Ester cleavage by *S*,*S*-(+)-tetrandrine derivative bearing thiol groups

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Dedicated to Professor Eusebio Juaristi on the occasion of his 55th birthday
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Abstract
A chiral cyclophane–type macrocycle bearing two thiol groups has been prepared by chemical modification of *S*,*S*-(+)-tetrandrine. Cleavage of *p*-nitrophenyl acetate by deprotonated form of this macrocycle proceeds with a rate constant expected for a simple thiol anion of similar basicity, but the reactivity toward *p*-nitrophenyl esters of *N*-protected amino acids is one order of magnitude higher than expected. This increased reactivity is attributed to a pre-association of voluminous hydrophobic amino acid substrates with a dimeric form of the macrocycle, which shows a small enantioselectivity in favor of L-amino acid ester (k_D/k_L=0.8). Ester cleavage by the monomeric form of the macrocycle proceeds slower, but shows higher and inverted enantioselectivity (k_D/k_L=3.4).

Keywords: Cyclophane, thiolysis, ester cleavage, kinetics

Introduction

Macrocycles with attached nucleophilic functionalities have been studied extensively as models for hydrolytic enzymes.[1] Among them macrocycles bearing thiol groups attracted attention as analogs of thiol proteases. Crown ethers bearing cysteinyl residues[2] and chiral crown ethers

functionalized with alkylthiol groups[3] were employed for enantioselective cleavage of activated esters. Crown ethers possessing two thiol groups were proposed as catalysts for peptide synthesis.[4] In these systems esters of general structure RCH(NH_3^+)COOAr were employed as substrates and their recognition by catalysts was achieved via complexation of the substrate ammonium group with the catalyst crown ether moiety. More recently alkaline-earth metal complexes of thiol-pendant crown ethers were studied as enzyme-like catalysts for methanolysis of *p*-nitrophenyl acetate.[5]

Chiral alkaloids are often used in asymmetric catalytic reactions, ***e.g.*** in enantioselective Michael addition[6] or hydrogenation.[7] Previously we have reported the enantioselective cleavage of activated amino acid esters by a natural cyclophane-like bis-isoquinoline type chiral macrocyclic alkaloid (+)-tubocurarine, which possesses two nucleophilic phenolate groups.[8] Since thiols are stronger nucleophiles than alcohols towards esters with good leaving groups[9],[10] we expected a better catalytic performance with a similar macrocycle, but bearing attached thiol groups. In this paper we report the synthesis and kinetic study of a thiol derivative of tetrandrine, another bis-isoquinoline type chiral macrocyclic alkaloid related to tubocurarine, but allowing more simple chemical modification via alkylation of two ternary nitrogen atoms. Recently we have prepared and characterized the binding properties of the bisbenzylated dicationic tetrandrine derivative,[11] which differ from the compound discussed in this paper just by the absence of two methanethiol groups in *para* positions of benzyl rings. Basing on these results we expected that the new catalyst would show selectivity towards neutral *N*-protected amino acid esters of general structure RCH(NHCOR')COOAr, which therefore were used as substrates in this study.

Results and Discussion

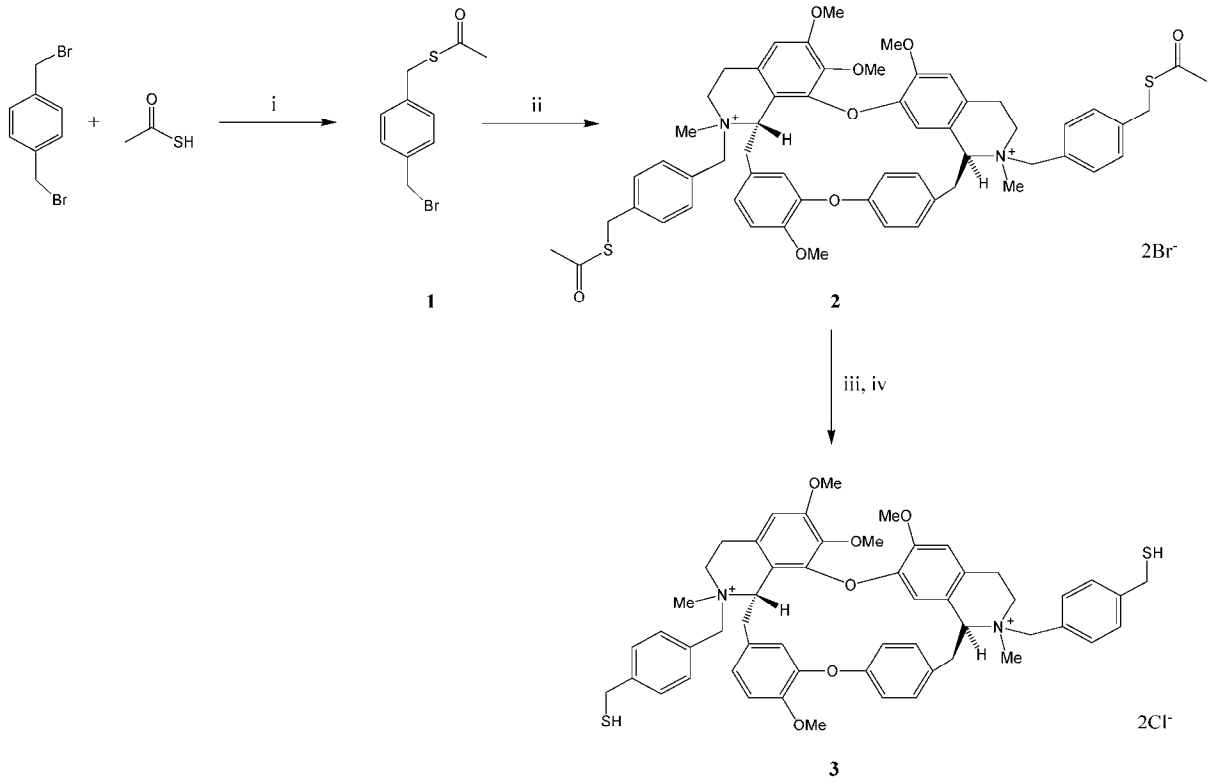
Synthesis of a thiol derivative of S,S-(+)-tetrandrine

The synthesis leading to the thiol functionalized macrocycle **3** is outlined in Scheme 1. The coupling reaction of α,α'-dibromo-*p*-xylene with thiolacetic acid, followed by chromatography on silica gel column gave compound **1** in 21% yield. Then the semisynthetic cyclophane type compound **2** was obtained in 80% yield by quaternization of two ternary nitrogen atoms of the bisisoquinoline alkaloid *S*,*S*-(+)-tetrandrine with compound **1**. Finally, compound **2** was converted to the thiol derivative of tetrandrine **3,** in *ca*. 100% yield, by deprotection of thiol groups with NaOH under N_2 followed by addition of concentrated HCl.

In contrast to natural tetrandrine, compound **3** in its dicationic form is highly soluble in polar solvents like water, MeOH and EtOH. Acid and neutral aqueous solutions of **3** are stable towards oxidation by air even at relatively high concentrations of **3**, but at pH values higher than 7 solutions of **3** rapidly become turbid apparently due to oxidation of the deprotonated thiol groups affording a less soluble probably polymeric compound with disulphide bonds between macrocyclic units. More diluted millimolar solutions of **3** prepared in freshly boiled water under

nitrogen were however stable even in basic solutions during several hours, sufficiently long for kinetic and spectrophotometric measurements.

Quaternization of nitrogen atoms produces two new chiral centers and in principle the compound **3** may be a mixture of stereoisomers. The ^{1}H NMR spectrum of macrocycle **3** as well as of its precursor **2** shows however a single set of signals for all hydrogens, indicating the formation of a single stereoisomer. Figure 1 shows the aromatic region of the ^{1}H NMR spectrum of macrocycle **3** in CD_3OD, where nine of the ten aromatic protons of cyclophane structure are in the range 6.2-7.17 ppm, and signals for hydrogens of the two benzyl units plus the tenth aromatic hydrogen of the cyclophane structure are in the range 7.36-7.60 ppm. Partial assignment of signals is given in the Experimental Section.



Scheme 1. Reagents and conditions: (i) THF, rt, 10 h (**1**: 21%); (ii) tetrandrine (0.4 eq.), acetone, reflux, 16 h (**2**: 80%); (iii) 0.2 M NaOH, N_2, rt, 3 h (iv) conc. HCl (**3**: *ca.* 100%).

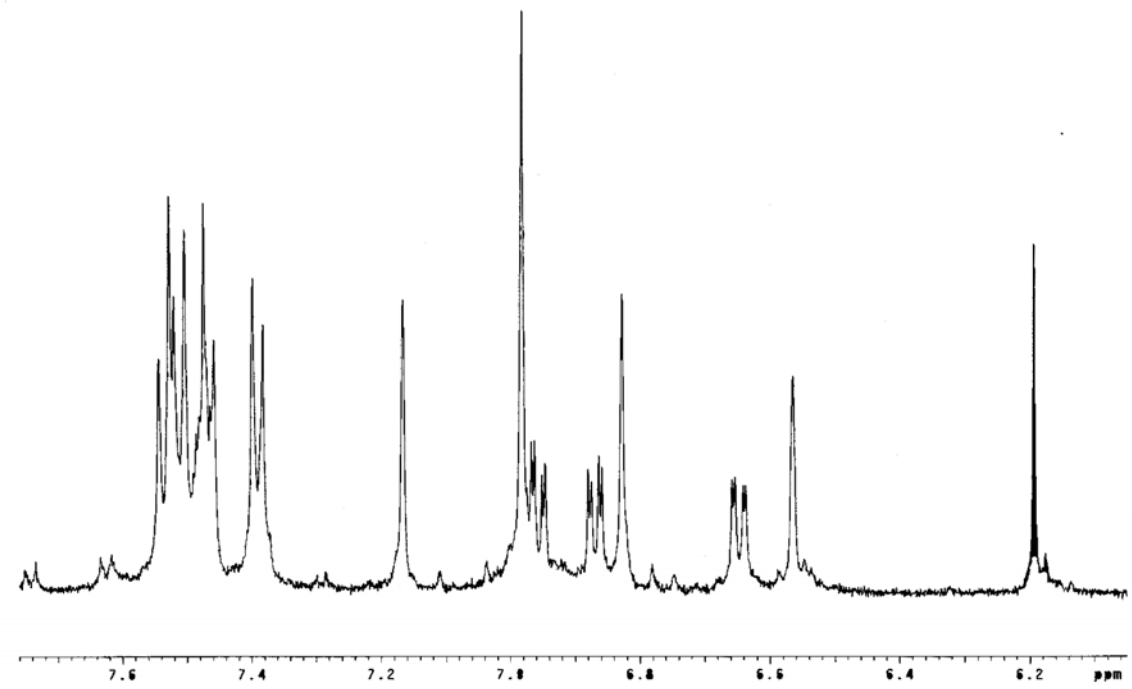


Figure 1. Aromatic region of the ^{1}H NMR spectrum of macrocycle **3** in CD_3OD (see Experimental Section for signal assignments).

Determination of pK_a values of 3

The pK_a values of both thiol groups were determined by spectrophotometric titration of **3** in 20% vol. aqueous acetonitrile (the reaction medium employed for kinetic studies) in the pH range 2-11. The absorption spectrum of **3** shows a maximum at 280 nm (ε =3360 M^{-1} cm^{-1} at pH 7) and the absorbance decreases in more acid media and increases significantly on increase in pH. A typical titration curve is illustrated in Figure 2. Titration results at three wavelengths in the range 260-290 nm were fitted to the equation (1) where A is the absorbance at a given wavelength and ε_{HH}, ε_H and ε are the molar absorptivities of diprotonated, monoprotonated and unprotonated forms of **3** respectively. The calculated pK_a values were averaged affording pK_{a1} = 4.81 ± 0.01 and pK_{a2} = 9.46 ± 0.04.

$$A = (\varepsilon_{HH}[H^+]^2/K_{a1}K_{a2} + \varepsilon_H[H^+]/K_{a2} + \varepsilon)[\mathbf{3}]/(1 + [H^+]/K_{a2} + [H^+]^2/K_{a1}K_{a2}) \quad (1)$$

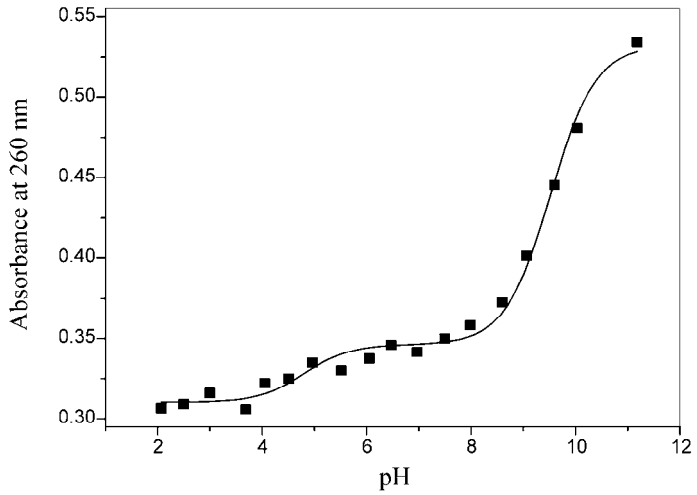


Figure 2. The spectrophotometric titration curve of 0.08 mM **3** at 260 nm in 20% vol. MeCN.

Successive deprotonation steps convert initially dicationic species **3** to a monocation and then to a neutral bis-zwitterionic species. The second pK_a is close to that for thiobenzyl alcohol (pK_a=9.43 in water[9] and 9.8 in 20% MeCN, see below), but the first pK_a of **3** is decreased much stronger than one would expect on basis of just the inductive effect of a tetraalkylammonium group. Apparently the first deprotonated mercapto group is stabilized by an intramolecular ion pairing with one of the ammonium groups in **3**.

Kinetics of ester cleavage

Kinetics of thiolysis of activated esters was studied in details by Hupe and Jencks[9] and in order to compare the reactivity of **3** with that of other thiols toward the same substrate we measured first kinetics of the cleavage of **NPA** (see Chart 1) employed in ref.[9] Reaction rates were measured in pH range 7.5-9.0 and with varied concentrations of **3** between 0.4 and 0.9 mM. Under these conditions the reaction kinetics followed a simple second-order rate equation (see below, however) and the second-order rate constants k_{2obs} obtained at different pH values were fitted to the equation (2) with fixed value of pK_{a2} = 9.46 (Supplementary Data, Figure 1S).

$$k_{2obs} = (k_{2H}[H^+]/K_{a2} + k_2)/(1 + [H^+]/K_{a2}) \quad (2)$$

The equation (2) is derived for a scheme in which the ester cleavage proceeds via two parallel routes involving mono-cationic and zwitterionic forms of **3** with the rate constants k_{2H} and k_2 respectively. From these results we obtained the second-order rate constants for **NPA** cleavage by both forms of **3** equaling k_{2H} = 0.30±0.07 and k_2=18.3±0.5 $M^{-1}s^{-1}$, respectively.

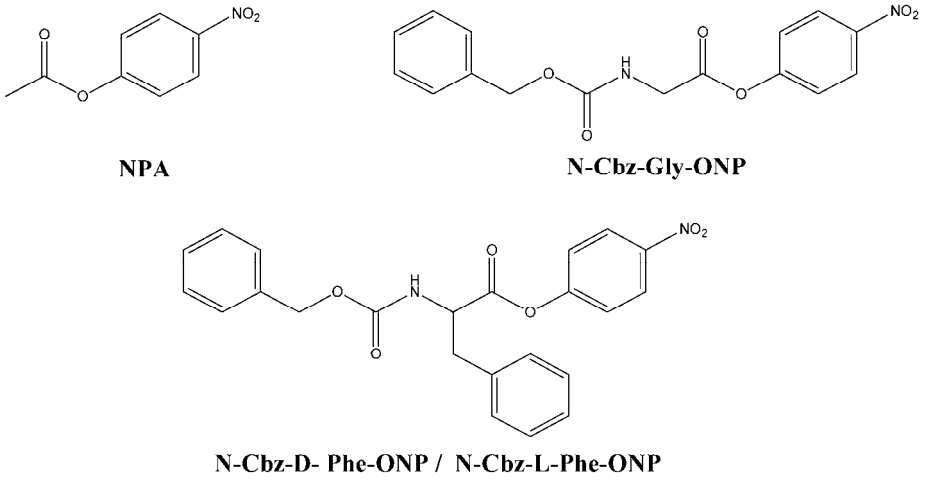


Chart 1. Chemical structures of esters employed as substrates in kinetic studies.

For comparison the reactivity of thiobenzyl alcohol was studied under similar conditions and from these results we obtained the second-order rate constant for **NPA** cleavage by the mercapto anion k_2=18.6±1.5 $M^{-1}s^{-1}$ and pK_a=9.8±0.1. Slightly increased pK_a value in 20 % MeCN as compared to that in water is an expected result due a lower dielectric constant of the mixed solvent. The reactivity of mercapto anions towards activated esters depends on their basicity: the slope of the Bronsted plot for thiols with pK_a>8 is small (β=0.27 for **NPA**), but for more acid thiols β=0.84.[9] Thus the difference in pK_a values for **3** and thiobenzyl alcohol ΔpK_a=0.34 corresponds to a difference in rate constants by just 20% and one may conclude that the reactivity of doubly deprotonated **3** is exactly the same as expected for a mercapto anion of such basicity. On the other hand, the rate constant expected for the monoprotonated **3** in accordance with its basicity is only 0.013 $M^{-1}s^{-1}$, an order of magnitude lower than experimentally measured k_{2H}. A possible explanation of this difference will be given below.

The absence of any specific effect of the macrocycle on the reactivity towards a simple substrate agrees with very low affinity of tubocurarine and *N*,*N*'-dibenzylated *S*, *S*-(+)-tetrandrine to neutral benzene derivatives.[8,11b] We expected, however, that more hydrophobic esters may show a higher affinity to the macrocycle and for this reason chose nitrophenyl esters of *N*-protected enantiomers of phenylalanine (see Chart 1). In addition, the use of such substrates as amino acid esters could allow one to test possible enantioselectivity of hydrolysis by a chiral thiol **3**.

Table 1 collects observed rate constants corrected for the background hydrolysis for the cleavage of D- and L-enantiomers of *p*-nitrophenyl esters of *N*-Cbz phenylalanine (***N*-Cbz-D-Phe-ONP** and ***N*-Cbz-L-Phe-ONP)** as well as a non-chiral substrate ***N*-Cbz-Gly-ONP** in the presence of **3** at pH 9.0. These esters are *ca*. one order of magnitude more reactive than **NPA** and this allowed us to study the reaction kinetics in a wider concentration range of the nucleophile, in particular at lower concentrations of **3**. With all three substrates the dependence of k_{obs} on the

concentration of **3** is approximately quadratic at low concentrations, but becomes more linear at higher concentrations, as illustrated graphically in Figure 3 for the thiolysis of ***N*-Cbz-Gly-ONP**. This behavior may be attributed to the self-association of **3** in such a way that associated, probably dimeric, species are much more reactive than the monomeric species. The existence of at least two different types of reactive species is also evident from relative reactivities of the macrocycle toward different substrates at low and high concentrations of **3**: with 0.01 mM **3** the cleavage of ***N*-Cbz-D-Phe-ONP** is faster than that of ***N*-Cbz-L-Phe-ONP** and cleavage of both substrates is *ca*. 4 times faster that that of ***N*-Cbz-Gly-ONP**, but with 0.2 mM **3** the enantioselectivity for ***N*-Cbz-D-Phe-ONP** and ***N*-Cbz-L-Phe-ONP** is inverted and the cleavage of these substrates proceeds only some 20-30 % faster than that of ***N*-Cbz-Gly-ONP**.

Table 1. Observed first-order rate constants (s^{-1}) corrected for the background hydrolysis for the cleavage of *p*-nitrophenyl esters of *N*-Cbz amino acids in the presence of **3** at pH 9.0

[**3**], mM	*N*-Cbz-L-Phe-ONP	*N*-Cbz-D-Phe-ONP	*N*-Cbz-Gly-ONP
0.01	5.1×10^{-4}	7.4×10^{-4}	1.7×10^{-4}
0.02	0.00248	0.00247	0.00146
0.05	0.0126	0.0112	0.00832
0.1	0.0307	0.0306	0.0231
0.2	0.0802	0.0658	0.0561

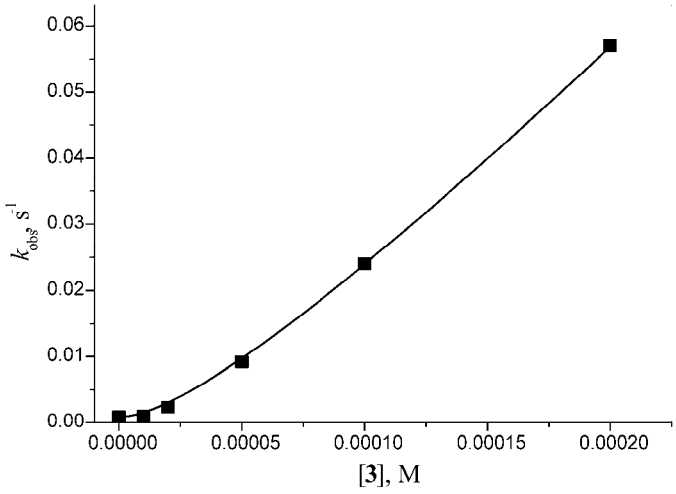


Figure 3. Observed first-order rate constants (s^{-1}) for the cleavage of ***N*-Cbz-Gly-ONP** *vs.* concentration of **3** at pH 9.0. The solid line is the fitting curve in accordance with the equation (6).

The self-association of bis-benzylated tetrandrine, a dicationic analog of **3** lacking mercapto groups, has been demonstrated previously.[11a] The dimerization constant K_D=42 M^{-1} at

0.1 M ionic strength was rather low, but with neutral **3** it may be much higher due to the absence of electrostatic repulsion between monomeric units. Qualitatively a significant self-association of **3** is manifested in broadening of NMR signals in water, but too low solubility of zwitterionic form of **3** in water did not allow us to study the process by this technique quantitatively.
The respective reaction scheme is shown below (S is the ester substrate, M is the monomeric form of **3** and D is the dimeric form of **3**):

$$2\ M \underset{}{\overset{K_D}{\rightleftharpoons}} D \qquad (3)$$

$$M + S \xrightarrow{k_M} \text{cleavage products} \qquad (4)$$

$$D + S \xrightarrow{k_D} \text{cleavage products} \qquad (5)$$

The kinetic equation, which corresponds to this mechanism takes the form ($[\mathbf{3}]_T$ is the total concentration of the macrocycle: $[\mathbf{3}]_T = [M] + 2[D]$):

$$k_{obs} = 0.5k_D[\mathbf{3}]_T + 0.125(2k_M - k_D)((1+8\ K_D[\mathbf{3}]_T)^{0.5}-1) \qquad (6)$$

Fitting of the experimental results to the equation (6) requires adjustment of three independent parameters. In order to reduce the number of parameters we analyzed first the results at lowest concentrations of **3** and estimated from these results the rate constant for the monomeric form. At low concentrations of **3** when the degree of dimerization is small and the concentration of free monomer is approximately equal to total **3** concentration, a simplified equation (7) is applicable.

$$k_{obs} = k_M[\mathbf{3}] + k_DK_D[\mathbf{3}]^2 \qquad (7)$$

In accordance with (7) a plot of $k_{obs}/[\mathbf{3}]$ *vs.* $[\mathbf{3}]$ should be linear with the intercept equal to k_M, as is seen from the equation (8).

$$k_{obs}/[\mathbf{3}] = k_M + k_DK_D[\mathbf{3}] \qquad (8)$$

Figure 4 shows the results from Table 1 at three lowest concentrations of **3** in the coordinates of equation (8) and Table 2 gives the respective second-order rate constants for the monomeric form.

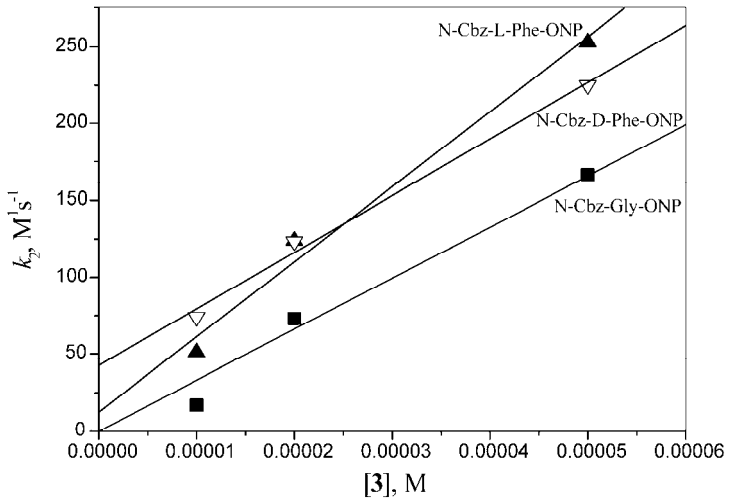


Figure 4. Second-order rate constants for the cleavage of of *p*-nitrophenyl esters of *N*-Cbz amino acids at low concentration of **3** at pH 9.0.

Table 2. Second-order rate constants ($M^{-1}s^{-1}$) for the cleavage of *p*-nitrophenyl esters of *N*-Cbz amino acids by monomeric (k_M) and dimeric (k_D) forms of **3** and by $PhCH_2SH$ at pH 9.0

	N-Cbz-L-Phe-ONP	*N*-Cbz-D-Phe-ONP	*N*-Cbz-Gly-ONP
k_M, $M^{-1}s^{-1}$	12.6±7.4	42.6±4.1	- [a]
k_D, $M^{-1}s^{-1}$ [b]	630±20	512±17	452±7
$k_{2obs}PhCH_2SH$	42.8±2.1	43.6±2.1	52.4±2.7

[a] Zero intercept in limits of errors.
[b] Per one thiol group.

Thus determined k_M values were used then as fixed parameters for the non-linear fitting of the entire concentration profiles to the equation (6) affording k_D values given in Table 2 and the average K_D=17000±2000 M^{-1}. The solid line in Figure 3 provides an example of the fitting curve in accordance with the equation (6). Increased by *ca*. two orders of magnitude K_D as compared with that for bis-benzylated tetrandrine corresponds to a decrease in the association free energy by *ca*. 12 kJ/mol, a value which reasonably agrees with expected ΔG for repulsion between two doubly charged species.[12]

Returning to results with **NPA** as a substrate one can see that they refer to the reactivity of the dimeric form of **3**, which predominates in the concentration range employed with this substrate. It is evident from Figure 3 that the dependence of k_{obs} on total concentration of **3** is linear above 0.2 mM and this explains the apparent second-order kinetics observed in **NPA** cleavage (see above).

Results in Table 2 show that the monomeric form of **3** has the reactivity toward amino acid substrates similar to that of thiobenzyl alcohol like in the case of **NPA**. The reactivity of the thiol

functionalized macrocycle is as expected higher than that of a macrocycle of a similar structure bearing phenolate groups: with (+)-tubocurarine zwitterion k_M equals 3.17 $M^{-1}s^{-1}$ and 2.02 $M^{-1}s^{-1}$ for ***N*-Cbz-L-Phe-ONP** and ***N*-Cbz-D-Phe-ONP** respectively.[8] The dimerization of **3** strongly enhances its reactivity, but reduces the enantioselectivity. Cleavage of phenylalanine esters by the monomeric form of the macrocycle is enantioselective (k_D/k_L=3.4), but for the dimeric form the enantioselectivity practically disappears and even is slightly inverted (k_D/k_L=0.8), see Table 2. Previously studied binding of *N*-Ac-Phe to the dication of bis-benzylated tetrandrine was not enantioselective.[11b] The size of the macrocycle cavity is too small to accommodate the phenyl ring and the binding is enantioselective only with smaller amino acids like alanine. [11b] The observed enantioselectivity for the monomeric form of **3** is manifested in a decreased reactivity for the L-enantiomer while the reactivity for the D-enantiomer is "normal", as compared to thiobenzyl alcohol. Probably there is some misfit for the transition state of the L-substrate.

The conformation of bis-benzylated tetrandrine was studied in details previously [11b] and we assumed that a reliable three-dimensional structure of **3** can be obtained starting from this structure after addition of CH_2SH groups to *para*-positions of benzylic rings and subsquent MM minimization. Molecular mechanics simulations were performed with Hypercube's Hyperchem package, using the mm+ force field as implemented in the 6.03 version of the program. Figures 2S and 3S (Supplementary Data) show the minimized structures of bis-benzylated tetrandrine and dication of **3**, which indeed are very much similar. The deprotonation of thiol groups leads to expected moving away of mercapto anions from each other and a minor change in the macrocycle conformation (Figure 4S, Supplementary Data). Then this simulated structure of the zwitterion was used to generate a possible structure of a dimeric form shown in Figure 5.

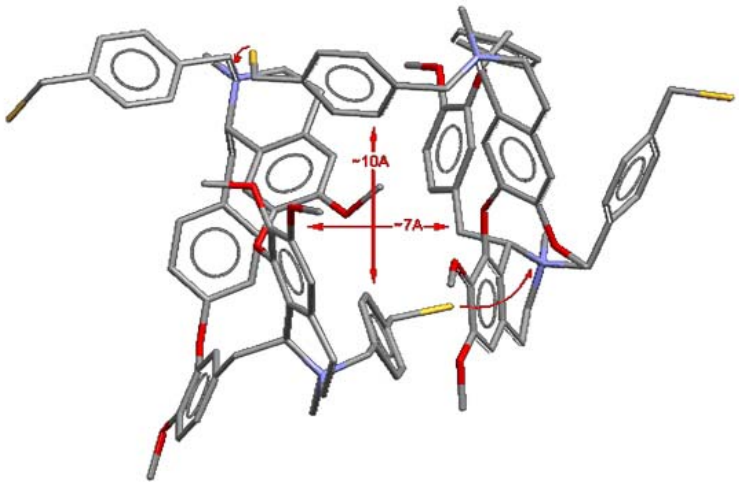


Figure 5. Simulated three-dimensional structure of the dimer of the zwitterionic form of **3** (hydrogens omitted). Red arrows show the intermolecular salt bridges and an approximate cavity size.

The dimerization of **3** involves ion pairing between thiolate anionic groups of one macrocycle and cationic ammonium groups of another one. In terms of this model one can explain both the increased reactivity of the dimeric form and strong differentiating in observed pK_a values for two thiol groups. Indeed, in accordance with estimated dimerization constant K_D=17000 M^{-1} the titration experiment (Figure 2) was performed with 60% of **3** as a dimer and therefore the decreased first pK_a can be attributed to thiol groups which form ion pairs within the dimer. At the same time, the dimerization creates a hydrophobic cavity of a size similar to that of α-cyclodextrin, Figure 5, which can accommodate one of phenyl groups of amino acid substrates. This pre-association with the substrate apparently is not strong since we do not observe a "saturation" in the cleavage kinetics up to the highest 0.2 mM concentration of **3** employed, which means that the respective binding constant should be lower than 10^3 M^{-1}. However, the pre-association with a binding constant of this order of magnitude would be sufficient to achieve an increased reactivity toward these substrates as compared to simple acetic acid ester **NPA**. In its turn, **NPA** can be included in the cavity via its nitrophenyl ring, but such included ester apparently will not be accessible to more basic and more reactive external thiol groups. However, this inclusion can explain the observed enhanced reactivity of less basic thiol groups of **3** toward **NPA** (see above).

Conclusions

Chemical modification of *S*,*S*-(+)-tetrandrine allowed us to obtain a new chiral cyclophane–type macrocycle bearing two thiol groups, which possesses fairly high nucleophilic reactivity toward activated esters. Although the enantioselectivity of ester cleavage is low, the macrocycle can discriminate between esters of different hydrophobicity. The kinetic study of ester cleavage indicates that the reactive form of the macrocycle is its deprotonated form, which undergoes a self-association affording a dimeric species and that these dimeric species are responsible for increased reactivity with more hydrophobic substrates. An important aspect of this study is an observation that quaternization of amino groups of the parent alkaloid leads to formation of a single stereoisomer. Thus further application of this class of natural compounds as chiral building blocks for preparation of new semi-synthetic receptors seems promising.

Experimental Section

General Procedures. In order to minimize problems from oxidation, all data were obtained in solutions of freshly boiled water. Melting points were taken using an electrothermal melting point apparatus and are uncorrected. Ultraviolet/Visible spectra were obtained with a Hewlett Packard 8453 spectrophotometer. NMR spectra were recorded on UNITY INOVA 400 and 500 MHz VARIAN spectrometers. Chemical shifts (δ) are reported in ppm relative to

tetramethylsilane and *J* values are given in hertz. Mass spectra were recorded on a high resolution VG 70-SE spectrometer in FAB technique. Least-squares fitting of titration and kinetic results were performed with the Microcal Origin 5.0 program.

Materials. *S*,*S*-(+)-Tetrandrine, thiolacetic acid, 1,8-diazabicyclo[5.4.0]undec-7-ene, α,α'-dibromo-p-xylene, thiobenzyl alcohol, *p*-nitrophenyl acetate, *N*-carbobenzyloxy-glycine *p*-nitrophenyl ester, and D- and L-enantiomers of *p*-nitrophenyl esters of *N*-carbobenzyloxy phenylalanine, solvents, inorganic salts and components of buffer solutions were purchased from Aldrich and Sigma and used without further purification. Silica gel 60 used on column chromatography and silica gel (with fluorescent indicator) coated on aluminium sheets for analytical thin layer chromatography (TLC) were supplied by Merck. All aqueous solutions were prepared in purified (Milli-Q Reagent Water System) water.

Determination of pK_a values. Spectrophotometric pH-titration of **3** was performed with 0.08 mM **3** in equimolar (0.032 M) mixture of acetate, phosphate and borate buffers. Nitrogen flushing was used at each step of the experiment.

Kinetic methods. Kinetics of the cleavage of esters were studied spectrophotometrically by the appearance of the 4-nitrophenolate anion at 400 nm in 20% vol. acetonitrile, 25 ºC and ionic strength 0.1 M sodium chloride. Buffers were 0.032 M acetate, phosphate, borate, trizma base and bis-tris propane. The reaction was initiated by addition of 50 µl of the substrate stock solution prepared in MeCN (typically 1 mM) to 2.45 ml of thiol solution at desired pH placed into the thermostatted cell of a spectrophotometer. The majority of experiments were performed with high excess of the nucleophile over the substrate provided the first-order reaction conditions and the respective rate constants were calculated by the integral method. With most diluted thiol solutions rate constants were calculated from the initial rates.

Compound 1 ($C_{10}H_{11}BrOS$). To a solution of α,α'-dibromo-*p*-xylene (1 g, 3.79 mmol) in THF (25 ml) was slowly added at room temperature, a solution of thiolacetic acid (0.437 g, 5.74 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.84 g, 5.52 mmol) in THF (5 ml). The resulting mixture was stirred for 10 h at rt. The solid precipitate was filtered off and the filtrate was removed on a rotary evaporator with vacuum. The residue was chromatographed on silica gel column eluted with hexane-acetone (20:1) to give 0.206 g of compound **1** (21%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.38 (s, 3H); 4.12 (s, 2H); 4.5 (s, 2H); 7.28 (m, 4H).

Compound 2 ($C_{58}H_{64}O_8N_2S_2Br_2$). To a solution of *S*,*S*-(+)-tetrandrine (0.203 g, 0.326 mmol) in acetone (60 ml) a solution of product **1** (0.21 g, 0.811 mmol) in acetone (20 ml) was added and the mixture was refluxed for 16 h. The solid precipitate was washed with acetone and dried with vacuum affording **2** (0.298 g, 80%). MP: 200-204 ºC. FAB-MS: *m/z* 490.4 ([**2**-2Br] – $2Br^-$, $\mathbf{2}^{2+}$). ^{1}H NMR (DMSO-d_6, 500 MHz): A single set of signals for all hydrogens was observed, indicating the formation of a single stereoisomer. Partial assignment of signals in DMSO-d_6 was made by comparison with the spectra of tetrandrine in the same solvent (and with the spectra of previously obtained *N*,*N*′-dibenzylated *S*,*S*-(+)-tetrandrine):[11a] Signals for N-CH_3 and O-CH_3 found at δ 2.57 (s, 3H); 3.17 (s, 3H); 3.32 (s, 3H); 3.46 (s, 3H) and 3.8 (6H), (while signals for

the same protons of natural tetrandrine are at δ 2.18, 2.52, 3.05, 3.28, 3.68 and 3.81). Signals for hydrogens of methyl units in the thiol protecting groups are at δ 2.35 (s, 3H) and 2.40 (s, 3H). Nine of the ten aromatic protons of cyclophane structure of **2** are in the range 6.08-7.12: δ 6.08 (s, 1H); 6.44 (d, *J* 1.5, 1H); 6.55 (dd, *J* 1.5 and 8.5, 1H); 6.75 (dd, *J* 3.0 and 8.5, 1H); 6.81 (s, 1H); 6.95 (dd, *J* 3.0 and 8.5, 1H); 6.99 (d, *J* 8.5, 1H); 7.09 (dd, *J* 1.5 and 8.5, 1H) and 7.12 (s, 1H). Finally, in the range 7.24-7.6 the spectra showed a complex set of signals for hydrogens of the two benzyl units plus the tenth aromatic hydrogen of the cyclophane structure.

Compound 3. A mixture of a 0.2 M solution of NaOH (150 ml) and 0.2 g of **2** (0.175 mmol) were introduced into a flask equipped with a magnetic stirrer and nitrogen inlet tube.[13] The mixture was stirred at room temperature under nitrogen for 3 h and then concentrated hydrochloric acid was added dropwise to the stirred mixture until pH was adjusted about 4. The solution was concentrated until a considerable amount of a light pink solid appeared. The solid was filtrated, recrystallized in ethanol and dried in vacuum to give **3**, in quantitative yield. MP: 200-204 ºC. FAB-MS: *m/z* 448.6 ([**3**-2Cl] – $2Cl^-$, $\mathbf{3}^{2+}$). ^{1}H NMR spectra were recorded in CD_3OD because of rapid oxidation of thiol groups to the disulfide in DMSO-d_6 and were similar to that for **2** in DMSO-d_6 but, as expected, no signals for methyl units of the thiol protecting groups were observed (found for **2**, in DMSO-d_6, at 2.35 and 2.40); N-CH_3 and O-CH_3 hydrogens were found at δ 2.63 (s, 3H), 3.41 (s, 3H), 3.6 (s, 3H), 3.8 (s, 3H) and 3.84 (s, 6H). Signals for nine of the ten aromatic hydrogens of cyclophane structure of **3** are at δ 6.20 (s, 1H); 6.57 (s, 1H); 6.65 (dd, *J* 3.0 and 8.5, 1H); 6.82 (s, 1H); 6.87 (dd, *J* 3.0 and 8.5, 1H); 6.96 (dd, *J* 3.0 and 8.5, 1H); 6.98 (s, 2H) and 7.17 (s, 1H). Signals for the tenth aromatic hydrogen of cyclophane structure and those for hydrogens of both benzyl units are in the range 7.36-7.60.

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