

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/224868035>

Pentacycloundecane-diol-Based HIV-1 Protease Inhibitors: Biological Screening, 2 D NMR, and Molecular Simulation Studies

ARTICLE in CHEMMEDCHEM · JUNE 2012

Impact Factor: 2.97 · DOI: 10.1002/cmde.201100512 · Source: PubMed

CITATIONS

13

READS

68

10 AUTHORS, INCLUDING:



[Sachin Ambadas Pawar](#)

GVK Bio

12 PUBLICATIONS 35 CITATIONS

[SEE PROFILE](#)



[Katja Petzold](#)

Karolinska Institutet

21 PUBLICATIONS 260 CITATIONS

[SEE PROFILE](#)



[Per I Arvidsson](#)

Karolinska Institutet

121 PUBLICATIONS 2,410 CITATIONS

[SEE PROFILE](#)



[Gert Kruger](#)

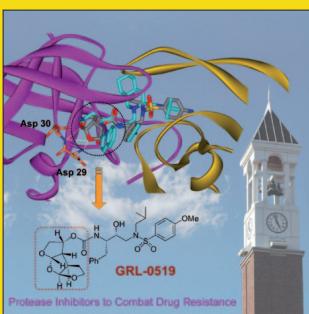
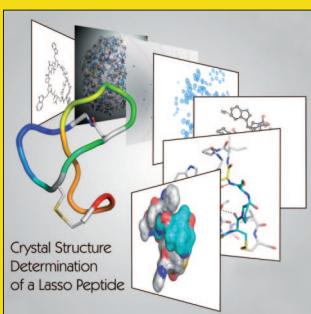
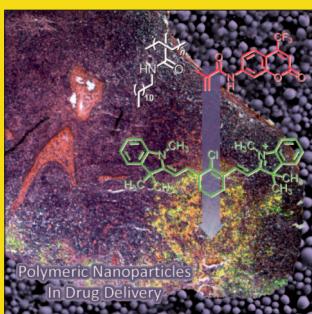
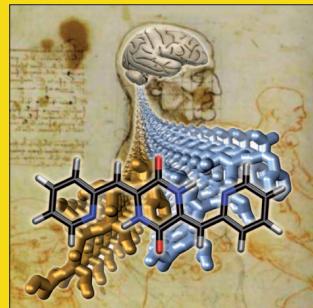
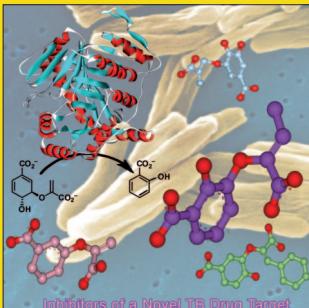
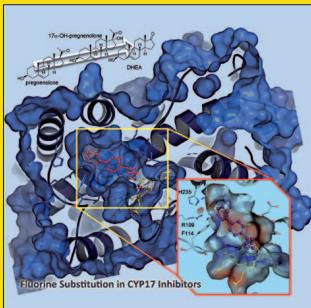
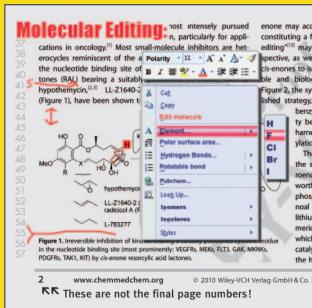
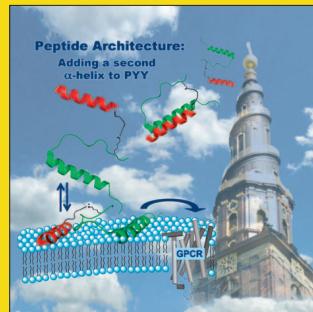
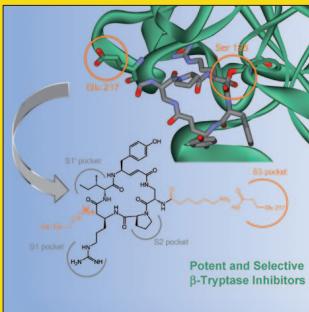
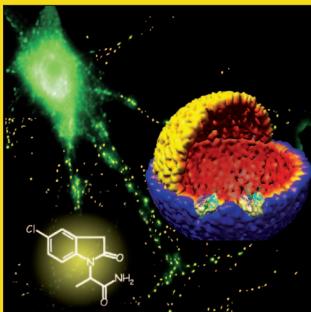
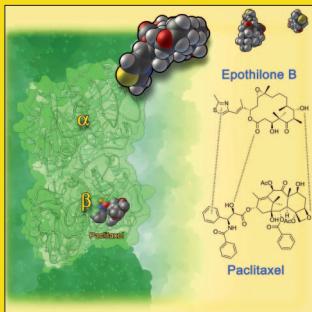
University of KwaZulu-Natal

302 PUBLICATIONS 1,603 CITATIONS

[SEE PROFILE](#)

CHEM MED CHEM

CHEMISTRY ENABLING DRUG DISCOVERY



Reprint

© Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



ChemPubSoc
Europe

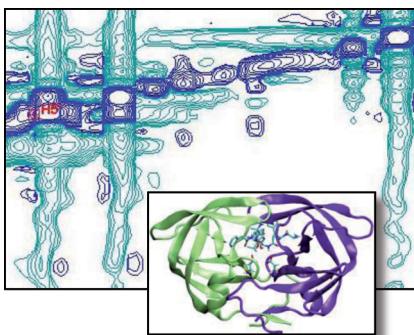
WILEY-VCH

Table of Contents

B. Honarpourvar, M. M. Makatini,
S. A. Pawar, K. Petzold, M. E. S. Soliman,
P. I. Arvidsson, Y. Sayed, T. Govender,
G. E. M. Maguire,* H. G. Kruger*

1009 – 1019

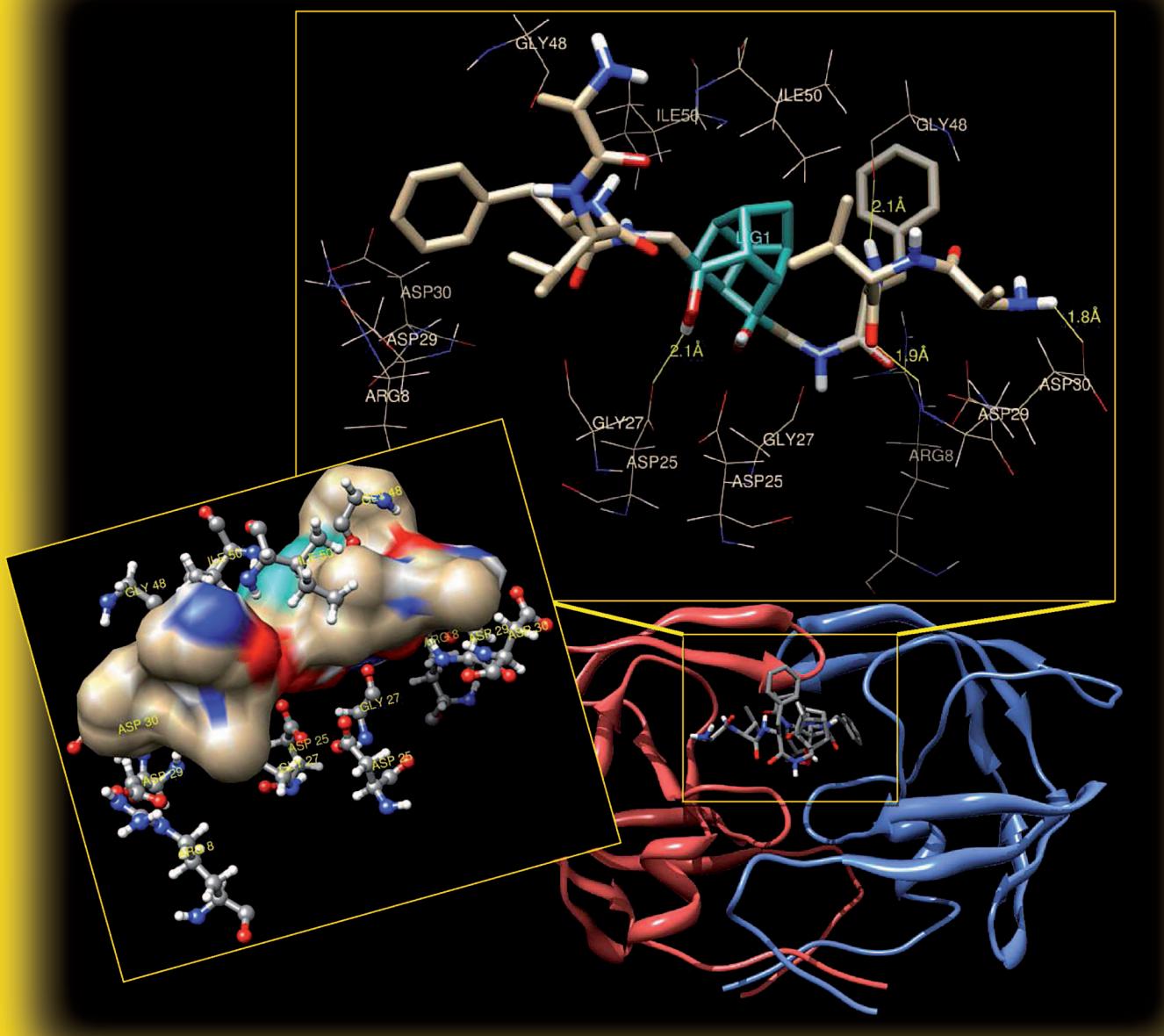
Pentacycloundecane-diol-Based HIV-1
Protease Inhibitors: Biological
Screening, 2D NMR, and Molecular
Simulation Studies



Cage peptides unleashed: The combination of drug design with in vitro assays, NMR techniques, and molecular modeling has enabled us to rationalize the observed inhibitory activities toward HIV protease by the various cage peptides in this series. EASY-ROESY NMR data show that the entire range of inhibitors exhibit a relatively stable interaction between the cage side chain and the cage protons.

CHEM MED CHEM

CHEMISTRY ENABLING DRUG DISCOVERY



6/2012

A Journal of



ChemPubSoc
Europe

The inside cover picture shows conserved hydrogen bonds between the pentacycloundecane (PCU) cage peptide and catalytic triad (Asp25, Ile26, Gly27) of C-South African (C-SA) HIV-1 protease predicted by computational techniques. Three inhibitors identified display promising IC₅₀ values against the target, with toxicity toward human MT-4 cells significantly lower than current therapies. For more details, see the Full Paper by Glenn E. M. Maguire, Hendrik G. Kruger et al. on p. 1009 ff.

www.chemmedchem.org

WILEY-VCH

Pentacycloundecane-diol-Based HIV-1 Protease Inhibitors: Biological Screening, 2D NMR, and Molecular Simulation Studies

Bahareh Honarpourvar,^[a] Maya M. Makatini,^[b] Sachin A. Pawar,^[b] Katja Petzold,^[a] Mahmoud E. S. Soliman,^[b, c] Per I. Arvidsson,^[b, d, e] Yasien Sayed,^[f] Thavendran Govender,^[b] Glenn E. M. Maguire,^{*[a]} and Hendrik G. Kruger^{*[a]}

Novel compounds incorporating a pentacycloundecane (PCU) diol moiety were designed, synthesized, and evaluated as inhibitors of the wild-type C-South African (C-SA) HIV-1 protease. Seven compounds are reported herein, three of which displayed IC₅₀ values in the 0.5–0.6 μM range. The cytotoxicity of PCU cage peptides toward human MT-4 cells appears to be several orders of magnitude less toxic than the current antiviral medications ritonavir and lopinavir. NMR studies based on the observed through-space ¹H, ¹H distances/contacts in the EASY-ROESY spectra of three of the considered PCU peptide inhibi-

tors enabled us to describe their secondary solution structure. Conserved hydrogen bonding interactions were observed between the hydroxy group of the PCU diol inhibitors and the catalytic triad (Asp25, Ile26, Gly27) of HIV protease in docking and molecular dynamics simulations. The biological significance and possible mode of inhibition by PCU-based HIV protease inhibitors discussed herein facilitates a deeper understanding of this family of inhibitors and their potential application to a vast number of alternative diseases related to proteases.

Introduction

The ease of transmittance and fatal prognosis of human immunodeficiency virus (HIV) infections demands new and effective antiretroviral therapies.^[1] A well-known method concerns the design and synthesis of HIV protease inhibitors^[2] based on the transition-state analogue approach, which has produced various highly potent peptide inhibitors of HIV protease in recent years.^[3–6] These compounds mimic the transition state of natural peptide substrates of the protease, and the key interaction is with the carboxyl groups of the catalytic aspartic acid residues of this enzyme.^[7] HIV protease is a C₂-symmetric active homodimer responsible for the cleavage of gag and gag-pol polyproteins into functional constituent proteins. Thus, proper activity of this enzyme in the viral cycle is crucial for the assembly and maturation of polyproteins to produce infectious virions.^[8,9] Inactivation of the protease results in the production of noninfectious virions and a consequent decrease in the spread of the infection to susceptible cells.^[10] A strategy for inhibiting HIV protease has been to prepare C₂-symmetric inhibitors in the form of peptidomimetics. One family of active compounds are symmetric 1,2-diols flanked by short peptide units, such as A-75925 and A-74704 (Figure 1).^[11,12] This family of inhibitors were designed after the hydroxyethylene isosteres provided the first highly potent inhibitors against HIV measured in a cell-based assay.^[13–15] It was found that the contribution from the second hydroxy group is negligible, as suggested by similar binding affinities of hydroxyethylene- and dihydroxyethylene-based transition-state analogues.^[16] Crystallographic studies showed that these diol inhibitors form hydrogen bonds

with the aspartic acid residues in the active site of the enzyme.^[17,18]

In spite of recent progress in the development of therapeutics for the treatment of acquired immune deficiency syndrome (AIDS), a number of problems persist with most synthetic antiretroviral peptidomimetic drugs. They suffer from

[a] Dr. B. Honarpourvar, Dr. K. Petzold, Dr. G. E. M. Maguire, Prof. H. G. Kruger

School of Chemistry

University of KwaZulu-Natal, Durban 4001 (South Africa)

E-mail: kruger@ukzn.ac.za

Maguireg@ukzn.ac.za

[b] Dr. M. M. Makatini, S. A. Pawar, Dr. M. E. S. Soliman, Prof. P. I. Arvidsson,

Dr. T. Govender

School of Pharmacy and Pharmacology

University of KwaZulu-Natal, Durban 4001 (South Africa)

[c] Dr. M. E. S. Soliman

Department of Pharmaceutical Organic Chemistry

Zagazig University, Zagazig 44519 (Egypt)

[d] Prof. P. I. Arvidsson

Organic Pharmaceutical Chemistry, Uppsala Biomedical Centre

Uppsala University, Box 574, 751 23 Uppsala (Sweden)

[e] Prof. P. I. Arvidsson

Project Management, CNSP iMed, AstraZeneca R&D Södertälje

Västra Mälarehamnen 9, 151 85 Södertälje (Sweden)

[f] Dr. Y. Sayed

Protein Structure–Function Research Unit

School of Molecular and Cell Biology

University of the Witwatersrand, Wits 2050 (South Africa)

 Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/cmdc.201100512>: detailed experimental procedures and analytical data; docked inhibitor–enzyme complex results and specific 3D peptide structures in .PDB file format.

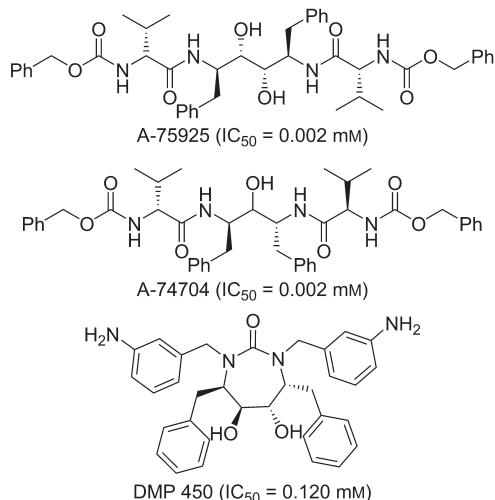


Figure 1. Structures of C₂-symmetric 1,2-diol inhibitors A-75925,^[16] A-74704,^[16] and DMP 450.^[35]

low membrane permeability, which enhances degradation to form toxic by-products, and due to mutations of the virus, these drugs have also lost activity.^[10,19,20] Thus, there is still an urgent demand for the design of novel HIV drugs, particularly for those with improved membrane permeability.^[11]

Over the past few years, it was postulated that the incorporation of polycyclic hydrophobic cage frameworks into biologically active molecules would enhance their activity^[21–24] and retard biodegradation.^[25,26] Various cage systems have shown biological activity^[22,27–29] and enhanced drug transport across cell membranes.^[22,30–32]

In our previous reports,^[33,34] a pentacycloundecane (PCU) lactam was used as a non-cleavable transition-state analogue. The most active PCU-lactam-based inhibitor, PCU-Glu-Ala-Ile-Ser (or PCU-EAIS) exhibited an *in vitro* IC₅₀ value of 0.078 μM against HIV protease and it exhibited negligible cytotoxicity toward human cells. We wanted to see if more potent HIV protease cage peptide inhibitors could be designed with alternative PCU compounds. This study also investigates the effect of protecting the terminal amino acid residue with protecting groups of various solubilities. Benzyloxycarbonyl (Cbz) and 2-pyrimidinylethanethioic acid (2-Peta) groups were used for this purpose.

Variation of activity due to the regiospecificity of cage-derived compounds is well documented.^[22,36–39] A number of chiral cage bioactive compounds have been reported, but they did not exhibit enantioselective-based activity.^[40,41] We recently reported^[33,34] a family of PCU lactam^[42–45] peptides, of which some (containing opposite cage lactam enantiomers) varied considerably in their inhibitory activities toward HIV protease. The cage lactam enantiomers exhibited different peptide side chain conformational preferences. It was shown that the cage peptides with the most stable conformations, experiencing significant through-space proton–proton nuclear Overhauser effect (NOE) interactions between the side chain and the cage, also exhibited the highest activities against HIV protease. However, computational studies of the interaction between the in-

hibitors and HIV protease suggested that the solution structure is lost upon complexation inside the enzyme active site. These results imply an induced-fit mechanism of complexation. A study on a PCU-derived cage ether was also reported.^[46]

In the study reported herein, the cage moiety was again employed as a core molecule owing to its lipophilic nature. Published results suggest that the lipophilic character of the cage should potentially improve membrane permeability.^[22,32] The C₂-meso cage 1,2-diol^[47,48] character of cages **1** and **2** avoids the synthesis of diastereomeric peptides (Figure 2). Another advantage of the PCU cage diol moiety is that it allows the synthesis of two peptide arms to the cage, as opposed to our previous one-arm PCU lactam peptide inhibitors.

The binding energy and specificity of the cage dipeptide can, in principle, increase because each additional hydrogen bond between the second peptide arm and the protease contributes to an additional 3–6 kcal mol⁻¹ stabilization^[49] of the complex. Peptides **1** and **2** will also aid in the study of the effect of C→N versus N→C coupling of the peptide to the cage diol (Figure 2).

During the synthesis of the cage diol structure for peptides **2**, an asymmetric cage diol was also isolated. This enabled us to synthesize the cage diol inhibitor **3** (Figure 3) bearing a single peptide arm. This should make it possible to determine what effect a second peptide side arm has on the inhibition of HIV protease by the cage diol peptides, and therefore increase our knowledge of the structure–activity relationship of this class of compounds.

Herein we report the synthesis, biological activity, NMR results [including efficient adiabatic symmetrized ROESY (EASY-ROESY)]^[50] and molecular modeling of seven PCU-derived HIV protease inhibitors (Table 1). Docking studies and molecular

Table 1. Inhibition of wild-type C-SA HIV protease by the PCU peptide derivatives.

Compd	R ¹	IC ₅₀ [μM] ^[a]	Yield [%]	Log P ^[b]
1 a	Phe-Ile-Ala-Ala-OMe	5	38	2.59 ± 0.92
1 b	Phe-Val-Ala-Ala-OMe	5	59	3.65 ± 0.92
1 c	Val-Ala-Ala-OMe	ND ^[d]	48	-0.56 ± 0.85
	R ²			
2 a	Phe-Val-Ala-Cbz ^[c]	7.5	19	6.47 ± 0.96
2 b	Phe-Val-Ala-2-Peta ^[c]	0.5	22	3.80 ± 0.93
2 c	Phe-Val-Ala-NH ₂	0.5	17	1.68 ± 0.89
	R ³			
3 a/b	Phe-Val-Ala-Cbz ^[c] Lopinavir	0.6 0.025 ± 0.002	44	1.67 ± 0.85

[a] Data represent the average of three experiments. [b] Calculated with ACD/labs software v 11.0. [c] Cbz = benzyloxycarbonyl; 2-Peta = 2-pyrimidinylethanethioic acid. [d] Not determined (> 10 μM).

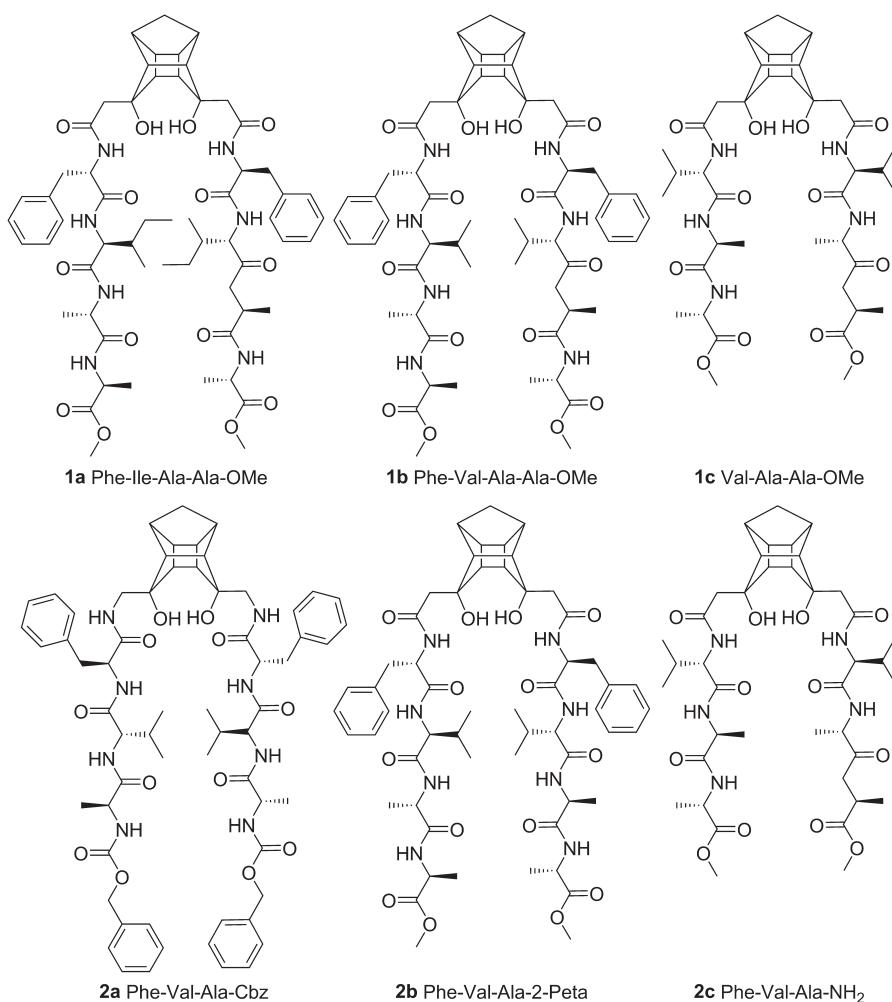


Figure 2. Cage diol inhibitors **1** and **2**.

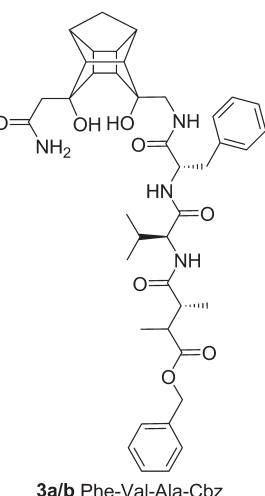


Figure 3. Structure of the cage diol peptide **3a/b**.

dynamic (MD) simulations of selected inhibitors inside the enzyme active site were performed to provide information on the modes of interaction between these inhibitors and HIV protease.

Results and Discussion

Design strategy of PCU diol inhibitors

The core functionality of the protease is to effect hydrolysis of the natural protein substrates by means of catalyzed nucleophilic attack through a solvent water molecule. Brik and Wong^[12] suggested that upon binding of the inhibitor to the catalytic site, the distortion of the scissile bond decreases the double bond character of the C–N bond and polarizes the carbonyl group, rendering it more electrophilic toward the incoming nucleophilic water molecule. The distortion of the amide bond is stabilized by interactions of the substrate with the extended binding cleft, mostly by hydrogen bond interactions between the interior side of the flap and the substrate carbonyl groups on either side of the scissile bond, as shown in Figure 4.^[12]

This study involved reformulation of the ritonavir precursor A-75925 (Figure 1) by introducing well-defined and systematic

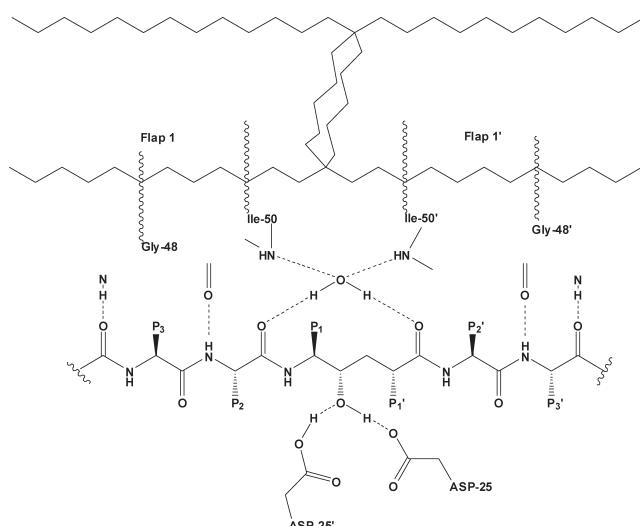


Figure 4. Schematic representation of two flaps of HIV protease and the hydrogen bonds with a solvent water molecule and a typical inhibitor.^[12]

changes. These changes are introduced by substituting the core diol with a cage diol moiety and altering the side chain amino acid residues. Inclusion of the cage diol is aimed at po-

tentially increasing the stereospecificity of the peptide side chains as well as the ability of the diol to form more effective hydrogen bonds with the Asp(25)/Asp(25') residues in the HIV protease active site.

Single-crystal X-ray analysis of the PCU diacid **1** coupled to one amino acid showed that one arm of the cage is positioned over the diols.^[51] If this structural behavior is also present in solution, it could support our previous findings with the cage lactam peptides,^[33,34] that a particular side chain orientation appears to be linked to increased HIV protease activity. The most active cage lactam peptides exhibited an interaction between the side chain and the cage (observed with NMR and MD simulations); this conformation is lost upon complexation with HIV protease, suggesting an induced-fit mechanism.

The binding affinity of the inhibitor to the mutated HIV protease is affected by the inability of the P₃ group of the inhibitor to bind to the enzyme S₃/S_{3'} subsite.^[19] This is caused by a decrease in the size of the S₃ pocket in the mutated protease. It is therefore important to design inhibitors with appropriate P₃ groups. Lee et al.^[19] reported alanine as an appropriate amino acid to use in the P₃ position. With this in mind, all the inhibitors synthesized in this study have alanine at the P₃ position.

To achieve the aims of this study, three cage compounds with variable functionalization options and spacer lengths were chosen (Figure 5). Cage diols **1** and **2** are both *meso* and will therefore not result in diastereomeric peptides, as was the

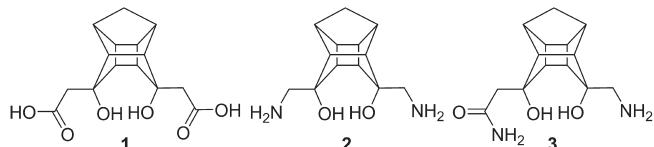


Figure 5. The structures of pentacycloundecane-diol moieties reported in this work.

case with the asymmetric cage lactam.^[33,34] The cage nevertheless becomes chiral (10 of the 11 carbon atoms) upon linkage with a chiral peptide on either side. The cage diol **3** is also asymmetric and will lead to the synthesis of diastereomeric cage peptides.

One of our first attempts for the cage diol peptide inhibitor was to use the natural substrate sequence of the most active cage lactam peptide (PCU-EAIS) reported previously.^[33,34] However, the cage diol peptide analogue (SIAE-1-EAIS) did not exhibit promising HIV protease activity (data not shown).

Seven inhibitors, namely **1a–c**, **2a–c**, and **3a/b**, were synthesized. Inhibitors **1a–c** consist of the cage diacid **1**^[52] and the peptide inhibitors **2a–c** and **3a/b** consist of the cage diamine **2** and cage **3** moieties, respectively. Cages **2** and **3** are both novel and are presented herein for the first time. All inhibitors except **1c** were designed such that the cage diol would potentially fit into the catalytic site of the enzyme, while phenylalanine, valine, alanine, alanine/carbonbenzyloxy are proposed to

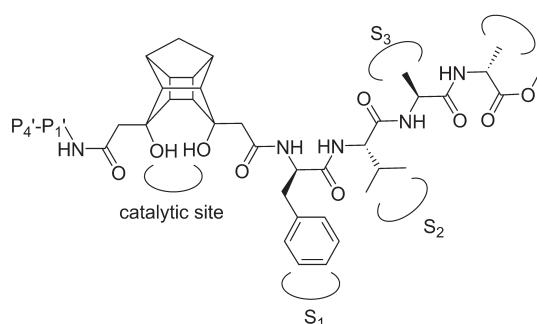


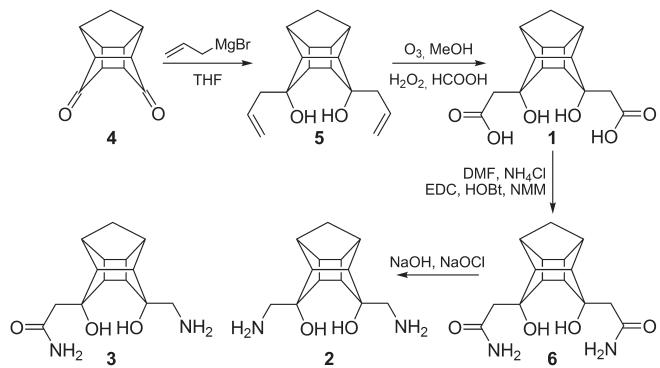
Figure 6. Schematic representation of how the symmetric cage diol inhibitor may bind HIV protease. Standard nomenclature P_{1'}–P_{4'} is used to designate the same amino acid residues as presented on the other side arm. The corresponding binding sites on the protease are referred to as the S₁–S₃ and S_{1'}–S_{3'} subsites.^[9]

occupy the S₁, S₂, S₃, and S₄ sites of the HIV enzyme pockets, respectively, thereby increasing specificity (see Figure 6).

Compound **1c** (Val-Ala-Ala side chains) was designed to investigate the ability of the cage to fit into the S₁ pocket of the enzyme. The S₁ pocket has shown a tendency to bind bulky groups.^[12] The absence of phenylalanine in the peptide side arm of compound **1c** enables us to investigate the ability of the cage to potentially bind to this site.

Peptide **2a** was synthesized to investigate the effect of the exact sequence used by Lee et al.^[19] (Phe-Val-Ala-Cbz) when coupled to the cage. Compound **2a** was also designed to investigate what effect the positioning of the carbonyl group next to the scissile bond has on binding to HIV protease. With cage **1**, the carbonyl group is three bonds away from the diols, whereas on cage **2** the carbonyl is four bonds away. The proposed mechanism for the binding of HIV protease to inhibitors illustrates the significance of the potential binding of the carbonyl group, next to the scissile bond, to the flaps of the protease.

The cage diacid **1**^[52] was the precursor in the synthesis of the novel cage moieties **2** and **3**. The cage acid was synthesized as illustrated in Scheme 1. Cages **2** and **3** were obtained from Hofmann rearrangement of the diamide, which was obtained by reacting the diacid with ammonium chloride and



Scheme 1. Synthesis of cage diol compounds **1–3** from the cage dione.^[52]

various coupling agents (see Supporting Information for the complete experimental details).

The peptide side chains to fit into the S₁/S_{1'}–S₄/S_{4'} sites of the enzyme pocket were synthesized by automated solid-phase peptide synthesis on 2-chlorotriyl resin (see Supporting Information). Peptides to be coupled to the diacid **1** moiety were cleaved from the resin. The C terminus of the peptide side chain was converted into a methyl ester before coupling the amine terminal to the cage diacid. Various reagents were tested for the formation of the amide bond between the cage acid and the peptide amine. First, the diacid was converted into an acid chloride and coupled with the N terminus of the peptide in presence of base. Second, the cage diacid and peptide were coupled in the presence of either HBTU/DIPEA¹ or EDC/NMM/HOB². The acid chloride route afforded the product in low yield with many impurities. The HBTU/DIPEA coupling reagents afforded product with only one side coupled. The HATU/DIPEA approach afforded the product in low yield with impurities. The EDC/NMM/HOB² resulted in good yields of **1a–c** with fewer impurities.

For coupling of the peptide acids to the cage diamine **2** and cage amine **3**, three different coupling reagents were used: HBTU/DIPEA, HATU/DIPEA, and EDC/NMM/HOB². The latter set also gave good yields with fewer impurities. All the synthesized compounds were then purified by semi-preparative HPLC.

IC₅₀ and 2D NMR correlation

All the designed inhibitors were synthesized and tested against wild-type C-SA protease (see Figures 2 and 3). The inhibitory activity (IC₅₀) of the PCU diol peptide derivatives against wild-type C-SA HIV protease are listed in Table 1, and according to the results, inhibitors **1a–c** exhibited weak activity. Inhibitor **1c** showed the lowest activity, and this was initially attributed to the absence of the phenylalanine ring, which is proposed to interact with the S1/S1' subsites for efficient binding.

The diamine inhibitor **2a** (Phe-Val-Ala-Cbz side chains) exhibited poor solubility at pH 5 in the sodium acetate buffer used for the protease assay and thus the accuracy of the observed activity (IC₅₀ = 7.5 μM) is questionable. We then substituted the benzyl group with the more soluble (2-pyrimidinylthio) acetic acid moiety in peptide **2b**. The IC₅₀ value improved from 7.5 μM to 0.5 μM. We then decided to remove the benzyl group completely (forming **2c**) to decrease the size and use the free amine group of alanine to increase solubility. The IC₅₀ value obtained for **2c** (Phe-Val-Ala-NH₂ side chains) was the same as for **2b**. Although the last amino acid in the chain of inhibitor **3a/b** was protected with a benzyl group, it exhibited much higher activity than **2a** with the same peptide sequence. The free amide on the other side of the cage of **3a/b** (one

Phe-Val-Ala-Cbz side chain) most likely ensures increased solubility.

The log P values of the best inhibitors (**2b**: 3.8 and **2c**: ~1.7) are in the preferred range^[53] of between 1.3 and 4.1 for the majority of orally available drugs. The variance in HIV activities for **2a** (~6.5) and **2b** (~3.8), in which the Cbz terminal group is replaced with a 2-Peta group, is also reflected in the log P values, which suggests solubility limitations for compound **2a**.

We previously reported that the cage peptides are much less toxic (6000–8500-fold) to human cells than lopinavir (IC₅₀ = 0.035 μM). The diastereomeric mixture **3a/b** (IC₅₀ = 390 μM) was ~11 000-fold less toxic than the same control. Interestingly, the most active cage lactam inhibitor previously studied (PCU-Glu-Ala-Ile-Ser)^[33] was considerably more toxic to human cells (IC₅₀ = 218 μM) than the cage diol peptides. Ritonavir is much less toxic (IC₅₀ = 57 μM)^[11] than lopinavir, but still much more toxic than the cage peptides.

Because the cage diol protease inhibitors were synthesized for the first time, we used EASY-ROESY^[50] to obtain information about their 3D structures from through-space ¹H,¹H distances/contacts. The EASY-ROESY NMR technique was designed to enable observation of through-space NOE interactions for smaller molecules, invisible by standard NOESY experiments. Structural conformation of a selection of these peptides (**1b** = 5 μM, **1c** > 10 μM, **2c** = 0.5 μM, and **3a/b** = 0.6 μM) in solution was obtained with EASY-ROESY NMR techniques.

The ROESY spectrum of **1b** (Phe-Val-Ala-Ala-OMe side chains) showed the presence of two conformations. In one conformation the phenyl ring (H-p4) interacts with cage protons H-1/7, which are situated at the back of the cage. This conformation therefore allows for the diol to be exposed in order to interact with the catalytic site. In the second conformation, the phenyl ring (H-p4) interacts with H-9/10 thus the peptide chain extends to the front and blocks the diol.

From the ROESY spectrum of **1c** (Val-Ala-Ala-OMe side chains) it was noted that one of the valine methyl protons (H-6' or H-7') interacts with cage protons H-9/10 (labeled structure, Figure 7).

Note that **2b** did not exhibit any NOE interactions between the side chain protons and the cage protons. The EASY-ROESY spectrum of inhibitor **3a/b** showed a long-range correlation between the methylene protons of phenylalanine H-5' and the cage protons H-5 (Figure 7 and Figure 8). Although these interactions were observed with deuterated dimethyl sulfoxide (DMSO) as solvent, we previously showed^[33] that the same solution structure is essentially maintained in the aqueous buffer solution used for the HIV protease activity tests.

The NOESY data from three of the four inhibitors with varying degrees of HIV protease activity (**1b**: 5 μM, **1c**: > 10 μM, and **3**: 0.6 μM) essentially show strong interaction between the peptide side chain and the respective cage protons. This is different from what we observed with the cage lactam peptides, for which only the more active inhibitors demonstrated this behavior.^[33] If our hypothesis about the requirement of a relatively rigid peptide side chain conformation for good HIV protease inhibition is correct, then these current results suggest that either the cage, and/or the cage peptide combination do

¹ HBTU = O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate; DIPEA = N,N-diisopropylethylamine.

² EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NMM = N-methylmorpholine; HOB² = 1-hydroxybenzotriazole.

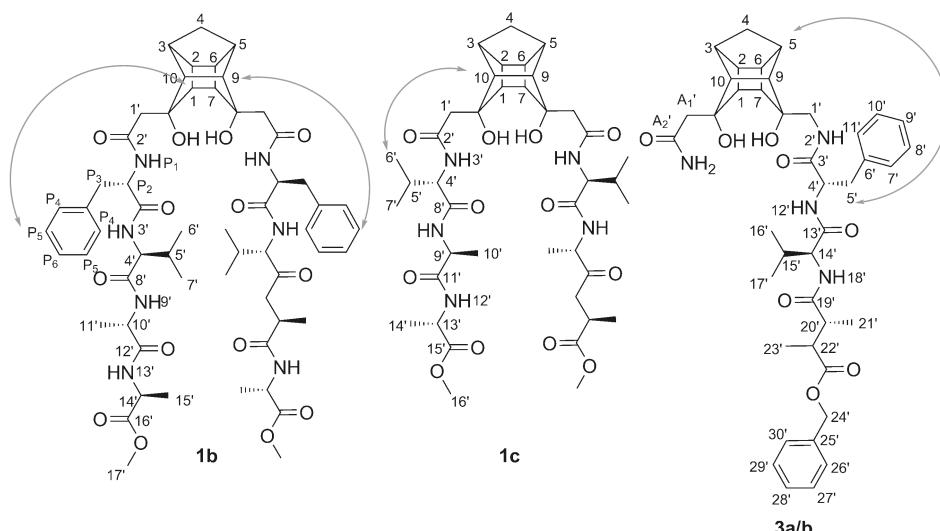


Figure 7. Simplified presentation of cage peptides **1b**, **1c**, and **3a/b** with arrows indicating the long-range correlations observed in the respective EASY-ROESY spectra. Folding of the side chains is required to facilitate these interactions.

not undergo a sufficient fit in the enzyme active site. Alternatively, there may be subtle but important requirements for the cage/side chain interactions of which we are yet unaware. Because the length of the link between the cage moiety and the side arms can be conveniently altered,^[54–57] future studies should explore modeling of the interaction between such inhibitors (with various side arm lengths) and the protease, to see if more active inhibitors theoretically exist.

of ligand–receptor complex structures and also to rank the ligand molecules based on the binding energies of the corresponding ligand–enzyme complexes. The objective of our docking study was to elucidate the potential interaction mode of the PCU diol peptide derivatives with C-SA HIV protease and to see if there is a correlation between the binding energies and the observed IC₅₀ results. The binding energies of the docked diastereomeric PCU peptide inhibitors are listed in Table 2.

The calculated binding energies for the two diastereomeric peptides **3a** and **3b** (one Phe-Val-Ala-Cbz side chain) suggest

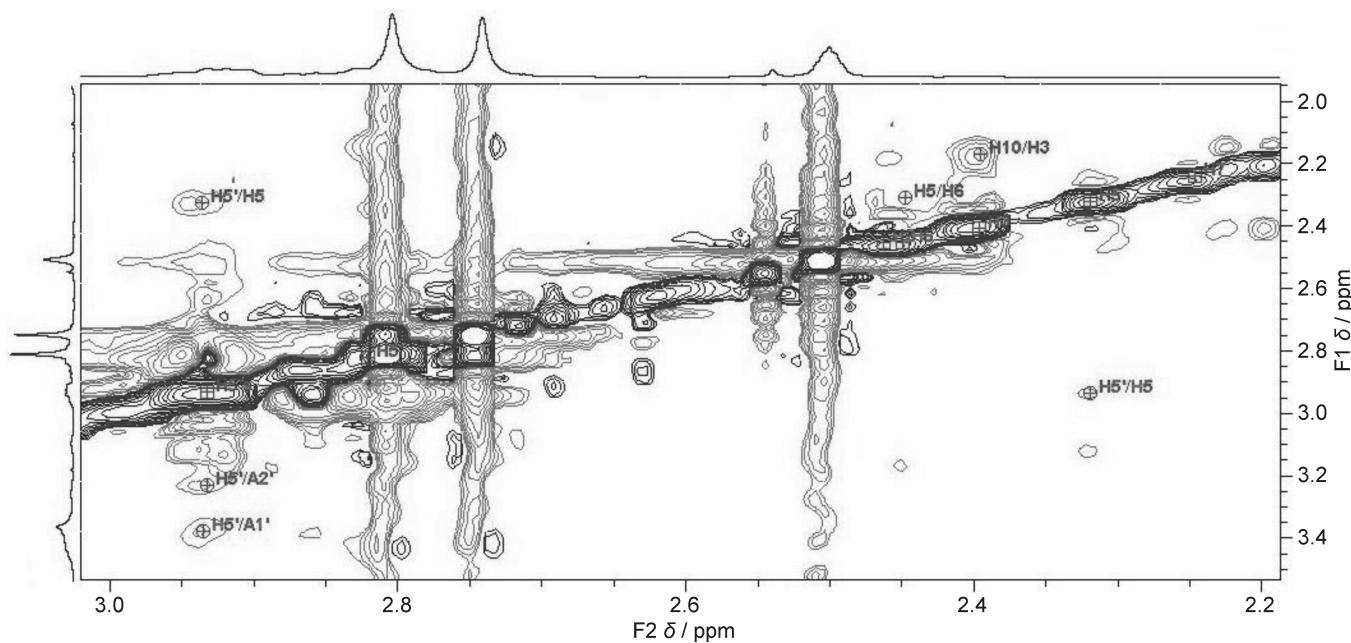


Figure 8. Portion of the EASY-ROESY spectrum of **3a/b**.

Table 2. Selected automated docking results for the synthesized PCU diol-derived peptide inhibitors and comparison with experimental HIV protease inhibition results.^[a]

Compd	Side chain(s)	Docked E_{bind} [kcal mol ⁻¹]	IC_{50} [μM] ^[b]
1 c	Val-Ala-Ala-OMe ^[d]	-8.63	>10
2 a	Phe-Val-Ala-Cbz ^[d]	-6.53	7.5
2 b	Phe-Val-Ala-2-Peta ^[d]	-8.30	0.5
2 c	Phe-Val-Ala-NH ₂ ^[d]	-9.86	0.5
3 a^[c]	Phe-Val-Ala-Cbz ^[e]	-9.33	
3 b^[c]	Phe-Val-Ala-Cbz ^[e]	-8.37	0.6

[a] All of the docked inhibitor–enzyme complex results are available in PDB format and are provided with the Supporting Information. [b] IC_{50} : concentration required to inhibit 50% of protease activity. [c] These peptides are isolated as two inseparable diastereomers; the specific 3D structures assigned to each peptide are available in PDB format with the Supporting Information. [d] Two side chains on either side of the cage diol. [e] One side chain attached to the cage diol.

that the peptide with one PCU enantiomer (**3 a**) is more active than the other. Peptide **3 b** on its own is expected to exhibit weak activity, similar to **1 c**. The docking results are in reasonable agreement with the measured IC_{50} values. Docking studies clearly show the importance of conserved hydrogen bonds between the cage hydroxy groups and Asp25/Asp25' of the dimeric catalytic triad residues, Asp25-Thr26-Gly27 (A/B chains) (Figure 9 and the Supporting Information). Such hydrogen bonds anchor the cage skeleton to the S1/S1' subsite.

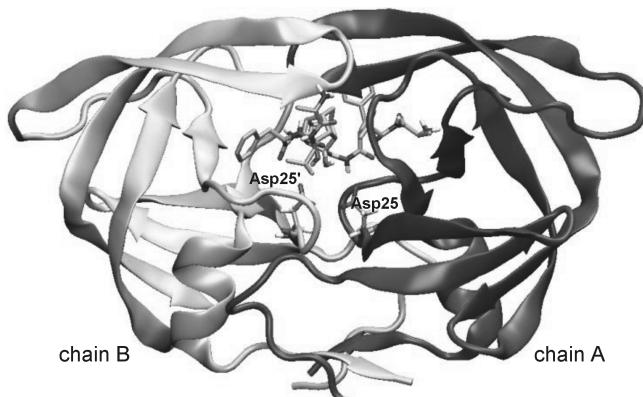


Figure 9. Lowest-energy docked structure for compound **2c** with C-SA HIV protease. The two protease monomers are shown as light- and dark-grey ribbons. The inhibitor and the two Asp25 residues are indicated in stick representation (3D coordinates for the computational results are available in the Supporting Information).

The docked binding energies previously calculated for the lactam peptides^[33] correlate reasonably well to the binding energies calculated in this study for cage diol peptides with similar IC_{50} values. The most active lactam peptide (PCU-Glu-Ala-Ile-Ser, $\text{IC}_{50}=0.075 \mu\text{M}$) exhibited a stronger calculated binding energy ($-10.23 \text{ kcal mol}^{-1}$ for the "a" diastereomer) than all the peptides in this study. This is expected, as the observed HIV protease activities for the peptides in this study are at least an order of magnitude weaker than that of PCU-Glu-Ala-

Ile-Ser. In all our studies so far, the cage heteroatoms form a strong hydrogen bonding interaction with the enzymatic aspartic acid (Asp25/Asp25') residues.

The docking calculations provided us with a general static picture of the most energetically favorable binding orientation of inhibitors to the enzyme. To obtain further insight into the dynamic changes of the docked inhibitors within the enzyme active site pocket over time, the lowest-energy docked complex of the most active inhibitor, **2c**, was subjected to unconstrained MD simulations (1 ns). The MD simulations clearly show that the inhibitor fits easily into the active enzyme pocket. To assess the quality of our MD simulations, energetic and structural properties were monitored along the entire 1 ns MD trajectory of the complex (Figure 10). The 1 ns averaged backbone RMSD for the **2c**-enzyme complex is 1.09 Å, and this is an indication that the generated MD trajectory of the complex is quite stable.

As evident from the analysis of the hydrogen bonding interactions along the MD trajectories, the inhibitor forms hydrogen bonds between the cage hydroxy groups and at least one of the two Asp25 carboxyl groups (Figure 10c). The post-dynamic monitoring of the overall hydrogen bond networks and the electrostatic interaction between the PCU diol peptide inhibitor and nearby residues clearly reveal that although these inhibitors settle comfortably inside the C-SA HIV protease active site (Figure 11), little movement of the inhibitor occurs during the MD simulation.

As was the case before, the solution structure of these inhibitors as observed with ROESY techniques, is not conserved upon complexation with the HIV protease active pocket, indicating an induced-fit mechanism of complexation. The basic orientation of the cage diol in relation to the two Asp(25)/Asp(25') residues is very similar to that of the cage lactam previously reported.

Conclusions

Peptide inhibitors attached to the cage diol moieties show promising activity against wild-type C-SA HIV protease. The PCU diacid-derived inhibitors (**1 a–c**) exhibited less activity than the PCU diamine (**2 a–c**) and PCU amino amide (**3 a/b**) moieties. Phenylalanine was observed to be an appropriate residue for the P_1 position of these cage peptide inhibitors, as inhibitors with less bulky amino acids at the P_1 position proved to be less active. The direction of attachment of the peptide to the cage (C→N versus N→C) appears to be important as well, because the former (peptides **2 a–c**) gave better activities than the latter (peptides **1 a–c**). The calculated log P values of the most active inhibitors fall within the ideal range (1.3–4.1) required for optimum oral bioavailability. It is also a significant improvement on the previous best lactam inhibitor (PCU-Glu-Ala-Ile-Ser), which had a log P value of about -3.5, which is an indication that the compound is too hydrophilic for optimum oral availability. The most active cage diol peptide (**2c**, with Phe-Val-Ala-NH₂ side chains, $\text{IC}_{50}=0.5 \mu\text{M}$) is only an order of magnitude less active against HIV protease than the best cage lactam peptide ($\text{IC}_{50}=0.078 \mu\text{M}$) previously reported, but a po-

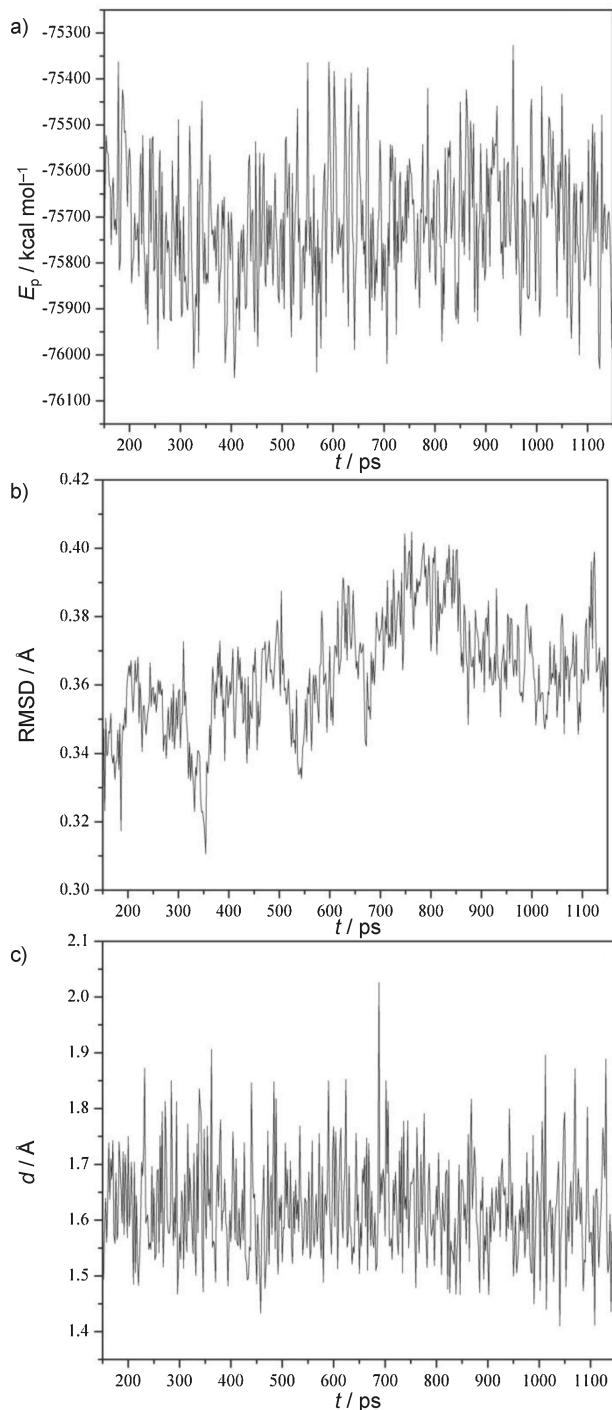


Figure 10. a) The potential energy of **2c** (Phe-Val-Ala-NH₂ side chains) C-SA HIV protease complex observed in MD simulations as a function of time. b) Root-mean-square deviations (RMSD) observed in MD simulations as a function of time. c) Hydrogen bond distance between the hydroxy group located at C6 and the Asp25 carboxyl group.

tential increased oral bioavailability may compensate somewhat for that. The cage diol peptide (**3a/b**, with one Phe-Val-Ala-Cbz side arm) is about sevenfold less toxic than ritonavir and 11 000-fold less toxic than lopinavir.

The combination of drug design with *in vitro* assays, NMR techniques, and molecular modeling studies has enabled us to

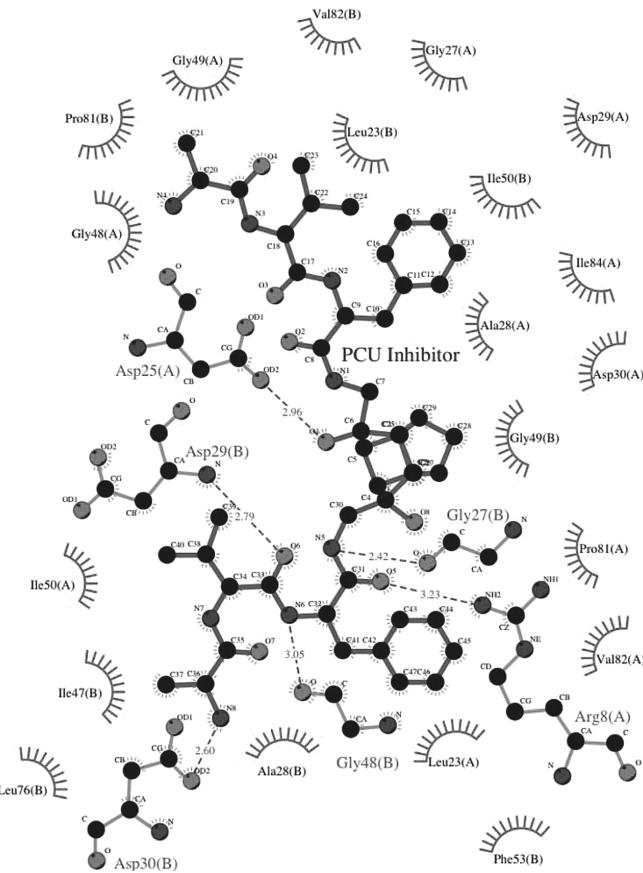


Figure 11. Detailed plot of electrostatic and hydrogen bonding interaction between **2c** (Phe-Val-Ala-NH₂ side chains) and the nearby residues of C-SA HIV protease active site. This plot was created with Ligplot software. (The 3D coordinates for computational results are available in the Supporting Information.)

rationalize the observed HIV protease inhibition for the various cage peptides in this series. The EASY-ROESY NMR data show that the whole range of inhibitors exhibit a relatively stable interaction between the cage side chain and the cage protons. These results are seemingly in contrast to those of our previous study with cage lactam peptides, in which only the most active inhibitors exhibited such interactions in solution. The docking and MD simulations show that the most active cage diol peptide fits quite easily into the active pocket of HIV protease. These results can be interpreted in at least three different ways. First, the hypothesis that a relatively rigid interaction between the cage and the peptide side chain is an important requirement for better HIV protease inhibition is only true for the family of cage lactam peptides previously studied, and not for the cage diol family of peptides. Second, the hypothesis may still be true, but there are subtle features of this cage/side chain interaction that we have not yet realized. Third, an improved cage diol dipeptide inhibitor can still be designed by changing the length of the link between the cage moiety and the side chain peptides. Two peptide arm inhibitors (**2b** and **2c**) exhibited the same activity as the one peptide arm inhibitor (**3a/b**). This is an important result, as the ability to decrease the molecular weight of an inhibitor while retaining potency is

important for commercial drugs. Ongoing studies in our research groups will attempt to optimize our knowledge and to find answers to the remaining questions.

Experimental Section

Peptide analysis

Analysis of the peptides was performed on an Agilent 1100 HPLC instrument at a flow rate of 1 mL min^{-1} with a Waters Xbridge C₁₈ column (150 mm × 4.6 mm × 5 μm) coupled to a UV detector (λ 215 nm) and an Agilent VL ion trap mass spectrometer operating in positive mode. Semi-preparative HPLC was carried out on a Shimadzu instrument at a flow rate of 17 mL min^{-1} on an Ace C₁₈ column (150 mm × 21.2 mm × 5 μm) with a UV/Vis detector (λ 215 nm) and an automated fraction collector. A two-buffer system was employed, with formic acid as the ion-pairing agent. Buffer A consisted of 0.1% formic acid/H₂O (v/v), and buffer B consisted of 0.1% formic acid/CH₃CN (v/v). High-resolution mass spectrometric analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode with internal calibration. Peptides were synthesized on an automated CEM Liberty microwave peptide synthesizer (conditions are provided in the Supporting Information). All ¹H, ¹³C, HSQC, COSY and HMBC NMR data were recorded on a Bruker AVANCE III 400 MHz spectrometer. The ROESY data were recorded on a Bruker AVANCE III 600 MHz spectrometer. More details about the NMR experimental setup are provided in the Supporting Information.

Synthesis of PCU diol peptide inhibitors

The synthesis and analytical data for the inhibitors are fully described in the Supporting Information. The peptides were synthesized in a microwave peptide synthesis instrument, and coupling of the peptides to the cage diacid was performed in solution.

In vitro HIV protease activity^[58–60]

Synthesis and purification of HIV protease type C were performed as described in the Supporting Information. The catalytic activity of the protease was monitored at λ 300 nm within the enzymatic hydrolysis of a chromogenic peptide substrate, Ala-Arg-Val-Nle-(*p*-nitro-Phe)-Glu-Ala-Nle-NH₂, in the presence of the inhibitors at λ 300 nm using an Analytic Jena Specord 210 spectrophotometer. Activity was standardized using commercially available drugs. Atazanavir and lopinavir were used as positive control compounds.

Molecular modeling studies

A comprehensive computational simulation scenario adopted in this work was reported previously,^[46] however, a summarized procedure is presented herein.

Preparation of PCU diol peptide inhibitors: The 3D structures of the PCU diol peptides were constructed, and the geometries were optimized using the MMFF94 force field implemented in the Avogadro software package.^[61] The minimized structures were then subjected to docking studies.

C-SA protease model system: Because the X-ray crystal structure of the South African HIV-1 protease subtype C (C-SA) has not yet been reported, the initial 3D structure of the enzyme was taken from the reported X-ray data of subtype B HIV protease (PDB code: 1HXW).^[62] C-SA HIV protease differs from subtype B at eight posi-

tions: T12S, I15V, L19I, M36I, R41K, H69K, L89M, and I93L.^[60] Mutations at the eight positions were manually induced. Throughout this work, the modeled C-SA HIV protease was used for docking and MD simulations. This constructed enzyme was successfully used in our previous study on cage lactam peptides.^[33,34]

XTT screening assay^[63,64]

The in vitro toxicology XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide) Kit (Sigma-Aldrich, St. Louis, MO, USA) was used to determine the cytotoxicity of one of the most active compounds (**3a/b**), as reported before.^[33] Cytotoxicity was determined by calculating the inhibitory concentration at 50% (IC₅₀). The assay was performed in triplicate with lopinavir as a reference drug. MT-4 cells were obtained from Dr. Douglas Richman at the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: H9/HTLV-IIIB NIH 1983.

Docking of the inhibitors into the C-SA protease model

Energy-minimized inhibitors were used in docking simulations. The effect of ionization states of docked compounds to the binding scores has been discussed earlier.^[65–67] The simulations were performed under physiological pH conditions, which require the correct protonation state of ionizable groups. In particular, one of the aspartates (Asp25) of the catalytic site exhibits an increased pK_a value of 5.2 in the inhibitor-bound protease,^[12] while a normal pK_a value was reported for the free form of the protease (pK_a = 4.5).^[68] Therefore, a monoprotonated state of Asp25/Asp25' was induced in the active site to be consistent with experimental conditions at physiological pH. However, to ensure that the results of the simulations do not critically depend on the protonation state, a control simulation of a cage-bound C-SA HIV protease was performed in which both the acid functions of the Asp25/Asp25' residues were deprotonated. We previously reported that no significant difference was observed for docking of the cage lactam peptides with the different protonation states of the enzyme.^[33,34] Docking studies were performed using AutoDock.^[69] Gasteiger charges were computed, and the AutoDock atom types were defined using the AutoDock Tools graphical user interface supplied by MGL Tools.^[70] The Cartesian coordinates of the docked structures with the best binding energies are available in the Supporting Information.

MD simulations of the inhibitor–enzyme complex

MD simulations were performed for the inhibitor–enzyme complex of the most active compound **2c**. Partial charges and the force field parameters for the inhibitor were generated using the Antechamber program^[71] in the Amber10 package.^[72,73] These were described by the general Amber force field (GAFF).^[71] All hydrogen atoms of the proteins were added using the Leap module in Amber10. The standard Amber force field for bioorganic systems (ff03)^[74] was used to describe the HIV protease parameters. Counterions were added to neutralize the complex. The system was then solvated using atomistic TIP3P water^[75] in a cubic box with 8.0 Å distance around the complex.

The molecular dynamics package of Amber10 was used for minimization and equilibration protocols. Cubic periodic boundary conditions were imposed, and the long-range electrostatic interactions were treated with the particle-mesh Ewald method^[76] implemented in Amber10 with a nonbonding cutoff distance of 10.0 Å. The energy minimization was first conducted using the steepest de-

scent method in Amber10 for 1000 iterations switched to conjugate gradient for 2000 steps with a restraint potential of 2 kcal mol⁻¹ Å² applied to the solute. Then the total system was freely minimized for 1000 iterations. For the equilibration and subsequent production run, the SHAKE algorithm^[77] was employed on all atoms covalently bonded to a hydrogen atom, allowing for an integration time step of 2 fs. Harmonic restraints with force constants of 2.0 kcal mol⁻¹ Å² were applied to all solute atoms. A canonical ensemble (NVT) MD was carried out for 70 ps, during which the system was gradually annealed from 0 to 300 K using a Langevin thermostat with a coupling coefficient of 1.0 ps. Subsequently, the system was equilibrated at constant volume and temperature (300 K) with a 2 fs time step for 100 ps while maintaining the force constants on the restrained solute. With no restraints imposed, a production run was performed for 1 ns in an isothermal isobaric (NPT) ensemble using a Berendsen barostat^[78] with a target pressure of 1 bar and a pressure coupling constant of 2 ps. The coordinate file was saved every 1 ps, and the trajectory was analyzed at every 1 ps using the P traj module implemented in Amber10.

Acknowledgements

This research was supported by the NRF (South Africa) TG (GUN: 66319), KP (GUN: 69728), CHPC (<http://www.chpc.ac.za/>), HGK, and PIA (South Africa–Sweden bilateral grant) and Aspen Pharmacare. Y.S. thanks the NRF (South Africa) for financial support. The authors thank Prof. Jürgen Schleucher (Department of Medical Biophysics and Biochemistry, Umeå University, Sweden) and Mr. Dilip Jagjivan for their assistance with NMR experiments and Dr. P. Govender (Department of Biochemistry, University of Kwa-Zulu-Natal, South Africa) for assistance with *in vitro* HIV protease experiments.

Keywords: HIV-1 protease • inhibitors • molecular docking • peptides • transition-state analogues

- [1] D. J. Kempf, K. C. Marsh, J. F. Denissen, E. McDonald, S. Vasavanonda, C. A. Flentge, B. E. Green, L. Fino, C. H. Park, X. P. Kong, N. E. Wideburg, A. Saldivar, L. Ruiz, W. M. Kati, H. L. Sham, T. Robins, K. D. Stewart, A. Hsu, J. J. Plattner, J. M. Leonard, D. W. Norbeck, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2484–2488.
- [2] R. J. Pomerantz, D. L. Horn, *Nat. Med.* **2003**, *9*, 867–873.
- [3] N. A. Roberts, J. A. Martin, D. Kinchington, A. V. Broadhurst, J. C. Craig, I. B. Duncan, S. A. Galpin, B. K. Handa, J. Kay, A. Krohn, R. W. Lambert, J. H. Merrett, J. S. Mills, K. E. B. Parkes, S. Redshaw, A. J. Ritchie, D. L. Taylor, G. J. Thomas, P. J. Machin, *Science* **1990**, *248*, 358–361.
- [4] D. H. Rich, J. Green, M. V. Toth, G. R. Marshall, S. B. H. Kent, *J. Med. Chem.* **1990**, *33*, 1285–1288.
- [5] D. H. Rich, C. Q. Sun, J. Prasad, A. Pathiasseri, M. V. Toth, G. R. Marshall, M. Clare, R. A. Mueller, K. Houseman, *J. Med. Chem.* **1991**, *34*, 1222–1225.
- [6] G. B. Dreyer, J. C. Boehm, B. Chamera, R. L. Desjarlais, A. M. Hassell, T. D. Meek, T. A. Tomaszek, *Biochemistry* **1993**, *32*, 937–947.
- [7] X. N. Chen, A. Tropsha, *J. Med. Chem.* **1995**, *38*, 42–48.
- [8] S. J. Desolms, E. A. Giuliani, J. P. Guare, J. P. Vacca, W. M. Sanders, S. L. Graham, J. M. Wiggins, P. L. Darke, I. S. Sigal, J. A. Zugay, E. A. Emini, W. A. Schleif, J. C. Quintero, P. S. Anderson, J. R. Huff, *J. Med. Chem.* **1991**, *34*, 2852–2857.
- [9] M. T. Reetz, C. Merk, G. Mehler, *Chem. Commun.* **1998**, 2075–2076.
- [10] M. L. West, D. P. Fairlie, *Trends Pharmacol. Sci.* **1995**, *16*, 67–75.
- [11] A. Wlodawer, J. Vondrasek, *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249–284.
- [12] A. Brik, C. H. Wong, *Org. Biomol. Chem.* **2003**, *1*, 5–14.
- [13] G. B. Dreyer, B. W. Metcalf, T. A. Tomaszek, T. J. Carr, A. C. Chandler, L. Hyland, S. A. Fakhouri, V. W. Magaard, M. L. Moore, J. E. Strickler, C. Debouck, T. D. Meek, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9752–9756.
- [14] T. D. Meek, D. M. Lambert, G. B. Dreyer, T. J. Carr, T. A. Tomaszek, M. L. Moore, J. E. Strickler, C. Debouck, L. J. Hyland, T. J. Matthews, B. W. Metcalf, S. R. Petteway, *Nature* **1990**, *343*, 90–92.
- [15] G. B. Dreyer, D. M. Lambert, T. D. Meek, T. J. Carr, T. A. Tomaszek, A. V. Fernandez, H. Bartus, E. Cacciavillani, A. M. Hassell, M. Minnich, S. R. Petteway, B. W. Metcalf, M. Lewis, *Biochemistry* **1992**, *31*, 6646–6659.
- [16] D. J. Kempf, H. L. Sham, *Curr. Pharm. Des.* **1996**, *2*, 225–246.
- [17] A. G. Tomasselli, S. Thaisrivongs, R. L. Heinrikson, *Adv. Antivir. Drug Des.* **1996**, *2*, 173–228.
- [18] A. Mühlman, J. Lindberg, B. Classon, T. Unge, A. Hallberg, B. Samuelsson, *J. Med. Chem.* **2001**, *44*, 3407–3416.
- [19] T. Lee, G. S. Laco, B. E. Torbett, H. S. Fox, D. L. Lerner, J. H. Elder, C. H. Wong, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 939–944.
- [20] R. I. Mahato, A. S. Narang, L. Thoma, D. D. Miller, *Crit. Rev. Ther. Drug Carrier Syst.* **2003**, *20*, 153–214.
- [21] D. W. Oliver, T. G. Dekker, F. O. Snyckers, T. G. Fourie, *J. Med. Chem.* **1991**, *34*, 851–854.
- [22] W. J. Geldenhuys, S. F. Malan, J. R. Bloomquist, A. P. Marchand, C. J. Van der Schyf, *Med. Res. Rev.* **2005**, *25*, 21–48.
- [23] B. James, S. Vijji, S. Mathew, M. S. Nair, D. Lakshmanan, R. A. Kumar, *Tetrahedron Lett.* **2007**, *48*, 6204–6208.
- [24] D. W. Oliver, S. F. Malan, *Med. Chem. Res.* **2008**, *17*, 137–151.
- [25] K. Bisetty, F. J. Corcho, J. Canto, H. G. Kruger, J. J. Perez, *J. Pept. Sci.* **2006**, *12*, 92–105.
- [26] F. M. Ito, J. M. Petroni, D. P. de Lima, A. Beatriz, M. R. Marques, M. O. de Moraes, L. V. Costa-Lotufo, R. C. Montenegro, H. I. F. Magalhaes, C. do O. Pessoa, *Molecules* **2007**, *12*, 271–282.
- [27] K. Aigami, Y. Inamoto, N. Takaishi, Y. Fujikura, A. Takatsuki, G. Tamura, *J. Med. Chem.* **1976**, *19*, 536–540.
- [28] Y. Inamoto, K. Aigami, T. Kadono, H. Nakayama, A. Takatsuki, G. Tamura, *J. Med. Chem.* **1977**, *20*, 1371–1374.
- [29] D. W. Oliver, T. G. Dekker, F. O. Snyckers, *Arzneim.-Forsch.* **1991**, *41*, 549–552.
- [30] R. S. Schwab, A. C. England, Jr., D. C. Poskanzer, R. R. Young, *J. Am. Med. Assoc.* **1969**, *208*, 1168.
- [31] J. L. Neumeyer in *Principles of Medicinal Chemistry* (Eds.: W. O. Foye, T. L. Lemke, D. A. Williams), Lea and Febiger, Philadelphia, **1989**, pp. 223.
- [32] K. B. Brookes, P. W. Hickmott, K. K. Jutle, C. A. Schreyer, *S. Afr. J. Chem.* **1992**, *45*, 8–11.
- [33] M. M. Makatini, K. Petzold, S. N. Sriharsha, N. Ndlovu, M. E. S. Soliman, B. Honarpvar, R. Parboosing, A. Naidoo, P. I. Arvidsson, Y. Sayed, P. Govender, G. E. M. Maguire, H. G. Kruger, T. Govender, *Eur. J. Med. Chem.* **2011**, *46*, 3976–3985.
- [34] M. M. Makatini, K. Petzold, S. N. Sriharsha, M. E. S. Soliman, B. Honarpvar, P. I. Arvidsson, Y. Sayed, P. Govender, G. E. M. Maguire, H. G. Kruger, T. Govender, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2274–2277.
- [35] P. K. Jadhav, P. Ala, F. J. Woerner, C. H. Chang, S. S. Garber, E. D. Anton, L. T. Bacheler, *J. Med. Chem.* **1997**, *40*, 181–191.
- [36] R. T. Rapala, R. J. Kraay, K. Gerzon, *J. Med. Chem.* **1965**, *8*, 580.
- [37] K. Gerzon, D. Kau, *J. Med. Chem.* **1967**, *10*, 189.
- [38] A. N. Voldeng, C. A. Bradley, R. D. Kee, E. L. King, F. L. Melder, *J. Pharm. Sci.* **1968**, *57*, 1053.
- [39] O. K. Onajope, P. Govender, P. D. van Heiden, H. G. Kruger, G. E. M. Maguire, I. Wiid, T. Govender, *Eur. J. Med. Chem.* **2010**, *45*, 2075–2079.
- [40] S. F. Malan, G. Dockendorf, J. J. Van der Walt, J. M. van Rooyen, C. J. Van der Schyf, *Pharmazie* **1998**, *53*, 859–862.
- [41] E. Grobler, A. Grobler, C. J. Van der Schyf, S. F. Malan, *Bioorg. Med. Chem.* **2006**, *14*, 1176–1181.
- [42] F. J. C. Martins, A. M. Viljoen, H. G. Kruger, J. A. Joubert, P. L. Wessels, *Tetrahedron* **1994**, *50*, 10783–10790.
- [43] H. G. Kruger, F. J. C. Martins, A. M. Viljoen, *J. Org. Chem.* **2004**, *69*, 4863–4866.
- [44] F. J. C. Martins, A. M. Viljoen, H. G. Kruger, P. L. Wessels, *Magn. Reson. Chem.* **2004**, *42*, 402–408.
- [45] G. A. Boyle, T. Govender, H. G. Kruger, N. I. Ndlovu, *Acta Crystallogr. Sect. E Struct. Rep. Online* **2007**, *63*, o3906.
- [46] R. Karpoormath, Y. Sayed, P. Govender, G. E. M. Maguire, H. G. Kruger, T. Govender, M. E. S. Soliman, *Bioorg. Chem.* **2012**, *40*, 19–29.

- [47] T. Govender, H. K. Hariprakasha, H. G. Kruger, A. P. Marchand, *Tetrahedron: Asymmetry* **2003**, *14*, 1553–1557.
- [48] H. G. Kruger, R. Ramdhani, *S. Afr. J. Chem.* **2006**, *59*, 71–U28.
- [49] J. March, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, John Wiley & Sons, New York, **1992**.
- [50] C. M. Thiele, K. Petzold, J. Schleucher, *Chem. Eur. J.* **2009**, *15*, 585–588.
- [51] R. Karpoormath, P. Govender, H. G. Kruger, T. Govender, G. E. M. Maguire, *Acta Crystallogr. Sect. E* **2010**, *66*, O2537–U2268.
- [52] O. K. Onajole, M. M. Makatini, P. Govender, T. Govender, G. E. M. Maguire, H. G. Kruger, *Magn. Reson. Chem.* **2010**, *48*, 249–255.
- [53] A. K. Ghose, V. N. Viswanadhan, J. J. Wendoloski, *J. Comb. Chem.* **1999**, *1*, 55–68.
- [54] T. G. Levitskaia, B. A. Moyer, P. V. Bonnesen, A. P. Marchand, K. Krishnudu, Z. B. Chen, Z. L. Huang, H. G. Kruger, A. S. McKim, *J. Am. Chem. Soc.* **2001**, *123*, 12099–12100.
- [55] G. A. Boyle, T. Govender, H. G. Kruger, G. E. M. Maguire, *Tetrahedron: Asymmetry* **2004**, *15*, 2661–2666.
- [56] G. A. Boyle, T. Govender, R. Karpoormath, H. G. Kruger, *Acta Crystallogr. Sect. E* **2007**, *63*, O4797–U5875.
- [57] G. A. Boyle, T. Govender, R. Karpoormath, H. G. Kruger, *Acta Crystallogr. Sect. E* **2007**, *63*, O3977–U2352.
- [58] R. M. Klabe, L. T. Bacheler, P. J. Ala, S. Erickson-Viitanen, J. L. Meek, *Biochemistry* **1998**, *37*, 8735–8742.
- [59] Y. F. Tie, P. I. Boross, Y. F. Wang, L. Gaddis, F. L. Liu, X. F. Chen, J. Tozser, R. W. Harrison, I. T. Weber, *FEBS J.* **2005**, *272*, 5265–5277.
- [60] S. Mosebi, L. Morris, H. W. Dirr, Y. Sayed, *J. Virol.* **2008**, *82*, 11476–11479.
- [61] Avogadro: An open-source molecular builder and visualization tool, v. 2011.
- [62] A. Velaquez-Campoy, S. Vega, E. Fleming, U. Bacha, Y. Sayed, H. W. Dirr, E. Freire, *AIDS Rev.* **2003**, *5*, 165–171.
- [63] D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827–4833.
- [64] N. W. Roehm, G. H. Rodgers, S. M. Hatfield, A. L. Glasebrook, *J. Immunol. Methods* **1991**, *142*, 257–265.
- [65] T. A. Soares, D. S. Goodsell, R. Ferreira, A. J. Olson, J. M. Briggs, *J. Mol. Recognit.* **2000**, *13*, 146–156.
- [66] T. M. Frimurer, G. H. Peters, L. F. Iversen, H. S. Andersen, N. P. H. Moller, O. H. Olsen, *Biophys. J.* **2003**, *84*, 2273–2281.
- [67] C. W. Locuson, P. M. Gannett, R. Ayscue, T. S. Tracy, *J. Med. Chem.* **2007**, *50*, 1158–1165.
- [68] R. Smith, I. M. Brereton, R. Y. Chai, S. B. H. Kent, *Nat. Struct. Biol.* **1996**, *3*, 946–950.
- [69] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998**, *19*, 1639–1662.
- [70] M. F. Sanner, *J. Mol. Graph. Model.* **1999**, *17*, 57–61.
- [71] J. Wang, W. Wang, P. A. Kollman, D. A. Case, *J. Mol. Graphics Modell.* **2006**, *25*, 247–260.
- [72] J. M. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **2004**, *25*, 1157–1174.
- [73] D. A. Case, T. E. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz, A. Onufriev, C. Simmerling, B. Wang, R. J. Woods, *J. Comput. Chem.* **2005**, *26*, 1668–1688.
- [74] Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. M. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. M. Wang, P. Kollman, *J. Comput. Chem.* **2003**, *24*, 1999–2012.
- [75] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, *J. Chem. Phys.* **1983**, *79*, 926–935.
- [76] U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee, L. G. Pedersen, *J. Chem. Phys.* **1995**, *103*, 8577–8593.
- [77] J. P. Ryckaert, G. Ciccotti, H. J. C. Berendsen, *J. Comput. Phys.* **1977**, *23*, 327–341.
- [78] H. J. C. Berendsen, J. P. M. Postma, W. F. Vangunsteren, A. Dinola, J. R. Haak, *J. Chem. Phys.* **1984**, *81*, 3684–3690.

Received: November 3, 2011

Revised: March 22, 2012

Published online on April 27, 2012