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Functionalized Depsipeptides, Substrates and Inhibitors of β-Lactamases and DD-Peptidases

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Abstract—A series of derivatives of phenyl phenylacetylglycinates (aryl phenaceturates) with a carboxylate substituent *meta* to the oxygen of the phenoxide leaving group and a functionalized methylene group in the *ortho*- or *para*-position have been synthesized. These molecules possess a latent *o*- or *p*-quinone methide electrophile which could be unmasked during enzymic turnover and could react with an active site nucleophile. This chemistry does seem to occur in solution where a common hydrolysis product, independent of the benzylic leaving group, presumably *o*- or *p*-hydroxymethylphenol, was observed. These depsipeptides are substrates of class A and C β-lactamases, particularly of the latter, comparable with the parent *m*-carboxyphenyl phenaceturate. They also have modest inhibitory activity against these enzymes and against the serine DD-peptidase of *Streptomyces* R61. The inhibition of a class C β-lactamase was turnover dependent, as expected of mechanism-based inhibitor, but the small leaving group dependence of the inhibition suggested that the quinone methide, if it was in fact responsible for the inhibition, was generated in solution subsequent to release of the product phenol from the active site.

Introduction

The antibiotic activity of β-lactams is largely dictated by their interactions with two groups of bacterial enzymes. β-Lactams inhibit the DD-peptidase/transpeptidases which are responsible for the final steps of bacterial cell wall biosynthesis, but are themselves destroyed by the β-lactamases which thereby provide much of the resistance of bacteria to these antibiotics. Detailed investigations of the structure and active-site chemistry of these two groups of enzymes have shown that their active sites have much in common.[1] In particular, β-lactamases of class A, C and D and the biosynthetic DD-peptidases both possess a conserved serine residue in the active site whose side chain hydroxyl group constitutes the primary nucleophile which attacks substrates. Catalysis in both cases therefore involves a double-displacement reaction sequence with an acyl-enzyme intermediate. β-Lactams inhibit DD-peptidases by acylation of the same serine hydroxyl group.[2] The major distinction between DD-peptidases and β-lactamases with respect to their interaction with β-lactams resides in the lifetime of the acyl-enzyme formed, long in the case of the former enzymes, short in the latter.

The catalytic mechanism of serine β-lactamases is not completely understood despite the availability of crystal structures of representative class A and class C enzymes.[3] For example, there is no consensus as to the identity of the amino acid residue providing the general base catalyst that is probably required to assist nucleophilic attack by the active-site serine hydroxyl function on the substrate: candidates include Lys 73 and Glu 166 (the latter possibly assisted by a water molecule) in class A β-lactamases. and Lys 67 and Tyr 150 in class C.[4,3b]

Mechanism-based inhibitors/suicide substrates could, in principle, reveal more of the nature and reactivity of these active site catalytic groups. Latent functionality in such inhibitors becomes unveiled during their turnover and may trap functional groups in their active form.[5] Many effective inhibitors of this type have in fact been discovered and devised for β-lactamases, but no functional group beyond the active site serine hydroxyl group has been trapped and identified.[6] Although clavulanic acid and the penicillin sulfones appear to modify at least one other functional group[7] no unequivocal identification of a modified residue has yet been achieved.[8]

A variety of depsipeptides, and in particular aryl phenylacetylglycinates (aryl phenaceturates) with a carboxylate group *meta* to the oxygen of the phenoxide leaving group, are β-lactamase substrates.[9,10] The structure of these molecules lends itself to the preparation of derivatives possessing a latent *ortho*- or *para*-quinone methide electrophile, as described below.

Most mechanism-based inhibitors of serine proteinases have a cyclic structure so as to tether the unmasked electrophilic group in the active site during the lifetime of the acyl-enzyme intermediate.[5b,11] For instance, functionalized derivatives of 3,4-dihydrocoumarins, β-lactams and cyclopeptides which possess latent quinone methides or quinoniminium methide cations are efficient inactivators of proteinases.[12] However, in at least one instance, that of a series of zinc metalloproteinases, where no covalent intermediate is thought to occur during substrate turnover, effective acyclic mechanism-based inhibitors have been devised.[13] Inactivation is thought to be effected by the rearranged leaving group prior to its

departure from the active site. In the case of glycosidases[14] and a tyrosine phosphatase[15] effective acyclic inactivators possessing latent quinone methide function have also been obtained. On the basis of these precedents we have prepared the depsipeptides **1**. We hoped that electrostatic attraction between the carboxylate of the phenol leaving group **2** and the positive potential of the β-lactamase or DD-peptidase active site,[4d] represented in the reaction Scheme I by the symbol A+ (where this may largely represent the local effect of a single functional group, e.g. Lys 234 of the class A β-lactamases and its analogs in the other enzymes) might ensure retention of the product phenol for a sufficient length of time in the active site to permit the generation and subsequent reaction there of the electrophilic quinone methide **3**. This paper therefore describes the synthesis of compounds of general structure **1** and their inhibitory activity against typical β-lactamases and a DD-peptidase.

Results and Discussion

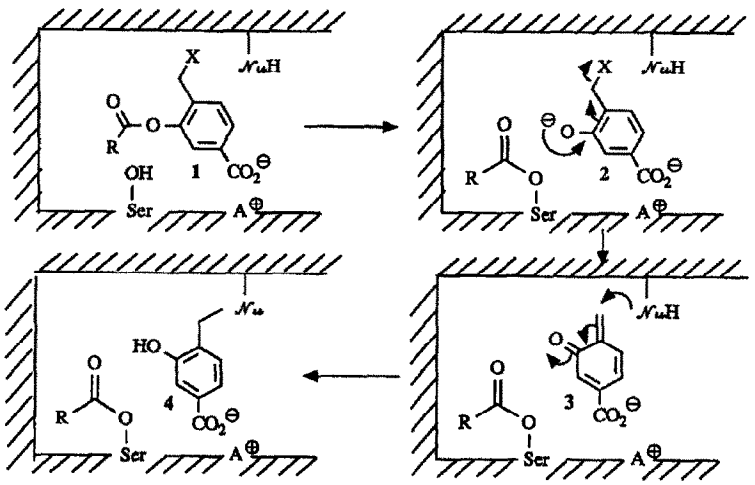
Synthesis

The synthesis of substituted aryl phenaceturates **1** or **1'** (*para*-analogs of molecules **1**) poses three different problems: the instability of the starting substituted phenol, the ready cyclization of the phenaceturic acid, and the decomposition of the aryl phenaceturate product during chromatographic purification.

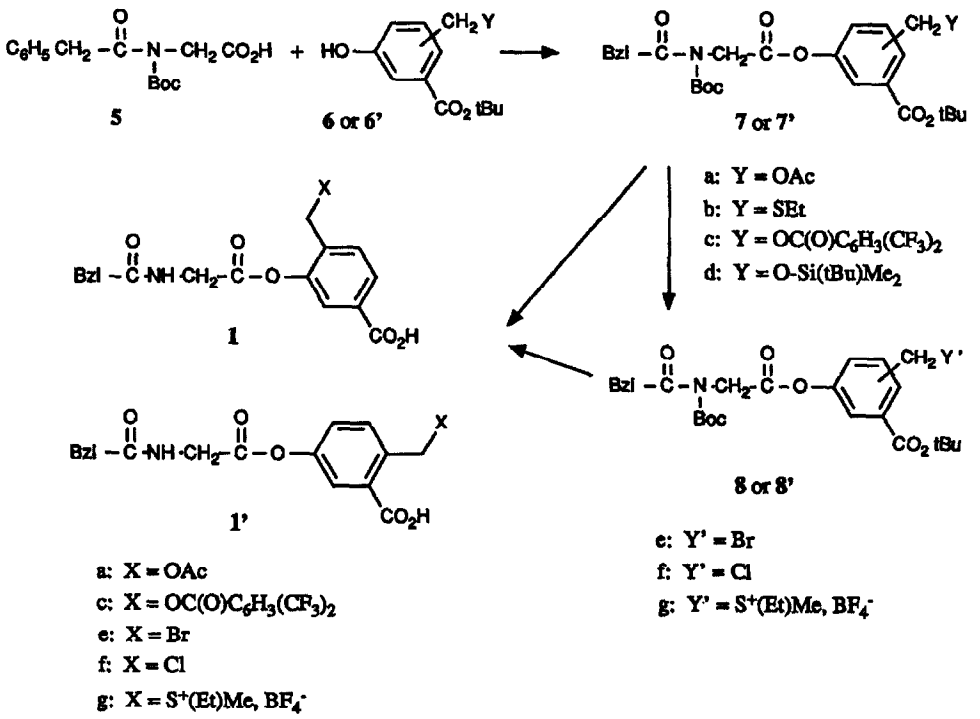
o- and *p*-Hydroxybenzyl derivatives having a good leaving group are unstable compounds, particularly in alkaline media: an elimination reaction gives *o*- or *p*-quinone methides which rapidly react with ambient nucleophiles or polymerize.[16] 2-Hydroxy-5-nitrobenzyl chloride has been condensed with *N*-Z-glycine using DCC in ethyl acetate to give the corresponding ester in 42 % yield.[17a] However, activated derivatives of *N*-acyl glycines cyclize much more easily than their *N*-alkoxycarbonyl counterparts to give 5(4*H*)-oxazolones which are sluggish in their further condensation with phenols. For the preparation of phenaceturates we recently suggested the application of the acid catalyzed DCC esterification in pyridine.[18] Furthermore, transient *N*-*tert*-butoxycarbonylation of the amide group of the starting acid avoided the intramolecular reaction leading to the oxazolone and furnished stable aryl *N*-Boc phenaceturates which can be chromatographed on SiO_2. The N-protecting group could be removed with a slight excess of trifluoroacetic acid. For phenols bearing electron withdrawing substituents, this indirect route is more efficient than the direct Holmberg procedure.[19] In the case of *tert*-butoxycarbonyl substituted compounds **7**, **7'**, **8** and **8'**, mild treatment with TFA could cleave both the *N*-Boc group and the *tert*-butyl ester function to give the functionalized depsipeptides **1** and **1'** (Scheme II).

Preparation of the starting substituted phenols. First, a series of phenols possessing an *m*-*tert*-butoxycarbonyl substituent and a functionalized methylene group in the *o*- or *p*-position have been prepared.

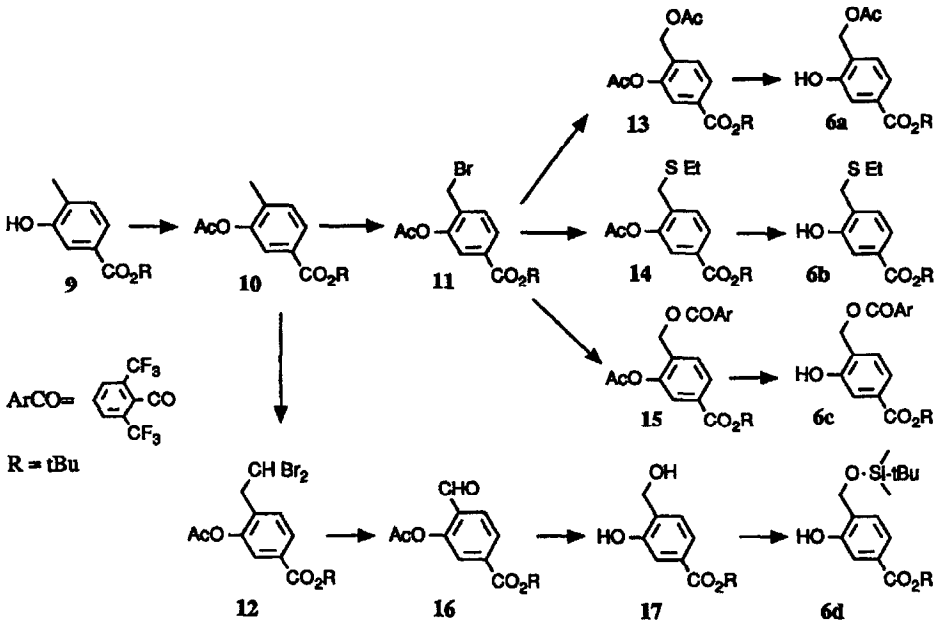
ortho-*Series*. Selective esterification of 3-hydroxy-4-methylbenzoic acid with *N,N*-dimethylformamide di-*tert*-butyl acetal,[20] followed by DMAP-catalyzed acetylation of the phenol function of compound **9** gave the *tert*-butyl 3-acetoxy-4-methylbenzoate **10** (Scheme III). Treatment of this ester with 1.1 or 2.2 equivalents of *N*-bromosuccinimide furnished the *tert*-butyl 3-acetoxy-4-bromomethylbenzoate **11** and 3-acetoxy-4-dibromomethylbenzoate **12**. From the monobromide **11**, nucleophilic substitutions with acetate, ethylthiolate[21] or 2,6-di-trifluoromethylbenzoate[22] ions gave the 3-acetoxy-4-acetoxymethylbenzoate, 3-acetoxy-4-ethylthiomethylbenzoate or 3-acetoxy-4-(2',6'-ditrifluoromethylbenzoxy)-methyl-benzoate **13**, **14** or **15**, respectively. Cleavage of the aryl acetate group of these molecules, by means of pyrrolidine in dichloromethane,[23] led to the free phenols **6a**, **6b** or **6c**. The aminolysis was selective in the case of the diacetate **13** and no rearranged *tert*-butyl 3-acetoxy-4-hydroxymethylbenzoate was observed.[24] Reduction of the substituted salicylaldehyde **16**, obtained from the dibromide **12**,[25] with sodium borohydride gave the phenol **17**. Then, selective silylation of the alcohol function of compound **17** in the presence of imidazole gave the silyl ether **6d**.



Scheme I. Postulated mechanism for the reaction of a functionalized depsipeptide **1** with an active site nucleophile NuH of a serine β-lactamase.

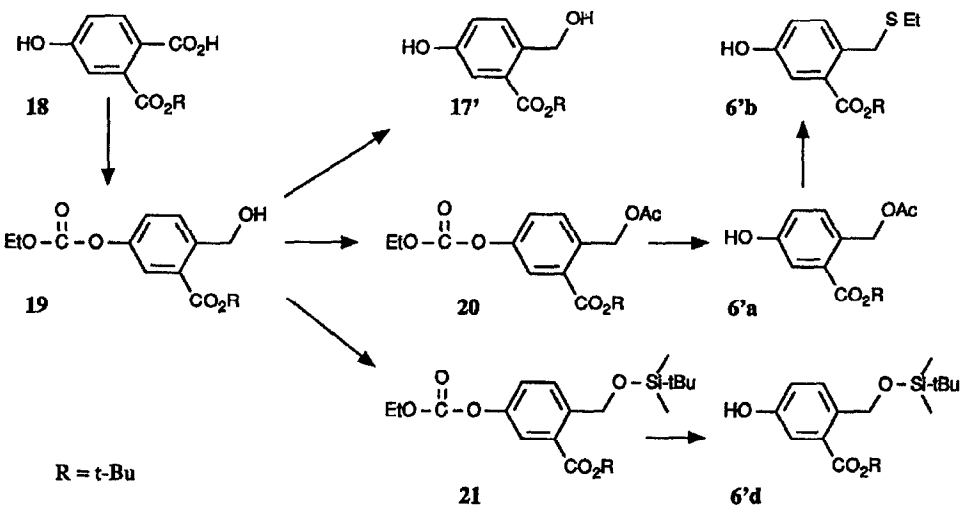


Scheme II. Synthetic scheme for the synthesis of the functionalized depsipeptides 1 and 1'.



Scheme III. Synthetic scheme for the synthesis of the substituted phenols 6a–d.

para-*Series*. A sequence of reactions **9'→10'→11'→15'→6'c** (not shown), analogous to that of the *ortho* series, unambiguously gave the *para*-substituted phenol **6'c** (see Experimental). This route required the preparation of the starting 3-hydroxy-6-methylbenzoic acid from 3-nitro-6-methylbenzoic acid by reduction and diazotation.[26] Esterification of the commercially available 4-hydroxyphthalic acid[27] with *N,N*-dimethylformamide di-*tert*-butyl acetal gave a mixture of monoesters which were separated by chromatography. Treatment of ester **18** with excess ethyl chloroformate/triethylamine, then selective borohydride reduction of the mixed anhydride intermediate furnished the alcohol **19** (Scheme IV). Cleavage of the carbonate group yielded the phenol **17'**. On the other hand, acetylation of alcohol **19** followed by a selective aminolysis of the phenolic carbonate function of compound **20** led to the phenol **6'a**. Then, nucleophilic substitution with



Scheme IV. Synthetic scheme for the synthesis of the substituted phenols **6'a**, **6'b** and **6'd**.

ethylthiolate anion gave the phenol **6'b**. Silylation of alcohol **19** led to the silyl ether **21** and then to the phenol **6'd** by treatment with pyrrolidine. Comparison of the ^{1}H and ^{13}C NMR spectra of products **6'a**, **6'b** and **6'd** with that of compound **6'c** supported the structural assignments shown in Scheme IV.

Condensation with N-*Boc-phenaceturic acid, substitution of the benzylic group and trifluoroacetolysis.* The reaction of the *N*-Boc-phenaceturic acid with the fragile substituted phenols **6a**, **6b**, **6d**, **6'a**, **6'b** and **6'd**, in the presence of DCC and *p*-TSA in pyridine, occurred without decomposition of the phenol and gave the aryl *N*-Boc-phenaceturates **7a**, **7b**, **7d**, **7'a**, **7'b** and **7'd**, which were purified by chromatography. Esterification of *N*-Boc-phenaceturic acid could also be achieved in good yield by using DCC and DMAP.

Alkylation of the thioethers **7b** and **7'b** with methyl iodide in the presence of silver tetrafluoroborate furnished the sulfonium salts **8g** and **8'g**. On the other hand, treatment of the silyl ethers **7d** and **7'd** with either $(C_6H_5)_3PBr_2$[28] or $(C_6H_5)_3PCl_2$ directly led to the formation of the corresponding bromides **8e** and **8'e**, or chlorides **8f** and **8'f**.

We planned to next remove both *tert*-butyl-based protecting groups in a single step. Effectively, trifluoracetic acid was able to simultaneously cleave the Boc N-protecting group and the *tert*-butyl ester function of compounds **7a**, **7c**, **7'a**, **7'c**, **8e–g** and **8'e–g** to give the free functionalized aryl phenaceturates **1a**, **1c**, **1e**, **1f**, **1g**, **1'a**, **1'c**, **1'e**, **1'f** and **1'g** in near quantitative yields.

Enzymology

The depsipeptides **1** and **1'** are both substrates and inhibitors of typical class A and class C β-lactamases, as seen in the data of Tables 1 and 2. Such behavior is anticipated of mechanism-based inhibitors/suicide substrates.

As judged by the k_{cat}/K_m parameters of Table 2, determined for **1e** and **1'e**, the compounds could be comparably effective as substrates of the class C *Enterobacter cloacae* P99 β-lactamase as the unsubstituted parent compound (**1**, X = H[10]). On the other hand, they may be rather poorer substrates of class A β-lactamases, as judged by their performance with the TEM plasmid β-lactamase and the *Staphylococcus aureus* PCl β-lactamase, than the parent compound, the difference amounting to a factor of about five for the latter enzyme and ten (**1'e**) or one hundred (**1e**) for the former. This observation is in accord with the generally broader specificity of class C β-lactamases.

An *ortho*-substituent, which might be presumed to lie, in the productive enzyme–substrate complex, closer to the functional groups of the active site than a *para*-substituent, certainly has no generally negative effect on reactivity—**1e** is at least as good a substrate as **1'e** for two of the three enzymes of Table 1. Similarly, perhaps, *o*- and *p*-carboxyphenyl phenaceturates were found to be comparably effective substrates of the P99 β-lactamase, although distinctly poorer than the *m*-analog.[10] It seems likely then that *o*- and *p*-substituents in the leaving group of aryl phenaceturates do not significantly disrupt the active site machinery. Conversely however, these substituents may not have direct access to active site functional groups after their rearrangement into inhibitory form as described below.

^{1}H NMR experiments revealed not only that **1** and **1'** were β-lactamase substrates but also that the elimination reaction forming the quinone methide **3** (Scheme I), subsequent to ester hydrolysis, was facile in certain cases, in free solution at least. Thus, the addition of the P99 β-lactamase (*ca* 0.1 μg) to a solution of **1e** (0.5 mL, 5mM) immediately (1 min) gave a solution of the hydrolysis products, one of which, from its NMR spectrum, was phenaceturate. The other product, **17** (R = H), (^{1}H NMR, $^{2}H_2O$, $^{2}HCO_3^-$: 4.65 (s, 2H, CH_2), 7.4–7.6 (m, 8H, ArH)) was characterized by a benzylic methylene resonance counterintuitively downfield from its original position in

Table 1. Inhibition of β-lactamases by depsipeptides **1** and **1'**[a]

Inhibitor	% Inhibition			
	Enzyme			
	Class A		Class B	Class C
	TEM	PC1	BCII	P99
1a	4	-	-	40
1c	0	-	-	53[b]
1e	26	11	14	54
1f	21	-	-	61
1g	8	-	-	8
1'a	11	-	-	13
1'c	19	-	-	40[c]
1'e	11	0	5	61
1'f	17	-	-	18
1'g	54	-	-	41

[a]After incubation of 2 μM enzyme with 5 mM (unless otherwise noted), **1** or **1'** at 25 °C for 1 h in 20 mM MOPS buffer, pH 7.5; [b] 0.73 mM for solubility reasons; [c]1.33 mM for solubility reasons.

Table 2. Depsipeptides **1e** and **1'e** as β-lactamase substrates[a]

Enzyme	k_{cat}/K_m (s^{-1} mM^{-1})	
	1e	**1'e**
TEM	0.12	1.7
PC1	0.24	0.22
P99	96	42

[a]20 mM MOPS buffer, pH 7.5, 25 °C.

1e (4.4). This experiment with **1c** yielded the same two products plus an aromatic species, presumably (see below) 2,6-bis(trifluoromethyl)benzoate. A slightly different phenomenon was observed with **1a**. Here, the initial products were phenaceturate and **6a** (R = H) (^{1}H NMR, ^2H_2O, $^2HCO_3^-$: 2.10 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 7.4–7.6 (m, 8H, ArH)) where the benzylic methylene resonance is upfield of its position in **1a**. Over several hours however, **6a** (R = H) changed slowly to a mixture of **17** (R = H) and acetate (1.90).

The likely interpretation of these observations is that enzyme-catalyzed hydrolysis of **1** initially generates, as expected, a mixture of phenaceturate and the phenoxide **2**. When a good leaving group is present in **2** at the benzylic carbon, such as Br (**2e**) or 2,6-bis(trifluoromethyl)benzoate (**2c**), its elimination occurs rapidly ($t_{1/2}$< 10 s. under our conditions), either at the active site of the β-lactamase, or, after discharge, in free solution, to yield **3**. The addition of water to **3** must also be very fast, again either at the active site or in solution, to give **17** (R = H) as the common product from **2e** and **2c**. No sign of the olefinic resonances of a quinone methide was observed in the NMR spectra. When a poorer leaving group is present, such as acetate, the initial product (**2a**) accumulates in solution (**6a**; R = H) and only slowly undergoes the elimination reaction to form, eventually, **17** (R = H). Thus, it seems likely that the chemistry of Scheme I is, in principle, available to **1** and **1'**.

In practice, the compounds **1** and **1'** were inhibitors of the serine β-lactamases, although, as indicated by the data of Table 1, not especially effective ones. They appeared to be somewhat more effective against the class C than against class A enzyme, but this difference may be illusory since complete turnover of the inhibitor as a substrate would not have occurred for the class A enzymes in the time interval of the incubation, i.e. the apparent difference may only reflect the fact that compounds **1** are rather better substrates of the class C enzyme.

This interpretation would of course assume that the inhibition was of the mechanism-based variety and directly related to the turnover of **1** as a substrate. In the instances of **1e** and **1c** as inhibitors of the P99 enzyme, evidence was obtained for turnover-related inhibition. At a fixed inhibitor concentration, the extent of inhibition was observed to vary inversely with the enzyme concentration (data not shown). It appeared that for these two compounds 3000–4000 turnovers were required for inhibition.

The effect of **1e** against the P99 β-lactamase at pH 9.5 was also tested in the hope that the lysine amines of the active site might be more accessible. The extent of inhibition

observed did not increase however. Hydroxide ion competition for the quinone methide may have compensated for any increase in availability of an enzyme nucleophile.

Also briefly examined were the susceptibilities to **1** of the class B (metallo) β-lactamase of *Bacillus cereus* and the serine DD-peptidase of *Streptomyces* R61. Although the former of these enzymes probably does not employ an acyl-enzyme intermediate as part of its catalytic mechanism,[29] the putative inhibition mechanism of Scheme I does not require one, only that the quinone methide be formed and react with an active site nucleophile before it diffuses into solution. In the event however, little inhibition of the class B enzyme was observed (Table 1). Similarly, a small amount of inhibition of the R61 DD-peptidase by **1e** (32 %) and by **1'e** (9 %) was observed.

We can conclude therefore that the strategy of Scheme I is not very effective with compounds **1** and **1'** and the β-lactamases despite the fact that these depsipeptides are substrates. The main reason for their ineffectiveness as inhibitors is probably that the initial product, the phenoxide **2**, diffuses from the active site into solution faster than it can undergo the elimination reaction at the active site and/or react with an active site nucleophile.[31] Once the phenoxide is in solution, the elimination reaction does occur, but the enzyme, at low concentration, would compete poorly with solvent for the quinone methide produced; presumably the latter has only small non-covalent affinity for the enzyme active site. This mechanism of inhibition by methide from solution may explain the observation that **1a** is almost as effective an inhibitor as **1e**, i.e. that the extent of inhibition is essentially independent of leaving group ability at the benzylic carbon. Furthermore, with the exception of the sulfonium derivatives **1g** and **1'g**,[32] the *ortho* and *para* isomers of **1** have quite similar inhibitory activities.

It therefore seems likely that some intramolecular tethering would be needed to achieve an effective β-lactamase inhibitor or β-lactam antibiotic of this type.

Experimental

Enzymes

The β-lactamases were purchased from the Centre for Applied Microbiology and Research, Porton Down, Wilts, U. K. The DD-peptidase of *Streptomyces* R61 was the generous gift of Drs J.-M. Ghuysen and J.-M. Frère of the University of Liège, Liège, Belgium.

Analytical and kinetic methods

All β-lactamase kinetics measurements were made in 20 mM MOPS buffer, pH 7.5, 25 °C. The inhibitory activity of **1** and **1'** against all enzymes was determined by following the loss of enzyme activity in incubation mixtures of enzyme and inhibitor: small aliquots were removed at appropriate times and assayed against benzylpenicillin.[30] The reactions of the R61 DD-peptidase was studied in 10 mM phosphate buffer containing 0.2 % gelatin at pH 7.0 and 37 °C, and the enzyme activity against *m*-carboxyphenyl phenaceturate determined. Estimates of k_{cat}/K_m values for **1** as β-lactamase substrates were obtained from spectrophotometrically determined pseudo-first order rate constants of the hydrolysis reactions at low substrate concentrations (40–80 μM). The products of β-lactamase action on **1** were identified by ^{1}H NMR experiments as previously described.[10]

Synthesis

^{1}H spectra were recorded on a Bruker AC 200E at 200 MHz and ^{13}C spectra at 50 MHz with TMS as internal standard. TLC was performed on silica gel 60F-254 (Merck) and visualized with UV light. Column chromatography was carried out on silica gel 60 (70–230 mesh). Unless otherwise stated, the eluent used is the same for TLC and for column chromatography purification.

t-*Butyl 3-hydroxy-4-methylbenzoate* ***9***. To a refluxing solution of 1.934 g (12.7 mmol) of 3-hydroxy-4-methylbenzoic acid in 20 mL of THF, 12.2 mL (50.9 mmol) of *N,N*-dimethylformamide di-*t*-butyl acetal was added dropwise. The solution was allowed to reflux for a further 30 min and then evaporated to remove the THF. The residue was purified by chromatography (pentane/EtOAc 99/1 to 9/1), affording 1.987 g (75 % yield) of product **9** (R_f 0.34, pentane/EtOAc 9/1). mp 106–107 °C; ^{1}H NMR ($CDCl_3$) δ: 1.50 (s, 9H, *t*-Bu), 2.21 (s, 3H, $ArCH_3$), 6.52 (s, br 1H, OH), 7.06 (d, $J_{5,6}$ = 7.8 Hz, 1H, H_5), 7.37 (dd, $J_{2,6}$ = 1.4 Hz, $J_{5,6}$ = 7.8 Hz, 1H, H_6), 7.53 (d, $J_{2,6}$ = 1.4 Hz, 1H, H_2) ppm. ^{13}C NMR ($CDCl_3$) δ: 16.1 (CH_3), 28.3 (*t*-Bu), 81.3 (CO_2C), 115.7, 121.5, 129.9, 130.5 and 130.7 (Ar), 154.3 (C_3), 166.7 (CO_2*t*-Bu) ppm. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.36; H, 7.72.

t-Butyl 3-*t*-butoxy-4-methylbenzoate, 286 mg (8.5 % yield) was isolated as a side product (R_f 0.84, pentane/EtOAc 9/1). ^{1}H NMR ($CDCl_3$) δ: 1.34 (s, 9H, ArO*t*-Bu), 1.51 (s, 9H, CO_2*t*-Bu), 2.20 (s, 3H, $ArCH_3$), 7.10 (d, $J_{5,6}$ = 7.8 Hz, 1H, H_5), 7.48 (dd, 1H, H_6), 7.57 (d, $J_{2,6}$ = 1.5 Hz, 1H, H_2) ppm.

t-*Butyl 3-acetoxy-4-methylbenzoate* ***10***. A solution of 1.987 g (9.55 mmol) of **9**, 0.99 mL (10.5 mmol) of Ac_2O, 1.33 mL (9.55 mmol) of Et_3N and 1.165 g (9.55 mmol) of DMAP in 170 mL of CH_2Cl_2 was stirred for 1 h at room temperature. The reaction was quenched with 3 mL of MeOH and the mixture was washed twice with water, dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography (pentane/EtOAc 99/1 to 95/5), affording 2.226 g (93 % yield) of **10** as an oil (R_f 0.64, pentane/EtOAc 9/1). ^{1}H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.22 (s, 3H, $ArCH_3$), 2.35 (s, 3H, OAc), 7.27 (d, $J_{5,6}$ = 7.9 Hz, 1H, H_5), 7.61 (d, $J_{2,6}$ = 1.4 Hz, 1H, H_2), 7.79 (dd, 1H, H_6) ppm. ^{13}C NMR ($CDCl_3$) δ: 16.0 (CH_3), 20.3 (OAc), 27.8 (*t*-Bu), 80.7 (CO_2C), 122.7, 126.7, 130.6, 130.9 and 135.0 (Ar), 148.9 (C_3), 164.5 (CO_2*t*-Bu), 168.6 (Ac) ppm. Anal. calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.02; H, 7.36.

t-*Butyl 3-acetoxy-4-bromomethylbenzoate* ***11***. A mixture of 1.375 g (5.5 mmol) of **10**, 1.077 g (6.05 mmol) of NBS and 25 mg of benzoyl peroxide in 20 mL of CCl_4 was refluxed for 4 h under argon. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and the residue was purified by chromatography (pentane/CH_2Cl_2 1/1), affording 1.226 g (70 % yield) of pure product **11** as an oil (R_f 0.39). ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.38 (s, 3H, OAc), 4.40 (s, 2H, $ArCH_2Br$), 7.47 (d, $J_{5,6}$ = 8.1 Hz, 1H, H_5), 7.72 (d, $J_{2,6}$ = 1.5 Hz, 1H, H_2), 7.85 (dd, 1H, H_6) ppm. ^{13}C NMR ($CDCl_3$) δ: 20.8 (OAc), 26.6 ($ArCH_2Br$), 28.0 (*t*-Bu), 81.5 (CO_2C), 124.0, 127.1, 130.5, 133.6 and 133.9 (Ar), 148.6 (C_3), 164.1 (CO_2*t*-Bu), 168.6 (Ac) ppm. Anal. calcd for $C_{14}H_{17}O_4Br$: C, 51.77; H, 5.21; Br, 24.28. Found: C, 51.74; H, 5.49; Br, 24.18.

The dibrominated compound **12** (see below) was also obtained: 0.29 g (13 % yield) (R_f 0.55).

t-*Butyl 3-acetoxy-4-dibromomethylbenzoate* ***12***. A mixture of 377 mg (1.51 mmol) of **10**, 592 mg (3.3 mmol) of NBS and 8 mg of benzoyl peroxide in 6 mL of CCl_4 was refluxed for 6 h under argon. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and the residue was purified by chromatography (pentane/CH_2Cl_2 1/1), affording 578 mg (94 % yield) of pure product **12** as an oil (R_f 0.55). ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.41 (s, 3H, OAc), 6.81 (s, 1H, $ArCHBr_2$), 7.69 (s, 1H, H_2), 7.91 (q, $J_{A,B}$ = 8.0 Hz, 2H, H_5 and H_6) ppm. ^{13}C NMR ($CDCl_3$) δ: 20.9 (OAc), 28.0 (*t*-Bu), 33.2 (ArCHBr), 81.8 (CO_2C), 123.9, 127.2, 129.5, 134.3 and 136.7 (Ar), 145.1 (C_3), 163.7 (CO_2*t*-Bu), 168.1 (Ac) ppm. Anal. calcd for $C_{14}H_{16}O_4Br_2$: C, 41.20; H, 3.95; Br, 39.16. Found C, 41.07; H, 3.75; Br, 39.45.

t-*Butyl 3-acetoxy-4-acetoxymethylbenzoate* ***13***. A mixture of 135 mg (0.41 mmol) of **11** and 201 mg (2.05 mmol) of anhydrous KOAc in 1 mL of DMF was stirred at room temperature for 1 h. The DMF was evaporated under vacuum at 45 °C and the residue was distributed between CH_2Cl_2 and water. The CH_2Cl_2 solution was dried and evaporated. The crude product was purified by chromatography (CH_2Cl_2), giving 112 mg (89 % yield) of the product **13** as an oil (R_f 0.23). ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.09 (s, 3H, CH_2OAc), 2.35 (s, 3H, ArOAc), 5.10 (s, 2H, $ArCH_2O$), 7.49 (d, $J_{5,6}$ = 8.0 Hz, 1H, H_5), 7.68 (d, $J_{2,6}$ = 1.5 Hz, 1H, H_2), 7.88 (dd, 1H, H_6) ppm. ^{13}C NMR ($CDCl_3$) δ: 20.6 (2 × OAc), 27.9 (*t*-Bu), 60.8 ($ArCH_2O$), 81.4 (CO_2C), 123.5, 127.0, 129.6, 132.5 and 133.3 (Ar), 148.5 (C_3), 164.2 (CO_2*t*-Bu), 168.9 (ArOAc), 170.3 (CH_2OAc) ppm. Anal. calcd for $C_{16}H_{20}O_6$ C, 62.32; H, 6.54. Found: C, 62.66; H, 6.64.

t-*Butyl 3-hydroxy-4-acetoxymethylbenzoate* ***6a***. To a solution of 0.561 g (1.82 mmol) of **13** in 5 mL of CH_2Cl_2, 177 µL (2 mmol) of pyrrolidine was added. The mixture was stirred at room temperature for 1.5 h. The reaction was quenched with 0.5 N HCl and the mixture was extracted with CH_2Cl_2. The organic layer was dried and evaporated. The crude product was purified by chromatography (CH_2Cl_2 followed by CH_2Cl_2/MeOH 99.7/0.3), giving 440 mg (91 % yield) of **6a** (R_f 0.34, CH_2Cl_2/MeOH 99/1). mp 106–107 °C. ^1H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.12 (s, 2H, OAc), 5.16 (s, 2H, $ArCH_2O$), 7.30 (d, $J_{5,6}$ = 7.9 Hz, 1H, H_5), 7.52 (dd, 1H, H_6), 7.57 (d, $J_{2,6}$ = 1.5 Hz, 1H, H_2), 7.90 (s, 1H, OH) ppm. ^{13}C NMR ($CDCl_3$) δ: 20.8 (OAc), 28.0 (*t*-Bu), 62.0 ($ArCH_2O$), 81.4 (CO_2C), 114.3, 121.1, 126.5, 130.4 and 133.5 (Ar), 154.9 (C_3), 165.6 (CO_2*t*-Bu), 172.4 (OAc) ppm. Anal. calcd for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 63.03; H, 6.87.

t-*Butyl 3-acetoxy-4-ethylthiomethylbenzoate* ***14***. To a solution of 33 mg (0.1 mmol) of **11** and 8 µL (0.11 mmol) of thioethanol in 0.3 mL of toluene, 16.5 µL (0.11 mmol) of DBU was added. The reaction mixture was stirred at room temperature for 15 min and filtered. The filtrate was diluted with CH_2Cl_2, washed with water and dried (Na_2SO_4). After evaporation, the crude product was purified by a preparative TLC (CH_2Cl_2), giving 16 mg (59 % yield) of the title product **14** (oil), (R_f 0.34). ^1H NMR ($CDCl_3$) δ: 1.21 (t, J = 7.4 Hz, 3H, SCH_2CH_3), 1.58 (s, 9H, *t*-Bu), 2.36 (s, 3H, OAc), 2.41 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.66 (s, 2H, $ArCH_2S$), 7.42 (d, $J_{5,6}$ = 7.8 Hz, 1H, H5), 7.66 (d, $J_{2,6}$ = 1.4 Hz, 1H, H_2), 7.83 (dd, 1H, H_6) ppm. MS: 309 (M^+–1), 249 (M^+–SEt), 267 (M^+–$COCH_3$).

8 mg (30 % yield) of product **6b** (see below) (R_f 0.18) was also isolated.

t-*Butyl 3-hydroxy-4-ethylthiomethylbenzoate* ***6b***. To a solution of 300 mg (0.912 mmol) of **11** and 74 µL (1 mmol) of thioethanol in 3 mL of toluene, 149 µL (1 mmol) of DBU was added. After stirring for 15 min, 100 µL (1.2 mmol) of pyrrolidine was added. The reaction was stirred for 20 min, diluted with EtOAc and filtered. The filtrate was washed with 10 % HCl, water then 3 % $NaHCO_3$. After drying and evaporation of the filtrate, the crude resultant product was purified by chromatography (pentane/EtOAc 95/5), affording 229 mg (94 % yield) of the title product **6b** (R_f 0.49, pentane/EtOAc 9/1). mp 88–89 °C. ^1H NMR ($CDCl_3$) δ: 1.22 (t, J = 7.4 Hz, 3H, SCH_2CH_3), 1.58 (s, 9H, *t*-Bu), 2.40 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.84 (s, 2H, $ArCH_2S$), 6.80 (s, 1H, OH), 7.14 (d, $J_{5,6}$ = 8.3 Hz, 1H, H_5), 7.48–7.52 (m, 2H, H_2 and H_6) ppm. ^{13}C NMR ($CDCl_3$) δ: 14.1 (SCH_2CH_3), 24.9 (SCH_2CH_3), 28.0 (*t*-Bu), 30.9 ($ArCH_2S$), 81.2 (CO_2C), 117.3, 121.3, 128.7, 130.1 and 132.0 (Ar), 154.7 (C_3), 165.8 (CO_2*t*-Bu) ppm. Anal. calcd for $C_{14}H_{20}O_3S$: C, 62.65; H, 7.51; S, 11.95. Found: C, 62.44; H, 7.68; S, 11.83.

t-*Butyl 3-acetoxy-4-(2',6'-di-trifluoromethylbenzoxy)-methylbenzoate* ***15***. A mixture of 131.6 mg (0.4 mmol) of **11**, 124 mg (0.48 mmol) of 2,6-di-trifluoromethylbenzoic acid and 61.5 mg (1.06 mmol) of KF in 0.4 mL of DMF was stirred at room temperature for 64 h.

Ether was added and the mixture was washed with water. The ether solution was dried and evaporated. The crude product was purified by chromatography (pentane/EtOAc 9218), giving 194 mg (96 % yield) of the product **15** (R_f 0.34). mp (EtOAc): 119–200 °C. ^{1}H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.36 (s, 3H, OAc), 5.35 (s, 2H, $ArCH_2O$), 7.54 (d, $J_{5,6}$ = 8.0 Hz, 1H, H_5), 7.71 (t, $J_{3',4'}$ and $J_{4',5'}$ = 7.9 Hz, 1H, $H_{4'}$), 7.74 (d, $J_{2,6}$ = 1.5 Hz, 1H, H_2), 7.88 (dd, 1H, H_6), 7.91 (d, 2H, $H_{3'}$, $H_{5'}$) ppm. ^{13}C NMR ($CDCl_3$) δ: 20.4 (OAc), 27.8 (*t*-Bu), 62.9 ($ArCH_2O$), 81.4 (CO_2C), 122.7 (q, $^1J_{C,F}$ = 274.2 Hz, CF_3), 128.7 (q, $^2J_{C,F}$ = 44 Hz, $C_{2'}$, $C_{6'}$), 129.7 (m, $^3J_{C,F}$ = 5 Hz, $C_{1'}$, $C_{3'}$, $C_{5'}$), 123.5, 126.9, 130.1, 130.4, 130.7 and 133.8 (Ar and $C_{4'}$), 148.8 (C_3), 164.1 (CO_2t-Bu), 164.5 (CO_2CH_2), 166.8 (Ac) ppm. ^{19}F NMR ($CDCl_3$) δ: –59.5 (CF_3) ppm. Anal. calcd for $C_{23}H_{20}F_6O_6$: C, 54.55; H, 3.98. Found: C, 54.45; H, 4.15.

t-*Butyl 3-hydroxy-4-(2',6'-di-trifluoromethylbenzoxy)-methylbenzoate* ***6c***. To a solution of 115 mg (0.23 mmol) of **15** in 0.6 mL of toluene was added 19 µL (0.3 mmol) of pyrrolidine. The mixture was stirred at room temperature for 20 min. The reaction was quenched with 0.5 N HCl and the mixture was extracted with EtOAc, washed with water, dried and evaporated, affording 110 mg of crude product **6c** (R_f 0.33 CH_2Cl_2/MeOH 99/1), which was further dried over P_2O_5 under vacuum. The product obtained **6c** is unstable on silica gel and cannot be chromatographed.The crude product has been used in the preparation of **7c**.^{1}H NMR ($CDCl_3$) δ: 1.59 (s, 9H, *t*-Bu), 5.46 (s, 2H, CH_2O), 7.38 (d, J_{5-6} = 7.9 Hz, 1H, H_5), 7.54 (d, J_{2-6} = 1.5 Hz, 1H, H_2), 7.59 (dd, 1H, H_6), 7.62 (t, $J_{3',4'}$ and $J_{4',5'}$ = 7.9 Hz, 1H, $H_{4'}$), 7.91 (d, 2H, $H_{3'}$ and $H_{5'}$).

t-*Butyl 3-hydroxy-4-formylbenzoate* ***16***. To a solution of 870 mg (6.9 mmol) of oxalic acid and 775 mg (13.8 mmol) of KOH in 8 mL of H_2O, a solution of 750 mg (1.84 mmol) of **12** in 8 mL of EtOH was added. The mixture was refluxed overnight and evaporated to remove the ethanol. The residue was distributed between EtOAc and water. The organic phase was dried and evaporated. The crude product was purified by chromatography (CH_2Cl_2/pentane 1/1 then 4/1), affording 240 mg (61 % yield) of **16** (R_f 0.44, CH_2Cl_2/pentane 4/1). mp 74–75 °C. ^{1}H NMR ($CDCl_3$) δ: 1.59 (s, 9H, *t*-Bu), 7.56–7.60 (m, 3H, H_2, H_5 and H_6), 9.96 (s, 1H, CHO), 11.0 (s,1H, OH) ppm. ^{13}C NMR ($CDCl_3$) δ: 27.8 (*t*-Bu), 81.9 (CO_2C), 116.6, 120.1, 122.4, 133.3 and 139.0 (Ar), 160.9 (C_3), 163.9 (CO_2t-Bu), 196.3 (CHO) ppm. Anal. calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found C, 64.64; H, 6.48.

The column was further eluted with CH_2Cl_2/MeOH 95/5, affording 110 mg (36 % yield) of 3-hydroxy-4-formylbenzoic acid (R_f 0.1, CH_2Cl_2/MeOH 95/5). ^{1}H NMR (CD_3OD) δ: 7.24 (s, 1H, OH), 7.56 (s, 1H, H_2), 7.59 (d, $J_{5,6}$ = 8.0 Hz, 1H, H_6), 7.74 (d, 1H, H_5), 9.94 (s, 1H, CHO) ppm.

t-*Butyl 3-hydroxy-4-hydroxymethylbenzoate* ***17***. A solution of 48 mg (0.22 mmol) of **16** in MeOH (1 mL) was reacted with 40 mg (1.08 mmol) of $NaBH_4$ at room temperature for 20 min. The reaction mixture was then neutralized with 10 % HCl to pH 7 and extracted with MeOH/CH_2Cl_2 1/9. After evaporation of the solvents, 43 mg (89 % yield) of the title product **17** was obtained (R_f 0.12, CH_2Cl_2/MeOH 99/1). mp 123–124 °C, ^{1}H NMR ($CDCl_3$) δ: 1.58 (s, 9H, *t*-Bu), 2.67 (s, br 1H, OH), 4.89 (s, 2H, CH_2O), 7.07 (d, $J_{5,6}$ = 7.7 Hz, 1H, H_5), 7.46 (dd, $J_{2,6}$ = 1.4 Hz, 1H, H_6), 7.47 (d, 1H, H_2), 7.62 (br, 1H, OH) ppm. ^{13}C NMR (acetone-d_6) δ: 28.2 (*t*-Bu), 60.9 (CH_2OH), 80.9 (CO_2C), 116.2, 121.2, 127.8, 132.4 and 133.3 (Ar), 155.4 (C_3), 165.9 (CO_2t-Bu) ppm. Anal. calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found C, 64.05; H, 7.21.

t-*Butyl 3-hydroxy-4-*t*-butyldimethylsilyloxymethyl-benzoate* ***6d***. A solution of 182 mg (0.81 mmol) of **17**, 134 mg (0.89 mmol) of *t*-butyldimethylsilyl chloride and 165 mg (2.53 mmol) of imidazole in 0.4 mL of DMF was stirred under argon for 2 h. The reaction was quenched with water and extracted with ether. The ether solution was dried and evaporated. The crude product was purified by chromatography (CH_2Cl_2/pentane 1/3, 1/1 then CH_2Cl_2), affording 242 mg (88 % yield) of the title product **6d** (R_f 0.26, CH_2Cl_2/pentane 1/1). mp 63–64 °C. ^{1}H NMR ($CDCl_3$) δ: 0.13 (s, 6H, 2 × $SiCH_3$), 0.92 (s, 9H, Si*t*-Bu), 1.57 (s, 9H, *t*-Bu), 4.91 (s, 2H, $ArCH_2O$), 7.04 (d, $J_{5,6}$ = 7.7 Hz, 1H, H_5), 7.46–7.47 (m, 2H, H_2 and H_6), 8.18 (s, 1H, OH) ppm. ^{13}C NMR ($CDCl_3$) δ: –5.49 ($SiCH_3$), 18.0 (SiC), 25.8 (Si*t*-Bu), 28.1 (*t*-Bu), 64.9 ($ArCH_2O$), 80.8 (CO_2C), 117.3, 120.8, 126.3, 128.9 and 132.5 (Ar), 155.9 (C_3), 165.5 (CO_2t-Bu) ppm. Anal. calcd for $C_{18}H_{30}O_4Si$: C, 63.86; H, 8.93. Found: C, 63.88; H, 9.10.

8 mg (2.2 % yield) of a disilylated product (R_f 0.58, CH_2Cl_2/pentane 1/1) was also isolated. ^{1}H NMR ($CDCl_3$) δ: 0.10 (s, 6H, 2 × $SiCH_3$), 0.25 (s, 6H, 2 × $SiCH_3$), 0.95 and 1.01 (2s, 2 × 9H, 2 × Si*t*-Bu), 1.58 (s, 9H, *t*-Bu), 4.47 (s, 2H, CH_2O), 7.38 (d, $J_{2,6}$ = 1.4 Hz, 1H, H_2), 7.49 (d, $J_{5,6}$ = 8.0 Hz, 1H, H_5), 7.50 (dd, 1H, H_6) ppm. MS: 451 (M^+–1), 395 (M–*t*-Bu), 321 (M^+–OSiMe$_2t$-Bu), 265 (M^+–*t*-Bu -OSiMe$_2t$-Bu).

2-t-*Butyl-4-hydroxyphthalate* ***18***. The procedure described by Widmer[20] for the formation of *t*-butyl esters was modified for the preparation of the monoester **18**. 1.82 g (10 mmol) of 4-hydroxyphthalic acid was dissolved in 40 mL of dry THF, 30 mL of toluene were added, and the resulting solution was heated at 60 °C. *N,N*-Dimethylformamide di-*t*-butyl acetal 2.24 g (11 mmol) in toluene (30 mL) was added dropwise within 2 h. The solution was maintained at 60 °C for a further 30 min, then evaporated. The residue was purified by chromatography (CH_2Cl_2/MeOH 9/1) giving 1.05 g of compound **18** (44 % yield), R_f 0.41; mp 169 °C. ^{1}H NMR (CD_3OD) δ: 1.47 (s, 9H, *t*-Bu), 6.71 (d, $J_{3,5}$ = 2.4 Hz, 1H, H_3), 6.73 (dd, $J_{5,6}$ = 8.7 Hz, 1H, H_5), 7.56 (d, 1H, H_6) ppm; ^{13}C NMR (CD_3OD) δ: 28.1 (*t*-Bu), 83.3 (CO_2C), 156.0 (C4), 161.8

(CO_2H), 169.9 (CO_2t-Bu) ppm. Anal. calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.73; H, 6.16.

In this reaction, the 1-*t*-butyl 4-hydroxyphthalate was also obtained with almost the same yield and was recovered. R_f 0.23 (CH_2Cl_2/MeOH 9/1), ^1H NMR (CD_3OD) δ: 1.52 (s, 9H, *t*-Bu), 6.76 (dd, $J_{5,6}$ = 8.7 Hz, $J_{3,5}$ = 2.4 Hz, 1H, H_5), 6.79 (d, 1H, H_3), 7.62 (d, 1H, H_6) ppm. This ester can be easily cleaved by trifluoroacetic acid to give the starting hydroxyphthalic acid. Di- *t*-butyl 4-hydroxyphthalate was observed and isolated when a larger excess of acetal was used in the reaction.

t-*Butyl 3-ethyloxycarbonyloxy-6-hydroxymethylbenzoate* ***19***. To a solution of 475 mg (2 mmol) of the acid **18** in THF (20 mL) triethylamine 0.61 mL (4.4 mmol) was added. The solution was cooled at 0 °C before addition of ethyl chloroformate 0.42 mL (4.4 mmol). The triethylamine hydrochloride was eliminated by filtration, then sodium borohydride 168 mg (4.4 mmol) dissolved in methanol (5 mL) was added. After stirring for 10 min at 0 °C, the reaction mixture was poured into a 10 % HCl solution. The product was extracted with dichloromethane, concentrated and the residue purified by chromatography (pentane/EtOAc 7/3), giving **19**, 460 mg (78 % yield), R_f 0.60. ^1H NMR ($CDCl_3$) δ: 1.33 (t, J = 7.0 Hz, 3H, CH_3), 1.53 (s, 9H, *t*-Bu), 4.26 (q, 2H, Et), 4.67 (s, 2H, $ArCH_2$), 7.26 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.3 Hz, 1H, H_4), 7.38 (d, 1H, H_5), 7.65 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.1 (CH_3), 28.0 (*t*-Bu), 63.9 (CH_2OH), 65.0 (OCH_2), 82.5 (CO_2C), 150.0 (OCO_2), 153.2 (C_3), 166.1 (CO_2t-Bu) ppm. Anal. calcd for $C_{15}H_{20}O_6$: C, 60.79; H, 6.80. Found: C, 60.77; H, 6.84.

t-*Butyl 3-hydroxy-6-hydroxymethylbenzoate* ***17'***. Pyrrolidine, 71 mg (1 mmol) was added to a solution of compound **19**, 296 mg (0.5 mmol) in dichloromethane (5 mL). The mixture was stirred overnight at room temperature, more dichloromethane was then added, and the solution washed with 10 % HCl, dried and evaporated. Purification by chromatography (pentane/EtOAc 7/3), gave **17'**, 108 mg (96 % yield), R_f 0.34. mp 86–88 °C. ^1H NMR (CD_3OD) δ: 1.52 (s, 9H, *t*-Bu), 4.57 (s, 2H, $ArCH_2$), 6.84 (dd, $J_{2,4}$ = 2.8 Hz, $J_{4,5}$ = 8.2 Hz, 1H, H_4), 7.16 (d, 1H, H_5), 7.32 (d, 1H, H_2) ppm; ^{13}C NMR (CD_3OD) δ: 28.4 (*t*-Bu), 63.5 (CH_2OH), 82.7 (CO_2C), 157.6 (C_3), 168.3 (CO_2t-Bu) ppm. Anal. calcd for $C_{12}H_{16}O_4$: C, 64.26; H, 7.19. Found: C, 64.03; H, 6.92.

t-*Butyl 3-hydroxy-6-acetoxymethylbenzoate* ***6'a***. To a solution of 390 mg (1.32 mmol) of compound **19** in dichloromethane (15 mL), 0.150 mL (1.58 mmol) of acetic anhydride, 0.128 mL (1.58 mmol) of pyridine and 10 mg of DMAP were added. After 2 h at room temperature, the solution was washed with water and diluted HCl. Addition of 187 mg (2.64 mmol) of pyrrolidine to the solution and stirring for 1 h gave a crude product which was purified by chromatography (pentane/ether 1/1) to give 312 mg (89 % yield) of the title product **6'a**, R_f 0.61. ^1H NMR ($CDCl_3$) δ: 1.51 (s, 9H, *t*-Bu), 2.03 (s, 3H, CH_3), 5.29 (s, 2H, $ArCH_2$), 6.88 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.4 Hz, 1H, H_4), 7.22 (d, 1H, H_5), 7.36 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 21.1 (CH_3), 28.1 (*t*-Bu), 65.0 (CH_2), 82.2 (CO_2C), 156.0 (C_3), 166.9 (CO_2t-Bu), 171.8 (Ac) ppm. Anal. calcd for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 62.94; H, 6.93.

t-*Butyl 3-hydroxy-6-ethylthiomethylbenzoate* ***6'b***. The acetoxymethyl derivative **6'a**, 92 mg (0.35 mmol), was dissolved in DMF (1 mL), and added to a solution of EtSNa (1.05 mmol) in DMF (1 mL). After 30 min, the solution was poured into water and the product extracted with ether. The ether solution was washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified by chromatography (pentane/ether 1/1) giving **6'b** 88 mg (95 % yield), R_f 0.69. ^1H NMR ($CDCl_3$) δ: 1.12 (t, J = 7.4 Hz, 3H, CH_3), 1.53 (s, 9H, *t*-Bu), 2.34 (q, 2H, SCH_2), 3.92 (s, 2H, $ArCH_2$), 6.80 (dd, $J_{2,4}$ = 2.8 Hz, $J_{4,5}$ = 8.3 Hz, 1H, H_4), 7.05 (d, 1H, H_5), 7.33 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.5 (CH_2CH_3), 25.5 (CH_2CH_3), 26.1 (*t*-Bu), 33.8 (CH_2S), 82.4 (CO_2C), 154.7 (C_3), 167.4 (CO_2t-Bu) ppm. Anal. calcd for $C_{14}H_{20}O_3S$: C, 62.65; H, 7.51; S, 11.95. Found: C, 62.68; H, 7.37; S, 11.78.

t-*Butyl 3-hydroxy-6-*t*-Butyldimethylsilyloxymethyl-benzoate* ***6'd***. 148 mg (0.5 mmol) of compound **19** were dissolved in DMF (1 mL). After addition of imidazole 102 mg (1.5 mmol) and *t*-butyldimethylsilyl chloride 83 mg (0.55 mmol), the reaction was stirred at room temperature for 30 min. The mixture was then diluted with ethyl acetate, washed with water, dried and evaporated. The crude product was dissolved in dichloromethane (2 mL) and pyrrolidine 71 mg (1 mmol) was added. The reaction was stirred for 3 h, then the solvent was evaporated and the product purified by chromatography (pentane/EtOAc 9/1) giving 140 mg of **6'd** (83 % yield), R_f 0.64; mp 81 °C. ^1H NMR ($CDCl_3$) δ: 0.01 (s, 6H, $SiCH_3$), 0.90 (s, 9H, Si*t*-Bu), 1.53 (s, 9H, *t*-Bu), 4.96 (s, 2H, $ArCH_2$), 6.95 (dd, $J_{2,4}$ = 2.7 Hz, $J_{4,5}$ = 8.5 Hz, 1H, H_4), 7.35 (d, 1H, H_2), 7.58 (d, 1H, H_5) ppm; ^{13}C NMR ($CDCl_3$) δ: −5.9 ($SiCH_3$), 18.4 (SiC), 25.9 (Si*t*-Bu), 28.2 (*t*-Bu), 63.1 (CH_2O), 81.9 (CO_2C), 154.3 (C_3), 166.9: (CO_2t-Bu) ppm. Anal. calcd for $C_{18}H_{30}O_4Si$: C, 63.86; H, 8.93. Found: C, 64.07; H, 9.04.

Compounds **9'**,**10'**,**11'**,**15'** and **6'c** have been prepared to obtain the compound **7'c** by a second route so as to confirm the relative positions of the ester and carboxyl functions in the monoester **18**.

t-*Butyl 3-hydroxy-6-methylbenzoate* ***9'***. Starting from 3-hydroxy-6-methylbenzoic acid, the reaction conditions were the same as those used for the preparation of the ester **18**. Compound **9'** was obtained in a 37 % yield. ^1H NMR ($CDCl_3$) δ: 1.50 (s, 9H, *t*-Bu), 2.38 (s, 3H, CH_3), 6.81 (dd, J_{2-4} = 3.2 Hz, J_{4-5} = 8.4 Hz, 1H, H_4), 6.97 (d, 1H, H_5), 7.33 (d, 1H, H_2).

t-*Butyl 3-acetoxy-6-methylbenzoate* ***10'***. 86 mg (0.41 mmol) of the phenol **9'** were dissolved in 4 mL of dichloromethane, then were added successively, DMAP 5 mg (0.04 mmol), triethylamine 0.58 mL (4.1 mmol) and

acetic anhydride 44 µL (0.46 mmol). The solution was stirred for 30 min, then washed with water, dried and evaporated. After chromatography (CH_2Cl_2), 93 mg of product **10'** was obtained (90 % yield), R_f 0.60. ^1H NMR ($CDCl_3$) δ: 1.54 (s, 9H, *t*-Bu), 2.36 (s, 3H, CH_3), 2.51 (s, 3H, $COCH_3$), 7.05 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.3 Hz, 1H, H_4), 7.18 (d, 1H, H_5), 7.49 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 20.9 and 21.1 (CH_3), 28.1 (*t*-Bu), 81.3 (CO_2C), 148.2 (C_3), 165.9 (CO_2*t*-Bu), 169.3 (Ac) ppm.

t-*Butyl 3-acetoxy-6-bromomethylbenzoate* ***11'***. A solution of compound **10'**, 84 mg (0.335 mmol) in CCl_4 was refluxed for 4 h in the presence of *N*-bromosuccinimide 66 mg (0.37 mmol) and benzoyl peroxide (5 mg). The solvent was evaporated and the product purified by chromatography (pentane/EtOAc 9/1), giving 87 mg of **11'** (79 % yield), R_f 0.54. ^1H NMR ($CDCl_3$) δ: 1.50 (s, 9H, *t*-Bu), 2.19 (s, 3H, $COCH_3$), 4.78 (s, 2H, CH_2Br), 7.07 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.4 Hz, 1H, H_4), 7.31 (d, 1H, H_5), 7.48 (d, 1H, H_2) ppm. ^{13}C NMR ($CDCl_3$) δ: 21.1 (CH_3), 28.1 (*t*-Bu), 31.0 (CH_2Br), 82.5 (CO_2C), 150.2 (C_3), 165.0 (CO_2*t*-Bu), 168.3 (Ac) ppm. In this reaction the formation of *t*-butyl 3-acetoxy-6-dibromomethylbenzoate was also observed.

t-*Butyl 3-acetoxy-6-(2',6'-di-trifluoromethylbenzoxy)methyl benzoate* ***15'***. A mixture of 80 mg (0.24 mmol) of the bromide **11'** and 62 mg (0.24 mmol) of 2,6-di-trifluoromethylbenzoic acid and 14 mg (0.48 mmol) of KF in 0.4 mL of DMF was stirred at room temperature for 40 h. The solvent was eliminated under reduced pressure and the crude product purified by chromatography (pentane/EtOAc 9/1), giving 104 mg of **15'** (85 % yield), R_f 0.39. ^1H NMR ($CDCl_3$) δ: 1.59 (s, 9H, *t*-Bu), 2.32 (s, 3H, $COCH_3$), 5.84 (s, 2H, CH_2O), 7.25 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.5 Hz, 1H, H_4), 7.61 (d, 1H, H_5), 7.69 (d, 1H, H_2), 7.70 (t, $J_{3',4'}$ and $J_{4',5'}$ = 8.0 Hz, 1H, $H_{4'}$), 7.93 (d, 2H, $H_{3'}$ and $H_{5'}$) ppm; ^{19}F NMR ($CDCl_3$) δ: –59.4 (CF_3) ppm; ^{13}C NMR ($CDCl_3$) δ: 20.9 (CH_3), 28.0 (*t*-Bu), 66.3 (CH_2O), 82.2 (CO_2C), 122.7 (q, $^1J_{CF}$ = 214.4 Hz, CF_3), 128.9 (q, $^2J_{CF}$ = 32.5 Hz, $C_{2'}$ and $C_{6'}$), 149.9 (C_3), 164.5 (OCO), 164.7 (CO_2*t*-Bu), 169.1 (Ac) ppm.

t-*Butyl 3-hydroxy-6-(2',6'-di-trifluoromethylbenzoxy)-methyl benzoate* ***6'c***. 63 mg of compound **15'** (0.127 mmol) were dissolved in 1 mL of toluene and reacted with 10 µL of pyrrolidine. After stirring for 15 min, the solution was diluted with diethyl ether and washed with 5 % HCl, water and dried (Na_2SO_4). The obtained product **6'c** is unstable on silica gel and cannot be chromatographed. The crude product was used in the preparation of **7'c**.^1H NMR ($CDCl_3$) δ: 1.57 (s, 9H, *t*-Bu), 5.74 (s, 2H, CH_2O), 6.96 (dd, $J_{2,4}$ = 2.5 Hz, $J_{4,5}$ = 8.5 Hz, 1H, H_4), 7.41 (d, 1H, H_5), 7.45 (d, 1H, H_2), 7.71 (t, $J_{3',4'}$ and $J_{3',5'}$ = 7.4 Hz, 1H, $H_{4'}$), 7.91 (d, 2H, $H_{3'}$ and $H_{5'}$) ppm; ^{19}F NMR ($CDCl_3$) δ: –59.4 (CF_3) ppm.

General procedure for coupling reactions. To a mixture of a phenol **6** or **6'** and 1.5 equivalent of the acid **5** in dry pyridine (1 mL/mmol) was added 10 mg/mmol of *p*-TsOH·H_2O and 1.5 equivalents of DCC. The resulting mixture was stirred at room temperature for 16 h. The reaction was diluted with ethyl acetate, and the resultant mixture washed with 10 % HCl and water, dried (Na_2SO_4) and evaporated. This procedure was modified for phenols **6c** and **6'c**.

*3-*t*-Butoxycarbonyl-6-acetoxymethylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***7a***. The crude product from the reaction of 147 mg (0.5 mmol) of **5** with 146.3 mg (0.55 mmol) of **6a** was purified by chromatography (CH_2Cl_2/MeOH 99/1), giving 225 mg (83 % yield) of **7a** as an oil (R_f 0.63).^1H NMR ($CDCl_3$) δ: 1.54 (s, 9H, Boc), 1.58 (s, 9H, *t*-Bu), 2.05 (s, 3H, OAc), 4.33 (s, 2H, $PhCH_2$), 4.77 (s, 2H, NCH_2), 5.06 (s, 2H, $ArCH_2O$), 7.23–7.31 (m, 5H, Ph), 7.48 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.68 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.84 (dd, 1H, H_4) ppm; ^{13}C NMR ($CDCl_3$) δ: 20.6 (Ac), 27.8 and 28.0 (Boc and *t*-Bu), 43.9 ($PhCH_2$), 45.4 (NCH_2), 60.7 ($ArCH_2O$), 81.5 (CO_2C), 84.4 (NCO_2C), 123.2, 126.7, 127.3, 128.2, 129.4, 129.7, 132.6, 133.3 and 134.5 (Ph and Ar), 148.1 (C_1), 151.7 (NCO_2), 164.1 (CO_2*t*-Bu), 167.2 (CO_2), 170.3 (Ac), 173.6 (CON) ppm. Anal. calcd for $C_{29}H_{35}NO_9$: C, 64.31; H, 6.51; N, 2.59. Found: C, 64.58; H, 6.42; N, 2.31.

*3-*t*-Butoxycarbonyl-6-ethylthiomethylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***7b***. The crude product from the reaction of 87.9 mg (0.3 mmol) of **5** with 53.6 mg (0.2 mmol) of **6b** was purified by chromatography (pentane/EtOAc 98/2 and 95/5), giving 106 mg (97.6 % yield) of the product **7b** (R_f 0.53, pentane/EtOAc 9/1). mp (MeOH): 67–68 °C. ^1H NMR ($CDCl_3$) δ: 1.16 (t, J = 7.4 Hz, 3H, SCH_2CH_3), 1.53 (s, 9H, Boc), 1.56 (s, 9H, *t*-Bu), 2.38 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 3.61 (s, 2H, $ArCH_2S$), 4.32 (s, 2H, $PhCH_2$), 4.76 (s, 2H, NCH_2), 7.22–7.30 (m, 5H, Ph), 7.64 (d, $J_{4,5}$ = 8.1 Hz, 1H, H_5), 7.44 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.84 (dd, 1H, H_4) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.2 (SCH_2CH_3), 25.4 (SCH_2CH_3), 27.9 and 28.1 (Boc and *t*-Bu), 30.1 ($ArCH_2S$), 44.0 ($PhCH_2$), 45.6 (NCH_2), 81.4 (CO_2C), 84.5 (NCO_2C), 123.3, 126.8, 127.2, 128.3, 129.5, 130.4, 132.1, 134.5 and 135.6 (Ph and Ar), 148.1 (C_1), 151.6 (NCO_2), 164.4 (CO_2*t*-Bu), 167.2 (CO_2), 173.7 (CON) ppm. Anal. calcd for $C_{29}H_{37}O_7SN$: C, 64.06; H, 6.86; N, 2.58; S, 5.90. Found: C, 64.27; H, 6.70; N, 2.51; S, 5.87.

*3-*t*-Butoxycarbonyl-6-(2',6'-di-trifluoromethylbenzoxy)-methylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***7c***. To a solution of 118 mg (0.4 mmol) of acid **5** in 0.2 mL of anhydrous CH_2Cl_2 was added 82.4 mg (0.4 mmol) of DCC and 12.2 mg (0.1 mmol) of DMAP. After 5 min the crude product **6c** (0.23 mmol) dissolved in 0.26 mL of CH_2Cl_2 was added. The reaction mixture was stirred at room temperature for 2.5 h, then EtOAc and 5 % HCl were added. The white precipitate (DCU) was removed by filtration. The filtrate was separated and the organic phase washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified by chromatography (CH_2Cl_2), 40 mg (34 %) of the starting product **15** was recovered (R_f

0.49) and 85.5 mg (51 % yield) of the title product **7c** was obtained as an oil (R_f 0.39).^{1}H NMR ($CDCl_3$) δ: 1.53 (s, 9H, Boc), 1.58 (s, 9H, *t*-Bu), 4.32 (s, 3H, $ArCH_2$), 4.79 (s, 2H, NCH_2), 5.35 (s, 2H, $ArCH_2O$), 7.23–7.30 (m, 5H, Ph), 7.56 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.73 (t, $J_{3',4'}$ and $J_{4',5'}$ = 7.9 Hz, 1H, $H_{4'}$), 7.74 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.90 (m, 3H, H_4, $H_{3'}$, $H_{5'}$) ppm; ^{13}C NMR ($CDCl_3$) δ: 27.8 and 28.0 (*t*-Bu and Boc), 44.0 ($ArCH_2$), 45.6 (NCH_2), 62.6 ($ArCH_2O$), 81.6 (CO_2C), 84.5 (NCO_2C), 122.7 (q, $^1J_{C,F}$ = 274.3 Hz, CF_3), 128.9 (q, $^2J_{C,F}$ = 32.7 Hz, $C_{2'}$, $C_{6'}$), 129.8 (m, $^3J_{C,F}$ = 4.8 Hz, $C_{1'}$, $C_{3'}$, $C_{5'}$), 123.1, 126.7, 127.3, 128.3, 129.5, 129.9, 130.3, 130.8, 133.8, 134.6 and 134.8 (Ph, Ar and $C_{4'}$), 148.2 (C_1), 151.8 (NCO_2), 164.1 (CO_2t-Bu), 164.5 (CO_2CH_2), 167.2 (CO_2), 173.7 (CON) ppm. ^{19}F NMR ($CDCl_3$) δ: –59.3 (CF_3) ppm; Anal. calcd for $C_{36}H_{35}F_6NO_9$ C, 58.45; H, 4.77; N, 1.89. Found: C, 58.67, H, 4.95, N, 1.72.

10 mg (14.5 % yield) of *t*-butyl 3-(2',6'-di-trifluoromethylbenzoxy)-4-hydroxymethyl benzoate was obtained as a by product (R_f 0.64). mp 180 °C (dec.). ^{1}H NMR ($CDCl_3$) δ: 1.57 (s, 9H, *t*-Bu), 4.32 (s, 2H, $ArCH_2O$), 6.40 (br, 1H, OH), 7.18 (d, $J_{5,6}$ = 7.2 Hz, 1H, H_5), 7.26 (s, 1H, H_2), 7.31 (m, 2H, H_6 and $H_{4'}$), 7.47 (d, $J_{3',4'}$ and $J_{4',5'}$ = 7.4 Hz, 2H, $H_{3'}$, $H_{5'}$) ppm; ^{19}F NMR ($CDCl_3$) δ: –59.5 (CF_3) ppm.

3-t-*Butoxycarbonyl-6*-t-*butyldimethylsilyloxymethylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***7d***. The crude product from the reaction of 312 mg (1.06 mmol) of **5** with 235 mg (0.7 mmol) of **6d** was purified by chromatography (CH_2Cl_2/pentane 3/2 followed by CH_2Cl_2), giving 372 mg (87.5 % yield) of the product **7d** as an oil (R_f 0.48, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 0.06 (s, 6H, 2 × $SiCH_3$), 0.92 (s, 9H, Si*t*-Bu), 1.53 (s, 9H, Boc), 1.57 (s, 9H, *t*-Bu), 4.31 (s, 2H, $PhCH_2$), 4.66 (s, 2H, CH_2OSi), 4.73 (s, 2H, NCH_2), 7.22–7.30 (m, 5H, Ph), 7.59 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.63 (d, $J_{4,5}$ = 8.1 Hz, 1H, H_5), 7.90 (dd, 1H, H_4) ppm; ^{13}C NMR ($CDCl_3$) δ: –5.5 ($SiCH_3$), 18.2 (SiC), 25.8 (Si*t*-Bu), 27.8 and 28.1 (*N*-Boc and *t*-Bu), 44.0 ($PhCH_2$), 45.6 (NCH_2), 59.6 ($ArCH_2O$), 81.2 (CO_2C), 84.4 (NCO_2C), 122.3, 126.8, 127.1, 127.4, 128.3, 129.5, 131.8, 134.5 and 138.5 (Ph and Ar), 146.4 (C_1), 151.8 (NCO_2), 164.6 (CO_2t-Bu), 167.1 (CO_2), 173.6 (CON) ppm. Anal. calcd for $C_{33}H_{47}O_8NSi$: C, 64.57; H, 7.72; N, 2.29. Found: C, 64.31; H, 7.57; N, 2.23.

3-t-*Butoxycarbonyl-6-bromomethylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***8e***. To a suspension of 115 mg (0.274 mmol) of Ph_3PBr_2 in 0.1 mL of CH_2Cl_2, a solution of 140 mg (0.228 mmol) of **7d** in 0.2 mL of CH_2Cl_2 was added. The resulting mixture was stirred under argon at room temperature. for 30 min and diluted with CH_2Cl_2, washed twice with water, dried and evaporated. The residue was purified by chromatography (pentane/EtOAc 92/8 and 9/1), giving 92 mg (72 % yield) of the product **8e** (R_f 0.49, pentane/EtOAc 9/1). mp (EtOAc): 102–103 °C. ^{1}H NMR ($CDCl_3$) δ: 1.55 (s, 9H, Boc), 1.58 (s, 9H, *t*-Bu), 4.35 (s, 2H, $PhCH_2$), 4.38 (s, 2H, $ArCH_2Br$), 4.82 (s, 2H, NCH_2), 7.24–7.33 (m, 5H, Ph), 7.48 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.72 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.88 (dd, 1H, H_4) ppm. ^{13}C NMR ($CDCl_3$) δ: 26.3 ($ArCH_2Br$), 27.9 and 28.0 (Boc and *t*-Bu), 44.0 ($PhCH_2$), 45.7 (NCH_2), 81.6 (CO_2C), 84.6 (NCO_2C), 123.6, 126.8, 127.5, 128.3, 129.5, 130.7, 133.7, 134.1 and 134.5 (Ph and Ar), 148.1 (C_1), 151.8 (NCO_2), 164.0 (CO_2t-Bu), 166.9 (CO_2), 173.9 (CON) ppm. Anal. calcd for $C_{27}H_{32}BrNO_7$: C, 57.65; H, 5.73; N, 2.49; Br, 14.21. Found: C, 57.80; H, 5.86; N, 2.13; Br, 13.92.

3-t-*Butoxycarbonyl-6-chloromethylphenyl* N-*Boc*-N-*phenylacetylglycinate* ***8f***. To a suspension of 142 mg (0.44 mmol) of Ph_3PCl_2 in 0.2 mL of CH_2Cl_2 a solution of 203 mg (0.4 mmol) of **7d** in 0.24 mL of CH_2Cl_2 was added. The resulting mixture was stirred under argon at room temperature for 30 min and diluted with CH_2Cl_2, washed twice with water, dried and evaporated. The residue was purified by chromatography (pentane/EtOAc 9/1), giving 120 mg (71 % yield) of the product **8f** (R_f 0.30). mp (EtOAc): 68–69 °C. ^{1}H NMR ($CDCl_3$) δ: 1.54 (s, 9H, Boc), 1.58 (s, 9H, *t*-Bu), 4.34 (s, 2H, $PhCH_2$), 4.49 (s, 2H, $ArCH_2Cl$), 4.79 (s, 2H, NCH_2), 7.24–7.31 (m, 5H, Ph), 7.51 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.70 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.89 (dd, 1H, H_4) ppm; ^{13}C NMR ($CDCl_3$) δ: 27.8 and 28.0 (Boc and *t*-Bu), 40.1 ($ArCH_2Cl$), 44.0 ($PhCH_2$), 45.6 (NCH_2), 81.6 (CO_2C), 84.6 (NCO_2C), 123.4, 126.8, 127.5, 128.3, 129.5, 130.3, 133.6, 133.7 and 134.4 (Ph and Ar), 148.0 (C_1), 151.8 (NCO_2), 164.0 (CO_2t-Bu), 167.0 (CO_2), 173.9 (CON) ppm. Anal. calcd for $C_{27}H_{32}ClNO_7$: C, 62.60; H, 6.23; N, 2.70; Cl, 6.85. Found: C, 62.43; H, 6.33; N, 2.57; Cl, 7.01.

3-t-*Butoxycarbonyl-6-ethylmethylsulfoniomethylphenyl* N-*Boc*-N-*phenylacetylglycinate tetrafluoroborate* ***8g***. To a solution of 179 mg (0.33 mmol) of **7b** in 0.25 mL of acetonitrile, 0.103 mL (1.65 mmol) of CH_3I and 64 mg (0.33 mmol) of $AgBF_4$ were added. The resulting mixture was stirred under argon in the dark at room temperature for 6 h. The reaction mixture was chromatographed on a small silica gel column eluted first with CH_2Cl_2, then with ether and finally with acetone, affording 90 mg (43 % yield) of **8g** as an oil (R_f 0.19, Et_2O/acetone 7/3). ^{1}H NMR (acetone-d_6) δ: 1.36 (t, J = 7.4 Hz, 3H, $S^+CH_2CH_3$), 1.58 (s, 9H, Boc), 1.59 (s, 9H, *t*-Bu), 2.88 (s, 2H, S^+CH_3), 3.39 (m, J = 7.4 Hz, J_{AB} = 13.3 Hz, 2H, $S^+CH_2CH_3$), 4.37 (s, 2H, $PhCH_2$), 4.71 (q, J_{AB} = 13 Hz, 2H, $ArCH_2S^+$), 4.89 (s, 2H, NCH_2), 7.29–7.34 (m, 5H, Ph), 7.81–7.85 (m, 2H, H_5 and H_2), 7.96 (dd, $J_{2,4}$ = 1.5 Hz, $J_{4,5}$ = 8.1 Hz, 1H, H_4) ppm; ^{13}C NMR (acetone-d_6) δ: 9.0 ($S^+CH_2CH_3$), 21.8 (S^+CH_3), 27.9 and 28.1 (Boc and *t*-Bu), 37.1 ($S^+CH_2CH_3$), 40.8 ($ArCH_2S^+$), 44.5 ($PhCH_2$), 47.1 (NCH_2), 82.6 (CO_2C), 85.4 (NCO_2C), 124.5, 126.3, 127.5, 128.4, 129.0, 130.6, 133.7, 135.9 and 136.0 (Ph and Ar), 150.4 (C_1), 152.7 (NCO_2), 164.3 (CO_2t-Bu), 168.6 (CO_2), 175.5 (CON) ppm. MS (FAB): 558 (M^+–BF_4), 458 (M^+–Boc), 382 (M^+–Boc–MeSEt), 326 (M^+–Boc–MeSEt–*t*-Bu), 176, 151. HRMS (FAB^+): calcd for [$C_{30}H_{40}O_7NS^+$] 558.2525. Found 558.2513.

3-t-Butoxycarbonyl-4-acetoxymethylphenyl N-*Boc*-N-*phenylacetylglycinate* ***7'a***. The crude product from the reaction of 41 mg (0.15 mmol) of **6'a** with 68 mg (0.23 mmol) of **5** was purified by chromatography on silica gel (pentane/EtOAc 8/2), giving 77 mg (93 % yield) of **7'a**, R_f 0.44, mp 117 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H, N-Boc), 1.51 (s, 9H, *t*-Bu), 2.06 (s, 3H, OAc), 4.26 (s, 2H, $PhCH_2$), 4.66 (s, 2H, NCH_2), 5.40 (s, 2H, $ArCH_2O$), 7.17 (dd, $J_{2,6}$ = 2.4 Hz, $J_{5,6}$ = 8.5 Hz, 1H, H_6), 7.41 (d, 1H, H_5), 7.54 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 20.9 (Ac), 27.8 and 28.1 (*N*-Boc and *t*-Bu), 44.0 ($PhCH_2$), 45.5 (NCH_2), 64.1 ($ArCH_2O$), 82.1 (CO_2C), 84.4 (NCO_2C), 149.5 (C_1), 151.8 (NCO_2),164.9 (CO_2*t*-Bu), 167.3 (CO_2), 170.5 (Ac), 173.7 (CON) ppm. Anal. calcd for $C_{29}H_{35}NO_9$: C, 64.31; H, 6.51; N, 2.59. Found: C, 64.32; H, 6.65; N, 2.46.

3-t-Butoxycarbonyl-4-ethylthiomethylphenyl N-*Boc*-N-*phenylacetylglycinate* ***7'b***. The crude product from the reaction of 75 mg (0.255 mmol) of **5** with 45 mg (0.17 mmol) of **6'b** was purified by chromatography on silica gel (pentane/Et_2O: 3/1), giving 70 mg (77 % yield) of the product **7'b**, R_f 0.51, as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.13 (t, J = 7.4 Hz, 3H, SCH_2CH_3), 1.43 (s, 9H, *N*-Boc), 1.51 (s, 9H, *t*-Bu), 2.36 (q, J = 7.4 Hz, 2H, SCH_2CH_3), 4.00 (s, 2H, $ArCH_2S$), 4.25 (s, 2H, $PhCH_2$), 4.64 (s, 2H, NCH_2), 7.04 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H_6), 7.23 (d, 1H, H_5), 7.44 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.4 (SCH_2CH_3), 25.6 (SCH_2CH_3), 27.8 and 28.1 (*N*-Boc and *t*-Bu), 33.5 ($ArCH_2S$), 43.8 ($PhCH_2$), 45.5 (NCH_2), 81.9 (CO_2C), 84.4 (NCO_2C), 148.7 (C_1), 151.8 (NCO_2), 165.6 (CO_2*t*-Bu), 167.3 (CO_2), 173.7 (CON) ppm. Anal. calcd for $C_{29}H_{37}NO_7S$: C, 64.06; H, 6.86; N, 2.58; S, 5.90. Found: C, 64.02; H, 6.87; N, 2.60; S, 5.82.

3-t-Butoxycarbonyl-4-(2',6'-di-trifluoromethylbenzoxy)-methylphenyl N-*Boc*-N-*phenylacetylglycinate* ***7'c***. a) From **8'f**: To a solution of 74.7 mg (0.133 mmol) of **8'f** in 0.4 mL of DMF was added 17 mg (0.29 mmol) of KF and 40 mg (0.146 mmol) of 2,6-di-trifluoromethyl benzoic acid. The resulting mixture was stirred under argon in the dark at room temperature for 24 h. The solvent was evaporated under reduced pressure, the reaction mixture was chromatographed on a small silica gel column and eluted with pentane/EtOAc 85/15, affording 85 mg (87 % yield) of **7'c**, as an oil, R_f 0.40. ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 9H, *N*-Boc), 1.51 (s, 9H, *t*-Bu), 4.26 (s, 2H, $PhCH_2$), 4.66 (s, 2H, NCH_2), 5.76 (s, 2H, $ArCH_2$), 7.15 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.6 Hz, 1H, H_6), 7.52 (d, 1H, H_5), 7.59 (d, 1H, H_2), 7.64 (t, 1H, H_4'), 7.86 (d, 2H, H_3' and H_5') ppm; ^{13}C NMR ($CDCl_3$) δ: 28.0 and 28.2 (*N*-Boc and *t*-Bu), 44.1 ($PhCH_2$), 45.7 (NCH_2), 66.4 (CH_2O), 82.4 (CO_2C), 84.5 (NCO_2C), 149.7 (C_1), 151.9 (NCO_2), 164.7 (CO_2*t*-Bu), 167.3 and 167.4 (CO_2), 173.8 (CON) ppm; ^{19}F NMR ($CDCl_3$) δ: −59.5 (CF_3) ppm. Anal. calcd for $C_{36}H_{35}NO_9F_6$: C, 58.45; H, 4.77; N, 1.89. Found: C, 58.74; H, 5.04; N, 1.87. b) From **6'c**: 2 equivalents of **5** in dichloromethane (0.5 mL), 2 equivalents of DCC and 0.1 equivalent of DMAP were added to **6'c**. The reaction mixture was stirred under an argon atmosphere for 45 min, then poured into 5 % HCl, extracted with ether, washed twice with water, dried and the solvent evaporated. After purification, **7'c** was obtained in a 79 % yield. The products obtained by the two routes have the same characteristics.

3-t-Butoxycarbonyl-4-t-butyldimethylsilyloxymethylphenyl N-*Boc*-N-*phenylacetylglycinate* ***7'd***. The crude product from the reaction of 198 mg (0.675 mmol) of **5** with 152 mg (0.45 mmol) of **6'd** was purifed by chromatography on silica gel (pentane/EtOAc 9/1) giving 229 mg (83 % yield) of product **7'd**, R_f 0.69 (oil). ^{1}H NMR ($CDCl_3$) δ: 0.06 (s, 6H, 2 $SiCH_3$), 0.91 (s, 9H, Si*t*-Bu), 1.46 (s, 9H, *N*-Boc), 1.51 (s, 9H, *t*-Bu), 4.28 (s, 2H, $PhCH_2$), 4.68 (s, 2H, NCH_2), 5.02 (s, 2H, CH_2OSi), 7.18 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.6 Hz, 1H, H_6), 7.54 (d, 1H, H_2), 7.75 (d, 1H, H_5) ppm; ^{13}C NMR ($CDCl_3$) δ: −5.6 ($SiCH_3$), 18.3 (SiC), 25.9 (Si*t*-Bu), 27.8 and 28.1 (*N*-Boc and *t*-Bu), 44.0 ($PhCH_2$), 45.5 (NCH_2), 62.9 (CH_2O), 81.6 (CO_2C), 84.3 (NCO_2C), 148.4 (C_1), 151.8 (NCO_2), 165.1 (CO_2*t*-Bu), 167.5 (CO_2), 173.7 (CON) ppm. Anal. calcd for $C_{33}H_{47}NO_8Si$: C, 64.57; H, 7.72; N, 2.29. Found: C, 64.43; H, 7.61; N, 2.30.

3-t-Butoxycarbonyl-4-bromomethylphenyl N-*Boc*-N-*phenylacetylglycinate* ***8'e***. To a suspension of 141 mg (0.333 mmol) of Ph_3PBr_2 in 0.1 mL of dichloromethane was added a solution of 186 mg (0.303 mmol) of **7'd** in 0.5 mL of the same solvent. The resulting mixture was stirred under argon at room temperature for 20 min then diluted with dichloromethane and washed twice with water, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (pentane/EtOAc 9/1), giving 123 mg (76 % yield) of the product **8'e**, R_f 0.41. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 9H, *N*-Boc), 1.49 (s, 9H, *t*-Bu), 4.24 (s, 2H, $PhCH_2$), 4.60 (s, 2H, NCH_2), 4.78 (s, 2H, $ArCH_2Br$), 7.06 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H_6), 7.31 (d, 1H, H_5), 7.46 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 27.8 and 28.0 (*N*-Boc and *t*-Bu), 30.7 (CH_2Br), 44.0 ($PhCH_2$), 45.5 (NCH_2), 82.4 (CO_2C), 84.4 (NCO_2C), 149.8 (C_1), 151.7 (NCO_2), 164.7 (CO_2*t*-Bu), 167.1 (CO_2), 173.6 (CON) ppm. Anal. calcd for $C_{27}H_{32}NO_7Br$: C, 57.65; H, 5.73; N, 2.49; Br, 14.21. Found: C, 57.75; H, 5.71; N, 2.33; Br, 14.10.

3-t-Butoxycarbonyl-4-chloromethylphenyl N-*Boc*-N-*phenylacetylglycinate* ***8'f***. To a suspension of 54.4 mg (0.17 mmol) of Ph_3PCl_2 in 0.3 mL of dichloromethane was added a solution of 86 mg (0.14 mmol) of **7'd** in 0.3 mL of the same solvent. The resulting mixture was stirred under argon at room temperature for 60 min and diluted with dichloromethane, washed twice with water, dried and evaporated. The residue was purified by chromatography on silica gel (pentane/EtOAc 9/1), giving 57 mg (79 % yield) of the product **8'f**, R_f 0.41. ^{1}H NMR ($CDCl_3$) δ: 1.54 (s, 9H, *N*-Boc), 1.60 (s, 9H, *t*-Bu), 4.33 (s, 2H, $PhCH_2$), 4.73 (s, 2H, NCH_2), 4.99 (s, 2H, $ArCH_2Cl$), 7.22 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.5 Hz, 1H, H_6), 7.52 (d, 1H, H_5), 7.60 (d, 1H, H_2) ppm; ^{13}C NMR ($CDCl_3$) δ: 27.8 and 28.0 (*N*-Boc

and *t*-Bu), 43.8 (CH_2Cl), 44.0 ($PhCH_2$), 45.5 (NCH_2), 82.4 (CO_2C), 84.5 (NCO_2C), 149.8 (C_1), 151.7 (NCO_2), 165.6 (CO_2t-Bu), 167.2 (CO_2), 173.7 (CON) ppm. Anal. calcd for $C_{27}H_{32}NO_7Cl$: C, 62.60; H, 6.23; N, 2.70. Found: C, 62.65; H, 6.33; N, 2.57.

3-t-Butoxycarbonyl-4-ethylmethylsulfoniomethylphenyl N-Boc-N-phenylacetylglycinate tetrafluoroborate ***8'g***. To a solution of 60 mg (0.11 mmol) of **7'b** in 0.3 mL of acetonitrile was added 0.034 mL (0.55 mmol) of CH_3I and 22.6 mg (0.116 mmol) of $AgBF_4$. The resulting mixture was stirred under argon in the dark at room temperature for 7 h. The reaction mixture was chromatographed on a small silica gel column, and eluted first with ether and then with ether/acetone 1/1, affording 33 mg (46 % yield) of **8'g**, as an oil, R_f 0.30. ^1H NMR (acetone-d_6) δ: 1.65 (t, J = 7.3 Hz, 3H, $S^+CH_2CH_3$), 1.60 (s, 9H, *N*-Boc), 1.68 (s, 9H, *t*-Bu), 2.73 (s, 3H, S^+CH_3), 3.72 (q, 2H, $S^+CH_2CH_3$), 4.39 (s, 2H, $PhCH_2$), 5.14 (q, J_{AB} = 12.7 Hz, 2H, $ArCH_2S^+$), 4.87 (s, 2H, NCH_2), 7.56 (dd, $J_{2,6}$ = 2.4 Hz, $J_{5,6}$ = 8.3 Hz, 1H, H_6), 7.89 (d, 1H, H_5), 7.91 (d, 1H, H_2) ppm; ^{13}C NMR (acetone-d_6) δ: 9.45 (S^+CH_2CH3), 22.9 (S^+CH_3), 28.1 and 28.3 (*N*-Boc and *t*-Bu), 38.0 ($S^+CH_2CH_3$), 44.5 ($PhCH_2$), 46.1 ($ArCH_2S^+$), 46.4 (NCH_2), 84.2 (CO_2C), 85.0 (NCO_2C), 152.6 (C_1), 152.9 (NCO_2), 165.8 (CO_2t-Bu), 168.5 (CO_2), 174.2 (CON) ppm. MS (FAB): 558 (M^+–BF_4), 458 (M^+–Boc), 326, 176, 151. HRMS (FAB^+): calcd for [$C_{30}H_{40}O_7NS^+$] 558.2525. Found 558.2524.

General procedure for the final deprotection. The products **7**, **7'**, **8** and **8'** were respectively treated with CF_3COOH (0.1 mL/mmol) at room temperature for 1 h. The reaction mixture was evaporated at reduced pressure, toluene was added and evaporated to remove the remaining trifluoroacetic acid. The resulting residue was dried over P_2O_5 and KOH in a desiccator under vacuum. The obtained solid was washed with pentane or dichloromethane to remove the soluble products, or crystallized in acetone or methanol. These products, obtained in near quantitative yield, are not stable enough to be purified on silica gel.

6-Acetoxymethyl-3-carboxyphenyl phenylacetylglycinate ***1a***. mp (MeOH): 180–181 °C (dec.). ^1H NMR (acetone-d_6) δ: 2.07 (s, 3H, OAc), 3.67 (s, 2H, $PhCH_2$), 4.34 (s, 2H, NCH_2), 5.14 (s, 2H, $ArCH_2O$), 7.24–7.44 (m, 5H, Ph), 7.59 (d, $J_{4,5}$ = 7.9 Hz, 1H, H_5), 7.82 (d, $J_{2,4}$ = 1.4 Hz, 1H, H_2), 7.95 (dd, 1H, H_4) ppm; ^{13}C NMR (acetone-d_6) δ: 21.2 (Ac), 42.0 (NCH_2), 43.1 ($PhCH_2$), 61.3 ($ArCH_2O$), 124.6, 127.5, 128.0, 128.9, 129.2, 130.0, 130.5, 132.7, 134.4 and 136.3 (Ph and Ar), 149.5 (C_1), 166.8 (CO_2), 169.2 (CO_2H), 171.0 (Ac), 172.7 (CON) ppm. Anal. calcd for $C_{20}H_{19}NO_7$: C, 62.34; H, 4.94; N, 3.64. Found: C, 62.17; H, 4.99; N, 3.48.

6-(2',6'-Di-trifluoromethylbenzoxy)methyl-3-carboxyphenyl phenylacetylglycinate ***1c***. mp (MeOH) 170–171 °C. ^1H NMR (CD_3OD + DMF-d_7) δ: 3.53 (s, 3H, $PhCH_2$), 4.20 (s, 2H, NCH_2), 5.33 (s, 2H, $ArCH_2O$), 7.10–7.22 (m, 5H, Ph), 7.56 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.77 (d, $J_{2,4}$ = 1.4 Hz, 1H, H_2), 7.82 (t, $J_{3',4'}$ and $J_{4',5'}$ = 8.0 Hz, 1H, $H_{4'}$), 8.00 (m, 3H, $H_{4'}$, $H_{3'}$, $H_{5'}$) ppm. ^{13}C NMR (CD_3OD + DMF-d_7) δ: 42.4 (NCH_2), 43.3 ($PhCH_2$), 63.9 ($ArCH_2O$), 124.3 (q, $^1J_{C,F}$ = 273.9 Hz, CF_3), 129.4 (q, $^2J_{C,F}$ = 31.5 Hz, $C_{2'}$, $C_{6'}$), 130.9 (m, $C_{1'}$), 131.9 (m, $C_{3'}$, $C_{5'}$), 124.8, 127.8, 128.3, 129.6, 130.3, 131.7, 132.6, 132.7, 133.9, 136.7 and 136.8 (Ph, Ar and $C_{4'}$), 150.2 (C_1), 165.8 ($Ar'CO_2CH_2$), 167.9 (CO_2), 169.7 (CO_2H), 174.3 (CON) ppm; ^{19}F NMR (CD_3OD + DMF-d_7) δ: –59.1 (CF_3) ppm. Anal. calcd for $C_{27}H_{19}F_6NO_7$: C, 55.59; H, 3.28; N, 2.40. Found: C, 55.18; H, 3.41; N, 2.18.

6-Bromomethyl-3-carboxyphenyl phenylacetylglycinate ***1e***. mp 215–216 °C (dec.). ^1H NMR (DMF-d_7) δ: 3.48 (s, 2H, $PhCH_2$), 4.38 (d, $J_{CH,NH}$ = 5.6 Hz, 2H, NCH_2), 4.73 (s, 2H, $ArCH_2Br$), 7.24–7.41 (m, 5H, Ph), 7.73–7.99 (m, 3H, H_2, H_5 and H_4), 8.03 (s, 1H, CO_2H), 8.35 (t, 1H, NH) ppm; ^{13}C NMR (DMF-d_7) δ: 28.0 (CH_2Br), 42.8 (NCH_2), 43.5 ($PhCH_2$), 125.2, 127.8, 128.5, 129.5, 130.5, 132.7, 133.9, 136.5 and 137.4 (Ph and Ar), 150.1 (C_1), 167.5 (CO_2), 169.9 (CO_2H), 172.9 (CON) ppm. Anal. calcd for $C_{18}H_{16}NO_5Br$: C, 53.22; H, 3.97; N, 3.45; O, 19.63. Found: C, 53.32; H, 4.01; N, 3.15; O, 19.57.

6-Chloromethyl-3-carboxyphenyl phenylacetylglycinate ***1f***. mp 206–208 °C (dec.). ^1H NMR (CD_3OD + DMF-d_7) δ: 3.65 (s, 2H, $PhCH_2$), 4.31 (s, 2H, NCH_2), 4.67 (s, 2H, $ArCH_2Cl$), 7.23–7.38 (m, 5H, Ph), 7.50 (d, $J_{4,5}$ = 7.9 Hz, 1H, H_5), 7.81 (s, 1H, H_2), 8.11 (d, 1H, H_4) ppm; ^{13}C NMR (CD_3OD + DMF-d_7) δ: 41.1 (CH_2Cl), 42.5 (NCH_2), 43.4 ($PhCH_2$), 125.3, 127.9, 128.6, 129.6, 130.3, 131.9, 133.7, 136.0 and 136.7 (Ph and Ar), 149.9 (C_1), 167.7 (CO_2), 169.6 (CO_2H), 174.3 (CON) ppm. Anal. calcd for $C_{18}H_{16}NO_5Cl$: C, 59.76; H, 4.46; N, 3.87; Cl, 9.80; O, 22.11. Found: C, 59.51; H, 4.44; N 3.74; Cl, 9.81; O, 21.98.

6-Ethylmethylsulfoniomethyl-3-carboxyphenyl phenylacetylglycinate tetrafluoroborate ***1g***. (oil) ^1H NMR (CD_3OD) δ: 1.31 (t, J = 7.4 Hz, 3H, $S^+CH_2CH_3$), 2.70 (s, 2H, S^+CH_3), 3.23 (m, J = 7.4 Hz, J_{AB} = 13.7 Hz, 2H, $S^+CH_2CH_3$), 3.52 (s,2H, $PhCH_2$), 4.24 (s, 2H, NCH_2), 4.42 (q, J_{AB} = 13.0 Hz, 2H, $ArCH_2S^+$), 7.21–7.34 (m, 5H, Ph), 7.66 (d, $J_{4,5}$ = 8.0 Hz, 1H, H_5), 7.86 (d, $J_{2,4}$ = 1.5 Hz, 1H, H_2), 7.96 (dd, 1H, H_4) ppm. ^{13}C NMR (CD_3OD) δ: 9.2 ($S^+CH_2CH_3$), 22.2 (S^+CH_3), 37.7 ($S^+CH_2CH_3$), 41.3 ($ArCH_2S^+$), 43.1 ($PhCH_2$ and NCH_2), 125.8, 126.8, 129.2, 129.7, 130.4, 133.6, 135.5 and 136.6 (Ph and Ar), 151.1 (C_1), 167.6 (CO_2), 170.1 (CO_2H), 175.1 (CON) ppm. MS (FAB): 402 (M^+–BF_4), 227 (phenoxy$^+$), 151. HRMS (FAB^+): calcd for [$C_{21}H_{24}O_5NS^+$] 402.1375. Found 402.1372.

4-Acetoxymethyl-3-carboxyphenyl phenylacetylglycinate ***1'a***. mp (MeOH) 133–135 °C; ^1H NMR (CD_3OD) δ: 2.04 (s, 3H, OAc), 4.25 (s, 2H, $PhCH_2$), 4.65 (s, 2H, NCH_2), 5.39 (s, 2H, $ArCH_2O$), 7.17 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.5 Hz, 1H, H_6), 7.41 (d, 1H, H_5), 7.54 (d, 1H, H_2) ppm; ^{13}C NMR (CD_3OD) δ: 20.8 (Ac), 42.4 (NCH_2), 43.4 ($PhCH_2$), 65.3 (CH_2O), 151.2 (C_1), 161.4 (CO_2H),

169.7 (CO_2), 172.5 (CON), 174.8 (Ac) ppm. Anal. calcd for $C_{20}H_{19}NO_7$: C, 62.33; H, 4.97; N, 3.63. Found: C, 62.33; H, 4.96; N, 3.53.

4-(2',6'-Di-trifluoromethylbenzoxy)methyl-3-carboxyphenyl phenylacetylglycinate 1'c. mp 139 °C .^{1}H NMR ($CDCl_3$) δ: 3.67 (s, 2H, $PhCH_2$), 4.24 (d, J_{CH-NH} = 5.4 Hz, 2H, NCH_2), 5.76 (s, 2H, CH_2O), 6.19 (t, 1H, NH), 7.20 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.5 Hz, 1H, H_6), 7.57 (d, 1H, H_5), 7.64 (t, $J_{3',4'}$ and $J_{4',5'}$ = 8.0 Hz, 1H, $H_{4'}$), 7.80 (d, 1H, H_2), 7.85 (d, 2H, $H_{3'}$ and $H_{5'}$) ppm; ^{13}C NMR ($CDCl_3$) δ: 42.9 (NCH_2), 43.1 ($PhCH_2$), 66.3 (CH_2O), 149.7 (C_1), 164.6 (CO_2H), 167.9 (CO_2Ar_F), 169.6 (CO_2Ar), 172.7 (CON) ppm; ^{19}F NMR ($CDCl_3$) δ: −59.6 (CF_3) ppm. Anal. calcd for $C_{27}H_{19}NO_7F_6$: C, 55.58; H, 3.28; N, 2.40. Found: C, 55.87; H, 3.39; N, 2.15.

4-Bromomethyl-3-carboxyphenyl phenylacetylglycinate 1'e. mp 122–124 °C; ^{1}H NMR (acetone-d_6) δ: 3.61 (s, 2H, $PhCH_2$), 4.25 (2H, NCH_2), 5.11 (s, 2H, $ArCH_2Br$), 7.33 (dd, $J_{2,6}$ = 2.5 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H_6), 7.64 (d, 1H, H_5), 7.75 (d, 1H, H_2) ppm; ^{13}C NMR (acetone-d_6) δ: 30.9 (CH_2Br), 42.1 (NCH_2), 43.2 ($PhCH_2$), 151.3 (C_1), 166.9 (CO_2H), 169.2 (CO_2), 171.9 (CON) ppm. Anal. calcd for $C_{18}H_{16}NO_5Br$: C, 53.22; H, 3.97; N, 3.45; Br, 19.67. Found: C, 53.11; H, 4.18; N, 3.22; Br, 19.5.

4-Chloromethyl-3-carboxyphenyl phenylacetylglycinate 1'f. mp 130 °C; ^{1}H NMR (acetone-d_6) δ: 3.53 (s, 2H, $PhCH_2$), 4.17 (s, 2H, NCH_2), 5.05 (s, 2H, $ArCH_2Cl$), 7.32 (dd, $J_{2,6}$ = 2.3 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H_6), 7.55 (d, 1H, H_5), 7.65 (d, 1H, H_2) ppm; ^{13}C NMR (acetone-d_6) δ: 42.1 (NCH_2), 43.2 ($PhCH_2$), 44.1 (CH_2Cl), 151.3 (C_1), 162.3 (CO_2H), 169.2 (CO_2), 172.1 (CON) ppm. Anal. calcd for $C_{18}H_{16}NO_5Cl$: C, 59.75; H, 4.46; N, 3.87; Found: C, 59.70; H, 4.70; N, 3.44.

4-Ethylmethylsulfoniomethyl-3-carboxyphenyl phenylacetylglycinate tetrafluoroborate 1'g. ^{1}H NMR (CD_3OD) δ: 1.67 (t, J = 7.4, 3H, CH_3), 3.06 (s, 3H, S^+CH_3), 3.60 (q, 2H, S^+CH_2), 3.79 (s, 2H, $PhCH_2$), 4.40 (s, 2H, NCH_2), 5.06 (dd, 2H, $ArCH_2$), 7.62 (dd, $J_{2,6}$ = 2.4 Hz, $J_{5,6}$ = 8.2 Hz, 1H, H_6), 7.81 (d, 1H, H_5), 8.14 (d, 1H, H_2) ppm; ^{13}C NMR (CD_3OD) δ: 9.2 (SCH_3), 22.9 (CH_3), 38.3 (CH_2S), 42.5 (NCH_2), 43.4 ($PhCH_2$), 47.0 (SCH_2), 153.4 (C_3), 163.5 (CO_2H), 169.6 (CO_2), 174.9 (CON) ppm. MS (FAB): 402 (M^+–BF_4), 227 (phenoxy$^+$), 176, 151. HRMS (FAB$^+$): calcd for [$C_{21}H_{24}O_5NS^+$] 402.1375. Found 402.1377.

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31. There is also the question of the timing of its protonation and whether this occurs at the active site; a proton donor should be present in order to assist departure of the leaving group of a β-lactam substrate. It is also possible that chemical modification of the enzyme does occur efficiently but, with the functional groups of the active site protected by the acyl moiety in the acyl-enzyme, this modification has little effect on activity. This situation is in fact observed during the hydrolysis of 2-chloromethyl-4-nitrophenyl esters by chymotrypsin and papain.[17]

32. Both the TEM and P99 β-lactamases are preferentially inhibited by the *p*-isomer **1'g**, but this selectivity may reflect low turnover of the ortho compound because of an unfavorable electrostatic interaction between the positively-charged *o*-substituent and the electropositive enzyme active sites.[4d]