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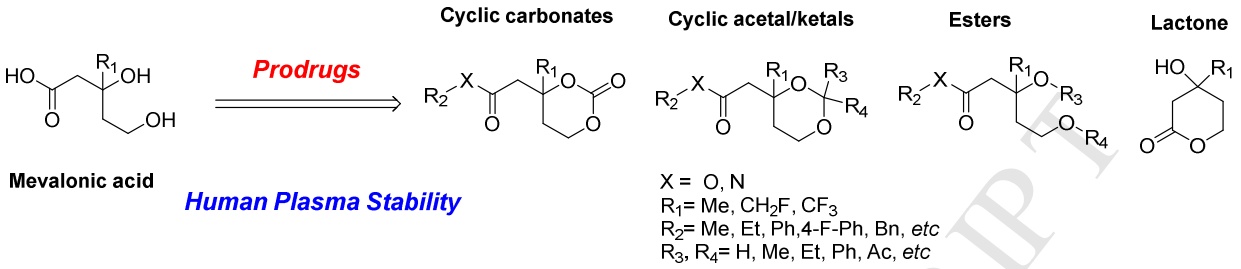
Graphical Abstract



Synthesis of Mevalonate- and Fluorinated Mevalonate Prodrugs and Their *in vitro* Human Plasma Stability

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KEYWORDS: mevalonate, 6-fluoromevalonate, 6,6,6-trifluoromevalonate, prodrugs, plasma stability

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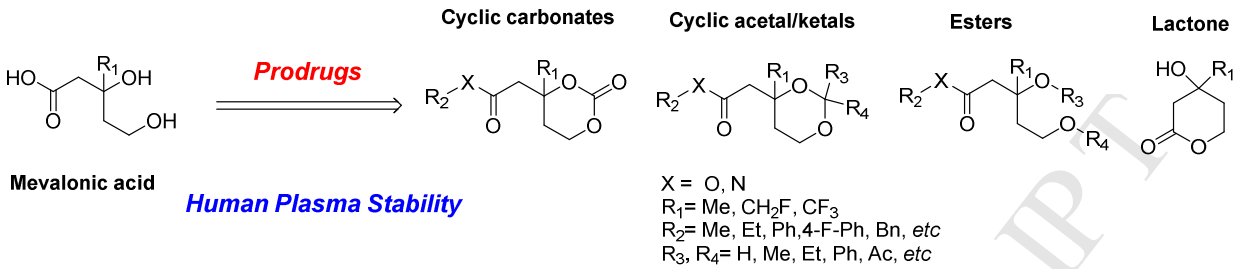
Graphical Abstract



Highlights

- Prodrug analogues of mevalonate, 6-fluoromevalonate, and 6,6,6-trifluoromevalonate synthesized and characterized
- Human blood plasma stabilities of ester, amide, carbonate, acetal, and ketal promoieties of these analogues determined
- A wide range of half-lives were obtained
- MIC values determined to evaluate antibacterial activity of select prodrugs

ABSTRACT: The mevalonate pathway is essential for the production of many important molecules in lipid biosynthesis. Inhibition of this pathway is the mechanism of statin cholesterol-lowering drugs, as well as the target of drugs to treat osteoporosis, to combat parasites, and to inhibit tumor cell growth. Unlike the human mevalonate pathway, the bacterial pathway appears to be regulated by diphosphomevalonate (DPM). Enzymes in the mevalonate pathway act to produce isopentenyl diphosphate, the product of the DPM decarboxylase reaction, utilize phosphorylated (charged) intermediates, which are poorly bioavailable. It has been shown that fluorinated DPMs (6-fluoro- and 6,6,6-trifluoro-5-diphosphomevalonate) are excellent inhibitors of the bacterial pathway; however, highly charged DPM and analogues are not bioavailable. To increase cellular permeability of mevalonate analogues, we have synthesized various prodrugs of mevalonate and 6-fluoro- and 6,6,6-trifluoromevalonate that can be enzymatically transformed to the corresponding DPM or fluorinated DPM analogues by esterases or amidases. To probe the required stabilities as potentially bioavailable prodrugs, we measured the half-lives of esters, amides, carbonates, acetals, and ketal promoieties of mevalonate and the fluorinated mevalonate analogues in human blood plasma. Stability studies showed that the prodrugs are converted to the mevalonates in human plasma with a wide range of half-lives. These studies provide stability data for a variety of prodrug options having varying stabilities and should be very useful in the design of appropriate prodrugs of mevalonate and fluorinated mevalonates.

Introduction

The mevalonate pathway (Figure 1) is an important cellular metabolic pathway present in all higher eukaryotes and many bacteria. Isopentenyl diphosphate (IPP), an intermediate in this pathway, is an important precursor of isoprenoids, which leads to many biologically active small molecules, including cholesterol, steroid hormones, and vitamin A.[1] Therefore, it is not surprising that enzymes in the mevalonate pathway are targets for a variety of drug discovery programs.[2] The statin cholesterol-lowering drugs target 3-hydroxy-3-methylglutaryl CoA reductase, the enzyme that produces mevalonate;[3] the osteoporosis drug alendronate inhibits the synthesis of farnesyl diphosphate from IPP;[4] enzymes in the mevalonate pathway are also targeted for cancer[5] and parasites.[6] Leyh and co-workers discovered that the mevalonate pathway in *S. pneumoniae* is regulated by 5-diphosphomevalonate (DPM).[7] They showed that DPM is a feedback inhibitor of mevalonate kinase (MK), and binds tightly to an allosteric site[8] of the pneumococcal MK. However, human MK is not inhibited by DPM at concentrations that effectively inhibit the S. *pneumoniae* system.[9] Therefore, DPM can be a lead compound for the development of new anti-pneumococcal antibiotics that do not perturb human metabolism.

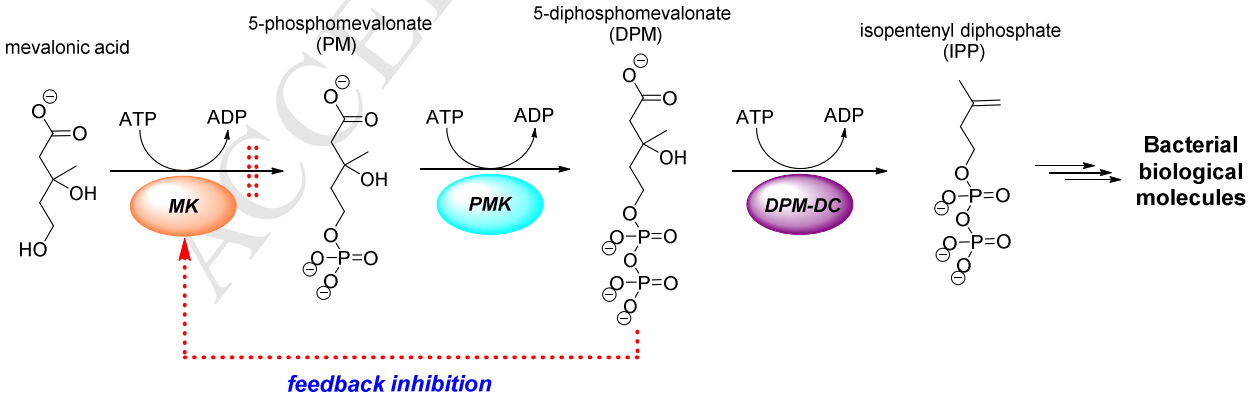


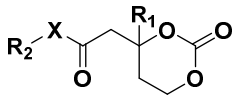
Figure 1. The bacterial mevalonate pathway. Conversion of mevalonic acid to isopentenyl diphosphate occurs in three ATP-dependent steps. DPM is a feedback inhibitor of MK: MK, mevalonate kinase; PMK, phosphomevalonate kinase; DPM-DC, diphosphomevalonate decarboxylase.

In early studies of DPM analogues,[10] it was found that 6,6,6-trifluoromevalonate was converted into the corresponding diphosphate enzymatically, which inhibited DPM decarboxylase (DPM-DC) and led to the accumulation of DPM in rat liver homogenates.[11] Moreover, 6-fluoromevalonate causes the accumulation of phosphorylated mevalonates and completely blocks the related bioactivity of mevalonate at 200 μM concentration.[12] However, whereas a functional mevalonate pathway is essential for the survival of bacteria, suppression of this pathway in humans results in minimal side effects, as evidenced by the common use of statin drugs, which block cholesterol biosynthesis at a step prior to DPM-DC, and by antiproliferative drugs, such as bisphosphonates, which block farnesyltransferase.[13] Furthermore, antibacterial treatment is short in duration, which should not have a serious effect on the products of this pathway. Nonetheless, diphosphate compounds are generally not suitable for use as drugs; because of their highly charged structure (4-), they are not expected to penetrate the negatively charged bacterial cell membrane.[14] Also phosphatases can degrade the diphosphate group easily.

Because of the importance of mevalonate and phosphorylated metabolites to drug discovery, neutral and less polar prodrugs, chemically modified molecules of the pharmacologically active moiety that are transformed into the active form *in vivo*,[15] were designed to avoid these potential bioavailability problems. The charged carboxylic acid was protected as an ester, lactone, or amide to make it neutral. To explore the influence of polarity of the prodrug on human

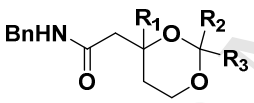
absorption and bacterial cell permeation, the two hydroxyl groups of the mevalonate were converted to carbonate, acetal, and ketal prodrugs. These esters,[16] lactones,[17] amides,[18] carbonates,[19] acetals,[20,21] and ketals,[22] having half-lives ranging from a couple of minutes to several days in human plasma, were chosen as the promoieties of the carboxyl and hydroxyl functionalities of mevalonate. These analogues should be enzymatically hydrolyzed to their original mevalonate or fluorinated mevalonates in both humans and bacteria, and then the fluorinated mevalonates can be enzymatically converted to the phosphorylated forms.[23] The stabilities of diverse analogues (Figure 2) in human blood plasma were studied to develop an armamentarium of promoieties for further *in vitro* and *in vivo* studies.

Cyclic carbonates



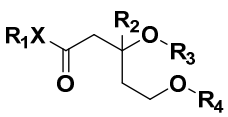
	X	R_1	R_2
4	O	Me	Bn
9a	O	Me	Ph
9b	O	Me	4F-Ph
10a	NH	Me	Ph
10b	NH	Me	Bn
10c	NH	Me	4F-Bn
16	O	CH_2F	Bn
18a	O	CH_2F	4F-Bn
18b	O	CH_2F	2,4-diF-Bn
23	O	CF_3	Bn
25a	O	CF_3	4F-Bn
25b	O	CF_3	2,4-diF-Bn

Cyclic acetals/ketals



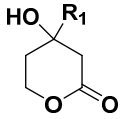
	R_1	R_2	R_3
30a	Me	H	H
30b	CF_3	H	H
31	Me	Me	Me
32a	Me	H	Ph
32b	CF_3	H	Ph
32c	CF_3	H	4-MeO-Ph
32d	CF_3	H	2,4-diMeO-Ph

Amide/ester/carbonate



	R_1X	R_2	R_3	R_4
33a	BnNH	Me	H	Boc
33b	BnNH	CF_3	H	Boc
34	$C_6F_5CH_2NH$	Me	H	H
35	$C_6F_5CH_2NH$	Me	H	Ac
36	$C_6F_5CH_2NH$	Me	Ac	Ac
38a	BnNH	CH_2F	H	Ac
38b	MeO	CH_2F	H	Ac
38c	EtO	CH_2F	H	Ac

Lactones



	R
1	Me
26	CF_3
28	CH_2F

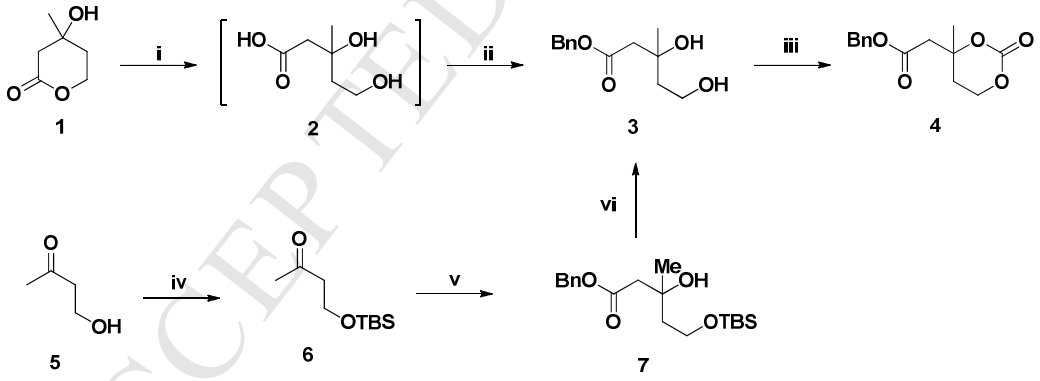
Figure 2. Cyclic carbonate, cyclic acetal/ketal, ester, and lactone prodrugs of mevalonate

Results and Discussion

Cyclic carbonate prodrug **4** was prepared from mevalonolactone (**1**) using the synthetic route described in Scheme 1. The hydrolysis of **1** with aqueous KOH afforded a solution of **2**, which was neutralized to pH 7-8 with aqueous HCl and lyophilized to remove water. If neutralization

was not carried out, starting material **1** was regenerated during lyophilization. The crude carboxylic acid (**2**) was converted to the corresponding benzyl ester (**3**) via treatment with benzyl bromide and tetrabutylammonium bromide. Although a portion of ester **3** was converted to the starting material (**1**) during column chromatography with silica gel, **3** was isolated as the major product (69% yield). Ester **3** was easily converted to the cyclic carbonate (**4**) via treatment with triphosgene. An alternative route to the benzyl ester (**3**) is also shown in Scheme 1; TBS protection of the hydroxyl group of 4-hydroxy-2-butanone (**5**), followed by an aldol reaction with benzyl acetate using LDA, afforded **7** in excellent yields. Deprotection of the TBS group in compound **7**, with tetrabutylammonium fluoride and two equivalents of acetic acid at 0 ºC, afforded desired alcohol **3**. The reaction of crude product **3** with triphosgene yielded cyclic carbonate **4;** lactone **1** was still generated gradually before **3** disappeared completely.

Scheme 1. Synthesis of compound **4**[a]

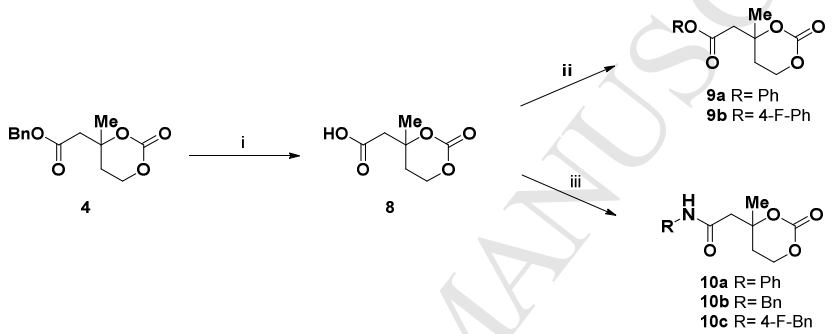


[a]Reagents and Conditions: (i) aq. KOH, 40 °C; aq. HCl, (ii) BnBr, TBAB, THF, 50 °C (69% for 2 steps), (iii) triphosgene, pyridine, 0 °C (88%); (iv) TBSCl, imidazole, DMF (90%), (v) benzyl acetate, LDA, THF, -78 °C (93%), (vi) TBAF, AcOH, THF, 0 °C (72%).

Cyclic carbonate analogues **9a,b** and **10a-c** were synthesized from benzyl ester **4** after removal of the benzyl group via palladium-catalyzed hydrogenolysis (Scheme 2). The coupling reaction

of carboxylic acid **8** with various phenols and amines using EDCI or HBTU provided the desired esters (**9a,b**) and amides (**10a-c**) in moderate to good yields (Scheme 2). The obtained benzyl amide derivatives were expected to be more stable than the ester derivatives in plasma. To confirm the relative stability of other less stable amides (due to the electron withdrawing effect), a phenyl amide (**10a**) and a 4-fluorobenzyl amide (**10c**) were prepared.

Scheme 2. Synthesis of **9a-b** and **10a-c**[a]

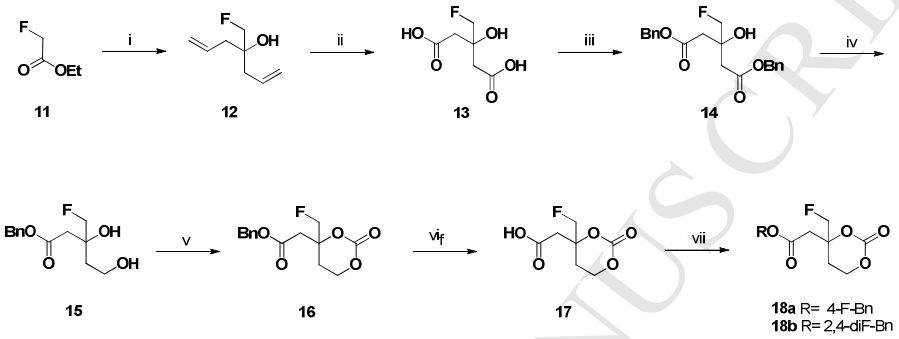


[a]Reagents and Conditions: (i) Pd/C, H_2, quantitative; (ii) EDCI, DMAP, CH_2Cl_2, ROH R = Ph (**9a**, 54%), 4-F-Ph (**9b**, 65%); (iii) R-NH_2, HBTU, DIEA, DMF, R = Ph (**10a**, 55%), Bn (**10b**, 61%), 4-F-Ph (**10c**, 38%)

The synthetic route for the 6-fluoromethyl cyclic carbonate analog is shown in Scheme 3. The addition of allylmagnesiun bromide (1.95 equiv) to ethyl fluoroacetate **11** at 0 ºC for 30 min afforded diolefin **12**. This reaction was sensitive to the duration and the equivalents of Grignard reagent; an addition of excess allymagnesium bromide or prolonged reaction times resulted in undesired side product generation. Ozonolysis of crude product **12**, followed by oxidation with H_2O_2 gave dicarboxylic acid **13**. The benzylation of crude product **13** was conducted to give diester **14** in more than 50% yield for four steps. Partial reduction of **14** with DIBAL-H (3-4 equiv) at 0 ºC in THF afforded the desired compound (**15**). Because **15** easily underwent intramolecular lactone formation on silica gel, the crude mixture was allowed to react with

triphosgene without further column purification to obtain cyclic carbonate **16** in moderate yields. The benzyl deprotection of **16**, followed by esterification with 4-fluorobenzyl bromide, or 2,4-difluorobenzyl bromide, provided the corresponding esters (**18a-b**), respectively.

Scheme 3 Synthesis of Compounds **18a-b**[a]

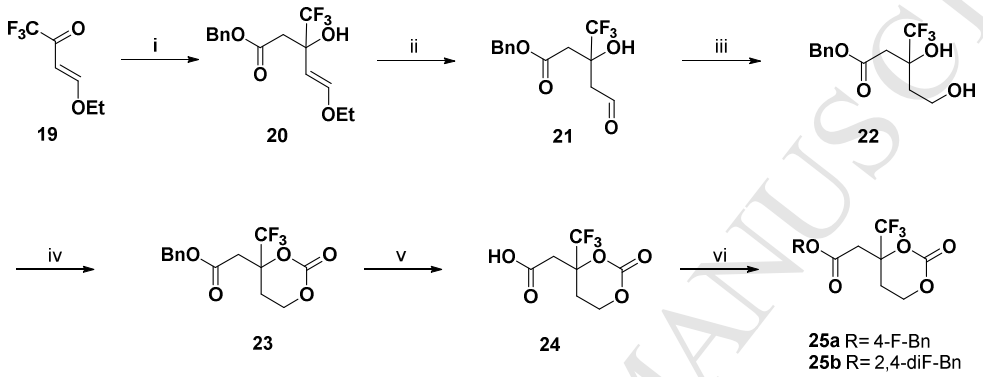


[a]Reagents and Conditions: (i) allylmagnesium bromide (1.95 equiv), Et_2O, 0 °C, < 30 min. (ii) (1) ozone, CH_2Cl_2, -78 °C (2) aq. H_2O_2, AcOH, H_2SO_4, reflux, (iii) BnBr, K_2CO_3, DMF, 54% for 4 steps (69% for 2 steps), (iv) DIBAL-H, THF, 0 °C, (v) $(Cl_3CO)_2CO$, pyridine, CH_2Cl_2, 0 °C (37% for 2 steps), (vi) H_2, Pd/C, AcOEt (99%), (vii) $NaHCO_3$, DMF, R-X; R= 4-F-BnBr (**18a**, 77%), 2,4-diF-BnBr (**18b**, 72%).

4-Trifluoromethyl carbonate analogues were synthesized from commercially available 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (Scheme 4). The aldol reaction of benzyl acetate and enone **19** with LDA gave **20**. The produced enol ether was hydrolyzed to the corresponding aldehyde (**21**) under acidic conditions, which was reduced to alcohol **22** by sodium triacetoxyborohydride. When sodium borohydride was used, the benzyl ester group was gradually reduced to the hydroxyl group; perhaps the reactivity of the ester group was increased by the electron withdrawing effect of the trifluoromethyl group. Cyclic carbonate **23** was obtained by treatment of the crude diol (**22**) with triphosgene. The benzyl group of **23** was removed by palladium-catalyzed hydrogenolysis in ethyl acetate under a hydrogen atmosphere. When MeOH was used

as a solvent in this reaction, the carbonate group decomposed. Carboxylic acid **24** was esterified with 4-fluorobenzyl bromide, or 2,4-difluorobenzyl bromide using sodium bicarbonate as a base, to give **25a-b**. When potassium carbonate was used for the esterification of **24**, the cyclic carbonate group decomposed.

Scheme 4. Synthesis of **25a-b**[a]

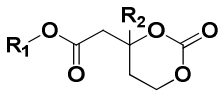


[a]Reagents and Conditions: (i) benzyl acetate, LDA, THF, -78 °C (88%), (ii) aq. HCl/acetone, 0 °C (80%), (iii) $NaBH(OAc)_3$, benzene, (iv) triphosgene, pyridine, CH_2Cl_2, 0 °C (68% for 2 steps), (v) H_2, Pd/C, AcOEt (98%), (vi) $NaHCO_3$, DMF, R-X = 4-F-BnBr (**25a**, 88%), 2,4-diF-BnBr (**25b**, 85%).

Stability of the cyclic carbonate prodrugs in PBS buffer. The stabilities of several cyclic carbonate analogues in PBS buffer (pH 7.4) were tested to determine baseline level of drug decomposition by the medium. The cyclic carbonate analogues (**4**, **16**, **18a-b**, **23**, **25a-b**) were UV active, which allowed us to determine the amount of drug in the culture media by HPLC with UV detection. After the mixture of the compounds and PBS buffer were incubated at 37 ºC, the degradation of the compounds over time was monitored. The half-life ($t_{1/2}$) of **4**, which is a mevalonate prodrug, was more than 48 h (Table 1). The $t_{1/2}$ of the 4-monofluoromethyl analogues (**16**, **18a-b**) were around 25 h, and the $t_{1/2}$ of the 4-trifluoromethyl analogues (**23**, **25a-**

b) were around 5 h. As the 4-substituent became increasingly more electron deficient, the $t_{1/2}$ decreased dramatically. However, electron-withdrawing groups on the aromatic ring of the ester (benzyl, 4-fluorobenzyl, or 2,4-difluorobenzyl) did not impact the half-lives of the molecules, as long as the R_2- substituent was constant (CH_3, CH_2F, or CF_3).

Table 1. Half-lives of the diverse carbonates in PBS buffer at 37 ºC.



#	R_1	R_2	$t_{1/2}$ (h)
4	Bn	CH_3	>48
16	Bn	CH_2F	25
18a	4-F-Bn	CH_2F	26
18b	2,4-*di*-F-Bn	CH_2F	22
23	Bn	CF_3	5
25a	4-F-Bn	CF_3	6
25b	2,4-*di*-F-Bn	CF_3	5

HPLC and ^{1}H NMR analysis of trifluoromethyl analogue **23** during incubation with PBS buffer was carried out to delineate its decomposition pathway and related intermediates (Figure 3). As the peak for **23** decreased, the peak for benzyl alcohol increased, but the peak for **22**, which results from decomposition of the carbonate moiety, was not observed by HPLC. ^{1}H NMR analysis of the incubation mixture after lyophilization showed carboxylic acid **24** was only a minor component; desired final product **27** was the major product. Further stability tests of prospective intermediates were carried out. By monitoring the ^{1}H NMR spectrum of synthesized **24** in PBS buffer it was found that the $t_{1/2}$ of **24** was about 3 h, shorter than the $t_{1/2}$ of **23** (5 h). The existence of about 8% of lactone **26** was also observed. Pure **22** was incubated with PBS buffer to confirm the decomposition pathway of **23** via **22**. Interestingly, the obtained $t_{1/2}$ for **22**

was very short (< 1 min), so even through little **22** was formed during the decomposition of **23**, whatever small amount was formed would quickly undergo further degradation to **26** or **27**. A similar experiment using lactone **26** was carried out. The spectra showed that lactone **26** was converted to carboxylic acid **27** rapidly ($t_{1/2}$ in PBS buffer was about 1 min). This indicates that the decomposition through **22** and **26** is also possible even though **22** was not detected.

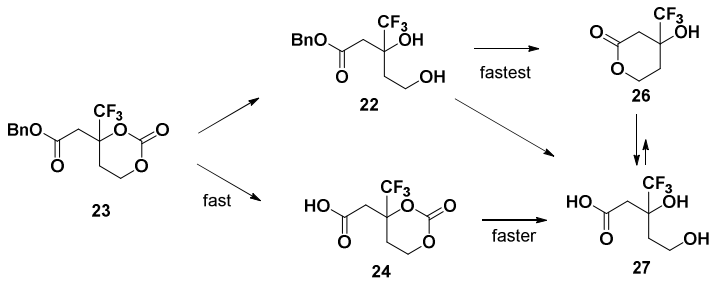
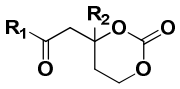


Figure 3. Pathways for the decomposition of trifluoromethyl benzyl ester carbonate **23**

Stability of the cyclic carbonate prodrugs in human plasma. Human plasma stabilities were evaluated,[24] for the cyclic carbonate analogues (**4**, **9a-b**, **10a-c**, **16**, **18a-b**, **23**, **25a-b**), and reported as their corresponding half-lives (Table 2). In all cases, quantification was performed in at least duplicate (<25% error) using HPLC in the presence of the plasma-stable internal standard haloperidol.[25] The mixture of test compounds and human plasma was incubated at 37 ºC, and the degradation of the compounds was monitored by HPLC. Slight differences in the $t_{1/2}$ were observed for the various ester groups, but the $t_{1/2}$ for **4** was 4 min and the $t_{1/2}$ for the remaining esters (**9a-b**, **16**, **18a-b**, **23**, **25a-b**) were less than 3 min. While the benzyl and phenyl ester moieties were unstable, the cyclic carbonate moiety was relatively more stable, and the peaks of the corresponding diols (**3**, **15**, or **22**), which correspond to the products when the carbonate moiety is hydrolyzed faster than the ester moiety, were not observed. The $t_{1/2}$ of these benzyl and phenyl ester analogues was very short. The $t_{1/2}$ for *N*-phenyl amide **10a** was also less than 3 min.

Interestingly, the $t_{1/2}$ values for benzyl amide analogues **10b** and **10c** were 8 min, resulting from the hydrolysis of the carbonate moiety rather than the amide moiety. No benzylamine peak was observed by HPLC through 12 h incubation, illustrating the high stability of the benzyl amide moiety in human plasma. These results demonstrate that the cyclic carbonate moiety of these analogues is more stable than the benzyl ester moiety but less stable than the benzyl amide moiety.

Table 2. Various half-lives of the diverse carbonate analogues in human plasma at 37 ºC.



#	R_1	R_2	$t_{1/2}$ (min)
4	BnO	CH_3	4
9a	PhO	CH_3	< 3
9b	4-F-PhO	CH_3	< 3
10a	PhNH	CH_3	< 3
10b	BnNH	CH_3	8[a]
10c	4-F-BnNH	CH_3	8[a]
16	BnO	CH_2F	2
18a	4-F-BnO	CH_2F	2
18b	2,4-*di*-F-BnO	CH_2F	1
23	BnO	CF_3	< 1
25a	4-F-BnO	CF_3	< 1
25b	2,4-*di*-F-BnO	CF_3	<1

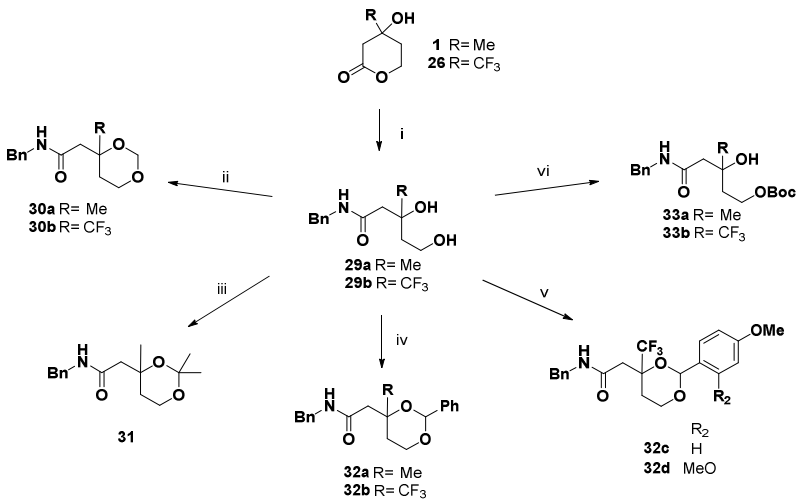
[a]Hydrolysis occurred only at carbonate promoieties

The enantiomeric ratio of the synthesized mevalonate derivatives was monitored by chiral chromatography and LC/MS during incubation with human plasma to determine the influence of stereochemistry on the hydrolysis rate.[26] At $t_{1/4}$ and $t_{1/2}$, a portion of the incubation mixtures of **4** and **10b** were quenched with acetonitrile and injected into a Chiral Cel OD-H column attached to a HPLC system. The observed integration ratio of each enantiomer of **4** was 35/65 at 2 min, and

41/59 at 4 min. In the case of compound **10b**, the enantiomeric ratio was 48/52 at 4 min and 46/54 at 8 min. Within the margin of error, hydrolysis of the carbonate was not significantly affected by the stereochemistry. Although a stereospecific hydrolysis was observed during incubation of a racemic mevalonate ester with human plasma, the difference did not seriously affect the overall hydrolysis rate.

Syntheses of acetal/ketal prodrugs. The synthesis of the acetal/ketal analogues shown in Scheme 5 was initiated by the ring-opening of **1** and **26** with benzylamine. The introduction of the MOM group, followed by treatment with BF_3•Et_2O, gave methylene acetals **30a-b**. Reaction of **29a** with 2,2-dimethoxypropane and a catalytic amount of camphorsulfonic acid (CSA) gave acetonides **31**. Treatment of **29a,b** with benzaldehyde dimethoxy acetal and catalytic CSA in CH_2Cl_2 gave diastereomeric mixtures **32a-b**. Diol **29b** was converted to the *p*-methoxybenzylidene acetal **32c-d** by treatment with anisaldehyde dimethyl acetal. Because electron-rich benzylidene acetals are known to undergo hydrolysis more quickly, 4-methoxybenzylidene derivative **32c** and 2,4-dimethoxybenzylidene derivative **32d** were expected to decompose more easily than **32b**. Compounds **29a-b** were converted to Boc-protected **33-b** by treatment with an excess amount of Boc_2O in anhydrous acetonitrile.

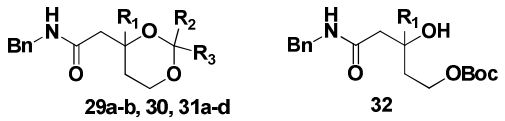
Scheme 5. Synthesis of Compounds **30-33** [a]



[a]Reactions and Conditions: (i) $BnNH_2$, DMF, 80 °C (80-88%), (ii) (a) MOMCl, CH_2Cl_2, 0 °C to room temperature, (b) BF_3OEt_2, CH_2Cl_2, 0 °C to room temperature, 77% yield for **30a**, 69% yield for **30b**, (iii) 2,2-dimethoxypropane, CSA, CH_2Cl_2, 27% yield, (iv) benzaldehyde dimethylacetal, CSA, CH_2Cl_2, 34% yield for **32a**, 42% yield for **32b**, (v) anisaldehyde dimethyl acetal, CSA, CH_2Cl_2, reflux, 60% yield for **32c**; 2,4-dimethoxybenzaldehyde, CSA, MS 4Å, benzene, reflux, 6% yield for **32d**, (vi) Boc_2O, DMAP, acetonitrile, reflux, 88% yield for **33a**, 68% yield for **33b**

Stability of the acetal/ketal amide prodrugs in human plasma. Prodrugs with the acetal, ketal, and carbonate promoieties were further investigated for their stability in human plasma (Table 3). The $t_{1/2}$ values for the acetal, ketal, and carbonate derivatives (**30a-b, 31, 32a-d, 33a-b**) in human plasma were much greater than 48 h. An electron-withdrawing group (trifluoromethyl) at C4 did not affect the stability of the acetal groups. These results show that the methylene, isopropylidene, and benzylidene acetal groups of the tested compounds may be useful if the mevalonate prodrug requires long-term stability.

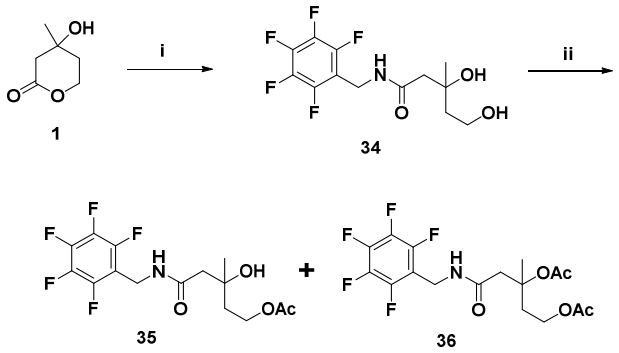
Table 3. Stability of the acetal/ketal amides in human plasma at 37 ºC



#	R_1	R_2	R_3	$t_{1/2}$ (h)
30a	CH_3	H	H	> 48
31	CH_3	CH_3	CH_3	> 48
32a	CH_3	Ph	H	> 48
30b	CF_3	H	H	> 48
32b	CF_3	Ph	H	> 48
32c	CF_3	4-MeO-Ph	H	> 48
32d	CF_3	2,4-$(MeO)_2$-Ph	H	> 48
33a	CH_3			> 48
33b	CF_3			> 48

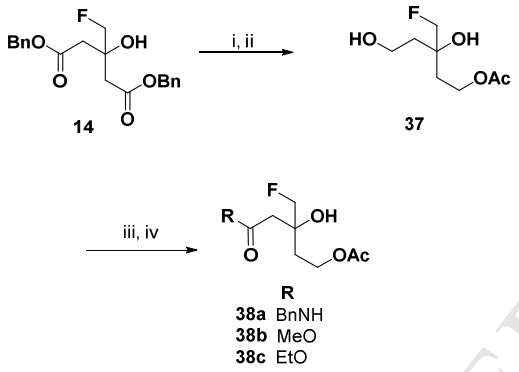
Syntheses of ester prodrugs. Syntheses of diverse ester and amide analogues are shown in Scheme 6 and Scheme 7. The ring opening of **1** was carried with various electron deficient amines, and the introduction of the acetyl group was performed to give diverse acetyl esters (**35, 36**). The reaction of **34** with excess acetic anhydride in pyridine gave both mono- and di-acetylated products (**35, 36**). The 4-monofluoromethyl analogues (**38a-c**) were prepared from dibenzyl ester **14** (Scheme 7). The dibenzyl ester was reduced to the triol using $LiBH_4$, and addition of one equivalent of acetic anhydride in pyridine yielded mono-acetylated intermediate **37**. That intermediate underwent oxidation by pyridinium dichromate (PDC), and was then coupled with various alcohols and an amine to obtain the corresponding products in moderate yields (**38a-c**).

Scheme 6[a]



[a]Reagents and Conditions: (i) 2,3,4,5,6-pentafluorobenzylamine, DMF, 80 °C, 53% yield, (ii) Ac_2O, pyridine, 41% yield for **35**, 20% yield for **36**

Scheme 7[a]

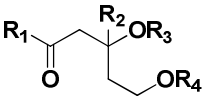


[a]Reagents and Conditions: (i) $LiBH_4$ (3 eq.), THF, 0 °C (70%) (ii) Ac_2O, DMAP, pyridine (45%), (iii) PDC, DMF, 24 h, room temperature (iv) RNH_2,EDCI, K_2CO_3, or R-X, K_2CO_3, DMF, two steps 11~25%.

Stability of the ester promoiety in PBS buffer. The stability of ethyl ester analogue **38c** in deuterated PBS buffer (pH 7.4) at 37 °C was monitored by ^{1}H NMR spectroscopy as a control to determine the decomposition of the ester prodrug by the medium. The half-life ($t_{1/2}$) of **38c** in PBS was > 48 h.

Stability of the ester promoiety in human plasma. The stabilities of the electron-deficient benzyl amide and ester analogues (**34-36**, **38a-c**) in human plasma were determined (Table 4). The half-life of the 2,3,4,5,6-pentafluorobenzyl amide (**34**) in human plasma was greater than 2 h; therefore, the amide of the parent compounds is expected to be useful if the prodrug requires long-term stability. The half-life of the acetyl groups on **35** and **38a** was 20 - 30 min, suggesting that this ester is a promising promoiety for the alcohols if the prodrug needs moderate stability in human plasma. The half-lives of the monofluoromethyl alkyl esters containing an acetyl moiety were around 10 min, suggesting that the alkyl ester group is a viable promoiety for the carboxylic acid and for moderate stability.

Table 4 Stability of the benzyl amide analogues in human plasma at 37 ºC



#	R_1	R_2	R_3	R_4	$t_{1/2}$ (min)
34	$C_6F_5CH_2NH$	CH_3	H	H	> 120
35	$C_6F_5CH_2NH$	CH_3	H	Ac	20[a]
36	$C_6F_5CH_2NH$	CH_3	Ac	Ac	12
38a	BnNH	CH_2F	H	Ac	30[a]
38b	MeO	CH_2F	H	Ac	10
38c	EtO	CH_2F	H	Ac	11

[a]Hydrolysis occurred only at the acetyl promoiety

Inhibition activity of diverse prodrugs against *Streptococcus pneumoniae*.

Diverse prodrugs of fluoromevalonates were evaluated for their *in vitro* antibacterial activity against *S. pneumoniae* (Table 5) in THB medium using a well-established technique,[27] and the minimum inhibitory concentration values (MICs) were determined. Carbonate prodrugs, which undergo relatively fast hydrolysis (1-4 min), were only marginally active (**25a**) or generally

inactive (**16**, **18a)**. Moderately stable (10-20 min) ester prodrugs (**38a-c**) and a quite stable acetal prodrug (**32b**) were found to lack activity. Lactones (**26** and **28**[28]), which were determined to undergo the fastest hydrolysis, were the most active compounds among these diverse prodrugs against *S. pneumoniae* in THB media; 6-fluoromevalolactone (**28**) is 4-fold more potent (MIC = 200 µM) than 6,6,6-trifluoromevalolactone (**26**, MIC = 800 µM). *S. pneumoniae* active compounds **25a**, **26**, and **28** were further evaluated against *Streptococcus pyogenes* (GAS 5448), *Streptococcus agalactiae* (GBS COH1), *Staphylococcus aureus* (TCH1516), vancomycin resistant *Enterococcus* (VRE), and *E. coli K12*. Only **26** displayed activity, albeit low, against *Streptococcus pyogenes* (MIC = 200 µM) and *Streptococcus agalactiae* (MIC = 800 µM); the others exhibited MIC values >1.6 mM concentration. For all of these additional organisms, vancomycin and penicillin G exhibited MIC values <0.0125 mM. No evidence of general cytotoxicity was observed for **25a**, **26**, and **28** against RAW murine macrophages and HaCaT human keratinocytes at concentrations up to 1.6 mM for 24 h as measured by lactate dehydrogenase (LDH) release vs media alone. Although these experiments do not consider clinical parameters of host bioavailability such as oral absorption, these results suggest that unstable prodrugs of fluoromevalonates are more beneficial than stable prodrugs to penetrate the bacterial cell and interact with enzymes of the mevalonate pathway. It is possible that the other relatively stable prodrugs, which probably have a better opportunity to penetrate the bacterial cell, are not hydrolyzed efficiently in the bacteria to reach their minimum effective concentration for a desired pharmacological effect in the bacteria.

Table 5. Antibacterial activity of diverse prodrugs toward *S. pneumoniae*

name	MIC[a] (mM)

16	>1.6
18a	>1.6
25a	1.6
26	0.8
28	0.2
32b	>1.6
38a	>1.6
38b	>1.6
38c	>1.6
Vancomycin	

[a]Minimum inhibitory concentration (MIC): lowest drug concentration that reduced growth by 80% or more.

Conclusion

Stability studies of diverse analogues of mevalonate and fluorinated mevalonates using human blood plasma and PBS buffer show that they are converted to mevalonate or fluorinated mevalonates via hydrolysis, mediated by human plasma or solvent. We also found that decomposition of the cyclic carbonates and esters is faster than that of the amides and cyclic acetals/ketals in the tested analogues. In general, an aliphatic ester promoiety is converted to the desired carboxylic acid with a $t_{1/2}$ of 10-20 min in human plasma. Although the cyclic carbonate decomposes relatively rapidly (4-8 min), and cyclic ketal, acetal, and amide moieties decompose relatively slowly in human plasma, this study shows that the $t_{1/2}$ in human plasma for each functional group is controllable by modifying the electron density of the promoiety. These plasma stability studies demonstrate that ester, amide, carbonate, acetal, or ketal promoieties have the potential to enhance biopermeability. We have observed that only rapidly hydrolyzed prodrugs of fluorinated mevalonates possess intracellular antibacterial activity against *S. pneumoniae*. Slowly hydrolyzed prodrugs may not have the appropriate pharmacokinetics with *S.*

pneumoniae. Other prodrug analogues may be more effective with different bacteria, or for use against human enzymes.

Experimental Section

General Chemistry Procedures All reagents were obtained from commercial suppliers and used without further purification. All solvents were distilled and stored under argon or a nitrogen atmosphere before use. All reactions were performed under an argon atmosphere unless otherwise noted. Analytical thin layer chromatography was visualized by ultraviolet light or by using phosphomolybdic acid as a universal stain. Flash chromatography was carried out under a positive pressure of nitrogen. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker AVANCE III 500 (500 MHz) spectrometer; chemical shift (δ) values were referenced to tetramethylsilane as internal standard and reported as follows: (δ) shift (multiplicity, coupling constants, proton count). Coupling constants were taken directly from spectra and are uncorrected. ^{13}C NMR spectra were recorded at 125 MHz, and all chemical shifts are reported in ppm on the δ scale with an internal reference of δ 77.16 or 49.0 for $CDCl_3$ or CD_3OD, respectively. High-resolution mass spectra (HRMS) were measured with an Agilent 6210 LC-TOF (ESI, APCI, APPI) mass spectrometer. The purity of the synthesized final compounds was determined by HPLC analysis to be ≥95%. The column used was a Phenomenex Luna 5 μm 200 Å, 4.6 × 250 mm.

Synthesis and characterization of compounds

Benzyl 3,5-dihydroxy-3-methylpentanoate (3). (±)-Mevalonolactone **1** (268 mg, 2.0 mmol) was added to a solution of KOH (123 mg, 2.2 mmol) in H_2O (4 mL). The solution was stirred at 40 ºC for 1 h, adjusted to pH 7-8 with aqueous HCl (0.1 M), and lyophilized. The residue was mixed with benzyl bromide (363 μL, 3.0 mmol) and tetrabutylammonium bromide (967 mg, 3.0 mmol) in THF (8 mL), and stirred at 50 ºC for 4 h. After the mixture was diluted with AcOEt and brine, the organic layer was partitioned, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **3** (329 mg, 69%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.38 (m, 5H, aromatic), 5.17 (s, 2H, benzyl-CH_2), 4.04 (br s, 1H, OH), 3.88 (m, 1H, CH_2CH_2O), 3.81 (m, 1H, CH_2CH_2O), 2.90 (br s, 1H, OH), 2.69 (d, 1H, OC(O)CH_2, *J* = 15.5 Hz), 2.52 (d, 1H, OC(O)CH_2, *J* = 15.5 Hz), 1.80 (m, 1H, CH_2CH$_2$O), 1.74 (m, 1H, CH_2CH$_2$O), 1.32 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 135.3, 128.7, 128.5, 128.4, 72.2, 66.7, 59.4, 45.1, 42.1, 26.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{18}NaO_4$: 261.1103, found: 261.1105.

Benzyl 2-(4-methyl-2-oxo-1,3-dioxan-4-yl)acetate (4) To a solution of **3** (436 mg, 1.83 mmol) in CH_2Cl_2 (15 mL) was added pyridine (224 μL, 2.75 mmol), and the mixture was stirred at 0 ºC for 15 min. A solution of triphosgene (98%, 665 mg, 2.20 mmol) in CH_2Cl_2 (5 mL) was added to the mixture, and the resulting mixture was stirred at 0 ºC for 30 min. The reaction was quenched with saturated aqueous NH_4Cl and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **4** (425 mg, 88%) as a light yellow oil.

Synthesis from **7**: To a solution of **7** (324 mg, 0.919 mmol) in THF (8 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 1.10 mL, 1.10 mmol) and AcOH (1.0 M

solution in THF, 2.20 mL, 2.2 mmol) at 0 ºC, and the mixture was stirred at 0 ºC for 24 h. The mixture was diluted with AcOEt and washed with aqueous $NaHCO_3$. The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified quickly by silica gel column chromatography (50% to 80% AcOEt in hexane) to give compound **6**. After the residue was dissolved in CH_2Cl_2 (8 mL), pyridine (163 μL, 2.0 mmol) was added to the solution, and the mixture was cooled at 0 ºC. To the mixture was added a solution of triphosgene (98%, 306 mg, 1.0 mmol) in CH_2Cl_2 (1 mL), and the resulting mixture was stirred at 0 ºC for 30 min. The reaction was quenched with addition of saturated aqueous NH_4Cl and extracted with AcOEt. The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (33% to 50% AcOEt in hexane) to give **4** (155 mg, 64% for 2 steps) as a light yellow oil, and **1** was recovered (39 mg, 12%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.40 (m, 5H, aromatic), 5.14 (s, 2H, benzyl-CH_2), 4.42 (m, 2H, CH_2CH_2O), 2.83 (s, 2H, $OC(O)CH_2$), 2.36 (m, 1H, CH_2CH_2O), 2.08 (m, 1H, CH_2CH_2O), 1.57 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 148.5, 135.1, 128.6, 128.5, 128.4, 81.0, 66.9, 64.5, 44.8, 30.4, 25.8; HRMS (pos. ion ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{16}NaO_5$: 287.0890, found: 287.0899.

4-((*tert*-butyldimethylsilyl)oxy)butan-2-one (6). To a solution of 4-hydryoxy-2-butanone (273 μL, 3.0 mmol) in DMF (20 mL) was added *tert*-butylchlorodimethylsilane (559 mg, 3.6 mmol) and imidazole (490 mg, 7.2 mmol) at 0 ºC, and the mixture was stirred at room temperature for 12 h. After the addition of MeOH, the mixture was diluted with Et_2O and washed with H_2O (x 3). The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (5% AcOEt in hexane) to give **6** (548 mg, 90%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (t, 2H, CH_2CH_2O, J = 6.3 Hz), 2.62 (t, 2H, CH_2CH_2O, J = 6.3 Hz), 2.19 (s, 3H, CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 0.05 (s,

6H, Si(CH_3)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.2, 58.8, 46.5, 30.9, 25.8, 18.2, –5.5; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{10}H_{22}NaO_2Si$: 225.1287, found: 225.1280.

Benzyl 5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-3-methylpentanoate (7). To a solution of lithium diisopropylamide (1.8 M solution in heptane/THF/ethyl benzene, 7.93 mL, 14.3 mmol) in THF (105 mL) was added benzyl acetate (2.04 mL, 14.3 mmol) at –78 ºC, and the mixture was stirred at –78 ºC for 30 min. A solution of **6** (2.41 g, 11.9 mmol) in THF (5 mL) was added to the mixture via cannula at –78 ºC, and the resulting mixture was stirred at –78 ºC for 1 h. After addition of sat. aq. NH_4Cl, the mixture was warmed to room temperature and evaporated. The residue was partitioned between AcOEt and sat. aq. NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (8% AcOEt in hexane) to give **7** (3.92 mg, 93%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.35 (m, 5H, aromatic), 5.17 (d, 1H, benzyl-CH_2, *J* = 7.5 Hz), 5.12 (d, 1H, benzyl-CH_2, *J* = 7.5 Hz), 4.20 (s, 1H, OH), 3.86 (t, 2H, CH_2CH_2O, *J* = 3.6 Hz), 2.66 (d, 1H, OC(O)CH_2, *J* = 15.6 Hz), 2.60 (d, 1H, OC(O)CH_2, *J* = 15.6 Hz), 1.82 (m, 2H, CH_2CH_2O), 1.30 (s, 3H, CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$); HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{19}H_{32}NaO_4Si$: 375.1962, found: 375.1965.

2-(4-Methyl-2-oxo-1,3-dioxan-4-yl)acetic acid (8). To a solution of **4** (340 mg, 1.29 mmol) in AcOEt (15 mL) was added Pd/C (10%, 33 mg), and the mixture was stirred under H_2 gas at room temperature for 30 min. The resulting mixture was filtered through Celite with acetone, and the filtrate was evaporated to give **8** (224 mg, quant.) as a colorless solid; mp. 83-85 ºC; ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (m, 2H, CH_2CH_2O), 2.82 (d, 1H, OC(O)CH_2, *J* = 15.5 Hz), 2.78 (d, 1H, OC(O)CH_2, *J* = 15.5 Hz), 2.48 (m, 1H, CH_2CH_2O), 2.12 (m, 1H, CH_2CH_2O), 1.57 (s, 3H,

CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 172.5, 151.9, 83.3, 66.2, 45.2, 31.3, 26.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_7H_{10}NaO_5$: 197.0420, found: 197.0426.

Phenyl 2-(4-methyl-2-oxo-1,3-dioxan-4-yl)acetate (9a). To a solution of **8** (35 mg, 0.20 mmol) in CH_3CN/CH_2Cl_2 (1/1, 1.5 mL) was added phenol (85 mg, 0.90 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (38 mg, 0.20 mmol), and DMAP (20 mg, 0.16 mmol) at 0 ºC, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt and partitioned between AcOEt and aq. HCl (0.5 M). The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (33% to 50% AcOEt in hexane) to give **9a** (27 mg, 54%) as a white solid; mp. 61-63 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ 7.40 (t, 2H, aromatic, *J* = 7.9, 8.0 Hz), 7.26 (t, 1H, aromatic, *J* = 8.0 Hz), 7.09 (d, 2H, aromatic, *J* = 7.9 Hz), 4.49 (t, 2H, CH_2CH_2O, *J* = 5.1 Hz), 3.05 (s, 2H, $OC(O)CH_2$), 2.47 (m, 1H, CH_2CH_2O), 2.19 (m, 1H, CH_2CH_2O), 1.68 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6, 150.0, 148.4, 129.6, 126.3, 121.3, 81.0, 64.5, 44.9, 30.6, 25.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{14}NaO_5$: 273.0733, found: 273.0731.

4-Fluorophenyl 2-(4-methyl-2-oxo-1,3-dioxan-4-yl)acetate (9b). 9b (35 mg, 65%, white solid) was prepared from **8** (35 mg, 0.20 mmol) as described for the preparation of **9a** using 4-fluorophenol instead of phenol; mp. 68-69 ºC; ^1H NMR (500 MHz, $CDCl_3$) δ 7.06-7.08 (m, 4H, aromatic), 4.48 (m, 2H, CH_2CH_2O), 3.03 (s, 2H, $OC(O)CH_2$), 2.48 (m, 1H, CH_2CH_2O), 2.17 (m, 1H, CH_2CH_2O), 1.67 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6, 160.4(d), 148.4, 145.8, 122.8(d), 116.3(d), 80.9, 64.5, 44.9, 30.6, 25.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{13}FNaO_5$: 291.0639, found: 291.0648.

2-(4-Methyl-2-oxo-1,3-dioxan-4-yl)-*N*-phenylacetamide (10a). To a solution of **8** (29 mg, 0.17 mmol) in DMF (1 mL) was added aniline (45 μL, 0.50 mmol), HBTU (64 mg, 0.17 mmol), and *N*,*N*-diisopropylethylamine (59 μL, 0.34 mmol) at 0 ºC, and the mixture was stirred at room temperature for 8 h. The mixture was diluted with AcOEt and partitioned between AcOEt and aq. HCl (0.5 M). The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **10a** (23 mg, 55%) as a colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (br s, 1H, NH), 7.54 (d, 2H, aromatic, *J* = 7.8 Hz), 7.33 (t, 2H, aromatic, *J* = 7.8, 8.2 Hz), 7.13 (t, 1H, aromatic, *J* = 8.2 Hz), 4.51 (m, 2H, CH_2CH_2O), 2.85 (s, 2H, $OC(O)CH_2$), 2.52 (m, 1H, CH_2CH_2O), 2.20 (m, 1H, CH_2CH_2O), 1.64 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 148.9, 137.4, 129.0, 124.7, 120.1, 82.4, 65.0, 48.4, 30.7, 25.6; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{15}NNaO_4$: 272.0893, found: 272.0894.

***N*-Benzyl-2-(4-methyl-2-oxo-1,3-dioxan-4-yl)acetamide (10b). 10b** (27 mg, 61%, colorless oil) was prepared from **8** (29 mg, 0.17 mmol) as described for the preparation of **10a** using benzylamine instead of aniline. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.35 (m, 5H, aromatic), 6.47 (br s, 1H, NH), 4.38-4.46 (m, 4H, CH_2CH_2O and benzyl-CH_2), 2.68 (d, 1H, $OC(O)CH_2$, *J* = 14.5 Hz), 2.63 (d, 1H, $OC(O)CH_2$, *J* = 14.5 Hz), 2.46 (m, 1H, CH_2CH_2O), 2.09 (m, 1H, CH_2CH_2O), 1.55 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 148.7, 137.8, 128.8, 127.8, 127.7, 82.0, 64.9, 47.5, 43.8, 30.7, 25.7; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{17}NNaO_4$: 286.1055, found: 286.1052.

***N*-(4-Fluorobenzyl)-2-(4-methyl-2-oxo-1,3-dioxan-4-yl)acetamide (10c). 10c** (19 mg, 38%, colorless oil) was prepared from **8** (29 mg, 0.17 mmol) as described for the preparation of **10a**

using 4-fluorobenzylamine instead of aniline. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24-7.27 (m, 2H, aromatic), 6.99-7.02 (m, 2H, aromatic), 6.70 (br s, 1H, NH), 4.37-4.44 (m, 4H, CH_2CH_2O and benzyl-CH_2), 2.66 (s, 2H, $OC(O)CH_2$), 2.45 (m, 1H, CH_2CH_2O), 2.10 (m, 1H, CH_2CH_2O), 1.54 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 162.2(d), 147.8, 134.0(d), 127.46 , 115.6(d), 81.8, 64.2, 46.5, 43.1, 29.2, 25.0; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{16}FNNaO_4$: 304.0956, found: 304.0960.

Dibenzyl 3-(fluoromethyl)-3-hydroxypentanedioate (14). To a solution of ethyl fluoroacetate (1.94 mL, 20.0 mmol) in Et_2O (120 mL) was added allylmagnesium bromide (1.0 M solution in Et_2O, 39.0 mL, 39.0 mmol) at 0 ºC, and the mixture was stirred at 0 ºC for 20 min. After addition of saturated aqueous NH_4Cl, the organic layer was separated, washed with brine, dried with Na_2SO_4, and evaporated. The residue (**12**) was used in the next reaction without further purification. The crude product **12** (2.60 g) was dissolved in CH_2Cl_2 (40 mL) and cooled to –78 ºC. Ozone was bubbled into the solution at –78 ºC for 30 min until the color of the solution turned to light purple. Oxygen was bubbled into the solution for 20 min to remove ozone, and the solution was warmed to room temperature. Acetic acid (20 mL) was added to the solution and then the solvent was reduced *in vacuo* until the amount of the solution was a few milliliters. To the residue was added acetic acid (15 mL), H_2O (15 mL), conc .H_2SO_4 (0.40 mL), and aq. H_2O_2 (30%, 9.0 mL), and the mixture was stirred under reflux for 4 h. After cooling to room temperature, the mixture was neutralized with $BaCO_3$ (1.5 g) and filtered through Celite with acetone. To the filtrate was added Pd/C (30 mg), and the mixture was stirred at room temperature for 8 h to decompose the H_2O_2. The mixture was filtered through Celite with acetone to remove the Pd/C, and the filtrate was evaporated. The residue was co-evaporated with H_2O (x 2) and toluene (x 3) to give crude dicarboxylic acid **13** as a brown oil. To a solution of crude

product **13** in DMF (80 mL) was added benzyl bromide (98%, 4.85 mL, 40 mmol) and K_2CO_3 (5.53 g, 40 mmol), and the mixture was stirred at room temperature for 8 h. After filtration through Celite to remove K_2CO_3, the solvent was evaporated. The residue was partitioned between AcOEt and aq. HCl (0.5 M). The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (10% to 20% AcOEt in hexane) to give diester **14** (3.87 g, 54% for 4 steps) as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.38 (m, 10H, aromatic), 5.14 (s, 4H, benzyl-CH_2 x2), 4.42 (d, 2H, C*H*$_2$F, *J* = 47 Hz), 4.25 (s, 1H, OH), 2.78 (m, 4H, C(O)C*H*$_2$ x2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 135.3, 128.6, 128.5, 128.3, 86.4(d), 71.1, 66.8, 39.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{20}H_{21}FNaO_5$: 383.1265, found: 383.1276.

Benzyl 2-(4-(fluoromethyl)-2-oxo-1,3-dioxan-4-yl)acetate (16). To a solution of **14** (280 mg, 0.777 mmol) in THF (8 mL) was added DIBAL-H (1.0 M solution in THF, 0.932 mL, 0.932 mmol) at 0 ºC, and the mixture was stirred at 0 ºC for 10 min. DIBAL-H (1.0 M solution in THF, 1.86 mL, 1.86 mmol) was added to the mixture at 0 ºC, and the resulting mixture was stirred at 0 ºC for 15 min. The reaction was quenched with addition of aq. HCl (1.5 M) that was saturated with NaCl and extracted with AcOEt. The organic layer was washed with aq. HCl (1.5 M), which was saturated with NaCl, sat. aq. $NaHCO_3$, and brine, and then dried (Na_2SO_4) and evaporated. After the residue (**15**) was dissolved in CH_2Cl_2 (60 mL), pyridine (285 μL, 3.50 mmol) was added to the solution and the mixture was cooled at 0 ºC. To the mixture was added a solution of triphosgene (706 mg, 2.33 mmol) in CH_2Cl_2 (4 mL), and the resulting mixture was stirred at 0 ºC for 30 min. The reaction was quenched with addition of sat. aq. NH_4Cl and extracted with AcOEt. The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (33% to 50% AcOEt

in hexane) to give **16** (81 mg, 37% for 2 steps) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.40 (m, 5H, aromatic), 5.15 (s, 2H, benzyl-CH_2), 4.62 (dd, 1H, C*H*$_2$F, *J* = 10.0, 12.6 Hz), 4.54 (dd, 1H, C*H*$_2$F, *J* = 10.0, 12.2 Hz), 4.40 (m, 2H, CH_2CH_2O), 2.86 (m, 2H, C(O)C*H*$_2$), 2.37 (m, 1H, C*H*$_2$$CH_2O$), 2.30 (m, 1H, C*H*$_2$$CH_2O$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0, 148.1, 134.9, 128.7, 128.7, 128.5, 85.0(d), 81.1, 67.2, 64.1, 39.8, 25.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{15}FNaO_5$: 305.0796, found: 305.0800.

2-(4-(Fluoromethyl)-2-oxo-1,3-dioxan-4-yl)acetic acid (17). 17 (249 mg, 99%, colorless oil) was prepared from **16** (368 mg, 1.30 mmol) as described for the preparation of **8**. ^{1}H NMR (500 MHz, acetone-d_6) δ 4.72 (d, 2H, C*H*$_2$F, *J* = 48 Hz), 4.48 (m, 2H, CH_2CH_2O), 2.94 (m, 2H, C(O)C*H*$_2$), 2.47 (m, 1H, C*H*$_2$$CH_2O$), 2.32 (m, 1H, C*H*$_2$$CH_2O$); ^{13}C NMR (125 MHz, acetone-d_6) δ 170.6, 148.7, 86.4(d), 82.3, 64.9, 39.4, 26.4; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_7H_9FNaO_5$: 215.0326, found: 215.0330.

4-Fluorobenzyl 2-(4-(fluoromethyl)-2-oxo-1,3-dioxan-4-yl)acetate (18a). 18a (41 mg, 77%, colorless oil) was prepared from **17** (34 mg, 0.18 mmol) as described for the preparation of **9a** using 4-fluorobenzyl bromide instead of iodomethane. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.36 (m, 2H, aromatic), 7.05-7.08 (m, 2H, aromatic), 5.12 (s, 2H, benzyl-CH_2), 4.61 (dd, 1H, C*H*$_2$F, *J* = 10.0, 14.0 Hz), 4.52 (dd, 1H, C*H*$_2$F, *J* = 10.0, 13.6 Hz), 4.42 (m, 2H, CH_2CH_2O), 2.86 (m, 2H, C(O)C*H*$_2$), 2.35 (m, 1H, C*H*$_2$$CH_2O$), 2.28 (m, 1H, C*H*$_2$$CH_2O$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0, 162.8(d), 148.0, 130.8(d), 130.7(d), 115.7(d), 85.0(d), 81.0, 66.5, 64.1, 40.0, 26.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{14}F_2NaO_5$: 323.0702, found: 323.0707.

2,4-Difluorobenzyl 2-(4-(fluoromethyl)-2-oxo-1,3-dioxan-4-yl)acetate (18b). 18b (36 mg, 72%, colorless oil) was prepared from **17** (30 mg, 0.16 mmol) as described for the preparation of

9a using 2,4-difluorobenzyl bromide instead of iodomethane. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (m, 1H, aromatic), 6.83-6.92 (m, 2H, aromatic), 5.17 (s, 2H, benzyl-CH_2), 4.62 (dd, 1H, C*H*$_2$F, *J* = 10.0, 13.1 Hz), 4.52 (dd, 1H, C*H*$_2$F, *J* = 10.0, 12.8 Hz), 4.42 (m, 2H, CH_2C*H*$_2$O), 2.85 (m, 2H, C(O)C*H*$_2$), 2.37 (m, 1H, C*H*$_2$$CH_2$O), 2.29 (m, 1H, C*H*$_2$$CH_2$O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 163.4(d), 161.4(d), 148.0, 132.2(m), 118.2(d), 111.7(m), 104.2(t), 85.0(d), 81.0, 64.2, 60.6, 39.9, 26.0; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{13}F_3NaO_5$: 341.0607, found: 341.0612.

Benzyl 5-ethoxy-3-hydroxy-3-(trifluoromethyl)pent-4-enoate (20). To a solution of lithium diisopropylamide (1.8 M solution in heptane/THF/ethyl benzene, 3.05 mL, 5.50 mmol) in THF (55 mL) was added benzyl acetate (784 μL, 5.50 mmol) at –78 ºC, and the mixture was stirred at –78 ºC for 30 min. A solution of 4-ethoxy-1,1,1,- trifluoro-3-buten-2-one (**19**, 712 μL, 5.00 mmol) in THF (5 mL) was added to the mixture via cannula at –78 ºC, and the resulting mixture was stirred at –78 ºC for 30 min. After the addition of sat. aq. NH_4Cl, the mixture was warmed to room temperature, and evaporated. The residue was partitioned between AcOEt and sat. aq. NH_4Cl. The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (6% AcOEt in hexane) to give **20** (1.36 g, 86%) as a light yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.40 (m, 5H, aromatic), 6.74 (d, 1H, CH=C*H*OEt, *J* = 12.6 Hz), 5.20 (d, 1H, benzyl-CH_2, *J* = 14.1 Hz), 5.16 (d, 1H, benzyl-CH_2, *J* = 14.1 Hz), 4.76 (s, 1H, OH), 4.71 (d, 1H, C*H*=CHOEt, *J* = 12.6 Hz), 3.70 (q, 2H, C*H*$_2$$CH_3$), 2.86 (d, 1H, C(O)C*H*$_2$, *J* = 15.6 Hz), 2.71 (d, 1H, C(O)C*H*$_2$, *J* = 15.6 Hz), 1.27 (t, 3H, CH_2C*H*$_3$, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 151.8, 134.8, 128.7, 128.5, 128.3, 124.6(q), 99.4, 73.3(q), 67.5, 65.5, 38.9, 14.7; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{15}H_{17}F_3NaO_4$: 341.0977, found: 341.0978.

Benzyl 4,4,4-trifluoro-3-formyl-3-hydroxybutanoate (21). To a solution of **20** (334 mg, 1.05 mmol) in acetone (16 mL) was added aq. HCl (12 M, 4 mL) at 0 ºC, and the mixture was stirred vigorously at 0 ºC for 8 min. To the mixture was added sat. aq. $NaHCO_3$ to neutralize and extracted with AcOEt. The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (20% AcOEt in hexane) to give **21** (243 mg, 80%) as a light yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (s, 1H, CHO), 7.34-7.40 (m, 5H, aromatic), 5.35 (s, 1H, OH), 5.19 (s, 2H, benzyl-CH_2), 2.75-2.93 (m, 4H, C*H*$_2$CHO and C(O)C*H*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.0, 170.8, 134.5, 128.8, 128.7, 128.5, 124.8(q), 73.3(q), 67.7, 46.1, 36.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{13}F_3NaO_4$: 313.0658, found: 313.0660.

Benzyl 2-(2-oxo-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetate (23). To a solution of **21** (320 mg, 1.10 mmol) in benzene (10 mL) was added sodium triacetoxyborohydride (95%, 701 mg, 3.31 mmol) at 0 ºC, and the mixture was stirred at room temperature for 2 h. The mixture was quenched with addition of sat. aq. $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated. After the residue was dissolved in CH_2Cl_2 (10 mL), pyridine (125 μL, 1.54 mmol) was added to the solution, and the mixture was cooled at 0 ºC. To the mixture was added a solution of triphosgene (98%, 400 mg, 1.32 mmol) in CH_2Cl_2 (2 mL) at 0 ºC, and the resulting mixture was stirred at 0 ºC for 30 min. The reaction was quenched with addition of sat. aq. NH_4Cl, and extracted with AcOEt. The organic layer was washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (33% AcOEt in hexane) to give **23** (281 mg, 80% for 2 steps) as a colorless solid; mp. 87-88 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.41 (m, 5H, aromatic), 5.18 (s, 2H, benzyl-CH_2), 4.43 (m, 1H, CH_2CH_2O), 4.37 (m, 1H, CH_2CH_2O), 3.10 (d,

1H, C(O)CH_2, *J* = 16.5 Hz), 2.87 (d, 1H, C(O)CH_2, *J* = 16.5 Hz), 2.74 (m, 1H, CH_2CH$_2$O), 2.33 (m, 1H, CH_2CH$_2$O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 146.8, 134.8, 128.8, 128.7, 128.5, 123.6(q), 80.2(q), 67.3, 64.1, 37.9, 24.2; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{13}F_3NaO_5$: 341.0607, found: 341.0613.

2-(2-Oxo-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetic acid (24) **24** (176 mg, 98%, colorless oil) was prepared from **23** (250 mg, 0.786 mmol) as described for the preparation of **8**. ^{1}H NMR (500 MHz, D_2O) δ 4.61 (m, 2H, CH$_2$CH_2O), 3.22 (d, 1H, C(O)CH_2, *J* = 16.5 Hz), 3.12 (d, 1H, C(O)CH_2, *J* = 16.5 Hz), 2.76 (m, 1H, CH_2CH$_2$O), 2.59 (m, 1H, CH_2CH$_2$O); ^{13}C NMR (125 MHz, D_2O) δ 171.4, 150.4, 123.4(q), 81.1(q), 65.3, 36.8, 23.5;HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_7H_7F_3NaO_5$: 251.0138, found: 251.0140.

4-Fluorobenzyl 2-(2-oxo-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetate (25a). Ester **25a** (48 mg, 88%, white solid) was prepared from **24** (39 mg, 0.17 mmol) as described for the preparation of **18a**; mp. 84-85 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 2H, aromatic), 7.07 (m, 2H, aromatic), 5.14 (s, 2H, benzyl-CH$_2$), 4.37-4.47 (m, 2H, CH$_2$CH_2O), 3.09 (d, 1H, C(O)CH_2, *J* = 16.4 Hz), 2.86 (d, 1H, C(O)CH_2, *J* = 16.4 Hz), 2.73 (m, 1H, CH_2CH$_2$O), 2.33 (m, 1H, CH_2CH$_2$O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.1, 162.9(d), 146.7, 130.8(d), 130.7, 123.6(q), 115.6(d), 80.1(q), 66.8, 64.0, 37.7, 24.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{12}F_4NaO_5$: 359.0519, found: 359.0518.

2,4-Difluorobenzyl 2-(2-oxo-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetate (25b). Ester **25b** (45 mg, 85%, colorless oil) was prepared from **24** (36 mg, 0.16 mmol) as described for the preparation of **18b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (m, 1H, aromatic), 6.88 (m, 2H, aromatic), 5.21 (d, 1H, benzyl-CH$_2$, *J* = 12.2 Hz), 5.17 (d, 1H, benzyl-CH$_2$, *J* = 12.2 Hz), 4.45

(m, 2H, CH_2CH_2O), 3.09 (d, 1H, C(O)CH_2, J = 16.4 Hz), 2.87 (d, 1H, C(O)CH_2, J = 16.4 Hz), 2.75 (m, 1H, C$H_2$$CH_2O$), 2.35 (m, 1H, C$H_2$$CH_2O$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 163.4(d), 161.4(d), 146.6, 132.3(m), 123.5(q), 118.0(d), 111.6(d), 104.2(t), 80.0(q), 64.1, 60.9, 37.6, 24.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{11}F_5NaO_5$: 377.0419, found: 377.0427.

4-Hydroxy-4-(trifluoromethyl)tetrahydro-2*H*-pyran-2-one (26). To a solution of **23** (267 mg, 0.919 mmol) in benzene (10 mL) was added sodium triacetoxyborohydride (95%, 615 mg, 2.76 mmol) at 0 ºC, and the mixture was stirred at room temperature for 2 h. The mixture was quenched with addition of sat. aq. $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated. After the residue was dissolved in CH_2Cl_2 (10 mL), trifluoroacetic acid (1 mL) was added to the solution at 0 ºC, and the mixture was stirred at room temperature for 2 h. The mixture was diluted with AcOEt and evaporated. The residue was purified by silica gel column chromatography (33% AcOEt in hexane) to give **26** (135 mg, 80% for 2 steps) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.61 (m, 1H, CH_2CH_2O), 4.45 (m, 1H, CH_2CH_2O), 2.86 (d, 1H, CH_2C(O), J = 14.7 Hz), 2.84 (br s, 1H, OH), 2.78 (d, 1H, CH_2C(O), J = 14.7 Hz), 2.24 (m, 1H, C$H_2$$CH_2O$), 2.07 (m, 1H, C$H_2$$CH_2O$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 124.7(q), 71.5(q), 64.4, 36.9, 28.6; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_6H_7F_3NaO_3$: 207.0239, found: 207.0233.

3,5-Dihydroxy-3-(trifluoromethyl)pentanoic acid (27). To a solution of **26** (10 mg, 0.054 mmol) in H_2O (1 mL) was added KOH (>90%, 3.4 mg, 0.052 mmol) at room temperature, and the mixture was stirred at 40 ºC for 2 h. The pH of the solution was lowered to about pH 7-8 (detected by pH indicator paper) with aq. HCl (0.1 M). The solvent was evaporated and lyophilized to give **27** (15 mg) as a white powder, including KCl. ^{1}H NMR (500 MHz, D_2O) δ

3.79 (m, 2H, CH_2CH_2O), 2.60 (d, 1H, C(O)CH_2, J = 15.5 Hz), 2.52 (d, 1H, C(O)CH_2, J = 15.5 Hz), 2.04 (m, 1H, C$H_2$$CH_2O$), 1.96 (m, 1H, C$H_2$$CH_2O$); ^{13}C NMR (125 MHz, D_2O) δ 178.4, 125.9(q), 73.2(q), 56.4, 38.3, 35.9; LRMS (ESI) m/z = 225 $[M+Na]^+$.

***N*-Benzyl-3,5-dihydroxy-3-methylpentanamide (29a)** To a solution of (±)-mevalonolactone (**1**, 97%, 134 mg, 1.00 mmol) in DMF (1 mL) was added benzyl amine (131 μL, 1.20 mmol) at room temperature, and the mixture was stirred at 80 ºC for 12 h. After being evaporated, the residue was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue (**29a**, light yellow oil) was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.18 (m, 5H, aromatic), 6.68 (br s, 1H, NH), 4.39 (d, 2H, PhCH_2N, J = 5.8 Hz), 3.82 (ddd, 1H, CH_2CH_2O, J = 11.5, 7.4, 4.3 Hz), 3.74 (ddd, 1H, CH_2CH_2O, J = 11.1, 6.7, 4.4 Hz), 2.48 (d, 1H, NHC(O)CH_2, J = 14.6 Hz), 2.26 (d, 1H, NHC(O)CH_2, J = 14.6 Hz), 1.74 (ddd, 1H, C$H_2$$CH_2O$, J = 14.5, 7.4, 4.4 Hz, 1H), 1.66 (ddd, 1H, C$H_2$$CH_2O$, J = 14.6, 6.7, 4.3 Hz), 1.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 138.0, 128.7, 127.6, 127.5, 72.3, 59.2, 46.6, 43.3, 42.0, 26.9; LRMS (ESI) m/z = 237.1 $[M+H]^+$.

***N*-Benzyl-2-(4-methyl-1,3-dioxan-4-yl)acetamide (30a).** To a solution of the crude product **29a** (26 mg, ≤0.11 mmol) in CH_2Cl_2 was added chloromethyl methyl ether (84 μL, 1.1 mmol), *N*,*N*-diisopropylethylamine (383 μL, 2.2 mmol), and DMAP (1.2 mg) at 0 ºC, and the mixture was stirred at room temperature for 2 h. After dilution with AcOEt, the mixture was partitioned between AcOEt and 0.5 M aq. HCl. The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated. The residue was dissolved in CH_2Cl_2 (17 mL), and BF_3•Et_2O (30 μL, 0.24 mmol) was added at 0 ºC. The mixture was stirred at room temperature for 8 h. After addition of sat. aq. $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was

washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **30a** (21 mg, 77% for 3 steps) as a white solid; mp. 82-84 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.35 (m, 5H, aromatic), 6.80 (br s, 1H, NH), 4.92 (d, 1H, OC*H*$_2$O, *J* = 6.5 Hz), 4.85 (d, 1H, OC*H*$_2$O, *J* = 6.5 Hz), 4.48 (d, 2H, benzyl-CH_2, *J* = 5.5 Hz), 3.95 (m, 1H, CH_2C*H*$_2$O), 3.89 (m, 1H, CH_2C*H*$_2$O), 2.60 (d, 1H, NHC(O)C*H*$_2$, *J* = 14.5 Hz), 2.47 (d, 1H, NHC(O)C*H*$_2$, *J* = 14.5 Hz), 2.02 (m, 1H, C*H*$_2$$CH_2$O), 1.48 (m, 1H, C*H*$_2$$CH_2$O), 1.40 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 138.5, 128.6, 127.5, 127.3, 87.8, 72.1, 62.8, 49.0, 43.3, 34.7, 20.9; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{19}NNaO_3$: 272.1257, found: 272.1259.

***N*-Benzyl-2-(2,2,4-trimethyl-1,3-dioxan-4-yl)acetamide (31).** To a solution of the crude product **29a** (55 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was added 2,2-dimethoxypropane (112 μL, 0.91 mmol) and camphor sulfonic acid (4 mg, 0.02 mmol) at 0 ºC, and the mixture was stirred at room temperature for 16 h. After addition of sat. aq. $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **31** (17 mg, 27% for 2 steps) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.35 (m, 5H, aromatic), 6.97 (br s, 1H, NH), 4.53 (dd, 1H, benzyl-CH_2, *J* = 6.0, 14.8 Hz), 4.39 (dd, 1H, benzyl-CH_2, *J* = 5.3, 14.8 Hz), 4.02 (m, 1H, CH_2C*H*$_2$O), 3.83 (m, 1H, CH_2C*H*$_2$O), 2.49 (d, 1H, NHC(O)C*H*$_2$, *J* = 14.4 Hz), 2.44 (d, 1H, NHC(O)C*H*$_2$, *J* = 14.4 Hz), 1.91 (m, 1H, C*H*$_2$$CH_2$O), 1.51 (m, 1H, C*H*$_2$$CH_2$O), 1.41 (s, 6H, C($CH_3$)$_2$), 1.23 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 138.4, 128.6, 127.7, 127.4, 98.5, 72.0, 56.5, 51.1, 43.4, 33.3, 29.9, 26.7, 25.7; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{16}H_{23}NNaO_3$: 300.1570, found: 300.1586.

***N*-Benzyl-2-(4-methyl-2-phenyl-1,3-dioxan-4-yl)acetamide (32a).** To a solution of the crude product **29a** (54 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was added benzaldehyde dimethyl acetal (51 μL, 0.34 mmol) and camphor sulfonic acid (4 mg, 0.02 mmol) at 0 ºC, and the mixture was stirred at room temperature for 16 h. After addition of sat. aq. $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (33-50% AcOEt in hexane) to give **32a** (25 mg, 34% for 2 steps) as a white solid; mp. 108-110 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20-7.32 (m, 10H, aromatic), 6.83 (br s, 1H, NH), 5.72 (s, 1H, benzyl-CH), 4.47 (dd, 1H, benzyl-CH_2, J = 5.8, 14.6 Hz), 4.38 (dd, 1H, benzyl-CH_2, J = 5.3, 14.6 Hz), 4.14 (m, 2H, CH_2CH_2O), 2.55 (s, 2H, NHC(O)CH_2), 2.16 (m, 1H, CH_2CH_2O), 1.53 (s, 3H, CH_3), 1.46 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 138.3, 138.1, 128.9, 128.7, 128.4, 127.9, 127.4, 125.8, 95.3, 73.4, 63.2, 50.8, 43.6, 33.6, 20.3; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{20}H_{23}NNaO_3$: 348.1570, found: 348.1569.

5-(Benzylamino)-3-hydroxy-3-methyl-5-oxopentyl *tert*-butyl carbonate (33a). To a solution of crude product **29a** (24 mg, ≤0.10 mmol) in acetonitrile (1 mL) was added $(Boc)_2O$ (99 mg, 0.45 mmol) and DMAP (1.2 mg, 0.01 mmol) at room temperature, and the mixture was stirred under reflux conditions for 16 h. After evaporated, the residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **32a** (30 mg, 88% for 2 steps) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.36 (m, 5H, aromatic), 6.23 (br s, 1H, NH), 4.65 (s, 1H, OH), 4.46 (m, 2H, benzyl-CH_2), 4.22 (m, 2H, CH_2CH_2O), 2.45 (d, 1H, NHC(O)CH_2, J = 14.5 Hz), 2.34 (d, 1H, NHC(O)CH_2, J = 14.5 Hz), 1.90 (t, 2H, CH_2CH_2O, J = 6.8 Hz), 1.45 (s, 9H, $C(CH_3)_3$), 1.28 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 153.4, 137.8, 128.8, 127.8,

127.7, 82.2, 70.5, 63.5, 46.4, 43.5, 40.2, 27.8, 27.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{18}H_{27}NNaO_5$: 360.1781, found: 360.1785.

***N*-Benzyl-3,5-dihydroxy-3-(trifluoromethyl)pentanamide (29b).** To a solution of **26** (120 mg, 0.652 mmol) in DMF was added benzyl amine (142 μL, 1.30 mmol), and the mixture was stirred at 80 ºC for 12 h. After the solvent was evaporated, the residue was purified by silica gel column chromatography (50-100% AcOEt in hexane) to give **29b** (189 mg, 99%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.37 (m, 2H, aromatic), 7.26-7.32 (m, 3H, aromatic), 6.62 (s, 1H, OH), 6.29 (br s, 1H, NH), 4.49 (dd, 1H, benzyl-CH_2, *J* = 6.0, 14.5 Hz), 4.43 (dd, 1H, benzyl-CH_2, *J* = 5.5, 14.5 Hz), 3.95 (m, 1H, CH_2CH_2O), 3.89 (m, 1H, CH_2CH_2O), 2.73 (d, 1H, NHC(O)CH_2, *J* = 14.7 Hz), 2.51 (m, 1H, OH), 2.49 (d, 1H, NHC(O)CH_2, *J* = 14.7 Hz), 2.09 (m, 1H, CH_2CH_2O), 1.84 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 137.2, 128.9, 128.7, 127.9, 125.7(q), 75.0(q), 58.4, 43.7, 38.0, 35.3; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{13}H_{16}F_3NNaO_3$: 314.0974, found: 314.0991.

***N*-Benzyl-2-(4-(trifluoromethyl)-1,3-dioxan-4-yl)acetamide (30b). 30b** (26 mg, 69% for 2 steps, colorless oil) was prepared from **29b** (36 mg, 0.12 mmol) as described for the preparation of **30a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.36 (m, 5H, aromatic), 6.43 (br s, 1H, NH), 5.06 (d, 1H, OCH_2O, *J* = 6.3 Hz), 4.94 (d, 1H, OCH_2O, *J* = 6.3 Hz), 4.55 (dd, 1H, benzyl-CH_2, *J* = 6.1, 14.9 Hz), 4.42 (dd, 1H, benzyl-CH_2, *J* = 6.0, 14.9 Hz), 3.99 (m, 2H, CH_2CH_2O), 2.76 (d, 1H, NHC(O)CH_2, *J* = 14.4 Hz), 2.56 (d, 1H, NHC(O)CH_2, *J* = 14.4 Hz), 2.37 (m, 1H, CH_2CH_2O), 1.98 (dt, 1H, CH_2CH_2O, *J* = 3.7, 3.7, 14.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 137.9, 129.1, 128.7, 128.6, 128.0, 127.5, 125.7(q), 89.9, 73.4(q), 62.5, 43.8, 41.2, 25.7; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{14}H_{16}F_3NNaO_3$: 326.0974, found: 326.0970.

***N*-Benzyl-2-(2-phenyl-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetamide (32b).** To a solution of **29b** (60 mg, 0.20 mmol) in CH_2Cl_2 (2 mL) was added benzaldehyde dimethyl acetal (151 μL, 1.00 mmol) and camphor sulfonic acid (4.7 mg, 0.02 mmol) at room temperature, and the mixture was stirred under reflux for 12 h. After addition of sat. aq. $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (10-25% AcOEt in hexane) to give *major diastereomer*-**32b** (25 mg, 33%) as a colorless oil or *minor diastereomer*-**32b** (7 mg, 9%) as a white solid. major-**32b:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.35 (m, 8H, aromatic), 7.16-7.18 (m, 2H, aromatic), 6.46 (br s, 1H, NH), 5.92 (s, 1H, benzylidene-CH), 4.41 (d, 2H, benzyl-CH_2, *J* = 5.6 Hz), 4.18 (m, 2H, CH_2CH_2O), 2.73 (d, 1H, NHC(O)CH_2, *J* = 14.3 Hz), 2.57 (d, 1H, NHC(O)CH_2, *J* = 14.3 Hz), 2.50 (m, 1H, CH_2CH_2O), 1.97 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 137.5, 129.4, 128.7, 128.5, 128.0, 127.5, 125.5(q), 98.0, 74.7(q), 63.2, 43.9, 42.8, 25.0; HRMS (pos. ion ESI) *m/z* calcd for $(M+Na)^+$ $C_{20}H_{20}F_3NNaO_3$: 402.1293. Found: 402.1285. minor-**32b:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.38 (m, 8H, aromatic), 7.21-7.23 (m, 2H, aromatic), 6.29 (br s, 1H, NH), 5.78 (s, 1H, benzylidene-CH), 4.49 (dd, 1H, benzyl-CH_2, *J* = 5.9, 14.6 Hz), 4.36 (dd, 1H, benzyl-CH_2, *J* = 5.3, 14.6 Hz), 4.27 (m, 1H, CH_2CH_2O), 4.19 (m, 1H, CH_2CH_2O), 3.01 (d, 1H, NHC(O)CH_2, *J* = 15.2 Hz), 2.94 (d, 1H, NHC(O)CH_2, *J* = 15.2 Hz), 2.36 (m, 1H, CH_2CH_2O), 2.02 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 137.5, 137.0, 129.4, 128.8, 128.4, 127.9, 127.7, 125.9(q), 96.4, 76.0(q), 62.5, 44.0, 36.6, 25.5; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{20}H_{20}F_3NNaO_3$: 402.1287, found: 402.1283.

***N*-Benzyl-2-(2-(4-methoxyphenyl)-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetamide (32c**). *major diasteromer* **32c** (30 mg, 35%, white solid) and *minor diasteromer* **32c** (22 mg, 25%, colorless oil,) were prepared from **29b** (62 mg, 0.21 mmol) as described for the preparation of

32b using anisaldehyde dimethyl acetal instead of benzaldehyde demethyl acetal: major-**32c** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27-7.31 (m, 3H, aromatic), 7.17-7.20 (m, 4H, aromatic), 6.75 (d, 2H, aromatic, *J* = 8.8 Hz), 6.50 (br s, 1H, NH), 5.86 (s, 1H, benzylidene-CH), 4.42 (d, 2H, benzyl-CH_2, *J* = 5.6 Hz), 4.15 (m, 2H, CH_2CH_2O), 3.79 (s, 3H, OCH_3), 2.73 (d, 1H, NHC(O)CH_2, *J* = 14.4 Hz), 2.56 (d, 1H, NHC(O)CH_2, *J* = 14.4 Hz), 2.46 (m, 1H, CH_2CH_2O), 1.96 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 160.2, 137.6, 129.8, 128.7, 128.1, 127.5, 125.5(q), 113.8, 97.9, 74.7(q), 63.2, 55.3, 43.9, 42.8, 25.0; HRMS (ESI) *m/z* calcd for $(M+Na)^+$ $C_{21}H_{22}F_3NNaO_4$: 432.1399. Found: 432.1408. minor-**32c** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.30 (m, 5H, aromatic), 7.22 (m, 2H, aromatic), 6.84 (d, 2H, aromatic, *J* = 8.8 Hz), 6.30 (br s, 1H, NH), 5.73 (s, 1H, benzylidene-CH), 4.51 (dd, 1H, benzyl-CH_2, *J* = 6.0, 14.6 Hz), 4.35 (dd, 1H, benzyl-CH_2, *J* = 5.2, 14.6 Hz), 4.25 (m, 1H, CH_2CH_2O), 4.17 (m, 1H, CH_2CH_2O), 3.80 (s, 3H, OCH_3), 2.99 (d, 1H, NHC(O)CH_2, *J* = 15.3 Hz), 2.95 (d, 1H, NHC(O)CH_2, *J* = 15.3 Hz), 2.34 (m, 1H, CH_2CH_2O), 1.98 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 160.3, 137.5, 129.4, 128.8, 127.9, 127.6, 124.9(q), 113.8, 96.3, 76.1(q), 62.5, 55.3, 44.0, 36.5, 25.5; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{21}H_{22}F_3NNaO_4$: 432.1393, found: 432.1389.

***N*-Benzyl-2-(2-(2,4-dimethoxyphenyl)-4-(trifluoromethyl)-1,3-dioxan-4-yl)acetamide (32d)** To a solution of **29b** (74 mg, 0.25 mmol) in benzene (3 mL) was added 2,4-dimethoxybenzaldehyde (98%, 52 mg, 0.31 mmol), camphor sulfonic acid (4.7 mg, 0.02 mmol), and 4Å molecular sieves (powder, 18 mg) at room temperature, and the mixture was stirred under reflux for 36 h. After addition of sat. aq. $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (50% AcOEt in hexane x 2) to give **31d** (7 mg, 6%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, 1H, aromatic, *J* = 8.5 Hz), 7.24-7.26 (m, 3H,

aromatic), 7.17-7.19 (m, 2H, aromatic), 6.57 (br s, 1H, NH), 6.52 (d, 1H, aromatic, *J* = 8.5 Hz), 6.32 (s, 1H, aromatic), 6.10 (s, 1H, benzylidene-CH), 4.44 (d, 2H, benzyl-CH_2, *J* = 5.4 Hz), 4.24 (m, 1H, CH_2CH_2O), 4.14 (m, 1H, CH_2CH_2O), 3.80 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.12 (d, 1H, $NHC(O)CH_2$, *J* = 15.3 Hz), 2.90 (d, 1H, $NHC(O)CH_2$, *J* = 15.3 Hz), 2.28 (m, 1H, CH_2CH_2O), 1.88 (m, 1H, CH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.7, 161.7, 157.3, 137.7, 129.4, 128.6, 128.4, 127.5, 127.3, 124.6(q), 117.9, 104.9, 98.2, 91.1, 76.1(q), 62.8, 55.4, 43.9, 36.6, 26.0; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{22}H_{24}F_3NNaO_5$: 462.1499, found: 462.1503.

5-(Benzylamino)-3-hydroxy-5-oxo-3-(trifluoromethyl)pentyl *tert*-butyl carbonate (33b). 33b (50 mg, 68%, colorless oil) was prepared from **30b** (55 mg, 0.19 mmol) as described for the preparation of **33a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.38 (m, 5H, aromatic), 6.55 (s, 1H, OH), 6.05 (br s, 1H, NH), 4.53 (dd, 1H, benzyl-CH_2, *J* = 6.0, 14.7 Hz), 4.42 (dd, 1H, benzyl-CH_2, *J* = 5.5, 14.7 Hz), 4.29 (m, 2H, CH_2CH_2O), 2.69 (d, 1H, , *J* = 15.3 Hz, $NHC(O)CH_2$), 2.50 (d, 1H, , *J* = 15.3 Hz, $NHC(O)CH_2$), 2.15 (m, 1H, CH_2CH_2O), 1.99 (m, 1H, CH_2CH_2O), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 153.1, 137.0, 128.9, 127.9, 127.6, 125.6(q), 82.5, 73.7(q), 61.8, 43.8, 37.1, 33.6, 27.7; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{18}H_{24}F_3NNaO_5$: 414.1504, found: 414.1500.

3,5-Dihydroxy-3-methyl-*N*-((perfluorophenyl)methyl)pentanamide (34). 34 (173 mg, 53%, colorless oil) was prepared from (±)-mevalonolactone and 2,3,4,5,6-pentafluorobenzylamine as described for the preparation of **29a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (bs, 1H, OH), 4.57 (s, 2H, benzyl-CH_2), 4.01 – 3.80 (m, 2H, CH_2CH_2OH), 2.54 (d, J = 14.9 Hz, 1H, $NHC(O)CH_2$), 2.30 (dd, J = 14.9, 1H, $NHC(O)CH_2$), 1.86 – 1.62 (m, 2H, CH_2CH_2OH), 1.29 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.07, 146.28, 144.30, 139.92, 138.50, 136.49, 111.44, 72.33, 59.55,

46.84, 41.66, 30.96, 26.72; HRMS (ESI) m/z calcd for $[M+Na]^+$ $C_{13}H_{14}F_5NNaO_3$: 350.0786, found: 350.0788.

3-Hydroxy-3-methyl-5-oxo-5-(((perfluorophenyl)methyl)amino)pentyl acetate (35) and **3-Methyl-5-oxo-5-(((perfluorophenyl)methyl)amino)pentane-1,3-diyl diacetate (36).** To a solution of **34** (163 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added acetyl chloride (142 µL, 2.0 mmol) and pyridine (242 µL, 3 mmol) at 0 ºC, and the mixture was stirred at room temperature for 48 h. After dilution with CH_2Cl_2 (15 mL), the mixture was partitioned between CH_2Cl_2 and 0.1 M aq. HCl (20 mL). The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (50% to 100% AcOEt in hexane) to give **35** (76 mg, 41%, colorless oil) and **36** (41 mg, 20%, colorless oil). **35**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (s, 1H, OH), 4.73 – 4.39 (m, 2H, benzyl-CH_2), 4.29 – 4.03 (m, 2H, CH_2CH_2OH), 2.49 – 2.28 (m, 2H, NHC(O)CH_2), 2.01 (s, 3H, $COCH_3$), 1.87 – 1.75 (m, 2H, CH_2CH_2OH), 1.21 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.04 , 171.25 , 147.39 – 143.21 (m), 142.41 – 139.45 (m), 139.14 – 135.47 (m), 111.42 (td, *J* = 17.6, 3.9 Hz), 70.46 , 60.80 , 46.25 , 39.97 , 30.89 , 26.89 , 20.92; HRMS (ESI) m/z calcd for $[M+Na]^+$ $C_{15}H_{16}F_5NNaO_4$: 392.0892, found: 392.0896. **36**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.26 (s, 1H, OH), 4.52 (d, *J* = 5.7 Hz, 2H, benzyl-CH_2), 4.17 (td, *J* = 7.0, 2.8 Hz, 2H, CH_2CH_2O), 2.98 – 2.70 (m, 2H, NHC(O)CH_2), 2.37 (dt, *J* = 14.1, 6.7 Hz, 1H, CH_2CH_2O), 2.18 – 2.10 (m, 1H, CH_2CH_2O), 2.04 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3COOCH_2), 1.55 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.03 , 170.98 , 168.76 , 146.84 – 143.78 (m), 142.37 – 139.63 (m), 139.01 – 135.86 (m), 111.45 (t, *J* = 18.1 Hz), 81.24 , 60.19 , 45.17 , 37.14 , 31.26 , 24.22 , 22.14 , 20.96; HRMS (ESI) m/z calcd for $[M+Na]^+$ $C_{17}H_{18}F_5NNaO_5$: 434.1003, found: 434.1006.

3-(Fluoromethyl)-3,5-dihydroxypentyl acetate (37). To a solution of **14** (500 mg, 1.39 mmol) in THF (10 mL) was added $LiBH_4$ (0.067 g, 3.08 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The reaction was quenched with addition of aq. HCl (1.5 M). The solution was co-evaporated with toluene, the product was extracted from the dry slurry with THF, and the solvent was evaporated to give the crude diol as a colorless oil. To a solution of the crude product (250 mg, 1.64 mmol) in THF (5 mL) was added pyridine (0.132 mL, 1.64 mmol) at room temperature. To the mixture was added acetic anhydride (0.24 mL, 2.46 mmol), and the mixture was stirred for 8 h. The reaction was quenched with aq. HCl (1.5 M) and extracted with AcOEt. The organic layer was washed with aq. HCl (1.5 M), sat. $NaHCO_3$, and brine, and then dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (50% to 75% AcOEt in hexane) to give **37** (239 mg, 75%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.40 (m, 1H, CH*H*$_2$F), 4.29 (m, 3H, CH*H*$_2$F and C*H*$_2$OAc), 3.94 (m, 2H, C*H*$_2$OH), 2.06 (s, 3H, CH_3CO), 1.97 (m, 2H, C*H*$_2$CH$_2$OAc), 1.85 (m, 2H, C*H*$_2$CH$_2$OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 87.3, 85.9, 73.2(d), 60.3, 59.3, 36.8, 35.6, 21.1; LRMS (ESI) *m*/*z* = 195.10 $[M+Na]^+$.

5-(Benzylamino)-3-(fluoromethyl)-3-hydroxy-5-oxopentyl acetate (38a). To a solution of 6-fluoromevalonate (200 mg, 1.35 mmol) in DMF (5 mL) was added benzylamine (0.18 mL, 1.62 mmol) at room temperature, and the reaction was stirred at 60 °C for 24 h. The reaction was quenched by aq. HCl (1.5 M) and extracted with AcOEt. The organic layer was washed with aq. HCl (1.5 M) and brine, then dried ($MgSO_4$) and evaporated. After the residue was dissolved in THF, pyridine (0.11 mL, 1.35 mmol) was added at room temperature. To the solution was added acetic anhydride (0.25 mL, 2.7 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched with aq. HCl (1.5 M) and extracted with AcOEt. The organic layer

was washed with aq. HCl (1.5 M) and brine, then dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (15% to 25% AcOEt in hexane) to give **38a** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (m, 5H), 6.03 (s, 1H), 4.98 (s, 1H), 4.43 – 4.35 (m, 2H), 4.25 (s, 1H), 4.24 – 4.17 (m, 2H), 4.16 (s, 1H), 2.41 (m, 2H), 1.96 (s, 3H), 1.87 – 1.84 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 170.9, 137.4, 128.9, 127.8(δ), 87.1, 85.7, 71.3 (δ) 59.8, 43.6, 40.7, 35.8, 21.0; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{15}H_{20}FNNaO_4$: 320.1269, found: 320.1268.

Methyl 5-acetoxy-3-(fluoromethyl)-3-hydroxypentanoate (38b). To a solution of **37** (300 mg, 1.54 mmol) in DMF (8 mL) was added pyridinium dichromate at room temperature, and the mixture was stirred at room temperature for 24 h. The reaction was quenched with aq. HCl (1.5 M) and extracted with AcOEt. The organic layer was washed with aq. HCl (1.5 M) and brine, then dried ($MgSO_4$) and evaporated. The crude pro duct was dissolved in DMF (5 mL), K_2CO_3 (638 mg, 4.62 mmol) was added to the mixture followed by the addition of methyl iodide (0.21 mL, 3.08 mmol), and the mixture was stirred for 24 h. The reaction was quenched with aq. HCl (1.5 M) and extracted with AcOEt. The organic layer was washed with aq. HCl (1.5 M) and brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (15% to 25% AcOEt in hexane) to give **38b** (105 mg, 31%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.41(dd, 1H, *J* = 9.91 Hz) 4.33 - 4.24 (m, 3H), 3.92 (s, 1H), 2.72 – 2.62 (m, 2H), 2.07 (s, 3H), 1.95 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 170.9, 87.3, 85.9, 71.2 (d), 59.7, 52.1, 39.5(d), 35.2, 21.0; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_9H_{15}FNaO_5$: 245.0796, found: 245.0933.

Ethyl 5-acetoxy-3-(fluoromethyl)-3-hydroxypentanoate (38c). 38c (26 mg, 7%, colorless oil) was prepared from **36** as described for the preparation of **38b** using ethyl iodide instead of methyl iodide. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.37 (dd, 1H), 4.30 – 4.23 (m, 3H), 4.19 (q, 2H, *J* = 7.19), 3.99 (s, 1H), 2.62 (dq, 2H), 2.04 (s, 3H), 1.93 (m, 2H), 1.28 (t, 3H, *J* = 7.19 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 170.9, 87.3, 85.9, 71.2(δ), 61.2, 59.8, 39.7(d), 35.2(d), 21.0, 14.1; HRMS (ESI) *m/z* calcd for $[M+Na]^+$ $C_{10}H_{17}FNaO_5$: 259.0952, found: 259.1080.

Evaluation of stability of the ester promoiety in PBS buffer (pH 7.4). Deuterated PBS buffer (pH 7.4) was prepared by dissolving NaCl (80 mg, 137 mM), KCl (2.0 mg, 2.7 mM), Na_2HPO_4 (14.4 mg, 10.1 mM), and KH_2PO_4 (2.4 mg, 1.8 mM) in 10 mL of deuterium oxide. Compound **38c** (1.0 mg, 4 μmol) was dissolved in deuterated PBS buffer (pH 7.4, 800 μL), placed in an NMR tube, and incubated at 37 °C. ^{1}H NMR spectra were taken at 30 min, 1 h, 4 h, 24 h, and 48 h to determine its stability in PBS buffer. The decomposition was less than 5% after 48 h on the basis of the integration ratio of impurities and **38c**.

Evaluation of stability of all other promoieties tested in PBS buffer (pH 7.4). A stock solution of the test compound (10 mM in acetonitrile, 50 μL) was added to PBS buffer (pH 7.4, 450 μL), and the mixture was incubated at 37 ºC. The final incubation volume was 0.5 mL, Aliquots (10 μL) of the samples were taken from the incubation solution at various times and were immediately injected into the HPLC. These tests were conducted twice for each compound. The $t_{1/2}$ (the time for disappearance of half of the starting material) values were calculated using Prism Version 5 a one-phase decay model with the equation $t_{1/2}$= ln(2)/b. Here b is the slope in the linear fit of the natural logarithm (ln) of the remaining fraction of the parent molecule in HPLC verses incubation time.

Evaluation of stability in human plasma. The stability of compounds in human plasma was performed after slight modification of a referenced protocol[24]; human blood plasma (Aldrich) was diluted with distilled water until the indicated volume, and then the solution was pre-incubated for 5 min at 37 ºC. The stock solution of the test compound (100 mM in acetonitrile, 20 µL) was added to the human plasma (480 µL), and the mixture was incubated at 37 ºC. The incubations were terminated at 1, (5), (10), 15, 30, and 60 min, by removing aliquots (80 µL) of the plasma samples and mixing them with an equal volume of acetonitrile. For stable compounds, 48 h of incubation and analysis was also performed. The mixture was stirred vigorously and centrifuged (5500 rpm, 5 min). The supernatant was filtered, and the filtrate was analyzed by HPLC. These tests were conducted two times for each compound. HPLC analysis was performed on a Phenomenex® Luna C18 column (250 x 4.6 mm) eluting with a gradient of acetonitrile and H_2O (90% to 10% water over 30 min) Detection was by UV absorbance at 254 nm or 220 nm. The flow rate was 1.0 mL/min. Data analysis was similar to that of PBS buffer stability, performed using Prism Version 5, a non-linear fit one-phase decay model.

Minimal inhibitory concentration (MIC) assay. The MICs of the prodrugs required to inhibit growth of *Streptococcus pneumoniae* strain TIGR4 was determined using a previously described method with minor modifications.[27] Overnight *S. pneumoniae* culture was diluted 1:10 into fresh THB and grown to logarithmic phase (OD600 of 0.4). The bacterial culture was washed and diluted in PBS. Bacteria (5 µL) was added to individual wells of a 96-well plate containing 195 µL of THB and the prodrugs (12.5 to 1600 µM) were serially diluted. The final concentration of bacteria was 1 x 105 CFU/well. The plate was incubated for 24 h at 37 °C, and the absorbance of the samples at 600 nm was read with a spectrophotometric plate reader. The MIC was defined as the lowest concentration of the drugs that inhibited bacterial growth.

ASSOCIATED CONTENT

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ABBREVIATIONS

DMP, diphosphomevalonate; MK, mevalonate kinase; PMK, phosphomevalonate kinase; DPM, 6-fluoro- and 6,6,6-trifluoro-5-diphosphomevalonate; DPM-DC, diphosphomevalonate decarboxylase; IPP, Isopentenyl diphosphate; EDCI, *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride; HBTU, *N*,*N*,*N*,*N*-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate; MIC, Minimal inhibitory concentration (MIC); THB, tissue homogenization buffer

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Highlights

- Prodrug analogues of mevalonate, 6-fluoromevalonate, and 6,6,6-trifluoromevalonate synthesized and characterized
- Human blood plasma stabilities of ester, amide, carbonate, acetal, and ketal promoieties determined
- A wide range of half-lives were obtained
- MIC values determined to evaluate antibacterial activity of select prodrugs
- Two of the prodrug analogues exhibited weak antibacterial activity