Design, Synthesis, and Proposed Active Site Binding Analysis of Monocyclic 2-Azetidinone Inhibitors of Prostate Specific Antigen

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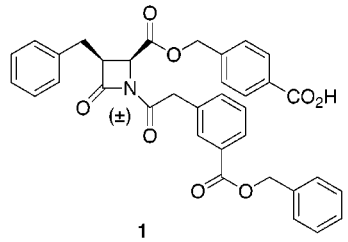
Received March 30, 2000

A homology derived molecular model of prostate specific antigen (PSA) was created and refined. The active site region was investigated for specific interacting functionality and a binding model postulated for the novel 2-azetidinone acyl enzyme inhibitor **1** ($IC_{50} = 8.98 \pm 0.90\ \mu M$) which was used as a lead compound in this study. A single low energy conformation structure II (Figure 2) was adopted as most likely to represent binding after minimization and dynamics calculations. Systematic analysis of the binding importance of all three side chains appended to the 2-azetidinone was conducted by the synthesis of several analogues. A proposed salt bridge to Lys-145 with **4** ($IC_{50} = 5.84 \pm 0.92\ \mu M$) gave improved inhibition, but generally the binding of the N-1 side chain in a specific secondary aromatic binding site did not tolerate much structural alteration. A hydrophobic interaction of the C-4 side chain afforded inhibitor **6** ($IC_{50} = 1.43 \pm 0.19\ \mu M$), and polar functionality could also be added in a proposed interaction with Gln-166 in **5** ($IC_{50} = 1.34 \pm 0.05\ \mu M$). Reversal of the C-4 ester connectivity furnished inhibitors **7** ($IC_{50} = 1.59 \pm 0.15\ \mu M$), **11** ($IC_{50} = 3.08 \pm 0.41\ \mu M$), and **13** ($IC_{50} = 2.19 \pm 0.36\ \mu M$) which were perceived to bind to PSA by a rotation of 180° relative to the C-4 ester of normal connectivity. Incorporation of hydroxyl functionality into the C-3 side chain provided **16** ($IC_{50} = 348 \pm 50$ nM) with the greatest increase in PSA inhibition by a single modification. Multiple copy simultaneous search (MCSS) analysis of the PSA active site further supported our model and suggested that **18** would bind strongly. Asymmetric synthesis yielded **18** ($IC_{50} = 226 \pm 10$ nM) as the most potent inhibitor of PSA reported to date. It is concluded that our design approach has been successful in developing PSA inhibitors and could also be applied to the inhibition of other enzymes, especially in the absence of crystallographic information.

Introduction

Prostate specific antigen (PSA) is a serine protease produced in the epithelial cells of the prostate gland.[1] It exhibits proteolytic action upon semenogelin and fibronectin, resulting in the liquifaction of seminal fluid.[2] In addition, PSA has also been shown to be responsible for the degradation of insulin-like growth factor binding protein-3 (IGFBP-3) in seminal fluid.[3] This stimulates mitogenesis by insulin-like growth factors I and II (IGF-I, II) in prostate epithelial cells which are normally blocked by IGFBP-3.[4] Such action may be responsible for enhanced growth of the prostatic cancer cell line DU-145,[5] and PSA may play a causative rather than merely descriptive role in prostate cancer.[6,7a] (Recent work has shown that this may not be the case.[7b]) This, and the implication of PSA in female breast tumors[8,9] and bone metastasis,[10] makes the design of PSA inhibitors important synthetic targets. We have previously communicated a series of PSA inhibitors.[11] In this report, we detail our active site analysis of a homology derived model of PSA, with subsequent design and synthesis of a range of novel monocyclic 2-azetidinone inhibitors of PSA.

Our approach to the inhibition of PSA was based upon the lead compound **1** ($IC_{50} = 8.98 \pm 0.90\ \mu M$.). This slow binding, time dependent inhibitor was shown by electrospray ionization mass spectrometry and peptide mapping experiments to form a stable 1:1 acyl enzyme complex at the active site with Ser-189. Computer



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simulated docking and subsequent molecular modeling calculations allowed us to create a description of binding of monocyclic 2-azetidinones to the active site of PSA and design modified structures predicted to bind more strongly. Such target molecules were then synthesized,

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10.1021/jm000145g CCC: $20.00
Published on Web 04/03/2001

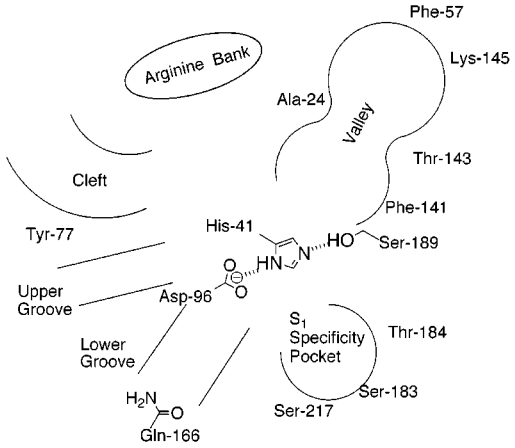


Figure 1.

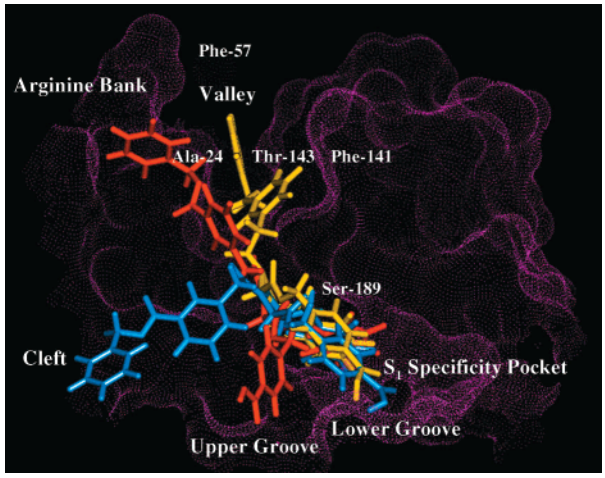


Figure 2.

and their inhibitory value against PSA was assessed. By treating this as an iterative process, biological data could be used to refine our model and further design compounds predicted to be more potent inhibitors.

Molecular Modeling

A homology derived molecular model of PSA was created by appropriate substitution for PSA from porcine kallikrein using AMBER[12,13] (version 3.0A). Two main loop insertions were necessary, and conformations were twisted to point hydrophilic groups toward the outside of the enzyme. Minimization and dynamics calculations were simulated within a monolayer of water with the substrate molecules SGAWYYVPLG or casein trypsin cleavage peptide inserted in the active site to maintain rigidity. Where residues were conserved, the hydrogen bonding structure was also conserved, and no major steric clashes were observed during this refinement procedure. Other homology derived models for PSA have been created by similar procedures.[14−16] A Ramachandran plot was generated within QUANTA17 (version 4.0) on a Silicon Graphics R4400 Indigo workstation and showed little deviation from the allowed φ and ψ angles of the protein backbone.[18] Inspection of our PSA model revealed that the active site comprising the catalytic residues His-41, Asp-96, and Ser-189 was contained within a crevice along the enzyme surface. On each side of this crevice was a domain of six-stranded antiparallel β-barrels as typical of serine proteases. The main binding subsite, the S_1 specificity pocket, was clearly identified proximal to the active site with Ser-183 located at the very base of this pocket, thus explaining the restricted chymotrypsin-like activity of PSA.[19,20] A solvent accessible surface, using a probe radius of 1.4 Å and centered on the oxygen of Ser-189, was calculated according to the method of Connolly.[21] The simplified topology of the active site area is represented in Figure 1. The cleft and valley regions form the active site crevice with the former being quite open but the latter displaying a more narrow opening in the enzyme surface, especially at one point where Ala-24 and Thr-143 residues protrude. Two less distinguished and shallow grooves in the enzyme surface were also detected close to the active site. Finally, in a flexible loop region which would be able to fold over the active site were a series of arginine residues (Arg-21, 23, and 44) which formed a region we termed the "arginine bank." The role of each location around the active site on docking of inhibitor structures will be discussed.

The structure of each enantimomer of compound **1** was minimized to an energy gradient tolerance of 0.01 using CHARMm 21.3[17] on a Silicon Graphics R4600 Indy workstation. The low energy conformations were manually docked into the active site of PSA using two criteria. First, the 2-azetidinone carbonyl was directed toward the oxyanion hole of PSA with the carbonyl carbon proximal to the hydroxyl group of Ser-189. Second, it was expected that one of the three 2-azetidinone side chains would occupy the S_1 specificity pocket and, subject to the first constraint, only the C-3 benzyl side chain could be fitted into this binding subsite. Such filling of the S_1 specificity pocket with the C-3 side chain of a 2-azetidinone has also been proposed in the inhibition of human leukocyte elastase[22,23] and thrombin.[24] The flexible nature of **1** allowed the N-1 side chain to be fitted either in the valley or cleft regions or toward the arginine bank. The remaining C-4 side chain naturally orientated toward the groove regions near the active site, and which groove was occupied depended upon the N-1 side chain orientation.

The six fitted structures of **1** (three for each enantiomer) were minimized to an energy gradient tolerance of 0.01 using CHARMm 21.3[17] within a constrained model of PSA. Dynamics calculations were then performed again, only allowing the inhibitor structure to move. Typically the system was heated to 300 K over 30 ps followed by a 10 ps equilibration period at this temperature. From the resulting structure, 10−30 ps simulations were performed, and low energy structures thus identified were further subjected to minimizations. The final conformer structures, shown in Figures 2 and 3 for the (3*S*,4*S*), (3*R*,4*R*) enantiomers, respectively, were analyzed for the position of each side chain and, in the case of the C-4 side chain, whether a polar interaction with the enzyme could be detected (Table 1). The distance from the carbonyl carbon to the closest NH group of the oxyanion hole (D_{oh}, Table 1) was measured with only conformer structure II exhibiting a formal hydrogen bonding distance.[25] Each inhibitor conformer structure was removed from the enzyme and allowed to freely release for 50 steps of minimization to gauge how far they were in energy from the free

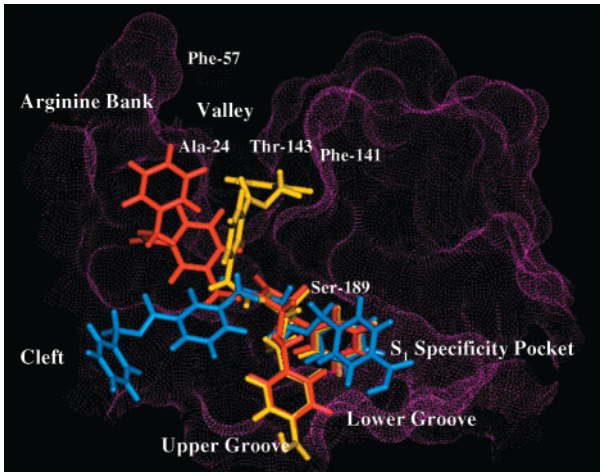


Figure 3.

Table 1

Property \ Conformer	I (3S,4S)	II (3S,4S)	III (3S,4S)	IV (3R,4R)	V (3R,4R)	VI (3R,4R)
C-3 Side Chain	S_1 Specificity Pocket					
N-1 Orientation	bank	valley	cleft	bank	valley	cleft
C-4 Groove Occupied	upper	lower	lower	upper	upper	lower
D_{oh} (Å)	2.10	1.90	3.22	3.32	3.33	2.08
Δ_{bind} (kcalmol^{-1})	46.6	35.8	41.5	31.5	49.4	46.8
C-4 Interaction Distance (Å) (Residue)	none	2.37 Gln 166	1.97 Ser 217 3.09 Gln 166	none	none	2.87 Ser 217

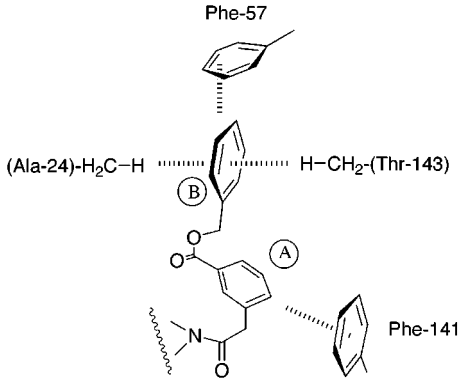
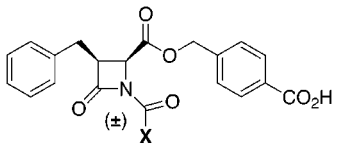
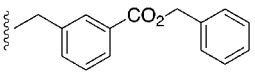
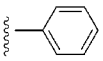
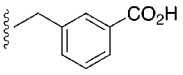
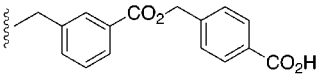


Figure 4.

Table 2



Compound	X	IC_{50} (μM)
1		8.98 ± 0.90
2		24.3 ± 3.9
3		>30
4		5.84 ± 0.92

minimized form. This energy difference (Δ_{bind}) reflects the unfavorable conformational strain energy, which is countered by a favorable van der Waals energy difference between free and bound states.[26] Conformer structure IV was immediately discarded as an unrealistic binding possibility since the inhibitor was orientated above rather than within the active site. This again left conformer structure II as potentially the best description of inhibitor binding with the lowest conformational strain on binding.

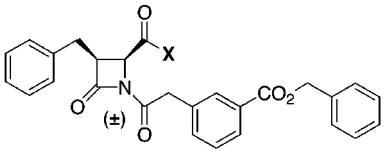
Discussion

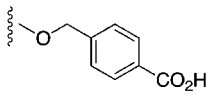
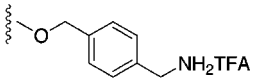
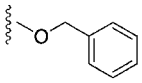
Our initial modeling experiments indicated that conformer structure II, indicating the (3*S*,4*S*) enantiomer, should be used as the basis for modification of lead inhibitor **1**. In addition to strong interaction of the 2-azetidinone carbonyl in the oxyanion hole and the C-3 benzyl side chain in the S_1 specificity pocket, the extended N-1 side chain proved a snug fit in the valley region (Figure 2). With four aromatic rings in inhibitor **1** we expected that aromatic−aromatic interaction would be important in binding.[27] Notably, conformer structure II shows edge to face aromatic interaction of the A and B rings with Phe-141 and Phe-57, respectively, (Figure 4). Further, the B ring appeared firmly lodged between protons from the methyl group of both Ala-24 and Thr-143 in the manner found for the first hydration sphere of benzene from Monte Carlo simulation experiments.[28]

In an attempt to both verify and utilize our active site analysis, compounds **2**−**4** were synthesized (Table 2). Without the second aromatic B ring on the nitrogen side chain as in compounds **2** and **3**, poorer binding to PSA was predicted, as reflected in significantly decreased IC_{50} values. Incorporation of a second carboxyl group in **4** was envisaged to allow salt bridging with Lys-145 in the valley region without dislodging either the A or B ring binding interactions, and an improvement in PSA inhibition was achieved.

In conformer structure II, the C-4 side chain was forced by the geometric constraints of the 2-azetidinone ring to lie in the lower groove. Although a formal hydrogen bond was not detected with Gln-166, conformational changes on binding and acyl enzyme formation could permit a strong interaction with the carboxyl group. Investigation of inhibitor−PSA interaction at the C-4 side chain was conducted with compounds **5** and **6** (Table 3). First, by changing the carboxyl to the more flexible aminomethylene group in **5**, a better distance and directionality of perceived hydrogen bonding to Gln-166 could be achieved, and indeed a significant lowering of the IC_{50} value was obtained. This vindicated the decision to base structure modification on binding conformation II. Conformer structure III could be

Table 3



Compound	X	IC_{50} (μM)
1		8.98 ± 0.90
5		1.34 ± 0.05
6		1.43 ± 0.19

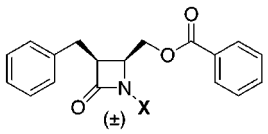
argued to explain the potency of inhibitor **4** via an interaction of the new carboxyl group at the "arginine bank" despite a poorer fit in the oxyanion hole and greater conformational strain on binding. However, in conformer structure III the geometric constraints of the 2-azetidinone ring forces the C-4 substituent into the upper groove area where no possible polar interactions could be detected, hence the potency of **5** could only be explained by binding in the manner of conformer structure II. Alteration of the substitution pattern of the aryl ring resulted in much poorer inhibitors. Large conformational changes would be necessary to allow hydrogen bonding to Gln-166 in this instance, and occupation of the lower groove region is sterically prohibited for either ortho or meta substituents, resulting in a significant decrease of binding potential of the C-4 side chain.

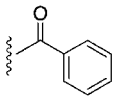
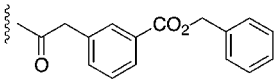
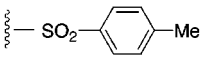
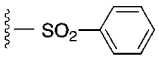
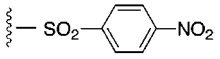
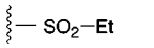
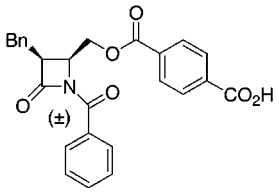
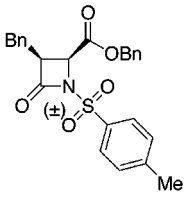
Since there appeared to be strict distance requirements for interaction with Gln-166 at the end of the lower groove, compound **6** was prepared which could utilize hydrophobic interactions only within the groove, without attempting to create a polar interaction. Compound **6** showed significant improvement over the lead inhibitor **1**. It is probable that if a precise interaction with Gln-166 is not possible, then polar functionalities will experience an attraction out of the enzyme toward external solvation, explaining the potency of compound **6**.

Further information concerning structural and chemical requirements were elucidated by biological testing of various synthetic intermediates. As expected, potent inhibitors containing amino or carboxyl functionality were inactive if these groups were chemically protected. Also, the 2-azetidinones which were unsubstituted at nitrogen did not display any PSA activity, presumably due not only to a lack of a side chain binding interaction but also to a low reactivity of the 2-azetidinone carbonyl toward acylation in the absence of an electron withdrawing group at N-1.

The C-4 ester group allowed the possibility of polar binding interactions to PSA. No such interaction was obvious from inspection of our model, and the carbonyloxymethylene group was reversed to probe binding interactions. The simple azetidinone **7** was found to be

Table 4



Compound	X	IC_{50} (μM)
7		1.59 ± 0.15
8		>100
10		4.79 ± 0.65
11		3.08 ± 0.41
12		11.5 ± 3.5
13		2.19 ± 0.36
9 IC_{50} > 100 μM		
14 IC_{50} > 30 μM		

a potent inhibitor of PSA. We attempted to combine this "reverse ester" functionality with the extended N-1 side chain and C-4 carbonyl functionality from our lead inhibitor **1** to give compounds **8** and **9**, respectively. Unfortunately neither compound exhibited realistic PSA inhibition (Table 4).

It was probable that "reverse ester" **7** had a different mode of binding to that of our previous inhibitors which we termed "normal esters." By 180° rotation around the 2-azetidinone carbonyl of **7** in the binding conformation II, the N-1 side chain could be positioned within the S_1 specificity pocket. This binding hypothesis was supported by comparison with the crystal structure of tosyl-α-chymotrypsin where the tosyl group occupies the S_1 specificity pocket.[29] Since reverse esters of cis relative stereochemistry were prepared, such a manipulation would necessitate the opposite enantiomer (i.e., (3*R*,4*R*) as shown in Figure 5 for **10**) to achieve a conformation able to bind at the active site. Such a manipulation suggests that the (3*R*,4*R*) enantiomer would have a more energetically favorable binding conformation for this reverse ester compared to the predicted (*3S*,*4S*) enantiomer for the normal ester compounds. The compounds were prepared as cis racemates to prevent the exclusion of a possible binding conformer. Thus, tosyl functionality attached at N-1 of a reverse ester would also be expected to inhibit PSA.

In accord with this model, **10** showed good PSA inhibition. Molecular models showed that the angle

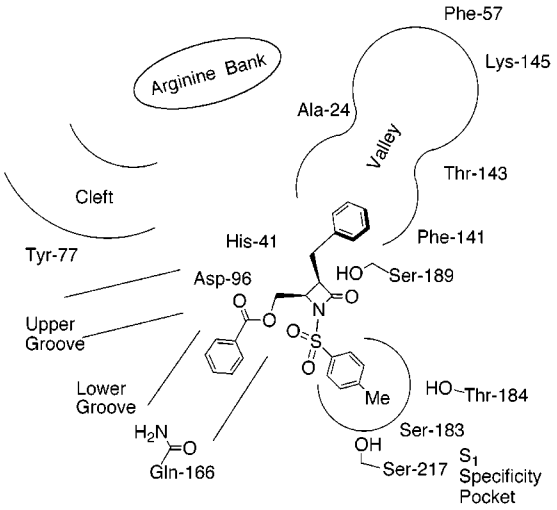


Figure 5.

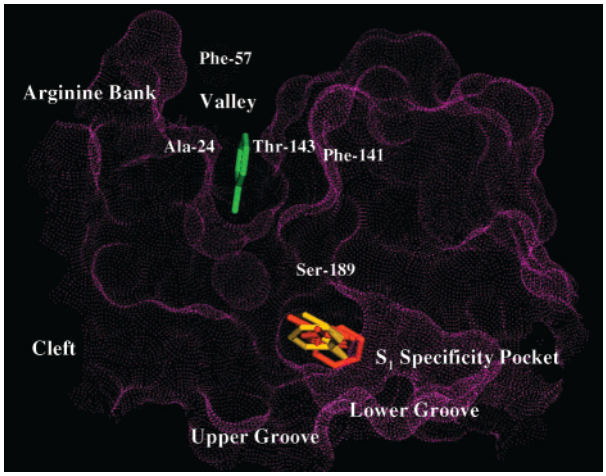


Figure 6.

subtended between the N-1 substituent and the average plane of the 2-azetidinone ring was not as steep as for the C-3 substituent. Therefore, when the N-1 substituent of **10** was oriented in the direction of the S_1 specificity pocket, the angle of approach over the lip of the pocket caused the *p*-methyl substituent to violate the solvent accessible boundary. Conformational changes that occur on acylation could alleviate this problem, but we considered the unsubstituted analogue **11**, which would create less steric clashes. Slight, but not greatly improved, potency was observed. However, introduction of a polar functionality such as a nitro group in **12** did decrease PSA inhibition significantly. This would be expected to result from the bulkier nitro versus hydrogen substituent. Also the para substituent cannot reach the polar region at the base of the specificity pocket because of the angle of entry dictated by the pyramidal nitrogen atom. The electronic nature of *para* substituents in **10**−**12** did not indicate any trend in inhibition values, presumably since all contained a suitably reactive 2-azetidinone carbonyl functionality to allow acylation of the enzyme. However, with a more flexible hydrophobic ethyl group, a further improvement in PSA inhibition by **13** was found.

It was now apparent that we did indeed have two distinct series of monocyclic 2-azetidinone inhibitors of PSA which bound to the active site in a different manner. In further confirmation, normal ester **14** was prepared with tosyl functionality at N-1 and was found to be largely inactive against PSA. This clearly showed that the characteristic binding groups from these normal and reverse esters could not be transposed in a structure−activity relationship analysis, and it strongly indicated different binding modes for these types of esters.

Multiple Copy Simultaneous Search

A de novo design approach to the inhibition of PSA was undertaken using a multiple copy simultaneous search (MCSS) method.[30] A sphere of radius 15 Å centered on the amide proton of Gly-187 was selected such that the active site and possible binding subsites were included. The functional groups methanol, acetate, methylammonium, toluene, *N*-methylacetamide, ether, propane, methionine, nitro, and threonine were all used. For each ligand, 1000 copies were used initially with a convergence criterion of 0.216, and the procedure was iterated three times to allow complete searching of the active site. In most cases the MCSS functionality maps were inconclusive for the identification of local minima due to large numbers of clusters. However, in the case of toluene, only two low energy binding sites were identified as depicted in Figure 6 on the Connolly surface. In view of the PSA inhibitors synthesized thus far, the importance of the binding of such aromatic functionality is evident. The lowest energy toluene cluster (red and yellow) shows a strong aromatic binding potential within the S_1 specificity pocket as utilized by the C-3 benzyl side chain in the normal ester series of PSA inhibitors and the N-1 aryl sulfonyl side chain in the reverse ester series in our model. The other toluene binding site (green) identified by the MCSS technique corresponds to the proposed B ring binding site of conformer structure II. An overlay of the toluene MCSS results and conformer structure II shows a good fit, further verifying this conformation as representative of binding in the normal ester series of inhibitors (Figure 7).

It was clear that the C-3 benzyl aromatic ring could fit further into the S_1 specificity pocket to give stronger binding. Several residues (Ser-183, Ser-217, and Thr-184) at the bottom of this pocket create a hydrogen bonding network. One of many sites identified by the MCSS method for the methanol ligand was in this area, suggesting that a *p*-hydroxyl substitution of the aromatic ring would improve binding. The conformer structure II of the (3*S*,4*S*) enantiomer of the lead inhibitor **1** was altered to include such a *p*-hydroxyl group as in **15** and was minimized within the active site of PSA. The resulting model of **15** is depicted within all possible binding regions within the active site of PSA in Figure 8. After energy minimization calculations the C-3 aromatic ring was indeed pulled further into the S_1 specificity pocket at the expense of interaction in the lower groove with Gln-166. Consequently, although **15** was a potent PSA inhibitor, compound **16** with no polar substituent at C-4 was predicted to improve PSA binding and consequently inhibition. Biological data (Table 5) confirmed this hypothesis. Indeed the sole introduction of the *p*-hydroxyl group converted a completely inactive compound into the good inhibitor **17**,

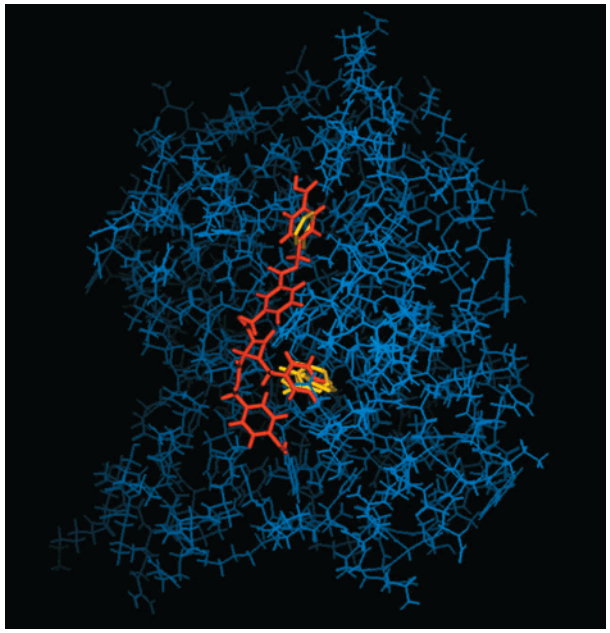


Figure 7.

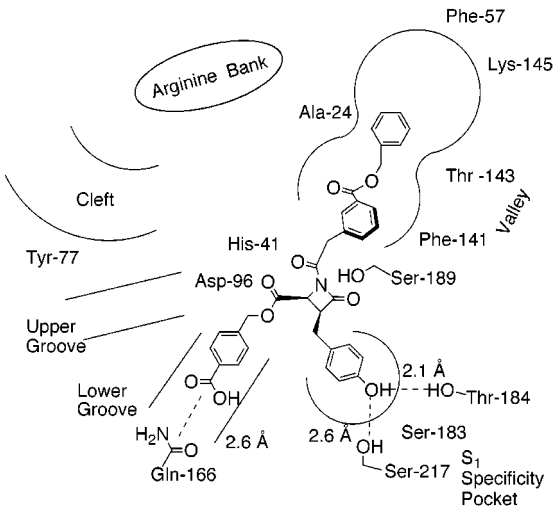


Figure 8.

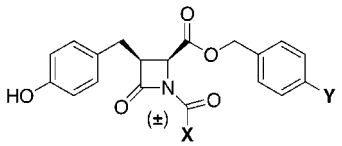
even in the absence of an extended secondary aromatic binding group.

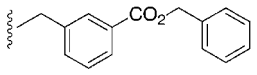
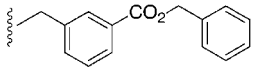
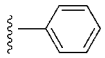
The conformer structure II, used in the design of more potent PSA inhibitors, represents only the (3*S*,4*S*) enantiomer. The calculated conformers of the (3*R*,4*R*) enantiomer are not able to utilize the secondary toluene binding subsite identified by MCSS calculations. Thus we were able to predict that the (3*S*,4*S*) enantiomer of the cis racemic normal ester compounds that we had prepared would be more active than their (3*R*,4*R*) counterparts. Assuming little inhibitory activity of the (3*R*,4*R*) enantiomers, an increase of up to 50% inhibition over the racemic mixture could be achieved by the preparation of a single (3*S*,4*S*) enantiomer. In practice, the homochiral (3*S*,4*S*)-2-azetidinone **18** exhibited significant lowering (35%) of the IC_{50} value for inhibition, emphasizing the predictive accuracy of our model.

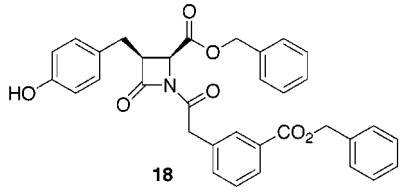
Chemistry

The monocyclic 2-azetidinones **23** and **24**, with cis relative stereochemistry, were prepared via a Staüdinger reaction using a modification of the method used by Alcaide (Scheme 1).[31] Protection of 3-(4-hydroxyphenyl)-propionic acid **20**, as phenolic acetate **21**, using acetic anhydride, then allowed acid chloride formation using oxalyl chloride with a catalytic quantity of *N*,*N*-dimethylformamide.[32] Hydrocinnamic acid **19** was converted to its acid chloride using phosphorus pentachloride. 1,4-Bis(4′-Methoxyphenyl)-1,4-diazabuta-1,3-diene **22**[33] was prepared and underwent Staüdinger reaction, with acid chlorides derived from **19** and **21**, respectively, and subsequent imine hydrolysis to give **23** and **24** in good yields. A moderate temperature range of 35−40 °C was found necessary to minimize competing

Table 5



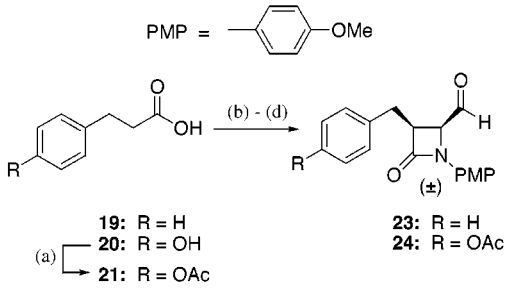
Compound	Y	X	IC_{50} (μM)
15	CO_2H		3.5 ± 0.4
16	H		0.348 ± 0.05
17	H		9.14 ± 0.86



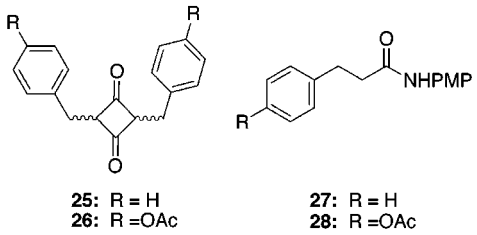
18

(3S,4S) IC_{50} = 0.226 ± 0.01 μM

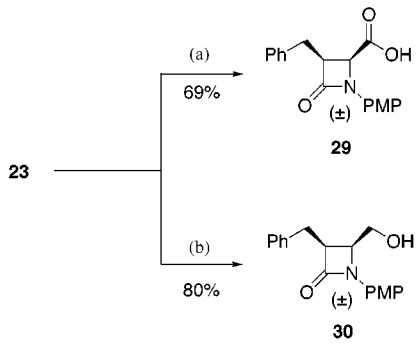
Scheme 1[a]



[a] (a) NaOH, H_2O, Ac_2O, 0 °C, 80%; (b) R = H, PCl_5, 100 °C, 80%; R = OAc, $(COCl)_2$, DCM, cat. DMF, 90%; (c) NEt_3, (*p*-MeO-C_6H_4-N=CH)$_2$ (**22**), 35−40 °C; (d) 5% aq. HCl, R = H, 61%; R = OAc, 85%.

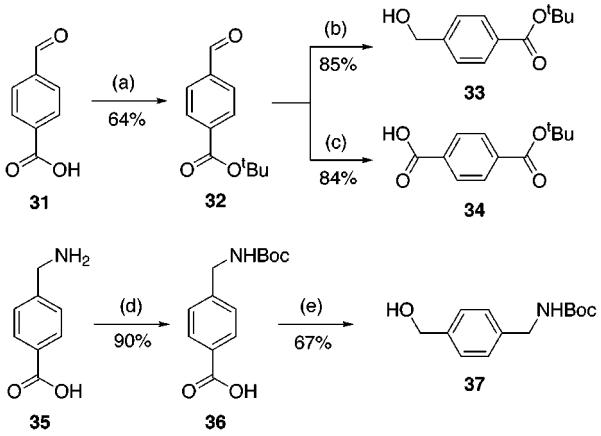


Scheme 2[a]



[a] (a) $CrO_3-H_2SO_4$, acetone−H_2O, 0 °C; (b) $NaBH_4$, MeOH.

Scheme 3[a]



[a] (a) $(^tBuO)_2CHNMe_2$, PhH, reflux; (b) $NaBH_4$, MeOH, 0 °C; (c) $CrO_3-H_2SO_4$, acetone−H_2O, 0 °C; (d) Boc_2O, NaOH, H_2O−dioxan, 0 °C; (e) BH_3−THF, THF, 25 °C.

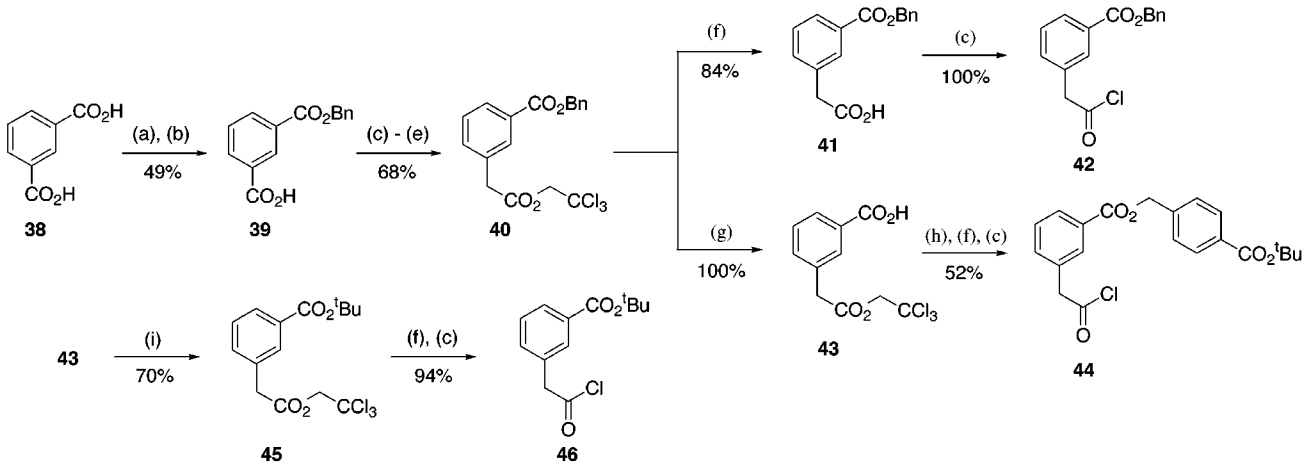
ketene dimerization to the 1,3-cyclobutadiones **25** and **26** and bias formation of 2-azetidinones **23** and **24** over amides **27** and **28**. No *trans*-2-azetidinones were detected from these transformations.

The required C-3 benzyl functionalities were now in place, and functionalization at C-4 was conducted next. Aldehyde **23** could be oxidized to acid **29** using a slight excess of Jones reagent.[34] In the presence of excess Jones reagent, cis to trans epimerization, presumably at the C-4 position, resulted. Alternatively, sodium borohydride in methanol selectively reduced the aldehyde group to give alcohol **30** in good yield (Scheme 2).[35] Consequently, diverse modification of the C-4 side chain could be easily incorporated into our synthetic plan.

For both normal and reverse ester compounds, 4-carboxybenzaldehyde **31** was used as a starting material. The carboxyl functionality was protected as the *tert*-butyl ester using *N*,*N*-dimethylformamide di-*tert*-butyl acetal.[36] For the preparation of gram quantities of **32**, *N*,*N*-dimethylformamide dineopentyl acetal and *tert*-butyl alcohol were used with only a small reduction in yield (56%). Then either sodium borohydride in methanol afforded alcohol **33**, or Jones oxidation[34] gave acid **34**, both in high yields (Scheme 3). Notably, no ester hydrolysis was detected under the latter acidic reaction conditions despite the ease of removal of the *tert*-butyl group with trifluoroacetic acid. To minimize reaction steps, the amine functionality of 4-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]benzoic acid **35** was protected with a *tert*-butyloxycarbonyl group which could also be removed under trifluoroacetic acid conditions. Acid **36** underwent borane reduction to give alcohol **37** in good yield (Scheme 3).

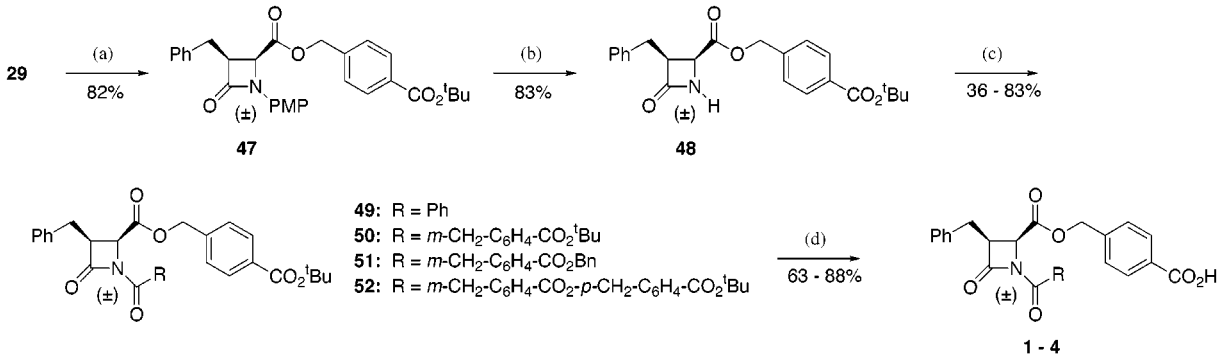
The N-1 side chains were prepared from isophthalic acid which contained the meta substitution pattern required to realize secondary subsite binding (Scheme 4). Modification of the literature procedure allowed preparation of acid **41**.[37] Monoesterification of **38** could be achieved in moderate yield to give **39** with recovery of unreacted **38** (and diester) which could be recycled. Arndt−Eistert homologation was conducted using 2,2,2-trichloroethanol to trap the intermediate ketene and form **40** which enabled purification on silica gel. Each acid functionality could be selectively revealed due to the orthogonal nature of the protecting groups. Zinc reduction in acetic acid[38] followed by acid chloride formation gave the required acid chloride **42**. Alternatively, hydrogenation quantiatively yielded **43** which was re-esterified with alcohol **33** allowing formation of acid chloride **44**. Acid **43** could also be reprotected as the *tert*-butyl ester **45** using *O*-*tert*-butyl trichloroacetimidate.[39] Treatment of **43** with *N*,*N*-dimethylformamide di-*tert*-butyl acetal resulted in enamine

Scheme 4[a]



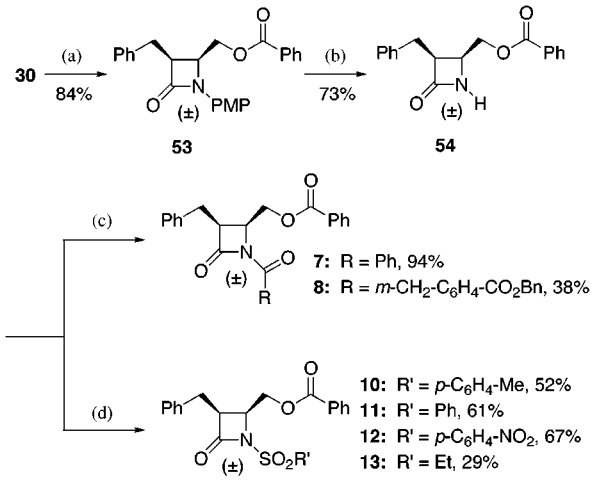
[a] (a) NEt_3, MeOH; (b) BnBr, DMF, 100 °C; (c) $(COCl)_2$, cat. DMF, DCM; (d) CH_2N_2, Et_2O, 0 °C−RT; (e) Ag_2O, Cl_3CCH_2OH, 60 °C; (f) Zn, AcOH−H_2O, THF; (g) H_2, Pd−C, EtOAc; (h) DCCI, (**33**), cat. DMAP, THF; (i) $Cl_3CC(O^tBu)=NH$, excess $BF_3{\cdot}OEt_2$, DCM−cyclohexane.

Scheme 5[a]



[a] (a) DCCI, (**33**), cat. DMAP, THF; (b) $CeIV(NH_4)_2(NO_3)_6$, $MeCN-H_2O$, 0 °C; (c) $NaN(SiMe_3)_2$, THF, −78 °C then RCOCl, −78 °C; (d) CF_3CO_2H, 0 °C.

Scheme 6[a]

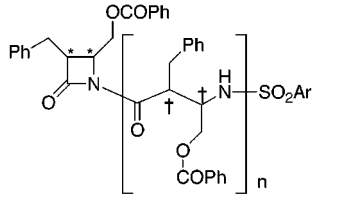


[a] (a) PhCOCl, cat. DMAP, NEt_3, DCM; (b) $CeIV(NH_4)_2(NO_3)_6$, $MeCN-H_2O$, 0 °C; (c) $NaN(SiMe_3)_2$, THF, −78 °C then RCOCl, −78 °C; (d) $NaN(SiMe_3)_2$, THF, −78 °C then R′SO_2Cl, −78 °C.

formation at the benzylic position in addition to the desired ester formation. Subsequent zinc reduction and acid chloride formation gave **46** in high yield.

Manipulation of the 2-azetidinone C-4 side chain proceeded smoothly (Scheme 5). Dicyclohexylcarbodiimide mediated esterification of **29** with **33** gave the N-1 protected 2-azetidinone **47**. Oxidative nitrogen dearylation with ceric ammonium nitrate[40] gave 2-azetidinone **48** in good yield, and a variety of acyl groups were attached by deprotonation with sodium bis(trimethylsilylamide) and reaction with acid chlorides. Variable yields of **49** to **52** were obtained for such acylations, presumably due to the steric bulk of the acid chlorides used and the presence of acidic impurities from their formation. Storage under high vacuum overnight immediately following preparation of the acid chloride, prior to reaction, generally afforded higher yields although these reactions were not optimized. Deprotection with trifluoroacetic acid gave target compounds **1**−**4** in good yields. Reaction times were generally less than 20 min, otherwise ring opening of the reactive 2-azetidinone occurred. Other normal esters with different functionalities at C-4 (e.g., **5** and **6**) were prepared by analogous reaction steps.

The synthesis of reverse ester inhibitors proceeded by similar reaction methods (Scheme 6). Esterification of **30** at C-4 gave **53** using benzoyl chloride and a catalytic quantity of (dimethylamino)pyridine. Nitrogen dearylation with ceric ammonium nitrate gave 2-azetidinone **54**, which was both acylated to give **7** and **8** and sulfonylated to give **10**−**13**. The aryl sulfonlyation reaction initially gave poor yields due to competing oligomerization reactions for the formation of dimers **55** and trimers **56**.[41] It was found that the aryl sulfonylation reactions proceeded in good yields in the presence of excess aryl sulfonyl chloride at low concentrations of 2-azetidinone **54**.[41] The formation of **13** proceeded in poor yield due to the poor reactivity of ethanesulfonyl chloride toward S_N2 displacement reactions. The 2-azetidinones **9** and **14** were prepared by analogous reaction sequences.

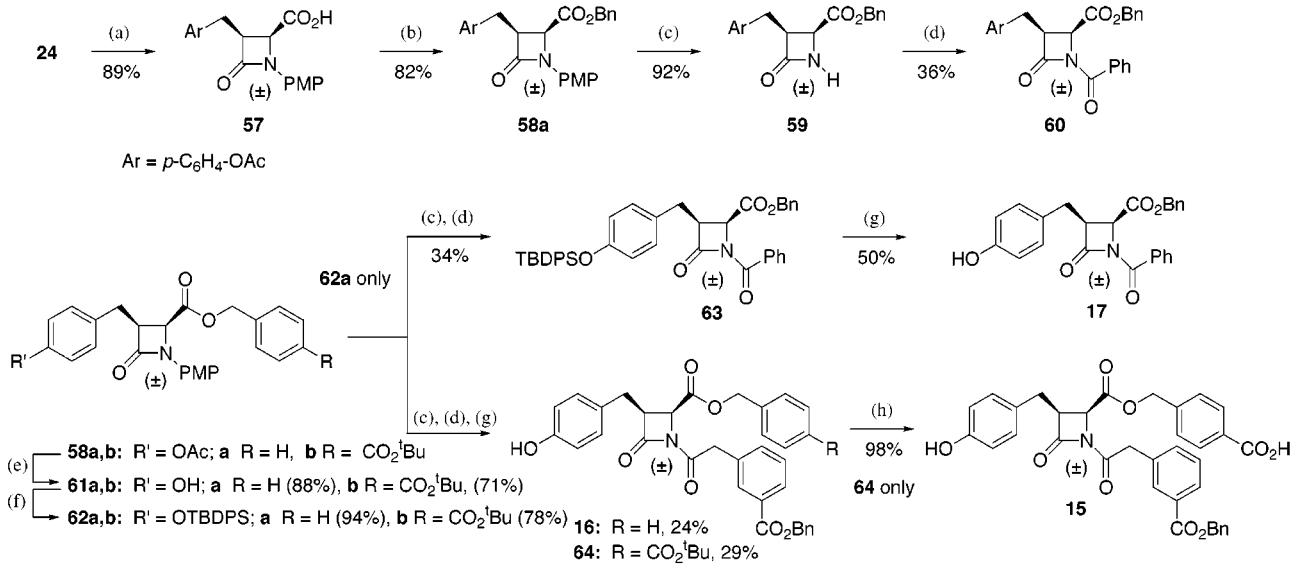


*,† denotes *(R,R)* or *(S,S)* stereochemistry

55: n = 1
56: n = 2

In the case of C-3 *p*-hydroxybenzyl 2-azetidinones, additional synthetic steps were required (Scheme 7). The acetyl protecting group was found suitable to withstand Jones oxidation to give acid **57**, esterification, and also nitrogen dearylation to give 2-azetidinone **59**. However, after N-1 acylation afforded the activated 2-azetidinone **60**, it was not found possible to remove the acetyl protecting group without ring opening of the reactive 2-azetidinone. Thus the acetyl group was exchanged for an organosilicon protecting group after investigation of several deacylation procedures. Alkaline hydrolysis (LiOH, K_2CO_3) of the acetyl group in **58a** resulted in competing hydrolysis of the C-4 benzyl ester. Sodium borohydride[42] and potassium trimethylsilanoate[43] also resulted in unselective hydrolysis, although **61a** was always the main product. However, using a mild activated zinc procedure, excellent yields of **61a**,**b** were obtained after 4 days.[44] Silyl protection to give **62a**,**b** then allowed selective oxidative removal of the N-1 aromatic ring. The *tert*-butyldiphenylsilyl protecting group was used in preference to *tert*-butyldimethylsilyl, as the latter did not withstand the acidic dearylation conditions. Acylation with benzoyl chloride

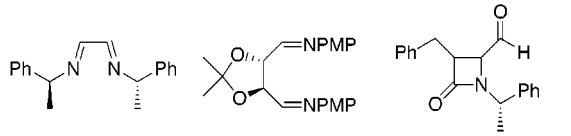
Scheme 7[a]



[a] (a) $CrO_3-H_2SO_4$, acetone$-H_2O$, 0 °C; (b) DCCI, BnOH, cat. DMAP, THF; (c) $CeIV(NH_4)_2(NO_3)_6$, MeCN$-H_2O$, 0 °C; (d) $NaN(SiMe_3)_2$, THF, −78 °C then RCOCl, −78 °C; (e) activated Zn, MeOH−THF, 35 °C; (f) TBDPSCl, imidazole, DMF, 40 °C; (g) $NH_4F{\cdot}HF$, NMP−DMF; (h) CF_3CO_2H, 0 °C.

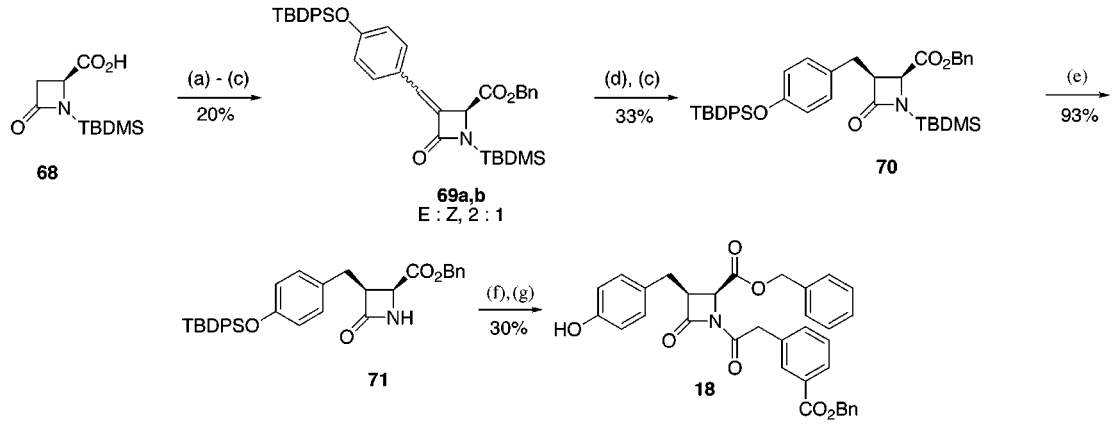
gave imide **63** which was used as a model for attempted desilylation reactions. Decomposition resulted when trifluoroacetic acid or 48% hydrogen fluoride was used. However, the mild desilylating agent ammonium hydrogen difluoride was found to afford **17** in 50% yield.[45] The desired extended N-1 side chain was attached, and ammonium hydrogen difluoride was used again successfully without 2-azetidinone ring opening to give phenols **16** and **64**, and also **15**, after an additional trifluoroacetic acid deprotection step.

Initial attempts to convert our cis racemic 2-azetidinone synthesis into an asymmetric process using the chiral diimines **65**[46] and **66**[47] in an asymmetric Staüdinger reaction were unsuccessful. A high yield (82%) of a 1:1 diastereomeric mixture **67** could be obtained using **65** at a moderate temperature (35−40 °C), but the diastereomers were found to be inseparable on silica gel. Lower temperatures, which are generally required for diastereoselectivity, resulted in poor chemical yield. Subsequent elaboration to a hydroxymethyl and phenylcarbonyloxymethyl substituent at C-4 also did not lead to separable diastereomers by chromatography or crystallization. Failure of **66** to undergo the reaction was attributed to the need of a more reactive imine component to allow the reaction to proceed at temperatures lower than that which caused extensive dimerization of the acid chloride used (<40 °C).



Consequently, a new synthetic route was adopted for the preparation of **18** (Scheme 8). The homochiral 2-azetidinone **68** with the required (4*S*) stereochemistry was prepared from L-aspartic acid by the literature method.[48] The C-3 arylidene 2-azetidinones **69a**,**b** were prepared from a Peterson reaction with 4-*tert*-butyldiphenylsilyloxybenzaldehyde and the (3*S*) stereochemistry created in a subsequent hydrogenation step.[49] The

Scheme 8[a]



[a] (a) i. LDA (2.1 equiv), THF, −9 ° C, ii. TMSCl (1.1 equiv), THF, −9 °C, iii. LDA (1.1 equiv), THF, −9 °C to 0 °C; (b) *p*-TBDPSO-C_6H_4-CHO, THF, 0 °C; (c) EDCI, BnOH, cat. DMAP, DCM; (d) H_2, Pd−C, THF; (e) 48% aq. HF, MeCN; (f) $NaN(SiMe_3)_2$, THF, −78 °C then *m*-BnO_2C-C_6H_4-CH_2COCl (**42**), −78 °C to RT, 45%; (g) $NH_4F{\cdot}HF$, NMP−DMF.

(4*S*) benzyloxycarbonyl group was thought to sterically prevent addition of hydrogen from one face of the 2-azetidinone, and subsequent rebenzylation afforded **70** as the sole isolable product. Both *E* and *Z* isomers of **69** could be isolated and converted to **70**. The overall yield for conversion of **68** to **70** (6% over seven steps) was disappointing, and material losses were ascribed to partial desilylation at N-1 during purification procedures. Consequently, N-1 desilylation of **70** proved facile and selective in respect of the *tert*-butyldiphenylsilyl ether at C-3, using 48% hydrogen fluoride, giving 2-azetidinone **71** in excellent yield. Comparison of the ^{1}H (500 MHz) NMR spectra of **71**, the cis racemic material, and a doped sample of both, all in the presence of 3 mol % of Eu(hfc)$_3$,[50] clearly showed the enantiopurity of **71**. Finally, acylation and desilylation procedures as described for the racemic synthesis afforded **18** in good yield.

Conclusion

In summary, a homology derived model was created for PSA which led to the design and selection of a number of novel monocyclic 2-azetidinone PSA inhibitor targets, predicted to show strong binding properties with the active site of PSA. A further, distinct, reverse ester series of monocylic 2-azetidinones were also prepared on the basis of this model and by the use of simple structure−activity analysis (SAR). These reverse esters were found to be inhibitors of PSA, and a different mode of binding was proposed. Furthermore, MCSS calculations were conducted to enhance interpretation of the homology model for normal ester (3*S*,4*S*)-2-azetidinone inhibitors, and the C-3 *p*-hydroxybenzyl substituent was predicted to exhibit greatest binding at the active site of PSA. Subsequent synthesis of the pure enantiomer **18** realized increased PSA inhibition ($IC_{50} = 226 \pm 10$ nM), further vindicating the active site binding model. Thus, by use of molecular modeling, we were able to augment SAR analysis and prepare the most potent inhibitors of PSA known to date. Further structural modification of the 2-azetidinone side chains could yield other potent PSA inhibitors. In addition, such a design process may prove successful in predicting inhibitors of other enzymes, particularly when crystallographic structures are not available.

Experimental Section

Biology. IC_{50} values were run on an individual as opposed to batch basis. As such, each IC_{50} value has its own quoted standard error limits; thence these values are not strictly quantitative. A spectrophotometric assay was used to determine the ability of compounds to inhibit PSA cleavage of *p*-nitroaniline (*p*-NA) from MeO-Suc-Arg-Pro-Tyr-*p*-NA·HCl, a chromogenic substrate for chymotrypsin (Pharmacia Hepar Chromogenix S-2586). All experiments were carried out in flat bottom 96-well plates coated with 10 μL of PBS/BSA (6.3 μg/mL BSA) and analyzed by a microtiter plate reader (Bio-Tec Instruments). A total of 1 to 5 mg of each compound was dissolved in DMSO and made up to 300 μM stock solutions. Serial dilutions of inhibitors with DMSO were evaluated (300 to 0.01 μM). A total of 10 μL of control or inhibitor was preincubated with 10 μL of PSA (40 nM) for 2 h at 37 °C. The reaction was initiated with 70 μL of 1 mM substrate. Twenty plate scans during a 10 min period at 450 and 405 nm were used to monitor the progress of enzyme cleavage. Software for the Bio-Tek plate reader (CERES UV900HDi) plotted reaction time on the *X*-axis versus mO.D. on the *Y*-axis and calculated a slope (reaction rate) for each inhibitor concentration. The reaction rates (mO.D. min^{-1}) for each inhibitor concentration were transferred to JMP for nonlinear analysis using a four-parameter logistic model. Estimated IC_{50} values were defined as the serial dilution required to produce a half-maximal effect.

All data for active compounds that have been reported were acquired using the spectrophotometric assay, but for preliminary screening of many compounds an HPLC assay was also used. In an HPLC assay, the peptide substrate (SGAWYYV-PLG) was dissolved in PBS to a concentration of 0.1 mg/mL. PSA stock was diluted to a concentration of 0.1 mg/mL with PBS containing 50% glycerol. A total of 1 to 5 mg of each compound was dissolved in DMSO and made up to 300 and 30 μM stock solutions. Serial dilutions of inhibitors with DMSO were prepared (30, 10, 3, 1 μM), and PSA was added. After incubation at 37 °C for 2 h, the reaction was terminated by addition of 100 μL of trifluoroacetic acid. A 100 μL sample was injected onto a reversed-phase HPLC column (Vydac C-18 solid phase with particle size of 5 mm.) A gradient elution using the mobile phase A = 0.1% TFA in water, B = 0.1% TFA in acetonitrile was conducted as follows:

time (min)	%B
0	15
5	15
35	30
36	15
40	15

Enzyme cleavage was monitored by detection at 280 nm. Estimated IC_{50} values were defined as the serial dilution required to produce a half-maximal effect.

Chemistry. All solvents were distilled before use. Anhydrous dichloromethane (DCM), toluene, benzene, acetonitrile, DMSO, 1-methyl-2-pyrrolidinone (NMP), and *tert*-butyl alcohol were obtained by refluxing over calcium hydride followed by distillation under an inert atmosphere of argon. Anhydrous methanol was obtained by distillation from magnesium methoxide. Anhydrous diethyl ether and anhydrous tetrahydrofuran (THF) were obtained by distillation from sodium/benzophenone ketyl under nitrogen immediately prior to use. LP and LP 40-60 refer to the fractions of light petroleum ether which boil within the ranges 30−40 °C and 40−60 °C, respectively. Solvents were evaporated under reduced pressure on a Büchi RE111 rotavapor; high boiling solvents were evaporated on a Büchi RE111 rotavapor fitted with a dry ice condenser at less than 2 mmHg. Kugelröhr distillations were performed at the recorded oven temperature and pressure.

All other reagents were used as obtained from commercial sources or purified by methods described in D. P. Perrin and W. L. F. Armarego's *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1988. All chemicals were handled in accordance with safety instructions as detailed in *Good Laboratory Practice*; Dyson Perrins Laboratory: Oxford, 1993−1996.

All reactions were performed under an inert atmosphere of argon unless otherwise stated. Low temperatures were obtained either by the use of dry ice/acetone or dry ice/2-propanol cooling baths, or by the use of a Julabo FT901 Immersion Cooler.

Thin-layer chromatography was performed on glass plates precoated with Merck silica gel 60F$_{254}$ 0.2 mm, which were visualized by quenching of ultraviolet fluorescence (λ_{max} 254 nm), by staining with iodine vapor, or by staining with 5% w/v phosphomolybdic acid in ethanol, 10% w/v ammonium molybdate in 2 N sulfuric acid, or 10% aqueous bromocreosol green, all followed by heat. R_f values are quoted to the nearest 0.05. Flash chromatography was performed on Janssen silica (0.035−0.070 nm) or Sorbsil C60 silica gel (30−60 mm).

Melting points (mp) were determined using a Büchi 510 or a Cambridge Instruments Galen III Köfler Block apparatus. Optical rotations ($[\alpha]_D^t$) were determined at temperature *t* using a Perkin-Elmer 241 polarimeter in 1 dm path length cells at 589 nm. Values are given in 10^{-1} deg cm^2 g with

concentrations given in g/100 mL. Ultraviolet spectra were recorded on a Perkin-Elmer Lambda 2 UV−vis spectrophotometer with absorbances given as λ_{max}. Infrared spectra were recorded on a Perkin-Elmer 781 spectrometer or a Perkin-Elmer 1750 Fourier Transform spectrophotometer. Only selected absorbances are listed.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini 200 operating at 200 MHz, a Brüker AC200 operating at 200 MHz, or a Brüker AM500 or AMX500 operating at 500 MHz. The spectra were referenced to residual protonated solvent residues, for example, $CHCl_3$ δ_H = 7.27 ppm, as an internal standard. Proton decoupled carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on either a Varian Gemini 200 operating at 50.3 MHz or a Brüker AMX500 operating at 125.7 MHz. The spectra were referenced to solvent carbon residues. Distortionless enhancement by polarization transfer (DEPT) editing was generally used for spectra obtained at 50.3 MHz. All chemical shifts (δ_H, δ_C) are quoted in parts per million (ppm) downfield from tetramethylsilane, in the solvent indicated in parentheses. Values are quoted to 0.01 ppm for δ_H and δ_C. Signal multiplicities are described as s − singlet, d − doublet, t − triplet, and q − quartet, with through *n* bond coupling constants ^nJ being reported to the nearest 0.5 hertz (Hz). The symbol br indicates that the peak was broad.

Low resolution mass spectra (MS) were recorded on either a V. G. ZAB1F, V. G. Masslab 20-250, V. G. TRIO1 (GCMS), V. G. Platform, or a V. G. BIO-Q instrument, with the modes of ionization being indicated as direct chemical ionization (DCI), probe chemical ionization (CI), atmospheric pressure chemical ionization (APCI), fast atom bombardment (FAB), or electrospray (ES). Intensities were recorded as percentages of the largest signal and only major peaks were recorded, as the mass-to-charge (*m*/*z*) ratio. APCI measurements were obtained using a mixed solvent system [MeOH:MeCN:H_2O, 40:40:20].

3-(4′-Acetoxyphenyl)propionic Acid (21). To a stirred suspension of phloretic acid [3-(4′-hydroxyphenyl)propionic acid] **20** (24.9 g, 15.0 mol) in water (120 mL) was added powdered sodium hydroxide (15.0 g, 37.5 mol), and a clear pale brown solution resulted after stirring for 30 min. Ice (150 mL) was added to cool the solution, and after this had melted, acetic anhydride (23.0 g, 22.5 mol) was added in one portion. After the mixture was stirred for 17 h, a brown precipitate had formed. The mixture was extracted with ether (2 × 120 mL) to remove the precipitate, and the aqueous layer was gradually acidified by careful addition of 5% aqueous hydrochloric acid. The solution was extracted at each integral pH with ether (2 × 120 mL). It was noted that on reaching pH 4 a white precipitation occurred. The ether solutions obtained at pH 4−5 were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a pale brown solid. Recrystallization from ether-LP 40-60 afforded **21** as white needles (24.9 g, 80%): mp 97−98 °C; IR (KBr, cm^{-1}) 2480−3280, 1756, 1704; MS (CI, NH_3) *m*/*z* 226 [MNH_4^+, 100], 208 [M^+, 8], 166 [30], 107 [73]. Anal. ($C_{11}H_{12}O_4$) C, H.

***cis*-3-Benzyl-4-formyl-1-(4′-methoxyphenyl)-2-azetidinone (23).** Hydrocinnamic acid **19** (60.0 g, 400 mmol) was carefully added to phosphorus pentachloride (83.3 g, 400 mmol) and heated to 100 °C. The reaction was *extremely vigorous*, and the resulting solution was stirred under reflux for 1 h. Phosphorus oxychloride was distilled off under atmospheric pressure (107 °C), and then distillation under reduced pressure afforded hydrocinnamoyl chloride as a colorless oil (53.6 g, 80%): [bp 146 °C (25 mmHg), lit.[51] 107 °C (11 mmHg)]; IR (NaCl, cm^{-1}) 3030, 2931, 1795. To a stirred suspension of 1,4-bis(4′-methoxyphenyl)-1,4-diazabuta-1,3-diene[46] (**22**) (3.22 g, 12.0 mmol) and triethylamine (2.43 g, 24.0 mmol) in dry toluene (110 mL) was added hydrocinnamoyl chloride (4.05 g, 24.0 mmol) dissolved in toluene (10 mL) via slow addition over 10 min, maintaining the temperature within the range 35−40 °C. The color changed from brown to orange as the reaction mixture was stirred for 1 h. Aqueous hydrochloric acid (5%, 100 mL) was added and the resulting heterogeneous mixture stirred vigorously for a further 3 h. The solution was washed with 5% aqueous hydrochloric acid (2 × 50 mL), water (100 mL), and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a black oil. The oil was passed through a silica pad, eluting with DCM-ether (10:1) to yield a yellow solid. Recrystallization from DCM-LP afforded **23** as a white crystalline solid (2.16 g, 61%): (R_f 0.45, DCM); mp 98−100 °C; IR (KBr, cm^{-1}) 3031, 2935, 1748, 1724; MS (CI, NH_3) *m*/*z* 296 [MH^+, 100], 149 [67], 134 [27], 124 [23]. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

***cis*-3-(4′-Acetoxybenzyl)-4-formyl-1-(4′-methoxyphenyl)-2-azetidinone (24).** To a stirred solution of acid (**21**) (17.0 g, 81.6 mmol) in DCM (250 mL) were added DMF (3 drops) then oxalyl chloride (45.1 g, 355 mmol). The resulting yellow solution was stirred for 1.5 h and concentrated under reduced pressure to give an orange oil. Distillation under reduced pressure afforded the 3-(4′-acetoxyphenyl)propionyl chloride as a colorless oil (16.7 g, 90%): bp 141 °C (0.06 mmHg); IR (NaCl, cm^{-1}) 3039, 2936, 1796, 1763). To a stirred suspension of diimine (**22**) (3.22 g, 12.0 mmol) and triethylamine (2.43 g, 24.0 mmol) in dry toluene (110 mL) at 35−40 °C was added 3-(4′-acetoxyphenyl)propionyl chloride (5.44 g, 24.0 mmol) in toluene (5 mL), dropwise over 2.5 h. The color changed from brown to orange as the reaction mixture was stirred for a further 1.5 h at 35−40 °C. Then 5% aqueous hydrochloric acid (150 mL) was added and the resulting heterogeneous mixture stirred for 2.5 h at room temperature. The solution was diluted with toluene (50 mL) and the aqueous layer separated then extracted with toluene (2 × 50 mL). The organic layers were combined and washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a black gum. Ether was added, and after scratching the glass flask, a pale brown solid precipitated and was filtered from the solution. Further purification was possible using flash chromatography (SiO_2, DCM-ether, 25:1) to afford **24** as a pale yellow crystalline solid (3.61 g, 85%): (R_f 0.45, DCM-ether; 10:1); mp 114.5−116 °C; IR (KBr, cm^{-1}) 3007, 2955, 2934, 1746; MS (CI, NH_3) *m*/*z* 371 [MNH_4^+, 100], 354 [MH^+, 32], 222 [28]. Anal. ($C_{20}H_{19}NO_5$) C, H, N.

General Procedure for the Preparation of Acids 29 and 57: *cis*-3-Benzyl-4-carboxy-1-(4′-methoxyphenyl)-2-azetidinone (29). To a stirred solution of aldehyde **23** (2.50 g, 8.5 mmol) in acetone (65 mL), at 0 °C, was added freshly prepared Jones reagent dropwise until an orange color persisted. [Jones reagent was prepared by dissolving chromium trioxide (6.7 g) in 12.5 mL of distilled water and adding 5.8 mL of concentrated sulfuric acid.] The reaction mixture was concentrated under reduced pressure, and the residue was then dissolved in ethyl acetate (50 mL) and washed with water (3 × 30 mL). The resulting orange solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield an orange solid. Trituration with ether afforded **29** as a yellow crystalline solid (1.82 g, 69%); mp 207−208 °C; IR (KBr, cm^{-1}) 3240−2420, 1729, 1687; MS (CI, NH_3) *m*/*z* 329 [MNH_4^+, 5], 312 [MH^+, 100], 296 [3], 239 [18], 134 [18]. Anal. ($C_{18}H_{17}NO_4$) C, H, N.

***cis*-3-(4′-Acetoxybenzyl)-4-carboxy-1-(4′-methoxyphenyl)-2-azetidinone (57).** The title compound was prepared in 89% isolated yield from aldehyde **24** (9.37 mmol scale) following a procedure similar to that described above for **29**: mp 193−194 °C; IR (KBr, cm^{-1}) 3640−2760, 3008, 2933, 1767, 1747, 1727; MS (ES) *m*/*z* 368 [$(M - H)^-$, 100]. Anal. ($C_{20}H_{19}NO_6$) C, H, N.

***cis*-3-Benzyl-4-hydroxymethyl-1-(4′-methoxyphenyl)-2-azetidinone (30).** A stirred suspension of aldehyde **23** (886 mg, 3.00 mmol) in methanol (12 mL) was cooled to 0 °C. Sodium borohydride (113 mg, 2.99 mmol) was added portionwise over 10 min, allowing dissolution to occur after each addition. A white precipitate soon formed, and the reaction was allowed to stir at 0 °C for a further 1 h. Water (7 mL) was added and the reaction mixture allowed to warm to room temperature. Methanol was removed under reduced pressure and the remaining solution extracted with ethyl acetate (3 × 15 mL). The solution was washed with water (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure

to yield a white solid. Flash chromatography (SiO_2, LP-ethyl acetate, 3:1; 1:1, gradient elution) afforded **30** as a white crystalline solid (717 mg, 80%): (R_f 0.15, LP-ethyl acetate; 4:1); mp 115 °C; IR (KBr, cm^{-1}) 3474, 3081, 1708; MS (CI, NH_3) *m/z* 315 [MNH_4^+, 10], 298 [MH^+, 100]. Anal. ($C_{18}H_{19}NO_3$) C, H, N.

4-(*tert*-Butyloxycarbonyl)benzaldehyde (32). To a stirred solution of 4-carboxybenzaldehyde **31** (1.20 g, 7.99 mmol) in refluxing benzene (15 mL) was added *N*,*N*-dimethylformamide di-*tert*-butylacetal (6.50 g, 32.0 mmol) over 1 h. The mixture was refluxed for a further 30 min, then allowed to cool, and washed with water (15 mL), 5% aqueous sodium hydrogen carbonate (2 × 10 mL), and brine (10 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Flash chromatography (SiO_2, LP-ether, 5:1) afforded **32** as a white crystalline solid (1.05 g, 64%): (R_f 0.7, LP-ether; 1:1); mp 52 °C; IR (KBr, cm^{-1}) 1707, 1700; MS (GCMS, CI, NH_3) *m/z* 224 [MNH_4^+, 12], 207 [MH^+, 100], 133 [15], 74 [7], 57 [3]. Anal. ($C_{12}H_{14}O_3$) C, H.

4-(*tert*-Butyloxycarbonyl)benzyl Alcohol (33). A stirred solution of aldehyde **32** (4.12 g, 20.0 mmol) in methanol (30 mL) was cooled in an ice bath at 0 °C. Sodium borohydride (832 mg, 22.0 mmol) was then added in ca. 50 mg portions over 10 min, allowing dissolution to occur after each addition, and the reaction was allowed to stir at 0 °C for a further 1 h. Solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (300 mL). The solution was washed with water (2 × 250 mL) and 5% aqueous sodium hydrogen carbonate (200 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Flash chromatography (SiO_2, LP-ether, 3:2) afforded **33** as a white crystalline solid (3.55 g, 85%): (R_f 0.4, LP-ether; 1:1); mp 51−52 °C; IR (KBr, cm^{-1}) 3600−3200, 2975, 1713; MS (CI, NH_3) *m/z* 226 [MNH_4^+, 25], 209 [MH^+, 82], 170 [70], 153 [42], 135 [100]. Anal. ($C_{12}H_{16}O_3$) C, H.

Terephthalic Acid-mono(*tert*-butyl)ester (34). To a stirred solution of aldehyde **32** (412 mg, 2.0 mmol) in acetone (15 mL), in an ice bath, was added freshly prepared Jones reagent dropwise until an orange color persisted.[34] The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (30 mL) and washed with water (3 × 20 mL) until the organic layer was colorless. The resulting solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Recrystallization from LP-ether afforded **34** as white needles (374 mg, 84%): (R_f 0.4, LP-ether; 1:1); mp 238−240 °C; IR (KBr, cm^{-1}) 3600−2290, 2977, 1714, 1690; MS (CI, NH_3) *m/z* 240 [MNH_4^+, 100], 223 [MH^+, 12], 74 [28]. Anal. ($C_{12}H_{14}O_4$) C, H.

4-[*N*-(*tert*-Butyloxycarbonyl)aminomethyl]benzoic Acid (36). To a stirred solution of 4-(aminomethyl)benzoic acid (5.00 g, 33.1 mmol) in 1,4-dioxan (60 mL), water (30 mL), and 1 M NaOH (34 mL, 34 mmol), at 0 °C, was added di-*tert*-butylpyrocarbonate(7.94 g, 36 mmol), and the mixture was stirred at 0 °C for 40 min. The solution was concentrated under reduced pressure to 40 mL, then ethyl acetate (80 mL) was added, and the mixture was acidified to pH 4 with 1 M $KHSO_4$. The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield a white solid. Recrystalization from ethyl acetate gave **36** as white crystalline needles (7.46 g, 90%); mp 167−168 °C; IR (KBr, cm^{-1}) 3356, 2982, 2931, 1686; MS (APCI, NH_3) *m/z* 252 [MH^+, 100%], 222 [30], 172 [25]. Anal. ($C_{13}H_{17}NO_4$) C, H, N.

4-(*tert*-Butyloxycarbonylaminomethyl)benzyl Alcohol (37). A stirred solution of acid **36** (1.51 g, 6.0 mmol) in THF (60 mL) was prepared in a flask immersed in a water bath at ca. 25 °C. Borane−THF complex (15 mL of a 1 M solution, 15 mmol) was carefully added dropwise over 10 min, and evolution of hydrogen was observed. After being stirred overnight (20 h), the reaction mixture was quenched with water (30 mL) and extracted with ethyl acetate (2 × 60 mL). The organic layers were combined and washed sequentially with water (30 mL), 5% aqueous sodium hydrogencarbonate (2 × 30 mL), and water (30 mL), then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Flash chromatography (SiO_2, LP-ether; 2:1; 1:2, gradient elution) afforded **37** as a white crystalline solid (955 mg, 67%): (R_f 0.45, LP-ether; 1:5); mp 100−101 °C; IR (KBr, cm^{-1}) 3348, 2981, 2924, 1685; MS (APCI, NH_3) *m/z* 255 [MNH_4^+, 14], 238 [MH^+, 4], 199 [100], 138 [67], 106 [22]. Anal. ($C_{13}H_{19}NO_3$) C, H, N.

Isophthalic Acid-monobenzylester (39).[37] To a stirred solution of isophthalic acid (**38**) (1.66 g, 10 mmol) in a methanol (20 mL)−water (2 mL) mixture was added triethylamine (1.02 g, 10.1 mmol) dissolved in methanol (10 mL). After the mixture was stirred overnight, the solvent was removed under reduced pressure and the residue dissolved in DMF (25 mL). Benzyl bromide (1.88 g, 11.0 mmol) was added dropwise and the mixture stirred for 2 h at 100 °C. After cooling, the mixture was poured into 5% aqueous sodium hydrogen carbonate (50 mL) and extracted with ethyl acetate (3 × 30 mL). The aqueous layer was acidified with 1 N hydrochloric acid to pH 6 and further extracted with ethyl acetate (3 × 30 mL) to remove diester impurities. Further acidification of the aqueous layer to the range pH 5−pH 3 allowed ethyl acetate extraction of the monoester product. Then acidification to pH 1 permitted recovery of unreacted **38**. The organic layers containing monoester were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Recrystallization from ethanol−water afforded **39** as a white crystalline solid (1.25 g, 49%): (R_f 0.25, LP-ether; 1:1); mp 107 °C; IR (KBr, cm^{-1}) 3300−2400, 1719, 1705, 1690; MS (CI, NH_3) *m/z* 274 [MNH_4^+, 24], 257 [MH^+, 4], 149 [60], 108 [94], 91 [100]. Anal. ($C_{15}H_{12}O_4$) C, H.

2,2,2-Trichloroethyl-(3-benzyloxycarbonyl)phenylacetate (40).[37] To a stirred solution of monoester **39** (2.60 g, 10.1 mmol) in DCM (30 mL) was added 2 drops of DMF. Oxalyl chloride (6.35 g, 50.0 mmol) was added, and after being stirred for 1 h at room temperature the reaction mixture was concentrated under reduced pressure to afford 3-(benzyloxycarbonyl)benzoyl chloride as a colorless thick oil which was used without further purification [(R_f 0.5, LP-ether; 1:1); IR (NaCl, cm^{-1}) 3069, 3035, 2957, 1762, 1724.]

An alcohol free, ethereal solution of diazomethane (ca. 3.4 g, 0.32 M prepared from 24.5 g of Diazald) was cooled with stirring in an ice−salt bath to a temperature between −5 and 0 °C. The acid chloride dissolved in benzene (30 mL) was added dropwise over 15 min, and stirring continued at a temperature below 0 °C for a further 30 min. The mixture was then allowed to warm to room temperature and stirred overnight. Excess diazomethane was removed under reduced pressure (bubbling gases were passed through an acetic acid reservoir) and then the reaction mixture concentrated under reduced pressure to dryness, affording benzyl-3-(diazomethylcarbonyl)benzoate in quantitative yield as a bright yellow solid which was used without further purification.

The solid was dissolved in 2,2,2-trichloroethanol (35 mL, 365 mmol) and heated to 50−60 °C. Silver oxide (ca. 2 g) [freshly prepared from silver nitrate and sodium hydroxide precipitate, thoroughly washed sequentially with water, acetone, and ether then dried under vacuum over P_2O_5] was added in 250 mg portions to the reaction mixture until addition no longer resulted in evolution of nitrogen. Heating was continued for a further 30 min, and then the mixture was allowed to cool to room temperature after a total of 210 min. Chloroform (30 mL) was added to dilute the mixture which was filtered through Celite with chloroform (100 mL) to remove silver residues and concentrated under reduced pressure to yield an orange oil. Flash chromatography (SiO_2, LP-ether, 10:1; 2:1, gradient elution) afforded **40** as a colorless oil (2.75 g, 68%): (R_f 0.75, LP-ether; 1:1); IR (NaCl, cm^{-1}) 3035, 2955, 1755, 1718; MS (CI, NH_3) *m/z* MNH_4^+: {424 [0.5%], 422 [3], 420 [9], 418 [$^{35}Cl_3MNH_4^+$,10]}, 293 [22], 295 [20], 297 [7], 108 [87], 91 [100]. Anal. ($C_{18}H_{15}Cl_3O_4$) C, H.

3-(Benzyloxycarbonyl)phenylacetic Acid (41).[37] To a stirred solution of trichloroethylester **40** (2.44 g, 6.08 mmol) in THF (180 mL), in an ice bath at 0 °C, was added 90% aqueous acetic acid (30 mL). Zinc dust (24.0 g, 367 mmol) was added portionwise every 10 min over 1 h. After 3 h the reaction

mixture was filtered through Celite and partitioned with DCM (200 mL)-water (200 mL). The aqueous layer was separated and further extracted with DCM (2 × 100 mL). The organic extacts were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Recrystallization from ether afforded **41** as a white crystalline solid (1.38 g, 84%): (R_f 0.45, LP-ether; 1:1); mp 95 °C; IR (KBr, cm^{-1}) 3240−2200, 2955, 1720, 1700; MS (CI, NH_3) *m/z* 288 [MNH_4^+, 23], 271 [MH^+, 5], 163 [22], 108 [82], 91 [100]. Anal. ($C_{16}H_{14}O_4$) C, H.

3-(Benzyloxycarbonyl)phenylacetyl Chloride (42). To a stirred solution of acid **41** (108 mg, 0.40 mmol) in DCM (50 mL), was added DMF (2 drops) then oxalyl chloride (254 mg, 2.00 mmol). The resulting orange solution was stirred for 3 h and concentrated under reduced pressure to yield 3-(benzyloxycarbonyl) phenylacetyl chloride **42** as a gray solid (quant) which was used without further purification [IR (NaCl, cm^{-1}) 3034, 1798, 1718].

2,2,2-Trichloroethyl-(3-carboxyphenyl)acetate (43). The trichloroethyl ester **40** (100 mg, 0.25 mmol) was dissolved in ethyl acetate (5 mL). To this stirred solution was added palladium on activated carbon (10 mg, 10% w/w), and the reaction vessel sequentially evacuated and flushed with hydrogen 3 times. After 3 h of stirring under an atmosphere of hydrogen (balloon pressure), the reaction mixture was filtered through Celite and concentrated under reduced pressure to afford **43** as a white crystalline solid (78 mg, quant): (R_f 0.35, LP-ether; 1:1); mp 113−114 °C; IR (KBr, cm^{-1}) 2959, 2232, 2072, 1753, 1694; MS (DCI, NH_3) *m/z* MNH_4^+ {334 [4%], 332 [33], 330 [92], 328 [$^{35}Cl_3MNH_4^+$, 100]}, 294 [15], 226 [25], 152 [33]. Anal. ($C_{11}H_9Cl_3O_4$) C, H.

3-[4′-(*tert*-Butyloxycarbonyl)benzyloxycarbonyl]-phenylacetyl Chloride (44). To a stirred solution of acid **43** (75 mg, 0.24 mmol) in dry THF (4 mL) cooled in an ice bath was added 4-(*tert*-butyloxycarbonyl)benzyl alcohol **33** (50 mg, 0.24 mmol) and DMAP (ca. 1 mg). Dicyclohexylcarbodiimide (53 mg, 0.26 mmol) was then added in ca. 10 mg portions over 10 min and the solution stirred overnight. A white precipitate of dicyclohexylurea was removed by filtration and the filtrate concentrated under reduced pressure. The residue was redissolved in DCM (10 mL), washed with water (3 × 8 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Flash chromatography (SiO_2, LP-ether; 4:1; 2:1, gradient elution) afforded 2,2,2-trichloroethyl-{3-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]phenyl}acetate as a white crystalline solid (81 mg, 67%): (R_f 0.75, LP-ether; 1:1); mp 69 °C; IR (KBr, cm^{-1}) 3050, 2933, 1757, 1718; MS (CI, NH_3) *m/z* MNH_4^+ {524 [4%], 522 [24], 520 [73], 518 [$^{35}Cl_3MNH_4^+$, 65]}, 468 [5], 466 [35], 464 [100], 462 [92], 428 [27], 152 [34]. Anal. ($C_{23}H_{23}Cl_3O_6$) C, H.

To a stirred solution of the above trichloroethylester (513 mg, 1.02 mmol) in THF (38 mL), in an ice bath at 0 °C, was added 90% aqueous acetic acid (5 mL). Zinc dust (2.70 g, 41.3 mmol) was added in ca. 500 mg portions every 10 min over 1 h. A further amount of 90% aqueous acetic acid (2 mL) and zinc dust (500 mg, 7.65 mmol) was added after 150 min. After 3 h, the reaction mixture was filtered through Celite and partitioned with DCM (50 mL)-water (50 mL). The aqueous layer was further extracted with DCM (2 × 50 mL). The organic extacts were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Recrystallization from ether afforded 3-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]phenylacetic acid as a white crystalline solid (295 mg, 78%): (R_f 0.65, LP-ether; 1:1); mp 122 °C; IR (KBr, cm^{-1}) 3600−2400, 3015, 2979, 1716, 1707, 1695; MS (DCI, NH_3) *m/z* 388 [MNH_4^+, 65], 332 [100], 163 [11]. Anal. ($C_{21}H_{22}O_6$) C, H.

To a stirred solution of the above acid (148 mg, 0.40 mmol) in DCM (50 mL) was added DMF (2 drops) then oxalyl chloride (254 mg, 2.00 mmol). The resulting orange solution was stirred for 3 h and concentrated under reduced pressure to yield **44** as a gray solid (quant) which was used without further purification [IR (NaCl, cm^{-1}) 2979, 1800, 1713].

2,2,2-Trichloroethyl-[(3-*tert*-butyloxycarbonyl)phenyl]-acetate (45). To a stirred solution of acid **43** (78 mg, 0.25 mmol) in DCM (1 mL) was added *O-(tert*-butyl)-trichloroacetimidate (110 mg, 0.503 mmol) in cyclohexane (2 mL). Upon addition of boron trifluoride etherate (5 mL), the reaction mixture became cloudy. After the mixture was stirred for 22 h, solid sodium hydrogencarbonate (5 mg) was added and the resulting solution filtered through a short flash silica pad. Concentration under reduced pressure afforded **45** as a colorless oil (64 mg, 70%): (R_f 0.45, LP-ether; 5:1); IR (NaCl, cm^{-1}) 2978, 1758, 1713; MS (CI, NH_3) *m/z*: {388 [31], 386 [100], 384 [$^{35}Cl_3MNH_4^+$, 100]}, 332 [20], 330 [74], 328 [71], 152 [28]. HRMS (CI, $C_{15}H_{21}{}^{35}Cl_3NO_4^+$): found, 384.0536; requires, 384.0536.

3-*(tert*-Butyloxycarbonyl)phenylacetyl Chloride (46). To a stirred solution of trichloroethylester **45** (218 mg, 0.60 mmol) in THF (16 mL), in an ice bath at 0 °C, was added 90% aqueous acetic acid (3 mL). Zinc dust (2.36 g, 36.1 mmol) was added in ca. 400 mg portions every 10 min over 1 h. A further portion of zinc dust (1.18 g, 18.0 mmol) and 90% acetic acid (1.5 mL) was added after 90 min. After 3 h the reaction mixture was filtered through Celite and partitioned with DCM (50 mL)-water (50 mL). The aqueous layer was separated and further extracted with DCM (2 × 50 mL). The organic extacts were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Recrystallization from ether afforded 3-*(tert*-butyloxycarbonyl)phenylacetic acid as a white crystalline solid (132 mg, 94%): (R_f 0.25, LP-ether; 5:1); mp 76−78 °C; IR (KBr, cm^{-1}) 3420−2460, 2977, 2927, 1716, 1705; MS (CI, NH_3) *m/z* 254 [45%, MNH_4^+], 198 [100, $MNH_4^+ - C_4H_8$]. Anal. ($C_{13}H_{16}O_4$) C, H.

To a stirred solution of the above acid (78 mg, 0.330 mmol) in DCM (50 mL) were added DMF (2 drops) then oxalyl chloride (190 mg, 1.50 mmol). The resulting orange solution was stirred for 3 h and concentrated under reduced pressure to yield 3-(*tert*-butyloxycarbonyl)phenylacetyl chloride **46** as a gray solid (quant) which was used without further purification [IR (NaCl, cm^{-1}) 2980, 1800, 1712].

General Procedure for the Preparation of C-4 Esters: *cis*-3-Benzyl-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-1-(4′-methoxyphenyl)-2-azetidinone (47). To a stirred solution of acid **29** (1.56 g, 5.02 mmol) in dry THF (75 mL), cooled in an ice bath, was added 4-(*tert*-butyloxycarbonyl)benzyl alcohol **33** (1.05 g, 5.05 mmol) and DMAP (ca. 10 mg). Dicyclohexylcarbodiimide (1.12 g, 5.48 mmol) was added portionwise over 10 min and the solution stirred overnight. A white precipitate of dicyclohexylurea was removed by filtration and the filtrate concentrated under reduced pressure. The residue was redissolved in DCM (50 mL), washed with water (3 × 30 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield an orange solid. Flash chromatography (SiO_2, LP-ether, 3:2) afforded **47** as a white crystalline solid (2.07 g, 82%): (R_f 0.6, LP-ether; 1:1); mp 98−99 °C; IR (KBr, cm^{-1}) 3027, 2970, 2933, 1751, 1735, 1711; MS (DCI, NH_3) *m/z* 519 [MNH_4^+, 12%], 501 [M^+, 21], 446 [100], 238 [28]. Anal. ($C_{30}H_{31}NO_6$) C, H, N.

***cis*-3-(4′-Acetoxybenzyl)-4-benzyloxycarbonyl-1-(4′-methoxyphenyl)-2-azetidinone (58a).** The title compound was prepared in 82% isolated yield (7.72 mmol scale) from acid **57** and benzyl alcohol following a procedure similar to that described above for **47**: (R_f 0.55, LP-ether; 1:5); mp 136−137 °C; IR (KBr, cm^{-1}) 3025, 2955, 1759, 1736; MS (CI, NH_3) *m/z* 477 [MNH_4^+, 6], 460 [MH^+, 15], 107 [66], 91 [100]. Anal. ($C_{27}H_{25}NO_6$) C, H, N.

***cis*-3-(4′-Acetoxybenzyl)-4-[4′-(*tert*-butyloxycarbonyl)-benzyloxycarbonyl]-1-(4′-methoxyphenyl)-2-azetidinone (58b).** The title compound was prepared in 75% isolated yield (10.5 mmol scale) from acid **57** and alcohol **33** following a procedure similar to that described above for **47**: (R_f 0.7, DCM-ether; 10:1); mp 46−48 °C; IR (KBr, cm^{-1}) 2980, 1761, 1711; MS (DCI, NH_3) *m/z* 577 [MNH_4^+, 16], 559 [M^+, 25], 521 [28], 504 [29], 326 [46], 296 [35], 135 [78], 107 [100], 91 [49], 56 [30]. Anal. ($C_{32}H_{33}NO_8$) C, H, N.

General Procedure for N-1 Dearylation of 2-Azetidinones: *cis*-3-Benzyl-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-2-azetidinone (48). The protected 2-azetidinone **47** (1.51 g, 3.01 mmol) was dissolved in acetonitrile (70 mL) and cooled in an ice−salt bath to between −5 and 0 °C. At this temperature a solution of ceric ammonium nitrate (4.93 g, 8.99 mmol) in water (40 mL) was added over 3 min. The reaction mixture was stirred for a further 10 min then diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The organic solution was then washed sequentially with 5% aqueous sodium hydrogen carbonate (20 mL), 10% aqueous sodium sulfite (3 × 30 mL), and water (40 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield an orange solid. Flash chromatography (SiO_2, DCM-ether, 6:1) afforded **48** as a white foam (993 mg, 83%): (R_f 0.3, LP-ether; 1:5); mp 112 °C; IR ($CHCl_3$, cm^{-1}) 3020, 1776, 1748, 1709; MS (DCI, NH_3) *m/z* 413 [MNH_4^+, 100], 357 [59], 340 [18], 131 [17]. Anal. ($C_{23}H_{25}NO_5$) C, H, N.

***cis*-3-Benzyl-4-phenylcarbonyloxymethyl-2-azetidinone (54).** The title compound was prepared in 73% isolated yield (0.60 mmol scale) from 2-azetidinone **53** following a procedure similar to that described above for **48**: (R_f 0.3, LP-ethyl acetate; 2:1); mp 117−119 °C; IR (KBr, cm^{-1}) 3207 (NH), 3087, 2925, 1751, 1713; MS (CI, NH_3) *m/z* 313 [MNH_4^+, 47], 296 [MH^+, 100], 130 [26], 105 [45], 91 [3]. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

General Procedure for the Prepartion of N-1 Acylated 2-Azetidinones: *cis*-3-Benzyl-4-benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-2-azetidinone (6). *cis*-3-Benzyl-4-benzyloxycarbonyl-1-(4′-methoxyphenyl)-2-azetidinone was prepared in 69% isolated yield (3.00 mmol scale) from acid **29** and benzyl alcohol following a procedure similar to that described above for **47**: (R_f 0.3, LP-ether; 3:2); mp 113−114 °C; IR (KBr, cm^{-1}) 2970, 1737; MS (DCI, NH_3) *m/z* 402 [MH^+, 100], 238 [48], 91 [48]. Anal. ($C_{25}H_{23}NO_4$) C, H, N.

cis-3-Benzyl-4-benzyloxycarbonyl-2-azetidinone was prepared in 88% isolated yield (1.49 mmol scale) from *cis*-3-benzyl-4-benzyloxycarbonyl-1-(4′-methoxyphenyl)-2-azetidinone following a procedure similar to that described above for **48**: (R_f 0.55, LP-ether; 3:2); mp 111 °C; IR (KBr, cm^{-1}) 3209 (NH), 3102, 2974, 1752; MS (CI, NH_3) *m/z* 313 [MNH_4^+, 11], 296 [MH^+, 72], 268 [55], 91 [100]. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

To a stirred solution of *cis*-3-benzyl-4-benzyloxycarbonyl-2-azetidinone (118 mg, 0.40 mmol) in THF (12 mL), at −78 °C was added dropwise sodium bis(trimethylsilyl)amide (0.40 mL of a 1.0 M solution in THF, 0.40 mmol). After 20 min, the acid chloride **42**(0.40 mmol), dissolved in THF (2 mL), was added dropwise with stirring continued at −78 °C. Water (1 mL) was added to the reaction at −78 °C after 2 h followed by ethyl acetate (30 mL), and the solution was allowed to warm to room temperature with stirring. The mixture was washed with water (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Flash chromatography (SiO_2, LP-ether, 3:1; 2:1, gradient elution) afforded the unreacted 2-azetidinone (50 mg, 42%) and **6** as a pale green gummy solid (87 mg, 40%): (R_f 0.2, LP-ether; 1:1); mp 60−61 °C; IR (NaCl, cm^{-1}) 3065, 3033, 2954, 1796, 1746, 1714; MS (DCI, NH_3) *m/z* 565 [MNH_4^+, 100], 548 [MH^+, 21], 350 [5], 198 [9], 108 [35], 91 [68]. HRMS (CI, $C_{34}H_{33}N_2O_6^+$): found, 565.2340; requires, 565.2339. Anal. ($C_{34}H_{29}NO_6$) C, H, N.

***cis*-1-Benzoyl-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)-benzyloxycarbonyl]-2-azetidinone (49).** The title compound was prepared in 83% isolated yield (0.30 mmol scale) from 2-azetidinone **48** and benzoyl chloride following a procedure similar to that described above for **6**: (R_f 0.3 LP-ether; 1:1); IR (NaCl, cm^{-1}) 3064, 2977, 1801, 1748, 1711, 1676; MS (APCI) *m/z* 522 [M + Na^+, 4], 475[11], 444[12], 443[15], 264[38], 222[12], 220[10], 122[100]. Anal. ($C_{30}H_{29}NO_6$) C, H, N.

***cis*-3-Benzyl-4-[(4′-*tert*-butyloxycarbonyl)benzyloxycarbonyl]-1-{3′-[*tert*-butyloxycarbonyl]phenylacetate)-2-azetidinone (50).** The title compound [and unreacted **48** (14%)] was prepared in 42% isolated yield (0.30 mmol scale) from 2-azetidinone **48** and acid chloride **46** following a procedure similar to that described above for **6**: (R_f 0.2, LP-ether; 2:1); mp 52−53 °C; IR (NaCl, cm^{-1}) 3065, 2978, 2933, 1798, 1749, 1713; MS (DCI, NH_3) *m/z* 631 [MNH_4^+, 30], 575 [7], 519 [39], 197 [94], 154 [93], 91 [100], 78 [33], 58 [99]. HRMS ($C_{36}H_{43}N_2O_8^+$): found, 631.3020; requires, 631.3019.

***cis*-3-Benzyl-4-[(4′-*tert*-butyloxycarbonyl)benzyloxycarbonyl]-1-{3′-[benzyloxycarbonyl]phenylacetate)-2-azetidinone (51).** The title compound was prepared in 38% isolated yield (0.40 mmol scale) from 2-azetidinone **48** and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.3 LP-ether; 4:6); IR (NaCl, cm^{-1}) 2977, 2933, 1797, 1748, 1715; MS (APCI) *m/z* 670[M + Na^+, 8], 592[12], 574[52], 438[8], 124[24], 122[100]. Anal. ($C_{39}H_{37}NO_8$) C, H, N.

***cis*-3-Benzyl-4-[(4′-*tert*-butyloxycarbonyl)benzyloxycarbonyl]-1-{3′-[(4″-*tert*-butyloxycarbonyl)benzyloxycarbonyl]phenylacetate}-2-azetidinone (52).** The title compound [and unreacted **48** (18%)] was prepared in 36% isolated yield (0.40 mmol scale) from 2-azetidinone **48** and acid chloride **44** following a procedure similar to that described above for **6**: (R_f 0.35, LP-ether; 1:1); mp 105−106 °C; IR (KBr, cm^{-1}) 2980, 1786, 1744, 1730, 1714; MS (FAB) *m/z* 636 [MH^+ − (C_4H_8 × 2), 3], 618 [8], 484 [6], 269 [7], 135 [100], 118 [13], 91 [22], 57 [99]. Anal. ($C_{44}H_{45}NO_{10}$) C, H, N.

***cis*-1-Benzoyl-3-benzyl-4-phenylcarbonyloxymethyl-2-azetidinone (7).** The title compound was prepared in 94% isolated yield (0.68 mmol scale) from 2-azetidinone **54** and benzoyl chloride following a procedure similar to that described above for **6**: R_f 0.65, LP-EtAc; 1:2); mp 124−126 °C; IR (KBr, cm^{-1}) 3058, 3028, 2954, 1790, 1720, 1671; MS (APCI) *m/z* 400[M + H^+, 34], 296[6], 278[13], 174[7], 157[6], 122[13], 105[100]. Anal. ($C_{25}H_{21}NO_4$) C, H, N.

***cis*-3-Benzyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-4-phenylcarbonyloxymethyl-2-azetidinone (8).** The title compound was prepared in 38% isolated yield (0.70 mmol scale) from 2-azetidinone **54** and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.25, LP-ethyl acetate; 4:1); IR (NaCl, cm^{-1}) 3032, 1789, 1719; MS (CI, NH_3) *m/z* 565 [MNH_4^+, 74], 548 [MH^+, 54], 296 [33], 108 [37], 105 [100], 91 [40], 78 [12]. HRMS (CI, $C_{34}H_{30}NO_6^+$): found, 548.2070; requires, 548.2073.

***cis*-1-Benzoyl-4-benzyloxycarbonyl-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone (63).** *cis*-4-Benzyloxycarbonyl-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone was prepared in 63% isolated yield (0.016 mmol scale) from 2-azetidinone **62a** following a procedure similar to that described above for **48**: (R_f 0.25, DCM-ether; 10:1); mp 47−48 °C; IR (NaCl, cm^{-1}) 3304, 3078, 2932, 1766, 1259, 1114; MS (DCI, NH_3) *m/z* 567 [MNH_4^+, 10], 550 [MH^+, 4], 91 [100]. HRMS (CI, $C_{34}H_{39}N_2O_4Si^+$): found, 567.2680; requires, 567.2679. Anal. ($C_{34}H_{35}NO_4Si$) C, H, N.

The title compound **63** was prepared in 54% isolated yield (0.346 mmol scale) from *cis*-4-benzyloxycarbonyl-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone and benzoyl chloride following a procedure similar to that described above for **6**: (R_f 0.75, DCM); IR (NaCl, cm^{-1}) 3071, 2932, 1799, 1747, 1258, 1114; MS (DCI, NH_3) *m/z* 671 [6%, MNH_4^+], 654 [MH^+, 18], 312 [29], 286 [30], 122 [41], 105 [72], 91 [100], 78 [33]. HRMS (CI, $C_{41}H_{40}NO_5Si^+$): found, 654.2680; requires, 654.2676.

General Procedure for Deprotections Using Trifluoroacetic Acid: *cis*-3-Benzyl-4-[(4′-carboxy)benzyloxycarbonyl]-1-{3′-[(4″-carboxy)benzyloxycarbonyl]phenylacetate}-2-azetidinone (4). To diester **52** (22 mg, 0.029 mmol) stirred at 0 °C was added trifluoroacetic acid (0.5 mL). After 25 min, **52** had been consumed and the solution was concentrated under reduced pressure. Sequential trituration with ether and concentration under reduced pressure afforded **4** as a white crystalline solid (16 mg, 87%): (R_f 0.3, LP-ether; 1:5); mp 206 °C; IR (KBr, cm^{-1}) 3560−2420, 2927, 1799, 1746, 1718, 1694; MS (ES) *m/z* 635 [M^-, 36], 634 [($M - H$)$^-$, 100].

***cis*-3-Benzyl-4-[(4′-carboxy)benzyloxycarbonyl]-1-[3′-(benzyloxycarbonyl)phenylacetate]-2-azetidinone (1).** The title compound was prepared in 88% isolated yield (0.067 mmol scale) from diester **51** following a procedure similar to that described above for **4**: (R_f 0.35, LP-ethyl acetate; 2:3) mp 136

°C (dec); IR (NaCl cm^{-1}) 3600−3000, 3065, 3032, 1797, 1747, 1714; MS (APCI) *m/z* 590[M − H^+, 100], 410[6], 338[8], 187[22], 186[16]. Anal. ($C_{35}H_{29}NO_8$) C, H, N.

***cis*-1-Benzoyl-3-benzyl-4-[(4′-carboxy)benzyloxycarbonyl]-2-azetidinone (2).** The title compound was prepared in 84% isolated yield (0.16 mmol scale) from diester **49** following a procedure similar to that described above for **4**: (R_f 0.35, LP-ethyl acetate; 2:3); mp 99 °C (dec); IR (NaCl, cm^{-1}) 3600−2700, 3064, 3300, 1798, 1748, 1686; MS (APCI) *m/z* 442[M − H^+], 262[13], 187[28].

***cis*-3-Benzyl-4-[(4′-carboxy)benzyloxycarbonyl]-1-[3′-carboxyphenylacetate]-2-azetidinone (3).** The title compound was prepared in 63% isolated yield (0.043 mmol scale) from diester **50** following a procedure similar to that described above for **4**: (R_f 0.6, LP-ether; 1:5); mp 214 °C (dec); IR (KBr, cm^{-1}) 3600−2480, 2920, 1782, 1739, 1718, 1694; MS (ES) *m/z* 501 [M^-, 32], 500 [(M − H)$^-$, 100].

***cis*-3-Benzyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-4-[4′-(ammoniummethyl)benzyloxycarbonyl]-2-azetidinone, Trifluoroacetate (5).** *cis*-3-Benzyl-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]benzyloxycarbonyl}-1-(4′-methoxyphenyl)-2-azetidinone was prepared in 89% isolated yield (0.73 mmol scale) from acid **29** and alcohol **37** following a procedure similar to that described above for **47**: (R_f 0.4, LP-DCM; 1:20); mp 93−94 °C; IR (KBr, cm^{-1}) 3382, 3061, 1741, 1696; MS (FAB, Na) *m/z* 553 [MNa^+, 6], 530 [M^+, 38], 429 [13], 164 [100], 91 [29], 57 [99]. Anal. ($C_{31}H_{34}N_2O_6$) C, H, N.

cis-3-Benzyl-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]-benzyloxycarbonyl}-2-azetidinone was prepared in 71% isolated yield (1.80 mmol scale) from *cis*-3-benzyl-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]benzyloxycarbonyl}-1-(4′-methoxyphenyl)-2-azetidinone following a procedure similar to that described above for **48**: (R_f 0.25, LP-ether; 1:5); mp 109−111 °C; IR (KBr, cm^{-1}) 3373, 3355, 2981, 1771, 1724, 1687; MS (DCI, NH_3) *m/z* 442 [MNH_4^+, 3], 369 [59], 341 [56], 325 [100], 120 [55]. Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

cis-3-Benzyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]benzyloxycarbonyl}-2-azetidinone was prepared in 61% isolated yield (0.45 mmol scale) from *cis*-3-benzyl-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]benzyloxycarbonyl}-2-azetidinone and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.3, DCM-ethyl acetate; 20:1); mp 94−95 °C; IR (KBr, cm^{-1}) 3346, 1781, 1733, 1725, 1713, 1686; MS (FAB) *m/z* 677 [MH^+, 20], 577 [76], 164 [100], 91 [96]. Anal. ($C_{40}H_{40}N_2O_8$) C, H, N.

The title compound **5** was prepared in 99% isolated yield (0.075 mmol scale) from *cis*-3-benzyl-1-[3′-(benzyloxycarbonyl)-phenylacetate]-4-{4′-[*N*-(*tert*-butyloxycarbonyl)aminomethyl]-benzyloxycarbonyl}-2-azetidinone following a procedure similar to that described above for **4**: mp 58−59 °C; IR (KBr, cm^{-1}) 3620−3245, 2860, 1798, 1745, 1713; MS (ES) *m/z* 577 [MH^+, 100].

***cis*-1-Benzoyl-3-benzyl-4-(4′-carboxyphenylcarbonyloxymethyl)-2-azetidinone (9).** To a stirred solution of acid **34** (111 mg, 0.50 mmol) and alcohol **30** (164 mg, 0.55 mmol) in dry DCM (10 mL), in an ice bath at 0 °C, were added DMAP (ca. 2 mg) and EDCI (105 mg, 0.55 mmol). After being stirred for 22 h, the solution was allowed to warm to room temperature. Further EDCI (20 mg, 0.10 mmol) was added, and the reaction mixture was stirred for a further 2 h. The reaction mixture was washed with water (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white foam. Flash chromatography (SiO_2, LP-ether, 2:1; 1:2, gradient elution) afforded *cis*-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-1-(4′-methoxyphenyl)-2-azetidinone as a white crystalline solid (143 mg, 57%): (R_f 0.3, LP-ether; 1:1); mp 122−123 °C; IR (KBr, cm^{-1}) 3063, 2981, 2934, 1740, 1729, 1714; MS (CI, NH_3) *m/z* 519 [MNH_4^+, 9], 502 [MH^+, 92], 446 [37], 298 [41], 280 [75], 150 [98], 149 [100], 124 [86]. Anal. ($C_{30}H_{31}NO_6$) C, H, N.

cis-3-Benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-2-azetidinone was prepared in 67% isolated yield (1.80 mmol scale) from *cis*-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-1-(4′-methoxyphenyl)-2-azetidinone following a procedure similar to that described above for **48**: (R_f 0.35, LP-ethyl acetate; 1:1); mp 202−203 °C; IR (KBr, cm^{-1}) 3193, 2981, 1737, 1722, 1705; MS (CI, NH_3) *m/z* 413 [MNH_4^+, 54], 396 [MH^+, 39], 357 [52], 340 [66], 264 [26], 174 [100], 130 [88]. HRMS (CI, $C_{23}H_{25}NO_5^+$): found, 396.1811; requires, 396.1811.

cis-1-Benzoyl-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-2-azetidinone was prepared in 66% isolated yield (0.250 mmol scale) from *cis*-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-2-azetidinone and benzoyl chloride following a procedure similar to that described above for **6**: (R_f 0.4, LP-ethyl acetate; 4:1); mp 115−116 °C; IR (KBr, cm^{-1}) 2978, 2925, 1802, 1723, 1704; MS (CI, NH_3) *m/z* 517 [MNH_4^+, 13], 500 [MH^+, 74], 122 [100], 105 [91], 91 [20], 58 [18]. Anal. ($C_{30}H_{29}NO_6$) C, H, N.

The title compound **9** was prepared in 71% isolated yield (0.086 mmol scale) from *cis*-1-benzoyl-3-benzyl-4-[4′-(*tert*-butyloxycarbonyl)phenylcarbonyloxymethyl]-2-azetidinone following a procedure similar to that described above for **4**: (R_f 0.55, LP-ethyl acetate; 1:4); mp 178 °C; IR (KBr, cm^{-1}) 3630−2720, 3066, 2925, 1813, 1717; MS (CI, NH_3) *m/z* 461 [MNH_4^+, 5], 444 [MH^+, 23], 400 [12], 280 [55], 190 [100], 176 [28], 122 [89], 105 [88], 91 [28]. HRMS (CI, $C_{26}H_{22}NO_6^+$): found, 444.1450; requires, 444.1447. Anal. ($C_{26}H_{21}NO_6$) C, H, N.

***cis*-1-[3′-(Benzyloxycarbonyl)phenylacetate]-4-(4′-carboxybenzyloxycarbonyl)-3-(4′-hydroxybenzyl)-2-azetidinone (15).** The title compound was prepared in 98% isolated yield (0.0235 mmol scale) from ester **64** following a procedure similar to that described above for **4**: (R_f 0.2, DCM-ether; 10:1); mp 78−80 °C; IR (KBr, cm^{-1}) 3362, 3440−2480, 3061, 2927, 1799, 1743, 1717; MS (ES) *m/z* 606 [(M − H)$^-$, 100], 561 [10].

***cis*-3-Benzyl-4-phenylcarbonyloxymethyl-1-(4′-methoxyphenyl)-2-azetidinone (53).** To a stirred solution of alcohol **30** (4.55 g, 15.3 mmol) in dry DCM (150 mL), at room temperature, were added DMAP (ca. 5 mg) and triethylamine (1.55 g, 15.3 mmol). Benzoyl chloride (2.15 g, 15.3 mmol) was added dropwise over 5 min and the solution stirred for 20 h. Further amounts of triethylamine (310 mg, 3.06 mmol) and benzoyl chloride (430 mg, 3.06 mmol) were added to the reaction mixture, and stirring was continued for 24 h. The reaction mixture was washed with water (3 × 60 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a white solid. Flash chromatography (SiO_2, LP-ether, 2:1; 1:2, gradient elution) afforded **53** as a white crystalline solid (5.18 g, 84%): (R_f 0.5, LP-ether; 1:2); mp 113 °C; IR (KBr, cm^{-1}) 3027, 2937, 1741, 1707; MS (CI, NH_3) *m/z* 419 [MNH_4^+, 5], 402 [MH^+, 100], 270 [11], 149[12], 105 [37]. Anal. ($C_{25}H_{23}NO_4$) C, H, N.

General Procedure for N-Arylsulfonylation of 2-Azetidinones: *cis*-3-Benzyl-4-phenylcarbonyloxymethyl-1-(4″-toluenesulfonyl)-2-azetidinone (10) and *cis*-3-Benzyl-1-{2′-benzyl-1′-oxo-4′-phenylcarbonyloxy-3′-[*N*-(4″-toluenesulfonyl)amine]butyl}-4-phenylcarbonyloxymethyl-2-azetidinone (55), (Ar = *p*-C_6H_4-Me). To a stirred solution of 2-azetidinone **54** (93.6 mg, 0.32 mmol) in THF (20 mL), at −72 °C, was added dropwise sodium bis(trimethylsilyl)amide (0.32 mL of a 1.0 M solution in THF, 0.32 mmol). After 25 min, *p*-toluenesulfonyl chloride (604 mg, 3.2 mmol), dissolved in THF (1 mL), was added all in one portion and stirring continued for 21 h at −72 °C. Water (2 mL) was added to the reaction and the mixture allowed to warm to room temperature with stirring. The mixture was diluted with water (15 mL) and extracted with ethyl acetate (2 × 20 mL), and the organic extracts were combined, washed with water (3 × 15 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a green oil. Flash chromatography (SiO_2, DCM; DCM-ether, neat; 10:1, gradient elution) afforded the title compounds: **10** as a white foam (73.2 mg, 52%) and **55** (Ar = *p*-C_6H_4-Me) as a white foam (23.5 mg, 20%). **10**: (R_f 0.4, LP-ethyl acetate; 2:1); mp 99−100 °C; IR (KBr, cm^{-1}) 3063, 2921, 1793, 1728, 1360, 1171; MS (CI, NH_3) *m/z* 467 [MNH_4^+, 20], 450 [MH^+, 2], 345 [18], 328 [36], 189 [100], 174 [96], 108 [32], 105 [82], 91 [37], 78 [23]. HRMS (CI, $C_{25}H_{27}N_2O_5S^+$): found, 467.1641; requires, 467.1641. Anal. ($C_{25}H_{23}NO_5S$) C, H, N. **55**:

(Ar = *p*-C_6H_4-Me) (R_f 0.25, LP-ethyl acetate; 2:1); mp 84−86 °C; IR (KBr, cm^{-1}) 3282, 3033, 2924, 1793, 1719, 1704, 1339, 1161; MS (FAB, NH_3) *m*/*z* 745 [MH^+, 94], 623 [12], 105 [100]. HRMS (FAB, $C_{43}H_{41}N_2O_8S^+$): found, 745.253; requires, 745.258.

***cis*-1-Benzenesulfonyl-3-benzyl-4-phenylcarbonyloxymethyl-2-azetidinone (11) and *cis*-3-Benzyl-1-{2′-benzyl-1′-oxo-3′-[*N*-(benzenesulfonyl)amine]-4′-[phenylcarbonyloxy]butyl}-4-phenylcarbonyloxymethyl-2-azetidinone (55), (Ar = Ph).** The title compounds, **11** and **55** (Ar = Ph), were prepared in 61% and 8% isolated yields, respectively (0.30 mmol scale), from 2-azetidinone **54** following a procedure similar to that described above for **10** and **55** (Ar = *p*-C_6H_4-Me). **11**: (R_f 0.35, LP-ethyl acetate; 2:1); mp 44−46 °C; IR (NaCl, cm^{-1}) 3034, 1793, 1722, 1315, 1171; MS (CI, NH_3) *m*/*z* 453 [MNH_4^+, 77], 436 [MH^+, 5], 331 [15], 314 [18], 130 [29], 105 [100]. HRMS (CI, $C_{24}H_{25}N_2O_5S^+$): found, 453.1484; requires, 453.1484. Anal. ($C_{24}H_{21}NO_5S$) C, H, N. **55**: (Ar = Ph) (R_f 0.25, LP-ethyl acetate; 2:1); IR (NaCl, cm^{-1}) 3282, 3031, 2929, 1790, 1722, 1704, 1339, 1163; MS (CI, NH_3) *m*/*z* 748 [MNH_4^+, 0.3], 731 [MH^+, 0.6], 626 [3], 609 [15], 452 [37], 428 [32], 314 [47], 296 [38], 175 [41], 105 [100]. HRMS (CI, $C_{42}H_{39}N_2O_8S^+$): found, 731.2430; requires, 731.2427.

***cis*-3-Benzyl-1-(4′-nitrobenzenesulfonyl)-4-phenylcarbonyloxymethyl-2-azetidinone (12).** The title compound was prepared in 67% isolated yield from 2-azetidinone **54** (0.25 mmol scale) following a procedure similar to that described above for **10**: (R_f 0.55, LP-ethyl acetate; 2:1); mp 60−62 °C; IR (KBr, cm^{-1}) 3105, 2955, 1796, 1724, 1350, 1175; MS (DCI, NH_3) *m*/*z* 498 [MNH_4^+, 25], 359 [22], 105 [100]. Anal. ($C_{24}H_{20}N_2O_7S$) C, H, N.

***cis*-3-Benzyl-1-ethanesulfonyl-4-phenylcarbonyloxymethyl-2-azetidinone (13).** The title compound was prepared in 29% isolated yield from 2-azetidinone **54** (0.42 mmol scale) following a procedure similar to that described above for **10**: (R_f 0.25, DCM; DCM-ether, neat; 10:1); IR (NaCl, cm^{-1}) 2936, 1794, 1723, 1355, 1156; MS (CI, NH_3) *m*/*z* 405 [MNH_4^+, 55], 388 [MH^+, 11], 283 [37], 266 [53], 105 [100]. HRMS (CI, $C_{20}H_{25}N_2O_5S^+$): found, 405.1484; requires, 405.1484.

***cis*-3-Benzyl-4-benzyloxycarbonyl-1-(4′-toluenesulfonyl)-2-azetidinone (14).** The title compound was prepared in 61% isolated yield (0.30 mmol scale) from *cis*-3-benzyl-4-benzyloxycarbonyl-2-azetidinone following a procedure similar to that described above for **10**: (R_f 0.5, DCM); mp 129−130 °C; IR (KBr, cm^{-1}) 2927, 1808, 1744, 1351, 1163; MS (CI, NH_3) *m*/*z* 467 [MNH_4^+, 100], 131 [35], 108 [44], 91 [82]. Anal. ($C_{25}H_{23}NO_5S$) C, H, N.

General Procedure for the Preparation of Phenols 61a and 61b: *cis*-4-Benzyloxycarbonyl-3-(4′-hydroxybenzyl)-1-(4′-methoxyphenyl)-2-azetidinone (61a). To a stirred solution of acetate **58a** (525 mg, 1.14 mmol) in a methanol (35 mL)-THF (20 mL) solution, at 35 °C, was added activated zinc. [Activated zinc was prepared by stirring zinc dust in 10% aqueous hydrochloric acid (50 mL) for 4 min. The zinc was filtered, washed sequentially with acetone (2 × 40 mL), ether (3 × 40 mL), and then methanol (2 × 40 mL), and added as a moist slurry in methanol.[44]] The reaction mixture was stirred for 4 days at 35 °C and then filtered through Celite to remove excess zinc. The solvent was removed under reduced pressure and the resulting gray oil dissolved in an ethyl acetate (20 mL)-water (20 mL) mixture. The solution was acidified to pH 3, extracted with ethyl acetate (3 × 40 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a gray solid. Flash chromatography (SiO_2, DCM-ether, 25:1) afforded **61a** as a white crystalline solid (419 mg, 88%): (R_f 0.45, DCM-ether; 10:1); mp 147.5−148.5 °C; IR (KBr, cm^{-1}) 3343, 2962, 1735; MS (CI, NH_3) *m*/*z* 435 [MNH_4^+, 3], 418 [MH^+, 100], 342 [20], 284 [19], 124 [50], 108 [41], 91 [59], 78 [11]. Anal. ($C_{25}H_{23}NO_5$) C, H, N.

***cis*-4-[4′-(*tert*-Butyloxycarbonyl)benzyloxycarbonyl]-3-(4′-hydroxybenzyl)-1-(4′-methoxyphenyl)-2-azetidinone (61b).** The title compound [and also 4-(*tert*-butyloxycarbonyl)benzyl alcohol **33** (17%)] was prepared in 71% isolated yield (0.643 mmol scale) from 2-azetidinone **58b** following a procedure similar to that described above for **61a**: (R_f 0.45, DCM-ether; 10:1); mp 158−159 °C; IR (KBr, cm^{-1}) 3293, 2977, 1739, 1713; MS (DCI, NH_3) *m*/*z* 462 [$MH^+ - C_4H_8$, 6], 342 [8], 328 [8], 284 [20], 124 [83], 107 [100], 91 [72]. Anal. ($C_{30}H_{31}NO_7$) C, H, N.

General Procedure for the Preparation of Silyl Ethers 62a and 62b: *cis*-4-Benzyloxycarbonyl-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-1-(4′-methoxyphenyl)-2-azetidinone (62a). To a stirred slurry of phenol **61a** (1.74 g, 4.17 mmol) in DMF (4 mL), heated at 40 °C, were added imidazole (708 mg, 10.4 mmol) and *tert*-butyldiphenylsilyl chloride (1.37 g, 4.98 mmol). After being stirred at 40 °C for 17 h, the reaction mixture was diluted with water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with water (40 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a colorless oil. Flash chromatography (SiO_2, DCM) afforded **62a** as a white gummy foam (2.57 g, 94%): (R_f 0.8, ether-LP; 1:1); mp 33−35 °C; IR (NaCl, cm^{-1}) 2932, 1757, 1753, 1251, 1115; MS (DCI, NH_3) *m*/*z* 656 [MH^+, 10], 91 [100], 78 [12]. Anal. ($C_{41}H_{41}NO_5Si$) C, H, N.

***cis*-3-(4′-*tert*-Butyldiphenylsilyloxybenzyl)-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-1-(4′-methoxyphenyl)-2-azetidinone (62b).** The title compound was prepared in 78% isolated yield from 2-azetidinone **61b** (0.435 mmol scale) following a procedure similar to that described above for **62a**: (R_f 0.7, DCM-ether; 10:1); mp 63−65 °C; IR (KBr, cm^{-1}) 2932, 2858, 1761, 1713, 1251, 1114; MS (FAB, Na) *m*/*z* 778 [MNa^+, 84], 755 [M^+, 15], 722 [14], 564 [28], 537 [25], 521 [35], 509 [47], 497 [67], 481 [64], 469 [83], 455 [68], 441 [71], 413 [100]. Anal. ($C_{46}H_{49}NO_7Si$) C, H, N.

General Procedure for the Preparation of Phenols 16−18 and 64: *cis*-1-Benzoyl-4-benzyloxycarbonyl-3-(4′-hydroxybenzyl)-2-azetidinone (17). To a stirred solution of silyl ether **63** (100 mg, 0.153 mmol) in DMF (4 mL) and *N*-methylpyrolidin-2-one (1.5 mL) was added ammonium hydrogen difluoride (87 mg, 1.53 mmol). The resulting suspension was stirred at room temperature for 16 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layers were combined, washed with water (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a colorless oil. Flash chromatography (SiO_2, DCM-ether, 25:1) afforded **17** as a white crystalline solid (31.7 mg, 50%): (R_f 0.7, DCM-ether, 10:1); mp 162−163 °C; IR (KBr, cm^{-1}) 3415, 1802, 1737; MS (DCI, NH_3) *m*/*z* 433 [MNH_4^+, 2], 416 [MH^+, 12], 147 [40], 122 [41], 105 [100], 91 [77], 78 [22]. HRMS (CI, $C_{25}H_{22}NO_5^+$): found, 416.1498; requires, 416.1498. Anal. ($C_{25}H_{21}NO_5$) C, H, N.

***cis*-1-[3′-(Benzyloxycarbonyl)phenylacetate]-4-benzyloxycarbonyl-3-(4′-hydroxybenzyl)-2-azetidinone (16).** *cis*-4-Benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone was prepared in 56% isolated yield (0.80 mmol scale) from *cis*-4-benzyloxycarbonyl-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone (36% recovery) and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.3, DCM); mp 47−48 °C; IR (KBr, cm^{-1}) 3033, 2931, 2857, 1796, 1747, 1714, 1269, 1112; MS (DCI, NH_3) *m*/*z* 819 [MNH_4^+, 8], 802 [MH^+, 2], 91 [100]. Anal. ($C_{50}H_{47}NO_7Si$) C, H, N.

The title compound **16** was prepared in 68% isolated yield from *cis*-4-benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-2-azetidinone (0.14 mmol scale) following a procedure similar to that described above for **17**: (R_f 0.2, DCM-ether, 25:1); mp 43−45 °C; IR (KBr, cm^{-1}) 3434, 2923, 1795, 1746, 1717, 1703; MS (FAB) *m*/*z* 564 [MH^+, 46], 546 [3], 287[19], 147 [11], 107 [9], 91 [100]. Anal. ($C_{34}H_{29}NO_7$) C, H, N.

(3*S*,4*S*)-4-Benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-[4′-hydroxybenzyl]-2-azetidinone (18). (3*S*,4*S*)-4-Benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-[4′-(*tert*-butyldiphenylsilyloxy)benzyl]-2-azetidinone was prepared in 45% isolated yield (0.11 mmol scale) from 2-azetidinone **71** and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.3, DCM); $[\alpha]_D^{22.5}$

−2.5 (*c* 0.88, $CHCl_3$); IR (NaCl, cm^{-1}) 3071, 2932, 2858, 1797, 1747, 1715, 1269, 1113; MS (APCI, NH_3) *m/z* 824 [MNa^+, 8], 819 [MNH_4^+, 22], 802 [MH^+, 45], 664 [12], 345[14], 122 [100]. The title compound **18** was prepared in 67% isolated yield (0.35 mmol scale) from (3*S*,4*S*)-4-benzyloxycarbonyl-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-[4′-(*tert*-butyldiphenylsilyloxy)benzyl]-2-azetidinone following a procedure similar to that described above for **17**: (R_f 0.2, DCM-ether, 25:1); $[\alpha]_D^{22.5}$ −14.1 (*c* 0.18, $CHCl_3$); Spectral data as for **16**.

***cis*-1-[3′-(Benzyloxycarbonyl)phenylacetate]-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-3-(4′-hydroxybenzyl)-2-azetidinone (64).** *cis*-3-(4′-*tert*-Butyldiphenylsilyloxybenzyl)-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-2-azetidinone was prepared in 68% isolated yield (0.34 mmol scale) from 2-azetidinone **62b** following a procedure similar to that described above for **48**: (R_f 0.25, DCM-ether; 10:1); mp 62−64 °C; IR (KBr, cm^{-1}) 3397, 3072, 2932, 2858, 1773, 1714, 1257, 1114; MS (DCI, NH_3) *m/z* 667 [MNH_4^+, 100], 611 [28], 594 [6], 477 [52], 460 [87], 345 [51], 193 [52], 119 [66], 78 [48]. Anal. ($C_{39}H_{43}NO_6Si$) C, H, N.

cis-1-[3′-(Benzyloxycarbonyl)phenylacetate]-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-4-(4′-*tert*-butyloxycarbonylbenzyloxycarbonyl)-2-azetidinone was prepared in 52% isolated yield (0.21 mmol scale) from *cis*-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-4-[4′-(*tert*-butyloxycarbonyl)benzyloxycarbonyl]-2-azetidinone and acid chloride **42** following a procedure similar to that described above for **6**: (R_f 0.3, DCM; DCM-ether, neat; 50:1); mp 74−75 °C; IR (KBr, cm^{-1}) 2930, 1797, 1751, 1714, 1269, 1113; MS (APCI, NH_3) *m/z* 919 [MNH_4^+, 23], 846 [100], 828 [78], 690 [56], 662 [83], 624 [63], 570 [65]. Anal. ($C_{55}H_{55}NO_9Si$) C, H, N.

The title compound **64** was prepared in 82% isolated yield (0.09 mmol scale) from *cis*-1-[3′-(benzyloxycarbonyl)phenylacetate]-3-(4′-*tert*-butyldiphenylsilyloxybenzyl)-4-(4′-*tert*-butyloxycarbonylbenzyloxycarbonyl)-2-azetidinone following a procedure similar to that described above for **17**: (R_f 0.3, DCM-ether, 10:1); mp 59−61 °C; IR (KBr, cm^{-1}) 3436, 3034, 2977, 2931, 1800, 1750, 1714; MS (FAB) *m/z* 664 [MH^+, 34], 608 [50], 590 [43], 500 [8], 197 [18], 135, [13], 107 [19], 91 [100]. Anal. ($C_{39}H_{37}NO_9$) C, H, N.

(4*S*)-4-Benzyloxycarbonyl-1-(*tert*-butyldimethylsilyl)-3-[*Z*-4′-(*tert*-butyldiphenylsilyloxy)phenylmethylene]-2-azetidinone (69a) and (4*S*)-4-Benzyloxycarbonyl-1-(*tert*-butyldimethylsilyl)-3-[*E*-4′-(*tert*-butyldiphenylsilyloxy)-phenylmethylene]-2-azetidinone (69b). To a stirred solution of di-isopropylamine (1.67 g, 2.31 mL, 16.5 mmol) in dry THF (16.3 mL) cooled to 0 °C, was added *n*-butyllithium (11.4 mL of a 1.45 M solution in hexanes, 16.5 mmol). After being stirred for 5 min at 0 °C, the reaction mixture containing lithium di-*iso*-propylamide was cooled to −78 °C. A solution of (4*S*)-1-(*tert*-butyldimethylsilyl)-4-carboxy-2-azetidinone **68**[48] (1.15 g, 5.01 mmol) dissolved in dry THF (50 mL) was stirred at −9 °C, freshly prepared lithium di-isopropylamide solution (20 mL, 11.0 mmol) was added, and stirring of the resulting brown solution continued at −9 °C for 15 min. Trimethylsilyl chloride (0.599 g, 5.52 mmol) was added, resulting in a yellow solution, which was stirred for 15 min at −9 °C. A further amount of freshly prepared lithium di-isopropylamide solution (10 mL, 5.5 mmol) was added, and stirring of the resulting brown solution continued for 5 min, allowing the solution to warm to 0 °C. A solution of 4′-*tert*-butyldiphenylsilyloxy benzaldehyde (1.99 g, 5.52 mmol) in THF (10 mL) was added dropwise over 5 min which resulted in a temporary deep red coloration, and stirring was continued at 0 °C for a further 1 h. The reaction mixture was removed from the cooling bath, diluted with water (50 mL), then acidified with 5% aqueous hydrochloric acid to pH 3, and extracted with ethyl acetate (2 × 50 mL). The organic extracts were combined and washed with brine (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a brown foam. This foam was dissolved in dry DCM (50 mL) and stirred at room temperature. Benzyl alcohol (0.596 g, 5.51 mmol) and DMAP (ca.2 mg) were added, and then EDCI (1.06 g, 5.53 mmol) was added portionwise over 10 min. After being stirred for a further 3 h, the reaction mixture was washed with brine (3 × 30 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a green gum. Flash chromatography (SiO_2, DCM) afforded **69a** as a colorless gum (246 mg, 7%), and **69b** as a white foam (436 mg, 13%). **69a**: (R_f 0.75, DCM); $[\alpha]_D^{21}$ −33.3 (*c* 0.91, $CHCl_3$); UV λ_{max} ($CHCl_3$) 316 nm (ϵ 13500); IR (NaCl, cm^{-1}) 3071, 2956, 2931, 2858, 1740, 1675, 1252; MS (APCI, NH_3) *m/z* 694 [MH^+ + MeOH, 5], 662 [MH^+, 100], 634 [12], 345 [15]. HRMS (CI, $C_{40}H_{48}NO_4Si_2^+$): found, 662.3120; requires, 662.3122. Anal. ($C_{40}H_{47}NO_4Si_2$) C, H. **69b**: (R_f 0.2, DCM); mp 43−44 °C; $[\alpha]_D^{21}$ +81.1 (*c* 0.92, $CHCl_3$); UV λ_{max} ($CHCl_3$) 310 nm (ϵ 18130); IR (NaCl, cm^{-1}) 3071, 2956, 2931, 2858, 1749, 1683, 1257; MS (APCI, NH_3) *m/z* 694 [MH^++MeOH, 6], 662 [MH^+, 100], 634 [38], 401 [8], 345 [9]. HRMS (CI, $C_{40}H_{48}NO_4Si_2^+$): found, 662.3120; requires, 662.3122. Anal. ($C_{40}H_{47}NO_4Si_2$) C, H.

(3*S*,4*S*)-4-Benzyloxycarbonyl-1-(*tert*-butyldimethylsilyl)-3-[4′-(*tert*-butyldiphenylsilyloxy)benzyl]-2-azetidinone (70). The *E*-alkene **69b** (0.298 g, 0.45 mmol) was dissolved in THF (5 mL). To this stirred solution was added palladium on activated carbon (67 mg, 10% w/w), and the reaction vessel was evacuated and flushed with hydrogen. After being stirred for 30 h under an atmosphere of hydrogen, the reaction solution was filtered through Celite and concentrated under reduced pressure to afford a brown foam. This foam was dissolved in dry DCM (10 mL) and stirred at room temperature. Benzyl alcohol (54.1 mg, 0.50 mmol) and DMAP (ca. 2 mg) were added, then EDCI (95.9 mg, 0.50 mmol) was added portionwise over 10 min. After being stirred for a further 14 h, the reaction mixture was washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a yellow oil. Flash chromatography (SiO_2, DCM) afforded **70** as a colorless gum (98 mg, 33%): (R_f 0.55, DCM); $[\alpha]_D^{20.5}$ +4.5 (*c* 0.96, $CHCl_3$); IR (NaCl, cm^{-1}) 3034, 2956, 2931, 2858, 1753, 1256; MS (APCI, NH_3) *m/z* 686 [MNa^+, 18], 664 [MH^+, 99], 636 [100], 550 [12], 435 [74], 345 [46], 122 [46]. HRMS (CI, $C_{40}H_{50}NO_4Si_2^+$): found, 664.3280; requires, 664.3278. A similar procedure was conducted using the *Z*-alkene **69a**, affording **70** in 30% yield with spectroscopic data as above.

(3*S*,4*S*)-4-Benzyloxycarbonyl-3-[4′-(*tert*-butyldiphenylsilyloxy)benzyl]-2-azetidinone (71). To a stirred solution of 2-azetidinone **70** (84.2 mg, 0.127 mmol) in acetonitrile (3.5 mL), at room temperature, was added 48% aqueous hydrogen fluoride (3 drops). After being stirred for 20 min, the reaction mixture was diluted with water (3.5 mL), neutralized with 5% aqueous sodium hydrogen carbonate, and extracted with ethyl acetate (2 × 20 mL). The organic extracts were combined, washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield **71** as a colorless gum (64.8 mg, 93%): (R_f 0.25, DCM-ether; 10:1); $[\alpha]_D^{20.5}$ +9.8 (*c* 0.62, $CHCl_3$); IR (NaCl, cm^{-1}) 3402, 3072, 2931, 1747, 1259, 1114; MS (APCI, NH_3) *m/z* 567 [MNH_4^+, 100], 550 [MH^+, 5], 345 [13], 122 [30]. HRMS (CI, $C_{34}H_{39}N_2O_4Si^+$): found, 567.2679; requires, 567.2679.

Acknowledgment. We thank the EPSRC (formerly SERC) for a studentship to W.M., Eli Lilly and Company for CASE support, the EPSRC mass spectrometry service (Swansea) for high resolution mass spectra, and J. G. Vaughan for the preparation of **68**.

Supporting Information Available: NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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