 4.82 (brs, 1H), 6.05 (s, 1H), 6.21 (brs, 1H), 7.32–7.44 (m, 2H), 7.57 (d, 1H, J = 8.7 Hz); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.83, 14.61, 22.75, 27.57, 28.52, 30.67, 31.53, 40.54, 47.83, 49.84, 49.92, 52.51, 55.19, 85.94, 90.48, 124.06, 126.29, 127.22, 128.64, 129.02, 133.39, 146.45, 147.29, 154.39, 159.31, 169.08; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{38}ClN_9O_5S_2$ $[M+H]^+$: 656.2199, found: 656.2170.

_N_-[(1_S_)-1-{7-[(2-Aminoethyl)amino]-5-[(3_S_)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide (30a)

To a solution of **27** (120 mg, 0.38 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) was added 1-Boc-ethylenediamine (0.30 mL, 1.91 mmol) and sodium bicarbonate (0.32 g, 3.81 mmol). After stirring at room temperature for 20 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced

pressure. The residue was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [2-({5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}amino)ethyl]carbamate (**28c**) (40 mg, 0.10 mmol, 27%) as a colorless amorphous.

To a solution of **28c** (40 mg, 0.10 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (30 mg, 0.12 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added triethylamine (0.070 mL, 0.50 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (50 mg, 0.13 mmol). After stirring at room temperature for 2 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl {2-[(5-chloro-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-7-yl)amino]ethyl}carbamate (33 mg, 0.052 mmol, 52%) as a colorless amorphous.

To a solution of *tert*-butyl {2-[(5-chloro-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-7-yl)amino]ethyl}carbamate (33 mg, 0.052 mmol) in 1-methyl-2-pyrrolidone (1.0 mL) was added triethylamine (0.12 mL, 0.83 mmol) and (*S*)-pyrrolidin-3-amine (0.037 mL, 0.42 mmol). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl {2-[(5-[(3*S*)-3-aminopyrrolidin-1-yl]-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-7-yl)amino]ethyl}carbamate (**29c**) (14 mg, 0.021 mmol, 40%) as a colorless powder.

To a solution of **29c** (14 mg, 0.021 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (1.0 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure to obtain **30a** (11 mg, 0.019 mmol, 91%) as a colorless powder.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.94–1.03 (m, 3H), 1.65–2.27 (m, 7H), 2.82 (s, 3H), 2.91–3.03 (m, 5H), 3.22–3.31 (m, 1H), 3.51–3.83 (m, 7H), 4.52–4.63 (m, 1H), 6.06 (s, 1H), 6.08–6.17 (m, 1H), 7.32–7.43 (m, 2H), 7.52–7.60 (m, 1H); ^{13}C-NMR (151 MHz,

DMSO-d_6) δ ppm 10.73, 13.99, 22.37, 30.22, 32.90, 40.05, 41.14, 43.59, 47.95, 50.38, 51.41, 56.97, 88.43, 90.58, 118.61, 120.78, 125.71, 126.07, 128.38, 131.26, 132.44, 146.51, 154.07, 158.99, 170.57; HRMS ESI/APCI dual *m/z* calcd for $C_{25}H_{36}ClN_9O_3S$ $[M+H]^+$: 578.2423, found: 578.2381.

_N_-[(1_S_)-1-(5-[(3_S_)-3-Aminopyrrolidin-1-yl]-6-methyl-7-{[2-(methylamino)ethyl]amino}pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-_N_-methylbenzamide (30b)

The title compound **30b** was synthesized according to the procedure described for **30a** from **27** (31% yield).

Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.94–1.02 (m, 3H), 1.70–1.79 (m, 1H), 1.94–2.24 (m, 6H), 2.48 (s, 3H), 2.83 (s, 3H), 2.86–2.99 (m, 5H), 3.22–3.31 (m, 1H), 3.53–3.81 (m, 7H), 4.52–4.65 (m, 1H), 6.02–6.12 (m, 2H), 7.32–7.43 (m, 2H), 7.53–7.59 (m, 1H); ^{13}C-NMR (151 MHz, DMSO-d_6) δ ppm 10.75, 14.19, 22.61, 30.47, 32.01, 34.41, 40.05, 42.56, 47.85, 50.06, 50.93, 51.85, 55.87, 79.14, 90.54, 122.11, 125.99, 127.20, 128.50, 128.80, 132.00, 146.75, 147.45, 154.23, 159.09, 170.04; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{38}ClN_9O_3S$ $[M+H]^+$: 592.2580, found: 592.2546.

第 2 章

Methyl 5-methyl-2-[4-(methylamino)butoxy]benzoate hydrochloride (33b)

To a solution of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (1.0 g, 4.92 mmol) in chloroform (4.9 mL) was added triethylamine (2.1 mL, 14.8 mmol, 3.0 eq) and methanesulfonyl chloride (0.57 mL, 7.38 mmol, 1.5 eq) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried over a phase separator and concentrated under reduced pressure. To a solution of the residue in *N*,*N*-dimethylformamide (9.8 mL) was added methyl 5-methylsalicylate (1.4 mL, 9.83 mmol, 2.0 eq) and potassium carbonate (2.7 g, 19.7 mmol, 4.0 eq). After stirring at 90 °C for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 5%–30% ethyl acetate

in hexane) to obtain methyl 2-{4-[*tert*-butoxycarbonyl(methyl)amino]butoxy}-5-methyl benzoate (**32b**) (1.5 g, 4.15 mmol, 84%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.67–1.85 (m, 4H), 2.30 (s, 3H), 2.86 (s, 3H), 3.24–3.33 (m, 2H), 3.87 (s, 3H), 3.98–4.07 (m, 2H), 6.82–6.88 (m, 1H), 7.20–7.25 (m, 1H), 7.56–7.60 (m, 1H); MS (ESI/APCI dual) *m/z*: 374 $[M+Na]^+$.

To a solution of **32b** (1.46 g, 3.07 mmol) in 1,4-dioxane (5.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (5.0 mL) and the mixture was stirred for 20 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **33b** (1.09 g, 3.78 mmol, 91%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.71–1.81 (m, 4H), 2.26 (s, 3H), 2.54 (s, 3H), 2.89–3.01 (m, 2H), 3.79 (s, 3H), 3.97–4.07 (m, 2H), 7.01–7.07 (m, 1H), 7.29–7.37 (m, 1H), 7.46 (s, 1H), 8.58 (brs, 2H); MS (ESI/APCI dual) *m/z*: 252 $[M+H]^+$.

Methyl 2-(4-aminobutoxy)-5-methylbenzoate hydrochloride (33e)

According to the procedure described for **33b**, the title compound was obtained as a colorless powder using *tert*-butyl *N*-(4-hydroxybutyl) carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (84% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.00–2.14 (m, 4H), 2.29 (s, 3H), 3.20 (t, J = 6.3 Hz, 2H), 3.88 (s, 3H), 4.09 (t, J = 5.1 Hz, 2H), 6.83–6.87 (m, 1 H), 7.24–7.29 (m, 1H), 7.61–7.68 (m, 1H); MS (ESI/APCI dual) *m/z*: 238 $[M+H]^+$.

Methyl 5-methyl-2-[3-(methylamino)propoxy]benzoate hydrochloride (33a)

According to the procedure described for **33b**, the title compound was obtained as a colorless oil using *tert*-butyl *N*-(3-hydroxypropyl)-*N*-methyl carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (71% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.33 (s, 3H), 2.41–2.50 (m, 2H), 2.76–2.85 (m, 3H), 3.25 (brs, 2H), 3.70 (s, 3H), 3.87 (s, 3H), 4.16–4.23 (m, 2H), 6.86 (d, J = 8.5 Hz, 1H), 7.34 (dd, J = 8.5, 1.9 Hz, 1H), 7.77 (d, J = 1.9 Hz, 1H), 9.84 (brs, 2H); MS (ESI/APCI dual) *m/z*: 238 $[M+H]^+$.

Methyl 5-methyl-2-{[5-(methylamino)pentyl]oxy}benzoate hydrochloride (33c)

According to the procedure described for **33b**, the title compound was obtained as a colorless powder using *tert*-butyl (5-hydroxypentyl)(methyl) carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (77% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.43–1.53 (m, 2H), 1.58–1.78 (m, 4H), 2.25 (s, 3H), 2.53 (s, 3H), 2.82–2.92 (m, 2H), 3.77 (s, 3H), 3.95–4.04 (m, 2H), 6.99–7.07 (m, 1H), 7.28–7.35 (m, 1H), 7.44 (s, 1H), 8.48 (brs, 2H); MS (ESI/APCI dual) *m/z*: 266 $[M+H]^+$.

Methyl 5-methyl-2-{[6-(methylamino)hexyl]oxy}benzoate hydrochloride (33d)

According to the procedure described for **33b**, the title compound was obtained as a colorless powder using *tert*-butyl *N*-(6-hydroxyhexyl)-*N*-methyl carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (77% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.31–1.50 (m, 4H), 1.55–1.73 (m, 4H), 2.25 (s, 3H), 2.84 (t, J = 7.6 Hz, 2H), 3.31 (s, 3H), 3.77 (s, 3H), 3.99 (t, J = 6.2 Hz, 2H), 7.02 (d, J = 8.5 Hz, 1H), 7.31 (dd, J = 8.5, 1.6 Hz, 1H), 7.43 (d, J = 1.6 Hz, 1H), 8.69 (brs, 2H); MS (ESI/APCI dual) *m/z*: 280 $[M+H]^+$.

5,7-Dichloro-2-[(2*S*)-piperidin-2-yl]pyrazolo[1,5-a]pyrimidine hydrochloride (36b)

To a solution of *tert*-butyl (2*S*)-2-(5-amino-1*H*-pyrazol-3-yl)piperidine-1-carboxylate (15.0 g, 56.3 mmol) in ethanol (280 mL) was added diethyl malonate (13 mL, 84.5 mmol, 1.5 eq) and 20% sodium ethoxide in ethanol (120 mL, 282 mmol, 5.0 eq), and the mixture was stirred at 90 °C for 18 h. The reaction mixture was acidified using 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain *tert*-butyl (2*S*)-2-(7-hydroxy-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)piperidine-1-carboxylate (21 g, 61.3 mmol, 100%) as a pale-yellow amorphous, which was used for the next reaction without further purification. MS (ESI/APCI dual) *m/z*: 303 $[M-H]^-$.

A solution of *tert*-butyl (2*S*)-2-(7-hydroxy-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)piperidine-1-carboxylate (21 g, 61.3 mmol) in 4 M hydrogen chloride in 1,4-dioxane (150 mL) was stirred for 3 h at room temperature. Then, the reaction mixture was

concentrated under reduced pressure to obtain 7-hydroxy-2-[(2*S*)-2-piperidyl]-4*H*-pyrazolo[1,5-a]pyrimidin-5-one hydrochloride (17 g, 63.0 mmol, 100%) as a yellow powder, which was used for the next reaction without further purification. MS (ESI/APCI dual) *m/z*: 235 $[M+H]^+$.

The mixture of 7-hydroxy-2-[(2*S*)-2-piperidyl]-4*H*-pyrazolo[1,5-a]pyrimidin-5-one hydrochloride (8.5 g, 31.0 mmol) and phosphorus oxychloride (83 mL, 910 mmol, 29 eq) was stirred at 90 °C overnight. Then, the reaction mixture was concentrated under reduced pressure and washed with 2-propanol/diisopropyl ether (1:4) to obtain **36b** (8.5 g, 28.0 mmol, 88%) as a brown powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.58–1.95 (m, 5H), 2.13–2.25 (m, 1H), 2.99–3.17 (m, 1H), 3.28–3.41 (m, 1H), 4.57–4.70 (m, 1H), 7.11 (s, 1H), 7.77 (s, 1H), 9.37–9.59 (m, 2H); MS (ESI/APCI dual) *m/z*: 271 $[M+H]^+$.

(1*S*)-1-(5,7-Dichloropyrazolo[1,5-a]pyrimidin-2-yl)-*N*-methylpropan-1-amine hydrochloride (36a)

To a solution of **11c** (6.1 g, 23.8 mmol) in methanol (45 mL) was added dimethyl propanedioate (4.1 mL, 35.7 mmol, 1.5 eq) and 28% sodium methoxide in methanol (24 mL, 119 mmol, 5.0 eq), and the mixture was stirred at 90 °C for 4 h. The reaction mixture was acidified using 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried over a phase separator and concentrated under reduced pressure to obtain *tert*-butyl *N*-[(1*S*)-1-(7-hydroxy-5-oxo-4*H*-pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-*N*-methyl carbamate (7.6 g, 23.5 mmol, 99%) as a pale-yellow amorphous, which was used for the next reaction without further purification.

MS (ESI/APCI dual) *m/z*: 323 $[M+H]^+$.

The mixture of *tert*-butyl *N*-[(1*S*)-1-(7-hydroxy-5-oxo-4*H*-pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-*N*-methyl carbamate (1.1 g, 3.46 mmol) and phosphorus oxychloride (83 mL, 910 mmol, 29 eq) was stirred at 110 °C for 2 h. Then, the reaction mixture was concentrated under reduced pressure to obtain **36a** (2.3 g, 3.53 mmol, 100%) as a brown oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.77–0.86 (m, 3H), 1.94–2.21 (m, 2H), 2.42–2.48 (m, 3H), 4.32–4.51 (m, 1H), 7.10 (s, 1H), 7.76 (s, 1H), 9.28–9.67 (m, 2H); MS (ESI/APCI

dual) *m/z*: 259 $[M+H]^+$.

Methyl 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methyl-amino)butoxy]-5-methylbenzoate (37h)

To a solution of **36b** (1.0 g, 2.93 mmol) in ethanol (29 mL) was added **33b** (1.0 g, 3.51 mmol, 1.2 eq) and triethylamine (4.1 mL, 29.3 mmol, 10 eq), and the mixture was stirred at 65 °C for 0.5 h. The reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **37h** (1.2 g, 2.43 mmol, 83%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45–1.71 (m, 4H), 1.79–2.03 (m, 6H), 2.30 (s, 3H), 2.73–2.83 (m, 1H), 3.12–3.19 (m, 1H), 3.23 (s, 3H), 3.79–3.87 (m, 4H), 3.99–4.05 (m, 2H), 4.07–4.17 (m, 2H), 5.87 (s, 1H), 6.33 (s, 1H), 6.78–6.86 (m, 1H), 7.20–7.25 (m, 1H), 7.56–7.63 (m, 1H); MS (ESI/APCI dual) *m/z*: 486 $[M+H]^+$.

(23a*S*)-18-Chloro-8,16-dimethyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one (38h)

To a solution of **37h** (1.2 g, 2.43 mmol) in methanol (10 mL) and tetrahydrofuran (10 mL) was added 1 M aqueous sodium hydroxide (15 mL) , and the mixture was stirred at 65 °C for 0.5 h. The reaction mixture was acidified using 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methylamino)butoxy]-5-methylbenzoic acid (1.3 g, 2.75 mmol, 100%) as a colorless amorphous, which was used for the next reaction without further purification.

To a solution of 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methyl-amino)butoxy]-5-methylbenzoic acid (1.2 g, 2.44 mmol) in *N*,*N*-dimethylformamide (120 mL, 0.020 M) was added triethylamine (2.7 mL, 19.5 mmol, 8.0 eq) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-

oxide hexafluorophosphate (1.9 g, 4.87 mmol, 2.0 eq). After stirring at room temperature overnight, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **38h** (1.5 g, 3.22 mmol, 100%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31–1.87 (m, 6H), 1.89–2.08 (m, 2H), 2.12–2.33 (m, 4H), 2.34–2.50 (m, 1H), 2.74–2.85 (m, 0.3H), 3.05 (s, 2.1H), 3.08 (s, 0.9H), 3.37–3.52 (m, 2.1H), 3.63–3.73 (m, 0.3H), 3.80–3.92 (m, 0.3H), 3.92–4.01 (m, 0.7H), 4.01–4.14 (m, 1H), 4.19–4.31 (m, 0.7H), 4.60–4.71 (m, 0.6H), 5.07–5.13 (m, 0.3H), 5.86 (s, 1H), 6.16 (s, 0.3H), 6.30 (s, 0.7H), 6.34–6.39 (m, 0.7H), 6.68–6.74 (m, 0.3H), 6.76–6.82 (m, 0.7H), 7.02–7.15 (m, 2H); MS (ESI/APCI dual) *m/z*: 454 $[M+H]^+$.

(23a*S*)-18-[(3*S*)-3-Aminopyrrolidin-1-yl]-8,16-dimethyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one hydrochloride (39h)

To a solution of **38h** (0.20 g, 0.441 mmol) in 1-methyl-2-pyrrolidone (2.2 mL) was added triethylamine (0.74 mL, 5.29 mmol, 12.0 eq) and *tert*-butyl *N*-[(*3S*)-pyrrolidin-3-yl]carbamate (0.49 g, 2.64 mmol, 6.0 eq). After stirring at 150 °C under microwave irradiation for 1 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (50%–100% ethyl acetate in hexane) to obtain *tert*-butyl {(3*S*)-1-[(23a*S*)-8,16-dimethyl-6-oxo-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-18-yl]pyrrolidin-3-yl}carbamate (0.18 g, 0.298 mmol, 68%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.28–2.02 (m, 18H), 2.10–2.33 (m, 5H), 2.41–2.57 (m, 1H), 2.84–2.99 (m, 3.2H), 3.01–3.13 (m, 0.8H), 3.32–3.46 (m, 2H), 3.54–3.70 (m, 3H), 3.73–3.84 (m, 1H), 3.87–3.99 (m, 1H), 4.00–4.16 (m, 2H), 4.26–4.39 (m, 1H), 4.60–4.76 (m, 1H), 4.99–5.04 (m, 0.2H), 5.06 (s, 0.2H), 5.10 (s, 0.8H), 5.82 (s, 0.2H), 5.96 (s, 0.8H), 6.27–6.35 (m, 0.8H), 6.67–6.73 (m, 0.2H), 6.74–6.80 (m, 0.8H), 7.01–7.06 (m,

0.4H), 7.07–7.13 (m, 1.6H); MS (ESI/APCI dual) *m/z*: 604 $[M+H]^+$.

The mixture of *tert*-butyl {(3*S*)-1-[(23a*S*)-8,16-dimethyl-6-oxo-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-18-yl]pyrrolidin-3-yl}carbamate (0.18 g, 0.298 mmol) and 4 M hydrogen chloride in 1,4-dioxane (3.0 mL) was stirred for 1 h at room temperature, and the reaction mixture was concentrated under reduced pressure to obtain **39h** (0.16 g, 0.296 mmol, 99%) as a colorless powder.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.20–1.34 (m, 2H), 1.36–1.79 (m, 5H), 1.83–1.92 (m, 1H), 2.01–2.11 (m, 1H), 2.12–2.24 (m, 2H), 2.26 (s, 3H), 2.33–2.41 (m, 1H), 3.15 (s, 3H), 3.25–3.32 (m, 1H), 3.37–3.43 (m, 1H), 3.44–3.94 (m, 5H), 3.95–4.06 (m, 4H), 4.85–4.88 (m, 0.2H), 5.22–5.26 (m, 1H), 6.04–6.09 (m, 0.8H), 6.19 (brs, 0.2H), 6.34 (brs, 0.8H), 6.84–6.87 (m, 0.2H), 6.95–6.98 (m, 0.8H), 7.04–7.07 (m, 0.8H), 7.07–7.09 (m, 0.2H), 7.10–7.13 (m, 0.2H), 7.15–7.18 (m, 0.8H), 8.68 (brs, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.77, 19.93, 23.49, 25.44, 26.04, 28.74, 29.36, 43.71, 46.52, 47.17, 49.18, 51.91, 54.26, 62.76, 66.30, 66.68, 88.51, 112.60, 125.11, 126.23, 127.68, 129.45, 130.39, 150.11, 150.33, 151.92, 155.94, 167.43; HRMS ESI/APCI dual *m/z* calcd for $C_{28}H_{37}N_7O_2$ $[M+H]^+$: 504.3082, found: 504.3059.

Methyl 2-{3-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]propoxy}-5-methylbenzoate (37a)

According to the procedure described for **37h**, **36a** (0.41 g, 1.0 eq) and **33a** (0.23 g, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 30%–100% ethyl acetate in hexane) to obtain **37a** (0.15 g, 48%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84–0.91 (m, 3H), 1.70–1.90 (m, 2H), 2.22–2.33 (m, 5H), 2.35 (s, 3H), 3.30 (s, 3H), 3.59–3.65 (m, 1H), 3.88 (s, 3H), 4.06–4.12 (m, 2H), 4.23–4.31 (m, 2H), 5.90 (s, 1H), 6.28 (s, 1H), 6.79–6.85 (m, 1H), 7.21–7.27 (m, 1H), 7.60–7.65 (m, 1H); MS (ESI/APCI dual) *m/z*: 460 $[M+H]^+$.

(16*S*)-3-Chloro-16-ethyl-5,12,15-trimethyl-5,6,7,8,15,16-hexahydro-14*H*-17,1-(metheno)pyrimido[6,1-f][1,5,7,8,11]benzoxatetraazacyclotetradecin-14-one (38a)

According to the procedure described for **38h**, the title compound **38a** was obtained (0.11 g, 87%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.10–1.16 (m, 3H), 1.84–2.27 (m, 4H), 2.30 (s, 3H), 2.69 (s, 3H), 3.13 (s, 3H), 3.89–3.97 (m, 1H), 4.03–4.11 (m, 1H), 4.18–4.30 (m, 1H), 5.01–5.13 (m, 1H), 5.82 (s, 1H), 6.11–6.19 (m, 1H), 6.30 (s, 1H), 6.68–6.75 (m, 1H), 7.08–7.16 (m, 2H); MS (ESI/APCI dual) *m/z*: 428 $[M+H]^+$.

(16*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-16-ethyl-5,12,15-trimethyl-5,6,7,8,15,16-hexahydro-14*H*-17,1-(metheno)pyrimido[6,1-f][1,5,7,8,11]benzoxatetraazacyclotetradecin-14-one (39a)

To a solution of **38a** (40 mg, 0.093 mmol) in 1-methyl-2-pyrrolidone (0.93 mL) was added triethylamine (0.13 mL, 0.935 mmol, 10.0 eq) and (*S*)-3-aminopyrolidine (0.041 mL, 0.467 mmol, 5.0 eq). After stirring at 150 °C under microwave irradiation for 1 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane to 5% methanol in chloroform) to obtain **39a** (12 mg, 0.025 mmol, 27%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.08–1.15 (m, 3H), 1.75–2.26 (m, 6H), 2.26–2.32 (m, 3H), 2.63 (s, 2.7H), 2.80 (s, 0.3H), 2.89 (s, 0.3H), 3.03 (s, 2.7H), 3.23–3.33 (m, 1H), 3.50-3.61 (m, 1H), 3.63–3.79 (m, 3H), 3.86–3.96 (m, 1H), 3.97–4.05 (m, 1H), 4.09–4.21 (m, 1H), 4.56–4.72 (m, 1H), 5.06 (s, 0.9H), 5.26 (s, 0.1H), 5.96 (s, 0.1H), 5.99 (s, 0.9H), 6.04–6.10 (m, 1H), 6.67–6.73 (m, 0.9H), 6.84–6.90 (m, 0.1H), 7.06–7.13 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.51, 19.89, 22.71, 28.38, 30.10, 33.98, 38.16, 44.96, 49.42, 50.66, 51.81, 54.97, 64.85, 76.79, 88.27, 111.06, 126.73, 127.68, 129.14, 130.11, 148.72, 151.43, 152.14, 154.19, 155.27, 169.18; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{35}N_7O_2$ $[M+H]^+$: 478.2925, found: 478.2915.

Methyl 2-{4-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]butoxy}-5-methylbenzoate (37b)

According to the procedure described for **37h**, **36a** (0.80 g, 1.0 eq) and **33b** (0.94 g, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **37b** (0.60 g, 47%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84–0.90 (m, 3H), 1.72–1.89 (m, 4H), 1.91–2.01 (m, 2H), 2.30 (s, 3H), 2.34 (s, 3H), 3.24 (s, 3H), 3.58–3.65 (m, 1H), 3.84 (s, 3H), 3.99–4.05 (m, 2H), 4.10–4.18 (m, 2H), 5.86 (s, 1H), 6.28 (s, 1H), 6.80–6.84 (m, 1H), 7.20–7.25 (m, 1H), 7.57–7.61 (m, 1H); MS (ESI/APCI dual) *m/z*: 474 $[M+H]^+$.

(17*S*)-3-Chloro-17-ethyl-5,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (38b)

According to the procedure described for **38h**, the title compound **38b** was obtained (0.59 g, 100%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.95–1.04 (m, 1H), 1.10–1.21 (m, 2H), 1.64–2.26 (m, 5H), 2.28–2.31 (m, 3H), 2.32–2.53 (m, 1H), 2.60 (s, 2H), 2.80 (s, 1H), 3.08 (s, 2H), 3.10 (s, 1H), 3.20–3.30 (m, 0.6H), 3.64–3.74 (m, 0.4H), 3.77–3.87 (m, 0.4H), 3.87–3.96 (m, 0.6H), 4.02–4.12 (m, 1H), 4.53–4.64 (m, 0.4H), 4.67–4.79 (m, 1H), 5.80–5.85 (m, 1H), 6.12–6.21 (m, 1H), 6.26 (s, 0.4H), 6.34 (s, 0.6H), 6.73–6.81 (m, 1H), 7.01–7.13 (m, 2H); MS (ESI/APCI dual) *m/z*: 442 $[M+H]^+$.

(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-5,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one hydrochloride (39b)

According to the procedure described for **39h**, the title compound **39b** was obtained (0.17 g, 84%) as a colorless amorphous.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.79–0.88 (m, 1H), 0.98–1.06 (m, 2H), 1.45–2.05 (m, 4H), 2.07–2.43 (m, 7H), 2.54 (s, 2H), 2.66 (s, 1H), 3.13–3.23 (m, 3H), 3.24–4.10 (m, 7.6H), 4.16–4.30 (m, 1H), 4.45–4.53 (m, 0.4H), 4.54–4.67 (m, 0.4H), 5.18 (s, 0.4H), 5.22 (s, 0.6H), 5.83–5.92 (m, 0.6H), 6.29–6.37 (m, 1H), 6.90–7.02 (m, 2H), 7.12–7.19 (m, 1H),

8.62 (brs, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.35, 10.93, 19.89, 19.92, 23.43, 23.51, 23.97, 24.03, 26.00, 27.17, 28.76, 30.91, 46.46, 49.20, 51.37, 51.87, 54.20, 57.24, 62.78, 64.97, 66.86, 88.87, 89.05, 111.72, 112.71, 125.57, 127.01, 127.20, 127.66, 128.64, 129.61, 130.05, 130.21, 149.24, 149.91, 151.88, 152.54, 154.57, 156.02, 168.35, 169.40; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{37}N_7O_2$ $[M+H]^+$: 492.3082, found: 492.3058.

Methyl 2-({5-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]pentyl}oxy)-5-methylbenzoate (37c)

According to the procedure described for **37h**, **36a** (0.60 g, 1.0 eq) and **33c** (0.22 g, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–100% ethyl acetate in hexane to 10% methanol in chloroform) to obtain **37c** (0.26 g, 86%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84–0.89 (m, 3H), 1.47–1.70 (m, 2H), 1.74–1.91 (m, 6H), 2.30 (s, 3H), 2.35 (s, 3H), 3.22 (s, 3H), 3.60–3.68 (m, 1H), 3.86 (s, 3H), 3.96–4.02 (m, 2H), 4.03–4.10 (m, 2H), 5.86 (s, 1H), 6.30 (s, 1H), 6.80–6.87 (m, 1H), 7.20–7.25 (m, 1H), 7.56–7.61 (m, 1H); MS (ESI/APCI dual) *m/z*: 488 $[M+H]^+$.

(18*S*)-3-Chloro-18-ethyl-5,14,17-trimethyl-5,6,7,8,9,10,17,18-octahydro-16*H*-19,1-(metheno)pyrimido[6,1-h][1,7,9,10,13]benzoxatetraazacyclohexadecin-16-one (38c)

According to the procedure described for **38h**, the title compound **38c** was obtained (0.20 g, 85%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91–0.97 (m, 0.6H), 1.08–1.15 (m, 2.4H), 1.49–1.82 (m, 3H), 1.83–2.25 (m, 3H), 2.29 (s, 3H), 2.59 (s, 2.4H), 2.93 (s, 0.6H), 3.07 (s, 0.6H), 3.08 (s, 2.4H), 3.17–3.28 (m, 0.2H), 3.57–3.68 (m, 0.8H), 3.86–3.95 (m, 1H), 4.02–4.12 (m, 1H), 4.54–4.64 (m, 0.2H), 4.69–4.76 (m, 0.2H), 4.90–5.00 (m, 0.8H), 5.88 (s, 1H), 6.09–6.17 (m, 0.8H), 6.26 (s, 0.2H), 6.39 (s, 0.8H), 6.76–6.82 (m, 1H), 7.01–7.12 (m, 2H); MS (ESI/APCI dual) *m/z*: 456 $[M+H]^+$.

(18*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-18-ethyl-5,14,17-trimethyl-5,6,7,8,9,10,17,18-octahydro-16*H*-19,1-(metheno)pyrimido[6,1-h][1,7,9,10,13]benzoxatetraazacyclohexadecin-16-one (39c)

According to the procedure described for **39a**, the title compound **39c** was obtained (45 mg, 68%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.79–0.86 (m, 1H), 1.06–1.15 (m, 2H), 1.35–2.25 (m, 10H), 2.28 (s, 3H), 2.59 (s, 2H), 2.88 (s, 1H), 2.93 (s, 1H), 2.98 (s, 2H), 3.22–3.44 (m, 1.7H), 3.49–3.62 (m, 1H), 3.65–3.79 (m, 3H), 3.81–3.91 (m, 0.7H), 3.92–4.07 (m, 1.3H), 4.07–4.16 (m, 0.3H), 4.59–4.66 (m, 0.3H), 4.71–4.83 (m, 0.7H), 5.12 (s, 0.7H), 5.19 (s, 0.3H), 6.00–6.10 (m, 1H), 6.73–6.78 (m, 0.7H), 6.79–6.84 (m, 0.3H), 7.00–7.12 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.79, 19.91, 21.84, 23.71, 26.06, 27.71, 30.97, 33.88, 37.92, 44.94, 50.64, 51.83, 54.88, 58.08, 66.32, 78.18, 89.39, 112.58, 127.28, 127.50, 129.29, 129.97, 149.68, 151.17, 151.73, 153.78, 155.17, 168.54; HRMS ESI/APCI dual *m/z* calcd for $C_{28}H_{39}N_7O_2$ $[M+H]^+$: 506.3238, found: 506.3227.

Methyl 2-({6-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]hexyl}oxy)-5-methylbenzoate (37d)

According to the procedure described for **37h**, **36a** (0.33 g, 1.0 eq) and **33d** (0.21 g, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–100% ethyl acetate in hexane) to obtain **37d** (0.12 g, 42%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84–0.91 (m, 3H), 1.33–1.43 (m, 2H), 1.47–1.57 (m, 2H), 1.69–1.90 (m, 6H), 2.29 (s, 3H), 2.34 (s, 3H), 3.21 (s, 3H), 3.60–3.65 (m, 1H), 3.86 (s, 3H), 3.95–4.01 (m, 2H), 4.01–4.07 (m, 2H), 5.84 (s, 1H), 6.28 (s, 1H), 6.81–6.85 (m, 1H), 7.20–7.25 (m, 1H), 7.56–7.60 (m, 1H); MS (ESI/APCI dual) *m/z*: 502 $[M+H]^+$.

(19*S*)-3-Chloro-19-ethyl-5,15,18-trimethyl-6,7,8,9,10,11,18,19-octahydro-5*H*,17*H*-20,1-(metheno)pyrimido[6,1-i][1,8,10,11,14]benzoxatetraazacycloheptadecin-17-one (38d)

According to the procedure described for **38h**, the title compound **38d** was obtained (96 mg, 70%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91–0.96 (m, 1H), 0.97–1.03 (m, 2H), 1.05–2.10 (m, 10H), 2.25–2.32 (m, 3H), 2.69 (s, 1.5H), 2.80 (s, 1.5H), 3.06–3.12 (m, 3H), 3.52–4.13 (m, 4H), 4.82–4.94 (m, 0.2H), 5.49–5.60 (m, 0.5H), 5.85–5.93 (m, 1H), 6.04–6.13 (m, 0.8H),

6.45 (s, 0.5H), 6.65–6.79 (m, 1H), 7.02–7.12 (m, 2H); MS (ESI/APCI dual) *m/z*: 470 $[M+H]^+$.

(19*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-19-ethyl-5,15,18-trimethyl-6,7,8,9,10,11,18,19-octahydro-5*H*,17*H*-20,1-(metheno)pyrimido[6,1-i][1,8,10,11,14]benzoxatetraazacycloheptadecin-17-one (39d)

According to the procedure described for **39a**, the title compound **39d** was obtained (16 mg, 44%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.94–1.03 (m, 3H), 1.04–1.90 (m, 7H), 1.92–2.11 (m, 3H), 2.14–2.24 (m, 1H), 2.25–2.31 (m, 3H), 2.34–2.42 (m, 1H), 2.69 (s, 2H), 2.84 (s, 1H), 2.98–3.05 (m, 3H), 3.24–3.62 (m, 4H), 3.67–3.81 (m, 3H), 3.82–3.91 (m, 1H), 4.75–4.84 (m, 0.2H), 5.06–5.15 (m, 1H), 5.31–5.45 (m, 0.8H), 5.48–5.62 (m, 0.2H), 5.69 (s, 0.2H), 5.95–6.04 (m, 0.8H), 6.11 (s, 0.8H), 6.63–6.72 (m, 1H), 7.02–7.11 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.85, 17.18, 19.87, 23.85, 24.60, 25.52, 28.82, 30.75, 33.02, 38.38, 44.78, 48.43, 49.38, 52.15, 54.04, 67.89, 78.74, 89.85, 111.50, 126.75, 127.66, 129.21, 130.11, 149.93, 150.97, 151.96, 153.08, 155.03, 167.95; HRMS ESI/APCI dual *m/z* calcd for $C_{29}H_{41}N_7O_2[M+H]^+$: 520.3395, found: 520.3389.

Methyl 2-[4-({5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}amino)butoxy]-5-methylbenzoate (37e)

According to the procedure described for **37h**, **36a** (0.15 g, 1.0 eq) and **33e** (0.12 g, 1.1 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–80% ethyl acetate in hexane) and reversed-phase preparative HPLC to obtain **37e** (0.11 g, 56%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.83–0.90 (m, 3H), 1.71–1.87 (m, 2H), 1.95–2.08 (m, 4H), 2.31 (s, 3H), 2.33 (s, 3H), 3.51–3.63 (m, 3H), 3.88 (s, 3H), 4.06–4.12 (m, 2H), 5.95 (s, 1H), 6.27 (s, 1H), 6.63–6.71 (m, 1H), 6.82–6.88 (m, 1H), 7.22–7.28 (m, 1H), 7.62–7.66 (m, 1H); MS (ESI/APCI dual) *m/z*: 460 $[M+H]^+$.

(17*S*)-3-Chloro-17-ethyl-13,16-dimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (38e)

According to the procedure described for **38h**, the title compound **38e** was obtained (9.0 mg, 16%) as a colorless amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.14–1.24 (m, 3H), 1.85–2.28 (m, 6H), 2.29–2.34 (m, 3H), 2.60 (s, 1.5H), 2.69 (s, 1.5H), 3.14–3.26 (m, 0.5H), 3.30–3.49 (m, 1.5H), 4.07–4.15 (m, 1.5H), 4.30–4.38 (m, 0.5H), 5.04–5.12 (m, 0.5H), 5.82 (s, 0.5H), 5.86 (s, 0.5H), 5.89–5.97 (m, 0.5H), 6.14 (s, 0.5H), 6.26 (s, 0.5H), 6.88–6.95 (m, 0.5H), 7.01–7.09 (m, 1H), 7.10–7.18 (m, 2H), 8.49–8.56 (m, 0.5H); MS (ESI/APCI dual) *m/z*: 428 $[M+H]^+$.

(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-13,16-dimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (39e)

According to the procedure described for **39a**, the title compound **39e** was obtained (4.3 mg, 43%) as a pale pink amorphous.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 0.92–0.97 (m, 1H), 0.99–1.05 (m, 2H), 1.58–2.19 (m, 8H), 2.25 (s, 2H), 2.27 (s, 1H), 2.44 (s, 2H), 2.63 (s, 1H), 2.99–3.57 (m, 8H), 3.69–4.03 (m, 3H), 4.60–4.66 (m, 0.4H), 5.17 (s, 0.4H), 5.26 (s, 0.6H), 5.67–5.72 (m, 0.6H), 5.80 (s, 0.4H), 5.84 (s, 0.6H), 6.78–6.83 (m, 0.6H), 6.92–7.18 (m, 3H), 7.42–7.47 (m, 0.4H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.69, 10.99, 19.87, 19.96, 22.57, 22.79, 26.04, 26.13, 26.39, 27.69, 30.59, 34.02, 42.30, 43.41, 44.94, 50.66, 52.19, 55.01, 68.49, 73.33, 79.12, 87.86, 88.37, 113.43, 127.42, 127.58, 127.74, 129.61, 130.01, 147.74, 152.62, 153.60, 154.15, 155.61, 169.40; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{35}N_7O_2$ $[M+H]^+$: 478.2925, found: 478.2927.

Methyl 2-[4-({5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}amino)butoxy]-5-methylbenzoate (37f)

According to the procedure described for **37h**, **27** (0.10 g, 1.0 eq) and **33e** (92 mg, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 80%–100% ethyl acetate in hexane) and reversed-phase preparative HPLC to obtain **37f** (98 mg, 64%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.83–0.90 (m, 3H), 1.72–1.90 (m, 2H), 1.90–2.02 (m, 4H), 2.30 (s, 3H), 2.35 (s, 3H), 2.45 (s, 3H), 3.59–3.66 (m, 1H), 3.82–3.90 (m, 5H), 4.05–

4.10 (m, 2H), 6.24 (s, 1H), 6.54–6.62 (m, 1H), 6.80–6.87 (m, 1H), 7.21–7.28 (m, 1H), 7.59–7.64 (m, 1H); MS (ESI/APCI dual) *m/z*: 475 $[M+H]^+$.

(17*S*)-3-Chloro-17-ethyl-4,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (38f)

According to the procedure described for **38h**, the title compound **38f** was obtained (61 mg, 71%) as a colorless powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.99–1.07 (m, 3H), 1.47–2.16 (m, 6H), 2.20 (s, 3H), 2.26 (s, 3H), 2.42 (s, 3H), 3.51–3.67 (m, 1H), 3.80–3.99 (m, 1.5H), 4.21–4.36 (m, 1H), 4.44–4.58 (m, 0.5H), 5.78–5.89 (m, 1H), 6.32 (s, 1H), 6.89–6.97 (m, 2H), 7.11–7.17 (m, 1H), 7.33–7.42 (m, 1H); MS (ESI/APCI dual) *m/z*: 442 $[M+H]^+$.

(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-4,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (39f)

According to the procedure described for **39a**, the title compound **39f** was obtained (21 mg, 50%) as a pink amorphous.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.01–1.07 (m, 0.6), 1.08–1.16 (m, 2.4H), 1.51–2.06 (m, 6H), 2.06–2.25 (m, 5H), 2.28 (s, 3H), 2.43 (s, 2.4H), 2.85 (s, 0.6H), 3.13–3.25 (m, 0.8H), 3.25–3.34 (m, 0.2H), 3.37–3.72 (m, 5H), 3.72–3.85 (m, 1.8H), 3.91–4.00 (m, 1H), 4.05–4.13 (m, 0.2H), 4.78–4.87 (m, 0.2H), 5.50–5.65 (m, 1H), 5.94 (s, 0.2H), 6.03–6.14 (m, 1.6H), 6.70–6.77 (m, 0.8H), 6.80–6.86 (m, 0.2H), 6.99–7.12 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 10.63, 11.09, 13.54, 13.99, 19.91, 22.75, 24.24, 25.62, 26.33, 27.05, 27.57, 29.12, 30.33, 33.92, 34.06, 43.79, 45.76, 48.05, 48.17, 50.78, 50.87, 51.71, 57.94, 58.12, 58.24, 65.31, 67.89, 86.56, 88.81, 89.69, 111.90, 112.89, 126.07, 127.30, 127.48, 129.53, 130.03, 146.61, 147.61, 147.77, 148.58, 152.36, 152.84, 153.96, 158.81, 158.85, 168.25, 168.88; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{37}N_7O_2$ $[M+H]^+$: 492.3082, found: 492.3065.

Methyl 2-{4-[{5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]butoxy}-5-methylbenzoate (37g)

According to the procedure described for **37h**, **27** (0.10 g, 1.0 eq) and **33b** (0.11 g, 1.2 eq) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 80%–100% ethyl acetate in hexane) and reversed-phase preparative HPLC to obtain **37g** (0.16 g, 100%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.83–0.91 (m, 3H), 1.72–1.92 (m, 6H), 2.29 (s, 3H), 2.33 (s, 3H), 2.35 (s, 3H), 3.15 (s, 3H), 3.57–3.64 (m, 2H), 3.64–3.70 (m, 1H), 3.84 (s, 3H), 3.93–4.00 (m, 2H), 6.36 (s, 1H), 6.73–6.81 (m, 1H), 7.17–7.23 (m, 1H), 7.54–7.60 (m, 1H); MS (ESI/APCI dual) *m/z*: 488 $[M+H]^+$.

(17*S*)-3-Chloro-17-ethyl-4,5,13,16-tetramethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (38g)

According to the procedure described for **38h**, the title compound **38g** was obtained (0.14 g, 88%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.99–1.07 (m, 0.9H), 1.09–1.18 (m, 2.1H), 1.42–1.68 (m, 2H), 1.75–2.21 (m, 4H), 2.29 (s, 3H), 2.33 (s, 3H), 2.58 (s, 2.1H), 2.80 (s, 3H), 2.95 (s, 0.9H), 3.14 (s, 3H), 3.23–3.34 (m, 0.7H), 3.57–3.70 (m, 1.3H), 3.73–3.84 (m, 1H), 3.91–4.05 (m, 1H), 4.79–4.87 (m, 0.3H), 6.17–6.24 (m, 0.7H), 6.26 (s, 0.3H), 6.41 (s, 0.7H), 6.68–6.79 (m, 1H), 7.01–7.12 (m, 2H); MS (ESI/APCI dual) *m/z*: 456 $[M+H]^+$.

(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-4,5,13,16-tetramethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (39g)

According to the procedure described for **39a**, the title compound **39g** was obtained (0.12 g, 77%) as a colorless powder.

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 0.96–1.02 (m, 0.9H), 1.07–1.15 (m, 2.1H), 1.54–2.23 (m, 11H), 2.28 (s, 3H), 2.61 (s, 2H), 2.95 (s, 1H), 3.04–3.22 (m, 5H), 3.34–3.46 (m, 1.7H), 3.59–4.05 (m, 5.3H), 4.75–4.80 (m, 0.3H), 5.97 (s, 0.3H), 6.09 (s, 0.7H), 6.11–6.15 (m, 0.7H), 6.72–6.76 (m, 1H), 7.02–7.10 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 10.61, 10.83, 11.03, 15.69, 15.90, 19.90, 22.49, 23.55, 23.81, 24.01, 24.72, 25.56, 26.69, 30.67, 32.80, 34.14, 34.20, 47.91, 48.05, 48.15, 50.89, 50.95, 51.03, 51.71, 52.92, 58.04,

58.12, 65.42, 67.55, 79.12, 89.95, 95.72, 111.80, 112.56, 127.24, 127.44, 127.78, 128.36, 129.39, 129.77, 130.03, 148.18, 149.64, 152.14, 152.90, 153.98, 159.39, 169.02; HRMS ESI/APCI dual *m/z* calcd for $C_{28}H_{39}N_7O_2$ $[M+H]^+$ 506.3238, found: 506.3228.

第 3 章

Methyl 2-{2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy}-5-fluorobenzoate (44)

To a solution of *tert*-butyl *N*-[2-(2-hydroxyethoxy)ethyl]carbamate (2.0 g, 9.74 mmol) in chloroform (20 mL) was added triethylamine (4.1 mL, 29.2 mmol, 3.0 eq) and methanesulfonyl chloride (1.1 mL, 14.6 mmol, 1.5 eq) at 0 °C. After stirring at 0 °C for 20 min, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure to obtain 2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethyl methanesulfonate (3.5 g, 12.3 mmol, 100%) as a pale yellow oil. This compound was used in the next reaction without further purification.

To a solution of 2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethyl methanesulfonate (2.0 g, 7.06 mmol) in *N*,*N*-dimethylformamide (14 mL) was added methyl 5-fluoro-2-hydroxybenzoate (1.2 g, 7.06 mmol, 1.0 eq) and potassium carbonate (3.9 g, 28.2 mmol, 4.0 eq). After stirring at 90 °C for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 30%–60% ethyl acetate in hexane) to obtain **44** (1.9 g, 5.26 mmol, 75%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.43 (s, 9H), 3.29–3.40 (m, 2H), 3.63 (t, J = 5.07 Hz, 2H), 3.83–3.86 (m, 2H), 3.89 (s, 3H), 4.14–4.18 (m, 2H), 5.11 (brs, 1H), 6.93–6.98 (m, 1H), 7.13–7.19 (m, 1H), 7.49–7.54 (m, 1H).

Methyl 2-(2-{2-[*tert*-butoxycarbonyl(methyl)amino]ethoxy}ethoxy)-5-fluorobenzoate (45)

To a solution of **44** (0.79 g, 2.21 mmol) in *N*,*N*-dimethylformamide (4.4 mL) was added silver (I) oxide (2.6 g, 11.1 mmol, 5.0 eq) and iodomethane (0.69 mL, 11.1 mmol, 5.0 eq). After stirring at 90 °C for 2 h, the mixture was filtered through a pad of Celite®, and the

filtrate was concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 30%–60% ethyl acetate in hexane) to obtain **45** (0.82 g, 2.20 mmol, 99%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 2.90 (brs, 3H), 3.41 (brs, 2H), 3.67 (brs, 2H), 3.83 (t, J = 5.01 Hz, 2H), 3.88 (s, 3H), 4.15 (t, J = 4.77 Hz, 2H), 6.94–6.99 (m, 1H), 7.11–7.19 (m, 1H), 7.46–7.52 (m, 1H).

Methyl 5-fluoro-2-{2-[2-(methylamino)ethoxy]ethoxy}benzoate hydrochloride (46)

To a solution of **45** (0.81 g, 2.19 mmol) in 1,4-dioxane (2.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (2.0 mL) and the mixture was stirred for 2.5 h at room temperature. The reaction mixture was then concentrated under reduced pressure to obtain **46** (0.67 g, 2.17 mmol, 99%) as a colorless powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.55 (s, 3H), 3.09 (t, J = 5.38 Hz, 2H), 3.74–3.84 (m, 7H), 4.18 (t, J = 4.40 Hz, 2H), 7.18–7.25 (m, 1H), 7.37–7.50 (m, 2H), 8.68 (brs, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 32.5, 47.4, 52.1, 65.8, 68.7, 69.1, 116.0, 116.8, 119.8, 121.5, 153.8, 156.3, 165.0; MS (ESI/APCI dual) *m/z*: 272 $[M+H]^+$.

Methyl 2-{4-[*tert*-butoxycarbonyl(methyl)amino]butoxy}-5-fluorobenzoate (41)

Compound **41** was prepared from *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methylcarbamate according to the procedure described for **44** to obtain a colorless oil in a reaction with an 80% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H), 1.68–1.85 (m, 4H), 2.86 (s, 3H), 3.29 (t, J = 6.66 Hz, 2H), 3.88 (s, 3H), 4.02 (t, J = 5.99 Hz, 2H), 6.86–6.95 (m, 1H), 7.09–7.19 (m, 1H), 7.44–7.55 (m, 1H); MS (ESI/APCI dual) *m/z*: 378 $[M+Na]^+$.

Methyl 5-fluoro-2-[4-(methylamino)butoxy]benzoate hydrochloride (42)

Using the procedure described for **46**, the title compound was obtained as a colorless powder in a reaction with a 79% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.03–2.12 (m, 2H), 2.19–2.27 (m, 2H), 2.74 (s, 3H), 3.13 (t, J = 6.48 Hz, 2H), 3.89 (s, 3H), 4.15 (t, J = 5.07 Hz, 2H), 6.93–6.98 (m, 1H), 7.19–7.25 (m, 1H), 7.56–7.61 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 22.3, 25.6,

32.2, 47.8, 52.1, 68.5, 115.6, 116.6, 119.7, 121.3, 153.9, 156.2, 165.1; MS (ESI/APCI dual) *m/z*: 256 $[M+H]^+$.

tert-Butyl _N_-(2-{2-[_tert_-butyl(dimethyl)silyl]oxyethylamino}ethyl)-_N_-methylcarbamate (48)

To a solution of *tert*-butyl *N*-(2-aminoethyl)-*N*-methylcarbamate (1.4 g, 8.15 mmol, 1.5 eq) in acetonitrile (14 mL) was added 2-bromoethoxy-*tert*-butyldimethylsilane (1.3 g, 5.43 mmol, 1.0 eq) and potassium carbonate (3.8 g, 27.2 mmol, 5.0 eq). After stirring at 80 °C for 16 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 0%–7% methanol in chloroform) to obtain **48** (1.3 g, 3.76 mmol, 69%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.06 (s, 6H), 0.89 (s, 9H), 1.45 (s, 9H), 2.73 (t, J = 5.32 Hz, 2H), 2.78 (t, J = 6.66 Hz, 2H), 2.88 (s, 3H), 3.33 (brs, 2H), 3.71 (t, J = 5.32 Hz, 2H).

tert-Butyl _N_-[2-(benzyloxycarbonyl-{2-[_tert_-butyl(dimethyl)silyl]oxyethyl}amino)ethyl]-_N_-methylcarbamate (49)

To a solution of **48** (1.0 g, 3.04 mmol) in chloroform (30 mL) was added triethylamine (2.5 mL, 18.2 mmol, 6.0 eq) and benzyl chloroformate (1.3 mL, 9.11 mmol, 3.0 eq) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was poured into saturated aqueous ammonium chloride and extracted with chloroform. The residue was purified using silica gel column chromatography (OH 10%–50% ethyl acetate in hexane) to obtain **49** (1.6 g, 3.32 mmol, 100%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm −0.04–0.04 (m, 6H), 0.83–0.90 (m, 9H), 1.41–1.47 (m, 9H), 2.69–2.82 (m, 1.5H), 2.85–2.92 (m, 1.5H), 3.26–3.42 (m, 4H), 3.42–3.52 (m, 2H), 3.63–3.70 (m, 1H), 3.72–3.79 (m, 1H), 5.10–5.15 (m, 2H), 7.28–7.43 (m, 5H); MS (ESI/APCI dual) *m/z*: 467 $[M+H]^+$.

tert-Butyl _N_-{2-[benzyloxycarbonyl(2-hydroxyethyl)amino]ethyl}-_N_-methylcarbamate (50)

To a solution of **49** (1.4 g, 3.04 mmol) in tetrahydrofuran (15 mL) was added TBAF (9.1 mL, 9.13 mmol, 3.0 eq) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was poured into saturated aqueous ammonium chloride and extracted with chloroform. The residue was purified using silica gel column chromatography (OH 50%–95% ethyl acetate in hexane) to obtain **50** (0.87 g, 2.46 mmol, 81%) as a colorless oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.43 (s, 9H), 2.64–2.97 (m, 3H), 3.29–3.54 (m, 6H), 3.66–3.87 (m, 2H), 5.05–5.19 (m, 2H), 7.29–7.41 (m, 5H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 27.9, 33.8, 45.0, 46.8, 49.8, 59.2, 66.2, 78.4, 127.3, 127.5, 127.7, 128.3, 136.8, 154.6, 155.2; MS (ESI/APCI dual) *m/z*: 352 $[M+H]^+$.

Methyl 2-[2-(benzyloxycarbonyl-{2-[_tert_-butoxycarbonyl(methyl)amino]ethyl}amino)ethoxy]-5-fluorobenzoate (51)

Compound **51** was prepared from **50** according to the procedure described for **44** to obtain a colorless oil in a reaction with a 38% yield.
1H NMR (400 MHz, $CDCl_3$) δ ppm 1.39–1.48 (m, 9H), 2.70–2.94 (m, 3H), 3.32–3.47 (m, 2H), 3.56–3.75 (m, 4H), 3.85 (s, 3H), 4.04–4.11 (m, 1H), 4.14–4.20 (m, 1H), 5.13 (s, 2H), 6.74–6.82 (m, 1H), 6.88–6.95 (m, 0.5H), 7.00–7.19 (m, 0.5H), 7.29–7.42 (m, 5H), 7.45–7.55 (m, 1H); MS (ESI/APCI dual) *m/z*: 505 $[M+H]^+$.

Methyl 2-(2-{benzyloxycarbonyl-[2-(methylamino)ethyl]amino}ethoxy)-5-fluorobenzoate hydrochloride (52)

Compound **52** was prepared from **51** according to the procedure described for **46** to obtain a colorless oil in a reaction with a 100% yield. This compound was used in the next reaction without further purification.
MS (ESI/APCI dual) *m/z*: 405 $[M+H]^+$.

Methyl 2-(2-_tert_-butoxy-2-oxoethoxy)-5-fluorobenzoate (55a)

To a solution of methyl 5-fluoro-2-hydroxybenzoate (2.0 g, 11.8 mmol) in acetonitrile

(30 mL) was added *tert*-butyl 2-bromoacetate (2.8 g, 14.1 mmol, 1.2 eq) and potassium carbonate (6.5 g, 47.0 mmol, 4.0 eq). After stirring at 70 °C for 1 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 2% ethyl acetate in hexane) to obtain **55a** (3.4 g, 12.0 mmol, 100%) as a colorless oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9H), 3.91 (s, 3H), 4.57 (s, 2H), 6.83–6.89 (m, 1H), 7.11–7.18 (m, 1H), 7.50–7.56 (m, 1H); MS (ESI/APCI dual) *m/z*: 307 $[M+Na]^+$.

Methyl 2-(2-{2-[*tert*-butoxycarbonyl(methyl)amino]ethylamino}-2-oxoethoxy)-5-fluorobenzoate (57a)

To a solution of **55a** (3.4 g, 12.0 mmol) in chloroform (20 mL) was added trifluoroacetic acid (20 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo to obtain 2-(4-fluoro-2-methoxycarbonylphenoxy) acetic acid (3.44 g, 15.1 mmol, 100%) as a colorless powder. This compound was used in the next reaction without further purification.

To a solution of 2-(4-fluoro-2-methoxycarbonylphenoxy) acetic acid (1.7 g, 7.45 mmol) in *N*,*N*-dimethylformamide (25 mL) was added *tert*-butyl *N*-(2-aminoethyl)-*N*-methylcarbamate (1.6 g, 8.94 mmol, 1.2 eq), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (3.4 g, 8.94 mmol, 1.2 eq) and triethylamine (7.3 mL, 52.2 mmol, 7.0 eq), and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with saturated aqueous sodium bicarbonate and extracted with ethyl acetate/toluene (1:1). The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 40%–50% ethyl acetate in hexane) to obtain **57a** (1.9 g, 4.99 mmol, 67%) as a yellow oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.41 (s, 9H), 2.92 (s, 3H), 3.35–3.47 (m, 2H), 3.50–3.59 (m, 2H), 3.93 (s, 3H), 4.54 (s, 2H), 6.84–6.93 (m, 1H), 7.18–7.29 (m, 3H), 7.59–7.67 (m, 1H), 8.17 (brs, 0.5H), 8.38 (brs, 0.5H); MS (ESI/APCI dual) *m/z*: 407 $[M+Na]^+$.

Methyl 5-fluoro-2-{2-[2-(methylamino)ethylamino]-2-oxoethoxy}benzoate hydrochloride (58a)

A mixture of **57a** (1.9 g, 4.99 mmol) and 4 M hydrogen chloride in 1,4-dioxane (30 mL) was stirred at room temperature for 1.5 h, and the reaction mixture was concentrated under reduced pressure to obtain **58a** (1.5 g, 4.55 mmol, 91%) as a colorless powder. This compound was used in the next reaction without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.79 (s, 3H), 3.25 (brs, 2H), 3.81 (brs, 2H), 3.90 (s, 3H), 4.66 (s, 2H), 6.85–6.99 (m, 1H), 7.16–7.25 (m, 1H), 7.53–7.68 (m, 1H), 8.79 (s, 1H), 9.66 (brs, 2H); MS (ESI/APCI dual) *m/z*: 285 $[M+H]^+$.

Methyl 2-(2-*tert*-butoxy-2-oxoethoxy)-5-chlorobenzoate (55b)

The title compound **55b** was synthesized according to the procedure described for **55a** from **53** and **54b** (98% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.91 (s, 3H), 4.59 (s, 2H), 6.80 (d, J = 8.80 Hz, 1H), 7.39 (dd, J = 8.80, 2.57 Hz, 1H), 7.80 (d, J = 2.57 Hz, 1H); MS (ESI/APCI dual) *m/z*: 323 $[M+Na]^+$.

Methyl 2-(2-*tert*-butoxy-2-oxoethoxy)-5-methylbenzoate (55c)

The title compound **55c** was synthesized according to the procedure described for **55a** from **53** and **54c** (100% yield).

MS (ESI/APCI dual) *m/z*: 281 $[M+H]^+$.

Methyl 2-(2-{2-[*tert*-butoxycarbonyl(methyl)amino]ethylamino}-2-oxoethoxy)-5-chlorobenzoate (57b)

The title compound **57b** was synthesized according to the procedure described for **57a** from **55b** (99% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42 (s, 9H), 2.91 (s, 3H), 3.41 (brs, 2H), 3.48–3.59 (m, 2H), 3.92 (s, 3H), 4.54 (s, 2H), 6.87 (d, J = 8.80 Hz, 1H), 7.47 (d, J = 8.44 Hz, 1H), 7.90 (s, 1H), 8.06–8.19 (m, 0.5H), 8.25–8.41 (m, 0.5H); MS (ESI/APCI dual) *m/z*: 401 $[M+H]^+$.

Methyl 2-[2-({2-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}amino)-2-oxoethoxy]-5-methylbenzoate (57c)

The title compound **57c** was synthesized according to the procedure described for **57a** from **55c** (84% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42 (s, 9H), 2.32 (s, 3H), 2.91 (s, 3H), 3.41 (brs, 2H), 3.48–3.58 (m, 2H), 3.90 (s, 3H), 4.54 (s, 2H), 6.82 (d, J = 8.44 Hz, 1H), 7.30 (d, J = 8.07 Hz, 1H), 7.72 (s, 1H), 8.17–8.33 (m, 0.5H), 8.39–8.54 (m, 0.5H); MS (ESI/APCI dual) *m/z*: 381 $[M+H]^+$.

Methyl 5-chloro-2-{2-[2-(methylamino)ethylamino]-2-oxoethoxy}benzoate hydrochloride (58b)

The title compound **58b** was synthesized according to the procedure described for **58a** from **57b** (100% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.76 (brs, 3H), 3.23 (brs, 2H), 3.77–3.85 (m, 2H), 3.89 (s, 3H), 4.66 (s, 2H), 6.89 (d, J = 8.68 Hz, 1H), 7.44 (d, J = 8.44 Hz, 1H), 7.86 (s, 1H), 8.64 (brs, 1H), 9.56 (brs, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 32.2, 34.8, 47.5, 52.4, 68.1, 116.7, 121.4, 124.8, 130.2, 133.3, 155.9, 164.7, 168.0; MS (ESI/APCI dual) *m/z*: 301 $[M+H]^+$.

Methyl 5-methyl-2-(2-{[2-(methylamino)ethyl]amino}-2-oxoethoxy)benzoate hydrochloride (58c)

The title compound **58c** was synthesized according to the procedure described for **58a** from **57c** (100% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.27 (s, 3H), 2.55 (t, J = 5.26 Hz, 3H), 2.96–3.06 (m, 2H), 3.46–3.53 (m, 2H), 3.83 (s, 3H), 4.59 (s, 2H), 7.06 (d, J = 8.44 Hz, 1H), 7.34–7.40 (m, 1H), 7.54–7.60 (m, 1H), 8.26 (t, J = 5.81 Hz, 1H), 8.96 (brs, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 32.3, 34.8, 45.4, 47.5, 52.0, 68.2, 114.9, 119.5, 130.3, 131.2, 134.4, 155.1, 168.5; MS (ESI/APCI dual) *m/z*: 281 $[M+H]^+$.

Methyl 2-[2-(benzyloxycarbonylamino)ethoxy]-5-fluorobenzoate (60)

To a solution of sodium hydride (60%, dispersion in Paraffin Liquid) (0.39 g, 9.70 mmol) in tetrahydrofuran (12 mL) was added methyl 5-fluoro-2-hydroxybenzoate (1.5 g, 8.82 mmol). After stirring at room temperature for 1 h, benzyl *N*-(2-bromoethyl)carbamate

(2.5 g, 9.70 mmol) was added to the reaction mixture and the mixture was heated under reflux for 12 h. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was separated using a phase separator and concentrated in vacuo. The residue was purified using silica gel column chromatography (OH 20% ethyl acetate in hexane) to obtain **60** (1.1 g, 3.25 mmol, 37%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.63 (q, J = 5.26 Hz, 2H), 3.88 (s, 3H), 4.11 (t, J = 4.89 Hz, 2H), 5.13 (s, 2H), 5.92 (brs, 1H), 6.88–6.96 (m, 1H), 7.12–7.20 (m, 1H), 7.27–7.39 (m, 5H), 7.49–7.55 (m, 1H); MS (ESI/APCI dual) *m/z*: 348 $[M+H]^+$.

Methyl 2-[2-({2-[*tert*-butoxycarbonyl(methyl)amino]acetyl}amino)ethoxy]-5-fluorobenzoate (62)

To a solution of **60** (1.1 g, 3.25 mmol) in methanol (30 mL) was added 5% palladium on carbon (0.30 g). The reaction was flushed with hydrogen and stirred under a hydrogen atmosphere at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite® and concentrated under reduced pressure to obtain methyl 2-(2-aminoethoxy)-5-fluorobenzoate (**61**) (1.1 g, 5.11 mmol, 100%.) as a colorless powder. This compound was used in the next reaction without further purification.

To a solution of **61** (0.69 g, 3.26 mmol) in *N*,*N*-dimethylformamide (15 mL) was added 2-[*tert*-butoxycarbonyl(methyl)amino]acetic acid (0.62 g, 3.26 mmol, 1.0 eq), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (1.9 g, 4.88 mmol, 1.5 eq) and triethylamine (1.8 mL, 13.0 mmol, 4.0 eq), and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with saturated aqueous sodium bicarbonate and extracted with ethyl acetate/toluene (1:1). The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 40% ethyl acetate in hexane) to obtain **62** (1.3 g, 3.36 mmol, 100%.) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.30–1.49 (m, 9H), 2.94 (s, 3H), 3.68–3.77 (m, 2H), 3.85–3.96 (m, 5H), 4.12 (t, J = 4.77 Hz, 2H), 6.91–6.98 (m, 1H), 7.14–7.23 (m, 1H), 7.50–7.56 (m, 1H); MS (ESI/APCI dual) *m/z*: 385 $[M+H]^+$.

Methyl 5-fluoro-2-(2-{[2-(methylamino)acetyl]amino}ethoxy)benzoate hydrochloride (63)

A mixture of **62** (1.3 g, 3.66 mmol) and 4 M hydrogen chloride in 1,4-dioxane (30 mL) was stirred at room temperature for 2 h, and the reaction mixture was concentrated under reduced pressure to obtain methyl **63** (1.1 g, 3.40 mmol, 100%) as a colorless powder. This compound was used in the next reaction without further purification.

MS (ESI/APCI dual) *m/z*: 285 $[M+H]^+$.

***tert*-Butyl *N*-[2-(chloromethylsulfonylamino)ethyl]-*N*-methylcarbamate (64)**

To a solution of *tert*-butyl *N*-(2-aminoethyl)-*N*-methylcarbamate (1.3 g, 7.17 mmol) in chloroform (15 mL) was added triethylamine (2.0 mL, 14.3 mmol, 2.0 eq) and chloromethanesulfonyl chloride (0.71 mL, 7.89 mmol, 1.1 eq) at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 20%–100% ethyl acetate in hexane) to obtain **64** (1.8 g, 6.21 mmol, 87%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 2.92 (s, 3H), 3.32–3.49 (m, 4H), 4.50 (s, 2H), 5.65 (brs, 1H); MS (ESI/APCI dual) *m/z*: 309 $[M+Na]^+$.

Methyl 2-{2-[*tert*-butoxycarbonyl(methyl)amino]ethylsulfamoylmethoxy}-5-fluorobenzoate (65)

To a solution of **64** (1.8 g, 6.21 mmol) in *N*,*N*-dimethylformamide (30 mL) was added methyl 5-fluoro-2-hydroxybenzoate (1.2 g, 6.83 mmol, 1.1 eq) and potassium carbonate (2.6 g, 18.6 mmol, 3.0 eq). After stirring at 80 °C for 3 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 50%–100% ethyl acetate in hexane) to obtain **65** (0.25 g, 0.595 mmol, 9.6%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 2.93 (s, 3H), 3.29–3.39 (m, 2H), 3.43 (t,

J = 6.11 Hz, 2H), 3.91 (s, 3H), 5.02 (s, 2H), 5.88–5.97 (m, 1H), 7.12–7.24 (m, 2H), 7.51–7.58 (m, 1H); MS (ESI/APCI dual) *m/z*: 443 $[M+Na]^+$.

Methyl 5-fluoro-2-[2-(methylamino)ethylsulfamoylmethoxy]benzoate hydrochloride (66)

To a solution of **65** (0.25 g, 0.595 mmol) in 1,4-dioxane (1.0 mL) and methanol (0.5 mL) was added 4 M hydrogen chloride in 1,4-dioxane (1.0 mL). After stirring for 1 h at room temperature, the reaction mixture was concentrated under reduced pressure to obtain **66** (0.25 g, 0.690 mmol, 100%.) as a colorless powder. This compound was used in the next reaction without further purification.

MS (ESI/APCI dual) *m/z*: 321 $[M+H]^+$.

***tert*-Butyl *N*-[2,2,2-trifluoro-1-(hydroxymethyl)ethyl]carbamate (68)**

To a solution of methyl 2-(*tert*-butoxycarbonylamino)-3,3,3-trifluoropropanoate (0.91 g, 3.53 mmol) in tetrahydrofuran (18 mL) was added lithium borohydride (0.23 g, 10.6 mmol, 3.0 eq). After stirring at room temperature for 30 min, the reaction mixture was poured into 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 10%–45% ethyl acetate in hexane) to obtain **68** (0.75 g, 3.28 mmol, 93%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9H), 1.74 (brs, 1H), 3.80–3.89 (m, 1H), 3.90–4.00 (m, 1H), 4.21–4.43 (m, 1H), 5.16 (brs, 1H); MS (ESI/APCI dual) *m/z*: 252 $[M+Na]^+$.

Methyl 2-[2-(*tert*-butoxycarbonylamino)-3,3,3-trifluoropropoxy]-5-fluorobenzoate (69)

To a solution of **68** (0.74 g, 3.24 mmol) in chloroform (6.5 mL) was added triethylamine (1.4 mL, 9.71 mmol, 3.0 eq) and methanesulfonyl chloride (0.38 mL, 4.86 mmol, 1.5 eq) at 0 °C. After stirring at 0 °C for 20 min, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure to obtain [2-(*tert*-butoxycarbonylamino)-3,3,3-trifluoropropyl]methanesulfonate as a yellow oil. This

compound was used in the next reaction without further purification.

To a solution of [2-(*tert*-butoxycarbonylamino)-3,3,3-trifluoropropyl]methanesulfonate (3.24 mmol) in *N*,*N*-dimethylformamide (16 mL) was added methyl 5-fluoro-2-hydroxybenzoate (0.61 g, 3.56 mmol, 1.1 eq) and potassium carbonate (1.8 g, 12.9 mmol, 4.0 eq). After stirring at 90 °C for 1 h, the reaction mixture was poured into brine and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH ethyl acetate in hexane) to obtain **69** (0.26 g, 0.691 mmol, 21%) as a yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9H), 3.93 (s, 3H), 4.05–4.13 (m, 1H), 4.44–4.52 (m, 1H), 4.59 (brs, 1H), 6.14–6.25 (m, 1H), 6.88–6.93 (m, 1H), 7.16–7.23 (m, 1H), 7.55–7.61 (m, 1H); MS (ESI/APCI dual) *m/z*: 404 $[M+Na]^+$.

Methyl 2-(2-{2-[*tert*-butoxycarbonyl(methyl)amino]ethylamino}-3,3,3-trifluoropropoxy)-5-fluorobenzoate (71)

To a solution of **69** (0.26 g, 0.682 mmol) in 1,4-dioxane (2.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (2.0 mL). After stirring at room temperature for 8 h, the reaction mixture was concentrated under reduced pressure to obtain methyl 2-(2-amino-3,3,3-trifluoropropoxy)-5-fluorobenzoate hydrochloride (0.22 g, 0.698 mmol, 100%) as a pale yellow powder. This compound was used in the next reaction without further purification.

To a solution of methyl 2-(2-amino-3,3,3-trifluoropropoxy)-5-fluorobenzoate hydrochloride (0.22 g, 0.698 mmol) and *tert*-butyl *N*-methyl-*N*-(2-oxoethyl)carbamate (0.12 g, 0.698 mmol, 1.0 eq) in chloroform (3.5 mL) was added sodium triacetoxyborohydride (0.44 g, 2.10 mmol, 3.0 eq). After stirring at room temperature for 30 min, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 10%–50% ethyl acetate in hexane) to obtain **71** (0.26 g, 0.600 mmol, 86%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42 (s, 9H), 2.89 (s, 3H), 2.94–3.03 (m, 2H), 3.24–3.59 (m, 3H), 3.89 (s, 3H), 4.10–4.19 (m, 1H), 4.21–4.29 (m, 1H), 6.91–6.97 (m, 1H),

7.14–7.22 (m, 1H), 7.51–7.57 (m, 1H); MS (ESI/APCI dual) *m/z*: 439 $[M+H]^+$.

Methyl 5-fluoro-2-{3,3,3-trifluoro-2-[2-(methylamino)ethylamino]propoxy}benzoate dihydrochloride (72)

To a solution of **71** (0.22 g, 0.496 mmol) in 1,4-dioxane (2.0 mL) was added 4 M hydrogen chloride in 1,4-dioxane (2.0 mL). After stirring at room temperature for 2.5 h, the reaction mixture was concentrated under reduced pressure to obtain **72** (0.24 g, 0.575 mmol, 100%) as a yellow oil. This compound was used in the next reaction without further purification.

MS (ESI/APCI dual) *m/z*: 339 $[M+H]^+$.

Methyl 2-{2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methylamino)ethoxy]ethoxy}-5-fluorobenzoate (73b)

To a solution of **36b** (0.20 g, 0.455 mmol) in ethanol (4.5 mL) was added **46** (0.14 g, 0.455 mmol, 1.0 eq) and triethylamine (0.63 mL, 4.55 mmol, 10 eq), and the mixture was stirred at 70 °C for 0.5 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 60%–100% ethyl acetate in hexane) to obtain **73b** (0.21 g, 0.419 mmol, 92%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46–1.71 (m, 6H), 1.86–1.93 (m, 1H), 1.97–2.04 (m, 1H), 2.76–2.85 (m, 1H), 3.15–3.21 (m, 1H), 3.22 (s, 3H), 3.78 (t, J = 4.77 Hz, 2H), 3.83–3.90 (m, 6H), 4.04 (t, J = 4.52 Hz, 2H), 4.34 (t, J = 5.20 Hz, 2H), 5.91 (s, 1H), 6.34 (s, 1H), 6.85–6.93 (m, 1H), 7.11–7.19 (m, 1H), 7.47–7.53 (m, 1H); MS (ESI/APCI dual) *m/z*: 506 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-11-methyl-6,7,10,11,19,20,21,22-octahydro-9*H*,18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzodioxatetraazacyclohexadecin-24-one (75b)

To a solution of **73b** (0.20 g, 0.395 mmol) in methanol (2.0 mL) and tetrahydrofuran (2.0 mL) was added 1 M aqueous sodium hydroxide (2.0 mL), and the mixture was stirred

at 70 °C for 0.5 h. The reaction mixture was acidified using 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 2-{2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methyl-amino)ethoxy]ethoxy}-5-fluorobenzoic acid (**74b**) (0.920 g, 0.401 mmol, 100%) as a colorless powder. This compound was used in the next reaction without further purification.

To a solution of **74b** (0.20 g, 0.401 mmol) in *N*,*N*-dimethylformamide (40 mL, 0.01 M) was added triethylamine (0.28 mL, 2.00 mmol, 5.0 eq) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (0.23 g, 0.601 mmol, 1.5 eq). After stirring at room temperature for 18 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **75b** (0.13 g, 0.27 mmol, 69%) as a colorless amorphous substance.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38–1.69 (m, 3H), 1.71–1.82 (m, 1H), 1.96–2.12 (m, 1H), 2.21–2.33 (m, 1H), 3.15–3.21 (m, 3H), 3.32–3.51 (m, 2H), 3.52–3.67 (m, 1H), 3.70–3.76 (m, 0.2H), 3.82–3.98 (m, 2H), 4.08–4.19 (m, 1.9H), 4.20–4.29 (m, 1.8H), 4.38–4.49 (m, 0.9H), 4.70–4.79 (m, 0.1H), 5.03–5.09 (m, 0.1H), 5.20–5.29 (m, 0.1H), 5.86–5.92 (m, 1H), 5.99 (s, 0.1H), 6.21–6.26 (m, 0.9H), 6.33 (s, 0.9H), 6.69–6.75 (m, 0.1H), 6.80–6.87 (m, 0.9H), 6.93–7.08 (m, 2H); MS (ESI/APCI dual) *m/z*: 474 $[M+H]^+$.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-6,7,10,11,19,20,21,22-octahydro-9*H*,18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzodioxatetraazacyclohexadecin-24-one (76b)

To a solution of **75b** (62 mg, 0.131 mmol) in 1-methyl-2-pyrrolidone (1.3 mL) was added triethylamine (0.36 mL, 2.62 mmol, 20 eq) and azetidine (0.088 mL, 1.31 mmol, 10 eq). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was purified using reversed-phase preparative HPLC to obtain **76b** (55 mg, 0.112 mmol, 86%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35–1.52 (m, 2H), 1.64–1.77 (m, 2H), 1.89–2.04 (m, 1H), 2.06–2.15 (m, 0.1H), 2.19–2.29 (m, 0.9H), 2.32–2.44 (m, 2H), 3.00–3.09 (m, 3H), 3.33–3.52 (m, 2.8H), 3.74–4.01 (m, 2.4H), 4.04–4.20 (m, 6.8H), 4.20–4.36 (m, 1.9H), 4.63–4.72 (m, 0.1H), 4.91–4.97 (m, 1H), 4.98–5.06 (m, 0.1H), 5.77 (s, 0.1H), 5.99 (s, 0.9H), 6.14–6.21 (m, 0.9H), 6.74–6.86 (m, 1H), 6.92–7.07 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 16.0, 20.2, 25.5, 29.7, 40.5, 44.1, 47.9, 50.5, 53.0, 68.4, 69.6, 70.3, 76.1, 89.3, 113.9, 114.3, 114.5, 116.2, 128.2, 150.4, 150.6, 151.9, 154.5, 157.2, 158.9, 166.1; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{31}FN_6O_3$ $[M+H]^+$ 495.2514, found 495.2499.

Methyl 2-{2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methyl-amino)ethylamino]-2-oxoethoxy}-5-fluorobenzoate (73c)

To a solution of **36b** (0.43 g, 1.25 mmol) in ethanol (12 mL) was added **58a** (0.44 g, 1.37 mmol, 1.1 eq) and triethylamine (1.7 mL, 12.5 mmol, 10 eq), and the mixture was stirred at 70 °C for 0.5 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 70% ethyl acetate in hexane) to obtain **73c** (0.36 g, 0.694 mmol, 56%) as a colorless amorphous substance.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46–1.71 (m, 4H), 1.79–1.92 (m, 1H), 1.95–2.03 (m, 1H), 2.75–2.83 (m, 1H), 3.14–3.21 (m, 1H), 3.23 (s, 3H), 3.74 (q, *J* = 6.07 Hz, 2H), 3.80–3.86 (m, 4H), 4.34 (s, 2H), 4.40 (t, *J* = 5.99 Hz, 2H), 5.90 (s, 1H), 6.23 (s, 1H), 6.74–6.82 (m, 1H), 7.16–7.24 (m, 1H), 7.54–7.60 (m, 1H), 8.35–8.44 (m, 1H); MS (ESI/APCI dual) *m/z*: 519 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (75c)

To a solution of **73c** (0.36 g, 0.694 mmol) in tetrahydrofuran (7.0 mL) and 2-propanol (7.0 mL) was added 1 M aqueous sodium hydroxide (14 mL, 13.9 mmol, 20 eq), and the mixture was stirred at 80 °C for 0.5 h. The reaction mixture was acidified with 1 M

aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure to obtain 2-{2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methyl-amino)ethylamino]-2-oxoethoxy}-5-fluorobenzoic acid (**74c**) (0.40 g, 0.792 mmol, 100%) as a colorless amorphous substance. This compound was used in the next reaction without further purification.

To a solution of **74c** (0.40 g, 0.792 mmol) in *N*,*N*-dimethylformamide (79 mL, 0.01 M) was added triethylamine (0.55 mL, 3.96 mmol, 5.0 eq) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (0.45 g, 1.19 mmol, 1.5 eq). After stirring at room temperature for 15 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate/toluene (1:1). The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 85% ethyl acetate in hexane to 7% methanol in chloroform) to obtain **75c** (0.25 g, 0.518 mmol, 65%) as a yellow amorphous substance.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.41–2.21 (m, 5H), 2.31–2.42 (m, 0.4H), 2.85–2.99 (m, 0.6H), 3.01–3.33 (m, 6.3H), 4.21–4.58 (m, 3.1H), 4.75–4.85 (m, 0.6H), 4.97–5.03 (m, 0.6H), 5.03–5.15 (m, 0.4H), 5.16–5.28 (m, 0.6H), 6.06 (s, 0.6H), 6.11 (s, 0.4H), 6.21 (s, 0.6H), 6.30–6.37 (m, 0.4H), 6.56 (s, 0.4H), 6.80–6.89 (m, 1H), 6.98–7.11 (m, 2H), 7.65–7.78 (m, 0.4H), 8.69–8.81 (m, 0.6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.7, 24.7, 29.8, 36.3, 37.3, 43.5, 46.9, 48.9, 54.0, 70.6, 79.1, 91.0, 113.8, 116.4, 119.3, 130.1, 149.5, 150.2, 150.3, 150.8, 155.2, 165.6, 167.3; MS (ESI/APCI dual) *m/z*: 487 $[M+H]^+$.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (76c)

To a solution of **75c** (0.10 g, 0.205 mmol) in 1-methyl-2-pyrrolidone (1.5 mL) was added triethylamine (0.57 mL, 4.11 mmol, 20 eq) and azetidine (0.14 mL, 2.05 mmol, 10 eq). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was purified using reversed-phase preparative HPLC to obtain **76c** (53 mg, 0.104 mmol, 51%)

as a colorless powder.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.39–1.77 (m, 4H), 1.81–1.98 (m, 1H), 2.05–2.12 (m, 0.7H), 2.15–2.21 (m, 0.3H), 2.26–2.35 (m, 2H), 2.83 (s, 0.9H), 2.89 (s, 2.1H), 2.97–3.20 (m, 2.7H), 3.35–3.42 (m, 0.3H), 3.80–3.89 (m, 0.3H), 3.93–4.10 (m, 6H), 4.47–4.55 (m, 1.7H), 4.63–4.76 (m, 1.7H), 5.30 (s, 0.7H), 5.37 (m, 0.3H), 5.87 (s, 0.7H), 5.96–6.00 (m, 0.3H), 6.08 (s, 0.3H), 7.09–7.15 (m, 0.7H), 7.15–7.28 (m, 1.6H), 7.30–7.37 (m, 0.7H), 7.48–7.57 (m, 0.3H), 8.97 (d, J = 6.61 Hz, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 24.7, 29.5, 36.4, 37.9, 43.6, 46.9, 48.3, 50.1, 54.0, 71.2, 78.0, 89.4, 113.7, 116.5, 120.0, 130.7, 149.6, 150.3, 151.6, 153.4, 156.9, 158.5, 165.4, 167.5; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{30}FN_7O_3$ $[M+H]^+$ 508.2467, found 508.2444.

Methyl 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methylamino)butoxy]-5-fluorobenzoate (73a)

The title compound **73a** was synthesized according to the procedure described for **73c** from **36b** and **42** (86% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46–1.68 (m, 4H), 1.80–2.03 (m, 6H), 2.73–2.85 (m, 1H), 3.13–3.20 (m, 1H), 3.24 (s, 3H), 3.80–3.87 (m, 4H), 3.99–4.05 (m, 2H), 4.07–4.20 (m, 2H), 5.88 (s, 1H), 6.34 (s, 1H), 6.84–6.91 (m, 1H), 7.10–7.18 (m, 1H), 7.44–7.55 (m, 1H); MS (ESI/APCI dual) *m/z*: 490 $[M+H]^+$.

Methyl 5-chloro-2-[2-({2-[{5-chloro-2-[(2*S*)-piperidin-2-yl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]ethyl}amino)-2-oxoethoxy]benzoate (73d)

The title compound **73d** was synthesized according to the procedure described for **73c** from **36b** and **58b** (85% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.41–1.75 (m, 4H), 1.84–1.93 (m, 1H), 1.95–2.03 (m, 1H), 2.75–2.84 (m, 1H), 3.15–3.21 (m, 1H), 3.22 (s, 3H), 3.74 (q, J = 6.07 Hz, 2H), 3.80–3.86 (m, 4H), 4.35 (s, 2H), 4.39 (t, J = 5.99 Hz, 2H), 5.90 (s, 1H), 6.23 (s, 1H), 6.76 (d, J = 8.80 Hz, 1H), 7.44 (dd, J = 8.80, 2.69 Hz, 1H), 7.84 (d, J = 2.69 Hz, 1H), 8.31–8.40 (m, 1H); MS (ESI/APCI dual) *m/z*: 535 $[M+H]^+$.

Methyl 2-[2-({2-[{5-chloro-2-[(2*S*)-piperidin-2-yl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]ethyl}amino)-2-oxoethoxy]-5-methylbenzoate (73e)

The title compound **73e** was synthesized according to the procedure described for **73c** from **36b** and **58c** (86% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45–1.75 (m, 4H), 1.84–1.92 (m, 1H), 1.96–2.03 (m, 1H), 2.32 (s, 3H), 2.75–2.85 (m, 1H), 3.15–3.24 (m, 4H), 3.74 (q, J = 6.07 Hz, 2H), 3.80–3.87 (m, 4H), 4.33–4.41 (m, 4H), 5.86–5.90 (m, 1H), 6.23 (s, 1H), 6.72 (d, J = 8.44 Hz, 1H), 7.24–7.29 (m, 1H), 7.64 (d, J = 1.96 Hz, 1H), 8.48–8.57 (m, 1H); MS (ESI/APCI dual) *m/z*: 515 $[M+H]^+$.

Methyl 2-[2-({2-[{5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]acetyl}amino)ethoxy]-5-fluorobenzoate (73f)

The title compound **73f** was synthesized according to the procedure described for **73c** from **36b** and **63** (84% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35–2.03 (m, 6H), 2.64–2.75 (m, 1H), 3.07–3.14 (m, 1H), 3.21 (s, 3H), 3.61–3.69 (m, 4H), 3.70–3.79 (m, 3H), 4.08–4.18 (m, 3H), 4.70 (s, 2H), 5.97 (s, 1H), 6.26 (s, 1H), 6.87–6.96 (m, 1H), 7.12–7.21 (m, 1H), 7.37–7.45 (m, 1H), 7.98–8.11 (m, 1H); MS (ESI/APCI dual) *m/z*: 519 $[M+H]^+$.

Methyl 2-{2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methylamino)ethylamino]-3,3,3-trifluoropropoxy}-5-fluorobenzoate (73g)

The title compound **73g** was synthesized according to the procedure described for **73c** from **36b** and **72** (71% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45–1.71 (m, 4H), 1.82–1.92 (m, 1H), 1.94–2.02 (m, 1H), 2.72–2.83 (m, 1H), 3.11–3.25 (m, 6H), 3.44–3.58 (m, 1H), 3.78–3.87 (m, 4H), 4.03–4.19 (m, 3H), 4.23–4.37 (m, 1H), 5.89 (s, 1H), 6.29–6.33 (m, 1H), 6.84–6.91 (m, 1H), 7.14–7.20 (m, 1H), 7.48–7.55 (m, 1H); MS (ESI/APCI dual) *m/z*: 573 $[M+H]^+$.

Methyl 2-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methylamino)ethylsulfamoylmethoxy]-5-fluorobenzoate (73h)

The title compound **73h** was synthesized according to the procedure described for **73c** from **36b** and **66** (89% yield).
MS (ESI/APCI dual) *m/z*: 555 $[M+H]^+$.

Methyl 2-(2-{benzyloxycarbonyl-[2-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methylamino)ethyl]amino}ethoxy)-5-fluorobenzoate (73i)

The title compound **73i** was synthesized according to the procedure described for **73c** from **36b** and **52** (100% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38–1.71 (m, 4H), 1.80–1.90 (m, 1H), 1.92–2.01 (m, 1H), 2.64–2.80 (m, 1H), 3.00–3.05 (m, 1.2H), 3.05–3.16 (m, 1H), 3.24 (s, 1.8H), 3.31–3.37 (m, 1H), 3.66–3.73 (m, 2.2H), 3.74–3.78 (m, 1.8H), 3.79–3.85 (m, 1H), 3.85–3.97 (m, 3H), 4.08–4.24 (m, 2H), 4.31–4.51 (m, 1H), 5.05 (s, 1.2H), 5.11 (s, 0.8H), 5.78 (s, 0.4H), 5.95 (s, 0.6H), 6.31–6.39 (m, 1H), 6.64–6.72 (m, 0.6H), 6.84–6.91 (m, 0.4H), 6.99–7.09 (m, 0.6H), 7.10–7.18 (m, 0.4H), 7.23–7.37 (m, 5H), 7.45–7.54 (m, 1H); MS (ESI/APCI dual) *m/z*: 639 $[M+H]^+$.

(23a*S*)-18-Chloro-8-fluoro-16-methyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one (75a)

The title compound **75a** was synthesized according to the procedure described for **75c** from **73a** (100% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31–1.89 (m, 6H), 1.89–2.08 (m, 2H), 2.17–2.33 (m, 1H), 2.35–2.49 (m, 1H), 2.76–2.86 (m, 0.2H), 3.05 (s, 2.4H), 3.08 (s, 0.6H), 3.33–3.52 (m, 2.4H), 3.64–3.74 (m, 0.2H), 3.79–4.00 (m, 1H), 4.01–4.14 (m, 1H), 4.18–4.31 (m, 0.8H), 4.57–4.70 (m, 0.4H), 5.03–5.10 (m, 0.2H), 5.87 (s, 1H), 6.18 (s, 0.2H), 6.28–6.36 (m, 1.6H), 6.72–6.79 (m, 0.2H), 6.81–6.88 (m, 0.8H), 6.93–7.08 (m, 2H); MS (ESI/APCI dual) *m/z*: 458 $[M+H]^+$.

(18a*S*)-2,13-Dichloro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (75d)

The title compound **75d** was synthesized according to the procedure described for **75c** from **73d** (99% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.44–2.22 (m, 5.5H), 2.31–2.41 (m, 0.5H), 2.86–2.93 (m, 0.5H), 3.02–3.32 (m, 6H), 4.21–4.57 (m, 3H), 4.75–4.83 (m, 0.5H), 4.97–5.11 (m, 1H), 5.15–5.26 (m, 0.5H), 6.06 (s, 0.5H), 6.11 (s, 0.5H), 6.21 (s, 0.5H), 6.30–6.37 (m, 0.5H), 6.56 (s, 0.5H), 6.76–6.85 (m, 1H), 7.24–7.34 (m, 2H), 7.65–7.74 (m, 0.5H), 8.56–8.65 (m, 0.5H); MS (ESI/APCI dual) *m/z*: 503 $[M+H]^+$.

(18a*S*)-13-Chloro-2,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (75e)

The title compound **75e** was synthesized according to the procedure described for **75c** from **73e** (92% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42–2.18 (m, 5.4H), 2.26–2.39 (m, 3.6H), 2.84–2.98 (m, 0.6H), 3.02–3.37 (m, 6H), 4.20–4.44 (m, 2H), 4.45–4.55 (m, 0.8H), 4.76–4.86 (m, 0.6H), 5.00–5.12 (m, 1H), 5.18–5.31 (m, 0.6H), 6.04 (s, 0.6H), 6.09 (s, 0.4H), 6.19 (s, 0.6H), 6.32–6.39 (m, 0.4H), 6.54 (s, 0.4H), 6.72–6.80 (m, 1H), 7.08–7.17 (m, 2H), 7.70–7.81 (m, 0.4H), 8.68–8.78 (m, 0.6H); MS (ESI/APCI dual) *m/z*: 483 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-11-methyl-7,8,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-9,24(6*H*)-dione (75f)

The title compound **75f** was synthesized according to the procedure described for **75c** from **73f** (57% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.33–1.72 (m, 3H), 1.72–1.84 (m, 1H), 2.00–2.15 (m, 1H), 2.15–2.31 (m, 1H), 3.06–3.67 (m, 7H), 3.69–4.20 (m, 4H), 4.95–5.01 (m, 0.2H), 6.02 (s, 0.2H), 6.08 (s, 0.8H), 6.16 (s, 0.2H), 6.35–6.41 (m, 0.8H), 6.46 (s, 0.8H), 6.82–6.90 (m, 1H), 6.98–7.09 (m, 2H), 7.60–7.78 (m, 1H); MS (ESI/APCI dual) *m/z*: 487 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-11-methyl-7-(trifluoromethyl)-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-24-one (75g)

The title compound **75g** was synthesized according to the procedure described for **75c** from **73g** (81% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.34–2.17 (m, 5.3H), 2.22–2.32 (m, 0.7H), 2.89–3.68 (m, 8.4H), 4.04–4.36 (m, 2.3H), 4.42–4.58 (m, 0.6H), 4.61–4.82 (m, 0.4H), 4.90–5.08 (m, 0.4H), 5.81–5.95 (m, 1H), 5.96–6.10 (m, 0.7H), 6.22–6.32 (m, 0.6H), 6.35–6.40 (m, 0.6H), 6.73–6.80 (m, 0.3H), 6.86–6.96 (m, 0.7H), 6.98–7.10 (m, 2H), 7.11–7.31 (m, 1H); MS (ESI/APCI dual) *m/z*: 541 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)-7λ^6-pyrido[2,1-l]pyrimido[6,1-h][1,3,4,7,9,10,13]benzoxathiapentaazacyclohexadecine-7,7,24(6*H*)-trione (75h)

The title compound **75h** was synthesized according to the procedure described for **75c** from **73h** (57% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42–1.63 (m, 2H), 1.79–1.91 (m, 2H), 1.99–2.12 (m, 1H), 2.34–2.43 (m, 1H), 2.92–3.06 (m, 1H), 3.11 (s, 3H), 3.27–3.39 (m, 2H), 3.43–3.53 (m, 1H), 3.61–3.75 (m, 1H), 4.97–5.10 (m, 3H), 6.07 (s, 1H), 6.26–6.33 (m, 1H), 6.47 (s, 1H), 6.98–7.15 (m, 3H); MS (ESI/APCI dual) *m/z*: 523 $[M+H]^+$.

Benzyl (18a*S*)-13-chloro-2-fluoro-11-methyl-24-oxo-6,7,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-8(9*H*)-carboxylate (75i)

The title compound **75i** was synthesized according to the procedure described for **75c** from **73i** (92% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.32–1.87 (m, 4H), 1.96–2.10 (m, 1H), 2.27–2.41 (m, 1H), 2.89 (s, 2H), 2.94–3.05 (m, 0.7H), 3.09 (s, 1H), 3.14–3.23 (m, 0.3H), 3.27–3.51 (m, 1.4H), 3.57–4.27 (m, 6.6H), 4.57–4.74 (m, 0.7H), 5.03–5.29 (m, 2.3H), 5.91–5.97 (m, 1H), 6.24–6.32 (m, 1H), 6.36–6.44 (m, 1H), 6.74–6.89 (m, 1H), 6.94–7.08 (m, 2H), 7.31–

7.43 (m, 5H); MS (ESI/APCI dual) *m/z*: 607 $[M+H]^+$.

(23a*S*)-18-(Azetidin-1-yl)-8-fluoro-16-methyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one (76a)

The title compound **76a** was synthesized according to the procedure described for **76c** from **75a** (58% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.19–1.99 (m, 8H), 2.08–2.14 (m, 0.2H), 2.15–2.21 (m, 0.8H), 2.23–2.34 (m, 3H), 2.60–2.69 (m, 0.4H), 2.88–2.94 (m, 3H), 3.14–3.24 (m, 1.6H), 3.49–3.57 (m, 0.8H), 3.58–3.64 (m, 0.2H), 3.69–3.81 (m, 1H), 3.91–4.03 (m, 5H), 4.03–4.08 (m, 0.8H), 4.35–4.42 (m, 0.2H), 4.75–4.79 (m, 0.2H), 5.11 (s, 0.2H), 5.13 (s, 0.8H), 5.79 (s, 0.2H), 5.91 (s, 0.8H), 5.97–6.02 (m, 0.8H), 6.92–6.96 (m, 0.2H), 7.03–7.08 (m, 0.8H), 7.10–7.22 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 23.4, 25.4, 25.7, 29.5, 37.8, 40.0, 43.8, 47.5, 50.1, 52.9, 67.3, 75.9, 88.8, 113.7, 114.0, 115.8, 127.9, 150.4, 150.5, 151.4, 151.7, 154.1, 158.5, 165.7; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{31}FN_6O_2$ $[M+H]^+$ 479.2565, found 479.2558.

(18a*S*)-13-(Azetidin-1-yl)-2-chloro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (76d)

The title compound **76d** was synthesized according to the procedure described for **76c** from **75d** (100% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.39–1.76 (m, 4H), 1.81–2.00 (m, 1H), 2.05–2.12 (m, 0.7H), 2.14–2.20 (m, 0.3H), 2.25–2.35 (m, 2H), 2.83 (s, 0.9H), 2.88 (s, 2.1H), 3.02–3.13 (m, 2.3H), 3.14–3.20 (m, 0.3H), 3.36–3.46 (m, 0.7H), 3.78–3.87 (m, 0.3H), 3.94–4.04 (m, 5H), 4.09–4.14 (m, 0.7H), 4.45–4.57 (m, 1.7H), 4.63–4.71 (m, 1H), 4.71–4.74 (m, 0.7H), 5.29 (s, 0.7H), 5.38 (s, 0.3H), 5.89 (s, 0.7H), 5.94–5.98 (m, 0.3H), 6.08 (s, 0.3H), 7.10 (d, J = 8.67 Hz, 0.7H), 7.19 (d, J = 8.67 Hz, 0.3H), 7.40–7.45 (m, 1.3H), 7.50–7.53 (m, 1H), 8.80–8.85 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 24.7, 29.5, 36.5, 38.1, 40.0, 47.1, 48.4, 50.1, 54.0, 70.3, 78.0, 89.6, 119.1, 126.4, 127.1, 129.8, 130.6, 150.3, 151.6, 152.0, 153.4, 158.5, 165.3, 167.4; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{30}ClN_7O_3$ $[M+H]^+$ 524.2171, found: 524.2156.

(18a*S*)-13-(Azetidin-1-yl)-2,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (76e)

The title compound **76e** was synthesized according to the procedure described for **76c** from **75e** (77% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.33–1.82 (m, 4.7H), 1.86–1.96 (m, 0.3H), 2.04–2.11 (m, 0.7H), 2.15–2.21 (m, 0.3H), 2.25–2.34 (m, 5H), 2.83 (s, 0.9H), 2.89 (s, 2.1H), 3.01–3.12 (m, 2.3H), 3.17–3.24 (m, 0.3H), 3.36–3.48 (m, 0.7H), 3.75–3.86 (m, 0.3H), 3.90–4.08 (m, 5.7H), 4.43–4.54 (m, 1.7H), 4.60–4.72 (m, 1H), 4.72–4.75 (m, 0.7H), 5.30 (s, 0.7H), 5.37 (s, 0.3H), 5.89 (s, 0.7H), 5.97–6.00 (m, 0.3H), 6.07 (s, 0.3H), 6.95 (d, J = 8.26 Hz, 0.7H), 7.03 (d, J = 8.26 Hz, 0.3H), 7.08–7.11 (m, 0.3H), 7.13–7.20 (m, 1.7H), 7.53–7.59 (m, 0.3H), 8.95–9.01 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 20.1, 24.8, 29.6, 36.5, 37.8, 40.0, 48.3, 48.6, 50.1, 53.9, 70.9, 78.0, 89.4, 117.9, 126.9, 129.1, 130.3, 132.7, 150.3, 151.1, 151.6, 153.5, 158.6, 167.0, 167.8; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{33}N_7O_3$ $[M+H]^+$ 504.2718, found: 504.2702.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-7,8,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-9,24(6*H*)-dione (76f)

The title compound **76f** was synthesized according to the procedure described for **76c** from **75f** (53% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.27–1.67 (m, 4H), 1.83–1.93 (m, 1H), 1.99–2.07 (m, 1H), 2.25–2.36 (m, 2H), 2.92 (s, 2.5H), 2.96 (s, 0.5H), 3.12–3.25 (m, 1H), 3.25–3.35 (m, 1H), 3.48–3.71 (m, 2.8H), 3.73–3.79 (m, 0.2H), 3.93–4.07 (m, 4.2H), 4.11–4.22 (m, 1.8H), 4.40–4.47 (m, 0.2H), 4.64–4.69 (m, 0.2H), 5.09–5.19 (m, 0.8H), 5.20 (s, 0.2H), 5.26 (s, 0.8H), 5.79 (s, 0.2H), 5.94 (s, 0.8H), 5.95–5.98 (m, 0.8H), 6.98–7.03 (m, 0.2H), 7.10–7.25 (m, 2.8H), 7.85–7.93 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.5, 25.3, 30.4, 38.4, 40.0, 43.9, 47.3, 50.1, 56.0, 66.0, 77.1, 89.3, 113.7, 113.9, 115.8, 128.0, 149.7, 149.9, 151.2, 155.1, 156.9, 158.5, 165.7, 168.9; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{30}FN_7O_3$ $[M+H]^+$ 508.2467, found: 508.2445.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-7-(trifluoromethyl)-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-24-one (76g)

The title compound **76g** was synthesized according to the procedure described for **76c** from **75g** (78% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ 1.31–1.68 (m, 4H), 1.85–2.20 (m, 2.2H), 2.24–2.34 (m, 2.3H), 2.51–2.57 (m, 0.5H), 2.73–3.28 (m, 6.2H), 3.33–3.43 (m, 0.9H), 3.49–3.66 (m, 1.5H), 3.82–3.91 (m, 0.4H), 3.92–4.03 (m, 3.9H), 4.05–4.31 (m, 2.4H), 4.41–4.52 (m, 0.2H), 4.69–4.73 (m, 0.1H), 4.79–4.83 (m, 0.1H), 4.88–4.98 (m, 0.5H), 5.03–5.05 (m, 0.2H), 5.08–5.14 (m, 0.8H), 5.59–5.67 (m, 0.1H), 5.72–5.75 (m, 0.2H), 5.90–5.98 (m, 1.5H), 7.07–7.32 (m, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 25.3, 29.7, 38.6, 40.0, 44.2, 45.8, 47.4, 50.1, 51.5, 58.0, 65.2, 75.6, 89.0, 114.1, 114.2, 114.8, 116.0, 128.3, 149.7, 149.8, 151.8, 154.1, 155.8, 158.6, 165.4; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{31}F_4N_7O_2$ $[M+H]^+$ 562.2548, found: 562.2531.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)-7λ^6-pyrido[2,1-l]pyrimido[6,1-h][1,3,4,7,9,10,13]benzoxathiapentaazacyclohexadecine-7,7,24(6*H*)-trione (76h)

The title compound **76h** was synthesized according to the procedure described for **76c** from **75h** (85% yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.14–1.58 (m, 3H), 1.62–1.68 (m, 1H), 1.90–1.98 (m, 1H), 2.16–2.22 (m, 1H), 2.28–2.35 (m, 2H), 2.96 (s, 3H), 3.17–3.29 (m, 2H), 3.35–3.43 (m, 1H), 3.54–3.63 (m, 1H), 3.67–3.76 (m, 1H), 3.91–4.05 (m, 5H), 5.23 (s, 1H), 5.29 (d, J = 12.39 Hz, 1H), 5.45 (d, J = 12.39 Hz, 1H), 5.93–5.97 (m, 1H), 6.02 (s, 1H), 7.22 (t, J = 5.37 Hz, 1H), 7.24–7.31 (m, 2H), 7.37–7.42 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 25.2, 28.7, 37.8, 40.0, 41.2, 44.1, 47.7, 49.6, 50.1, 76.8, 77.4, 89.8, 113.7, 115.9, 116.1, 128.3, 148.9, 150.0, 151.7, 154.2, 156.1, 158.6; HRMS ESI/APCI dual *m/z* calcd for $C_{25}H_{30}FN_7O_4S$ $[M+H]^+$ 544.2137, found: 544.2123.

Benzyl (18a*S*)-13-(azetidin-1-yl)-2-fluoro-11-methyl-24-oxo-6,7,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-8(9*H*)-carboxylate (76i)

The title compound **76i** was synthesized according to the procedure described for **76c** from **75i** (98% yield).

MS (ESI/APCI dual) *m/z*: 628 $[M+H]^+$.

(18a*S*)-13-Chloro-2-fluoro-8,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (77)

To a solution of **75c** (37 mg, 0.076 mmol) in *N*,*N*-dimethylformamide (0.76 mL) was added sodium hydride (60%, dispersion in Paraffin Liquid) (9.2 mg, 0.228 mmol, 3.0 eq) at 0 °C. After stirring at room temperature for 30 min, iodomethane (14.2 μL, 0.228 mmol, 3.0 eq) was added to the reaction mixture. After stirring at room temperature for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **77** (38 mg, 0.076 mmol, 100%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.34–1.63 (m, 2H), 1.63–1.89 (m, 2.2H), 1.96–2.13 (m, 0.8H), 2.21–2.30 (m, 0.2H), 2.33–2.44 (m, 0.8H), 2.74–3.12 (m, 6H), 3.13–3.51 (m, 3H), 3.52–3.68 (m, 0.3H), 3.83–3.99 (m, 0.3H), 4.03–4.18 (m, 0.7H), 4.22–4.69 (m, 0.7H), 4.70–4.86 (m, 2H), 5.06–5.15 (m, 0.2H), 5.28–5.41 (m, 0.8H), 5.48–5.61 (m, 0.2H), 6.00–6.10 (m, 1H), 6.21 (s, 0.2H), 6.28–6.34 (m, 0.8H), 6.45 (s, 0.8H), 6.94–7.10 (m, 2.2H), 7.15–7.21 (m, 0.8H); MS (ESI/APCI dual) *m/z*: 501 $[M+H]^+$.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-8,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (78)

Compound **78** was prepared from **77** according to the procedure described for **76c** to

obtain a colorless amorphous substance in a reaction with a 35% yield.
^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.29–1.79 (m, 4H), 1.82–2.22 (m, 2H), 2.25–2.36 (m, 2H), 2.76–3.24 (m, 7H), 3.26–3.45 (m, 1H), 3.71–3.82 (m, 1.3H), 3.88–4.19 (m, 6H), 4.46–4.89 (m, 3H), 5.15–5.39 (m, 1H), 5.71–6.02 (m, 1.7H), 7.06–7.31 (m, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 15.6, 19.8, 25.3, 28.4, 33.7, 37.4, 44.0, 46.9, 47.6, 50.1, 51.0, 69.7, 77.0, 79.1, 89.4, 114.6, 116.4, 117.1, 128.3, 150.1, 150.4, 151.3, 153.4, 154.3, 158.4, 165.3, 167.4; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{32}FN_7O_3$ $[M+H]^+$ 522.2623, found: 522.2605.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-11-methyl-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-24-one (79)

To a solution of **76i** (69 mg, 0.109 mmol) in methanol (0.5 mL) was added 10% palladium on carbon (69 mg). The reaction was flushed with hydrogen and stirred under a hydrogen atmosphere at room temperature for 1.5 h. The reaction mixture was filtered through a pad of Celite® and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 0%–10% methanol in chloroform) to obtain **79** (40 mg, 0.082 mmol, 75%) as a colorless powder.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.19–1.91 (m, 4H), 1.93–2.05 (m, 1H), 2.08–2.16 (m, 0.3H), 2.17–2.26 (m, 0.7H), 2.31–2.44 (m, 2H), 2.81–2.94 (m, 2H), 2.98 (s, 2.1H), 3.01–3.17 (m, 3.5H), 3.24–3.33 (m, 0.7H), 3.37–3.46 (m, 0.7H), 3.91–4.18 (m, 7.4H), 4.61–4.71 (m, 0.3H), 4.89–4.94 (m, 0.3H), 4.97 (s, 0.3H), 5.01 (s, 0.7H), 5.07–5.20 (m, 0.3H), 5.82 (s, 0.3H), 6.04 (s, 0.7H), 6.21–6.27 (m, 0.7H), 6.73–6.80 (m, 0.3H), 6.81–6.89 (m, 0.7H), 6.93–7.07 (m, 2H); HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{32}FN_7O_2$ $[M+H]^+$ 494.2674, found: 494.2655.

(18a*S*)-13-(Azetidin-1-yl)-2-fluoro-8-(methanesulfonyl)-11-methyl-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-24-one (80)

To a solution of **79** (7.1 mg, 0.014 mmol) in chloroform (0.48 mL) was added triethylamine (12 μL, 0.086 mmol, 6.0 eq) and methanesulfonyl chloride (3.4 μL, 0.043

mmol, 3.0 eq) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was poured into saturated aqueous ammonium chloride and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 25%–100% ethyl acetate in hexane) to obtain **80** (7.5 mg, 0.013 mmol, 91%) as a pale yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.04–2.18 (m, 5H), 2.25–2.46 (m, 3H), 2.91 (s, 2.1H), 2.95 (s, 0.9H), 3.04–3.30 (m, 4.7H), 3.47–3.92 (m, 5H), 3.99–4.27 (m, 6H), 4.46–4.71 (m, 1.3H), 4.96–5.02 (m, 0.6H), 5.05 (s, 0.7H), 5.83 (s, 0.3H), 6.07 (s, 0.7H), 6.16–6.23 (m, 0.7H), 6.80–6.90 (m, 1H), 6.93–7.10 (m, 2H); HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{34}FN_7O_4S$ $[M+H]^+$ 572.2450, found: 572.2433.

(18a*S*)-2-Fluoro-13-(3-hydroxyazetidin-1-yl)-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (81a)

To a solution of **75c** (23 mg, 0.047 mmol) in 2-propanol (0.47 mL) was added 1 M aqueous sodium hydroxide (0.47 mL, 0.472 mmol, 10 eq) and 3-hydroxyazetidine hydrochloride (52 mg, 0.472 mmol, 10 eq). After stirring at 130 °C under microwave irradiation for 30 min, the reaction mixture was extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 0%–6% methanol in chloroform) to obtain **81a** (11 mg, 0.021 mmol, 44%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37–2.15 (m, 5H), 2.25–2.37 (m, 1H), 2.85–3.05 (m, 4H), 3.07–3.29 (m, 2H), 3.92–4.02 (m, 2H), 4.11–4.25 (m, 0.7H), 4.26–4.62 (m, 4.6H), 4.66–4.94 (m, 3.4H), 5.12 (s, 0.7H), 5.18 (s, 0.3H), 5.91 (s, 0.7H), 6.22 (s, 0.3H), 6.23–6.27 (m, 0.3H), 6.80–6.92 (m, 1H), 6.96–7.09 (m, 2H), 7.78 (d, J = 8.44 Hz, 0.3H), 9.07 (d, J = 7.21 Hz, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.5, 36.4, 37.9, 46.9, 48.3, 54.0, 60.0, 60.4, 71.2, 78.4, 79.1, 89.4, 113.7, 116.5, 120.1, 130.8, 149.6, 150.3, 151.6, 153.4, 157.0, 158.5, 165.4, 167.6, HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{30}FN_7O_4$ $[M+H]^+$ 524.2416, found: 524.2401.

(18a*S*)-13-(3-Aminoazetidin-1-yl)-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (81b)

To a solution of **75c** (0.13 g, 0.267 mmol) in 1-methyl-2-pyrrolidone (1.3 mL) was added triethylamine (0.37 mL, 2.67 mmol, 10 eq) and *tert*-butyl *N*-(azetidin-3-yl)carbamate (0.23 g, 1.34 mmol, 5.0 eq). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 10%–100% ethyl acetate in hexane) to obtain *tert*-butyl {1-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidin-3-yl}carbamate (0.14 g, 0.230 mmol, 86%) as a colorless amorphous substance.
MS (ESI/APCI dual) *m/z*: 623 $[M+H]^+$.

To a solution of *tert*-butyl {1-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidin-3-yl}carbamate (0.14 g, 0.230 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (0.53 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 0%–9% methanol in chloroform) to obtain **81b** (0.13 g, 0.242 mmol, 100%) as a colorless amorphous substance.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.01–2.17 (m, 5.7H), 2.28–2.37 (m, 0.3H), 2.84–3.30 (m, 6H), 3.69–3.80 (m, 2H), 3.93–4.03 (m, 1H), 4.11–4.25 (m, 0.7H), 4.25–4.61 (m, 4.6H), 4.67–4.92 (m, 2.4H), 5.12 (s, 0.7H), 5.19 (s, 0.3H), 5.89 (s, 0.7H), 6.20 (s, 0.3H), 6.23–6.27 (m, 0.3H), 6.80–6.92 (m, 1H), 6.96–7.08 (m, 2H), 7.74–7.83 (m, 0.3H), 9.05–9.13 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.9, 24.7, 29.5, 36.4, 37.9, 40.0, 42.9, 48.3, 54.0, 60.4, 67.6, 71.2, 78.5, 89.4, 113.5, 116.3, 120.1, 130.8, 149.6, 150.3,

151.7, 153.4, 157.0, 158.6, 165.4, 167.6; HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{31}FN_8O_3$ $[M+H]^+$ 523.2576, found: 523.2555.

(18a*S*)-2-Fluoro-11-methyl-13-[3-(methylamino)azetidin-1-yl]-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (81c)

To a solution of **75c** (29 mg, 0.060 mmol) in 2-propanol (0.60 mL) was added 1 M aqueous sodium hydroxide (0.60 mL, 0.596 mmol, 10 eq) and *tert*-butyl *N*-(azetidin-3-yl)-*N*-methylcarbamate hydrochloride (0.13 g, 0.596 mmol, 10 eq). After stirring at 100 °C under microwave irradiation for 1 h, the reaction mixture was extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using reversed-phase preparative HPLC to obtain *tert*-butyl {1-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidin-3-yl}methylcarbamate (44 mg, 0.069 mmol, 100%) as a colorless oil.
MS (ESI/APCI dual) *m/z*: 637 $[M+H]^+$.

To a solution of *tert*-butyl {1-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidin-3-yl}methylcarbamate (44 mg, 0.069 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (0.16 mL), and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using reversed-phase preparative HPLC to obtain **81c** (9.3 mg, 0.017 mmol, 25%) as a colorless powder.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.11–2.38 (m, 6H), 2.43–2.49 (m, 3H), 2.84–3.04 (m, 4H), 3.08–3.30 (m, 2H), 3.69–3.79 (m, 1H), 3.80–3.92 (m, 2H), 4.08–4.40 (m, 4H), 4.43–4.62 (m, 1.4H), 4.66–4.93 (m, 2.3H), 5.13 (s, 0.7H), 5.20 (s, 0.3H), 5.88 (s, 0.7H), 6.19 (s, 0.3H), 6.22–6.28 (m, 0.3H), 6.79–6.91 (m, 1H), 6.96–7.10 (m, 2H), 7.75–7.82 (m,

0.3H), 9.01–9.13 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.5, 32.8, 36.4, 37.9, 46.9, 48.3, 49.5, 54.0, 57.0, 71.2, 78.4, 79.1, 89.4, 113.7, 116.5, 120.1, 130.8, 149.6, 150.3, 151.7, 153.4, 157.0, 158.6, 165.4, 167.6; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{33}FN_8O_3$ $[M+H]^+$ 537.2732, found: 537.2720.

(18a*S*)-13-[(3*S*)-3-Aminopyrrolidin-1-yl]-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (81d)

To a solution of **75c** (0.15 g, 0.308 mmol) in 1-methyl-2-pyrrolidone (1.5 mL) was added triethylamine (0.31 mL, 3.08 mmol, 10 eq) and (3*S*)-pyrrolidin-3-amine (0.13 g, 1.54 mmol, 5.0 eq). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was purified using reversed-phase preparative HPLC to obtain **81d** (82 mg, 0.153 mmol, 50%) as a colorless powder.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.41–2.06 (m, 7H), 2.06–2.12 (m, 0.7H), 2.17–2.23 (m, 0.3H), 2.85 (s, 0.9H), 2.91 (s, 2.1H), 2.97–3.49 (m, 5.3H), 3.51–3.62 (m, 3H), 3.81–3.91 (m, 0.3H), 3.94–4.06 (m, 1H), 4.06–4.11 (m, 0.7H), 4.46–4.56 (m, 1.7H), 4.63–4.75 (m, 1.7H), 5.43 (s, 0.7H), 5.51 (s, 0.3H), 5.85 (s, 0.7H), 5.96–6.00 (m, 0.3H), 6.04 (s, 0.3H), 7.10–7.15 (m, 0.7H), 7.16–7.27 (m, 1.6H), 7.32–7.36 (m, 0.7H), 7.54–7.59 (m, 0.3H), 8.99–9.06 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.9, 24.7, 29.4, 33.9, 36.3, 36.6, 37.9, 45.0, 48.3, 54.0, 55.0, 71.2, 79.1, 79.7, 89.0, 113.7, 116.3, 120.1, 130.8, 149.6, 150.1, 151.7, 153.3, 155.4, 156.9, 165.3, 167.6; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{33}FN_8O_3$ $[M+H]^+$ 537.2732, found 537.2725.

(18a*S*)-13-(Azetidin-3-yl)-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (82)

To a solution of 1-Boc-3-iodoazetidine (0.25 g, 0.883 mmol, 5.0 eq) in *N*,*N*-dimethylacetamide (2.0 mL) was added zinc powder (59 mg, 0.901 mmol, 5.1 eq) at 65 °C. After stirring for 20 min, a solution of **75c** (86 mg, 0.177 mmol) in *N*,*N*-dimethylacetamide (2.0 mL) was placed in another flask, and copper iodide (I) (6.7 mg, 0.035 mmol, 0.20 eq) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II)

(78 mg, 0.106 mmol, 0.60 eq) were added. After stirring at 85 °C for 5 min, the reaction mixture was added to the prepared zinc reagent. After stirring at 85 °C for 5 min, the reaction mixture was poured into saturated aqueous ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain *tert*-butyl 3-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidine-1-carboxylate (79 mg, 0.130 mmol, 73%) as a pale brown amorphous substance.

To a solution of *tert*-butyl 3-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]azetidine-1-carboxylate (78 mg, 0.128 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (1.0 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using reversed-phase preparative HPLC to obtain **82** (27 mg, 0.054 mmol, 42%) as a colorless powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.19–1.72 (m, 3H), 1.74–1.95 (m, 2H), 1.96–2.24 (m, 1.6H), 2.34–2.45 (m, 0.4H), 2.86–3.32 (m, 6H), 3.87–4.08 (m, 4H), 4.18–4.32 (m, 1.4H), 4.34–4.60 (m, 2H), 4.72–4.82 (m, 0.6H), 4.90–5.14 (m, 1.6H), 6.06 (s, 0.6H), 6.10 (s, 0.4H), 6.27 (s, 0.6H), 6.33–6.38 (m, 0.4H), 6.60 (s, 0.4H), 6.80–6.90 (m, 1H), 6.97–7.10 (m, 2H), 7.78 (d, J = 8.70 Hz, 0.4H), 8.96 (d, J = 7.90 Hz, 0.6H); HRMS ESI/APCI dual *m/z* calcd for $C_{26}H_{30}FN_7O_3$ $[M+H]^+$ 508.2467, found: 508.2450.

(18a*S*)-13-[(1*s*,3*R*)-3-Aminocyclobutyl]-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (84a, *cis* isomer) and (18a*S*)-13-[(1*r*,3*S*)-3-aminocyclobutyl]-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-

l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (84b, *trans* isomer)

According to the procedure described for **82**, the title compound **83** (a mixture of **84a** and **84b**) was obtained as a brown amorphous substance in a reaction with a 44% yield using *tert*-butyl *N*-(3-iodocyclobutyl)carbamate instead of 1-Boc-3-iodoazetidine.

Then, **83** was separated using chiral HPLC (CHIRALPAK OD 35 μm, 4.6 × 150 mm; flow, 1 mL/min, 50% ethanol in hexane; detection wavelength, 254 nm; *cis*–isomer **84a** t_R = 5.21 min; *trans*–isomer **84b** t_R = 3.60 min) to obtain **84a** and **84b** as colorless powders.

84a: ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.40–2.04 (m, 7H), 2.15–2.20 (m, 0.7H), 2.23–2.29 (m, 0.3H), 2.44–2.54 (m, 3H), 2.93 (s, 0.9H), 2.98 (s, 2.1H), 3.00–3.52 (m, 4H), 3.88–4.09 (m, 2H), 4.20–4.27 (m, 0.3H), 4.47–4.58 (m, 1.7H), 4.60–4.66 (m, 0.3H), 4.82–4.93 (m, 1.4H), 6.06–6.11 (m, 0.3H), 6.19 (s, 0.7H), 6.24 (s, 0.3H), 6.33 (s, 0.7H), 6.56 (s, 0.3H), 7.08–7.13 (m, 0.7H), 7.16–7.29 (m, 1.6H), 7.34–7.39 (m, 0.7H), 7.51–7.56 (m, 0.3H), 8.85–8.90 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.7, 33.8, 36.0, 36.4, 36.6, 38.0, 40.0, 44.2, 46.9, 48.1, 54.0, 67.6, 71.0, 91.0, 113.7, 116.5, 119.8, 130.6, 149.6, 151.6, 154.1, 156.9, 164.7, 165.5, 167.5; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{32}FN_7O_3$ $[M+H]^+$ 522.2623, found: 522.2620.

84b: ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.42–2.11 (m, 7H), 2.15–2.21 (m, 0.7H), 2.23–2.31 (m, 0.3H), 2.42–2.54 (m, 3H), 2.92 (s, 0.9H), 2.97 (s, 2.1H), 3.00–3.65 (m, 4H), 3.87–4.10 (m, 2H), 4.18–4.29 (m, 0.3H), 4.48–4.58 (m, 1.7H), 4.60–4.65 (m, 0.3H), 4.82–4.93 (m, 1.4H), 6.06–6.11 (m, 0.3H), 6.16 (s, 0.7H), 6.21 (s, 0.3H), 6.35 (s, 0.7H), 6.57 (s, 0.3H), 7.08–7.13 (m, 0.7H), 7.15–7.29 (m, 1.6H), 7.33–7.39 (m, 0.7H), 7.51–7.57 (m, 0.3H), 8.85–8.92 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.7, 34.9, 36.0, 36.4, 36.6, 37.6, 38.0, 40.0, 43.6, 45.5, 48.1, 54.0, 67.6, 71.0, 91.2, 113.6, 116.4, 119.8, 128.3, 149.6, 151.6, 154.1, 156.9, 165.5, 167.5; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{32}FN_7O_3$ $[M+H]^+$ 522.2623, found: 522.2613.

Ethyl 3-(3-benzyloxycyclobutyl)-3-oxopropanoate (86)

To a solution of 3-benzyloxycyclobutanecarboxylic acid (5.3 g, 26 mmol) in tetrahydrofuran (86 mL) was added 1,1'-carbonyldiimidazole (6.3 g, 39 mmol, 1.5 eq). After stirring at room temperature for 2.5 h, the reaction mixture was added to potassium

3-ethoxy-3-oxo-propanoate (8.7 g, 51 mmol, 2.0 eq) and magnesium chloride (4.9 g, 51 mmol, 2.0 eq). After stirring at 65 °C for 2.5 h, the reaction mixture was cooled to room temperature and filtered through a pad of Celite®, and the filtrate was concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 5%–20% ethyl acetate in hexane) to obtain **86** (3.1 g, 11.5 mmol, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.23–1.32 (m, 3H), 2.14–2.34 (m, 2H), 2.39–2.59 (m, 2H), 2.82–2.95 (m, 1H), 3.38–3.45 (m, 2H), 3.93–4.05 (m, 1H), 4.13–4.24 (m, 2H), 4.37–4.47 (m, 2H), 7.27–7.37 (m, 5H); MS (ESI/APCI dual) *m/z*: 277 $[M+H]^+$.

tert-Butyl (2*S*)-2-[5-(3-benzyloxycyclobutyl)-7-oxo-4H-pyrazolo[1,5-a]pyrimidin-2-yl]piperidine-1-carboxylate (87)

To a solution of **86** (3.1 g, 11 mmol, 1.2 eq) in acetic acid (23 mL) was added *tert*-butyl (2*S*)-2-(5-amino-1*H*-pyrazol-3-yl)piperidine-1-carboxylate (**34**) (2.5 g, 9.4 mmol). After stirring at 100 °C for 2.5 h, the reaction mixture was cooled to room temperature and was concentrated under reduced pressure.

To a solution of the resulting residue in chloroform (31 mL) was added triethylamine (2.6 mL, 19 mmol, 2.0 eq) and di-*tert*-butyl dicarbonate (2.0 g, 9.4 mmol, 1.0 eq). After stirring for 2 h at room temperature, the reaction mixture was poured into 0.5 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried through a phase separator and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 50%–100% ethyl acetate in hexane) to obtain **87** (4.0 g, 8.3 mmol, 88%) as a colorless amorphous substance.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31–1.80 (m, 13H), 2.12–2.26 (m, 2H), 2.42–2.56 (m, 0.8H), 2.57–2.88 (m, 3.4H), 3.01–3.17 (m, 0.8H), 3.45–3.59 (m, 0.2H), 3.89–4.30 (m, 2.8H), 4.39–4.52 (m, 2H), 5.34–5.46 (m, 1H), 5.51 (s, 0.8H), 5.60–5.81 (m, 1.2H), 7.28–7.43 (m, 5H), 10.51–11.37 (m, 1H); MS (ESI/APCI dual) *m/z*: 479 $[M+H]^+$.

tert-Butyl (2*S*)-2-{5-[(1*s*,3*R*)-3-(benzyloxy)cyclobutyl]-7-chloropyrazolo[1,5-a]pyrimidin-2-yl}piperidine-1-carboxylate (88a) and _tert_-butyl (2*S*)-2-{5-[(1*r*,3*S*)-3-(benzyloxy)cyclobutyl]-7-chloropyrazolo[1,5-a]pyrimidin-2-yl}piperidine-1-carboxylate (88b)

A solution of 4-dimethylaminopyridine (1.8 g, 15.0 mmol, 1.1 eq) in pyridine (68 mL) was cooled to 0 °C, and phosphorus oxychloride (8.7 mL, 95.5 mmol, 7.0 eq) and **87** (6.5 g, 13.6 mmol) were added. After stirring at 65 °C for 0.5 h, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH ethyl acetate) and (OH 15% ethyl acetate in hexane) to obtain **88a** (3.8 g, 7.76 mmol, 65%) as a pale yellow oil and **88b** (0.90 g, 1.80 mmol, 15%) as a pale yellow oil.

88a: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37–1.71 (m, 13H), 1.82–1.96 (m, 1H), 2.34 (q, J = 9.01 Hz, 2H), 2.50 (d, J = 13.20 Hz, 1H), 2.66–2.76 (m, 2H), 2.91 (t, J = 11.80 Hz, 1H), 3.07–3.20 (m, 1H), 4.02–4.18 (m, 2H), 4.49 (s, 2H), 5.62 (brs, 1H), 6.49 (s, 1H), 6.80 (s, 1H), 7.27–7.40 (m, 5H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.3, 24.7, 27.8, 28.0, 31.8, 35.7, 35.8, 68.1, 69.0, 78.9, 95.0, 107.3, 127.4, 127.6, 128.1, 137.1, 138.3, 149.7, 154.5, 158.2, 163.3; MS (ESI/APCI dual) *m/z*: 497 $[M+H]^+$.

88b: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38–1.71 (m, 13H), 1.81–1.97 (m, 1H), 2.45–2.57 (m, 3H), 2.61–2.72 (m, 2H), 2.85–2.98 (m, 1H), 3.58–3.71 (m, 1H), 4.01–4.17 (m, 1H), 4.32–4.41 (m, 1H), 4.47 (s, 2H), 5.63 (brs, 1H), 6.49 (s, 1H), 6.74 (s, 1H), 7.27–7.39 (m, 5H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.4, 21.7, 27.3, 28.0, 31.0, 33.8, 34.1, 44.2, 53.6, 69.2, 70.8, 79.2, 86.9, 92.2, 127.4, 127.6, 128.2, 138.3, 142.5, 152.5, 154.4, 155.3, 155.9, 156.7; MS (ESI/APCI dual) *m/z*: 497 $[M+H]^+$.

tert-Butyl (2*S*)-2-{5-[(1*s*,3*R*)-3-(benzyloxy)cyclobutyl]-7-[(2-{2-[4-fluoro-2-(methoxycarbonyl)phenoxy]acetamido}ethyl)(methyl)amino]pyrazolo[1,5-a]pyrimidin-2-yl}piperidine-1-carboxylate (89a)

To a solution of **88a** (0.21 g, 0.425 mmol) in 1-methyl-2-pyrrolidone (4.2 mL) was added triethylamine (0.59 mL, 4.25 mmol, 10 eq) and **58a** (0.19 g, 0.594 mmol, 1.4 eq). After stirring at 150 °C under microwave irradiation for 30 min, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 30%–70% ethyl acetate in hexane) to obtain **89a** (0.11 g, 0.145 mmol, 34%) as a colorless amorphous substance.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.36–1.66 (m, 12H), 1.76–1.89 (m, 1H), 2.23–2.34 (m, 2H), 2.35–2.49 (m, 1H), 2.59–2.71 (m, 2H), 2.78–2.91 (m, 1H), 2.94–3.06 (m, 1H), 3.21 (s, 3H), 3.71–3.85 (m, 5H), 3.93–4.28 (m, 5H), 4.47 (s, 4H), 5.53 (brs, 1H), 5.83 (s, 1H), 6.17 (s, 1H), 6.77–6.89 (m, 1H), 7.13–7.22 (m, 1H), 7.28–7.39 (m, 5H), 7.52–7.61 (m, 1H), 8.24–8.36 (m, 1H); MS (ESI/APCI dual) *m/z*: 745 $[M+H]^+$.

tert-Butyl (2*S*)-2-{5-[(1*s*,3*R*)-3-(benzyloxy)cyclobutyl]-7-[(2-{2-[4-chloro-2-(methoxycarbonyl)phenoxy]acetamido}ethyl)(methyl)amino]pyrazolo[1,5-a]pyrimidin-2-yl}piperidine-1-carboxylate (89b)

To a solution of **88a** (6.7 g, 13.5 mmol) in *N*,*N*-dimethylformamide (68 mL) was added triethylamine (15.1 mL, 108 mmol, 8.0 eq) and **58b** (6.4 g, 19.0 mmol, 1.4 eq). After stirring at 80 °C for 1 h, the reaction mixture was cooled and was poured into water and extracted with ethyl acetate/toluene (4/1). The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 25%–100% ethyl acetate in hexane) to obtain **89b** (9.1 g, 11.9 mmol, 88%) as a colorless amorphous substance.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.33–1.69 (m, 12H), 1.75–1.90 (m, 1H), 2.22–2.34 (m, 2H), 2.35–2.49 (m, 1H), 2.60–2.72 (m, 2H), 2.78–2.91 (m, 1H), 2.94–3.07 (m, 1H), 3.20 (s, 3H), 3.70–3.86 (m, 5H), 3.92–4.29 (m, 5H), 4.41–4.55 (m, 4H), 5.53 (brs, 1H), 5.83 (s, 1H), 6.17 (s, 1H), 6.81 (d, J = 8.68 Hz, 1H), 7.28–7.46 (m, 6H), 7.83 (s, 1H), 8.22–8.31 (m, 1H); MS (ESI/APCI dual) *m/z*: 761 $[M+H]^+$.

tert-Butyl (2*S*)-2-{5-[(1*s*,3*R*)-3-(benzyloxy)cyclobutyl]-7-[(2-{2-[2-(methoxycarbonyl)-4-methylphenoxy]acetamido}ethyl)(methyl)amino]pyrazolo[1,5-a]pyrimidin-2-yl}piperidine-1-carboxylate (89c)

According to the procedure described for **89a**, the title compound **89c** was obtained as a colorless oil in a reaction with a 65% yield using **58c** instead of **58a**.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35–1.65 (m, 13H), 1.76–1.89 (m, 1H), 2.22–2.35 (m, 5H), 2.39–2.50 (m, 1H), 2.56–2.73 (m, 2H), 2.78–2.91 (m, 1H), 2.93–3.05 (m, 1H),

3.20 (s, 3H), 3.73–3.82 (m, 5H), 3.94–4.10 (m, 2H), 4.11–4.19 (m, 2H), 4.43–4.51 (m, 4H), 5.53 (brs, 1H), 5.77–5.85 (m, 1H), 6.16–6.20 (m, 1H), 6.77 (d, *J* = 8.44 Hz, 1H), 7.27–7.39 (m, 6H), 7.66 (s, 1H), 8.33–8.45 (m, 1H); MS (ESI/APCI dual) *m/z*: 741 $[M+H]^+$.

(18a*S*)-13-[(1*s*,3*R*)-3-(benzyloxy)cyclobutyl]-2-fluoro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (91a)

To a solution of **89a** (0.11 g, 0.145 mmol) in methanol (0.5 mL) and tetrahydrofuran (0.5 mL) was added 1 M aqueous sodium hydroxide (1.0 mL), and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was acidified using 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 2-{2-[2-({5-(3-benzyloxycyclobutyl)-2-[(2*S*)-1-*tert*-butoxycarbonyl-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methylamino)ethylamino]-2-oxoethoxy}-5-fluorobenzoic acid (0.10 g, 0.141 mmol. 97%) as a colorless oil. This compound was used in the next reaction without further purification.

To a solution of 2-{2-[2-({5-(3-benzyloxycyclobutyl)-2-[(2*S*)-1-*tert*-butoxycarbonyl-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}methylamino)ethylamino]-2-oxoethoxy}-5-fluorobenzoic acid (0.10 g, 0.141 mmol) in 1,4-dioxane (0.24 mL) was added 4 M hydrogen chloride in 1,4-dioxane (0.47 mL). After stirring for 1 h at room temperature, the reaction mixture was concentrated under reduced pressure to obtain **90a** (96 mg, 0.144 mmol, 100%) as a colorless powder. This compound was used in the next reaction without further purification.

To a solution of **90a** (96 mg, 0.141 mmol) in *N*,*N*-dimethylformamide (7.2 mL, 0.02 M) was added triethylamine (0.16 mL, 1.15 mmol, 8.0 eq) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (0.11 g, 0.288 mmol, 2.0 eq). After stirring at room temperature for 2 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with brine, dried over

magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–90% ethyl acetate in hexane) to obtain **91a** (72 mg, 0.118 mmol, 82%) as colorless amorphous substance.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.63–1.92 (m, 3H), 1.96–2.44 (m, 4H), 2.63–2.75 (m, 2H), 2.86–3.32 (m, 8H), 4.02–4.32 (m, 2H), 4.33–4.60 (m, 4H), 4.71–4.81 (m, 0.6H), 4.87–5.10 (m, 2H), 5.99 (s, 0.6H), 6.04 (s, 0.4H), 6.25 (s, 0.6H), 6.31–6.38 (m, 0.4H), 6.58 (s, 0.4H), 6.80–6.89 (m, 1H), 6.97–7.09 (m, 2H), 7.29–7.40 (m, 5H), 7.75–7.82 (m, 0.4H), 8.95–9.02 (m, 0.6H); MS (ESI/APCI dual) *m/z*: 613 $[M+H]^+$.

(18a*S*)-13-[(1*s*,3*R*)-3-(Benzyloxy)cyclobutyl]-2-chloro-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (91b)

According to the procedure described for **91a**, the title compound **91b** was obtained from **89b** as a colorless amorphous substance in a reaction with an 89% yield.
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.63–1.93 (m, 3H), 1.95–2.43 (m, 4H), 2.61–2.77 (m, 2H), 2.84–3.32 (m, 8H), 4.02–4.31 (m, 2H), 4.33–4.59 (m, 4H), 4.70–4.80 (m, 0.6H), 4.84–5.06 (m, 2H), 5.98 (s, 0.6H), 6.04 (s, 0.4H), 6.26 (s, 0.6H), 6.31–6.37 (m, 0.4H), 6.57 (s, 0.4H), 6.77–6.86 (m, 1H), 7.28–7.39 (m, 7H), 7.72–7.80 (m, 0.4H), 8.80–8.91 (m, 0.6H); MS (ESI/APCI dual) *m/z*: 629 $[M+H]^+$.

(18a*S*)-13-[(1*s*,3*R*)-3-(Benzyloxy)cyclobutyl]-2,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (91c)

According to the procedure described for **91a**, the title compound **91c** was obtained from **89c** as a colorless amorphous substance in a reaction with an 89% yield.
MS (ESI/APCI dual) *m/z*: 609 $[M+H]^+$.

(18a*S*)-2-Fluoro-13-[(1*s*,3*R*)-3-hydroxycyclobutyl]-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (92a)

To a solution of **91a** (72 mg, 0.118 mmol) in methanol (1.2 mL) was added palladium hydroxide on carbon (36 mg). The reaction was flushed with hydrogen and stirred under a hydrogen atmosphere at 60 °C for 3 h. The reaction mixture was cooled to room temperature and filtered through a pad of Celite®, then concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH chloroform) to obtain **92a** (56 mg, 0.11 mmol, 91%) as a colorless amorphous substance. ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.40–1.85 (m, 4H), 1.86–2.05 (m, 1H), 2.07–2.30 (m, 3H), 2.44–2.55 (m, 1H), 2.89–3.23 (m, 6H), 3.34–3.53 (m, 1H), 3.88–3.97 (m, 0.3H), 3.98–4.09 (m, 2.4H), 4.20–4.27 (m, 0.3H), 4.47–4.57 (m, 1.7H), 4.60–4.66 (m, 0.3H), 4.82–4.94 (m, 1.4H), 5.09–5.14 (m, 1H), 6.07–6.10 (m, 0.3H), 6.15–6.19 (m, 0.7H), 6.21–6.24 (m, 0.3H), 6.32–6.36 (m, 0.7H), 6.56–6.59 (m, 0.3H), 7.08–7.12 (m, 0.7H), 7.15–7.28 (m, 1.6H), 7.34–7.38 (m, 0.7H), 7.52–7.56 (m, 0.3H), 8.85–8.90 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.7, 31.9, 36.4, 36.6, 38.0, 40.0, 43.6, 48.1, 54.0, 61.5, 71.0, 91.0, 92.4, 113.7, 116.5, 119.8, 130.6, 149.3, 149.6, 151.6, 154.2, 156.9, 164.3, 165.5, 167.5; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{31}FN_6O_4$ $[M+H]^+$ 523.2464, found: 523.2454.

(18a*S*)-2-Chloro-13-[(1*s*,3*R*)-3-hydroxycyclobutyl]-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (92b)

To a solution of **91b** (4.5 g, 7.20 mmol) in acetonitrile (72 mL) was added chlorotrimethylsilane (4.6 mL, 36.0 mmol, 5.0 eq) and sodium iodide (5.4 g, 36.0 mmol, 5.0 eq). After stirring at 65 °C for 1 h, the reaction mixture was poured into saturated aqueous sodium thiosulfate/saturated aqueous sodium bicarbonate (1/1) and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 0%–20% methanol in chloroform) and silica gel column chromatography (NH 0%–12% methanol in chloroform) to obtain **92b** (3.6 g, 6.75 mmol, 94%) as colorless amorphous substance.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42–2.29 (m, 6.6H), 2.34–2.43 (m, 0.4H), 2.72–2.85 (m, 2H), 2.86–3.32 (m, 8H), 4.17–4.59 (m, 4.4H), 4.70–4.80 (m, 0.6H), 4.88–5.10 (m,

1.6H), 5.95 (s, 0.6H), 6.01 (s, 0.4H), 6.25 (s, 0.6H), 6.30–6.37 (m, 0.4H), 6.57 (s, 0.4H), 6.77–6.86 (m, 1H), 7.27–7.34 (m, 2H), 7.76 (d, *J* = 7.82 Hz, 0.4H), 8.84 (d, *J* = 7.58 Hz, 0.6H); MS (ESI/APCI dual) *m/z*: 539 $[M+H]^+$.

(18a*S*)-13-[(1*s*,3*R*)-3-Hydroxycyclobutyl]-2,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (92c)

According to the procedure described for **92a**, the title compound **92c** was obtained from **91c** as a colorless amorphous substance in a reaction with an 87% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.60–2.43 (m, 7H), 2.73–3.36 (m, 10H), 4.17–4.58 (m, 4.4H), 4.73–4.83 (m, 0.6H), 4.90–5.14 (m, 1.6H), 5.92–5.96 (m, 0.6H), 5.97–6.01 (m, 0.4H), 6.24 (s, 0.6H), 6.34–6.41 (m, 0.4H), 6.57 (s, 0.4H), 6.77 (d, *J* = 8.07 Hz, 1H), 7.07–7.17 (m, 2H), 7.77–7.86 (m, 0.4H), 8.91–9.03 (m, 0.6H); MS (ESI/APCI dual) *m/z*: 519 $[M+H]^+$.

(18a*S*)-2-Fluoro-13-[(1*r*,3*S*)-3-hydroxycyclobutyl]-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (93a)

To a solution of **92a** (55 mg, 0.11 mmol) in tetrahydrofuran (1.1 mL) was added 4-nitrobenzoic acid (26 mg, 0.158 mmol, 1.5 eq), 2.2 M diethyl azodicarboxylate in toluene (0.29 mL, 0.632 mmol, 6.0 eq) and triphenylphosphine (0.17 g, 0.632 mmol, 6.0 eq). After stirring at 65 °C for 30 min, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain (1*S*,3*r*)-3-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]cyclobutyl 4-nitrobenzoate (74 mg, 0.11 mmol, 100%) as colorless amorphous substance.

MS (ESI/APCI dual) *m/z*: 672 $[M+H]^+$.

To a solution of (1*S*,3*r*)-3-[(18a*S*)-2-fluoro-11-methyl-7,24-dioxo-6,7,8,9,10,11,19,20,21,22-decahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-

l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecin-13-yl]cyclobutyl 4-nitrobenzoate (73 mg, 0.11 mmol) in tetrahydrofuran (1.1 mL) was added 1 M aqueous sodium hydroxide (1.1 mL), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was neutralized by 1 M aqueous hydrochloric acid and extracted with chloroform. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 0%–5% methanol in chloroform) to obtain **93a** (41 mg, 0.078 mmol, 72%) as a colorless amorphous substance.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.41–1.86 (m, 4H), 1.86–2.06 (m, 1H), 2.14–2.30 (m, 3H), 2.44–2.55 (m, 1H), 2.93 (s, 0.9H), 2.98 (s, 2.1H), 3.01–3.22 (m, 3H), 3.34–3.53 (m, 1.6H), 3.87–3.97 (m, 0.3H), 3.98–4.10 (m, 1.4H), 4.20–4.29 (m, 0.3H), 4.33–4.43 (m, 1H), 4.48–4.58 (m, 1.4H), 4.60–4.65 (m, 0.3H), 4.82–4.95 (m, 1.4H), 5.03–5.09 (m, 1H), 6.06–6.10 (m, 0.3H), 6.16 (s, 0.7H), 6.22 (s, 0.3H), 6.35 (s, 0.7H), 6.58 (s, 0.3H), 7.09–7.13 (m, 0.7H), 7.16–7.29 (m, 1.6H), 7.35–7.38 (m, 0.7H), 7.53–7.56 (m, 0.3H), 8.88 (d, J = 7.02 Hz, 0.7H); ^{13}C NMR (151 MHz, DMSO-d_6) δ ppm 19.8, 24.7, 29.7, 33.9, 36.6, 37.7, 38.0, 40.0, 43.6, 48.1, 54.0, 63.9, 71.0, 91.3, 92.5, 113.7, 116.5, 119.8, 130.5, 149.6, 150.4, 151.6, 154.2, 156.9, 165.5, 165.7, 167.5; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{31}FN_6O_4$ $[M+H]^+$ 523.2464, found: 523.2450.

(18a*S*)-2-Chloro-13-[(1*r*,3*S*)-3-hydroxycyclobutyl]-11-methyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (93b)

According to the procedure described for **93a**, the title compound **93b** was obtained from **92b** as a colorless amorphous substance in a reaction with a 54% yield.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 1.43–1.85 (m, 4H), 1.87–1.96 (m, 0.7H), 1.97–2.05 (m, 0.3H), 2.14–2.29 (m, 3H), 2.44–2.55 (m, 1H), 2.93 (s, 0.9H), 2.98 (s, 2.1H), 3.03–3.23 (m, 2.7H), 3.37–3.57 (m, 1.6H), 3.86–3.94 (m, 0.3H), 4.01–4.08 (m, 0.7H), 4.11 (d, J = 14.04 Hz, 0.7H), 4.16–4.22 (m, 0.3H), 4.34–4.44 (m, 1H), 4.50–4.56 (m, 1.7H), 4.65 (d, J = 14.86 Hz, 0.3H), 4.83–4.91 (m, 1.4H), 5.04–5.08 (m, 1H), 6.05–6.09 (m, 0.3H), 6.16 (s, 0.7H), 6.22 (s, 0.3H), 6.36 (s, 0.7H), 6.58 (s, 0.3H), 7.08 (d, J = 8.67 Hz, 0.7H), 7.19 (d, J = 8.67 Hz, 0.3H), 7.39–7.46 (m, 1.3H), 7.51–7.55 (m, 1H), 8.73 (d,

J = 7.43 Hz, 0.7H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$) δ ppm 19.8, 24.7, 29.7, 33.9, 36.7, 37.7, 38.1, 43.6, 48.2, 54.1, 63.9, 70.0, 79.1, 91.3, 92.6, 118.8, 126.4, 129.8, 130.4, 149.5, 151.6, 151.9, 154.1, 155.5, 165.4, 165.6, 167.3; HRMS ESI/APCI dual *m/z* calcd for $C_{27}H_{31}ClN_6O_4$ $[M+H]^+$ 539.2168, found: 539.2152.

(18a*S*)-13-[(1*r*,3*S*)-3-Hydroxycyclobutyl]-2,11-dimethyl-8,9,10,11,19,20,21,22-octahydro-18a*H*,24*H*-18,15-(metheno)pyrido[2,1-l]pyrimido[6,1-h][1,4,7,9,10,13]benzoxapentaazacyclohexadecine-7,24(6*H*)-dione (93c)

According to the procedure described for **93a**, the title compound **93c** was obtained from **92c** as a colorless amorphous substance in a reaction with a 96% yield.

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ ppm 1.37–1.88 (m, 4.7H), 1.92–2.02 (m, 0.3H), 2.07–2.26 (m, 3H), 2.28 (s, 0.9H), 2.30 (s, 2.1H), 2.45–2.55 (m, 1H), 2.90–3.01 (m, 3H), 3.02–3.17 (m, 2.6H), 3.20–3.26 (m, 0.3H), 3.36–3.58 (m, 1.4H), 3.84–3.92 (m, 0.3H), 3.97–4.08 (m, 1.7H), 4.16–4.24 (m, 0.3H), 4.33–4.43 (m, 0.7H), 4.44–4.49 (m, 1H), 4.53–4.62 (m, 1H), 4.83–4.92 (m, 1.4H), 5.03–5.15 (m, 1H), 6.07–6.11 (m, 0.3H), 6.14–6.18 (m, 0.7H), 6.20–6.23 (m, 0.3H), 6.33–6.38 (m, 0.7H), 6.54–6.58 (m, 0.3H), 6.92–6.95 (m, 0.7H), 7.01–7.04 (m, 0.3H), 7.10–7.20 (m, 2H), 7.54–7.60 (m, 0.3H), 8.86–8.92 (m, 0.7H); ^{13}C NMR (151 MHz, DMSO-*d*$_6$) δ ppm 19.8, 20.1, 24.9, 29.8, 33.9, 36.6, 37.7, 40.0, 43.5, 48.1, 54.0, 63.9, 70.7, 79.1, 91.2, 92.5, 117.6, 126.9, 128.8, 130.4, 132.7, 149.5, 151.1, 151.6, 154.3, 165.7, 167.1, 167.7; HRMS ESI/APCI dual *m/z* calcd for $C_{28}H_{34}N_6O_4$ $[M+H]^+$ 519.2714, found: 519.2706.

REMD simulation

The conformations of the compounds were sampled using REMD simulation[59] run on GROMACS 5.0.4.[60] For each compound, eight independent REMD simulations were performed under NVT conditions for 10 ns each, to sample sufficient conformational space. Temperatures of the replicas were set at 310.0, 366.5, 433.2, 512.1, 605.4, 715.6, 845.9, and 1000 K. GAFF forcefiled[63] and GBSA model[64] were applied to the compounds and the solvent, respectively. The dihedral angle between the pyrazolo[1,5-a]pyrimidine ring and the amide plane was calculated for each conformation sampled every 10 ps at 310.0 K.

Molecular docking simulations

Molecular docking simulations were performed using the CDOCKER algorithm in Discovery Studio 2017 R2.[61] The input coordinates of RSV A2 were obtained from the X-ray coordinates of RSV A2 complexed with its inhibitor JNJ-2408068 (PDB entry 5EA3). The "Input Site Sphere" parameter for CDOCKER was defined using the position of JNJ-2408068. Hydrogen atoms were added, and the ionization states were assigned using the Protonate-3D function of the Molecular Operating Environment program (MOE)[62] ; the positions of the hydrogen atoms were then optimized using the Amber10 forcefield implemented in MOE. For D486N, after manually correcting the coordinates from wild-type to D486N, the positions of the amino acid residues within 4.5 angstroms of JNJ-2408068 were optimized using the Amber10 forcefield implemented in MOE.

Molecular dynamics simulation

The molecular dynamics (MD) simulation was performed using the Standard Dynamics Cascade algorithm in Discovery Studio 2017 R2.[61] The input coordinates were obtained from the result of the molecular docking simulation of RSV A2 and compound **93b**. A CHARMm forcefield and GBSW model were applied to the compounds and solvent, respectively. A production MD of 1.0 ns was performed using the well-equilibrated system at a temperature of 300 K.

EXSY analysis

For the EXSY (EXchange SpectroscopY) analysis,[65,66] each compound was dissolved in DMSO-d_6 at 10 mg/mL. Spectra were recorded using a JNM-ECA500 spectrometer (JEOL, Tokyo, Japan) with a mixing time of 0.5 s and relaxation delay of 10 s. Compounds **76c** and **93b** produced clear exchange signals sufficient for analysis at room temperature. Furthermore, the exchange rates for **40,** which showed no exchange signals at room temperature, were predicted by extrapolating an Arrhenius plot from 60 °C to 90 °C. The EXSY spectra are shown below (**Figures S1–S4**).

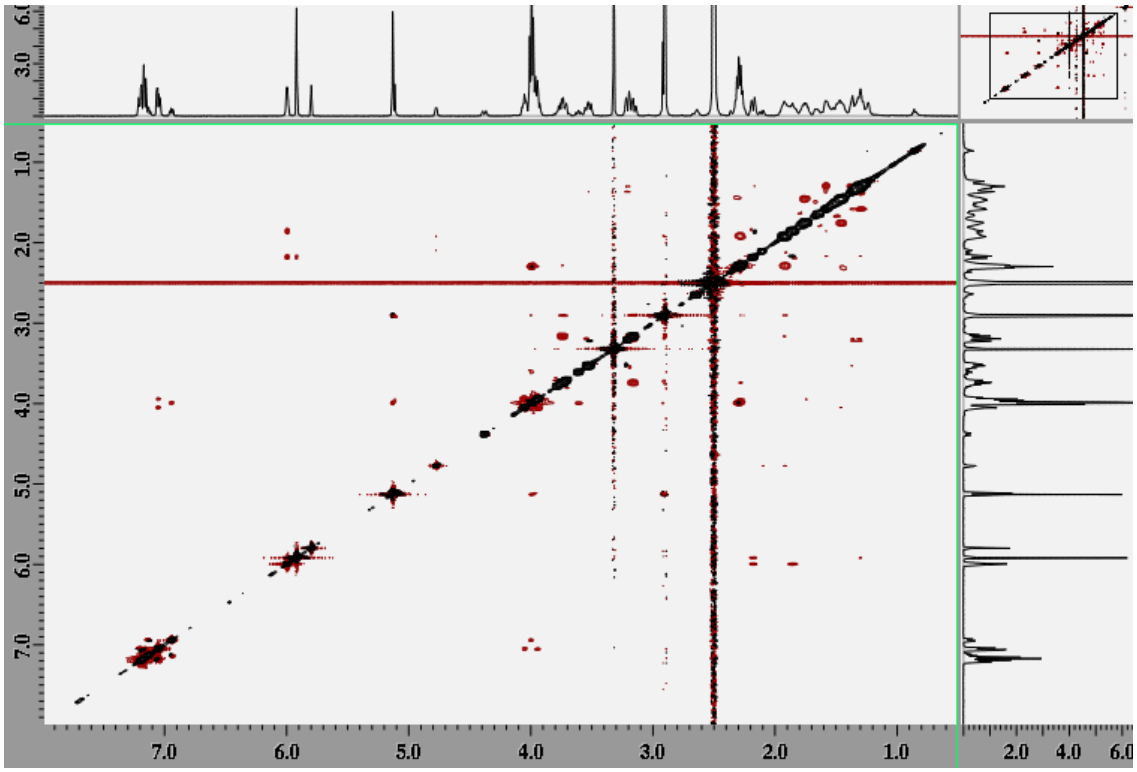

Figure S1. The EXSY spectrum of **40** at room temperature. NOEs were shown in red and diagonal or exchange signals were shown in black (exchange signals weren't observed).

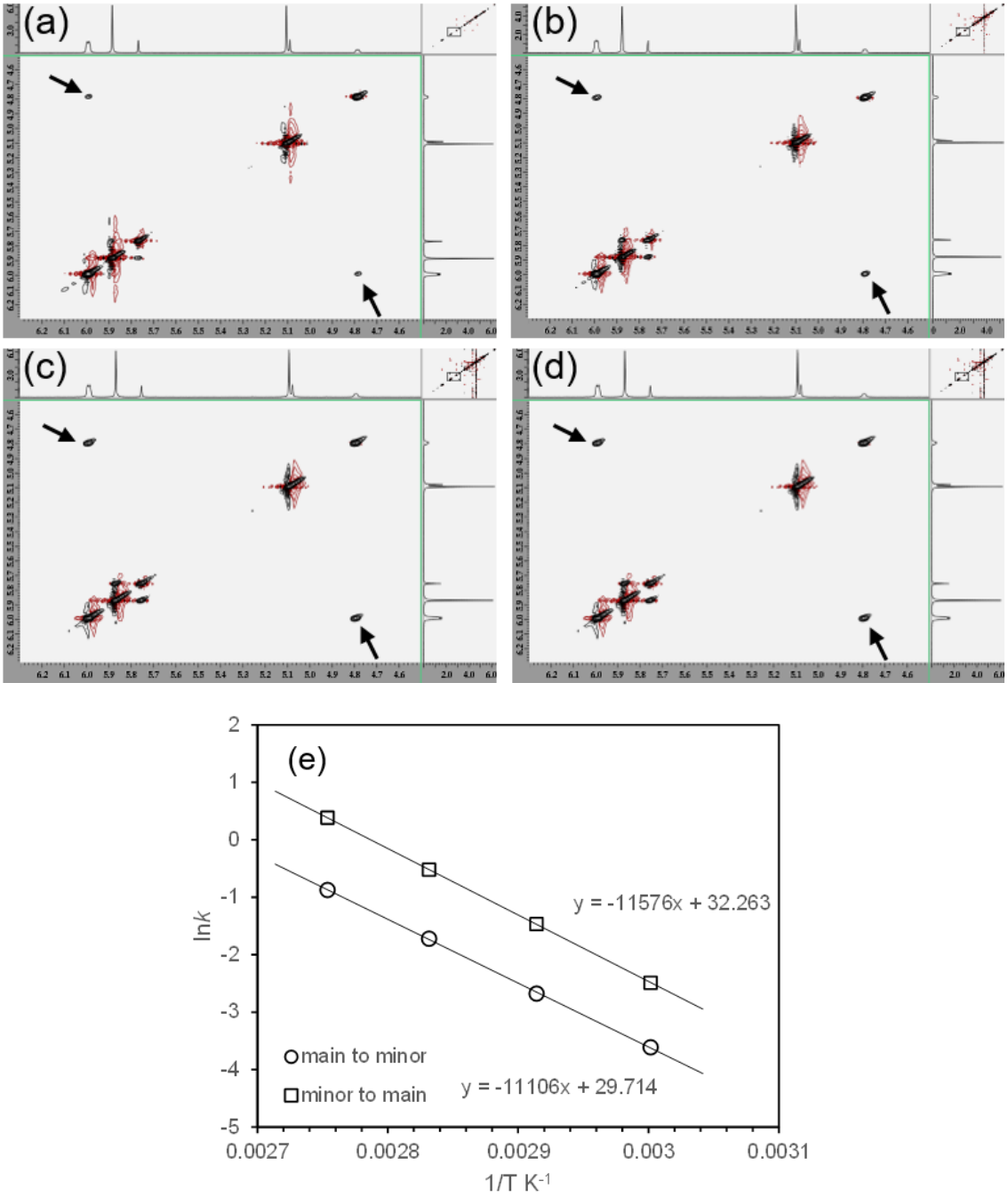

Figure S2. Expanded EXSY spectra of **40** at 60 ºC (a), 70 ºC (b), 80 ºC (c), 90 ºC (d). NOEs were shown in red and diagonal or exchange signals were shown in black. The exchange rates at each temperature were calculated from the intensity of Diagonal and exchange (arrowed) signals.[65,66] After that, those at room temperature were predicted by extrapolating an Arrhenius plot (e). The equilibrium $t_{1/2}$ were calculated from exchange rates and ratio on ^{1}H-NMR.

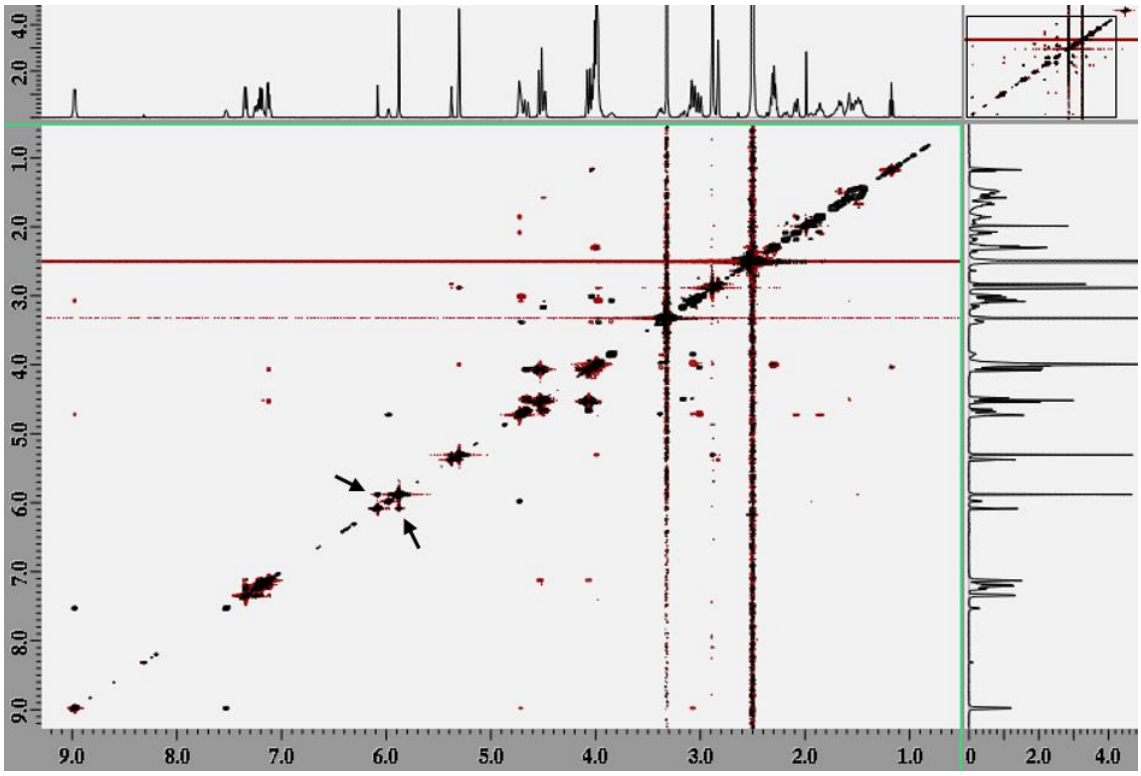

Figure S3. The EXSY spectrum of **76c** at room temperature. NOEs were shown in red and diagonal or exchange signals were shown in black. Two signals at 5.88 (main) and 6.08 (minor) ppm were used for exchange rate calculation (exchange signals were arrowed).

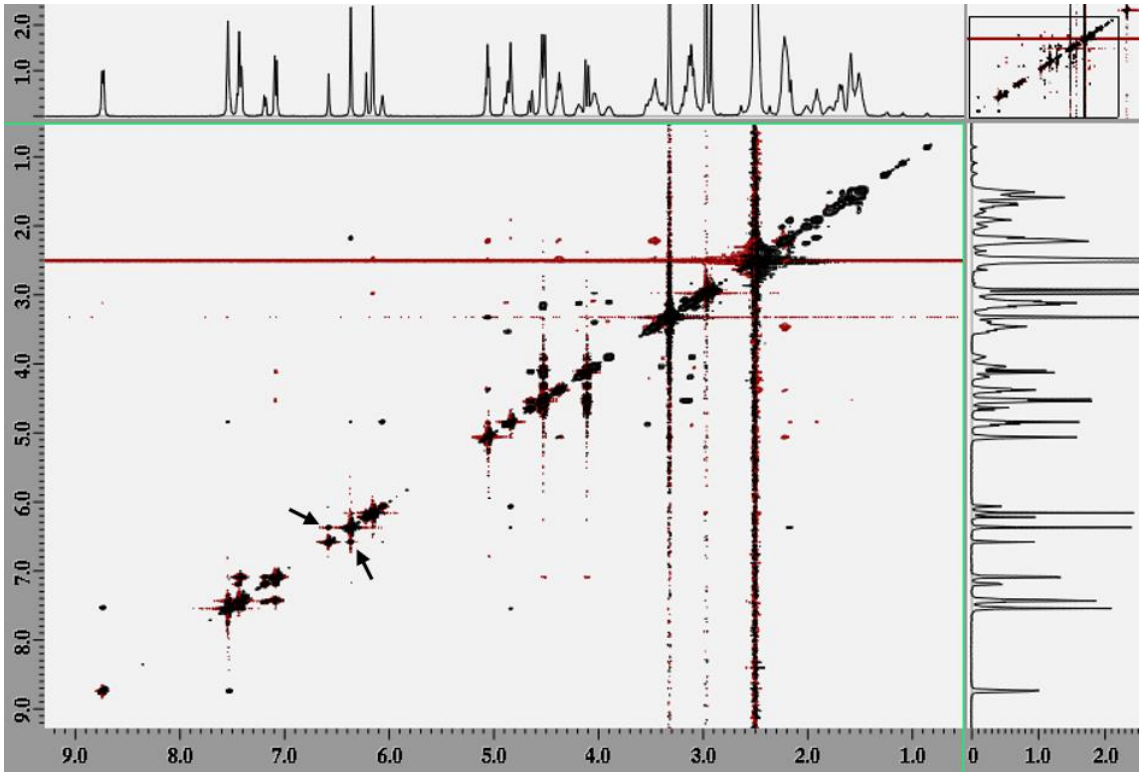

Figure S4. The EXSY spectrum of **93b** at room temperature. NOEs were shown in red and diagonal or exchange signals were shown in black. Two signals at 6.37 (main) and 6.58 (minor) ppm were used for exchange rate calculation (exchange signals were arrowed).

Cells and viruses

HEp-2 cells were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 50 μg/mL gentamicin, and 600 μg/mL L-glutamine. RSV A2 (ATCC VR-1540) was purchased from the American Type Culture Collection (Manassas, VA, USA). RSV A2 with the D486N mutation in F protein was selected by serial passage in the presence of a pyrazolo[1,5-a]pyrimidine derivative **7**. The mutant was confirmed to have no other mutations in the F gene by a genotypic analysis.

Antiviral assay

HEp-2 cells were cultured in 96-well plates overnight, and the test compounds were added after dilution with MEM supplemented with 2% FBS, 100 units/mL penicillin, 100

μg/mL streptomycin and 300 μg/mL L-glutamine. The cells were then infected with RSV A2 or D486N. After incubation at 37 °C, 5% CO_2 for 4 d, the RSV-induced CPE was determined by adding XTT reagent. The concentration of the test compound required to inhibit the CPE by 50% (EC_{50}) was calculated using the least squares method.

Parallel artificial membrane permeability assay (PAMPA)

Membrane permeability was evaluated using the PAMPA Evolution instrument (pION Inc., Billerica, MA, USA). The permeation of a test compound across an artificial membrane was quantified using a UV plate reader after 4 h of incubation at room temperature. The apparent permeability at pH6.2 was calculated using PAMPA Evolution software (pION Inc.).

Metabolism-dependent inhibition (MDI)

A test compound was pre-incubated for 0 or 30 min with 10 × HLMs (0.5 mg protein/mL) at 37 °C in sodium-potassium phosphate buffer (pH7.4) containing a β-nicotinamide-adenine dinucleotide phosphate (NADPH)-regenerating system. At the end of the pre-incubation period, an aliquot of the reaction mixture was diluted (10-fold) into a secondary incubation buffer containing a probe substrate for CYP3A (testosterone, 250 μM) and a β-NADPH-regenerating system. Secondary incubation was performed for 10 min, and the reaction was terminated by the addition of acetonitrile containing an internal standard. The precipitated protein was removed by centrifugation, and the supernatant was subjected to liquid chromatography/tandem mass spectrometry. The percent inhibition of probe metabolism with 0-min or 30-min of pre-incubation was calculated, and the metabolic-dependent inhibition was calculated as the percent inhibition difference between 0-min and 30-min of pre-incubation.

Plasma protein binding

The protein binding of the test compounds in human and mouse plasma was evaluated using the equilibrium dialysis method. Equilibrium dialysis was conducted on a 96-well Equilibrium Dialysis Device (HTDialysis, LLC, Gales Ferry, CT, USA) with a 12 to 14-kDa cutoff dialysis membrane. A test compound was dissolved in DMSO and spiked into

the blank plasma at a final concentration of 1 μg/mL. The plasma sample was equilibrated with phosphate buffer (pH7.4) at 37 °C in 5% CO_2 for 4 h. After dialysis, the concentrations of the test compound in plasma and phosphate buffer were determined using a liquid chromatography-tandem mass spectrometry method. The protein binding (%) was calculated based on the instruction manual for the 96-well plate.

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主論文目録

本学位論文内容は下記の発表論文による

1. Yamaguchi, S. T.; Tamura, Y.; Ogata, Y.; Kawaguchi, T.; Kurosaka, J.; Sugaya, Y.; Iwakiri, K.; Busujima, T.; Takahashi, R.; Ueda, Y. N.; Tanigawa, E.; Abe, K. T.; Sugiyama, H.; Kanuma, K. Design and Synthesis of 2-(1-Alkylaminoalkyl)pyrazolo[1,5-a]pyrimidines as New Respiratory Syncytial Virus Fusion Protein Inhibitors. *Chem. Pharm. Bull.* **2020**, *68*, 345−362.

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主査 千葉大学大学院教授（薬学研究院） 薬学博士 根本 哲宏
副査 千葉大学大学院講師（薬学研究院） 薬学博士 原田 慎吾
副査 千葉大学大学院准教授（薬学研究院） 薬学博士 高屋 明子
副査 千葉大学大学院准教授（薬学研究院） 工学博士 星野 忠次