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Graphical Abstract



A series of 3-substituted-1,4-diaryl-2-azetidinones were asymmetrically synthesized and SARs revealed that the stereochemistry were critically important for the antiproliferative activity.

Design, Synthesis, Biological Evaluation and Cocrystal Structures with Tubulin of Chiral β -Lactam Bridged Combretastatin A-4 Analogues as Potent Antitumor Agents

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ABSTRACT

A diverse of chiral β -lactam bridged analogues of combretastatin A-4 (CA-4), 3-substituted 1,4-diaryl-2-azetidinones, were asymmetrically synthesized and biologically evaluated, leading to identify a number of potent anti-proliferative compounds represented by **14b** and **14c** with IC₅₀ values of 0.001-0.021 μ M, against four human cancer cell lines (A2780, Hela,

SKOV-3 and MDA-MB-231). Structure-activity relationship (SAR) studies on all stereoisomers of **14b** and **14c** revealed that the absolute configurations of the chiral centers at 3- and 4-position were critically important for the activity and generally a *trans* configuration between the "A" and "B" rings is optimal. In addition, **14b** and **14c** displayed less cytotoxicity on normal human oviduct epithelial cells than malignant cells indicating good selectivity in vitro. Further biochemical evaluation and cocrystal structures with tubulin demonstrated that both compounds disrupted tubulin polymerization through interacting at the colchicine-binding site, suppressed angiogenesis in vitro and in vivo, blocked cell cycle progression at mitotic phase and induced cellular apoptosis. The in vivo assays verified that both compounds inhibited xenograft tumor growth in nude mice with acceptable therapeutic window, showing promising potentials for further clinical development.

Keywords: 1,4-diaryl-2-azetidinone; combretastatin A-4; tubulin polymerization; antitumor

1. Introduction

Microtubule is an important component of cytoskeleton, which is polymerized by α - and β -subunit. Microtubule plays a role of separating the daughter chromosomes to the opposite poles during the mitosis. The disruption of microtubule will result in the interruption of mitosis and lead to the apoptosis of cell [1]. After Colchicine is the first discovered compound which binds to the dimer and destabilizes microtubule [2], Combretastatin A-4 (CA-4, 1, Figure 1) is a natural product isolated from *Combretum caffrum* which can bind to the colchicine binding-site, and shows extremely strong depolymerization of microtubule in vitro [3]. Its water soluble phosphate prodrug CA-4P (2), is now in phase III clinical trials [4]. Since the *cis*-double bond is critically important for the activity of **1** and the spontaneous isomerization from cis-isomer to trans-isomer leads to its chemical instability [5], a large number of conformationally restricted analogues of CA-4 have been reported, in which the *cis* double bond is replaced by a heterocycle [6]. Among them, β -lactam bridged analogues of CA-4 are the most attractive and a number of substituted 1,4-diaryl-2-azetidinones have been reported with significant suppressions on tubulin polymerization and cellular proliferation [7]. Due to two chiral carbon atoms of azetidin-2-one scaffold and the existence of two pairs of enantiomers, accumulating studies have been focused on the relationship between stereo-chemical structure and anticancer activity. The trans-form of C-3 and C-4 turned out to be favor by the work of Meegan and Coccetti groups [7d,7f]. But the absolute configurations of chiral centers remained unclear until compounds 3/4 were synthesized asymmetrically by our group and firstly identified the favorable orientation of phenyl at 4-position on β -lactam ring which demonstrated critically important for the antiproliferative

activity [8]. In this study, we would like to report a series of optically pure β -lactam linked CA-4 analogues to define their SARs systematically, especially the influence of the absolute configurations of C-3 and C-4 on the activities.



Figure 1. Small molecules that interact with tubulin.

2. Results and discussion

2.1 Chemistry

A series of chiral 3-methylene-2-azetidinones were synthesized asymmetrically according to literature method [9] (Scheme 1). Racemic MBH adducts 6 were obtained by Morita-Baylis-Hillman reaction of corresponding aromatic aldehydes 5 with ethyl acrylate in the presence of DABCO in 3-14 days, depending on the reactivity of the carbonyl group of aldehydes. After protecting the hydroxyl groups of 6 with acetic anhydride, 7 were submitted to the asymmetric allylic amination using aromatic amines 8 as the nucleophiles under the catalysis of $Pd_2(dba)_3$ and 9a, affording the corresponding optically active amination products 10 in high yields (up to 94%) and enantioselectivities (up to 99% *ee*). Finally 10 can be readily transformed into their corresponding β -lactam derivatives 11a-r in good yields (37-92%) without loss of enantioselectivity by reaction with $Sn[N(TMS)_2]_2$ in refluxing toluene.

Scheme 1. Synthesis of (S)-1,4-Diaryl-3-methylene-2-azetidinones 3 and 11a-r^a



^{*a*}Reagents and conditions: (a) DABCO, ethyl acrylate, MeOH, 25 °C, 3-14 days, 37-99%; (b) Ac₂O, Et₃N, CH₂Cl₂, 0 °C, 1 h, 37-91%; (c) i) Zn, AcOH, MeOH, reflux, 30 min; ii) Boc₂O, THF, reflux, 16 h, 41% for 2 steps; (d) Pd₂(dba)₃ (1%), **8a~e** (1.5 equiv), **9a** (2.5%), K₂CO₃ (aq.), CH₂Cl₂, 25 °C, 3 h, 52-94%; (e) Sn[N(TMS)₂]₂, toluene, reflux, 3 h, 37-92%, 95-99% ee; (f) Et₃N, acryloyl chloride, CH₂Cl₂, 25 °C, 1 h, 76%; (g) TBAF, THF, 0 °C, 1 h, 81-90%.

Previous research has indicated clearly that the C-3 substituent is relatively flexible [6b,7a,7c,7d,8,10]. With 3-methylene-2-azetidinones in hand, modifications of 3-methylene group allowed us to further examine the role of 3-substituent as well as the *cis/trans/*absolute configurations of azetidin-2-ones on their antiproliferative effects. Accordingly, a diversity of derivatives of **3** were designed and synthesized, and these transformations could be readily realized with 3-methylene as latent functional group (**Schemes 2-4**).

Various hydroxyl-protected compounds **12a-h** were obtained by acylation or alkylation of **3** with corresponding acyl chlorides or alkyl halides in good yields (**Scheme 2**). Then olefin cross-metathesis of **3** were performed under the catalysis of Grubbs II to give 3-benzylidene substituted compounds **13a-b**, and the newly formed C=C bonds were identified as *Z* configurations as indicated by NOESY. The Michael additions of **3** or 11g with corresponding nucleophiles afforded 3-hydroxymethyl or aminomethyl substituted target compounds **14a-j**. The *trans*-diastereoisomer of **4**, **14c** and **14g** could be obtained by bromination of **14b** or **14e** followed by hydrogenation under the catalysis of Pd/C in the presence of AcONa.

Scheme 2. Synthesis of Azetidinones 12-14^{*a*}



^{*a*}Reagents and conditions: (a) acyl chlorides, anhydrides or alkyl halides, Et₃N or K₂CO₃, CH₂Cl₂, 78-97%; (b) styrene or 1-(*tert*-butyl)-4-vinylbenzene (2.5 equiv), Grubbs II (15% equiv), 1,2-dichloroethane, reflux, 48 h, 30-51%; (c) for **14a** and **14b**: i) B₂(pin)₂ (1.3 equiv), CuCl (5%), MeOH (1.5 equiv), PPh₃ (15%), *t*-BuOLi (10%), THF, 25 °C, 12 h; ii) NaBO₃·4H₂O, H₂O, THF, 25 °C, 2 h, 46% and 21% for 2 steps; for **14d** and **14e**: i) B₂(pin)₂ (1.3 equiv), CuCl (5%), MeOH (1.5 equiv), PPh₃ (15%), *t*-BuOLi (10%), THF, 25 °C, 12 h; ii) NaBO₃·4H₂O, H₂O, THF, 25 °C, 2 h, 42% and 17% for 2 steps; for **14f**: Pd/C, H₂, EtOH, 25 °C, 12 h, 89%; for **14h**: MeONa, MeOH, 25 °C, 12 h, 66%; for **14i**: Me₂NH·HCl, Et₃N, MeOH, reflux, 6 h, 75%; for **14j**: BnNH₂, EtOH, reflux, 12 h, 30%; (d) i) BnCl, K₂CO₃, MeCN, reflux, 12 h; ii) CBr₄, PPh₃, THF, 25 °C, 6 h; iii) Pd/C, AcONa, H₂, EtOH, 25 °C, 12 h; iv) TFA, DCM, 25 °C, 1 h, 18% for 4 steps.

In order to further diversify the substitution of C-3, the dihydroxylation of **12e** proceeded under the catalysis of $K_2OsO_4 \cdot 2H_2O$ with 4-methylmorpholine *N*-oxide (NMO) as the oxidant, yielding 3-hydroxy-3-hydroxymethyl substituted compound **15**, whose configuration was determined by NOESY (**Scheme 3**). The direct catalytic hydrogenolysis or methylation followed by catalytic hydrogenolysis of **15** provided products **16a-b**. Oxidation with NaIO₄ and subsequent reduction with NaBH₄ of **15** afforded *cis*-(3*R*,4*S*)-3-hydroxy substituted

analogue **17**, whose configuration was determined as indicated by ¹H NMR. The alkylation or esterification followed by the debenzylation of **17** produced 3-alkoxy or 3-acyloxy substituted analogues **18a-b**. The sulfonylation of **17** with mesyl chloride gave 3-mesyloxy substituted product **19** in 86% yield.

OMe OMe OMe OMe MeO MeO MeO MeC MeO MeO MeC MeC а С е OH ЮH ΌMs ОH MeC MeO MeC MeC ÒBn ÒBn ÒBn ÒBn 12e 15 19 17 ļЬ d OMe OMe MeO MeC MeC MeO OR ÓR OR MeO MeC ÔН ÔН 18a, R = Me 16a, R = H 16b, R = Me 18b, R= COCH₂CH₂COOH

Scheme 3. Synthesis of Azetidinones 15-19^{*a*}

^{*a*}Reagents and conditions: (a) $K_2OsO_4 \cdot 2H_2O$ (3%), NMO (50%, aq.), acetone, H₂O, 25 °C, 12 h, 92%; (b) for **16a**: Pd/C, H₂, EtOH, 25 °C, 12 h, 96%; for **16b**: i) Me₂SO₄, K₂CO₃, acetone, reflux, 6 h; ii) Pd/C, H₂, EtOH, 25 °C, 12 h, 73% for 2 steps; (c) i) NaIO₄, MeOH, H₂O, 25 °C, 4 h; ii) NaBH₄, MeOH, 0 °C, 1 h, 85% for 2 steps; (d) for **18a**: i) Me₂SO₄, K₂CO₃, acetone, reflux, 6 h; ii) Pd/C, H₂, EtOH, 25 °C, 12 h, 82% for 2 steps; for **18b**: i) Succinic anhydride (1.2 equiv), DIPEA, DMAP, CH₂Cl₂, 25 °C, 6 h; ii) Pd/C, H₂, EtOH, 25 °C, 12 h, 86%.

Nucleophilic substitution of **19** with tetrabutylammonium bromide (TBAB) under the condition of microwave yielded trans-(3S,4S)-3-bromo substituted product **20** and its

configuration was identified as indicated by ¹H NMR (Scheme 4). Debenzylation of 20 with H_2 catalyzed by Pd/C resulted in the expected product 21 and the further debrominated product 22 in the yields of 62% and 28%, respectively. Nucleophilic substitution of 19 with NaN₃ at 120 °C in DMF provided *trans*-(3*S*,4*S*)-3-azido substituted product 23, whose configuration was confirmed by ¹H NMR. Then 23 was submitted to azide-alkyne click reaction with propargyl alcohol afforded 3-(1,2,3-triazol-1-yl) substituted analogue 24. Reduction of 23 with SnCl₂ produced *trans*-(3*S*,4*S*)-3-amino substituted compound 25. Derivatization of the amino of 25 with acid anhydride or acyl chloride gave the 3-acylamino or 3-sulphonylamino substituted analogues 26a-c.





^{*a*}Reagents and conditions: (a) TBAB (3.5 equiv), DMF, microwave, 170 °C, 6 h, 42%; (b) Pd/C, H₂, EtOH, 25 °C, 8 h, 62% for **21** and 28% for **22**; (c) NaN₃ (1.5 equiv), DMF, 120 °C, 24 h, 53%; (d) i) CuSO₄· 5H₂O (5%), *L*-ascorbic acid sodium salt (15%), propargyl alcohol (1.1 equiv), EtOH, H₂O, 25 °C, 8 h; ii) Pd/C, H₂, EtOH, 25 °C, 12 h, 93%; (e) SnCl₂ (3

equiv), 9% HCl (aq.), MeOH, reflux, 4 h, 64%; (f) i) acid anhydride or acyl chloride, DIPEA, CH_2Cl_2 , 0 °C, 1 h; ii) Pd/C, H₂, EtOH, 25 °C, 12 h, 58-99%.

The synthesis of (*R*)-1,4-diaryl-3-methylene-2-azetidinone **29** was achieved by the similar method of **3**, with **9b** rather than **9a** as the ligand (**Scheme 5**). The C-3 modified analogues **30a-d** were synthesized just as the corresponding enantiomers **14a-c** and **4**, respectively.

Scheme 5. Synthesis of Azetidinones 28-30^{*a*}



^{*a*}Reagents and conditions: (a) $Pd_2(dba)_3$, **9b**, K_2CO_3 (aq.), CH_2Cl_2 , 25 °C, 3 h, 96%; (b) $Sn[N(TMS)_2]_2$, toluene, reflux, 6 h, 91%, 99% ee; (c) TBAF, THF, 0 °C, 1 h, 71%; (d) for **30a** and **30b**: i) $B_2(pin)_2$, CuCl, MeOH, PPh₃, *t*-BuOLi, THF, 25 °C, 12 h; ii) NaBO₃·4H₂O, H₂O, THF, 25 °C, 2 h, 52% and 19% for **30a** and **30b** respectively; for **30d**: 10% Pd/C, EtOH, H₂, 25 °C, 12 h, 92%; (e) i) BnCl, K_2CO_3 , MeCN, reflux, 12 h; ii) CBr₄, PPh₃, THF, 25 °C, 6 h; iii) 10% Pd/C, AcONa, EtOH, H₂, 25 °C, 12 h, 20% for 3 steps.

2.2 Biological results and discussion

2.2.1 Activities of Anti-proliferation in Vitro. The newly synthesized chiral β -lactam analogues of CA-4 were screened for anti-proliferative activities against four human cancer cell lines A2780, Hela, SKOV-3, and MDA-MB-231 with CA-4 (1) and paclitaxel (PTX) as positive control. As summarized in **Table 1**, most of target compounds display moderate to

potent inhibition on cell growth. Among them, compounds **11g**, **11h**, **12f**, **14b**, **14c**, **14e**, **14g**, **21**, **22**, **24**, **26a** and **26b** exhibited considerable activities with IC₅₀ values less than 100 nM, therein compound **14c** exhibited similar activity with the referenced agents CA-4 (**1**) and PTX. In addition, we also tested the cytotoxicity of **14b** and **14c** in a normal ovarian epithelial cell line HOSE, and the result shows that both compounds achieve less 50% inhibition ratio at 1 μ M, indicating the low toxicity of **14b** and **14c** against normal cells.

Compound	$IC_{50} (\mu M)^a$						
Compound	$A2780^{b}$	MDA-MB-231 ^c	SKOV- 3^d	Hela ^e			
11a	> 20	> 20	> 20	> 20			
11b	> 20	> 20	> 20	> 20			
11c	0.127	0.193	0.265	0.209			
11d	> 20	> 20	> 20	> 20			
11e	0.121	0.112	0.136	0.131			
11f	0.316	0.512	0.341	0.371			
11g	0.031	0.040	0.036	0.032			
11h	0.065	0.034	0.063	0.125			
11j	10.472	6.643	14.490	15.021			
11k	0.215	0.784	0.365	0.547			
111	0.287	0.560	0.414	0.657			
11n	0.677	1.368	1.388	2.172			
11p	> 20	> 20	> 20	> 20			
11r	0.112	0.125	0.175	0.295			
12a	0.854	1.018	2.260	3.157			
12b	0.854	0.958	1.135	2.032			
12c	3.417	5.623	5.257	6.888			
12d	11.330	15.930	18.030	> 20			
12e	> 20	> 20	> 20	> 20			
12f	0.081	0.133	0.104	0.116			
12g	> 20	12.845	12.080	17.785			
12h	0.133	0.240	0.177	0.224			
13 a	2.545	3.165	2.988	4.732			
13b	> 20	> 20	> 20	> 20			
14a	0.220	0.182	0.408	0.358			
14b	0.014	0.016	0.021	0.021			
14c	0.002	0.003	0.003	0.001			

Table 1. Antiproliferative Activities on Different Cell Lines

14d	0.327	0.107	0.054	0.328
14e	0.035	0.027	0.008	0.044
14f	0.081	0.063	0.059	0.215
14g	0.021	0.017	0.008	0.035
14h	1.772	4.466	2.995	3.419
14i	1.753	2.648	2.472	2.559
14j	1.021	1.791	1.666	2.269
16a	1.545	2.323	2.074	2.099
16b	17.723	> 20	> 20	> 20
18 a	1.221	1.956	2.212	1.794
18b	0.079	0.102	0.210	0.178
21	0.008	0.010	0.009	0.013
22	0.022	0.028	0.024	0.030
24	0.015	0.028	0.083	0.033
26a	0.033	0.047	0.032	0.035
26b	0.027	0.047	0.045	0.024
26c	0.164	0.254	0.231	0.192
30a	>20	>20	>20	>20
30b	0.364	0.383	0.206	0.332
30c	0.771	1.082	0.650	0.807
30d	5.752	5.137	3.270	3.918
1	0.006	0.008	0.012	0.010
PTX	0.001	0.003	0.002	0.003

^{*a*}The anti-proliferation activities of individual compound to tumor cells were determined by the MTT assay. The data are the mean of triplicate determinations. ^{*b*, *d*}A2780 and SKOV-3 are human ovarian cancer cell lines. ^{*c*}MDA-MB-231 is a human breast cancer cell line. ^{*e*}Hela is a human cervical carcinoma cell line.

2.2.2 Structure–Activity Relationships Study. According to the activities mentioned above, the preliminary SARs of these β -lactam analogues can be summarized.

Comparing the data of **4** and **14a-g** with **30a-d** (Scheme 6), S-configuration of C-4 is critically important for the activity, which showed the same conclusion as the reported literature [8]. 3-Hydroxymethyl substituted products **14b** and **14e** with *trans-(3R,4R)* configuration displayed about 4-20 fold more potent than cis-(3S,4R) diastereoisomers **14a** and **14d**. 3-Methyl substituted compounds **14c** and **14g** with *trans-(3S,4R)* configuration also displayed about 4-63 fold more potent than cis-(3R,4R) diastereoisomers **4** and **14f**.

Analogues (21, 24, 26a-b) with *trans*-orientation also showed excellent antiproliferative activities. In conclusion, *trans*-orientation of substituents at 3,4-positions of β -lactam scaffold benefit the activity, which coincide with the results reported by Coccetti and Meegan groups [7d,7f].

Scheme 6. The anti-proliferative activities of 3-hydroxymethyl and 3-methyl substituted stereoisomers.



Many of 3-methylene substituted analogues (**11e**, **11g**, **11h**) showed good activities, but 3-(*Z*)-benzylidene analogues (**13a-b**) were inactive, indicating that 3-methylene is tolerant but bulky steric hindrance in the same plane is forbidden. The 3,4,5-trimethoxyphenyl turned out to be the best choice for *N*-1 substitution, which is consistent with CA-4 and previous research results. The analogue with 3,5-dimethoxy substituted *N*-phenyl ring (**11r**) exhibited a little loss of activity, whereas **11n** and **11p** with only one MeO- group on their *N*-phenyl rings showed considerable loss in potency. The 4-methoxy of the phenyl ring at C-4 was very important for the activity and the lack of 4-methoxy (**11a**, **11b**, **11j**) resulted in loss of activity, whereas the 3-hydroxy of 4-phenyl ring can tolerate various arrangements. Removal or replacement of 3-hydroxy of 4-phenyl ring with other bioisosteres, such as F, Cl and NH₂,

as well as the acylation of 3-hydroxy can maintain the activity (**11e**, **11f**, **11g**, **11h**, **12f**, **12h**). In the cases of the alkylated derivatives (**12a-e**) of 3-hydroxy at 4-phenyl ring, the bulkier steric hindrance of alkyl group the less potent the activity is.

2.2.3 Disruption of Tubulin Assembly. Representative compounds 14b and 14c were tested for inhibitory effects on tubulin assembly, using isolated pig brain tubulin. As shown in Figure 2A, 14b and 14c suppressed in vitro tubulin polymerization in a dose-dependent manner, compared to the solvent (DMSO) control. When incubated with 6 μ M of the two compounds, tubulin polymerization was almost completely inhibited. The IC₅₀ values were listed in Table 2. Both compounds exhibited stronger effects than positive control Colchicine.



Figure 2. (A) Effects of compounds **14b** and **14c** on tubulin polymerization in vitro. (B) **14b** (500 nM) and **14c** (500 nM) induce depolymerization of tubulin in HeLa cells. The supernatant faction (S) contains unpolymerized tubulin and the pellet fraction (P) contains polymerized tubulin. DMSO (0.2%) was used as negative control. (C) Immunofluorescence assay of SKOV-3 cells shows the effect of compounds **14b** and **14c** on the organizations of

cellular microtubule network. CA-4 (1) served as tubulin depolymerized control, whereas **PTX** was used as tubulin stabilized control. DMSO (0.2%) was used as negative control. Scale bars: $20 \mu m$.

Table 2. IC ₅₀	Values of	14b and	l 14c on	Inhibition of	f Tubulin Po	olymerization
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Compounds	14b	14c	Colchicine		
a,b IC ₅₀ (μ M)	3.5 ± 0.1	1.7 ± 0.0	8.6 ± 0.9		
^{<i>a</i>} IC ₅₀ values were	calculated by inhibition	ratio of 9 concentrations	of each compound.		

^bThe values present as mean \pm SD from at least two independent experiments.

Next, the effects of **14b** and **14c** on the inhibition of cellular tubulin assembly were also determined. After treatment with **14b**, **14c**, **1** or PTX respectively, the cells were lysed with detergents, and centrifuged. The pellet (P, polymerized tubulin) and supernatant (S, depolymerized tubulin) fraction were collected and analyzed by western blotting (**Figure 2B** and **S1**). Treatment of **1** significantly decreased polymerized microtubules while treatment of PTX increased polymerized tubulin in Hela cells. As expected, cells treated with the two tested compounds showed dramatically reduction of polymerized tubulin.

Furthermore, we employed cellular immunofluorescence assay to visualize the effects of β -lactam analogues **14b** and **14c** on microtubule structure. As shown in **Figure 2C**, when treated with solvent (DMSO) control, the microtubule network (green) of SKOV-3 cells were slim and hair-like, distributing around nucleus (blue). However, when treated cells with **1**, **14b** or **14c**, the fibrous microtubule structures were disorganized and their density was also significantly reduced. In contrast, cells treated with PTX exhibited a spindle shaped microtubule network and the density of microtubule was significantly increased [11]. Taken together, these results clearly demonstrated that our β -lactam bridged CA-4 analogues strongly inhibit tubulin assembly in both purified protein and cancer cells.

2.2.4 Anti-angiogenic Effects in Vitro and in Vivo. Targeting angiogenesis has been one of the strategies for increasing the efficacy of cancer therapeutics. Since many tubulin binding agents, including CA-4, have been reported to interfere with vascular formation [12], **14b** and **14c** were evaluated for their effects against angiogenesis in vitro and in vivo in this study. In capillary-like tube formation assay, vascular endothelial cells could form capillary-like structures on matrigel [13], an extracellular matrix rich in proangiogenic factors. As shown in **Figure 3A**, the HUVEC cells only treated with diluent (DMSO) produced a complete network structure. However, both **14b** and **14c** could effectively alter the tubule-like structures after 12 h incubation. At the concentration of 50 nM, **14c** almost completely disrupted the capillary-like tube formation.

Furthermore, the in vivo anti-vascular activities of **14b** and **14c** were also tested using matrigel plug assay [14]. In this assay, matrigel supplemented with human VEGF (100 ng/mL) was mixed with **14b** (100 nM), **14c** (100 nM) or diluent (DMSO), followed by injection into nude mice subcutaneously. One week later, the growth of capillary blood vessel in the plug was massively induced by VEGF, when matrigel was only mixed with diluent (**Figure 3B** upper line). However, the existence of compound **14b** or **14c** attenuated the VEGF-induced capillary blood vessel in the matrigel plug. The relative level of angiogenesis was also presented by hemoglobin content in the matrigel plug. As shown in **Figure 3B** lower line, the levels of hemoglobin in matrigel were reduced by **14b** and **14c** treatment compared with diluent treated control. Collectively, these results demonstrated that **14b** and **14c** suppressed VEGF-induced angiogenesis in vitro and in vivo.





Figure 3. Effects of compounds 14b and 14c on angiogenesis in vitro and in vivo. (A) 14b and 14c disrupted capillary-like tubule formed by HUVECs. CA-4 (1) served as positive control. (B) Matrigel plug assay showed that 14b and 14c inhibited growth of capillary vascular in nude mice.

2.2.5 Inhibition of Colony Formation and Cell Cycle Arrest. In the following experiments, we further confirmed the cytotoxic effects of 14b and 14c by colony formation

assay. As shown in **Figure 4A** and **4B**, the colonies formed by MDA-MB-231 cells were dose-dependently reduced by the exposure to **14b** and **14c**.

To further characterize the inhibitory effects of **14b** and **14c** on cell growth, cell cycle progression was analyzed by quantitating DNA content, since chemical agents generally decrease growth and proliferation of cancer cells through altering the regulation of cell cycle [15]. As expected, results showed that, similar to the positive compound **1**, both compounds **14b** and **14c** arrest Hela cells in the G2/M phase (**Figure 4C** and **S2**). In the DMSO treated group, only about 24% of cells were distributed in the G2/M phase. However, after being treated with **14c** or **1** at the dose of 50 nM, nearly 100% of cells were arrested in G2/M phase. Moreover, immunoblotting showed that **14b** and **14c** markedly up-regulated the expression of cyclin B1, which is synthesized at G2-phase and reaches maximum level at metaphase. In addition, **14b** and **14c** treatment also induced hyper-phosphorylation of the mitotic checkpoint kinase BubR1 and mitotic trigger histone H3 in a dose-dependent manner (**Figure 4D**). These results clearly demonstrate that, consistent with other reported microtubule inhibitors [16], β -lactam analogues **14b** and **14c** significantly induce cellular mitotic arrest in Hela cells.



Figure 4. (A) Treatment with **14b** and **14c** does-dependently suppressed cell colony formation of MDA-MB-231 cells. (B) Qualification of the numbers of colonies. (C) Cell cycle distribution of HeLa cells after treatment with indicated concentrations of **14b** and **14c** for 24 h. (D) Western blot analysis of mitosis regulated proteins. ** P<0.01; *** P<0.001.

2.2.6 Induction of Cellular Apoptosis. Numerous studies have demonstrated that tubulin polymerization inhibitors possess the abilities to induce cellular apoptosis [17]. For this reason, we assessed whether the active analogues **14b** and **14c** could induce apoptosis of Hela cells by double staining with Annexin V-FITC and propidium iodide (PI). The flow cytometric data (**Figure 5A**) showed the high activities of **14b** and **14c** on the induction of cellular apoptosis. As qualified in **Figure S3**, the total proportions of apoptotic cells, including Annexin V+/PI- (the right lower quadrant representing early apoptosis) and

Annexin V+/PI+ (the right upper quadrant representing late apoptosis or necrosis), were 38.7%, 57.5% and 70.7% after treatment with 50, 100 and 200 nM of **14b**, and were 52.7%, 65.1% and 71.3% after treatment with 50, 100 and 200 nM of **14c**, respectively. Exposure to positive control **1** (100 nM) leaded to totally 62.5% cell apoptosis, however, only 2.7% apoptotic cells were detected in solvent (DMSO) treated group. Subsequently, we further examined apoptosis associated proteins by immunoblotting analysis. As indicated in **Figure 5B**, pro-apoptotic protein Bax and p53 was up-regulated by both **14b** and **14c** treatment in a dose-dependent manner. In addition, both compounds also dose-dependently promoted cleavage of poly(ADP-ribose) polymerase-1 (PARP-1), a marker of cells undergoing apoptosis. These results implied that the β -lactam analogues exhibited their anti-tumor effects through, at least partly, induction of cellular apoptosis.



Figure 5. (A) Representative flow cytometry profiles showed that **14b** and **14c** does-dependently induced apoptosis of Hela cells. (B) Western blot analysis of apoptosis-related proteins. Cells were treated with indicated concentrations of **14b**, **14c**, **1** or diluent (0.2% DMSO) for 48 h.

2.2.7 Anti-tumor Activities in Vivo. Firstly, we performed single-dose acute toxicity assay to assess the safety of compounds **14b** and **14c**. ICR mice (n=10, half male and half female) were injected intraperitoneally with various dosages of **14b** (95, 70, 50, 35 and 25 mg/kg) or **14c** (275, 200, 150, 125 and 100 mg/kg), or vehicle control. As summarized in **Table 3**, none of the mice died in the two days after treatment with **14b**, whereas the death of

mice mainly occurred in the second day after treatment with **14c**. With LD_{50} values of 136.5 kg/mg, **14c** exhibited less toxicity than **14b**, whose LD_{50} value was calculated as 61.5 mg/kg.

	Dose No. of (mg/kg) mice	No. of dead mice					Total	$\mathbf{S}_{\mathbf{u}}$	
Compd.		mice	1	2	3	4	5-14	dooth	Survival (%)
			day	days	days	days	days	ueatii	Off day 14
	95	10	0	0	6	1	1	8	20
	70	10	0	0	4	2	1	7	30
14b	50	10	0	0	1	0	0	1	90
	35	10	0	0	1	0	0	1	90
	25	10	0	0	0	0	0	0	100
14c	275	10	2	4	2	1	0	9	10
	200	10	1	4	2	0	0	7	30
	150	10	2	4	0	0	0	6	40
	125	10	0	2	1	0	0	3	70
	100	10	0	0	1	0	0	1	90
vehicle		10	0	0	0	0	0	0	100

Table 3. Acute Toxicity of 14b and 14c in Mice

14b: LD₅₀ = 61.5 mg/kg; **14c**: LD₅₀ = 136.5 mg/kg.

Next, we evaluated the in vivo anti-tumor effects of compounds **14b** and **14c** using human ovarian cancer xenograft mice model, which is established by subcutaneous inoculation of A2780 cells in the female Balb/C nude mice. After the mean value of tumor volumes reached approximately 100 mm³, the mice were randomly divided into 4 groups and administrated with 7.5 mg/kg of **14b**, 8 mg/kg of **14c**, 10 mg/kg of PTX (as positive control) or vehicle (negative control) by intraperitoneal injection once every two days. As shown in **Figure 6A**, treatment of both **14b** and **14c** significantly suppressed the tumor growth, achieving 78.9% and 76.3% reduction in tumor size at the end of the observation period, respectively. When the observation time ended, the mice were sacrificed and the tumors were excised and weighed. The results were illustrated in **Figures 6B** and **6D**, and again confirmed the anti-tumor activities of the two compounds. Slight weight loss was observed in **14b** treated

animals, but was not statistically significant (**Figure 6C**). Furthermore, H&E staining of tumor sections showed extensive areas of necrosis or cell death (indicated by black arrow in **Figure 6E**) in both **14b** and **14c** treated groups, however, none of abnormal areas were observed in the vehicle treatment group. In addition, we also examined organs of agents treated and vehicle treated mice, including liver, kidney and spleen, using H&E staining (**Figure 6F**). No detectable abnormalities were observed in the organs examined, indicating the safety of **14b** and **14c** at the therapeutic dosage. Taken together, considering its LD₅₀ value, it could be proposed that compound **14c** possesses a satisfied therapeutic window and is worth for further validation.





organic sections. No obvious alternation was observed in the organs examined, including liver, kidney and spleen after treatment with **14b** and **14c**. ** P<0.01; *** P<0.001.

2.2.8 The Cocrystal Structure of Tubulin in Complex with Compounds 14b and 14c.

To reveal the specific details of binding, as well as to provide solid basis for further structural optimization, compounds 14b and 14c were soaked into the crystals of a protein complex (T2R-TTL) consisting of two α,β -tubulin heterodimers, the stathmin-like protein RB3, and the tubulin tyrosine ligase. Finally the co-crystal structures of these two compounds with tubulin were determined at 2.56 Å for 14b (PDB code 5XAG) and 2.55 Å for 14c (PDB code 5XAF), respectively (Figure 7). The data collection and refinement statistics was summarized in Table S1. As expected, 14b and 14c occupied the binding site of colchicine with satisfactory electron density. To determine the impact of ligand binding against the overall conformation of tubulin, these two complex structures were subsequently superimposed to the tubulin structure in the absence of ligand (PDB code 5JQG). The RMSD values for the superimposed structures of 14b and 14c were 0.603 Å over 1892 Ca atoms and 0.608 Å over 1917 Ca atoms, respectively, indicating that these two compounds did not affect the global conformation of tubulin. However, the major conformational changes come from two loops of tubulin near the binding site after ligand binding. Similar conformational changes were also observed in the structure of tubulin with colchicine, indicating that 14b and 14c may possess the same inhibitory mechanism as colchicine. In addition, as shown in Figure S4, the conformations of these two loops were not the same in three structures, suggesting that the flexibility of this binding site might be further exploited. Both hydrophobic contacts and hydrogen bonds were observed in the structures of tubulin with 14b and 14c. As shown in Figure 7G, 14b formed a direct hydrogen bond with β -Ala250, as

well as water-bridged hydrogen bonds with β -Asn349. However, the main driving force of binding was thought to be shape matching between the compound and surrounding hydrophobic residues, including the residues Leu242, Leu248, Ala250, Leu255, Met259, Val315, Ala316, Ala317, Ile318 of β -tubulin. Notably, as shown in **Figure 7I**, **14b** and **14c** were more deeply buried than colchicine in the binding site, which is consistent with their better potency against tubulin polymerization. However, the acetamido group of colchicine was not overlaid in the superimposed structures, providing us a new possible direction for further structural modification.

Figure 7. Crystal structures of the tubulin-14b and tubulin-14c complex.



(A) Overall view of the complex formed between 14b and tubulin. α -tubulin is shown as green cartoon, and β -tubulin is shown as cyan cartoon. **14b** is shown in yellow sphere representation (PDB code 5XAG). (B) Overall view of the complex formed between 14c and tubulin. α -tubulin is shown as green cartoon, and β -tubulin is shown as cyan cartoon. **14c** is shown in yellow sphere representation (PDB code 5XAF). (C) 2Fo-Fc electron density (blue) for 14b, and the contour level is set to 1.0 sigma. (D) 2Fo-Fc electron density (blue) for 14c, and the contour level is set to 1.0 sigma. (E) **14b** binds to the colchicine site of β -tubulin, which is shown in surface representation. (F) 14c binds to the colchicine site of β -tubulin, which is shown in surface representation. (G) Close-up views of the hydrogen bonds and hydrophobic contacts formed between 14b (yellow sticks) and tubulin. Hydrogen bonds are shown in yellow dashed lines, and corresponding distances were labeled in angstrom. Interacting residues of tubulin are shown in white stick representation, and crystal waters are shown in red sphere representation. (H) Close-up views of the hydrogen bonds and hydrophobic contacts formed between 14c (yellow sticks) and tubulin. Hydrogen bonds are shown in yellow dashed lines, and corresponding distances were labeled in angstrom. Interacting residues of tubulin are shown in white stick representation, and crystal waters are shown in red sphere representation. (I) Superimposition of 14b (cyan stick, PDB code 5XAG) and 14c (green stick, PDB code 5XAF) with colchicine (salmon stick, PDB code 4O2B).

3. Conclusion

In summary, a diverse of chiral β -lactam bridged combretastatin A-4 analogues have been synthesized and biologically evaluated. Most of the target compounds displayed moderate to potent anti-proliferative activities against four human cancer cell lines (A2780, Hela, SKOV-3 and MDA-MB-231). The studies on SARs revealed that the absolute configurations of the chiral C-4 were critically important for the activity, more specifically, (*S*)-configuration for 3-methylene substituted series (**11**) and the same orientation for other analogues. On this basis, *trans*-configuration of substituents at 3,4-positions of β -lactam scaffold benefit the antiproliferative activity. Among all synthesized compounds, **14b** and **14c** turned out to be most potent and were selected for further pharmacological studies after comprehensive consideration. The co-crystal structures of tubulin in complex with **14b** and **14c** were determined by X-ray crystallography, which showed that they bind to the same site

as colchicine with similar binding mode. Further biochemical evaluation demonstrated that both compounds disrupted tubulin assembly in isolated protein level and in cellular level, suppressed angiogenesis in vitro and in vivo, blocked cell cycle progression at mitotic phase and induced cellular apoptosis. Importantly, both compounds inhibited xenografts tumor growth in nude mice with acceptable therapeutic window, showing promising potentials for further clinical development.

4. Experimental section

All reagents were commercially available and were used without further purification. Melting points were measured on a SGW X-4 apparatus and uncorrected. Optical rotations were measured at the sodium D line, using a Jasco P-1020 automatic digital polarimeter and a 100 mm cell. ¹H and ¹³C spectra were obtained by a Varian 400 MHz or a Bruker 600 MHz NMR spectrometer at 303 K, using tetramethylsilane as internal standard. MS was measured on Agilent 6120 Quadrupole LC/MS. HRMS determinations for all new compounds were performed on AB SCIWX TRIPLETOF 5600+ or Agilent 6224 TOF LC/MS, respectively. Flash chromatography was carried out using standard silica gel 60 (300–400 mesh) and detected with UV light monitor at 254 nM. The purity of the biological tested compounds was determined by reverse-phase HPLC (SB-CN column (5 Micro, 4.6 mm × 250 mm) using a Waters 1525 Binary HPLC Pump and a Waters 2489 UV/visible Detector). The mobile phase was MeOH/H₂O (85:15, v/v) and with a total flow rate of 1 mL/min. The purity was determined by monitoring at 254 nm. The purities of all the activity tested compounds were confirmed to be \geq 95% by this HPLC analysis. **4.1 General Method for MBH Reaction.** A 100 mL round-bottom flask was charged with appropriate benzaldehyde (10 mmol), ethyl acrylate (10 mmol), DABCO (10 mmol) and MeOH (5 mmol). The solution was stirred at room temperature for several days. The mixture was directly purified by flash column chromatography to give the corresponding MBH product.

4.1.1 Ethyl 2-(Hydroxy(phenyl)methyl)acrylate (**6a**). React for 1 week. Colorless oil; yield 65%. ¹H NMR (400 MHz, CDCl₃): δ 7.29-7.14 (m, 5H), 6.25 (s, 1H), 5.82 (s, 1H), 5.45 (s, 1H), 4.03-4.01 (m, 2H), 1.16-1.08 (m, 3H).

4.1.2 Ethyl 2-(Hydroxy(*p*-tolyl)methyl)acrylate (6b). React for 2 weeks. Colorless oil; yield 73%. ¹H NMR (400 MHz, CDCl₃): δ 7.25 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 6.32 (s, 1H), 5.83 (s, 1H), 5.52 (d, J = 5.4 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 3.07 (d, J = 5.4 Hz, 1H), 2.33 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H).

4.1.3 Ethyl 2-(Hydroxy(4-methoxyphenyl)methyl)acrylate (6c). React for 2 weeks.
Colorless oil; yield 44%. ¹H NMR (400 MHz, CDCl₃): δ 7.22 (d, J = 7.4 Hz, 2H), 6.80 (d, J = 7.4 Hz, 2H), 6.26 (s, 1H), 5.81 (s, 1H), 5.45 (s, 1H), 4.16-3.98 (m, 2H), 3.73 (s, 3H), 1.18 (td, J = 7.1, 1.0 Hz, 3H).

4.1.4 Ethyl 2-((3-Fluoro-4-methoxyphenyl)(hydroxy)methyl)acrylate (6d). React for 5 days. Colorless oil; yield 71%. ¹H NMR (400 MHz, CDCl₃): δ 7.13-7.02 (m, 2H), 6.93-6.88 (m, 1H), 6.32 (s, 1H), 5.82 (br s, 1H), 5.46 (d, *J* = 5.6 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.86 (s, 3H), 3.17 (d, *J* = 5.6 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H).

4.1.5 Ethyl 2-((3-Chloro-4-methoxyphenyl)(hydroxy)methyl)acrylate (6e). React for 4 days. Colorless oil; yield 38%. ¹H NMR (400 MHz, CDCl₃): δ 7.37 (d, J = 2.1 Hz, 1H), 7.23

(dd, J = 8.5, 2.1 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.34 (s, 1H), 5.83 (s, 1H), 5.47 (d, J = 5.5 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.88 (s, 3H), 3.10 (d, J = 5.5 Hz, 1H), 1.25 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 165.6, 153.9, 141.2, 134.0, 127.9, 125.5, 125.3, 121.7, 111.2, 71.8, 60.4, 55.5, 13.4. ESI-MS (m/z): 293.1 (M + Na⁺). ESI-HRMS (m/z): calcd for C₁₃H₁₅ClO₄ + Na⁺ [M + Na⁺], 293.0551; found, 293.0553.

4.1.6 Ethyl 2-(Hydroxy(4-methoxy-3-nitrophenyl)methyl)acrylate (6f). React for 3 days. Colorless oil; yield 99%. ¹H NMR (400 MHz, CDCl₃): δ 7.85 (d, J = 2.1 Hz, 1H), 7.57 (d, J = 8.7, 2.1 Hz, 1H), 7.07 (d, J = 8.7 Hz, 1H), 6.37 (s, 1H), 5.90 (s, 1H), 5.53 (d, J = 5.6 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.95 (s, 3H), 3.43 (d, J = 5.6 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, DMSO-d₆): δ 166.1, 152.5, 141.6, 139.4, 134.3, 132.7, 126.5, 124.1, 113.6, 72.1, 72.0, 61.4, 56.8, 14.2. ESI-MS (m/z): 304.1 (M + Na⁺). ESI-HRMS (m/z): calcd for C₁₃H₁₅NO₆ + NH₄⁺ [M + NH₄⁺], 299.1238; found, 299.1238.

4.1.7 Ethyl 2-((4-((*tert***-Butyldimethylsilyl)oxy)phenyl)(hydroxy)methyl)acrylate (6g). React for 1 week. Colorless oil; yield 37%. ¹H NMR (400 MHz, CDCl₃): \delta 7.11 (t, J = 7.8 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 6.81 (s, 1H), 6.69 (dd, J = 7.8, 2.0 Hz, 1H), 6.24 (s, 1H), 5.79 (s, 1H), 5.42 (d, J = 5.2 Hz, 1H), 4.05 (q, J = 7.1 Hz, 2H), 3.76 (d, J = 5.2 Hz, 1H), 1.14 (t, J = 7.1 Hz, 3H), 0.95 (s, 9H), 0.15 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): \delta 182.1, 177.8, 168.5, 152.9, 152.2, 142.3, 142.2, 138.4, 133.4, 132.8, 132.2, 85.7, 73.7, 38.5, 33.9, 33.8, 31.1, 26.9, 8.4. ESI-MS (m/z): 336.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₁₈H₃₂NO₄Si + NH₄⁺ [M + NH₄⁺], 354.2095; found, 354.2089.**

2-((3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(hydroxy)methyl)acrylate (6h).

React for 1 week. Colorless oil; yield 42%, with 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**5h**) recovered (recovery 30%). ¹H NMR (400 MHz, CDCl₃): δ 6.92 (dd, J = 8.3, 2.1 Hz, 1H), 6.85 (d, J = 2.1 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.70-6.76 (m, 1 H), 6.31 (s, 1H), 5.77 (d, J = 1.1 Hz, 1H), 5.47 (d, J = 5.0Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.79 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H), 0.98 (s, 9H), 0.13 (s, 6H).

4.1.9 Ethyl 2-(Hydroxy(3,4,5-trimethoxyphenyl)methyl)acrylate (6i). React for 5 days at 45 °C. White solid; yield 42%; mp 90-91 °C. ¹H NMR (400 MHz, CDCl3): δ 6.59 (s, 2H), 6.32 (s, 1H), 5.86 (s, 1H), 5.49 (d, J = 5.2 Hz, 1H), 4.20-4.14 (m, 2H), 3.83-3.81 (m, 9H), 1.26 (t, J = 7.2 Hz, 3H).

4.2 General Method for the Acetylation of MBH Products. A 250 mL round-bottom flask was charged with appropriate MBH product (10 mmol), triethylamine (20 mmol) and dichloromethane (50 mL). The solution was cooled to 0 $^{\circ}$ C by an ice bath, then acetic anhydride (20 mmol) was added dropwise into the flask within 10 minutes. After stirred for 1 h, 50 mL of water was added into the solution, and the mixture was extracted with dichloromethane (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give the title compounds.

4.2.1 Ethyl 2-(Acetoxy(phenyl)methyl)acrylate (7a). Colorless oil; yield 88%. ¹H NMR (400 MHz, CDCl₃): δ 7.37-7.29 (m, 5H), 6.67 (s, 1H), 6.37 (s, 1H), 5.80 (s, 1H), 4.15 (q, J = 7.1 Hz, 2H), 2.10 (s, 3H), 1.22 (t, J = 7.0 Hz, 3H).

4.2.2 Ethyl 2-(Acetoxy(*p*-tolyl)methyl)acrylate (7b). Colorless oil; yield 83%. ¹H NMR
(400 MHz, CDCl₃): δ 7.28 (d, J = 7.9 Hz, 2H), 7.15 (d, J = 7.9 Hz, 2H), 6.67 (s, 1H), 6.39 (s, 1H), 5.84 (s, 1H), 4.15 (q, J = 7.0 Hz, 2H), 2.33 (s, 3H), 2.09 (s, 3H), 1.22 (t, J = 7.0 Hz, 3H).

4.2.3 Ethyl 2-(Acetoxy(4-methoxyphenyl)methyl)acrylate(**7c**). Colorless oil; yield 85%. ¹H NMR (400 MHz, CDCl₃): δ 7.33-7.27 (m, 2H), 6.99-6.83 (m, 2H), 6.63 (s, 1H), 6.37 (t, J = 0.9 Hz, 1H), 5.86-5.81 (m, 1H), 4.19-4.10 (m, 2H), 3.79 (s, 3H), 2.09 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H).

4.2.4 Ethyl 2-(Acetoxy(3-fluoro-4-methoxyphenyl)methyl)acrylate (**7d**). Colorless oil; yield 85%. ¹H NMR (400 MHz, CDCl₃): δ 7.12-7.07 (m, 2H), 6.91 (t, *J* = 8.6 Hz, 1H), 6.59 (s, 1H), 6.39 (s, 1H), 5.85 (s, 1H), 4.20-4.10 (m, 2H), 3.87 (s, 3H), 2.09 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 169.3, 164.8, 153.2, 150.8, 147.7, 147.5, 139.4, 130.7, 130.7, 125.3, 124.0, 123.9, 115.5, 115.3, 112.9, 72.3, 61.0, 56.1, 21.1, 14.0. ESI-MS (*m*/*z*): 319.1 (M + Na⁺). ESI-HRMS (*m*/*z*): calcd for C₁₅H₁₇FO₅ + Na⁺ [M + Na⁺], 319.0952; found, 319.0954.

4.2.5 Ethyl 2-(Acetoxy(3-chloro-4-methoxyphenyl)methyl)acrylate (**7e**). Colorless oil; yield 79%. ¹H NMR (400 MHz, CDCl₃): δ 7.36 (d, *J* = 2.1 Hz, 1H), 7.27 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.57 (s, 1H), 6.39 (s, 1H), 5.87 (s, 1H), 4.20-4.08 (m, 2H), 3.87 (s, 3H), 2.09 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 168.8, 164.2, 154.3, 138.8, 130.4, 128.9, 127.1, 124.7, 121.7, 111.1, 71.7, 60.4, 55.5, 20.5, 13.4. ESI-MS (*m*/*z*): 335.1 (M + Na⁺). ESI-HRMS (*m*/*z*): calcd for C₁₅H₁₇ClO₅ + Na⁺ [M + Na⁺], 335.0657; found, 335.0659.

4.2.6 Ethyl 2-(Acetoxy(4-methoxy-3-nitrophenyl)methyl)acrylate (**7f**). Colorless oil; yield 76%. ¹H NMR (400 MHz, CDCl₃): δ 7.84 (d, J = 2.2 Hz, 1H), 7.59 (dd, J = 8.7, 2.2 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H), 6.60 (s, 1H), 6.43 (s, 1H), 5.95 (s, 1H), 4.19-4.10 (m, 2H), 3.94 (s, 3H), 2.10 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H). ESI-MS (m/z): 346.1 (M + Na⁺). ¹³C NMR (100 MHz, CDCl₃): δ 169.2, 164.5, 152.8, 139.3, 138.8, 134.0, 130.5, 125.7, 124.9, 113.4, 71.8, 61.2, 56.6, 21.0, 14.0. ESI-HRMS (m/z): calcd for C₁₅H₁₇NO₇ + NH₄⁺ [M + NH₄⁺], 341.1343; found, 341.1344.

4.2.7

Ethyl

2-(Acetoxy(3-(*tert*-butoxycarbonyl)amino)-4-methoxyphenyl)methyl)acrylate (7g). To a solution of 7f (1.7 g, 5.2 mmol) and zinc powder (1.03 g, 16 mmol) in MeOH (20 mL) was slowly added acetic acid (3.2 g, 52 mmol), followed by heating to reflux for 30 minutes. The reaction mixture was then allowed to cool down to room temperature and filtered. The solvent was removed under reduced pressure and the residue was added to 30 mL of saturated sodium bicarbonate solution. The mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure to get the crude product. The crude product and di-*tert*-butyl dicarbonate (740 mg, 3.4 mmol) was dissolved in 10 mL of THF. The reaction mixture was heated to reflux for 16 h. The solvent was removed under vacuum and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:3, v/v) to give the title compound **7g** as colorless oil (853 mg, yield 41%). ¹H NMR (400 MHz, CDCl₃): δ 8.10 (br s, 1H), 7.07 (br s, 1H), 7.01 (dd, J = 8.3, 2.0 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 6.62 (s, 1H), 6.39 (s, 1H), 5.85 (s, 1H), 4.20-4.11 (m, 2H), 3.85 (s, 3H), 2.10 (s, 3H),
1.52 (s, 9H), 1.24 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 168.9, 164.5, 151.9, 146.8, 139.2, 129.8, 127.6, 124.9, 121.4, 116.6, 108.9, 72.7, 60.3, 55.1, 27.7, 20.6, 13.4. ESI-MS (m/z): 416.1 (M + Na⁺). ESI-HRMS (m/z): calcd for C₂₀H₂₇NO₇ + NH₄⁺ [M + NH₄⁺], 411.2126; found, 411.2128.

4.2.8 Ethyl 2-((3-(*tert*-Butyldimethylsilyloxy)phenyl)(hydroxy)methyl)acrylate (7h). Colorless oil; yield 37%. ¹H NMR (400 MHz, CDCl₃): δ 7.17 (t, J = 7.8 Hz, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.83 (s, 1H), 6.78-6.73 (m, 1H), 6.62 (s, 1H), 6.37 (s, 1H), 5.77 (s, 1H), 4.14 (q, J = 7.1 Hz, 2H), 2.07 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H), 0.96 (s, 9H), 0.17 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 182.1, 177.8, 168.5, 152.9, 152.2, 142.3, 142.2, 138.4, 133.4, 132.8, 132.2, 85.7, 73.7, 38.5, 33.9, 33.8, 31.1, 26.9, 8.4. ESI-MS (m/z): 401.2 (M + Na⁺). ESI-HRMS (m/z): calcd for C₂₀H₃₀O₅Si + NH₄⁺ [M + NH₄⁺], 396.2201; found, 396.2201.

4.2.9

Ethyl

2-(Acetoxy(3-(*(tert***-butyldimethylsilyl)oxy)-4-methoxyphenyl)methyl)acrylate** (**7i**). [9a] Yellow oil; yield 83%. ¹H NMR (400 MHz, CDCl₃): δ 6.93 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.79 (d, *J* = 8.3 Hz, 1H), 6.58 (s, 1H), 6.36 (s, 1H), 5.77 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 2.09 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 3H), 0.98 (s, 9H), 0.13 (s, 6H).

4.2.10 Ethyl 2-(Acetoxy(3,4,5-trimethoxyphenyl)methyl)acrylate (7j). Colorless oil; yield 91%. ¹H NMR (400 MHz, CDCl3): δ 6.63 (s, 1H), 6.60 (s, 2H), 6.40 (s, 1H), 5.84 (s,1H), 4.22-4.14 (m, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 2.12 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H).

4.3 General Method for the Palladium Catalyzed Enantioselective Allylic Amination. A 250 mL round-bottom flask was charged with 50 mL of dichloromethane and the solvent was deoxygenized with nitrogen bubbling for 15 min. Tris(dibenzylideneacetone)dipalladium

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(92 mg, 0.1 mmol) and **9a** (165 mg, 0.25 mmol) was added into the flask. The resulted purple solution was stirred under nitrogen atmosphere for 30 min at room temperature. Then aniline (15 mmol), K_2CO_3 (1.0 M aq. solution, 30 mL, 30 mmol) and appropriate acetylated MBH product (10 mmol, dissolved in 20 mL of oxygen free dichloromethane) were added under a steam of nitrogen. The solution was stirred for 5 h at room temperature. Water (50 mL) was added into the solution, and the mixture was extracted with dichloromethane (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give the title compounds.

4.3.1 (S)-Ethyl 2-(Phenyl(phenylamino)methyl)acrylate (10a). Colorless oil; yield
89%. ¹H NMR (400 MHz, CDCl3): δ 7.38-7.27 (m, 5H), 7.16 (t, J = 8.4 Hz, 2H), 6.72 (t, J = 7.2Hz, 1H), 6.57 (d, J = 8.8 Hz, 2H), 6.38 (s, 1H), 5.94 (s, 1H), 5.40 (d, J = 4.8 Hz, 1H),
4.19-4.09 (m, 3H), 1.20 (t, J = 7.2 Hz, 3H).

4.3.2 (S)-Ethyl 2-(Phenyl(3,4,5-trimethoxyphenylamino)methyl)acrylate (10b).
Colorless oil; yield 85%. ¹H NMR (400 MHz, CDCl3): δ 7.38-7.24 (m, 5H), 6.39 (s, 1H), 5.95 (s, 1H), 5.82 (s, 2H), 5.40 (s, 1H), 4.19-4.10 (m, 3H), 3.73 (s, 9H), 1.20 (t, J = 7.2 Hz, 3H).

4.3.3 (*S*)-Ethyl 2-(*p*-Tolyl((3,4,5-trimethoxyphenyl)amino)methyl)acrylate (10c). Colorless oil; yield 52%. ¹H NMR (400 MHz, CDCl₃): δ 7.26 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 2H), 6.38 (s, 1H), 5.95 (s, 1H), 5.82 (s, 2H), 5.35 (s, 1H), 4.21-4.05 (m, 2H), 3.76 (s, 6H), 3.75 (s, 3H), 2.33 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 166.3, 153.8, 143.6, 140.8, 137.7, 137.5, 130.5, 129.4, 127.3, 125.7, 91.2, 61.1, 60.8, 59.1,

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55.9, 21.1, 14.1. ESI-MS (*m*/*z*): 386.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₂H₂₇NO₅ + H⁺ [M + H⁺], 386.1962; found, 386.1964.

4.3.4 (S)-Ethyl 2-((4-Methoxyphenyl)(3,4,5-trimethoxyphenylamino)methyl)acrylate
(10d). Colorless oil; yield 90%. ¹H NMR (400 MHz, CDCl3): δ 7.28 (d, J = 8.8 Hz, 2H),
6.84 (d, J = 8.8 Hz, 2H), 6.35 (s,1H), 5.94 (s, 1H), 5.82 (s, 2H), 5.35 (s, 1H), 4.23 (br s, 1H),
4.18-4.09 (m, 2H), 3.74-3.72 (m, 12H), 1.21 (t, J = 7.2 Hz, 3H).

4.3.5

(S)-Ethyl

2-((3-Fluoro-4-methoxyphenyl)((3,4,5-trimethoxyphenyl)amino)methyl)acrylate (10e). Yellow oil; yield 69%. ¹H NMR (400 MHz, CDCl₃): δ 7.11 (t, J = 2.3 Hz, 1H), 7.08 (s, 1H), 6.91 (t, J = 8.3 Hz, 1H), 6.38 (s, 1H), 5.92 (s, 1H), 5.81 (s, 2H), 5.31 (s, 1H), 4.21-4.13 (m, 2H), 3.87 (s, 3H), 3.76 (s, 6H), 3.74 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 166.0, 153.8, 153.5, 151.0, 147.1, 147.0, 143.3, 140.3, 133.6, 133.6, 130.4, 126.1, 123.1, 115.1, 114.9, 113.3, 91.1, 61.1, 60.9, 58.5, 56.2, 55.8, 14.0. ESI-MS (m/z): 420.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₂H₂₆FNO₆ + H⁺ [M + H⁺], 420.1817; found, 420.1817.

4.3.6

(S)-Ethyl

2-((3-Chloro-4-methoxyphenyl)((3,4,5-trimethoxyphenyl)amino)methyl)acrylate (10f). Yellow oil; yield 65%. ¹H NMR (400 MHz, CDCl₃): δ 7.37 (d, J = 2.1 Hz, 1H), 7.24 (dd, J = 8.8, 2.1 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 6.39 (s, 1H), 5.94 (s, 1H), 5.81 (s, 2H), 5.30 (s, 1H), 4.21-4.13 (m, 2H), 3.89 (s, 3H), 3.77 (s, 6H), 3.75 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 165.5, 153.9, 153.2, 142.7, 139.6, 133.2, 129.8, 128.5, 126.3, 125.6, 122.0, 111.4, 90.5, 60.5, 60.4, 57.8, 55.6, 55.3, 13.5. ESI-MS (m/z): 436.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₂H₂₆ClNO₆ + H⁺ [M + H⁺], 436.1521; found, 436.1523.

4.3.7

(S)-Ethyl

2-((3-((*tert***-Butoxycarbonyl)amino)-4-methoxyphenyl)((3,4,5-trimethoxyphenyl)amino) methyl)acrylate (10g)**. Yellow oil; yield 81%. ¹H NMR (400 MHz, CDCl₃): δ 8.03 (br s, 1H), 7.02 (br s, 1H), 6.91 (dd, J = 8.4, 2.2 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.30 (s, 1H), 5.89 (s, 1H), 5.75 (s, 2H), 5.27 (s, 1H), 4.16-4.02 (m, 2H), 3.78 (s, 3H), 3.70 (s, 6H), 3.68 (s, 3H), 1.45 (s, 9H), 1.16 (t, J = 7.1 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 165.8, 153.1, 152.0, 146.4, 143.0, 140.1, 132.7, 129.7, 127.8, 125.1, 120.6, 116.5, 109.2, 90.6, 79.8, 60.4, 60.1, 58.4, 55.3, 55.1, 27.7, 13.5. ESI-MS (m/z): 517.3 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₇H₃₆N₂O₈ + H⁺ [M + H⁺], 517.2544; found, 517.2544.

4.3.8

(S)-Ethyl

2-((3-((*tert*-Butyldimethylsilyl)oxy)phenyl)((3,4,5-trimethoxyphenyl)amino)methyl)acryl ate (10h). Yellow oil; yield 94%. ¹H NMR (400 MHz, CDCl₃): δ 7.19 (t, J = 7.7 Hz, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.83 (t, J = 2.0 Hz, 1H), 6.75 (dd, J = 7.7, 2.0 Hz, 1H), 6.37 (s, 1H), 5.91 (s, 1H), 5.82 (s, 2H), 5.33 (s, 1H), 4.21-4.12 (m, 2H), 3.76 (s, 6H), 3.75 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H), 0.95 (s, 9H), 0.15 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 166.2, 156.0, 153.8, 143.5, 142.1, 140.8, 130.5, 129.7, 126.0, 120.4, 119.4, 119.2, 91.4, 61.1, 60.9, 59.2, 55.9, 25.7, 18.2, 14.1, -4.4. ESI-MS (m/z): 502.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₇H₃₉NO₆Si + H⁺ [M + H⁺], 502.2620; found, 502.2619.

2-((3-((tert-Butyldimethylsilyl)oxy)-4-methoxyphenyl)((3,4,5-trimethoxyphenyl)amino)m

ethyl)acrylate (10i) [8]. Yellow gel; yield 86%. ¹H NMR (400 MHz, CDCl₃): δ 6.91 (dd, J = 8.3, 2.1 Hz, 1H), 6.83 (d, J = 2.1 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.34 (s, 1H), 5.88 (s, 1H), 5.81 (s, 2H), 5.28 (s, 1H), 4.15 (q, J = 7.1 Hz, 2H), 3.78 (s, 3H), 3.76 (s, 6H), 3.74 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.96 (s, 9H), 0.11 (s, 6H).

4.3.10

(S)-Ethyl

2-((3,4,5-Trimethoxyphenyl)(3,4,5-trimethoxyphenylamino)methyl)acrylate (10j). Colorless oil; yield 92%. ¹H NMR (400 MHz, CDCl3): δ 6.62 (s, 2H), 6.40 (s, 1H), 5.98 (s, 1H), 5.86 (s, 2H), 5.34 (s, 1H), 4.29 (s, br, 1H), 4.20 (m, 2H), 3.82-3.74 (m, 18H), 1.25 (t, J = 7.2 Hz, 3H).

4.3.11

(S)-Ethyl

2-((3-((*tert***-Butyldimethylsilyl)oxy)-4-methoxyphenyl)((3-methoxyphenyl)amino)methyl) acrylate (10k)**. Yellow oil; yield 87%. ¹H NMR (400 MHz, CDCl₃): δ 7.07 (t, *J* = 8.2 Hz, 1H), 6.92 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.84 (d, *J* = 2.0 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 6.30 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.21 (d, *J* = 8.2 Hz, 1H), 6.14 (t, *J* = 2.0 Hz, 1H), 5.90 (s, 1H), 5.30 (s, 1H), 4.18-4.12 (m, 2H), 3.80 (s, 3H), 3.76 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H), 0.99 (s, 9H), 0.14 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 165.7, 160.1, 149.9, 147.6, 144.4, 139.9, 132.6, 129.2, 124.7, 120.1, 119.7, 111.5, 105.9, 102.2, 98.9, 60.1, 57.8, 54.9, 54.4, 25.1, 17.8, 13.5, -5.2. ESI-MS (*m*/*z*): 472.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₆H₃₇NO₅Si + H⁺ [M + H⁺], 472.2514; found, 472.2514.

4.3.12

(S)-Ethyl

2-((3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)((4-methoxyphenyl)amino)methyl) acrylate (10l). Yellow oil; yield 73%. ¹H NMR (400 MHz, CDCl₃): δ 6.91 (dd, J = 8.2, 1.8 Hz, 1H), 6.84 (d, J = 1.8 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 8.8 Hz, 2H).), 6.53 (d, J = 8.8 Hz, 2H), 6.33 (s, 1H), 5.88 (s, 1H), 5.22 (s, 1H), 4.23-4.07 (m, 2H), 3.89 (s, 1H), 3.78 (s, 3H), 3.73 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.97 (s, 9H), 0.12 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 167.4, 151.8, 151.5, 144.4, 141.9, 141.4, 127.1, 126.8, 123.6, 121.8, 114.2, 111.2, 60.3, 55.2, 54.8, 41.4, 25.1, 25.0, 17.7, 13.7, -5.4. ESI-MS (m/z): 472.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₆H₃₇NO₅Si + H⁺ [M + H⁺], 472.2514; found, 472.2515.

4.3.13

(S)-Ethyl

2-((3-((*tert***-Butyldimethylsilyl)oxy)-4-methoxyphenyl)((3,5-dimethoxyphenyl)amino)met hyl)acrylate (10m)**. Yellow oil; yield 88%. ¹H NMR (400 MHz, CDCl₃): δ 6.89 (dd, J = 8.3, 2.1 Hz, 1H), 6.82-6.78 (m, 2H), 6.35 (s, 1H), 5.88 (d, J = 2.1 Hz, 2H), 5.77 (d, J = 2.1 Hz, 2H), 5.28 (s, 1H), 4.21-4.05 (m, 2H), 3.78 (s, 3H), 3.72 (s, 6H), 1.20 (t, J = 7.1 Hz, 3H), 0.97 (s, 9H), 0.12 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 166.2, 161.6, 150.5, 148.7, 145.1, 140.5, 133.2, 125.4, 120.7, 120.3, 112.2, 92.4, 90.1, 60.7, 58.5, 55.5, 55.1, 25.7, 18.5, 14.3, -4.6. ESI-LRMS (m/z): 502.2 (M + H⁺). ESI-HRMS (m/z): calcd for [C₂₇H₃₉NO₆Si + H⁺ [M + H⁺], 502.2619; found, 502.2619.

4.4 General Method for the Cyclization of Allylic Amination Products. To a 250 mL Schlenk flask equipped with a cold finger was added appropriate allylic amination product **10** (10 mmol), Sn[N(TMS)₂]₂ (6.6 g, 15 mmol) and dry toluene (100 mL). The mixture was heated to reflux for 3 h under nitrogen atmosphere. The solution was cooled and directly purified by flash chromatography to give the title compound.

4.4.1 (*S*)-3-Methylene-1,4-diphenylazetidin-2-one (11a). White solid; yield 85%; mp 149-150 °C; $[\alpha]_D^{20} = +98.9$ (*c* 1.00, CHCl₃), 96% *ee* [determined by HPLC analysis using a

Chiralcel OD-H column; *n*-Hex / *i*-PrOH = 95:5, 1.0 mL/min, 254 nm; t_R (minor) = 8.22 min; t_R (major) = 10.43 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.31 (m, 7H), 7.23 (t, *J* = 8.0 Hz, 2H), 7.03 (t, *J* = 7.6 Hz, 1H), 5.81 (s, 1H), 5.38 (s, 1H), 5.12 (s, 1 H). ESI-MS (*m*/*z*): 236.1 [M + H⁺].

4.4.2 (*S*)-3-Methylene-4-phenyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (11b). White solid; yield 74%; mp 149-150 °C; $[\alpha]_D^{20} = +53.7$ (*c* 1.28, CHCl₃), 96% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 85:15, 1.0 mL/min, 254 nm; t_R (minor) =10.00 min; t_R (major) = 11.54 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.41-7.33 (m, 5H), 6.60 (s, 2H), 5.82 (s, 1H), 5.37 (s, 1H), 5.15 (s, 1H), 3.76 (s, 3H), 3.70 (s, 6H). ESI-MS (*m*/*z*): 348.1 [M + Na⁺].

4.4.3 (*S*)-3-Methylene-4-(*p*-tolyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (11c). Colorless oil; yield 89%; $[\alpha]_D^{20} = +87.5$ (*c* 0.12, CHCl₃), 97% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 85:15, 1.0 mL/min, 254 nm; t_R (minor) = 9.04 min; t_R (major) = 11.43 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.29 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 2H), 6.58 (s, 2H), 5.83 (t, *J* = 1.7 Hz, 1H), 5.33 (s, 1H), 5.16-5.12 (m, 1H), 3.76 (s, 3H), 3.72 (s, 6H), 2.35 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.9, 153.5, 149.9, 138.9, 134.7, 133.8, 133.5, 129.8, 126.8, 110.7, 94.9, 63.9, 60.9, 56.1, 21.2. ESI-MS (*m*/*z*): 340.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₁NO₄ + H⁺ [M + H⁺], 340.1543; found, 340.1550.

4.4.4 (S)-4-(4-Methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (11d). Colorless oil; yield 74%; $[\alpha]_D^{20} = +41.5$ (c 1.00, CHCl₃), 96% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 90:10, 1.0 mL/min, 254

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nm; t_R (minor) = 18.05 min; t_R (major)= 25.54 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.34 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.61 (s, 2H), 5.82 (t, J = 1.6 Hz, 1H), 5.34 (s, 1H), 5.15 (t, J = 1.6 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H). ESI-MS (m/z): 378.1 [M + Na⁺].

4.4.5

(*S*)-4-(3-Fluoro-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-on e (11e). Yellow oil; yield 58%. $[\alpha]_D^{20} = +107.0$ (*c* 0.17, CHCl₃), 97% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 85:15, 1.0 mL/min, 254 nm; t_R (minor) = 14.68 min; t_R (major) = 21.68 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.15-7.10 (m, 2H), 6.96 (t, *J* = 8.5 Hz, H), 6.57 (s, 2H), 5.84 (s, 1H), 5.30 (s, 1H), 5.16 (s, 1H), 3.88 (s, 3H), 3.76 (s, 3H), 3.74 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.0, 153.0, 152.9, 151.2, 148.9, 147.6, 147.5, 134.2, 133.0, 128.7, 128.7, 122.3, 122.2, 113.9, 113.8, 113.07, 110.4, 94.2, 62.5, 60.3, 55.7, 55.5. ESI-MS (*m*/*z*): 374.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H_{14c}NO₅ + H⁺ [M + H⁺], 374.1398; found, 374.1400.

4.4.6

(*S*)-4-(3-Chloro-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-on e (11f). White solid; yield 68%; mp 119-120 °C; $[\alpha]_D^{20} = +35.1$ (*c* 0.29, CHCl₃), 97% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 80:20, 1.0 mL/min, 254 nm; t_R (minor) = 10.62 min; t_R (major) = 13.97 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.41 (d, *J* = 2.0 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.56 (s, 2H), 5.83 (s, 1H), 5.28 (s, 1H), 5.16 (s, 1H), 3.88 (s, 3H), 3.75 (s, 3H), 3.73 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.1, 154.8, 153.0, 148.8, 134.2, 133.0, 128.9, 128.2, 125.7, 122.6, 111.8, 110.5, 94.2, 62.3, 60.3, 55.6, 55.5. ESI-MS (m/z): 390.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₀H₂₀ClNO₅ + H⁺ [M + H⁺], 390.1103; found, 390.1103.

4.4.7

(*S*)-4-(3-Amino-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-on e (11g). Synthesized using 10g as starting material. Yellow oil; yield 37%; $[\alpha]_D^{20} = +50.0$ (*c* 0.14, CHCl₃), 97% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 80:20, 1.0 mL/min, 254 nm; t_R (minor) = 19.58 min; t_R (major) = 17.42 min]. ¹H NMR (400 MHz, CDCl₃): δ 6.79 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.71 (d, *J* = 1.9 Hz, 1H), 6.62 (s, 2H), 5.80 (t, *J* = 1.6 Hz, 1H), 5.24 (s, 1H), 5.15 (t, *J* = 1.6 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.74 (s, 6H). ¹³C NMR (CDCl₃): δ 161.1, 153.5, 150.0, 147.8, 136.8, 134.6, 134.0, 129.0, 117.5, 112.5, 110.5, 110.3, 94.9, 64.0, 60.9, 56.1, 55.5. ESI-MS (*m*/*z*): 371.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₂N₂O₅ + H⁺ [M + H⁺], 371.1601; found, 371.1605.

4.4.8

(S)-N-(2-Methoxy-5-(3-methylene-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl) acrylamide (11h). A 50 mL round-bottom flask was charged with 11g (20 mg, 0.054 mmol), TEA (15 μ L, 0.108 mmol), acryloyl chloride (10 mg, 0.081 mmol) and anhydrous CH₂Cl₂ (2 mL). The solution was stirred for 1 h at room temperature. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (16 mg, yield 66%); mp 143-144 °C; $[\alpha]_D^{20} = -96.4$ (*c* 0.11, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 8.70 (s, 1H, NH), 7.93 (s, 1H), 7.05 (dd, J = 8.5, 1.9 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.62 (s, 2H), 6.43 (d, J = 16.8 Hz, 1H), 6.29 (dd, J = 16.8, 10.1 Hz, 1H), 5.83 (s, 1H), 5.79 (d, J = 10.1 Hz, 1H), 5.36 (s, 1H), 5.19 (s, 1H), 3.89 (s, 3H), 3.75 (s, 3H), 3.74 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 162.8, 160.3, 152.9, 149.1, 147.4, 133.9, 133.2, 130.6, 128.4, 127.3, 127.2, 121.0, 118.7, 110.2, 110.0, 94.2, 63.1, 60.3, 55.5, 55.3. ESI-MS (m/z): 425.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₃H₂₄N₂O₆ + NH₄⁺ [M + NH₄⁺], 442.1973; found, 442.1975.

4.4.9

(*S*)-4-(3-((*tert*-Butyldimethylsilyl)oxy)phenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)az etidin-2-one (11i). Colorless oil; yield 76%. ¹H NMR (400 MHz, CDCl₃): δ 7.27-7.20 (m, 1H), 6.99 (d, *J* = 7.7 Hz, 1H), 6.83-6.78 (m, 2H), 6.57 (s, 2H), 5.81 (t, *J* = 1.7 Hz, 1H), 5.30 (s, 1H), 5.16-5.15 (m, 1H), 3.75 (s, 3H), 3.71 (s, 6H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.7, 156.4, 153.6, 149.6, 138.0, 134.8, 133.7, 130.1, 120.7, 119.8, 118.2, 110.7, 95.0, 63.7, 60.9, 56.0, 25.6, 18.2, -4.4, -4.5. ESI-MS (*m*/*z*): 456.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₅H₃₃NO₅Si + H⁺ [M + H⁺], 456.2201; found, 456.2201.

4.4.10 (*S*)-3-Methylene-1,4-bis(3,4,5-trimethoxyphenyl)azetidin-2-one (11k). White solid; yield 80%; mp 160-161 °C; $[\alpha]_D{}^{20} = +28.7$ (*c* 1.00, CHCl₃), 98% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 80:20, 1.0 mL/min, 254 nm; t_R (minor) =11.81 min; t_R (major) = 13.90 min]. ¹H NMR (400 MHz, CDCl₃): δ 6.63-6.62 (m, 4H), 5.85 (t, *J* = 2.0Hz, 1H), 5.30 (s, 1H), 5.22 (t, *J* = 1.6 Hz, 1H), 3.85-3.84 (m, 9H), 3.78 (s, 3H), 3.75 (s, 6H). ESI-MS (*m*/*z*): 438.1 [M + Na⁺].

4.4.11

(*S*)-4-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxy phenyl)azetidin-2-one (111) [8]. Colorless oil; yield 92%; $[\alpha]_D{}^{20} = +37.6$ (c 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.95 (dd, J = 8.3, 2.1 Hz, 1H), 6.85-6.81 (m, 2H), 6.58 (s, 2H), 5.80 (s, 1H), 5.26 (s, 1H), 5.13 (s, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.71 (s, 6H), 0.92 (s, 9H), 0.06 (d, J = 4.1 Hz, 6H). ESI-MS (m/z): 486.1 (M + H⁺).

4.4.12

(*S*)-4-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3-methoxyphenyl)-3-methyl eneazetidin-2-one (11m). Colorless oil; yield 69%. ¹H NMR (400 MHz, CDCl₃): δ 7.15 (t, *J* = 8.3 Hz, 1H), 7.04 (s, 1H), 6.97 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.86-6.84 (m, 3H), 6.61 (dd, *J* = 8.3, 1.8 Hz, 1H), 5.84 (s, 1H), 5.30 (s, 1H), 5.17 (s, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 0.96 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.5, 159.5, 150.8, 149.4, 144.8, 138.1, 129.2, 128.1, 119.6, 118.7, 111.6, 110.1, 109.5, 108.9, 102.4, 62.8, 54.9, 54.6, 25.1, 17.8, -5.3. ESI-MS (*m*/*z*): 426.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₄H₃₁NO₄Si + H⁺ [M + H⁺], 426.2095; found, 426.2096.

4.4.13

(S)-4-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(4-methoxyphenyl)-3-methyl eneazetidin-2-one (110). Colorless oil; yield 77%. ¹H NMR (400 MHz, CDCl₃): δ 7.28 (d, J = 9.1 Hz, 2H), 6.93 (dd, J = 8.3, 1.8 Hz, 1H), 6.83-6.77 (m, 4H), 5.78 (s, 1H), 5.25 (s, 1H), 5.11 (s, 1H), 3.79 (s, 3H), 3.74 (s, 3H), 0.94 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.1, 155.6, 150.8, 149.5, 144.8, 130.5, 128.2, 119.7, 118.8, 117.9,

113.7, 111.6, 109.3, 62.7, 54.8, 25.1, 17.8, -5.2, -5.3. ESI-MS (m/z): 426.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₄H₃₁NO₄Si + H⁺ [M + H⁺], 426.2095; found, 426.2096.

4.4.14

(*S*)-4-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)-1-(3,5-dimethoxyphenyl)-3-met hyleneazetidin-2-one (11q). Colorless oil, 76% yield. ¹H NMR (400 MHz, CDCl₃): δ 6.94 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.83-6.81 (m, 2H), 6.53 (d, *J* = 2.2 Hz, 2H), 6.16 (t, *J* = 2.2 Hz, 1H), 5.81 (t, *J* = 1.7 Hz, 1H), 5.25 (s, 1H), 5.18-5.10 (m, 1H), 3.79 (s, 3H), 3.70 (s, 6H), 0.94 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 161.2, 161.1, 151.4, 150.1, 145.6, 139.2, 128.8, 120.2, 119.3, 112.3, 110.7, 96.7, 95.8, 63.6, 55.5, 55.3, 25.7, 18.4, -4.7. ESI-MS (*m*/*z*): 456.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₅H₃₃NO₅Si + H⁺ [M + H⁺], 456.2201; found, 456.2202.

4.5 General Method for the Deprotection of TBS. A 250 mL round-bottom flask was charged with appropriate TBS protected β -lactam compound (2 mmol) and THF (10 mL). The solution was cooled to 0 °C by an ice base, then TBAF (0.68 g, 2.6 mmol) was added into the flask. After stirred for 10 min at 0 °C, 30 mL of water was added into the solution. The mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give the title compounds.

4.5.1 (*S*)-4-(3-Hydroxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (11j). White solid; yield 86%; mp 150-151 °C; $[\alpha]_D^{20} = +101.5$ (*c* 0.13, CHCl₃), 98% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 80:20, 1.0

mL/min, 254 nm; t_R (minor) = 12.09 min; t_R (major) = 8.43 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.59 (s, 1H), 7.22-7.21 (m, 1H), 6.94 (d, J = 7.6 Hz, 1H), 6.88-6.86 (m, 2H), 6.56 (s, 2H), 5.73 (s, 1H), 5.31 (s, 1H), 5.14 (dd, J = 1.7, 1.2 Hz, 1H), 3.74 (s, 3H), 3.66 (s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 161.4, 157.4, 153.5, 148.7, 137.7, 133.5, 130.3, 119.1, 116.6, 113.0, 111.5, 95.1, 64.1, 60.9, 56.0. ESI-MS (m/z): 342.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₁₉H₁₉NO₅ + H⁺ [M + H⁺], 342.1336; found, 342.1337.

4.5.2

(S)-4-(3-Hydroxy-4-methoxyphenyl)-1-(3-methoxyphenyl)-3-methyleneazetidin-2-one

(11n). White solid; yield 81%; mp 104-105 °C; $[\alpha]_D^{20} = +61.0$ (*c* 0.21, CHCl₃), 99% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 75:25, 1.0 mL/min, 254 nm; t_R (minor) = 10.45 min; t_R (major) = 12.04 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.14 (t, *J* = 8.2 Hz, 1H), 7.04 (s, 1H), 6.95 (d, *J* = 1.9 Hz, 1H), 6.89 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.84-6.82 (m, 2H), 6.60 (dd, *J* = 8.2, 1.6 Hz, 1H), 5.82 (s, 1H), 5.73 (br s, 1H), 5.29 (s, 1H), 5.15 (s, 1H), 3.88 (s, 3H), 3.75 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 161.1, 160.1, 145.0, 147.0, 146.2, 138.7, 129.9, 129.6, 118.6, 112.8, 110.9, 110.8, 110.1, 109.5, 103.1, 63.5, 56.0, 55.3. ESI-MS (*m*/*z*): 334.1 (M + Na⁺). ESI-HRMS (*m*/*z*): calcd for C₁₈H₁₇NO₄ + H⁺ [M + H⁺], 312.1230; found, 312.1232.

4.5.3

(S)-4-(3-Hydroxy-4-methoxyphenyl)-1-(4-methoxyphenyl)-3-methyleneazetidin-2-one

(11p). White solid; yield 86%; mp 180-181 °C; $[\alpha]_D^{20} = +113.4$ (*c* 0.10, CHCl₃), 97% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 75:25, 1.0 mL/min, 254 nm; t_R (minor) = 15.28 min; t_R (major) = 19.43 min]. ¹H NMR (400 MHz, CDCl₃): δ 7.28 (d, J = 8.9 Hz, 2H), 6.94 (d, J = 1.9 Hz, 1H), 6.87 (dd, J = 8.1, 1.9 Hz, 1H), 6.83-6.77 (m, 3H), 5.77 (s, 1H), 5.77 (s, 1H), 5.26 (s, 1H), 5.10 (s, 1H), 3.87 (s, 3H), 3.73 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.0, 155.6, 149.5, 146.3, 145.5, 130.5, 129.0, 117.9, 117.9, 113.8, 112.3, 110.3, 109.4, 62.7, 55.3, 54.8; ESI-MS (m/z): 334.1 (M + Na⁺). ESI-HRMS (m/z): calcd for C₁₈H₁₇NO₄ + H⁺ [M + H⁺], 312.1230; found, 312.1231.

4.5.4

(*S*)-1-(3,5-Dimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-3-methyleneazetidin-2-on e (11r). Colorless oil, yield 90%; $[\alpha]_D^{20} = +49.6$ (*c* 0.12, CHCl₃), 99% *ee* [determined by HPLC analysis using a Chiralcel AD-H column; *n*-Hex / *i*-PrOH = 80:20, 1.0 mL/min, 254 nm; t_R (minor) = 13.41 min; t_R (major) = 14.97 min]. ¹H NMR (400 MHz, CDCl₃): δ 6.93 (d, J = 2.0 Hz, 1H), 6.87 (dd, J = 8.2, 2.0 Hz, 1H), 6.81 (d, J = 8.2 Hz, 1H), 6.53 (d, J = 2.2 Hz, 2H), 6.15 (t, J = 2.2 Hz, 1H), 5.94 (s, 1H), 5.80 (t, J = 1.2 Hz, 1H), 5.25 (s, 1H), 5.13 (t, J =1.2 Hz, 1H), 3.84 (s, 3H), 3.70 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.6, 160.5, 149.3, 146.5, 145.6, 138.6, 128.9, 118.0, 112.2, 110.4, 110.3, 95.9, 95.2, 63.0, 55.3, 54.7. ESI-MS (*m*/*z*): 342.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₁₉H₁₉NO₅ + H⁺ [M + H⁺], 342.1336; found, 342.1337.

4.5.5

(S)-4-(3,4-Dimethoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-one

(12a). A 50 mL round-bottom flask was charged with 3 (20 mg, 0.054 mmol), K_2CO_3 (15 mg, 0.108 mmol), dimethylsulfate (10 μ L, 0.106 mmol) and acetone (2 mL). The solution was heated to reflux for 1 h. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was

separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (20 mg, yield 96%); mp 138-139 °C; $[\alpha]_D^{20} = +35.6$ (*c* 0.21, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.00 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 1.8 Hz, 1H), 6.60 (s, 2H), 5.84 (s, 1H), 5.31 (s, 1H), 5.17 (s, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.76 (s, 3H), 3.73 (s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 161.3, 153.8, 150.1, 150.0, 149.9, 134.9, 134.2, 129.1, 120.2, 111.4, 111.2, 109.3, 95.1, 64.3, 61.3, 56.3. ESI-MS (*m*/*z*): 386.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₁H₂₃NO₆ + H⁺ [M + H⁺], 386.1598; found, 386.1598.

4.5.6

(*S*)-4-(3-Ethoxy-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-on e (12b). A 50 mL round-bottom flask was charged with 3 (20 mg, 0.054 mmol), K₂CO₃ (15 mg, 0.108 mmol), bromoethane (8 μ L, 0.108 mmol) and DMF (1.5 mL). The solution was stirred overnight at room temperature. Then 30 mL of ethyl acetate was added into the solution, and the mixture was washed with H₂O (30 mL × 3), brine (30 mL), dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (23 mg, yield 97%); mp 92-93 °C; $[\alpha]_D^{20} = +34.0$ (*c* 0.27, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.98 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.83 (d, *J* = 2.0 Hz, 1H), 6.60 (s, 2H), 5.83 (s, 1H), 5.29 (s, 1H), 5.16 (s, 1H), 4.02 (q, *J* = 7.0 Hz, 2H), 3.86 (s, 3H), 3.75 (s, 3H), 3.72 (s, 6H), 1.41 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 161.2, 153.6, 150.0, 149.9, 149.1, 134.8, 134.0, 128.9, 112.0, 111.5, 111.1, 110.6, 94.9, 64.6, 64.2, 61.2, 56.2, 56.1, 14.8. ESI-MS (m/z): 400.2 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₂H₂₅NO₆ + H⁺ [M + H⁺], 400.1755; found, 400.1756.

4.5.7

(S)-4-(4-Methoxy-3-propoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-

one (12c). 12c was synthesized via the same procedure with 12b, using 1-bromopropane as the alkylating reagent. White solid (19 mg, yield 85%); mp 90-91 °C; $[\alpha]_D^{20} = +40.0$ (*c* 0.07, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.98 (dd, J = 8.2 Hz, J = 1.4 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 1.4 Hz, 1H), 6.60 (s, 2H), 5.84 (s, 1H), 5.30 (s, 1H), 5.17 (s, 1H), 3.90 (t, J = 6.8 Hz, 2H), 3.86 (s, 3H), 3.76 (s, 3H), 3.73 (s, 6H), 1.87-1.76 (m, 2H), 1.00 (t, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 161.4, 153.8, 150.3, 150.1, 149.5, 134.9, 134.2, 129.0, 120.1, 111.8, 111.1, 110.9, 95.1, 70.8, 64.4, 61.3, 56.3, 22.7, 10.7. ESI-MS (*m*/*z*): 436.2 (M + Na⁺). ESI-HRMS (*m*/*z*): calcd for C₂₃H₂₇NO₆ + H⁺ [M + H⁺], 414.1911; found, 414.1912.

4.5.8

(*S*)-4-(3-Isopropoxy-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (12d). 12d was synthesized via the same procedure with 12b, using 2-bromopropane as the alkylating reagent. White solid (21 mg, yield 94%); mp 146-147 °C; $[\alpha]_D^{20} = +13.1$ (*c* 0.21, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.98 (d, J = 8.2 Hz, 1H), 6.91-6.81 (m, 2H), 6.60 (s, 2H), 5.83 (s, 1H), 5.29 (s, 1H), 5.16 (s, 1H), 4.50–4.44 (m, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H), 1.29 (dd, J = 17.7, 6.0 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 160.4, 152.9, 150.4, 149.3, 147.2, 134.1, 133.2, 128.0, 119.5, 133.3, 111.4, 110.1, 70.1, 63.3, 60.3, 55.4, 55.3, 21.3, 21.2. ESI-MS (m/z): 436.2 (M + Na⁺). ESI-HRMS (m/z): calcd for C₂₃H₂₇NO₆ + H⁺ [M + H⁺], 414.1911; found, 414.1916.

4.5.9

(*S*)-4-(3-(Benzyloxy)-4-methoxyphenyl)-3-methylene-1-(3,4,5-trimethoxyphenyl)azetidin -2-one (12e). 12e was synthesized via the same procedure with 12b, using benzyl bromide as the alkylating reagent. White solid; mp 133-134 °C; $[\alpha]_D^{20} = +61.2$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.40-7.23 (m, 5H), 6.98 (dd, J = 8.2, 1.6 Hz, 1H), 6.92-6.82 (m, 2H), 6.54 (s, 2H), 5.78 (s, 1H), 5.24 (s, 1H), 5.11 (s, 2H), 5.07 (s, 1H), 3.89 (s, 3H), 3.77 (s, 3H), 3.70 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.3, 152.9, 149.7, 149.1, 147.9, 135.9, 134.0, 133.2, 128.1, 127.9, 127.3, 126.7, 119.6, 111.5, 111.2, 110.1, 94.1, 70.4, 63.2, 60.3, 55.4. ESI-MS (*m*/*z*): 462.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₇H₂₇NO₆ + H⁺ [M + H⁺], 462.1911; found, 462.1909.

4.5.10

(S)-2-Methoxy-5-(3-methylene-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl

Acetate (12f). A 100 mL round-bottom flask was charged with 3 (0.25 g, 0.673 mmol), triethylamine (0.13 mL, 0.94 mmol) and dichloromethane (10 mL). The solution was cooled to 0 °C by an ice bath, and then acetic anhydride (0.09 mL, 0.094 mmol) was added dropwise into the flask within 10 minutes. After stirred for 30 min, 20 mL of water was added into the solution, and the mixture was extracted with dichloromethane (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 2:1, v/v) to give the title compound as

white solid (0.23 g, yield 83%); mp 122-123 °C; $[\alpha]_D^{20} = +72.8$ (*c* 1.0, CHCl₃). ¹H NMR (CDCl₃): δ 7.25 (br d, J = 8.4 Hz, 1H), 7.10 (br s, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.57 (s, 1H), 5.84 (s, 1H), 5.30 (s, 1H), 5.18 (s, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.73 (s, 6H), 2.29 (s, 3H). ¹³C NMR (CDCl₃): δ 168.0, 160.2, 153.0, 151.0, 149.0, 139.6, 134.1, 133.1, 128.3, 124.6, 121.1, 112.2, 110.4, 94.2, 62.7, 60.3, 55.5, 55.4, 20.0. ESI-MS (*m*/*z*): 414.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₂H₂₃NO₇ + H⁺ [M + H⁺], 414.1547; found, 414.1546.

4.5.11

(S)-2-Methoxy-5-(3-methylene-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl

4-Methylbenzenesulfonate (12g). A 50 mL round-bottom flask was charged with **3** (20 mg, 0.054 mmol), TEA (15 μ L, 0.108 mmol), 4-methylbenzenesulfonyl chloride (16 mg, 0.084 mmol) and anhydrous dichloromethane (2 mL). The solution was stirred for 1 h at room temperature. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (27 mg, yield 95%); mp 162-163 °C; $[\alpha]_D^{20}$ = +53.1 (*c* 0.16, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.25-7.19 (m, 3H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.55 (s, 2H), 5.82 (s, 1H), 5.28 (s, 1H), 5.15 (s, 1H), 3.78 (s, 3H), 3.76 (s, 6H), 3.60 (s, 3H), 2.43 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 160.0, 153.0, 151.80, 148.9, 144.7, 138.1, 134.2, 133.0, 132.4, 128.8, 128.3, 127.8, 125.6, 122.2, 112.7, 110.4, 94.2, 62.3, 60.3, 55.5, 55.3, 21.0. ESI-MS (*m*/*z*): 548.1 (M + Na⁺). ESI-HRMS (*m*/*z*): calcd for C₂₇H₂₇NO₈S + NH₄⁺ [M + NH₄⁺], 543.1796; found, 543.1794.

4.5.12

(S)-2-Methoxy-5-(3-methylene-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-2-yl)phenyl

sulfamate (12h). A 50 mL round-bottom flask was charged with **3** (20 mg, 0.054 mmol), chlorosulfonamide (31 mg, 0.27 mmol) and anhydrous dichloromethane (2 mL). The solution was stirred for 1 h at room temperature. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (23 mg, yield 95%); mp 199-200 °C; $[α]_D^{20} = -14.3$ (*c* 0.10, DMSO). ¹H NMR (400 MHz, DMSO-d₆): *δ* 7.98 (d, *J* = 2.2 Hz, 1H), 7.83 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.12 (s, 2H), 6.23 (t, *J* = 1.7 Hz, 1H), 6.09 (s, 1H), 5.70 (s, 1H), 4.30 (s, 3H), 4.15 (s, 6H), 4.07 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): *δ* 160.1, 153.1, 151.8, 149.2, 138.6, 133.9, 132.9, 128.6, 125.9, 122.7, 113.7, 111.6, 94.6, 61.6, 60.0, 55.7, 55.6. ESI-MS (*m*/*z*): 451.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₂N₂O₈S + NH₄⁺ [M + NH₄⁺], 468.1435; found, 468.1435.

4.6 General Method for the Olefin Cross-metathesis of 3. A 50 mL Schlenk tube was charged with **3** (16 mg, 0.043 mmol), corresponding aryl ethylene (0.11 mmol), Grubb's 2nd generation catalyst (5 mg, 0.006 mmol) and 1,2-dichloroethane (1 mL). The resulting purple solution was stirred under nitrogen atmosphere for 12 h at 60 °C. Water (5 mL) was added into the solution, and the mixture was extracted with dichloromethane (15 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by

flash column chromatography (elute with EtOAc/hexane = 4:1, v/v) to give the title compounds with some **3** recovered.

4.6.1

$(S,Z) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 1-(3,4,5-trimethoxyphenyl) azetidin (S,Z) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 1-(3,4,5-trimethoxyphenyl) azetidin (S,Z) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 1-(3,4,5-trimethoxyphenyl) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 1-(3,4,5-trimethoxyphenyl) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 1-(3,4,5-trimethoxyphenyl) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenyl) \hbox{-} 3-Benzylidene-4-(3-hydroxy-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-methoxyphenylidene-4-m$

-2-one (13a). Yellow solid; yield 51% (with 3 recovered, recovery 31%); mp 168-169 °C; $[\alpha]_D^{20} = +102.3$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.99 (d, J = 7.2 Hz, 2H), 7.41-7.31 (m, 3H), 7.02 (d, J = 2.0 Hz, 1H), 6.96 (dd, J = 8.2, 2.0 Hz, 1H), 6.86 (d, J = 8.2Hz, 1H), 6.68 (s, 2H), 6.29 (s, 1H), 5.70 (s, 1H), 5.28 (s, 1H), 3.89 (s, 3H), 3.78 (s, 3H), 3.76 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 159.7, 152.9, 146.4, 145.6, 139.9, 133.9, 133.5, 133.4, 130.2, 129.5, 129.3, 129.0, 128.0, 118.2, 112.4, 110.3, 94.0, 61.8, 60.4, 55.4. ESI-MS (m/z): 448.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₆H₂₅NO₆ + H⁺ [M + H⁺], 448.1755; found, 448.1754.

4.6.2

(*S*,*Z*)-3-(4-(*tert*-Butyl)benzylidene)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyp henyl)azetidin-2-one (13b). Yellow solid; yield 30% (with 3 recovered, recovery 50%); mp 77-78 °C; $[α]_D^{20} = +131.8$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.92 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 1.9 Hz, 1H), 6.95 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.68 (s, 2H), 6.28 (s, 1H), 5.68 (s, 1H), 5.27 (s, 1H), 3.89 (s, 3H), 3.77 (s, 3H), 3.76 (s, 6H), 1.31 (s, 9H). ¹³C NMR (150 MHz, CDCl₃): δ 159.9, 152.9, 152.4, 146.4, 145.6, 139.1, 133.8, 133.6, 130.8, 130.0, 129.6, 129.1, 125.0, 118.2, 112.5, 110.3, 94.0, 76.6, 76.4, 76.2, 61.8, 60.4, 55.4, 55.4, 34.2, 30.5. ESI-MS (*m*/*z*): 504.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₃₀H₃₃NO₆ + H⁺ [M + H⁺], 504.2381; found, 504.2376.

4.7

(3S,4R)-4-(3-Hydroxy-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one(14a)

(3*R*,4*R*)-4-(3-hydroxy-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (14b). A 50 mL Schlenk tube was charged with 3 (0.11 g, 0.3 mmol), bis(pinacolato)diboron (0.1 g, 0.4 mmol), PPh₃ (12 mg, 0.045 mmol), t-BuOLi (2.4 mg, 0.03 mmol), CuCl (1.5 mg, 0.015 mmol), MeOH (15 µL, 0.45 mmol) and anhydrous THF (5 mL). The mixture was stirred under nitrogen atmosphere for 12 h at room temperature. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in THF/H₂O (5 mL/5 mL). Then NaBO₃·4H₂O (0.23 g, 1.5 mmol) was added into the mixture and the resulting solution was stirred for 2 h at room temperature. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 2:1, v/v) to give the title compounds.

14a. White gel; yield 46%; $[\alpha]_D^{20} = +101.2$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.94-6.77 (m, 3H), 6.55 (s, 2H), 5.75 (s, 1H), 5.16 (d, *J* = 5.2 Hz, 1H), 3.90 (s, 3H), 3.87-3.61 (m, 12H). ¹³C NMR (150 MHz, CDCl₃): δ 165.2, 153.6, 146.7, 146.1, 134.7, 133.6, 127.1, 118.5, 112.9, 110.9, 95.1, 61.0, 58.2, 57.3, 56.7, 56.2, 56.0. ESI-MS (m/z): 390.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₀H₂₃NO₇ + H⁺ [M + H⁺], 390.1547; found, 390.1546.

14b. White gel; yield 21%; $[\alpha]_D^{20} = +15.7$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.97 (d, J = 1.5 Hz, 1H), 6.91 (dd, J = 8.3, 1.5 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 6.56 (s, 2H), 5.70 (s, 1H), 4.91 (d, J = 1.8 Hz, 1H), 4.00 (dd, J = 11.9, 3.3 Hz, 1H), 3.90 (s, 3H), 3.76 (s, 3H), 3.73 (br s, 7H), 3.28 (d, J = 2.3 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 165.2, 153.6, 146.7, 146.1, 134.7, 133.6, 127.1, 118.5, 112.9, 110.9, 95.1, 61.0, 58.2, 57.3, 56.7, 56.2, 56.0. ESI-MS (*m*/*z*): 390.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₃NO₇ + H⁺ [M + H⁺], 390.1547; found, 390.1546.

4.8

(35,4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2 -one (14c). A 50 mL round-bottom flask was charged with 14b (0.1 g, 0.26 mmol), K_2CO_3 (53 mg, 0.39 mmol), BnCl (36 µL, 0.31 mmol) and MeCN (5 mL). The mixture was stirred reflux for 12 h. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in anhydrous THF (5 mL). Then CBr₄ (0.26 g, 0.78 mmol) and PPh₃ (0.2 g, 0.78 mmol) were added into the mixture and the resulting solution was stirred for 6 h at room temperature. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was then dissolved in 2 mL of ethanol, 10% Pd/C (10 mg) and AcONa (0.1 g, 1.3 mmol) were added and the solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:2, v/v) to give the title compounds as white solid (20 mg, yield 21%); mp 54-55 °C; $[\alpha]_D^{20}$ = +12.8 (*c* 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.93 (d, *J* = 1.5 Hz, 1H), 6.87 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.54 (s, 2H), 5.69 (s, 1H), 4.44 (d, *J* = 2.2 Hz, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H), 3.11 (qd, *J* = 7.3, 2.2 Hz, 1H), 1.45 (d, *J* = 7.3 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 167.7, 152.9, 146.1, 145.6, 133.7, 133.5, 130.5, 117.1, 111.4, 110.3, 94.1, 62.2, 60.3, 55.4, 54.5, 12.4. ESI-MS (*m*/*z*): 374.0 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₃NO₆ + H⁺ [M + H⁺], 374.1589; found, 374.1602.

4.9

(3*S*,4*R*)-4-(3-Amino-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl)az etidin-2-one (14d) and

(3*R*,4*R*)-4-(3-amino-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl)az etidin-2-one (14e). A 50 mL Schlenk tube was charged with 11g (0.1 g, 0.27 mmol) bis(pinacolato)diboron (0.89 g, 0.35 mmol), PPh₃ (11 mg, 0.04 mmol), *t*-BuOLi (2.2 mg, 0.027 mmol), CuCl (1.4 mg, 0.014 mmol), MeOH (16 μ L, 0.4 mmol) and anhydrous THF (3 mL) were added in. The mixture was stirred under nitrogen atmosphere for 12 h at room temperature. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in THF/H₂O (5 mL/1 mL). Then NaBO₃·4H₂O (0.12 g, 0.81 mmol)

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was added into the mixture and the resulting solution was stirred for 2 h at room temperature. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compounds.

14d. White gel; yield 42%; $[\alpha]_D^{20} = +109.3$ (*c* 0.37, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.77 (d, J = 8.1 Hz, 1H), 6.68-6.64 (m, 2H), 6.57 (s, 2H), 5.11 (d, J = 5.4 Hz, 1H), 3.85 (s, 3H), 3.81-3.75 (m, 5H), 3.72 (s, 6H), 3.68 (dd, J = 10.5, 3.1 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 164.7, 152.9, 146.7, 136.2, 133.9, 133.2, 125.8, 115.9, 111.8, 109.9, 94.3, 60.3, 57.6, 56.9, 56.1, 55.5, 54.9. ESI-MS (*m*/*z*): 389.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₅N₂O₆ + H⁺ [M + H⁺], 389.1713; found, 389.1718.

14e. White gel; yield 17%; $[\alpha]_D^{20} = +19.6$ (*c* 0.35, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.78-6.75 (m, 2H), 6.72 (br s, 1H), 6.57 (s, 2H), 4.85 (d, J = 2.3 Hz, 1H), 4.13 (dd, J = 12.0, 4.1 Hz, 1H), 3.99 (dd, J = 12.0, 3.4 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 3.72 (s, 6H), 3.27 (dd, J = 6.7, 4.1 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 165.3, 152.8, 146.9, 136.4, 133.8, 133.23, 129.4, 115.8, 111.1, 109.8, 94.2, 61.5, 60.3, 58.3, 57.1, 55.4, 54.9. ESI-MS (*m/z*): 389.1 (M + H⁺). ESI-HRMS (*m/z*): calcd for C₂₀H₂₅N₂O₆ + H⁺ [M + H⁺], 389.1713; found, 389.1720.

4.10

(3*R*,4*R*)-4-(3-Amino-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-o ne (14f). A 50 mL round-bottom flask was charged with 11g (10 mg, 0.027 mmol), 10% Pd/C (2 mg) and ethanol (2 mL). The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 2:1, v/v) to give the title compounds as white solid (9 mg, yield 89%); mp 103-104 °C; $[\alpha]_D^{20} = +86.7$ (*c* 0.9, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.74 (d, *J* = 8.1 Hz, 1H), 6.59-6.56 (m, 4H), 5.01 (d, *J* = 5.8 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.71 (s, 6H), 3.63-3.54 (m, 1H), 0.91 (d, *J* = 7.6 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 168.1, 152.8, 146.5, 135.7, 133.7, 133.5, 126.6, 116.5, 112.5, 109.6, 94.3, 60.3, 58.0, 55.5, 54.9, 48.7, 9.0. ESI-MS (*m*/*z*): 373.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₅N₂O₅ + H⁺ [M + H⁺], 373.1763; found, 373.1767.

4.11

(35,4*R*)-4-(3-Amino-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2-o ne (14g). A 50 mL round-bottom flask was charged with 14e (20 mg, 0.052 mmol), Boc₂O (22 mg, 0.1 mmol), Et₃N, (14 μ L, 0.1 mmol) and THF (5 mL). The mixture was stirred reflux for 24 h. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in anhydrous THF (5 mL). Then CBr₄ (86 g, 0.26 mmol) and PPh₃ (67 mg, 0.78 mmol) were added into the mixture and the resulting solution was stirred for 6 h at room temperature. Water (10 mL) for 3 times. The organic layer was separated, washed with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with ethyl acetate (10 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was then dissolved in 2 mL of ethanol, 10% Pd/C (3 mg) and AcONa (43 mg, 0.52 mmol) were added and the solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was dissolved in DCM (5 mL). 0.1 mL of TFA was added into the solution, and it was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:2, v/v) to give the title compounds as colorless gel (3.5 mg, yield 18%); $[\alpha]_D{}^{20} = +10.3$ (*c* 0.2, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.93 (d, *J* = 1.5 Hz, 1H), 6.77-6.73 (m, 2H), 6.69 (d, *J* = 1.6 Hz, 1H), 6.56 (s, 2H), 4.40 (d, *J* = 2.1 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 3.72 (s, 6H), 3.11 (dd, *J* = 7.2, 2.0 Hz, 1H), 1.44 (d, *J* = 7.4 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 167.9, 152.8, 146.8, 136.3, 133.7, 133.6, 129.8, 115.6, 110.9, 109.8, 94.3, 94.0, 62.5, 60.3, 55.4, 54.9, 54.4, 12.5. ESI-MS (*m*/*z*): 373.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₅N₂O₅ + H⁺ [M + H⁺], 373.1763; found, 373.1765.

4.12

(3*S*,4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(methoxymethyl)-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (14h). A 25 mL round-bottom flask was charged with 3 (20 mg, 0.054 mmol), MeONa (7 mg, 0.135 mmol) and MeOH (1 mL). The solution was stirred at room temperature for 12 h. 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 3:1, v/v) to give the title compounds as white gel (14 mg, yield 66%); $[\alpha]_D^{20}$ = +71.8 (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.88-6.73 (m, 3H), 6.54 (s, 2H), 5.76

(s, 1H), 5.12 (d, J = 5.7 Hz, 1H), 3.87 (s, 3H), 3.85-3.78 (m, 1H), 3.75 (s, 3H), 3.70 (s, 6H), 3.48 (dd, J = 10.1, 4.5 Hz, 1H), 3.24 (dd, J = 10.1, 8.6 Hz, 1H), 3.00 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 164.4, 152.9, 145.9, 145.1, 133.9, 133.1, 126.6, 118.3, 112.7, 109.9, 94.4, 66.3, 60.3, 58.0, 57.1, 55.5, 55.3, 54.6, 29.1. ESI-LRMS (m/z): 404.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₁H₂₅NO₇ + H⁺ [M + H⁺], 404.1704, found, 404.1707.

4.13

(3R,4R)-3-((Dimethylamino)methyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethox yphenyl)azetidin-2-one (14i). A 25 mL Schlenk tube was charged with 3 (16 mg, 0.043 mmol), dimethylamine hydrochloride (11 mg, 0.13 mmol), Et₃N (19 µL, 0.13 mmol) and MeOH (1 mL). The solution was heated to reflux for 6 h. 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 3:1, v/v) to give the title compounds as light yellow solid (13 mg, yield 75%); mp 79-81 °C; $[\alpha]_D^{20} = +86.8$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.85-6.83 (m, 2H), 6.77 (dd, J = 8.2, 1.2 Hz, 1H), 6.55 (s, 2H), 5.12 (d, J =5.8 Hz, 1H), 3.90 (s, 3H), 3.82-3.74 (m, 4H), 3.71 (s, 6H), 2.44 (dd, J = 13.3, 6.8 Hz, 1H), 2.25 (dd, J = 13.3, 5.8 Hz, 1H), 2.17 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 166.0, 152.8, 146.1, 145.3, 133.8, 133.1, 126.9, 118.4, 112.9, 110.1, 94.3, 60.3, 57.6, 55.4, 55.3, 53.8, 53.0, 44.9. ESI-MS (m/z): 417.1 $(M + H^{+})$. ESI-HRMS (m/z): calcd for $C_{22}H_{28}N_2O_6 + H^{+}[M + H^{+}]$, 417.2023; found, 417.2025.

(3R,4R)-3-((Benzylamino)methyl)-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyp henyl)azetidin-2-one (14j). A 50 mL round-bottom flask was charged with 3 (13 mg, 0.035 mmol), benzylamine (4 µL, 0.039 mmol) and MeOH (2 mL). The solution was heated to reflux for 24 h. 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compounds as yellow solid (5 mg, yield 30%); mp 75-77 °C; $[\alpha]_D^{20}$ = +65.5 (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.26-7.16 (m, 4H), 7.05 (d, J = 6.6 Hz, 1H), 6.85 (d, J = 1.4 Hz, 1H), 6.81-6.76 (m, 2H), 6.53 (s, 2H), 5.10 (d, J = 5.6 Hz, 1H), 3.90 (s, 3H), 3.88-3.74 (m, 4H), 3.71 (s, 6H), 3.63 (d, J = 13.2 Hz, 1H), 3.48 (d, J = 13.2 Hz, 1H), 2.79 (dd, J = 12.3, 6.3 Hz, 1H), 2.60 (dd, J = 12.3, 9.2 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): *δ* 165.7, 152.9, 146.1, 145.4, 138.8, 133.8, 133.0, 127.7, 127.3, 126.6, 126.3, 118.0, 112.4, 110.2, 94.3, 60.3, 56.9, 55.5, 55.3, 54.1, 53.2, 44.0. ESI-MS (m/z): 479.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₇H₃₀N₂O₆ + H⁺ [M + H⁺], 479.2177; found, 479.2175.

4.15

(3R,4S)-4-(3-(Benzyloxy)-4-methoxyphenyl)-3-hydroxy-3-(hydroxymethyl)-1-(3,4,5-trim ethoxyphenyl)azetidin-2-one (15). A 100 mL round-bottom flask was charged with 12e (0.93 g, 2 mmol), potassium osmate (VI) dihydrate (10 mg, 0.03 mmol), NMO (50% aq., 0.62 mL, 3 mmol), acetone (15 mL) and H₂O (0.6 mL). The solution was stirred for 12 h at room temperature. 20 mL of water was added into the solution, and the mixture was extracted with

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ethyl acetate (50 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:3, v/v) to give the title compounds as white solid (0.91 g, yield 92%); mp 123-124 °C; $[\alpha]_D^{20}$ = +61.8 (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.34-7.21 (m, 5H), 6.90-6.77 (m, 2H), 6.72 (s, 1H), 6.43 (s, 2H), 5.10 (s, 2H), 4.94 (s, 1H), 4.53 (br d, *J* = 30.9 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.63 (s, 6H), 3.53 (dd, *J* = 12.3, 4.3 Hz, 1H), 3.27 (dd, *J* = 12.3, 5.5 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 166.5, 152.8, 149.5, 147.4, 135.9, 134.3, 132.3, 127.9, 127.4, 126.6, 124.3, 119.1, 112.0, 111.3, 95.0, 85.7, 70.4, 66.9, 61.6, 60.3, 55.4, 55.4. ESI-MS (*m*/*z*): 496.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₇H₂₉NO₈ + H⁺ [M + H⁺], 496.1966; found, 496.1978.

4.16

(*3R*,4*S*)-3-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimeth oxyphenyl)azetidin-2-one (16a). A 50 mL round-bottom flask was charged with 15 (17mg, 0.034 mmol), 10% Pd/C (2mg) and ethanol (2 mL). The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 2:1, v/v) to give the title compounds as white solid (13 mg, yield 96%); mp 96-97 °C; $[\alpha]_D^{20} = +79.7$ (*c* 2.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.84-6.82 (m, 2H), 6.76 (br d, *J* = 8.1 Hz, 1H), 6.52 (s, 2H), 5.80 (s, 1H), 5.01 (s, 1H), 4.53 (br s, 1H), 3.89 (s, 3H), 3.82-3.62 (m, 10H), 3.45 (d, *J* = 12.3 Hz, 1H), 2.49 (br s, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 166.6, 152.9, 146.2, 145.4, 134.3, 132.3, 125.2, 117.8, 112.1, 110.3, 95.1, 85.7,

76.6, 76.4, 76.2, 66.8, 61.6, 60.3, 55.5, 55.3. ESI-MS (m/z): 406.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₀H₂₃NO₈ + H⁺ [M + H⁺], 406.1496; found, 406.1505.

4.17

(3R,4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-methoxy-3-(methoxymethyl)-1-(3,4,5-trimet hoxyphenyl)azetidin-2-one (16b). A 50 mL round-bottom flask was charged with 15 (25mg, 0.048 mmol), K₂CO₃ (26 mg, 0.19 mmol), dimethylsulfate (18 µL, 0.19 mmol) and acetone (5 mL). The solution was heated to reflux for 12 h. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in 1 mL of EtOH. 10% Pd/C (3 mg) was added into the mixture. The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (15 mg, yield 73%); mp 58-59 °C; $[\alpha]_D^{20} =$ +71.0 (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.85 (d, J = 1.4 Hz, 1H), 6.82 (d, J = 8.3 Hz, 1H), 6.77 (dd, J = 8.3, 1.4 Hz, 1H), 6.58 (s, 2H), 5.06 (s, 1H), 3.89 (s, 3H), 3.78 (s, 3 3H), 3.72 (s, 6H), 3.63 (s, 3H), 3.51 (d, J = 11.0 Hz, 1H), 3.37 (d, J = 11.0 Hz, 1H), 3.03 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 163.7, 152.9, 145.9, 145.0, 134.3, 132.3, 125.7, 118.4, 112.6, 109.8, 95.1, 91.8, 76.6, 76.4, 76.2, 68.8, 63.1, 60.3, 58.7, 55.5, 55.3, 53.1. ESI-MS (m/z): 434.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₂H₂₇NO₈ + H⁺ [M + H⁺], 434.1809; found, 434.1808.

(3R,4S)-4-(3-(Benzyloxy)-4-methoxyphenyl)-3-hydroxy-1-(3,4,5-trimethoxyphenyl)azeti din-2-one (17). A 100 mL round-bottom flask was charged with 15 (0.2 g, 0.41 mmol), NaIO₄ (0.13 mg, 0.62 mmol), MeOH (4 mL) and H₂O (1 mL). The solution was stirred for 3 h at room temperature. 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved with MeOH (4 mL). After cooled to 0 °C with an ice bath, NaBH₄ (18 mg, 0.47 mmol) was added into the system. The mixture was stirred for 1 h at 0 °C. Water (10 mL) was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (0.16 g, yield 85%); mp 149-150 °C; $[\alpha]_D^{20}$ = +61.8 (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.39-7.22 (m, 5H), 6.94-6.88 (m, 2H), 6.82 (s, 1H), 6.52 (s, 2H), 5.14 (s, 2H), 5.12 (d, J = 5.5 Hz, 1H), 5.08 (dd, J = 8.8, 5.5 Hz, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.69 (s, 6H), 2.15 (d, J = 8.8 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 166.5, 152.8, 149.5, 147.4, 135.9, 134.3, 132.3, 127.9, 127.4, 126.6, 124.3, 119.1, 112.0, 111.3, 95.0, 85.7, 70.4, 66.9, 61.6, 60.3, 55.42, 55.36. ESI-MS (m/z): 466.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₆H₂₇NO₇ + H⁺ [M + H⁺], 466.1860; found, 466.1862.

4.19

(3R,4S)-4-(3-Hydroxy-4-methoxyphenyl)-3-methoxy-1-(3,4,5-trimethoxyphenyl)azetidin

65

-2-one (18a). A 50 mL round-bottom flask was charged with 17 (20mg, 0.043 mmol), K₂CO₃ (12 mg, 0.086 mmol), dimethylsulfate (8 µL, 0.086 mmol) and acetone (5 mL). The solution was heated to reflux for 12 h. Then 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in 1 mL of EtOH. 10% Pd/C (3 mg) was added into the mixture. The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:1, v/v) to give the title compound as white solid (13 mg, yield 82%); mp 137-138 °C; $[\alpha]_D^{20} = +55.7$ (c0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.99 (d, J = 1.9 Hz, 1H), 6.91 (dd, J = 8.3, 1.9 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.58 (s, 2H), 5.64 (s, 1H), 5.08 (d, J = 4.8 Hz, 1H), 4.77 (d, J = 4.8 Hz, 1H), 3.90 (s, 3H), 3.76 (s, 3H), 3.73 (s, 6H), 3.24 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 163.5, 152.9, 146.2, 145.1, 134.2, 132.7, 125.7, 119.2, 113.6, 109.9, 94.7, 84.0, 61.0, 60.3, 57.9, 55.5, 55.3; ESI-MS (m/z): 390.1 $(M + H^{+})$. ESI-HRMS (m/z): calcd for $C_{20}H_{23}NO_7 + H^+ [M + H^+]$, 390.1547; found, 390.1547.

4.20

4-(((2*S*,3*R*)-2-(3-Hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3 -yl)oxy)-4-oxobutanoic acid (18b). A 50 mL round-bottom flask was charged with 17 (23 mg, 0.049 mmol), succinic anhydride (6 mg, 0.059 mmol), DIPEA (15 μ L, 0.09 mmol), DMAP (1 mg) and dichloromethane (1 mL). After stirred for 6 h at room temperature, 10 mL of water was added into the solution, and the mixture was extracted with dichloromethane (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in 1 mL of EtOH. 10% Pd/C (3 mg) was added into the mixture. The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with DCM/MeOH = 10:1, v/v) to give the title compounds as white solid (10 mg, yield 45%); mp 96-98 °C; $[\alpha]_D^{20} = -10.5$ (*c*1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.89-6.80 (m, 3H), 6.58 (s, 2H), 5.95 (d, *J* = 4.8 Hz, 1H), 5.25 (d, *J* = 4.8 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.73 (s, 6H), 2.54-2.44 (m, 1H), 2.42-2.35 (m, 2H), 2.29-2.16 (m, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 175.4, 169.9, 161.0, 153.0, 146.5, 145.1, 134.6, 132.3, 124.4, 119.2, 113.4, 110.0, 94.8, 75.6, 60.7, 60.3, 55.5, 55.4, 27.7. ESI-MS (*m*/*z*): 476.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₃H₂₅NO₁₀ + H⁺ [M + H⁺], 476.1551; found, 476.1561.

4.21

(2*S*,3*R*)-2-(3-(Benzyloxy)-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3yl methanesulfonate (19). A 100 mL round-bottom flask was charged with 17 (0.2 g, 0.43 mmol), triethylamine (0.09 mL, 0.64 mmol) and dichloromethane (4 mL). After cooled to 0 $^{\circ}$ C with an ice bath, methanesulfonyl chloride (50 µL, 0.64 mmol) was added into the system. The mixture was stirred for 1 h at 0 $^{\circ}$ C. 10 mL of water was added into the solution, and the mixture was extracted with dichloromethane (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 5:1, v/v) to give the title compounds as white solid (0.2 g, yield 86%); mp 76-77 °C; $[\alpha]_D^{20} = +95.3$ (*c* 2.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.36 (d, J = 7.7 Hz, 2H), 7.33-7.20 (m, 3H), 6.99-6.84 (m, 3H), 6.47 (s, 2H), 5.76 (d, J = 5.0 Hz, 1H), 5.21 (d, J = 5.0 Hz, 1H), 5.13 (s, 2H), 3.88 (s, 3H), 3.77 (s, 3H), 3.67 (s, 6H), 2.66 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 160.3, 153.7, 150.8, 148.3, 136.8, 135.5, 132.6, 128.8, 128.2, 127.5, 123.8, 121.5, 113.7, 111.9, 95.4, 79.4, 71.2, 61.7, 61.2, 56.3, 6.2, 38.9. ESI-MS (*m*/*z*): 544.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₇H₂₉NO₉S + H⁺ [M + H⁺], 544.1636; found, 544.1640.

4.22

(35,45)-4-(3-(Benzyloxy)-4-methoxyphenyl)-3-bromo-1-(3,4,5-trimethoxyphenyl)azetidi n-2-one (20). A 5 mL microwave reaction tube was charged with 19 (0.55 g, 1 mmol), TBAB (1.1g, 3.4 mmol) and DMF (2 mL). The solution was heated to 170 °C with the microwave reaction machine for 6 h. After cooled to room temperature, 10 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (20 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:2, v/v) to give 20 as white solid (0.22 g, yield 42%); mp 55-56 °C; $[\alpha]_D^{20} = -29.4$ (*c* 2.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.27 (m, 5H), 6.97 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.46 (s, 2H), 4.92 (d, *J* = 1.5 Hz, 1H), 4.53 (d, *J* = 1.5 Hz, 1H), 3.90 (s, 3H), 3.77 (s, 3H), 3.66 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.6, 153.5, 150.8, 148.8, 136.4, 135.2, 133.1, 128.6, 128.1, 127.4, 127.3, 119.5, 112.2, 111.5, 95.1, 71.2, 66.1, 60.9, 56.1, 56.1, 50.1. ESI-MS (m/z): 550.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₆H₂₆NBrO₆ + H⁺ [M + H⁺], 550.0836; found, 550.0850.

4.23

(3S,4S)-3-Bromo-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-

one	(21)	and	(S)

4-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (22). A 50 mL round-bottom flask was charged with **20** (30 mg, 0.057 mmol), 10% Pd/C (4 mg) and EtOH (1 mL). The solution was stirred for 8 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was purified by column chromatography (elute with EtOAc/hexane = 1:3, v/v) to give the title compounds.

21: white solid; yield 62%; mp 43-44 °C; $[\alpha]_D^{20} = -10.7$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.93 (d, J = 1.8 Hz, 1H), 6.90 (dd, J = 8.2, 1.8 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.54 (s, 2H), 5.73 (s, 1H), 4.87 (d, J = 1.8 Hz, 1H), 4.59 (d, J = 1.8 Hz, 1H), 3.91 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.7, 153.6, 147.5, 146.5, 135.3, 133.0, 128.1, 118.2, 112.1, 111.1, 95.4, 77.2, 77.0, 76.8, 66.1, 63.2, 60.9, 56.1, 56.1. ESI-MS (*m*/*z*): 438.2 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₁₉H₂₀BrNO₆ + H⁺ [M + H⁺], 438.0552; found, 438.0558.

22: white solid; yield 28%; mp 45-46 °C; $[\alpha]_D^{20} = +49.8$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.96 (d, J = 2.1 Hz, 1H), 6.89 (dd, J = 8.3, 2.1 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 6.55 (s, 2H), 5.67 (s, 1H), 4.87 (dd, J = 5.5, 2.6 Hz, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H), 3.51 (dd, J = 15.2, 5.5 Hz, 1H), 2.93 (dd, J = 15.2, 2.6 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 163.9, 152.9, 146.1, 145.7, 133.8, 133.5, 130.7, 117.1, 111.4, 110.3, 94.0, 60.3,

55.43, 55.39, 53.5, 46.4. ESI-MS (m/z): 360.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₁₉H₂₁NO₆ + H⁺ [M + H⁺], 360.1442; found, 360.1442.

4.24

(3S,4S)-3-Azido-4-(3-(benzyloxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (23). A 100 mL round-bottom flask was charged with 19 (0.5 g, 0.92 mmol), NaN₃ (90 mg, 1.38 mmol) and DMF (10 mL). After stirring for 24 h at 120 °C, 20 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (50 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 5:1, v/v) to give the title compound as yellow oil (0.24 g, yield 53%); $[\alpha]_{D}^{20} = -50.4$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.23 (m, 5H), 6.94-6.88 (m, 2H), 6.80 (br s, 1H), 6.43 (s, 2H), 5.12 (s, 2H), 4.67 (d, J = 1.4 Hz, 1H), 4.39 (d, J = 1.4 Hz, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.65 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 160.7, 152.9, 150.0, 148.1, 135.8, 134.5, 132.2, 127.9, 127.4, 126.8, 126.6, 118.8, 111.5, 111.0, 94.5, 71.7, 70.5, 62.5, 60.3, 55.5, 55.4. ESI-MS (m/z): 491.1 (M + H⁺). ESI-HRMS (m/z): calcd for C₂₆H₂₆N₄O₆ + H⁺ [M + H⁺], 491.1925; found, 491.1924.

4.25

(3*S*,4*S*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one (24). A 250 mL round-bottom flask was charged with 23 (30 mg, 0.076 mmol), prop-2-yn-1-ol (5 μ L, 0.084 mmol), CuSO₄·5H₂O (1 mg, 5% mmol), *L*-ascorbic acid sodium salt (2.2 mg, 15% mmol), EtOH (0.7 mL) and H₂O (0.3 mL).
After stirring for 8 h at room temperature, 5 mL of water was added into the solution, and the mixture was extracted with ethyl acetate (5 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in 1 mL of EtOH. 10% Pd/C (2 mg) was added into the mixture. The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 1:4, v/v) to give the title compound as white solid (26 mg, yield 93%); mp 93-95 °C; $[\alpha]_D^{20} = -96.9$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.75 (s, 1H), 6.98 (d, *J* = 1.5 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.59 (s, 2H), 5.76 (s, 1H), 5.55 (d, *J* = 1.6 Hz, 1H), 5.31 (d, *J* = 1.7 Hz, 1H), 4.85 (s, 2H), 3.92 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 159.0, 153.7, 147.6, 146.7, 135.5, 132.5, 127.6, 121.5, 118.4, 112.0, 111.2, 100.0, 95.7, 72.0, 63.5, 61.0, 56.7, 56.2, 56.1. ESI-MS (*m*/*z*): 457.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₂H₂₄N₄O₇ + H⁺ [M + H⁺], 457.1718; found, 457.1718.

4.26

(3S,4S)-3-Amino-4-(3-(benzyloxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)azetidin -2-one (25). A 100 mL round-bottom flask was charged with 23 (0.23 g, 0.47 mmol), SnCl₂ (0.28 g, 1.4 mmol), HCl (9% aq., 2 mL) and MeOH (8 mL). After refluxing for 4 h, 10 mL of saturated NaHCO₃ (aq.) was added into the solution, and the mixture was extracted with ethyl acetate (50 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (elute with EtOAc/hexane = 3:1, v/v) to give the title compound as white solid (0.14 g, yield 64%); mp 64-65 °C; $[\alpha]_D^{20} = +7.9$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.39-7.21 (m, 5H), 6.97-6.82 (m, 3H), 6.47 (s, 2H), 5.12 (s, 2H), 4.50 (s, 1H), 3.98 (s, 1H), 3.89 (s, 3H), 3.77 (s, 3H), 3.66 (s, 6H). ¹³C NMR (150 MHz, CDCl₃): δ 167.9, 153.5, 150.1, 148.6, 136.6, 134.6, 133.6, 129.2, 128.5, 128.0, 127.3, 119.2, 112.1, 111.6, 95.0, 71.1, 69.7, 66.8, 60.9, 56.1, 56.0. ESI-MS (*m/z*): 465.1 (M + H⁺). ESI-HRMS (*m/z*): calcd for C₂₆H₂₈N₂O₆ + H⁺ [M + H⁺], 465.2020; found, 465.2035.

4.27 General Method for the Acylation of 25. A 50 mL round-bottom flask was charged with **25** (29 mg, 0.062 mmol), DIPEA (16 μ L, 0.093 mmol) and dichloromethane (2 mL). The solution was cooled to 0 °C by an ice bath, then corresponding anhydride or acyl chloride (0.08 mmol) was added into the flask. After the reaction completed, 10 mL of water was added into the solution, and the mixture was extracted with dichloromethane (30 mL) for 3 times. The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and filtered. The solvent was removed under reduced pressure and the residue was dissolved in EtOH (2 mL). 10% Pd/C (3 mg) was added into the mixture. The solution was stirred for 12 h under H₂ atmosphere (1 atm). Pd/C was filtrated, then the solvent was removed under reduced pressure and the residue was removed under the residue was purified by flash column chromatography to give the title compounds.

4.27.1

N-((2*S*,3*S*)-2-(3-Hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3yl)acetamide (26a). White solid; yield 58%; mp 105-107 °C; $[\alpha]_D^{20} = +19.7$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.93-6.78 (m, 3H), 6.52-6.49 (m, 3H), 5.75 (br s, 1H), 4.83 (br s, 1H), 4.62 (d, *J* = 7.0 Hz, 1H), 3.88 (s, 3H), 3.75 (s, 3H), 3.69 (s, 7H), 2.05 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 169.8, 163.4, 152.8, 146.4, 145.7, 134.1, 132.8, 128.7, 117.6, 111.5, 110.4, 94.6, 65.3, 62.7, 60.3, 55.4, 28.6, 22.2. ESI-MS (*m*/*z*): 417.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₁H₂₄N₂O₇ + H⁺ [M + H⁺], 417.1656; found, 417.1656.

4.27.2

N-((2*S*,3*S*)-2-(3-Hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3yl)benzamide (26b). A mixture of *E*- and *Z*-isomer. White solid; yield 80%; mp 102-103 °C; $[\alpha]_D^{20} = -30.0 \ (c \ 1.0, \ CHCl_3)$. ¹H NMR (400 MHz, CDCl_3): δ 7.84 (d, *J* = 7.4 Hz, 2H), 7.47-7.41 (m, 3H), 6.97-6.77 (m, 3H), 5.74 (d, *J* = 3.9 Hz, 1H), 4.95 (d, *J* = 1.9 Hz, 1H), 4.82 (dd, *J* = 6.8, 1.9 Hz, 1H), 3.88 (s, 3H), 3.74 (s, 3H), 3.68 (s, 6H). ¹³C NMR (150 MHz, CDCl_3): δ 166.7, 163.4, 153.1, 152.8, 146.4, 145.7, 134.1, 132.8, 132.2, 131.6, 128.8, 128.2, 128.1, 126.6, 126.5, 117.7, 111.6, 110.5, 94.6, 65.7, 62.8, 60.3, 55.4, 55.3, 55.2. ESI-MS (*m/z*): 479.1 (M + H⁺). ESI-HRMS (*m/z*): calcd for C₂₆H₂₆N₂O₇ + H⁺ [M + H⁺], 479.1818; found, 479.1813.

4.27.3

N-((2*S*,3*S*)-2-(3-Hydroxy-4-methoxyphenyl)-4-oxo-1-(3,4,5-trimethoxyphenyl)azetidin-3yl)methanesulfonamide (26c). White solid; yield 99%; mp 87-88 °C; $[\alpha]_D^{20} = -10.7$ (*c* 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.86-6.83 (m, 3H), 6.36 (s, 2H), 6.03 (d, *J* = 9.2 Hz, 1H), 5.72 (s, 1H), 4.68 (d, *J* = 2.0 Hz, 1H), 4.44 (dd, *J* = 9.2, 2.0 Hz, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.68 (s, 6H), 3.14 (s, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 162.3, 152.8, 152.8, 146.6, 145.8, 133.8, 132.4, 127.8, 117.6, 111.3, 110.5, 94.6, 94.5, 67.1, 63.8, 60.4, 55.4, 41.7, 28.6. ESI-MS (*m*/*z*): 453.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₄N₂O₈S + H⁺ [M + H⁺], 453.1326; found, 453.1327.

4.27.4

(R)-Ethyl

2-((3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)((3,4,5-trimethoxyphenyl)amino)m
ethyl)acrylate (27) [8]. Colorless oil, yield 96%. ¹H NMR (400 MHz, CDCl₃): δ 6.91 (dd, J = 8.3, 2.1 Hz, 1H), 6.83 (d, J = 2.1 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.34 (s, 1H), 5.88 (s, 1H), 5.81 (s, 2H), 5.28 (s, 1H), 4.21-4.11 (m, 2H), 4.07 (br s, 1H), 3.79 (s, 3H), 3.76 (s, 6H), 3.74 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.97 (s, 9H), 0.12 (s, 6H).

4.28

(*3R*,*4R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (30a). 30a was synthesized similar with 14a starting from 29. White solid; yield 52%; mp 180-181 °C; $[\alpha]_D^{20} = -116.9 (c \ 1.0, CHCl_3)$. ¹H NMR (400 MHz, CDCl_3): δ 6.88 (d, *J* = 1.5 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.79 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.54 (s, 2H), 5.88 (br d, *J* = 20.7 Hz, 1H), 5.15 (d, *J* = 5.3 Hz, 1H), 3.89 (s, 3H), 3.85-3.73 (m, 5H), 3.71 (br s, 7H), 3.61 (dd, *J* = 11.2, 7.9 Hz, 1H). ¹³C NMR (150 MHz, CDCl_3): δ 164.8, 152.9, 146.2, 145.5, 134.0, 133.0, 126.5, 117.9, 112.4, 110.3, 94.4, 60.3, 60.0, 57.4, 56.7, 56.1, 55.5, 55.3. ESI-MS (*m*/*z*): 390.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₃NO₇ + H⁺ [M + H⁺], 390.1547; found, 390.1551.

4.29

(3*S*,4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-(hydroxymethyl)-1-(3,4,5-trimethoxyphenyl) azetidin-2-one (30b). 30b was synthesized similar with 14b starting from 29. White gel; yield 19%; $[\alpha]_D^{20} = -21.0$ (*c* 1.6, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.96 (s, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.56 (s, 2H), 5.78 (br d, *J* = 15.5 Hz, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 3.99 (d, *J* = 11.7 Hz, 1H), 3.89 (s, 3H), 3.76 (s, 3H), 3.72 (br s, 7H), 3.27 (s, 1H), 3.27 (d, J = 2.4 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃): δ 165.2, 152.8, 146.2, 145.7, 133.9, 133.1, 130.1, 117.3, 111.6, 110.4, 94.3, 61.5, 60.3, 59.8, 58.1, 56.9, 55.4. ESI-HRMS (m/z): calcd for C₂₀H₂₃NO₇ + H⁺ [M + H⁺], 390.1547; found, 390.1549.

4.30

(3*R*,4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2 -one (30c). 30c was synthesized similar with 14c starting from 30b. White gel; yield 20%; $[\alpha]_D^{20} = -14.5$ (*c* 0.65, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.97-6.82 (m, 3H), 6.54 (s, 2H), 5.69 (br s, 1H), 4.43 (d, *J* = 2.1 Hz, 1H), 3.89 (s, 3H), 3.75 (s, 3H), 3.72 (s, 6H), 3.10 (q, *J* = 7.6 Hz, 1H), 1.44 (d, *J* = 7.5 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 167.7, 152.9, 146.1, 145.7, 133.7, 133.5, 130.4, 117.0, 111.4, 110.3, 94.1, 62.2, 60.3, 55.4, 54.4, 12.4. ESI-MS (*m*/*z*): 374.0 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₃NO₆ + H⁺ [M + H⁺], 374.1589; found, 374.1601.

4.31

(3*S*,4*R*)-4-(3-Hydroxy-4-methoxyphenyl)-3-methyl-1-(3,4,5-trimethoxyphenyl)azetidin-2 -one (30d). 30d was synthesized similar with 4 starting from 29. White gel; yield 92%; $[\alpha]_D^{20}$ = -112.8 (*c* 1.64, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.82 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 1.8 Hz, 1H), 6.72 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.56 (s, 2H), 5.75 (br d, *J* = 8.0 Hz, 1H), 5.05 (d, *J* = 5.8 Hz, 1H), 3.88 (s, 3H), 3.76 (s, 3H), 3.72 (s, 6H), 3.66-3.57 (m, 1H), 0.90 (d, *J* = 7.6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 167.9, 152.9, 145.8, 145.2, 133.8, 133.3, 127.3, 118.1, 112.6, 110.0, 94.4, 60.3, 59.8, 57.8, 55.5, 55.3, 48.6, 26.3. ESI-MS (*m*/*z*): 374.1 (M + H⁺). ESI-HRMS (*m*/*z*): calcd for C₂₀H₂₃NO₆ + H⁺ [M + H⁺], 374.1589; found, 374.1601.

4.32 In Vitro Cell Growth Inhibition Assay. Human ovarian cancer cell lines A2780 and SKOV-3, breast cancer cell line MDA-MB-231 and cervical cancer cell line Hela were purchased from American Type Culture Collection (ATCC). Above-mentioned cells were cultured in Dulbecco's modified Eagle medium (DMEM) or RPMI-1640, supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in a humidified incubator at 37 °C in an atmosphere of 5 % CO₂.

In vitro cell growth inhibition was assessed by the MTT reagent as described previously [18]. Briefly, 5000 cells per well were seeded into 96-well plate and allowed to adhere overnight. Cells were treated with various concentrations of tested compounds or DMSO (0.2%, as negative control) for 48 h. Then, the medium along with tested compounds or DMSO was discarded and 200 μ L per well MTT containing medium (0.5 mg/mL) was added. After incubation at 37 °C for 4 h, the MTT-containing medium was replaced by DMSO (150 μ L per well) to dissolve the formazan crystals. Absorbance of the resulting solution was measured by microplate reader (Biotech ELx800) at 540 nm wavelength. Growth inhibition rates were calculated with the following equation:

Inhibition ratio (%) =
$$\frac{OD_{DMSO} - OD_{compd}}{OD_{DMSO} - OD_{blank}} \times 100\%$$

Half maximal inhibitory concentration (IC₅₀) of each compound was calculated using GraphPad Prism, version 6.0.

4.33 In Vitro Tubulin Polymerization Assay. Pig brain tubulin was obtained commercially and stored in aliquots at -70 °C. In this assay, tubulin protein was incubated with indicated concentrations of compounds **14b**, **14c**, colchicine as positive control, or diluent (0.2% DMSO) as negative control in general tubulin buffer (100 mM PIPES, 1.0 mM

MgCl₂, 1 mM EGTA, 1 mM GTP and 5 % glycerol). The absorbance of wavelength at 340 nm was detected every 1 min for 20 min by Spectra Max 190 spectrophotometer (Molecular Device) at 37 °C. The results were indicated as mean values from three independent determinations.

4.34 Immunoblotting Analysis. In general, after treatment with indicated concentrations of **14b**, **14c** or diluent (0.2% DMSO), Hela cells were harvested, washed twice with PBS and lysed with cell lysis buffer (Beyotime, China). Then the concentrations of total protein were determined by BCA protein assay kit (Beyotime, China). Equally amount of protein samples were subjected to SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore). The membrane was blocked by 5 % BSA in TBST and interacted with specific primary antibodies (Proteintech), followed by incubation with horseradish peroxidase (HRP)–conjugated secondary antibodies (Abnova, Taipei, China). Finally, the protein bands were detected using the chemiluminescent reagents (Millipore).

The immunoblotting analysis of tubulin polymerization was performed as described previously with some modifications [19]. Briefly, after being treated with diluent (0.2% DMSO), tested compound, **1** or PTX for 6 h, Hela cells were lysed with microtubule-stabilizing buffer, containing 100 mM PIPES, pH 6.8, 1 mM MgCl₂, 2 mM EGTA, 0.5% NP-40, 2 M glycerol, 5 μ M **1** and protease inhibitors cocktail (Roche), and the lysate was centrifuged at 15,000 rpm for 15 min. Supernatant containing depolymerized tubulin was carefully collected, whereas pellet (polymerized tubulin) was further dissolved in SDS-lysis buffer (Beyotime, China). Equal amounts of supernatant and dissolved pellet were

subjected to immunoblotting analysis as described above using α -tubulin antibody (Proteintech).

4.35 Immunofluorescent Analysis. To visualize the morphological character of tubulin, immunofluorescence staining was performed according to a previously reported protocol [17a]. In general, after treatment with diluent (0.2% DMSO) or tested compounds at indicated concentrations for 24 h, SKOV-3 cells were fixed with methanol, permeabilized with 0.1% Triton X-100 in PBS and blocked by goat serum. Then the cells were incubated with α -tubulin antibody at 4 °C overnight, followed by incubation with Alexa 488 labeled secondary antibody (Jackson ImmunoResearch) at RT for 1 h. After being stained with DAPI, cells were examined and photographed with a Leica SP5 co-focal fluorescence microscope.

4.36 Capillary-like Tube Formation Assay. Capillary-like tube formation assay was carried out following the procedures published previously [14,18]. Briefly, 60 μ L matrigel (Corning) per well was added to 96-well plate and pre-incubated at 37 °C for 1 h for gelation. 3×10^4 human umbilical veinendothelial cells (HUVECs) in 100 μ L medium per well containing indicated concentrations of **14b**, **14c**, **1** as positive control, or diluent (0.2% DMSO) as negative control, respectively, were seeded to 96-well plates on the matrigel layer, followed by continual incubation at 37 °C for 12 h to allow capillary-like tube formation. Images were captured with a CCD Sensicam camera mounted on an Olympus inverted microscope. Representative data from three independent experiments were shown.

4.37 Matrigel Plug Assay. 6 weeks old female Balb/C nude mice were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). Matrigel plug assay was

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performed according to the literatures with some modifications [20]. Matrigel containing 100 ng/mL human recombinant VEGF-A165 (Peprotech, Rocky Hill, NJ) were mixed with indicated concentrations of **14b**, **14c**, or diluent (0.2% DMSO) as negative control at 4°C. Then, 0.5 mL of the matrigel mixture per mouse was injected subcutaneously into nude mice (n = 4) in the dorsal region to generate matrigel plugs. 2 weeks later, the mice were sacrificed and matrigel plugs were recovered. Representative data from three independent experiments were shown. The animal experimental protocols were approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

4.38 Colony Formation Assay. 1000 MDA-MB-231 cells per well were seeded into 6-well plate at a single cell density and were cultured at 37 °C for 48 h. After being treated with indicated concentrations of **14b**, **14c**, **1** as positive control, or diluent (0.2% DMSO) as negative control for 48 h, the agents containing medium was replaced by fresh medium to allow cell growth for additional 7-10 days. Then the cells were fixed with methanol and stained with gentian violet for 30 min. The number of colonies which consisted of more than 50 cells were counted. The results were indicated as mean values from three independent determinations.

4.39 Cell Cycle Analysis. 2×10^5 Hela cells per well were seeded into six-well plate and allowed to adhere overnight at 37 °C. After treatment with indicated concentrations of **14b**, **14c**, **1** as positive control or diluent (0.2% DMSO) as negative control for 24 h, cells were harvested, washed twice with PBS and fixed with 75% ethanol at -20 °C overnight. Then cells were stained with propidium iodide dye (BD Biosciences) for 15 min in dark conditions at room temperature, followed by subjected to flow cytometry (Cytomics FC 500MPL,

Beckman Coulter) detection. The results were analyzed by Multicycle AV (for Windows, version 320) software and indicated as mean values from three independent determinations.

4.40 Cell Apoptosis Analysis. Cell apoptosis was detected using FITC Annexin V Apoptosis Detection Kit (BD Biosciences) according to manufacturer's instructions. Briefly, cells were incubated with indicated concentrations of **14b**, **14c**, **1** as positive control or diluent (0.2% DMSO) as negative control for 48 h. Then the cells were harvested, washed twice with PBS, and re-suspended in binding buffer. After being stained with Annexin-V and PI for 15 min in the dark place, cells were subjected to flow cytometry (Cytomics FC 500 MPL, Beckman Coulter) analysis. The results were indicated as mean values from three independent determinations.

4.41 Acute Toxicity Assay. KM mice were housed individually in a specific pathogen free facility. Groups of mice (n = 10 per group, half male and half female) were injected intraperitoneally once with various dosages of **14b** (95, 70, 50, 35 and 25 mg/kg) or **14c** (500, 425, 350, 275 and 200 mg/kg), or vehicle (8.3 % cremohorp EL and 8.3 % alcohol in PBS) as negative control, respectively. **14b** and **14c** was dissolved in cremeohore EL and alcohol mixture (1:1, v/v) and further diluted with PBS (1:5, v/v). The death of mice were monitored daily and recorded up to 14 days after injection. The animal experimental protocols were approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

4.42 Xenografts Tumor Growth Assay in Nude Mice. 6 weeks old female Balb/c nude mice were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). 2×10^6 A2780 cells suspended in 0.2 mL PBS per mouse was injected subcutaneously into the nude mice. When the average tumor volume reached 100 mm³, mice were randomly divided

into 4 groups (n = 10) and administered intraperitoneally with 7.5 mg/kg compound 14b, 12.5 mg/kg 14c, 5 mg/kg PTX (Taxol, Bristol-Myers Squibb Company) injection or vehicle control (same as described in acute toxicity assay), respectively. Administration of agents or vehicle, or agents and measurement of tumor volumes with a digital caliper was done once every 2 days. Tumor volumes were calculated as volume = shortest diameter (W)² × longest diameter (L) × 0.52. When the average tumor volumes of vehicle treated group reached 2000 mm³ in diameter, the mice were sacrificed and the tumors were isolated and weighed. Visceral organs of the mice as well as solid tumors were further analyzed by H&E staining. The animal experimental protocols were approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

4.43 Statistical Analysis. Comparisons between control and treated groups were determined by *t* testor one-way ANOVA followed by Tukey's multiple comparison tests. Results were considered statistically significant at the p < 0.05 level.

Associated content

Supporting Information

Supplementary data related to this article can be found at XXXXXX.

Accession Codes

The PDB code of the complex of tubulin and compound **14b** is 5XAG. The PDB code of the complex of tubulin and compound **14c** is 5XAF. The authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare no competing financial interest.

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Abbreviations used

SAR, structure-activity relationship; DABCO, 1,4-Diazabicyclo[2.2.2]octane; TBAF, Tetrabutylammonium fluoride; NMO, 4-Methylmorpholine N-oxide; TBAB, Tetrabutylammonium bromide; VEGF, vascular endothelial growth factor; PI, propidium iodide; FITC, fluorescein isothiocyanate; PARP, poly ADP-ribose polymerase; PTX, paclitaxel; H&E staining, hematoxylin-eosin staining; PIPES, piperazine-1,4-bisethanesulfonic acid; DMEM, Dulbecco minimum essential medium; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; PVDF, polyvinylidene fluoride; DAPI, 4',6-diamidino-2-phenylindole.

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Highlights:

A diverse of chiral β -lactam analogues of CA-4 were asymmetrically synthesized. Some potent anti-proliferative compounds were identified in vitro and in vivo. Cocrystal structures with tubulin were obtained by X-ray crystallography. The relationship between chiral centers and SARs were studied comprehensively.