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Design, Synthesis, Structure–Function Relationship, Bioconversion, and Pharmacokinetic Evaluation of Ertapenem Prodrugs

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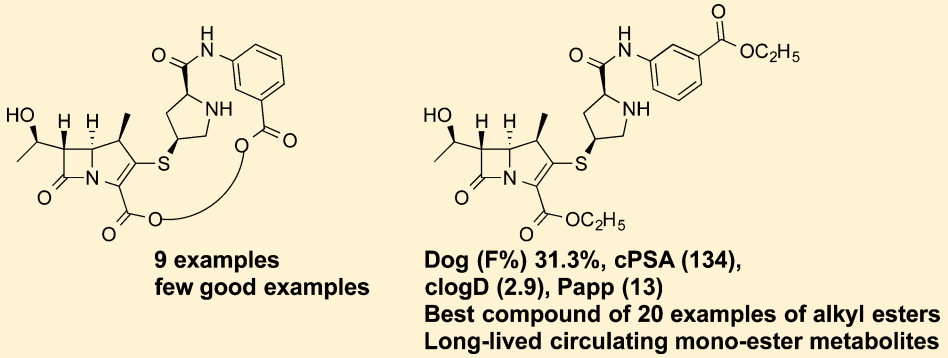
Supporting Information



ABSTRACT: Described here are synthesis and biological evaluations of diversified groups of over 57 ertapenem prodrugs which include alkyl, methylenedioxy, carbonate, cyclic carbonate, carbamate esters, and esters containing active transport groups (e.g., carboxyl, amino acid, fatty acids, cholesterol) and macrocyclic lactones linking the two carboxyl groups. Many of the prodrugs were rapidly hydrolyzed in rat plasma but not in human plasma and were stable in simulated gastrointestinal fluid. The diethyl ester prodrug showed the best total absorption (>30%) by intredeudenal dosing in dogs, which could potentially be improved by formulation development. However, its slow rate of the hydrolysis to ertapenem also led to the presence of large amounts of circulating monoester metabolites, which pose significant development challenges. This study also suggests that the size of susbtituents at C-2 of carbapenem (e.g., benzoic acid of ertapenem) has significant impact on the absorption and the hydrolysis of the prodrugs.

Carbapenems are a class of β-lactam antibiotics useful for treatment of serious and life-threatening Gram-positive and Gram-negative infections.[1] Thienamycin (**1**) was the first member of the carbapenem class of antibiotics discovered from *Streptomyces cattleya* in 1976. At the time, thienamycin discovery and development presented unique challenges and opportunities. It was unstable, particularly at higher solution concentrations in the fermentation broth, making scale-up by fermentation inherently challenging. Perhaps the most critical factor impacting thienamycin's stability was the intramolecular ring opening of the β-lactam by its pendent primary amine. As direct sequelae to these stability deficits, total synthesis was the only viable option to enable discovery and development of a stable derivative with commercial viability. After tremendous efforts, Merck scientists synthesized imipenem by total synthesis during which they masked the primary amine as an amidine; this compound retained useful antibacterial activity and spectrum while conferring adequate stability. In 1985, an imipenem (**2**) and cilastatin combination was approved as an antibiotic for treatment of serious bacterial infections in hospital setting. In vivo, imipenem is hydrolyzed by renal dehydropeptidase (DHP). Cilastatin is an inhibitor of DHP that stabilizes imipenem in vivo by preventing its hydrolysis. Subsequently, it was discovered that introduction of a β-methyl group at C-1 of carbapenem renders them resistant to DHP while retaining most of the antibiotic activity and spectrum. Because of these useful properties, all subsequent carbapenem antibiotics, excluding panipenem (**3**)/betamipron, that were developed contain a β-methyl group at C-1. There have been six carbapenem antibiotics approved after imipenem (Chart 1). Panipenem/betamipron was approved in Japan in 1993, and that was followed by the U.S. FDA approval of ertapenem (**4**, 2001) and by Japanese approval of biapenem (**5**, 2001). Meropenem (**6**) and doripenem (**7**) were approved by the U.S. FDA in 2005 and 2007, respectively. Orapenem (**8**), a prodrug

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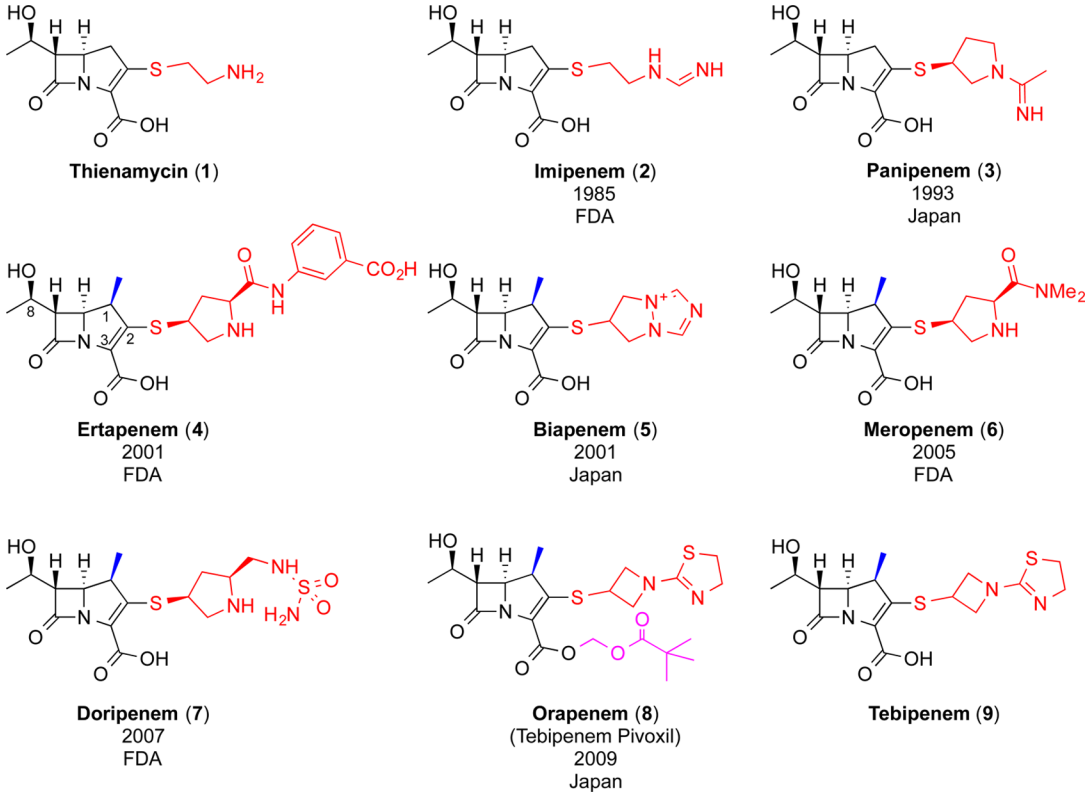
Chart 1. Structures of Carbapenem Antibiotics



of tebipenem (**9**), was the last carbapenem antibiotic that was approved by Japanese regulators in 2009.

As noted, carbapenems are highly effective antibiotics, typically administered by intravenous infusion in a hospital setting. Once the bacterial burden of a patient is reduced and their symptoms are improved, the patient is sent home with a non-carbapenem oral antibiotic. While this is not an issue for most patients, it does present a challenge in some cases wherein patients return to hospital because of lack of effectiveness or for tolerance issues. Carbapenem antibiotics are not orally absorbed because of their high polarity, large polar suface area, and negative log *D*. We envisaged that a prodrug approach could improve lipophilicity (increasing clogD) and absorption. All carbapenems, at a minimum, contain at least one carboxyl moiety and a secondary hydroxyl group. Ester derivatives derived from these carbapenem substituents potentially could confer a viable prodrug strategy for oral formulation that would allow for step down therapy. Step down therapy represents an attractive opportunity to effectively treat opportunistic infections outside of a clinical setting. When we initiated this program, there was a paucity of information in the literature related to carbapenem prodrugs. Orapenem (tebipenem pivoxil, **8**) was an exception. It is a pivaloxymethyl ester prodrug of tebipenem (**9**).[2−6] It has been approved for clinical use only in Japan. In addition, during the course of our studies, syntheses of several meropenem (**6**) prodrugs were reported.[7]

Ertapenem (**4**) is a prominent member of the carbapenem class of antibiotics and is used broadly. Importantly, ertapenem is the only carbapenem antibiotic administered once daily.[1,8,9] The core structure of ertapenem and other β-methyl antibiotics, including tebipenem, is common. They are differentiated only by the structure of their side chain at C-2. Carbapenem antibiotics, including tebipenem, possess basic side chains at C-2 with either a net positive charge or net zwitterionic, with the exception of ertapenem, which consists of a prolyl-3-aminobenzoic acid. Clearly, the net acidic charge of ertapenem contributes to higher protein binding (92−94%), longer plasma half-life, and longer duration of action. Therefore, ertapenem was selected for prodrug studies with the expectation that once-daily dosing of the prodrug will be maintained. It is envisaged that the prodrug will rapidly hydrolyze in plasma to produce ertapenem with minimal or no circulating metabolites, leading to clear and abbreviated path for development and registration. In order to accomplish this goal, a careful research operating plan was designed and executed in which prodrugs were evaluated for plasma hydrolysis, stability in simulated gastric and intestinal fluids, and fit for purpose intraduodenal (ID) pharmacokinetic rat and dog models. In addition, carbapenem chemistry is synthetically demanding not only because of their poor stability at both ends of the pH ranges leading to purification challenges but also because of high polarity and high degree of chemical reactivity. Therefore, special care needs to be applied for synthesis of carbapenem analogs. Details of the design, synthesis, structure−function relationship, bioconversion, and pharmacokinetic evaluation of a series of prodrugs of ertapenem are described herein.

RESULTS AND DISCUSSIONS

Design. Ertapenem possesses four polar groups amenable to derivatization by prodrug moieties. Specifically, **4** is substituted with a C-8 hydroxyl, a secondary pyrrolidine amine, and two carboxyl groups. We focused our major attention on the synthesis of ester prodrugs of both carboxyl groups to increase log *D* substantially. Examples of prodrugs available clinically provided the initial basis for the selection of prodrug moieties. Since ertapenem is a potent antibiotic with potential to inhibit

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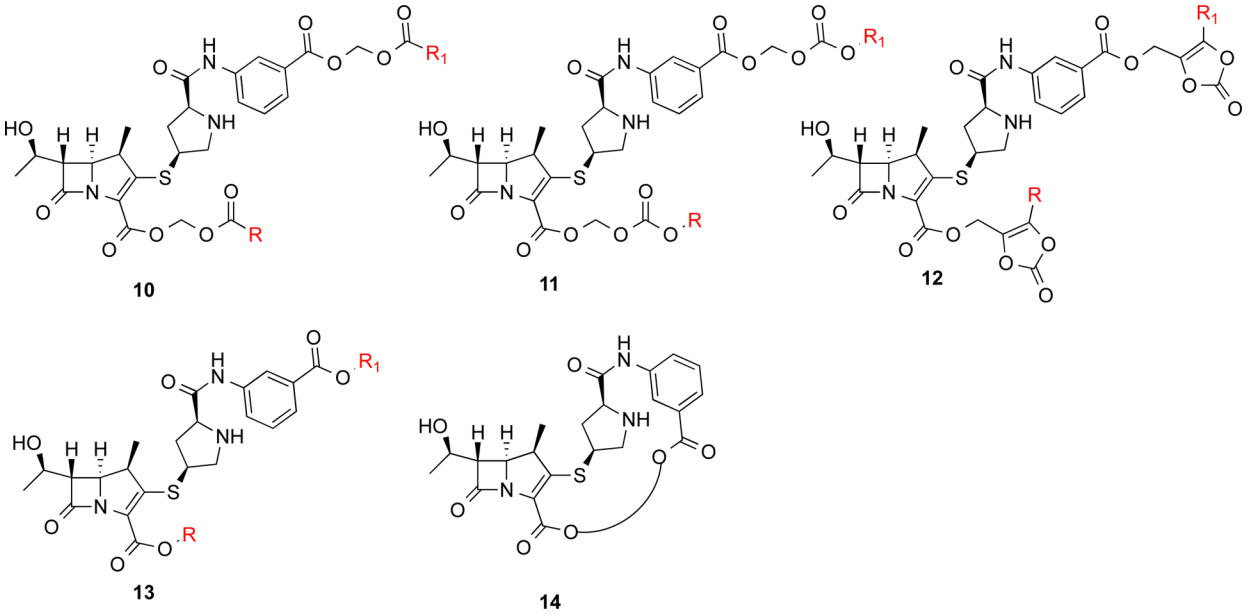
Chart 2. General Structures of Five Classes of Prodrugs



growth of commensurate bacteria in the gut, our design strategy required that the prodrugs must have reduced or no antibacterial activity. As a free carboxyl group at C-3 is essential for potent antibacterial activity[10] by all carbapenems, we posited that a prodrug with an esterified C-3 carboxyl group would be devoid of antibacterial activity. Thus, our main focus was to synthesize bis-esters and lactone prodrugs. Lesser emphasis was directed toward the synthesis of acyl derivatives of the C-8 secondary hydroxy group or carbamate derivatives of the pyrrolidine amino group of diesters to evaluate their potential role in absorption. Prodrug ester moieties that are part of successful clinical agents include pivaloxymethyl (e.g., adefovir dipivoxil, cefetamet pivoxil),[11,12] carbonate esters (e.g., tenoforvir disoproxil fumarate),[11] (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (e.g., olmesartan medoxomil),[11,13] ethyl (e.g., xemilofiban),[14] and lactones (e.g., lovastatin and simvastatin).[15] While preparing ertapenem prodrugs, we have incorporated the concepts of all of the promoieties and synthesized five (**10**–**14**) groups of prodrugs (Chart 2). The prodrugs are bis-acyloxylmethyl esters (**10**), bis-(alkoxycarbonyl)oxy methyl esters (**11**), bis(alkoxy-2-oxo-1,3-dioxol-4-yl) methyl esters (**12**), bis-alkyl esters (**13**), and macrocyclic-lactones (**14**). We recently reported a preliminary account of the synthesis and evaluation of a member (**10a**, **12a**, **13a**, and **14a**) of each group except for the carbonate series **11**.[16] Prodrugs with varying lypophilicity (clogD), calculated polar surface area (cPSA), and calculated apparent permeability coefficient (cPapp) were synthesized to study structure–function relationships. Despite low and unfavorable clogD or calculated permeability numbers, in each ester series, we also generated C-3 monoesters and in some cases both C-3 and benzoate monoesters to test the potential role of intestinal organic acid transporters (OATP) for the absorption of monoacids. These esters also served as bioanalytical reference standards for metabolites originating from bis-esters.

In order to elucidate the structure–function relationship (SFR) of acyloxylmethyl esters, the experience of *tert*-butyl group of (POM) acyloxylmethyl esters (**10a**) was extended (Table 1) by synthesis to include analogs bearing the simplest and smallest group (methyl ester **10k**), intermediate isopropyl (**10f**), cyclohexyl (**10i**), and POM methyl analog of **10a** (**10m**). Both monoesters of *tert*-butyl (**10b** and **10c**) and isopropyl (**10g** and **10h**) and a C-3 cyclohexyl monoester (**10j**) were also prepared. Similarly, bis- and monoesters in the carbonate series (**11a**–**j**) were also synthesized (Table 2). As expected, the carbonate esters showed increased polar surface area compared to corresponding acyloxylmethyl esters. A limited number of analogs (**12a**–**d**) were also prepared in the bis(alkoxy-2-oxo-1,3-dioxol-4-yl) methyl ester series as represented by isopropyl and *tert*-butyl groups (Table 3). An extensive study was undertaken to study SFR of the alkyl ester series, since the diethyl ester **13a** had shown the best absorption.[16] Twenty compounds (**13a**–**t**) representing this series are presented in Table 4. We prepared a range of esters with varied properties, including those with electron withdrawing groups such as trifluoroethyl esters (**13l**–**n**), small chain alkyl *n*-propyl (**13e**), *n*-butyl (**13i**), medium chain fatty acid (e.g., octanoate, **13j**), larger chain fatty acid (e.g., palmitate, **13k**), and cholesteryl ester (**13o**). To test the role of the remaining polar groups of **13a** for absorption, we generated the C-8 acetate (**13p**), propionate (**13q**), and valinyl (**13r**) analogs. In addition, we also studied the alkyl (**13s**) and carbamate (**13t**) analogs of pyrrolidine with (alkoxy-2-oxo-1,3-dioxol-4-yl)methyl group. The latter group was selected for carbamate derivatization because of its rapid hydrolysis (e.g., **12a**) in the human plasma.[16]Addition of two prodrug moieties in the bis-ester series unfortunately increased the molecular size of the prodrug substantially. This MW increase is expected to present challenges for absorption, especially for ertapenem prodrugs, as ertapenem is already endowed with relatively high molecular weight. We envisaged circumventing this challenge by macrocyclization of the two carboxyl groups with appropriate smaller linkers. Therefore, we prepared *n*-propyl linked macrocycle (**14a**), extended the macrocycle with one carbon and made the *n*-butyl linked analog (**14b**), and rigidified the linker by making *Z*-butene analog (**14c**). In addition, we synthesized the cyclic carbonate macrocyclic analog **14d** to test whether the cyclic carbonate displays a faster rate of hydrolysis such as that

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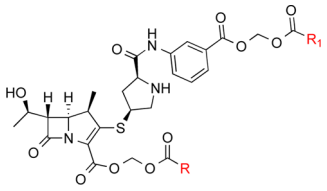
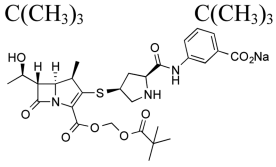
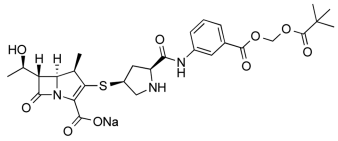
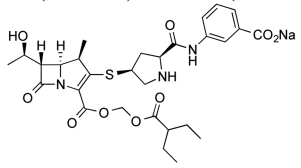
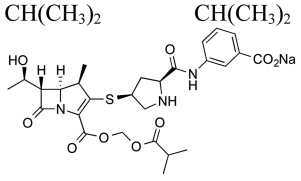
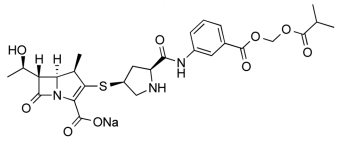
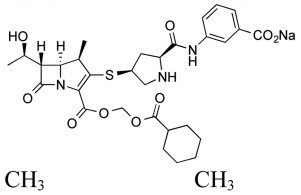
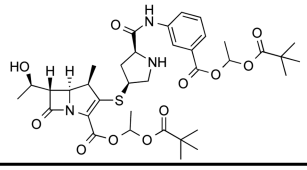
Table 1. Results of Plasma Hydrolysis and Calculated Properties of Acyloxylmethyl Esters



Cpds	R	R_1	Plasma hydrolysis (rat) after 60 min incubation[a]		Plasma hydrolysis (human) after 60 min incubation[a]		MW	cPSA	clogD[b]	cPapp
			% R	% C	% R	% C				10^{-6} cm/sec
4[c]	-	-	100	0	100	0	475.51	156	-5.9	1.8
10a	$C(CH_3)_3$	$C(CH_3)_3$	2	46	36	1	703.8	187	3	7.2
10b			0	66	45	4	611.66	172	-3.2	3.2
10c			0	86	53	19	611.66	172	-3.4	4.5
10d	$CH(CH_2CH_3)_2$	$CH(CH_2CH_3)_2$	3	31	83	0	731.85	187	4.9	5.2
10e			0	23	82	2	625.68	172	-2	2.9
10f	$CH(CH_3)_2$	$CH(CH_3)_2$	1	57	8	21	675.75	187	2.2	6.1
10g			0	100	15	78	597.63	172	-3.6	2.7
10h			0	20	26	44	597.63	172	-3.8	3.6
10i	C_6H_{11}	C_6H_{11}	10	42	60	13	755.87	187	4.6	4.8
10j			0	31	20	47	637.69	172	-2.4	2.7
10k	CH_3	CH_3	0	100	0	34	619.64	187	0.5	5.7
10l	CH_2CH_3	CH_2CH_3	0	100	0	60	647.69	187	2.2	6.6
10m			2	46	46	2	731.85	187	3.6	6.6

[a]R = remaining, C = conversion to ertapenem from respective prodrugs in column 1. [b]ACD = clogP at pH7.4. [c]Disodium salt.

observed for **12a**. We also extended the *N*-carbamate analogs of the olefinic macrocyles **14e−g**. Since we had detected a poor or incomplete plasma hydrolysis of **14a**, we envisaged and designed macrocyclic analog **14h** in which the hydrolysis of

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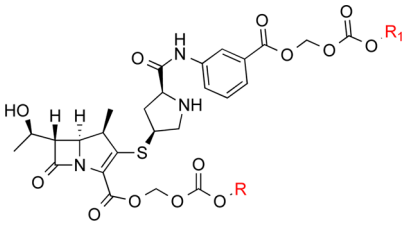
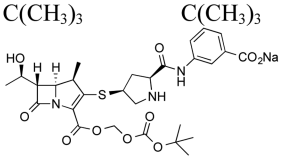
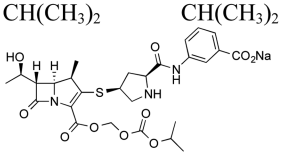
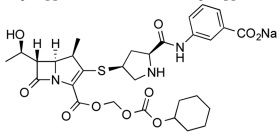
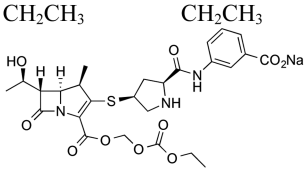
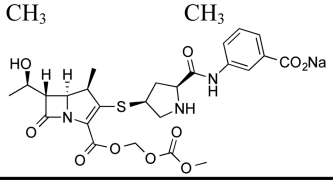
Table 2. Results of Plasma Hydrolysis and Calculated Properties of (Alkoxycarbonyl)oxy Methyl Esters



Cpd#	R	R_1	Plasma hydrolysis (rat) after 60 min incubation[a]		Plasma hydrolysis (human) after 60 min incubation[a]		MW	cPSA	clogD[b]	cPapp
			% R	% C	% R	% C				10^{-6} cm/sec
11a	$C(CH_3)_3$	$C(CH_3)_3$	8	37	67	4	735.8	205	2.9	7.4
11b			0	76	40	33	627.66	181	-3.3	3
11c	$CH(CH_3)_2$	$CH(CH_3)_2$	3	100	28	6	707.75	205	2.7	6.5
11d			0	100	62	18	613.63	181	-3.1	2.7
11e	C_6H_{11}	C_6H_{11}	10	90	96	3	787.87	205	4.5	6
11f			0	100	46	16	653.69	181	-2.5	2.9
11g	CH_2CH_3	CH_2CH_3	0	100	1	27	679.69	205	2.1	6.1
11h			0	97	10	60	583.6	172	-3.4	2.4
11i	CH_3	CH_3	0	100	0	26	651.64	205	1	7.1
11j			0	44	33	36	569.58	172	-4.5	2.6

[a]R = remaining, C = conversion to ertapenem from respective prodrugs in column 1. [b]ACD = clogP at pH 7.4.

either ester group is expected to collapse the molecule to ertapenem. To reduce the polarity further, we also acetylated C-8 hydroxy group of **14h** to produce C-8 acetate analog **14i**, which exhibited one of the best permeability cPapp values (calculated) of 11. Synthesis and systematic structure−function relationship exploration of each of the five prodrug classes are described herein.

Chemistry. *Synthesis of Bis-ester Prodrugs of Ertapenem (**10**−**13**).* All bis-esters were synthesized starting from ertapenem sodium by first protecting the pyrrolidine secondary amine by reaction with allyl chloroformate in phosphate buffer at room temperature to yield an *N*-allylcarbamate (**15**) in over 95% yield (Scheme 1). The intermediate **15** was lyophilized at pH 7 and used directly without further purification to avoid decomposition. This material was reacted with 2−5 equiv of appropriate halides (RCO_2CH_2X for **10**, $ROCO_2CH_2X$ for **11**, (alkoxy-2-oxo-1,3-dioxol-4-yl)CH_2X for **12**, and alkyl iodide for **13**) using *N*-ethyldiisopropylamine and phase transfer catalyst benzyltriethylammonium chloride to afford respective N-protected esters (**16**) which was deprotected with phenylsilane and purified by reversed phase HPLC to furnish final deprotected esters **10**−**13** (Tables 1−4). Similar reaction of **15** with 1-pivoloxylethyl iodide ($((CH_3)_3CCO_2CH(CH_3)I$) followed by deprotection with phenylsilane afforded the pivoloxyl-1-ethyl ester of ertapenem **10m** in 19% overall two-step yield (Scheme 1, Table 1).

*Synthesis of Macrocyclic Lactone Prodrugs of Ertapenem (**14**).* Macrocyclic lactones were synthesized following a similar reaction sequence as described for the synthesis of bis-esters (Scheme 2). The allylcarbamate protected ertapenem sodium salt was reacted with appropriate dihalide to yield protected macrocyclic lactones (**17**), which upon deprotection by phenylsilane and tetrakis(triphenylphosphine)palladium or by

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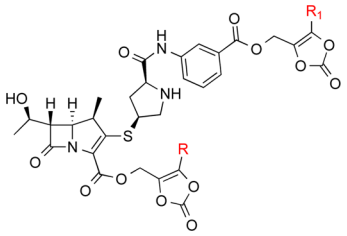
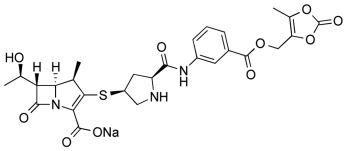
Table 3. Results of Plasma Hydrolysis and Calculated Properties of (Alkoxy-2-oxo-1,3-dioxol-4-yl) Methyl Esters



Cpd #	R	R_1	Plasma hydrolysis (rat) after 60 min incubation[a]		Plasma hydrolysis (human) after 60 min incubation[a]		MW	cPSA	clogD[b]	cPapp
			% R	% C	% R	% C				10^{-6} cm/sec
12a	CH_3	CH_3	0	89	1	98	699.68	205	-0.3	6.7
12b			0	84	12	84	609.6	181	-5	3.2
12c	$CH(CH_3)_2$	$CH(CH_3)_2$	6	78	8	32	755.79	205	1.6	6.4
12d	$C(CH_3)_3$	$C(CH_3)_3$	0	99	10	46	783.84	205	2.3	7.3

[a]R = remaining, C = conversion to ertapenem from respective prodrugs in column 1. [b]ACD = clogP at pH 7.4.

hydrogenolysis followed by reversed phase HPLC purification afforded **14** (Table 5).

Synthesis of Monoesters. The C-3 pivaloxylmethyl ester (**10b**) was prepared by reacting pivaloxylmethyl iodide with ertapenem sodium in DMF at 0 °C for 30 min followed by purification by reverse phase HPLC. The free acid tended to decompose and was stabilized by storing it as a sodium salt. The benzoate ester **10c** was prepared by selective alkylation of C-3 *p*-nitrobenzyl ester (**18**) intermediate obtained from ertapenem synthesis.[17] The pyrrolidine NH was protected as allylcarbamate to give **19**, which was then reacted with pivaloxylmethyl iodide to give $\mathbf{20c}_{10}$. Phenylsilane deprotection gave $\mathbf{21c}_{10}$, which was hydrogenolyzed and lyophilized to afford sodium salt **10c** (Scheme 3). The other benzoate monoesters were prepared similarly using Scheme 3. In most instances, hydrogenolysis of intermediate **20** directly gave the final product.

The monoalkyl esters were prepared by following Scheme 1. The reactions were closely monitored and stopped in about 4 h when both monoesters (**16**, R, R_1 = H, ester) were predominantly present. Intermediate esters **16** were purified directly on reversed phase HPLC and deprotected by phenylsilane and purified by HPLC to afford monoesters (**10**–**13**, Tables 1–4).

Synthesis of 3-Trifluoroethyl-cholesteryl-acetyl-ertapenem-benzoate. Ertapenem *N*-allylcarbamate (**15**) was reacted with trifluoroethyl triflate under phase transfer conditions and DIEA producing monoester $\mathbf{16m}_{13}$. This monoester was alkylated with cholesteryl iodoacetate (prepared from cholesteryl chloroacetate and NaI) in similar alkylation conditions except for using Na_2CO_3 as a base producing the protected diester, which was immediately deprotected by phenylsilane to give highly lipophilic cholesteryl diester **13o** (Scheme 4).

Synthesis of 8-Acylated Ertapenem Diesters. N-protected diethyl ester of ertapenem ($\mathbf{16a}_{13}$) was acetylated with acetyl chloride to yield acetylated product **22a**, which was N-deprotected by phenylsilane to produce **13p** (Scheme 5). Propionyl ertapenem diethyl ester was prepared by EDCI coupling of propionic acid and the N-protected diethyl ester ($\mathbf{16a}_{13}$) to give **22b**, which was deprotected with phenylsilane to afford **13q**. Coupling of allylcarbamate-(*S*)-valine with $\mathbf{16a}_{13}$ produced **22c** which was treated with phenylsilane to remove both allyl carbanates, affording **13r**.

Synthesis of N-Derivatized Diesters. The bis-ethyl ester (**13a**) was alkylated with a reaction of (methyl-2-oxo-1,3-dioxol-4-yl)methyl chloride and DIEA in DMF to give N-alkylated derivative **13s**. A similar reaction of diethyl ester (**13a**) with (methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate produced carbamate **13t** (Scheme 6, Table 4).

Synthesis of 8-Acylated Ertapenem Macrocyclic Lactone. The macrocyclic lactone ($\mathbf{17h}_{14}$) was acetylated by coupling of acetic acid using EDCI to give acetylated derivative **23** which was deprotected by reaction with phenylsilane catalyzed by tetrakis(triphenylphosphine)palladium to afford acetylated macrocyclic compound **14i** (Scheme 7).

Synthesis of N-Carbamate Substituted Macrocylic Compounds (14e–g). *N*-Carbamate substituted macrocyles were synthesized by reacting ertapenem with the 5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate to give a common intermediate **24** which was cyclized with requisite dihalides to give carbamate substituted macrocycles (**14e**–**g**) (Scheme 8).

Overview of Research Operating Plan. The program goal was to find a compound that is stable in the gastrointestinal (GI) tract, absorbed through the intestinal walls, and rapidly hydrolyzed to parent ertapenem in blood. A thorough and careful evaluation strategy was developed for prodrugs as a part of the research-operating plan. Carbapenems are generally inherently unstable. Therefore, all prodrugs were tested for their stability in phosphate buffer (pH 7), simulated

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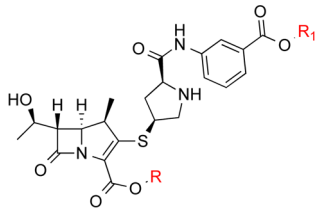
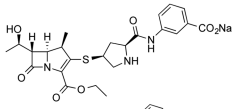
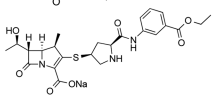
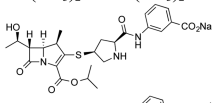
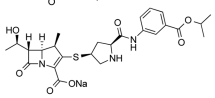
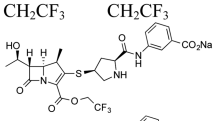
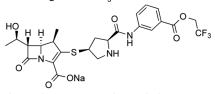
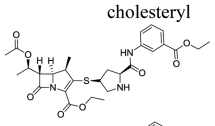
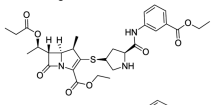
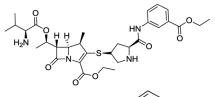
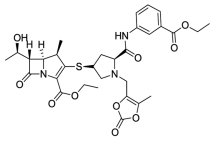
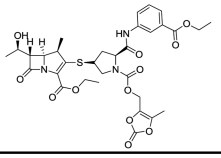
Table 4. Results of Plasma Hydrolysis and Calculated Properties of Alkyl Esters



Cpd#	R	R_1	Plasma hydrolysis (rat) after 60 min incubation[a]		Plasma hydrolysis (human) after 60 min incubation[a]		MW	cPSA	clogD[b]	cPapp
			% R	% C	% R	% C				10^{-6} cm/sec
13a	CH_2CH_3	CH_2CH_3	0	2	5	0	531.62	134	2.9	6.9 (13)
13b			ND	ND	ND	ND	525.57	145	-3.1	2.2
13c			ND	ND	ND	ND	525.57	145	-3.2	2.9
13d	CH_3	CH_3	0	2	0	0	503.57	134	1.8	8
13e	C_3H_7	C_3H_7	0	5	7	0	559.67	134	3.9	7
13f	$CH(CH_3)_2$	$CH(CH_3)_2$	0	1	16	0	559.67	134	3.5	7.3
13g			ND	ND	ND	ND	539.59	145	-2.8	2.5
13h			ND	ND	ND	ND	539.59	145	-2.9	2.9
13i	C_4H_9	C_4H_9	2	9	1	0	587.73	134	5	7.9
13j	C_8H_{17}	C_8H_{17}	4	2	80	1	699.94	134	9.2	4.8
13k	$C_{16}H_{33}$	$C_{16}H_{33}$	89	0	89	0	924.37	134	17.7	4.3
13l	CH_2CF_3	CH_2CF_3	3	100	6	0	639.56	134	1.8	9.3
13m			7	59	80	1	557.54	145	-3.9	2.6
13n			ND	ND	ND	ND	579.54	145	-4	3.7
13o	CH_2CF_3	CH_2CO-cholesteryl	97	0	92	0	984.21	161	12	4
13p			0	0	37	0	573.66	140	3.2	8.7
13q			0	0	22	0	587.68	140	3.7	9
13r			0	0	38	0	630.75	166	3	4.8
13s			0	0	19	0	643.7	161	2.7	11
13t			0	0	9	0	687.71	187	3.1	13

[a]R = remaining, C = conversion to ertapenem from respective prodrugs in column 1. ND = not determined. [b]ACD = clogP at pH 7.4.

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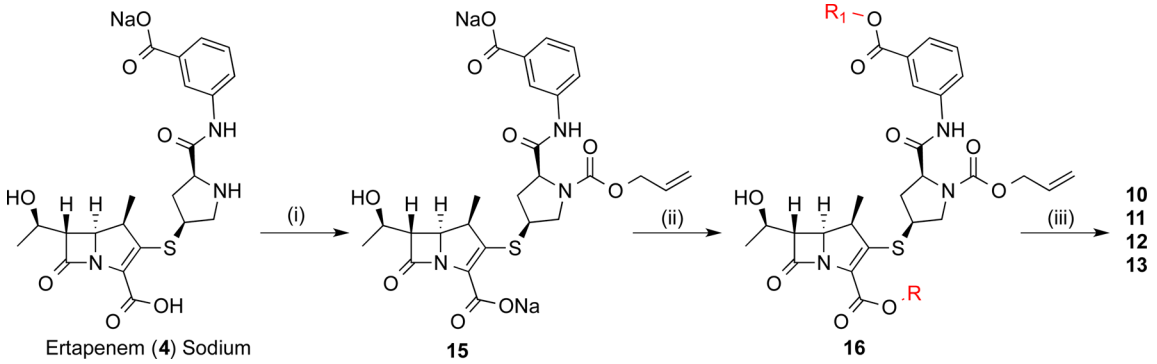
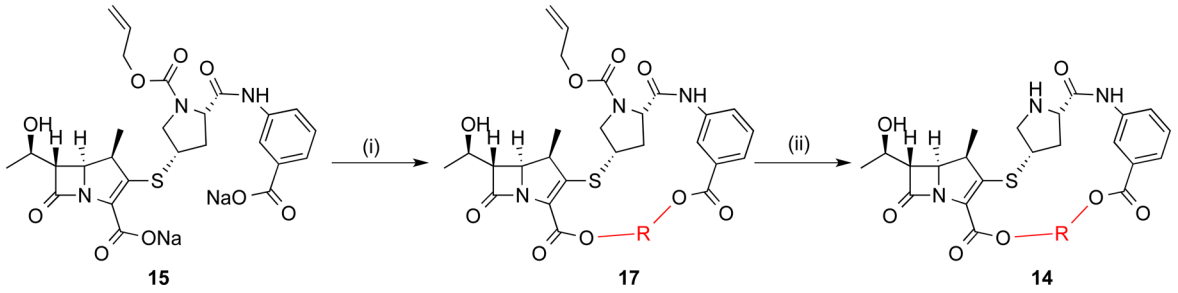
Scheme 1. Synthesis of Bis-esters[a]



[a]Reagents: (i) allyl chloroformate, water, acetone, phosphate buffer (pH 7), room temperature, 30 min; (ii) appropriate halide, benzyltriethylammonium chloride, DIEA, DMF, 40−45 °C, 4−18 h; (iii) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 10−180 min, or H_2, Pd/C, THF, room temperature.

Scheme 2. Synthesis of Macrocylic Ester Prodrugs of Ertapenem[a]



[a]Reagents: (i) alkyl dihalide, benzyltriethylammonium chloride, Na_2CO_3, DMF, 50 °C, 4−18 h; (ii) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 10−180 min, or H_2, Pd/C, THF, water, room temperature, 1 h.

gastrointestinal fluid (FaSSIF, pH 6.5), and simulated gastric fluid (SGF, pH 2.0). All tested compounds showed instability in SGF with over 25% loss to β-lactam ring open product in 60 min. Therefore, at the later stage of the program, this test was abandoned. The stability in the upper intenstine was evaluated by incubating all compounds in simulated gastrointestinal fluid (FaSSIF, pH 6.5). Samples from all stability tests were analyzed by LC−MS−MS. All compounds were soluble and stable (>95% remaining) in phosphate buffer (pH 7) and FaSSIF at 50 μM for greater than 3 h.

After the stability test, all compounds were evaluated for the hydrolysis of prodrugs to ertapenem by rat and human plasma, measuring for the disappearance of the prodrug and production of ertapenem. It is well established that rat plasma has additional esterases (e.g., carboxylesterases, cholinesterases) compared to higher species.[18] Higher mammalian species, including humans, express many of these esterases in liver.[19] On the basis of the esterase distribution in different tissue types or body fluid in rat compared to higher species, it is expected that the in vitro hydrolysis of the prodrugs in rat plasma may be more pronounced than the hydrolysis in plasmas of the other mammalian species. Therefore, any prodrug that is stable in the rat plasma may not be suitable for further evaluation.

Because of expression differences of esterases in different mammalian species, particularly expression of many estarases only in the liver in many higher species, selected prodrugs were evaluated for their hydrolysis in rat, human, dog, and monkey liver microsomal preparations (with or without NADPH) and hepatocytes. The results of **13a** were described earlier[16] and serve as an illustration. However, these studies did not show a clear discernible pattern in these experimental models and were not followed up further.

A selected group of prodrugs that showed rapid hydrolysis in the rat plasma with rapid conversion (with a few exceptions) to ertapenem were selected for rat pharmacokinetic (PK) studies. As explained above, these classes of compounds are known to be highly unstable at low pH, which was experimentally demonstrated by measuring stability in SGF. Therefore, it is expected that these compounds will not survive stomach acids. Hence, it became critical to find an alternative dosing regimen for dosing prodrugs that either physically protected drugs from exposure to stomach acid or bypassed the stomach altogether. Prevention of exposure of drugs to stomach acid can be accomplished by appropriate formulation (e.g., enteric coating of the tablets/capsules). However, it is not practical or cost-effective to enterically coat each compound in the discovery phase of a program. This is not only because of high cost/investment required for formulation efforts but also because of the large amount of compound required for formulation development, which is particularly challenging for difficult-to-synthesize targets. Therefore, it was imperative to identify and implement an alternative-dosing regimen that bypassed the stomach for successful execution of this program. Intraduodenal dosing regimen emerged as a viable option that accomplished this goal, which was readily adopted for conducting rat and dog PK experiments for these compounds. A development compound would be expected to be forumuated by enteric coating for oral delivery of the prodrug.

Evaluation of Prodrugs. As described in the research-operating plan, all prodrugs that were stable in phosphate buffer

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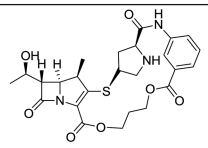
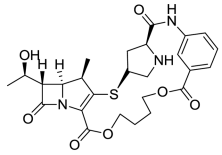
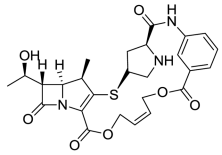
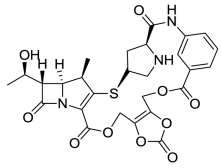
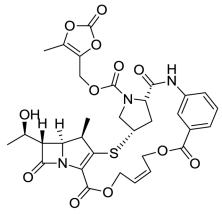
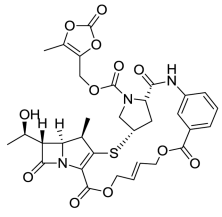
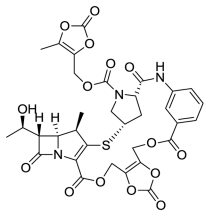
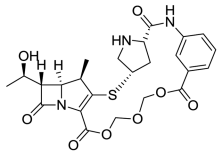
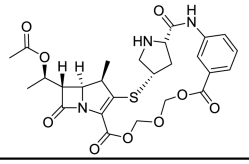
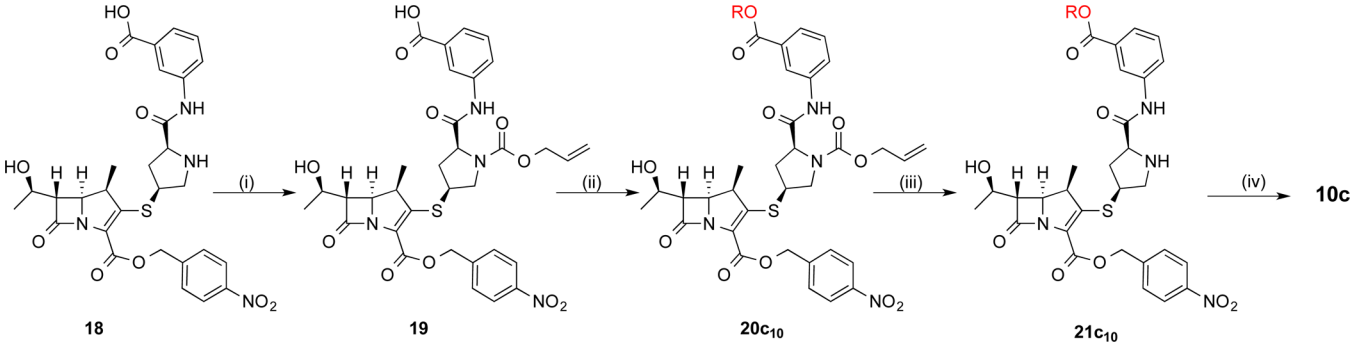
Table 5. Results of Plasma Hydrolysis and Calculated Properties of Macrocyclic Lactones

Cpd#	Structure	Plasma hydrolysis (rat) after 60 min incubation[a]		Plasma hydrolysis (human) after 60 min incubation[a]		MW	cPSA	clogD[b]	cPapp
		% R	% C	% R	% C				10^{-6} cm/sec
14a		0	100	34	0	515.58	134	1.1	8.2
14b		0	0	4	0	529.61	134	1.7	7.6
14c		0	0	21	0	527.59	134	1.4	7.8
14d		0	41	0	39	585.58	170	-1.3	6.5
14e		0	0	20	0	683.68	187	1.9	9.9
14f		0	0	17	0	683.68	187	1.9	9.9
14g		0	64	0	56	741.68	223	-0.8	8.9
14h		0	3	60	16	517.55	143	0.6	8.2
14i		0	0	30	0	559.59	150	1.5	11

[a]R = remaining, C = conversion to ertapenem from respective prodrugs in column 1. [b]ACD = clogP at pH 7.4.

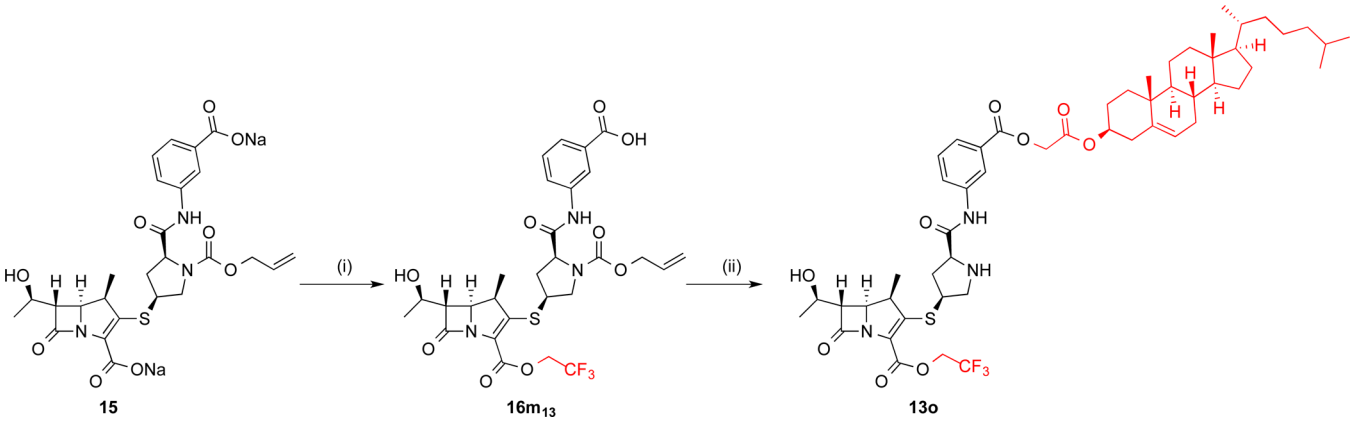
dx.doi.org/10.1021/jm500879a | J. Med. Chem. 2014, 57, 8421−8444

Scheme 3. Synthesis of Pivaloxylmethyl-benzoate Ertapenem (10c)[a]



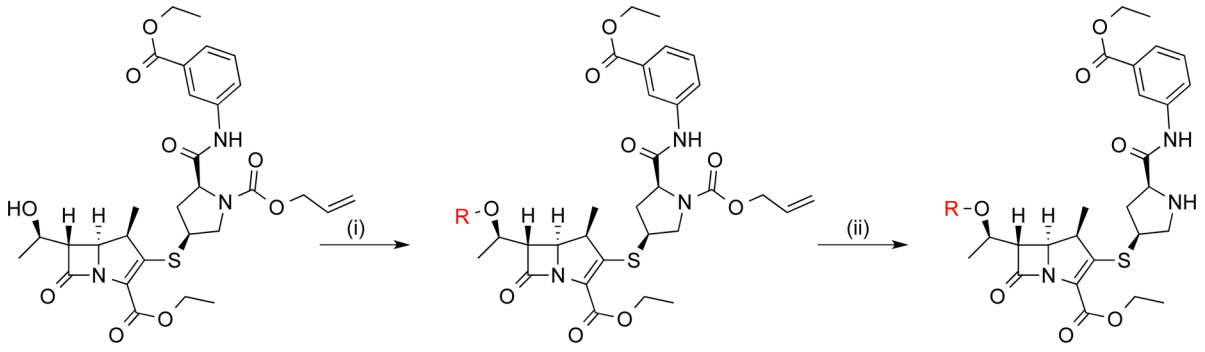
[a]Reagents: (i) allyl chloroformate, water, acetone, phosphate buffer (pH 7), room temperature, 3 h; (ii) pivaloxylmethyl iodide, DIEA, DMF, room temperature, 30 min; (iii) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 30 min; (iv) H_2, $NaHCO_3$, Pd/C, THF, water, room temperature, 1.5 h.

Scheme 4. Synthesis of Cholesteryl Ester (13o)[a]



[a]Reagents: (i) trifluoroethyl triflate, DIEA, DMF, 45 °C, 3 h; (ii) (a) cholesteryl 2-iodoacetate, Na_2CO_3, benzyltriethylammonium chloride, DMSO, 50 °C, 18 h; (b) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 10 min.

Scheme 5. Synthesis of 8-Acylated Ertapenem Diethyl Esters[a]



[a]Reagents: (i) acetyl chloride, DMAP, pyridine; or propionic acid or *N*-Alloc-(*S*)-valine, EDCI, DMAP, DCM; (ii) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 10−60 min.

and FASSIF were incubated in rat and human plasma followed by PK in rat and dog by ID route. As a control, ertapenem was incubated in rat and human plasma and found to be completely stable in both plasmas for 60 min. Prodrugs were incubated in a similar fashion. For prodrugs, we monitored the disappearance of prodrug and concomitant appearance of ertapenem. In selected cases we also monitored the level of monoesters formed from partial hydrolysis of the prodrugs.

Plasma Hydrolysis of Prodrugs. Incubation of acyloxyl methyl esters (**10**) and linear carbonates (**11**) series (Tables 1 and 2) in rat plasma showed rapid disappearance of prodrugs in almost all cases. However, incubation of bis-esters with only

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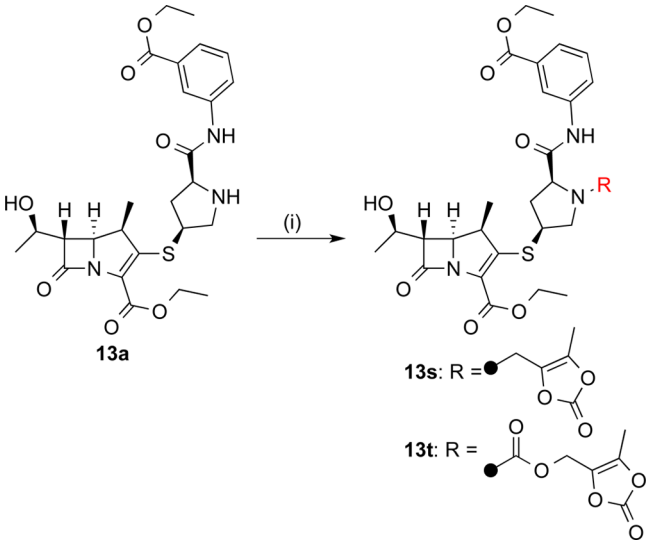
Scheme 6. Synthesis of N-Derivatized Diethyl Esters[a]

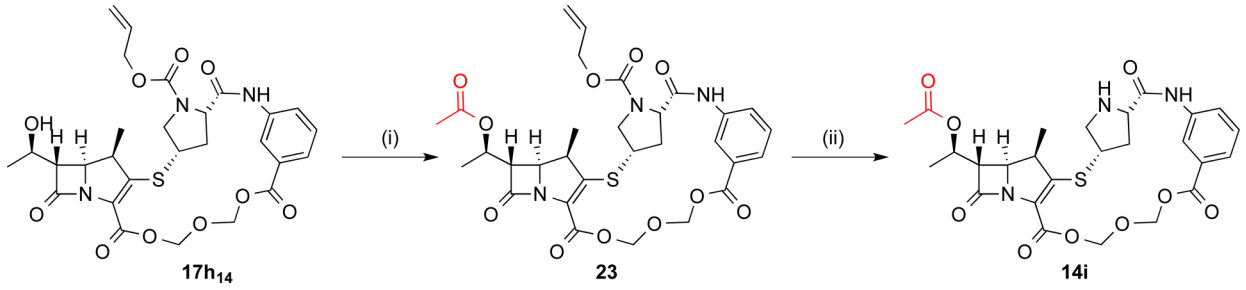


[a]Reagents: (i) (methyl-2-oxo-1,3-dioxol-4-yl)methyl chloride or (methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate, DIEA, DMF, 50 °C, 2 h.

smaller groups (e.g., methyl (**10k**, **11i**), ethyl (**10l**, **11g**)) produced >90% ertapenem in 60 min. Relatively lower percentages of conversion of ertapenem were observed from the prodrugs containing hindered prodrug moieties (**10a**, **10d**, **10f**, **10i**, **10m**, **11a**) in both the **10** and **11** series, but the effect was much more pronounced for acyloxyl methyl ester (**10**) series as evidenced by the results of **11c** and **10f**. The isopropyl carbonate ester **11c** showed rapid disappearance of the prodrug moieties with rapid and proportional conversion to ertapenem, whereas incubation of **10f** showed rapid disappearance of the prodrug but only 57% conversion to ertapenem. The hydrolysis pattern of the monoesters was less clear-cut. We could not acertain which of the two ester groups (C-3 or benzoate) hydrolyzed faster. The hydrolysis of the **10** and **11** series prodrugs by human plasma was generally less efficient other than for really small esters such as the bis-methyl (**10k**) and bis-ethyl (**10l**) esters of acyloxyl methyl esters. These two compounds showed rapid disappearance of the prodrug, but only 34 and 60% ertapenem was produced in 60 min from **10k** and **10l**, respectively (Table 1). While hydrolysis and corresponding conversion to ertapenem of linear carbonates were highly efficient in rat plasma, it was generally less efficient in the human plasma (Table 2). The cyclic carbonates (**12a**–**d**) were efficiently hydrolyzed by rat plasma with rapid conversion to ertapenem in high yields (Table 3). Essentially equivalent hydrolysis was also observed in human plasma, but efficient conversion to ertapenem was only noted for the less hindered methyl carbonates **12a** and **12b**. Isopropyl (**12c**) and *tert*-butyl (**12d**) substituted carbonates showed lower ertapenem conversions, 32% and 46%, respectively. As we have reported earlier[16] for the bis-ethyl ester **13a**, the hydrolysis of bis-alkyl esters **13b**–**t** (Table 4) with (**13b**–**o**, **13s**, **13t**) or without (**13p**–**r**) free OH group at C-8 by rat plasma was generally efficient with respect to disappearance of the prodrug but not for the formation of ertapenem. Exceptions to this observation included the bis-palmitate (**13k**) and cholesteryl ester (**13o**), which were remarkably stable in rat plasma. Bis-esters with electron withdrawing groups (e.g., **13l**) were rapidly hydrolyzed in the rat plasma with rapid quantitative conversion to ertapenem. Similar general hydrolysis patterns and conversions to ertapenem were also observed for alkyl esters by human plasma except for octanoyl ester **13j** and monoester **13m**, which did not show significant hydrolysis in human plasma. While the bis-trifluoroethyl ester (**13l**) was rapidly hydrolyzed by human plasma at rates similar to rat plasma, no ertapenem was produced in contrast to results seen using rat plasma. Most of the macrocyclic lactones (**14a**–**i**, Table 5) rapidly disappeared when incubated in either rat or human plasma with the exception of the two cyclic analogs **14h** and **14i**, which showed lesser hydrolytic efficiency in human plasma. Conversion efficiency to ertapenem from macrolactones was generally poor in both rat and human plasma hydrolytic reactions except for **14a**, which showed excellent ertapenem conversion in rat plasma. The cyclic carbonate (**14d**, **14g**) showed moderate ertapenem conversion. Surprisingly, the cyclic compound **14h** showed better conversion to ertapenem in human plasma than in rat plasma. The C-8 acetate analog of the cyclic compound **14i** was rapidly hydrolyzed in rat plasma without conversion to any ertapenem in 60 min similar to the parent **14h**. The rate of hydrolysis of **14i** in human plasma was somewhat better than the parent **14h**, but conversion to ertapenem was not observed.

Pharmacokinetics. As per selection criteria layed out in the research-operating plan, a selected group of the prodrugs were evaluated for their PK characteristics using intraduodenal dosing in rat. It has been shown earlier[16] from the studies of compounds **10a**, **12a**, and **13a** that the Sprague–Dawley (SD) rat ID PK model, which was first selected for these studies because of obvious reasons of lower cost and lower compound requirement, was not ideal for evaluation because of unveiling poor absorption (Table 6). However, before abandoning the rat PK meaurment for prioritization of compounds, we elected to

Scheme 7. Synthesis of 8-Acetyl Macrocyclic Lactone (14i)[a]



[a]Reagents: (i) acetic acid, EDCI, DMAP, DCM, −5 °C, 1 h; (ii) $Pd(PPh_3)_4$, phenylsilane, DMF, room temperature, 10 min.

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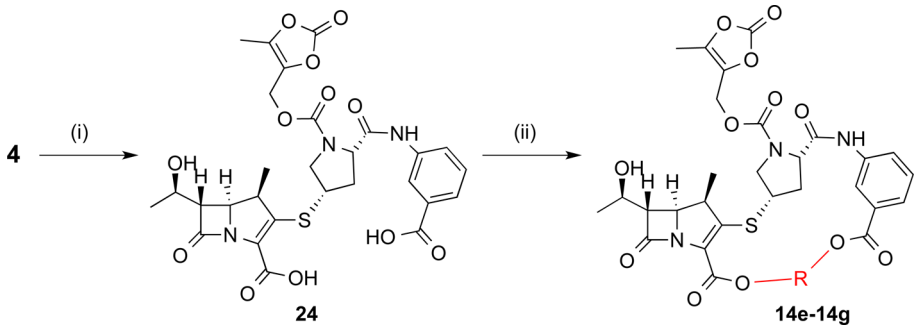
Scheme 8. Synthesis of N-Substituted Macrocycles (14e−g)[a]



[a]Reagents: (i) (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate, $NaHCO_3$, acetone, water, room temperature, 1 h, 50 °C, 2 h; (ii) dihalide, Na_2CO_3, DMF, 50−55 °C, 4−18 h.

evaluate several new analogs in this model to determine whether the earlier observation was a compound specific phenomenon. Therefore, two additional compounds with differing characteristics (hydrolysis and/or physical properties) in each of the three series were evaluated for rat ID PK. The two isopropyl esters **10f** and **10g** were selected from the acyloxylmethyl series. The former compound showed rat plasma hydrolysis and cPapp, clogD, and cPSA properties similar to **10a** but exhibited slightly improved human plasma hydrolysis (Table 1). The latter compound exhibited overall better plasma hydrolysis properties than other compounds from this series while evincing less favorable calculated properties (cPSA, clogD, and cPapp), indicative of potentially poor absorption.[20,21] However, **10g** contained a free benzoic acid that allowed for testing the hypothesis as to whether the benzoid acid can act as substrate for intestinal organic acid anion uptake transporters (OATPs). Both of these compounds were dosed at 10 mg/kg by ID route, and the presence of the parent prodrug, monoester metabolites, and ertapenem was measured in the rat plasma at various time intervals for 8 h. These compounds showed only marginal, if any, improvement from **10a** in total absorption (Table 6), suggesting that smaller ester groups or potential anion transporters do not affect overall absorption in rats, at least for these compounds. The compounds **12c** and **12d** of the cyclic carbonate series were tested for rat PK to supplement information gleaned from **12a**. Bulkier isopropyl and *tert*-butyl groups in **12c** and **12d** substituted the methyl group of **12a**, leading to larger molecular weights but improved clogD and similar cPapp values (Table 3). Unfortunately, total production of ertapenem from **12c** and **12d** was marginally decreased compared to **12a** (Table 6). As reported earlier,[16] the most promising prodrug series continued to be bis-alkyl series of **13a** type. Two additional bis-esters, the bis-isopropyl ester **13f** and bis-trifluoroethyl ester **13l**, with improved cPapp and similar clogD values (Table 4) were evaluated in rat PK by ID routes of administration. The total absorption of ertapenem was below the level of quantification for the hindered ester **13f** and was about 4-fold lower for the ester-containing electron withdrawing group **13l** (Table 6). Ertapenem showed 156-fold lower absorption by ID dosing in rat compare to iv dosing (Table 6). These studies with diverse group of nine compounds confirm that poor rat absoption of the original three compounds **10a**, **12a**, and **13a** was not specific to just those compounds but to the entire ertapenem prodrug series. Since the absorption of prodrugs in rat by ID dosing was very poor, no in vitro/in vivo correlation could be established in rats. Therefore, rat PK model was abandoned in favor of beagle dog for further evaluations.

We have reported earlier that the absorption of many of these compounds was species dependent.[16] For example, the total absorption of bis-acyloxylmethyl ester **10a** and bis-ethyl ester **13a** was improved by approximately 8× and 5×, respectively, in beagle dogs when dosed by ID administration (Table 7). We extended the dog PK studies with additional compounds from each of the prodrug series to more fully elucidate absorption structure function relationship. ID dosing of the intermediate sized bis-isoproyl acyloxylmethyl ester **10f** resulted in 3.6% total absorption all in the form of ertapenem with 2× improvement from rat (F_T = 1.8%), which happens to be the same level improvement observed for **10a** (Table 7). The total absoption of the smaller bis-ethyl acyloxylmethyl ester **10l** was 2.9% similar to the large (bulky) bis-ester **10a**, suggesting that the bis-isopropyl ester may be of optimal size for this series. The cyclic carbonate *tert*-butyl ester **12d**, with the best clogD and cPapp values of the series, showed very poor total absoption (F_T = 0.4%) in the dog PK study (Table 7). None of the other carbonates were tested for dog PK.

The simple alkyl esters appeared to be most effective prodrugs of ertapenem, as exemplified by bis-ethyl ester **13a**. Therefore, series **13** compounds that showed rapid hydrolysis in rat plasma with or without rapid conversion to ertapenem was systematically studied for beagle dog ID PK starting with the smaller bis-methyl ester **13d**, bis-*n*-propyl ester **13e**, moderately hindered bis-isopropyl ester **13f**, bis-*n*-butyl ester **13i**, a mid-chain fatty acid ester bis-octanoate ester **13j**, and bis-trifluoroethyl ester **13l**. Of this group, the smallest methyl ester (**13d**) showed the best total absorption of 24.2%, bis-*n*-propyl ester (**13e**) 11.0%, bis-*n*-butyl ester (**13i**) 16.9%, bis-isopropyl ester (**13f**) 5.1%, bis-octanoyl ester (**13j**) 1.1%, and bis-trifluoroethy ester (**13l**) 12.0% (Table 7). The bis-ethyl ester (**13a**) remained the best compound exhibiting 31.3% total absorption. However, the bis-ester prodrugs formed long lasting circulating monoesters in vivo (Table 7). Since the monoesters formed after dosing of the bis-ester prodrugs were not measured for all compounds, precise structure−function relationship cannot be ascertained but a general trend can be established. C_1−C_4 linear esters are reasonably absorbed with ethyl being the best. Isopropyl ester is less favored, and the medium chain (C_8) fatty esters are very poorly absorbed. The long chain (C_{16}) fatty ester (**13k**) was insoluble in the vehicles and was not dosed. While bis-trifluoroethyl ester (**13l**) showed quantitative conversion to ertapenem in rat plasma but not human plasma (Table 4), it failed to improve overall absorption

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Table 6. Sprague–Dawley Rat (Fasted) Pharmacokinetic Measurement of Prodrugs by Intraduodenal (ID) Infusion Dosing[a]

compd	dosing route (dose, mg/kg)	prodrug $AUC_{(0-8)}$, μM·h	**4** $AUC_{(0-8)}$, μM·h	8-OH, C-3 ester $AUC_{(0-8)}$, μM·h	8-OH, benzoate ester $AUC_{(0-8)}$, μM·h	F(**4**),[b] %	F_T(total),[c] %
4	iv (1)[d]	NA	7.8	NA	NA	NA	NA
4	ID (10)[e]	NA	0.5	NA	NA	0.7	0.7
10a	ID (10)[f]	NQ[h]	0.2	NQ[h]	NQ[h]	0.4	0.4
10f	ID (10)[f]	NQ[h]	0.9	NQ[h]	NQ[h]	1.8	1.8
10g	ID (10)[f]	NQ[h]	0.7	NQ[h]	NA	1.2	1.2
12a	ID (7.4)[f]	NQ[h]	0.3	ND	ND	0.8	0.8
12c	ID (10)[f]	NQ[h]	0.4	ND	ND	0.9	0.9
12d	ID (10)[f]	NQ[h]	0.2	ND	ND	0.3	0.3
13a	ID (4.41)[g]	0.06	1.5	0.1	0.4	5.0	6.6
13f	ID (10)[f]	0.09	<0.1	ND	ND	<0.1	0.1
13l	ID (10)[f]	NQ[h]	1.7	ND	ND	3.0	3.0

[a]NA: not applicable. ND: not determined. [b]Calculated by dividing normalized AUC of ertapenem formed from prodrug/normalized AUC of ertapenem dosed iv. [c]Combined total AUC of parent, monoester metabolites, and ertapenem calculated by dividing normalized AUC of circulating parent prodrug, ertapenem, and two monoesters formed from prodrug/normalized AUC of ertapenem dosed iv. [d]Dosing vehicle (30% Captisol, clear solution). [e]Dosing vehicle (0.5% methylcellulose [MC], clear solution). [f]Dosing vehicle (0.5% MC, homogeneous suspension). [g]Dosing vehicle (0.5% MC, nearly clear solution). [h]Not quantifiable (absolute data were below level of quantitation, 5 nM). The difference between $AUC_{(0-\infty)}$ and $AUC_{(0-8)}$ was ±20%.

in dog compared to the bis-ethyl ester **13a** (Table 7). Addition of acetate (**13p**) or propionate (**13q**) at C-8 of bis-ethyl ester (**13a**) did not improve total absorption and exhibited total absorption of only 23.3% and 20.2%, respectively. The cholesterol ester (**13o**) showed very poor total absorption of only <0.1% and all intact prodrug. No ertapenem or monoester **13m** was detected. Clearly, cholesterol transporters played no role in the absorption of this prodrug. Likewise, the amino acid transporters played no role in the absorption of the C-8 valinyl ester (**13r**). It showed about half (14.9%) of the total absorption compared with its parent **13a**. Substitution of proton of pyrrolidine NH by a cyclic carbonate carbamate (**13t**) of **13a** with cPapp of **13** led to about 2-fold reduction in the total absorption (14.4% vs 31.3%).

The absorption of the macrocyclic esters containing a propyl group (**14a**) or cyclic carbonate linked macrocycle (**14d**) was generally less than 1%. However, the cyclic acetal acetate **14i**, with an intermediate cPapp value of 11, showed significantly better total absorption of 16%. These studies did not provide good correlation between rat and human plasma hydrolyses as well as correlation in vivo. ID dog PK performed well for the measurement of absorption of these prodrugs and could be used for absorption measurement of compounds that show instability in gastric fluid.

Careful analysis of the physical properties and absorption (Figures 1 and 2) allowed for formulation of some general conclusions. The molecular size of the prodrugs has a more substantial impact on absorption than other parameters such as cPapp values as evident from *XY* plots shown in Figures 1 and 2. The data suggest that molecular weights of the bis-ester prodrugs lower than 600 show better absorption. The esters (**13e** and **13f**) with odd number of carbons in alkyl chain appear to be exceptions. For example, the ester with the even alkyl chain (C-4, **13i**) showed better absorption than esters with odd alkyl chain even if they were of a smaller size (C-3, **13e** and **13f**) (Figure 1). A careful correlation of the total absorption and cPapp values indicated that cPapp values of 7 or higher showed absorption of over 10% except when either steric factors were involved (e.g., isopropyl ester **13f**) or the compound was a carbamate derivative (**13s**). As expected, prodrugs with lower cPapp values show lower absorptions than those with higher cPapp values for this series of compounds (Figure 2). Unfortunately ertapenem, the parent, is endowed with high molecular weight (475.5 Da), larger polar surface area (156), and very low clogD (−5.9) and low cPapp (1.8), so it fares rather poorly when compared to tebipenem (MW = 383.5 Da, cPSA = 93, clogD = 2.4, and cPapp = 5.2). Differences in these properties are likely attributed to favorable absorption of tebipenem pivoxil. Meropenem POM ester also showed >25% absorption in rat even with MW greater than 643.[7] This would suggest benzoic acid structural moiety present as part of substitution at C-2 in ertapenem negatively impacted the absorption regardless of the class of prodrugs.

Additionally, tebipenem pivoxil is transported by intestinal uptake transporters OATB1A2 and OATBB1, which positively impacts absoption.[22] To build transporter affinity in a molecule is challenging; however, we did attempt to design molecules that can exploit amino acid transporters (e.g., valinyl ester, **13r**), fatty acid esters (**13j** and **13k**), cholestryl ester (**13o**), and free carboxyl monoester (**13f**). These compounds failed to show improved absorption. The bis-ethyl ester (**13a**) remained the best compound of ertapenem prodrugs showing 31.3% total absorption. The current study established that only simple alkyl

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Table 7. Beagle Dog (Fasted) Pharmacokinetic Measurement of Prodrugs by Intraduodenal (ID) Infusion Dosing[a]

compd	dosing route (dose, mg/kg)	prodrug $AUC_{(0-8)}$, μM·h	**4** $AUC_{(0-8)}$, μM·h	8-OH, C-3 ester $AUC_{(0-8)}$, μM·h	8-OH, benzoate ester $AUC_{(0-8)}$, μM·h	F(**4**),[b] %	F_T(total),[c] %
4	iv (1)[d]	NA	8.9	NA	NA	NA	NA
4	ID (10)[d]	NA	1.4	NA	NA	1.6	1.6
10a	ID (10)[e]	NQ[k]	1.8	<0.1	NQ[k]	3.0	3.07
13a	ID (10)[f]	5.0	14.3	2.2	3.3	18.1	31.3
14a	ID (10)[g]	0.1	0.4	ND	ND	0.5	0.7
10f	ID (10)[h]	NQ[k]	2.2	NQ[k]	NQ[k]	3.6	3.6
10l	ID (10)[h]	NQ[k]	1.6	NQ[k]	ND	2.9	2.9
12d	ID (10)[i]	NQ[k]	0.2	ND	ND	0.4	0.4
13d	ID (10)[i]	0.8	12.0	3.9	3.6	14.3	24.2
13e	ID (10)[h]	1.0	7.3	ND	ND	9.8	11.0
13f	ID (6.18)[e]	1.6	0.2	0.2	0.3	0.5	5.1
13i	ID (10)[i]	2.8	9.4	ND	ND	13.1	16.9
13j	ID (10)[h]	0.6	NQ[k]	ND	ND	NQ[k]	1.1
13l	ID (10)[i]	0.2	6.7	0.8	0.2	10.2	12.0
13o	ID (10)[i]	<0.1	NQ[k]	NQ[k]	ND	NQ[k]	0.1
13p	ID (10)[h]	<0.1	11.3	1.8	2.2	15.4	23.3[l]
13q	ID (10)[j]	NQ[k]	9.1	1.3	1.6	12.6	20.2[l]
13r	ID (10)[j]	NQ[k]	6.5	0.9	1.0	9.7	14.9[l]
13t	ID (10)[i]	NQ[k]	3.4	0.7	3.1	6.4	14.4[l]
14d	ID (10)[i]	NQ[k]	0.7	ND	ND	1.0	1.0
14i	ID (10)[j]	0.1	10.1	ND	ND	13.4	16.0[l]

[a]NA: not applicable. ND: not determined. [b]Calculated by dividing normalized AUC of ertapenem formed from prodrug/normalized AUC of ertapenem dosed iv. [c]Combined total AUC of parent, monoester metabolites, and ertapenem calculated by dividing normalized AUC of circulating parent prodrug, ertapenem, and two monoesters formed from prodrug/normalized AUC of ertapenem dosed iv. [d]Dosed as disodium salt, dosing vehicle (30% Captisol, clear solution). [e]Vehicle (0.5% MC, homogeneous opaque suspension). [f]Dosing vehicle (10% Tween, nearly clear solution). [g]Dosing vehicle (PEG400, hazy suspension). [h]100% PEG400, clear solution. [i]40% PEG400−10% Tween 80−50% water, clear solution. [j]Dosing vehicle 10% Tween 80, clear solution. [k]Not quantifiable (absolute data were below level of quantitation, 5 nM). The difference between $AUC_{(0-\infty)}$ and $AUC_{(0-8)}$ was ±20%. [l]Including other possible metabolites excluding β-lactam hydrolyzed products. Tebipenem pivoxil, dog ID PK: parent (<0.1%), tebipenem (60.7%).

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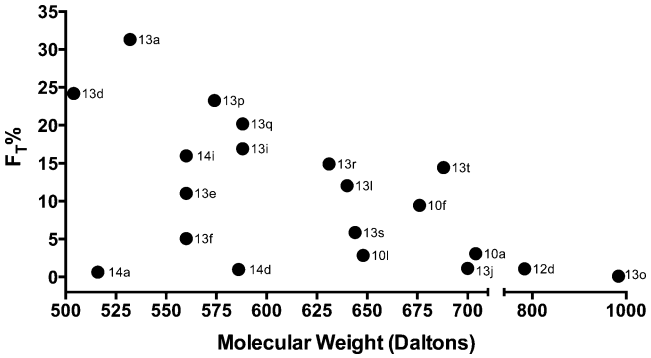

Figure 1. Percentage of total absorption versus molecular weights of prodrugs in beagle dogs by intradeuodenal dosing.

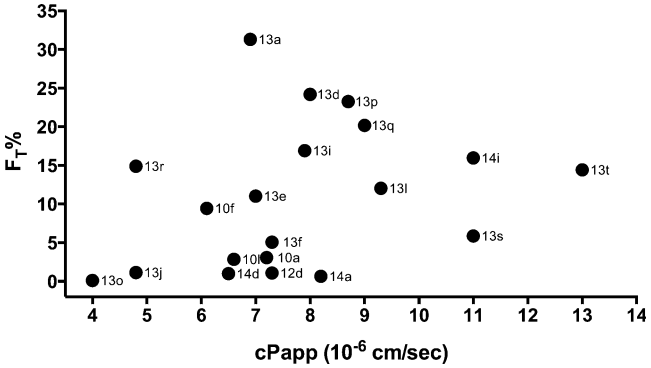

Figure 2. Percentage of total absorption versus cPapp of prodrugs in beagle dogs by intradeuodenal dosing.

esters with small and unhindered groups with relatively smaller molecular weight are favored for absorption. Going forward, a streamlined research-operating plan could be envisaged for evaluation of the compounds of this class involving measurement of disappearance of prodrug and appearance of carbapenem in rat plasma followed by ID dog PK with a special emphasis given to smaller molecular size, preferably less than 600 Da, and cPapp > 10.

In summary, we have described a detailed study of the synthesis and evaluation of ertapenem prodrugs to address the lack of oral absorption of ertapenem. Five classes of prodrugs were studied using intraduodenal dosing to bypass and avoid the effect of stomach acid and approximately mimic oral absorption. The simplest bis-ethyl and bis-methyl ester prodrugs were shown to be the most promising, providing 31.3% and 24.2% total absorption in dog, respectively, versus the 1.6% absorption observed when dosing ertapenem. Unfortunately, the best compound **13a** showed slow hydrolysis and conversion to parent ertapenem in vivo in dog and rats and showed the presence of significant amounts of long-lived circulating monoester metabolites. These properties of the prodrugs were undesirable for a development compound and did not meet the developmental objective, leading to the termination of **13a** and the program from further development. Therefore, no efforts were expended to develop expensive enteric-coated formulation and testing of **13a** by oral dosing. The macrocyclic lactone acetate acetal prodrug **14i** showed some promise with 16.0% absorption of ertapenem in dogs and warrants additional investigation. The study showed that intradeudenal dosing is a very effective method for PK measurement for compounds unstable in gastric fluid. The prodrugs in this study showed no correlation between rat and human plasma hydrolysis as well as in vivo in rat or dog. This study did demonstrate that the benzoic acid substitution at C-2 of ertapenem played a detrimental role in prrodrug absorption and hydrolysis. In addition, the study suggested the smaller C-2 carbapenem substitution is better for prodrug absorption and hydrolysis (e.g., tebipenem and meropenem).

EXPERIMENTAL SECTION

Solvents, reagents, and intermediates that are commercially available were used as received. Reagents and intermediates that were not commercially available were prepared in the manner as described below. ^{1}H NMR spectra were measured on either a Varian VNMR system 400 or Bruker Avance 400 spectrometer at 400 MHz and are reported as ppm downfield from Me_4Si with number of protons, multiplicities, and coupling constants in hertz indicated parenthetically. Where LC/MS data are presented, analyses were performed using an Agilent 6110A MSD or an Applied Shimadzu 2020MSD. The parent ion is given. Purification was conducted with reversed phase HPLC (Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) or Phenomenex Synergi C_{18} (250 mm × 50 mm, 10 μm) column, elution of acetonitrile/water from 100% acetonitrile to 0% acetonitrile) or silica gel column chromatography (SAN PONT, ZXC II). Animal hepatocytes were prepared at WuXi AppTec Co., Ltd. All other reagents were from commercial sources. All compounds (with the exception of a few compounds) tested in the bioassays were of ≥95% purity as measured by HPLC (A = 220 nm) and ^{1}H NMR. Specific HPLC % purity of all compounds is listed with the compound synthesis.

Synthesis of *N*-Allyloxycarbonyl-ertapenem (15). To a suspension of ertapenem **4** (20 g, 40 mmol) in a mixture of H_2O (20 mL), acetone (250 mL), and NaH_2PO_4–Na_2HPO_4 buffer (pH 7, 7.20 mL) was slowly added allyl chloroformate (5.0 mL, 48 mmol). The mixture was stirred at room temperature for 30 min and diluted with water. The reaction mixture was adjusted to pH 7 by addition of aqueous solution of 1 N $NaHCO_3$. The solution was lyophilized to give **15** as a yellow solid which was directly used in the next reaction without further purification (23 g, 94.8% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.92–8.02 (m, 1H), 7.75–7.80 (m, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.30 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.75–6.05 (m, 1H), 5.05–5.40 (m, 2H), 3.35–4.60 (m, 9H), 3.20–3.25 (m, 1H), 2.70–2.85 (m, 1H), 1.95–2.05 (m, 1H), 1.15–1.30 (m, 6H). ESI MS m/z 560.5 $[M + H]^+$. HPLC purity: 93.6%.

Synthesis of Ertapenem Bis(pivaloyloxy)methyl Ester (10a). Iodomethyl pivalate (5.1 g, 20.4 mmol) was slowly added to a solution of compound **15** (3.0 g, 5.1 mmol), *N*-ethyldiisopropylamine (1.38 g, 10.2 mmol), and benzyltriethylammonium chloride (392 mg, 1.72 mmol) in DMF (10 mL), and the mixture was heated at 45 °C for 18 h. After addition of EtOAc (300 mL), the combined organic layers were washed with H_2O (100 mL × 3) and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography, eluting with DCM/MeOH (40:1). Fractions containing the product were concentrated under vacuo to give the desired bis(pivaloyloxy)methyl ester **16a$_{10}$** (R = R_1 = CH_2OCO-*t*-Bu) (2.0 g, 49.3%) as a pale yellow oil. ESI MS m/z 788.5 $[M + H]^+$.

To a solution of **16a$_{10}$** (2.0 g, 2.54 mmol) in DMF (8.0 mL) were added phenylsilane (213 mg, 5.08 mmol) and tetrakis(triphenylphosphine)palladium (80 mg, 0.069 mmol), and the mixture was stirred at room temperature for 10 min. EtOAc (100 mL) was added, and the organic solution was washed with H_2O (40 mL × 3) and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) column eluted with a water and acetonitrile gradient (mobile phase A, water; mobile phase B, acetonitrile; gradient 52–72% B, 0–8 min; 100% B, 8.5–10.5 min; 5% B, 11–12 min) followed by lyophilization to afford the desired compound **10a** (600 mg, 33.7%) as a colorless powder. ^{1}H NMR (400

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MHz, CD_3OD) δ 8.30 (s, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.45 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 6.00 (s, 2H), 5.75−5.85 (m, 2H), 3.75−4.25 (m, 4H), 3.40−3.58 (m, 2H), 3.25−3.30 (m, 1H), 2.65−2.95 (m, 2H), 1.82−1.90 (m, 1H), 1.25 (t, J = 6.4 Hz, 6H), 1.15 (s, 18H). ESI MS m/z 704.3 $[M + H]^+$. HPLC purity: 95.1%.

Synthesis of Ertapenem C-3-Methyl Pivalate (10b). Iodomethyl pivalate (2.42 g, 10 mmol) was added dropwise to a solution of ertapenem **4** (5.0 g, 10 mmol) in DMF (30 mL) at 0 °C for 30 min. The mixture was directly purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Synergi C_{18} (250 mm × 50 mm, 10 μm) using water (0.1% formic acid) and acetonitrile as eluents (mobile phase A, water (0.1% formic acid); Mmobile phase B, acetonitrile; gradient, B from 25% to 50% in 25 min; flow rate, 80 mL/min; detective wavelength, 220 nm) followed by freeze-drying to afford free acid of C-3 monoester **10b** (250 mg, 4.2%) as a white solid. Structure was determined by HMBC. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.40 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.83 (d, J = 5.6 Hz, 1H), 5.77 (d, J = 5.6 Hz, 1H), 4.19−4.22 (m, 1H), 3.99−4.12 (m, 2H), 3.79−3.85 (m, 1H), 3.48−3.53 (m, 2H), 3.22−3.30 (m, 1H), 2.95−2.99 (m, 1H), 2.73−2.81 (m, 1H), 1.84−1.91 (m, 1H), 1.24−1.27 (m, 6H), 1.15 (s, 9H). ESI MS m/z 590.2 $[M + H]^+$. HPLC purity: 96.5%.

Free acid form of **10b** (250 mg, 0.42 mmol) was dissolved in MeCN (20 mL)−H_2O (20 mL), and 0.01 N $NaHCO_3$ aqueous solution (42 mL, 0.42 mmol) was added dropwise. The mixture was concentrated by freeze-drying. The residue was purified by reversed phase HPLC followed by freeze-drying to give sodium salt **10b** (206 mg, 80.0%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.40 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.83 (d, J = 5.6 Hz, 1H), 5.77 (d, J = 5.6 Hz, 1H), 4.19−4.22 (m, 1H), 3.99−4.12 (m, 2H), 3.79−3.85 (m, 1H), 3.48−3.53 (m, 2H), 3.22−3.30 (m, 1H), 2.95−2.99 (m, 1H), 2.73−2.81 (m, 1H), 1.84−1.91 (m, 1H), 1.24−1.27 (m, 6H), 1.15 (s, 9H). ESI MS m/z 590.2 $[M + H]^+$, 612.2 $[M + Na]^+$. HPLC purity: 95.0%.

Synthesis of Ertapenem C-3-Sodium Carboxyl Methyl Pivalate (10c). To a solution of **18** (8.54g, 14.0 mmol) in H_2O (12 mL), acetone (110 mL), and NaH_2PO_4−Na_2HPO_4 buffer (pH 12 mL) was added allyl chloroformate (2.4 g, 0.02 mol) dropwise, and then the mixture was stirred at room temperature for 3 h. After concentration by vacuo followed by dilution with H_2O, the resulting solid was filtered and dried to give **19** (9.5 g, 97.7%) as a pale white solid which was directly used in the next reaction without further purification. ESI MS m/z 695 $[M + H]^+$.

To a solution of compound **19** (4.5 g, 6.5 mmol) and iodomethyl pivalate (3.1 g, 12.8 mmol) was added *N*-ethyldiisopropylamine (0.84 g, 6.5 mmol), and the mixture was stirred at room temperature for 30 min. The reaction was quenched with H_2O (500 mL), extracted with EtOAc (80 mL × 3), and the organic layer was concentrated and purified by silica gel column chromatography, eluting with DCM/MeOH (40:1). It was concentrated by vacuo to give **20c$_{10}$** (R_1 = CH_2OCO-*t*-Bu) (1.8 g, 34.4%) as a pale yellow oil. ESI MS m/z 809 $[M + H]^+$.

A solution of *N*-allyoxycarbonyl **20c$_{10}$** (1.8 g, 2.2 mmol), phenylsilane (475 mg, 4.4 mmol), and $Pd(PPh_3)_4$ (100 mg) in DMF (20 mL) was stirred at room temperature for 30 min. After filtration, the filtrate was concentrated and purified by silica gel column chromatography, eluting with DCM/MeOH (40:1) and concentrated by vacuo to give des-allyloxy compound **21c$_{10}$** (R_1 = CH_2OCO-*t*-Bu) (900 mg, 55.8%) as a pale yellow solid. ESI MS m/z 725 $[M + H]^+$.

10 % Pd−C (110 mg) was added to a solution of **21c$_{10}$** (1.1 g, 1.5 mmol) and $NaHCO_3$ (63 mg, 0.75 mmol) in THF (50 mL)−H_2O (50 mL), and the mixture was stirred at room temperature under H_2 for 1.5 h. After filtration, the filtrate was concentrated by freeze-drying. The residue was purified by reversed phase-HPLC followed by freeze-drying to give **10c** (R = Na, R_1 = CH_2OCO-*t*-Bu) (268 mg, 30%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.37 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.44 (dd, $J_1 = J_2$ = 8.0 Hz, 1H), 5.92 (s, 2H), 4.95 (brs, 1H), 3.93−3.95 (m, 1H), 3.85−3.88 (m, 1H), 3.76−3.80 (m, 1H), 3.38−3.44 (m, 1H), 3.19−3.25 (m, 1H), 3.07−3.11 (m, 1H), 2.98−3.01 (m, 1H), 2.60−2.64 (m, 1H), 1.50−1.57 (m, 1H), 1.21 (s, 9H), 1.07−1.12 (m, 3H), 1.02 (d, J = 6.8 Hz, 3H). ESI MS m/z 590.2 $[M + H]^+$, 612.2 $[M + Na]^+$. HPLC purity: 95.7%.

Synthesis of Ertapenem Bis((2-ethylbutanoyl)oxy)methyl Ester (10d). Reaction of 2-ethylbutanoyl oxymethylchloride with **15** in a similar manner gave **16d$_{10}$** (R = R_1 = $CH_2OCOCH(Et)_2$) as described above, which was followed by deprotection and purification by reversed phase HPLC to yield the desired diester **10d** in 32.2% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.09 (d, J = 7.6 Hz, 1H), 8.05 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.43 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 6.01 (s, 2H), 5.89 (s, 2H), 4.20−4.25 (m, 2H), 3.95−4.05 (m, 1H), 3.60−3.68 (m, 1H), 3.45−3.50 (m, 1H), 3.30−3.38 (m, 1H), 3.23−3.25 (m, 1H), 2.88−2.91 (m, 1H), 2.75−2.83 (m, 1H), 2.22−2.30 (m, 3H), 1.96−2.02 (m, 1H), 1.49−1.70 (m, 8H), 1.34 (d, J = 6.4 Hz, 3H), 1.26 (d, J = 7.6 Hz, 3H), 0.84−0.89 (m, 12H). ESI MS m/z 732.3 $[M + H]^+$. HPLC purity: 99.9%.

Synthesis of Ertapenem C-3-Methyl 2-Ethylbutanoate Sodium Salt (10e). Reaction of iodomethyl 2-ethylbutanoate with ertapenem **4** followed by formation of sodium salt and purification by reversed phase HPLC gave 5.2% yield of desired C-3 monoester sodium salt **10e** (R = $CH_2OCOCH(Et)_2$, R_1 = Na) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.24 (s, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.35 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.80 (d, J = 6.0 Hz, 1H), 5.75 (d, J = 6.0 Hz, 1H), 5.10 (brs, 1H), 4.19−4.22 (m, 1H), 3.94−3.97 (m, 1H), 3.83−3.86 (m, 1H), 3.40−3.60 (m, 3H), 3.23−3.25 (m, 2H), 2.59−2.67 (m, 2H), 2.18−2.24 (m, 1H),1.65−1.68 (m,1H), 1.39−1.52 (m, 4H), 1.14−1.17 (m, 6H), 0.77−0.80 (m, 6H). ESI MS m/z 604.3 $[M + H]^+$, 626.3 $[M + Na]^+$. HPLC purity: 99.4%.

Synthesis of Ertapenem Bis(isobutanoyloxy)methyl Ester (10f). Chloromethyl isobutyrate was reacted with *N*-allyloxy carbamate **15** to give **16f$_{10}$** (R = R_1 = CH_2OCO-*i*-Pr) followed by deprotection with phenylsilane and purification by reversed phase HPLC to provide 2.9% yield of the diester **10f** as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.37 (dd, $J_1 = J_2$ = 8.0 Hz, 1H), 5.93 (s, 2H), 5.85 (d, J = 5.6 Hz, 1H), 5.77 (d, J = 5.6 Hz, 1H), 4.15−4.19 (m, 2H), 3.93−3.95 (m, 1H), 3.57−3.61 (m, 1H), 3.40−3.44 (m, 1H), 3.26−3.30 (m, 1H), 3.16−3.19 (m, 1H), 2.83−2.87 (m, 1H), 2.70−2.76 (m, 1H), 2.48−2.59 (m, 2H), 1.92−1.98 (m, 1H), 1.28 (d, J = 6.4 Hz, 3H), 1.20 (d, J = 7.6 Hz, 3H), 1.09−1.13 (m, 12H). ESI MS m/z 676.3 $[M + H]^+$. HPLC purity: 99.3%.

Synthesis of Ertapenem C-3-Methyl Isobutyratesodium Salt (10g). Reaction of iodomethyl isobutyrate with ertapenem **4** followed by formation of sodium salt and purification by reversed phase HPLC gave desired C-3 monoester sodium salt **10g** (R = CH_2OCO-*i*-Pr, R_1 = Na) in 2.2% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.12 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.31 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.81 (d, J = 5.6 Hz, 1H), 5.71 (d, J = 5.6 Hz, 1H), 5.11 (brs, 1H), 4.20−4.23 (m, 1H), 3.94−3.98 (m, 1H), 3.81−3.85 (m, 1H), 3.24−3.66 (m, 5H), 2.67−2.70 (m, 2H), 1.63−1.70 (m, 1H), 1.04−1.18 (m, 12H). ESI MS m/z 576.2 $[M + H]^+$, 598.2 $[M + Na]^+$. HPLC purity: 95.8%.

Synthesis of Ertapenem C-3-Sodium Carboxyl (Isobutyloxy)-methylbenzoate (10h). Reaction of 1.2 equiv of chloromethyl isobutyrate (705 mg, 5.19 mmol) with compound **19** (3.0 g, 4.32 mmol) followed by purification by silica gel column chromatography afforded desired benzoate **20h$_{10}$** (R_1 = CH_2OCO*i*-Pr) (1.3 g, 37.9%) as a red oil. To a solution of **20h$_{10}$** (124 mg, 0.17 mmol) and $NaHCO_3$ (12 mg, 0.14 mmol) in THF (6.0 mL)−H_2O (6.0 mL) was added 10% Pd−C (110 mg), and the mixture was stirred at room temperature under H_2 for 1 h. After filtration, the filtrate was concentrated by freeze-drying. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water (0.2% $HCOONH_4$) and acetonitrile as eluents (mobile phase A, water (0.2% $HCOONH_4$); mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by freeze-drying to give **10h** (6.8 mg, 7.2%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.37 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.67 (d, J =

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8.0 Hz, 1H), 7.49 (dd, $J_1 = J_2 = 8.0$, 1H), 5.95 (s, 2H), 5.06 (brs, 1H), 4.14−4.17 (m, 1H), 3.94−3.96 (m, 1H), 3.85−3.89 (m, 1H), 3.58−3.62 (m, 2H), 3.19−3.21 (m, 2H), 2.58−2.73 (m, 3H), 1.64−1.74 (m, 1H), 1.14−1.16 (m, 12H). ESI MS m/z 576.2 $[M + H]^+$, 598.2 $[M + Na]^+$. HPLC purity: 95.3%.

Synthesis of Ertapenem Bis((cyclohexanecarbonyl)oxy)-methyl Ester (10i). Reaction of chloromethyl cyclohexanecarboxylate with N-protected **15** produced **16i$_{10}$** (R = R_1 = $CH_2OCOC_6H_{11}$) followed by deprotection of allylcarbamate and purification by reversed phase HPLC afforded diester **10i** in 13.8% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.06−8.09 (m, 2H), 7.81 (d, J = 8.0 Hz, 1H), 7.43 (dd, $J_1 = J_2$ = 8.0 Hz, 1H), 5.98 (s, 2H), 5.89 (d, J = 5.6 Hz, 1H), 5.82 (d, J = 5.6 Hz, 1H), 4.22−4.25 (m, 2H), 4.02−4.05 (m, 1H), 3.63−3.68 (m, 1H), 3.48−3.51 (m, 1H), 3.33−3.37 (m, 1H), 3.23−3.25 (m, 1H), 2.88−2.92 (m, 1H), 2.77−2.82 (m, 1H), 2.28−2.39 (m, 2H), 1.98−2.03 (m, 1H), 1.55−1.96 (m, 11H), 1.12−1.46 (m, 17H). ESI MS m/z 756.3 $[M + H]^+$. HPLC purity: 99.4%.

Synthesis of Ertapenem C-3-((Cyclohexanecarbonyl)oxy)-methyl Ester Sodium Salt (10j). Reaction of 1.0 equiv of chloromethyl cyclohexanecarboxylate (1.76 g, 9.96 mmol) with compound **15** (5.81 g, 10.0 mmol) followed by purification by reversed phase HPLC afforded desired C-3-((cyclohexanecarbonyl)-oxy)methyl ester **16j$_{10}$** (R = CH_2OCOc-Hex, R_1 = H) (450 mg, 6.5%) as white solid. **16j$_{10}$** was deprotected by using phenylsilane and purified by reversed phase HPLC to afford **10j** (240 mg, 60.9%) as white powder, which was converted to sodium salt using $NaHCO_3$ and purified by neutral HPLC as described earlier to give 35.1% yield of the desired sodium salt **10j** (40 mg) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.10 (s, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.30 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.81 (d, J = 6.0 Hz, 1H), 5.71 (d, J = 6.0 Hz, 1H), 5.12 (brs, 1H), 4.20−4.23 (m, 1H), 3.95−3.98 (m, 1H), 3.81−3.85 (m, 1H), 3.24−3.65 (m, 5H), 2.58−2.70 (m, 2H), 1.76−1.79 (m, 2H), 1.60−1.69 (m, 3H), 1.48−1.51 (m, 1H), 1.10−1.36 (m, 11H). ESI MS m/z 616.2 $[M + H]^+$, 638.3 $[M + Na]^+$. HPLC purity: 96.8%.

Synthesis of Ertapenem Bis-acetoxymethyl Ester (10k). Reaction of acetoxymethyl chloride with **15** afforded **16k$_{10}$** (R = R_1 = CH_2OCOMe) followed by upon deprotection and purification by reversed phase HPLC yielded the diacetoxy diester **10k** in 2.3% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 8.04 (s, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.43 (dd, J_1 = 8.4 Hz, J_2 = 7.6 Hz, 1H), 5.97 (s, 2H), 5.86 (d, J = 5.6 Hz, 1H), 5.81 (d, J = 5.6 Hz, 1H), 4.20−4.23 (m, 2H), 3.97−4.01 (m, 1H), 3.60−3.67 (m, 1H), 3.45−3.49 (m, 1H), 3.30−3.37 (m, 1H), 3.22−3.24 (m, 1H), 2.88−2.93 (m, 1H), 2.73−2.81 (m, 1H), 2.38 (brs, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 1.98−2.08 (m, 1H), 1.79 (brs, 1H), 1.34 (d, J = 6.4 Hz, 3H), 1.25 (d, J = 7.2 Hz, 3H). ESI MS m/z 620.2 $[M + H]^+$. HPLC purity: 95.9%.

Synthesis of Ertapenem Bis(propionyloxy)methyl Ester (10l). Propionyloxyl methylchloride was reacted with *N*-allyloxy carbamate **15**, which furnished **16l$_{10}$** (R = R_1 = CH_2OCOEt), followed by deprotection with phenylsilane and purification by reversed phase HPLC to provide the desired diester **10l** in 4.7% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.03−8.09 (m, 2H), 7.80 (d, J = 7.6 Hz, 1H), 7.42 (dd, $J_1 = J_2$ =7.6 Hz, 1H), 5.98 (s, 2H), 5.88 (d, J = 5.6 Hz, 1H), 5.82 (d, J = 5.6 Hz, 1H), 4.18−4.24 (m, 2H), 3.99−4.03 (m, 1H), 3.60−3.68 (m, 1H), 3.45−3.50 (m, 1H), 3.32−3.36 (m, 1H), 3.22−3.24 (m, 1H), 2.89−2.93 (m, 1H), 2.73−2.81 (m, 1H), 2.33−2.42 (m, 4H), 1.98−2.05 (m, 1H), 1.33 (d, J = 6.0 Hz, 3H), 1.25 (d, J = 7.6 Hz, 3H), 1.09−1.16 (m, 6H). ESI MS m/z 648.2 $[M + H]^+$. HPLC purity: 95.5%.

Synthesis of Ertapenem Bis-1-(pivaloyloxy)ethyl Ester (10m). Reaction of 1-chloroethyl pivalate with **15** provided **16m$_{10}$** (R = R_1 = -CH(Me)OCO-*t*-Bu), which was followed by phenylsilane deprotection and reversed phase HPLC purification to furnish the diester **10m** in 4.4% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (d, J = 5.6 Hz, 1H), 8.02−8.13 (m, 2H), 7.78 (d, J = 7.6 Hz, 1H), 7.42 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 7.06−7.09 (m, 1H), 6.88−6.96 (m, 1H), 4.20−4.25 (m, 2H), 4.01−4.04 (m, 1H), 3.58−3.68 (m, 1H), 3.46−3.51 (m, 1H), 3.23−3.46 (m, 2H), 2.75−2.86 (m, 2H), 1.93−2.05 (m, 1H), 1.61 (d, J = 5.2 Hz, 3H), 1.52−1.53 (m, 3H), 1.33−1.36 (m, 3H), 1.16−1.27 (m, 21H). ESI MS m/z 732.3 $[M + H]^+$. HPLC purity: 98.3%.

Synthesis of Ertapenem Bis((*tert*-butoxycarbonyl)oxy)-methyl Ester (11a). *tert*-Butyl (chloromethyl)carbonate was reacted with *N*-allyloxy carbamate **15**, which provided N-protected dicarbonate **16a$_{11}$** (R = R_1 = -CH_2OCOO-*t*-Bu), which was likewise deprotected with phenylsilane followed by reversed phase HPLC purification and lyophilization to afford the desired dicarbonate **11a** in 14.0% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.30 (s, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.54 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.89 (s, 2H), 5.76 (d, J = 6.0 Hz, 1H), 5.67 (d, J = 6.0 Hz, 1H), 5.13 (brs, 1H), 4.23−4.30 (m, 2H), 3.90−3.97 (m, 2H), 3.70−3.75 (m, 1H), 3.26−3.53 (m, 2H), 3.04−3.09 (m, 1H), 2.86−2.89 (m, 1H), 1.87−1.94 (m, 1H), 1.42 (s, 9H), 1.40 (s, 9H), 1.12−1.18 (m, 6H). ESI MS m/z 736.3 $[M + H]^+$. HPLC purity: 97.7%.

Synthesis of Ertapenem C-3-((*tert*-Butylcarbonyl)oxy)methyl Ester Sodium Salt (11b). Reaction of 1.0 equiv of *tert*-butyl (chloromethyl)carbonate (2.90 g, 17.5 mmol) with compound **15** (10.0 g, 17.2 mmol) followed by purification by reversed phase HPLC afforded desired C-3-((*tert*-butylcarbonyl)oxy)methyl ester **16b$_{11}$** (R = -CH_2OCOO-*t*-Bu, R_1 = H) (820 mg, yield 6.5%) as a white solid. **16b$_{11}$** was deprotected by using phenylsilane and purified by reversed phase HPLC to afford **11b** (700 mg, 51.8%) as white powder, which was converted to sodium salt using $NaHCO_3$ to give 55.7% yield of the desired sodium salt **11b** (113 mg) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.30 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.42 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.76 (d, J = 6.0 Hz, 1H), 5.65 (d, J = 6.0 Hz, 1H), 5.09 (brs, 1H), 4.21−4.23 (m, 1H), 3.92−3.99 (m, 1H), 3.83−3.87 (m, 1H), 3.63−3.67 (m, 1H), 3.50−3.55 (m, 1H), 3.24−3.41 (m, 2H), 2.55−2.71 (m, 2H), 1.62−1.72 (m, 1H), 1.41 (s, 9H), 1.12−1.20 (m, 6H). ESI MS m/z 606.2 $[M + H]^+$, 628.2 $[M + Na]^+$. HPLC purity: 95.2%.

Synthesis of Ertapenem Bis((isopropoxycarbonyl)oxy)-methyl Ester (11c). *N*-Ethyldiisopropylamine (690 mg, 5.34 mmol) and benzyltriethylammonium chloride (1.2 g, 5.26 mmol) were added to a solution of compound **15** (3.0 g, 5.16 mmol) and chloromethyl isopropyl carbonate (1.6 g, 10.48 mmol) in DMF (10 mL), and the mixture was stirred at 45 °C for 4 h. After cooling to room temperature and addition with EtOAc, the combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reversed phase HPLC to give the desired N-protected dicarbonate ester **16a$_{11}$** (R = R_1 = -CH_2OCOO-*i*-Pr) as a white solid (1.4 g, 34.3%). ESI MS m/z 792 $[M + H]^+$.

To a solution of compound **16a$_{11}$** (600 mg, 0.75 mmol) in DMF (2.0 mL) were added phenylsilane (162 mg, 1.50 mmol) and tetrakis(triphenylphosphine)palladium (60 mg, 0.052 mmol), and the mixture was stirred at room temperature for 10 min. EtOAc was added, and the organic solution was washed with H_2O and brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by reverse phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) column, eluting with a water and acetonitrile gradient (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to give the desired compound **11c** (210 mg, 39.6%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.08−8.10 (m, 2H), 7.82 (d, J = 7.6 Hz, 1H), 7.44 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.99 (s, 2H), 5.89 (d, J = 5.6 Hz, 1H), 5.83 (d, J = 5.6 Hz, 1H), 4.87−4.95 (m, 2H), 4.21−4.26 (m, 2H), 3.99−4.03 (m, 1H), 3.63−3.67 (m, 1H), 3.46−3.50 (m, 1H), 3.33−3.37 (m, 1H), 3.23−3.25 (m, 1H), 2.89−2.94 (m, 1H), 2.75−2.80 (m, 1H), 1.99−2.06 (m, 1H), 1.25−1.35 (m, 18H). ESI MS m/z 708.2 $[M + H]^+$. HPLC purity: 95.1%.

Synthesis of Ertapenem C-3-((Isopropoxycarbonyl)oxy)-methyl Estersodium Salt (11d). To a solution of compound **15** (5.81 g, 10.0 mmol) in DMF (60 mL) were added DIPEA (1.26 g,

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9.76 mmol), NaI (1.49 g, 9.94 mmol), and chloromethyl isopropyl carbonate (1.52 g, 9.96 mmol), and then the mixture was stirred at 45 °C for 5 h. The mixture was directly purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Synergi C_{18} (250 mm × 50 mm, 10 μm) using water (0.2% formic acid) and acetonitrile as eluents (mobile phase A, water (0.2% formic acid); mobile phase B, acetonitrile; gradient, B from 25% to 50% in 25 min; flow rate, 80 mL/min; detective wavelength, 220 nm.) followed by freeze-drying to give desired product **16d$_{11}$** (R = CH_2OCOO-*i*-Pr, R_1 = H) (380 mg, 5.6%) as a white solid.

The mixture of compound **16d$_{11}$** (380 mg, 0.56 mmol), phenylsilane (120 mg, 1.12 mmol), and $Pd(PPh_3)_4$ (45 mg) in DMF (4 mL) was stirred at room temperature for 10 min and then purified by reversed phase HPLC followed by freeze-drying to afford **11d** (200 mg, 60.1%, R_1 = H) as a white powder. To a solution of the free acid of **11d** (200 mg, 0.34 mmol) in CH_3CN (20 mL) was slowly added 100 mL of H_2O containing 28 mg of $NaHCO_3$ (0.33 mmol) at 0−5 °C. Freeze-drying followed by purification by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water and acetonitrile as eluents (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by freeze-drying afforded the desired sodium carboxylate **11d** (88 mg, 42.5%, R_1 = Na) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.37 (s, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.50 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.86 (d, *J* = 6.0 Hz, 1H), 5.76 (d, *J* = 6.0 Hz, 1H), 5.16 (d, *J* = 5.2 Hz, 1H), 4.84−4.89 (m, 1H), 4.27−4.30 (m, 1H), 4.02−4.06 (m, 1H), 3.90−3.94 (m, 1H), 3.71−3.74 (m, 1H), 3.56−3.62 (m, 1H), 3.27−3.50 (m, 2H), 3.05−3.13 (m, 1H), 2.60−2.80 (m, 1H), 1.72−1.79 (m, 1H), 1.27−1.32 (m, 12H). ESI MS *m*/*z* 592.2 [M + H]$^+$, 614.2 [M + Na]$^+$. HPLC purity: 98.4%.

Synthesis of Ertapenem Bis((cyclohexylcarbonyl)oxy)methyl Ester (11e). Reaction of chloromethyl cyclohexyl carbonate with **15** by using NaI and DIEA in DMF at 40 °C for 4 h gave **16e$_{11}$** (R = R_1 = $CH_2OCOOC_6H_{13}$), which was followed by deprotection and purification by reversed phase HPLC to yield the desired dicarbonate **11e** in 1.4% yield as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 8.10 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.43 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.99 (s, 2H), 5.89 (d, *J* = 5.6 Hz, 1H), 5.83 (d, *J* = 5.6 Hz, 1H), 4.63−4.71 (m, 2H), 4.24−4.26 (m, 2H), 3.54−3.72 (m, 2H), 3.31−3.39 (m, 1H), 3.24−3.25 (m, 1H), 2.98−3.08 (brs, 1H), 2.78−2.88 (m, 1H), 1.98−2.08 (m, 1H), 1.68−1.96 (m, 9H), 1.13−1.58 (m, 20H). ESI MS *m*/*z* 788.3 [M + H]$^+$, 810.2 [M + Na]$^+$. HPLC purity: 99.7%.

Synthesis of Ertapenem C-3-Methyl Cycloxeyl Carbonate Sodium Salt (11f). To a solution of chloromethyl cyclohexyl carbonate (1.92 g, 10 mmol) in acetone (60 mL) was added NaI (4.47 g, 30 mmol), and the mixture was stirred at reflux for 18 h. The mixture was filtered, concentrated, and then diluted with DCM and filtered again. The filtrate was concentrated to give the desired product cyclohexyl (iodomethyl) carbonate (4.0 g, 91.5% yield) as a brown oil which was used directly in the next step without further purification.

Reaction of cyclohexyl (iodomethyl) carbonate with ertapenem **4** followed by formation of sodium salt and purification by reversed phase HPLC gave 4.1% yield of desired C-3 monocarbonate sodium salt **11f** (R = -CH_2OCOOc-C_6H_{13}, R_1 = Na) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.28 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.45 (dd, J_1 = J_2 = 8.0 Hz, 1H), 5.81 (d, *J* = 6.0 Hz, 1H), 5.71 (d, *J* = 6.0 Hz, 1H), 5.10 (d, *J* = 5.2 Hz, 1H), 4.52−4.60 (m, 1H), 4.22−4.25 (m, 1H), 3.97−4.00 (m, 2H), 3.70−3.80 (m, 1H), 3.48−3.52 (m, 2H), 2.58−2.85 (m, 2H), 1.61−1.83 (m, 6H), 1.22−1.46 (m, 7H), 1.13−1.18 (m, 6H). ESI MS *m*/*z* 632.2 [M + H]$^+$, 654.2 [M + Na]$^+$. HPLC purity: 95.0%.

Synthesis of Ertapenem Bis((ethoxycarbonyl)oxy)methyl Ester (11g). Chloromethyl ethyl carbonate was reacted with compound **15**, which furnished **16g$_{11}$** (R = R_1 = $CH_2OCOOEt$), which was followed by phenylsilane deprotection and reversed phase HPLC purification to furnish 11.3% yield of the diethoxy carbonate **11g** as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.08−8.09 (m, 2H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.44 (dd, J_1 = 8.8 Hz, J_2 = 7.6 Hz, 1H), 6.00 (s, 2H), 5.89 (d, *J* = 5.6 Hz, 1H), 5.84 (d, *J* = 5.6 Hz, 1H), 4.21−4.29 (m, 6H), 3.98−4.02 (m, 1H), 3.63−3.66 (m, 1H), 3.45−3.49 (m, 1H), 3.30−3.37 (m, 1H), 3.23−3.25 (m, 1H), 2.89−2.91 (m, 1H), 2.74−2.80 (m, 1H), 1.98−2.05 (m, 1H), 1.25−1.34 (m, 12H). ESI MS *m*/*z* 680.2 [M + H]$^+$. HPLC purity: 99.3%.

Synthesis of Ertapenem C-3-Methyl Propionate Carbonate Sodium Salt (11h). Reaction of iodomethyl propionate with ertapenem **4** followed by formation of sodium salt and purification by reversed phase HPLC gave 0.43% yield of desired C-3 monoester sodium salt **11h** (R = $CH_2OCOOEt$, R_1 = Na) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1 H), 7.89 (s, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.17 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.77 (d, *J* = 5.6 Hz, 1H), 5.69 (d, *J* = 5.6 Hz, 1H), 5.11 (brs, 1 H), 4.17−4.20 (m, 1H), 3.82−3.93 (m, 1H), 3.76−3.79 (m, 1H), 3.62−3.64 (m, 1H), 3.46−3.52 (m, 1H), 3.20−3.22 (m, 1H), 2.67−2.70 (m, 2H), 2.28−2.38 (m, 3H), 1.53−1.60 (m, 1H), 1.15−1.18 (m, 6H), 0.87−0.92 (m, 3H). ESI MS *m*/*z* 562.2 [M + H]$^+$, 584.2 [M + Na]$^+$. HPLC purity: 97.0%.

Synthesis of Ertapenem Bis((methoxycarbonyl)oxy)methyl Ester (11i). Chloromethyl methyl carbonate was reacted with *N*-allyloxy carbamate **15**, which afforded **16i$_{11}$** (R = R_1 = -$CH_2OCOOMe$), followed by deprotection with phenylsilane and purification by reversed phase HPLC to provide the desired dimethoxy carbonate **11i** in 11.8% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.07−8.09 (m, 2H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.44 (dd, J_1 = J_2 = 7.6 Hz, 1H), 6.00 (s, 2H), 5.89 (d, *J* = 5.6 Hz, 1H), 5.84 (d, *J* = 5.6 Hz, 1H), 4.22−4.26 (m, 2H), 3.98−4.02 (m, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.63−3.66 (m, 1H), 3.45−3.49 (m, 1H), 3.33−3.37 (m, 1H), 3.23−3.25 (m, 1H), 2.82−2.94 (m, 1H), 2.74−2.79 (m, 1H), 1.99−2.06 (m, 1H), 1.32 (d, *J* = 6.4 Hz, 3H), 1.24 (d, *J* = 7.2 Hz, 3H). ESI MS *m*/*z* 652.2 [M + H]$^+$. HPLC purity: 98.5%.

Synthesis of Ertapenem C-3-Methyl Acetate Carbonate Sodium Salt (11j). Reaction of iodomethyl acetate with ertapenem **4** followed by formation of sodium salt and purification by reversed phase HPLC gave desired C-3 monoester sodium salt **11j** (R = CH_2OCOMe, R_1 = Na) in 0.44% yield as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.27 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.40 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.72 (d, *J* = 6.0 Hz, 1H), 5.68 (d, *J* = 6.0 Hz, 1H), 5.07 (d, *J* = 4.4 Hz, 1H), 4.17−4.20 (m, 1H), 3.91−3.94 (m, 1H), 3.79−3.83 (m, 1H), 3.60−3.64 (m, 1H), 3.47−3.51 (m, 1H), 3.21−3.23 (m, 2H), 2.55−2.70 (m, 2H), 2.07 (s, 3H),1.63−1.70 (m, 1H), 1.04−1.18 (m, 6H). ESI MS *m*/*z* 548.1 [M + H]$^+$, 570.1 [M + Na]$^+$. HPLC purity: 97.6%.

Synthesis of Ertapenem Bis(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester (12a). After addition of 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (2.29 g, 15.5 mmol) to a solution of compound **15** (3.0 g, 5.1 mmol) in DMF (15 mL) at room temperature, *N*-ethyldiisopropylamine (2.0 g, 15.5 mol) was added to the mixture. The mixture was stirred at 40 °C for 4 h. After cooling to room temperature and addition with EtOAc, the combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo and the residue was purified by reversed phase HPLC to give the desired N-protected dicyclic carbonate ester **16a$_{12}$** (R = R_1 = -CH_2-*c*-(C(OCOO)=C-Me) as a white solid (600 mg, 14.8%). ESI MS *m*/*z* 784 [M + H]$^+$.

To a solution of **16a$_{12}$** (600 mg, 0.77 mmol) in DMF (3.0 mL) was added phenylsilane (166 mg, 1.54 mmol) and tetrakis(triphenylphosphine)palladium (112 mg, 0.097 mmol), and the mixture was stirred at room temperature for 10 min. EtOAc was added, and the organic solution was washed with H_2O and brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) column, eluting with a water and acetonitrile gradient (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to afford the desired compound **12a** (91 mg, 17%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.08

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(s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.40 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.05 (s, 2H), 4.82−4.96 (m, 2H), 4.15−4.22 (m, 2H), 3.90−4.00 (m, 1H), 3.15−3.60 (m, 4H), 2.70−2.92 (m, 2H), 2.18 (s, 3H), 2.12 (s, 3H), 1.95−2.04 (m, 1H), 1.28 (d, J = 6.4 Hz, 3H), 1.18 (d, J = 7.2 Hz, 3H). ESI MS m/z 700.2 $[M + H]^+$. HPLC purity: 95.1%.

Synthesis of Ertapenem C-3-Sodium Carboxyl(4-methyl-5-methyl-1,3-dioxol-2-one)benzoate (12b). Reaction of 1.0 equiv of 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (300 mg, 2.0 mmol) with compound **19** (1.46 g, 2.0 mmol) followed by purification by silica gel column chromatography afforded desired benzoate **20b$_{12}$** (R = CH_2-c-(C(OCOO)=C-Me) (400 mg, 23.7%) as a white solid. ESI MS m/z 807.2 $[M + H]^+$. **20b$_{12}$** was deprotected by hydrogenolysis and purified by reversed HPLC to afford **12b** (11 mg, 1.3%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.42 (dd, J_1 = J_2 = 7.6 Hz, 1H), 4.96 (s, 2H), 4.13−4.16 (m, 1H), 3.88−3.94 (m, 2H), 3.37−3.48 (m, 2H), 3.20−3.22 (m, 1H), 2.73−2.78 (m, 1H), 2.60−2.67 (m, 2H), 2.07 (s, 3H), 1.69−1.74 (m, 1H), 1.09−1.18 (m, 6H). ESI MS m/z 588.1 $[M + H]^+$, 610.2 $[M + Na]^+$. HPLC purity: 95.2%.

Synthesis of Ertapenem Bis(5-isopropyl-2-oxo-1,3-dioxol-4-yl)methyl Ester (12c). Reaction of 4-(bromomethyl)-5-isopropyl-1,3-dioxol-2-one[23] with **15** provided **16c$_{12}$** (R = R_1 = -CH_2-c-(C(OCOO)=C-i-Pr), which was followed by deprotection and purification by reversed phase HPLC to yield the desired dicyclic carbonate ester **12c** in 9.4% yield as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.48 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.20 (s, 2H), 5.00−5.09 (m, 3H), 4.19−4.21 (m, 1H), 3.92−4.04 (m, 2H), 3.71−3.77 (m, 1H), 3.46−3.59 (m, 2H), 3.23−3.25 (m, 1H), 3.08−3.15 (m, 1H), 2.98−3.06 (m, 1H), 2.79−2.85 (m, 1H), 1.76 (brs, 1H), 1.06−1.20 (m, 18H). ESI MS m/z 756.2 $[M + H]^+$, 778.2 $[M + Na]^+$. HPLC purity: 95.4%.

Synthesis of Ertapenem Bis(5-*tert*-butyl-2-oxo-1,3-dioxol-4-yl)methyl Ester (12d). Reaction of 4-(bromomethyl)-5-(*tert*-butyl)-1,3-dioxol-2-one[23] with **15** gave **16d$_{12}$** (R = R_1 = -CH_2-c-(C(OCOO)=C-t-Bu), which was followed by deprotection and purification by reversed phase HPLC to yield the desired dicyclic carbonate ester **12d** in 14.3% yield as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.06 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.42 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.18 (s, 2H), 5.07 (d, J = 14.0 Hz, 1H), 5.02 (d, J = 14.0 Hz, 1H), 4.20−4.22 (m, 2H), 3.98−4.08 (m, 1H), 3.58−3.68 (m, 1H), 3.45−3.52 (m, 1H), 3.28−3.38 (m, 1H), 3.20−3.23 (m, 1H), 2.82−2.94 (m, 1H), 2.75−2.80 (m, 1H), 1.97−2.00 (m, 1H), 1.33 (s, 9H), 1.28 (s, 9H), 1.23−1.36 (m, 6H). ESI MS m/z 784.2 $[M + H]^+$, 806.3 $[M + Na]^+$. HPLC purity: 96.8%.

Synthesis of Ertapenem Diethyl Ester (13a). Iodoethane (805 mg, 5.16 mmol) was added to a solution of compound **15** (1.0 g, 1.66 mmol), *N*-ethyldiisopropylamine (430 mg, 3.32 mmol), and benzyltriethylammonium chloride (378 mg, 1.66 mmol) in DMF (10 mL), and the mixture was stirred at 45 °C for 18 h. After addition of EtOAc (300 mL), the combined organic layer was washed with H_2O (100 mL × 3) and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography, eluting with DCM/MeOH (40:1). Fractions containing the product were combined and concentrated under vacuo to give the desired diethyl ester **16a$_{13}$** (R = R_1 = Et) as a pale yellow solid (820 mg, 80.4%). ESI MS m/z 616 $[M + H]^+$.

To a solution of **16a$_{13}$** (820 mg, 1.33 mmol) in DMF (4.0 mL) were added phenylsilane (288 mg, 2.66 mmol) and tetrakis(triphenylphosphine)palladium (80 mg, 0.069 mmol), and the mixture was stirred at room temperature for 10 min. EtOAc (100 mL) was added, and the organic solution was washed with H_2O (40 mL × 3) and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) column, eluting with a water and acetonitrile gradient (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to afford the desired diethyl ester **13a** (80 mg, 11.3%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.07 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.41 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 4.20−4.40 (m, 6H), 3.95−4.06 (m, 1H), 2.74−3.64 (m, 6H), 1.98−2.07 (m, 1H), 1.23−1.42 (m, 12H). ESI MS m/z 532.2 $[M + H]^+$. HPLC purity: 96.8%.

Synthesis of Ertapenem C-3-Ethyl Ester (13b) and Ethyl Benzoate (13c). *N*-Ethyldiisopropylamine (433 mg, 3.44 mol) was slowly added to a solution of compound **15** (5.8 g, 10.0 mmol) and iodoethane (1.4 g, 10.0 mmol) in DMF (30 mL), and the mixture was stirred at 45 °C for 4 h. The mixture was directly purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Synergi C_{18} (250 mm × 50 mm, 10 μm) using water (0.1% formic acid) and acetonitrile as eluents (mobile phase A, water (0.1% formic acid); mobile phase B, acetonitrile; gradient, B from 25% to 50% in 25 min; flow rate, 80 mL/min; detective wavelength, 220 nm) followed by freeze-drying to give **16b$_{13}$** (R = Et, R_1 = H) (460 mg, 7.8%) and **16c$_{13}$** (R = H, R_1 = Et) (420 mg, 6.9%) as a white solid. **16b$_{13}$**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.22−8.25 (m, 1H), 7.84−7.86 (m, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.43 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.72−5.92 (m, 1H), 4.94−5.36 (m, 3H), 3.71−4.61 (m, 11H), 3.23−3.33 (m, 2H), 2.71−2.87 (m, 1H), 1.71−1.88 (m, 1H), 1.14−1.33 (m, 9H). ESI MS m/z 588 $[M + H]^+$. **16c$_{13}$**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.23−8.27 (m, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.46 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.72−5.94 (m, 1H), 5.00−5.36 (m, 3H), 4.28−4.52 (m, 5H), 3.77−4.20 (m, 4H), 3.45−3.52 (m, 2H), 3.23−3.33 (m, 2H), 2.67−2.70 (m, 1H), 1.76−1.88 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.05−1.22 (m, 6H). ESI MS m/z 588 $[M + H]^+$.

N-Allyloxycarbonyl **16b$_{13}$** (200 mg, 0.34 mmol) was hydrogenolyzed as described for **10c**, affording **13b** (10 mg, 5.9%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.30 (s, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.43 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.09 (d, J = 3.6 Hz, 1H), 4.08−4.20 (m, 3H), 3.90−3.97 (m, 1H), 3.82−3.88 (m, 1H), 3.61−3.66 (m, 1H), 3.22−3.24 (m, 2H), 2.60−2.75 (m, 3H), 1.69−1.81 (m, 1H), 1.12−1.39 (m, 9H). ESI MS m/z 504.1 $[M + H]^+$. HPLC purity: 91.3%.

Similar deprotection of *N*-allyloxycarbonyl **16$_{c13}$** (200 mg, 0.34 mmol) by hydrogenolysis afforded **13c** (25 mg, 14.7%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1 H), 8.32 (s, 1 H), 7.87 (d, J = 8.0 Hz, 1 H), 7.61−7.68 (d, J = 7.6 Hz, 1H), 7.48 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1 H), 5.12 (brs, 1H), 4.31 (q, J = 7.2 Hz, 2H), 4.02−4.18 (m, 2H), 3.88−3.97 (m, 2H), 3.74−3.76 (m, 1H), 3.21−3.23 (m, 2H), 2.87−2.91 (m, 1H), 2.70−2.76 (m, 1H), 1.74−1.81 (m, 1H), 1.31 (t, J = 7.2 Hz, 3H), 1.14−1.18 (m, 6H). ESI MS m/z 504.2 $[M + H]^+$. HPLC purity: 90.1%.

Synthesis of Ertapenem Dimethyl Ester (13d). Reaction of the carboxylic acid **15** (1.9 g, 18 mmol) and iodomethane (5.1 g, 36 mmol) gave dimethyl ester **16d$_{13}$** (R = R_1 = Me) (1.4 g, 27.7%) as a colorless oil. ESI MS m/z 588 $[M + H]^+$. The *N*-allyoxycarbonyl carbamate **16d$_{13}$** was deprotected by phenylsilane followed by reversed phase HPLC purification to afford the desired dimethyl ester **13d** (400.0 mg, 33.3%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.09 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.41 (dd, J_1 = J_2 = 7.6 Hz, 1H), 4.20−4.27 (m, 2H), 3.99−4.03 (m, 1H), 3.90 (s, 3H), 3.77 (s, 3H), 3.60−3.65 (m, 1H), 3.45−3.50 (m, 1H), 3.23−3.36 (m, 2H), 2.90−2.95 (m, 1H), 2.73−2.82 (m, 1H), 2.00−2.07 (m, 1H), 1.34 (d, J = 6.4 Hz, 3H), 1.24 (d, J = 7.6 Hz, 3H). ESI MS m/z 504.2 $[M + H]^+$. HPLC purity: 99.3%.

Synthesis of Ertapenem Di-*n*-propyl Ester (13e). Reaction of 1-iodopropanewith the *N*-allyloxy carbonate **15** produced the di-*n*-propyl ester **16e$_{13}$** (R = R_1 = n-C_3H_7), which was deprotected accordingly by phenylsilane and purified by reversed phase HPLC to give 4.5% yield of di-*n*-propyl ester **13e** as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.07 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.42 (dd, J_1 = J_2 = 7.6 Hz, 1H), 4.10−4.30 (m, 6H), 3.99−4.03 (m, 1H), 3.60−3.63 (m, 1H), 3.46−3.50 (m, 1H), 3.23−3.33 (m, 2H), 2.90−2.95 (m, 1H), 2.75−2.80 (m, 1H),

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1.99−2.05 (m, 1H), 1.50−1.90 (m, 4H), 1.36 (d, J = 6.0 Hz, 3H), 1.26 (d, J = 6.8 Hz, 3H), 1.03 (t, J = 7.6 Hz, 3H), 0.94 (t, J = 7.6 Hz, 3H). ESI MS m/z 560.2 $[M + H]^+$. HPLC purity: 99.0%.

Synthesis of Ertapenem Diisopropyl Ester (13f). Isopropyl iodide was reacted with *N*-allyloxycarbonyl ertapenem **15** to give diisoproyl ester **16f**$_{13}$ (R = R_1 = *i*-$CH(CH_3)_2$) which was deprotected with phenylsilane and purified by reversed phase HPLC to furnish 16.1% overall yield of **13f** as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.02 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.38 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.20−5.26 (m, 1H), 5.06−5.12 (m, 1H), 4.18−4.25 (m, 2H), 3.97−4.01 (m, 1H), 3.54−3.62 (m, 1H), 3.45−3.49 (m, 1H), 3.20−3.32 (m, 2H), 2.87−2.92 (m, 1H), 2.73−2.80 (m, 1H), 1.97−2.04 (m, 1H), 1.23−1.36 (m, 18H). ESI MS m/z 560.2 $[M + H]^+$. HPLC purity: 97.6%.

Synthesis of Ertapenem C-3-Isopropyl Ester (13g) and Isopropyl Benzoate (13h). Reaction of 2-iodopropane with compound **15** followed by separation afforded **16g**$_{13}$ (R = *i*-Pr, R_1 = H) and **16h**$_{13}$ (R = H, R_1 = *i*-Pr) as described for the synthesis of **13b** and **13c**. Hydrogenolysis of **16g**$_{13}$ followed by reversed HPLC provided 0.23% yield of **13g** as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1 H), 8.28 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 7.6 Hz, 1H), 7.47 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.09−5.17 (m, 2H), 4.15−4.18 (m, 1H), 3.80−4.03 (m, 2H), 3.66−3.77 (m, 2H), 3.21−3.22 (m, 2H), 2.81−2.89 (m, 1H), 2.67−2.72 (m, 1H), 1.71−1.78 (m, 1H), 1.16−1.36 (m, 12H). 3.20−4.18 (m, 8H), 2.67−2.89 (m, 2H), 1.71−1.78 (m, 1H), 1.28−1.36 (m, 6H), 1.16−1.18 (m, 6H). ESI MS m/z 518.2 $[M + H]^+$. HPLC purity: 84.5%.

Hydrogenolysis of **16h**$_{13}$ followed by reversed HPLC furnished **13h** (0.54%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.30 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.43 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.09 (brs, 1H), 4.88−4.97 (m, 1H), 4.18−4.20 (m, 1H), 3.89−3.98 (m, 2H), 3.63−3.68 (m, 2H), 3.22−3.24 (m, 2H), 2.74−2.79 (m, 1H), 2.59−2.67 (m, 1H), 1.72−1.78 (m, 1H), 1.10−1.28 (m, 12H). ESI MS m/z 518.2 $[M + H]^+$. HPLC purity: 81.6%.

Synthesis of Ertapenem Di-*n*-butyl Ester (13i). Reaction of 1-iodobutane with **15** furnished **16i**$_{13}$ (R = R_1 = *n*-Bu), which was followed by deprotection and purification by reversed phase HPLC to yield the desired di-*n*-butyl ester **13i** in 1.7% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H), 8.00 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.35 (dd, J_1 = J_2 = 7.6 Hz, 1H), 4.08−4.27 (m, 6H), 3.92−4.00 (m, 1H), 3.52−3.58(m, 1H), 3.38−3.45 (m, 1H), 3.17−3.26 (m, 2H), 2.84−2.88 (m, 1H), 2.68−2.75 (m, 1H), 1.92−1.98 (m, 1H), 1.25−1.78 (m, 9H), 1.29 (d, J = 6.0 Hz, 3H), 1.19 (d, J = 7.2 Hz, 3H), 0.91 (t, J = 7.6 Hz, 3H), 0.82 (t, J = 7.6 Hz, 3H). ESI MS m/z 588.3 $[M + H]^+$. HPLC purity: 96.6%.

Synthesis of Ertapenem Dioctyl Ester (13j). Reaction of 1-iodooctane with **15** gave **16j**$_{13}$ (R = R_1 = *n*-C_8H_{17}), which was followed by deprotection and purification by reversed phase HPLC to give 17.1% yield of diester **13j** as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.30 (s, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.41 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.05 (d, J = 5.2 Hz, 1H), 4.10−4.26 (m, 3H), 3.90−4.08 (m, 3H), 3.71−3.82 (m, 1H), 3.51−3.58 (m, 1H), 3.35−3.48 (m, 1H), 3.12−3.19 (m, 1H), 2.45−2.70 (m, 2H), 1.62−1.67 (m, 3H), 1.10−1.52 (m, methylene), 0.65−0.75 (m, 6H). ESI MS m/z 700.4 $[M + H]^+$. HPLC purity: 99.7%.

Synthesis of Ertapenem Dihexadecyl Ester (13k). Reaction of **15** with hexadecyl iodide gave **16k**$_{13}$ (R = R_1 = *n*-$C_{16}H_{33}$), which was followed by deprotection with phenylsilane and reversed phase HPLC purification to produce 1.7% yield of diester **13k** as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.34 (s, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.63 (d, J = 7.2 Hz, 1H), 7.43 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.07 (d, J = 4.8 Hz, 1H), 4.16−4.36 (m, 3H), 3.95−4.07 (m, 3H), 3.82−3.86 (m, 1H), 3.58−3.68 (m, 2H), 3.11−3.15 (m, 1H), 2.67−2.72 (m, 2H), 1.67−1.74 (m, 3H), 1.14−1.52 (m, methylenes), 0.80−0.90 (m, 6H). ESI MS m/z 925.5 $[M + H]^+$. HPLC purity: 98.7%.

Synthesis of Ertapenem Ditrifluoroethyl Ester (13l). Reaction of **15** with 1,1,1-trifluoro-2-iodoethane gave **16l**$_{13}$ (R = R_1 = CH_2CF_3), which was treated with phenylsilane and purified by reversed phase HPLC to afford **13l** in 8.5% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.40 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.51 (dd, J_1 = J_2 = 8.0 Hz, 1H),), 5.11 (d, J = 5.2 Hz, 1H), 4.97−5.04 (m, 2H), 4.77−4.83 (m, 2H), 4.24 (d, J = 9.6 Hz, 1H), 3.82−4.02 (m, 2H), 3.87 (brs, 1H), 3.26−3.72 (m, 3H), 2.55−2.72 (m, 2H), 1.70−1.74 (m, 1H), 1.14−1.19 (m, 6H). ESI MS m/z 640.1 $[M + H]^+$. HPLC purity: 99.1%.

Synthesis of Ertapenem C-3-Trifluoroethyl Ester (13m) and Trifluoroethyl Benzoate Ester (13n). Reaction of 2,2,2-trifluoroethyl trifluoromethanesulfonate with compound **15** gave **16m**$_{13}$ (R = CH_2CF_3, R_1 = H) and **16n**$_{13}$ (R = H, R_1 = CH_2CF_3), which were separated as described for the synthesis of **13b** and **13c**. Deprotection of **16m**$_{13}$ by hydrogenolysis followed by purification by reversed HPLC provided 2.8% yield of **13m** as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 8.40 (s, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.56 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.10 (brs, 1H), 4.98−5.05 (m, 2H), 4.32−4.44 (m, 1H), 4.19−4.21 (m, 1H), 3.65−3.96 (m, 4H), 3.22−3.24 (m, 1H), 3.04−3.08 (m, 1H), 2.82−2.92 (m, 1H), 1.85−1.90 (m, 1H), 1.10−1.20 (m, 6H). ESI MS m/z 558.1 $[M + H]^+$. HPLC purity: 76.5%.

Deprotection of **16n**$_{13}$ by hydrogenolysis followed by purification by reversed HPLC provided 0.41% yield of **13n** as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.29 (s, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.44 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 1H), 5.11 (d, J = 4.8 Hz, 1H), 4.77−4.85 (m, 2H), 4.24−4.27 (m, 1H), 3.88−3.99 (m, 2H), 3.68−3.74 (m, 2H), 3.50−3.56 (m, 2H), 2.75−2.80 (m, 1H), 2.62−2.70 (m, 1H), 1.20−1.28 (m, 1H), 1.16−1.22 (m, 6H). ESI MS m/z 558.2 $[M + H]^+$. HPLC purity: 82.7%.

Synthesis of C-3-Trifluoroethyl-cholesteryl-acetyl-ertapenem-benzoate (13o). To a solution of compound **15** (11.6 g, 20.0 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (4.6 g, 20.0 mmol) in DMF (30 mL) was slowly added *N*-ethyldiisopropylamine (5.0 g, 30.0 mmol), and the mixture was heated at 45 °C for 3 h. The mixture was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Synergi C_{18} (250 mm × 50 mm, 10 μm) using water (0.1% formic acid) and acetonitrile as eluents (mobile phase A, water (0.1% formic acid); mobile phase B, acetonitrile; gradient, B from 25% to 50% in 25 min; flow rate, 80 mL/min; detective wavelength, 220 nm) followed by lyophilization to afford the desired C-3-trifluoroethyl **16m**$_{13}$ (2.0 g, 15.6%) as a yellow solid.

To a solution of cholesteryl 2-chloroacetate (10.5 g, 22.68 mmol) in acetone (300 mL) was added NaI (6.758 g, 45.36 mmol), and the mixture was refluxed for 18 h. After filtration, the filtrate was concentrated under reduced pressure to afforded the desired 2-iodoacetate which was used directly in the next step without further purification (10 g, 80% yield).

The mixture of **16m**$_{13}$ (2.0 g, 3.12 mmol), cholesteryl 2-iodoacetate (1.72 g, 3.12 mmol), Na_2CO_3 (0.66 g, 6.24 mmol), and benzyltriethylammonium chloride (0.70 g, 3.12 mmol) in DMSO (40 mL) was stirred at 50 °C for 18 h. The reaction was quenched with water (150 mL), extracted with DCM (100 mL × 3), dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluting with DCM/MeOH (40:1) and lyophilized to afford 1.0 g of cholesteryl-acetyl-ertapenem-benzoate **16o**$_{13}$ (R = CH_2CF_3, R_1 = CH_2CO-cholesteryl) as a yellow solid. *N*-Allyoxycarbonyl cholesteryl-acetyl-ertapenem-benzoate **16o**$_{13}$ (1.0 g, 0.94 mmol) was deprotected with phenylsilane and purified by reversed phase HPLC to provide **13o** (300 mg, 8.9% in 2 steps) as a pale brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.14 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.45 (dd, J_1 = J_2 = 7.6 Hz, 1H), 5.30−5.35 (m, 1H), 4.60−4.80 (m, 4H), 4.23−4.26 (m, 2H), 3.98−4.04 (m, 1H), 3.63−3.70 (m, 1H), 3.45−3.52 (m, 1H), 3.32−3.40 (m, 1H), 3.24−3.28 (m, 1H), 2.92−2.95 (m, 1H), 2.74−2.82 (m, 1H), 2.28−2.32 (m, 2H), 1.76−2.06 (m, 8H), 0.84−1.70 (m, 36H), 0.98 (s, 3H), 0.65 (s, 3H). ESI MS m/z 985.3 $[M + H]^+$. HPLC purity: 92.7%.

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Synthesis of 8-Acetyl-ertapenem Diethyl Ester (13p). Acetyl chloride (0.32 g, 4.8 mmol) was added to a solution of compound **16a**$_{13}$ (1.0 g, 1.6 mmol) and DMAP (20 mg) in pyridine (25 mL), and the mixture was stirred at room temperature for 4 h. After addition of water, the mixture was extracted with EtOAc, washed with H_2O, dried (Na_2SO_4), and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluting with DCM/MeOH (40:1), and then it was concentrated under vacuo to give the desired product 8-acylated compound **22a** (460 mg, 43.8%) as a pale yellow solid. ESI MS m/z 658 $[M + H]^+$. The *N*-allyloxycarbonyl group of compound **22a** was removed by upon deprotection with phenylsilane followed by reversed phase HPLC purification and lyophilization to afford the 8-acetyl diethyl ester **13p** (199 mg, 37.8%) as a colorless amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1 H), 8.31 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.43 (dd, $J_1 = J_2$ = 8.0 Hz, 1H), 5.08−5.15 (m, 1H), 4.29 (q, J = 7.2 Hz, 2H), 4.18−4.21 (m, 1H), 4.04−4.11 (m, 2H), 3.80−3.84 (m, 1H), 3.55−3.62 (m, 2H), 3.46−3.51 (m, 1H), 3.28−3.38 (m, 1H), 2.50−2.69 (m, 2H), 2.00 (s, 3H), 1.61−1.71 (m, 1H), 1.30 (t, J = 7.2 Hz, 3H), 1.24 (d, J = 6.4 Hz, 3H), 1.11−1.18 (m, 6H). ESI MS m/z 574.2 $[M + H]^+$. HPLC purity: 98.3%.

Synthesis of 8-Propyonyl-ertapenem Diethyl Ester (13q). To a solution of **16a**$_{13}$ (1.5 g, 2.5 mmol) in DCM (25 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 3.8 g, 19.8 mmol), DMAP (300 mg, 2.46 mmol), and then propionic acid (740 mg, 10 mmol). The mixture was stirred at −20 °C for 1 h. The reaction was quenched with water (80 mL), and it was extracted with DCM (30 mL × 3), washed with brine, dried over Na_2SO_4, and filtered. After concentration of filtrate by vacuo, it was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water (0.2% $HCOONH_4$) and acetonitrile as eluents (mobile phase A, water (0.2% $HCOONH_4$); mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by freeze-drying to give the desired propionate **22b** (1.5 g. 94%) as a yellow solid. ESI MS m/z 672 $[M + H]^+$.

N-Allyloxycarbonyl group of compound **22b** was removed by upon deprotection with phenylsilane, followed by reversed phase HPLC purification and lyophilization to afford the 8-propyonyl ertapenem diethyl ester **13q** (1.0 g, 77%) as a colorless amorphous compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.06 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.41 (dd, $J_1 = J_2$ = 8.0 Hz,1H), 5.25−5.29 (m, 1H), 4.38 (q, J = 7.2 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 4.16−4.19 (m, 1H), 3.98−4.02 (m, 1H), 3.58−3.64 (m, 1H), 3.45−3.50 (m, 1H), 3.36−3.38 (m, 1H), 3.24−3.30 (m, 1H), 2.89−2.94 (m, 1H), 2.72−2.82 (m, 1H), 2.34 (q, J = 7.6 Hz, 2H), 2.01−2.05 (m, 1H), 1.38−1.42 (m, 6H), 1.24−1.30 (m, 6H), 1.15 (t, J = 7.6 Hz, 3H). ESI MS m/z 588.2 $[M + H]^+$, 610.2 $[M + Na]^+$. HPLC purity: 98.3%.

Synthesis of 8-(*S*)-Valinyl-ertapenem Diethyl Ester (13r). Condensation of (*S*)-2-(((allyloxy)carbonyl)amino)-3-methylbutanoic acid (1.4 g, 6.4 mmol) with compound **16a**$_{13}$ (1.0 g, 1.6 mmol) by using the above procedure followed by silica gel column purification, eluting with DCM/MeOH (40:1), and concentration by vacuo afforded the desired 8-(*S*)-*N*-allyloxycarbonyl-valinyl-ertapenem **22c** (1.0 g, 72%) as a yellow solid. ESI MS m/z799 $[M + H]^+$. The two allyloxycarbonyl groups in compound **22c** (1.0 g, 1.2 mmol) were removed upon deprotection with phenylsilane followed by reversed phase HPLC purification and lyophilization to afford the 8-(*S*)-valinyl-ertapenem diethyl ester **13r** (100 mg, 13%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.05 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.39 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.28−5.35 (m, 1H), 4.36 (q, J = 7.2 Hz, 2H), 4.24 (q, J = 7.2 Hz, 2H), 4.13−4.16 (m, 1H), 3.96−4.04 (m, 1H), 3.52−3.64 (m, 1H), 3.46−3.50 (m, 1H), 3.26−3.36 (m, 3H), 2.89−2.93 (m, 1H), 2.72−2.82 (m, 1H), 1.98−2.05 (m, 2H), 1.36−1.41 (m, 6H), 1.22−1.28 (m, 6H), 0.98 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H). ESI MS m/z 631.3 $[M + H]^+$, 653.3 $[M + Na]^+$. HPLC purity: 92.7%.

Synthesis of Ertapenem *N*-(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Diethyl Ester (13s). To a solution of ertapenem diethyl ester **13a** (1.33 g, 2.5 mmol) in DMF (10 mL) were added DIPEA (645 mg, 5.0 mmol) and 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (555 mg, 3.75 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water (0.2% $HCOONH_4$) and acetonitrile as eluents (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to afford the desired compound **13s** (336 mg, 20.8%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.24 (s, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.43 (dd, $J_1 = J_2$ = 8.0 Hz,1H), 4.38 (q, J = 7.2 Hz, 2H), 4.24−4.32 (m, 4H), 3.84 (brs, 1H), 3.68 (d, J = 15.2 Hz, 1H), 3.57 (d, J = 15.2 Hz, 1H), 3.36−3.40 (m, 1H), 3.20−3.28 (m, 3H), 3.05−3.09 (m, 1H), 2.82−2.90 (m, 1H), 2.10 (s, 3H), 2.01−2.07 (m, 1H), 1.51−1.53 (m, 1H), 1.35−1.44 (m, 6H), 1.23−1.30 (m, 6H). ESI MS m/z 644.3 $[M + H]^+$, 666.2 $[M + Na]^+$. HPLC purity: 97. 1%.

Synthesis of Ertapenem (5-Methyl-2-oxo-1,3-dioxol-4-yl)-methyl *N*-carbamate Diethyl Ester (13t). Reaction of 1.0 equiv (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate (prepared as described by Alexander et al.[24]) (885 mg, 3.0 mmol) with ertapenem diethyl ester **13a** (1.6 g, 3.0 mmol) followed by reversed phase HPLC purification and lyophilization afforded the desired compound **13t** (605 mg, 29.2%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (brs, 1H), 8.01 (brs, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.41 (dd, $J_1 = J_2$ = 7.6 Hz,1H), 4.80−5.04 (m, 2H), 4.45−4.58 (m, 1H), 4.37 (q, J = 7.2 Hz, 2H), 4.20−4.30 (m, 4H), 3.92−4.02 (m, 1H), 3.75−3.85 (m, 1H), 3.38−3.64 (m, 1H), 3.25−3.31 (m, 2H), 2.57−2.78 (m, 2H), 2.02−2.22 (m, 4H), 1.30−1.41 (m, 6H), 1.23−1.29 (m, 6H). ESI MS m/z 688.2 $[M + H]^+$, 710.2 $[M + Na]^+$. HPLC purity: 96.9%.

Synthesis of Ertapenem Macrocyclic Propyl-bis-lactone (14a). Reaction of 1,3-dibromopropane with *N*-allylcarbamate **15** gave protected cyclic product **17a** (R = $CH_2CH_2CH_2$), which was followed by deprotection with phenylsilane followed by reversed phase HPLC purification to afford the macrocyclic bis-lactone **14a** in 3.7% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.61−7.67 (m, 2H), 7.46 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.06 (d, J = 4.7 Hz, 1H), 4.56−4.65 (m, 1H), 4.38−4.41 (m, 1H), 4.23−4.26 (m, 1H), 3.80−4.03 (m, 5H), 3.38−3.45 (m, 2H), 3.18−3.24 (m, 2H), 2.54−2.65 (m, 2H), 2.24−2.30 (m, 1H), 1.82−1.91 (m, 2H), 1.11−1.14 (m, 6H). ESI MS m/z 516.2 $[M + H]^+$. HPLC purity: 97.0%.

Synthesis of Ertapenem Macrocylic butyl-bis-lactone (14b). 1,4-Dibromobutane was reacted with intermediate **15** and afforded **17b** (R = $CH_2CH_2CH_2CH_2$), which was followed by deprotection with phenylsilane and purification by reversed phase HPLC purification to furnish the desired macrocyclic bis-lactone **14b** in 0.92% yield as an amorphous powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.77 (s, 1H), 7.62 (d, J = 7.2 Hz, 1H), 7.46 (dd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 1H), 5.05 (d, J = 4.0 Hz, 1H), 4.34−4.43 (m, 2H), 4.17−4.22 (m, 2H), 3.88−3.96 (m, 2H), 3.78−3.80 (m, 2H), 3.38−3.45 (m, 2H), 3.20−3.21 (m, 1H), 3.05−3.08 (m, 1H), 2.50−2.66 (m, 2H), 1.63−1.90 (m, 5H), 1.08−1.15(m, 6H). ESI MS m/z 530.2 $[M + H]^+$. HPLC purity: 98.1%.

Synthesis of Ertapenem Macrocylic (*Z*)-But-2-ene-bis-lactone (14c). Reaction of (*Z*)-1,4-dichlorobut-2-ene with **15** furnished **17f** (R = $CH_2CH{=}CH{-}CH_2$), which was followed by deprotection with phenylsilane and purification by reversed phase HPLC to give 0.96% yield of desired bis-lactone **14c** as white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.17 (brs, 1H), 8.08 (brs, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.51 (dd, $J_1 = J_2$ = 7.6 Hz, 1H), 5.78−5.93 (m, 2H), 5.33−5.38 (m, 1H), 5.09−5.10 (m, 1H), 4.88−4.94 (m, 1H), 4.74−4.78 (m, 1H), 4.45−4.49 (m, 1H), 4.21−4.24 (m,1H), 3.89−3.97 (m, 3H), 3.46−3.49 (m, 2H), 3.14−3.24 (m, 3H), 2.67−2.74 (m, 1 H), 1.86−1.92 (m, 1H), 1.10−1.20 (m, 6H). ESI MS m/z 528.2 $[M + H]^+$. HPLC purity: 88.8%.

Synthesis of Ertapenem Macrocylic (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl-bis-lactone (14d). To a solution of 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (1.48 g, 10 mmol) in

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CCl_4 (60 mL) were added AIBN (164 mg, 1.0 mmol) and NBS (1.78 g, 10 mmol). Then the mixture was stirred at reflux for 12 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column, eluting with PE/EtOAc (50:1) to give the desired product 4-(bromomethyl)-5-(chloromethyl)-1,3-dioxol-2-one (1.13 g, 50.2% yield) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.38 (s, 2H), 4.21 (s, 2H).

Reaction of 4-(bromomethyl)-5-(chloromethyl)-1,3-dioxol-2-one with the N-protected intermediate **15** gave the desired bis-lactone **17c** (R = CH_2-*c*-(C(OCOO)=C-)CH_2) which was hydrogenolyzed over 5% Pd/C with 1 atm of hydrogen in THF−water at room temperature for 1 h. The mixture was filtered and purified by reversed phase HPLC to afford **14d** in 2.7% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.53 (dd, J_1 = J_2 = 8.0 Hz, 1H), 5.61 (d, J = 14.0 Hz, 1H), 5.30−5.38 (m, 2H), 5.10 (d, J = 5.2 Hz, 1H), 4.85 (d, J = 14.0 Hz, 1H), 4.21 (d, J = 5.2 Hz, 1H), 4.05 (s, 1H), 3.88−4.01 (m, 2H), 3.76−3.78 (m, 1H), 3.49−3.53 (m, 1H), 3.21−3.23 (m, 1H), 3.10−3.13 (m, 1H), 2.67−2.75 (m, 2H), 1.77−1.81 (m, 1H), 1.14−1.18 (m, 6H). ESI MS m/z 586.1 $[M + H]^+$. HPLC purity: 98.2%.

Synthesis of Ertapenem Macrocyclic (*Z*)-1,4-But-2-ene (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl *N*-Carbamate (14e). A mixture of ertapenem sodium carboxylate **4** (4.75g, 10 mmol), (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (4-nitrophenyl) carbonate[24] (2.95g, 10 mmol), and $NaHCO_3$ (840 mg, 10 mmol) in acetone (25 mL)−H_2O (25 mL) was stirred at room temperature for 1 h, and it was heated at 50 °C for 2 h. The reaction solvent was evaporated and the mixture was directly purified by reversed phase HPLC to give ertapenem (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl *N*-carbamate **24** (500 mg, 7.4%) as a white solid. To a solution of compound **24** (200 mg, 0.32 mmol) in DMF (10 mL) was slowly added Na_2CO_3 (74 mg, 0.70 mmol), and it was stirred at room temperature for 20 min. After addition of (*Z*)-1,4-dichlorobut-2-ene (37 mg, 0.30 mmol), it was stirred at 50−55 °C for 18 h, and then it was filtered. The filtrate was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water (0.2% $HCOONH_4$) and acetonitrile as eluents (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to afford the desired macrocycle **14e** (20 mg, 10%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98−10.02 (m, 1H), 8.00−8.04 (m, 1H), 7.78−7.82 (m, 1H), 7.64−7.68 (m, 1H), 7.40−7.49 (m, 1H), 5.75−5.90 (m, 2H), 5.32−5.42 (m, 1H), 5.06−5.07 (m, 1H), 4.72−5.01 (m, 4H), 4.33−4.44 (m, 2H), 4.21−4.23 (m, 1H), 3.91−3.98 (m, 3H), 3.56−3.59 (m, 1H), 3.36−3.42 (m, 1H), 3.21−3.23 (m, 1H), 2.78−2.88 (m, 1H), 2.00−2.12 (m, 4H), 1.10−1.14 (m, 6H). ESI MS m/z 684.2 $[M + H]^+$, 706.2 $[M + Na]^+$. HPLC purity: 92.2%. Tautomer is observed.

Synthesis of Ertapenem Macrocyclic (*E*)-1,4-But-2-ene-bis-lactone (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl *N*-Carbamate (14f). Reaction of 1.0 equiv (*E*)-1,4-dichlorobut-2-ene (37 mg, 0.30 mmol) with ertapenem (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl *N*-carbamate **24** followed by reversed phase HPLC purification and lyophilization afforded the desired compound **14f** (20 mg, 10%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.47 (s, 1H), 7.61−7.70 (m, 2H), 7.45−7.49 (m, 1H), 6.00−6.02 (m, 2H), 5.06−5.15 (m, 2H), 4.68−5.02 (m, 3H), 4.53−4.60 (m, 2H), 4.45−4.48 (m, 1H), 4.18−4.21 (m, 1H), 3.92−4.02 (m, 3H), 3.60−3.66 (m, 1H), 3.37−3.44 (m, 1H), 3.19−3.21 (m, 1H), 2.82−2.95 (m, 1H), 1.98−2.12 (m, 4H), 1.06−1.13 (m, 6H). ESI MS m/z 684.1 $[M + H]^+$, 706.1 $[M + Na]^+$. HPLC purity: 93.2%.

Synthesis Ertapenem Macrocyclic (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl-bis-lactone (5-Methyl-2-oxo-1,3-dioxol-4-yl)-methyl *N*-Carbamate (14g). Reaction of 4-(bromomethyl)-5-(chloromethyl)-1,3-dioxol-2-one (110 mg, 0.48 mmol) with ertapenem (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl *N*-carbamate **24** (300 mg, 0.47 mmol) followed by reversed phase HPLC purification and lyophilization afforded the desired compound **14g** (13 mg, 3.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06−10.10 (m, 1H), 8.04−8.07 (m, 1H), 7.68−7.72 (m, 2H), 7.47−7.53 (m, 1H), 5.56−5.61 (m, 1H), 5.29−5.42 (m, 2H), 4.81−5.11 (m, 4H), 4.45−4.48 (m, 1H), 4.24−4.26 (m, 1H), 3.97−4.09 (m, 3H), 3.64−3.68 (m, 1H), 3.38−3.47 (m, 1H), 3.22−3.24 (m, 1H), 2.84−2.98 (m, 1H), 2.04−2.15 (m, 4H), 1.02−1.18 (m, 6H). ESI MS m/z 742.2 $[M + H]^+$. HPLC purity: 96.0%.

Synthesis of Ertapenem Macrocyclic Methyleneoxymethylene-bis-lactone (14h). To a solution of *N*-allyloxy carbamate **15** (8.0 g, 15 mmol) in DMF (100 mL) were added bromo(bromomethoxy)methane[25] (3.0 g, 15 mmol) and DIEA (4.7 g, 38 mmol) at room temperature, and it was stirred at 50 °C for 4 h. After the solution was cooled to 0 °C, the reaction was quenched by addition of saturated aqueous NaCl (1500 mL), extracted with EtOAc (300 mL × 5), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) using water (0.2% $HCOONH_4$) and acetonitrile as eluents (mobile phase A, water (0.2% $HCOONH_4$); mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by freeze-drying to give N-protected macrocyclic bis-lactone $\mathbf{17h_{14}}$ (R = -CH_2OCH_2-) (1.0 g, 11%) as a white solid. ESI MS m/z 602 $[M + H]^+$.

To a solution of *N*-allyloxycarbonyl bis-lactone $\mathbf{17h_{14}}$ (200 mg, 0.33 mmol) in DMF (2.0 mL) were added phenylsilane (72 mg, 0.66 mmol) and tetrakis(triphenylphosphine)palladium (140 mg, 0.12 mmol), and the mixture was stirred at room temperature for 3 h. The reaction was quenched with saturated brine, extracted with EtOAc, and the combined organic extracts were dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a GILSON 281 instrument fitted with a Phenomenex Gemini C_{18} (250 mm × 21.2 mm, 5 μm) column, eluting with a water and acetonitrile gradient (mobile phase A, water; mobile phase B, acetonitrile; gradient, 52−72% B, 0−8 min; 100% B, 8.5−10.5 min; 5% B, 11−12 min) followed by lyophilization to give the desired compound **14h** (38 mg, 24.5%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (brs, 1H), 7.96−8.00 (m, 1H), 7.81−7.84 (m, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.36−7.41 (m, 1H), 5.73 (d, J = 6.8 Hz, 1H), 5.68 (d, J = 4.8 Hz, 1H), 5.35 (d, J = 6.8 Hz, 1H), 5.24 (d, J = 4.8 Hz, 1H), 4.19−4.22 (m, 1H), 4.01−4.11 (m, 2H), 3.79−3.81 (m, 1H), 3.54−3.56 (m, 1H), 3.29−3.34 (m, 2H), 3.13−3.15 (m, 1H), 2.50−2.75 (m, 1H), 2.13−2.17 (m, 1H), 1.26 (d, J = 6.0 Hz, 3H), 1.19 (d, J = 7.2 Hz, 3H). ESI MS m/z 518.1 $[M + H]^+$. HPLC purity: 96.1%.

Synthesis of 8-Acetyl Macrocyclic Methyleneoxymethylene Bis-lactone (14i). Acetic acid (0.40 g, 6.66 mmol) was condensated with *N*-allyoxycarbonyl macrocyclic methyleneoxymethylene bis-lactone $\mathbf{17h_{14}}$ (1.0 g, 1.67 mmol) by EDCI and DMAP method followed by silica gel column purification, eluting with DCM/MeOH (40:1), and concentration in vacuo to give the desired 8-acetyl ertapenem **23** (0.83 g, 78.1%) as a pale yellow solid.

N-Allyloxycarbonyl group of compound **23** was removed by phenylsilane followed by reversed phase HPLC purification and lyophilization to afford the 8-acetyl macrocyclic methyleneoxymethylene bis-lactone **14i** (172 mg, 23.8%) as a white powder.^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.42 (dd, J_1 = J_2= 8.0 Hz, 1H), 5.78 (d, J = 6.4 Hz, 1H), 5.73 (d, J = 5.2 Hz, 1H), 5.40 (d, J = 6.4 Hz, 1H), 5.32 (d, J = 5.2 Hz, 1H), 5.20−5.28 (m, 1H), 4.16−4.19 (m, 1H), 3.92−3.97 (m, 1H), 3.75−3.78 (m, 1H), 3.48−3.51 (m, 1H), 3.24−3.35 (m, 3H), 2.66−2.74 (m, 1H), 2.08−2.14 (m, 1H), 2.05 (s, 3H), 1.38 (d, J = 6.3 Hz, 3H), 1.21 (d, J = 7.0 Hz, 3H). ESI MS m/z 560.2 $[M + H]^+$. HPLC purity: 100.0%.

Plasma Stability Assay. Plasma was prewarmed at 37 °C in water bath for 5 min. Prodrugs were incubated in plasma at 50 μM with an incubation volume of 100 μL for each time point sample. The reaction solutions were kept at 37 °C. Time samples (0, 5, 15, 30, and 60 min) were removed from the water bath and immediately mixed with 500 μL of acetonitrile containing internal standard. Some prodrugs were quenched by ethanol containing internal standard. After centrifugation the supernatant was collected and diluted with mobile phase or

ultrapure water for LC−MS/MS analysis. Tebipenem pivoxil was used as positive control. Standard curves were used to calculate the concentrations of the prodrugs, monoesters, and parent drugs. One of the following LC−MS/MS conditions was used for quantitative analysis. (i) The LC−MS/MS system consisted of HPLC (Shimadzu LC 20AD) and API 4000 (Applied Biosystems) instruments with autosampler of CTC PAL. The HPLC mobile phases consisted of 2 mM ammonium acetate in water and acetonitrile. Chromatographic separation was achieved on a pHlex ODS column (5 μm, 2.1 mm × 50 mm, Boston Separations). (ii) The LC−MS/MS system consisted of UPLC (Waters Acquity) and API 4000 (Applied Biosystems) instruments. The UPLC mobile phases consisted of 2 mM ammonium acetate in water and acetonitrile. Chromatographic separation was achieved on a pHlex ODS column (5 μm, 2.1 mm × 50 mm, Boston Separations). (iii) The LC−MS/MS system consisted of HPLC (Shimadzu LC 20AD) and API 4000 (Applied Biosystems) instruments with autosampler of CTC PAL. The HPLC mobile phases consisted of 2 mM ammonium acetate in water and acetonitrile. Chromatographic separation was achieved on Synergi, Hydro-RP, C_{18} column (4 μm, 2.10 mm × 30 mm, Phenomenex). (iv) The LC−MS/MS system consisted of HPLC (Shimadzu LC 20AD) and API 4000 (Applied Biosystems) instruments with autosampler of CTC PAL. The HPLC mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Chromatographic separation was achieved on Synergi, Hydro-RP, C_{18} column (4 μm, 2.10 mm × 30 mm, Phenomenex). All analytes were detected using either positive or negative ESI (electrospray ionization) with both Q1 and Q3 operated under unit resolution. The ion source conditions were optimized as the following: 600 °C source temperature; 5500 V for positive ionspray voltage and −4200 V for negative ionspray voltage; 3 L/min nebulizer gas; 15 L/min drying gas.

Stability Measurement of Prodrugs in Simulated Gastric Fluid (SGF), Simulated Gastrointestinal Fluid (FaSSIF), and Phosphate Buffer. Prodrugs and parent were dissolved in DMSO, and a 10 mM stock solution was prepared and stored at −20 °C. A 25 μL aliquot was added to 4975 μL of simulated gastric fluid (pH 2.0), simulated intestinal fluid (pH 6.5), phosphate buffer (pH 7.0), and acetonitrile−water (1:1) to make 50 μM concentration samples. A 50 μL aliquot of each solution was further diluted 50-fold by addition of 2450 μL of the corresponding media to provide 1 μM concentration samples. DMSO control was prepared similarly from 25 μL of DMSO without prodrug. Acetonitrile−water samples served as a stability control for the prodrug and parent, and DMSO control served as blank control. Each sample was incubated at 37 °C for 3−4 h, and 5 μL aliquot was removed (SGF, 10, 20, 60, 90, 120 min; FaSSIF and phosphate buffer, 15, 30, 60, 120, 180 min) from each incubated sample and diluted with 5 μL of acetonitrile and 990 μL of 1:1 acetonitrile−water containing 10 ng/mL of tolbutamide and 1 ng/mL of buspirone (internal standards). Samples were analyzed by LC−MS/MS using the experimental condition (iv) described for plasma hydrolysis. Prodrugs and parent were quantified using calibration curves based on peak area ration of analyte and two internal standards.

Dosing Solution Preparation for Rat and Dog Studies. Dosing solutions were prepared and administered within an hour of formulation. The iv formulations were filtered with 0.22 μM filter before dosing. The vehicles were 30% captisol for iv and 0.5% methylcellulose for ID for rat (Table 6). For dog studies, evaluations of solubility and redispersibility in a variety of potential vehicles including PEG 400, 40% PEG400/10% Tween 80/50% water, 30% Captisol, 10% Tween 80, 50% Imwitor/50% Tween were conducted to select the optimal vehicle for the prodrugs (Table 7).

Husbandry and Surgical Preparation of Rats and Dogs. All animal studies were approved by the Animal Care Committees of Merck and WuXi. Male Sprague−Dawley Rats (200−300 g) were obtained from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). They were housed in stainless steel cages and had free access to water and certified rodent diet (catalog no. M-01F, SLAC Laboratory Animal Co. Ltd., Shanghai, China). Rats for intraveneous (iv) bolus dosing were surgically implanted with catheters in both the jugular vein and carotid artery using polyethylene tubing (carotid artery, Harvard-720192, 0.4 mm internal diameter; jugular vein, Harvard-598322, 0.28 mm internal diameter) according to generally accepted procedures. Rats for intraduodenal (ID) dosing were implanted with a catheter (Access Technologies-BC-3P French size 3, gauge 20) which was inserted into the duodenum according to generally accepted procedures. The animals were also cannulated at the carotid artery for blood sampling as described above. The rats were allowed to recover at least 3 days after surgery. Male beagle dogs (6−10 kg) were obtained from Marshall Bioresources (Beijing, China) and were fed certified dog diet (Beijing Vital Keao Feed Co., Ltd. Beijing, P. R. China). For the dogs dosed via ID administration, a cannula made of silicone tubing (0.025 in. i.d. × 0.047 in. o.d., catalog no. SIL-3.5-25, Instech Solomon) was placed within the first 1.5−2 cm of duodenum after the pylorus, as close as possible to pylorus, according to generally accepted procedures. The cannula was enveloped and placed outside with 1−2 cm exterior to the skin. The dogs were allowed to recover 7 days following surgery.

Pharmacokinetic Evaluation in Rat (Dosing and Sample Processing). Rats were fasted overnight prior to drug administration and had access to water ad libitum and access to chow 4 h postdose. For iv studies, the jugular vein cannula was used for administration at 1 mpk in a dose formulation of 0.5 mg/mL and the carotid artery catheter was used for blood sampling. For ID studies, the duodenal catheter was used for dosing at 10 mpk in a dose formulation of 2 mg/mL at a rate of 5 mL/h using an infusion pump (Harvard Apparatus PHD 2000) and the carotid artery cannula used for blood sampling. Approximately 0.20 mL of blood was collected at 0 (predose to serve as a blank), 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose from carotid artery via a catheter after iv and ID infusion administration of each compound. All blood samples were transferred into plastic microcentrifuge tubes containing 4 μL of 0.5 M K_2-EDTA anticoagulant, placed on wet ice, and then immediately centrifuged to get plasma. To plasma, an equal volume of 1 M MOPS buffer, pH 7.0, was added under ice-cold conditions to stabilize the carbapemens and then mixed. After that, the mixed samples were immediately quenched by addition of a 4-fold volume of acetonitrile containing internal standard. The processed plasma samples were stored in polypropylene tubes, quick-frozen over dry ice, and kept at −70 ± 10 °C until LC−MS/MS analysis.

Pharmacokinetic Evaluation in Dogs. Dogs were fasted overnight prior to drug administration and had access to water ad libitum and access to chow 4 h postdose. For iv studies, the cephalic vein was used for administration at 1 mpk in a dose formulation of 1 mg/mL. For ID studies, the duodenal catheter was used for dosing at 10 mpk in a dose formulation of 2 or 5 mg/mL for at least 15 min using an infusion pump (Harvard Apparatus PHD 2000). Approximately 0.20 mL of blood was collected at 0 (predose to serve as a blank), 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose from the cephalic vein or saphenous vein after iv and ID infusion administration of each compound. All blood samples were transferred into plastic microcentrifuge tubes containing 4 μL of 0.5 M of K_2-EDTA anticoagulant, placed on wet ice, and then immediately centrifuged to get plasma. To plasma, an equal volume of 1 M MOPS buffer, pH 7.0, was added under ice-cold conditions to stabilize the carbapemens and then mixed. After that, the mixed samples were immediately quenched by addition of a 4-fold volume of precipitant (80% ACN/EtOH) containing internal standard. The processed samples were stored in polypropylene tubes, quick-frozen over dry ice, and kept at −70 ± 10 °C until LC−MS/MS analysis.

Quantitative Analysis. Dose formulation retains were assayed in duplicate for each dose using LC/UV or LC−MS/MS methodologies with a calibration curve at least six points. Each processed plasma sample was assayed using LC−MS/MS with an internal standard using a minimum of eight-point standard curves. No formal method validation was conducted, but a minimum of six QC samples were included in each analytical run and needed to be within ±25% of their nominal values when back-calculated from the standard curve linear regression analysis to ensure assay performance. The BA methods for PK samples' analysis were optimized individually for different compounds.

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Data Analysis and Calculations. Plasma concentrations versus time data were analyzed by noncompartmental approaches using the WinNonlin software program (version 5.2, Pharsight Corporation, Mountain View, CA).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of all final prodrugs. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

ID, intradeuodenal; OATP, organic acid transporter; OATB1A2, organic acid transporter B1A2; OATBB1, organic acid transporter BB1

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