Double Cyclization of Bis(α-hetarylmethyl)amino Esters to Optically Active Bridged N-Heterocycles of HIV-Inhibiting Activity

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Dedicated to Prof. Dr. Johann Mulzer on the occasion of his 60th birthday

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Anellated 1-azabicyclo[3.3.1]nonanes **6** were synthesized by several routes starting from natural α-amino esters **2** and *o*-haloaryl- or *o*-bromohetarylmethyl bromides **1**. *N*-Alkylation of the starting amino esters to **5** and **3** was followed by halogen/lithium exchange and double cyclization. The cyclization products **6** exhibit interesting inhibition of RNase H and DNA-polymerase activity of reverse transcriptase (RT) of HIV-1 at concentrations where human cellular DNA polymerases are not affected.

Introduction

Achiral 1-azadibenzo[*c*,*f*]bicyclo[3.3.1]nonanes have attracted interest as antihistaminic compounds [1] and as starting materials in the synthesis of *Amaryllidaceae* alkaloids.[2,3] Such compounds as well as thieno analogues were synthesized by acid-catalyzed double cyclization of α-(dibenzylamino)aldehydes, acetals,[1,2,4,5] ketones [6,7] or *N*,*N*-dibenzyl-2-propynylamines.[8] Alternatively, suitably functionalized isoquinolines can undergo electrophilic cyclization to 1-azadibenzo[*c*,*f*]bicyclo[3.3.1]nonanes.[3,9−12] 1-Azadibenzo[*c*,*f*]bicyclo[3.3.1]nonanes were also obtained by treatment of 4-aryltetrahydroisoquinolines with formaldehyde.[13] So far, optically active members of this class of compounds could only be obtained by optical resolution.[2] We recently found a novel stereoselective chiral pool synthesis of heterocyclic analogues **6** of 1-azadibenzo[*c*,*f*]bicyclo[3.3.1]nonanes (most of the analogues were β-carboline derivatives).[14] Our synthesis starts with amino esters **2**, which are dialkylated by *o*-bromobenzyl halides or their heterocyclic analogues **1** to afford **5**. After the exchange of bromide by lithium with butyllithium, double cyclization occurs under very mild conditions (−100 °C) to afford chiral polycyclic hydroxytetrahydropyridines **6** via intermediate monocyclization products **8**. Here, we disclose the scope and limitations of this synthesis, the extension of this method to bis(*o*-iodobenzyl)amino esters, as well as full experimental details. We further report the highly interesting activity of bridged polycyclic hydroxytetrahydropyridines **6** as inhibitors of RNase H and DNA-polymerase activity of reverse transcriptase (CT) of HIV 1.

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Results and Discussion

Synthesis

A variety of brominated and iodinated *N*,*N*-bis(hetarylmethyl)-2-amino esters **5** were obtained by reaction of the corresponding α-amino ester **2** with 2 equiv. of the corresponding benzyl halide or its heterocyclic analogue **1** (Scheme 1, Table 1). Asymmetrically substituted dialkylation products **5k**−**m** with different aryl rings ($Ar^1 \neq Ar^2$) were synthesized in a two-step mode, i.e. by the isolation of monoalkylation products **3**. Higher yields of the 3-bromoindol-2-yl-substituted products **5** (Ar^1 = indole, $Ar^2 \neq Ar^1$) were obtained when the 3-bromoindol-2-ylmethyl substituent was introduced first (formation of corresponding **3a** with Ar^1 = indole) followed by *N*-alkylation with the other *o*-bromobenzyl halide or heterocyclic analogue **1**, rather than with the reversed sequence. To prevent competing formation of the dialkylation product **5** with identical aryl rings ($Ar^1 = Ar^2$) in the monoalkylation to **3a**, an excess of

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amino ester **2** was used. X-ray crystal analysis of the *N*,*N*-diskatylvaline derivative **5f** exhibits a sterically crowded arrangement around the indole rings, which is somewhat relieved by arranging these two rings in an edge to face manner (Figure 1).

Table 1. (*S*)-*N*,*N*-Bis(hetarylmethyl)-2-amino esters **5**

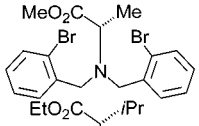
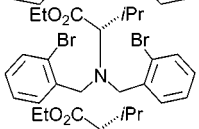
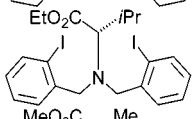
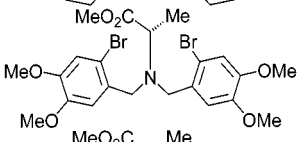
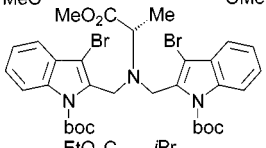
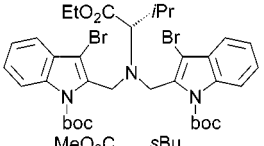
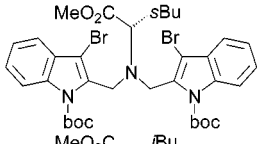
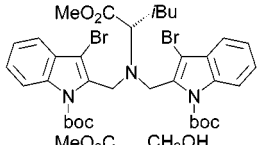
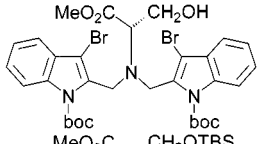
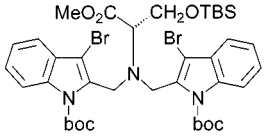
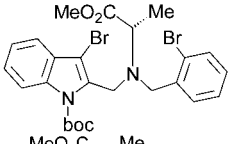
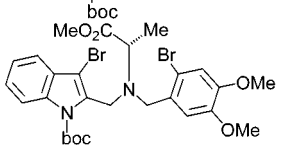
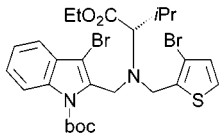
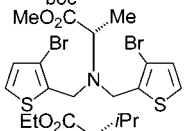
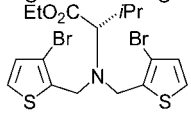
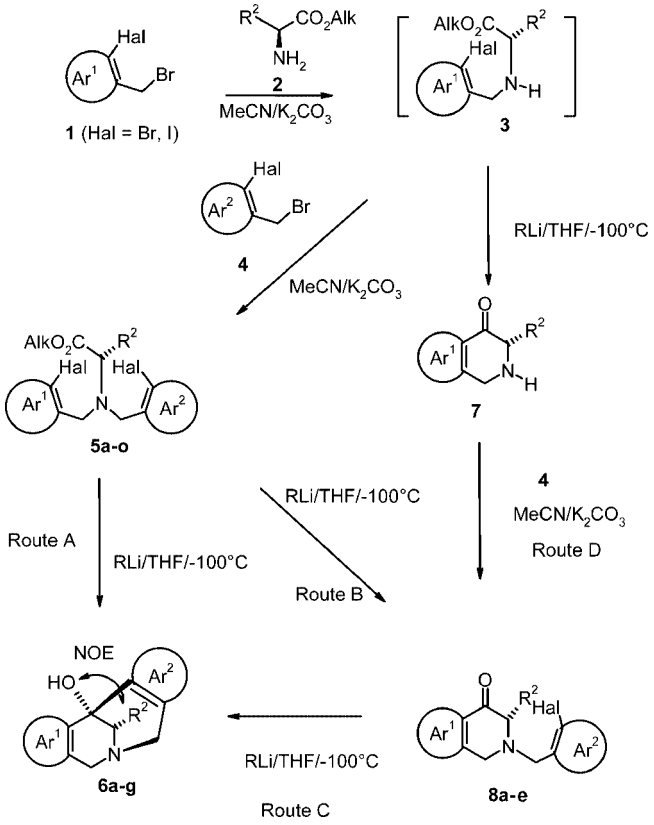
5		Yield
a		77%
b		62%
c		77%
d		73%
e		74%
f		64%
g		73%
h		61%
i		75%
j		91%
k		74%
l		79

Table 1 *(continued)*

5		Yield
m		84
n		44
o		18



Scheme 1

The cyclization of the *N*,*N*-dialkylated products **5** to 1-azadibenzo[*c*,*f*]bicyclo[3.3.1]nonanes and their heterocyclic analogues **6** (see Table 2) was generally achieved by implementing the halo/lithium exchange with *n*BuLi at −100 °C (Route A). In this context it is worth mentioning that reaction of just 1 equiv. of *n*BuLi with the dialkylation product **5m** gave a monocyclization product **8e** rather than low yields of the corresponding double cyclization product **6f** (Route B). Further treatment of **8e** with *n*BuLi (Route C) implemented the second cyclization to **6f**. This result also demonstrates the higher reactivity of the bromoindole ring in bromo/lithium exchange reactions relative to that of the bromothiophene substituents.

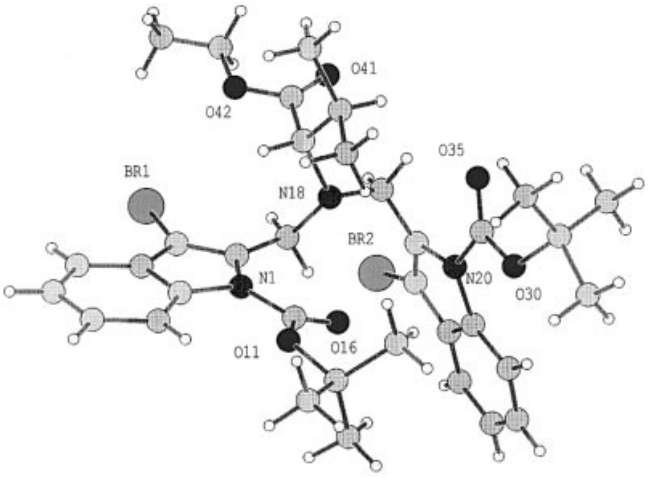
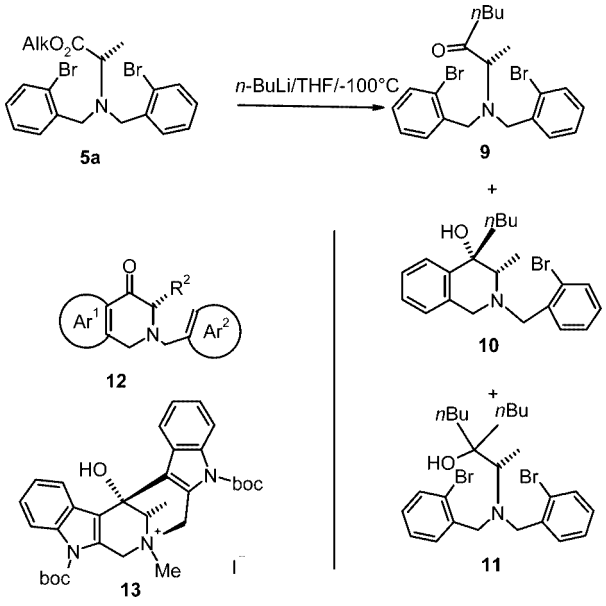


Figure 1. X-ray crystal analysis of *N*,*N*-diskatylvaline ester **5f**

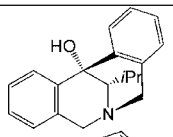
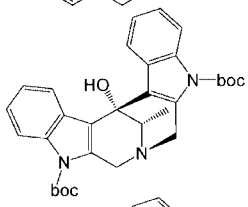
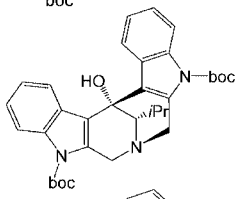
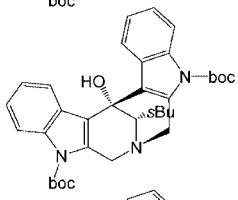
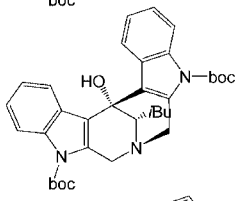
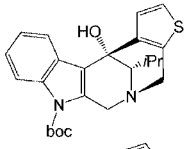
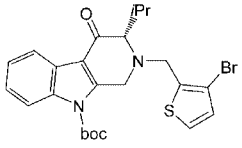
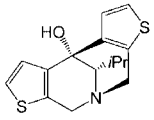
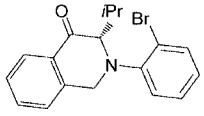
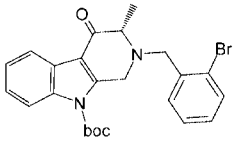
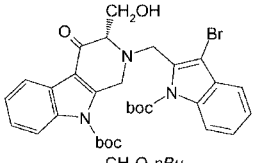
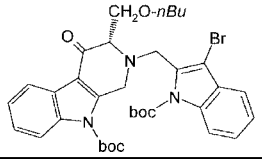
The *N*,*N*-bis(*o*-bromobenzyl)valine ester **5b** afforded the monocyclization product **8a** when treated with 2 equiv. of butyllithium (Route B), i.e. it resisted a second bromo/lithium exchange and cyclization. Similar monocyclization products **7** were obtained before, when mono(*o*-bromobenzyl)amino esters or heterocyclic analogues **3** were treated with butyllithium at low temperatures.[15,16] Only unchanged starting material **5b** and the monocyclization product **8a** were found in the reaction mixture when the bis(*o*-bromobenzyl)amino ester **5b** was treated with 2 equiv. of *t*BuLi. Obviously, the *o*-bromobenzyl moieties are not as reactive as the bromo-substituted heterocyclic rings. This was also observed in the treatment of *N*,*N*-bis(*o*-bromobenzyl)alanine ester **5a** with *n*BuLi, which afforded a mixture of bromobenzylated products **9**, **10** and **11** and in which BuLi had attacked the carbonyl group while the bromo substituents survived (Scheme 2). Remarkably, a double cyclization to the envisaged **6a** could be achieved starting from the more reactive *N*,*N*-(*o*-iodobenzyl)valine ester **5c** through route A, although in low yields. Treatment of the tetramethoxy-substituted *N*,*N*-bis(*o*-bromobenzyl)-alanine ester **5d** with *n*BuLi resulted in the re-isolation of the starting material. We suppose that the bromo/Li exchange is hampered by the high electron density in the aryl ring, or that the chelating properties of the dimethylcatechyl moiety caused complexation of the Li ion thus preventing bromo/Li exchange. In some of the successful double cyclizations of bis(*o*-bromohetarylmethyl)amino esters **5** to **6**, halogen-lacking monocyclization products **12** were observed as by-products in low yield. These by-products could be formed from monocyclization product **8** by further bromo/lithium exchange. But the second cyclization to **6** failed, and thus a proton was introduced upon quenching with water. The second cyclization was also unsuccessful, when the serine ester **5i** was used as starting material. Treatment of this compound with 3 equiv. of *n*BuLi gave a mixture of monocyclization product **8c** and the corresponding *O*-butyl ether (formula **8** with R^2 = $BuOCH_2$), obviously derived from alkylation by butyl bromide formed in the bromo/lithium exchange. Therefore, **5i** was protected by TBDMSCl to afford **5j**, which was further treated with 2 equiv. of *n*BuLi. Again, only monocyclization occurred, and the debrominated β-carboline **12** (Ar^1 = Ar^2 = indole; R^2 = CH_2OTBS) was obtained.



Scheme 2

The structures of the cyclization products **6** and **8** were elucidated by spectroscopic methods, in particular by NMR spectroscopy. An NOE was observed between the hydroxy proton and the *i*Pr group in **6g**, thus proving the proximity of both groups. The stereochemical mode of attack in the second cyclization step is likely to be *anti* with respect to the substituent R^2, as found in intermolecular 1,2-additions of Grignard reagents or hydride to the carbonyl group of condensed dihydropyridones **7**.[16,17] However, this mode only has consequences toward the final configuration if products **6** are formed in which Ar^1 and Ar^2 are different from each other, i.e. **6f**. Since the latter compound could also be synthesized starting form **8e**, the *syn* attack would lead to the given structure **6f** (see Scheme 1 and Table 2). HPLC and NMR spectroscopy of the crude double cyclization products **6** did not show any sign of racemization or epimerization. Thus, a peak for the (−) enantiomer of **6c** was totally absent in the chromatogram (HPLC at CHIRALCEL OD phase) of compound **6c**. This result corresponds to an *ee* > 99%, i.e. the configuration of the chiral center in the starting amino esters **2** was not affected throughout the transformations into the final product. The diindolo product **6b** could be methylated with methyl iodide to afford the quaternary ammonium salt **13** in high yield,

Table 2. 5-Hydroxy-1-azabicyclo[3.3.1]nonanes **6** and monocyclization products **8**

Reactant **5**	Product **6**	Yield of **6**/ Method	Product **8**	Yield of **8**/ Method
5c	**6a** 	10%/A		
5e	**6b** 	59%/A		
5f	**6c** 	75%/A		
5g	**6d** 	73%/A		
5h	**6e** 	72%/A		
5m	**6f** 	48%/A[a] 56%/C	**8e** 	17%/A[c] 58%/B 95%/D
5o	**6g** 	53%/A		
5b			**8a** 	48%/A[b]
5k			**8b** 	56%/B
5i			**8c** 	30%/B[d]
5i			**8d** 	19%/B[d]

[a] +17% **8e**. [b] *t*BuLi. [c] + 48% **6f**. [d] 30% **8c** + 19% **8d** + 18% **5i**.

Table 3. Quantification of polymerization products in the presence of inhibitor

Inhibitor μM	**6b**[a]	**6d**[a]	**6e**[a]
100	87	89	88
32	75	85	88
8	0	46	33
2	0	0	0
0.8	0	0	0

[a] Values represent percentage of inhibition.

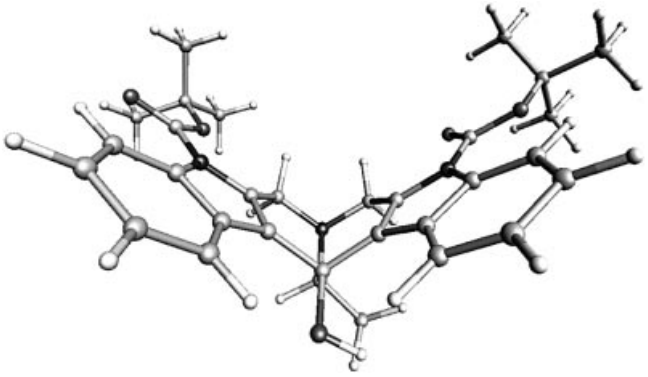


Figure 2. PM3 geometry-optimized structure of **6b** (UniChem. 4.0)

which might be useful as in asymmetric phase-transfer catalysis. The double cyclization products **6** are butterfly-shaped (for a PM3 geometry-optimized structure see Figure 2). Since other butterfly-shaped molecules were reported as anti-HIV agents,[18] we tested some products **6** for possible antiviral properties.

Antiviral Testing

RT Inhibition

The enzyme reverse transcriptase (RT) is a pivotal enzyme in the life cycle of human immunodeficiency virus 1 (HIV-1), the causative agent of AIDS. It catalyzes the conversion of the single-stranded RNA genome into double-stranded DNA, which is then incorporated into the genome of the host cell. Antiviral substances that have been developed to inhibit this enzyme lead to the development of a resistant virus. Thus, the design of new antivirals to fight the virus is essential.

In order to determine the inhibitory effect of **6b**, **6d**, and **6e** on the DNA polymerization activity of HIV-1 RT, we performed RT activity assays with a heteropolymeric DNA template. This substrate mimics the synthesis of the second DNA strand during reverse transcription. A 5′[^{32}P] radioactively labeled primer hybridized onto single-stranded M13 DNA was elongated in the presence of HIV-1 RT and dNTPs. The length of the polymerization products was visualized on a denaturing polyacrylamide gel by phosphorimaging (Figure 3). Quantification of the elongation products in the presence of inhibitor (Table 3) shows that with all three inhibitors, concentrations of around 32 μM are sufficient to severely impair the polymerization activity; 74%, 85% and 88% of inhibition can be achieved at this concentration. The main difference between the polymerization products in the absence or presence of the inhibitors appears to be the quantity of the products but not their length. This indicates that the inhibitor reduces the polymerization activity of the enzyme but does not reduce its processivity, i.e. the number of nucleotides incorporated before the enzyme dissociates from the substrate.

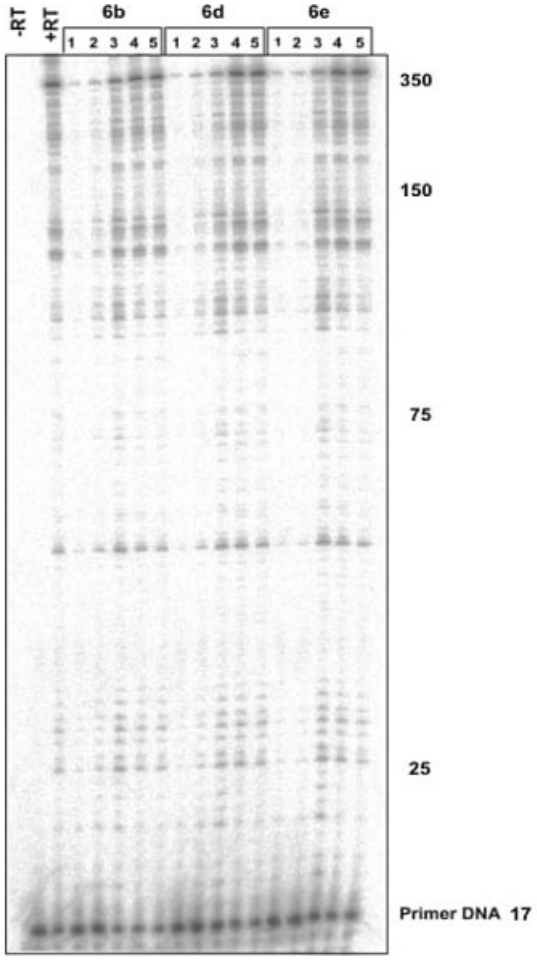


Figure 3. Analysis of polymerization products in the presence of inhibitors; HIV-1 RT (10 nM) was preincubated with dNTPs (250 μM) in the absence of inhibitor (lane +RT) or in the presence of **6b**, **6d** or **6e** at the following concentrations: 100 μM (lanes 1), 32 μM (lanes 2), 8 μM (lanes 3), 2.0 μM (lanes 4), 0.8 μM (lanes 5); lane −RT = substrate without HIV-1 RT; approximate sizes (in nucleotides) of the elongation products are indicated on the right; reactions were started by the addition of a radioactively labeled M13/primer substrate; after incubation at 37 °C for 20 min, reactions were stopped and the samples were applied on a 10% denaturing urea/polyacrylamide gel; the polymerization products were visualized by phosphorimaging

Inhibition of RNase H Activity

The RNase H domain of the dimeric HIV RT is located at the C-terminus of the larger 66 kDa subunit of the enzyme and hydrolyzes the RNA strand in the RNA/DNA hybrid that is created during the first step of reverse transcription of the viral RNA genome. This activity is also

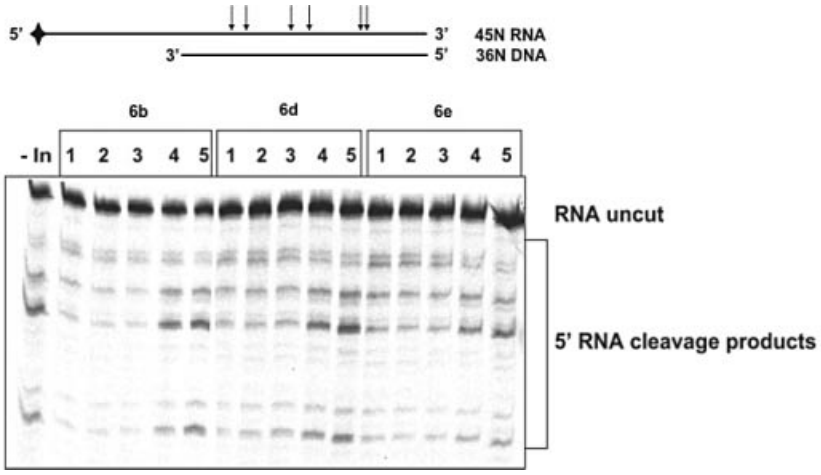


Figure 4. Inhibition of RNase H activity; 20 nM of HIV-1 RT was incubated with **6b**, **6d** or **6e** at the following concentrations: 200 μM (lanes 1), 100 μM (lanes 2), 32 μM (lanes 3), 8 μM (lanes 4) or 2 μM (lanes 5); RNase H cleavage reactions were started by the addition of a fluorescent-labeled RNA/DNA hybrid; a schematic representation of the hybrid is shown on top; the asterisk on the 5′ end of the RNA indicates the Cy5 fluorescent label; potential cleavage sites in the RNA are indicated by arrows; cleavage products were separated on 10% denaturing urea/polyacrylamide gels; the fluorescent bands were visualized using a fluorimaging device

essential in the life cycle of the virus. Since no RNase H inhibitors are in clinical use yet, RNase H is an interesting target for HIV inhibition.

To mimic the RNA/DNA substrate in vitro we used an RNA that contained a fluorescent label at the 5′ end, and hybridized it to an oligodeoxynucleotide. Upon cleavage of the RNA by the RNase H activity of RT, the labeled 5′ RNA reaction products can be analyzed on a denaturing polyacrylamide gel.

Figure 4 shows the cleavage products by HIV-1 RT in the presence of inhibitors. Clearly, with all three inhibitors (**6b**, **6d** and **6e**) a strong inhibition of more than 50% of the RNase H activity is already visible at inhibitor concentrations of 8 μM (Table 4). These concentrations are even lower than those necessary for inhibition of the polymerase activity (Figure 3). Since our results indicate that both the polymerase as well as the RNase H activity of RT are reduced in the presence of inhibitor, inhibition by these substances may be even more effective in vivo. Furthermore, inhibition of the two enzyme activities implies conformational changes of the enzyme upon inhibitor binding, which influence its two active sites.

Table 4. Quantification of the RNase H cleavage products in the presence of inhibitor

Inhibitor μM	**6b**[a]	**6d**[a]	**6e**[a]
200	83	81	80
100	83	81	80
32	87	81	80
8	57	72	74
2	48	56	44

[a] Values represent percentage of inhibition.

Toxicity

We also determined if these substances had a toxic effect on human cells. This was tested on the human HeLa cell line with increasing amounts of inhibitors. Our results indicate that **6d** was least toxic. Even at the highest concentration of 300 μM of **6d**, only about 30% of the cells died, whereas with **6b** and **6e** about 40−50% of the cells died at a concentration of 100 μM.

Effect on Cellular DNA Polymerases

To determine whether the inhibitors are specific for HIV RT but do not impair human cellular polymerases the two inhibitors of HIV-RT-polymerase **6b** and **6d**, dissolved in DMSO, were tested for a main replicative DNA polymerase, exemplified as pol δ, and for an essential repair DNA polymerase, exemplified as pol β (Figure 5). For both tested inhibitors **6b** and **6d** with concentrations of 16 μM, pol δ is inhibited by about 10%, and with a concentration of 32 μM by about 40%. When pol β was tested with these two inhibitors, it is even less sensitive (with a concentration of 32 μM for the inhibitors, only 10% inhibition with **6b** or 20% with **6d**) than pol δ. The influence of both substances on pol λ, another repair pol, was similar to that on pol β (data not shown). The final concentration of DMSO as the solvent for the chemical substances in the reactions was 5%, and was taken as the zero control to measure the effect of DMSO alone.

Conclusion

A highly stereoselective synthesis of chiral polycyclic hydroxytetrahydropyridines **6** has been accomplished through a double cyclization of bis(haloheteroarylmethyl)amino esters by bromo or iodo/lithium exchange with BuLi.

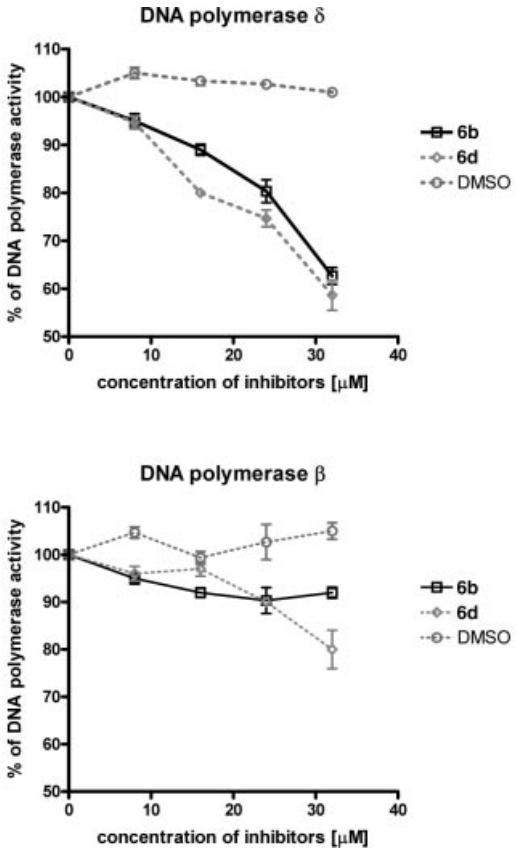


Figure 5. Effect of the two different inhibitors **6b** (corresponds to number 1316) and **6d** (corresponds to number 1317) on DNA polymerases δ (A) and DNA polymerase β (B); the DNA polymerase assays were carried out as outlined in Materials and Methods (Exp. Sect.); the results represent the mean of triplicate experiments

The synthesis can conveniently be implemented as a one-pot procedure but is also possible in two steps. The two heterocyclic rings anellated to the final hydroxytetrahydropyridine can be identical or different. Bromobenzyl substituents are not as suitable for BuLi-mediated double cyclization as heterocyclic analogues. *N*-Alkylation of the products **6** produces quaternary ammonium salts. The double cyclization products **6** possess butterfly-shaped structures. They exhibit remarkable, inhibitory effects on the polymerase as well as on the RNase H activity of HIV-1 RT. On the other hand, these products appear to exert low toxicity and affect human DNA-polymerases to a lower extent than HIV-1 RT. The repair DNA polymerase β is even less affected. Thus, these compounds might represent a promising lead for the development of new drugs in the HIV therapy.

Experimental Section

a) Synthesis

General Remarks: All reactions were carried out under argon in oven-dried glassware. Solvents were dried and deoxygenated by standard procedures. Starting materials were purchased from Aldrich and Merck. (Bromomethyl)arenes and heteroarenes **1** and **4** are new and were obtained by adopting known procedures for the synthesis of analogues.[18−21] TLC analysis was performed on Merck silica gel 60F_{254} plates and visualized with UV illumination and charring with phosphomolybdic acid in EtOH (5%, v/v) or 0.3% ninhydrin in EtOH. Column chromatography was conducted with Merck silica gel 60 (400−639 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively, with a Bruker AC-300 in $CDCl_3$ with TMS as internal standard. Mass spectra were measured at 70 eV. Optical rotations were determined with a Perkin−Elmer polarimeter 241 (d = 2 mm). Enantiomeric purity was proved by analytical HPLC on cellulose carbamate (Chiral PAK AD).

Starting Materials 1

***N*-Boc-3-bromo-2-(bromomethyl)indole (1a):** NBS (4.45 g, 0.025 mol) and a catalytic amount of AIBN were added to a solution of *N*-Boc-3-bromo-2-methylindole (7.75 g, 0.025 mol) in dry CCl_4 (150 mL). The mixture was slowly heated to 80 °C and kept at this temperature for 2 h. After cooling to room temperature, the resulting succinimide was filtered off, and the filtrate was concentrated under vacuum. The remainder was purified by column chromatography (hexane/EtOAc, 9:1; R_f = 0.56) to afford the colorless product in 98% yield. ^{1}H NMR ($CDCl_3$): δ = 1.98 (s, 9 H), 5.31 (s, 2 H), 7.13−7.35 (m, 3 H), 8.01 (d, J = 8.4 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 25.4 (CH_2), 28.4 (CH_3), 85.9 (C), 104.1 (C), 116.3 (CH), 120.2 (CH), 124.1 (CH), 127.0 (CH), 128.2 (C), 133.1 (C), 136.5 (C), 149.5 (CO) ppm. $C_{14}H_{15}Br_2NO_2$ (389.08): calcd. C 43.22, H 3.89, N 3.60; found C 43.61, H 3.72, N 3.41.

3-Bromo-2-(bromomethyl)thiophene (1b): 3-Bromo-2-methylthiophene (2.60 g, 0.0147 mol) was brominated with NBS (2.62 g, 0.0147 mol) in CCl_4 in the same manner (vide supra) to afford the product as yellow oil (R_f = 0.43; hexane/EtOAc, 9:1) in 86% yield. ^{1}H NMR ($CDCl_3$): δ = 4.56 (s, 2 H), 6.82 (d, J = 5.4 Hz, 1 H), 7.19 (d, J = 5.4 Hz, 1 H) ppm. ^{13}C NMR: ($CDCl_3$): δ = 25.2 (CH_2), 112.7 (C), 126.7 (CH), 130.5 (CH), 134.6 (C) ppm.

***tert*-Butyl 3-Bromo-2-({[(1*S*)-1-(methoxycarbonyl)-2-methylpropyl]-amino}methyl)-1*H*-indol-1-carboxylate (3a):** A solution of 1-Boc-3-bromo-2-(bromomethyl)indole (5.84 g, 0.015 mol), methyl valinate (3.93 g, 0.03 mol), and K_2CO_3 (2.07 g, 0.015 mol) in MeCN (200 mL) was refluxed for 3 h. The resulting suspension was filtered, and the filtrate was concentrated under vacuum. The remainder was purified by column chromatography. Yellow oil, 62% yield, R_f (hexane/ethyl acetate, 98:2) = 0.22, $[\alpha]_D^{20}$ = +2.3 (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.81 (d, J = 6.8 Hz, 3 H, CH_3), 0.86 (d, J = 6.3 Hz, 3 H, CH_3), 1.66 [s, 9 H, $(CH_3)_3C_q$], 1.71−1.80 [m, 1 H, $CH(CH_3)_2$], 2.09 (s, 1 H, NH), 2.98 (d, J = 5.6 Hz, 1 H, CHN), 3.19 (s, 3 H, CH_3O), 4.11 (d, J = 14.7 Hz, 1 H, CH_2N), 4.24 (d, J = 14.7 Hz, 1 H, CH_2N), 7.19−7.28 (m, 2 H, CH_{ar}), 7.42 (d, J = 7.2 Hz, 1 H, CH_{ar}), 8.02 (d, J = 7.9 Hz, 1 H, CH_{ar}) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.4 (CH_3), 19.1 (CH_3), 28.4 (C), 31.9 (CH), 44.7 (CH_2−N), 51.2 (CH_3), 65.5 (CH), 84.8 (C), 102.3 (C), 115.7 (CH), 118.3 (CH), 119.9 (CH), 123.3 (CH), 127.9 (C), 135.5 (C), 135.7 (C), 149.8 (CO), 175.3 (CO) ppm. $C_{20}H_{27}BrN_2O_4$ (439.34): calcd. C 54.68, H 6.19, Br 18.19, N 6.38; found C 54.73, H 6.08, Br 18.57, N 6.46.

***tert*-Butyl (3*S*)-3-Isopropyl-4-oxo-1,2,3,4-tetrahydro-9*H*-β-carbolin-9-carboxylate (7a):** A solution of **3a** (0.658 g, 1.5 mmol) in dry THF (80 mL) under argon was cooled to −100 °C. 1.7 M *t*BuLi in pentane (3.15 mmol) was added dropwise while the temperature was kept below −98 °C. After complete addition, the mixture was stirred at −100 °C for 4 h and was quenched with satd. aqueous NH_4Cl. The mixture was stirred at room temperature until all pre-

cipitate had dissolved. The mixture was extracted with CH_2Cl_2 three times, and the combined organic layers were dried (Na_2SO_4). After removal of the solvent under vacuum, the remainder was purified by column chromatography. Colorless solid, 61% yield. M. p. 168 °C, R_f (CH_2Cl_2/acetone, 8:2) = 0.36, $[\alpha]_D^{20} = -11.4$ (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.95 (d, J = 6.8 Hz, 3 H, CH_3), 1.01 (d, J = 6.9 Hz, 3 H, CH_3), 1.63 [s, 9 H, $(CH_3)_3C_q$], 2.06 (s, 1 H, NH), 2.36−2.49 [m, 1 H, $CH(CH_3)_2$], 3.10 (d, J = 5.5 Hz, 1 H, CHN), 4.29 (d, J = 19.0 Hz, 1 H, CH_2N), 4.54 (d, J = 19.0 Hz, 1 H, CH_2N), 7.25 −7.28 (m, 2 H, CH_{ar}), 8.06−8.13 (m, 1 H, CH_{ar}), 8.17−8.21 (m, 1 H, CH_{ar}) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.5 (CH_3), 20.3 (CH_3), 27.1 (CH), 28.6 (CH_3), 44.2 (CH_2), 67.6 (CH), 86.1 (C), 115.6 (CH), 116.1 (C), 121.9 (CH), 124.9 (CH), 125.5 (CH), 126.1 (C), 136.0 (C), 149.9 (CO), 151.3 (C), 196.2 (CO) ppm. $C_{19}H_{24}N_2O_3$ (328.406): calcd. C 69.49, H 7.37, N 8.53; found C 69.27, H 7.07, N 8.55.

General Procedure for the Synthesis of Symmetric Dialkylation Products 5: The amino ester **2** (6.43 mmol) and K_2CO_3 (4.15 g, 30 mmol) were added to a solution of the arylmethyl bromide or hetarylmethyl bromide **1** (13 mmol) in MeCN (250 mL). The mixture was refluxed for 2−5 h. After complete reaction, the suspension was allowed to cool down, and the solid was filtered off. The filtrate was concentrated under vacuum and the remainder purified by column chromatography.

General Procedure for the Synthesis of Asymmetric Dialkylation Products 5k−5m: The arylmethyl bromide or hetarylmethyl bromide **1** (5.5 mmol) and K_2CO_3 (0.69 g, 5 mmol) were added to a solution of the [(*o*-bromoindolyl)methyl]amino ester **3** (Ar^1 = indole)[15] (5 mmol) in MeCN (150 mL). After 6 h of reflux, the cold reaction mixture was filtered. The filtrate was concentrated under vacuum and the remainder was purified by column chromatography.

Methyl (2*S*)-2-[Bis(2-bromobenzyl)amino]propanoate (5a): Colorless oil, 77% yield. R_f (hexane/ethyl acetate, 9:1) = 0.48. $[\alpha]_D^{20} = -48.35$ (c = 1.15, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 1.28 (d, J = 7.2 Hz, 3 H), 3.47 (q, J = 7.2 Hz, 1 H), 3.63 (s, 3 H,) 3.78 (d, J = 15.1 Hz, 2 H), 3.85 (d, J = 15.1 Hz, 2 H), 6.89−6.95 (m, 2 H, CH), 7.08−7.11 (m, 2 H, CH), 7.35 (dd, J_1 = 7.9, J_2 = 1.2 Hz, 2 H), 7.44 (dd, J_1 = 7.7, J_2 = 1.5 Hz, 2 H) ppm.^{13}C NMR ($CDCl_3$): δ = 15.3 (CH_3), 51.9 (CH_3), 55.1 (2 CH_2), 58.3 (CH), 124.6 (2 C), 127.6 (2 CH), 128.7 (2 CH), 130.8 (2 CH), 132.9 (2 CH), 138.9 (2 C), 174.5 C ppm. MS: m/z = 441 (1) [M^+], 384 (12), 382 (23), 380 (12), 212 (16), 210 (13), 185 (19), 183 (17), 171 (100), 170 (10), 169 (95), 104 (14), 102 (11), 91 (27), 90 (54), 89 (46), 82 (12), 80 (11), 77 (15), 63 (17), 59 (10), 56 (15), 55 (12). $C_{18}H_{19}Br_2NO_2$ (441.16): calcd. C 49.01, H 4.34, Br 36.22, N 3.18; found C 48.72, H 4.29, Br 36.53, N 3.47.

Ethyl (2*S*)-2-[Bis(2-bromobenzyl)amino]-3-methylbutanoate (5b): Colorless oil, 62% yield. R_f (hexane/ethyl acetate, 9:1) = 0.44. ^{1}H NMR ($CDCl_3$): δ = 0.88 (d, J = 6.5 Hz, 3 H, CH_3), 1.11 (d, J = 6.6 Hz, 3 H), 1.38 (t, J = 7.1 Hz, 3 H,), 2.19−2.27 (m, 1 H), 2.99 (d, J = 10.6 Hz, 1 H), 3.93 (s, 4 H), 4.30 (q, J = 7.1 Hz, 2 H,), 7.07−7.12 (m, 2 H), 7.25−7.32 (m, 2 H, CH), 7.52 (d, J = 7.9 Hz, 2 H, 2 H), 7.64 (d, J = 7.7 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.0 (CH_3-CH_2), 20.4 (CH_3), 20.5 (CH_3), 28.3 (CH), 55.2 (2 CH_2), 60.5 (CH_2), 69.8 (CH), 124.7 (2 C), 127.6 (2 CH), 128.6 (2 CH), 130.4 (2 CH), 133.1 (2 CH), 138.6 (2 C), 172.5 (C) ppm. $C_{21}H_{25}Br_2NO_2$ (483.24): calcd. C 52.19, H 5.21, Br 33.07, N 2.90; found C 51.93, H 5.27, Br 33.20, N 2.85.

Ethyl (2*S*)-2-[Bis(2-iodobenzyl)amino]-3-methylbutanoate (5c): Colorless solid, 77% yield. R_f (hexane/ethyl acetate, 7:3) = 0.37. $[\alpha]_D^{20}$ = −41.7. (c = 1.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.87 (d, J = 5.3 Hz, 3 H, CH_3), 0.89 (d, J = 5.3 Hz, 3 H, CH_3), 1.19 (t, J = 7.1 Hz, 3 H, CH_3), 1.87 (m, 1 H, CH), 2.92 (d, J = 8.2 Hz, 1 H, CH), 3.55 (d, J = 13.9 Hz, 2 H, CH_2), 3.76 (d, J = 13.9 Hz, 2 H, CH_2), 4.08 (q, J = 7.1 Hz, 2 H, CH_2), 6.84 (m, 2 H, CH), 7.23 (m, 4 H, CH), 7.71 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.5 (CH_3), 18.7 (CH_3), 19.4 (CH_3), 31.8 (CH), 56.9 (CH_2), 60.4 (CH_2), 66.7 (CH), 99.9 (2 C), 128.1 (2 CH), 128.7 (2 CH), 129.6 (2 CH), 2 × 139.3 (CH), 142.1 (2 C), 174.9 (CO) ppm.

Methyl (2*S*)-2-[Bis(2-bromo-4,5-dimethoxybenzyl)amino]propanoate (5d): Colorless oil, 73% yield. R_f (hexane/ethyl acetate, 7:3) = 0.25. $[\alpha]_D^{20} = -49.9$ (c = 2.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 1.51 (d, J = 7.1 Hz, 3 H), 3.72 (q, J = 7.1 Hz, 1 H), 3.89 (s, 3 H,) 3.92 (s, 12 H), 3.94 (s, 4 H, 2 CH_2), 7.02 (s, 2 H, CH), 7.17 (s, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.4 (CH_3), 51.5 (CH_3), 54.6 (2 CH_2), 55.9 (2 CH_3), 56.1 (2 CH_3), 58.6 (CH), 113.2 (2 CH), 113.8 (2 C), 115.2 (2 CH), 130.6 (2 C), 148.2 (2 C), 174.1 (C) ppm. MS: m/z = 502 (11), 231 (89), 230 (11), 229 (100), 151 (17), 107 (34), 105 (11), 92 (10), 91 (11), 89 (15), 79 (15), 77 (26), 63 (11), 56 (11). $C_{22}H_{27}Br_2NO_6$ (561.26): calcd. C 47.08, H 4.85, Br 28.47, N 2.50; found C 47.15, H 4.29, Br 27.43, N 2.38.

Methyl (2*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]amino}-propanoate (5e): Colorless solid, 74% yield. R_f (hexane/ethyl acetate, 9:1) = 0.25. $[\alpha]_D^{20} = -47.4$ (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 1.32 (d, J = 7.3 Hz, 3 H, CH_3CH), 1.52 [s, 18 H, 2 $(CH_3)_3C$], 3.61 (q, J = 7.3 Hz, 1 H, CH), 3.63 (s, 3 H, CH_3), 4.38 (s, 4 H, 2 CH_2), 7.09−7.25 (m, 6 H, CH), 7.72 (d, J = 7.6 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.3 (C), 28.6 (2 $(CH_3)_3$], 47.2 (2 CH_2), 51.7 (CH_3), 59.5 (CH), 84.3 (2 C), 103.5 (2 C), 115.6 (2 CH), 119.5 (2 CH), 123.1 (2 CH), 125.2 (2 CH), 128.6 (2 C), 135.9 (4C), 149.7 (2 C), 174.9 (C) ppm. MS: m/z = 355 (13), 353 (15), 254 (10), 252 (11), 210 (78), 209 (12), 208 (79), 130 (43), 129 (41), 128 (38), 102 (22), 101 (16), 77 (10), 57 (89), 56 (34), 55 (16), 50 (11). $C_{32}H_{37}Br_2N_3O_6$ (719.46): calcd. C 53.42, H 5.18, N 5.84; found C 53.05, H 5.24, N 6.00.

Ethyl (2*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]amino}-3-methylbutanoate (5f): Colorless solid, 64% yield. R_f (hexane/ethyl acetate, 9:1) = 0.2. $[\alpha]_D^{20} = -110.5$ (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.59 [d, J = 6.4 Hz, 3 H, $(CH_3)_2$], 0.67 [d, J = 6.5 Hz, 3 H, $(CH_3)_2$], 1.34 (t, J = 7.1 Hz, 3 H, CH_3), 1.46 [s, 18 H, (2 $CH_3)_3$], 2.18−2.23 (m, 1 H, CH), 2.63 (d, J = 10.8 Hz, 1 H, CH), 4.06 (d, J = 13.6 Hz, 2 H, 2 CH_2), 4.20−4.29 (m, 2 H, CH_2), 4.58 (d, J = 13.6 Hz, 2 H, 2 CH_2), 7.15−7.19 (m, 4 H, CH), 7.38−7.40 (m, 2 H, CH), 7.81 (d, J = 7.3 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.1 (C), 20.2 (C), 20.9 (C), 27.5 (C), 28.6 (2 C), 47.2 (2 CH_2), 60.2 (CH_2), 69.9 (CH), 84.3 (2 C), 104.5 (2 C), 115.7 (2 CH), 119.7 (2 CH), 123.1 (2 CH), 125.3 (2 CH), 128.7 (2 C), 135.7 (2 C), 136.2 (2 C), 149.5 (2 C), 172.4 (C) ppm. $C_{35}H_{43}Br_2N_3O_6$ (761.54): calcd. C 55.20, H 5.69, N 5.52; found C 54.84, H 5.68, N 5.23. ***rac*-5f** was obtained by starting from racemic valine ester.

X-ray Crystal Analysis of 5f:[22] Empirical formula: $C_{35}H_{43}Br_2N_3O_6$, formula mass: 761.54, temperature: 295 (2) K, wavelength: 0.71073 Å, crystal system: orthorhombic, space group: $P2_12_12_1$, unit cell dimensions: a = 14.501(18), b = 15.941(3), c = 16.072(3) Å, volume: 3715.4(10) Å^3, Z = 4, density (calcd.): 1.361 Mg/m^3, absorption coefficient: 2.227 mm^{-1}, F(000) = 1568, crystal size: 0.98 × 0.96 × 0.80 mm, θ range for data collection: 1.80−25.05°, limiting indices: $-4 \le h \le 17$, $0 \le k \le 18$, $0 \le l \le 19$; reflections collected: 5485, unique: 4544 [R (int) = 0.0446], completeness to θ = 25.05°, absorption correction: empirical (ψ-scan), max. and min. transmission: 0.9994 and 0.8232, refine-

ment method: full-matrix least squares on F^2, data/restraints/parameters: 3351/1/376, goodness-of-fit on F^2: 1.156, final R_{int} [$I > 2\sigma(I)$]: $R1 = 0.0536$, $wR2 = 0.1039$; R_{int} (all data): $R1 = 0.1175$, $wR2 = 0.1400$, absolute structure parameter: −0.030(18), extinction coefficient: 0.0017(4), largest diff. peak and hole: 0.417 and −0.485 e A^{-3}.

Methyl (2*S*,3*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]-amino}-3-methylpentanoate (5g): Colorless solid, 76% yield. R_f (hexane/ethyl acetate, 9:1) = 0.28. $[\alpha]_D^{20} = -55.8$ (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.46 (t, J = 7.3 Hz, 3 H, CH_3), 0.63 (d, J = 6.6 Hz, 3 H, CH_3), 1.48 (s, 18 H, 2 $(CH_3)_3$], 1.49−1.74 (m, 2 H, CH_2), 1.91−2.12 (m, 1 H, CH_3), 2.87 (d, J = 10.6 Hz, 1 H, CH), 3.71 (s, 3 H, CH_3), 4.13 (d, J = 13.7 Hz, 2 H, CH_2), 4.51 (d, J = 13.7 Hz, 2 H, CH_2), 7.14 −7.19 (m, 4 H, CH), 7.36−7.39 (m, 2 H, CH), 7.81 (d, J = 7.5 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.6 (C), 16.0 (C), 25.8 (C), 28.6 (2 C), 33.2 (C), 47.3 (2 CH_2), 51.0 (CH_3), 68.4 (CH), 84.3 (2 C), 104.3 (2 C), 115.8 (2 CH), 119.7 (2 CH), 123.2 (2 CH), 125.4 (2 CH), 128.7 (2 C), 135.7 (2 C), 136.2 (2 C), 149.6 (2 C), 172.8 (C) ppm. $C_{35}H_{43}Br_2N_3O_6$ (761.54): calcd. C 55.20, H 5.69, N 5.52; found C 54.96, H 5.51, N 5.43.

Methyl (2*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]amino}-4-methylpentanoate (5h): Colorless solid, 61% yield. R_f (hexane/ethyl acetate, 9:1) = 0.26. $[\alpha]_D^{20} = -81.5$ (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.32 (d, J = 6.4 Hz, 3 H, CH), 0.70 (d, J = 6.5 Hz, 3 H, CH), 1.38−1.46 (m, 1 H, CH), 1.51 (s, 18 H, 2 CH), 1.72−1.84 (m, 2 H, CH_2), 3.31−3.40 (m, 1 H, CH), 3.64 (s, 3 H, CH_3), 4.38 (d, J = 13.8 Hz, 2 H, CH_2), 4.44 (d, J = 13.8 Hz, 2 H, CH_2), 7.09−7.19 (m, 4 H, CH), 7.32−7.35 (m, 2 H, CH), 7.77−7.80 (m, 2 H, CH) ppm. MS: m/z = 397 (15), 395 (15), 254 (11), 252 (12), 210 (30), 208 (31), 130 (11), 129 (12), 128 (13), 57 (100), 44 (12) ppm. ^{13}C NMR ($CDCl_3$): δ = 21.7 (C) 23.6 (C), 24.9 (C), 28.7 2(C), 38.5 (C), 47.1 (2 CH_2), 51.5 (CH_3), 61.1 (CH), 84.4 (2 C), 103.9 (2 C), 115.8 (2 CH), 119.6 (2 CH), 123.2 (2 CH), 125.4 (2 CH), 128.6 (2 C), 135.8 (2 C), 136.0 (2 C), 149.7 (2 C), 174.5 (C) ppm. $C_{35}H_{43}Br_2N_3O_6$ (761.54): calcd. C 55.20, H 5.69, Br 20.98, N 5.52; found C 55.14, H 5.72, Br 21.08, N 5.73.

Methyl (2*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]amino}-3-hydroxypropanoate (5i): Colorless solid, 75% yield. R_f (hexane/ethyl acetate, 9:1) = 0.16. ^{1}H NMR ($CDCl_3$): δ = 1.59 [s, 18 H, 2 $(CH_3)_3$], 3.30−3.33 (m, 1 H, CH), 3.72−3.81 (m, 2 H, CH_2), 3.85 (s, 3 H, CH_3), 4.53 (d, J = 14.7 Hz, 1 H, CH_2), 4.55 (d, J = 14.7 Hz, 1 H, CH), 7.19−7.30 (m, 4 H, CH), 7.39−7.46 (m, 2 H, CH), 7.81 (d, J = 8.0 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.6 (2 C), 46.8 (2 CH_2), 51.7 (CH_3), 59.4 (CH_2), 64.9 (CH), 84.9 (2 C), 105.0 (2 C), 116.1 (2 CH), 119.8 (2 CH), 123.4 (2 CH), 125.6 (2 CH), 128.6 (2), 134.9 (2 C), 135.9 (2 C), 150.1 (2 × C), 172.1 (C) ppm. $C_{32}H_{37}Br_2N_3O_7$ (735.46): calcd. C 52.26, H 5.07, N 5.71; found C 52.04, H 5.22, N 5.83.

Methyl (2*S*)-2-{Bis[(1-Boc-3-bromo-1*H*-indol-2-yl)methyl]amino}-3-(*tert*-butyldimethylsilyloxy)propanoate (5j): Colorless solid, 91% yield. R_f (hexane/ethyl acetate, 9:1) = 0.69 ppm. ^{13}C NMR ($CDCl_3$): δ = −5.4 (CH_3), −5.1 (CH_3), 18.6 (C), 26.2 (C), 28.6 (2 C), 48.2 (2 CH_2), 51.4 (CH_3), 64.0 (CH_2), 66.2 (CH), 84.3 (2 C), 103.6 (2 C), 115.7 (2 CH), 119.5 (2 CH), 123.1 (2 CH), 125.3 (2 CH), 128.6 (2 C), 135.8 (2 C), 135.9 (2 C), 149.6 (2 C), 172.5 (C) ppm. $C_{38}H_{51}Br_2N_3O_7Si$ (849.72): calcd. C 53.71, H 6.05, Br 18.81, N 4.95; found C 54.04, H 5.87, Br 19.22, N 4.79.

***tert*-Butyl 3-Bromo-2-({(2-bromobenzyl)[(1*S*)-2-methoxy-1-methyl-2-oxoethyl]amino}methyl)-1*H*-indole-1-carboxylate (5k):** Colorless oil, 74% yield. R_f (hexane/ethyl acetate, 9:1) = 0.29. $[\alpha]_D^{20} = -3.0$ (c = 1, CH_2Cl_2).^{1}H NMR ($CDCl_3$): δ = 1.66 (d, J = 7.3 Hz, 3 H, CH_3), 2.02 [s, 9 H, $(CH_3)_3$], 4.01 (q, J = 7.3 Hz, 1 H, CH), 4.04 (s, 3 H, CH_3), 4.17 (d, 1 H, CH_2), 4.26 (d, 1 H, CH_2), 4.71 (d, J = 13.5 Hz, 1 H, CH_2), 4.86 (d, J = 13.5 Hz, 1 H, CH_2), 7.03−7.27 (m, 2 H, CH), 7.45−7.69 (m, 5 H, CH), 8.13 (d, J = 8.1 Hz, 1 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.6 (CH_3), 28.3 (C), 48.6 (CH_2), 51.4 (CH_3), 54.1 (CH_2), 59.9 (CH), 84.2 (C), 103.1 (C), 115.0 (CH), 119.4 (CH), 122.8 (CH), 123.3 (C), 125.1 (CH), 126.4 (CH), 127.5 (CH), 127.8 (C), 129.8 (CH), 132.1 (CH), 134.7 (C), 135.4 (C), 139.1 (C), 149.4 (C), 174.2 (C) ppm. $C_{25}H_{28}Br_2N_2O_4$ (580.31): calcd. C 51.74, H 4.86, Br 27.54, N 4.83; found C 51.54, H 4.41, Br 27.24, N 4.80.

***tert*-Butyl 3-Bromo-2-({(2-bromo-4,5-dimethoxybenzyl)[(1*S*)-2-methoxy-1-methyl-2-oxo-ethyl]amino}methyl)-1*H*-indole-1-carboxylate (5l):** Colorless solid, 79% yield. R_f (hexane/ethyl acetate, 7:3) = 0.32. ^{1}H NMR ($CDCl_3$): δ = 1.62 (d, J = 7.2 Hz, 3 H, CH_3), 1.98 [s, 9 H, $(CH_3)_3$], 3.89 (s, 3 H, CH_3), 3.99 (s, 3 H, CH_3), 4.03 (s, 3 H, CH_3), 4.08 (d, 1 H, CH_2), 4.16 (d, 1 H, CH_2), 4.43 (q, J = 7.2 Hz, 1 H, CH), 4.73 (d, 1 H,CH_2), 4.80 (d, 1 H, CH_2), 7.02 (s, 1 H, CH), 7.05 (s, 1 H, CH), 7.48−7.63 (m, 2 H, CH), 7.69−7.78 (m, 1 H, CH), 8.18 (d, J = 7.7 Hz, 1 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.2 (CH_3), 28.3 (C), 49.1 (CH_2), 51.4 (CH_3), 54.0 (CH_2), 55.7 (CH_3), 56.0 (CH_3), 60.2 (CH), 84.5 (C), 102.9 (C), 112.7 (CH), 112.9 (C), 114.9 (CH), 115.09 (CH), 119.4 (CH), 123.1 (CH), 125.5 (CH), 128.0 (C), 131.4 (C), 135.4 (C), 135.5 (C), 147.9 (C), 148.0 (C), 149.5 (C), 174.2 (C) ppm. $C_{27}H_{32}Br_2N_2O_6$ (640.36): calcd. C 50.64, H 5.04, N 4.37; found C 50.54, H 4.92, N 4.56.

***tert*-Butyl 3-Bromo-2-({[(3-bromo-2-thienyl)methyl][(1*S*)-1-(ethoxycarbonyl)-2-methylpropyl]amino}methyl)-1*H*-indole-1-carboxylate (5m):** Yellowish oil, 84% yield. R_f (hexane/ethyl acetate, 9:1) = 0.43. ^{1}H NMR ($CDCl_3$): δ = 0.90 (d, J = 6.6 Hz, 3 H, CH), 1.03 (d, J = 6.5 Hz, 3 H, CH), 1.39 (t, J = 7.1 Hz, 3 H, CH), 1.73 (s, 9 H, C), 2.16−2.21 (m, 1 H, CH), 3.13 (d, J = 10.5 Hz, 1 H, CH), 3.96 (d, J = 15.9 Hz, 1 H, CH_2), 4.12 (d, J = 15.9 Hz, 1 H, CH_2), 4.29 (q, J = 7.1 Hz, 2 H, CH), 4.42 (d, J = 13.5 Hz, 1 H, CH_2), 4.49 (d, J = 13.5 Hz, 1 H, CH_2), 6.66 (d, J = 5.3 Hz, 1 H, CH), 6.93 (d, J = 5.3 Hz, 1 H, CH), 7.23−7.34 (m, 2 H, CH), 7.46−7.48 (m, 1 H, CH), 7.93−7.96 (m, 1 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.0 (C), 20.4 (C), 21.0 (C), 28.4 (C), 28.8 (C), 49.3 (CH_2), 49.8 (CH_2), 60.4 (CH_2), 72.4 (CH), 84.7 (C), 104.3 (C), 108.1 (C), 115.8 (CH), 119.9 (CH), 123.3 (CH), 124.5 (CH), 125.7 (CH), 128.4 (C), 129.5 (CH), 134.9 (C), 135.9 (C), 140.0 (C), 149.8 (C), 172.5 (C) ppm. $C_{26}H_{32}Br_2N_2O_4S$ (628.42): calcd. C 49.69, H 5.13, N 4.46, S 5.10; found C 49.75, H 5.26, N 4.64, S 5.01.

Methyl (2*S*)-2-{Bis[(3-bromo-2-thienyl)methyl]amino}propanoate (5n): Yellowish oil, 44% yield. R_f (hexane/ethyl acetate, 9:1) = 0.35. ^{1}H NMR ($CDCl_3$): δ = 1.31 (d, J = 7.2 Hz, 3 H, CH), 3.53 (q, J = 7.2 Hz, 1 H, CH), 3.68 (s, 3 H, CH_3), 3.83 (d, J = 15.2 Hz, 2 H, CH_2), 4.03 (d, J = 15.2 Hz, 2 H, CH_2), 6.8 (d, J = 5.3 Hz, 2 H, CH), 7.15 (d, J = 5.3 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.8 (C), 49.5 (2 CH_2), 51.9 (CH_3), 57.0 (CH), 109.3 (2 C), 125.6 (2 CH), 130.1 (2 CH), 139.1 (2 C) 174.1 (C) ppm. $C_{14}H_{15}Br_2NO_2S_2$ (453.21): calcd. C 37.10, H 3.34, Br 35.26, N 3.09, S 14.15; found C 37.13, H 3.44, Br 35.55, N 3.28, S 14.17.

Ethyl (2*S*)-2-{Bis[(3-bromo-2-thienyl)methyl]amino}-3-methylbutanoate (5o): Light yellow oil, 19% yield. R_f (hexane/ether, 9:1) = 0.43. ^{1}H NMR ($CDCl_3$): δ = 0.80 (d, J = 6.4 Hz, 3 H, CH), 1.05 (d, J = 6.6 Hz, 3 H, CH), 1.28 (t, J = 7.1 Hz, 3 H, CH), 2.06−2.24 (m, 1 H, CH), 2.93 (d, J = 10.6 Hz, 1 H, CH), 3.87 (s, 4 H, 2 CH_2), 4.19 (q, J = 7.1 Hz, 2 H, CH), 6.82 (d, J = 5.3 Hz, 2 H, CH), 7.16 (d, J = 5.3 Hz, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.0 (C), 20.2 (CH_3), 20.4 (CH_3), 28.3 (C), 49.7 (2 CH_2), 60.7 (CH_2), 68.8

(CH), 109.6 (2 C), 125.5 (2 CH), 130.1 (2 CH), 138.6 (2 C), 172.4 (C) ppm. $C_{17}H_{21}Br_2NO_2S_2$ (495.29): calcd. C 41.22, H 4.27, N 2.83; found 41.45, H 4.40, N 3.11.

General Procedure for the Synthesis [*c*,*f*]-Difused 1-Azabicyclo[3-3-1]nonanes 6 and Monocyclization Products 8

Route A/B: A solution of the dialkylamino ester **5** (2 mmol) in dry THF (130 mL) was cooled to −100 °C under argon. 1.6 M *n*BuLi in hexane or 1.7 M *t*BuLi in pentane (4.2 mmol) was added dropwise while the temperature was kept below −98 °C. After complete addition, the solution was stirred at −100 °C for 6 h and was quenched with saturated aqueous NH_4Cl. The mixture was allowed to warm up to room temperature whilst stirring. After all precipitate had dissolved, the mixture was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were dried with Na_2SO_4 and concentrated under vacuum. The remainder was purified by column chromatography.

Route D/C: 3-Bromo-2-(bromomethyl)thiophene (0.304 g, 1.18 mmol) and K_2CO_3 (0.163 g, 1.18 mmol) were added to a solution of the corresponding monocyclized β-carbolinone **7a** (Ar^1 = indole) in MeCN (30 mL). The mixture was refluxed for 4 h. The cold suspension was filtered, and the filtrate was concentrated under vacuum. The remaining material was purified by column chromatography (hexane/EtOAc, 9:1) providing pure *N*-alkyl-β-carbolinone **8e** (0.435 g, 95% yield). This product was submitted to Route A with only 1.1 equiv. of *n*BuLi to afford 56% of the final product **6f** after column chromatography (hexane/ethyl acetate, 9:1).

(17*S*)-17-Isopropyl-9-azatetracyclo[7.7.1.$0^{2,7}$.$0^{11,16}$]heptadeca-2,4,6,11,13,15-hexaen-1-ol (6a): Yellow-brownish oil, 10% yield. R_f (hexane/ethyl acetate, 7:3) = 0.31. $[\alpha]_D^{20}$ = −37.75. (*c* = 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 0.98 (d, *J* = 6.4 Hz, 3 H, CH_3), 1.00 (d, *J* = 6.4 Hz, 3 H, CH_3), 2.04 (m, 1 H, CH), 2.51 (s, 1 H, OH), 3.03 (d, *J* = 7.5 Hz, 1 H, CH), 4.17 (m, 4 H, 2 CH_2), 6.93 (m, 4 H, CH), 7.04 (m, 2 H, CH), 7.25 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 19.9 (CH_3), 20.3 (CH_3), 27.1 (CH), 59.7 (2 CH_2), 69.9 (C), 76.7 (CH), 126.9 (2 CH), 128.1 (2 CH), 128.5 (4CH), 139.5 (2 C), 141.9 (2 C).) ppm. $C_{31}H_{35}N_3O_5$ (529.63): calcd. C 70.30, H 6.66, N 7.93; found C 69.98, H 6.41, N 7.62.

Di-*tert*-butyl (23*S*)-1-Hydroxy-23-methyl-9,12,15-triazahexacyclo[10.10.1.$0^{2,10}$.$0^{3,8}$.$0^{14,22}$. $0^{16,21}$]tricosa-2(10),3,5,7,14(22),16,18,20-octaene-9,15-dicarboxylate (6b): Colorless solid, 59% yield. M.p.161−163 °C, R_f (CH_2Cl_2/acetone, 95:5) = 0.14. $[\alpha]_D^{20}$ = +55.2 (*c* = 1, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 1.21 (d, *J* = 6.8 Hz, 3 H, CH), 1.56 [s, 9 H, $(CH_3)_3$], 1.57 [s, 9 H, $(CH_3)_3$], 2.39 (s, 1 H, OH), 3.38 (q, *J* = 6.8 Hz, 1 H, CH), 4.06 (d, *J* = 19.6 Hz, 1 H, CH_2), 4.18 (d, *J* = 18.8 Hz, 1 H, CH_2), 4.63 (d, *J* = 19.6 Hz, 1 H, CH_2), 4.71 (d, *J* = 18.6 Hz, 1 H, CH_2), 7.06−7.20 (m, 4 H, CH), 7.92−7.99 (m, 2 H, CH), 8.09−8.21 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.7 (C), 28.7 [2(CH_3)$_3$], 51.5 (CH_2), 57.8 (CH_2), 60.0 (CH), 71.5 (C), 84.4 (C), 84.5 (C), 115.9 (2 CH), 120.9 (CH), 121.0 (CH), 121.6 (C), 123.3 (2 CH), 123.8 (CH), 123.9 (CH), 126.3 (C), 126.9 (C), 127.8 (C), 132.9 (C), 134.0 (C), 136.1 (2 C), 150.7 (C), 150.8 (C) ppm. $C_{31}H_{35}N_3O_5$ (529.63): calcd. C 70.03, H 6.66, N 7.93; found C 69.84, H 6.38, N 7.72.

Di-*tert*-butyl (23*S*)-1-Hydroxy-23-isopropyl-9,12,15-triazahexacyclo[10.10.1.$0^{2,10}$.$0^{3,8}$. $0^{14,22}$.$0^{16,21}$]tricosa-2(10),3,5,7,14(22),16,18,20-octaene-9,15-dicarboxylate (6c): Colorless solid, 75% yield. R_f (hexane/ethyl acetate, 8:2) = 0.34. $[\alpha]_D^{20}$ = +117.1 (*c* = 1, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 1.05−1.19 (m, 6 H, CH), 1.56 [s, 9 H, $(CH_3)_3C$], 1.57 [s, 9 H, $(CH_3)_3C$], 1.68−1.81 (m, 1 H, CH), 2.21 (s, 1 H, OH), 2.79 (d, *J* = 9.5 Hz, 1 H, CH), 3.95 (d, *J* = 19.8 Hz, 1 H, CH_2), 4.17 (d, *J* = 18.7 Hz, 1 H, CH_2), 4.50 (d, *J* = 19.8 Hz, 1 H, CH_2), 4.61 (d, *J* = 18.7 Hz, 1 H, CH_2), 7.07−7.21 (m, 4 H, CH), 7.92−8.25 (m, 4 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 22.4 (C), 22.5 (C), 28.2 (C), 28.6 (2 C), 28.7 (C), 51.8 (CH_2), 58.6 (CH_2), 70.4 (CH), 73.8 (C), 84.3 (2 C), 115.7 (CH), 115.8 (CH), 121.0 (CH), 121.3 (CH), 122.0 (C), 123.2 (CH), 123.3 (CH), 123.6 (CH), 123.9 (CH), 127.0 (C), 127.4 (C), 127.9 (C), 133.9 (C), 134.3 (C), 136.0 (C), 136.3 (C), 150.7 (C), 150.8 (C) ppm. $C_{33}H_{39}N_3O_5$ (557.68): calcd. C 71.07, H 7.05, N 7.53; found C 70.84, H 6.84, N 7.32. HPLC determination of the enantiomeric excess: Column: CHIRALCEL OD, eluent: hexane/*i*PrOH (98:2), flow rate: 0.5 mL/min, detection: UV 254 nm, temperature 20 °C. Racemic mixture ***rac*-6c**, obtained starting from ***rac*-5f**, revealed for the (−) enantiomer R_t = 13 min and for the (+) enantiomer R_t = 14.5 min. Compound **6c** showed just one peak at 14.5 min, i.e. *ee* > 99%.

Di-*tert*-butyl (23*S*)-1-Hydroxy-23-[(1*R*)-1-methylpropyl]-9,12,15-triazahexacyclo[10.10.1.$0^{2,10}$.$0^{3,8}$.$0^{14,22}$.$0^{16,21}$]tricosa-2(10),3,5,7,14(22),16,18,20-octaene-9,15-dicarboxylate (6d): Light yellow solid, 73% yield. M.p. 134 °C, R_f (hexane/ethyl acetate, 9:1) = 0.09. $[\alpha]_D^{20}$ = +128.2 (*c* = 1, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 0.85 (t, *J* = 7.4 Hz, 3 H, CH), 1.05 (d, *J* = 6.6 Hz, 3 H, CH), 1.28−1.49 (m, 2 H, CH), 1.56 [s, 9 H, $(CH_3)_3$], 1.57 [s, 9 H, $(CH_3)_3$], 1.62−1.80 (m, 1 H, CH), 2.24 (s, 1 H, OH), 2.90 (d, *J* = 9.8 Hz, 1 H, CH), 3.95 (d, *J* = 19.9 Hz, 1 H, CH_2), 4.17 (d, *J* = 18.9 Hz, 1 H, CH_2), 4.49 (d, *J* = 19.9 Hz, 1 H, CH_2), 4.59 (d, *J* = 18.9 Hz, 1 H, CH_2), 7.05 −7.25 (m, 4 H, CH), 7.92−8.26 (m, 4 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.0 (C), 18.0 (C), 27.8 (C), 28.7 (2 C), 33.8 (C), 51.9 (CH_2), 58.4 (CH_2), 68.0 (CH), 73.7 (C), 84.3 (2 C), 115.7 (CH), 115.8 (CH), 121.1 (CH), 121.3 (CH), 121.9 (C), 123.2 (CH), 123.3 (CH), 123.6 (CH), 123.9 (CH), 127.0 (C), 127.6 (C), 127.9 (C), 133.9 (C), 134.3 (C), 136.0 (C), 136.3 (C), 150.7 (C), 150.8 (C) ppm. $C_{34}H_{41}N_3O_5$ (571.71): calcd. C 71.43, H 7.23, N 7.35; found C 71.28, H 7.46, N 7.20.

Di-*tert*-butyl (23*S*)-1-Hydroxy-23-isobutyl-9,12,15-triazahexacyclo[10.10.1.$0^{2,10}$.$0^{3,8}$. $0^{14,22}$.$0^{16,21}$]tricosa-2(10),3,5,7,14(22),16,18,20-octaene-9,15-dicarboxylate (6e): Light yellow solid, 72% yield. R_f (hexane/ethyl acetate, 7:3) = 0.43. $[\alpha]_D^{20}$ = +41.6 (*c* = 1, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 1.05 (d, *J* = 6.6 Hz, 6 H, 2 CH_3), 1.49 (m, 1 H, CH), 1.67 (s, 18 H, 2(CH_3)$_3$], 2.05 (m, 2 H, CH_2), 2.21 (s, 1 H, OH), 3.42 (m, 1 H, CH), 4.15 (d, *J* = 19.6 Hz, 1 H, CH_2), 4.32 (d, *J* = 18.9 Hz, 1 H, CH_2), 4.62 (d, *J* = 19.6 Hz, 1 H, CH_2), 4.77 (d, *J* = 18.9 Hz, 1 H, CH_2), 7.24 (m, 4 H, CH), 8.11 (m, 2 H, CH), 8.26 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 22.4 (CH_3), 24.4 (CH_3), 25.8 (C), 28.7 (C), 34.7 (CH_2), 51.4 (CH_2), 58.1 (CH_2), 62.8 (CH), 71.4 (C), 84.5 (C), 115.8 (2 CH), 120.9 (CH), 121.0 (CH), 122.1 (C), 123.3 (C), 128.8 (CH), 123.9 (CH), 126.6 (CH), 126.9 (CH), 127.0 (C), 127.6 (C), 127.8 (C), 133. (C), 134.3 (C), 135.9 (C), 136.1 (C), 150.7 (C), 150.8 (C) ppm. $C_{34}H_{41}N_3O_5$ (571.71): calcd. C 71.43, H 7.23, N 7.35; found C 71.14, H 7.13, N 6.96.

***tert*-Butyl (19*S*)-1-Hydroxy-19-isopropyl-15-thia-9,12-diazapentacyclo[10.6.1.$0^{2,10}$.$0^{3,8}$. $0^{14,18}$]nonadeca-2(10),3,5,7,14(18),16-hexaene-9-carboxylate (6f):** Yellow oil, 48% yield (Route A), 56% yield (Route C). R_f (hexane/ethyl acetate, 9:1) = 0.23. ^1H NMR ($CDCl_3$): δ = 1.05 (d, *J* = 6.7 Hz, 3 H, CH_3), 1.09 (d, *J* = 6.5 Hz, 3 H, CH_3), 1.57 (s, 9 H, C), 2.17 (s, 1 H, OH), 2.78 (d, *J* = 9.5 Hz, 1 H, CH), 3.88−3.97 (m, 2 H, 2 CH_2), 4.53 (d, *J* = 19.1 Hz, 1 H, CH_2), 6.92 (d, *J* = 5.1 Hz, 1 H, CH), 7.12−7.18 (m, 3 H, CH), 6.94−8.03 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 22.1 (CH_3), 22.2 (CH_3), 27.7 (C), 28.3 (C), 51.11 (CH_2), 57.2 (CH_2), 69.4 (CH), 73.5 (C), 83.9 (C), 115.4 (CH), 120.5 (CH), 121.4 (C), 122.8 (CH), 123.4 (CH), 123.5 (2 CH), 127.1 (C), 133.6 (C), 134.1 (C), 135.9 (C),

147.3 (C), 150.4 (C) ppm. $C_{24}H_{28}N_2O_3S$ (424.56): calcd. C 67.90, H 6.65, N 6.60, S 7.55; found C 67.52, H 6.45, N 6.49, S 7.78.

(15*S*)-15-Isopropyl-5,11-dithia-8-azatetracyclo[6.6.1.0^{2,6}.0$^{1\circ,14}$]-pentadeca-2(6),3,10(14),12-tetraen-1-ol (6g): Yellow oil, 53% yield. R_f (hexane/ethyl acetate, 9:1) = 0.12. ^{1}H NMR ($CDCl_3$): δ = 0.99 (d, J = 6.6 Hz, 3 H, CH_3), 1.05 (d, J = 6.6 Hz, 3 H, CH_3), 1.62−1.69 (m, 1 H, CH), 2.11 (s, 1 H, OH), 2.72 (d, J = 9.3 Hz, 1 H, CH), 3.69 (d, J = 18.3 Hz, 1 H, CH_2); 3.89 (d, J = 17.4 Hz, 1 H, CH_2), 4.41 (d, J = 18.3 Hz, 1 H, CH_2), 4.47 (d, J = 17.4 Hz, 1 H, CH_2), 6.94−7.18 (m, 4 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 22.3 (CH_3), 22.5 (CH_3), 28.2 (CH), 50.8 (CH_2), 57.8 (CH_2), 69.7 (CH), 73.9 (C), 123.3 (CH), 123.5 (CH), 123.7 (CH), 124.2 (CH), 134.3 (C), 134.6 (C), 142.2 (C), 146.8 (C) ppm. $C_{15}H_{17}NOS_2$ (291.43): calcd. C 61.82, H 5.88, N 4.81, S 22.01; found C 61.49, H 5.73, N 4.85, S 21.91.

Corresponding By-Product 5-Isopropyl-6-(2-thienylmethyl)-6,7-dihydrothieno[2,3-*c*]pyridin-4(5*H*)-one (12): Yellow oil, 26% yield. R_f (hexane/ethyl acetate, 9:1) = 0.21. $[\alpha]_D^{20}$ = +2.0 (c = 1.55, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.86 (d, J = 6.6 Hz, 3 H, CH_3), 1.12 (d, J = 6.5 Hz, 3 H, CH_3), 1.86−1.96 (m, 1 H, CH), 2.78 (d, J = 10.5 Hz, 1 H, CH); 3.73−3.99 (m, 3 H, 2 × CH_2), 4.34 (d, J = 18.2 Hz, 1 H, CH_2), 6.76−7.36 (m, 5 H, CHar) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.0 (CH_3), 21.8 (CH_3), 27.8 (CH), 45.6 (CH_2), 54.3 (CH_2), 74.5 (CH), 124.5 (CH), 125.0 (CH), 125.7 (CH), 125.8 (CH), 126.1 (CH), 135.5 (C), 143.4 (C), 150.5 (C), 194.7 (CO) ppm.

(3*S*)-2-(2-Bromobenzyl)-3-isopropyl-2,3-dihydro-4(1*H*)-isoquinolinone (8a): Colorless solid, 48% yield. R_f (hexane/ethyl acetate, 9:13) = 0.34. ^{1}H NMR ($CDCl_3$): δ = 0.83 (d, J = 6.6 Hz, 3 H, CH_3), 1.11 (d, J = 6.6 Hz, 3 H, CH_3), 1.94−2.02 (m, 1 H, CH), 2.85 (d, J = 10.5 Hz, 1 H, CH), 3.66 (d, J = 17.9 Hz, 1 H, CH_2), 3.75 (d, J = 14.4 Hz, 1 H, CH_2), 3.86 (d, J = 14.3 Hz, 1 H, CH_2), 4.32 (d, J = 17.9 Hz, 1 H, CH_2), 7.05−7.56 (m, 7 H, CH), 7.94 (dd, J_1 = 7.8, J_2 = 1.0 Hz, 1 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.0 (CH_3), 21.6 (CH_3), 27.4 (C), 47.7 (CH_2), 58.7 (CH_2), 125.1 (C), 126.8 (CH), 127.6 (CH), 127.7 (CH), 129.1 (CH), 130.4 (CH), 131.0 (CH), 133.3 (CH), 134.3 (CH), 138.4 (C), 138.6 (C), 140.1 (C), 199.4 (CO). ppm. $C_{19}H_{20}BrNO$ (358.27): calcd. C 63.70, H 5.63, N 3.91; found C 63.44, H 5.41, N 3.74.

***tert*-Butyl (3*S*)-2-(2-Bromobenzyl)-3-methyl-4-oxo-1,2,3,4-tetrahydro-9*H*-β-carbolin-9-carboxylate (8b):** Colorless solid,56% yield. R_f (hexane/ethyl acetate, 7:3) = 0.27. $[\alpha]_D^{20}$ = −11.9 (c = 1, CH_3CN). ^{1}H NMR ($CDCl_3$): δ = 1.32 (d, J = 7.1 Hz, 3 H, CH), 1.54 [s, 9 H, $(CH_3)_3$,] 3.49 (q, J = 7.1 Hz, 1 H, CH), 3.82 (d, J = 14.7 Hz, 1 H, CH_2), 3.91 (d, J = 14.7 Hz, 1 H, CH_2), 4.26 (s, 2 H, CH_2), 7.04−7.30 (m, 4 H, CH), 7.37 (d, J = 7.2 Hz, 1 H, CH), 7.43 (d, J = 7.4 Hz, 1 H, CH), 8.02−8.09 (m, 1 H, CH), 8.15−8.20 (m, 1 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.7 (CH_3), 28.1 (C), 46.3 (CH_2), 57.3 (CH_2), 63.5 (CH), 85.7 (C), 114.2 (C), 115.3 (CH), 121.5 (CH), 124.6 (CH), 124.7 (C), 125.1 (CH), 125.6 (C), 127.5 (CH), 128.9 (CH), 130.8 (CH), 132.9 (CH), 136.0 (C), 137.5 (C), 147.9 (C), 149.4 (C), 196.4 (CO) ppm. $C_{24}H_{25}BrN_2O_3$ (469.37): calcd. C 61.41, H 5.37, N 5.97; found C 61.24, H 5.28, N 5.84.

***tert*-Butyl (3*S*)-2-{[3-Bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl]methyl}-3-(hydroxymethyl)-4-oxo-1,2,3,4-tetrahydro-9*H*-β-carbolin-9-carboxylate (8c):** Colorless solid, 18% yield. R_f (hexane/ethyl acetate, 7:3) = 0.19. $[\alpha]_D^{20}$ = −12.4 (c = 1, CH_2Cl_2) ppm. ^{13}C NMR ($CDCl_3$): δ = 26.3 (C), 26.5 (C), 42.2 (CH_2), 45.5 (CH_2), 57.3 (CH_2), 68.7 (CH), 83.7 (C), 84.2 (C), 104.7 (C), 113.3 (C), 113.6 (CH), 114.1 (CH), 117.8 (CH), 119.8 (CH), 121.8 (CH), 122.2 (C), 123.0 (CH), 123.5 (CH), 124.2 (CH), 125.9 (C), 131.5 (C), 133.8 (C), 139.3 (C), 146.1 (C), 147.8 (C), 148.5 (C), 193.2 (C) ppm. $C_{31}H_{34}BrN_3O_6$ (624.52): calcd. C 59.62, H 5.49, N 6.73; found C 59.34, H 5.38, N 6.64.

***tert*-Butyl (3*S*)-2-{[1-(*tert*-Butoxycarbonyl)-1*H*-indol-2-yl]methyl}-3-(butoxymethyl)-4-oxo-1,2,3,4-tetrahydro-9*H*-β-carbolin-9-carboxylate (8d):** Colorless solid, 19% yield. R_f (hexane/ethyl acetate, 7:3) = 0.12. ^{13}C NMR ($CDCl_3$): δ = 14.6 (CH_3), 24.3 (CH_2), 28.4 (CH_3), 28.6 (CH_3), 29.5 (CH_2), 46.0 (CH_2), 53.2 (CH_2), 59.9 (CH_2), 68.1 (CH_2), 70.7 (CH), 84.9 (C), 86.5 (C), 111.1 (CH), 115.7 (CH), 115.8 (CH), 116.1 (C), 120.8 (CH), 121.9 (CH), 123.3 (CH), 124.7 (CH), 125.2 (CH), 125.5 (C), 125.8 (CH), 128.9 (C), 136.4 (C), 137.2 (C), 138.0 (C), 148.0 (C), 149.6 (C), 150.8 (C), 193.8 (C) ppm.

***tert*-Butyl (3*S*)-2-[(3-Bromo-2-thienyl)methyl]-3-isopropyl-4-oxo-1,2,3,4-tetrahydro-9*H*-β-carbolin-9-carboxylate (8e):** Colorless solid, 95% yield. M. p. 147 °C. R_f (hexane/ethyl acetate, 9:1) = 0.24. $[\alpha]_D^{20}$ = −20.37 (c = 0.54, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.89 (d, J = 6.6 Hz, 3 H, CH_3), 1.15 (d, J = 6.6 Hz, 3 H, CH_3), 1.57 (s, 9 H, C), 1.81−1.98 (m, 1 H, CH), 2.79 (d, J = 10.5 Hz, 1 H, CH), 3.79 (d, J = 14.7 Hz, 1 H, CH), 4.02 (d, J = 14.7 Hz, 1 H, CH_2), 4.24 (d, J = 19.7 Hz, 1 H, CH_2), 4.39 (d, J = 19.7 Hz, 1 H, CH_2), 6.83 (d, J = 5.3 Hz, 1 H, CH), 7.17 (d, J = 5.3 Hz, 1 H, CH), 7.27−7.30 (m, 2 H, CH), 8.03−8.21 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 19.6 (CH_3), 21.6 (CH_3), 28.1 (C), 45.7 (CH_2), 53.6 (CH_2), 75.2 (CH), 85.8 (C), 109.3 (C), 114.4 (C), 115.3 (CH), 121.6 (CH), 124.6 (CH), 125.0 (CH), 125.4 (CH), 125.6 (CH), 129.8 (CH), 135.9 (C), 138. (C), 146.3 (C), 149.5 (C), 196.3 (CO) ppm. $C_{24}H_{27}BrN_2O_3S$ (503.45): calcd. C 57.26, H 5.41, Br 15.87, N 5.56, S 6.37; found C 56.95, H 5.67, Br 16.01, N 5.46, S 6.50.

(2*S*)-2-[Bis(2-Bromobenzyl)amino]-3-heptanone (9): Colorless oil, 18% yield. R_f (hexane/ethyl acetate, 9:1) = 0.50. ^{13}C NMR ($CDCl_3$): δ = 8.1 (C), 14.2 (C), 23.7 (C), 25.9 (CH_2), 40.2 (C), 56.0 (2 CH_2), 63.2 (CH), 125.5 (2 C), 127.7 (2 CH), 128.9 (2 CH), 131.2 (2 CH), 133.1 (2 CH), 138.8 (2 C), 212.6 (CO) ppm. $C_{21}H_{25}Br_2NO$ (467.24): calcd. C 53.98, H 5.39, N 3.00; found C 54.00, H 5.32, N 3.09.

(3*S*,4*S*)-2-(2-Bromobenzyl)-4-butyl-3-methyl-1,2,3,4-tetrahydro-4-isoquinolinol (10): Colorless solid, 41% yield. Diastereomeric ratio 78:22 (major stereoisomer separable), R_f (hexane/ethyl acetate, 9:1; major stereoisomer) = 0.25. ^{1}H NMR ($CDCl_3$): δ = 0.73 (t, J = 7.3 Hz, 3 H, CH_3), 0.95 (d, J = 6.6 Hz, 3 H, CH_3), 1.06−1.18 (m, 2 H, CH_2), 1.66−1.79 (m, 2 H, CH_2), 1.96−2.06 (m, 2 H, CH_2), 3.02 (q, J = 6.6 Hz, 1 H, CH), 3.58−3.81 (m, 4 H, 2 CH_2), 6.89 (d, J = 7.4 Hz, 1 H, CH), 7.02−7.21 (m, 4 H, CH), 7.34 (dd, J_1 = 7.6, J_2 = 1.6 Hz, 1 H, CH), 7.45−7.49 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 6.3 (CH_3), 14.5 (CH_3), 23.5 (CH_2), 26.0 (CH_2), 42.3 (CH_2), 50.9 (CH_2), 57.7 (CH) 58.8 (CH_2), 75.7 (C), 125.5 (C), 125.9 (CH), 126.5 (CH), 126.9 (CH), 127.5 (CH), 129.0 (CH), 131.7 (CH), 133.2 (CH), 133.3 (CH), 134.1 (C), 138.4 (C), 141.4 (C) ppm. $C_{21}H_{26}BrNO$ (388.34): calcd. C 64.95, H 6.75, N 3.61, Br 20.58; found C 65.09, H 6.66, N 3.68, Br 20.71.

5-{(1*S*)-1-[Bis(2-bromobenzyl)amino]ethyl}-5-nonanol (11): Colorless oil, 16% yield. R_f (hexane/ethyl acetate, 9:1) = 0.47. ^{13}C NMR ($CDCl_3$): δ = 9.5 (C), 14.5 (2 C), 22.8 (C), 24.0 (C), 25.4 (CH_2), 26.3 (CH_2), 36.2 (C), 36.8 (C), 54.9 (2 CH_2), 58.3 (CH), 75.9 (C), 124.9 (2 C), 127.8 (2 CH), 129.0 (2 CH), 132.0 (2 CH), 133.2 (2 CH), 138.6 (2 C) ppm. $C_{25}H_{35}Br_2NO$ (525.36): calcd. C 57.15, H 6.72, N 2.67 found C 57.22, H 6.66, N 2.72.

(23*S*)-9,15-Bis(*tert*-butoxycarbonyl)-1-hydroxy-12,23-dimethyl-9,15-diaza-12-azonia-hexacyclo[10.10.1.0^{2,10}.0^{3,8}.0^{14,22}.0^{16,21}]tricosa-2(10),3,5,7,14(22),16,18,20-octaene Iodide (13): MeI (0.36 mL, 5.66 mmol) was added to a solution of **6b** (1.15 mmol) in CH_2Cl_2

(30 mL). After 30 min of refluxing, the volatile components were evaporated under vacuum to afford the product as a yellow solid in 98% yield. R_f ($CHCl_3$/MeOH, 9:1) = 0.18. $[\alpha]_D^{20}$ = +80.5 (*c* = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.58−1.61 (m, 21 H, CH_3), 3.82 (s, 3 H, CH_3) 4.98 (d, 1 H, CH_2), 5.06 (d, 1 H, CH_2), 5.24−5.45 (m, 2 H, CH_2), 5.71 (d, 1 H, CH_2), 7.08−7.29 (m, 4 H, CH, 7.78−7.86 (m, 2 H, CH), 8.04−8.27 (m, 2 H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ = 9.7 (C), 28.20 $(CH_3)_3$, 28.23 $(CH_3)_3$, 56.9 (CH_3), 63.4 (CH_2), 68.6 (CH_2), 69.8 (CH), 70.1 (C), 86.1 (C), 86.2 (C), 115.2 (CH), 115.4 (CH), 121.1 (CH), 121.2 (C), 122.0 (CH), 122.4 (C), 123.6 (2 C), 123.7 (CH), 123.8 (CH), 124.8 (C), 125.4 (2 CH), 126.0 (C), 135.4 (C), 135.5 (C), 150.7 (C), 150.8 (C) ppm. $C_{32}H_{38}IN_3O_5$ (671.57): calcd. C 57.23, H 5.70, N 6.26; found C 57.05, H 5.87, N 6.52.

b) Biological Testing

Methods

Purification of Recombinant HIV-1 RT: The enzyme purification was described previously.[23−25]

Inhibitors: The inhibitors **6b**, **6c** and **6e** were dissolved and diluted in 100% DMSO. The inhibitor solution (2 μL, different concentrations) was added to the assay solution to reach a final DMSO concentration of 2%.

RNase H Assay: The RNase H activity was examined with a 45/36mer RNA/DNA hybrid. The 45mer RNA was synthesized chemically and possessed a fluorescent Cy5 label attached to its 5′ end (IBA GmbH, Göttingen, Germany). The sequence of the template RNA was 5′ Cy5-CUAAUUCCCCUUUCCCCCUCUC-CUGGUGAUCCUUUCCAUCCCUGU 3′. The sequence of the DNA primer was complementary to the last 36 nucleotides of the 3′ end of the RNA. Hybridization was performed as described previously.[26] The RNase H cleavage was examined in RT buffer (50 mM Tris-HCl pH = 8, 80 mM KCl, 5 mM DTT, 6 mM $MgCl_2$). 20 nM HIV-1 RT was pre-incubated with different concentrations of inhibitor for 2 min at 37 °C. Reactions were started by the addition of 80 nM primer/template and stopped by the addition of formamide buffer [80% (v/v) formamide in 90 mM Tris-HCl pH = 8.3, 90 mM boric acid, 3 mM EDTA]. Reaction products were separated in the dark on denaturing 20% polyacrylamide gels, containing 7 M urea. The fluorescent bands were visualized at an excitation wavelength of 635 nm with the use of a fluorimaging device (Fuji FLA 5000). Emission was measured with a cut-off filter (665 nm).

Analysis of Polymerization Products in the Presence of Inhibitors: DNA-dependent polymerization on a single-stranded bacteriophage M 13 DNA template was determined as described previously[27] with the following modifications: Briefly, an18mer oligodeoxynucleotide labeled radioactively at the 5′ end with ^{32}P was hybridized to single-stranded M13 DNA in RT buffer described above. 8 nM of HIV-1 RT was pre-incubated in RT buffer with different amounts of inhibitor and 250 μM dNTPs at 37 °C for 5 min in a final assay volume of 50 μL. 1 μL of the inhibitor solution was added to the assay solution. The final DMSO concentration was 2%. Reactions were started by the addition of the primer/template substrate to a final concentration of 10 nM. Reactions were stopped after 20 min by the addition of 25 μL of formamide buffer. 15-μL aliquots were loaded on 10% denaturing polyacrylamide gels containing 7 M urea. The bands were visualized using a phosphorimaging device.

Toxicity on Human Cells: The toxicity of the inhibitors was tested with the human HeLa cell line. HeLa cells were seeded on a 24-well plate and incubated overnight in DMEM medium with 10% fetal calf serum at 37 °C. Then the medium was exchanged and inhibitors were added at different concentrations. After 2 d, the medium was removed and fresh medium containing the same inhibitor concentrations was added. After incubation for another 3 d, the cells were trypsinated, washed with PBS buffer (137 mM NaCl, 2.68 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH = 7.4) and resuspended in 100 μL of PBS. 20 μL of the cell suspension was mixed with 20 μL of Trypan Blue to detect dead cells. Cells were counted in a Neubauer chamber.

Materials and Methods for DNA Polymerase Assay

Chemicals: [^{3}H]dTTP (40 Ci/mmol) was from Amersham Biosciences; unlabeled dNTPs, poly(dA), and oligo(dT)12-18 were from Roche Molecular Biochemicals. Whatman was the supplier of the GF/C filters. All other reagents were of analytical grade and were purchased from Merck or Fluka.

DNA Polymerase Assay: DNA polymerase (pol) β activity on poly(dA)/oligo(dT) 10:1 (0.5 μg) was assayed in a final volume of 25 μL containing 50 mM Tris-HCl (pH = 7.6), 0.25 mg/mL bovine serum albumin, 1 mM DTT, 0.5 mM $MnCl_2$, and 25 μM [^{3}H]dTTP (5 Ci/mmol). All reaction mixtures were incubated at 37 °C for 15 min, and the DNA was precipitated with 10% trichloroacetic acid. Insoluble radioactive material was determined by scintillation counting as described;[28] pol δ was assayed as described for pol β with the exception that instead of 0.5 mM $MnCl_2$, 6 mM $MgCl_2$ was used in the reaction, in addition to 40 nM of proliferating cell nuclear antigen (PCNA).

Enzymes: pol δ and pol β were purified as described.[29,30] PCNA was purified as previously described.[31]

Quantification of the Polymerization Products: This was performed by phosphorimaging (Figure 3). The amount of extended primer in the absence of inhibitor (65%) was set as 100%. The amount of products in the presence of inhibitor was quantified as a ratio thereof.

Quantification of the RNase H Cleavage Products: This was performed by phosphorimaging (Figure 4). The amount of cleaved 5′ RNA fragments in the absence of inhibitor (54%) was set as 100%. The amount of products in the presence of inhibitor was quantified as a ratio thereof.

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