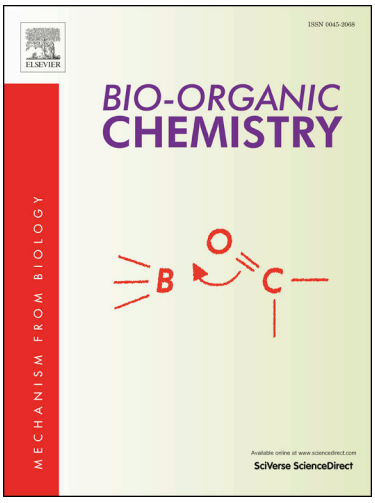
Journal Pre-proofs



Development of carbapenem-based fluorogenic probes for the clinical screening of carbapenemase-producing bacteria

Juhyeon Kim, Yihoon Kim, Ahmed Z. Abdelazem, Hoon Seok Kim, Jung Ok Kim, Yeon-Joon Park, Sun-Joon Min

PII: S0045-2068(19)30957-5
DOI: https://doi.org/10.1016/j.bioorg.2019.103405
Reference: YBIOO 103405

To appear in: *Bioorganic Chemistry*

Received Date: 18 June 2019
Revised Date: 18 September 2019
Accepted Date: 26 October 2019

Please cite this article as: J. Kim, Y. Kim, A.Z. Abdelazem, H. Seok Kim, J. Ok Kim, Y-J. Park, S-J. Min, Development of carbapenem-based fluorogenic probes for the clinical screening of carbapenemase-producing bacteria, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103405

Development of carbapenem-based fluorogenic probes for the clinical screening of carbapenemase-producing bacteria

Juhyeon Kim[a,b], Yihoon Kim[c], Ahmed Z. Abdelazem[d], Hoon Seok Kim[e], Jung Ok Kim[e], Yeon-Joon Park[e], Sun-Joon Min[c,f,*]

[a]*Department of Chemistry, Korea University, Seoul, 02841, Republic of Korea*

[b]*Center for Neuro-Medicine, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea*

[c]*Department of Applied Chemistry, Hanyang University, Ansan, Gyeonggi-do, 15588, Republic of Korea*

[d]*Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt*

[e]*Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, 06591, Republic of Korea*

[f]*Department of Chemical & Molecular Engineering, Hanyang University, Ansan, Gyeonggi-do, 15588, Republic of Korea*

email: sjmin@hanynag.ac.kr

Abstract

This report describes the synthesis of a library of fluorogenic carbapenemase substrates consisting of carbapenem derivatives, fluorescence dyes, and active cleavable linkers and their evaluation for specifically detecting carbapenemase-producing organisms (CPOs). We synthesized a series of compounds having three different types of linkers such as benzyl ether, carbamate, and amine using hydroxymethyl carbapenem **7a** and hydroxyally carbapenem **7b**

as key intermediates. Probe **1b** exhibited high stability and a prompt turn-on fluorescence signal upon hydrolysis by carbapenemases. In particular, the screening of clinical samples indicated that the probe **1b** exhibited excellent selectivity to the CPOs over other β-lactamases or non-carbapenemase producing bacteria, which may be of clinical use for the rapid and accurate detection of CPOs for timely diagnosis and treatment.

Keywords: carbapenems; carbapenemase-producing bacteria; linker; fluorescence; probes

1. Introduction

Carbapenems are an important class of last-resort antibiotics [1,2] used for the treatment of severe and life-threatening bacterial infections due to their broad spectrum of activity and exceptional stability to β-lactamases, including extended spectrum β-lactamases (ESBLs) (**Fig. 1a**) [3]. However, the carbapenem resistance is frequently occuring as a result of the overuse and misuse of this antibiotic and rapid transfer of carbapenemase gene through plasmids in pathogenic strains. The major mechanism of pathogenic resistance to carbapenem is related to the acquisition and expression of carbapenemases [4]. Since the first carbapenemase in *Enterobacteriaceae* was identified in 1993 [5], the number of carbapenemase-producing bacteria has increased worldwide. Currently, the spread of carbapenemase-producing species is the most important clinical issue in antibiotic resistance to pathogenic bacteria, and it must be prevented or mitigated.

Three major classes of β-lactamases are involved in carbapenem resistance: Ambler class A, B, and D [6]. On the basis of their hydrolysis mechanisms, class A and D carbapenemases are serine-hydrolyzing enzymes possessing a serine residue in the active site for hydrolysis. Class B carbapenemases belong to metallo-β-lactamases in which zinc ions in the active site play a crucial role in hydrolytic activity [7,8]. Most carbapenemases expressed from *Enterobacteriaceae* deactivate almost all β-lactam antibiotics, including carbapenems, because they have a very broad substrate profile. The bacterial infectionrelated mortality is significantly increased and the problem is intensified by the lack of efficient early-stage detection methods and insufficient new antibiotic development. Therefore, recent research has focused on solving these issues.

Current detection methods of carbapenem-resistant bacteria are mainly classified into phenotypic and genotypic methods [9,10]. The Clinical Laboratory Standards Institute (CLSI) guideline recommends two phenotypic methods, the CarbaNP test and the mCIM (modified

carbapenem inactivation method) test. However, the CarbaNP test has several limitations; the special reagents are required and can be stored in a short period time, invalid results occur with some isolates, and OXA-48 type carbapenemases are not consistently detected. The mCIM test is readily performed in most laboratories, but it requires overnight incubation [11]. Currently, colorimetric based assay kits such as RAPIDEC CARBA NP (bioMérieux) and β-CARBA test (Bio-Rad) which requires only 2 h and 30 min, respectively, are also commercially available. In addition, a new MALDI-TOF MS (Matrix-assisted laser desorption ionization-time of flight mass spectrometry) based carbapenem hydrolysis assay has been developed, but it is not yet used as routine protocol to detect carbapenemases from clinical samples.[12] Genotypic methods, such as PCR-based molecular assays, enable rapid detection with high sensitivity and specificity. In spite of their high accuracy, genotypic methods are less useful because they require special equipment and expertise to perform, and cannot detect unknown carbapenemase genes [13,14].

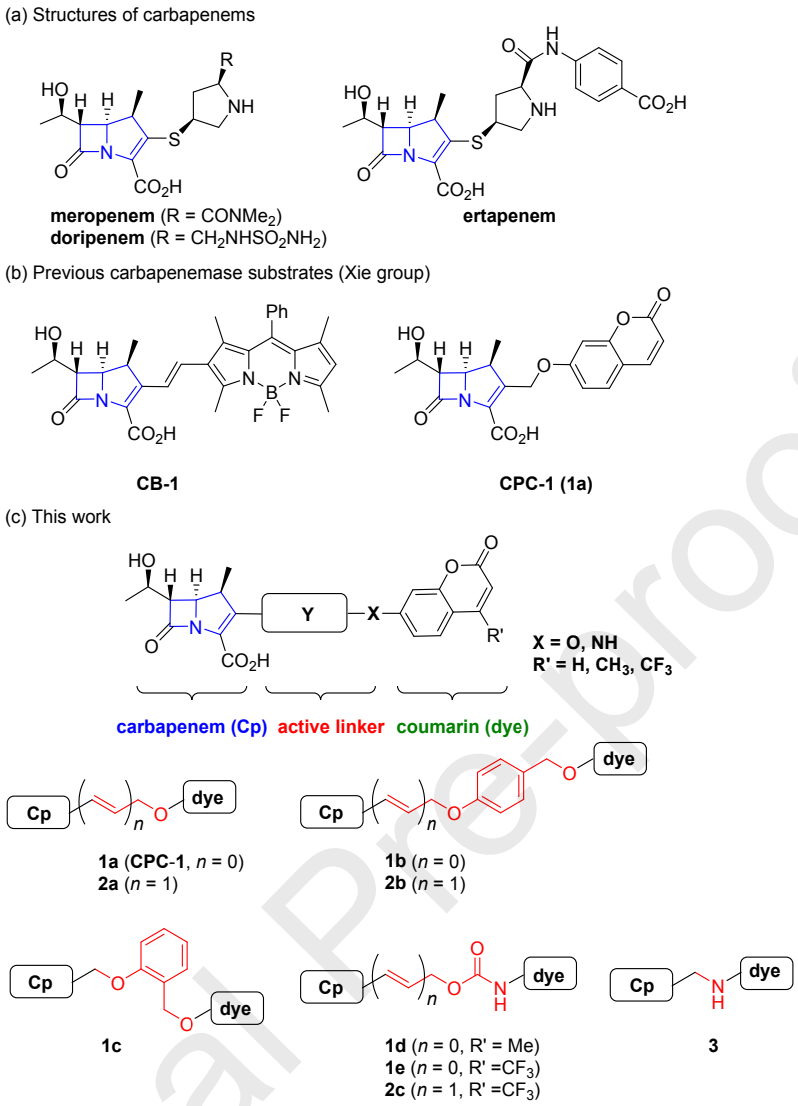


Fig. 1. Design of a chemical library of carbapenem-based fluorogenic probes.

Fluorescence-based assays have garnered considerable attention recently because of the ease of the procedure, high sensitivity, cost effectiveness, and rapid detection time. Since the use of a fluorescent chemical probe for detecting β-lactamases was first reported by the Tsien group [15], the development of β-lactamase fluorogenic probes has been studied by many researchers [16-18]. However, most reported fluorescence-based probes in detection of β-lactam resistance are based on a core structure of cephalosporin antibiotics, which are easily hydrolyzed by most β-lactamases. Thus, cephalosporin-based substrates are not suitable for selective detection of

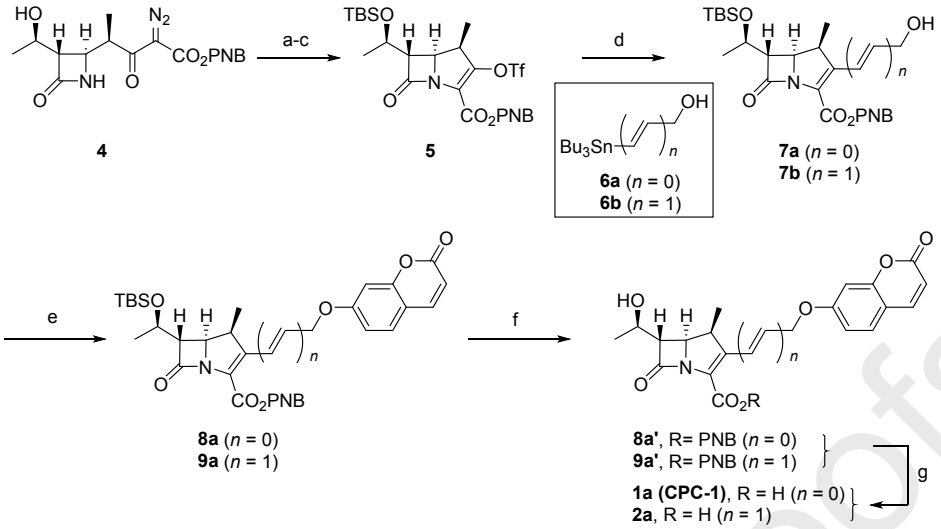
carbapenemase-producing bacteria.

Recently, Xie and colleagues reported carbapenem-based substrates CB-1 and CPC-1 as fluorogenic probes for detecting carbapenemase-producing bacteria (**Fig. 1b**) [19,20]. Both probes demonstrated that fluorogenic substrates derived from a carbapenem core structure are well-recognized by carbapenemases. Although they exhibited high specificity to metallo-carbapenemases (VIM-27, NDM-1, and IMP-1) over other β-lactamases such as TEM-1 and CTX-M-9, it is still necessary to develop a method to detect a broad range of carbapenemases including KPC or OXA-type carbapenemases and explore its clinical applications.

Based on these chemical probes and our studies on β-lactamase probes [21-23], we designed a chemical library of fluorogenic probes, consisting of a carbapenem core structure, an active linker, and a fluorophore to identify new fluorogenic probes specific to all types of carbapenemases (**Fig. 1c**). In this approach, we envisaged that the different types of the active linker would operate as a functional spacer between the enzyme recognition site and fluorescent dye, which may facilitate the enzymatic acceptance of our probes as substrates, so that a wide range of carbapenemases would be detectable. Additionally, we expect that their physicochemical properties, such as molecular stability in an aqueous solution, would improve. Herein, we describe a chemical library of carbapenem-based fluorogenic probes, which are clinically evaluated for the specific detection of carbapenemase-producing bacteria.

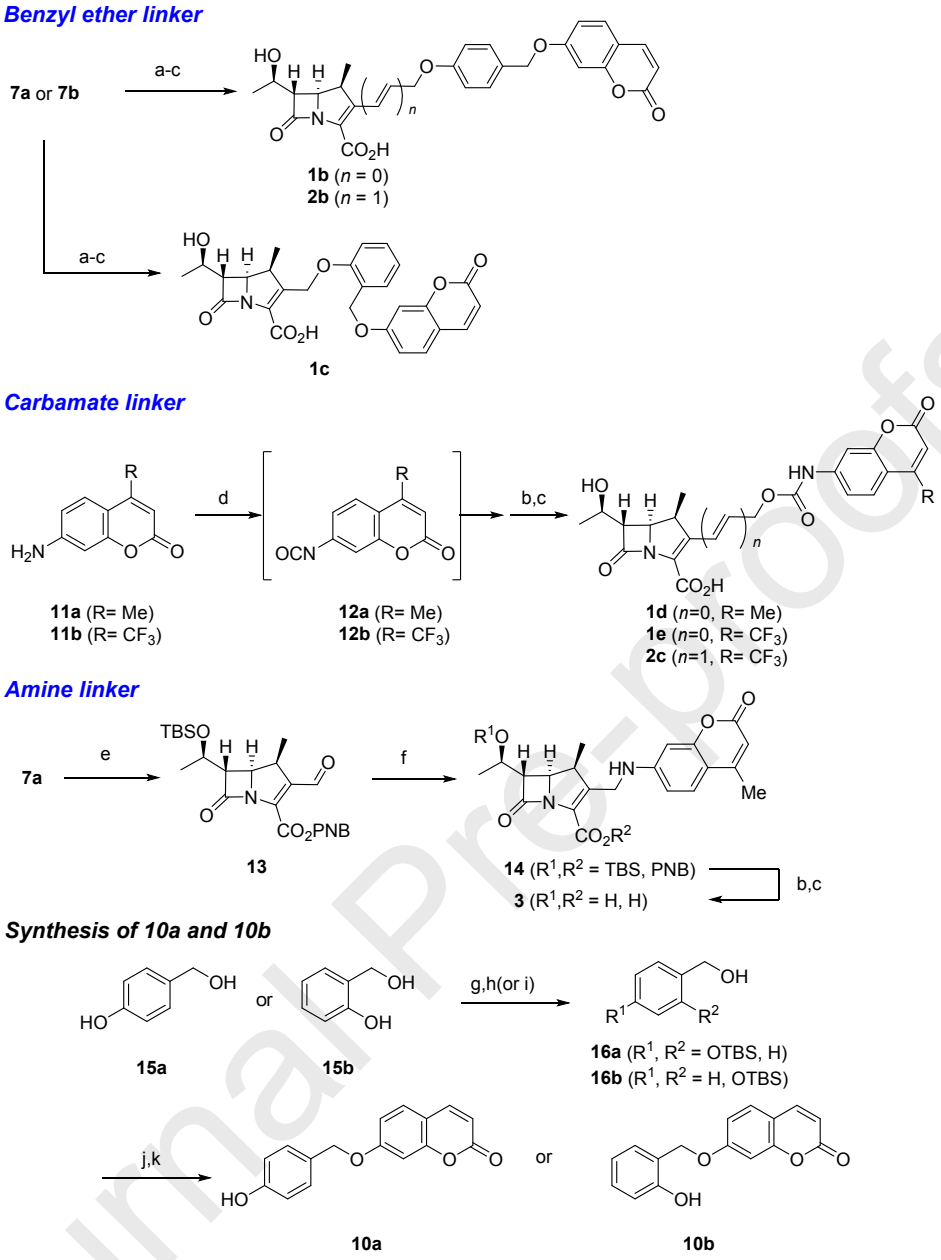
2. Results and discussion

The synthesis of carbapenem-based fluorogenic probes is illustrated in **Scheme 1**. Following the procedure reported in previous literature [24,25], we first synthesized hydroxymethyl carbapenem **7a** and hydroxyallyl carbapenem **7b** as key versatile intermediates for preparation of the library. Thus, hydroxy diazo β-lactam **4** was transformed to the corresponding triflate **5** in good yield through the following three steps: TBS protection, intramolecular insertion, and treatment of trifilic anhydride. Then, triflate **5** was coupled with hydroxymethyl stannane **6a** [26] through the Stille coupling reaction in the presence of $Pd_2(dba)_3 \cdot CHCl_3$ afforded hydroxymethyl carbapenem **7a** as a whited solid. The allyl alcohol derivative **7b** was also prepared in a higher yield using (*E*)-3-(tributylstannyl)prop-2-en-1-ol **6b** [27] as a coupling partner under the modified reaction condition. With alcohols **7a** and 7**b** in hand, we initially attempted to synthesize probes **1a** and **2a** containing ether moiety as a linker. Although the synthesis of **CPC-1** has already been reported, we synthesized this compound as well in order to use it as a control substrate. Based on the reported procedure [28,29], we performed the Mitsunobu reaction of **7a** with umbelliferone and subsequent removal of TBS group and PNB group to yield compound **1a**, which was purified by semi-prep HPLC, dried by lyophilization, and stored at a low temperature. Using the same reaction sequence, we were able to obtain the desired carbapenem derivative **2a** without difficulty.



Scheme 1. Synthesis of the carbapenemase-based fluorogenic probes **1a** and **2a**. Reagents and conditions: a) TBSCl, imidazole, DMF, rt; b) $Rh_2(OAc)_4$, $ZnCl_2$, CH_2Cl_2, 60 °C (in pressure tubes); c) TMP, DIPEA, Tf_2O, −50 °C, 92% (three steps from **4**); d) **6a** or **6b**, $Pd_2(dba)_3 \cdot CHCl_3$, P(2-furyl)$_3$, $ZnCl_2$, HMPA or NMP, 70 °C, 52% (**7a**) and 74% (**7b**); e) umbelliferone, DIAD, PPh_3, toluene, 0 °C, 90% (**8a**) and 91% (**9a**); f) NH_4HF_2, NMP/DMF, rt, 82% (**8a'**) and 75% (**9a'**); g) Rh/C, H_2, 1 M $NaHCO_3$, THF/H_2O, rt, 73% (**1a**) and 44% (**2a**).

Next, we expanded these optimized procedures to synthesize a series of carbapenemase substrates with different types of active linkers as described in **Scheme 2**. The synthesis of chemical probes **1b**, **2b**, and **1c** having benzyl ether as an active linker was achieved by the Mitsunobu reaction of **7a** or **7b** with coumarin-attached phenols **10a** and **10b** (See Scheme S1 in ESI for the synthesis of **10a** and **10b**) followed by the deprotection of both protecting groups. The reaction of **7a** or **7b** with **12a** or **12b** isocyanate, generated *in-situ* from the treatment of **11a** or **11b** with triphosgene [30] afforded the corresponding carbamate intermediates, which underwent protecting group eliminations to provide **1d**, **1e**, and **2c**. Finally, the carbapenem-based probe **3** having an amine linker was prepared from alcohol **7a** via Dess-Martin oxidation, reductive amination [31], and selective deprotection reaction sequences. Therefore, we effectively constructed a chemical library of nine different carbapenem-based probes, including **CPC-1** (**1a**) [32].



Scheme 2. Synthesis of the carbapenem-based fluorogenic probes with different linkers such as benzyl ether, carbamates, and amine. Reagents and conditions: a) **10a** or **10b**, DIAD, PPh_3, toluene, 0 °C, 1 h, 50-84%; b) NH_4HF_2, NMP/DMF, rt, 3 d, 48-72%; c) Rh/C, H_2, 1M $NaHCO_3$, THF/H_2O, rt, 2 h, 24-60%; d) i) triphosgene, TEA, DCE, 80 °C, 2 h; ii) **7a** or **7b**, TEA, THF, rt, 12 h, 33-78%; e) Dess-Martin periodinane, DCM, rt, 64%; f) **11a**, AcOH, $NaBH(OAc)_3$, rt, 12 h, 48%; g) TBSCl, Imidazole, DMF, rt, 12 h, 99% (**15a'**) and 76% (**15b'**); h) NIS, MeOH, rt, 2 h, 88% (for **16a**); i) $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 12 h, 72% (for **16b**); j) umbelliferone, DIAD, PPh_3, toluene, rt, 1 h, 98% (**16a'**) and 77% (**16b'**); k) AcOH, TBAF, THF, rt, 2 h, 91% (**10a**) and 98% (**10b'**).

Having synthesized new fluorescent probes, we then examined their intrinsic stability in aqueous solution by measuring their fluorescence intensity in phosphate buffered saline (PBS, pH 7.4) for 12 h (**Fig. 2**). Unlike the control **1a** having good stability, allyl ether probe **2a** slowly decomposed up to 27% in PBS solution. We found that probes **1b** and **1c** containing benzyl ether linkers exhibit excellent stability with fluorescence increases lower than 15%, whereas carbamate-linked probes **1d**, **1e,** and **2c** were easily hydrolyzed to generate high fluorescent signals within a short period of time. The low stability of these probes is presumably due to the higher susceptibility of the carbamate moiety to nucleophilic attack [33]. We also confirmed that probe **3** is more stable when we observed its remaining amount by the analysis of HPLC, but it cannot be used as a turn-on fluorogenic probe because there was no difference in fluorescence intensity between probe **3** and aminocoumarin **11a**.

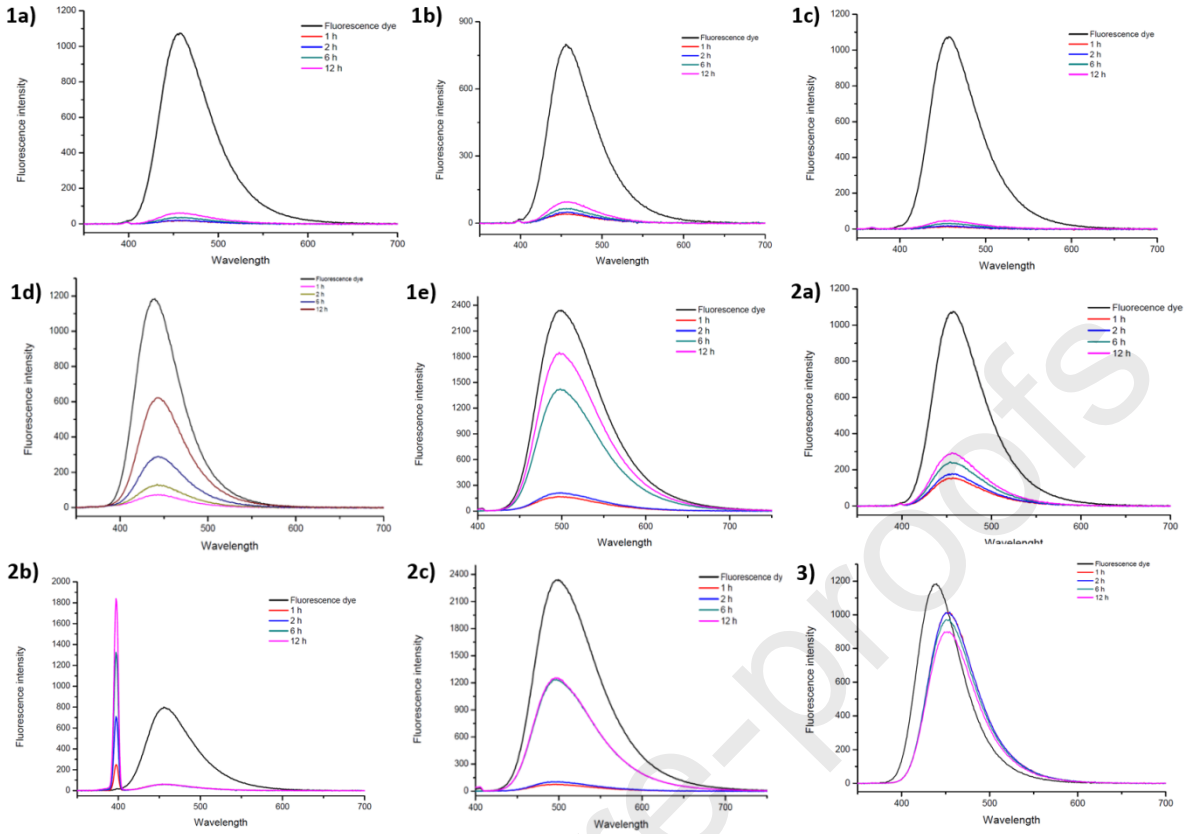


Fig. 2. Intrinsic stability of fluorescence probes **1a-1e, 2a-2c** and **3** in PBS. The enhanced fluorescence intensity of probes (10 μM) was measured for 12 hours in PBS (pH 7.4). λ_{ex} = 365 nm (**1a-1c**) and 375 nm (**1d-3**).

Next, we investigated the absorbance and fluorescence of **1b** and **1c** before and after treatment by carbapenemase (**Fig. 3**). Probe **1b** displayed a strong absorption peak at 320 nm. After the treatment of NDM-1, the maximum absorption wavelength shifted to 360 nm, which is identical to the characteristic absorption band of deprotonated umbelliferone (Fig. 2a). A similar absorbance shift was observed upon incubation of probe **1c** with NDM-1 (not shown). When probes **1b** and **1c** were treated with NDM-1, they showed over 150-fold fluorescence signal enhancement at 460 nm (**Fig. 3b** and **3c**).

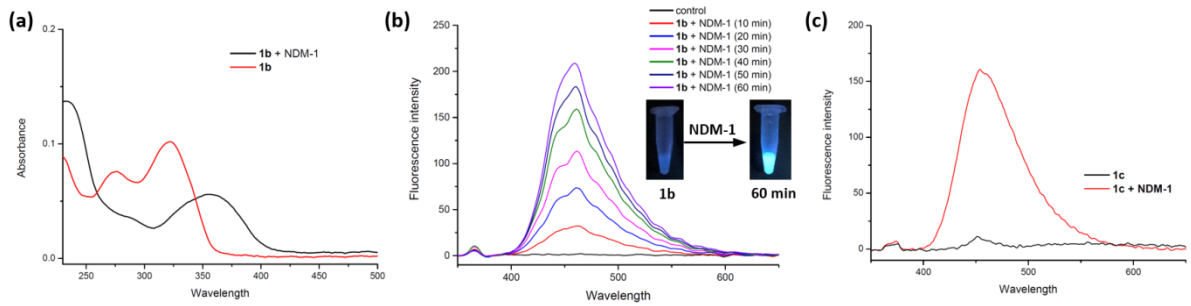


Fig. 3. Absorbance and fluorescence of **1b** and **1c**. (a) Changes in absorption spectra for probe **1b** before (red) and after (black) incubation with NDM-1 (100 nM) at 25 °C. (b) The fluorescence emission profile of **1b** (10 μM) upon incubation with NDM-1 (100 nM) at 25 °C. (c) The fluorescence emission profile of **1c** (10 μM) upon incubation with NDM-1 (100 nM) at 25 °C.

To verify the possibility of using the newly synthesized compounds as fluorogenic probes to detect carbapenemases, we conducted the enzyme assay of probes **1a** (**CPC-1**), **1b** and **1c** (**Fig. 4**). Four of the most clinically widespread carbapenemases, including two metallo-β-lactamses (IMP-1, NDM-1), one class A β-lactamase (KPC-3), and one class D β-lactamase (OXA-48), were prepared. We observed a significant increase in fluorescence signals when all carbapenemases were treated with probe **1b** for one hour (**Fig. 4a**). However, treatment with probe **1c** under the same condition showed a slower signal induction, which may be due to the high stability of **1c** (**Fig. 4b**). In particular, probe **1b** exhibited higher detection efficiency against NDM-1, IMP-1, and KPC-3 than with compound **1a** (**Fig. 4c**). Additionally, TEM-1, a serine β-lactamase (Class A) most commonly found in gram-negative bacteria, was utilized as a negative control. As expected, no fluorescence enhancement was observed in TEM-1 (**Fig. 4d**). We further confirmed the detection of TEM-1 as the fluorogenic probe reported in the literature [18] was used as a positive control. (**Fig. S1**). These results indicate that probe **1b** is useful for the specific detection of carbapenemases.

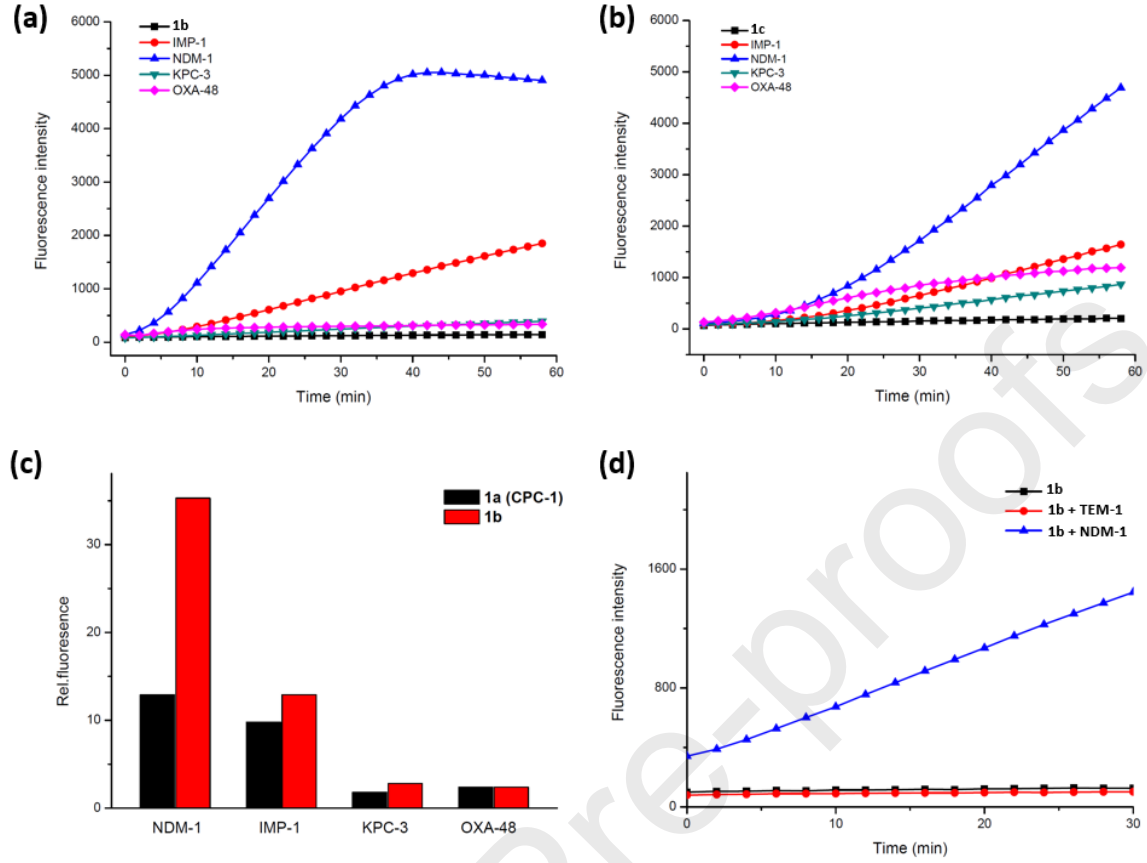


Fig. 4. Fluorescence response of probes **1a** (**CPC-1**), **1b** and **1c** to carbapenemases. (a) and (b) Time dependence of the fluorescence of **1b** and **1c** (10 μM in PBS, pH 7.4) upon incubation with the indicated carbapenemase. (c) Fluorescent enhancement of **1a** (**CPC-1**) and **1b** (10 μM in PBS, pH 7.4) upon incubation with a series of carbapenemases for 60 min. (d) Time dependence of the fluorescence of **1b** in the presence of NDM-1 and TEM-1 for 30 min.

Finally, we tested the two probes (**1a** and **1b**) for detecting carbapenemase-producing *Enterobacteriaceae* (CPE) isolated from clinical specimens. A total of 42 previously characterized isolates of *Enterobacteriaceae* were used: 24 CPEs (9 KPC, 9 NDM, and 6 OXA) and 18 carbapenem-susceptible *Enterobacteriaceae* (CSE) isolates. The isolates were collected from various clinical samples from multiple Korean hospitals. The identification of bacterial isolates was performed using MALDI-TOF MS analysis via Vitek MS (bioMérieux, Marcy L'Etoile, France). They were previously characterized by PCR amplification and molecular

sequencing as described in the literatures [34,35]. The detailed characteristics of isolates including minimum inhibitory concentrations (MIC) of carbapenems are shown in **Table S1** (Supplementary Material). In addition, the manual Carba NP test was conducted as described in the CLSI guidelines [11]. As a result, probe **1b** exhibited higher sensitivity (91.7%) than **1a** (**CPC-1**) (58.3%) for detecting CPE (**Table 1**). The probe **1a** (**CPC-1**) exhibited poor sensitivity (44.4%) in detecting KPC enzyme, which reflects the findings of a previous report [18], whereas probe **1b** showed high sensitivity (100%) for detecting KPC enzyme. In addition, probe **1b** exhibited higher sensitivity (66.7%) for detection of OXA-48 like carbapenemases compared to probe **1a** (**CPC-1**) (16.7%) and the manual Carba NP test (16.7%), which is known to exhibit low sensitivity for detection of OXA-48 type carbapenemases [36]. The fluorescence signals of the probes according to the carbapenemase types are presented in **Fig. 5**.

Table 1. Results of the fluorogenic assay using **1a** (**CPC-1)** and probe **1b**, and the Carba NP test in 24 CPE and 18 CSE isolates.

Classification	Gene	No. of isolates	**1a (CPC-1)**	Pos.%	**1b**	Pos.%	Carba NP	Pos.%
Class A	KPC	9	4	44.4	9	100	9	100
Class B	NDM	9	9	100	9	100	9	100
Class D	OXA	6	1	16.7	4	66.7	1	16.7
Total	CPE	24	14	58.3	22	91.7	19	79.2
Total	CSE	18	0	0	0	0	0	0

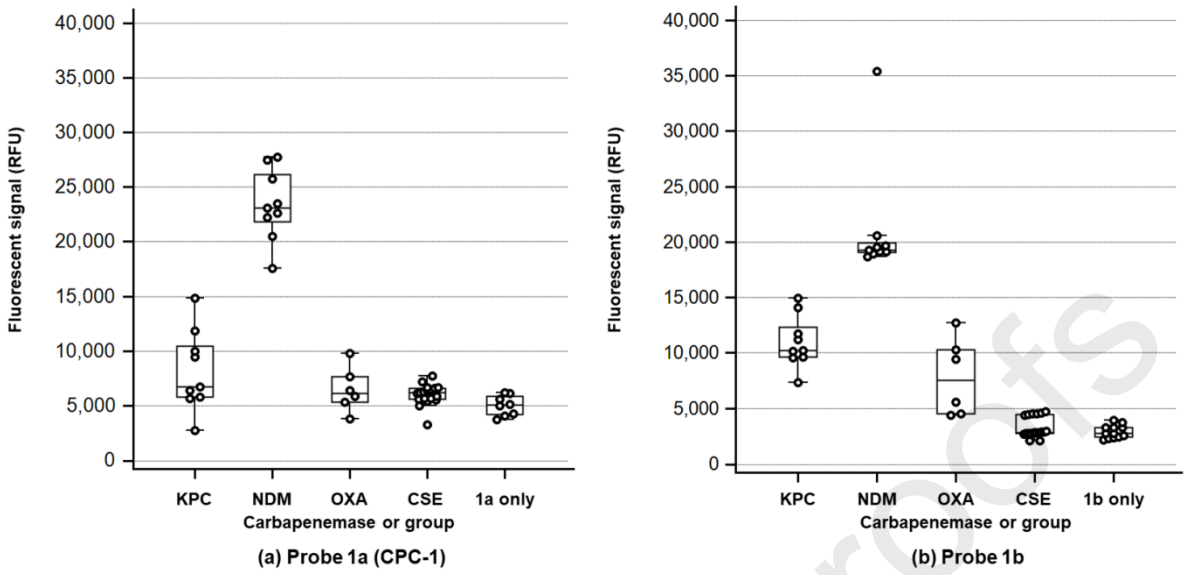


Fig. 5. Fluorescence signals after treatment of each gene (or group) with probes **1a** (**CPC-1**) and **1b**.

3. Conclusion

In summary, we have described a library of carbapenem-based fluorogenic probes with different active linkers for the detection of carbapenemase-producing bacteria. Among the synthesized fluorescent probes, compound **1b** with a benzyl ether linker generated an enhanced fluorescence signal upon hydrolysis by different types of carbapenemases, which proved that the active linker plays a crucial role in the stability of the probes and their ability to potentiate the activity of carbapenemases. Finally, we confirmed that probe **1b** could detect pathogenic bacteria expressing carbapenemase with excellent sensitivity and specificity. Therefore, probe **1b** can be clinically used as a potential substrate for diagnosing CPO-induced diseases.

4. Experimental Section

4.1 Chemistry

General: All reactions were conducted using oven-dried glassware under an atmosphere of argon (Ar). All commercially available reagents and anhydrous solvents were obtained from Sigma Aldrich, TCI, Alfa, Junsei, Samchun, DaeJung Chemical and were used without further purification. Solvents CH_2Cl_2 was dried and distilled following usual protocols. Organic solvents were evaporated with reduced pressure using a rotary evaporator. Reactions were followed by TLC analysis using silica gel 60 F_{254} with fluorescent indicator using UV lamp and ninhydrin solution with heat as visualizing agents. Flash chromatography was carried out using Merck silica gel 60 (0.063-0.200 mm) and KANTO silica gel 60N (spherical, neutral). The ^{1}H NMR spectra and ^{13}C NMR spectra were measured with Bruker AVANCE III HD 400. ^{1}H NMR chemical shifts are expressed in parts per million (δ) downfield to $CHCl_3$ (δ = 7.26), ^{13}C NMR chemical shifts are expressed in parts per million (δ) relative to the central $CDCl_3$ resonance (δ = 77.0). Coupling constants in ^{1}H NMR are in Hz. The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet. $CDCl_3$ was used as NMR solvent and standard material TMS (tetramethylsilane) wasn't contained. LC/MS analyses were performed on Agilent 6125 SQ LCMS system.

*4.1.1. 4-Nitrobenzyl (R)-4-((2R,3S)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl)-2-diazo-3-oxopentanoate (**4'**)* [25]

To a solution of alcohol **4** (5.00 g, 12.8 mmol) in anhydrous DMF (43.0 mL) cooled at 0 °C was added imidazole (4.42 g, 64.8 mmol) and *tert*-butyldimethylsilyl chloride (7.72 g, 51.2 mmol). The mixture was allowed to warm up to room temperature over 12 h. After completion of the reaction (monitored by TLC), it was quenched with saturated aqueous NH_4Cl, extracted

with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:2) to afford target compound **4'** (6.16 g, 95%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 5.89 (s, 1H), 5.37 (d, J = 13.2 Hz, 1H), 5.33 (d, J = 13.2 Hz, 1H), 4.21-4.16 (m, 1H), 3.90-3.89 (m, 2H), 2.96 (d, J = 2.9 Hz, 1H), 1.19 (d, J = 6.0 Hz, 3H), 1.17 (d, J = 5.9 Hz, 3H), 0.85 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.29, 168.15, 160.72, 148.09, 141.90, 128.77, 124.08, 65.60, 65.08, 61.16, 51.48, 43.30, 25.75, 22.61, 17.94, 12.28, -4.28, -4.98.

*4.1.2. 4-Nitrobenzyl (4R,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-(((trifluoromethyl)sulfonyl)oxy)-1-azabicyclo[3.2.0]heptane-2-carboxylate (**5**)* [25]

A sealed tube was charged with diazo carbonyl compound **4'** (100 mg, 0.198 mmol) in dichloromethane (0.600 mL). Rhodium acetate dimer (1 mg, 0.002 mmol) and zinc chloride (1 mg, 0.006 mmol) was added to the mixture. The reaction mixture was allowed to stir at 60 °C for 1 h. After completion of the reaction (monitored by TLC), 2,2,6,6-tetramethyl piperidine (40 µL, 0.24 mmol) and N,N-diisopropylethylamine (17 µL, 0.10 mmol) was added to the mixture at -78 °C and trifluoromethanesulfonic anhydride (37 µL, 1.68 mmol) was dropwise for 5 min. After 1 h, the resulting residue was purified by flash column chromatography on neutral silica gel (dichloromethane only) to afford triflate **5** (117 mg, 97%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21(d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7Hz, 2H), 5.42 (d, J = 13.6 Hz, 1H), 5.35 (d, J = 13.6 Hz, 1H), 4.35 (dd, J = 3.4, 11.0 Hz, 1H), 4.30-4.24 (m, 1H), 3.41-3.33 (m, 2H), 1.29 (d, J = 7.3 Hz, 3H), 1.22 (d, J = 6.2 Hz, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H).

*4.1.3. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-3-(hydroxymethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (**7a**)* [25]

To a solution of tri(2-furyl)phosphine (11.4 mg, 0.049 mmol), $Pd_2(dba)_3$-$CHCl_3$ (25.4 mg, 0.025 mmol), zinc chloride (22.4 mg, 0.164 mmol), in anhydrous HMPA (0.5 mL) was slowly added triflate **5** (100 mg, 0.164 mmol) in HMPA (0.1 mL) and hydroxymethyl stannane **6a** (211 mg, 0.656 mmol) in HMPA (0.1 mL) at 70 °C. After 2 h, it was quenched with saturated aqueous NH_4Cl at 5 °C, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:8 to 1:2) to afford target compound **7a** (42.0 mg, 52%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 5.46 (d, J = 13.9 Hz, 1H), 5.27 (d, J = 13.9 Hz, 1H), 4.55 (d, J = 15.0 Hz, 1H), 4.39 (d, J = 14.6 Hz, 1H), 4.27-4.22 (m, 2H), 3.30-3.23 (m, 2H), 3.14 (s, 1H), 1.24 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 7.4 Hz, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.14, 161.88, 153.43, 147.67, 142.48, 128.06, 127.44, 123.79, 65.73, 65.70, 60.34, 57.68, 55.73, 41.65, 25.68, 22.35, 17.96, 15.37, -4.20, -5.02.

*4.1.4. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-3-((E)-3-hydroxyprop-1-en-1-yl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (**7b**)*

To a solution of tri(2-furyl)phosphine (19.1 mg, 0.082 mmol), $Pd_2(dba)_3$-$CHCl_3$ (42.5 mg, 0.041 mmol), zinc chloride (10.9 mg, 0.182 mmol), in anhydrous *N*-Methylpyrrolidine (10.3 mL) was added triflate **5** (500 mg, 0.821 mmol) and (*E*)-3-(tributylstannyl)prop-2-en-1-ol **6b** (428 mg, 1.232 mmol) at room temperature. After 12 h, it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:4 to 1:2) to afford target compound

7b (315 mg, 74%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 16.3 Hz, 1H), 6.20 (dt, J = 5.5, 16.2 Hz, 1H), 5.44 (d, J = 13.9 Hz, 1H), 5.27 (d, J = 13.9 Hz, 1H), 5.27 (d, J = 13.9 Hz, 1H), 4.32 (bs, 2H), 4.29-4.23 (m, 1H), 4.20 (dd, J = 2.7, 9.4 Hz, 1H), 3.41-3.33 (m, 1H), 3.23 (dd, J = 2.7, 5.6 Hz, 1H), 1.27 (d, J = 6.2 Hz, 3H), 1.22 (d, J = 7.3 Hz, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.17, 174.90, 160.88, 148.60, 147.58, 142.97, 137.57, 128.09, 125.22,123.71, 122.40, 65.96, 65.21, 63.25, 59.12, 56.19, 39.41, 30.68, 29.59, 25.67, 17.64, -4.22, -4.99.

*4.1.5. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8a**)* [19]

To a solution of alcohol **7a** (15.5 mg, 0.032 mmol) in toluene (0.6 mL) was added umbelliferone (6.2 mg, 0.038 mmol) and triphenylphosphine (10.1 mg, 0.038 mmol) at 0 °C. After 2 min, diisopropyl azodicarboxylate (8 μL, 0.047 mmol) was added to the mixture. The mixture was warm up to room temperature over 30 min. After completion of the reaction (monitored by TLC), it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:2) to afford target compound **8a** (18.2 mg, 90%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 9.6 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 2.1 Hz, 1H), 6.83 (s, 1H), 6.27 (d, J = 9.5 Hz, 1H), 5.54 (d, J = 14.4 Hz, 1H), 5.48 (d, J = 13.8 Hz, 1H), 5.29 (d, J = 13.8 Hz, 1H), 4.78 (d, J = 14.4 Hz, 1H), 4.28-4.13 (m, 2H), 3.44-3.35 (m, 1H), 3.31-3.23 (m, 1H), 1.25 (d, J = 6.2 Hz, 3H), 1.22 (d, J = 6.6 Hz, 3H), 0.85 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C-NMR (100

MHz, $CDCl_3$) δ 175.18, 161.25, 161.04, 160.75, 155.78, 147.66, 146.91, 143.31, 142.51, 129.05, 128.13, 127.87, 123.78, 113.58, 113.10, 112.53, 101.82, 65.65, 65.5, 63.0, 60.8, 55.4, 40.3, 25.7, 22.3, 17.9, 15.5, -4.2, -5.0.

*4.1.6. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8a'**)* [19]

To a solution of silyl ether **8a** (65.4 mg, 0.103 mmol) in NMP:DMF =1:3 (2.1 mL) was added ammonium hydrogen fluoride (23.5 mg, 0.412 mmol). The reaction mixture was allowed to stir at the same temperature for 30 h. After completion of the reaction (monitored by TLC), the mixture was diluted with saturated aqueous $NaHCO_3$, extracted with EtOAc. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 3:1) to afford alcohol **8a'** (44.0 mg, 82%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 9.4 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 6.82 (s, 1H), 6.28 (d, J = 9.4 Hz, 1H), 5.54 (d, J = 14.7 Hz, 1H), 5.29 (d, J = 13.5 Hz, 1H), 4.80 (d, J = 14.4 Hz, 1H), 4.29-4.25 (m, 2H), 3.45-3.38 (m, 1H), 3.36-3.33 (m, 1H), 1.35 (d, J = 6.2 Hz, 3H), 1.27 (d, J = 7.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.18, 161.25, 161.04, 160.75, 155.78, 147.66, 146.91, 143.31, 142.51, 129.05, 128.13, 127.87, 123.78, 113.58, 113.10, 112.53, 101.82, 65.65, 65.50, 63.00, 60.75, 55.43, 40.33, 25.70, 22.28, 17.96, 15.49, -4.24, -4.99.

*4.1.7. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**1a**, **CPC-1**)* [19]

A mixture of compound **8a′** (30.0 mg, 0.057 mmol) and 5% Rh/C (3.6 mg) in a mixed solution of THF/H_2O (1.9 mL, 2:1) under hydrogen atmosphere (1 atm) were stirred at room

temperature for 2 h. The mixture were passed through a PTFE syringe filter to remove precipitate and the filtrate was washed with ethyl acetate. The water phase was purified with preparative RP-HPLC with CH_3CN-H_2O as mobile phase. Lyophilization afforded compound **1a** (**CPC-1**, 72.7 mg, 73%) as a white solid. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.86 (d, J = 9.5 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 6.98 (dd, J = 2.4, 8.6 Hz, 1H), 6.95 (d, J = 2.1 Hz, 1H), 6.22 (d, J = 9.5 Hz, 1H), 5.64 (d, J = 13.5 Hz, 1H), 4.79 (d, J = 13.5 Hz, 1H), 4.08-4.04 (m, 2H), 3.24-3.20 (m, 1H), 3.19 (dd, J = 2.9, 7.3 Hz, 1H), 1.26 (d, J = 6.3 Hz, 3H), 1.18 (d, J = 7.3 Hz, 3H); HRMS (ESI) m/z calcd for $C_{26}H_{34}N_2O_7$ $(M\text{-}C_6H_{16}N)^-$ 486.2366, found 384.1096.

*4.1.8. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((E)-3-((2-oxo-2H-chromen-7-yl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9a**)*

Following the same procedure as that used for the synthesis of **8a**, the reaction of allyl alcohol **7b** (100 mg, 0.194 mmol), umbelliferone (34.6 mg, 0.213 mmol), triphenylphosphine (68.0 mg, 0.233 mmol) and diisopropyl azodicarboxylate (49 μL, 0.290 mmol) in toluene (3.9 mL) gave target compound **9a** (119 mg, 91%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 1:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 9.6 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.85 (dd, J = 2.3, 8.6 Hz, 1H), 6.81 (d, J = 7.0 Hz, 1H), 6.28-6.17 (m, 2H), 5.45 (d, J = 13.8 Hz, 1H), 5.28 (d, J = 14.0 Hz, 1H), 4.74 (d, J = 5.8 Hz, 2H), 4.27-4.21 (m, 2H), 3.42-3.37 (m, 1H), 3.25 (dd, J = 2.6, 5.4 Hz, 1H), 1.26 (d, J = 6.2 Hz, 6H), 0.85 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.70, 161.42, 161.13, 160.74, 155.78, 147.62, 147.29, 143.35, 142.80, 131.20, 128.90, 128.11, 126.40, 125.57, 123.73, 113.41, 113.00, 112.89, 101.78, 68.80, 65.85, 65.35, 59.26, 56.17, 39.28, 25.66, 22.41, 17.62, 16.80, -4.22, -5.01.

*4.1.9. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((E)-3-((2-oxo-2H-chromen-7-yl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9a'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **9a** (40.1 mg, 0.059 mmol) and ammonium hydrogen fluoride (13.6 mg, 0.238 mmol) in a mixed solution of NMP/DMF (1.2 mL, 1:3) gave target compound **9a'** (24.0 mg, 75%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 9.6 Hz, 1H), 7.45 (d, J = 16.3 Hz, 1H), 7.37 (d, J = 8.6 Hz, 1H), 6.83 (dd, J = 2.4, 8.6 Hz, 1H), 6.80 (d, J = 2.3 Hz, 1H), 6.28 (d, J = 4.8 Hz, 1H), 6.20 (dt, J = 5.7, 16.3 Hz, 1H), 5.49 (d, J = 13.8 Hz, 1H), 5.25 (d, J = 13.8 Hz, 1H), 4.73 (d, J = 5.8 Hz, 2H), 4.26-4.23 (m, 2H), 3.47-3.43 (m, 1H), 3.28 (dd, J = 2.6, 6.9 Hz, 1H), 1.37 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 7.2 Hz, 3H).

*4.1.10. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((E)-3-((2-oxo-2H-chromen-7-yl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**2a**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **9a'** (36.0 mg, 0.066 mmol) and 5% Rh/C (4.2 mg) in a mixed solution of THF/H_2O (2.1 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **2a** (12.0 mg, 44%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.87 (d, J = 9.5 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 16.3 Hz, 1H), 6.97 (dd, J = 2.4, 8.6 Hz, 1H), 6.93 (d, J = 2.3 Hz, 1H), 6.22 (d, J = 9.5 Hz, 1H), 6.03 (dt, J = 6.1, 16.2 Hz, 1H), 4.74 (d, J = 6.0 Hz, 2H), 4.10-4.05 (m, 2H), 3.37-3.26 (m, 1H), 3.15 (dd, J = 2.4, 7.5 Hz, 1H), 1.28 (d, J = 6.3 Hz, 3H), 1.27 (d, J = 7.2 Hz, 3H); HRMS (ESI) m/z calcd for $C_{22}H_{21}NO_7$ $(M+Na)^+$ 434.1210, found 434.1212.

*4.1.11. tert-Butyl((4-((tert-butyldimethylsilyl)oxy)benzyl)oxy)dimethylsilane (**15a'**)*

To a solution of *para*-hydroxybenzyl alcohol **15a** (500 mg, 4.03 mmol) in anhydrous DMF (20.2 mL) was added imidazole (1.10 g, 16.1 mmol) and *tert*-butyldimethylsilyl chloride (2.42 g, 16.1 mmol) at 0 °C. The mixture was allowed to warm up to room temperature over 12 h. After completion of the reaction (monitored by TLC), it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:15) to afford target compound **15a'** (1.40 g, 99%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 4.67 (s, 2H), 0.98 (s, 9H), 0.93 (s, 9H), 0.18 (s, 6H), 0.08 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.60, 134.17, 127.42, 119.83, 64.80, 26.00, 25.72, 18.46, 18.23, -4.41, -5.17.

*4.1.12. (4-((tert-butyldimethylsilyl)oxy)phenyl)methanol (**16a**)*

To a solution of bis-silyl ether **15a'** (35.3 mg, 0.10 mmol) in MeOH (0.3 mL) was added N-iodosuccinimide (1.1 mg, 0.005 mmol) at room temperature. After 12 h, it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:8 to 1:4) to afford target compound **16a** (21.0 mg, 88%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J = 8.5 Hz, 2H), 6.82 (dt, J = 2.3, 9.0 Hz, 2H), 4.60 (s, 2H), 0.98 (s, 9H), 0.19 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.28, 133.74, 128.58, 120.17, 65.05, 25.71, 18.24, -4.41.

*4.1.13. 7-((4-((tert-Butyldimethylsilyl)oxy)benzyl)oxy)-2H-chromen-2-one (**16a'**)*

To a solution of benzyl alcohol **16a** (1.50 g, 5.34 mmol) in toluene (108 mL) was added

umbelliferone (961 mg, 5.93 mmol) and triphenylphosphine (1.89 mg, 6.47 mmol) at 0 °C. After 2 min, diisopropyl azodicarboxylate (1.37 mL, 8.08 mmol) was added to the mixture. The mixture was warm up to room temperature over 30 min. After completion of the reaction (monitored by TLC), it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:8) to afford target compound **16a** (2.02 g, 98%) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 9.5 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 2.3 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.4 Hz, 1H), 6.25 (d, J = 9.4 Hz, 1H), 5.03 (s, 2H), 0.98 (s, 9H), 0.20 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.99, 161.20, 155.91, 155.82, 143.44, 129.26, 128.79, 128.38, 120.32, 113.25, 113.12, 112.65, 101.85, 70.39, 25.68, 18.21, -4.39.

*4.1.14. 7-((4-Hydroxybenzyl)oxy)-2H-chromen-2-one (**10a**)*

Under Ar atmosphere, to a solution of **16a'** (1.00 g, 2.61 mmol) in THF (anhydrous, 26.1 mL) at 0°C were added slowly AcOH (1.05 mL, 18.3 mmol) and TBAF (1M solution in THF, 13 mL, 13.0 mmol). The reaction mixture were stirred at room temperature for 3 h, and then concentrated under reduced pressure. The residues were purified by chromatography on silica gel column (Acetone:*n*-hexane = 1:2) to afford the title compound **10a** (635 mg, 91%) as a white solid. ^{1}H-NMR (400 MHz, Acetone) δ 7.89 (d, J = 9.5 Hz, 1H), 7.68 (d, J = 9.3 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 6.98-6.96 (m, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.21 (d, J = 9.5 Hz, 1H), 5.13 (s, 2H); ^{13}C-NMR (100 MHz, Acetone) δ 162.16, 160.07, 157.54, 155.98, 143.67, 129.79, 129.24, 127.23, 115.29, 112.95, 112.75, 112.63, 101.51, 70.24.

*4.1.15. tert-Butyl((2-((tert-butyldimethylsilyl)oxy)benzyl)oxy)dimethylsilane (**15b'**)*

Following the same procedure as that used for the synthesis of **15a'**, the reaction of *ortho*-hydroxybenzyl alcohol **15b** (500 mg, 4.03 mmol), imidazole (686 mg, 10.1 mmol) and *tert*-butyldimethylsilyl chloride (1.52 g, 10.1 mmol) in anhydrous DMF (20.2 mL) gave target compound **15b'** (1.08 g, 76%) as a colorless oil after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 1:15). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.46 (dt, J = 1.0, 9.4 Hz, 1H), 7.11 (td, J = 2.0, 9.6 Hz, 1H), 6.97 (td, J = 1.2, 9.3 Hz, 1H), 6.74 (dd, J = 1.2, 10.0 Hz, 1H), 4.76 (s, 2H), 1.01 (s, 9H), 0.95 (s, 9H), 0.22 (s, 6H), 0.10 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 151.93, 132.23, 127.23, 126.97, 121.09, 117.81, 60.58, 26.01, 25.75, 18.49, 18.25, -4.19, -5.33.

*4.1.16. (2-((tert-Butyldimethylsilyl)oxy)phenyl)methanol (**16b**)*

To a solution of bis-silyl ether **15b'** (636 mg, 1.80 mmol) in acetonitrile (25.8 mL) was added cerium chloride heptahydrate (1.34 g, 3.61 mmol) at 90 °C. After 12 h, it was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate and washed with brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:8 to 1:4) to afford target compound **16b** (49.0 mg, 72%) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, J = 2.0, 9.3 Hz, 1H), 7.18 (td, J = 2.1, 9.7 Hz, 1H), 6.96 (dt, J = 1.2, 9.3 Hz, 1H), 6.82 (d, J = 10.1 Hz, 1H), 4.68 (s, 2H), 1.02 (s, 9H), 0.26 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 1153.50, 131.49, 128.85, 128.63, 121.36, 118.41, 61.96, 25.76, 18.19, -4.15.

*4.1.17. 7-((2-((tert-Butyldimethylsilyl)oxy)benzyl)oxy)-2H-chromen-2-one (**16b'**)*

Following the same procedure as that used for the synthesis of **16a'**, the reaction of benzyl alcohol **16b** (68.0 mg, 0.285 mmol), umbelliferone (50.8 mg, 0.314 mmol), triphenylphosphine

(100 mg, 0.342 mmol) and diisopropyl azodicarboxylate (0.072 mL, 0.428 mmol) in toluene (5.80 mL) gave target compound **16b'** (84.0 mg, 77%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 1:8). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 11.8 Hz, 1H), 7.39 (dd, J = 1.9, 9.5 Hz, 1H), 7.36 (d, J = 10.5 Hz, 1H), 7.22 (td, J = 2.0, 9.7 Hz, 1H), 6.97 (td, J = 1.2, 9.4 Hz, 1H), 6.91-6.85 (m, 3H), 6.24 (d, J = 11.8 Hz, 1H), 5.12 (s, 2H), 0.97 (s, 9H), 0.25 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 162.15, 161.30, 155.91, 153.52, 143.45, 129.47, 128.75, 126.31, 121.32, 118.67, 113.16, 113.08, 112.57, 101.69, 66.12, 25.71, 21.97, 18.22, -4.16.

*4.1.18. 7-((2-Hydroxybenzyl)oxy)-2H-chromen-2-one (**10b**)*

Following the same procedure as that used for the synthesis of **10a**, the reaction of **16b'** (427 mg, 1.12 mmol), AcOH (0.447 mL, 7.81 mmol) and TBAF (1M solution in THF, 5.6 mL, 5.58 mmol) in THF (anhydrous, 11.2 mL) gave compound **10b** (292 mg, 98%) as a white solid after purification by column chromatography on silica gel (acetone:*n*-hexane = 1:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 11.8 Hz, 1H), 7.58 (d, J = 10.4 Hz, 1H), 7.41 (dd, J = 1.8, 9.4 Hz, 1H), 7.20 (td, J = 1.9, 9.6 Hz, 1H), 7.01-6.98 (m, 2H), 6.94 (d, J = 9.4 Hz, 1H), 6.88 (td, J = 1.1, 9.3 Hz, 1H), 6.21 (d, J = 11.9 Hz, 1H), 5.25 (s, 2H); ^{13}C-NMR (100 MHz, acetone-D_6) δ 162.22, 161.13, 160.11, 156.00, 155.17, 143.84, 129.75, 129.25, 122.62, 119.68, 115.34, 112.87, 112.72, 112.06, 101.39, 65.45.

*4.1.19. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8b**)*

Following the same procedure as that used for the synthesis of **8a**, the reaction of alcohol **7a**

(94 mg, 0.192 mmol), phenol compound **10a** (56.7 mg, 0.211 mmol), triphenylphosphine (67.3 mg, 0.230 mmol) and diisopropyl azodicarboxylate (49 μL, 0.288 mmol) in toluene (3.8 mL) gave target compound **8b** (120 mg, 84%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 2:5:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 9.0 Hz, 2H), 7.63 (d, J = 9.8 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.35 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 6.91-6.87 (m, 2H), 6.25 (d, J = 9.4 Hz, 1H), 5.47 (d, J = 14.6 Hz, 1H), 5.47 (d, J = 13.8 Hz, 1H), 5.27 (d, J = 13.8 Hz, 1H), 5.05 (s, 2H), 4.72 (d, J = 15.1 Hz, 1H), 4.29-4.25 (m, 2H), 3.50-3.42 (m, 1H), 3.30 (dd, J = 3.2, 5.2 Hz, 1H), 1.24 (d, J = 6.2 Hz, 6H), 0.86 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.34, 161.87, 161.23, 160.82, 158.37, 155.82, 148.49, 147.67, 143.43, 142.61, 129.47, 128.82, 128.10, 123.79, 114.74, 113.30, 101.85, 70.21, 65.64, 65.53, 62.58, 60.66, 55.54, 40.40, 25.70, 22.32, 21.97, 17.98, 15.50, -4.23, -4.97.

*4.1.20. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8b'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **8b** (95.0 mg, 0.128 mmol) and ammonium hydrogen fluoride (29.3 mg, 0.513 mmol) in a mixed solution of NMP/DMF (2.4 mL, 1:3) gave target compound **8b'** (58.0 mg, 72%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.8 Hz, 2H), 7.67-7.62 (m, 3H), 7.38-7.33 (m, 3H), 6.92-6.89 (m, 3H), 6.86 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 9.4 Hz, 1H), 5.52 (d, J = 13.6 Hz, 1H), 5.47 (d, J = 14.8 Hz, 1H), 5.26 (d, J = 13.8 Hz, 1H), 5.06 (s, 2H), 4.74 (d, J = 14.8 Hz, 1H), 4.29-4.24 (m, 2H), 3.52-3.44 (m, 1H), 3.33 (dd, J = 3.0, 6.3 Hz, 1H), 1.35 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.72. 161.86. 161.28. 160.70. 158.21. 155.80. 149.47. 147.73. 143.47. 142.56. 129.39. 128.82. 128.63, 128.24.127.05.

123.85. 114.75. 113.39. 113.22. 112.71. 101.88. 70.19. 65.65. 62.57. 59.87. 55.90. 40.57. 22.04. 21.79. 15.44.

*4.1.21. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**1b**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **8b′** (26.0 mg, 0.041 mmol) and 5% Rh/C (2.6 mg) in a mixed solution of THF/H_2O (1.4 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **1b** (10.0 mg, 50%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.87 (d, J = 9.6 Hz, 1H), 7.52 (d, J = 9.3 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 6.98-6.96 (m,4 H), 6.23 (d, J = 9.5 Hz, 1H), 5.54 (d, J = 13.5 Hz, 1H), 5.08 (s, 2H), 4.68 (d, J = 13.4 Hz, 1H), 4.08-4.02 (m, 2H), 3.47-3.46 (m, 1H), 3.17 (dd, J = 2.8, 7,5 Hz, 1H), 3.12-3.11 (m, 1H), 1.25 (d, J = 6.3 Hz, 3H), 1.17 (d, J = 7.1 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD) δ 175.50, 161.15, 162.38, 16199, 158.72, 155.69, 144.39, 138.33, 134.92, 129.19, 129.02, 128.40, 114.42, 113.13, 112.70, 111.97, 101.34, 70.03, 65.51, 62.64, 59.03, 56.15, 39.38, 20.34, 14.36; HRMS (ESI) m/z calcd for $C_{27}H_{25}NO_8$ $(M+Na)^+$ 514.1472, found 514.1474.

*4.1.22. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((E)-3-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9b**)*

Following the same procedure as that used for the synthesis of **8a**, the reaction of allyl alcohol **7b** (20 mg, 0.039 mmol), phenol **10a** (11.5 mg, 0.043 mmol), triphenylphosphine (13.7 mg, 0.047 mmol) and diisopropyl azodicarboxylate (9.8 μL, 0.058 mmol) in toluene (0.8 mL) gave target compound **9b** (22 mg, 74%) as a white solid after purification by column

chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 2:5:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 9.5 Hz, 1H), 7.43 (d, J =16.3 Hz, 1H), 7.38-7.34 (m, 3H), 6.93-6.84 (m, 4H), 6.25 (d, J = 9.5 Hz, 1H), 6.24-6.19 (m, 1H), 5.44 (d, J = 13.9 Hz, 1H), 5.27 (d, J = 13.9 Hz, 1H), 5.05 (s, 2H), 4.69 (d, J = 5.7 Hz, 2H), 4.27-4.24 (m, 1H), 4.20 (dd, J = 2.6, 9.4 Hz, 1H), 3.43-3.35 (m, 1H), 3.24 (dd, J = 2.6, 5.6 Hz, 1H), 1.26 (d, J = 6.2 Hz, 3H), 1.23 (d, J = 7.3 Hz, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H).

*4.1.23. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((E)-3-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9b'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **9b** (44.0 mg, 0.057 mmol) and ammonium hydrogen fluoride (13.1 mg, 0.229 mmol) in a mixed solution of NMP/DMF (1.2 mL, 1:3) gave target compound **9b'** (17.0 mg, 51%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 9.7 Hz, 1H), 7.43 (d, J =16.5 Hz, 1H), 7.38-7.33 (m, 3H), 6.92-6.85 (m, 4H), 6.26-6.19 (m, 2H), 5.48 (d, J = 13.8 Hz, 1H), 5.25 (d, J = 13.8 Hz, 1H), 5.05 (s, 2H), 4.69 (d, J = 5.4 Hz, 2H), 4.28-4.21 (m, 2H), 3.48-3.41 (m, 1H), 3.28 (dd, J = 2.4, 6.7 Hz, 1H), 1.38 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 7.5 Hz, 3H).

*4.1.24. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((E)-3-(4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**2b**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **9b′** (40.0 mg, 0.061 mmol) and 5% Rh/C (3.6 mg) in a mixed solution of THF/H_2O (2.0 mL,

2:1) under hydrogen atmosphere (1 atm) gave target compound **2a** (19.0 mg, 60%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.87 (d, J = 9.4 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), 7.42 (d, J =16.2 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 6.98-6.95 (m, 4H), 6.22 (d, J = 9.5 Hz, 1H), 6.01 (dt, J = 6.0, 16.1 Hz, 1H), 5.09 (s, 2H), 4.65 (d, J = 5.4 Hz, 2H), 4.08-4.03 (m, 2H), 3.14-3.12 (m, 2H), 1.28 (d, J = 6.3 Hz, 3H), 1.15 (d, J = 7.2 Hz, 3H); HRMS (ESI) m/z calcd for $C_{29}H_{27}NO_8$ $(M+Na)^+$ 540.1629, found 540.1630.

*4.1.25. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((2-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]*hept-2-ene-*2-carboxylate (**8c**)*

Following the same procedure as that used for the synthesis of **8a**, the reaction of alcohol **7a** (117 mg, 0.238 mmol), compound **10b** (70.4 mg, 0.262 mmol), triphenylphosphine (85.5 mg, 0.286 mmol) and diisopropyl azodicarboxylate (60 μL, 0.357 mmol) in toluene (4.8 mL) gave target compound **8c** (87.6 mg, 50%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 2:5:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 10.8 Hz, 2H), 7.68-7.58 (m, 3H), 7.42 (d, J = 9.5 Hz, 1H), 7.37 (d, J = 11.6 Hz, 1H), 7.32 (t, J = 9.1 Hz, 1H), 7.02 (t, J = 9.2 Hz, 1H), 6.95-6.89 (m, 3H), 6.26 (d, J = 11.8 Hz, 1H), 5.53 (d, J = 18.2 Hz, 1H), 5.48 (d, J = 17.2 Hz, 1H), 5.28 (d, J = 17.2 Hz, 1H), 5.05 (s, 2H), 4.80 (d, J = 18.4 Hz, 1H), 4.27-4.20 (m, 2H), 3.41-3.33 (m, 1H), 3.27 (t, J = 5.0 Hz, 1H), 1.21 (d, J = 5.8 Hz, 3H), 1.20 (d, J = 4.3 Hz, 3H), 0.83 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.35, 162.05, 141.10, 160.80, 155.89, 155.81, 148.05, 147.69, 143.36, 142.59, 130.00, 129.60, 128.80, 128.08, 127.25, 124.00, 123.77, 121.38, 113.23, 113.17, 112.66, 111.41, 101.59, 65.92, 65.54, 65.42, 62.82, 60.71, 55.32, 40.52, 29.71, 25.67, 17.95, 15.51, -4.30, -5.02.

*4.1.26. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((2-(((2-oxo-2H-chromen-7-yl)oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8c'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **8c** (69.9 mg, 0.094 mmol) and ammonium hydrogen fluoride (21.6 mg, 0.378 mmol) in a mixed solution of NMP/DMF (1.9 mL, 1:3) gave target compound **8c'** (28.1 mg, 48%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). ^{1}H-NMR (400 MHz, CDCl3) δ 8.21 (d, *J* = 10.8 Hz, 2H), 7.67-7.59 (m, 3H), 7.42 (d, *J* = 9.5 Hz, 1H), 7.37 (d, *J* = 11.6 Hz, 1H), 7.32 (t, *J* = 9.1 Hz, 1H), 7.01 (t, *J* = 9.2 Hz, 1H), 6.96-6.89 (m, 3H), 6.25 (d, *J* = 11.8 Hz, 1H), 5.52 (d, *J* = 18.2 Hz, 1H), 5.48 (d, *J* = 17.2 Hz, 1H), 5.28 (d, *J* = 17.2 Hz, 1H), 5.06 (s, 2H), 4.79 (d, *J* = 18.4 Hz, 1H), 4.28-4.21 (m, 2H), 3.41-3.43 (m, 1H), 3.28 (dd, *J* = 3.0, 6.3 Hz, 1H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.25 (d, *J* = 7.2 Hz, 3H).

*4.1.27. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((2-(((2-oxo-2H-chromen-7-yl) oxy)methyl)phenoxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**1c**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **8c'** (22.0 mg, 0.035 mmol) and 5% Rh/C (2.2 mg) in a mixed solution of THF/H_2O (1.2 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **1c** (10.7 mg, 31%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) *δ* 7.88 (d, *J* = 9.5 Hz, 1H), 7.54 (d, *J* = 9.3 Hz, 1H), 7.39 (d, *J* = 7.4 Hz, 1H), 7.32-7.27 (m, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 7.00-6.92 (m, 3H), 6.23 (d, *J* = 9.4 Hz, 1H), 5.60 (d, *J* = 13.8 Hz, 1H), 5.20 (s, 2H), 4.74 (d, *J* = 13.9 Hz, 1H), 4.07-4.00 (m, 1H), 3.97 (dd, *J* = 2.8, 10.0 Hz, 1H), 3.24-3.20 (m, 1H), 3.15-3.12 (m, 1H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.12 (d, *J* = 7.3 Hz, 3H); HRMS (ESI) m/z calcd for $C_{27}H_{25}NO_8$ $(M+Na)^+$

514.1472, found 514.1467.

*4.1.28. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-3-((((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8d**)*

To a solution of 7-amino-4-methylcoumarin **11a** (224 mg, 1.28 mmol) in 6.4 mL dry dichloroethane was added triethylamine (392 μL, 2.81 mmol). After 10 min, triphosgene (129 mg, 1.28 mmol) was added and the mixture was stirred at 90 °C for 6 h. The reaction was completed at the end of 6 h, as indicated by TLC. Excess of solvent was removed by distillation in vacuum. The crude isocyanate product was used next step without purification. To a solution of crude isocyanate in 1.6 mL dry THF was added alcohol **7a** (157 mg, 0.320 mmol) and triethylamine (134 μL, 0.960 mmol). The solution was stirred at room temperature for 12 h. Excess of solvent was removed by distillation in vacuum. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 2:4:5) to afford carbamate **8d** (72.0 mg, 33%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, J = 2.3, 8.6 Hz, 2H), 7.66 (d, J =10.9 Hz, 2H), 7.54 (d, J =10.9 Hz, 1H), 7.41 (d, J =10.1 Hz, 2H), 6.95 (s, 1H), 6.20 (d, J = 1.4 Hz, 1H), 5.59 (d, J =17.7 Hz, 1H), 5.46 (d, J =17.2 Hz, 1H), 5.29 (d, J =17.3 Hz, 1H), 4.93 (d, J = 17.8 Hz, 1H), 4.29-4.25 (m, 2H), 3.33-3.29 (m, 2H), 2.41 (d, J =1.3 Hz, 3H), 1.23 (d, J =7.6 Hz, 3H), 1.22 (d, J =3.1 Hz, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.90, 161.04, 160.46, 154.40, 152.48, 152.31, 147.69, 145.56, 142.55, 141.14, 128.66, 128.18, 125.51, 123.77, 115.77, 114.40, 113.32, 106.03, 65.63, 65.58, 60.68, 59.50, 55.63, 40.49, 25.69, 22.31, 18.61, 17.96, 15.50, -4.23, -4.98.

4.1.29. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-((((4-methyl-2-oxo-2H-

*chromen-7-yl)carbamoyl)oxy)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8d'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **8d** (82.0 mg, 0.119 mmol) and ammonium hydrogen fluoride (27.0 mg, 0.474 mmol) in a mixed solution of NMP/DMF (1.2 mL, 1:3) gave target compound **8d'** (35.0 mg, 51%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 10.9 Hz, 2H), 7.66 (d, J =10.8 Hz, 2H), 7.54 (d, J =10.8 Hz, 1H), 7.41-7.40 (m, 2H), 6.96 (s, 1H), 6.20 (d, J = 1.3 Hz, 1H), 5.58 (d, J =17.8 Hz, 1H), 5.51 (d, J =17.0 Hz, 1H), 5.25 (d, J =17.1 Hz, 1H), 4.93 (d, J = 18.1 Hz, 1H), 4.31-4.26 (m, 2H), 3.40-3.26 (m, 2H), 2.38 (d, J =10.2 Hz, 3H), 1.35 (d, J =7.8 Hz, 3H), 1.25 (d, J =9.1 Hz, 3H).

*4.1.30. (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-((((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**1d**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **8d′** (35.0 mg, 0.061 mmol) and 5% Rh/C (3.8 mg) in a mixed solution of THF/H_2O (2.1 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **1d** (7.0 mg, 26%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.71 (d, J = 11.0 Hz, 1H), 7.63 (d, J =2.4 Hz, 1H), 7.44 (dd, J =2.5, 10.8 Hz, 1H), 6.22 (s, 1H), 5.55 (d, J = 16.9 Hz, 1H), 4.96 (d, J =17.0 Hz, 1H), 4.17-4.10 (m, 2H), 3.24-3.22 (m, 2H), 2.47 (s, 3H), 1.30 (d, J =7.9 Hz, 3H), 1.21 (d, J =9.1 Hz, 3H); HRMS (ESI) m/z calcd for $C_{22}H_{22}N_2O_8$ $(M+Na)^+$ 465.1268, found 465.1269.

4.1.31. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1-azabicyclo

*[3.2.0]hept-2-ene-2-carboxylate (**8e**)*

To a solution of 7-amino-4-trifuloromethylcoumarin **11b** (50.0 mg, 0.218 mmol) in 2.2 mL dry dichloroethane was added triethylamine (60 μL, 0.436 mmol). After 10 min, triphosgene (65.0 mg, 0.218 mmol) was added and the mixture was stirred at 90 °C for 6 h. The reaction was completed at the end of 6 h, as indicated by TLC. Excess of solvent was removed by distillation in vacuum. The crude isocyanate product was used next step without purification. To a solution of crude isocyanate in 0.5 mL dry THF was added alcohol **7a** (27.0 mg, 0.055 mmol) and triethylamine (23 μL, 0.165 mmol). The solution was stirred at room temperature for 12 h. Excess of solvent was removed by distillation in vacuum. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 1:4:3) to afford carbamate **8e** (32.0 mg, 78%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.66-7.62 (m, 4H), 7.33 (dd, J = 2.0, 8.9 Hz, 1H), 7.01 (s, 1H), 6.68 (s, 1H), 5.60 (d, J =14.1 Hz, 1H), 5.46 (d, J = 13.8 Hz, 1H), 5.28 (d, J = 13.8 Hz, 1H), 4.94 (d, J = 14.1 Hz, 1H), 4.30-4.25 (m, 2H), 3.34-3.28 (m, 2H), 1.24-1.22 (m, 6H), 0.85 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 179.09, 164.41, 163.59, 159.26, 156.82, 151.53, 150.24, 150.05, 147.14, 146.50, 145.46 (d, ^2J = 33.5 Hz), 132.09, 129.87, 127.62, 125.40 (d, ^1J = 274.14 Hz), 119.22, 117.01, 112.52, 110.02 (d, ^3J = 5.96 Hz), 64.47, 69.40, 64.37, 63.31, 59.42, 44.42, 29.48, 26.06, 21.79, 19.26, -0.46, -1.24.

*4.1.32. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8e'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **8e** (80.0 mg, 0.107 mmol) and ammonium hydrogen fluoride (24.5 mg, 0.439 mmol) in a mixed solution of NMP/DMF (2.1 mL, 1:3) gave target compound **8e'** (40.0 mg, 59%) as a white solid

after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 2:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.6 Hz, 2H), 7.67-7.62 (m, 4H), 7.33 (dd, J = 1.9, 8.8 Hz, 1H), 7.10 (s, 1H), 6.69 (s, 1H), 5.58 (d, J =14.2 Hz, 1H), 5.50 (d, J = 13.6 Hz, 1H), 5.27 (d, J = 13.7 Hz, 1H), 4.95 (d, J = 14.2 Hz, 1H), 4.31-4.25 (m, 2H), 3.39-3.31 (m, 2H), 1.35 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 7.2 Hz, 3H).

*4.1.33. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**1e**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **8e′** (25.0 mg, 0.040 mmol) and 5% Rh/C (2.1 mg) in a mixed solution of THF/H_2O (1.2 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **1e** (7.0 mg, 35%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.66-7.62 (m, 2H), 7.41-7.38 (m, 1H), 6.70 (s, 1H), 5.46 (d, J =13.7 Hz, 1H), 4.82 (d, J = 13.8 Hz, 1H), 4.11-4.08 (m, 2H), 3.24-3.21 (m, 1H), 3.20-3.15 (m, 1H), 1.16 (d, J = 6.3 Hz, 3H), 1.08 (d, J = 7.2 Hz, 3H); HRMS (ESI) m/z calcd for $C_{22}H_{19}F_3N_2O_8$ $(M+Na)^+$ 514.1472, found 514.1467.

*4.1.34. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((E)-3-(((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9c**)*

To a solution of 7-amino-4-trifuloromethylcoumarin **11b** (99.5 mg, 0.434 mmol) in 2.2 mL dry dichloroethane was added triethylamine (133 μL, 0.955 mmol). After 10 min, triphosgene (128.8 mg, 0.434 mmol) was added and the mixture was stirred at 90 °C for 6 h. The reaction was completed at the end of 6 h, as indicated by TLC. Excess of solvent was removed by distillation in vacuum. The crude isocyanate product was used next step without purification.

To a solution of crude isocyanate in 0.5 mL dry THF was added allyl alcohol **7b** (56.3 mg, 0.109 mmol) and triethylamine (46 μL, 0.327 mmol). The solution was stirred at room temperature for 12 h. Excess of solvent was removed by distillation in vacuum. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane:$CHCl_3$ = 1:4:3) to afford carbamate **9c** (61.5 mg, 73%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.4 Hz, 2H), 7.68-7.66 (m, 4H), 7.41 (d, J = 16.2 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 7.16 (s, 1H), 6.69 (s, 1H), 6.14 (dt, J =5.9, 16.1 Hz, 1H), 5.44 (d, J = 13.9 Hz, 1H), 5.27 (d, J = 14.0 Hz, 1H), 4.84 (d, J = 5.8 Hz, 2H), 4.28-4.21 (m, 2H), 3.42-3.33 (m, 1H), 3.26-3.24 (m, 1H), 1.26 (d, J = 6.0 Hz, 1H), 1.23 (d, J = 7.3 Hz, 3H), 0.85 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.73, 160.80, 159.16, 155.48, 152.34, 147.64, 147.42, 142.74. 142.37, 141.33 (d, ^2J = 30.2 Hz), 130.65, 128.05, 126.44, 126.17, 125.76, 123.76, 121.51 (d, ^1J = 273.7 Hz), 115.13, 113.68 (d, ^3J = 5.74 Hz), 108.99, 106.28, 65.84, 65.76, 65.36, 59.30, 56.18, 39.28, 25.68, 22.44, 17.94, 16.82, -4.19, -5.00.

*4.1.35. (4S,5R,6S)-6-((R)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-4-methyl-7-oxo-3-((E)-3-(((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid (**9c'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **9c** (25.0 mg, 0.032 mmol) and ammonium hydrogen fluoride (7.4 mg, 0.120 mmol) in a mixed solution of NMP/DMF (1.2 mL, 1:3) gave target compound **9c'** (14.0 mg, 65%) as a white solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 2:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.67-7.62 (m, 4H), 7.39 (d, J = 16.0 Hz, 1H), 7.34 (d, J = 2.1 Hz, 1H), 6.67 (s, 1H), 6.14 (dt, J =5.9, 16.2 Hz, 1H), 5.48 (d, J = 13.8 Hz, 1H), 5.25 (d, J = 13.8 Hz, 1H), 4.83 (d, J = 5.7 Hz, 2H), 4.28-4.22 (m, 2H), 3.46-3.39 (m, 1H), 3.28 (dd, J = 2.6, 6.7 Hz, 1H), 1.37 (d, J = 6.2 Hz, 3H), 1.23 (d, J = 7.3 Hz, 3H).

*4.1.36. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-((E)-3-(((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbamoyl)oxy)prop-1-en-1-yl)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**2c**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **9c′** (48.0 mg, 0.073 mmol) and 5% Rh/C (3.8 mg) in a mixed solution of THF/H_2O (2.4 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **2a** (17.0 mg, 45%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) 7.75 δ (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 1.8, 8.9 Hz, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 6.71 (s, 1H), 5.97 (dt, J =5.8, 16.3 Hz, 1H), 4.76 (d, J = 5.3 Hz, 2H), 4.09-4.03 (m, 2H), 3.34-3.33 (m, 1H), 3.15-3.11 (m, 1H), 1.28 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 7.2 Hz, 3H); HRMS (ESI) m/z calcd for $C_{24}H_{21}F_3N_2O_8$ $(M+Na)^+$ 545.1142, found 514.1145.

*4.1.37. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-3-formyl-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**13**)*

To a solution of compound **7a** (20 mg, 0.045 mmol) in dichloromethane (0.3 mL) were added Dess-Martin periodinane (19.1 mg, 0.045 mmol). The reaction mixture were stirred at room temperature for 1 h. After extracted with ethyl acetate at 5 °C, the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:8) to afford aldehyde **13** (12.7 mg, 64%) as a yellowish solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.4, (s, 1H), 8.24 (d, J = 10.8 Hz, 2H), 7.66 (d, J =10.8 Hz, 2H), 5.50 (d, J =17.1 Hz, 1H), 5.36 (d, J = 17.1 Hz, 1H), 4.36 (dd, J =4.3, 13.0 Hz, 1H), 4.33-4.27 (m, 1H), 3.55-3.47 (m, 1H), 3.41 (t, J =5.0 Hz, 1H), 1.24 (d, J =9.4 Hz, 3H), 1.22 (d, J =8.0 Hz, 3H), 0.85 (s, 9H), 0.08 (s, 3H),

0.07 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.32, 172.83, 159.27, 147.89,143.10, 141.70, 140.04, 128.41, 123.86, 66.42, 65.09, 60.90, 56.14, 38.00, 25.64, 22.04, 17.95, 16.29, -4.22, -5.14.

*4.1.38. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-methyl-3-(((4-methyl-2-oxo-2H-chromen-7-yl)amino)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**14**)*

To a solution of 7-amino-4-methylcoumarin **11a** (56.7 mg, 0.324 mmol) and aldehyde **13** (105.4 mg, 0.216 mmol) in 1.7 mL dry dichloroethane was added acetic acid (12 μL, 0.216 mmol). After 3 h, sodium triacetoxyborohydride (137 mg, 0.648 mmol) was added and mixture was stirred at room temperature for 12 h. After completion of the reaction (monitored by TLC), the mixture was diluted with saturated aqueous $NaHCO_3$, extracted with EtOAc. The organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:2) to afford benzyl amine **14** (66.6 mg, 48%) as a yellowish solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 10.7 Hz, 2H), 7.67 (d, J =10.6 Hz, 2H), 7.34 (d, J =11.2 Hz, 1H), 6.58-6.50 (m, 3H), 5.98 (s, 1H), 5.48 (d, J = 17.4 Hz, 1H), 5.30 (d, J =17.4 Hz, 1H), 4.72 (d, J =20.4 Hz, 1H), 4.24 (t, J =7.1 Hz, 1H), 4.18 (dd, J = 3.5, 12.8 Hz, 1H), 3.98 (d, J = 20.5 Hz, 1H), 3.26-3.17 (m, 2H), 2.33 (s, 3H), 1.20 (d, J =8.2 Hz, 6H), 0.83 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.96, 161.85, 161.09, 155.84, 152.99, 151.03, 150.01, 147.67, 142.60, 128.15, 128.03, 125.77, 123.77, 111.20, 110.32,109.91, 98.45, 65.58, 65.54, 60.50, 55.30, 41.09, 39.71, 25.70, 25.27, 18.56, 17.95, 15.69, -4.28, -4.97

*4.1.39. 4-Nitrobenzyl (4S,5R,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-(((4-methyl-2-oxo-2H-chromen-7-yl)amino)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**14'**)*

Following the same procedure as that used for the synthesis of **8a'**, the reaction of silyl ether **14** (107 mg, 0.155 mmol) and ammonium hydrogen fluoride (35.4 mg, 0.621 mmol) in a mixed solution of NMP/DMF (3.2 mL, 1:3) gave target compound **14'** (48.1 mg, 54%) as a yellowish solid after purification by column chromatography on silica gel (EtOAc:*n*-hexane = 3:1). as a yellowish solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 10.8 Hz, 2H), 7.65 (d, J =10.8 Hz, 2H), 7.30 (d, J =10.8 Hz, 1H), 6.48 (dd, J = 2.8, 10.8 Hz, 1H), 6.42 (d, J = 2.7 Hz, 1H), 5.95 (s, 1H), 5.51 (d, J = 17.42 Hz, 1H), 5.25 (d, J =17.2 Hz, 1H), 4.71 (d, J =20.8 Hz, 1H), 4.19-4.15 (m, 2H), 3.95 (d, J = 20.9 Hz, 1H), 3.25-3.18 (m, 2H), 2.33 (s, 3H), 1.29 (d, J =7.8 Hz, 3H), 1.18 (d, J = 9.1 Hz, 3H).

*4.1.40. (4S,5R,6S)-6-((R)-1-Hydroxyethyl)-4-methyl-3-(((4-methyl-2-oxo-2H-chromen-7-yl) amino)methyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**3**)*

Following the same procedure as that used for the synthesis of **1a**, the reaction of compound **14′** (40.5 mg, 0.076 mmol) and 5% Rh/C (6.4 mg) in a mixed solution of THF/H_2O (2.4 mL, 2:1) under hydrogen atmosphere (1 atm) gave target compound **3** (7.3 mg, 24%) as a white solid after purification by preparative RP-HPLC with CH_3CN-H_2O as mobile phase and lyophilization. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.46 (d, J = 11.1 Hz, 1H), 6.67 (dd, J =2.5, 10.9 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 5.91 (s, 1H), 5.48 (s, 1H), 4.78 (d, J = 20.1 Hz, 1H), 4.04 (t, J =8.5 Hz, 1H), 3.98 (dd, J = 3.4, 12.3 Hz, 1H), 3.88 (d, J = 20.2 Hz, 1H), 3.13-3.05 (m, 2H), 2.36 (s, 3H), 1.24 (d, J =7.8 Hz, 3H), 1.14 (d, J =9.1 Hz, 3H); HRMS (ESI) m/z calcd for $C_{21}H_{22}N_2O_6$ $(M+Na)^+$ 421.1370, found 421.1372.

4.2. Evaluation of stability

For stability studies of the compounds, the 10 μM solution of each fluoregenic probe **1-3** in PBS (pH = 7.4) was prepared. The fluorescent intensity of each solution was measured

immediately and continuously monitored in every 1 h, 2 h, 6 h, and 12 h upon excitation 365 nm or 375nm by QEpro high performance spectrometer (Ocean optics).

4.3. Biological evaluation

4.3.1. Enzyme assay

The recombinant carbapenemases (NDM-1, IMP-1, KPC-3, OXA-48) were provided from Prof. H. Xie group.[19] The carbapenemase activity assay of new fluorogenic probe **1-3** was performed at PBS (100 μL, PH 7.4) containing 0.1% CHAPS. After new fluorogenic probe **1-3** (10 μM) was incubated with indicated carbapenemase (100 nM) for 10 min at room temperature, fluorescence intensities of the mixtures were were collected immediately and then at 5 min interval for 1 h in microplate reader (Synergy H1 hybrid multi-mode reader, $\lambda_{ex/em}$ = 365/465 nm, 37 °C). TEM-1 assay of compound **1a** and **1b** was performed using the similar protocol as described previously. [18]

4.3.2. Fluorogenic assay of clinical isolates

For the fluorogenic assay, a 2 μl loopful of bacterial isolates were lysed with 150 μL B-PER solution in Tris-HCl buffer (Thermo Scientific Pierce), vortexed for 1 min, incubated at room temperature for 30 min, and centrifuged at 10,000 g for 5 min. The 30 μL of supernatant was mixed with 100 μL PBS and 13 μl of 1 mM fluorogenic probe **1a** or **1b**. Fluorescence was measured (λ_{ex} = 360 nm/ λ_{em} = 465 nm) every 5 min for 50 min using a fluorimeter (Infinite F200pro, Tecan Group Ltd.). The strains for the screening were 24 CPEs (carbapenem-producing Enterobacteriaceae) and 18 carbapenem-susceptible isolates.

4.3.3. manual Carba NP test

The manual Carba NP test was conducted as described in the 29th edition of the CLSI

guidelines (2019) [11]. Solution A and solution B (solution A + 6 mg/mL imipenem) were prepared as done in the CLSI. A 1 μL loopful of the test isolates was resuspended in 100 μL B-PER II in each of three 1.5 ml microtubes: 'a', and 'b'. After vortexing the tubes for 5 sec, we added 100 μL of solution A to tube 'a' and solution B to tube 'b'. The tubes were vortexed well and incubated at 35 °C and read every 30 min for 2 h.

4.3.4. Statistical analysis

The performance of the tests for the detection of carbapenemase-producing *Enterobacteriaceae* was evaluated by using molecular results previously obtained from characterized isolates. For each test, the sensitivity was calculated as the number of true-positive organisms, while the specificity was calculated as the number of true-negative organisms. The receiver operating characteristics (ROC) curve analysis was used to determine the cut-off value of the fluorogenic assay. Statistical analysis was performed using MedCalc Statistical Software version 18.9 (MedCalc Software bvba, Ostend, Belgium).

Notes

The authors declare no competing financial interest.

Acknowledgements

This study was financially supported by KHIDI (HI17C1037, HI16C1677) and NRF (2019R1A2B1012277). We would like to thank Prof. H. Xie and Dr. Ki Tae Kim for generously providing us carbapenemases.

References

[1] K. M. Papp-Wallace, A. Endimiani, M.A. Taracila, R. A. Bonomo, Carbapenems: Past, Present, and Future, Antimicrob. Agents Ch. 55 (11) (2011) 4943-4960.

[2] M. Mckenna, Antibiotic resistance: The last resort, Nature 499 (7459) (2013) 394-396.

[3] S. Shaikha, J. Fatimab, S. Shakil, S. M. D Rizvia, M. A. Kamal, Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment, Saudi J. Biol. Sci. 22 (1) (2015) 90-101.

[4] J. H. Jeon, J. H. Lee, J. J. Lee, K. S. Park, A. M. Karim, C.-R. Lee, B. C. Jeong, S. H. Lee, Structural Basis for Carbapenem-Hydrolyzing Mechanisms of Carbapenemases Conferring Antibiotic Resistance, Int. J. Mol. Sci. 16 (5) (2015) 9654-9692.

[5] T. Naas, P. Nordmann, Analysis of a carbapenem-hydrolyzing class A, β-lactamase from Enterobacter cloacae and of its LysR-type regulatory protein, Proc. Natl. Acad. Sci. USA. 91(16) (1994) 7693-7697.

[6] G. Gautier, T. Guillard, B. Podac, B. Bercot, V. Vernet-Garnier, C. de Champs, Detection of different classes of carbapenemases: Adaptation and assessment of a phenotypic method applied to Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii, and proposal of a new algorithm, J. Microbiol. Methods. 147 (2018) 26-35.

[7] W. Fast, L. D. Sutton, Metallo-β-lactamase: inhibitors and reporter substrates. Biochim. Biophys. Acta. Proteins Proteomics. 1834 (8) (2013) 1648-1659.

[8] C. M. Rotondo, G. D. Wright, Inhibitors of metallo-β-lactamases, Curr. Opin. Microbiol. 39 (2017) 96-105.

[9] M. Georgios, B. Maria, Beta-lactamase and Carbapenemase Detection Methods: An Overview of Recent Patents, Recent Pat. Anti-Infect. Drug Discovery, 9 (1) (2014) 1-5.

[10] D. Hammoudi, C. A. Moubareck, D. K. Sarkis, How to detect carbapenemase producers? A literature review of phenotypic and molecular methods, J. Microbiol. Methods. 107 (2014) 106-118.

[11] Anonymous. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

[12] C. G. Monteferrante, S. Sultan, M. T. Ten Kate, L. J. Dekker, K. Sparbier, M. Peer, M. Kostzrewa, T. M. Luider, W. H. Goessens, P. C. Burgers, Evaluation of different pretreatment protocols to detect accurately clinical carbapenemaseproducing Enterobacteriaceae by MALDI-TOF, J. Antimicrob. Chemother. 71(10) (2016) 2856-2867.

[13] M. Aitamimi, A. Alsalamah, M. Alkhulaifi, H. Alajlan, Comparison of phenotypic and PCR methods for detection of carbapenemases production by Enterobacteriaceae. Saudi J. Biol. Sci. 24 (1) (2017) 155-161.

[14] H. Savli, A. Karadenizli, F. Kolayli, S. Gundes, U. Ozbek, H. Vahaboglu, Expression stability of six housekeeping genes: A proposal for resistance gene quantification studies of Pseudomonas aeruginosa by real-time quantitative RT-PCR. J. Med. Microbiol. 52 (5) (2003) 403-408.

[15] W. Gao, B. Xing, R. Y. Tsien, J. Rao, Novel Fluorogenic Substrates for Imaging β-Lactamase Gene Expression, J. Am. Chem. Soc. 125 (37) (2003) 11146-11147.

[16] H. Yao, M.-K. So, J. Rao, A bioluminogenic substrate for in vivo imaging of beta-lactamase activity, Angew. Chem. Int. Ed. 46 (37) (2007) 7031-7034.

[17] J. Zhang, Y. Shen, S. L. May, D. C. Nelson, S. Li, Ratiometric Fluorescence Detection of Pathogenic Bacteria Resistant to Broad-Spectrum β-Lactam Antibiotics, Angew. Chem. Int. Ed. 51 (8) (2012) 1865-1868.

[18] H. B. D. Thai, J. K. Yu, B. S. Park, Y.-J. Park, S.-J. Min, D.-R. Ahn, A fluorogenic substrate of beta-lactamases and its potential as a probe to detect the bacteria resistant to

the third-generation oxyimino-cephalosporins, Biosens. Bioelectron. 77 (2016) 1026-1031.

[19] W. Mao, Y. Wang, X. Qian, L. Xia and H. Xie, A Carbapenem-Based Off-On Fluorescent Probe for Specific Detection of Metallo-β-Lactamase Activities ChemBioChem, 2019, **20 (4)**, 511-515.

[20] W. Mao, L. Xia, H. Xie, Detection of Carbapenemase-Producing Organisms with a Carbapenem-Based Fluorogenic Probe, Angew. Chem. Int. Ed. 56 (16) (2017) 4468-4472.

[21] B. Xing, A. Khanamiryan, J. Rao, Cell-Permeable Near-Infrared Fluorogenic Substrates for Imaging β-Lactamase Activity, J. Am. Chem. Soc. 127 (12) (2005) 4158-4159.

[22] W. Mao, X. Qian, J. Zhang, L. Xia, H. Xie, Specific Detection of Extended-Spectrum β-Lactamase Activities with a Ratiometric Fluorescent Probe, ChemBioChem, 18 (20) (2017) 1990-1994.

[23] H. Xie, J. Mire, Y. Kong, M. Chang, H. A. Hassounah, C. N. Thornton, J. C. Sacchettini, J. D. Cirillo, J. Rao, Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe, Nat. Chem. 4 (10) (2012) 802-809.

[24] M. S. Jensen, C. Yang, Y. Hsiao, N. Rivera, K. M. Wells, J. Y. L. Chung, N. Yasuda, D. L. Hughes, P. J. Reider, Synthesis of an Anti-Methicillin-Resistant *Staphylococcus aureus* (MRSA) Carbapenem via Stannatrane-Mediated Stille Coupling, Org. Lett. 2 (8) (2000) 1081-1084

[25] N. Yasuda, C. Yang, K. M. Wells, M. S. Jensen, D. L. Hughes, Preparation of crystalline*p*-nitrobenzyl 2-hydroxymethyl carbapenem as a key intermediate for the anti-MRS carbapenem L-786,392, Tetrahedron Lett. 40 (3) (1999) 427-430.

[26] N. Lohse-Fraefel, E. M. Carreira, Polyketide Building Blocks via Diastereoselective Nitrile Oxide Cycloadditions with Homoallylic Alcohols and Monoprotected Homoallylic Diols, Chem. Eur. J. 15 (44) (2009) 12065-12081.

[27] P. Li, J. Li, F. Arikan, W. Ahlbrecht, M. Dieckmann, D. Menche, Total Synthesis of Etnangien, J. Am. Chem. Soc. 131 (33) (2009) 11678-11679.

[28] J. C. Amould, F. Land, M. J. Paaquet, New applications of the mitsunobu reaction in the synthesis of C-2 N-methyl carbapenems, Tetrahedron Lett. 33 (47) (1992) 7133-7136.

[29] R. W. Ratcliffe, R. R. Wilkening, K. J. Wildonger, S. T. Waddell, G. M. Santorelli, D. L. Parker, J. D. Morgan, T. A. Blizzard, M. L. Hammond, J. V. Heck, J. Huber, J. Kohler, K. L. Dorso, E. St. Rose, J. G. Sundelof, W. J. May, G. G. Hammond, Synthesis and properties of 2-(naphthosultamyl)methyl-carbapenems with potent anti-MRSA activity: Discovery of L-786,392, Bioorg. Med. Chem. Lett. 9 (5) (1999) 679-684.

[30] H.-J. Jin, J. Lu, X. Wu, Development of a new enzyme-responsive self-immolative spacer conjugate applicable to the controlled drug release, Bioorg. Med. Chem. 20 (11) (2012) 3465-3469.

[31] A. F. Abdel-Magid, S. J. Mehrman, A Review on the Use of Sodium Triacetoxyborohydride in the Reductive Amination of Ketones and Aldehydes, Org. Proc. Res. Dev. 10 (5) (2006) 971-1031.

[32] Introduction of 4-aminobenzyl ether or carbonate linker to our probes were unsuccessful due to their low stability.

[33] In case of probe **2b**, we observed unexpected fluorescent emission at a different wavelength and precipitation of solid material during the experiment. Thus, the use of probe **2b** is excluded for further study.

[34] N. Woodford, E. J. Fagan, M. J. Ellington, Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases, J. Antimicrob. Chemother. 57 (1) (2006) 154-155.

[35] L. Poirel, T. R. Walsh, V. Cuvillier, P. Nordmann, Multiplex PCR for detection of acquired carbapenemase genes, Diagn. Microbiol. Infect. Dis. 70 (1) (2011) 119-123.

[36] P. D. Tamma, B. N. Opene, A. Gluck, K. K. Chambers, K. C. Carroll, P. J. Simner, Comparison of 11 Phenotypic Assays for Accurate Detection of Carbapenemase-Producing *Enterobacteriaceae*, J. Clin. Microbiol. 55 (4) (2017) 1046-1055.

Development of carbapenem-based fluorogenic probes for the clinical screening of carbapenemase-producing bacteria

Juhyeon Kim[a,b], Yihoon Kim[c], Ahmed Z. Abdelazem[d], Hoon Seok Kim[e], Jung Ok Kim[e], Yeon-Joon Park[e], Sun-Joon Min[c,f,*]

[a]*Department of Chemistry, Korea University, Seoul, 02841, Republic of Korea*

[b]*Center for Neuro-Medicine, Korea Institute of Science and Technology (KIST), Seoul, 02792, Republic of Korea*

[c]*Department of Applied Chemistry, Hanyang University, Ansan, Gyeonggi-do, 15588, Republic of Korea*

[d]*Biotechnology and Life Sciences Department, Beni-Suef University, Egypt*

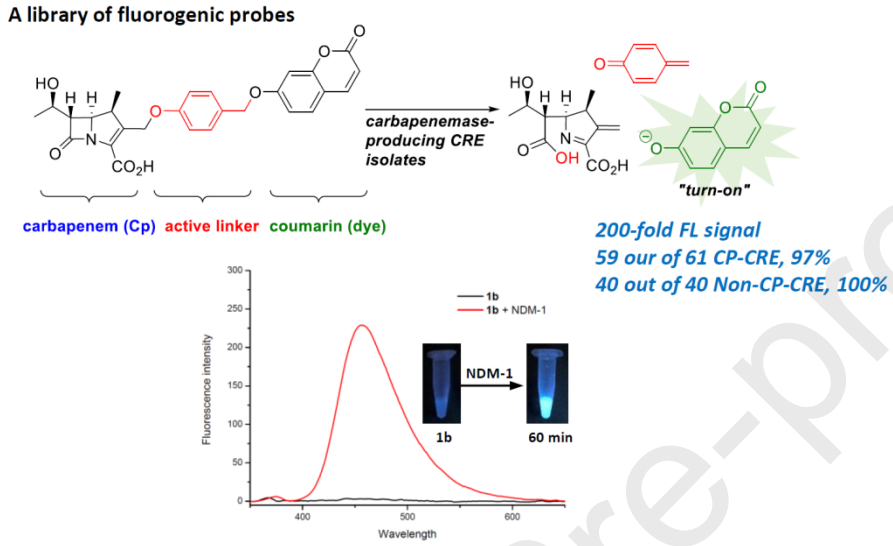
[e]*Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, 06591, Republic of Korea*

[f]*Department of Chemical & Molecular Engineering, Hanyang University, Ansan, Gyeonggi-do, 15588, Republic of Korea*

email: sjmin@hanynag.ac.kr

Graphical Abstract

A library of cabapenem-based fluorogenic probes were synthesized and evaluated for specific detection of carbapenemase-producing carbapenem resistant bacteria.



Highlights

1. A library of carbapenem-based fluorogenic probes having different active linkers were synthesized.

2. Probe **1b** showed high stability and rapid fluorescence signal in the carbapenemase assay.

3. Probe **1b** exhibited excellent selectivity to the carbapenemase-producing carbepenem-resistant *Enterobacteriaceae* (CP-CRE) in clinical screening.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: