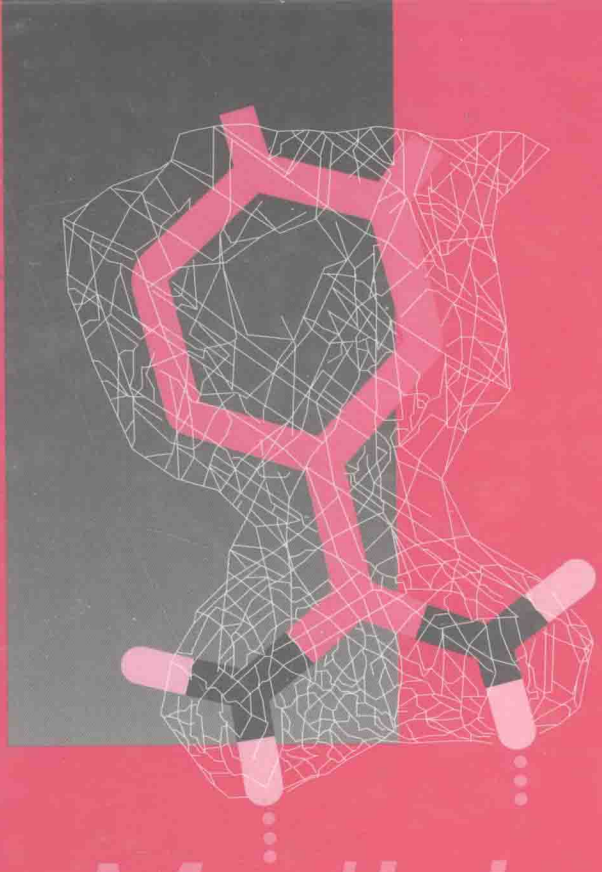


RS•C
ROYAL SOCIETY OF CHEMISTRY

Medicinal Chemistry

INTO THE MILLENNIUM

edited by MALCOLM M. CAMPBELL
and IAN S. BLAGBROUGH

Medicinal Chemistry into the Millennium

Edited by

M.M. Campbell
University of Bath, UK

I.S. Blagbrough
University of Bath, UK

RS•C
ROYAL SOCIETY OF CHEMISTRY

Proceedings of the European Federation of Medicinal Chemistry Symposium held in Edinburgh on 6–11 September, 1998.

Special Publication No. 264

ISBN 0-85404-769-7

A catalogue record for this book is available from the British Library

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK
Registered Charity No. 207890

For further information see our web site at www.rsc.org

Printed by Athanaeum Press Ltd, Gateshead, Tyne and Wear, UK

Preface

The XVth ISMC continued the now well established tradition of biennial medicinal chemistry symposia organised throughout Europe by the hosting Society under the auspices of the European Federation of Medicinal Chemistry. Judged by its multinational participation (over 1000 delegates from 48 different countries) and effective blend of industrialists, academics and students, Edinburgh undoubtedly enjoyed the success of preceding symposia and contributed to their international prestige. Comprising nine major themes, it is impossible to single out just one that is of particular importance, although supporting technologies, metabolism and pharmacokinetics studies underpin many of the advances made in specific therapeutic areas and are thus crucial to the successful exploitation of the plethora of newly discovered molecular targets.

The New Technologies in Drug Discovery session is particularly relevant to the pharmaceutical industry, and indeed all industries dependent on the discovery of biologically active molecules (*e.g.* pesticides, flavours and fragrances). With increasing numbers of molecular targets derived through genomic sequencing there has been a strong drive to enrich the quality and range of chemical libraries used to generate new leads, while at the same time enhancing the rate at which such libraries can be screened and hit molecules validated. Techniques such as library design, construction, analysis, quality control, robotisation, high throughput screening and informatics all feature strongly in modern lead identification.

Accompanying the New Technologies sessions were seven themes devoted to structurally distinct molecular targets. Such a selection can never be exhaustive, but those targets chosen cover areas perceived by the pharmaceutical industry to be therapeutically important. Undoubtedly a considerable amount of new data were presented during these and their supporting poster sessions, effectively contributing to the dissemination of current knowledge. While the Growth Factor and Glycochemistry and Glycobiology areas are less well established than the others, they are by no means less relevant to emerging therapeutic targets. It is perhaps in these areas that understanding at the molecular level is lacking, as opposed to the more established structural types where extensive research has paid significant dividends.

Having identified good leads on novel molecular targets one of the major problems confronting the pharmaceutical industry is the need to convert these into viable drugs. Drug metabolism and pharmacokinetics (DMPK) have always been a limitation in drug development, primarily because they lacked predictivity and were traditionally investigated late in the discovery programme. There is now a strong drive to develop predictive DMPK paradigms and to use these effectively early in the discovery process. The ninth session addressed some of the more pertinent problems and afforded insights into a number of the available solutions.

A large number of people contributed to the success of this symposium and it is not possible to thank them all. One person though, who through illness was forced to relinquish his chairmanship late in the organisational stage, does deserve a specific mention. Professor Malcolm Campbell worked hard to make Edinburgh a reality and its success is largely due to his insights and tremendous effort.

Derek Buckle
Chairman of the Organising Committee

Contents

Growth Factors

Intracellular Signalling

Protease Inhibition

Glycochemistry and Glycobiology

Nitric Oxide Synthase Inhibition

Predicting DMPK

New Technologies for Drug Discovery

DESIGN OF MACROCYCLIC PEPTIDASE INHIBITORS: THE RELATED ROLES OF STRUCTURE-BASED APPROACHES AND LIBRARY CHEMISTRY

Paul A. Bartlett,* Naeem Yusuff, Hyung-Jung Pyun, Alice C. Rico, J. Hoyt Meyer, Whitney W. Smith and Matthew T. Burger

Department of Chemistry, University of California, Berkeley, California 94720-1460

1 INTRODUCTION

New and improved approaches to the discovery and invention of biologically active compounds follow each other in rapid succession. Rational design approaches have at various times encompassed strategies that involve covalent and irreversible inactivation of receptors or enzymes with affinity labels, that exploit enzyme mechanism with suicide inhibitors and transition state analogs, or that rely on 3-dimensional characterization of the binding site or ligand for structure-based design. The currently fashionable combinatorial or library-based approaches would appear to represent a swing of the pendulum away from these "rational" methods. However, strategies that share a common goal are inevitably going to find common ground. There are elements of logic and serendipity in all of these approaches: mechanistic and structural insight can be used to advantage in the design of combinatorial libraries and the interpretation of screening results, and library screening can provide a key starting point in a structure-based optimization process. As we seek to demonstrate in this overview, one approach often sets the stage for another, and the newer strategies can provide insights and tools that enable the older methods to be applied more effectively.

2 MACROCYCLIC PEPTIDASE INHIBITORS

Our evolving interest in transition state analogs of peptidase inhibitors illustrates how these ideas can be applied to the design of potent inhibitors and used to understand some of the fundamental principles of protein-ligand binding. The underlying tenet of transition state analogy is embodied in Equations 1 and 2 (where TS and GS refer to transition and ground states, respectively).[1]

$$K_{TS}=(K_{GS}/k_{cat})k_{noncat} \qquad \text{Equation 1}$$

$$\log K_I \propto \log K_{TS} = \log(K_m/k_{cat}) + \text{constant} \qquad \text{Equation 2}$$

Linear relationships between inhibitor K_i and substrate K_m/k_{cat} values have enabled us to demonstrate transition state mimicry by phosphonamidate and phosphonate peptide inhibitors of zinc peptidases, such as thermolysin[2-4] and carboxypeptidase A,[5-7] and the aspartic peptidase pepsin[8,9] (Figure 1). The potency of these inhibitors, in one case reaching 11 fM,[6] and their conformational and electronic similarities to the transition state structures have made them valuable subjects for crystallographic analysis.[10-15] As a result, we have extensive structural information on how they bind to the target enzymes.

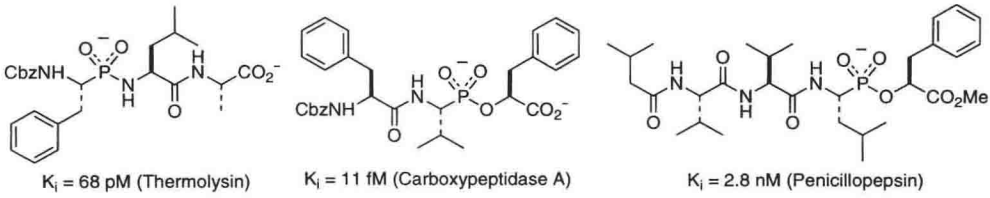


Figure 1 *Representative phosphorus-containing peptidase inhibitors*

2.1 Macrocyclic Inhibitors of Thermolysin

In the bound conformations of phosphorus-containing inhibitors of thermolysin, represented by the complex with Cbz-Phe^P-Leu-Ala (K_i = 68 pM),[11] both the P1 side chain and the terminal carboxylate at P2' approach each other near the opening of the active site (Figure 2a). Their proximity stimulated us to design a rigidified analog in which the α-carbons at P1 and P2' are bridged by a bicyclic chroman unit.[16] This compound, **1**, is a potent inhibitor of thermolysin and binds as anticipated, with the aromatic ring spanning the narrow opening of the active site (Figure 2b). However, an attempt to determine how much of the binding enhancement for this analog (relative to an unbridged phosphonamidate **3**) arises from conformational constraint and how much is due to interactions between the enzyme and the bridging unit itself was undermined by the finding that the acyclic comparison compound **2** binds differently than **1**, with the bicyclic moiety rotated out of the active site (Figure 2c).

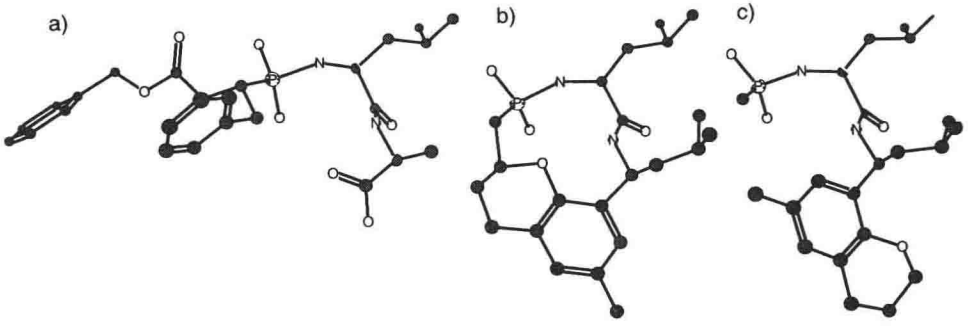
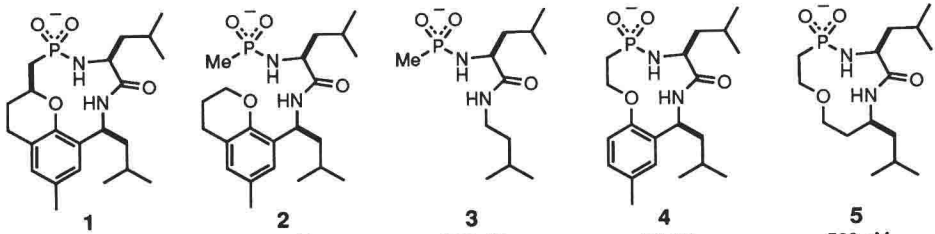


Figure 2 *Conformations of macrocyclic and acyclic inhibitors bound to thermolysin. a) Cbz-Phe^P-Leu-Ala; b) rigid tricycle **1**; c) seco-analog **2***



1 4 nM
2 80 nM
3 200 nM
4 50 nM
5 500 nM
K_i (Thermolysin)

The complication of alternative binding modes has been minimized in the series of three macrocycles **1**, **4**, and **5**, which span two orders of magnitude in binding affinity but differ only in the size and conformational flexibility of the bridging units. For this series of inhibitors, we have been able to distinguish the relative contributions that conformational mobility (or lack thereof) and hydrophobic interactions make to the differences in binding affinity. The bound conformations of the three analogs have been determined by Debbi Holland and Doug Juer in Brian Matthews' group in Oregon; **1** and **4** are essentially identical, except for the two-carbon segment that is missing from the latter (Figure 3a). The ring of **5** is slightly different because sp^3 hybridization and an additional degree of rotational freedom allow a more puckered conformation and a different rotamer of the isobutyl substituent corresponding to P2' (Figure 3b).

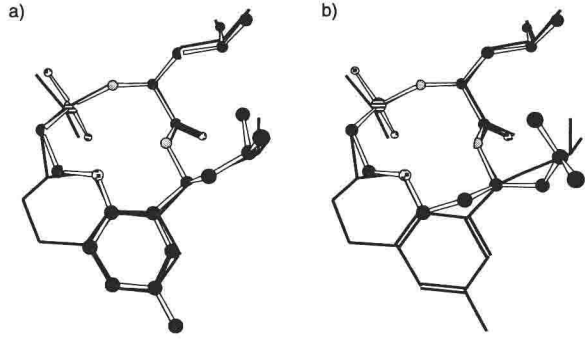


Figure 3 *Conformations of macrocyclic inhibitors bound to thermolysin; comparison of **1** (line) with a) **4** and b) **5** (ball-and-stick)*

The conformational properties of the three cyclic analogs were determined in solution, using a combined NMR and modeling method.[17] The findings are understandable, but nonetheless striking. The tricyclic analog **1** is essentially rigid, adopting the same conformation in solution that it does in the thermolysin active site (Figure 4a). The bicyclic derivative **4** has two comparable, low-energy conformations, differing at the O–C–C–P dihedral angle (Figure 4b); one corresponds to the bound conformation. Finally, the monocycle **5** is highly flexible in solution, particularly in the region of the bridging chain; a sample of low-energy conformers is shown in Figure 4c.

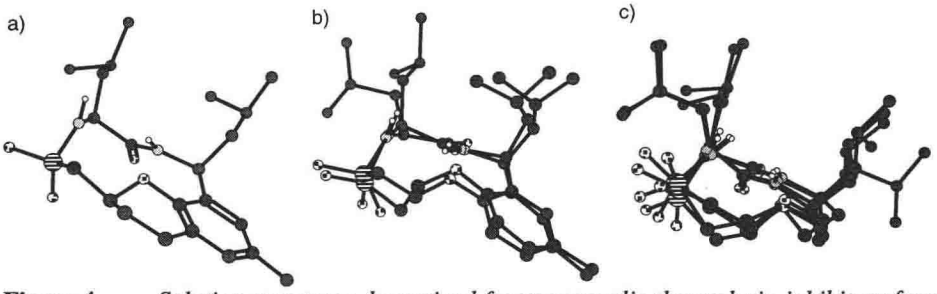


Figure 4 *Solution structures determined for macrocyclic thermolysin inhibitors from NMR and modeling: a) single conformation for **1**, b) two conformations for **4**; c) ensemble of low-energy conformations for **5***

The hydrophobic contributions to the differences in binding affinities of the three macrocycles were assessed through modeling as well as experimentally. The amount of hydrophobic surface area, on both enzyme and inhibitor, removed from contact with solvent on association was readily determined from the crystal structures of the native and inhibited enzymes (Table 1). The results are striking and unequivocal: while **1** and **4** differ slightly in size, there is almost the same reduction in exposed surface area when they bind to thermolysin. Since the extra ethylene unit of **1** does not contact the enzyme, it is exposed in both the bound and unbound states and thus does not contribute to the binding interaction. In contrast, monocycle **5** has significantly less contact with the enzyme than **1** or **4**; indeed, the reduction in hydrophobic contact surface for **1** represents a loss of 2.3 kcal/mole in binding affinity, much more than the observed difference of 1.4 kcal/mole versus **4** or **5**.

Table 1 *Calculations of Surface Area Buried on Binding*

Inhibitor Complex	Surface area of I ($Å^2$)	Surface area of E ($Å^2$)	Surface area of E·I complex ($Å^2$)	$\Delta Å^2$ (E+I → E·I)	$\Delta\Delta Å^2$	$\Delta\Delta G°$ (kcal/mole)[a]
1	653.6	12703.8	12565.2	–792		
					2.5	<0.1
4	641.1	12703.8	12555.2	–790		
					97.1	2.3
5	544.4	12703.8	12555.5	–693		

[a] Calculated on the basis of 24 cal/mole/$\Delta Å^2$.[18]

Breslow's recent use of solvation effects to probe hydrophobic contact surfaces in organic reaction transition states[19] led us to attempt a similar approach to assess hydrophobic binding contributions experimentally. The inhibition constants for the three cyclic inhibitors, plus an acyclic phosphinate, Cbz-Gly^P-(C)Leu-Ala, were determined in the presence of increasing concentrations of ethanol, up to 12%. As the water content of the solvent decreases, the hydrophobic contributions to binding become less significant,

and the binding becomes weaker. This effect is quite dramatic, leading to more than a 10-fold increase in K_i for the bi- and tricyclic inhibitors in the range 0-9% ethanol (Figure 5).

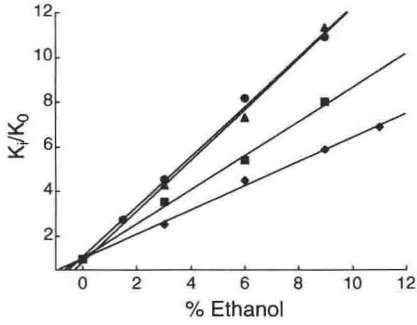


Figure 5 *Effect of solvent composition on inhibitor binding to thermolysin:* ● = *tricycle* ***1***, ▲ = *bicycle* ***4***, ■ = *monocycle* ***5***, ♦= *Cbz-Gly*^{P}*-(C)Leu-Ala*

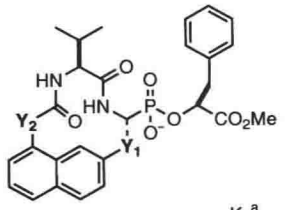
Importantly, the effect is less prominent for the monocyclic and acyclic analogs. There is thus a remarkable correlation between the modeling results of Table 1 and the experimentally observed "anti-hydrophobic" solvent effect.

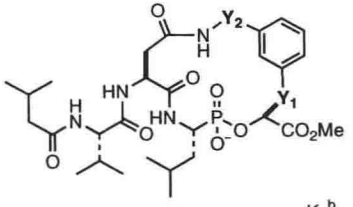
From these results, it is clear that there is no hydrophobic contribution to the difference in binding affinities between **1** and **4**; the factor of 10 therefore arises entirely from the increased conformational flexibility of **4**, and the possibility that the bound conformation is higher in energy than the other one observed. (However, no significant difference in energy between the two conformers of the ring systems of **4** is apparent from the modeling,) For the monocyclic inhibitor **5**, the reduced hydrophobic contribution accounts for *more* than the observed difference with **4**, even before the added conformational flexibility of **5** is taken into account. This conundrum is explained by the different conformations that the inhibitors adopt around the P2' units in the active site, which result in different interactions with protein. The orientation of the isobutyl side chain of the more flexible inhibitor **5** must lead to an interaction that is more favorable than those of **1** and **4**, largely compensating for the differences in size and flexibility.

2.2 Macrocyclic Inhibitors of Penicillopepsin

Although the zinc and aspartic peptidases differ in mechanism and active site configurations, they both catalyze direct addition of water to the peptide linkage and are potently inhibited by phosphonate analogs. As described above for thermolysin, the structure of the complex between penicillopepsin and the phosphonate inhibitor, isovaleryl-Val-Val-Leu^P-(O)Phe-OMe (K_i = 2.8 nM),[15] provided the foundation for design of more rigid derivatives and another opportunity to assess the quantitative value of this approach. Since the peptide analog adopts an extended conformation in the active site, alternate side chains adopt parallel orientations and thus can be linked in macrocyclic structures. The program CAVEAT[20] was used to identify molecular fragments that could bridge from the P3 to the P1 side chains, and from P2 to P1', without bumping into the protein. For the P2-P1' linked derivative, a variety of designs were evaluated to ensure that the desired conformer was favored in the low energy population. Two macrocycles were designed and synthesized: **6**, bridged between the P3 and P1 side chains,[21] and **9**, bridged between the P2

and P1' positions,[22] along with acyclic comparison compounds from which a methylene group had been removed. Relative to the macrocycles, these *seco* analogs have only slightly greater surface area and can more readily adopt similar conformations.





	K_i [a]
6: $Y_1 = Y_2 = CH_2$	800 nM
7: $Y_1 = CH_2$; Y_2 = H,H	7,600 nM
8: Y_1 = H,H; $Y_2 = CH_2$	110,000 nM

	K_i [b]
9: $Y_1 = Y_2 = CH_2$	0.1 nM
10: $Y_1 = CH_2$; Y_2 = H,H	42 nM
11: Y_1 = H,H; $Y_2 = CH_2$	1,300 nM

K_i (Penicillopepsin): [a] pH 3.5, [b] pH 4.5

Within each set of compounds, the observed binding affinities reflect the relative conformational flexibility expected: the macrocycles are the most potent, and among the acyclic analogs, the least-branched are the weakest. However, both the absolute affinity and the impact of the macrocyclic constraint are most pronounced for the P2-P1' linked analog **9**, which is much more potent than **6** and 3-4 orders of magnitude more tightly bound than the acyclic derivatives **10** and **11**.

The solution conformations of both **6** and **9** were determined (Figure 6); each appears to be well-defined, with the peptide backbone closely approximating the bound conformation of the original inhibitor (isovaleryl-Val-Val-Leu^P-(O)Phe-OMe). However, while the conformation found for **9** is very similar to the design model, in **6**, the naphthalene ring is in a different orientation than originally envisaged, up and out of the plane of the macrocycle. In this conformation, the naphthalene ring of **6** could not fit into the P3-P1 binding pocket: either the macrocycle or the protein would have to deform in order for it to bind, which explains why **6** is such a weak inhibitor.

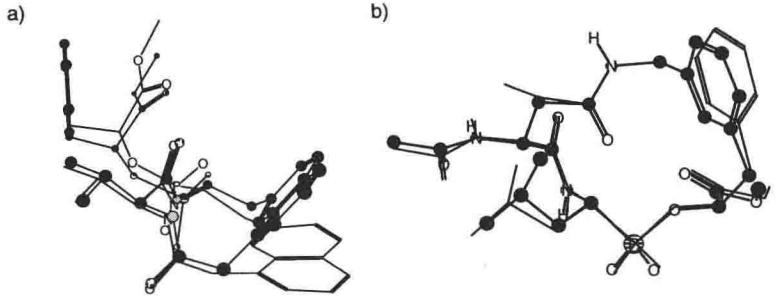


Figure 6 *Solution conformations of macrocycles **6** and **9**; a) comparison of original model (line) of **6** bound to enzyme with solution conformation (ball-and-stick) determined from NMR and modeling; b) comparison of solution conformation of **9** (ball-and-stick) with bound conformation of isovaleryl-Val-Val-Leu^P-(O)Phe-OMe (line)*

The structures of the penicillopepsin complexes of **6**, **7**, and **8**,[23] and of **9** and **10**,[24] reveal both exciting and unanticipated results, and enable us to make sense out of the binding data. First, the bound conformation of the P3-P1-linked analog **6** is identical to that determined in solution: it is the *protein* that deforms to accommodate the unfavorable shape! The mobile flap that usually closes down over the active site when substrates and inhibitors bind is forced upward by the naphthalene ring, reducing the favorable contacts with the rest of the inhibitor and explaining the poor affinity of the analog. In the acyclic derivative **7**, the naphthalene ring is not constrained in a macrocycle and can swing into a more favorable position in the binding pocket, thus allowing the protein flap to close into its usual position (Figure 7a). As a result, the active site interactions for **7** are considerably improved and the loss of binding affinity from conformational flexibility is largely overcome. The second surprise was the completely unexpected orientation found for the other acyclic analog, **8**, which binds out of register, with the phosphonate located where the P2-P1 peptide bond should be (Figure 7b). Since every interaction between inhibitor and enzyme is different for **8** than for the other analogs, its binding affinity cannot be related to those of the others. It is still unclear why **8** adopts this orientation; while it is hard to fathom why this abnormal position is more stable, it is unlikely that the normal orientation was blocked kinetically, since the complex was formed by co-crystallization.

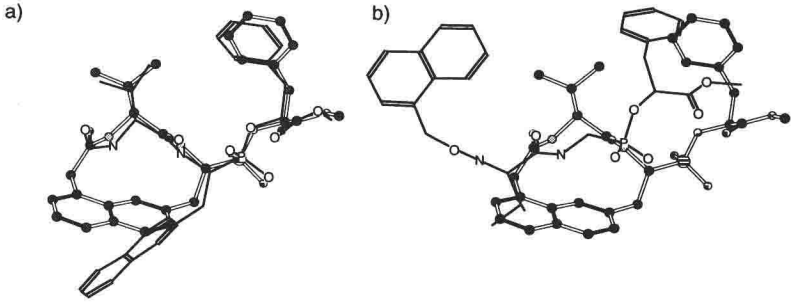


Figure 7 *Conformations of macrocycle **6** (ball and stick) and acyclic comparison compounds (line) a) **7** and b) **8** bound to penicillopepsin*

The recent results from structural analysis of the penicillopepsin complexes of macrocycle **9** and the acyclic comparison **10** are much less ambiguous (Figure 8).[24] The bound and unbound conformations of **9** are very similar, but in this case, it is the inhibitor that deforms on interaction with the enzyme. The meta-substituted aromatic ring of the P1' residue is displaced slightly toward the plane of the macrocycle on binding, and the amide linkage between the P2 and P1' sidechains is rotated 1800 (Figure 8a); however, the peptide backbone and the P2 side chain are essentially unaffected. The energy penalty for this deformation is modest; the two amide rotamers differ by less than one kcal/mole.