Paracyclophanes: A Novel Class of Water-Soluble Inhibitors of HIV Proteinase†

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A versatile synthesis of functionalized para- and metacyclophanes (macrocycles with one or more aromatic rings incorporated; ansa-compounds) has been developed. Cyclophanes constitute a novel building block for potent human immunodeficiency virus (HIV) protease inhibitors. The synthesis of the macrocyclic ring system was achieved by regio- and stereospecific ring opening of N-protected 4-amino-2,3-epoxy-5-phenylpentanoates with appropriate α,ω-diamines and consecutive ring closure under high dilution conditions. The resulting macrocyclic building blocks enabled further broad and flexible derivation. Paracyclophanes, containing oxyethylene substructures, were found to dissolve in phosphate-buffered saline at concentrations as high as 3 mg/mL at physiological pH. Several derivatives with K_i values lower than 10 nM and antiviral activities in the range of 15−50 nM have been obtained. The influence of the ring size and of the substitution pattern of the cyclophane moiety on enzyme inhibition, antiviral activity, and water solubility are discussed. Preliminary data on oral bioavailability in mice are given for selected compounds.

Introduction

Recently, we reported on the synthesis of 2-heterosubstituted 4-amino-3-hydroxy-5-phenylpentanoic acid as a novel building block for the design of HIV proteinase (HIV PR) inhibitors.[1] Stereochemical control and convergent synthesis of the core-unit of the inhibitors together with maximum synthetic flexibility for derivation allowed the preparation of different types of PR inhibitors (Figure 1). Compound **1**, a representative of numerous potent derivatives, was obtained by a novel synthetic route. K_i values for inhibition of HIV-1 proteinase by chemotype 1 were typically below 5 nM, and antiviral activity with IC_{50} values below 30 nM was demonstrated.[1] The characteristic structural feature of inhibitors of type 1 based on a 2-heterosubstituted statine moiety is a benzimidazole substituent covering the P3′-subsite.[2] However, compounds of this type lack oral bioavailability in different animal species.[3] Oral uptake in several animal species of compounds of type **1** was achieved by truncation of the C-terminus of the inhibitor, and by introduction of 1(*S*)-amino-2(*R*)-hydroxyindane as P2′-substituent, which yielded PR inhibitor **2**.[4] This compound, designated SDZ PRI 053, exhibits exceptionally high oral bioavailability in mice, rats, and dogs.[4] However, the antiviral activity of SDZ PRI 053 (**2**) was somewhat lower when compared to inhibitor **1** (Table 5).

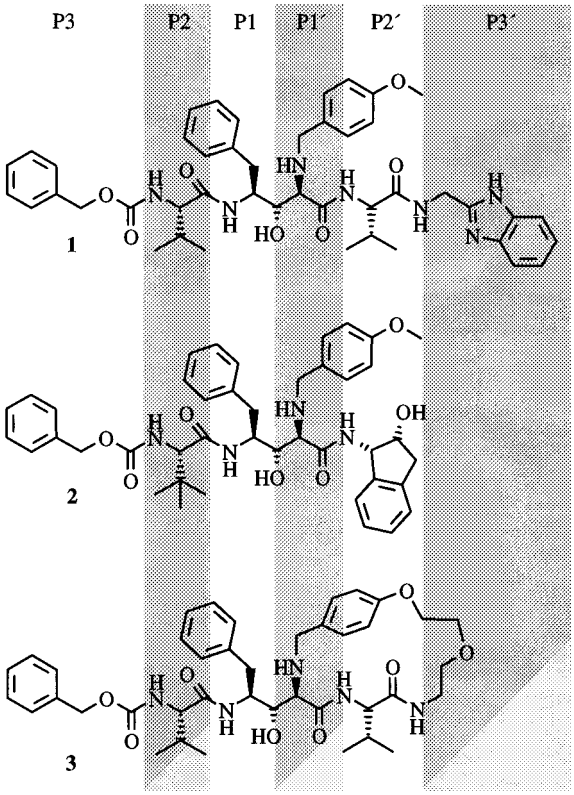

Figure 1. Chemical structures of HIV PR inhibitors **1**−**3**.

Therefore, we have investigated strategies to improve antiviral activity while retaining oral bioavailablity of the compounds.[4b,5] One of our approaches was prompted by reports that increased aqueous solubility of inhibitors facilitated oral absorption. This statement was originally based on an analysis of the bioavailability of sparingly water soluble, albeit potent, peptidomimetic PR inhibitors.[6,7] Few nonpeptidic HIV PR inhibitors have been reported to be significantly water-soluble. Furthermore, their antiviral activities are poor compared to peptide-based inhibitors.[8] We chose to design

† Abbreviations: The abbreviations for the natural amino acids (three-letter code) are in accord with the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9−37). In addition: AcN (acetonitrile), BOP (1-(benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate, Boc (*tert*-butyloxycarbonyl; 1,1-dimethylethoxycarbonyl), DCC (dicyclohexylcarbodiimide), DMAP (4-(dimethylamino)pyridine), DMF (dimethylformamide), DMSO (dimethyl sulfoxide), EDC·HCl (*N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride), HOBt (1-hydroxy-benzotriazole), HODhbt (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine), HPTLC (high performance thin-layer chromatography), NMM (*N*-methylmorpholine), Ph (phenyl), PyBOP (1-(benzotriazolyloxy)tripyrrolidinophosphonium hexafluorophosphate), RP-HPLC (reversed-phase high-performance liquid chromatography), rt (room temperature), *tert*-leucine ((2*S*)-amino-3,3-dimethylbutanoic acid), THF (tetrahydrofuran), TLC (thin-layer chromatography), Tle (*tert*-leucine; (2*S*)-amino-3,3-dimethylbutanoic acid), TsCl (4-methylbenzenesulfonyl chloride), TsOH (4-methylbenzenesulfonic acid), Z (benzyloxycarbonyl).

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peptidomimetic inhibitors containing ether-type macrocycles (**3**) which would (i) increase the hydrophilicity of the compounds by introduction of polar structural elements in positions of the inhibitors which do not interfere with the hydrophobic active site of the HIV PR, and (ii) would at least partially fill the S3′ pocket that had remained unoccupied by **2**. Various examples for cyclic inhibitors of renin have been described.[9] In addition, two reports of macrocyclic, peptide-based inhibitors of HIV PR were published while this work was in progress.[10] These macrocyclic inhibitors offer the further advantages of improved binding affinity to the enzyme resulting from entropic energy gain of conformationally constrained structures, greater stability against proteolytic enzymes, and enhanced specificity for the target enzyme. Herein we report the design and synthesis of a series of cyclophane-containing HIV PR inhibitors. The structural parameters determining enzyme inhibition, anti HIV activity, oral bioavailability, and aqueous solubility of this novel chemotype of an HIV PR inhibitor are also discussed.

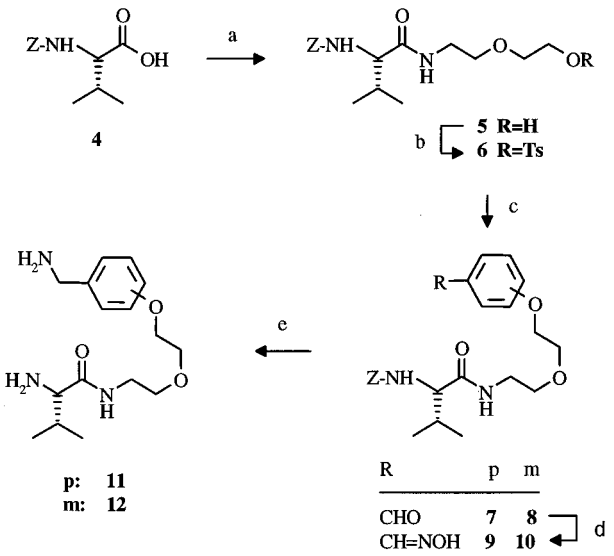
Inhibitor Design

To support the concept that compounds like **3** could represent novel HIV PR inhibitors, modeling studies were performed. The conformation of the 2-aminobenzylstatine-containing compound **1**, docked in the active site of the HIV-1 PR,[11] was used as a template for the construction of various macrocyclic inhibitors incorporating oligo ethylene glycol ether linkages between P1′ and P3′. The benzimidazole moiety in **1** was replaced by the appropriate linkers which were attached both in para and meta position to the benzene ring in P1′. Energy minimization[12] of these structures was followed by the prediction of biological activity (enzyme inhibition) using the 3D QSAR model described recently.[11] The calculated K_i value (40 to 50 nM) for paracyclophane **3** encouraged us to develop a synthetic route to these type of macrocycle to further investigate their physicochemical properties.

The crucial step in the synthesis of the cyclophanes is the regio- and stereoselective nucleophilic ring-opening of oxirane **13**[1] (Scheme 2) with diamines. The straightforward synthesis of the diamines **11**, **12** is outlined in Scheme 1. The appropriate amino alcohol was coupled to *N*-carbobenzyloxy valine using EDC·HCl and HOBt. The resulting alcohol **5** was converted to the tosylate **6** which was subsequently introduced in a phenolic O-alkylation of m- or p-hydroxybenzaldehyde. The best yields of crystalline aldehydes **7** and **8** were obtained when the reaction was carried out in DMSO with K_2CO_3 and 10% tetrabutylammonium iodide. DMSO was superior to DMF since fewer side products were formed.[13] Upon treatment of the aldehydes (**7**, **8**) with hydroxylamine and subsequent catalytic hydrogenation of the corresponding oximes (**9**, **10**; 10% Pd/C in methanol containing 4% formic acid), the carbobenzyloxy protecting group was simultaneously removed to give the desired diamines **11** and **12**.

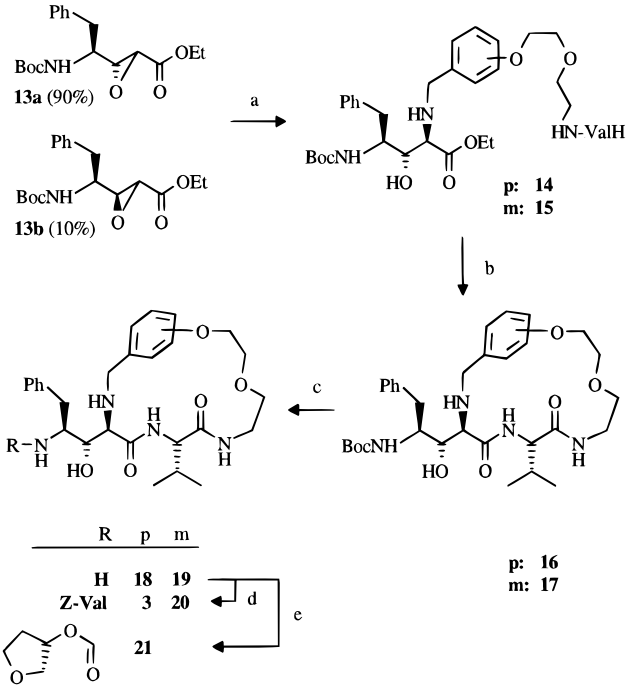
Synthesis of the seco acid esters **14** and **15** and subsequent cyclization follows the route described in Scheme 2. Upon treatment of oxiranes **13a**,**b**[1] with a diamine (**11**, **12**) in ethanol, only the sterically less hindered more nucleophilic nitrogen atom of the diamine reacts with the 2,3-epoxy ester to give the α-alkylamino

Scheme 1. Synthesis of the Diamines **11** and **12**[a]



[a]Conditions: (a) 2-(2-aminoethoxy)ethanol, HOBt, EDC·HCl, DMF, rt; (b) *p*-TsCl, pyridine, CH_2Cl_2, rt; (c) hydroxybenzaldehyde, K_2CO_3, tetrabutylammonium iodide, DMSO, 70 °C; (d) NH_2OH·HCl, NaOAc, H_2O, EtOH, 60 °C; (e) 10% Pd/C, H_2, MeOH, 4% HCOOH, rt.

Scheme 2. Epoxide Opening and Cyclization[a]



[a]Conditions: (a) **11** or **12**, EtOH, 80 °C; (b) (i) 0.4 M LiOH, dioxane, rt, (ii) 4 equiv of HODhbt, 4 equiv of EDC·HCl, 1 mM in THF, rt; (c) 6 N HCl, dioxane, rt; (d) Z-Val, HODhbt, EDC·HCl, dioxane, rt; (e) (+)-3(*S*)-hydroxytetrahydrofuran, phosgene in toluene, pyridine, CH_2Cl_2, rt.

β-hydroxy esters (**14**, **15**). The products were obtained in a diasteriomerically pure form after chromatographic workup. The regioselectivity of the nucleophilic attack and the inversion of configuration at the C-2 chiral center were determined previously with other examples from the 2-heterosubstituted statine ester series.[1] A 9:1 mixture of the diasteromeric oxiranes **13a** and **13b** is readily available via a three-step procedure starting from *N-tert*-butyloxycarbonyl phenylalaninol.[1] As shown previously, this mixture can be used in the next step without further purification.

Table 1. Macrocyclization: Yields of **16** as Determined by RP-HPLC

variations	solvent	*c*[mM] seco acid	reagent	yield[a] % **16**
solvent	DMF	1.3	HODhbt/EDC·HCl[b]	45
	AcN	1.0	HODhbt/EDC·HCl[b]	54
	EtOAc	1.3	HODhbt/EDC·HCl[b]	5
solvent and seco	THF	1.3	HODhbt/EDC·HCl[b]	67
acid concn	THF	2.7	HODhbt/EDC·HCl[b]	37
	CH_2Cl_2	2.7	HODhbt/EDC·HCl[b]	29
reagent	THF	1.3	HOBt/EDC·HCl[c]	55
	THF	1.3	HODhbt/DCC[d]	77
	THF	1.3	HOBt/DCC[e]	28
	THF	1.0	BOP/DMAP[f]	71
	THF	1.0	NMM[g]	49
	THF	1.0	PyBOP/DMAP[h]	72

[a] The kinetics of the macrocyclization was monitored by RP-HPLC: RP18 Column 150 × 3.9 mm, 10 mM ammonium acetate (pH = 7)/AcN 1:1 (v/v), flow rate 1 mL/min, rt, UV-detection at 210 nm. The yields represent the maximum turnover obtained after approximately 30 h. [b] 4 equiv of HODhbt, 4 equiv of EDC·HCl. [c] 4 equiv of HOBt, 4 equiv of EDC·HCl. [d] 4 equiv of HODhbt, 4 equiv DCC. [e] 4 equiv of HOBt, 4 equiv DCC. [f] 2 equiv of BOP, 3 equiv of DMAP. [g] 2 equiv of BOP, 3 equiv of NMM. [h] 2 equiv of PyBOP, 3 equiv of DMAP.

14 and **15** were saponified, and the macrocyclic amide bond was formed upon treatment with 4 equiv of EDC·HCl/HODhbt in dry THF. This macrocyclization step was optimized for the paracyclophane **16**. Although the seco-acid precursor of **16** hardly dissolves in THF, this solvent was superior to DMF, AcN, EtOAc, and dichloromethane (Table 1). The optimal concentration of the seco acid was found to be 1.3 mM. Doubling the concentration lowered the yield by a factor of two. After testing several coupling reagents it was found that HODhbt/DCC, HODhbt/EDC·HCl, and PyPOB/DMAP gave almost the same yields (67−77%).

Removal of the Boc protecting group of the cyclophanes **16** and **17** using 6 N HCl in dioxane yielded the central building blocks **18** and **19** for further derivation in P3 and P2. Coupling of the N-terminal 3(*S*)-hydroxytetrahydrofuran moiety with the deprotected cyclophane **18** was performed by prereacting a solution of phosgene with the appropriate alcohol. Addition of **18** to tetrahydrofuranyl chloroformate prepared *in situ* yielded the paracyclophane **21** in 80% yield.

Biological Results and Discussion

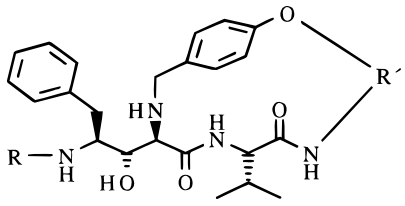
For all cyclophane derivatives reported here, the inhibition constant (K_i) was measured for HIV-1 proteinase. Antiviral activity (IC_{50}) was determined in an assay observing the HIV-1 strain IIIB induced cytopathic effect in *de novo* infected MT4 cells. Aqueous solubility was evaluated in phosphate-buffered saline at physiological pH and for selected compounds in the pH profile 4−8 (see Experimental Section for details).

First, we studied the effects of variation of the ring size of para- and metacyclophanes on biological activity. For each macrocycle, both the derivatives spanning the S3 to S3′ receptor binding site ("type P3-P3′", R = Cbz-Val; **3**, **22**−**24** and **28**, **20**, **29**) and their respective precursors with the *tert*-butyloxycarbonyl group occupying the P2 pocket ("type P2-P3′", R = Boc; **16**, **25**−**27** and **30**, **17**, **31**) were tested (Tables 2 and 3).

Increasing the ring size from a [13]- to a [20]-cyclophane and modifying the substitution pattern on the aromatic ring from para to meta (Table 2 and 3: **3**, **22**−**24** and **28**, **20**, **29**) did not effect the K_i values (6.6 to 9.4 nM) to a significant extent for the "type P3-P3′" inhibitors. These findings corroborate the modeling studies which had suggested that the linkers between the benzylamine in P1′ and P3′ would mediate contact to the solvent outside of the active site, and that the overall conformation of the residues occupying the S1′ and S2′ pockets should not be altered by the size of the rings.

Whereas, a slight increase of the antiviral activity was observed in the paracyclophane series when the ring size was altered from [13] (**22**) to [17] (**23**) (Table 2), the metacyclophane series activity dropped by a factor of approximately seven (Table 3: **28**, **20**, **29**). Even though K_i and IC_{50} values were nearly unaffected by the variation of the ring size and the substitution pattern, a larger difference between meta- and paracyclophanes was observed with regard to water solubility. The [14]-paracyclophane (**3**) is at least 50-fold more soluble than the [14]-metacyclophane (**20**). Contrary to expectation, an increasing number of oxyethylene moieties did not increase aqueous solubility. The [20]-paracyclophane (**24**) was found to be 70-fold less soluble than the [14]-paracyclophane **3** at physiological pH.

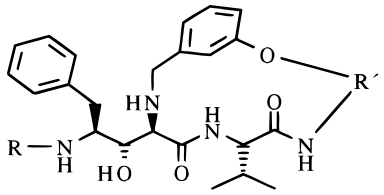
Table 2. Paracyclophanes: Inhibition of HIV-1 Protease, Antiviral Activity against HIV-1, IIIB, in MT4 Cells, and Aqueous Solubility at pH 7.4



no.	R	R′	ring size	K_i (nM)[a]	IC_{50} (nM)[b]	solubility (μM)	formula[c]
22	Z-Val	$(CH_2)_4$	[13]	6.6	27 ± 6 (6)	155	$C_{40}H_{53}N_5O_7$
3	Z-Val	$(CH_2)_2O(CH_2)_2$	[14]	7.9	21 ± 5 (6)	336	$C_{40}H_{53}N_5O_8 \cdot 0.8H_2O$
23	Z-Val	$(CH_2)_2O(CH_2)_2O(CH_2)_2$	[17]	6.9	16 ± 8 (6)	38	$C_{42}H_{57}N_5O_9 \cdot 0.9H_2O$
24	Z-Val	$(CH_2)_2O(CH_2)_2O(CH_2)_2O(CH_2)_2$	[20]	7.5	52 ± 3 (4)	5	$C_{44}H_{61}N_5O_{10} \cdot 0.6H_2O$
25	Boc	$(CH_2)_4$	[13]	17	1810 (1)	788	$C_{32}H_{46}N_4O_6 \cdot 0.6H_2O$
16	Boc	$(CH_2)_2O(CH_2)_2$	[14]	14	450 ± 213 (5)	1667	$C_{32}H_{46}N_4O_7 \cdot 0.9H_2O$
26	Boc	$(CH_2)_2O(CH_2)_2O(CH_2)_2$	[17]	12.6	169 ± 91 (4)	1049	$C_{34}H_{50}N_4O_8 \cdot 0.5H_2O$
27	Boc	$(CH_2)_2O(CH_2)_2O(CH_2)_2O(CH_2)_2$	[20]	23.2	1170 ± 14 (2)	1421	$C_{36}H_{54}N_4O_9 \cdot 0.8H_2O$

[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained.

Table 3. Metacyclophanes: Inhibition of HIV-1 Protease, Antiviral Activity against HIV-1, IIIB, in MT4 Cells, and Aqueous Solubility at pH 7.4



no.	R	R′	ring size	K_i (nM)[a]	IC_{50} (nM)[b]	solubility (μM)	formula[c]
28	Z-Val	$(CH_2)_4$	[13]	9.4	29 ± 17 (6)	1	$C_{40}H_{53}N_5O_7 \cdot 0.6H_2O$
20	Z-Val	$(CH_2)_2O(CH_2)_2$	[14]	7.6	60 ± 30 (5)	6	$C_{40}H_{53}N_5O_8$
29	Z-Val	$(CH_2)_2O(CH_2)_2O(CH_2)_2$	[17]	7.7	211 ± 116(5)	5	$C_{42}H_{57}N_5O_9 \cdot 0.8H_2O$
30	Boc	$(CH_2)_4$	[13]	16.1	335 ± 110 (5)	48	$C_{32}H_{46}N_4O_6$
17	Boc	$(CH_2)_2O(CH_2)_2$	[14]	68.8	935 ± 432 (6)	323	$C_{32}H_{46}N_4O_7$
31	Boc	$(CH_2)_2O(CH_2)_2O(CH_2)_2$	[17]	207.7	3000 (1)	607	$C_{34}H_{50}N_4O_8 \cdot 0.5H_2O$

[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained.

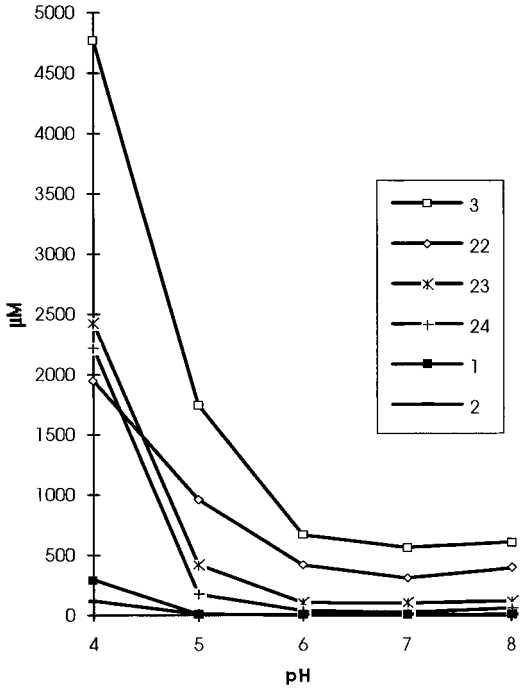


Figure 2. pH-dependency of the water solubility of the paracyclophanes **3**, **22**, **23**, **24** as well as **1** and **2** in Merck standard buffers (see Experimental Section for details).

When the aqueous solubilities of the [13], [14], [17], and [20]-paracyclophanes were measured at different pH in the range of 5 to 8, the same order **3** > **22** > **23** > **24** was observed (see Figure 2). At pH 4, where protonation of the secondary amine in the ring strongly contributes to solubility, **3** still is more soluble than **22**, **23**, and **24,** which are 20-fold more soluble than **1** and **2**.

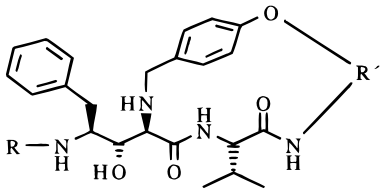
"Type P2-P3′" paracyclophanes (R = Boc, Table 2: **16**, **25**−**27**) still show notable protease inhibition with K_i values in the range of 12.6 to 23.2 nM. As before, the ring size can be regarded as essentially irrelevant to the thermodynamics of enzyme binding. This is not the case for the "type P2-P3′" metacyclophanes where the K_i value of the 17-membered ring is 12-fold higher than that of the 13-membered ring (Table 3: **31**, **30**). Though the antiviral activity of these "type P2-P3′" inhibitors is significantly lower than with the "type P3-P3′" compounds, the [17]paracyclophane (**26**) exhibits a remarkable antiviral potency (IC_{50} = 170 nM) and aqueous solubility (1 mM) at physiological pH.

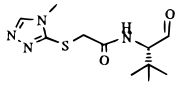
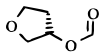
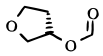
To summarize the results of the variation of ring size in para- and metacyclophanes, the paracyclophanes clearly proved superior to the metacyclophanes with regard to antiviral activity and aqueous solubility. Modifying the ring size in the paracyclophanes had only little effect on enzyme inhibition and antiviral activity, whereas the aqueous solubility decreased when the ring size was larger than 14. On the basis of the observation that multiple oxyethylene moieties decreased aqueous solubility, and the fact that the tetramethylene linker-containing paracyclophane **22** is >100 times more soluble than **1** or **2** at physiological pH, we conclude that the favorable hydrophilic characteristic is an inherent feature of the paracyclophane. A possible explanation could be the better accessibility of the polar atoms of the ring structure by the surrounding water molecules compared to the acyclic structures **1** and **2**.

Consequently, papracyclophanes **3**, **16**, and **26** were further modified in P3 and P2 (Table 4). Replacement of valine in P2 in **3** by *tert*-leucine yielded a [14]-paracyclophane (**32**) with almost the same solubility and affinity to the enzyme, but a slightly increased antiviral activity. Substitution of the N-terminal benzyloxycarbonyl group in **3** by the more polar triazolylthioacetyl moiety (**33**) led to the expected enhancement of the aqueous solubility by a factor of five. While this compound still is a good HIV PR inhibitor (K_i = 11 nM), it failed to inhibit virus growth in a cellular assay at concentrations as high as 3 μM. This finding illustrates again that antiviral activity is determined not only by inhibitory potency against the proteinase, but also by other poorly understood factors such as cellular uptake, stability, or intracellular distribution.[1]

Recently, it has been reported that 3(*S*)-tetrahydrofuranol is a good mimic for the P2-amino acid of selected protease inhibitors.[14] Encouraged by the potency of the paracyclophanes with a *tert*-butoxycarbonyl group in P2, we decided to replace this protecting group in **16** and **26** by the more hydrophilic tetrahydrofuran group. We expected that this replacement would result in even higher solubility. Indeed, with aqueous solubilities of 1.5 mg/mL and 2.1 mg/mL at pH 7.4, 14-paracyclophane **21** and [17]-paracyclophane **34** displayed a 1.5 and 3 times higher aqueous solubility compared to **16** and **26**.

Table 4. Paracyclophanes Modified in P3-P2: Inhibition of HIV-1 Protease, Antiviral Activity against HIV-1, IIIB, in MT4 Cells, and Aqueous Solubility at pH 7.4



no.	R	R´	K_i (nM)[a]	IC_{50} (nM)[b]	Solubility (μM)	Formula[c]
3	Z-Val	$(CH_2)_2$-O-$(CH_2)_2$	7.9	21±5 (6)	336	$C_{40}H_{53}N_5O_8 \cdot 0.8H_2O$
32	Z-Tle	$(CH_2)_2$-O-$(CH_2)_2$	8	15±9(6)	323	$C_{41}H_{55}N_5O_8 \cdot 1.2H_2O$
33		$(CH_2)_2$-O-$(CH_2)_2$	11.1	>3000 (2)	1708	$C_{38}H_{54}N_8O_7S$[d]
16	Boc	$(CH_2)_2$-O-$(CH_2)_2$	14	450±213 (5)	1667	$C_{32}H_{46}N_4O_7 \cdot 0.9H_2O$
21		$(CH_2)_2$-O-$(CH_2)_2$	8.2	157±15 (3)	2562	$C_{32}H_{44}N_4O_8$
26	Boc	$(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$	12.6	169±112 (5)	1049	$C_{34}H_{50}N_4O_8 \cdot 0.5H_2O$
34		$(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$	13.8	198±45 (4)	3350	$C_{34}H_{48}N_4O_9 \cdot 0.5H_2O$

[a]Usual standard deviation ±20%. [b]Mean value ± standard deviation. Number of determinations given in parentheses. [c]Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [d]N: calcd, 14.61; found, 13.29.

Table 5. Activity of Aminobenzylstatine-Containing Inhibitors against HIV-1, HIV-2, Human Cathepsin D and against HIV-1, IIIB, and HIV-2, EHO Replication in MT4 Cells and Aqueous Solubility at pH 7.4, and Oral Bioavailability

	HIV-1		HIV-2		IC_{50} (nM)	solubility	$AUC_{(0\rightarrow\infty)}$
no.	K_i (nM)[a]	IC_{50} (nM)[b]	K_i (nM)[a]	IC_{50} (nM)[b]	human cathepsin D	(μM)	(μM·h)
1	3.4	29 ± 19 (18)	120	460	380	<10	<0.4
2	9.5	242 ± 114 (10)	50	843 ± 335 (3)	>10000	<10	82.5
3	7.9	21 ± 5 (6)	19	20 ± 2 (3)	>10000	336	2.2
23	6.9	16 ± 8 (6)	27	37 ± 22(2)	>10000	38	<0.4
32	8.0	15 ± 9 (6)	16	53 ± 7 (2)	>10000	323	4.7

[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses.

The beneficial effect of this mimic of the P2-amino acid on enzyme inhibition and antiviral activity[14] was not observed for these paracyclophanes to a significant extent.

A selection of paracyclophanes (**3**, **23**, and **32**) was characterized in more detail (see Table 5). The compounds do not inhibit the mammalian aspartic proteinase cathepsin D which was affected by earlier compounds like **1** that span the P3 to P3′ subsites. The paracyclophanes are 2- to 4-fold weaker inhibitors of HIV-2 proteinase than of the HIV-1 enzyme. The difference in inhibition of the two enzymes is less pronounced than with other aminobenzylamino-substituted statine derivatives (e.g., **1**; Table 5). Inhibition of HIV-2 replication in MT4 cells is inhibited at similar (compound **3**) or 2- to 4-fold higher (**23**, **32**) concentrations than that of HIV-1.

Compound **23** was also tested against HIV in primary cells. In primary T4-lymphocytes, the paracyclophane showed IC_{50} = 3.1 nM for inhibition of HIV-1, strain IIIB, and IC_{50} = 17 nM in the case of HIV-2, strain MS. For inhibition of a monocytotropic variant of HIV-1, namely strain BaL, an IC_{50} value of 40 nM in primary monocytes was measured. These results may be compared with IC_{50} values for Saquinavir (= Ro31-8959[15]) of 4.3 and 4.9 nM for inhibition of HIV-1, IIIB and BaL, respectively, obtained in parallel experiments.

Paracyclophanes **3**, **23**, and **32** were administered orally to mice and plasma levels of the compounds were analyzed by HPLC (Table 5). At a dose of 125 mg/kg, the compounds showed only low oral bioavailability in mice compared to compound **2**, despite better aqueous solubility.

Summary

We have designed a versatile synthesis of HIV-proteinase inhibitors which contain a cyclophane ring system as the characteristic structural feature covering the S1′-S3′ subsites[2] of the enzyme. This central part of the inhibitors is available by regio- and stereospecific ring opening of N-protected 4-amino-2,3-epoxy-5-phenylpentanoic acid ester with appropriate α,ω-diamines followed by macrocyclization, and allows flexible derivation. Substitution of this building block with Z protected amino acids as P2-P3 substituent provided HIV-inhibitors which, in terms of anti-HIV activity, are comparable to Saquinavir (= Ro31-8959[15]). Remarkably, the ring size of the macrocycle has very little influence on enzyme inhibition and antiviral activity in both series of cyclophanes (meta and para). In terms of antiviral activity, the paracyclophanes turned out to be more active compared to their meta-substituted congeners. Besides high anti-HIV activity of several derivatives synthesized, the most striking feature of this novel type

of inhibitor is the good solubility in water at physiological pH. Solubilities at pH 7.4 as high as 2.1 mg/mL could be achieved while still inhibiting virus growth with an IC_{50} of 157 nM. Three protease inhibitors from this class (**3**, **23**, and **32**), were selected for their antiviral activity and were tested for oral bioavailability. Contrary to expectation, enhanced aqueous solubility (at least 3−10 times higher than **2**) did not result in enhanced systemic uptake in mice.

Experimental Section

Chemistry. ^{1}H-NMR spectra were recorded with a Bruker WC-250 or AMX-500 spectrometer at 300 K unless otherwise noted; chemical shifts are reported in ppm (δ) relative to internal Me_4Si. All J values are given in hertz (Hz). Elemental analyses were performed by Analytical Department, Sandoz Basle, Switzerland, and Mikroanalytisches Laboratorium, Institut für Physikalische Chemie der Universität Wien, and are within 0.4% of the theoretical value. All compounds gave correct FAB-MS spectra. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} glass plates (HPTLC, Merck). Preparative column chromatography was performed on silica gel (40−63 μm) under pressure (~0.2 mPa). Solvents were AR grade and were used without further purification. All reagents were obtained from commercial suppliers and were used without further purification. Evaporations were carried out *in vacuo* with a rotary evaporator. Melting points were determined with a thermovar apparatus (Reichert-Jung) or capillary melting point apparatus (neoLab) and are not corrected.

Representative methods for all compounds synthesized according to Scheme 1 are described. 2-[2-(2-Aminoethoxy)-ethoxy]ethanol and 2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]-ethanol were prepared from tri- and tetraethylene glycol by monotosylation and aminolysis with aqueous ammonia.[16]

2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]-ethanol (5). 2-(2-Aminoethoxy)ethanol (22.5 g, 0.213 mol), HOBt (27.7 g, 0.181 mol), and EDC·HCl (37.5 g, 0.196 mol) were added to an ice-cooled solution of Z-Val (49.1 g, 0.195 mol) in 500 mL of DMF. The reaction mixture was stirred for 24 h at room temperature. After evaporation of the solvent, ethyl acetate and water were added. The organic layer was washed with 1 N HCl, 1 N NaOH, and brine, dried, and concentrated *in vacuo*. Addition of cyclohexane precipitated 54.8 g of **5** (83%) as a white solid: mp 123−125 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.36−7.27 (m, 5H); 6.94 (bs, 1H); 5.62 (d, J = 9 Hz, 1H); 5.09 (bs, 2H); 3.95 (dd, J = 7, 9 Hz, 1H); 3.71−3.68 (m, 2H); 3.55−3.35 (m, 6H); 3.18 (bs, 1H); 2.10 (oct, J = 7 Hz, 1H); 0.96 and 0.93 (2d, J = 7 Hz, 6H).

Toluene-4-sulfonic Acid 2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]ethyl Ester (6). An ice-cooled solution of **5** (54.8 g, 0.162 mol) and pyridine (65 mL) in 550 mL of dichloromethane was treated with toluene-4-sulfonic acid chloride (30.9 g, 0.162 mol) in 50 mL of dichloromethane and stirred for 12 h while gradually warming to room temperature. The reaction mixture was washed with water, 1 N HCl, saturated aqueous sodium hydrogen carbonate, and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The product crystallized after trituration with diethyl ether: yield 61 g of **6** (76%) (chromatographic purification (eluent: ethyl acetate/*n*-hexane = 2/1) of the mother liquid yielded additional 6.3 g of **6**; overall yield 84%): mp 74−75 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.17 (d, J = 8 Hz, 2H); 7.36−7.27 (m, 7H); 6.44 (bs, 1H); 5.49 (d, J = 9 Hz, 1H); 5.11 (s, 2H); 4.19−4.15 (m, 2H); 4.02 (dd, J = 6, 9 Hz, 1H); 3.65−3.61 (m, 2H); 3.50−3.42 (m, 4H); 2.44 (s, 3H); 2.13 (oct, J = 7 Hz, 1H); 0.95 and 0.91 (2d, J = 7 Hz, 6H). Anal. ($C_{24}H_{32}N_2O_7S$·$0.3H_2O$) C, H, N.

4-[2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]-ethoxy]benzaldehyde (7). A solution of 4-hydroxybenzaldehyde (14 g, 0.115 mol) and **6** (56.5 g, 0.115 mol) in 500 mL of dimethyl sulfoxide was treated with K_2CO_3 (38.6 g, 0.279 mol) and 4.2 g (11.4 mmol) of tetrabutylammonium iodide. After stirring for 4 h at 70 °C, the solvent was removed *in vacuo*. Ethyl acetate and 1 N aqueous sodium hydroxide were added, and the organic layer was dried over Na_2SO_4 and concentrated. After addition of *n*-hexane the product crystallized: yield 42.5 g of **7** (83%) (chromatographic purification (ethyl acetate/*n*-hexane = 1/1) of the mother liquid yielded additional 6.5 g of **7**; overall yield: 96%); mp 115−118 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 9.89 (s, 1H); 7.83 and 7.00 (2d, J = 9 Hz, 4H); 7.37−7.30 (m, 5H); 6.31 (bs, 1H); 5.41 (d, J = 9 Hz, 1H); 5.09 (s, 2H); 4.20−4.16 (m, 2H); 3.96 (dd, J = 6, 9 Hz, 1H); 3.84−3.81 (m, 2H); 3.64−3.45 (m, 4H); 2.09 (oct, J = 7 Hz, 1H); 0.94 and 0.90 (2d, J = 7 Hz, 6H). Anal. ($C_{24}H_{30}N_2O_6$·$0.3H_2O$) C, H, N.

3-[2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]-ethoxy]benzaldehyde (8). Preparation was as described for **7**: yield 3.97 g of **8** (45%); mp 91−94 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 9.97 (s, 1H); 7.47−7.41 (m, 2H); 7.36−7.27 (m, 6H); 7.26−7.18 (m, 1H); 6.37 (bs, 1H); 5.42 (d, J = 9 Hz, 1H); 5.09 (s, 2H); 4.19−4.15 (m, 2H); 3.98 (dd, J = 6, 9 Hz, 1H); 3.84−3.80 (m, 2H); 3.63−3.47 (m, 4H); 2.09 (oct, J = 7 Hz, 1H); 0.96 and 0.90 (2d, J = 7 Hz, 6H). Anal. ($C_{24}H_{30}N_2O_6$·$0.1H_2O$) C, H, N.

4-[2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]-ethoxy]benzaldehyde Oxime (9). **7** (48.4 g, 0.109 mol) was dissolved in 200 mL of ethanol (gentle warming) and treated with hydroxyammonium chloride (11.4 g, 0.164 mol) and 13.5 g (0.165 mol) sodium acetate dissolved in 100 mL of water. The reaction mixture was stirred at 60 °C, for 90 min, the solvent was removed *in vacuo*, ethyl acetate and water were added, and the organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over Na_2SO_4, and concentrated. The title compound precipitates after addition of *n*-hexane: yield 50.0 g of **9** (quantitative); mp 71−73 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 9.70 (s, 1H); 8.08 (s, 1H); 7.49 and 6.87 (2d, J = 9 Hz, 4H); 7.38−7.23 (m, 5H); 6.45 (bs, 1H); 5.59 (d, J = 9 Hz, 1H); 5.13 and 5.06 (AB, J = 12 Hz, 2H); 4.12−4.08 (m, 2H); 3.96 (dd, J = 7, 9 Hz, 1H); 3.82−3.79 (m, 2H); 3.68−3.40 (m, 4H); 2.09 (oct, J = 7 Hz, 1H); 0.90 and 0.87 (dd, J = 7 Hz, 6H). Anal. ($C_{24}H_{31}N_3O_6$) C, H, N.

3-[2-[2-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]ethoxy]-ethoxy]benzaldehyde Oxime (10). Preparation was as described for **9**: yield 3.1 g of **10** (86%); mp 114−117 °C; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 9.61 (s, 1H); 8.15 (s, 1H); 7.49 (s, 1H); 7.35−7.22 (m, 6H); 7.04 (d, J = 8HZ, 1H); 6.96−6.92 (m, 2H); 5.78 (d, 1H); 5.14 and 5.07 (AB, J = 12 Hz, 2H); 4.17−4.14 (m, 2H); 3.98 (dd, J = 7, 9 Hz, 1H); 3.86−3.53 (m, 5H); 3.38−3.23 (m, 1H); 2.06 (oct, J = 7 Hz, 1H); 0.94 and 0.93 (dd, J = 7 Hz, 6H). Anal. ($C_{24}H_{31}N_3O_6$) C, H, N.

4-[2-[2-[(Valyl)amino]ethoxy]ethoxy]benzylamine (11). A solution of **9** (49.5 g, 0.108 mol) in 1 L of methanol and 60 mL of formic acid was treated with 4 g of 10% Pd/C and stirred under atmospheric pressure of hydrogen for 3 h. After filtration over Celite the solvents were removed *in vacuo*. The oily residue was suspended in dichloromethane and sufficient 1 N NaOH added to maintain in the aqueous phase at pH > 8. The organic layer was dried over Na_2SO_4, the solvent was removed, and the residue was chromatographed on silica gel (eluent: dichloromethane/methanol/aqueous ammonia = 10/1/0.1): yield 15.1 g of **11** (44%) as a waxlike solid; ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.50 (bs, 1H); 7.21 and 6.87 (2d, J = 9 Hz, 4H); 4.12−4.08 (m, 2H); 3.82−3.79 (m, 4H); 3.65−3.60 (m, 2H); 3.51−3.44 (m, 2H); 3.18 (d, J = 4 Hz, 1H); 2.24 (dhept, J = 4, 7 Hz, 1H); 1.53 (bs, 4H); 0.96 and 0.80 (2d, J = 7 Hz, 6H). Anal. ($C_{16}H_{27}N_3O_3$·$0.4H_2O$) C, H, N.

3-[2-[2-[(Valyl)amino]ethoxy]ethoxy]benzylamine (12). Preparation was as described for **11**: yield 1.43 g of **12** (48%); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.52 (bs, 1H); 7.22 (t, J = 8 Hz, 1H); 6.94−6.83 (m, 3H); 6.78 (dd, J = 2, 8 1Hz, 1H); 4.13−4.09 (m, 2H); 3.82 (s, 2H), 3.82−3.78 (m, 2H); 3.67−3.55 and 3.55−3.40 (2m, 3H); 3.16 (d, J = 4 Hz, 1H); 2.22 (dhept, J = 7 Hz, 4Hz, 1H); 1.58 (bs, 4H); 0.94 and 0.79 (2d, J = 7 Hz, 6H). Anal. ($C_{16}H_{27}N_3O_3$·$0.44H_2O$) C, H, N.

(2*R*,3*S*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-2-[4-[2-[2-[(valyl)amino]ethoxy]ethoxy]benzylamino]-5-phenylpentanoic Acid Ethyl Ester (14). A solution of **11** (14.5 g, 46.9 mmol) and **13**[1] (11.78 g, 35.1 mmol) in 400 mL of ethanol was kept at 80 °C for 24 h. The solvent

was evaporated *in vacuo*, and the residue was purified by silica gel chromatography (eluent: ethyl acetate/*n*-hexane = 1/1): yield 11.55 g of **14** (51%); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.49 (bs, 1H); 7.26 and 6.85 (2d, J = 7 Hz, 4H); 7.30−7.18 (m, 5H); 4.91 (d, J = 9 Hz, 1H); 4.23−4.13 (m, 2H); 4.10−4.08 (m, 2H); 4.05 (bq, J = 8 Hz, 1H); 3.80 (dd, J = 3, 4 Hz, 2H); 3.70 and 3.52 (AB, J = 12 Hz, 2H); 3.68 (d, J = 8 Hz); 3.65−3.58 (m, 2H); 3.52−3.45 (m, 2H); 3.29 (d, J = 8 Hz, 1H); 3.18 (d, J = 6 Hz, 1H); 2.95−2.80 (m, 2H); 2.23 (m, 1H); 1.37 (s, 9H); 1.26 (t, J = 7 Hz, 3H); 0.96 and 0.82 (2d, J = 7 Hz, 6H). Anal. ($C_{34}H_{52}N_4O_8 \cdot 0.5H_2O$) C, H, N.

(2*R*,3*S*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-2-[3-[2-[2-[(valyl)amino]ethoxy]ethoxy]benzylamino]-5-phenylpentanoic Acid Ethyl Ester (15). Preparation was as described for **14**: yield 1.05 g of **15** (55%); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.55 (bs, 1H); 7.30−7.06 (m, 6H); 6.90−6.70 (m, 3H); 5.10 (d, J = 8 Hz, 1H); 4.05−4.18 (m, 5H); 3.79 (dd, J = 3 Hz, 5Hz, 2H); 3.70−3.40 (m, 8H); 3.27 (d, J = 7 Hz, H); 3.16 (d, J = 4 Hz, H); 2.90−2.80 (m, 2H); 2.20 (dhept, J = 4 Hz, 7Hz, H); 2.00 (bs, 5H); 1.35 (s, 9H); 1.23 (t, J = 7 Hz, H); 0.94 and 0.79 (2d, J = 7 Hz, 6 H).

(1′*S*,2′*S*,9*S*,12*R*)-12-[2′-[[(1,1-Dimethylethoxy)carbonyl]amino]-1′-hydroxy-3′-phenylprop-1′-yl]-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo[14]paracyclophane (16). **14** (13.2 g, 20.5 mmol) was dissolved in 240 mL of dioxane and treated with aqueous LiOH (76.7 mL of a 0.4 M solution, 30.7 mmol). The reaction mixture was stirred for 16 h at room temperature (completeness of reaction was judged by HPTLC analysis), treated with 1 N HCl (30.7 mL), and freeze-dried. The freeze-dried seco acid (7 g, 10.29 mmol) was suspended in 7.5 L of dry tetrahydrofuran, cooled in an ice-bath, and treated with HODhbt (6.72 g, 41.2 mmol) and DCC (8.49 g, 41.2 mmol). After stirring for 5 days at room temperature, the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate. The suspension was filtered, and the filtrate was washed with 1 N NaOH and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Pure **16** was obtained as an amorphous solid by chromatography on silica gel (eluent: gradient ethyl acetate/methanol = 1/0 to 1/0.05): yield 3.51 g (57%); mp 109−114 °C; ^{1}H-NMR (500 MHz; DMSO-d_6; 373 K) δ 7.34 (bs, 1H); 7.29−7.24 (m, 4H); 7.19−7.16 (m, 1H); 6.98 and 6.78 (2d, J = 8 Hz, 4H); 6.66 (d, J = 8 Hz, 1H); 5.85 (bs, 1H); 4.30 (q, J = 8 Hz, 1H); 4.23−4.15 (m, 3H); 3.95 (dd, j = 5, 8 Hz, 1H); 3.83 and 3.37 (AB, J = 15 Hz, 2H); 3.75−3.61 (m, 4H); 3.52−3.48 (m, 1H); 3.17 (bt, 1H); 2.92 (d, J = 10 Hz, 1H); 2.87−2.77 (m, 3H); 1.86 (dhept, J = 5, 7 Hz, 1H); 1.41 (s, 9H); 0.79 and 0.78 (2d, J = 7 Hz, 6H). Anal. ($C_{32}H_{46}N_4O_7 \cdot 0.9H_2O$) C, H, N.

(1′*S*,2′*S*,9*S*,12*R*)-12-[2′-[[(1,1-Dimethylethoxy)carbonyl]amino]-1′-hydroxy-3′-phenylprop-1′-yl]-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo[14]metacyclophane (17). Preparation was as described for **16**. Instead of DCC, EDC·HCl was used: yield 180 mg of **17** (19%); mp 155−156 °C; ^{1}H-NMR (250 MHz; $CDCl_3/D_2O$; 373 K) δ 7.29−7.11 (m, 5H); 7.12 (t, J = 8 Hz, 1H); 6.81−6.78 (m, 3H); 4.17−4.07 (m, 4H); 3.69−3.35 (m, 8H); 2.95−3.10 (m, 2H); 2.77 and 2.72 (AB-part of ABX, J = 13 Hz, 2H); 1.95 (oct, J = 7 Hz, 1H); 1.31 (s, 9H); 0.82 and 0.81 (2d, J = 7 Hz, 6H). Anal. ($C_{32}H_{46}N_4O_7$) C, H, N.

(1′*S*,2′*S*,9*S*,12*R*)-12-(2′-Amino-1′-hydroxy-3′-phenylprop-1′-yl)-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo-[14]paracyclophane Dihydrochloride (18·2HCl). **16** (3.5 g, 5.85 mmol) was dissolved in 10 mL of dioxane and treated with an equal amount of 18% aqueous HCl. The solvents were immediately evaporated. Completeness was judged by HPTLC analysis. The oily residue was dissolved in water and lyophilized (the free base (**18**) is sparingly soluble in ethyl acetate and in dichloromethane) to give an amorphous solid: yield 3.34 g of **18**·2HCl (100%); mp 175−180 °C; ^{1}H-NMR (500 MHz; D_2O) δ 7.92 (dd, J = 8, 4 Hz, 1H); 7.50−7.47 (m, 2H); 7.45−7.42 (m, 1H); 7.37−7.35 (m, 2H); 7.28 and 6.93 (2d, J = 9 Hz, 4H); 4.53 and 4.00 (AB, J = 14 Hz, 2H); 4.28 and 4.22 (AB-part of ABXY, J = 12, 6, 2 Hz, 2H); 4.18 (t, J = 5 Hz, 1H); 3.98 (d, J = 6 Hz, 1H); 3.96−3.89 (m, 2H); 3.89−3.83 (m, 1H); 3.83−3.67 (m, 4H); 3.79 (d, J = 4Hz, 1H); 3.14 and 2.98 (AB-part of ABX, J = 14, 9, 6 Hz, 2H); 3.04−2.98 (m, 1H); 1.81 (dhept, J = 7, 4 Hz, 1H); 0.76 and 0.70 (2d, J = 7 Hz, 6H). Anal. ($C_{27}H_{40}Cl_2N_4O_5 \cdot 2H_2O$) C, H, N.

(1′*S*,2′*S*,9*S*,12*R*)-12-(2′-Amino-1′-hydroxy-3′-phenylprop-1′-yl)-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo-[14]metacyclophane (19). Preparation was as described for **18**. The free base was obtained by treating a solution of the dihydrochloride of **19** in a small amount of water with saturated aqueous K_2CO_3 solution, and extraction of the free base with ethyl acetate: yield 200 mg of **19** (100%); ^{1}H-NMR (250 MHz; D_2O) δ 8.20−8.11 (m, 1H); 7.49−7.28 (m, 6H); 7.14−7.07 (m, 2H); 6.91 (s, 1H); 4.48 and 4.12 (AB, J = 14 Hz, 2H); 4.26 (t, J = 6 Hz, 1H); 4.16 (d, J = 5 Hz, 1H); 4.28−4.15 (m, 2H); 3.86 (d, J = 6 Hz, 1H); 3.91−3.64 (m, 6H); 3.12 and 2.94 (ABX; J = 14, 9, 5 Hz, 2H); 3.14−3.02 (m, 1H); 1.96 (oct, J = 7 Hz, 1H); 0.88 and 0.82 (2d, J = 7 Hz, 6H). Anal. ($C_{27}H_{38}N_4O_5 \cdot 1.7H_2O$) C, H, N.

(1′*S*,2′*S*,2″*S*,9*S*,12*R*)-12-[2″-[[*N*-[(Benzyloxy)carbonyl]-valyl]amino]-1′-hydroxy-3′-phenylprop-1′-yl]-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo[14]paracyclophane (3). An ice-cooled solution of **18**·2HCl (100 mg, 0.175 mmol) in 10 mL of dioxane was treated with Z-Val (93 mg, 0.351 mmol), HODhbt (57 mg, 0.349), EDC·HCl (67 mg, 0.35 mmol), and triethylamine (147 μL, 1.05 mmol). After stirring overnight at room temperature, the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and 0.1 M HCl, and the organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (dichloromethane/methanol = 10/1) yielded the title compound as an amorphous solid: yield: 65 mg of **3** (50%); mp 107−110 °C; ^{1}H-NMR (500 MHz; $CDCl_3$; 330 K) δ 7.35−7.34 (m, 3H); 7.34−7.26 (m, 2H); 7.26−7.25 (m, 4H); 7.19−7.14 (m, 1H); 7.03 and 6.83 (2d, J = 9 Hz, 4H); 6.56 (d, J = 8 Hz, 1H); 6.33 (d, J = 9 Hz, 1H); 5.31−5.26 (m, 1H); 5.26 (d, J = 8 Hz, 1H); 5.17−5.10 (AB, J = 12 Hz, 2H); 4.65 (q, J = 8 Hz, 1H); 4.20 (t, J = 3 Hz, 2H); 3.87 (d, J = 8 Hz, 1H); 3.80−3.59 (m, 9H); 3.51−3.48 (m, 1H); 3.03 (d, J = 9 Hz, 1H); 3.00 and 2.93 (AB-part of ABX, J = 14, 8, 2 Hz, 2H); 2.90−2.85 (m, 1H); 1.97 (dhept, J = 7, 5 Hz, 1H); 0.98 (s, 9H); 0.78 and 0.75 (2d, J = 7 Hz, 6H). Anal. ($C_{41}H_{55}N_5O_8 \cdot 1.2H_2O$) C, H, N.

(1′*S*,2′*S*,2″*S*,9*S*,12*R*)-12-[2″-[[*N*-[(Benzyloxy)carbonyl]-valyl]amino]-1′-hydroxy-3′-phenylprop-1′-yl]-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo[14]metacyclophane (20). Preparation was as described for **3**: yield 158 mg of **20** (45%); mp 86−92 °C; ^{1}H-NMR (500 MHz; $CDCl_3$; 330 K) δ 6.83 (d, J = 8 Hz, 1H); 7.36−7.23 (m, 10H); 7.20−7.17 (m, 2H); 7.06 (t, J = 2 Hz, 1H); 6.83 (d, J = 8 Hz, 1H); 6.80−6.78 (m, 1H); 6.42 (d, J = 9 Hz, 1H); 6.20 (bs, 1H); 5.11 and 5.08 (AB, J = 12Hz, 2H); 5.03 (d, J = 8 Hz, 1H); 4.70 (bs, 1H); 4.49 (q, J = 8 Hz, 1H); 4.26 (dd, J = 5, 9 Hz, 1H); 4.21−4.19 (m, 2H); 3.94 (dd, J = 1, 10 Hz, 1H); 3.90 (dd, J = 5, 8 Hz, 1H); 3.78−3.72 (m, 2H); 3.68−3.64 (m, 2H); 3.52−3.46 (m, 3H); 3.22 (d, J = 9.5 Hz, 1H); 3.10−3.02 (m, 1H); 3.00 and 2.96 (AB-part of ABX, J = 14, 8 Hz, 2H); 2.24 (2dh, J = 5, 7 Hz, 2H); 0.90 and 0.85 and 0.79 and 0.73 (4d, J = 7 Hz, 12H). Anal. ($C_{40}H_{53}N_5O_8$) C, H, N.

(1′*S*,2′*S*,3″*S*,9*S*,12*R*)-12-[2′-[[(3″-Tetrahydrofuranyl-oxy)carbonyl]amino]-1′-hydroxy-3′-phenylprop-1′-yl]-9-(1-methylethyl)-7,10,13-triaza-1,4-dioxa-8,11-dioxo[14]-paracyclophane (21). A cooled solution (0 °C) of phosgene (420 μL, 1.93 M solution in toluol) and pyridine (64 μL) in 5 mL of dichloromethane were treated with a solution of (+)-3(*S*)-hydroxytetrahydrofuran (54 μL, 0.61 mmol in 5 mL of dichloromethane) via a syringe pump at a rate of 2.5 mL/h. **18** (200 mg, 0.40 mmol) was suspended in 5 mL of dichloromethane and 64 μL of pyridine, cooled to 0 °C, and treated with 7 mL of the above described solution. After stirring for 16 h at room temperature, ethyl acetate and saturated aqueous hydrogen carbonate were added. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (gradient: ethyl acetate/methanol 20/1 to 10/1) yielded 180 mg of **21** (75%); mp 175−178 °C; ^{1}H-NMR (500 MHz; DMSO-d_6; 410 K) δ 7.29−7.25 (m, 4H); 7.19−7.16 (m, 1H); 7.07 (bs, 1H); 6.99−6.80 (2d,

J = 8 Hz, 4H); 6.62 (d, $J = J$ = 8 Hz, 1H); 6.09 (d, J = 9 Hz, 1H); 5.14−5.10 (m, 1H); 4.31 (dq, J = 7, 2 Hz, 1H); 4.24−4.17 (m, 3H); 3.96 (dd, J = 8, 5 Hz, 1H); 3.86 and 3.38 (AB, J = 15 Hz, 2H); 3.82 (q, J = 8 Hz, 1H); 3.79 (dd, J = 10, 5 Hz, 1H); 3.76−3.66 (m, 3H); 3.64−3.58 (m, 3H); 3.52−3.48 (m, 1H); 3.28 (bd, 1H); 2.97 (d, J = 9 Hz, 1H); 2.89 and 2.82 (ABX, J = 14, 8 Hz, 2H); 2.92−2.83 (m, 1H); 2.43 (bs, 1H); 2.13 (oct, J = 7 Hz, 1H); 2.93−1.84 (m, 2H); 0.81 and 0.80 (2d, J = 7 Hz, 6H). Anal. ($C_{32}H_{44}N_4O_8$) C, H, N.

Biology. HIV-1 proteinase was expressed in *Escherichia coli* and was purified to homogeneity as described.[17] Enzymatic activity was measured by following cleavage of the substrate H-Lys-Ala-Arg-Val-Leu-pNph-Glu-Ala-Nle-NH_2, originally described by Richards et al.[18] with the modifications reported previously.[19] Recombinant HIV-2 proteinase was kindly supplied by P. Strop, Prague, and was assayed at pH 4.7 using the same substrate and similar condition as for HIV-1 proteinase. All test compounds were dissolved in dimethyl sulfoxide to 10 mM and diluted into assay buffer; the concentration of dimethyl sulfoxide did not exceed 5%.

The HIV-1 strain IIIB and the HIV-2 strain EHO have been described.[20,21] Inhibition of virus-induced cytopathic effect was determined in MT4 cells by measuring the viability of both HIV- and mock-infected cells essentially as in Pauwels et al.[22] Viability was assessed spectrophotometrically via in situ reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Compounds were added postadsorption. Concentrations of test compounds reducing cell density of mock infected cultures by 50% (CC_{50}) and those reducing virus-induced cytopathic effect by 50% (IC_{50}) were determined by viability staining 5 days after infection.

Primary T4-lymphocytes were purified from human spleens obtained from healthy donors by using a commercial kit ("Lympho-Kwik") as described recently.[19] Cells were stimulated with phytohemagglutinin (PHA) and were infected with HIV-1, IIIB, or HIV-2, MS, as described, and incubated in medium containing IL-2.[19] Test compound was added after stimulation and virus adsorption. Every 3−4 days half of the supernatant of the infected cultures was removed and replaced by fresh medium containing IL-2 and the test compound at the particular concentration. Preparation and infection of monocytes were performed essentially as in Perno et al., 1989,[23] with the modifications described.[24] Mononuclear cells were isolated from healthy, HIV negative donors using Ficoll density separation and adherence to tissue culture plates for 5 days. Preparations obtained by this procedure were >95% positive for nonspecific esterase; cell viability was always >95%. The cells were infected with HIV-1, strain BaL, and were cultivated in the presence of different drug concentrations. Every 3−4 days the supernatant of the infected cultures was removed and replaced by fresh medium containing the test compound at the particular concentration. IC_{50} and IC_{90} values were calculated by comparing p24 antigen concentrations (determined by ELISA, Coulter) in supernatants of treated, infected cells to those of untreated, infected cells at days postinfection, when p24 production was increasing exponentially.

For oral bioavaiability studies, female Balb/c mice (n = 2 per time point) were used. For oral administration (by gavage) the animals were fasted for 24 h prior to the start of and throughout the experiment; water was given *ad libitum* and the compounds were administered in a microemulsion formulation. Blood analysis, involving solid-phase extraction and HPLC determination, was performed as described.[3]

Solubilities in phosphate-buffered saline (PBS, pH 7.4) and in Merck standard buffers (pH: 4, 5, 6, 7, 8) were determined as follows: A weighed amount (about 1 mg) of inhibitor in amorphous form (lyophilized out of dioxane to constant weight) was combined with 1 mL of PBS (buffer). Samples were vortexed vigorously for 1 min and sonicated at 37 °C for 30 min. The suspensions were filtered through a G3 sinter-funnel (or alternatively a pasteur pipette filled with a cotton plug), and the filtrate was centrifuged at 100000 rpm for 30 min. The supernatant was analyzed by HPLC (column: RP-C18; isocratic elution with 10 mM aqueous NH_4OAc/AcN = 50/50; flow rate: 1 mL/min; UV detection at 210 nm; rt). The concentration of the compound was calculated by external standardization of peak area versus concentration for each sample. Minimal detectable concentration: 0.1 μg/mL; standard deviation: ±10%.

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References

(1) Scholz, D.; Billich, A.; Charpiot, B.; Ettmayer, P.; Lehr, P.; Rosenwirth, B.; Schreiner, E.; Gstach, H. Inhibitors of HIV-1 Proteinase Containing 2-Heterosubstituted 4-Amino-3-hydroxy-5-phenylpentanoic Acid: Synthesis, Enzyme Inhibition, and Antiviral Activity. *J. Med. Chem.* **1994**, *37*, 3079−3089.

(2) The nomenclature used for describing the individual amino acid residues (P3, P2, P1, P1′, P2′, etc.) of a substrate and the corresponding subsites (S3, S2, S1, S1′, S2′, etc.) of a protease is that of Schechter and Berger: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−162.

(3) Billich, A.; Charpiot, B.; Fricker, G.; Gstach, H.; Lehr, P.; Peichl, P.; Scholz, D.; Rosenwirth, B. HIV Proteinase Inhibitors Containing 2-Aminobenzylstatine as a Novel Scissile Bond Replacement: Biochemical and Pharmacological Characterization. *Antivir. Res.* **1994**, *25*, 215−233.

(4) (a) Billich, A.; Fricker, G.; Müller, I.; Donatsch, P.; Ettmayer, P.; Gstach H.; Lehr P.; Peichl P.; Scholz D.; Rosenwirth B. SDZ PRI 053, an Orally Bioavailable Human Immunodeficiency Virus Type 1 Proteinase Inhibitor Containing the 2-Aminobenzylstatine Moiety. *Antimicrob. Agents Chemother.* **1995**, *39*, 1406−1413. (b) Lehr, P.; Billich, A.; Charpiot, B.; Gstach, H.; Ettmayer, P.; Scholz, D.; Rosenwirth, B. Inhibitors of HIV-1 Protease Containing 2-Aminobenzyl Substituted 4-Amino-3-hydroxy-5-phenylpentanoic Acid (AHPPA): Synthesis, Activity, and Oral Bioavailability. *J. Med. Chem.* **1996**, 39, 2060−2067.

(5) Billich, A.; Scholz, D.; Charpiot, B.; Gstach, H.; Lehr P.; Peichl, P.; Rosenwirth B. Potent and Orally Bioavailable HIV-1 Proteinase Inhibitors Containing the 2-Aminobenzylstatine Moiety. *Antivir. Chem. Chemother.* **1995**, 6(5), 327−336.

(6) Kempf, D. J.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P.; Sham, H. L. Symmetry-based Inhibitors of HIV Protease. Structure−Activity Studies of Acylated 2,4-Diamino-1,5-diphenyl-3-hydroxypentane and 2,5-Diamino-1,6-diphenylhexane-3,4-diol. *J. Med. Chem.* **1993,** *36*, 320−330.

(7) Kageyama, S.; Anderson, B. D.; Hoesterey, B. L.; Hayashi, H.; Kiso, Y.; Flora, K. P.; Mitsuya, H. Protein Binding of Human Immunodeficiency Virus Protease Inhibitor KNI-272 and Alteration of its in Vitro Antiretroviral Activity in the Presence of High Concentrations of Proteins. *Antimicrob. Agents Chemother.* **1994**, *38*, 1107−1111.

(8) Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palu, G.; Blair, E. D. A Bioactive Fullerene Peptide. *J. Med. Chem.* **1994**, *37*, 4558−4562. Brinckworth, R.; Woon, T.; Fairlie, D. Inhibition of HIV-1 Proteinase by non-Peptide Carboxylates. *Biochem Biophys. Res. Commun.* **1991**, *176*, 241−246. DesJarlais, R.; Seibel, G.; Kuntz, I.; Furth, P.; Alvarez, J.; Ortiz de Montellano, P.; DeCamp, D.; Babe, L.; Craik, C.; Structure Based Design of Nonpeptide Inhibitors Specific for the Human Immunodeficieny Virus 1 Protease. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6644−6648.

(9) Weber, A. E.; Steiner, M. G.; Krieter, P. A.; Colletti, A. E.; Tata, J. R.; Halgren, T. A.; Ball, R. G.; Doyle, J. J.; Schorn, T. W.; Stearns, R. A. Highly Potent, Orally Active Diester Macrocyclic Human Renin Inhibitors. *J. Med. Chem.* **1992**, *35*, 3755−73. Reily, M. D.; Thanabal, V.; Lunney, E. A.; Repine, J. T.; Humblet, C. C.; Wagner, G. Design, Synthesis and Solution Structure of a Renin Inhibitor. Structural Constraints from NOE, and Homonuclear and Heteronuclear Coupling Constants Combined with Distance Geometry Calculations. *FEBS Lett.* **1992**, *302*, 97−103. Weber, A. E.; Halgren, T. A.; Doyle, J. J.; Lynch, R. J.; Siegl, P. K.; Parsons, W. H; Greenlee, W. J.; Patchett, A. A. Design and Synthesis of P2-P1′-linked Macrocyclic Human Renin Inhibitors. *J. Med. Chem.* **1991**, *34*, 2692−2701. Fesik, S. W.; Bolis, G.; Sham, H. L.; Olejniczak, E. T. Structure Refinement of a Cyclic Peptide from Two-Dimensional NMR Data and Molecular Modeling. *Biochemistry* **1987**, *26*, 1851−1859.

(10) Podlogar, B. L.; Farr, R. A.; Friedrich, D.; Tarnus, C.; Huber E. W.; Cregge, R. J.; Schirlin, D. Design, Synthesis, and Conformational Analysis of a Novel Macrocyclic HIV-Protease Inhibitor. *J. Med. Chem.* **1994**, *37*, 3684−3692. Smith, R. A.; Coles, P. J.;

Chen, J. J.; Robinson, V. J.; Macdonald, I. D.; Carriere, J.; Krantz, A. Design, Synthesis, and Activity of Conformationally-Constrained Macrocyclic Peptide-Based Inhibitors of HIV Protease. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2217−2222.

(11) For full details of the docking experiments see: Krömer, R. T.; Ettmayer, P.; Hecht, P. 3D Qantitative Structure-Activity Relationships of Human Immunodeficiency Virus Type-1 Proteinase Inhibitors: Comparative Molecular Field Analysis of 2 Heterosubstituted Statine Derivatives - Implications for the Design of Novel Inhibitors. *J. Med. Chem.* **1995**, *38*, 4917−4928.

(12) The structures were minimized in a two-step procedure: (A) using the standard Tripos Force Field without electrostatics applying the Powell minimization technique. The convergence criteria were set to be an energy change of <0.05 kcal/mol in two consecutive steps; (B) the resulting structure was minimized under the same conditions in the protease environment (brookhaven: 4HVP without inhibitor) which was defined as completely rigid. Distance constraints corresponding to a set of hydrogen bonds between inhibitor and protein were applied as described in footnote 11. If the conformation of the modeled structure displayed unfavorable interactions or did not fulfill the distance constrain requirements to a large extent, the starting conformation was manually modified and the new conformer was subjected again to minimization procedures A and B.

(13) The phenolic O-alkylation was done in analogy to: Chung, J. Y. L.; Zhao, D.; Hughes, D. L.; Grabowski, E. J. J. A Practical Synthesis of Fibrinogen Receptor Antagonist MK-383. Selective Functionalisation of (S)-Tyrosine. *Tetrahedron* **1993**, *49*, 5767−5776.

(14) Ghosh. A. K.; Thompson, W. J.; McKee, S. P.; Duong, T. T.; Lyle, T. A.; Chen, J. C.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson P. S. 3-Tetrahydrofuran and Pyran Urethanes as High-Affinity P2-Ligands for HIV-1 Protease Inhibitors. *J. Med. Chem.* **1993**, *36*, 292−294.

(15) Vella, S. Update on a Proteinase Inhibitor. *AIDS* **1994**, 8 (suppl 3), S25−S29.

(16) Kobler, A.; Malter, M. Quinoline Carboxylic Acid Amides. CIBA U.S.P: 2798873 (1953).

(17) Billich, A.; Hammerschmid, F.; Winkler, G. Purification, Assay and Kinetic Features of HIV-1 proteinase. *Biol. Chem. Hoppe-Seyler* **1990**, *371*, 265−272.

(18) Richards, A.; Phylip, L. H.; Farmerie, W. G.; Scarborough, P. E.; Alvarez, A.; Dunn, B. M.; Hirel, P. H.; Konvalinka, J.; Strop, P.; Pavlickova, L.; Kostka, V.; Kay, J. Sensitive, Soluble Chromogenic Substrates for HIV-1 Proteinase. *J. Biol. Chem.* **1990**, *265*, 7733−7736.

(19) Rosenwirth, B.; Billich, A.; Datema, R.; Donatsch, P.; Hammerschmid, F.; Harrison, R.; Hiestand, P.; Jaksche, H.; Mayer, P.; Peichl, P.; Quesniaux, V.; Schatz, F.; Schuurman, H. H.; Traber, R.; Wenger, R.; Wolff, B.; Zenke, G.; Zurini, M. Inhibition of HIV-1 Replication by SDZ NIM 811, a Non-Immunosuppressive Cyclosporin A Analog. *Antimicrob. Agents Chemother.* **1994**, *38*, 1763−1772.

(20) Gartner, S.; Markovits, P.; Markovits, D. M.; Kaplan, M. H.; Gallo, R. C.; Popovic, M. The Role of Mononuclear Phagocytes in HTLV-III/LAV Infection. *Science* **1986**, *233*, 215−219.

(21) Rey, M. A.; Krust, B.; Laurent, A. G.; Guetard, D.; Montagnier, L.; Hovanessian, A. G. Characterization of an HIV-2 Related Virus with a Smaller Sized Extracellular Envelope Glycoprotein. *Virology* **1989**, *173*, 258−267.

(22) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clerq, E. Rapid and Automated Tetrazolium-Based Colorimetric Assay for the Detection of anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309−321.

(23) Perno, C. F.; Yarchoan, R.; Cooney, D. A.; Hartman, N. R.; Webb, D. S. A.; Hao, Z.; Mitsuya, H.; Johns, D. G.; Broder, S. Replication of Human Immuno-Deficiency Virus in Monocytes. *J. Exp. Med.* **1989**, *169*, 933−951.

(24) DeClerq, E.; Yamamoto, N.; Pauwels, R.; Balzarini, J.; Witvrouw, M.; De Vreese, K.; Debyser, Z.; Rosenwirth, B.; Peichl, P.; Datema, R.; Thornton, D.; Skerlj, R.; Gaul, F.; Padmanabhan, S.; Bridger, G.; Henson, G.; Abrams, M. Highly Potent and Selective Inhibition of Human Immunodeficiency Virus by the Bicylam Derivative JM3100. *Antimicrob. Agents Chemother.* **1994**, *38*, 668−674.

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