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The Discovery of MK-6169, A Potent Pan-genotype Hepatitis C Virus NS5A Inhibitor with Optimized Activity Against Common Resistance-Associated Substitutions

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ABSTRACT: We describe the discovery of MK-6169, a potent and pan-genotype hepatitis C virus NS5A inhibitor with optimized activity against common resistance-associated substitutions. SAR studies around the combination of changes to both the valine and aminal carbon region of elbasvir lead to the discovery of a series of compounds with substantially improved potency against common resistance-associated substitutions in the major genotypes, as well as good pharmacokinetics in both rat and dog. Through further optimization of key leads from this effort, MK-6169 (**21**) was discovered as a preclinical candidate for further development.

INTRODUCTION

In the recently published *Global Hepatitis Report 2017* from the World Health Organization, it was estimated that 71 million people worldwide were chronically infected with hepatitis C virus (HCV) as of 2015, representing approximately 1% of the global population.[1] Studies have suggested that the

incidence of HCV infection has decreased since the second half of the 20th century; however, there were still 1.75 million new infections in 2015, mainly due to unsafe health-care practices (including unsafe healthcare injections) and injection drug use.[2] Without treatment, chronic HCV infection could lead to severe liver damage and life-threatening disease. For example, the risk of cirrhosis of the liver among patients with chronic HCV infection is between 15–30% within 20 years.[3] In 2015, there were 1.34 million deaths worldwide attributable to viral hepatitis, with 30% a result of complications of chronic HCV infection, mostly from cirrhosis and hepatocellular carcinoma.[4] The treatment for HCV infection has evolved significantly during the last decade. Early treatment involved interferon injections and use of ribavirin for a long treatment duration. The regimen suffered from low sustained virologic response (SVR) rates (40~65%) and many debilitating side effects. Addition of first-generation HCV non-structural (NS) 3/4A protease inhibitors (boceprevir or telaprevir) to the interferon/ribavirin standard-of-care improved the SVR rate to 65~85% but the side effects of interferon remained. Current treatment for HCV infection involves interferon-free all oral direct-acting antiviral agent (DAA)[5-7] combinations chosen from three different categories: NS3/4A protease inhibitors, non-structural protein 5A (NS5A) replication complex inhibitors, and nucleoside or non-nucleoside NS5B polymerase inhibitors.

HCV NS5A protein is a multifunctional RNA binding protein that interacts with several host proteins and is essential for HCV replication.[7,8] The protein has no enzymatic activity or a known human orthologue from which a function can be inferred. A detailed understanding of how NS5A directly influences HCV replication is lacking and therefore the mechanism of inhibitors remain unclear. While crystal structures of the N-terminal region of the protein have been reported,[9,10] the noted differences in the dimer orientation and the absence of an inhibitor-protein complex render a structure-based rational drug design approach pretty difficult. In the absence of enzymatic activity for NS5A, structure-activity relationships (SARs) for inhibitors have been driven by cell-based replicon activities. First generation NS5A inhibitors showed a low resistance barrier to genotype 1 subtype a

(GT1a) as well as weak activity against several other genotypes.[11] Thus, the need for identification of more potent NS5A inhibitors against a wider variety of genotypes as well as NS5A resistance mutations particularly in the prevalent GT1a became a focus for many groups. Six HCV NS5A inhibitors have been approved for chronic HCV treatment, in combination with other DAA's: daclatasvir (BMS-790052),[11,12] ledipasvir (GS-5885),[13] ombitasvir (ABT-267),[14] elbasvir (MK-8742).[15,16] velpatasvir (GS-5816),[17] and pibrentasvir (ABT-530).[18]

Our efforts in the research on HCV NS5A inhibitors have progressed over time.[19-21] Multiple NS5A inhibitors, including MK-4882,[15] MK-8325,[22] and MK-8742,[15,16] have been identified. MK-8742 (elbasvir, Figure 1) is one of the two components in Zepatier which has gained FDA approval for the treatment of GT1 and GT4 chronic HCV infection. Through our continued effort in optimization of NS5A inhibitors, we aim to identify an inhibitor with a 'flat' potency profile, which exhibits less than a 10-fold potency reduction from the wild type genotype 1a (GT1a) virus relative to other genotypes and resistance-associated substitutions (RASs). Since no X-ray structures of inhibitor and protein complexes are available to facilitate a structure-based inhibitor design approach, we plan to modify every part of the MK-8742 molecule to identify SAR that could improve the potency against the least sensitive RASs, such as GT1a Y93H and GT2b L31M (a naturally resistant substitution). Our experience has shown that activity vs. these variants could often lead to improved flatness across a broad range over genotypes and mutations. Previously, we reported SAR studies towards an improved "core",[23,24] "cap",[25] proline,[26] and "Z" group region (Figure 2).[27-30] Key findings from SAR studies included potency improvements against GT2b L31M through introducing a tetrahydro-2H-pyran (THP) group in the "cap" region,[25] potency improvements against both GT1a Y93H and GT2b L31M through introducing a fluorine substitution at the C-1 position of the tetracyclic indole core,[24] or a relatively large aryl Z group, such as the 4-cyclopropylphenyl,[28] 4-diphenyl,[28] and 7'-chromane.[29] More recently, substituted thiazole and thiophene as "Z" groups were also identified to improve the potency against

NS5A RASs.[30] Through these efforts we identified MK-8408 (ruzasvir).[30-32] Herein, we disclose the discovery of an additional pan-genotype HCV NS5A inhibitor, preclinical candidate MK-6169.

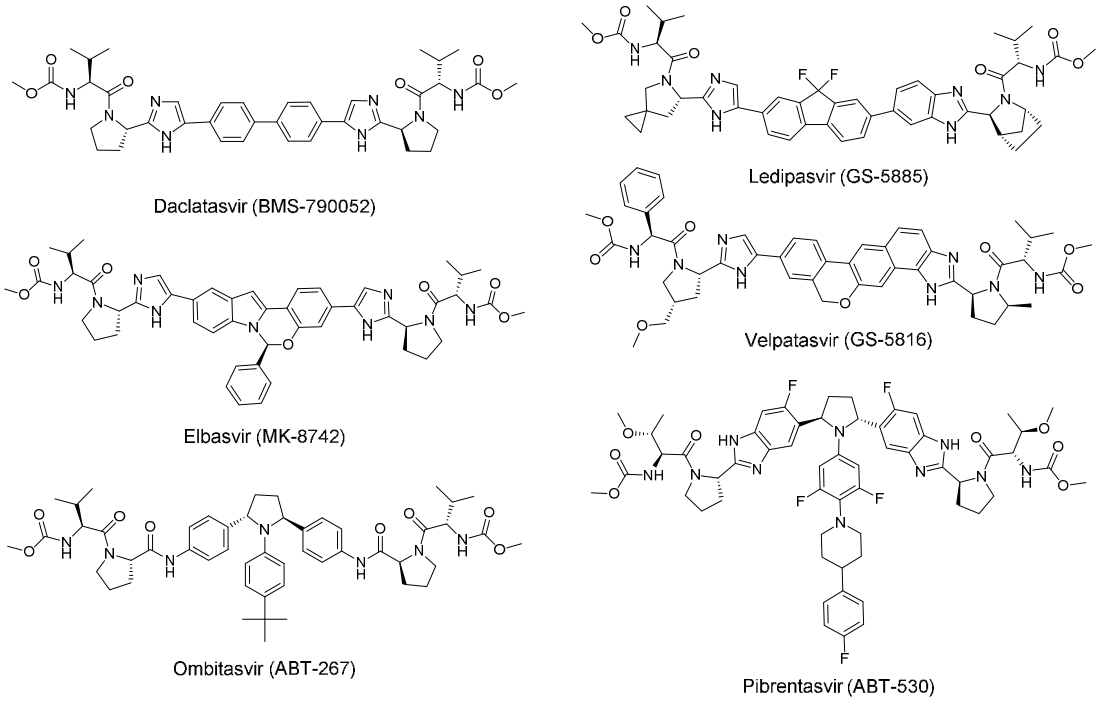


Figure 1. HCV NS5A inhibitors approved by FDA

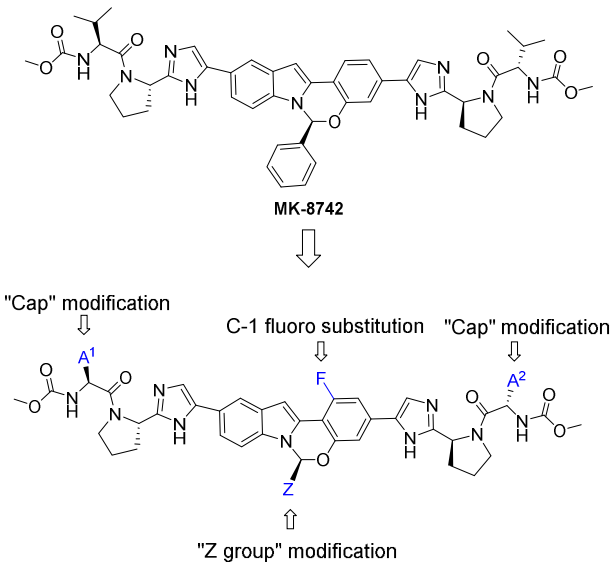


Figure 2. Strategy for the modification of MK-8742 molecule.

RESULTS AND DISCUSSION

As reported previously, we found that replacing the isopropyl group of the valine "cap" of elbasvir with a (*S*)-THF group was able to improve the activity on GT2b L31M while maintaining potency against the other NS5A RASs.[25] We therefore wanted to incorporate this SAR with the "Z" groups identified more recently in an attempt to improve the activity on GT1a Y93H and GT2b L31M. Thus, the (*S*)-THP group was initially incorporated on the left-hand-side of the molecule with a range of "Z" groups; the *in vitro* potencies (replicon EC_{90}) are summarized in Table 2. Incorporation of the (*S*)-THP "cap" with chromane as "Z" group (**1**-**2**) showed a 10x improvement against GT1a Y93H and GT2b L31M vs. the corresponding bis-valine analogs.[29] Based on the EC_{90} values in Table 2, we observed a reduced shift in potency of the wild type GT1a vs. GT1a Y93H and GT2b L31M which for **1** is 9X and 4X, respectively. A similar result was observed for **3** whose "Z" group is 4-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazine. Encouraged by these data, a series of compounds with substituted thiophene moieties as the "Z" group (**4**-**12**) was prepared. The substituents on the thiophene included methyl (**4**), ethyl (**5**), *n*-propyl (**6**), *iso*-propyl (**7**), cyclopropyl (**8**), di-F-methyl (**9**), and tri-F-methyl (**10**). Analogs with benzothiophene (**11**) and 5,6-dihydro-4H-cyclopenta[b]thiophene (**12**) were also prepared. All these compounds showed a potency shift less than 10X from GT1a wild type to the other genotypes and mutants tested, except **11** whose "Z" group is a benzothiophenyl group. Compound **11** showed a slightly larger potency shift (20X) against GT1a Y93H. Compounds with the (*S*)-THP on the right hand side of the molecules were also prepared (**13**-**14**) and their *in vitro* potencies are summarized in Table 3. Both compounds, with the "Z" group as either chromane or 4-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazine, showed single or double digit pM EC_{90} values for all the genotypes and mutants tested. It was interesting to observe that compound **14** showed 15-fold potency loss against GT1a relative to compound **3** in which the THP group is on the left-hand side.

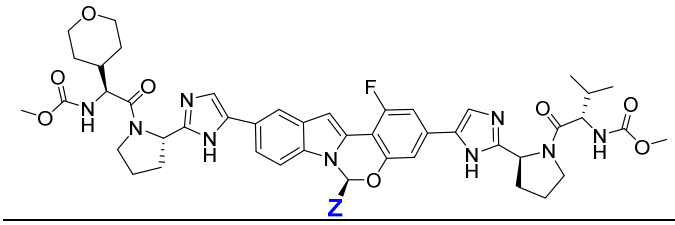
Table 1. *In vitro* potency profiles of Elbasvir.[15]

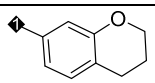
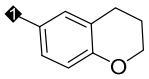
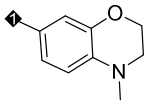
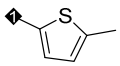
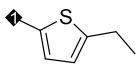
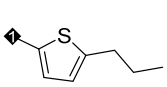
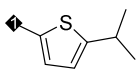
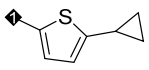
ID	Replicon EC_{90} (nM)[a]							
	GT1a[b]	GT1b	GT2a	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
Elbasvir	0.006	0.006	0.019	11	0.12	0.016	28	1

[a] EC_{90} is the concentration of inhibitor that reduces the effective response by 90%.

[b] see abbreviation for the full name of genotype and mutant names.

Table 2. NS5A inhibitors with a (*S*)-THP group on the left-hand side



ID	Z	Replicon EC_{90} (nM)						
		GT1a	GT1b	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
1		0.004	0.006	0.019	0.008	0.007	0.035	0.005
2		0.005	0.003	1.069	0.009	0.006	0.356	0.007
3		0.005	0.004	0.069	0.038	0.009	0.047	0.006
4		0.003	0.006	0.024	0.006	0.004	0.019	0.010
5		0.004	0.003	0.020	0.005	0.003	0.008	0.003
6		0.002	0.006	0.012	0.005	0.002	0.009	0.003
7		0.004	0.002	0.036	0.003	0.004	0.010	0.003
8		0.004	0.005	0.014	0.008	0.005	0.018	0.006

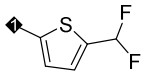
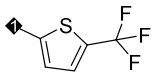
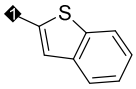
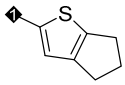
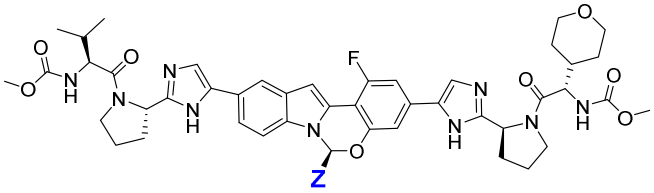
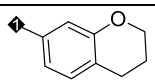
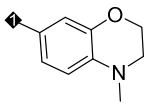
9		0.004	0.006	0.016	0.010	0.004	0.015	0.005
10		0.003	0.005	0.010	0.006	0.003	0.023	0.005
11		0.005	0.003	0.057	0.011	0.004	0.111	0.008
12		0.005	0.005	0.064	0.022	0.004	0.039	0.013

Table 3. NS5A inhibitors with a (*S*)-THP group on the right-hand side



ID	Z	Replicon EC_{90} (nM)						
		GT1a	GT1b	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
13[a]		0.002	0.003	0.005	0.003	0.002	0.008	0.001
14		0.071	0.007	0.054	0.017	0.009	0.073	nt[b]

[a] EC_{50} value, which is the concentration of inhibitor that reduces the effective response by 50%.

[b] nt = not tested.

The rat and dog PK profiles of selected compounds in Table 3 are summarized in Table 4. Unfortunately, all compounds tested exhibited low exposure in rat with their AUC normally less than 1 μM.h at 10 mpk oral dosing. The oral bioavailability of these compounds is quite low as well. This is expected due to their high plasma protein binding and low permeability. Compound **3** displayed a relatively higher exposure with oral AUC = 1.23 μM.h at 10 mpk, but its bioavailability was only 1.5%. Compound **3** had a low PK exposure in dog as well. Surprisingly, both compounds **4** and **8**

showed good dog PK exposure even though their rat PK exposures were low. The single rising dose rat PK study of **8** is summarized in Table 5, which revealed a linear increase in exposure from 10 to 100 mpk whereas a less than dose-proportional increase in exposure was observed from 100 to 300 mpk.

Table 4. Pharmacokinetic profiles of **1**, **3-5**, **7**, **8**, **13**, and **14** in rat or dog[a]

ID	Species	iv		po				
		$T_{1/2}$ (h)	CL (mL/min/Kg)	Dose (mpk)	AUC (μM.h)	C_{max} (nM)	C_{24} (nM)	F%
1	Rat	3.2	11	10	0.13	0.032	0.001	0.8
3	Rat	5.5	2	10	1.23	0.162	0.005	1.5
4	Rat	4.2	24	10	0.31	0.031	0.002	4.1
5	Rat	5.3	12	10	0.53	0.048	0.005	3.9
7	Rat	6.1	13	10	0.46	0.050	0.002	3.4
8	Rat	4.0	18	10	0.29	0.034	0.002	3.1
13	Rat	1.9	19	10	0.05	0.010	0.001	0.5
14	Rat	3.8	13	10	0.10	0.016	0.001	0.8
3	dog	6.1	8.6	5	0.14	0.022	0.002	1.1
4	dog	4.5	4.1	5	1.32	0.23	0.004	6.2
8	dog	4.2	3.6	5	2.61	0.46	0.022	11.0

[a] Fasted male Wistar Han rats or Beagle dogs were used. IV dose was formulated with 60% PEG200 and the compounds were dosed at 2 mg/kg in rat or 1 mg/kg in dog. P.O. dose was formulated with 10% TWEEN and the compounds were dosed as indicated in the table.

Table 5. Rat single rising dose PK profile of **8**[a]

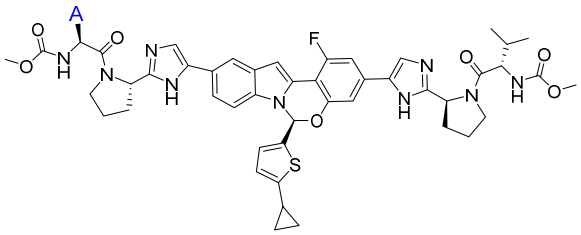
Dose (mpk)	PO AUC (μM.h)	C_{max} (μM)	C_{24h} (μM)	T_{max} (h)	MRT (h)
10	0.14	0.02	0.001	3	7.9

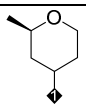
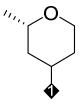
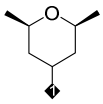
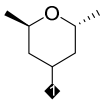
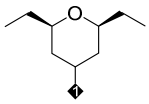
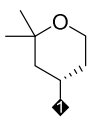
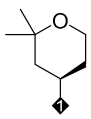
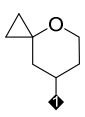
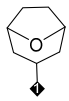
100	1.64	0.20	0.011	4	8.3
300	3.99	0.36	0.032	4	9.9

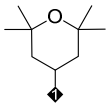
[a] Fed male Wistar Han rats were used. Compound was dosed at indicated doses P.O. in 10% TWEEN.

As reported previously, the bis-valine analog with chromane as the "Z group" showed reasonable exposure in rat with AUC (po) at 10 mpk being 1.65 μM.h.[29] However, replacing the isopropyl group of the valine moiety with a tetrahydro-2H-pyran (THP) group, either in the left-hand side (**1**) or right-hand side (**13**), resulted in a significant drop of PK exposure in rat. Therefore the THP group was believed to be responsible for the poor rat PK of compounds in Table 4. A metabolite study on a similar compound not shown in this publication revealed that a possible hydroxylation occurred on the carbon next to the oxygen that led to the ring opening of the THP group. To prevent this potential metabolic pathway, analogs with a variety of α-substituted THP groups were synthesized and their replicon EC_{90} data are listed in Table 6. It should be noted that compounds in Table 6 have multiple diastereomers. All diastereomers were separated by chiral SFC, but only one or two diastereomers with the best potency are reported here. Their absolute stereochemistry was either introduced from the starting material with known stereochemistry, determined by VCD with confidence, or assigned arbitrarily. Please see the experimental section for details. Both (*R*)- or (*S*)-2-methyl-THP analogs (**15**-**16**) exhibited good activity on the genotypes tested, and similar potency profiles were observed for both *cis*- and *trans*-2,6-dimethyl THP analogs (**17**-**18**). However, *cis*-2,6-diethyl THP analog **19** exhibited reduced potency against all the genotypes tested, especially on GT2b L31M with a ~100-fold loss in potency as compared with the corresponding *cis*-2,6-dimethyl THP analog **17**, indicating that a larger substituent was not tolerated. 2,2-Di-methyl substituted THP analogs (**20**-**21**) as well as the 2-spiro-cyclopropyl THP analog (**22**) all displayed good potency against all the genotypes tested. Additionally, the 8-oxabicyclo[3.2.1]octane substituted analog **23** and the 2,2,6,6-tetramethyl-THP analog **24** also displayed good potency on the genotypes tested.

Table 6. NS5A inhibitors with a substituted-THP group



ID	A	Replicon EC_{90} (nM)						
		GT1a	GT1b	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
15		0.004	0.004	0.014	0.007	0.010	0.009	0.003
16		0.005	0.007	0.023	0.008	0.007	0.013	0.002
17		0.004	0.004	0.025	0.008	0.007	0.027	0.005
18		0.004	0.004	0.012	0.007	0.007	0.015	0.004
19		0.017	nt[a]	2.701	0.062	nt	0.642	nt
20		0.004	0.007	0.027	0.009	0.007	0.018	0.002
21		0.004	0.004	0.018	0.001	0.006	0.033	0.004
22		0.006	0.004	0.036	0.008	0.004	0.049	0.007
23		0.005	0.004	0.019	0.012	0.008	0.028	0.006

24		0.004	0.002	0.073	0.007	0.004	0.047	0.004

[a] nt = not tested.

The PK parameters of all compounds except **19** in Table 6 are summarized in Table 7. Both mono-methyl substituted analogs **15** and **16** showed low PK exposure in rat at 10 mpk oral dosing. The 2,6-dimethyl substituted analogs **17** and **18** had higher exposures in rat than **15** and **16**. Interestingly, while one diastereomer (**20**) of the 2,2-di-methyl substituted THP analogs displayed low rat PK exposure, one of the other diastereomers (**21**) exhibited a much improved PK exposure in rat with AUC (po) at 10 mpk being 1.65 μM.h. It also showed improved C_{24h} relative to the other compounds. The rat PK exposure of the 2-spiro-cyclopropyl THP analog (**22**) was low, and so were the rat PK exposures of **23** and **24**. The dog PK profiles of **17**, **18**, and **21** were also examined. All of them displayed good dog PK exposures at 5 mpk oral dosing. They also had low clearance and relatively higher bioavailability than in rat. Rat single rising dose PK study of compound **17**, **18**, and **21** are summarized in Table 8. All three compounds showed less than dose-proportional increase in exposure from 10 to 300 mpk.

Table 7. Pharmacokinetic profiles of **15-18** and **20-24** in rat or dog[a]

		iv			po				
ID	Species	Dose (mpk)	$T_{1/2}$ (h)	CL (mL/min/Kg)	Dose (mpk)	AUC (μM.h)	C_{max} (μM)	C_{24} (μM)	F%
15	Rat	2	4.7	11	10	0.21	0.025	0.001	1.4
16	Rat	2	5.5	11	10	0.19	0.021	0.001	1.3
17	Rat	2	5.1	11	10	0.92	0.067	0.003	5.9
18	Rat	2	3.6	11	10	0.93	0.119	0.004	6.3
20	Rat	2	5.2	11	10	0.24	0.025	0.001	1.6

21	Rat	2	5.4	7	10	1.65	0.183	0.011	6.6
22	Rat	2	5.7	5	10	0.58	0.057	0.004	1.9
23	Rat	2	3.9	13	10	0.33	0.024	0.003	2.5
24	Rat	2	7.7	7	10	0.70	0.076	0.005	3.1
17	dog	1	7.2	3.0	5	2.42	0.33	0.011	8.5
18	dog	1	11.3	2.6	5	5.05	0.64	0.087	18.7
21	dog	1	5.8	2.9	5	4.19	0.66	0.029	14.9

[a] Fasted male Wistar Han rats or Beagle dogs were used. IV dose was formulated with 60% PEG200. P.O. dose was formulated with 10% TWEEN.

Table 8. Rat single rising dose PK profiles of **17**, **18**, **21**[a]

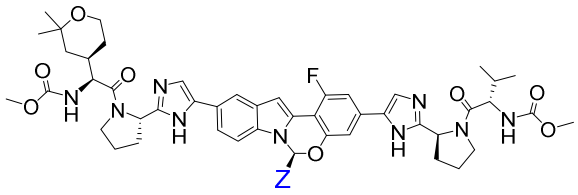
ID	Dose (mpk)	AUC (μM.h)	C_{max} (μM)	C_{24h} (μM)	T_{max} (h)	MRT (h)
	10	0.33	0.08	0.005	4	9.0
17	100	1.95	0.22	0.027	2	17
	300	4.08	0.39	0.040	6	11
	10	0.31	0.05	0.001	3	6.0
18	100	2.07	0.24	0.009	3	7.6
	300	5.58	0.51	0.029	7	9.3
	10	0.95	0.15	0.003	3.0	6.5
21	100	7.82	0.76	0.072	2.0	12.4
	300	14.7	1.06	0.164	5.0	10.7

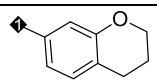
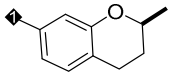
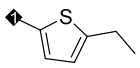
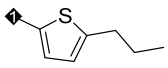
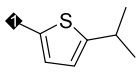
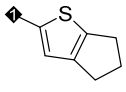
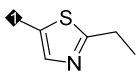
[a] Fed male Wistar Han rats were used. Compounds were formulated with 10% TWEEN and dosed orally.

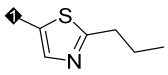
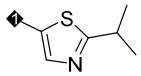
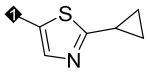
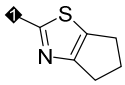
The 2,2-dimethyl-THP isomer employed in **21** was used as a test to determine its impact in combination with other "Z groups" that had previously been identified to show good potency. Compounds **25-35** have the dimethyl -THP cap on the left-hand side and their replicon EC_{90} data are

listed in Table 9. Not surprisingly, most of them showed "flat" potency profile against all genotypes and mutants tested, with the exception of **26**, **30**, **33**, and **35** which exhibited slightly reduced potency against GT1a Y93H with the corresponding EC_{90} values in the triple digit pM range. Two compounds (**36**, **37**) with the same 2,2-dimethyl-THP isomer on the right-hand side were also prepared. Their EC_{90} data are listed in Table 10. Both compounds were pretty potent against the genotypes and mutants been tested, except **36** exhibited slightly reduced potency in GT1a, GT2b L31M, and GT1a Y93H.

Table 9. NS5A inhibitors with a 2,2-dimethyl-THP group on the left-hand side



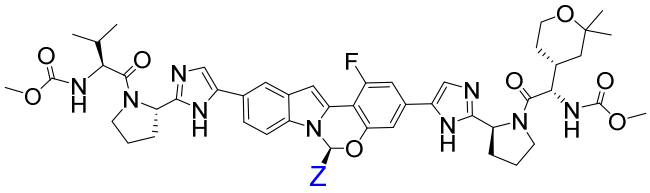
ID	Z	Replicon EC_{90} (nM)						
		GT1a	GT1b	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
25		0.003	0.005	0.017	0.007	0.005	0.048	0.004
26[a]		0.005	0.007	0.102	0.008	0.008	0.348	0.005
27		0.005	0.006	0.028	0.009	0.007	0.038	0.003
28		0.010	0.009	0.084	0.016	0.011	0.078	0.006
29		0.004	0.005	0.038	0.006	0.006	0.036	0.004
30		0.005	0.006	0.043	0.009	0.006	0.117	0.006
31		0.006	0.005	0.041	0.006	0.004	0.017	0.005

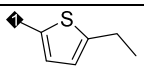
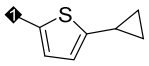
32		0.004	0.005	0.015	0.008	0.006	0.018	0.006
33		0.006	0.006	0.019	0.038	0.007	0.329	0.004
34		0.006	0.006	0.015	0.015	0.007	0.029	0.006
35		0.005	0.004	0.174	0.013	0.007	0.590	nt[b]

[a] Single chromane isomer, absolute stereochemistry assigned arbitrarily.

[b] nt = not tested.

Table 10. NS5A inhibitors with a 2,2-dimethyl-THP group on the right-hand side



ID	Z	Replicon EC_{90} (nM)						
		GT1a	GT1b	GT2b L31M	GT3a	GT4a	GT1a Y93H	GT1a L31V
36		0.033	0.067	0.349	0.082	0.106	0.155	0.046
37		0.006	0.005	0.031	0.013	0.004	0.036	0.001

The rat PK profile of selected compounds in both Table 9 and 10 are summarized in Table 11. While the majority of these compounds showed a moderate to low PK exposure in rat, **30** had comparable PK exposure in rat as **21**, as well as reasonable bioavailability.

Table 11. Pharmacokinetic profiles of compounds **25**, **27**, **29-32**, **34**, **36**, and **37** in rat

ID	iv	po

	$T_{1/2}$ (h)	CL (mL/min/Kg)	AUC (μM.h)	C_{max} (μM)	C_{24} (μM)	F%
25	3.8	25	0.08	0.01	0.001	1.2
27	5.2	8	0.78	0.11	0.004	3.5
29	9.6	5	0.80	0.10	0.004	2.7
30	4.3	9	1.90	0.29	0.006	10.2
31	5.3	29	0.05	0.01	0.001	0.8
32	4.5	21	0.08	0.02	0.001	1.0
34	4.0	9	0.51	0.06	0.004	2.8
36	5.9	9	0.23	0.02	0.002	1.2
37	6.0	9	0.45	0.04	0.004	2.0

[a] Fasted male Wistar Han rats were used. IV dose was formulated with 60% PEG200 and the compounds were dosed at 2 mg/kg. P.O. dose was formulated with 10% TWEEN and the compounds were dosed at 10 mg/kg.

With all the potency and PK data in hand, **21** emerged as a lead with both "flat" potency and acceptable PK profile in both rat and dog. Comparing with other compounds, **21** shows the best C_{24h} in both rat and dog. To reduce the HCV resistance, it is important to achieve adequate C_{24h} in human to cover the EC_{90} values for as many HCV NS5A genotypes and RASs as possible. Compound **21** has the best potential to achieve that in relatively low dose with QD dosing. Its potency profile against additional HCV NS5A genotypes and RASs are summarized in Table 12. Compound **21** showed <10X shift on a majority of the genotypes and mutants tested, other than several mutants, such as GT3a Y93H and GT3a L31V, with the corresponding EC_{90} values still in the single digit nM range. As NS5A inhibitors are often developed in combination with other DAAs such as NS3 inhibitors, we tested the potency of **21** on NS3 RASs. As anticipated, compound **21** retained its potency and showed good potency on prototypical GT1a NS3 RASs such as GT1a R155K and GT1a V36M+R155K double substitution, as well as prototypical GT1b NS3 RASs such as GT1b A156T and GT1b D168Y. These

data suggested that a cross resistance is unlikely. The potency profile of **21** is among the most flat profiles we have achieved so far.

The in vitro cytotoxicity potential of compound **21** was tested in Huh-7 cells up to a maximum concentration of 25 μM by monitoring GAPDH mRNA levels. No cytotoxicity was observed.

Table 12. *In vitro* potency profile of compound **21**

Replicons	EC_{90} (nM)	Shift[a]	Replicons	EC_{90} (nM)	Shift	Replicons	EC_{90} (nM)	Shift
1A	0.0042	-	1B	0.0044	1X	2B L31M	0.018	4X
1A (A92P)[b]	0.00014	<1X	1B (A156T)[c]	0.0040	1X	2B (A146S)	0.0034	1X
1A (K24R)	0.00034	<1X	1B (A92K)	0.026	6X	2B (M31V)	0.019	5X
1A (L31M:Y93C)	0.18	43X	1B (A92T)	0.0039	1X	3A	0.0066	2X
1A (L31V)	0.0043	1X	1B (D168Y)[b]	0.0018	<1X	3A (L31F)	0.29	69X
1A (M28K)	0.42	100X	1B (L28M:Y93H)	0.065	15X	3A (A30K)	0.018	4X
1A (M28T)	0.0020	<1X	1B (L31I)	0.021	5X	3A (A30T)	0.0036	1X
1A (Q30R)	0.12	28X	1B (L31M)	0.0016	<1X	3A (L31V)	3.2	762X
1A (R155K)[c]	0.0019	<1X	1B (L31M:Y93H)	0.19	45X	3A (Y93H)	2.4	571X
1A (R155K:V36M)[c]	0.0025	<1X	1B (L31V)	0.0058	1X	4A	0.0060	2X
1A (R81W)	0.00032	<1X	2A	0.0027	<1X	4A (L30H)	0.072	17X
1A (Y93H)	0.033	8X	2A (F28L)	0.00050	<1X	4A (P32L)	0.0044	1X
1A (Y93N)	0.069	16X	2A (F28Y)	0.069	16X	4A (L31V)	0.0077	2X
1A R81S	0.00033	<1X	2A (L31M)	0.025	6X			

[a] Shift from GT1a EC_{90} value

[b] The single amino acid code is indicated for all substitutions e.g. 1A (A92P) refers to a genotype 1a substitution where an alanine at position 92 is replaced with a proline

[c] HCV NS3 RAS

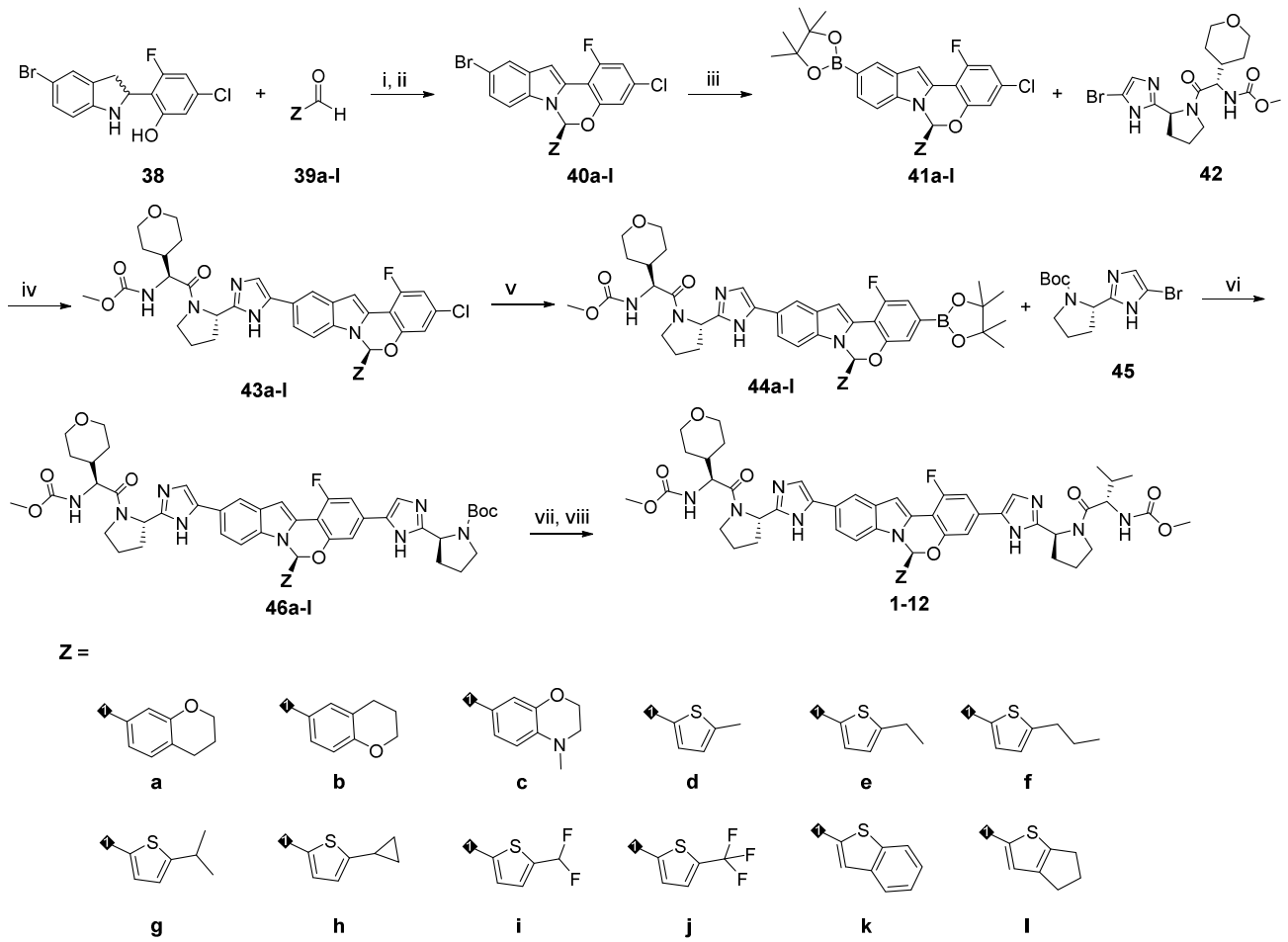
The structure of compound **21** has been fully determined. The stereochemistry of the six chiral centers either originated from known chiral starting materials or was determined by Vibrational Circular Dichroism (VCD) spectroscopy with confidence (see experimental section for details). Given its superior potency and pharmacokinetic properties, compound **21** was recommended as a preclinical candidate for further development.

CONCLUSION

We incorporated (*S*)-THP amino acid "cap" with varying substituents at the aminal carbon (the "Z" group) and identified HCV NS5A inhibitors with significantly improved potency against the least sensitive RASs of elbasvir, such as GT1a Y93H and GT2b L31M. These leads also showed an excellent *in vitro* profile in many other genotypes and mutants. Some of the compounds we identified possess "flat" potency profiles, with EC_{90} values against a majority of the mutants tested within a 10-fold range of the EC_{90} value for GT1a H77 (wild type). Compounds with an unsubstituted (*S*)-THP amino acid "cap" exhibited low PK exposure in rat, likely attributable to oxidative metabolism. Small alkyl substituted (*S*)-THP replacements for the isopropyl group of valine showed improved PK exposure in both rat and dog. Among them, the compound with the (*S,R*)-2,2-dimethyl-THP amino acid "cap" (compound **21**) had the best PK profile in both rat and dog, and an increased exposure-dose response up to 300 mpk in rat as well. Compound **21** was recommended as a preclinical candidate (**MK-6169**) for further development.

CHEMISTRY

The chemistry for the preparation of compounds **1-12** is outlined in Scheme 1. The preparation of compound **38** was described previously.[16] Aldehydes **39a-l** were either purchased or prepared based on known procedures.[30-32] Treatment of **38** with aldehydes **39a-l** under acidic condition to give the tetracyclic indoline intermediates which were in turn oxidized to the corresponding racemic indoles. These compounds were separated by chiral SFC to give **40a-l**, representing the enantiomers that give the more potent final compounds. The stereochemistry of the aminal carbons in **40a-l** was not determined, but we believe it's reasonable to assume it is "*S*" since in previous studies we have determined that the "*S*" isomers at the aminal carbon of the tetracyclic indole cores always give the more potent final compounds including MK-8742 and MK-8408.[15,30] Compounds **40a-l** were converted to the corresponding pinnacol boronate esters **41a-l** which upon treatment under Suzuki coupling conditions with **42** afforded intermediates **43a-l**. Compound **42** was prepared based on published procedures.[16] Treatment of **43a-l** under $Pd_2(dba)_3$/X-Phos conditions afforded pinnacol boronate esters **44a-l** that coupled with **45**[16] to give **46a-l**. The Boc groups of **46a-l** were removed and a methoxycarbonyl-L-valine moiety was introduced to give compounds **1-12**. Compound **13** and **14** were also prepared in a similar method by switching the valine and (*S*)-THP "caps" in the synthesis.

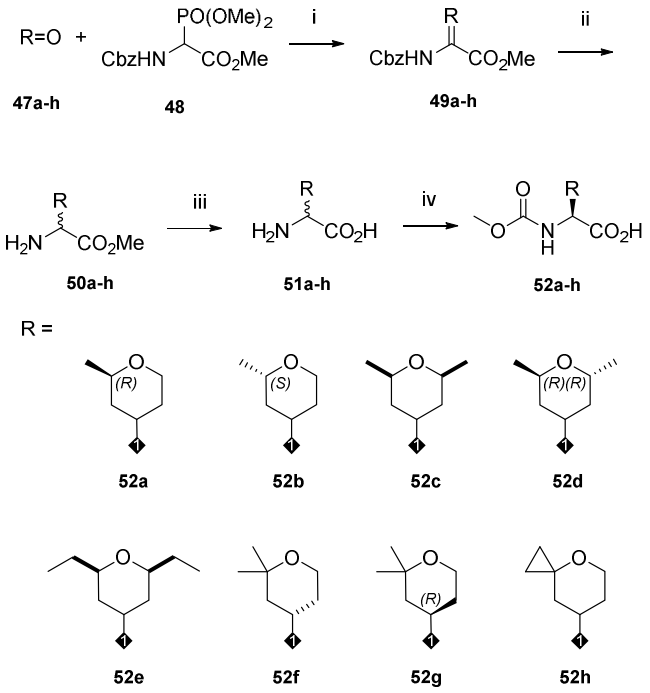


Scheme 1 Preparation of compounds **1-12**

Reagents and conditions: (i) TFA, CH_3CN, 25 ^oC, 6 h; (ii) DDQ, toluene, 115 ^oC, 2 h, then chiral SFC separation; (iii) Bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, dioxane, 90 ^oC, 16 h; (iv) **42**, Pd(dppf)Cl_2, K_2CO_3, dioxane/H_2O (10:1), 90 ^oC, 16 h; (v) Bis(pinacolato)diboron, $Pd_2(dba)_3$, X-Phos, dioxane, 110 ^oC, 2 h; (vi) **45**, Pd(dppf)Cl_2, K_2CO_3, dioxane/H_2O (10:1), 90 ^oC, 16 h; (vii) HCl, MeOH, 0.5 h. (viii) (*S*)-2-((methoxycarbonyl)amino)-2-(tetrahydro-2H-pyran-4-yl)acetic acid, BOP, DIPEA, DMF, 10 h.

The preparation of **52a-h** was outlined in Scheme 2. Ketone intermediates **47a-j** were either purchased from commercial resources or prepared (Schemes 4-7). Treatment of **47a-h** with **48** under DBU conditions gave condensed products **49a-h**, which were reduced by hydrogen under Pd/C to afford amine intermediates **50a-h**. Compounds **50a-h** were hydrolyzed to give racemic amino acids **51a-h**, to which a methyl carbamate functionality was introduced and the diastereomers were separated

by chiral SFC to provide **52a-h**, representing the diastereomers which lead to the most potent final compounds. The stereochemistry of the R group on the amino acid was not determined except **52 g**. We believe it is reasonable to assume they have the "*S*" configuration since in previous studies we have determined that (*S*)-2-amino-2-(tetrahydro-2H-pyran-4-yl)acetic acid always lead to the more potent final compounds than (*R*)-2-amino-2-(tetrahydro-2H-pyran-4-yl)acetic acid.[25] The stereochemistry of the chiral centers on the THP groups were either introduced via starting materials (see Schemes 4-7) or assigned arbitrarily, except **52g**, whose stereochemistry were fully determined by VCD with confidence (see experimental section).

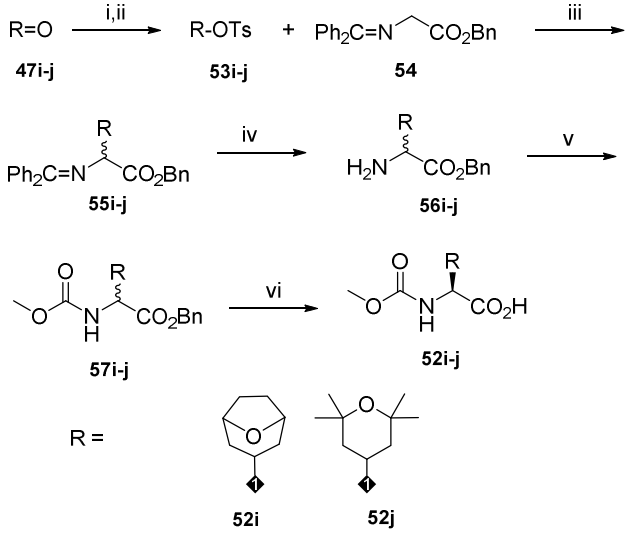


Scheme 2 Preparation of compounds **52a-h**

Reagents and conditions: (i) DBU, CH_2Cl_2, 25 ^oC, 5 h; (ii) Pd/C (10%), H_2 (35 psi), MeOH, 25 ^oC, 8 h; (iii) LiOH, MeOH, H_2O, 25 ^oC, 15 h; (iv) methyl chloroformate, LiOH, H_2O, 25 ^oC, 3 h; then chiral SFC separation.

Compounds **52i** and **52j** were prepared by a different synthesis outlined in Scheme 3. Ketone **47i-j** were reduced to the corresponding alcohols and then converted to tosylates **53i-j**. Replacement of the tosyl groups of **53i-j** by the enolate generated from compound **54** afforded compounds **55i-j**, which

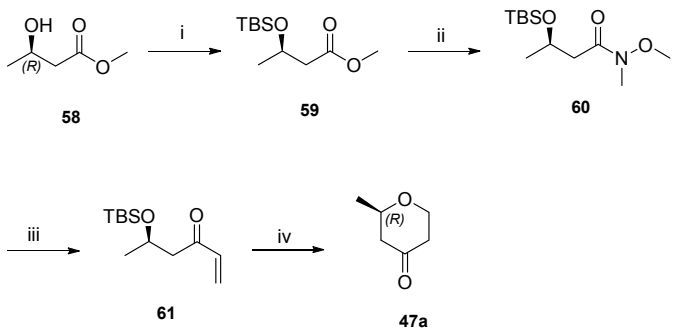
were deprotected under acidic conditions to provide amine **56i-j**, which were subsequently converted to methyl carbamates **57i-j** and further hydrolyzed to give acids **52i-j**.



Scheme 3 Preparation of compounds **52i-j**

Reagents and conditions: (i) L-selectride, THF, -78 to 25 ^{o}C, 12 h, then NaOH, H_2O; (ii) TsCl, pyridine, DCM, 25 ^{o}C, 12 h; (iii) **54**, LiHMDS, toluene, 100 ^{o}C, 4 h; (iv) HCl, THF, 25 ^{o}C, 2 h; (v) methyl chloroformate, DCM, Et_3N, 25 ^{o}C, 12 h; (vi) Pd/C (10%), H_2 (40 psi), MeOH, 25 ^{o}C, 12 h.

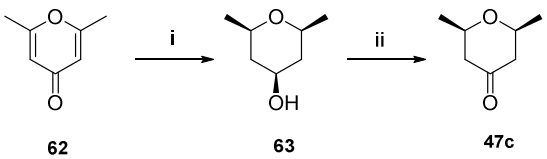
The preparation of (*R*)-2-methyltetrahydro-4H-pyran-4-one (**47a**) is described in Scheme 4. Commercially available methyl (*R*)-3-hydroxybutanoate (**58**) was protected with a TBS group to give compound **59**, which was converted to Weinreb amide **60**. Addition of a vinyl group to compound **60** gave α,β–unsaturated ketone **61**, which was deprotected and provided ketone **47a**. (*S*)-2-methyltetrahydro-4H-pyran-4-one (**47b**) was prepared in a similar manner starting with commercially available methyl (*S*)-3-hydroxybutanoate.



Scheme 4 Preparation of compounds **47a**

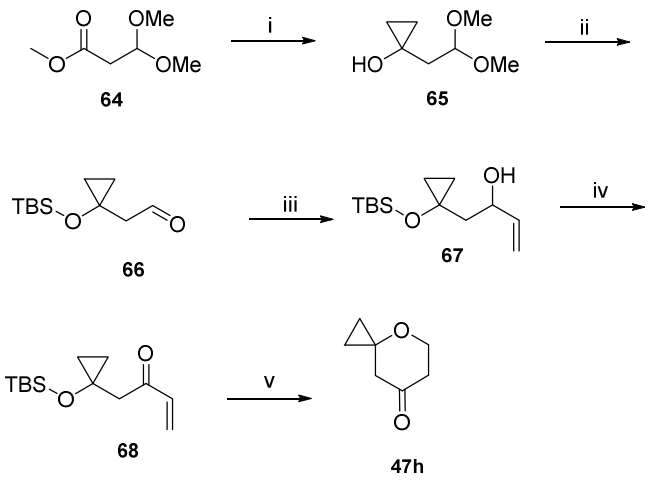
Reagents and conditions: (i) TBSCl, imidazole, EtOAc, 0 to 25 ^{o}C, 12 h, 95%; (ii) N,O-dimethyl hydroxylamine, *i*-PrMgCl, THF, -20 ^{o}C, 2 h, 72%; (iii) vinyl magnesium bromide, THF, 0 to 5 ^{o}C, 1 h, 79%; (iv) Amberlyst 15 resin, $CHCl_3$, 25 ^{o}C, 15 h, used without purification.

The preparation of *cis*-2,6-dimethyltetrahydro-4H-pyran-4-one (**47c)** is described in Scheme 5. Reduction of compound **62** led to *cis*-alcohol **63**, which was oxidized to ketone **47c**. The preparation of ketone **47h** is described in Scheme 6. Cyclopropylation of **64** was achieved under Ti(*i*-PrO)$_4$/EtMgBr conditions to afford compound **65**, which was protected with a TBS group. The dimethyl acetal also fell off under the TBSOTf condition and aldehyde **66** was obtained. Addition of a vinyl group to the aldehyde **66** led to alcohol **67**, which was oxidized to α,β–unsaturated ketone **68**. Deprotection of the TBS group led to the formation of desired ketone **47h**. Ketone **47i** was prepared in a two-step synthesis (Scheme 7). Reaction between furan and 1,1,3,3-tetrabromopropan-2-one under Zn/(EtO)$_3$B conditions gave di-bromo ketone **69**, which was reduced to the desired ketone **47i** under Pd catalyzed hydrogenation.



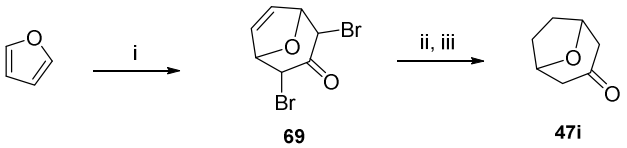
Scheme 5 Preparation of compounds **47c**

Reagents and conditions: (i) Pd/C (10%), H_2 (50 psi), EtOH, 35 ^{o}C, 17 h, 99%; (ii) TEMPO, $NaHCO_3$, KBr, NaClO, DCM, H_2O, 25 ^{o}C, 5 h, 99%.



Scheme 6 Preparation of compounds **47h**

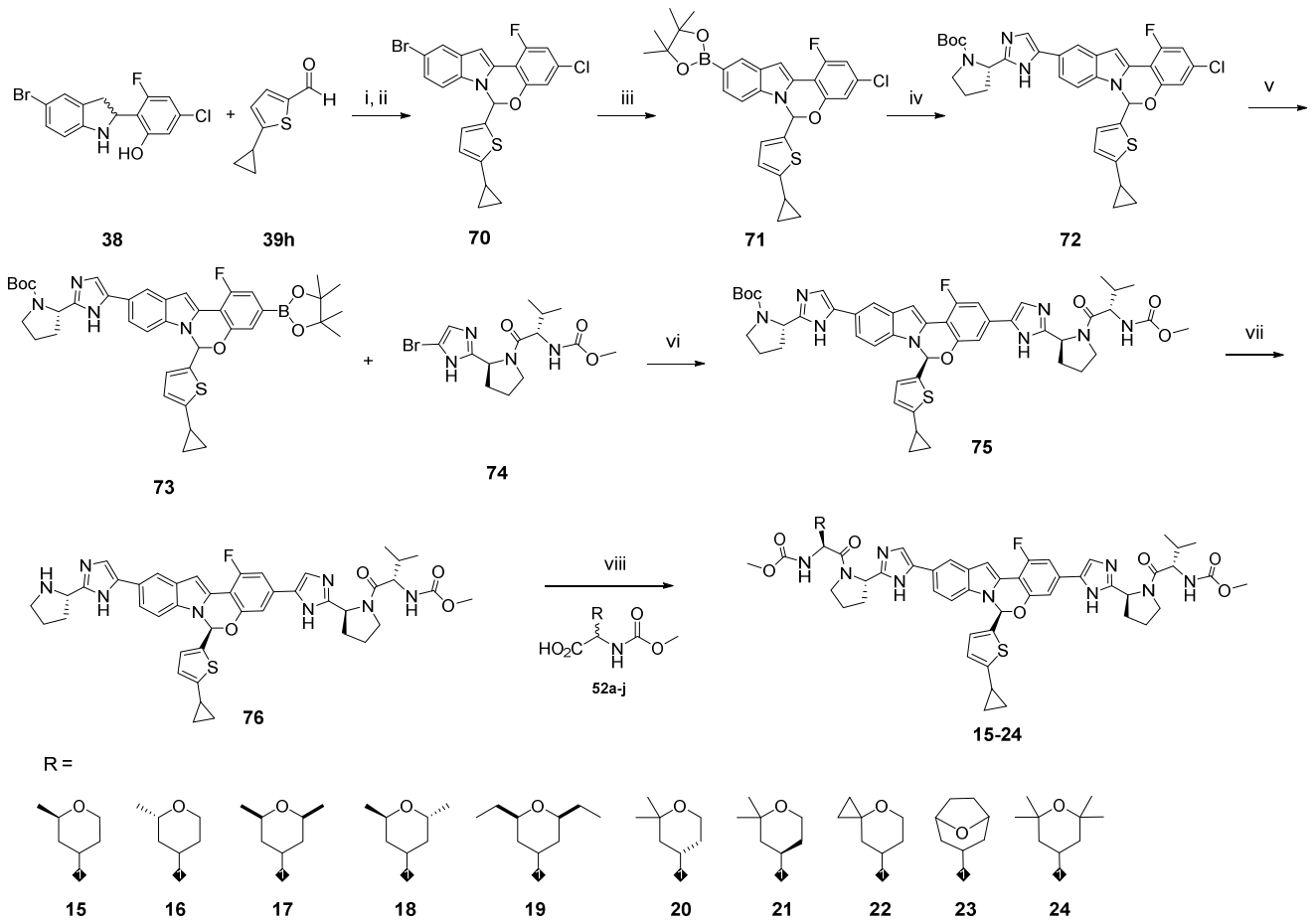
Reagents and conditions: (i) Ti(*i*-PrO)$_4$, EtMgBr, THF/Et$_2$O (1:1), 0 to 25 ^{o}C, 12 h, 89%; (ii) TBSOTf, 2,6-lutidine, DCM, 10 ^{o}C, 1 h, 41%; (iii) vinyl magnesium bromide, THF, -78 ^{o}C, 0.5 h, 41%; (iv) IBX, CH$_3$CN, 80 ^{o}C, 2 h, 50%; (v) Amberlyst 15 resin, DCM, reflux, 4 h, used without purification.



Scheme 7 Preparation of compounds **47i**

Reagents and conditions: (i) 1,1,3,3-tetrabromopropan-2-one, Zn, (EtO)$_3$B, THF, 25 ^{o}C, 17 h; (ii) Zn, CuCl, MeOH, 25 ^{o}C, 19 h, 30% for two steps.

With **52a-j** in hand, compounds **15-24** were synthesized as described in Scheme 8. The preparation of **75** from **38** and **39h** was similar to the chemistry described in Scheme 1. Compound **75** was resolved by chiral SFC. The active isomer, which was identified by carrying both isomers to the final compounds and comparing their replicon activity, was deprotected to give amine **76**. The substituted THP "cap" was introduced by BOP mediated amide coupling reaction to provide final compounds **15-24**



Scheme 8 Preparation of compounds **15-24**

Reagents and conditions: (i) TFA, CH_3CN, 25 ^oC, 6 h, 81%; (ii) DDQ, toluene, reflux, 2 h, 88%; (iii) Bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, dioxane, 90 ^oC, 16 h, 88%; (iv) **45**, Pd(dppf)Cl_2, K_2CO_3, dioxane/H_2O (10:1), 90 ^oC, 16 h, 77%; (v) Bis(pinacolato)diboron, $Pd_2(dba)_3$, X-Phos, dioxane, 110 ^oC, 2 h, 89%; (vi) **72**, Pd(dppf)Cl_2, K_2CO_3, dioxane/H_2O (10:1), 90 ^oC, 16 h, 74%; then chiral SFC separation, 26%. (vii) HCl, MeOH, 2 h, 98%. (viii) **52a-j**, BOP, DIPEA, DMF, 10 h.

Compounds **25-37** were synthesized by similar methods as described in Scheme 1 by replacing the unsubstituted (*S*)-THP "cap" with **52g**.

EXPERIMENTAL SECTION

Chemicals purchased from commercial suppliers were used as received unless specified. Silica gel column chromatography was carried out on ISCO CombiFlash Companion. Prepacked silica gel cartridges were used. TLC (thin layer chromatography) visualization was performed under 254 nm ultraviolet light. Reverse-phase preparative HPLC purifications were done on a Gilson 215 Liquid Handler with Unpoint software, typically with a Sun Fire Prep C18 OBD 5 am 19 × 50 mm column. The following method was typically used: Run time: 15 min. flow rate: 14 mL/min. Mobile phase: 10% to 90% CH_3CN/H_2O, with 0.1%TFA. UV detection at 254 nm or 210 nm was typically used. SFC separations were normally done on a TharSFC instrumentation. Columns and separation conditions were specified in each separation.

Nuclear magnetic resonance spectra were obtained on a Varian 400 MHz, 500 MHz or Bruker 400 MHz spectrometers. Spectra were taken at ambient temperature. Chemical shifts were assigned by using residual solvent signal as internal standard and are reported in parts per million (ppm). Resonance patterns are reported by using the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet).

The purity of all final compounds were determined to be >95% according to the LCMS data obtained on Agilent 1200 Series HPLC equipped with DAD & 6110 single quadrupole MSD & ELSD with Agilent TC-C18, 50×2.1mm, 5μm for acid methods, Waters X-Bridge ShieldRP18, 50x2.1mm 5μm for basic methods. HRMS analysis was performed on Waters Acquity UPLC, Column: Acquity UPLC BEH C18 1.7 μm, and Waters Xevo G2 Qtof with ionization mode as positive ESI+ for all compounds except **21**. The HRMS analysis of **21** was carried on Thermo LTQ obitrap mass spectrometer with ionization mode as positive ESI+ and HPLC system consisting of a Phenomenex Kinetex C18 column, 150 x 4.6 mm, 2.6 micron particle size. The flow rate was 1.0 ml/min. The column temperature was 30º C. The mobile phase was water and acetonitrile with 0.1% formic acid added to each. A linear gradient from 20% to 90% acetonitrile in 8 minutes was used.

Virology assay

Detailed assay method were published previously.[31,32,34,35] All EC_{50} or EC_{90} data are average values of at least two measurements, with a maximum variation of 3-fold.

PK Assessment

Rat and dog PK studies were done in 24 hour period. Overnight fasted or fed rats and dogs were used. Dose was administered either by IV or PO with two animals in each dosing group. Plasma was obtained by centrifugation of the blood collected. Compound concentrations in plasma were determined by LC/MS analysis.

Aldehyde **39a** was prepared based on published procedures.[31-33] Aldehydes **39b-c** were purchased from commercial resources. Aldehyde **39d-l** were either purchased from commercial resources or prepared based on published procedures[29,31-33]

Methyl ((*S*)-2-((*S*)-2-(5-((*S*)-6-(chroman-7-yl)-1-fluoro-3-(2-((*S*)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (1). Compound **1** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.933 (s, 1 H), 7.81 (s, 1 H), 7.68 (s, 1 H), 7.74-7.08 (m, 6 H), 6.88 (d, *J*=8.0 Hz, 1 H), 6.41 (d, *J*=8.4 Hz, 1 H), 6.29 (s, 1H), 5.23-5.18 (m, 2 H), 4.29 (d, 7.6 Hz, 1 H), 4.21 (d, *J*=6.4 Hz, 1 H), 4.08-3.88 (m, 8 H), 3.64 (d, *J*=20 H, 6 H), 3.38-3.31 (m, 2 H), 2.65-2.53 (m, 2 H), 2.51-2.50 (m, 2 H), 2.26-2.02 (m, 8 H), 1.83-1.85 (m, 2 H), 1.55-1.35 (m, 4 H), 0.90 (dd, *J*=6.8, 20 Hz, 6 H). HRMS (ESI+) *m/z* calcd for C54H61FN9O9 $[M+H]^+$, 998.4576; found, 998.4587.

Methyl ((*S*)-2-((*S*)-2-(5-((*S*)-6-(chroman-6-yl)-1-fluoro-3-(2-((*S*)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (2). Compound **2** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.00 (s, 1 H), 7.88 (s,1 H), 7.72 (s, 1 H), 7.45 (d, *J*=4.8 Hz, 2 H), 7.32 (d, *J*=11.2 Hz, 1 H), 7.25-7.21 (m, 3 H), 6.74 (d, *J*=7.2 Hz, 2 H), 6.61 (d, *J*=9.2 Hz, 1 H), 5.24-5.16 (m, 2 H), 4.26 (d, *J*=8.0 Hz, 1 H), 4.19 (d, *J*=7.6 Hz, 1 H), 4.10-4.07 (m, 4 H), 3.93-3.82 (m, 4 H), 3.64 (s, 6 H), 3.40-3.30 (m, 2 H), 2.61-2.51 (m, 4 H), 2.40-2.13 (m, 8 H), 2.03-1.87 (m, 2 H), 1.56-1.27 (m, 4 H), 0.90-0.85 (m, 6 H). HRMS (ESI+) *m/z* calcd for C54H61FN9O9 $[M+H]^+$, 998.4576; found, 998.4586.

Methyl ((*S*)-2-((*S*)-2-(5-((*S*)-1-fluoro-3-(2-((*S*)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1*H*-imidazol-5-yl)-6-(4-methyl-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-7-yl)-6*H*-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1*H*-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2*H*-pyran-4-yl)ethyl)carbamate (3). Compound **3** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.86-7.80 (m, 1 H), 7.75-7.74 (m, 1 H), 7.60-7.59 (m, 1 H), 7.32-6.89 (m, 6 H), 6.49-6.36 (m, 3 H), 5.22-5.15 (m, 2 H), 4.28 (d, *J*=7.2 Hz, 1 H), 4.20 (d, *J*=6.8 Hz, 1 H), 4.08 (s, 4 H), 3.87-3.85 (m, 4 H), 3.62 (s, 6 H), 3.35-3.30 (m, 2 H), 3.13 (s, 2 H), 2.73 (d, *J*=3.2 Hz, 3 H), 2.49-2.47 (m, 2 H), 2.22-2.03 (m, 8 H), 1.55-1.33 (m, 4 H), 0.92-0.85 (m, 6 H). HRMS (ESI+) *m/z* calcd for C54H62FN10O9 $[M+H]^+$, 1013.4685; found, 1013.4699.

Methyl ((S)-2-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (4). To a solution of the compound **46d** (2.1 g, 2.32 mmol) in dry dioxane (8 mL) was added HCl-dioxane (4M, 6 mL) through syringe and stirred at 25 °C for 2 hours, then concentrated and dried under high vacuum

to give HCl salt of methyl ((*S*)-2-((*S*)-2-(5-((*S*)-1-fluoro-6-(5-methylthiophen-2-yl)-3-(2-((*S*)-pyrrolidin-2-yl)-1H-imidazol-4-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (1.87g, 98%). LC/MS Calcd. For $[M+H]^+$ C43H45FN8O5S: 805.3; found 805.4. To a mixture of the methyl ((*S*)-2-((*S*)-2-(5-((*S*)-1-fluoro-6-(5-methylthiophen-2-yl)-3-(2-((*S*)-pyrrolidin-2-yl)-1H-imidazol-4-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (500 mg, 0.62 mmol), (methoxycarbonyl)-L-valine (108.5 mg, 0.62 mmol) and DIPEA (160 mg, 1.24 mmol) in DMF (3 mL) was added HATU (235.6 mg, 0.62 mmol). The resulting mixture was stirred at 25 °C for 2 hours before the solution was applied to HPLC purification to afford the desired compound **4** (340 mg, 57%). ^{1}H NMR (MeOD) δ: 8.02 (d, *J*=4 Hz, 1H), 7.94 (s, 1H), 7.83 (s, 1H), 7.76 (d, *J*=4 Hz, 1H), 7.52 (s, 2 H), 7.40-7.39 (m, 1 H), 7.32-7.31 (m, 1 H), 7.20 (s, 1 H), 6.52-6.49 (m, 2 H), 5.24-5.20 (m, 2 H), 4.29-4.27 (m, 2 H), 4.20-4.10 (m, 2 H), 3.97-3.90 (m, 4 H), 3.86 (s, 6 H), 3.55-3.45 (m, 2 H), 2.60-2.52 (m, 2 H), 2.32-2.01 (m, 11 H), 1.62-1.31 (m, 4 H), 0.92-0.85 (m, 6 H). HRMS (ESI+) *m/z* calcd for C50H57FN9O8S $[M+H]^+$, 962.4035; found, 962.4055.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-ethylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (5). Compound **5** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.99 (s, 1 H), 7.88 (s, 1 H), 7.81 (s, 1 H), 7.73 (s, 1 H), 7.50-7.46 (m, 2 H), 7.37-7.34 (m, 1 H), 7.28 (s, 1 H), 7.16 (s, 1 H), 6.55-6.49 (m, 2 H), 5.24-5.16 (m, 4 H), 4.27 (d, *J*=8.4 Hz, 1 H), 4.20 (d, *J*=7.2 Hz, 1 H), 4.18-4.10 (m, 2 H), 4.07-3.70 (m, 5 H), 3.63 (s, 6 H), 3.40-3.29 (m, 2 H), 2.70-2.65 (m, 2 H), 2.60-2.53 (m, 2 H), 2.35-1.85 (m, 8 H), 1.65-1.34 (m, 4 H), 1.13 (t, *J*=7.6 Hz, 3 H), 0.92-0.86 (m, 6 H). HRMS (ESI+) *m/z* calcd for C51H59FN9O8S $[M+H]^+$, 976.4191; found, 976.4222.

Methyl ((S)-2-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-propylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (6). Compound **6** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.01 (s, 1 H), 7.92 (s, 1 H), 7.82 (s, 1 H), 7.75 (s, 1 H), 7.51-7.49 (m, 2 H), 7.39-7.31 (m, 2 H), 7.20 (s, 1 H), 6.56-6.52 (m, 2 H), 5.24-5.20 (m, 2 H), 4.29-4.20 (m, 2 H), 4.13-4.10 (m, 2 H), 3.94-3.66 (m, 4 H), 3.65 (s, 5 H), 3.56-3.41 (m, 3 H), 2.66-2.64 (m, 2 H), 2.62-2.55 (m, 2 H), 2.28-2.26 (m, 2 H), 2.18-2.16 (m, 4 H), 2.05-2.03 (m, 2 H), 1.59-1.53 (m, 3 H), 1.51-1.34 (m, 3 H), 0.97-0.84 (m, 9 H). HRMS (ESI+) *m/z* calcd for C52H61FN9O8S $[M+H]^+$, 990.4348; found, 990.4362.

Methyl ((S)-2-((S)-2-(5-((S)-1-fluoro-6-(5-isopropylthiophen-2-yl)-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (7). Compound **7** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.01 (s, 1 H), 7.91 (s, 1 H), 7.83 (s, 1 H), 7.76 (s, 1 H), 7.55-7.53 (m, 2 H), 7.50-7.41 (m, 1 H), 7.38-7.30 (m, 1 H), 7.19-7.18 (m, 1 H), 6.61-6.53 (m, 2 H), 5.26-5.21 (m, 2 H), 4.32-4.24 (m, 2 H), 4.13-4.12 (m, 2 H), 3.96-3.88 (m, 4 H), 3.68 (s, 5 H), 3.43-3.32 (m, 2 H), 3.05-3.02 (m, 1 H), 2.58-2.55 (m, 2 H), 2.30-2.05 (m, 9 H), 1.65-1.56 (m, 1 H), 1.55-1.38 (m, 3 H), 1.20-1.19 (m, 6 H), 0.99-0.91 (m, 6 H). HRMS (ESI+) *m/z* calcd for C52H61FN9O8S $[M+H]^+$, 990.4348; found, 990.4361.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (8). Compound **8** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.01 (s, 1 H), 7.93 (s, 1 H), 7.81 (s, 1 H), 7.75 (s, 1 H), 7.52-7.48 (m, 2

H), 7.40-7.37 (m, 1 H), 7.30 (s, 1 H), 7.19-7.18 (m, 1 H), 6.51-6.45 (m, 2 H), 5.23-5.18 (m, 2 H), 4.29-4.20 (m, 2 H), 4.13-4.10 (m, 2 H), 3.94-3.86 (m, 4 H), 3.66-3.65 (m, 6 H), 3.40-3.36 (m, 2 H), 2.55-2.54 (m, 2 H), 2.28-1.93 (m, 9 H), 1.59-1.34 (m, 4 H), 0.93-0.88 (m, 8 H), 0.55-0.53 (m, 2 H). HRMS (ESI+) *m/z* calcd for C52H59FN9O8S $[M+H]^+$, 988.4191; found, 988.4212.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-(difluoromethyl)thiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (9). Compound **9** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.98 (d, *J* = 5.6 Hz, 2 H), 7.83 (s, 1 H), 7.74 (s, 1 H), 7.60-7.56 (m, 2 H), 7.40-7.29 (m, 2 H), 7.18 (s, 1 H), 7.17-7.06 (m, 1 H), 6.88 (t, *J* = 56 Hz, 1 H), 6.62 (m, 1 H), 5.25-5.16 (m, 2 H), 4.27-4.21 (m, 2 H), 4.19-4.08 (m, 2 H), 3.90-3.84 (m, 4 H), 3.64 (s, 6 H), 3.36-3.31 (m, 2 H), 2.54-2.50 (m, 2 H), 2.28-1.99 (m, 8 H), 1.56-1.35 (m, 4 H), 0.92-0.87 (m, 6 H). HRMS (ESI+) *m/z* calcd for C50H55F3N9O8S $[M+H]^+$, 998.3846; found, 998.3872.

Methyl ((S)-2-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-(trifluoromethyl)thiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (10). Compound **10** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.88 (m, 2 H), 7.53 (m, 1 H), 7.42 (m, 2 H), 7.36 (s, 1 H), 7.28 (m, 2H), 7.22 (m, 2H), 5.15 (m, 2 H), 4.30 (m, 1H), 4.22 (m, 1H), 3.83-4.14 (m, 4H), 3.65 (s, 6H), 3.46-3.36(m, 3H), 1.90-2.40 (m, 13H), 1.44-1.68 (m, 5H), 0.94 (m, 8 H). HRMS (ESI+) *m/z* calcd for C50H54F4N9O8S $[M+H]^+$, 1016.3752; found, 1016.3757.

Methyl ((S)-2-((S)-2-(5-((S)-6-(benzo[b]thiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (11). Compound **11** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.83 (m, 1 H), 7.59 (m, 1 H), 7.09-7.49 (m, 6 H), 6.98 (m, 1 H), 5.15 (m, 2 H), 4.30 (m, 1H), 4.23(m, 1H), 3.85-4.09 (m, 4H), 3.65 (s, 6H), 2.73 (s, 2H), 2.52 (s, 2H), 1.73-2.42 (m, 13H), 1.22-1.68 (m, 10H), 0.94 (m, 8 H). LCMS (ESI+) *m/z* calcd for C53H57FN9O8S $[M+H]^+$, 998.40; found, 999.21.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (12). Compound **12** was prepared based on the same procedures as compound **4**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.90 (m, 2 H), 7.70 (m, 1 H), 7.59 (m, 1 H), 7.20-7.50 (m, 6 H),7.10 (s, 1 H), 6.80 (m, 2H), 5.15 (m, 2 H), 4.30 (m, 1H), 4.20 (m, 1H), 3.81-4.13 (m, 4H), 3.65 (s, 6H), 1.80-2.40 (m, 13H), 1.20-1.68 (m, 8H), 0.92 (m, 8 H). LCMS (ESI+) *m/z* calcd for C52H59FN9O8S $[M+H]^+$, 988.41; found,989.20.

Methyl ((S)-1-((S)-2-(5-((S)-6-(chroman-7-yl)-1-fluoro-3-(2-((S)-1-((S)-2-((methoxycarbonyl)amino)-2-(tetrahydro-2H-pyran-4-yl)acetyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (13). Compound **13** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.92 (s, 1 H), 7.80 (s, 1 H), 7.69 (s, 1 H), 7.46-7.42 (m, 2 H),7.29-7.25 (m, 2 H), 7.14 (s, 1 H), 7.09 (s, 1 H), 6.90 (s, 1 H), 6.43-6.44 (m, 1 H), 6.26 (s, 1 H), 5.23-5.15 (m, 2 H), 4.27-4.25 (m, 2 H), 4.24-4.22 (m, 2 H), 4.10-4.00 (m, 2 H), 3.88-3.86 (m, 4 H),

3.64 (s, 6 H), 3.34-3.31 (m, 2 H), 2.65-2.62 (m, 2 H), 2.55-2.51 (m, 2 H), 2.25-2.26 (m, 2 H), 2.19-2.11 (m, 4 H), 2.09-2.05 (m, 2 H), 1.86-1.83 (m, 2 H), 1.55 (s, 1 H), 1.36-1.34 (m, 3 H), 0.95-0.89 (m, 6 H). HRMS (ESI+) *m/z* calcd for C54H61FN9O9 $[M+H]^+$, 998.4576; found, 998.4570.

Methyl ((S)-1-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((S)-2-((methoxycarbonyl)amino)-2-(tetrahydro-2H-pyran-4-yl)acetyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(4-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (14). Compound **14** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (s, 1 H), 7.88 (s, 1 H), 7.74 (s, 1 H), 7.48-7.46 (m, 1 H), 7.41 (s, 1 H), 7.33-7.31 (m, 2 H), 7.28-7.20 (m, 2 H), 6.54-6.52 (m, 1 H), 6.44-6.38 (m, 2 H), 5.26-5.16 (m, 2 H), 4.27-4.22 (m, 2H), 4.15-4.12 (m, 2 4), 3.93-3.85 (m, 5 H), 3.65 (s, 6 H), 3.19 (s, 2 H), 2.80 (s, 3 H), 2.58-2.53 (m, 2 H), 2.27-2.03 (m, 10 H), 1.33-1.31 (m, 5 H), 0.98-0.89 (m, 6 H). HRMS (ESI+) *m/z* calcd for C54H62FN10O9 $[M+H]^+$, 1013.4685; found, 1013.4720.

Methyl ((1S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((2R)-2-methyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (15). Compound **15** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.03 (s, 1 H), 7.91 (s, 1 H), 7.79-7.77 (m, 2 H), 7.56-7.54 (m, 1 H), 7.49-749 (m, 1 H), 7.39-7.37 (m, 1 H), 7.29 (s, 1 H), 7.17 (s, 1 H), 6.51-6.45 (m, 2 H), 5.29-5.19 (m, 2 H), 4.29-4.22 (m, 2H), 4.12-4.11 (m, 2 H), 3.95-3.87 (m, 3 H), 3.66 (s, 6 H), 3.42-3.35 (m, 2 H), 2.58-2.56 (m, 2 H), 2.27-1.94 (m, 9 H), 1.54 (s, 1 H), 1.37-1.31 (m, 2 H), 1.09-1.07 (m, 4 H), 0.94-0.89 (m, 8 H), 0.55-0.54 (m, 2 H). HRMS (ESI+) *m/z* calcd for C53H61FN9O8S $[M+H]^+$, 1002.4348; found, 1002.4362.

Methyl ((1S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((2S)-2-methyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (16). Compound **16** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (s, 1 H), 7.91 (s, 1 H), 7.80-7.76 (m, 2 H), 7.55-7.47 (m, 2 H), 7.40-7.37 (m, 1 H), 7.29 (s, 1 H), 7.18 (s, 1 H), 6.51-6.46 (m, 2 H), 5.26-5.19 (2 H), 4.30-4.22 (m, 2 H), 4.12-4.11 (m, 2 H), 3.92-3.86 (m, 3 H), 3.66 (s, 6 H), 3.41-3.36 (m, 2 H), 2.55 (s, 2 H), 2.28-1.94 (m, 9 H), 1.60 (s, 1 H), 1.38-1.33 (m, 2 H), 1.30-0.89 (m, 12 H), 0.55-0.54 (d, J = 4.8 Hz, 2 H). HRMS (ESI+) *m/z* calcd for C53H61FN9O8S $[M+H]^+$, 1002.4348; found, 1002.4367.

Methyl ((1S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((2R,6S)-2,6-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (17). Compound **17** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.06 (s, 1 H), 7.91 (s, 1 H), 7.80 (s, 1 H), 7.74 (s, 1 H), 7.57-7.55 (m, 1 H), 7.49-7.46 (m, 1 H), 7.39-7.36 (m, 1 H), 7.30 (s, 1 H), 7.18-7.17 (m, 1 H), 6.51-6.45 (m, 2 H), 5.36-5.33 (m, 1 H), 5.20 (s, 1 H), 4.29 (d, J = 8 Hz, 1 H), 4.21 (d, J = 8 Hz, 1 H), 4.11-4.08 (m, 2 H), 3.85-3.83 (m, 2 H), 3.65 (s, 3 H), 3.59 (s, 3 H), 3.55-3.48 (m, 2 H), 2.56-2.52 (m, 2 H), 2.20-1.93 (m, 9 H), 1.76-1.73 (m, 1 H), 1.60-1.57 (m, 1 H), 1.17 (d, J = 6 Hz, 6 H), 1.07-0.88 (m, 10 H), 0.55-0.53 (m, 2 H). HRMS (ESI+) *m/z* calcd for C54H63FN9O8S $[M+H]^+$, 1016.4504; found, 1016.4539.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((2R,6R)-2,6-dimethyltetrahydro-2H-pyran-4-

yl)-2-oxoethyl)carbamate (18). Compound **18** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.03 (s, 1 H), 7.91 (s, 1 H), 7.80-7.79 (m, 2 H), 7.52-7.47 (m, 2 H), 7.38-7.35 (m, 1 H), 7.30 (s, 1 H), 7.18 (s, 1 H), 6.49-6.44 (m, 2 H), 5.22-5.18 (m, 2 H), 4.19-4.15 (m, 4 H), 4.08-4.07 (m, 1 H), 3.85-3.81 (m, 2 H), 3.38 (d, *J* = 11 Hz, 6 H), 2.57-2.53 (m, 2 H), 2.24-2.15 (m, 6 H), 2.13-1.93 (m, 2 H), 1.59-1.54 (m, 1 H), 1.43-1.42 (m, 1 H), 1.26-1.18 (m, 4 H), 1.10 (s, 1 H), 1.00-0.96 (m, 4 H), 0.91-0.87 (m, 8 H), 0.53 (d, *J* = 4 Hz, 2 H). HRMS (ESI+) *m/z* calcd for $C_{54}H_{63}FN_9O_8S$ $[M+H]^+$, 1016.4504; found, 1016.4554.

Methyl ((1S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((2R,6S)-2,6-diethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (19). Compound **19** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (s, 1 H), 7.91 (s, 1 H), 7.78-7.76 (m, 2 H), 7.54-7.51 (m, 2 H), 7.37-7.35 (m, 1 H), 7.28 (s, 1 H), 7.17 (s, 1 H), 6.49-6.45 (m, 2 H), 5.22-5.19 (m, 2 H), 4.26-4.20 (m, 2 H), 4.19-4.09 (m, 2 H), 3.86-3.83 (m, 2 H), 3.64 (s, 6 H), 3.12-3.11 (m, 2 H), 2.55-2.54 (m, 2 H), 2.25-2.14 (m, 6 H), 2.03-1.92 (m, 3 H), 1.44-1.29 (m, 6 H), 0.96-0.78 (m, 16 H), 0.53-0.51 (m, 2 H). HRMS (ESI+) *m/z* calcd for $C_{56}H_{67}FN_9O_8S$ $[M+H]^+$, 1044.4817; found, 1044.4835.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((S)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (20). Compound **20** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.00 (s, 1 H), 7.90 (s, 1 H), 7.79 (s, 1 H), 7.75 (s, 1 H), 7.51-7.47 (m, 2 H), 7.38-7.35 (m, 1 H), 7.18 (d, *J* = 3 Hz, 1 H), 6.50-6.44 (m, 2 H), 5.23-5.18 (m, 2 H), 4.20-4.16 (m, 4 H), 3.86-3.82 (m, 2 H), 3.64-3.57 (m, 8 H), 2.55-2.53 (m, 2 H), 2.26-2.11 (m, 8 H), 2.01-1.93 (m, 1 H),

1.62-1.59 (m, 1 H), 1.28-1.04 (m, 10 H), 0.97-0.86 (m, 8 H), 0.55-0.51 (m, 2 H). HRMS (ESI+) *m/z* calcd for C54H63FN9O8S $[M+H]^+$, 1016.4504; found, 1016.4490.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (21). To a mixture of **76h** (120 mg, 0.15 mmol), (*S*)-2-((*R*)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (**52g**, 37 mg, 0.15 mmol) and HATU (58 mg, 0.15 mmol) in DMF (4 ml) was added DIPEA (39 mg, 0.30 mmol). The resulting mixture was stirred at 20℃ overnight, and the reaction solution was subjected directly to reverse phase HPLC purification to afford **21** (81 mg, 53%). ^{1}H NMR (DMSO-d_6) δ: 8.23-8.21 (m, 2 H), 8.13 (s, 1 H), 7.99 (s, 1 H), 7.75-7.73 (m, 2 H), 7.67-7.66 (m, 2 H), 7.26-7.22 (m, 2 H), 7.10 (s, 1 H), 6.56-6.55 (m, 2 H), 5.18-5.16 (m, 2 H), 3.98-4.22 (m, 4 H), 3.82 (s, 1 H), 3.62-3.54 (m, 2 H), 3.51-3.49 (m, 10 H), 2.19-2.44 (m, 7 H), 2.18-2.08 (m, 1 H), 1.94-2.06 (m, 3 H), 1.25-1.08 (m, 12 H), 0.89-0.84 (m, 6 H), 0.77 (d, *J* = 2 Hz, 3 H), 0.53-0.51 (m, 2 H). ^{13}C NMR: (DMSO-d_6) δ: 170.91, 170.33, 159.34, 157.68, 156.88, 150.53, 149.98, 149.94, 149.84, 148.65, 135.01, 134.06, 133.06, 128.55, 127.06, 125.79, 121.80, 121.29, 120.19, 118.13, 116.52, 113.55, 110.97, 107.22, 107.07, 102.37, 80.07, 70.74, 60.09, 57.84, 56.89, 52.90, 51.54, 51.49, 47.15, 38.21, 32.21, 31.58, 31.51, 31.03, 30.94, 28.93, 27.17, 24.97, 24.85, 22.11, 19.52, 17.70, 10.71, 9.90, 9.88 HRMS (ESI+) *m/z* Calcd. For C54H64FN9O8S $[M+2H]^{2+}$: 508.7277; found 508.7286.

The absolute stereochemistry of the aminal carbon of **21** was determined to be "*S*" in a different synthetic scheme, by which **21** was prepared from (6*S*)-3-bromo-10-chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole, whose stereochemistry was determined by Vibrational Circular Dichroism (VCD) spectroscopy with confidence.

Methyl ((1S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(4-oxaspiro[2.5]octan-7-yl)ethyl)carbamate (22). Compound **22** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, DMSO-d_6) δ: 8.11 (s, 1 H), 8.04 (s, 1 H), 7.97 (s, 1 H), 7.70-7.68 (m, 1 H), 7.59-7.53 (m, 2 H), 7.45-7.42 (m, 1 H), 7.30 (d, *J* = 8 Hz, 1 H), 7.10 (s, 1 H), 6.55-6.54 (m, 1 H), 6.48 (s, 1 H), 5.14-5.08 (m, 2 H), 4.23-4.20 (m, 1 H), 4.12-4.08 (m, 1 H), 3.87-3.54 (m, 10 H), 3.37-3.31 (m, 2 H), 2.50-2.42 (m, 1 H), 2.41-2.29 (m, 1 H), 2.14-2.02 (m, 4 H), 1.99-1.96 (m, 5 H), 1.82 (m, 1 H), 1.43-1.23 (m, 3 H), 0.90-0.87 (m, 5 H), 0.86-0.75 (m, 3 H), 0.64-0.63 (m ,1 H), 0.52-0.51 (m, 2 H), 0.50-0.45 (m, 2 H), 0.08 (s, 1 H). HRMS (ESI+) *m/z* calcd for C54H61FN9O8S $[M+H]^+$, 1014.4348; found, 1014.4390.

Methyl ((1S)-1-(8-oxabicyclo[3.2.1]octan-3-yl)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (23). Compound **23** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, DMSO-d_6) δ: 8.34 (s, 1 H), 8.24-8.21 (m, 2 H), 8.00 (s, 1 H), 7.83-7.81 (m, 1 H), 7.78-7.76 (m, 2 H), 7.49 (s, 1 H), 7.26 (d, *J* = 8 Hz, 1 H), 7.25-7.23 (s, 1 H), 7.13 (s, 1 H), 5.17-5.13 (m, 2 H), 4.17-4.13 (m, 2 H), 4.11-4.00 (m, 2 H), 3.98-3.82 (m, 3 H), 3.61-3.47 (m, 6 H), 3.12-3.09 (m, 1 H), 2.49-2.38 (m, 2 H), 2.36-2.24 (m, 5 H), 2.20-2.18 (m, 1 H), 2.09- 2.00 (m, 2 H), 1.22-1.15 (m, 4 H), 1.14-1.12 (m, 8 H), 1.09-1.05 (m, 5 H), 0.83-0.81 (m, 3 H), 0.75-0.73 (m, 3H). HRMS (ESI+) *m/z* calcd for C54H61FN9O8S $[M+H]^+$, 1014.4348; found, 1014.4337.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(2,2,6,6-tetramethyltetrahydro-2H-pyran-4-yl)ethyl)carbamate (24). Compound **24** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.03 (s, 1 H), 7.56 (s, 1 H), 7.79 (s, 2 H), 7.54-7.52 (m, 1 H), 7.48-7.46 (m, 1 H), 7.43-7.37 (m, 1 H), 7.35-7.29 (m, 1 H), 7.17-7.16 (m, 1 H), 6.50-6.43 (m, 2 H), 5.26-5.18 (m, 2 H), 4.21-4.4.17 (m, 3 H), 4.16-4.15 (m, 1 H), 3.85 (s ,2 H), 3.65-3.64 (m, 6 H), 3.39-3.29 (m, 2 H),2.56-2.50 (m,2 H), 2.25-2.22 (m, 4 H), 2.16-2.14 (m, 3 H), 2.04-2.03 (m, 1 H), 1.94-1.93 (m, 1 H), 1.70-1.65 (m, 1 H), 1.24- 1.03 (m, 13 H), 0.92-0.87 (m, 8 H), 0.54-.053 (m, 2 H). HRMS (ESI+) *m/z* calcd for $C_{56}H_{67}FN_9O_8S$ $[M+H]^+$, 1044.4817; found, 1044.4840.

Methyl ((S)-2-((S)-2-(5-((S)-6-(chroman-7-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (25). Compound **25** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.08 (s, 1 H), 7.92 (s, 1 H), 7.76 (s, 1 H), 7.53-7.51 (m, 2 H), 7.35-7.27 (m, 3 H), 7.16 (s, 1 H), 6.91 (d, J = 8 Hz, 1 H), 6.43-6.41 (m, 1 H), 6.30 (s, 1 H), 5.25-5.19 (m, 2 H), 4.23 (s, 2 H), 4.22-3.89 (m, 7 H), 3.69-3.65 (m, 6 H), 3.38-3.35 (m, 2 H),2.66-2.49 (m, 5 H), 2.29-2.15 (m, 9 H), 1.87 (s, 2 H), 1.53-1.51 (m, 1 H), 1.12-1.10 (m, 6 H), 0.93-0.87 (m, 6 H). HRMS (ESI+) *m/z* calcd for $C_{56}H_{65}FN_9O_9$ $[M+H]^+$, 1026.4889; found, 1026.4894.

Methyl ((1S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((2S)-2-(5-((6S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(2-methylchroman-7-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (26). Compound **26** was prepared based on the same procedures as compound **21**.

^{1}H-NMR (400MHz, CD_3OD) δ: 8.08 (m, 1 H), 7.91 (m, 1 H), 7.81 (m, 1 H), 7.62 (m, 2 H), 7.40-7.18 (m, 4 H), 6.96 (m, 1 H), 6.48 (m, 1 H), 6.24 (m, 1 H), 5.21 (m, 1 H), 4.22-3.78 (m, 8 H), 3.66 (m, 6 H), 2.75-2.56 (m, 3 H), 2.38-1.82 (m, 9 H), 1.65 (m, 2 H), 1.32-0.86 (m, 21H). HRMS (ESI+) *m/z* calcd for C57H67FN9O9 $[M+H]^+$, 1040.5046; found, 1040.5046.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-6-(5-ethylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (27). Compound **27** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.03 (s, 1 H), 7.89 (s, 1 H), 7.82 (s, 1 H), 7.77 (s, 1 H), 7.52 (m, 2 H), 7.36 (m, 2 H), 7.17 (d, *J* = 7.2 Hz, 1 H), 6.54-6.48 (m, 2H), 5.22 (m, 2 H), 4.20-4.06 (m, 4 H), 3.85 (m, 2 H), 3.66 (m, 8 H), 2.64 (q, *J* = 8.0 Hz, 2 H), 2.52 (m, 2 H), 2.28-1.98 (m, 8 H), 1.54 (m, 1 H), 1.23-1.04 (m, 12 H), 0.89 (m, 6 H). HRMS (ESI+) *m/z* calcd for C53H63FN9O8S $[M+H]^+$, 1004.4504; found, 1004.4503.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-propylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (28). Compound **28** was prepared based on the same procedures as compound **21**. HRMS (ESI+) *m/z* calcd for C54H65FN9O8S $[M+H]^+$, 1018.4661; found, 1018.4680.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-1-fluoro-6-(5-isopropylthiophen-2-yl)-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (29). Compound **29** was prepared based on the same procedures as compound **21**.

^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (s, 1 H), 7.93 (s, 1 H), 7.85 (s, 1 H), 7.77 (s, 1 H), 7.52 (m, 2 H), 7.40-7.33 (m, 2 H), 7.22 (s, 1 H), 6.59-6.52 (m, 2 H), 5.22 (m, 2 H), 4.21-4.08 (m, 4 H), 3.87-3.65 (m, 10 H), 3.41 (m, 1 H), 3.02 (m, 1 H), 2.48 (m, 2 H), 2.28-2.04 (m, 9 H), 1.85 (m, 1 H), 1.63 (m, 1 H), 1.31-1.17 (m, 12 H), 0.88 (m, 6 H). HRMS (ESI+) *m/z* calcd for C54H65FN9O8S $[M+H]^+$, 1018.4661; found, 1018.4698.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (30). Compound **30** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.06 (s, 1 H), 7.96 (s, 1 H), 7.81-7.87 (m, 2 H), 7.57-7.51 (m, 2 H), 7.39-7.32 (m, 2 H), 7.19 (s, 1 H), 6.33 (s, 1 H), 5.21 (m, 2 H), 4.19-3.83 (m, 7 H), 3.64 (s, 6 H), 2.72 (m, 2 H), 2.52 (m, 4 H), 2.32-2.15 (m, 8 H), 1.58 (m, 1 H), 1.27-0.86 (m, 18 H). HRMS (ESI+) *m/z* calcd for C54H63FN9O8S $[M+H]^+$, 1016.4504; found, 1016.4545.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-6-(2-ethylthiazol-5-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (31). Compound **31** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (m, 2 H), 7.92 (1, 1 H), 7.76 (s, 1 H), 7.62 (m, 2 H), 7.43-7.31 (m, 2 H), 7.20 (m, 2 H), 5.22 (m, 2 H), 4.23-4.19 (m, 4 H), 3.88 (m, 2 H), 3.66 (m, 8 H), 3.42 (m, 1 H), 2.86 (q, J = 7.6 Hz, 2 H), 2.56 (m, 2 H), 2.27-2.16 (m, 8 H), 1.65 (m, 1 H), 1.22-1.14 (m, 11 H), 0.89 (m, 6 H). HRMS (ESI+) *m/z* calcd for C52H62FN10O8S $[M+H]^+$, 1005.4457; found, 1005.4467.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(2-propylthiazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (32). Compound **32** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.02 (m, 2 H), 7.91 (s, 1 H), 7.62 (s, 1 H), 7.59 (m, 2 H), 7.43-7.34 (m, 2 H), 7.22 (m, 2 H), 5.22 (m, 2 H), 4.23-4.19 (m, 4 H), 3.88 (m, 2 H), 3.66 (m, 8 H), 2.82 (m, 2 H), 2.55 (m, 2 H), 2.27-2.16 (m, 8 H), 1.64 (m, 3 H), 1.28-1.14 (m, 8 H), 0.93-0.85 (m, 10 H). HRMS (ESI+) *m/z* calcd for C53H64FN10O8S $[M+H]^+$, 1019.4613; found, 1019.4628.

Methyl ((S)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((S)-2-(5-((S)-1-fluoro-6-(2-isopropylthiazol-5-yl)-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (33). Compound **33** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.34 (s, 1 H), 8.21 (m, 1 H), 8.01 (s, 1 H), 7.83-7.70 (m, 3 H), 7.49 (s, 1 H), 7.24-7.13 (m, 2 H), 5.24 (m, 2 H), 4.17-3.98 (m, 4 H), 3.82-3.49 (m, 8 H), 3.12 (m, 1 H), 2.51 (m, 6 H), 2.38-2.02 (m, 9 H), 1.22-1.06 (m, 15 H), 0.83-0.73 (m, 5 H). HRMS (ESI+) *m/z* calcd for C53H64FN10O8S $[M+H]^+$, 1019.4613; found, 1019.4666.

Methyl ((S)-2-((S)-2-(5-((S)-6-(2-cyclopropylthiazol-5-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (34). Compound **34** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.80 (s, 2 H), 7.54- 7.43 (m, 3 H), 7.28 (m, 2 H), 7.12-6.90 (m, 3 H), 5.20 (m, 2 H), 4.23 (m, 2 H), 4.08 (m, 2 H), 3.89 (m, 2 H), 3.60 (s, 6 H), 2.45-1.92 (m, 12 H), 1.58 (m,

1 H), 1.27-0.84 (m, 20 H). HRMS (ESI+) *m/z* calcd for C53H62FN10O8S $[M+H]^+$, 1017.4457; found, 1017.4453.

Methyl ((S)-2-((S)-2-(5-((S)-6-(5,6-dihydro-4H-cyclopenta[d]thiazol-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-1-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-oxoethyl)carbamate (35). Compound **35** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 8.06 (s, 1 H), 7.96 (s, 1 H), 7.78 (m, 1 H), 7.53 (m, 2 H), 7.32 (m, 2 H), 7.19 (m, 1 H), 6.33 (s, 1 H), 5.22 (m, 2 H), 4.19-4.08 (m, 4 H), 3.85 (m, 2 H), 3.63 (m, 6 H), 2.72 (m, 2 H), 2.52 (m, 4 H), 2.32-1.92 (m, 9 H), 1.58 (m, 1 H), 1.27-0.86 (m, 18 H). HRMS (ESI+) *m/z* calcd for C53H62FN10O8S $[M+H]^+$, 1017.4457; found, 1017.4486.

Methyl ((S)-1-((S)-2-(5-((S)-3-(2-((S)-1-((S)-2-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-ethylthiophen-2-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (36). Compound **36** was prepared based on the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.99 (s, 1 H), 7.89 (m, 2 H), 7.81 (s, 1 H), 7.74 (s, 1 H), 7.51-7.41 (m, 2 H), 7.35-7.18 (m, 3 H), 6.49 (m, 2 H), 5.22 (m, 2 H), 4.26-4.08 (m, 4 H), 3.84 (m, 2 H), 3.67 (m, 7 H), 2.72-2.48 (m, 4 H), 2.18-2.02 (m, 8 H), 1.60 (m, 1 H), 1.27-1.04 (m, 12 H), 0.91 (m, 6 H). HRMS (ESI+) *m/z* calcd for C53H63FN9O8S $[M+H]^+$, 1004.4504; found, 1004.4541.

Methyl ((S)-1-((S)-2-(5-((S)-6-(5-cyclopropylthiophen-2-yl)-3-(2-((S)-1-((S)-2-((R)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (37). Compound **37** was prepared based on

the same procedures as compound **21**. ^{1}H-NMR (400MHz, CD_3OD) δ: 7.94 (s, 1 H), 7.87 (s, 1H), 7.71-7.68 (m, 2 H), 7.48-7.12 (m, 5 H), 6.51 (m, 1 H), 5.22 (m, 2 H), 4.21 (m, 2 H), 4.08 (m, 2 H), 3.84 (m, 2 H), 3.64 (m, 8 H), 2.52 (m, 2 H), 2.26-2.06 (m, 8 H), 1.82 (m, 1 H), 1.55 (m, 1 H), 1.21-0.86 (m, 18 H), 0.51 (m, 2 H). HRMS (ESI+) *m/z* calcd for C54H63FN9O8S $[M+H]^+$, 1016.4504; found, 1016.4510.

(*S*)-10-Bromo-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (40d). To a solution of 2-(5-bromoindolin-2-yl)-5-chloro-3-fluorophenol[16] (**38**, 10 g, 29 mmol) and 5-methylthiophene-2-carbaldehyde (**39a**, 4.4 g, 35 mmol) in MeCN (50 mL) was added TFA (990 mg, 8.7 mmol), and the mixture was stirred at 25 ℃ for 1 h. Solid appeared. The solid was collected by filtration, washed with MeCN, dried under vacuum to give 10-bromo-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole as white solid (11.0 g, 85%). To the solution of compound 10-bromo-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (11 g, 24.4 mmol) in dry toluene (100 mL) was added DDQ (8.3 g, 36.6 mmol). After refluxing for 2 hours under N_2, the reaction mixture was concentrated and diluted with EtOAc. The organic layer was washed with saturated $Na_2S_2O_3$ aqueous solution, brine, dried over Na_2SO_4, filtered, and concentrated. The crude mixture was washed with MeOH (20 mL) and filtered to give 10-bromo-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (10.0 g, 92%). LC/MS: Calcd. For C20H12BrClFNOS $[M+H]^+$: 450.0; found 450.0 10-Bromo-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (5.9 g) was separated by chiral SFC to give **40d** (2.9 g, 49% yield) and another isomer. LC/MS: Calcd. For $[M+H]^+$ C20H12BrClFNOS: 450.0; found 450.0. SFC conditions: Column, Chiralpak AS-H 150x4.6 mm I.D., 5 μm; Mobile phase, methanol (0.05% DEA) in CO_2 from5% to 40%; Flow rate, 3 mL/min; Wavelength: 220 nm.

(*S*)-3-Chloro-1-fluoro-6-(5-methylthiophen-2-yl)-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (41d). A suspension of **40d** (5.9 g, 13.1 mmol), bis(pinacolato)diboron (4 g, 15.8 mmol), KOAc (2.6 g, 26.2 mmol) and Pd(dppf)Cl_2 (0.48 g, 0.655 mmol) in dioxane (120 mL) was degassed and stirred at 100 °C under N_2 for 2 hours. The reaction mixture was cooled and concentrated. EtOAc and saturated aqueous $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was purified by chromatographed on silica gel (petroleum ether/ EtOAc from 100:1 to 20:1) to give **41d** (6.0 g, 92%). LC/MS: Calcd. For C26H25BClFNO3S $[M+H]^+$: 496.1; found 496.2.

Methyl ((*S*)-2-((*S*)-2-(5-((*S*)-3-chloro-1-fluoro-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (43d). A suspension of **41d** (3.5 g, 7.06 mmol), **42**[16] (3.2 g, 7.77 mmol), Na_2CO_3 (1.5 g, 14.12 mmol) and Pd(dppf)Cl_2 (258 mg, 0.35 mmol) in THF/H_2O (36 mL, 5:1) was degassed, filled with N_2, and stirred at 100°C under N_2 overnight. The reaction mixture was cooled and concentrated. EtOAc and saturated aqueous $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was purified by chromatographed on silica gel (petroleum ether/ethyl acetate = 1/5 to DCM/MeOH 50/1) to give **43d** (4.5 g, 90.6%). LC/MS: Calcd. For C36H36ClFN5O5S $[M+H]^+$: 704.2; found 704.2.

Methyl ((*S*)-2-((*S*)-2-(5-((*S*)-1-fluoro-6-(5-methylthiophen-2-yl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-2-oxo-1-(tetrahydro-2H-pyran-4-yl)ethyl)carbamate (44d). To a 50 mL flask was added **43d** (1.0 g, 1.42 mmol), bis(pinacolato)diboron (397 mg, 1.56 mmol), KOAc (278 mg, 2.84 mmol), $Pd_2(dba)_3$ (130 mg, 0.14 mmol), and X-Phos (135 mg, 0.284 mmol). The flask was degassed, filled with N_2, and dry dioxane (12 mL) was added. The mixture was stirred at 100 °C under N_2 for

overnight. The reaction mixture was cooled and concentrated. EtOAc and saturated aqueous $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was purified by chromatographed on silica gel (DCM/MeOH from 100:1 to 50:1) to afford the **44d** (0.79 g, 70%). LC/MS: Calcd. For C42H48BFN5O7S $[M+H]^+$: 796.3; found 796.4.

***tert*-Butyl (*S*)-2-(4-((*S*)-1-fluoro-10-(2-((*S*)-1-((*S*)-2-((methoxycarbonyl)amino)-2-(tetrahydro-2H-pyran-4-yl)acetyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)-6-(5-methylthiophen-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-3-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (46d).** A suspension of the **44d** (3.5 g, 4.4 mmol), *tert*-butyl (*S*)-2-(4-bromo-1H-imidazol-2-yl)pyrrolidine-1-carboxylate[16] (**45**, 1.7 g, 5.28 mmol), Na_2CO_3 (933 mg, 8.8 mmol) and $Pd(dppf)Cl_2$ (161 mg, 0.22 mmol) in THF/H_2O (48 mL, 5:1) was degassed and stirred at 100 °C under N_2 overnight. The reaction mixture was cooled and concentrated. EtOAc and saturated aqueous $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was purified by chromatographed on silica gel (DCM/MeOH from 100:1 to 50:1) to give the **46d** (2.1 g, 53%). LC/MS: Calcd. For C48H54FN8O7S $[M+H]^+$: 905.4; found 905.5.

(*R*)-2-methyltetrahydro-4H-pyran-4-one (47a). To a solution of **61** (180 g, 789 mmol) in chloroform (500 mL) was added Amberlyst 15 resin (100 g). The mixture was stirred at 25 ℃ for overnight. The mixture was filtered and the filtrate was concentrated to give crude **47a** which was used for the next step without further purification.

(2*R*,6*S*)-2,6-dimethyltetrahydro-4H-pyran-4-one (47c). To a solution of **63** (74.7 g, 0.57 mol) in DCM (750 mL) was added a solution of $NaHCO_3$ (4.83 g, 57 mmol) and KBr (6.84 g, 57 mmol) in water (200 mL). TEMPO (0.9 g, 5.7 mmol) was added. The mixture was treated at 0 ℃ under vigorous

stirring with aqueous NaClO solution (47.1 g, 0.63 mol, 5% ~ 7%) was added over a period of 1 hour. The reaction mixture was stirred at 25℃ for another 5 hours and the aqueous layer was extracted with DCM. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and the solvent evaporated to give **47c** as pale yellow oil (72.2 g, 99 %). ^{1}H NMR: ($CDCl_3$) δ: 3.75-3.70 (m, 2 H), 2.33 (d, *J* = 16 Hz, 2 H), 2.19 (t, *J* = 24 Hz, 2 H), 1.31 (d, *J* = 6 Hz, 6 H).

4-oxaspiro[2.5]octan-7-one (47h). To a solution of **67** (4.8 g, 20 mmol) in DCM (20 mL) was added Amberlyst 15 (2 g). The mixture was refluxed for 4 hours, filtered, and the filtrate was concentrated to give **47h** (2.5 g, 100%). ^{1}H NMR: ($CDCl_3$) δ: 3.86 (t, *J* = 5.6 Hz, 2 H), 2.43-2.46 (m, 2 H), 2.38 (s, 2 H), 0.74-0.76 (m, 2 H), 0.40-0.41 (m, 2 H).

8-oxabicyclo[3.2.1]octan-3-one (47i). To a solution of **69** (26.1 g, 93 mmol) in MeOH (30 mL) was added Zn powder (36.3 g, 558 mmol), CuCl (4.6 g, 46.5 mmol), and NH_4Cl (34.5 g, 0.64 mol) in MeOH (80 mL). The reaction mixture was maintained below 15 ℃ during reagent addition. The mixture was stirred at 25 ℃ for 19 hours. The reaction mixture was concentrated. EtOAc and saturated aqueous $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to give **47i** (3.5g, 30.4% yield). ^{1}H NMR: ($CDCl_3$) δ: 6.23 (s, 2 H), 5.00 (d, *J* = 5.2 Hz, 2 H), 2.73 (dd, *J* = 16.4, 4.8 Hz, 2 H), 2.32 (d, *J* = 16.4 Hz, 2 H).

Methyl 2-(((benzyloxy)carbonyl)amino)-2-(2,2-dimethyltetrahydro-4H-pyran-4-ylidene)acetate (49f). To a solution of **48** (1.163 g, 3.52 mmol) in dry DCM (20 mL) at 0 ^{o}C was added DBU (0.534 g, 3.52 mmol) followed by a solution of **47f** (1.8 g, 14.08 mmol) in dry DCM (20 mL) dropwise. The reaction mixture was stirred at 25 ^{o}C for 3 days. After removal of the solvent, the residue was purified by SiO_2 chromatography (petroleum ether/ ethyl acetate = 5:1 to 3:1) to afford **49f** as white solid (0.15

g, 13% yield). ^{1}H NMR ($CDCl_3$): δ 7.30-7.35 (m, 5 H), 5.11 (s, 2 H), 3.82-3.88 (m, 3 H), 3.09-3.16 (m, 2 H), 1.84 (s, 2 H), 1.48 (s, 2 H).

Methyl 2-amino-2-(2,2-dimethyltetrahydro-2H-pyran-4-yl)acetate (50f). To a solution of **49f** (3 g, 9.01 mmol) in MeOH (100 mL) was added Pd/C (0.6 g) carefully under N_2. The reaction mixture was stirred at 25 ^{o}C under H_2 (45 psi) for 3 hours. Pd/C was filtered and the solvent was removed to give **50f** as colorless oil (1.8 g, 99% yield). LC/MS: Calcd. For C10H20NFO3 $[M+H]^+$: 202.1; found: 202.1.

Methyl 2-(2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetate (51f). To a solution of **50f** (1.7 g, 8.46 mmol) in dry DCM (40 mL) was added DIPEA (1.65 g, 12.69 mmol) and methyl chloroformate (0.964 g, 10.15 mmol) dropwise at 0 ^{o}C. The reaction solution was stirred at 25 ^{o}C for 2 hours. Water and DCM was added. The organic phase was separated, washed with brine, dried over Na_2SO_4, and concentrated to give **51f** as colorless oil (1.9 g, 87% yield). LC/MS: Calcd. For C12H22NFO5 $[M+H]^+$: 260.1; found: 260.2.

(*S*)-2-((methoxycarbonyl)amino)-2-((2*R*,4*S*)-2-methyltetrahydro-2H-pyran-4-yl)acetic acid (52a). 2-((methoxycarbonyl)amino)-2-((2*R*)-2-methyltetrahydro-2H-pyran-4-yl)acetic acid (10 g, prepared by following a similar procedures as **52f**) was separated by SFC by the following conditions to give four diastereomers. **52a** (2 g, 20% yield) is the first eluted peak. LC/MS: Calcd. For C10H17NO5 $[M+H]^+$: 232.11; found 232.1. SFC conditions: Column, AS-H 250x4.0 mm, mobile phase, 35% MeOH (0.05% DEA) in CO_2. Flow rate: 2.35 mL/min. The absolute stereochemistry was assigned arbitrarily except the chiral center introduced from the starting material.

(*S*)-2-((methoxycarbonyl)amino)-2-((2*S*,4*S*)-2-methyltetrahydro-2H-pyran-4-yl)acetic acid (52b). 2-((methoxycarbonyl)amino)-2-((2*S*)-2-methyltetrahydro-2H-pyran-4-yl)acetic acid (prepared by following a similar procedures as **52f**) was separated by chiral SFC by using the same condition as **52a**. Four diastereomers were obtained and **52b** is the first eluted peak in SFC. The absolute stereochemistry was assigned arbitrarily except the chiral center introduced from the starting material.

(2*S*)-2-(*cis*-2,6-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (52c). 2-(cis-2,6-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (16 g, prepared by following a similar procedures as **52f**) was separated by SFC by the following conditions to give two diastereomers. **52c** (5.4 g, 34% yield) is the first eluted peak. LC/MS: Anal. Calcd. For $[M+H]^+$ C11H19NFO5: 246.1; found: 246.1. SFC conditions: Column: AY-5, 150×4.6 mm, 5 um, mobile phase, 5% to 40% MeOH (0.05% DEA) in CO_2, Flow rate: 2.5 mL/min. The absolute stereochemistry was assigned arbitrarily except the chiral center introduced from the starting material.

(*S*)-2-((2*R*,6*R*)-2,6-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (52d). 2-((2R,6R)-2,6-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (1.4 g, prepared by following a similar procedures as **52f**) was separated by SFC by the following conditions to give two diastereomers. **52d** (0.5 g, 35% yield) is the first eluted peak. LC/MS: Anal. Calcd. For $[M+H]^+$ C11H19NO5: 246.13; found 246.53. SFC conditions: Column, Chiralpak AS-H 250x4.0 mm, mobile phase, 5% to 40% MeOH (0.05% DEA) in CO_2. Flow rate: 2.35 mL/min. The absolute stereochemistry was assigned arbitrarily except the chiral center introduced from the starting material.

(2*S*)-2-(*cis*-2,6-diethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (52e). 2-(cis-2,6-diethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (2.5 g, prepared by

following a similar procedures as **52f**) was separated by SFC by the following conditions to give two diastereomers. **52e** (0.9 g, 36% yield) is the first eluted peak. LC/MS: Anal. Calcd. For $[M+H]^+$ C13H23NO5: 274.16; found 274.12. SFC conditions: Column, Chiralpak AD-H 150x4.6 mm, mobile phase, 5% to 40% MeOH (0.05% DEA) in CO_2. Flow rate: 2.5 mL/min.

Methyl 2-(((benzyloxy)carbonyl)amino)-2-(2,2-dimethyltetrahydro-4H-pyran-4-ylidene)acetate (52f) and (*S*)-2-((*R*)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid (52g). To a solution of **51f** (1.9 g, 7.34 mmol) in THF/H_2O (20 mL/4 mL) was added LiOH (0.264 g, 11.00 mL) and the reaction was stirred at 25 ^oC for 4 hours. 1 N HCl was added to adjust the pH value to 6. The aqueous layer was extracted by DCM. The organic phase was washed with brine, dried over Na_2SO_4. After removal of the solvent, the crude was purified by HPLC (C18 column, $CNCH_3$/water: 10% to 70%, with addition of 0.1% TFA) to give a mixture of four isomers as white solid (1.0 g, 56% yield). The mixture (1.0 g) was separated by SFC under the following condition to give **52f** (second peak eluted, 170 mg, 17% yield), LC/MS: calcd. For C11H20NFO5 $[M+H]^+$: 245.13; found: 246.1; **52g** (first peak eluted, 230 mg, 23% yield), LC/MS: calcd. For C11H20NFO5 $[M+H]^+$: 245.13; found: 246.1; and two other isomers. Instrument: Thar SFC; Column: AS-H, 150×4.6 mm, 5 μm; Mobile phase B: EtOH (0.05% DEA); gradient: 5% to 40% B.

The absolute stereochemistry of **52g** was determined to be (*S*)-2-((*R*)-2,2-dimethyltetrahydro-2H-pyran-4-yl)-2-((methoxycarbonyl)amino)acetic acid by Vibrational Circular Dichroism (VCD) spectroscopy with confidence.

(*S*)-2-((methoxycarbonyl)amino)-2-((*R*)-4-oxaspiro[2.5]octan-7-yl)acetic acid (52h). 2-((methoxycarbonyl)amino)-2-(4-oxaspiro[2.5]octan-7-yl)acetic acid (prepared from 47h by following a similar procedures as **52f**) was separated by chiral SFC by using the same condition as **52f**. Four

diastereomers were obtained and **52h** is the second eluted peak in SFC. The absolute stereochemistry was assigned arbitrarily.

2-(8-oxabicyclo[3.2.1]octan-3-yl)-2-((methoxycarbonyl)amino)acetic acid (52i). To a solution of **57i** (60 mg, 0.18 mmol) in MeOH (10 mL) was added Pd/C (10 mg, 0.03 mmol). The mixture was stirred at 25 ℃ under 40 psi of H_2 for 12 hours. The reaction mixture was filtered and the filtrate was concentrated to afford 2-(8-oxabicyclo[3.2.1]octan-3-yl)-2-((methoxycarbonyl)amino)acetic acid (40 mg, 93% yield). ^{1}H NMR (MeOD): δ 4.35 (s, 2 H), 3.95 (d, *J* = 4.4Hz, 1 H), 3.62 (s, 3 H), 1.85-1.95 (m, 2 H), 1.70-1.80 (m, 2 H), 1.39-1.60(m, 4 H), 1.23-1.35 (m, 1 H), 1. 16 (t, *J* = 7.2Hz,, 1 H). 2-(8-oxabicyclo[3.2.1]octan-3-yl)-2-((methoxycarbonyl)amino)acetic acid (40 mg) was separated by chiral SFC to give two isomers and **52i** is the first eluted peak. SFC conditions: Column: AY-5, 150×4.6 mm, 5 um, mobile phase, 5% to 40% MeOH (0.05% DEA) in CO_2, Flow rate: 2.5 mL/min. The absolute stereochemistry was assigned arbitrarily.

(*S*)-2-((methoxycarbonyl)amino)-2-(2,2,6,6-tetramethyltetrahydro-2H-pyran-4-yl)acetic acid (52j). Benzyl 2-((methoxycarbonyl)amino)-2-(2,2,6,6-tetramethyltetrahydro-2H-pyran-4-yl)acetate(**57j**, 250 mg, prepared from **47j** by following a similar procedures as **57i**) was separated by chiral SFC to give two isomers. SFC conditions: Column: Chiralpak AS-H 250×4.6mm I.D. Mobile phase: 5% to 40% ethanol (0.05% DEA) in CO_2; Flow rate: 2.35 mL/min. The second eluted peak (70 mg) in 20 mL MeOH was added 10 mg Pd/C and stirred at 25℃ for 16 hours under H_2 (50 psi). The mixture was filtered and the filtrate was concentrated in vacuo to give **52j** (52 mg, 99 % yield). LC/MS: Anal. Calcd. For $[M+H]^+$ C13H23NO5: 274.16; found:274.2.

8-Oxabicyclo[3.2.1]oct-6-en-3-yl 4-methylbenzenesulfonate (53i). To a solution of **47i** (700 mg, 5.64 mmol) in THF (20 mL) was added L-selectride (1 M in THF, 11.3 mL, 11.3 mmol) at -78 ℃ over 100

min. After the mixture was stirred under N_2 at -78 ℃ for 1 h, it was warmed to 25℃ for 12 hours. Then the mixture was cooled to 0℃, and NaOH (1N in water, 5 mL) and H_2O (5 mL) was added. After the mixture was stirred at 25 ℃ for another 1 hour, it was quenched with 3N HCl, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated to give 8-oxabicyclo[3.2.1]oct-6-en-3-ol (320 mg, 45.4% yield). ^{1}H NMR: ($CDCl_3$) δ: 6.42 (s, 2 H), 4.68 (d, *J* = 4.0 Hz, 2 H), 3.89-3.93 (m, 1 H), 2.24 (dd, *J* = 14.4, 4.0 Hz, 2 H), 1.64 (d, *J* = 14.4 Hz, 2 H). To a solution of 8-oxabicyclo[3.2.1]oct-6-en-3-ol (320 mg, 2.56 mmol) and pyridine (820 mg, 10.24 mmol) in DCM (20 mL) was added TsCl (973 mg, 5.12 mmol) at 0 ℃. The mixture was stirred under N_2 at 25 ℃ for 12 hours before it was poured into water and extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate. After filtration and concentration, the residue was purified by SiO2 column chromatography (Petroleum Ether: EtOAc = 5:1) to give **53i** (330 mg, 45.9% yield). ^{1}H NMR: ($CDCl_3$) δ: 7.67 (d, *J* = 8.0 Hz, 2 H), 7.25 (d, *J* = 8.0 Hz, 2 H), 6.18 (s, 2 H), 4.68-4.72 (m, 1 H), 4.57 (d, *J* = 4.0 Hz, 2 H), 2.38 (s, 3 H), 2.11 (dd, *J* = 15.6, 4.0 Hz, 2 H), 1.64 (d, *J* = 15.6 Hz, 2 H).

Benzyl 2-(8-oxabicyclo[3.2.1]oct-6-en-3-yl)-2-((diphenylmethylene)amino)acetate (55i). To a microwave tube was charged with **53i** (330 mg, 1.18 mmol) and benzyl 2-(diphenylmethyleneamino) acetate (**54**, 466 mg, 1.4 mmol) in toluene (15 mL) was added LiHMDS (1.5 mL, 1.5 mmol) dropwise under N_2. The mixture was stirred at 100 ℃ under microwave for 4 hours. It was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate. After filtration and concentration, the residue was purified by SiO_2 column (Petroleum Ether: EtOAc =5:1) to give **55i** (330 mg, 65% yield). LC/MS: Calcd. For C29H28NO3 $[M+H]^+$:438.2; found:438.2.

Benzyl 2-amino-2-(8-oxabicyclo[3.2.1]oct-6-en-3-yl)acetate (56i). To a solution of **55i** (330 mg, 0.75 mmol) in THF (20 mL) was added 2N HCl (5 mL) at 0 ℃. The mixture was stirred under N_2 at 25 ℃

for 2 hours before it was poured into a saturated aqueous $NaHCO_3$ solution and extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentration to give **56i** (150 mg, 72.8% yield). LC/MS: Calcd. For C16H20NO3 $[M+H]^+$: 274.1; found:274.1.

Benzyl 2-(8-oxabicyclo[3.2.1]oct-6-en-3-yl)-2-((methoxycarbonyl)amino)acetate (57i). To a solution of **56i** (150 mg, 0.55 mmol) and Et_3N (221 mg, 2.2 mmol) in DCM (10 mL) was added MocCl (96 mg, 0.95 mmol) at 0 ℃. The mixture was stirred under N_2 at 25 ℃ for 12 hours before it was poured into water and extracted with DCM. The organic layer was washed with brine, dried over sodium sulfate. After filtration and concentration, the residue was purified by SiO_2 column (eluting with Petroleum Ether: EtOAc =1:1) to give **57i** (60 mg, 60% yield). LC/MS: Calcd. For C18H22NO5 $[M+H]^+$: 332.1; found:332.1.

Methyl (*R*)-3-((tert-butyldimethylsilyl)oxy)butanoate (59). Methyl (*R*)-3-hydroxybutanoate **(58**, 174 g, 1475 mmol) and imidazole (109 g, 1622 mmol) were dissolved in ethyl acetate (1 L). After cooling to 0 ^oC, tert-butyl chlorodimethylsilane (242 g, 1662 mmol) in ethyl acetate (300 mL) was added dropwise and kept the internal temperature below 10 ^oC. After stirring the thick suspension at 25 ^oC for 16 hours, water (800 mL) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (450 mL) one more time. The combined organic layers were washed with brine (450 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give **59** as colorless oil (322 g, 95 % yield). ^1H NMR ($CDCl_3$) δ: 4.28-4.13 (m, 1H), 3.60 (s, 3H), 2.47-2.36 (m, 1H), 2.35-2.28 (m, 1H), 1.13 (d, J = 6.0 Hz, 3H), 0.80 (s, 9H), 0.00 (d, J = 9.5 Hz, 6H).

(*R*)-3-((tert-butyldimethylsilyl)oxy)-N-methoxy-N-methylbutanamide (60). Compound **59** (322 g, 1.4 mol) was dissolved in THF (250 mL) and N, O-dimethyl hydroxylamine hydrochloride salt (155 g, 1.6 mol) was added. After cooling the slurry to -20 ℃, a solution of isopropyl magnesium chloride in

THF (2.0 M, 1.6 L, 3.2 mol) was added dropwise over 1 hour, maintaining the reaction temperature below 0℃. The reaction was stirred at -20℃ for 2 additional hour. The reaction was quenched with saturated aqueous ammonium chloride (2 L) and extracted with ethyl acetate (500 mLx2). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by SiO_2 chromatography elution with petroleum ether/ethyl acetate = 20:1 to afford **60** as colorless oil (260 g, 72% yield). ^{1}H NMR (CDCl3)δ: 4.39-4.24 (m, 1H), 3.66 (s, 3H), 3.13 (s, 3H), 2.73 (m, 1H), 2.31 (dd, *J* = 4, 12 Hz, 1H), 1.17 (d, *J* = 8 Hz, 3H), 0.82 (s, 9H), 0.01 (d, *J* = 12 Hz, 6H)

(*R*)-5-((tert-butyldimethylsilyl)oxy)hex-1-en-3-one (61). To a solution of vinyl magnesium bromide in THF (1.0 M, 1.6 L, 1.6 mol) at 0 ℃ under nitrogen atmosphere was added **60** (260 g, 1 mol) dropwise over 30 minutes, maintaining the reaction temperature below 5 ℃. The reaction was stirred between 0 to 5℃ for 1 additional hour, then was poured into a stirred mixture of ice, saturated aqueous citric acid (1 L), and ammonium chloride (1000 mL). The layers were separated, the aqueous layer was extracted with ethyl acetate (1 L). The combined organic layers were washed with saturated aqueous sodium carbonate and brine, then dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by SiO_2 chromatography, eluting with a 1:100 mixture of ethyl acetate and petroleum ether to provide **61** as colorless oil (180 g, 79% yield). ^{1}H NMR ($CDCl_3$) 6.36 (dd, J_1 = 17.7, J_2 = 10.5 Hz, 1H), 6.22 (dd, J_1 = 17.7, J_2 = 1.3 Hz, 1H), 5.85 (dd, J_1 = 10.5, J_2 = 1.3 Hz, 1H), 4.37-4.33 (m, 1H), 2.85 (dd, J_1 = 14.9, J_2 =7.2 Hz, 1H), 2.54 (dd, J_1 = 14.9, J_2 = 5.4 Hz, 1H), 1.20 (d, *J* = 6.2 Hz, 3H), 0.86 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H).

(*Cis*)-2,6-dimethyltetrahydro-2H-pyran-4-ol (63). To a solution of 2,6-dimethyl-4H-pyran-4-one (**62**, 73 g, 0.59 mol) in ethanol was added Pd/C (10%, 4 g) and the reaction was stirred at 35 ℃ under H_2 (50 psi) for 17 hours. The reaction was filtered through celite and the volatiles were removed in

vacuo to afford **63** (76 g, 99% yield). ^{1}H NMR: ($CDCl_3$) δ: 3.75 (s, 1 H), 3.44-3.40 (m, 2 H), 1.88 (d, *J* = 16 Hz, 2 H), 1.19 (d, *J* = 8 Hz, 6 H), 1.14-1.08 (m, 2 H).

1-(2,2-dimethoxyethyl)cyclopropan-1-ol (65). To a solution of methyl 3,3-dimethoxypropanoate (**64**, 100 g, 0.52 mol) in THF/Et_2O (600/600 mL) was added Titanium isopropoxide (29 g, 0.1 mol) at 0 ℃ with stirring. After stirring for 10 minutes, ethyl magnesium bromide (434 mL, 3M in Et_2O, 1.3 mol) was added dropwise at 10~15℃. After addition complete, the solution was stirred at 25℃ for 30 minutes. The reaction was quenched with 6 mL of water. The mixture was filtered. The filtrate was concentrated in vacuum. The residue was purified by column chromatography (Petroleum Ether / EtOAc = 20:1- 4:1) to give **65** (80 g, 89% yield). ^{1}H NMR: ($CDCl_3$) δ: 4.61 (t, *J* = 6.0 Hz, 1 H), 3.32 (s, 6 H), 1.81 (d, *J* = 6.0 Hz, 2 H), 0.69-0.70 (m, 2 H), 0.37-0.38 (m, 2 H).

2-(1-((tert-butyldimethylsilyl)oxy)cyclopropyl)acetaldehyde (66). To a solution of **65** (80 g, 0.46 mol) in dichloromethane (600 mL) was added 2,6-lutidine (246 g, 2.3 mol), TBSOTf (121 g, 0.46 mol) at 10 ℃. The reaction was stirred at 10 ℃ for 30 minutes, then TMSOTf (153 g, 0.7 mol) was added. The reaction was stirred at 10 ℃ for 1 more hour, it was poured into water, extracted with ethyl acetate (3 times). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by column (Petroleum Ether / EtOAc = 100:1- 20:1) to give **66** (40 g, 41% yield). ^{1}H NMR: ($CDCl_3$) δ:9.84 (t, *J* = 2.8 Hz, 1H), 2.37 (d, *J* = 2.8 Hz, 2H), 0.79 (m, 2H), 0.77 (s, 9H), 0.52 (m, 2H), 0.00 (s, 6H).

1-(1-((tert-butyldimethylsilyl)oxy)cyclopropyl)but-3-en-2-ol (67). To a solution of **66** (40 g, 180 mmol) in THF (400 mL) was added vinyl magnesium bromide (175 mL, 1.6 M in THF, 280 mmol) at -78 ℃. After the reaction was stirred at -78 ℃ for 30 minutes, it was quenched with aqueous NH_4Cl and extracted with ethyl acetate (3 times). The combined organic phases were dried over anhydrous

Na_2SO_4, filtered, and concentrated. The residue was purified by SiO_2 column (Petroleum Ether / EtOAc, 10:1- 5:1) to give **67** (20 g, 41% yield). LC/MS: Calcd. For C13H27O2Si $[M+H]^+$: 243.2; found: 243.3.

1-(1-((tert-butyldimethylsilyl)oxy)cyclopropyl)but-3-en-2-one (68). To a solution of **67** (20 g, 82 mmol) in acetonitrile (200 mL) was added IBX (46 g, 165 mmol) at 25℃. The mixture was stirred at 80 ℃ for 2 hours, filtered, and concentrated. The crude product was purified by SiO_2 column chromatography chromatography (300 g, EtOAc/Hexane 0% to 50%) give **68** (10 g, 50% yield). ^{1}H NMR: ($CDCl_3$) δ: 6.59 (dd, *J* = 16.0, 9.6 Hz, 1 H), 6.19 (d, *J* = 16.0 Hz, 1 H), 5.68 (d, *J* = 9.6 Hz, 1 H), 2.64 (s, 2 H), 0.73-0.74 (m, 2 H), 0.73 (s, 9 H), 0.52-0.53 (m, 2 H), 0.07 (s, 6 H).

2,4-dibromo-8-oxabicyclo[3.2.1]oct-6-en-3-one (69). To a solution of furan (1.3 g, 18.77 mmol) and Zn powder (2 g, 31 mmol) in THF (3 mL) was added 1,1,3,3-tetrabromopropan-2-one (10.5 g, 28 mmol) and triethyl borate (5.48 g, 38 mmol) dropwise at 25 ℃ during 1 hour period in the dark. The resulting dark brown mixture was stirred at 25 ℃ for 17 hours. The mixture was cooled to -15 and H_2O (30 mL) was added. The mixture was stirred at 25 ^{o}C for 30 min and extracted with EtOAc. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated to give **69** (26.1 g, 100% yield) which was used without further purification.

10-Bromo-3-chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (70). To a mixture of **39h** (2.7 g, 17.5 mmol) and **38** (4.0 g, 11.7 mmol) in anhydrous CH_3CN (50 mL) was added TFA (1 mL). The mixture was stirred at 20 ℃ for 6 hours. The reaction mixture became a clear solution and then a solid appeared. The solid was collected by filtration and washed with CH_3CN to give 10-bromo-3-chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (4.5 g, 81%). ^{1}H NMR ($CDCl_3$) δ: 7.21 (s, 2 H), 6.52 - 6.78 (m, 6

H), 5.07 (d, *J* = 9.6 Hz, 1H), 3.50 (dd, *J* = 16.4, 9.2 Hz, 1 H), 3.19 (d, *J* = 16.4 Hz, 1 H), 1.91 - 1.96 (m, 1 H), 0.88 - 0.93 (m, 2 H), 0.63 - 0.65 (m, 2 H). To a solution of 10-bromo-3-chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-12,12a-dihydro-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (4.5 g, 9.4 mmol) in dry toluene (50 mL) was added DDQ (3.2 g, 14.2 mmol). After the reaction mixture was stirred under refluxing for 2 hours, it was concentrated and diluted with EtOAc. The organic layer was washed with saturated aqueous Na_2SO_3 and brine, dried over Na_2SO_4, filtered and concentrated. The residue was washed with MeOH (20 mL) to give a **70** (4.0 g, 88%). LC/MS: Calcd. For C22H15BrClFNOS $[M+H]^+$: 476.0; found:476.1.

3-Chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indole (71). A degassed suspension of **70** (4.0 g, 8.4 mmol), bis(pinacolato)diboron (2.6 g, 10.1 mmol), KOAc (2.1 g, 21.1 mmol) and Pd(dppf)Cl_2 (310 mg, 0.42 mmol) in dioxane (50 mL) was stirred at 100 °C for 2 hours under N_2. The reaction mixture was cooled and concentrated in vacuum, and the residue was purified by SiO_2 chromatography (80 g, EtOAc/Hexane 0% to 5%) to give **71** (4.0 g, 88% yield). LC/MS: Calcd. For C28H27BClFNO3S $[M+H]^+$: 522.1; found:522.2.

tert-Butyl (2*S*)-2-(5-(3-chloro-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (72). A degassed suspension of **71** (4.4 g, 8.4 mmol), **45** (3.2 g, 10.2 mmol), Na_2CO_3 (2.2 g, 21.0 mmol) and Pd(dppf)Cl_2 (310 mg, 0.42 mmol) in THF/H_2O (v/v=5/1, 120 mL) was stirred at 80 °C overnight under N_2. After cooling down, water was added and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. After concentrated under vacuum, the residue was purified by SiO_2 chromatography (80 g, EtOAc/Hexane,

10% to 50%) to give **72** (4.5 g, 77%). LC/MS: Calcd. For $C_{34}H_{33}ClFN_4O_3S$ $[M+H]^+$: 631.2; found 631.3.

tert-Butyl (2*S*)-2-(5-(6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (73). To a degassed mixture of **72** (4.5 g, 6.5 mmol), bis(pinacolato)diboron (2.0 g, 7.8 mmol), KOAc (1.6 g, 16.3 mmol), $Pd_2(dba)_3$ (338 mg, 0.33 mmol), X-Phos (312 mg 0.65 mmol) was added dry dioxane under N_2. The mixture was stirred at 100 ^oC overnight. After cooling to 20 ℃, the reaction mixture was filtered and concentrated in vacuum. The residue was purified by SiO_2 chromatography (3 g, Hexane/EtOAc, 20% to 50%) to give **73** (4.6 g, 89%). LC/MS: Calcd. For $C_{40}H_{45}BFN_4O_5S$ $[M+H]^+$: 723.3; found 723.5.

tert-Butyl (*S*)-2-(5-((*S*)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((*S*)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-4-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (75). A suspension of **73** (4.2 g, 5.8 mmol), **74**[16] (3.25 g, 8.7 mmol), Na_2CO_3 (1.5 g, 14.5 mmol) and $Pd(dppf)Cl_2$ (222 mg, 0.29 mmol) in THF/H_2O (72 mL, 5:1) was stirred at 100°C under N_2 protection overnight. After filtration, the filtrate was washed with water (50 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with brine and dried over Na_2SO_4. After filtration and concentration, the residue was purified by SiO2 chromatography (2 g, MeOH/DCM 0% to 20%) to give tert-butyl (2S)-2-(5-(6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-4-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (3.8 g, 74%). LC/MS: Calcd. For $C_{48}H_{54}FN_8O_6S$ $[M+H]^+$: 889.4; found 889.4. tert-Butyl (2S)-2-(5-(6-(5-cyclopropylthiophen-2-yl)-1-fluoro-3-(2-((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-4-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-10-yl)-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (3.8 g) was

separated by SFC by using the following conditions to give **75** (1.0 g, 26.3%). Column: Chiralpak AS-H 250×4.6mm I.D., 5um; Mobile phase: 40% of iso-propanol (0.05% DEA) in CO_2.

methyl ((*S*)-1-((*S*)-2-(4-((*S*)-6-(5-cyclopropylthiophen-2-yl)-1-fluoro-10-(2-((S)-pyrrolidin-2-yl)-1H-imidazol-5-yl)-6H-benzo[5,6][1,3]oxazino[3,4-a]indol-3-yl)-1H-imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (76). To a solution of **75** (1.0 g, 1.1 mmol) in dry dioxane (10 mL) was added HCl-dioxane (20 mL) through syringe and the reaction mixture was stirred at 20℃ for 2 hours. It was concentrated and dried under high vacuum to give the HCl salt of **76** (0.9 g, 98%. It was assumed that **76** formed 3HCl salt. The actual number of HCl salt was not determined). LC/MS: Calcd. For $C_{43}H_{46}FN_8O_4S$ $[M+H]^+$: 789.3; found 789.5.

Supporting Information

^{1}H NMR spectra of compounds **1**−**27** and **29**−**37**

^{13}C NMR spectra of compound **21**

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Abbreviations used

BOP ((Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate), DEA (diethyl amine), DIAD (Diisopropyl azodicarboxylate), DIEA or DIPEA (N, N-diisopropylethylamine), EtOAc or EA (ethyl acetate), HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate), IPA (isopropyl alcohol), MeOH (methanol), mpk (mg per kg), PE (Petroleum ether), PIFA ([Bis(trifluoroacetoxy)iodo]benzene), GT1a (genotype 1 subtype a), GT1b (genotype 1 subtype b), GT2a (genotype 2 subtype a), GT2b (genotype 2 subtype), GT3a (Genotype 3

subtype a), GT4 –(genotype 4 subtype a), GT1a Y93H (genotype 1 subtype a with a tyrosine (Y) to histidine (H) amino acid substitution at position 93), GT1a L31V (genotype 1 subtype a with a leucine (L) to valine (V) amino acid substitution at position 31).

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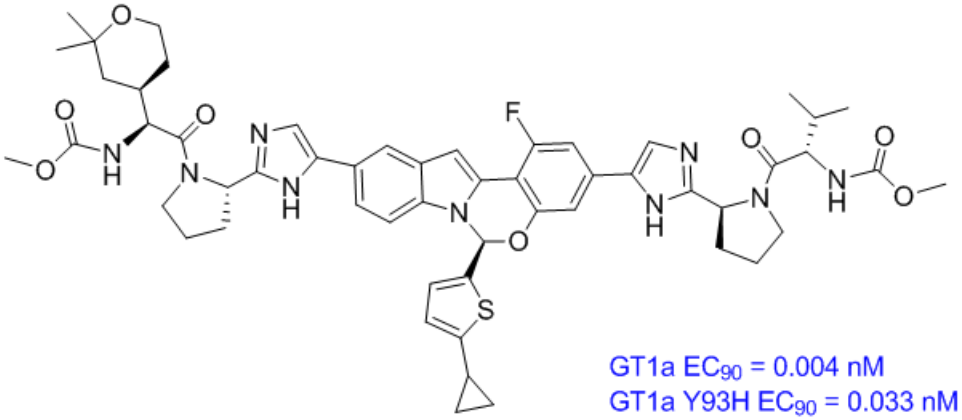
Table of Contents graphic



21
(MK-6169)

GT1a EC_{90} = 0.004 nM
GT1a Y93H EC_{90} = 0.033 nM
GT1a L31V EC_{90} = 0.004 nM
GT2b L31M EC_{90} = 0.018 nM