

SYNTHESIS AND SAR OF THIOESTER AND THIOL INHIBITORS OF IMP-1 METALLO- β -LACTAMASE

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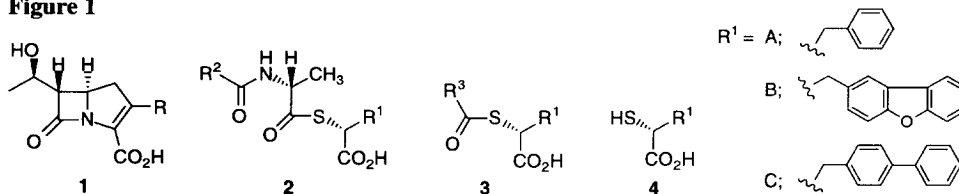
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Abstract: Potent thioester and thiol inhibitors of IMP-1 metallo- β -lactamase have been synthesized employing a solid-phase Mitsunobu reaction as the key step. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction: Carbapenems, such as imipenem and meropenem, are β -lactam antibiotics noted for their broad-spectrum of activity and stability to most β -lactamases. However, bacterial resistance to these clinically important antibiotics is on the rise, in part due to the increasing incidence of metallo- β -lactamases (MBLs).¹ Unlike the more common serine β -lactamases, MBLs are active-site zinc enzymes which efficiently hydrolyze carbapenems, as well as penicillin and cephalosporin members of the β -lactam family. Once rare and regarded as clinically unimportant, these enzymes have now been identified from more than 20 bacterial sources, including clinical isolates of a number of pathogenic bacterial species.^{1,2} Of the MBLs identified to date, the IMP-1 enzyme appears to pose the greatest threat, due to the transferable nature of its gene.³ Indeed, the dissemination of IMP-1 among gram-negative bacterial species, notably *P. aeruginosa* and *S. marcescens*, has been observed in Japan.³ Recently, carbapenem resistant organisms producing MBLs related to IMP-1 have been reported in Singapore^{4a} and Italy.^{4b} Thus, the development of inhibitors of MBLs, particularly IMP-1, is important in order to counter this emerging threat to antimicrobial chemotherapy with β -lactam antibiotics.

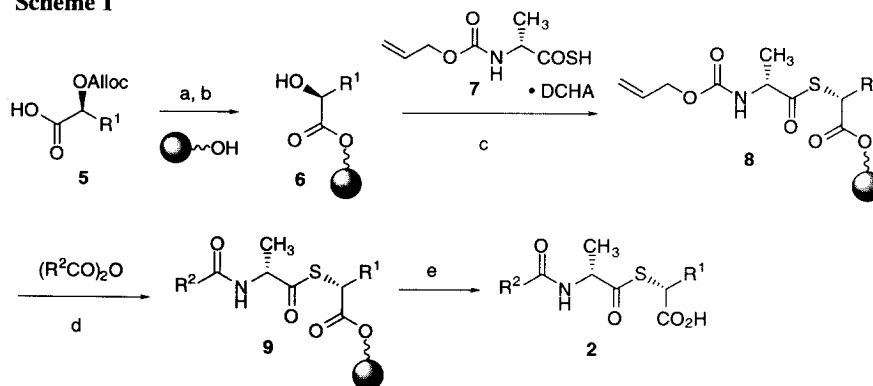
The structural information available to date on MBLs indicates that they are a heterogeneous group of enzymes, although substantial active-site homology exists among them.⁵ In general, they appear to function with a binuclear zinc catalytic center, although the details of the mechanism by which they hydrolyze β -lactams have not been elucidated. These metalloenzymes are not inactivated by inhibitors of the serine β -lactamases such as clavulanic acid or sulbactam, but several recent reports of compounds that inhibit some MBLs have appeared.^{6,7} However, only two of these studies described inhibitors of IMP-1,^{7a,c} despite its demonstrated potential for widespread dispersal. Simple thiodepsipeptides **2** ($R^1 = H$ or Ph) have been reported to be inhibitors of several MBLs, but with widely varying potencies.⁶ Thioesters **2** bear a structural resemblance to the β -lactam targets of these enzymes (e.g., **1**) and in one case ($R^1 = H$), the inhibition was shown to be mechanism-based, involving hydrolysis of the thioester to the thiol.^{6a} In the present study, we have found that thiodepsipeptides **2** are inhibitors of the IMP-1 MBL and that introduction of more hydrophobic C-terminal substituents (Figure 1; $R^1 = A, B, C$) greatly enhances this activity.⁸ Furthermore, the simpler thioesters **3** ($R^3 = CH_3, Ph$) have been discovered to be even more active inhibitors of IMP-1. Thioesters **2** and **3** have been determined to be substrates for the IMP-1 enzyme and as such are hydrolyzed to thiols **4**, which are themselves potent inhibitors of IMP-1.⁹ Finally, the (*R*)-stereochemistry of the α -mercapto-acid center has been shown to be important for the inhibitory activity of these compounds.

Figure 1



Chemistry: The thiopeptides **2** were synthesized using a solid-phase Mitsunobu reaction of a resin-bound α -hydroxy ester (**6**) and alloc-D-thioalanine (**7**) as the key step (Scheme 1). The starting protected α -hydroxy acids **5** were synthesized by Evans' asymmetric hydroxylation methodology.^{10,11} For the solid support, Rapp TentaGel® S-NH₂ resin with a mild acid-cleavable HMPB linker¹² was chosen as being compatible with the labile thioester functionality. After attachment of substrate **5** to the resin,^{13,14} removal of the alloc protecting group provided **6**. Mitsunobu reaction of **6** with alloc-D-thioalanine¹⁵ employing a modified Volante procedure¹⁶ proceeded readily to yield thioesters **8**. As has been noted with other Mitsunobu reactions, this reaction was accelerated by using the electron-deficient tris(4-chlorophenyl)phosphine in place of the more common triphenylphosphine and by the addition of an amine base (e.g., *i*-Pr₂NEt).¹⁷ Furthermore, the dicyclohexylamine salt of thioacid **7** could be conveniently employed in this reaction to achieve the same accelerating effect without addition of an exogenous base.

Scheme 1

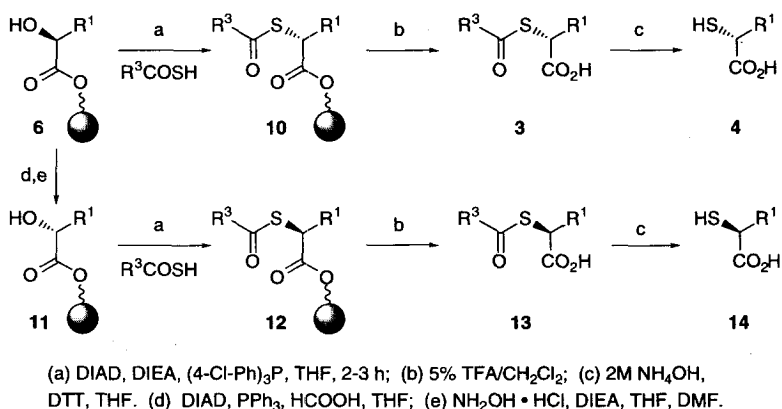


(a) TentaGel-HMPB resin, DIC, DMAP, DMF; (b) Pd(PPh₃)₄, N-methylmorpholine, HOAc, NMP, 2-4 h; (c) DIAD, (4-Cl-Ph)₃P, THF, 1-3 h; (d) Pd(PPh₃)₄, PhSiH₃, CH₂Cl₂; (e) 5% TFA in CH₂Cl₂.

Introduction of a variety of R² acyl substituents was accomplished via a "transacylation" protocol, based on the procedure of Guibé et. al.,¹⁸ which was fully compatible with the thioester functionality. Symmetrical anhydrides were found to be the acylating agents of choice for this reaction,¹⁹ and a number of commercially available or readily prepared symmetrical anhydrides were successfully employed. Furthermore, it was found that the anhydride could be prepared and used in situ (R²CO₂H, *t*-BuN=C=NEt, CH₂Cl₂),²⁰ thus greatly extending the scope of this transacylation reaction. After cleavage from the resin, the thioesters were generally obtained in 80–95% yield and >90% purity by HPLC.^{21–23} Using this procedure,^{24,25} a variety of thiopeptides **2** were prepared (Table 1).

The syntheses of thioesters **3** and thiols **4** were accomplished as shown in Scheme 2. Mitsunobu reaction of **6** with thioacetic acid or thiobenzoic acid gave the thioesters **10**. After cleavage from the resin, thioesters **3** ($R^3 = \text{CH}_3, \text{Ph}$) were obtained in 80–90% yield and >95% purity by HPLC.^{22,23} Removal of the acetyl group of thioester **3** ($R^3 = \text{CH}_3$) was then accomplished with aqueous ammonia under conditions reported to give minimal racemization²⁶ providing thiols **4**. This reaction was carried-out in the presence of dithiothreitol (DTT) to suppress disulfide formation. Compounds epimeric at the thiol center were prepared from the epimeric resin-bound α -hydroxy ester, **11**, which was obtained either by starting with the enantiomer of α -acyloxy acid **5** in Scheme 1 or by inverting the hydroxy center of **6** as indicated in Scheme 2.

Scheme 2



Biological Activity: Compounds were assayed for activity against the IMP-1 and CcrA (*B. fragilis*) metallo- β -lactamases.^{27,28} As shown in Table 1, thiodepsipeptides **2** were generally good inhibitors of IMP-1, but were poorly active against the CcrA enzyme. Comparing compounds **2a**,²⁹ **2d**, **2g**, and **2k** reveals that increased hydrophobicity in the R^1 group significantly enhances activity against IMP-1. In addition, the dibenzofuranyl substituent ($R^1 = \text{B}$) was found to confer a slight activity advantage over the corresponding biphenyl substituent ($R^1 = \text{C}$). On the other hand, the R^2 group was found to generally have a less pronounced effect on activity. This was investigated most thoroughly in the more readily available $R^1 = \text{C}$ series (**2i–2al**) where the most potent compound was **2z**, with an IC_{50} against IMP-1 of 0.25 μM . The importance of the (*R*)-stereochemistry at the thiol center is dramatically illustrated by comparing the activity of **2g** to its (*2S*)-epimer **2h**, which was more than 300-fold less active against IMP-1.

The thiol components (**4**) of thiodepsipeptides **2** were found to be even more potent inhibitors of the IMP-1 enzyme (Table 2). This suggested that these thioesters might be operating as substrates for IMP-1, releasing thiols **4** as the active inhibitors. Indeed, several of the thioesters in Table 1 have been shown to be efficiently hydrolyzed by the IMP-1 enzyme.³⁰ Thus, the differences in activity seen among the thioesters in Table 1 may be partially due to differences in the rate of hydrolysis by the enzyme. Interestingly, the simple acetyl and benzoyl thioesters shown in Table 2 were substantially more active than the thiodepsipeptides of Table 1, and in some cases were significantly more active than the thiols themselves. For example, the thiobenzoate **3f** was a 0.4 nM inhibitor of IMP-1 and was thus >100-fold more active than its thiol component **4c**. This suggests that at least in this case, the thioester serves as more than simply a pro-drug form of the active thiol component. The trend towards enhanced activity with a more hydrophobic R^1 substituent was

maintained in the thioesters and thiols in Table 2. A dramatic preference for the (*R*)-stereochemistry was also observed, although the magnitude of the effect was reduced with the thiols relative to the thioesters. As was the case with the thiodipeptides, the thioesters and thiols in Table 2 were much more active against IMP-1 than against CcrA, although **4b** did show significant activity against the latter enzyme.

Table 1. Inhibitory Activity of Thiodipeptides Against IMP-1 and CcrA

Compd	R ¹	R ²	IC ₅₀ (μM)		Compd	R ¹	R ²	IC ₅₀ (μM)	
			IMP-1	CcrA				IMP-1	CcrA
2a	H	Ph-	240	>1000	2t	C	HO ₂ CCH ₂ SCH ₂ -	13	--
2b	A	CH ₃ -	63	>1000	2u	C	HO ₂ C(CH ₂) ₃ -	91	--
2c	A	H ₂ C=CHCH ₂ O-	64	>1000	2v	C	PhCH ₂ -	5.8	>1500
2d	A	Ph-	20	>1000	2w	C	PhOCH ₂ -	1.0	930
2e	B	CH ₃ -	2	>1000	2x	C	PhCH ₂ CH ₂ -	15	--
2f	B	H ₂ C=CHCH ₂ O-	2.9	>1500	2y	C	(<i>E</i>)-PhCH=CH-	11	--
2g	B	Ph-	1.5	680	2z	C	PhCOCH ₂ CH ₂ -	0.25	--
2h^a	B	Ph-	430	--	2aa	C	PhCONHCH ₂ -	50	--
2i	C	CH ₃ -	18	1000	2ab	C	4-HO-PhCH ₂ -	3.1	--
2j	C	H ₂ C=CHCH ₂ O-	4.4	1000	2ac	C	4-MeO-PhCH ₂ -	12	--
2k	C	Ph-	3.6	1000	2ad	C	4-(Me ₂ N)-PhCH ₂ -	55	--
2l	C	CH ₃ CH ₂ -	9.5	>1500	2ae	C	2-BnO-PhCH ₂ -	24	--
2m	C	CH ₃ CH ₂ CH ₂ -	9.3	--	2af	C	(3-pyridyl)-CH ₂ -	32	--
2n	C	CH ₃ (CH ₂) ₃ -	4.8	--	2ag	C	(1-naphthyl)-CH ₂ -	12	>1500
2o	C	CH ₃ (CH ₂) ₄ -	13	--	2ah	C	4-MeO-Ph-	29	--
2p	C	(CH ₃) ₂ CH-	9.7	>1500	2ai	C	3-MeO-Ph-	38	--
2q	C	(CH ₃) ₂ CHCH ₂ -	5.3	--	2aj	C	3-(Me ₂ N)-Ph-	15	--
2r	C	(<i>E</i>)-CH ₃ CH=CH-	33	--	2ak	C	2,4,6-(MeO) ₃ -Ph-	46	--
2s	C	HO ₂ C(CH ₂) ₂ -	4.7	>1500	2al	C	2-naphthyl-	18	--

(a) Stereochemistry at C-2 is (*S*).

Table 2. Inhibitory Activity of Thioesters and Thiols Against IMP-1 and CcrA

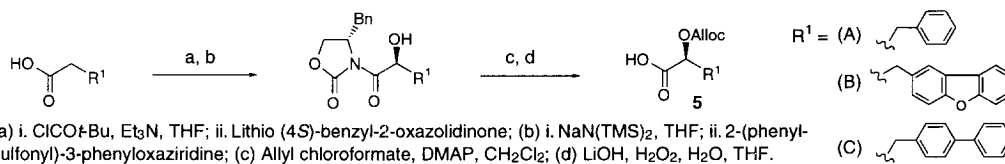
Compd	R ¹	R ⁴	R/S	IC ₅₀ (μM)		Compd	R ¹	R ⁴	R/S	IC ₅₀ (μM)
				IMP-1	CcrA					
3a	A	CH ₃ CO	R	12	>1000	13a	A	CH ₃ CO	S	740
3b	B	CH ₃ CO	R	0.045	>500	13b	B	CH ₃ CO	S	160
3c	C	CH ₃ CO	R	0.084	750	13c	C	CH ₃ CO	S	74
3d	A	PhCO	R	0.064	>1000	13d	A	PhCO	S	2.5
3e	B	PhCO	R	0.0013	>500	13e	B	PhCO	S	49
3f	C	PhCO	R	0.00041	180	13f	C	PhCO	S	120
4a	A	H	R	0.086	690	14a	A	H	S	3.7
4b	B	H	R	0.023	9.9	14b	B	H	S	0.25
4c	C	H	R	0.057	750	14c	C	H	S	1.2

Conclusions: A series of thioesters and thiols has been synthesized employing a novel solid-phase Mitsunobu reaction. These compounds were potent inhibitors of the IMP-1 metallo-β-lactamase, but were only weakly active against the CcrA enzyme. A further characterization of the properties of the inhibitors described herein, including the kinetics of inhibition and a demonstration of synergy with β-lactam antibiotics against IMP-1 producing bacteria, will be the subject of a separate report.³¹

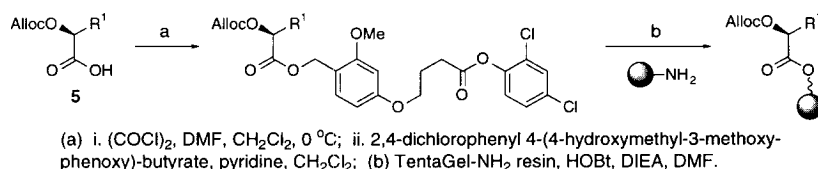
Acknowledgment: We thank Dr. James V. Heck for encouragement and helpful discussions during this work.

References and Notes

- (a) Livermore, D. M. *J. Antimicrob. Chemother.* **1998**, *41* (Suppl. D), 25. (b) Livermore, D. M. *J. Antimicrob. Chemother.* **1997**, *39*, 673.
- (a) Bush, K. *Clin. Infect. Dis.* **1998**, *27* (Suppl 1), S48. (b) Rasmussen, B. A.; Bush, K. *Antimicrob. Agents Chemother.* **1997**, *41*, 223.
- (a) Ito, H.; Arakawa, Y.; Ohsuka, S.; Wacharotayankun, R.; Kato, N.; Ohta, M. *Antimicrob. Agents Chemother.* **1995**, *39*, 824. (b) Senda, K.; Arakawa, Y.; Ichiyama, S.; Nakashima, K.; Ito, H.; Ohsuka, S.; Shimokata, K.; Kato, N.; Ohta, M. *J. Clin. Microbiol.* **1996**, *34*, 2909.
- (a) Koh, T. H.; Babini, G. S.; Woodford, N.; Sng, L.-H.; Hall, L. M. C.; Livermore, D. M. *Lancet* **1999**, *353*, 2162. (b) Cornaglia, G.; Riccio, M. L.; Mazzariol, A.; Lauretti, L.; Fontana, R.; Rossolini, G. M. *Lancet* **1999**, *353*, 899.
- (a) Concha, N. O.; Rasmussen, B. A.; Bush, K.; Herzberg, O. *Structure* **1996**, *4*, 823. (b) Carfi, A.; Duée, E.; Galleni, M.; Frère, J.-M.; Dideberg, O. *Acta Cryst.* **1998**, *D54*, 313. (c) Ullah, J. H.; Walsh, T. R.; Taylor, I. A.; Emery, D. C.; Verma, C. S.; Gamblin, S. J.; Spencer, J. *J. Mol. Biol.* **1998**, *284*, 125. (d) Fitzgerald, P. M. D.; Wu, J. K.; Toney, J. H. *Biochemistry* **1998**, *37*, 6791.
- (a) Payne, D. J.; Bateson, J. H.; Gasson, B. C.; Proctor, D.; Khushi, T.; Farmer, T. H.; Tolson, D. A.; Bell, D.; Skett, P. W.; Marshall, A. C.; Reid, R.; Ghosez, L.; Combret, Y.; Marchand-Brynaert, J. *Antimicrob. Agents Chemother.* **1997**, *41*, 135. (b) Payne, D. J.; Bateson, J. H.; Gasson, B. C.; Khushi, T.; Proctor, D.; Pearson, S. C.; Reid, R. *FEMS Microb. Lett.* **1997**, *157*, 171.
- (a) Goto, M.; Takahashi, T.; Yamashita, F.; Koreeda, A.; Mori, H.; Ohta, M.; Arakawa, Y. *Biol. Pharm. Bull.* **1997**, *20*, 1136. (b) Toney, J. H.; Fitzgerald, P. M. D.; Grover-Sharma, N.; Olson, S. H.; May, W. J.; Sundelof, J. G.; Vanderwall, D. E.; Cleary, K. A.; Grant, S. K.; Wu, J. K.; Kozarich, J. W.; Pompliano, D. L.; Hammond, G. G. *Chem. Biol.* **1998**, *5*, 185. (c) Page, M. I.; Laws, A. P. *Chem. Commun.* **1998**, 1609. (d) Walter, M. W.; Felici, A.; Galleni, M.; Soto, R. P.; Adlington, R. M.; Baldwin, J. E.; Frère, J.-M.; Gololobov, M.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2455. (e) Nagano, R.; Adachi, Y.; Hashizume, T.; Morishima, H. 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, Sept 24–27, 1998, Abstract #F55.
- These compounds were originally targeted at PBP2a, the bacterial enzyme which mediates resistance to β -lactams in MRSA. Thus, hydrophobic R¹-substituents of the type known to enhance the binding of carbapenems to PBP2a were introduced. cf: Meurer, L. C.; Guthikonda, R. N.; Huber, J. L.; DiNinno, F. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 767.
- Simple thiol compounds, including mercaptoacetic acid and mercaptopropionic acid, have previously been reported to be inhibitors of IMP-1 (ref 7a).
- Starting materials **5B** and **5C** were synthesized as shown below. Compound **5A** (R¹ = -CH₂Ph) was prepared directly from (S)-phenyllactic acid (via steps c and d below).



- Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346.
- Flörsheimer, A.; Riniker, B. In *Peptides 1990. Proceedings of the 21st European Peptides Symposium*; Giralt, E., Andreu, D., Eds; ESCOM: Leiden, 1991; pp 131–133.
- Higher resin loadings could be achieved by coupling the substrate to the HMPB linker (activated as a 2,4-dichlorophenyl ester) prior to its attachment to the resin as shown below (cf ref 14).



14. Chao, H.-G.; Bernatowicz, M. S.; Reiss, P. D.; Klimas, C. E.; Matsueda, G. R. *J. Am. Chem. Soc.* **1994**, *116*, 1746.
15. Alloc-D-thioalanine was prepared from alloc-D-alanine by the method described in the following ref: Yamashiro, D.; Li, C. H. *Int. J. Peptide Protein Res.* **1988**, *31*, 322.
16. (a) Volante, R. P. *Tetrahedron Lett.* **1981**, *22*, 3119. (b) Strijteven, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664.
17. (a) Campbell, D. A.; Bermak, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 6039. (b) Richter, L. S.; Gadek, T. R. *Tetrahedron Lett.* **1994**, *35*, 4705.
18. (a) Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibé, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741. (b) Thieriet, N.; Alsina, J.; Giralt, E.; Guibé, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275.
19. Other means of acyl-activation that were examined with less success included: DIC/HOBT, PyBOP/DIEA, CDI and EtCO₂Cl/DIEA as well as use of pre-formed Ac-OPfp and Ac-OSu esters.
20. Use of N-*t*-butyl-N'-ethylcarbodiimide gives a soluble urea by-product which is compatible with the solid-phase reaction; cf Izdebski, J.; Pawlak, D. *Polish J. Chem.* **1994**, *68*, 403.
21. With deactivated anhydrides (cf entries 2ah and 2ak, Table 1) varying amounts of unacylated amine and N-allylamine by-products were observed.
22. HPLC conditions: Column, LiChrospher 100 RP-18 (5 μ m), 4 x 250 mm; Mobile phase A = MeCN, mobile phase B = 0.1% TFA in H₂O, isocratic elution with A:B ranging from 50:50 to 75:25; Flow rate, 1.0 mL/min; UV detection at 210 nm.
23. Satisfactory ¹H NMR (500 MHz) and MS data were obtained for all compounds described herein.
24. A representative procedure for the Mitsunobu reaction: A 0.950 g (0.228 mmol) portion of biphenyl resin **6C** (R¹ = -CH₂-*p*-biphenyl) was swelled with 7 mL of dry THF under N₂ and then washed 4 x 5 mL with dry THF. In a separate flask tris(4-chlorophenyl)phosphine (0.417 g, 5.0 equiv) was dissolved in 4 mL of THF, cooled to 0 °C and diisopropyl azodicarboxylate (0.224 mL, 5 equiv) was added dropwise during 5 min. The cooling bath was removed and the yellow solution was stirred for 15 min. Recrystallized alloc-D-thioalanine dicyclohexylamine salt (0.422 g, 5 equiv) was added and it dissolved with stirring during 2–3 min. The solution was added to the above drained resin and the reaction was mixed for 1.5 h. The solution was drained and the resin was washed with THF (3x), DMF (3x), THF (3x), MeOH (3x) and CH₂Cl₂ (5x) and then dried in vacuo. A small portion of resin was cleaved with 5% TFA/CH₂Cl₂ and the resulting product analyzed by HPLC which indicated a purity of >97%.
25. A representative procedure for the transacylation reaction: A 25 mg (0.0060 mmol) portion of the above resin (**8C**) was swelled with 0.5 mL of dry CH₂Cl₂ under N₂ and then washed 3 x 0.5 mL with dry CH₂Cl₂. To the drained resin was added 0.1 mL of a 0.5 M solution of anhydride in CH₂Cl₂ (8 equiv) [freshly prepared from 3-phenylpropionic acid (158 mg, 1.05 mmol) and N-*t*-butyl-N'-ethylcarbodiimide (77.5 μ L, 0.50 mmol) in 1.0 mL of CH₂Cl₂ for 1 h]. This was followed after 1 min by addition of 0.1 mL of a CH₂Cl₂ solution containing 0.25 equiv of Pd(PPh₃)₄, 0.5 equiv of PPh₃ and 5 equiv of PhSiH₃. The reaction was allowed to proceed for 1 h, mixing periodically, and some gas evolution was observed. The resin was drained and washed with CH₂Cl₂ (3x), 2% Et₂NCS₂Na/NMP (1x), DMF (3x), THF (3x), MeOH (3x), and CH₂Cl₂ (5x). The product was cleaved from the resin with 5% TFA/CH₂Cl₂ (5 x 0.25 mL) and the solution evaporated to give 2.7 mg of **2x** as an oil (97% yield, 98% HPLC purity).
26. Hof, R. P.; Kellogg, R. M. *J. Chem. Soc. Perkin Trans. 1* **1995**, 1247.
27. The 50% inhibitory concentration (IC₅₀) for each compound was determined following a 15 min incubation at 37 °C with either IMP-1 (0.75 nM in 50 mM pH 7 MOPS, 2 mM CHAPS) or CcrA (4 nM in 50 mM pH 7 MOPS). Using initial velocity as a measure of activity, inhibition was monitored spectrophotometrically at 490 nm in a Molecular Devices SPECTRAMax™ 250 96-well plate reader employing nitrocefin as the reporter substrate at approximately K_m concentrations (60 μ M and 18 μ M for IMP-1 and CcrA, respectively). For the enzyme preparations see ref 28 (CcrA) and ref 31 (IMP-1).
28. Toney, J. H.; Wu, J. K.; Overbye, K. M.; Thompson, C. M.; Pompliano, D. L. *Protein Expr. Purif.* **1997**, *9*, 355.
29. Prepared from benzoyl-D-alanine and mercaptoacetic acid: Grais, H.-J. *Angew. Chem. Int. Ed. Eng.* **1977**, *16*, 244.
30. Established both semi-quantitatively by HPLC analysis (ref 22) and quantitatively using Ellman's reagent to measure thiol formation spectrophotometrically (ref 31). Background chemical hydrolysis of these thioesters was found to be negligible under the conditions of the enzyme assay.
31. Hammond, G. G.; Huber, J. L.; Greenlee, M. L.; Laub, J. B.; Young, K.; Silver, L. L.; Balkovec, J. M.; Pryor, K. D.; Wu, J. K.; Leiting, B.; Pompliano, D. L.; Epstein-Toney, J. H. *FEMS Microb. Lett.* **1999**, in press.